Oxazolidinone Mediated Sequence Determination of One Bead One Compound Cyclic Peptide Libraries

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Supporting Information

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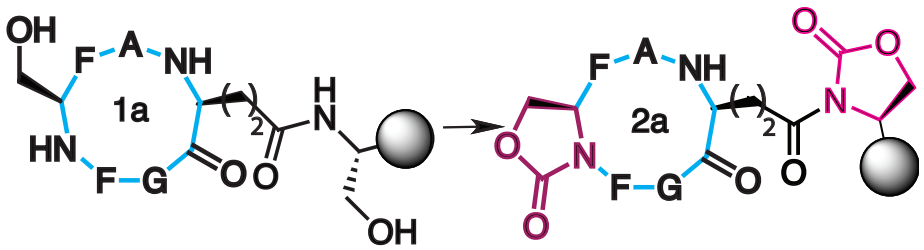
Table S1. Optimization of the activation of cyclic peptide *cyc*(Gly-Phe-Ser-Phe-Ala-Glu)-Ser-*Rink* **1a** on solid support.[a]



entry	DSC (equiv)	temp. (°C)	time (h)	conversion (%)[b]	
				2a	**2a'**
1	10	rt	15	50	50
2	15	rt	15	75	25
3	20	rt	15	>99	-
4	20	rt	6	90	10
5	20	65	6	ND	ND
6	20	65	15	ND	ND

[a]Cyclic peptide **1a** (50 mg) was reacted with DSC (10-20 equiv), DIEA (10-20 equiv) and a crystal of DMAP in DMF for (3-15 h). [b]Conversion to desired two oxazolidinone-containing cyclic peptide cyc(GF-Oxd-FAE)-Oxd **2a** and one oxazolidinone-containing cyclic peptide **2a'** was calculated from the absorbance at 220 nm using HPLC after release from solid support (Supporting Information). ND = not detected.

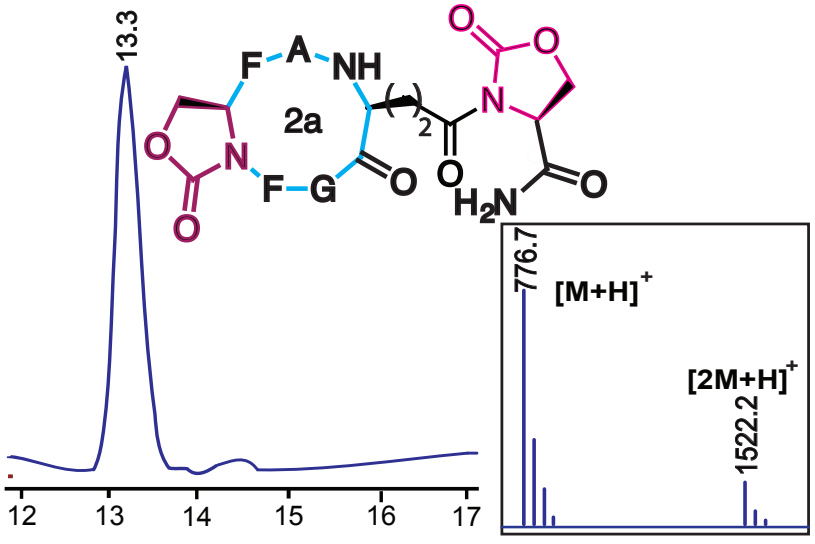
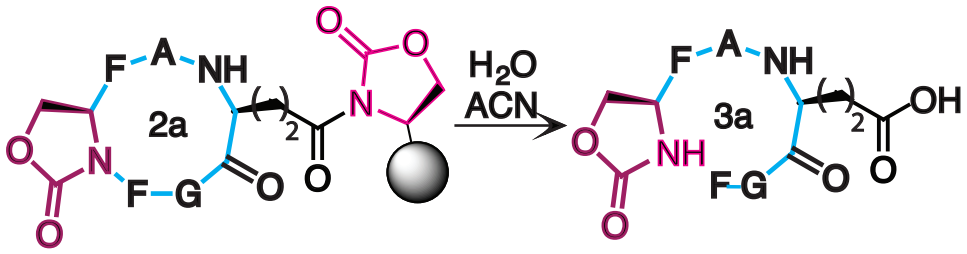


Figure S1. HPLC and MS of *cyc*(GF-*Oxd*-FAE)-*Oxd* **2a**

Table S2. Optimization of the reaction conditions for dual ring-opening/cleavage of cyclic peptide **2a** to its linear counterpart **3a** on solid support.[a]



entry	base	temp. (°C)	time (h)	conv.(%)[b]
1	-	rt	15	10
2	DIEA	rt	4	70
3	DIEA	rt	8	97
4	DIEA	rt	15	99
5	DIEA	65	4	>99
6	DIEA	65	2	99

[a]Activated cyclic peptide **2a** (50 mg) was treated with water:ACN (1:1) 1 mL and DIEA (40 μL). [b]Conversion and release of resulting linear peptide *Oxd*-FAEGF **3a** into the solution was calculated from the absorbance at 220 nm using HPLC (Supporting Information).

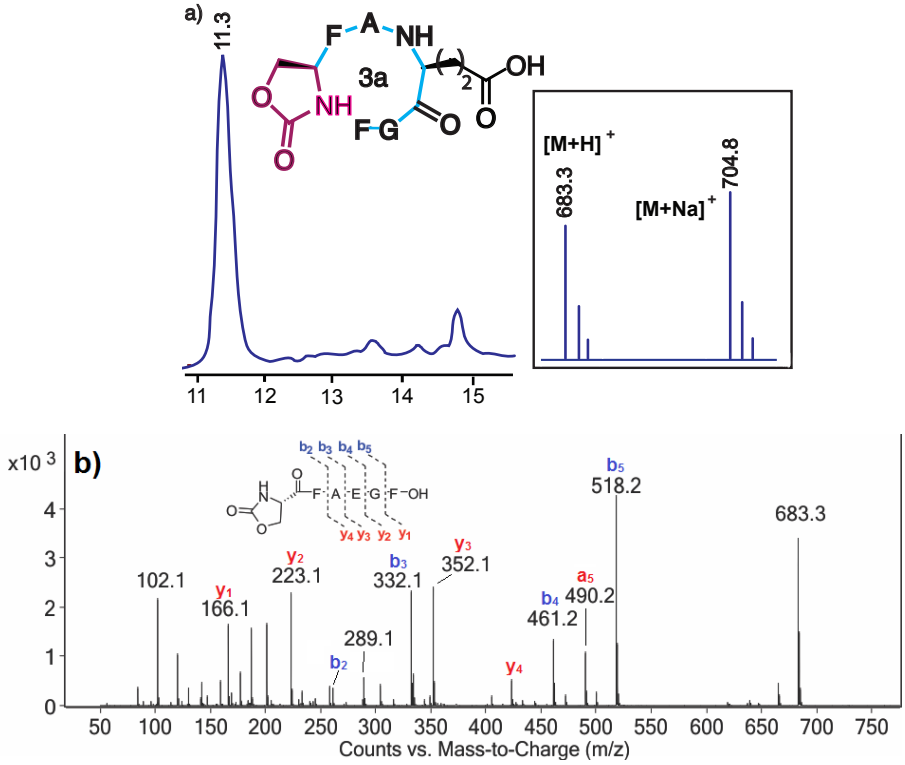
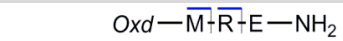
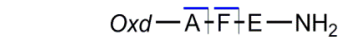
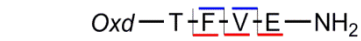
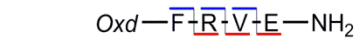
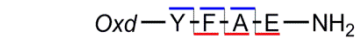
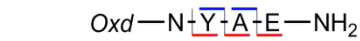
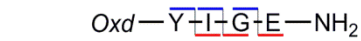
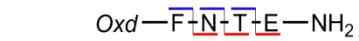
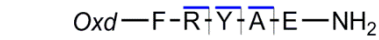
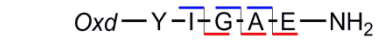
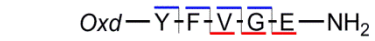
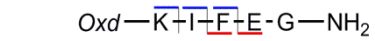
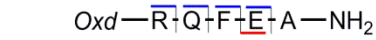
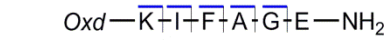
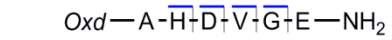
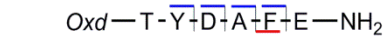
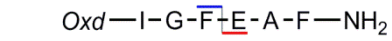
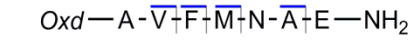
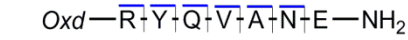


Figure S2. a) HPLC and MS of linear peptide *Oxd*-FAEGF **3a,** b) MS/MS spectra of peptide *Oxd*-FAEGF.

Table S3. High throughput screening of various cyclic peptides with varying ring sizes and composition on beads.

entry	cyclic peptide sequence	MS/MS sequence coverage[a]	calculated[b] $[M+H]^+$	observed[b] $[M+H]^+$	conversion (%)[c]
1	SMRE		547.2293	547.2303	>99
2	SAFE		478.1932	478.1918	>99
3	STFVE		607.2722	607.2716	>99
4	SFRVE		662.3257	662.3265	>99
5	SYFAE		641.2566	641.2561	>99
6	SNYAE		608.2311	608.2335	>99
7	SYIGE		593.2566	593.2584	99
8	SFNTE		622.2467	622.2461	>99
9	SVRFAE		733.3628	733.3651	>99
10	SFRYAE		797.3577	797.3595	>99
11	SNYAAE		679.2682	679.2676	>99
12	SYIGAE		664.2937	664.2947	99
13	SYFVGE		726.3093	726.3115	99
14	SKIFEG		705.3566	705.3576	>99
15	SHVDEF		758.3104	758.3105	>99
16	STLDEF		736.3148	736.3156	>99
17	SRQFEA		762.3529	762.3531	99
18	SKIFAGE		776.3937	776.3944	99
19	SAHDVGE		739.3006	739.2987	99
20	STYDAFE		857.3312	857.3307	>99
21	STFKRVE		891.4683	891.4704	>99
22	SIGFEAF		795.3672	795.3683	>99
23	SRVDYGAE		921.4061	921.4053	>99
24	SAVFMNAE		893.3822	893.3811	>99
25	SKVRNYAE		496.2514[d]	496.2513[d]	99
26	SRYQVANE		496.2332[d]	496.2330[d]	99
27	SKARFGVE		459.7432[d]	459.7430[d]	99
28	SAQVFRFTE		1109.5374	1109.5393	99
29	SYTFNDKLE		1141.5160	1141.5174	>99
30	SRVKNGFEA		1032.5221	1032.5232	99
31	SYLFDWREK		1268.6058	1268.6067	>99
32	SGYVKDFRAE		1196.5695	1196.5672	99
33	SAYKFGPSAE		541.2511[d]	541.2519[d]	99
34	SATKFESRVE		589.7937[d]	589.7917[d]	>99

[a]MS/MS sequence coverage indicates observed y and b ions as right (red) and left (blue) cleavages, respectively. [b]Calculated and observed $[M+H]^+$ are for ring-opened peptides. [c]Reaction conditions: Conversion was determined by cleavage of the pre-treated resin under TFA cleavage conditions. [d]Doubly charged ions, $[M+2H]^2$

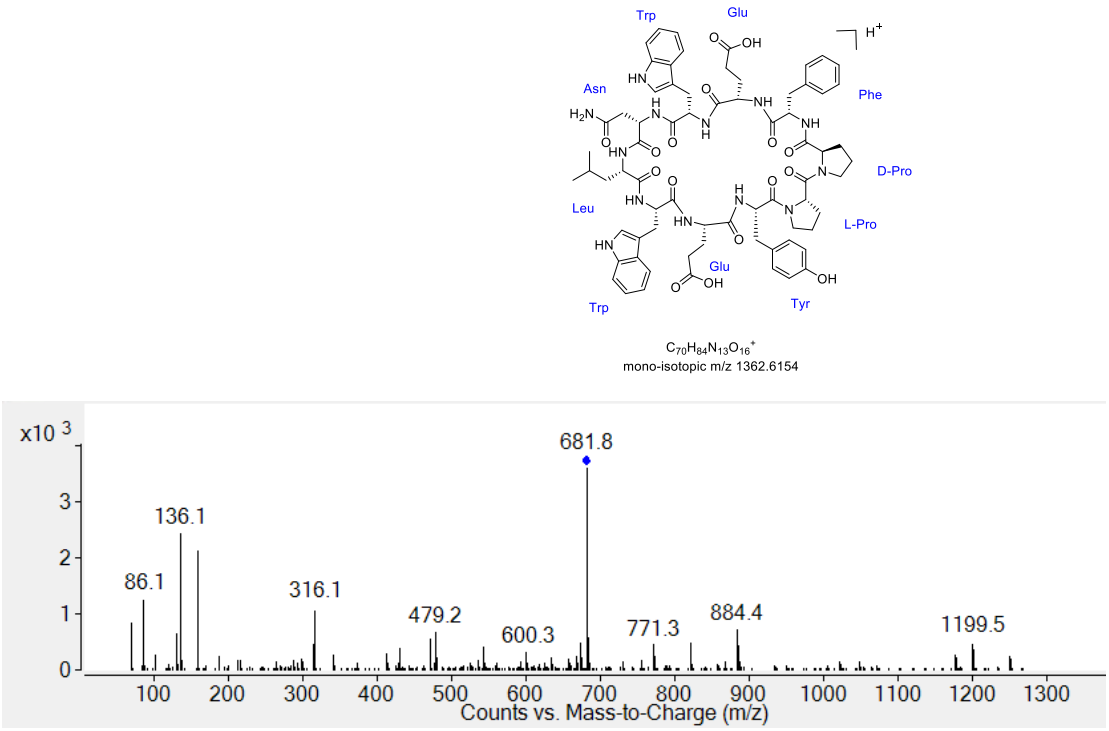


Figure S3. MS of cyclic peptide p53 analogue.

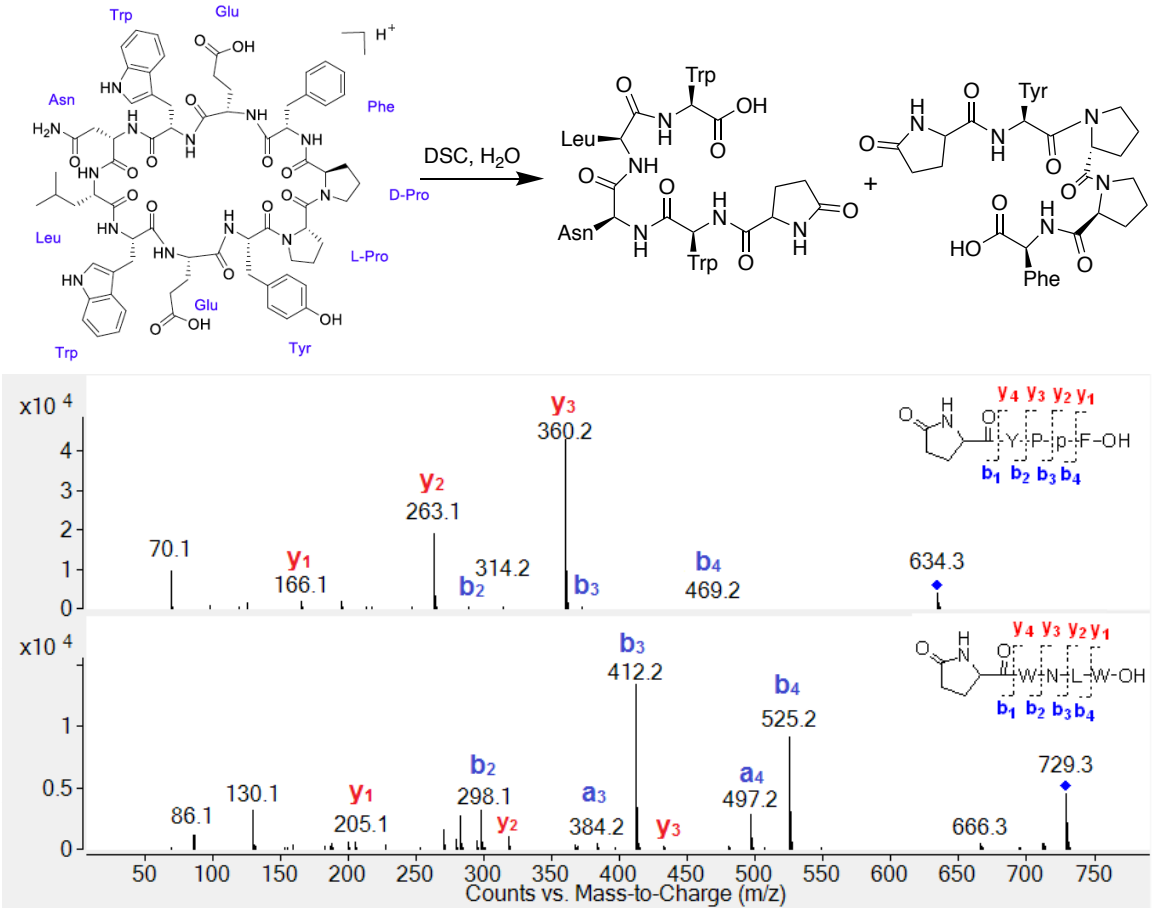


Figure S4. a) Reaction of cyclic peptide p53 analogue with DSC, DIEA, DMAP in DMF followed by hydrolysis. MS/MS spectra of cleaved fragments at N-terminus of Glu residues.

IV. General. All commercial materials (Sigma-Aldrich, Fluka, and Novabiochem) were used without further purification. All solvents were reagent or HPLC (Fisher) grade. Diethyl ether, CH_2Cl_2, and DMF were obtained from a dry solvent system (passed through column of alumina) and used without further drying. Conversions were obtained by comparison of HPLC peak areas of products and starting materials. HPLC was used to monitor reaction progress.

V. Materials. Fmoc-amino acids were obtained from Novabiochem (EMD Millipore Corporation, Billerica, Massachusetts) and CreoSalus (Louisville, Kentucky). Rink amide resin was obtained from ChemPep Inc (Wellington, Florida). N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) was obtained from CreoSalus (Louisville, Kentucky). N,N′-Disuccinimidyl carbonate (DSC) was obtained from Novabiochem (EMD Millipore Corporation, Billerica, Massachusetts). 4-Dimethylaminopyridine (DMAP) was obtained from Merck KGaA (Darmstadt, Germany). N,N-Dimethylformamide (DMF) was obtained from Macron Fine Chemicals (Center Valley, Pennsylvania). Acetonitrile, N,N- Diisopropylethylamine (DIEA), and N,N'-diisopropylcarbodiimide (DIC) were purchased from Novabiochem (EMD Millipore Corporation, Billerica, Massachusetts). Piperidine was purchased from Alfa Aesar (Ward Hill, Massachusetts). Trifluoroacetic acid (TFA) was purchased from VWR (100 Matsonford Road Radnor, Pennsylvania). Diethyl ether was obtained from Sigma-Aldrich (St. Louis, Missouri). Water was purified using a Millipore Milli-Q water purification system.

VI. Purification. Semi-preparative chromatography was performed using a Beckman Coulter equipped with a System Gold 168 detector, a 125P solvent module, and a 10 mm C-18 reversed-phase column. All separations involved a mobile phase of 0.1% formic acid (v/v) in water (solvent A) and 0.1% formic acid (v/v) in acetonitrile (solvent B). The semi-preparative HPLC method employed a linear gradient of 0–80% solvent B over 30 minutes at ambient temperature with a flow rate of 3.0 mL min^{-1}. The separation was monitored by UV absorbance at both 220 and 254 nm unless otherwise noted.

VII. Analytical Methods.

HPLC:
Peptide compositions were evaluated by high performance liquid chromatography (HPLC) on an Agilent 1200 series HPLC equipped with a 4.6 mm C-18 reversed-phase column. All separations used mobile phases of 0.1% formic acid (v/v) in water (solvent A) and 0.1% formic acid (v/v) in acetonitrile (solvent B). A linear gradient of 0–80% solvent B in 30 minutes at room temperature with a flow rate of 1.0 mL min^{-1} was used. The eluent was monitored by UV absorbance at 220 nm unless otherwise noted.

LC-MS:
Mass spectrometry to check reaction mixtures was performed using an Agilent 1100 Series

HPLC with MSD VL mass spectrometer using positive polarity electrospray ionization (+ESI).

HRMS:
HRMS data were recorded on an Agilent 1290 UHPLC with 6560 ion mobility Q-ToF mass spectrometer using positive polarity electrospray ionization (+ESI).

LC-IMS-MS/MS for sequencing peptides:
An Agilent 1290 UHPLC and 6560 ion mobility Q-ToF mass spectrometer using positive polarity electrospray ionization (+ESI) were used to sequence peptides. LC-IMS-MS conditions are provided in **Table S4**. Collision induced dissociation (CID) was used to generate MS/MS spectra using N_2 as collision gas and energies ramped from 20 to 60 V.

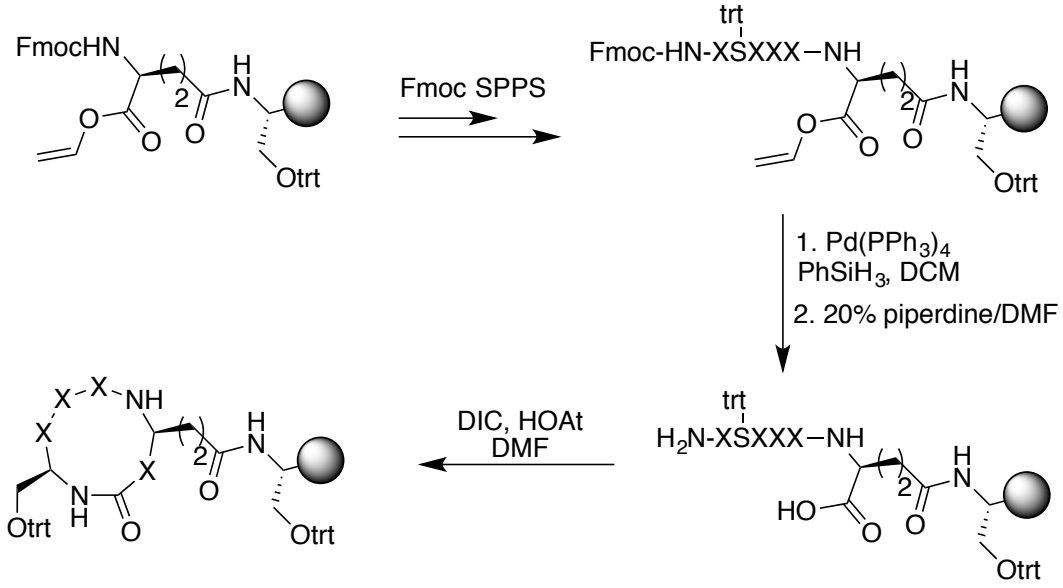
Table S4. LC-IMS-MS conditions

Parameter	Condition		
Column	Waters Acquity CSH C18, 1.7 μm particle size, 50 mm x 2.1 mm I.D.		
Column temperature	50°C		
Mobile phase A	0.1% formic acid in water		
Mobile phase B	acetonitrile		
Gradient	time (mins)	% A	% B
	0.00	95	5
	0.65	1	99
	0.75	1	99
	0.76	95	5
	1.00	95	5
Injection volume	1.0 μL		
Flow rate	1.0 mL/min		
Ionization	(+)ESI using Agilent's jet stream source		
Voltages	capillary = 3.5 kV, fragmentor = 175 V, nozzle = 1100 V		
Drying gas	N_2, 300°C, 10 L/min		
Nebulizer	45 psig		
Sheath gas	N_2, 275°C, 12 L/min		
Mass range	m/z 150 to 3200		
IM trap	fill time = 40,000 μs, release time = 150 μs		
Acquisition rate	frames = 1/s, IM transients = 19/frame, max drift time = 50 ms		

VIII. Fmoc Solid-Phase Peptide Synthesis.[1] Peptides were synthesized manually on a 0.25 mm scale using Rink amide, Wang, Chem Matrix and TentaGel resins. Fmoc was deprotected using 20% piperidine–DMF for 20 min to obtain a deprotected peptide-resin. Fmoc-protected amino acids (0.75 mm) were sequentially coupled on the resin using HBTU (0.75mm) and DIEA (0.75mm) for 2 h at room temperature. Peptides were synthesized using standard protocols.[1] Peptides were cleaved from the resin using a cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropyl silane:water for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting solid was purified by semi-preparative chromatography.

IX. General Procedure for Cyclization of Linear Peptides on Solid Support. To a peptide containing Glu(Oallyl)OH amino acid on the solid support (50 mg, 0.69 mm/g) was added a solution of Tetrakis(triphenylphosphine)palladium(0), $Pd(PPh_3)_4$ (20 mg), phenylsilane (72 μL) in DCM (3 mL), and the peptide was left on the shaker for 40 minutes. The resin was washed with DCM (3 X 2 min). The above reaction was repeated, and then the resin was washed with DCM (3 X 2 min), MeOH (3 X 2 min), and DMF (3 X 2 min). The palladium catalyst was removed from the resin by washing it with DIEA (2 X 2 min), followed by washing with DMF (2 X 2 min). Next, the peptide was treated with 20% piperdine/DMF to remove the Fmoc protecting group. Macrocyclization was achieved by exposing the resin to DIC, HOAt, and a catalytic amount of DMAP in DMF on a shaker for 15 h. The solution was drained, and the resin was washed with DMF. To confirm the complete macrocyclization, the Kaiser test was performed. Yellow bead coloration indicated absence of free amines, while blue coloration indicated incomplete macrocyclization. To further confirm formation of cyclic peptides, the resin was cleaved using a cocktail of 95:2.5:2.5 trifluoroacetic acid:triisopropyl silane:water (v/v/v) for 2 h. The resin was then removed by filtration, and the resulting solution was evaporated. Cyclic peptides were purified by semi-preparative chromatography and analyzed by MS.

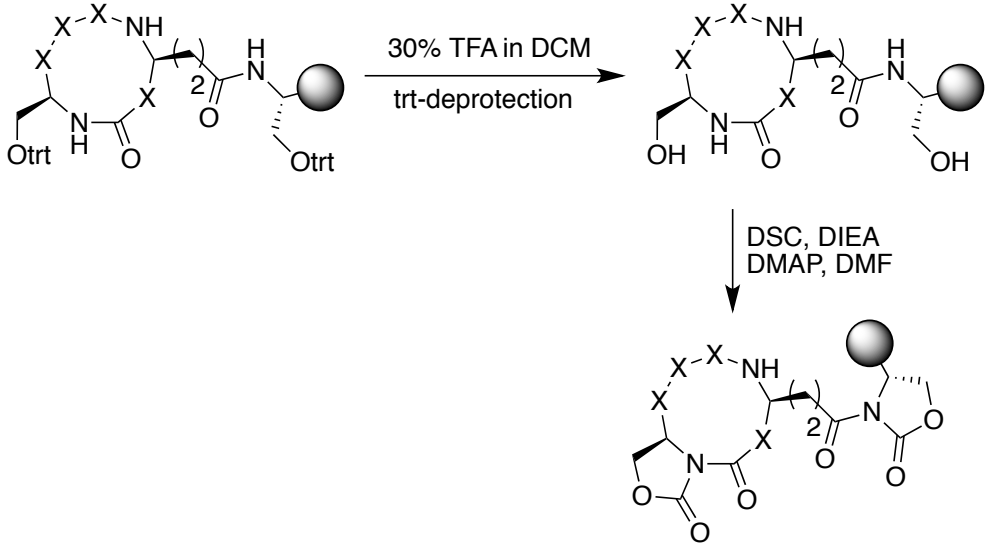
Scheme S1. Cyclization of linear peptides on solid support



X. General Procedure for Serine Activation to Cyclic Urethane Moiety within Cyclic Peptides on Solid Support: The trityl protecting groups on serine residues were selectively removed by exposing the peptide on solid support to 30% TFA in DCM (3 X 5 min). Both serine groups (one as a linker and the other within the macrocycle) were activated on solid support (25-100 mg, 0.25-0.69 mm/g) using a solution of DSC (25 equiv), DIEA (25 equiv), and a catalytic amount of DMAP in DMF on shaker for 17 h. The solution was drained, and the resin was washed with DMF, followed by peptide cleavage

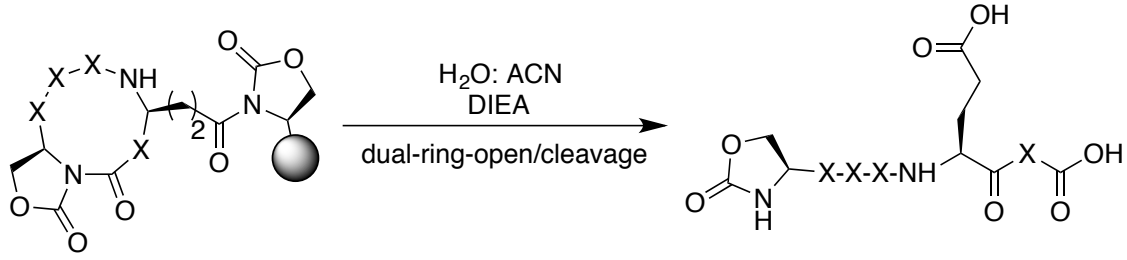
using a cocktail of 95:2.5:2.5 trifluoroacetic acid:triisopropyl silane:water (v/v/v) for 2 h. The resin was removed by filtration, and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting solid was purified by semi-preparative chromatography for MS analysis.

Scheme S2. Serine activation to cyclic urethane moiety within cyclic peptides on solid support



XI. General Procedure for Dual Ring-Opening/Cleavage of Cyclic Peptides on Solid Support for MS/MS Sequencing: A solution of 1 mL H_2O:ACN (1:1, v/v) and 40 μL of DIEA was added to the resin containing the activated cyclic urethane-containing macrocyclic peptide. This reaction was left on a heated shaker at 65 °C for 4 h. The resin was then filtered, followed by solvent removal under vacuum. The resulting ring-opened peptide acid was then sequenced by LC-IMS-MS/MS.

Scheme S3. Dual ring-opening/cleavage of cyclic peptides on solid support



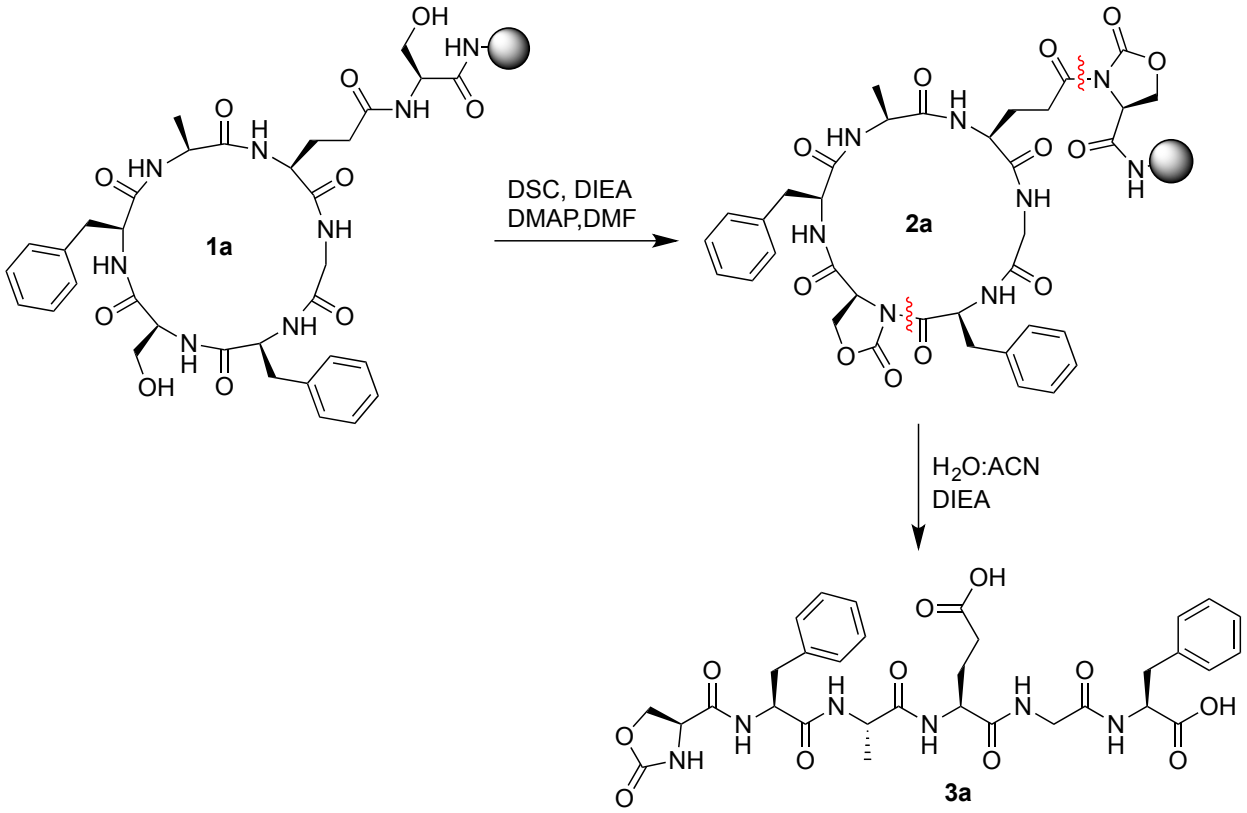
General procedure for macrocyclization in solution phase. Peptide was cleaved from the chlorotrityl resin with a 20% v/v HFIP solution in DCM. The DCM was evaporated, and the linear peptide was purified by chromatography. Cyclization of the linear peptide was carried out with 20 mg HOAt, 20 μL DIC in dry DMF per 2 mg of peptide. Macrocyclization was confirmed by MS analysis. Side chain protecting groups were then deprotected by addition of TFA:TES:water (95/2.5/2.5, v/v/v) and incubating the solution for 2 h at room temperature. The macrocyclic peptide was then purified by reversed-phase analytical HPLC.

General Procedure for the activation of glutamic acid in cyclic peptide in solution phase. To a 5-mL round-bottom flask containing peptide 2-20mg (1 equiv) in 0.5-2 mL dimethylformamide (DMF) was added a solution of DSC (15 equiv), DIEA (15 equiv) and crystal of DMAP in DMF (0.2-0.5 mL). The mixture was stirred at room temperature for 10 h. The reaction was concentrated under vacuum and resulting peptide was dissolved in water: acetonitrile (1:1) solution and purified by HPLC.

General Procedure for ring opening of modified cyclic peptide in solution phase. To a modified cyclic peptide, 1 mL phosphate buffer (pH 7.4) was added. The reaction was stirred at 25 °C and monitored by analytical HPLC at regular intervals followed by analysis of the ring opened peptide using LC-MS/MS.

XII. Peptide Characterization and HPLC Traces

Figure S5. Macrocyclic ring-opening and resin-cleavage of peptide **1a**, *cyc*(GFSFAE)-S-*Rink*



***cyc*(Gly-Phe-Ser-Phe-Ala-Glu)-Ser-CONH$_2$ (1a).** LC-MS: *m/z* 724.8 (calcd $[M+H]^+$ = 725.3), *m/z* 746.8 (calcd $[M+Na]^+$ = 747.3), Purity: >95% (HPLC analysis at 220 nm). Retention time: 10.19 min

***cyc*(Gly-Phe-*Oxd*-Phe-Ala-Glu)-*Oxd*-CONH$_2$ (2a).** LC-MS: *m/z* 776.7 (calcd $[M+H]^+$ = 777.2), *m/z* 798.7 (calcd $[M+Na]^+$ = 799.2), 1552.2 (calcd $[2M+H]^+$ = 1554.4), 1574.3 (calcd $[2M+Na]^+$ = 1576.4), Purity: >95% (HPLC analysis at 220 nm). Retention time: 13.5 min

***Oxd*-Phe-Ala-Glu-Gly-Phe (3a).** LC-MS: *m/z* 682.7 (calcd $[M+H]^+$ = 683.2), *m/z* 704.8 (calcd $[M+Na]^+$ = 705.2), Purity: >95% (HPLC analysis at 220 nm). Retention time: 11.3 min

Figure S6. HPLC trace and mass spectrum of peptide **1a**, *cyc*(GFSFAE)-S-$CONH_2$ after resin cleavage

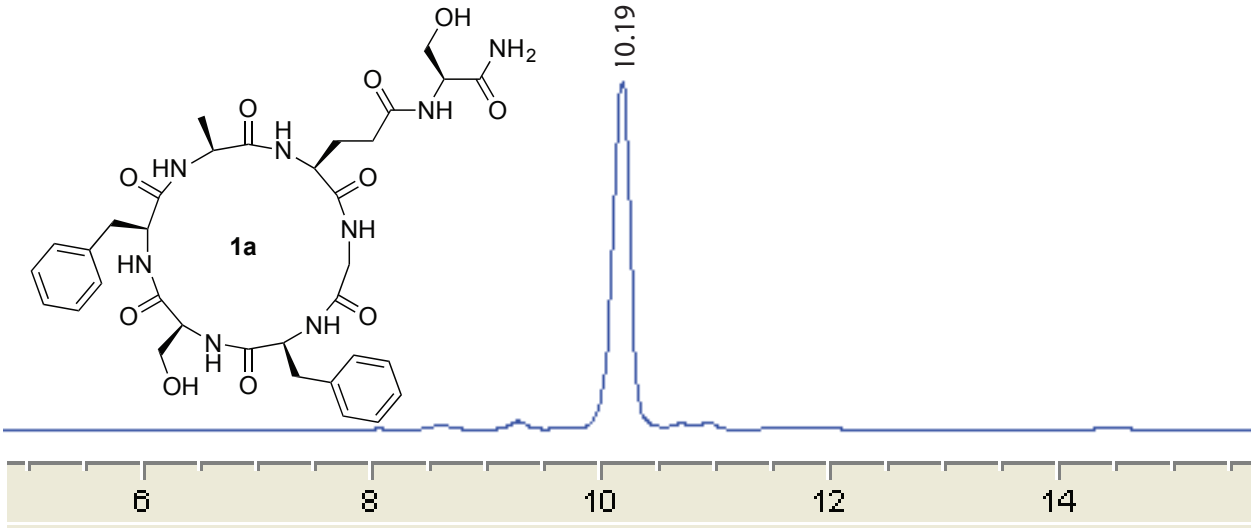


Figure S7. HPLC trace and mass spectrum of peptide **2a**, *Cyc*(GF-*Oxd*-FAE)-*Oxd*-$CONH_2$ after resin cleavage

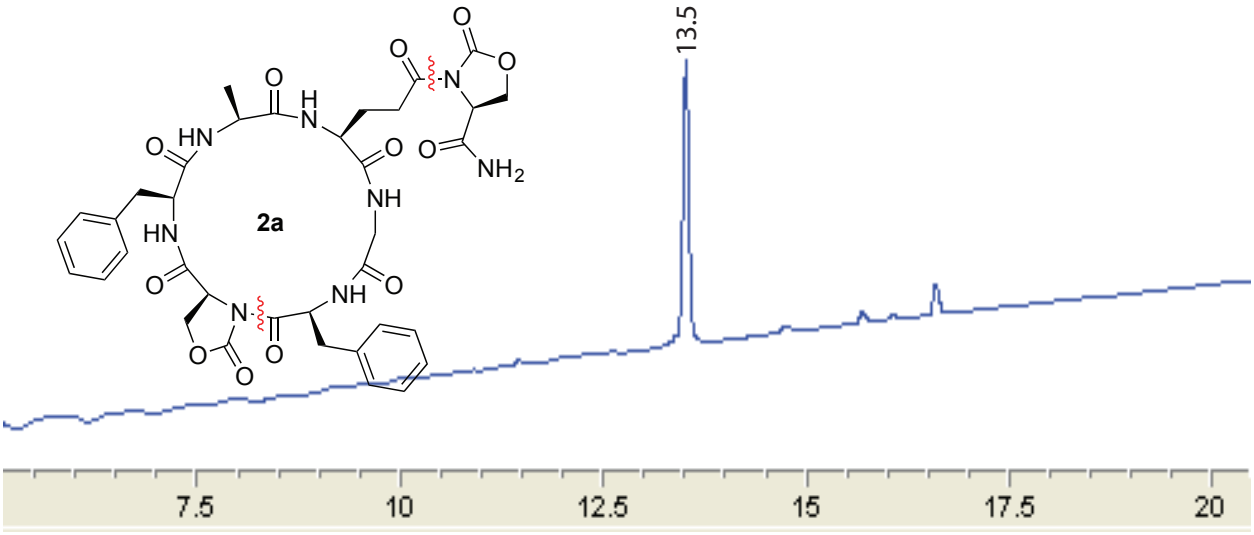


Figure S8. HPLC trace and mass spectrum of peptide **3a**, *Oxd*-FAEGF-OH

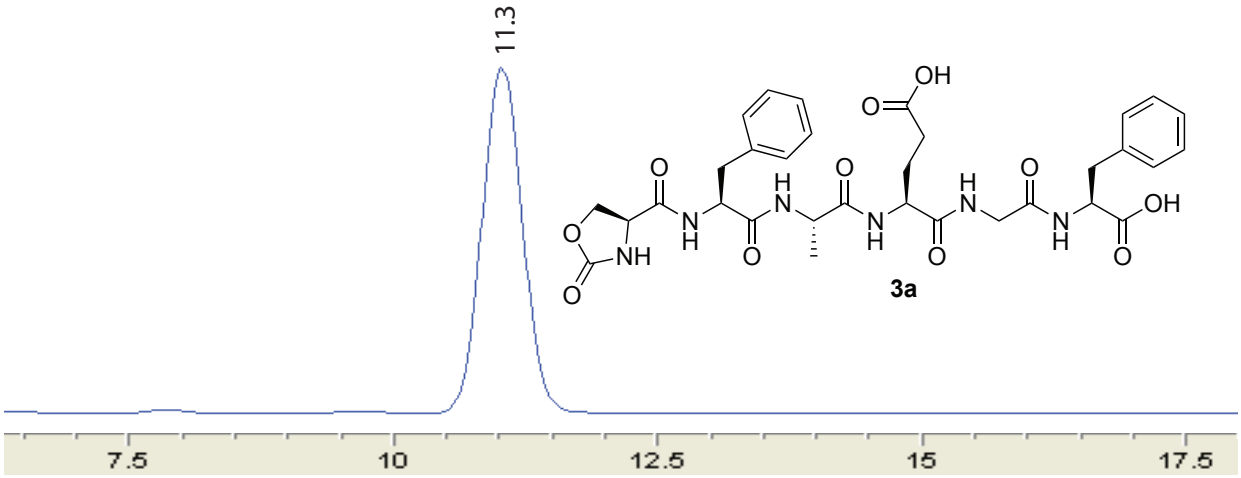


Figure S9. LC-IM-MS/MS data for linear peptide **3a**, *Oxd*-FAEGF-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 683.2671, Found $[M+H]^+$ = 683.2670

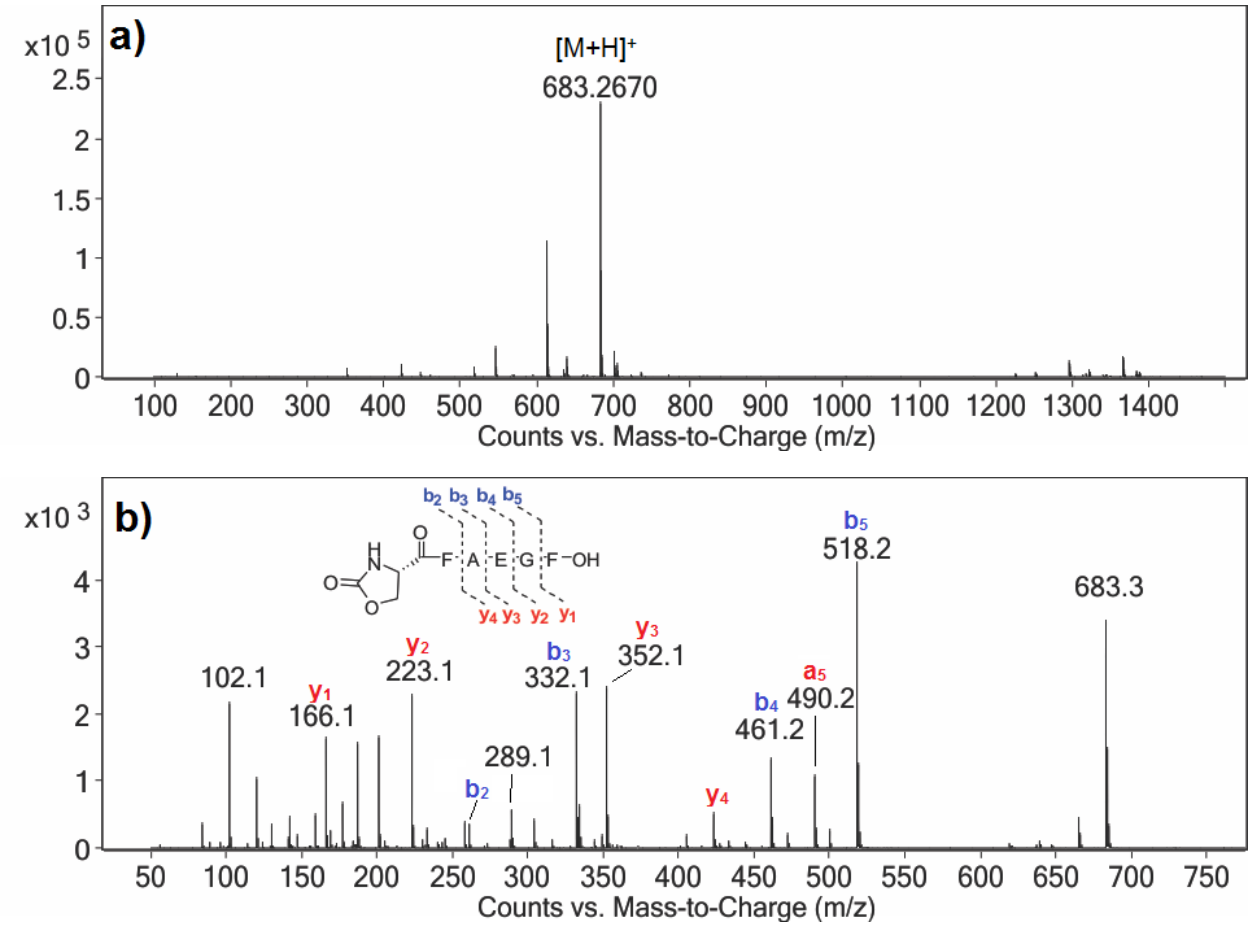
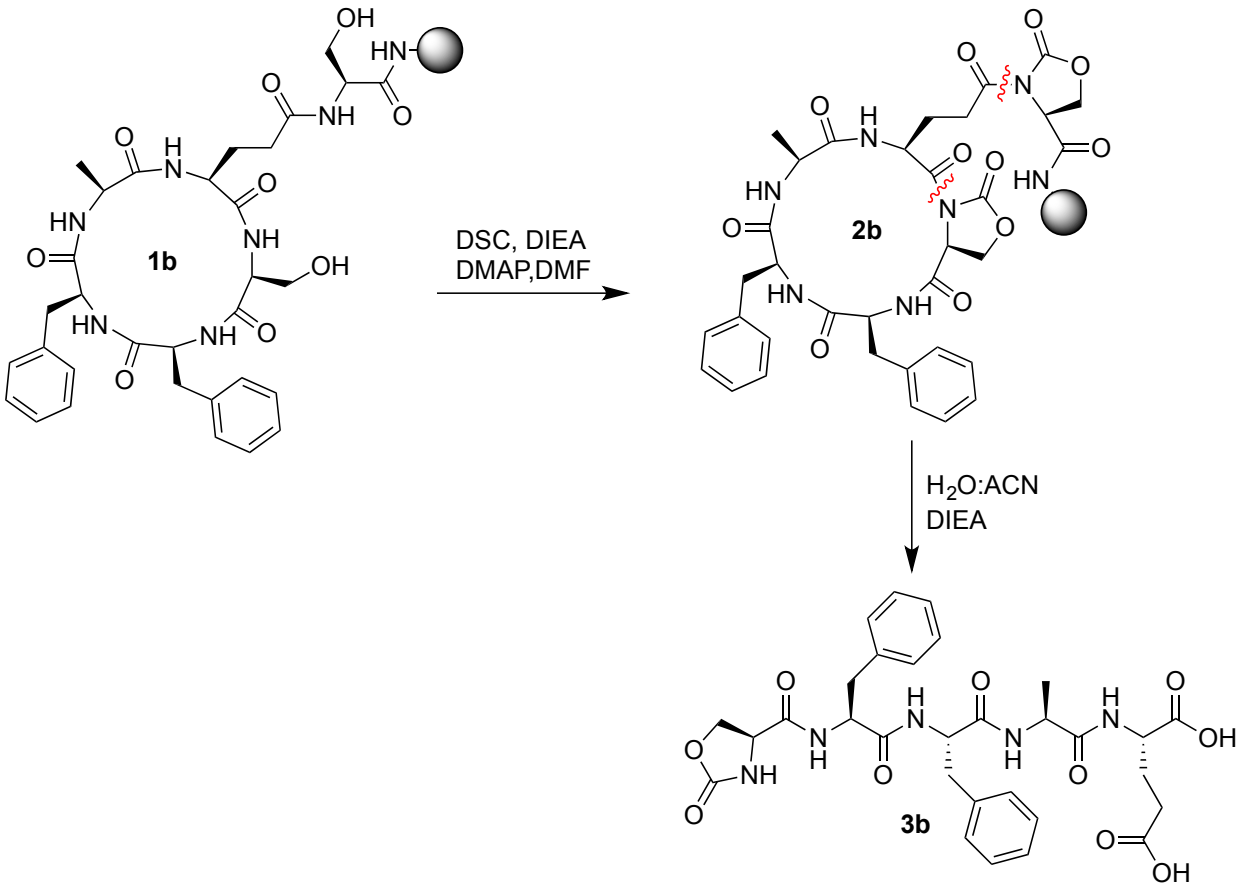


Figure S10. Macrocyclic ring-opening and resin-cleavage of peptide **1b**, *cyc*(SFFAE)-S-*Rink*



***cyc*(Ser-Phe-Phe-Ala-Glu)-Ser-CONH$_2$ (1b).** LCMS: *m/z* 668.0 (calcd $[M+H]^+$ = 668.3), *m/z* 690.3 (calcd $[M+Na]^+$ = 690.3), Purity: >95% (HPLC analysis at 220 nm). Retention time: 13.96

***Oxd*-Phe-Phe-Ala-Glu (3b).** LCMS: *m/z* 625.8 (calcd $[M+H]^+$ = 626.24), *m/z* 647.5 (calcd $[M+Na]^+$ = 648.24), Purity: >95% (HPLC analysis at 220 nm). Retention time: 11.71 min

Figure S11. HPLC trace and mass spectrum of peptide **1b**, *cyc*(SFFAE)-S-$CONH_2$ after resin cleavage

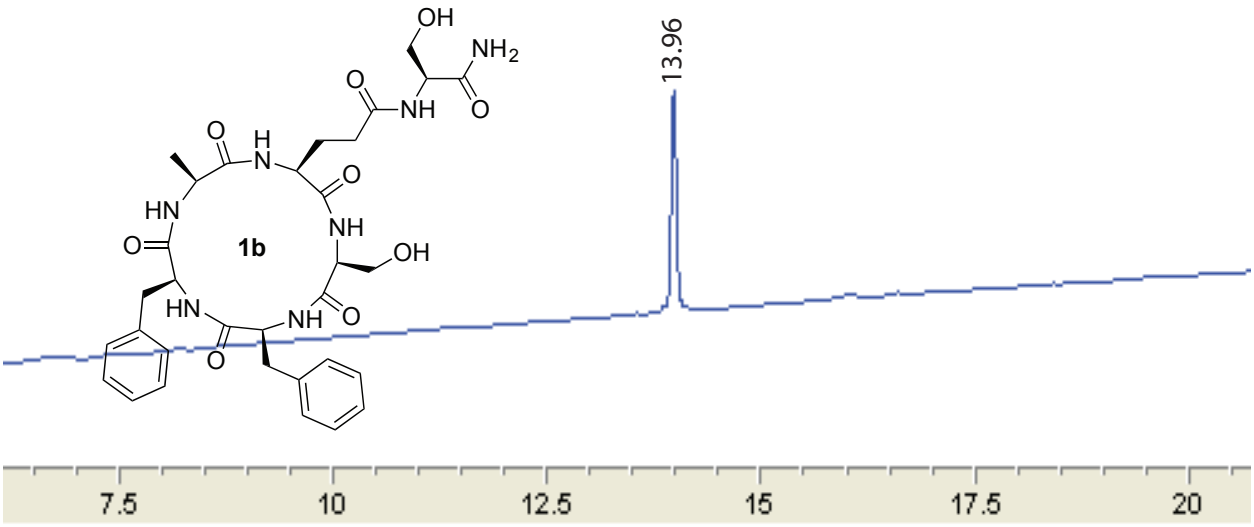


Figure S12. HPLC trace and mass spectrum of peptide **3b**, *Oxd*-SFFAE-OH

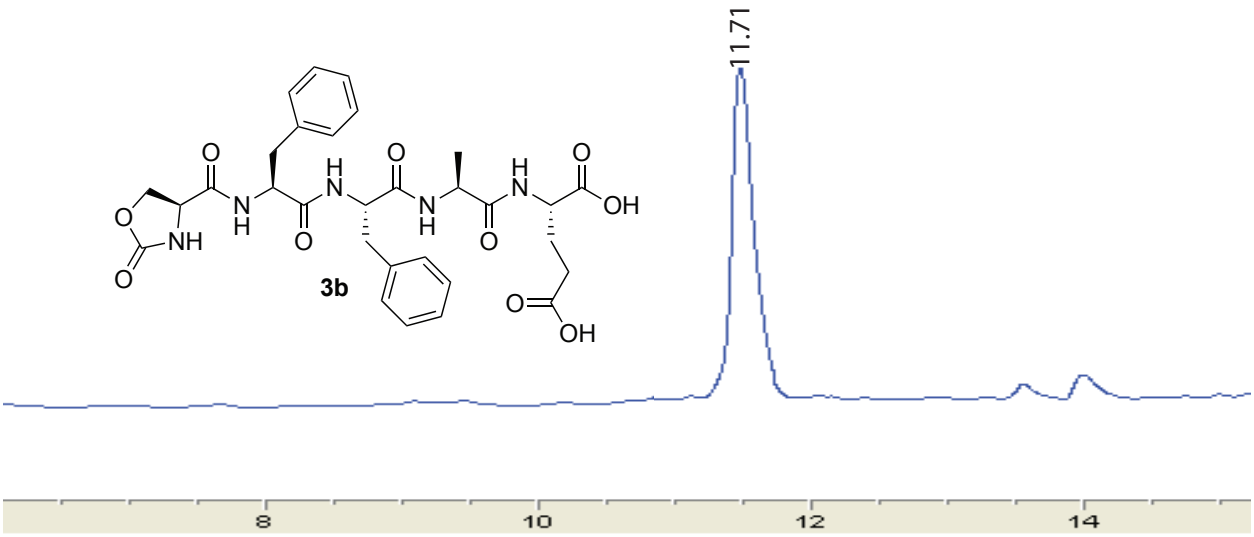


Figure S13. LC-IM-MS/MS data for linear peptide **3b**, *Oxd*-FFAE-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 626.2457, Found $[M+H]^+$ = 626.2450

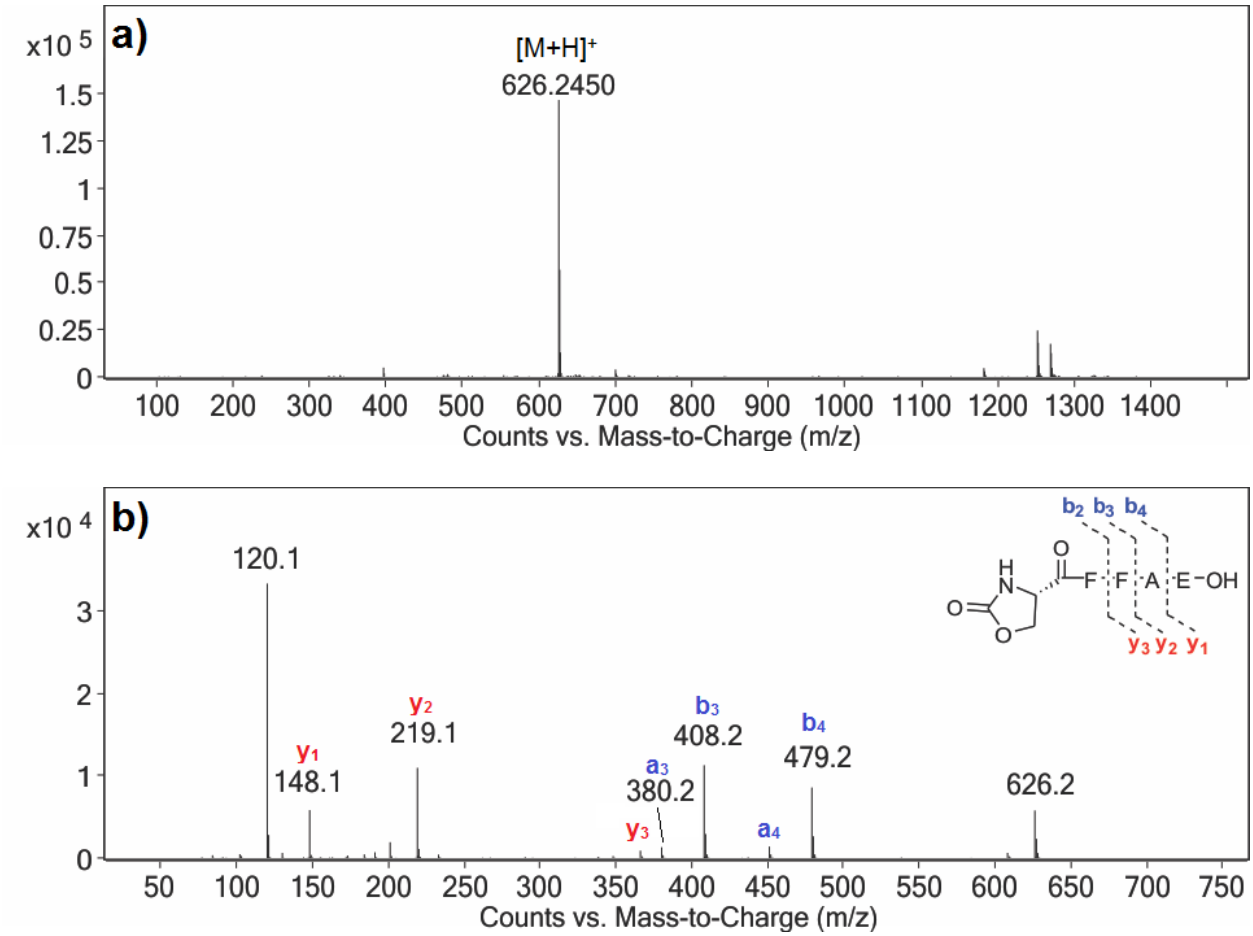
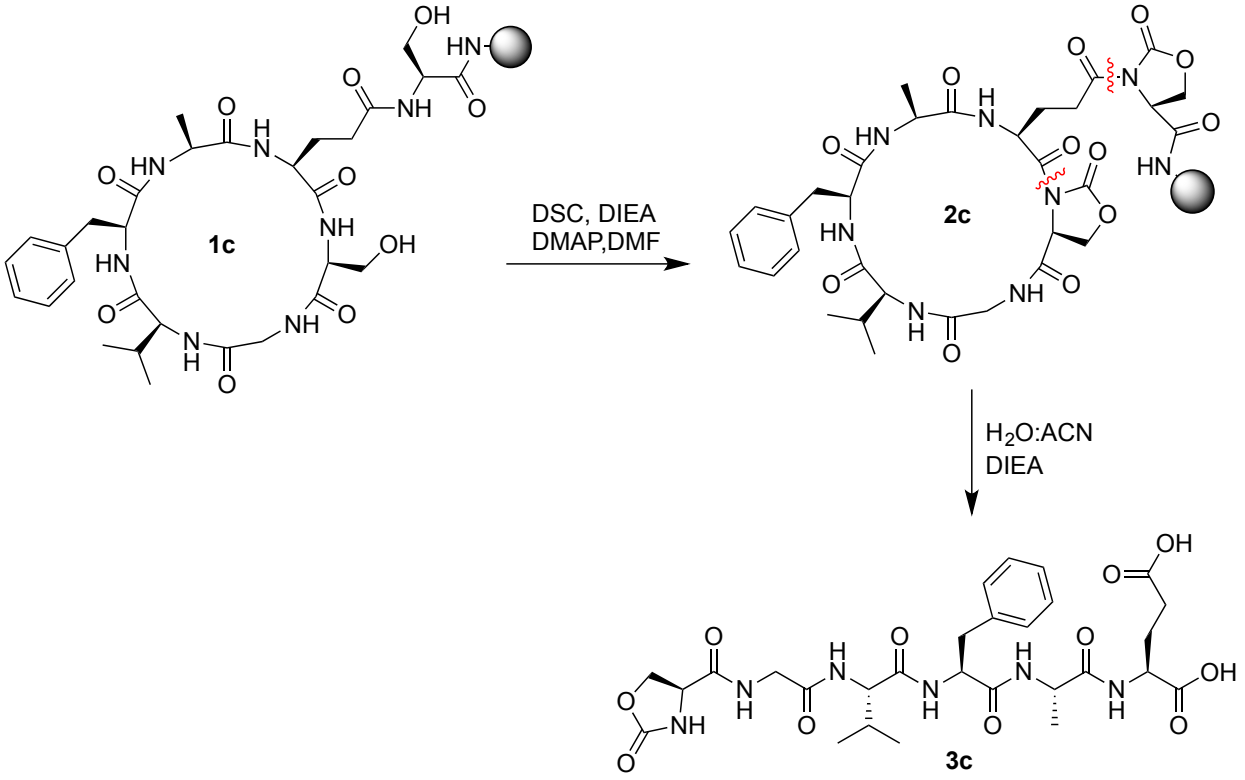


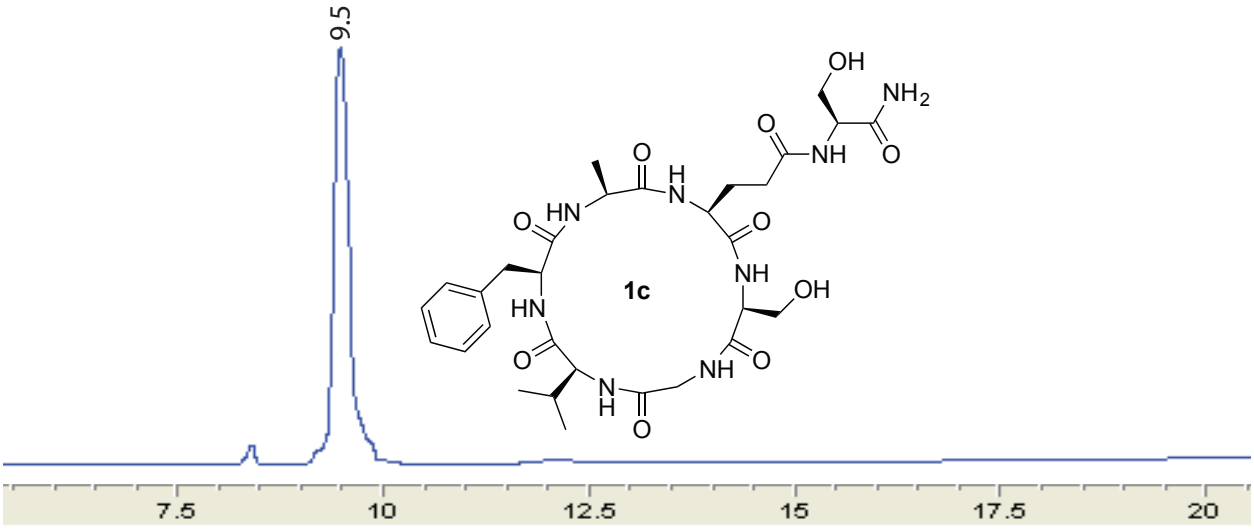
Figure S14. Macrocyclic ring-opening and resin-cleavage of peptide **1c**, *cyc*(SGVFAE)-S-*Rink*



***cyc*(Ser-Gly-Val-Phe-Ala-Glu)-Ser-CONH$_2$ (1c).** LCMS: *m/z* 676.70 (calcd $[M+H]^+$ = 677.3), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.5 min

***Oxd*-Gly-Val-Phe-Ala-Glu (3c).** LCMS: *m/z* 635.0 (calcd $[M+H]^+$ = 635.6), Purity: >95% (HPLC analysis at 220 nm). Retention time: 7.9 min

Figure S15. HPLC trace and mass spectrum of peptide **1c**, *cyc*(SGVFAE)-S-$CONH_2$ after resin cleavage



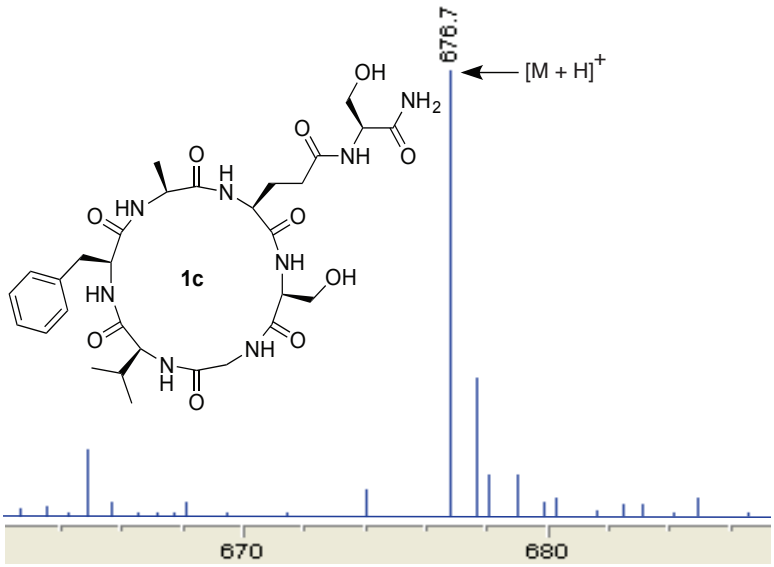


Figure S16. HPLC trace of peptide **3c**, *Oxd*-GVFAE-OH

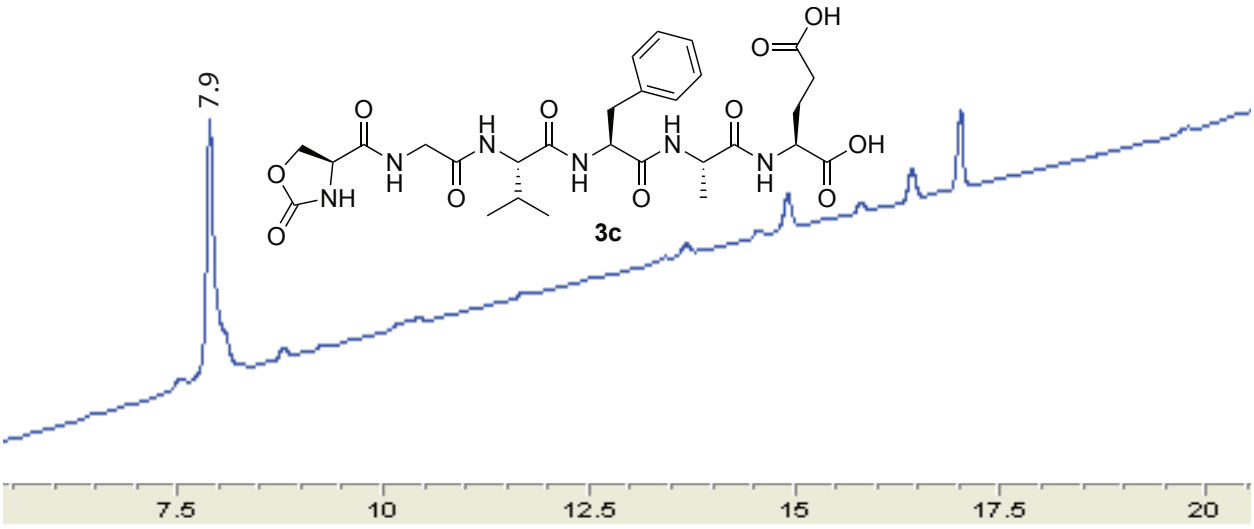
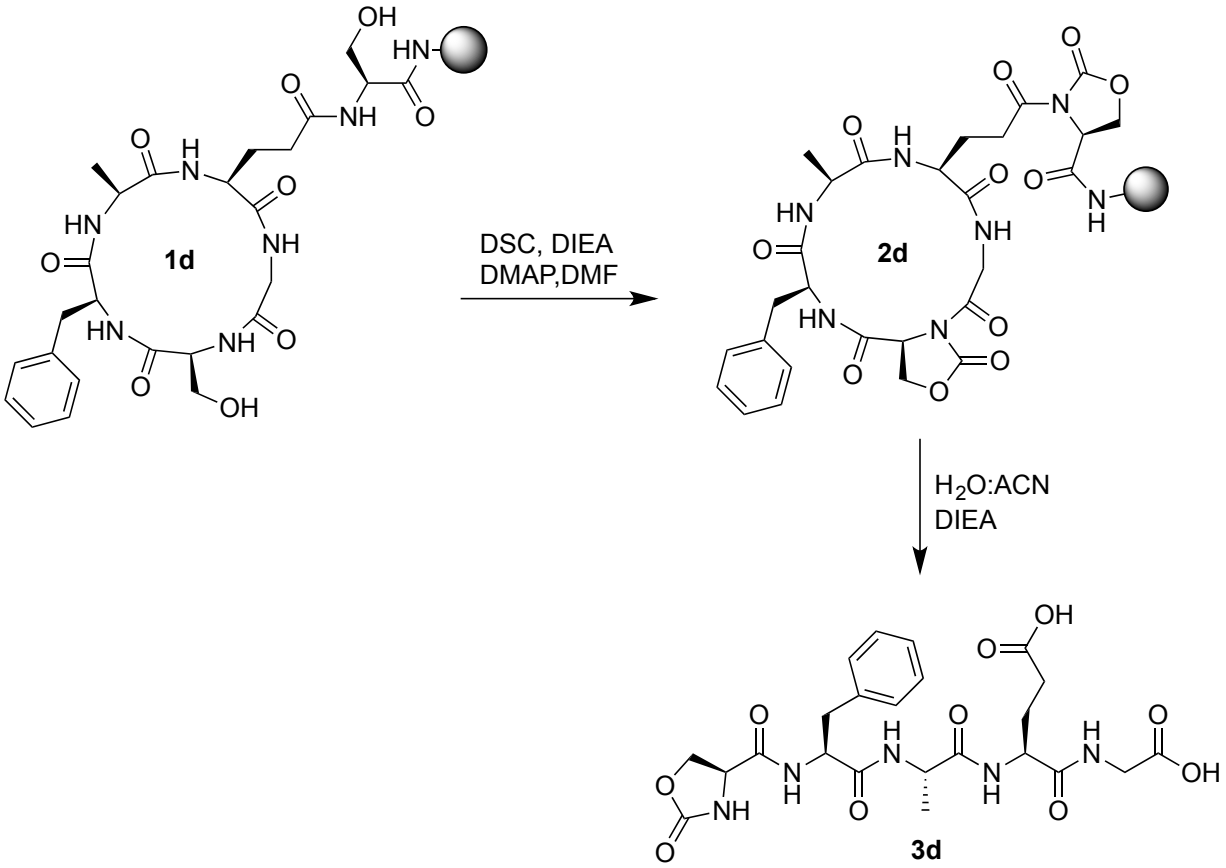


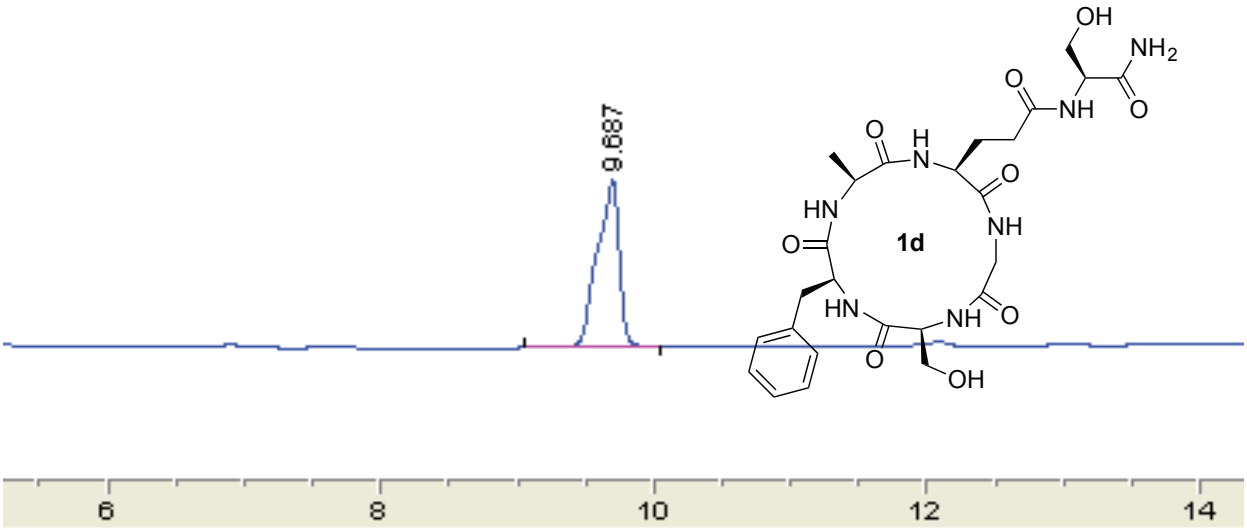
Figure S17. Macrocyclic ring-opening and resin-cleavage of peptide **1d**, *cyc*(GSFAE)-S-*Rink*



***cyc*(Gly-Ser-Phe-Ala-Glu)-Ser-CONH$_2$ (1d).** LCMS: *m/z* 578.0 (calcd $[M+H]^+$ = 578.2), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.68 min

***Oxd*-Phe-Ala-Glu-Gly (3d).** LCMS: *m/z* 536.0 (calcd $[M+H]^+$ = 536.1), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.55 min

Figure S18. HPLC trace and mass spectrum of peptide **1d**, *cyc*(GSFAE)-S-$CONH_2$ after resin cleavage



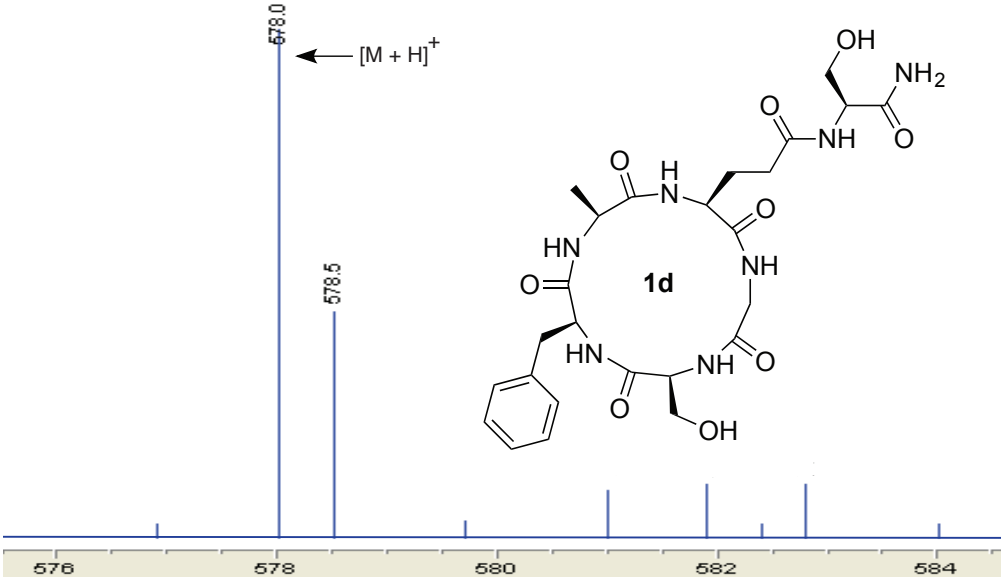


Figure S19. HPLC trace of peptide **3d**, *Oxd*-FAEG-NH_2

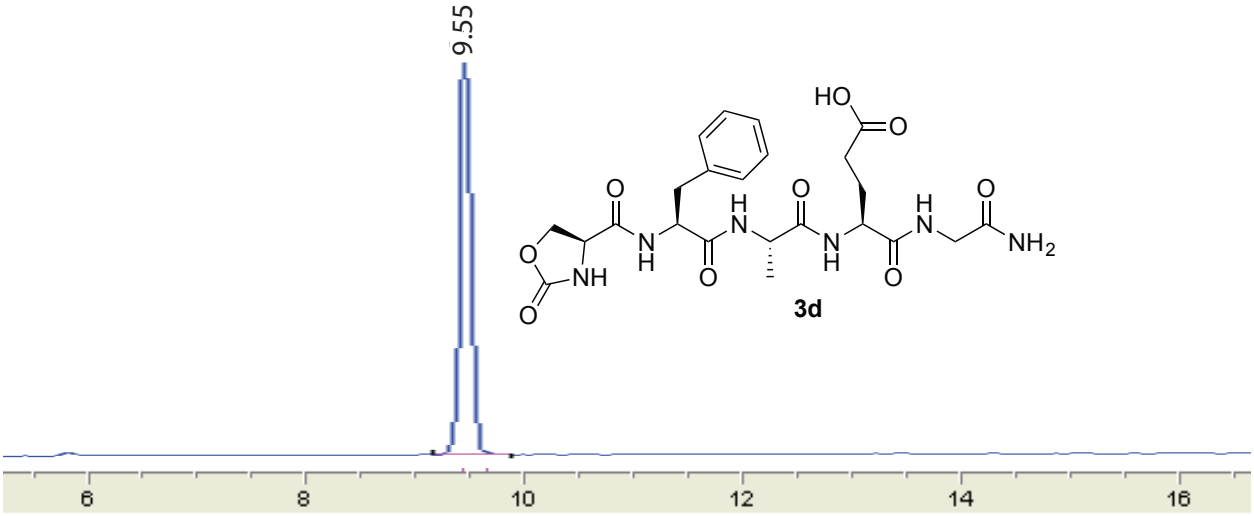
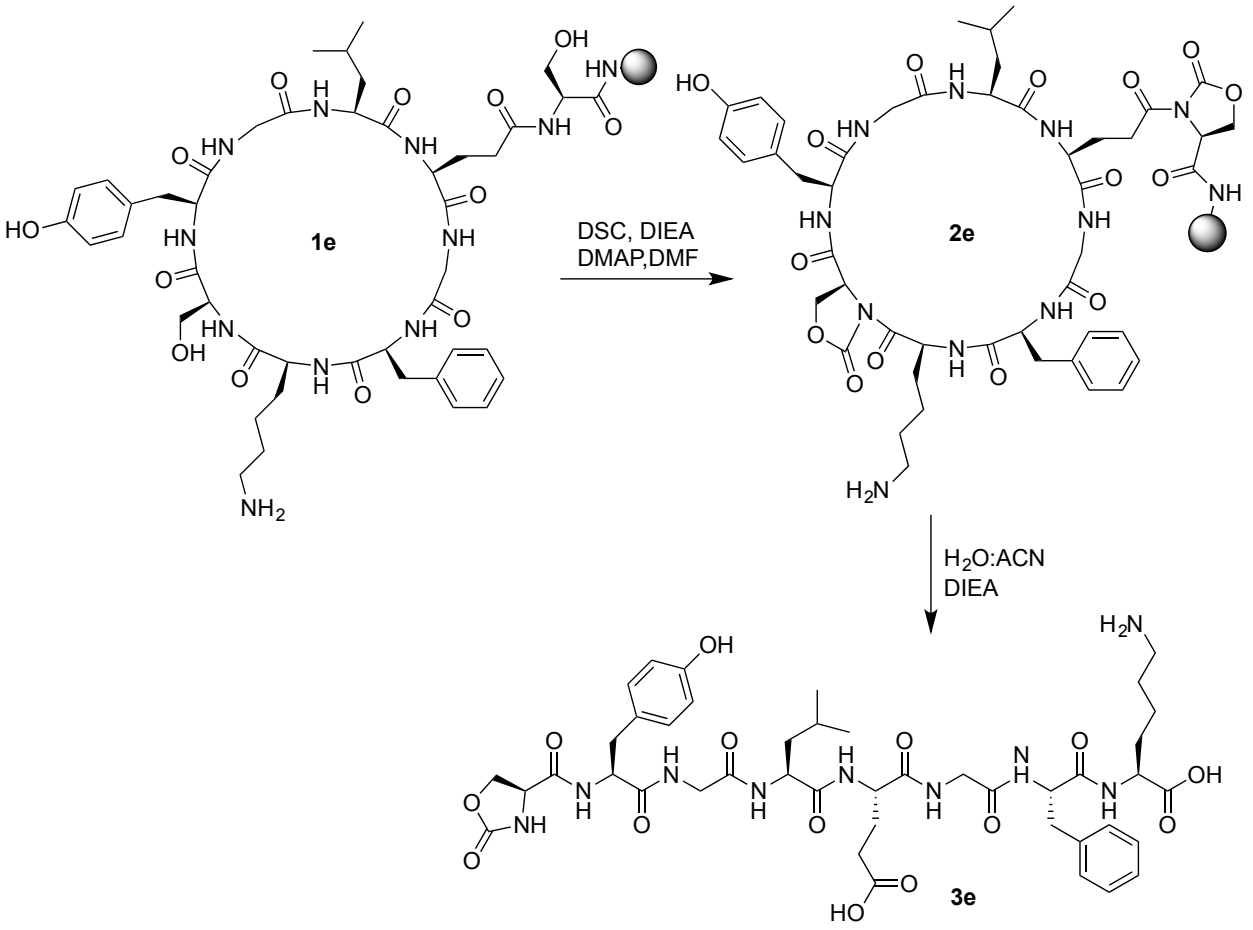


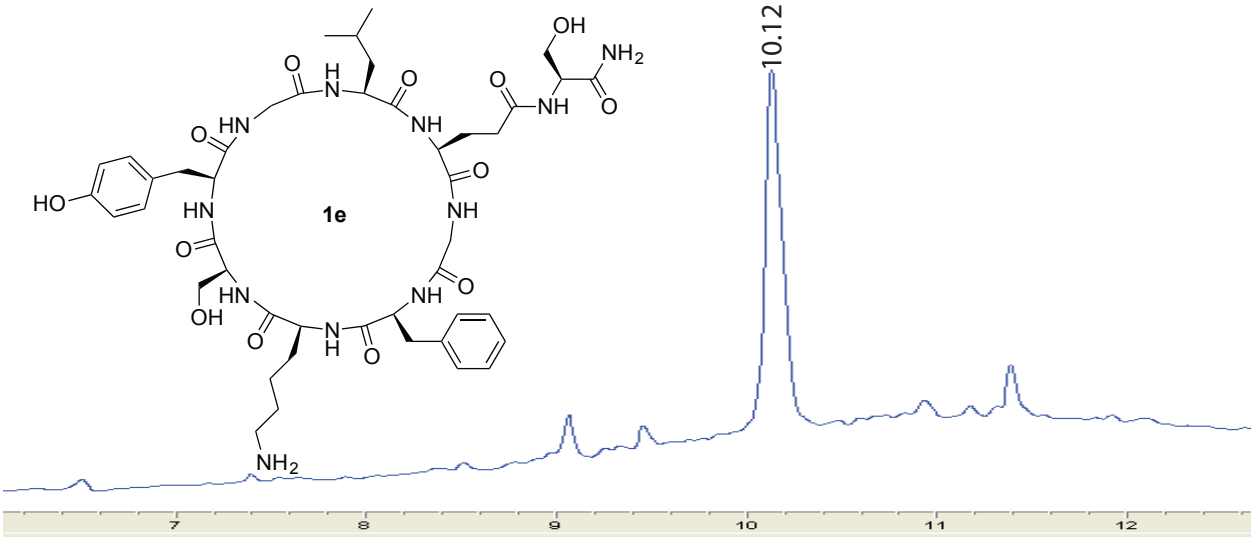
Figure S20. Macrocyclic ring-opening and resin-cleavage of peptide **1e**, *cyc*(GFKSYGLE)-S-*Rink*



***cyc*(Gly-Phe-Lys-Ser-Tyr-Gly-Leu-Glu)-Ser-CONH$_2$ (1e).** LCMS: *m/z* 967.7 (calcd $[M+H]^+$ = 968.4), *m/z* 989.7, (calcd $[M+Na]^+$ = 990.4), *m/z* 484.5 (calcd $[(M+2)/2]^+$ = 484.7), Purity: >95% (HPLC analysis at 220 nm). Retention time: 10.12

***Oxd*-Tyr-Gly-Leu-Glu-Gly-Phe-Lys (3e).** LCMS: *m/z* 925.7 (calcd $[M+H]^+$ = 926.4), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.5 min

Figure S21. HPLC trace and mass spectrum of peptide **1e**, *cyc*(GFKSYGLE)-S-$CONH_2$ after resin cleavage



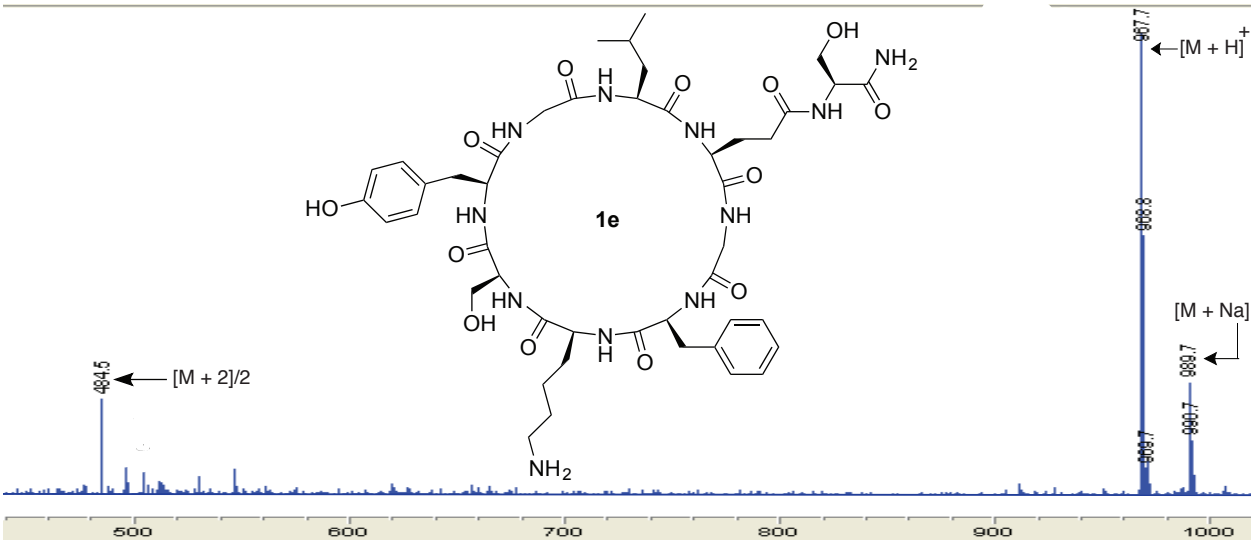


Figure S22. HPLC trace of peptide **3e**, *Oxd*-YGLEGFK-OH

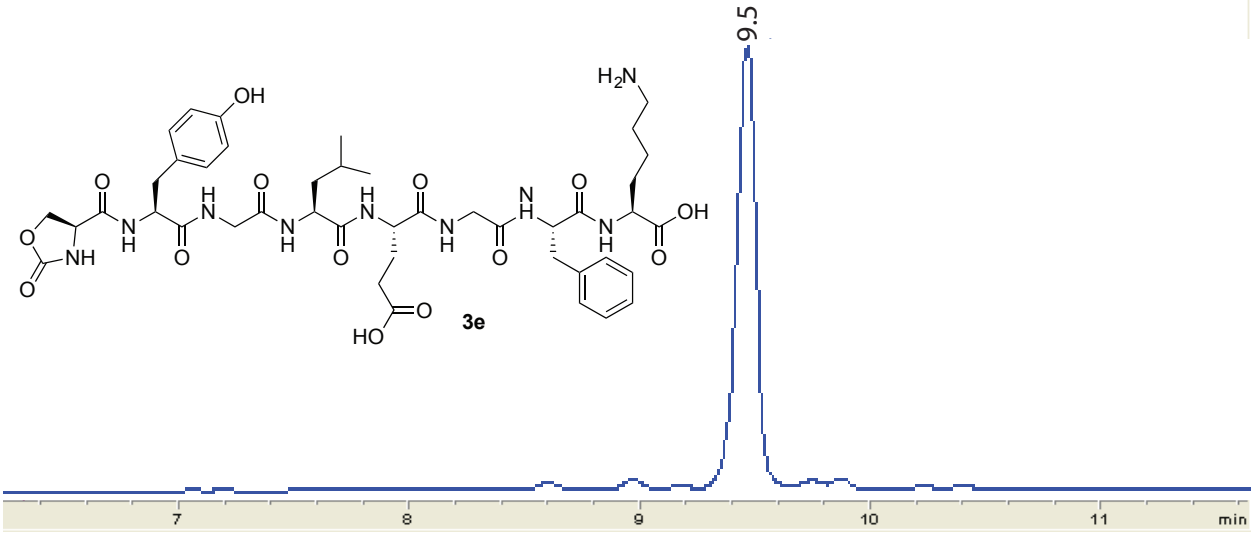
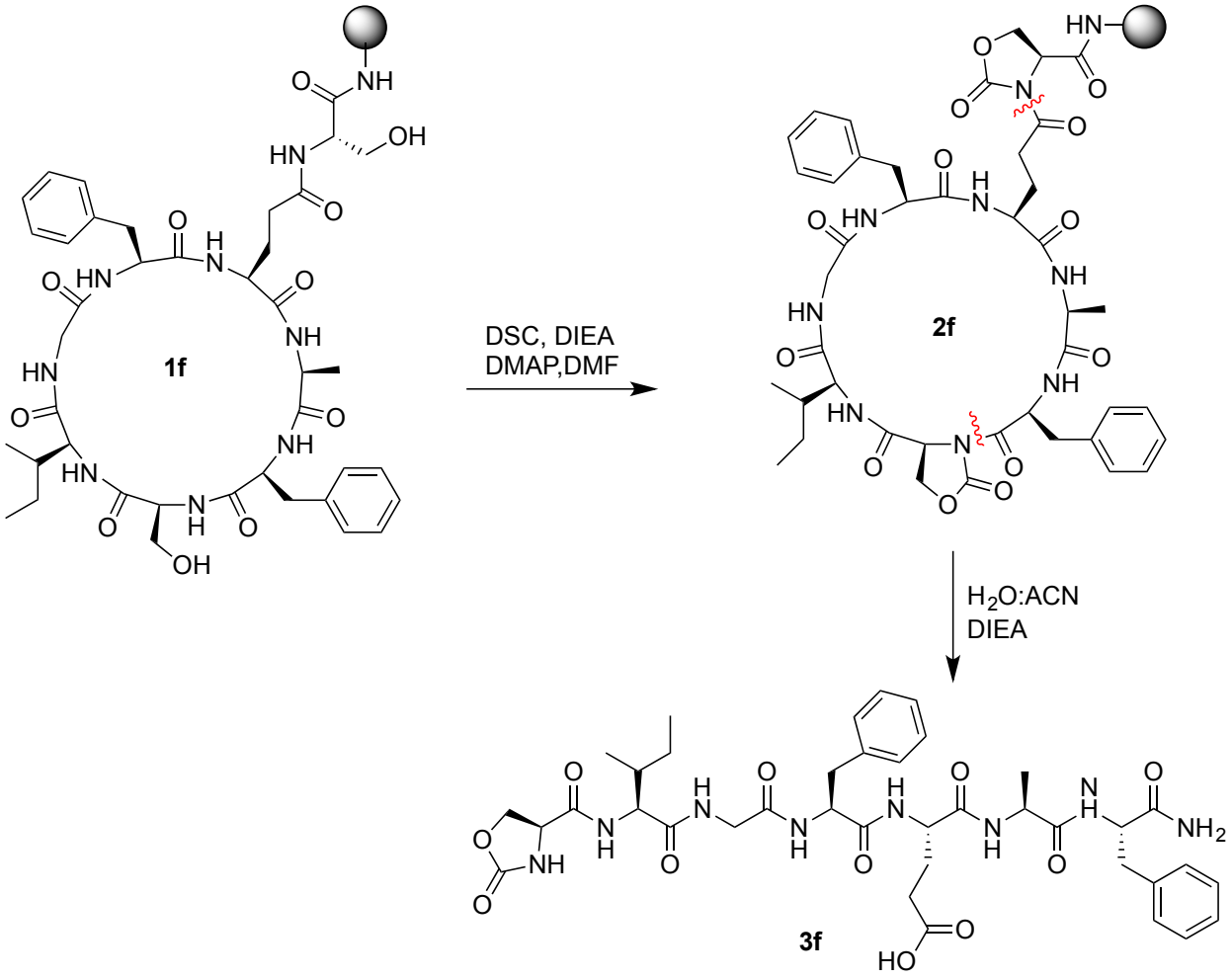


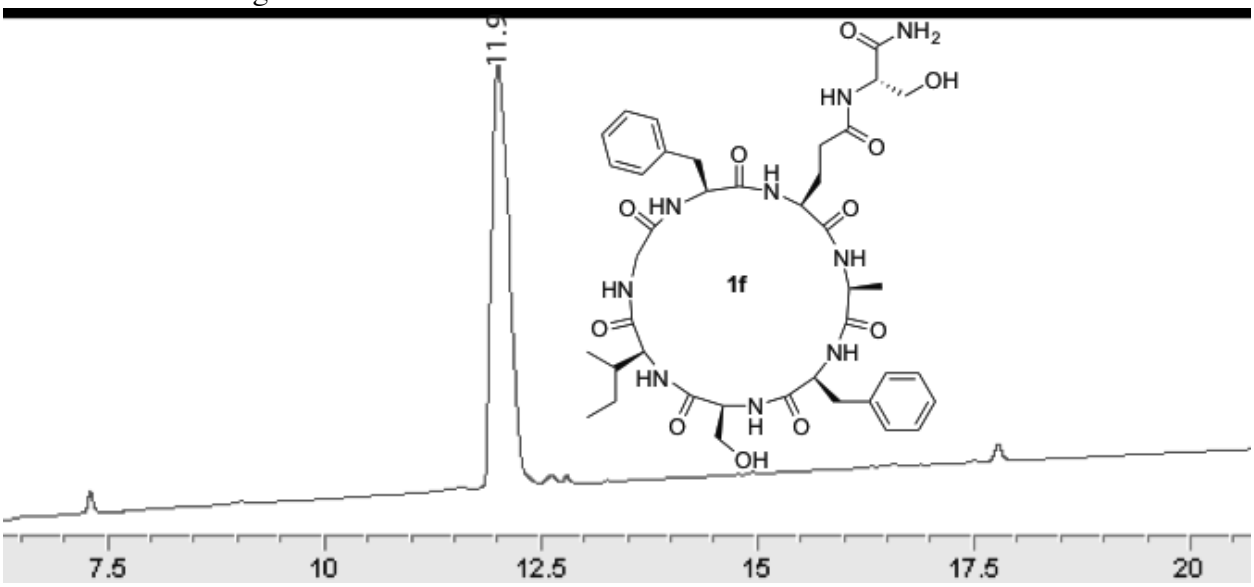
Figure S23. Macrocyclic ring-opening and resin-cleavage of peptide **1f**, *cyc*(AFSIGFE)-S-*Rink*



***cyc*(Ala-Phe-Ser-Ile-Gly-Phe-Glu)-Ser-CONH$_2$ (1f).** LCMS: *m/z* 837.8 (calcd $[M+H]^+$ = 838.4), *m/z* 859.7 (calcd $[M+Na]^+$ = 860.4), Purity: >95% (HPLC analysis at 220 nm). Retention time: 11.9

***Oxd*-Ile-Gly-Phe-Glu-Ala-Phe (3f).** LCMS: *m/z* 795.8 (calcd $[M+H]^+$ = 795.3), *m/z* 818.7, (calcd $[M+Na]^+$ = 817.3), Purity: >95% (HPLC analysis at 220 nm). Retention time: 12.8 min

Figure S24. HPLC trace and mass spectrum of peptide **1f**, *cyc*(AFSIGFE)-S-$CONH_2$ after resin cleavage



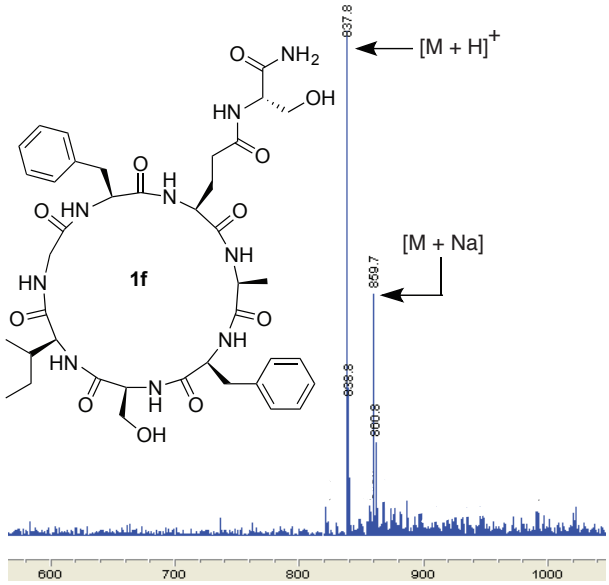


Figure S25. HPLC trace and mass spectrum of peptide **3f**, *Oxd*-IGFEAF-NH_2

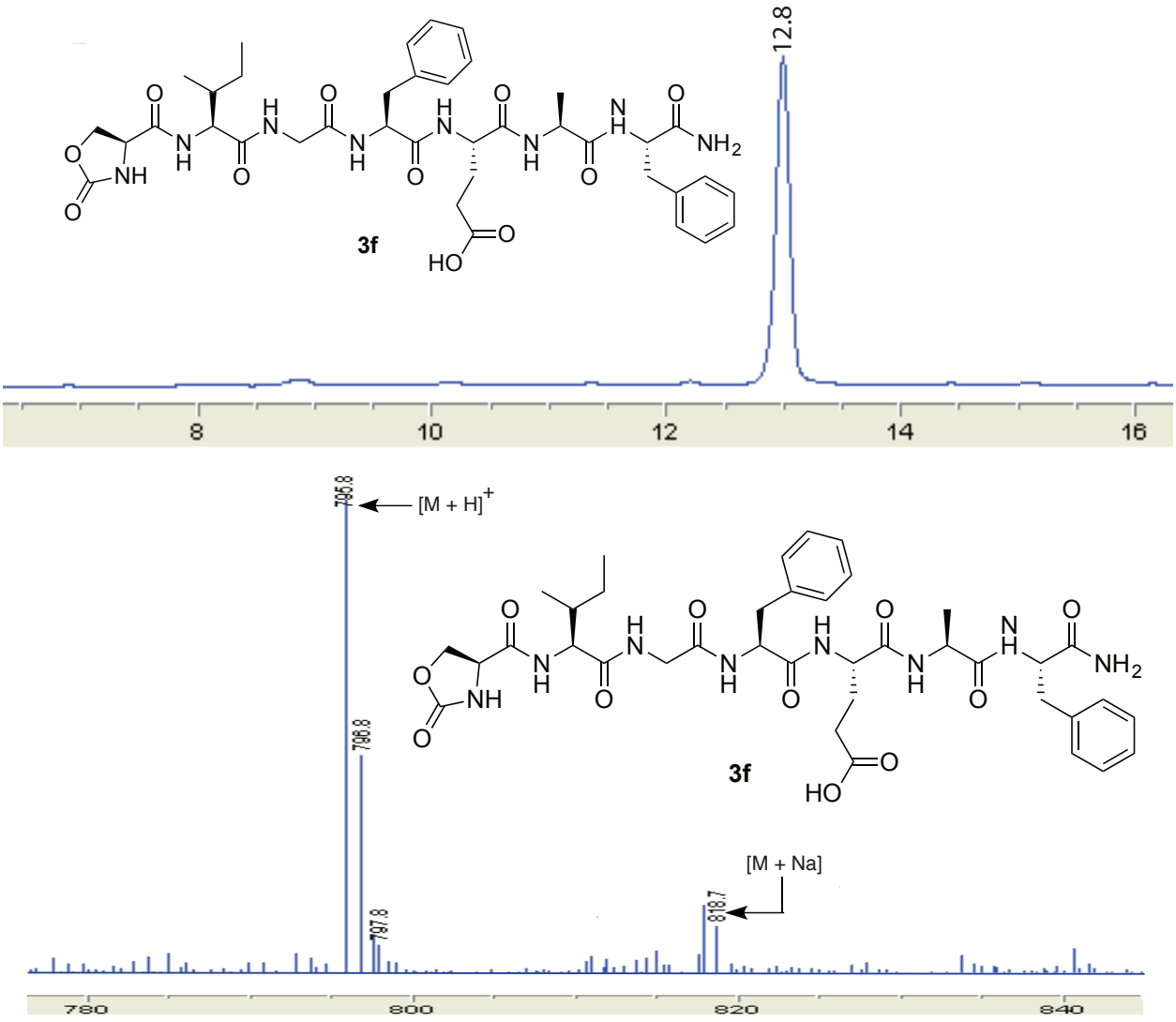
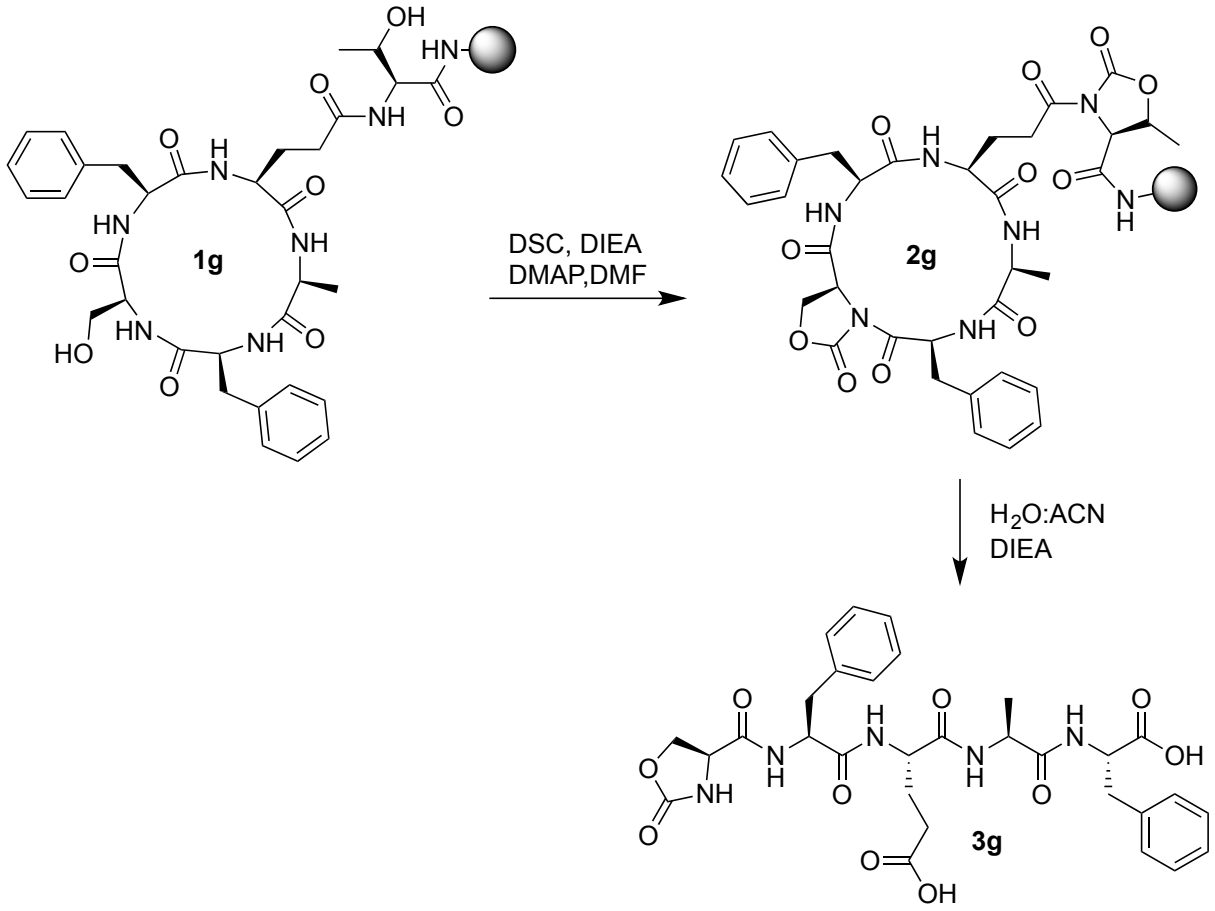


Figure S26. Macrocyclic ring-opening and resin-cleavage of peptide **1g**, *cyc*(AFSFE)-T-*Rink*



***cyc*(<u>Ala-Phe-Ser-Phe-Glu</u>)-Thr-CONH$_2$ (1g).** LCMS: *m/z* 681.90 (calcd $[M+H]^+$ = 682.3), *m/z* 1363.7 (calcd $[2M+H]^+$ = 1364.6), Purity: >95% (HPLC analysis at 220 nm). Retention time: 12.07

***Oxd*-Phe-Glu-Ala-Phe (3g).** LCMS: *m/z* 625.8 (calcd $[M+H]^+$ = 626.2), Purity: >95% (HPLC analysis at 220 nm). Retention time: 11.31 min

Figure S27. HPLC trace and mass spectrum of peptide **1g**, *cyc*(AFSFE)-T-$CONH_2$ after resin cleavage

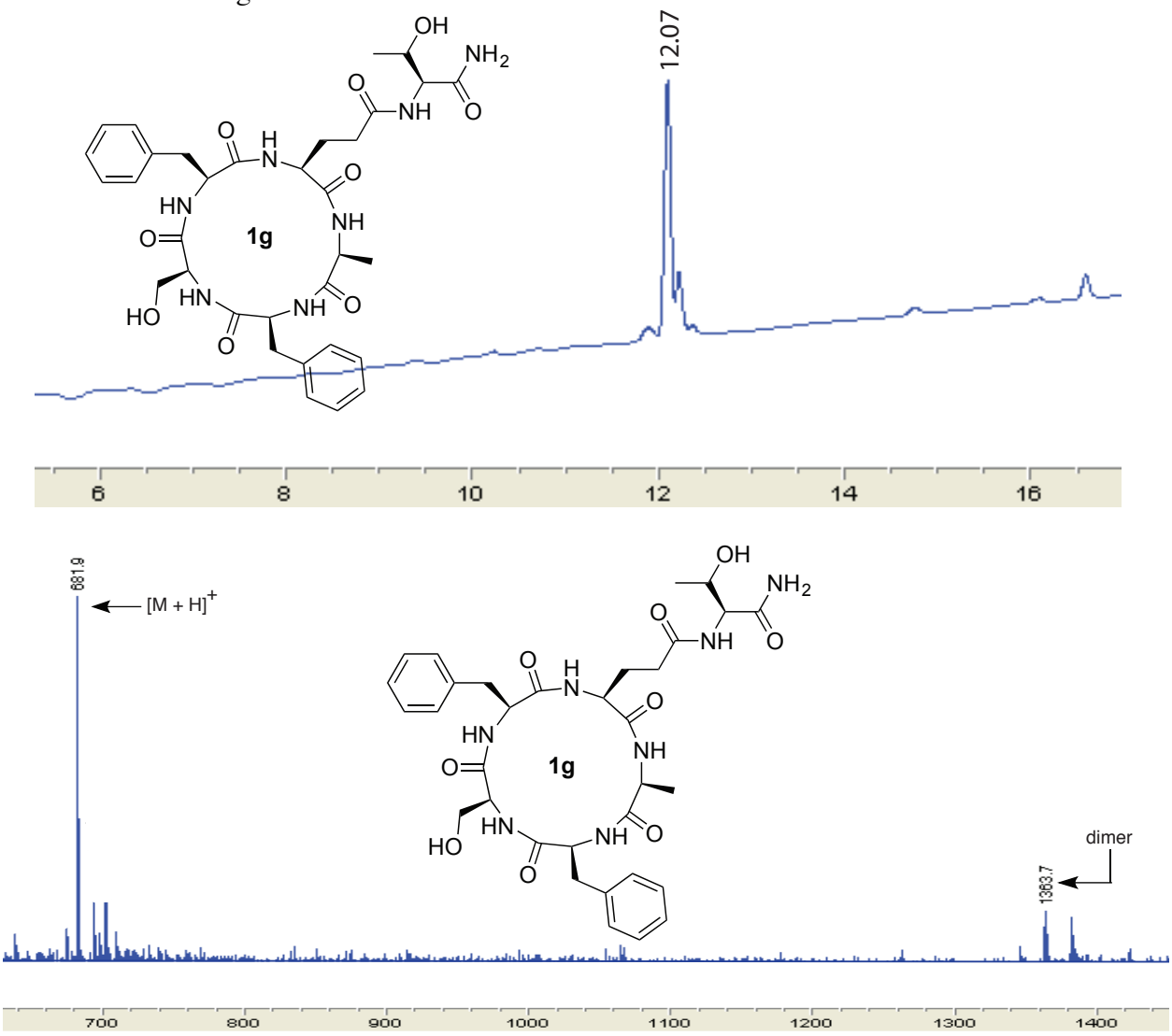
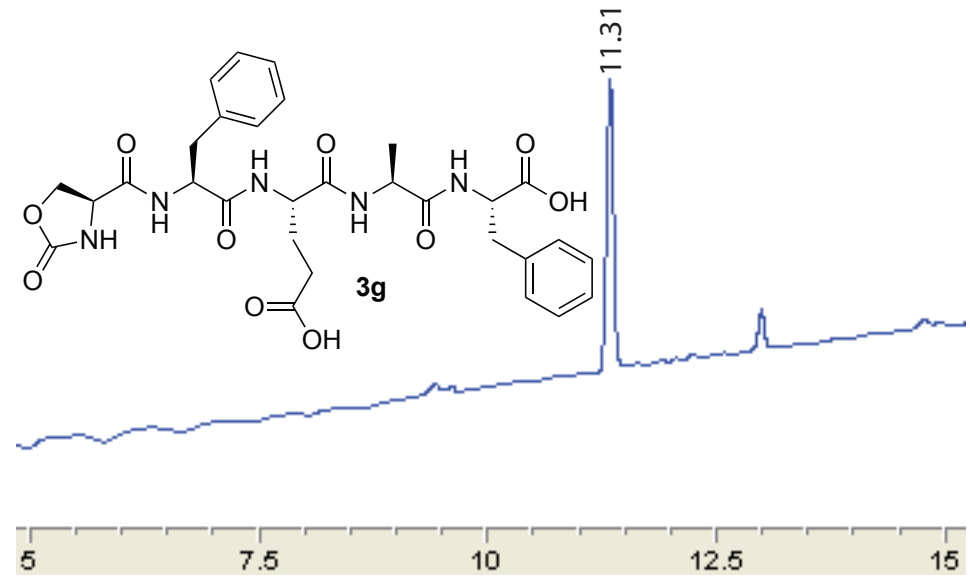


Figure S28. HPLC trace and mass spectrum of peptide **3g**, *Oxd*-FEAF-OH



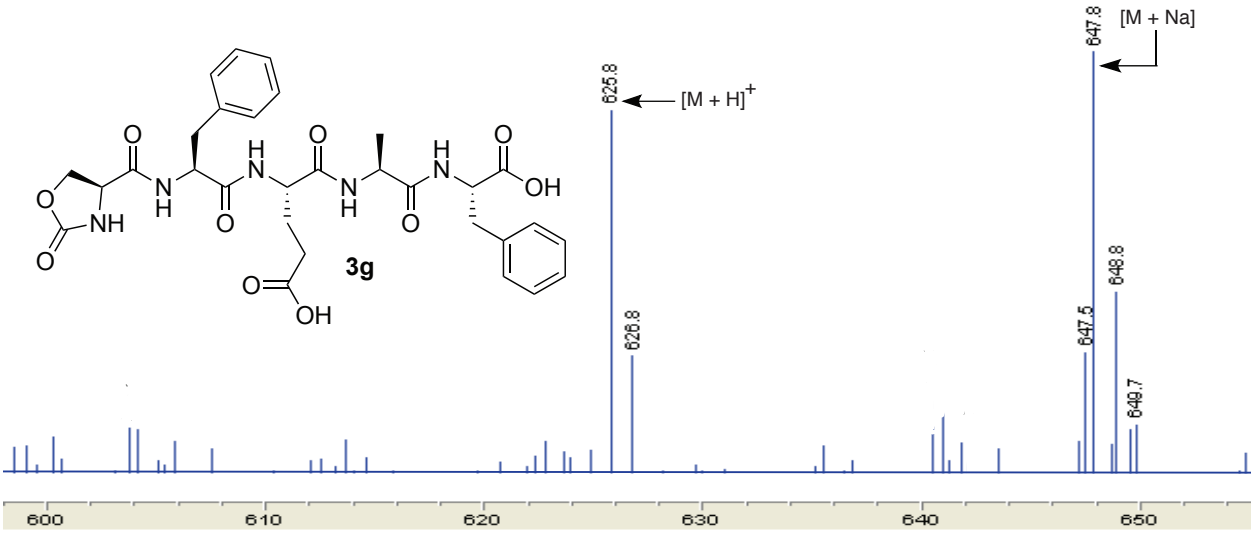
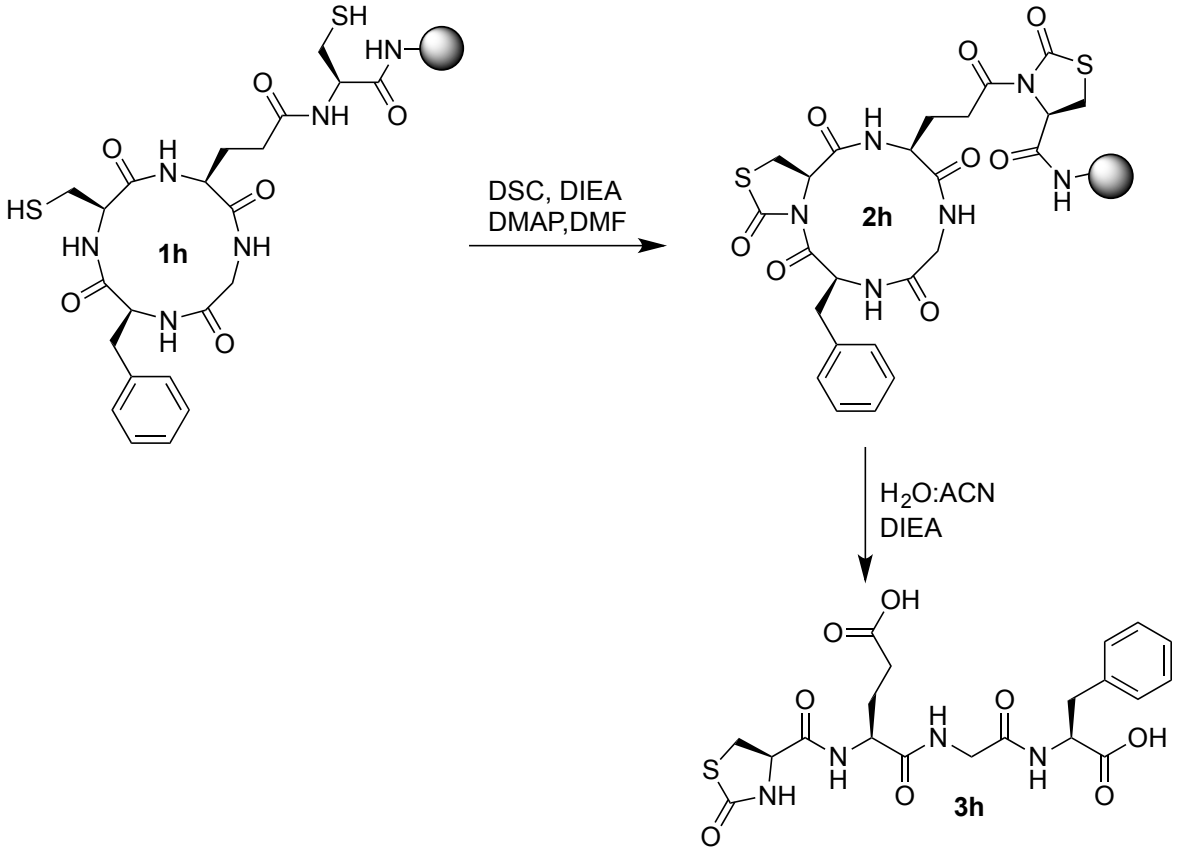


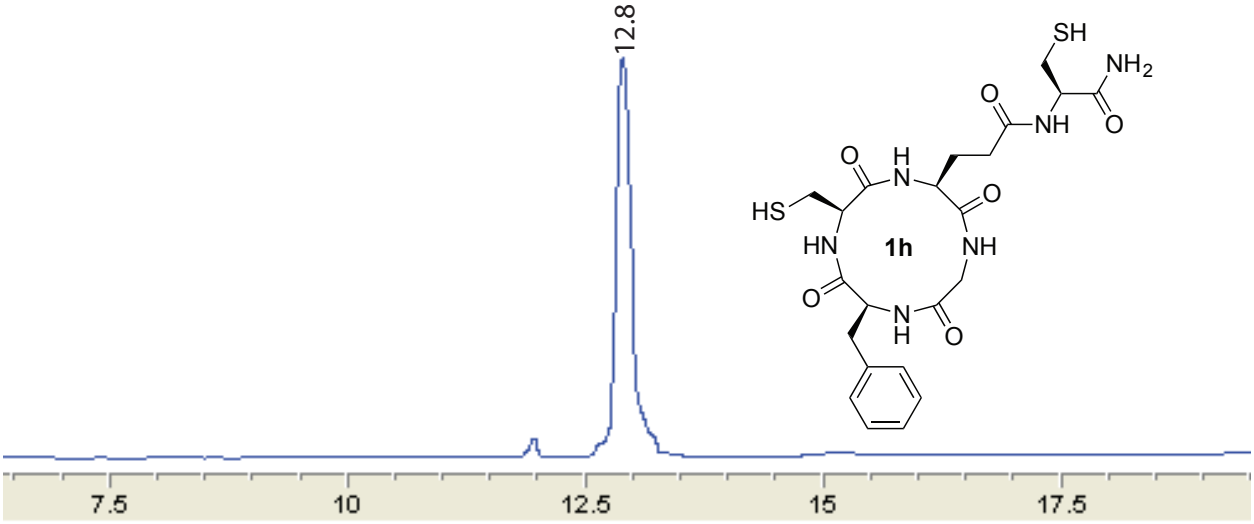
Figure S29. Macrocyclic ring-opening and resin-cleavage of peptide **1h**, *cyc*(GFCE)-C-*Rink*



***cyc*(Gly-Phe-Cys-Glu)-Cys-$CONH_2$ (1h).** LCMS: *m/z* 539.0 (calcd $[M+H]^+$ = 539.1), *m/z* 1076.0, (calcd $[dimer]^+$ = 1076.2) *m/z* 1098.5 (calcd $[dimer+Na]^+$ = 1099.2), Purity: >95% (HPLC analysis at 220 nm). Retention time: 12.8

***Thz*-Glu-Gly-Phe (3h).** LCMS: *m/z* 480.9 (calcd $[M+H]^+$ = 481.1), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.1 min

Figure S30. HPLC trace and mass spectrum of peptide **1h**, *cyc*(GFCE)-S-$CONH_2$ after resin cleavage



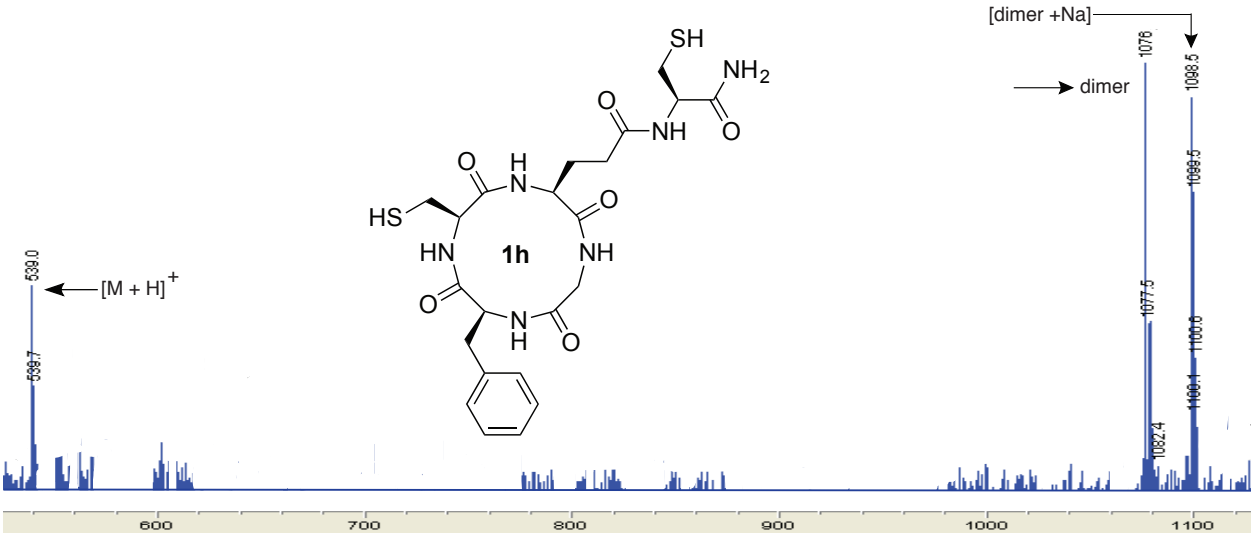
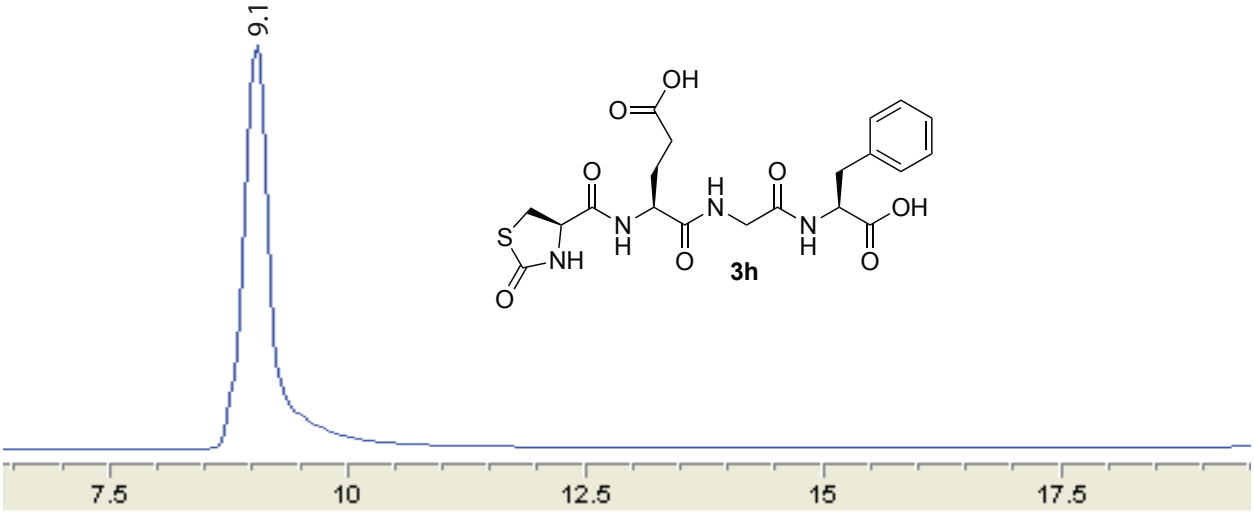


Figure S31. HPLC trace and mass spectrum of peptide **3h**, *Thz*-EGF-OH



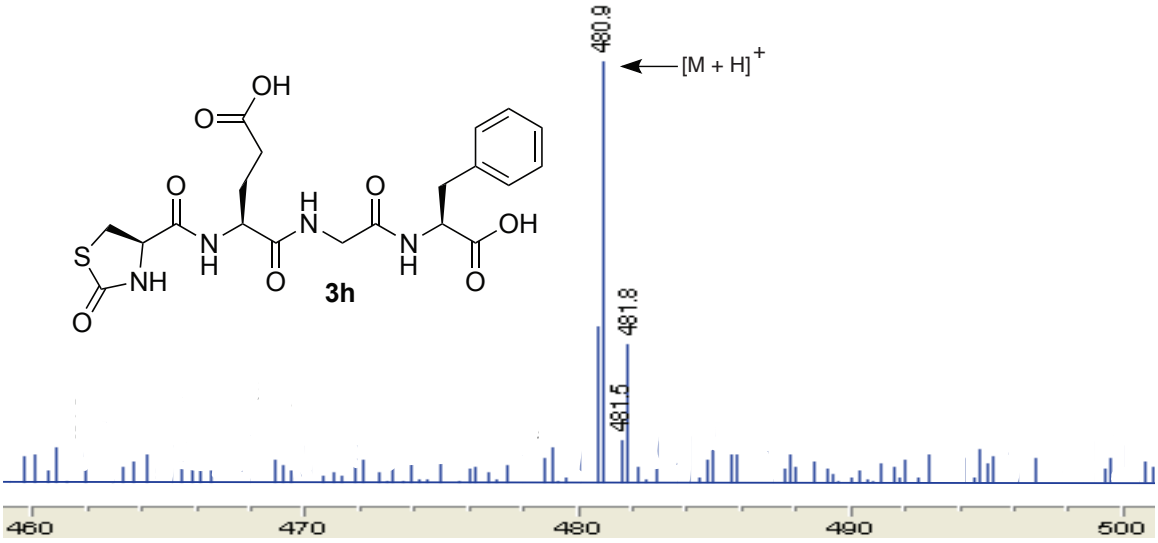
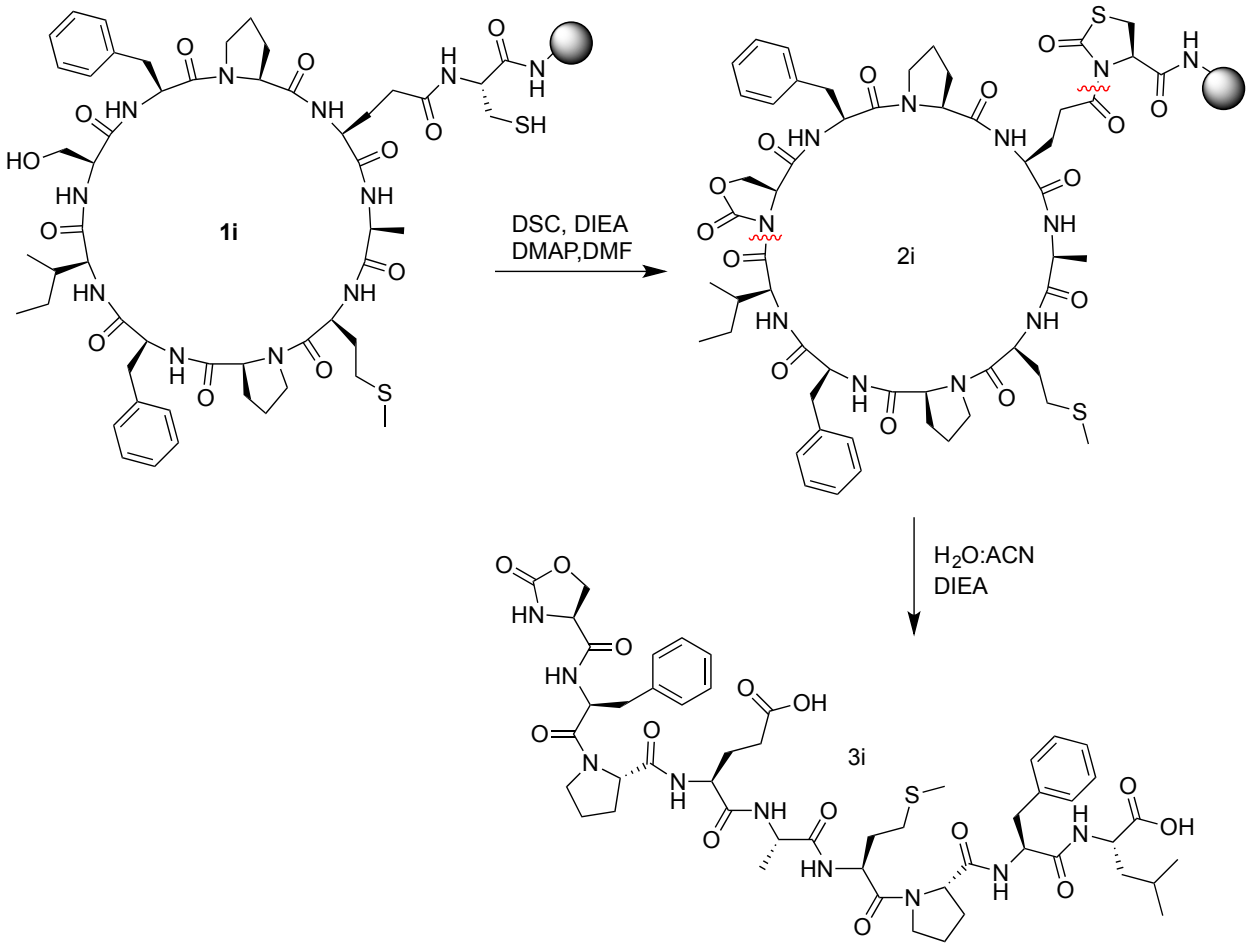


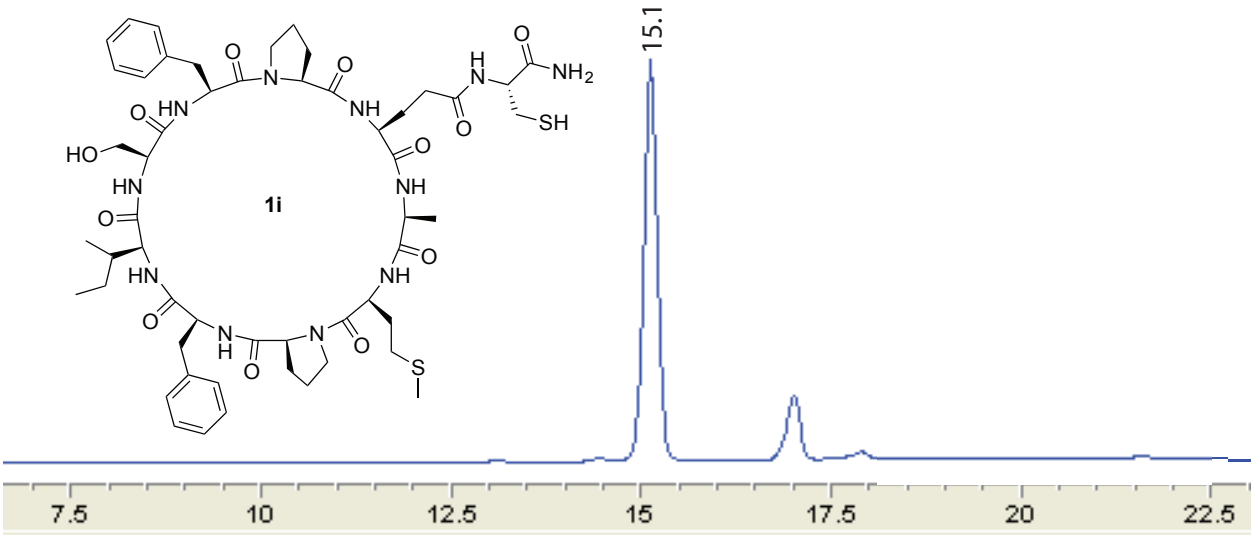
Figure S32. Macrocyclic ring-opening and resin-cleavage of peptide **1i**, *cyc*(AMPFISFPE)-C-*Rink*



***cyc*(Ala-Met-Pro-Phe-Ile-Ser-Phe-Pro-Glu)-Cys-CONH$_2$ (1i).** LCMS: *m/z* 1121.7 (calcd [*M*+H]$^+$ = 1122.5), *m/z* 1143.7, (calcd [*M*+Na]$^+$ = 1144.5) 561.5 (calcd [*M*+Na]$^+$ = 561.7), Purity: >95% (HPLC analysis at 220 nm). Retention time: 15.1 min

***Oxd*-Phe-Pro-Glu-Ala-Met-Pro-Phe-Ile (3i).** LCMS: *m/z* 1063.6 (calcd [*M*+H]$^+$ = 1064.4), *m/z* 1087.8, (calcd [*M*+Na]$^+$ = 1086.4) *m/z* 532.5 (calcd [*M*+Na]$^+$ = 532.7), Purity: >95% (HPLC analysis at 220 nm). Retention time: 14.5 min

Figure S33. HPLC trace and mass spectrum of peptide **1i**, *cyc*(AMPFISFPE)-C-CONH$_2$ after resin cleavage



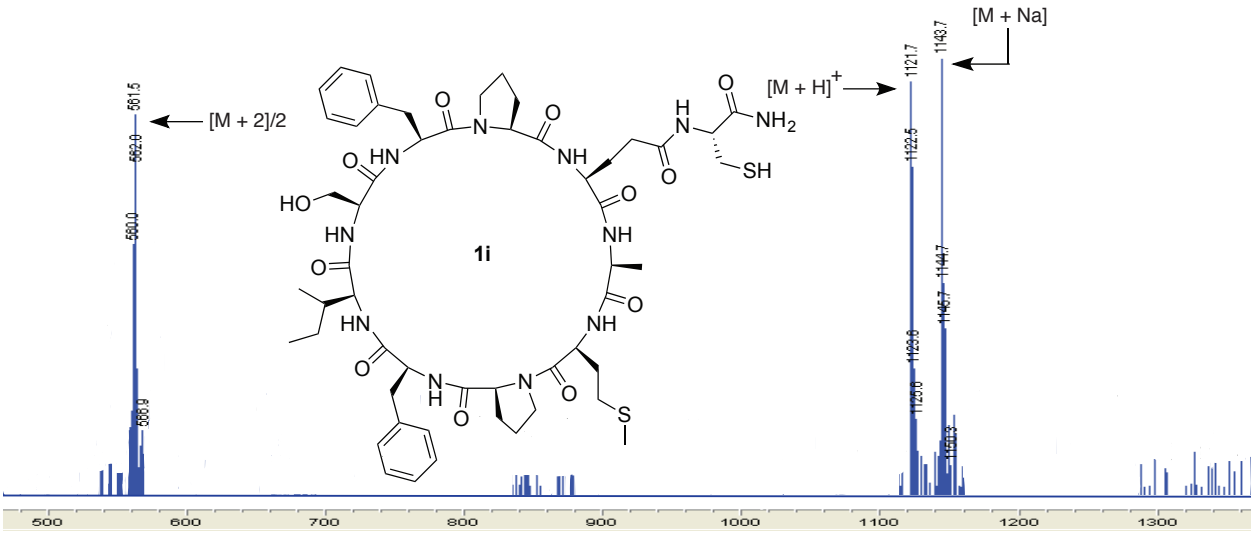
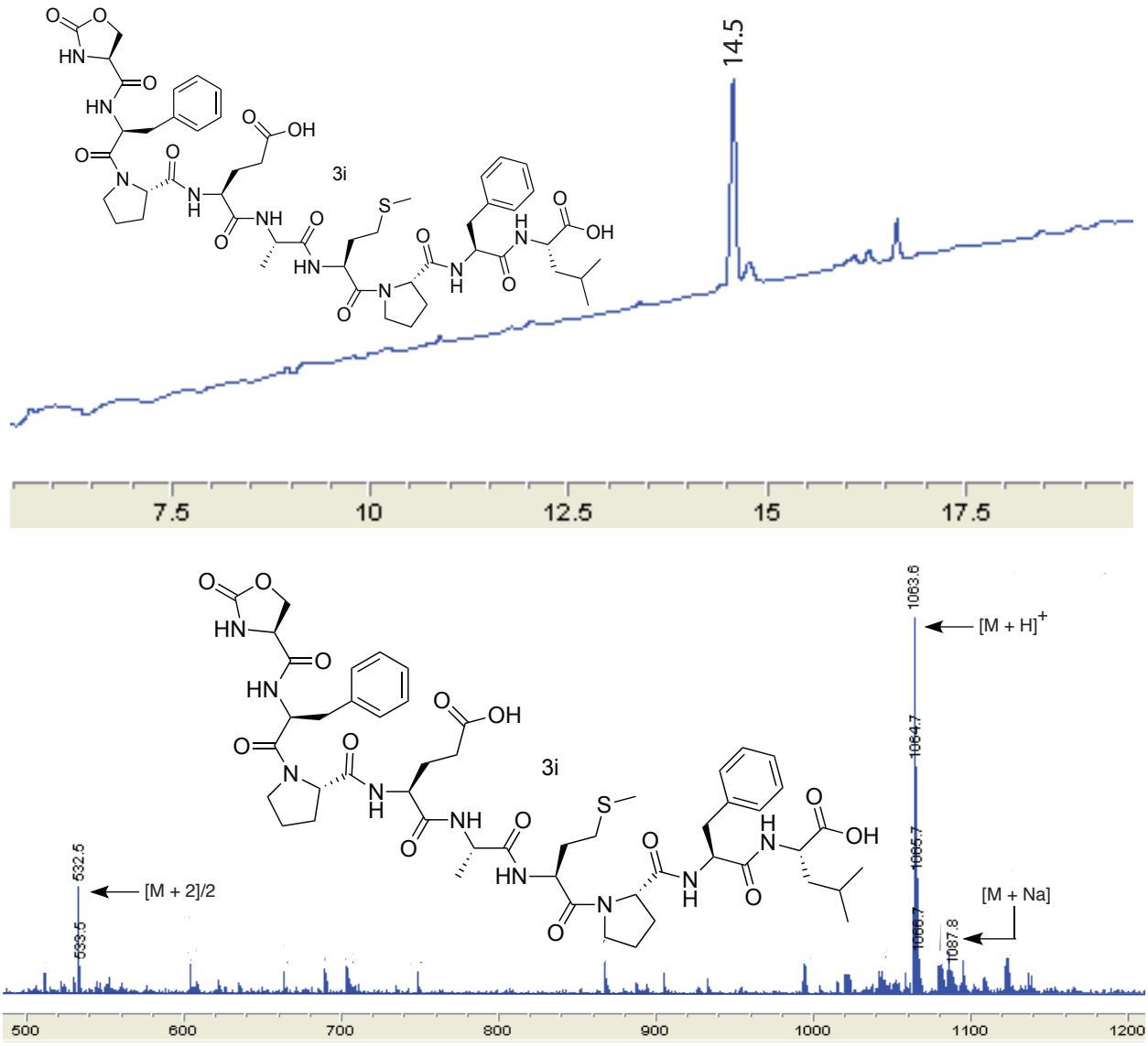


Figure S34. HPLC trace and mass spectrum of peptide **3i**, *Oxd*-FPEAMPFI-OH



XIII. MS/MS Sequencing Data

Figure S35. LC-IM-MS/MS data for linear peptide **3a**, *Oxd*-FAEGF-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 683.2671, Found $[M+H]^+$ = 683.2670

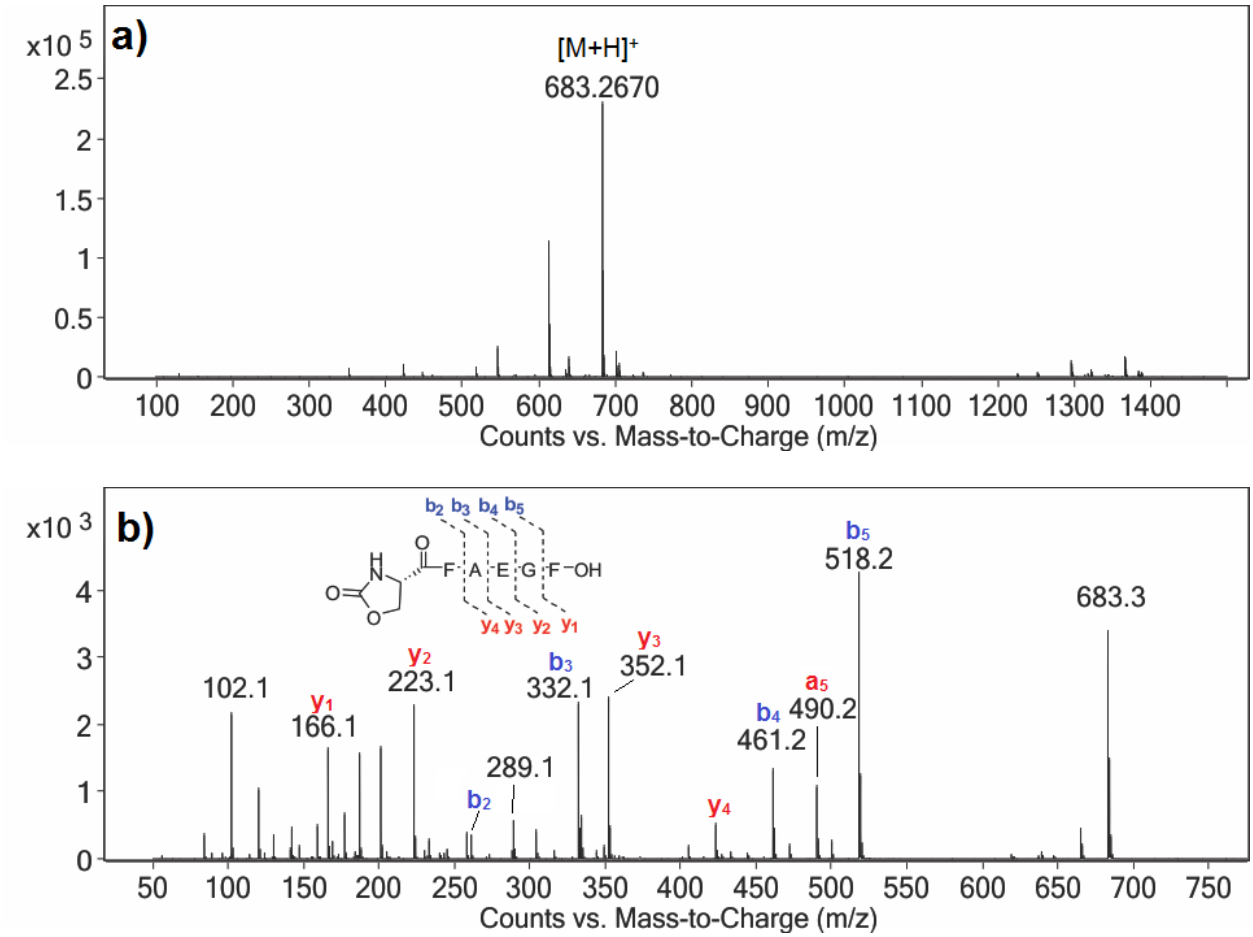


Figure S36. LC-IM-MS/MS data for linear peptide **3b**, *Oxd*-FFAE-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 626.2457, Found $[M+H]^+$ = 626.2450

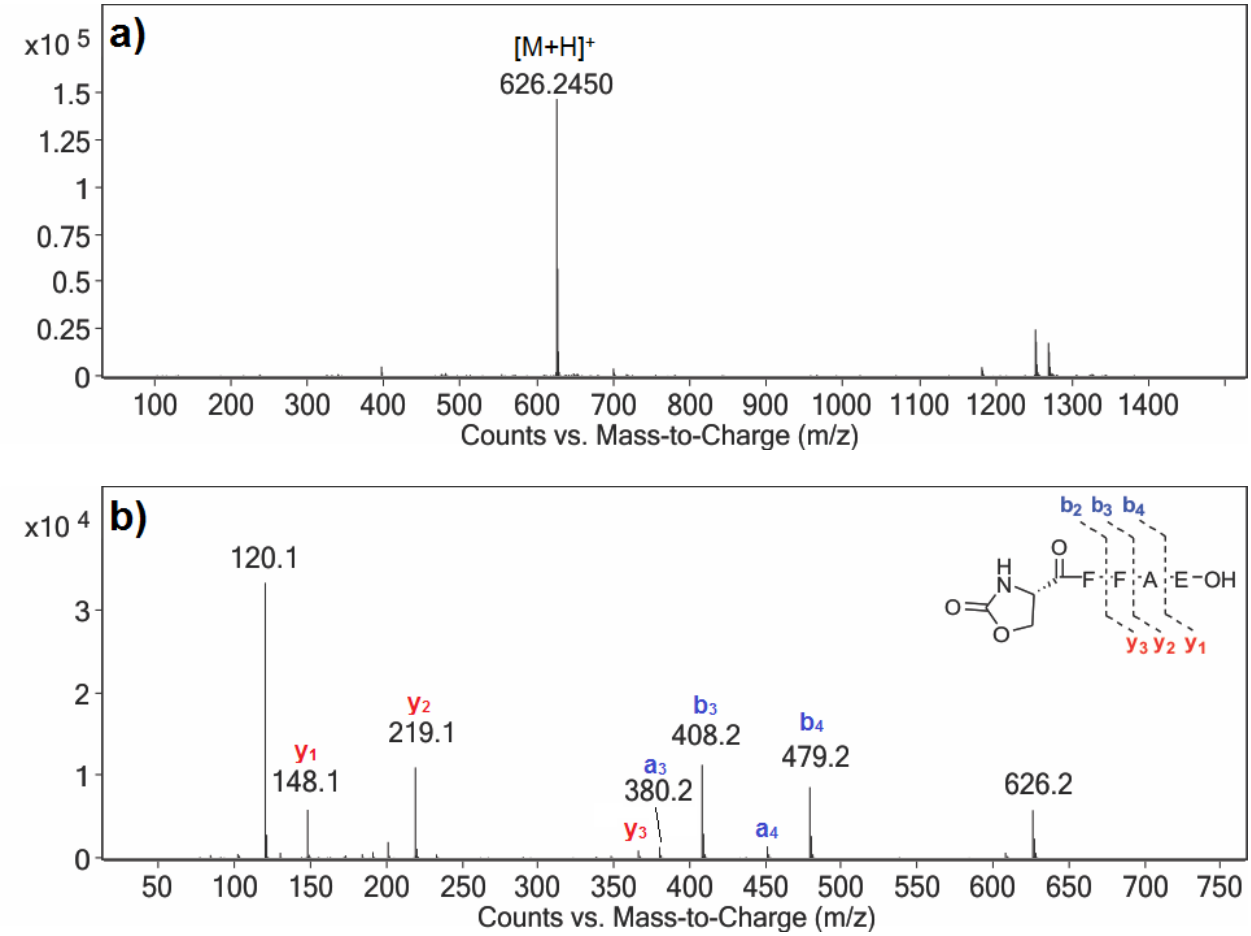


Figure S37. LC-IM-MS/MS data for linear peptide **3c**, *Oxd*-GVFAE-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 635.2671, Found $[M+H]^+$ = 635.2663

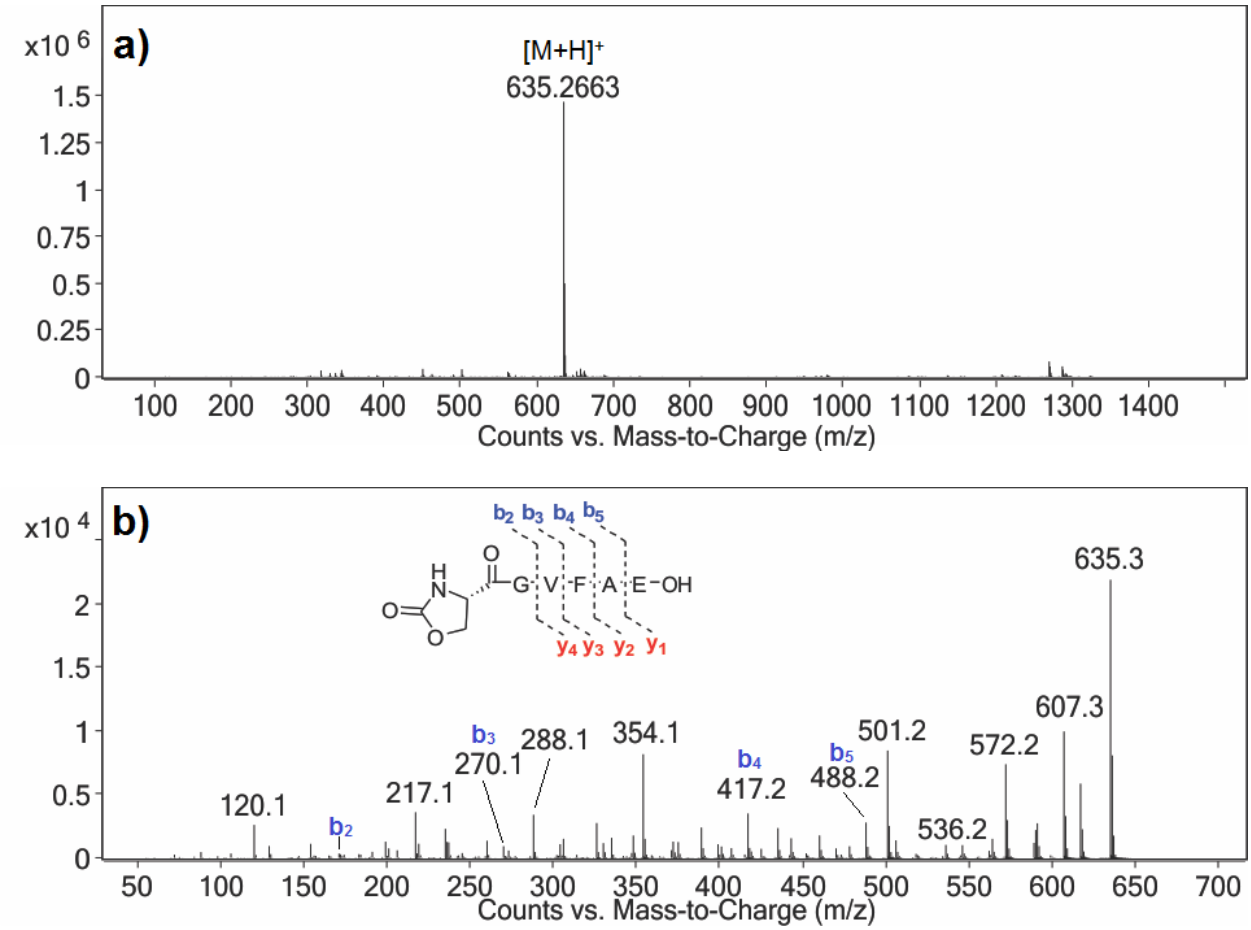


Figure S38. LC-IM-MS/MS data for linear peptide **3d**, *Oxd*-FAEG-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 536.1987, Found $[M+H]^+$ = 536.1993

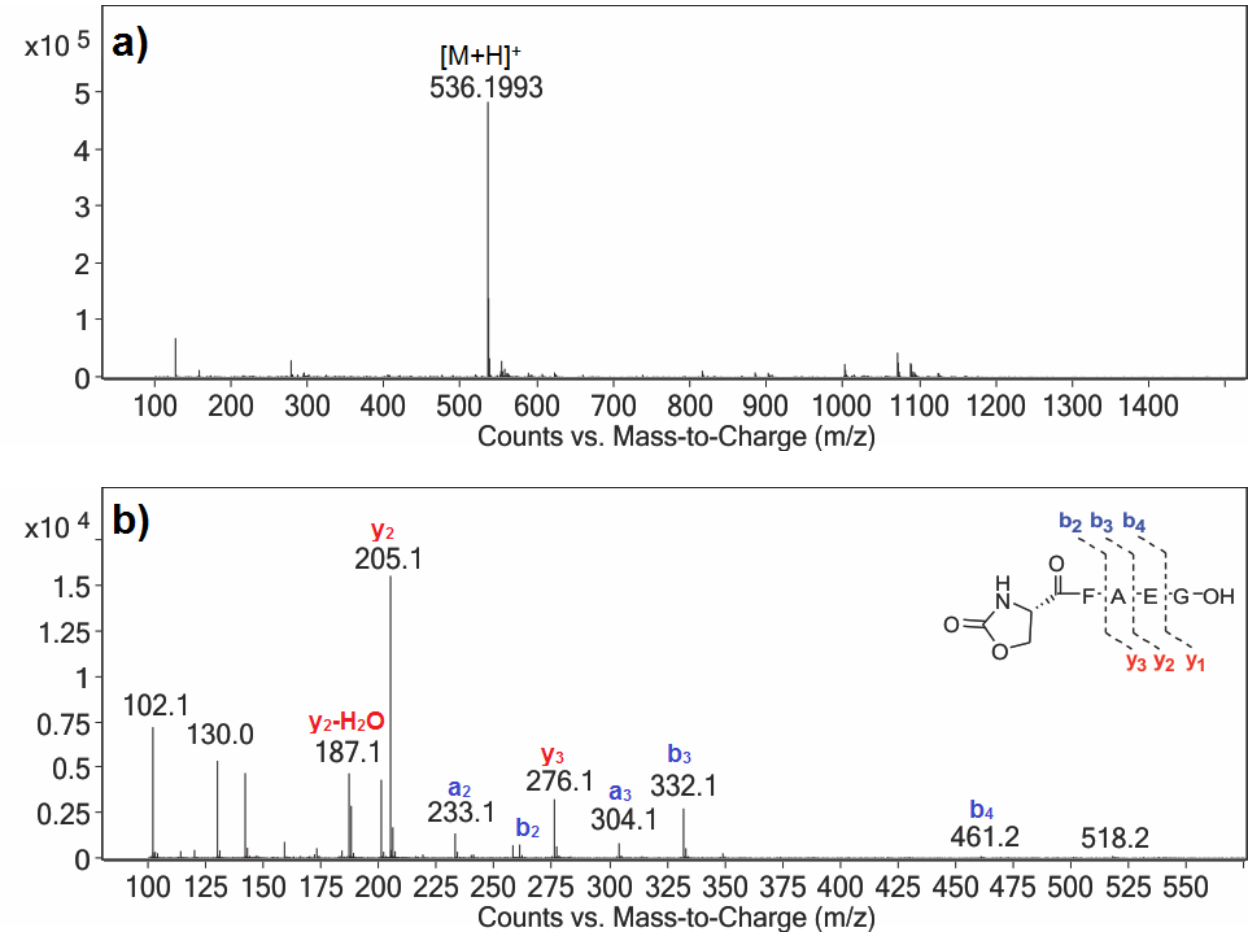


Figure S39. LC-IM-MS/MS data for linear peptide **3e**, *Oxd*-YGLEGFK-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 926.4254, Found $[M+H]^+$ = 926.4267

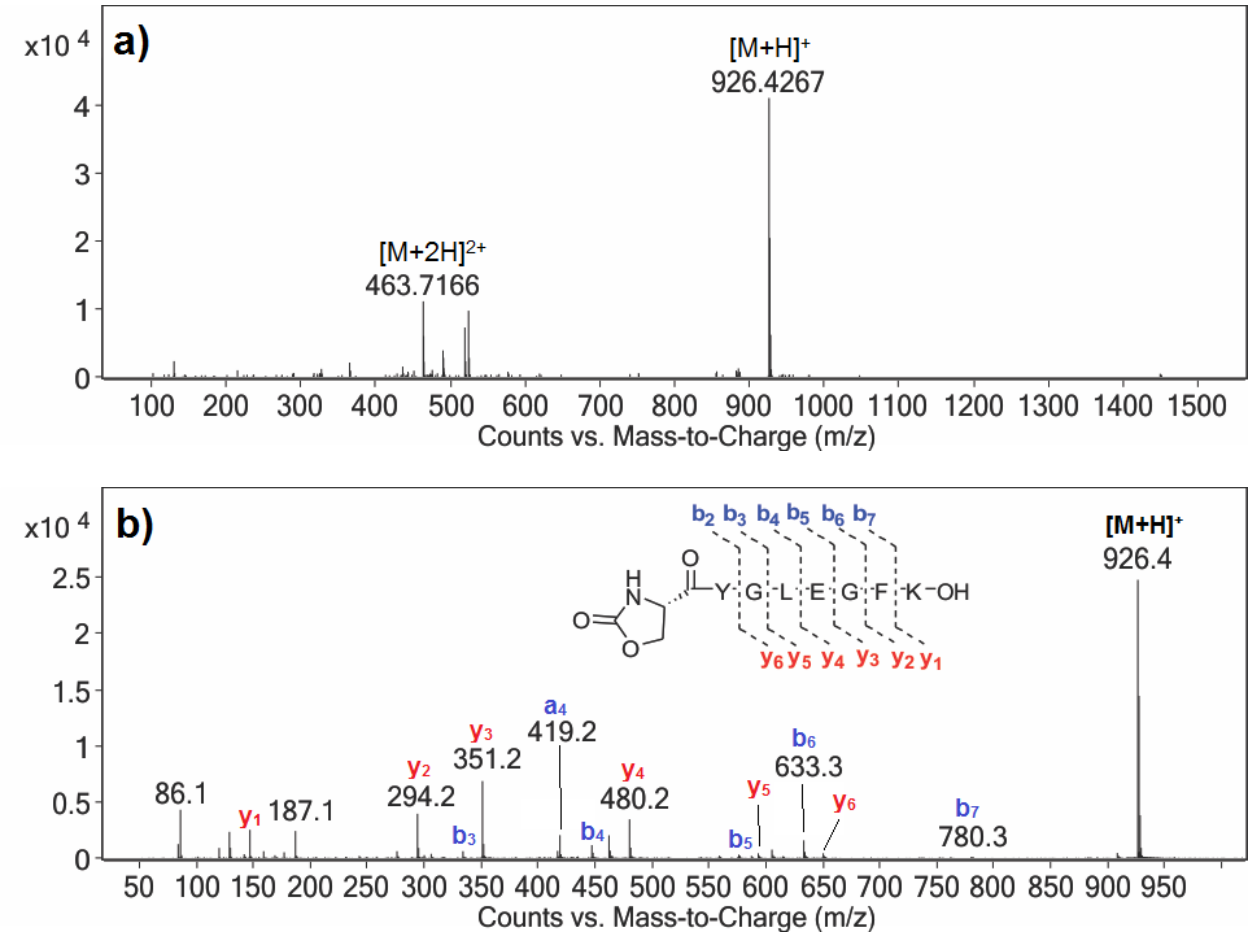


Figure S40. LC-IM-MS/MS data for linear peptide **3f**, *Oxd*-IGFEAF-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 796.3512, Found $[M+H]^+$ = 796.3514

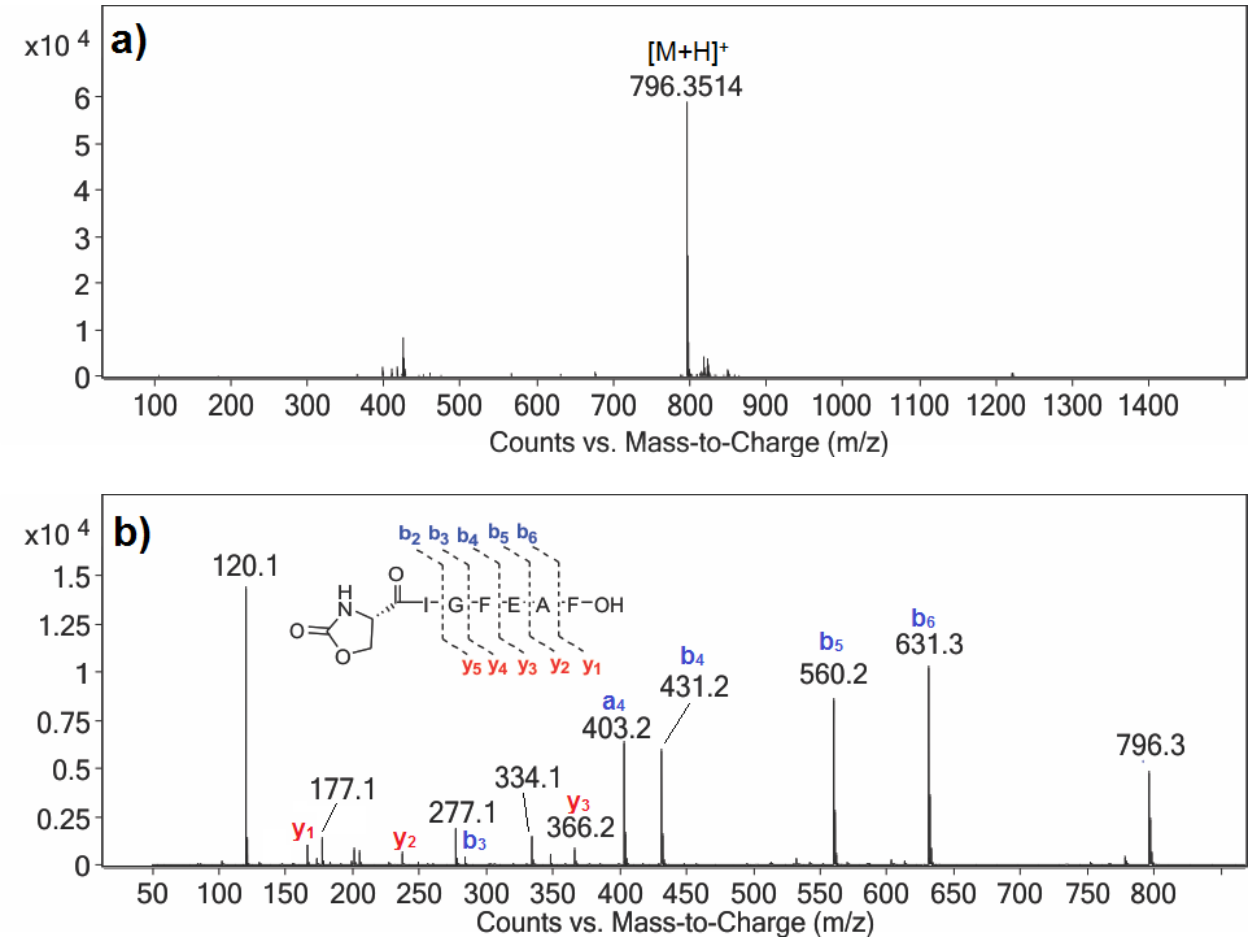


Figure S41. LC-IM-MS/MS data for linear peptide **3g**, *Oxd*-FEAF-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 626.2457, Found $[M+H]^+$ = 626.2445

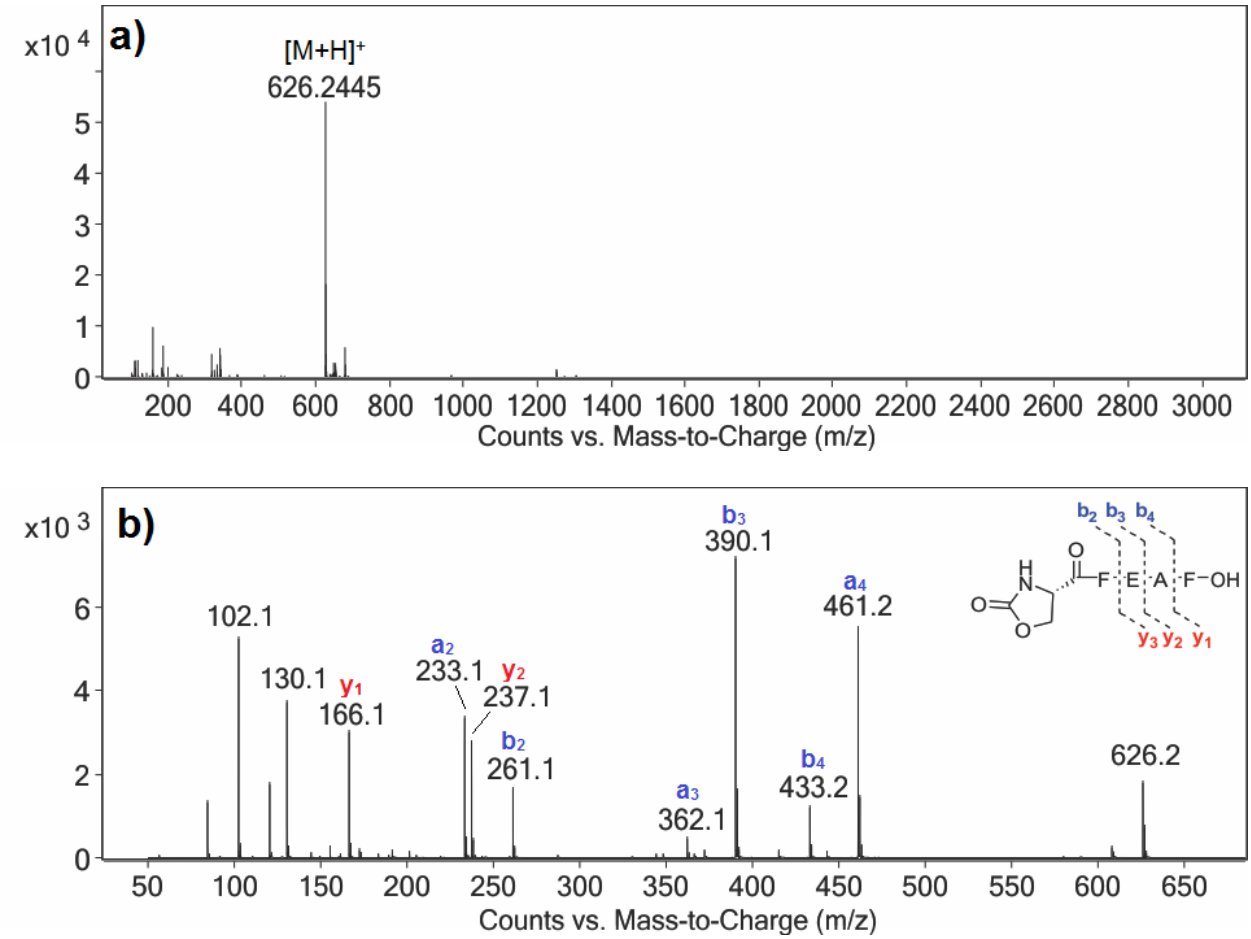


Figure S42. LC-IM-MS/MS data for linear peptide **3h**, *Thz*-EGF-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 481.1388, Found $[M+H]^+$ = 481.1361

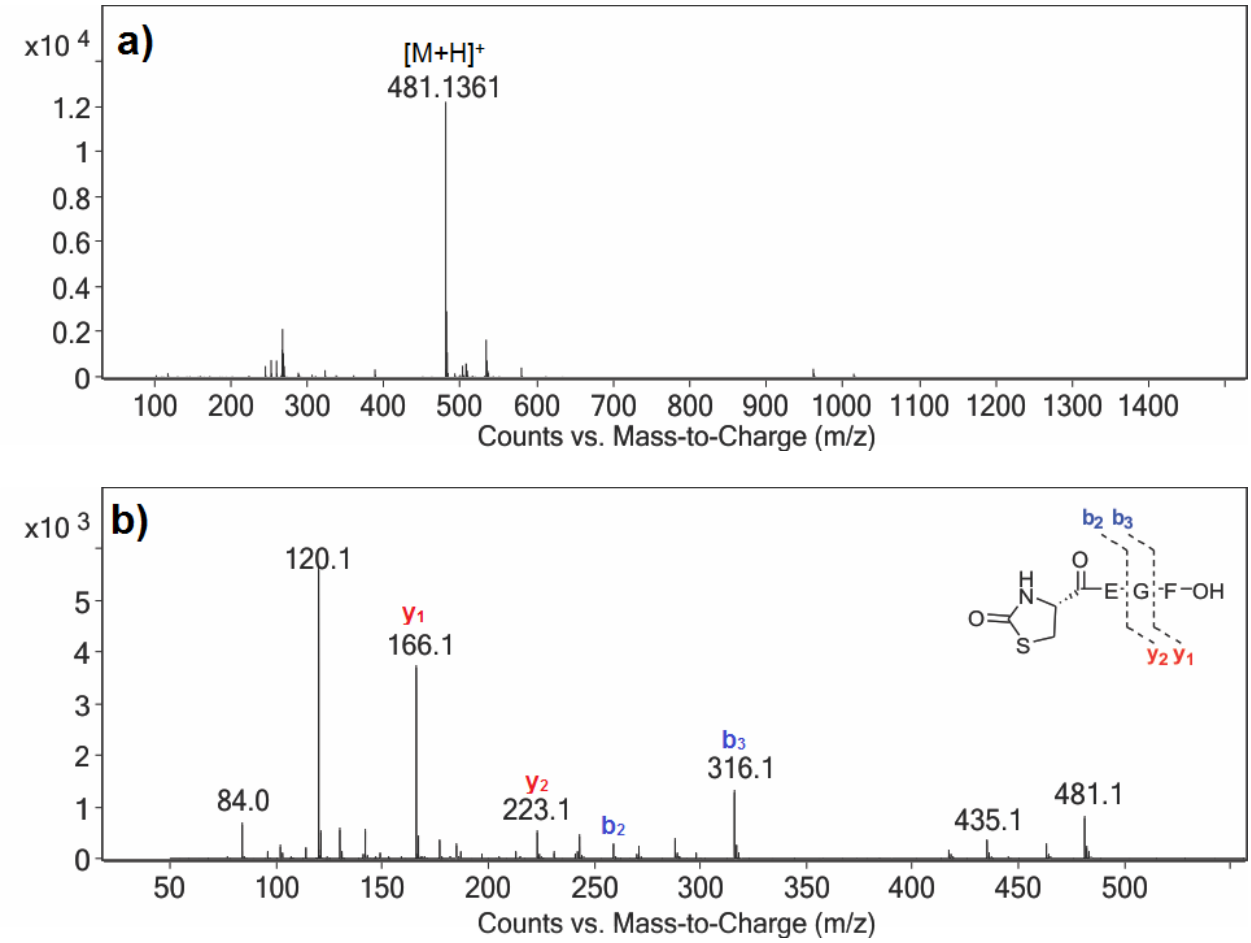


Figure S43. LC-IM-MS/MS data for linear peptide **3i**, *Oxd*-FPEAMPFI-OH. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time
Calcd $[M+H]^+$ = 1064.4757, Found $[M+H]^+$ = 1064.4731

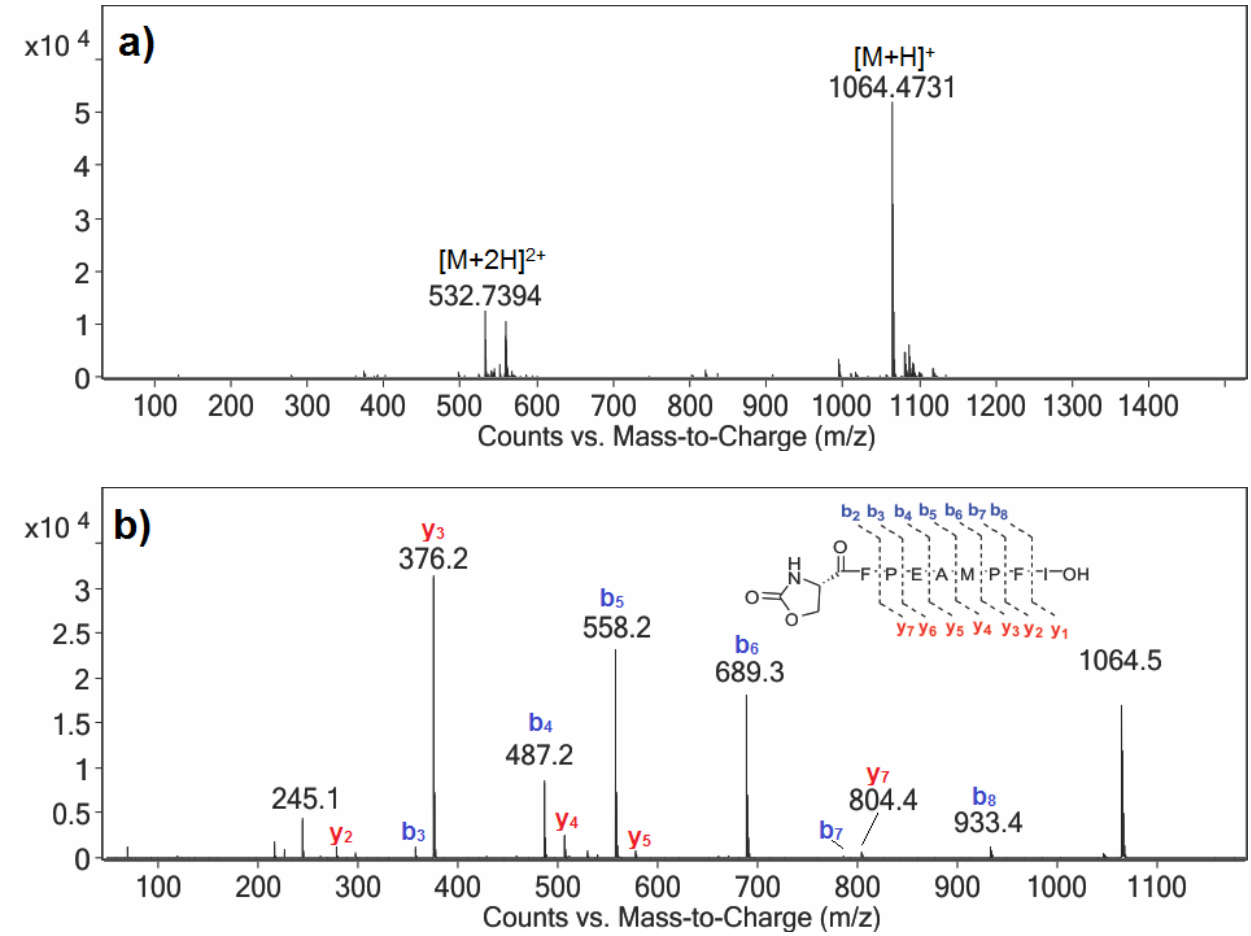


Figure S44. LC-IM-MS/MS data for linear peptide **(Table S3, entry 1)**, *Oxd*-MRE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 547.2293, Found $[M+H]^+$ = 547.2303

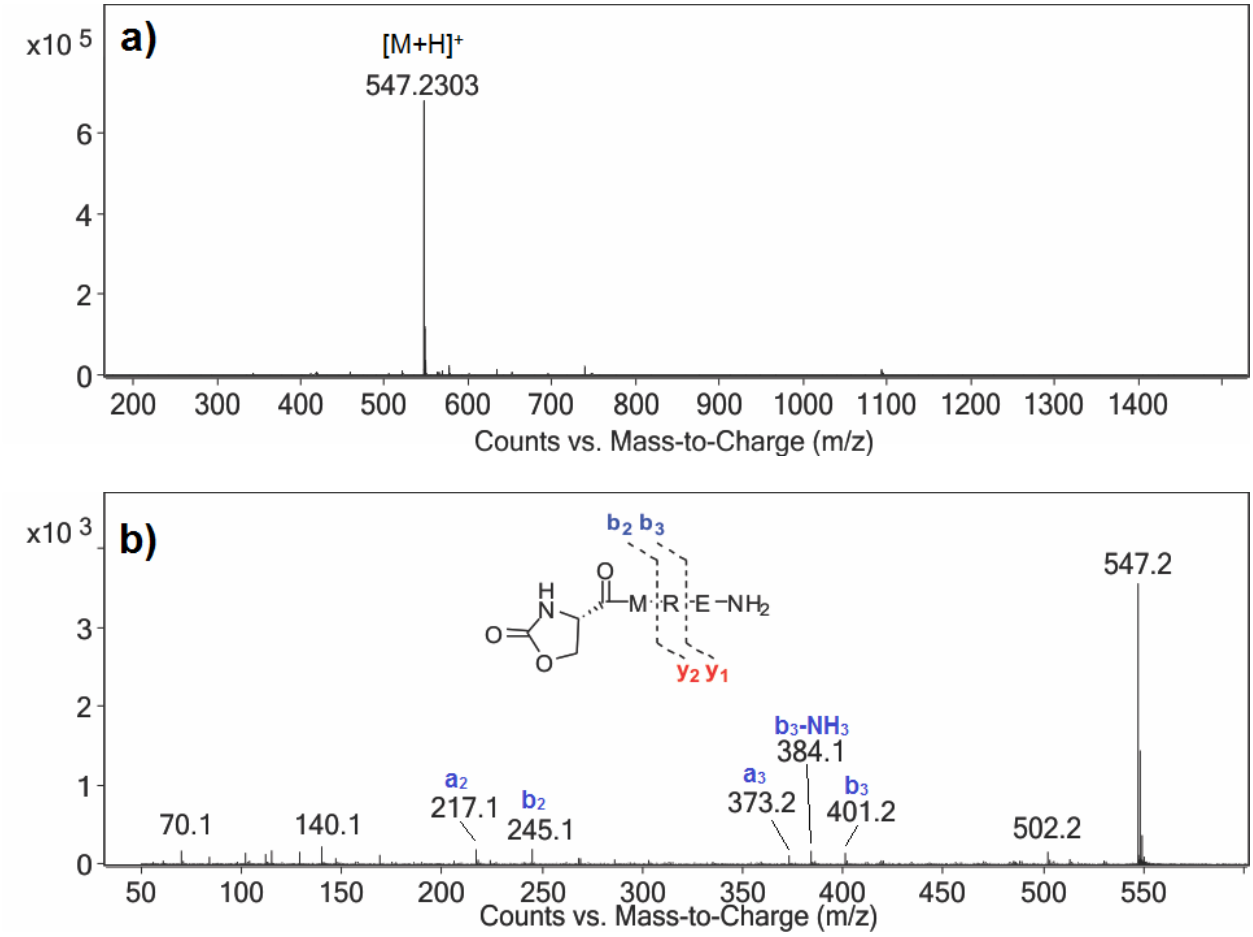


Figure S45. LC-IM-MS/MS data for linear peptide **(Table S3, entry 2)**, *Oxd*-AFE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time Calcd $[M+H]^+$ = 478.1932, Found $[M+H]^+$ = 478.1918

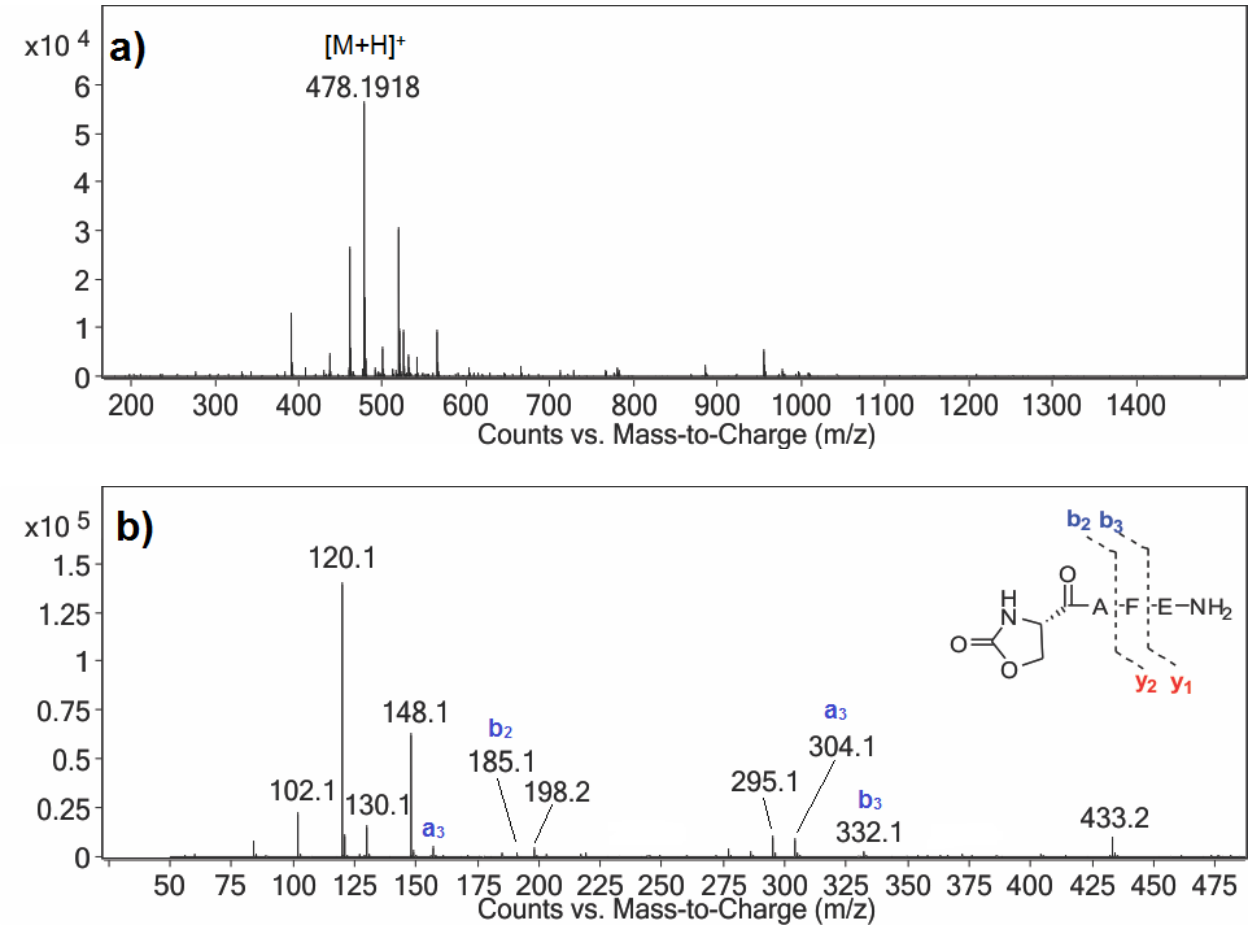


Figure S46. LC-IM-MS/MS data for linear peptide **(Table S3, entry 3)**, *Oxd*-TFVE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 607.2722, Found $[M+H]^+$ = 607.2716

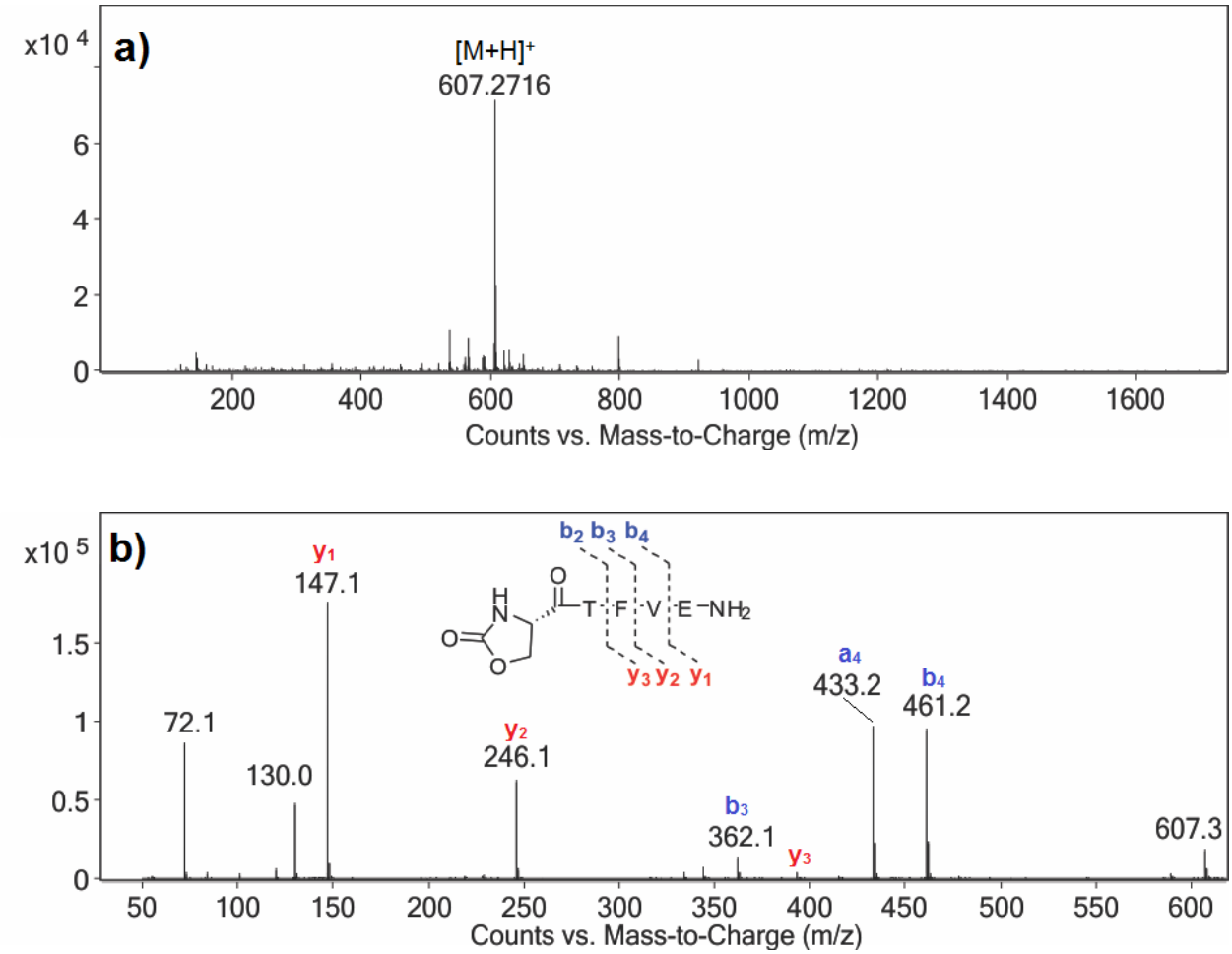


Figure S47. LC-IM-MS/MS data for linear peptide **(Table S3, entry 4)**, *Oxd*-FRVE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 662.3257, Found $[M+H]^+$ = 662.3265

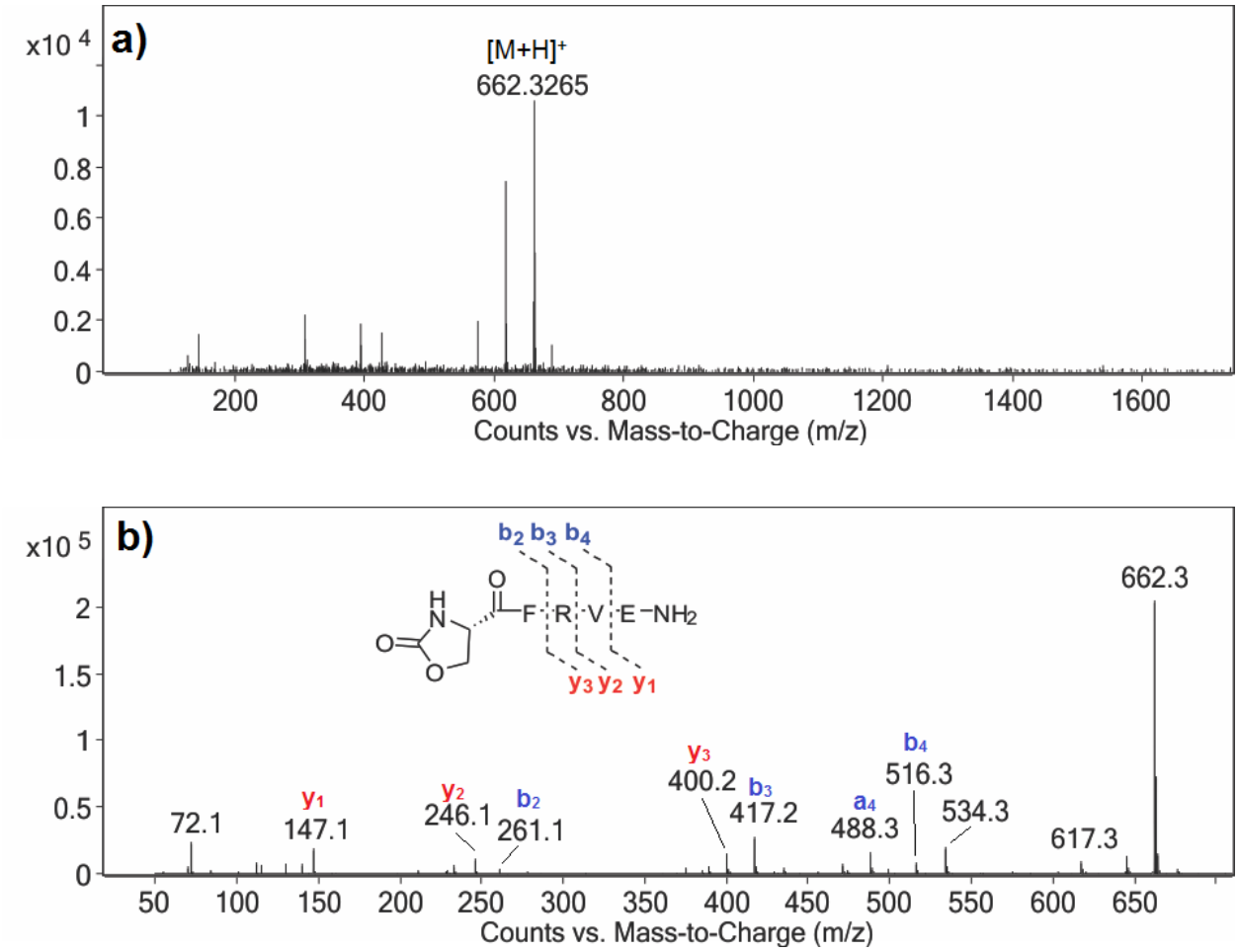


Figure S48. LC-IM-MS/MS data for linear peptide **(Table S3, entry 5)**, *Oxd*-YFAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 641.2566, Found $[M+H]^+$ = 641.2561

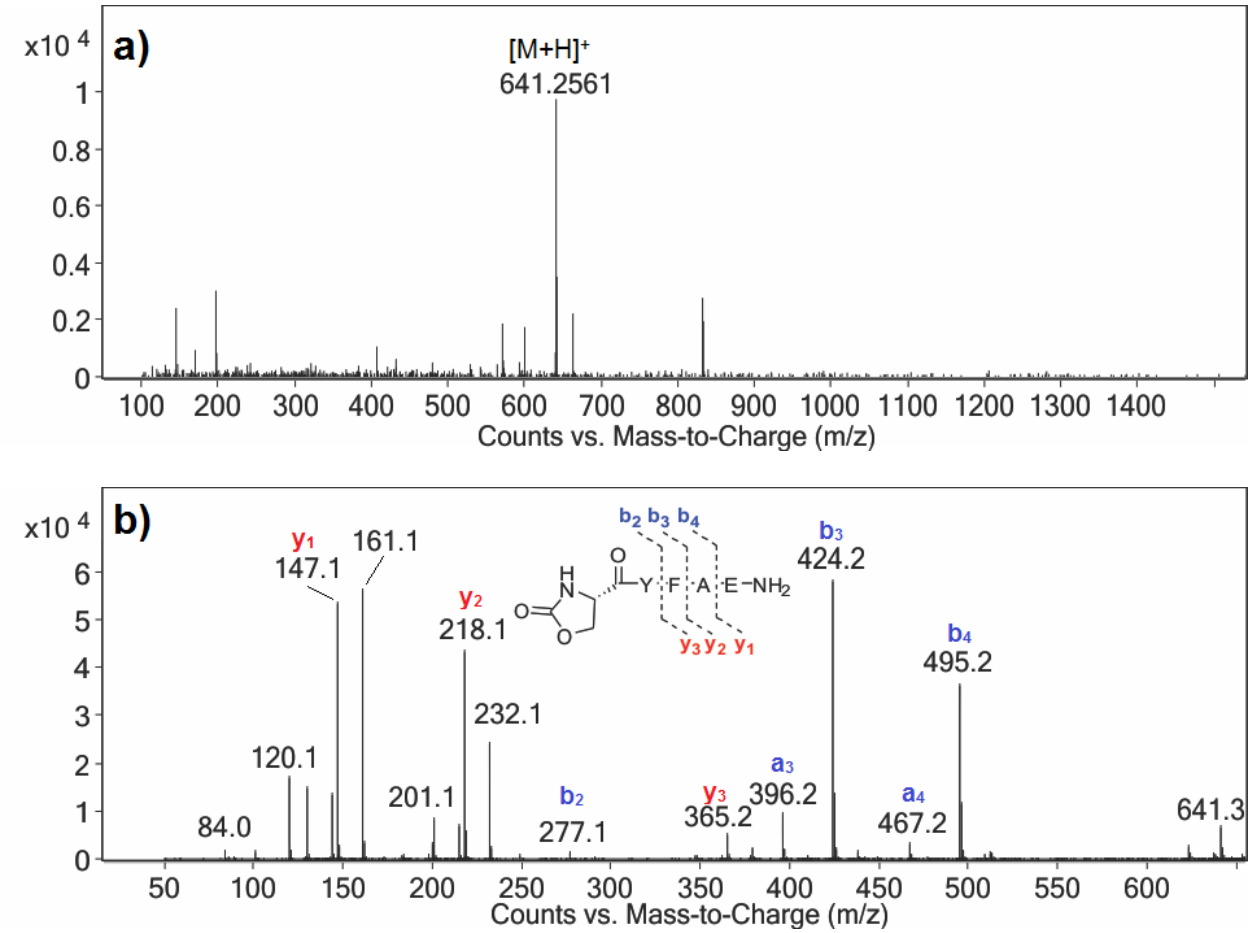


Figure S49. LC-IM-MS/MS data for linear peptide **(Table S3, entry 6),** *Oxd*-NYAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 608.2311, Found $[M+H]^+$ = 608.2335

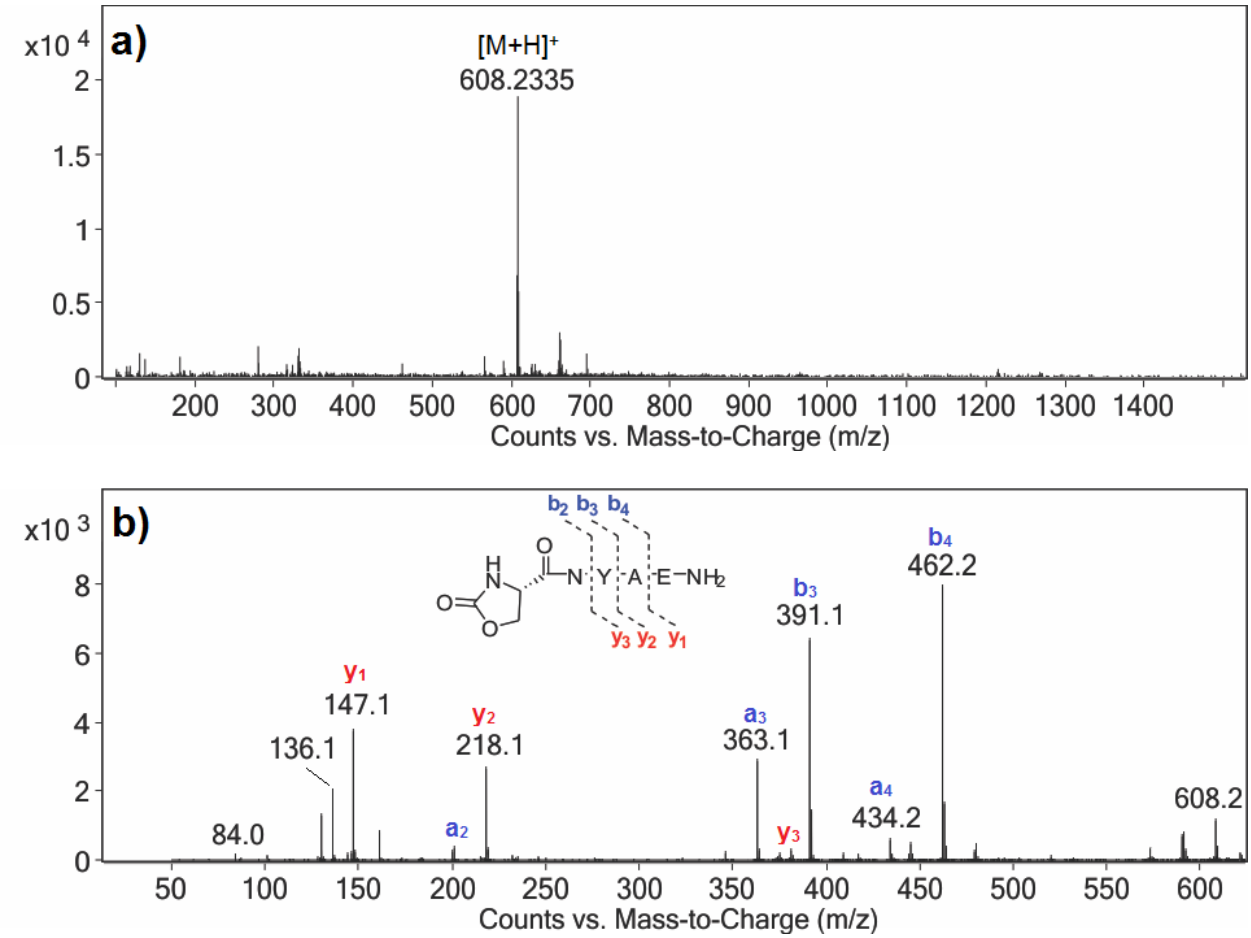


Figure S50. LC-IM-MS/MS data for linear peptide **(Table S3, entry 7)**, *Oxd*-YIGE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 593.2566, Found $[M+H]^+$ = 593.2584

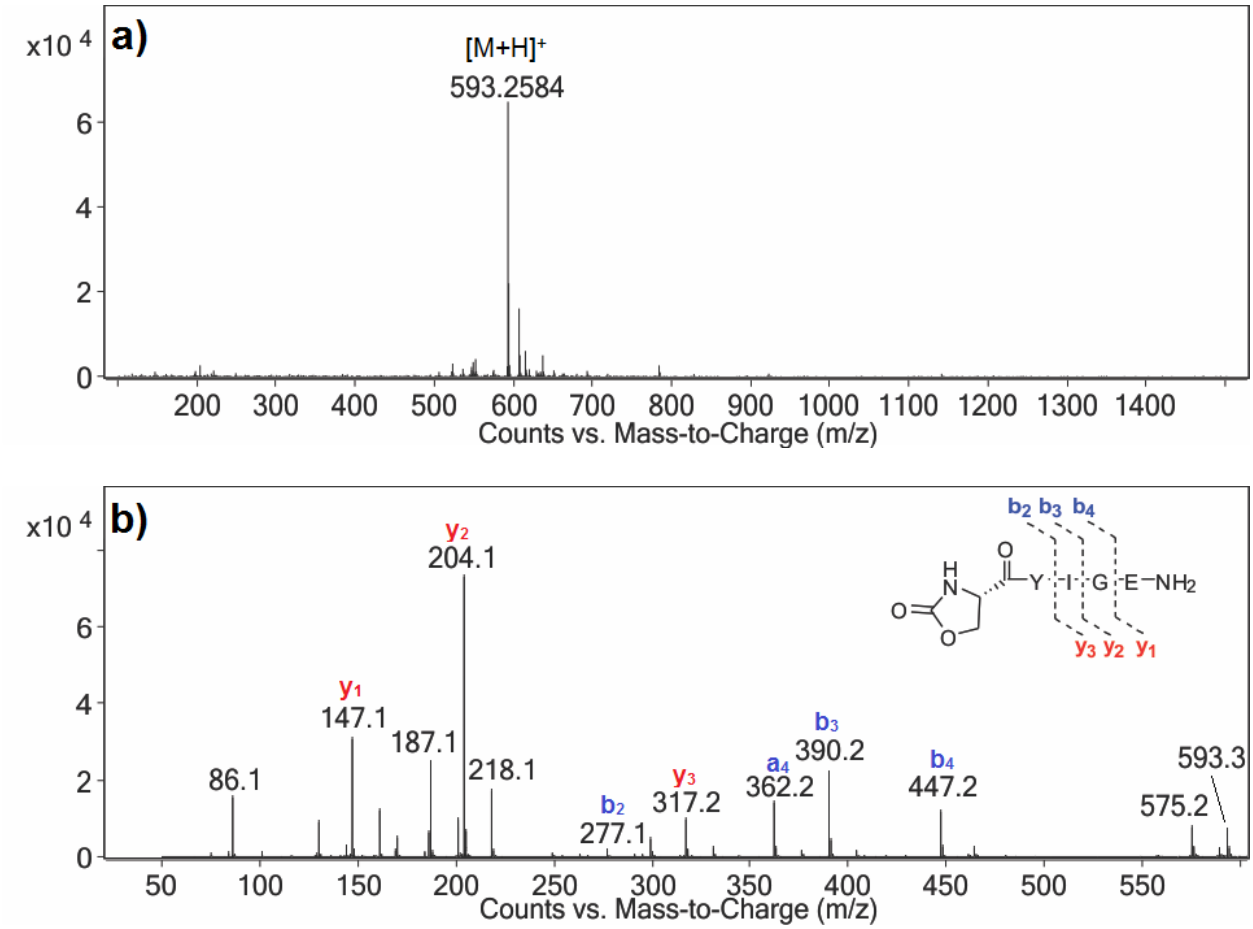


Figure S51. LC-IM-MS/MS data for linear peptide **(Table S3, entry 8),** *Oxd*-FNTE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 622.2467, Found $[M+H]^+$ = 622.2461

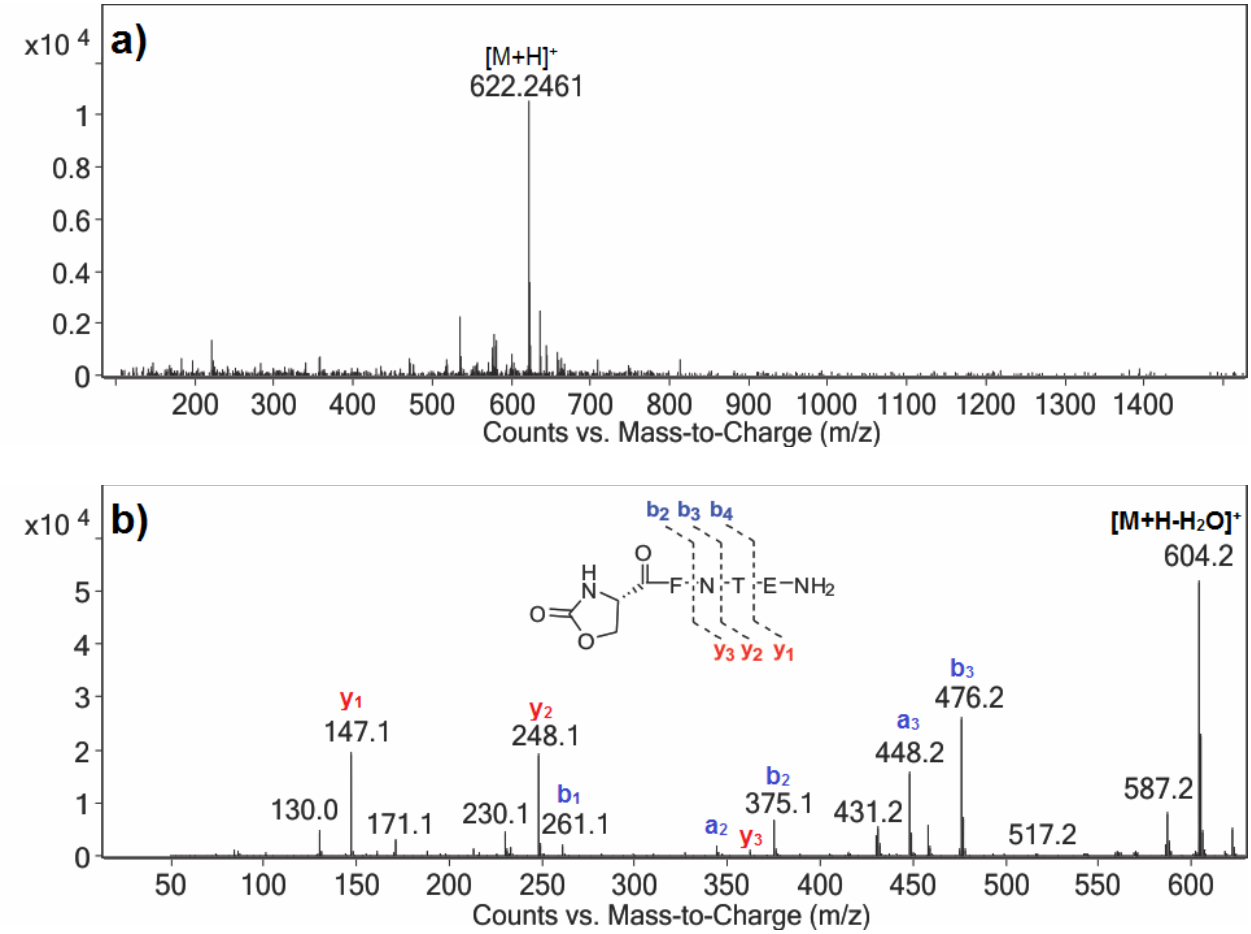


Figure S52. LC-IM-MS/MS data for linear peptide **(Table S3, entry 9)**, *Oxd*-VRFAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 733.3628, Found $[M+H]^+$ = 733.3651

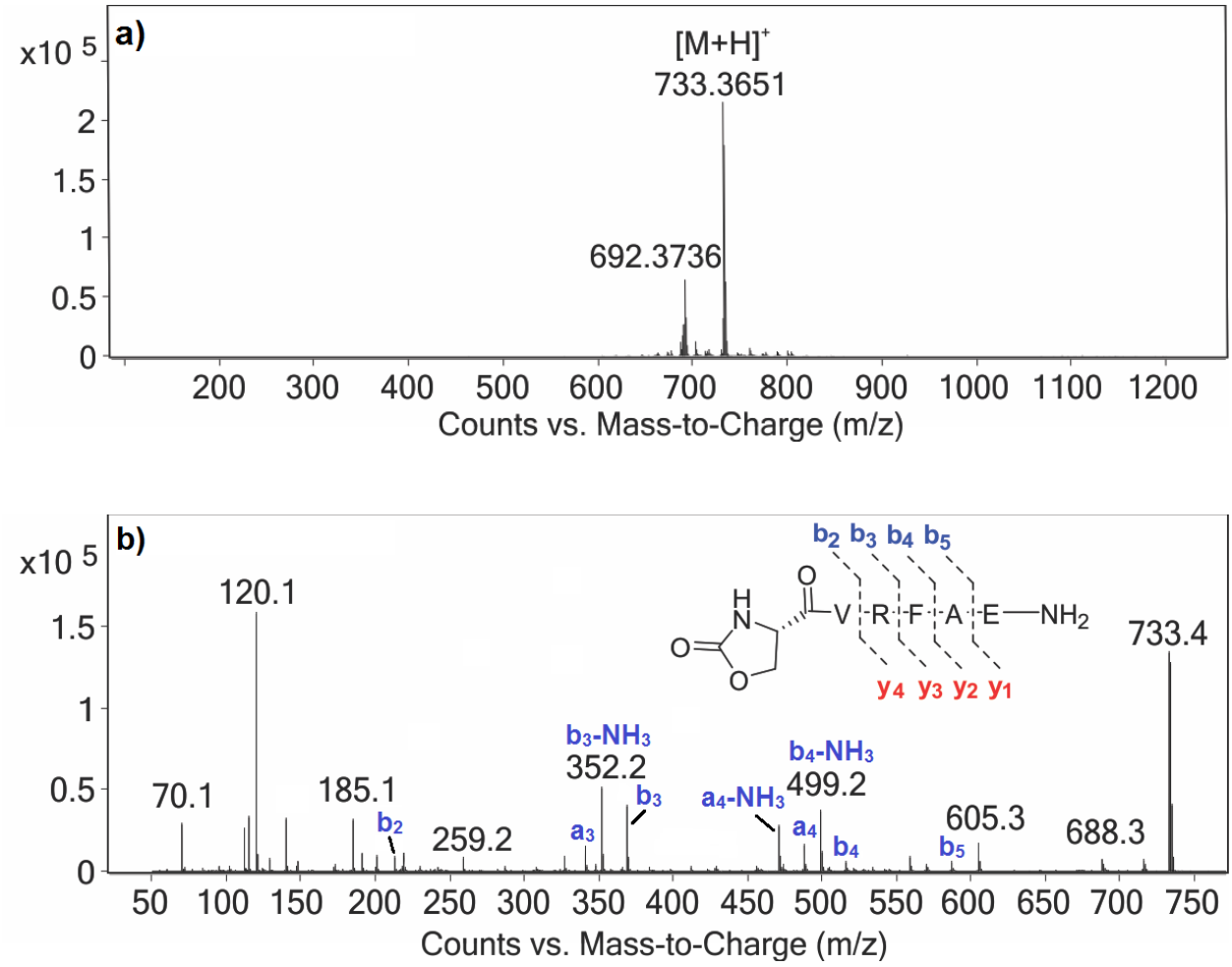


Figure S53. LC-IM-MS/MS data for linear peptide (**Table S3, entry 10**), *Oxd*-FRYAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 797.3577, Found $[M+H]^+$ = 797.3595

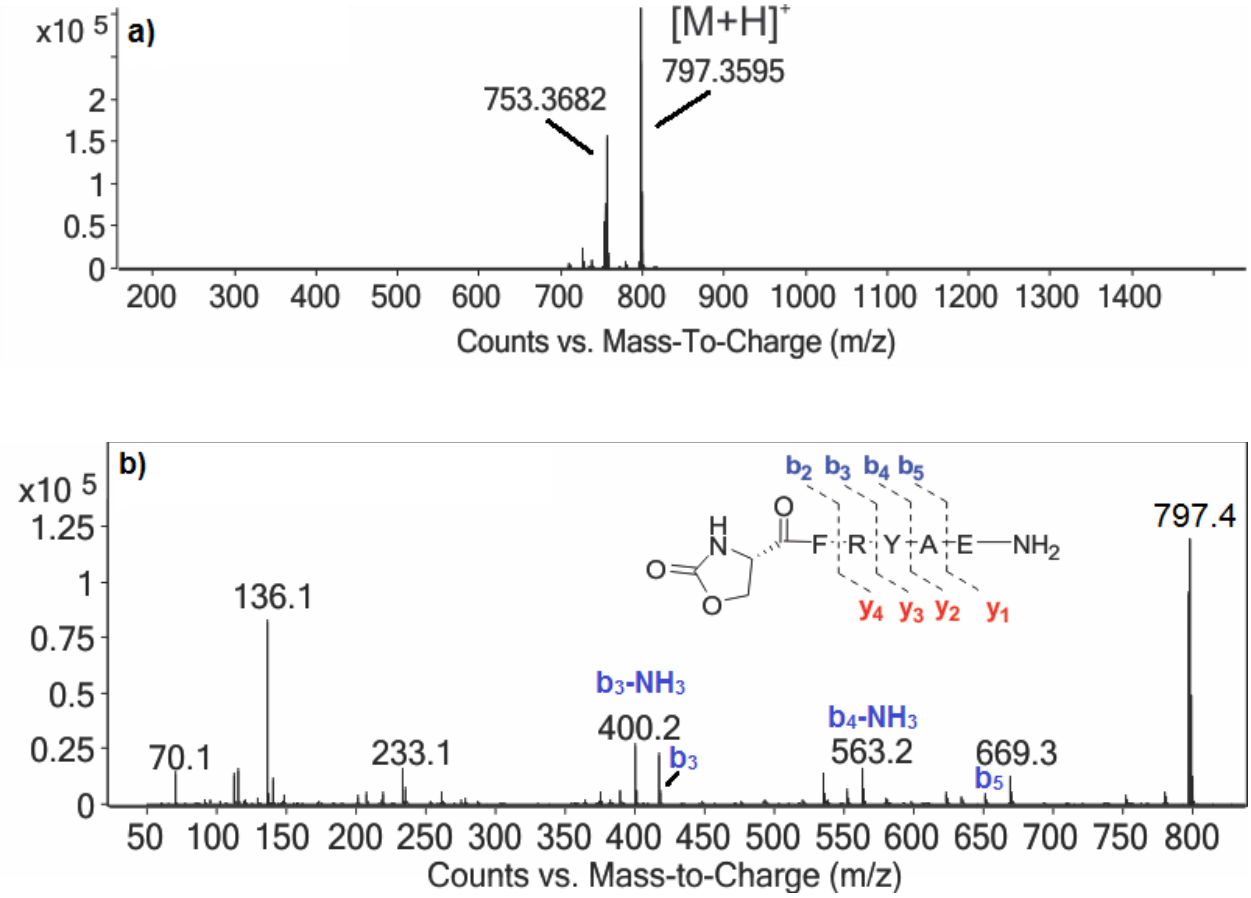


Figure S54. LC-IM-MS/MS data for linear peptide (**Table S3, entry 11**), *Oxd*-NYAAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 679.2682, Found $[M+H]^+$ = 679.2676

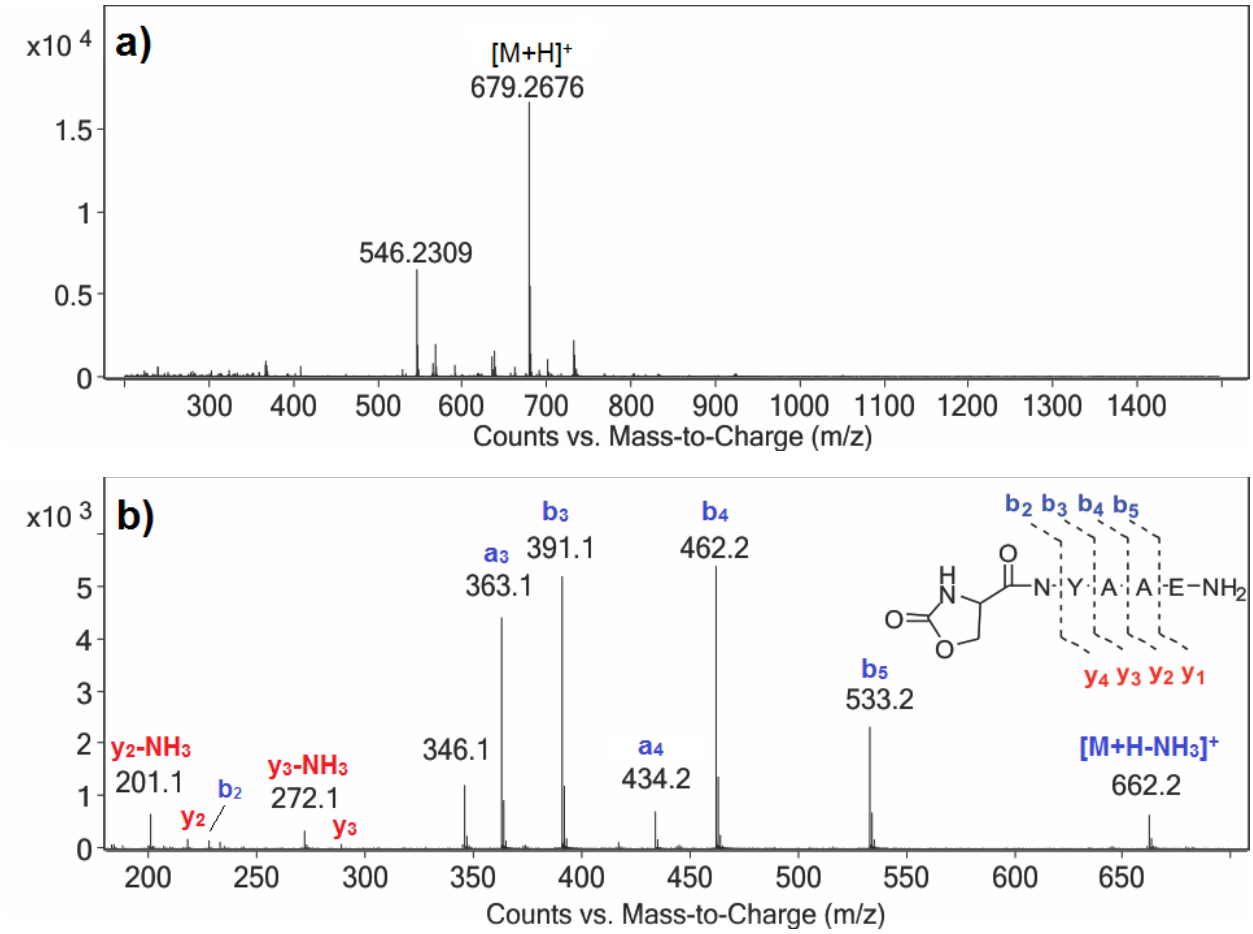


Figure S55. LC-IM-MS/MS data for linear peptide (**Table S3, entry 12**), *Oxd*-YIGAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 664.2937, Found $[M+H]^+$ = 664.2947

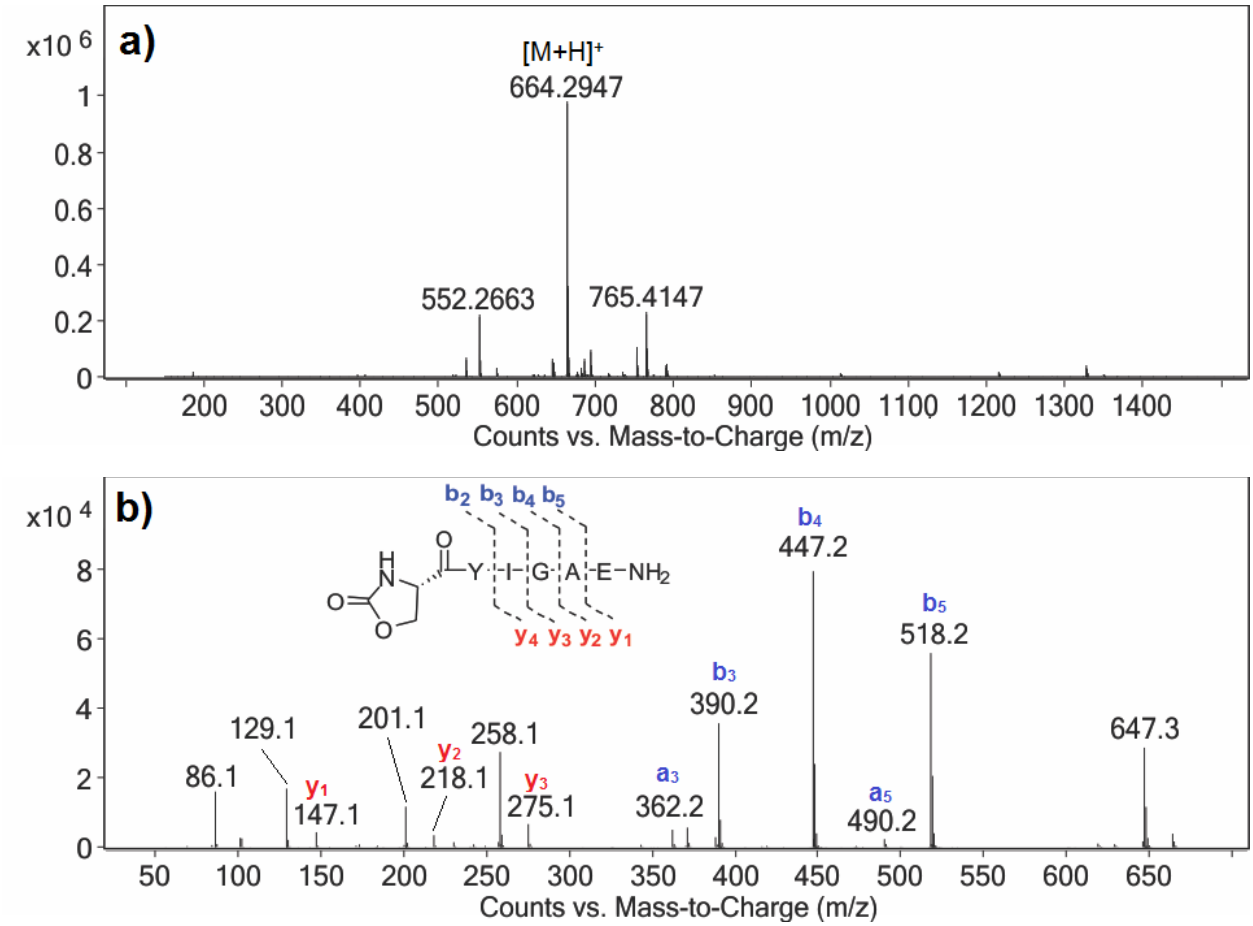


Figure S56. LC-IM-MS/MS data for linear peptide (**Table S3, entry 13)**, *Oxd*-YFVGE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 726.3093, Found $[M+H]^+$ = 726.3115

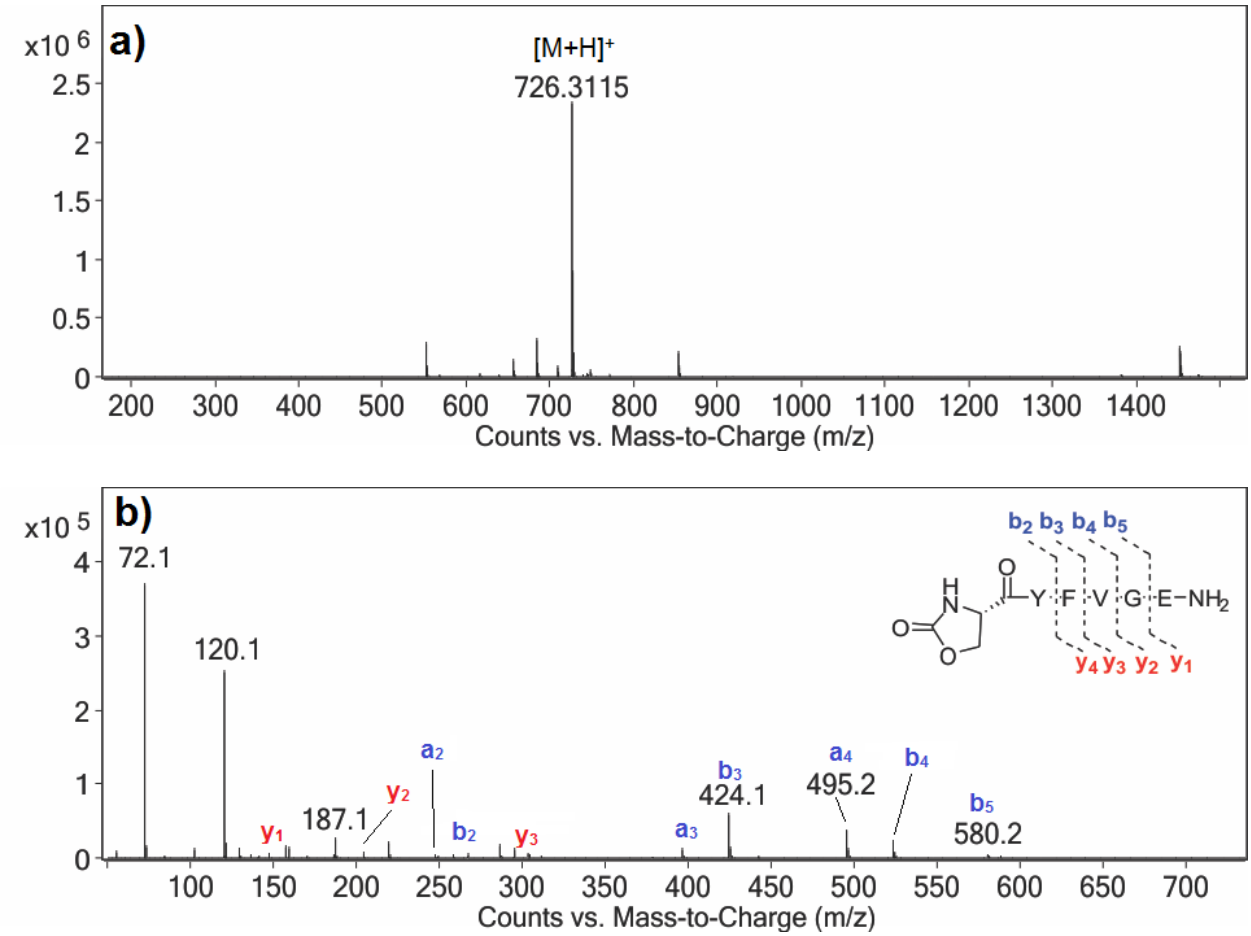


Figure S57. LC-IM-MS/MS data for linear peptide (**Table S3, entry 14)**, *Oxd*-KIFEG-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 705.3566, Found $[M+H]^+$ = 705.3576

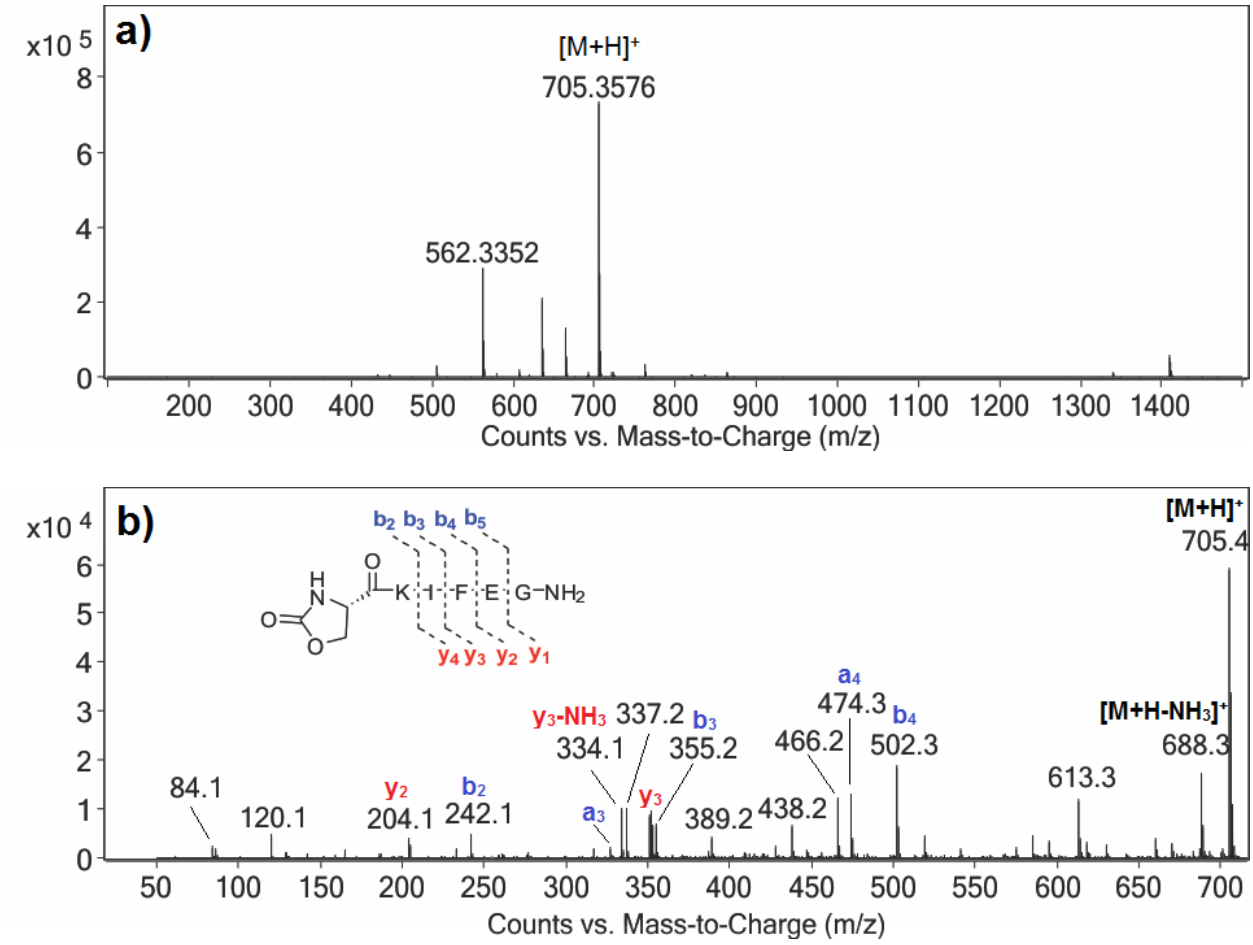


Figure S58. LC-IM-MS/MS data for linear peptide (**Table S3, entry 15**), *Oxd*-HVDEF-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 758.3104, Found $[M+H]^+$ = 758.3105

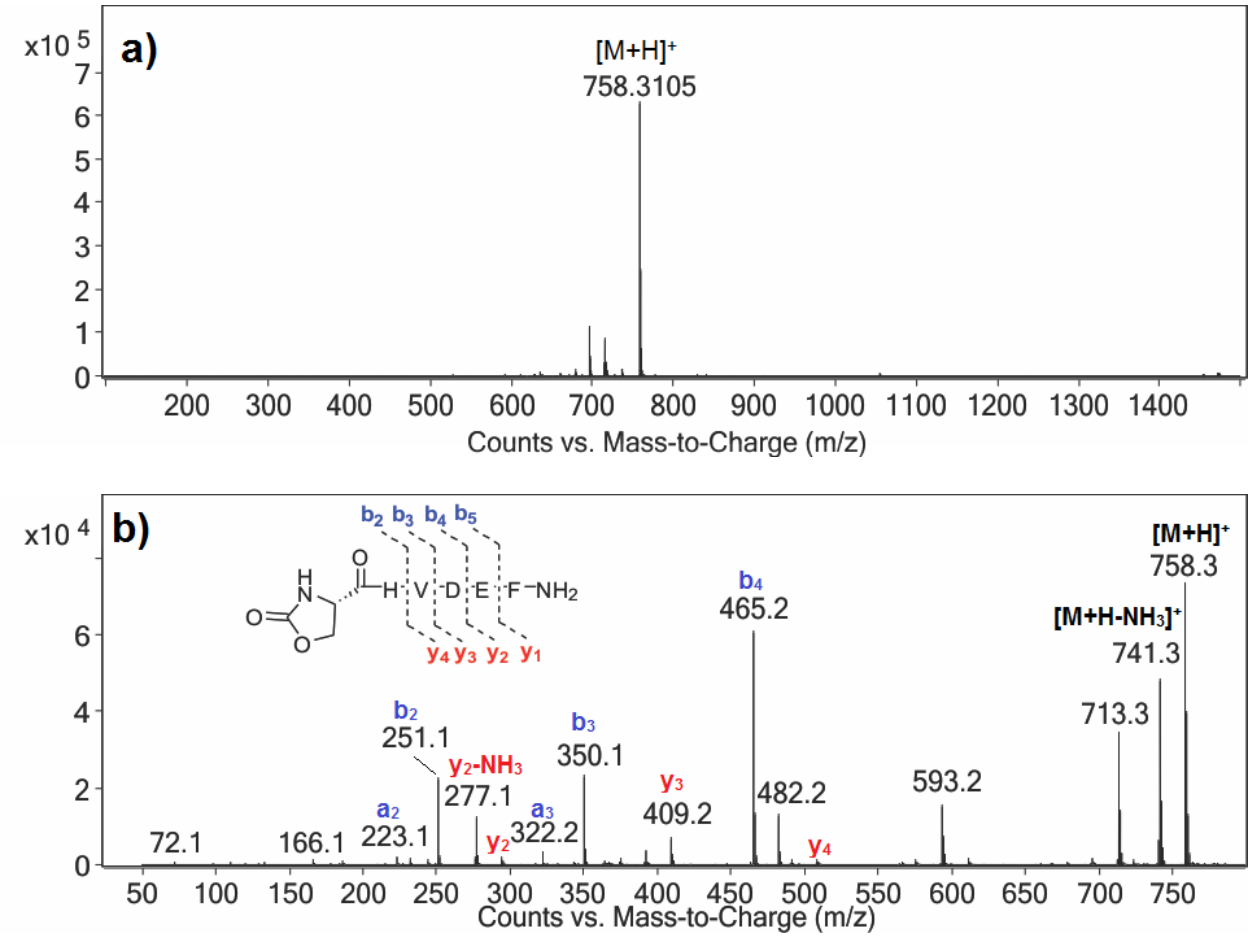


Figure S59. LC-IM-MS/MS data for linear peptide (**Table S3, entry 16**), *Oxd*-TLDEF-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 736.3148, Found $[M+H]^+$ = 736.3156

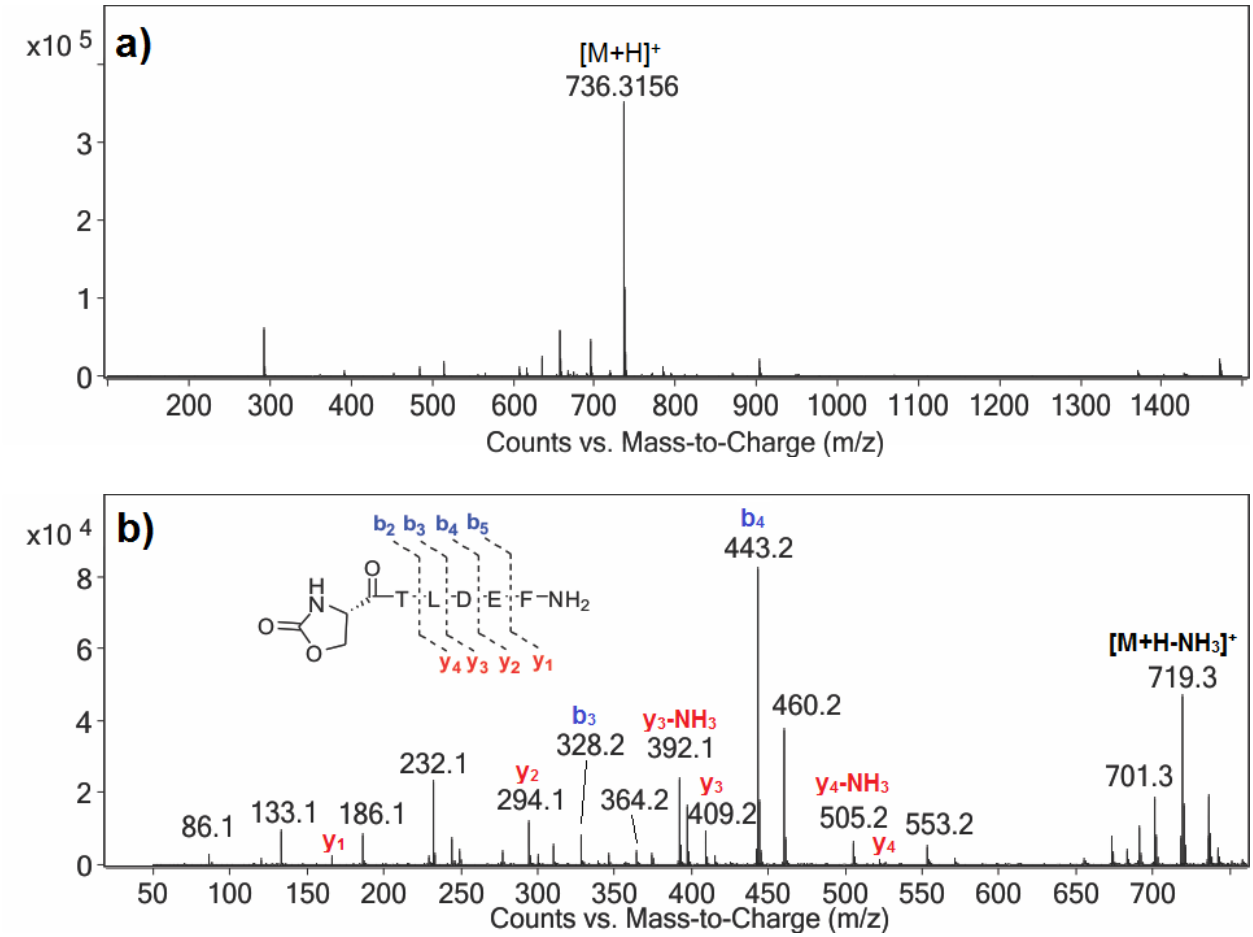


Figure S60. LC-IM-MS/MS data for linear peptide (**Table S3, entry 17)**, *Oxd*-RQFEA-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 762.3529, Found $[M+H]^+$ = 762.3531

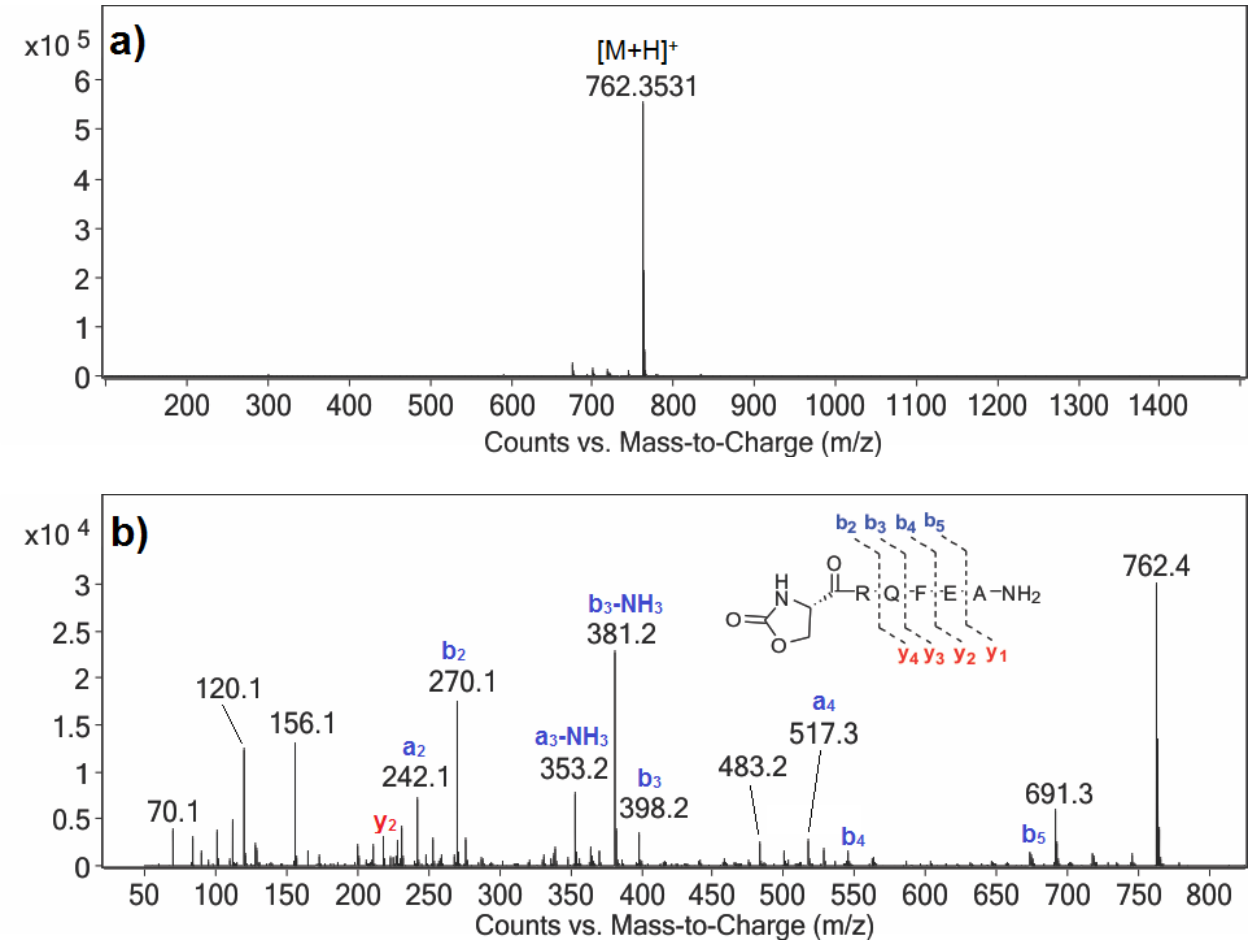


Figure S61. LC-IM-MS/MS data for linear peptide (**Table S3, entry 18)**, *Oxd*-KIFAGE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 776.3937, Found $[M+H]^+$ = 776.3944

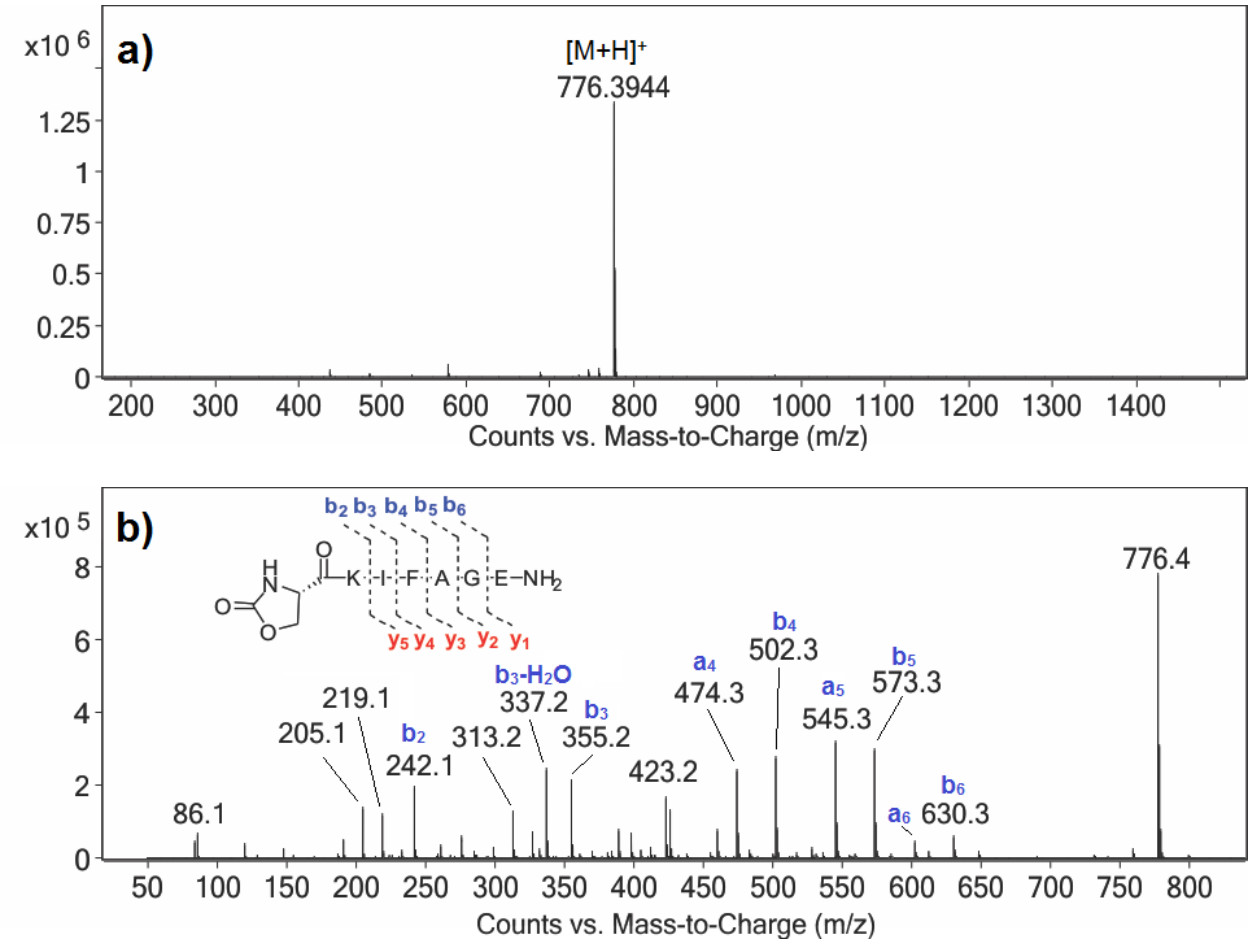


Figure S62. LC-IM-MS/MS data for linear peptide (**Table S3, entry 19)**, *Oxd*-AHDVGE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 739.3006, Found $[M+H]^+$ = 739.2987

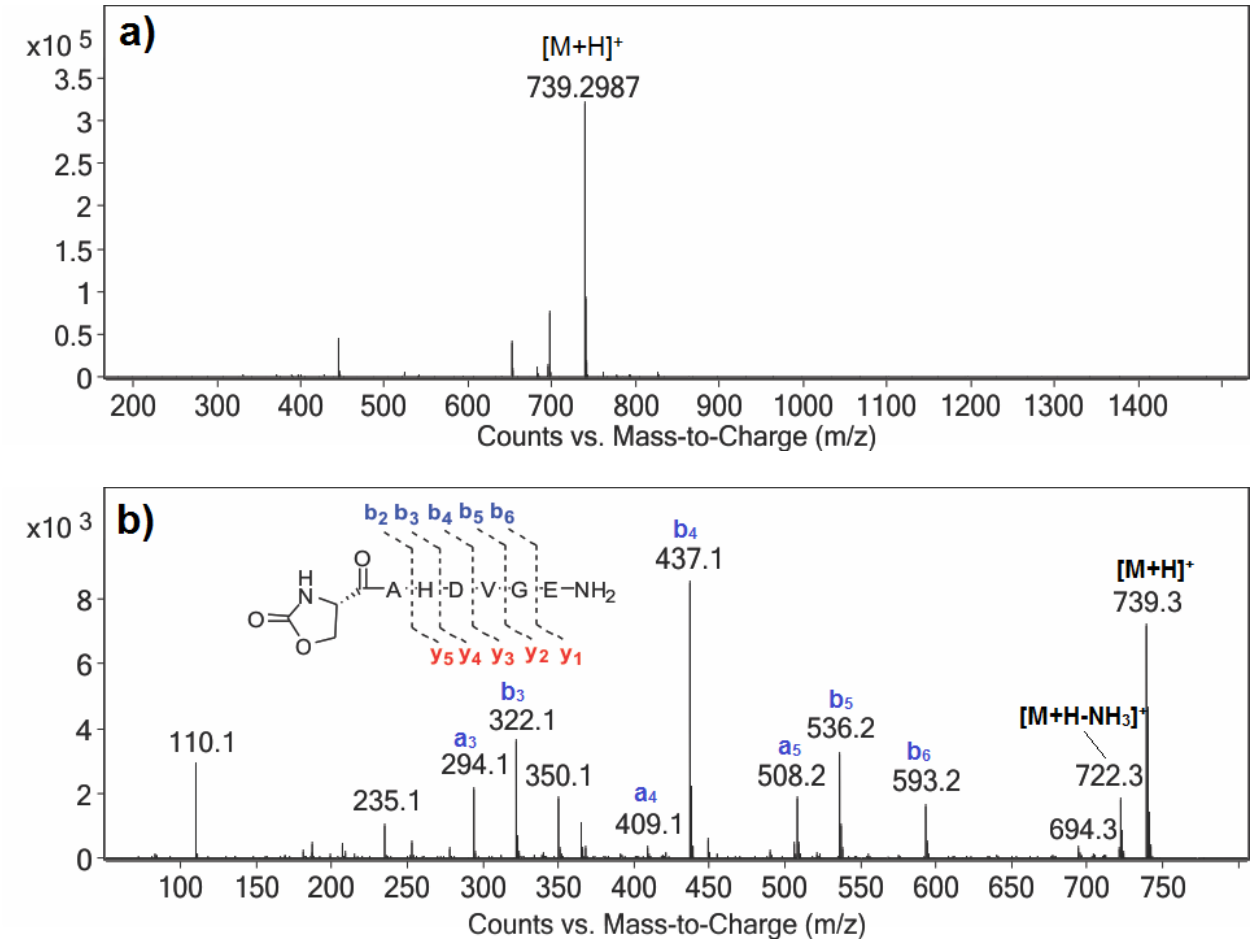


Figure S63. LC-IM-MS/MS data for linear peptide (**Table S3, entry 20)**, *Oxd*-TYDAFE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 857.3312, Found $[M+H]^+$ = 857.3307

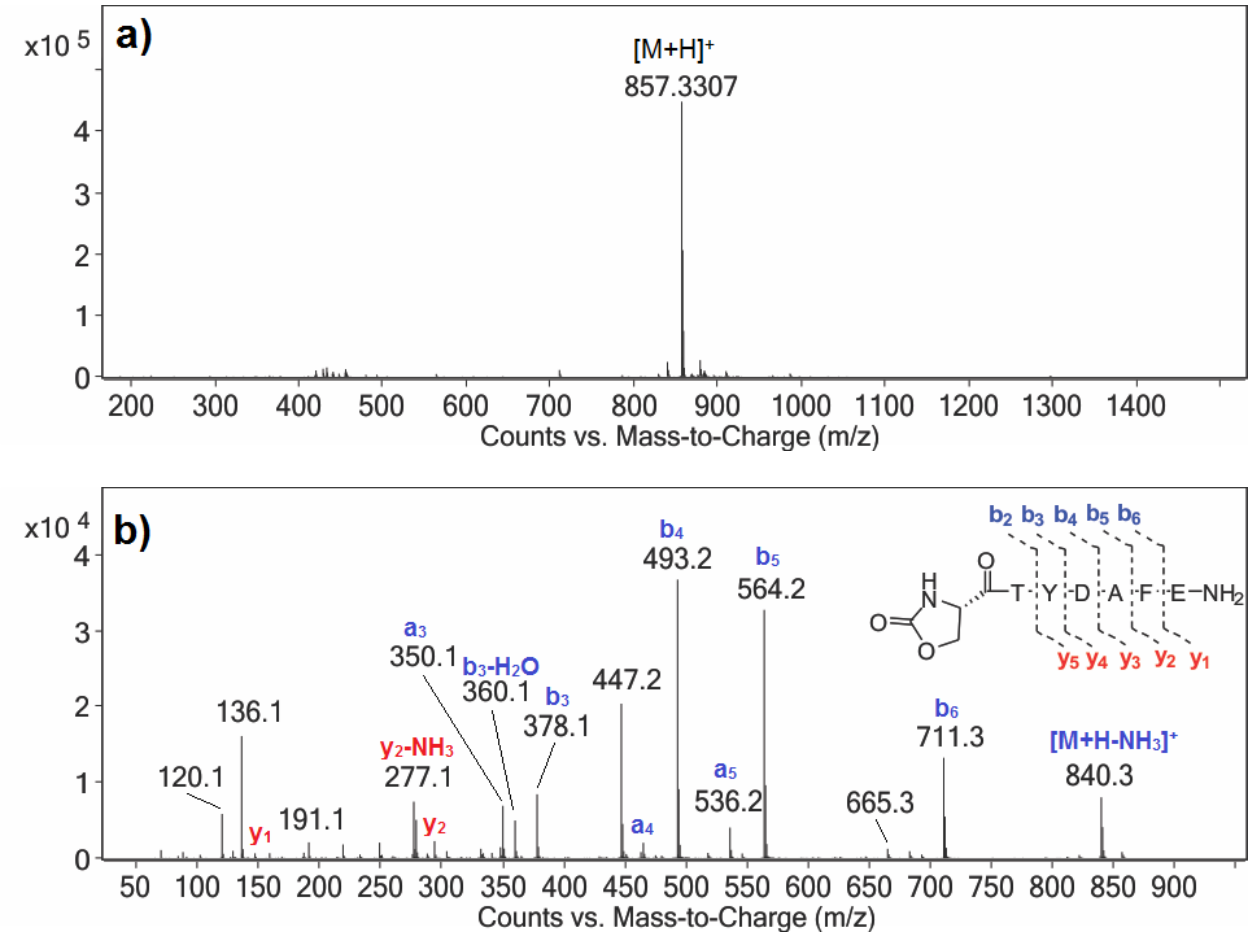


Figure S64. LC-IM-MS/MS data for linear peptide **(Table S3, entry 21)**, *Oxd*-TFKRVE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 891.4683, Found $[M+H]^+$ = 891.4704

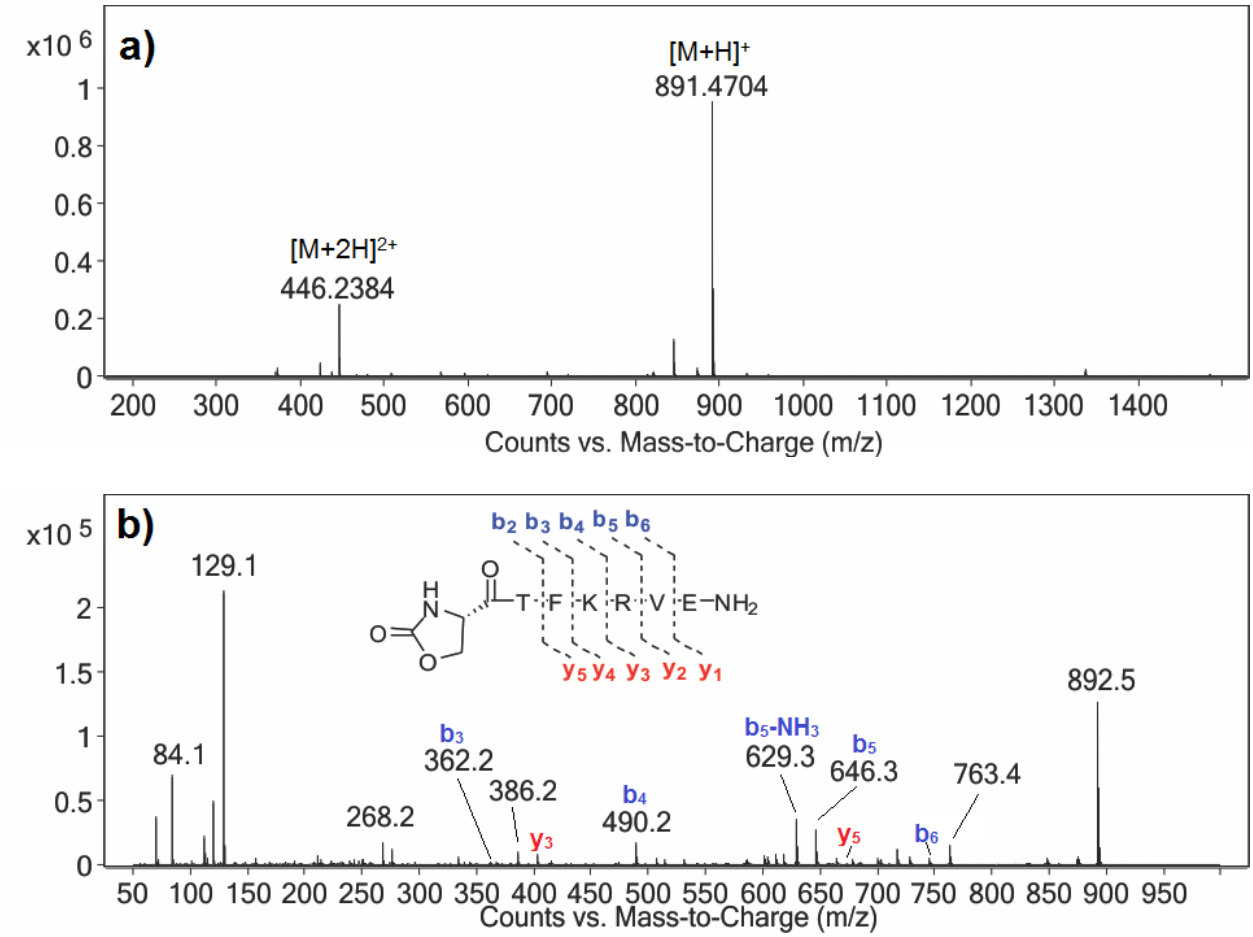


Figure S65. LC-IM-MS/MS data for linear peptide (**Table S3, entry 22)**, *Oxd*-IGFEAF-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 795.3672, Found $[M+H]^+$ = 795.3683

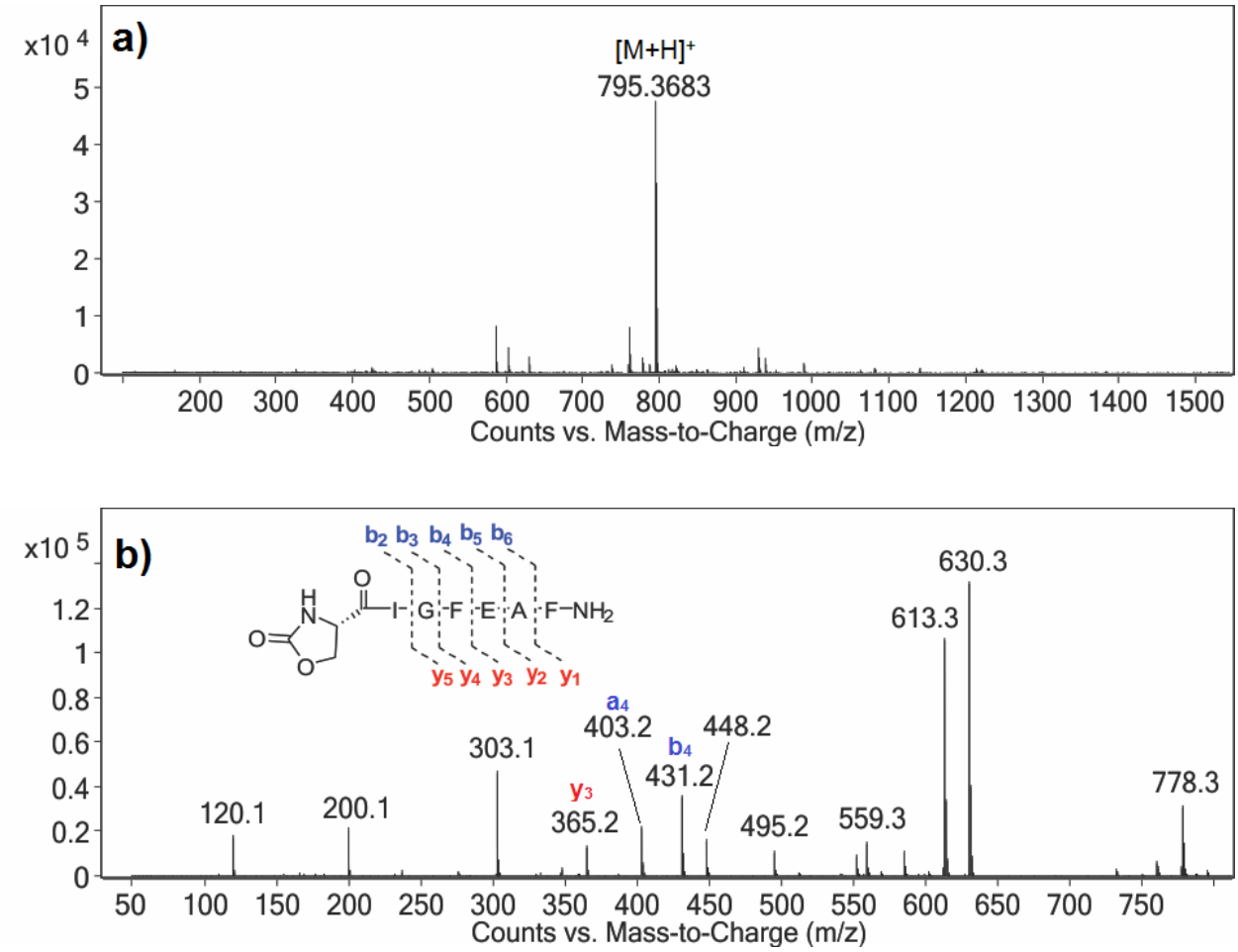


Figure S66. LC-IM-MS/MS data for linear peptide (**Table S3, entry 23)**, *Oxd*-RVDYGAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 921.4061, Found $[M+H]^+$ = 921.4053

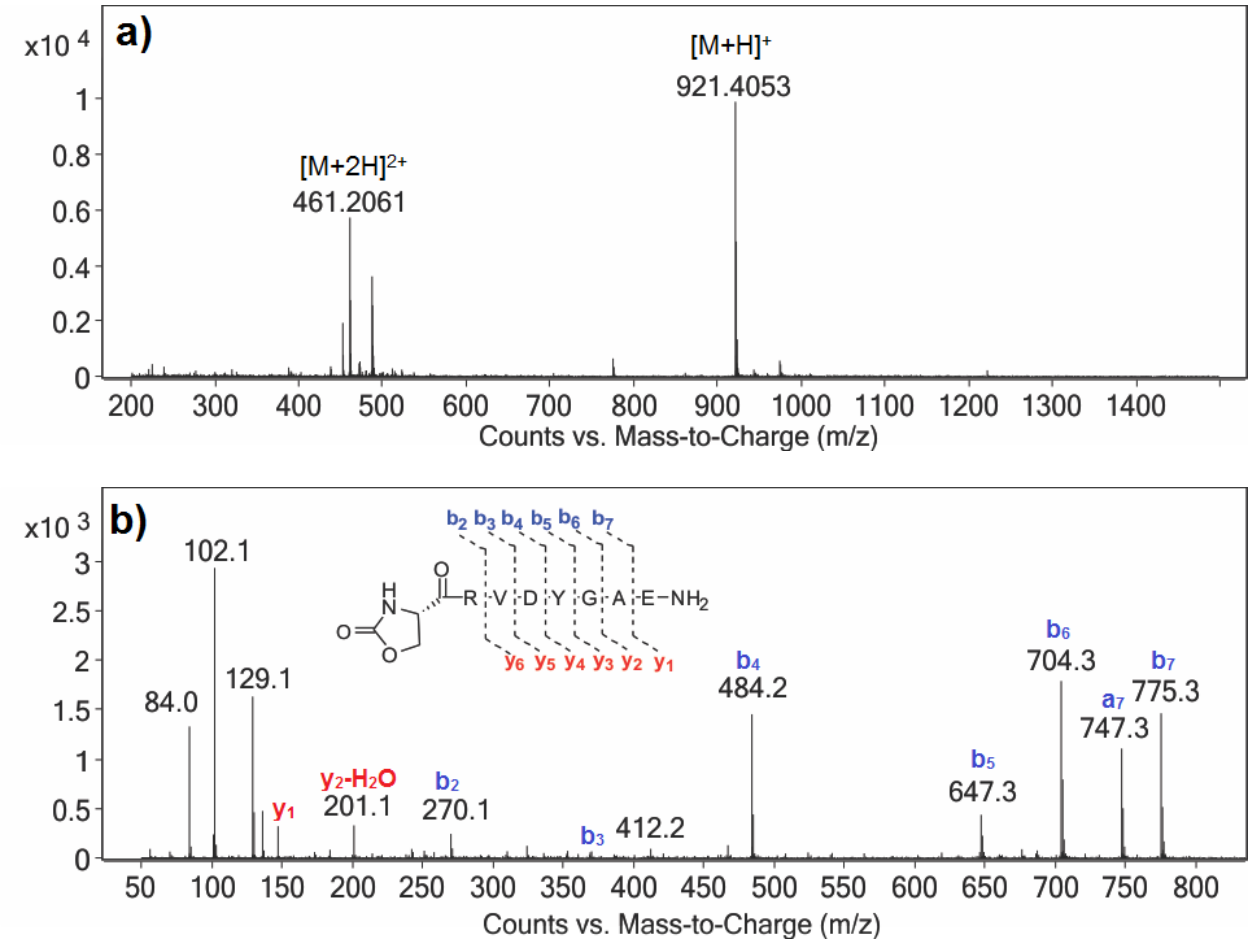


Figure S67. LC-IM-MS/MS data for linear peptide (**Table S3, entry 24)**, *Oxd*-AVFMNAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 893.3822, Found $[M+H]^+$ = 893.3811

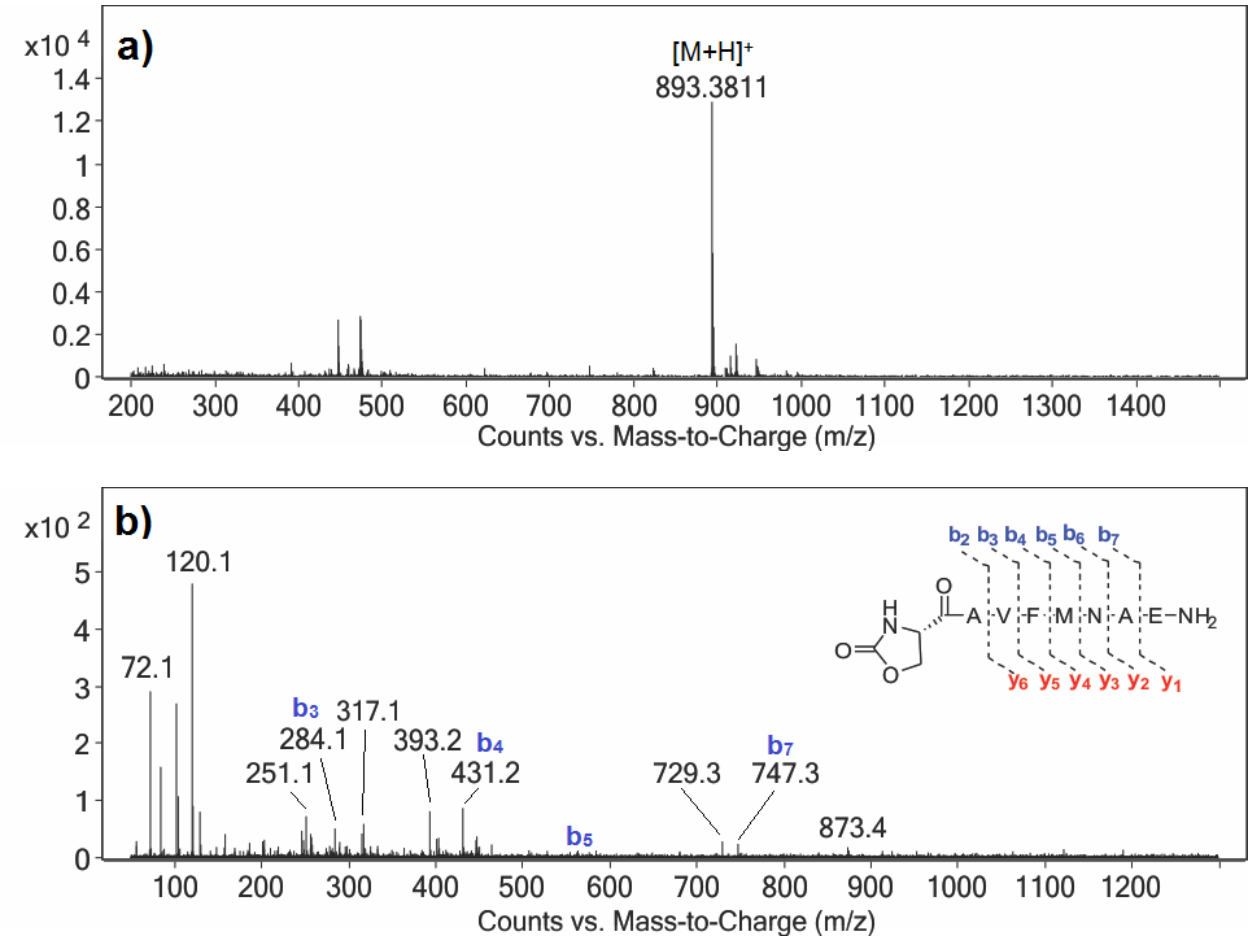


Figure S68. LC-IM-MS/MS data for linear peptide (**Table S3, entry 25)**, *Oxd*-KVRNYAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+2H]^{2+}$ = 496.2514, Found $[M+2H]^{2+}$ = 496.2513

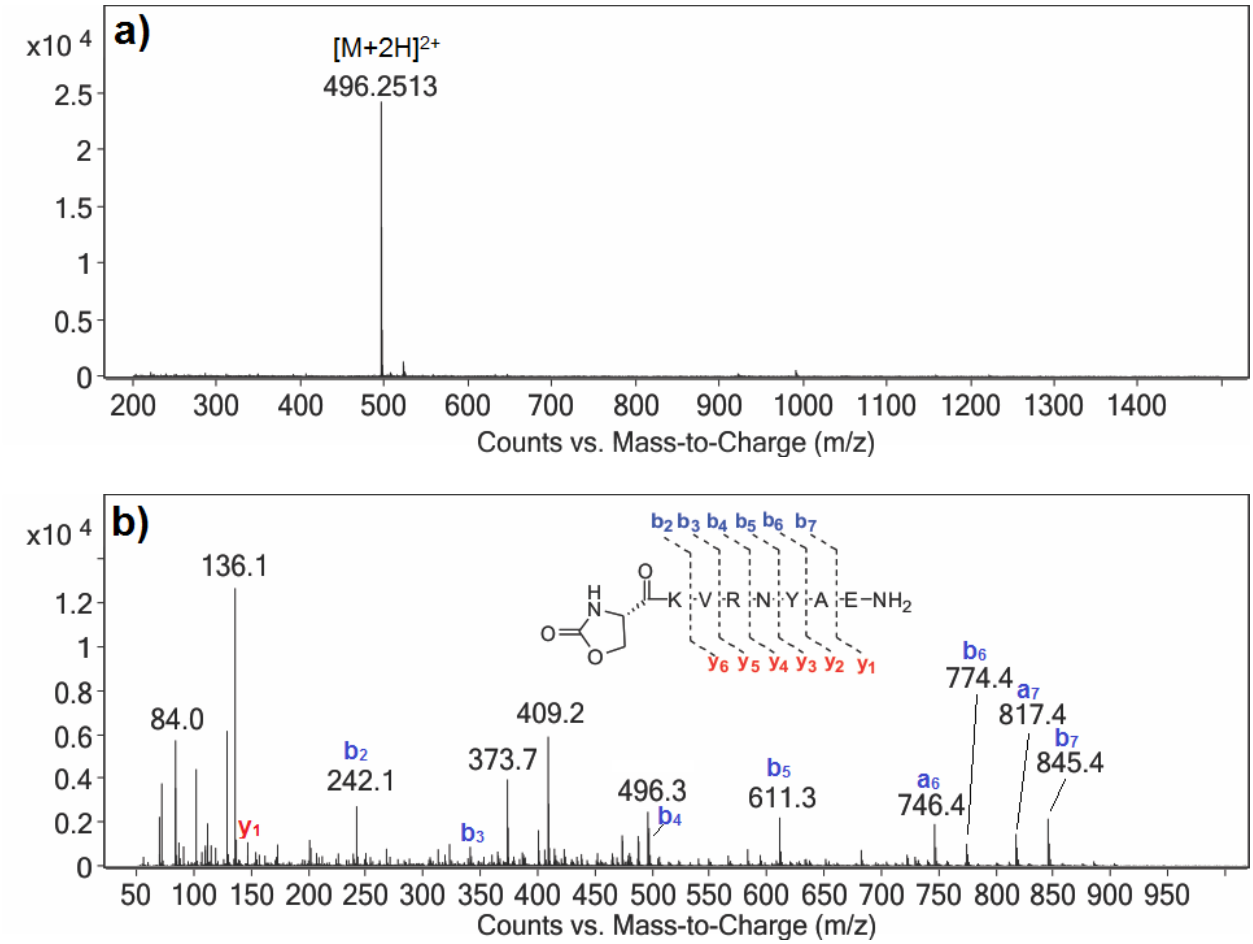


Figure S69. LC-IM-MS/MS data for linear peptide (**Table S3, entry 26)**, *Oxd*-RYQVANE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+2H]^{2+}$ = 496.2332, Found $[M+2H]^{2+}$ = 496.2330

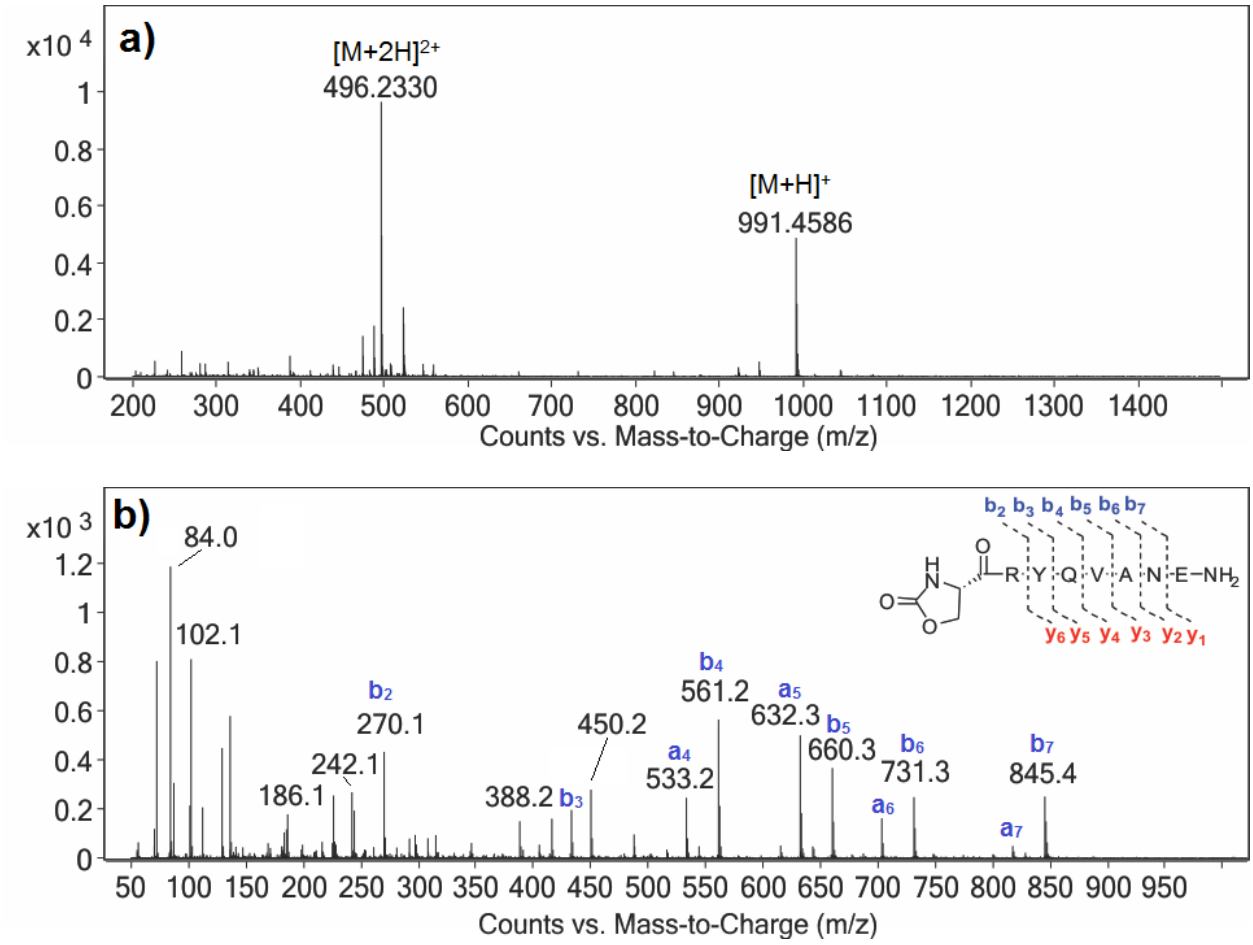


Figure S70. LC-IM-MS/MS data for linear peptide (**Table S3, entry 27)**, *Oxd*-KARFGVE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+2H]^{2+}$ = 459.7432, Found $[M+2H]^{2+}$ = 459.7430

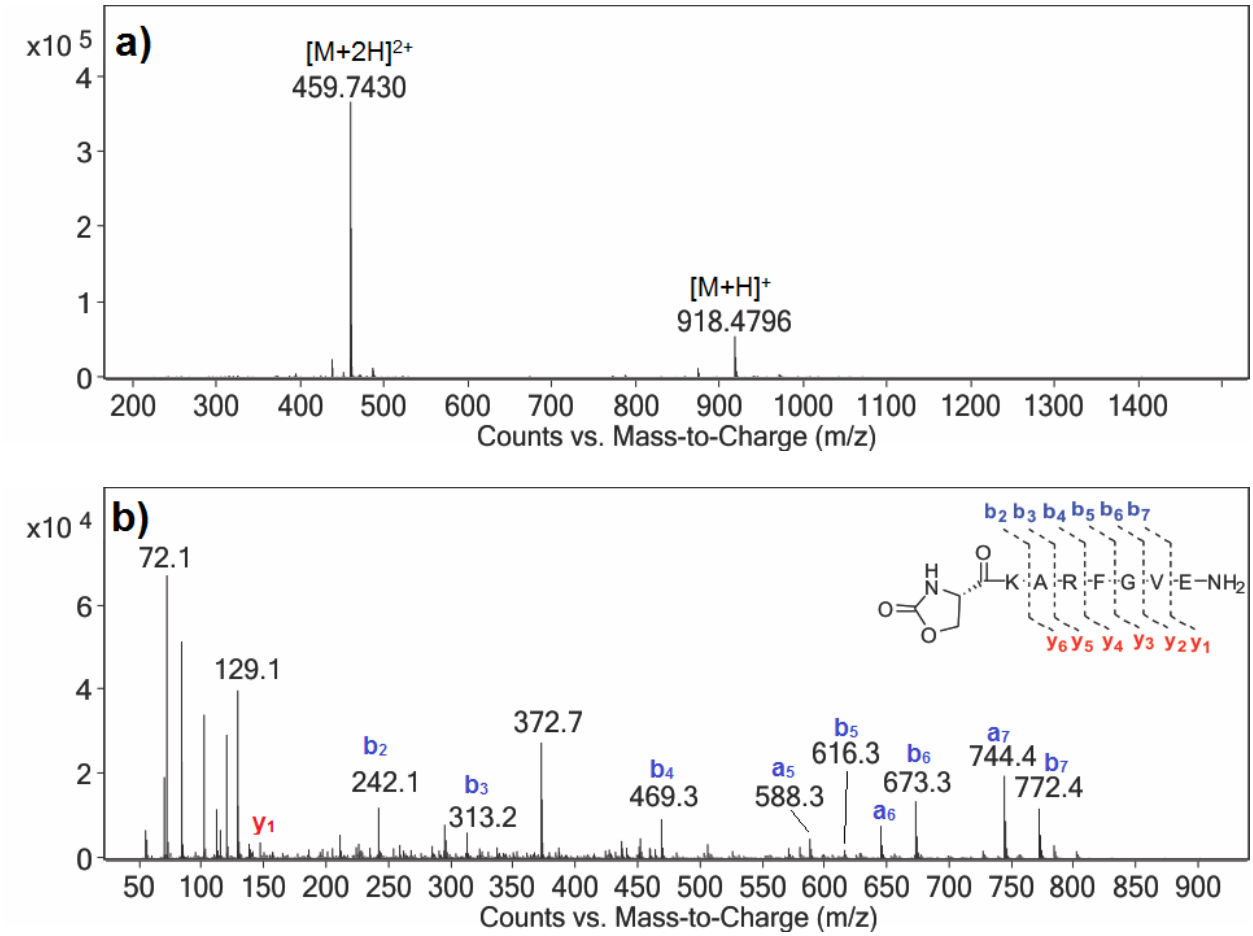


Figure S71. LC-IM-MS/MS data for linear peptide (**Table S3, entry 28)**, *Oxd*-AQVFRFTE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 1109.5374, Found $[M+H]^+$ = 1109.5393

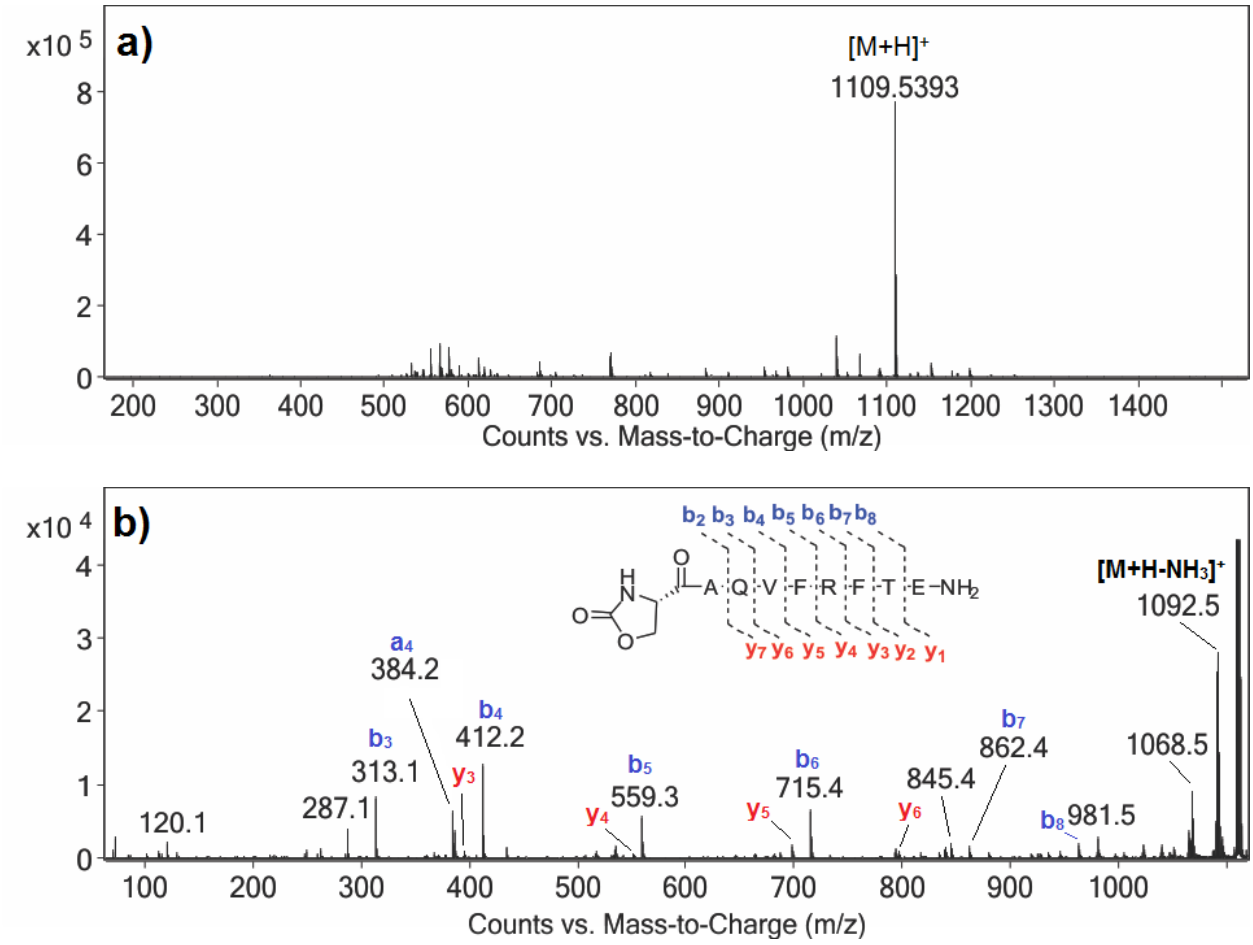


Figure S72. LC-IM-MS/MS data for linear peptide (**Table S3, entry 29)**, *Oxd*-YTFNDKLE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 1141.5160, Found $[M+H]^+$ = 1141.5174

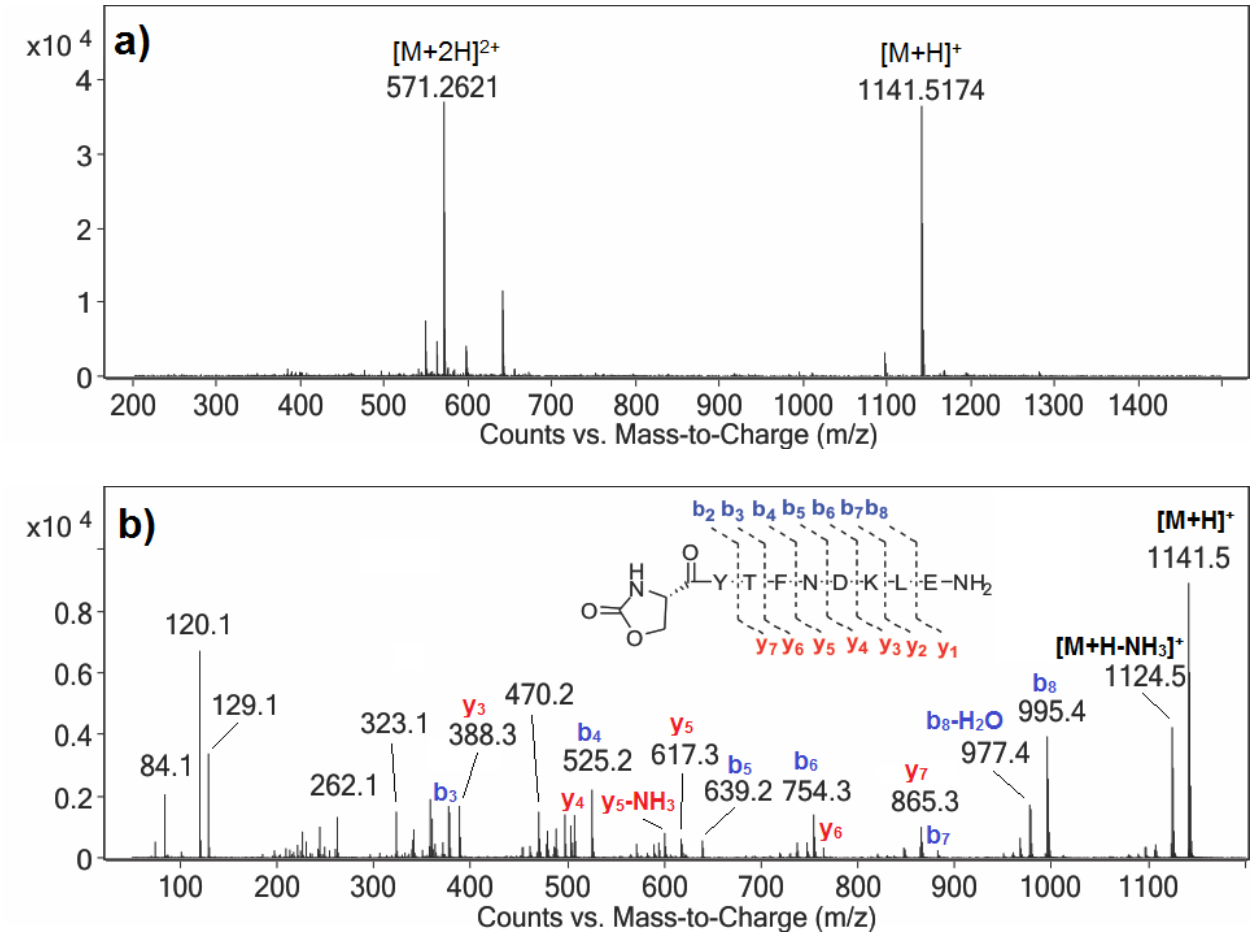


Figure S73. LC-IM-MS/MS data for linear peptide (**Table S3, entry 30)**, *Oxd*-RVKNGFEA-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 1032.5221, Found $[M+H]^+$ = 1032.5232

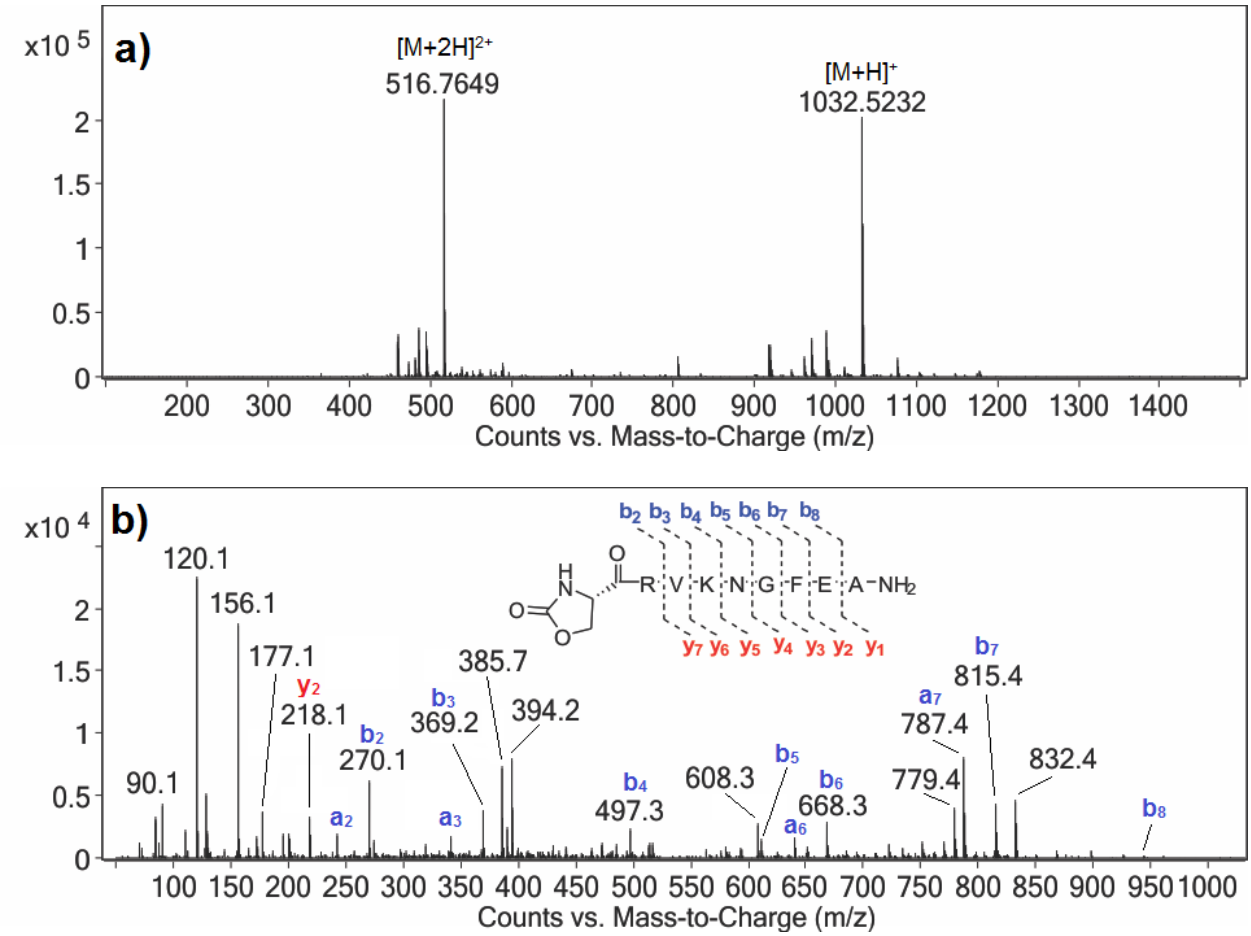


Figure S74. LC-IM-MS/MS data for linear peptide (**Table S3, entry 31)**, *Oxd*-YLFDWREK-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 1268.6058, Found $[M+H]^+$ = 1268.6067

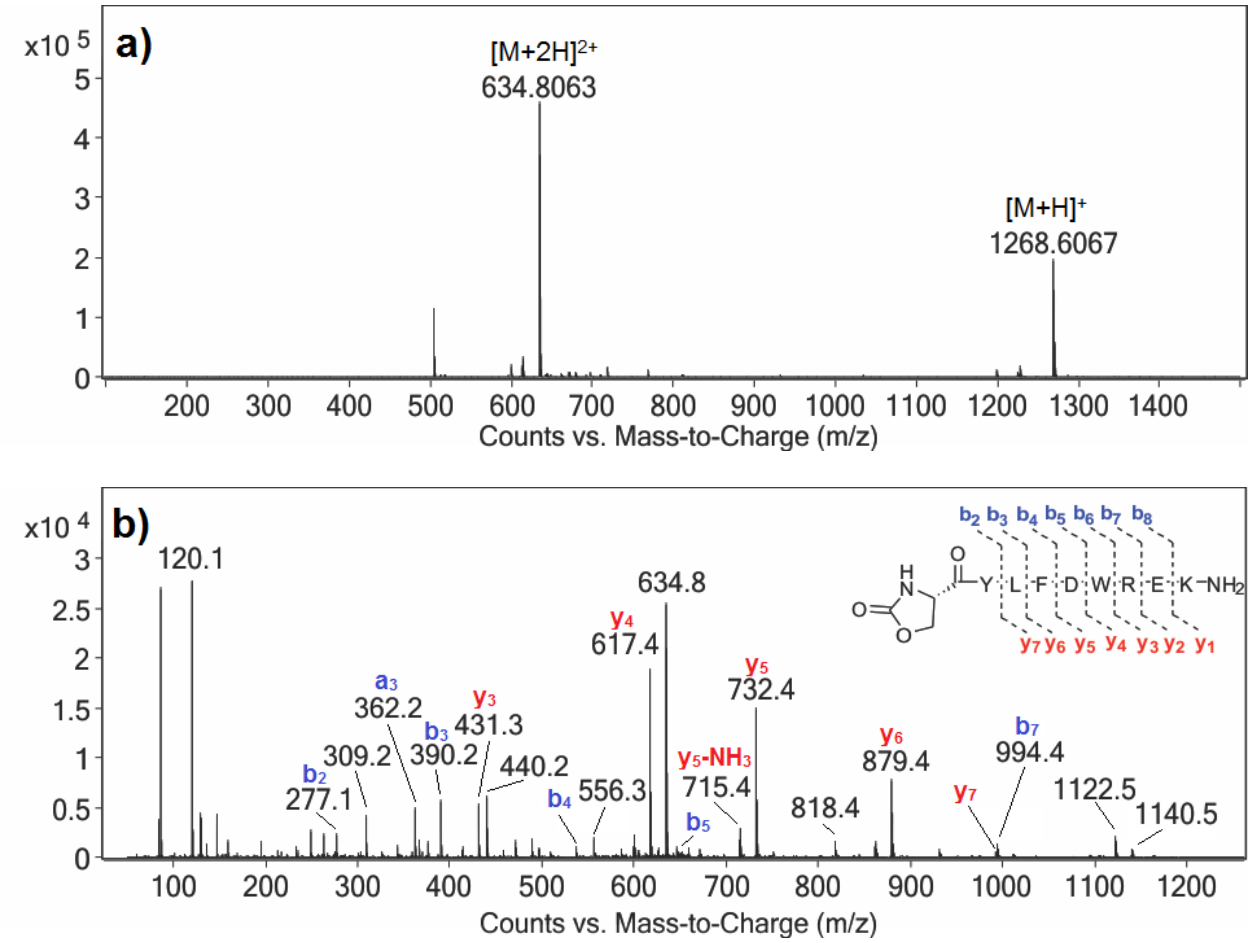


Figure S75. LC-IM-MS/MS data for linear peptide (**Table S3, entry 32**), *Oxd*-GYVKDFRAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+H]^+$ = 1196.5695, Found $[M+H]^+$ = 1196.5672

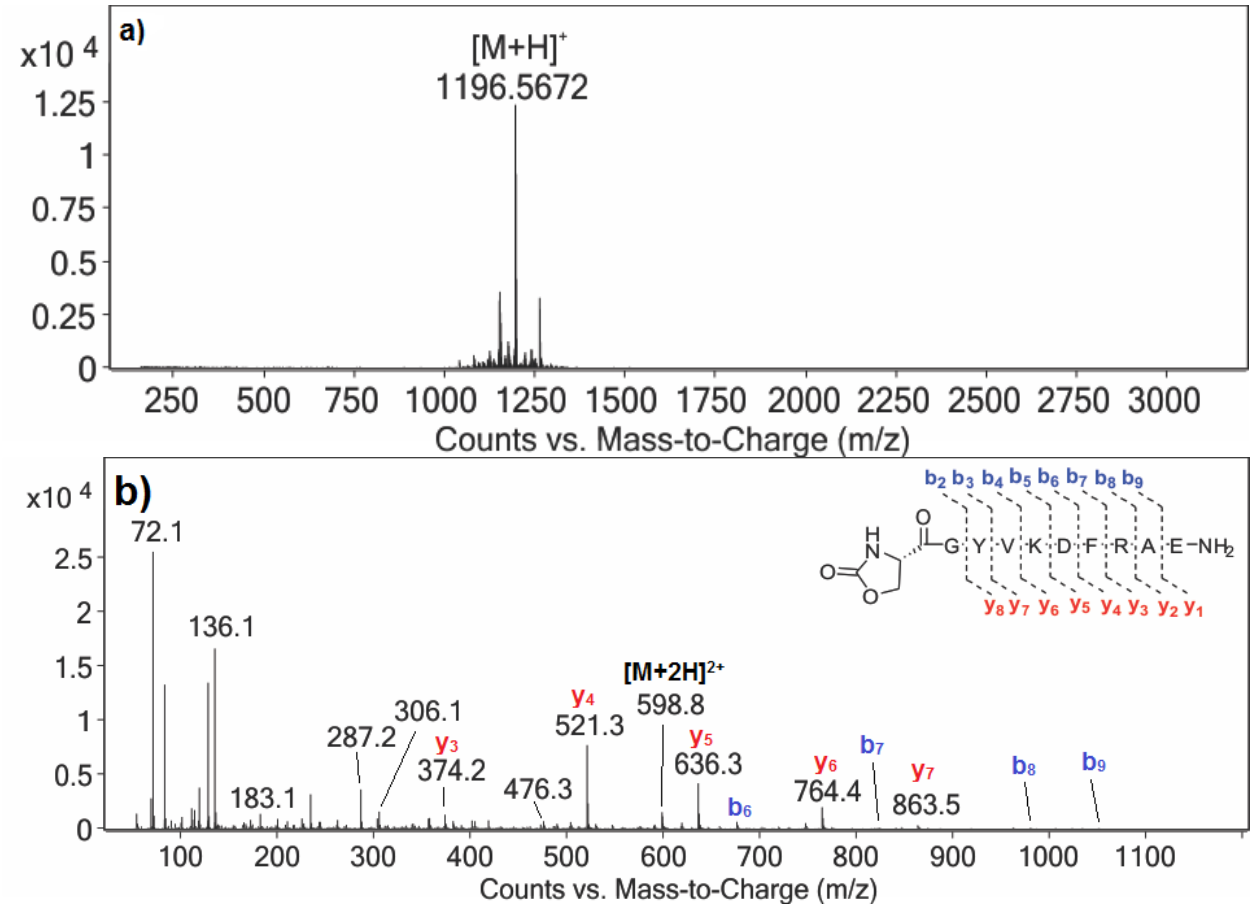


Figure S76. LC-IM-MS/MS data for linear peptide (**Table S3, entry 33**), *Oxd*-AYKFGPSAE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+2H]^{2+}$ = 541.2511, Found $[M+2H]^{2+}$ = 541.2519

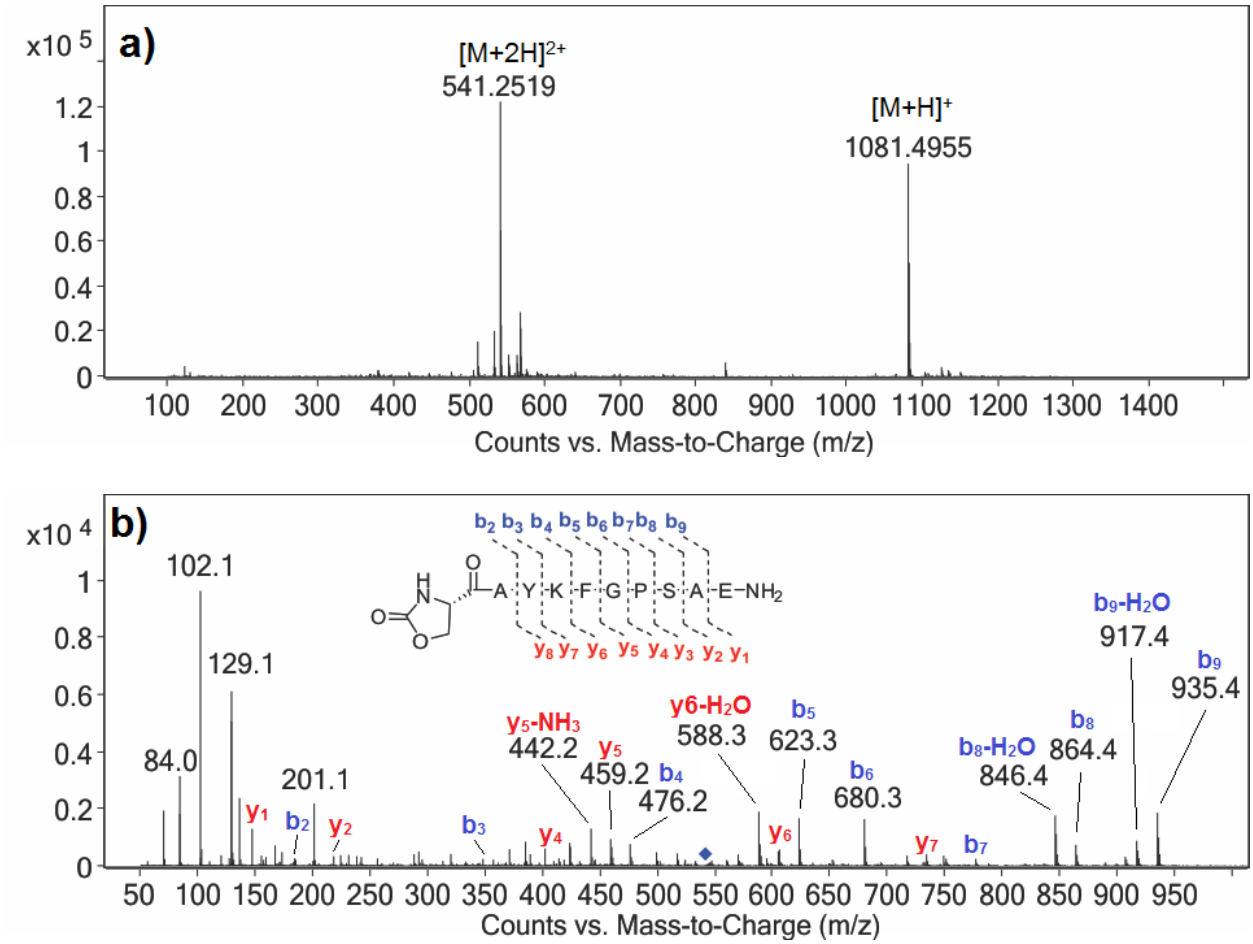
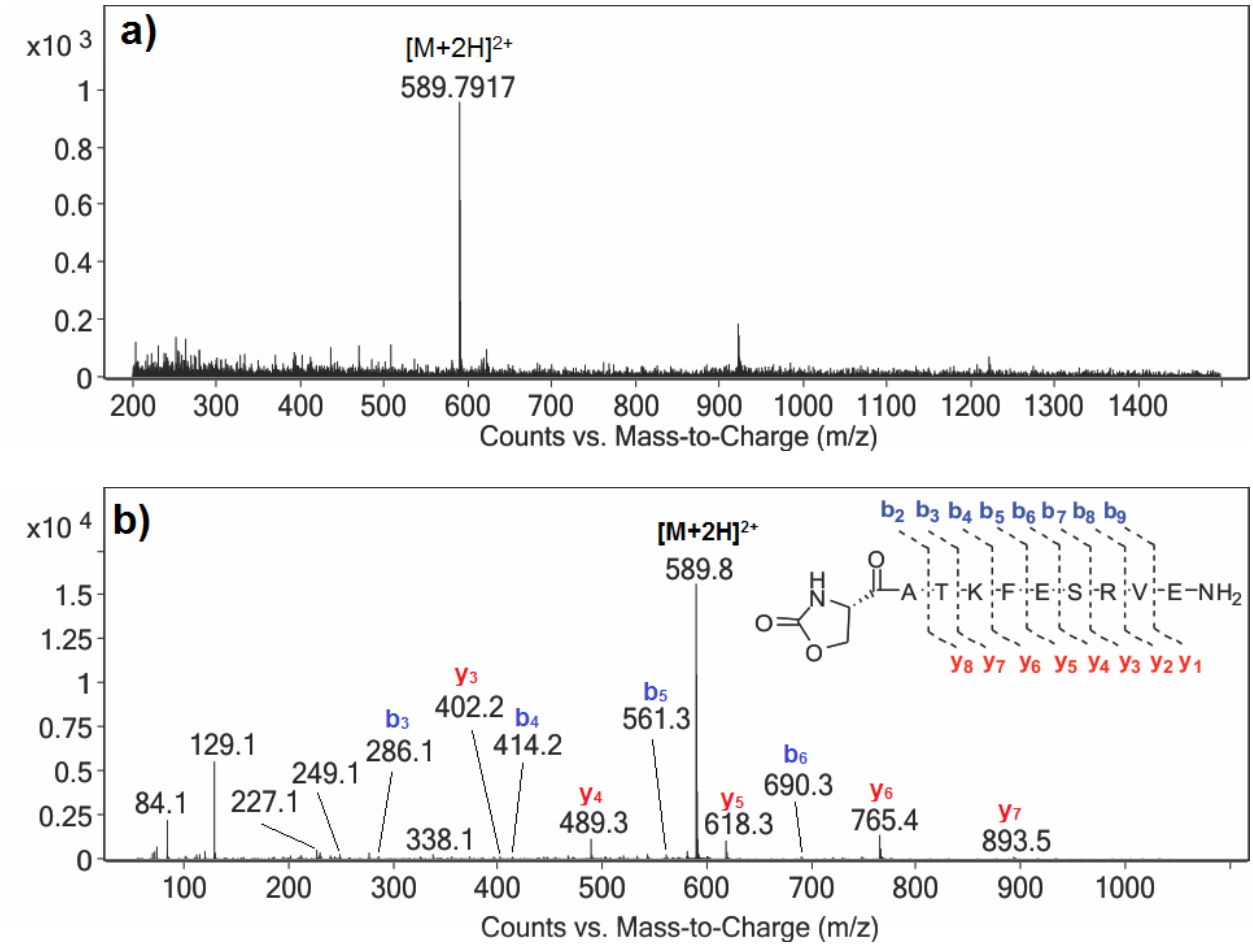


Figure S77. LC-IM-MS/MS data for linear peptide (**Table S3, entry 34)**, *Oxd*-ATKFESRVE-NH_2. a) extracted MS spectrum, and b) filtered MS/MS spectrum based on product peak drift time; Calcd $[M+2H]^{2+}$ = 589.7937, Found $[M+2H]^{2+}$ = 589.7917



References:

[1] Chan, W.C.; White, P.D. *Fmoc solid phase peptide synthesis: a practical approach*; Oxford University Press: New York, 2000.