Supporting Information

Separation of ^{13}C- and ^{15}N-Isotopologues of Amino Acids with a Primary Amine without Mass Resolution by means of O-Phthalaldehyde Derivatization and Collision Induced Dissociation

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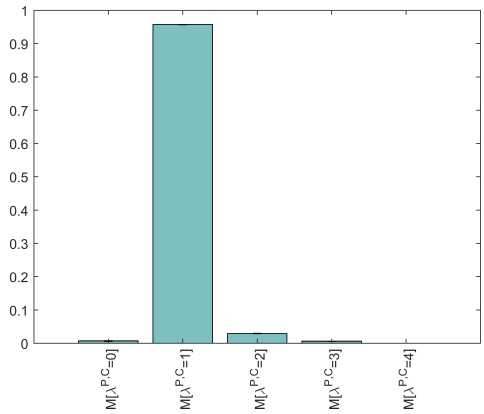
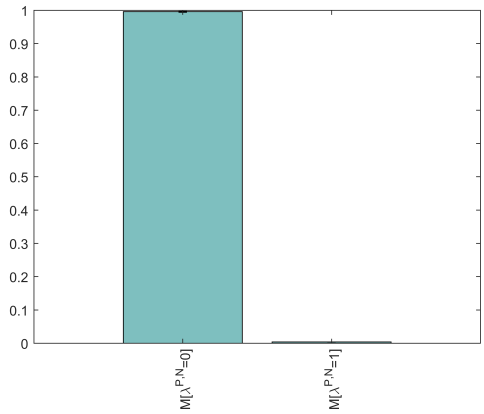
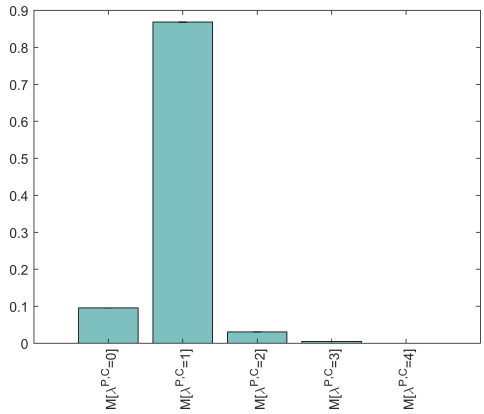
S1 Carbon and nitrogen enrichments for defined mixtures of [4-^{13}C]Asp and [^{15}N]Asp



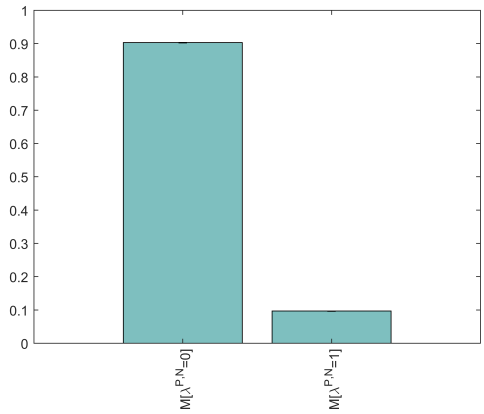
^{13}C-MID for 100 % [4-^{13}C]Asp



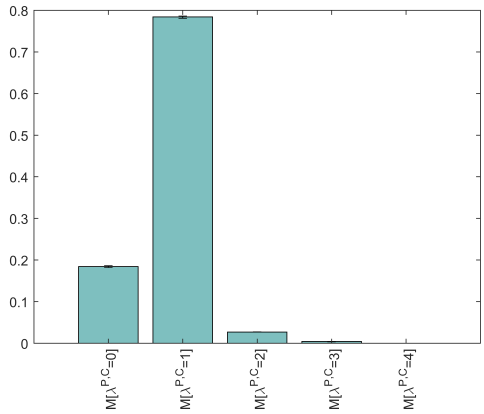
^{15}N-MID for 100 % [4-^{13}C]Asp



^{13}C-MID for 90 % [4-^{13}C]Asp/ 10 % [^{15}N]Asp



^{15}N-MID for 90 % [4-^{13}C]Asp/ 10 % [^{15}N]Asp



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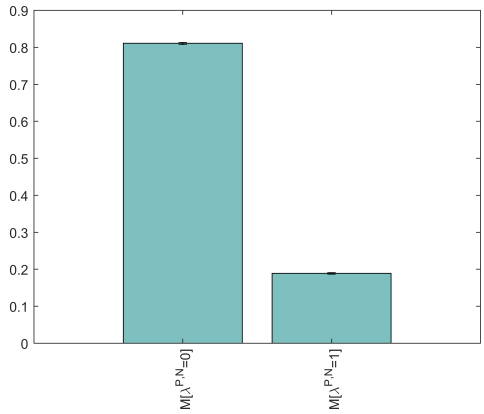
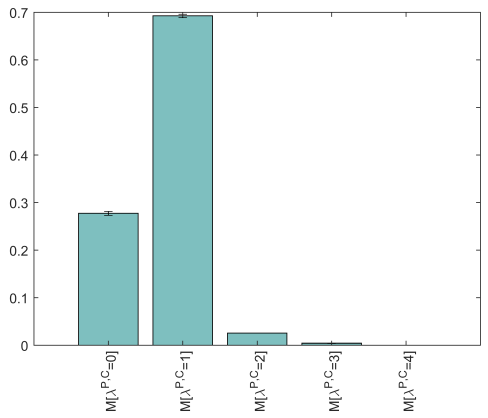
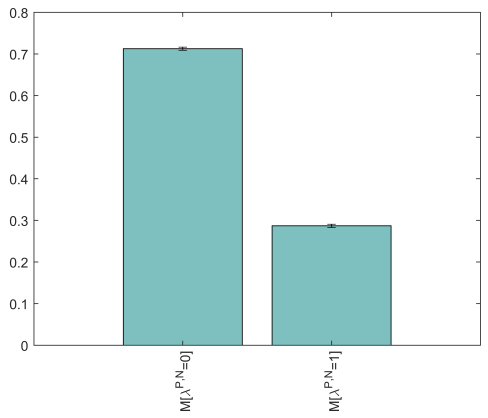


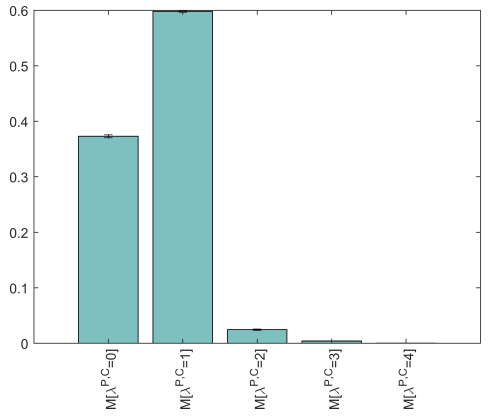
^{15}N-MID for 80 % [4-^{13}]Asp/ 20 % [^{15}N]Asp



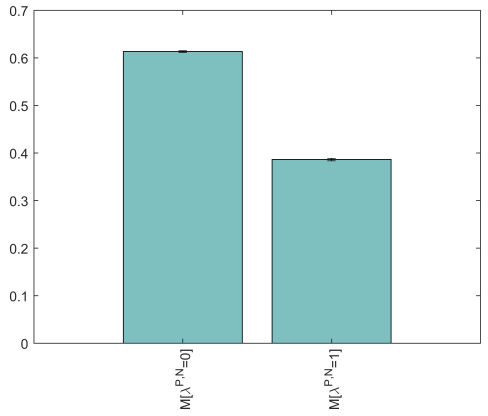
^{13}C-MID for 70 % [4-^{13}C]Asp/ 30 % [^{15}N]Asp



^{15}N-MID for 70 % [4-^{13}C]Asp/ 30 % [^{15}N]Asp



^{13}C-MID for 60 % [4-^{13}C]Asp/ 40 % [^{15}N]Asp



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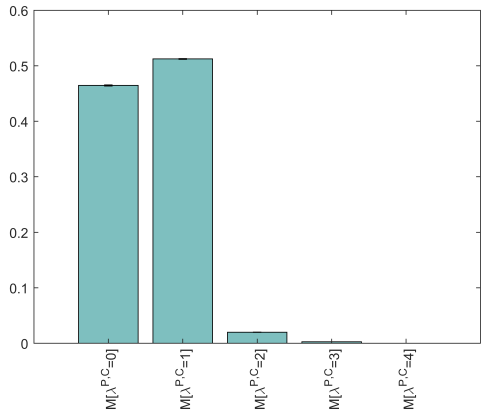

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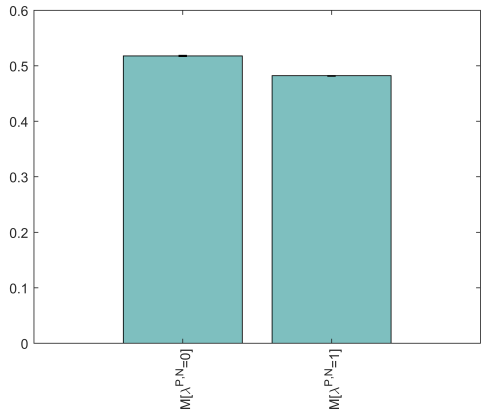
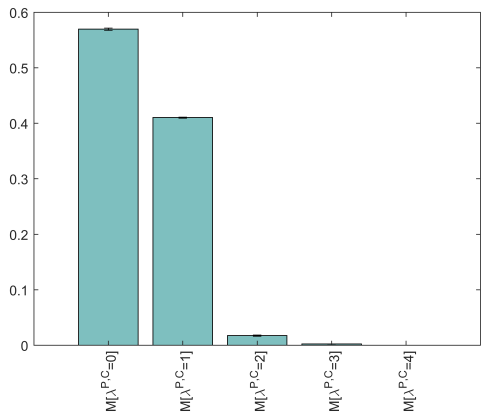
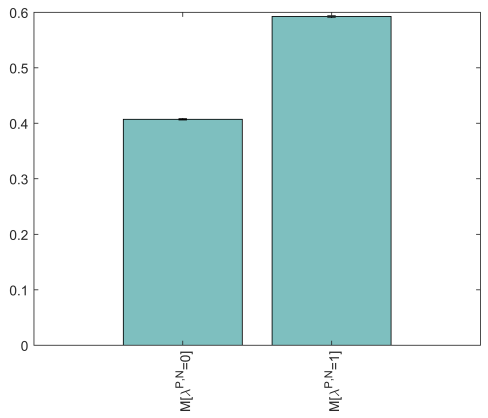


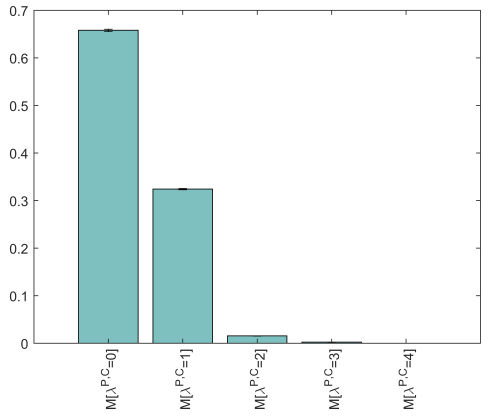
^{15}N-MID for 50 % [4-^{13}]Asp/ 50 % [^{15}N]Asp



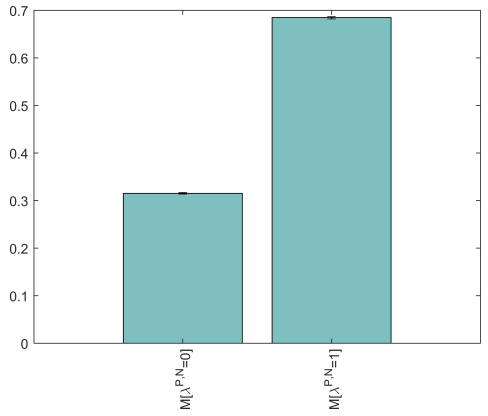
^{13}C-MID for 40 % [4-^{13}C]Asp/ 60 % [^{15}N]Asp



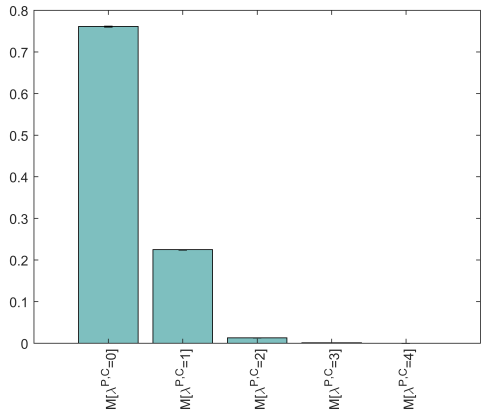
^{15}N-MID for 40 % [4-^{13}C]Asp/ 60 % [^{15}N]Asp



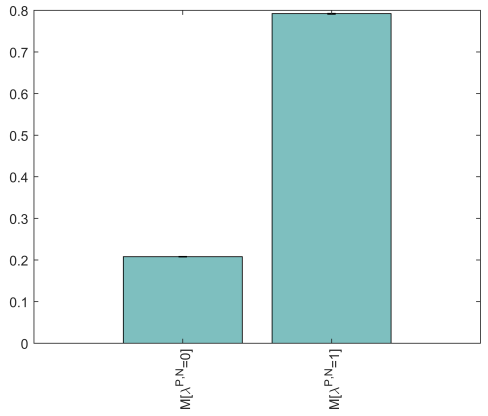
^{13}C-MID for 30 % [4-^{13}C]Asp/ 70 % [^{15}N]Asp



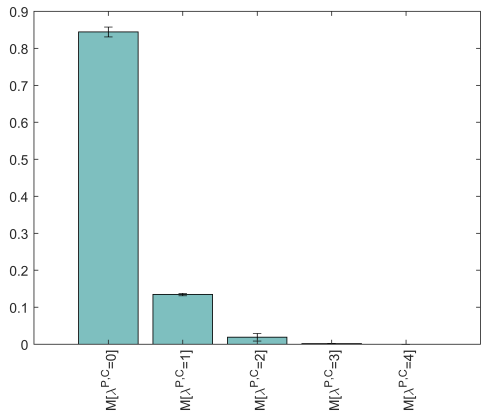
^{15}N-MID for 30 % [4-^{13}C]Asp/ 70 % [^{15}N]Asp



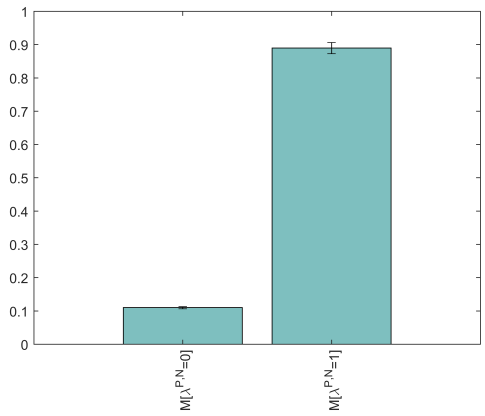
^{13}C-MID for 20 % [4-^{13}]Asp/ 80 % [^{15}N]Asp



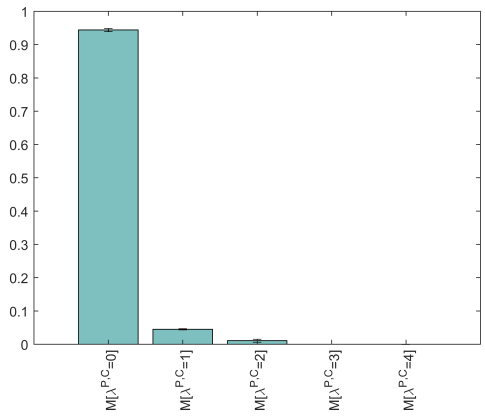
^{15}N-MID for 20 % [4-^{13}]Asp/ 80 % [^{15}N]Asp



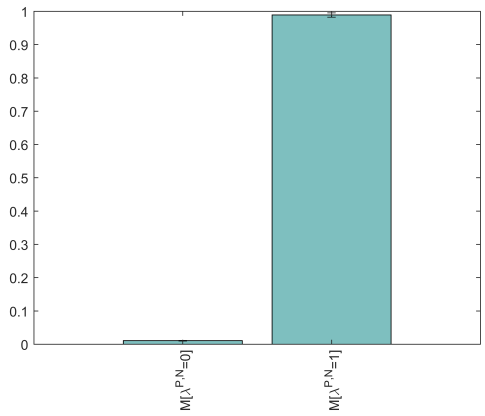
^{13}C-MID for 10 % [4-^{13}C]Asp/ 90 % [^{15}N]Asp



^{15}N-MID for 10 % [4-^{13}C]Asp/ 90 % [^{15}N]Asp



^{13}C-MID for 100 % [^{15}N]Asp



^{15}N-MID for 100 % [^{15}N]Asp

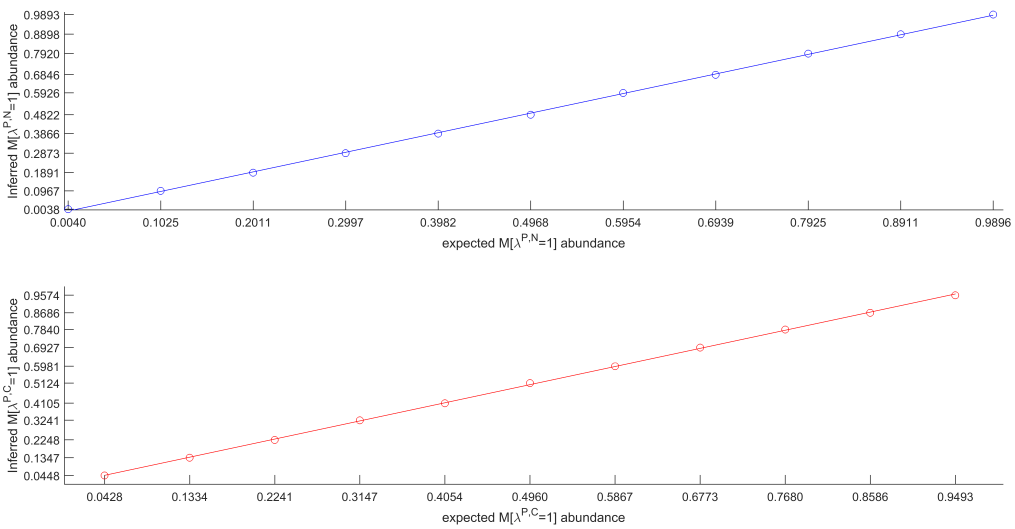


Figure S-1: Inferred enrichment of the M[$\lambda^{P,N}$=1] and M[$\lambda^{P,C}$=1] against expected enrichment.

S2 Fragmentation of amino acid adducts

S2.1 Amino acids and Li adducts

Table S-1: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms. The intensity was quantified relative to the most intense product ion over a CE-ramp ± 5-50 eV.

Amino acid	Neutral loss	Precursor ion					
		$[M+H]^+$		$[M-H]^-$		$[M+Li]^{+a}$	
		peak *m/z*	rel. Intensity	peak *m/z*	rel. Intensity	peak *m/z*	rel. Intensity
Ala	$-NH_3$	73.0284	0	71.0139	1.71e-03	79.0336	3.76e-03
	$-NH_3-H_2O$	55.0178	6.17e-04	53.0033	0	61.0260	0
Asn	$-2NH_3$	99.0077	3.82e-03	96.9931	0	105.0158	5.06e-03
	$-2NH_3-H_2O$	80.9971	0	—[b]	—	87.0053	0
Asp	$-NH_3$	117.0182	0	115.0037	7.02e-02	123.0264	3.84e-03
	$-NH_3-H_2O$	99.0077	4.23e-04	96.9931	3.76e-05	105.0158	7.36e-04
Cys	$-NH_3$	105.0005	3.19e-02	102.9859	0	111.0087	3.66e-01
	$-NH_3-H_2S$	71.0128	0	68.9982	0	77.0209	0
	$-NH_3-H_2O$	86.9899	3.08e-02	84.9754	0	92.9981	0
Glu	$-NH_3$	131.0339	4.91e-03	129.0193	1.12e-03	137.0421	4.64e-02
	$-NH_3-H_2O$	113.0233	4.11e-04	111.0088	0	119.0315	0
	$-NH_3-2H_2O$	95.0128	1.23e-04	92.9982	0	101.0209	0
Gln	$-2NH_3$	113.0233	4.63e-04	111.0088	0	119.0315	7.29e-04
	$-2NH_3-H_2O$	95.0128	1.29e-03	92.9982	0	101.0209	0
Gly	$-NH_3$	59.0128	6.36e-04	56.9982 [c]	0	65.0209 [c]	3.18e-04
	$-NH_3-H_2O$	41.0022	0	—[b]	—	—[b]	—
Hom	$-NH_3$	103.0390	0	101.0244	4.70e-04	109.0471	3.51e-03
	$-NH_3-H_2O$	85.0284	0	83.0139	0	91.0366	1.05e-04

Table S-1 Continued: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms.

	$-NH_3-2H_2O$	67.0178	0	65.0033	0	73.0260	0
Isoleu	$-NH_3$	115.0754	1.27e-04	113.0608	0	121.0835	1.23e-02
	$-NH_3-H_2O$	97.0648	2.65e-04	95.0502	0	103.0730	0
Leu	$-NH_3$	115.0754	1.52e-05	113.0608	5.95e-04	121.0835	2.55e-02
	$-NH_3-H_2O$	97.0648	1.00e-04	95.0502	0	103.0730	2.86e-05
Lys	$-2NH_3$	113.0597	4.17e-04	111.0452	0	119.0679	2.96e-03
	$-2NH_3-H_2O$	95.0491	1.76e-03	93.0346	0	101.0573	5.55e-05
Met	$-NH_3$	133.0318	5.44e-01	131.0172	0	139.0400	4.69e-02
	$-NH_3-H_2O$	115.0212	8-79e-04	113.0067	0	121.03	0
Orn	$-2\ NH_3$	99.0441	0	97.0295	0	n.d.	n.d.
	$-2\ NH_3-H_2O$	81.0335	0	79.0189	0	n.d.	n.d.
Phe	$-NH_3$	149.0597	1.58e-02	147.0452	1.95e-01	155.0679	1.13e-02
	$-NH_3-H_2O$	131.0491	1.65e-02	129.0346	0	137.0573	0
Pro	$-NH_3$	99.0441	0	97.0295	0	105.0522	5.58e-05
	$-NH_3\ -H_2O$	81.0335	0	79.0189	0	87.0417	0
Ser	$-NH_3$	89.0233	0	87.0088	0	95.0315	4.12e-04
	$-NH_3-H_2O$	71.0128	0	68.9982	0	77.0209	0
	$-NH_3-2H_2O$	53.0022	0	—[b]	—	59.0104 [c]	0
Thr	$-NH_3$	103.0390	0	101.0244	0	109.0471	0
	$-NH_3-H_2O$	85.0284	7.01e-04	83.0139	0	91.0366	0
	$-NH_3\ -2H_2O$	67.0178	0	65.0033	0	73.0260	0
Tyr	$-NH_3$	165.0546	4.71e-01	163.0401	1.46e-01	171.0628	1.21e-02
	$-NH_3-H_2O$	147.0441	9.37e-02	145.0295	0	153.05	0
Val	$-NH_3$	101.0597	3.84e-05	99.0452	0	107.0679	7.62e-03
	$-NH_3-H_2O$	83.0491	1.68e-04	81.0346	0	89.0573	0

[a] The product ion spectrum of the ^{7}Li- ion adduct was considered. [b] The product ion is not admissible given the elemental composition of the precursor ion. [c] The corresponding product ion is a double radical ion species.

S2.2 Methylcarbamoyl-amino acids

Table S-2: Relative intensities of product ions derived from the derivatization group and which contain the amino group nitrogen atom but no carbon backbone atom. The intensity was quantified relative to the most intense product ion over a CE-ramp -(5-50) eV.

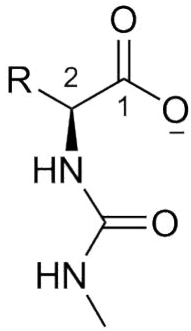
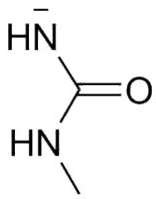
Amino acid	Precursor Ion		Fragment ions	
				
	m/z TOF	*m/z* found	*m/z* 41.9985	*m/z* 73.0407
L-Ala	145.0619	145.0617	1.10e-02	1.86e-03
L-Asn	188.0677	188.0672	2.26e-01	3.64e-03
L-Asp	189.0517	189.0514	5.19e-02	1.19e-03
L-Cys	177.0339	177.0339	1.01e-02	0
L-Glu	203.0673	203.0670	6.94e-03	0
L-Gln	202.0833	202.0838	8.13e-02	8.28e-05
Gly	131.0462	131.0454	9.42e-03	0
L-Hom	175.0724	175.0725	1.38e-02	4.21e-04
L-Ile	187.1088	187.1089	5.76e-03	4.92e-05
L-Leu	187.1088	187.1092	7.34e-03	1.85e-04
L-Lys[a]	259.1412	259.1409	4.40e-02	1.43e-03
L-Met	205.0652	205.0647	6.55e-03	0
L-Orn[b]	245.1255	245.1263	1.47e-03	1.67e-04

Table S-2 Continued: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms.

L-Phe	221.0932	221.0925	1.03e-02	3.11e-03
L-Pro	171.0775	171.0766	3.37e-03	0
L-Ser	161.0568	161.0563	6.61e-03	1.38e-04
L-Thr	175.0724	175.0728	9.95e.03	1.53e-05
L-Tyr	237.0881	n.i.	—	—
L-Val	173.0932	173.0930	7.95e-02	0

[a] N^2,N^6-bis(methylcarbamoyl)-L-Lys. [b] N^2,N^5-bis(methylcarbamoyl)-L-Orn.

S2.3 Fmoc-amino acids

Table S-3: Relative intensities of product ions derived from the derivatization group and which contain the amino group nitrogen atom but no carbon backbone atom. The intensity was quantified relative to the most intense product ion over a CE-ramp -(5-90) eV.

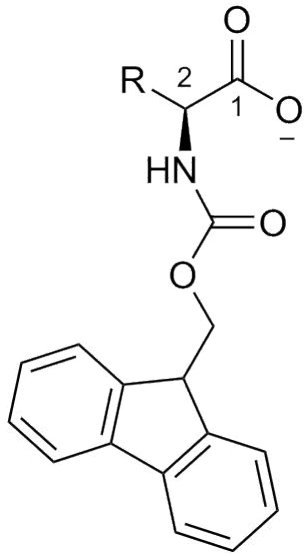
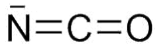
Amino acid	Precursor Ion		Fragment ions
			
	m/z TOF	*m/z* found	*m/z* 41.9985
L-Ala	310.1085	available as standard	1.98e-01
L-Asn	353.1143	available as standard	4.09e-01
L-Asp	354.0983	available as standard	1.92e-01
L-Cys	342.0806	available as standard	1.56e-02
L-Glu	368.1140	available as standard	1.69e-01
L-Gln	367.1299	available as standard	5.13e-01
Gly	296.0928	available as standard	1.01e-01
L-Ile	352.1554	available as standard	1.05e-01
L-Leu	352.1554	available as standard	1.18e-01

Table S-3 Continued: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms.

L-Lys[a]	589.2344	available as standard	5.36e-02
L-Met	370.1119	available as standard	6.19e-02
L-Orn[b]	575.2188	available as standard	8.59e-02
L-Phe	386.1398	available as standard	7.71e-02
L-Pro	336.1241	available as standard	1.98e-05
L-Ser	326.1034	available as standard	4.34e-02
L-Thr	340.1190	available as standard	8.27e-03
L-Tyr	402.1347	available as standard	1.83e-02
L-Val	338.1398	available as standard	1.18e-01

[a] N^2,N^6-bis(methylcarbamoyl)-L-Lys. [b] N^2,N^5-bis(methylcarbamoyl)-L-Orn.

S2.4 N-acetyl-amino acids

Table S-4: Relative intensities of product ions derived from the derivatization group and which contain the amino group nitrogen atom but no carbon backbone atom. The intensity was quantified relative to the most intense product ion over a CE-ramp -(5-50) eV.

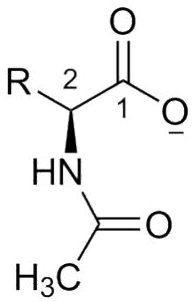
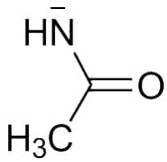
Amino acid	Precursor Ion		Fragment ions
			
	m/z TOF	*m/z* found	*m/z* 58.0298
L-Ala	130.0510	130.0513	3.67e-03
L-Asn	173.0568	173.0574	3.51e-01
L-Asp	174.0408	174.0409	8.11e-02
L-Cys [a]	204.0336	204.0342	1.55e-05
L-Glu	188.0564	188.0569	3.97e-02
L-Gln	187.0724	187.0730	2.46e-01
Gly	116.0353	116.0353	3.21e-04
L-Ile	172.0979	172.0984	1.23e-02
L-Leu	172.0979	172.0985	9.07e-03
L-Lys	[b] 229.1194	229.1195	1.19e-01
L-Met	190.0543	190.0547	5.20e-05
L-Orn [c]	215.1037	215.1041	2.14e-01
L-Phe	206.0823	206.0829	8.92e-02
L-Pro	156.0666	156.0661	3.56e-05

Table S-4 Continued: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms.

L-Ser	146.0459	146.0458	8.91e-03
L-Thr	160.0615	160.0619	1.30e-03
L-Tyr	222.0772	n.i. [d]	—
L-Val	158.0823	158.0827	8.69e-03

[a] The considered precursor is N,S-diacetyl-L-Cys. [b] The considered precursor is N^2,N^6-diacetyl-L-Lys. [c] The considered precursor is N^2,N^5-diacetyl-L-Orn. [d] No precursor ion could be identified.

S2.5 Dansylated amino acids

Table S-5: Relative intensities of product ions derived from the derivatization group and which contain the amino group nitrogen atom but no carbon backbone atom. The intensity was quantified relative to the most intense product ion over a CE-ramp -(5-90) eV.

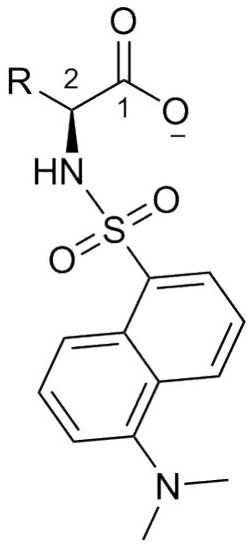
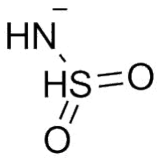
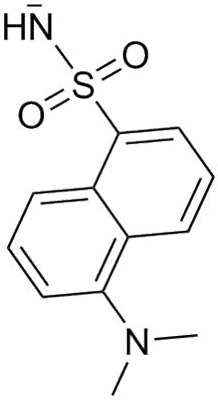
Dansyl chloride-derivatized amino acid	M-H Precursor Ion		Fragment ions	
				
	m/z TOF	*m/z* found	*m/z* 79.9812	*m/z* 249.0703
L-Ala	321.0915	321.0906	3.57e-03	2.33e-01
L-Asn	364.0973	364.0963	3.57e-03	1.77e-01
L-Asp	365.0813	365.0791	5.40e-03	1.93e-01
L-Cys	353.0653	n.i.	—	—
L-Glu	379.0969	379.0941	1.79e.03	7.26e-02
L-Gln	378.1129	378.1118	2.16e-03	8.38e-02
Gly	307.0758	307.0734	1.19e-03	5.99e-02
L-Hom	351.1020	351.1018	2.60e-03	1.08e-01
L-Ile	363.1384	363.1388	3.41e-03	1.91e-01

Table S-5 Continued: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms.

L-Leu	363.1384	363.1383	3.18e-03	1.93e-01
L-Lys [a]	611.2003	611.1994	4.64e-04	3.31e-02
L-Met	381.0948	381.0930	1.55e-03	8.18e-02
L-Orn [b]	597.1847	597.1828	9.01e-04	6.59e-02
L-Phe	397.1228	397.1220	3.09e-04	1.05e-01
L-Pro	347.1071	347.1073	0	4-66e-04
L-Ser	337.0864	337.0860	0	1.89e-02
L-Thr	351.1020	351.1020	2.76e-04	4.88e-03
L-Tyr	646.1687	646.1671	2.18e-04	2.93e-02
L-Val	349.1228	349.1205	3.05e-03	1.81e-01

[a] The considered precursor ion is N^2,N^6-Dansyl chloride-derivatized-L-Lys. [b] The considered precursor ion is N^2,N^5-Dansyl chloride-derivatized-L-Orn.

S2.6 PITC amino acids

Table S-6: Relative intensities of product ions derived from the derivatization group and which contain the amino group nitrogen atom but no carbon backbone atom. The intensity was quantified relative to the most intense product ion over a CE-ramp -(5-90) eV.

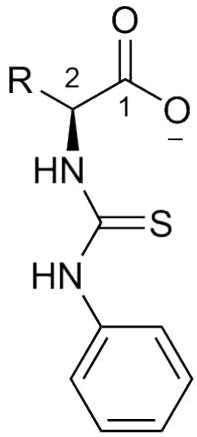
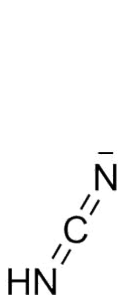
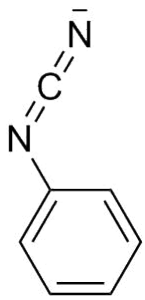
PITC-derivatized amino acid	Precursor Ion		Fragment Ions	
				
	m/z TOF	*m/z* found	*m/z* 41.0145	*m/z* 117.0458
L-Ala	223.0547	233.0543	3.84e-02	4.14e-01
L-Asn	266.0605	266.0621	1.82e-02	8.80e-02
L-Asp	267.0445	267.0451	3.91e-03	5.15-03
L-Cys	255.0267	255.0246	7.68e-03	1.51e-01
L-Glu	281.0602	281.0609	7.10e-03	1.35e-02
L-Gln	280.0761	280.0779	3.22e-02	3.43e-01
Gly	209.0390	209.0371	2.00e-02	3.30e-01
L-Hom	253.0652	253.0638	1.92e-02	1.80e-01
L-Ile	265.1016	265.1006	2.46e-02	6.02e-01
L-Leu	265.1016	265.1004	2.60e-02	6.91e-01
L-Lys [a]	415.1268	415.1246	8.02e-03	8.18e-01

Table S-6 Continued: Relative intensities of product ions of amino acids corresponding to neutral loss combinations that leave the carbon backbone intact but eliminate all nitrogen atoms.

L-Met	283.0580	283.0566	6.95e-03	2.14e-01
L-Orn [b]	401.1111	401.1081	1.05e-02	9.65e-01
L-Phe	299.0860	299.0854	7.92e-03	1.74e-01
L-Pro	249.0703	249.0685	3.37e-05	8.98e-04
L-Ser	239.0496	239.0493	3.03e-03	1.14e-02
L-Thr	253.0652	253.0636	3.03e-03	1.14e-02
L-Tyr	315.0809	315.0797	1.19e-02	6.40e-01
L-Val	251.0860	251.0831	3.06e-02	7.21e-01

[a] The considered precursor ion is N^2,N^6-PITC-derivatized-L-Lys. [b] The considered precursor ion is N^2,N^5-PITC-derivatized-L-Orn.

S3 Mass spectra of OPM-amino acids

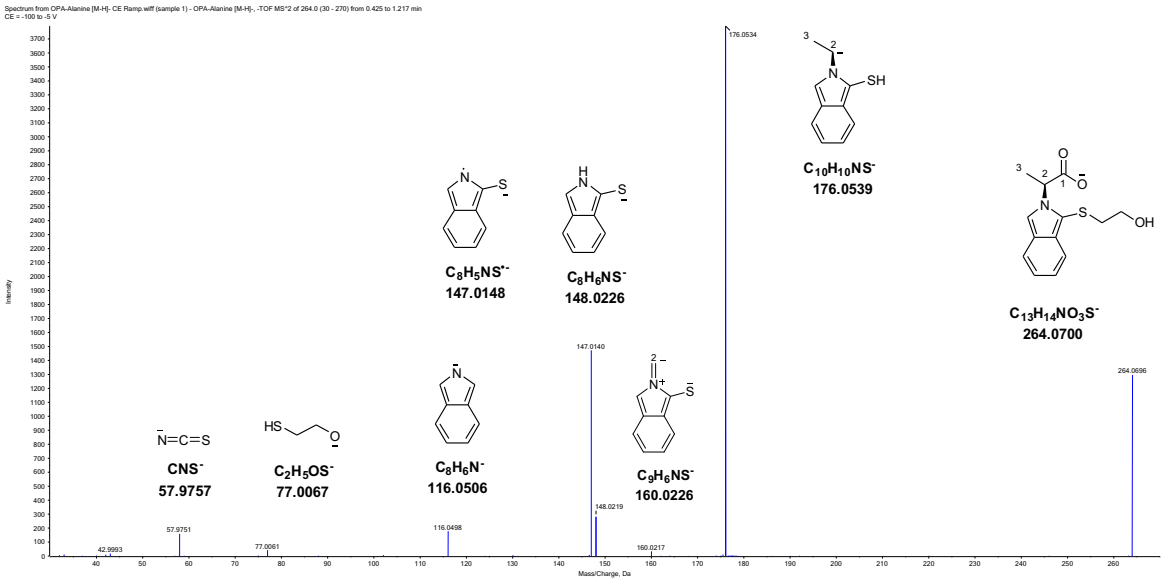


Figure S-2: The product ion spectrum of the [M-H]- ion of OPM-Ala.

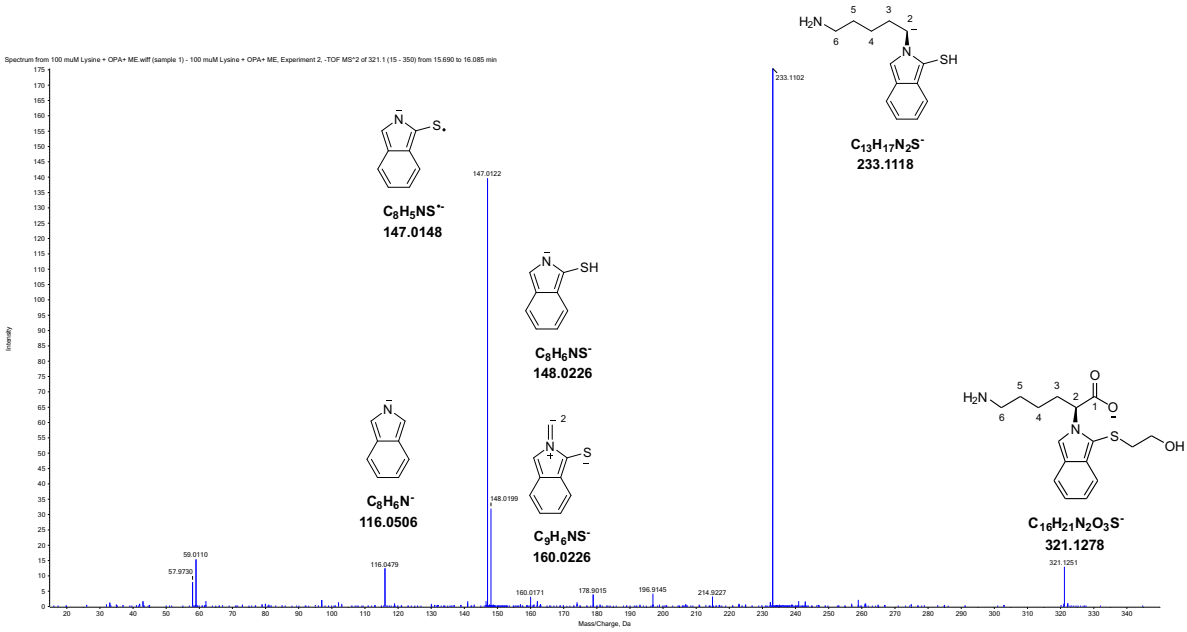


Figure S-3: The product ion spectrum of the [M-H]- ion of α-OPM-Lys.

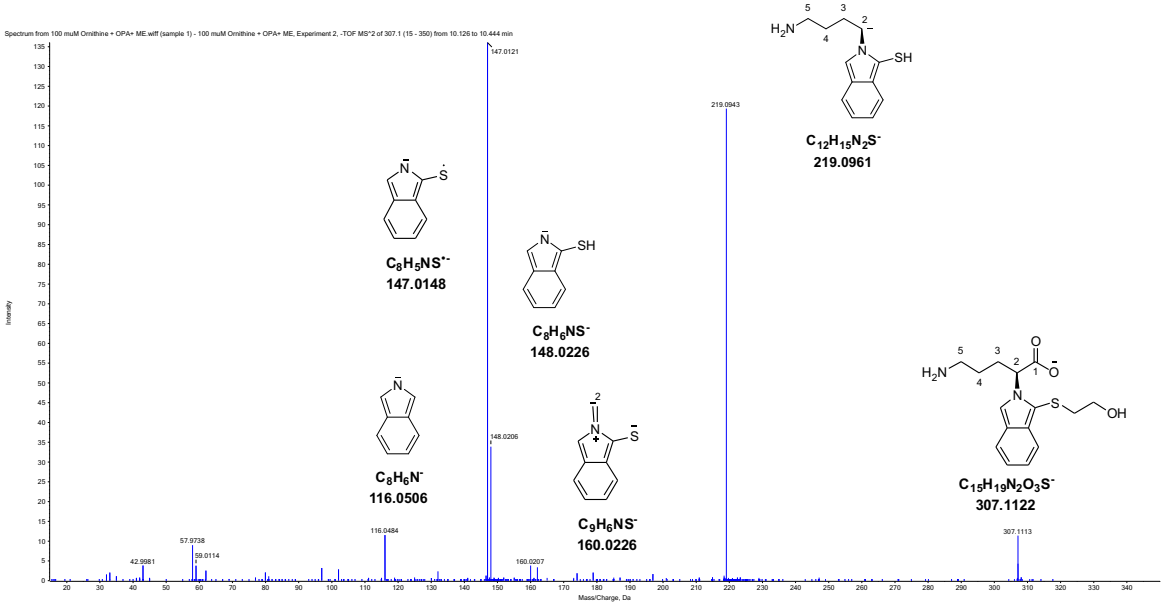


Figure S-4: The product ion spectrum of the [M-H]- ion of α-OPM-Orn.

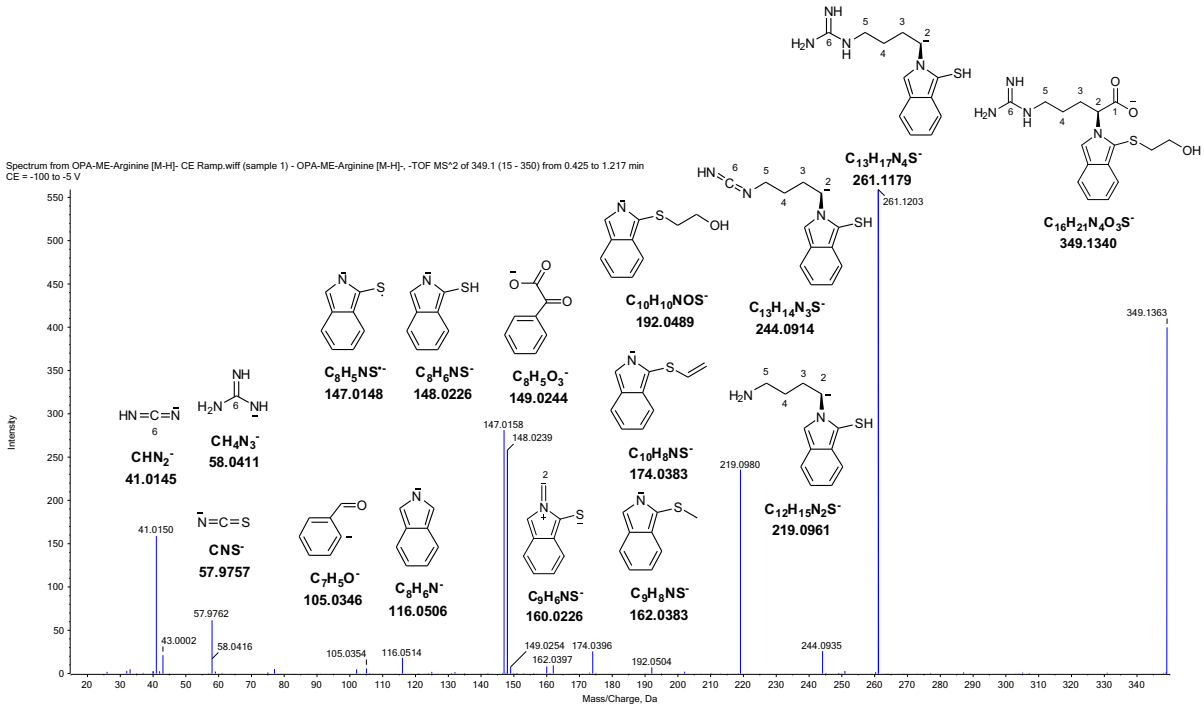


Figure S-5: The product ion spectrum of the [M-H]- ion of OPM-Arg.

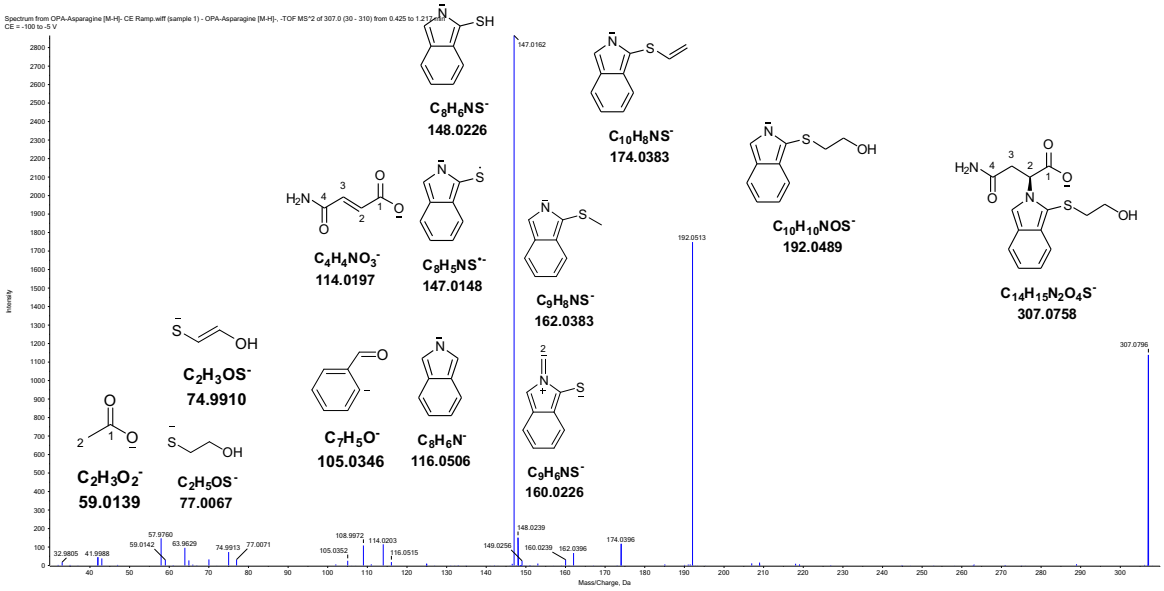


Figure S-6: The product ion spectrum of the [M-H]- ion of OPM-Asn.

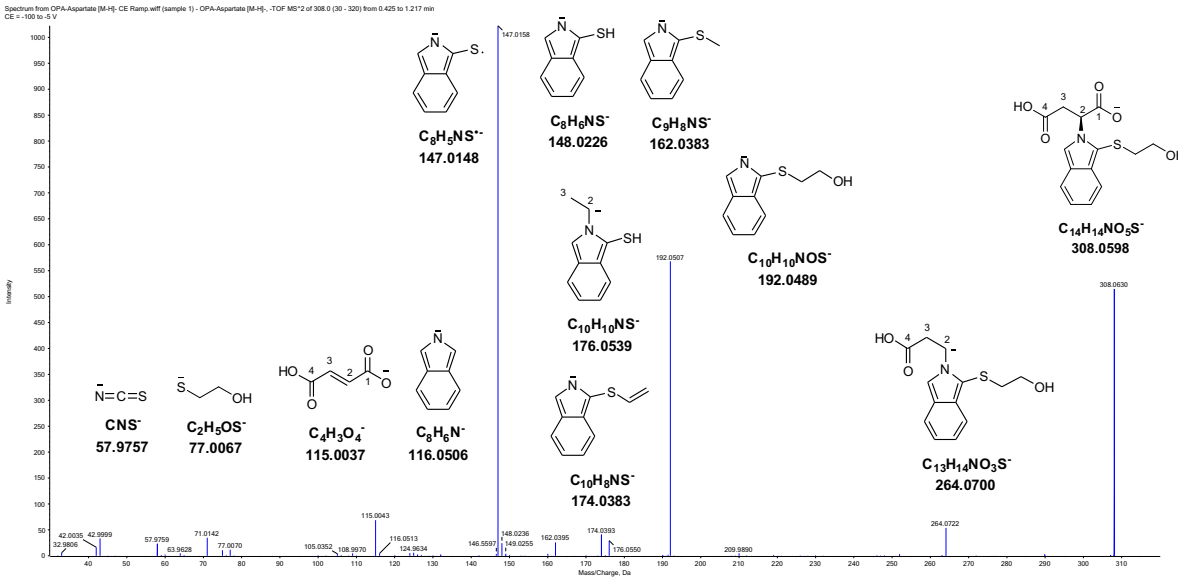


Figure S-7: The product ion spectrum of the [M-H]- ion of OPM-Asp.

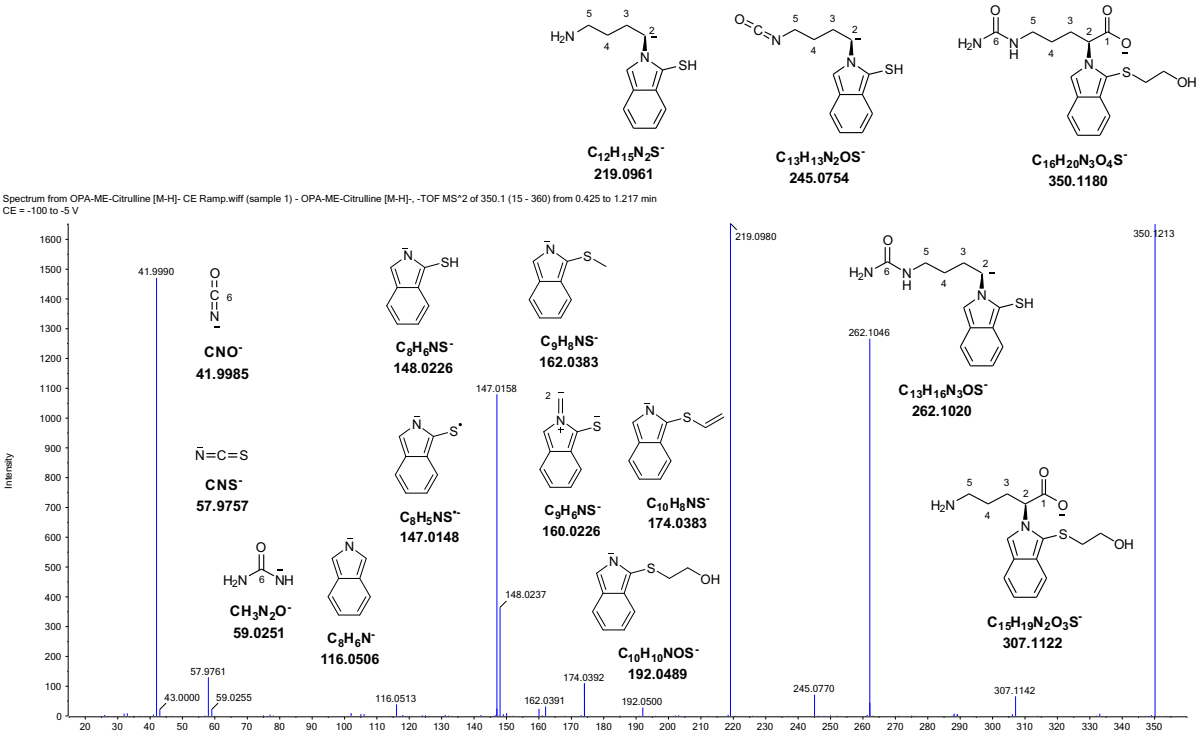


Figure S-8: The product ion spectrum of the [M-H]- ion of OPM-Citr.

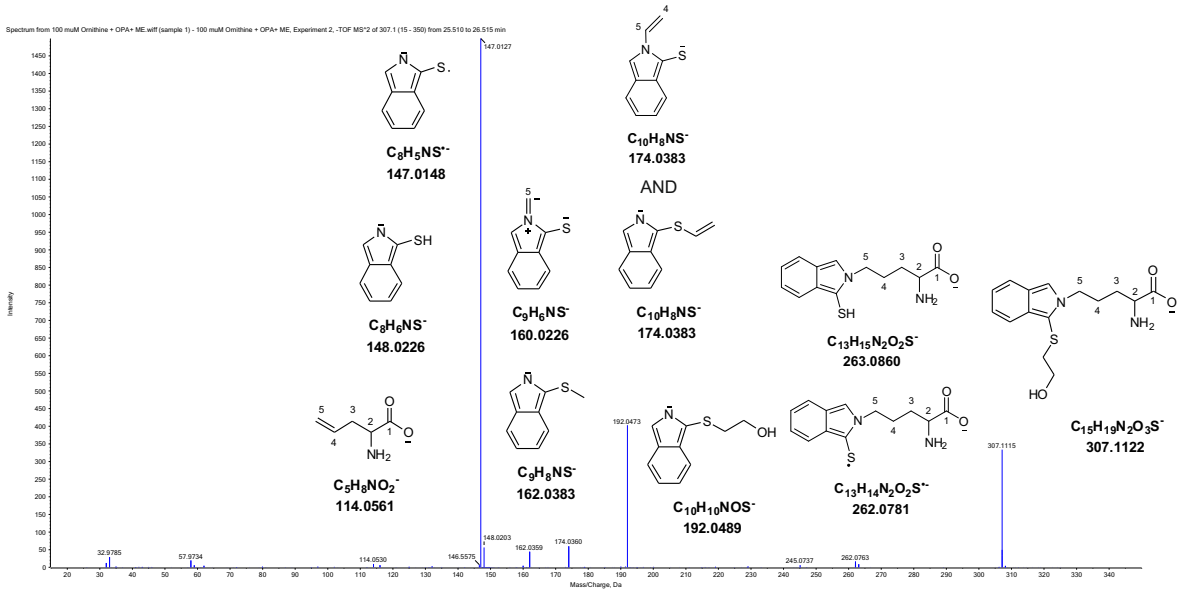


Figure S-9: The product ion spectrum of the [M-H]- ion of δ-OPM-Orn.

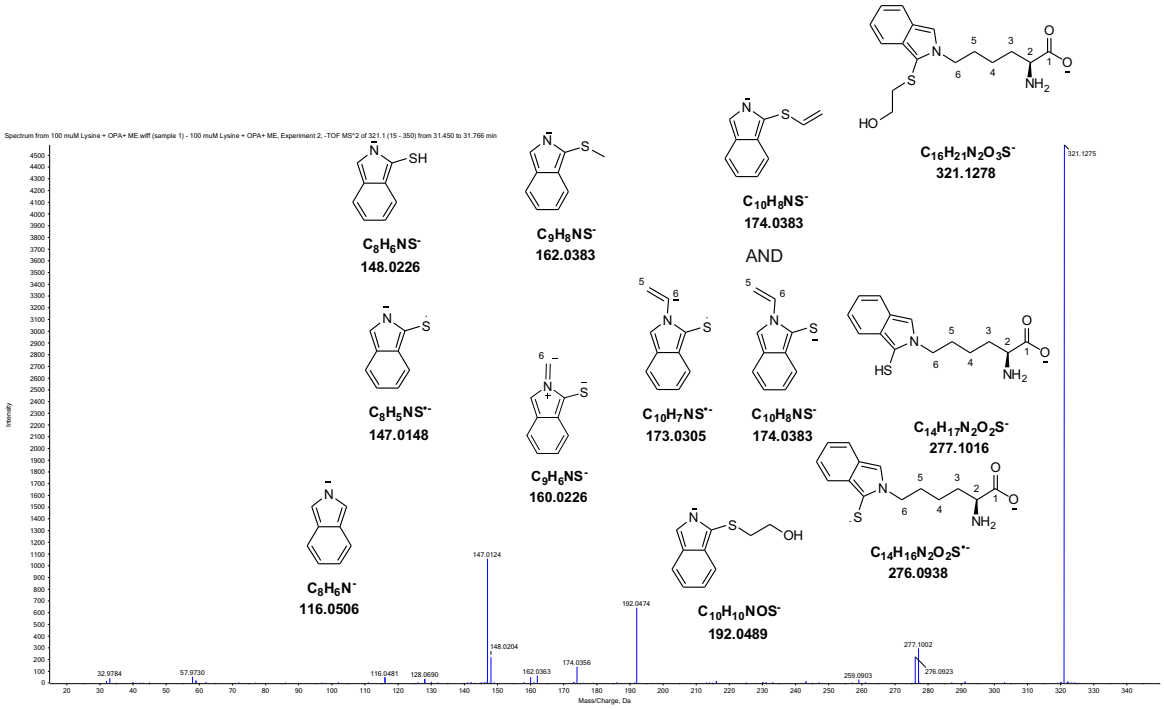


Figure S-10: The product ion spectrum of the [M-H]- ion of ϵ-OPM-Lys.

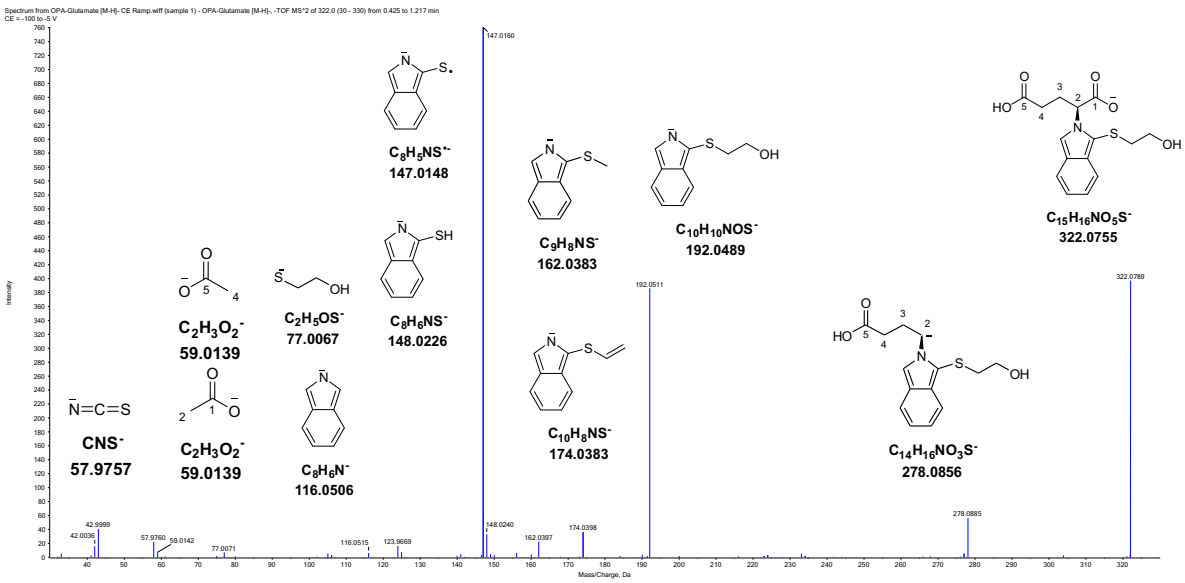


Figure S-11: The product ion spectrum of the [M-H]- ion of OPM-Glu.

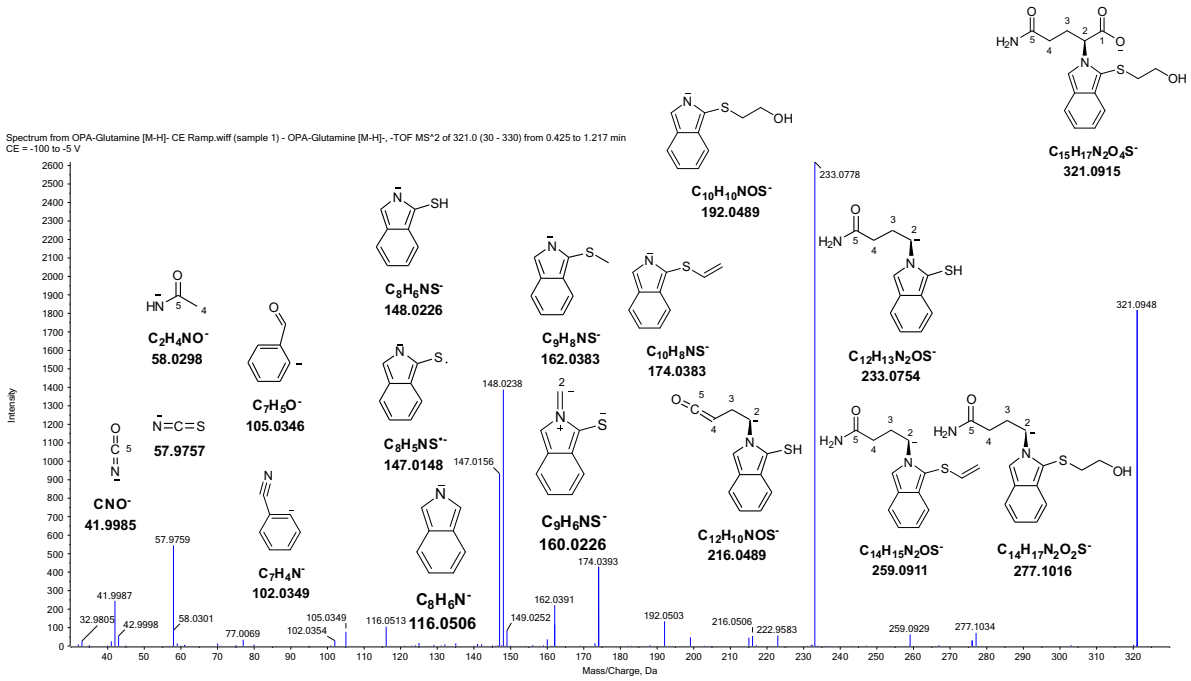


Figure S-12: The product ion spectrum of the [M-H]- ion of OPM-Gln.

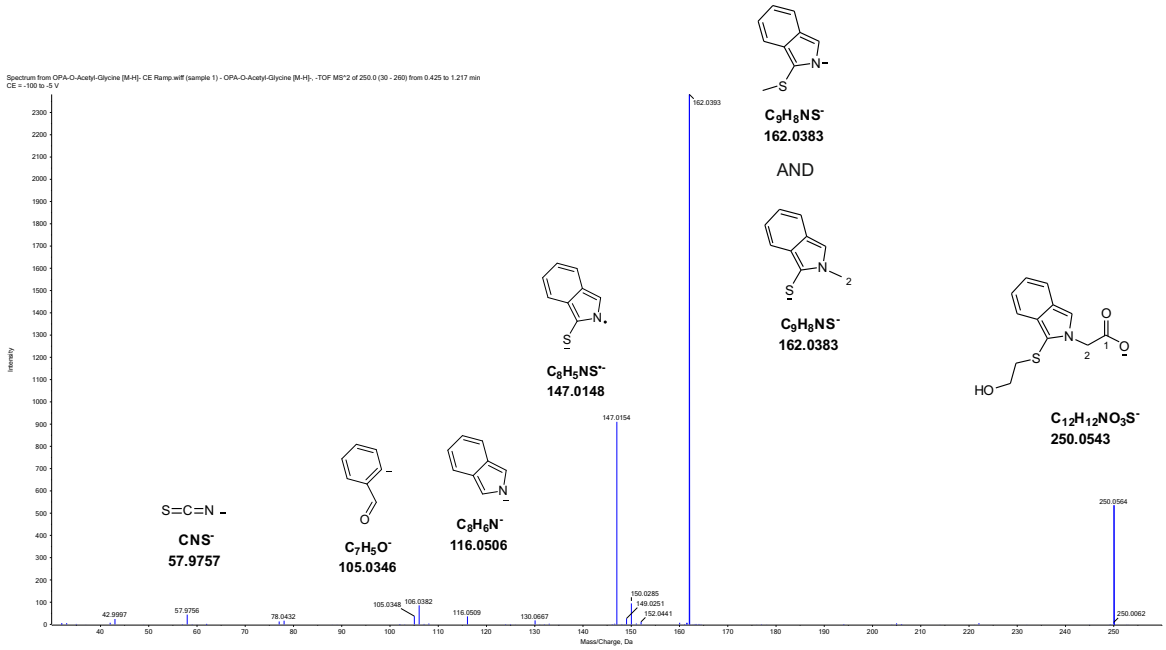


Figure S-13: The product ion spectrum of the [M-H]- ion of OPM-Gly.

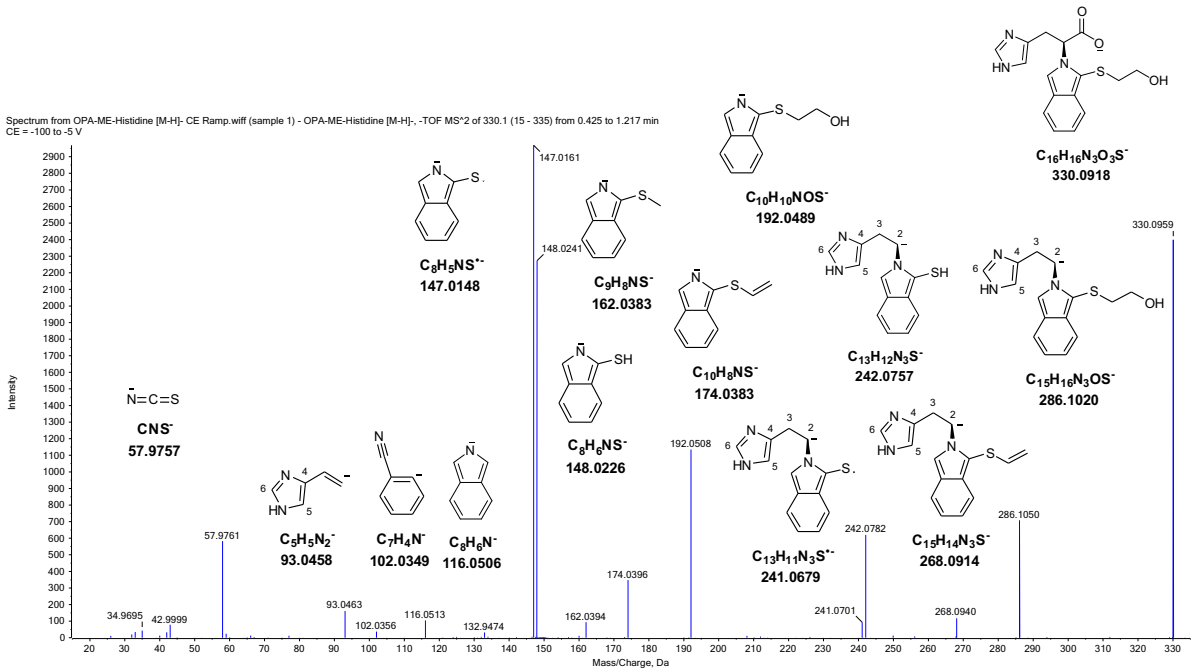


Figure S-14: The product ion spectrum of the [M-H]- ion of OPM-His.

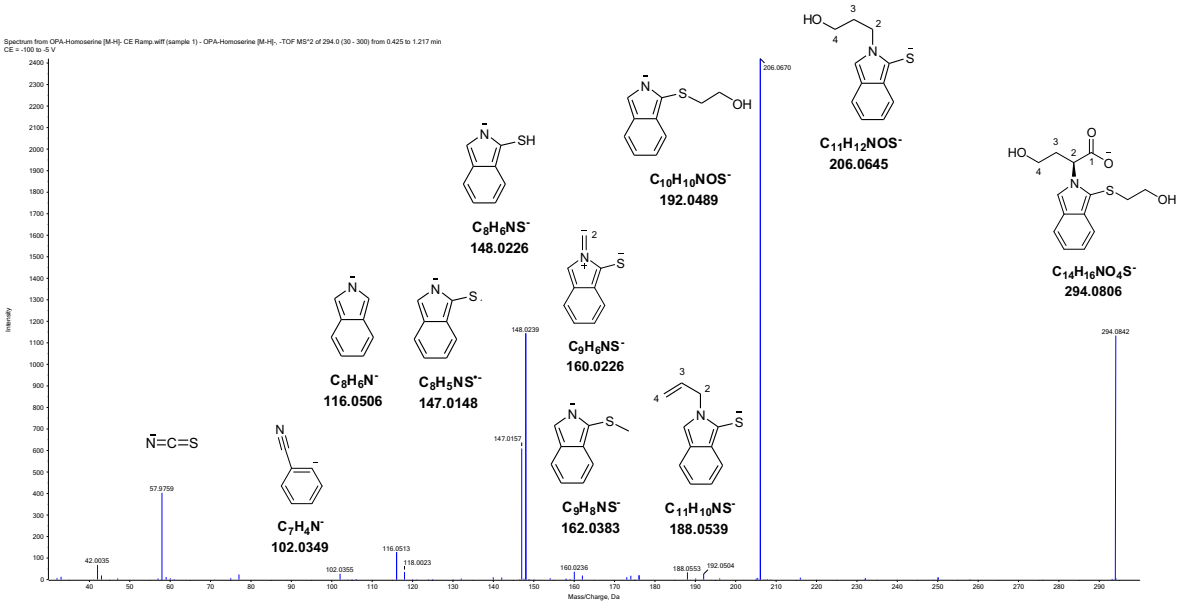


Figure S-15: The product ion spectrum of the [M-H]- ion of OPM-Hom.

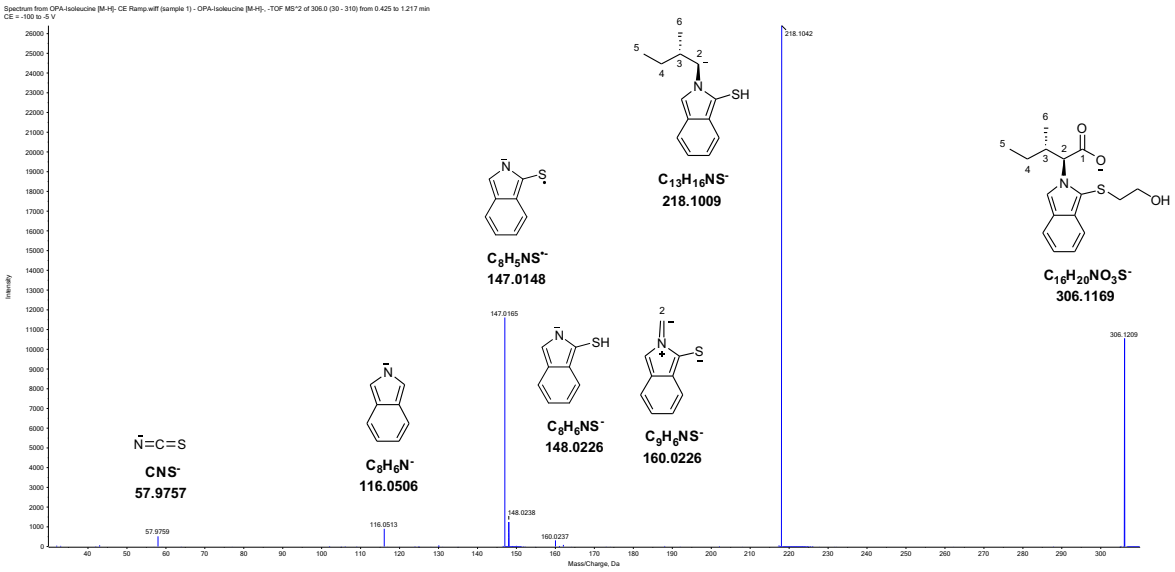


Figure S-16: The product ion spectrum of the [M-H]- ion of OPM-Ile.

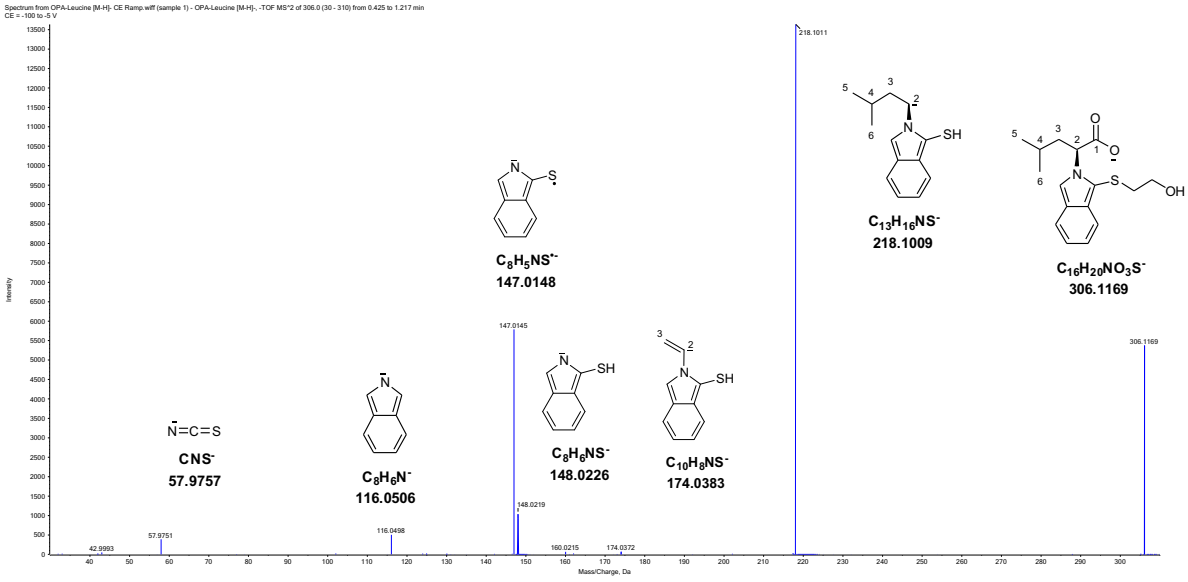


Figure S-17: The product ion spectrum of the [M-H]- ion of OPM-Leu.

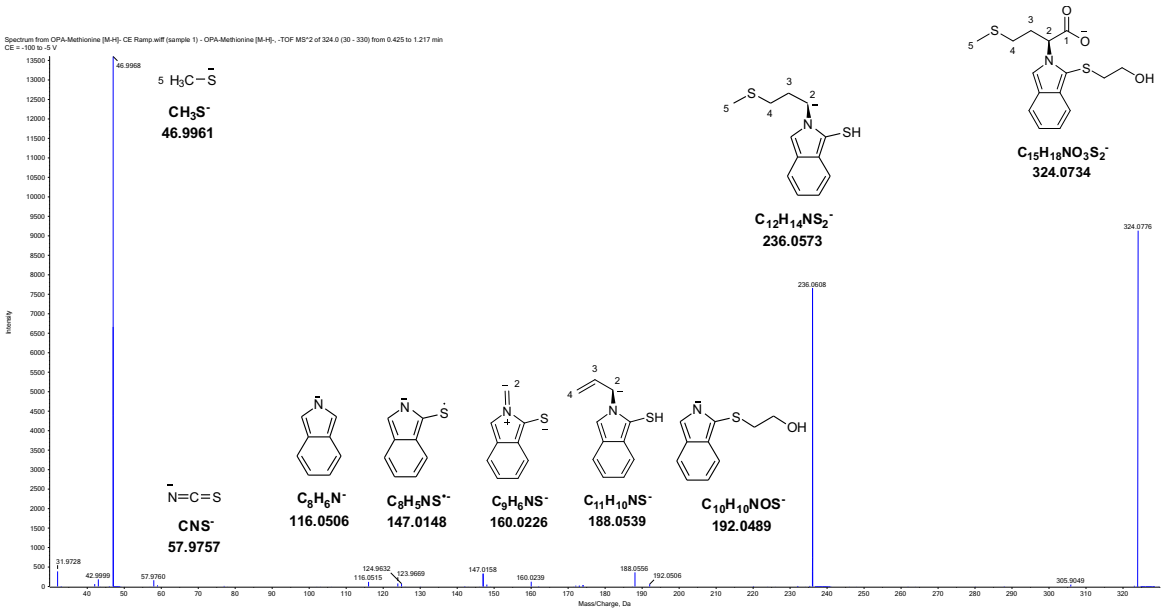


Figure S-18: The product ion spectrum of the [M-H]- ion of OPM-Met.

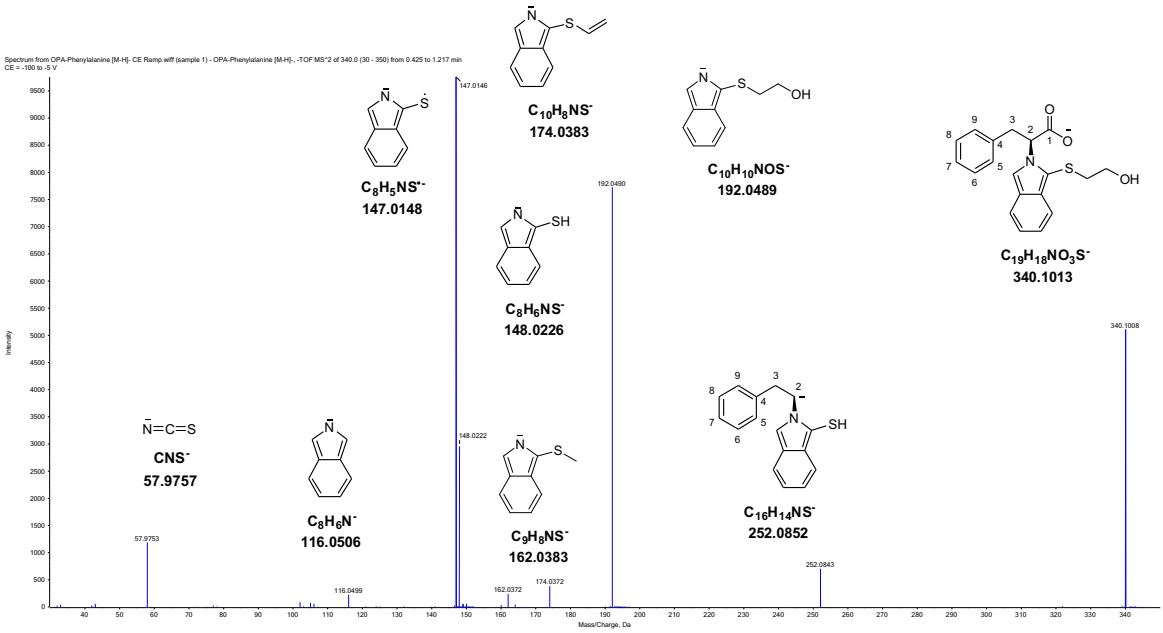


Figure S-19: The product ion spectrum of the [M-H]- ion of OPM-Phe.

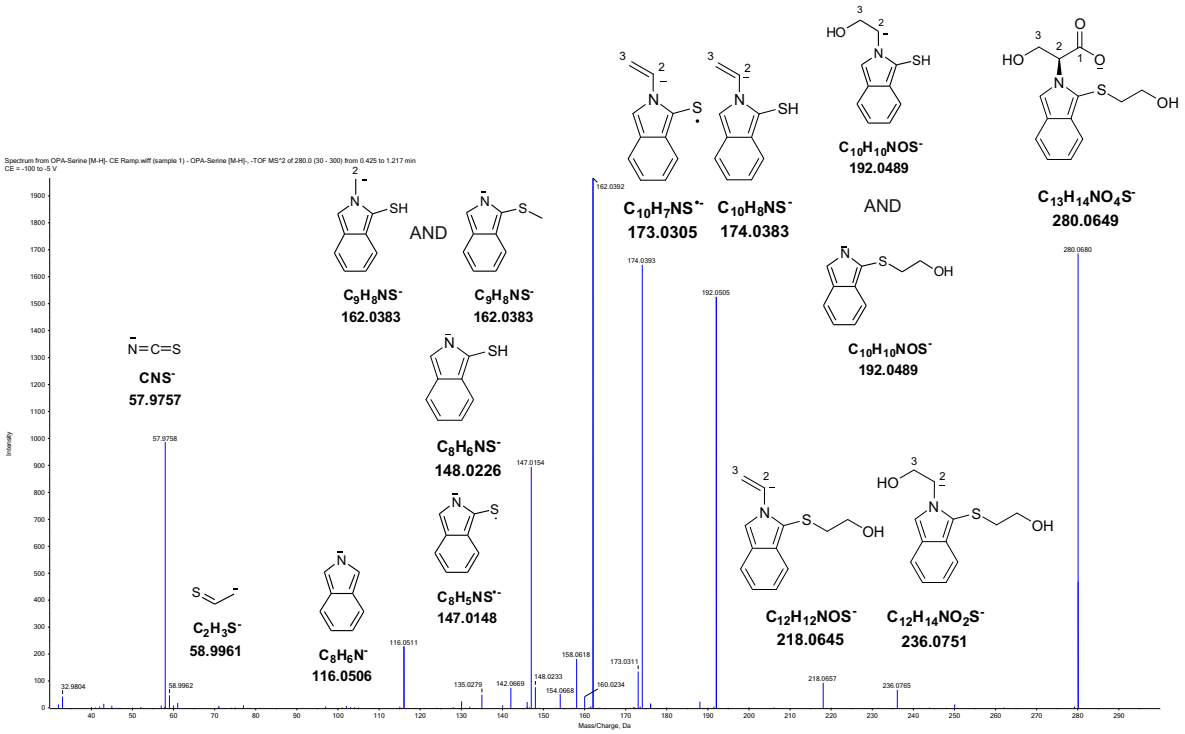


Figure S-20: The product ion spectrum of the [M-H]- ion of OPM-Ser.

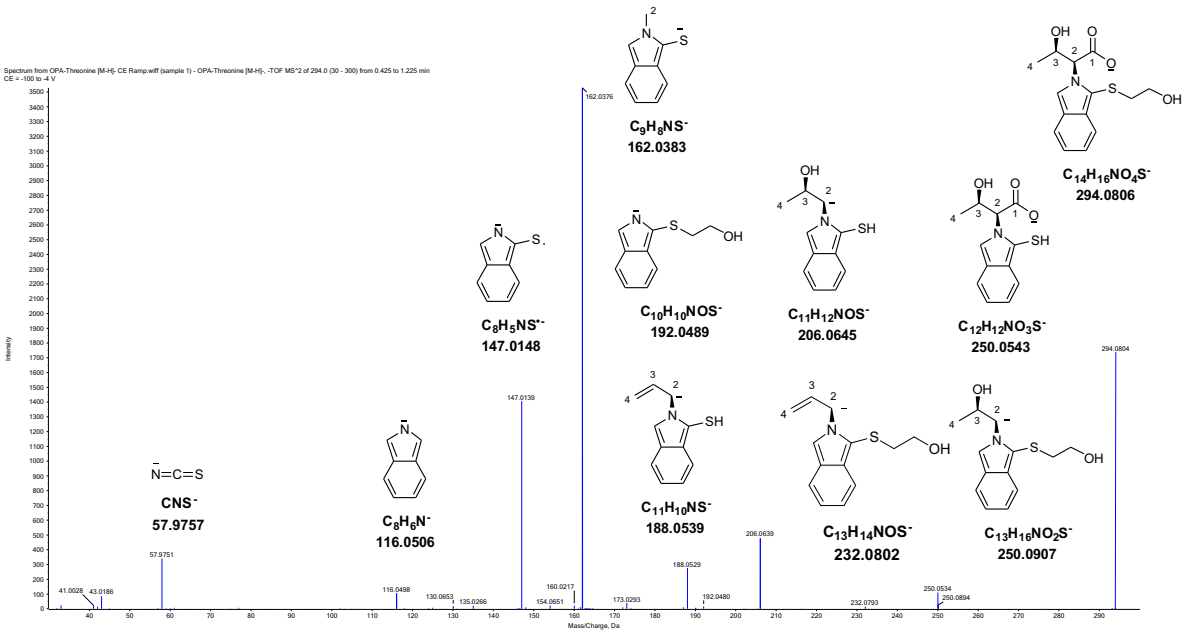


Figure S-21: The product ion spectrum of the [M-H]- ion of OPM-Thr.

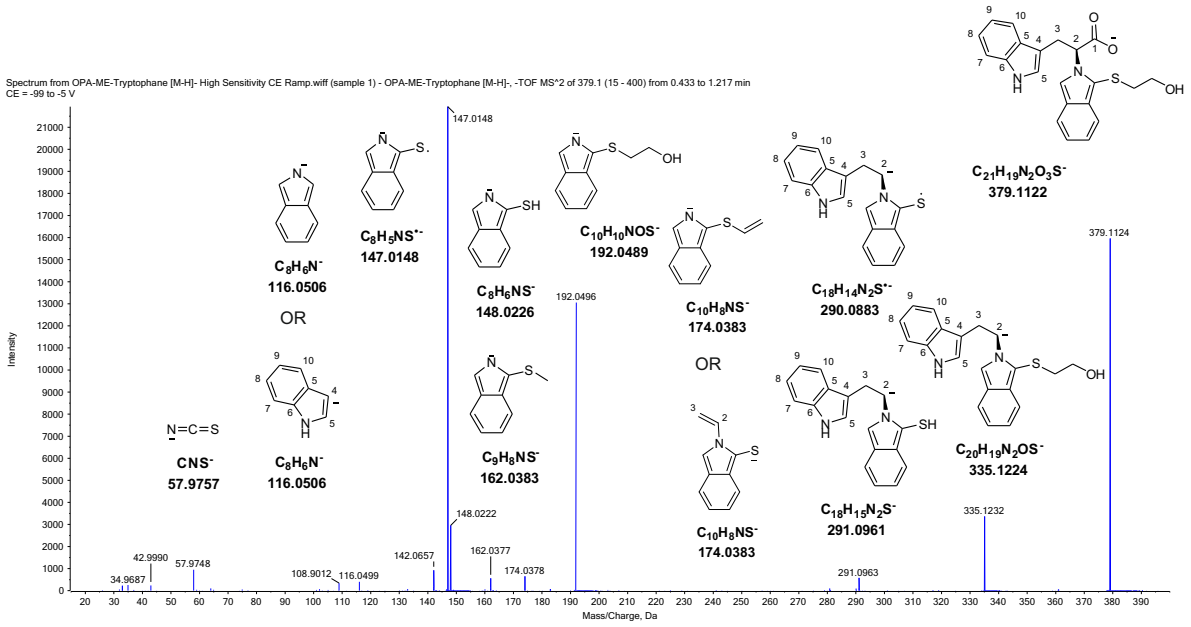


Figure S-22: The product ion spectrum of the [M-H]- ion of OPM-Trp.

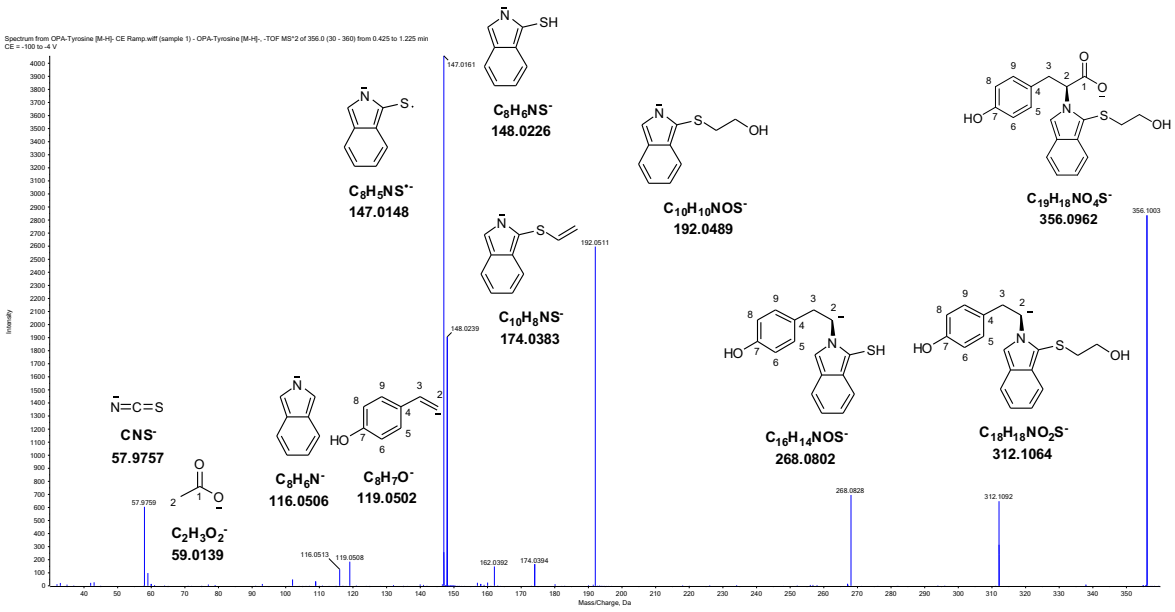


Figure S-23: The product ion spectrum of the [M-H]- ion of OPM-Tyr.

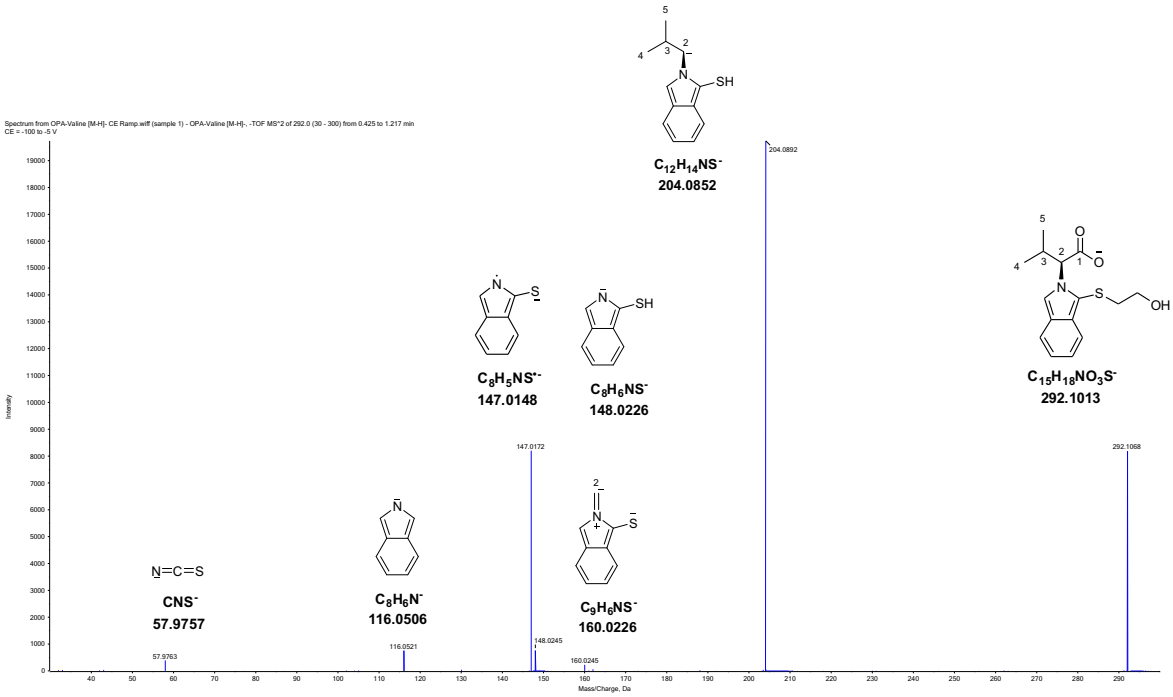


Figure S-24: The product ion spectrum of the [M-H]- ion of OPM-Val.

S4 Mass spectra of OPT-amino acids

Each spectrum was recorded as described in the Materials and Methods section of the main text. We verified each structure by derivatizing a fully ^{13}C- and ^{15}N-labeled cell-free amino acid mixture with the LC-MS/MS method described in the main text. The carbon atom numbering follows ordinary conventions in carbon atom numbering.

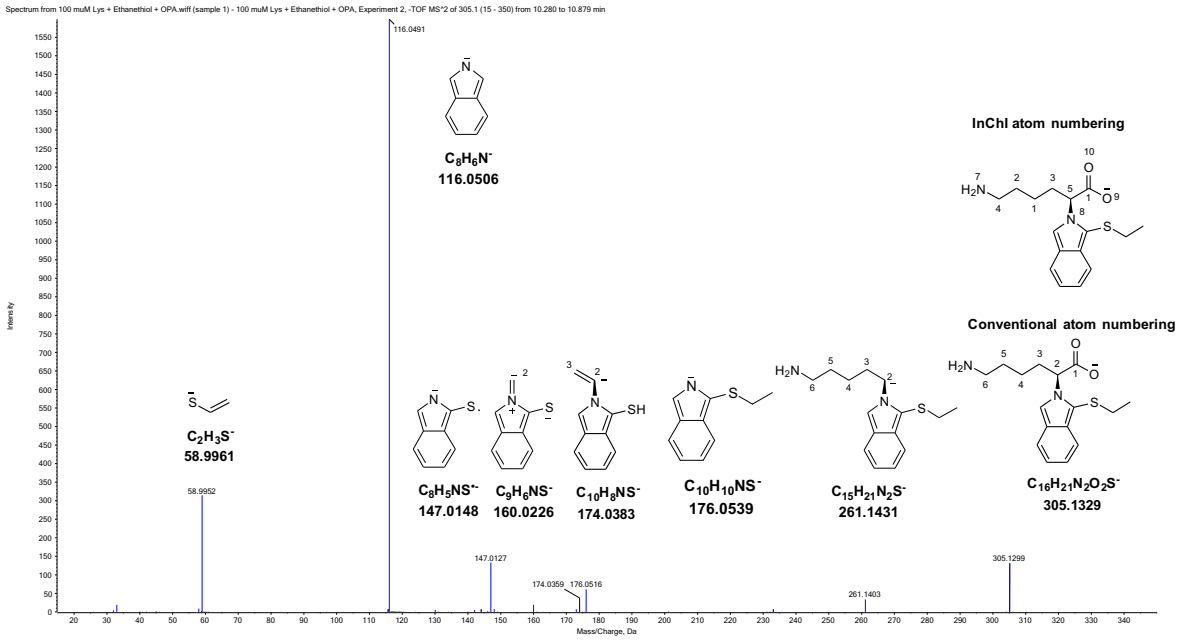


Figure S-25: The product ion spectrum of the [M-H]- ion of α-OPT-Lys.

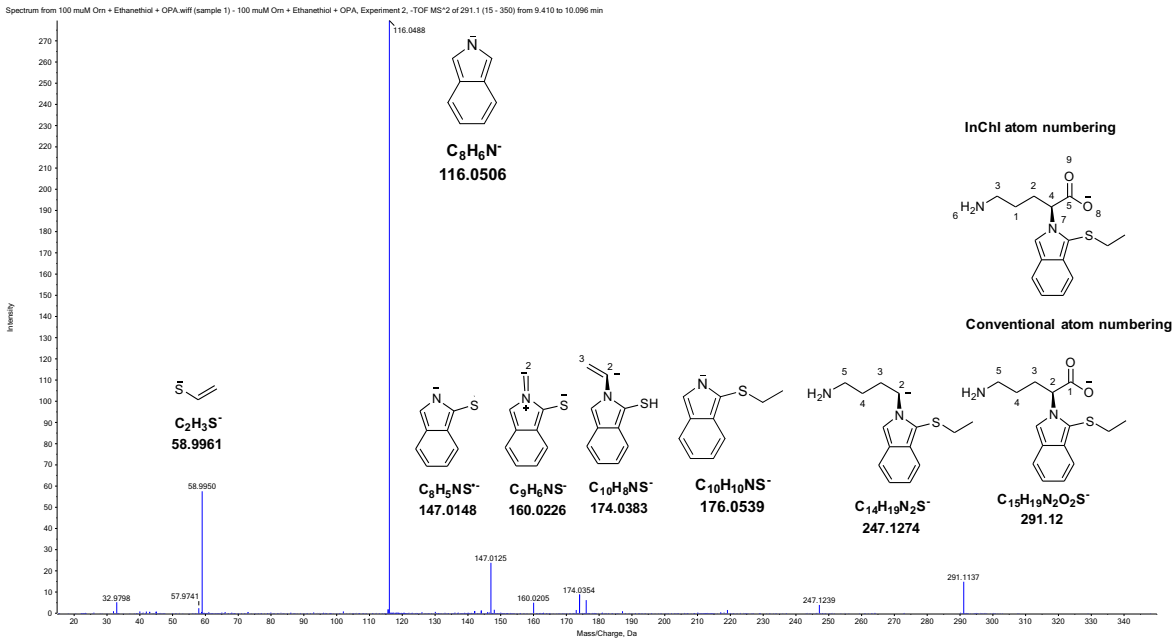


Figure S-26: The product ion spectrum of the [M-H]- ion of α-OPT-Orn.

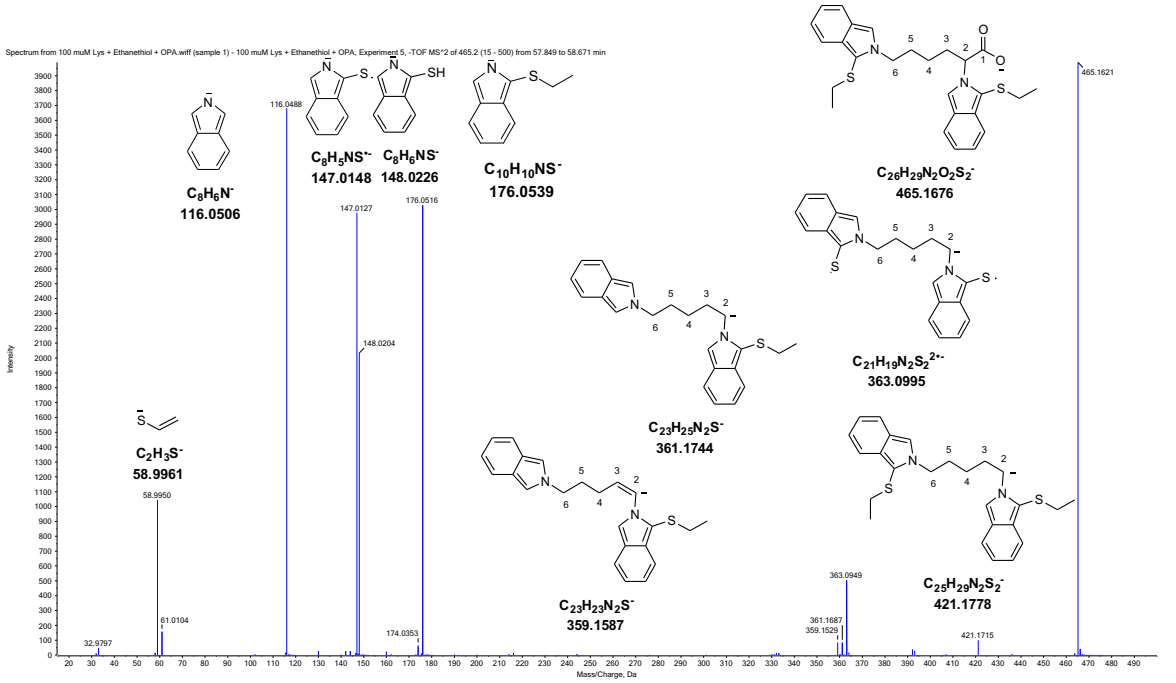


Figure S-27: The product ion spectrum of the [M-H]- ion of Bis-OPT-Lys.

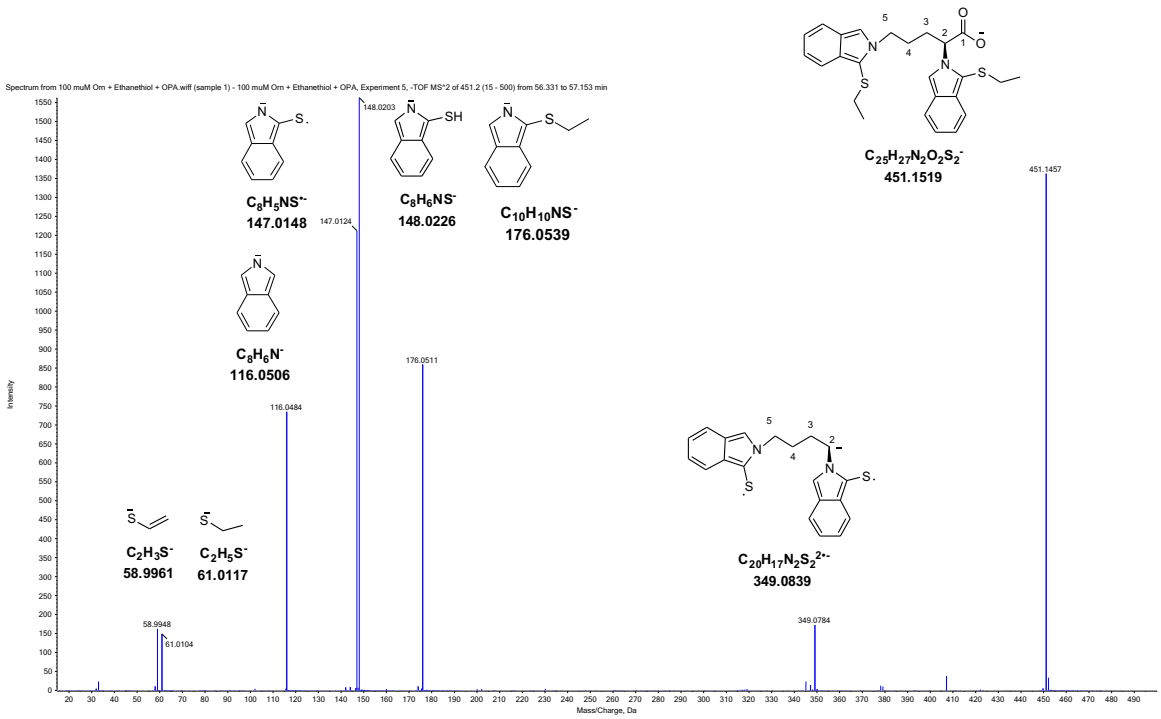


Figure S-28: The product ion spectrum of the [M-H]- ion of Bis-OPT-Orn.

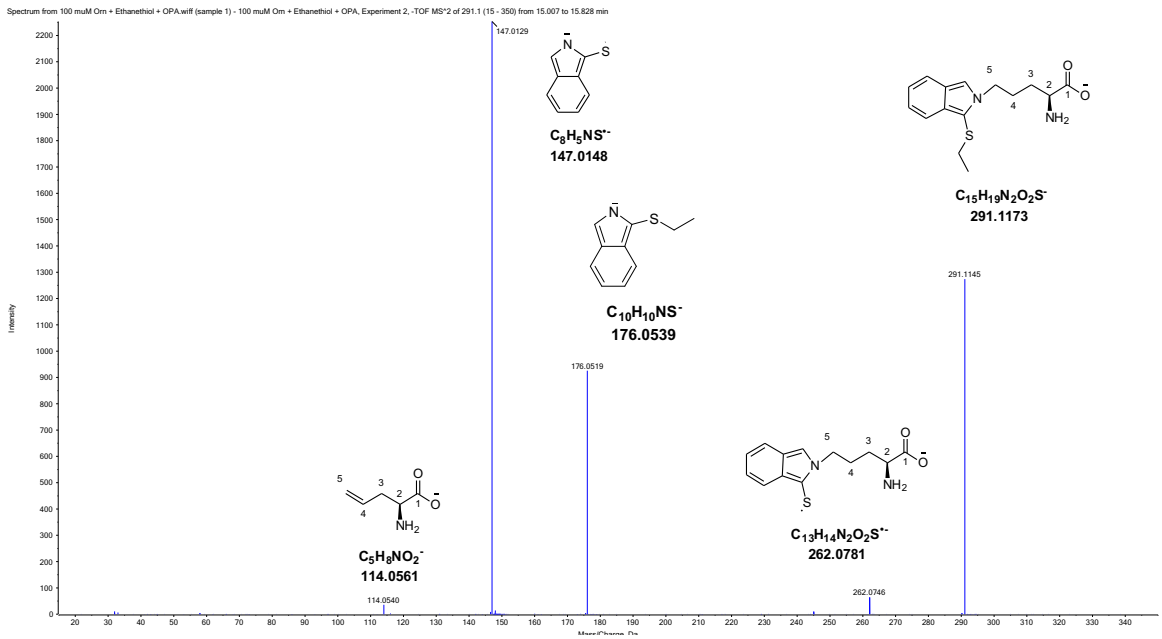


Figure S-29: The product ion spectrum of the [M-H]- ion of δ-OPT-Orn.

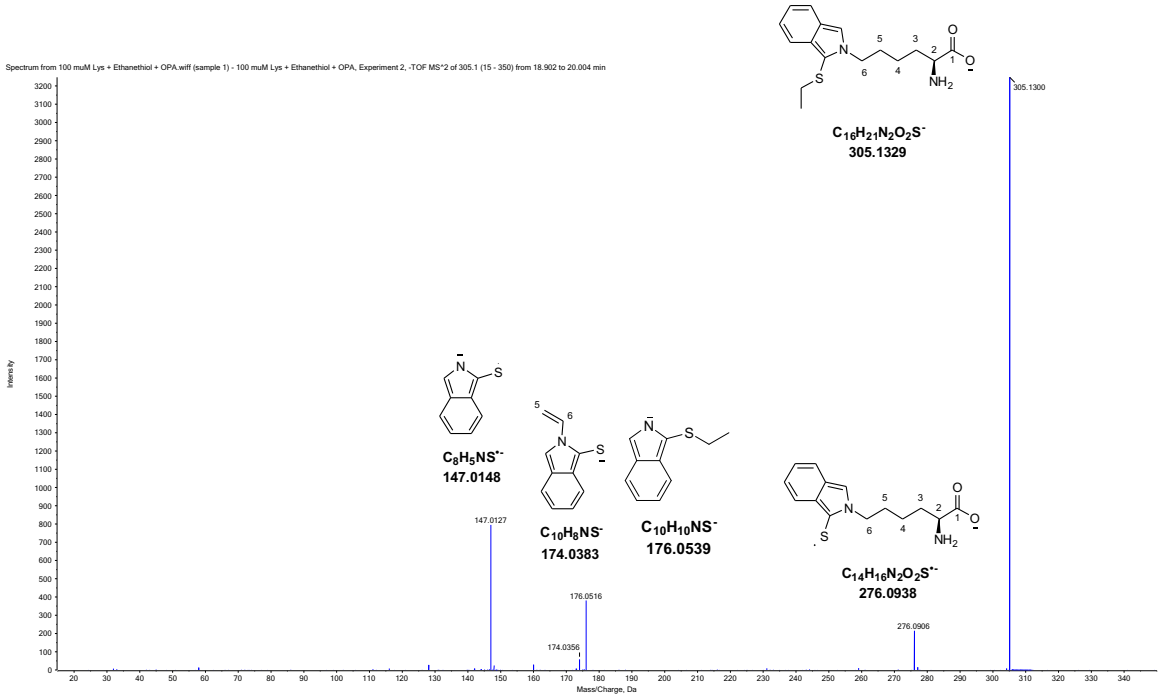


Figure S-30: The product ion spectrum of the [M-H]- ion of ϵ-OPT-Lys.

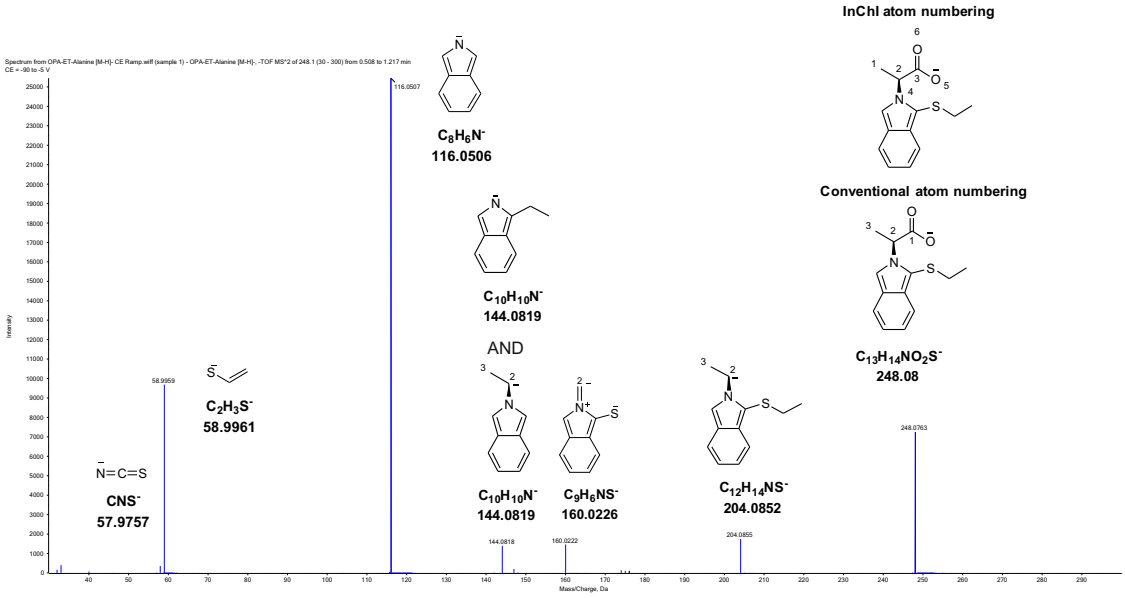


Figure S-31: The product ion spectrum of the [M-H]- ion of OPT-Ala.

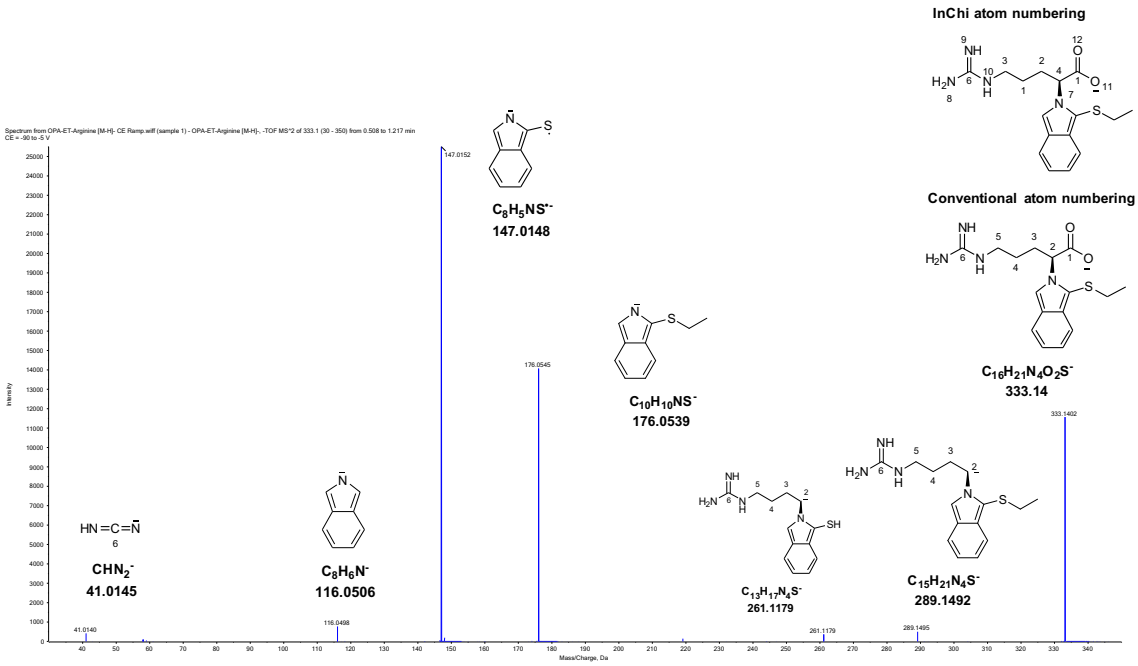


Figure S-32: The product ion spectrum of the [M-H]- ion of OPT-Arg.

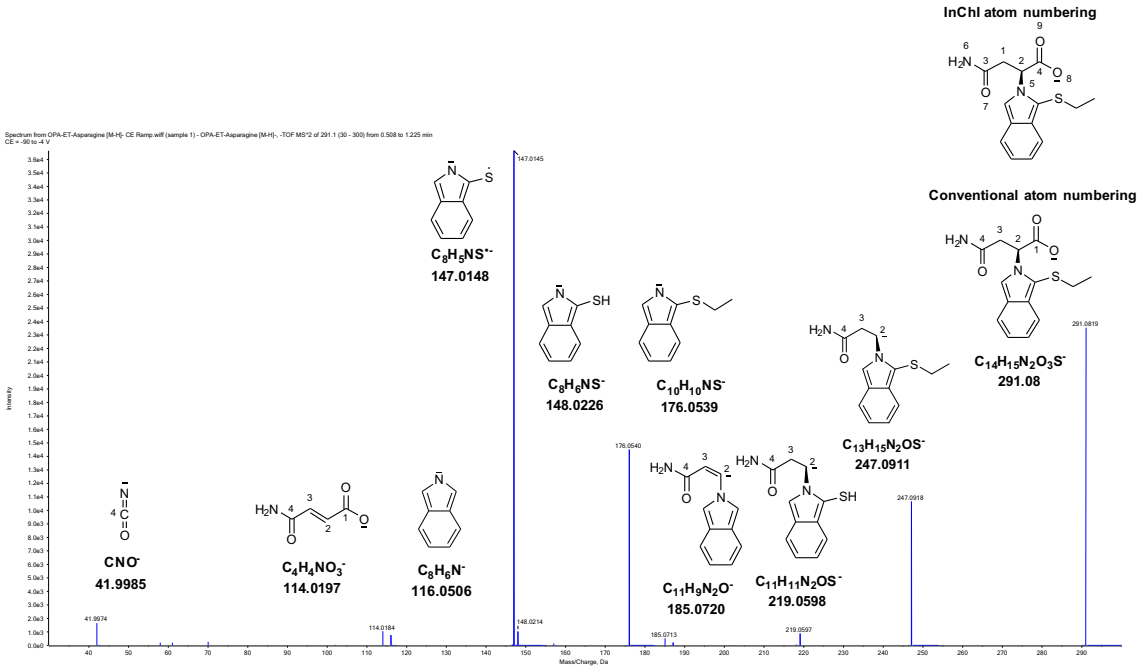


Figure S-33: The product ion spectrum of the [M-H]- ion of OPT-Asn.

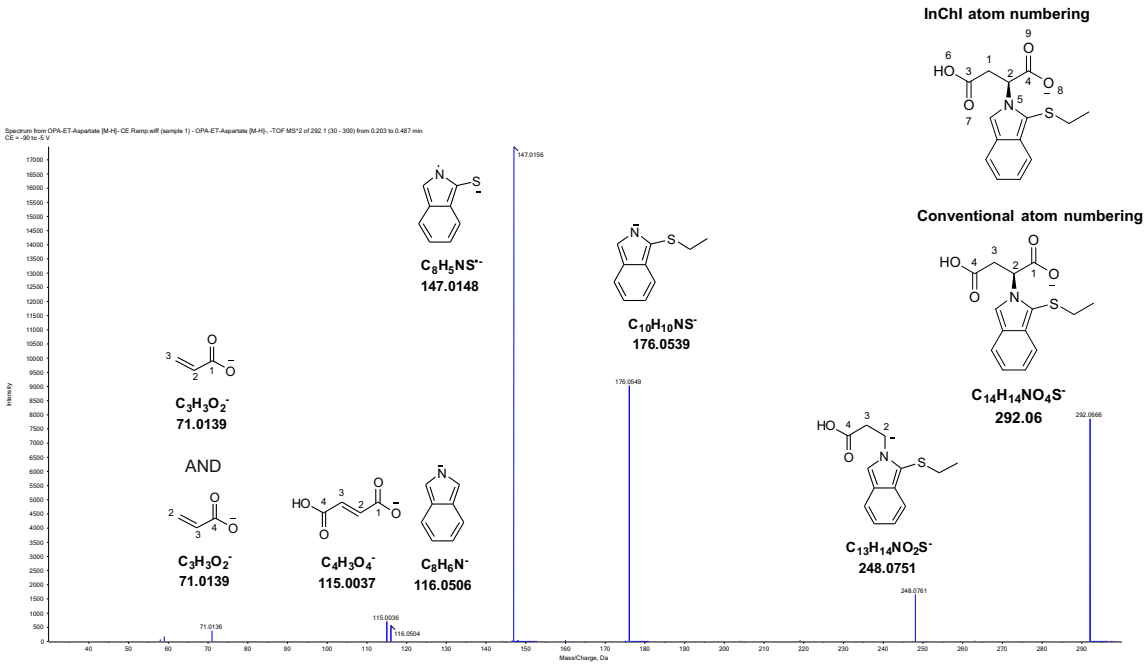


Figure S-34: The product ion spectrum of the [M-H]- ion of OPT-Asp.

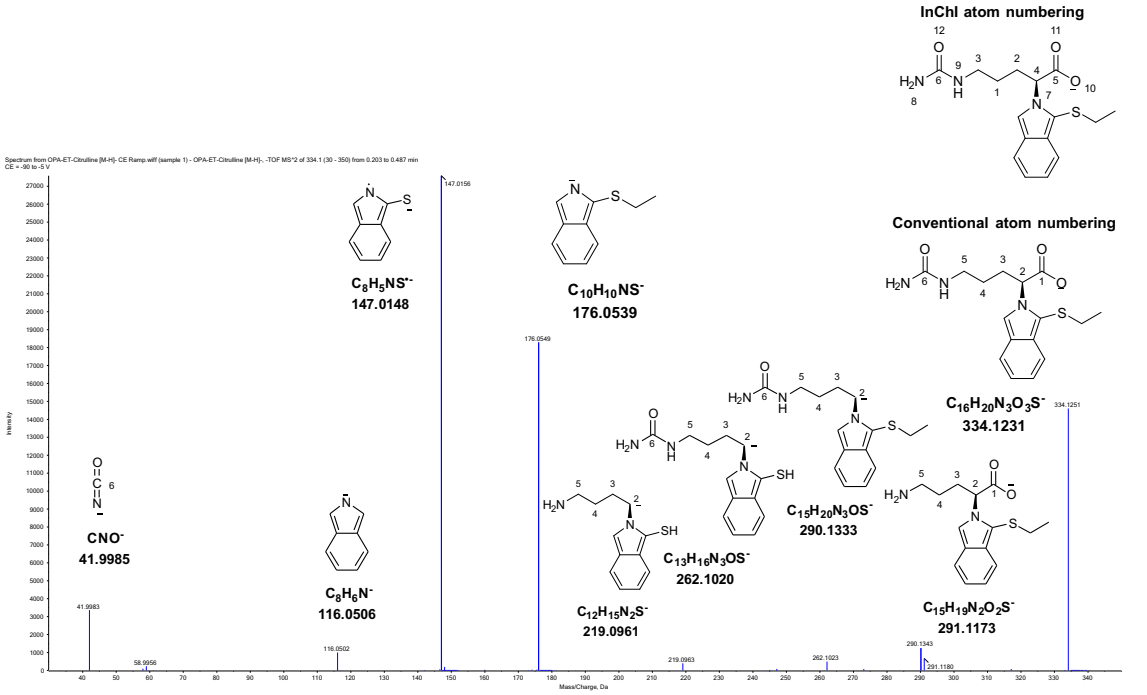


Figure S-35: The product ion spectrum of the [M-H]- ion of OPT-Citr.

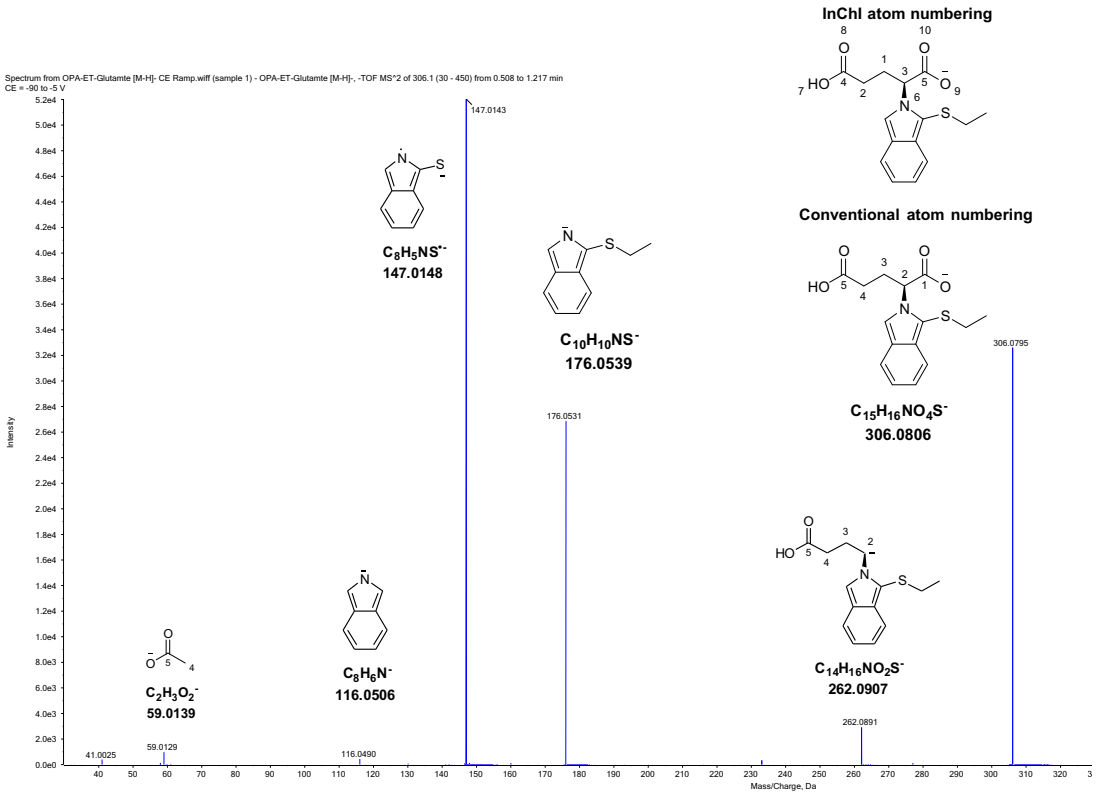


Figure S-36: The product ion spectrum of the [M-H]- ion of OPT-Glu.

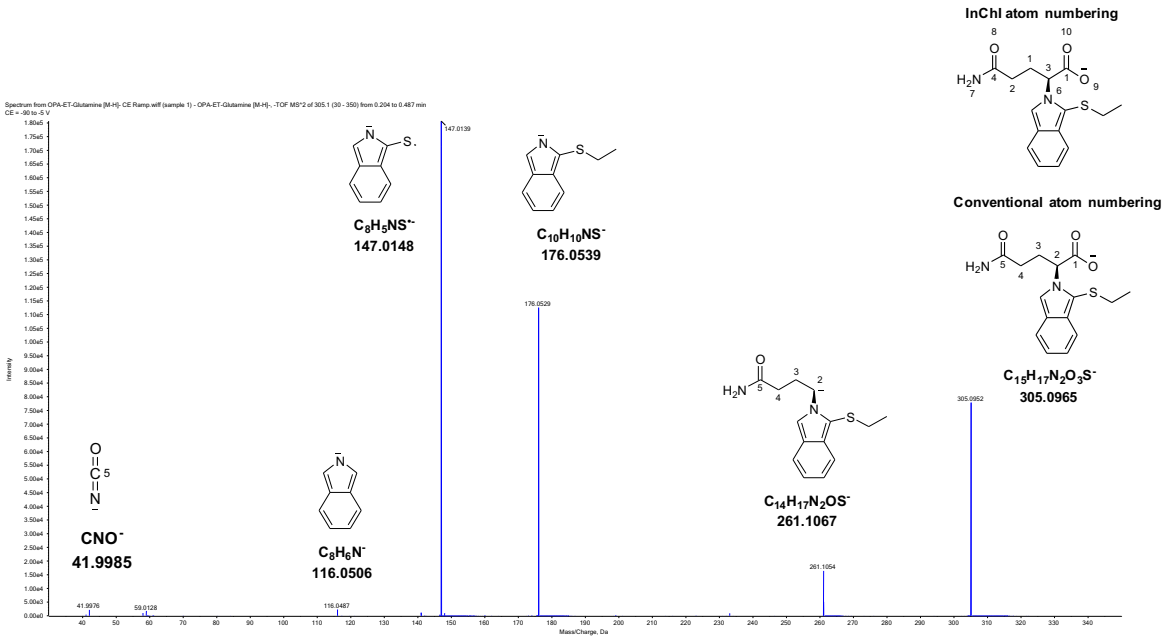


Figure S-37: The product ion spectrum of the [M-H]- ion of OPT-Gln.

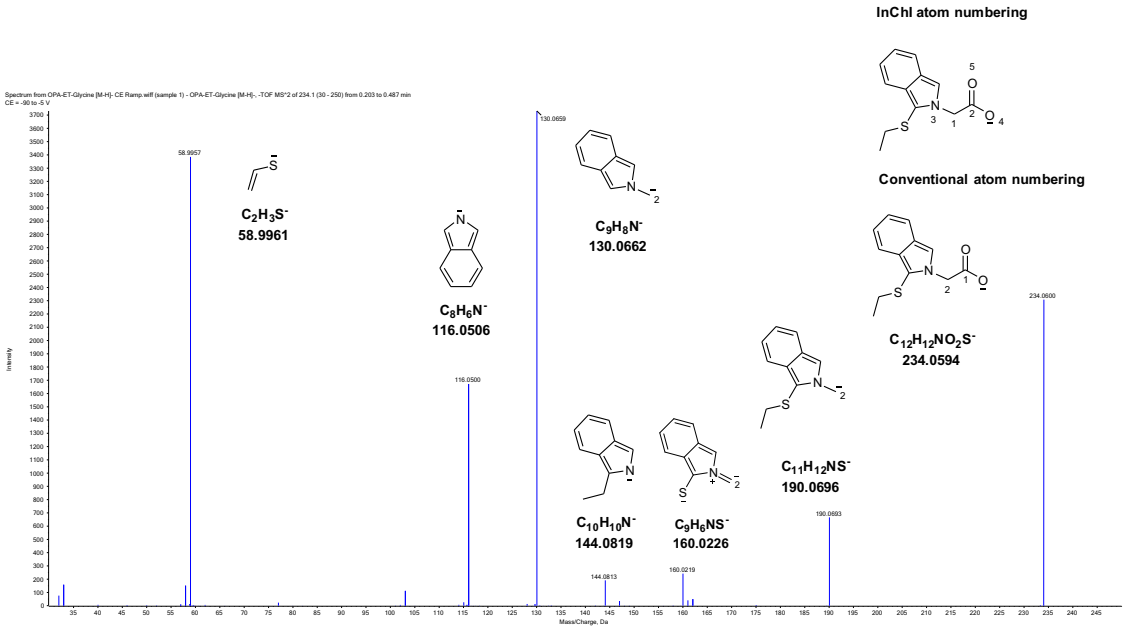


Figure S-38: The product ion spectrum of the [M-H]- ion of OPT-Gly.

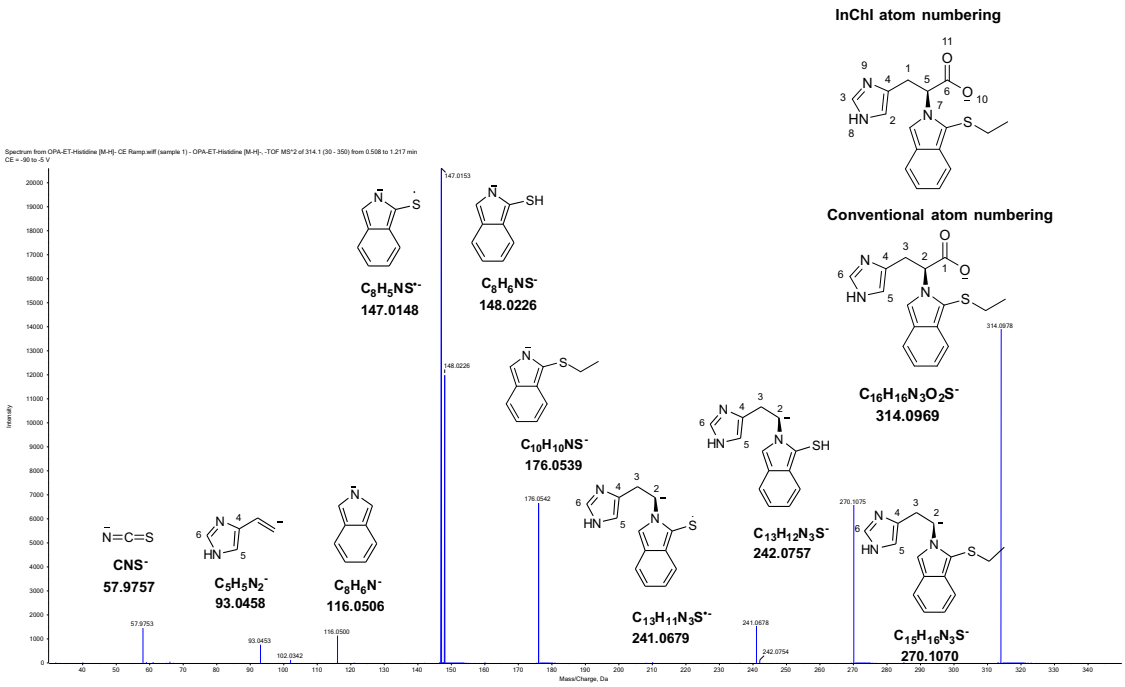


Figure S-39: The product ion spectrum of the [M-H]- ion of OPT-His.

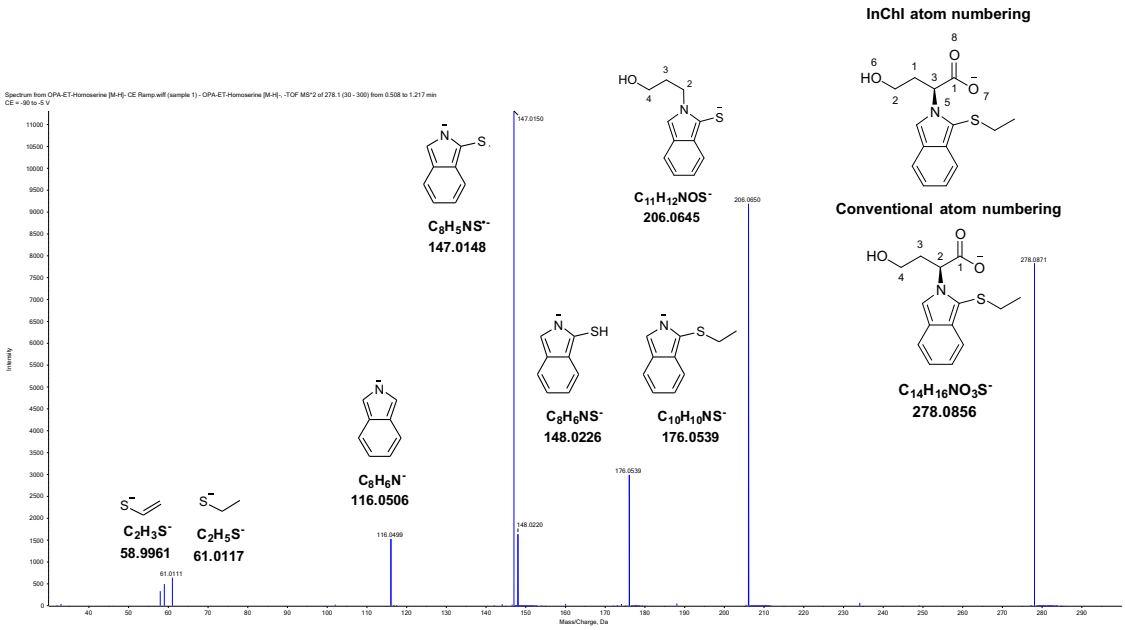


Figure S-40: The product ion spectrum of the [M-H]- ion of OPT-Hom.

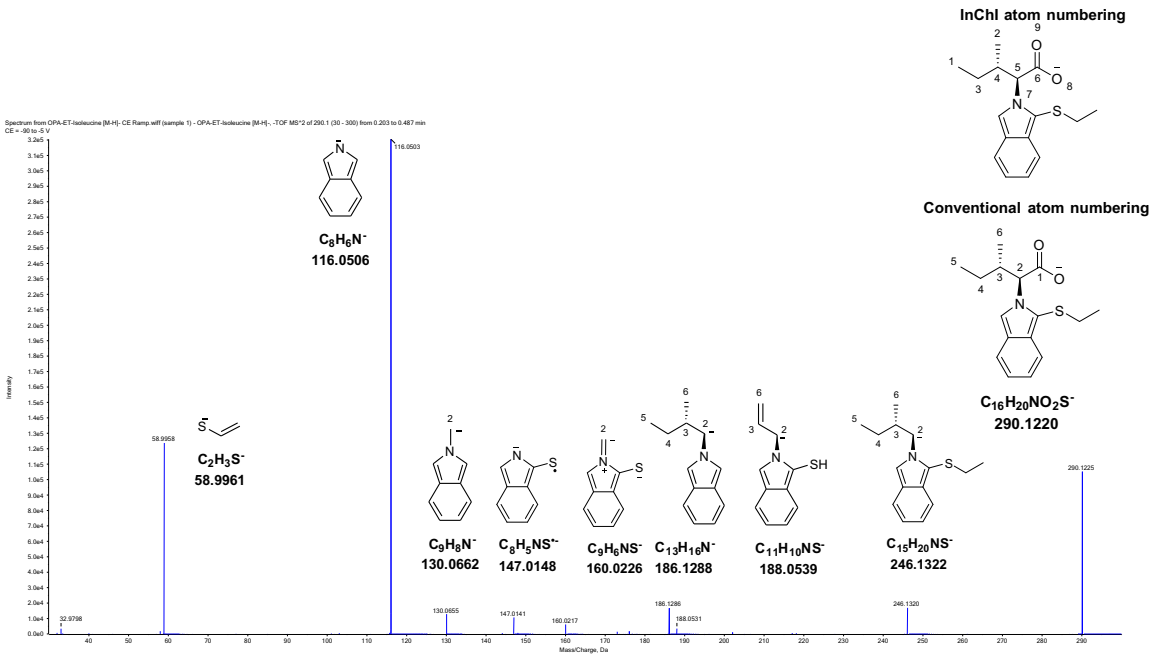


Figure S-41: The product ion spectrum of the [M-H]- ion of OPT-Ile.

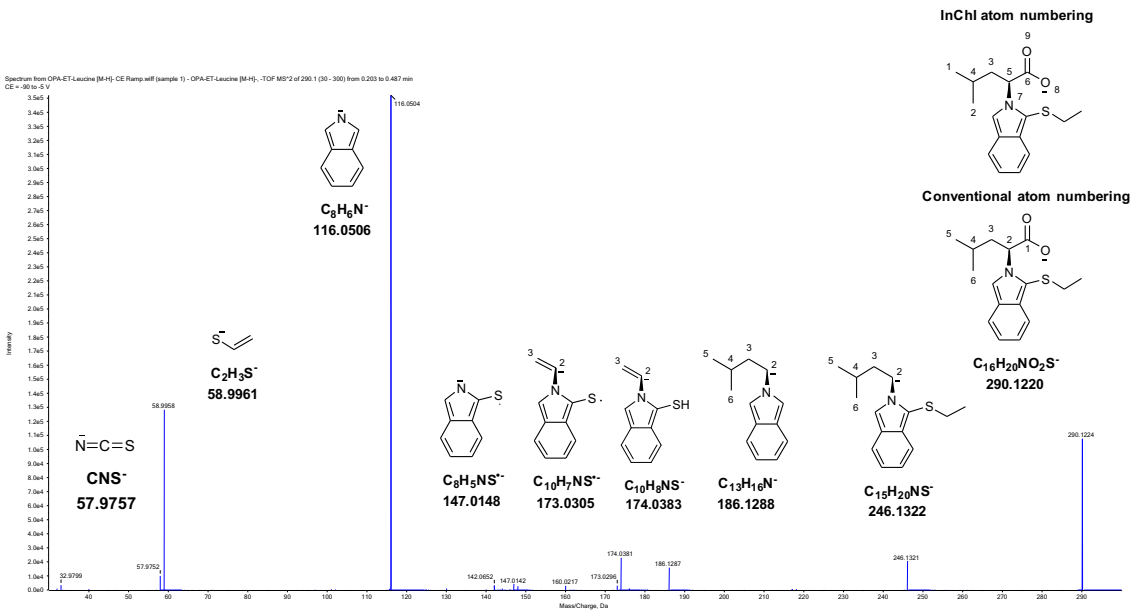


Figure S-42: The product ion spectrum of the [M-H]- ion of OPT-Leu.

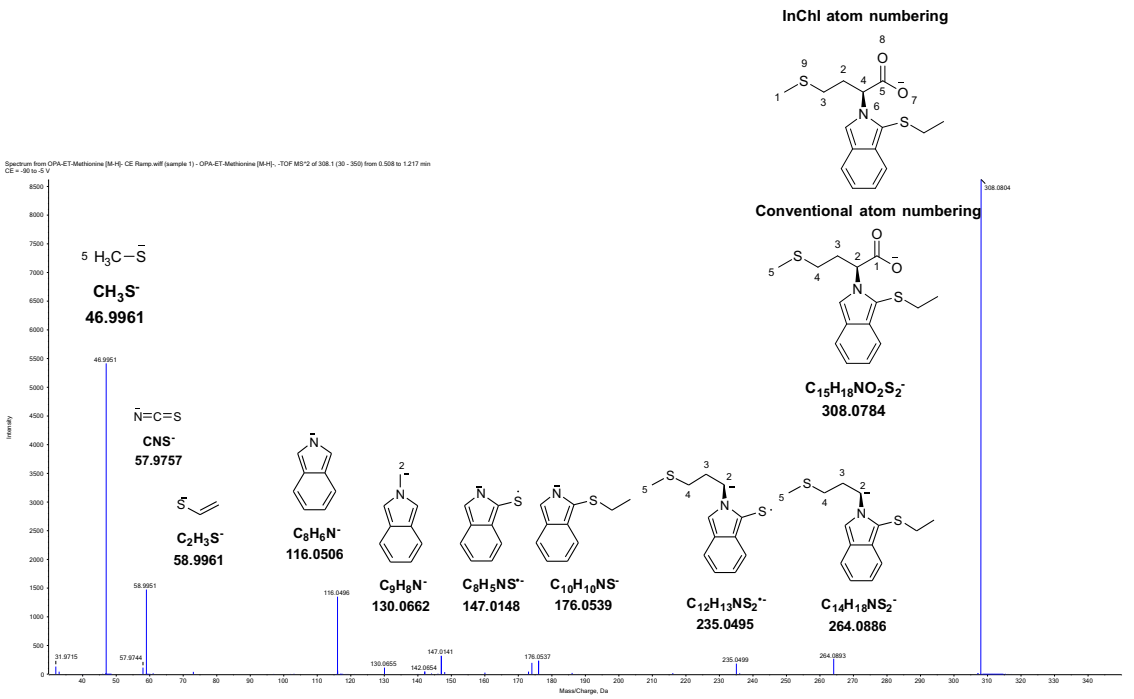


Figure S-43: The product ion spectrum of the [M-H]- ion of OPT-Met.

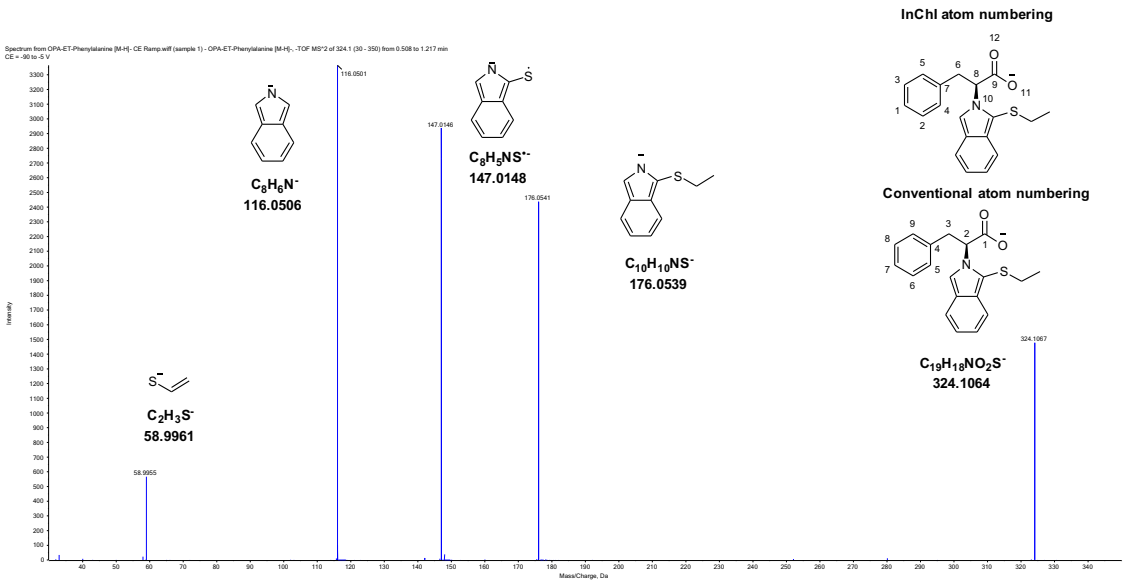


Figure S-44: The product ion spectrum of the [M-H]- ion of OPT-Phe.

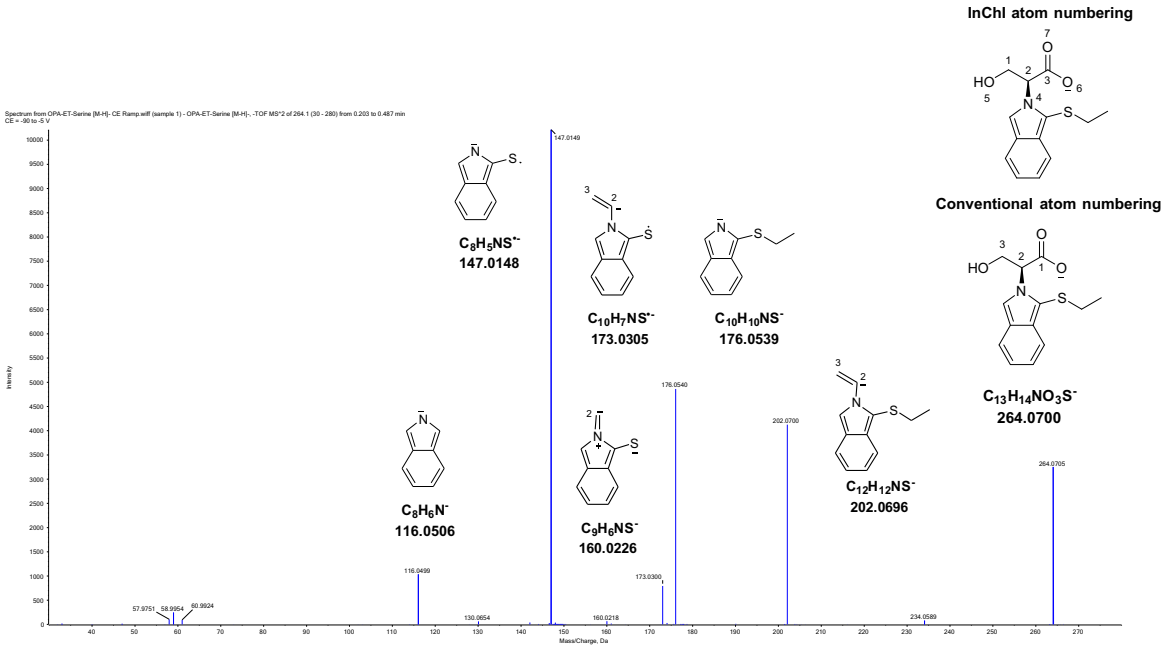


Figure S-45: The product ion spectrum of the [M-H]- ion of OPT-Ser.

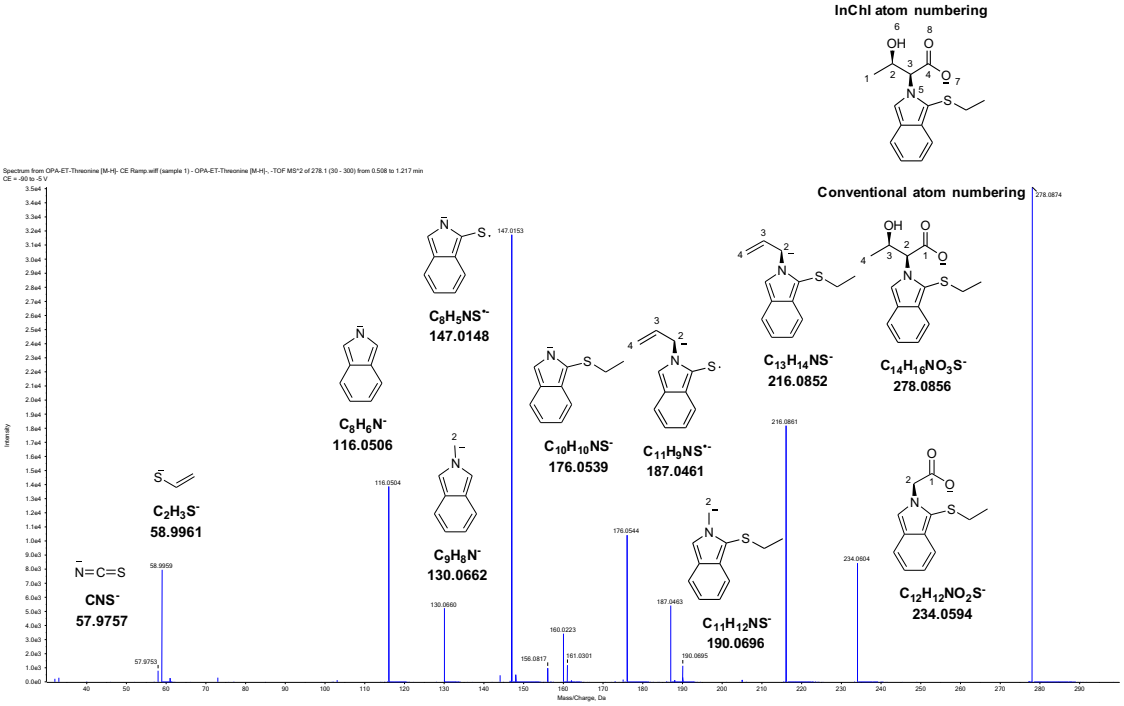


Figure S-46: The product ion spectrum of the [M-H]- ion of OPT-Thr.

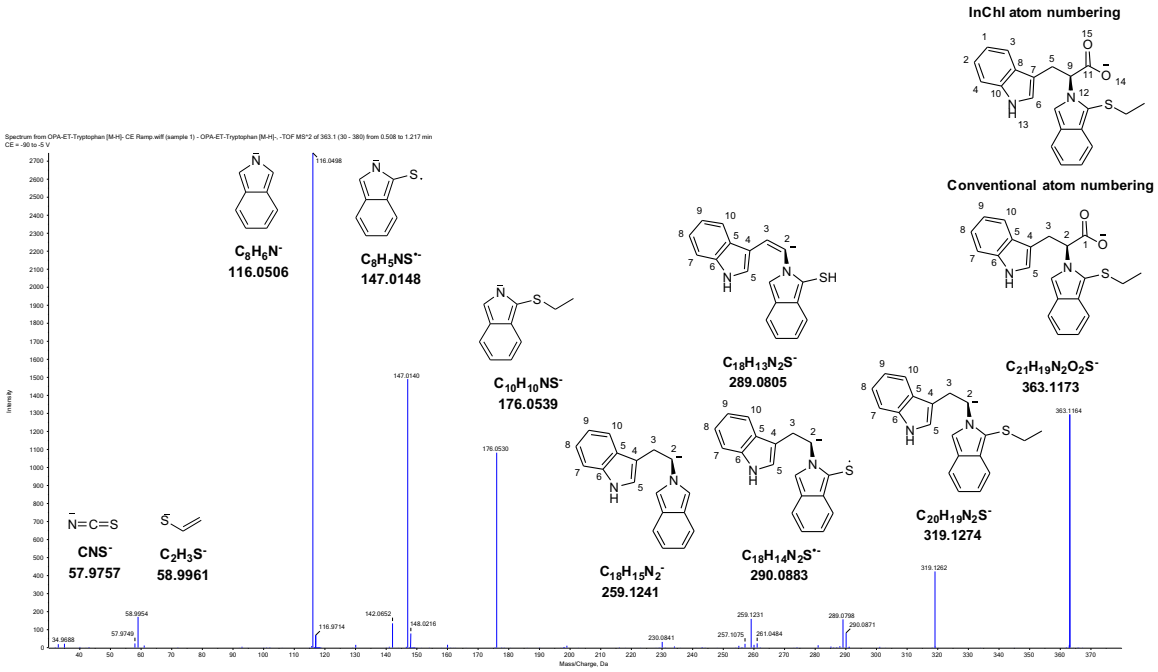


Figure S-47: The product ion spectrum of the [M-H]- ion of OPT-Trp.

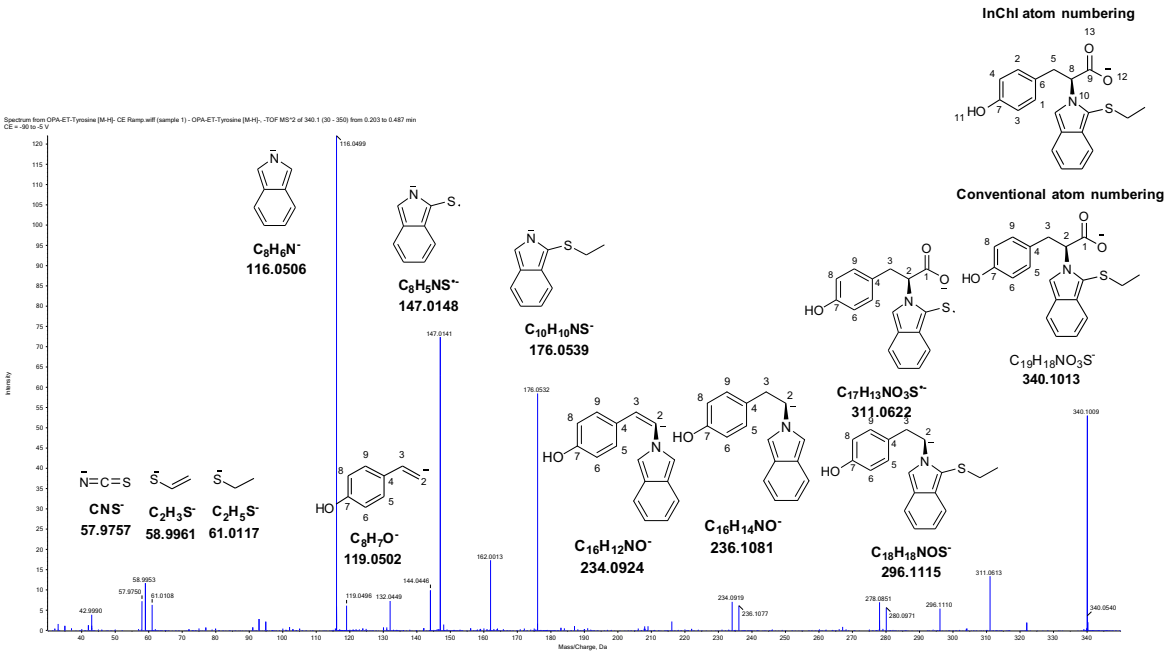


Figure S-48: The product ion spectrum of the [M-H]- ion of OPT-Tyr.

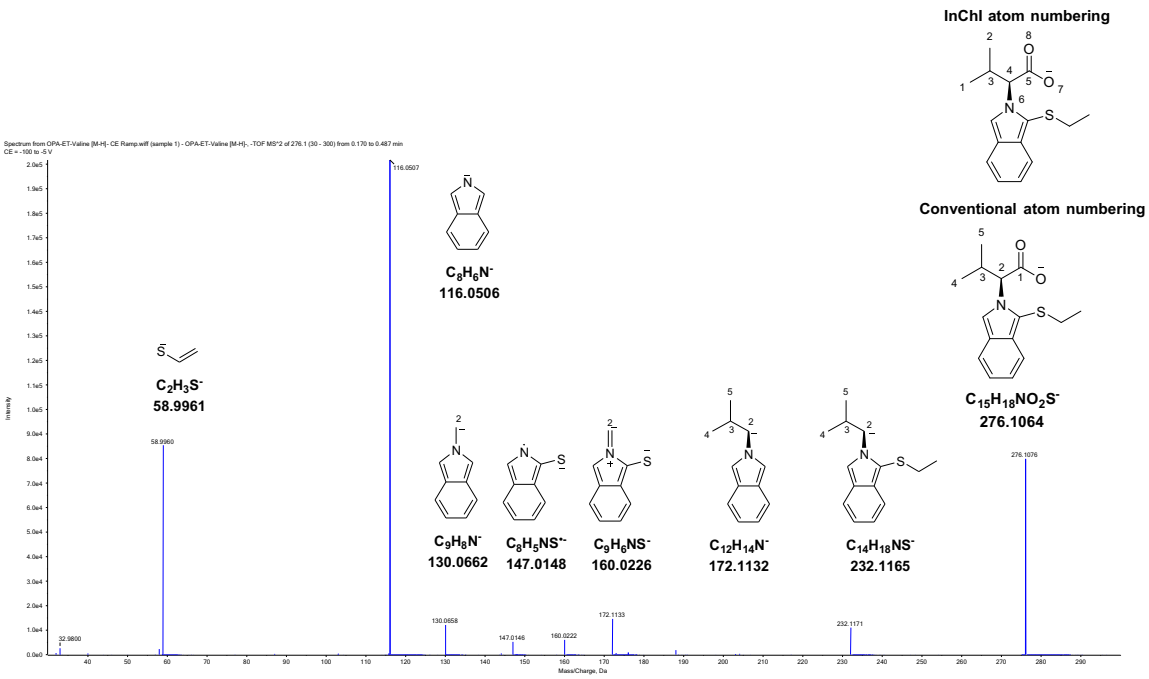


Figure S-49: The product ion spectrum of the [M-H]- ion of OPT-Val.

S5 Reaction products of L-lysine and L-ornithine with O-phthaldialdehyde and ethanethiol

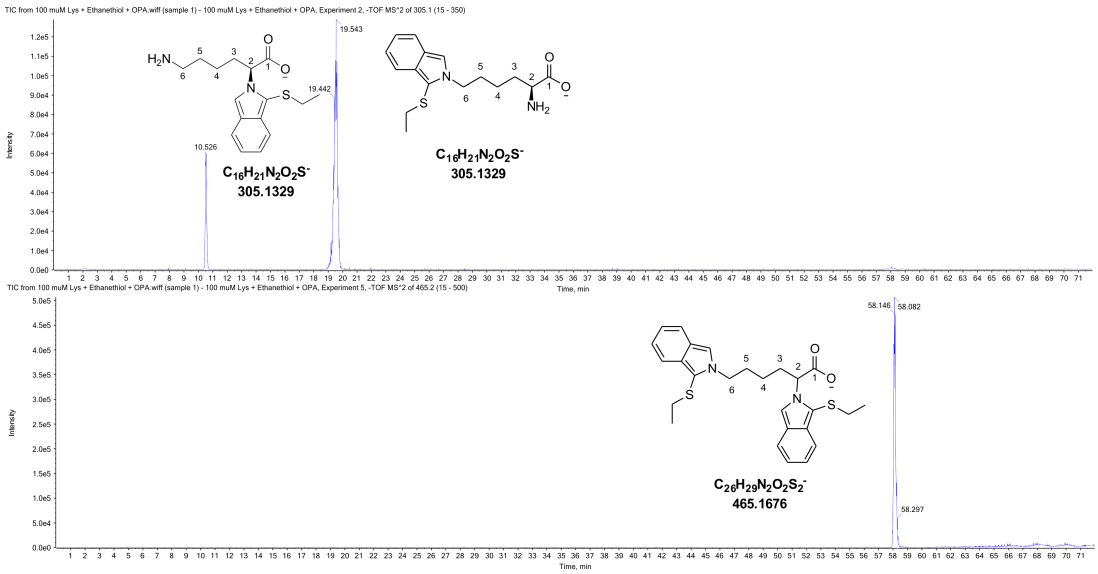


Figure S-50: Reaction products of Lys with OPA and ethanethiol. 20 μl of a 100 μM standard of Lys were derivatized with 0.5 μl reagent solution for 30 seconds and separated using the gradient program documented in the main text.

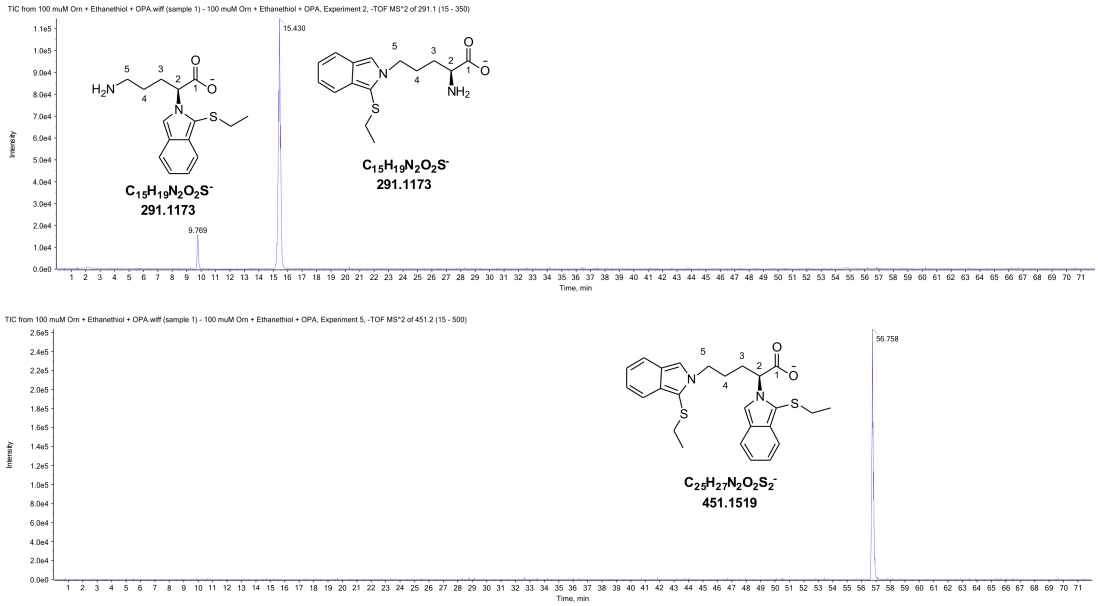


Figure S-51: Reaction products of Orn with OPA and ethanethiol. 20 μl of a 100 μM standard of Orn were derivatized with 0.5 μl reagent solution for 30 seconds and separated using the gradient program documented in the main text.

S6 Autosampler online-derivatization protocol

The derivatization was performed with an Agilent High Performance Autosampler 1260 G1367E-1 cooled to 15°C. The washing of the needle was performed in the needle port with 50 % methanol (LC-MS)/ 50 % MilliQ water.

- 1: DRAW 10 μl from sample
- 2: WASH NEEDLE with default wash parameters
- 4: DRAW 2 μl from reagent solution
- 4: WASH NEEDLE with default wash parameters
- 5: MIX 10 μl in air, six times
- 7: WAIT 1 min
- 8: INJECT

S7 Final acquisition method for OPT-amino acids

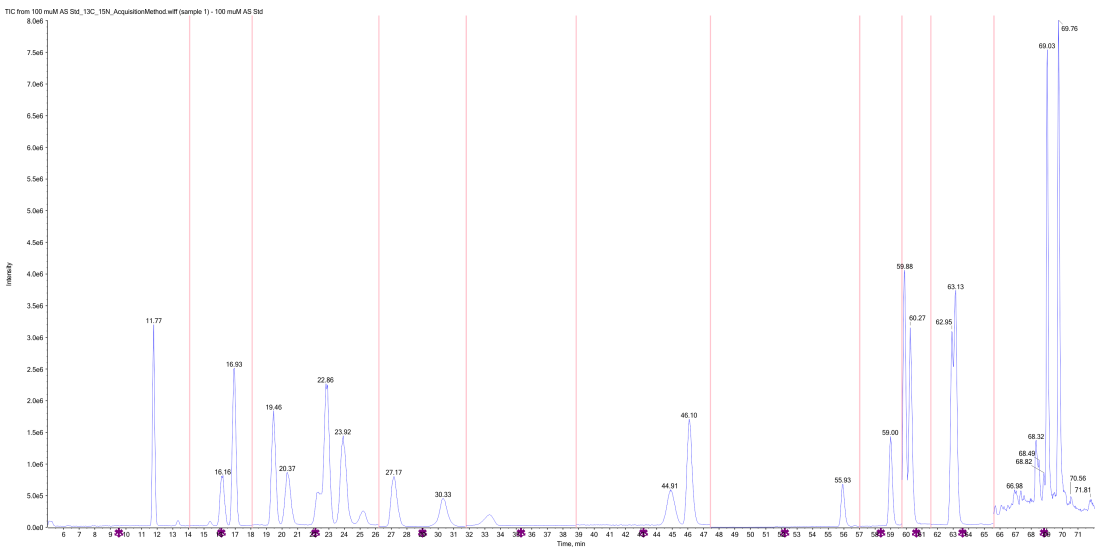


Figure S-52: Gradient elution of OPA/ethanethiol derivatized amino acids acquired with the final acquisition method. Each pale red bar represents the boundary between two periods in which only the MS2 scans corresponding to the labeling states of the OPT-amino acids eluting in this period were acquired.

Table S-7: Final acquisition method for OPT-amino acids. Scan type: negative ionization; scan mode: high sensitivity; GS1: 80; GS2: 50; CUR: 25; TEM: 600; ISVF: 4500.

Precursor Ion Mass Isotopomer	Retention Time	Q1 Window	DP	Targeted Ion	CE
OPT-His [M-H]- M0	11.81	314.1	-20	176.0539	-23
OPT-His [M-H]- M1		315.1			
OPT-His [M-H]- M2		316.1			
OPT-His [M-H]- M3		317.1			
OPT-His [M-H]- M4		318.1			
OPT-His [M-H]- M5		319.1			
OPT-His [M-H]- M6		320.11			
OPT-His [M-H]- M7		321.11			
OPT-His [M-H]- M8		322.11			
OPT-His [M-H]- M9		323.11			
α-OPT-Orn [M-H]- M0	13.37	291.12	-20	116.0506	-26
α-OPT-Orn [M-H]- M1		292.12			
α-OPT-Orn [M-H]- M2		293.12			
α-OPT-Orn [M-H]- M3		294.12			
α-OPT-Orn [M-H]- M4		295.12			
α-OPT-Orn [M-H]- M5		296.13			
α-OPT-Orn [M-H]- M6		297.13			
α-OPT-Orn [M-H]- M7		298.13			
α-OPT-Lys [M-H]- M0	15.39	305.13	-30	116.0506	-31
α-OPT-Lys [M-H]- M1		306.13			
α-OPT-Lys [M-H]- M2		307.13			
α-OPT-Lys [M-H]- M3		308.14			
α-OPT-Lys [M-H]- M4		309.14			
α-OPT-Lys [M-H]- M5		310.14			
α-OPT-Lys [M-H]- M6		311.15			
α-OPT-Lys [M-H]- M7		312.15			
α-OPT-Lys [M-H]- M8		313.15			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

OPT-Asn [M-H]- M0		291.08			
OPT-Asn [M-H]- M1		292.08			
OPT-Asn [M-H]- M2		293.08			
OPT-Asn [M-H]- M3	16.28	294.08	-30	176.0539	-21
OPT-Asn [M-H]- M4		295.09			
OPT-Asn [M-H]- M5		296.09			
OPT-Asn [M-H]- M6		297.09			
OPT-Arg [M-H]- M0		333.14			
OPT-Arg [M-H]- M1		334.14			
OPT-Arg [M-H]- M2		335.14			
OPT-Arg [M-H]- M3		336.14			
OPT-Arg [M-H]- M4		337.14			
OPT-Arg [M-H]- M5	17.01	338.14	-30	176.0539	-22
OPT-Arg [M-H]- M6		339.15			
OPT-Arg [M-H]- M7		340.15			
OPT-Arg [M-H]- M8		341.15			
OPT-Arg [M-H]- M9		342.15			
OPT-Arg [M-H]- M10		343.15			
OPT-Gln [M-H]- M0		305.1			
OPT-Gln [M-H]- M1		306.1			
OPT-Gln [M-H]- M2		307.1			
OPT-Gln [M-H]- M3	19.59	308.1	-30	176.0539	-19
OPT-Gln [M-H]- M4		309.1			
OPT-Gln [M-H]- M5		310.11			
OPT-Gln [M-H]- M6		311.11			
OPT-Gln [M-H]- M7		312.11			
OPT-Ser [M-H]- M0		264.07			
OPT-Ser [M-H]- M1		265.07			
OPT-Ser [M-H]- M2	20.53	266.07	-30	147.0148	-32
OPT-Ser [M-H]- M3		267.08			
OPT-Ser [M-H]- M4		268.08			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

OPT-Citr [M-H]- M0	23.01	334.12	-30	176.0539	-20
OPT-Citr [M-H]- M1		335.12			
OPT-Citr [M-H]- M2		336.12			
OPT-Citr [M-H]- M3		337.12			
OPT-Citr [M-H]- M4		338.13			
OPT-Citr [M-H]- M5		339.13			
OPT-Citr [M-H]- M6		340.13			
OPT-Citr [M-H]- M7		341.13			
OPT-Citr [M-H]- M8		342.13			
OPT-Citr [M-H]- M9		343.13			
OPT-Asp [M-H]- M0	22.67	292.06	-30	176.0539	-19
OPT-Asp [M-H]- M1		293.07			
OPT-Asp [M-H]- M2		294.07			
OPT-Asp [M-H]- M3		295.07			
OPT-Asp [M-H]- M4		296.08			
OPT-Asp [M-H]- M5		297.08			
OPT-Hom [M-H]- M0	24.07	278.09	-30	176.0539	-21
OPT-Hom [M-H]- M1		279.09			
OPT-Hom [M-H]- M2		280.09			
OPT-Hom [M-H]- M3		281.09			
OPT-Hom [M-H]- M4		282.09			
OPT-Hom [M-H]- M5		283.09			
OPT-Glu [M-H]- M0	27.28	306.08	-20	147.0148	-35
OPT-Glu [M-H]- M1		307.08			
OPT-Glu [M-H]- M2		308.08			
OPT-Glu [M-H]- M3		309.09			
OPT-Glu [M-H]- M4		310.09			
OPT-Glu [M-H]- M5		311.09			
OPT-Glu [M-H]- M6		312.09			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

δ-OPT-Orn [M-H]- M0		291.12			
δ-OPT-Orn [M-H]- M1		292.12			
δ-OPT-Orn [M-H]- M2		293.12			
δ-OPT-Orn [M-H]- M3	25.46	294.12	-20	176.0539	-19
δ-OPT-Orn [M-H]- M4		295.12			
δ-OPT-Orn [M-H]- M5		296.13			
δ-OPT-Orn [M-H]- M6		297.13			
δ-OPT-Orn [M-H]- M7		298.13			
OPT-Thr [M-H]- M0		278.09			
OPT-Thr [M-H]- M1		279.09			
OPT-Thr [M-H]- M2	30.39	280.09	-20	147.0148	-35
OPT-Thr [M-H]- M3		281.09			
OPT-Thr [M-H]- M4		282.1			
OPT-Thr [M-H]- M5		283.1			
OPT-Gly [M-H]- M0		234.06			
OPT-Gly [M-H]- M1	30.45	235.06	-20	116.0506	-55
OPT-Gly [M-H]- M2		236.06			
OPT-Gly [M-H]- M3		237.06			
ϵ-OPT-Lys [M-H]- M0		305.13			
ϵ-OPT-Lys [M-H]- M1		306.13			
ϵ-OPT-Lys [M-H]- M2		307.13			
ϵ-OPT-Lys [M-H]- M3		308.14			
ϵ-OPT-Lys [M-H]- M4	33.59	309.14	-20	176.0539	-21
ϵ-OPT-Lys [M-H]- M5		310.14			
ϵ-OPT-Lys [M-H]- M6		311.15			
ϵ-OPT-Lys [M-H]- M7		312.15			
ϵ-OPT-Lys [M-H]- M8		313.15			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

OPT-Ala [M-H]- M0		248.08			
OPT-Ala [M-H]- M1		249.08			
OPT-Ala [M-H]- M2	44.78	250.08	-20	116.0506	-37
OPT-Ala [M-H]- M3		251.08			
OPT-Ala [M-H]- M4		252.08			
OPT-Tyr [M-H]- M0		340.1			
OPT-Tyr [M-H]- M1		341.1			
OPT-Tyr [M-H]- M2		342.1			
OPT-Tyr [M-H]- M3		343.11			
OPT-Tyr [M-H]- M4		344.11			
OPT-Tyr [M-H]- M5	46.05	345.11	-20	176.0539	-16
OPT-Tyr [M-H]- M6		346.12			
OPT-Tyr [M-H]- M7		347.12			
OPT-Tyr [M-H]- M8		348.12			
OPT-Tyr [M-H]- M9		349.13			
OPT-Tyr [M-H]- M10		350.13			
OPT-Met [M-H]- M0		308.08			
OPT-Met [M-H]- M1		309.08			
OPT-Met [M-H]- M2		310.08			
OPT-Met [M-H]- M3	55.62	311.09	-20	116.0506	-50
OPT-Met [M-H]- M4		312.09			
OPT-Met [M-H]- M5		313.09			
OPT-Met [M-H]- M6		314.09			
OPT-Val [M-H]- M0		276.11			
OPT-Val [M-H]- M1		277.11			
OPT-Val [M-H]- M2		278.11			
OPT-Val [M-H]- M3	58.45	279.11	-20	116.0506	-36
OPT-Val [M-H]- M4		280.12			
OPT-Val [M-H]- M5		281.12			
OPT-Val [M-H]- M6		282.12			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

OPT-Trp [M-H]- M0		363.12			
OPT-Trp [M-H]- M1		364.12			
OPT-Trp [M-H]- M2		365.12			
OPT-Trp [M-H]- M3		366.12			
OPT-Trp [M-H]- M4		367.12			
OPT-Trp [M-H]- M5	59.47	368.13	-20	176.0539	-19
OPT-Trp [M-H]- M6		369.13			
OPT-Trp [M-H]- M7		370.13			
OPT-Trp [M-H]- M8		371.14			
OPT-Trp [M-H]- M9		372.14			
OPT-Trp [M-H]- M10		373.14			
OPT-Trp [M-H]- M11		374.15			
OPT-Trp [M-H]- M12		375.15			
OPT-Trp [M-H]- M13		376.15			
OPT-Phe [M-H]- M0		324.11			
OPT-Phe [M-H]- M1		325.11			
OPT-Phe [M-H]- M2		326.11			
OPT-Phe [M-H]- M3		327.11			
OPT-Phe [M-H]- M4		328.12			
OPT-Phe [M-H]- M5	59.89	329.12	-20	176.0539	-16
OPT-Phe [M-H]- M6		330.12			
OPT-Phe [M-H]- M7		331.13			
OPT-Phe [M-H]- M8		332.13			
OPT-Phe [M-H]- M9		333.13			
OPT-Phe [M-H]- M10		334.13			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

OPT-Ileu [M-H]- M0	62.02	290.12	-20	116.0506	-35
OPT-Ileu [M-H]- M1		291.12			
OPT-Ileu [M-H]- M2		292.13			
OPT-Ileu [M-H]- M3		293.13			
OPT-Ileu [M-H]- M4		294.13			
OPT-Ileu [M-H]- M5		295.14			
OPT-Ileu [M-H]- M6		296.14			
OPT-Ileu [M-H]- M7		297.14			
OPT-Leu [M-H]- M0	62.46	290.12	-20	116.0506	-35
OPT-Leu [M-H]- M1		291.12			
OPT-Leu [M-H]- M2		292.13			
OPT-Leu [M-H]- M3		293.13			
OPT-Leu [M-H]- M4		294.13			
OPT-Leu [M-H]- M5		295.14			
OPT-Leu [M-H]- M6		296.14			
OPT-Leu [M-H]- M7		297.14			
Bis-OPT-Orn [M-H]- M0	68.97	451.15	-30	176.0539	-26
Bis-OPT-Orn [M-H]- M1		452.15			
Bis-OPT-Orn [M-H]- M2		453.15			
Bis-OPT-Orn [M-H]- M3		454.16			
Bis-OPT-Orn [M-H]- M4		455.16			
Bis-OPT-Orn [M-H]- M5		456.16			
Bis-OPT-Orn [M-H]- M6		457.16			
Bis-OPT-Orn [M-H]- M7		458.16			

Table S-7 Continued: Final acquisition method for OPT-amino acids.

Bis-OPT-Lys [M-H]- M0		465.17			
Bis-OPT-Lys [M-H]- M1		466.17			
Bis-OPT-Lys [M-H]- M2		467.17			
Bis-OPT-Lys [M-H]- M3		468.17			
Bis-OPT-Lys [M-H]- M4	69.73	469.17	-30	176.0539	-26
Bis-OPT-Lys [M-H]- M5		470.18			
Bis-OPT-Lys [M-H]- M6		471.18			
Bis-OPT-Lys [M-H]- M7		472.18			
Bis-OPT-Lys [M-H]- M8		473.18			

S8 Linear dynamic range of OPT-amino acids

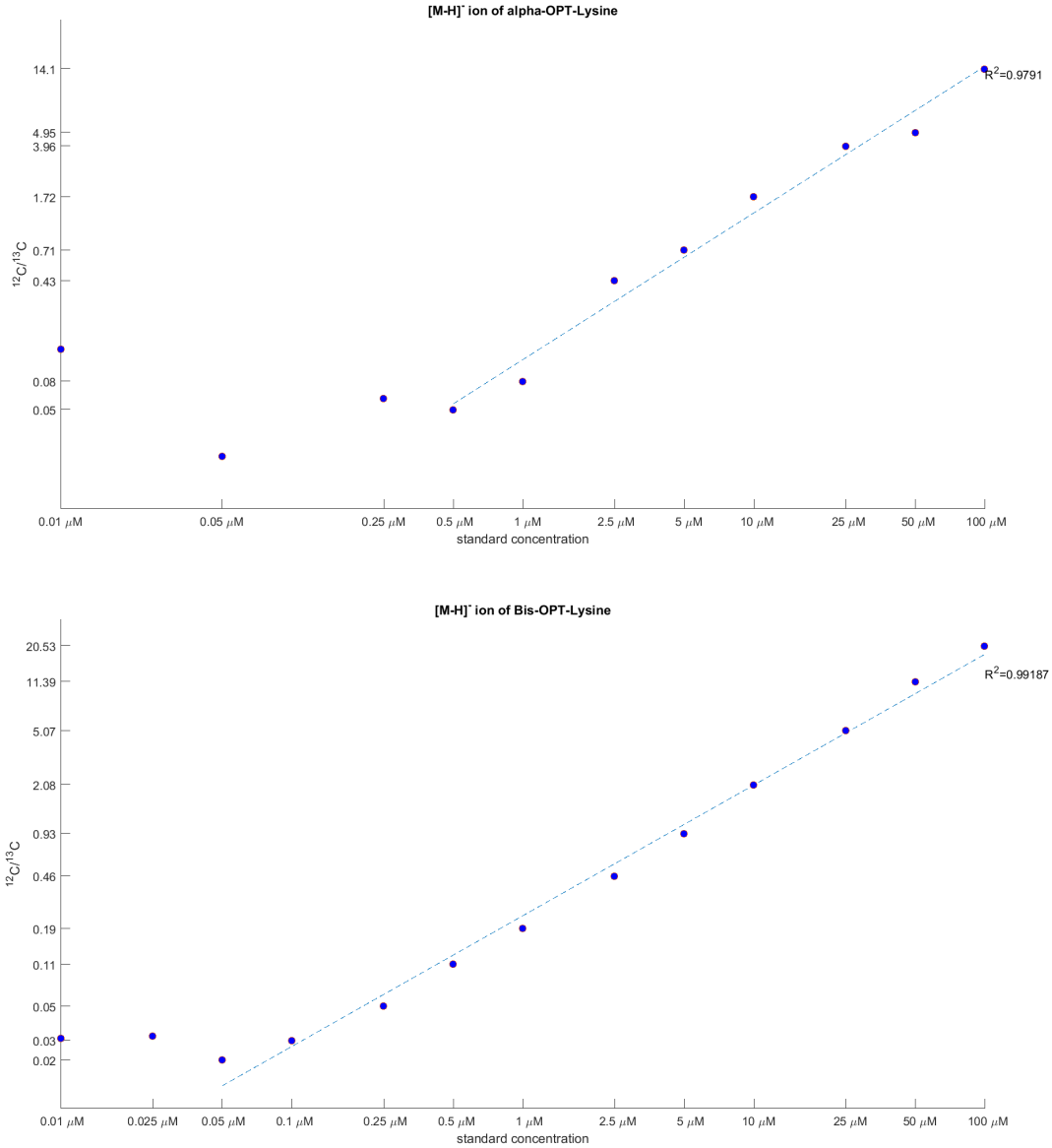


Figure S-53: Linear dynamic ranges of α-OPT-Lys and Bis-OPT-Lys

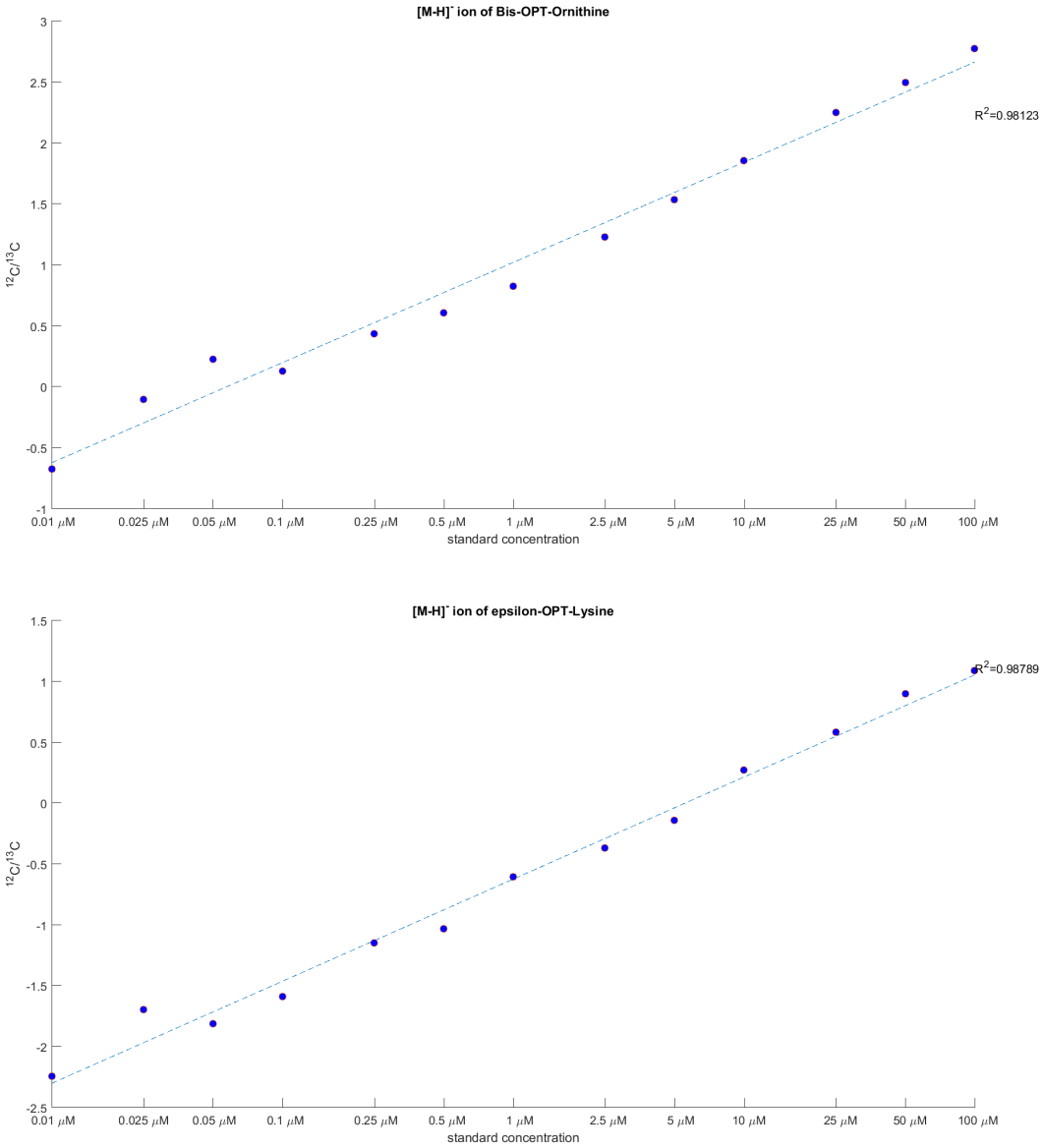


Figure S-54: Linear dynamic ranges of Bis-OPT-Orn and ϵ-OPT-Lys

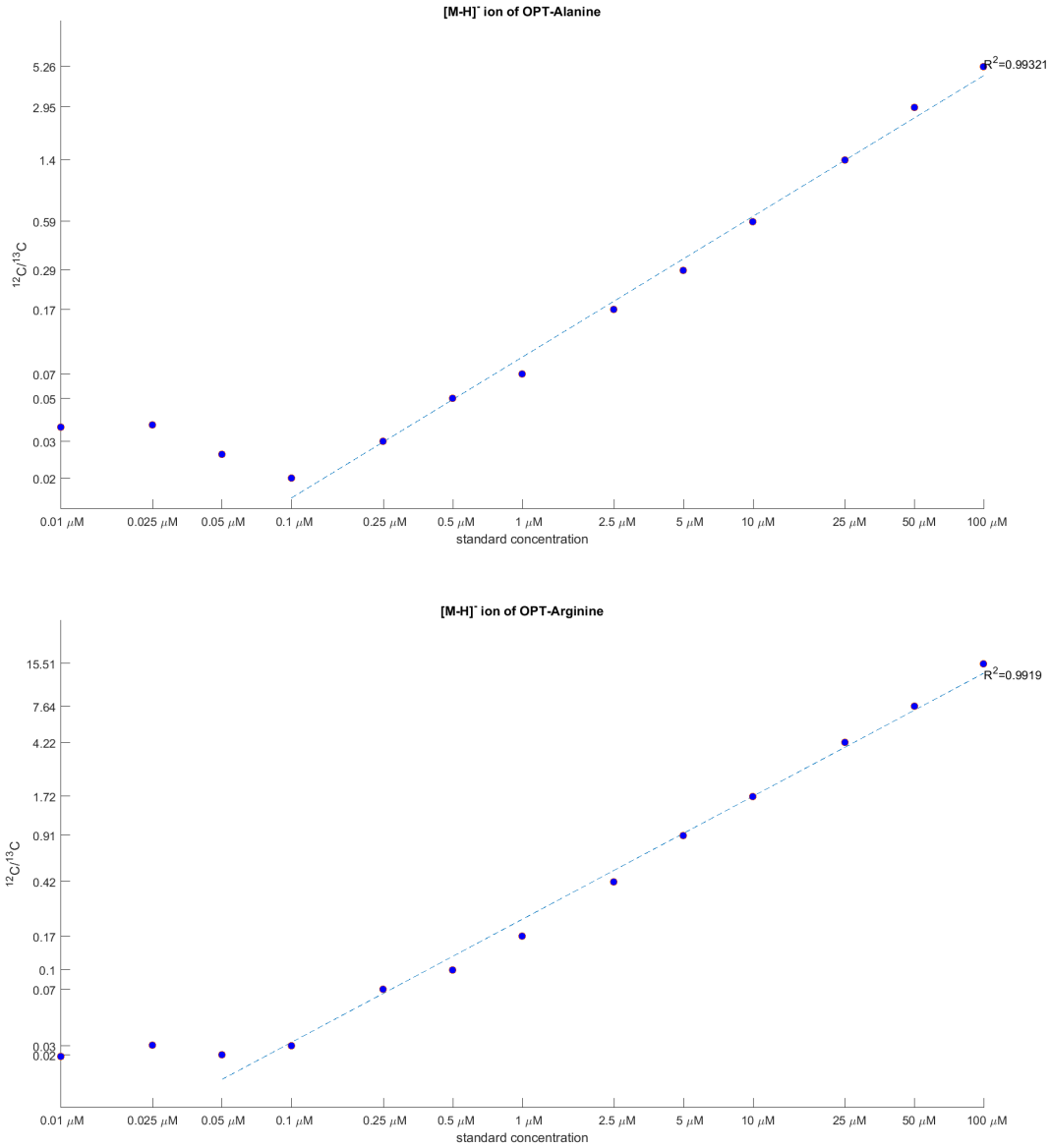


Figure S-55: Linear dynamic ranges of OPT-Ala and OPT-Arg.

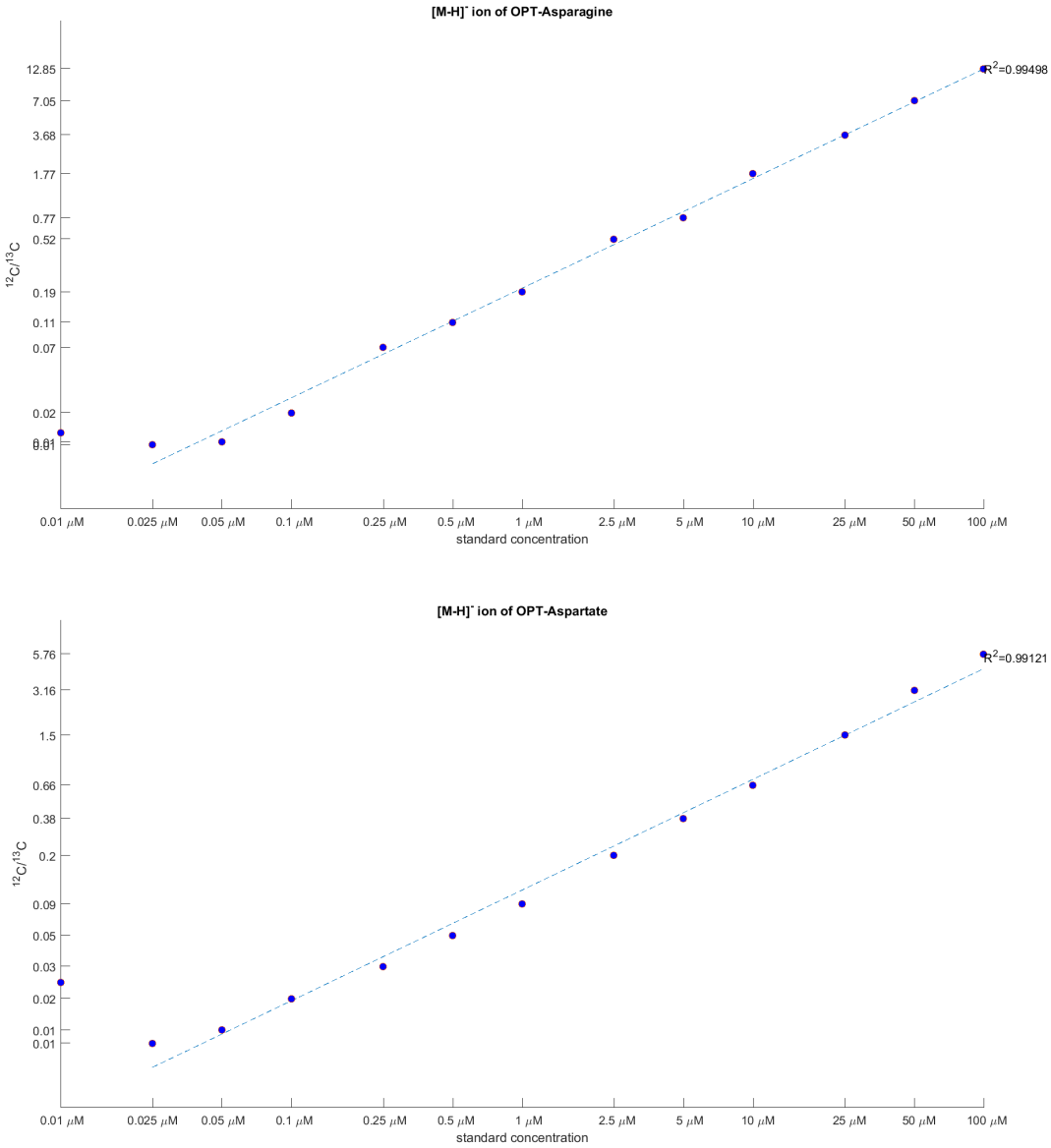


Figure S-56: Linear dynamic ranges of OPT-Asn and OPT-Asp.

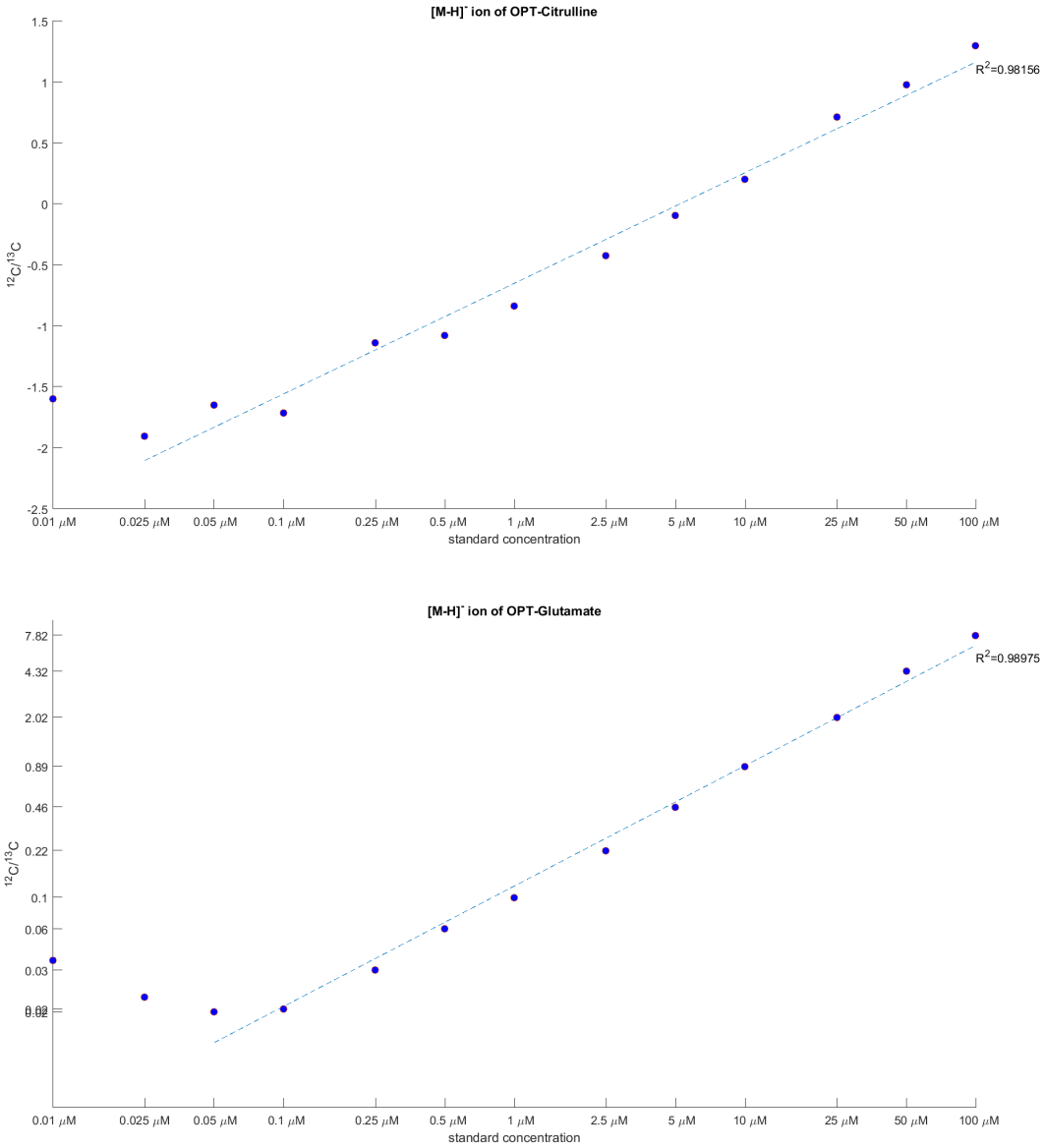


Figure S-57: Linear dynamic ranges of OPT-Citr and OPT-Glu.

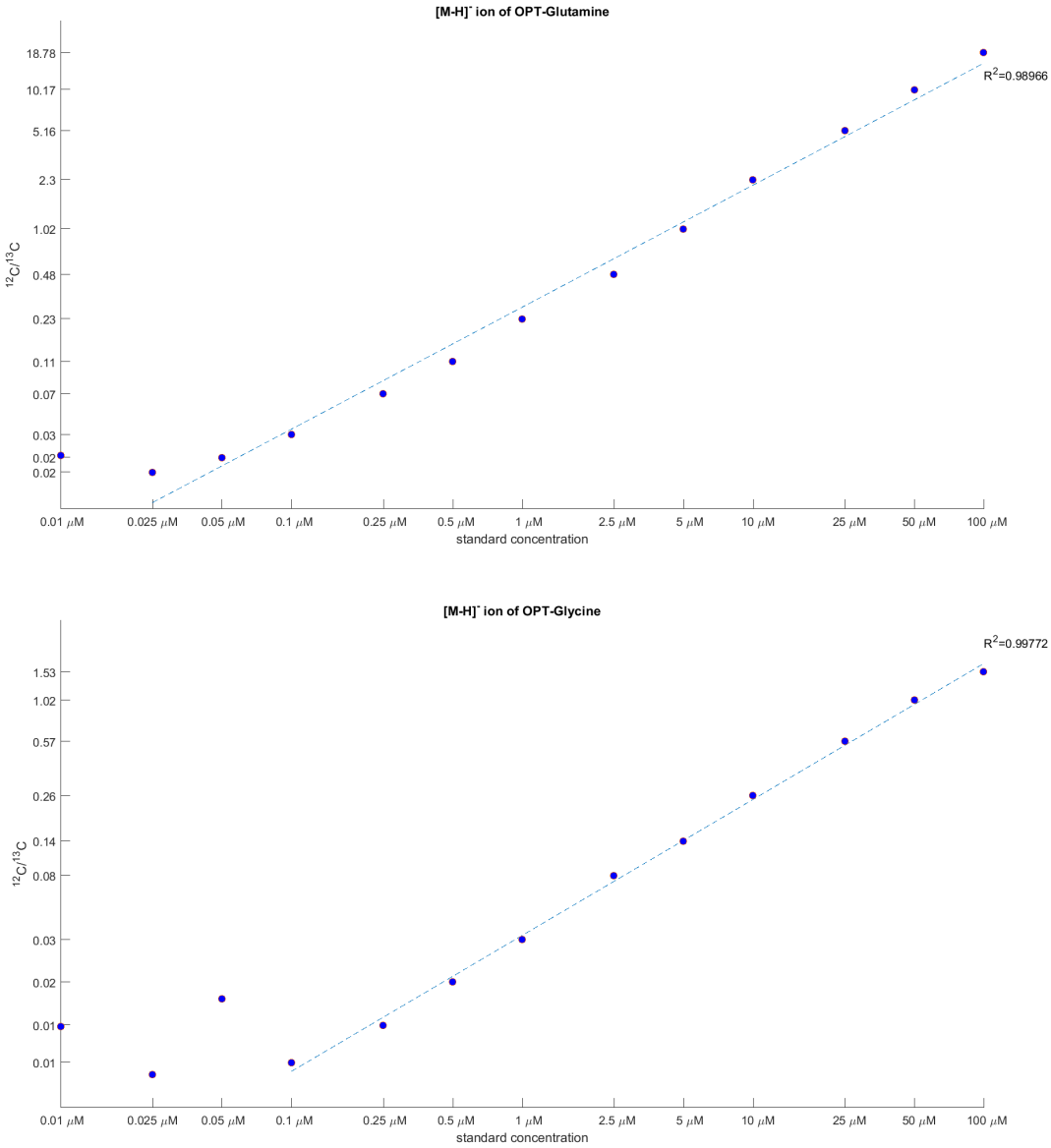


Figure S-58: Linear dynamic ranges of OPT-Gln and OPT-Gly.

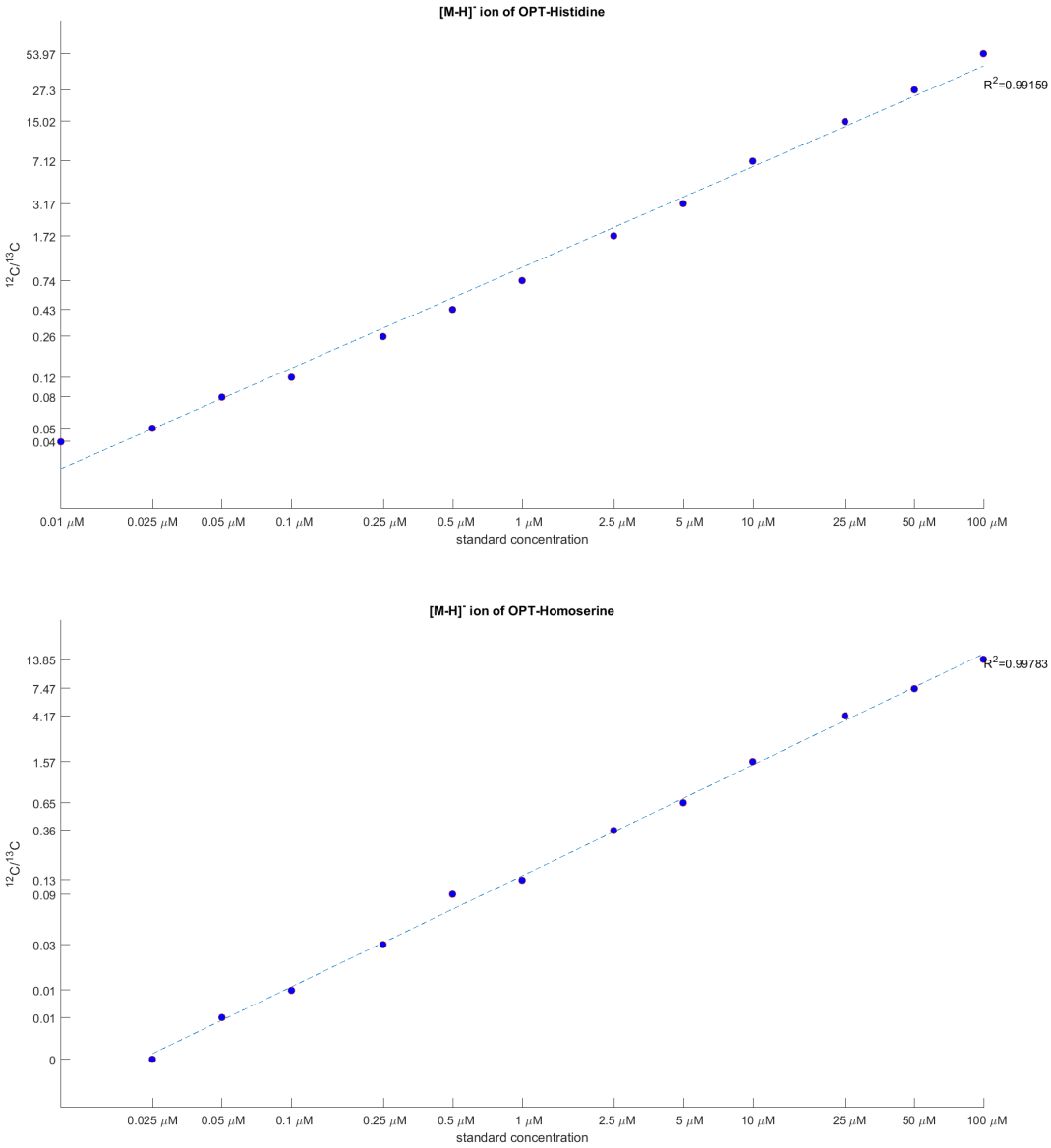


Figure S-59: Linear dynamic ranges of OPT-His and OPT-Hom.

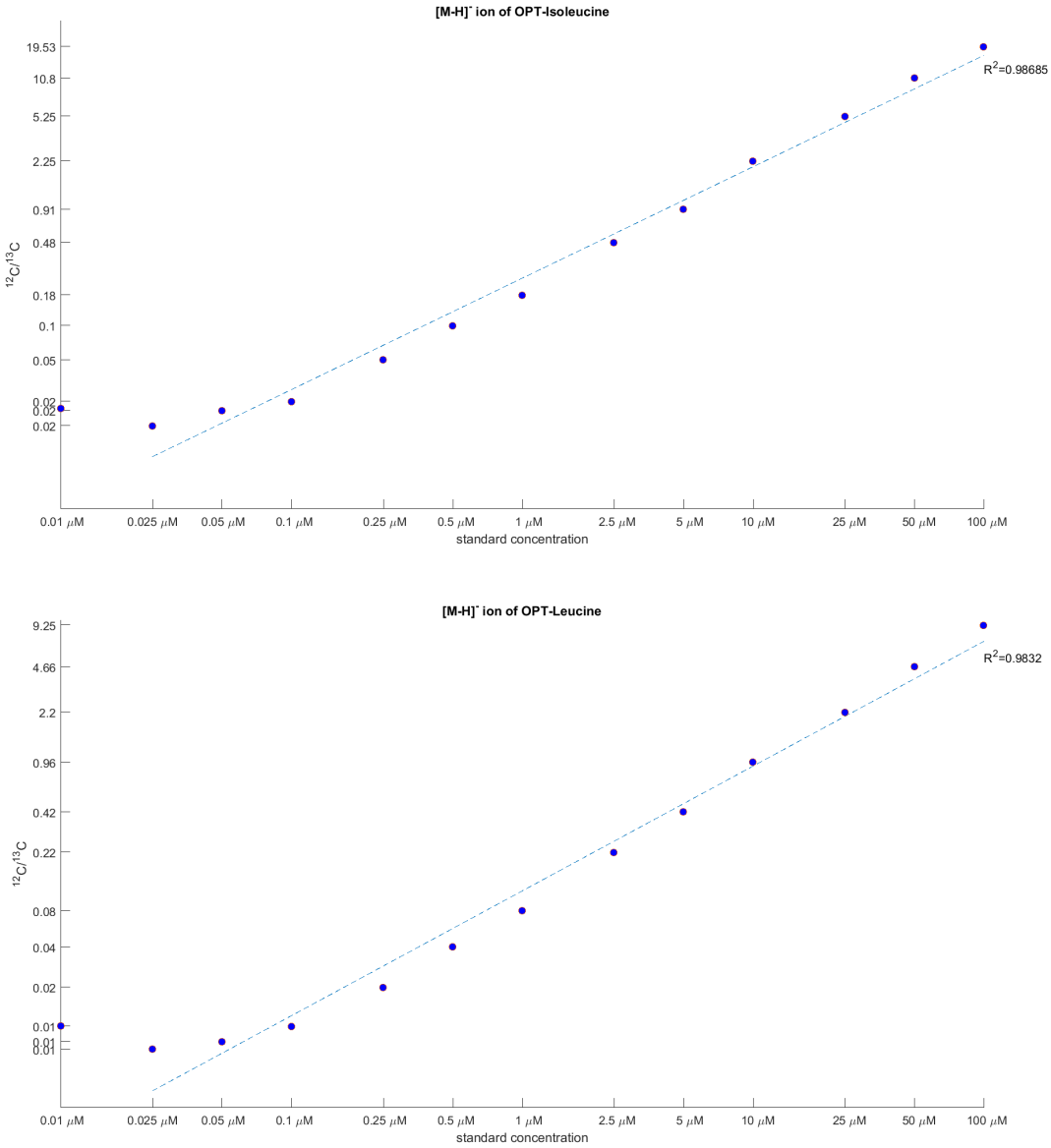


Figure S-60: Linear dynamic ranges of OPT-Ile and OPT-Leu.

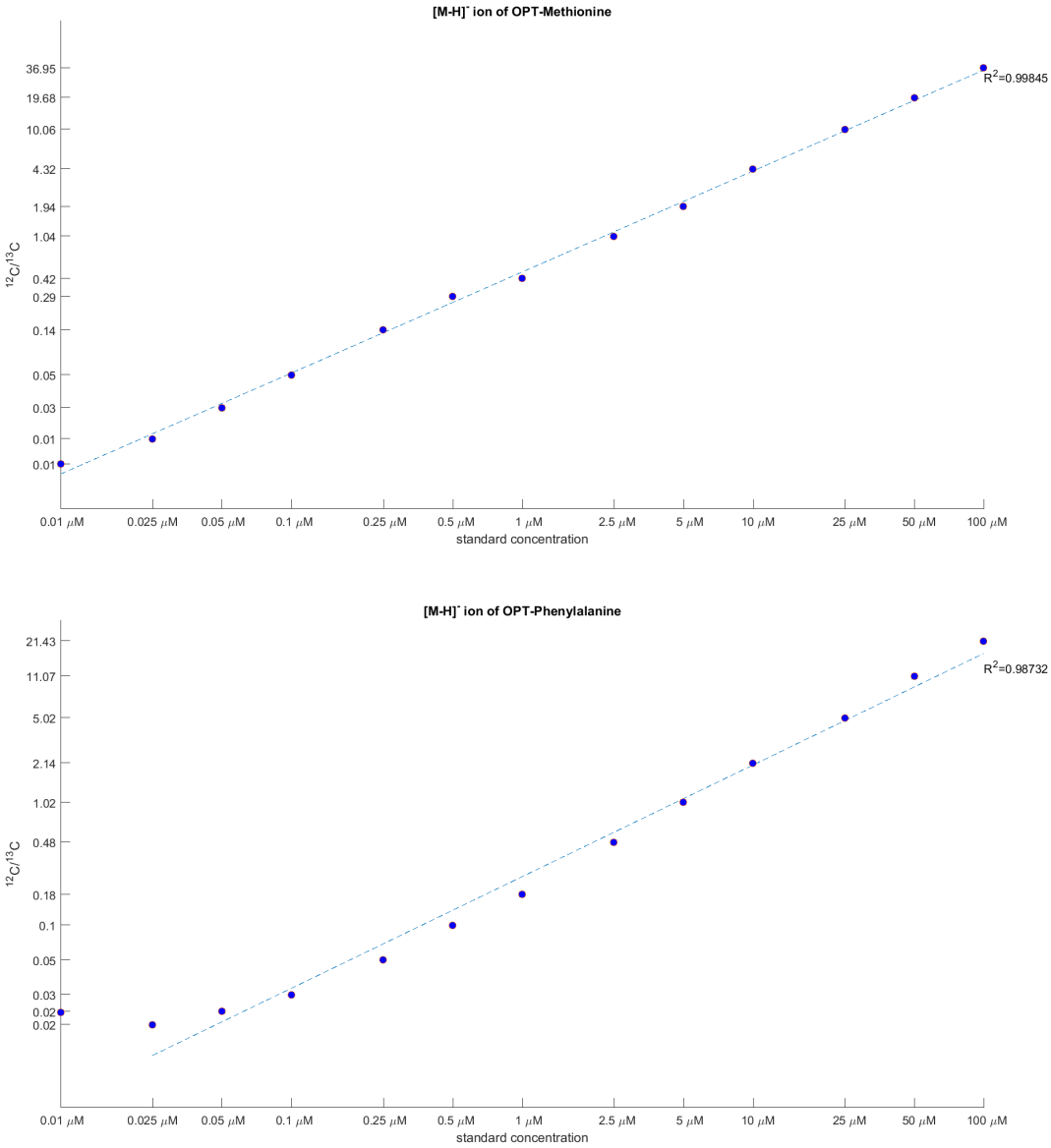


Figure S-61: Linear dynamic ranges of OPT-Met and OPT-Phe.

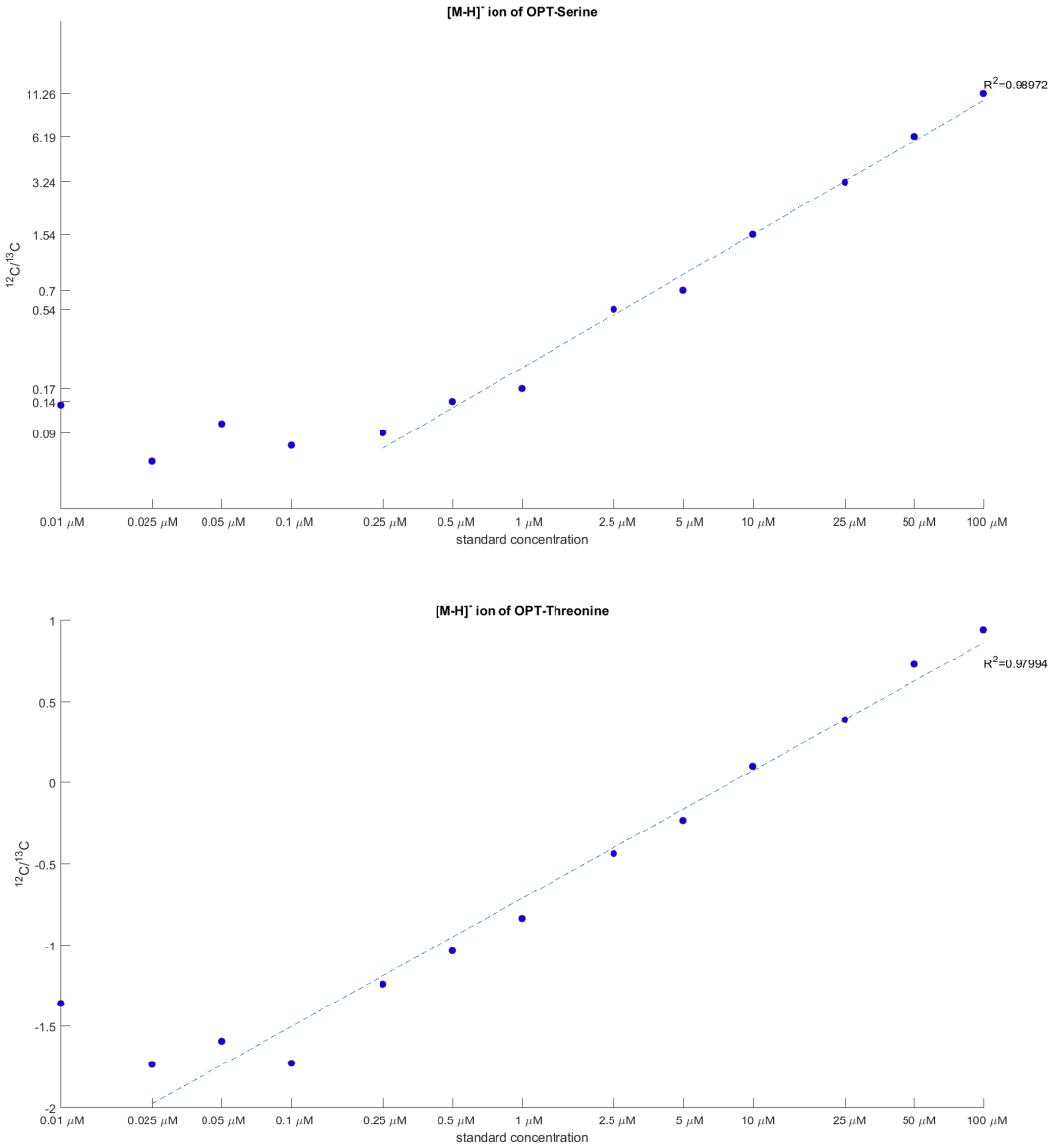


Figure S-62: Linear dynamic ranges of OPT-Ser and OPT-Thr.

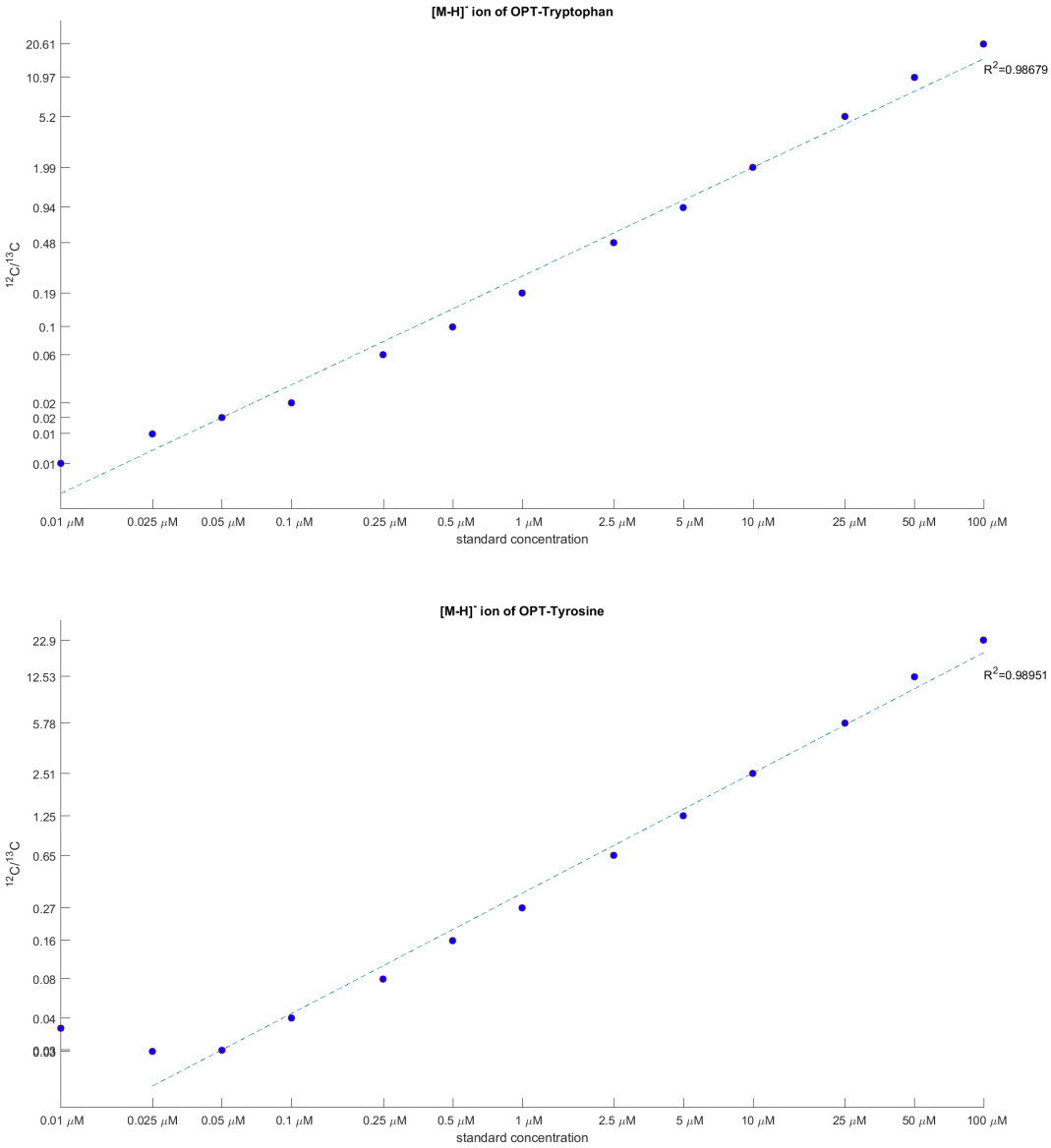


Figure S-63: Linear dynamic ranges of OPT-Trp and OPT-Tyr.

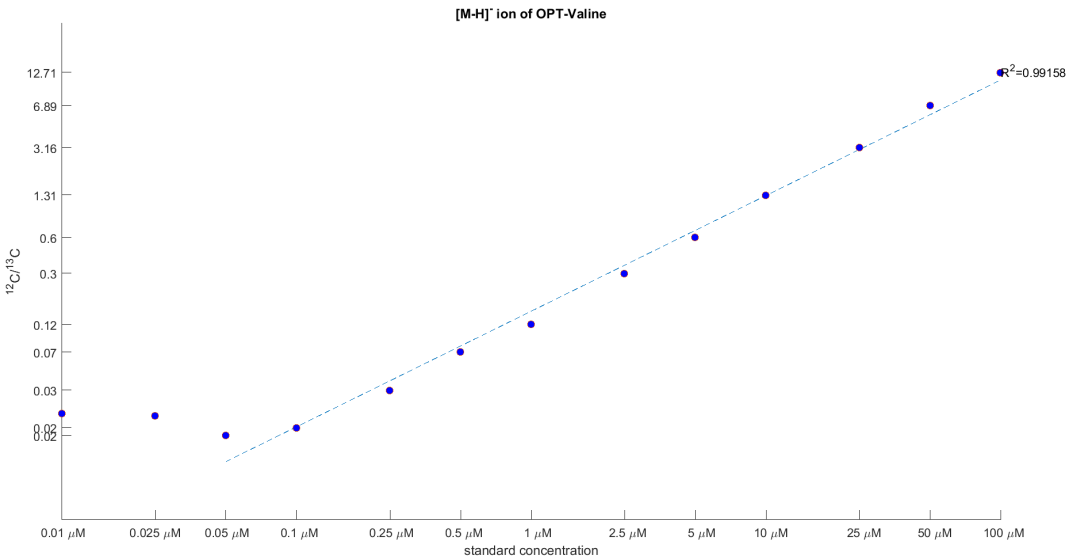


Figure S-64: Linear dynamic ranges of OPT-Val.

S9 Experiment to generate isotopic standards

Table S-8: Isotopic composition of the used carbon and nitrogen source. The standard deviation of the measurement is pooled across all measured enrichments and is therefore identical for all isotopic species.To separate the isotopes of ammonium sulfate, 50 μl of the ammonium sulfate stock solution (at a concentration equivalent to 200 g/l unlabeled ammonium sulfate) was diluted with 1.5 ml water and acidified with 20 μl of 0.2 M sulfuric acid. The NMR measurement was carried out at the analytical core facility of Forschungszentrum Jülich.

Isotope	Compound	
	mean enrichment	standard deviation
	Acetate	
^{12}C	24.48	0.671
1-^{13}C	25.61	0.671
2-^{13}C	24.83	0.671
U-^{13}C	25.07	0.671
	Ammonium sulfate	
^{14}N	50.26	0.159
^{15}N	49.74	0.159

S10 Isotope correction workflow

1. Integrate mass traces corresponding to all precursor and product ion labeling states for a given metabolite.

2. Write the raw ion trace areas for each replicate to the same text file. To denote a mass trace follow the notation of ICT. Separate the values of the replicates by commas. Specify the labeling state using M0.0, M1.0, M1.1, etc. Make no distinction between carbon and nitrogen labeling state of either fragment or precursor ion.

3. Write the chemical composition of the precursor and product ion into a text file called *chemdata.txt* (alternatively, use the reference version provided in S12). Specify the total number of labeled carbon and nitrogen atoms of the precursor and product ion as ^{13}C-labeled atoms. Specify the number of carbon atoms of the derivatization group as carbon atoms to correct for and include all atoms of elements other than carbon and nitrogen. Omit nitrogen from the correction by setting the number of nitrogen atoms to correct for to zero.

4. Call ICT (https://github.com/jungreuc/isotope_correction_toolbox) using the command line call

   - *perl <path to script>\ict.pl -c chemdata.txt -m <tof_measured_filename> -o <tof_corrected_filename>*

5. Compute the corrected tandem mass isotopomer distribution (TMID) from the corrected mass trace areas.

6. Perform averaging of the TMID over replicates.

7. To export corrected TMIDs to FluxML, skip steps 5. and 6. and instead execute the *ict2fluxml.py* script (S13) to calculate the TMIDs, determine their averages and standard deviations

   - *ict2fluxml.py <tof_corrected_filename>*

8. Copy the output to the <data>-tag of a FluxML model (e.g. the example given in S13).

S11 Measured fractional abundance in amino acids of defined label distribution

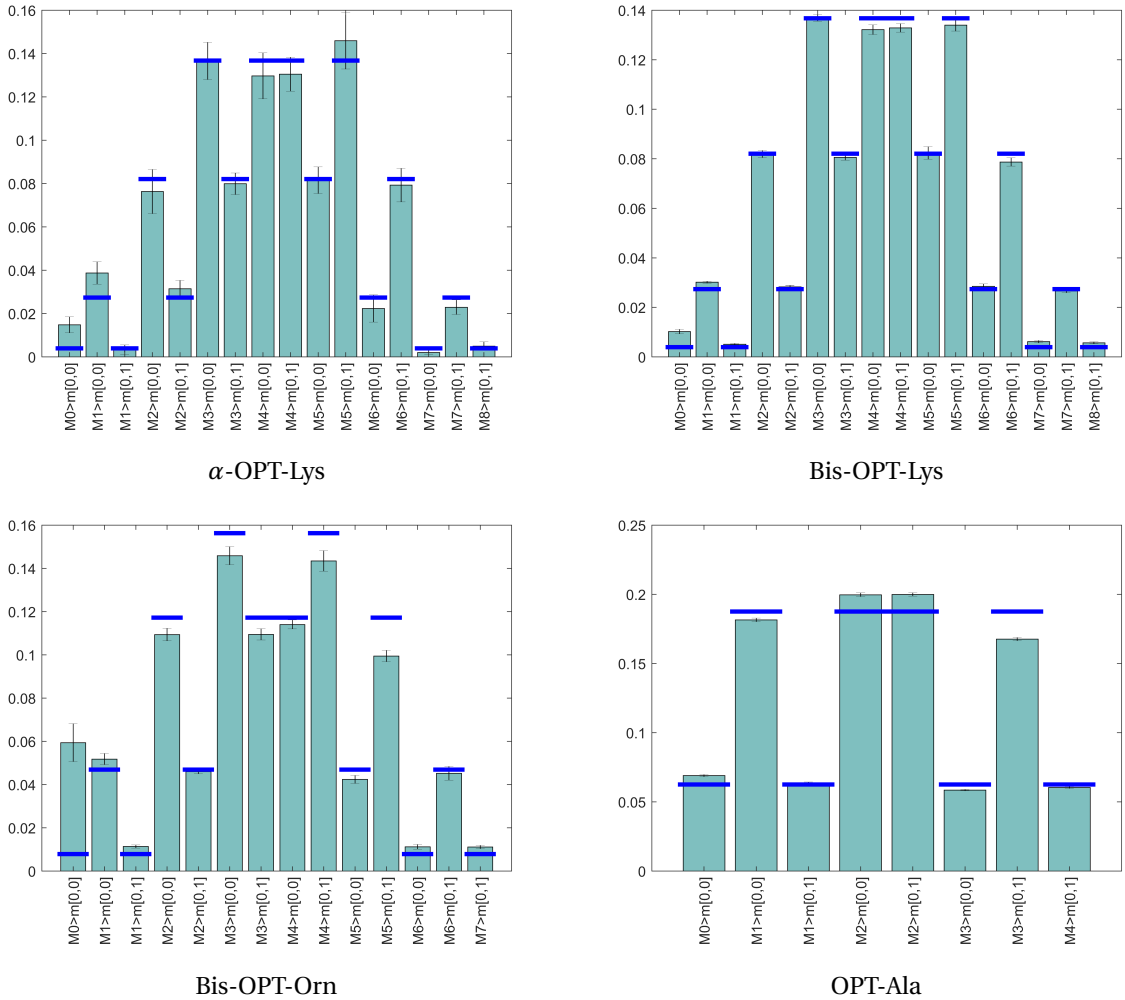
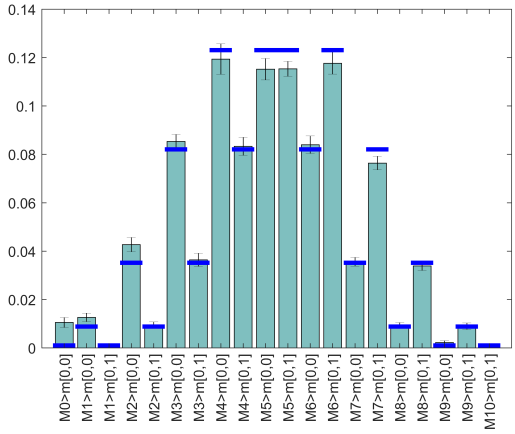
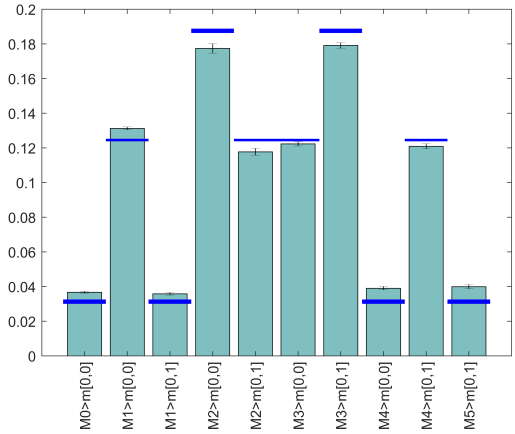


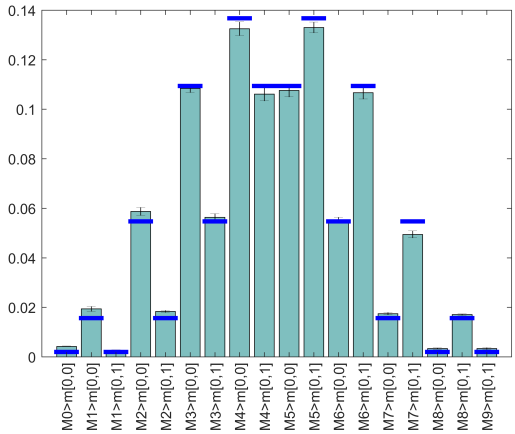
Figure S-65: Measured FA of the mass traces corresponding to labeling states of α-OPT-Lys, Bis-OPT-Lys, Bis-OPT-Orn, OPT-Ala. Blue horizontal lines indicate the expected relative mass trace intensity calculated from the binomial probability density function.



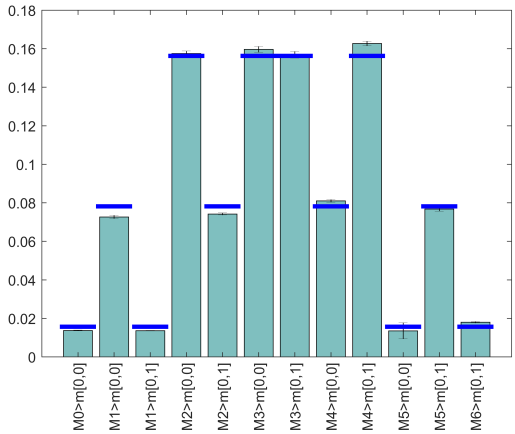
OPT-L-Arg



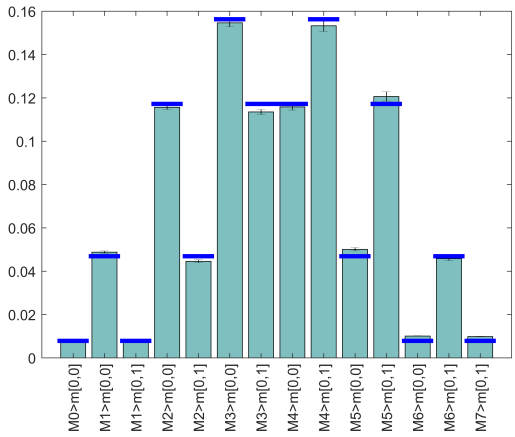
OPT-L-Asp



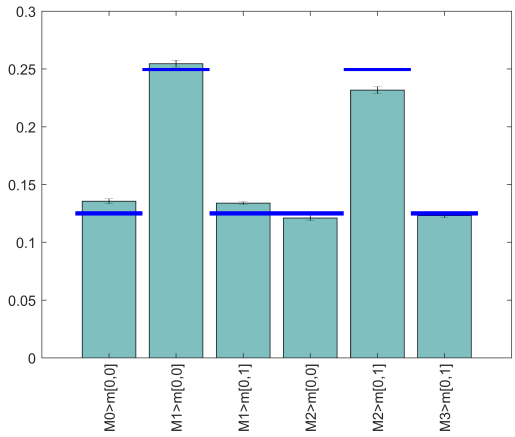
OPT-Citr



OPT-Glu

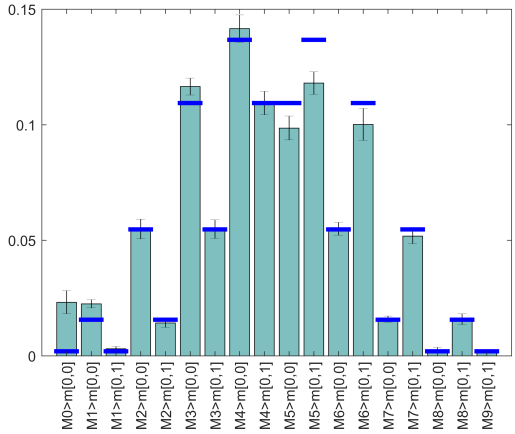


OPT-Gln

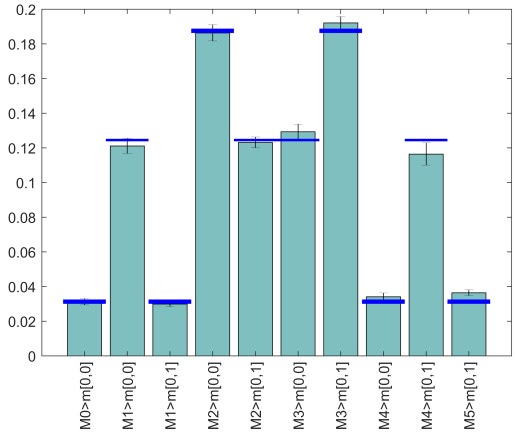


OPT-Gly

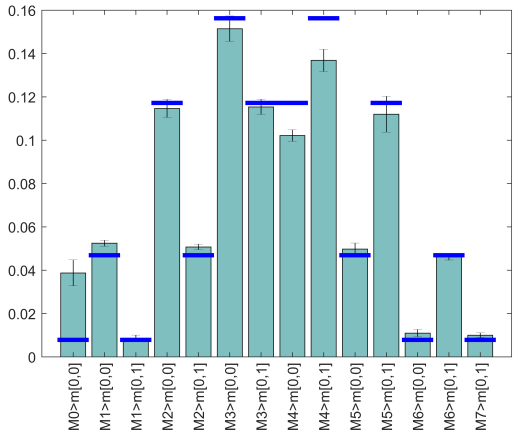
Figure S-66: Measured FA of the mass traces corresponding to labeling states of OPT-Arg, OPT-Asp, OPT-Citr, OPT-Glu, OPT-Gln, and OPT-Gly. Blue horizontal lines indicate the expected relative mass trace intensity calculated from the binomial probability density function.



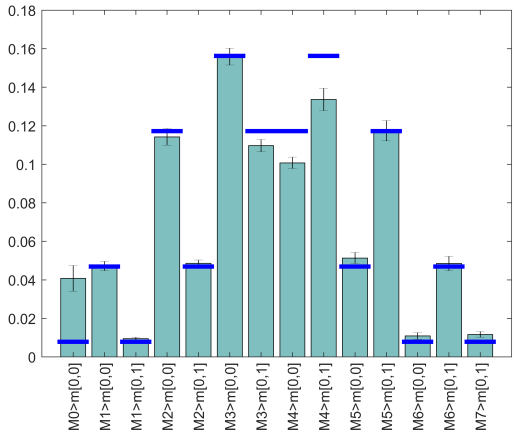
OPT-His



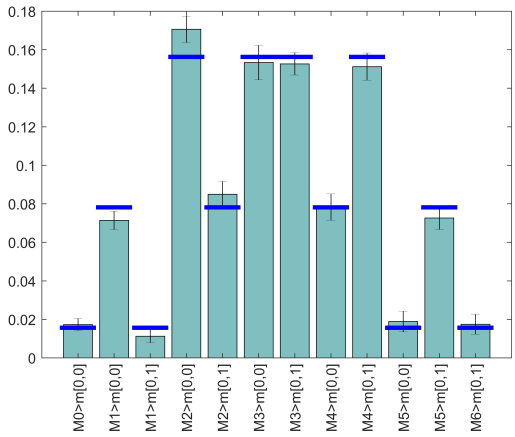
OPT-Hom



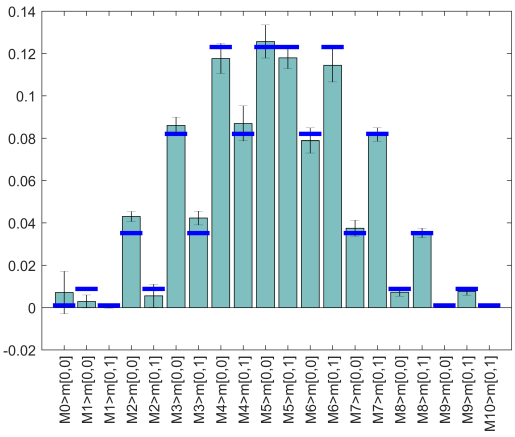
OPT-Isoleu



OPT-Leu



OPT-Met



OPT-Phe

Figure S-67: Measured FA of the mass traces corresponding to labeling states of OPT-His, OPT-Hom, OPT-Ile, OPT-Leu, OPT-Met, and OPT-Phe. Blue horizontal lines indicate the expected relative mass trace intensity calculated from the binomial probability density function.

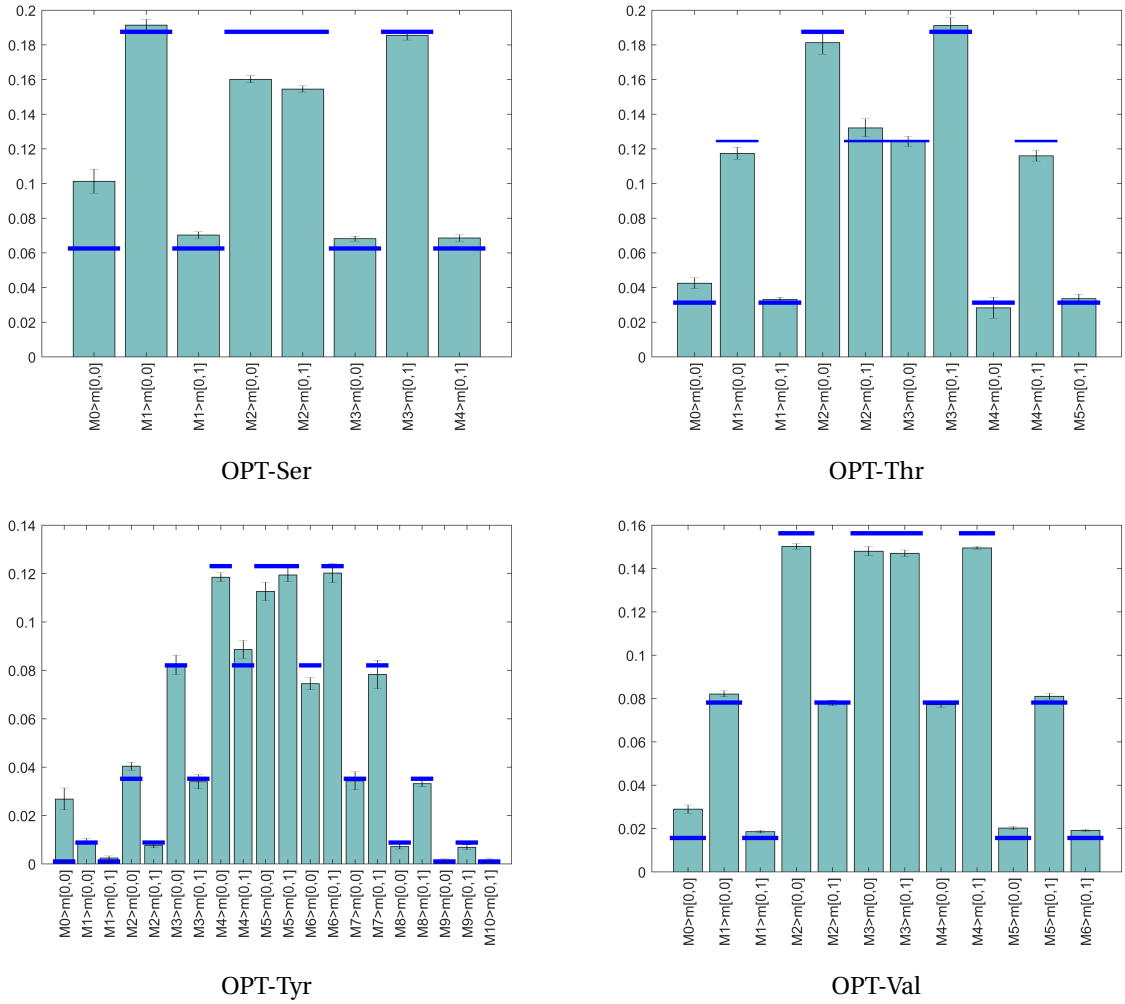


Figure S-68: Measured FA of the mass traces corresponding to labeling states of OPT-Ser, OPT-Thr, OPT-Tyr, and OPT-Val. Blue horizontal lines indicate the expected relative mass trace intensity calculated from the binomial probability density function.