Supporting Information

Formation and Occurrence of Iodinated Tyrosyl Dipeptides in Disinfected Drinking Water

Guang Huang,[†,§] Ping Jiang,[†,§] Lindsay K. Jmaiff Blackstock,[†] Dayong Tian,[†,‡] Xing-Fang Li[†,*]

[†]Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta Canada T6G 2G3

[‡]College of Chemical and Environmental Engineering, Anyang Institute of Technology, Anyang 455000, Henan, P. R. China

[*]Corresponding author: Xing-Fang Li

Phone: (780) 492-5094; fax: (780) 492-7800; e-mail: xingfang.li@ualberta.ca

[§]G.H. and P.J. contributed equally to this study.

Table of Contents

Section 1. Chloramination of Tyrosine

Because of the similarities between the structures of tyrosine and tyrosyl dipeptides, tyrosine was chloraminated with iodide to determine the possible iodinated products. We prepared the tyrosine solution by dissolving 110 μmol tyrosine in 20 mL of Optima water. The phosphate buffer was prepared by adding potassium phosphate monobasic (0.1200 g, 1 mmol) in 100 mL of Optima water, and adjusted to pH 7.0 with sodium hydroxide (1 M).

Monochloramine solution was freshly prepared prior to each experiment by adding sodium hypochlorite to an ammonium chloride solution at the desired 0.7 Cl/N molar ratio. The reaction was conducted in bicarbonate buffer (10 mM, pH 8.5), in an ice bath for 30 min, prior to use. The chloramination mixture contained 0.55 μmol tyrosine, 0.26 mg KI, and 1.10 μmol monochloramine in 100 mL of phosphate buffer (10 mM, pH7).[1,2] The reaction took place in the dark at room temperature for 24 h. Finally, the reaction was quenched by adding 0.5 mL of 50% formic acid (FA) according to our previous procedure.[3]

Solid phase extraction (SPE): The chloramination solution of tyrosine was desalted and concentrated by SPE with Waters Oasis HLB cartridges (6 mL, 200 mg per cartridge) mounted in a VISIPREP SPE manifold (Supelco, Bellefonte, PA) with flow control liners. The HLB cartridges were first rinsed twice with 6 mL of methanol containing 0.25% FA, and then rinsed twice with 6 mL of acidified water with 0.25% FA. The sample was drawn through the cartridge under vacuum at a flow rate of approximately 8 mL/min. After sample loading, the cartridge was washed twice with 6 mL of acidified water with 0.25% FA and then dried under vacuum for 10 min. The analytes on the cartridge were eluted with 10 mL of methanol with 0.25% FA, and then the methanol extract was divided into two equal portions. One portion was analyzed using the high resolution quadrupole time-of-flight (X500R QTOF System; Sciex, Concord, Ontario, Canada) mass spectrometer with direct infusion. The remaining 5 mL of methanol extract were evaporated down to 100 μL under a gentle nitrogen stream, and then reconstituted with Optima water to a volume of 1100 μL water/methanol (v/v 10/1). After brief sonication and vortexing, a 20-μL aliquot of the reconstituted SPE extract (1100 μL) was diluted with Optima water to 1000

μL. Finally, a 25-μL aliquot of the 1000 μL solution was further diluted with Optima water to a volume of 1000 μL prior to the analysis by the HPLC-MS/MS (MRM) method (Sciex QTRAP 5500), described in the manuscript.

Figure S1(a) shows a full scan high resolution mass spectrum of the extract of the reaction mixture of tyrosine (0.55 μmol), sodium iodide (2.0 mg/L), and monochloramine (1.10 μmol) after reaction in the dark at room temperature for 24 h. The peak of *m/z* 182.0811 corresponds to the protonated molecular ion ($[M+H]^+$) of tyrosine with mass accuracy of 0.4 ppm. A pair of new peaks, *m/z* 307.9771 and *m/z* 433.8736, differing from tyrosine by ~125.9 and 251.8 Da, match with $[M+H]^+$ of mono-iodinated and di-iodinated tyrosine (theoretical mass *m/z* 307.9778 and 433.8745), respectively. Based on the experimental accurate masses, the Sciex OS software predicted molecular formulas (**Table S4**), which matched with monoiodinated tyrosine and di-iodinated tyrosine, respectively.

Figure S1(b-c) are the extracted ion current chromatograms (EICs) of (b) tyrosine, (c) 3-I-tyrosine, and (d) 3,5-di-I-tyrosine from the chloramination solution of tyrosine and the standards. The retention time, paired MRM transitions, and the intensity ratio of the paired MRM transitions of monoiodinated tyrosine (**Figure S1(b)**) are the same as those of the 3-I-tyrosine standard (**Figure S1(c)**), which demonstrates that the monoiodinated tyrosine detected in the tyrosine chloramination solution is 3-I-tyrosine. Similarly, the di-iodinated tyrosine formed after chloramination of tyrosine was identified as 3,5-di-I-tyrosine (**Figure S1(d)**). Furthermore, the MS/MS spectrum of monoiodinated tyrosine (*m/z* 307.9771) (**Figure S2(a)**) shows the characteristic fragment (*m/z* 244.9615) that corresponds with the moiety of tyrosine's aromatic group containing one iodine atom. Similarly, the fragments (*m/z* 232.9454 and 387.8683) of di-iodinated tyrosine (*m/z* 433.8736) are characteristic peaks corresponding to moieties of two iodine atoms on the aromatic ring. Details for the fragments of iodinated tyrosine are summarized in **Table S4.**

Section 2. Ascorbic Acid Experiment for the Identification of Chlorinated Products

The standards of chlorinated dipeptides are not commercially available and they are difficult to

be purified from the reaction mixture. Thus, we sought a different way to elucidate the structures of chlorinated products.[4] Ascorbic acid can selectively reduce an *N*-Cl-peptide back to a peptide, but will not affect Cl-substitutions on the aromatic ring of the peptides. Two sets of seven dipeptides (0.1 mg each, total dipeptides, 2.5 μmol), 0.26 mg KI were mixed in 100 mL of phosphate buffer (10 mM, pH=7). Freshly prepared monochloramine (5.0 μmol) was added into each of the dipeptide mixture solutions at a molar ratio of monochloramine/total dipeptides of 2/1. After the reaction took place in the dark at room temperature for 24 h, one reaction solution was quenched by 0.5 mL of 50% FA. The other solution was quenched by 10.2 mg (i.e., 57.8 μmol) of ascorbic acid (VC, Vitamin C). Ten minutes later, 0.5 mL of 50% FA was added to the ascorbic-acid–quenched solution prior to SPE treatment. Finally, both solutions were extracted, diluted, and analyzed using the same SPE-HPLC-MS/MS-MRM method as described in **SI Section 1**. The results of chlorinated tyrosyl dipeptides quenched with FA or ascorbic acid are shown in **Figure S9-S15** and discussed in the main manuscript.

Section 3. Recovery of the Seven Tyrosyl Dipeptides and 3-I-/3,5-di-I-Tyr-Ala

The recovery of tyrosyl dipeptides was evaluated at concentrations of 30 nM and 100 nM in Optima water. Each dipeptide at 60 nmol and 200 nmol was separately dissolved in 2 L of Optima water containing 10 mL of 50% FA. Next, a 1-mL aliquot was set aside and the remaining solution was extracted using the same SPE procedures described above. The resulting methanol extract was evaporated down to 100 μL under a gentle stream of nitrogen and then reconstituted with Optima water to a final volume of 1100 μL. A 30-μL aliquot from the 1100 μL extract was then diluted with Optima water to a total volume of 900 μL. Finally, for the recovery analysis, a 100-μL aliquot from the 900 μL solution was further diluted with Optima water to 1000 μL. The final diluted extract and the 1-mL aliquot collected before SPE were separately analyzed by HPLC-MS/MS. The following recoveries were determined at 30 nM: Tyr-Ala (8%), Tyr-Gly (5%), Tyr-Val (5%); and at 100 nM: Tyr-Ala (56%), Tyr-Gly (14%), Tyr-Val (30%).

To evaluate the recovery of iodinated dipeptides, two commercially available standards, 3-I-Tyr-Ala and 3,5-di-I-Tyr-Ala, were examined at two concentrations. Two solutions of 3-I-Tyr-Ala at 0.2 nM and 1.6 nM and two solutions of 3,5-di-I-Tyr-Ala at 0.23 nM and 1.8 nM were prepared each in 2 L Optima water with 0.25% FA. The procedures for recovery workflow and analysis of these standard solutions including sample preparation and extraction dilution were the same as for the tyrosyl dipeptides, described above. The final diluted extract and the 1-mL aliquot collected before SPE were separately analyzed by HPLC-MS/MS. The following recoveries were determined for 3-I-Tyr-Ala: at 0.2 nM (10%) and 1.6 nM (10%), and for 3,5-di-I-Tyr-Ala: at 0.23 nM (84.5%) and 1.8 nM (92.5%).

This study demonstrates that the recovery of the dipeptides was low. We will develop a new extraction method to improve the recovery for future surveillance studies.

Section 4. Synthesis of Iodinated Peptides for the Stability Test

Seven dipeptides, Tyr-Ala, Tyr-Gly, Tyr-Val, Tyr-His, Tyr-Gln, Tyr-Glu, and Tyr-Phe (0.1 mg each; total dipeptides 2.5 μmol), were mixed and dissolved in 100 mL phosphate buffer (10 mM, pH=7.0) spiked with 2.0 mg/L KI. Excess monochloramine (5.0 μmol, freshly prepared) was used in the peptide reaction mixture at a molar ratio of monochloramine/total dipeptides of 2/1. After 24 h of chloramination in the dark at room temperature, 0.5 mL of 50% FA was added to quench the reaction. The iodinated peptides generated were extracted using the same SPE procedures described above. The methanol extract was evaporated down to 100 μL under a gentle stream of nitrogen, and then reconstituted with Optima water to a total volume of 1100 μL. After brief sonication and vortexing, a 30-μL aliquot of each diluted extract was further diluted with Optima water to a volume of 1100 μL. Finally a 25-μL aliquot of each 1100 μL solution was diluted with Optima water to 1000 μL. Phe-Gly (1 μg/L) was added to the final sample prior to the HPLC-MS/MS analysis in MRM mode (Sciex QTRAP 5500) as an internal standard for peak area normalization. The sample was maintained at 4 ^{o}C in the autosampler during analysis.

Section 5. Residual Tyrosyl Dipeptides and the Yields of 3-I-/3,5-di-I-Tyr-Ala from Chloramination

To measure the yields of iodinated products from the chloramination of tyrosyl dipeptides, we quantified 3-I-/3,5-di-I-Tyr-Ala using commercially available standards. We used HPLC-MS/MS to measure the peak areas from standard solutions of tyrosyl dipeptides at 0.3, 0.5, 1, 2, 5, and 10 μg/L; of 3-I-Tyr-Ala at 0.3, 0.5, 1, 2, 5, and 10 μg/L; and of 3,5-di-I-Tyr-Ala at 0.4, 0.67, 1.33, 2.67, 6.67, and 13.33 μg/L. The calibration curves of tyrosyl dipeptides and 3-I-/3,5-di-I-Tyr-Ala were obtained based on the peak area versus concentration.

Based on the calibration curves, we determined the concentrations of tyrosyl dipeptides and 3-I-/3,5-di-I-Tyr-Ala in the samples used for the stability test in **SI Section 4**. The concentrations of 3-I-Tyr-Ala and 3,5-di-I-Tyr-Ala were determined to be 8.89 and 10.2 μg/L, respectively. The remaining dipeptides in the chloramination solution were Tyr-Gly 7.7%, Tyr-Ala 2.9%, Tyr-Glu 9.7%, Tyr-Phe 38.5%, Tyr-His 0.2%, Tyr-Val 18.9%, Tyr-Gln 5.8%. The yields of 3-I-Tyr-Ala and 3,5-di-I-Tyr-Ala were 13.2% and 11.3%, respectively, when Tyr-Ala (0.1 mg) was chloraminated in the presence of KI (2 mg/L).

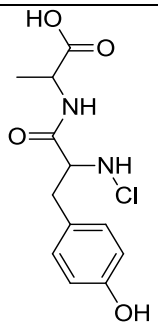
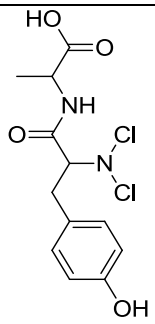
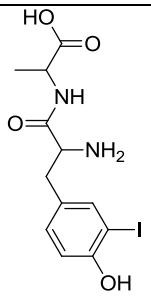
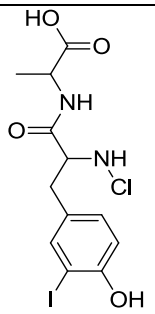
We also evaluated the residual dipeptides and their conversion to iodinated dipeptides in the reaction of low concentration of dipeptides chloramination (each 0.02 mg, in total 488 nmol) in the presence of a low concentration of KI (100 μg/L). Other conditions were kept the same as those described above. The remaining dipeptides in the chloramination solution were Tyr-Gly 24%, Tyr-Ala 5.6%, Tyr-Glu 27.2%, Tyr-Phe 48.9%, Tyr-His 0.7%, Tyr-Val 33.4%, Tyr-Gln 14.4%. The yields of 3-I-Tyr-Ala and 3,5-di-I-Tyr-Ala were 5.3% and 0.9%, respectively. These results demonstrated that iodinated dipeptides were produced with lower conversion yield from dipeptides to iodinated dipeptides, when dipeptides and iodide were at lower concentrations.

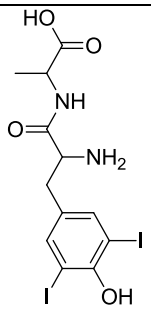
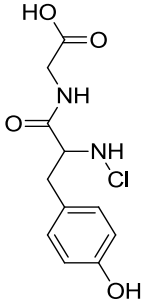
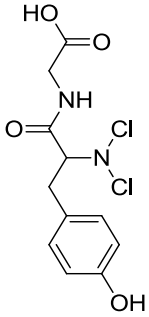
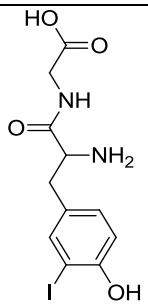
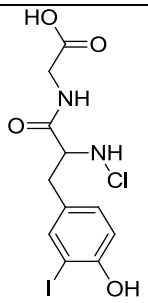
Table S1. MRM parameters for LC-MS/MS analysis of dipeptides and chlorinated dipeptides (positive ESI mode)

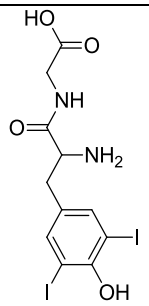
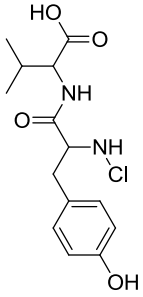
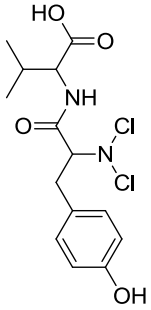
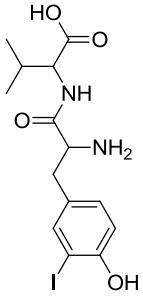
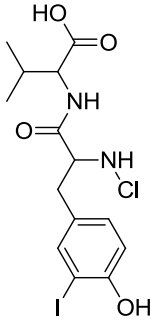
Compounds	Molecular ion	Product ion	DP (V)	EP (V)	CE (V)	CXP (V)
Tyrosine	182.1	123.0	81.2	8.5	23.9	17.8
		119.0	81.5	11.8	26.7	18.9
		136.0	81.1	11.3	17.9	18.6
3-I-tyrosine	308.0	262.0	67.3	12.7	23.4	27.2
		135.0	72.3	11.8	36.2	17.3
		291.0	75.3	11.6	16.1	30.0
3,5-di-I-tyrosine	433.9	387.9	80.9	11.4	25.5	35.7
		290.0	73.4	11.4	28.4	28.5
		261.0	72.6	11.0	41.5	30.0
Tyr-Ala	253.2	135.7	75.0	10	24.0	18.0
		119.1	75.0	10	36.0	18.0
3-I-Tyr-Ala	379.0	262.0	77.4	10	25.4	7.7
		135.0	79.1	9	44.3	12.0
3,5-di-I-Tyr-Ala	504.9	387.9	68.6	7.3	28.5	11.03
		261.0	81.7	8.0	47.7	24.5
		107.0	90.0	8.3	72.8	11.4
Tyr-Gly	239.1	136.1	56	10	21.0	12.0
		107.0	56	10	57.8	10.4
3-I-Tyr-Gly	365.0	262.0	78.8	11.9	23.3	7.3
		135.0	78.8	11.9	42.4	11.1
3,5-di-I-Tyr-Gly	490.9	387.9	75.2	6.9	29.8	11.4
		107.0	80.3	9.4	79.1	12.9
		261.0	75.0	7.5	46.7	22.2
Tyr-Val	280.8	135.7	139.0	10.0	26.0	18.0
		118.9	64.0	10.0	40.0	18.0
3-I-Tyr-Val	407.0	262.0	81.7	11.5	27.3	7.2
		72.0	77.5	8.8	48.1	8.4
		135.0	89.5	8.6	47.1	10.8
3,5-di-I-Tyr-Val	533.0	387.9	99.4	8.5	30.8	10.6
		261.0	97.6	7.7	52.2	24.7
		72.0	98.4	8.9	59.3	7.9
Tyr-His	319.1	110.1	228.0	10.0	32.0	14.0
		156.0	90.0	10.0	25.0	21.0
3-I-Tyr-His	445.1	110.0	134.5	11.0	51.7	9.7
		136.0	80.9	11.0	39.1	11.4
		107.0	80.9	11.0	102.5	12.3
3,5-di-I-Tyr-His	571.0	110.0	111.3	6.6	65.3	9.6
		107.0	96.4	5.5	82.9	11.3
		552.9	114.2	7.0	25.2	15.5
Tyr-Gln	310.1	136.0	80.0	10.0	30.0	18.0
		147.3	75.0	10.0	24.0	14.0
3-I-Tyr-Gln	436.1	262.0	143.2	12	31.6	7.2
		130.0	143.2	12	38.5	11.7
3,5-di-I-Tyr-Gln	562.0	387.9	143.4	8.9	35.6	11.8
		130.0	122.4	6.2	48.1	14.3
		107.0	77.7	5.8	91.2	14.3
Tyr-Glu	311.1	136.0	75.0	10.0	27.0	13.0
		147.9	75.0	10.0	21.0	14.0

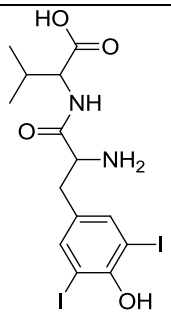
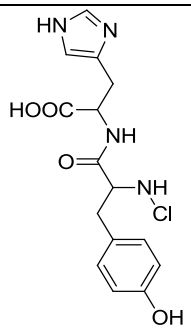
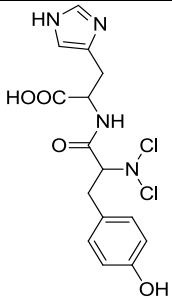
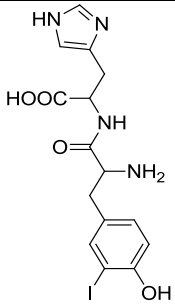
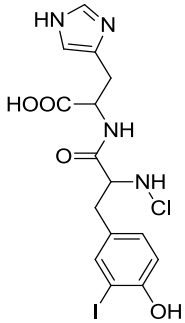
3-I-Tyr-Glu	437.1	262.0	136.5	12	30.4	7.3
		130.0	136.5	12	33.6	11.7
3,5-di-I-Tyr-Glu	563.0	387.9	72.4	5.9	34.1	12.1
		130.0	54.9	5.0	43.3	13.1
		107.0	77.9	4.4	86.9	11.6
Tyr-Phe	329.6	135.6	75.0	10.0	25.0	18.0
		119.1	75.0	10.0	32.0	18.0
3-I-Tyr-Phe	455.1	262.0	96.4	12.5	28.2	24.1
		120.1	94.2	9.9	52.4	11.4
3,5-di-I-Tyr-Phe	581.0	387.9	77.0	2.7	34.2	11.3
		120.0	98.2	3.4	59.4	10.6
		107.0	74.5	4.5	83.3	13.2

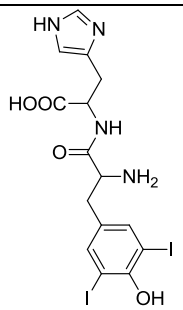
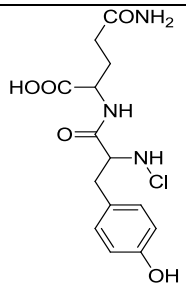
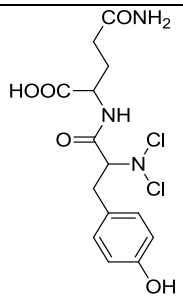
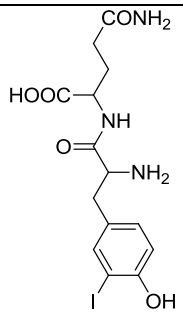
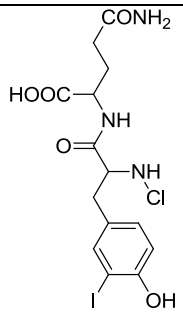
Table S2. Summary of 35 halogenated tyrosyl dipeptide byproducts detected after chloramination of seven tyrosyl dipeptides in the presence of iodide

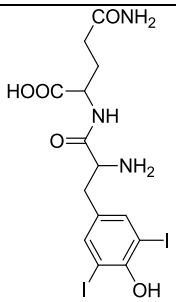
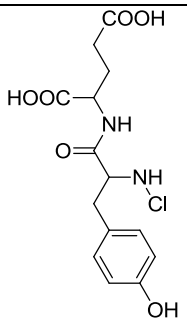
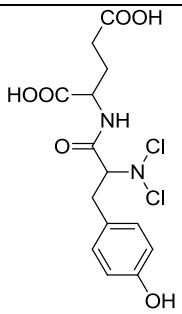
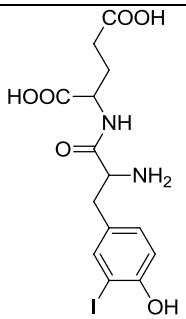
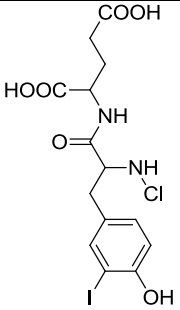
Compound	Structure	Theoretical $[M+H]^+$	Determined $[M+H]^+$	Formula	Δm (ppm)	S/N	Peak intensity
N-Cl-Tyr-Ala		287.0793	287.0791	$C_{12}H_{15}ClN_2O_4$	-0.7	71	133
N,N-di-Cl-Tyr-Ala		321.0403	321.0402	$C_{12}H_{14}Cl_2N_2O_4$	-0.3	28	50
3-I-Tyr-Ala		379.0149	379.0150	$C_{12}H_{15}IN_2O_4$	0.3	3453	6215
N-Cl-3-I-Tyr-Ala		412.9760	412.9764	$C_{12}H_{14}ClIN_2O_4$	1.0	36	64

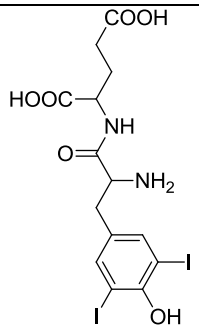
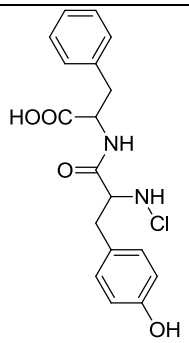
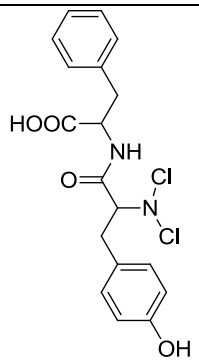
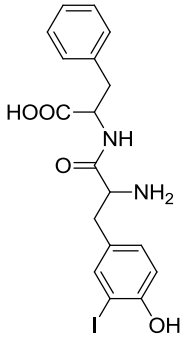
3,5-di-I-Tyr-Ala		504.9116	504.9119	$C_{12}H_{14}I_2N_2O_4$	0.6	4792	8625
N-Cl-Tyr-Gly		273.0637	273.0634	$C_{11}H_{13}ClN_2O_4$	-1.1	46	82
N,N-di-Cl-Tyr-Gly		307.0247	307.0244	$C_{11}H_{12}Cl_2N_2O_4$	-1.0	67	120
3-I-Tyr-Gly		364.9993	364.9993	$C_{11}H_{13}IN_2O_4$	0	2354	4238
N-Cl-3-I-Tyr-Gly		398.9603	398.9605	$C_{11}H_{12}ClIN_2O_4$	0.5	333	600

3,5-di-I-Tyr-Gly		490.8959	490.8964	$C_{11}H_{12}I_2N_2O_4$	1.0	3328	5991
N-Cl-Tyr-Val		315.1106	315.1105	$C_{14}H_{19}ClN_2O_4$	-0.3	19	34
N,N-di-Cl-Tyr-Val		349.0716	349.0717	$C_{14}H_{18}Cl_2N_2O_4$	0.3	29	53
3-I-Tyr-Val		407.0462	407.0464	$C_{14}H_{19}IN_2O_4$	0.5	6048	10886
N-Cl-3-I-Tyr-Val		441.0073	441.0076	$C_{14}H_{18}ClIN_2O_4$	0.7	48	87

3,5-di-I-Tyr-Val		532.9429	532.9435	$C_{14}H_{18}I_2N_2O_4$	1.1	6437	11586
N-Cl-Tyr-His		353.1011	353.1009	$C_{15}H_{17}ClN_4O_4$	-0.3	29	53
N,N-di-Cl-Tyr-His		387.0621	387.0621	$C_{15}H_{16}Cl_2N_4O_4$	0	39	70
3-I-Tyr-His		445.0367	445.0378	$C_{15}H_{17}IN_4O_4$	2.5	49	88
N-Cl-3-I-Tyr-His		478.9978	478.9982	$C_{15}H_{16}ClIN_4O_4$	0.8	6	10

3,5-di-I-Tyr-His		570.9334	570.9326	$C_{15}H_{16}I_2N_4O_4$	-1.4	3281	5905
N-Cl-Tyr-Gln		344.1008	344.1016	$C_{14}H_{18}ClN_3O_5$	2.3	6	10
N,N-di-Cl-Tyr-Gln		378.0618	378.0620	$C_{14}H_{17}Cl_2N_3O_5$	0.5	57	103
3-I-Tyr-Gln		436.0364	436.0367	$C_{14}H_{18}IN_3O_5$	0.7	567	1021
N-Cl-3-I-Tyr-Gln		469.9973	469.9977	$C_{14}H_{17}ClIN_3O_5$	0.9	8	14

3,5-di-I-Tyr-Gln		561.9331	561.9331	$C_{14}H_{17}I_2N_3O_5$	0	1887	3396
N-Cl-Tyr-Glu		345.0848	345.0846	$C_{14}H_{17}ClN_2O_6$	-0.6	16	28
N,N-di-Cl-Tyr-Glu		379.0458	379.0458	$C_{14}H_{16}Cl_2N_2O_6$	0	131	235
3-I-Tyr-Glu		437.0204	437.0204	$C_{14}H_{17}IN_2O_6$	0	2229	4013
N-Cl-3-I-Tyr-Glu		470.9814	470.9814	$C_{14}H_{16}ClIN_2O_6$	0	10	17.5

3,5-di-I-Tyr-Glu		562.9171	562.9186	$C_{14}H_{16}I_2N_2O_6$	2.7	4728	8511
N-Cl-Tyr-Phe		363.1106	363.1106	$C_{18}H_{19}ClN_2O_4$	0	52	93.5
N,N-di-Cl-Tyr-Phe		397.0716	397.0716	$C_{18}H_{18}Cl_2N_2O_4$	0	23	42
3-I-Tyr-Phe		455.0462	455.0463	$C_{18}H_{19}IN_2O_4$	0.2	3917	7051

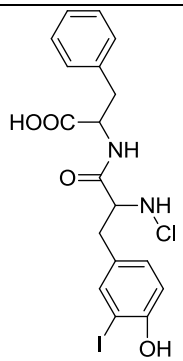
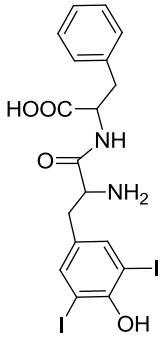
N-Cl-3-I-Tyr-Phe		489.0073	489.0071	$C_{18}H_{18}ClIN_2O_4$	-0.4	6	11
3,5-di-I-Tyr-Phe		580.9429	580.9439	$C_{18}H_{18}I_2N_2O_4$	1.7	2381	4285

Table S3. Measured accurate m/z, molecular formula, mass accuracy (Δm, ppm), and MS/MS fragments of iodinated Tyr-Ala

Compound	Full-scan			MS/MS		
	m/z	Formula	Δm (ppm)	m/z	Formula	Δm (ppm)
N-Cl-3-I-Tyr-Ala	412.9764	$[C_{12}H_{15}ClIN_2O_4]^+$	2.7	366.9694	$[C_{11}H_{13}ClIN_2O_2]^+$	2.9
				361.9875	$[C_{12}H_{13}INO_4]^+$	2.4
				330.9926	$[C_{11}H_{12}IN_2O_2]^+$	3.6
				295.9326	$[C_8H_8ClINO]^+$	2.5
				289.9666	$[C_9H_9INO_2]^+$	2.4
				272.9402	$[C_9H_6IO_2]^+$	1.8
				232.9451	$[C_7H_6IO]^+$	3.0
				88.0393	$[C_3H_6NO_2]^+$	3.4
				44.0492	$[C_2H_6N]^+$	5.4
3-I-Tyr-Ala	379.0134	$[C_{12}H_{16}IN_2O_4]^+$	4.0	361.9867	$[C_{12}H_{13}INO_4]^+$	4.6
				315.9820	$[C_{11}H_{11}INO_2]^+$	2.9
				272.9339	$[C_9H_6IO_2]^+$	3.8
				261.9712	$[C_8H_9INO]^+$	4.3
				246.9605	$[C_8H_8IO]^+$	3.9
				147.0434	$[C_9H_7O_2]^+$	4.7
				130.0494	$[C_5H_8NO_3]^+$	3.9
				107.0485	$[C_7H_7O]^+$	5.7
				84.0437	$[C_4H_6NO]^+$	8.1
3,5-di-I-Tyr-Ala	504.9114	$[C_{12}H_{15}I_2N_2O_4]^+$	9.3	487.8861	$[C_{12}H_{12}I_2NO_4]^+$	2.1
				458.9045	$[C_{11}H_{13}I_2N_2O_2]^+$	3.6
				387.8689	$[C_8H_8I_2NO]^+$	0.3
				272.9401	$[C_9H_6IO_2]^+$	2.3
				232.9459	$[C_7H_6IO]^+$	0.6
				130.0495	$[C_5H_8NO_3]^+$	2.8

Table S4. MS/MS fragments of iodinated tyrosine

Compound	Full-scan			MS/MS		
	m/z	Formula	Δm (ppm)	m/z	Formula	Δm (ppm)
3-I-tyrosine	307.9771	$[C_9H_{11}INO_3]^+$	2.5	290.9506	$[C_9H_8IO_3]^+$	2.5
				272.9399	$[C_9H_6IO_2]^+$	3.1
				261.9717	$[C_8H_9INO]^+$	2.6
				244.9453	$[C_8H_6IO]^+$	2.0
				163.0386	$[C_9H_7O_3]^+$	2.5
				134.0596	$[C_8H_8NO]^+$	3.2
				107.0486	$[C_7H_7O]^+$	4.7
3,5-di-I-tyrosine	433.8736	$[C_9H_{10}I_2NO_3]^+$	2.0	416.8473	$[C_9H_7I_2O_3]^+$	1.5
				387.8683	$[C_8H_8I_2NO]^+$	1.7
				288.9350	$[C_9H_6IO_3]^+$	2.1
				259.9562	$[C_8H_7INO]^+$	1.9
				232.9454	$[C_7H_6IO]^+$	1.7
				134.0598	$[C_8H_8NO]^+$	1.7

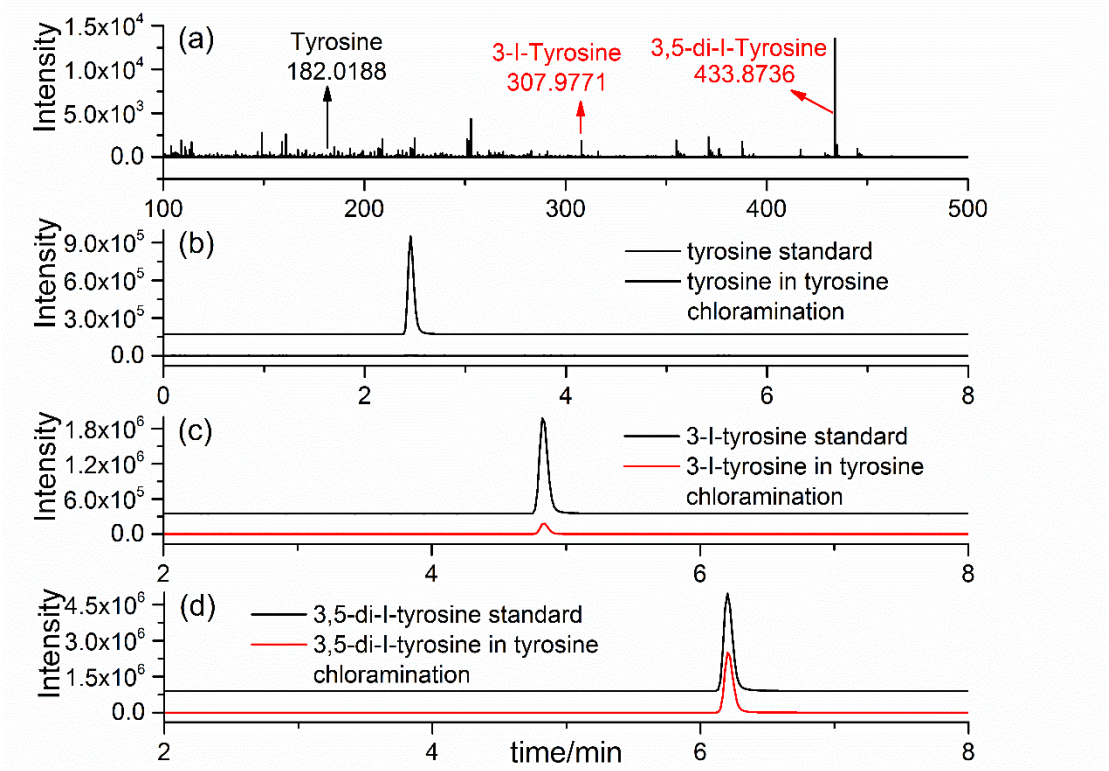


Figure S1. (a) High resolution full scan mass spectrometry of the extract of the chloramination mixture of tyrosine (0.55 μmol) spiked with sodium iodide (2.0 mg/L) and monochloramine (1.10 μmol) after reacting for 24 h. (b-c) Extracted ion current chromatograms (EICs) of the (b) tyrosine, (c) 3-I-tyrosine, and (d) 3,5-di-I-tyrosine from tyrosine chloramination solution and the standards.

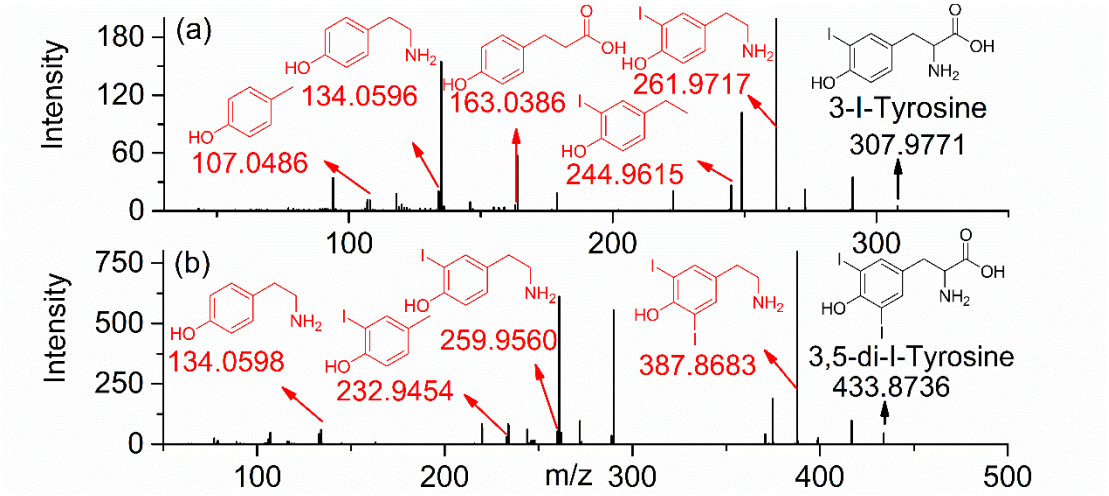


Figure S2. High resolution MS/MS spectra of (a) 3-I-tyrosine and (b) 3,5-di-I-tyrosine from tyrosine chloramination solution

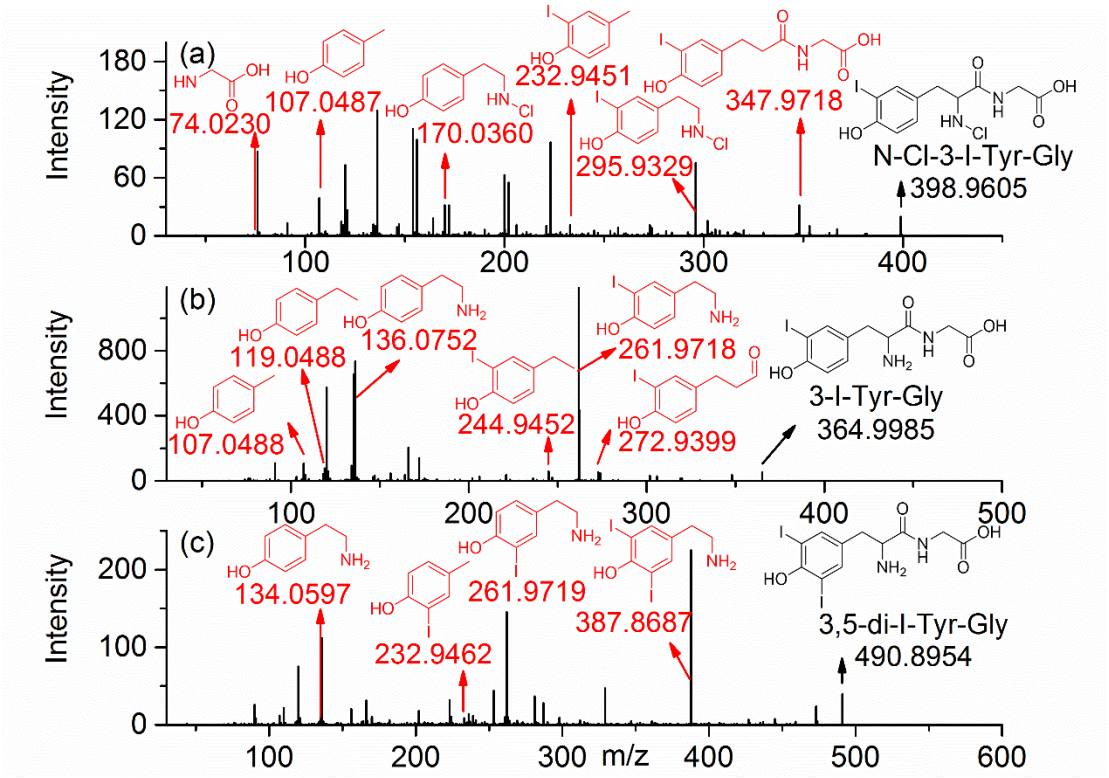


Figure S3. High resolution MS/MS spectra of (a) N-Cl-3-I-Tyr-Gly, (b) 3-I-Tyr-Gly, and (c) 3,5-di-I-Tyr-Gly from the reaction mixture containing seven tyrosyl dipeptides after chloramination in the presence of iodine.

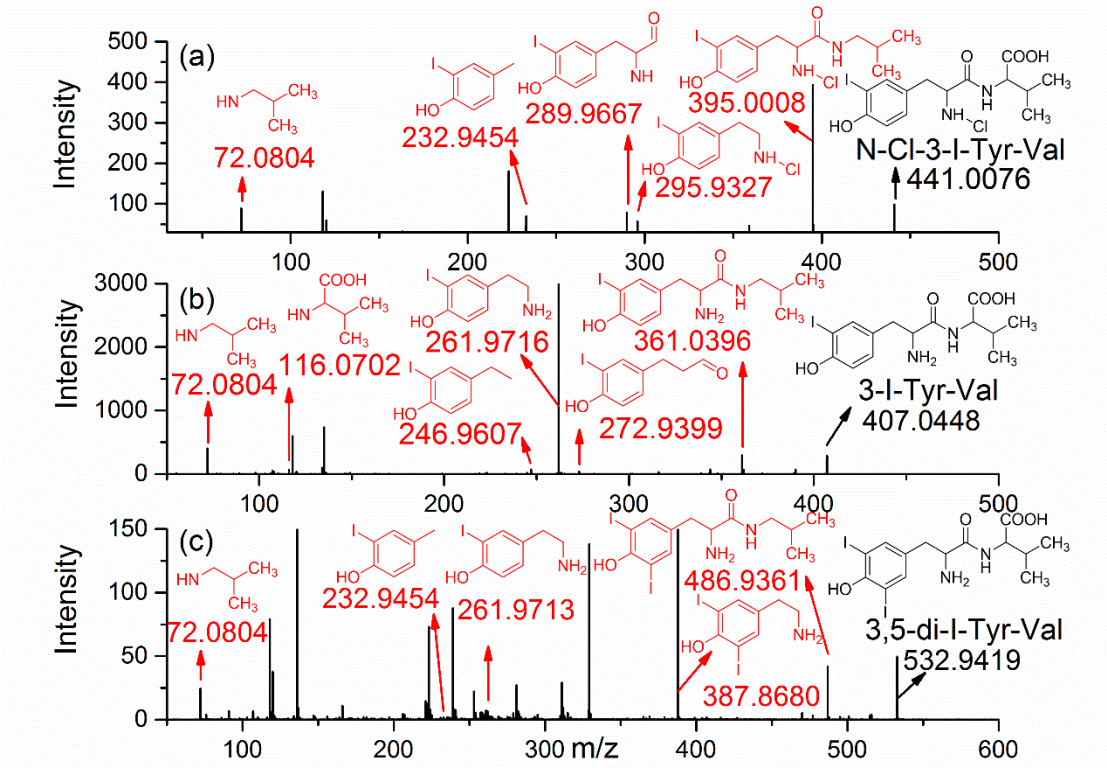


Figure S4. High resolution MS/MS spectra of (a) N-Cl-3-I-Tyr-Val, (b) 3-I-Tyr-Val, and (c) 3,5-di-I-Tyr-Val from the reaction mixture containing seven tyrosyl dipeptides after chloramination in the presence of iodine.

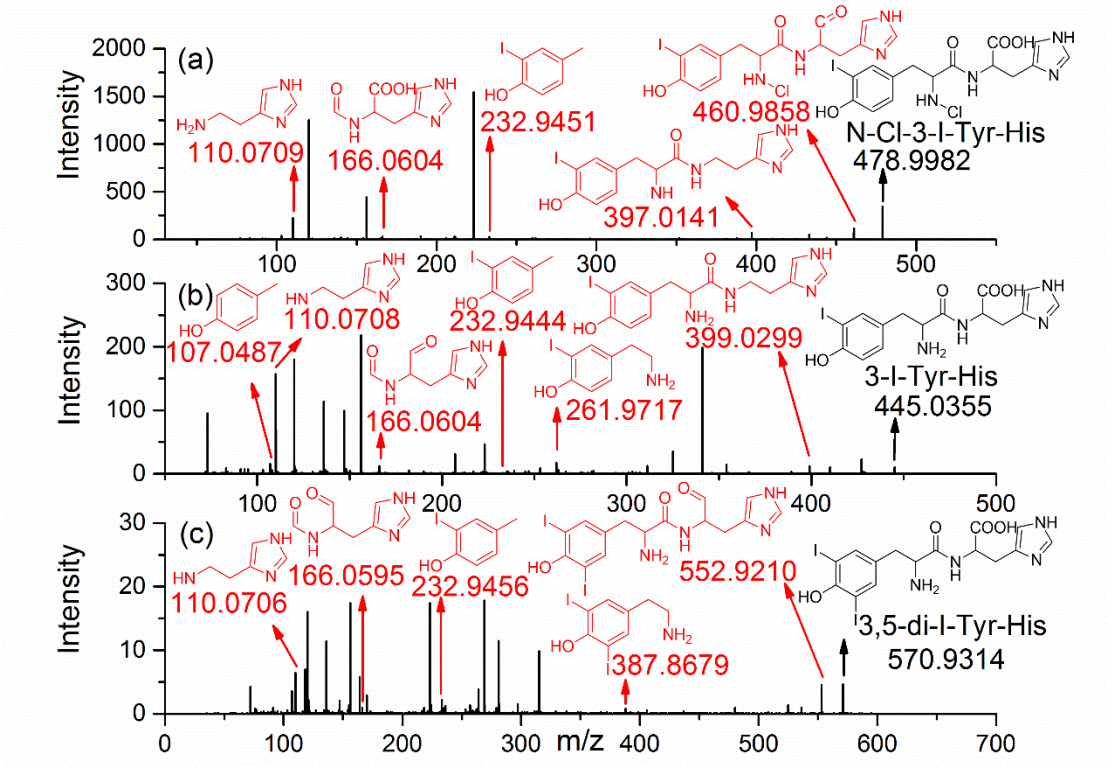


Figure S5. High resolution MS/MS spectra of (a) N-Cl-3-I-Tyr-His, (b) 3-I-Tyr-His, and (c) 3,5-di-I-Tyr-His from the reaction mixture containing seven tyrosyl dipeptides after chloramination in the presence of iodine.

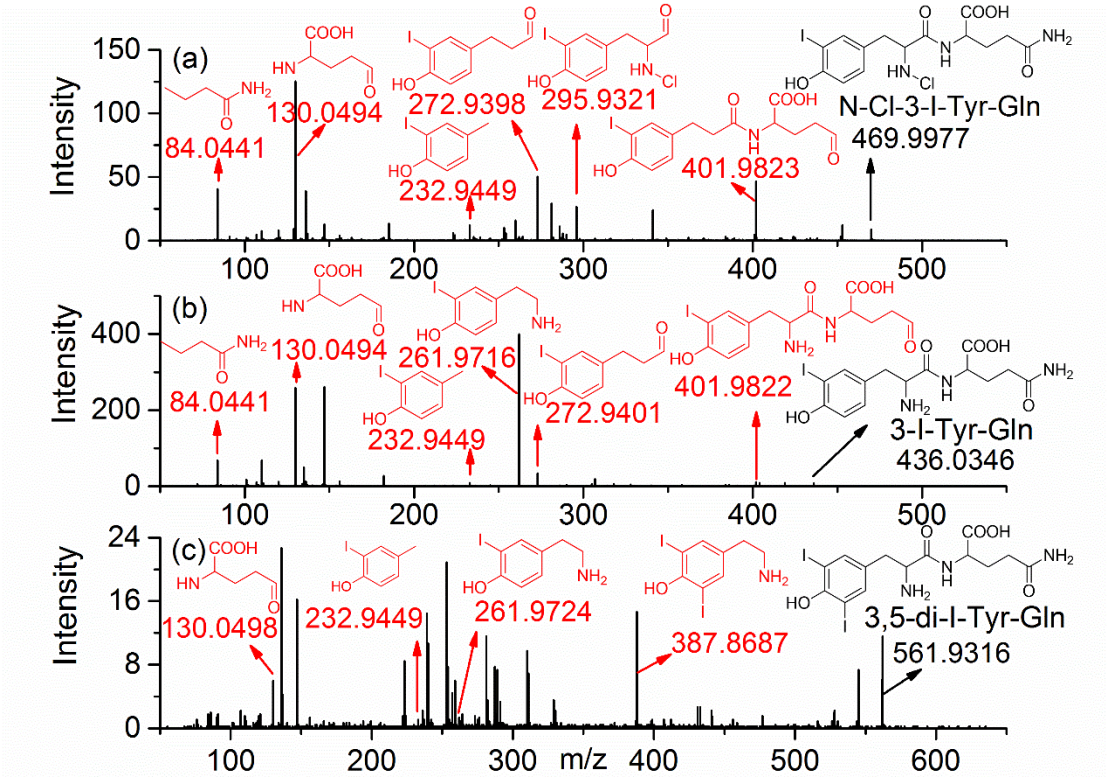


Figure S6. High resolution MS/MS spectra of (a) N-Cl-3-I-Tyr-Gln, (b) 3-I-Tyr-Gln, and (c) 3,5-di-I-Tyr-Gln from the reaction mixture containing seven tyrosyl dipeptides after chloramination in the presence of iodine.

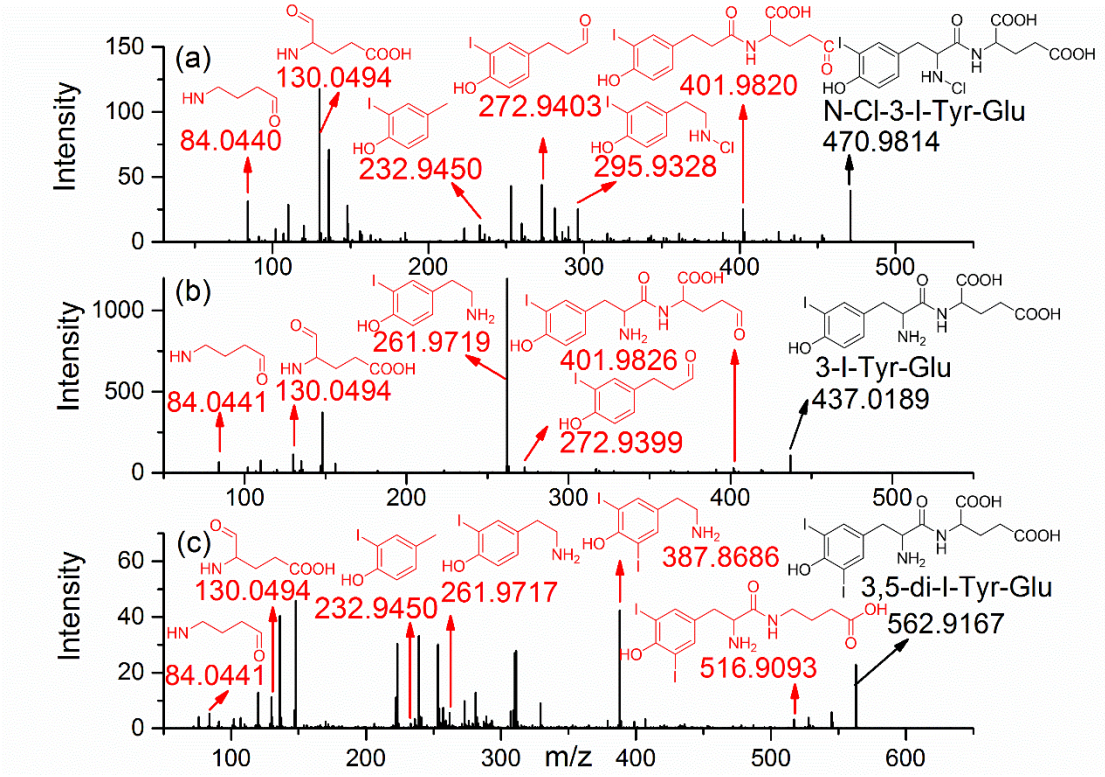


Figure S7. High resolution MS/MS spectra of (a) N-Cl-3-I-Tyr-Glu, (b) 3-I-Tyr-Glu, and (c) 3,5-di-I-Tyr-Glu from the reaction mixture containing seven tyrosyl dipeptides after chloramination in the presence of iodine.

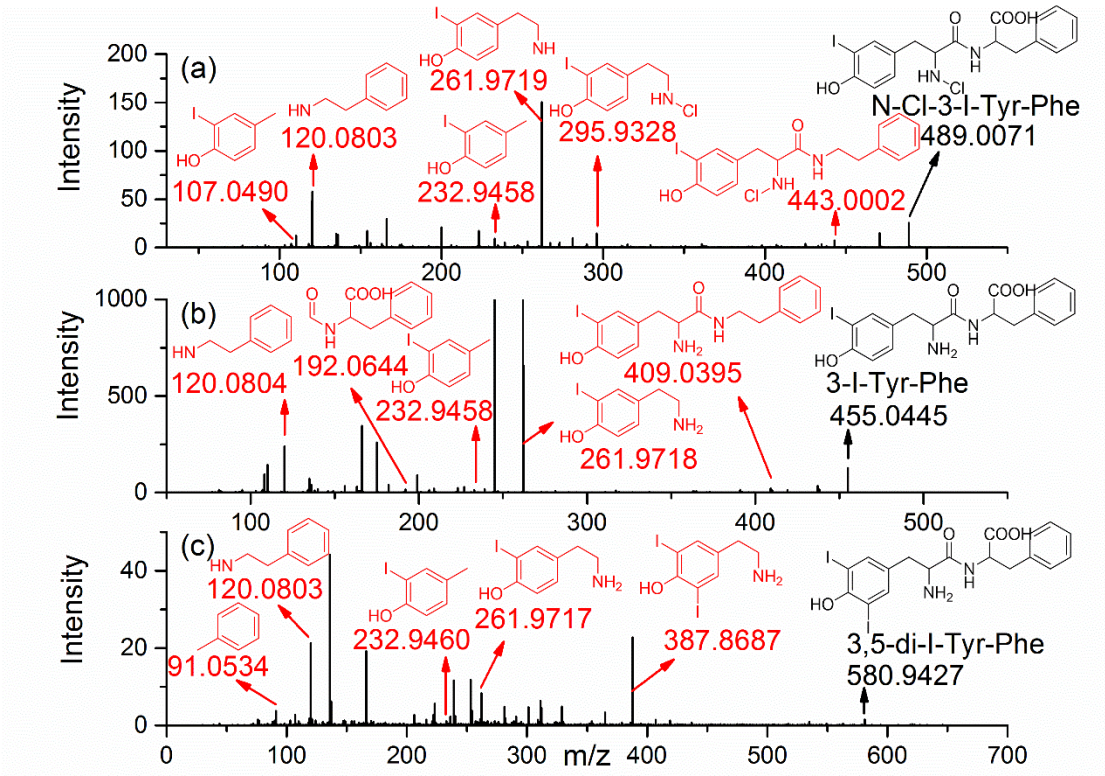


Figure S8. High resolution MS/MS spectra of (a) N-Cl-3-I-Tyr-Phe, (b) 3-I-Tyr-Phe, and (c) 3,5-di-I-Tyr-Phe from the reaction mixture containing seven tyrosyl dipeptides after chloramination in the presence of iodine.

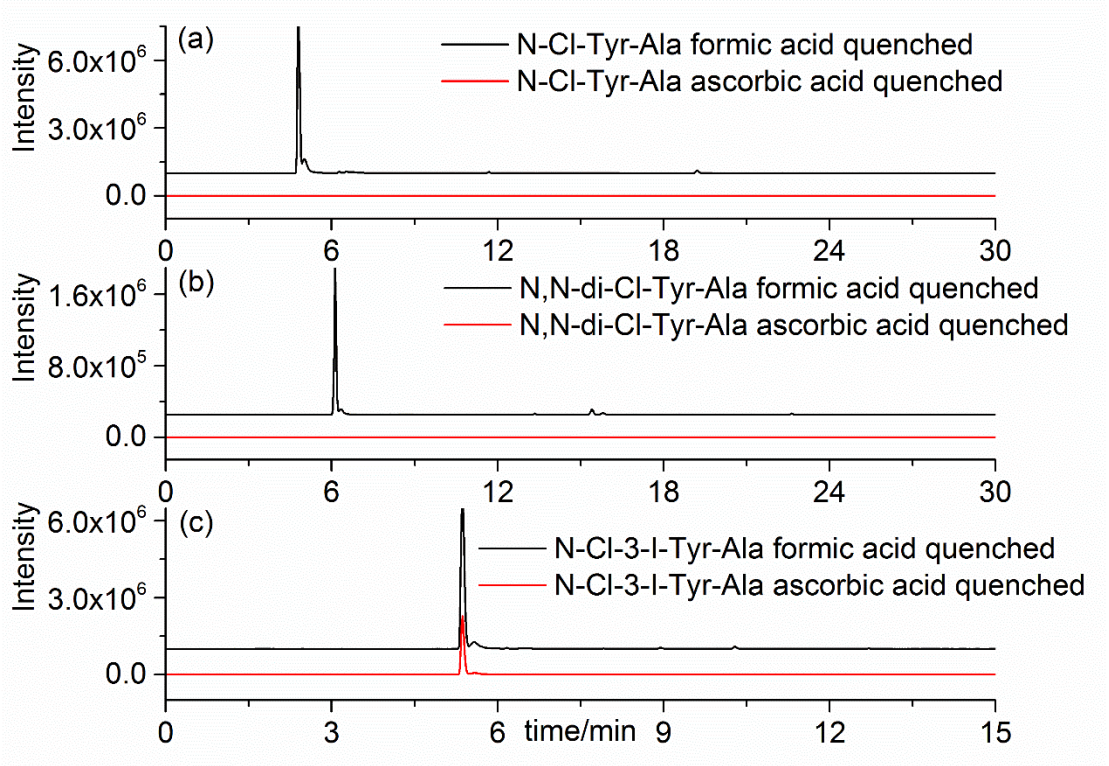


Figure S9. Detection of chlorinated Tyr-Ala from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

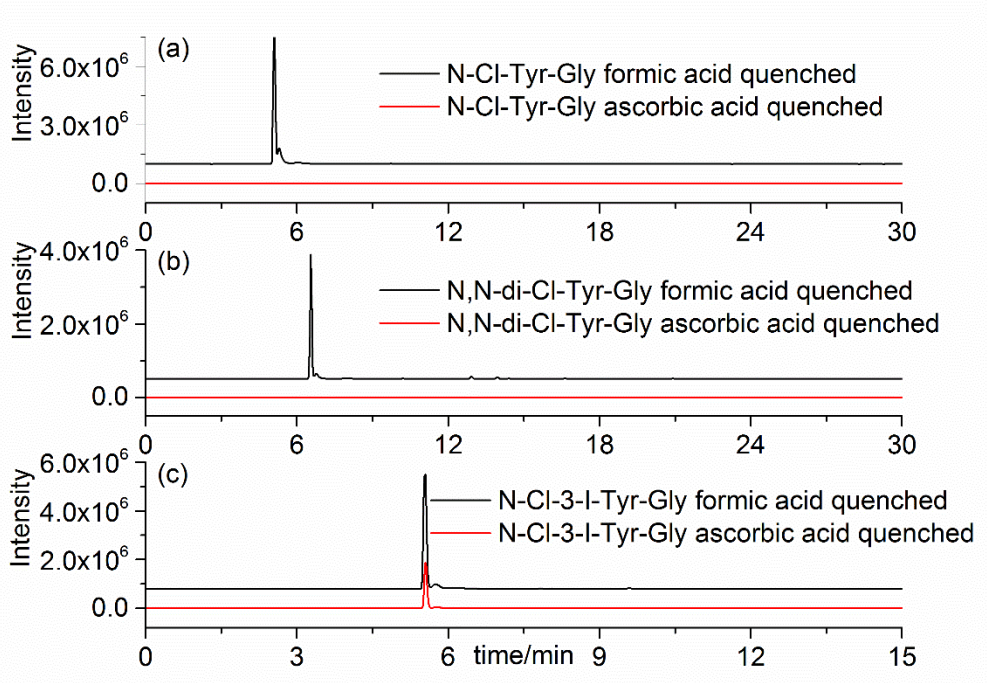


Figure S10. Detection of chlorinated Tyr-Gly from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

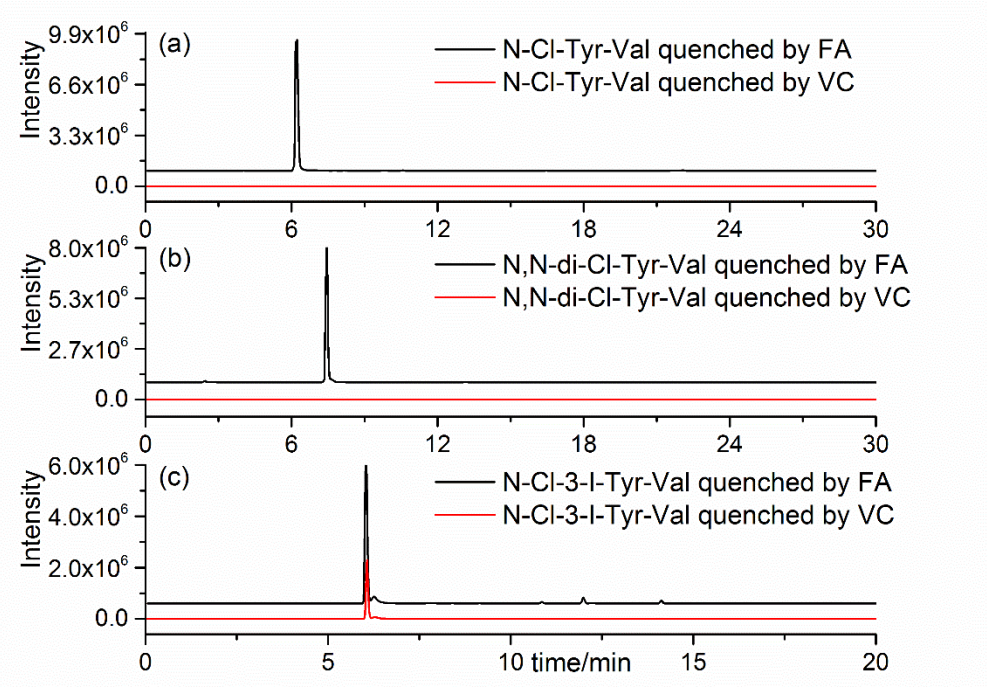


Figure S11. Detection of chlorinated Tyr-Val from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

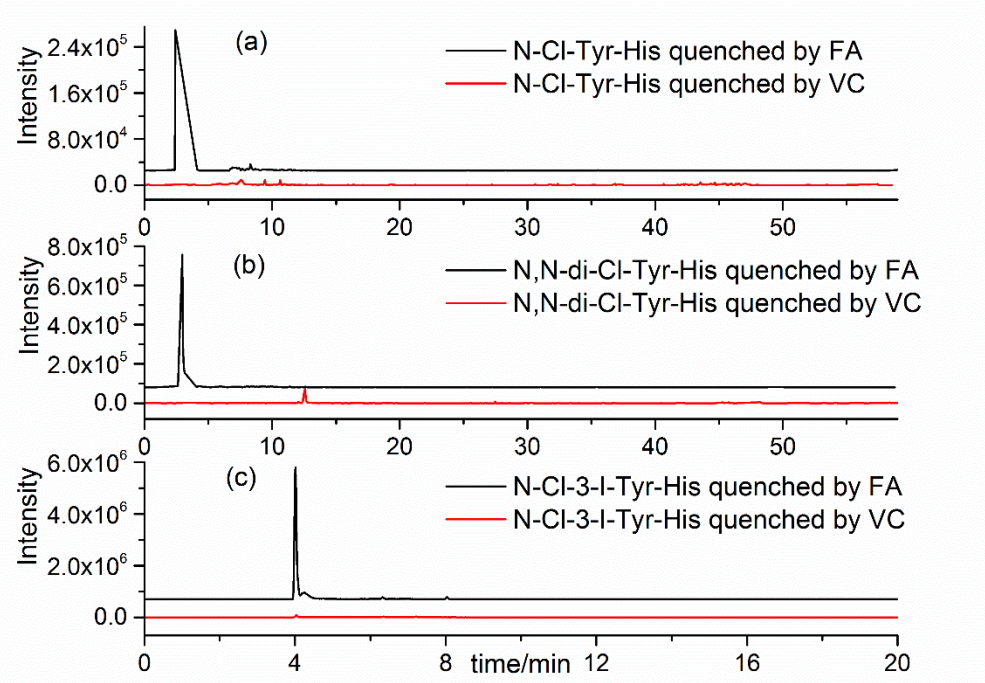


Figure S12. Detection of chlorinated Tyr-His from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

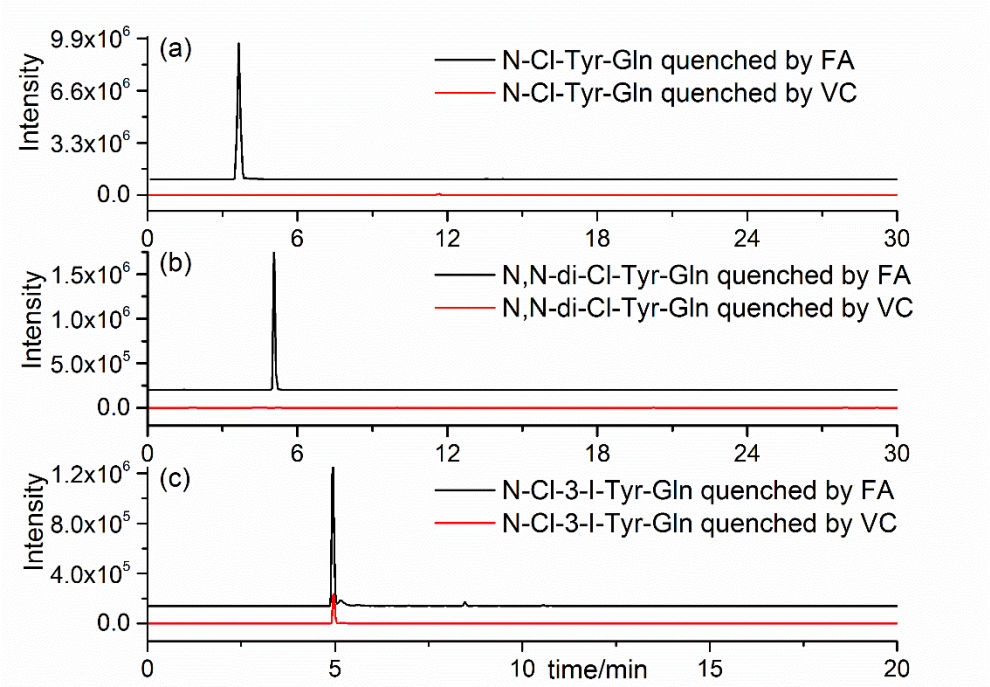


Figure S13. Detection of chlorinated Tyr-Gln from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

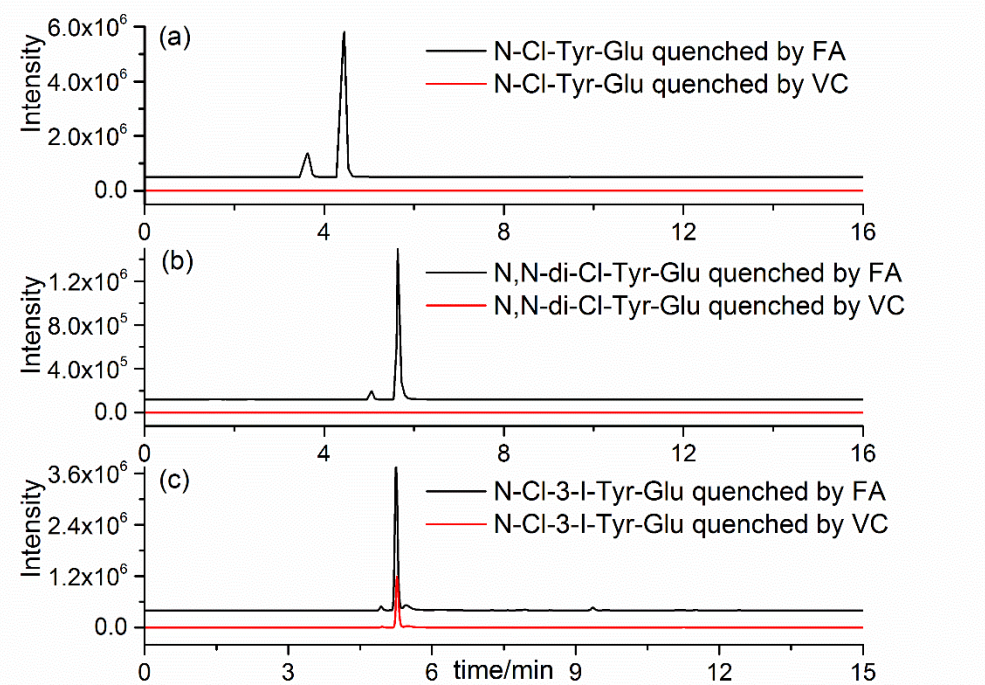


Figure S14. Detection of chlorinated Tyr-Glu from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

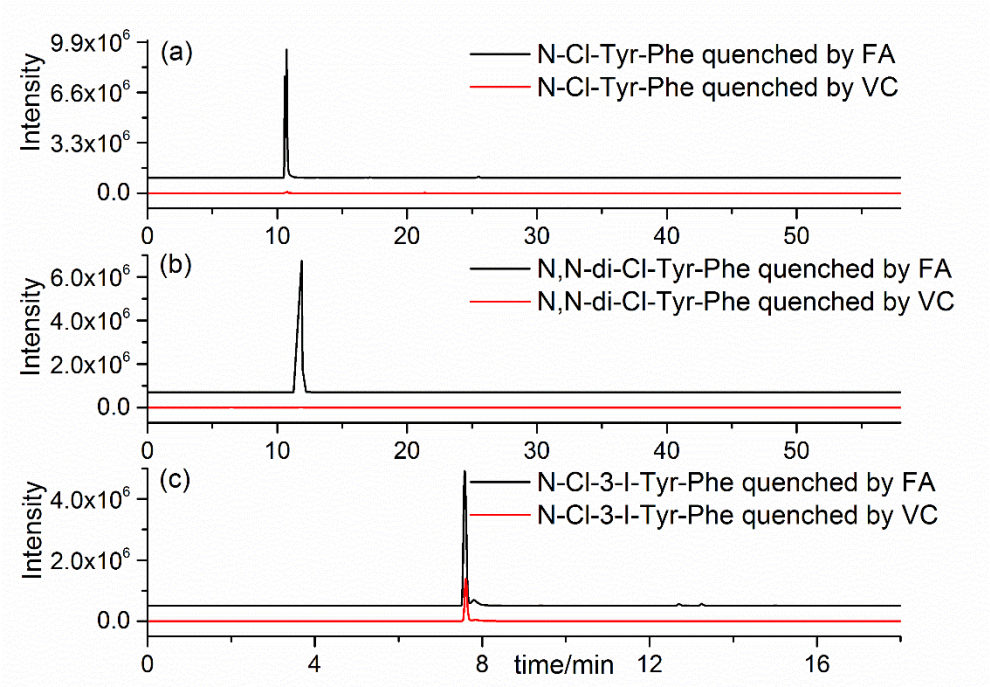


Figure S15. Detection of chlorinated Tyr-Phe from the chloramination of the seven dipeptides after the reactions were quenched by formic acid or ascorbic acid.

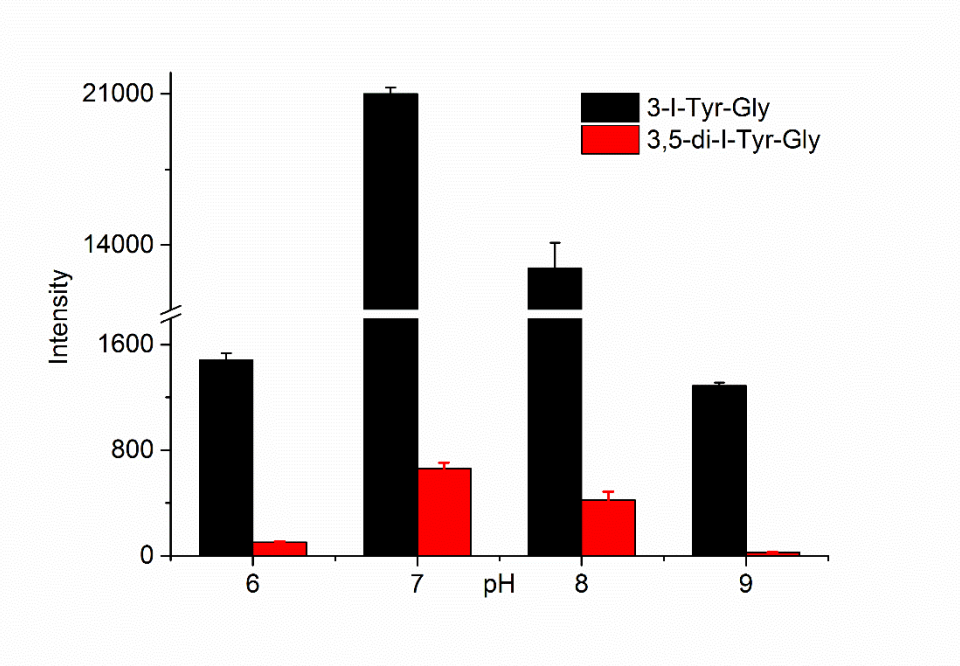


Figure S16. Formation of 3-I-Tyr-Gly and 3,5-di-I-Tyr-Gly under phosphate-buffered pH conditions.

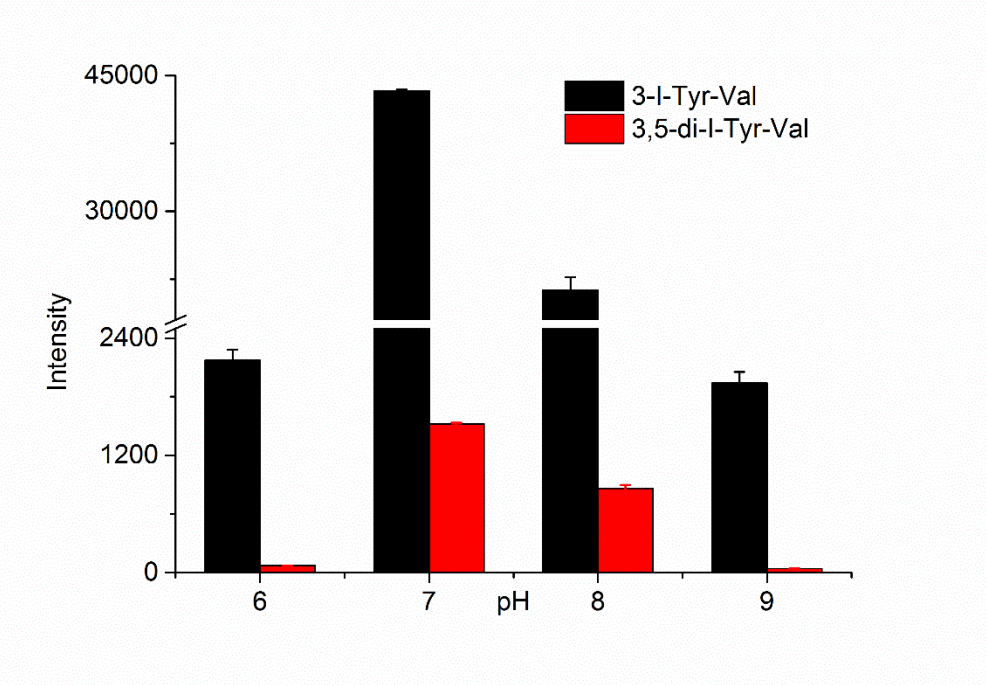


Figure S17. Formation of 3-I-Tyr-Val and 3,5-di-I-Tyr-Val under phosphate-buffered pH conditions.

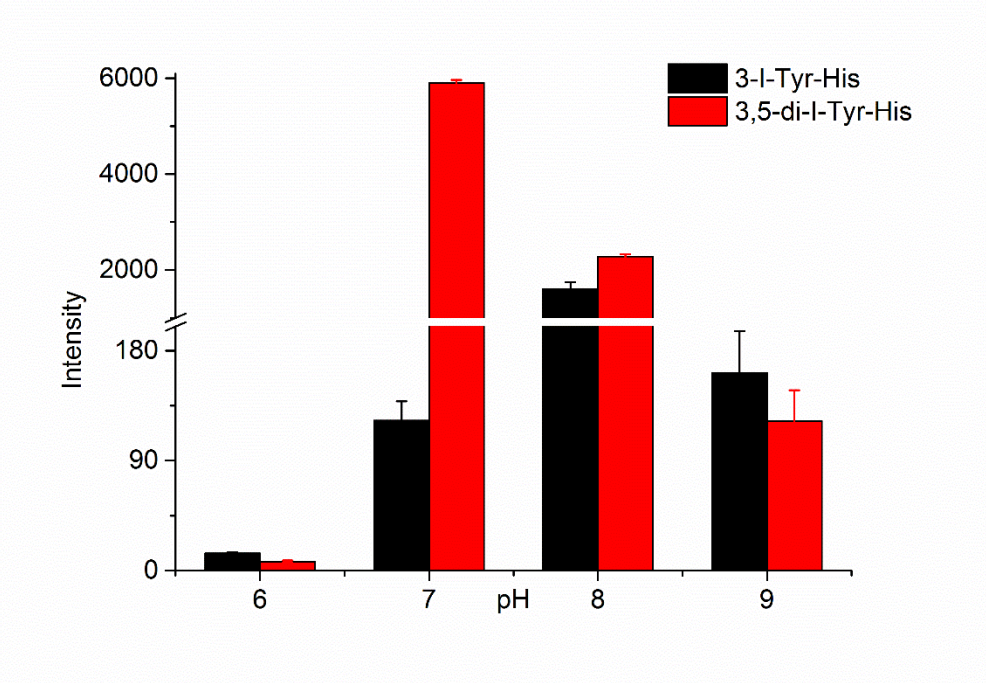


Figure S18. Formation of 3-I-Tyr-His and 3,5-di-I-Tyr-His under phosphate-buffered pH conditions.

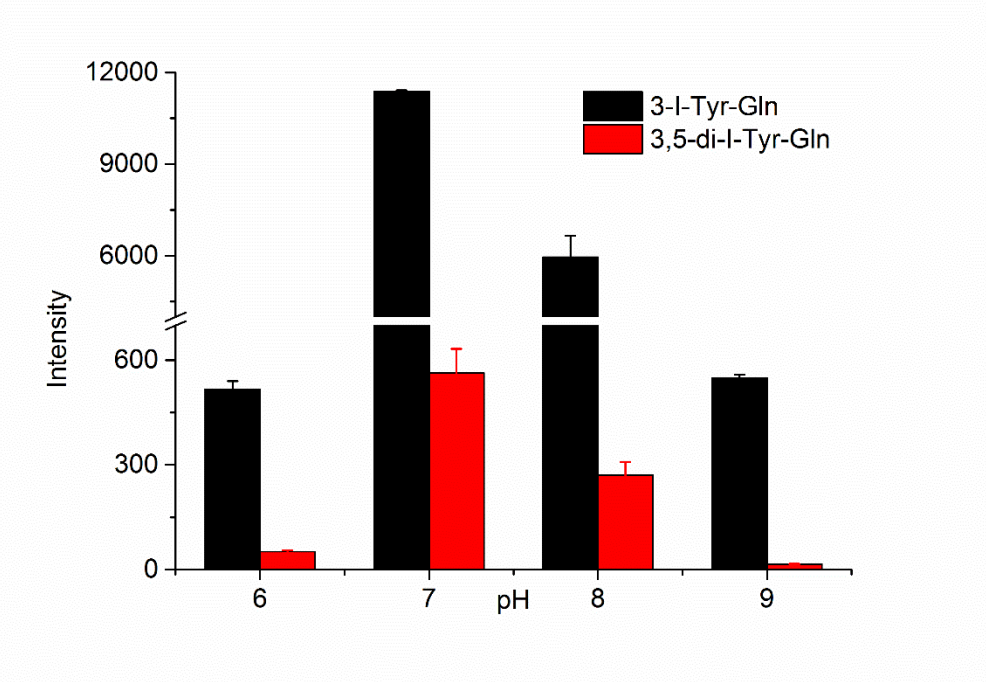


Figure S19. Formation of 3-I-Tyr-Gln and 3,5-di-I-Tyr-Gln under phosphate-buffered pH conditions.

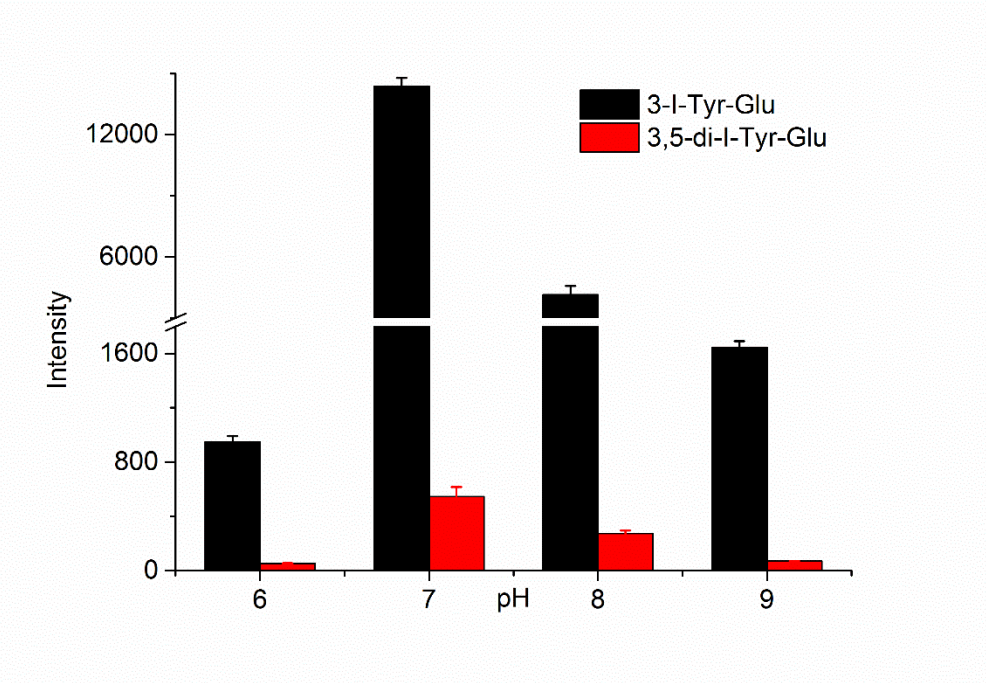


Figure S20. Formation of 3-I-Tyr-Glu and 3,5-di-I-Tyr-Glu under phosphate-buffered pH conditions.

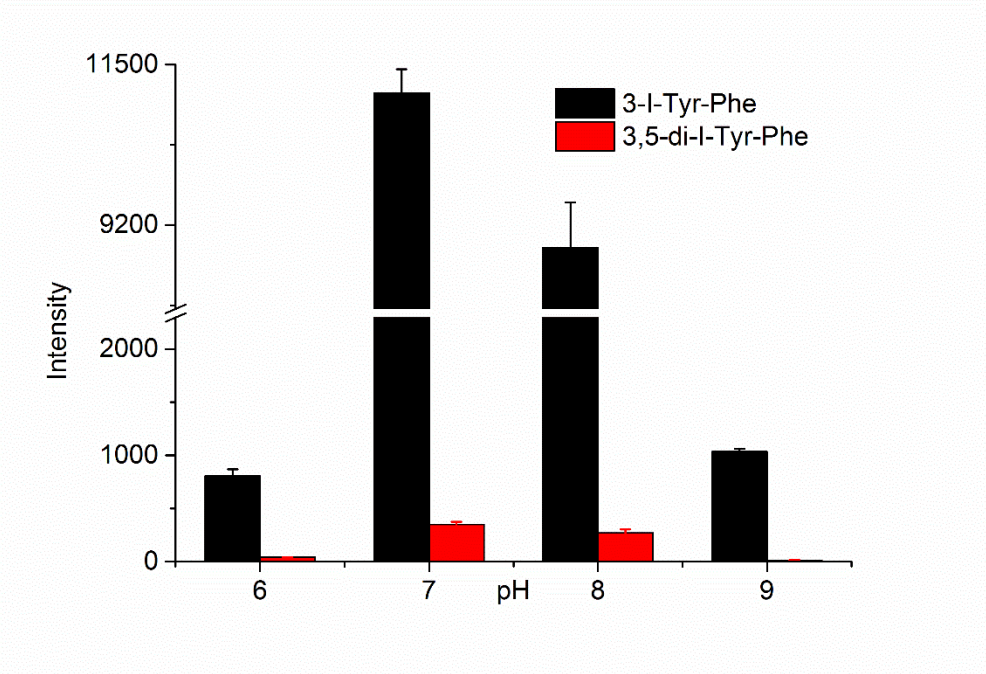


Figure S21. Formation of 3-I-Tyr-Phe and 3,5-di-I-Tyr-Phe under phosphate-buffered pH conditions.

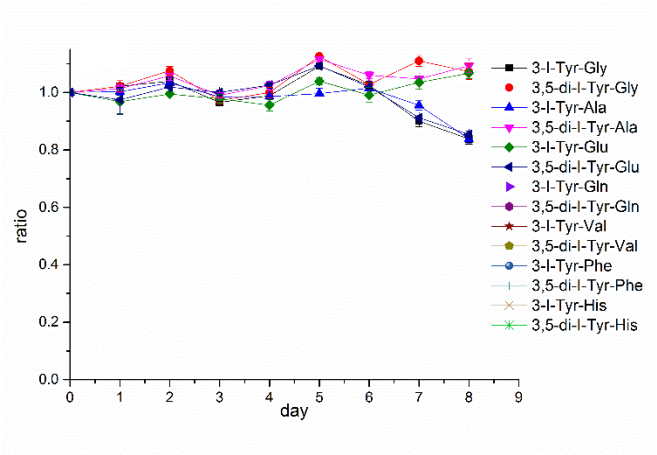


Figure S22. Stability of the synthesized iodinated tyrosyl dipeptides in Optima water over 8 days. The y-axis represents the ratio between the normalized signal intensity of day N over that of day zero.

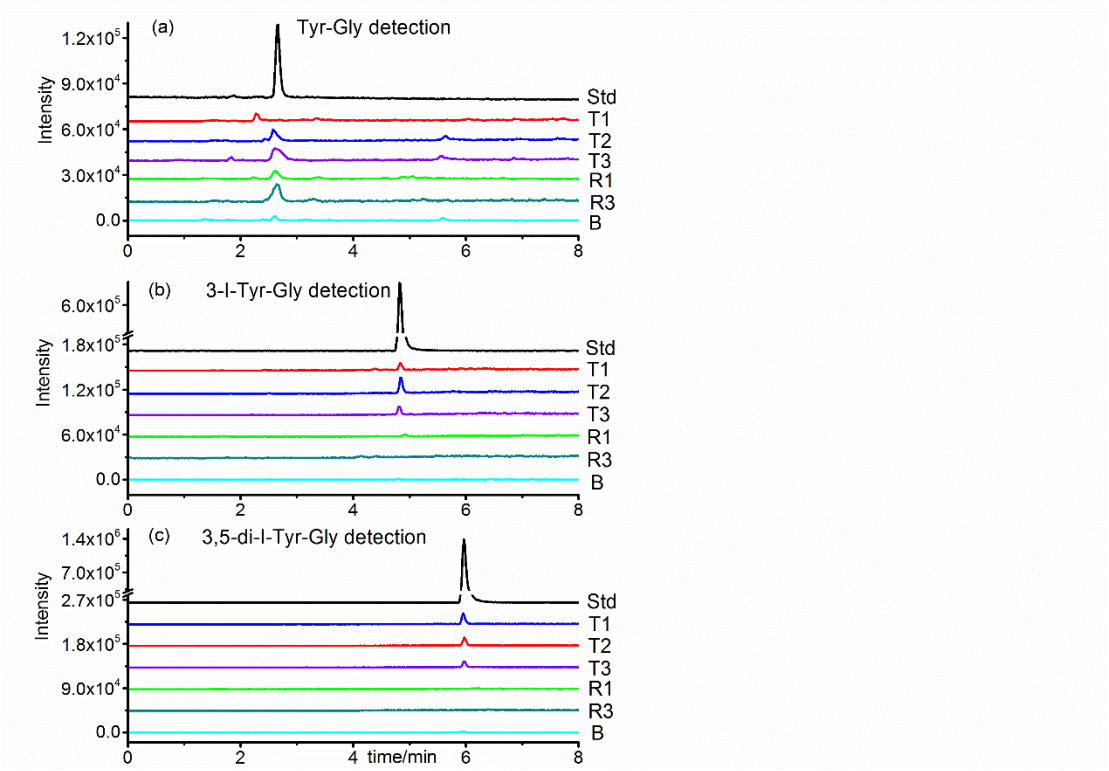


Figure S23. Detection of (a) Tyr-Gly, (b) 3-I-Tyr-Gly, and (c) 3,5-di-I-Tyr-Gly in tap waters T1 (City 1), T2 (City 2), and T3 (City 3); raw water R1 (City 1) and R3 (City 3) by HPLC-ESI-MS/MS.

References

(1) Postigo, C.; Cojocariu, C. I.; Richardson, S. D.; Silcock, P. J.; Barcelo, D. *Anal. Bioanal. Chem.* **2016**, *408*, 3401-3411.

(2) Duirk, S. E.; Lindell, C.; Cornelison, C. C.; Kormos, J.; Ternes, T. A.; Attene-Ramos, M.; Osiol, J.; Wagner, E. D.; Plewa, M. J.; Richardson, S. D. *Environ. Sci. Technol.* **2011**, *45*, 6845-6854.

(3) Zhao, Y.; Anichina, J.; Lu, X.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. *Water Res.* **2012**, *46*, 4351-4360.

(4) Jiang, P.; Huang, G.; Jmaiff Blackstock, L. K.; Zhang, J.; Li, X.-F. *Anal. Chem.* **2017**, 89, 13642-13650.