Supporting information for

Imidazolium-based iodoacetamide functional tags: Design, synthesis, and property study for cysteinyl-peptides analysis by mass spectrometry

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Chemicals and Reagents

1-Methylimidazole, 1-butylimidazole, 3-bromopropylamine hydrobromide, iodoacetic acid, iodoacetamide (IAA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), bovine serum albumin, and TPCK-treated trypsin (from bovine pancreas) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl) and peptides with the sequences ALVCEQEAR (m/z 1017.49), CDPGYIGSR (m/z 966.42), LEACTFRRP (m/z 1091.55), MECFG (m/z 585.19), and CKDECSLDG (m/z 968.36) were obtained from GL Biochem (Shanghai, China).

Trifluoroacetic acid (TFA) was from Acros organics (Geel, Belgium). α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from Bruker Daltonics (Bremen, Germany). HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). All inorganic reagents were analytical-reagent grade and other reagents were of HPLC grade. Water was purified by a Milli-Q system (Millipore, Molsheim, France).

Synthesis of 1-(3-aminopropyl)-3-methylimidazolium bromide

1-Methylimidazole (1.0 g, 12 mmol) and 3-bromopropylamine hydrobromide (1.8 g, 8 mmol) were firstly dissolved in 10 mL of anhydrous ethanol, followed by refluxing at 80 °C for 24 h under nitrogen atmosphere. Subsequently, ethanol was removed under vacuum and the crude residue was dissolved in 2 mL of water that is brought to pH ~8 by the addition of solid potassium hydroxide. Finally, the water was removed under vacuum again, and the crude residue was redissolved in 30 mL of tetrahydrofuran/methanol (1:1, v/v), filtered, dried under vacuum, and thoroughly washed with dichloromethane to yield the final product.

Synthesis of 1-(3-aminopropyl)-3-hexylimidazolium bromide

1-Hexylimidazole (1.1 g, 7 mmol) were synthesized [1] and dissolved in 13 mL of anhydrous ethanol. Subsequently, 3-bromopropylamine hydrobromide (1.1 g, 5 mmol) were added and the resulting mixture was refluxed at 80 °C for 24 h under nitrogen atmosphere. Then, ethanol was removed under vacuum and the crude residue was dissolved in 2 mL of water that is brought to pH ~8 by the addition of solid potassium hydroxide. Finally, the water was removed under vacuum again, and the crude residue was redissolved in 30 mL of tetrahydrofuran/methanol (1:1, v/v), filtered, dried under vacuum, and thoroughly washed with dichloromethane to yield the final product.

Synthesis of 1-[3-[(2-Iodo-1-oxoethyl)amino]propyl]-3-methylimidazolium bromide (IPMI)

1-(3-Aminopropyl)-3-methylimidazolium bromide (20 mg, 0.09 mmol) were firstly

dissolved in 0.5 mL of acetonitrile/water (4:1, v:v) at 0 °C. Then, iodoacetic acid (12 mg, 0.06 mmol) were added and the resulting mixture was stirred at 0 °C for 10 min. EDC•HCl (14 mg, 0.07 mmol) were sequentially added, and the resulting mixture was stirred at 0 °C for additional 1 h. Finally, the crude product was purified by reversed-phase high-performance liquid chromatography with acetonitrile-water containing 0.1% (v/v) TFA as the mobile phase to yield the final product. NMR spectra were obtained on a Bruker AVANCE III 600 MHz spectrometer, and HRMS spectra were determined on a Bruker apex ultra 7.0 T Fourier transform mass spectrometer (Bruker, Bremen, Germany). ^{1}H NMR (600 MHz, DMSO) δ= 9.08 (s, 1H), 8.36 (s, 1H), 7.76 (s, 1H), 7.71 (s, 1H), 4.17 (t, *J* = 6.7 Hz, 2H), 3.85 (s, 3H), 3.63 (s, 2H), 3.07 (m, 2H), 1.97-1.90 (m, 2H). HRMS, m/z: 308.02523.

Synthesis of 1-[3-[(2-Iodo-1-oxoethyl)amino]propyl]-3-hexylimidazolium bromide (IPHI)

1-(3-Aminopropyl)-3-hexylimidazolium bromide (22 mg, 0.08 mmol) were firstly dissolved in 0.22 mL of acetonitrile/water (3.5:1, v:v) at 0 °C. Then, iodoacetic acid (10 mg, 0.05 mmol) were added and the resulting mixture was stirred at 0 °C for 10 min. EDC•HCl (7 mg, 0.07 mmol) were sequentially added, and the resulting mixture was stirred at 0 °C for additional 1 h. Finally, the crude product was purified by reversed-phase high-performance liquid chromatography with acetonitrile-water containing 0.1% (v/v) TFA as the mobile phase to yield the final product. ^{1}H NMR (600 MHz, DMSO) δ=9.14 (s, 1H), 8.38 (s, 1H), 7.79 (d, *J* =12.7 Hz, 2H), 4.19-4.13 (m, 4H), 3.62 (s, 2H), 3.09-3.03 (m, 2H), 2.00-1.90 (m, 2H), 1.82-1.75 (m, 2H), 1.30-1.20 (m, 6H), 0.86 (t, 3H). HRMS, m/z: 378.10337.

Log P and gas-phase hydrogenation capacity prediction

The Log P value of IPMI, IPBI, and IPHI was calculated based on the on-line software Molinspiration (Website: http://www.molinspiration.com/cgi-bin/properties) and the gas-phase hydrogenation capacity was calculated based on the software ORCA 2.8 (Website:

http://www.thch.uni-bonn.de/tc/orca/) with method B3LYP/TZVP [2-4].

Peptide derivatization

In a typical experiment, 5 μL of 1 mg/mL peptides ALVCEQEAR, CDPGYIGSR, CKDECSLDG, LEACTFRRP or 0.5 mg/mL MECFG was initially mixed with 10 μL of 20 mM TCEP in 50 mM Tris-HCl buffer (pH~8.4). After the peptides were reduced at 56 °C for 1 h, an aliquot of 50 μL of 30 mM IPMI, IPHI, or 1-[3-[(2-Iodo-1-oxoethyl)amino]propyl]-3-butylimidazolium bromide (IPBI) were added, followed by further incubating at 37 °C in dark for 1 h with end-over-end rotation. The alkylation procedure of the peptides with IAA was similar to the peptide derivatization experiment with IPMI, IPHI or IPBI except that 30 mM of IAA was added.

Protein derivatization and digestion

In brief, 1.0 mg of bovine serum albumin were firstly dissolved in 500 μL of 8 M urea and then denatured at 56 °C for 1 h. Subsequently, 10 μg of proteins were reduced by 20 mM TCEP at 56 °C for 1 h. After cooling to room temperature, 50 μL of 30 mM IPHI were added, followed by further incubating at 37 °C in dark for 1 h with end-over-end rotation. The sample was diluted with 50 mM Tris-HCl (pH 8.4) till the urea concentration was less than 1 M. Then, trypsin was added with enzyme/protein ratio of 1:20 (w/w). The sample was further incubated at 37 °C overnight, desalted, and redissolved in 750 μL of water, ready for analysis.

The procedure of the protein derivatized with IAA was similar to the derivatization procedure with IPHI, except that 30 mM of IPHI was replaced by 30 mM of IAA.

MS analysis

MALDI-TOF MS experiments were performed on a Bruker Ultraflex III TOF/TOF mass spectrometer (Bruker, Bremen, Germany) in positive ion reflectron mode. An aliquot of 1 μL of native or derivatized peptides were directly deposited and dried on the polished steel target, followed by 1 μL of matrix solution (7 mg/mL CHCA in 0.1% TFA/60% acetonitrile)

deposition. MS spectra were processed and analyzed with the software FlexAnalysis (version 3.0) and BioTools (version 3.2) (Bruker, Bremen, Germany). The protein was identified by peptide mass fingerprints (PMF), and database searching was performed on MASCOT server (Matrix Science, London, UK) to search the Database of bovine, with parameters as following: (i) enzyme: trypsin; (ii) fixed modification: carbamidomethyl (C) or modifying cysteine residue with 249.1841 Da for IPHI derivatization; (iii) variable modification: oxidation (M); (iv) Peptide mass tolerance: 200 ppm; (v) missed cleavages: 2.

A Finnigan surveyor MS pump (Thermo Finnigan, San Jose, CA, USA) coupled with a LTQ linear ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) were used for peptide charge state determination.

Reference for the General Procedure section:

1 H. Zhao, F. W. Foss and R. Breslow, *J. Am. Chem. Soc.*, 2008, **130**, 12590-12591.

2 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648-5652.

3 A. D. Becke, *Phys. Rev. A*, 1988, **38**, 3098-3100.

4 O. Treutler and R. J. Ahlrichs, *J. Chem. Phys.*, 1995, **102**, 346-356.

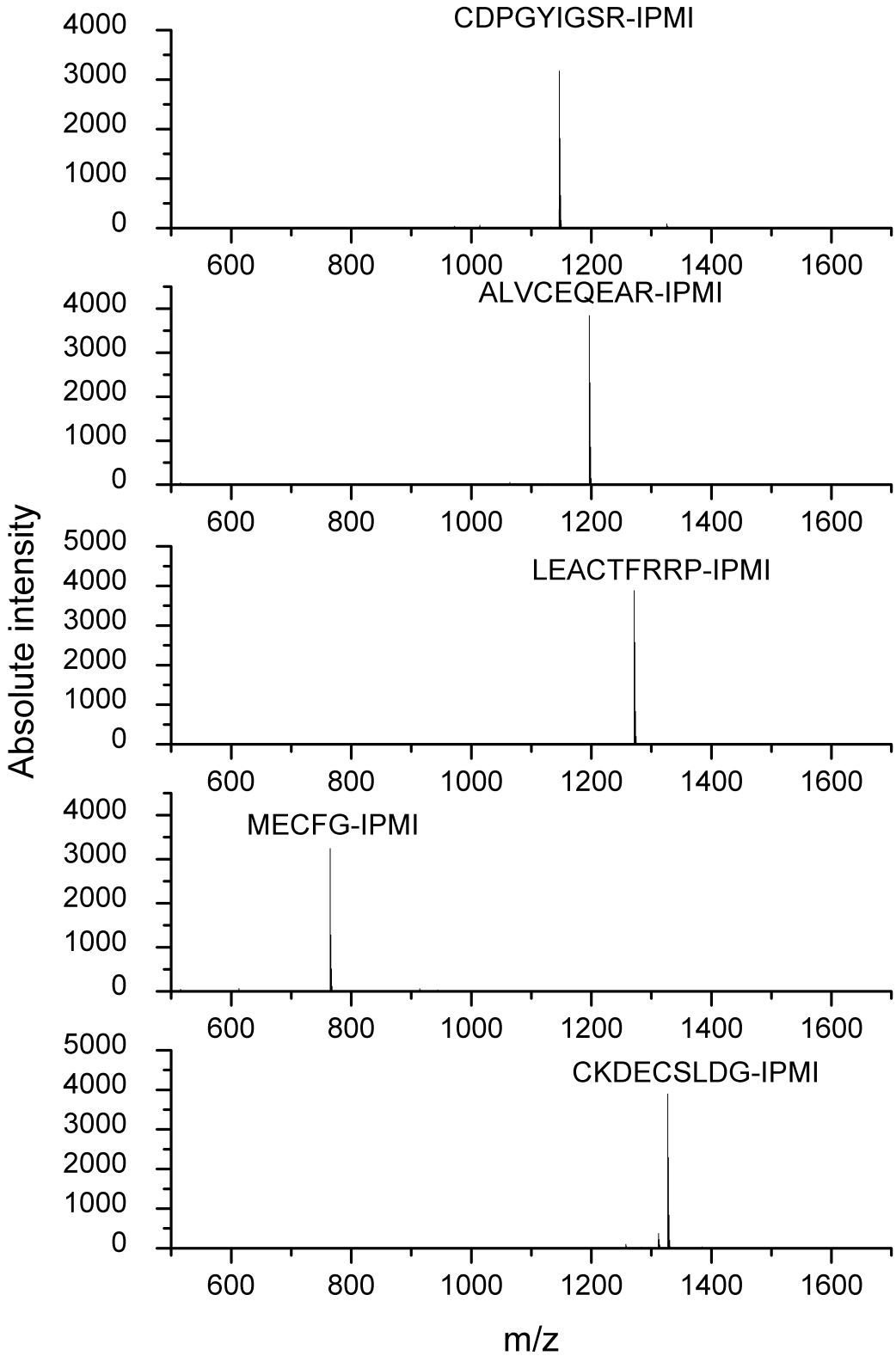

Fig. S1 MALDI-TOF MS spectra of peptides CDPGYIGSR, ALVCEQEAR, LEACTFRRP, MECFG, and CKDECSLDG respectively derivatized by IPMI.

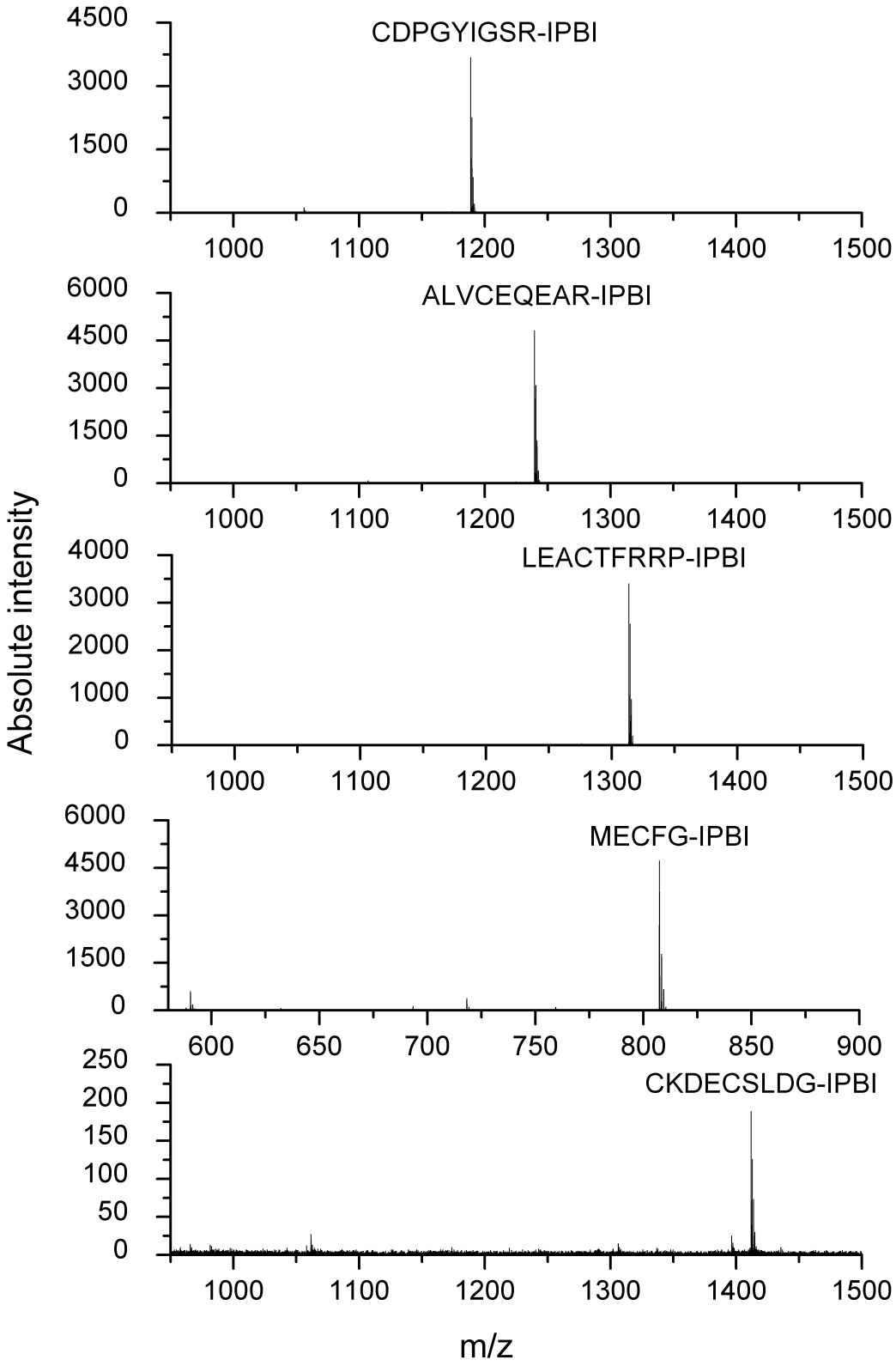

Fig. S2 MALDI-TOF MS spectra of peptides CDPGYIGSR, ALVCEQEAR, LEACTFRRP, MECFG, and CKDECSLDG respectively derivatized by IPBI.

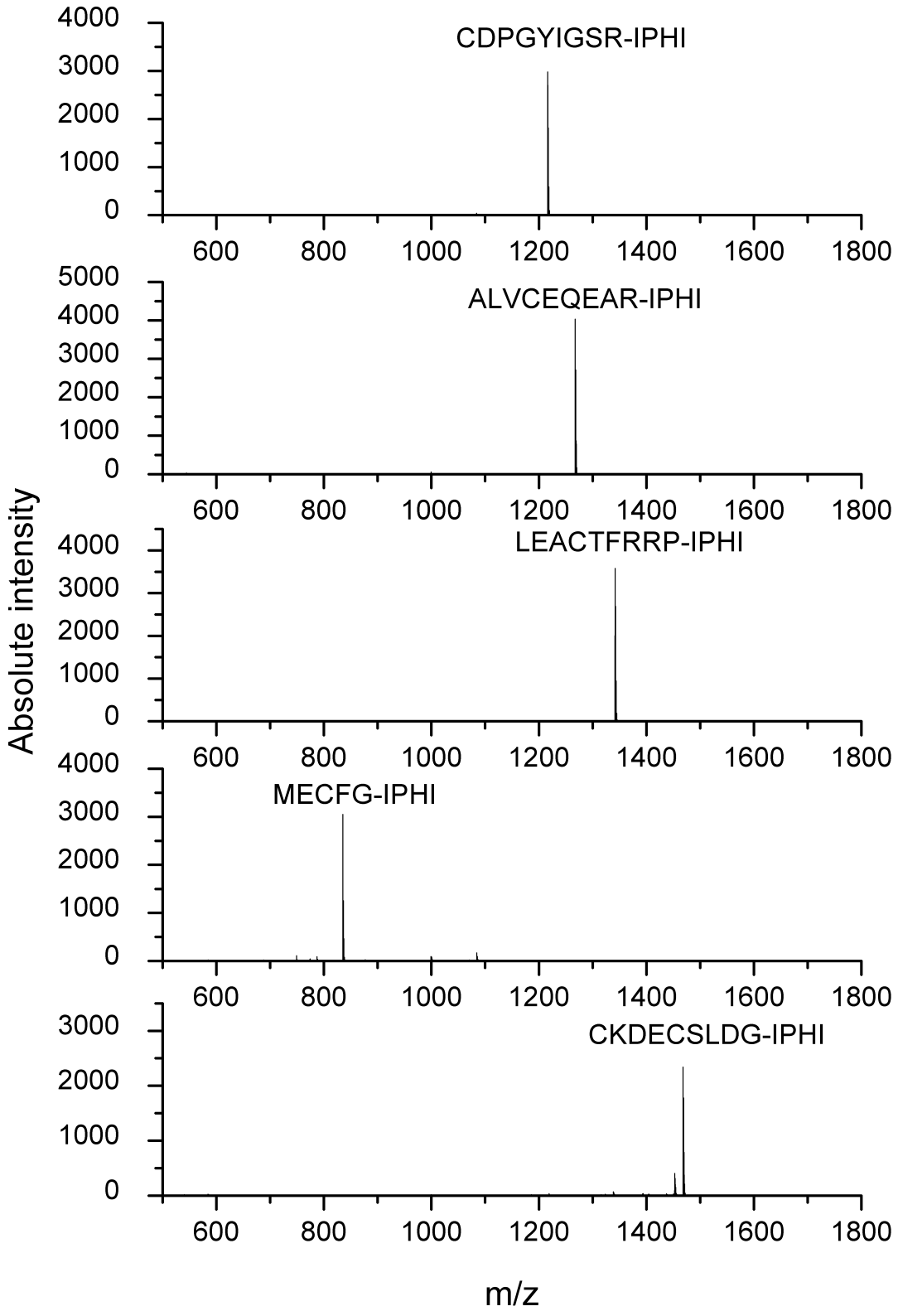

Fig. S3 MALDI-TOF MS spectra of peptides CDPGYIGSR, ALVCEQEAR, LEACTFRRP, MECFG, and CKDECSLDG respectively derivatized by IPHI.

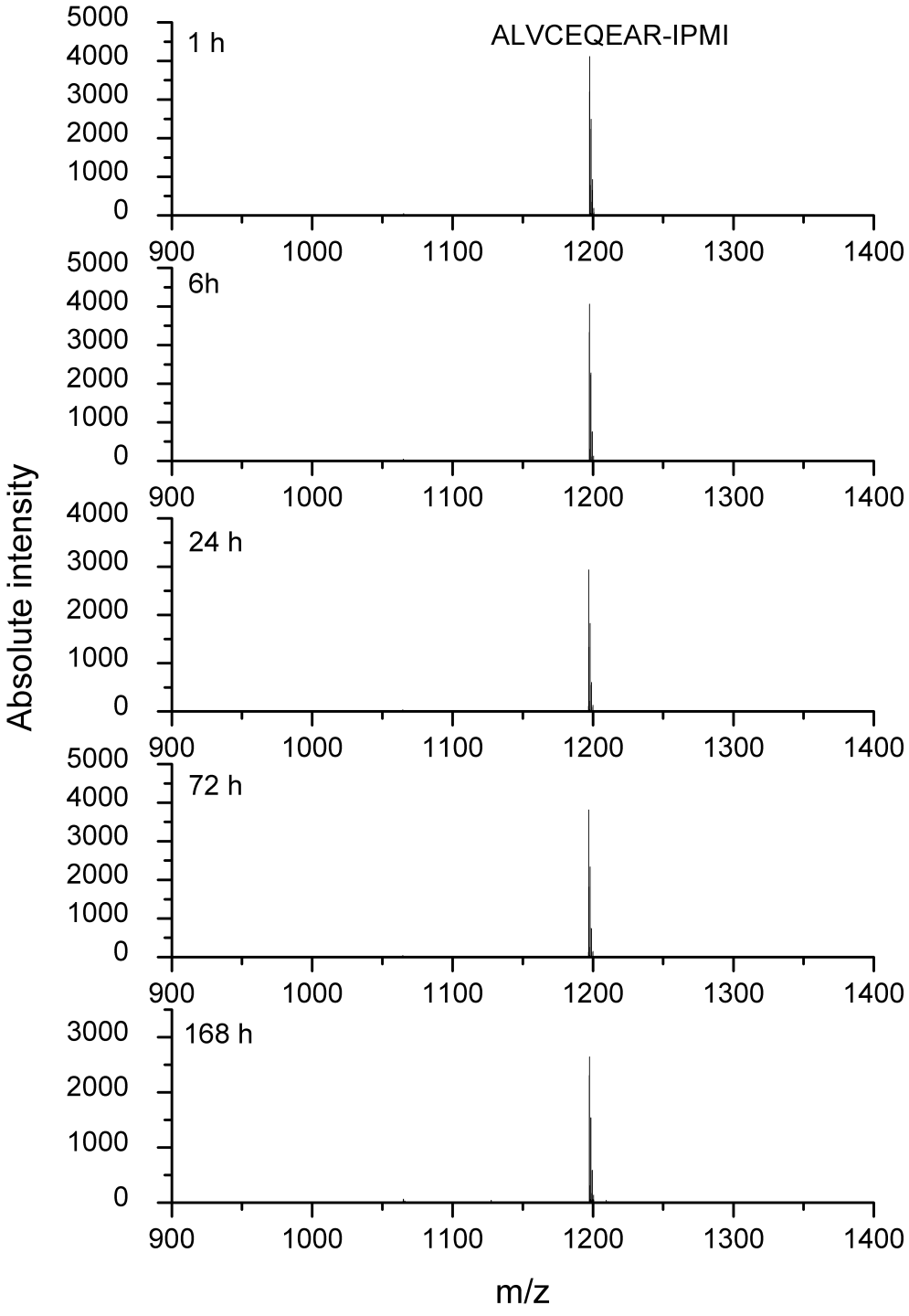

Fig. S4 MALDI-TOF MS spectra of IPMI-derivatized peptide ALVCEQEAR stored in the reaction buffer for 1, 6, 24, 72, and 168 h.

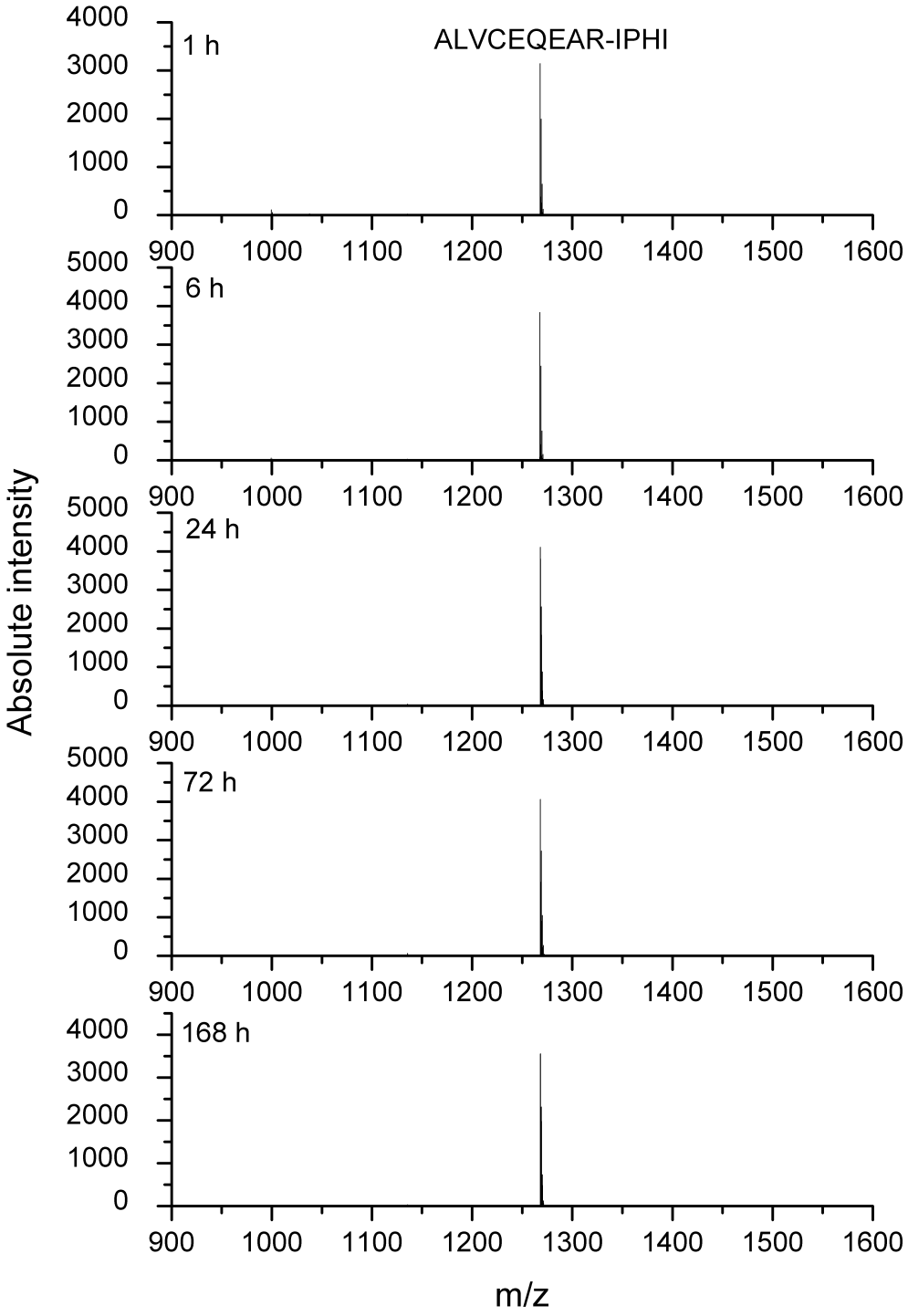

Fig. S5 MALDI-TOF MS spectra of IPHI-derivatized peptide ALVCEQEAR stored in the reaction buffer for 1, 6, 24, 72, and 168 h.

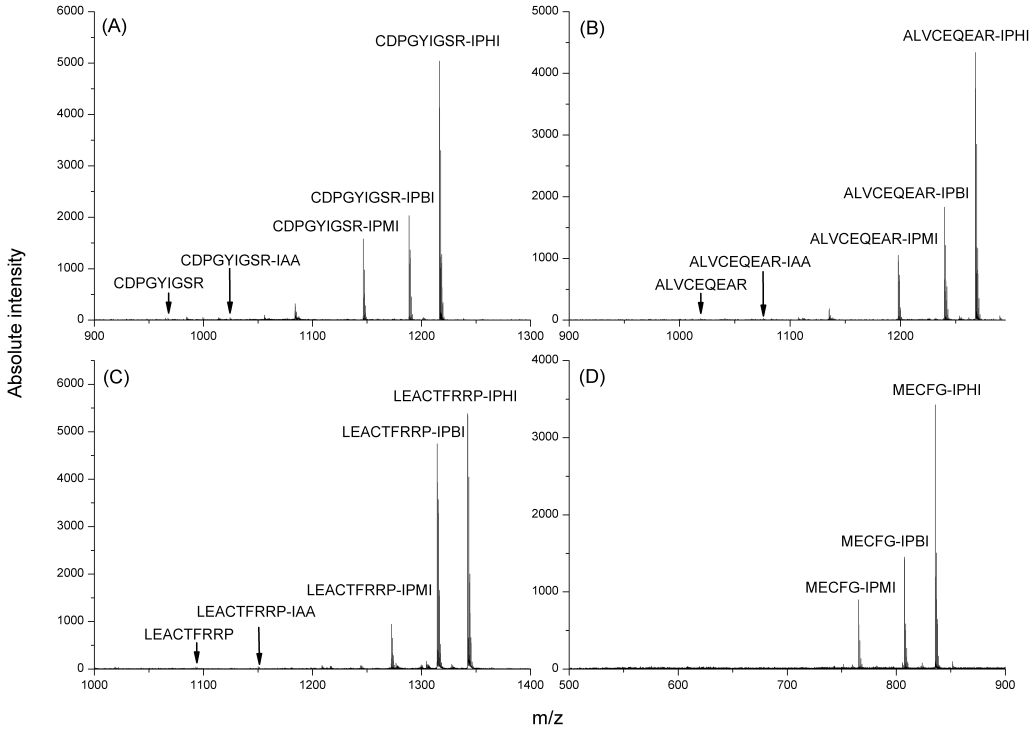

Fig. S6 MALDI-TOF MS spectra of equimolar mixture of IAA, IPMI, IPBI, and IPHI derivatized peptides CDPGYIGSR (A), ALVCEQEAR (B), LEACTFRRP (C), or MECFG (D) with its native cognates.

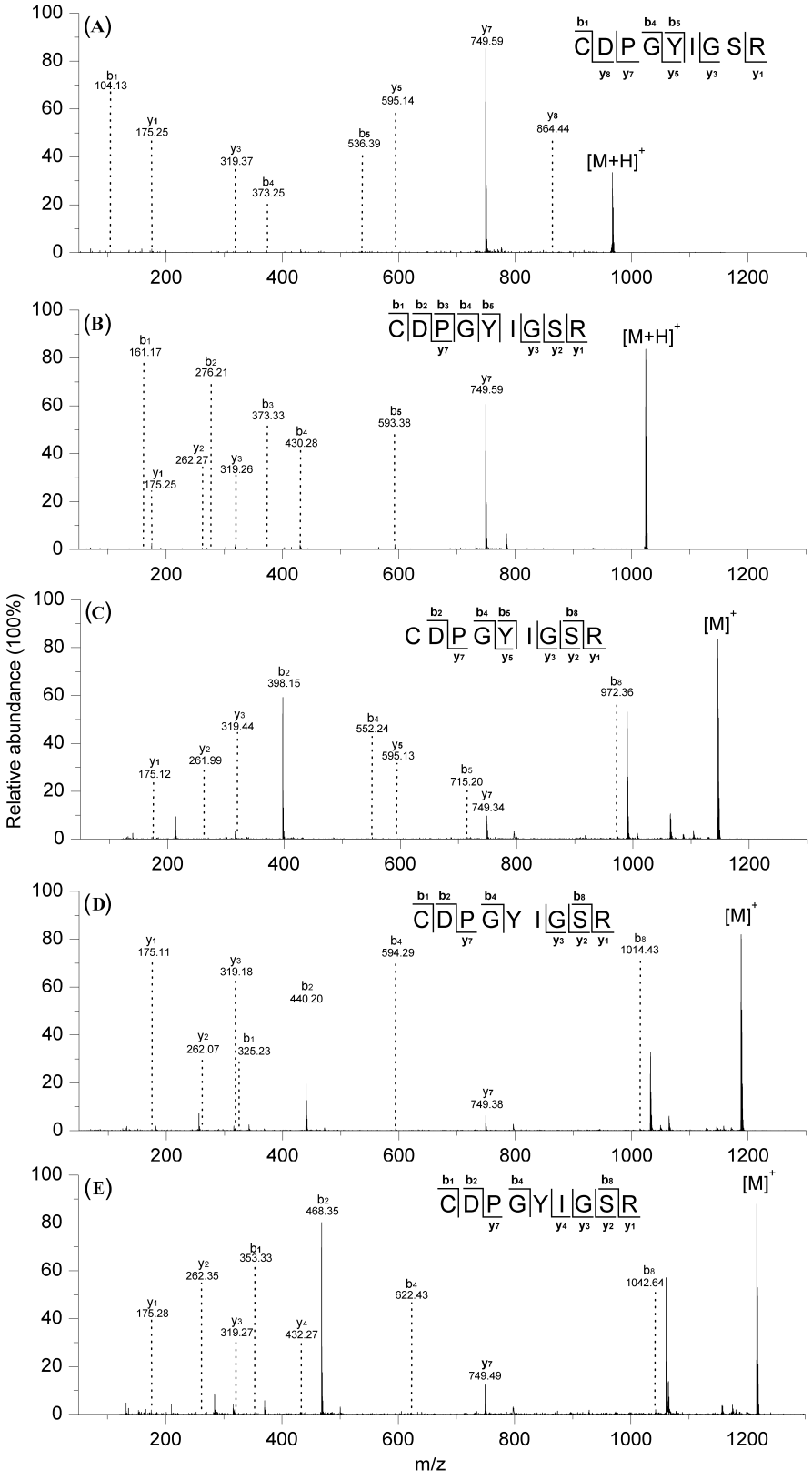

Fig. S7 Collision-induced dissociation (CID) product ion mass spectra of native peptide CDPGYIGSR (A) and that respectively derivatized by IAA (B), IPMI (C), IPBI (D), and IPHI (E).

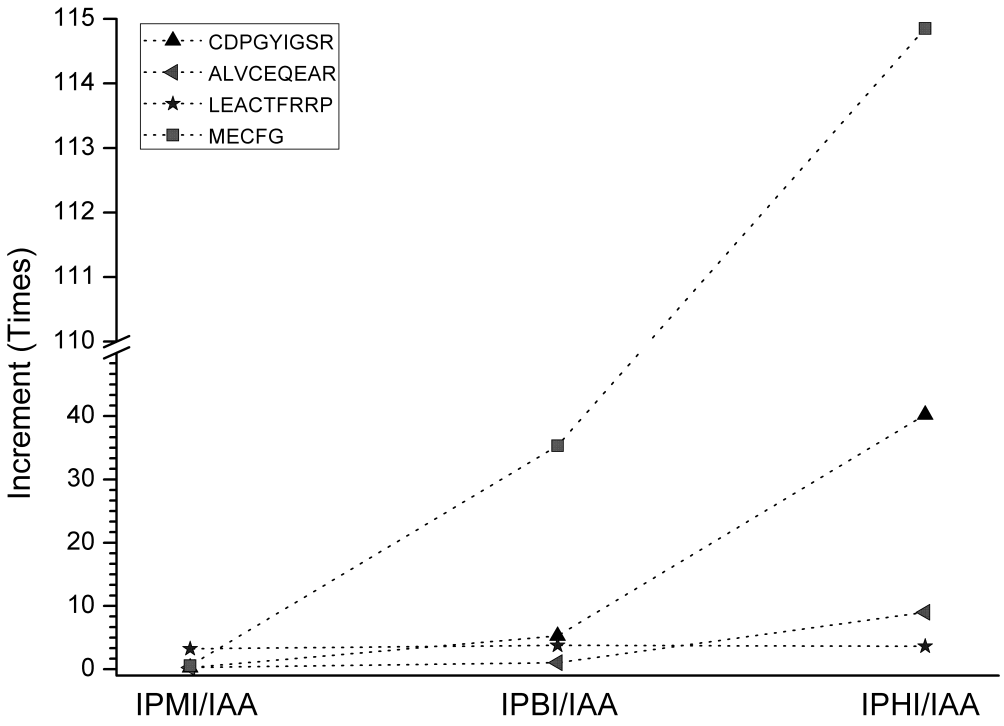

Fig. S8 Effect of derivatization on the ionization efficiency increment via IPMI, IPBI, and IPHI in ESI MS. Y axis represents the ratio of S/N of peptides derivatized by IPMI, IPBI, or IPHI to the S/N of the IAA modified counterparts.

Table S1. The identified cysteinyl-peptides from bovine serum albumin modified with IAA and IPHI

No	Position	Peptide sequence	No of cysteines	IAA	IPHI
1	76-88	K.TCVADESHAGCEK.S	2	√	√
2	89-100	K.SLHTLFGDELCK.V	1	√	√
3	106-117	R.ETYGDMADCCEK.Q	2		√
4	118-138	K.QEPERNECFLSHKDDSPDLPK.L	1	√	
5	139-151	K.LKPDPNTLCDEFK.A	1		√
6	139-155	K.LKPDPNTLCDEFKADEK.K	1	√	√
7	198-204	K.GACLLPK.I	1		√
8	223-228	R.CASIQK.F	1		√
9	264-285	K.VHKECCHGDLLECADDRADLAK.Y	3	√	
10	286-297	K.YICDNQDTISSK.L	1	√	
11	298-309	K.LKECCDKPLLEK.S	2	√	√
12	300-309	K.ECCDKPLLEK.S	2		√
13	310-340	K.SHCIAEVEKDAIPENLPPLTADFAEDKDVCK.N	2	√	
14	375-386	K.EYEATLEECCAK.D	2		√
15	387-399	K.DDPHACYSTVFDK.L	1		√
16	387-401	K.DDPHACYSTVFDKLK.H	1		√
17	413-420	K.QNCDQFEK.L	1	√	
18	460-468	R.CCTKPESER.M	2	√	√
19	469-482	R.MPCTEDYLSLILNR.L	1		√
20	483-489	R.LCVLHEK.T	1		√
21	483-495	R.LCVLHEKTPVSEK.V	1	√	√
22	483-498	R.LCVLHEKTPVSEKVTK.C	1	√	
23	499-507	K.CCTESLVNR.R	2	√	√
24	508-523	R.RPCFSALTPDETYVPK.A	1	√	√
25	581-597	K.CCAADDKEACFAVEGPK.L	3	√	
26	588-597	K.EACFAVEGPK.L	1		√