Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Suppression of Matrix Ions by N-Phosphorylation Labeling Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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EXPERIMENTAL SECTION

Materials and Reagents. L-Amino acids, D-(+)-glucosamine hydrochloride, agmatine sulfate salt, formic acid, magnesium sulfate ($MgSO_4$), trifluoroacetic acid (TFA), triethylamine (TEA), tetrachloromethane (CCl_4), α-cyano-4-hydroxycinnamic acid (CHCA), and 2, 5-dihydroxybenzoic acid (DHB) were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Diisopropyl phosphate (DIPP-H) and anhydrous ethanol were obtained from Alfa Aesar Chemical Ltd. (Tianjin, China). Peptide calibration standard used for calibration of MALDI-MS instrument was obtained from Bruker Daltonics (Bruker, Germany). Sep-Pak Vac C18 cartridges were purchased from Waters (MA, USA). Porous graphitic carbon (PGC) cartridges were obtained from Alltech Associates, Inc. (Deerfield, IL). Graphene nonopowder (8 nm flakes) was obtained from Graphene Laboratories Inc. (Reading, MA). HPLC-grade acetonitrile (ACN) and methanol were purchased from Tedia (Fairfield, OH, USA). Deionized water (18 MΩ) used in all experiments was prepared from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Synthesis of N-Diisopropyl Phosphoryl Alanine (DIPP-Ala). The title compound was synthesized according to a literature method.[S1] In a typical procedure, alanine (10 mmol) was dissolved in a mixture of water (5 mL), triethylamine (2.5 mL), and ethanol (2.5 mL) and cooled in an ice-salt bath. Then, DIPP-H (12 mmol) in 5 mL tetrachloromethane was added dropwise to the above solution over a period of approximately 30 min. After an additional 30 min of stirring at room temperature, the reaction mixture was concentrated under reduced pressure in order to remove the organic solvents. After removing the organic phase, 5 mL water was added and sequentially washed with ethyl ether (10 mL) and ethyl acetate (10 mL). The aqueous solution was adjusted to pH 2.0-3.0 by addition of 1 M HCl and extracted three times with ethyl acetate (3×10 mL). After drying the organic layer with $MgSO_4$, the solvent was removed in vacuum to afford target product as colourless oil. The crude product was purified by crystallization from ethyl acetate to give colourless tiny crystals (yield 82 %). NMR data for DIPP-Ala (Fig. S5, Supporting Information): ^{31}P NMR (162 MHz, $CDCl_3$): δ = 5.8 ppm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23-1.30 (m, 12 H), 1.40 (d, J = 7.2 Hz, 3 H), 3.79 (dq, J = 7.2, 14.4 Hz, 1 H), 4.51-4.60 (m, 2 H), 4.81 (dd, J = 8.0, 12.0 Hz, 1 H), 11.89 (br, 1 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.9 (d, J = 10.9 Hz), 71.5 (d, J = 5.6 Hz), 71.2 (d, J = 5.5 Hz), 49.7, 23.6-23.8 (m), 20.8 (d, J = 2.6 Hz)

ppm.

Preparation of Standard Solutions. The standard solutions of amino acids, D-(+)-glucosamine hydrochloride, and agmatine sulfate salt were prepared by dissolving corresponding chemicals in water (0.1 % acetic acid) at a concentration of 10 mM as stock solution in 1.5 mL polypropylene microcentrifuge tubes. Urine sample (100 μL) was collected from a healthy male volunteer and mixed with 3-fold amount of methanol. The precipitated proteins were removed by centrifuging at 16.0 k rpm for 30 min. The treated urine sample was stored at -20 ˚C. The mixture solutions were prepared by mixing and diluting the stock solutions with water to give the proper concentrations of each analytes. For N-phosphorylation derivatization, two reaction solutions were prepared as follow: (A) ethanol/TEA (1:1, v/v); (B) DIPP-H/CCl_4 (1:5, v/v). All stock solutions were kept at -20 ˚C for further use.

N-Phosphorylation Derivatization of Amino Acids, Glucosamine, Agmatine. A modified Atherton-Todd reaction with quantitative yield was used for N-phosphorylation derivatization.[S1] The individual solutions of amino acids (100 μL), mixture of model amino acids (100 μL), glucosamine (100 μL), agmatine (100 μL) and urine sample (100 μL) were mixed with solution A (100 μL) in 1.5 mL tube, respectively. The reaction mixture was cooled to 0 ˚C in an ice-water bath. Then, solution B (60 μL) was added slowly into the above reaction mixture, which was mixed periodically. ^{31}P NMR could be applied to trace the reaction process. The mixture was allowed to proceed 30 min for obtaining completion at room temperature. After the N-phosphorylation labeling of amino group-contained molecules, the excess DIPP-H reagent would be activated further to react with water to form TEA salts of phosphoric acids, which could be easily removed by subsequent solid-phase extraction (SPE) desalting. Then, the reaction mixture was evaporated to dryness under reduced pressure. The reaction residues of standard amino acids, agmatine, and urine sample were dissolved in 250 μL H_2O (0.1 % formic acid) and desalted by SPE using reverse-phase Sep-Pak Vac C18 cartridge (Waters, MA, USA) according to the manufacture's instructions prior to MS analysis. For N-phosphoryl-labeled glucosamine, PGC cartridges were used to purify the reaction mixture. Briefly, PGC cartridges were washed with 3.0 mL ACN/water (4:1, v/v) containing 0.1 % TFA followed by 3.0 mL water. The reaction mixture was dissolved in 250 μL H_2O and loaded on PGC cartridges and then washed with 3 mL water in order to remove salts. Then, N-phosphoryl glucosamine was eluted with ACN/water (1:1, v/v)

containing 0.1 % TFA and collected for MALDI-MS analysis.

Mass Spectrometry Analysis. MALDI-TOF MS analyses were performed on a Bruker Autoflex II mass spectrometer (Bruker Daltonics, Bremen, Germany) in positive reflection mode. The mass spectrometer was equipped with a pulsed nitrogen laser operated at 337 nm with 3 ns duration pulses and employed stainless steel targets (MTP 384 target ground steel, Bruker Daltonics). Voltage impressed on the ion one and two was 20.0 and 19.0 kV, respectively. The laser power energy was adjusted as needed. The acceleration voltage, grid voltage, and delayed extraction time were set as 19 kV, 90 %, and 150 ns, respectively. Each mass spectrum was acquired as an average of 500 laser shots at 10.0 Hz frequency and summed over at least 15 different locations on the target spot. Matrix CHCA was dissolved in ACN/water (1:1, v/v) containing 0.1 % TFA to get a saturated matrix solution. DHB matrix was prepared at a concentration of 20 mg/mL in ACN/water (1:1, v/v) with 0.1 % TFA. Graphene nanoparticles (2 mg) was dispersed in a 1 mL solution of ethanol/water (1:1, v/v) and sonicated for 3 min prior to use. Samples were prepared by applying 1 μL mixture solution (1:1, v/v) of sample and matrix onto a stainless steel MALDI target plate and allowing the droplet to dry in the air at room temperature before transferring into mass spectrometer.

REFERENCE

S1 G. J. Ji, C. B. Xue, J. N. Zeng, L. P. Li, W. G. Chai and Y. F. Zhao, *Synthesis* 1988, **6**, 444-448.

List of Figures:

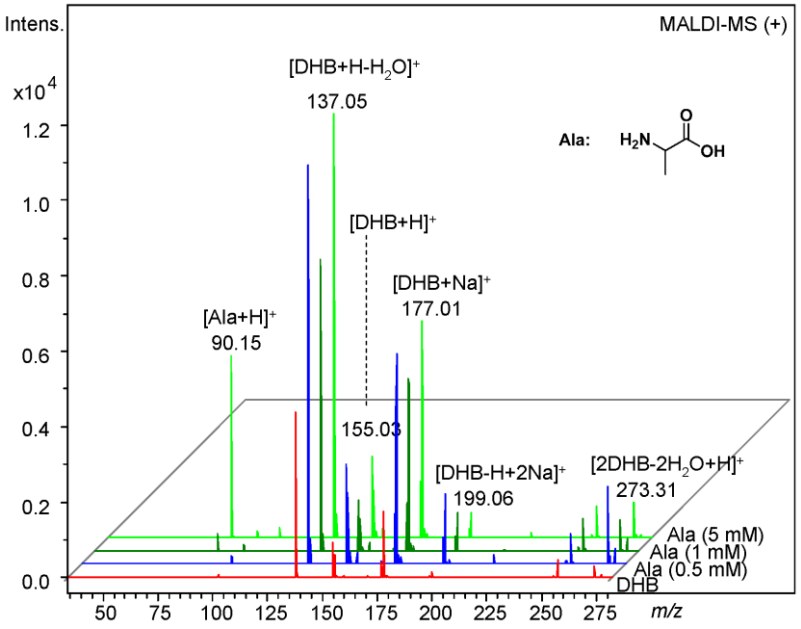

Fig. S1 MALDI-TOF MS spectra of Ala at different concentrations (0 mM, 0.5 mM, 1.0 mM, and 5.0 mM) with DHB as matrix in positive ion mode.

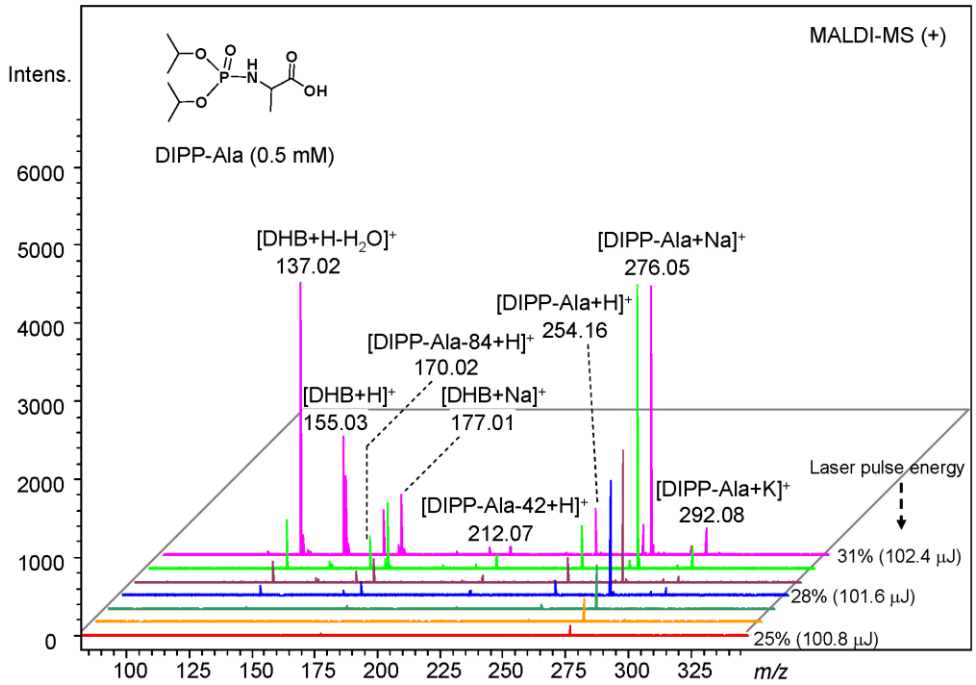

Fig. S2 MALDI-TOF MS spectra of DIPP-Ala (0.5 mM) under different laser pulse energy (25 % to 31 %) with DHB as matrix in positive ion mode.

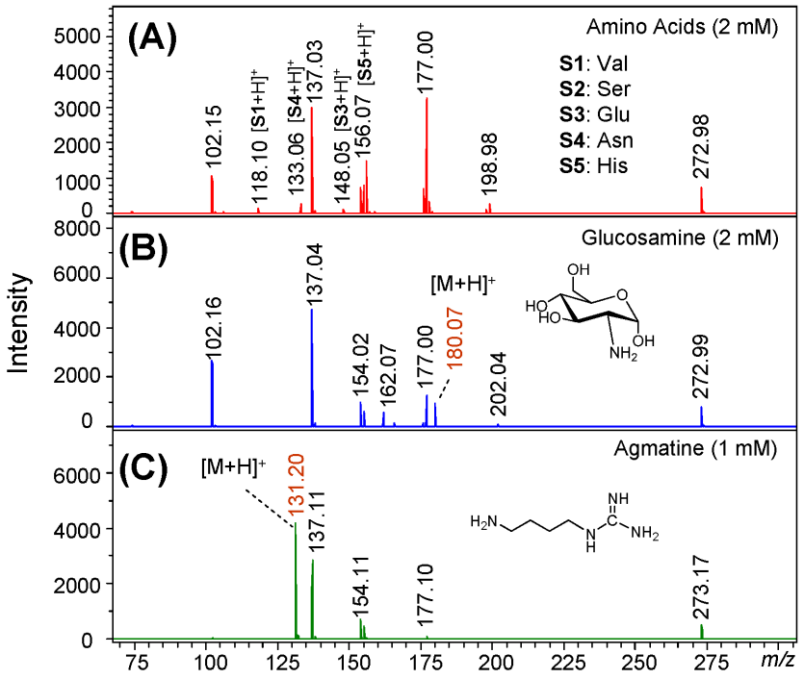

Fig. S3 MALDI-TOF MS spectra of amino acids (A), glucosamine (B), and agmatine (C) with DHB as matrix in positive ion mode.

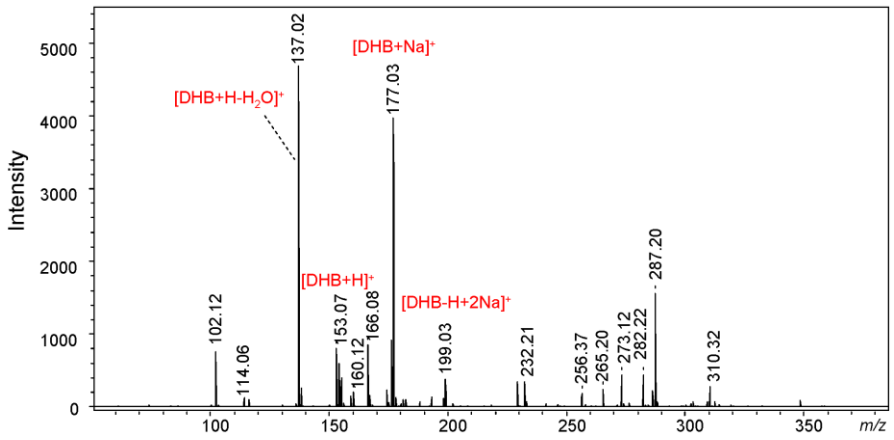

Fig. S4 MALDI-TOF MS spectrum of amino acids in urine sample with DHB as matrix in positive ion mode.

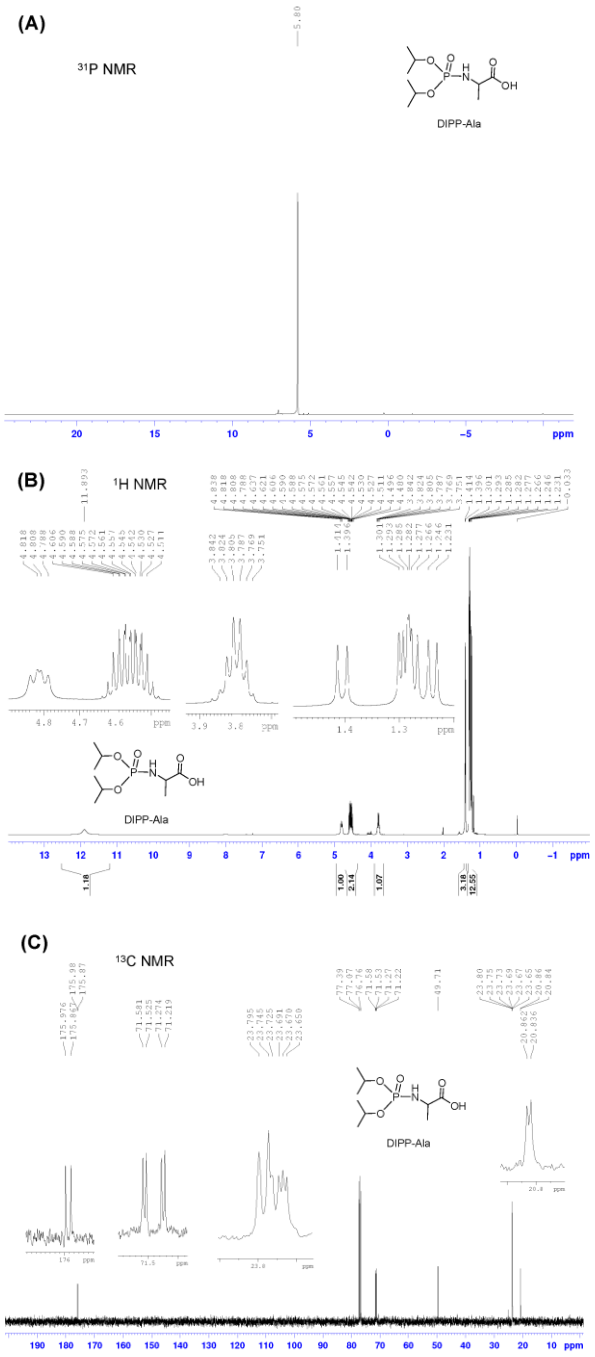

Fig. S5 NMR spectra of DIPP-Ala. (A) ^{31}P NMR spectrum; (B) ^{1}H NMR spectrum; (C) ^{13}C NMR spectrum. The insets are the chemical structures of DIPP-Ala.