A Plausible Model Correlates Prebiotic Peptide Synthesis with Primordial Genetic Code

Jianxi Ying,[a] Songsen Fu,[a] Xin Li,[a] Liubin Feng,[a] Pengxiang Xu,[a] Yan Liu,[a*] Xiang Gao,[d] and Yufen Zhao [a,b,c*]

[a] Department of Chemical Biology, Key Laboratory for Chemical Biology of Fujian Province and High-Field NMR Center, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, Fujian, China

[b] Institute of Drug Discovery Technology, Ningbo University, Ningbo 315211, Zhejiang, China

[c] Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, China

[d] School of Pharmaceutical Sciences, Xiamen University, Xiamen 361005, Fujian, China

* Corresponding authors: zhaoyufen@nbu.edu.cn (Yufen Zhao); stacyliu@xmu.edu.cn (Yan Liu)

Supporting Information

Contents

1 Materials

Proline (Pro), methionine (Met), glycine (Gly), valine (Val), histidine (His), isoleucine (Ile), leucine (Leu), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), alanine (Ala), serine (Ser), phenylalanine (Phe), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), lysine (Lys), adenosine (A), guanosine (G), cytidine (C), uridine (U) and dipeptides were obtained from Aladdin Ltd.. Unless otherwise noted, the amino acids used here are all L- configuration. Trimetaphosphate and Nonafluoropentanoic acid (NFPA) were purchased from Sigma Aldrich. Analytical reagents including methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from SpectrumChemical. Formic acid and ammonium acetate were purchased from Sigma Aldrich. Ultrapure water (18.2 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, MA) was used to prepare solutions and the mobile phase.

2 General preparations for trapping the nucleotide amidates (aa-*N*-NMPs)

0.1 mmol sodium trimetaphosphate (P_3m) and 0.1 mmol amino acids (a total of 20) were mixed with 0.1 mmol nucleosides (a total of 4) in 1 mL water, respectively. The pH of the reaction mixture was adjusted to 11 using 10 M NaOH. Then the reaction mixtures were placed at 50 °C for 4.5 h. The reaction was quenched with 10 M NaOH solution (0.01 mL).

3 General preparations for detecting dipeptides

0.1 mmol sodium trimetaphosphate (P_3m) and 0.1 mmol amino acids (a total of 5) were mixed with 0.1 mmol nucleosides (a total of 4) in 1 mL water, respectively. The pH of the reaction mixture was adjusted to 11 using 10 M NaOH. Then the reaction mixtures were placed at 50 °C for 2 days. The reaction was quenched with 6 M HCl solution.

4 General preparations for continuous peptide synthesis

0.02 mmol sodium trimetaphosphate (P_3m), 0.01 mmol Phe dipeptide (Phe_2), 0.01 mmol nucleosides (a total of 4) with or without 0.01 mmol Phe were mixed with in 1 mL water, respectively. The initial reaction conditions were listed in the following table.

Substrates	Initial reaction conditions		
	pH	Tem. (°C)	Time (d)
Phe_2+P_3m+A	11	50	2
Phe_2+P_3m+G	11	50	2
Phe_2+P_3m+C	11	50	2
Phe_2+P_3m+U	11	50	2
Phe_2+P_3m+A+Phe	11	50	2
Phe_2+P_3m+G+Phe	11	50	2
Phe_2+P_3m+C+Phe	11	50	2
Phe_2+P_3m+U+Phe	11	50	2

5 Analysis methods

NMR

The ^{31}P (or P-H COSY) and ^{1}H (H-H COSY) measurements were performed on Bruker 600 MHz and 850 MHz NMR spectrometers at ambient temperature, respectively. The 2'-Phe-*N*-AMP and 2'-Phe-*N*-UMP were isolated and purified by liquid chromatography.

HPLC-MS

MS and MS^2 were performed on a Bruker micrOTOF-Q Ⅱ system in positive mode. The MS instrument parameters were as follows: capillary voltage of 4500 V, nebulizer pressure of 2 bar, dry gas of 8 L• min^{-1}, dry temperature at 200 °C. Mass spectra were registered in the scan range from *m/z*=50 to 1000. For ESI-MS, about 1/10 of the eluate from LC was introduced through a splitting T valve. As for the on-line detection of the reaction product by HPLC-MS, we set up the divert valve of the MS instrument as follows: 1) when the divert valve was in waste position, the valve can be used for switching HPLC flows directly to waste for about 3 min; 2) after that, when the divert valve was in source position, the valve can be used for switching HPLC flows directly to MS.

The HPLC was performed on Agilent 1260 Infinity system and fitted with an Agilent TC- C18, 5 μm, 4.6 mm × 150 mm column. The column temperature was maintained at room temperature. The HPLC flow rate was 0.8 mL• min^{-1} with TC-C18.

According to the different properties of aa-*N*-NMPs, the following three chromatographic procedures were used. (Table S1)

Table S1 Methods of HPLC analysis

Chromatographic procedures	Solvent A	Solvent B	aa-*N*-NMP (total of 56)
0-14 min, 5 % B; 14-20 min, 5-60 % B; 20-22 min, 60 % B; 22-24 min, 60-5 % B; 24-30 min, 5% B. (a total of 39)	5 mM NH_4Ac	ACN	Phe-*N*-AMP, Ile-*N*-AMP, His-*N*-AMP, Met-*N*-AMP, Tyr-*N*-AMP, Ser-*N*-AMP, Gly-*N*-AMP, Thr-*N*-AMP, Gln-*N*-AMP, Cys-*N*-AMP, Trp-*N*-AMP, Leu-*N*-AMP, Arg-*N*-AMP, Val-*N*-AMP, Ala-*N*-AMP, Lys-*N*-AMP, Glu-*N*-AMP, Asn-*N*-AMP, Phe-*N*-GMP, Ile-*N*-GMP, His-*N*-GMP, Met-*N*-GMP, Tyr-*N*-GMP, Ser-*N*-GMP, Trp-*N*-GMP, Leu-*N*-GMP, Arg-*N*-GMP, Val-*N*-GMP, Lys-*N*-GMP, Phe-*N*-CMP, Ile-*N*-CMP, Gly-*N*-CMP, Trp-*N*-CMP, Leu-*N*-CMP, Glu-*N*-CMP, Phe-*N*-UMP,

			Ile-*N*-UMP, Trp-*N*-UMP, Leu-*N*-UMP
0-2 min, 5 % B; 2-22 min, 5-70 % B; 22-24 min, 70 % B; 24-26 min, 70-5 % B; 26-31 min, 5% B. (a total of 14)	2 mM Nonafluoropentanoic acid (NFPA)	ACN	Asp-*N*-AMP, Ala-*N*-AMP, His-*N*-CMP, Met-*N*-CMP, Tyr-*N*-CMP, Ser-*N*-CMP, Thr-*N*-CMP, Arg-*N*-CMP, Val-*N*-CMP, Ala-*N*-CMP, Asp-*N*-CMP, His-*N*-UMP, Met-*N*-UMP, Lys-*N*-UMP
0-10 min, 5 % B; 10-22 min, 5-70 % B; 22-24 min, 70 %B; 24-26 min, 0-5 %B; 26-31 min, 5% B (a total of 3)	2 mM NFPA	ACN	Tyr-*N*-UMP, Arg-*N*-UMP, Val-*N*-UMP

HPLC method of Fig. 3 and Fig. 4

Solvent A was 0.1 % formic acid and solvent B was methyl alcohol. A 30 min gradient method was used: 0-10 min, 5-30 % B; 10-20 min, 30-80 % B; 20-22 min, 80 % B, 22-24 min 80-5 % B, 24-30 min 5% B. Column temperature was maintained at room temperature.

HPLC method of Fig. 5

Solvent A was 5 mM ammonium acetate and solvent B was acetonitrile. A 30 min gradient method was used: 0-14 min, 5 % B; 14-20 min, 5-60 % B; 20-23 min, 60 % B, 23-25 min 60-5 % B, 25-30 min 5% B. The column temperature was maintained at room temperature. The reaction of 2'-Phe-*N*-AMP and Phe was carried out under alkaline aqueous.

The amount of purified 2'-Phe-*N*-AMP was very small and structurally unstable. In order to simultaneously detect the product Phe_2 and the possible residual 2'-Phe-*N*-AMP, we adjusted the liquid phase method to the above method which was different from the method of 2.

HPLC method of Fig. S25 (for detecting Phe3)

Solvent A was 5 mM ammonium acetate and solvent B was acetonitrile. A 40 min gradient method was used: 0-10 min, 5 % B; 10-28 min, 5-90 % B; 28-30 min, 90 % B, 30-34 min 90-5 % B, 34-40 min 5% B. The column temperature was maintained at room temperature.

6 Table S2 Accurate mass measurements of aa-*N*-NMPs.

aa	aa-*N*-NMP / *m/z*	aa- *N*-AMP [M+H]$^+$	aa- *N*-GMP [M+H]$^+$	aa- *N*-CMP [M+H]$^+$	aa- *N*-UMP [M+H]$^+$	aa	aa-*N*-NMP / *m/z*	aa- *N*-AMP [M+H]$^+$	aa- *N*-GMP [M+H]$^+$	aa- *N*-CMP [M+H]$^+$	aa- *N*-UMP [M+H]$^+$
Phe	*Cal*.[a]	495.1393	511.1342	471.1281	472.1121	Trp	*Cal.*	534.1502	550.1451	510.1390	511.1230
	Exp.[b]	495.1393	511.1342	471.1280	472.1119		*Exp.*	534.1500	550.1451	510.1389	511.1223
	Δ ppm[c]	0	0	0.2	0.4		*Δ ppm*	0.4	0	0.2	1.4
Ile	*Cal.*	461.1550	477.1499	437.1437	438.1278	Leu	*Cal.*	461.1550	477.1499	437.1437	438.1278
	Exp.	461.1550	477.1497	437.1430	438.1276		*Exp.*	461.1553	477.1489	437.1433	438.1270
	Δ ppm	0	0.4	1.6	0.5		*Δ ppm*	0.7	2.1	0.9	1.8
His	*Cal.*	485.1298	501.1247	461.1186	462.1026	Arg	*Cal.*	504.1720	520.1669	480.1608	481.1448
	Exp.	485.1297	501.1247	461.1180	462.1015		*Exp.*	504.1721	520.1668	480.1590	481.1485
	Δ ppm	0.2	0	1.3	2.4		*Δ ppm*	0.2	0.2	3.7	7.7
Met	*Cal.*	479.1114	495.1063	455.1002	456.0842	Val	*Cal.*	447.1393	463.1342	423.1281	424.1121
	Exp.	479.1114	495.1070	455.0994	456.0847		*Exp.*	447.1393	463.1343	423.1269	424.1123
	Δ ppm	0	1.4	1.8	1.1		*Δ ppm*	0	0.2	2.8	0.5
Tyr	*Cal.*	511.1342	527.1292	487.1230	488.1070	Ala	*Cal.*	419.1080	435.1029	395.0968	396.0808
	Exp.	511.1342	527.1290	487.1232	488.1070		*Exp.*	419.1079	435.1030	395.0961	ND[d]
	Δ ppm	0	0.4	0.4	0		*Δ ppm*	0.2	0.2	1.8	---
Ser	*Cal.*	435.1029	451.0979	411.0917	412.0757	Lys	*Cal.*	476.1659	492.1608	452.1546	453.1387
	Exp.	435.1033	451.0967	411.0923	ND		*Exp.*	476.1647	492.1607	ND	453.1361
	Δ ppm	0.9	2.7	1.5	---		*Δ ppm*	2.5	0.2	---	5.7
Gly	*Cal.*	405.0924	421.0873	381.0811	382.0652	Glu	*Cal.*	477.1135	493.1084	453.1023	454.0863
	Exp.	405.0916	ND	381.0799	ND		*Exp.*	477.1144	ND	453.1024	ND
	Δ ppm	2.0	---	3.1	---		*Δ ppm*	1.9	---	0.2	---
Thr	*Cal.*	449.1186	465.1135	425.1074	426.0914	Asp	*Cal.*	463.0979	479.0928	439.0866	440.0706
	Exp.	449.1171	ND	425.1068	ND		*Exp.*	463.0985	ND	439.0868	ND
	Δ ppm	3.3	---	1.4	---		*Δ ppm*	1.3	---	0.5	---
Gln	*Cal.*	476.1295	492.1244	452.1183	453.1023	Asn	*Cal.*	462.1138	478.1088	438.1026	439.0866
	Exp.	476.1289	ND	ND	ND		*Exp.*	462.1148	ND	ND	ND
	Δ ppm	1.3	---	---	---		*Δ ppm*	2.2	---	---	---
Cys	*Cal.*	451.0801	467.075	427.0689	428.0529	Pro	*Cal.*	445.1237	461.1186	421.1124	422.0965
	Exp.	451.0827	ND	ND	ND		*Exp.*	ND	ND	ND	ND
	Δ ppm	5.8	---	---	---		*Δ ppm*	---	---	---	---

[a] *Cal.*: The calculated value of the compound [M+H]$^+$. [b] *Exp.*: The experimental value the compound [M+H]$^+$.

[c] *Δ ppm*: The relative error between the calculated value and the experimental value. Most of *Δ ppm* are less than 5 ppm. [d] ND: The compound was not detected.

7 HPLC-MS spectra of aa-*N*-NMPs

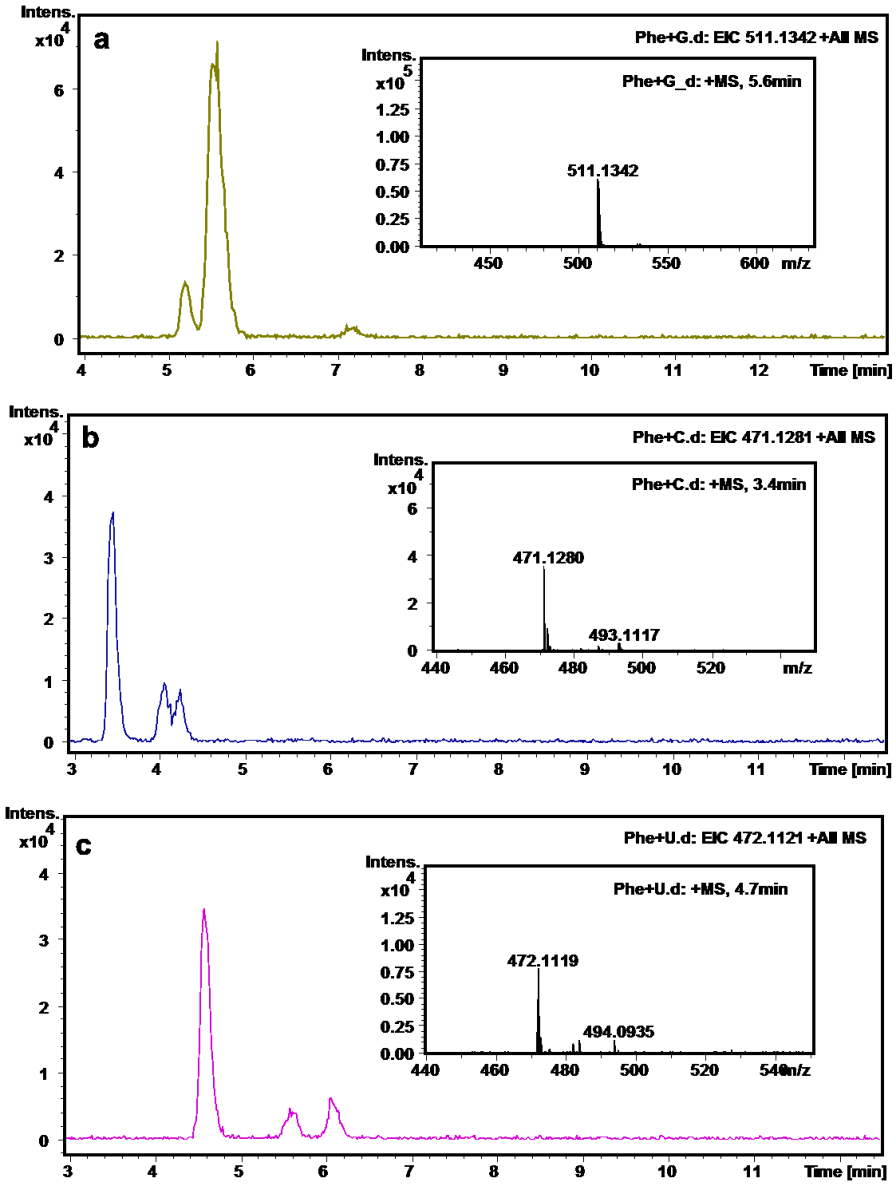

Fig. S1 HPLC-HRMS extracted ion chromatogram (EIC) spectra of $[M+H]^+$ for the product Phe-*N*-NMP of Phe with nucleosides. NMP: GMP, CMP and UMP. The EIC spectra of $[M+H]^+$ give three distinct peaks, which indicated that Phe-*N*-NMP have three isomers, namely 2'-Phe-*N*-NMP, 3'-Phe-*N*-NMP and 5'-Phe- *N*-NMP. a) reaction of Phe and P_3m with guanosine; b) reaction of Phe and P_3m with cytidine; c) reaction of Phe and P_3m with uridine.

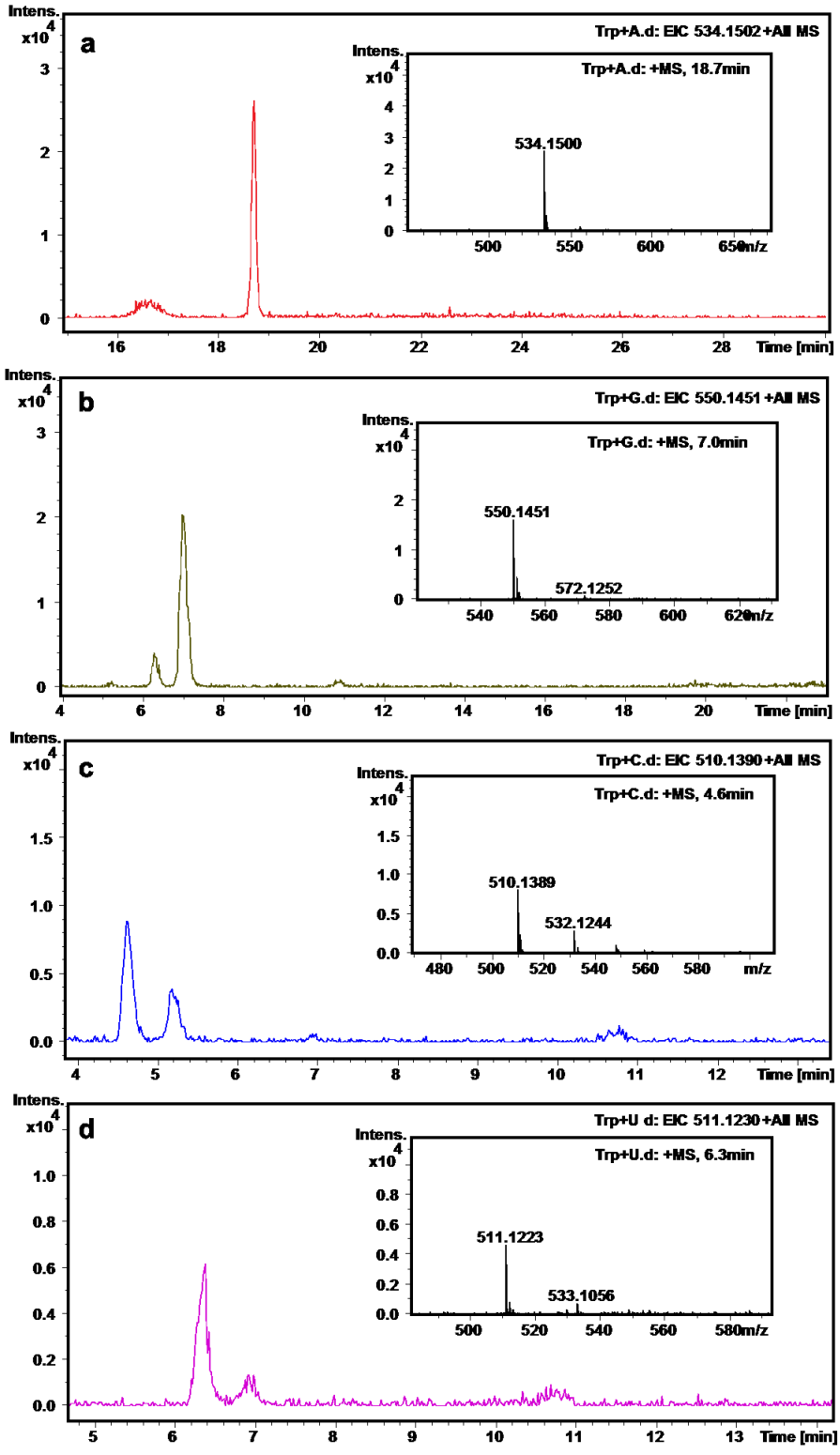

Fig. S2 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Trp-*N*-NMP of Trp with nucleosides. The EIC spectra of $[M+H]^+$ give 2~3 distinct peaks, which indicated that the Trp-*N*-NMP have three isomers and it might be that the polarity differences of some isomers are too small so that their peaks are overlapped. a) reaction of Trp and P_3m with adenosine; b) reaction of Trp and P_3m with guanosine; c) reaction of Trp and P_3m with cytidine; d) reaction of Trp and P_3m with uridine.

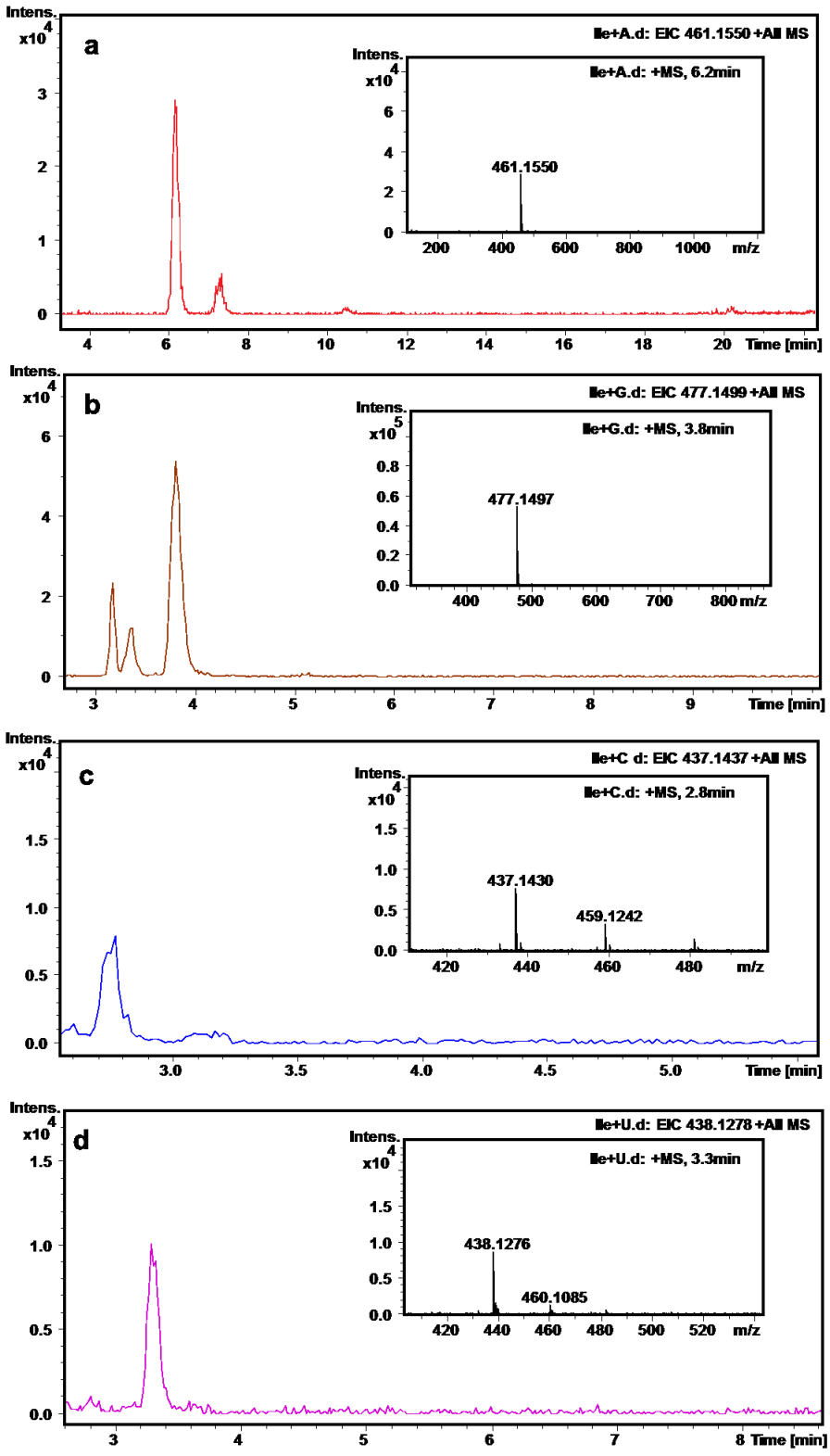

Fig. S3 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Ile-*N*-NMP of Ile with nucleosides. The EIC spectra of $[M+H]^+$ give 2~3 distinct peaks, which indicated that the Ile-*N*-NMP have three isomers and it might be that the polarity differences of some isomers are too small so that their peaks are overlapped. a) reaction of Ile and P_3m with adenosine; b) reaction of Ile and P_3m with guanosine; c) reaction of Ile and P_3m with cytidine; d) reaction of Ile and P_3m with uridine.

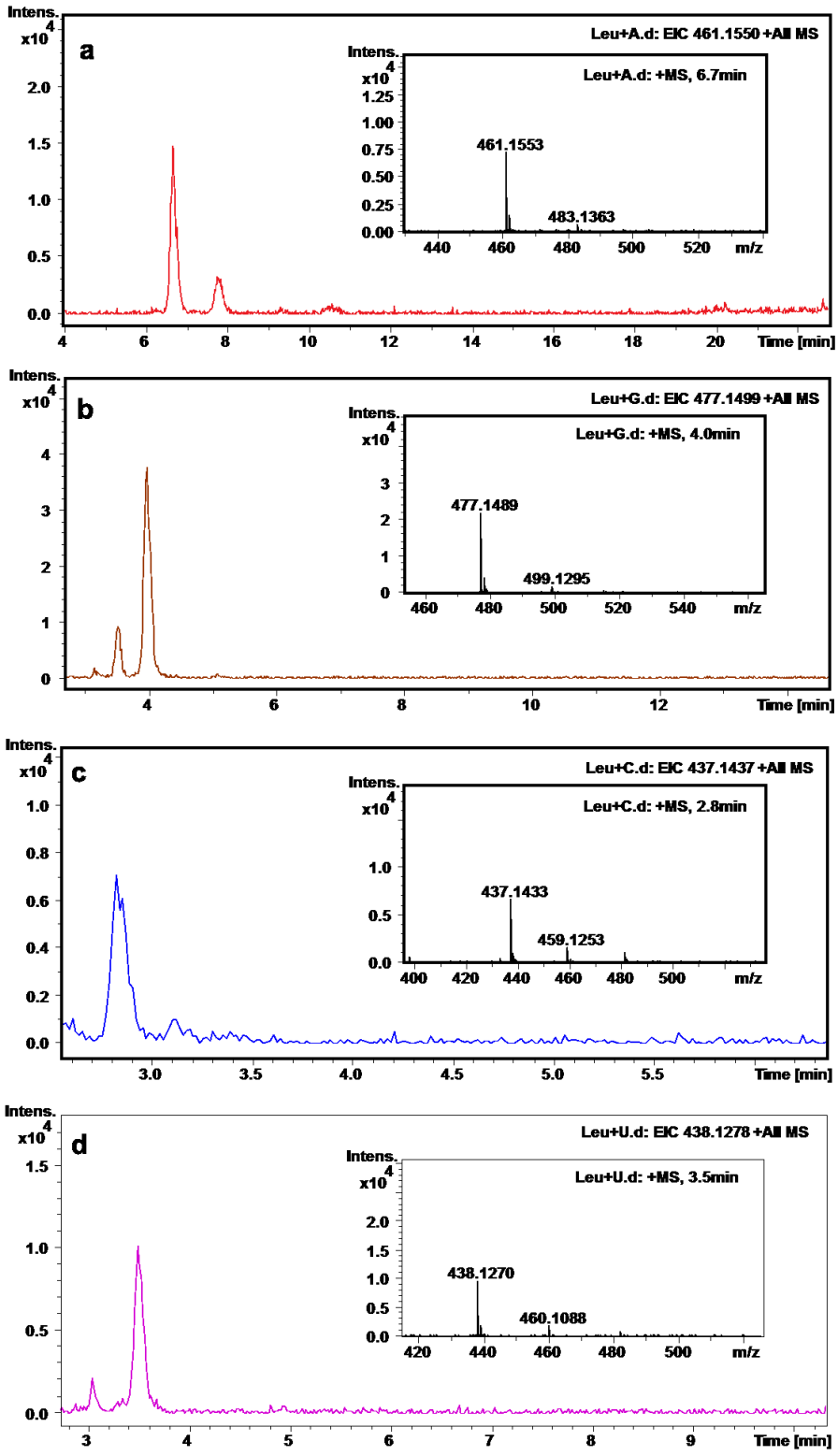

Fig. S4 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Leu-*N*-NMP of Leu with nucleosides. The EIC spectra of $[M+H]^+$ give 2~3 distinct peaks, which indicated that the Leu-*N*-NMP have three isomers and it might be that the polarity differences of some isomers are too small so that their peaks are overlapped. a) reaction of Leu and P_3m with adenosine; b) reaction of Leu and P_3m with guanosine; c) reaction of Leu and P_3m with cytidine; d) reaction of Leu and P_3m with uridine.

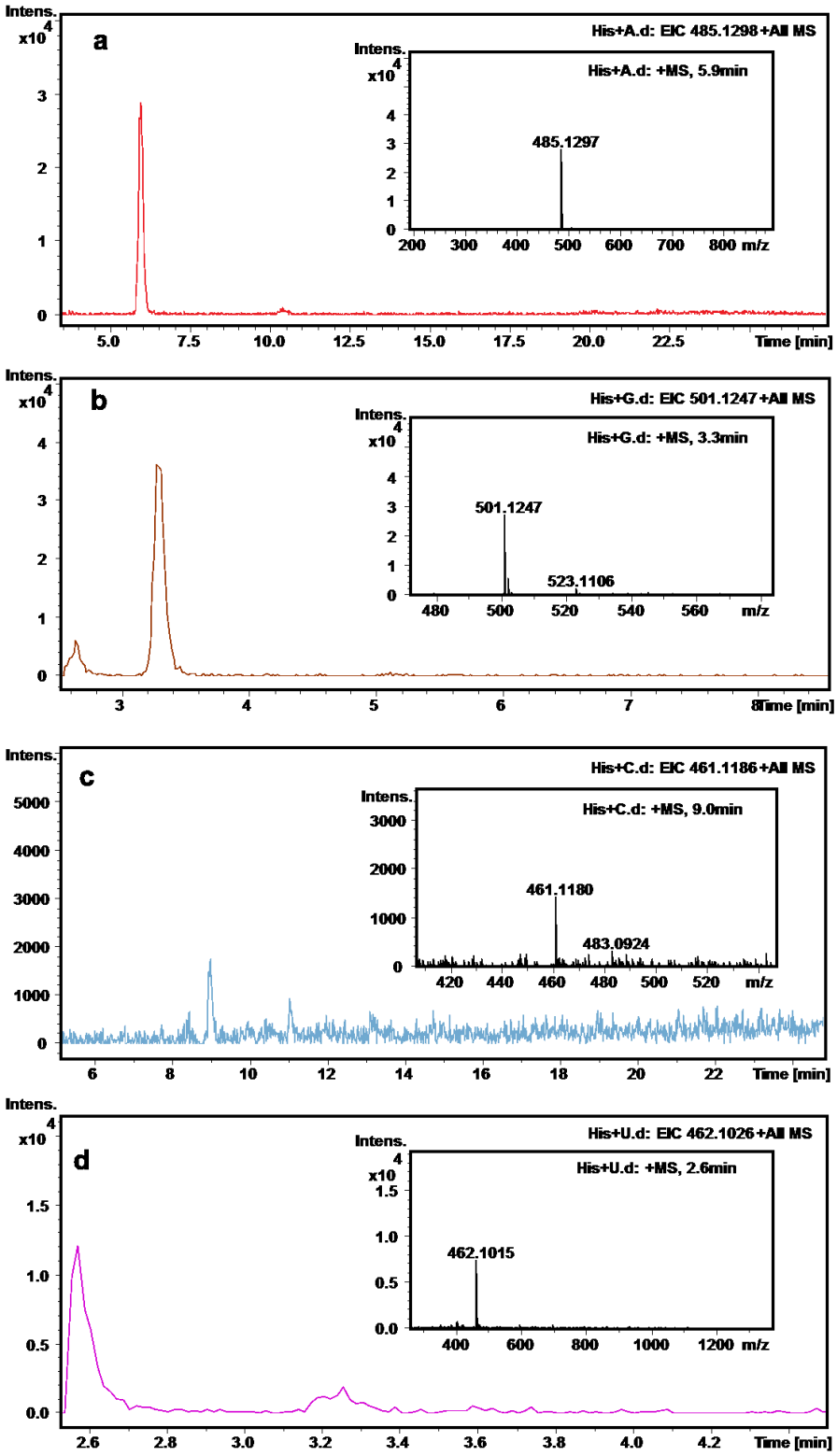

Fig. S5 HPLC-MS-EIC spectra of $[M+H]^+$ for the product His-*N*-NMP of His with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the His-*N*-NMP are too small so that their peaks are overlapped. a) reaction of His and P_3m with adenosine; b) reaction of His and P_3m with guanosine; c) reaction of His and P_3m with cytidine; d) reaction of His and P_3m with uridine.

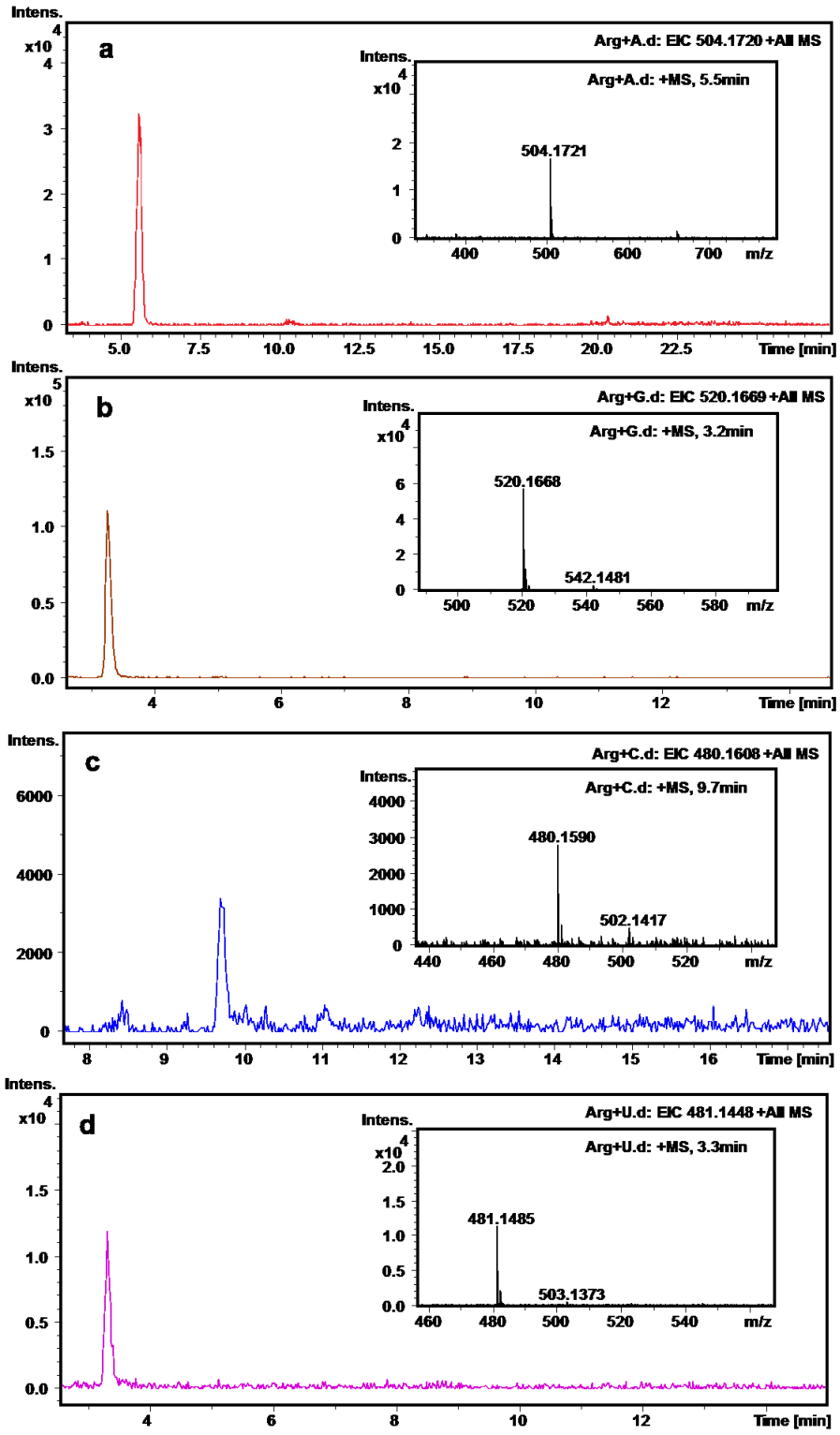

Fig. S6 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Arg-*N*-NMP of Arg with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Arg-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Arg and P_3m with adenosine; b) reaction of Arg and P_3m with guanosine; c) reaction of Arg and P_3m with cytidine; d) reaction of Arg and P_3m with uridine.

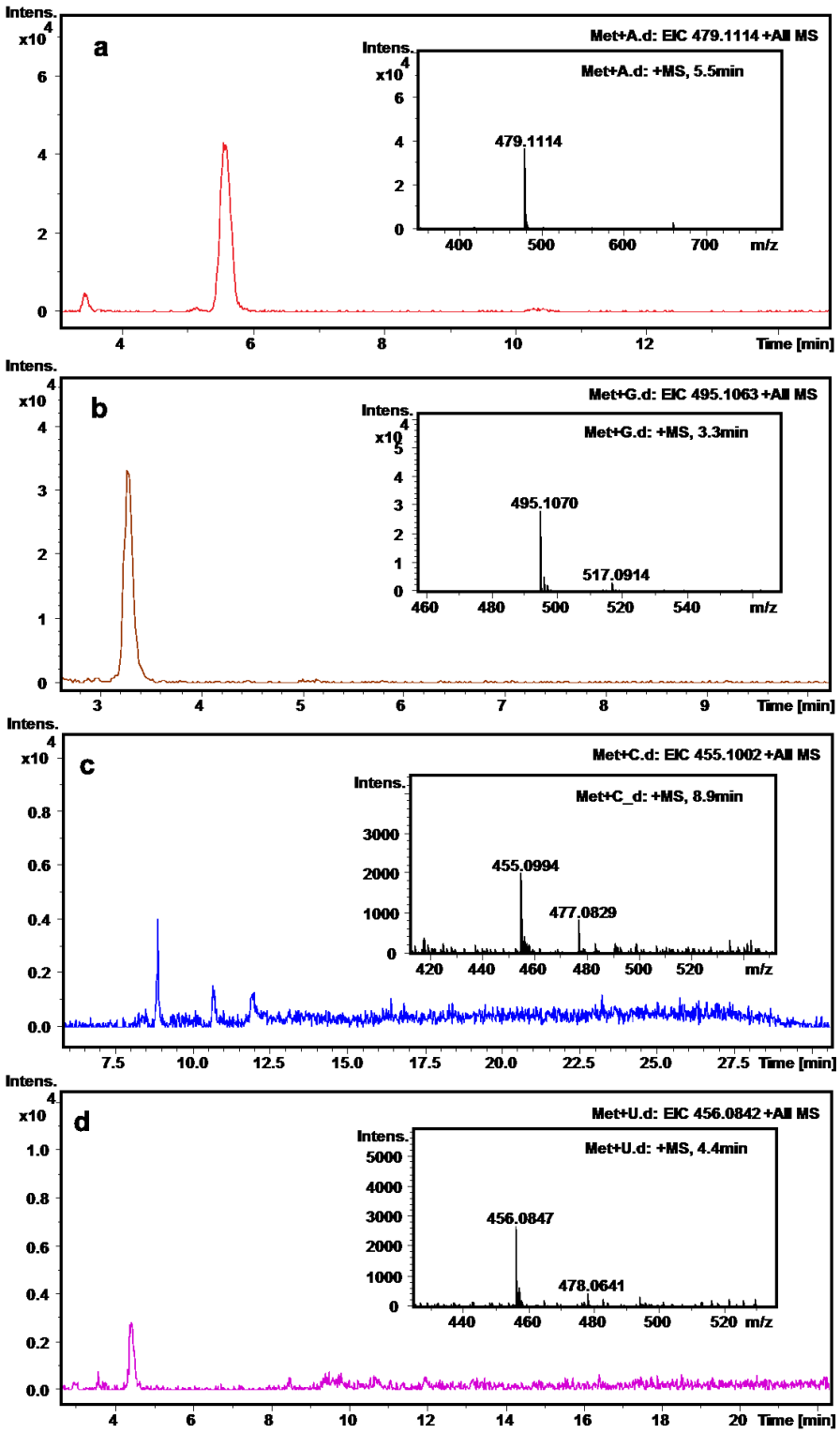

Fig. S7 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Met-*N*-NMP of Met with nucleosides. The EIC spectra of $[M+H]^+$ give 1~3 distinct peaks, which indicated that the Met-*N*-NMP have three isomers and it might be that the polarity differences of some isomers are too small so that their peaks are overlapped. a) reaction of Met and P_3m with adenosine; b) reaction of Met and P_3m with guanosine; c) reaction of Met and P_3m with cytidine; d) reaction of Met and P_3m with uridine.

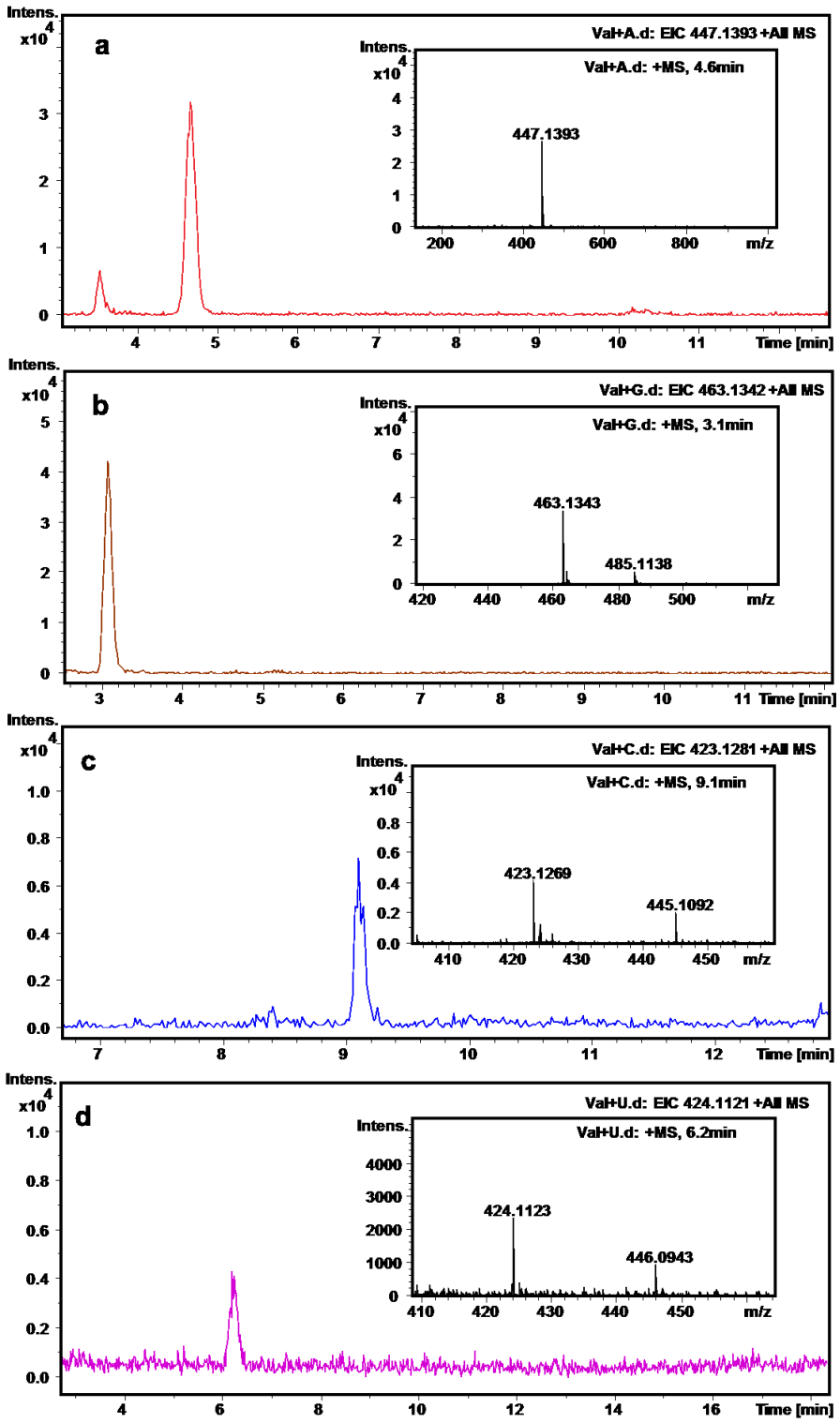

Fig. S8 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Val-*N*-NMP of Val with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Val-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Val and P_3m with adenosine; b) reaction of Val and P_3m with guanosine; c) reaction of Val and P_3m with cytidine; d) reaction of Val and P_3m with uridine.

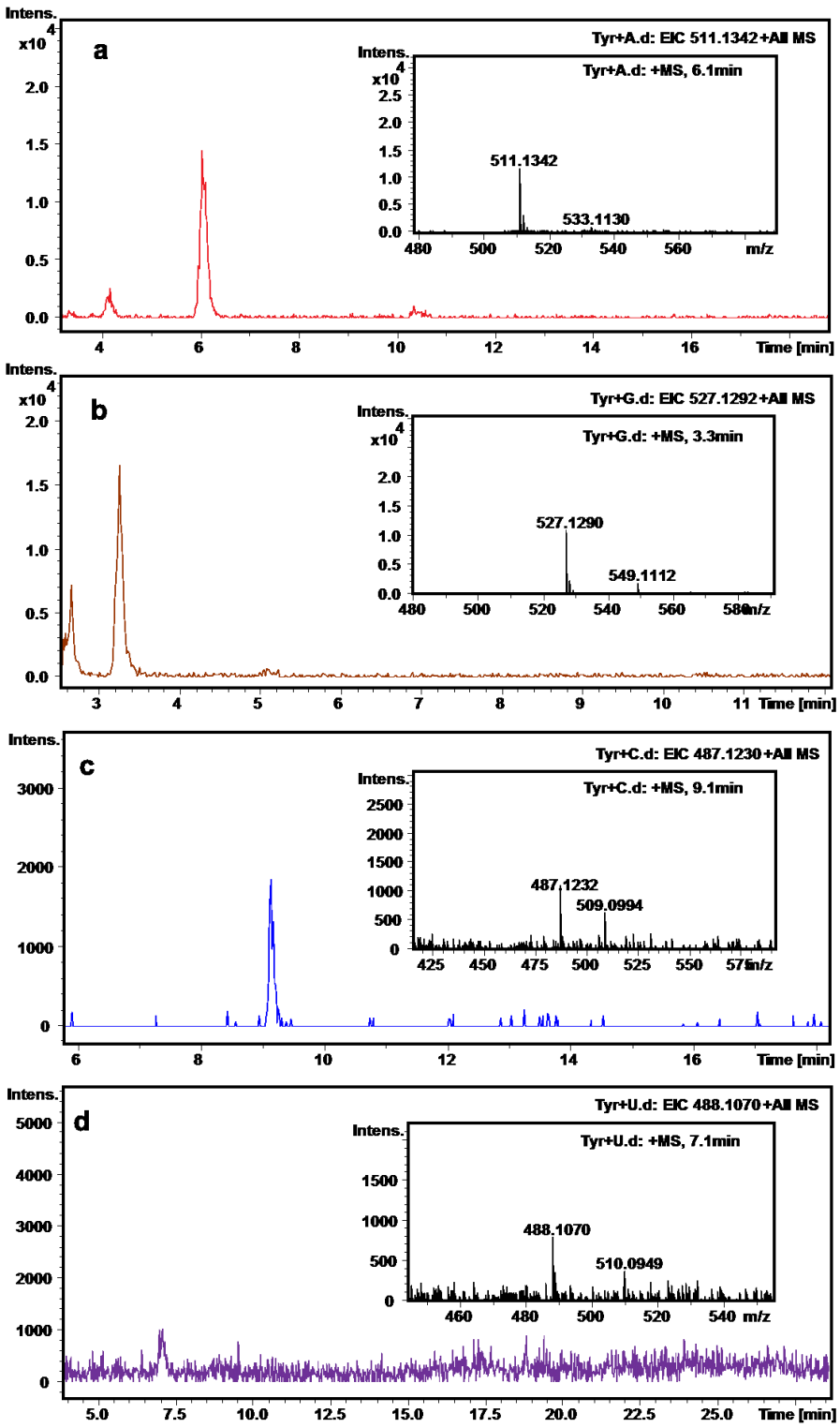

Fig. S9 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Tyr-*N*-NMP of Tyr with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Tyr-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Tyr and P_3m with adenosine; b) reaction of Tyr and P_3m with guanosine; c) reaction of Tyr and P_3m with cytidine; d) reaction of Tyr and P_3m with uridine.

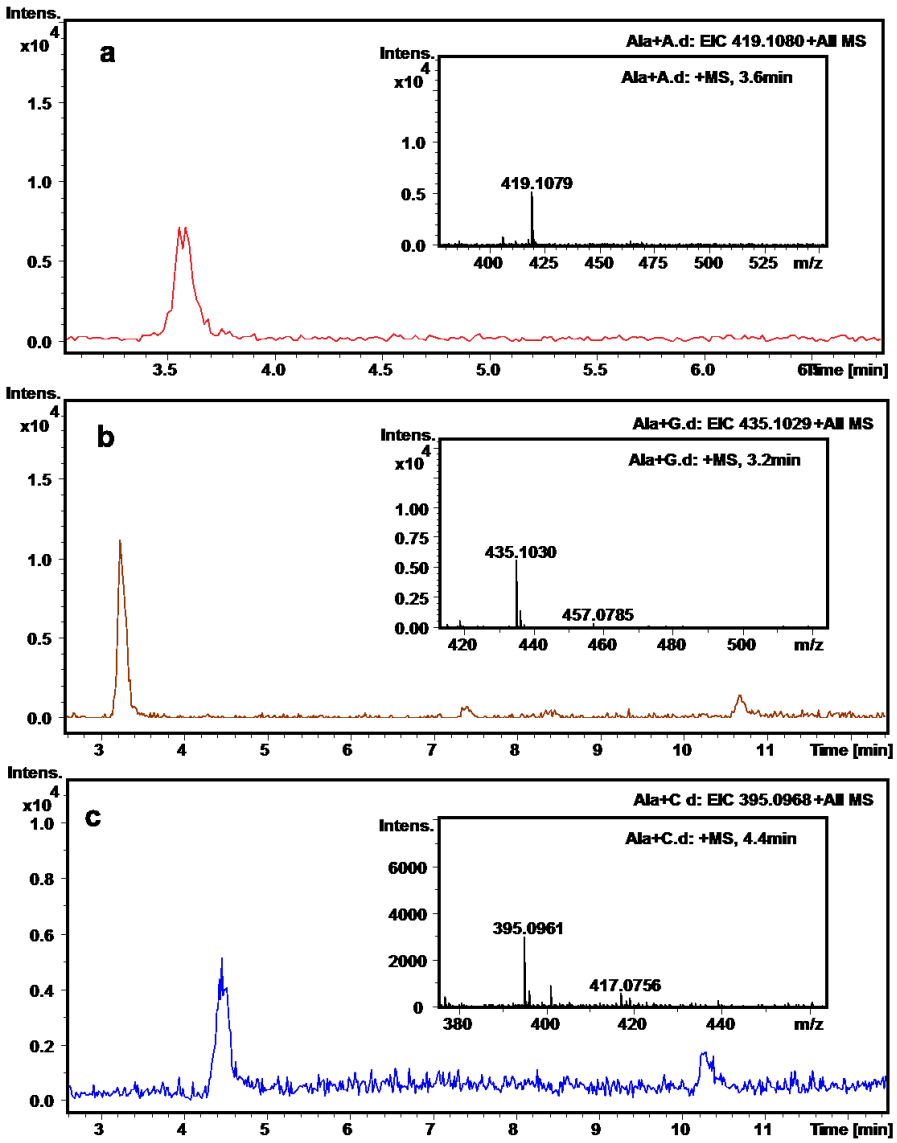

Fig. S10 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Ala-*N*-NMP of Ala with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Ala -*N*-NMP are too small so that their peaks are overlapped. a) reaction of Ala and P_3m with adenosine; b) reaction of Ala and P_3m with guanosine; c) reaction of Ala and P_3m with cytidine.

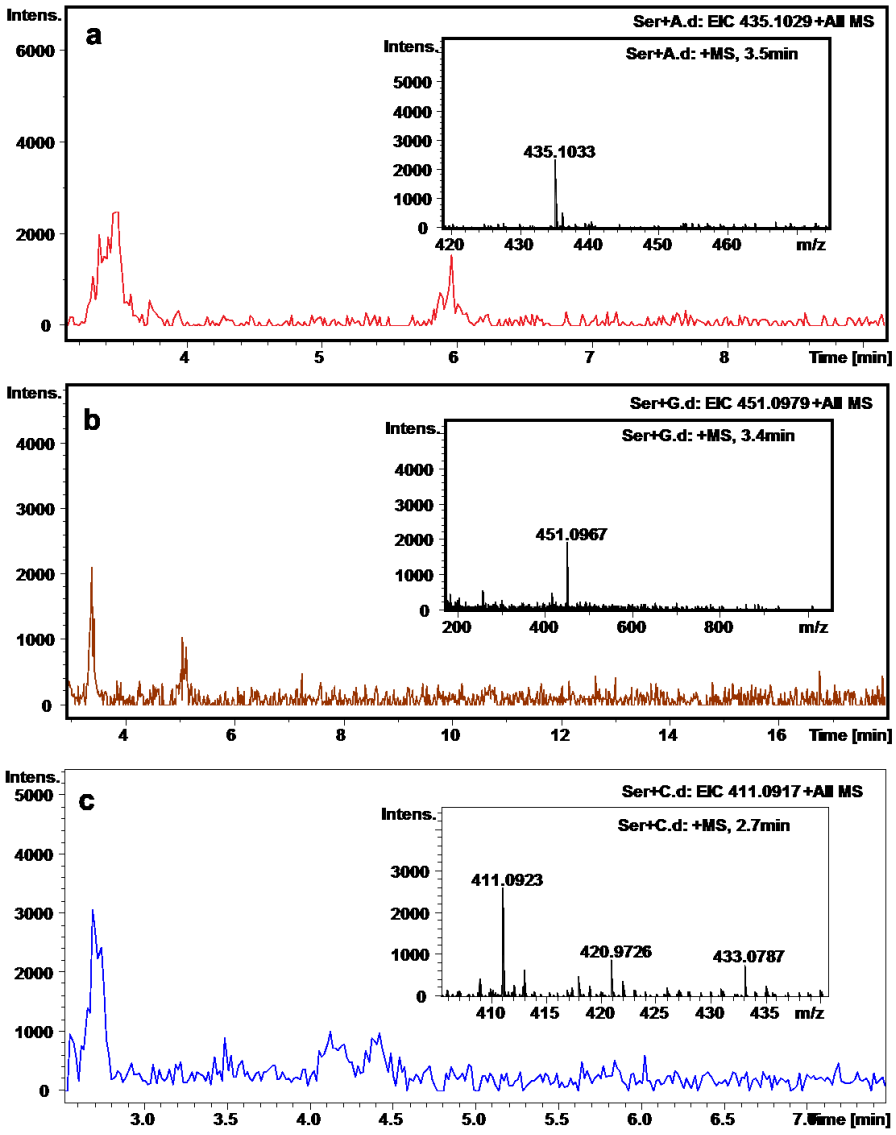

Fig. S11 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Ser-*N*-NMP of Ser with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Ser-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Ser and P_3m with adenosine; b) reaction of Ser and P_3m with guanosine; c) reaction of Ser and P_3m with cytidine.

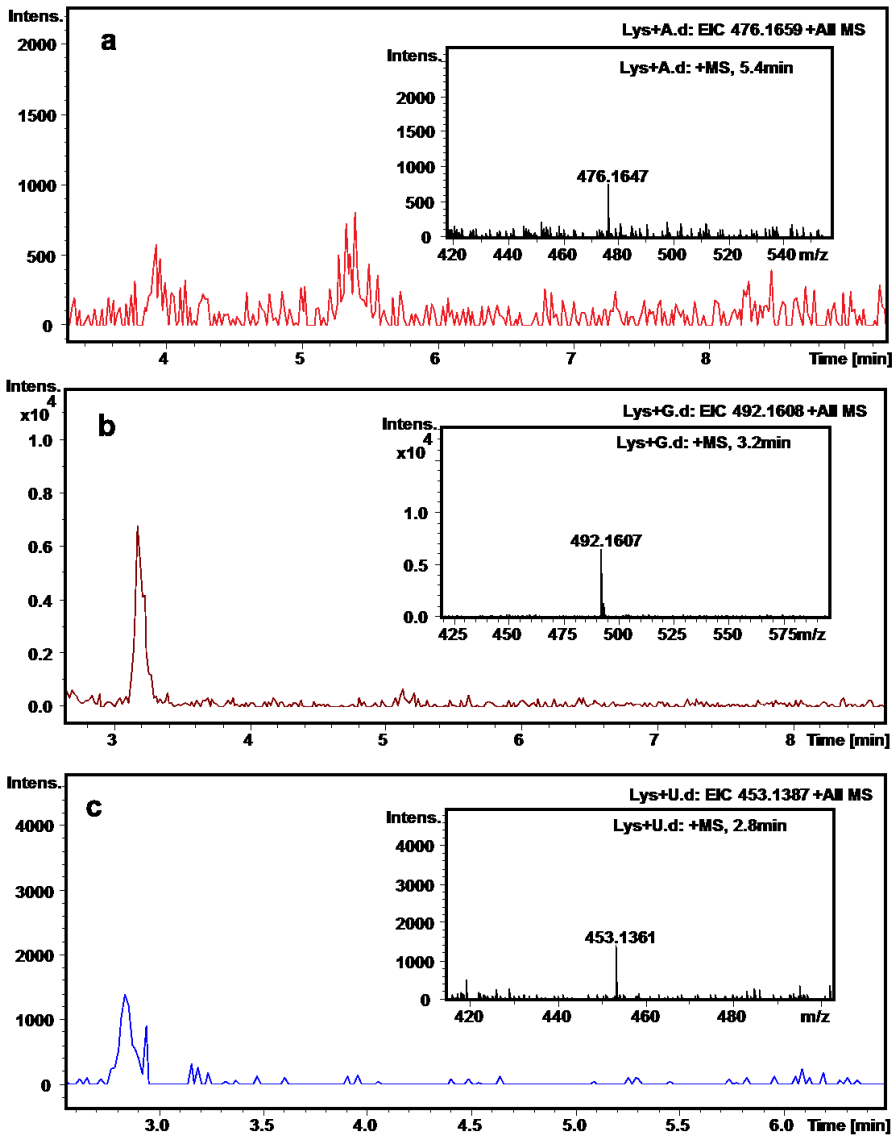

Fig. S12 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Lys-*N*-NMP of Lys with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Lys-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Lys and P_3m with adenosine; b) reaction of Lys and P_3m with guanosine; c) reaction of Lys and P_3m with uridine.

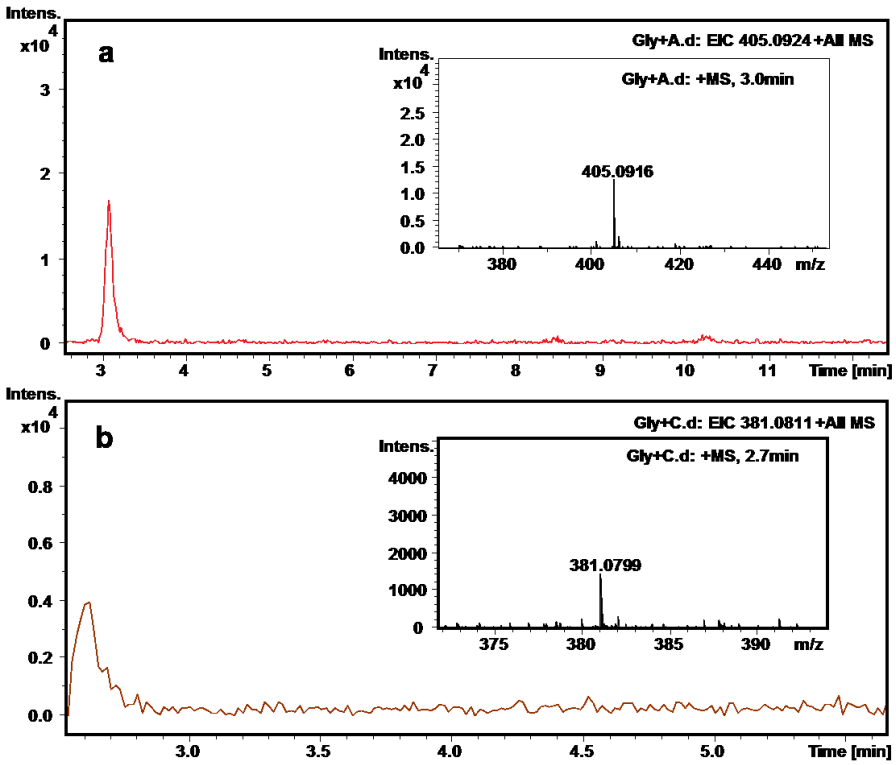

Fig. S13 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Gly-*N*-NMP of Gly with nucleosides. The EIC spectra of $[M+H]^+$ gives 1 distinct peak, which indicated that the polarity differences of some isomers of the Gly-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Gly and P_3m with adenosine; b) reaction of Gly and P_3m with cytidine.

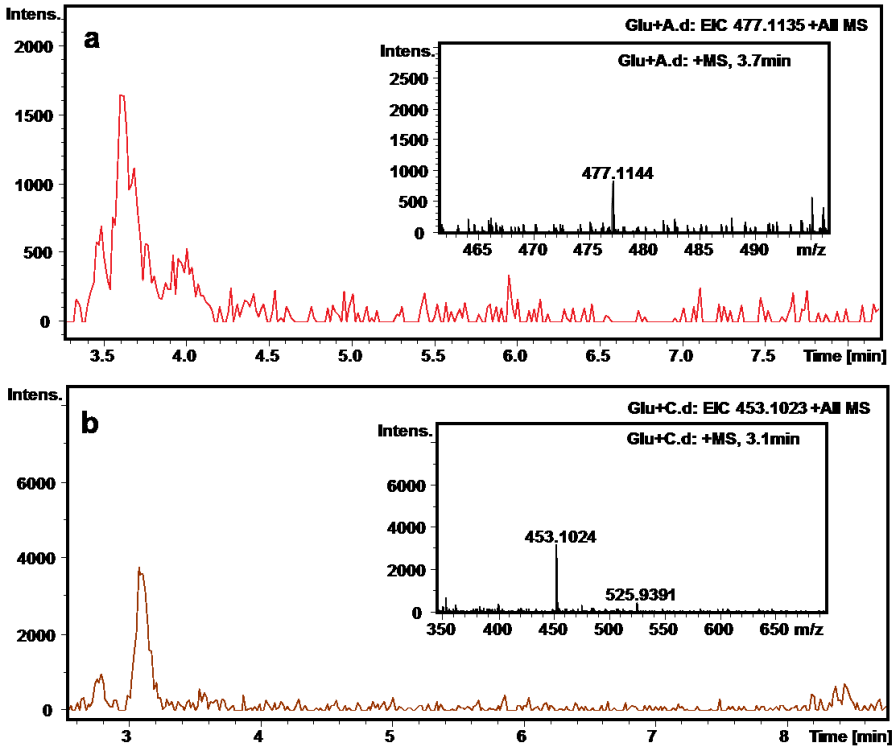

Fig. S14 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Glu-*N*-NMP of Glu with nucleosides. The EIC spectra of $[M+H]^+$ give 1~2 distinct peaks, which indicated that the polarity differences of some isomers of the Glu-*N*-NMP are too small so that their peaks are overlapped. a) reaction of Glu and P_3m with adenosine; b) reaction of Glu and P_3m with cytidine.

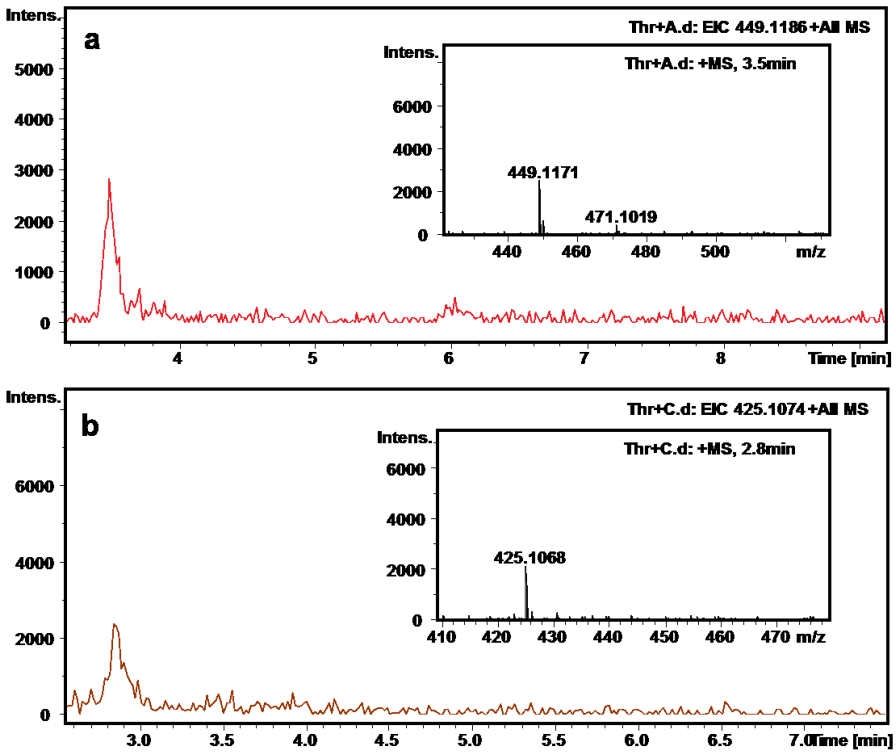

Fig. S15 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Thr-*N*-NMP of Thr with nucleosides. The EIC spectra of $[M+H]^+$ gives 1 distinct peak, which indicated that the polarity differences of some isomers of the Thr -*N*-NMP are too small so that their peaks are overlapped. a) reaction of Thr and P_3m with adenosine; b) reaction of Thr and P_3m with cytidine.

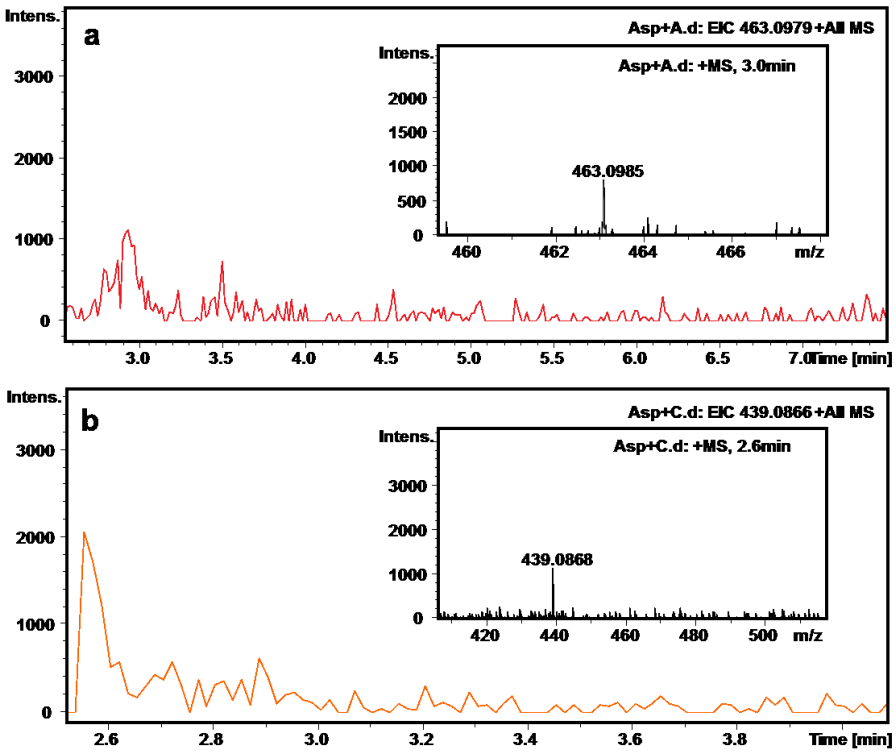

Fig. S16 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Asp-*N*-NMP of Asp with nucleosides. The EIC spectra of $[M+H]^+$ gives 1 distinct peak, which indicated that the polarity differences of some isomers of the Asp-*N*-NMP are too small to separate the chromatography. a) reaction of Asp and P_3m with adenosine; b) reaction of Asp and P_3m with cytidine.

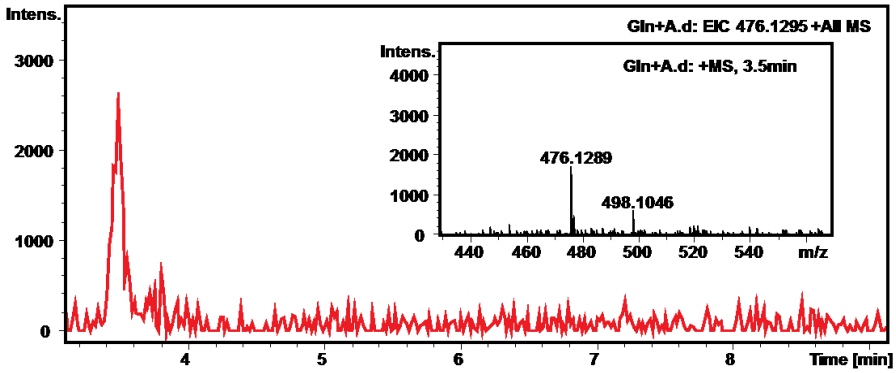

Fig. S17 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Gln-*N*-AMP of Gln with adenosine.

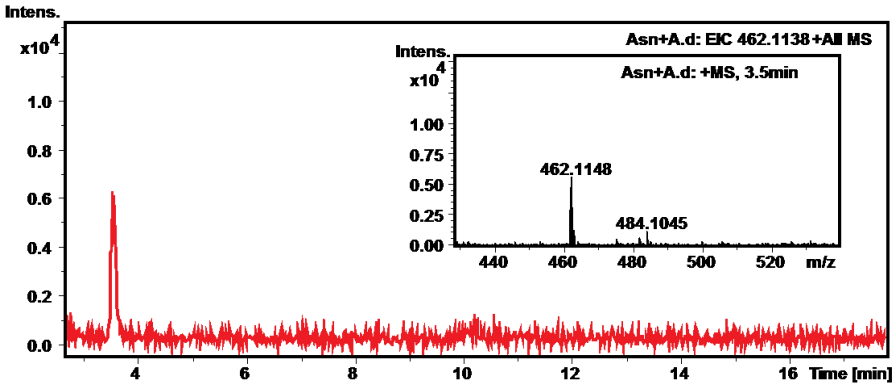

Fig. S18 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Asn-*N*-AMP of Asn with adenosine.

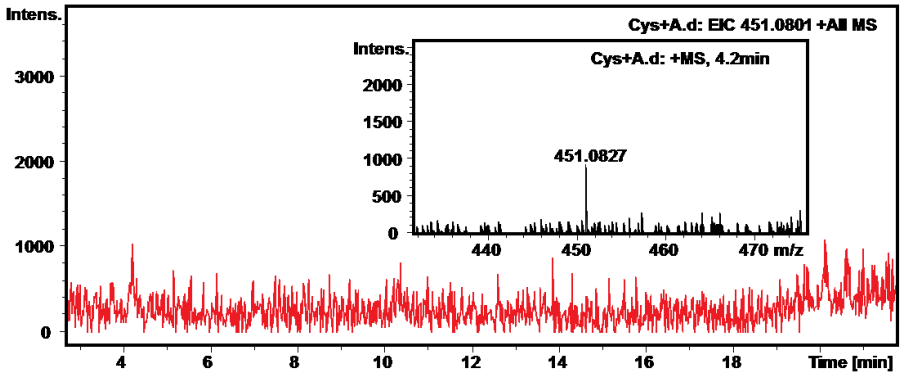

Fig. S19 HPLC-MS-EIC spectra of $[M+H]^+$ for the product Cys-*N*-AMP of Cys with adenosine.

8 Spectra analysis of product Peak 1 (Fig. 2 b, 2'-Phe-*N*-AMP)

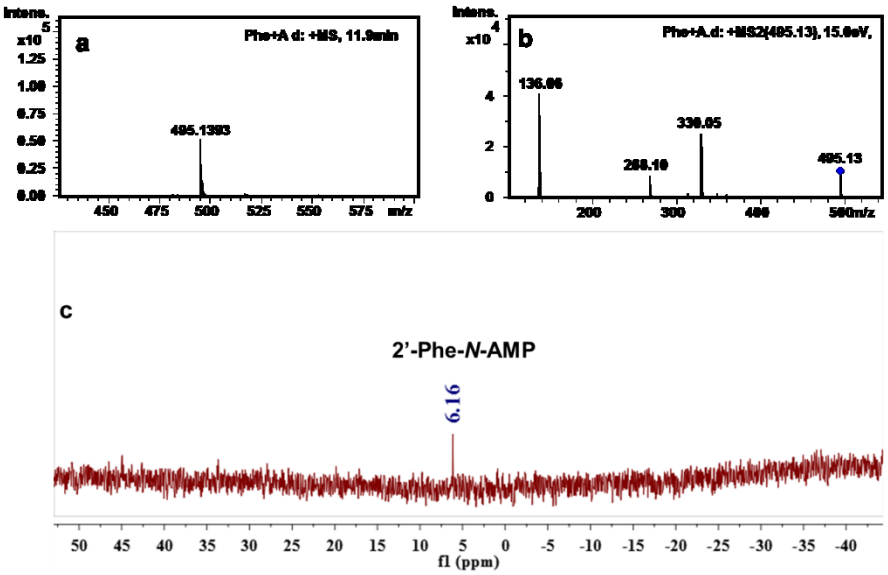

Fig. S20 Spectra analysis of fresh collected Peak 1 of Fig. 2 b.

a) the MS spectrum of Peak 1; b) the MS^2 spectrum of Peak 1; c) the ^{31}P NMR spectrum of Peak 1 (δ=6.16).

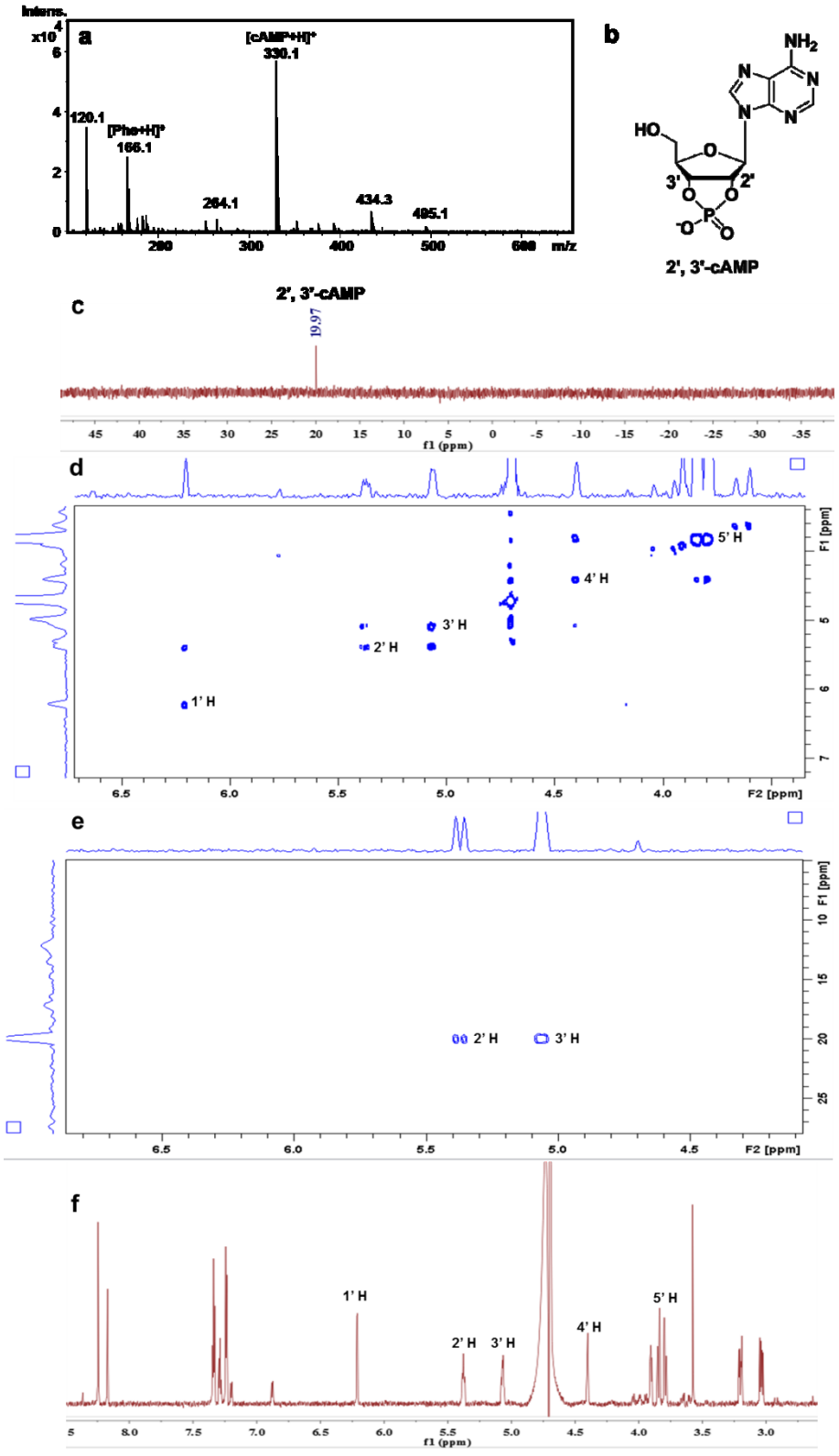

Fig. S21 Spectra analysis of Peak 1 being concentrated at room temperature. a) the MS spectrum. c) the ^{31}P NMR spectrum. d) the ^{1}H-^{1}H COSY spectrum. e) the ^{31}P-^{1}H HMBC spectrum. f) the ^{1}H NMR spectrum. These spectra indicated that the sample has been hydrolyzed to give 2', 3'-cAMP (^{31}P NMR, δ=19.97 ppm) and Phe. b) the structure of 2',3'-cAMP. The Phe-*N*-AMP is highly unstable and undergoes hydrolysis to give 2',3'-cAMP and Phe in the aqueous solution at room temperature.

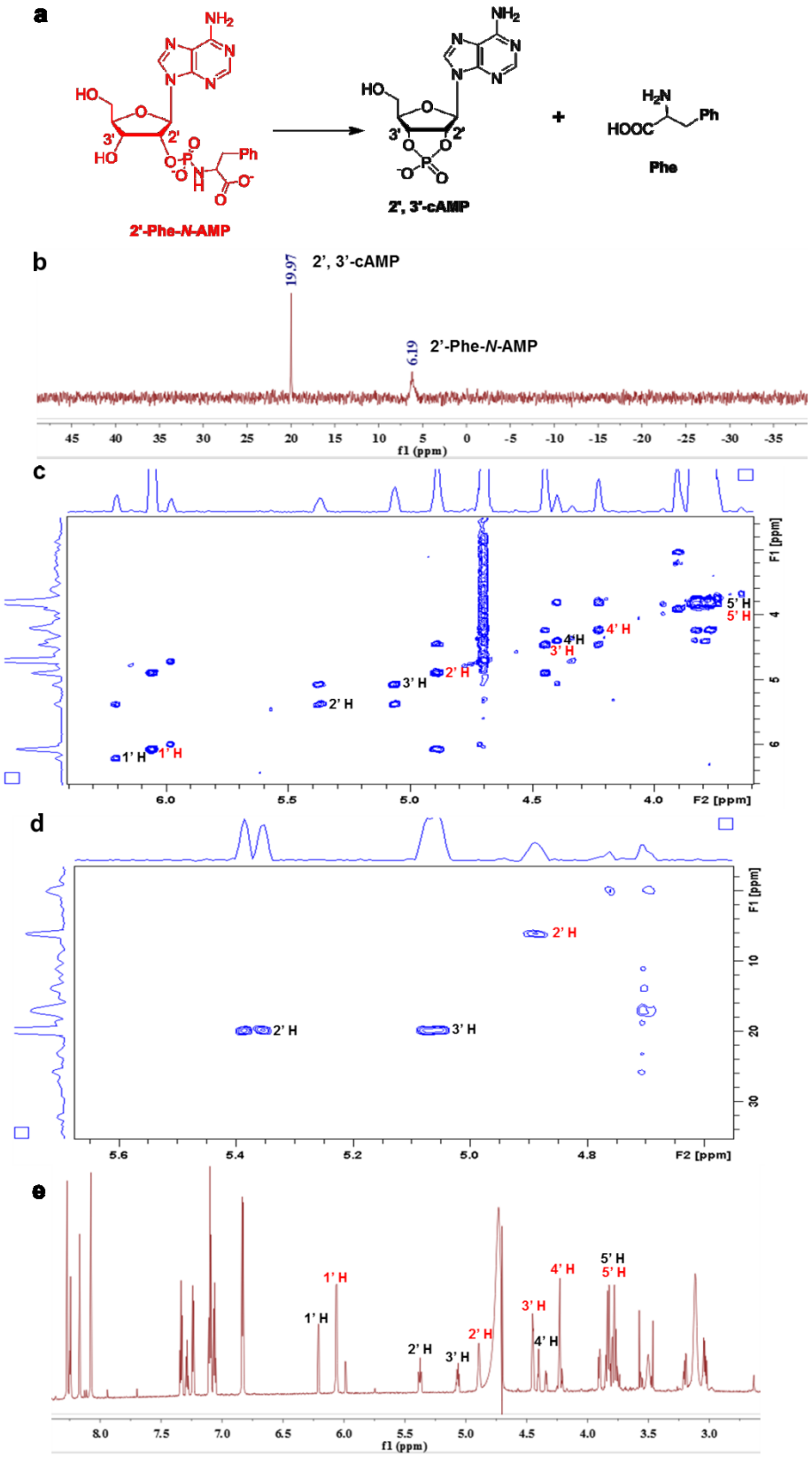

Fig. S22 Spectra analysis of Peak 1 being concentrated at 4 °C and pH 11.

a) the hydrolysis reaction of 2'-Phe-*N*-AMP. b) the ^{31}P NMR spectrum of Peak 1 being concentrated at 4 °C (^{31}P NMR, δ=6.19 ppm), which indicated that the sample has been partially hydrolyzed to give 2', 3'-cAMP (^{31}P NMR, δ=19.97 ppm) and Phe. c) the ^{1}H-^{1}H COSY spectrum. d) the ^{31}P-^{1}H HMBC spectrum. e) the ^{1}H NMR

spectrum. These spectra indicated that the structure of Peak 1 is 2'-Phe-*N*-AMP. The red mark represents the hydrogen atom on 2'-Phe-*N*-AMP. The black mark represents the hydrogen atom on 2',3'-cAMP which is the hydrolysis product of 2'- Phe-*N*-AMP.

9 The reaction of 2'-Phe-*N*-AMP and Phe

The chromatographically purified 2'-Phe-*N*-AMP was mixed with appropriate Phe in 0.25 mL D_2O. The pH of the reaction mixture was adjusted to 11 by using 10 M NaOH. Then the reaction mixtures were placed at 37 °C for 15 h.

10 Spectra analysis of 2'-Phe-*N*-UMP

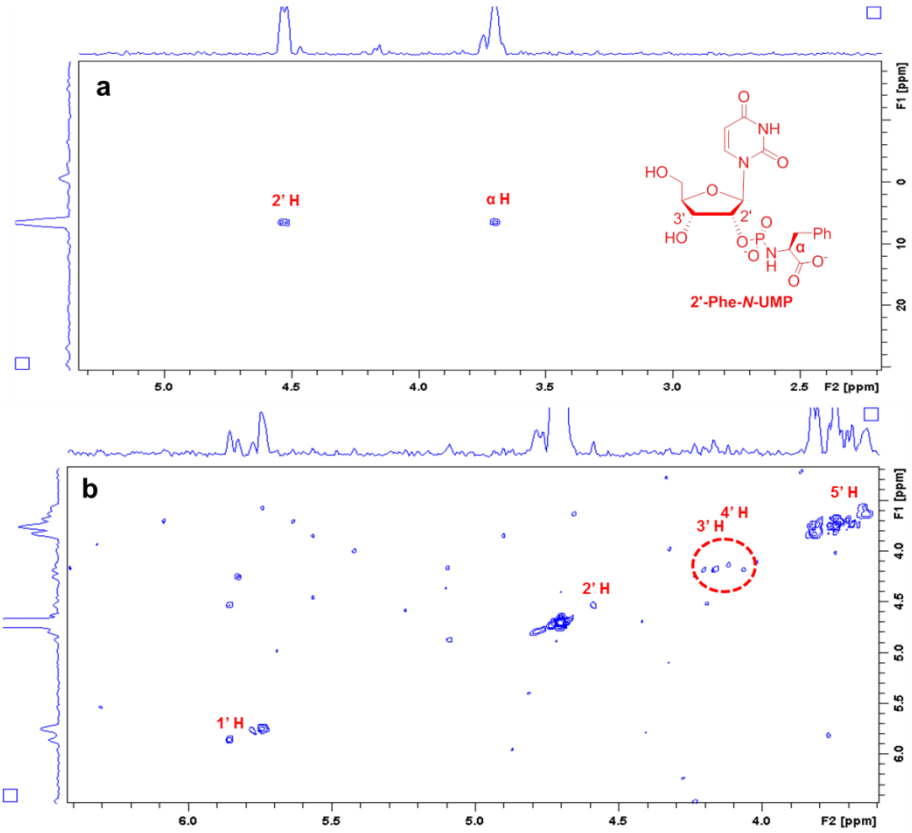

Fig. S23 Spectra analysis of 2'-Phe-*N*-UMP being concentrated at 4 °C and pH11.

a) the ^{31}P-^{1}H HMBC spectrum. b) the ^{1}H-^{1}H COSY spectrum.

The Phe-*N*-UMP is highly unstable and undergoes hydrolysis in the aqueous solution at 4 °C. Meanwhile, the anticipated product was formed in much lower amounts, so we were unable to definitively prove the structure. However, the chemical properties of Phe-*N*-AMP, an analogue of Phe-*N*-UMP, and HRMS and HMBC data strongly suggest that it is 2'-Phe-*N*-UMP (^{31}P NMR, δ=6.7 ppm).

11 The HPLC-MS analysis of dipeptides

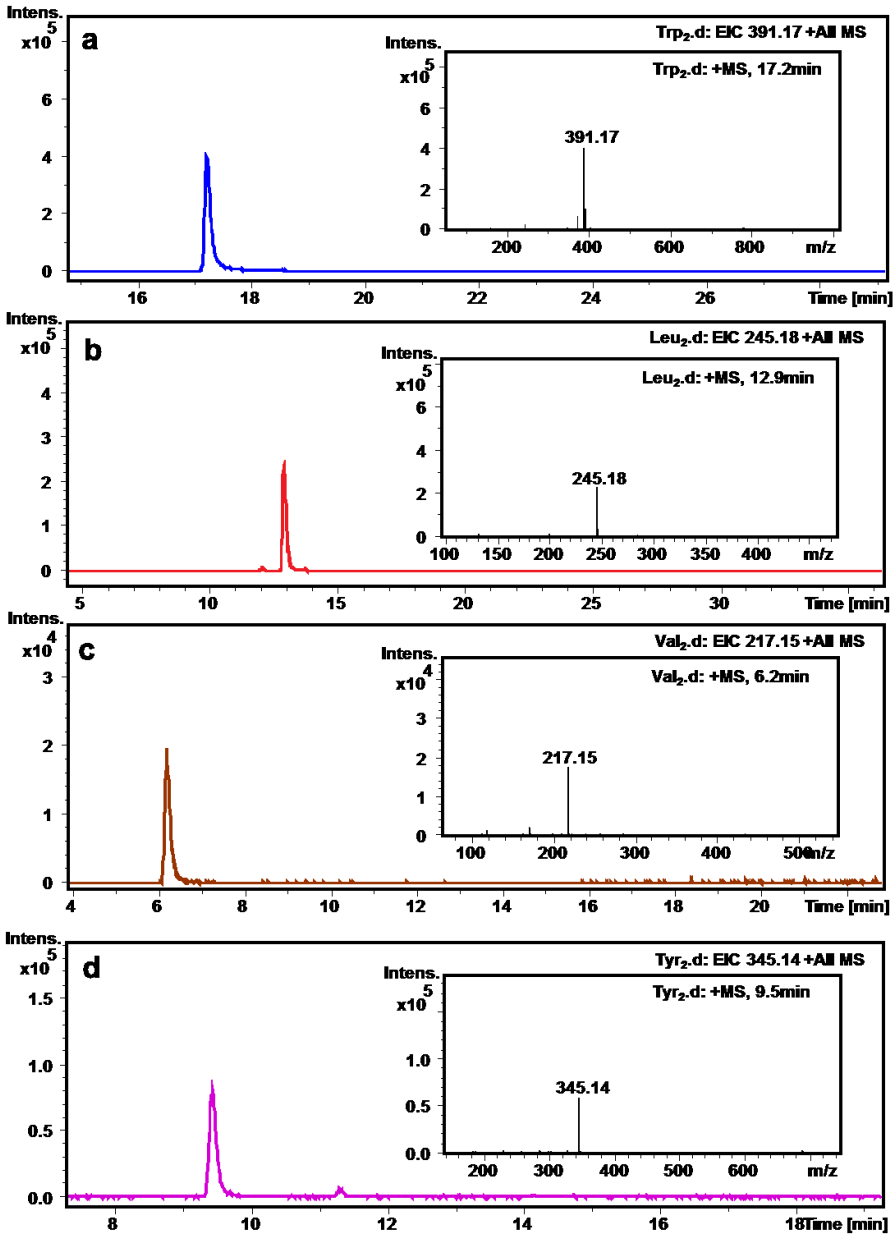

Fig. S24 HPLC-MS-EIC of the dipeptides $[M+H]^+$ for the reaction products of the amino acids and nucleosides in P_3m alkaline aqueous solution. a) the reaction product of Trp_2; b) the reaction product of Leu_2; c) the reaction product of Val_2 ; d) the reaction product of Tyr_2.

12 The HPLC-MS analysis of Phe tripeptides (Phe_3)

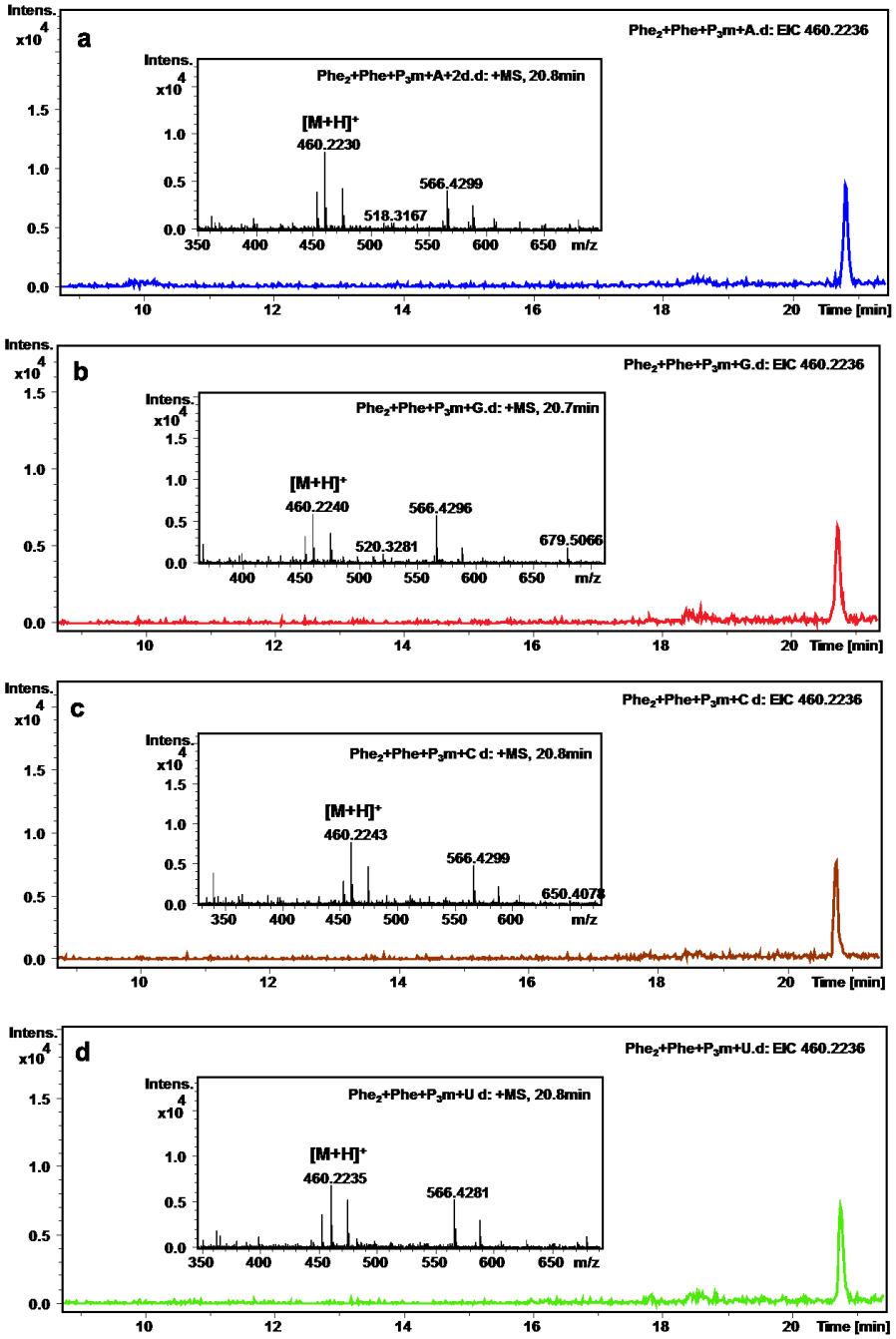

Fig. S25 HPLC-MS-EIC of the Phe tripeptides (Phe_3) $[M+H]^+$ for the reaction products of Phe, Phe_2 and nucleosides in P_3m alkaline aqueous solution. a) the reaction of Phe, Phe_2 and adenosine; b) the reaction of Phe, Phe_2 and guanosine; c) the reaction of Phe, Phe_2 and cytidine; d) the reaction of Phe, Phe_2 and uridine.