DOI: 10.1002/ejoc.201100234

Intermolecular Phosphoryl Transfer of *N*-Phosphoryl Amino Acids

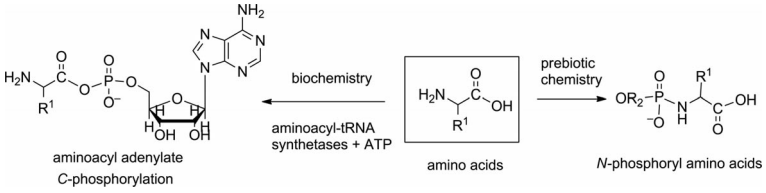
Xiang Gao,[a] Honggui Deng,[a] Guo Tang,[a] Yan Liu,[a] Pengxiang Xu,[a] and Yufen Zhao*[a,b]

Keywords: Reaction mechanisms / Phosphorus / Amino acids / Origin of life

N-Phosphoryl amino acids (NPAAs) are a novel series of *N*-terminal-activated amino acids that act as the energy source and phosphoryl donor in intra- and intermolecular phosphoryl transfer to form "high-energy" species, such as acetyl phosphate and aminoacyl phosphates, and in the self-assembled synthesis of polypeptides under mild aqueous conditions. In this work, the chemical reactivity of *N*-mono(methoxyphosphoryl)glycine as a representative was investigated in detail by using a combination of the stable-isotope-labeling (^{15}N) technique, ^{31}P NMR, ESI-MS/MS and LC-MS. The phosphoryl group of NPAAs can be transferred intermolecularly to the carboxy group of another molecule through intramolecular cyclic pentacoordinate phosphoric–amino acid anhydride intermediates. In addition to *C*-terminal activation by phosphate anhydride, amino acids can also be self-activated by *N*-phosphorylation. This information not only provides some interesting clues for understanding the active role of the phosphoryl group in living systems, but also shows that the origin of life might be attributed to the chemical evolution of *N*-phosphoryl amino acids.

Introduction

In contemporary biosynthesis, amino acids are first activated by *C*-terminal phosphorylation to form energy-rich aminoacyl adenylates for protein synthesis, catalysed by aminoacyl tRNA synthetases (Scheme 1).[1] Furthermore, amino acids can be activated to form aminoacyl phosphate anhydrides under a variety of potentially primordial conditions.[2] Because of the prevalence of these compounds, there have been many investigations into the inherent nature of the aminoacyl phosphate anhydride as key intermediate.[3] On the other hand, the biologically "high-energy" phosphoramidate bond (P–N bond) has attracted increasing attention because of its fundamental roles in metabolism and signalling.[4] In our research group, *N*-phosphoryl amino acids (NPAAs) have been investigated as model compounds under mild conditions.[5] In NPAAs, natural α-amino acids are activated by *N*-phosphorylation to a higher energy state through the intramolecular phosphoryl-carboxy mixed anhydride[6] and some interesting biomimetic reactions have been observed, including self-assembly oligopeptide formation, ester exchange on the phosphoryl group, carboxylic ester formation and an intramolecular phosphoryl group migration from nitrogen to oxygen.[7]



Scheme 1. Two pathways for amino acid activation: the *C*- and *N*-terminal phosphorylation of natural α-amino acids.

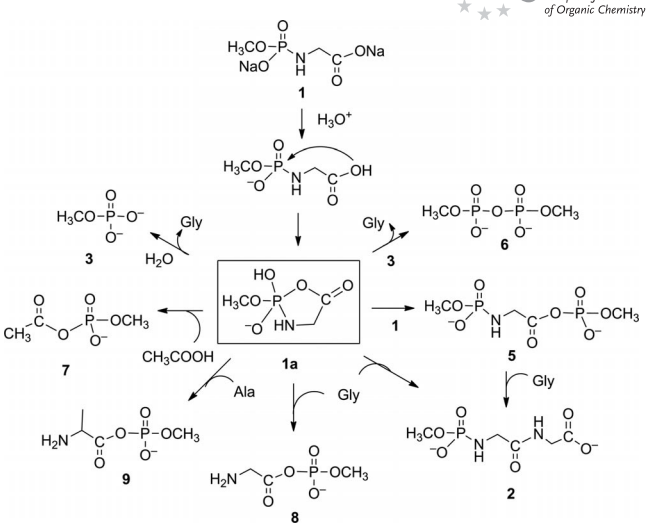
Indeed, NPAAs can be produced by the reaction of amino acids with prebiotically available linear polyphosphates or trimetaphosphate[8] in aqueous solution.[9] In addition, NPAAs have been considered as mini-chemical models in a study of intramolecular catalysis[10] and for the elucidation of the phosphoryl transfer mechanism in kinases.[11] These studies not only have shown that the origin

[a] Department of Chemistry and The Key Laboratory for Chemical Biology of Fujian Province, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, P. R. China
Fax: +86-592-2185780
E-mail: yfzhao@xmu.edu.cn
[b] The Key Laboratory for Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry, School of Sciences, Tsinghua University, Beijing 100084, P. R. China
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201100234.

of life might be attributed to the chemical evolution of *N*-phosphoryl amino acids, but have also provided some important clues for understanding the central role of phosphates in living systems.[12]

In recent decades, there has been significant progress in the design and development of amino acid phosphoramidate triesters as an effective pro-drug strategy for the intracellular delivery of charged anti-viral and anti-cancer nucleotide monophosphates.[13] Interestingly, in different biological media and animal species, the activation of phosphoramidate triester pro-dugs, mediated by carboxyesterase-type enzymes, has been used to form a high concentration of phosphoramidate conjugates of amino acids and nucleotides having P–N bonds and free carboxylic groups.[14] Recently, it has been demonstrated that amino acid phosphoramidates of nucleosides can mimic the natural triphosphate moiety effectively and be incorporated into growing DNA chains under catalysis by polymerases.[15] In addition, the thermochemistry and mechanism for the hydrolysis of aspartic acid phosphoramidate nucleotide as a model compound has been investigated by comparative theoretical calculations.[16] The gas-phase chemistry of amino acid phosphoramidates of adenosine and *N*-phosphoryl amino acids has been explored by electrospray ionization mass spectrometry.[17] However, to the best of our knowledge, the chemistry of amino acid phosphoramidates of nucleosides has remained relatively unexplored in spite of their potentially significant biological and pharmaceutical applications.[18]

Initially we found that the nucleotide portion of the molecule has undesirable chemical complexity because of its multiple functional groups. The desired reaction pathways need to emerge primarily from the *N*-phosphoryl amino acid moiety having two reactive centres, at the phosphoryl and carbonyl groups, independent of the nucleotide group. Based on these considerations, it appeared more expedient to study simpler monoesters of *N*-phosphoryl amino acids to retain the potentially chemically useful properties of amino acid phosphoramidates of nucleosides while avoiding the complexity of the nucleoside groups. The present study focuses on the reactions of the disodium salt of *N*-mono(methoxyphosphoryl)glycine (*N*-MMP-Gly, **1**) as a typical member of the general class in the context of phosphoryl group transfer (Scheme 2). The phosphoryl group of NPAAs can be transferred intermolecularly to the carboxy group of another molecule through intramolecular cyclic pentacoordinate phosphoric-amino acid anhydride intermediates. Besides *C*-terminal activation by phosphate anhydrides, amino acids can also be self-activated by *N*-phosphorylation. In parallel with the formation of polypeptides, "high-energy" phosphoester bonds are formed by phosphoryl transfer reactions that have been referred to as "the centrepiece of biochemical processes"[19] through the breaking of the P–N bond of an *N*-phosphoryl amino acid under mild aqueous conditions. Note that the biologically important divalent magnesium ion can catalyse the formation of pyrophosphate, which may have acted as an ancient energy carrier in place of ATP.[20]



Scheme 2. "High-energy" phosphoester bond and peptide formation of *N*-phosphorylglycine in aqueous solution.

Results and Discussion

The reaction of *N*-MMP-Gly in aqueous solution was monitored by ^{31}P NMR spectroscopy and LC-ESI-MS. Within a pD range of 8.0–9.0 and at a temperature of 40 °C, there was no degradation of *N*-MMP-Gly (0.2 M) observed after a period of 8 d (Figure 1, a). In contrast, as shown in part b of Figure 1 at pD 4.0–5.0, *N*-MMP-Gly (0.2 M) decomposes to generate products **2** [δ = 9.2 ppm, *N*-mono(methoxyphosphoryl)glycylglycine (*N*-MMP-Gly-Gly, confirmed by comparison with an authentic sample, Figure S1, Supporting Information)], **3** (δ = 1.7 ppm, methyl phosphate), **4** (δ = 0.1 ppm, PO_4^{3-}) and **6** (δ = –9.6 ppm, dimethyl pyrophosphate, confirmed by LC-MS; Figure S2, Supporting Information). This dependence of reactivity on mild acid conditions established the reactive species as the monosodium salt of the methyl phosphonamidate with the carboxylic acid un-ionized, as seen in earlier work by Blackburn and Kirby and their co-workers.[21] Detailed analysis of the reaction identifies an intermediate **5** with two phosphorus signals at δ = 8.7 and –5.9 ppm that appears immediately, increases to a maximum after 3.5 h and then slowly disappears over 18 h. A 1:1 ratio of **5**-P_N/**5**-P_C is seen by ^{31}P NMR spectroscopy during the formation of **5**. The singlet peak of **5**-P_N at δ = 8.7 ppm changes into multiple peaks ($J_{H\text{-}P}$ = 10.7 Hz, tq) in the ^{1}H-coupled ^{31}P NMR spectrum (Figure 2, b), and is similar to reactant **1** and *N*-MMP-Gly-Gly **2**, resulting from splitting by the methyl and methylene groups. To establish the reaction pathway, stable-isotope-labelled ^{15}N-Gly was incorporated into *N*-MMP-Gly. Under the same reaction conditions, the ^{1}H-decoupled ^{31}P NMR spectrum of the reaction mixture derived from ^{15}N-labelled *N*-MMP-Gly showed a doublet at δ = 8.7 ppm ($J_{N\text{-}P}$ = 35.6 Hz), indicative of a P–N bond in intermediate **5** (Figure 2, c). The quartet ($J_{H\text{-}P}$ = 11.2 Hz) and singlet observed in Figure 2, parts e and f, respectively, are as expected for the P_C signal of **5**. These NMR spectroscopic data are also in agreement with the reported values for the

structure of the mixed phosphoric-carboxylic anhydride.[2,3] Consequently, intermediate **5** is proposed as the structure of the phosphate anhydride of *N*-MMP-Gly with the formation of the "high-energy" phosphoester bond (Scheme 2). In addition, LC-ESI-MS analysis of the reaction mixture after 8 d incubation at 40 °C showed the formation of diglycine (G_2, 13.0% yield), triglycine (G_3, 1.2% yield) and trace amounts of tetraglycine (Table 1).

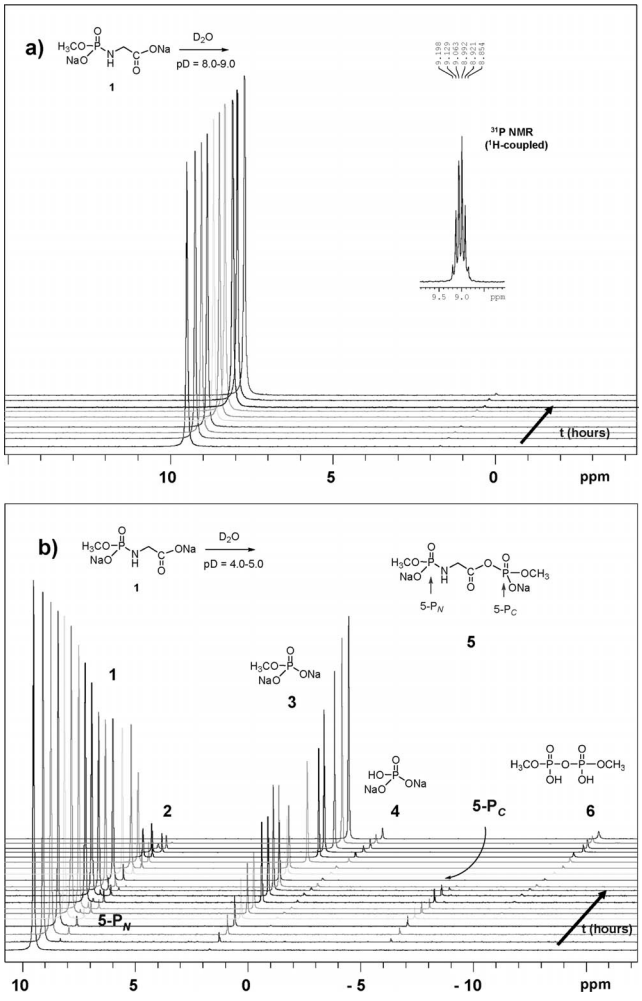


Figure 1. Time-dependent, stacked ^{31}P NMR spectra for the reactions of *N*-MMP-Gly in aqueous solution at 40 °C for 8 d. (a) *N*-MMP-Gly (0.2 M) at pD 8.0–9.0; (b) *N*-MMP-Gly (0.2 M) at pD 4.0–5.0.

To investigate the effect of the intermolecular phosphoryl transfer reaction, the reaction of *N*-MMP-Gly (0.2 M) with acetic acid (0.2 M) in aqueous solution was monitored (Figure 3, a). This showed that in addition to product **5**, acetyl phosphate **7** was detected (–6.15 ppm, quartet in the proton-coupled spectrum) as the reaction intermediate. In addition, the reaction of *N*-MMP-Gly with acetic acid was analysed by ESI-MS and ESI-MS/MS (Figure 4). Acetyl phosphate **7** was observed at *m*/*z* = 198.9 as the $[7 + Na]^+$ ion, which formed methyl phosphate (*m*/*z* = 156.9) and methyl metaphosphate (*m*/*z* = 124.9) by tandem MS/MS. This assignment was further confirmed by comparison of

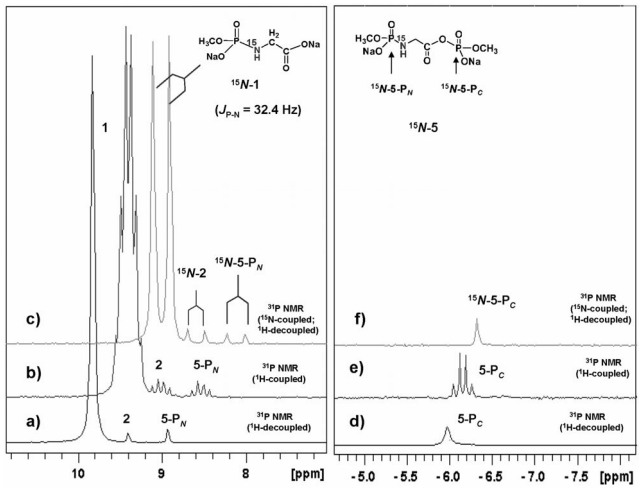


Figure 2. ^{31}P NMR analysis of the products **1**, **2** and **5** formed in the reaction of *N*-MMP-Gly (a,d and b,e) and stable-isotope-labeled ^{15}N-MMP-Gly (c,f) incubated in D_2O.

7 with a synthetic sample of acetyl phosphate (Figure S3, Supporting Information). Thus, the phosphoryl group of *N*-MMP-Gly can be transferred to acetic acid to form acetyl phosphate, and this can serve two different purposes, either to transfer its phosphoryl group into the phosphate pool or to supply its active acetyl group for the biosynthesis of the carbon structures.[22]

The reaction of *N*-MMP-Gly (0.2 M) with glycine (0.2 M) in D_2O was also tracked by ^{31}P NMR spectroscopy (Figure 3, b). Indeed, intermediate **8** is observed at –6.2 ppm, which is consistent with values reported for aminoacyl ethyl phosphate[4b] and shows similar kinetic behaviour. The structure of the mixed anhydride **8** was also confirmed by comparison with an authentic sample of glycyl methyl phosphate (Figure S4, Supporting Information). In the reaction of *N*-MMP-Gly with alanine, a carboxy-activated intermediate can be detected at δ = –6.1 ppm and its structure was confirmed as alaninyl methyl phosphate **9** by comparison with an authentic sample (Figure S5, Supporting Information).

It is thus evident that *N*-phosphoryl amino acids are not only self-activated to produce "high-energy" phosphoric-acetyl anhydride bonds, but also deliver reasonable yields of oligopeptides for several *N*-phosphoryl amino acids under different reaction conditions (Table 1 and Figure S6, Supporting Information). Variations in the chemical structure of the alkyl group and the concentration of *N*-MMP-Gly had no significant effect on the yields of phosphates and peptides (entries 1–3). However, the product yields depend markedly on the structures of the amino acid moieties of the *N*-phosphoryl amino acids. In the case of *N*-MMP-Val (entry 8), the yield of dipeptide Val-Val is very low (1.0%), whereas the yield of phosphate **4** is as high as 68.2%, as determined by ^{31}P NMR integration. This finding can be rationalized in terms of the steric effect of the hydrophobic side-chains of the amino acids that position the carboxylic acid and phosphoryl group closer and consequently facili-

Table 1. Yields of phosphates and peptides induced by *N*-phosphoryl amino acids.

Entry	Reactions[a] (M)	Yield of phosphate [%][b]		Yield of peptide [%][c]
		P_i	P_2	
1	MMP-Gly (0.2)	5.1	5.2	G_2 (13.0), G_3 (1.2), G_4 (0.1)
2	MMP-Gly (0.5)	5.0	6.6	G_2 (16.0), G_3 (1.7), G_4 (0.2)
3	MEP-Gly (0.2)[d]	3.0	5.2	G_2 (11.5), G_3 (1.0)
4	MMP-Gly (0.2) + Mg^{2+} (0.02)	5.7	6.3	G_2 (6.4), G_3 (0.3)
5	MMP-Gly (0.2) + Mg^{2+} (0.05)	4.1	11.8	G_2 (6.8), G_3 (0.3)
6	MMP-Gly (0.2) + Mg^{2+} (0.1)	4.4	17.2	G_2 (7.9), G_3 (0.4)
7	MMP-Ala (0.2)	40.4	5.7	A_2 (4.6), A_3 (0.2)
8	MMP-Val (0.2)	68.2	8.3	V_2 (1.0)
9	MMP-Gly (0.2) + Ala (0.2)	5.7	3.6	G_2 (3.8), GA (4.0),[e] AG (3.5), A_2 (3.9)

[a] Reaction conditions: incubation at 40 °C, pD = 4.0–5.0, reaction time: entries 1, 3–6 and 9: 8 d; entry 7: 10 d; entries 2 and 8: 12 d. For full experimental details, see the Supporting Information. [b] Yields were determined by integration of the ^{31}P NMR spectra. P_i: PO_4^{3-}; P_2: dimethyl pyrophosphate. [c] Peptide yields were determined relative to *N*-phosphoryl amino acids by HPLC. [d] MEP-Gly: sodium salt of *N*-(ethoxyphosphoryl)glycine. [e] Including a small amount of triglycine.

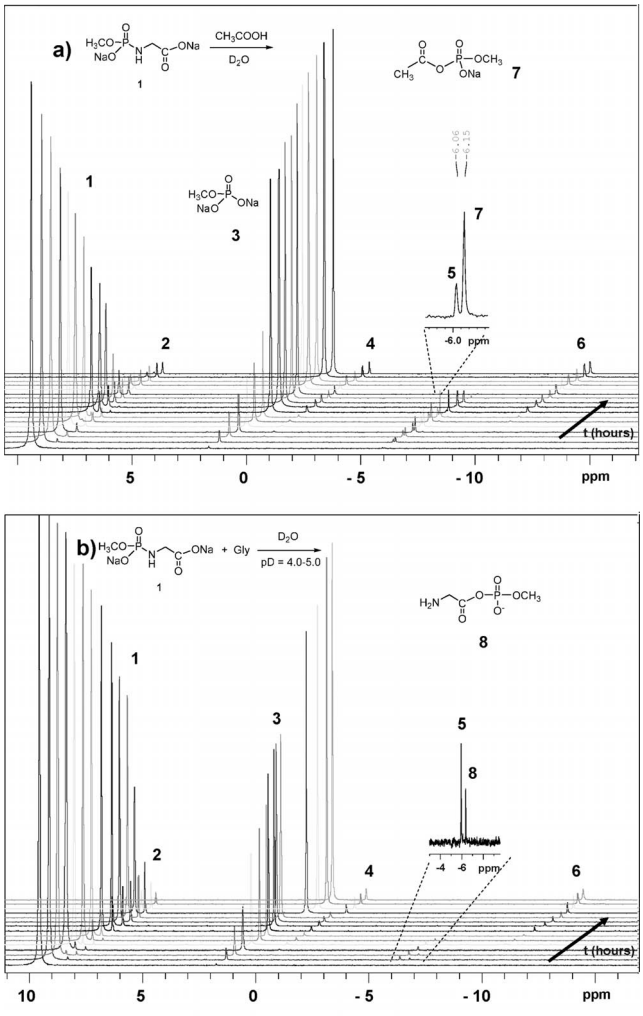


Figure 3. Time-dependent, stacked ^{31}P NMR spectra for the reactions of *N*-MMP-Gly in D_2O. (a) *N*-MMP-Gly (0.2 M) with acetic acid (0.2 M); (b) *N*-MMP-Gly (0.2 M) with glycine (0.2 M).

tate nucleophilic attack of the carboxy on the phosphorus to form pentacoordinate phosphate intermediate **1a** (Scheme 2). That leads to expulsion of a methanol with the formation of cyclic acylphosphoramidate (CAPA), which in turn is hydrolysed to generate phosphate **4**, rather than unfavourable attack by another molecule of amino acid with a large side-chain to generate an *N*-phosphoryl peptide.[10] Significantly, the Group II ion Mg^{2+} could catalyse the formation of dimethyl pyrophosphate **6** even at low concentration (5 mM). Yields of **6** were as high as 17.2% in the presence of 0.1 M Mg^{2+} with no formation of phosphate precipitates (entry 6). Furthermore, it is noteworthy that free natural amino acids such as alanine can also be activated by *N*-MMP-Gly to produce homo- and hetero-peptides in reasonable yields (entry 9). These observations indicate marked selectivity favouring the phosphoryl transfer reaction and the notable dependence on the intrinsic structures of amino acids for peptide formation.

A possible mechanism for this phosphoryl transfer reaction is proposed in Scheme 2. Under slightly acidic conditions, the phosphoryl group of an NPAA can undergo spontaneous intramolecular transfer to carboxylic acid to form pentacoordinate cyclic acylphosphoramidate intermediate **1a**. This can be attacked not only by the nucleophilic carboxylic group of acetic acid and amino acids at phosphorus to form "high-energy" anhydride phosphoester bonds, but also by the amino group of any other amino acid at the carbonyl group to produce *N*-phosphorylated peptides, which subsequently hydrolyse to form oligopeptides. Promoted by the neighbouring carboxy group as an essential component, the phosphoryl group of NPAAs may be attacked nucleophilically to form pentacoordinate phosphorane intermediates through an addition/elimination process ($A_N + D_N$ in the IUPAC nomenclature), which contrasts with the unimolecular S_N1-type mechanism ($D_N + A_N$) possibly involving a reactive monomeric metaphosphate intermediate.[23] Furthermore, it should be noted that this phosphoryl transfer of *N*-phosphoryl amino acids with the participation of the carboxylic acid group is different to the general reactions of phosphoramidates by the concerted mechanism (SN_2) routes. Indeed, the active five-membered cyclic pentacoordinate phosphoric-amino acid mixed anhydrides have been observed by ^{31}P NMR spectroscopy in anhydrous organic solvents.[6]

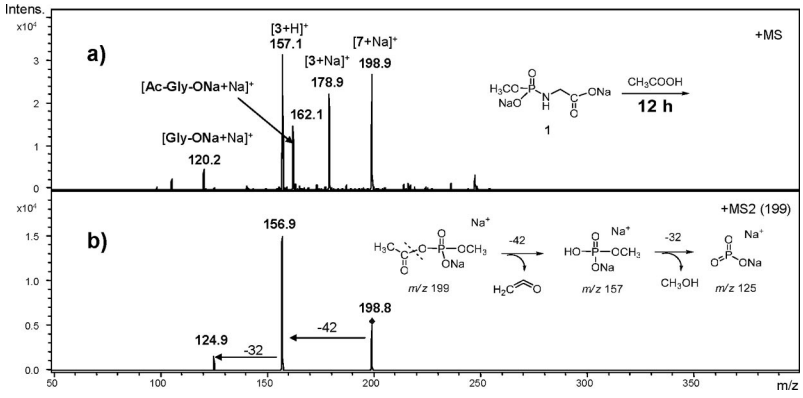


Figure 4. ESI-MS spectra of the products from the reaction of *N*-MMP-Gly with acetic acid in D_2O. (a) Mass spectrum acquired after 12 h; (b) ESI-MS/MS of [**7** + Na]$^+$ ion at m/z = 199.

Conclusions

These studies suggest that *N*-phosphoryl amino acids are a novel series of *N*-terminal-activated amino acids providing the energy source and phosphoryl donor not only for intra- and intermolecular phosphoryl transfers to form "high-energy" species (*C*-terminal-activated) such as pyrophosphates, acetyl phosphates and aminoacyl phosphates, but also for the self-assembled synthesis of peptides under mild aqueous conditions. Interestingly, unlike contemporary protein biosynthetic pathways that need specific enzymes to catalyse the formation of aminoacyl adenylates, NPAAs are capable of transferring their chemical energy through the self-activation process to biologically significant, energy-rich acyl phosphate species. Although the yields of peptide formation and phosphoryl group transfer are limited, the reactivities of *N*-phosphoryl amino acids as small chemical models are novel. It is therefore possible that the evolution of NPAAs may have been relevant to biochemical energy metabolism and possibly to prebiotic chemistry leading to the emergence of life. This study may also contribute to a better understanding of the mechanism of the intramolecular catalysis of biological phosphoryl transfer reactions through possible intermediate pentacovalent species and provide a chemical basis for the pharmacokinetics and metabolism of anti-viral and anti-cancer amino acid phosphoramidate agents that may serve as leads and scaffolds for medicinal research and development. Further developments, such as the research of nucleotide *N*-phosphoryl amino acids as novel chemical model systems to elucidate the basic molecular recognition between amino acids and nucleotides for the origin of the genetic code, are currently under investigation in our group.

Experimental Section

General: Commercial reagents were used as received. ^{15}N-Glycine was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). L-Amino acids were purchased from GL Biochem. Ltd. (Shanghai, China). Gly-Gly (G_2), Gly-Ala (GA), Ala-Gly (AG), Ala-Ala (A_2), Gly-Gly-Gly (G_3), nonafluoropentanoic acid (NFPA), *o*-phthaldialdehyde (OPA), 3-mercaptopropionic acid (MPA) and trifluoroacetic acid (TFA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Diphenyl phosphite was obtained from J&K Chemical Ltd. (Shanghai, China). HPLC-grade methanol and acetonitrile were purchased from Tedia Company Inc. (Fairfield, OH, USA). Strong acidic cation-exchange resin (Diaion SK1B) was purchased from H&E Co. Ltd. (Beijing, China). Water was produced by the Milli-Q system (18 MΩ; Millipore, Bedford, MA, USA). Unless specified otherwise, all chemicals and solvents were analytical reagents.

NMR Analysis: ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker 400 MHz NMR spectrometer. Chemical shifts for ^{1}H NMR are reported relative to internal tetramethylsilane (Me_4Si, δ = 0.00 ppm) with $CDCl_3$ as solvent and D_2O (H_2O, δ = 4.70 ppm). ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts for ^{13}C NMR spectra were measured relative to $CDCl_3$ (δ = 77.0 ppm). ^{31}P NMR chemical shifts were recorded at 162 MHz, and chemical shifts were relative to external 85% phosphoric acid (δ = 0.0 ppm). Chemical shifts are given in ppm and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), quint. (quintuplet) and m (multiplet).

ESI-MS and ESI-MS/MS Analysis: Mass spectra were acquired with a Bruker Dalton Esquire 3000 plus ion-trap mass spectrometer (Bruker Daltonik Co., Germany) equipped with a gas nebulizer probe capable of analysing ions up to m/z = 6000. The mass spectrometer was operated in the positive ion mode. Nitrogen was used as the dry gas at a flow rate of 4 L/min. The heated capillary temperature was 250 °C. The reaction samples dissolved in methanol were ionized by ESI and continuously infused into the ESI chamber at a flow rate of 0.24 mL/h by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Co., Vernon Hills, IL, USA). The precursor ions [M + H]$^+$ were selected by using an isolation width of 1.0–1.5 m/z units and analysed by multistage tandem mass spectrometry (MS^n) through collisions with helium.

High-resolution mass spectra were recorded with an ESI-Q-TOF-MS spectrometer (Micromass, Waters, England) equipped with an analytical electrospray source in the positive ion mode. The sample in CH_3OH was introduced into the source at a flow rate of 10 μL/min. The nebulizing gas flow was 0.5 L min^{-1} and the ion accumulation time was 10.0 ms.

LC-ESI-MS Analysis: To analyse the samples by LC-MS,[24] an ion-trap mass spectrometer (Bruker Esquire 3000 plus, Dalton Co., Germany) equipped with an electrospray ionization source was

coupled to an Agilent 1100 binary pumping system (Agilent 1100 technologies, Wilmington, DE). Chromatogram column: Supelcosil ABZ + plus 150 × 4.6 mm I.D., 5 μm (Supelco, Bellefonte, PA, USA). Solvent A: 2 mM NFPA in water; solvent B: acetonitrile. The column was thermostatted at 25 °C and eluted by using an eluent program with a flow rate of 1.0 mL/min at 4% B in 20 min. The injection volume was 20 μL. Operating conditions for ESI in the positive ion mode are listed below: spray voltage: 4000 V; target: m/z = 200; capillary temperature: 280 °C; dry gas (N_2): 10 L/min; nebulizer (N_2): 30 psi. Mass spectra were registered in the scan range from m/z = 50 to m/z = 600. For LC-ESI-MS, about 1/10 of the effluent from the UV absorbance detector was introduced into the electrospray through a splitting T valve in order to avoid too high a flow rate in the ion source.

HPLC Analysis: The amino acids and peptides of the reaction mixture were analysed by derivatization method with *o*-phthaldialdehyde (OPA)/3-mercaptopropionic acid (MPA) according to the literature.[25] (a) The stock solution of OPA/MPA (prepared every day): OPA (25 mg) + CH_3OH (0.5 mL) + borate buffer (3.5 mL, 100 mmol/L, pH 10.4) + MPA (25 μL). (b) Borate buffer (100 mmol/L, pH = 10.4). (c) Diluted reaction mixtures: the lyophilized samples were dissolved in Milli-Q water (0.5 mL) and then 10 μL of the solution were diluted to 1.0 mL with Mili-Q water. Derivatization procedure: 10 μL of (c) were added to solutions of 20 μL of (a) and 70 μL of (b). After 2 min, 25 μL aliquots were injected into HPLC.

Reactions of Sodium Salts of *N*-(Alkoxyphosphoryl) Amino Acids (*N*-MAP-AAs) in Aqueous Solution

(1) For Entries 1–3, 7 and 8: The sodium salts of *N*-MAP-AAs (0.1 mmol; 0.25 mmol for entry 2) were dissolved in D_2O (0.5 mL). The pD of the solution was about 10.0–11.0 and then adjusted to 4.0–5.0 by the addition of 1 M DCl. The solution was kept at 40 °C for several days and the pD of the solution was maintained at 4.0–5.0 by frequent addition of 1 M DCl. The reaction was traced periodically by ^{31}P NMR spectrometry and ESI-MS through the reaction process.

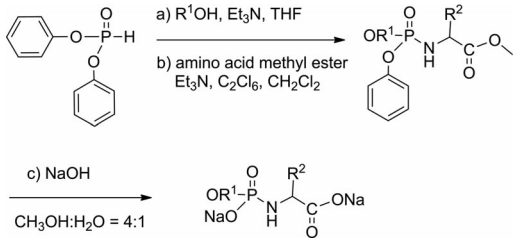
(2) For Entries 4–6: The sodium salt of *N*-MMP-Gly (0.1 mmol) was dissolved in D_2O (0.5 mL). $MgCl_2{\cdot}6H_2O$ (0.01–0.05 mmol) was added to the solution. The pD of the solution was adjusted to 4.0–5.0 by the addition of 1 M DCl. The solution was kept at 40 °C for 8 d. The reaction mixture was monitored by ^{31}P NMR spectroscopy and analysed by LC-MS.

(3) For entry 9: The sodium salt of *N*-MMP-Gly (0.1 mmol) was dissolved in D_2O (0.5 mL). Alanine (0.1 mmol) was added to the solution. The pD of the solution was adjusted to 4.0–5.0 by the addition of 1 M DCl. The solution was kept at 40 °C for 8 d. The reaction mixture was monitored by ^{31}P NMR spectroscopy and analysed by LC-MS.

All of the above reaction solutions were frozen, lyophilized and stored at –20 °C for further HPLC analysis.

Reaction of Sodium Salt of *N*-(Methoxyphosphoryl)glycine and Acetic Acid: The sodium salt of *N*-MMP-Gly (0.1 mmol) was dissolved in D_2O (0.5 mL). Acetic acid (0.1 mmol, about 7 μL) was added to the solution. The solution was kept at 40 °C for 8 d. The reaction was traced by ^{31}P NMR spectroscopy and ESI-MS. The reaction mixture was analysed by LC-MS.

General Procedure for the Synthesis of the Alkoxy Phenoxy Phosphoramidates of Amino Acid Methyl Esters and the Alkoxy Phenoxy Phosphoramidate of Glycylglycine Methyl Ester: The title compounds were synthesized according to a literature method (Scheme 3).[26] Diphenyl phosphite (2 mmol) and a catalytic amount of Et_3N (about five drops) were dissolved in dry THF (15 mL) and cooled to –5 °C. CH_3OH (1 mmol, 200 μL) in dry THF (10 mL) was added dropwise to the solution under argon. The resulting mixture was stirred at –5 °C for about 0.5 h and then warmed to room temperature over 1 h. The progress of the reaction was traced by ^{31}P NMR spectroscopy. Then the amino acid methyl ester hydrochloride (2 mmol), Et_3N (3 mmol, 0.31 g) and C_2Cl_6 (3.6 mmol) in CH_2Cl_2 (15 mL) were sequentially added to the resulting solution at 0 °C and the reaction mixture was stirred at room temperature for about 4 h. The solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether) to afford the target products.



Scheme 3. Synthesis of the sodium salts of *N*-monoalkyloxyphosphoryl amino acids.

Methoxy Phenoxy Phosphoramidate of Glycine Methyl Ester (1a): Colourless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.12–7.32 (m, 5 H), 3.81 (d, J = 17.2 Hz, 3 H), 3.73–3.78 (m, 2 H), 3.71 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0 (d, J = 8 Hz), 150.7 (d, J = 6 Hz), 129.6, 124.8, 120.1 (d, J = 5 Hz), 53.6 (d, J = 5 Hz), 52.3, 42.8 ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 4.44 ppm. IR (film): $\tilde{\nu}$ = 3228, 2960, 2842, 1590, 1436, 1270, 1212, 1142, 1038 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 260.0699; found 260.0685; calcd. for $[M + Na]^+$ 282.0507; found 282.0508.

Methoxy Phenoxy [^{15}N]Phosphoramidate of Glycine Methyl Ester (^{15}N-1a): Colourless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.11–7.32 (m, 5 H), 3.80 (d, J = 11.6 Hz, 3 H), 3.70–3.77 (m, 2 H), 3.69 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0 (d, J = 8 Hz), 149.7 (d, J = 7 Hz), 128.6, 123.8, 119.1 (d, J = 5 Hz), 52.6 (d, J = 5 Hz), 51.3, 41.8 (d, J = 9 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 4.43 (d, J = 45.4 Hz) ppm. IR (film): $\tilde{\nu}$ = 3207, 2955, 1760, 1598, 1494, 1254, 1146, 1042 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 261.0658; found 261.0654.

Methoxy Phenoxy Phosphoramidate of Alanine Methyl Ester (10a): The title compound was synthesized as a mixture of two diastereomers. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.12–7.28 (m, 5 H), 3.95–4.08 (m, 1 H), 3.81 (d, J = 11.6 Hz, 3 H), 3.71 (s, 3 H), 1.38 (d, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (d, J = 9 Hz), 150.7, 129.6 (d, J = 2 Hz), 124.8, 120.1 (d, J = 5 Hz), 53.6 (d, J = 6 Hz), 52.4, 50.1, 20.9 (d, J = 5 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 3.59, 3.49 ppm. IR (film): $\tilde{\nu}$ = 3211, 2977, 1942, 1760, 1598, 1441, 1378, 1254, 1146, 1096, 1042, 922 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 274.0844; found 274.0791; calcd. for $[M + Na]^+$ 296.0664; found 296.0657.

Methoxy Phenoxy Phosphoramidate of Valine Methyl Ester (11a): The title compound was synthesized as a mixture of two diastereomers. Colourless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.12–7.33 (m, 5 H), 3.75–3.82 (m, 4 H), 3.70 (s, 3 H), 3.45–3.51 (m, 1 H),

1.98–2.08 (m, 1 H), 0.87–0.94 (m, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.1, 150.8, 129.5, 124.7, 120.7, 59.9 (d, J = 11 Hz), 53.7 (d, J = 6 Hz), 52.0.1, 32.19 (d, J = 4 Hz), 18.8, 17.3 (d, J = 3 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 4.44 ppm. IR (film): $\tilde{\nu}$ = 3212, 2959, 1743, 1590, 1486, 1254, 1204, 1147, 1047, 926 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 302.1157; found 302.1152; calcd. for $[M + Na]^+$ 324.0977; found 324.0964.

Ethoxy Phenoxy Phosphoramidate of Glycine Methyl Ester (12a): Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.13–7.24 (m, 5 H), 4.16–4.23 (m, 2 H), 3.76–3.80 (m, 2 H), 3.71 (s, 3 H), 1.34 (dt, J = 0.8, 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9 (d, J = 9 Hz), 150.7, 129.6 (d, J = 2 Hz), 124.7, 120.1 (t, J = 5 Hz), 53.6 (d, J = 5 Hz), 52.4 (d, J = 2 Hz), 50.1 (d, J = 8 Hz), 20.9 (t, J = 5 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 2.94 ppm. IR (film): $\tilde{\nu}$ = 3228, 2950, 1756, 1590, 1486, 1212, 1146, 1047, 943 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 274.0844; found 274.0840; calcd. for $[M + Na]^+$ 296.0664; found 296.0648.

Methoxy Phenoxy Phosphoramidate of Glycylglycine Methyl Ester (2a): Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.12–7.51 (m, 5 H), 4.70 (m, 1 H), 3.94 (dd, J = 5.6, 10.0 Hz, 2 H), 3.79 (d, J = 11.2 Hz, 3 H), 3.66–3.71 (m, 5 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4 (d, J = 5.2 Hz), 170.1, 150.6 (d, J = 7 Hz), 129.7, 124.9, 120.1 (d, J = 4 Hz), 53.8 (d, J = 5 Hz), 52.1, 44.4, 40.9 ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 5.15 ppm. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + Na]^+$ 339.0722; found 339.0724.

General Procedure for the Synthesis of the Sodium Salts of *N*-Alkyloxyphosphoryl Amino Acids and *N*-(alkyloxyphosphoryl)glycylglycine: The title compounds were synthesized according to a literature method.[9d] A solution of 0.4 M NaOH (CH_3OH/H_2O = 1:1 or $EtOH/H_2O$ = 1:1, v/v, 10 mL) was added to the alkoxy phenoxy phosphoramidate of the amino acid ester (or the alkoxy phenoxy phosphoramidate of the glycylglycine methyl ester) (1 mmol). The deprotection reactions were carried out at room temperature while stirring under argon for 2–4 h. The course of the reaction was monitored by ^{31}P NMR until the disappearance of the starting material. Once the starting material had disappeared, the solvent was removed under reduced pressure to give an oily crude product, which was recrystallized from 95% ethanol (about 10 mL). The precipitate was washed three times with ethanol (3 mL) and dried under vacuum to give the products as a white solid.

Sodium Salt of *N*-(Methoxyphosphoryl)glycine (1): White solid; m.p. >300 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.39 (d, J = 8.4 Hz, 2 H), 3.51 (d, J = 10.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 179.2, 51.8, 44.9 ppm. ^{31}P NMR (162 MHz, D_2O): δ = 9.64 ppm. IR (KBr): $\tilde{\nu}$ = 3402, 2950, 1606, 1416, 1320, 1204, 1076 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 213.9857; found 213.9857; calcd. for $[M + Na]^+$ 235.9677; found 235.9673.

Sodium Salt of [^{15}*N*](Methoxyphosphoryl)glycine (^{15}N-1): White solid; m.p. >300 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.45 (d, J = 10.8 Hz, 3 H), 3.33 (d, J = 8.0 Hz, 2 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 179.2 (d, J = 9 Hz), 51.8, 45.0 (d, J = 7 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 9.67 (d, J = 32.4 Hz) ppm. ^{15}N NMR (40 MHz, D_2O): δ = 38.89 (d, J = 32.4 Hz) ppm. IR (KBr): $\tilde{\nu}$ = 3253, 2942, 1602, 1411, 1370, 1308, 1125, 1067 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 214.9828; found 214.9828.

Sodium Salt of *N*-(Methoxyphosphoryl)alanine (10): White solid; m.p. >300 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.55 (m, 1 H), 3.49 (d, J = 10.8 Hz, 3 H), 1.28 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 182.9, 52.4, 51.7 (d, J = 8.0 Hz), 21.3 ppm. ^{31}P NMR (162 MHz, D_2O): δ = 8.71 ppm. IR (KBr): $\tilde{\nu}$ = 3361, 2971, 1598, 1453, 1411, 1358, 1200, 1071 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 228.0014; found 228.0017.

Sodium Salt of *N*-(Methoxyphosphoryl)valine (11): White solid; m.p. >300 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.46 (d, J = 10.8 Hz, 3 H), 3.23 (dd, J = 9.6, 6 Hz, 1 H), 1.78–1.87 (m, 1 H), 0.91 (t, J = 6.8 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 182.0, 62.8, 51.6, 32.2 (d, J = 6.0 Hz), 18.8, 17.6 ppm. ^{31}P NMR (162 MHz, D_2O): δ = 9.2 ppm. IR (KBr): $\tilde{\nu}$ = 3402, 2954, 1586, 1411, 1200, 1076, 910 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 256.0327; found 256.0330.

Sodium Salt of *N*-(Methoxyphosphoryl)glycine (12): White solid; m.p. >300 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.78 (dq, J = 7.0, 8.4 Hz, 2 H), 3.33 (d, J = 8.0 Hz, 2 H), 1.16 (t, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 179.2 (d, J = 10 Hz), 61.17, 45.09, 15.7 (d, J = 7 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 8.41 ppm. IR (KBr): $\tilde{\nu}$ = 3299, 2967, 1586, 1399, 1308, 1254, 1208, 1067 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + Na]^+$ 249.9833; found 292.9850.

Sodium Salt of *N*-(Methoxyphosphoryl)glycylglycine (2): White solid; m.p. 179–181 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.75 (s, 2 H), 3.49 (d, J = 10.4 Hz, 2 H), 3.47 (d, J = 10.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 176.6, 174.6, 51.9, 44.5, 43.1 ppm. ^{31}P NMR (162 MHz, D_2O): δ = 9.17 ppm. IR (KBr): $\tilde{\nu}$ = 3398, 2934 1594, 1047, 1320, 1212, 1084, 1042 cm^{-1}. HRMS (ESI-TOF, positive ion mode): calcd. for $[M + H]^+$ 271.0072; found 271.0071; calcd. for $[M + Na]^+$ 292.9891; found 292.9883.

Disodium Salt of Methyl Phosphate (3): The title compound was synthesized according to a literature method.[27] White solid; m.p. 81–82 °C. ^{1}H NMR (400 MHz, D_2O): δ = 3.53 (d, J = 10.8 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 52.8 (d, J = 5 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 3.03 ppm.

Dimethyl Acetyl Phosphate: The title compound was synthesized according to a reported procedure.[28] Acetyl chloride (1.95 g, 0.025 mol) and sodium dimethyl phosphate (3.7 g, 0.025 mol) were suspended in dry tetrahydrofuran (25 mL). The resulting mixture was stirred for 2 d at room temperature under nitrogen. The reaction solution was filtered to remove sodium chloride. Removal of the solvent left the target compound as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.90 (d, J = 11.6 Hz, 6 H), 2.23 (d, J = 1.6 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.0 (d, J = 9.2 Hz), 55.3 (d, J = 5 Hz), 21.7 (d, J = 8 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = –6.2 ppm. MS (ESI, positive ion mode): m/z = 168.9 $[M + H]^+$, 190.9 $[M + Na]^+$.

Sodium Methyl Acetyl Phosphate (7): A solution of sodium iodide (2.4 g, 16 mmol) in dry acetone (15 mL) was added to a solution of acetyl dimethyl phosphate (2 g, 16 mmol) in dry acetone (10 mL). The solution stood overnight at room temperature. The precipitate was collected by filtration and washed with dry acetone followed by CH_2Cl_2. Then the crude product was dried under vacuum to give the target product as a white powder. ^{1}H NMR (400 MHz, D_2O): δ = 3.60 (d, J = 11.2 Hz, 3 H), 2.11 (d, J = 1.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 170.2 (d, J = 9.0 Hz), 53.6 (d, J = 6 Hz), 21.5 (d, J = 6 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ = –6.0 ppm. MS (ESI, positive ion mode): m/z (%) = 176.9 $[M + H]^+$. MS (ESI, negative ion mode): m/z = 152.8.

Methyl Phosphate Dicyclohexylammonium Salt: The title compound was synthesized according to a literature method.[29] Yield 55%. White solid; m.p. 203–205 °C. ^{1}H NMR (400 MHz, D_2O): δ

= 3.44 (d, J = 10.4 Hz, 3 H), 3.08–3.15 (m, 2 H), 1.95–1.97 (m, 4 H), 1.75–1.78 (m, 4 H), 1.62 (d, J = 12.8 Hz, 2 H), 1.24–1.37 (m, 8 H), 1.10–1.19 (m, 2 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 51.4 (d, J = 5 Hz), 50.2, 30.2, 24.2, 23.7 ppm. ^{31}P NMR (162 MHz, D_2O): δ = 9.64 ppm.

Aminoacyl Methyl Phosphate: The title compound was synthesized according to a literature method.[30] *N*,*N*′-Dicyclohexylcarbodiimide (DCC, 0.43 g, 2.1 mmol) was added to a flask containing *N*-Boc-amino acid (0.95 g, 4.4 mmol) in CH_2Cl_2 (30 mL) at room temperature. The resulting reaction mixture was stirred for 5 min under argon and methyl phosphate *N*,*N*′-diisopropyl-*N*-ethylammonium (DIEA) salt dissolved in CH_3CN (5 mL) was added. The reaction mixture was stirred at room temperature for 1 h. After the reaction was complete, the resulting mixture was filtered and washed with CH_2Cl_2 (10 mL). The CH_2Cl_2 solution was then extracted three times with water (18 mL). Then the aqueous phases were combined, frozen and lyophilized. The product was purified by short silica gel column chromatography using acetone as eluent to remove inorganic salts. The solvent was removed in vacuo to give *N*-Boc-amino acid methyl phosphate DIEA salt.

Aminoacyl methyl phosphate was obtained by adding a minimum amount of TFA to the *N*-Boc-glycyl methyl phosphate. After 30 min, an excess of diethyl ether was added to precipitate the product. Following centrifugation, the Et_2O was decanted, the precipitate was washed twice by agitation with Et_2O followed by centrifugation and decantation. The solid product was dried under vacuum.

Glycyl Methyl Phosphate (8): ^{1}H NMR (400 MHz, D_2O): δ = 3.90 (s, 2 H), 3.63 (d, J = 11.6 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 164.7 (d, J = 9.0 Hz), 54.1 (d, J = 5 Hz), 40.7 (d, J = 8 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ = –6.4 ppm. MS (ESI, positive ion mode): m/z = 191.8 $[M + H]^+$.

Alaninyl Methyl Phosphate (9): ^{1}H NMR (400 MHz, D_2O): δ = 4.19 (q, J = 7.6 Hz, 1 H), 3.61 (d, J = 11.6 Hz, 3 H), 1.54 (d, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 164.7 (d, J = 9.0 Hz), 66.0, 54.1, 40.7 (d, J = 8.0 Hz) ppm. ^{31}P NMR (162 MHz, D_2O): δ = –6.2 ppm. MS (ESI, positive ion mode): m/z = 205.9 $[M + H]^+$.

Supporting Information (see footnote on the first page of this article): LC-ESI-MS spectra of entry 1, ^{31}P NMR spiking spectra of products **2**, **7**, **8** and **9**, HPLC chromatograms of entries 1–9 and standard samples.

Acknowledgments

We are grateful for financial support from the National Natural Science Foundation of China (NSFC) (grant numbers 21075103, 20972130, 20805037, and 20732004). We thank Prof. G. M. Blackburn for helpful suggestions.

[1] a) A. R. Lewinsohn, M. Paecht-Horowitz, A. Katchalsky, *Biochim. Biophys. Acta* **1967**, *140*, 24–36; b) P. Schimmel, *Annu. Rev. Biochem.* **1987**, *56*, 125–158.

[2] a) J. P. Biron, R. Pascal, *J. Am. Chem. Soc.* **2004**, *126*, 9198–9199; b) J. P. Biron, A. L. Parkes, R. Pascal, J. D. Sutherland, *Angew. Chem. Int. Ed.* **2005**, *44*, 6731–6734; c) L. J. Leman, L. E. Orgel, M. R. Ghadiri, *J. Am. Chem. Soc.* **2006**, *128*, 20–21.

[3] a) F. H. Westheimer, *Science* **1987**, *235*, 1173–1178; b) N. S. M. D. Wickramasinghe, M. P. Staves, J. C. Lacey, *Biochemistry* **1991**, *30*, 2768–2772; c) R. Kluger, R. W. Loo, V. Mazza, *J. Am. Chem. Soc.* **1997**, *119*, 12089–12094; d) S. Tzvetkova, R. Kluger, *J. Am. Chem. Soc.* **2007**, *129*, 15848–15854.

[4] a) P. V. Attwood, M. J. Piggott, X. L. Zu, P. G. Besant, *Amino Acids* **2007**, *32*, 145–156; b) E. A. Ruben, M. S. Chapman, J. D. Evanseck, *J. Am. Chem. Soc.* **2005**, *127*, 17789–17798; c) K. Hakansson, D. B. Wigley, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1505–1510.

[5] a) Y. F. Zhao, P. S. Cao, *J. Biol. Phys.* **1994**, *20*, 283–287; b) W. H. Zhou, Y. Ju, Y. F. Zhao, Q. G. Wang, G. A. Luo, *Origins Life Evol. Biosphere* **1996**, *26*, 547–560; c) Z. Z. Chen, B. Tan, Y. M. Li, Y. F. Zhao, *J. Org. Chem.* **2003**, *68*, 4052–4058.

[6] H. Fu, Z. Z. Li, Y. F. Zhao, G. Z. Tu, *J. Am. Chem. Soc.* **1999**, *121*, 291–295.

[7] C. M. Cheng, X. H. Liu, Y. M. Li, Y. Ma, B. Tan, R. Wan, Y. F. Zhao, *Origins Life Evol. Biosphere* **2004**, *34*, 455–464.

[8] a) Y. Yamagata, H. Watanabe, M. Saitoh, T. Namba, *Nature* **1991**, *352*, 516–519; b) A. W. Schwartz, *Philos. Trans. R. Soc. London, Ser. B* **2006**, *361*, 1743–1749; c) M. A. Pasek, T. P. Kee, D. E. Bryant, A. A. Pavlov, J. I. Lunine, *Angew. Chem. Int. Ed.* **2008**, *47*, 7918–7920.

[9] a) J. Rabinowitz, J. Flores, R. Krebsbach, G. Rogers, *Nature* **1969**, *224*, 795–796; b) X. Gao, Y. Liu, P. X. Xu, Y. M. Cai, Y. F. Zhao, *Amino Acids* **2008**, *34*, 47–53; c) F. Ni, S. T. Sun, C. Huang, Y. F. Zhao, *Green Chem.* **2009**, *11*, 569–573; d) F. Ni, X. Gao, Z. X. Zhao, C. Huang, Y. F. Zhao, *Eur. J. Org. Chem.* **2009**, *18*, 3026–3035.

[10] a) S. J. Benkovic, P. A. Benkovic, *J. Am. Chem. Soc.* **1967**, *89*, 4714–4722; b) E. J. Sampson, J. Fedor, P. A. Benkovic, S. Benkovic, *J. Org. Chem.* **1973**, *38*, 1301–1306.

[11] F. Ni, W. Li, Y. M. Li, Y. F. Zhao, *Curr. Protein Pept. Sci.* **2005**, *6*, 437–442.

[12] a) L. F. Landweber, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11067–11068; b) R. Pascal, L. Boiteau, A. Commeyras, *Top. Curr. Chem.* **2005**, *259*, 69–122.

[13] a) C. R. Wagner, V. V. Iyer, E. J. McIntee, *Med. Res. Rev.* **2000**, *20*, 417–451; b) D. Cahard, C. McGuigan, J. Balzarini, *Mini-Rev. Med. Chem.* **2004**, *4*, 371–381; c) S. J. Hecker, M. D. Erion, *J. Med. Chem.* **2008**, *51*, 2328–2345; d) Y. Mehellou, J. Balzarini, C. McGuigan, *ChemMedChem* **2009**, *4*, 1779–1791.

[14] a) J. Balzarini, A. Karlsson, S. Aquaro, C. F. Perno, D. Cahard, L. Naesens, E. D. Clercq, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7295–7299; b) M. Derudas, D. Carta, A. Brancale, C. Vanpouille, A. Lisco, L. Margolis, J. Balzarini, C. McGuigan, *J. Med. Chem.* **2009**, *52*, 5520–5530; c) C. L. Chen, G. Yu, T. K. Venkatachalam, F. M. Uckun, *Drug Metab. Dispos.* **2002**, *30*, 1523–1531.

[15] a) O. Adelfinskaya, P. Herdewijn, *Angew. Chem. Int. Ed.* **2007**, *46*, 4356–4358; b) O. Adelfinskaya, M. Terrazas, M. Froeyen, P. Marliere, K. Nauwelaerts, P. Herdewijn, *Nucleic Acids Res.* **2007**, *35*, 5060–5072; c) M. Terrazas, P. Marliere, P. Herdewijn, *Chem. Biodiversity* **2008**, *5*, 31–39.

[16] S. Michielssens, N. T. Trung, M. Froeyen, P. Herdewijn, M. T. Nguyen, A. Ceulemans, *Phys. Chem. Chem. Phys.* **2009**, *11*, 7274–7285.

[17] a) H. Fu, L. Xu, Q. Lv, J. Z. Wang, H. Z. Xiao, Y. F. Zhao, *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1813–1822; b) X. Gao, F. Ni, J. Bao, Y. Liu, P. X. Xu, Y. F. Zhao, *J. Mass Spectrom.* **2010**, *45*, 779–787.

[18] T. Lonnberg, M. Ora, H. Lonnberg, *Mini-Rev. Org. Chem.* **2010**, *7*, 33–43.

[19] a) K. N. Allen, D. Dunaway-Mariano, *Trends Biochem. Sci.* **2004**, *29*, 495–503; b) P. P. Dzeja, R. Bortolon, C. Perez-Terzic, E. L. Holmuhamedov, A. Terzic, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10156–10161; c) J. R. Knowles, *Annu. Rev. Biochem.* **1980**, *49*, 877–919.

[20] I. S. Kulaev, V. M. Vagabov, T. V. Kulakovskaya, *The Biochemistry of Inorganic Polyphosphates*, 2nd ed., Wiley, Chichester, **2004**, pp. 194–204.

[21] a) G. M. Blackburn, M. J. Brown, *J. Am. Chem. Soc.* **1969**, *91*, 525–526; b) S. A. Khan, A. J. Kirby, M. Wakselman, D. P. Horning, J. M. Lawlor, *J. Chem. Soc. B* **1970**, 1182–1187.
[22] F. Lipmann, *Science* **1954**, *120*, 855–865.
[23] a) A. C. Hengge, *Acc. Chem. Res.* **2002**, *35*, 105–112; b) M. Ora, K. Mattila, T. Lonnberg, M. Oivanen, H. Lonnberg, *J. Am. Chem. Soc.* **2002**, *124*, 14364–14372; c) M. J. P. Harger, *Chem. Commun.* **2004**, 1952–1953.
[24] K. Petritis, S. Brussaux, S. Guenu, C. Elfakir, M. Dreux, *J. Chromatogr. A* **2002**, *957*, 173–185.
[25] a) B. N. Jones, S. Paabo, S. Stein, *J. Liq. Chromatogr.* **1981**, *4*, 565–586; b) A. Fiorino, G. Frigo, E. Cucchetti, *J. Chromatogr.* **1989**, *476*, 83–92.
[26] X. S. Li, H. Fu, Y. Y. Jiang, Y. F. Zhao, J. Y. Liu, *Synlett* **2004**, *14*, 2600–2602.
[27] P. K. Grzyska, P. G. Czyryca, J. Golightly, K. Small, P. Larsen, R. H. Hoff, A. C. Hengge, *J. Org. Chem.* **2002**, *67*, 1214–1220.
[28] R. Kluger, A. S. Grant, S. L. Bearne, M. R. Trachsel, *J. Org. Chem.* **1990**, *55*, 2864–2868.
[29] C. Dueymes, C. Pirat, R. Pascal, *Tetrahedron Lett.* **2008**, *49*, 5300–5301.
[30] R. Kluger, L. L. Cameron, *J. Am. Chem. Soc.* **2002**, *124*, 3303–3308.

Received: February 22, 2011
Published Online: May 2, 2011

