JOC Article

Novel Synthesis of Nucleoside 5′-Phosphoramidates through Reaction of Nucleoside Triphosphates with Amines Mediated by Trimethylsilyl Chloride

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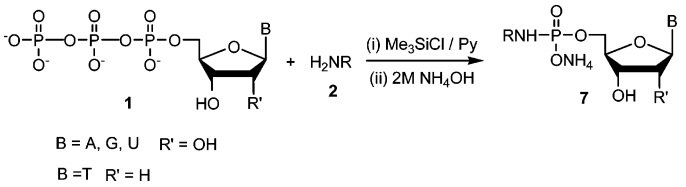

Reaction of nucleoside triphosphates (NTPs) with amines in pyridine mediated by trimethylsilyl chloride produced nucleoside 5′-phosphoramidates in moderate yields without any preprotection of nucleosides and amino acid methyl esters. The reaction pathway is very similar to the mechanism of the RNA capping reaction, DNA or RNA ligation reaction, and catalysis of hydrolases and nucleases involving the formation of covalent enzyme−NMP (nucleoside 5′-monophosphate) intermediates in biological systems, which could provide a valuable clue for the enzymatic reactions.

Introduction

Strand joining by polynucleotide ligase entails AMP transfer from ATP to the 5′-monophosphate end of DNA or RNA to form AppDNA or AppRNA (Figure 1a).[1,2] A similar reaction involving GMP transfer from GTP to the 5′-diphosphate RNA end to form the structure G(5′)-ppp(5′)RNA[3−5] occurs during RNA capping (Figure 1b). In both cases, nucleotidyl transfer occurs through covalent lysyl−NMP (nucleoside 5′-monophosphate) intermediates containing a P−N bond (Figure 2a).[6] Catalysis of many hydrolases and nucleases also undergoes phosphoramidate intermediates.[7] For example, the human fragile histidine triad protein Fhit catalyzes the Mg^{2+}-dependent hydrolysis of P^1-5′-*O*-adenosine-P^3-5′-*O*-adenosine triphosphate, Ap^3A, to AMP and ADP (Figure 1c). The reaction is thought to follow a two-step mechanism, in which the complex of Ap^3A and Mg^{2+} reacts in the first step with His96 of the enzyme to form a covalent Fhit−AMP phosphoramidate intermediate and release MgADP (Figure 2b).

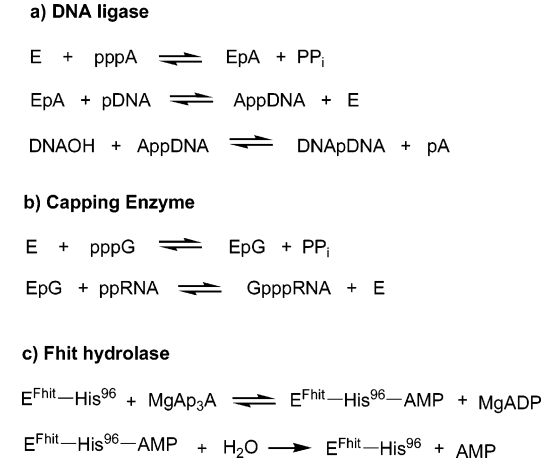

FIGURE 1. Covalent catalysis in nucleotidyl transfer by DNA ligase, capping enzyme, and Fhit hydrolase.

Nucleoside triphosphates (NTPs) as high-energy phosphate donors are able to transfer nucleoside monophos-

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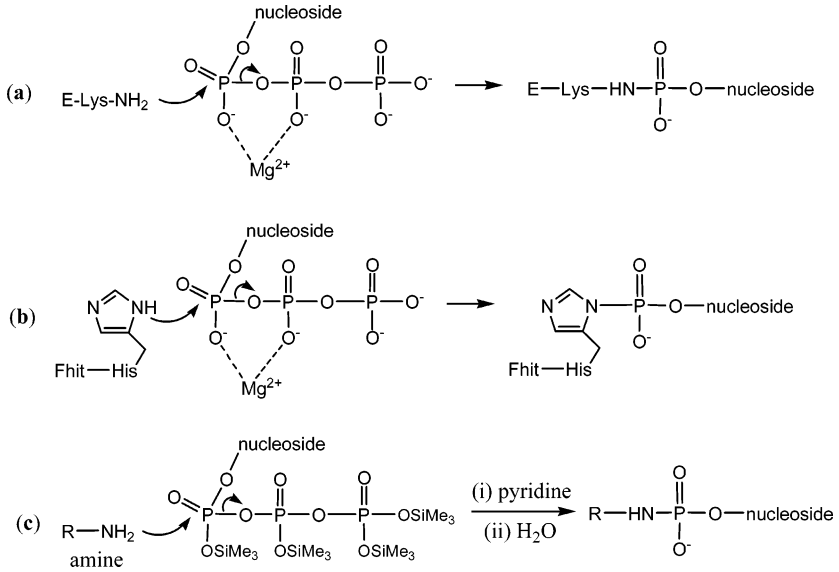
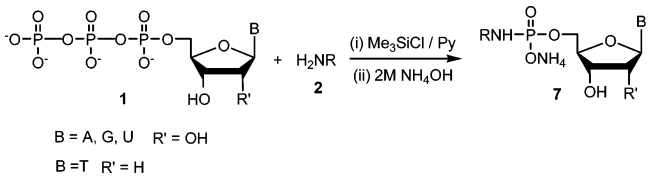


FIGURE 2. (a) The possible formation mechanism of the lysyl−NMP intermediates (phosphoramidates) for covalent catalysis in nucleotidyl transfer by DNA ligase and capping enzyme. (b) The nucleophilic attack by the imidazole ring of His96 on Ap_3A to form the covalent intermediate Fhit-AMP. (c) Our strategy: reaction of tetrasilyl-NTPs with amines in pyridine.

phates (NMPs) to the nitrogen nucleophiles, especially lysyl and histidyl residues in biological systems, and the formation of intermediates containing P−N bond speeds up the overall transfer of phosphates to oxygen nucleophiles. However, nucleotidyl or phosphoryl transfer reaction of nucleoside 5′-triphosphates (NTPs) with amines to form enzymatic intermediates containing a P−N bond had never been observed directly in solution, and there was no experimental basis for the nucleotidyl transfer model.[8] On the other hand, phosphoramidate pronucleotides have proven to be an effective strategy for the intracellular delivery of nucleoside 5′-monophosphates, and amino acid phosphoramidates of nucleosides have been shown to enhance antiviral activity and reduce cytotoxicity compared with their parent nucleosides.[9−11] Therefore, insights drawn from these studies have provided valuable references for the future design of phosphoramidate-based pronucleotides.[12,13] Chlorophosphate[9−11] and dicyclohexylcarbodiimide (DCC)[14]-mediated coupling methods are usually applied for the synthesis of this kind of phosphoramidates. Here, we attempted the reaction of NTPs with amines mediated by trimethylsilyl chloride (TMS−Cl) in pyridine under mild conditions, and the desired nucleoside phosphoramidates were obtained in moderate yields.

SCHEME 1. Reaction of NTPs with Amines Mediated by TMS−Cl in Pyridine



Results and Discussion

Attack of nucleophile on the NTP tetraanion is prevented by electrostatic repulsion in aqeous solution, and the enzyme, with the help of an Mg^{2+} ion, provides an environment that permits a nucleophilic attack on the triphosphate (Figure 2a,b). Encouraged by these results, we believe that nucleophilic attack of the amino group of amines on α-phosphorus can occur in the similar procedures given above if these negative charges on NTP are blocked by silylation with TMS−Cl in pyridine (Figure 2c).

Reaction of NTPs with amines mediated by TMS−Cl in pyridine was performed under very mild conditions (Scheme 1). TMS−Cl (2 equiv) was added dropwise to a mixture of NTP (nucleoside 5′-triphosphate disodium salt) and 2 equiv of amino acid methyl ester in anhydrous pyridine at room temperature under nitrogen atmosphere, and the solution was stirred continuously for 24 h. From the beginning to the end, there are white precipitates appearing in reaction system. The solvent and an excess of TMS−Cl were removed under reduced pressure, the residue was hydrolyzed in 2 M NH_4OH, and the resulting solution was washed with ether three times. The aqueous phase was lyophilized, and the residue was

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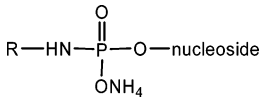
TABLE 1. Isolated Yields of Products through Reaction of NTPs with Amines Mediated by TMS−Cl in Pyridine



compd	amine	nucleoside	yield, %
7aa	*n*-pentylamine	A	48
7ab	LeuOMe	A	56
7ac	PheOMe	A	52
7ad	*β*-AlaOMe	A	48
7ae	Asp(OMe)$_2$	A	44
7af	SerOMe	A	45
7ba	LeuOMe	G	54
7bb	PheOMe	G	53
7bc	AlaOMe	G	48
7bd	Asp(OMe)$_2$	G	44
7be	SerOMe	G	43
7ca	LeuOMe	U	51
7cb	PheOMe	U	53
7cc	AlaOMe	U	50
7cd	Asp(OMe)$_2$	U	46
7ce	SerOMe	U	44
7da	LeuOMe	T	54
7db	PheOMe	T	55
7dc	AlaOMe	T	50
7dd	Asp(OMe)$_2$	T	49

subjected to column chromatography on silica gel with use of 2-propanol:NH_4OH:H_2O (13:1:2) as eluent. The products were obtained as a colorless solid in 43−56% yields (Table 1), and their structures were confirmed by ^{31}P, ^{1}H, and ^{13}C NMR and HR-MS.

Reaction Mechanism of NTPs with Amines Mediated by TMS−Cl in Pyridine. ^{31}P NMR spectroscopy provides a very convenient method for studying the reaction mechanism of organophosphorus compounds, and Figure 3A−E gives ^{31}P NMR spectra of ATP and its derivatives under different conditions. ATP has three peaks at −7.96 (*γ*-P), −10.24 (α-P), and −20.41 ppm (*β*-P) in water (Figure 3A). Reaction products of ATP with TMS−Cl in pyridine show four peaks at −15.92 (α-P), −20.63 and −24.09 (*γ*-P), and −29.71 ppm (*β*-P) (Figure 3B); the peaks at −20.63 and −24.09 ppm correspond to the products in which only one or two of two oxygen anions on *γ*-phosphorus were silylanized. Here, we chose the reaction of ATP with phenylalanine methyl ester as a typical example. TMS−Cl was added to ATP and phenylalanine methyl ester in pyridine, and the solution was stirred for 24 h at room temperature; two new peaks at 1.12 ppm and −7.38 ppm in Figure 3C, corresponding to compound **5** and **6** in Scheme 2, appeared. The resulting solution was evaporated to dryness under reduced pressure, and the residue was hydrolyzed; three peaks at 6.82 (**7ac**), 1.84 (salt of AMP), and 1.21 ppm (salt of H_3PO_4) were observed (Figure 3D). The target product (**7ac**) at 6.82 ppm (Figure 3E) was obtained after isolation by column chromatography. It is interesting that nucleophilic attack of amine only occurred on α- but not *β*- or *γ*-phosphorus. In fact, the ^{31}P NMR spectrum provided us a clue: ^{31}P NMR chemical shifts of compound **3** are $\delta_{\alpha-P} > \delta_{\gamma-P} > \delta_{\beta-P}$, and correspondingly, α-phosphorus has the lowest electron density, which is favorable for nucleophilic attack of amine on α-phosphorus. Silylation of NTP in pyridine canceled negative charges at the triphosphate moiety, deactivated the 3′-OH group of

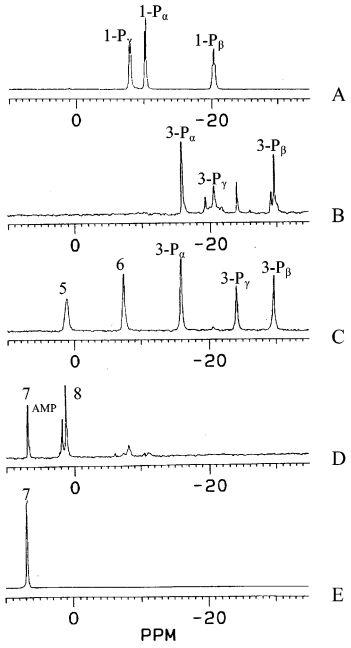


FIGURE 3. ^{31}P NMR spectra of the samples: (A) ATP in water; (B) reaction products of ATP with TMS−Cl in pyridine; (C) reaction products of ATP with phenylalanine methyl ester hydrochloride mediated by TMS−Cl in pyridine; (D) hydrolysis products of samples in Figure 1C; and (E) the isolated target product (**7c**).

nucleoside (preventing conversion to cNMP), and allowed for an attack of external nucleophlies such as the amino group of amines, with silylated pyrophosphate as the leaving group. It is important to notice that an excess of TMS−Cl did not deactivate amines since bis-silylated amines were not formed under these conditions. Therefore, α-phosphorus, as the sterically less hindered center, under these circumstances underwent attack by the amino function of amines, providing the target phosphoramidates as shown in Scheme 2. In addition, pyridine was used as the reaction medium and scavenger for hydrogen chloride, but it took as the nucleopile, providing transient and discrete intermediates such as the pyridinium of the pentacoordinated phosphoranes (**1′** and **2′**) in Scheme 3 undergoing further attack by NH_2−nucleophiles, and similar pyridinium intermediates had been reported in earlier research.[15,16]

We performed a control experiment. TMS−Cl was added to pyrophosphoric acid and phenylalanine methyl ester in pyridine, but no matter how long the stirring lasted, tetrasilylpyrophosphoric acid could not react with phenylalanine methyl ester because of the higher charge and steric exclusion on phosphorus of tetrasilylpyrophosphoric acid.

This synthetic method for nucleoside phosphoramidates has an evident advantage over DCC and chlorophosphate methods, i.e., it is not necessary to preprotect the function groups on nucleosides and amino acid methyl esters.

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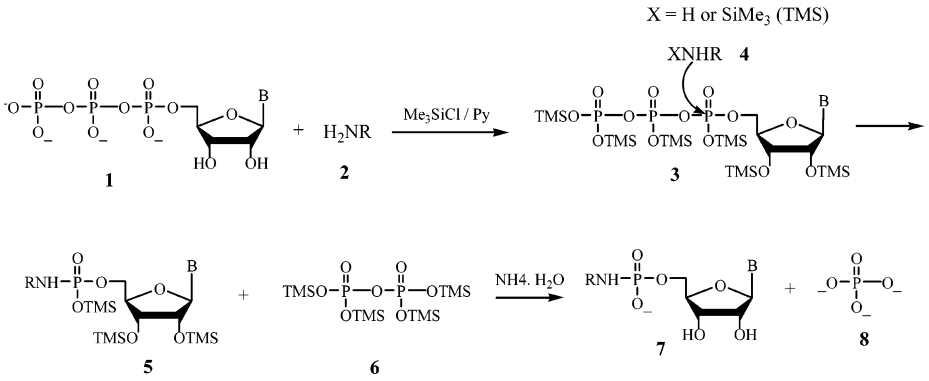
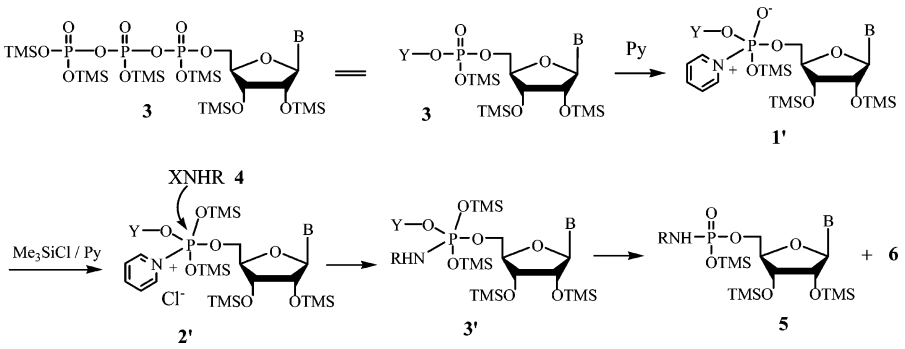
SCHEME 2. Possible Reaction Mechanism of NTPs with Amines Mediated by TMS−Cl in Pyridine



SCHEME 3. Catalysis of Pyridine for the Formation of Phosphoramidates in Scheme 2



Conclusions

Reaction of NTPs with amines yielded phosphoramidates of nucleosides in moderate yields under mild conditions: the reaction pathway is very similar to the mechanism of the RNA capping reaction, the DNA or RNA ligation reaction, and the catalysis of hydrolases and nucleases involving the formation of covalent enzyme−NMP intermediates, which could provide a valuable clue for the enzymatic reactions. In addition, reaction of NTPs with amines mediated by TMS−Cl in pyridine produced phosphoramidates of nucleosides in moderate yields without any preprotection of nucleosides and amino acid methyl esters, which also gave a very useful method for the synthesis of the phosphoramidates.

Experimental Section

Reaction of NTPs with Amines Mediated by TMS−Cl in Pyridine. Under a nitrogen atmosphere at 25 °C, 2 equiv of TMS−Cl was added dropwise to a mixture of NTP (nucleoside 5′-triphosphate disodium salt) (0.2 mmol) and amine or amine hydrochloride (0.4 mmol) in anhydrous pyridine, and the solution was stirred continuously for 24 h. From the beginning to the end, there are white precipitates in the reaction system. The solvent and excess of TMS−Cl were removed under reduced pressure. The residue was hydrolyzed in 2 M NH_4OH and then washed with ether three times. The aqueous phase was then lyophilized, and the residue was subjected to column chromatography on silica gel with 2-propanol:NH_4OH:H_2O (13:1:2) as eluent. The products were isolated as colorless solids (yields: 43−56%), and their structures were checked by ^{31}P, ^{1}H, and ^{13}C NMR and high-resolution mass spectrometry (HR-MS).

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Supporting Information Available: Characterization data for compounds **7aa**−**dd** and ^{1}H and ^{13}C NMR spectra of the representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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