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A General and Chemoselective Synthesis of Phosphoramidates through Reaction of Silylated Nucleoside Di- and Triphosphates with Silylated Amines Containing Multifunctional Groups

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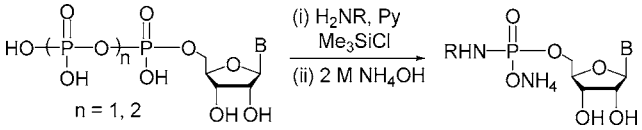

An approach to various phosphoramidates has been developed through one-pot chemoselective reaction of nucleoside di- and triphosphates with 3′-amino-3′-deoxythymidine, D-glucosamine, and peptides mediated by trimethylsilyl chloride, and the corresponding conjugates via the linkage of phosphate were obtained. The method could be generally applied for the synthesis of phosphoramidates without any preprotection of polyphosphates and amines containing multifunctional groups.

Oligonucleotide N3′→P5′ phosphoramidates have attracted considerable attention as a class of compounds of potential therapeutic value[1−6] since these oligonucleotide analogues are resistant toward various nucleases[2,7] and hybridize to complementary DNA or RNA targets with much higher affinity than their natural congeners do.[2] Some synthetic methods of these phosphoramidates have been developed such as the H-phosphonate method[8] and using phosphotriester chemistry[9] via the Staudinger-type reaction[10] or by the phosphoramidite method.[4] Phosphoramidate pronucleotides have also 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.[11,12] Therefore, insights drawn from these studies have provided valuable references for the future design of phosphoramidate-based pronucleotides.[13] Chlorophosphate,[12] *N*-chlorosuccinimide (NCS),[14] H-phosphonates,[15] and dicyclohexylcarbodiimide (DCC)[16]-mediated coupling methods are usually applied for the synthesis of this kind of phosphoramidates; however, most of these methods need preprotection for various functional groups. In our previous research, reactions of adenosine diphosphate (ADP) or nucleoside triphosphates with amino acid esters in pyridine mediated by trimethylsilyl chloride (TMS-Cl) were studied.[17] As a continuation of this program, we attempted the one-pot reaction of NDPs (nucleoside diphosphates) or NTPs (nucleoside triphosphates) with 3′-amino-3′-deoxythymidine, D-glucosamine, or peptides containing multifunctional groups mediated by TMS-Cl in pyridine under the mild conditions, and the desired nucleoside phosphoramidates were obtained in moderate yields.

Oligonucleotide N3′→P5′ phosphoramidates are useful target molecules; however, to the best of our knowledge, the previous methods for their synthesis usually need protection and deprotection procedures.[8−10] Herein, we initiated a program to develop an approach to dinucleotide N3′→P5′ phosphoramidates as shown in Scheme 1. Reaction of nucleoside di- or triphosphate

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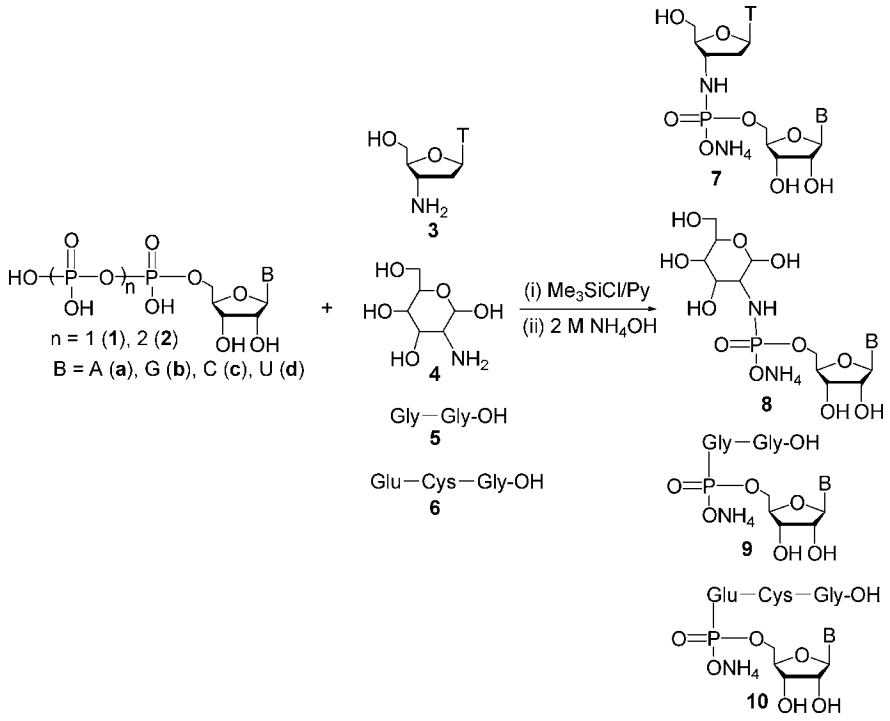
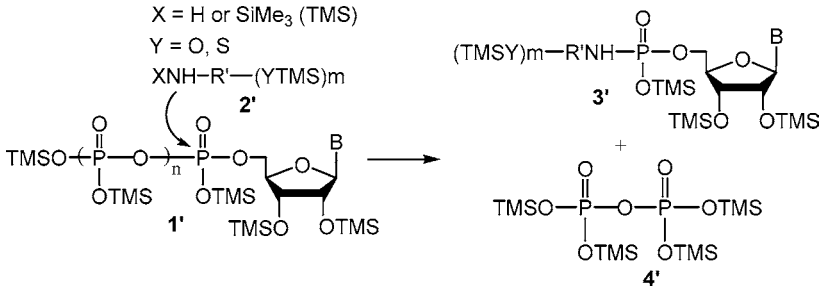
SCHEME 1. Reaction of Silylated Nucleoside Di- and Triphosphates with 3′-Amino-3′-deoxythymidine, D-Glucosamine, and Peptides



SCHEME 2. Possible Reaction Mechanism of Nucleoside Di- and Triphosphates with Amines Mediated by TMS-Cl in Pyridine



with 2 equiv of 3′-amino-3′-deoxythymidine, D-glucosamine, or peptides mediated by TMS-Cl in pyridine was performed at room temperature under nitrogen atmosphere. The amount of TMS-Cl depended on the number of functional groups in the reactants (nucleoside polyphosphate and amine); the ratio of total number of functional groups to TMS-Cl is 1:3. For example, in the reaction of ATP with 3′-amino-3′-deoxythymidine (see Table 1), the total number of functional groups in reactants is nine including seven hydroxyls and two amino groups. Bis-silylated amines cannot be formed under these conditions, so 27 equiv of TMS-Cl is necessary. The addition of excess trimethylsilyl chloride aims at removing the trace amount of water in solution. The reaction needs longer time because nucleoside polyphosphates appear in the form of disodium salts and are of low solubility in pyridine. The solution was stirred continuously for 36 h. The solvent and 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 for three times. The aqueous phase was lyophilized, and the residue was subjected to column chromatography on silica gel using 2-propanol/NH_4OH/H_2O (7:1:2) as eluent. The desired target products were obtained as colorless solid (Table 1), and their structures were confirmed by ^{31}P, ^{1}H, and ^{13}C NMR and HR-MS.

TABLE 1. Amount of TMS-Cl and Yields of Reaction Products of NDP or NTP with Amines

	NDP		NTP	
product	TMS-Cl (equiv)	yield (%)	TMS-Cl (equiv)	yield (%)
7a	24	52	27	53
7b	24	51	27	56
7c	24	52	27	54
7d	21	50	24	52
8a	33	45	36	46
8b	33	42	36	48
8c	33	42	36	45
8d	30	44	33	47
9a	24	51	27	52
9b	24	53	27	53
9c	24	52	27	55
9d	21	50	24	53
10a	30	41	33	45
10b	30	42	33	44
10c	30	47	33	43
10d	27	44	30	44

We made further extension for the reaction above; reactions of polyphosphates with D-glucosamine and peptides mediated by TMS-Cl in pyridine were also carried out using a similar procedure. Although these amines contain multifunctional

groups, for example, D-glucosamine has four hydroxyls and one amino group, Glu-Cys-Gly has one thiol, two carboxyls, and one amino group, the target phosphoramidates were also obtained through the procedure above.

Possible reaction mechanism of NDPs or NTPs with amines mediated by TMS-Cl in pyridine is similar to the previous research.[17] 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, and nucleophilic attack of amino or imino group of silylated amine only occurred on α-, not β- or γ-phosphorus of silylated nucleoside polyphosphate because of the lowest electron density of α-phosphorus relative to β- and γ-phosphorus (Scheme 2). These amines contain various functional groups including hydroxyl, carboxyl, thiol, and amino in a nucleoside base; however, the reaction only occurred on the aliphatic amino group because of its stronger basicity, so the highly chemoselective reactions can be applied to construct complex conjugates via the bridge of phosphate.

Conclusions

One-pot chemoselective reactions of nucleoside polyphosphates (NDPs and NTPs) with amines containing multifunctional groups (3′-amino-3′-deoxythymidine, D-glucosamine, and peptides) yielded the corresponding conjugates in moderate yields under mild conditions. The method could provide valuable reference for the construction of more complicated conjugates with biological and medicinal activity.

Experimental Section

General Procedure for the Reaction of Nucleoside Di- or Triphosphates with Amines Mediated by TMS-Cl. Under a nitrogen atmosphere at 25 °C, TMS-Cl was added by injector to a mixture of NDP (nucleoside 5′-diphosphate disodium salt) or NTP (nucleoside 5′-triphosphate disodium salt) and 2 equiv of amine in anhydrous pyridine (amount of TMS-Cl depended on the number of functional groups in the reactants, see Table 1), and the solution was then stirred continuously for 36 h. The solvent and the excess TMS-Cl were removed under reduced pressure, and the residue was hydrolyzed in 2 M NH_4OH. The resulting solution was then washed with ether three times, the aqueous phase was lyophilized, and the residue was subjected to column chromatography on silica gel using 2-propanol/NH_4OH/H_2O (7:1:2) as eluent. The products were obtained as colorless solids in 41−55% yields (see Table 1). Their structures were confirmed by ^{31}P, ^{1}H, and ^{13}C NMR and HR-MS.

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Supporting Information Available: Characterization data and ^{1}H and ^{13}C NMR spectra of compounds **7**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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