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Application of a Solid-Phase β-Triphosphitylating Reagent in the Synthesis of Nucleoside β-Triphosphates

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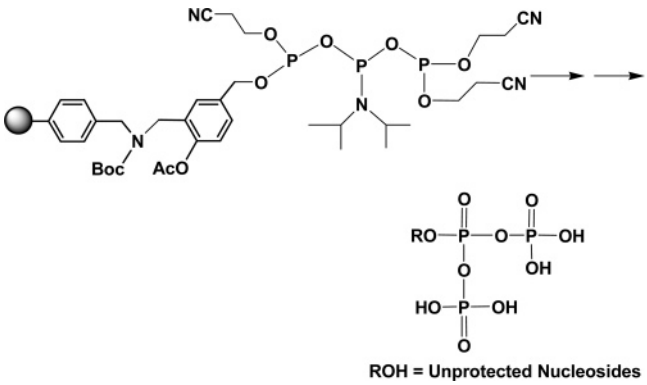

A β-triphosphitylating reagent was subjected to reaction with aminomethyl polystyrene resin-bound *p*-acetoxybenzyl alcohol to yield the corresponding polymer-bound β-triphosphitylating reagent. The solid-phase reagent was reacted with unprotected nucleosides (e.g., 3′-azido-3′-deoxythymidine, cytidine, thymidine, uridine, inosine, or adenosine) in the presence of 1*H*-tetrazole. Polymer-bound nucleosides underwent oxidation with *t*-butyl hydroperoxide, deprotection of cyanoethoxy groups with DBU, and the acidic cleavage, respectively, to afford only monosubstituted 5′-*O*-β-triphosphorylated nucleosides.

Nucleoside triphosphates are the building blocks for the synthesis of DNA and RNA. Naturally occurring deoxyribo- and ribonucleoside triphosphates are synthesized intracellularly from nucleosides in the presence of kinases.[1] Triphosphorylation is also essential for the biological activities of antiviral nucleosides.[2,3]

Modified nucleoside triphosphates have been investigated for potential therapeutic applications and in the study of a number of pharmacological and biochemical processes. Most of the attention has been on modifying and/or substitution on the base[4,5] or carbohydrate[6−11] moieties of the nucleotides. Research on designing nucleotides containing a modified triphosphate group has been focused on developing triphosphate analogues by replacing oxygens with heteroatoms, such as sulfur and nitrogen in thiotriphosphates[12−16] and imidotriphosphates,[17] respectively. These compounds are finding diverse applications in pharmacology, biochemistry, molecular biology, and nucleic acid research. Furthermore, unnatural triphosphate mimics that can be easily synthesized are urgently needed for application in these fields and antiviral research.

The synthesis of nucleoside β-triphosphates remains unexplored, possibly due to the challenges in their preparation. We describe the synthesis of a solid-phase β-triphosphitylating reagent and its application for the synthesis of nucleoside β-triphosphates as part of our ongoing research to develop nucleosides containing a modified triphosphate group. These compounds can have diverse applications in studying the enzymes that use natural nucleoside triphosphates. To the best of our knowledge, this is the first paper on the synthesis of a polymer-bound β-triphosphitylating reagent and nucleoside 5′-*O*-β-triphosphates.

Only monosubstituted nucleoside 5′-*O*-β-triphosphates were produced. The presence of a β-triphosphitylating reagent on a hindered and rigid solid support only allowed the reaction with the most reactive and exposed hydroxyl group in the nucleosides. The solid-phase strategy also offered the advantage of facile isolation and the recovery of products. The unprotected nucleosides were mixed with the polymer-bound reagent. Washing the support allowed for removal of unreacted reagents and starting materials. Furthermore, in the final reaction, the linker remained trapped on the resin, which facilitated the separation of the monosubstituted final products by filtration.

Scheme 1 illustrates the synthesis of the β-triphosphitylating reagent (**7**). Phosphorus trichloride (**1**, 10 mmol) was reacted with 3-hydroxypropionitrile (1 or 2 equiv) in the presence of triethylamine (1 or 2 equiv) to yield 2-cyanoethyl phosphorodichloridite (**2**) or bis(2-cyanoethyl)phosphorochloridite (**3**),

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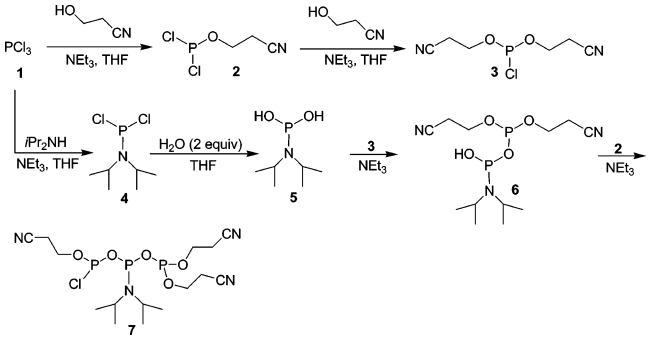
SCHEME 1. Synthesis of β-Triphosphitylating Reagent (7)



respectively. The parallel reaction of **1** (10 mmol) with diisopropylamine (10 mmol, 1 equiv) afforded diisopropylphosphoramidodichloridite (**4**). The addition of water (2 equiv) gave the compound **5** that was reacted with **3** (1 equiv) in the presence of triethylamine (1 equiv) to afford **6**. The reaction of equimolar amounts of **2** and **6** produced a β-triphosphitylating reagent (**7**) in 91% overall yield. The chemical structure of **7** was determined by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, and ^{31}P NMR) and high-resolution time-of-flight electrospray mass spectrometry. Stability studies using these spectroscopic methods showed that the compound remained stable even after 2 months storage at −20 °C and 10 days at room temperature in DMSO solution.

A *p*-hydroxy benzyl alcohol linker was previously designed for the solid-phase synthesis of nucleoside and carbohydrate monophosphates and monomethyl phosphates.[18,19] Bradley and colleagues developed a polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol (aminomethyl polystyrene resin linked through a reduced amide bond with *p*-acetoxybenzyl alcohol) as a safety-catch linker for solid-phase synthesis of a squalamine analogue.[20] Polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol with a reduced amide bond[20] and other safety-catch linkers, such as polymer-bound *p*-acetoxybenzyl alcohol containing an amide bond[21] and polymer-bound oxathiophospholane,[22] were used for the synthesis of carbohydrate and nucleoside monophosphates and monothiophosphates,[21,22] diphosphates, diphosphodithioates, triphosphates, or triphosphotrithioates.[23] These studies revealed that the *p*-acetoxybenzyl alcohol is an appropriate linker for attachment to solid-phase resins and can be used in a variety of reactions. The aminomethyl polystyrene resin linked through a reduced amide bond with *p*-acetoxybenzyl alcohol (**8**) was synthesized from aminomethyl polystyrene resin in multiple-step reactions according to the previously reported procedure.[20,21]

Scheme 2 shows the synthesis of nucleoside 5′-*O*-β-triphosphates (**14a**−**f**). The aminomethyl polystyrene resin-bound linker (**8**, 3.75 g, 0.72 mmol/g) was subjected to reaction with the β-triphosphitylating reagent (**7**, 10 mmol) in the presence of triethylamine (10 mmol) to produce the corresponding polymer-bound β-triphosphitylating reagent (**9**). Unprotected nucleosides (e.g., adenosine (a), uridine (b), 3′-azido-3′-deoxythymidine (c), thymidine (d), inosine (e), and cytidine (f)) were reacted with polymer-bound reagent **9** in the presence of 1*H*-tetrazole to yield **10a**−**f**. Oxidation with *t*-butyl hydroperoxide followed by removal of the cyanoethoxy group with DBU afforded the corresponding polymer-bound nucleoside 5′-*O*-β-triphosphotriester (**12a**−**f**). The cleavage of polymer-bound compounds was carried out under acidic conditions (TFA). The intramolecular cleavage mechanism of final products from (**12a**−**f**) is shown in Scheme 2. The linker-trapped resin (**15**) was separated from the final products by filtration. The crude products had a purity of 87−96% (Table 1) and were purified by using small C_{18} Sep-Pak cartridges and appropriate solvents to afford nucleoside 5′-*O*-β-triphosphates (**14a**−**f**) in 65−87% overall yield (calculated from **9** in the four-step reaction sequence) (Table 1). Only one type of monosubstituted compound was produced with high selectivity as a result of this sequence. The chemical structures of the final products (**14a**−**f**) were determined by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, and ^{31}P NMR), high-resolution time-of-flight electrospray mass spectrometry, and quantitative phosphorus analysis. Stability studies using ^{1}H NMR showed that all final compounds remained stable after 8 months storage in DMSO at −20 °C.

In conclusion, nucleoside 5′-*O*-β-triphosphates were synthesized by using a polymer-bound β-triphosphitylating reagent. Only one type of monosubstituted product was formed using the solid-phase strategy, probably due to the reaction of the sterically rigid polymer-bound reagent with the most exposed and reactive hydroxyl groups. The products were easily isolated from the polymer-bound trapped linker. To the best of our knowledge, this is the first paper on designing a polymer-bound β-triphosphitylating reagent and its application in the synthesis of nucleoside 5′-*O*-β-triphosphates. These compounds can have diverse applications in nucleic acid research and studying and/or inhibiting enzymes involved in the synthesis of nucleoside triphosphates.

Experimental Section

As a representative example, thymidine (2.0 mmol) and 1*H*-tetrazole (71 mg, 1.0 mmol) were added to **9** (790 mg, 0.51 mmol/g) in anhydrous THF (2 mL) and DMSO (3 mL). The mixture was shaken for 24 h at room temperature. The resin was collected by filtration and washed with DMSO, THF, and MeOH, respectively, and dried under vacuum to give **10d**. *t*-Butyl hydroperoxide in decane (5−6 M, 6.0 mmol) was added to resin **10d** in THF. After 1 h shaking at room temperature, the resin was collected by filtration and washed with THF and MeOH, respectively, and was dried overnight at room temperature under vacuum to give **11d**. To swelled resin **11d** in THF was added DBU (4.0 mmol). After 48 h shaking of the mixture at room temperature, the resin was collected by filtration and washed with THF and MeOH, respectively, and dried overnight at room temperature under vacuum to give **12d**. To swelled resin **12d** in anhydrous DCM was added DCM/TFA/water (74:24:2 v/v, 4 mL). After the mixture was shaken for 30 min at room temperature, the resin was collected by filtration and washed with DCM, THF, and MeOH, respectively. The solvents of the filtrate solution were immediately evaporated at −20 °C. The residue was mixed with Amberlite AG-50W-X8 (100−200 mesh, hydrogen form, 1.0 g) in water/dioxane (75:25 v/v, 5 mL) for 15 min. After filtration, the solvents were removed using lyophilization, and the crude product was purified using a C_{18} Sep-Pak to yield thymidine-5′-*O*-β-triphosphate (**14d**). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.74 (d, $J_{5-CH_3,6}$ = 1.1 Hz, 3H), 2.10−2.11 (m, 2H), 3.49−3.65 (m, 2H), 3.72−3.80 (m, 1H), 4.19−4.28 (m, 1H), 4.95−5.05 (m, 1H), 5.15−5.25 (m, 1H), 6.17 (t, $J_{1',2'}$ and $J_{1',2''}$

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SCHEME 2. Synthesis of Polymer-Bound *β*-Phosphitylating Reagent (9) and Nucleosides 5′-*O*-*β*-Triphosphates (14a−f) using Polymer-Bound Linker 8

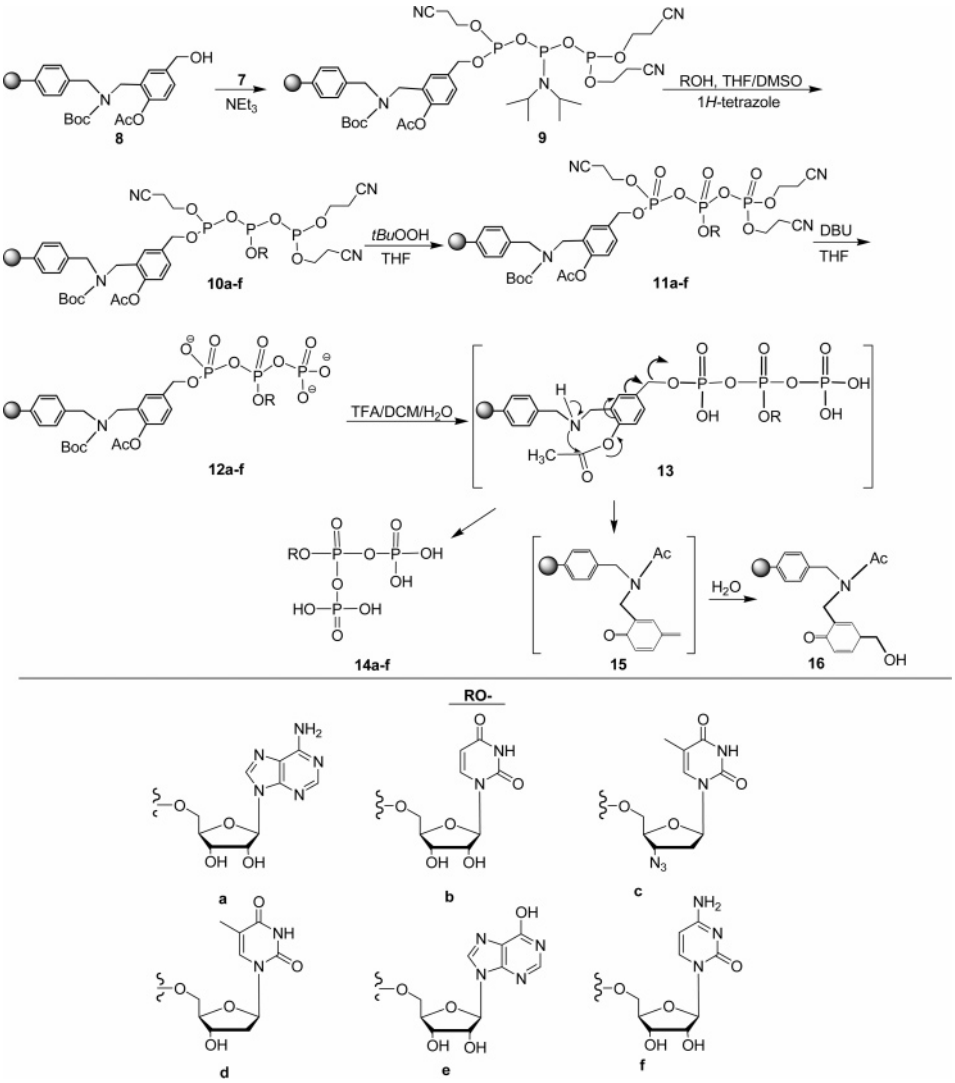

TABLE 1. Overall Isolated Yields and Purity of Crude Products for Nucleoside 5′-*O*-*β*-Triphosphates (14a−f)

no.	overall yield (%) calcd from **9**	purity of crude products
14a	79	90
14b	65	88
14c	87	96
14d	78	89
14e	63	87
14f	81	92

= 8.0 Hz, 1H), 7.69 (d, $J_{6,5\text{-}CH_3}$ = 1.1 Hz, 1H), 11.05−11.15 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 13.2, 40.8, 62.3, 71.5, 84.8, 88.2, 110.5, 137.2, 151.6, 164.9; ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): −17.83 (t, $J_{\beta,\alpha}$ = 11 Hz, β-P, 1P), 1.66 (d, $J_{\alpha,\beta}$ = 11 Hz, α-P, 2P); HR-MS (ESI-TOF) (*m*/*z*): calcd, 481.9893; found, 481.6183 $[M]^+$; Anal. Calcd, P 19.27%; found 19.52%.

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Supporting Information Available: Experimental procedures and characterization of resins with FT-IR and final compounds with ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution mass spectrometry. This material is available free of charge via the Internet at http://pubs.acs.org.

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