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Synthesis of Nucleoside Mono-, Di-, and Triphosphoramidates from Solid-Phase *cyclo*Saligenyl Phosphitylating Reagents

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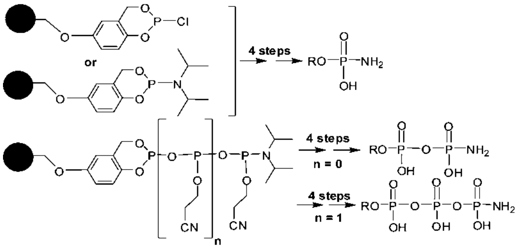
ABSTRACT



ROH = (a) 3′-azido-3′-deoxythymidine, (b) adenosine, (c) 3′ -fluoro-3′ -deoxythymidine, (d) 2′,3′-didehydro-2′,3′-dideoxythymidine, (e) thymidine, (f) 2′-deoxyadenosine, (g) 2′-deoxycytidine, (h) 2′-deoxyguanidine

Chloromethyl polystyrene resin was reacted with 5-hydroxysalicylaldehyde in the presence of potassium carbonate to afford polymer-bound 2-hydroxybenzaldehyde. Subsequent reduction with borane solution produced polymer-bound 2-hydroxybenzyl alcohol. The reaction of immobilized 2-hydroxybenzyl alcohol with appropriate phosphitylating reagents yielded solid-phase *cyclo*Saligenyl mono-, di-, and triphosphitylating reagents, which were reacted with unprotected nucleosides, followed by iodine oxidation, deprotection of cyanoethoxy groups, and the basic cleavage, respectively, to afford 5′-*O*-nucleoside mono-, di-, and triphosphoramidates in 52−73% overall yield.

Antiviral and antitumor nucleoside analogues undergo three phosphorylation steps by cellular kinases to generate nucleoside 5′-triphosphates that act as competitive inhibitors of DNA polymerases or incorporate into DNA and cause chain termination.[1] The first phosphorylation step is often the rate-limiting step. Thus, several nucleoside phosphoramidate derivatives have been synthesized as prodrugs with the aim of delivering the corresponding 5′-mononucleotide intracellularly and bypassing the initial phosphorylation step.[2−6] A number of phosphoramidate derivatives of antiviral and antitumor nucleosides have demonstrated to have enhanced activity and reduced cytotoxicity when compared with their corresponding parent nucleosides.[3,6−8]

Furthermore, oligonucleotide phosphoramidates have attracted considerable attention as potential antisense agents

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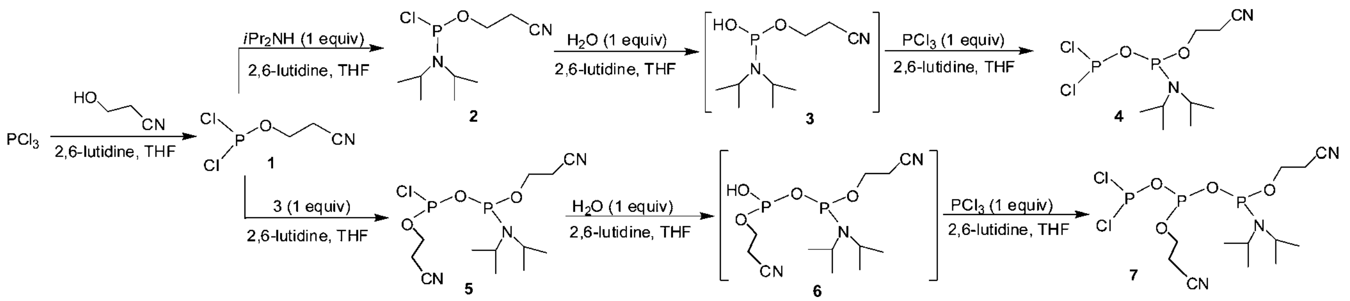
Scheme 1. Synthesis of Diphosphitylating and Triphosphitylating Reagents (**4** and **7**)



because of their stability toward nucleases and being able to form a duplex with complementary chains of DNA or RNA sequences with higher affinity.[9,10] Catalysis of many hydrolases and nucleases also occurs through nucleoside phosphoramidate intermediates.[11,12] Therefore, the synthesis of nucleoside phosphoramidates and phosphoramidate-based pronucleotides and oligonucleotides is the subject of considerable interest in nucleic acid research. The facile synthesis of larger quantities of phosphoramidate derivatives is essential for studying their biological properties.

The reported solution-phase methods for the synthesis of nucleoside 5′-phosphoramidates include the reaction of nucleoside diphosphates, triphosphates, chlorophosphates, H-phosphonates, or trimethaphosphates with amines[13−16] in the presence of a base and/or a coupling reagent (e.g., *N*-carbodiimide derivatives[13,17,18] or trimethylsilyl chloride[15,19]). Alternatively, highly reactive phosphoramidate precursors (e.g., phosphoryldichloride derivatives or bis(benzotriazolyl)phosphoramidates) have been used in reaction with nucleosides for the synthesis of nucleoside phosphoramidates.[6] These methods have one or more disadvantages, such as the requirement for the synthesis of precursor nucleoside phosphates or phosphoramidates, the poor solubility of precursors in organic solvents, tedious purification of final products from intermediates and starting reagents, and low or moderate overall yields. We have previously reported the solid-phase synthesis of nucleoside mono-, di-, and triphosphates with high regioselectivity using polymer-bound linkers of *p*-hydroxybenzyl alcohol or *p*-acetoxybenzyl alcohol.[20−24]

*cyclo*Saligenyl (*cyclo*Sal) phosphate triesters of several nucleoside analogues have been designed as a pH-driven nucleotide delivery system.[25−28] As part of our ongoing efforts to synthesize organophosphorus compounds,[29] we report the synthesis of immobilized *cyclo*Sal phosphitylating reagents and their application for the synthesis of nucleoside mono-, di-, and triphosphoramidates to circumvent one or more of the problems associated with the solution-phase methods. To the best of our knowledge, this is the first paper on the synthesis of polymer-bound *cyclo*Sal phosphitylating reagents. Mono-, di-, and triphosphitylating reagents were first immobilized on polystyrene resin-bound linker of 2-hydroxybenzyl alcohol. Coupling reactions of unprotected nucleosides with the immobilized reagent followed by iodine oxidation, deprotection, and basic cleavage afforded nucleoside mono-, di-, and triphosphoramidates.

The advantages of this solid-phase strategy included the following: (i) The immobilization of hindered phosphitylating reagents on a rigid polymer-bound linker allowed for the regioselective reaction with the most reactive hydroxyl group in the presence of an excess of unprotected nucleosides to afford monosubstituted final products. (ii) Unprotected nucleosides were used instead of precursor nucleoside phosphate derivatives. (iii) Excesses of nucleosides and unreacted reagents were removed in each step by washing the resins. Furthermore, the modified linker remained trapped on the resins. This facilitated isolation and purification of monosubstituted final products. (iv) This strategy allowed the synthesis of nucleoside 5′-*O*-mono-, di-, and triphosphoramidates from the same polymer-bound linker.

Scheme 1 illustrates the synthesis of diphosphitylating and triphosphitylating reagents (**4** and **7**). Phosphorus trichloride

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was subjected to reaction with 3-hydroxypropionitrile (1 equiv) in the presence of 2,6-lutidine in anhydrous THF to yield 2-cyanoethyl phosphorodichloridate (**1**). The subsequent reaction of **1** with diisopropylamine (1 equiv) in the presence of 2,6-lutidine afforded 2-cyanoethyl diisopropylchlorophosphoramidite **2**. Addition of water (1 equiv) and 2,6-lutidine gave the intermediate **3** that was reacted with phosphorus trichloride (1 equiv) in the presence of 2,6-lutidine to afford the diphosphitylating reagent (**4**, 93%).

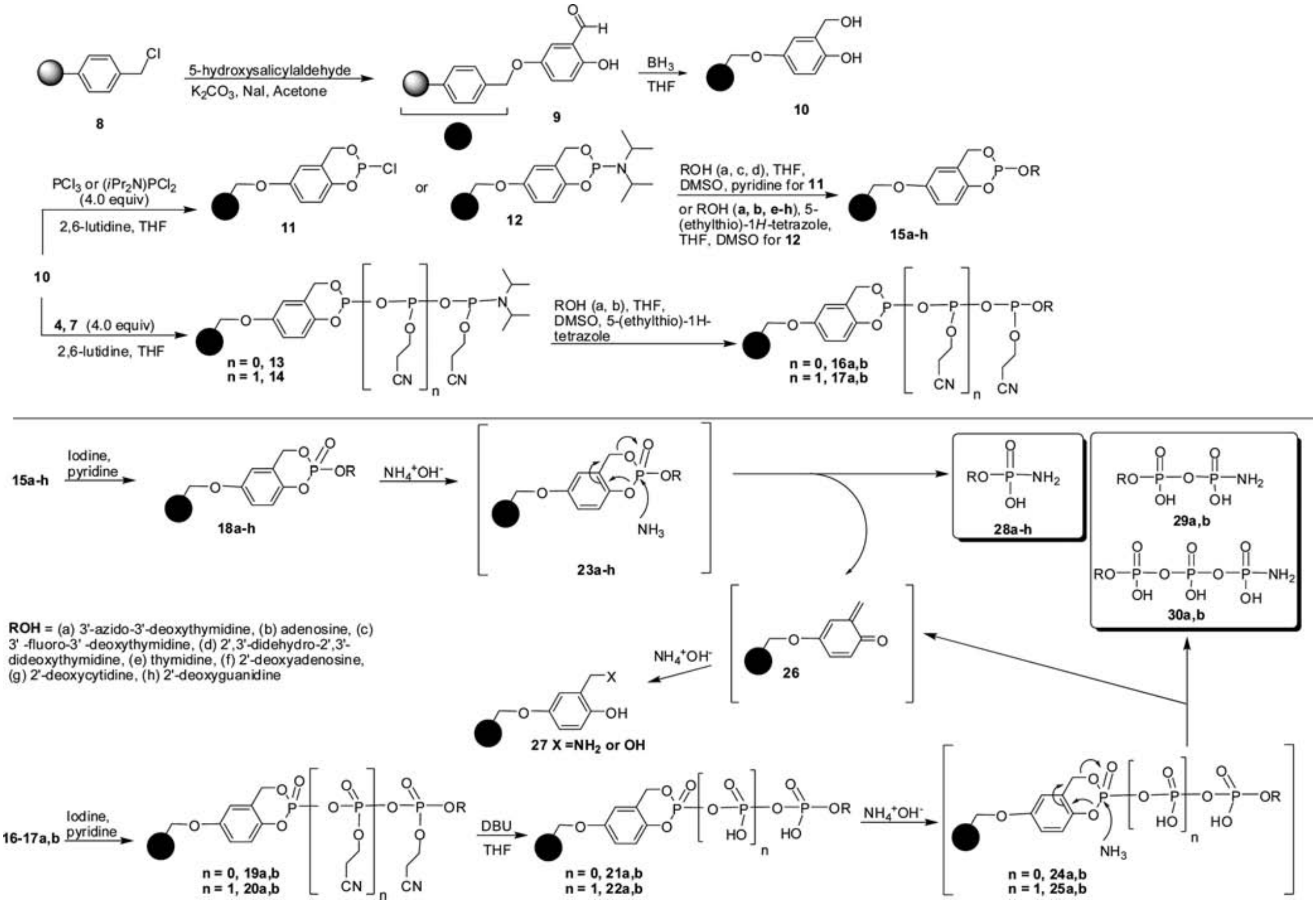
In a separate reaction, 2-cyanoethyl phosphorodichloridate (**1**) was reacted with the intermediate **3** (1 equiv) in the presence of 2,6-lutidine in anhydrous THF to yield **5**. Compound **5** was immediately treated with water (1 equiv) and phosphorus trichloride (1 equiv), respectively, in the presence of 2,6-lutidine to yield the triphosphitylating reagent (**7**, 87%).

The diphosphitylating and triphosphitylating reagents (**4** and **7**) were used immediately in coupling reactions with the polymer-bound 2-hydroxybenzyl alcohol. Compounds **4** and **7** were reacted with water, and the chemical structures of their dihydroxy forms were confirmed by high-resolution time-of-flight electrospray mass spectrometry.

Scheme 2 shows the synthesis of nucleoside mono-, di-, and triphosphoroamidates from polymer-bound 2-hydroxybenzyl alcohol (**10**). Chloromethyl polystyrene resin (**8**) was reacted with 5-hydroxysalicylaldehyde in the presence of sodium iodide and potassium carbonate to afford polymer-bound 2-hydroxybenzaldehyde (**9**). Reduction of the aldehyde group in **9** in the presence of borane solution (1 M) produced polymer-bound 2-hydroxybenzyl alcohol (**10**), which was reacted with phosphorus trichloride or *N*,*N*-diisopropyl phosphoramidous dichloride in the presence of 2,6-lutidine to produce the corresponding polymer-bound *cyclo*Sal monophosphitylating reagents **11** and **12**, respectively.

Similarly, the reaction of **10** with diphosphitylating and triphosphitylating reagents **4** and **7** in the presence of 2,6-lutidine produced polymer-bound *cyclo*Sal diphosphitylating and triphosphitylating reagents (**13** and **14**), respectively. The treatment of **11** or **12** with excess of unprotected nucleosides (e.g., 3′-azido-3′-deoxythymidine (**a**), adenosine (**b**), 3′-fluoro-3′-deoxythymidine (**c**), 2′,3′-didehydro-2′,3′-dideoxythymidine (**d**), thymidine (**e**), 2′-deoxyadenosine (**f**), 2′-deoxycytidine (**g**), and 3′-deoxyguanidine (**h**)) in the presence of pyridine or 5-(ethylthio)-1*H*-tetrazole, respectively, gave **15a−h**. Similarly, reaction of **13** and **14** with excess 3′-azido-3′-deoxythymidine (**a**) and adenosine (**b**) in the presence of 5-(ethylthio)-1*H*-tetrazole afforded **16−17a**,**b**. The most reactive hydroxyl group of unprotected nucleosides reacted

Scheme 2. Synthesis of Nucleoside Monophosphoramidates **28a−h**, Diphosphoramidates, and Triphosphoramidates **29−30a**,**b** on the Solid Phase Using Polymer-Bound Linker **10**



selectively with hindered polymer-bound reagents (**11**−**14**) when an excess of nucleoside was used in the coupling reaction.

Iodine oxidation of **15a**−**h** and **16**−**17a**,**b** yielded the corresponding polymer-bound nucleosides 5′-*O*-monophosphate (**18a**−**h**), diphosphate (**19a**,**b**), and triphosphate triester derivatives (**20a**,**b**). The removal of the cyanoethoxy group with DBU in **19**−**20a**,**b** afforded the corresponding polymer-bound nucleosides **21**−**22a**,**b**.

The cleavage of polymer-bound compounds **18a**−**h** and **21**−**22a**,**b** was carried out under basic conditions (NH_4OH). The intramolecular cleavage mechanism of final products from (**23a**−**h** and **24**−**25a**,**b**) is shown in Scheme 2. The cleavage relies on a nucleophilic attack on the phosphate triester by ammonia and a subsequent hydrolysis pathway to yield the nucleoside phosphoramidate derivatives. The reaction of ammonium hydroxide on resin **26** at the same time produced the linker-trapped resin (**27**), which was separated from the final products by filtration. The crude products had a purity of 68−92% and were purified on the C_{18} Sep-Pak cartridges to afford 5′-*O*-nucleoside monophosphoramidates, diphosphoramidates, and triphosphoramidates (**28a**−**h**, **29**−**30a**,**b**, Scheme 2) in 52−73% overall yield (calculated from **11**−**14**, Table S1, see the Supporting Information). The products were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution mass spectrometry (ESI-TOF).

This is the first report of the synthesis of solid-phase *cyclo*Sal phosphitylating reagents and their application for the preparation of nucleoside 5′-*O*-mono-, di-, and triphosphoramidates. The solid-phase strategy allowed facile synthesis and purification of nucleoside 5′-phosphoramidate derivatives from unprotected nucleosides by removing the unreacted reagents by washing in each step.

As a typical procedure (Scheme 2), 3′-azido-3′-deoxythymidine (**a**, 1 mmol, 4 equiv) and 5-(ethylthio)-1*H*-tetrazole (4 equiv) were dissolved in dry DMSO (3 mL) and were added to swollen **13** (229 mg, 0.25 mmol, 1.09 mmol/g) in THF (5 mL). The mixture was shaken for 28 h at room temperature. The resin was collected by filtration, washed with DMSO (2 × 10 mL) and THF (2 × 10 mL), respectively, and dried under vacuum to give **16a** (267 mg). Iodine solution in pyridine/water (98:2 v/v) (1.5 equiv, 1.5 mL, 0.5 M) was added to swollen resin **16a** in THF (5 mL). After 15 min of shaking at room temperature, the resin was collected by filtration and washed with pyridine (2 × 10 mL), THF (2 × 10 mL), and DCM (2 × 10 mL), respectively, and was dried overnight at room temperature under vacuum to give **19a** (273 mg). DBU (2 mmol) was added to swollen resin **19a** in THF (5 mL). After 48 h of shaking the mixture at room temperature, the resin was collected by filtration, washed with THF (3 × 15 mL) and DCM (3 × 15 mL), respectively, and dried overnight at room temperature under vacuum to give **21a** (244 mg). NH_4OH (30%, 3 mL) was added to swollen resin **21a** in THF (3 mL). After 75 min of shaking the mixture at room temperature, the resin was collected by filtration and washed with MeOH (2 × 10 mL). The solvents of filtrate solution were immediately evaporated at room temperature. The residue was mixed with Rexyn 101 (H) (hydrogen form, 500 mg, 5.72 meq/g) in water/dioxane (75:25 v/v, 3 mL) for 15 min. After filtration, the solvents were removed using lyophilization, and the crude products were purified on C_{18} Sep-Pak using appropriate solvents. The solvents were evaporated, and the residues were dried under vacuum at −20 °C to yield **29a**.

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Supporting Information Available: Experimental procedures and characterization of resins with IR and final compounds with 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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