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Solid-Phase Synthesis of Symmetrical 5′,5′-Dinucleoside Mono-, Di-, Tri-, and Tetraphosphodiesters

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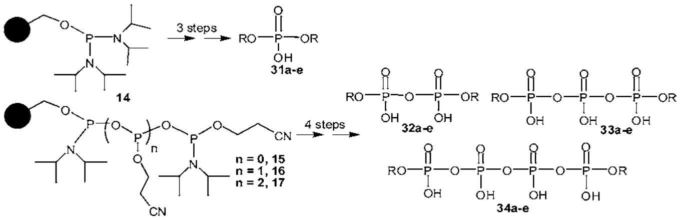
ABSTRACT



Four classes of phosphitylating reagents were subjected to reactions with aminomethyl polystyrene resin-bound *p*-acetoxybenzyl alcohol to yield the corresponding polymer-bound mono-, di-, tri-, and tetraphosphitylating reagents. The solid-phase reagents were reacted with unprotected nucleosides (e.g., thymidine, adenosine, 3′-azido-3′-deoxythymidine, cytidine, or inosine) in the presence of 5-(ethylthio)-1*H*-tetrazole. Polymer-bound nucleosides underwent oxidation with *tert*-butyl hydroperoxide, deprotection of cyanoethoxy groups with DBU, and the acidic cleavage, respectively, to afford 5′,5′-dinucleoside mono-, di-, tri-, and tetraphosphodiesters in 59–78% yield.

The biological properties and applications of various dinucleoside 5′-5′-phosphodiester derivatives ($\mathrm{Np}_x\mathrm{N}$, $x = 1{-}4$) have been previously reported.[1−11] Bis(2′-deoxynucleoside) 5′,5′-diphosphates, triphosphates, and tetraphosphates were shown to be substrates for several DNA polymerases of viral, bacterial, and human origin, such as HIV reverse transcriptase, *E. coli* DNA polymerase, Klenow fragment, and human DNA polymerase α.[1,2] $\mathrm{Ap}_x\mathrm{A}$ derivatives ($x = 3{-}6$) have been reported as active substances in neural and nonneural tissues.[3] $\mathrm{Ap}_4\mathrm{A}$ and $\mathrm{Ap}_5\mathrm{A}$ have been examined as potential therapeutic agents for inhibition of platelet aggregation[4,5] and regulation of vasoactivity.[6,7] Uridine dinucleotide ($\mathrm{Up}_4\mathrm{U}$) has been tested for the treatment of dry eye and chronic obstructive pulmonary diseases.[8,9] Organic and biological chemists investigating these fields are required to prepare many kinds of pure $\mathrm{Np}_x\mathrm{N}$ analogues in sufficient quantities to study their biochemical and therapeutic applications.

Dinucleoside oligophosphates have been synthesized from the corresponding nucleoside 5′-monophosphate in reaction with another nucleoside 5′-monophosphate, 5′-diphosphate, or 5′-triphosphate in the presence of different coupling reagents, such as triphenylphosphine and 2,2′-dipyridyldisulfide,[10] dicyclohexylcarbodiimide (DCC),[11,12] or 1,1′-car-

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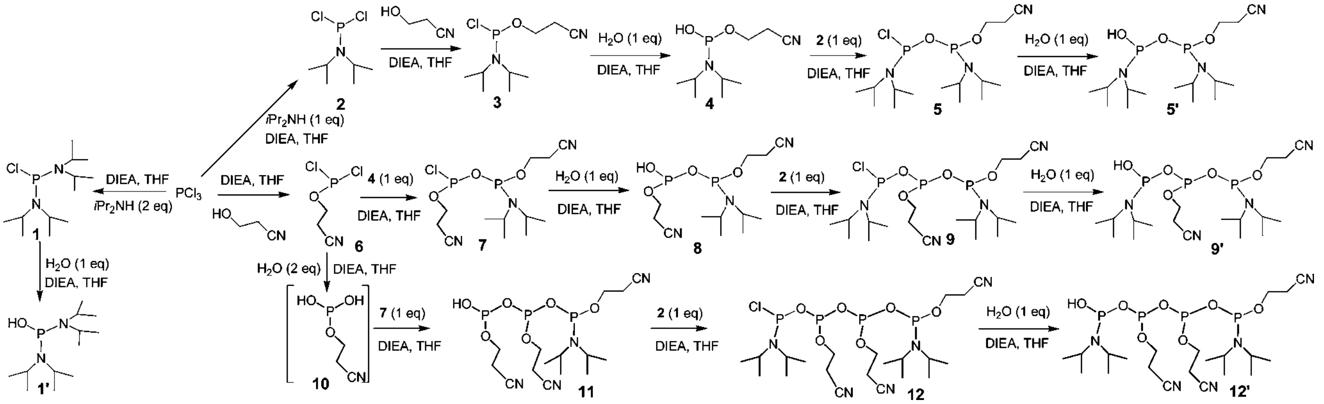
Scheme 1. Synthesis of Mono-, Di-, Tri-, and Tetraphosphitylating Reagents (**1**, **5**, **9**, and **12**)



bonyldiimidazole (CDI).[1] Furthermore, Np$_4$N analogues have been synthesized in modest yield by the reaction of a nucleoside triphosphate with a nucleotide activated as the morpholidate or imidazolate.[13−15] Recently, Han and colleagues used protected triacetyl adenosine or guanosine and trimetaphosphate chemistry to prepare dinucleoside tetra- and pentaphosphates.[16] Symmetrical dinucleoside monophosphates (NpN) have also been synthesized from 3′- or 5′-protected nucleosides and phosphitylating or phosphoramidite reagents.[17,18]

Some of the solution-phase strategies have been hampered by one or more of the following difficulties. (i) The nucleoside phosphate precursors, such as 5′-monophosphate, 5′-diphosphate, or 5′-triphosphate, need to be synthesized first. (ii) Some strategies involve protection and deprotection of nucleosides, thus are rather cumbersome. (iii) Extensive purification of final products from the starting precursors and other reagents are required.

Alternatively, enzymatic methods[19,20] have been used for the synthesis of Np$_x$N derivatives, such as the synthesis of diinosine polyphosphates from the corresponding diadenosine polyphosphates in the presence of 5′-adenylic acid deaminase.[21,22] Enzymatic approaches have limitations in the scale of products and use of natural nucleosides.

We have previously reported the solid-phase synthesis of dinucleoside phosphodiesters and phosphothiodiesters using polymer-bound *N*,*N*-diisopropylamino-1,3,2-oxathiophospholane.[23] The method was limited to the synthesis of NpN. As part of our ongoing efforts to synthesize organophosphorus compounds[24] and to minimize one or more of above-described problems associated with the solution-phase methods, we synthesized polymer-bound phosphitylating reagents for the synthesis of Np$_x$N ($x = 1-4$) analogues. This strategy offered several advantages. (i) A diverse number of compounds were synthesized in a short period without the need to use nucleoside phosphate precursors or protected nucleosides. (ii) This method allowed the synthesis of four classes of compounds (Np$_x$N, $x = 1-4$) from the same polymer-bound linker. (iii) Reactions using this strategy offered the advantage of facile isolation of final products from the resin-trapped linkers by filtration. (iv) Only one type of phosphodiester derivatives (i.e., 5′-5′-dinucleoside analogues) was synthesized because of the presence of the phosphitylating reagents on the solid support having a hindered structure, thereby allowing for the regioselective reaction. The most reactive hydroxyl group of unprotected nucleosides reacted selectively with hindered polymer-bound reagents when an excess of nucleoside was used. To the best of our knowledge, this is the first paper on the synthesis of Np$_x$N derivatives using polymer-bound phosphitylating reagents.

At first, the trifunctional monophosphitylating (**1**), diphosphitylating (**5**), triphosphitylating (**9**), and tetraphosphitylating (**12**) reagents were synthesized from phosphorus trichloride (PCl$_3$) (Scheme 1). Trifunctional phosphitylating reagents contain one chlorine group ($-$Cl) and two diisopropylamino groups ($-$N(*i*Pr)$_2$) that are replaced after the attachment of the reagent to the polymer-bound linker and after coupling reactions with two unprotected nucleosides, respectively.

PCl$_3$ (10 mmol) was reacted with diisopropylamine (*i*Pr$_2$NH, 2 equiv) in the presence of *N*,*N*-diisopropylethylamine (DIEA, 2 equiv) to yield bis(diisopropylamino)chloro-

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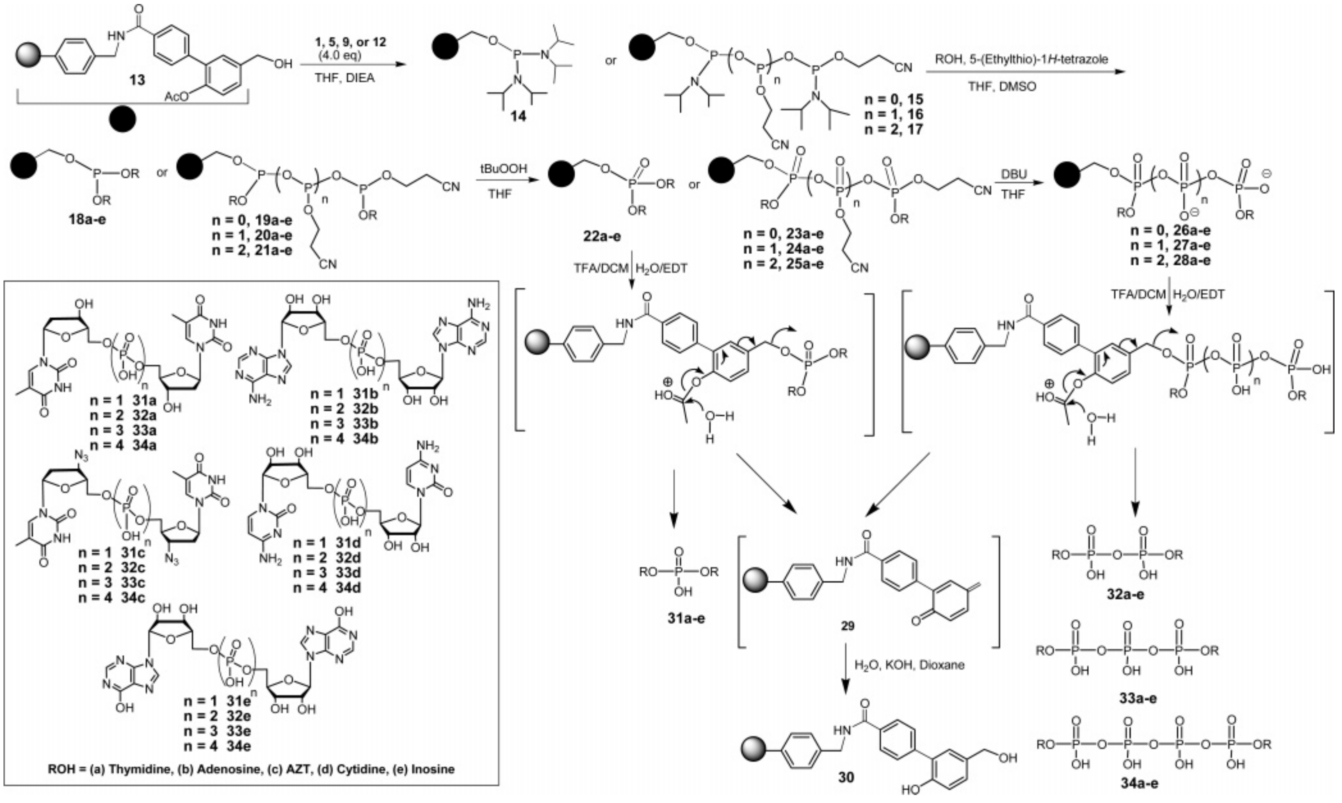
Scheme 2. Synthesis of 5′-5′-Dinucleoside Mono-, Di-, Tri- and Tetraphosphodiesters **31−34a−e** Using Polymer-Bound Linker **13**



phosphine (**1**) as the monophosphitylating reagent. Reaction of PCl_3 (10 mmol) with iPr_2NH (1 equiv) in the presence of DIEA (1 equiv) gave diisopropylphosphoramidous dichloride (**2**). The addition of hydroxypropionitrile (1 equiv) and DIEA (1 equiv) afforded 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (**3**) that was reacted with water (1 equiv) in the presence of DIEA (1 equiv) to afford **4**. Addition of another equiv of **2** to **4** in the presence of DIEA (1 equiv) afforded diphosphitylating reagent **5**. PCl_3 (10 mmol) was also reacted with 3-hydroxypropionitrile (1 equiv) in the presence of DIEA (1 equiv) to yield 2-cyanoethyl phosphorodichloridate (**6**). Subsequent addition of **4** (1 equiv) to **6** in the presence of DIEA (1 equiv) afforded **7**. Compound **7** was subjected to reactions with water (1 equiv) and **2** (1 equiv), respectively, to afford triphosphitylating reagent **9**. Finally, compound **6** (10 mmol) was reacted with water (1 equiv), **7** (1 equiv), and **2** (1 equiv), respectively, in the presence of DIEA to afford tetraphosphitylating reagent **12**. A small amount of **1**, **5**, **9**, and **12** was converted to the hydroxyphosphoramidite forms (**1′**, **5′**, **9′**, and **12′**) in the presence of water (1 equiv) and DIEA (1 equiv). The chemical structures of **1′**, **5′**, **9′**, and **12′** were determined by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution ESI mass spectrometry. Mass spectrometry studies showed that the hydroxyphosphoramidite forms remained stable even after 2 months storage at −20 °C in THF.

We have previously reported the synthesis of aminomethyl polystyrene resin-bound *p*-acetoxybenzyl alcohol (**13**, Scheme 2) from aminomethylated polystyrene resin in multistep reactions.[25] Our research on and the synthesis of organophosphorus and organosulfur compounds[23−25] revealed that the *p*-acetoxybenzyl alcohol is a good linker for attachment to solid-phase resins and application in a variety of reactions.

Four classes of synthesized phosphitylating reagents **1**, **5**, **9**, and **12** were immediately immobilized on **13** (3.63 g, 0.69 mmol/g) in the presence of DIEA (10 mmol) to yield the corresponding polymer-bound mono- (**14,** 94%, 0.62 mmol/g), di- (**15,** 94%, 0.53 mmol/g), tri- (**16**, 95%, 0.50 mmol/g), and tetra- (**17**, 92%, 0.42 mmol/g) phosphitylating reagents (Scheme 2).

Finally, polymer-bound reagents **14−17** were used for the synthesis of Np_xN ($x = 1-4$) analogues (**31−34a−e**). The synthetic strategy consisted of four steps (Scheme 2): (i) O-derivatization of the solid-phase reagents with unprotected nucleosides (e.g., thymidine (**a**), adenosine (**b**), 3′-azido-3′-deoxythymidine (**c**), cytidine (**d**), or inosine (**e**)) in the presence of 5-(ethylthio)-1*H*-tetrazole to yield **18−21a−e**; (ii) oxidation of polymer-bound nucleosides with *tert*-butyl hydroperoxide to afford **22−25a−e**; (iii) removal of the cyanoethoxy group(s) with DBU in case of **23−25a−e**; and (iv) the acidic cleavage. The cleavage mechanism of final products from **22a−e** and **26−28a−e** is shown in Scheme 2. The multistep cleavage mechanisms are shown in one step here for simple demonstration. The crude products had a purity of 75−95% and were purified by using small C_{18} Sep-

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Pak cartridges and appropriate solvents to afford 5′-5′-dinucleoside phosphodiesters (**31a**−**e**), diphosphodiesters (**32a**−**e**), triphosphodiesters (**33a**−**e**), and tetraphosphodiesters (**34a**−**e**) in 59−78% overall yield (calculated from **14**−**17**, Table S1, see Supporting Information). The linker remained trapped on the resin, which facilitated the separation of the final products by filtration. The final products were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, high-resolution time-of-flight electrospray mass spectrometry, and quantitative phosphorus analysis.

For a typical example (Scheme 2), thymidine (**a**, 4.0 mmol) and 5-(ethylthio)-1*H*-tetrazole (260 mg, 2.0 mmol) were added to **16** (946 mg, 0.50 mmol/g) in anhydrous THF (2 mL) and DMSO (3 mL). The mixture was shaken for 48 h at room temperature. The resin was collected by filtration and washed with DMSO, THF, and MeOH, respectively, and dried under vacuum to give **20a** (1055 mg). *tert*-Butyl hydroperoxide in decane (5−6 M, 1.5 mL, 7.5 mmol) was added to the resin (**20a**) in THF (5 mL). After 2.5 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 **24a** (1102 mg). To the swelled resin **24a** in THF was added DBU (6.0 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 **27a** (1055 mg). To the swelled resin (**27a**) in anhydrous DCM was added DCM/TFA/water/EDT (72.5:23:2.5:2 v/v/v/v, 5 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 filtrate solution were immediately evaporated at −20 °C. The residue was desalted with Amberlite AG-50W-X8 (100−200 mesh, hydrogen form, 1.0 g) and lyophilized. The crude products were purified on C_{18} Sep-Pak to yield **33a**.

In conclusion, this solid-phase strategy allows the synthesis of Np_xN analogues in a short synthetic route without the need for the nucleoside phosphate precursors or protected nucleosides and the purification of intermediates. To the best of our knowledge, this is the first report of the synthesis of Np_xN analogues by using polymer-bound phosphitylating reagents, which offer the advantages of 5′-O-substitution for nucleosides and facile recovery of final products from the trapped linkers on the resins by filtration. In addition, by using this strategy, Np_xN analogues ($x = 1-4$) were synthesized in a parallel format.

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Supporting Information Available: Experimental procedures and characterization of resins with IR and final compounds with NMR, high-resolution mass spectrometry, quantitative phosphorus analysis, and analytical HPLC profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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