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Solid-Phase Synthesis of Dinucleoside and Nucleoside-Carbohydrate Phosphodiesters and Thiophosphodiesters

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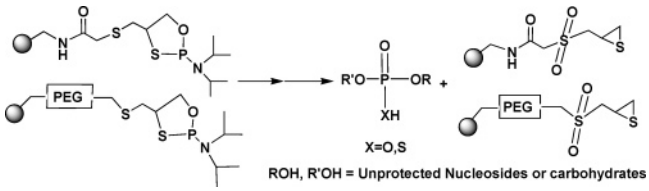

Unprotected nucleosides (ROH) were reacted with two polymers bound to *N*,*N*-diisopropylamino-1,3,2-oxathiaphospholane in the presence of 1*H*-terazole. Oxidation with *tert*-butyl hydroperoxide or sulfurization with Beaucage's reagent, followed by the 1,3,2-oxathiaphospholane ring opening with unprotected nucleosides or carbohydrates (R′OH) in the presence of DBU, afforded nucleoside-(5′-5′)-nucleoside or nucleoside-carbohydrate phosphodiester and thiophosphodiester derivatives through the elimination of polymer-bound ethylene episulfide. This strategy offers the advantages of facile isolation of final products and monosubstitution of unprotected nucleosides and carbohydrates.

Dinucleoside and nucleoside-carbohydrate phosphodiesters[1−7] and thiophosphodiesters[8−12] are of considerable interest in nucleic acid and carbohydrate research. These compounds have been the subject of investigation for the development of nucleosides and their prodrugs with improved cellular permeability[3,13] and enhanced antiviral[2−4] or anticancer[2] activities.

On entering the cell, a majority of antiviral nucleoside analogues are phosphorylated to monophosphate, diphosphate, and triphosphate forms, respectively, by cellular kinases to show activity.[14] In attempts to bypass the first rate-limiting phosphorylation step in the metabolic conversion of a number of nucleoside analogues, numerous prodrugs of 5′-monophosphate types, such as phosphodiesters and phosphotriesters, have been proposed[13,15] as potential pronucleotides. A number of these prodrugs showed the ability to liberate the nucleoside-monophosphate intracellularly by phosphodiesterases.[5,6] For example, the homodimeric 5′-5′-phosphodiester derivative of 3′-azido-3′-deoxythymidine (AZT) demonstrated an enhanced therapeutic index and lower cytotoxicity relative to AZT,[6] possibly due to the intracellular release of AZT-5′-monophosphate.[15]

Some carbohydrates, such as mannose 6-phosphate, can be used as a site-directing moiety toward mannosyl-binding proteins. Mannose-specific receptors in macrophages are the target of numerous pathogens, such as the human immunodeficiency virus (HIV).[16] Lipophilic mannose phosphodiester and triester derivatives of the antiviral nucleosides have been synthesized as membrane-soluble prodrugs directed toward cells carrying mannosyl receptors.[3,7]

Additionally, phosphodiesters have been used as precursors for the synthesis of phosphotriesters.[3,4,17−19] For example, phosphotriester derivatives of AZT have been synthesized from the AZT−mannose phosphodiester conjugate.[3]

Like phosphodiesters, thiophosphodiester analogues of nucleotides have become an indispensable tool for nucleic acids and antisense research.[8,9] Phosphorothioate oligonucleotides have been introduced as antisense molecules[11] because of their resistance to degradation by nucleases.[9] Phosphorothioate and phosphorodithioate oligonucleotides have a higher binding affinity to proteins compared to that of unmodified oligonucleotides and have the potential for further development as diagnostic reagents and therapeutics.[12]

A limited number of chemical strategies have been used for the synthesis of dinucleoside and nucleoside-carbohydrate phosphodiesters in solution, such as the reaction of protected nucleoside monophosphates,[1,4] nucleoside hydrogen phosphonates,[2,15] or carbohydrate phosphates[3,18] with protected nucleosides or carbohydrates in the presence of an appropriate coupling reagent. These synthetic methods have one or more of the following difficulties. (i) Reactions using unprotected polyhydroxylated starting materials lead to a mixture of different monosubstituted and/or polysubstituted intermediates and final products that need to be purified in each step and in the final

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workout. Therefore, all starting nucleosides and carbohydrates must be protected and final products need to be deprotected. (ii) Nucleoside or carbohydrate monophosphates need to be synthesized first. The monophosphate precursors usually have poor solubility in anhydrous organic solvents. (iii) Extensive purifications of intermediates and final products from other reagents are required.

We report the solid-phase synthesis of dinucleoside and nucleoside-carbohydrate phosphodiesters using a solid-phase approach to minimize problems associated to solution-phase methods. This strategy offered several advantages. (i) A diverse number of compounds were synthesized in a short period without the need to use protected nucleosides and carbohydrates or monophosphate precursors. (ii) The final products were easily isolated from resin-trapped linkers by filtration. (iii) Only one type of monosubstituted phosphodiester derivative (i.e., 5′-*O*-nucleoside analogues) was synthesized because of the presence of a phosphitylating reagent on the solid support having a hindered structure. The solid-supported reagent reacted only with the most exposed and reactive hydroxyl group in unprotected nucleosides and carbohydrates.

Oxathiaphospholane and dithiaphospholane have been previously synthesized in solution.[20−29] We have previously reported the synthesis of two classes of polymer-bound *N*,*N*-diisopropylamino-1,3,2-oxathiaphospholanes, **1** and **2**, derived from NovaSyn Tentagel and acetamidomethyl NovaGel resins, respectively (Scheme 1).[30] Herein, we report the application of these polymer-bound linkers for the synthesis of phosphodiester and thiophosphodiester derivatives. The synthetic strategy consisted of three steps (Scheme 1): (i) *O*-derivatization of polymer-bound oxathiaphospholanes, **1** and **2**, by reaction with unprotected nucleosides (e.g., thymidine (**a**), uridine (**b**), AZT (**c**), or adenosine (**d**)) in the presence of 1*H*-tetrazole to afford **3a**−**d** and **4a**−**d**, respectively; (ii) oxidation with *tert*-butyl hydroperoxide to yield **5a**−**d** and **6a**−**d**, respectively, or sulfurization with Beaucage's reagent (3*H*-1,2-benzotrithiole-3-one 1,1-dioxide)[31] to yield **7a**−**d** and **8a**−**d**, respectively; and (iii) 1,3,2-oxathiaphospholane ring opening and cleavage reaction with the same or different unprotected nucleosides (**a**−**d**) or carbohydrates (α,*β*-D-(+)-mannose, 6-*O*-α-D-galactopyranosyl-α,*β*-D-glucose (melibiose)) in the presence of DBU. The crude products had a purity of 65−95% and were purified by using small C_{18} Sep-Pak cartridges and appropriate solvents to afford phosphodiesters **9e**−**o** or thiophosphodiesters **10e**−**q** in 50−70% for dinucleosides and 42−51% for nucleoside−carbohydrate conjugates (overall yield calculated from **1** and **2**) (Table 1, Supporting Information). In total, by using different combinations of nucleosides and carbohydrates, 24 compounds were synthesized.

SCHEME 1. Synthesis of Dinucleoside and Nucleoside-Carbohydrate Phosphodiesters and Thiophosphodiesters on the Solid Phase Using Polymer-Bound *N*,*N*-Diisopropylamine Oxathiophospholanes 1 and 2

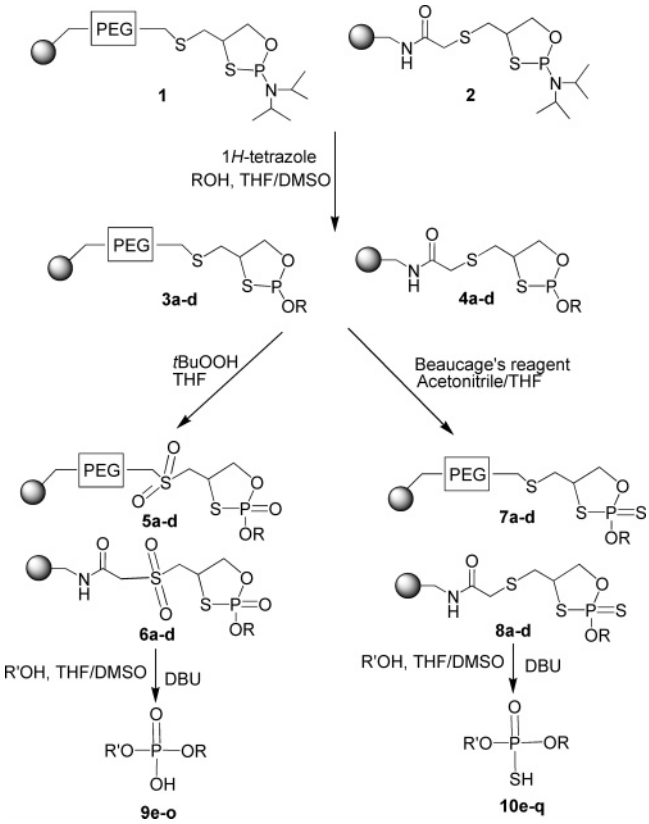

Figure 1 shows the chemical structures of the synthesized compounds. The final products were characterized by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, ^{31}P NMR), high-resolution time-of-flight electrospray mass spectrometry, and quantitative phosphorus analysis.

The cleavage mechanism of final thiophosphodiester products from **7a**−**d** and **8a**−**d** is shown in Scheme 2. The multistep cleavage mechanisms are shown in one step here for simple demonstration. 1,3,2-Oxathiaphospholane ring opening via cleavage of the endocyclic PS bond with nucleophilic attack by unprotected nucleosides and carbohydrates afforded the thiophosphodiester derivatives (**10e**−**q**) through the elimination of polymer-bound ethylene episulfide. All thiophosphodiester products (**10e**−**q**) were obtained as the mixtures of P-diastereomers as shown in ^{31}P NMR spectra. Ethylene episulfide remained trapped on the resins using both polymer-bound linkers, which facilitated the separation of the final products by filtration.

Products were compared for yield and purity. No multisubstitution of unprotected ROH and R′OH was observed. There were no significant differences in the purity and yields of the final products using resin-bound linkers **1** and **2**. However, the yield and purity of dinucleoside products were higher than those

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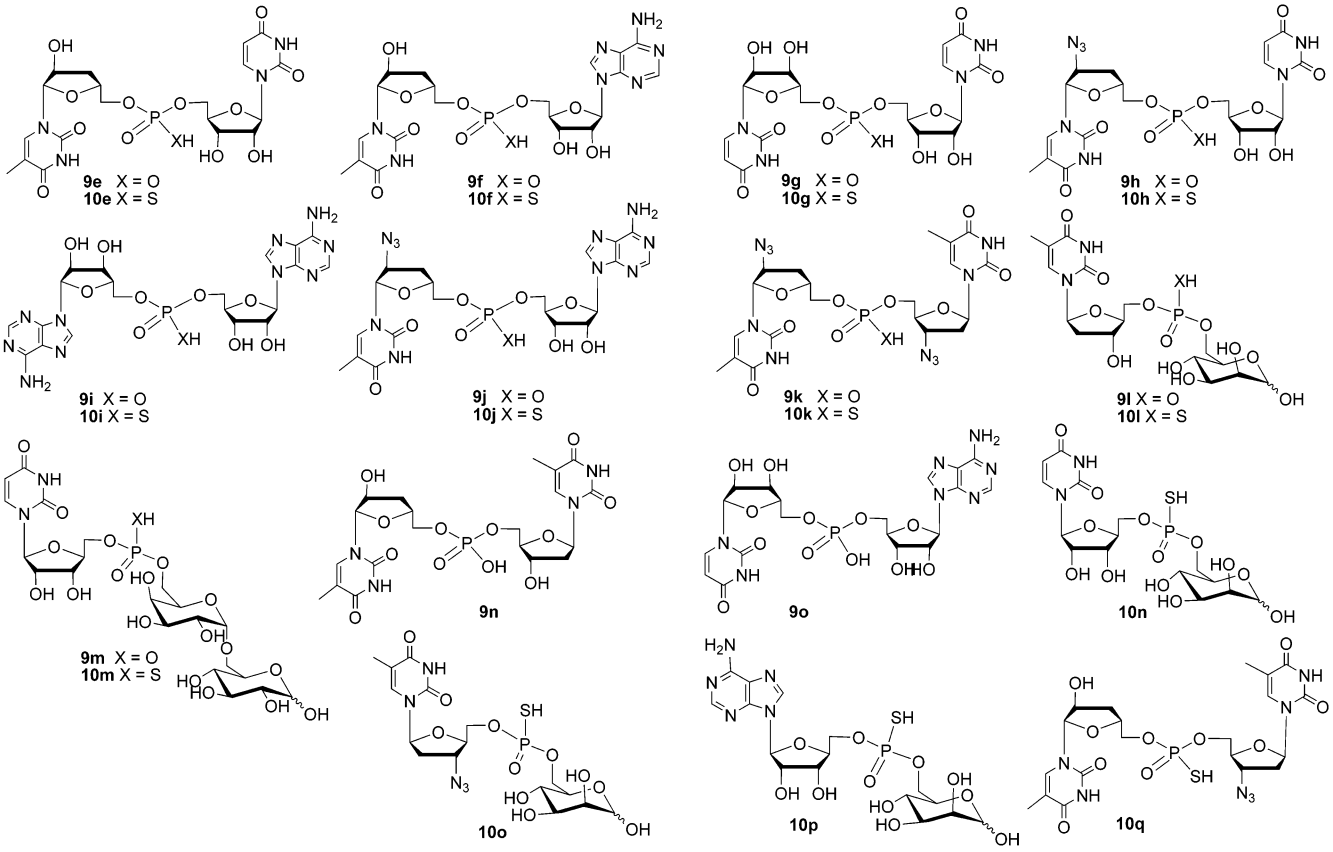
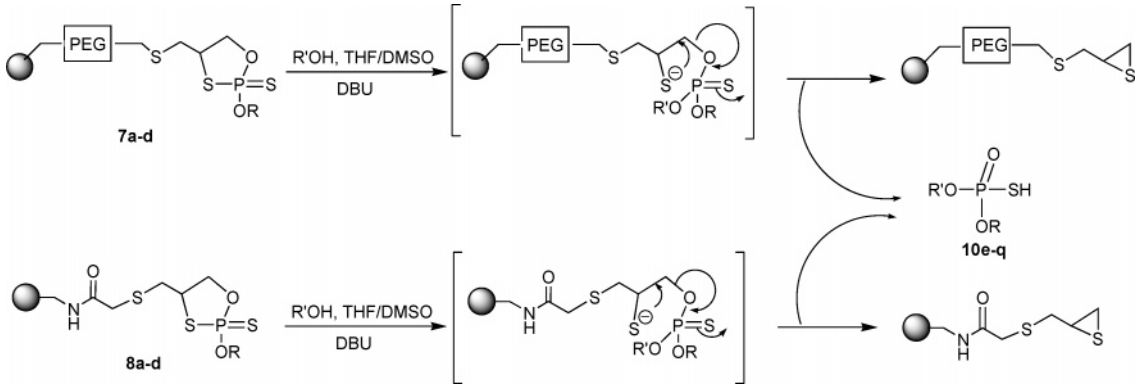


FIGURE 1. Structures of synthesized dinucleoside and nucleoside-carbohydrate phosphodiesters and phosphothiodiesters.

SCHEME 2. Cleavage Mechanism of Dinucleoside and Nucleoside-Carbohydrate Thiophosphodiesters (10e−q) from 7a−d and 8a−d



of nucleoside-carbohydrate derivatives (Table 1, Supporting Information).

In conclusion, this solid-phase strategy allows the synthesis of dinucleoside and nucleoside-carbohydrate phosphodiesters and thiophosphodiesters in a short synthetic route without the need for protection of starting materials, the synthesis of monophosphate precursors, and the purification of intermediates. To the best of our knowledge, this is the first report of the synthesis of phosphodiesters and thiophosphodiesters using polymer-bound oxathiophospholanes as phosphitylating reagents, which offer the advantages of monosubstitution for ROH and R′OH and facile isolation and recovery of final products. The linkers remained trapped on the resins, which facilitated the separation of the final products by filtration. In addition, by using this strategy, phosphodiester and thiophosphodiester derivatives of nucleosides and carbohydrates can be synthesized in a parallel format.

Experimental Section

For a typical example, thymidine (155.0 mg, 0.64 mmol) and 1*H*-tetrazole (34 mg, 0.48 mmol) were added to polymer-bound oxathiophospholane **1** (500 mg, 0.32 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 (3 × 15 mL), THF (2 × 10 mL), and MeOH (3 × 15 mL), respectively, and dried under vacuum to give **3a** (522.0 mg). *tert*-Butyl hydroperoxide in decane (5 M, 128 μL, 0.64 mmol) was added to the resin (**3a**, 522 mg) in THF (3 mL). After 1 h of shaking at room temperature, the resin was collected by filtration and washed with DMSO (10 mL), THF (2 × 10 mL), and MeOH (3 × 15

mL), respectively, and then dried under vacuum to give **5a**. To the swelled resin **5a** in anhydrous DMSO (2 mL) and THF (3 mL) was added DBU (96 μL, 0.64 mmol) and unprotected α,β-D-mannose (115.3 mg, 0.64 mmol). After 40 h of shaking the mixture at room temperature, the resin was collected by filtration and washed with DCM (2 × 15 mL), THF (2 × 15 mL), and MeOH (3 × 15 mL), respectively. The solvents of filtrate solutions were removed, and the residues were mixed with Amberlite AG-50W-X8 (100−200 mesh, hydrogen form, 500 mg, 1.7 meq/g) in water:dioxane (70:30 v/v, 3 mL) for 30 min at room temperature. After filtration, the solvents were evaporated and the crude product was purified using C_{18} Sep-Pak and acetonitrile/DCM/H_2O. The solvents were evaporated, and the residue was dried under vacuum to afford 6-α,β-D-(+)-mannopyranosyl 5′-thymidinyl phosphate (**9l**). ^{1}H NMR (D_2O, 400 MHz, δ ppm): δ 1.84 (s, 3H), 2.31−2.37 (m, 2H), 3.33−3.38 (m, 1H), 3.52−3.57 (m, 1H), 3.60−3.76 (m, 3H), 3.76−3.85 (m, 5H), 3.85−3.94 (m, 4H), 3.96−4.02 (m, 1H), 4.41−4.47 (m, 1H), 4.75−4.86 (m, 1H), 5.13−5.17 (m, 1H), 6.23 (t, $J_{1',2'} = 8.0$ Hz, 1H), 7.62 (s, 1H). ^{13}C NMR (D_2O, 100 MHz, δ ppm): 11.9, 38.9, 61.3, 61.5, 66.9, 67.1, 70.5, 70.7, 71.0, 71.5, 72.68, 73.35, 76.46, 85.32, 86.80, 93.97, 94.34, 111.60, 137.74, 151.89, 166.68. ^{31}P NMR (in D_2O and H_3PO_4, 85% in water as the external standard, 162 MHz, δ ppm): 4.48 (s). HR-MS (ESI-TOF) (*m*/*z*): calcd, 484.1094; found, 484.1532 [M^+], 486.1517 [$M^+ + H$]. Anal. calcd P: 6.39%. Found: 6.33%.

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

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