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Selective Diphosphorylation, Dithiodiphosphorylation, Triphosphorylation, and Trithiotriphosphorylation of Unprotected Carbohydrates and Nucleosides

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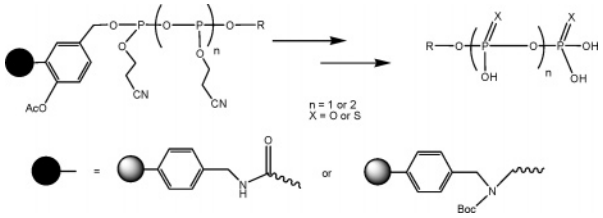
ABSTRACT



Aminomethyl polystyrene resin-bound linkers of *p*-acetoxybenzyl alcohol were subjected to reactions with diphosphitylating and triphosphitylating reagents to yield the corresponding polymer-bound diphosphitylating and triphosphitylating reagents, respectively. A number of unprotected carbohydrates and nucleosides were reacted with the polymer-bound reagents. Oxidation with *tert*-butyl hydroperoxide or sulfurization with Beaucage's reagent, followed by removal of cyanoethoxy group with DBU and the acidic cleavage, respectively, afforded only one type of monosubstituted nucleoside and carbohydrate diphosphates, dithiodiphosphates, triphosphates, and trithiotriphosphates with high regioselectivity.

The selective diphosphorylation, dithiodiphosphorylation, triphosphorylation, and trithiotriphosphorylation of unprotected carbohydrates are challenging goals for organic chemists. Thus, the chemical syntheses of carbohydrate diphosphates, dithiodiphosphates, triphosphates, and trithiotriphosphates have been rarely reported, and their biological properties remain unknown. On the other hand, nucleosides are intracellularly converted to nucleoside monophosphates, diphosphates, and triphosphates, respectively, in the presence of kinases.[1] Diphosphorylation and triphosphorylation are required for the synthesis of nucleotides and nucleic acids, thus producing biological activity of all nucleosides, as shown in several antiviral drugs.[2,3] Phosphorothioate and phosphorodithioate oligonucleotides have been used as diagnostic reagents and in therapeutics.[4]

Currently, there is no universal method for the selective diphosphorylation, dithiodiphosphorylation, triphosphorylation, and trithiotriphosphorylation of unprotected carbohydrates. However, a number of chemical strategies have been previously reported for diphosphorylation and triphosphorylation of nucleosides in solution,[5−7] such as the reaction of nucleoside phosphoramidates (e.g., morpholidate) or phosphorodichloridates with bis(tri-*n*-butylammonium) pyrophosphate derivatives[5] or the reaction of nucleoside diphosphates and their derivatives (e.g., morpholidate, imidazolidate) with phosphoric acid.[6] These synthetic strategies have been hampered by one or more of the following difficulties. (i) The reactions generally must be carried out in anhydrous organic solvents. Because of the poor solubility of most pre-

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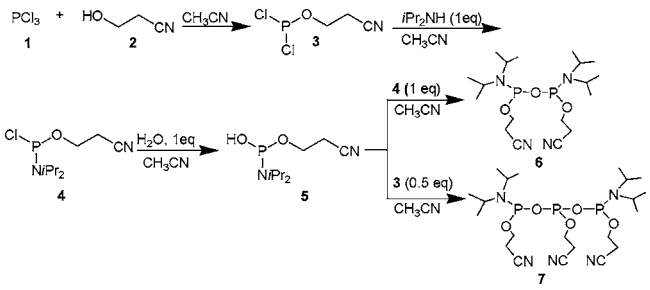
cursor phosphates in the reaction mixture, the yield is low in most cases. (ii) For triphosphorylation in solution, the phosphoramidates and phosphorodichloridates are needed to be synthesized first. (iii) Extensive purification of intermediates and final products from the reagents are required. (iv) These strategies involve protection and deprotection reactions for carbohydrates and lead in most cases to low overall yield due to the lack of regioselectivity. (v) The synthesis of dithiodiphosphate and trithiotriphosphate derivatives from the corresponding diphosphate and triphosphate derivatives in solution phase often leads to the incorporation of two sulfur atoms (disulfurization) on the terminal phosphorus atom.

To solve one or more of these problems, we report the solid-phase diphosphorylation, dithiodiphosphorylation, triphosphorylation, and trithiotriphosphorylation of unprotected carbohydrates and nucleosides. This strategy offered several advantages. (i) The main advantage of this chemical procedure was that it produced one type of monosubstituted derivatives. Similar reactions in solution phase yield a mixture of polysubstituted products. (ii) The alcohols (unprotected nucleosides and carbohydrates) were mixed with an immobilized reagent and were thereby "captured" as an immobilized compound. Washing the support allowed for removal of unreacted reagents and guaranteed that no unreacted starting materials remained. (iii) This approach made use of the presence of reagents on a rigid solid support having a hindered structure, thereby allowing for the regioselective reaction. The most reactive hydroxyl group of carbohydrates and nucleosides reacted selectively with hindered polymer-bound reagents when an excess of carbohydrates and nucleoside was used. (iv) Reactions using this strategy offered the advantage of facile isolation and the recovery of products. The linkers remained trapped on the resins, which facilitated the separation of the monosubstituted final products by filtration. (v) This method was used for synthesis of four classes of compounds, carbohydrate and nucleoside diphosphates, triphosphates, dithiodiphosphates, and trithiotriphosphates, from the same polymer-bound linker.

Scheme 1 illustrates the synthesis of diphosphitylating and triphosphitylating reagents (**6** and **7**). Phosphorus trichloride (**1**, 20 mmol) was reacted with 3-hydroxypropionitrile (**2**, 1 equiv) to yield 2-cyanoethylphosphorodichloridite (**3**). No base was required in this reaction since generated HCl was insoluble in acetonitrile and was bubbled out under dry nitrogen at atmospheric pressure. The subsequent reaction of **3** with diisopropylamine (20 mmol, 1 equiv) afforded 2-cyanoethyldiisopropylphosphoramidochloridite (**4**). Addition of water (1 equiv) gave the intermediate **5** that was reacted with **4** (1 equiv) or **3** (0.5 equiv) to yield the diphosphitylating (**6**, 97%) and triphosphitylating (**7**, 94%) reagents, respectively. The chemical structures of **6** and **7** were determined by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, ^{31}P NMR) and high-resolution time-of-flight electrospray mass spectrometry. Stability studies using spectroscopic methods showed that the compounds remained stable even after 2 weeks storage at −20 °C.

Scheme 1. Synthesis of Diphosphitylating (**6**) and Triphosphitylating Reagents (**7**)



Our research on the development of polymer-bound linkers[8] and the synthesis of monophosphates and monothiophosphates[9] revealed that the *p*-acetoxybenzyl alcohol is a good linker for attachment to solid-phase resins and application in a variety of reactions. Two polymer-bound linkers containing the *p*-acetoxybenzyl alcohol were selected and synthesized from aminomethyl polystyrene resin in multiple-step reactions.[9] The polymer-bound linkers included aminomethyl polystyrene resin linked through amide bond with *p*-acetoxybenzyl alcohol (**8A**) and aminomethyl polystyrene resin linked through reduced amide bond with *p*-acetoxybenzyl alcohol (**8B**) (Scheme 2).

Two classes of aminomethyl polystyrene resin-bound linkers of *p*-acetoxybenzyl alcohol, **8A** (3.05 g, 0.87 mmol/g) and **8B** (3.75 g, 0.72 mmol/g), were subjected to reactions with the diphosphitylating reagent (**6**, ∼10 mmol) in the presence of 1*H*-tetrazole to produce the corresponding polymer-bound diphosphitylating reagents (**9A** and **9B**). Several unprotected nucleosides (e.g., thymidine (**a**), uridine (**b**), 3′-azido-3′-deoxythymidine (**c**), adenosine (**d**)) and carbohydrates (e.g., α,β-D-mannose (**e**), β-D-galactopyranose (**f**), β-D-fructopyranose (**g**), melibiose (**h**)) (1.28 mmol) were reacted with the polymer-bound reagents (**9A** and **9B**) in the presence of 1*H*-tetrazole to yield **11a**−**h** and **12a**−**h**, respectively. Oxidation with *tert*-butyl hydroperoxide or sulfurization with Beaucage's reagent (3*H*-1,2-benzotrithiole-3-one-1,1-dioxide),[10] followed by removal of the cyanoethoxy group with DBU, afforded the corresponding polymer-bound diphosphodiesters, **23a**−**h** and **24a**−**h**, or diphosphodithioesters, **25a**−**h** and **26a**−**h**. The cleavage of polymer-bound compounds was carried out under acidic conditions (TFA). The crude products had a purity of 69−91% and were purified by using small C_{18} Sep-Pak cartridges and appropriate solvents to afford nucleoside and carbohydrate diphos-

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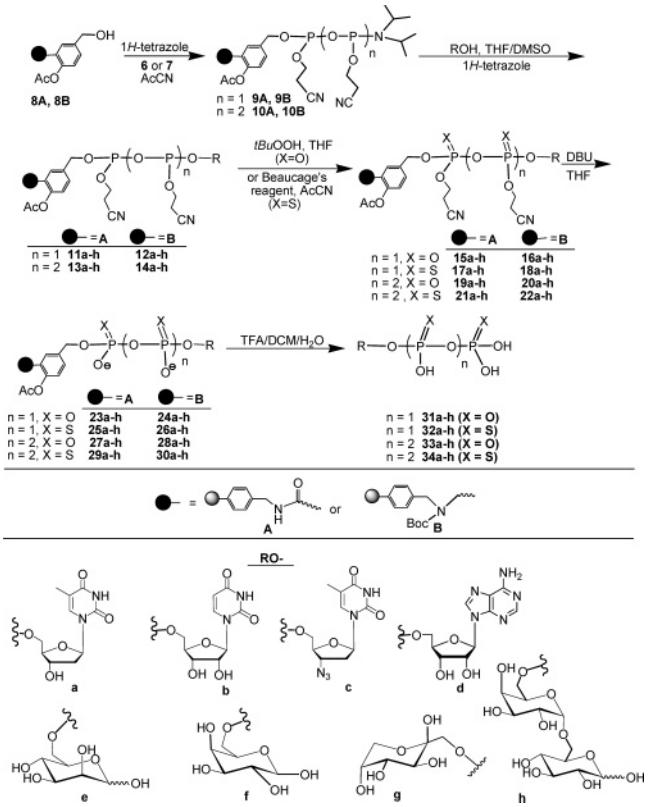
Scheme 2. Selective Synthesis of Nucleoside and Carbohydrate Diphosphates, Dithiodiphosphates, Triphosphates, and Trithiotriphosphates on Solid Phase Using Polymer-Bound Linkers **8A** and **8B**



phates (**31a**−**h**) and diphosphodithioates (**32a**−**h**) (Scheme 2) in 51−84% overall yield (calculated from **9A** and **9B** in four-step reaction sequence) (Table 1). Only one type of monosubstituted compounds was produced with high selectivity as a result of this sequence.

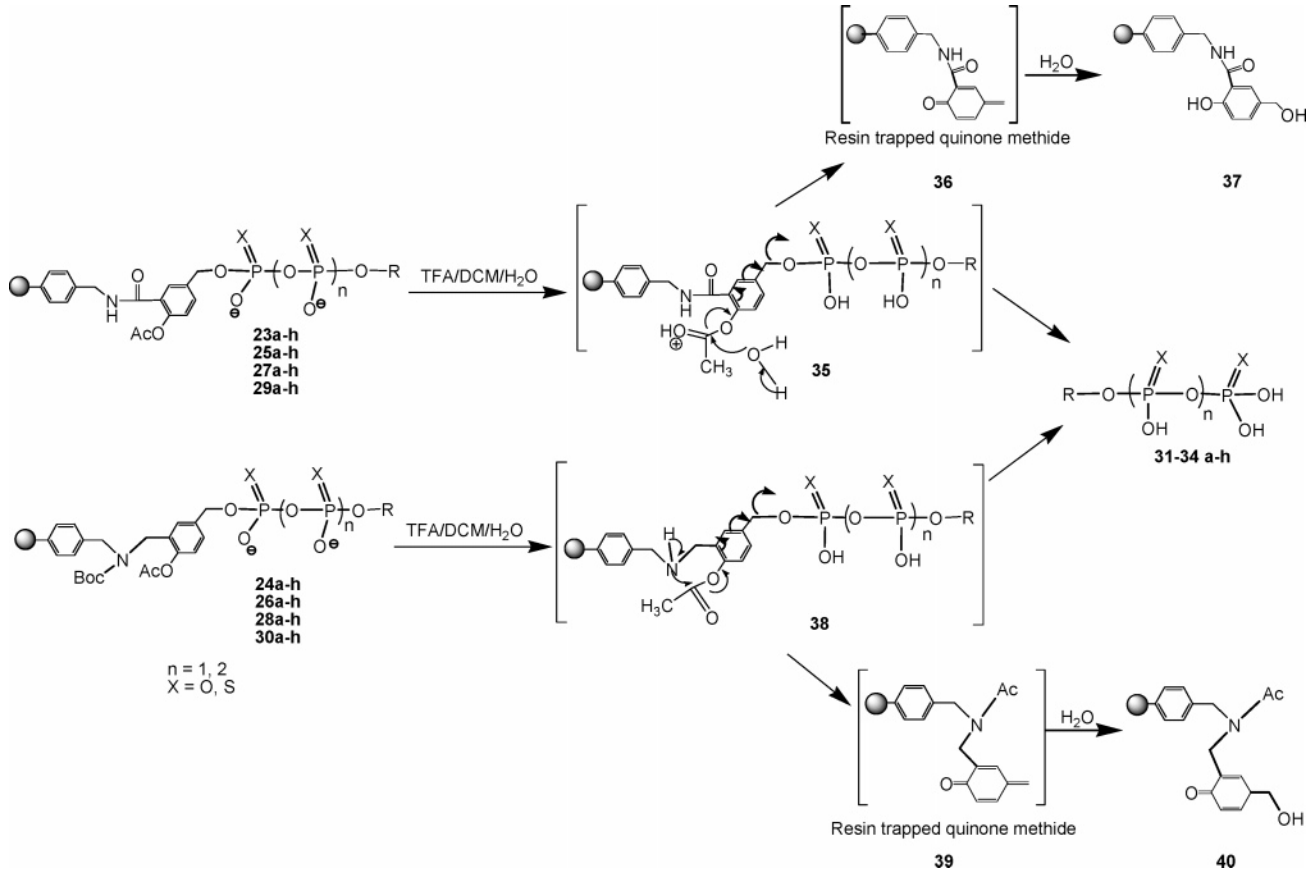
The synthetic approach for the selective solid-phase synthesis of triphosphates and triphosphotrithioates was similar to the diphosphorylation and dithiophosphorylation protocols (Scheme 2), respectively, except for the use of a triphosphitylating reagent (**7**), instead of a diphosphitylating reagent (**6**). The reaction of polymer-bound linkers **8A** and **8B** with the triphosphitylating reagent (**7**) in the presence of 1*H*-tetrazole afforded the polymer-bound triphosphitylating reagents **10A** and **10B**. A number of unprotected nucleosides (e.g., **a**−**d**) and carbohydrates (e.g., **e**−**h**) were reacted with the polymer-bound reagents. The resulting polymer-bound compounds, **13a**−**h** and **14a**−**h**, underwent oxidation and deprotection reactions to afford the polymer-bound triphosphodiesters, **27a**−**h** and **28a**−**h**, or triphosphotrithiodiesters, **29a**−**h** and **30a**−**h**. Cleavage from the resins under acidic conditions and purification of crude products (64−91% purity) by using small C_{18} Sep-Pak cartridges afforded the nucleoside and carbohydrate triphosphates (**33a**−**h**) and triphosphotrithioates (**34a**−**h**) with high selectivity in 42−79% overall yield (calculated from **10A** and **10B** in four steps) (Table 1).

Table 1. Overall Isolated Yields and Purity of Crude Products for Carbohydrate and Nucleoside Diphosphates (**31a**−**h**), Diphosphodithioates (**32a**−**h**), Triphosphates (**33a**−**h**), and Triphosphotrithioates (**34a**−**h**)

No.	overall yield (%) calcd from **9A** or **10A**	overall yield (%) calcd from **9B** or **10B**	purity of crude products (%) using **8A**	purity of crude products (%) using **8B**
31a	77	80	81	91
31b	75	77	83	89
31c	84	80	90	87
31d	68	64	82	83
31e	60	68	77	87
31f	66	60	89	79
31g	61	51	86	84
31h	51	54	69	72
32a	69	74	83	91
32b	72	77	88	85
32c	78	82	91	89
32d	63	72	75	87
32e	70	74	80	86
32f	72	61	87	76
32g	53	55	75	81
32h	57	57	74	79
33a	74	76	80	90
33b	71	73	78	87
33c	79	77	91	91
33d	63	60	79	79
33e	64	64	81	83
33f	58	51	76	72
33g	55	47	71	80
33h	43	42	64	67
34a	63	77	75	89
34b	69	70	86	86
34c	81	75	90	90
34d	67	59	77	76
34e	73	71	79	82
34f	65	54	76	69
34g	45	46	67	77
34h	47	50	71	67

For a typical example (Scheme 2), β-D-galactopyranose (**f**, 1.21 mmol) and 1*H*-tetrazole (68 mg, 0.96 mmol) were added to **10A** (0.59 mmol) 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 **13f**. *tert*-Butyl hydroperoxide in decane (5−6 M, 1.28 mmol) was added to the resin (**13f**) 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 **19f**. To the swelled resin **19f** in THF was added DBU (0.64 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 **27f**. To the swelled resin (**27f**) in anhydrous DCM was added DCM/TFA/water (24:74:2 v/v, 3 mL). After the mixture was shaken for 25 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

Scheme 3. Cleavage Mechanisms of Nucleoside and Carbohydrate Diphosphates, Dithiodiphosphates, Triphosphates, and Trithiotriphosphates **32**−**34** (**a**−**h**) from **23**−**30** (**a**−**h**)



at −20 °C. The residue was mixed with Amberlite AG-50W−X8 (100−200 mesh, hydrogen form, 500 mg) in water:dioxane (75:25 v/v, 3 mL) for 15 min. After filtration, the solvents were evaporated, and the crude product was purified using C18 Sep-Pak to yield **33f**.

The cleavage mechanisms of final products from **23**−**30** (**a**−**h**) using polymer-bound linkers **A** and **B** are shown in Scheme 3. The linkers remained trapped on the resins in both methods, which facilitated the separation of the final products by filtration. In general, the yield and purity of nucleoside products were higher than those of carbohydrate derivatives, but there was no significant difference between polymer-bound linkers **8A** and **8B**.

In conclusion, these solid-phase strategies offer the advantages of monosubstitution, high selectivity, facile isolation and purification of products, and trapping byproducts on resins. To the best of our knowledge, this is the first time that carbohydrate and nucleoside diphosphates, diphosphodithioates, triphosphates, and triphosphotrithioates have been synthesized by using solid-phase reagents. Washing of the resins allows for the removal of an excess of alcohols and unreacted reagents. This strategy ensures, as much as possible, that no unreacted reagent is present in the cleaved product. Another advantage of this chemical procedure is that, in principle, it can produce selectively monosubstituted derivatives. Additionally, for the dithiodiphosphorylation and trithiotriphosphorylation reactions in the solid phase, the terminal oxygen of the phosphorus is linked to the resin and does not react with Beaucage's reagent; therefore, only monosulfurization occurs on the terminal phosphorus atom. This procedure allows the synthesis of carbohydrate diphosphates, dithiodiphosphates, triphosphates, and trithiotriphosphates. Carbohydrate phosphate derivatives are known to serve as important intermediates in metabolism or biosynthesis of complex carbohydrates in several organisms, such as bacteria.[11,12] Synthesized carbohydrate diphosphates or triphosphates may have application in biosynthesis or metabolic studies of carbohydrates.

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Supporting Information Available: Experimental procedures and characterization of resins with 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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