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Polymer-Bound Oxathiaphospholane: A Solid-Phase Reagent for Regioselective Monothiophosphorylation and Monophosphorylation of Unprotected Nucleosides and Carbohydrates

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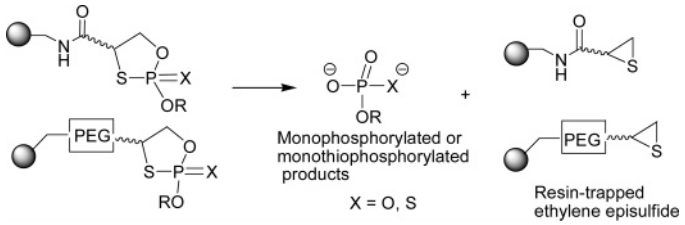
ABSTRACT



Two polymers bound to *N,N*-diisopropylamino-1,3,2-oxathiaphospholane were reacted with unprotected carbohydrates and nucleosides in the presence of 1*H*-tetrazole, followed by oxidation with *tert*-butyl hydroperoxide or sulfurization with Beaucage's reagent. The 1,3,2-oxathiaphospholane ring-opening with 3-hydroxypropionitrile, followed by treatment with DBU, afforded the corresponding monophosphate and monothiophosphate derivatives, respectively, through the elimination of polymer-bound ethylene episulfide. Reactions using this strategy offer the advantages of high regioselectivity, monosubstitution, and facile isolation and recovery of products.

Phosphorylated and thiophosphorylated alcohols are subjects of considerable interest due to their crucial biological roles. Phosphorylated nucleosides (e.g., 2′,3′-dideoxynucleosides as monophosphates and triphosphates),[1−3] carbohydrates (e.g., mannose-6-phosphate,[4,5] glycosyl phosphatidylinositols[6−8]), and proteins composed of phosphoserine, phosphothreonine, and/or phosphotyrosine residues,[9,10] are involved in several fundamental biological processes and pathways such as molecular recognition and signal transduction. Nucleoside phosphoromonothioates are important tools for studying the mechanisms of action of the nucleolytic enzymes.[11] Phosphorothioate oligodeoxyribonucleotides are resistant to degradation by nucleases[12] and, hence, have demonstrated their usefulness as antisense molecules[13] by inhibiting gene expression in vitro. Phosphorothioate oligonucleotides, with sulfur replacing one of the nonbridging

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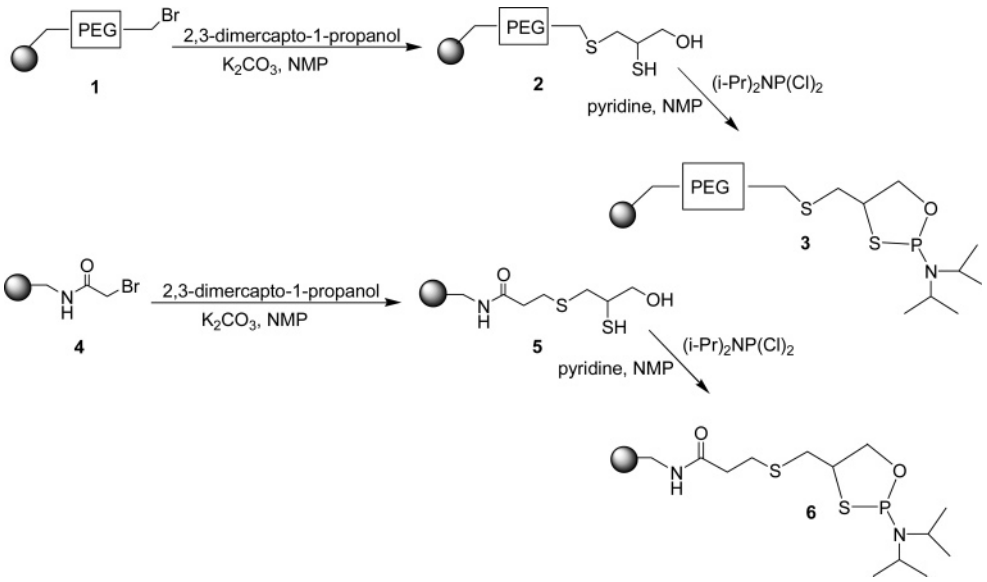
Scheme 1. Synthesis of Polymer-Bound 2,3-Dimercapto-1-propanols **2** and **5** and Polymer-Bound *N*,*N*-Diisopropylamine Oxathiaphospholanes **3** and **6**



phosphate oxygens, bind to proteins more tightly than unmodified oligonucleotides and have the potential to be used as diagnostic reagents and therapeutics.[14]

Organic chemists investigating these fields are required to prepare many kinds of pure phosphorylated and thiophosphorylated compounds in sufficient quantities. Several solution-phase strategies can be used for the synthesis of phosphorylated compounds such as the reaction of alcohols with P(III) species followed by oxidation,[15,16] activated P(IV) species,[17−19] or a mixed ester.[20] Several approaches have been introduced for the thiophosphorylation of alcohols. These include the reaction of alcohols with P(III) species, followed by oxidation by Beaucage's reagent (3*H*-1,2-benzodithiole-3-one 1,1-dioxide),[21,22] the treatment of appropriately protected nucleosides with thiophosphoryl tris-imidazolide,[12] the treatment of unprotected nucleosides with thiophosphoryl chloride in triethyl phosphate,[23] the treatment of nucleosides with phosphorous acid in the presence of DCC followed by sulfurization of the resulting nucleoside 5′-*O*-*H*-phosphonate with elemental sulfur,[24] and finally the treatment of phosphate triesters with polymer-supported sulfur-transfer reagent, 3-amino-1,2,4-dithiazole-5-thione (ADTT).[25] Despite several reported regioselective phosphorylations of nucleosides in solution,[18,26−29] extensive purifications of final products from the remaining reagents are required. Therefore, using these methods in creating diverse libraries of the nucleoside phosphates is rather cumbersome. In the case of unprotected carbohydrates, the monophosphorylation and monothiophosphorylation reactions are even more challenging, since multiply phosphorylated or multiply thiophosphorylated compounds are usually formed and the purification of monosubstituted products from multisubstituted compounds is required. Therefore, protection and deprotection of hydroxyl groups is needed, leading in most cases to low overall yield of the final products.

We have previously reported solid-phase monophosphorylation and methylphosphorylation of unprotected carbohydrates and nucleosides using different linkers and resins.[30−32] These strategies afforded monosubstituted alcohols with high regioselectivity. For example, monophosphorylated carbohydrates and nucleosides were synthesized in an overall yield of 41−62% in eight to nine steps starting from aminomethyl polystyrene resin that was used for the synthesis of polymer-bound *p*-hydroxylbenzyl alcohol.[30] Alternative polymer-

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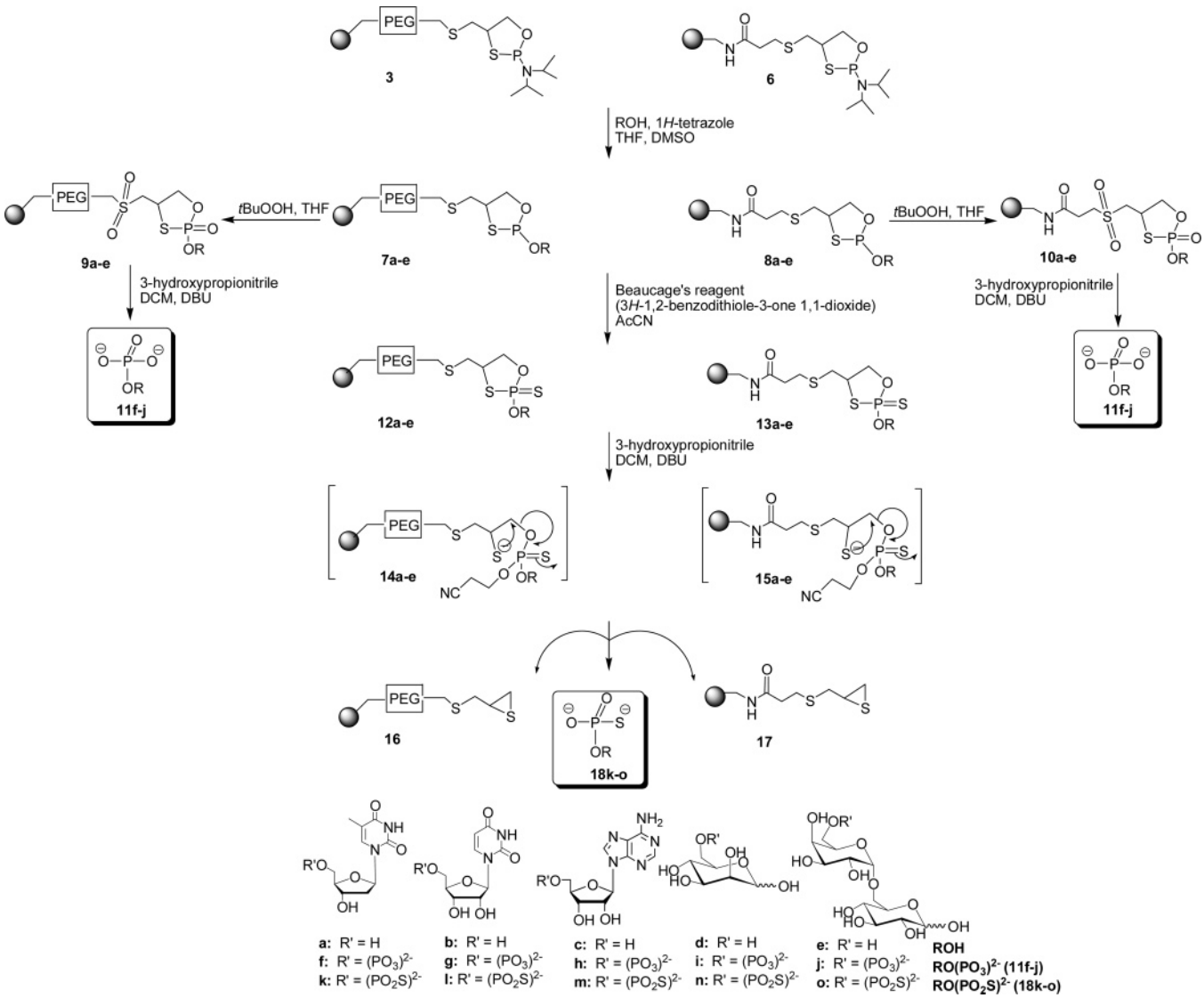
Scheme 2. Regioselective Monophosphorylation and Monothiophosphorylation of Unprotected Nucleosides and Carbohydrates on Solid-Phase Using **3** and **6**



bound linkers that can be used for the monothiophosphorylation in addition to the monophosphorylation of unprotected carbohydrates and nucleosides are needed. A solid-phase reagent was developed to reduce the number of steps for the preparation of polymer-bound linkers and phosphorylation reaction, to improve the overall yield compared to the previously reported solid-phase phosphorylation strategies,[30−32] and to extend the solid-phase method to accommodate the thiophosphorylation of unprotected carbohydrates and nucleosides. The reagent was designed on the basis of the previously reported use[33−37] of the 1,3,2-oxathiaphospholane (2-(*N*,*N*-diisopropylamino)-1,3,2-oxathiaphospholane) in the synthesis of phosphorothioates and phosphates in solution. Polymer-bound 1,3,2-oxathiaphospholane was used for the solid-phase synthesis of phosphorylated and thiophosphorylated alcohols to achieve the concomitant cleavage of product and removal of the linker group without any loss in overall synthetic efficiency.

As shown before, the type of the resin or linker can control the purity of the final products in solid-phase reactions.[38] Two resins, NovaSyn Tentagel bromo resin (**1**) and bromoacetamidomethyl NovaGel resin (**4**), were selected for the attachment to 1,3,2-oxathiaphospholane. Reactions of **1** and **4** with 2,3-dimercapto-1-propanol in the presence of potassium carbonate (K_2CO_3) afforded NovaSyn Tentagel resin

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Table 1. Comparison of Percentage Yields and Purity of Crude Products for Monophosphorylated and Monothiophosphorylated Products

compd	from polymer-bound linker **2** (yield, %)	from polymer-bound linker **3** (yield, %)	purity of crude products (%)	from polymer-bound linker **5** (yield, %)	from polymer-bound linker **6** (yield, %)	purity of crude products (%)
11f	71	75	92	67	71	83
11g	69	73	89	71	76	90
11h	75	78	91	70	75	82
11i	62	65	75	64	68	77
11j	57	60	80	55	58	73
18k	63	66	87	65	69	92
18l	66	69	84	70	74	88
18m	70	74	84	73	78	89
18n	56	59	73	56	60	76
18o	51	54	75	53	56	74

bound to 2,3-dimercapto-1-propanol (**2**) and acetamidomethyl NovaGel resin bound to 2,3-dimercapto-1-propanol (**5**), respectively. The treatment of resins bound to 2,3-dimercapto-1-propanol, **2** and **5**, with diisopropyl phosphoramidite dichloride [$(i\text{Pr})_2\text{NPCl}_2$] in the presence of pyridine afforded resins bound to oxathiaphospholane **3** and **6**, respectively (Scheme 1).

The phosphitylating precursors **3** and **6** were used for the regioselective monophosphorylation and monothiophosphorylation of carbohydrates and nucleosides. In general, the synthetic strategy consists of four steps: (i) O-derivatization of polymer-bound 1,3,2-oxathiaphospholane, **3** and **6**, in reaction with unprotected carbohydrates and nucleosides in the presence 1*H*-tetrazole, (ii) oxidation or sulfurization, (iii) opening of the 1,3,2-oxathiaphospholane ring with 3-hydroxypropionitrile, and (iv) removal of the β-cyanoethyl protecting group in the presence of DBU (Scheme 2).

Two polymer-bound oxathiaphospholanes, **3** and **6**, were reacted with unprotected carbohydrates and nucleosides, **a**−**e**, in the presence of 1*H*-tetrazole to yield **7a**−**e** and **8a**−**e**, respectively (Scheme 2).

For monophosphorylation, the oxidation of **7a**−**e** and **8a**−**e** with *tert*-butyl hydroperoxide afforded the polymer-bound phosphotriesters **9a**−**e** and **10a**−**e**, respectively. The ring opening of **9a**−**e** and **10a**−**e** in the presence of excess of 3-hydroxypropionitrile (4 equiv), a primary alcohol, and deprotection of the cyanoethoxy groups with DBU[33−35] yielded the corresponding phosphate analogues **11f**−**j** in 55−75% overall yield (calculated from **2** and **5**) (Scheme 2).

For monothiophosphorylation, the sulfurization of **7a**−**e** and **8a**−**e** with Beaucage's reagent[21] yielded the polymer-bound phosphothiotriesters **12a**−**e** and **13a**−**e**, respectively. 1,3,2-Oxathiaphospholane ring-opening via chemoselective and stereospecific cleavage of the endocyclic P−S bond with excess of 3-hydroxypropionitrile (4 equiv), followed by treatment with DBU,[33−35] afforded the thiophosphorylated alcohols **18k**−**o** in 51−73% overall yield (calculated from **2** and **5**) through the elimination of polymer-bound ethylene episulfide (Scheme 2).

In both phosphorylation and thiophosphorylation, the cleavage of cyclic phosphate diesters was based on the elimination of ethylene episulfide through a selective C−O bond cleavage mechanism and release of the final products (Scheme 2). Ethylene episulfide remained bound to the resin and trapped. The multistep cleavage mechanisms are shown in one step here for simple demonstration.

Products were compared for yield, purity, and regioselectivity. No multiphosphorylated products were observed, and both resin-bound linkers **2** and **5** afforded similar monophosphorylated and monothiophosphorylated alcohols with high regioselectivity. For example, the thiophosphorylation of 6-*O*-α-D-galactopyranosyl-α,β-D-glucose (melibiose), an unprotected disaccharide, by the developed solid-phase reagents yielded only the monothiophosphorylated product, melibiose-monothiophosphate (6-*O*-α-D-galactopyranosyl-6′-*O*-thiophosphate-α,β-D-glucose), with high regioselectivity. There were no significant differences in the regioselectivity and yields of the final products using these two resins (Table 1). This solid-phase strategy provides phosphorylated products in a shorter synthetic route with improved overall yield compared to the previously developed solid-phase reagents in our laboratory.[30−32] In addition, monothiophosphorylated nucleosides and carbohydrates were synthesized using this strategy.

In summary, polymer-bound oxathiaphospholane as phosphitylating reagents offered the advantages of high regioselectivity, monosubstitution, and facile isolation and recovery of final phosphorylated and thiophosphorylated products. The linkers remained trapped on the resins, which facilitated the separation of the final products by filtration. Washing of the resins allowed for the recovery of an excess of an alcohol and removal of unreacted reagents. Using this solid-phase strategy can also allow the thiophosphorylation of unprotected carbohydrates and nucleosides.

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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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