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Polymer-Supported Reagents for Methylphosphorylation and Phosphorylation of Carbohydrates

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Abstract—Two polymer-supported reagents for methylphosphorylation and phosphorylation of carbohydrates have been developed. *p*-Hydroxybenzyl alcohol and β-mercaptoethanol were immobilized on cross-linked divinylbenzene-polystyrene copolymer and conjugated with methyl *N*,*N*-diisopropylchlorophosphoramidite. Carbohydrates were reacted with polymer-bound phosphitylating reagents. Further oxidation, with or without the methoxy group deprotection, and cleavage yielded methylphosphorylated or phosphorylated carbohydrates, respectively. © 2002 Elsevier Science Ltd. All rights reserved.

The synthesis of phosphorylated compounds and their derivatives is a subject of considerable interest due to their crucial biological roles. Glycosyl phosphatidylinositols (GPIs) act as intracellular second messenger.[1] Mannose-6-phosphate (Man-6-P) is required for targeting some mammalian acid-hydrolases to lysosomes.[2] 2′,3′-Dideoxynucleosides triphosphates act as competitive inhibitors of human immunodeficiency virus reverse transcriptase (HIV-RT).[3,4] Phosphorylation of defined serine, threonine, and/or tyrosine residues in various proteins is involved in the regulation of different cellular processes and in signal transduction pathways. For the study of the events influenced by phosphorylated proteins, phosphopeptides that embody the characteristic phosphorylated partial structures of the parent proteins have proven to be invaluable reagents.[5]

Several strategies have been set up for the phosphorylation of alcohols. Chemical synthesis of a phosphate monoester may proceed in solution from the alcohol by reaction with an activated P(IV) species,[6] mixed ester[7] or by coupling with a P(III) species followed by oxidation.[8] Monophosphorylation of polyhydroxylated compounds in solution can involve protection and deprotection reactions leading in most cases to low overall yield and the need for purification of monophosphorylated from multi-phosphorylated compounds.

The synthesis of carbohydrate methylphosphates and phosphates by using two polymer-supported reagents is now described. This study is based on our previous success with capture phosphorylation of carbohydrates and nucleosides.[9] The preliminary data presents a general simple approach with first immobilization of linkers and phosphitylating reagents on solid support and subsequent reaction with carbohydrates. The polymer-bound phosphitylated precursors were subjected to oxidation reaction to produce the corresponding polymer-bound monophosphate triesters. Phosphorylated compounds were cleaved from resins in mild condition. The linkers are susceptible to intramolecular reaction with appropriate reagents. These two strategies differ in the linkers used and the mechanism of cleavage to final products from solid supports. The solid-phase approach can improve the selectivity of a phosphorylation reaction with polyhydroxylated alcohols due to the rigid and hindered structure of polymer-supported phosphorylating reagents. Another advantage of this chemical procedure is that, in principle, it can provide monophosphorylated alcohols, and polyphosphorylation is suppressed. Phosphorylation using the solid-phase strategy could also offer facile isolation and the recovery of phosphorylated compounds. Washing of resins allows for recovery of an excess of an alcohol and removal of

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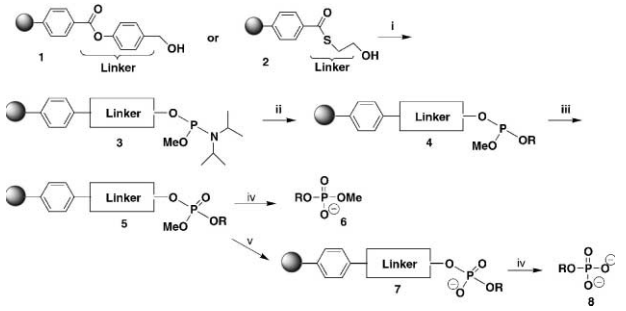
unreacted reagents. This strategy assures that no unphosphorylated alcohol is present in the cleaved product. A single-bead FT-IR microspectroscopy (SBFTIRM) technique and microanalyses were used for monitoring reactions carried out on solid supports. Electrospray MS and ^{1}H NMR (500 MHz, D_2O) were used to confirm the structure of final products.

Two linkers, *p*-hydroxylbenzyl alcohol and β-mercaptoethanol, were immobilized on 1% cross-linked divinylbenzene–polystyrene copolymers to give (i) carboxy-polystyrene polymer-bound *p*-hydroxylbenzyl alcohol (**1**) and (ii) carboxy-polystyrene polymer-bound β-mercaptoethanol (**2**) (SATE strategy). Scheme 1 shows the general protocol for the use of **3** as phosphitylating reagent. Mixing of an alcohol (ROH) with **3** in the presence of 1*H*-tetrazole in THF gives the phosphite triester **4**, which can then be oxidized to the immobilized phosphate triester **5** using *t*-butyl hydroperoxide or iodine in THF/pyridine/H_2O. The resulting phosphodiester **5** is cleaved with sodium methoxide to give methylphosphate derivative **6**. The methoxy group in **5** can be deprotected conventionally with thiophenol and triethylamine, and the resulting phosphodiester **7** is cleaved with sodium methoxide to yield phosphate derivative **8**.

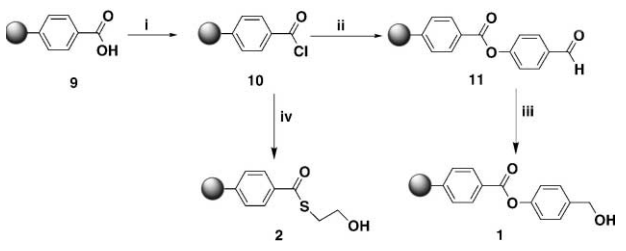
Scheme 2 shows the procedure for the preparation of polymer-bound linkers **1** and **2**. We have previously reported the preparation of **1** (2.74 mmol/g) from commercial carboxyl-polystyrene (**9**, 200 mesh, 1% DVB, 3.9 mmol/g) in three steps. Resin **1** was converted to polymer-bound cyanoethoxy *N*,*N*-diisopropylamine phosphine, which was used for capture phosphorylation of carbohydrates and nucleosides.[9] Treatment of **10** with β-mercaptoethanol in anhydrous benzene and pyridine gave **2** in 60% yield. A solution of β-mercaptoethanol in dry pyridine was added to a swelled solution of resin **10** (3.11 mmol/g) in dry benzene under argon and refluxing was continued for 24 h. The resin was filtered and washed with benzene, dioxane, water, and methanol, successively ending with dry ether and dried under vacuum to give **2** (60%, 1.88 mmol/g based on sulfur content determined by elemental analysis 6.0%). Both polymer-bound linkers **1** and **2** have a hydroxyl group that can be reacted with a bifunctional phosphitylating reagent, such as methyl *N*,*N*-diisopropylchlorophosphoramidite.

Scheme 3 shows the preparation of polymer-bound methoxy *N*,*N*-diisopropylamine phosphine (**14**) as a phosphitylating reagent from polymer-bound linker **1**. Methyl *N*,*N*-diisopropylchlorophosphoramidite was added dropwise to a stirred solution of **1** (2.74 mmol/g) and diisopropylethylamine in anhydrous THF and stirred for 24 h at room temperature under argon atmosphere. The resin was collected by filtration and washed successively with dichloromethane, THF, methanol, water, methanol, dichloromethane, THF, and dry ether and dried under vacuum overnight to give **14** (54%, 1.47 mmol/g based on total nitrogen content determined by elemental analysis 2.06%).

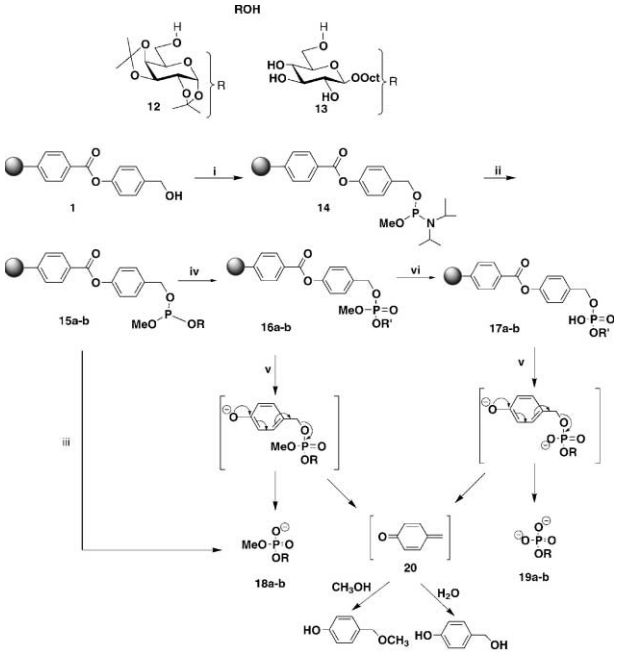
Resin **14** was used for the capture phosphorylation of carbohydrates (Scheme 3). In a general coupling reaction, mixing of a carbohydrate (ROH, **12** or **13**) with **14** in the presence of 1-*H*-tetrazole in THF gave the phos-



Scheme 1. Solid-phase phosphorylation strategies using linker-bound phosphitylating reagents. Reagents and conditions: (i) $(i\text{-Pr})_2NP(Cl)OCH_3$, DIPEA, THF (ii) ROH, THF, 1*H*-tetrazole; (iii) *t*BuOOH, THF or I_2, THF/pyridine/H_2O 7:2:1; (iv) NaOMe, dioxane, methanol; (v) thiophenol, DCM, triethylamine.



Scheme 2. Reagents and conditions: (i) oxalyl chloride, toluene, 120 °C, 80%; (ii) *p*-hydroxybenzaldehyde, Et_3N, DMAP, CH_2Cl_2, ~98%; (iii) $NaBH_4$, isopropanol, THF, 90%; (iv) β-mercaptoethanol, pyridine, benzene, 60%.



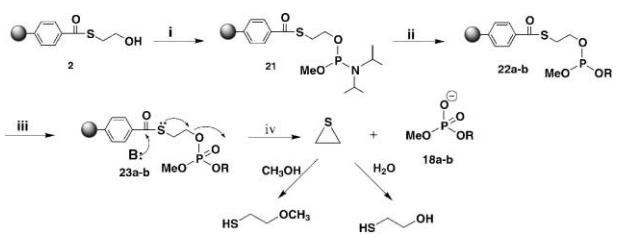
Scheme 3. Procedure and reagents: (i) $(i\text{-Pr})_2NP(Cl)OCH_3$, DIPEA, THF, 54%; (ii) ROH, THF, 1*H*-tetrazole; (iii) iodine in THF/pyridine/H_2O (7:2:1, 5 mL); (iv) *t*-butyl hydroperoxide, THF, quant; (v) NaOMe, dioxane, methanol; (vi) thiophenol, DCM, triethylamine.

phite triesters (**15a**–**b**). For example, 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**12**) and 1*H*-tetrazole were added to **14** (1.47 mmol/g) in anhydrous THF and stirred for 24 h at room temperature under argon atmosphere. The resin was collected by filtration and washed successively with THF, dichloromethane, methanol, THF, methanol, dichloromethane, THF, and dry ether and dried under vacuum to yield polymer-bound 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl methylphosphite triester (**15a**, 56%, 0.82 mmol/g based on the elemental analysis of total remaining percentage of nitrogen 0.91%). This general coupling procedure was used for the preparation of polymer-bound *n*-octyl-β-D-glucopyranosidyl methylphosphite triester (**15b**, 39%, 0.57 mmol/g based on the elemental analysis of total remaining percentage of nitrogen 1.26%).

The phosphite to phosphate conversion is nearly a quantitative process. The product composition could be largely controlled by the choice of oxidant. Oxidative cleavage of polymer-bound methoxy 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-phosphite triester (**15a**) with iodine gave the corresponding methylphosphate (**18a**) directly. As a general oxidation reaction, a 0.1 M solution of iodine (0.2 M) in THF/pyridine/water (7:2:1 v/v/v, 5 mL) was added to the resin **15a** (0.82 mmol/g). After 1.5 h stirring at room temperature, the resin was collected by filtration and washed successively with methanol, water, methanol, dichloromethane, methanol, and dichloromethane. The combined solvents were evaporated under reduced pressure to dryness. The residue was partitioned between ethyl acetate and aqueous sodium bisulfite (1%), and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The impurities were separated from the final products using a C_{18} Sep-Pak cartridge. After evaporation of solvents, the resulting white solids were dissolved in H_2O and filtrated through a syringe containing Amberlite AG-50W-X8 (H^+) and fitted with a Gelman nylon acrodisc 0.2 μm microfilter. The product was lyophilized to give 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-methylphosphate (**18a**, 56%). Similarly oxidative cleavage of **15b** (~0.57 mmol/g) with I_2 in THF/pyridine/water gave *n*-octyl-β-D-glucopyranosidyl-6-methylphosphate (**18b**, 55%). A milder oxidation condition was applied to avoid the direct oxidative cleavage of **15a**–**b** to methylphosphates **18a**–**b**. Polymer-bound methylphosphate triesters (**16a**–**b**) can be obtained using *t*-butyl hydroperoxide as an oxidation reagent. For example, *t*-butyl hydroperoxide in decane (5–6 M) was added to the resin **15a** (0.82 mmol/g) or **15b** (0.57 mmol/g) in THF. After 1 h stirring at room temperature, the resin was collected by filtration and washed successively with THF, dichloromethane and methanol, water, methanol, THF, and dry ether and dried under vacuum overnight to give polymer-bound 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl methylphosphate triester (**16a**, ~0.82 mmol/g) or *n*-octyl-β-D-glucopyranosidyl 6-methylphosphate triester (**16b**, ~0.57 mmol/g).

A general cleavage reaction was proceeded using sodium methoxide in dioxane and methanol to yield the carbohydrate methylphosphates (**18a**–**b**). As an example, to resin **16a** (~0.82 mmol/g) in anhydrous dioxane and methanol was added anhydrous sodium methoxide. After 24 h stirring at room temperature, water was added and stirring was continued for another 1 h. Amberlite AG-50W-X8 (H^+) was added until the pH became acidic. The product was eluted from the resin, which was washed successively with dioxane, water, dioxane and water. The solution containing the product was directly lyophilized. The purity of compound was above 90% after initial lyophilization of product. The ^1H NMR in this step indicated the presence of the *p*-hydroxymethyl or *p*-methoxymethylphenol. The impurities were separated from the final product with a C_{18} Sep-Pak cartridge using (i) water and (ii) water/methanol (95:5) as eluent solvents. The product was lyophilized to give 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-methylphosphate (**18a**, 45%). A similar cleavage procedure was used for **16b** to give *n*-octyl-β-D-glucopyranosidyl-6-methylphosphate (**18b**) in 38% yield. The cleavage reaction in both cases proceeded with the concomitant release of a methylene quinone (**20**), which reacted with either methanol or adventitious water to produce *p*-hydroxymethyl or *p*-methoxymethylphenol (Scheme 3).

The methylphosphate compounds **18a**–**b** can be converted to the phosphate derivatives **19a**–**b** in solution phase according to previous reported procedures.[10–12] The methoxy protecting group can be also cleaved conventionally in solid-phase reactions using thiophenol and triethylamine in dichloromethane. As an example of deprotection reaction, thiophenol and triethylamine were added to the polymer-bound *n*-octyl-β-D-glucopyranosidyl-6-methylphosphate triester (**16b**, ~0.57 mmol/g) in dry dichloromethane. After 9 h stirring at room temperature, the resin was collected by filtration and was washed successively with dichloromethane, THF, and dichloromethane and dried under vacuum to give polymer-bound *n*-octyl-β-D-glucopyranosidyl-6-phosphate diester (**17b**). The resulting phosphodiester **17b** was cleaved with sodium methoxide to yield carbohydrate phosphate **19b** (57%). In the case of resin **16a**, the demethylation was not complete and the electrospray mass spectroscopy confirmed the presence of a mixture of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-phosphate (**19a**) and 6-methylphosphate (**18a**) (30:70) probably due to the presence of the bulky groups of isopropylidene groups on the carbohydrate in proximity to the methylphosphate.



Scheme 4. Procedure and reagents: (i) $(i\text{-Pr})_2NP(Cl)OCH_3$, DIPEA; (ii) ROH, THF, 1*H*-tetrazole; (iii) iodine in THF/pyridine/H_2O (7:2:1, 5 mL); (iv) NaOMe, dioxane, methanol.

S-Acyl-2-thioethyl (SATE) strategy has been used with mononucleoside phosphotriester derivatives to deliver selectively the corresponding nucleoside 5′-monophosphates in infected cells.[13] This strategy was selected to be used in solid-phase organic synthesis of carbohydrates in order to facilitate the phosphorylation and the cleavage to final phosphorylated products. This SATE concept is based on phosphate triesters, such as **23a**–**b** that will eliminate ethylene episulfide through a selective C–O bond cleavage mechanism, as shown in Scheme 4. This intramolecular reaction will release the phosphorylated products in the final step. The episulfide will react with either methanol or adventitious water to produce β-mercaptoethanol or 2-methoxyethanethiol, respectively.

Scheme 4 shows the preparation of polymer-bound methoxy *N*,*N*-diisopropylamine phosphine (**21**) as a phosphitylating reagent. Technically, the resin bound to *S*-acyl-2-thioethyl (SATE) (**2**) was reacted with methyl *N*,*N*-diisopropylchlorophophoramidite to yield polymer-bound methoxy *N*,*N*-diisopropylamine phosphine (**21**, 56%, 1.04 mmol/g based on total nitrogen content determined by elemental analysis 1.46%). To demonstrate the utility and specificity of this solid-phase phosphorylation system, the polymer-bound phosphitylated precursor (**21**) was subjected to reaction with two carbohydrates (**12** and **13**) in the presence of 1*H*-terazole using the general coupling procedure explained for resin **14** to give polymer-bound 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-methylphosphite triester (**22a**, 52%, 0.54 mmol/g based on the elemental analysis of total remaining percentage of nitrogen 0.71%) and polymer-bound *n*-octyl-β-D-glucopyranosidyl-6-methylphosphite triester (**22b**, 63%; 0.65 mmol/g based on the elemental analysis of total remaining percentage of nitrogen 0.54%). The phosphitylated products **22a** and **22b** were washed with solvents and subsequent oxidation with I_2, THF/pyridine/H_2O 7:2:1 converted the phosphites (**22a**–**b**) in a quantitative yield to polymer-bound 1,2:3,4 -di-*O*-isopropylidene-D-galactopyranosyl-6-methylphosphate triester (**23a**, ~0.54 mmol/g) and polymer-bound *n*-octyl-β-D-glucopyranosidyl-6-methylphosphate triester (**23b**, 0.65 mmol/g), respectively. The unchanged sulfur percentage determined by elemental analysis (S 5.37%) indicated the stability of the thioester group to iodine oxidation. The cleavage of **23a** and **23b** was carried out using sodium methoxide in dioxane and methanol to yield **18a** (49%) and **18b** (50%), respectively.

In summary, this paper describes our preliminary efforts towards the development of a simple polymer-supported reagent for methylphosphorylation and phosphorylation of carbohydrates. Reagents **14** and **21** were shown to be practical polymer-supported reagents for the methylphosphorylation and phosphorylation of alcohols. This solid-phase assisted method was found to be highly selective at the phosphorylation site, and gave monophosphorylated products. We are yet to demonstrate and compare the usefulness of such polymer-supported reagents for phosphorylation other carbohydrates, nucleosides, and peptides. These approaches may be used to create a library of phosphorylated compounds.

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