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Solid-Phase Reagents for Selective Monophosphorylation of Carbohydrates and Nucleosides

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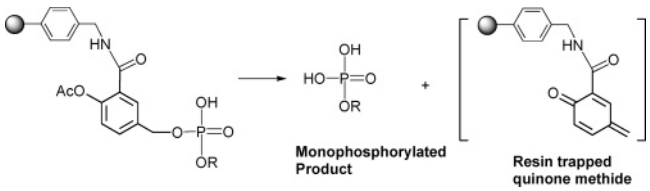

Two classes of aminomethyl polystyrene resin-bound linkers of *p*-acetoxybenzyl alcohol were subjected to reactions with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite to produce the corresponding polymer-bound phosphitylating reagents. These were reacted with a number of unprotected nucleosides and carbohydrates in the presence of 1*H*-tetrazole. Oxidation with *tert*-butyl hydroperoxide followed by removal of the cyanoethoxy group with 1,8-diazabicyclo-[5.4.0]undec-7-ene afforded the corresponding polymer-bound phosphate diesters. Acidic cleavage of the *p*-acetoxybenzyl alcohol linker yielded monophosphorylated products with high regioselectivity and trapped linkers on the resins that can be reused.

Ready access to phosphorylated alcohols such as carbohydrate phosphates (e.g., mannose-6-phosphate,[1] glycosyl phosphatidylinositols[2,3]), nucleosides (e.g., 2′,3′-dideoxynucleosides monophosphates and triphosphates),[4−7] and phosphopeptides composed of phosphoserine, phosphothreonine, and/or phosphotyrosine residues[8,9] is an important requirement for studying several fundamental biological processes and pathways such as molecular recognition and signal transduction. Organic chemists investigating these fields are required to prepare many kinds of pure phosphorylated alcohols in sufficient quantities.

Currently there is no universal method for regioselective monophosphorylation of multihydroxylated compounds and creating nucleoside and carbohydrate monophosphate libraries. Several strategies have been established for phosphorylating of alcohols in solution such as reaction of alcohols with an activated P(IV) species,[10] a mixed ester,[11] or a P(III) species followed by oxidation.[12] These strategies can involve protection and deprotection reactions leading in most cases to low overall yield. In the case of unprotected carbohydrates, multiply phosphorylated compounds are usually formed and the purification of monophosphorylated products from multi-phosphorylated compounds is required. Although regioselective phosphorylation of nucleosides have been previously reported in solution,[13−16] these methods are rather cumbersome to be used for generating nucleoside phosphate libraries needed in screening assays since extensive purifications of final products from remaining reagents are required.

Two versatile solid-phase approaches (methods A and B) for selective monophosphorylation of unprotected nucleosides and carbohydrates alcohols are now described. In these two strategies, the linkers were attached through amide or reduced amide bonds, respectively, to aminomethyl polystyrene resin. Both strategies present a general approach with initial immobilization of phosphitylating reagents on solid support using appropriate linkers and subsequent reaction with alcohols. Washing of the phosphitylated supports guaranteed the removal of unreacted reagents and that no unreacted alcohol remained in next steps. Oxidation and dealkylation reactions followed by cleavage led to release of phosphorylated products. The linkers were susceptible to intramolecular reactions in acidic conditions and remained trapped on resins. The resins-attached linkers can be reused with reaction with phosphitylating reagents and subsequent reaction with unprotected alcohols (see the Supporting Information). This work complements our earlier efforts with capture phosphorylation and methylphosphorylation of carbohydrates and nucleosides.[17−19] In the earlier work, the linkers did not remain trapped on resins and were released along with phosphorylated compounds in solution leading to contamination; there-

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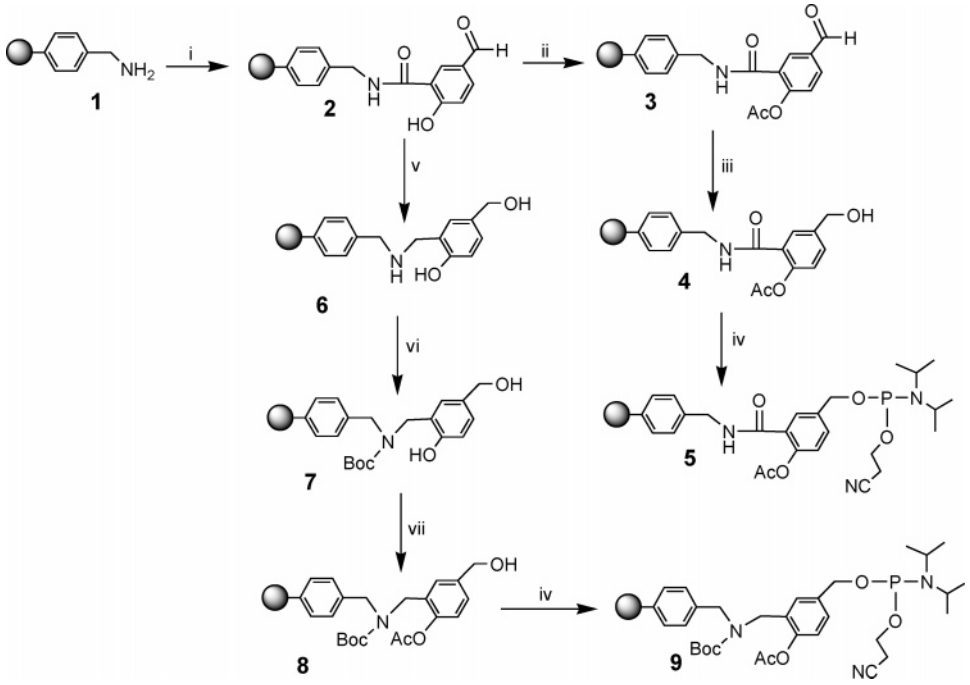
SCHEME 1. Synthesis of Resin-Bound Linkers (4 and 8) and Resin-Bound Phosphitylating Reagents (5 and 9)[a]



[a] Reagents: (i) 5-formylsalicylic acid, DMF, HOBt, DIC; (ii) Ac_2O, pyridine; (iii) (a) sodium borohydride, isopropyl alcohol, (b) NaOH (1 N), 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine, THF; (iv) $(i\text{-Pr})_2NP(Cl)OCH_2CH_2CN$, DIEA, THF; (v) BH_3, THF, 55−65 °C; (vi) Boc_2O, DCM; (vii) NaOH (1 N), 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine, THF.

fore, further purification of final products was needed. Additionally the overall yields were modest for unprotected carbohydrates. These two polymer-bound phosphorylating reagents were designed to circumvent these problems. The linkers remained trapped on resins upon cleavage, which facilitated the separation of final products with filtration. The yield and purity of final products, regioselectivity of phosphorylation, and monophosphorylation vs multiphosphorylation were compared for these reagents.

Scheme 1 shows the procedure for the preparation of resin-bound *p*-acetoxybenzyl alcohol linkers **4** and **8** and resin-bound phosphitylating reagents **5** and **9**. The reaction of 5-formylsalicylic acid with aminomethyl polystyrene resin in the presence of HOBt and DIC as coupling reagents afforded **2**. The reaction of **2** with acetic anhydride was used to cap the resin and to yield acetylated polymer-bound linker **3**. The reduction reaction with sodium borohydride followed by selective acetylation of phenolic group (produced from reduction of acetoxy) with 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine gave the resin-bound linker **4** (Scheme 1). The resin-bound linker with reduced amide bond, **8**, was synthesized according to previously reported procedure as shown in Scheme 1.[20] Reactions of **4** and **8** with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite gave the phosphite donors **5** and **9**, respectively, in 87% and 91% yields (Scheme 1).

Schemes 2 and 3 demonstrate some examples of the use of **5** (method A) and **9** (method B) as phosphitylating reagents for unprotected nucleosides, thymidine (**a**), uridine (**b**) and adenosine (**c**), and unprotected monosaccharide and disaccharides carbohydrates, α-D-mannose (**d**) and 6-*O*-α-D-galactopyranosyl-D-glucose (melibiose) (**e**), to yield monophosphorylated products **14f**−**j**. The polymer-bound phosphitylated precursors **5** and **9** were subjected to reactions with a variety of unprotected nucleosides and carbohydrates (**a**−**e**) in the presence of 1*H*-tetrazole to produce appropriate polymer-bound phosphite triesters **10a**−**e** (Scheme 2) and **16a**−**e** (Scheme 3), respectively. The resulting polymer-bound phosphite triesters were washed with solvents and then oxidized with *tert*-butyl hydroperoxide (*t*BuOOH) to give the polymer-bound phosphate triesters of the corresponding compounds **11a**−**e** (Scheme 2) and **17a**−**e** (Scheme 3), respectively. The removal of cyanoethoxy group in **11a**−**e** and **17a**−**e** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded **12a**−**e** (Scheme 2) and **18a**−**e** (Scheme 3), respectively.

The linkers are attached to aminomethyl polystyrene resins through amide or reduced amide bonds in these two strategies. Although the mechanisms for intramolecular final cleavage of phosphorylated alcohols from **12a**−**e** and **18a**−**e** are different, both reactions released the monophosphorylated products (**14f**−**j**) in the presence of 50% TFA, DCM (Schemes 2 and 3) without the liberation of the reactive quinone methide byproducts in solution. Acetic acid produced in the cleavage reaction of the ester group in **12a**−**e** (Scheme 2) was easily separated from the monophosphorylated products by evaporation and the linker remained trapped as quinone methide on the resin (see **15**). The Boc protecting group in **18a**−**e** (Scheme 3) was removed by treating with TFA/DCM to yield monophosphate derivatives **14f**−**j** through the intramolecular cleavage of **19a**−**e** and without the lib-

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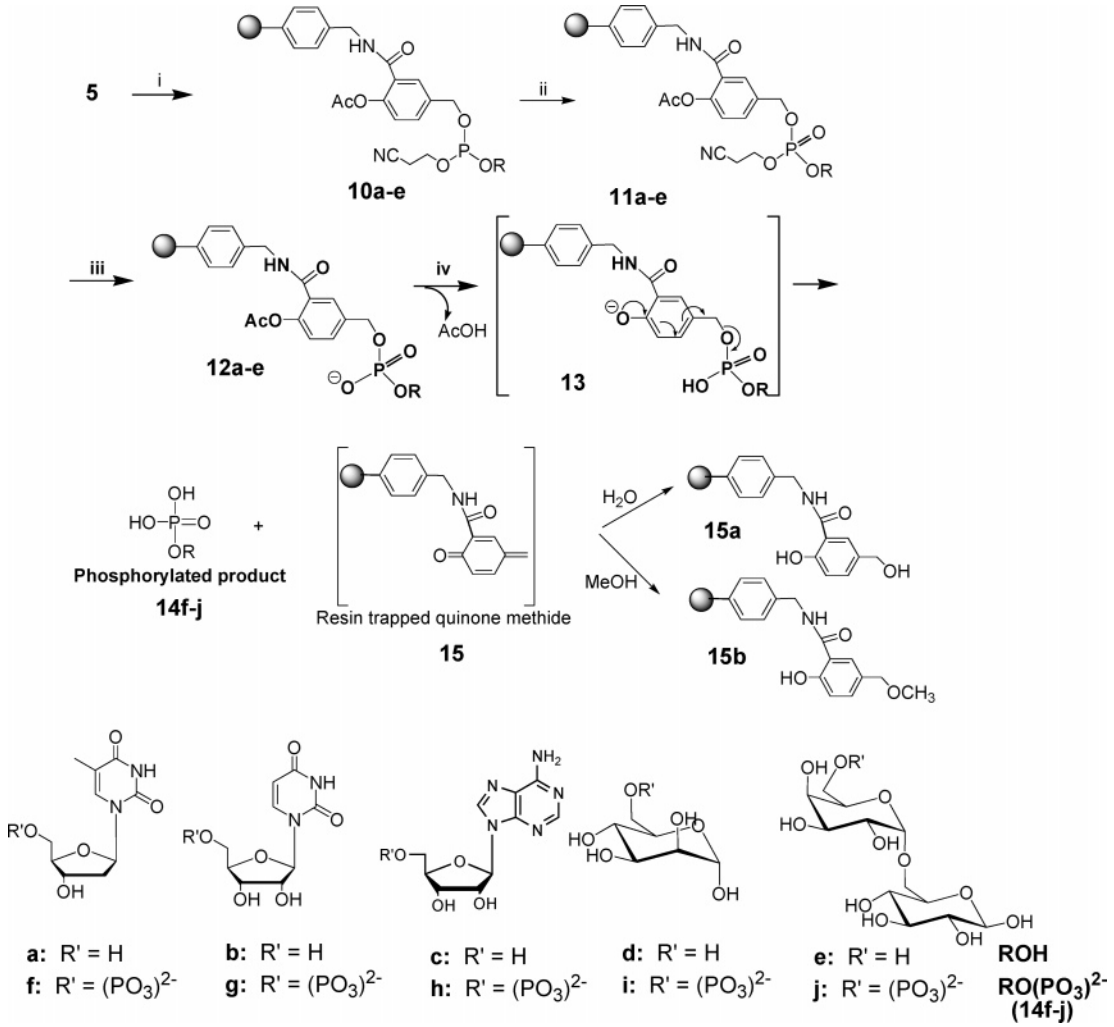
SCHEME 2. Synthesis of Carbohydrate and Nucleoside Monophosphates (14f–j) from 5 (Method A)[a]



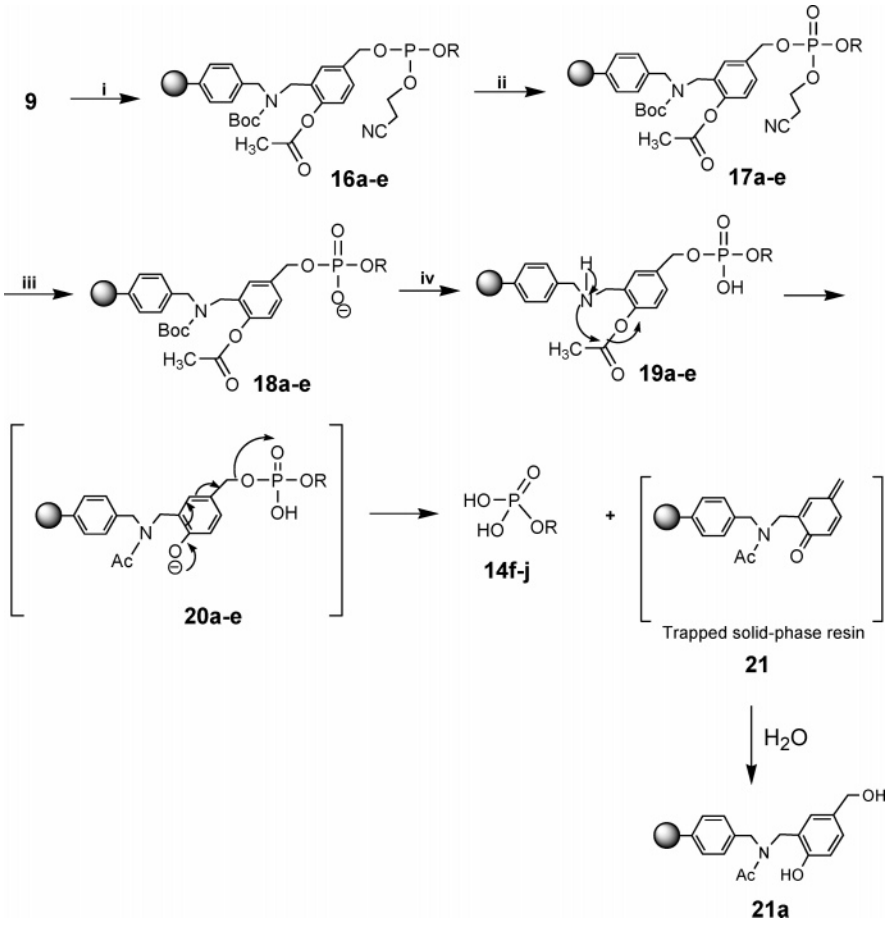
[a] Reagents: (i) ROH, THF, DMSO, 1*H*-tetrazole; (ii) *t*-BuOOH, THF, quant; (iii) DBU, THF; (iv) TFA/DCM (50%).

eration of the reactive quinone methide and byproducts (Scheme 3). The side products remained trapped on the resins (see **20a**–**e** and **21a**, Scheme 3). The chemical structures of final products were confirmed by high-resolution MS and NMR analysis.

For a typical example (Scheme 2), α-D-mannose (**d**) and 1*H*-tetrazole were added to **5** in anhydrous THF and DMSO. The mixture was shaken for 24 h at room temperature. The resin was collected by filtration, washed with DMSO, THF, and MeOH, respectively, and dried under vacuum to give **10d**. *tert*-Butyl hydroperoxide in decane (5–6 M) was added to the resin (**10d**) in THF. After 1 h shaking at room temperature, the resin was collected by filtration, washed with DMSO, THF, and MeOH, respectively, and dried under vacuum to give **11d**. To the swelled resin **11d** in THF was added DBU. After the mixture was shaken for 48 h at room temperature, the resin was collected by filtration, washed with THF and MeOH, respectively, and dried under vacuum to give **12d**. To the swelled resin (**12d**) in anhydrous DCM was added DCM/TFA (50:50 v/v). 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. The residue was mixed with Amberlite AG-50W–X8 (100–200 mesh, hydrogen form) in water/dioxane (70:30 v/v) for 30 min. After filtration, the solvents were evaporated, and the crude product was purified using C_{18} Sep-Pak to yield **14i**.

Products were compared for yield, purity, and regioselectivity. No multiphosphorylated products were observed, and both strategies afforded similar monophosphorylated alcohols with high regioselectivity. The total isolated yields were slightly higher in the second strategy than the first strategy for the phosphorylation of nucleosides (Table 1). For example, the phosphorylation of uridine (**b**) gave 53% (Scheme 2) and 65% (Scheme 3) of 5′-*O*-uridine monophosphate (**14g**), using methods A and B, respectively. No product composed of 2′ or 3′-uridine phosphates was formed. Phosphorylation of unprotected monosaccharides and disaccharides using two strategies gave comparable yields (Table 1). For example, these strategies afforded melibiose monophosphate (6-*O*-α-D-galactopyranosyl-6′-*O*-phosphate-D-glucose) (**14j**) in 41% (Scheme 2) and 45% (Scheme 3) yields, respectively. In general, monophosphorylated alcohols were generated in higher yields from polymer-bound phosphitylating reagent having reduced amide (Scheme 3) (45–67%) com-

SCHEME 3. Synthesis of Carbohydrate and Nucleoside Monophosphates (14f–j) from 9 (Method B)[a]



[a] Reagents: (i) ROH, THF, DMSO, 1*H*-tetrazole; (ii) *tert*-butyl hydroperoxide, THF, quant.; (iii) DBU, THF; (iv) TFA/DCM (50%).

TABLE 1. Comparison of Percentage Yields for Monophosphorylated Products Using Methods A and B

compd no.	method A (yield, %)	method B (yield, %)
14f	55	62
14g	53	65
14h	61	67
14i	44	48
14j	41	45

pared with the one with an amide bond (Scheme 2) (41–61%).

The regioselectivity of these reactions is high as shown in compounds **14f–j**, and no unreacted alcohols remained with the final products (all unreacted carbohydrates were recovered in the conjugation reaction). Since in the final cleavage the linkers remained trapped as quinone methide, the purification of final products was convenient. This is an advantage to our previously reported procedure[17] that required purification of monophosphorylated products from *p*-methoxyphenol and *p*-hydroxybenzyl alcohol linker products released in solution. The phosphorylation of carbohydrates such as **d** and **e** using solution-phase methods could potentially lead to different phosphorylated products. These solid-phase strategies afforded only monophosphorylated compounds with high regioselectivity. Trapped solid-phase resins **15a** and **21a** can be reused for generating the solid-phase phosphitylating reagents (see the Supporting Information).

In summary, two solid-phase phosphorylating regents were introduced that were capable of trapping the linkers and avoiding the contamination of final phophorylated products. Phosphorylation using these solid-phase strategies offered the advantages of facile isolation and the recovery of monophosphorylated alcohols. The phosphorylation procedures exhibited high regioselectivity for multi-hydroxylated compounds and only one monophosphorylated product was formed. These solid-phase phosphorylating reagents have the potential to be used for monophosphorylation of unprotected multihydroxylated compounds and generation of nucleoside and carbohydrate monophosphate libraries in a parallel format. Use of these solid-phase methodologies can also allow for the more expeditious development of valuable monophosphorylated alcohols.

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Supporting Information Available: Experimental procedures and characterization of resins with IR and final novel 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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