ORGANIC LETTERS

2001
Vol. 3, No. 2
307−309

A Solid Phase Reagent for the Capture Phosphorylation of Carbohydrates and Nucleosides

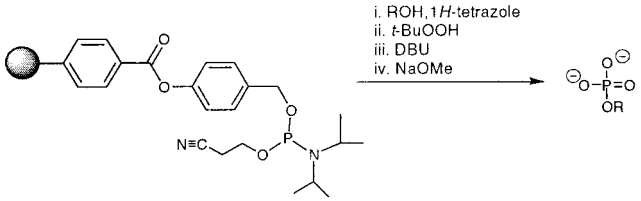
Keykavous Parang,*,†,‡ Eric J.-L. Fournier,† and Ole Hindsgaul†

Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2 Canada, and Department of Biomedical Sciences, College of Pharmacy, University of Rhode Island, Fogarty Hall, 41 Lower College Road, Kingston, Rhode Island 02881-0809

kparang@uri.edu

Received December 1, 2000

ABSTRACT



A 1% cross-linked divinylbenzene–polystyrene copolymer, containing cyanoethoxy *N*,*N*-diisopropylamine phosphine was prepared as a phosphitylating agent. The polymer-bound phosphitylated precursor was subjected to reaction with alcohols in the presence of 1*H*-tetrazole to produce the corresponding polymer-bound phosphite triesters. These were then oxidized with *tert*-butyl hydroperoxide to give the polymer-bound monophosphate triesters. Removal of cyanoethoxy on the resin with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) followed by basic cleavage of the *p*-hydroxybenzyl linker products yielded monophosphate derivatives.

Crucial biological roles of carbohydrate phosphates have long been recognized.[1] In mammalian cells they are found in phosphorylated inositols involved in protein-membrane attachment and in signal transduction,[1,2] and mannose 6-phosphate residues are required to target hydrolytic enzymes to the lysosomes.[3] In bacteria, they are much more abundant, being present in many different classes of cell-surface polysaccharides such as teichoic acids and polyribosylphosphates. In both types of cells, oligosaccharides are biosynthesized from sugar-nucleotides requiring sugar 1-phosphate to couple to nucleoside 5′-phosphates via their phosphotriesters.[4]

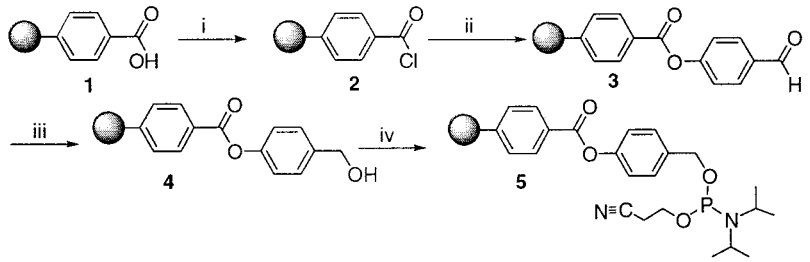
The direct phosphorylation of alcohols is generally accomplished by reaction with an activated P(IV) species[5] or mixed ester[6] or by coupling with a P(III) species followed by oxidation.[7] For example, several solution phase synthetic strategies have been reported in the preparation of 5-fluoro-2′-deoxyuridine monophosphate.[8,9] Solution monophosphorylation of diols and triols can involve a complicated process of protection and deprotection reactions leading in most cases to low overall yield and the need for purification. While several examples exist with significant regioselectivity in

† University of Alberta.
‡ University of Rhode Island.

(1) Watanabe, Y.; Yamamoto, T.; Ozaki, S. *J. Org. Chem.* **1996**, *61*, 14.
(2) Low, M. G.; Saltiel, A. R. *Science* **1988**, *239*, 268.
(3) Olson, L. J.; Zhang, J.; Lee, Y. C.; Dahms, N. M.; Kim, J. J. P. *J. Biol. Chem.* **1999**, *274*, 29889.
(4) Fisher, W.; Behr, T.; Hartmann, R.; Peterkatalinic, J.; Egge, H. *Eur. J. Biochem.* **1993**, *215*, 851.
(5) Uchiyama, M.; Asoy, N. R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373.
(6) Marugg, J. E.; McLaughlin, L. W.; Piel, N.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1983**, *24*, 3989.
(7) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925.
(8) Lorey, M.; Meier, C.; De Clercq, E.; Balzarini, J. *Nucleosides Nucleotides* **1997**, *16*(5&6), 789.
(9) Lorey, M.; Meier, C.; De Clercq, E.; Balzarini, J. *Nucleosides Nucleotides* **1997**, *16*(7−9), 1307.
(10) Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; Mcbride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J. Y. *Methods Enzymol.* **1987**, *154*, 287.

10.1021/ol0069498 CCC: $20.00
Published on Web 01/04/2001

Scheme 1[a]



[a] Reagents: (i) oxalyl chloride, toluene, 120 °C, 80%; (ii) *p*-hydroxybenzaldehyde, Et_3N, DMAP, CH_2Cl_2, ~98%; (iii) $NaBH_4$, 2-propanol, THF, 90%; (iv) $(i\text{-Pr})_2NP(Cl)OCH_2CH_2CN$, DIPEA, THF, 56%.

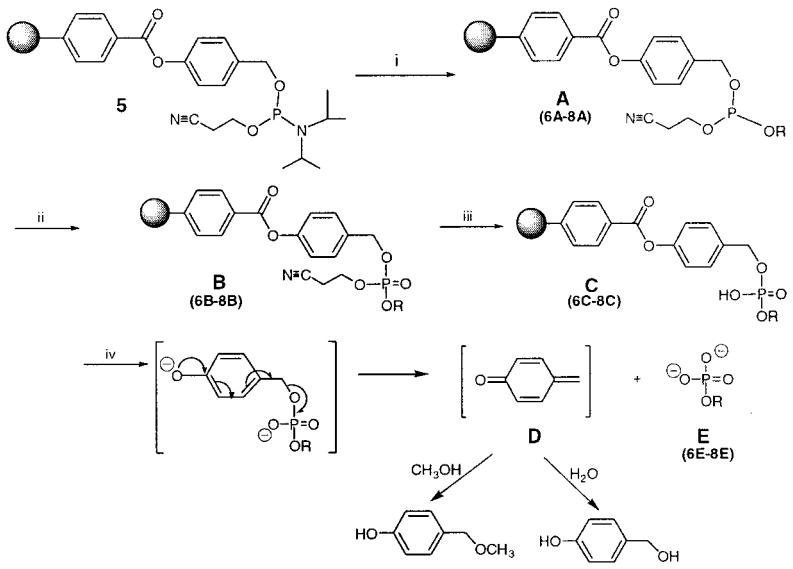
phosphorylation reactions, multiply phosphorylated compounds are usually formed.

We present here preliminary results on a method designed to produce carbohydrate and nucleoside monophosphates using a phosphorylating reagent that is immobilized on the solid phase. The novelty of the method lies in its simplicity. The substrate alcohol (or polyol) is mixed with the reagent which is an immobilized phosphitylating reagent. The alcohol is thereby "captured" as an immobilized phosphite, and washing the support guarantees that no unreacted alcohol remains. Intermolecular reactions are suppressed on the solid phase even when swelling resins are used and can in principle be completely eliminated using rigid resins, potentially resulting in mono-phosphitylation of polyols when the phosphitylation reagent is immobilized. Using chemistry developed for solid phase synthesis,[10] the immobilized phosphite is then oxidized, deprotected, and cleaved from the resin.

Scheme 1 displays the synthesis of the solid phase reagent **5**. Commercial carboxylpolystyrene (**1**) was converted to the acid chloride (**2**) in 80% yield on the basis of chloride content determined by elemental analysis. This was reacted with *p*-hydroxybenzaldehyde to produce **3** (98% based on recovery of base cleavable aldehyde). Reduction of **3** with sodium borohydride gave **4** (~90% based on base-cleavable *p*-hydroxybenzyl alcohol), which contained the cleavable linker required for attachment of the phosphitylating reagent. Reaction of **4** with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite finally gave the target reagent **5** in 56% yield on the basis of elemental analysis of nitrogen.

Scheme 2 shows the general protocol for the use of **5** as a phosphorylating reagent. Mixing of an alcohol (ROH) with **5** in the presence of 1*H*-tetrazole in an appropriate solvent (THF or THF/DMSO based on solubility of the alcohol) gives the phosphite triester **A** which can then be oxidized to the immobilized phosphotriester **B** using *tert*-butyl hydroperoxide. The cyanoethyl protecting group is then cleaved conventionally using DBU, and the resulting phosphodiester

Scheme 2[a]

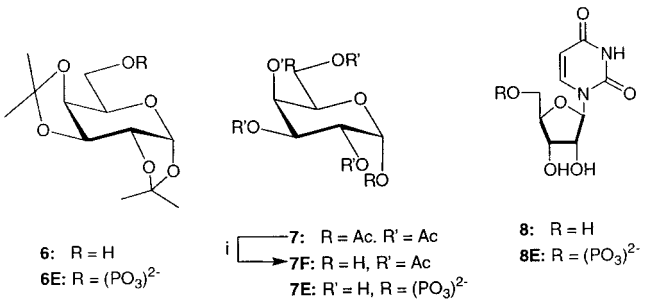


[a] Procedure and reagents: (i) ROH, THF or THF/DMSO, 1*H*-tetrazole; (ii) *tert*-butyl hydroperoxide, THF, quant.; (iii) DBU, THF, quant.; (iv) NaOMe, dioxane, MeOH, 90%.

(**C**) is cleaved with sodium methoxide. The cleavage proceeds with the concomitant release of a methylene quinone which reacts with either methanol or adventitious water to produce *p*-hydroxymethyl- or *p*-methoxymethylphenol.

Scheme 3 demonstrates some examples of the use of **5** as a phosphorylating reagent. Mixing 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**6**, 234 mg, 0.9 mmol) with **5** for 24 h in THF:DMSO 4:1 immobilized the alcohol. Oxidation (*t*-BuOOH/THF, 1 h) was followed by cleavage of the cyanoethyl group (DBU/THF, 48 h). After each step, the resin was washed sequentially with THF, MeOH, THF, and Et_2O. The phosphorylated product was then cleaved by reaction with NaOMe/dioxane for 24 h. Water was then added along with Amberlite-50W (H^+) until the solution became acidic (pH ~ 4−5). After filtration and washing of the Amberlite with dioxane and water, the solution was lyophilized to leave a white powder (**6E**, 62 mg, 79%) that was characterized by ^{1}H NMR and electrospray MS. Reaction of **5** with uridine **(8)** under similar conditions produced exclusively uridine 5′-monophosphate (**8E**, 67%) shown to be identical to the commercial product (Sigma). Preparation of uridine 5′-monophosphate in solution phase is laborious without protection of 2′- and 3′-hydroxy groups.

Scheme 3. Structure of Starting Materials and Products[a]



[a] (i) THF, Bn-NH_2.

Reagent **5** proved very convenient also for the preparation of sugar 1-phosphates. Stirring D-galactose pentaacetate (**7**, 333, 0.9 mmol) with 0.9 equiv of benzylamine in THF for 24 h resulted in the selective removal of the anomeric *O*-acetyl group to produce the reducing sugar **7F**. Reagent **5** was then added directly to this solution to capture the alcohol. The sequence of oxidation, deprotection, and cleavage from the resin with NaOMe, which simultaneously cleaved the remaining *O*-acetyl esters, then furnished α-D-galactopyranosyl 1-phosphate (**7E**) in 78% yield on a 37 mg scale. The preparation of α-sugar 1-phosphate donors in solution often require many steps including purification and give anomeric mixtures.[11] The solid phase method was found to afford specifically α-D-galactopyranosyl 1-phosphate when a solution approach[11] reported a major *β*-phosphorylated product.

In summary, reagent **5** was shown to be a practical reagent for the capture−phosphorylation of alcohols. The primary advantage of the reagent is that it ensures that no unphosphorylated alcohol is present in the cleaved product and that polyphosphorylation is suppressed.

Supporting Information Available: Experimental procedures and characterization of compounds. This material is available free of charge via the Internet at htttp://pubs.acs.org.

OL0069498

(11) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.