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Selective Phosphitylation of the Primary Hydroxyl Group in Unprotected Carbohydrates and Nucleosides

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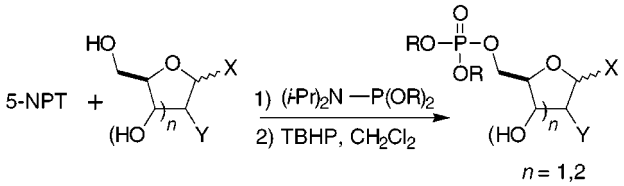
ABSTRACT



Carbohydrates and nucleosides containing a phosphate at the less-hindered primary hydroxyl group are often prepared using a protection/deprotection strategy. Herein we report that the phosphoramidite method can be used to *selectively* incorporate phosphorus at the primary hydroxyl group of *O*-unprotected carbohydrates and nucleosides; in situ oxidation of the resulting phosphite triester yields the phosphate triester.

A common method for the synthesis of 5′-nucleotides from nucleosides employs a protection/deprotection strategy[1] involving (1) selective protection of the less hindered primary hydroxyl group (5′-OH) using a sterically demanding reagent such as 4,4′-dimethoxytrityl (DMT) chloride or *tert*-butyldimethylsilyl chloride, (2) protection of the remaining secondary hydroxyl groups, (3) removal of the 5′-OH protecting group, (4) phosphorylation (or phosphitylation followed by oxidation) of the free 5′-hydroxyl, and (5) removal of the secondary hydroxyl protecting groups (Figure 1, right). In this "standard protocol" there are two concerns that must be addressed. First, the protecting groups must be chosen so that their incorporation and removal proceeds selectively and does not interfere with functionality already present in the molecule. Second, the protection/deprotection strategy is, by its very nature, inefficient in terms of time, cost, and particularly yield; even with an optimized yield of 90% for each of the five steps the overall isolated yield of 5′-phosphate **2** will not exceed 60%.

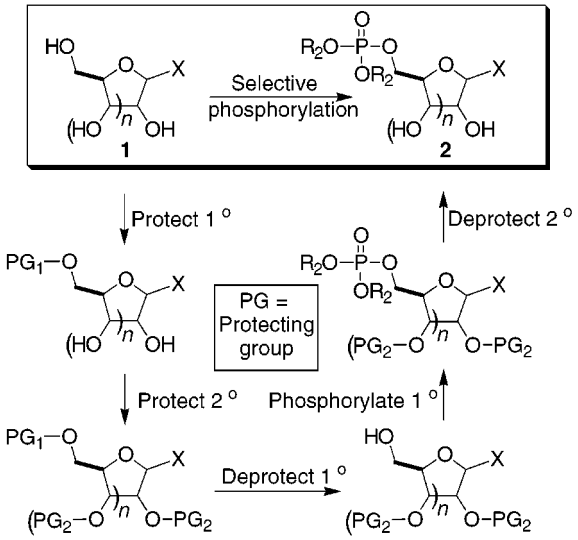


Figure 1.

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(1) (a) Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, *54*, 631. (b) Hutchinson, D. W. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum: New York, 1988; Vol. 2, pp 81−160.

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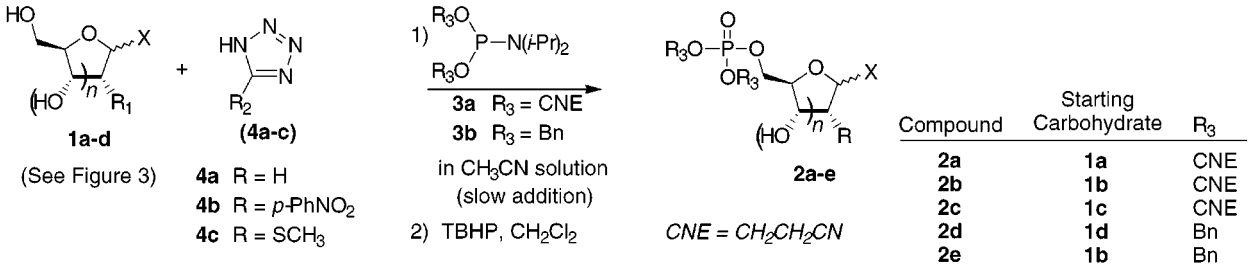
Table 1. Selective Phosphitylation/Oxidation of Carbohydrates/Nucleosides **1a**−**d**[a]



Compound	Starting Carbohydrate	R_3
2a	**1a**	CNE
2b	**1b**	CNE
2c	**1c**	CNE
2d	**1d**	Bn
2e	**1b**	Bn

no.	start. matl	tet.	phosphoramidite (equiv)	addn time	total time[b]	temp	product	isolated yield, %	selectivity[c]	% carbohydr recvry,[d] %
1	**1b**	**4a**	**3a** (1.0)	30	50	RT	**2b**	33	0.66	50
2	**1b**	**4a**	**3a** (1.2)	26	50	RT	**2b**	25	0.43	41
3	**1b**	**4a**	**3a** (1.4)	33	50	RT	**2b**	36	0.81	55
4	**1b**	**4a**	**3a** (1.6)	37	50	RT	**2b**	35	0.61	43
5	**1b**	**4a**	**3a** (1.8)	43	50	RT	**2b**	38	1.15	67
6	**1b**	**4a**	**3a** (2.0)	44	50	RT	**2b**	34	0.75	55
7	**1b**	**4a**	**3a** (0.8)	16	50	RT	**2b**	40	1.22	73
8	**1b**	**4a**	**3a** (0.8)	16	50	0 °C	**2b**	40	1.29	75
9	**1b**	**4a**	**3a** (0.8)	16	50	−20 °C	**2b**	33	1.03	75
10	**1b**	**4a**	**3a** (0.8)	16	50	−36 °C	**2b**	31	1.73	86
11	**1b**	**4b**	**3a** (0.8)	20	25	−36 °C	**2b**	59	2.48	81
12	**1b**	**4b**	**3a** (0.8)	16	300	−36 °C	**2b**	57	2.66	82
13	**1b**	**4b**	**3a** (0.8)	16	50	−36 °C	**2b**	60	3.04	83
14	**1b**	**4b**	**3a** (1.0)	35	75	−36 °C	**2b**	23	0.77	71
15	**1b**	**4b**	**3a** (1.0)	16	180	−36 °C	**2b**	31	1.28	76
16	**1b**	**4b**	**3a** (0.8)	16	50	0 °C	**2b**	50	1.47	70
17	**1b**	**4c**	**3a** (0.8)	16	50	−36 °C	**2b**	62	3.74	87
18	**1b**	**4b**	**3b** (0.8)	20	25	−36 °C	**2e**	60	2.37	80
19	**1a**	**4b**	**3a** (0.8)	20	25	−36 °C	**2a**	55	5.23	92
20	**1d**	**4b**	**3b** (0.8)	20	25	−36 °C	**2d**	69	2.33	75
21	**1c**	**4b**	**3a** (0.8)	60	60	−36 °C	**2c**	54	3.11	86
22[e]	**1b**	**4b**	**3a** (0.8)	16	50	−36 °C	**2b**	65	2.45	80

[a] Conditions: slow addition of phosphoramidite (~0.5 M in CH_3CN) to a solution of carbohydrate (0.3−0.4 mmol scale, 0.01 M in CH_3CN) and tetrazole **4** (2.4 equiv) and then oxidation with excess TBHP (20 min). [b] Total time prior to TBHP addition. [c] Desired product/"missing" material (see text). [d] (Desired product + recovered starting carbohydrate)/total starting carbohydrate. [e] Scale = 4.7 mmol.

Recently we had need of phosphate **2b** (Table 1) as part of a project involving the chemical synthesis of the calcium release agent cyclic adenosine diphosphate ribose (cADPR).[2] The original route to **2b** employed a protection/deprotection strategy (DMT for the 5′-OH; acetyl for the 2′- and 3′-OH groups). However, the 5′-DMT group proved surprisingly difficult to remove cleanly, thus prompting the current study.

Several methods exist for the selective 5′-phosphorylation of nucleosides using phosphoryl chlorides.[3,4] These methods are limited in that the phosphate produced is either anionic or the free acid, and aqueous ion-exchange chromatography must be used if high-purity phosphate is desired. In contrast, the phosphoramidite method[5] as used in solution phase synthesis can be used to produce fully esterified, neutral phosphotriesters, which can be purified using silica gel chromatography.[6] Though there are reports of *O*-selective versus *N*-selective phosphitylation using phosphoramidites[7] or other reagents,[8] the issue of differential hydroxyl phosphitylation was not addressed. Herein we report a method for the *selective* phosphitylation of the primary hydroxyl group of carbohydrates and nucleosides. Our method has the virtue of allowing for purification of phosphotriester **2** on silica gel,[9] and the yields are competitive with the protection/deprotection and selective phosphorylation strategies. If we allow for recovery of starting carbohydrate, then the yields are improved further, in most cases exceeding 80%.

(2) Lee, H.-C. *Physiol. Rev.* **1997**, *77*, 1133.
(3) (a) Sowa, T.; Ouchi, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2084. (b) Imai, K.-I.; Fujii, S.; Takanohashi, K.; Furukawa, Y.; Masuda, T.; Honjo, M. *J. Org. Chem.* **1969**, *34*, 1547. (c) Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505. (d) Hes, J.; Mertes, M. P. *J. Org. Chem.* **1974**, *39*, 3767.
(4) For a report of selective phosphorylation using H-phosphonates, see: Knerr, L.; Pannecoucke, X.; Schmitt, G.; Luu, B. *Tetrahedron Lett.* **1996**, *37*, 5123.
(5) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859.
(6) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(7) Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, *20*, 1879.
(8) (a)Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.-i.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710. (b) Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373.

The commonly accepted mechanism for phosphitylation of hydroxyl groups using the phosphoramidite method is shown in Figure 2.[10] Protonation of a phosphoramidite (**3**)

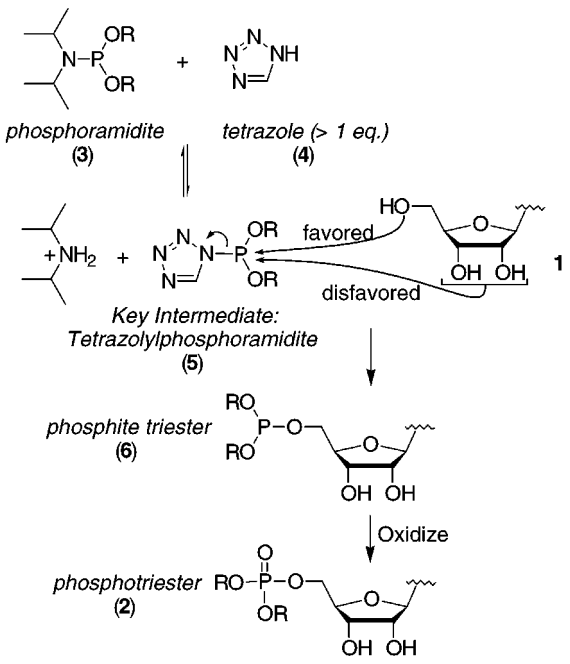


Figure 2. Mechanism of phosphitylation.

by the weak acid 1*H*-tetrazole (**4**), followed by nucleophilic attack by tetrazolide ion, gives a tetrazolylphosphoramidite (**5**). Nucleophilic attack of a hydroxyl group from **1** on **5** produces phosphite triester **6**; subsequent oxidation of **6** gives the desired protected phosphotriester **2**. We reasoned that under conditions where the nucleophilic attack of the hydroxyl group is rate limiting[10a] (i.e., where the concentration of tetrazolylphosphoramidite **5** is low), it should be possible to find conditions so that this attack is also selective for less hindered primary hydroxyl groups.

The data for the phosphitylation of carbohydrates/nucleosides **1a**−**d** is summarized in Table 1, and the structures of **1a**−**d** are shown in Figure 3. We presumed that the selectivity would be optimal if an acetonitrile solution of phosphoramidite **3** was added slowly (syringe pump) to a solution of **1** and the tetrazole. The initial attempts (entries 1−10) used pentenyl riboside[11] **1b** as the carbohydrate, **3a** as the phosphoramidite,[12] and 1*H*-tetrazole (**4a**) as catalyst,

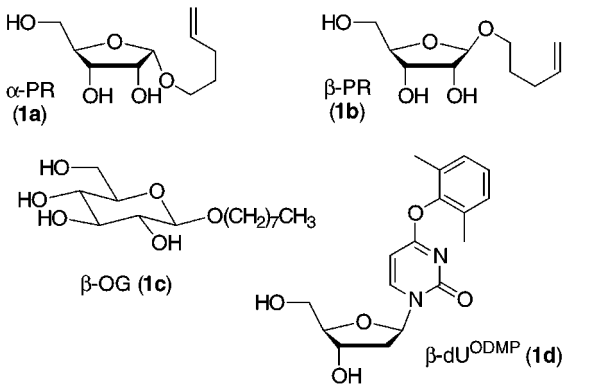


Figure 3. Structures of the carbohydrates/nucleosides used in this study.

but after systematically varying the amount of phosphoramidite, its time of addition, and the temperature of the reaction, an isolated yield of 40% and a selectivity of approximately 1.3 for the production of phosphotriester **2b** could not be improved upon.[13] Furthermore, it was difficult to monitor the outcome when an aqueous extraction was included in the reaction workup; apparently the starting carbohydrate, and to a lesser extent the product phosphotriester, display appreciable water solubility. The data in Table 1 were obtained using our standard workup whereby the reaction volume is simply concentrated on a rotary evaporator and then loaded onto the chromatography column (see Supporting Information).

Assuming the lack of selectivity and the low yield in the reactions catalyzed by tetrazole **4a** were due to insufficient discrimination in the rates of attack of primary versus secondary hydroxyl groups on tetrazolylphosphoramidite **5**, the next logical step was to increase the steric requirements of the reaction by using other tetrazoles. To this end 5-(*p*-nitrophenyl)-1*H*-tetrazole (5-NPT, **4b**)[14] and 5-methylthio-1*H*-tetrazole (5-MTT, **4c**)[15] were investigated. These tetrazoles were reported to give higher yields in the coupling step of solid-phase oligodeoxyribonucleotide synthesis be-

(9) To obtain high-purity phosphate from the silica gel-purified phosphotriesters, it is necessary that the phosphate not migrate during the deprotection reaction. As phosphotriesters **2** already have the phosphorus atom in the less hindered position and lack a hydroxyl group cis to the phosphorus, we expect the phosphate deprotection to proceed without significant phosphate migration. For example, see: Haines, A. H. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, R. S., Horton, D., Eds.; Academic: New York, 1976; Vol. 33, p 108.

(10) (a) Dahl, B. H.; Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 1729. (b) Berner, S.; Mühlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853. (c) Berner, S.; Mühlegger, K.; Seliger, H. *Nucleosides Nucleotides* **1988**, *7*, 763. (d) Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279.

(11) Chapeau, M.-C.; Marnett, L. J. *J. Org. Chem.* **1993**, *58*, 7258.

(12) (a) Pederson, R. L.; Esker, J.; Wong, C.-H. *Tetrahedron* **1991**, *47*, 2643. (b) Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175. (c) Uhlmann, E.; Engels, J. *Tetrahedron Lett.* **1986**, *27*, 1023.

(13) While the isolation of 5′-phosphotriesters **2** and recovered carbohydrate **1** using silica gel chromatography was uneventful, our ability to isolate "incorrectly" or multiply phosphorylated product proved to be dependent on the nature of the phosphate protecting group. In the cyanoethyl series (**2a**−**c**) the product phosphotriesters are more polar than their respective starting carbohydrates; multiply phosphorylated product is apparently too polar to be chromatographed. In contrast, the benzyl phosphotriesters (**2d**−**e**) are less polar than their respective starting carbohydrates. Additional preliminary data (not shown) for the conversion of **1d** to **2d** indicate the production of approximately 10% and 5% of 3′,5′-bisphosphorylated and 3′-monophosphorylated material, respectively. To compare the cyanoethyl and benzyl series, we simply assumed that after accounting for the amounts of **2** and **1** obtained the balance (or "missing") material was incorrectly or multiply phosphorylated carbohydrate. For our purposes, "selectivity" is therefore defined as the ratio of the moles of desired product to the moles of "missing" material.

(14) (a) Froehler, B.; Matteucci, M. D. *Tetrahedron Lett.* **1983**, *24*, 3171. (b) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1997**, *119*, 11758.

(15) Wright, P.; Lloyd, D.; Rapp, W.; Andrus, A. *Tetrahedron Lett.* **1993**, *34*, 3373.

cause of their enhanced acidity relative to **4a**. Though it seems counterintuitive to expect that a more active tetrazole would allow for enhanced selectivity of phosphitylation, it should be pointed out that the conditions for solid-phase oligodeoxyribonucleotide synthesis (excess phosphoramidite) are substantially different than those used here (approximately stoichiometric phosphoramidite; see below). Gratifyingly, we observed dramatic yield and selectivity improvements when a solution of phosphoramidite **3a** (0.8 equivalents)[16] was slowly added to a solution of tetrazole **4b** and carbohydrate **1b** at −36 °C[17] followed by oxidation with *tert*-butyl hydroperoxide/CH_2Cl_2. Using tetrazole **4b** (entry 11), phosphotriester **2b** was produced in 59% isolated yield with a minimal selectivity of approximately 2.5; if recovered starting carbohydrate is taken into account, then the yield improves to 81%.[18,19] The reaction appears to be complete by or soon after the addition of phosphoramidite, as increased reaction times (entries 12 and 13) gave similar results. Attempts to increase the amount of phosphoramidite to 1 equiv led to a significant decrease in the isolated yield of **2b** (entries 14 and 15). The other substituted tetrazole worked equally well (entry 17); **4c** and the same phosphoramidite/carbohydrate (**3a**/**1b**) pair gave phosphotriester **2b** in 62% isolated yield with a minimal selectivity of approximately 3.7 (87% based on recovered starting carbohydrate).

With these "optimum" conditions in hand, we explored the scope of the selective phosphitylation, utilizing different carbohydrates and phosphoramidites. The reaction is generally applicable, as the reaction of phosphoramidite **3a** and tetrazole **4b** with α-ribofuranosides (entry 19) and β-glucopyranosides (entry 21) gave **2a** and **2c**, respectively, with yields and selectivities similar to those above. The latter entry is remarkable in that there are three secondary hydroxyl groups present in the starting carbohydrate. Cyanoethyl phosphoramidite **3a** is not unique in its selectivity, as dibenzyl phosphoramidite **3b** (entries 18 and 20) behaved similarly.[13] Entry 20, using nucleoside **1d**,[20] also demonstrates that the selective phosphitylation works for nucleoside, as opposed to glycoside, carbohydrates. Finally, the reaction works on larger scales; entry 22 was run on a 10-fold larger scale than the other entries.

This procedure for selective phosphitylation, though useful, is not without limitations. First, the generality of the reaction may be hampered by problems of solubility. The starting carbohydrates and the tetrazoles[14,15] are only sparingly soluble in acetonitrile, and the reactions we conducted were often run at the limit of solubility of these components. The issue of carbohydrate solubility is a problem only with underivatized, highly polar compounds such as short-chain alkyl glycosides and the parent nucleosides. We have not yet investigated whether the commercially available *O*-unprotected *N*-protected nucleosides (e.g., *N*-benzoyl or *N*-acetyl) can be selectively phosphitylated using this procedure, but they are expected to display the requisite solubility in acetonitrile. Phosphitylation reactions in solvents other than acetonitrile, such as acetone,[21] are known, but their effect on the selective phosphitylation described herein has yet to be determined. Second, the observed selectivity in our reaction is only moderate. Here too it will be useful to investigate other solvents, but a more useful approach may be to further increase the steric bulk of tetrazolylphosphoramidite **5**. In one study of the effect of phosphoramidite substituents on the rate of phosphitylation,[10a] it was found that for each replacement of a cyanoethyl β-hydrogen by methyl there was a 2- to 3-fold reduction in the rate of phosphitylation. An intriguing possibility is that not only might the more hindered β-methyl- and dimethylcyanoethyl phosphoramidites display improved selectivity of primary hydroxyl group phosphitylation but that it may now be possible to do so *stereoselectively*.[10d,22]

In conclusion, we have found a convenient method that allows one to selectively phosphitylate a primary hydroxyl group in the presence of multiple secondary hydroxyl groups. Our efforts to improve the yield and selectivity of this reaction, the ability to extend it to other nucleoside substrates, and the stereoselective synthesis of chiral phosphates will be reported in due course.

Identity and Characterization. The structure and purity of phosphates **2a**−**e** were determined from analysis of their ^{1}H, ^{13}C, and ^{31}P spectra.

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Supporting Information Available: Full experimental procedures for the preparation of phosphates **2a**−**e**, including ^{1}H, ^{13}C, and ^{31}P NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) In principle, the highest selectivity would be achieved with a large excess of carbohydrate over phosphoramidite. Operationally, conditions were sought where the selectivity was sufficient to use a 1:1 ratio of carbohydrate to phosphoramidite, as this allows for more mass of product attainable in a single run. We have thus settled on the compromise value of 0.8 equiv of phosphoramidite per equivalent of carbohydrate.

(17) Tetrazole **4b** begins to precipitate below this temperature.

(18) The percent yield based on carbohydrate recovery is defined as (moles of **2b** isolated + moles of **1b** recovered)/moles of total starting **1b**.

(19) Identification of the primary hydroxyl as the site of phosphorylation was confirmed by analysis of the ^{13}C attached proton test (APT) NMR spectrum. Due to coupling with phosphorus (^2J and ^3J) some of the carbon signals were doublets. In compound **2b** only one of the methine carbon signals was a doublet; had the 3′ hydroxyl been phosphorylated there should have three methine doublet signals.

(20) The synthesis of **1d** will be reported in due course.

(21) Wolter, A.; Biernat, J.; Köster, H. *Nucleosides Nucleotides* **1986**, *5*, 65.

(22) Stec, W. J.; Grajkowski, A.; Kobylanska, A.; Karwowski, B.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019.