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A new method for the phosphorylation of nucleosides

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Abstract

A new phosphorylating reagent, 2-*O*-(4,4′-dimethoxytrityl)ethylsulfonylethan-2′-yl-phosphate (**1**), was developed for the phosphorylation of primary and secondary alcohols of nucleosides. In the many examples studied, yields in the phosphorylation step were excellent (~80 to 95%). There is potential for wide applicability of this procedure, not only in nucleoside and nucleotide chemistry, but also in the phosphorylation of biomolecules such as carbohydrates and amino acids.

Keywords: phosphorylation methodology; nucleosides; new reagent.

Phosphorylated nucleosides are of interest as biological probes in the study of various enzyme-catalyzed reactions and as potential therapeutic agents.[1] Technically, synthesis of these complex compounds is designed in a manner to avoid the handling of the phosphomonoester function until the very last step(s) when they are introduced through enzymatic or chemical phosphorylation. Chemical phosphorylation requires temporary esterification of the hydroxyl functions of the phosphate being introduced.[2] A requirement of the phosphate protecting groups is not only stability with respect to reaction conditions of synthesis, but also selective lability under relatively mild conditions at the termination of synthesis.

Chemical phosphorylation with P_2O_5 or $POCl_3$ in triethylphosphate or pyridine is used traditionally for direct one-step introduction of the monophosphate function into unprotected molecules, such as sugars and nucleosides.[2–4] The products of these phosphorylations, the free acids or their salts, are polar compounds and their purification is difficult and time consuming. In addition, $POCl_3$ does not work well when applied to compounds containing protecting groups that are acid labile. Some $POCl_3$ derivatives such as phosphoimidazolides and triazolides[5] or hydroxybenztriazolides[6] are milder phosphorylating reagents. Dibenzyl phosphorochloridate[7] readily phosphorylates the primary alcohol groups of nucleosides and the benzyl protection in the resulting phosphotriester can be removed by catalytic hydrogenation. However, the efficiency of phosphorylation of secondary alcohol groups with this reagent is not high. Tris-(8-quinolyl)phosphate[8] is also selective for primary alcohols of nucleosides, but reaction times for these phosphorylations are long and, in some cases, the conversions proceed with relatively low yields.

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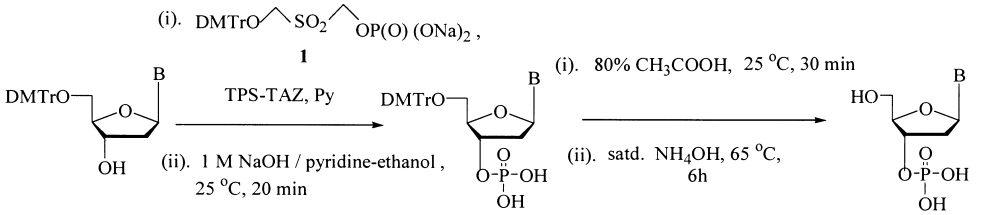
A major problem in phosphorylation reactions is the removal of protecting groups after the phosphorylation as they generally require harsh reaction conditions for disconnection. This can be circumvented with the use of base-labile β-substituted ethyl protecting groups which can be removed by β-elimination under mildly alkaline conditions.[9–14] However, these reactions are difficult to monitor. A phosphorylating reagent that contains the DMTr group for easy monitoring of incorporation of phosphate has been reported to give good yields (~75%) in the phosphorylation step.[15] Disadvantages of this method are the time consuming steps in the preparation of precursors and the two-step deprotection procedure which includes a troublesome periodate oxidation step. Another example of a phosphorylating agent that meets the criteria of ease of removal and of monitoring is (2-cyanoethyl)-2-[2′-*O*-(4,4′-dimethoxytrityl)oxy-ethylsulfonyl]ethyl-*N*,*N*-diisopropylphosphoramidite.[10] However, this approach has the disadvantage of working with unstable phosphoramidites. Other protecting groups such as trityloxyethylamino- have also been reported,[11] but these approaches require three additional steps after the initial phosphorylation, which results in low overall yields of the target compounds.

We describe herein a new method for the phosphorylation of nucleosides (Schemes 1 and 2) which utilizes the desirable features discussed above and reliable phosphodiester chemistry. The new phosphorylating reagent is 2′-*O*-(4,4′-dimethoxytrityl)ethylsulfonylethan-2-yl-phosphate (**1**). This reagent can be synthesized from sulfonyldiethanol by monotritylation followed by reaction with $POCl_3$/1,2,4-triazole/triethylamine and work-up with $NaHCO_3$. Activated by coupling reagents such as TPS-TAZ or TPS-NT, reagent **1** phosphorylates primary and secondary alcohol functions in nucleosides with remarkable efficiency. Monitoring of the progress of the reaction is easily performed by TLC. Deprotection of the phosphodiester intermediate is performed in only one step, but that step is actually in the work-up of the reaction.[19] The yields after work-up are between ~80–95% (Table 1). Protection of nucleosides is required prior to the phosphorylation step. This method of phosphorylation has the potential for wide applicability and can be extended to include ribonucleosides, oligonucleotides, carbohydrates and other biomolecules.



Base, B =

U
N-Bz-C
N-Ibu-G
N-Bz-A
T

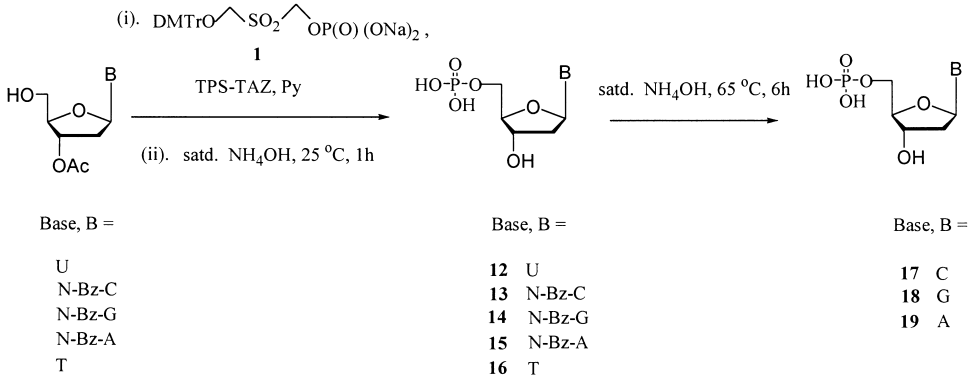
Base, B =

2 U
3 N-Bz-C
4 N-Ibu-G
5 N-Bz-A
6 T

Base, B =

7 U
8 C
9 G
10 A
11 T

Scheme 1.



Scheme 2.

Table 1
Yields of 3′- and 5′-phosphates utilizing the new phosphorylation procedure

Starting Material	Product	% Yield	Final Product	Compd. References
5′-ODMTr-dU	**2**	91	**7**	16
5′-ODMTr-N^4-BzdC	**3**	92	**8**	17
5′-ODMTr-N^2-iBudG	**4**	95	**9**	17
5′-ODMTr-N^6-BzdA	**5**	90	**10**	17
5′-ODMTr-dT	**6**	93	**11**	18
dU-3′-OAc	**12**	87	**12**	18
N^4-BzdC-3′-OAc	**13**	84	**17**	17
N^2-BzdG-3′-OAc	**14**	80	**18**	18
N^6-BzdA-3′-OAc	**15**	79	**19**	17,18
dT-3′-OAc	**16**	80	**16**	18

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19. General procedure for phosphorylation: The new phosphorylating reagent, 2′-*O*-(4,4′-dimethoxytrityl)ethyl-sulfonylethan-2-yl-phosphate (**1**) was synthesized from sulfonyldiethanol (Aldrich) as described previously by us.[1c] Phosphorylation was carried out on a 0.2 mmolar scale as follows: 0.2 mmol of starting nucleoside derivative to be phosphorylated, containing free 5′ or 3′ hydroxyl groups, was dried by repeated evaporation with anhydrous pyridine. Compound **1** (1 mmol, 600 mg), TPS-TAZ or TPS-NT (2 mmol) and 5 mL of pyridine were added under nitrogen. The reaction mixture was refrigerated for 1 h, then kept for an additional 2 h at 25 °C, partitioned between methylene chloride (100 mL) and saturated $NaHCO_3$ (10 mL) and the organic layer was dried and evaporated. In the case of compounds **2**–**6**, the residue was dissolved in pyridine (5 mL) and treated with 2 M NaOH in ethanol (5 mL). The reaction mixture was stirred at 25°C for 20 min. Deprotection of the DMTr-group on the phosphate was monitored by TLC. The reaction mixture was partitioned between methylene chloride (100 mL) and water (10 mL). The organic layer was evaporated, co-evaporated with toluene (basic pH has to be maintained; it is adjusted by adding triethylamine). The residue was purified on a short silica gel column. The target phosphorylated compounds were eluted with 10–15% MeOH in methylene chloride. Further purifications were carried out by reversed phase HPLC on a C-18 column with ethanol–water as the eluting solvent. For compounds **12**–**16**, the residue of the phosphorylation reaction was stirred with conc. NH_4OH (10 mL) at 25°C for 1 h. The reaction mixture was evaporated and purified by reversed-phase HPLC. Final deprotections for both series were carried out as indicated in Schemes 1 and 2. The products were characterized by spectral data. For example, for compound **2** (triethylammonium salt): UV (H_2O) 233 nm (14000), 264 nm (8000). ^{1}H NMR (MeOH-d_4), 7.82 (d, J = 7.8 Hz, 1H, H-6), 7.41 (d, J = 7.6 Hz, 2H), 7.30–7.22 (m, 7H) and 7.86 (d, J = 7.6 Hz, 4H) (DMTr), 6.30 (t, J = 6.4 Hz, 1H, 1′), 5.21 (d, J = 7.8 Hz, 1H, H-5), 5.04 (s, 1H, 3′), 4.29 (s, 1H 4′), 3.78 (s, 6H, CH_3O), 3.53–3.50 (m, 1H, 5′$_a$), 3.38–3.36 (m, 1H, 5′$_b$), 3.11 (s, 6H, CH_2N), 2.56–2.52 (m, 1H, 2′$_a$), 2.39 (qw, J = 7.6 Hz, 1H, 2′$_b$), 1.27 (s, 9H, CH_3CH$_2$N); (in $CDCl_3$), 8.69 (s. 1H, NH), 7.34–7.21 (m, 9H) and 6.83 (d, J = 11.2 Hz, 4H) (DMTr), 6.35 (t, J = 8.8 Hz, 1H, 1′), 5.38 (d, J = 10.8 Hz, 1H, H-5), 5.14 (br s, 1H, 3′), 4.16 (s, 1H, 4′), 3.77 (s, 6H, CH_3O), 3.30 (s, 2H, 5′), 2.98 (q, J = 9.8 Hz, 6H, CH_2N), 2.53–2.49 (m, 1H, 2′$_a$), 2.30–2.25 (m, 1H, 2′$_b$), 1.23 (t, J = 9.8 Hz, 9H, CH_3CH$_2$N). ^{13}C NMR (MeOH-d_4), 166.1 (C-4), 160.3 (unidentified), 152.0 (C-2), 142.5 (C-6), 146.0, 131.5, 131.4, 130.5, 129.5, 129.4, 128.9, 128.0, 114.2 and 113.9 (DMTr), 102.4 (C-5), 88.3 (Ph_3C), 87.1 and 87.0 (1′), 86.6 (4′), 76.4 (3′), 64.9 (5′), 55.7 (CH_3O), 47.6 (CH_2N), 40.7 (2′), 9.2 (CH_3CH_2N). ^{31}P NMR: (MeOH-d_4), 3.74 (s). FAB HRMS: $(M–H)^-$ calcd for $C_{30}H_{30}N_2O_{10}P$: 609.1638; found: 609.1631.