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Phosphonate-free phosphorylation of alcohols using bis-(*tert*-butyl) phosphoramidite with imidazole·hydrochloride and imidazole as the activator

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ABSTRACT

A variety of commercially available alcohols were converted to their bis-(*tert*-butyl) protected mono-phosphate pro-drugs. The improved procedure uses the unique combination of imidazole·hydrochloride and imidazole as the activator to suppress the formation of undesired phosphonate by-product frequently encountered with the bis-(*tert*-butyl) phosphoramidite reagents. The new activation procedure eliminates the need to use excess reagents.

1. Discussion

Mono-alkyl phosphates are recognized to play important roles in many biological processes.[1a] Derivatization of an alcohol moiety as the mono-phosphate esters have been employed as pro-drugs since they are readily ionizable, significantly increase aqueous solubility,[1b–d] are sufficiently stable in both the solid state and in aqueous solution for drug product manipulation and are readily cleaved in vivo by nonselective alkaline phosphatases, thus releasing the parent drug in vivo.[1d] Early synthetic methods involved the electrophilic dialkyl phosphoryl halides in their P(V) oxidation state (Scheme 1).

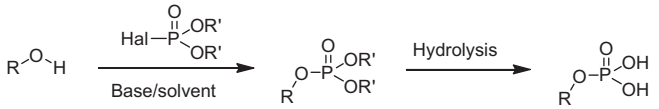
Although these methods gave direct access to the desired protected phosphates, many of the phosphoryl halide methods gave complex mixtures[2a] and/or low yields,[2b] or required toxic[1c] or exotic reagents.[2c] Protecting groups other than benzyl, *tert*-butyl or trimethylsilyl-ethoxy gave incomplete hydrolysis,[3c] probably due to non-regioselective hydrolysis pathways, thus low to moderate yields were observed at the final hydrolysis stage. Bis-benzyl and bis-*tert*-butyl phosphates were prepared using their corresponding phosphorohalidates (Scheme 1, R = *t*-Bu) but suffered from instability and low electrophilicity.[4]

Johns et al.[3] reported the phosphorylation using bis-alkyl phorphoramidites for the phosphitylation of alcohols. The in situ generated phosphites were then oxidized in the same pot to the desired phosphates (Scheme 2).

In the context of large-scale preparation of phosphate pro-drug development candidates, careful consideration must be given to the choice of the protecting group. The Pd-catalyzed hydrogenolysis of benzyl groups is less desirable due to the difficulties associated with the removal of Pd residues and the incompatibility with halogenated aromatics under standard hydrogenolysis conditions. The benzyl groups can also be cleaved under TMSBr and TMSI conditions[5] to name a few. Under these conditions, concomitant formation of noxious benzyl halides, a toxic by-product, also makes this approach less attractive for large scale. The trimethylsilyl-ethoxy protecting group has been widely used,[6c] especially for solid phase synthesis. However, the TFA or TBAF cleavage conditions makes this option less attractive in the context of large-scale preparation of pharmaceutical phosphate pro-drugs.

The bis-*tert*-butyl group, since it undergoes facile acidic cleavage, may be a superior candidate at circumventing the problems associated with the above protecting groups. The well known bis-*tert*-butyl *N,N*-dialkyl phosphoramidites have been extensively used for this transformation[3c] and the resulting phosphates have been easily deprotected to their mono-phosphates in high yields using TFA,[7a–c] HCl–dioxane,[3c] TMSCl[7d] and TMSCl/NaI[1c] conditions. However, it has been documented that the bis-*tert*-butyl phosphoramidite gives rise to an undesired phosphonate by-product[6,8] (Scheme 3).

The suppression of the phosphonate by-product has been reported[6a] by the use of excess phosphoramidite reagent (10–20 equiv), an unacceptable solution for large scale chemistry. For safety reasons, we modified the mode of addition of the original procedure[3] and we experienced increased levels of the phosphonate by-product with increasing scale when the phorphoramidite was added last (not shown). After investigation and isolation of the phosphonate by-product, we suspected that the low pK_a of 1*H*-tetrazole (pK_a = 4.89)[9] promoted the cleavage of one of the *tert*-butyl groups



Scheme 1.

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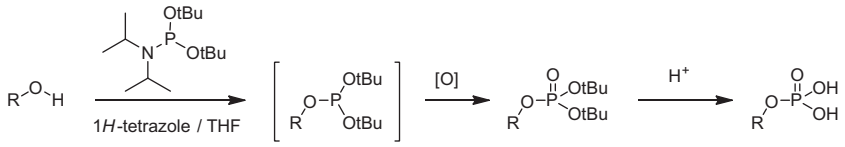
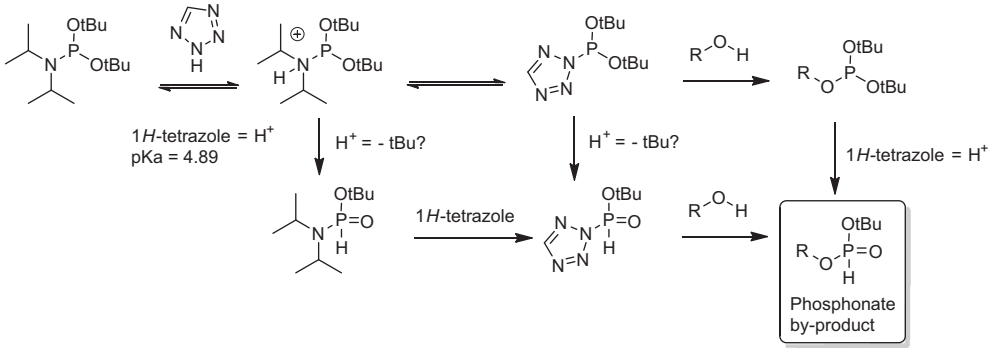


Scheme 2.



Scheme 3.

of the reagent and/or the intermediate phosphite.[6a] Indeed, it was alarming to see rising levels of undesired phosphonate with increasing scale. This was probably due to time dilation to control the exotherm on larger scale during the phosphoramidite addition (not shown). Furthermore, the activator 1*H*-tetrazole[6] has been recently reclassified as an explosive.[9,10] Consequently, accessing this reagent from commercial sources is now severely restricted.

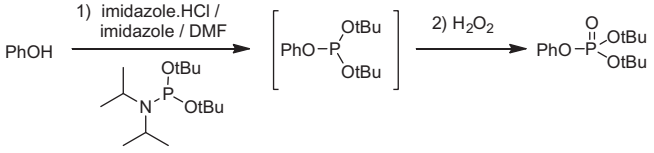
In recent reports,[9] the crucial activator 1*H*-tetrazole can be replaced by phenanthroline salts,[9a] anilinium salts,[9a] benzimidazolium salts,[9a,b] pyridinium salts[9a] and imidazolium salts.[9] The ease of activation is reported to be proportional to the degree of acidity of the activator.[9a] Of the above salts,[9a] anilinium salts have a pK_a of 4.44–5.34, pyridinium salts have a pK_a of 5.23 and imidazolium salts have a pK_a of 6.99. In the case of the activation of bis-*tert*-butyl phosphoramidites, we postulated that the use of imidazole·hydrochloride (imidazole·HCl) in combination with imidazole would give optimal results at suppressing the phosphonate by-product.

2. Results

The phosphorylation of phenol (Scheme 4) was optimized to identify the optimal stoichiometry of the reagents (Table 1). We quickly identified that it was mandatory to use 1.5 equiv of imidazole·HCl and phosphoramidite reagent for complete conversion of phenol to the phosphate. The synergistic effect of 1.0 equiv of imidazole to buffer the reaction media was crucial to nearly eliminate the occurrence of the phosphonate by-product (Table 3, entry 1).

3. Activator effect

Benzyl alcohol was used to study the effect of the activator in Table 2. We first compared our imidazole·HCl/imidazole conditions



Scheme 4.

(entry 1) with 1*H*-tetrazole (entry 2). Clearly, imidazole·HCl gave improved product distribution compared to 1*H*-tetrazole. The role of imidazole·HCl was tested in entry 3. As expected, this experiment confirmed that imidazole alone was not sufficient to activate the phosphitylation step.

4. Scope

Table 3 shows a variety of alcohols that were tested in our laboratories with our general procedure. Phenol (entry 1), benzyl alcohol (entry 2) and phenethyl alcohol (entry 3) were smoothly phosphorylated in good to high yields. Oxidation-sensitive *p*-methoxy congeners (entries 4 and 5) did not show over-oxidation by-products. The secondary benzylic alcohol (entry 7)[11] and 2-hydroxymethyl pyridine (entry 8) were phosphorylated in modest yields. For the 2-hydroxymethyl pyridine in entry 8, we suspect the modest yield is due to increased aqueous solubility and/or *N*-oxide by-products from exposure to hydrogen peroxide. Phosphorylation of more complex alcohols could also be performed. For example, serine derivative *N*-CBz serine benzyl ester (entry 9) was chemoselectively phosphorylated in good yield and the commercial drug Metronidazole (entry 10) was phosphorylated in good yield.

As can be seen in Table 3, the phosphonate by-product is less than 2% in most cases (Scheme 5).

We found, however, that the enhanced reactivity of the buffer activation system led to decreased chemoselectivity. For example, phosphorylation of tertiary alcohol (entry 7) proved challenging, either because of the lack of reactivity due to steric hindrance or because of in situ cleavage back to the alcohol through stabilized benzylic carbocation formation. Indeed, when treating the secondary benzylic alcohol (entry 6), we observed a subtle limitation to the method. Upon HPLC analysis of the reaction mixture, incomplete conversion of the alcohol to the intermediate phosphite was observed (80–86% conversion). After addition of hydrogen peroxide, the remaining alcohol had converted to the desired product.

This observation suggests that the limitation of the method is the phosphorylation of secondary benzylic alcohol where reaction completion of the phosphite intermediate should be considered at the phosphate stage (after hydrogen peroxide oxidation).

Table 1
Stochiometry optimization with phenol[a]

Entry	Equiv imidazole·HCl	Equiv phosphoramidite	Results[b]		
			Phenol	Phosphonate	Phosphate
1	1.4	1.4	33.9	<1	66.1
2	1.4	1.4	1.7	<1	97.8
3	1.5	1.5	1.3-1.5	<1	97.8–98.0
4	1.7	1.7	1.7	<1	98.3
5	2.0	2.0	1.9	<1	97.2

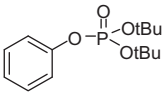
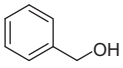
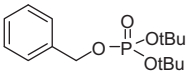
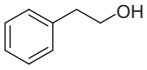
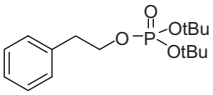
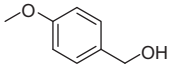
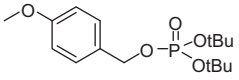
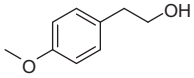
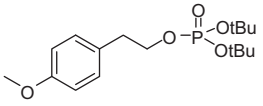
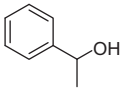
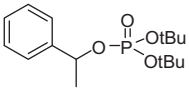
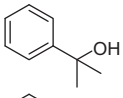
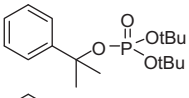
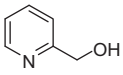
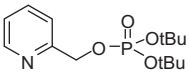
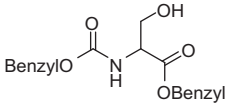
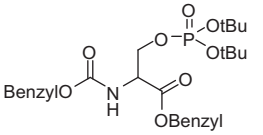
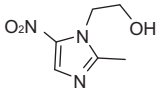
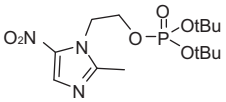
[a] For all entries, 1.0 equiv imidazole was used.
[b] HPLC area % after H_2O_2 oxidation.

Table 2
Activator effect with benzyl alcohol[a]

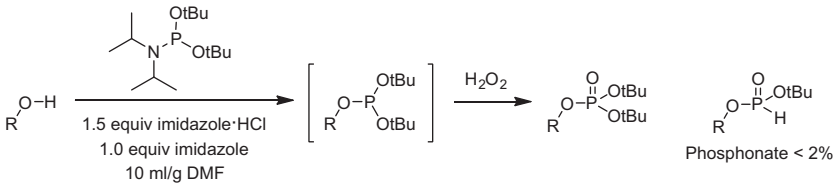
Entry	Equiv imidazole·HCl	Equiv 1*H*-tetrazole	Equiv imidazole	Results[b]		
				Benzyl Alcohol	Phosphonate	Phosphate
1	1.5		1.0	<1	<1	96.5
2		1.5		<1	1.6	87.0
3			2.5		No reaction	

[a] For both entries, 1.5 equiv of phosphoramidite was used.
[b] HPLC area % after H_2O_2 oxidation.

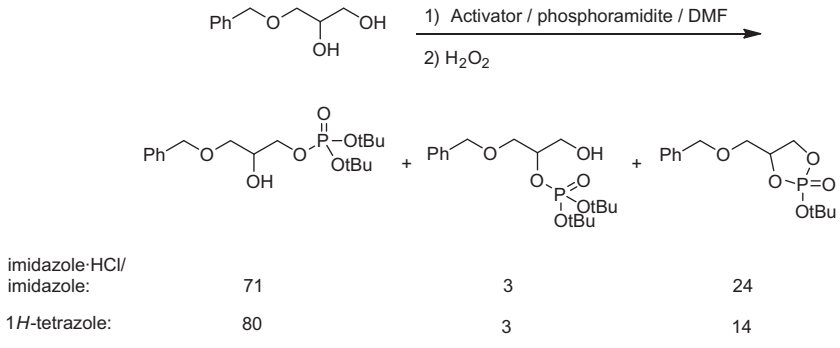
Table 3
Phosphorylation of a variety of alcohols[a]

Entry	Substrate	Product	Crude phosphonate[b]	Crude product[b]	Yield
1			2.8	95.7	82
2			<1	96.5	88
3			1.6	86.2	63
4			N/A[c]	N/A[c]	83
5			<1	96.2	73
6			2.4	98.0	82
7			Phosphate product too labile		
8			2.0	92.8	51
9			3.5	83.4	80
10			1.7	89.2	69

[a] For all entries, 1.5 equiv of phosphoramidite was used.
[b] HPLC area % after H_2O_2 oxidation.
[c] Starting material and product co-eluted by HPLC. Reaction conditions and isolation were performed as per the general procedure.



Scheme 5.



Scheme 6.

5. Phosphorylation of 1,2-diols

In the context of the phosphorylation of 1,2-diols with 1*H*-tetrazole, details from the literature on product distribution are limited.[12] The phosphorylation of diols with our imidazole·HCl/imidazole buffer system proved challenging and gave three main products. LC–MS of the reaction mixture suggested predominant phosphorylation of the primary alcohol over the secondary alcohol and the 1,2-cyclic phosphate as shown in Scheme 6.

Intrigued by this observation, we tested the phosphorylation with both the imidazole·HCl/imidazole system and 1*H*-tetrazole.[13] Indeed, modest selectivity was achieved with both systems. However, a slightly better selectivity was observed with 1*H*-tetrazole. This also demonstrates that the enhanced activation reactivity of the imidazole·HCl/imidazole buffer system led to decreased chemoselectivity compared to the well established 1*H*-tetrazole.

In conclusion, we discovered an improved activation system for the phosphorylation of alcohols using bis-*tert*-butyl phosphoramidites. The improved conditions using imidazole·HCl/imidazole as the activator gave less than 2% of phosphonate by-products in most cases. The methodology is applicable to a variety of alcohols with a few limitations. This improved the imidazole·HCl/imidazole activation method which is safer to use than the commonly used activator 1*H*-tetrazole,[13] a recently classified explosive.[9,10]Large quantities of imidazole·HCl and imidazole are both easy to obtain and inexpensive. The improved activation procedure will further expand the usefulness of the phosphorylation of alcohols using bis-*tert*-butyl phorphoramidites.

6. General procedure (using phenol as an example)

To a 50 mL round-bottom flask was charged phenol (1.00 g, 10.6 mmol), imidazole·HCl (1.67 g, 15.9 mmol) and imidazole (0.723 g, 10.6 mmol). The mixture was dissolved in DMF (10 mL) under magnetic stirring and nitrogen atmosphere. Once complete dissolution was achieved, *N*,*N*-diisopropyl bis-*tert*-butyl phosphoramidite (5.03 mL,15.9 mmol) was charged dropwise over 1–3 min. The reaction was stirred at room temperature until complete consumption of phenol was observed by HPLC (less than 1 h). The reaction mixture was cooled in an ice-water bath and treated with dropwise addition of 30 wt % hydrogen peroxide (3.01 mL, 26.6 mmol) and then warmed to room temperature. Once the oxidation reaction was judged complete by HPLC analysis (<2% phosphite, usually ~3 h), the reaction was cooled in an ice-water bath and carefully quenched with saturated aqueous sodium thiosulphate (10 mL).[14] The product was then partitioned between ethyl acetate (20 mL) and distilled water (20 mL) in a separatory funnel and the layers were separated. The top ethyl acetate layer was washed with brine (2 × 5 mL) and distilled water (5 mL). The ethyl acetate layer was concentrated on a rotary evaporator followed by purification on silica gel using heptane/ethyl acetate (3:1).[15] The pure fractions were concentrated on a rotary evaporator. Further drying under high vacuum to constant weight gave the phenyl bis-*tert*-butyl phosphate product as a colorless oil (2.51 g, 82% yield).

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11. The commercial alcohol was 47% pure by HPLC.
12. Literature precedents, mostly in patents often purify by prep-HPLC and no product distribution data are available.
13. Scifinder® search of the *N,N*-di-alkyl-bis-*tert*-butyl phosphoramidite as a reagent returned >100 references, all of the references were using 1*H*-tetrazole as the activator. Three exceptions, pyridine·HCl (WO 2001040236, no phosphonate discussion); 1,2,3-triazole (WO 2001074369 and WO 2001074368, no phosphonate discussion) and pyridine·HCl + pyridine (phosphonate discussion with vague product distribution details); pyridine·HCl giving phosphonate by-product (Barnwell, N.; Cheema, L; Cherryman, J.; Dubiez, J; Howells, G.; Wells, A. *Org. Process Res. Dev.* **2006**, *10*, 644); Pyridine·HCl and Pyridine·HCl/imidazole. (no phosphonate discussion, rather *O,N* selectivity): Kato, Y.; Oka, N.; Wada, T. *Tetrahedron Lett.* **2006**, *47*, 2501; Imidazolium Trifluoroacetate (no phosphonate discussion): Gomez, C.; Chen. J.; Wang, S. *Tetrahedron Lett.* **2009**, *50*, 6691–6692.
14. In some cases, less sodium thiosulphate is necessary to prevent decomposition of the crude phosphate product.
15. In some cases, multiple chromatographies were required to remove the *N,N*-diisopropyl bis-*tert*-butyl phosphoramidate: