Organocatalytic phosphorylation of alcohols using pyridine-*N*-oxide

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Abstract: Phosphorylation of alcohols by phosphoryl chlorides catalyzed by pyridine-*N*-oxide is reported. The utility of this method is demonstrated through phosphorylation of primary, secondary and a tertiary alcohol as well as phenols under mild reaction conditions and with low catalyst loading (5 mol%).

Key words: Phosphorylation, alcohols, pyridine-*N*-oxide, organocatalysis.

Phosphorylation of alcohol groups in *e.g.* peptides, proteins, inositols, glycerols and steroids plays a pivotal role in many physiologically important processes.¹ Disease states including cancers and immune system disorders are often characterized by perturbation of these processes.^{2,3} Phosphate derivatives are also commonly prepared in order to increase the aqueous solubility of alcohols *e.g.* in pro-drugs.^{4–6} The development of synthetic methods for the efficient, cost-effective and scalable phosphorylation of alcohol derivatives is therefore an important goal.

Alcohol phosphorylation (*i.e.* phosphate *mono*-ester formation) by direct acid catalyzed condensation with phosphoric acid is generally difficult to control for structurally diverse alcohols.⁷ Consequently, phosphorylation is more generally achieved either by phosphitylation then oxidation/deprotection [*i.e.* using a P(III) reagent *e.g.* a phosphoramidite] or by direct phosphorylation then deprotection [*i.e.* using a P(V) reagent *e.g.* dialkoxychlorophosphate].⁸ Phosphitylation reactions tend to be faster than phosphorylation reactions but require the substrate to display stability to the subsequent oxidation. Direct phosphorylation can be achieved with Brønsted/Lewis acid or nucleophilic catalysis. For example, Jones *et al.* have used Ti(O^{*i*}Bu)₄, TiCl₄ or Cu(OTf)₂ with phosphoryl chlorides^{9,10} and *N*-phosphoryl oxazolidinones,^{11–13} and more recently, Sculimbrene *et al.* have reported the use of Ti(O^{*i*}Bu)₄ with pyrophosphates.^{14,15} Pyridine,¹⁶ 4-DMAP¹⁷ and DABCO¹⁸ have been used as super-stoichiometric nucleophilic promoters of phosphorylation and Miller *et al.* have deployed *N*-methylimidazole (NMI)¹⁹ and 4-DMAP²⁰ sub-stoichiometrically with phosphoryl chlorides (Figure 1).

Figure 1. Catalytic methods for phosphorylation of alcohols.

Whilst the Lewis acid catalyzed methods have demonstrated good substrate scope and are moderate to high yielding, separation of the metal catalysts from the products can be problematic and costly *e.g.* in late stage pharmaceutical synthesis.^{21–24} The existing organonucleophile catalyzed processes do not appear to have been systematically investigated but our experience indicates that their scope/efficacy is inferior to the procedure described herein.

Pyridine-*N*-oxides have been used extensively as nucleophilic catalysts for the coupling of 3'-phosphorylated nucleotides with 5'-OH nucleotides in the synthesis of oligonucleotides^{25,26} and we have recently reported the use of 2-aryl-4-(dimethylamino)pyridine-*N*-oxides as catalysts for the chemoselective phosphorylation of α -amino acid derivatives, polyols and peptides.²⁷ During the course of these studies, it was noted that pyridine-*N*-oxide itself provided significant rate enhancement in alcohol phosphorylation reactions using phosphoryl chlorides. Herein, we report the use of pyridine-*N*-oxide as a competent nucleophilic catalyst for the phosphorylation of hydroxyl containing substrates using phosphoryl chlorides.

Our initial studies focused on evaluation of bases for the phosphorylation of 4-phenyl-1-butanol (**1**) by diphenyl phosphoryl chloride (DPPCl) using pyridine-*N*-oxide (**2**) as a catalyst (Table 1).

Table 1 Phosphorylation of 4-phenyl-1-butanol.

Entry	Catalyst	Base	Conv. (%) ^a
1	None	None	0
2	2	None	0
3	2	PPO	47
4	2	PS ®	>99 (98) ^b
5	2	NEt ₃	87
6	2	PMP	>99
7	None	PS ®	0

^aConversion to product **3** as determined by ¹H NMR of the crude reaction mixture. ^bIsolated yield after chromatographic purification. PPO = propylene oxide, PS® = 1,8-bis(NMe₂)-naphthalene (Proton Sponge®), PMP = 1,2,2,6,6-pentamethylpiperidine.

In the absence of base and/or catalyst no reaction was observed (entries 1, 2 and 7, Table 1). Whilst propylene oxide provided moderate levels of conversion (entry 3, Table 1), both Proton Sponge® and PMP provided quantitative conversion within 2 h (entries 4, 6, Table 1). With the aim of developing a general method for the economic and scalable phosphorylation of alcohols, Proton Sponge® was identified as optimal and these conditions were applied to a variety of alcohol substrates (Table 2).

Table 2 Pyridine-*N*-oxide catalyzed phosphorylation - substrate scope.

Entry	Product	Compound #	Time (h)	Yield (%) ^a
1		3b	2	98
2		3c	8	94
3		3d	8	~81 ^b
4		3e	24	57
5		3f	1	95 (17) ^c
6		3g	1	97 (21) ^c
7		3h	1	91 (14) ^c

^aIsolated yield after chromatographic purification. ^bProduct **3d** decomposes on silica gel (*cf.* ^{9,14}), impurities present, see Supporting Information. ^cConversion to product **3** as determined by ¹H NMR of the crude reaction mixture of the *uncatalyzed* reaction.

Benzyl alcohol was phosphorylated in good yield within 2 h (entry 1, Table 2). Secondary alcohols and *tert*-BuOH were also phosphorylated with moderate to good yields, albeit with increased reactions times (entries 2-4, Table 1). Other tertiary alcohols (*e.g.* α -terpineol and 2-phenyl-2-propanol) were unreactive to these conditions. Phenols were converted to phosphorylated products in high yields and short reaction times (1 h), irrespective of the electronics of other aryl substituents (entries 5-8, Table 2). Although these phenols are also phosphorylated in the absence of catalyst, rates are significantly increased in the presence of the pyridine-*N*-oxide catalyst.

Having established reaction conditions that appeared general for the phosphorylation of a range of hydroxyl containing substrates, including the preparation of phosphoryl derivatives known to be highly sensitive to adventitious nucleophiles (*e.g.* compounds **3b** and **3d**), phosphorylation of a selection of biologically relevant molecules and phosphate ester pro-drugs was evaluated (Table 3).

Table 3 Phosphorylation of biologically relevant molecules and phosphate pro-drugs.

Entry	Product	Compound #	Time (h)	Yield (%) ^a
1		4	2	85
2		5a R = Ph	1	74 (27) ^b
		5b R,R = <i>o</i> -XP	1	71 (15) ^b
3		6	2	83
4		7	2	61
5		8	2	77 (74) ^c
6		9	8	54
7		10a R = Ph	1	91 (59) ^b
		10b R,R = <i>o</i> -XP	1	93 (21) ^b

^aIsolated yield after chromatographic purification. ^bConversion to product as determined by ¹H NMR of the crude reaction mixture of the

uncatalyzed reaction. °Yield of reaction conducted on 20 mmol scale with respect to alcohol.

The allylic alcohol geraniol was phosphorylated in good yield within 2 h (entry 1, Table 3). Steroid derivatives containing phenolic and primary alcohols were also phosphorylated in good yield and with chemoselectivity over secondary and tertiary hydroxyl functionalities (entries 2 and 3, Table 3). Phosphorylation of the primary 5'-OH group of adenosine in the presence of the 2' and 3'-OH groups was also achieved (entry 4, Table 3); phosphorylation of the 2' and 3'-OH groups was not detected (by ^{31}P NMR). Phosphorylation of protected amino-acid residues Ser, Thr and Tyr was also achieved in moderate to good yields (entries 5-7, Table 3). Phosphorylation of Boc-Ser(OH)-OMe was also conducted on a 20 mmol (5 g) scale with comparable yield in the same timescale (entry 5, Table 3).

For the phosphorylation of the phenolic substrates,²⁸ *o*-xylenyl phosphoryl chloride (*o*-XPCl) was also employed in place of DPPCl (entries 2 and 7, Table 3).²⁹ This allows for selective deprotection by hydrogenolysis ($\text{H}_2/\text{Pd-C}$)^{27,30} or with acid (*e.g.* HBr/AcOH),³¹ which cannot be achieved for phenyl phosphate esters (or for products containing other phenyl moieties).³¹⁻³³ Thus, deprotection of phosphates **5b** and **8** by hydrogenolysis yielded the corresponding phosphates quantitatively (Scheme 1).

Scheme 1. Deprotection of phosphate esters **5b** and **8**.

To probe the mechanism of catalysis operating in the phosphorylation reactions, a 1:1 mixture of pyridine-*N*-oxide and DPPCl was analysed by NMR in CDCl_3 . ^1H and ^{31}P NMR spectra of this mixture revealed complete conversion of both starting materials to a new species in which the pyridyl C2/C6 protons had shifted by $\Delta\delta_{\text{H}} = +0.28$ ppm and the phosphorus signal by $\Delta\delta_{\text{P}} = -20.32$ ppm, suggesting the formation of an *O*-phosphorylated salt as expected during nucleophilic catalysis (Figure 2).

Figure 2 The aromatic regions of ^1H NMR spectra of pyridine-*N*-oxide (upper) and the proposed intermediate salt (lower). The peaks at $\delta_{\text{H}} \sim 8.3$ ppm and ~ 8.6 ppm are the C2/C6 protons.

Subsequent treatment of this salt with an alcohol results in rapid conversion to the phosphate *mono*-ester product. Our attempts to obtain crystals of the intermediate salt, suitable for single crystal X-ray structure determination, have however so far been unsuccessful.

In conclusion, we have demonstrated the use of pyridine-*N*-oxide as an effective and economical organocatalyst for the phosphorylation of primary and secondary alcohols and phenols, including several biologically relevant molecules. The reaction times and yields are comparable to those described for Lewis acid catalyzed conditions and superior to those of previously described simple nucleophilic organocatalysts (*e.g.* 4-DMAP and NMI). This method therefore provides a complimentary approach to catalytic phosphoryl transfer that obviates the need for metal catalysts and which may therefore be of particular utility in the late-stage production of phosphate esters for biological applications, *e.g.* in the preparation of pro-drugs. For difficult alcohol phosphorylation cases, *e.g.* the site selective phosphorylation of polyols, the use of a 2-aryl-4-DMAP-*N*-oxide catalyst will likely be warranted,²⁷ but the commercial availability and low cost of pyridine-*N*-oxide makes it an attractive first choice catalyst for evaluation.

Supporting Information for this article is available online at <http://www.thieme-connect.com/products/ejournals/journal/10.1055/s-00000083>.

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28. The *o*-XP group can also be introduced onto non-phenolic alcohols (see ref. 27), where its ease of removal can also be advantageous for certain applications.
29. *General Method*: To a solution of alcohol component (0.9 mmol), pyridine-*N*-oxide (0.045 mmol, 5 mol%) and Proton Sponge® (1.8 mmol) in CH₂Cl₂ (0.2 M) was added the specified phosphoryl chloride (1.0 mmol). The reaction mixture was stirred at room temperature for the time specified before the addition of MeOH (2 mL). The reaction mixture was then conc. *in vacuo* and crude material purified by flash chromatography to afford the product. e.g. 17- β -3-Xylenylphosphoryl estradiol (**5b**) - According to the *General Method*, 17- β -estradiol (246 mg, 0.9 mmol), pyridine-*N*-oxide (5 mg, 5 mol%), Proton Sponge® (392 mg, 2.0 mmol) and xylenyl phosphoryl chloride (217 mg, 1.0 mmol) followed by flash chromatography (eluent: 0–50% EtOAc/hexanes) afforded xylenylphosphate **5b** as a white solid (290 mg, 0.64 mmol, 71%). M.p. 146–147 °C. IR (ν_{max} /cm⁻¹): 3314, 3033, 3001, 2936, 1707, 1612, 1536, 1286, 1211, 1097, 875, 736, 467. ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.43–7.41 (m, 2H), 7.36–7.34 (m, 2H), 7.30–7.28 (m, 1H), 7.08–7.05 (m, 1H), 5.44–5.38 (m, 2H), 5.28–5.19 (m, 2H), 3.76 (t, *J* = 8.5 Hz, 1H), 2.88 (m, 2H), 2.39–2.31 (m, 1H), 2.26–2.11 (m, 2H), 1.99 (dt, *J* = 12.5, 3.3 Hz, 1H), 1.94–1.89 (m, 1H), 1.77–1.69 (m, 1H), 1.55–1.18 (m, 9H), 0.81 (s, 3H). ¹³C NMR (100 MHz, CDCl₃, ppm): δ 138.8, 135.2, 129.4, 129.1, 126.8, 119.6, 119.5, 116.8 114.2, 81.9, 68.9, 50.1, 44.1, 43.2, 38.5, 36.7, 30.6, 29.6, 27.0, 26.2, 23.1, 11.1. ³¹P NMR (161 MHz, CDCl₃, ppm): δ -6.94. HRMS (*m/z* +ES): Found: 455.1988 (M+H⁺ C₂₆H₂₈O₅P Requires: 455.1987). [α]_D¹⁹ - 87 (c 0.99, CHCl₃). (S)-Methyl 2-((*tert*-butoxycarbonyl)amino)-3-((diphenoxyphosphoryl)oxy)propanoate (**8**) - According to the *General Method*, Cbz-Ser(OH)-OMe (200 mg, 0.9 mmol), pyridine-*N*-oxide (5 mg, 5 mol%), Proton Sponge® (392 mg, 2.0 mmol) and diphenyl phosphoryl chloride (0.21 mL, 1.0 mmol) followed by flash chromatography (eluent: 0–10% EtOAc/hexanes) afforded diphenylphosphate **8** as a white solid (311 mg, 0.69 mmol, 77%). M.p. 61–63 °C. IR (ν_{max} /cm⁻¹): 3313, 3067, 3039, 2957, 1721, 1590, 1514, 1488, 1456, 1288, 1185, 1163, 948, 754, 688. ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.40–7.20 (m, 10H), 5.40 (d, *J* = 7.9 Hz, 1H), 4.67–4.65 (m, 1H), 4.60–4.51 (m, 1H), 3.71 (s, 3H), 1.46 (s, 9H). ¹³C NMR (100 MHz, CDCl₃, ppm): δ 169.4, 155.1, 150.4, 129.9, 129.8, 125.6, 120.1, 120.0, 80.4, 68.8, 53.8, 52.8, 28.3. ³¹P NMR (161 MHz, CDCl₃, ppm): δ -12.34. HRMS (*m/z* +ES): Found: 474.1300 (M+Na C₂₁H₂₆NO₈PNa Requires: 474.1294). [α]_D¹⁹ + 161 (c 0.96, CHCl₃).
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**Pyridine-*N*-oxide
phosphorylation****catalyzed****alcohol**