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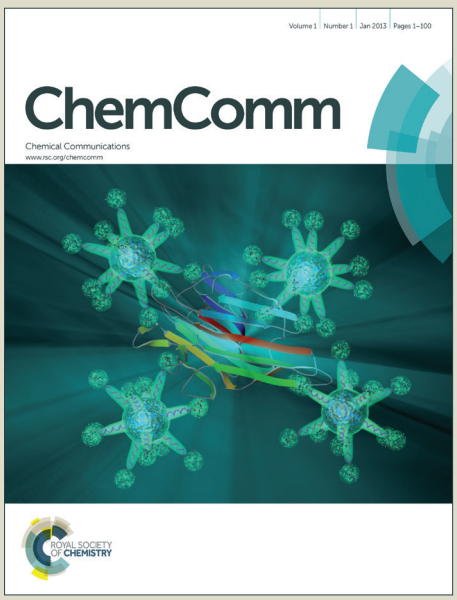

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Highly efficient and selective phosphorylation of amino acid derivatives and polyols catalysed by 2-aryl-4-(dimethylamino)pyridine-*N*-oxides – towards kinase-like reactivity

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The chemoselective phosphorylation of hydroxyl containing amino acid derivatives and polyols by phosphoryl chlorides catalyzed by 2-aryl-4-(dimethylamino)pyridine-*N*-oxides is described.

Signal transduction cascades in living systems are often controlled *via* the post-translational phosphorylation/dephosphorylation of proteins and other secondary metabolites.[1] *In vivo*, phosphorylation is catalyzed by protein kinases, which selectively phosphorylate serine (Ser), threonine (Thr) and tyrosine (Tyr) residues and hence play an important role in many disease states, including cancer and immune system disorders.[2] Despite significant interest in drugs which act on protein kinases,[3–7] there are currently no catalytic synthetic methods for the site-selective phosphorylation of hydroxyl-containing amino acid residues.[2,8]

Miller *et al*. have reported the use of *N*-methyl histidine-containing pentapeptide catalysts for asymmetric phosphorylation of inositol derivatives by phosphoryl chlorides,[9–17] *e.g*. in the synthesis of D-*myo*-inositol-1/3-phosphate[18] and for site-selective phosphorylation of teicoplanin.[19] They have also reported some analogous processes *via* P(III) phosphoramidite transfer catalysed by tetrazole-containing peptides followed by oxidation.[20,21]

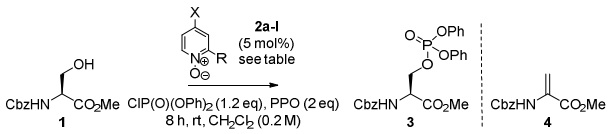
$Ti(O^tBu)_4$ and $Cu(OTf_2)$ catalyzed phosphorylation of alcohols by both phosphoryl chlorides[22] and *N*-phosphoryl oxazolidinones,[23] has been reported by Jones *et al*., and Sculimbrene *et al.* have used $Ti(O^tBu)_4$ with benzyl pyrrophosphate to phosphorylate Ser and Tyr derivatives.[24] Whilst these methods are moderate to high yielding, they require the use of metals and long reaction times and have not been used for site-selective phosphorylation of polyols.

Pyridine-*N*-oxides promote coupling of 3'-phosphorylated nucleotides with 5'-OH nucleosides for the preparation of oligonucleotides.[25,26] The *N*-oxides perform a dual catalytic role: mediating activation of the 3'-phosphate by an aryl sulfonyl chloride 'condensing agent' and then mediating substitution of this intermediate by the 5'-OH group. Efimov *et al.*[25,26] have demonstrated that pyridine-*N*-oxides with electron-donating 4-substituents provide significant rate enhancements in these reactions, with 4-dimethylaminopyridine-*N*-oxide (4-DMAP-*N*-oxide, catalyst **2d**, below) proving optimal.

We envisioned that pyridine-*N*-oxides would also act as efficient nucleophilic catalysts for the phosphorylation of OH-containing substrates by phosphoryl chlorides and that by positioning a sterically/electronically tuneable aryl group at the 2-position it might be possible to modulate the reactivity and selectivity of these catalysts. It was also our expectation that the low basicity of pyridine-*N*-oxides relative to *N*-based nucleophilic catalysts (*e.g*. pyridine, 4-DMAP, imidazoles, amidines *etc*.) would allow for the phosphorylation of alcohol substrates which are labile towards subsequent base catalysed elimination (*e.g*. Ser → dehydro-Ala).

Phosphorylation of Ser derivative **1** by diphenylphosphoryl chloride catalysed by simple pyridine-*N*-oxides **2a-e** and by 2-aryl pyridine-*N*-oxides **2f-l** was examined using propylene oxide (PPO) as a non-basic proton scavenger (Table 1).

Table 1. Phosphorylation of Ser 1: Catalyst evaluation



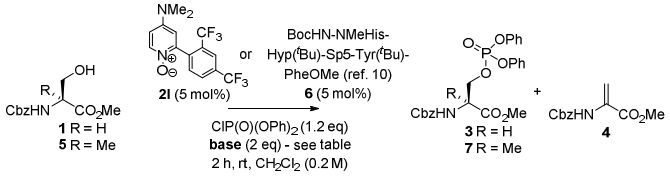
Entry	Catalyst	R	X	Conv.(%)[a]
1	None	-	-	0
2	**2a**	H	H	82 (79)[b]
3	**2b**	H	Cl	50
4	**2c**	Cl	H	0
5	**2d**	H	NMe_2	72
6	**2e**	Cl	NMe_2	78
7	**2f**	4-$MeOC_6H_4$	H	0
8	**2g**	4-$MeOC_6H_4$	NMe_2	27
9	**2h**	4-MeC_6H_4	H	47
10	**2i**	4-MeC_6H_4	NMe_2	28
11	**2j**	3,5-$(CF_3)_2C_6H_3$	H	11
12	**2k**	3,5-$(CF_3)_2C_6H_3$	NMe_2	86 (81)[b]
13	**2l**	2,4-$(CF_3)_2C_6H_3$	NMe_2	97 (95)[b]

[a]Conversion to product **3** as determined by ^{1}H NMR of crude reaction mixture. [b]Isolated yield after chromatographic purification. PPO = propylene oxide.

Interestingly, pyridine-*N*-oxide (**2a**) was a superior catalyst both to 4-DMAP-*N*-oxide (**2d**)[†] and the other non-arylated catalysts tested (**2b, 2c** and **2e**). By contrast, 2-aryl-4-DMAP-*N*-oxides (*e.g.* **2g** and **2k**) tended to be more active than their 2-aryl pyridine-*N*-oxide analogues (*e.g.* **2f** and **2j**) and moreover, although electronically neutral/rich 2-aryl substituents attenuated catalytic activity (*e.g.* **2g** and **2i**), electron-deficient 2-aryl substituents enhanced catalytic activity (*e.g.* **2k** and **2l**) relative to 4-DMAP-*N*-oxide itself and to a level higher than that of pyridine-*N*-oxide (**2a**). In particular, 2-[2,4-bis(trifluoromethyl)phenyl]-substituted 4-DMAP-*N*-oxide (**2l**, 5 mol%) was a very efficient catalyst giving 97% conversion to phosphorylated product **3** within 8 h (entry 13). We propose that the high catalytic activity of this derivative may result from synergistic enhancement of the nucleophilicity of the *N*-oxide oxygen by the 4-amino substituent and the enhancement of the nucleofugacity of the *N*-oxide by the 2-aryl substituent. These effects would be expected to lower the kinetic barriers both for initial nucleophilic addition of the *N*-oxide to the phosphoryl chloride and for subsequent substitution at phosphorus by the alcohol to regenerate the *N*-oxide, respectively during the catalytic cycle.[8]

Using optimal catalyst **2l**, a selection of bases were evaluated in place of the PPO for the transformation **1** → **3**, but with the reaction duration restricted to just 2 h (*cf.* 8 h in Table 1) to explore whether an increased rate of phosphorylation could be achieved without promoting subsequent elimination. Analogous reactions with α-MeSer **5** were also performed in order to reveal the rates of phosphorylation in the absence of the possibility of elimination (Table 2).

Table 2. Phosphorylation of Ser 1 (& α-MeSer 5): influence of base



Entry	Substrate	Cat.	Base/H$^+$ scavenger	Yield of **3** (or **7**) /%[a]	Yield of **4** /%[a]
1	**1** (**5**)	**2l**	None	0 (0)	0 (0)
2[b]	**1** (**5**)	**2l**	PPO	53[c] (60)	0 (0)
3	**1** (**5**)	**6**	PPO	18 (16)	0 (0)
4	**1** (**5**)	**2l**	PS©	86[c] (87)	0 (0)
5	**1** (**5**)	**2l**	PMP	98[c] (95)	0 (0)
6	**1** (**5**)	**2l**	NEt_3	46 (96)	54 (0)
7	**1** (**5**)	**6**	NEt_3	14 (55)	72 (0)
8	**1** (**5**)	**2l**	DBU	9 (92)	83 (0)

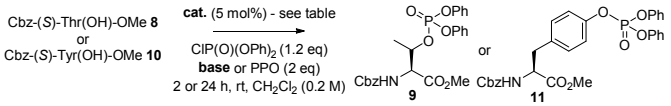
[a]Isolated yield after chromatographic purification. [b]Analogous catalysis (using **1**) was also successful in DMF, MeCN and DMSO, see ESI. [c]>98% ee by CSP-HPLC, see ESI. PS© = 1,8-bis(NMe_2)-naphthalene, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, PMP = 1,2,2,6,6-pentamethylpiperidine, Hyp = (2*S*,4*R*)-4-hydroxyproline, Sp5 = 1-amino-1-cyclopentane-carboxylic acid.

Proton Sponge (PS©) and particularly PMP proved to be more effective than PPO in promoting rapid phosphorylation of Ser derivative **1** without detectable elimination or racemisation (entries 4 & 5 *cf.* entry 2). By contrast, NEt_3 and particularly DBU promoted subsequent elimination to dehydro-Ala **4**, although in the absence of the possibility of elimination these bases provide comparable phosphorylation rates to PS© and PMP as evidenced by their efficient promotion of the phosphorylation of α-MeSer **5** (entries 6 & 8). There does not appear to be a straightforward correlation between the pK_a's of the conjugate acids of the *tert*-amine auxiliary bases evaluated here and their effectiveness as co-promoters in these reactions. This probably reflects the role of the amine in aiding proton transfer as well as scavenging HCl and promoting elimination.

Miller's *N*-methyl imidazole catalyst **6**[10] provided low levels of conversion relative to pyridine-*N*-oxide **2l** for phosphorylation of both Ser **1** (with PPO) and α-MeSer **5** (with PPO and NEt_3) as well as promoting more extensive elimination of Ser **1** to dehydroalanine **4** (with NEt_3) (entries 3 & 7). The low reactivity of catalyst **6** when combined with PPO probably reflects protonation of the *N*-methyl imidazole under these conditions (pK_a ~7, *cf.* pyridine-*N*-oxide pKa ~1) but with Et_3N, where imidazole protonation should not be significant, the intrinsic reactivity is lower than that of catalyst **2l** (55% *vs.* 96% conversion after 2 h, entries 7 & 6).

Phosphorylations of Thr **8** and Tyr **10** were investigated next with the aim of identifying catalyst/base combinations for selective phosphorylation of these *sec*-alcohol and phenol-containing substrates respectively (Table 3).

Table 3. Phosphorylation of Thr 8 & Tyr 10: Influence of catalyst and base



Entry	Substrate	Cat.	Time (h)	Base/H$^+$ scavenger	Conv. (%)[a]
1	**8**	**2a**	24	PPO	47
2	**8**	**2d**	24	PPO	17
3	**8**	**2k**	24	PPO	38
4	**8**	**2l**	24	PPO	54
5	**8**	**2l**	24	PMP	9
6	**8**	**2l**	24	PS©	>99 (96[b])
7	**10**	**2l**	2	PPO	0 (0[c])
8	**10**	**2l**	2	PMP	>99[d] (79[c])
9	**10**	**2l**	2	PS©	59 (21[c])
10	**10**	**2l**	2	NEt_3	>99[d] (95[b])

[a]Conversion to products **9** or **11** as determined by ^{1}H NMR of crude reaction mixture. [b]Isolated yield after chromatographic purification. [c]Conversion to products **9** or **11** of *uncatalysed* reaction. [d]Reaction complete within 1 h.

Despite the greater steric demand of *sec*-alcohol **8** relative to primary alcohol **1**, hindered *N*-oxide **2l** again proved to be the optimal catalyst in terms of rate when using PPO as proton scavenger (entries 1-4). Interestingly, PMP retarded this reaction (entry 5) whereas PS© provided a significant rate increase, allowing essentially quantitative phosphorylation within 24 h (entry 6). For phenol **10**, attempted phosphorylation with catalyst **2l** and PPO resulted in no reaction (entry 7), but both PMP and NEt_3 allowed essentially quantitative phosphorylation within 1 h (entries 8 and 10).

As the distinctive reactivity profiles displayed by alcohols **1**, **8** and **10** augured well for achieving site-selective phosphorylation reactions, triol **12**[27] was subjected to reaction conditions expected to show selectivity for phosphorylation of

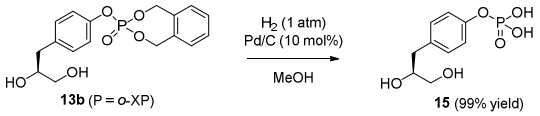
the primary and phenolic OH groups (Table 4). *o*-Xylenyl phosphoryl chloride (*o*-XPCl, **14**)[‡] was employed for the phenol selective reaction as selective deprotection can be accomplished either by hydrogenolysis[§] (H_2/Pd-C)[31] or with acid (*e.g.* HBr/AcOH).[30]

Pleasingly, high levels of selectivity for *mono*-phosphorylation of the primary OH group (**12** → **13a**) were achieved by using PPO with comparable yields for diphenyl phosphoryl chloride and *o*-XPCl over an 8 h reaction duration (94% and 87% yields respectively, entry 1). Good levels of selectivity for *mono*-phosphorylation of the phenol group (**12** → **13b**) were achieved by using PMP over a 1 h reaction duration (81% yield, entry 2), although some of the primary alcohol *mono*-phosphate **13a** was also isolated under these conditions (11% yield). Diphosphorylation of the primary and phenolic OH groups (**12** → **13c**) was achieved through use of PMP (2.2 eq.) over a 4 h reaction duration (86% yield, entry 3). No conditions were identified that could selectively *mono*-phosphorylate the *sec*-alcohol, but 'global' phosphorylation of all OH groups (**12** → **13d**) was achieved through the use of PS© (3.2 eq.) over a 24 h reaction duration (96% yield, entry 4).

The *mono*-phosphorylated products **13a** [P = $P(O)(OPh)_2$ and *o*-XP] and **13b** [P = $P(O)(OPh)_2$] were also individually resubjected to the reaction conditions with PPO, PMP and PS© for 24 h in the *absence* of phosphorylating agent; no changes could be detected by ^{31}P NMR in any of these control reactions, confirming that phosphoryl migration does not occur under these conditions.

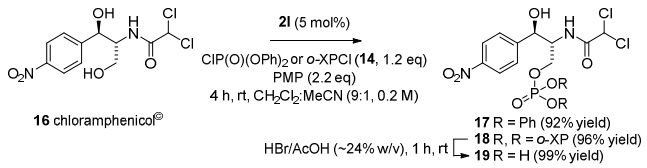
Deprotection of *o*-xylenyl phosphate **13b** was achieved by hydrogenolysis (H_2/Pd-C) in MeOH to give free phosphate **15** in almost quantitative yield (Scheme 1).

Scheme 1. Deprotection of xylenyl phosphate 13b



To further exemplify the utility of this organocatalytic site-selective phosphorylation/deprotection sequence, selective *mono*-phosphorylation of the primary alcohol in the antimicrobial agent chloramphenicol© (**16**) was investigated (Scheme 2).

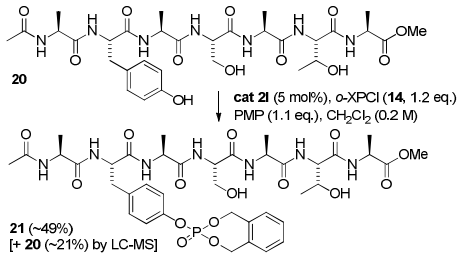
Scheme 2. Selective phosphorylation of chloramphenicol© (16)



Using catalyst **2l** and either diphenyl phosphoryl chloride or *o*-XPCl (**14**) in conjunction with PMP, selective *mono*-phosphorylation of the primary alcohol group could be achieved to give products **17** and **18** in 91% and 96% yields respectively. Control reactions using catalysts **2a** and **2d** provided phosphorylated derivative **17** in yields of just 57% and 45% respectively, underscoring the superiority of catalyst **2l**. Deprotection of the *o*-xylenyl phosphate **18**, in the presence of the labile *gem*-dichloro and nitro functions, using HBr in AcOH/MeOH gave free phosphate **19** quantitatively.

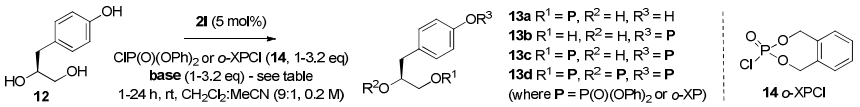
Finally, we sought to demonstrate the potential of 2-aryl-4-DMAP-*N*-oxide **2l** as a synthetic Tyr kinase mimetic. Tyr kinases constitute the largest class of mammalian kinases[32] and are involved in control of cellular processes such as cell metabolism, growth, mobility, survival, proliferation and differentiation.[33] The phosphorylation of Tyr selectively in the presence of Ser and Thr residues in peptides/proteins therefore represents an appealing challenge. We used the all-(*S*) configured synthetic peptide Ac-Ala-Tyr-Ala-Ser-Ala-Thr-Ala-OMe (**20**) as a model substrate (Scheme 3).

Scheme 3. Tyr kinase–like selectivity in the *mono*-phosphorylation of heptapeptide 20 using catalyst 2l



Following treatment of peptide **20** with *o*-XPCl (**14**), PMP and catalyst **21** in CH_2Cl_2 for 24 h, LC-MS analysis revealed ~49% conversion to product **21** (structure confirmed by MS^2) along with ~21% recovered substrate; other minor peaks (<10% each) also eluted but these could not be identified and did not appear to correspond to phosphorylation at the Ser or Thr positions or elimination products thereof (see ESI).

Table 4. Site-selective phosphorylation of triol 12



Entry	Base/H^+ scavenger (eq.)	Reaction Time (h)	Phosphorylating agent (eq.)	Product Yield (%)[a]			
				13a	**13b**	**13c**	**13d**
1	PPO (2.0)	8	$ClP(O)(OPh)_2$ (1.2)	94 (87)[b,c]	<5	-	-
2	PMP (1.0)	1	XPCl (1.0)	11	81	-	-
3	PMP (2.2)	4	$ClP(O)(OPh)_2$ (2.2)	<5	<5	86	-
4	PS© (3.2)	24	$ClP(O)(OPh)_2$ (3.2)	-	-	<5	96

[a]Isolated product yield after chromatographic purification. [b]Isolated product yield of analogous reaction using XPCl (**14**, 1.2 eq.). [c]Reaction conducted in CH_2Cl_2:DMF (9:1, 0.2 M) due to low solubility of reagents. '-' Indicates that this product was not detected by TLC analysis.

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In summary, 2-aryl-4-DMAP-*N*-oxides such as **2l** are highly efficient organocatalysts for the site-selective phosphorylation of alcohols. Their use is particularly advantageous for substrates prone to elimination and when selectivity for primary (over *sec* and phenolic) or for phenolic (over primary and *sec*) OH groups in polyols is required. Preliminary studies have also demonstrated that catalyst **2l** displays Tyr kinase-like selectivity for the *mono*-phosphorylation of a heptapeptide containing Tyr, Ser and Thr residues. These catalysts may therefore hold promise for the preparation of phospho-peptides for biomedical applications although clearly achieving selectivity in the presence of alternative nucleophiles and more polar solvents will prove challenging; further studies towards this end are ongoing in our laboratory.

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Notes and references

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† By contrast, Efimov[25,26] found that 4-DMAP-*N*-oxide (**2d**) outperformed pyridine-N-oxide (**2a**) for oligonucleotide coupling, presumably due to competing catalyst demands in the dual catalytic cycle.

‡ *o*-XPCl has been described for phosphorylation previously[31] but its use synthetically has not been pursued.

§ H_2/PtO_2 as used for Ph phosphate deprotection reduce phenols.[28–30]

Electronic Supplementary Information (ESI) available: Experimental procedures and compound characterization data for all new compounds. See DOI: 10.1039/b000000x/

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