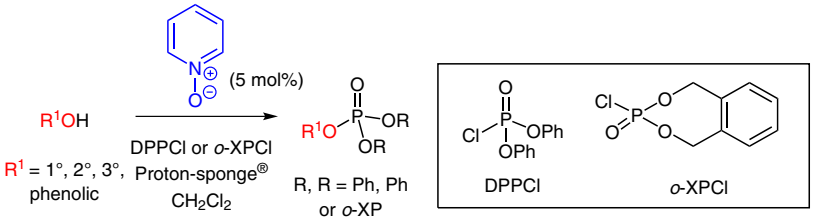
Synlett J. I. Murray et al. Letter

Organocatalytic Phosphorylation of Alcohols Using Pyridine-*N*-oxide

James I. Murray[a]
Rudiger Woscholski[a,b]
Alan C. Spivey*[a]

[a] Department of Chemistry, South Kensington Campus, Imperial College London, SW7 2AZ, UK
[b] Institute of Chemical Biology, Imperial College London, London, SW7 2AZ, UK
a.c.spivey@imperial.ac.uk



Received: 08.12.2014
Accepted after the revision: 06.01.2015
Published online: 10.02.2015
DOI: 10.1055/s-0034-1379993; Art ID: st-2014-d1015-l

Abstract Phosphorylation of alcohols by phosphoryl chlorides catalysed 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

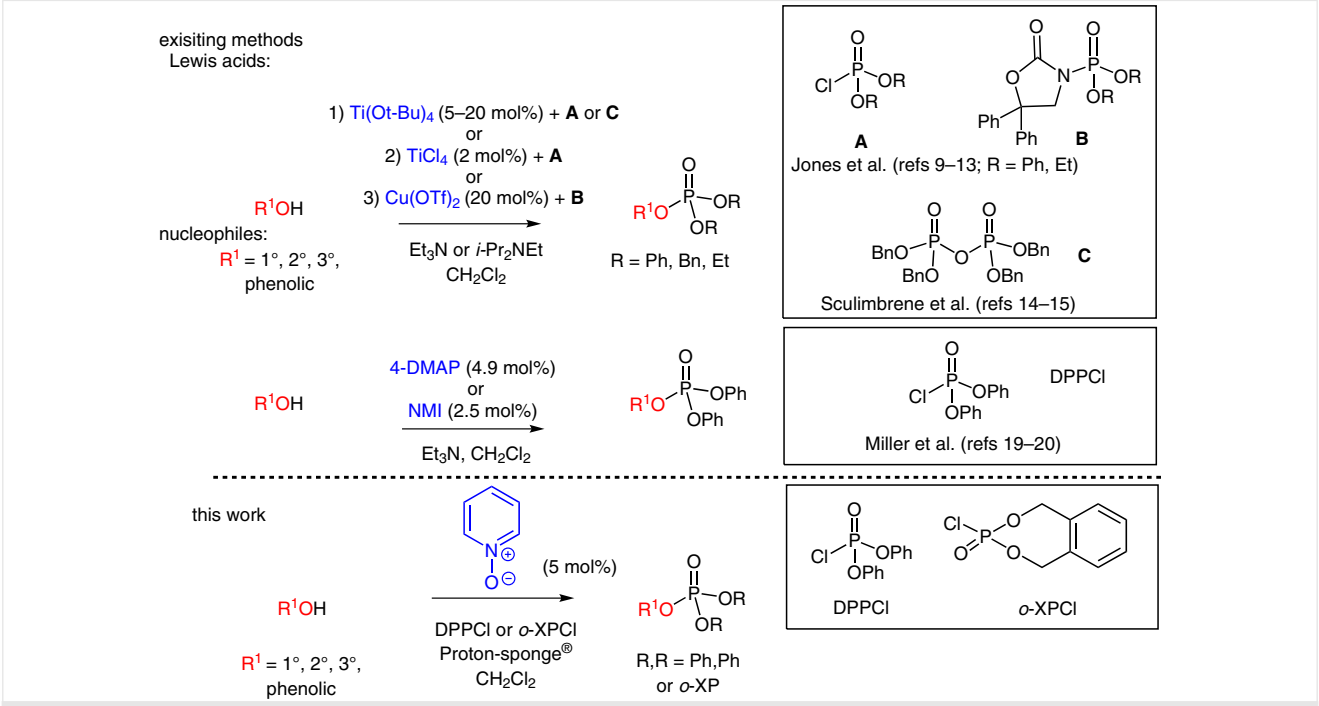
Phosphorylation of alcohol groups in e.g. peptides, proteins, inositols, glycerols and steroids plays a pivotal role in many physiologically important processes.[1] Disease states including cancers and immune system disorders are often characterised by perturbation of these processes.[2,3] Phosphate derivatives are also commonly prepared in order to increase the aqueous solubility of alcohols e.g. in prodrugs.[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 monoester formation) by direct acid-catalysed 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 such as a phosphoramidite] or by direct phosphorylation then deprotection [i.e., using a P(V) reagent such as a 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*t*-Bu)$_4$, TiCl$_4$ 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*t*-Bu)$_4$ with pyrophosphates.[14,15] Pyridine,[16] 4-DMAP[17] and DABCO[18] have been used as super-stoichiometric nucleophilic promotors of phosphorylation and Miller et al. have deployed *N*-methylimidazole (NMI)[19] and 4-DMAP[20] substoichiometrically with phosphoryl chlorides (Scheme 1).

Whilst the Lewis acid catalysed 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-catalysed processes do not appear to have been systematically investigated but our experience indicates that their scope/efficacy is inferior to the procedure described herein.

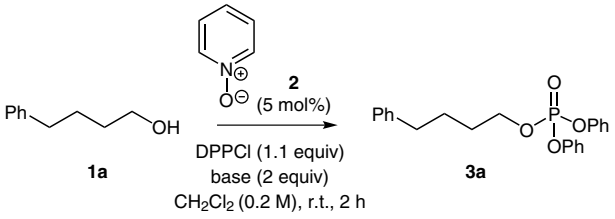
Pyridine-*N*-oxides have been used extensively as nucleophilic catalysts for the coupling of 3′-phosphorylated nucleotides with 5′-OH nucleotides in the synthesis of oligonucelotides[25,26] and we have recently reported the use of 2-aryl-4-(dimethylamino)pyridine-*N*-oxides as catalysts for the chemoselective 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).



Scheme 1 Catalytic methods for phosphorylation of alcohols

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**	Et_3N	87
6	**2**	PMP	>99
7	none	PS®	0

[a] Conversion to product **3** as determined by ^1H NMR of the crude reaction mixture.
[b] Isolated yield after chromatographic purification. PPO = propylene oxide, PS® = 1,8-bis(NMe_2)-naphthalene (Proton-sponge®), PMP = 1,2,2,6,6-pentamethylpiperidine.

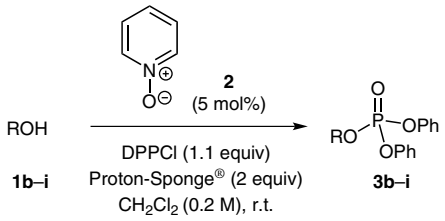
In the absence of base and/or catalyst no reaction was observed (entries 1, 2 and 7, Table 1). Whilst propylene oxide provided moderate levels of conversion (entry 3, Table 1), both Proton-sponge® and PMP provided quantitative conversion within two hours (entries 4 and 6, Table 1). With the aim of developing 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).

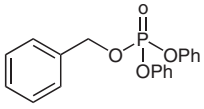
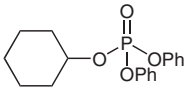
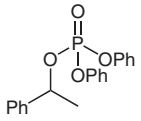
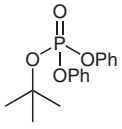
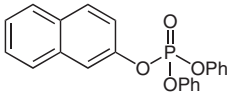
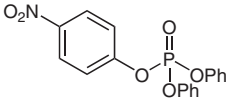
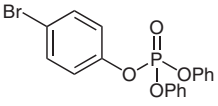
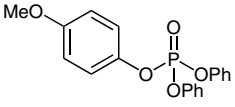
Benzyl alcohol was phosphorylated in good yield within two hours (entry 1, Table 2). Secondary alcohols and *tert*-butyl alcohol were also phosphorylated with moderate to good yields, albeit with increased reactions times (entries 2–4, Table 1). Other tertiary alcohols (e.g., α-terpineol and 2-phenyl-2-propanol) 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 hydroxy-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 prodrugs was evaluated (Table 3).

The allylic alcohol geraniol was phosphorylated in good yield within two hours (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 (en-

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	ca. 81[b]
4		**3e**	24	57
5		**3f**	1	95 (17)[c]
6		**3g**	1	97 (21)[c]
7		**3h**	1	91 (14)[c]
8		**3i**	1	82 (7)[c]

[a] Isolated yield after chromatographic purification.
[b] Product **3d** decomposes on silica gel (see refs. 9 and 14), impurities present, see Supporting Information.
[c] Conversion to product **3** as determined by ^{1}H NMR of the crude reaction mixture of the uncatalyzed reaction.

tries 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 (H_2, 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 hydrogenolyis yielded the corresponding phosphates quantitatively (Scheme 2).

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 $CDCl_3$. The ^{1}H NMR 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 $\Delta\delta_H$ = +0.28 ppm and the phosphorus signal by $\Delta\delta_P$ = –20.32 ppm, suggesting the formation of an O-phosphorylated salt as expected during nucleophilic catalysis (Figure 1).

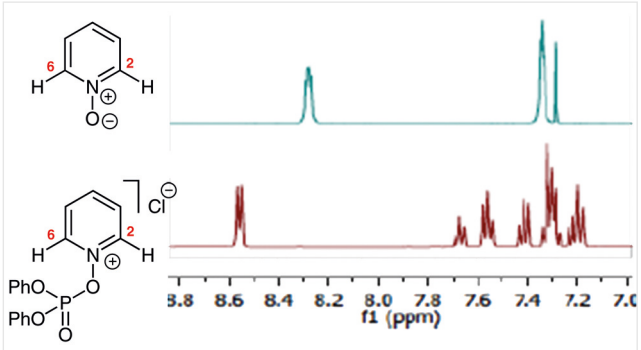
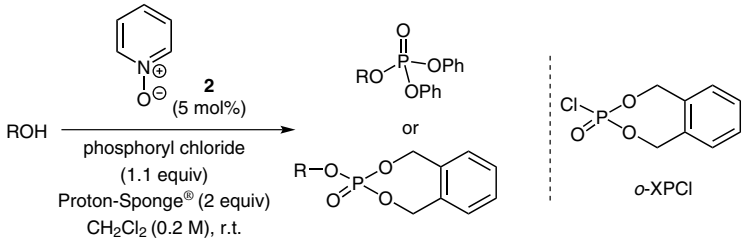


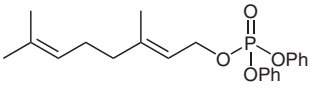
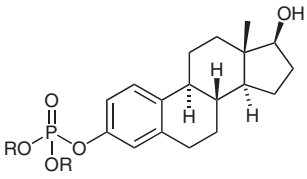
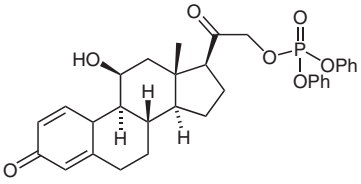
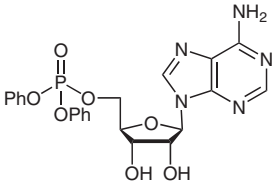
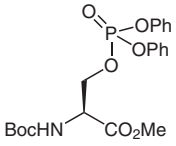
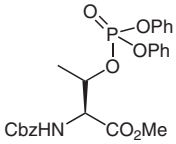
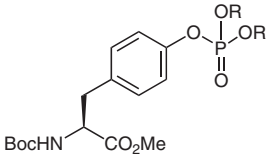
Figure 1 The aromatic regions of ^{1}H NMR spectra of pyridine-*N*-oxide (upper) and the proposed intermediate salt (lower); the peaks at $\delta_H \approx$ 8.3 ppm and ca. 8.6 ppm are the C2/C6 protons

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

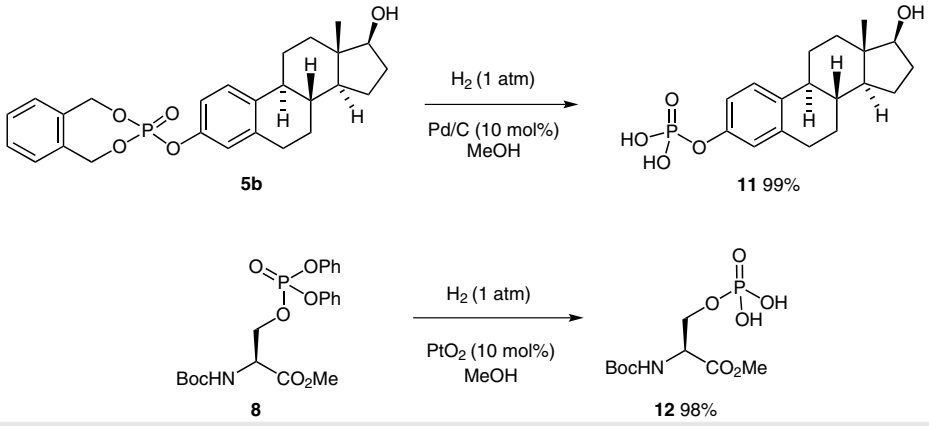
In conclusion, we have demonstrated the use of pyridine-*N*-oxide as an effective and economical organocatalyst for the phosphorylation of primary and secondary alcohols and phenols, including several biologically relevant molecules. The reaction times and yields are comparable to those described for Lewis acid catalysed 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 be of particular utility in the late-stage pro-

Table 3 Phosphorylation of Biologically Relevant Molecules and Phosphate Prodrugs



Entry	Product	Compound	Time (h)	Yield (%)[a]
1		**4**	2	85
2		**5a** R = Ph **5b** R–R = *o*-XP	1 1	74 (27)[b] 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 **10b** R–R = *o*-XP	1 1	91 (59)[b] 93 (21)[b]

[a] Isolated yield after chromatographic purification.
[b] Conversion to product as determined by ^{1}H NMR of the crude reaction mixture of the uncatalyzed reaction.
[c] Yield of reaction conducted on 20 mmol scale with respect to alcohol.



Scheme 2 Deprotection of phosphate esters **5b** and **8**

duction of phosphate esters for biological applications, e.g. in the preparation of prodrugs. For difficult alcohol phosphorylation cases, for example 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.

Acknowledgment

We thank the EPSRC and the Imperial College Institute of Chemical Biology for funding. J.I.M. is also grateful to the Society of Chemical Industry for a Messel Scholarship.

Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1379993.

References and Notes

(1) Corbridge, D. E. C. *J. Am. Chem. Soc.* **2000**, *122*, 10742.
(2) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science* **2002**, *298*, 1912.
(3) Jessen, H. J.; Ahmed, N.; Hofer, A. *Org. Biomol. Chem.* **2014**, 3526.
(4) Schultz, C. *Bioorg. Med. Chem.* **2003**, *11*, 885.
(5) Zawilska, J. B.; Wojcieszak, J.; Olejniczak, A. B. *Pharmacol. Reports* **2013**, *65*, 1.
(6) Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Järvinen, T.; Savolainen, J. *Nat. Rev. Drug Discovery* **2008**, *7*, 255.
(7) Uphues, G.; Ploog, U. US Patent US4921990A, **1990**.
(8) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925.
(9) Jones, S.; Selitsianos, D. *Org. Lett.* **2002**, *4*, 3671.
(10) Jones, S.; Selitsianos, D.; Thompson, K. J.; Toms, S. M. *J. Org. Chem.* **2003**, *68*, 5211.
(11) Jones, S.; Smanmoo, C. *Org. Lett.* **2005**, *7*, 3271.
(12) Jones, S.; Selitsianos, D. *Tetrahedron: Asymmetry* **2005**, *16*, 3128.
(13) Crook, S.; Parr, N. J.; Simmons, J.; Jones, S. *Tetrahedron: Asymmetry* **2014**, *25*, 1298.
(14) Fenton, O. S.; Allen, E. E.; Pedretty, K. P.; Till, S. D.; Todaro, J. E.; Sculimbrene, B. R. *Tetrahedron* **2012**, *68*, 9023.
(15) Coppola, K. A.; Testa, J. W.; Allen, E. E.; Sculimbrene, B. R. *Tetrahedron Lett.* **2014**, *55*, 4203.
(16) Mora, N.; Lacombe, J. M.; Pavia, A. A. *Tetrahedron Lett.* **1993**, *34*, 2461.
(17) Sabesan, S.; Neira, S. *Carbohydr. Res.* **1992**, *223*, 169.
(18) Pisarek, S.; Bednarski, H.; Gryko, D. *Synlett* **2012**, *23*, 2667.
(19) Sculimbrene, B. R.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10125.
(20) Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 11653.
(21) Garrett, C. E.; Prasad, K. *Adv. Synth. Catal.* **2004**, *346*, 889.
(22) Kucznierz, R.; Dickhaut, J.; Leinert, H.; Von Der Saal, W. *Synth. Commun.* **1999**, *29*, 1617.
(23) Guillaume, M.; Cuypers, J.; Dingenen, J. *Org. Process Res. Dev.* **2007**, *11*, 1079.
(24) Reeves, J. T.; Tan, Z.; Han, Z. S.; Li, G.; Zhang, Y.; Xu, Y.; Reeves, D. C.; Gonnella, N. C.; Ma, S.; Lee, H.; Lu, B. Z.; Senanayake, C. H. *Angew. Chem. Int. Ed.* **2012**, *51*, 1400.
(25) Efimov, V. A.; Chakhmakhcheva, O. G.; Ovchinnikov, Y. A. *Nucleic Acids Res.* **1985**, *13*, 3651.
(26) Efimov, V. A.; Buryakova, A. A.; Dubey, I. Y.; Polushin, N. N.; Chakhmakhcheva, O. G.; Ovchinnikov, Y. A. *Nucleic Acids Res.* **1986**, *14*, 6525.
(27) Murray, J. I.; Woscholski, R.; Spivey, A. C. *Chem. Commun.* **2014**, *50*, 13608.
(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 CH_2Cl_2 (0.2 M) was added the specified phosphoryl chloride (1.0 mmol). The reaction mixture was stirred at r.t. for the time specified before the addition of MeOH (2 mL). The reaction mixture was then concentrated in vacuo and the crude material was purified by flash chromatography to afford the product.
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%); mp 146–147 °C; $[\alpha]_D^{19}$ –87 (*c* = 0.99, $CHCl_3$). IR: 3314, 3033, 3001, 2936, 1707, 1612, 1536, 1286, 1211, 1097, 875, 736, 467 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.41–7.43 (m, 2 H), 7.34–7.36 (m, 2 H), 7.28–7.30 (m, 1 H), 7.05–7.08 (m, 1 H), 5.38–5.44 (m, 2 H), 5.19–5.28 (m, 2 H), 3.76 (t, *J* = 8.5 Hz, 1 H), 2.88 (m, 2 H), 2.31–2.39 (m, 1 H), 2.11–2.26 (m, 2 H), 1.99 (dt, *J* = 12.5, 3.3 Hz, 1 H), 1.89–1.94 (m, 1 H), 1.69–1.77 (m, 1 H), 1.18–1.55 (m, 9 H), 0.81 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 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. ^{31}P NMR (161 MHz, $CDCl_3$): δ = –6.94. HRMS (ES): *m*/*z* [M + H^+] calcd for $C_{26}H_{32}O_5P$: 455.1987; found: 455.1988.

(*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%); mp 61–63 °C; $[\alpha]_D^{19}$ + 161 (*c* = 0.96, $CHCl_3$). IR: 3313, 3067, 3039, 2957, 1721, 1590, 1514, 1488, 1456, 1288, 1185, 1163, 948, 754, 688 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.20–7.40 (m, 10 H), 5.40 (d, *J* = 7.9 Hz, 1 H), 4.65–4.67 (m, 1 H), 4.51–4.60 (m, 1 H), 3.71 (s, 3 H), 1.46 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): 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. ^{31}P NMR (161 MHz, $CDCl_3$): δ = –12.34. HRMS (ES): *m*/*z* [M + Na] calcd for $C_{21}H_{26}NO_8PNa$: 474.1294; found: 474.1300.

(30) Yamago, S. US Patent US2008177049A1, **2008**.
(31) Toth, G.; Kele, Z.; Varadi, G. *Curr. Org. Chem.* **2007**, *11*, 409.
(32) Jarowicki, K.; Kocienski, P. *J. Chem. Soc., Perkin Trans. 1* **1998**, *1*, 4005.
(33) McMurray, J. S.; Coleman, D. R.; Wang, W.; Campbell, M. L. *Biopolymers* **2001**, *60*, 3.