Mild and Chemoselective Phosphorylation of Alcohols Using a Ψ-Reagent

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Abstract. *An operationally simple, scalable, and chemoselective method for the direct phosphorylation of alcohols using a P(V)-approach based on the Ψ-reagent platform is disclosed. The method features a broad substrate scope of utility in both simple and complex settings and provides access to valuable phosphorylated alcohols that would be otherwise difficult to access.*

Main text. Phosphorylation of alcohol-containing biomolecules is one of Nature's most simple methods for regulating cell circuitry.[1] Introduction of a phosphate group can also be critical in the medicinal,[2] agrochemical,[3] and materials areas.[4] Biological enzymatic phosphorylation overcomes thermodynamic barriers to achieve selective functionalization through molecular recognition and by lowering the activation energy of the P–O bond forming step.[5] Current purely chemical alcohol phosphorylation methods all suffer from various limitations and/or multistep processes (Figure 1).[6-10] For example, the use of P(III)-based phosphoramidites require a three step process for installation including protecting group removal and oxidation.[7] P(V)-based strategies such as the use of $POCl_3$ and derivatives thereof can be problematic due to over reactivity (often producing mixtures of mono-, di-, and tri-alkylphosphates) and protecting group manipulations.[8] The direct use of phosphoric acid requires high temperatures and exhibits limited scope due to the high acidity and harsh conditions.[9] Activation methods used in concert with phosphoric acid or its salts have been employed with limited scope.[10] The recently reported bioinspired method based on an enzymatically produced P(V)-reagent (PEP-K) solves many of these problems despite requiring its use in excess at 100 °C.[11] However, like all known methods it suffers from a lack of chemoselectivity (in this case free amines are not tolerated). The recently disclosed P(V)-based Ψ-platform for the construction of P-linkages has been applied to the simplified synthesis of an ever growing, diverse range of compounds such as: cyclic dinucleotides,[12,13] stereopure anti-sense oligonucleotides,[12] methylphosphonates,[14] chiral phosphines,[14] DNA[5] and protein bioconjugates,[16] complex alkaloids,[17] and fully chemically modified oligonucleotides using a commercial automated synthesizer.[18] As part of the ongoing Ψ-platform development, Ψ^O (**1**) was identified as a suitable reagent for forging phosphodiester bonds.[18] This Letter builds on those findings to highlight how the chemoselective nature of Ψ-reagents can be leveraged to access phosphates from alcohols in a mild, scalable, and operationally simple (one-pot) fashion across a wide range of alcohol substrates.

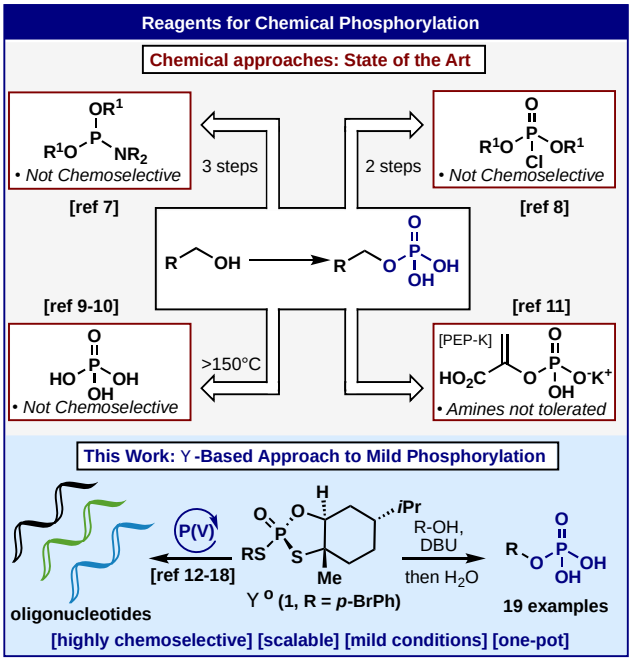
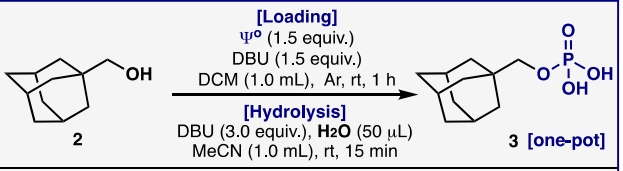


FIGURE 1. Alcohol phosphorylation: Literature precedent, limitations, and a mild solution using the Ψ^O reagent (**1**).

Previous work on the Ψ-platform demonstrated that these reagents facilitate formation of phosphate (or thiophosphate) linkages via stepwise nucleophilic addition of two different alcohols.[12] In a similar manner, appendage of an alcohol to the Ψ^O reagent to form intermediate Ψ-loaded adduct, followed by addition of water should in principle lead to the formation of monoalkyl phosphate. To explore the feasibility of this idea, alcohol **2** was chosen as a simple substrate to start optimization efforts (Table 1A). In fully optimized form, the reaction requires 1.5 equivalents of Ψ^O and DBU as lower quantities lead to diminished yield due to

formation of double addition products (dialkylphosphates, Table 1A, entry 2) or decreased conversion (Table 1A, entry 3). Consistent with prior findings, the reaction performs best with DBU as a base although DBN and DABCO furnish product in diminished yield (Table 1A, entries 4-8). Best conversions are achieved in anhydrous DCM although MeCN or DMF can be used with only slightly lower yields (Table 1A, entries 9-12). Unsurprisingly, control experiments confirm the need for Ψ^{O} and base for initial P–O bond formation, and H_2O for the hydrolysis step (Table 1A, entries 13-15). The reaction can be performed without using anhydrous solvent and open to air, but affords the desired product with diminished yield due to competing hydrolysis of Ψ^{o} reagent during loading step (Table 1A, entries 16 and 17).

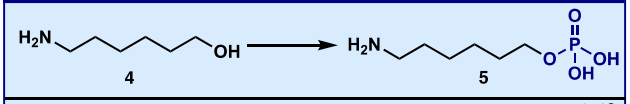
A. Invention and Optimization of Ψ-Based Alcohol Phosphorylation



2 3 [one-pot]

entry	deviation from above	yield (%)[a]
1	none	75 (68)
2	1.2 equiv. of Ψ^{o}	63
3	1.3 equiv. of DBU	36
4	DBN instead of DBU	43
5	DIPEA instead of DBU	<5
6	TMG instead of DBU	<5
7	DABCO instead of DBU	41
8	*N*-methylimidazole instead of DBU	<5
9	THF instead of DCM	13
10	DMF instead of DCM	48
11	MeCN instead of DCM	54
12	Toluene instead of DCM	47
13	no Ψ^{o}	N.D.
14	no DBU	N.D.
15	no H_2O	<5
16	non-anhydrous DCM	43
17	under air	52

B. Chemoselectivity Challenge: *O*- vs *N*-phosphorylation



4 5

entry	state-of-the-art phosphorylation methods	yield (%)[a]
1	H_3PO_4, 150 °C **[ref 19b]**	(51)
2	FmocCl; then $POCl_3$; then H_2O; then piperidine **[ref 19c]**	(64)
3	$iPr_2NP(OBn)_2$; then *m*CPBA; then H_2, Pd/C **[ref 7d]**	<10
4	$P(OMe)_3$, CBr_4; then TMSCl, NaI **[ref 7e]**	<10
5	$POCl_3$; then allyl alcohol; then $Pd(PPh_3)_4$, $PhSiH_3$ **[ref 8c]**	<10
6	$PO(OBn)_2Cl$; then H_2, Pd/C **[ref 8d]**	N.D.
7	$NBu_4H_2PO_4$, Cl_3CCN **[ref 10a]**	<5
8	PEP-K, NBu_4HSO_4, 100 °C **[ref 11a]**	N.D.
9	H_3PO_4, *N*-Bu-Imidazole, Bu_3N, 160 °C **[ref 9b]**	55
10	**Ψ^{o}, DBU; then H_2O [this work]**	**65 (49)**

TABLE 1. (A) Optimization of Ψ-based alcohol phosphorylation and (B) its use in the selective *O*-phosphorylation of aminoalcohol **4**. [a]Yields determined by quantitative ^{31}P NMR (see SI). Isolated yields in brackets.

ϖ-Aminoalkyl dihydrogen phosphates and their salts are used as active ingredients in cosmetics, promoting fibroblast proliferation and collagen biosynthesis.[19] They are also employed in biochemistry as linkers for bioconjugates.[20] However, their availability is hampered by inconvenient synthetic routes. For instance, compound **5** was previously synthesized in 51% isolated yield (only melting point and elemental analysis reported) by condensation with crystalline H_3PO_4 at 150 °C under high vacuum (Table 1B, entry 1).[20b] Milder routes to phosphate **5** require Fmoc-protection of the amine functionality, followed by phosphorylation by $POCl_3$, and deprotection (64% over 3 steps, entry 2).[20c] As it is known that Ψ reagents are exquisitely *O*-selective,[16] the chemoselectivity of the direct phosphorylation in the context of ϖ-aminoalcohol **4** was examined. We started by surveying 7 literature conditions and out of those reported protocols, six delivered little to no observable **5** with the main byproduct being both *N*- and *O*-phosphorylation as part of a complex mixture (Table 1B, entries 3-8). Only the harsh conditions of phosphoric acid at 160 °C delivered synthetically useful yields in our hands (entry 9) likely due to the *in situ* protonation of the amine. In stark contrast, our newly developed conditions using **1** followed by hydrolysis cleanly provided **5** at ambient temperature (Table 1B, entry 10). Importantly, no *N*-phosphorylated product could be observed by ^{31}P NMR.

The scope of this method was exemplified by the preparation of 19 different phosphorylated alcohols (Table 2). It is worth noting that, most of the older papers in this field present only analysis based on melting point and elemental analysis (occasionally ^{31}P NMR). More recent disclosures, with very few notable exceptions,[8c,9c,10a,11] usually do not include high resolution images of NMR spectra. It is also common that such methodology papers report only conversions not isolated yields, presumably due to the difficulty in handling those polar substances. To be sure, multiple methods have been reported for the purification of phosphates including: HPLC,[11a] HILIC,[10a] solid phase ion exchange,[9c,10a] and recrystallization.[10b] We found that in small scale experiments HPLC was superior (see SI for column and eluent conditions) whereas HILIC was the method of choice for larger scale preparations or for compounds that are extremely polar. In some cases, it was convenient to isolate phosphates as their ammonium salts. As indicated in Table 2A, simple amines are tolerated in this reaction (**5**, **14**, **15**) as well as basic heterocycles (**16, 19**). Alkynes (**7**), azides (**8**), thiophenes (**9**), aryl iodides (**10**), olefins (**11**), alkyl halides (**12**, **13**), nitro arenes (**17**, **19**), and indoline (**18**) were unscathed upon P-O bond formation. Finally, four medicinally relevant substrates were phosphorylated (Table 2B): metronidazole (**19**), AZT (**20**), cholesterol (**21**), and a tyrosine-containing peptide bearing histidine, aspartamide, and glutamide side-chains. Prior routes to some of these compounds were either laborious or contained limited experimental data such as the preparation of **7** (three steps,[21] utility in biomolecule functionalization),[22] **14** (three steps),[23] **20** (most methods

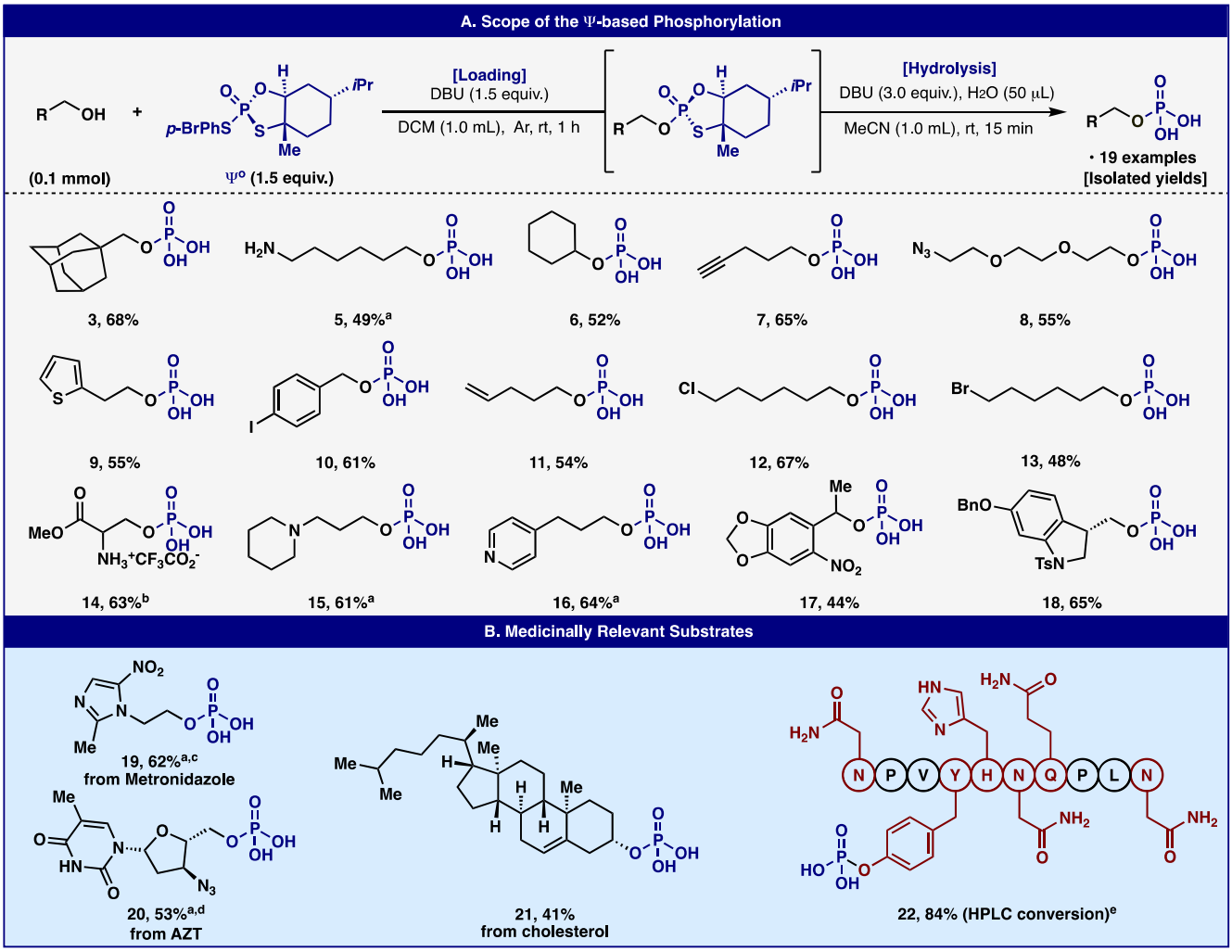


TABLE 2. Scope of the Ψ-based alcohol phosphorylation method. [a]Isolated as ammonium salt. [b]From *N*-Boc Serine. [c]Reaction in DMF. [d]Reaction in MeCN. [e]Ψ^{O} (5.0 equiv.), DBU (5.0 equiv.), reaction in DMF on a 1.0 μmol scale.

<35% yield[24a] or multistep procedures[24b,c] with one paper showing higher yield with $POCl_3$ and characterization based only on UV spectrum;[24d] enhanced HIV1 activity reported[25]), and **21** (one[10c] and three[26] step routes given with little characterization data). The limitations of this reaction (see SI for details) stem from lack of tolerance of preexisting functionality on the alcohol to basic conditions, and lower nucleophilicity of sterically hindered substrates (i.e. tertiary alcohols).

The intermediate Ψ-loaded adducts can also be used for the preparation of dialkyl phosphates rather than free phosphates. As shown in Figure 2, alcohol **2** could be loaded with reagent **1** followed by addition of BnOH to deliver dialkyl phosphate **23** in 42% isolated yield (without any additional optimization). The scalability of this reaction was also demonstrated using metronidazole wherein the standard protocol (0.1 mmol scale) could be increased 30-fold while maintaining efficiency.

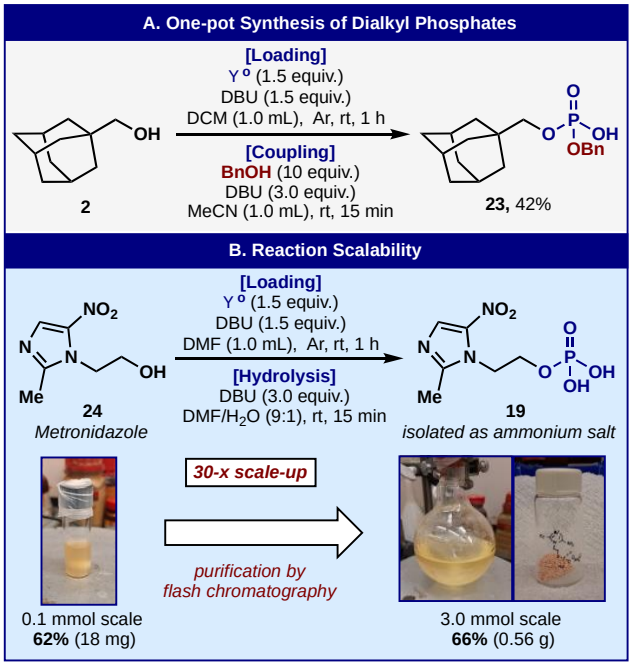


FIGURE 2. (A) One-pot synthesis of dialkyl phosphate **23** and (B) scale up of Ψ-based phosphorylation of metronidazole.

The operationally simple phosphorylation method described herein represents a useful addition to the toolkit for installing this important functional group in a chemoselective fashion and is yet another example of the versatility of the Ψ-platform in organic synthesis.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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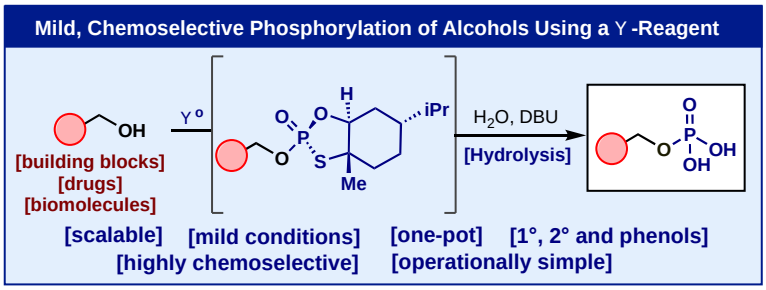
TOC Graphic:


Mild, Chemoselective Phosphorylation of Alcohols Using a Y -Reagent
OH
[building blocks]
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Y
O
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S
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iPr
Me
H2O, DBU
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O
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OH
OH
[scalable]
[mild conditions]
[one-pot]
[1°, 2° and phenols]
[highly chemoselective]
[operationally simple]