

# Catalytic Chemoselective O-Phosphorylation of Alcohols

K. Domon, M. Puripat, K. Fujiyoshi, M. Hatanaka, S. A. Kawashima, K. Yamatsugu,\* and M. Kanai\*



Cite This: *ACS Cent. Sci.* 2020, 6, 283–292



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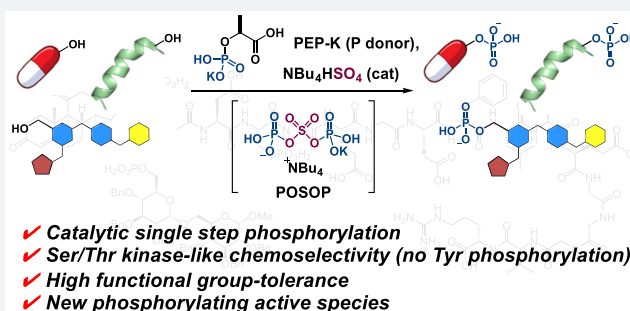
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**ABSTRACT:** Phosphorylation of alcohols is a fundamentally important reaction in both life science and physical science. Product phosphate monoesters play key roles in living organisms, natural products, pharmaceuticals, and organic materials. Most of the chemical methods to date for synthesizing phosphate monoesters, however, require multistep sequences or are limited to specific types of substrates possibly due to harsh conditions. An alternative way to enable the simple production of phosphate monoesters from highly functionalized precursor alcohols is, thus, highly desired. We report herein a catalytic phosphorylation of alcohols with high functional group tolerance using tetrabutylammonium hydrogen sulfate (TBAHS) and phosphoenolpyruvic acid monopotassium salt (PEP-K) as the catalyst and phosphoryl donor, respectively. This method enables the direct introduction of a nonprotected phosphate group to the hydroxy group of a diverse menu of alcohol substrates, including functionalized small molecules, carbohydrates, and unprotected peptides. Nuclear magnetic resonance, mass spectrometric, and density functional theory analyses suggest that an unprecedented mixed anhydride species, generated from PEP-K and TBAHS, acts as an active phosphoryl donor in this reaction. This operationally simple and chemoselective catalytic phosphorylation allows for the efficient production of densely functionalized O-phosphorylated compounds, which are useful in diverse fields including biology and medicine.



## INTRODUCTION

Phosphate monoesters are fundamental motifs in various molecules and play diverse and pivotal roles in life science and physical science. Phosphate monoester motifs are widely found in biomolecules, including proteins,<sup>1</sup> signal messengers,<sup>2</sup> and primary<sup>3</sup> and secondary<sup>4,5</sup> metabolites, and in human-made functional molecules, such as pharmaceuticals<sup>6</sup> and organic materials.<sup>7</sup> In proteins, for example, phosphorylation of the serine, threonine, and tyrosine hydroxy groups is among the most common and important post-translational modifications.<sup>1</sup> Such modifications may induce structural changes in the protein or lead to the formation of a new protein–protein interaction surface, triggering cellular signal cascades and constituting a fundamental regulatory mechanism of cell biology.<sup>8</sup> The introduction of a phosphate monoester motif can dramatically alter the function of a molecule and can play a key role in mediating the bioactivity of natural products<sup>9,10</sup> and the pharmacokinetics of pharmaceutical agents.<sup>6</sup> Thus, a simple and robust method to enable easy access to these important motifs would be highly desirable, as the ready availability of large quantities of these functional materials would facilitate further investigation of their biological and physical functions.<sup>11–13</sup>

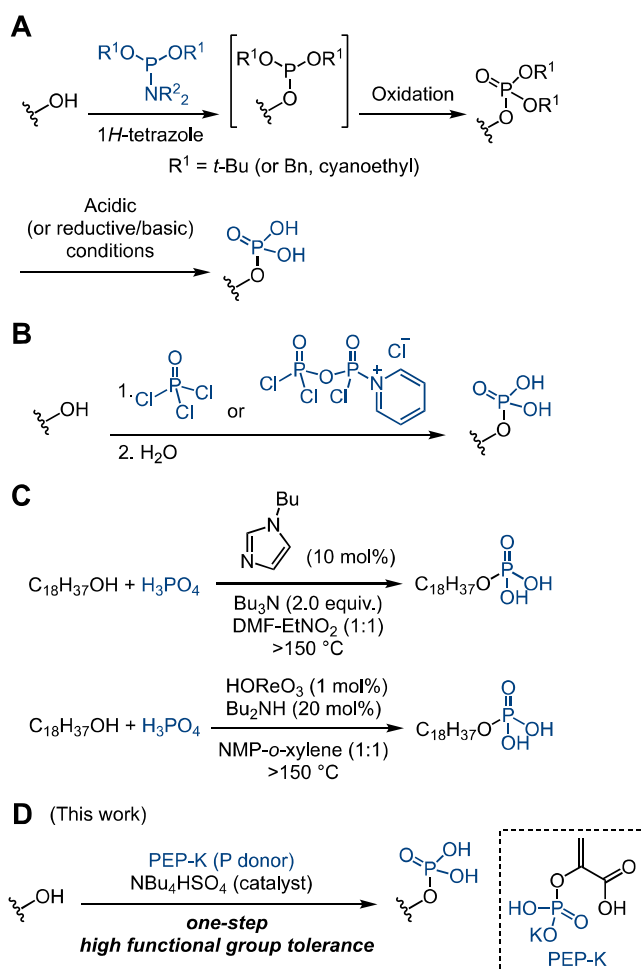
A straightforward way to access phosphate monoesters is via phosphorylation of alcohol starting materials. There are two major chemical approaches to the transformation of alcohols to phosphate monoesters: (1) the formation of trivalent

phosphite followed by oxidation and (2) the reaction with a pentavalent phosphoryl donor (Figure 1). A standard example of the first approach is depicted in Figure 1A. Thus, an alcohol is first reacted with a trivalent phosphoramidite reagent to produce a phosphite intermediate, which is then oxidized, typically in the same reaction vessel, to afford a protected phosphate triester. Finally, the removal of the protecting groups under acidic, reductive, or basic conditions ( $R^1 =$  alkyl, benzyl, or cyanoethyl, respectively) produces the desired phosphate monoester.<sup>14–16</sup> This method requires three steps and is sometimes incompatible with substrates that are sensitive to oxidation or to the (acidic, basic, or reducing) conditions required to remove the protecting groups, although several improved methods were reported.<sup>17,18</sup> A typical example of approach (2), outlined in Figure 1B, uses phosphoryl chloride and its derivatives as the phosphoryl donors.<sup>19–22</sup> Although the use of these phosphoryl chloride derivatives directly provides the desired phosphate monoester after hydrolysis, the high reactivity of the phosphoryl chloride derivatives sometimes render functional groups in a substrate

Received: December 12, 2019

Published: January 22, 2020





**Figure 1.** Chemical approaches for phosphate monoester synthesis from alcohols. (A) Step-wise phosphorylation with phosphoramidites, followed by oxidation and deprotection. (B) Phosphorylation with phosphoryl chloride and its derivative, followed by hydrolysis. (C) Dehydrative condensation approaches developed by Ishihara and co-workers.<sup>23,24</sup> (D) Catalytic direct phosphorylation of alcohols with phosphoenolpyruvic acid monopotassium salt (PEP-K) and tetrabutylammonium hydrogen sulfate developed in this study.

incompatible with the reaction conditions and complicate the reaction.

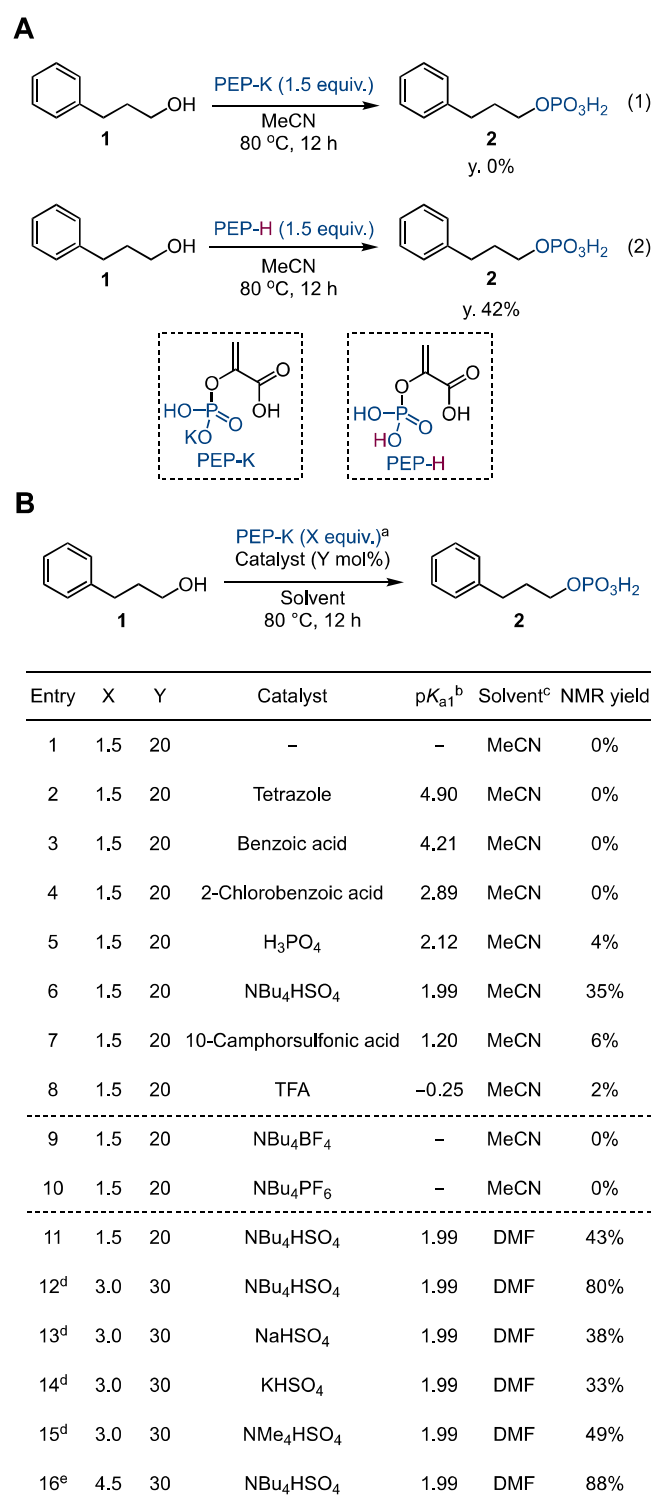
There is thus a clear need for a robust method that allows access to a diverse menu of nonprotected phosphate monoesters in a single step from alcohol precursors. Toward this end, Ishihara and co-workers have reported the synthesis of phosphate monoesters through azeotropic dehydrative condensation between phosphoric acid and alcohols promoted by *N*-butylimidazole and tributylamine (Figure 1C).<sup>23</sup> In 2007, the researchers further developed a rhenium(VII) oxide-catalyzed variant that proceeded at >150 °C (Figure 1C).<sup>24</sup> The substrate scope of those reactions, however, was mostly limited to alcohols containing simple alkyl chains, likely due to the high reaction temperature. Therefore, the development of a catalyst-promoted direct phosphorylation of alcohols with high functional group tolerance is an important goal in organic chemistry.

Kinases are capable of effectively catalyzing phosphoryl-transfer reactions in highly functionalized substrates, such as proteins, carbohydrates, and other metabolites using nucleoside 5'-triphosphates (e.g., adenosine triphosphate, ATP) or

phosphoenolpyruvate (PEP)<sup>25</sup> as high-energy phosphoryl donors.<sup>26</sup> The kinetic barrier of the phosphoryl-transfer reactions is generally high, but kinases (biocatalysts) provide enormous rate acceleration in part by efficiently activating the phosphoryl donor.<sup>26</sup> In our effort to achieve a direct phosphorylation of alcohols, we sought to exploit those high-energy phosphates because they are stable as they are but can become reactive if properly activated. Among the phosphoryl donors, PEP has the highest energy phosphate bond (Figure 1D).<sup>27</sup> We envisioned that, if an appropriate catalyst could be identified that is capable of activating PEP under mild conditions, then it should be possible to achieve chemoselective phosphorylation to directly access unprotected phosphate monoesters in a single step from alcohol substrates. Herein, we report a catalytic and chemoselective direct phosphorylation of a wide range of alcohol substrates, including functionalized small molecules, carbohydrates, and unprotected peptides, mediated by a phosphoenolpyruvic acid monopotassium salt (PEP-K)/tetrabutylammonium hydrogen sulfate (TBAHS) system (Figure 1D).

## RESULTS AND DISCUSSION

For our initial studies, we selected 3-phenyl-1-propanol (**1**) as a model substrate. Alcohol **1** was subjected to simple heating conditions in the presence of commercially available PEP-K in acetonitrile for 12 h. However, under these conditions, no phosphorylated product **2** was detected, and PEP-K was recovered (Figure 2A, eq 1). In contrast, when phosphoenolpyruvic acid (PEP-H, prepared by cation exchange resin from PEP-K; see the Supporting Information for details) was used as the phosphoryl donor under the same conditions, product **2** was obtained in 42% yield (Figure 2A, eq 2). Thus, we postulated that a Brønsted acid catalyst could protonate PEP-K and facilitate the formation of an active phosphorylating species through PEP-H. On the basis of this hypothesis, we investigated the catalytic activities of various Brønsted acids (20 mol %) in the phosphorylation of **1** using PEP-K (1.5 equiv) as a phosphoryl donor in acetonitrile at 80 °C (Figure 2B, Entries 1–8; for a complete list of reaction conditions evaluated, see Table S1). The  $pK_{a1}$  value of PEP-H in water is reported to be less than 2.<sup>28</sup> Brønsted acids of weak acidity, such as tetrazole ( $pK_{a1} = 4.9$ ) and benzoic acid derivatives ( $pK_{a1} = 2.9\text{--}4.2$ ), did not afford any phosphorylated product **2** (Figure 2B, Entries 2–4). Stronger Brønsted acids, such as phosphoric acid ( $pK_{a1} = 2.1$ ), 10-camphorsulfonic acid ( $pK_{a1} = 1.2$ ), and trifluoroacetic acid (TFA,  $pK_{a1} = -0.3$ ), gave product **2** in very low yields (2–6%, Figure 2B, Entries 5, 7, and 8). However, tetrabutylammonium hydrogen sulfate (TBAHS,  $pK_{a1} = 2.0$ ) did catalyze the phosphorylation, affording **2** in 35% yield (Figure 2B, Entry 6). We can thus conclude that catalytic activity does not directly correlate with Brønsted acidity (i.e.,  $pK_{a1}$  values). We next established that the hydrogen sulfate Brønsted acid is key to promoting the reaction; other quaternary ammonium salts without the hydrogen sulfate did not react to afford **2** at all (Figure 2B, Entries 9–10). A solvent screen revealed that, with DMF as solvent and an increased TBAHS catalyst loading of 30 mol %, a product yield of 80% could be obtained (Figure 2B, Entries 11–12, and Table S1). Hydrogen sulfate catalysts containing counterions other than the tetrabutylammonium ion produced less satisfactory results (Figure 2B, Entries 13–15). Finally, raising the temperature to 100 °C and using 4.5 equiv of PEP-



<sup>a</sup>The value of  $pK_{a1}$  of PEP-H is less than 2. <sup>b</sup>Values in water are shown for reference. <sup>c</sup>0.1 M for entries 1–11 and 0.2 M for entries 12–16. <sup>d</sup>24 h. <sup>e</sup>100 °C, 6 h

**Figure 2.** Initial trials and optimization of reaction conditions. (A) Phosphorylation of 3-phenyl-1-propanol with PEP-K (eq 1) and with PEP-H (eq 2). (B) Optimization of reaction conditions.

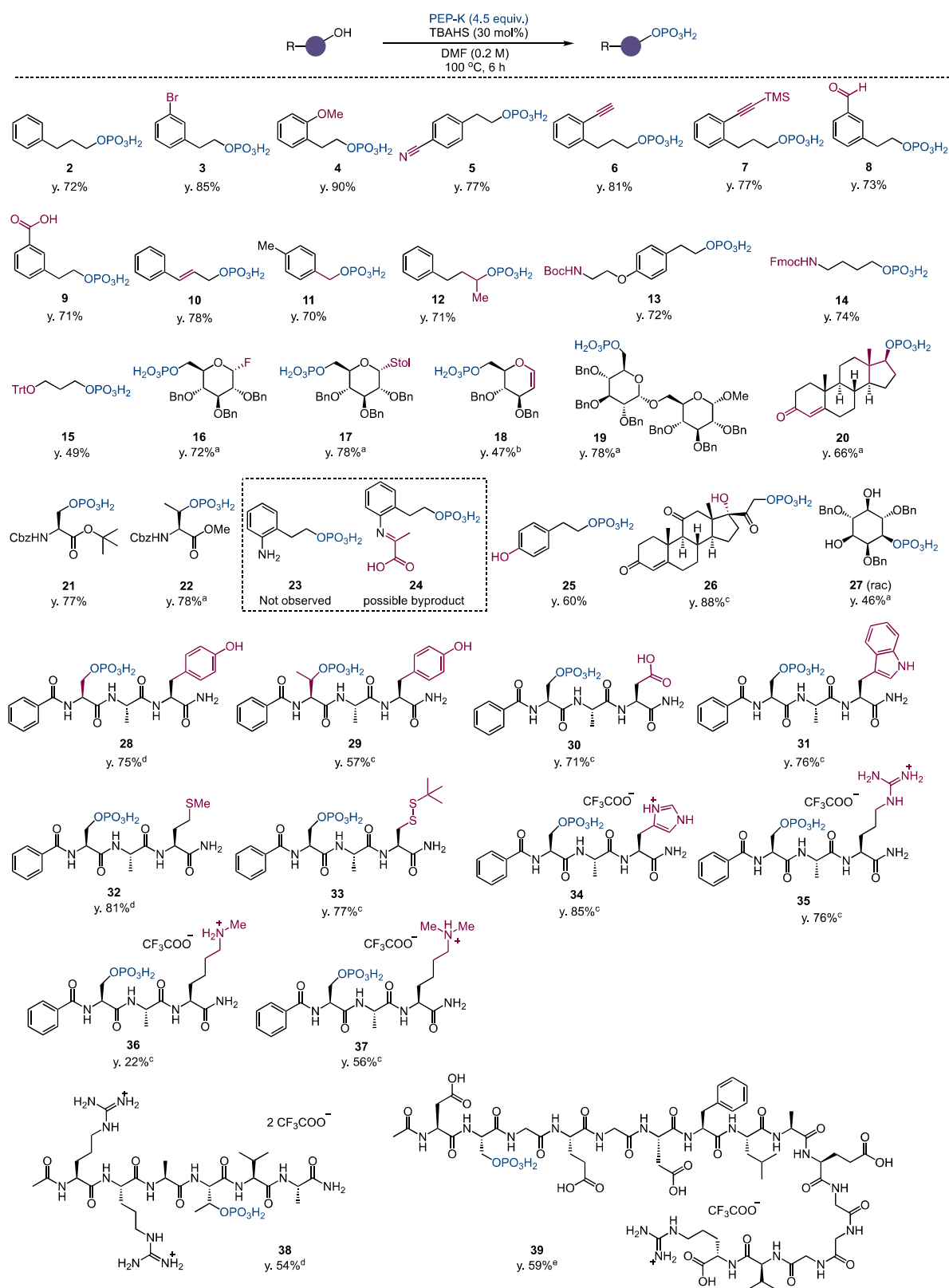
K led to an improved yield of **2** (88%) and shorter reaction times (6 h) (Figure 2B, Entry 16).

Having identified optimized reaction conditions, we next investigated the substrate scope of the transformation. As shown in Figure 3, a range of useful functional groups,

including bromide (**3**: 85% yield), methoxy (**4**: 90% yield), cyano (**5**: 77% yield), alkyne (**6**: 81% yield), trimethylsilyl (**7**: 77% yield), aldehyde (**8**: 73% yield), and carboxylic acid (**9**: 71% yield), were well-tolerated, reacting to afford the corresponding phosphorylation products in good to excellent yield. Allylic (**10**: 78% yield), benzylic (**11**: 70% yield), and secondary (**12**: 71% yield) alcohols also underwent direct phosphorylation in good yields. Importantly, phosphorylation of alcohols bearing acid-, base-, and/or oxidation-sensitive functional groups also proved possible, as Boc carbamate (**13**: 72% yield), Fmoc carbamate (**14**: 74% yield), and trityl group (**15**: 49% yield; moderate yield was due to low reactivity) were all readily accommodated in this transformation. Glycosyl donors with activated anomeric leaving groups were also found to be good substrates for the direct phosphorylation. Thus, phosphorylated glycosyl fluoride (**16**: 72% yield) and thioglycoside (**17**: 78% yield) were formed from their corresponding alcohols in good yield. In particular, the reaction of a glycal substrate containing an enol ether moiety to form **18** (47% yield) is notable, because this functional group is highly sensitive to both slightly acidic and oxidative conditions. The moderate yield of **18** was due to the gradual decomposition of the substrate under the current conditions. A disaccharide with an acid-sensitive glycosidic linkage (**19**: 78% yield) readily underwent phosphorylation. It is also noteworthy that a sterically hindered secondary alcohol was efficiently phosphorylated using this catalytic system (**20**: 66% yield). Protected serine (**21**: 77% yield) and threonine (**22**: 78% yield) underwent phosphorylation in good yield, although a substrate bearing a primary amine did not react to generate the desired phosphorylation product, **23**. Although <sup>1</sup>H nuclear magnetic resonance (NMR) analysis of the reaction indicated that phosphorylation of the hydroxy group did proceed in this case, imine formation between the primary amine moiety and pyruvic acid generated after phosphorylation complicated the reaction, producing **24** as a possible byproduct.

Chemo- and regioselective phosphorylation of substrates containing more than one OH group was also found to be possible (Figure 3). Thus, an aliphatic hydroxy group was selectively phosphorylated over a phenolic hydroxy group, affording **25** as the sole product in 60% yield. The primary hydroxy group in cortisone was selectively reacted over the tertiary hydroxy group to afford **26** in 88% yield. A *myo*-inositol derivative was regioselectively phosphorylated at the most reactive hydroxy groups to give monophosphorylated product **27** in 46% yield with 8% yield of a bisphosphorylated product.<sup>29</sup> The regioisomer was not detected.

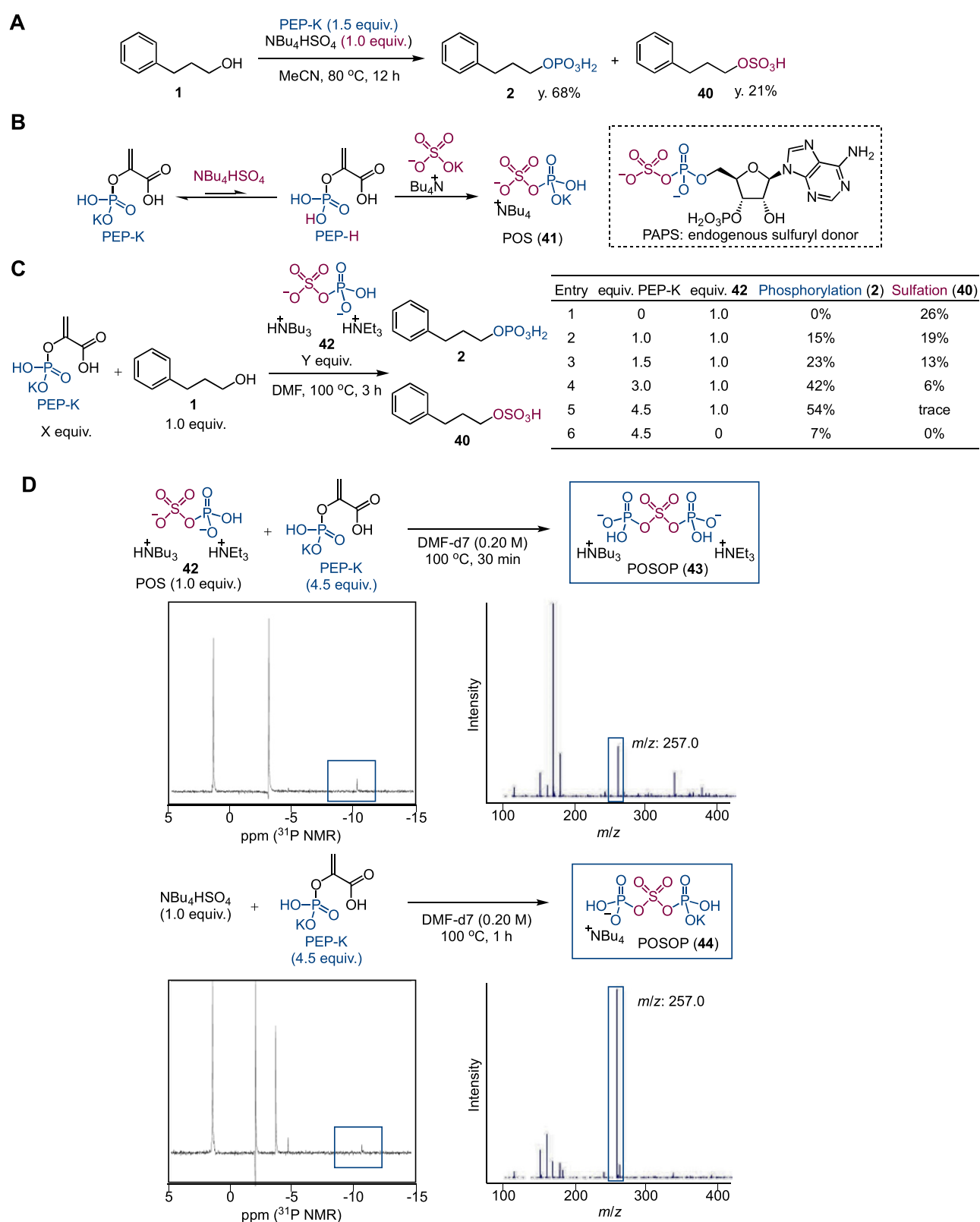
The high functional group tolerance of the PEP-K/TBAHS system allowed for the straightforward, single-step, chemo-selective synthesis of *O*-phosphorylated peptides with minimal use of protecting groups (Figure 3). Serine (**28**: 75% yield) and threonine (**29**: 57% yield) residues in tripeptides underwent selective *O*-phosphorylation in good yield even in the presence of a tyrosine residue on the peptide sequence. The presence of carboxylic acid (aspartic acid, **30**: 71% yield), indole (tryptophan, **31**: 76% yield), and sulfide (methionine, **32**: 81% yield) moieties on the peptide substrates did not adversely impact the reaction yields. Although the presence of an unprotected cysteine residue inhibited phosphorylation, the yield improved dramatically when the cysteine thiol group was converted to a disulfide (**33**, 77% yield). Peptides containing basic amino acids, such as histidine (**34**: 85% yield) and arginine (**35**: 76% yield), were also competent substrates when



**Figure 3.** Substrate scope of the catalytic phosphorylation of alcohols with PEP-K and TBAHS. The general conditions are shown in the reaction scheme. Modified conditions are as follows. a: PEP-K (6.0 equiv), TBAHS (60 mol %). b: PEP-K (6.0 equiv), TBAHS (60 mol %), 3 h. c: PEP-K (10 equiv), TBAHS (60 mol %), 4.5 h. d: PEP-K (10 equiv), TBAHS (60 mol %), 3 h. e: PEP-K (50 equiv), TBAHS (200 mol %), DMF (0.0125 M), 3 h. Isolated yields are shown.

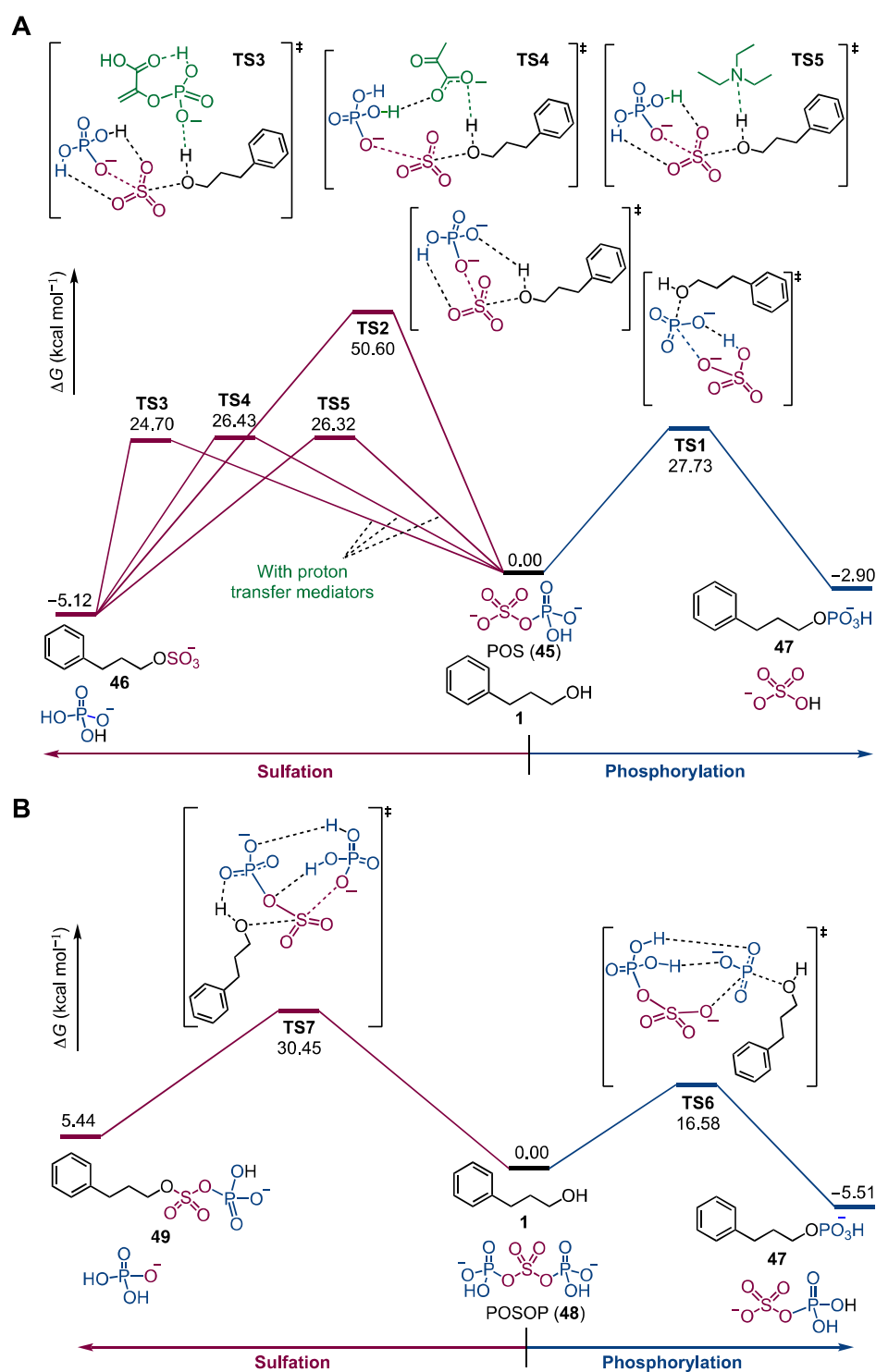
used as their trifluoroacetic acid salts. A peptide containing an unprotected lysine residue did not afford the desired product, likely because the *O*-phosphorylated product further reacted

with the *in situ* generated pyruvic acid at the primary amine of the lysine residue, as seen in **24**. Peptides containing monomethylated (**36**: 22% yield) and dimethylated lysines



(37: 56% yield), however, did react to afford the O-phosphorylated products in moderate yield due to the slower reaction of the products with pyruvic acid. The reaction was

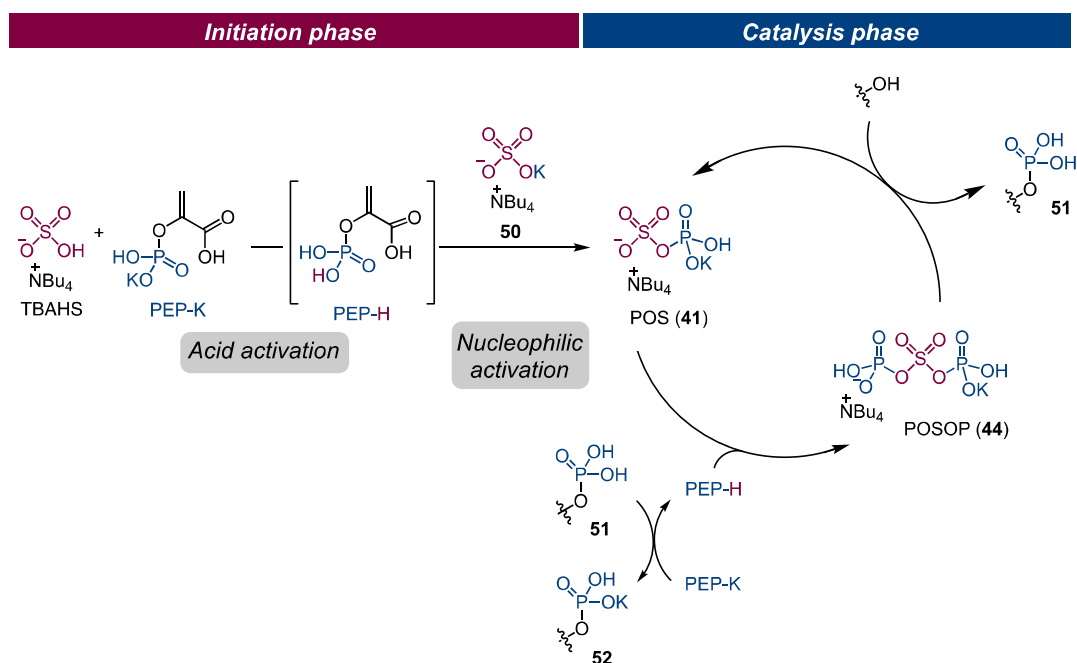
also applicable to longer oligopeptides. Phosphorylated hexapeptide 38, a useful substrate for a sensitive serine/threonine protein phosphatase assay,<sup>30</sup> was synthesized in 54%



**Figure 5.** DFT calculation to rationalize the contrasting reaction pattern between POS (41; sulfuryl donor) and POSOP (44; phosphoryl donor). Sulfation (left) and phosphorylation (right) reactions of alcohol 1 with POS (A) and POSOP (B) are shown. The references of relative Gibbs free energies  $\Delta G$  (in kcal mol<sup>-1</sup>) are 1 + 45 + proton-transfer mediators (A) and 1 + 48 (B). Detailed Gibbs free energy profiles are shown in Figures S8–S14.

yield from the corresponding starting material in a single step. A 15-mer oligopeptide containing multiple acidic and basic amino acids was phosphorylated to produce 39, a potential cancer biomarker,<sup>31</sup> in 59% yield, although more than the stoichiometric amount of TBAHS is required for the high conversion.

Next, we undertook several studies to gain a mechanistic insight into this catalytic phosphorylation reaction. Our observation that catalyst activity does not correlate with Brønsted acidity (Figure 2B) seemed to suggest a role for TBAHS beyond that of a simple Brønsted acid. Interestingly, during optimization of the reaction conditions, we found that, in the presence of 1.5 equiv of PEP-K and 1.0 equiv of



**Figure 6.** Postulated mechanism of the catalytic phosphorylation of alcohols with PEP-K and TBAHS.

TBAHS, sulfurylated compound **40** was produced as a byproduct (21% yield), together with the desired phosphorylated product **2** (68% yield) (Figure 4A). Sulfation of **1** did not proceed when PEP-K was excluded from the reaction (i.e., when TBAHS was used as the sole additive). Thus, both PEP-K and TBAHS are needed for the desired phosphorylation and the undesired sulfation to proceed under the conditions of Figure 4A. This result, coupled with a previous report indicating that an endogenous mixed anhydride, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), works as an *O*-sulfuryl donor,<sup>32</sup> led us to hypothesize that a new inorganic mixed anhydride, phosphosulfate (POS, **41**), is generated from PEP-K and TBAHS (Figure 4B) and that this species may function as a phosphoryl or sulfuryl donor. On the basis of this hypothesis, we synthesized POS **42** (Figure S1) and assessed its reactivity with alcohol **1** in the presence of varying amounts of PEP-K (Figure 4C). As shown in Figure 4C, Entry 1, only sulfurylated compound **40** was obtained in the presence of 1.0 equiv of **42** and the absence of PEP-K; thus, POS **42** acted as a sulfuryl donor in this reaction. In striking contrast, the yield of the phosphorylated product **2** increased with the amount of PEP-K employed (Figure 4C, Entries 2–5), reaching 54% yield in the presence of 4.5 equiv of PEP-K. In the absence of POS **42**, however, the reaction of **1** with 4.5 equiv of PEP-K produced only a 7% yield of **2** (Figure 4C, Entry 6). These results indicate that the hypothesized active species POS **41** is itself a sulfuryl donor and that this intermediate reacts with PEP-K to produce another true active phosphoryl donor species.

To identify the true active phosphoryl donor species, we conducted a series of 31-phosphorus NMR (<sup>31</sup>P NMR) and mass spectrometric (MS) experiments. When we monitored the reaction between POS **42** and PEP-K (4.5 equiv) by <sup>31</sup>P NMR in DMF at 100 °C, a new singlet peak, which is very close to but clearly different from that of **42** (−10.8 ppm, Figure S1), appeared at −10.1 ppm after 30 min (Figures 4D and S2–S5). MS analysis suggested the presence of a species with *m/z* of 257.0 (Figure 4D). This species corresponds to a

new mixed anhydride, POSOP **43**. Importantly, careful monitoring of the reaction between TBAHS and PEP-K by <sup>31</sup>P NMR and MS revealed that POS **41** was formed first (−11.3 ppm in <sup>31</sup>P NMR and *m/z* of 177.1 in MS, Figure S6A) and subsequently converted into POSOP **44** (−10.7 ppm in <sup>31</sup>P NMR and *m/z* of 257.0, Figures 4D and S6B). We further confirmed the presence of POS **41** and POSOP **44** using <sup>34</sup>S-labeled TBAHS in the MS analysis, because phosphorylation and <sup>32</sup>S-sulfation give the same mass shift, and POS **41** and pyrophosphate could not be differentiated under the MS conditions. As a result, species with *m/z* corresponding to PO<sup>34</sup>S (216.8 for monopotassium salt) and PO<sup>34</sup>SOP (319.4 for potassium sodium salt) were observed when <sup>34</sup>S-labeled TBAHS was used (Figure S7). These results suggest that the mixed anhydride species, POSOP, is formed through intermediate POS and that the POSOP species acts as an active phosphoryl donor in the PEP-K/TBAHS-promoted phosphorylation reaction.

We next investigated the origin of the differing reactivities of POS (sulfuryl donor) and POSOP (phosphoryl donor) using density functional theory (DFT) calculations combined with an automated reaction path search method called the “artificial force induced reaction” (AFIR) method (Figure 5).<sup>33–35</sup> In the reaction between alcohol **1** and POS **45**, sulfurylated product **46** was thermodynamically more stable than phosphorylated product **47** by 2.22 kcal mol<sup>−1</sup> (Figures 5A and S8–S12). The activation barriers for the transition states (TSs) in sulfation and phosphorylation were 50.60 and 27.73 kcal mol<sup>−1</sup>, respectively. The activation barrier for sulfation markedly decreased, however, in the presence of a molecule mediating the proton transfer from ROH **1** to the phosphate group of **45**. Thus, the activation barriers were 24.70, 26.43, and 26.32 kcal mol<sup>−1</sup> in the presence of PEP (TS3), pyruvic acid (for the conditions in Figure 4A, TS4), and triethylammonium ion (for the conditions in Figure 4C, TSS), respectively. Since the activation barrier difference (3.03 kcal/mol) between sulfation in the presence of proton-transfer mediators and phosphorylation corresponds to a 60-fold difference in the reaction rate

at 100 °C, sulfation was favored over phosphorylation for POS. The dramatic effects of proton-transfer mediators in reducing the activation barrier of sulfation with POS can be rationalized by considering the geometry of the TSs (Figure S15). In the TS of sulfation in the absence of a proton-transfer mediator (TS2 in Figure 5), the lengths of the formed  $O^{(ROH)}-S$  bond and the cleaved  $S-O^{(phosphate\ in\ POS)}$  bond were similar (2.40 and 2.55 Å, respectively; Figure S15B), indicating that this process can be categorized as an  $S_N2$  reaction. The phosphate group of POS existed proximate to ROH in TS2, because protonation of the phosphate group by ROH was a prerequisite for the elimination of the phosphate group. As a consequence, the  $O^{(ROH)}-S-O^{(phosphate\ in\ POS)}$  angle in TS2 (68.1°) deviated significantly from the ideal angle for an  $S_N2$  reaction (180°), leading to destabilization of TS2. In the presence of a proton-transfer mediator, however, the  $O^{(ROH)}-S-O^{(phosphate\ in\ POS)}$  angle was nearly ideal for an  $S_N2$  reaction (167.9–177.5° for TS3–TS5; Figure S15C–E), because the phosphate group was already protonated by the proton-transfer mediator.

In contrast, in the reaction between POSOP 48 and 1, the phosphorylation pathway was favored over sulfation, both thermodynamically and kinetically (Figures 5B and S13–S14). The activation barrier of phosphorylation (TS6) was only 16.58 kcal mol<sup>-1</sup>, which was much smaller than that of sulfation by 48 (30.45 kcal mol<sup>-1</sup>, TS7). The activation barrier of TS6 was also significantly smaller than that of sulfation by POS 45 in the presence of a proton-transfer mediator (24.70–26.43 kcal mol<sup>-1</sup>, TS3–TS5). This energy difference between TS6 (phosphorylation by POSOP) and TS3–TS5 (sulfation by POS) corresponds to about a 60 000-fold kinetic difference at 100 °C. Thus, the phosphorylation pathway through TS6 should be predominant compared to the sulfation pathway through TS3–TS5 even if POS and POSOP coexist in the reaction mixture (Figure 6).

The reactivity difference between POS 41 and POSOP 44 can be explained as follows. As noted above, *O*-sulfation of alcohols by POS can be categorized as an  $S_N2$  reaction. Protonation of the phosphate leaving group in POS by a proton-transfer mediator facilitated the sulfation pathway by enabling the TS geometry favorable for the  $S_N2$  reaction (Figure 5A). In contrast, *O*-phosphorylation by POSOP can be viewed as an  $S_N1$  reaction: the P–O bond in POSOP was cleaved prior to the formation of the  $O^{(ROH)}-P^{(phosphate)}$  bond in the reaction coordinate (Figures S13 and S16A). The exceptionally high ability of POS to act as a leaving group can rationalize this switching of the reaction mechanism. Indeed, the bond order of P–O in POSOP was markedly smaller (0.48) than that of other bonds (0.79, 0.56, and 0.67 for S–O in POSOP, P–O in POS, and S–O in POS, respectively; see Table S2). Moreover, POSOP was a better electrophile than POS: the HOMO–LUMO gap between an alcohol nucleophile and POSOP was smaller than that of POS (Figure S17). Taken together, we may conclude that alcohol *O*-phosphorylation by POSOP is the most favorable pathway among many possible pathways in the developed PEP-K/TBAHS system.

On the basis of these mechanistic investigations, a postulated mechanism for the catalytic phosphorylation of alcohols with the PEP-K/TBAHS system is shown in Figure 6. First, TBAHS catalyst works as a Brønsted acid to protonate PEP-K, generating PEP-H and sulfate  $SO_4^{2-}$  (50). PEP-H then reacts with 50 to afford POS intermediate 41 with the release of pyruvic acid (initiation phase). Although this POS

intermediate itself acts as a sulfuryl donor, in the presence of excess PEP-K, the sulfate anionic oxygen atom of POS 41 selectively reacts with a second PEP-H to produce the active phosphoryl donor, POSOP 44. Nucleophilic addition of the hydroxy group of the substrate alcohol to POSOP 44 occurs at the phosphorus atom to produce phosphate monoester 51 with a concomitant regeneration of POS 41. Finally, 51 protonates PEP-K to afford product phosphate 52 and PEP-H, the latter of which reacts with POS 41 to enter another catalytic cycle (catalysis phase).

Two major features of this method are its high functional group tolerance and single-step introduction of an unprotected phosphate group to various alcohols. Although temperature-sensitive substrates are incompatible with the reaction conditions (i.e., 100 °C), these characteristics should prove advantageous for the late-stage functionalization of bioactive molecules, such as pharmaceuticals.<sup>36</sup> Many bioactive compounds contain hydroxy groups, and phosphorylation of these groups can have a dramatic impact on biological activity and pharmacokinetics.<sup>6,9,10</sup> Therefore, the application of this method to bioactive compounds or their advanced synthetic intermediates is expected to deliver valuable compound libraries with new bioactivities.

Another attractive potential application of this method is the synthesis of radioactive *O*-phosphorylated peptides containing a <sup>32</sup>P-nucleus at a serine/threonine residue. Such peptides are useful for sensitive assays of protein phosphatase activity<sup>30</sup> and anticancer radiotherapy.<sup>37</sup> It is not currently realistic to access radioactive *O*-phosphorylated peptides via chemical synthesis. All available methods are time- and labor-intensive, requiring the preparation of protected serine/threonine building blocks containing <sup>32</sup>P-labeled protected phosphate groups, followed by the merger of these amino acid building blocks via solid-phase peptide synthesis, deprotection, cleavage from the solid phase, and finally HPLC purification. Therefore, the synthesis of radioactive *O*-phosphorylated peptides has thus far relied on kinase-mediated methods using a  $\gamma$ -<sup>32</sup>P-adenosine 5'-triphosphate (ATP) phosphoryl donor. The efficiency of enzymatic methods, however, depends on the nature of the peptide sequence.<sup>38</sup> This restriction significantly narrows the range of available <sup>32</sup>P-labeled peptide sequences. <sup>32</sup>P-labeled PEP is routinely synthesized from readily available  $\gamma$ -<sup>32</sup>P-ATP<sup>39</sup> with pyruvate kinase<sup>40</sup> or phosphoenolpyruvate carboxylase.<sup>41</sup> Using <sup>32</sup>P-PEP as a phosphoryl donor, the application of our phosphorylation method to preassembled peptides would directly produce <sup>32</sup>P-labeled peptides without significant sequence biases.

## CONCLUSIONS

We have developed the first catalytic and chemoselective phosphorylation of alcohols using PEP-K as a phosphoryl donor and TBAHS as a catalyst. This method displays high functional group tolerance and thus enables efficient access to a variety of phosphate monoesters, including functionalized small molecules, carbohydrates, and unprotected peptides. Mechanistic studies suggest that TBAHS plays a significant role as both a Brønsted acid and a nucleophilic activator. This dual activation mode generates an unprecedented mixed anhydride species, POSOP 44, as an active phosphoryl donor, enabling the functional group-tolerant *O*-phosphorylation reactions. Since functionalized phosphate monoesters are prevalent as important chemical entities ranging from biologically active compounds to functional materials in



diverse fields, this operationally simple and efficient synthetic method will broadly contribute to both life science and physical science.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acscentsci.9b01272>.

Materials and methods, Cartesian coordinates of the critical points, spectral data, synthesis of *O*-benzyl phosphosulfate and  $^{31}\text{P}$  NMR change,  $^{31}\text{P}$  NMR and MS spectra, MS analyses of POS and POSOS, Gibbs free energy profiles, geometries of TSs for phosphorylation and sulfation by POS and POSOP, frontier orbitals and their energy levels, list of reaction conditions for optimization of the phosphorylation of 3-phenyl-1-propanol (**1**), bond orders/distances of POS **45** and POSOP **48** (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Authors

**K. Yamatsugu** – Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan;

[orcid.org/0000-0002-4260-414X](https://orcid.org/0000-0002-4260-414X); Email: [yamatsugu@mol.fu-tokyo.ac.jp](mailto:yamatsugu@mol.fu-tokyo.ac.jp)

**M. Kanai** – Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan; [orcid.org/0000-0003-1977-7648](https://orcid.org/0000-0003-1977-7648); Email: [kanai@mol.fu-tokyo.ac.jp](mailto:kanai@mol.fu-tokyo.ac.jp)

### Other Authors

**K. Domon** – Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

**M. Puripat** – Institute for Research Initiatives, Division for Research Strategy, Nara Institute of Science and Technology (NAIST), Ikoma 630-0192, Japan

**K. Fujiyoshi** – Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

**M. Hatanaka** – Institute for Research Initiatives, Division for Research Strategy, Nara Institute of Science and Technology (NAIST), Ikoma 630-0192, Japan; Graduate School of Science and Technology, Data Science Center, NAIST, Ikoma 630-0192, Japan

**S. A. Kawashima** – Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan;

[orcid.org/0000-0002-2946-6627](https://orcid.org/0000-0002-2946-6627)

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acscentsci.9b01272>

### Author Contributions

K.D., S.A.K., K.Y., and M.K. conceived and designed the study. K.D., K.F., and K.Y. performed all the synthetic experiments. M.P. and M.H. performed the DFT calculations. K.D., K.F., M.H., S.A.K., K.Y., and M.K. analyzed the data. K.D., M.H., K.Y., and M.K. cowrote the paper with comments from all the authors.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Christopher W. Adamson for providing useful comments on the manuscript. We acknowledge the computer resources provided by the Academic Center for Computing

and Media Studies (ACCMS) at Kyoto University. This research was supported by JSPS KAKENHI Grant nos. JP17H06442 (M.K.), JP17H06445 (M.H.) (Hybrid Catalysis), and JP17K15420 (K.Y.). This manuscript is dedicated to Prof. Laura L. Kiessling for her 60th birthday.

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