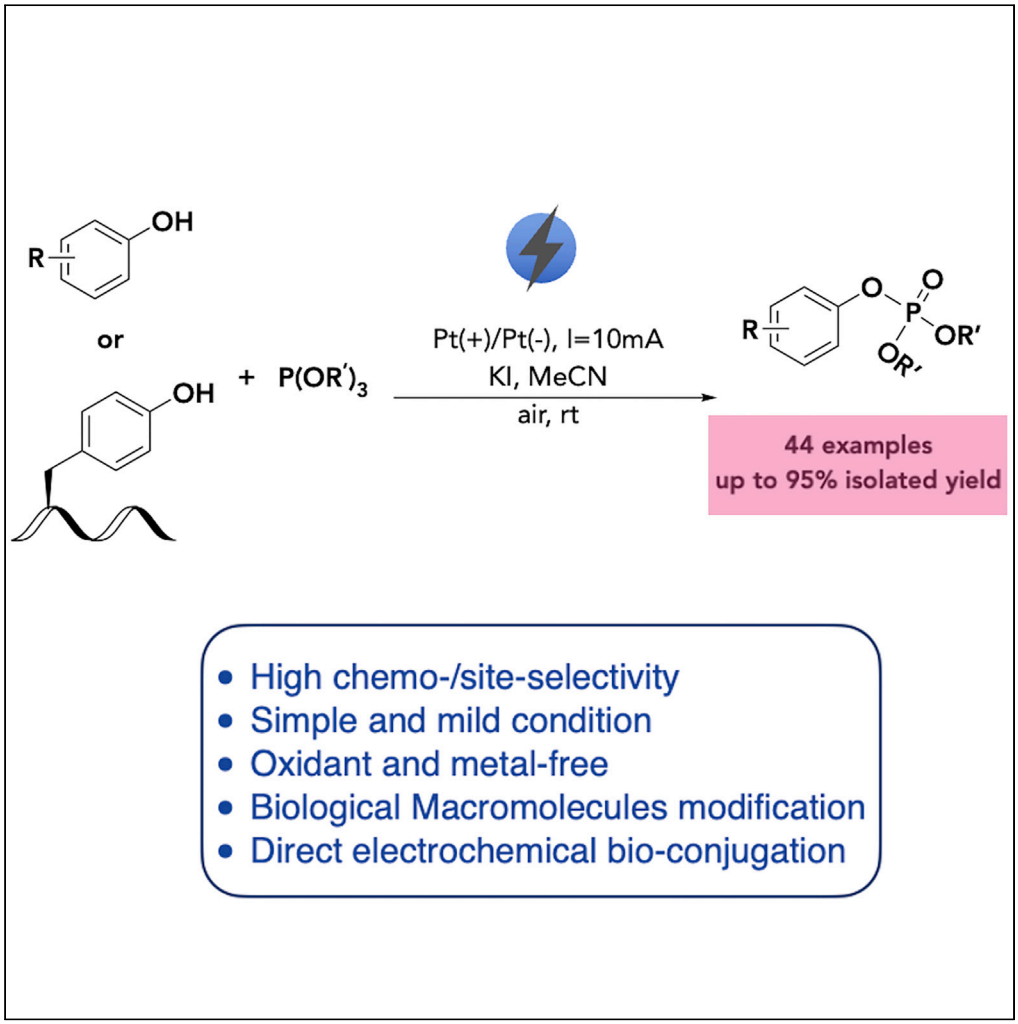
iScience

CellPress
OPEN ACCESS

Article

Electrochemical-induced phosphorylation of arenols and tyrosine containing oligopeptides



Rong Sun, Fan Hu, Haoyang Jiang, ..., Pan Wu, Dingyu Li, Yue Weng

wupan@hubu.edu.cn (P.W.)
dyrei@126.com (D.L.)
wengyue@hubu.edu.cn (Y.W.)

Highlights

High-yield phosphorylation: electrochemical method yields up to 95% organophosphates

Eco-friendly process: uses iodide salts, avoiding toxic oxidants, and bases

Versatile substrate scope: effective for various phenols and tyrosine peptides

Mechanistic insight: reaction proceeds via electrooxidative radical pathway

Sun et al., iScience 27, 110487
September 20, 2024
https://doi.org/10.1016/j.isci.2024.110487

iScience

CellPress
OPEN ACCESS

Article

Electrochemical-induced phosphorylation of arenols and tyrosine containing oligopeptides

Rong Sun,[1] Fan Hu,[1] Haoyang Jiang,[1] Wenbin Du,[1] Chaochao Zhang,[1] Tianhao Chang,[1] Yuling Zhou,[3] Pan Wu,[3,*] Dingyu Li,[2,*] and Yue Weng[1,4,*]

SUMMARY

A disclosed technique employs electrochemical dehydrogenative cross-coupling to create organophosphates, utilizing phosphites compounds with arenols. Inorganic iodide salts serve dual roles as redox catalysts and electrolytes in an undivided cell, omitting the need for external oxidants or bases. Initial mechanistic investigations indicate the reaction unfolds via an electro-oxidative radical pathway, facilitating the formation of P–O bonds, leading to the generation of oxygen radicals in the formation of acetylaminophenol. This environmentally friendly approach offers excellent tolerance to various functional groups, achieves high yields (up to 95% isolated yield), and accommodates a wide range of substrates, showcasing its utility for practical synthesis applications.

INTRODUCTION

Organophosphorus compounds, marked by their oxygen-phosphoryl (O-P) bond, are crucial across a wide spectrum of applications, ranging from medicinal to agricultural chemistry.[1] These compounds, including fosfomycin, a clinically used antibiotic, and the widely produced herbicides glyphosate and glufosinate, play a significant role in healthcare and food production.[2] Their use also extends to organic synthesis and catalysis, as demonstrated by their involvement in Horner-Wadsworth-Emmons reactions, which underlines their essentiality in chemical manufacturing.[2,3]

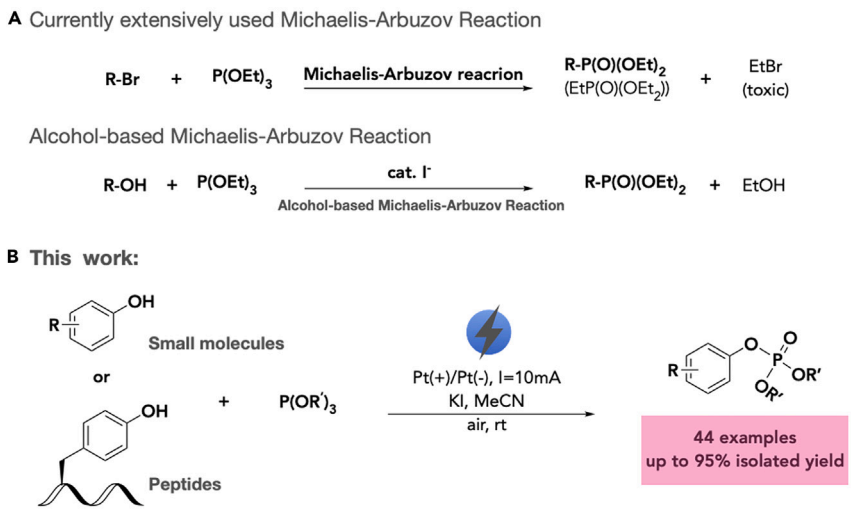
The application of organophosphorus compounds extends to material chemistry, where their effectiveness in metal extraction is well recognized. Additionally, their unique properties are leveraged in the development of eco-friendly fire retardants, reflecting a commitment to safer, and more sustainable chemical practices.[4,5] This wide-ranging utility highlights the critical need for advancing synthesis methods for organophosphorus compounds, particularly those that are environmentally considerate and efficient.

Historically, the synthesis of organophosphates and phosphoramidates-key subclasses of these compounds has depended on conventional methods like direct esterification/amidation. These techniques, often requiring the use of toxic and moisture-sensitive phosphoryl halides, present notable safety, and environmental hazards. Moreover, the Michaelis-Arbuzov reaction, despite its popularity, faces limitations such as the employment of toxic alkyl halides and the production of environmentally unfriendly side products, exacerbating concerns over sustainability and efficiency. In 2018, Prof. Han Li-Biao's team reported an efficient and environmentally friendly alcohol-based Michaelis-Arbuzov reaction.[5] Subsequent years saw further innovations, including Prof. Han Jianlin's team's 2019 report on the electrochemical dehydrogenative phosphorylation of alcohols,[6] and Prof. Wang Jianbo's 2019 report on catalyst-free phosphorylation of aryl halides through electrochemical reduction.[7] In 2021, Prof. Zhou Aihua's team reported on the electrochemical phosphorylation of arenols and anilines, leading to the synthesis of organophosphates and phosphoramidates (Scheme 1).[4]

Recently, electrochemical synthesis has gained traction as an innovative alternative, offering a cleaner, more efficient, and greener approach to complex molecule synthesis, including organophosphorus compounds.[8–10] This method eliminates the necessity for starting material pre-functionalization and the extensive use of toxic oxidants, offering a more streamlined, oxidant-free process.

The introduction of electrochemical techniques for forming P(O)-O bonds marks a significant leap forward in the synthesis of organophosphates. This strategy not only adheres to the principles of green chemistry by minimizing the environmental footprint of chemical processes but also broadens the efficiency and versatility of organophosphorus compound synthesis.[11–20] Considering their widespread applications in areas such as flame retardancy, pest control, pharmaceuticals, materials science, and semiconductor technology, developing more efficient and eco-friendly synthesis methods is crucial.

[1]Hubei Key Laboratory of Precision Manufacturing for Small-molecular Active Pharmaceutical Ingredients, School of Chemistry and Chemical Engineering, State Key Laboratory of Biocatalysis and Enzyme Engineering, Hubei University, Wuhan, P.R. China
[2]Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Qiaokou District, Wuhan, P.R. China
[3]State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Life Sciences, Hubei University, Wuhan 430062, P.R. China
[4]Lead contact
*Correspondence: wupan@hubu.edu.cn (P.W.), dyrei@126.com (D.L.), wengyue@hubu.edu.cn (Y.W.)
https://doi.org/10.1016/j.isci.2024.110487



Scheme 1. Reaction design

(A) Methods for organophosphonate synthesis.

(B) Electrochemically induced phosphorylation method.

RESULTS AND DISCUSSION

Here in, our study aims to establish an innovative, effective, and green electrochemical technique for producing organophosphates and apply it to the post modification of peptides. By applying electrochemical oxidative cross-coupling protocols, we utilize diethyl phosphite and arenols/tyrosine residue in the presence of potassium iodide, serving both as electrolyte and catalyst, to synthesize the desired products under gentle conditions. This method underscores the potential of electrochemical strategies to propel organophosphorus chemistry forward. Through our research, we contribute to the ongoing pursuit of sustainable and new synthetic strategies, ensuring the enduring significance and practicality of organophosphorus compounds in meeting the challenges of contemporary science and technology.

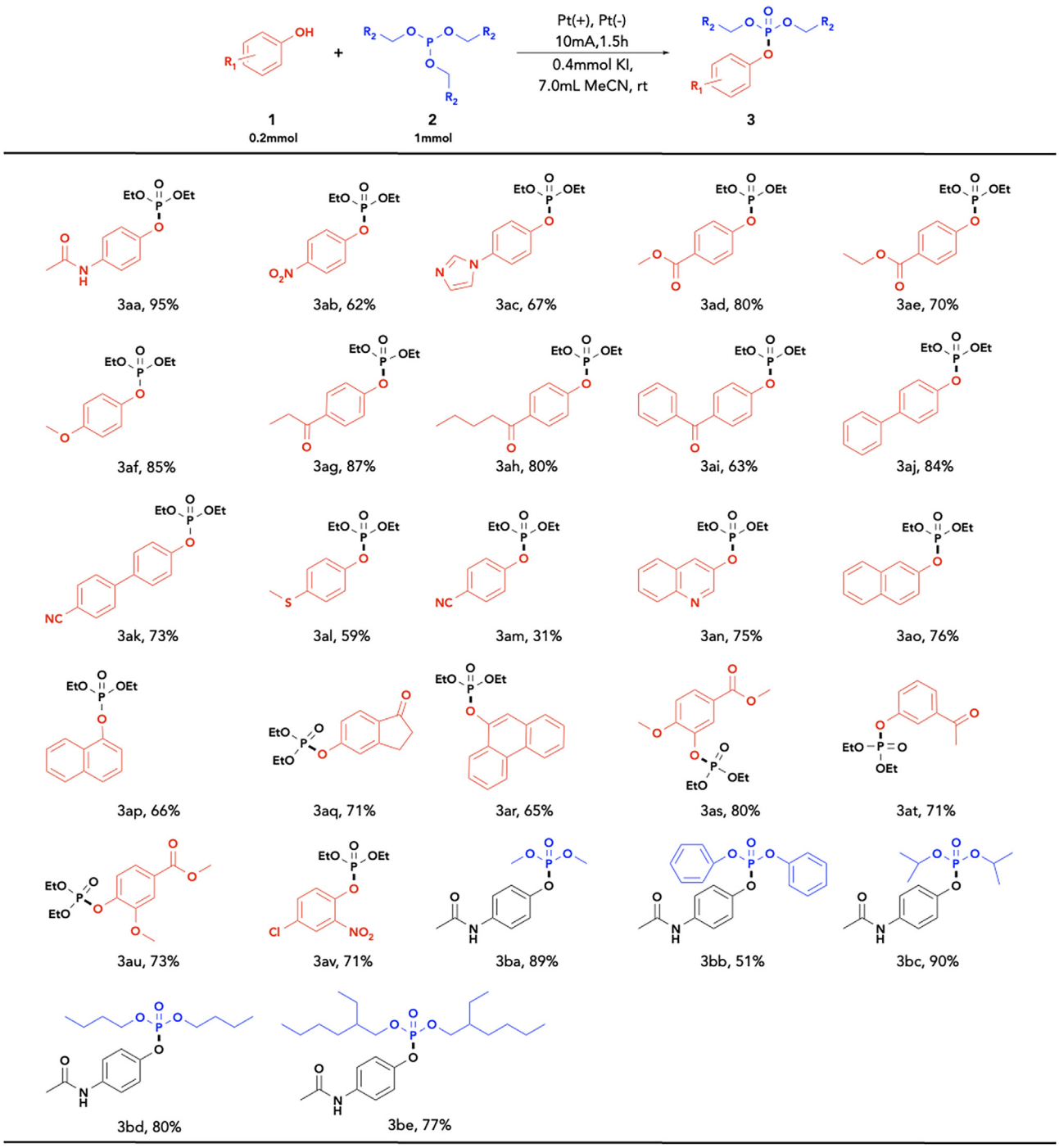
In order to explore the applicability of phenol derivatives, a diverse array of substituted phenols was subjected to reaction with triethyl phosphite under optimized conditions (Scheme 2). Initially, arenols featuring electron-donating or electron-withdrawing groups at the para-position underwent phosphoesterification, resulting in the formation of corresponding phosphates products (3aa-3a.m.) with yields ranging from 31% to 95%, indicating both excellent and good outcomes. Moreover, it was noted that modification of Aromatic ring compounds possessing phenolic hydroxyl groups with triethyl phosphite (3an-3ar) achieved moderate yields, specifically between 65% and 76%. In instances where ortho substituents presented slight steric hindrance to the dehydroesterification process, the resultant products (3as-3av) were still obtained with commendable yields of 71%–80%. Findings revealed that the reaction accommodated phosphites with diverse alkyl lengths, such as triisopropyl phosphite $P(O^{i}Pr)_3$ and tributyl phosphite $P(O^{n}Bu)_3$, both of which were suited for the reaction, producing the desired outcomes with moderate yields. However, when employing substrates with significant steric bulk like triphenyl phosphite $P(OPh)_3$, the efficiency in achieving the targeted product yields was observed to decrease.

Following the exploration of the scope of phenol and phosphite derivatives, the selectivity and tolerance of other amino acids containing tyrosine residues were subsequently investigated. Dipeptides incorporating tyrosine were subjected to the reaction, demonstrating favorable selectivity toward modification (Scheme 3). Notably, di-peptides synthesized with inert amino acids such as glycine (7a), alanine (7b), leucine (7c), isoleucine (7d), and valine (7e) yielded positive results.

Moreover, under electrochemical conditions, compatibility was extended to other oligopeptides, including phenylalanine with its aromatic ring, Boc-protected glutamic acid, Boc-protected aspartic acid, methionine containing a thiol group. This highlights the excellent tolerance and specificity of functional groups under electrochemical conditions. Furthermore, amino acids other than tyrosine were not modified, indicating that the electrosynthesis bioconjugation reaction proceeds with selective, clean, and efficient outcomes, achieving good separation yields.

To achieve modification of biomacromolecules, we investigated the application of electrochemical methods for peptide modification (Scheme 3). Our initial experiments employed the tyrosine-containing pentapeptide YAGFL as a substrate, which, upon reaction with phosphite, was fully converted to the phosphite-labeled product 7a. Subsequently, we explored the suitability of this electrolytic approach for other tyrosine-containing peptide drugs, including β-endorphin, β-endorphin (1–5) amide, conotoxin, thyrotropin-releasing hormone, angiotensin II, enkephalin, and the anticancer agent-2 (MP-2). These peptides, each possessing at least one tyrosine residue at the N-terminal, C-terminal, or within a loop structure, were found to be successfully labeled with phosphite within 20 min at room temperature for all cases (9b–9h).

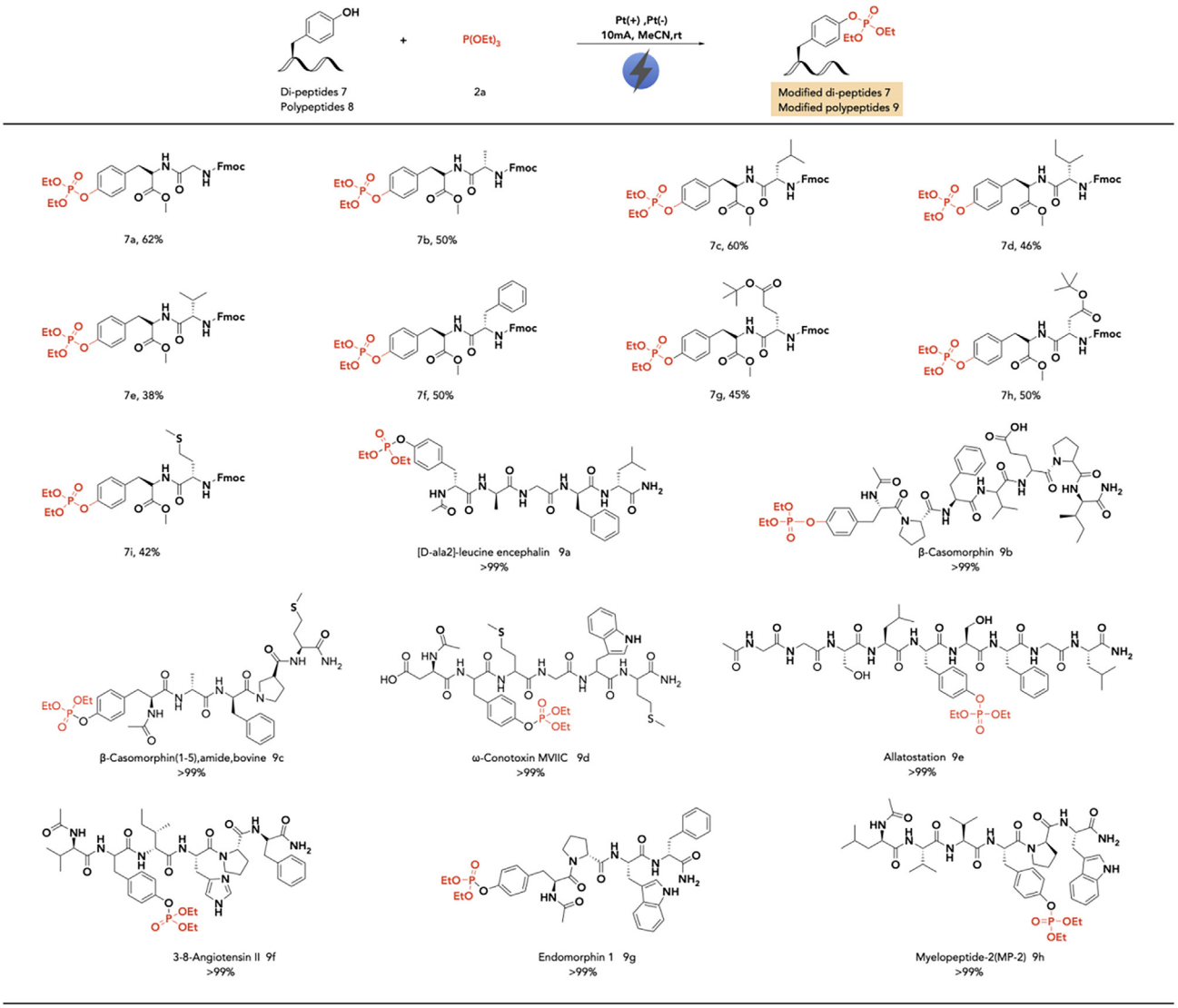
For further demonstrate the practicality and industrial applicability of the reaction, operations were scaled up to the gram level (Scheme 4). A reaction involving 6.0 mmol of 1a and 20 mmol of 2a was conducted with a current of 10 mA for a duration of 22 h. The outcome was notably



Scheme 2. Substrate scope and survey of chemo-selective and functional group tolerance

favorable, yielding 1.2 g of the cross-coupling product with a yield of 68%. The experimental results clearly indicate that the reaction maintains a relatively high efficiency even when scaled up to the gram level.

To investigate the reaction mechanism of interest, a series of control experiments were designed (Scheme 5). Initially, acetylaminophenol and triethyl phosphite $P(OEt)_3$ were employed as reactants under standard conditions, with the addition of radical scavengers DMPO and TEMPO. It was observed that the inclusion of these scavengers significantly reduced the yield, thereby substantiating the radical nature of the reaction. Subsequently, the reaction was conducted in the absence of electricity with iodine I_2 as an additive, yet the desired product was not obtained (iv). This indicates that the oxidation of KI to form iodine does not facilitate the reaction. Furthermore, when diethyl



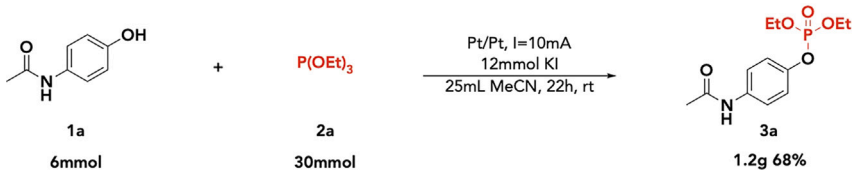
Scheme 3. Substrate scope and survey of oligopeptides

(1) reaction condition of di-peptides: constant current = 10 mA, tyrosine dipeptide 7 (0.20 mmol), triethyl phosphite 2a (1.00 mmol), electrolyte KI (0.4 mmol), reaction solvent MeCN (7.0 mL), non-separation cell, air, room temperature reaction for 2 h, (2) reaction condition of polypeptides: constant current = 10 mA, polypeptide 9 (5mg), triethyl phosphite 2a (30μl), electrolyte KI (20mg), reaction solvent MeCN (3.0 mL), non-separation cell, air, room temperature reaction for 15 min. The conversion rate was quantitatively analyzed using Thermo Proteome Discoverer 2.5 based on the data obtained from mass spectrometry.

phosphite $HPO(OEt)_2$ containing pentavalent phosphorus was reacted with acetylaminophenol under standard conditions, the anticipated phosphoesterification product was not formed (v). This suggests that the reaction does not proceed via oxidation of triethyl phosphite followed by a coupling reaction.

Moreover, cyclic voltammetry (CV) was employed to study the redox potentials of acetylaminophenol 1a, triethyl phosphite 2a, and potassium iodide (KI) as detailed in (Figure 1A). CV shows that potassium iodide presents double oxidation peaks at 0.934V and 1.46V relative to Ag/Ag+. The first oxidation potential of KI is lower than that of acetaminophen. The results show that potassium iodide is first anodized to form free radical intermediates. Consequently, we hypothesize that the iodide ion is oxidized first, which then activates the phenolic hydroxyl group of the acetylamino phenol, leading to the generation of oxygen radicals in the formation of acetylaminophenol.

Based on the results of these mechanistic experiments, a plausible electrooxidative radical reaction pathway was proposed (Figure 1B). Iodide ions are oxidized at the anode to form high-valent iodine species, which activate the phenolic hydroxyl group on tetraacetamide phenol, leading to the formation of the iodo-oxygen intermediate A. Intermediate A then undergoes a reversible homolytic cleavage to produce the oxygen radical intermediate B, which is captured by phosphite, yielding intermediate C. Finally, intermediate C eliminates an alkyl radical to form the target product.



Scheme 4. Gram-scale reaction

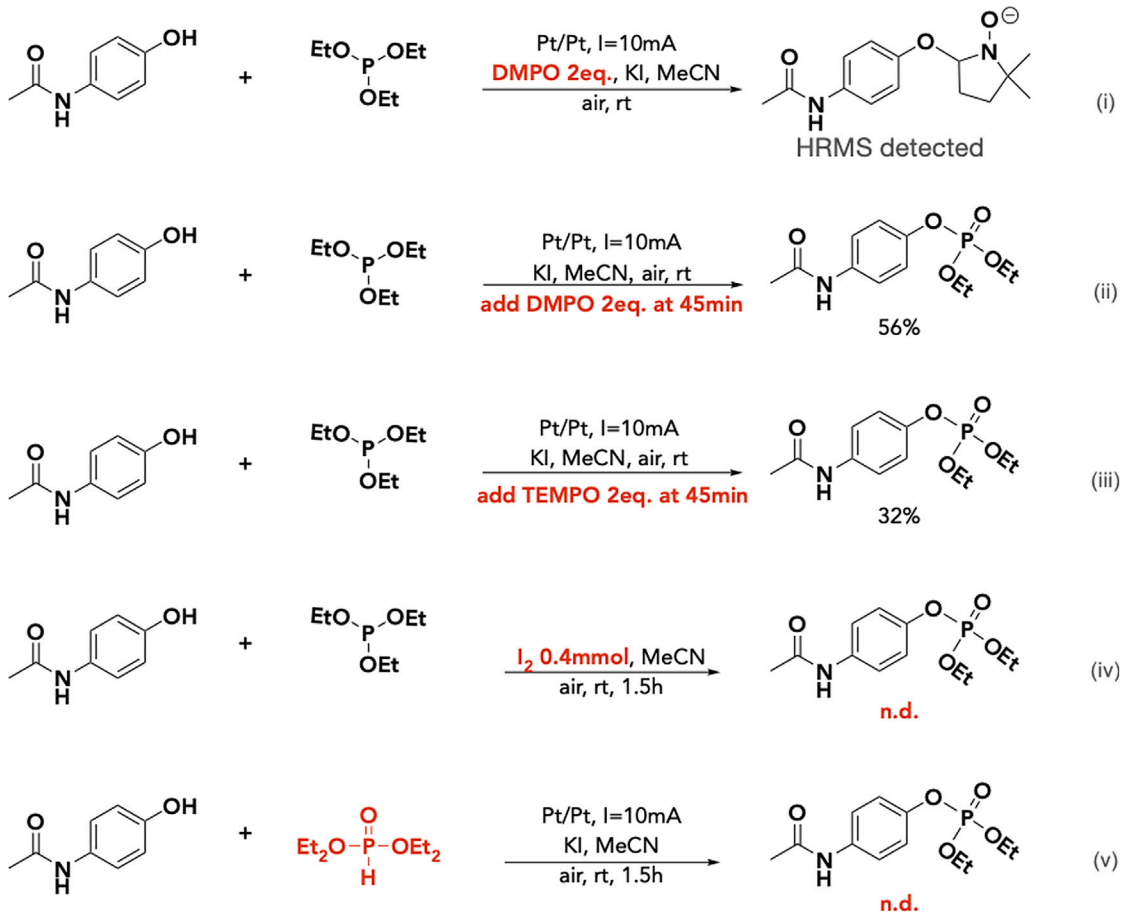
Conclusions

In summary, we have crafted an innovative and environmentally friendly method for producing organophosphates. This method employs an electrochemical oxidative cross-coupling technique, utilizing dialkyl phosphite alongside arenols and tyrosine-containing biomolecules. By incorporating iodide salts as both redox catalysts and electrolytes within acetonitrile, we achieve notable yields of the desired products. Our mechanistic analysis indicates that this reaction unfolds via an electro-oxidative radical pathway, facilitating the formation of P–O bonds. Key advantages of our approach include its rapid execution, the use of gentle conditions, a high tolerance for various functional groups, and a wide applicability to different substrates. Our team is actively pursuing further research into electrochemical transformations to expand upon these findings.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY



Scheme 5. Experiments of mechanism study

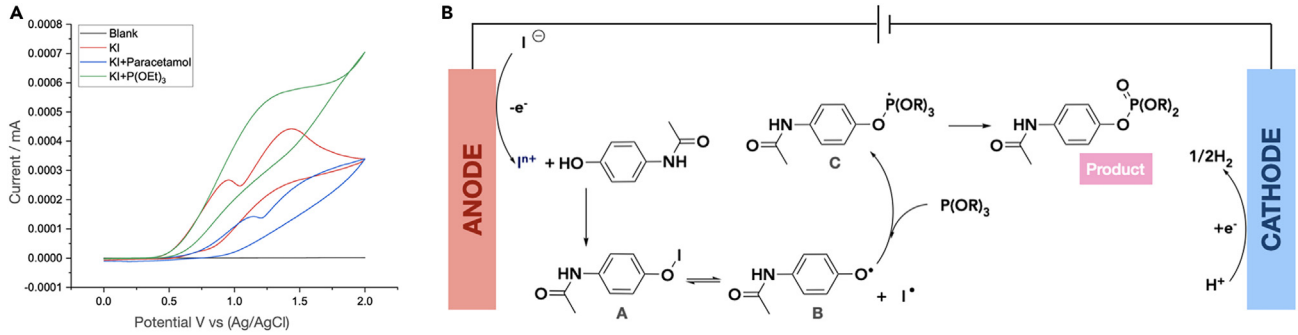


Figure 1. Proposed mechanism

(A) Cyclic voltammograms.

(B)The proposed reaction mechanism.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110487.

ACKNOWLEDGMENTS

The authors are grateful for financial support from the by Open Funding Project of the State Key Laboratory of Biocatalysis and Enzyme Engineering (grant no. SKLBEE2022009, SKLBEE2021027); National Natural Science Foundation of China (grant no. 22301069, 31900051); National & Local Joint Engineering Research Center of High-throughput Drug Screening Technology (K20201001).

AUTHOR CONTRIBUTIONS

R.S., F.H., H.J., W.D., and T.C. conducted the experiments. Y.Z. contributed as a collaborator, while P.W. and D.L. participated in the project. Y.W. oversaw the project, P.W., D.L., and Y.W. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: March 22, 2024
Revised: June 5, 2024
Accepted: July 8, 2024
Published: August 5, 2024

REFERENCES

Afagh, N.A., and Yudin, A.K. (2010). Chemoselectivity and the curious reactivity preferences of functional groups. Angew Chem Int Ed Engl. *49*, 262–310.Long, H., Huang, C., Zheng, Y.T., Li, Z.Y., Jie, L.H., Song, J.S., Zhu, S.B., and Xu, H.C. (2021). Electrochemical C–H phosphorylation of arenes in continuous flow suitable for late-stage functionalization. Nat Commun *12*, 6629.Wang, R., Dong, X., Zhang, Y., Wang, B., Xia, Y., Abdukader, A., Xue, F., Jin, W., and Liu, C. (2021). Electrochemical Enabled Cascade Phosphorylation of N−H/O−H/S−H Bonds with P−H Compounds: An Efficient Access to P(O)-X Bonds. Chem. Eur. J. *27*, 14931–14935.

Budnikova, Y.H., Dolengovsky, E.L., Tarasov, M.V., and Gryaznova, T.V. (2022). Recent advances in electrochemical C-H phosphorylation. Front Chem *10*, 1054116.Huang, Y., and Chen, Q. (2022). Recent Advances in C(sp3)—H Phosphorylation Based on Secondary Phosphine Oxides and Phosphite Esters. Chin. J. Org. Chem. *41*, 4138–4153.

3. Wang, R., Dong, X., Zhang, Y., Wang, B., Xia, Y., Abdukader, A., Xue, F., Jin, W., and Liu, C. (2021). Electrochemical Enabled Cascade Phosphorylation of N-H/O-H/S-H Bonds with P-H Compounds: An Efficient Access to P(O)-X Bonds. Chem *27*, 14931–14935.
4. Zhong, Z., Xu, P., and Zhou, A. (2021). Electrochemical phosphorylation of arenols

and anilines leading to organophosphates and phosphoramidates. Org. Biomol. Chem. *19*, 5342–5347.

5. Ma, X., Xu, Q., Li, H., Su, C., Yu, L., Zhang, X., Cao, H., and Han, L.-B. (2018). Alcohol-based Michaelis–Arbuzov reaction: an efficient and environmentally-benign method for C–P(O) bond formation. Green Chem. *20*, 3408–3413.
6. Deng, L., Wang, Y., Mei, H., Pan, Y., and Han, J. (2019). Electrochemical Dehydrogenative Phosphorylation of Alcohols for the Synthesis of Organophosphinates. J. Org. Chem. *84*, 949–956.
7. Wang, S., Yang, C., Sun, S., and Wang, J. (2019). Catalyst-free phosphorylation of aryl halides with trialkyl phosphites through electrochemical reduction. Chem. Commun. *55*, 14035–14038.
8. Yuan, Y., Liu, X., Hu, J., Wang, P., Wang, S., Alhumade, H., and Lei, A. (2022). Electrochemical oxidative N-H/P-H cross-coupling with H(2) evolution towards the synthesis of tertiary phosphines. Chem. Sci. *13*, 3002–3008.
9. Weng, Y., Wan, C., Sun, R., Jiang, S., Yang, G., and Lei, A. (2023). Electrochemical-induced cross-coupling for organic and bioorganic synthesis. Chem Catal. *3*, 100513.
10. Mackay, A.S., Payne, R.J., and Malins, L.R. (2022). Electrochemistry for the Chemoselective Modification of Peptides and Proteins. J. Am. Chem. Soc. *144*, 23–41.
11. Li, Q.Y., Swaroop, T.R., Hou, C., Wang, Z.Q., Pan, Y.M., and Tang, H.T. (2019). Electrochemical Dehydrogenative Coupling of Alcohols with Hydrogen Phosphoryl Compounds: A Green Protocol for P−O Bond Formation. Adv. Synth. Catal. *361*, 1761–1765.
12. Li, Y., Yang, Q., Yang, L., Lei, N., and Zheng, K. (2019). A scalable electrochemical dehydrogenative cross-coupling of P(O)H compounds with RSH/ROH. Chem. Commun. *55*, 4981–4984.
13. Liu, X., Yin, X., Wu, W., She, Y., Zhao, Q., Zhang, C., Li, M., and Shen, Z. (2022). Electrosynthesis of C−3 Phosphorylated 2H-Indazoles from Trialkyl Phosphites and 2H-Indazoles. J. Electrochem. Soc. *169*, 125501.
14. Wang, Z., Niu, K., Liu, Y., Song, H., and Wang, Q. (2022). Electrochemical alpha-C(sp(3))-H/O-H cross-coupling of isochromans and alcohols assisted by benzoic acid. Chem. Commun. *58*, 10949–10952.
15. Zhong, Z., Xu, P., Ma, J., and Zhou, A. (2021). Electrochemical cross-coupling reactions of sodium arenesulfinates with thioarenols and phenols. Tetrahedron *99*, 132444. https://doi.org/10.1016/j.tet.2021.132444.
16. Tian, Z., Gong, Q., Huang, T., Liu, L., and Chen, T. (2021). Practical Electro-Oxidative Sulfonylation of Phenols with Sodium Arenesulfinates Generating Arylsulfonate Esters. J. Org. Chem. *86*, 15914–15926.
17. Smeyne, D., Verboom, K., Bryan, M., LoBue, J., and Shaikh, A. (2021). Electrochemical esterification via oxidative coupling of aldehydes and alcohols. Tetrahedron Lett. *68*, 152898.
18. Jiang, X., Yang, L., Ye, Z., Du, X., Fang, L., Zhu, Y., Chen, K., Li, J., and Yu, C. (2020). Electrosynthesis of C3 Alkoxylated Quinoxalin-2(1H)-ones through Dehydrogenative C–H/O–H Cross-Coupling. European J. Org. Chem. *2020*, 1687–1694.
19. Song, C., Liu, K., Wang, Z., Ding, B., Wang, S., Weng, Y., Chiang, C.W., and Lei, A. (2019). Electrochemical oxidation induced selective tyrosine bioconjugation for the modification of biomolecules. Chem. Sci. *10*, 7982–7987.
20. Feng, P., Ma, G., Chen, X., Wu, X., Lin, L., Liu, P., and Chen, T. (2019). Electrooxidative and Regioselective C-H Azolation of Phenol and Aniline Derivatives. Angew. Chem. Int. Ed. Engl. *58*, 8400–8404.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Polypeptide drug		
triethyl phosphite	titanci-group	122-52-1
Acetonitrile	titanci-group	75-05-8
KI	titanci-group	7681-11-0

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Yue Weng (wengyue@hubu.edu.cn).

Materials availability

"This study did not generate new unique reagents."

Data and code availability

- Data: Data reported in this paper will be shared by the lead contact upon request.
- Code: This paper does not report original code.
- Additional: Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Reaction optimization

In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, Paracetanol (0.20 mmol), Triethyl phosphite (1.00 mmol), KI (0.40 mmol) and solvent (7 mL) were combined and added.The bottle was equipped Platinum Plate (15 mm × 15 mm × 0.3mm,about 15 mm immersion depth in solution) as the anode and Platinum Plate(15 mm × 15 mm × 0.3mm) as the cathode.The reaction mixture was stirred and electrolyzed at constant current under room temperature. When the reaction finished, the reaction mixture was concentrated.The pure product was obtained by flash column chromatography on silica gel (dichloromethane: methanol = 80:1).

Paracetanol scope and characterization

General Procedure for BioconJugation of Paracetanol and Triethyl phosphite:In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, protected Paracetanol(0.20 mmol), Triethyl phosphite(1.00 mmol), KI(0.40 mmol)and MeCN (7 mL) were combined and added. The bottle was equipped Platinum Plate (15 mm × 15 mm×0.3 mm immersion depth in solution) as the anode and Platinum Plate(15 mm × 15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolyzed at constant current under room temperature. When the reaction finished, the reaction mixture was concentrated. The pure product was obtained by flash column chromatography on silica gel (dichloromethane: methanol = 80:1).

Triethyl phosphite scope and characterization

General Procedure for BioconJugation of Paracetanol and Triethyl phosphite:In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, protected Paracetanol(0.20 mmol), Triethyl phosphite(1.00 mmol), KI(0.40 mmol)and MeCN (7 mL) were combined and added. The bottle was equipped Platinum Plate (15 mm × 15 mm×0.3 mm immersion depth in solution) as the anode and Platinum Plate(15 mm × 15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolyzed at constant current under room temperature. When the reaction finished, the reaction mixture was concentrated. The pure product was obtained by flash column chromatography on silica gel (dichloromethane: methanol = 100:1).

Dipeptide scope and characterization

General Procedure for BioconJugation of dipeptide and Triethyl phosphite:In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, dipeptides(0.20 mmol), Triethyl phosphite(1.00 mmol), KI(0.40 mmol)and MeCN (7 mL) were combined and added. The bottle was equipped Platinum Plate (15 mm × 15 mm×0.3 mm immersion depth in solution) as the anode and Platinum Plate(15 mm × 15 mm× 0.3 mm) as the cathode. The reaction mixture was stirred and electrolyzed at constant current under room temperature. When the reaction finished, the reaction mixture was concentrated. The pure product was obtained by flash column chromatography on silica gel (dichloromethane: methanol = 60:1).

Polypeptide scope and characterization

General Procedure for BioconJugation of polypeptides and Triethyl phosphite: In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, dipeptides(5mg), Triethyl phosphite(20μl), KI(20mg)and MeCN (3mL) were combined and added. The bottle was equipped Platinum Plate (10 mm × 10 mm×0.3 mm) as the anode and Platinum Plate(10 mm × 10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolyzed at constant current of 10 mA under room temperature for 15 min. After completion of the reaction, the solution was analyzed by MALDI-TOF-MS spectroscopy.The reaction was analyzed by reversed-phase HPLC on a 250 mm long ChromCore C18 5μm column using a gradient of 5%–50% buffer B within 30 min. HPLC analysis used buffers A (water +0.1% TFA) and B (9:1 acetonitrile: water +0.1% TFA). Conversion reported as a % conversion as determined.