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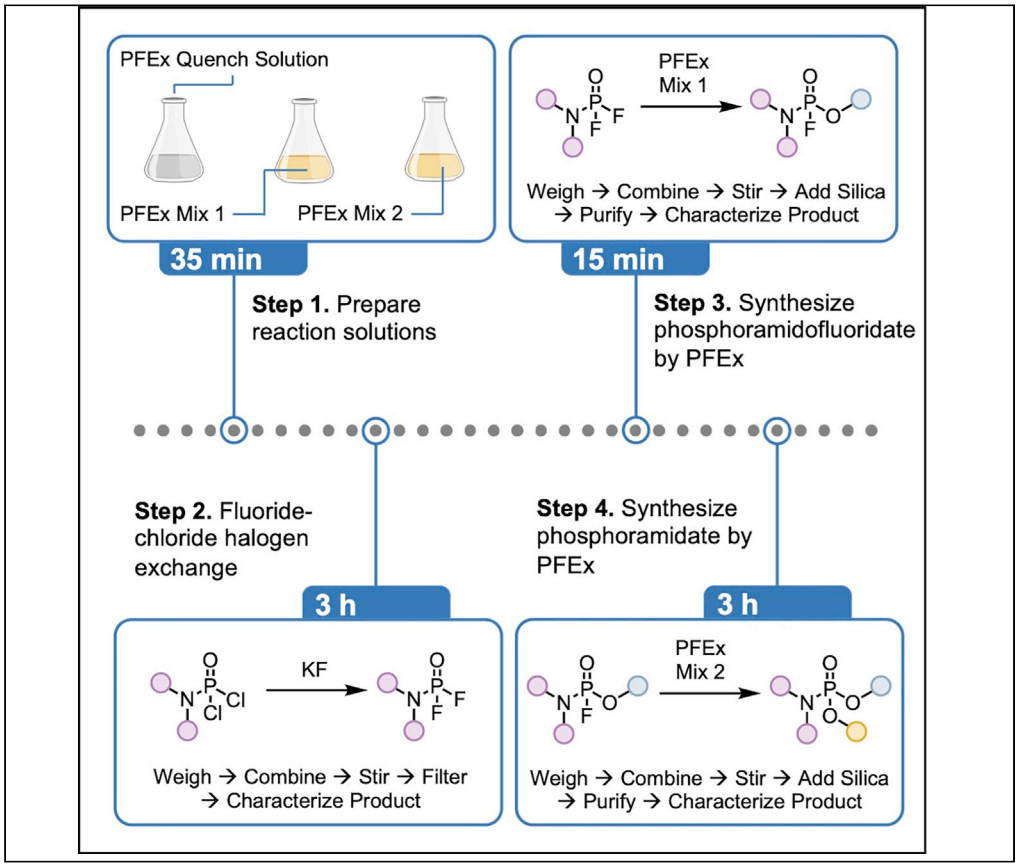
Protocol for producing phosphoramidate using phosphorus fluoride exchange click chemistry



Phosphorus fluoride exchange (PFEx) is a catalytic click reaction that involves exchanging high oxidation state P–F bonds with alcohol and amine nucleophiles, reliably yielding P–O- and P–N-linked compounds. Here, we describe steps for preparing a phosphoramidic difluoride and performing two sequential PFEx reactions to yield a phosphoramidate through careful catalyst selection. We then detail procedures for handling and quenching potentially toxic P–F-containing compounds to ensure user safety when conducting PFEx reactions.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Fluoride-chloride halogen exchange of a phosphoramidic dichloride

Steps outlining two sequential PFEx reactions to generate a phosphoramidate

Details provided regarding compound handling and effective quenching of PFEx substrates

Homer et al., STAR Protocols 5, 102824
March 15, 2024 https://doi.org/10.1016/j.xpro.2023.102824

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Protocol for producing phosphoramidate using phosphorus fluoride exchange click chemistry

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https://doi.org/10.1016/j.xpro.2023.102824

SUMMARY

Phosphorus fluoride exchange (PFEx) is a catalytic click reaction that involves exchanging high oxidation state P–F bonds with alcohol and amine nucleophiles, reliably yielding P–O- and P–N-linked compounds. Here, we describe steps for preparing a phosphoramidic difluoride and performing two sequential PFEx reactions to yield a phosphoramidate through careful catalyst selection. We then detail procedures for handling and quenching potentially toxic P–F-containing compounds to ensure user safety when conducting PFEx reactions.

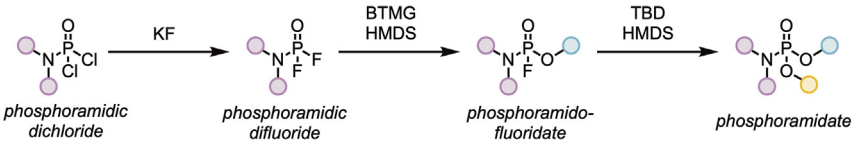
For complete details on the use and execution of this protocol, please refer to Sun et al.[1]

BEFORE YOU BEGIN

Click chemistry is a synthesis-based discovery method that accelerates the design and production of target functional molecules. Mirroring Nature's modular approach to constructing biopolymers via heteroatom bonds—as exemplified in DNA, RNA, proteins, and carbohydrates—click chemistry utilizes robust, efficient, and highly selective reactions to couple molecular modules through an expanding array of linkages.[2–8]

Prominent click reactions, specifically the copper-catalyzed azide-alkyne cycloaddition (CuAAC)[3,4] and sulfur(VI) fluoride exchange (SuFEx)[6,9–12] have emerged as cornerstone methodologies for an array of intricate and complex applications,[13] forging connections through 1,2,3-triazole- and sulfur-centered linkages, respectively. Diversity oriented clicking (DOC),[14–16] a strategy involving the combination of multiple click reactions in sequence, was developed for the application in the library synthesis of novel click structures for biological evaluation. By leveraging a comprehensive array of click transformations, DOC fosters the design of lead-like structures, thereby accelerating the discovery of functional molecules through the more divergent exploration of chemical space.[14,15]

Until recently, phosphorus-centered connections were notably absent from the click chemistry toolbox. The development of phosphorus fluoride exchange (PFEx) click chemistry in 2023[1] bridged this gap, enriching the strategies for molecular assembly and heralding potential advancements in chemical synthesis. The PFEx process showcases impressive versatility, and with careful selection of the right catalyst, PFEx can facilitate stepwise, consecutive P–F exchange reactions on multivalent P–F substrates. This impressive control is further heightened by PFEx's exceptional chemoselectivity, paving the way for sequential click reactions following the SuFEx → PFEx → CuAAC reaction sequence[1]—a feature ideally suited for DOC applications.



Scheme 1. General scheme for a phosphorus fluoride exchange (PFEx) reaction. BTMG = 2-*tert*-butyl-1,1,3,3-tetramethylguanidine, HMDS = hexamethyldisilazane, TBD = 1,5,7-triazabicyclo[4.4.0]dec-5-ene

This protocol delineates the methodological steps for transforming a prototypical phosphoramidic dichloride to its phosphoramidic difluoride counterpart. Subsequently, this compound undergoes a pair of sequential PFEx reactions, yielding the corresponding phosphoramidate, as illustrated in Scheme 1. As is the case with all click reactions, PFEx is typically robust and reliable, but optimal success hinges on the judicious selection of the catalyst and reaction conditions to ensure targeted, comprehensive reactivity and maximal yield.

For reference, we refer the reader to the seminal publication by Sun et al.,[1] where a comprehensive list of compounds synthesized via the PFEx reaction has been documented, including details regarding amine-based PFEx reactions not covered in this protocol. This seminal publication demonstrates the broad applicability of the PFEx reaction and serves as an invaluable resource for researchers navigating this field.

⚠ CRITICAL: The PFEx protocol mandates synthesizing and handling organophosphorus entities, particularly those with electrophilic P–X bonds. It's paramount to underscore that while organophosphorus compounds harbor considerable promise in drug development, they can also manifest pronounced toxicological profiles. Specifically, compounds possessing P–F bonds have the potential to act as covalent nerve agents. Such substances can interact with the active site serine residues of acetylcholinesterase (AChE), a crucial enzyme involved in the termination of synaptic transmission.[17,18] Consequently, this interaction disrupts the enzyme's functionality, which can induce muscle paralysis, seizures, and, in severe instances, fatal respiratory failure.

Therefore, before engaging in any operations involving P–F-containing compounds, researchers should thoroughly familiarize themselves with the pertinent literature[19] and strictly adhere to the safety and quenching protocols detailed within this document to ensure the success of the chemical transformations and the safety and well-being of those performing the work. Any deviations from this work should be accompanied by a thorough risk assessment, as one would for any chemical synthesis, and we encourage readers to refer to the American Chemical Society's policy on chemical management and safety (https://www.acs.org/policy/publicpolicies/sustainability/chemicalsmanagement.html).

Preparation of the reagents and equipment

Note: Please be advised that the following procedures, including the weighing of PFEx substrates, must be conducted within a fully functional fume hood to ensure proper ventilation and safety.

Preparation of PFEx quenching solution

Timing: 15 min

1. Add 750 mL of deionized (DI) water to a 2000 mL conical flask.
2. Carefully add an appropriately sized stir bar. We recommend a 50 mm × 8 mm stir bar.
3. Weigh out 60.0 g (1.50 mol) of NaOH and add this to the conical flask (Figure 1A).

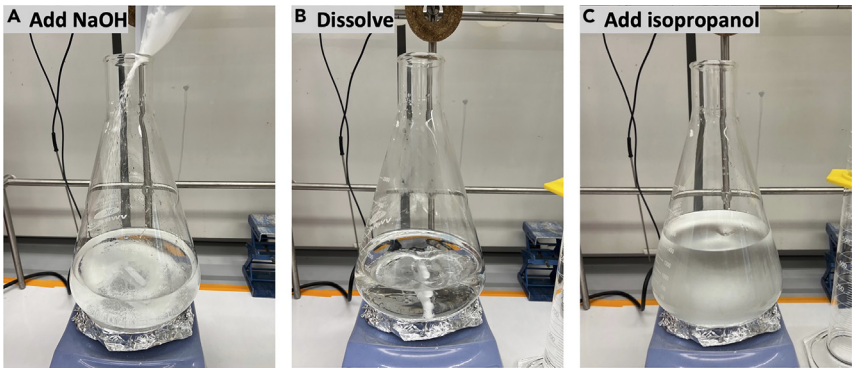


Figure 1. Preparation of the PFEx Quenching Solution

4. Stir the solution until the solid has completely dissolved (Figure 1B) – this solution has a concentration of 2.0 $molL^{-1}$.
5. Add 750 mL of isopropanol (Figure 1C), generating the PFEx Quenching Solution.

⚠ CRITICAL: The caustic quench solution is potentially hazardous and could cause skin burns and eye damage. Wear appropriate personal protective equipment (PPE), including safety glasses, gloves, and a lab coat, while handling to minimize the risk of exposure. Latex gloves are best suited when working with the PFEx Quenching Solution.

6. Prepare a plastic wash bottle (~250 mL) filled with the PFEx Quenching Solution and keep it readily accessible to quickly neutralize any spilled reaction mixtures.
7. Transfer the remaining solution (~1250 mL) into a large plastic tray, which should be maintained within a fume hood.
   a. All glassware and equipment used during this protocol should be soaked in a bath of the PFEx quenching solution for a minimum of 24 h to ensure adequate decomposition of electrophilic P–F compounds. Refer to supplementary information for relevant quenching experiments (Figure S1).

Note: The PFEx Quenching Solution may be safely stored under ambient conditions for up to one week (7 days). After usage, ensure proper disposal of the PFEx Quenching Solution by following institutional guidelines for hazardous waste management.

Preparation of PFEx mix 1 (BTMG + HMDS)

Timing: 10 min

8. Add 10 mL of MeCN to a 12 mL vial.
9. Add 161.2 µL (0.80 mmol) of BTMG.
10. Add 838.4 µL (4.0 mmol) of HMDS.
11. Seal the vial and invert slowly several times to ensure thorough mixing.
    a. Use 2.50 mL of PFEx Mix 1 for every 1.00 mmol of phosphoramidic difluoride.

Preparation of PFEx mix 2 (TBD + HMDS)

Timing: 10 min

12. Add 10 mL of MeCN to a 12 mL vial.
13. Add 111.4 mg (0.8003 mmol) of TBD.

14. Seal the vial and invert slowly several times until the catalyst fully dissolves.
15. Add 1006 μL (4.8 mmol) of HMDS.
16. Seal the vial and invert slowly several times to ensure thorough mixing.
    b. Use 2.50 mL of PFEx Mix 2 for every 1.0 mmol of phosphoramidofluoridate.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
KF, anhydrous 99%	Beantown Chemical (USA)	CAS: 7789-23-3
Acetone, anhydrous	VWR (USA)	CAS: 67-64-1
Phenol	Alfa Aesar (USA)	CAS: 108-95-2
2-*tert*-Butyl-1,1,3,3-tetramethylguanidine (BTMG)	Ambeed, Inc. (USA)	CAS: 29166-72-1
Hexamethyldisilazane (HMDS)	Fisher Scientific (USA)	CAS: 999-97-3
Acetonitrile, anhydrous	Thermo Scientific (USA)	CAS: 75-05-8
3,5-Dimethylphenol	TCI (USA)	CAS: 108-68-9
1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD)	Sigma-Aldrich (USA)	CAS: 5807-14-7
NaOH	VWR (USA)	CAS: 1310-73-2
Isopropanol	VWR (USA)	CAS: 67-63-0
Other		
Vial (12 mL)	VWR (USA)	P/N: 66011-530
Round-bottom flask (100 mL)	Fisher Scientific (USA)	P/N: 31501109
Conical flask (2000 mL)	VWR (USA)	P/N: 10545-844
Rotary evaporator	IKA (USA)	P/N: 0010005195
Vacuum pump	Edwards Vacuum (USA)	P/N: A65401906
Wash bottle (for PFEx Quenching Solution)	VWR (USA)	P/N: 414004-227
Plastic tray (for PFEx Quenching Solution)	Amazon (USA)	P/N: B01MR7L1VD
Magnetic stir bars (50 mm × 8 mm, 15 × 8 mm, 10 mm × 5 mm)	Amazon (USA)	P/N: B08VW4LPHS
Stirring/heating plate	IKA (USA)	P/N: 3810001
Biotage Selekt flash purification system[a]	Biotage (USA)	P/N: SEL-2SW5
Biotage Sfär silica D column (5 *g*)[a]	Biotage (USA)	P/N: FSRD-0445-0005
NMR tube, disposable, 7″ L, 100 MHz	Wilmad-LabGlass	P/N: WG-1000-7

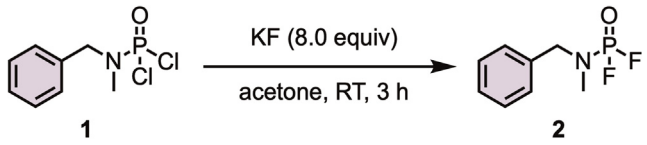
[a]Flash column chromatography techniques can be used as an alternate to automated purification systems.

STEP-BY-STEP METHOD DETAILS

Part 1: Synthesis of the phosphoramidic difluorides by fluoride-chloride halogen exchange

Timing: 3 h

The following section details the synthesis of a representative phosphoramidic difluoride for use as a PFEx substrate. The process begins with the preparation of phosphoramidic dichloride **1** according to a method described in the referenced literature.[20] Subsequently, a fluoride-chloride halogen exchange is performed, elaborated below, to generate phosphoramidic difluoride **2** (refer to Scheme 2). For a comprehensive understanding of this transformation, including its full scope, readers are encouraged to consult the original work by Sun and co-workers.[1]



Scheme 2. Synthesis of the phosphoramidic difluoride 2 by fluoride-chloride halogen exchange

Table 1. Preparation of phosphoramidic difluoride 2

Reagent	MW (g/mol)	m (g)	n (mmol)	Equiv	V (mL)	Conc (M)	Yield (%)
Phosphoramidic dichloride **1**[a]	238.05	1.19	5.00	1.0			
KF	58.10	2.32	40.0	8.0			
Acetone					40.0	0.125	
Phosphoramidic difluoride **2**	205.14	0.870	4.20				85

[a]Prepared according to the protocol described by Flader and co-workers.[20]

1. Prepare the phosphoramidic difluoride **2** via fluoride-chloride exchange (Table 1).

⚠ CRITICAL: It is important to note the potential toxicity of phosphoramidic difluorides. When preparing these compounds, one should avoid generating potentially low-boiling compounds. All phosphoramidic difluorides should be handled in a dedicated fume hood. Double gloving (i.e., wearing a latex glove over a nitrile glove) may be considered for added safety. All used glassware and equipment must be treated with the PFEx quenching protocol, as detailed below.

a. Weigh 1.19 g (5.00 mmol) of phosphoramidic dichloride **1** into a 100 mL round-bottom flask. Add an appropriately sized stir bar (Figure 2A). We recommend a 15 mm × 8 mm stir bar.
   i. Take care to prevent exposure to the substrate; weighing should occur in a pre-weighed and sealed reaction vessel and be moved between the balance and fume hood as needed.
b. Add 40.0 mL of anhydrous acetone (0.125 M) to the flask in a well-ventilated fume hood.
c. Turn on the stirrer plate, agitating the solution by stirring.
d. Once substrate **1** is completely dissolved, add 2.32 g (8.00 equiv, 40.0 mmol) of KF to the solution in one portion (Figure 2B).

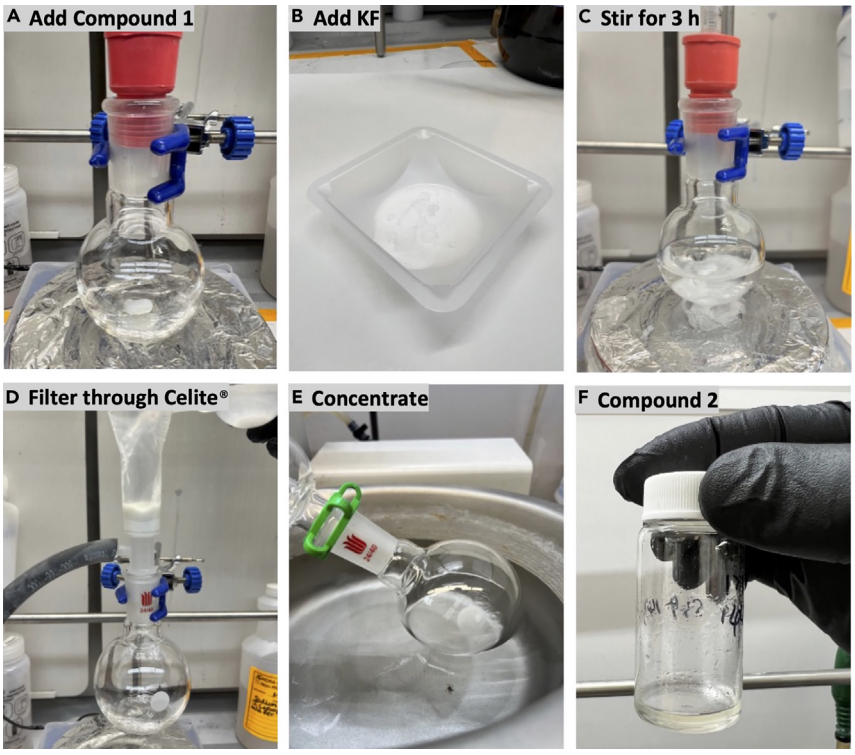


Figure 2. Preparation of phosphoramidic difluoride 2

e. Secure the reaction vessel in a nitrogen-rich environment by sealing it with a rubber septum and a balloon filled with nitrogen or through a direct connection to a nitrogen manifold.
f. Allow the reaction to stir at 21°C for 3 h (Figure 2C).
g. To prevent exposure to the P–F-containing compounds, monitoring the progress of the reaction by thin-layer chromatography (TLC) should be avoided. Instead, monitor the reaction by LC-MS analysis to confirm the complete consumption of the starting material. To do this, take a 50 μL aliquot from the reaction mixture and dilute this with 0.5 mL of MeCN.

Note: Depending on the chromatography column and/or run conditions used, a difference between the retention time of **1** and **2** may not be observed. Hence, we recommend monitoring the changes in the mass spectrum. For this example, the mass of starting material **1** (m/z $[M + H]^+$ = 238.00) disappears, and the mass of product **2** (m/z $[M + H]^+$ = 206.05) is formed.

h. Filter the reaction mixture through a short pad of Celite, collecting the filtrate in a round-bottom flask (Figure 2D). This will remove the suspended salts (unreacted KF and the KCl byproduct).
i. Keeping the flask sealed, move the reaction to a rotary evaporator dedicated to toxic reactions (i.e., located in a fume hood). Concentrate the reaction mixture under reduced pressure (Figure 2E).
    i. Remove residual solvents using a high vacuum located in a fume hood.
j. Characterize the resulting phosphoramidic difluoride **2** without further purification by ^{1}H, ^{31}P, and ^{19}F NMR analysis (Figure 2F).
    i. Disposable grade NMR tubes should be used. Tubes should be transported to the NMR machine using secondary containment to reduce the risk. Extreme care must be taken.

Note: The shelf-life of phosphoramidic difluorides is limited (several hours at 21°C or up to 3 days at −20°C). These compounds should be converted to the more stable phosphoramidofluoridates as soon as possible – see below for the PFEx procedure.

k. All glassware (including NMR tubes) and equipment used in the above protocol should be treated using the premade PFEx Quenching Solution for at least 24 h.

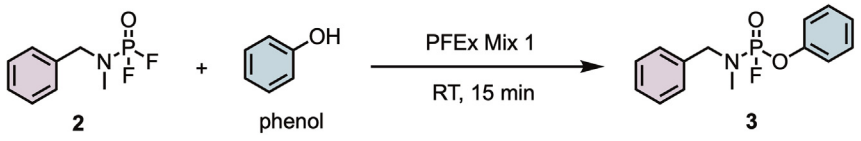
Part 2: Synthesis of phosphoramidofluoridates by PFEx reaction

Timing: 15 min

In this part, we detail the PFEx reaction of phosphoramidic difluorides with aryl alcohols to generate the corresponding phosphoramidofluoridates. Phosphoramidofluoridate **3** will be prepared as an example (Scheme 3). The full scope of this transformation is described in Sun et al.[1]

2. Prepare phosphoramidofluoridate **3** from phosphoramidic difluoride **2** (Table 2).

⚠ CRITICAL: It is essential to be aware of the potential toxicity of phosphoramidic difluorides and phosphoramidofluoridates when working with these compounds. To mitigate



Scheme 3. Synthesis of phosphoramidofluoridate 3 by PFEx reaction

Table 2. Preparation of phosphoramidofluoridate 3

Reagent	MW (g/mol)	m (mg)	n (mmol)	Equiv	V (mL)	Conc (M)	Yield (%)
Phosphoramidic difluoride **2**	205.14	205.1	1.000	1.00			
Phenol	94.11	94.1	1.00	1.00			
PFEx Mix 1					2.5	0.40	
Phosphoramidofluoridate **3**	279.25	253.0	0.9060				90

risks, avoid creating low-boiling derivatives that can easily vaporize. Always handle these compounds within a dedicated fume hood to prevent inhalation of toxic fumes. Double gloving (i.e., wearing a latex glove over a nitrile glove) may be considered for added safety. Furthermore, ensure that all glassware and equipment used in the preparation process are thoroughly quenched following the procedures outlined below.

a. Weigh 205.1 mg (1.000 mmol) of the required phosphoramidic difluoride **2** (prepared in Part 1) into a 12 mL vial, being careful to prevent exposure to the substrate.
   i. Pre-weigh the empty, capped vial and move the vessel between the balance and fume hood while weighing out compound **2**.
b. Add an appropriately sized stir bar (Figure 3A). We recommend a 10 mm × 5 mm stir bar.
c. Weigh 94.1 mg (1.00 mmol) of phenol (Figure 3B) and add this to the vial in one portion.
d. Add 2.50 mL of PFEx Mix 1 (containing BTMG and HMDS) to the vial (Figure 3C).
e. Begin agitating the reaction mixture at 21°C. The solution will initially be clear (Figure 3D).
f. Allow the reaction mixture to stir for 15 min. The solution will become cloudy (Figure 3E).
g. Monitor the progress of the reaction by LC-MS analysis to confirm the complete consumption of the starting material **2**. To do this, take a 50 µL aliquot from the reaction mixture and dilute this with 0.5 mL of MeCN.

Note: For this example, the mass of starting material **2** (m/z $[M + H]^+$ = 206.05) disappears, and the mass of product **3** (m/z $[M + H]^+$ = 280.09) is formed.

h. Add a small amount of silica gel (approximately 1 g is sufficient) to the reaction vial (Figure 3F). Transfer this slurry to a 100 mL round-bottom flask.
i. Move the reaction flask to a rotary evaporator dedicated to toxic reactions (i.e., located in a fume hood). Keep the reaction sealed when moving between fume hoods. Concentrate the reaction mixture under reduced pressure (Figure 3G).
j. Purify the product using a Biotage Selekt Flash Purification System (or equivalent) housed in a fume hood (Figure 3H), eluting with ethyl acetate and hexane. Flash column chromatography techniques can be used as an alternative.
   i. Dispose of Biotage cartridges and silica gel after use in a dedicated waste stream.
k. Characterize the resulting compound by ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR analyses and high-resolution mass spectrometry (HRMS).
   i. NMR tubes should be transported to the NMR machine in a secondary container to reduce the risk of spillage.
l. Dry the purified phosphoramidofluoridate **3** on a dedicated toxic rotary evaporator and high vacuum to avoid potentially exposing yourself to the P–X-containing compounds (Figure 3I).
m. Quench all the glassware (including NMR tubes) and equipment used in the above protocol by soaking in the premade PFEx Quenching Solution for a minimum of 24 h.

Part 3: Synthesis of phosphoramidates by PFEx reaction

Timing: 3 h

In this part, we detail the steps required to accomplish a successful PFEx reaction between a phosphoramidofluoridate and an aryl alcohol nucleophile. The reaction affords a phosphoramidate

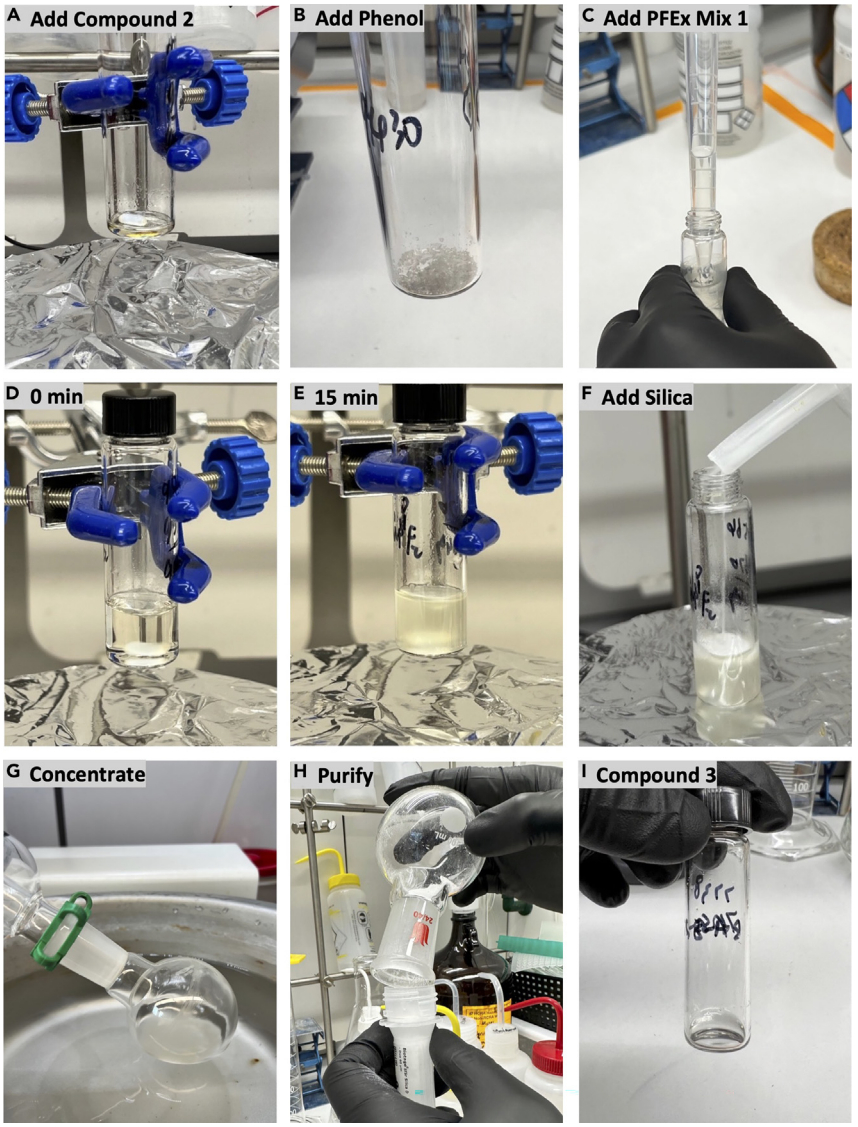
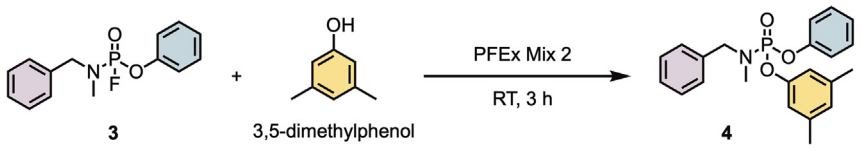


Figure 3. Preparation of phosphoramidofluoridate 3

product – the product of two successive PFEx reactions. Phosphoramidate **4** will be prepared as an example (Scheme 4). A video demonstration of this reaction can be found on YouTube. The full scope of this transformation is described in Sun et al.[1]

3. Preparation of phosphoramidate **4** from phosphoramidofluoridate **3** (Table 3).

⚠ CRITICAL: It is important to note the potential toxicity of phosphoramidofluoridates. When preparing these compounds, one should avoid generating low-boiling compounds,



Scheme 4. Synthesis of phosphoramidate 4 by PFEx reaction

Table 3. Preparation of phosphoramidate 4

Reagent	MW (g/mol)	m (mg)	n (mmol)	Equiv	V (mL)	Conc (M)	Yield (%)
Phosphoramidofluoridate **3**	279.25	223.3	0.7996	1.000			
3,5-Dimethylphenol	122.16	117.3	0.9602	1.201			
PFEx Mix 2					2.0	0.40	
Phosphoramidate **4**	381.41	283.8	0.7441				93

and all compounds should be handled in a dedicated fume hood. Double gloving (i.e., wearing a latex glove over a nitrile glove) may be considered for added safety. All glassware and equipment used should be quenched, as detailed below.

a. Weigh 223.3 mg (0.7996 mmol) of the required phosphoramidofluoridate **3** (prepared in Part 2) into a 12 mL vial, being careful to minimize exposure to the substrate.
   i. Pre-weigh the sealed vial and move the vessel between the balance and fume hood while weighing out compound **3.**
b. Add an appropriately sized stir bar. We recommend a 10 mm × 5 mm stir bar.
c. Weigh 117.3 mg (0.9602 mmol) of 3,5-dimethylphenol and add this to the reaction vial.
d. Add 2.00 mL of PFEx Mix 2 containing TBD and HMDS to the vial. Begin agitating the reaction mixture.
e. Allow the reaction to stir for 3 h. The solution will become yellow in color.
f. Monitor the reaction by LC-MS analysis to confirm the complete consumption of the starting material **3.** To do this, take a 50 μL aliquot from the reaction mixture and dilute this with 0.5 mL of MeCN.

Note: For this example, the mass of starting material **3** (m/z $[M + H]^+$ = 280.09) disappears, and the mass of the product **4** (m/z $[M + H]^+$ = 382.16) is formed.

g. When the reaction is deemed to be complete, add a small amount of silica gel (approximately 1 g is sufficient) to the reaction vial. Transfer this slurry to a 100 mL round-bottom flask.

Note: Now that all the electrophilic P–X bonds have been replaced with electron-donating aryl alcohol groups, the reactivity of the phosphorus core is significantly decreased, reducing the overall toxicity of the compounds.

h. Concentrate the reaction mixture under reduced pressure on a rotary evaporator.
i. Purify the product using a Biotage Selekt Flash Purification System (or equivalent), eluting with ethyl acetate and hexane. Flash column chromatography techniques can be used as an alternative.
j. Characterize the resulting compound by ^{1}H, ^{13}C, and ^{31}P NMR analyses and HRMS.

EXPECTED OUTCOMES

Following this protocol will see the generation of three compounds: phosphoramidic difluoride **2** (as a colorless oil in 85% yield (870 mg, 4.2 mmol), phosphoramidofluoridate **3** as a colorless oil in 90% yield (253 mg, 0.906 mmol) and phosphoramidate **4** as a colorless solid in 93% yield (284 mg, 0.745 mmol).

QUANTIFICATION AND STATISTICAL ANALYSIS

General information

^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded on a Bruker Ascend 400 (400 MHz) instrument as dilute solutions in the stipulated solvent. Disposable grade NMR tubes were used (refer to key resources table for product information). HRMS were obtained using an Agilent 6530 accurate-mass

Q-TOF LC/MS in electrospray ionization (ESI) mode or a Thermo Fisher Scientific Q Exactive HF utilizing a heated electrospray ionization (HESI-II) probe.

Note: Likely NMR impurities may include acetone (δ 2.17 (s)), MeCN (δ 2.10 (s)), ethyl acetate (δ 1.26 (t), 2.05 (s), 4.12 (q)), hexane (δ 0.88 (t), 1.26 (m)).

The recorded NMR spectra are included in the supplemental information.

Phosphoramidic difluoride 2

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.29 (m, 5H), 4.29 (d, *J* = 11.3 Hz, 2H), 2.67 (appt. dt, *J* = 10.8, 1.4 Hz, 3H) (Scheme 2).

^{31}P NMR (162 MHz, $CDCl_3$) δ 2.4 – -10.1 (m).

^{19}F NMR (376 MHz, $CDCl_3$) δ -79.3 (d, *J* = 1004.9 Hz).

Note: Limited stability precludes complete characterization. NMR analysis should be conducted promptly after synthesis.

Phosphoramidofluoridate 3

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (t, *J* = 8.0 Hz, 2H), 7.34–7.28 (m, 3H), 7.27–7.21 (m, 5H), 4.33–4.28 (m, 2H), 2.68 (dd, *J* = 10.4, 1.6 Hz, 3H) (Scheme 3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 150.1 (d, *J* = 6.0 Hz), 136.3 (d, *J* = 10.1 Hz), 130.1, 128.8, 128.2, 128.0, 125.7, 120.0 (d, *J* = 5.1 Hz), 53.1 (d, *J* = 5.6 Hz), 33.1 (d, *J* = 4.6 Hz).

^{31}P NMR (162 MHz, $CDCl_3$) δ 0.3 (d, *J* = 973.5 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -75.4 (d, *J* = 973.5 Hz).

HRMS (ESI$^+$) calculated for $C_{14}H_{16}FNO_2P$ $[M + H]^+$: m/z = 280.0897, m/z found 280.0895.

Phosphoramidate 4

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.32 (m, 2H), 7.29–7.27 (m, 2H), 7.26–7.23 (m, 3H), 7.20–7.15 (m, 3H), 6.88 (s, 2H), 6.81 (s, 1H), 4.36–4.24 (m, 2H), 2.67 (d, *J* = 10.4 Hz, 3H), 2.29 (s, 6H) (Scheme 4).

^{13}C NMR (101 MHz, $CDCl_3$) δ 151.0 (d, *J* = 7.0 Hz), 150.7 (d, *J* = 7.0 Hz), 139.6, 137.1 (d, *J* = 4.6 Hz), 129.8, 128.5, 128.3, 127.5, 126.7, 125.0, 120.4 (d, *J* = 4.9 Hz), 117.9 (d, *J* = 4.9 Hz), 113.2, 53.3 (d, *J* = 4.9 Hz), 33.4 (d, *J* = 3.6 Hz), 21.4.

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.1 (s).

HRMS (ESI$^+$) calculated for $C_{22}H_{25}NO_3P$ $[M + H]^+$: m/z = 382.1566, m/z found 382.1565.

LIMITATIONS

PFEx is a highly adaptable click reaction, and the outlined protocols are efficacious in coupling a variety of P–F-containing substrates with aryl alcohol nucleophiles. Nevertheless, as is common with many chemical processes, certain substrate combinations might necessitate extended reaction durations to reach completion. Furthermore, some particularly challenging substrate pairs might require enhanced reaction optimization strategies, such as increased temperatures or catalyst loadings. These scenarios should be assessed and addressed on an individual basis, tailoring the process to ensure optimal reaction efficiency and product yield.

For guidance on PFEx reactions involving other P–F substrate classes, refer to the work by Sun et al.[1]

TROUBLESHOOTING

Problem 1

Silica has a tendency to cause bumping when placed under vacuum on the rotary evaporator (relates to Part 2 and Part 3).

Potential solution

When reducing pressure on the rotary evaporator, do not go much lower than the boiling pressure of MeCN (226 mbar with a 40°C water bath). Drop the pressure slowly and ensure even, rapid rotation. As an extra layer of protection, use either a fritted adaptor or fill an adaptor with cotton wool to prevent silica from bumping up the rotary evaporator.

Problem 2

PFEx reaction of phosphoramidic difluoride **2** with phenol gives multiple compounds (relates to Part 2).

Potential solution

Ensure the correct PFEx solution is used (i.e., PFEx Mix 1). If PFEx Mix 2 is accidentally used, a competing second PFEx reaction could occur due to the more active TBD catalyst. Also, check the purity of the phosphoramidic difluoride substrate prior to use – these compounds are sometimes unstable and can degrade rapidly.

Problem 3

The PFEx reactions are incomplete (relates to Part 2 and Part 3).

Potential solution

If the PFEx reactions appear incomplete by LC-MS analysis after the stipulated reaction times, add an additional portion (equating to 50% of the original volume) of the PFEx solutions. This will deliver more catalyst and HMDS to the reaction mixture.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Professor Dr. John E. Moses (moses@cshl.edu).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contacts, Dr. Joshua A. Homer (homer@cshl.edu) and Dr. Shoujun Sun (ssun@cshl.edu).

Materials availability

Upon request, the lead contact will share the newly generated compounds associated with this protocol.

This study did not generate new unique reagents.

Data and code availability

The article published by Sun et al., and the attached supplemental information contains all data generated or analyzed during this study.[1]

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2023.102824.

ACKNOWLEDGMENTS

J.E.M. is grateful to the NCI Cancer Center Support Grant 5P30CA045508, the Cold Spring Harbor Laboratory (CSHL) Northwell Health Affiliation, the F.M. Kirby Foundation, the Sunshine Foundation, S.J. Edwards, the Starr Foundation, Swim Across America Inc., and the Wasily Family Foundation. This work was performed with assistance from the CSHL Mass Spectrometry and Metabolomics Core Facility, which is partly supported by the NCI Cancer Center Support Grant 5P30CA045508. The authors also thank Dr. Zifei Wang for assisting in preparing this protocol.

AUTHOR CONTRIBUTIONS

J.A.H., S.S., and J.E.M. designed and wrote the protocol. J.A.H., S.S., and R.A.K. performed the experiments.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Sun, S., Homer, J.A., Smedley, C.J., Cheng, Q.-Q., Sharpless, K.B., and Moses, J.E. (2023). Phosphorus fluoride exchange: Multidimensional catalytic click chemistry from phosphorus connective hubs. Chem *9*, 2128–2143.

2. Kolb, H.C., Finn, M.G., and Sharpless, K.B. (2001). Click chemistry: diverse chemical function from a few good reactions. Angew. Chem. Int. Ed. *40*, 2004–2021.

3. Rostovtsev, V.V., Green, L.G., Fokin, V.V., and Sharpless, K.B. (2002). A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. Angew. Chem. Int. Ed. *41*, 2596–2599.

4. Tornøe, C.W., Christensen, C., and Meldal, M. (2002). Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. J. Org. Chem. *67*, 3057–3064.

5. Moses, J.E., and Moorhouse, A.D. (2007). The growing applications of click chemistry. Chem. Soc. Rev. *36*, 1249–1262.

6. Dong, J., Krasnova, L., Finn, M.G., and Sharpless, K.B. (2014). Sulfur(VI) Fluoride Exchange (SuFEx): Another Good Reaction for Click Chemistry. Angew. Chem. Int. Ed. *53*, 9430–9448.

7. Barrow, A.S., Smedley, C.J., Zheng, Q., Li, S., Dong, J., and Moses, J.E. (2019). The growing applications of SuFEx click chemistry. Chem. Soc. Rev. *48*, 4731–4758.

8. Moorhouse, A.D., Homer, J.A., and Moses, J.E. (2023). The certainty of a few good reactions. Chem *9*, 2063–2077.

9. Liu, F., Wang, H., Li, S., Bare, G.A.L., Chen, X., Wang, C., Moses, J.E., Wu, P., and Sharpless, K.B. (2019). Biocompatible SuFEx Click Chemistry: Thionyl Tetrafluoride (SOF4)-Derived Connective Hubs for Bioconjugation to DNA and Proteins. Angew. Chem. Int. Ed. *58*, 8029–8033.

10. Li, S., Li, G., Gao, B., Pujari, S.P., Chen, X., Kim, H., Zhou, F., Klivansky, L.M., Liu, Y., Driss, H., et al. (2021). SuFExable polymers with helical structures derived from thionyl tetrafluoride. Nat. Chem. *13*, 858–867.

11. Giel, M.-C., Smedley, C.J., and Moses, J.E. (2021). Sulfur Fluoride Exchange (SuFEx). In Science of Synthesis: Click Chemistry, F.P.J.T. Rutjes, ed. (Thieme Chemistry), pp. 435–484.

12. Smedley, C.J., Homer, J.A., Gialelis, T.L., Barrow, A.S., Koelln, R.A., and Moses, J.E. (2022). Accelerated SuFEx Click Chemistry for Modular Synthesis. Angew. Chem. Int. Ed. *61*, e202112375.

13. Ottonello, A., Wyllie, J.A., Yahiaoui, O., Sun, S., Koelln, R.A., Homer, J.A., Johnson, R.M., Murray, E., Williams, P., Bolla, J.R., et al. (2023). Shapeshifting bullvalene-linked vancomycin dimers as effective antibiotics against multidrug-resistant gram-positive bacteria. Proc. Natl. Acad. Sci. USA *120*, e2208737120.

14. Smedley, C.J., Li, G., Barrow, A.S., Gialelis, T.L., Giel, M.-C., Ottonello, A., Cheng, Y., Kitamura, S., Wolan, D.W., Sharpless, K.B., and Moses, J.E. (2020). Diversity Oriented Clicking (DOC): Divergent Synthesis of SuFExable Pharmacophores from 2-Substituted-Alkynyl-1-Sulfonyl Fluoride (SASF) Hubs. Angew. Chem. Int. Ed. *59*, 12460–12469.

15. Cheng, Y., Li, G., Smedley, C.J., Giel, M.-C., Kitamura, S., Woehl, J.L., Bianco, G., Forli, S., Homer, J.A., Cappiello, J.R., et al. (2022). Diversity oriented clicking delivers β-substituted alkenyl sulfonyl fluorides as covalent human neutrophil elastase inhibitors. Proc. Natl. Acad. Sci. USA *119*, e2208540119.

16. Smedley, C.J. (2022). A diversity oriented clicking strategy: the stereoselective synthesis of highly-functionalised olefins from 2-substituted-alkynyl-1-sulfonyl fluorides. Chem. Commun. *58*, 11316–11319.

17. Delfino, R.T., Ribeiro, T.S., and Figueroa-Villar, J.D. (2009). Organophosphorus compounds as chemical warfare agents: a review. J. Braz. Chem. Soc. *20*, 407–428.

18. Franca, T.C.C., Kitagawa, D.A.S., Cavalcante, S.F.d.A., da Silva, J.A.V., Nepovimova, E., and Kuca, K. (2019). Novichoks: The Dangerous Fourth Generation of Chemical Weapons. Int. J. Mol. Sci. *20*, 1222.

19. Timperley, C. (2014). Best Synthetic Methods: Organophosphorus (V) Chemistry (Newnes).

20. Flader, C., Liu, J., and Borch, R.F. (2000). Development of Novel Quinone Phosphorodiamidate Prodrugs Targeted to DT-Diaphorase. J. Med. Chem. *43*, 3157–3167.