# Sulfur-Fluoride Exchange (SuFEx)

Literature Seminar
2019/11/28
M2 Katsuya Maruyama

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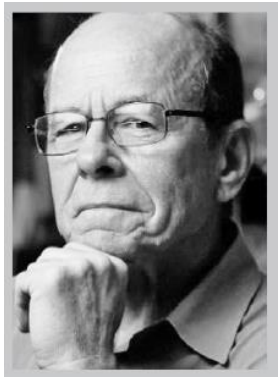
- Introduction
- Synthesis of S(VI)-F unit
- Applications of SuFEx

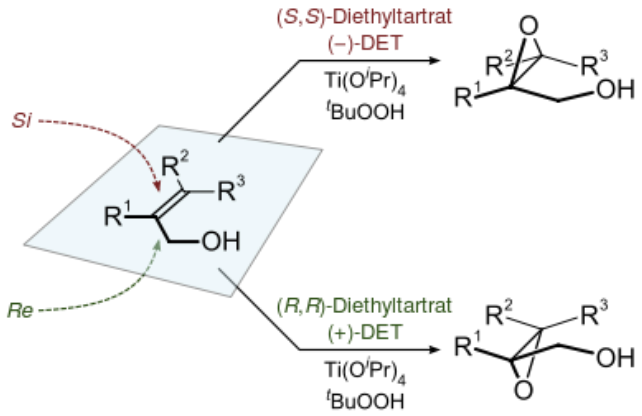
# K. B. Sharpless



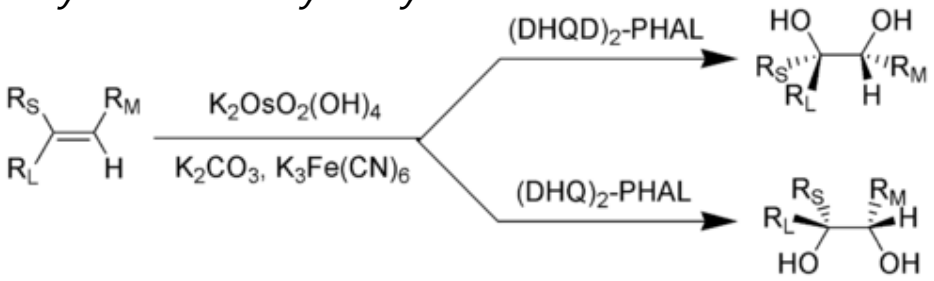
*Nobel Laureate K. Barry Sharpless became W. M. Keck Professor of Chemistry at The Scripps Research Institute and The Skaggs Institute of Chemical Biology in 1990. Previously a professor at MIT and Stanford, he was educated at Dartmouth College (BA 1963), Stanford (PhD 1968 with E. E. van Tamelen; postdoc 1969 with J. P. Collman), and Harvard (postdoc 1970, K. E. Bloch).*

The Nobel Prize in Chemistry (2001):
"for his work on chirally catalyzed oxidation reactions."

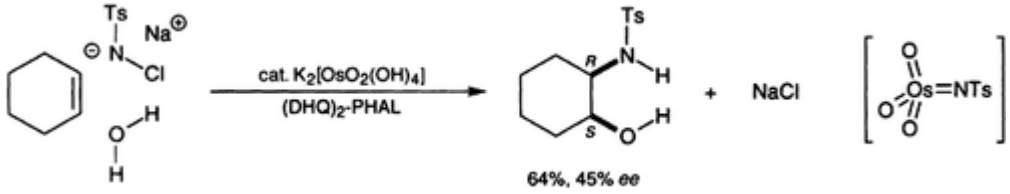
*Asymmetric epoxidation*



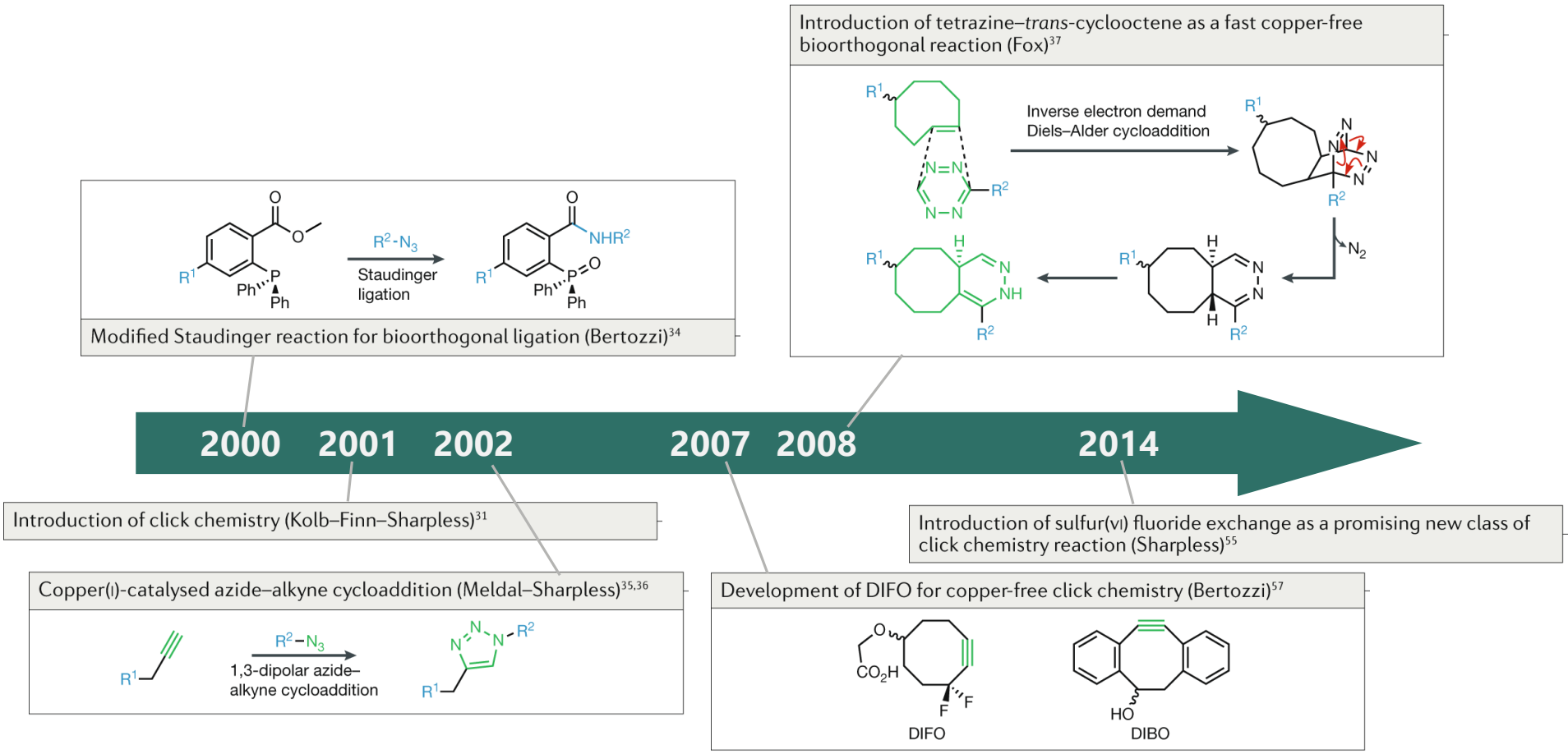

*Asymmetric dihydroxylation*




*Asymmetric aminohydroxylation*



# Click Chemistry

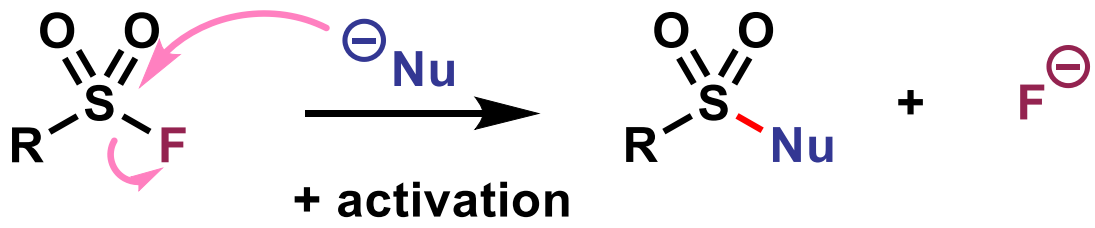



*Nat. Rev. Chem.* **2018**, *2*, 202.

- ***Robust connection***
- ***High functional group tolerance***
- ***Fast kinetics***
- ***Easy operation***

# SuFEx Reaction

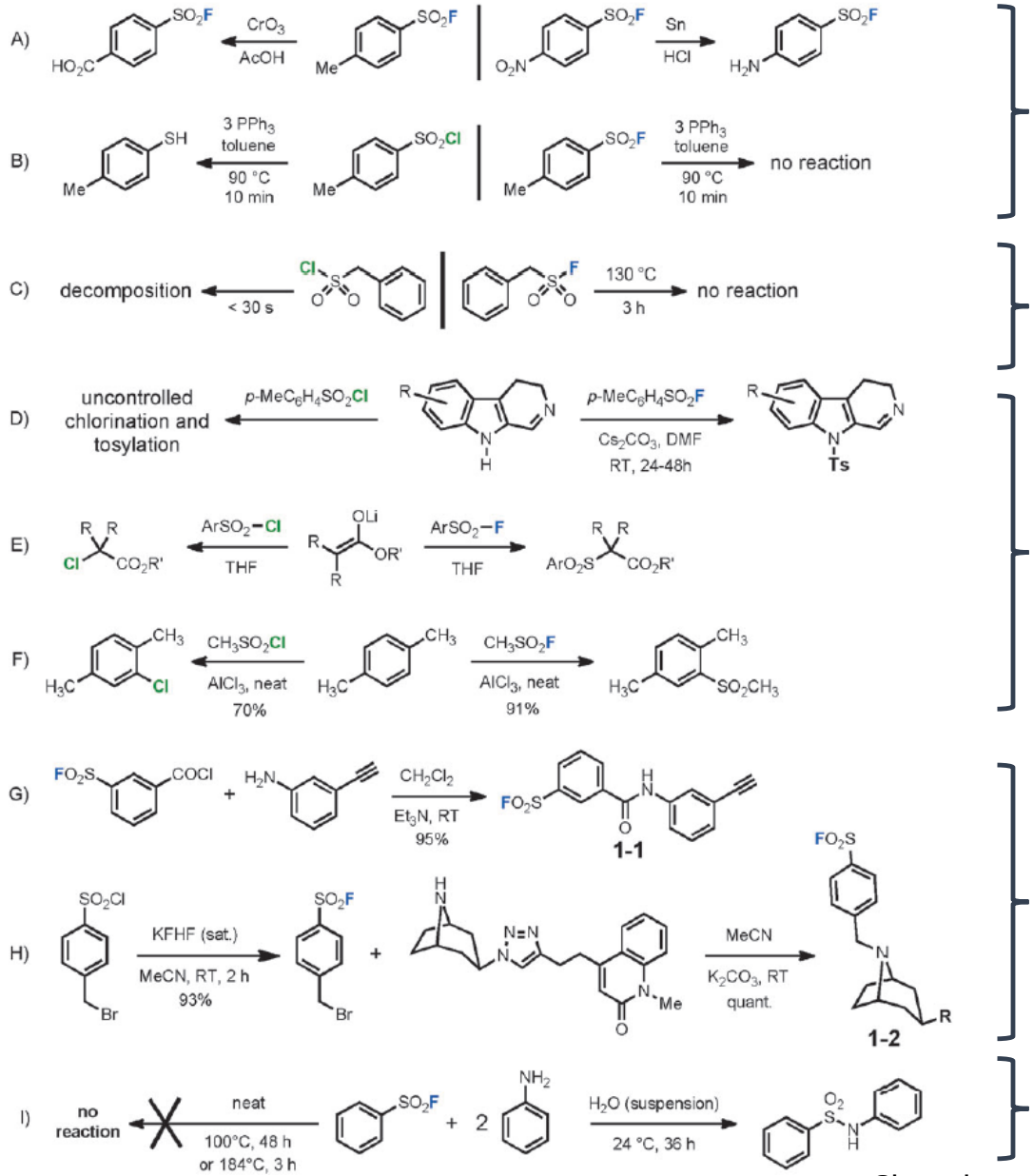
General scheme




Stable S-F bond (necessity of activation)
Metal free
diversity of linkage

"Context dependent" Click reaction

# Property of Sulfonyl Fluoride




Oxidation/reduction

Heating

Substitution at S(VI) center (no fluorination)

Nucleophilic attack

Activation with water

BDE

$SO_2F_2$ 90.5 kcal/mol

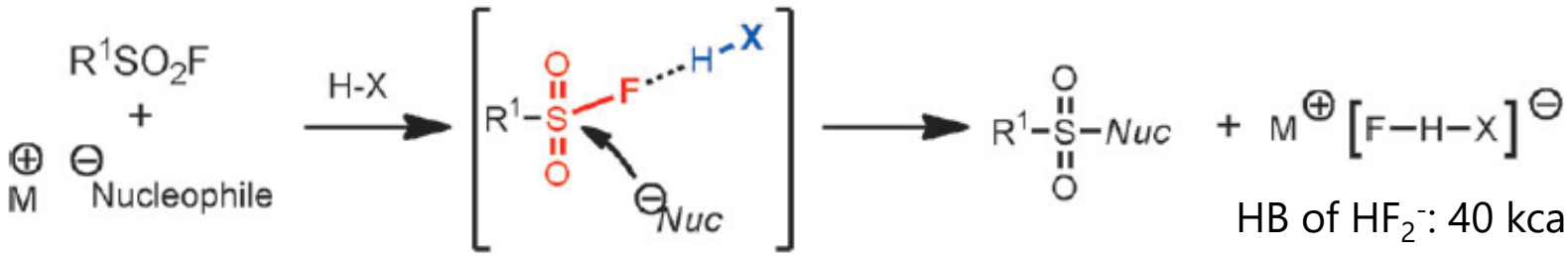
$SO_2Cl_2$ 46 kcal/mol

Sharpless, K. *et al.* *Angew. Chem. Int. Ed.* **2014**, *53*, 9430.

# SuFEx Reaction

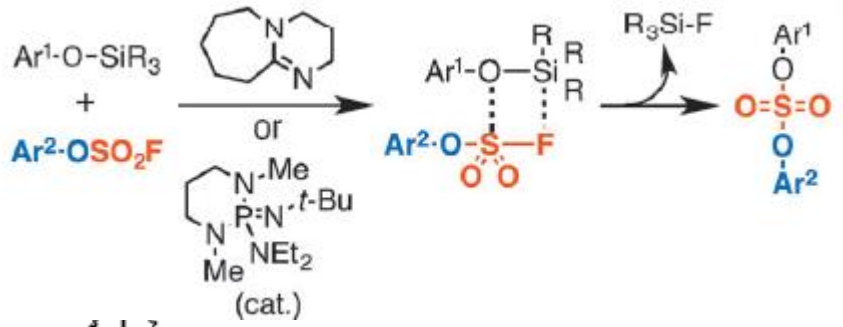
## Activation of S(VI)-F

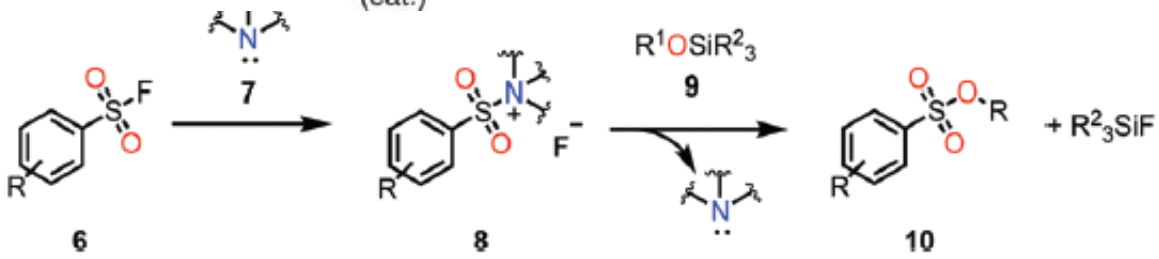
- Hydrogen bond donor (acid, HX)

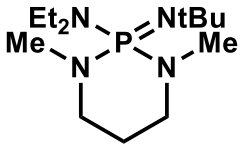



HB of $HF_2^-$: 40 kcal/mol

- Silylated nucleophile



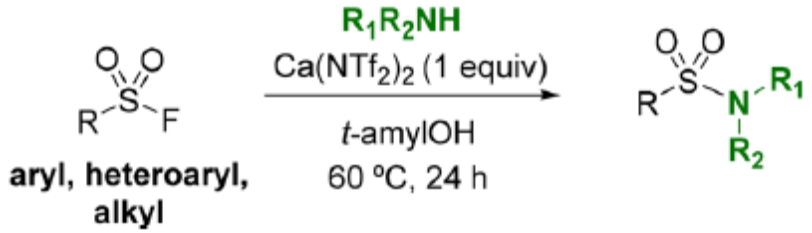



**BEMP**
**(2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine)**

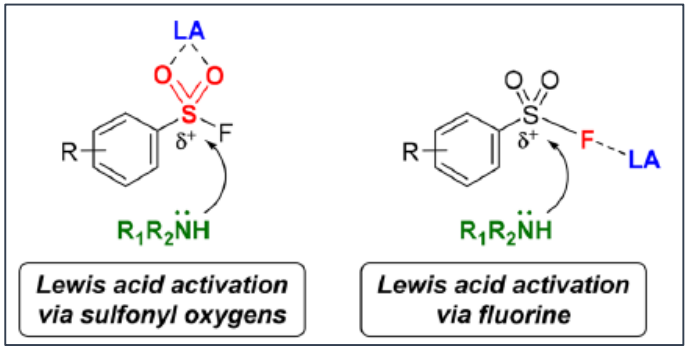
Reactivity: $Et_3N$ < DBU < BEMP

# SuFEx Reaction

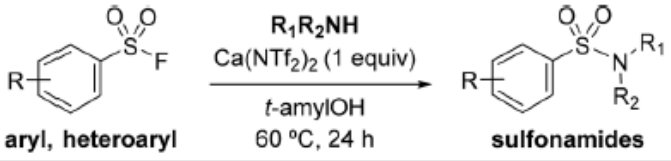
## Activation with $Ca(NTf)_2$ (Lewis acid)

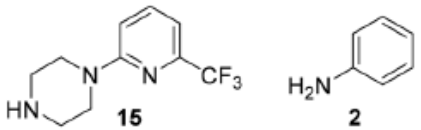


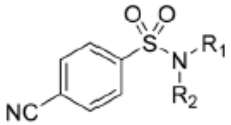

## Proposed mode of activation



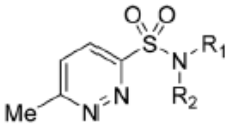

## Effect of Ca salt



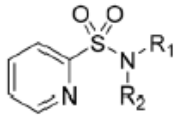



| | 21 | 26 |
|---|---|---|
| | with Ca: 82%<br>without Ca: 95% | with Ca: 85%<br>without Ca: N/R[a] |

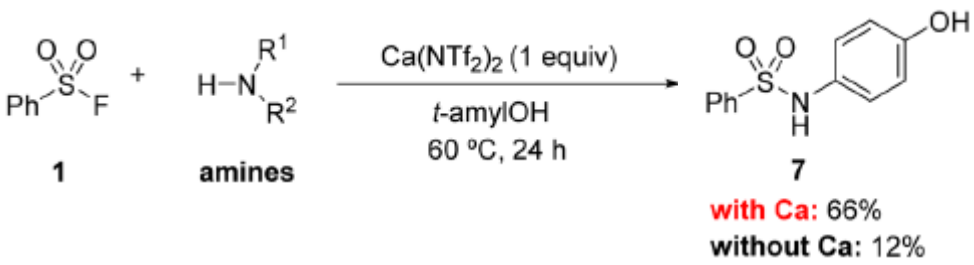


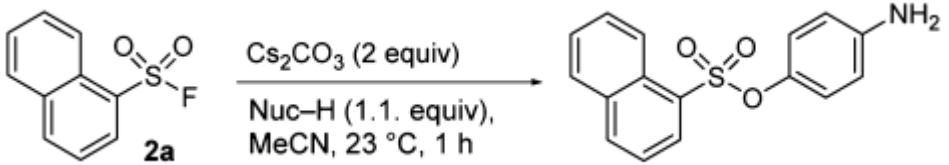
| | 22 | 27 |
|---|---|---|
| | with Ca: 90%<br>without Ca: 81% | with Ca: 88%<br>without Ca: trace[b] |



| | 23 | 28 |
|---|---|---|
| | with Ca: 95%<br>without Ca: 90% | with Ca: 98%<br>without Ca: trace[b] |

## Selectivity of nucleophile



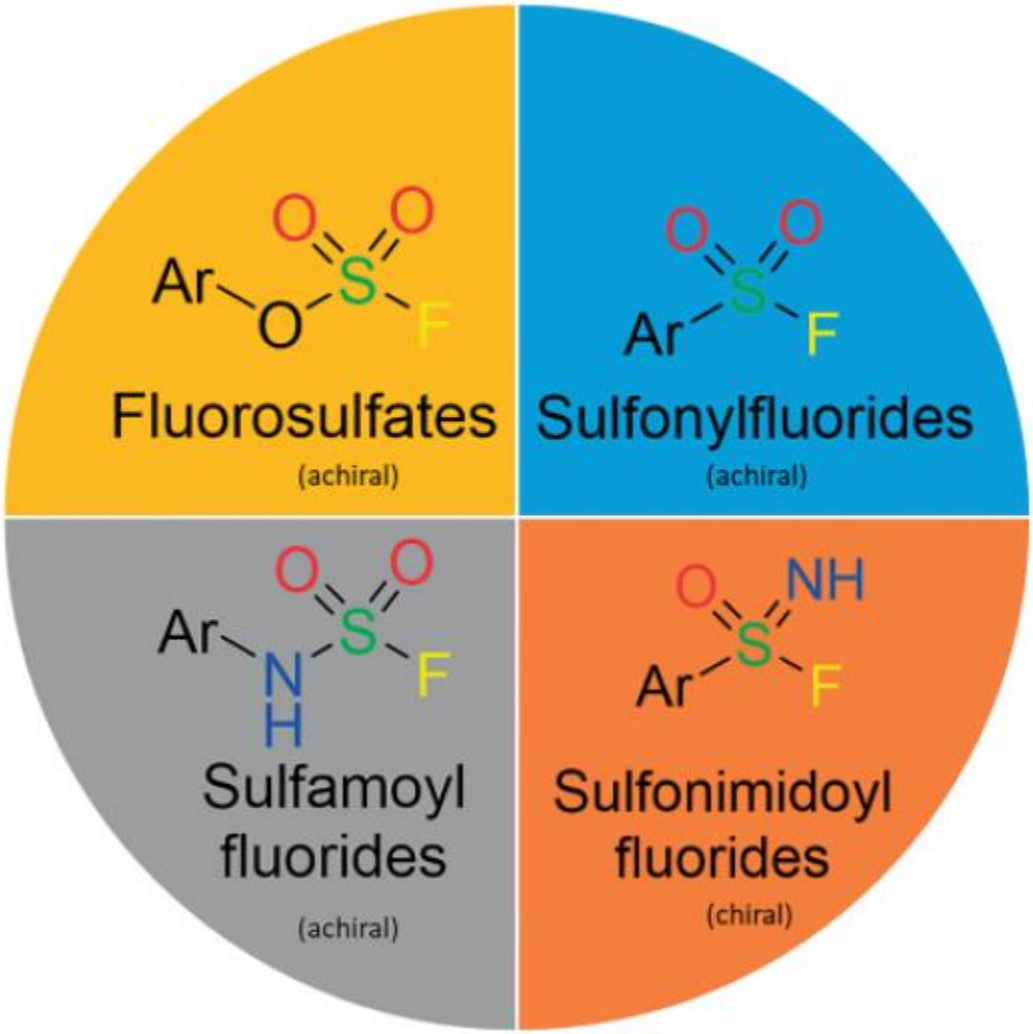




*JOC* **2017**, *82*, 2294.

Am Ende, C. W.; Ball, N. D. *et al. Org. Lett.* **2018**, *20*, 3943.

# Synthesis of SuFEx unit

# SuFEx Unit

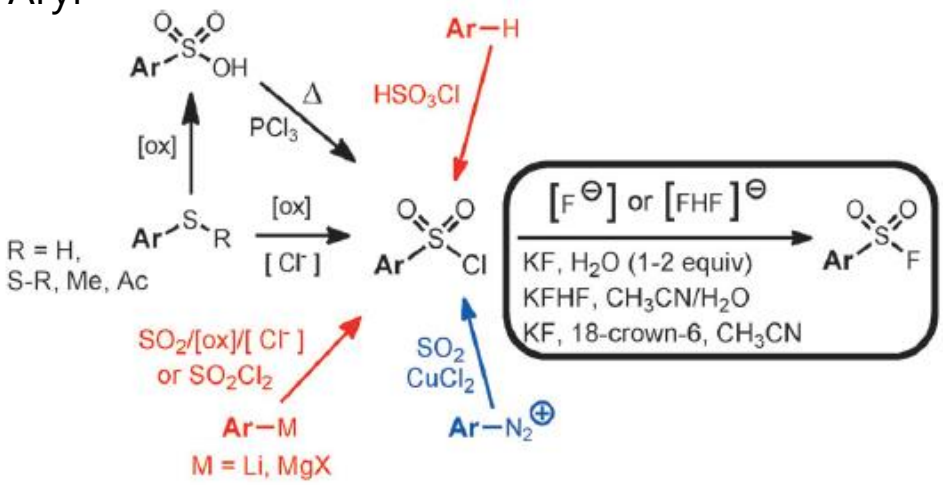



Chinthakindi, P. K.; Arvidsson, P. I. *Eur. J. Org. Chem.* **2018**, 3648.

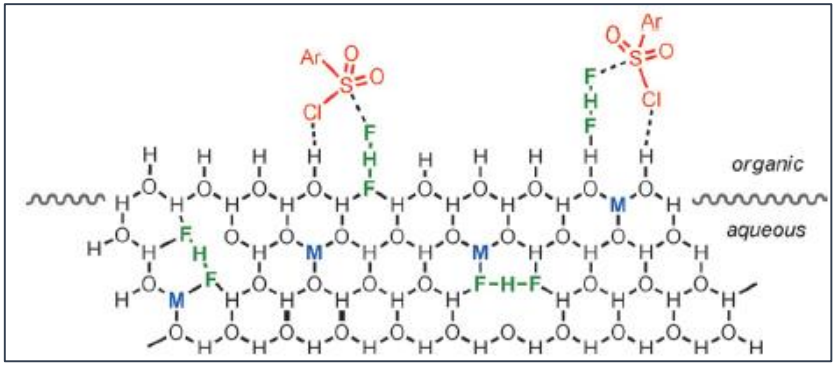
# Sulfonylfluoride ($RSO_2F$)

## Oldest conditions: via $RSO_2Cl$

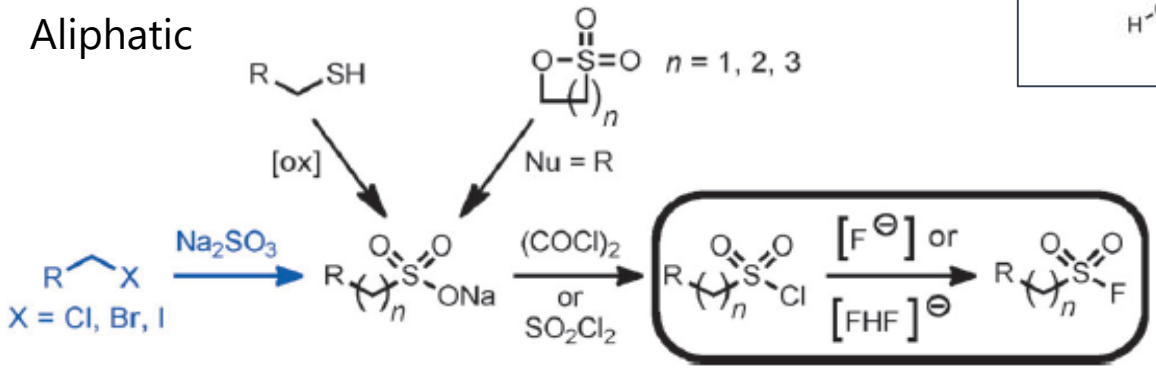
Aryl




Destabilized and nucleophilic $F^-$




Aliphatic

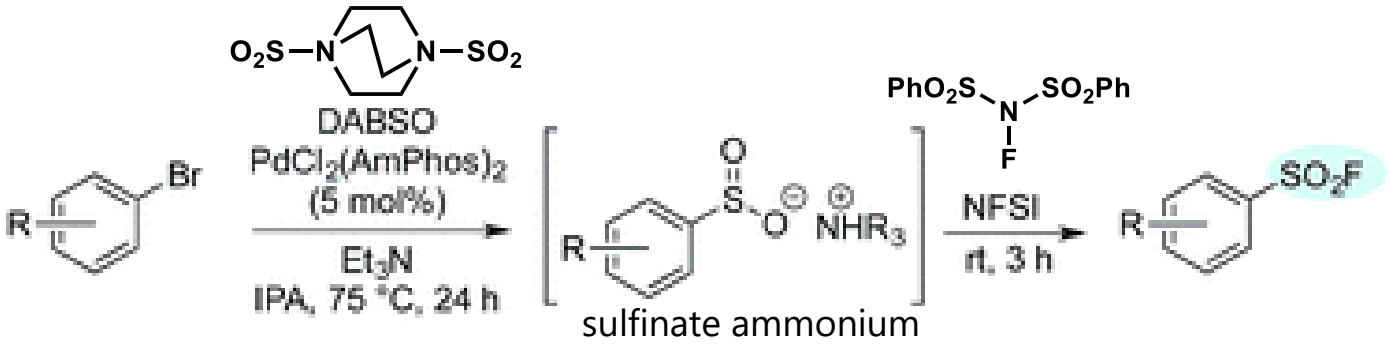



Harsh conditions

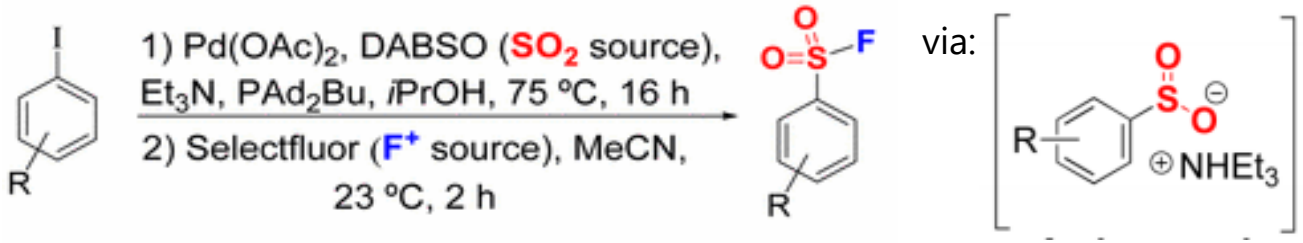
Sharpless, K. *et al. Angew. Chem. Int. Ed.* **2014**, *53*, 9430.

# Sulfonylfluoride ($RSO_2F$)

## Pd catalyzed $SO_2$ insertion

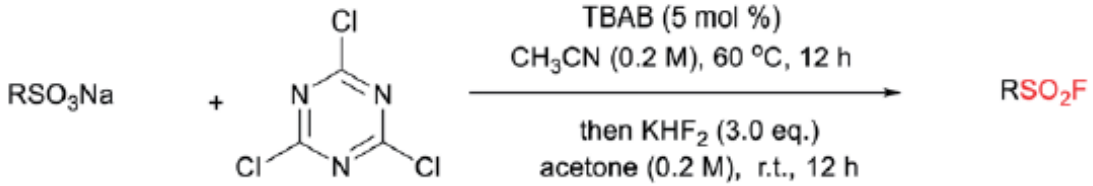



Baglev. S. W.: Willis, M. C. *et al. Chem. Sci.* **2017**, *8*, 1233.




Ball, N. D. *et al. J. Org. Chem.* **2017**, *82*, 2294.

## Via sulfonate salt

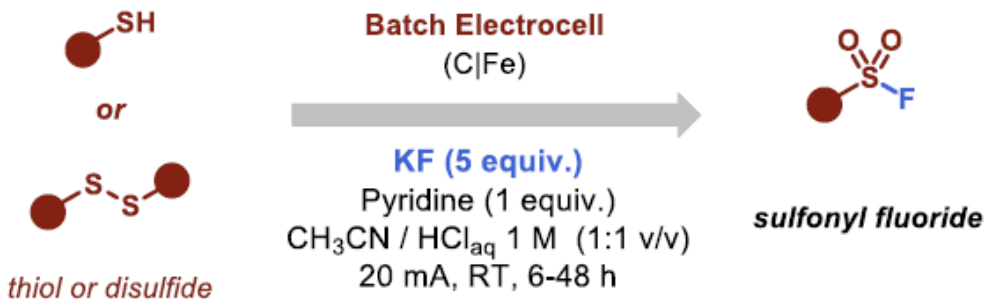



- transition metal free
- one-pot

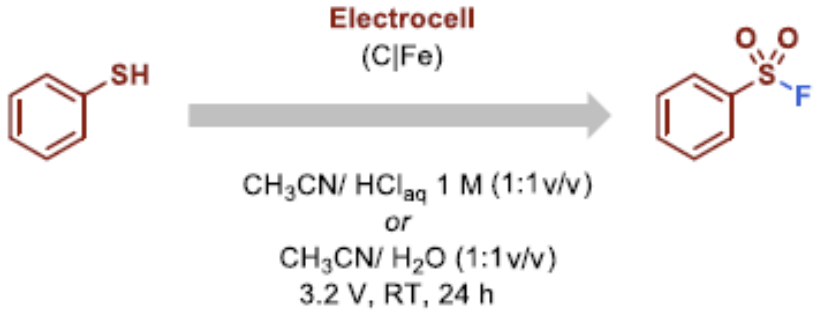
Sun, B.; Qin, H. L. *et al. RSC Adv.* **2019**, *9*, 13863.

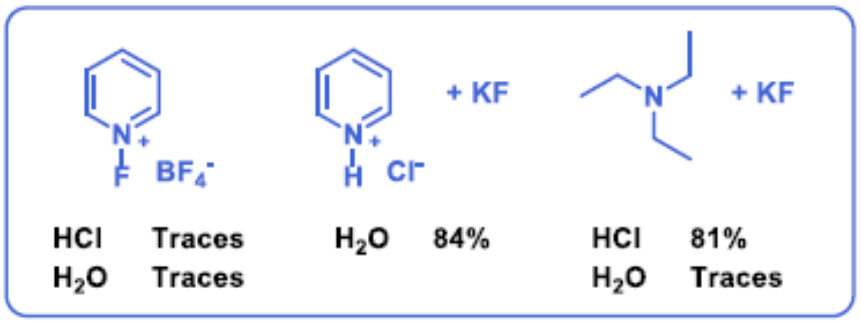
# Sulfonylfluoride ($RSO_2F$)

## Electrochemical synthesis

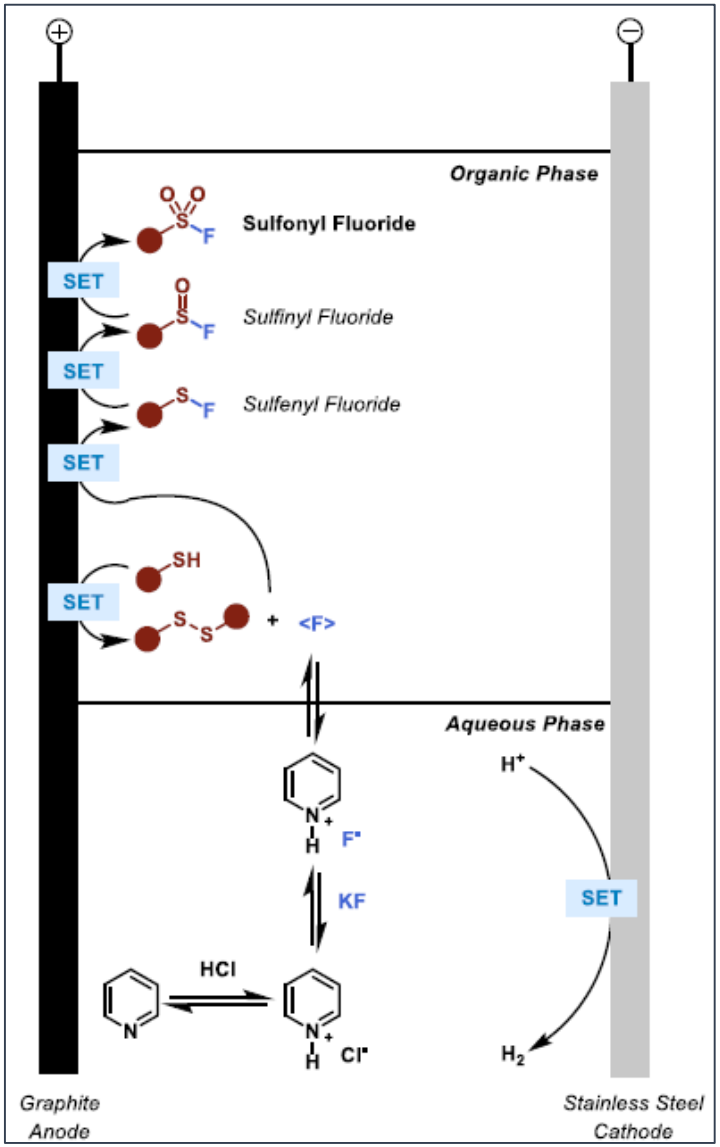



## Investigation of active species



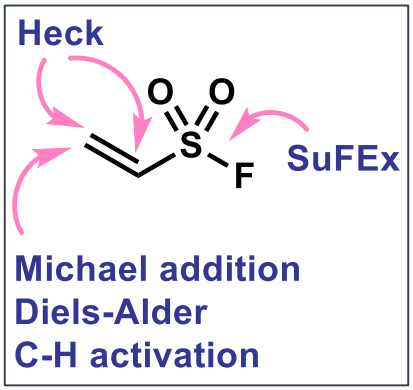

## Mechanism




Noël, T. *et al. J. Am. Chem. Soc.* **2019**, *141*, 11832.

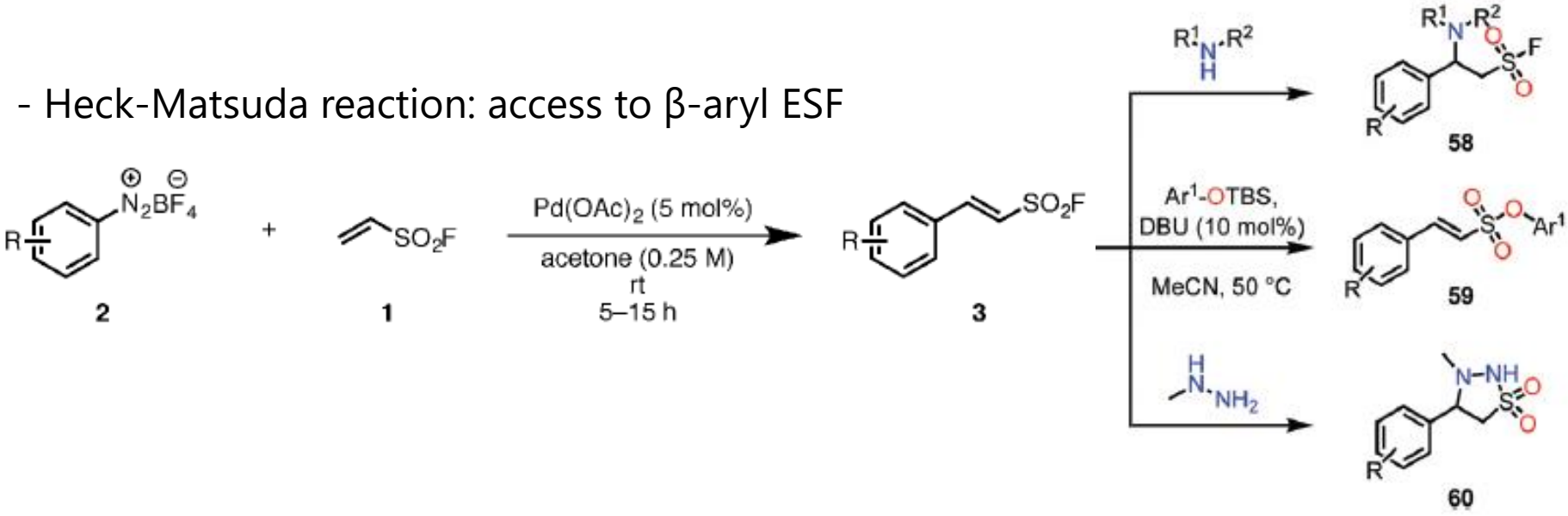
# SuFEx Hubs

## ESF: ethene sulfonyl fluoride




- Michael addition

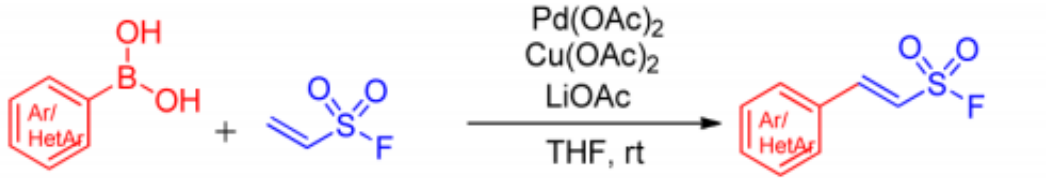

- Heck-Matsuda reaction: access to β-aryl ESF



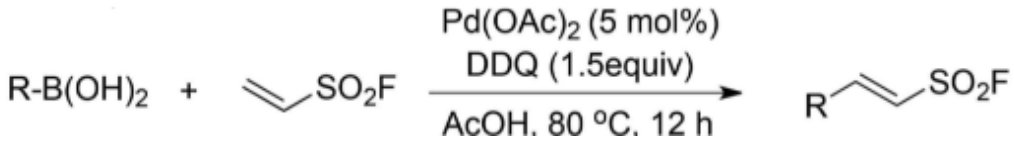

Wu, P.; Sharpless, K. B. *et al. Angew. Chem. Int. Ed.* **2016**, *55*, 14155.

# SuFEx Hubs

- Oxidative Heck reaction

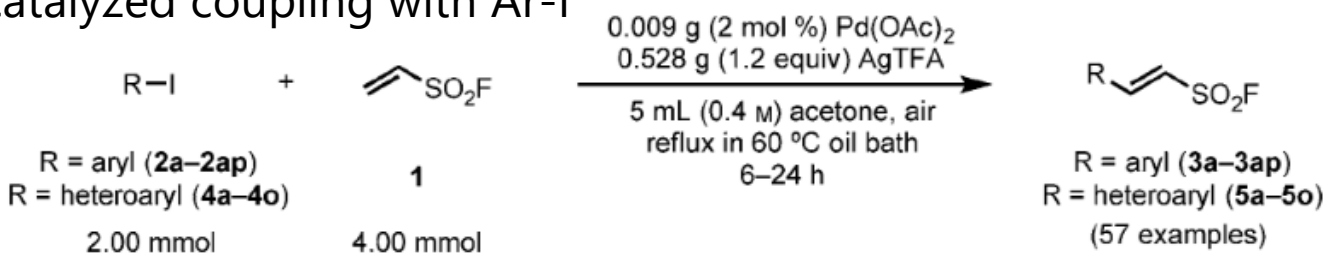



Arvidsson, P. I. *et al. Org. Lett.*, **2017**, *19*, 480.




Qin, H. L. *et al. Adv. Synth. Catal.*, **2017**, *359*, 3237.

- Pd catalyzed coupling with Ar-I



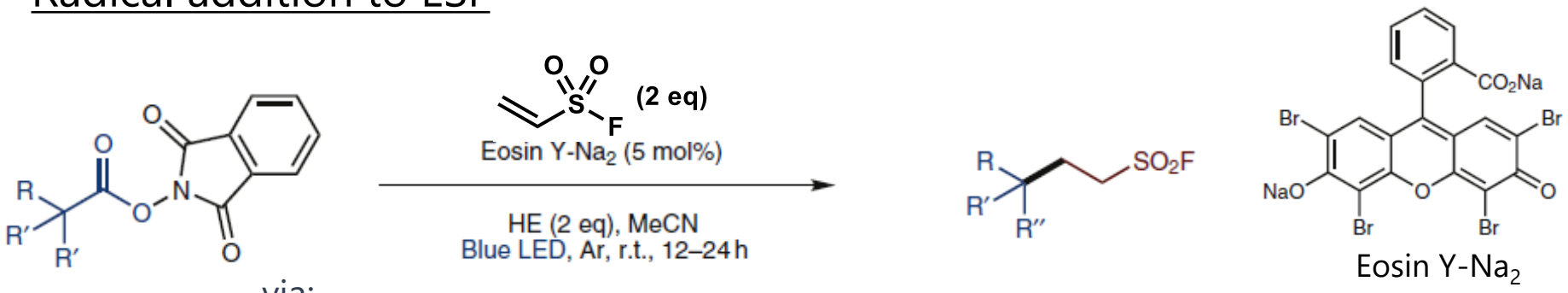

Qin, H. L.; Shalpless, K. B. *et al. Angew. Chem., Int. Ed.*, **2017**, *56*, 4849.

- C-H activation


*Org. Chem. Front.*, **2018**, *5*, 1411.
*Eur. J. Org. Chem.*, **2018**, 4407.
*Org. Lett.*, **2018**, *20*, 4699.
*Organometallics*, **2019**, *38*, 76.

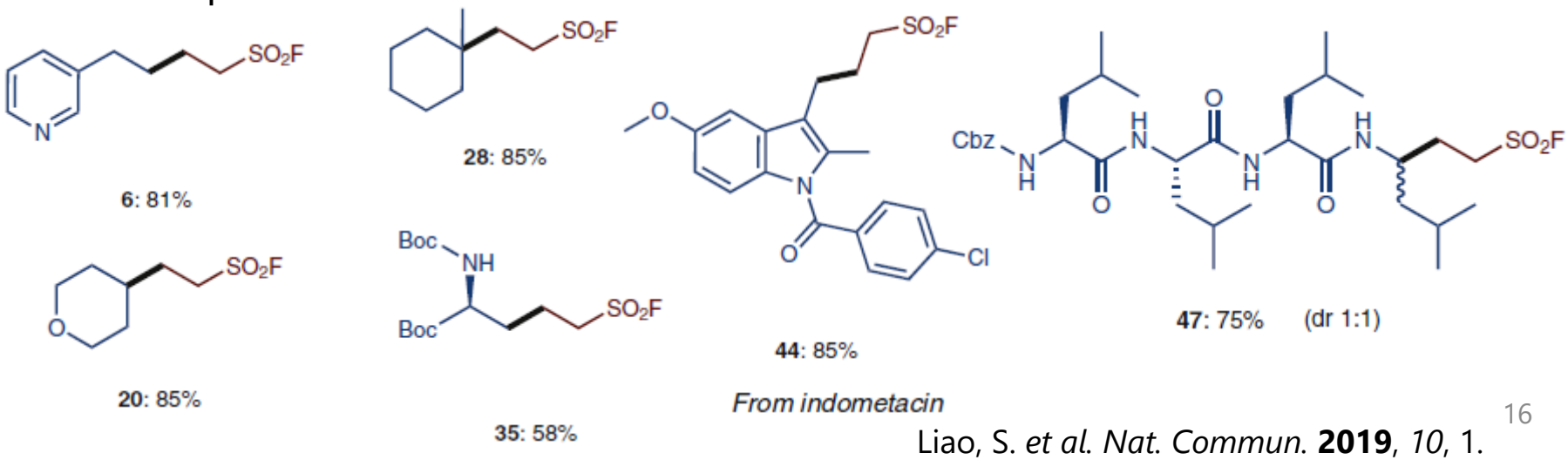
# SuFEx Hubs

## Radical addition to ESF




via:


## Selected scopes

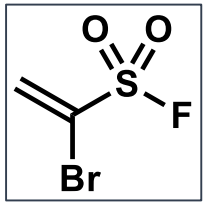




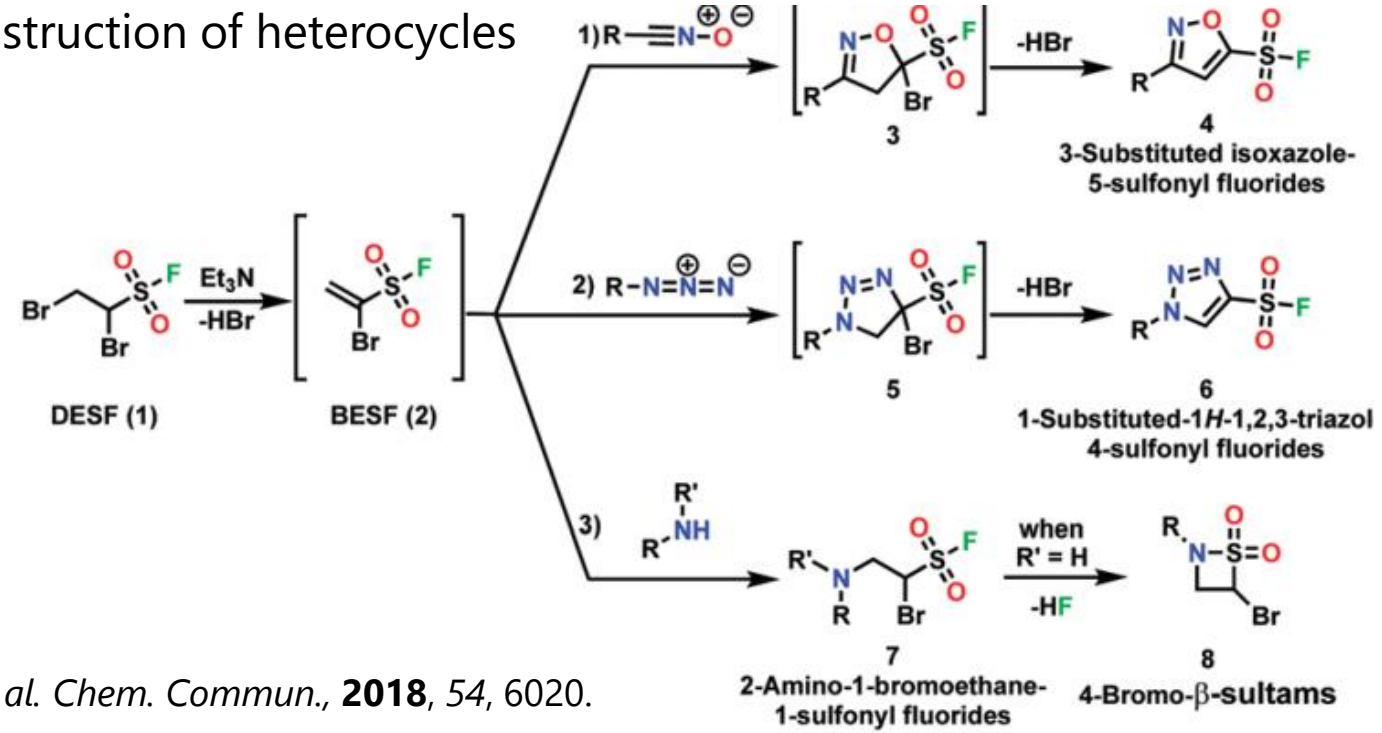

Liao, S. *et al. Nat. Commun.* **2019**, *10*, 1.

# SuFEx Hubs

## BESF: 1-bromoethene-1-sulfonyl fluoride

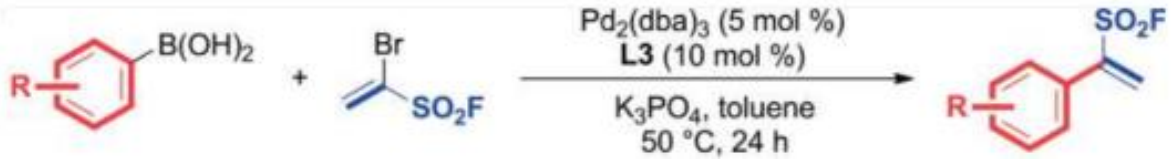


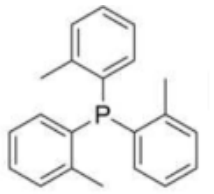
Construction of heterocycles




Moses, J. E. *et al. Chem. Commun.*, **2018**, *54*, 6020.

- Suzuki-Miyaura cross coupling: access to α-aryl ESF



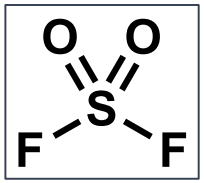



tri-*o*-tolylphosphine
L3

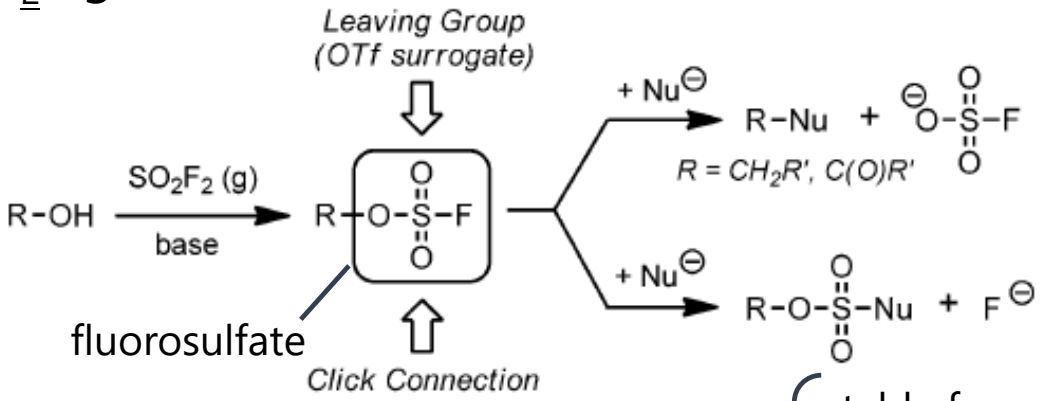
Qin, H. L. *et al. Eur. J. Org. Chem.* **2019**, 6101.

# Synthesis of Fluorosulfate ($ROSO_2F$)

Sulfuryl fluoride ($SO_2F_2$): gas



Reaction with alcohol/phenol




Aryl fluorosulfate is useful in terms of stability.

stable for
- months (neutral buffer)
- 2 weeks (buffer pH 10)

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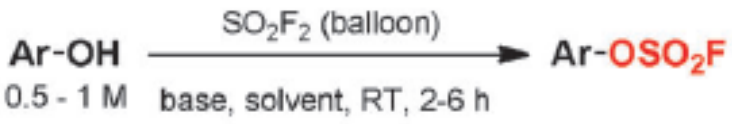
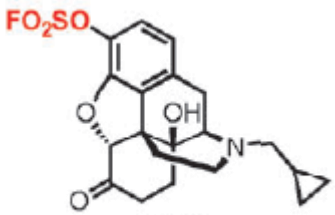


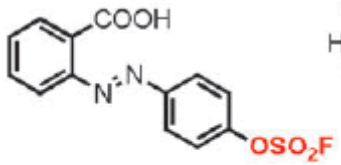

A) $Et_3N$ in $CH_2Cl_2$,
:for simple molecules

B) $Et_3N$ or $iPr_2NEt$ in biphasic mixture ($CH_2Cl_2$/water)
:for complex molecules

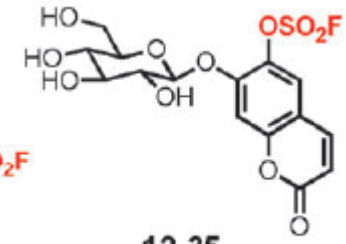
Selected scopes




12-31
82%




12-33
88%

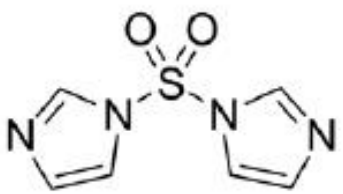



12-35
74%

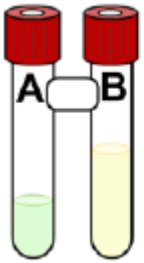
Sharpless, K. *et al. Angew. Chem. Int. Ed.* **2014**, *53*, 9430.

# *Ex Situ* Generation of $SO_2F_2$ Gas

Precursor of $SO_2F_2$

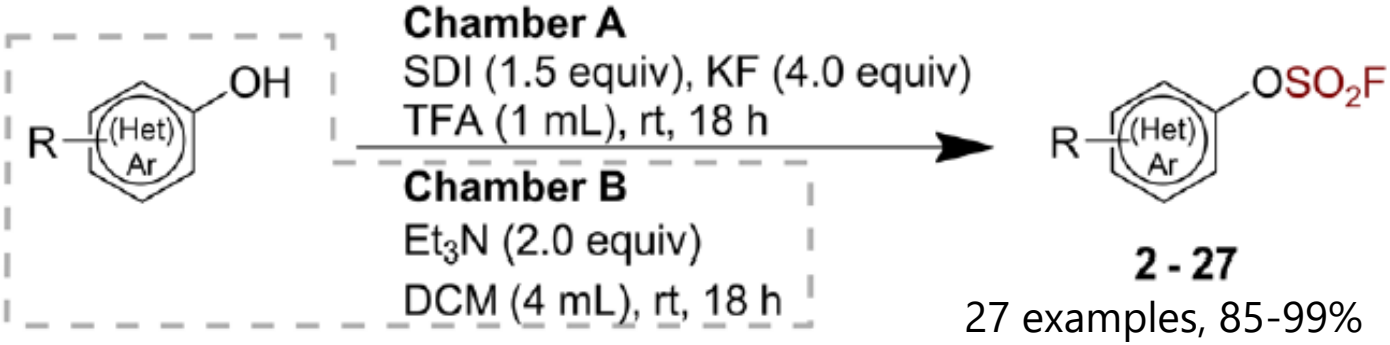


**1,1'-sulfonyldiimidazole (SDI)**




**A: $SO_2F_2$ generation**

**B: (Het)ArOH functionalization**



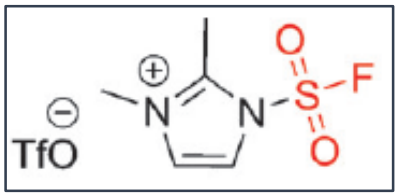

**2 - 27**

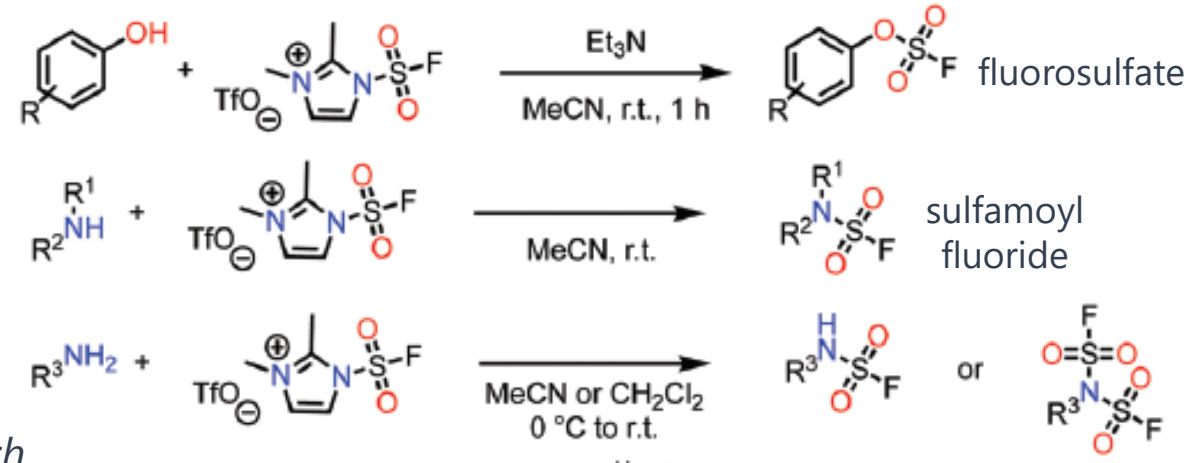
27 examples, 85-99%

De Borggraeve, W. M. *et al. Org. Lett.* **2017**, *19*, 5244.

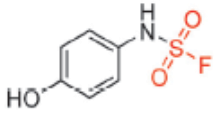
# $SO_2F_2$ Equivalents

## Fluorosulfuryl imidazolium salt




- *bench-stable solid*
- *higher reactivity than $SO_2F_2$*
- *commercially available from Aldrich*
- *applicable to 1° amine*

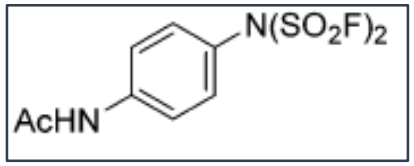


**9-18**[e]
91%

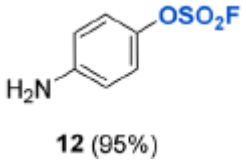
selectivity:
amine, aniline > phenol

Sharpless, K. B.; Dong, J. *et al. Angew. Chem. Int. Ed.* **2018**, *57*, 2605.

## AISF: [(acetylamino)phenyl]imidodisulfuryl difluoride







**12** (95%)

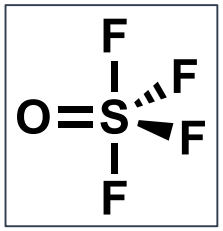
selectivity:
phenol > aniline

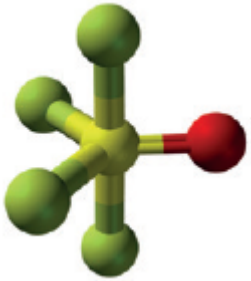
- *no need of $SO_2F_2$ for preparation*
- *bench-stable solid*
- *higher reactivity than $SO_2F_2$*
- *rapid kinetics*

Am Ende, C. W. *et al. Org. Lett.* **2018**, *20*, 812.

# Sulfonimidoyl Fluoride (R-SONHF)

## Thionyl tetrafluoride ($SOF_4$) gas

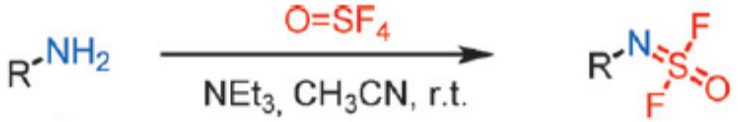


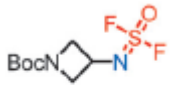

multidimensional hub

## 1st addition

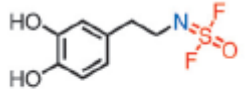
- 1° amine



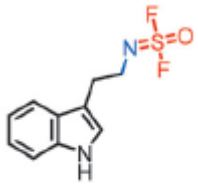

Selected scopes



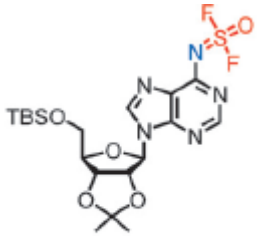

**2-8**
98%
250 mg




**2-16**
54%
64 mg

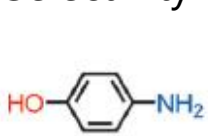



**2-17**
75%, 91 mg

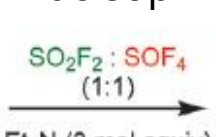


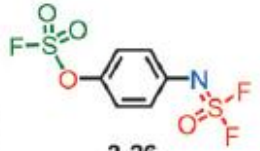

**2-24**
85%
214 mg

Selectivity of nucleophile




**1-26**




**2-26**
90%, 2.48 g
light yellow solid
mp: 50 °C

orthogonal reactivity
$SO_2F_2$: phenol > amine
$SOF_4$: amine > phenol

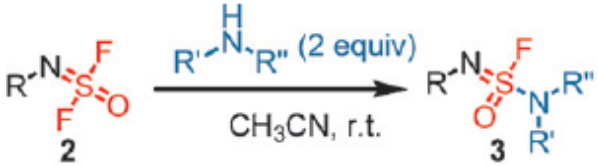


Sharpless, K. B. *et al. Angew. Chem. Int. Ed.* **2017**, *56*, 2903.

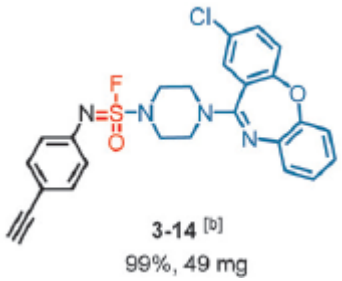
# Sulfonimidoyl Fluoride (R-SONHF)

2nd addition

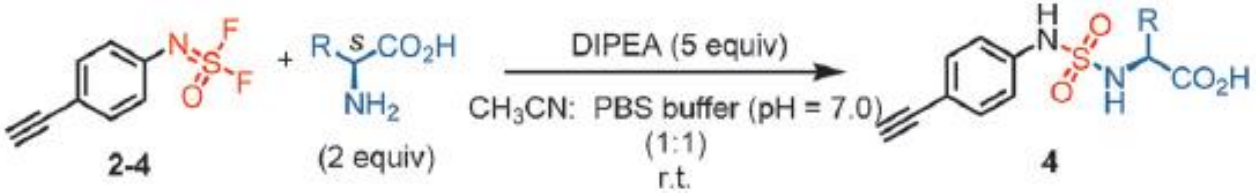
- 2° amine

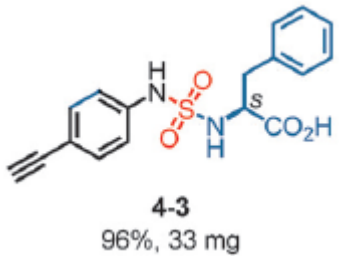



sulfonimidoyl fluoride

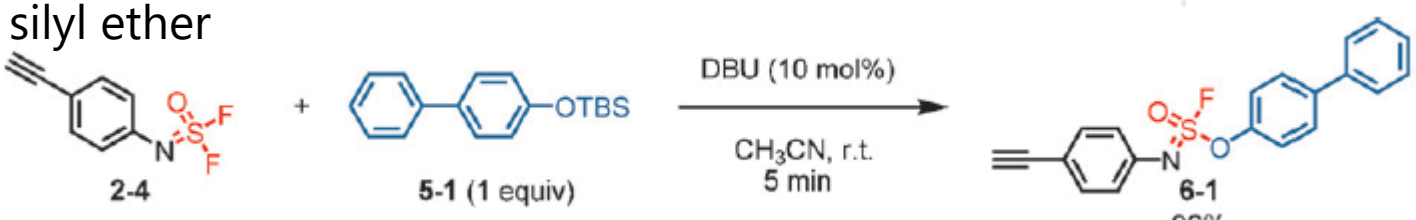



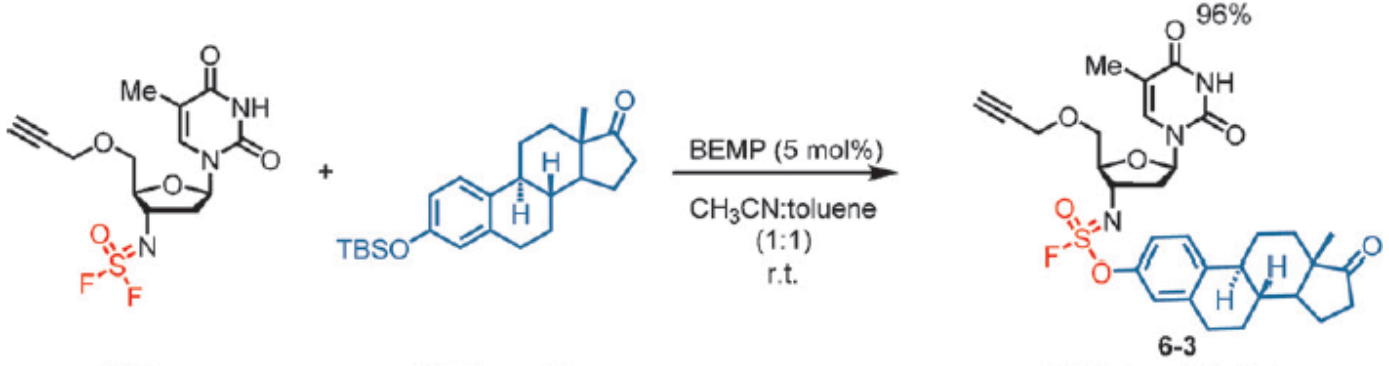

- Amino acid: hydrolytic loss of second S-F



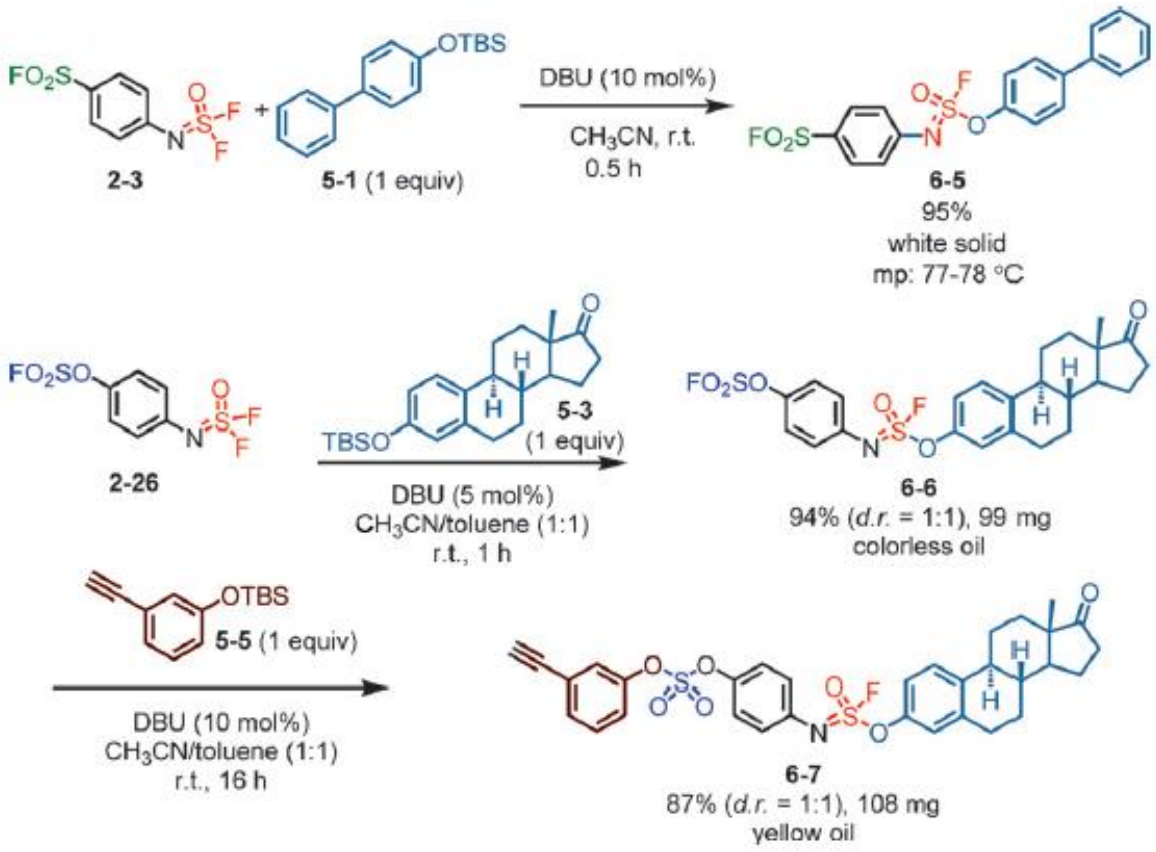

- Aryl silyl ether

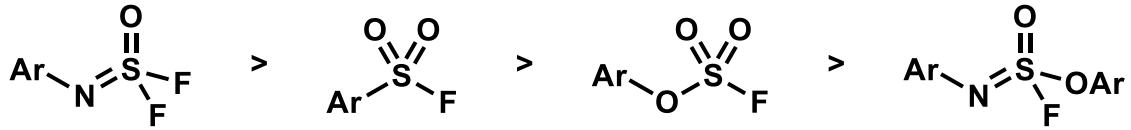


# Sulfonimidoyl Fluoride (R-N=S(O)$F_2$)

## Comparison of reactivity



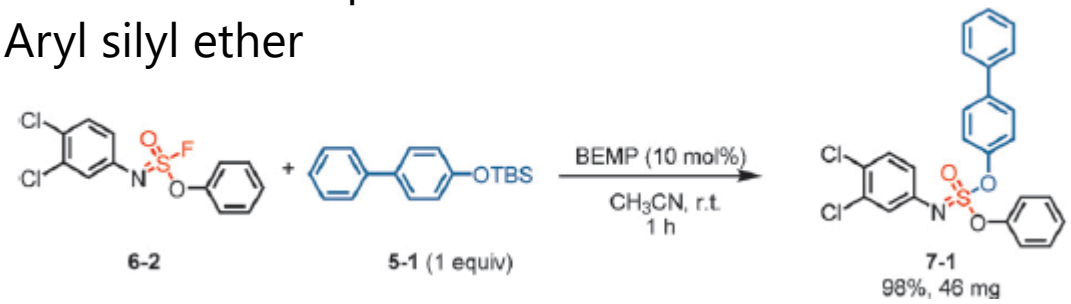




**sulfonimidoyl fluoride** > **sulfonyl fluoride** > **fluorosulfate** >

# Sulfonimidoyl Fluoride (R-SONHF)

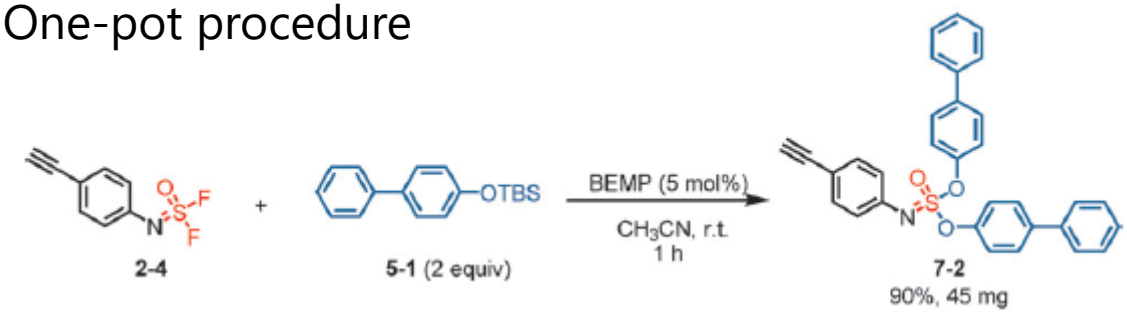
3rd addition BEMP as potent base to activate inert S-F bond

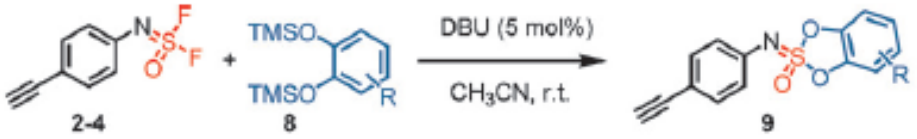
- Aryl silyl ether




- 2° amine


- One-pot procedure

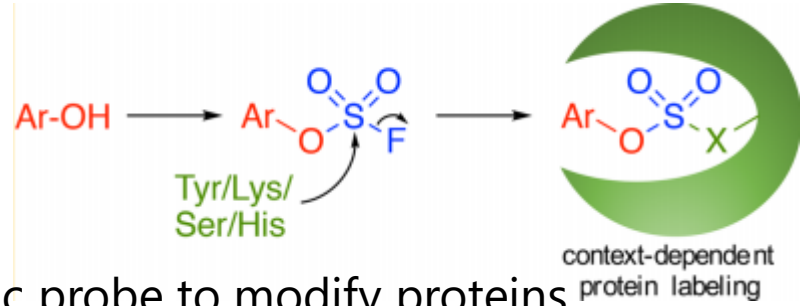


# Application

Medicinal chemistry
Chemical biology
New synthetic methodology
Material science

# $RSO_2F$ in Biological Context




electrophilic probe to modify proteins

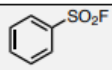
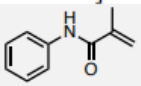
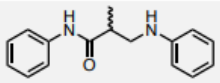
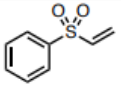
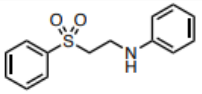
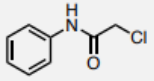
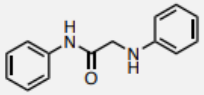
H+ mediated switch-on
-> specificity to microenvironment

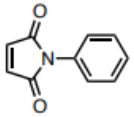
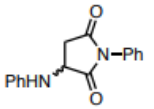
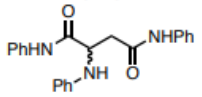
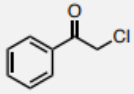
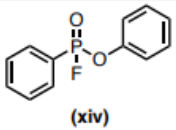
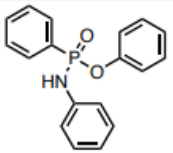
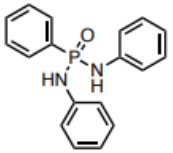
- Activation of S-F in special environment
- Proximity driven reaction
- Ser, Tyr, Lys, Thr, His, Cys

<u>Examples</u>
- Covalent drugs
- Target identification and validation
- Mapping of enzyme binding sites, substrates and protein–protein interactions.
- Late-stage functionalization (LSF) of bioactive molecules

# Inertness of Sulfonyl Fluoride

## Nucleophilic stability of electrophiles in chemical biology

| Entry | Electrophile | % remaining SM | Product (% HPLC yield) |
|---|---|---|---|
| 1 |  (ii) Sulfonyl fluoride | 100 | N/A |
| 2 |  (vii) Epoxide [(±)-styrene oxide] | 0 | Unidentified mixture |
| 3 |  (viii) Acrylamide | 59 |  (41) |
| 4 |  (ix) Vinyl sulfone | 0 |  (100) |
| 5 |  (x) α-chloroamide | 0 |  (>95) |
| 6 |  (xi) β-lactam | 0 | Unidentified mixture |

| Entry | Electrophile | % remaining SM | Product (% HPLC yield) |
|---|---|---|---|
| 7 |  (xii) maleimide | 0 |  (27)  (73) |
| 8 |  (xiii) α-chloroketone | 0 | Unidentified mixture |
| 9 |  (xiv) fluorophosphorate | 11 |  (55)  (34) |

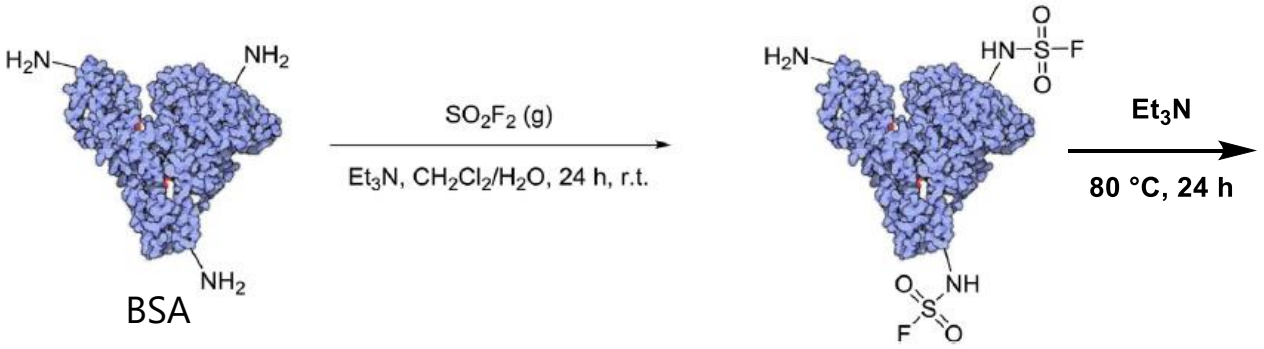
Conditions: electrophiles (1.0 mmol), aniline 1.3 mL (neat), 184 °C



Sharpless, K. B. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 18808.

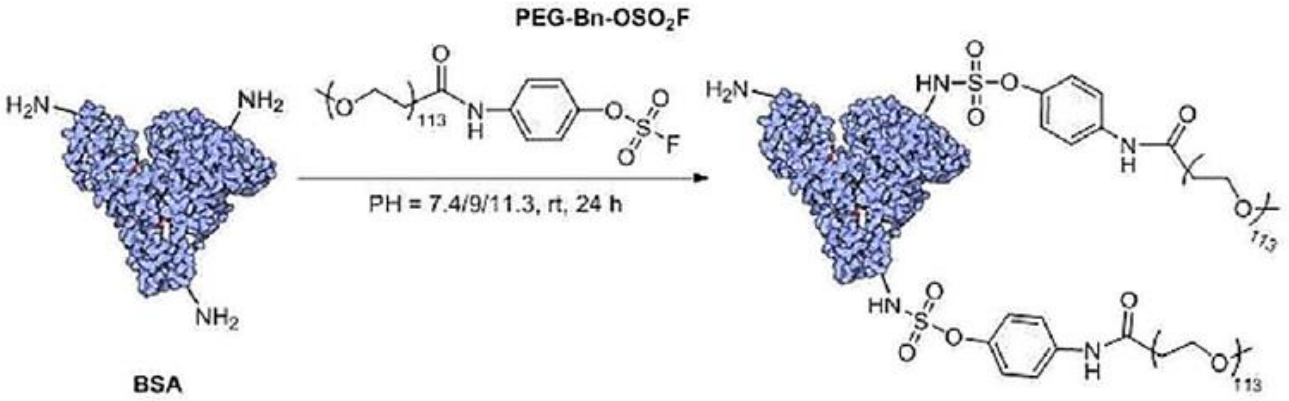
# Application to Bioconjugation

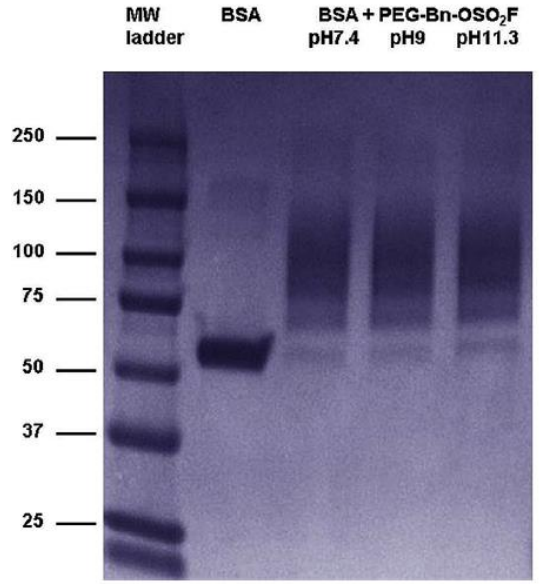
## Modification of BSA with $SO_2F_2$ gas




~4 modifications on Lys


## Modification of BSA with fluorosulfate

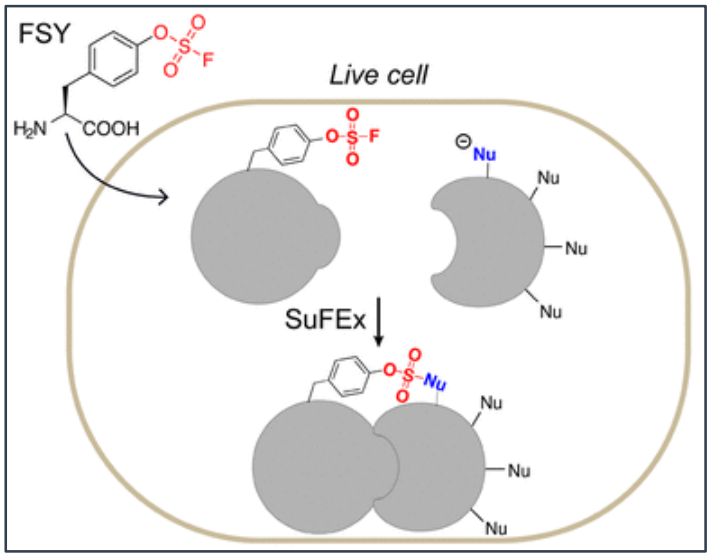



Averick, S. *et al. Polymer* **2016**, *99*, 7.

# SuFEx with Protein *in Vivo*

## Fluorosulfate-L-tyrosine (FSY)




### Incorporation of FSY

Pyrrolysyl-tRNA synthetase (PylRS) mutated library (mutation at A302, L305, Y306, L309, I322, N346, C348, Y384, V401, W417)

selection

6 hits PylRS mutant specific for FSY
(mutation 302I, 346T, 348I, 384L, 417K)
-> FSYRS (fluorosulfate tyrosyl-tRNA synthetase)

Coexpression of target protein gene containing TAG codon with $tRNA^{Pyl}_{CUA}$/FSYRS

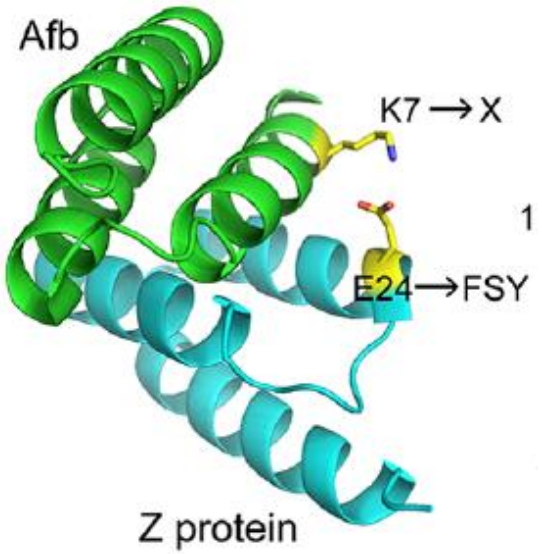
- FSY was successfully introduced in the protein of E. coli and HELA
- No toxicity to cells during the expression of FSY incorporated proteins.

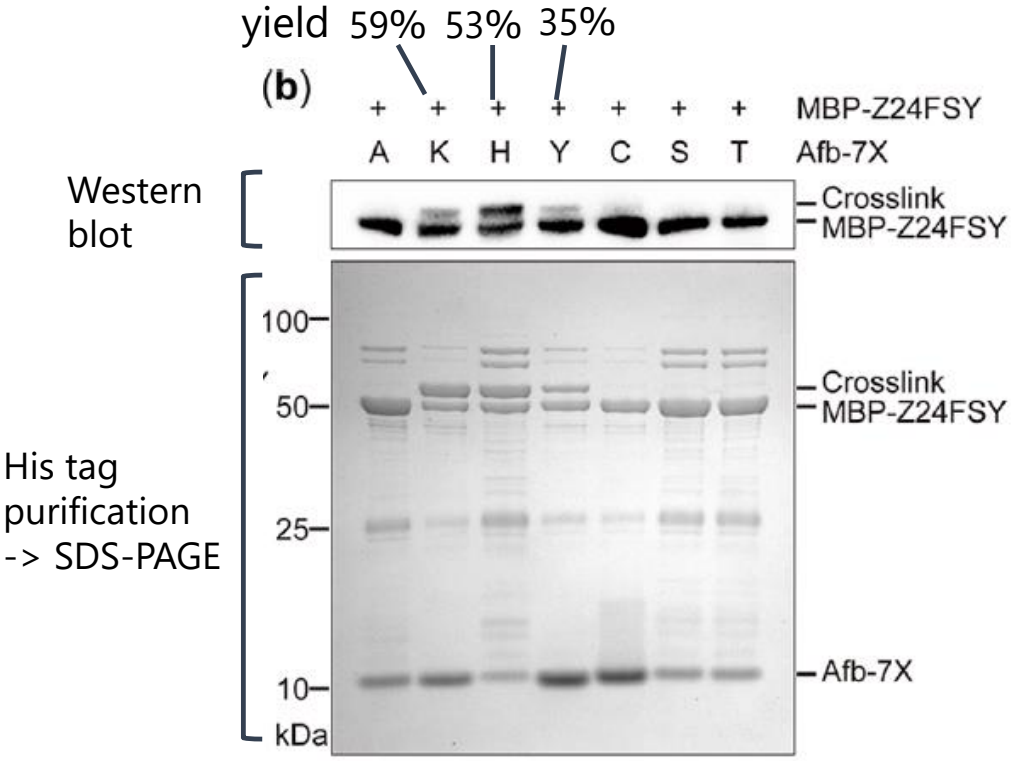
Wang, L. *et al. J. Am. Chem. Soc.* **2018**, *140*, 4995.

# SuFEx with Protein *in Vivo*

## Afb-Z protein

MBP-Z24FSY, Afb-7X were coexpressed in *E. coli*.

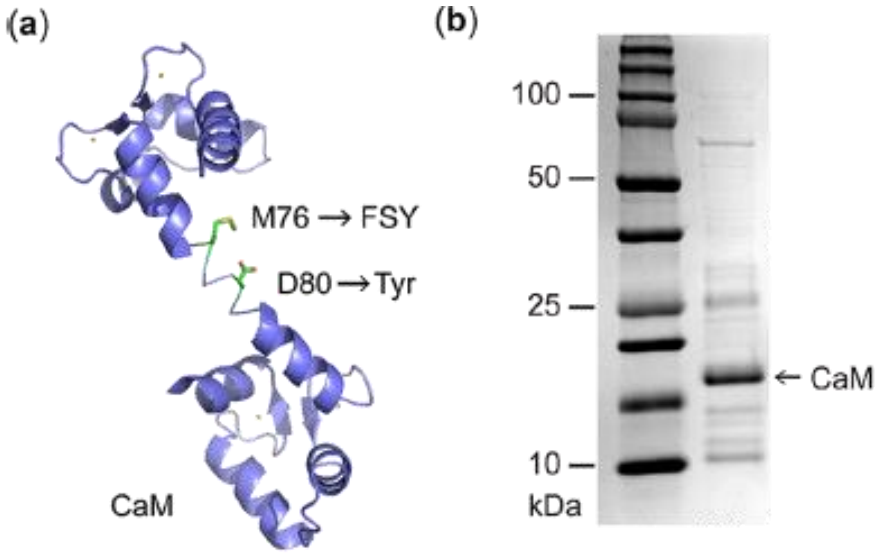



LC-MS/MS
Croslinking between FSY and Lys/His/Thr

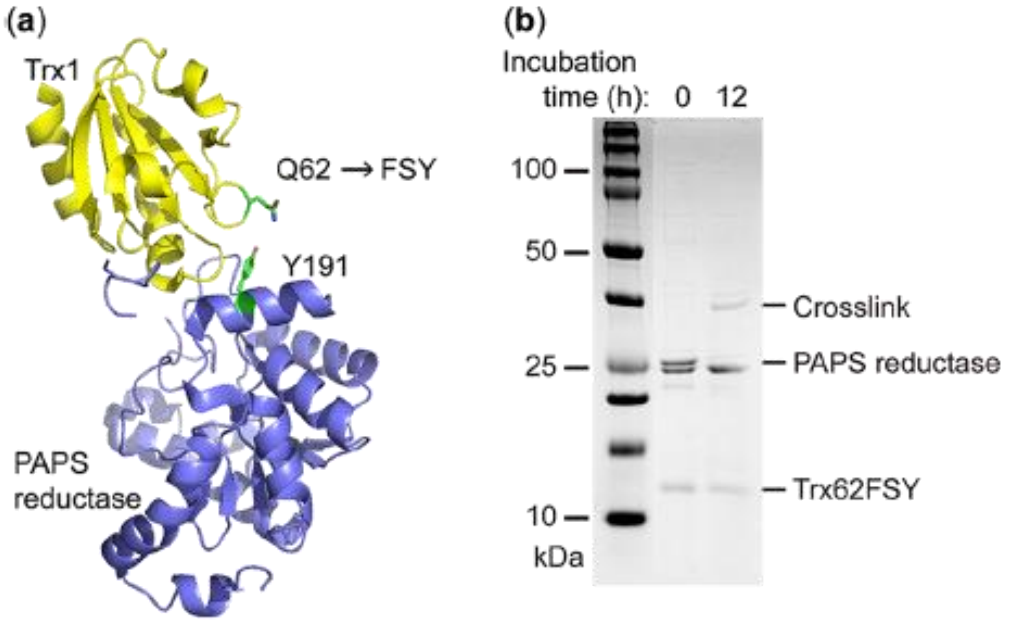
# SuFEx with Protein *in Vivo*

## Calmodulin




intramolecular crosslinking

## Trx1-PAPS reductase

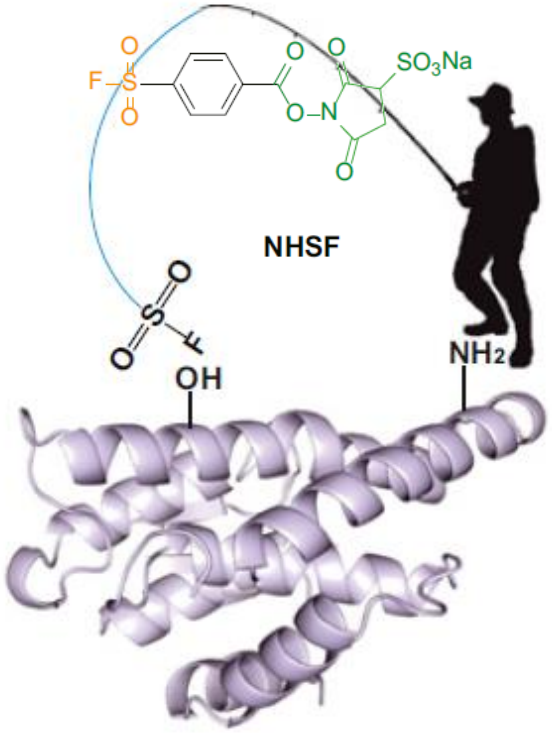


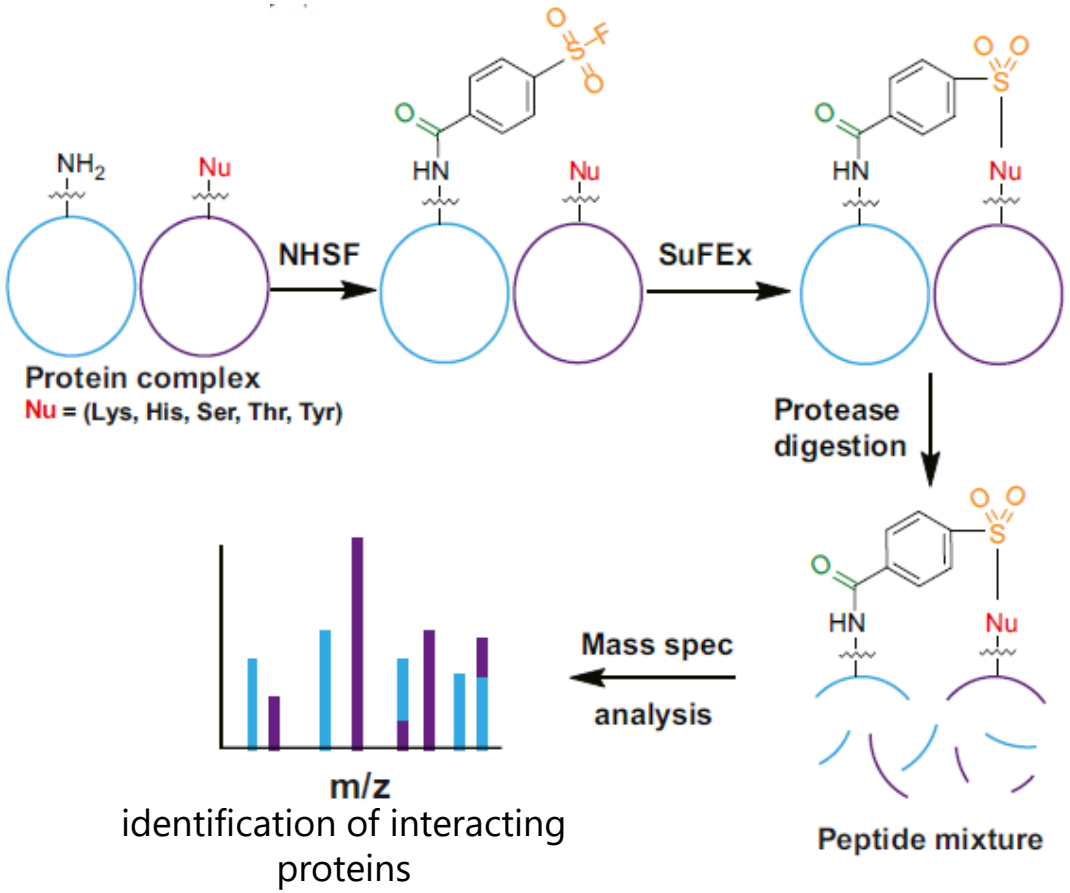

crosslinking with native Tyr

# SuFEx Cross-linking for Mass Spectrometry

Cross-linking mass spectrometry (CXMS)
Identification of protein interaction partners

"plant-and-cast" strategy

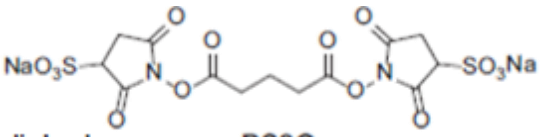


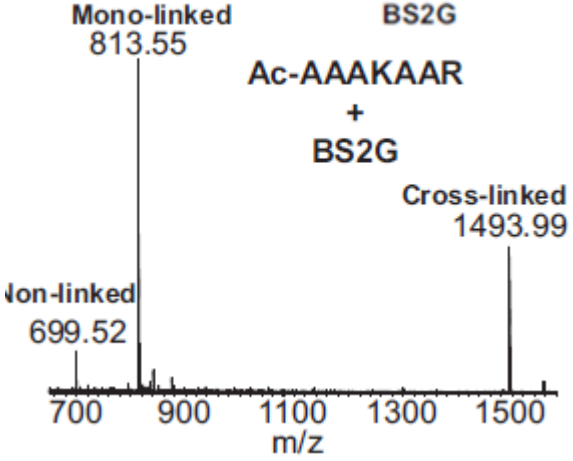



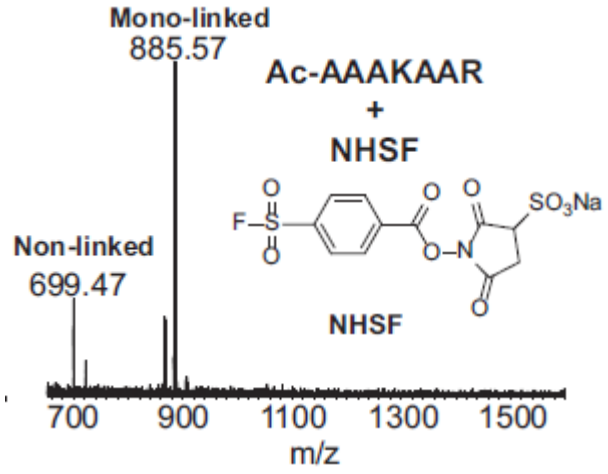

Wang, L. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 11162.

# SuFEx Cross-linking for Mass Spectrometry

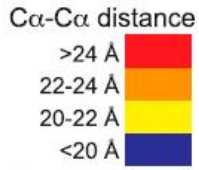
## Reaction with peptide



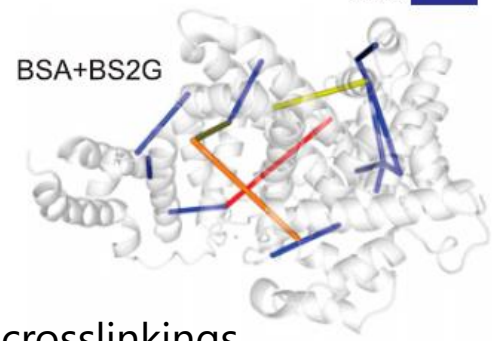



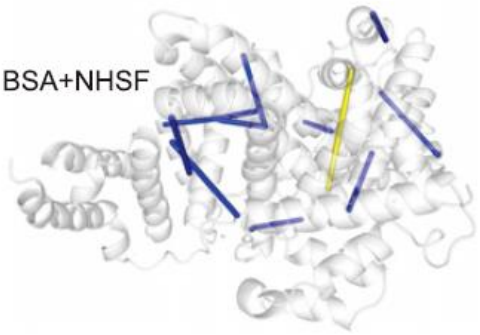

## CXMS analysis of BSA




18 crosslinkings

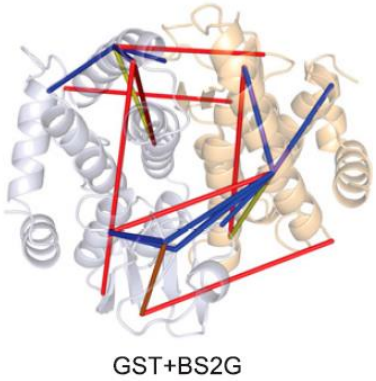


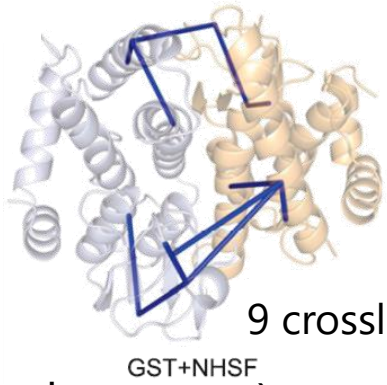

13 crosslinkings




## GST

15 crosslinkings




9 crosslinkings

= Cα-Cα distance (in the crystal structure) > side chain length*2 + linker length ~ 20 Å

Cross-linking occurred at different structure from crystal structure.

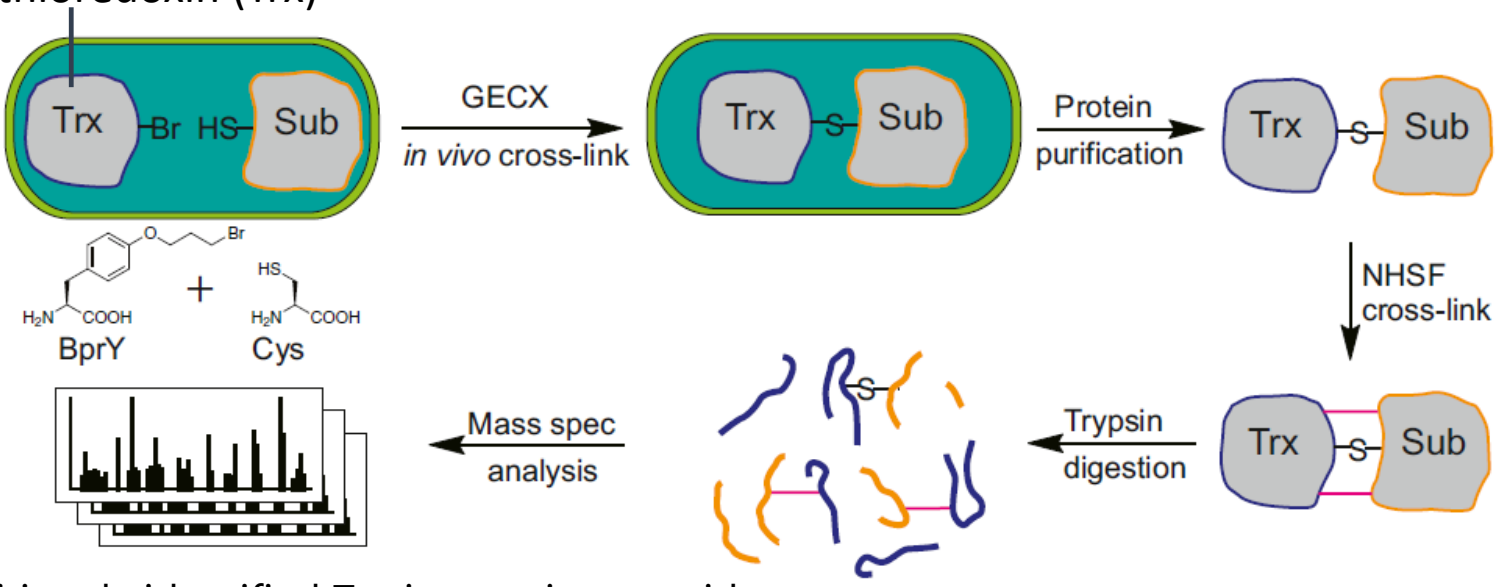
Non-specific cross-linking is observed with BS2G.

# SuFEx Cross-linking for Mass Spectrometry

## Analysis of Enzyme-Substrate Interaction

genetically encode chemical crosslinkers (GECX)

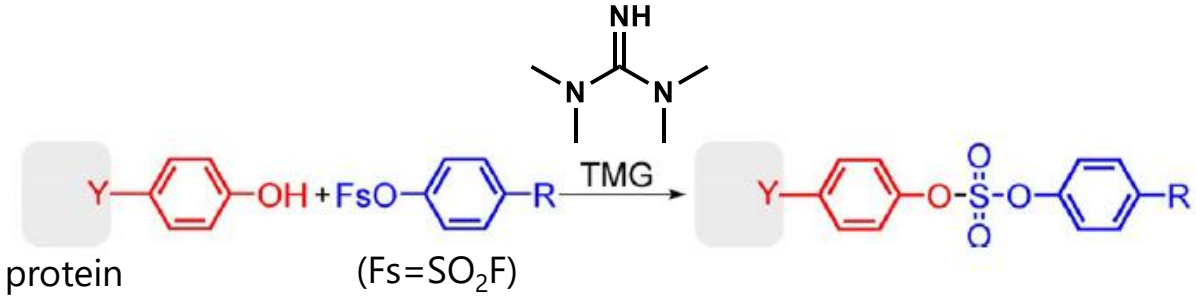
thioredoxin (Trx)



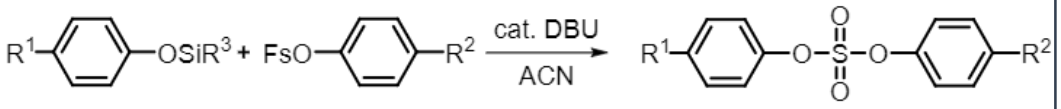

Additionaly identified Trx-interacting peptides

| Accession # | Gene | Description | Cross-linked peptides (cross-linked sites in red) |
|---|---|---|---|
| P43319 | yraK | Uncharacterized fimbrial-like protein | MSDKIIHLTDDSFDTDVLK-GAWVDGMGSPSTGDFHDLVK |
| P36566 | cmoM | tRNA 5-carboxymethoxyuridine methyltransferase | MSDKIIHLTDDSFDTDVLK-YCRQEPYITLGR |
| P16918 | rhsC | May involved in natural ecology of cell | EAAGEFSGEITGVTDGAGRHFR-SDKIIHLTDDSFDTDVLK |
| C4ZUA4 | menC | o-succinylbenzoate synthase | GIPTLLLFKNGEVAATK-EKVAK |
| P55138 | ygcE | Uncharacterized sugar kinase YgcE | GIPTLLLFKNGEVAATK-GYGIR |
| P0AGA2 | secY | Protein translocase subunit | GIPTLLLFKNGEVAATK-KANLK |
| P0AGC3 | slt | Soluble lytic murein transglycosylase | NGEVAATK-DLFKR |

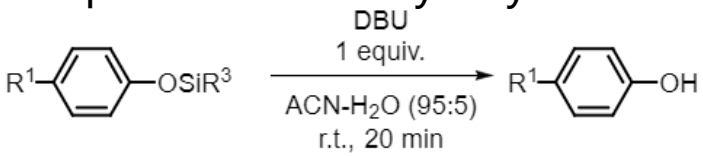
# Tyr Bioconjugation



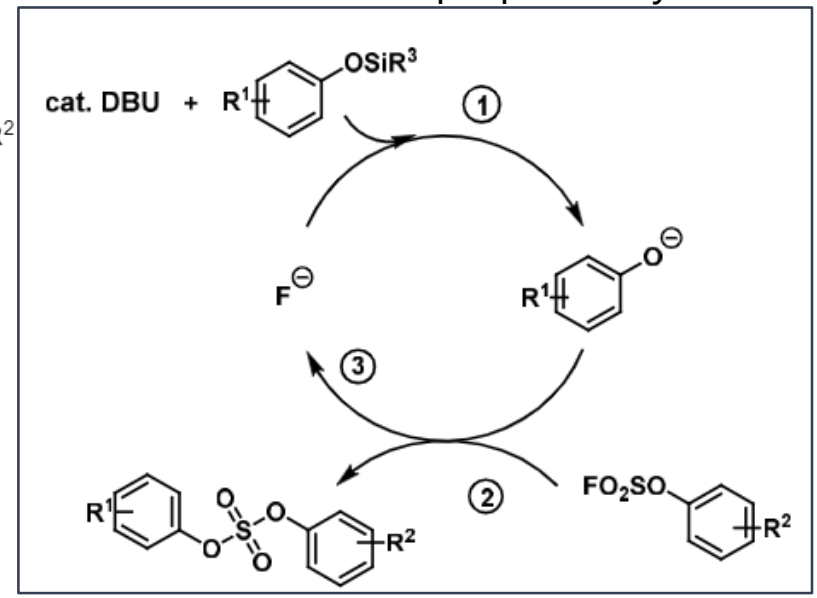

SuFEx




Deprotection of aryl silyl ether




Mechanism of SuFEx proposed by authors

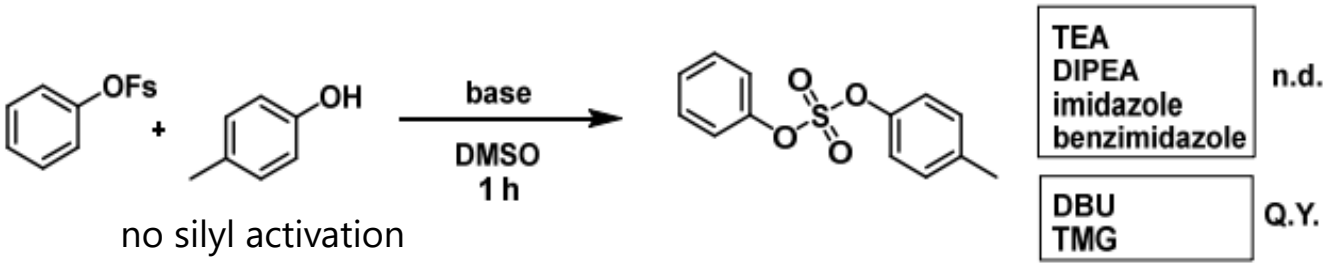



Use of stoichiometric amount of base avoids the silyl ether?

Lee, Y.; Kim, B. M. *et al. Chem. Eur. J.* **2018**, *24*, 10948.

# Tyr Bioconjugation

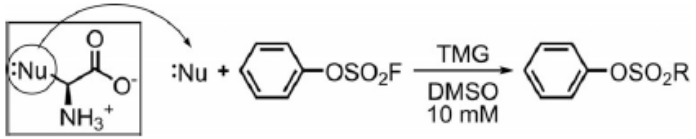
## Screening of base




no silyl activation

## Residue selectivity

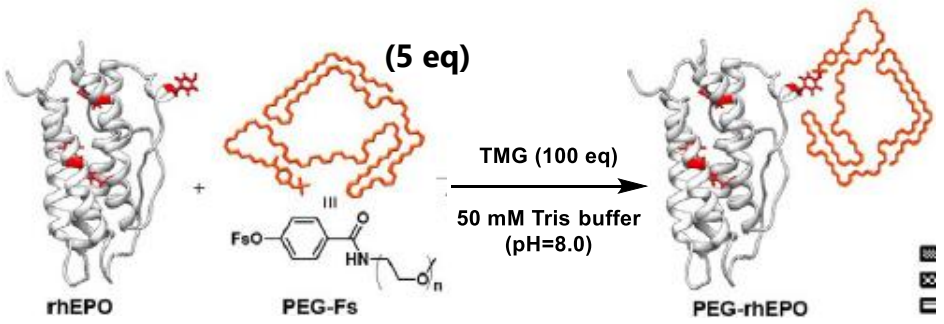
**Table 1.** Comparison of SuFEx reactivity in the reactions of various model nucleophiles representing amino acids with phenyl fosylate.

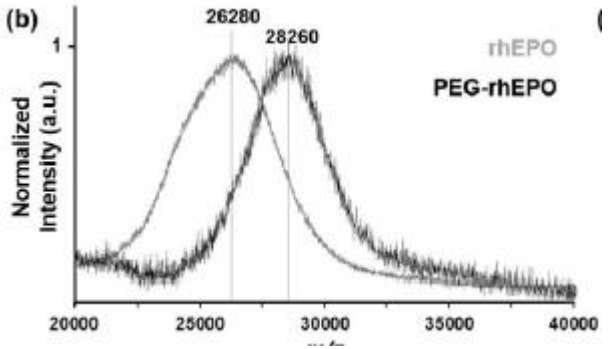



| Entry | Model nucleophile | Amino acid | Time (h) | Yield[a] (%) |
|---|---|---|---|---|
| 1 | *p*-cresol | Y | 1.5 | 93.5 |
| 2 | *n-butyl*amine | K | 12 | n.d. |
| 3 | Propanethiol | C | 12 | n.d. |
| 4 | Methanol | S | 12 | n.d. |
| 5 | *N*-propylguanidine | R | 12 | n.d. |
| 6 | 3-methylindole | W | 12 | 12.5 |
| 7[b] | 4-methylimidazole | H | 12 | trace |

[a] Yield of isolated product. [b] 0.25 equiv of $NiCl_2(H_2O)_6$ was added.

## Recombinant human erythropoietin (rhEPO)

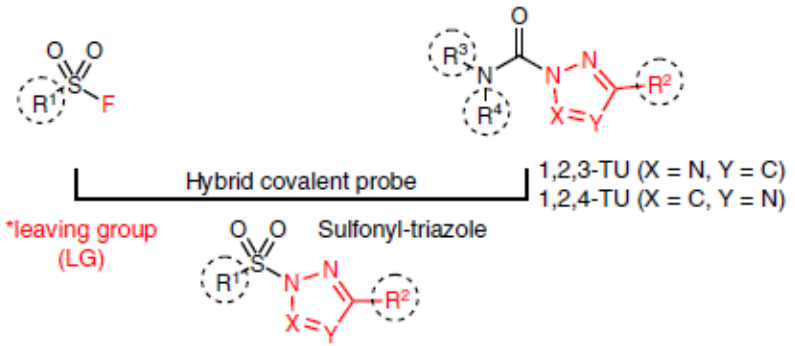


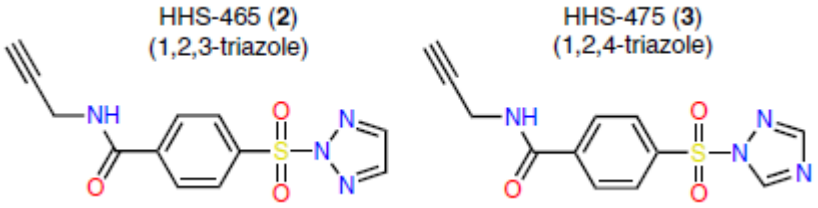

PEG
Mn=2372
Δm/z=1980

# Tyr-Reactive Probe

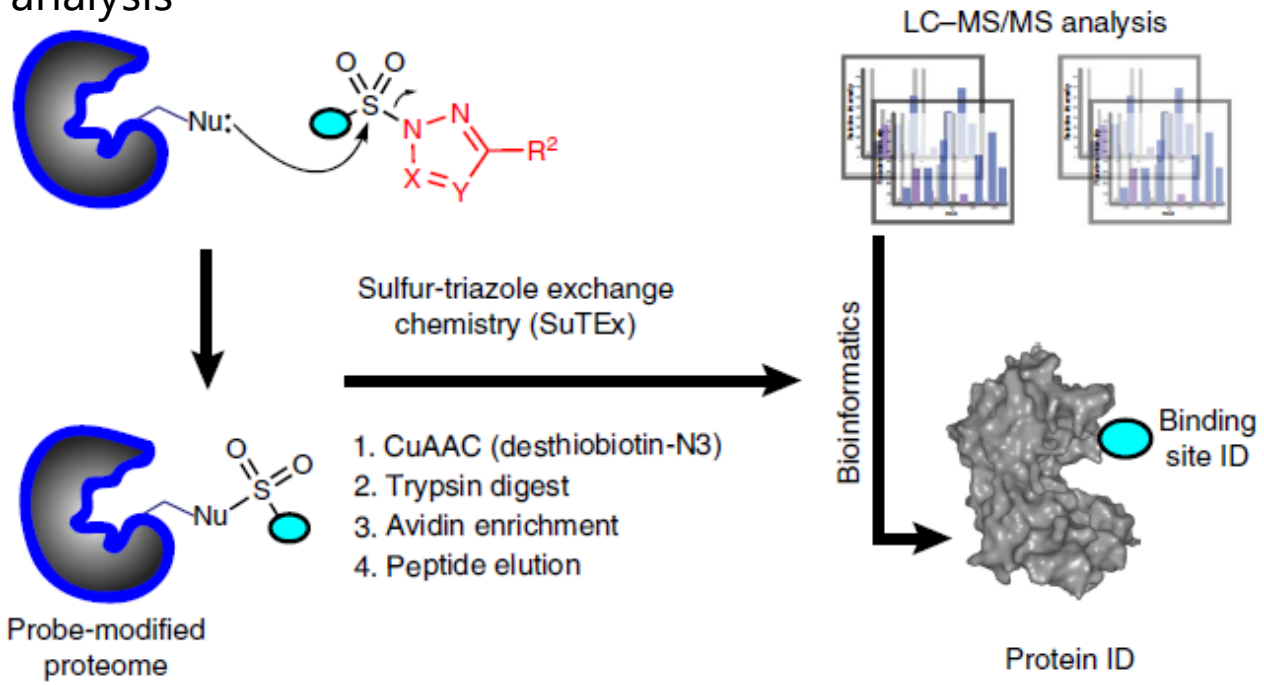
## Sulfur-Triazole EXchange (SuTEx)




Probes



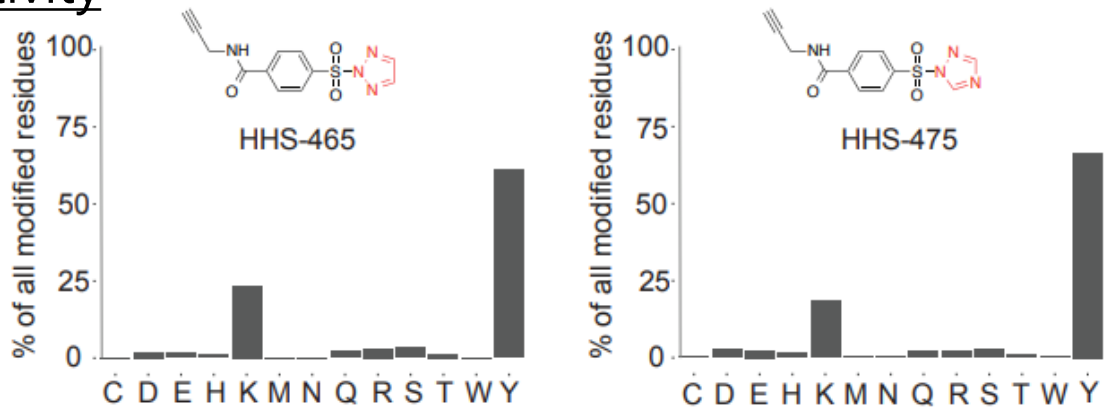

Proteome analysis



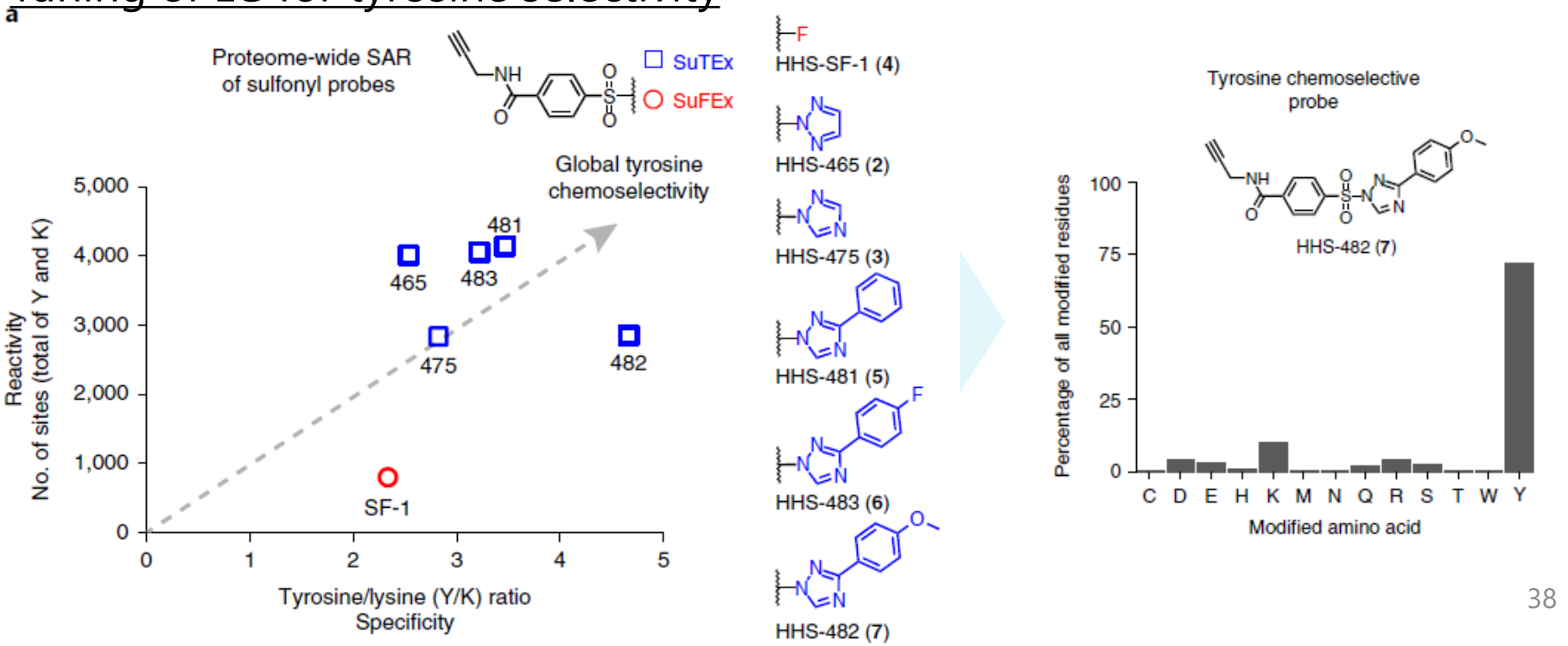

Hsu, K. *et al. Nat. Chem. Biol.* doi:10.1038/s41589-019-0404-5

# Tyr-Reactive Probe

## Residue selectivity



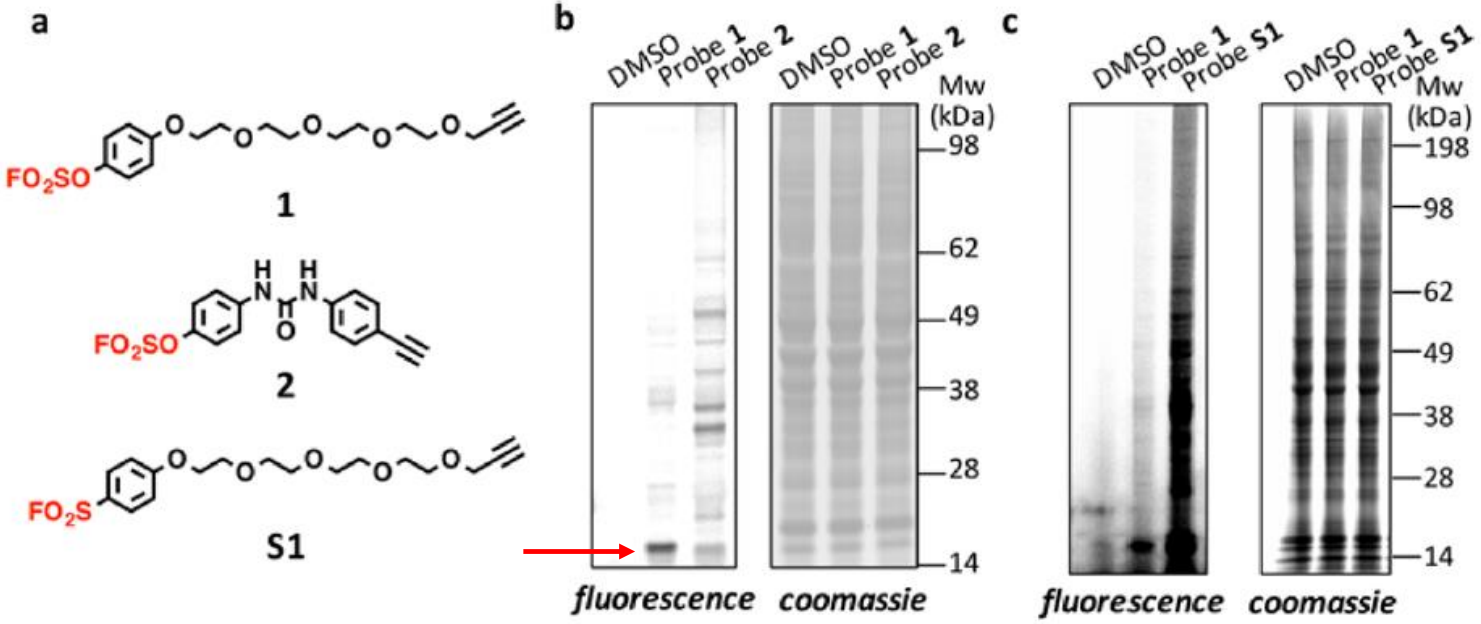

## Tuning of LG for tyrosine selectivity



# Covalent Inhibitor by SuFEx

Inhibitor of intracellular lipid binding protein (iLBP)

Evaluation of proteome reactivity of probes



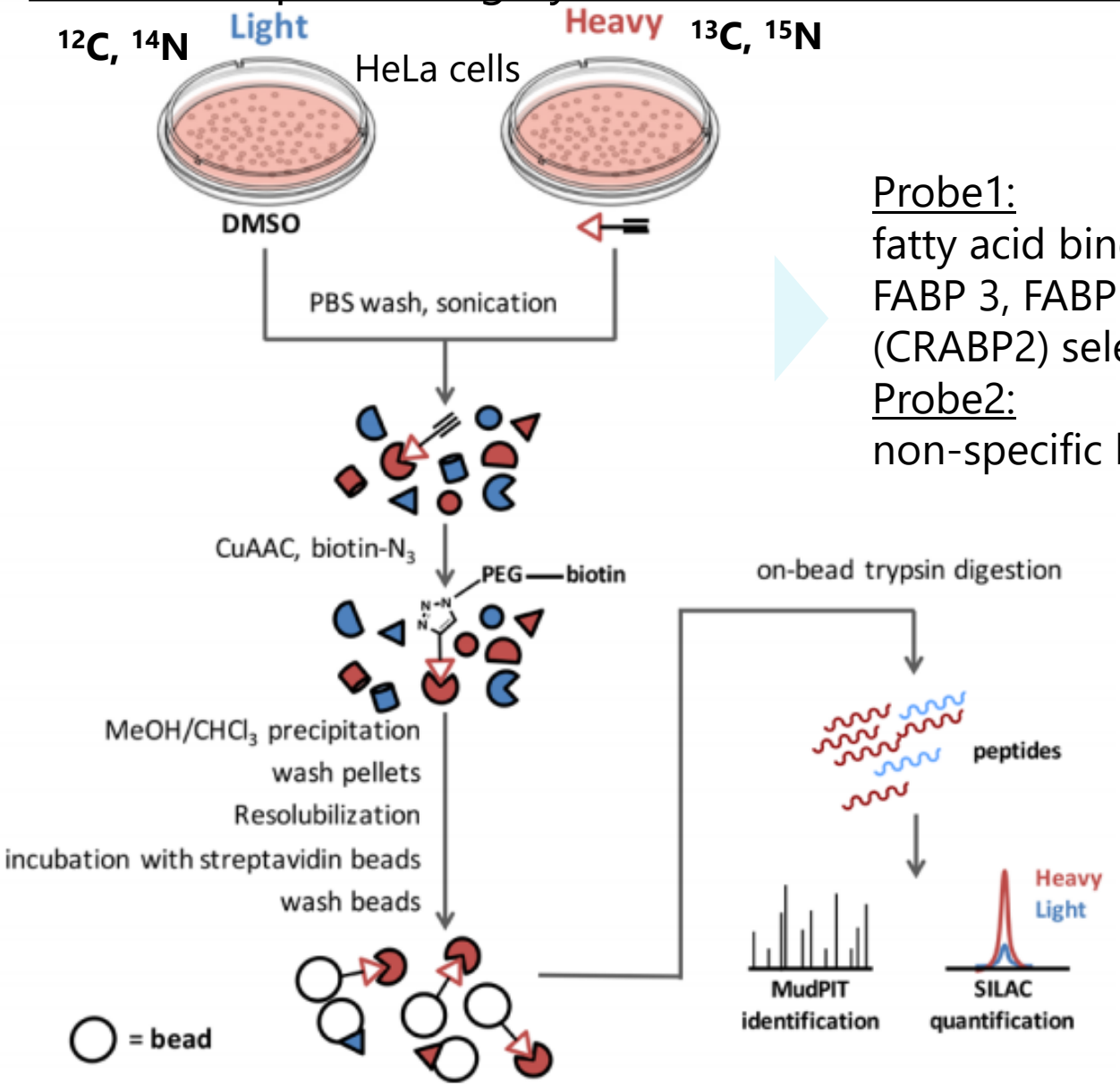

HeLa cell + blank/1/ 2 or S1 (40 uM, 37 °C, 16 h), CuAAC with cell lysate to attach fluolophore, SDS-PAGE

Sulfonyl fluoride: highly reactivive, resulting in non-specific reactivity

Sharpless, K. B.; Kelly, J. W. *et al.* *J. Am. Chem. Soc.* **2016**, *138*, 7353.

# Covalent Inhibitor by SuFEx

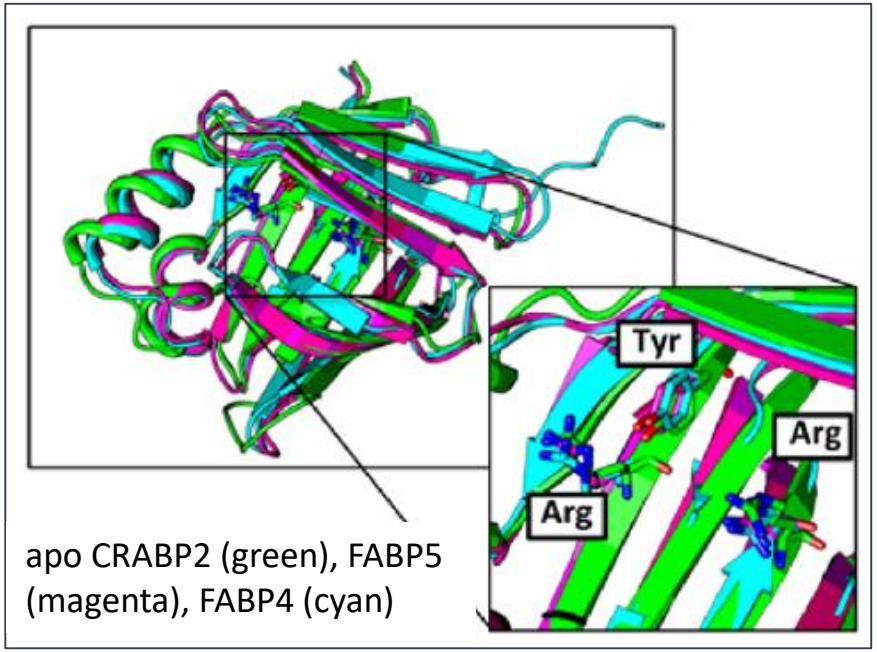
Stable isotope labeling by amino acids in cell culture (SILAC)




Probe1:
fatty acid binding protein (a family of iLBPs)
FABP 3, FABP 5,
(CRABP2) selective labeling
Probe2:
non-specific labeling

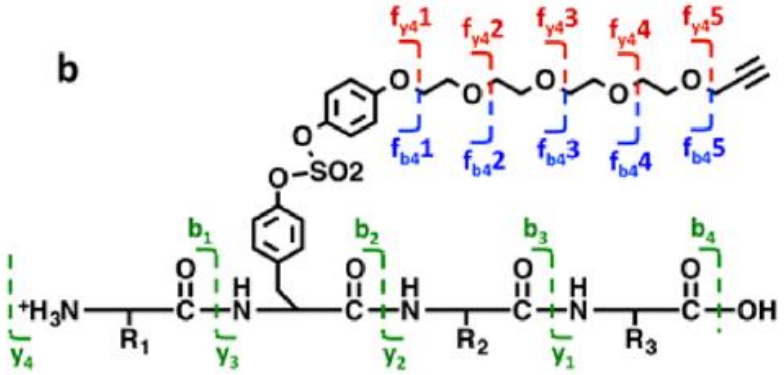
# Covalent Inhibitor by SuFEx

Conserved Arg~Arg~Tyr module in iLBPs

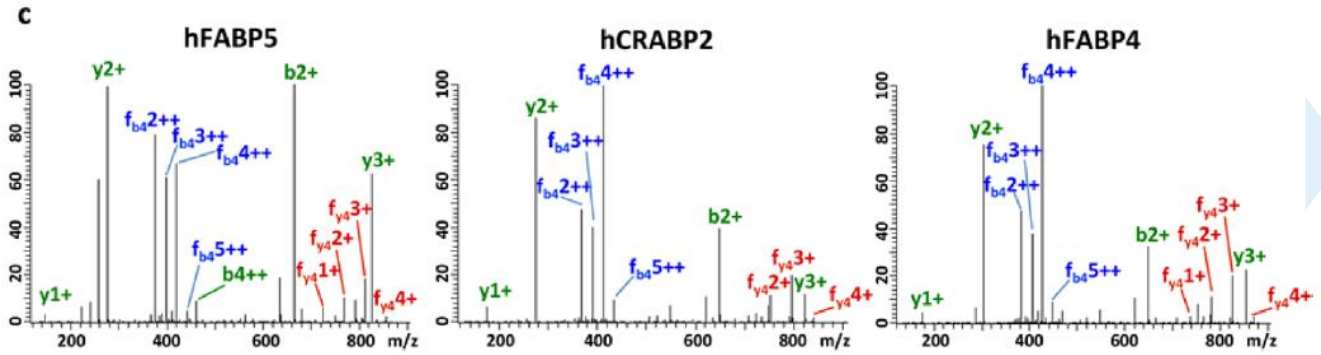



apo CRABP2 (green), FABP5 (magenta), FABP4 (cyan)

LC-MS/MS to determine reactive residue



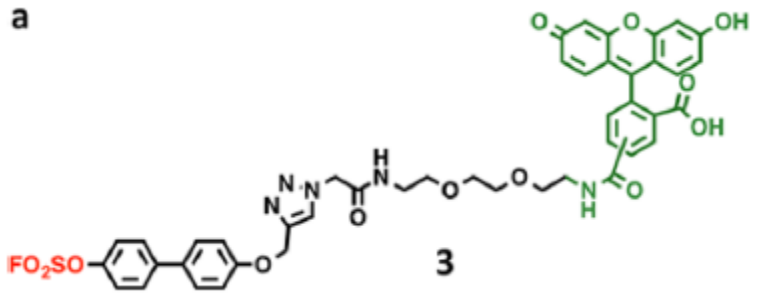

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| hFABP5 | Ile | Glu | Lys |
| hCRABP2 | Val | Val | Arg |
| hFABP4 | Val | Glu | Arg |




All reacted at Tyr in Arg~Arg~Tyr module.

# Covalent Inhibitor by SuFEx

## Introduction of biphenyl substructure

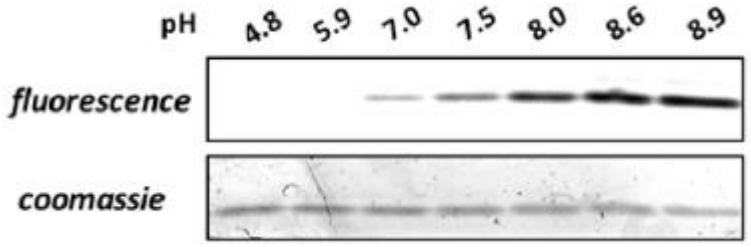


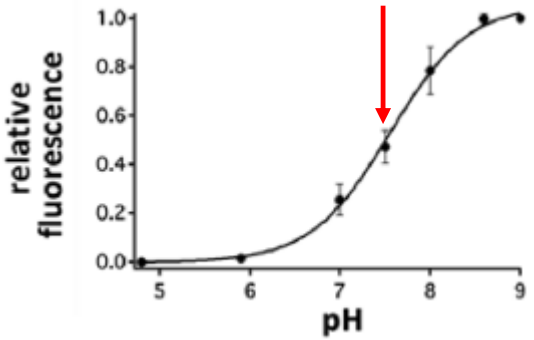

Improved reactivity for CRABP2
No reactivity with FABP3, 4, 5

## Mechanism analysis of selectivity for CRABP2

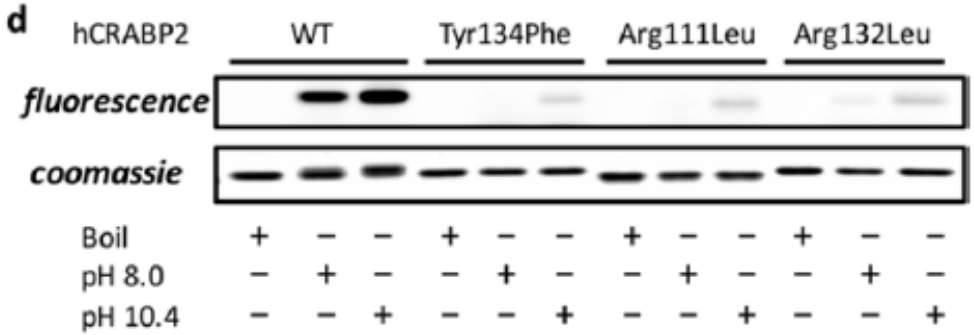
Tyr134 pKa ~ 7.6
effect of Arg111, Arg132?

- Buffer pH dependence



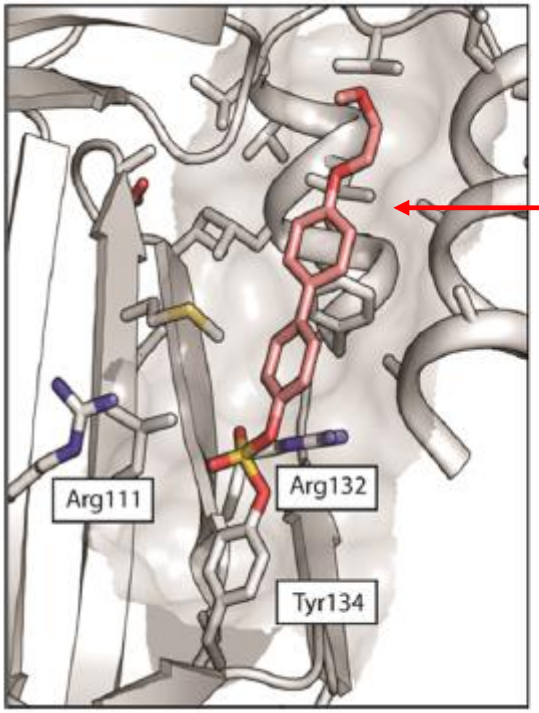

- Mutation experiment



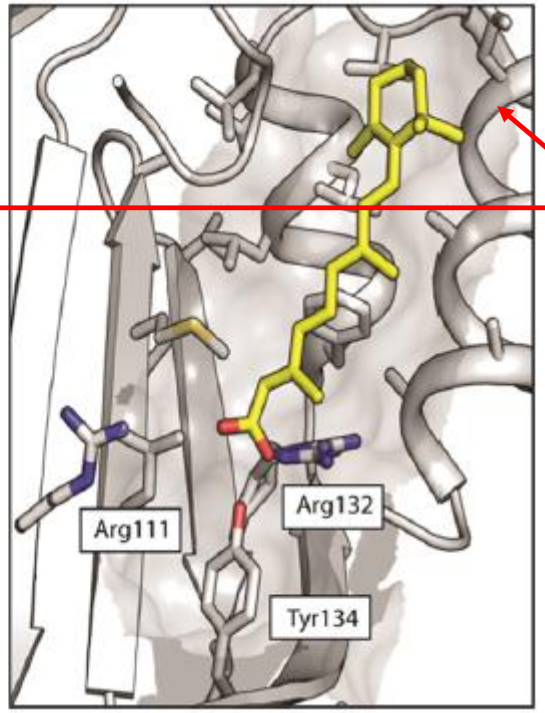
# Covalent Inhibitor by SuFEx

crystal structure

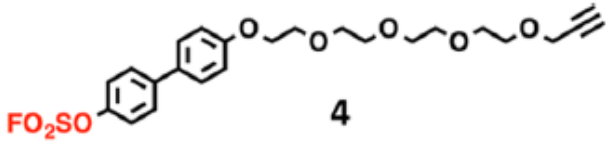
4-CRABP2



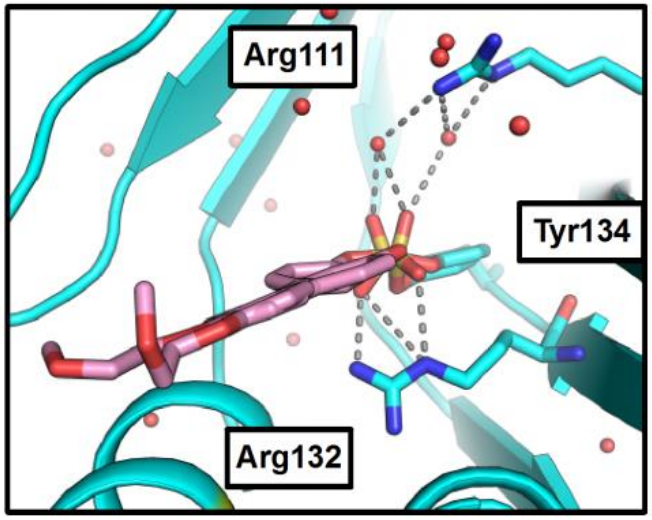

retinoic acid (RA)-CRABP2
(PDB: 2FR3)




large hydrophobic binding pocket to accommodate Phenyl ring/ trimethyl cyclohexene ring (selectivity of 4 to CRABP2)



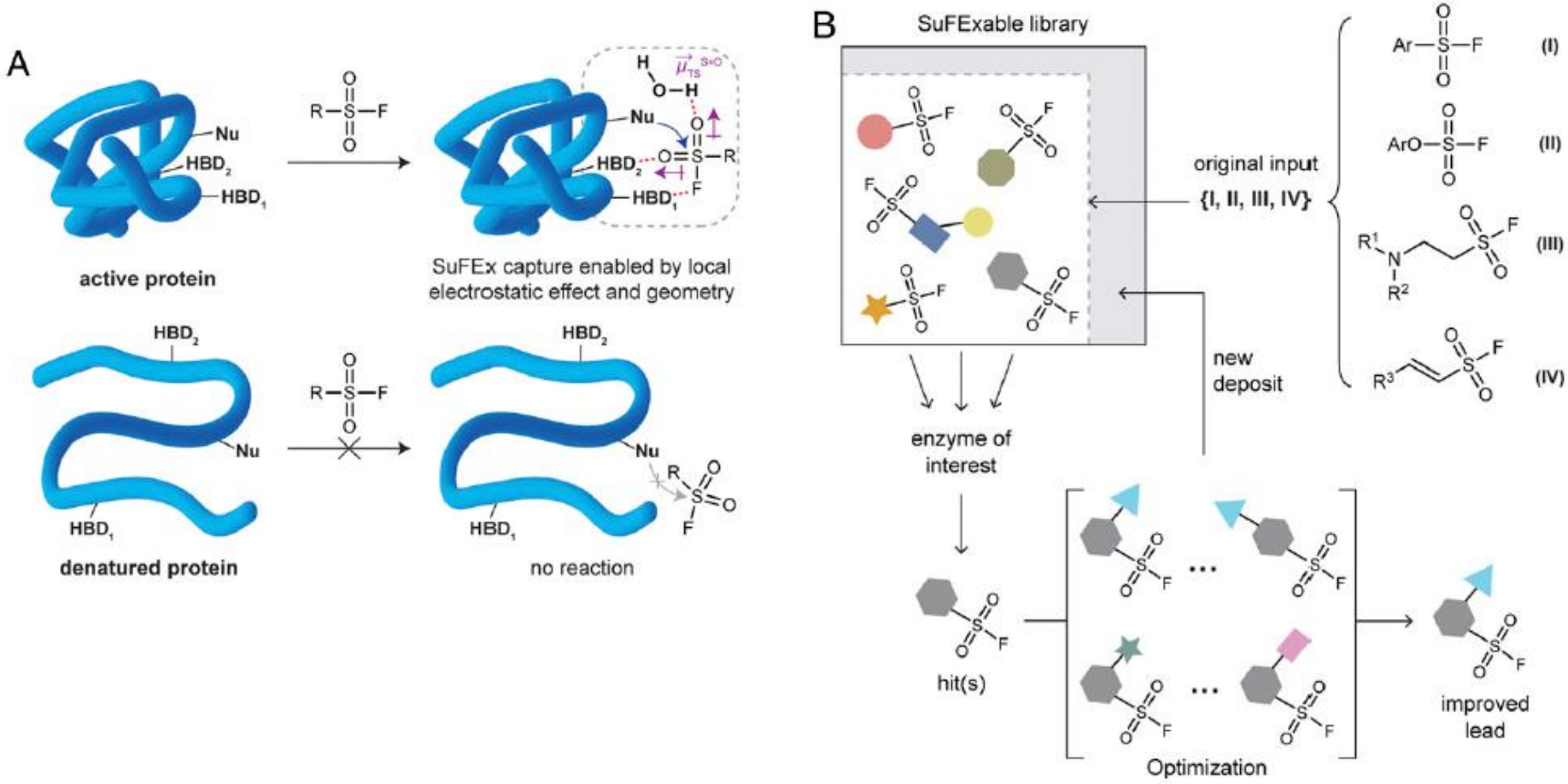

4



# Covalent Inhibitor by SuFEx

Covalent inhibitors of human neutrophil elastase (hNE).

PNAS 2019 116 18808




Sharpless, K. B. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 18808.

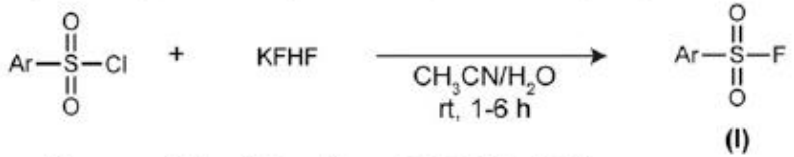
# Covalent Inhibitor by SuFEx

## Categolized SuFExable library

105 compounds

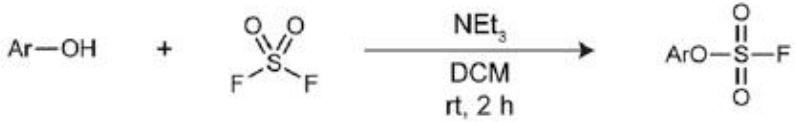
relative rate with ArOTBS

A aryl sulfonyl fluoride (33 entries, 90–100% yield)



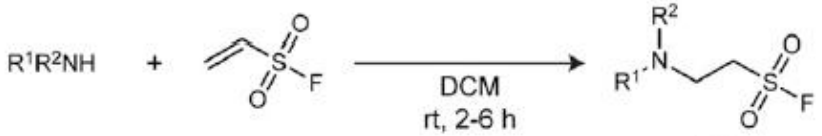

(I) 28.5

B aryl fluorosulfate (32 entries, 82–99% yield)



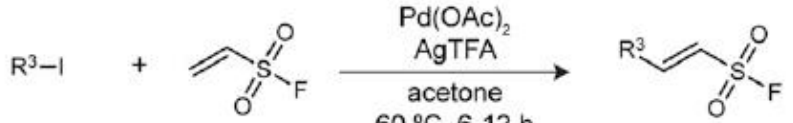

(II) 1

C alkyl sulfonyl fluoride (30 entries, 85–98% yield)




(III) 14.1

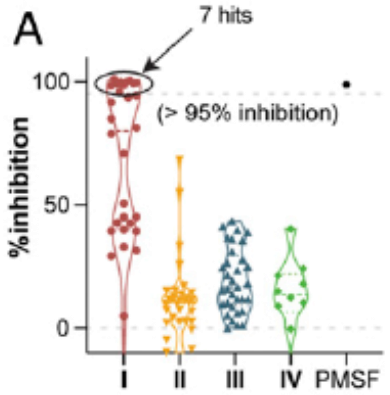
D vinyl sulfonyl fluoride (10 entries, 59–99% yield)




(IV) 4.1

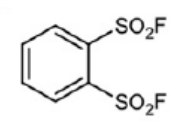
## 1st screen

Incubation with enzyme for 10 min
Addition of substrate peptide (MeOSuc-AAPV-AMC)
Measure increase in fluorescence

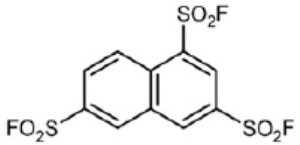


# Covalent Inhibitor by SuFEx

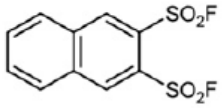
## Hit compounds



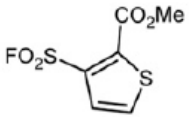
**1**
$IC_{50}$ = 3.3 ± 1.0 μM



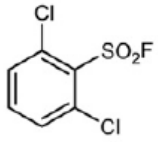
**2**
$IC_{50}$ = 17.5 ± 1.1 μM



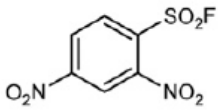
**3**
$IC_{50}$ = 6.8 ± 1.1 μM



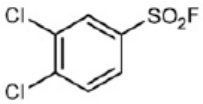
**4**
$IC_{50}$ = 17 ± 2 μM



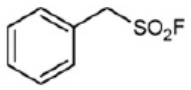
**5**
$IC_{50}$ = 5.4 ± 0.5 μM



**6**
$IC_{50}$ = 5.9 ± 1.1 μM

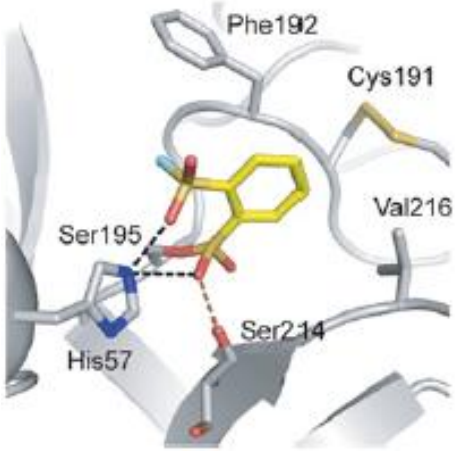


**7**
$IC_{50}$ = 49 ± 3 μM

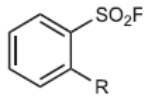


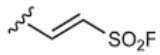
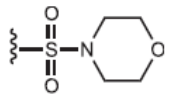
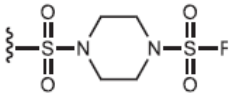
PMSF
$IC_{50}$ = 24 ± 1 μM

## Crystal structure hNE+1



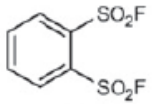
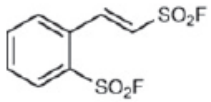
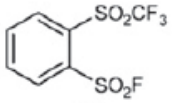
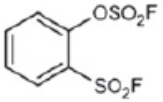
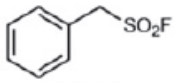

## 2nd screen



| Compound | R = | $IC_{50}$ (μM)* |
|---|---|---|
| **1** | $SO_2F$ | 3.3 ± 1.0 |
| **8** | F | ~120 |
| **9** | Cl | 82 ± 16 |
| **10** | Br | 20 ± 10 |
| **11** | I | 9.7 ± 1.2 |
| **12** | Me | >200 |
| **13** | OMe | 73 ± 4 |
| **14** | CN | 13.3 ± 0.5 |
| **15** | $CF_3$ | 60 ± 8 |
| **16** | $NO_2$ | 20 ± 1 |
| **17** | $CO_2Me$ | 37 ± 2 |
| **18** | Ph | 27 ± 1 |
| **19** |  | 2.2 ± 0.7 |
| **20** |  | 84.4 ± 0.6 |
| **21** |  | >200 |
| **22** | $SO_2CF_3$ | 1.1 ± 0.1 |
| **23** | $SO_2(CF_2)_2CF_3$ | 48 ± 2 |
| **24** | $OSO_2F$ | 0.24 ± 0.02 |

# Covalent Inhibitor by SuFEx

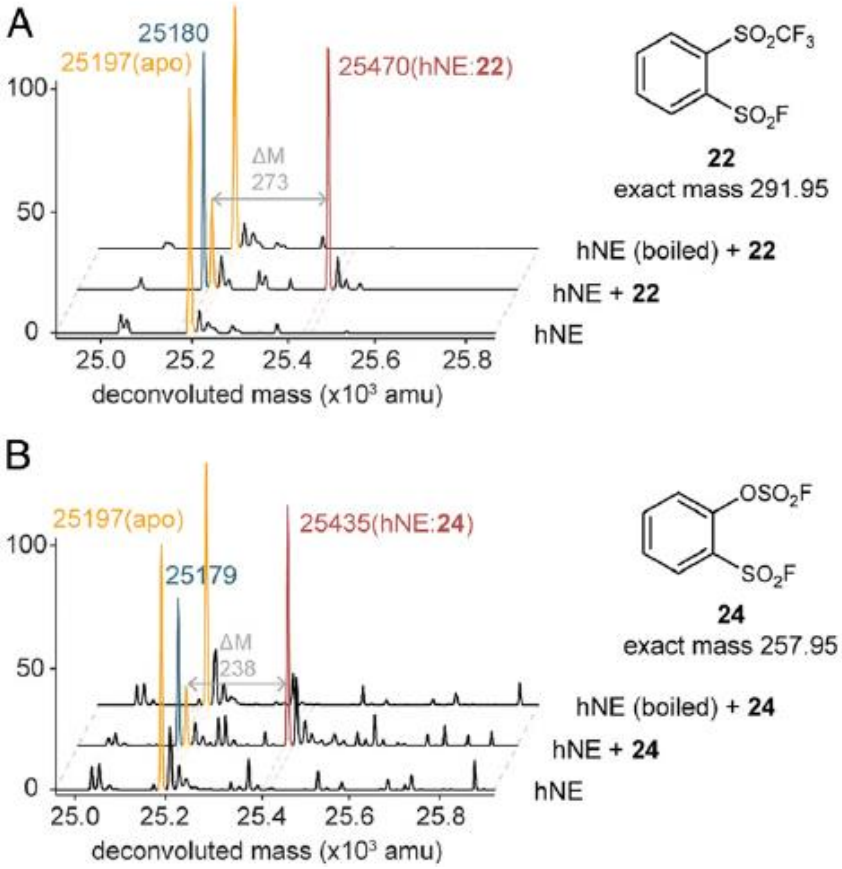
## Selectivity between serine proteases

| Compound | hNE $IC_{50}$ (μM)* | hCG $IC_{50}$ (μM)* | $S^{\dagger}$ |
|---|---|---|---|
|  **1** | 3.3 ± 1.0 | 190 ± 40 | 58 |
|  **19** | 2.2 ± 0.7 | 6.0 ± 0.7 | 2.7 |
|  **22** | 1.1 ± 0.1 | >200 | >182 |
|  **24** | 0.24 ± 0.02 | >200 | >833 |
|  PMSF | 24 ± 1 | 69 ± 6 | 2.5 |

*$IC_{50}$ values were measured based on 10-min incubation and are shown in mean ± SD ($n \geq 3$).
†$S$ value denotes the selectivity, defined by the ratio of $IC_{50}$ (hCG) over $IC_{50}$ (hNE).

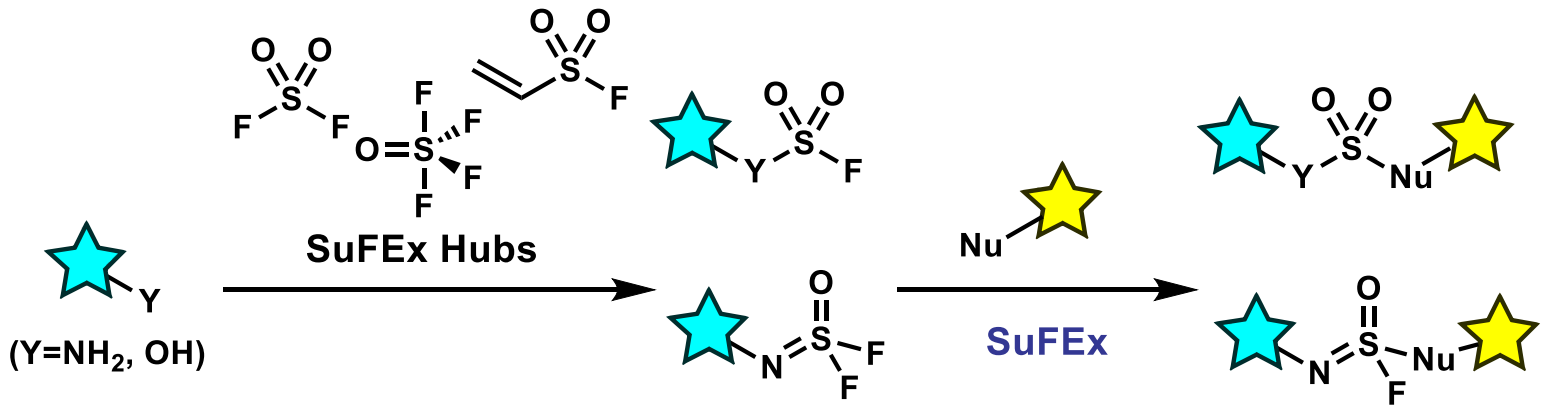
## Effect of denaturation

MALDI-TOF-MS



# Summary

## SuFEx




- Advocated by Sharpless in 2014.
- Stable S(VI)-F bond
- Context dependent activation

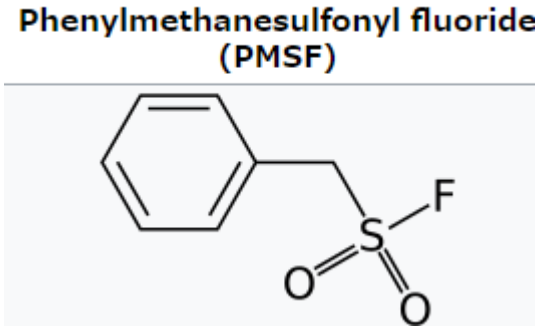
New synthetic methodology
Proteome analysis
Analysis of PPI
Medicinal chemistry
Polymer synthesis

<u>Challenges</u>
Mild/sharp activation of S-F bond
Tuning of leaving group instead of $F^-$
Elucidation of mechanism

# Appendix

# Unstable Sulfonyl Fluoride

PMSF: serine protease inhibitor

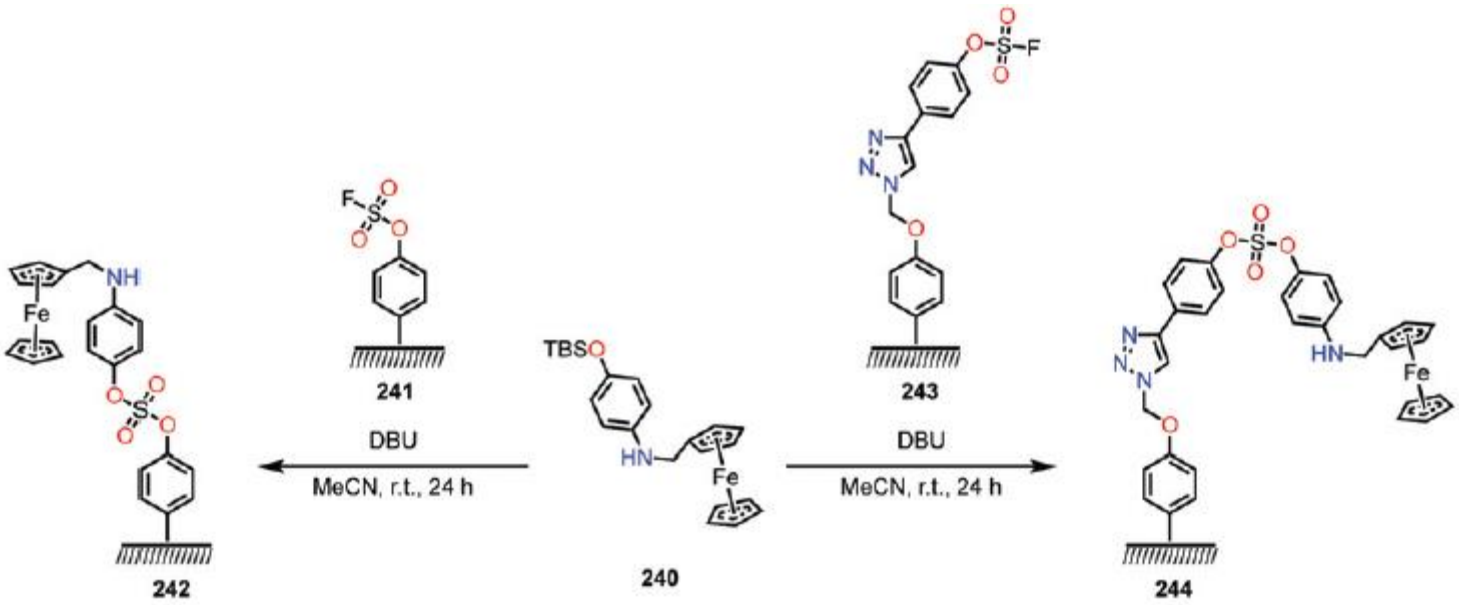



short half-life under aqueous conditions
pH 7: 110 min
pH 7.5: 55 min
pH 8: 35 min

unstable under basic conditions
(forms sulfene via $E_1cB$ elimination)

Aliphatic sulfonyl fluoride encounter the risk of hydrolysis under basic conditions.

# Stability under Electrolytic Conditions

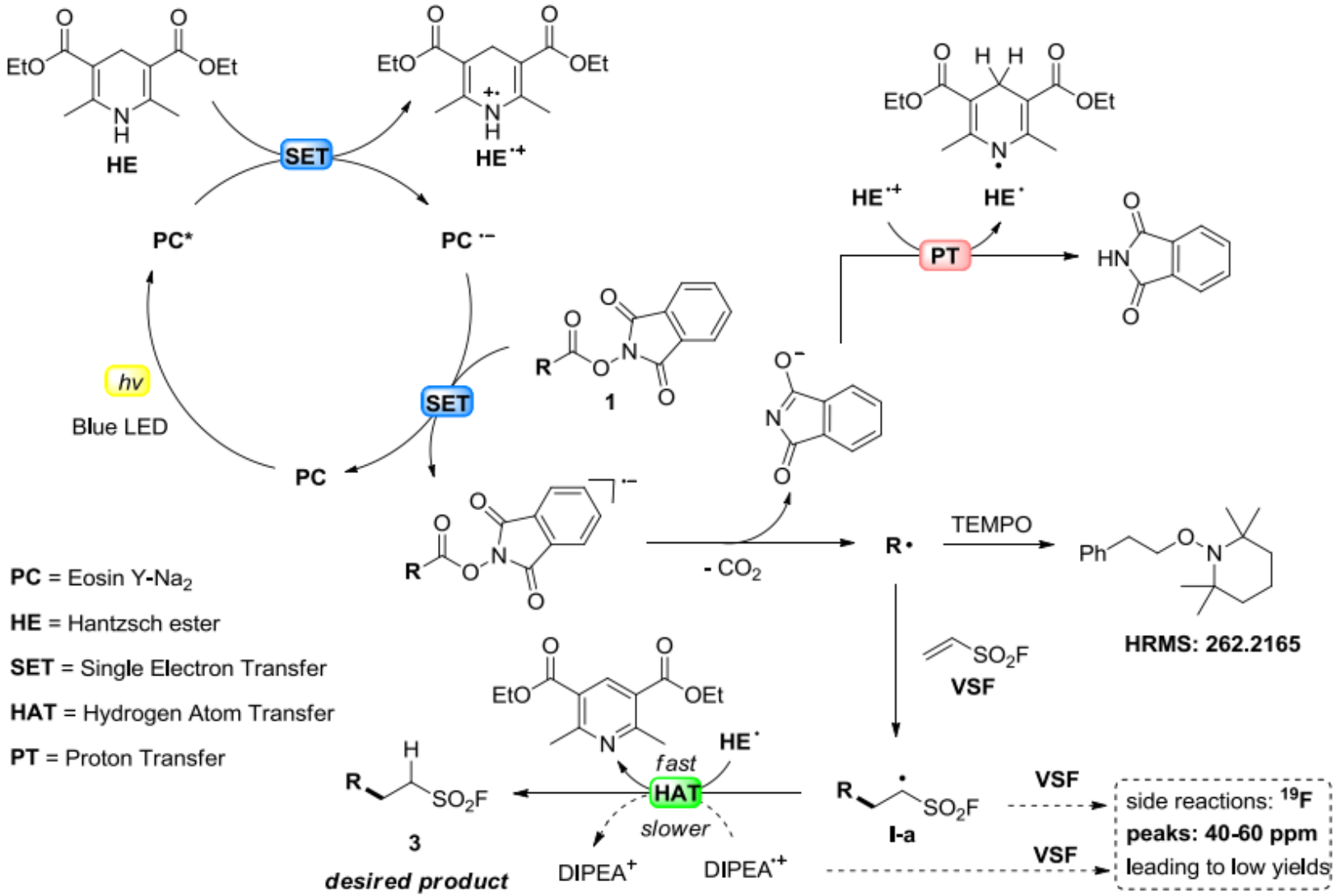



Scheme 48 Modification of carbon fibers by SuFEx click chemistry.

Electrochemical instability of 1,2,3-triazole is demonstrated with CV.

# Radical Addition to ESF

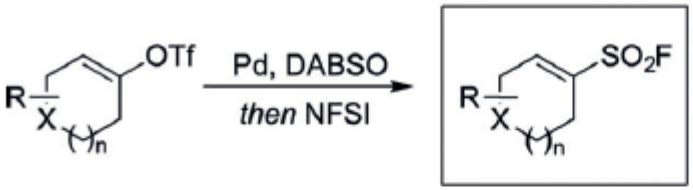
## Entire mechanism



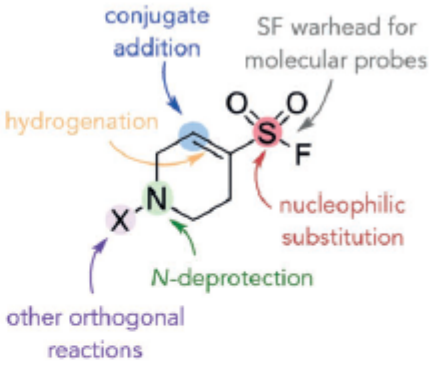

Liao, S. *et al. Nat. Commun.* **2019**, *10*, 1.

# Sulfonylfluoride (RSO2F)

Cyclic alkenylsulfonyl fluoride



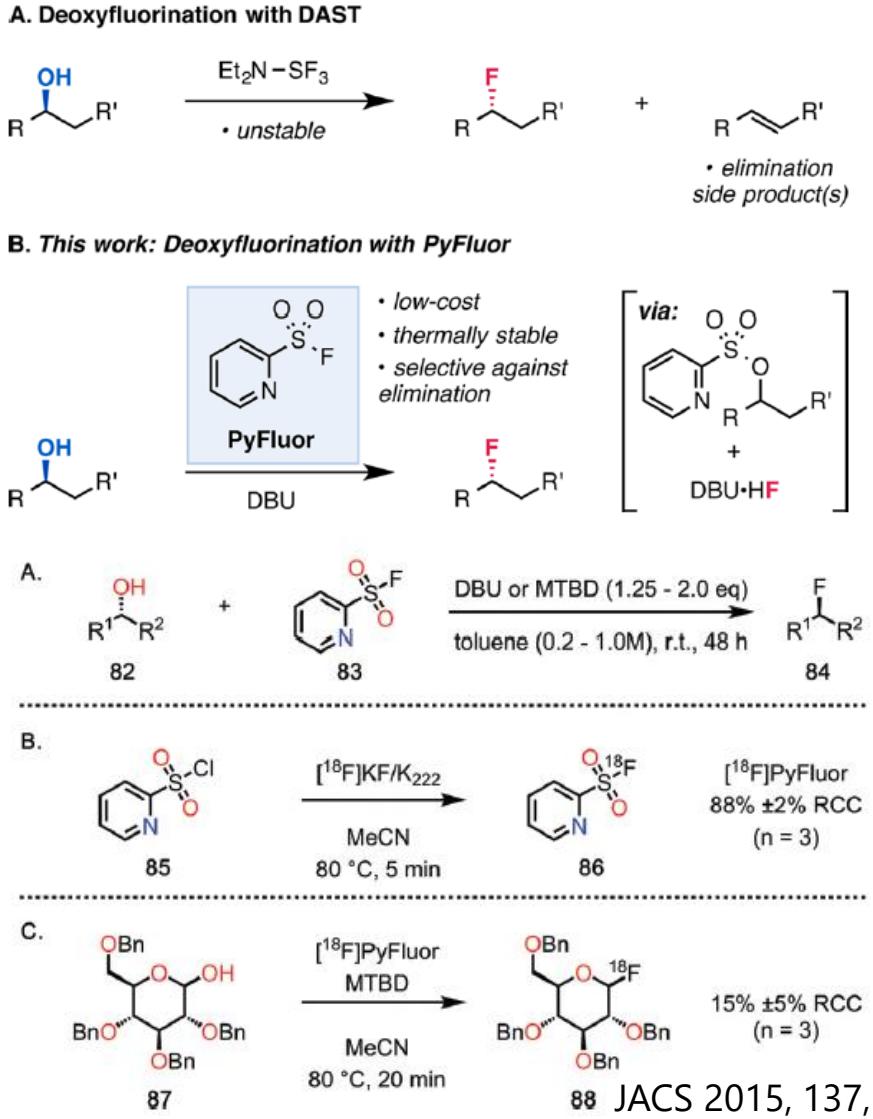

- Readily available starting materials
- Compact molecular scaffold
- Multiple reactive sites



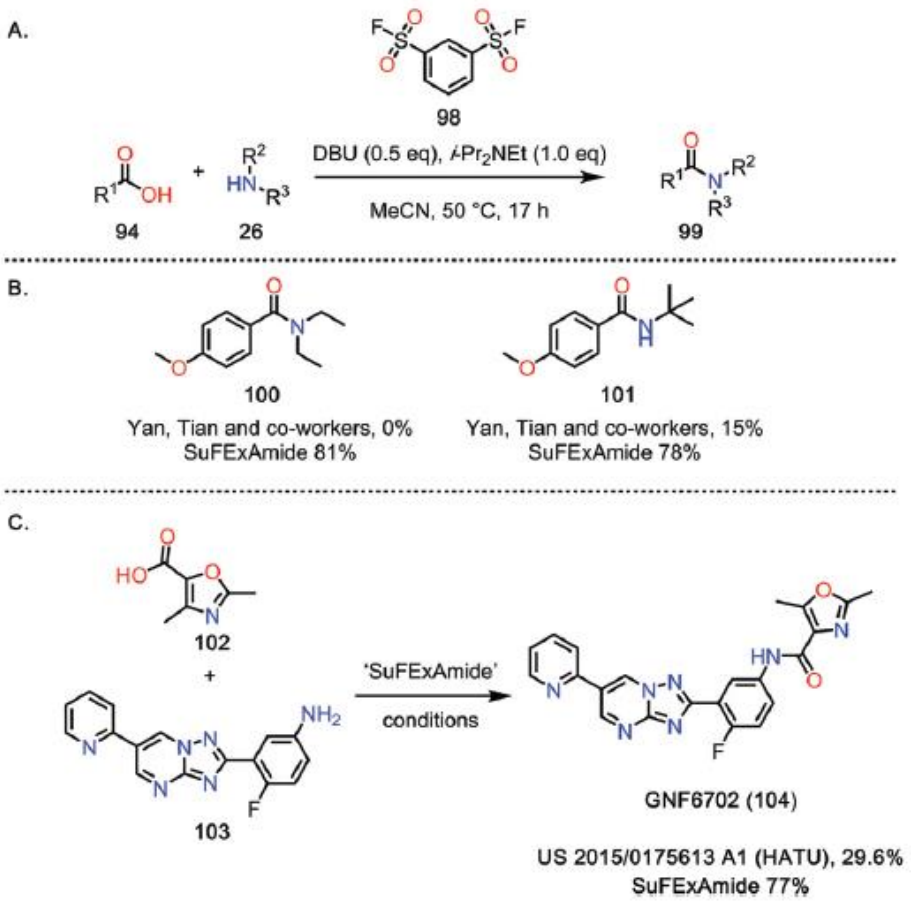

**A family of compact** low-molecular-weight cyclic alkenylsulfonyl fluorides can be readily prepared from the corresponding alkenyl triflates using palladium catalysis. These densely functionalized reagents undergo a diverse range of derivatization reactions, including substitution at sulfur, conjugate addition, and N-functionalization.

# RSO2F as Synthetic Reagent

## PyFluor: deoxyfluorination




## Coupling reagent

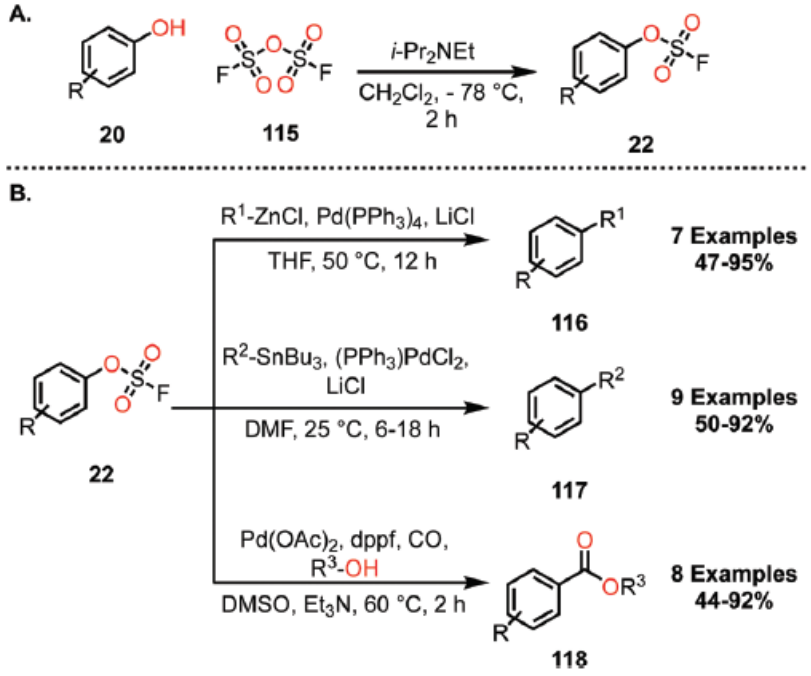



JACS 2015, 137, 9571

# RSO2F as Synthetic Reagent

## ArOSO2F as cross-coupling partner

toxitic




SO2F2, AISF, imidazolium salt can be alternative.

✓ high reactivity

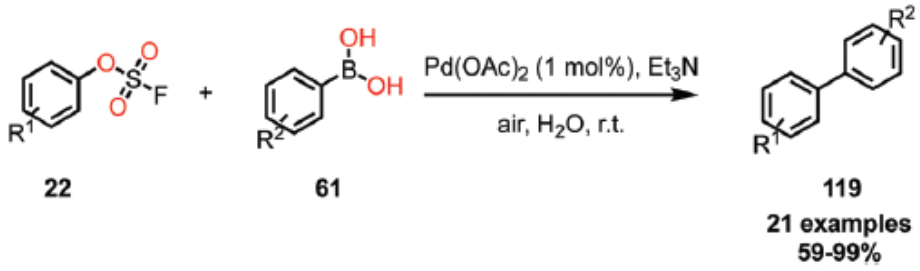
-OSO2F ~ -OTf, -Br > -OTs, -OMs

by Hanley

-Br ≥ -OTf > -OSO2F > -Cl

by Sharpless

## Additive free Suzuki-Miyaura coupling



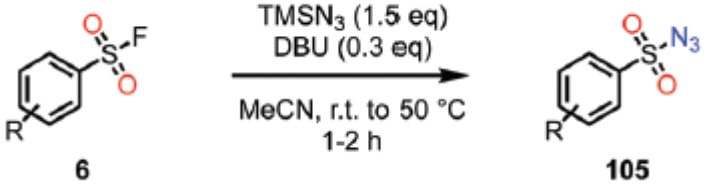
# RSO2F as Synthetic Reagent

1,1-Dihydrofluoroalkylation of Amines


- no reaction with –I, -OTs, -OMs

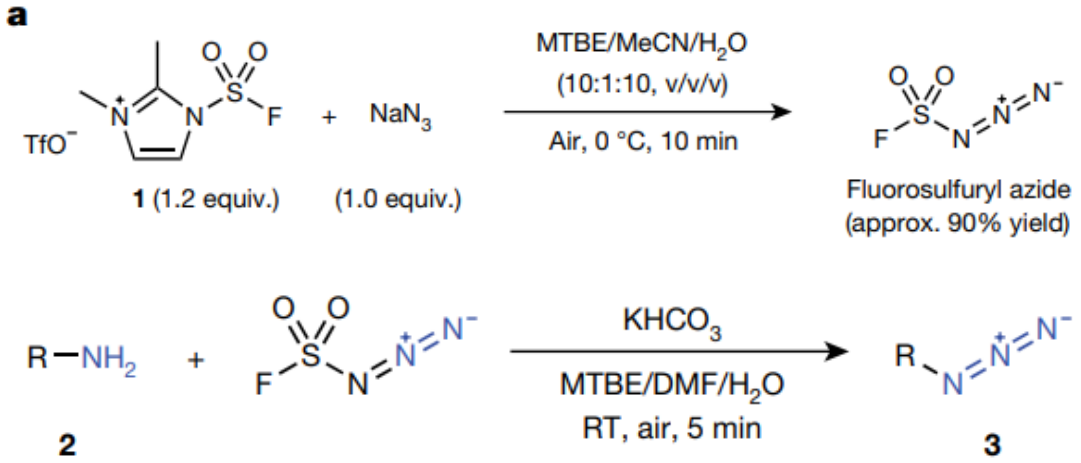
Synthesis of sulfonyl azide

Synlett 2016, 27, 1840

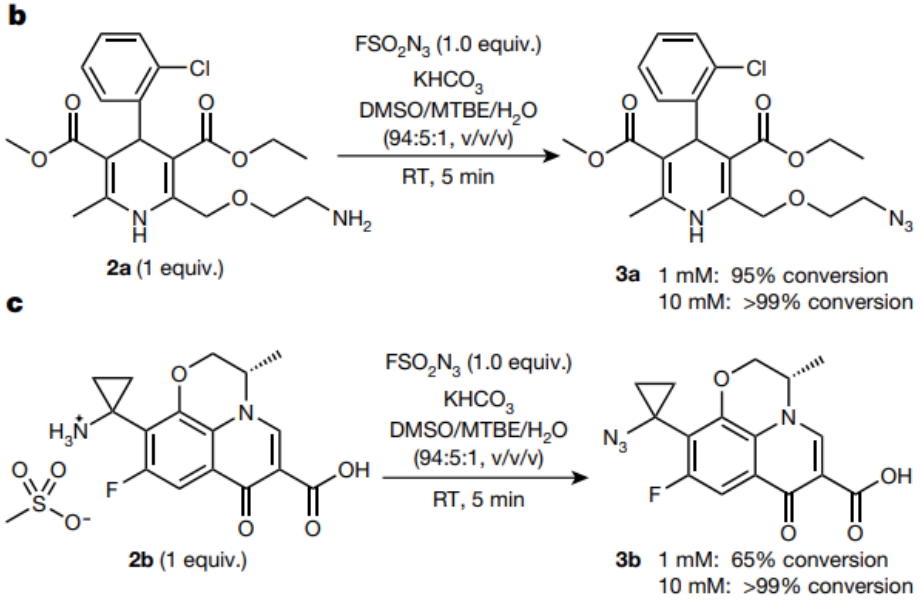


# RSO2F as Synthetic Reagent

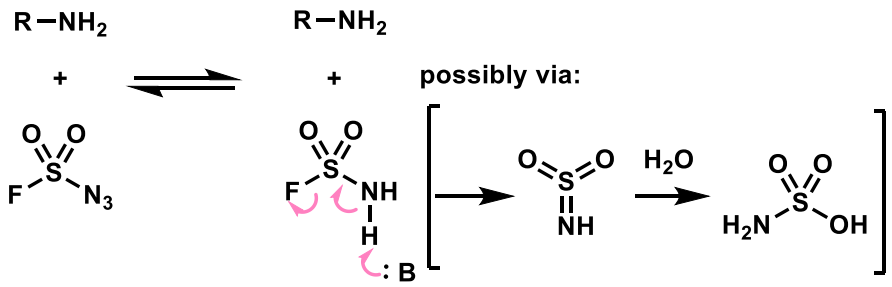
Diazotizing reagent: primary amine/sulfonamide to azide/sulfonylazide




> 40 examples
orthogonal to most of function
(including 2°/3° amines)

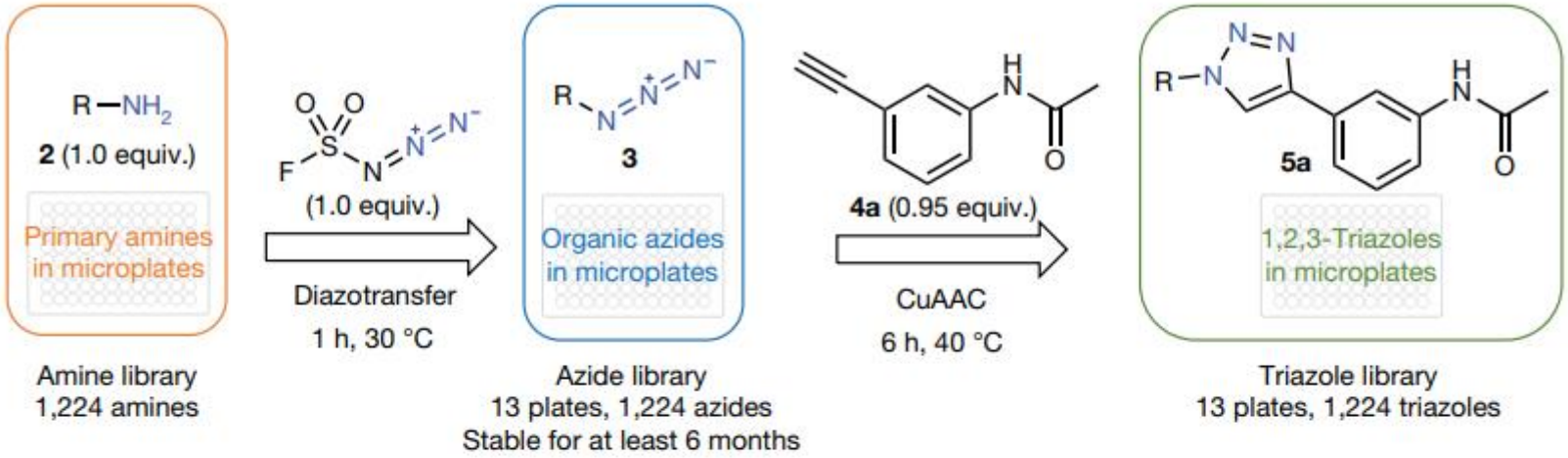


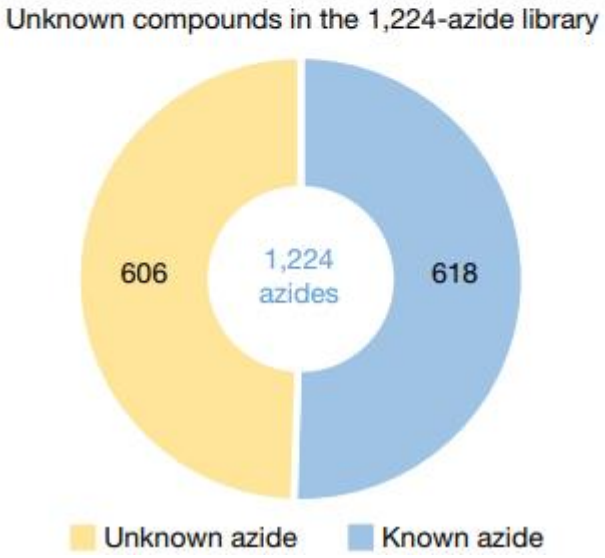

diazotransfer is reversible

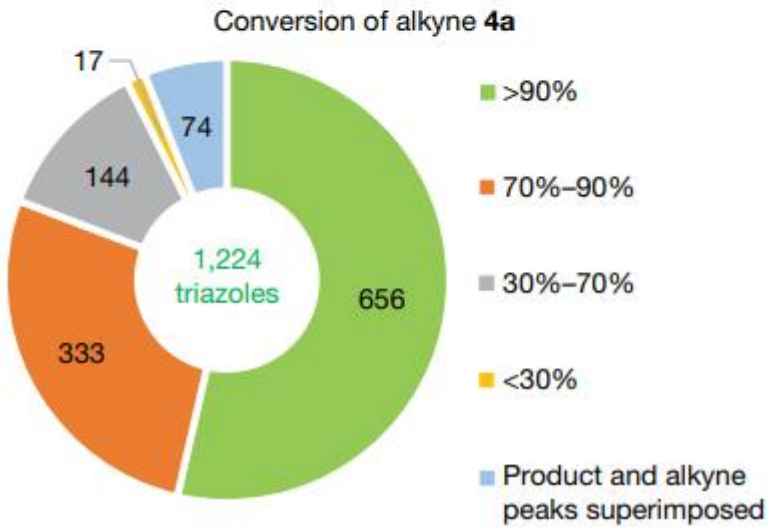



Sharpless, K. B.; Dong, J. *et al. Nature* **2019**, *574*, 86.

# RSO2F as Synthetic Reagent



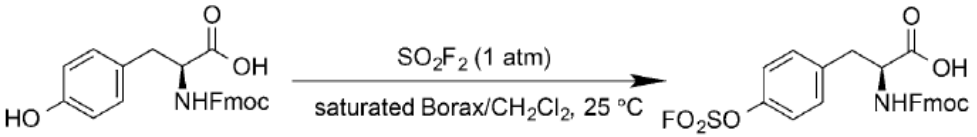



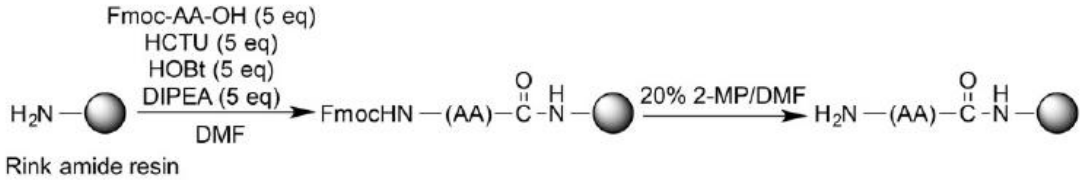


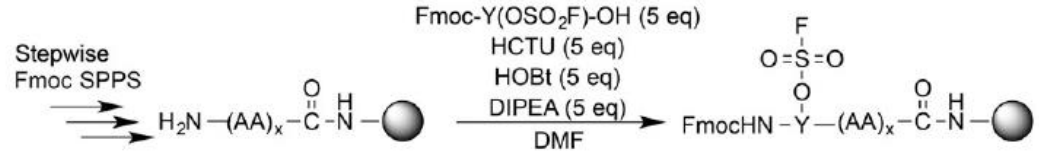

Sharpless, K. B.; Dong, J. *et al. Nature* **2019**, *574*, 86.

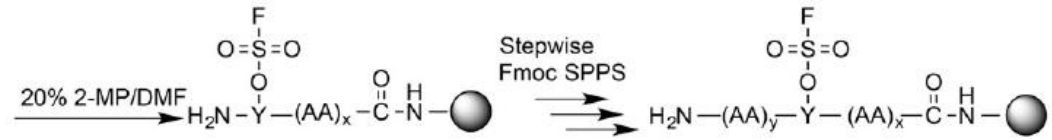
# Synthesis of Sulfotyrosine

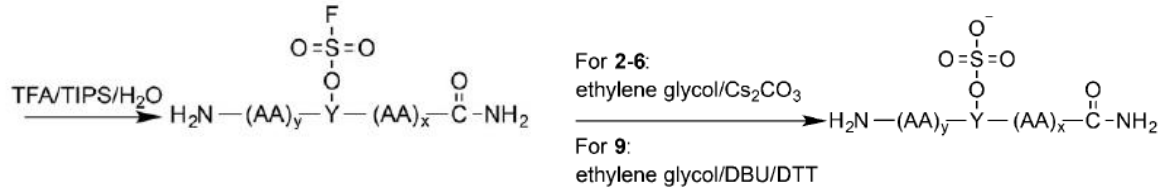
Tyr O-sulfation: seen in PTMs

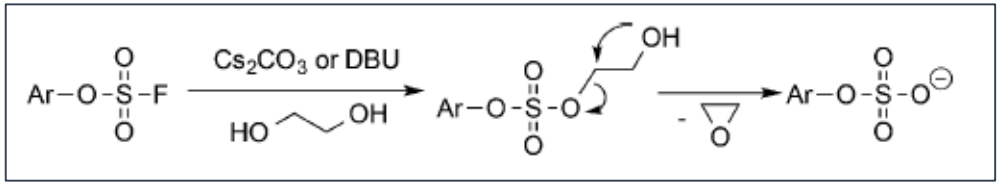


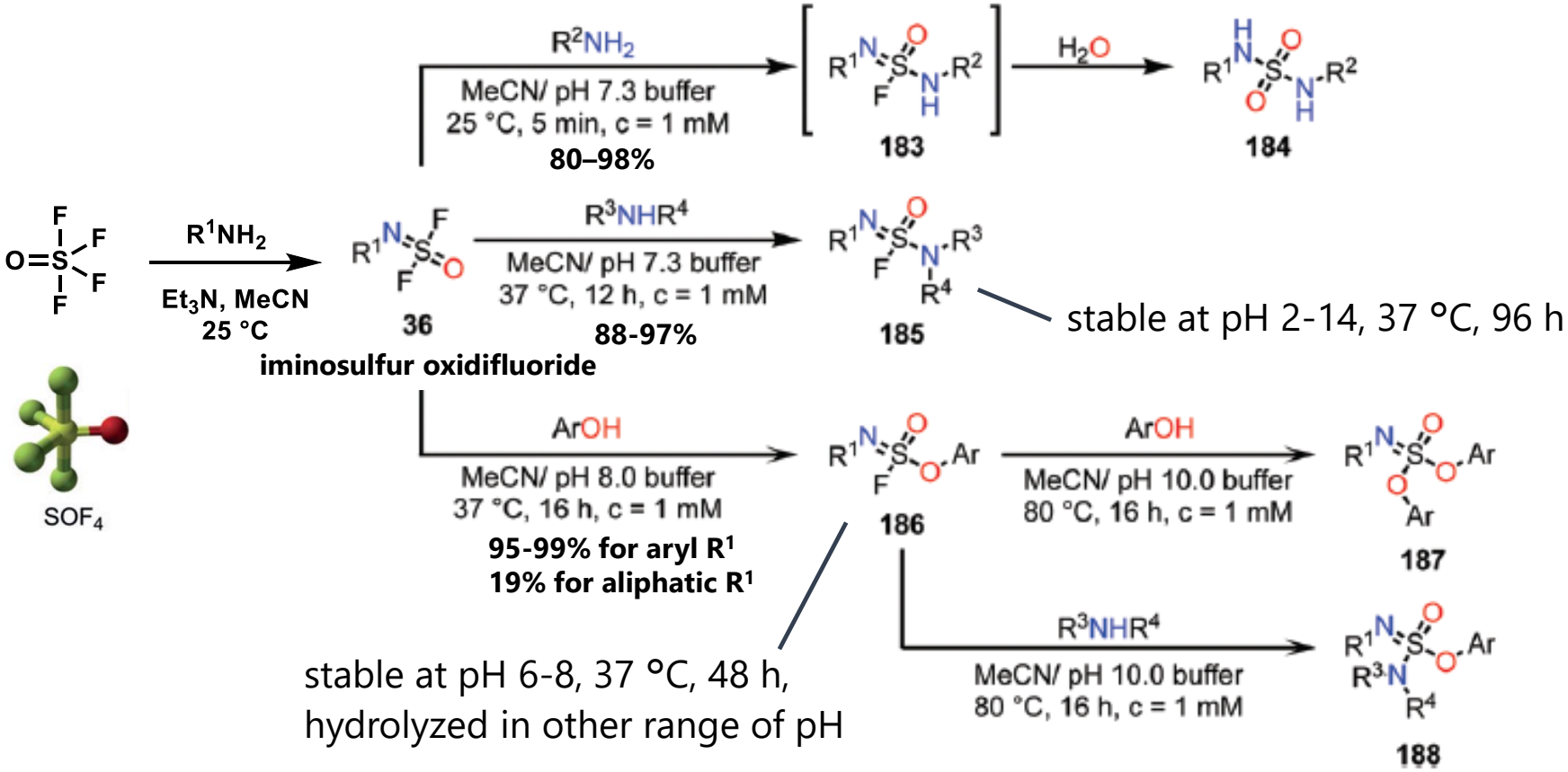




ACIE 2016, 55, 1835

# Iminosulfur Oxidifluoride under Aqueous Conditions

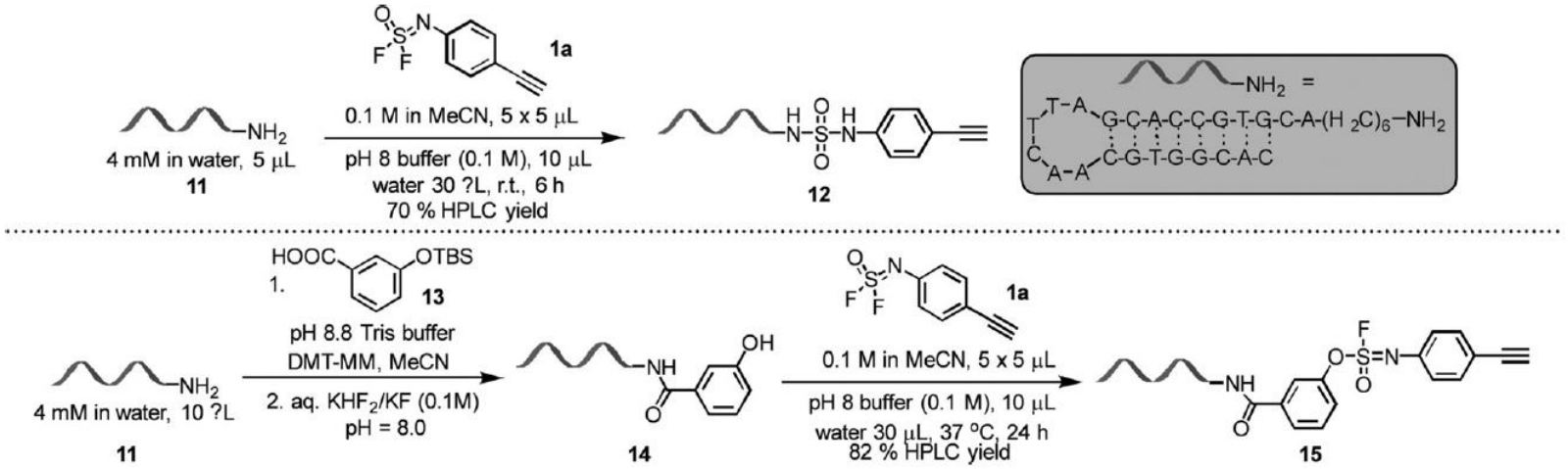
Derivatization under aqueous conditions

ACIE 2019, 58, 8



# Iminosulfur Oxidifluoride under Aqueous Conditions

## DNA modification




## Protein modification