
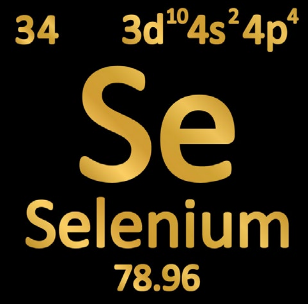


# The Biochemistry of Selenocysteine(Sec)


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College of Chemistry and Molecular Engineering

Jun. 29th, 2024

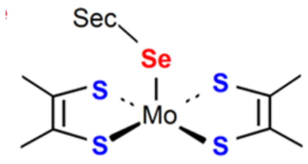
# Contents

- Discovery of Sec and its Insertion Mechanism
- Sec vs Cys in Proteins: Why Selenium?
- Sec Probes and Bioconjugate Chemistry
- Deselenative Modifications for Sec

# Timeline of Selenium Biochemistry

**1817**
**Discovery of element Se**
Berzelius

**1954**
**Se is essential for the activity of formate dehydrogenases in *E. Coli.***
Pinsent




**1986**
**Recognition of UGA as codon for Sec**
Bock

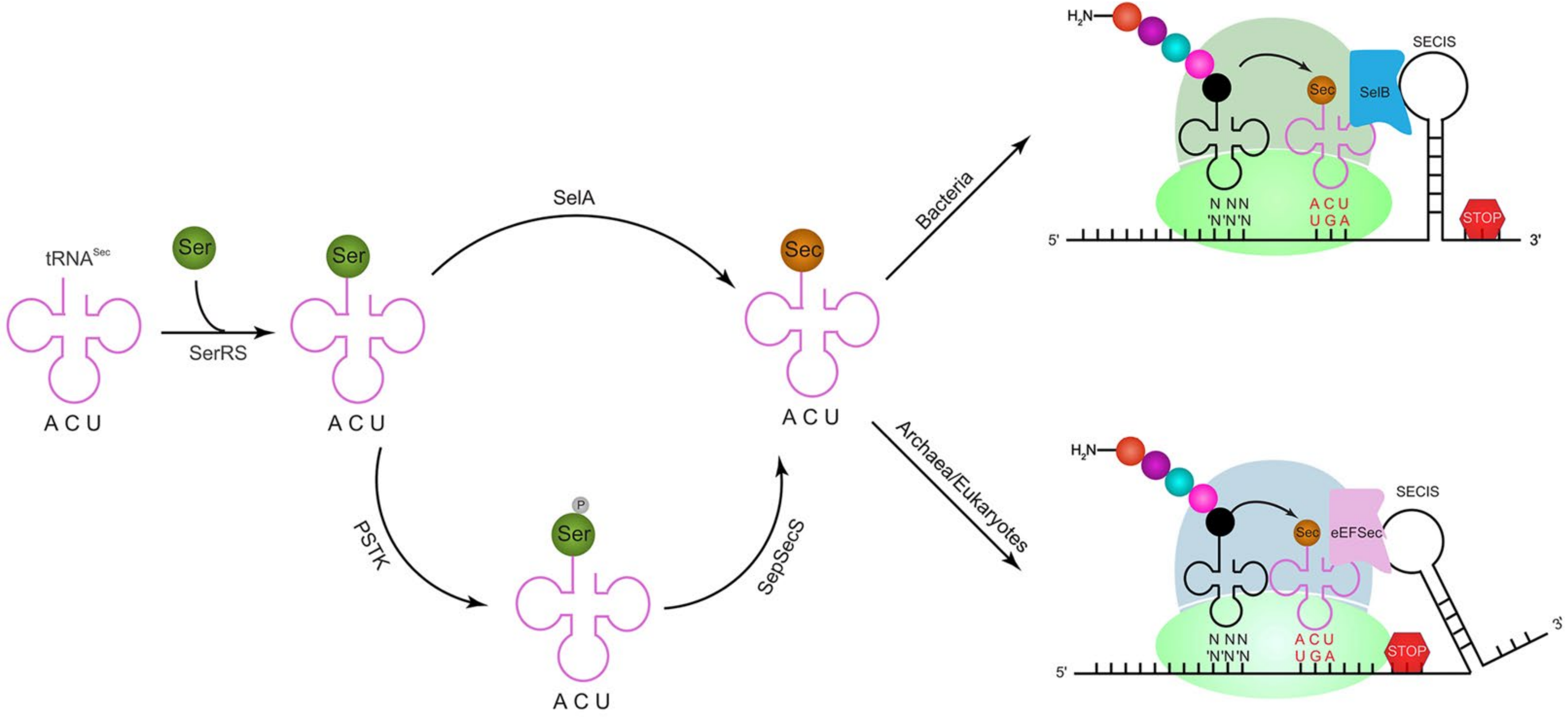
**1936**
**Se "adsorbed on protein" in stuffs leading to alkali disease for mules**
Franke

**1976**
**Identification of Sec as the organoselenium moiety**
**through hydrolysis of glycine reductase**
Stadtman

*Astragalus sp.*

Berzelius, J. "*Lettre de M. Berzelius à M. Berthollet sur deux métaux nouveaux*" **1818**
Franke, K.; Painter, E. *Cereal Chem.* **1936**.*13*, 67.
Pinsent, J. *Biochem. J.* **1954**, *57*, 10.
Cone, J.; Del Río, R.; Davis, J.; Stadtman, T. *Proc. Natl. Acad. Sci.* **1976**, *73*, 2659.
Zinoni, F; Birkmann, A.; Stadtman, T.; Bock, A. *Proc. Natl. Acad. Sci.* **1986**, *83*, 4650.

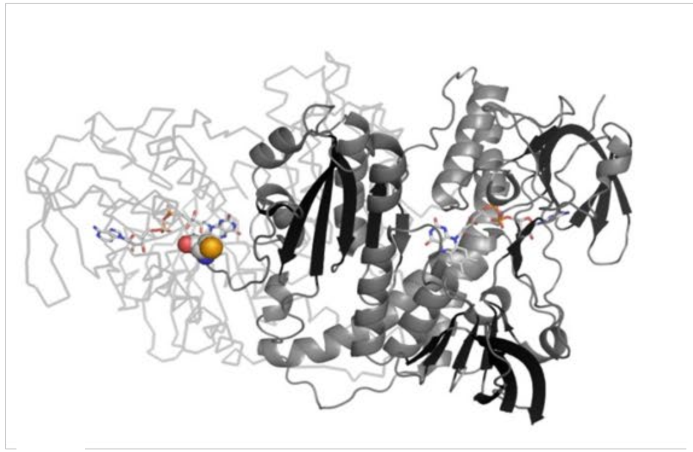
# Native Sec(U) Insertion Mechanisms



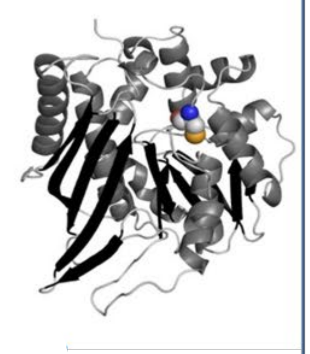

J, Liu.; F, Zheng.; R, Cheng.; S, Li.; S, Rozovsky.; Q, Wang.; L, Wan. *J. Am. Chem. Soc.* **2018**, *140*, 8807.

# Structure and Functions of some Selenoproteins

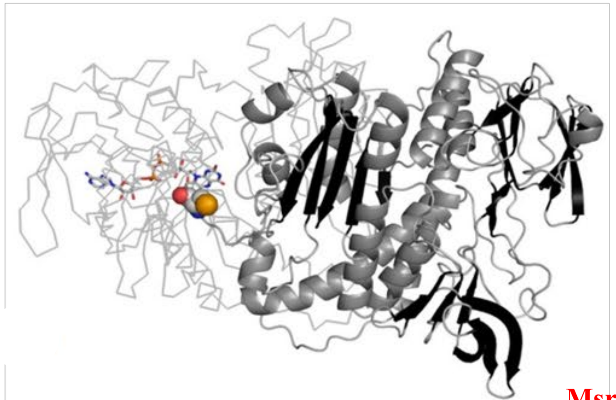
Functions: thioredox signaling, removal of peroxides, protein folding, selenoprotein synthesis, selenium transport…



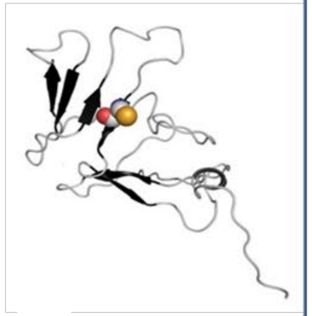
**TR1** (U498) protein size 499 (homodimer)
reduction of cytosolic thioredoxin



**SPS2** (U60) protein size 448
synthesis of selenoproteins

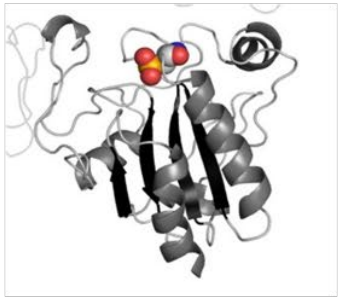


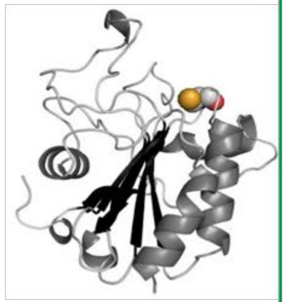
**TR3** (U522) protein size 523 (homodimer)
reduction of mitochondrial thioredoxin



**MsrB1** (U95) protein size 116
reduction of oxidized Met residues

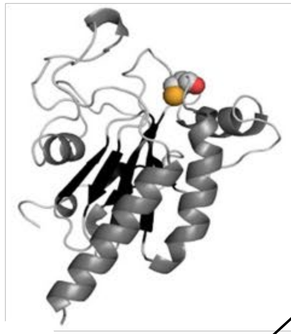
**GPX1** (U47) protein size 201
cytosolic glutathione peroxidase



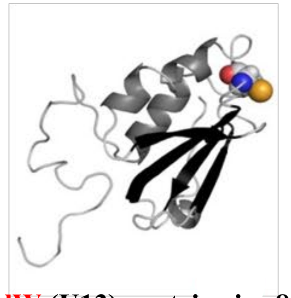


**GPX2** (U40) protein size 190
gastrointestinal glutathione peroxidase

**GPX3** (U73) protein size 226
Plasma glutathione peroxidase

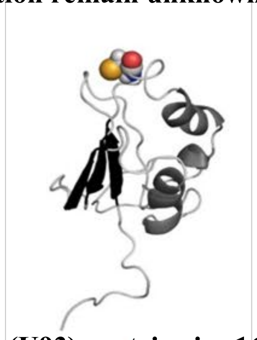


**GPX4** (U73) protein size 197
Phospholipid hydroperoxide glutathione peroxidase



**SelW** (U13) protein size 87
function remain unknown

**SelM** (U48) protein size 145
function remain unknown



**Sep15** (U93) protein size 162
control of protein folding (ER)

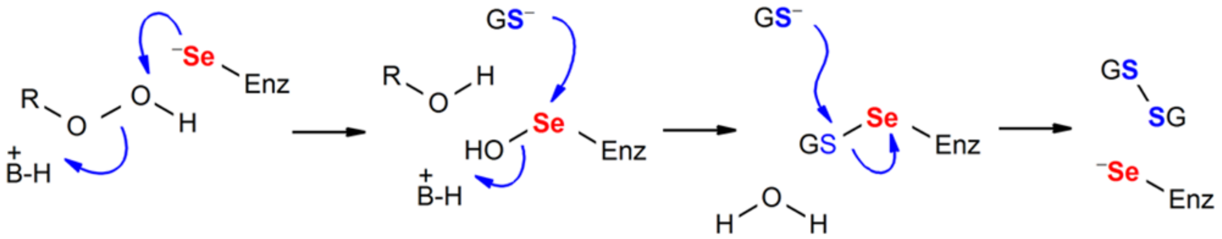


V, Labunskyy.; D, Hatfield.; V, Gladyshev. *Physiol. Rev.* **2014**, *94*, 739.

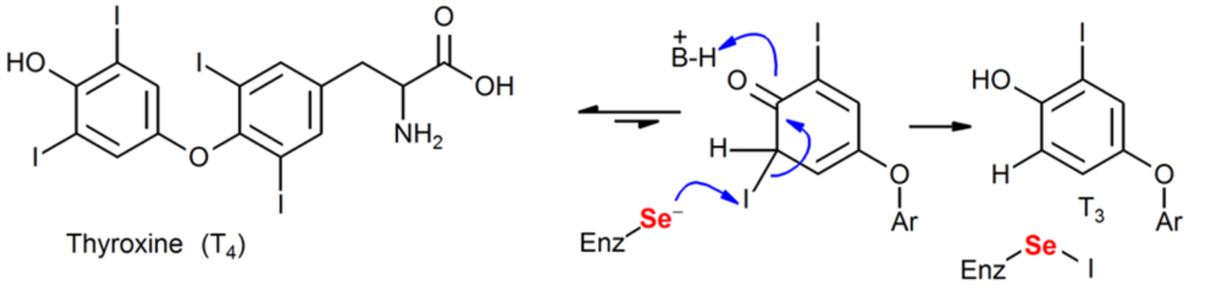
# Contents

# Roles of Sec; Difference between Se and S

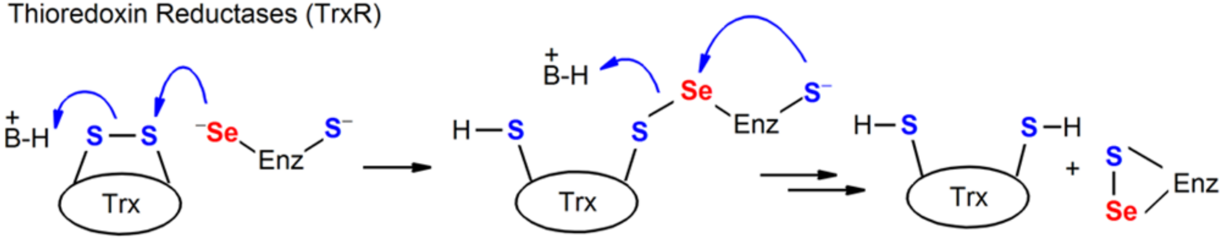
Glutathione peroxidase (Gpx)



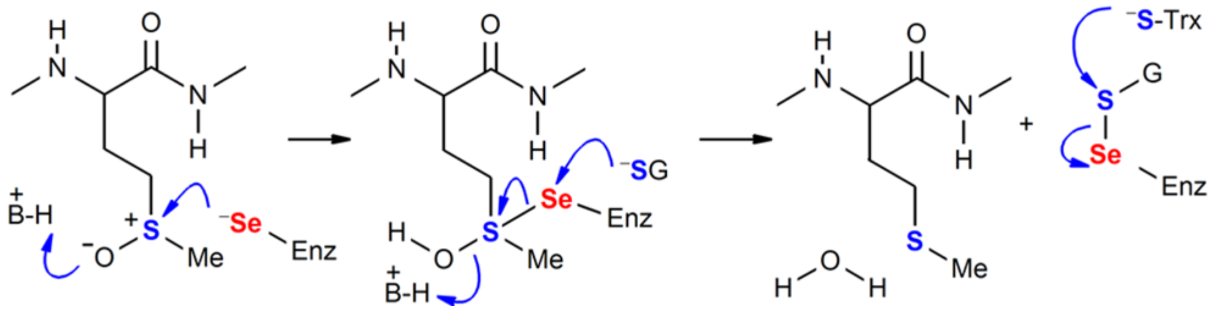

Iodothyronine Deiodinase (DIO)

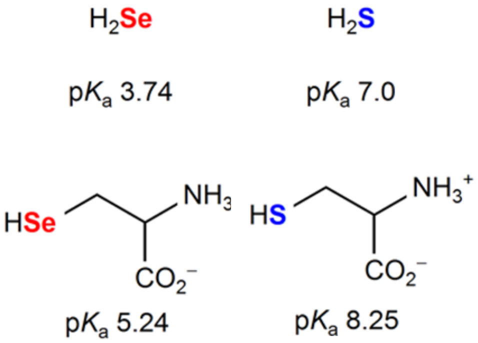


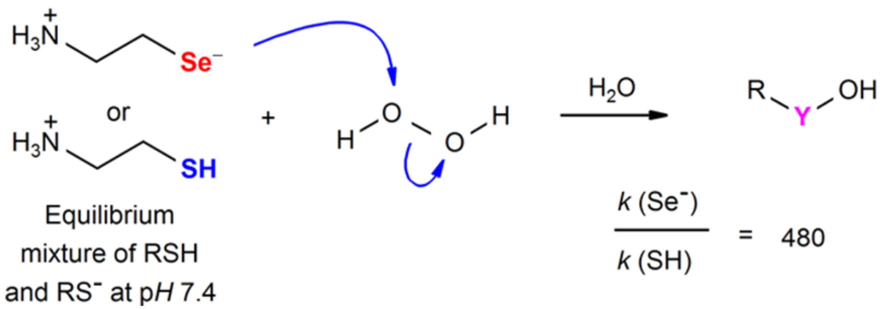

Thioredoxin Reductases (TrxR)

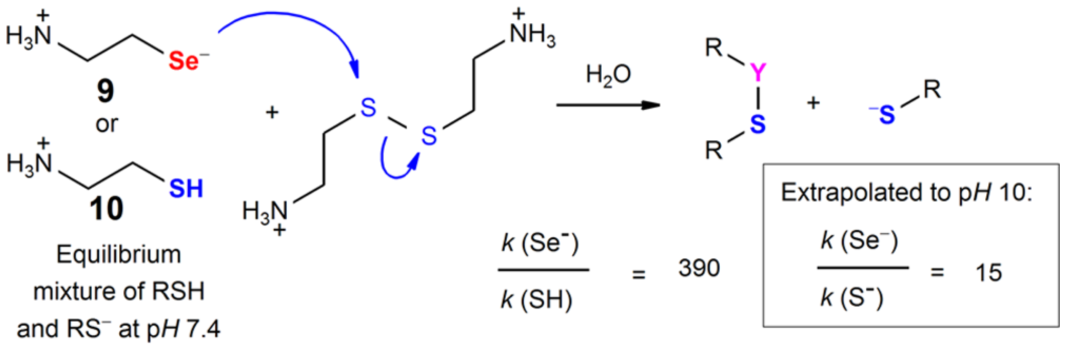



Methionine sulfoxide reductase (Msr)



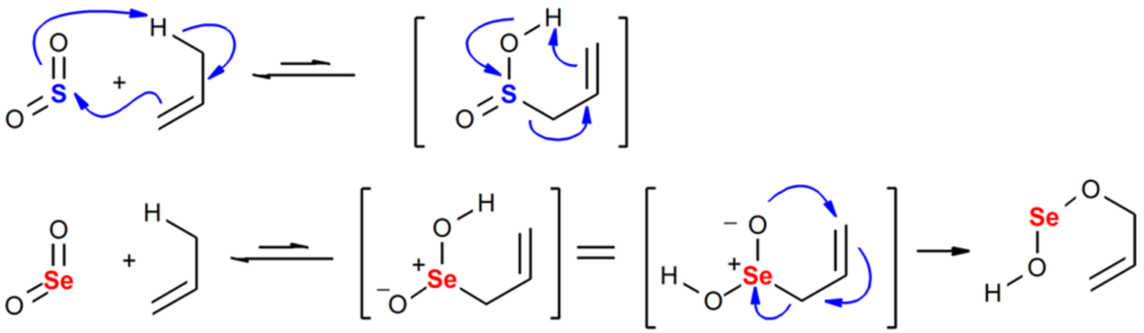



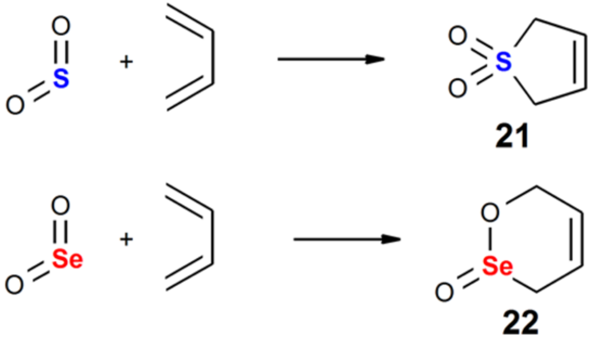



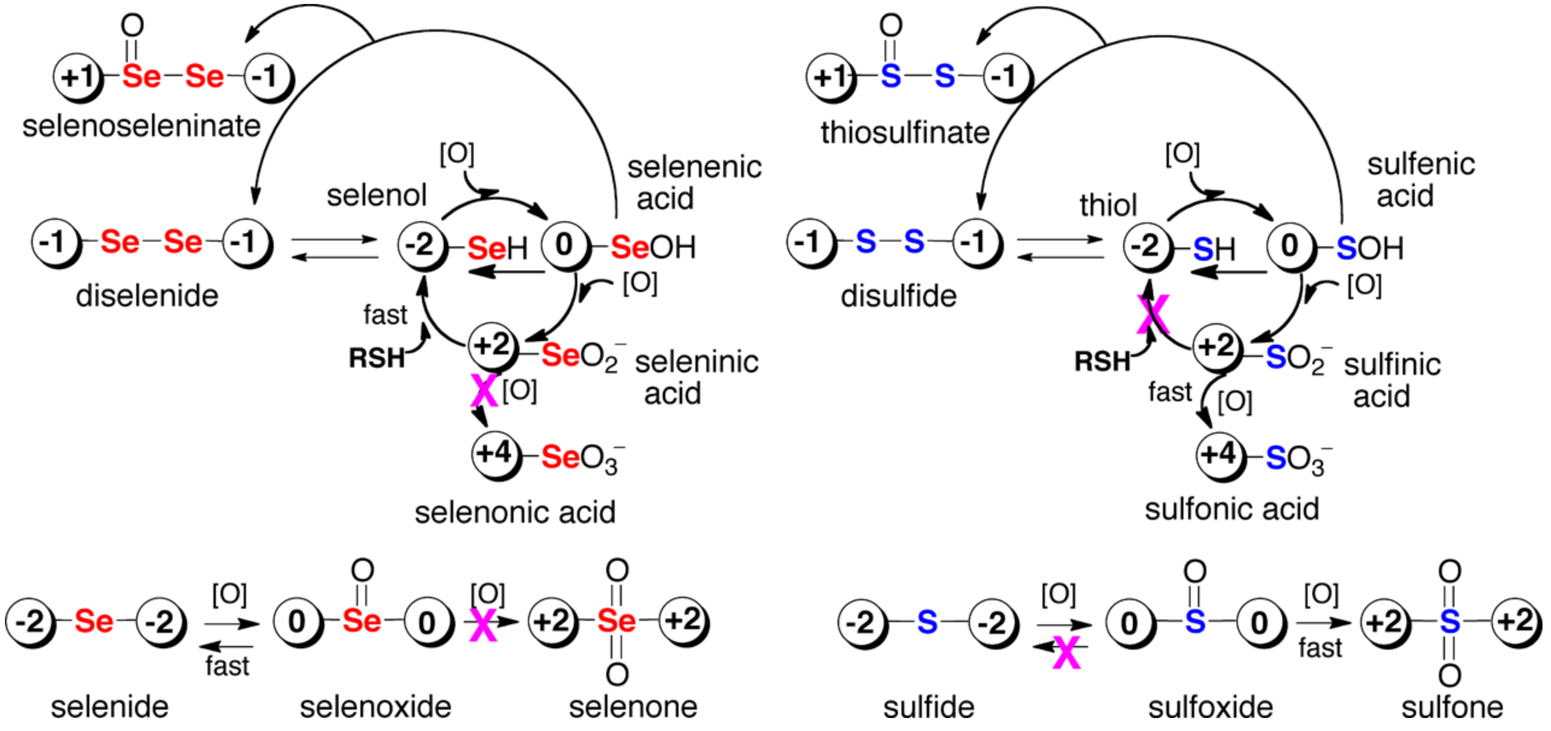




Z, Zhao.; S, Laps.; J, Gichtin.; N, Metanis. *Nat. Rev. Chem.* **2024**, *8*, 211.

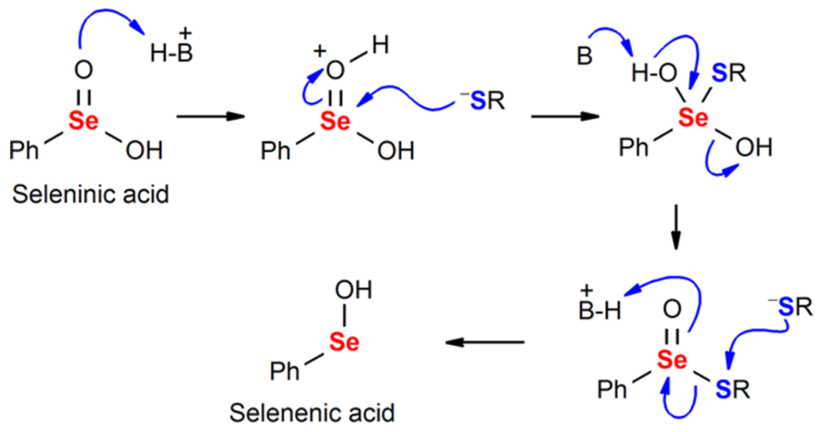
# Redox Chemistry as the most Distinctive Factor



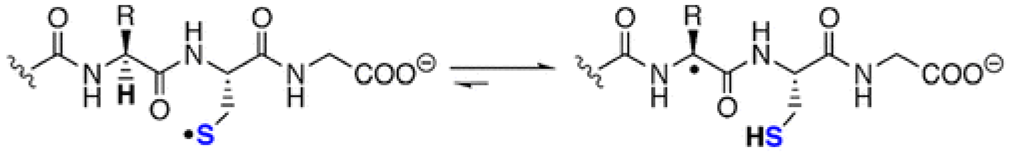




Reduction of seleninic acid by thiols: extremely fast!

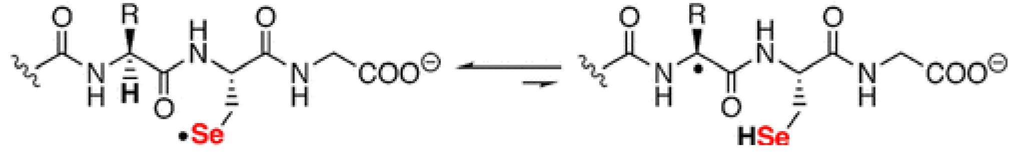


# Se: Resistance to Oxidation and Inactivation

**Inertness in one-electron redox processes:**

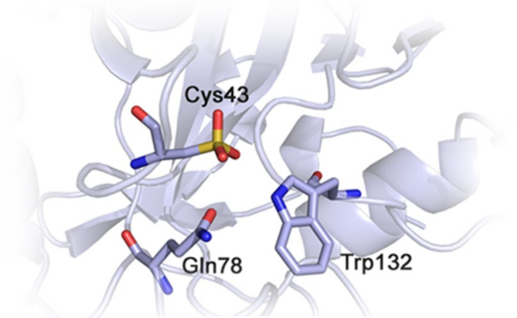



RS• / RSH: E°′ = 0.92 V
a thiyl radical is capable of oxidizing Tyr and Trp




RSe• /  RSeH:  E°′ = 0.43 V
longer half-life, preventing damage propagation

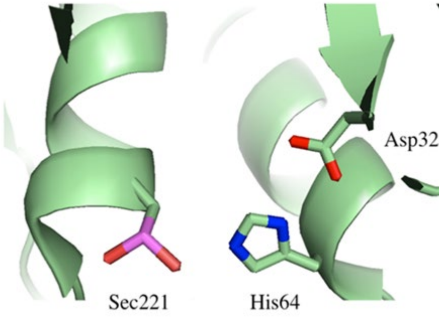
**GPX4 U43C**

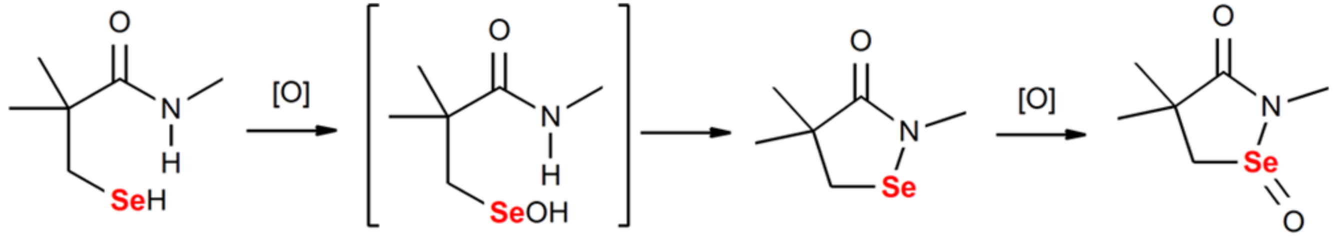



GPX1 Cys-mutant:  activity $10^3$-fold smaller than that of the natural enzyme
very easily inactivated by hydroperoxides

*Schistosoma mansoni* GPX4 consisting of U43C oxidized to a **sulfonic acid**
(inactive at all stages, even though reducing agents were present)

**Selenosubtilisin with U221 in seleninic acid form**



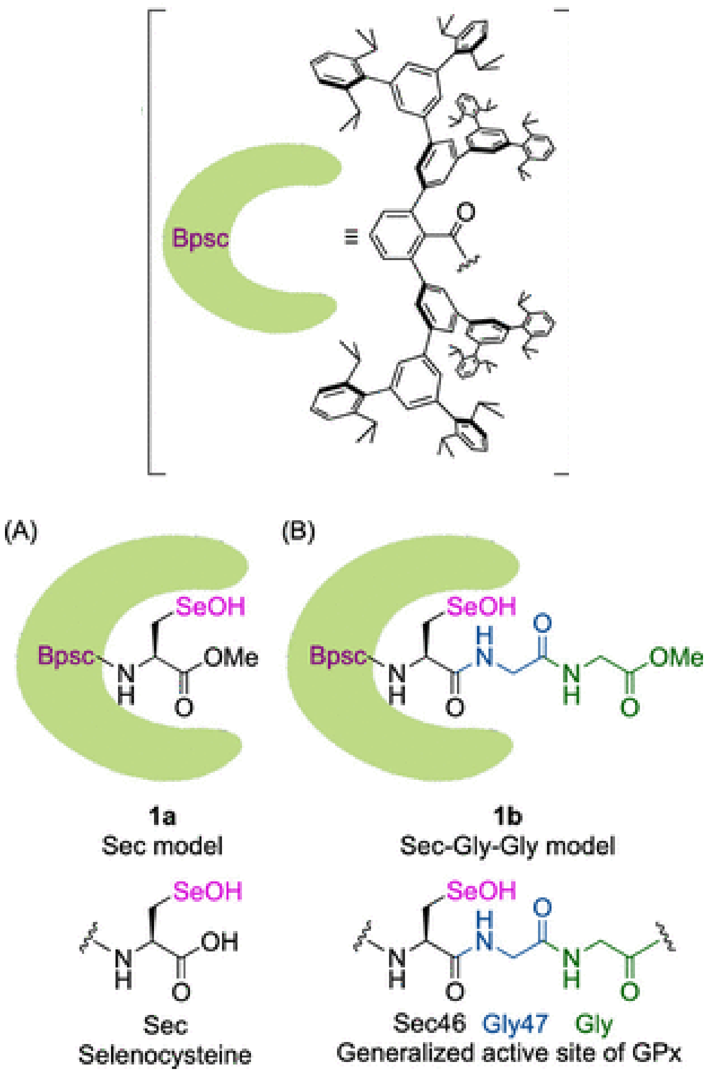



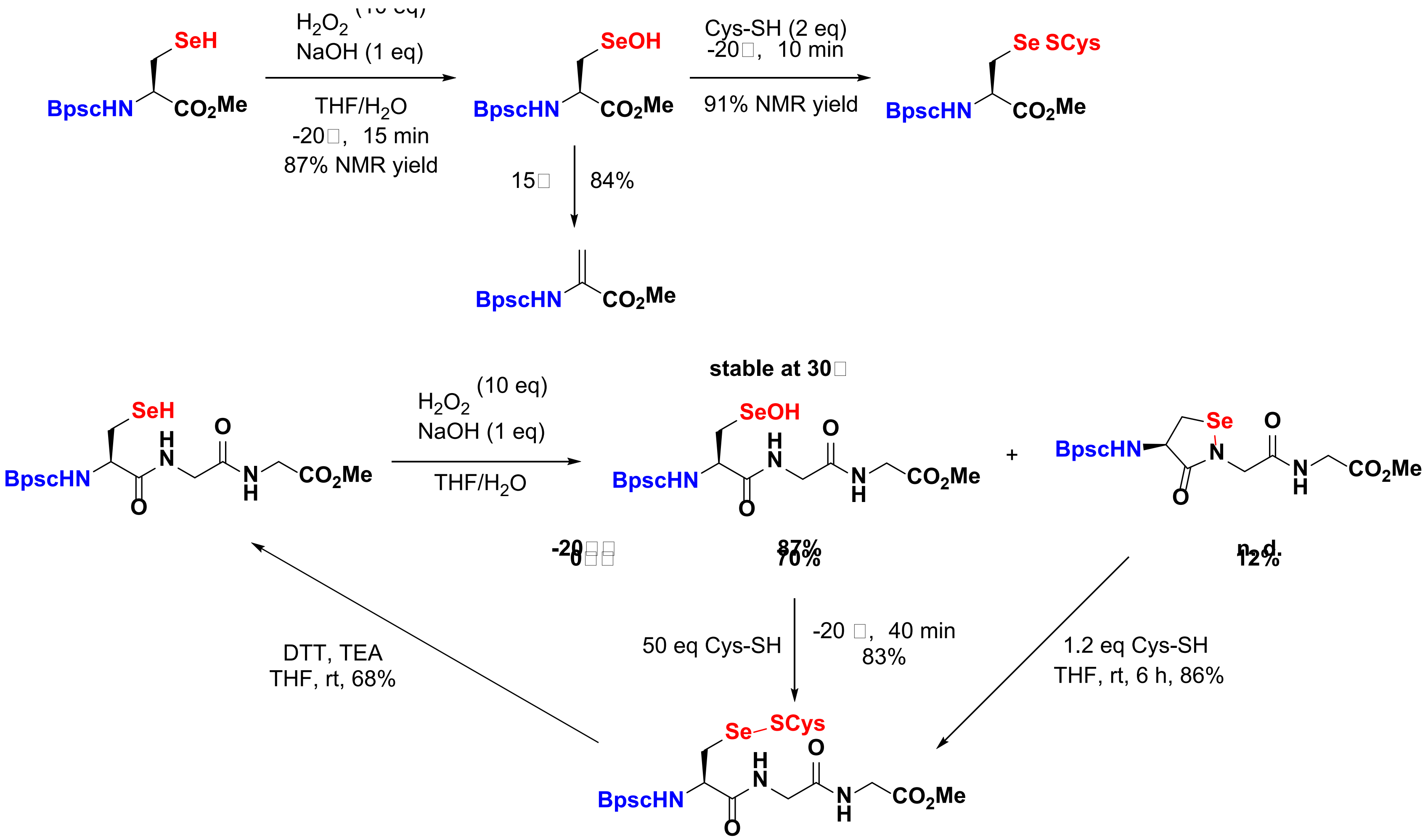

GPX4 → $H_2O_2$ → trypsin digestion → LCMS:
**Selenous acid not observed, but signal of selenamide instead.**


Z, Wu.; D, Hilvert. *J. Am. Chem. Soc.* **1990**, *112*, 5647.; H, Reich.; C, Jasperse. *J. Am. Chem. Soc.* **1987**, *109*, 5549.; G, Snider.; E, Ruggles.; N, Khan.; R, .Hondal. *Biochemistry*, **2013**, *52*, 5472.; C, Rocher.; J, Lalanne.; J, Chaudieŕe. *Eur. J. Biochem.* **1992**, *205*, 955.; D, Dimastrogiovanni.; M, Anselmi.; A, Miele.; G, Boumis.; L, Petersson.; F, Angelucci.; A, Nola.; M, Brunori.; A, Bellelli. *Proteins: Struct. Funct. Genet.* **2010**, *78*, 259.

# Direct Characterization the Selenic Acid and Selenenyl Amide

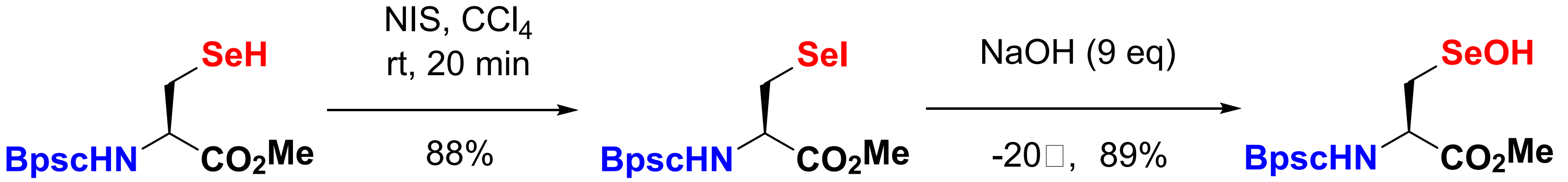
## Large molecular cavity as a protective cradle

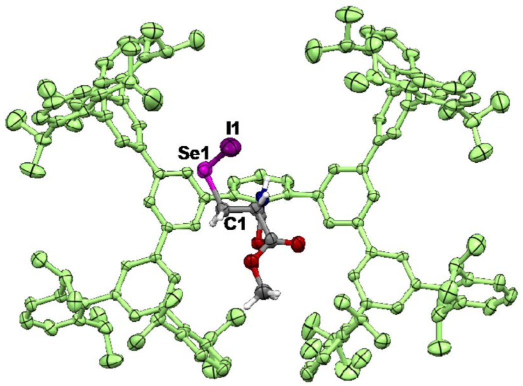


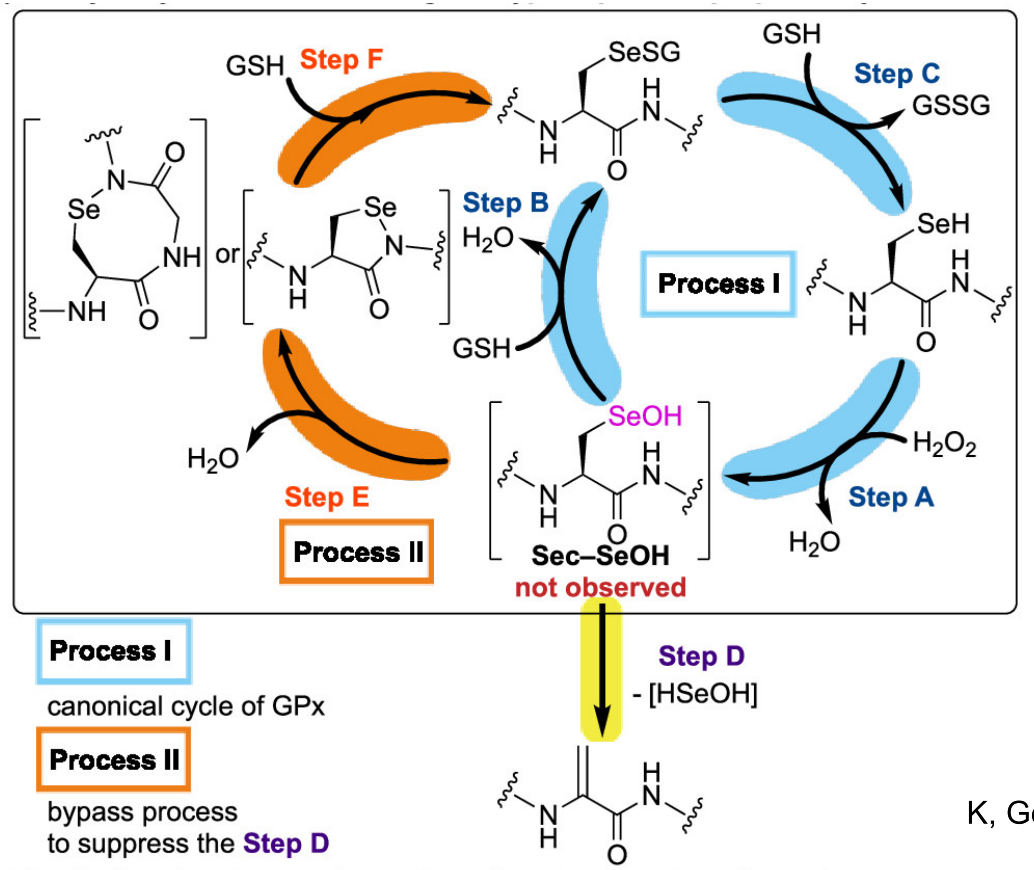



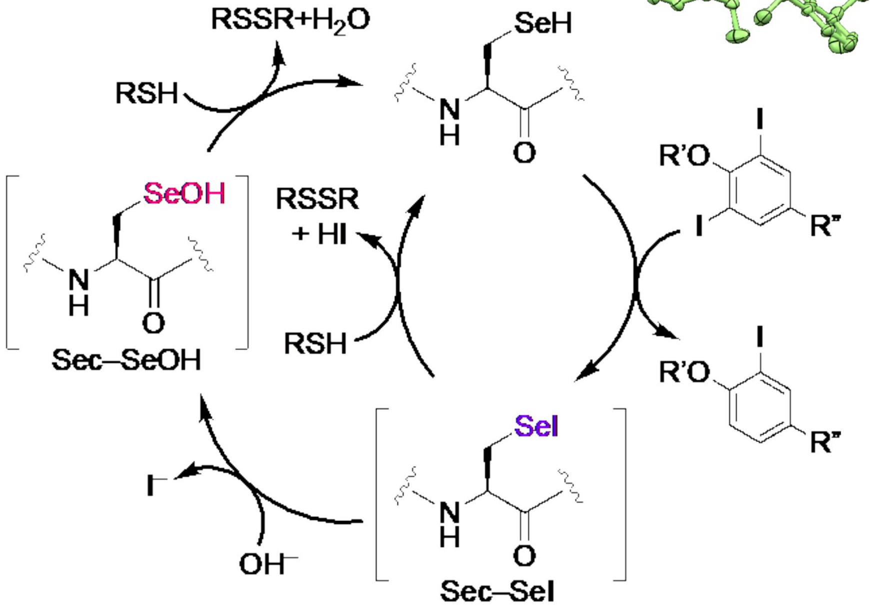

R, Masuda.; R, Kimura.; T, Karasaki.; T, Karasaki.; S, Sase.; K, Goto. *J. Am. Chem. Soc.* **2021**, *143*, 6345.

# Proposed Catalytic Cycle of GPX and DIO



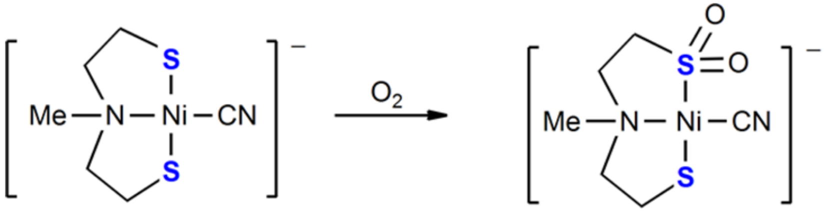



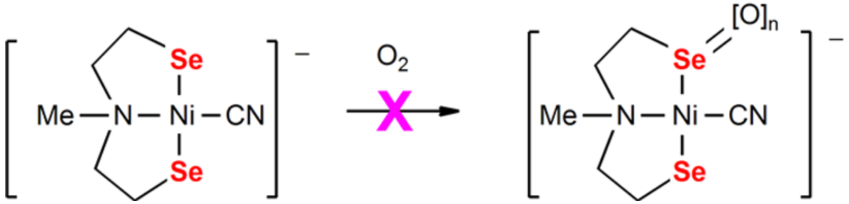




K, Goto.; R, Kimura.; R, Masuda.; T, Karasaki.; S, Sase. *Molecules* **2023**, *28*, 7972.

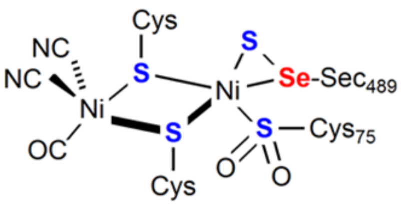
# Selenium in Hydrogenases: Oxygen Tolerance Provided

Maroney: Ni-containing mimics of the active site that had either sulfur or selenium ligands.



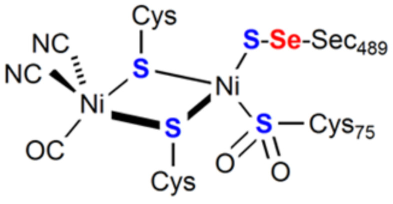




Matias: structure of [NiFeSe] hydrogenase from *Desulfovibrio vulgaris Hildenborough*



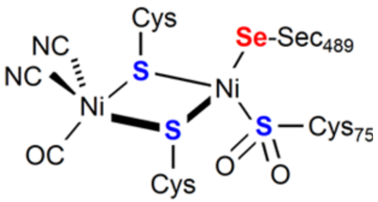

**Structure I**

oxidized form (70%)



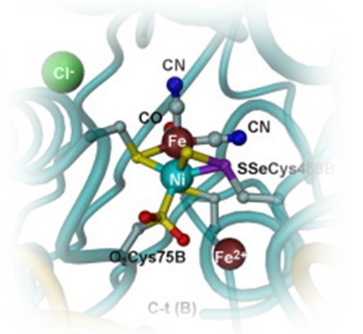

**Structure II**

intermediate (15%)

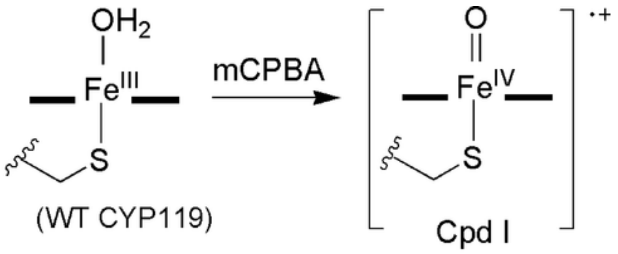


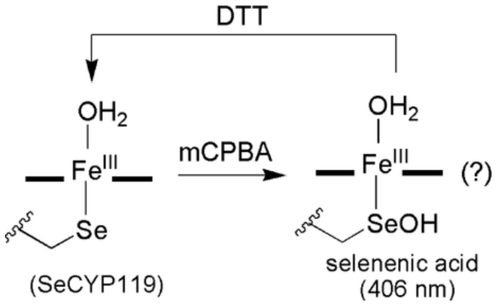

**Structure III**

reduced form (15%)




Montellano: introduction of Sec into P450 monooxygenase: protect against oxidative heme destruction.



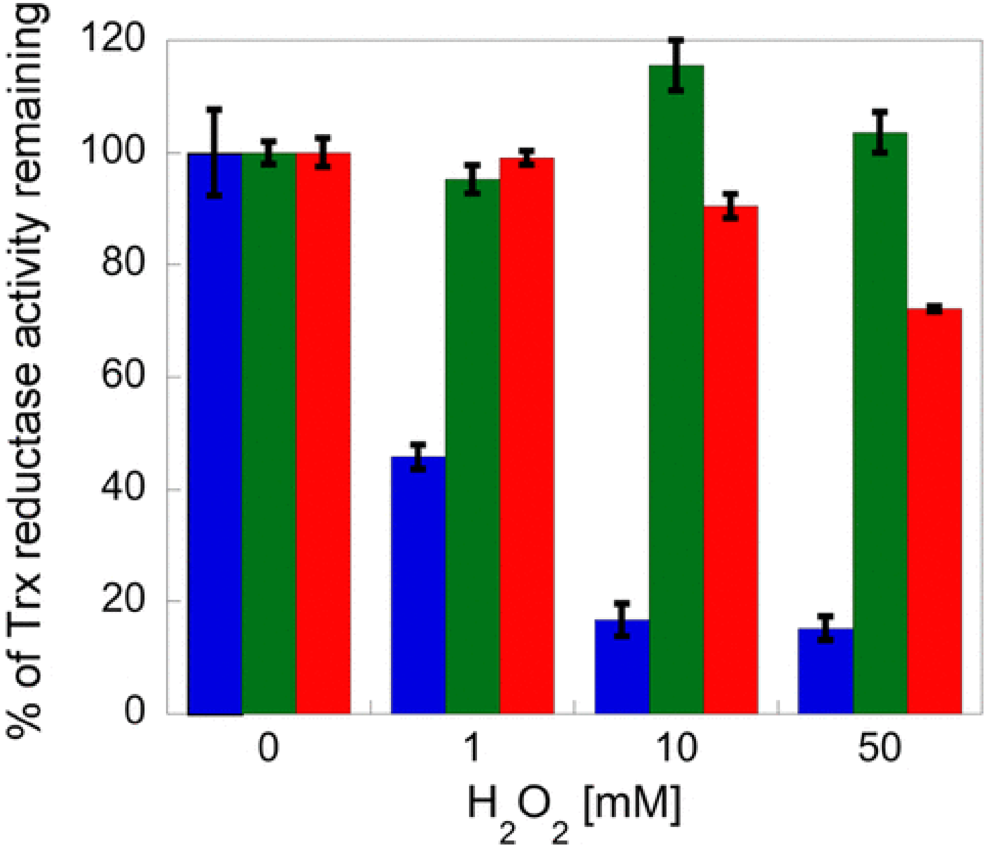



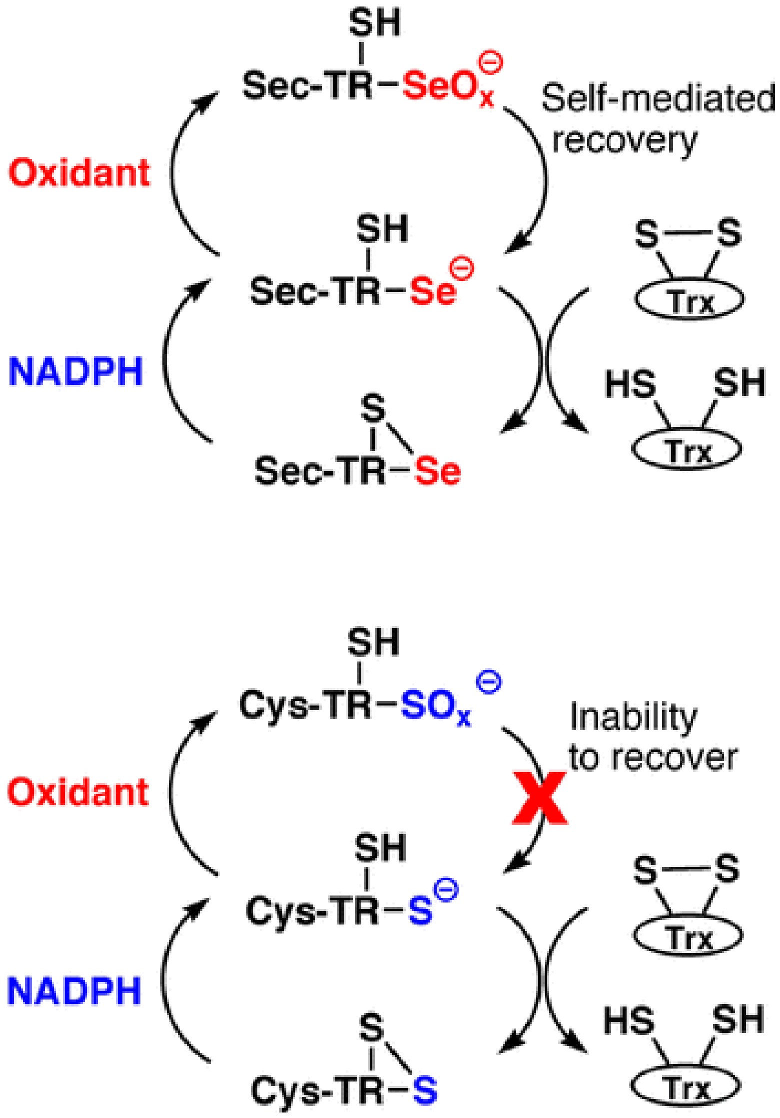

Oxidized form of Sec identified with magnetic circular dichroism spectrum (CYP119 from *S. acidocaldarius*)

S, Choudhury.; M, Pressler.; S, Mirza.; R, Day.; M, Maroney. *Inorg. Chem.* **1994,** *33*, 4831.
S, Sivaramakrishnan.; H, Ouellet.; J, Du.; K, McLean.; K, Medzihradszky.; J, Dawson.; A, Munro.; P, Ortiz de Montellano, *Biochemistry*, **2011**, *50*, 3014.
M, Teixeira.; M, Fauque.; G, Moura.; I, Lespinat.; P, Berlier.; Y, Prickril.; B, Peck.; H, Xavier.; A, Le Gall.; J, Moura. *Eur. J. Biochem.* **1987**, *167*, 47.
M, Marques; R, Coelho.; A, De Lacey.; I, Pereira.; P, Matias. *J. Mol. Biol.* **2010**, *396*, 893.

# “Sec-Rescue”-TrxR Resistance to Oxidative Inactivation

**Comparison of ability to resist oxidative inactivation:**
thioredoxin reductase (TrxR) from mouse mitochondria in red
its Cys-ortholog from from *D. melanogaster* (DmTR) in blue
Cys→Sec mutant of DmTR in green




G, Snider.; E, Ruggles.; N, Khan.; R, Hondal. *Biochemistry*, **2013**, *52*, 5472.

# Summary I: Why Selenium?

- **Enhanced chemical reactivity to accelerate enzymatic reactions**

Almost all reactions involving selenium are faster in comparison to the same reaction with sulfur, attributing to lower pKa and its “softness” (polarizability), which lead to weaker bond strengths.

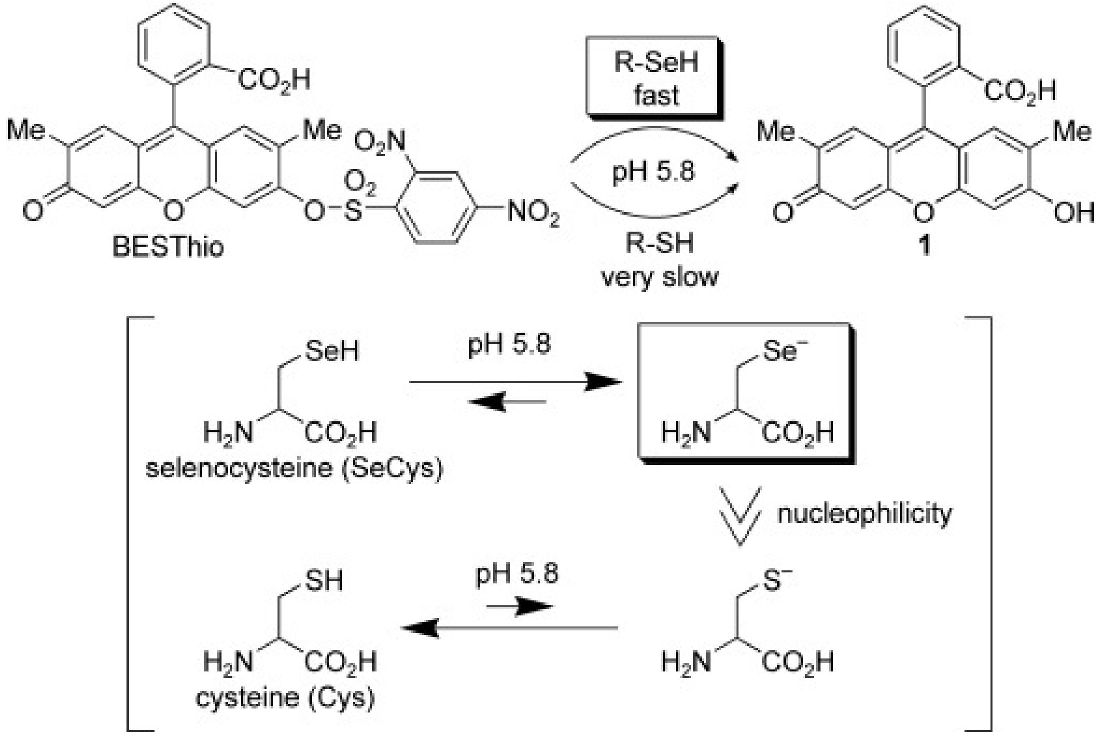
- **Unique ability to react with oxygen and related ROS in a readily reversible manner**

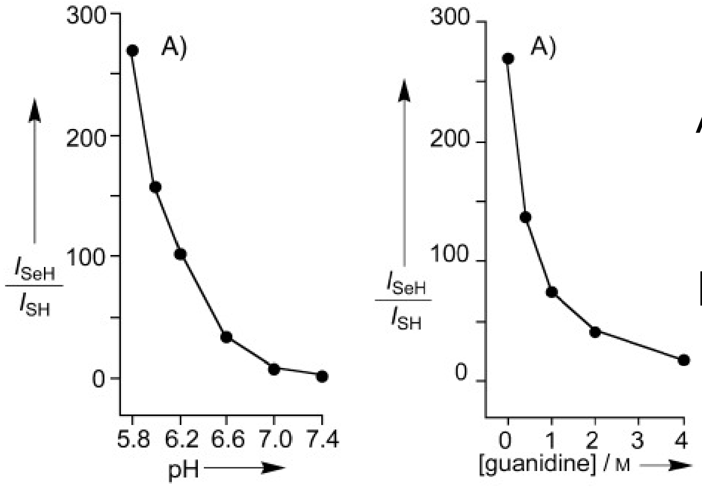
Due to weak π-bonding in Se-oxides, they are prone to be rapidly reduced back to the original state in comparison to S-oxides.

# Contents

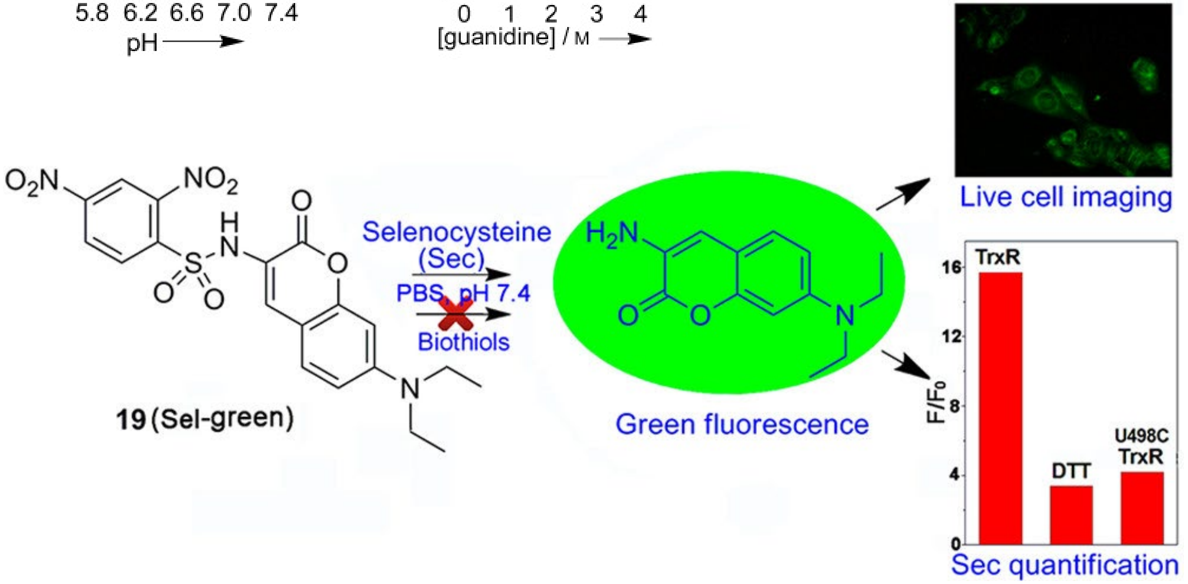
- Discovery of Sec and its Insertion Mechanism
- Sec vs Cys in Proteins: Why Selenium?
- Sec Probes and Bioconjugate Chemistry
- Deselenative Modifications for Sec

# BESThio at Lower pH and Sel-green in Neutral Conditions



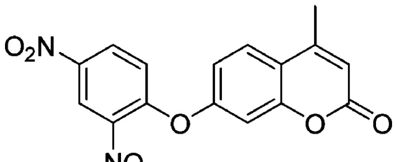




A. pH-dependent changes in the selectivity ($I_{SeH}/I_{SH}$).
B. effects of guanidine upon selectivity ($I_{SeH}/I_{SH}$)




H, Maeda.; K, Katayama.; H, Matsuno.; T, Uno. *Angew. Chem. Int. Ed.* **2006**, *45*, 1810.
B, Zhang.; C, Ge.; J, Yao.; Y, Liu.; H, Xie.; J, Fang. *J. Am. Chem. Soc.* **2015**, *137*, 757.

# Discovery of Sel-green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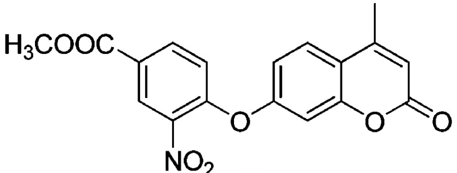
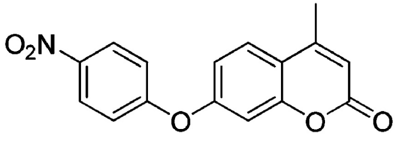
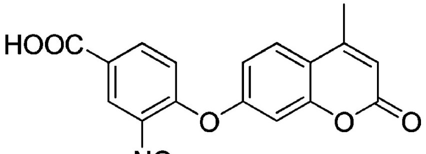

2b


2c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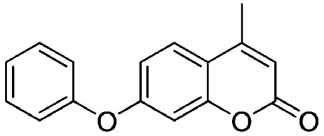
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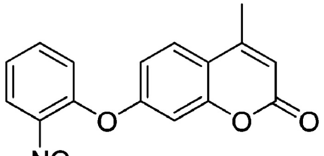
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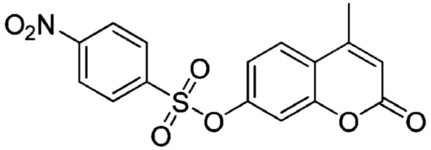
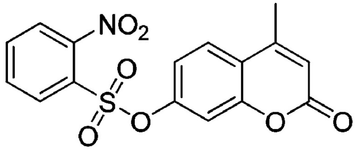
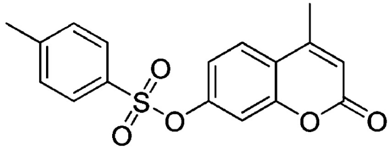
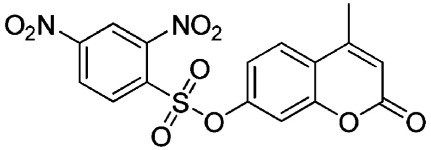
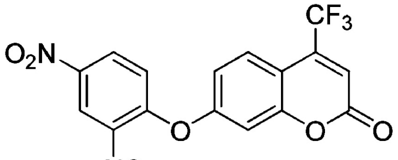
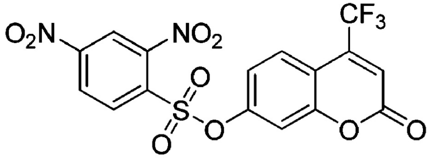

3a


3b


3c


3d


5a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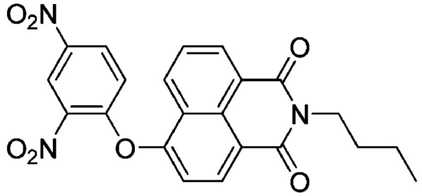
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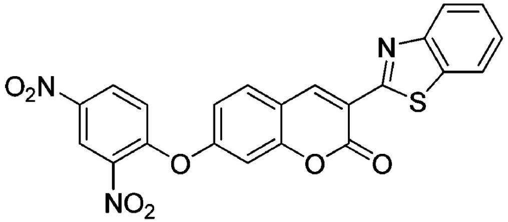
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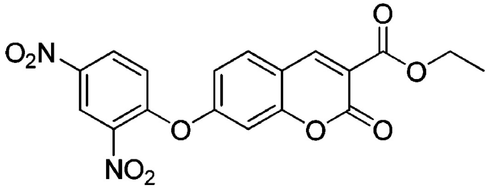
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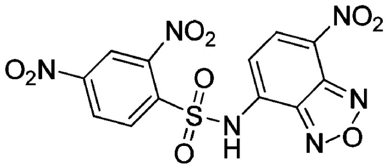
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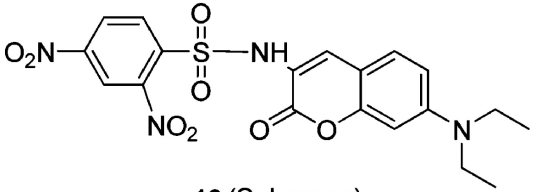
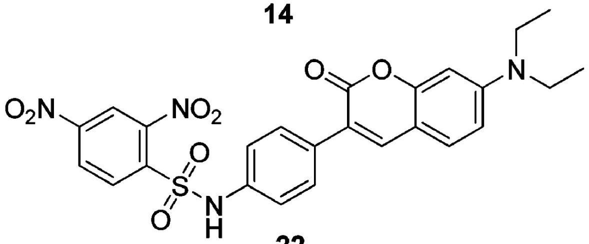
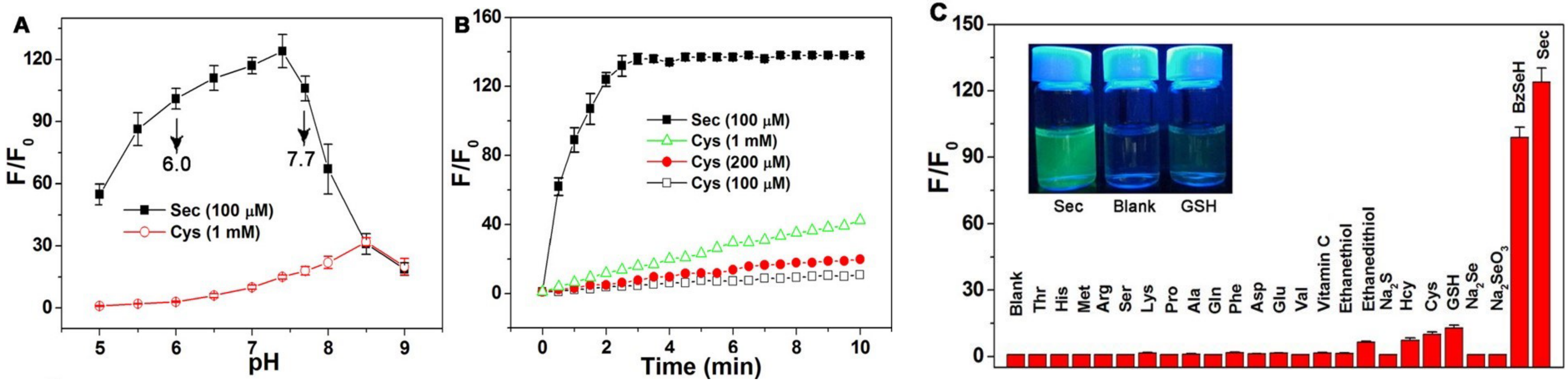
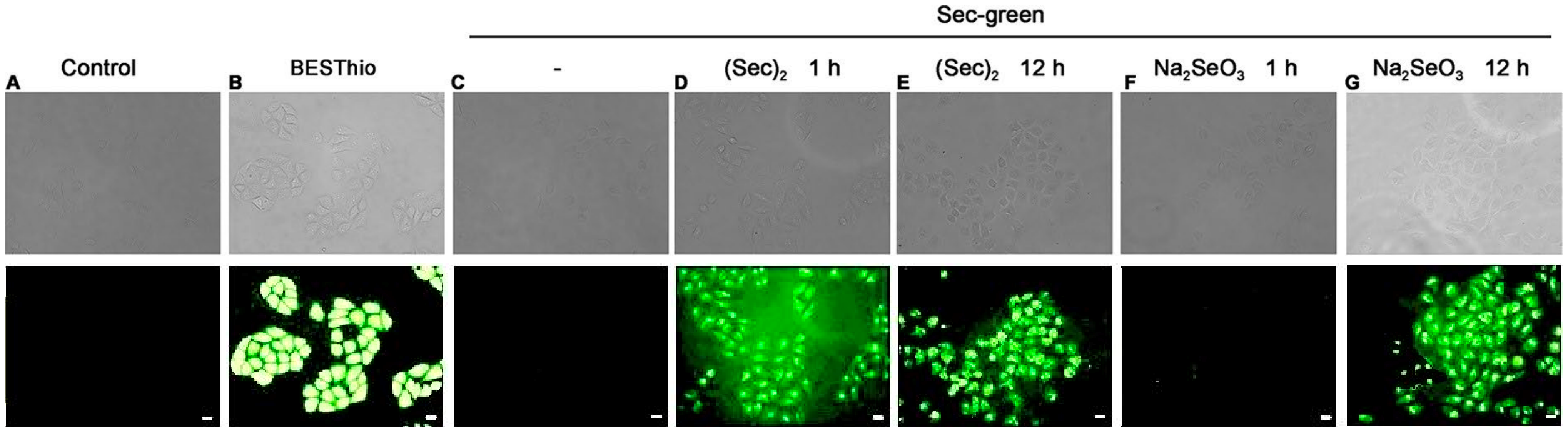

**19** (Sel-green)


22

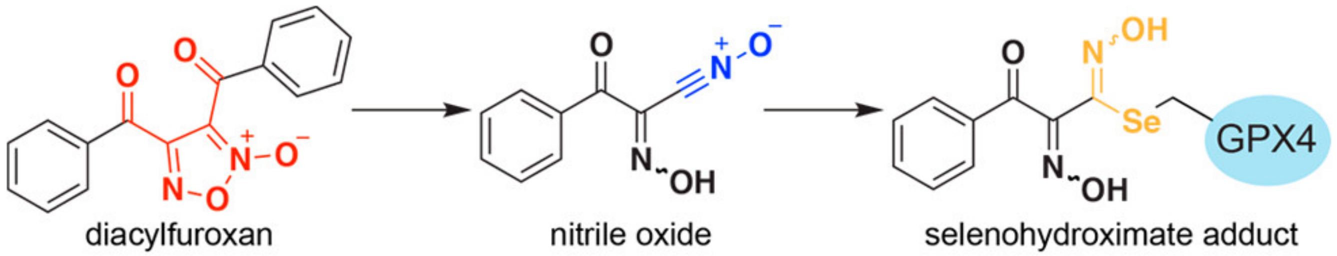
| probes | Sec[b] $(F/F_0)$ | DTT[c] $(F/F_0)$ | selectivity (Sec/DTT) |
|---|---|---|---|
| **2a** | 16.5 | 1.5 | 11.0 |
| **2b** | 1.1 | 0.9 | 1.2 |
| **2c** | 1.0 | 1.2 | 0.8 |
| **2d** | 0.8 | 1.1 | 0.7 |
| **2e** | 0.9 | 1.2 | 0.8 |
| **2f** | 1.2 | 1.3 | 0.9 |
| **3a** | 2.0 | 1.6 | 1.2 |
| **3b** | 1.3 | 1.0 | 1.3 |
| **3c** | 1.1 | 0.9 | 1.2 |
| **3d** | 10.1 | 11.0 | 0.9 |
| **5a** | 20.1 | 2.0 | 10.0 |
| **5b** | 37.0 | 33.2 | 1.1 |
| **9** | 25.0 | 1.2 | 20.8 |
| **12** | 72.1 | 1.5 | 48.1 |
| **14** | 8.8 | 1.3 | 6.8 |
| **16** | 25.8 | 2.3 | 11.2 |
| **19** (Sel-green) | 125 | 6.2 | 20.2 |
| **22** | 2.96 | 1.1 | 2.7 |

# Live Cell Imaging in HepG2 Cells



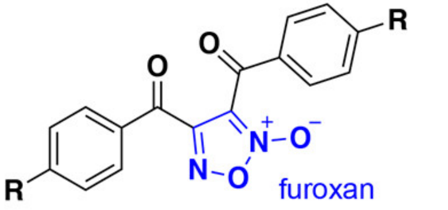



# Diacylfuroxans: Masked Nitrile Oxides which Covalently Inhibit GPX4

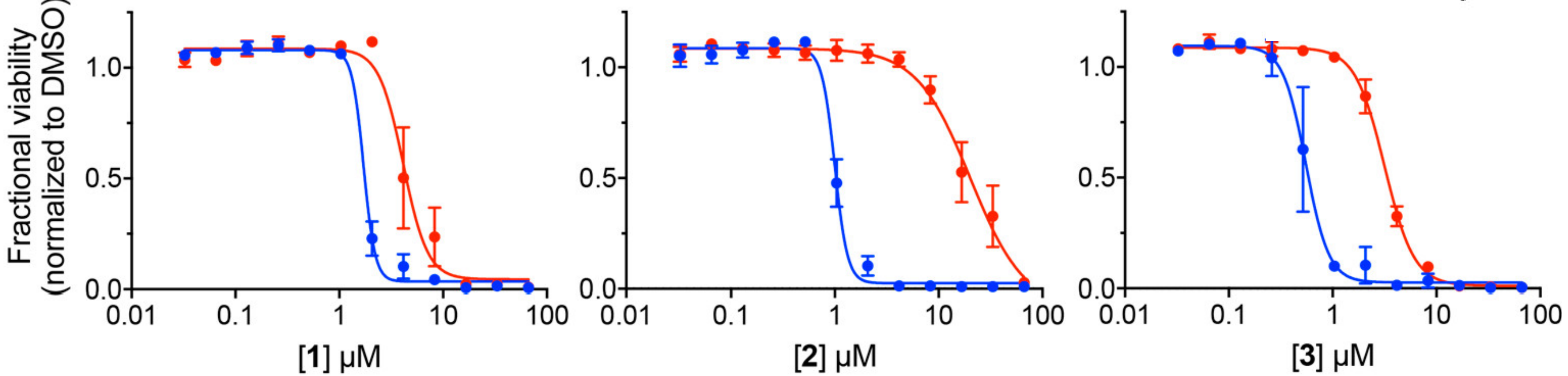


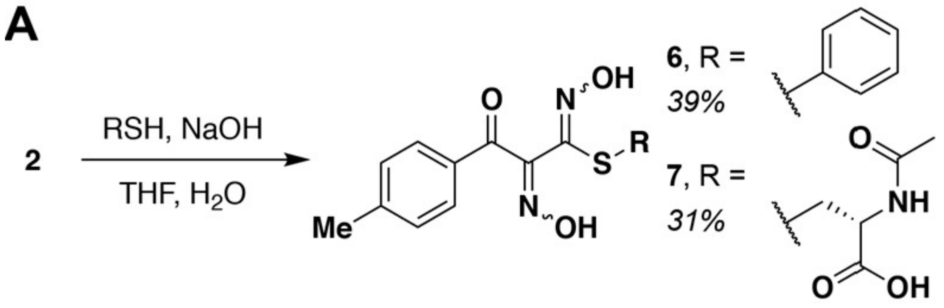

Cotreatment with **fer-1** rescues the cell-killing effects of **1**, **2**, and **3** in LOX-IMVI cells:

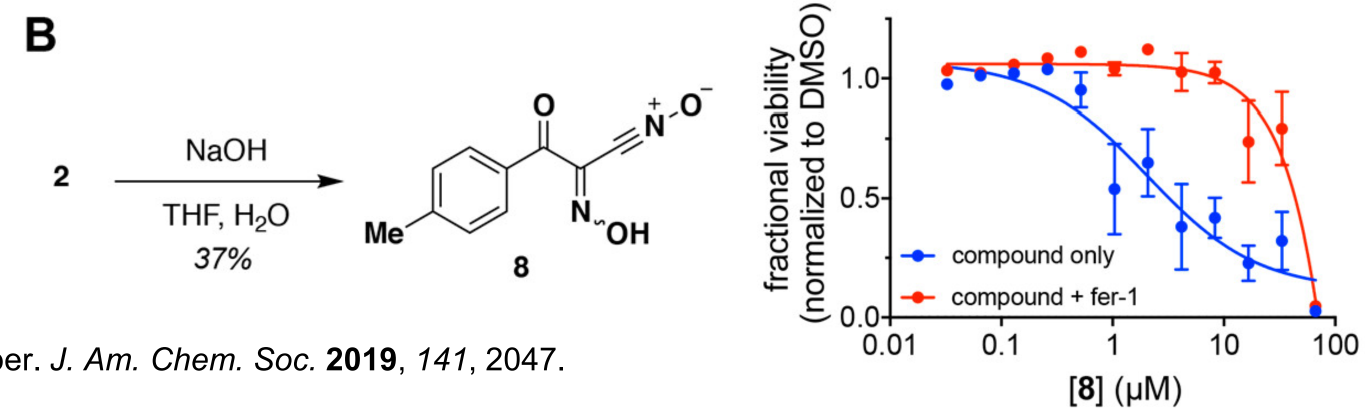
compound only
compound + fer-1




**1**, R = -OMe (NSC144988)
**2**, R = -Me
**3**, R = -H

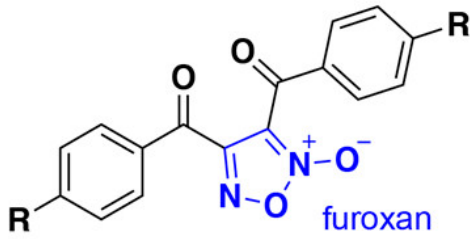


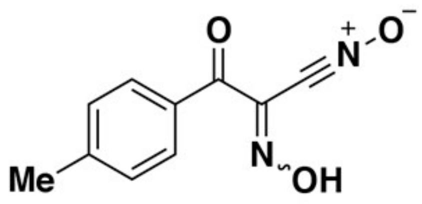




J, Eaton.; R, Ruberto.; A, Kramm.; V, Viswanathan.; S, Schreiber. *J. Am. Chem. Soc.* **2019**, *141*, 2047.

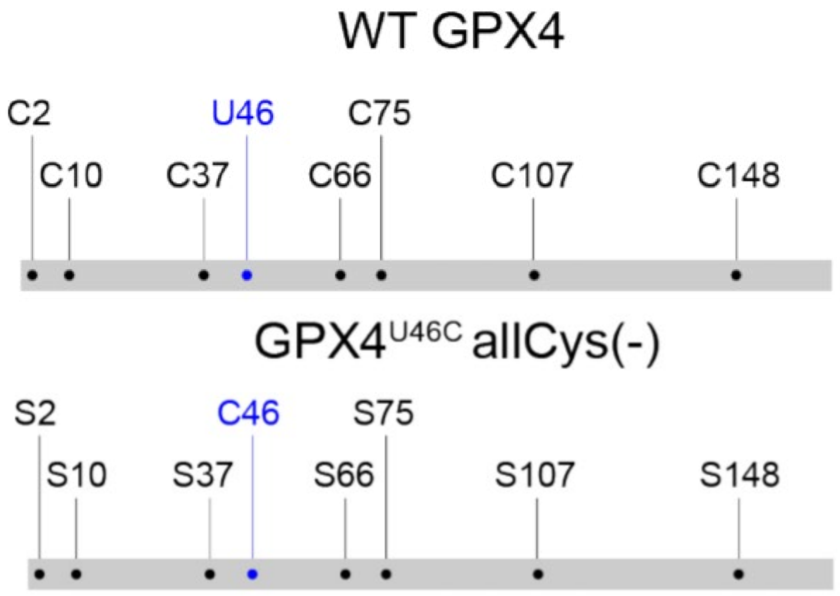
# Reactions with GPX4$^{U48C}$allCys(-)




**1**, R = -OMe (NSC144988)
**2**, R = -Me
**3**, R = -H

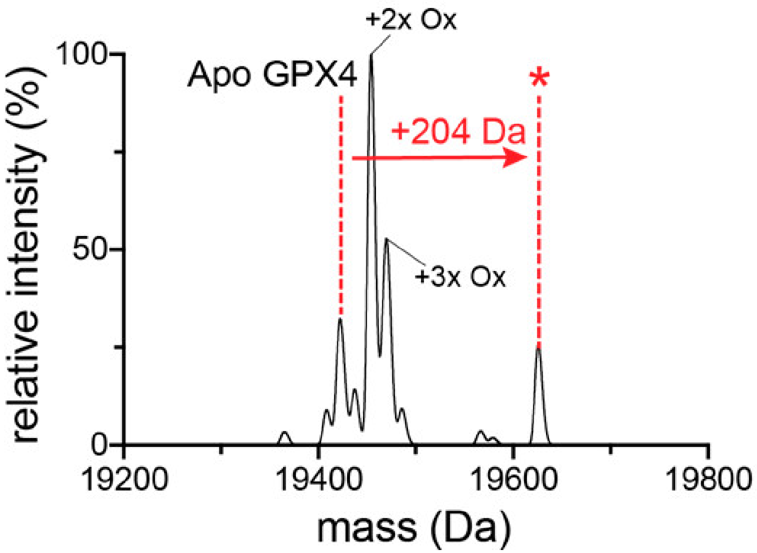



**8**



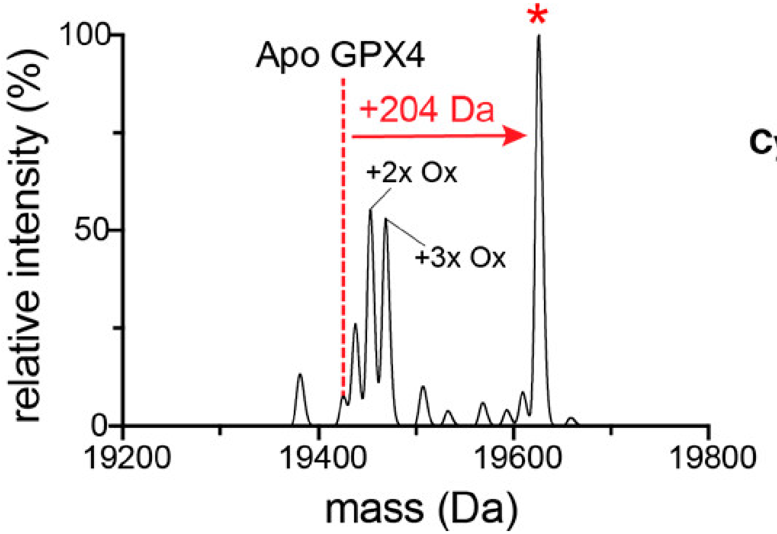

**E**

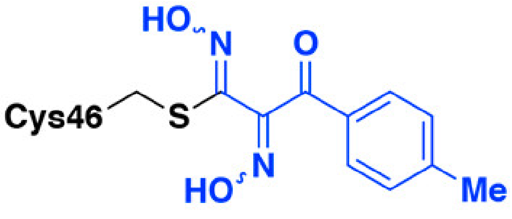
**2** + GPX4$^{U46C}$ allCys(-)




**F**

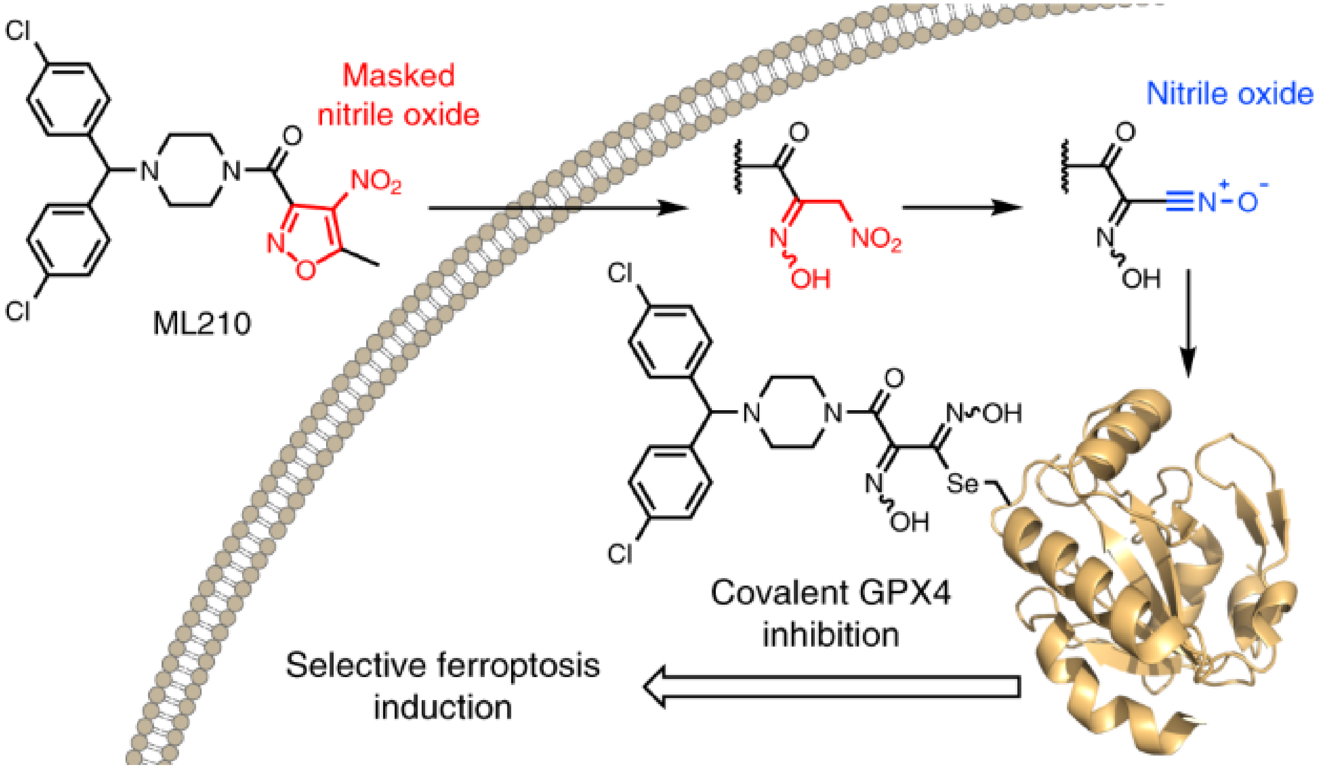
**8** + GPX4$^{U46C}$ allCys(-)

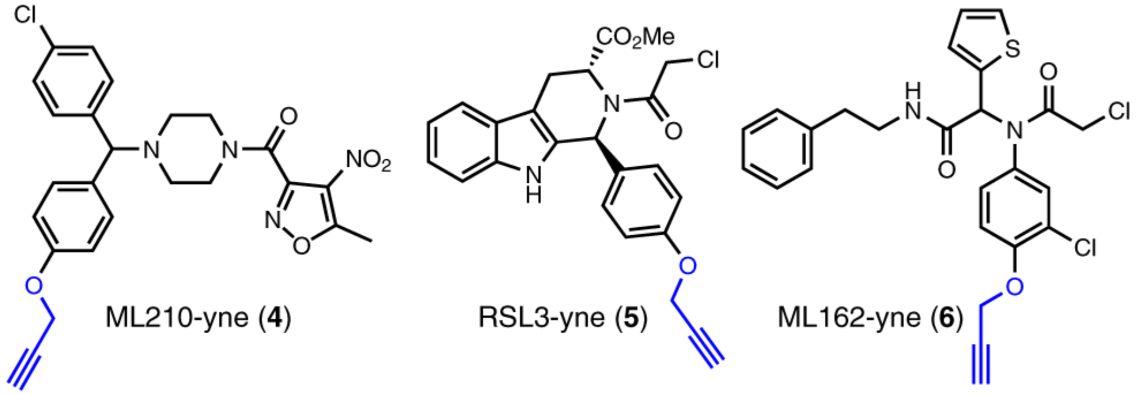


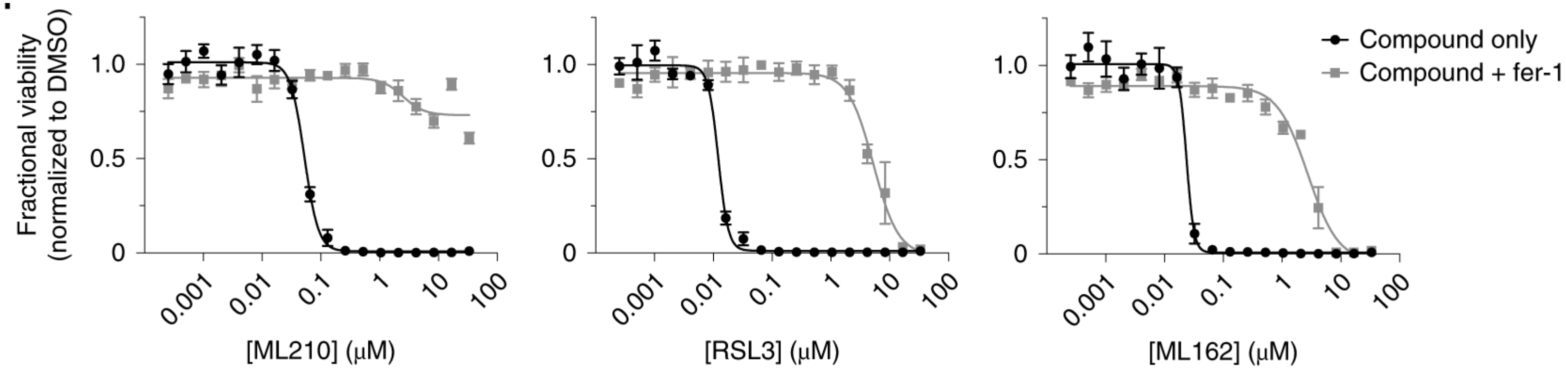



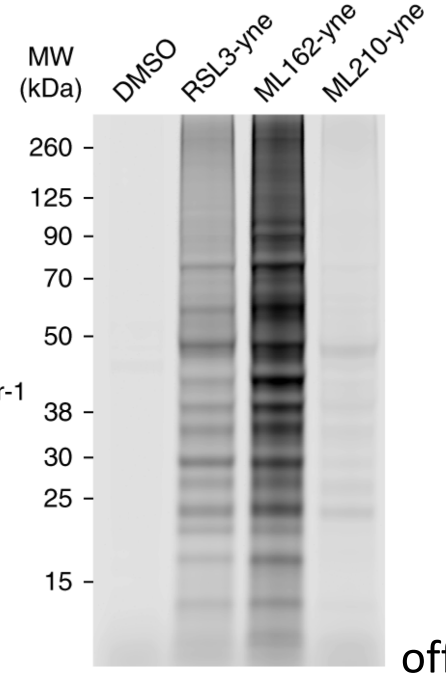

+204 Da thiohydroximate adduct

# ML210: Reduced Reactivity and Enhanced Selectivity



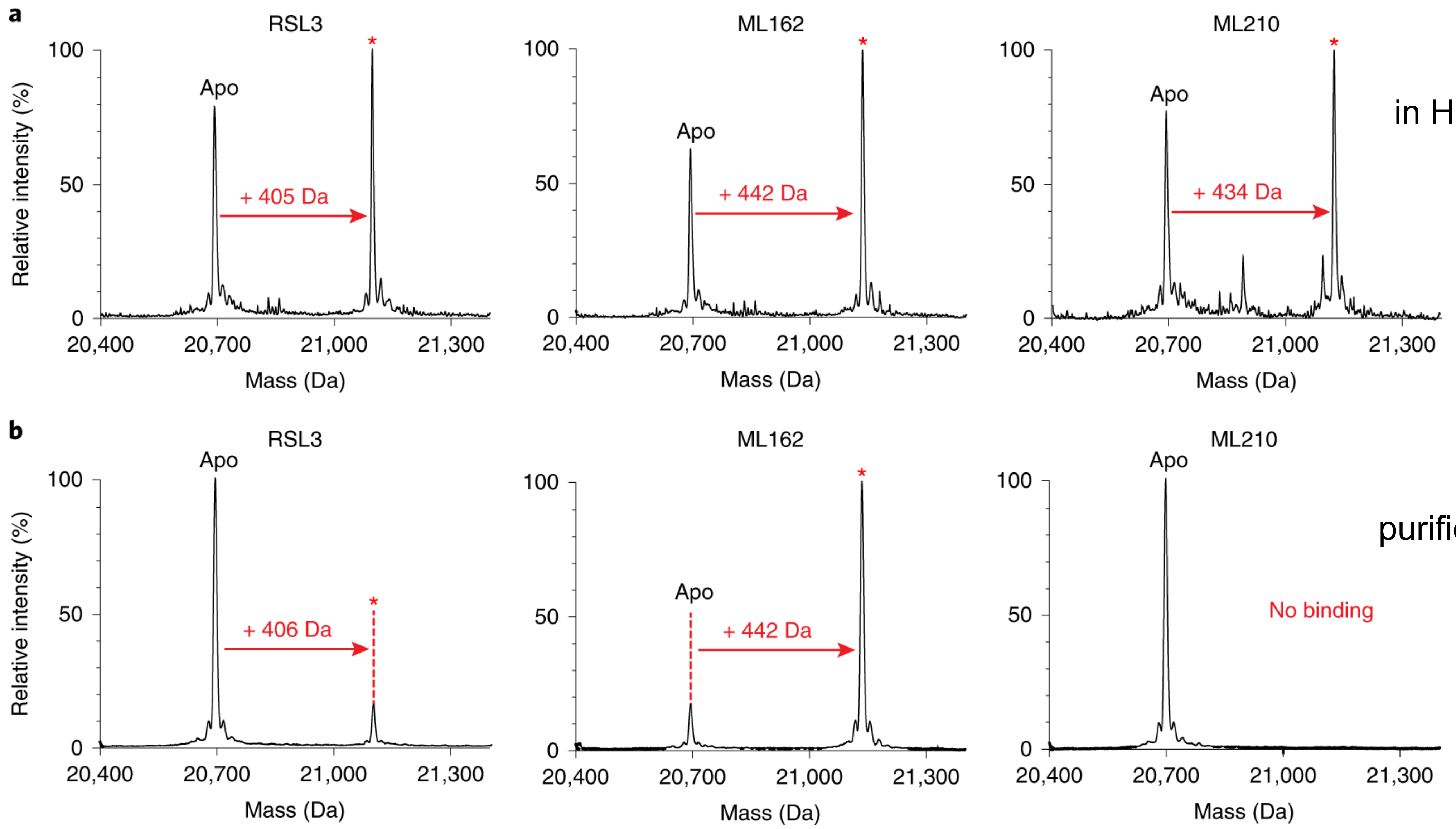

LC-MS/MS identified proteins enriched (~50 kDa band)

| protein name | gene |
|---|---|
| tubulin beta chain | TUBB |
| actin, cytoplasmic 1 | ACTB |
| tubulin beta-4B chain | TUBB4B |
| heme oxygenase 2 | HMOX2 |
| tubulin alpha-1B chain | TUBA1B |
| tubulin alpha-1C chain | TUBA1C |
| tubulin beta-6 | TUBB6 |

off-targets: highly abundant

J, Eaton.; L, Furst.; R, Ruberto.; D, Moosmayer.; A, Hilpmann.; M, Ryan.; K, Zimmermann.; L, Cai.; M, Niehues.; V, Badock.; A, Kramm.; S, Chen.; R, Hillig.; P, Clemons.; S, Gradl.; C, Montagnon.; K, Lazarski.; S, Christian.; B, Bajrami.; R, Neuhaus.; A, Eheim.; V, Viswanathan.; S, Schreiber. *Nat. Chem. Bio.* **2020**, *16*, 497.

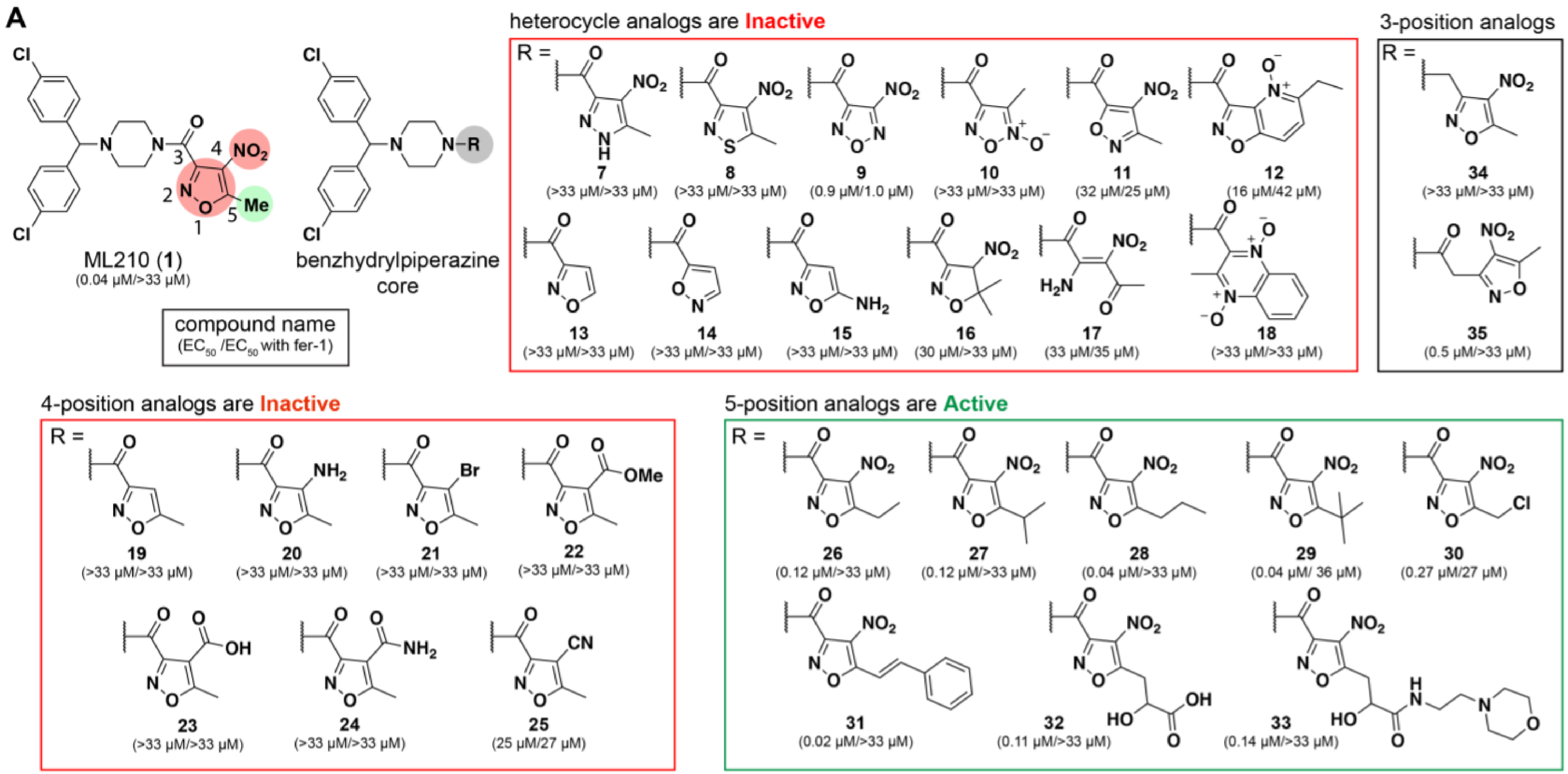
# Intact-cell Context Required for ML210 to bind GPx4



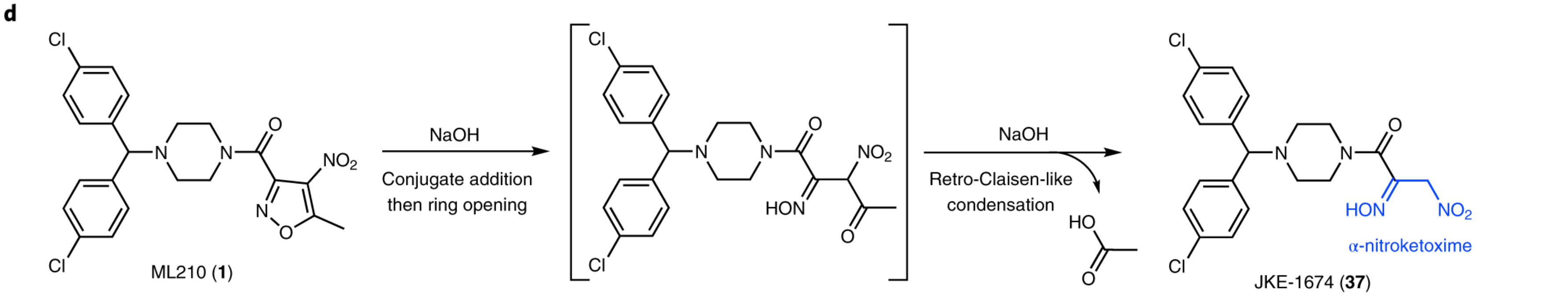

in HEK293-6E cells

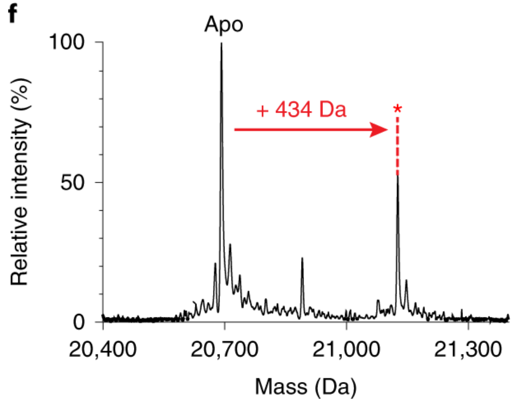
purified FLAG-GPX4$^{WT}$

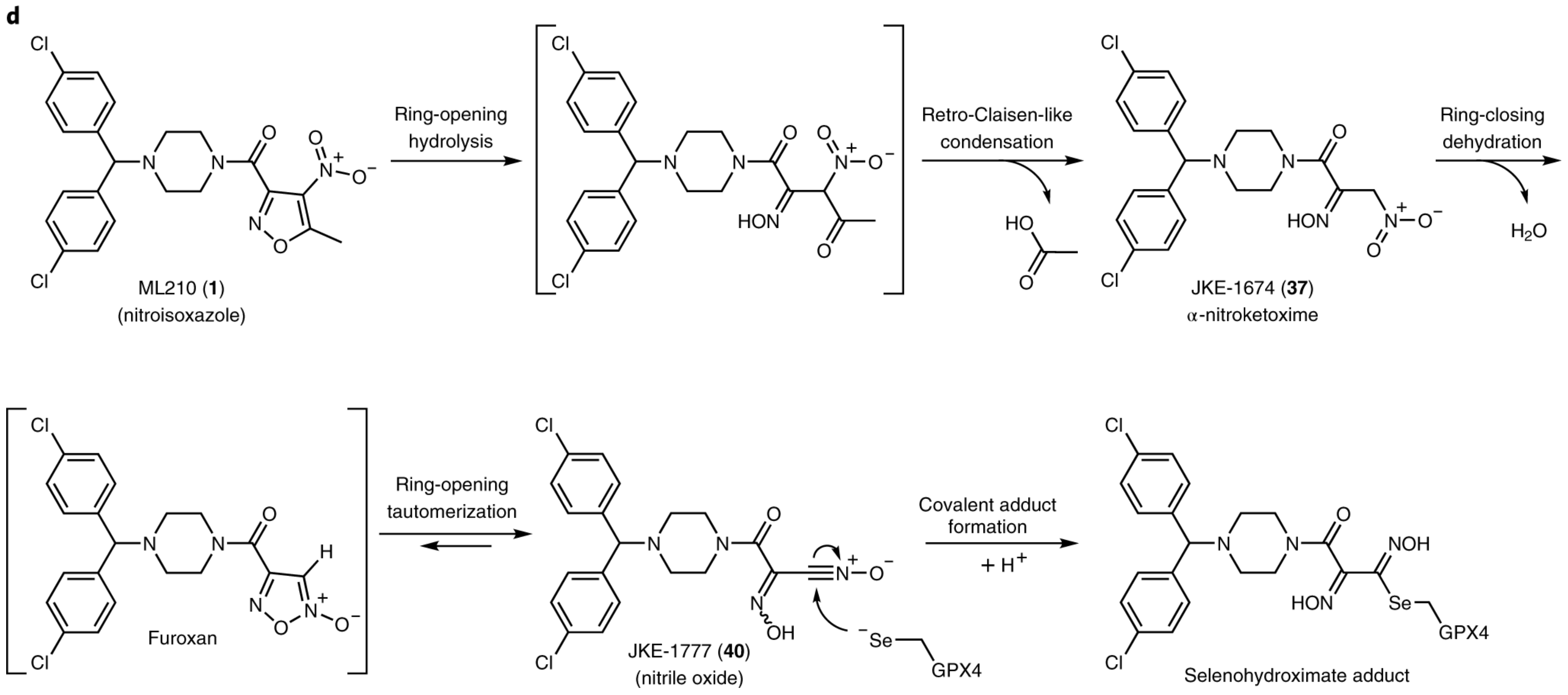
# Effects of Substituents on Potency

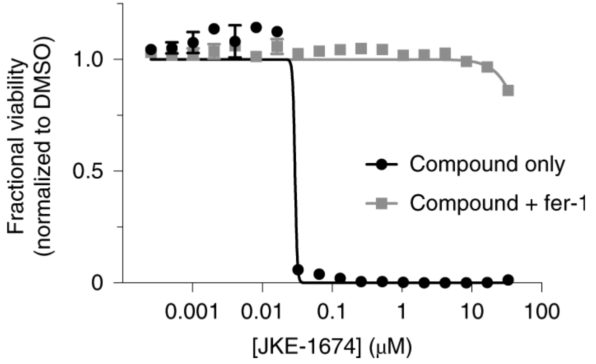


# Identification of the Nitroketoxime Intermediate

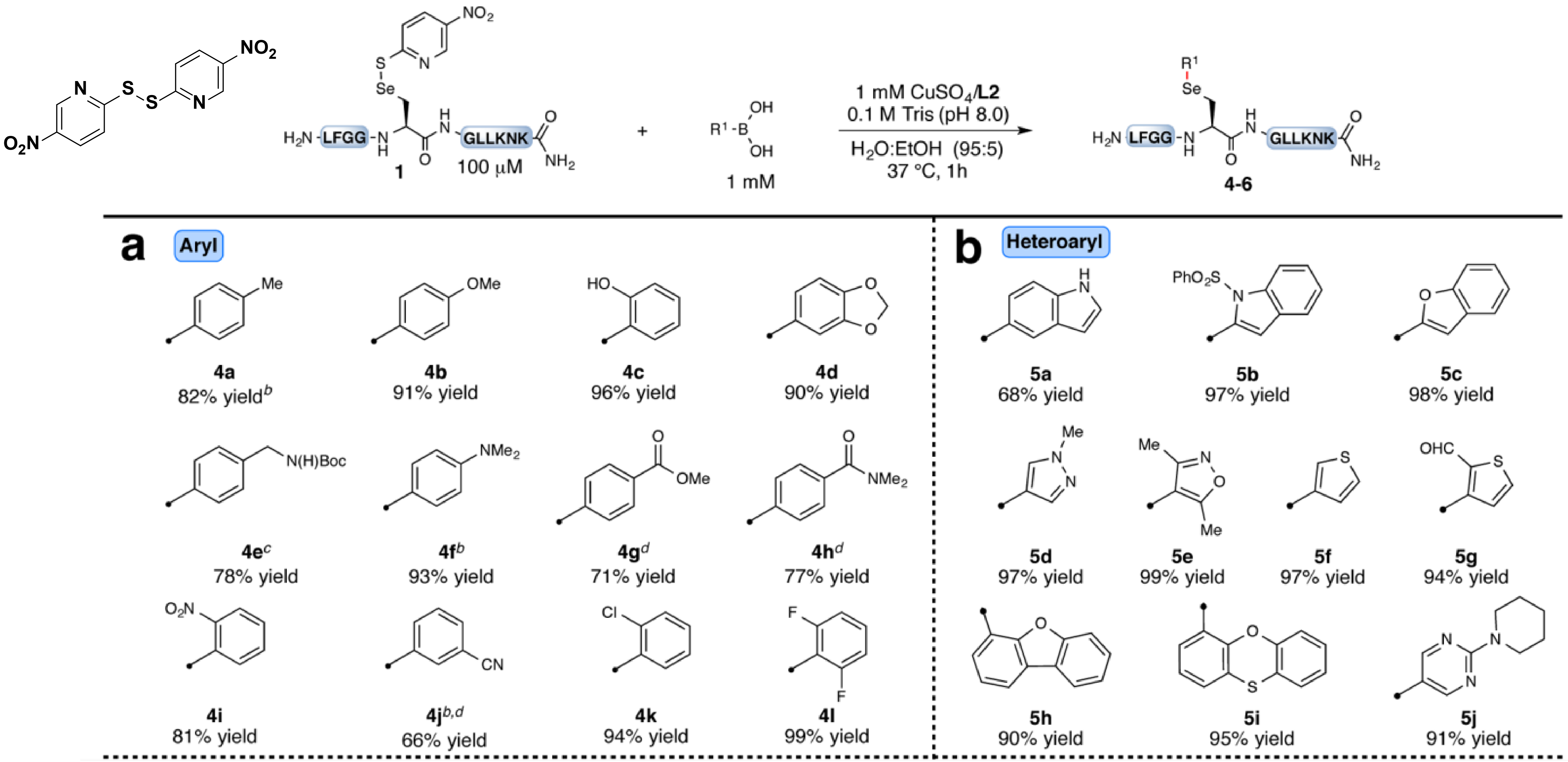


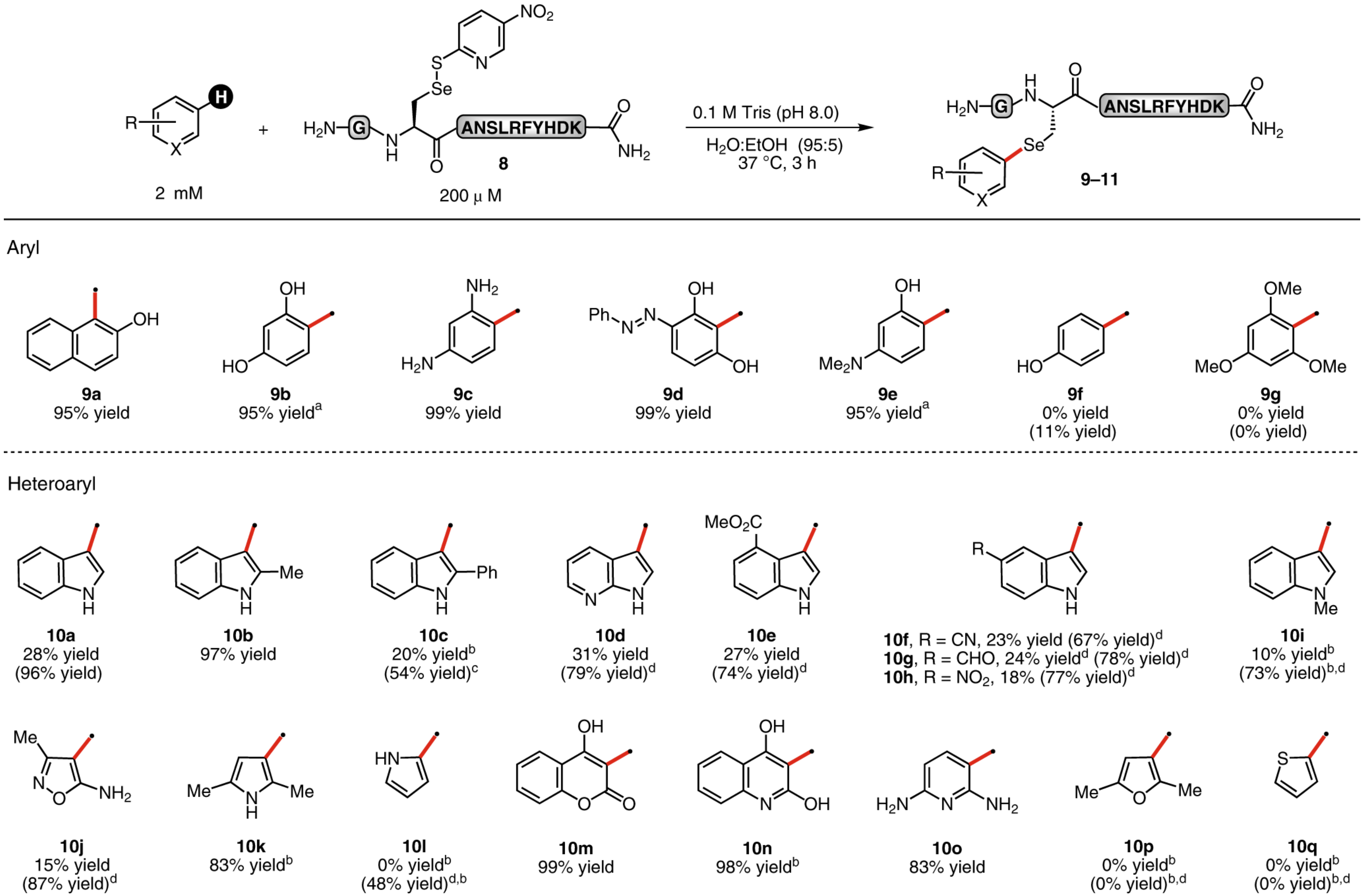



# Cu-catalyzed Sec Arylation in Peptides




D, Cohen.; C, Zhang.; B, Pentelute.; S, Buchwald. *J. Am. Chem. Soc.* **2015**, *137*, 9784.

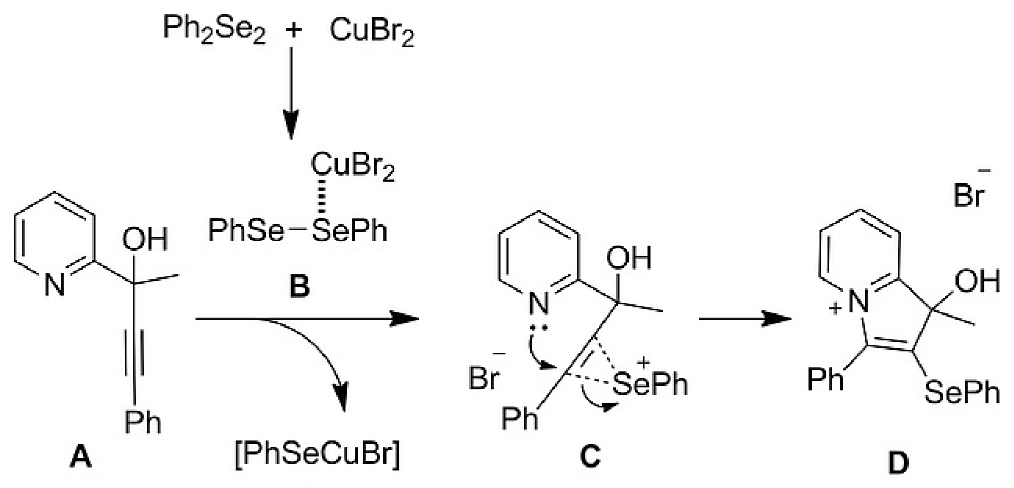
# Direct Conjugation *via* Aromatic Electrophilic Substitution

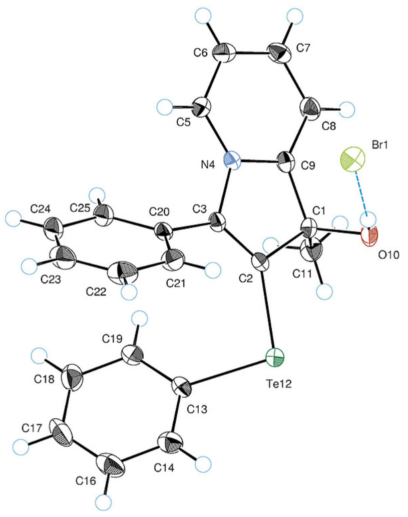


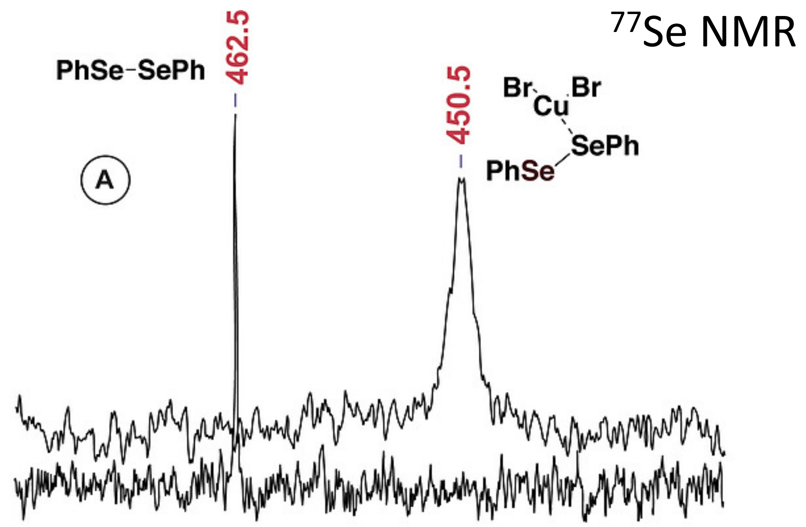


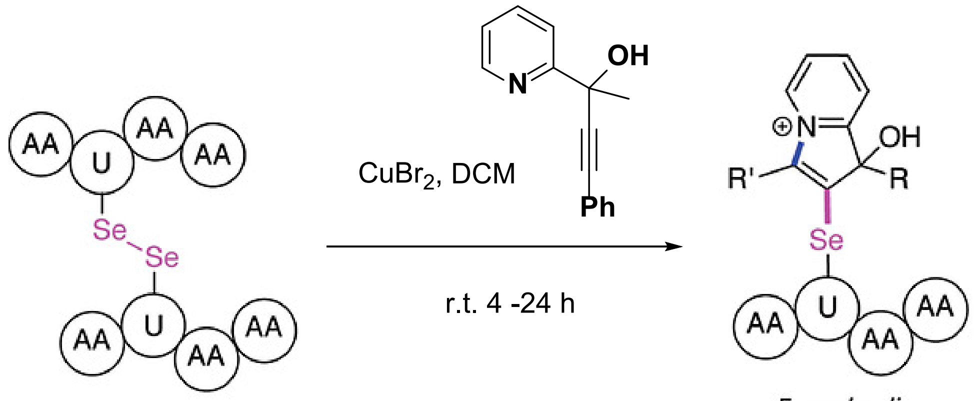

D, Cohen.; C, Zhang.; C, Fadzen.; A, Mijalis.; L, Hie.; K, Johnson.; Z, Shriver.; O, Plante.; S, Miller.; S, Buchwald.; B, Pentelute. *Net. Chem.* **2019**, *11*, 78.

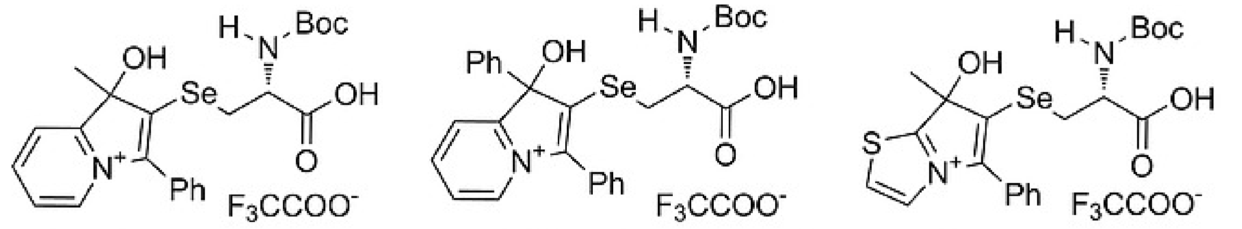
# Selenocysteine Electrophile for Alkynyl Substrates



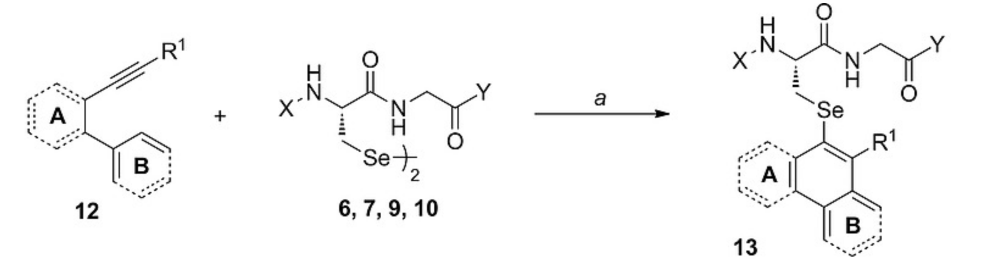



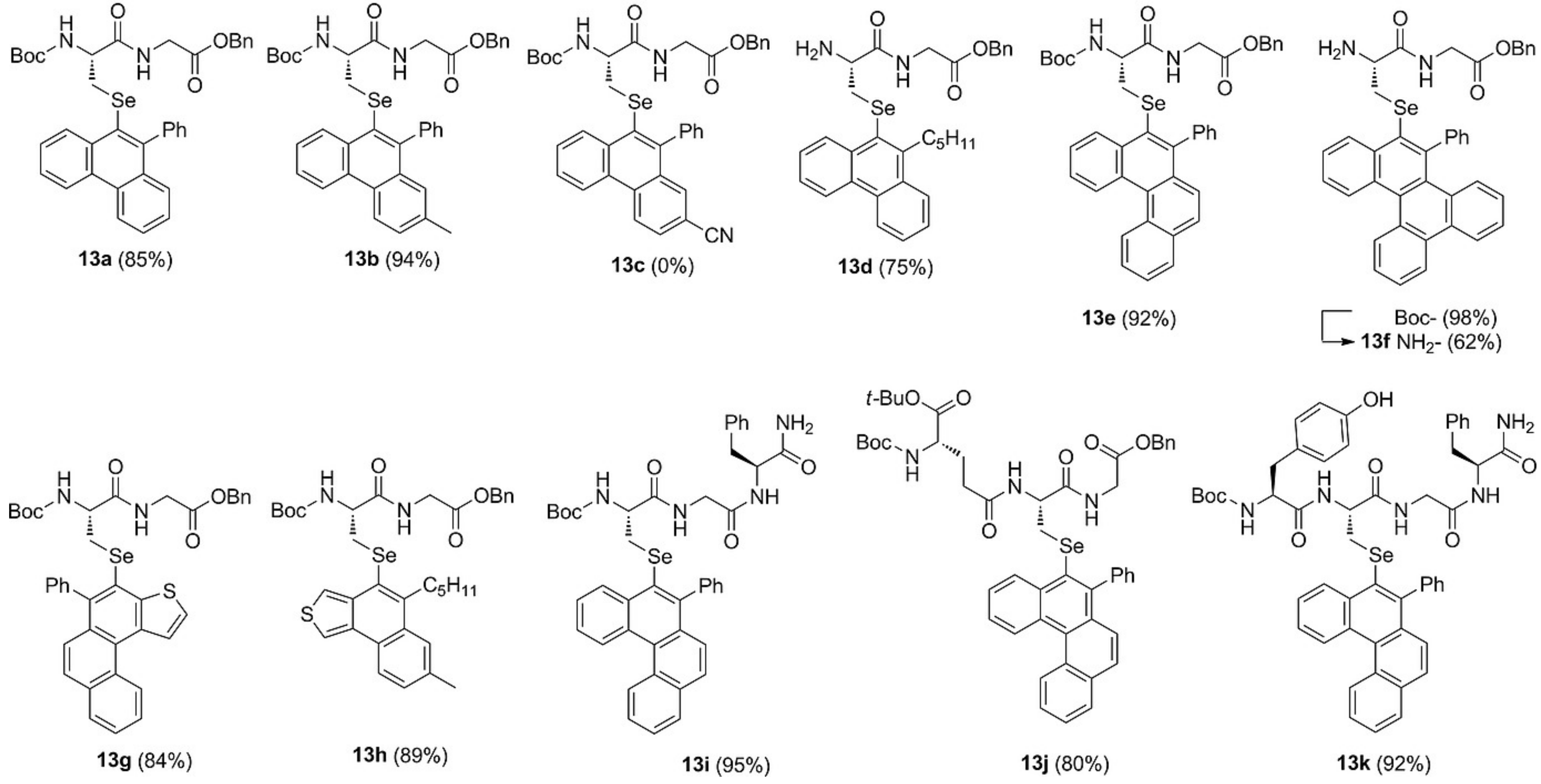




P, Arsenyan.; S, Lapcinska.; A, Ivanova.; J, Vasiljeva. *Eur. J. Org. Chem.* **2019**, 4951.

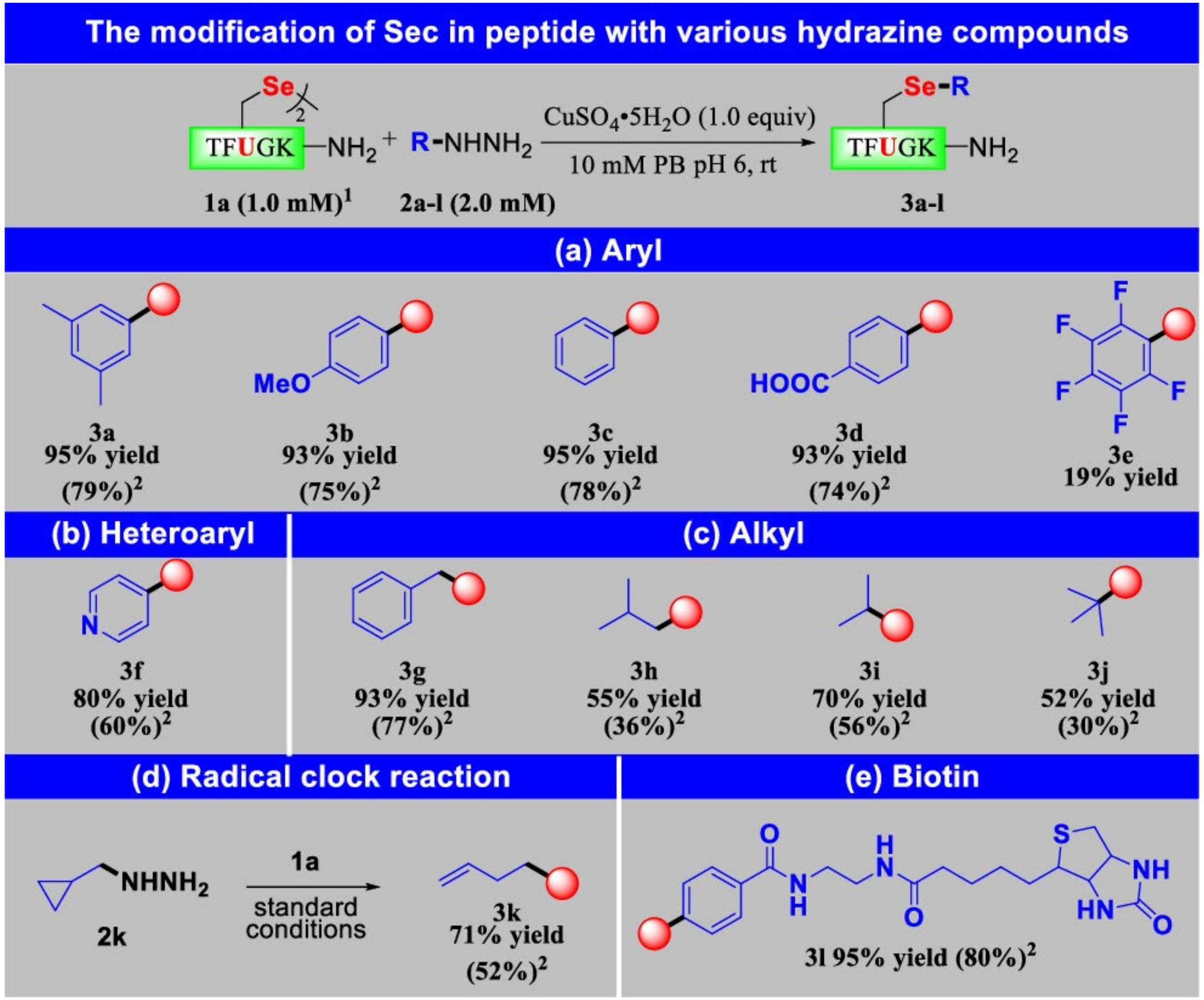
# *6-endo-dig* Cyclizations

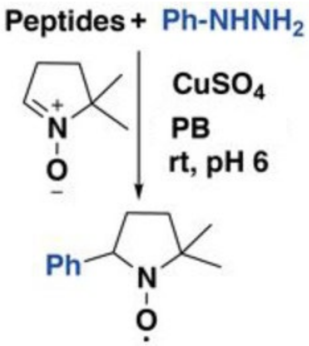



Condition: $CuBr_2$, DCM, rt, 4 - 24 h

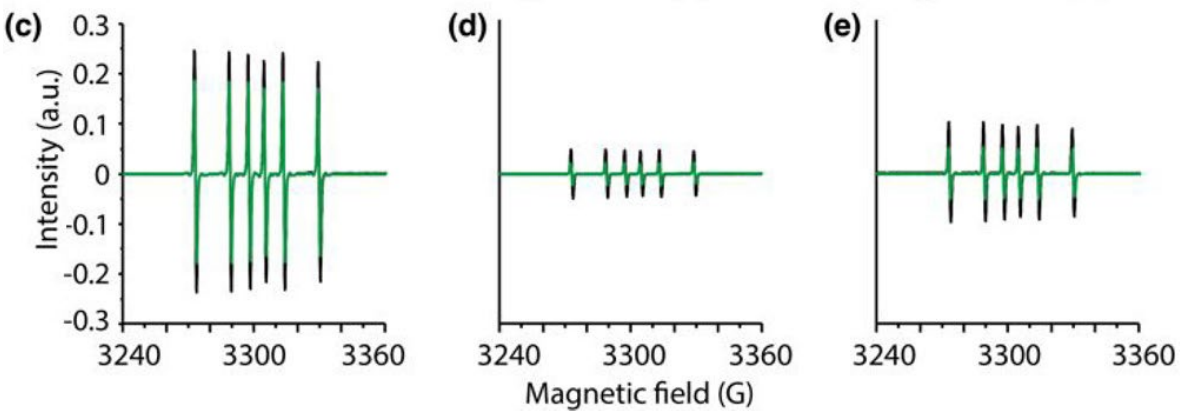


# Hydrazine-based Sec Arylation and Alkylations



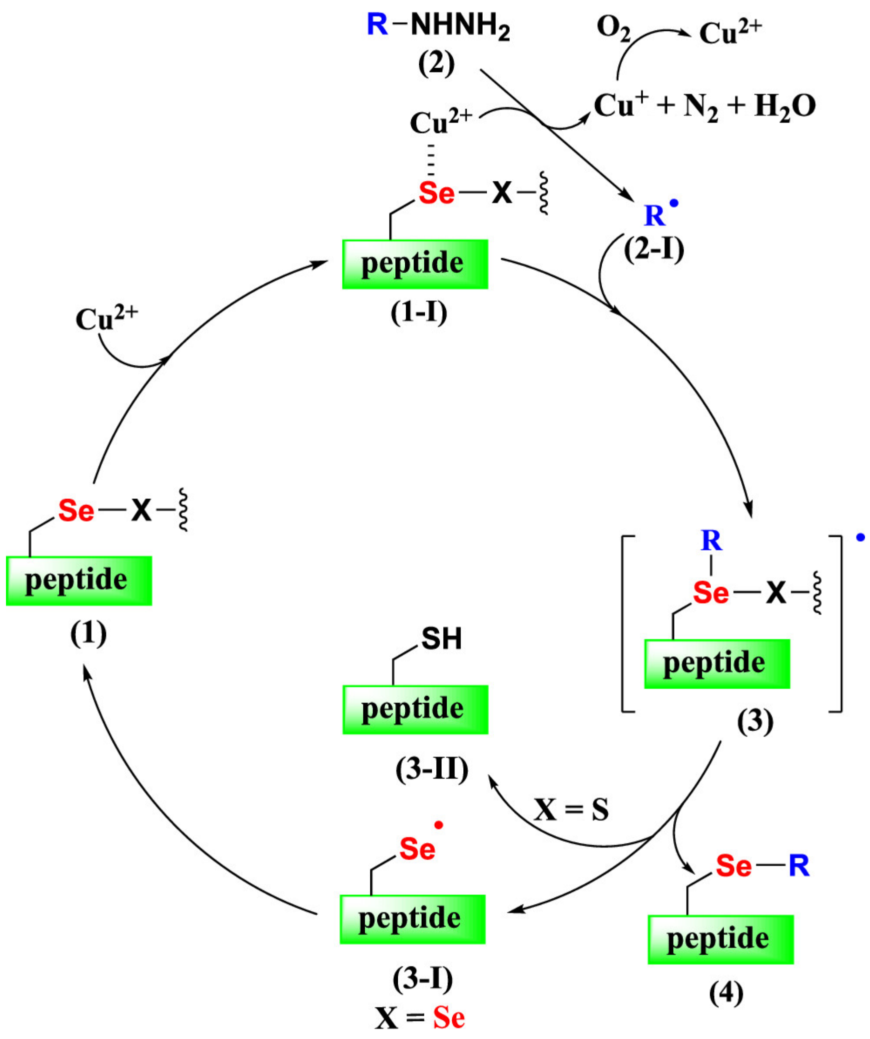

Se atom interacts with the formed Ph radical and partially stabilizes it

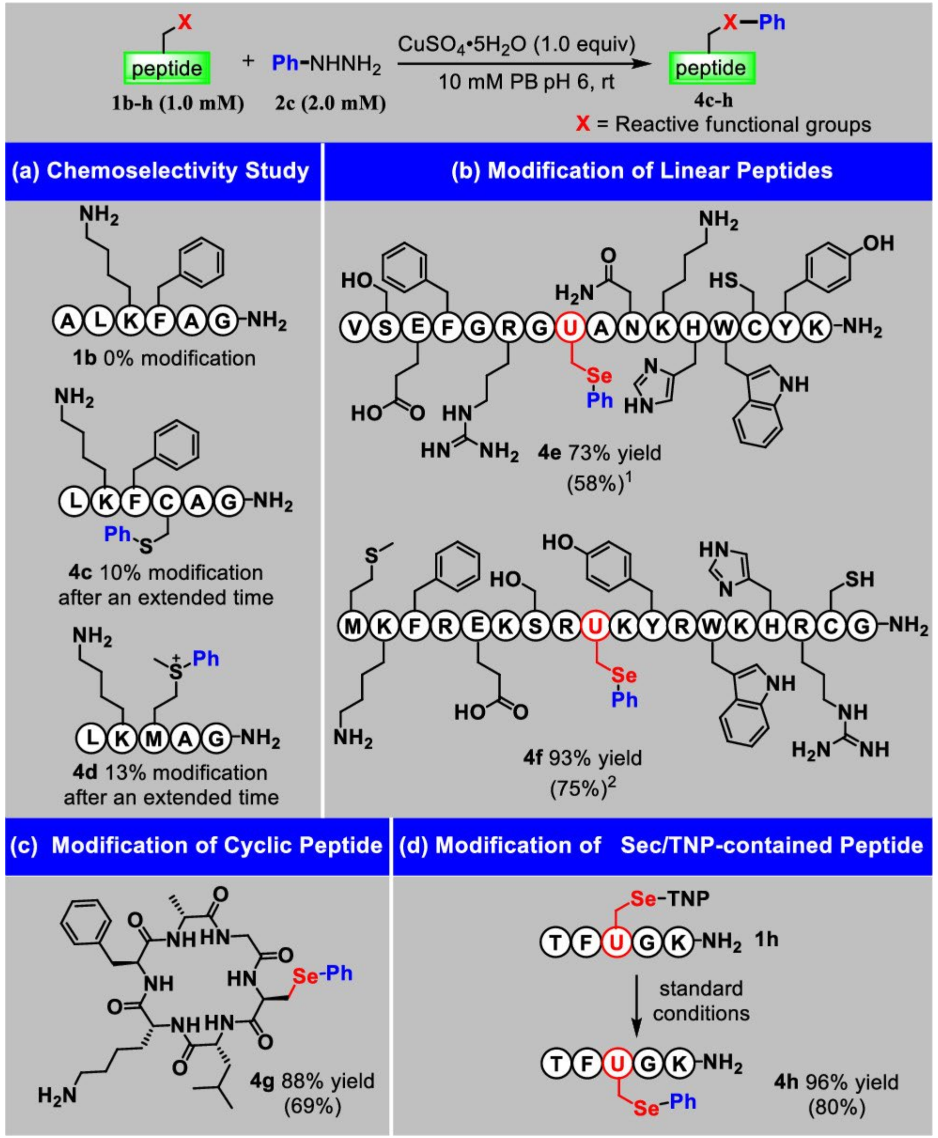


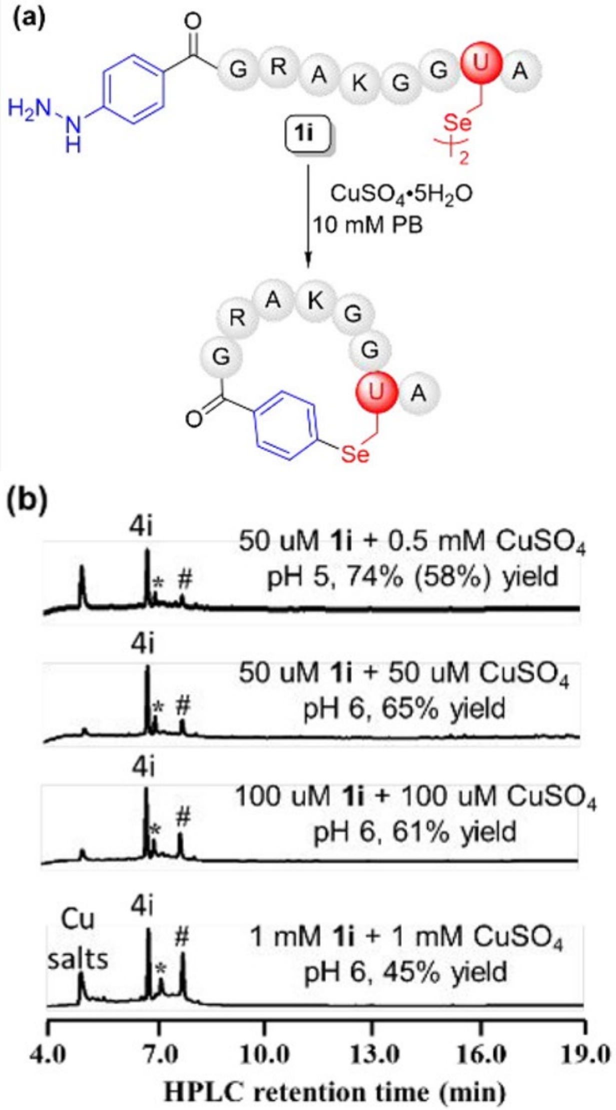

**TFUGK** **ALKFAG** **LKFCAG**

Z, Zhao.; D, Shimon.; N, Metanis. *J. Am. Chem. Soc.* **2021**, *143*, 12817.

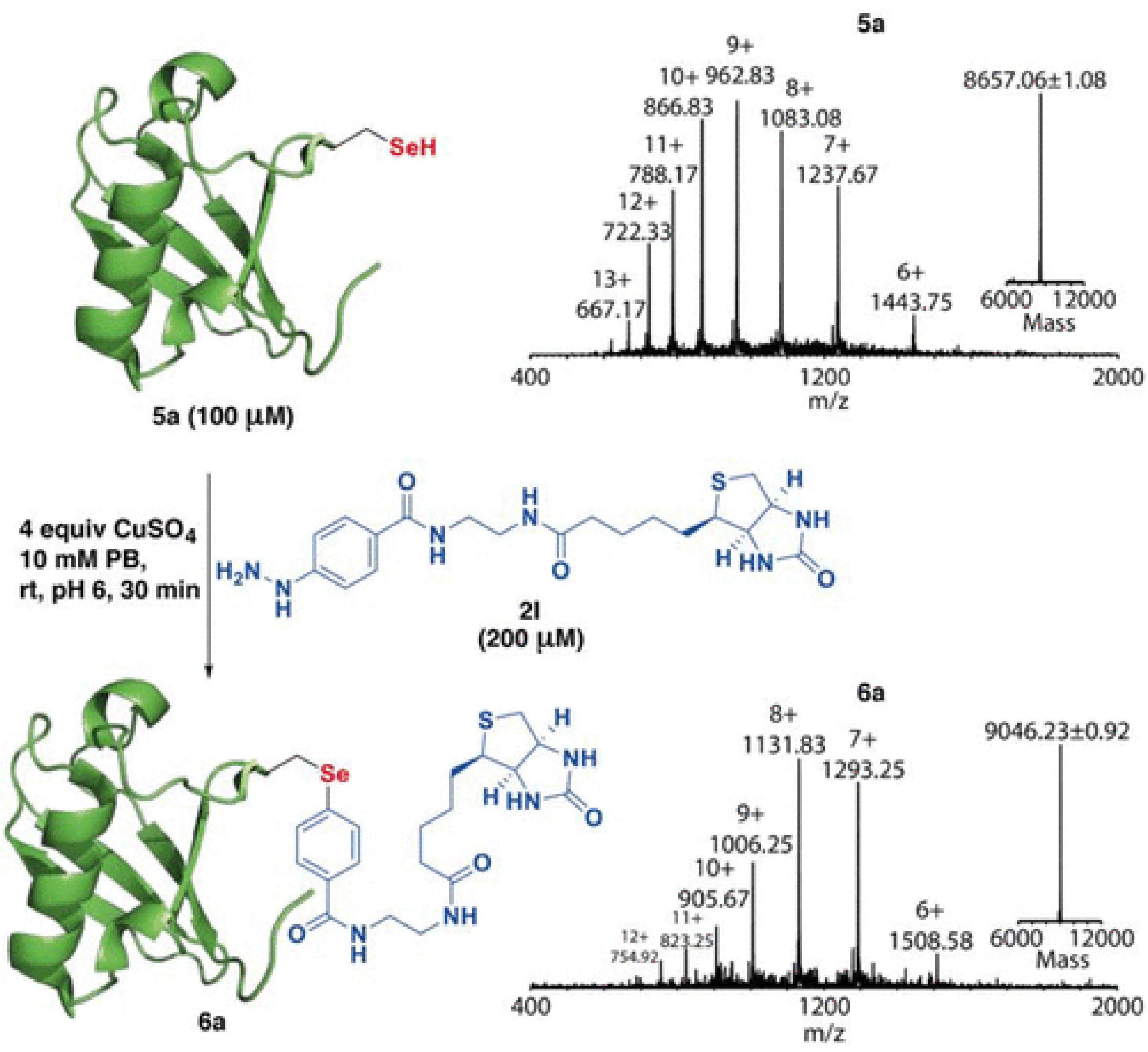
# Substrate Scope



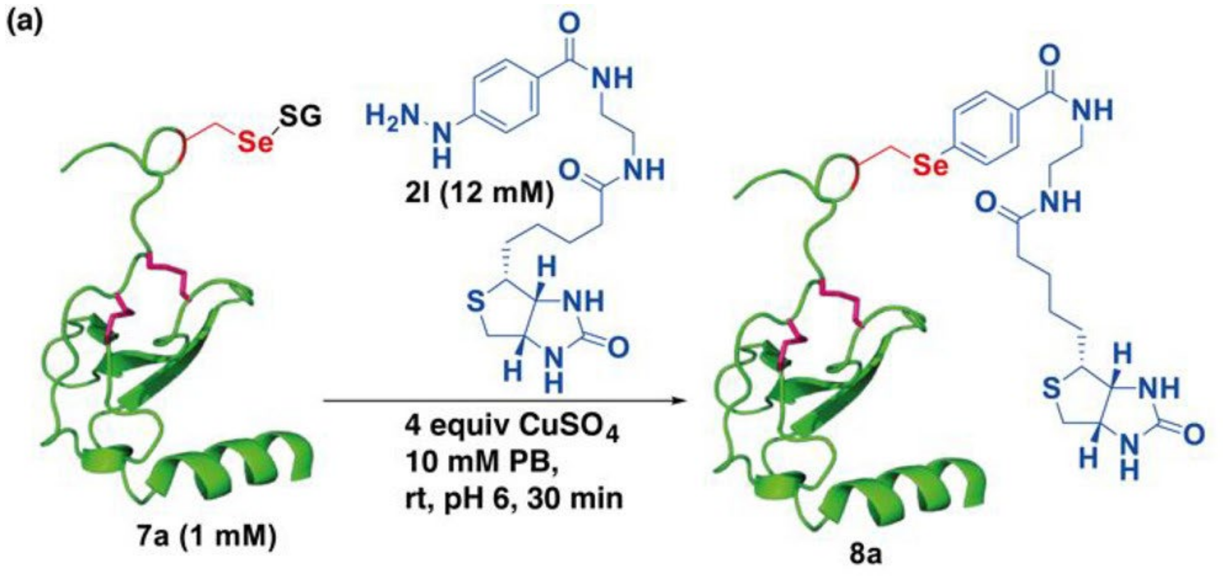



# Modification of Proteins




Ub G47U: almost quantitative biotinylation / 60% yield

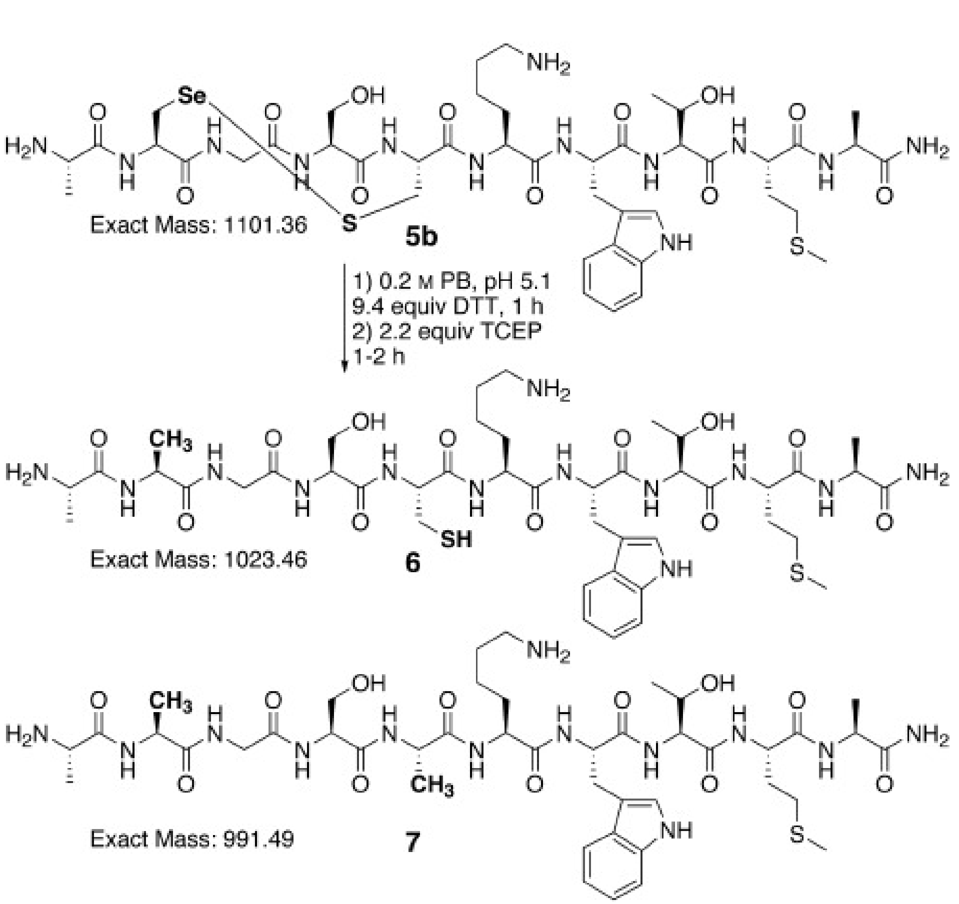


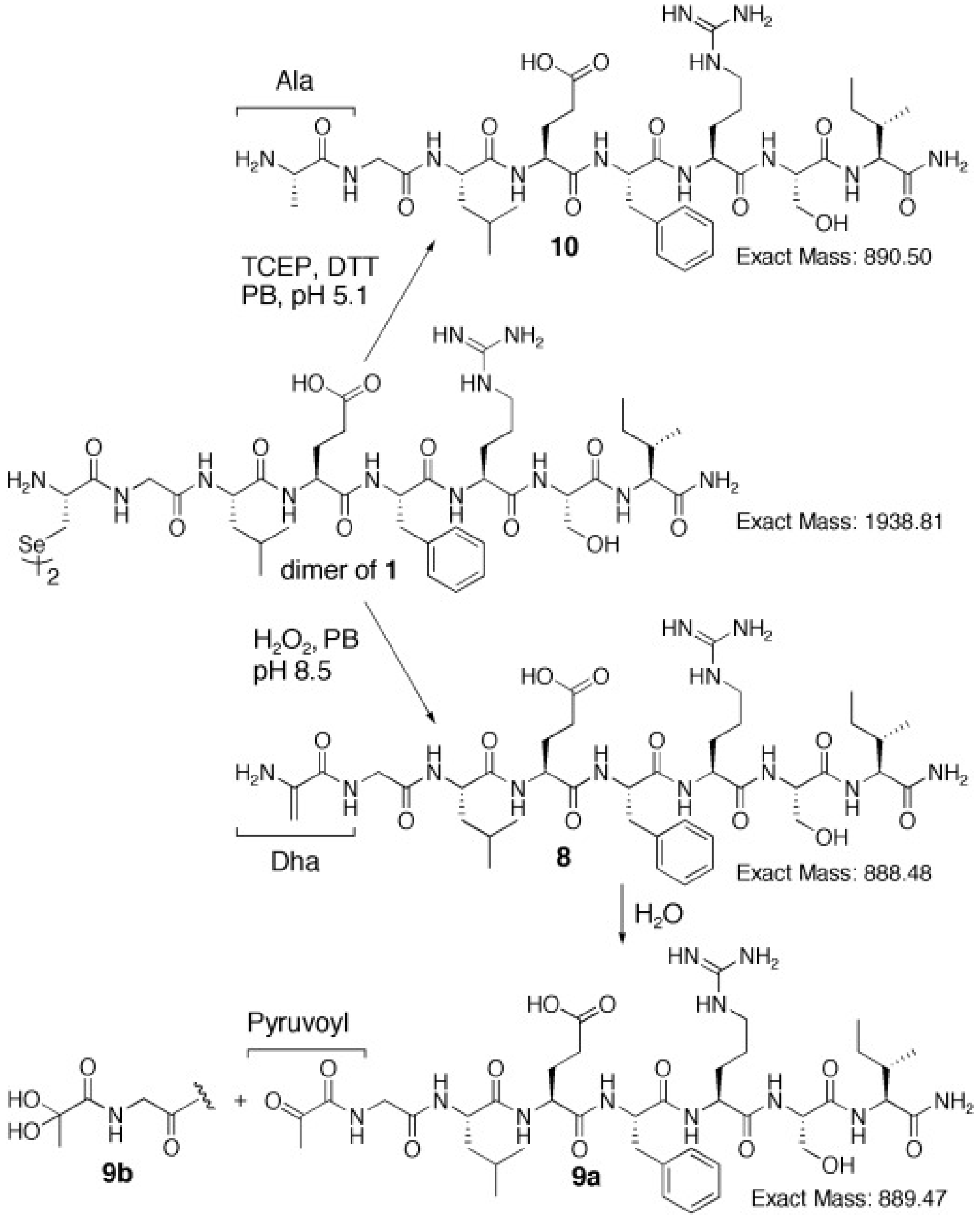

Modification of stromal cell-derived factor-1 (**SDF1**) with 2 disulfide bonds: 70% conversion / 43% yield

# Contents

- Discovery of Sec and its Insertion Mechanism
- Sec vs Cys in Proteins: Why Selenium?
- Sec Probes and Bioconjugate Chemistry
- Deselenative Modifications for Sec

# Selective Deselenization under the Presence of Cysteine

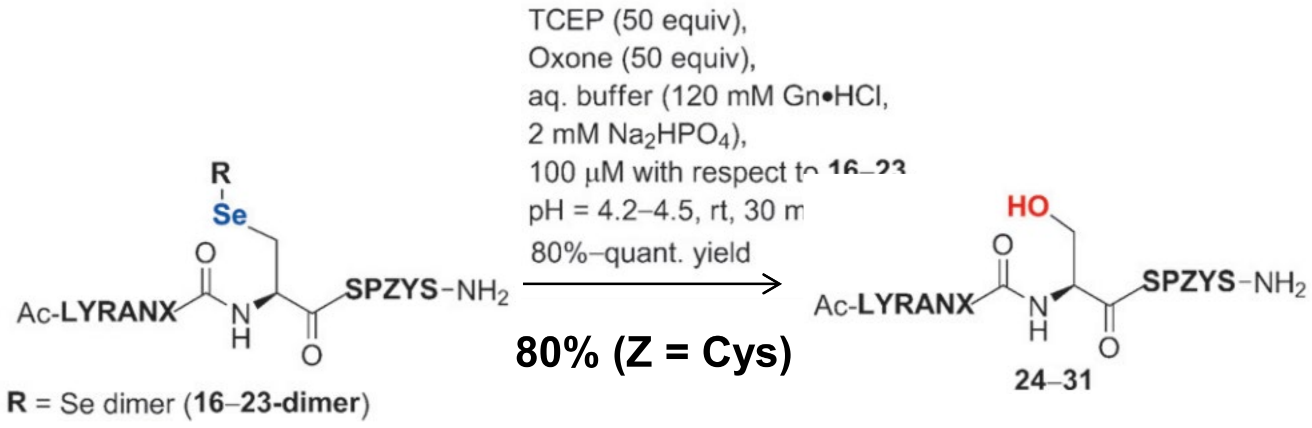


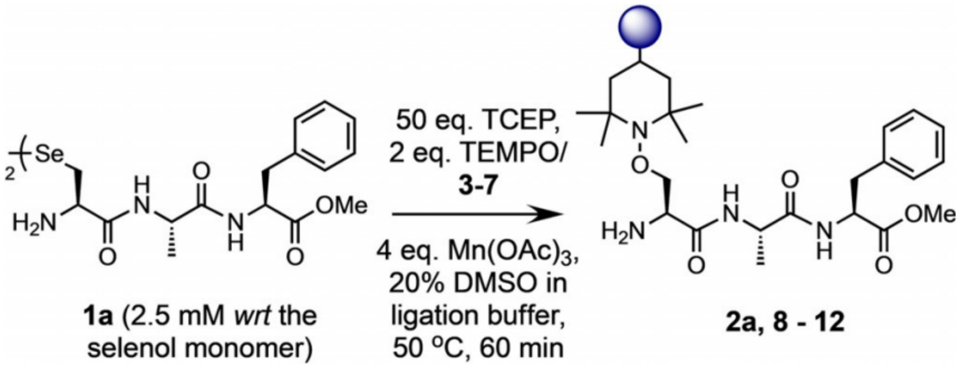




Selectivity achieved by using TCEP in the absence of radical initiators. Only trace amount of **7** observed.

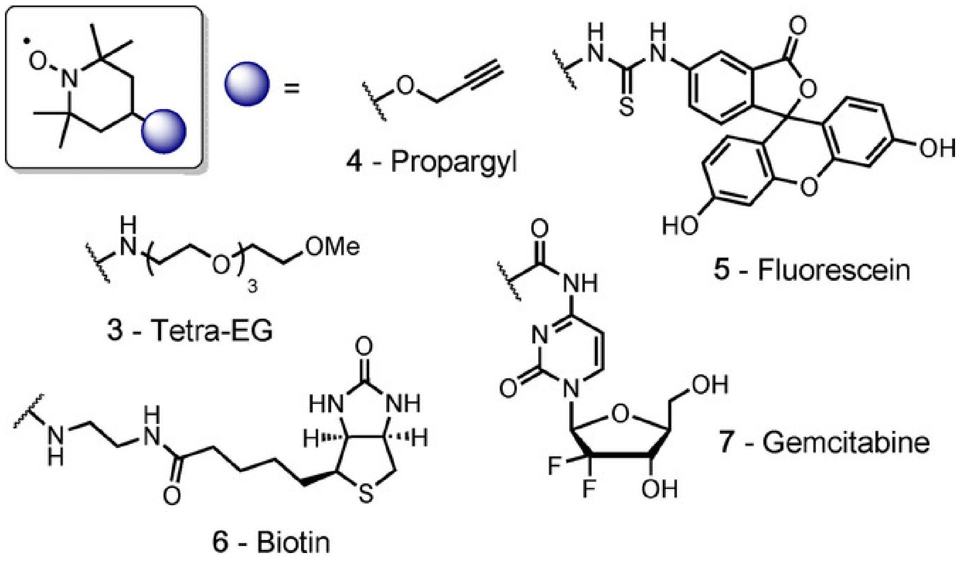
N, Metanis.; E, Keinan.; P, Dawson. *Angew. Chem. Int. Ed.* **2010**, *49*, 7049.

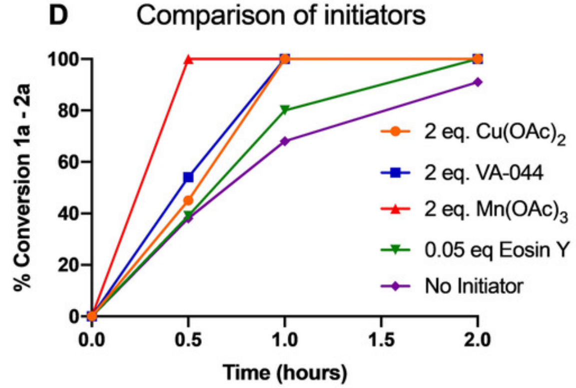
# Deselenative $C_\beta$ Radical Generation



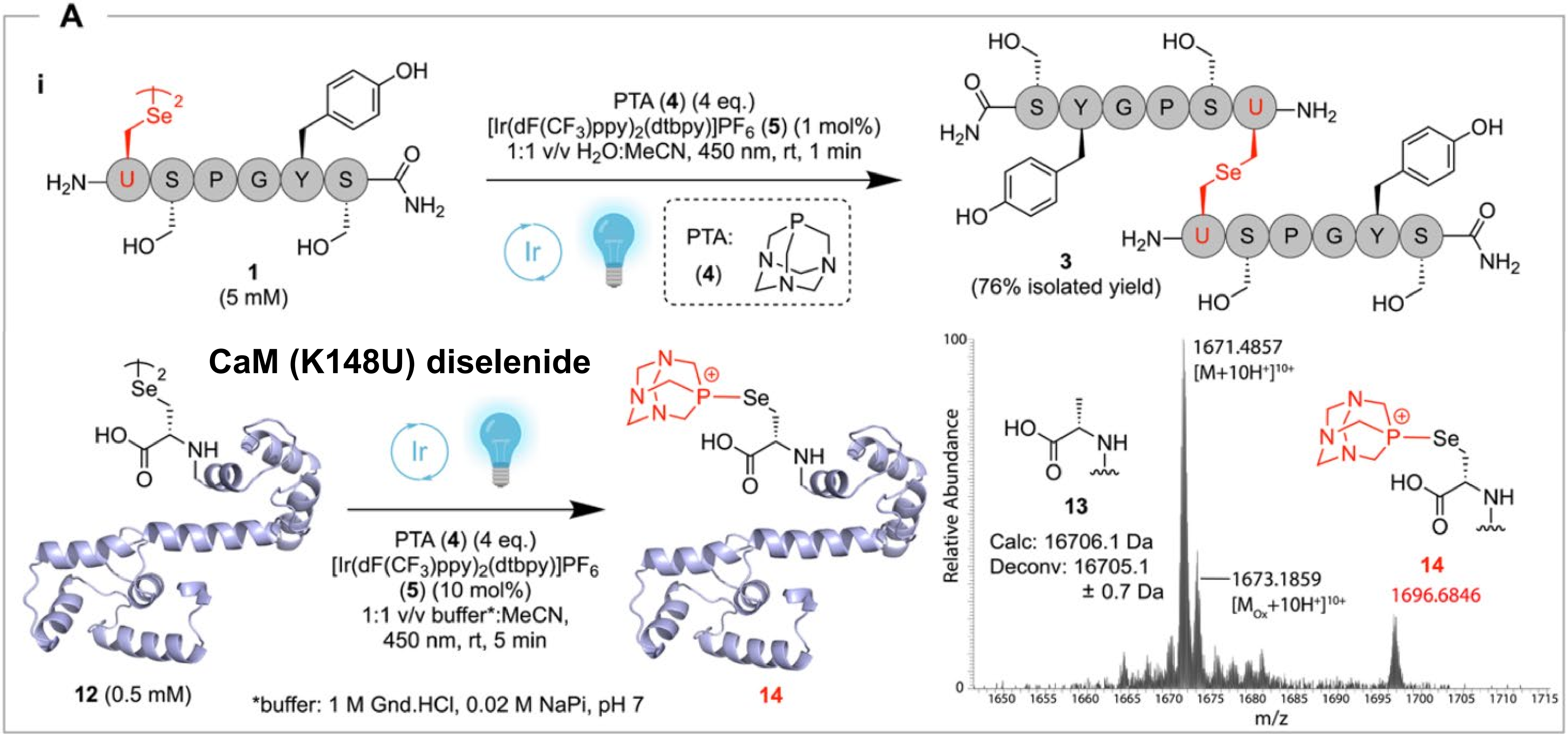

| Entry | Product | Trap | Yield [%] |
|---|---|---|---|
| 13 | **2a** | TEMPO | 83 |
| 14 | **8** | tetra-EG (**3**) | 72 |
| 15 | **9** | propargyl (**4**) | 80 |
| 16 | **10** | fluorescein (**5**) | 69 |
| 17 | **11** | biotin (**6**) | 78 |
| 18 | **12** | gemcitabine (**7**) | 67 |



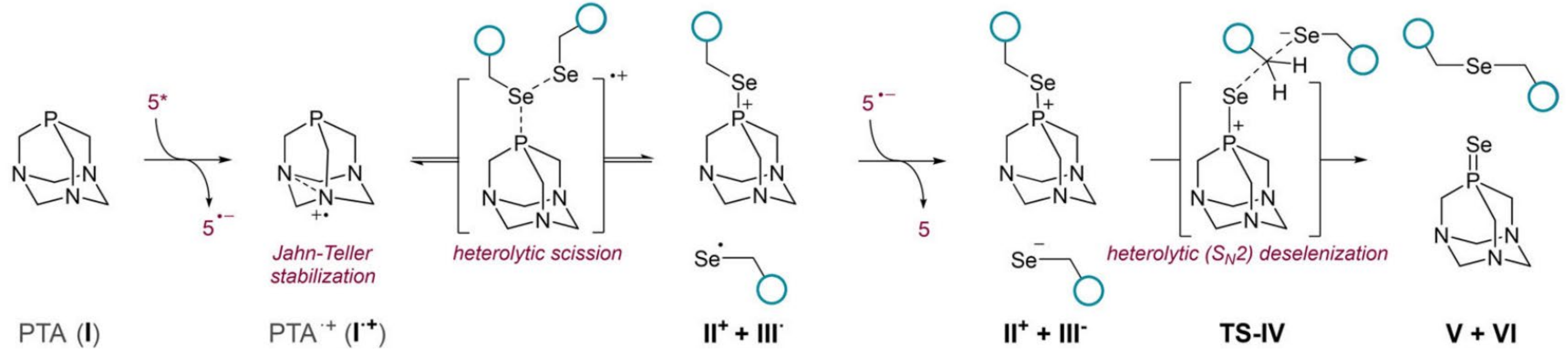

L, Malins.; N, Mitchell.; S, McGowan.; R, Payne. *Angew. Chem. Int. Ed.* **2015**, *54*, 12716.
R, Griffiths.; F, Smith.; J, Long.; H, Williams.; R, Layfield.; N, Mitchell. *Angew. Chem. Int. Ed.* **2020**, *59,* 23659.

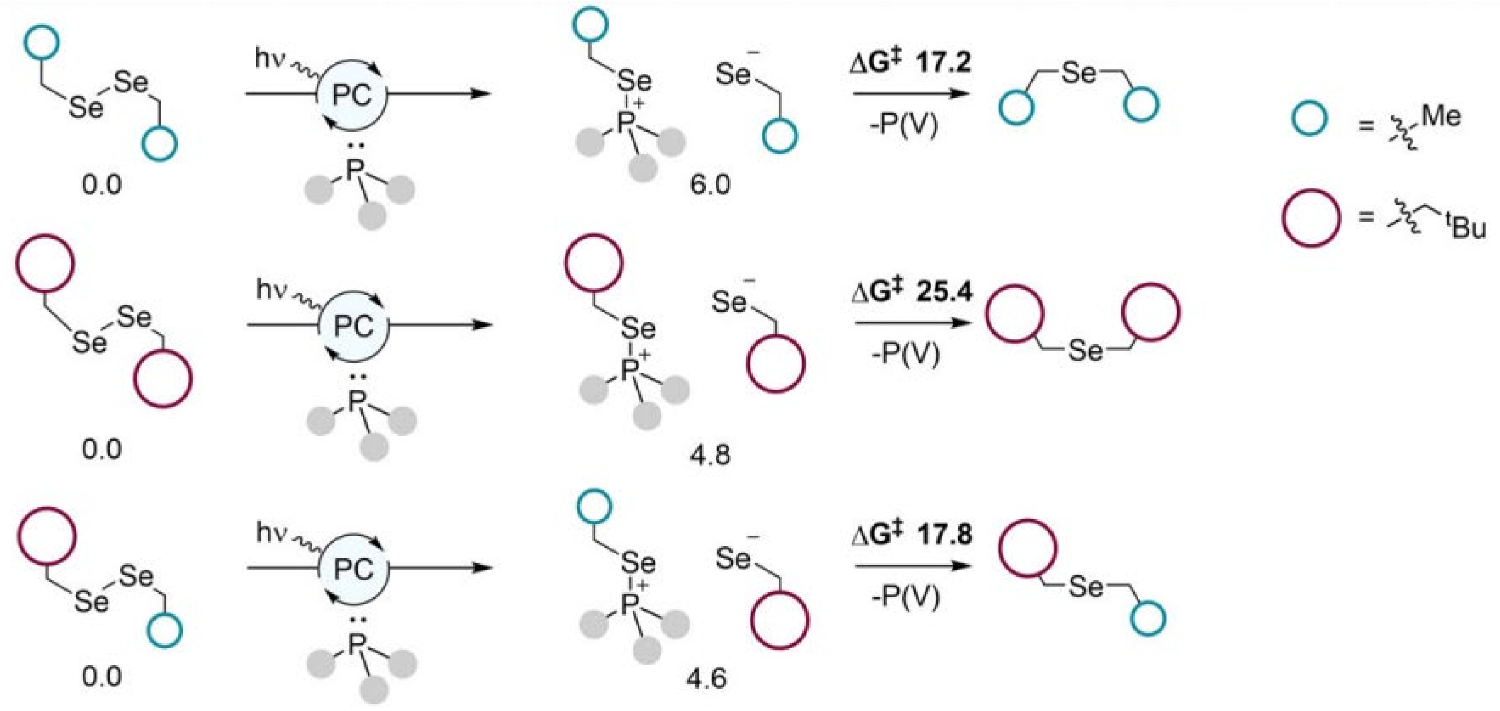
# Photocatalytic Diselenide Contraction



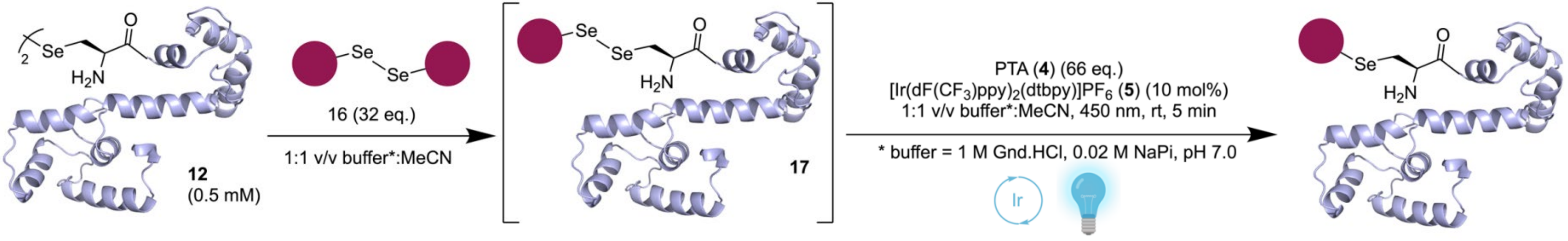

L, Dowman.; S, Kulkarni.; J, Alegre-Requena.; A, Giltrap.; A, Norman.; A, Sharma.; L, Gallegos.; A, Mackay.; A, Welegedara.; E, Watson.; D, Raad.; G, Niederacher.; S, Huhmann.; N, Proschogo.; K, Patel.; M, Larance.; C, Becker.; J, Mackay.; G, Lakhwani.; T, Huber.; R, Paton.; R, Payne. *Nat. Commun.* **2022**, *13*, 6885.

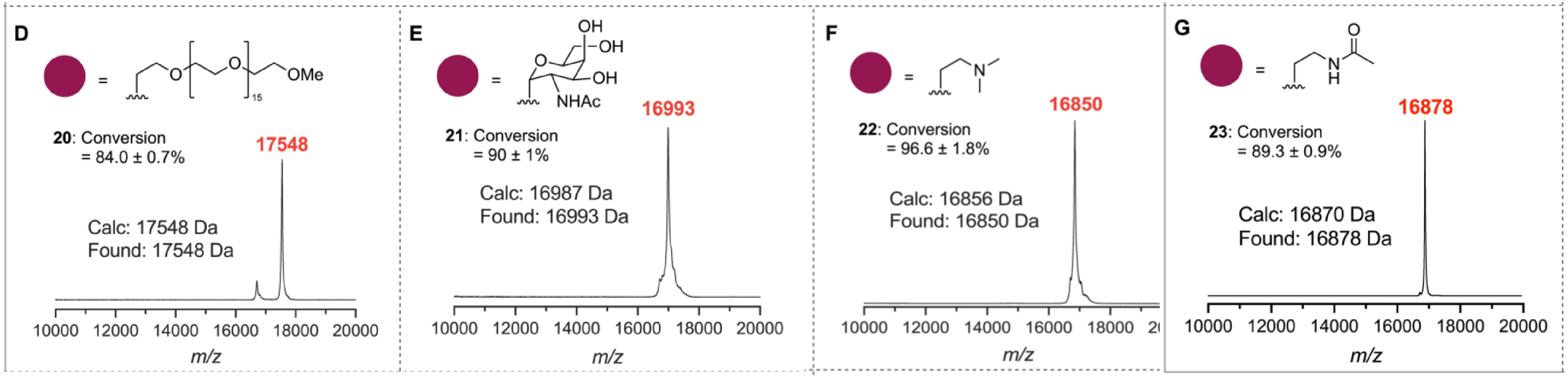
# Explanations for the Inability to Dimerize



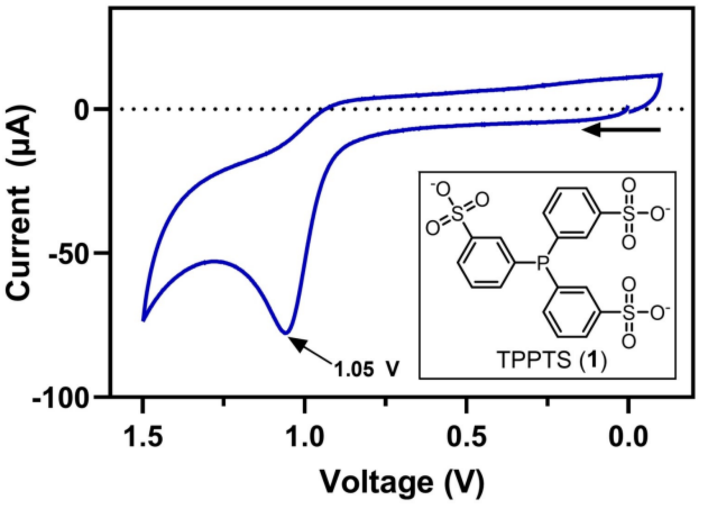



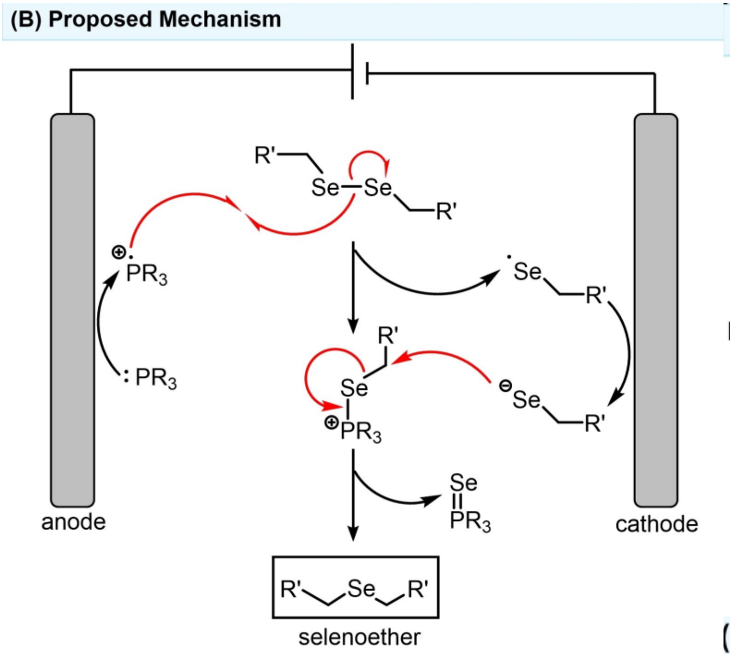
# Binary Contraction for Selenide Etherifications

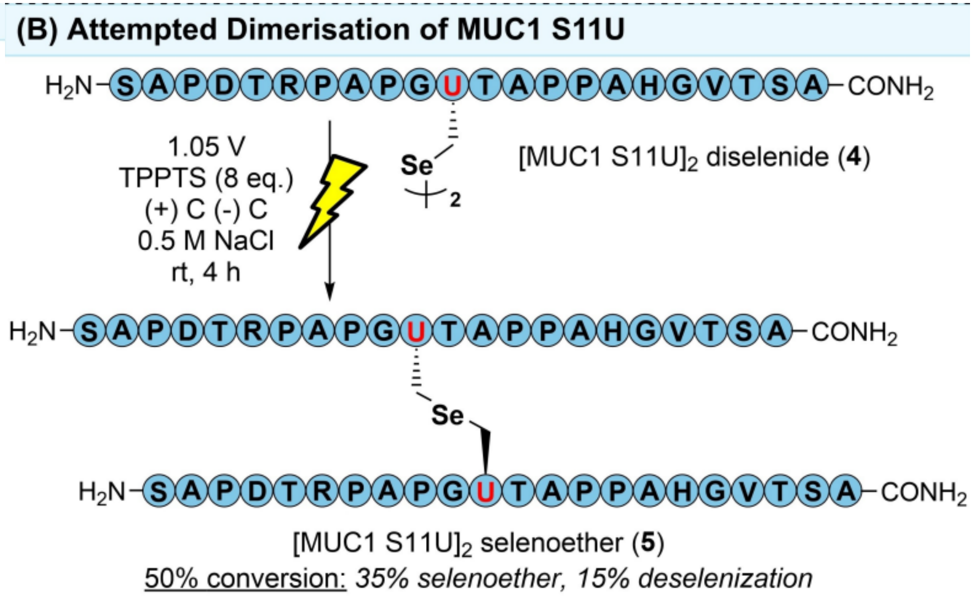


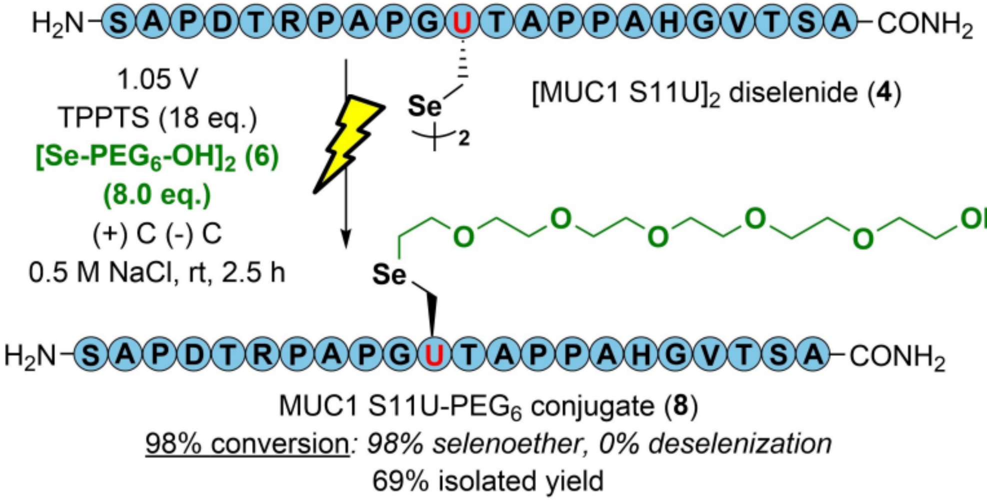



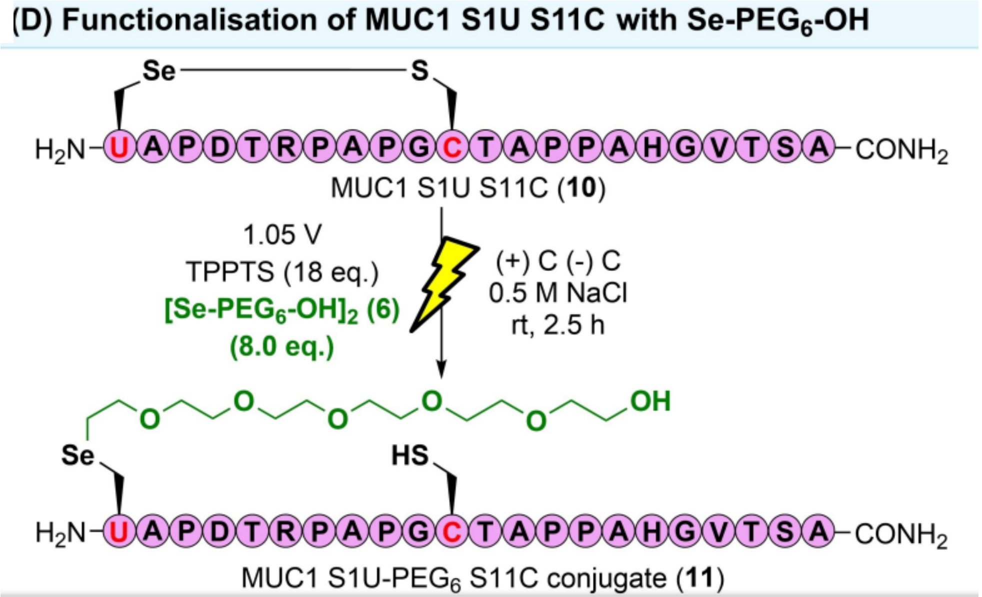
# Analogous Electrochemical Reaction

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# Summary II: Post-translational Modifications for Sec

- **Conjugative Sec Probes**

  BESThio (at pH = 5.8); Sel-green at neutral pH and live cell imaging

  Masked nitrile oxides: diacylfuroxans and ML210 (improved selectivity)

- **Sec Arylation**

  Cu-catalyzed coupling with boric acids

  Direct electrophilic substitutions (aromatics, alkynes)

  Reactions with phenyl radical generated from hydrazines

- **Deselenation**

  Phosphine-induced $C_\beta$-Se bond homolysis

  Photochemical / electrochemical deselenative contractions