Synthesis and Applications of Fluorinated α-Amino Acids

Overview and Recent Advances

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MacMillan Group Meeting
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11/15/2017

Outlines

- **Introduction**
  - Interest of artificial amino acids
  - Unique properties of the C-F bond
  - Natural fluorinated amino acid

- **Applications of fluorinated α-amino acids**
  - Protein engineering
  - Medicinal chemistry
  - ^{19}F-NMR
  - ^{18}F-PET

- **Incorporation of fluorinated α-amino acids**

- **Synthesis of fluorinated α-amino acids**
  - Strategy I
  - Strategy II
  - Strategy III
  - Strategy IV
  - Strategy V
  - Strategy VI

Introduction

Interest of Artificial α-Amino Acids

- Peptides and proteins occupy innumerous biological functions
  - Metabolic
  - Structural
- Serve as inspiration in many research fields
  - New therapeutic agents
  - Biomaterials
- Appealing features
  - Low toxicity
  - Structural simplicity
  - Low immune response
- Intrinsic limitations
  - Enzymatic degradation (protease)
  - Thermic stability
  - Denaturation in organic solvents
- The use of non-canonical amino acids may overcome some of those limitations
  - D-α-amino acids
  - β-amino acids
  - **Artificial amino acids**
- Artificial amino acids expand the functional diversity and open the way to tailored protein design

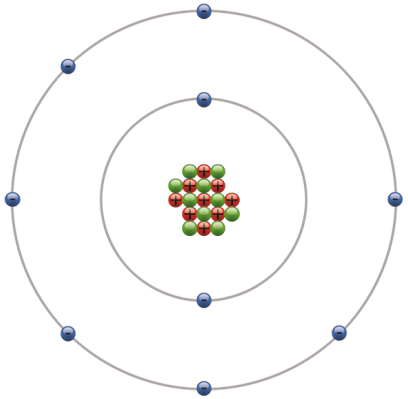
C-H → C-F Bond Substitution

- **Minor steric impact**
  F: smallest Van der Waals radius after H and Ne

- **Major electronic impact**
  F: most electronegative element
  Inverted polarization vs C-H

- **Unique features of the C-F bond**
  Often described as the strongest organic bond (BDE up to 544 KJ/mol)
  Most polar covalent bond
  Can influence polarization on adjacent bonds
  Low polarizability of C-F can give rise to “Fluorous effect”

- **Fluorous effect**
  Auto-segregation of fluorine-rich organic species
  Intermolecular: fluorous phase
  Intermolecular: fluorophile domain on protein



Can those unique features be explored in peptide/protein engineering ?

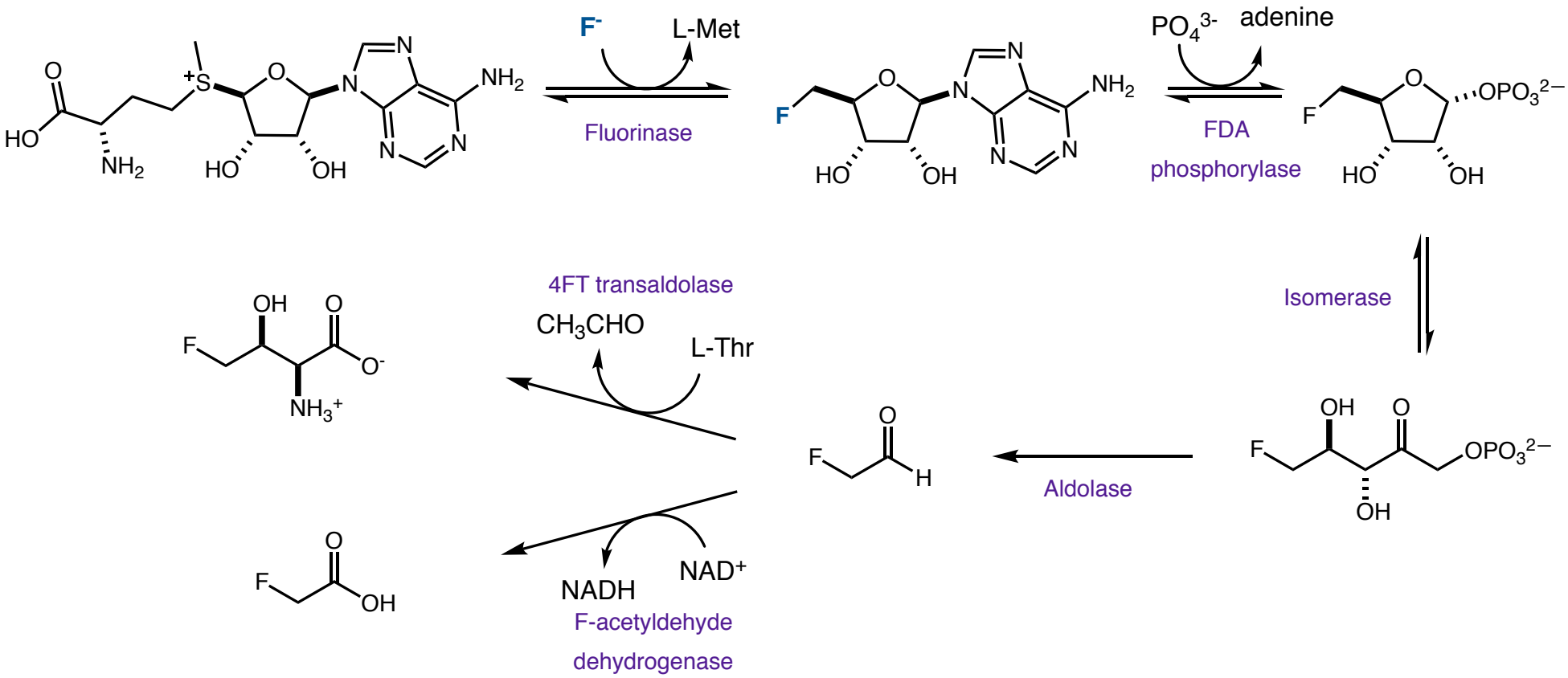
Fluorinated α-amino acids (α-AA$_F$)

Buer, B. C.; Marsh, E. N. G. *Protein Sci.* **2012**, *21*, 453.
Cametti, M.; Crousse, B.; Metrangolo, P.; Milani, R.; Resnati, G. *Chem. Soc. Rev.* **2012**, *41*, 31.

Natural Fluorinated α-Amino Acid

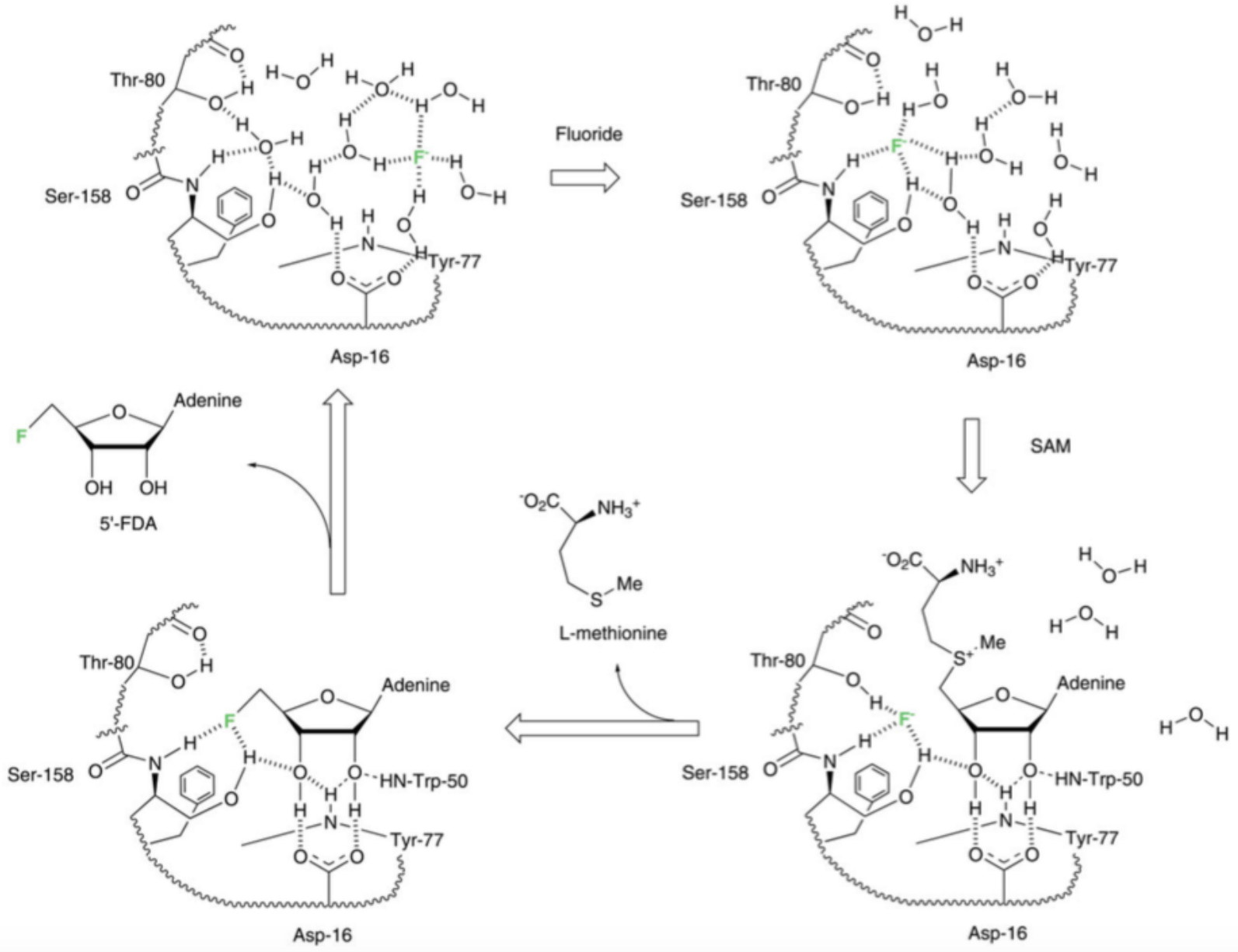
- 4-Fluoro-L-threonine (4F-Thr): only natural α-AA_F known
  - Produced by *Streptomyces cattleya* (Gram-positive)
  - Antibiotic agent
  - Not identified in any peptide or protein so far

- Biosynthesis involves incorporation of fluoride by a fluorinase enzyme



Odar, C.; Winkler, M.; Wiltschi, B. *Biotechnol. J.* **2015**, *10*, 427.

Incorporation of Inorganic Fluoride by Fluorinase Enzyme



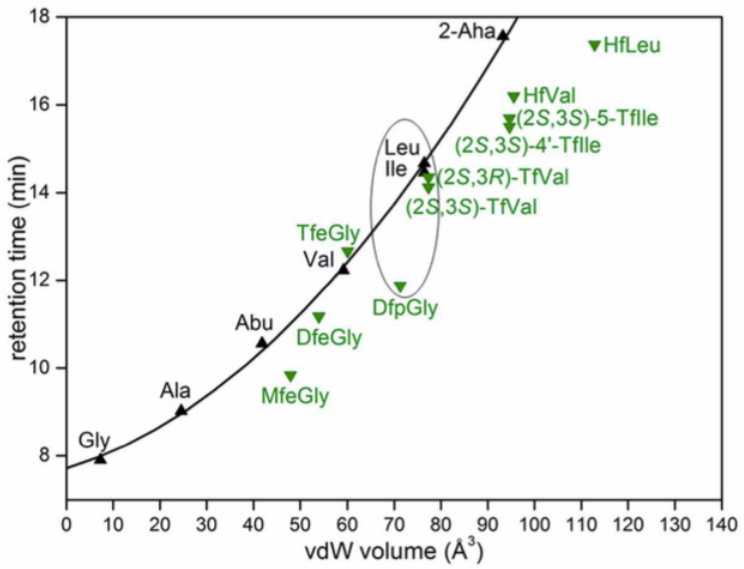
Odar, C.; Winkler, M.; Wiltschi, B. *Biotechnol. J.* **2015**, *10*, 427.

Application of α-AA$_F$

Protein Engineering: General Considerations

- Many properties can be influenced by the substitution of canonical α-amino acids by fluorinated analogues

  Protein folding
  Protein-protein interactions
  Ribosomal translation
  Lipophilicity
  Acidity/basicity character
  Optimal pH
  Stability: temperature, protease, organic solvents



- Example: lipophilicity vs.VdW volume of side chains

  a-AA$_F$ can fill a desired space but with a different lipophilicity from α-AA

- Often claimed to generally improve stability and properties in out-of-field literature

- But, not that simple !

« Fluorine has been shown to impart often favourable but seldom predictable properties to peptides and proteins »

-Beate Koksch (2017)-

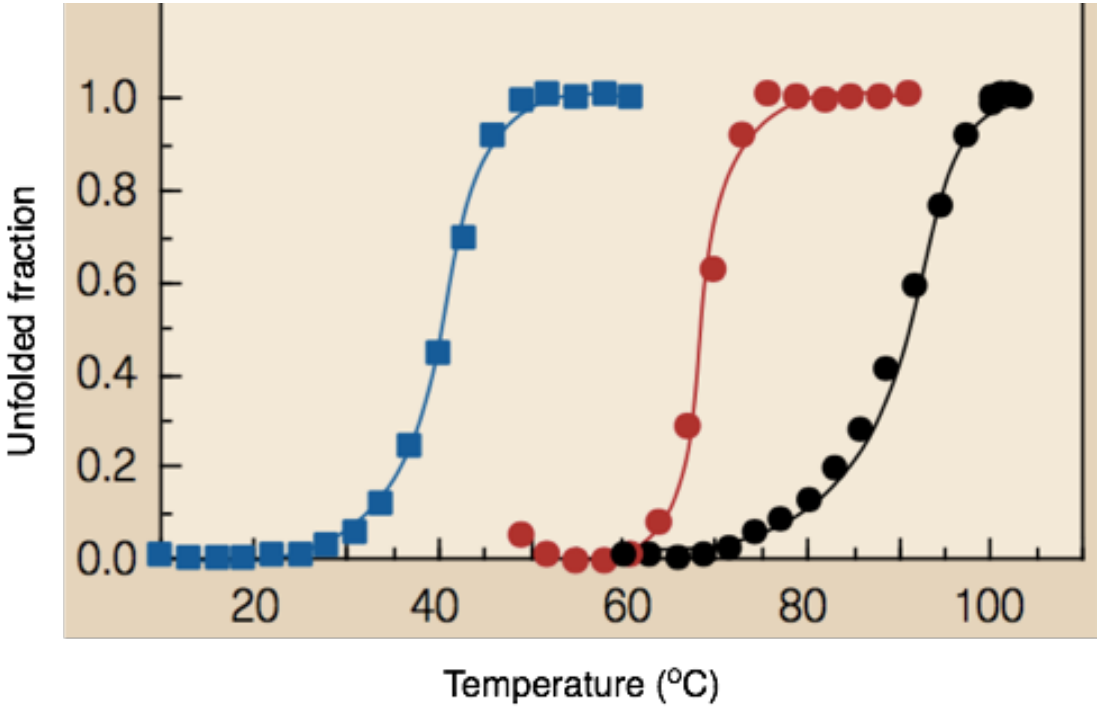
Merkel, L.; Schauer, M.; Antranikian, G.; Budisa, N. *ChemBioChem* **2010**, *11*, 1505.

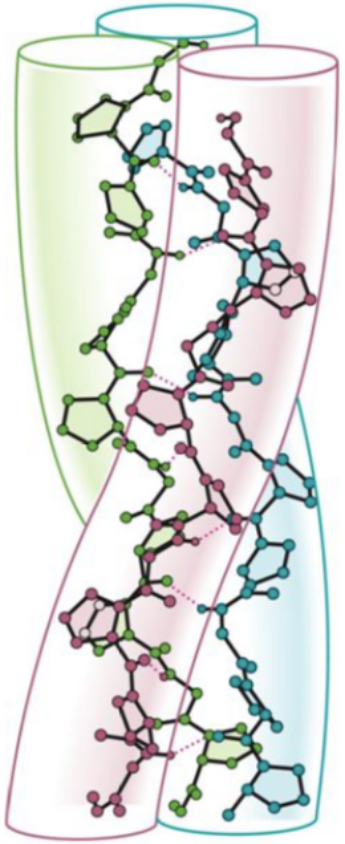
Berger, A. A.; Völler, J.-S.; Budisa, N.; Koksch, B. *Acc. Chem. Res.* **2017**, *50*, 2093.

Applications of α-AA_F

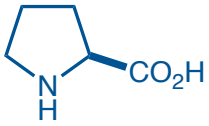
Protein Engineering

- Classic example: stabilization of the folded triple helix structure of collagen at high temperature

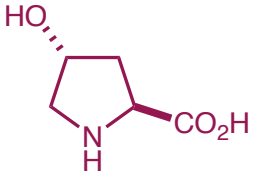




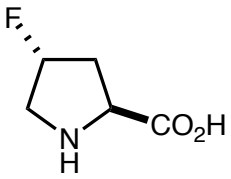
(ProProGly)$_{10}$
(ProHypGly)$_{10}$
(ProFlpGly)$_{10}$



Pro



Hyp



Flp

Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666.

Applications of α-AA$_F$

Medicinal Chemistry of Peptides

- **Interest for peptides in medicinal chemistry**
  - Low toxicity
  - Simple structure / synthesis
  - Low immune response

- **Drawbacks of peptides in medicinal chemistry**
  - Biodisponibility
  - Low membrane permeability
  - Enzymatic cleavage

Can α-AA$_F$ help to overpass those limitations ?

- **Impact of fluorine in medicinal chemistry**
  - 150 fluorinated small molecules reaching the market since 1950
  - In 2010, 20% of all administered drugs
  - **30% by 2017**

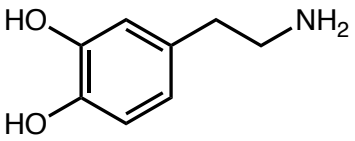
Can this impact be translated to peptides ?

Berger, A. A.; Völler, J.-S.; Budisa, N.; Koksch, B. *Acc. Chem. Res.* **2017**, *50*, 2093.

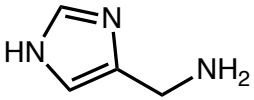
Applications of α-AA$_F$

Inhibitors

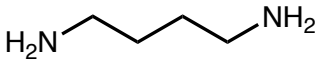
- Because of their resemblance with canonical a-amino acids, some α-AA$_F$ can act as inhibitors
  - Known action on amino acid decarboxylase enzymes
  - Inhibition of the production of biologically relevant amines, including:



Dopamine

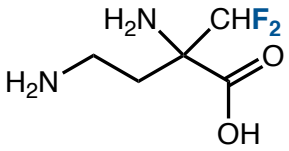


Histamine



Putrescine

- α-(difluoromethyl)ornithine (DMDO)
  - Commercial name: Eflornithine
  - Treatment of hirsutism and African trypanosomiasis (sleeping sickness)
  - Inhibitor or ornithine decarboxylase
  - Acts on putrescine biosynthesis



DMDO

Kollonitsch, J.; et al. *Nature* **1978**, *274*, 906.

Seki, M.; Suzuki, M.; Matsumoto, K. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1024.

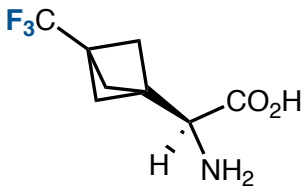
Applications of α-AA$_F$

^{19}F-NMR Spectroscopy

- Widely used non-native NMR-active nucleus for interpretation of biological structure and functions

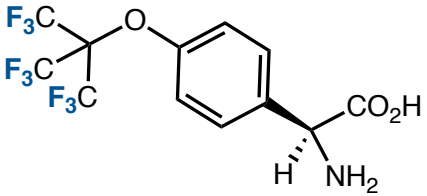
- Many advantages
  - No marking necessary
  - No background in biological systems
  - Sensible
  - Can be used at biorelevant concentrations

- Some α-AA$_F$ specifically designed as NMR probes



CF_3-Bpg

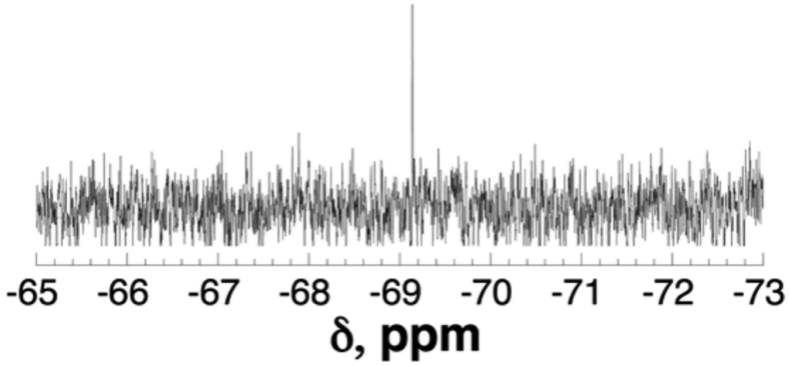
Ulrich



Tyr(C_4H_9)

Zondlo

^{19}F-NMR spectra of a peptide bearing a Tyr(C_4H_9) at 500nM



Salwiczek, M.; Mikhailiuk, P. K.; Afonin, S.; Komarov, I. V.; Ulrich, A. S.; Koksch, B. *Amino Acids* **2010**, *39*, 1589.

Tressler, C. M.; Zondlo, N. J. *Org. Lett.* **2016**, *18*, 6240.

Applications of α-AAF

^{18}F-Positron Emission Tomography (^{18}F-PET)

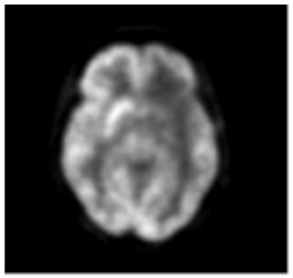
- $_{18}$F : $t_{1/2}$ = 109.77 min

- $_{18}$F-PET mainly used in oncology

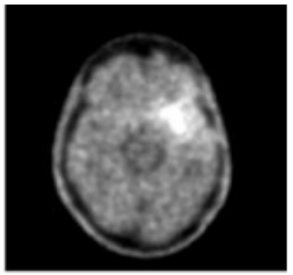
- Main marker used: $_{18}$F-FDG

  ($_{18}$F)-fluorodeoxyglucose

  Discrimination of cancer cells due to their higher metabolism



FDG



F-TYR

- Consumption of α-amino acids also higher in cancer cells

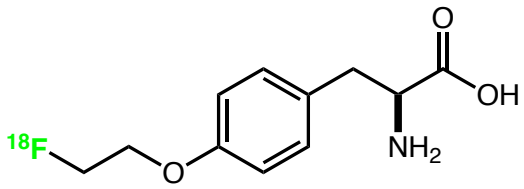
  α-AAF explored as new markers

  Better resolution in some cases

- α-AA$_F$ could also be used for more refine analysis

  Marking based on active transport events

  Transport protein LAT1 upregulated in cancer cells



[^{18}F]-F-TYR

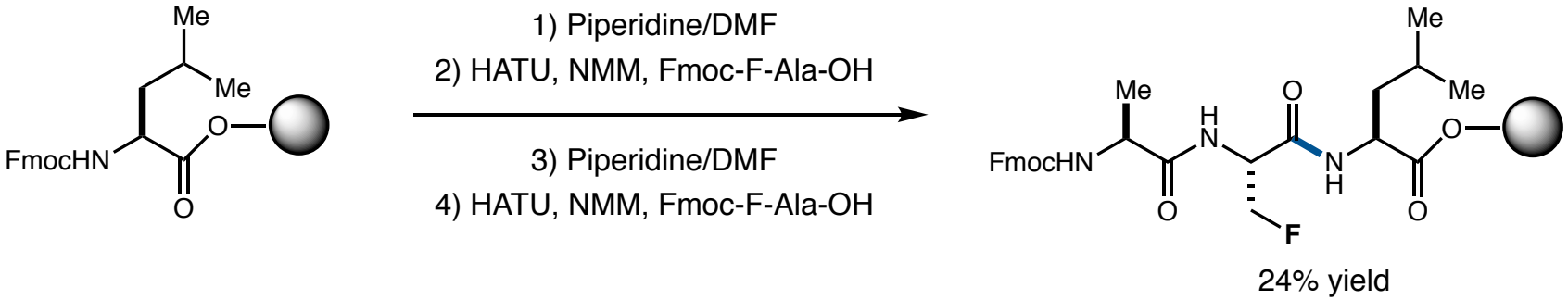
Kotzerke, J.; et al. *Eur. J. Nucl. Med. Mol. Imag.* **2003**, *30*, 1004.

Nodwell, M. B.; Britton, R.; et al. *J. Am. Chem. Soc.* **2017**, *139*, 3595.

Incorporation of Fluorinated α-Amino Acids in Peptides and Proteins

Synthetic Incorporation with Solid-Phase Peptide Synthesis (SPSS)

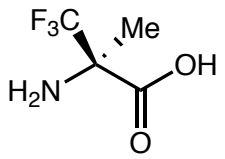
■ A lot of α-AA_F can be used in the context of SPSS



24% yield

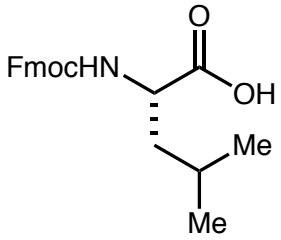
■ Coupling in solution can overpass compatibility issues with problematic α-AA_F

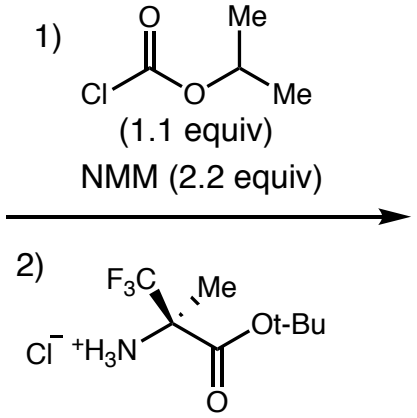
(*R*)-α-Tfm-Ala-OH

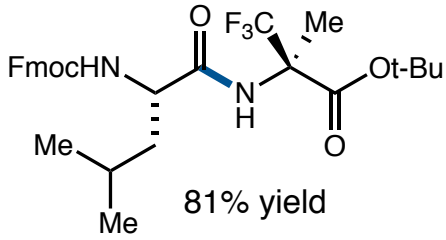


Poorly Nu- amine

Not suitable for standard SPPS







81% yield

Protected dipeptide

Suitable for SPPS

Carpentier, C.; Godbout, R.; Otis, F.; Voyer, N. *Tetrahedron Lett.* **2015**, *56*, 1244.

Devillers, E.; Pytkowicz, J.; Chelain, E.; Brigaud, T. *Amino Acids* **2016**, *48*, 1457.

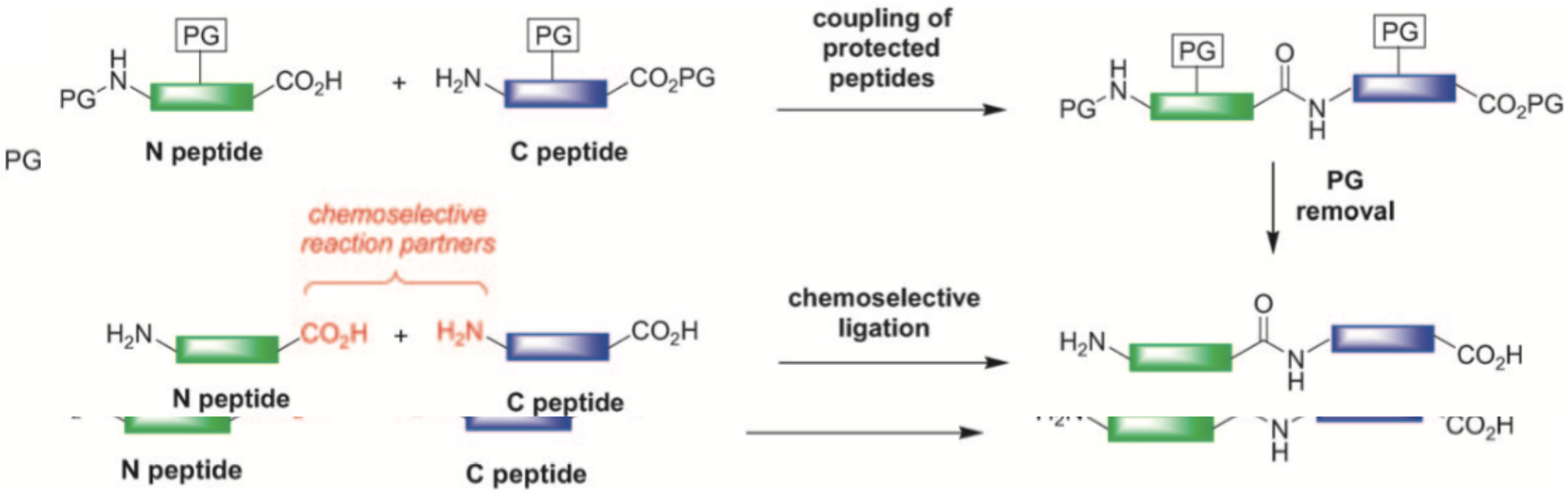
Incorporation of Fluorinated α-Amino Acids in Peptides and Proteins

Synthetic Incorporation Into Larger Peptide/Proteins

- Limitations of SPSS

Compatibility issues with some α-AA$_F$
Specific protecting groups required
Not suitable for more than 50-100 α-AA

- Coupling of fragments in solution may be used for longer sequences



Hackenberger, C. P. R.; Schwarzer, D. *Angew. Chem., Int. Ed.* **2008**, *47*, 10030.

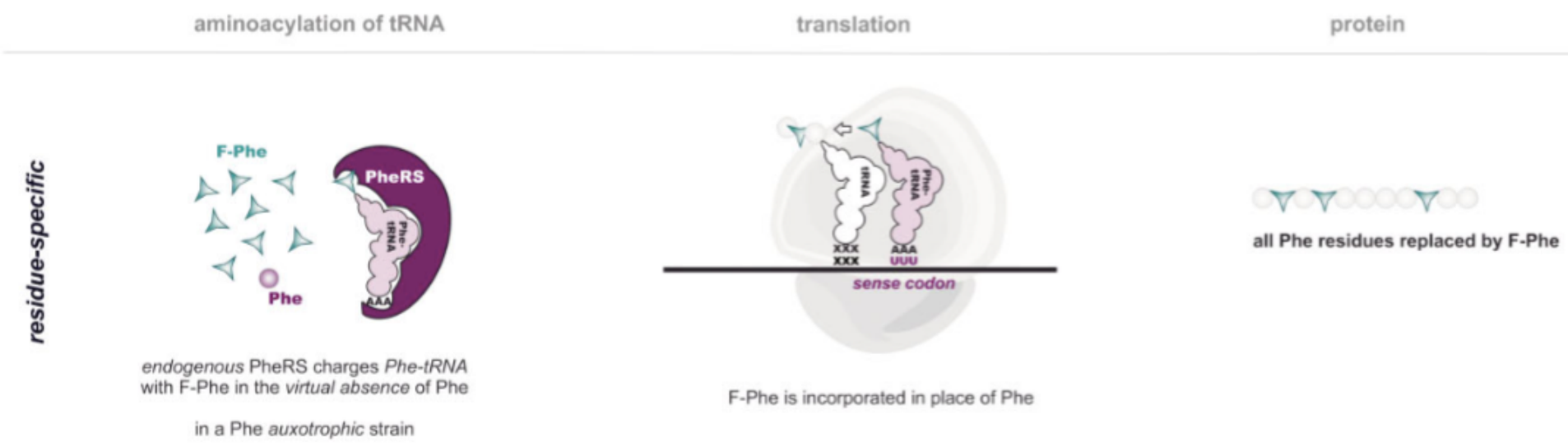
Incorporation of Fluorinated α-Amino Acids Into Peptides and Proteins

In Vivo Ribosomal Translation of Non-Canonical α-Amino Acids

■ Residue specific

Relies on native translational machinery of the cell
Incorporates the modified α-AA at every position occupied by the parental α-AA

Needs strains of bacteria that are auxotrophic for the parental α-AA
α-AA$_F$ has to be sufficiently similar to its natural analog
Particularly suitable for monoofluorated α-AA$_F$



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Incorporation of Fluorinated α-Amino Acids Into Peptides and Proteins

In Vivo Ribosomal Translation of Non-Canonical α-Amino Acids

■ Site specific

Nonsense suppression: associate one of the three stop codons with an artificial α-AA

Design of an appropriately engineered pair: **aminoacyl *t*RNA synthetase (aaRS)** / **Suppressor *t*RNA**

Corresponding sequence needs to be implemented in the genetic code in order to express the desired protein

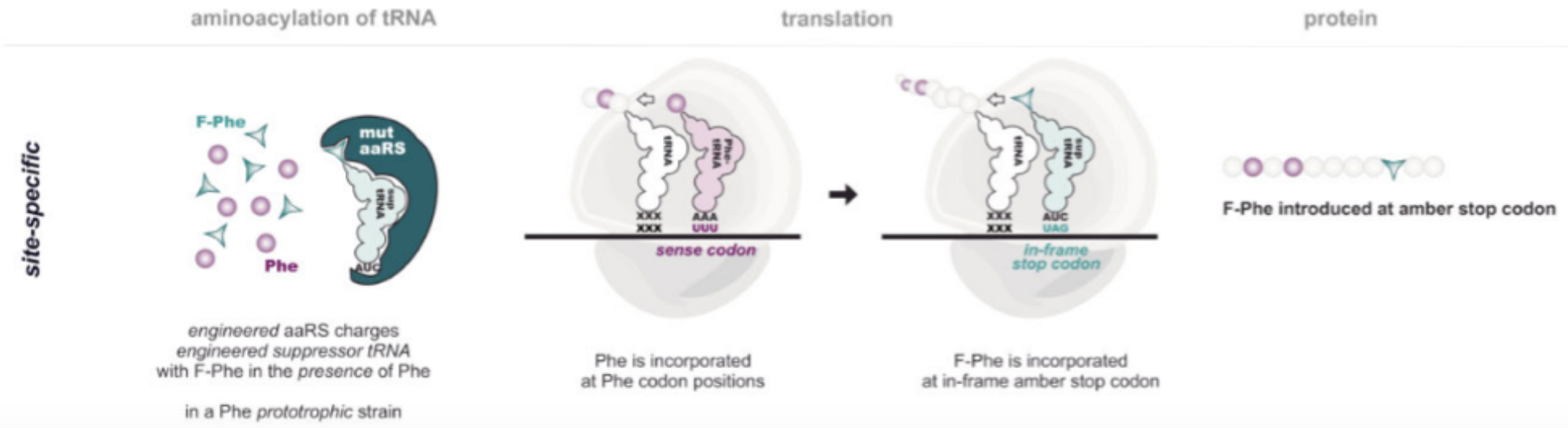
Suppressor *t*RNA

Recognizes stop codon (via anti-codon)

Incorporates artificial α-AA in the protein

Mutant aaRS

Brings together artificial α-AA and suppressor *t*RNA



Odar, C.; Winkler, M.; Wiltschi, B. *Biotechnol. J.* **2015**, *10*, 427.

Biava, H.; Budisa, N. *Engineering in Life Sciences* **2014**, *14*, 340.

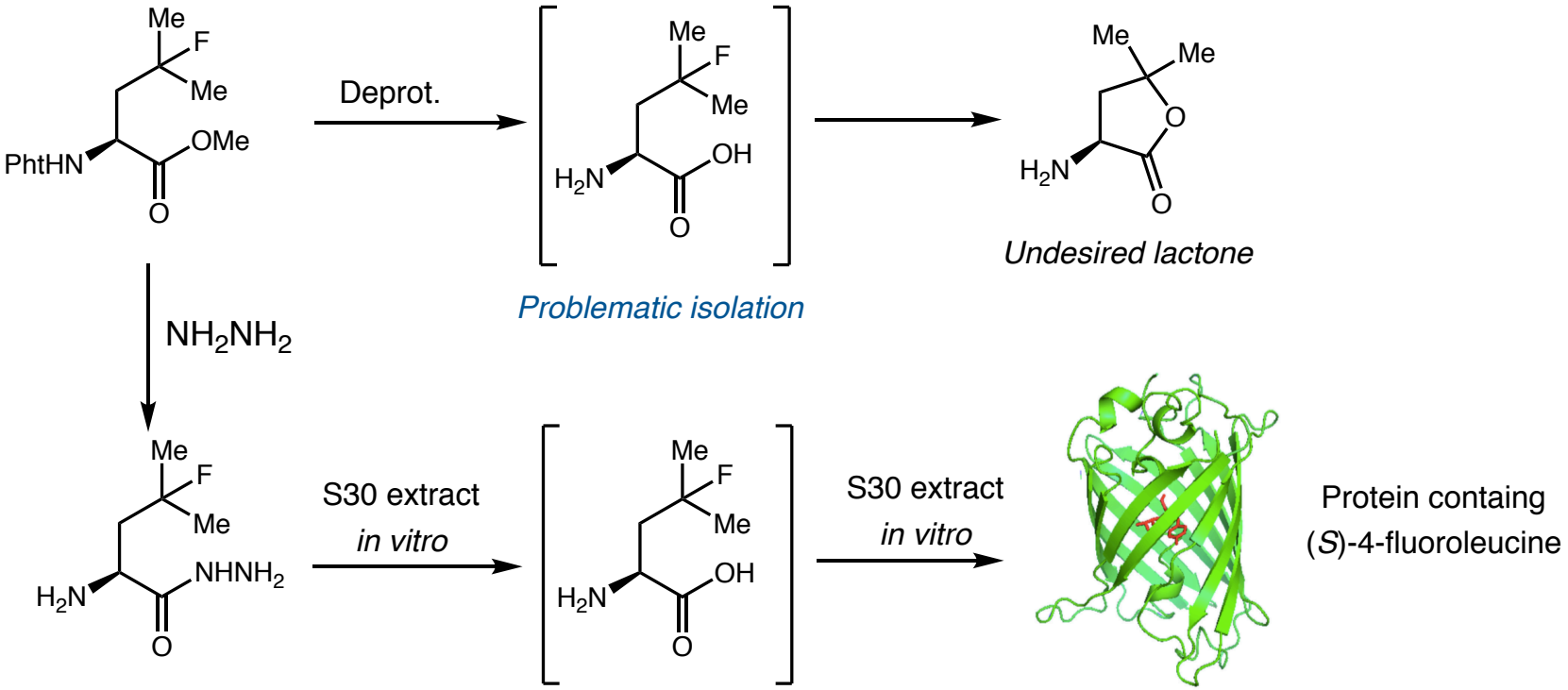
Incorporation of Fluorinated α-Amino Acids Into Peptides and Proteins

Cell-Free Ribosomal Translation of Non-Canonical α-Amino Acids

- Recently developed as *in vitro* alternatives to the *in vivo* approaches

Rely on the same translational machinery
Need prior extraction and partial purification of cellular material
More tunable systems

- Other enzymes can be added to promote other transformations



Easton, C. J.; et al. *Chem.–Eur. J.* **2013**, *19*, 6824.

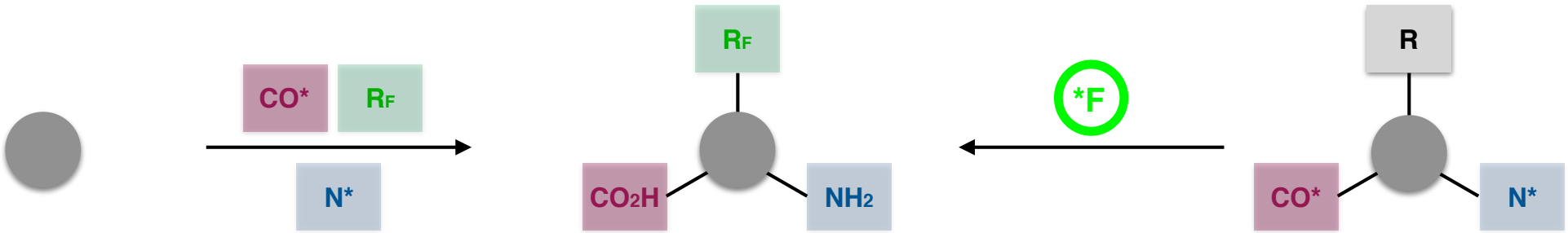
Synthesis of α-AA$_F$: Proposed Classification

Approach A
Installation of functionalities

(Strategies 1-5)

Approach B
Fluorination of side chain

(Strategy 6)

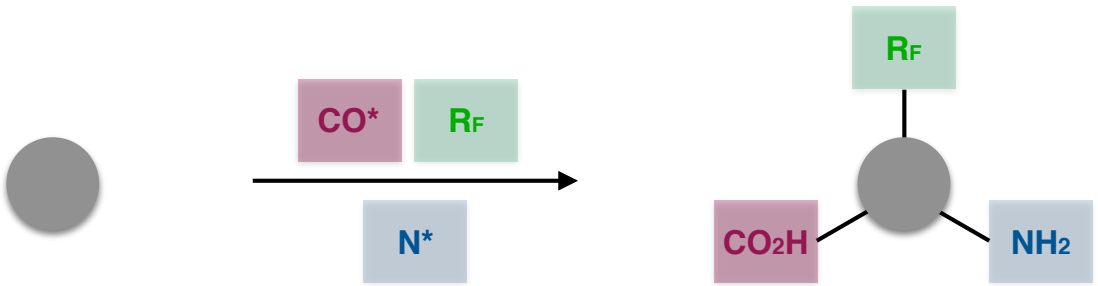


N*	Amine or *nitrogen function*	R_F	Fluorinated side chain	*F	Fluorinating agent
CO*	Carbox. acid or oxygenated function	R	Non fluorinated side chain		Amino acid Cα

Synthesis of α-AA$_F$: Proposed Classification

Approach A
Installation of functionalities

(Strategies 1-5)



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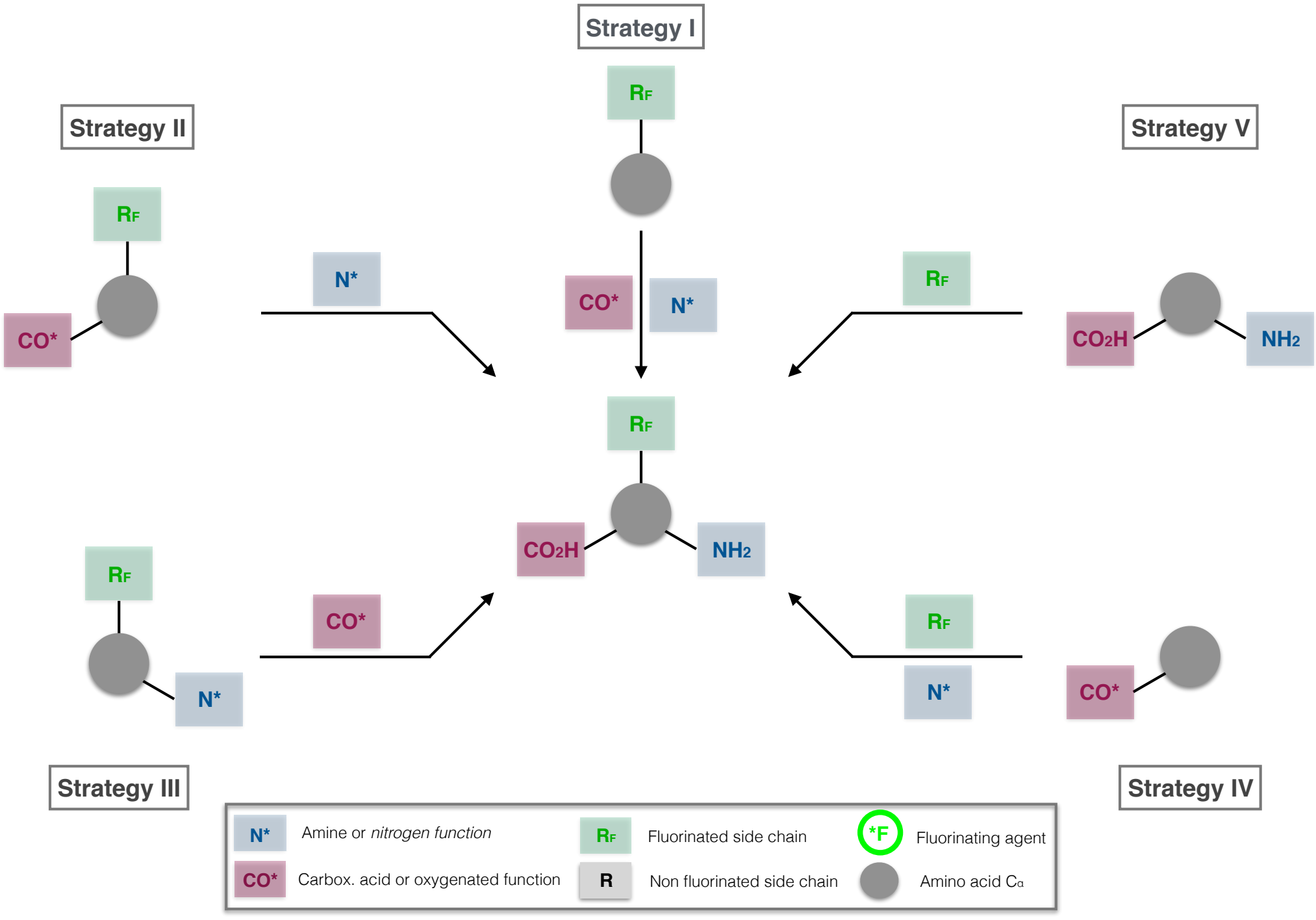
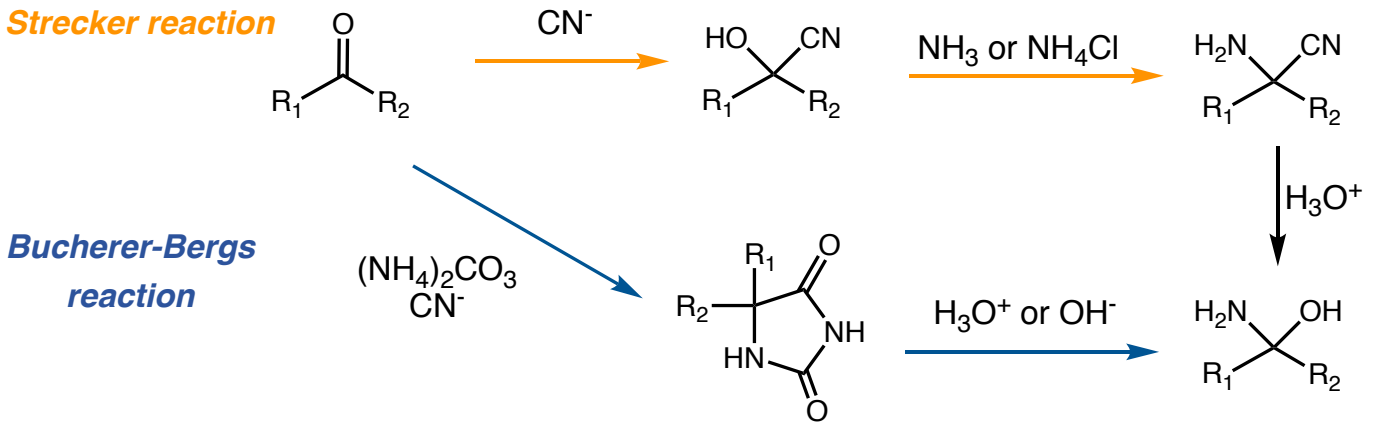
Synthesis of α-AA$_F$: Proposed Classification [Approach A]



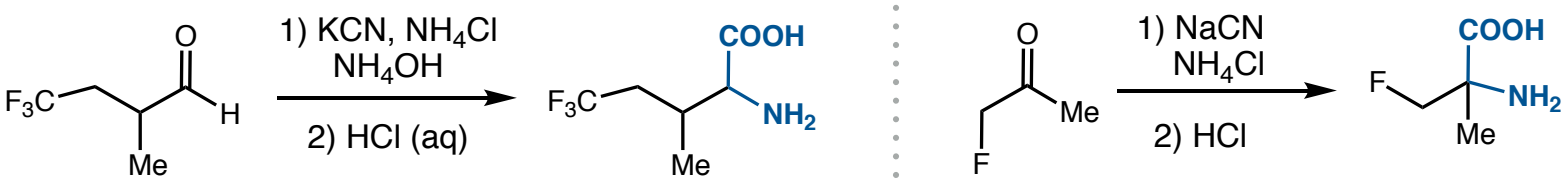
Strategy I

Methodologies from Classic α-Amino Acid Chemistry

- Installation of carboxylic acid and amine functionalities on a carbonyl precursor



- Examples in the synthesis of α-AAF**:** Strecker reaction



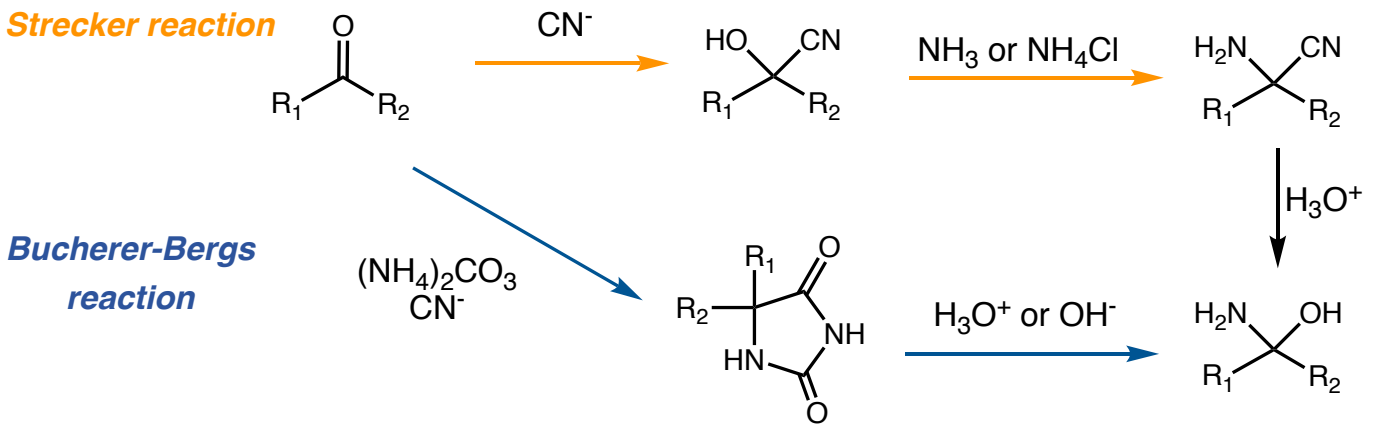
Bergmann, E. D.; Shani, A. *J. Chem. Soc.* **1963**, 3462.

Muller, N. *J. Fluorine Chem.* **1987**, *36*, 163.

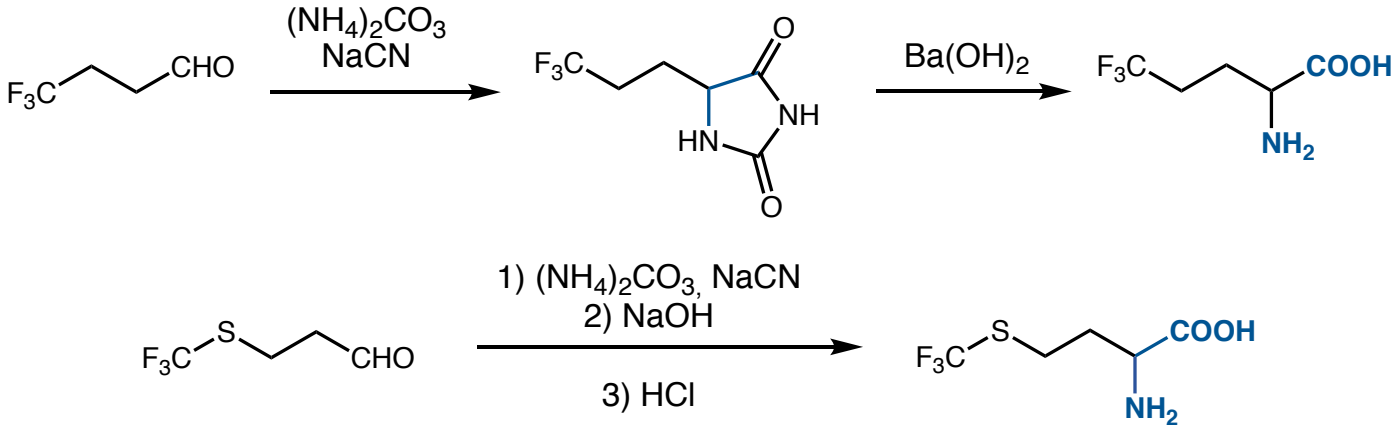
Strategy I

Methodologies from Classic α-Amino Acid Chemistry

- Installation of carboxylic acid and amine functionalities on a carbonyl precursor



- Examples in the synthesis of α-AA_F: Bucherer-Bergs reaction

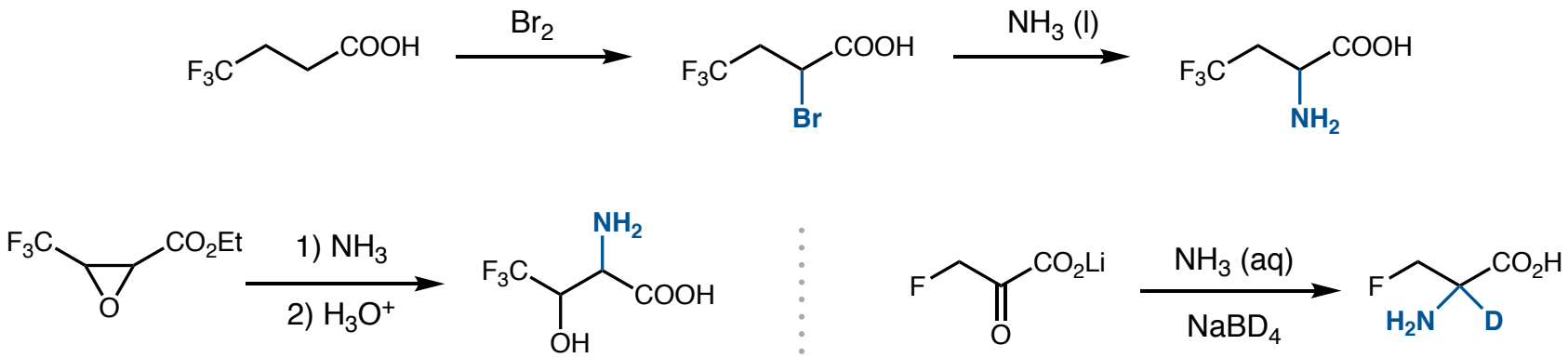


Tolman, V. *Amino Acids* **1996**, *11*, 15.

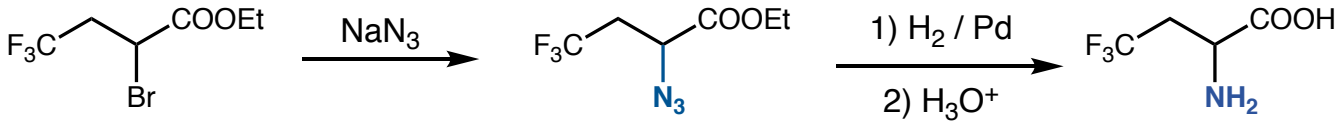
Strategy II

Classic Amination Reactions

Direct amination with ammonia



Azide as nucleophilic aminating agent



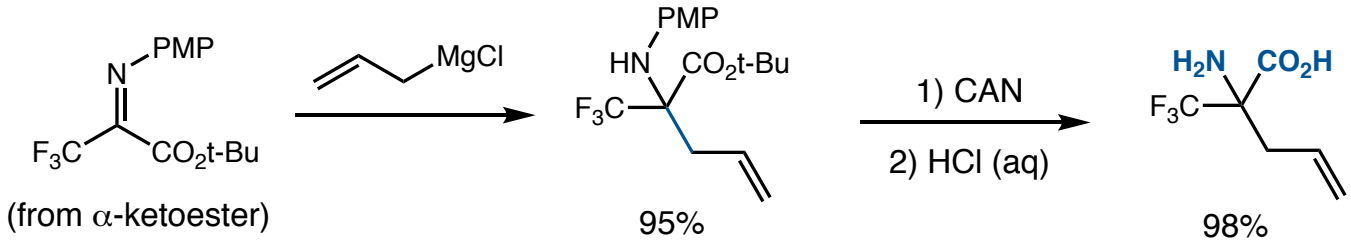
Tolman, V. *Amino Acids* **1996**, *11*, 15.

Walborsky, H. M.; Baum, M. E. *J. Org. Chem.* **1956**, *21*, 538.

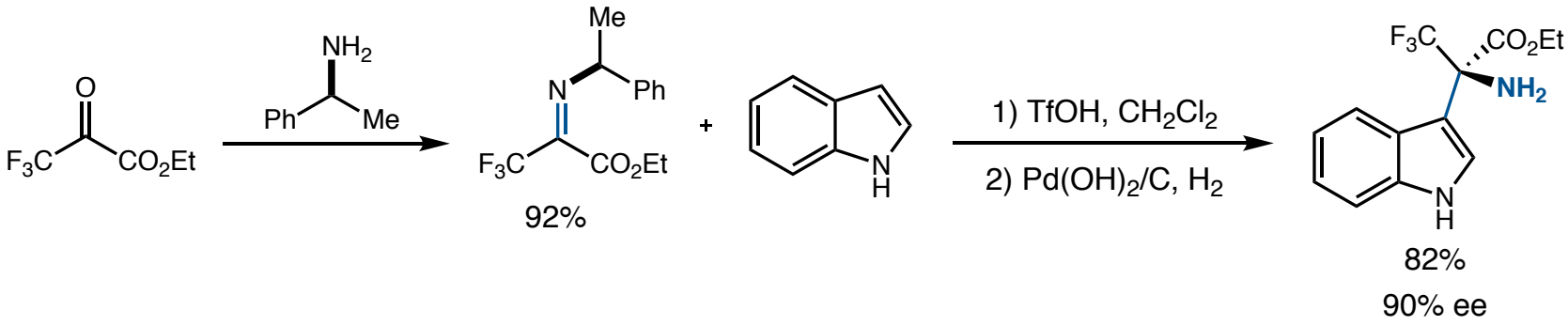
Strategy II

Via the Formation of an Imine

- Imines can be reduced by organomettallic reagents



- Chiral imines can influence stereoselectivity on following reaction at Cα



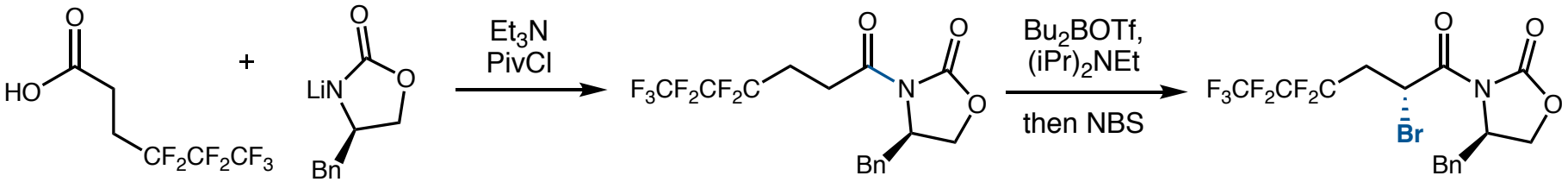
Uneyama, K.; Katagiri, T.; Amii, H. *J. Synth. Org. Chem. Jpn* **2002**, *60*, 1069.

Abid, M.; Teixeira, L.; Torok, B. *Org. Lett.* **2008**, *10*, 933.

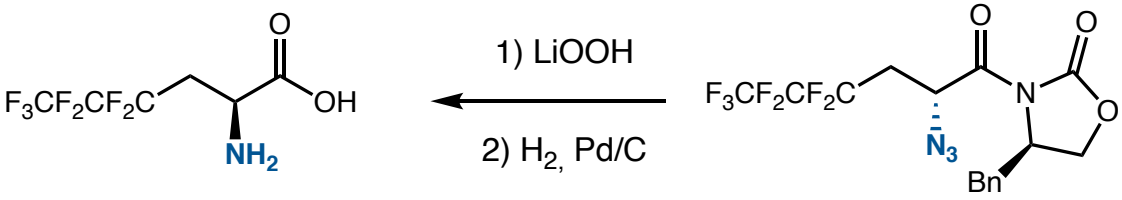
Strategy II

Stereoselective Amination

- Amination directed by Evans auxilairy



TMGA



3 other α-AA_F prepared

77-97% yield

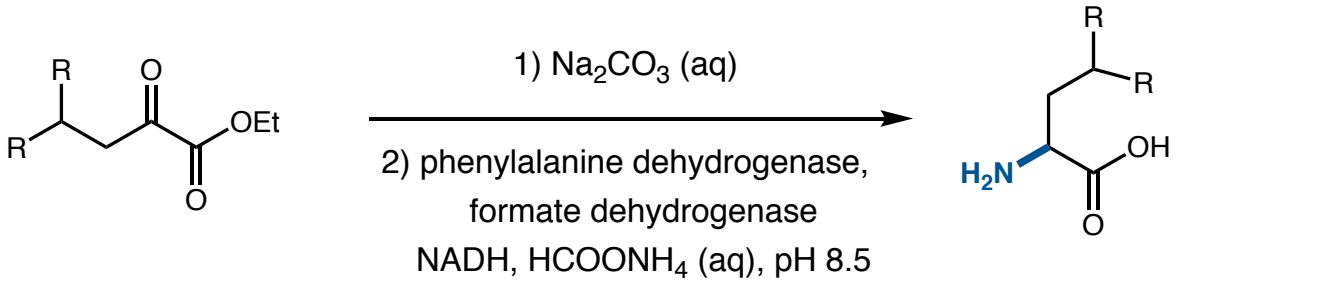
> 98:2 dr

Larsson, U.; Carlson, R.; Leroy, J. *Acta Chem. Scand.* **1993**, *47*, 380.

Strategy II

Obtention of Single Stereoisomer with Biocatalysts

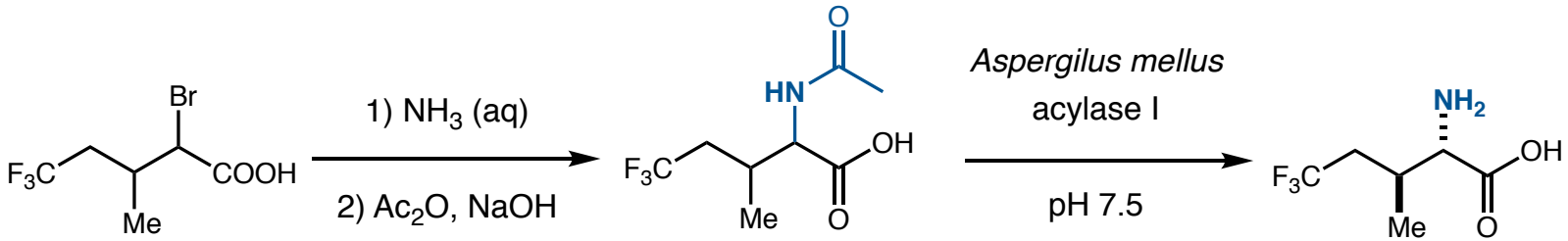
■ Ezymatic transamination



39%, 99% ee (R = CF_3)

46%, 98% ee (R = CF_2H)

■ Kinetic resolution



48%, 2 steps

29%

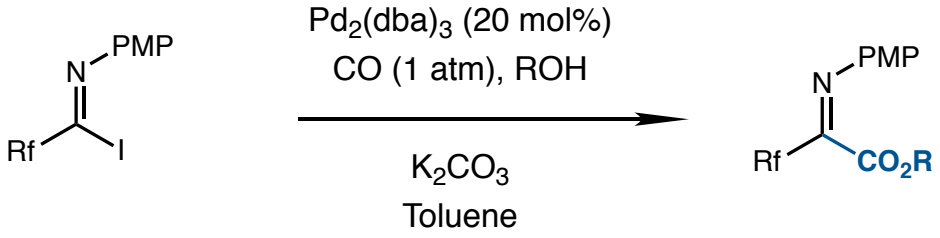
Chiu, H. P.; Cheng, R. P. *Org. Lett.* **2007**, *9*, 5517.

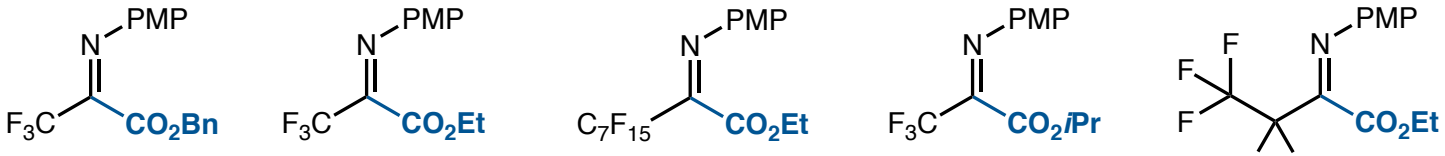
Biava, H.; Budisa, N. *J. Fluorine Chem.* **2013**, *156*, 372.

Strategy III

Palladium-Catalyzed Alkoxycarbonylation

- Installation of an ester on an imidoyl halide





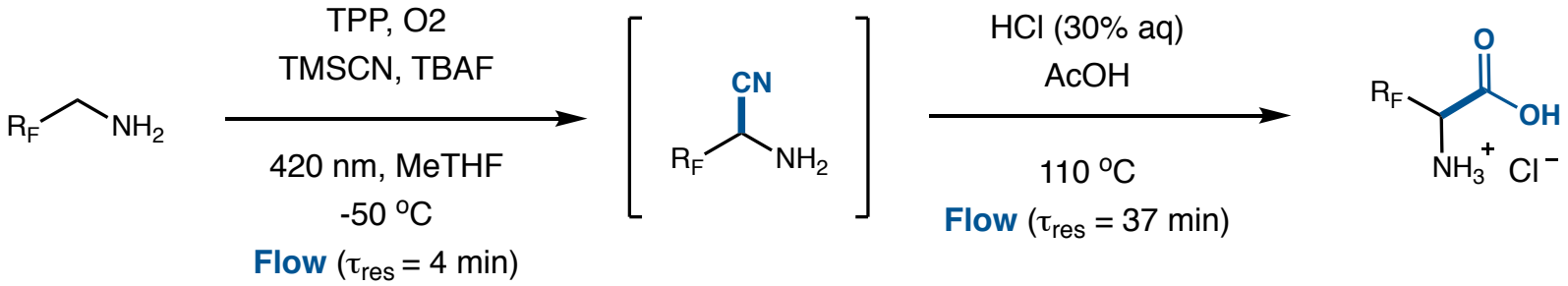
Biava, H.; Budisa, N. *W*atanabe, H.; Hashizume, Y.; Uneyama, K. *Tetrahedron Lett.* **1992**, *33*, 4333.

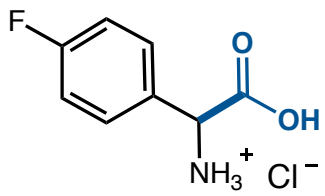
*A*mii, H.; Kishikawa, Y.; Kageyama, K.; Uneyama, K. *J. Org. Chem.* **2000**, *65*, 3404.

Strategy III

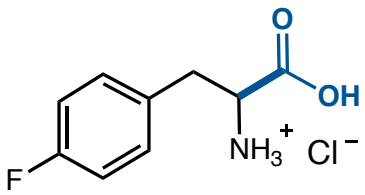
Photooxidative Cyanation

- Formation and hydrolysis of an α-aminonitrile in a semi-continuous process

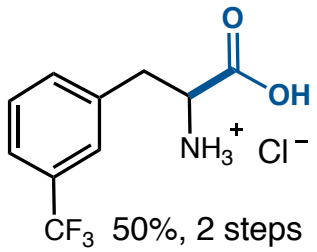




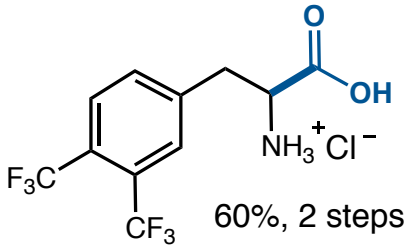
64%, 2 steps



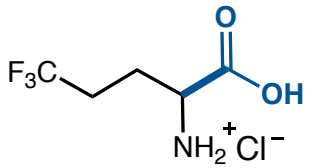
67%, 2 steps



50%, 2 steps



60%, 2 steps



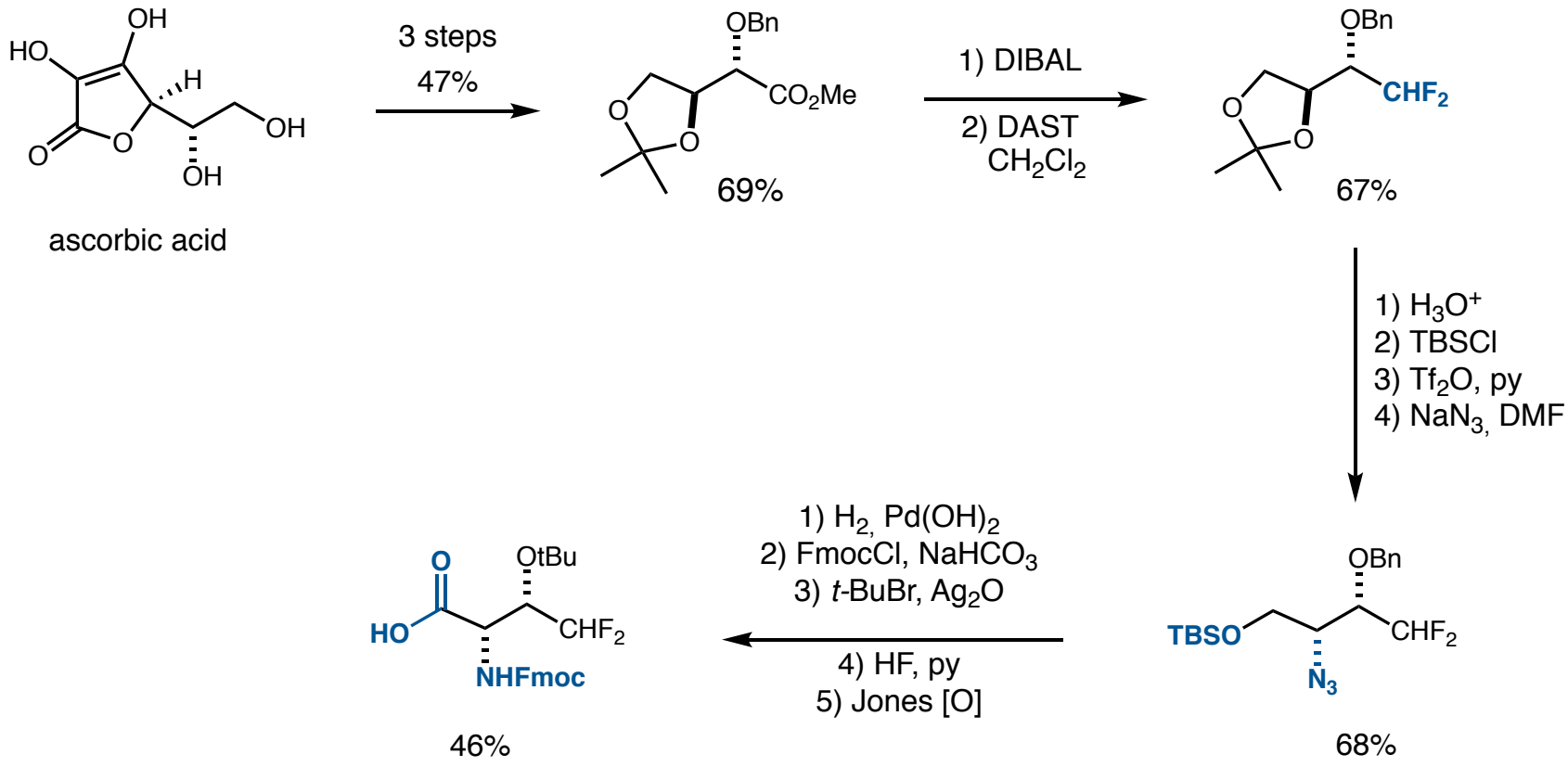
63%, 2 steps

Vukelić, S.; Ushakov, D. B.; Gilmore, K.; Koksch, B.; Seeberger, P. H. *Eur. J. Org. Chem.* **2015**, *2015*, 3036.

Strategy IV

Deoxofluoration Followed by Amination

- Preparation of protected γ-difluorothreonine from ascorbic acid

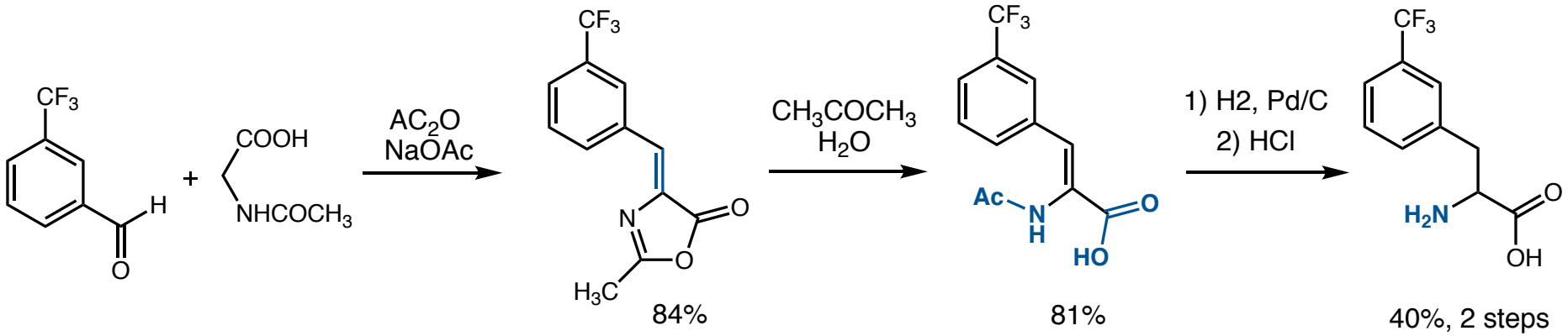


Li, G. Y.; van der Donk, W. A. *Org. Lett.* **2007**, *9*, 41.

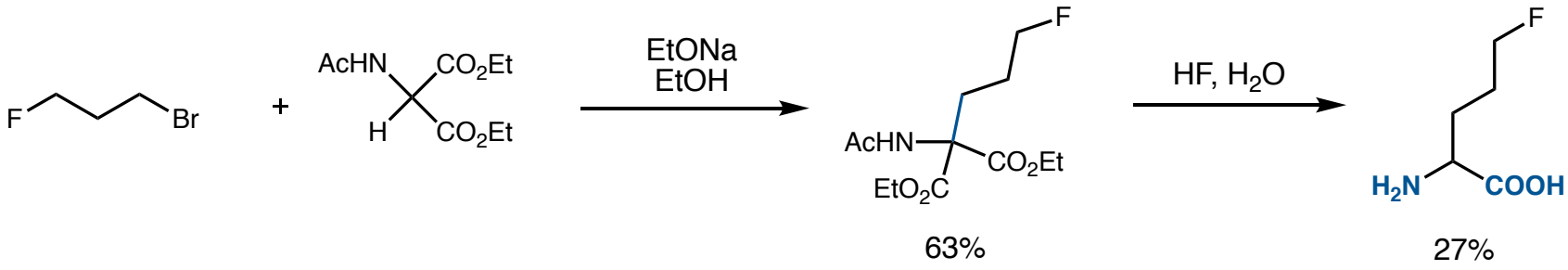
Strategy V

Exploiting Basicity of Cα on the Glycine Moiety

- Erlenmeyer synthesis (azlactone) - First α-AAF synthesis (1932)



- S_N2 reaction with an alkyl halide



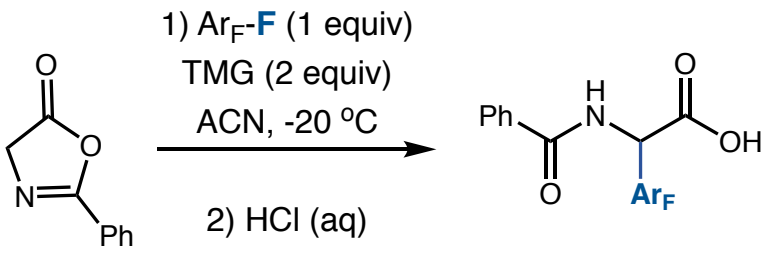
Raasch, M. S. *J. Org. Chem.* **1958**, *23*, 1567.

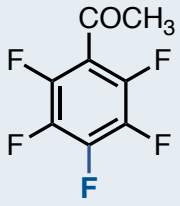
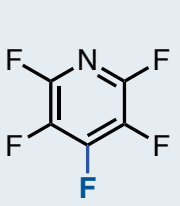
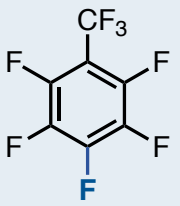
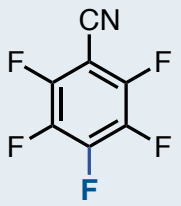
Tolman, V. *Amino Acids* **1996**, *11*, 15.

Strategy V

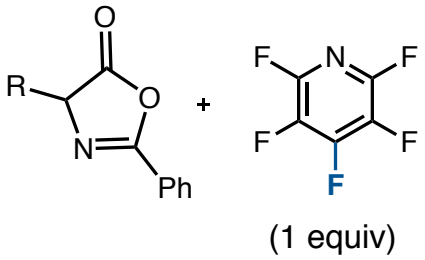
Use of Oxazolone as α-Amino Acid Template

- Oxazolone enolates can be used to perform nucleophilic aromatic substitution



Ar_F-F				
α-AA_F (%)	87	86	82	78

- Fully substituted α-amino esters also accessible



80% (R = Me)
74% (R = i-Bu)
79% (R = CH_2CH_2SMe)
71% (R = Bn)

Teegardin, K. A.; Weaver, J. D. *Chem. Commun.* **2017**, *53*, 4771.

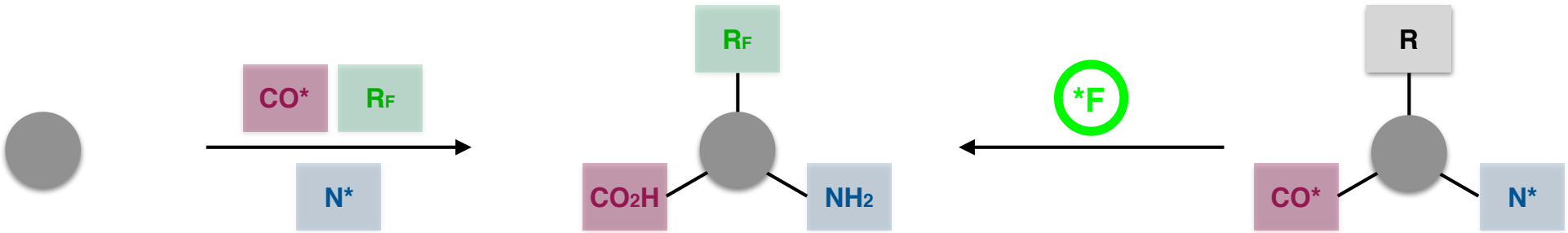
Synthesis of α-AA$_F$: Proposed Classification

Approach A
Installation of functionalities

(Strategies 1-5)

Approach B
Fluorination of side chain

(Strategy 6)

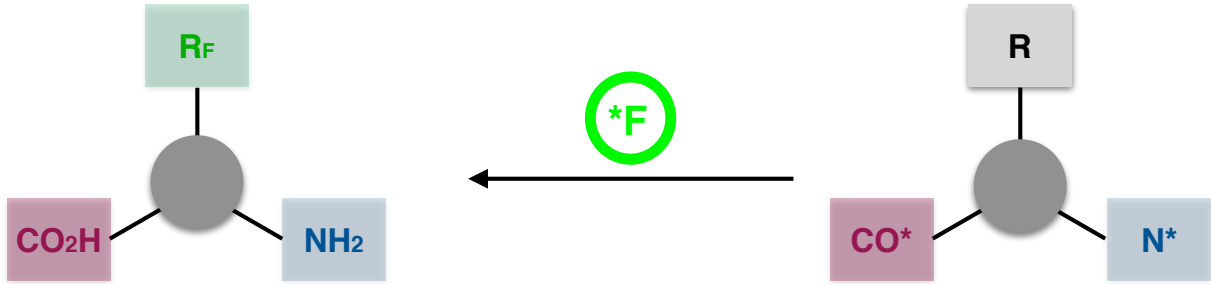


N*	Amine or *nitrogen function*	R_F	Fluorinated side chain	*F	Fluorinating agent
CO*	Carbox. acid or oxygenated function	R	Non fluorinated side chain		Amino acid Cα

Synthesis of α-AA$_F$: Proposed Classification

Approach B
Fluorination of side chain

(Strategy 6)

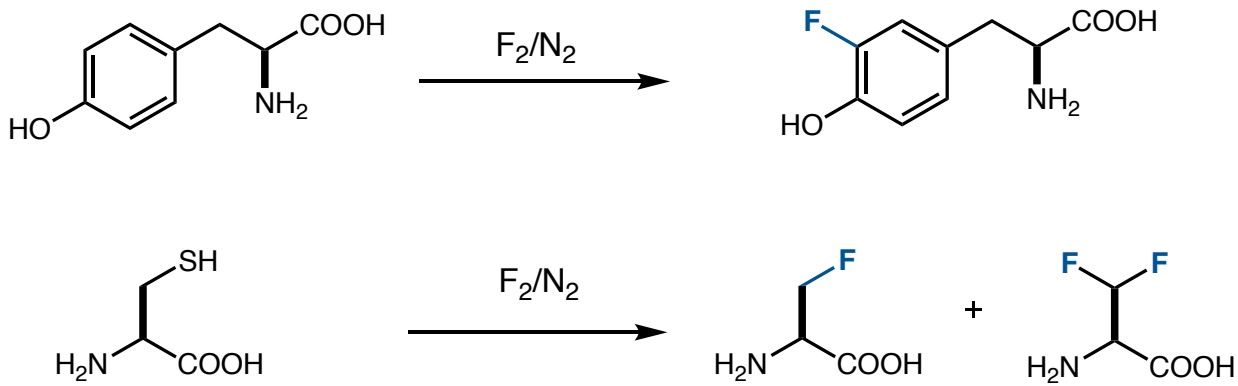


N*	Amine or *nitrogen function*	R_F	Fluorinated side chain	*F	Fluorinating agent
CO*	Carbox. acid or oxygenated function	R	Non fluorinated side chain		Amino acid Cα

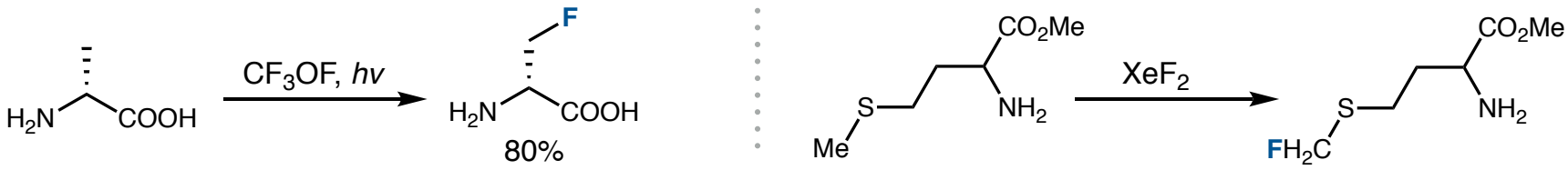
Strategy VI

Pioneering Works

- Fluorination of canonical α-AA with F_2



- XeF_2 and CF_3OF also explored



Smith, R. W.; et al *J. Fluorine Chem.* **1987**, *37*, 267.

Kollonitsch, J.; Marburg, S.; Perkins, L. M. *J. Org. Chem.* **1976**, *41*, 3107.

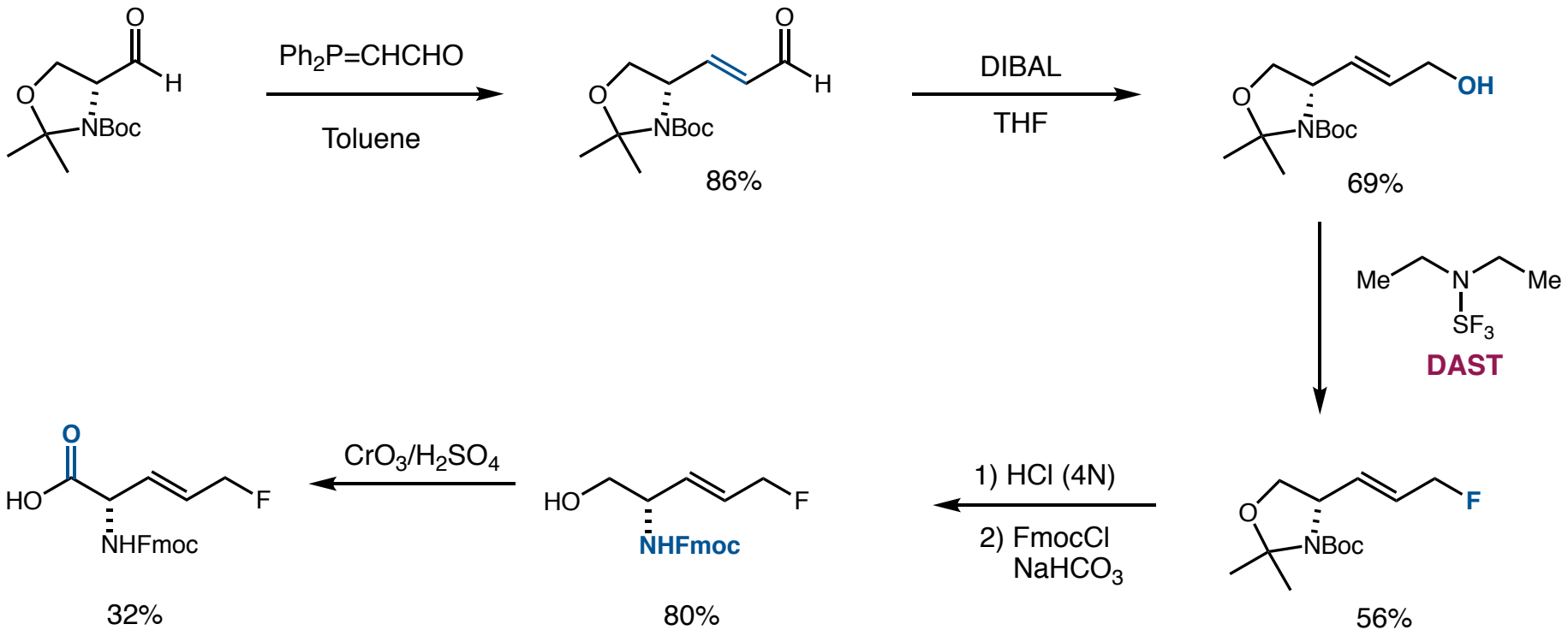
Kollonitsch, J.; Barash, L. *J. Am. Chem. Soc.* **1976**, *98*, 5591.

Janzen, A. F.; Wang, P. M. C.; Lemire, A. E. *J. Fluorine Chem.* **1983**, *22*, 557.

Strategy VI

Deoxofluorination on Side Chain

- Preparation of α-AA$_F$ from Garner's aldehyde

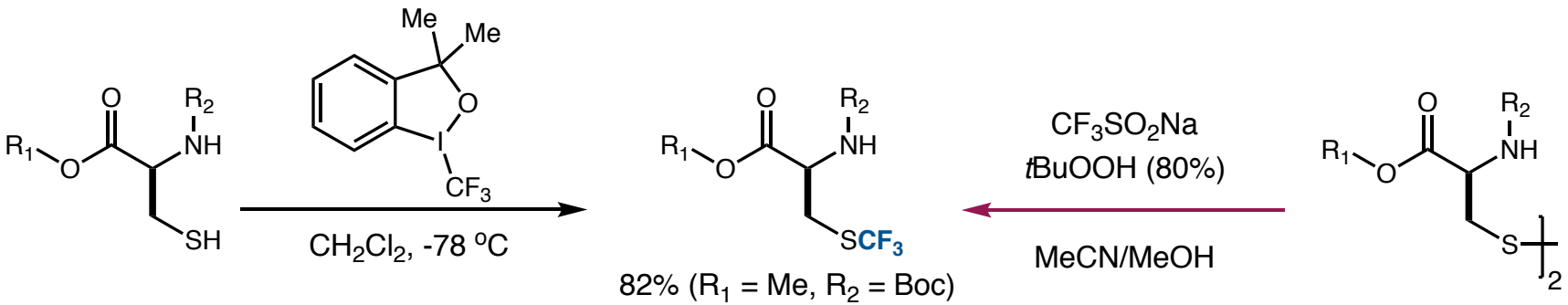


Zhang, X. G.; Ni, W. J.; van der Donk, W. A. *J. Org. Chem.* **2005**, *70*, 6685.

Strategy VI

Addition of Trifluoromethyl Radical

■ Trifluoromethylation of L-cysteine



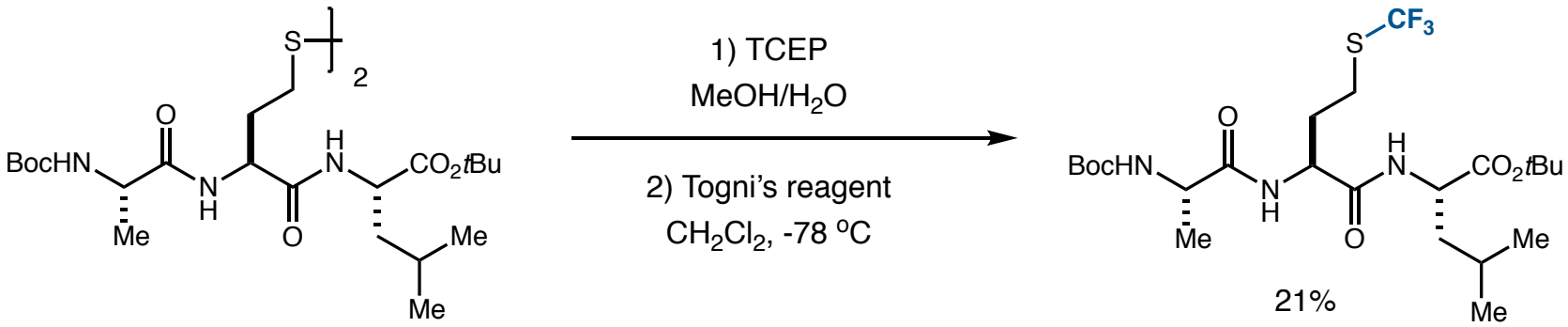
82% (R_1 = Me, R_2 = Boc)

99% (R_1 = Et, R_2 = H)

84% (R_1 = Me, R_2 = Ac)

42% (R_1 = Bn, R_2 = Boc)

■ Togni's reagent has shown potential for late-stage functionnalisation on tripeptides



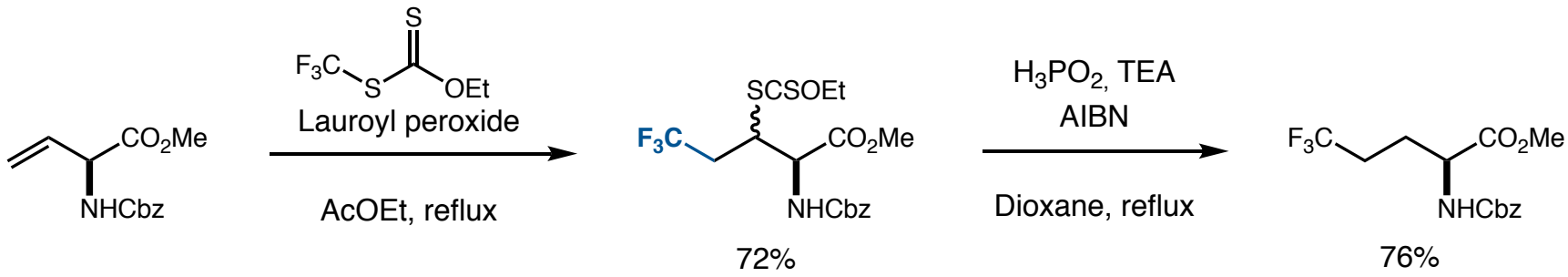
*K*ieltsch, I.; Eisenberger, P.; Togni, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 754.

Gadais, C.; Saraiva-Rosa, N.; Chelain, E.; Pytkowicz, J.; Brigaud, T. *Eur. J. Org. Chem.* **2017**, *2017*, 246.

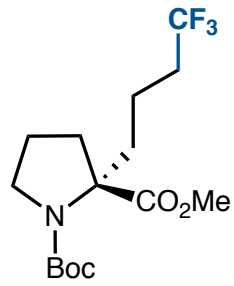
Strategy VI

Via the Addition of a Xanthate

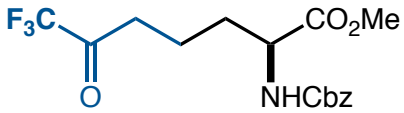
- Trifluoromethylation of protected vinyl glycine by a CF_3 radical generated from a xanthate



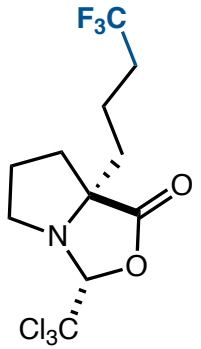
- Other type of fluorinated side chains accessed from different olefins



60%, 2 steps



53%, 2 steps



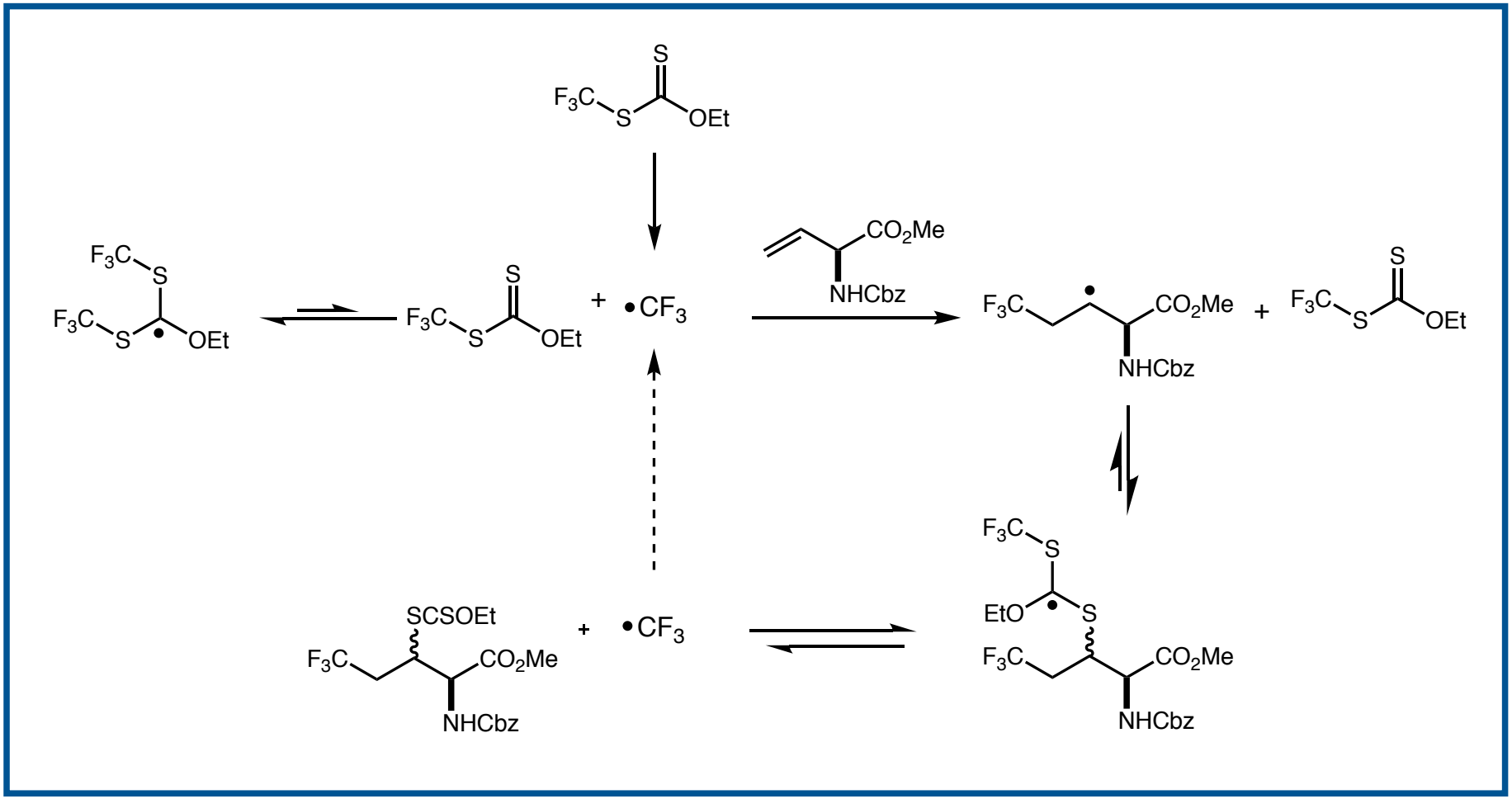
52%, 2 steps

Li, S.-G.; Portela-Cubillo, F.; Zard, S. Z. *Org. Lett.* **2016**, *18*, 1888.

Strategy VI

Via the Addition of a Xanthate

- Xanthate acts as a CF_3 ''resevoir''

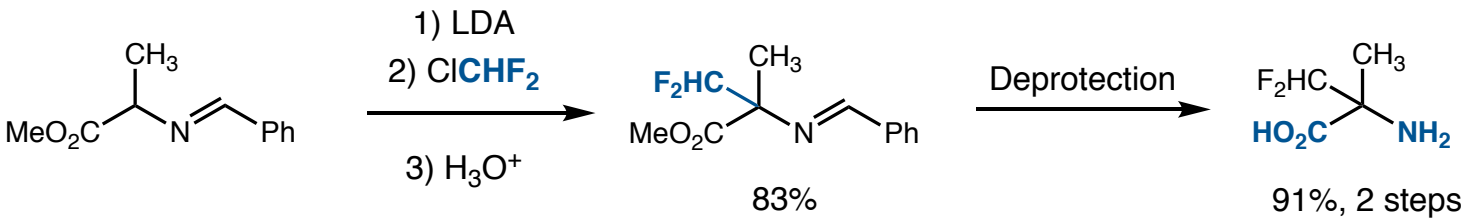


Li, S.-G.; Portela-Cubillo, F.; Zard, S. Z. *Org. Lett.* **2016**, *18*, 1888.

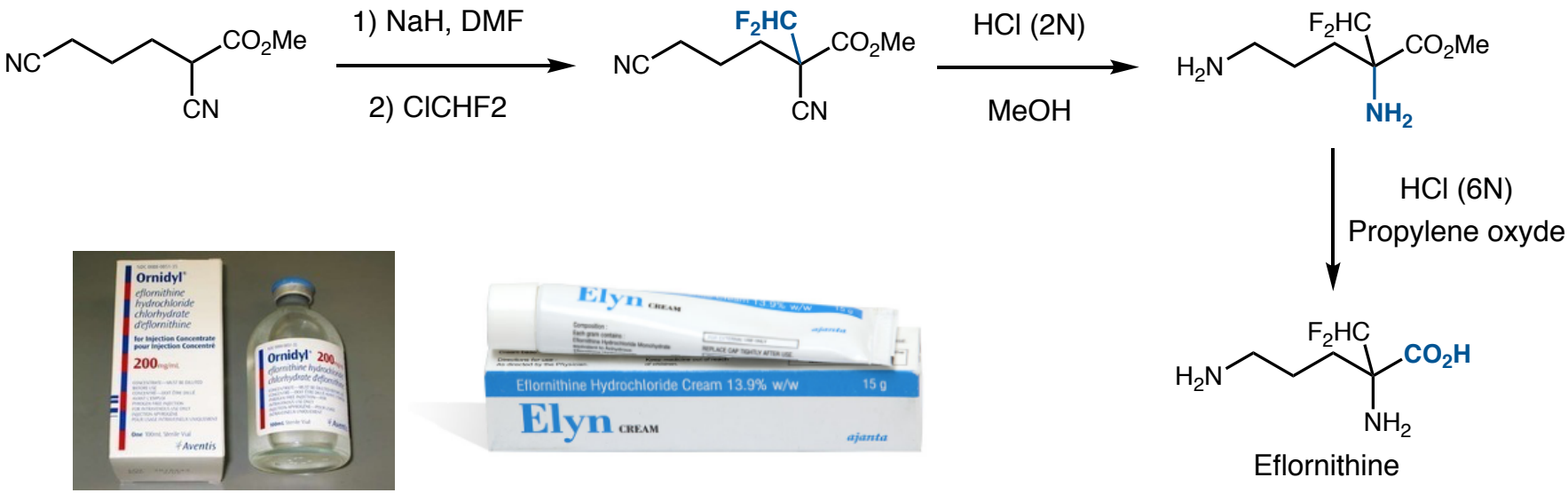
Strategy VI

Difluoromethylation with Difluorocarbene Source

- Trapping of a difluorocarbene by a carbanion generated at Cα



- Strategy employed in the synthesis of eflornithine



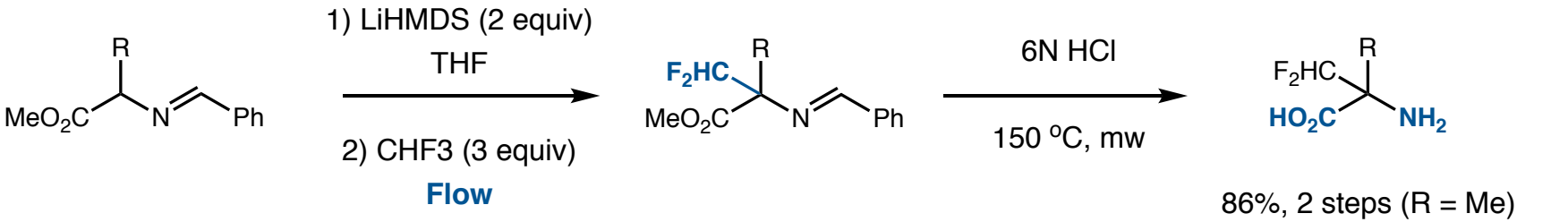
Bey, P.; Vevert, J. P.; Van Dorsselaer, V.; Kolb, M. *J. Org. Chem.* **1979**, *44*, 2732.

Seki, M.; Suzuki, M.; Matsumoto, K. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1024.

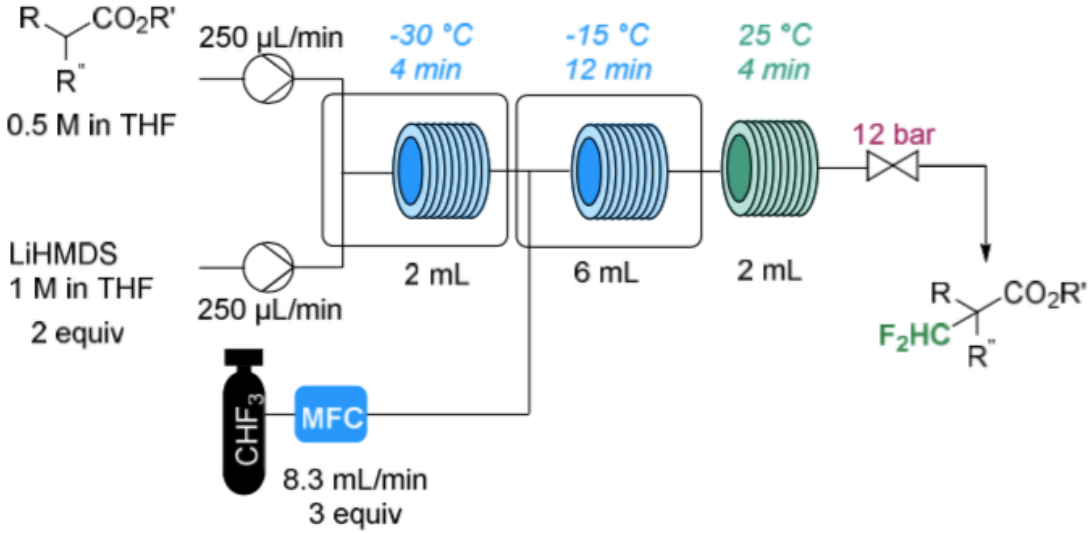
Strategy VI

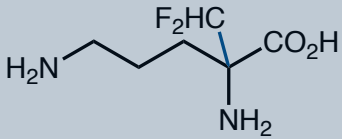
Difluoromethylation with Difluorocarbene Source

■ Use of fluoroform as a CHF_2 source



86%, 2 steps (R = Me)
76%, 2 steps (R = *i*Pr)
87%, 2 steps (R = *i*Bu)
86%, 2 steps (R = CH_2Ph)





Eflornithine

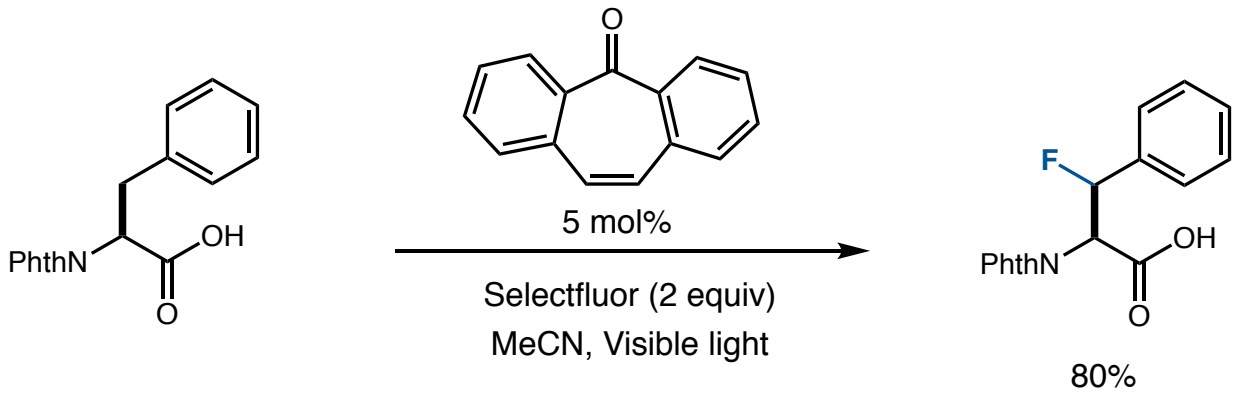
76%, 2 steps

Kockinger, M.; Ciaglia, T.; Bersier, M.; Hanselmann, P.; Gutmann, B.; Kappe, C. O. *Green Chem.* **2017**, *accepted.*

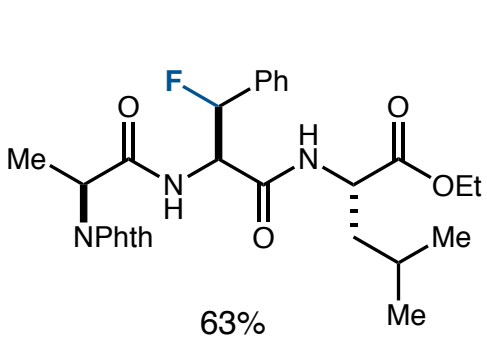
Strategy VI

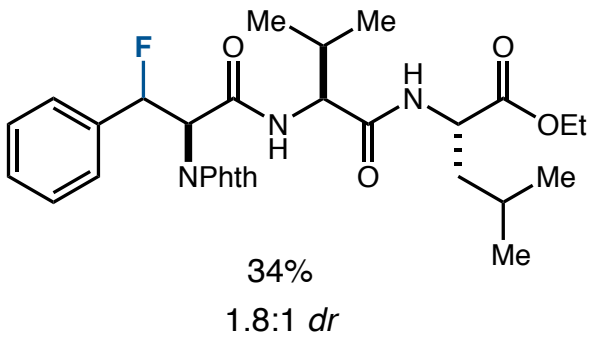
Benzylic C-H Fluorination

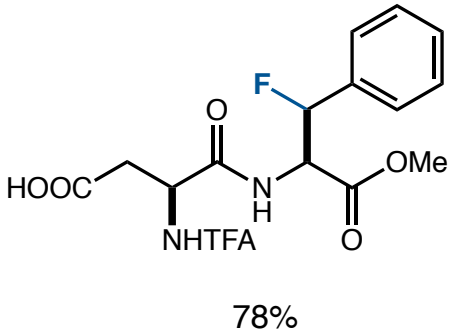
- Visible-light sensitized benzlic C-H fluorinated with Selectfluor



- Selective late-stage fluorination of benzylic position in peptides







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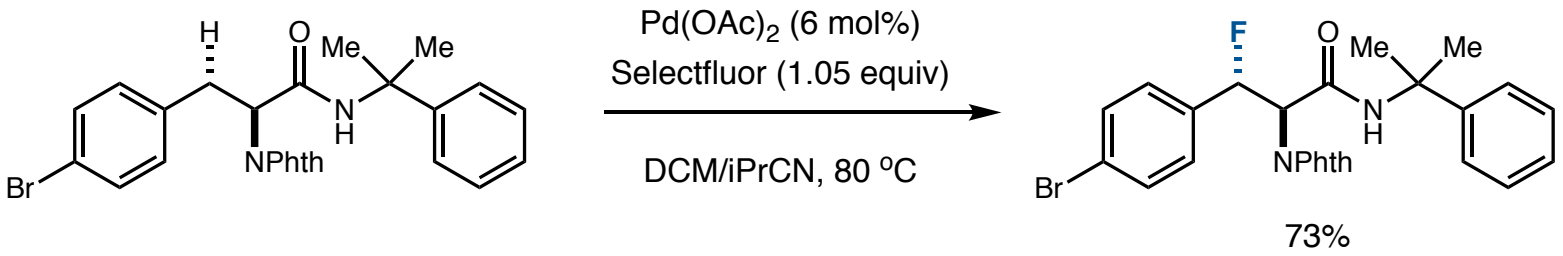
Strategy VI

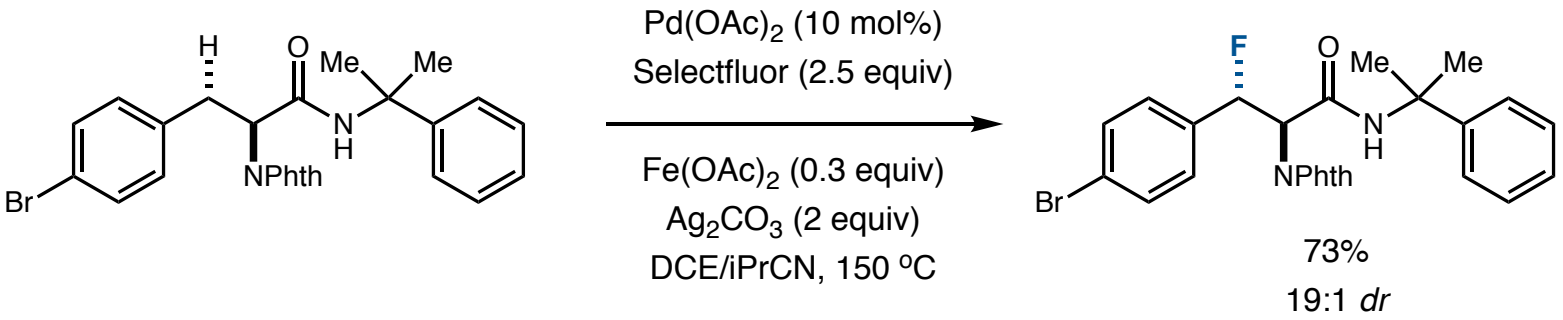
Benzylic C-H Fluorination

- Pd-catalyzed fluorination using 2-(pyridin-2-yl)isopropyl (PIP) as protecting and directing group

  - Shi (JACS, 2015)



  - Ge (Org Lett, 2015)



*Z*hang, Q.; Yin, X.-S.; Chen, K.; Zhang, S.-Q.; Shi, B.-F. *J. Am. Chem. Soc.* **2015**, *137*, 8219.

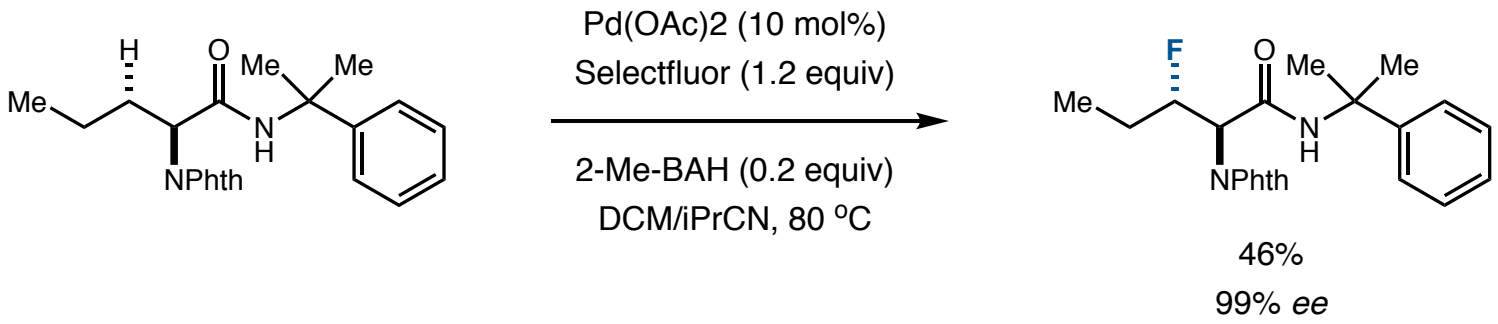
Miao, J.; Yang, K.; Kurek, M.; Ge, H. *Org. Lett.* **2015**, *17*, 3738.

Strategy VI

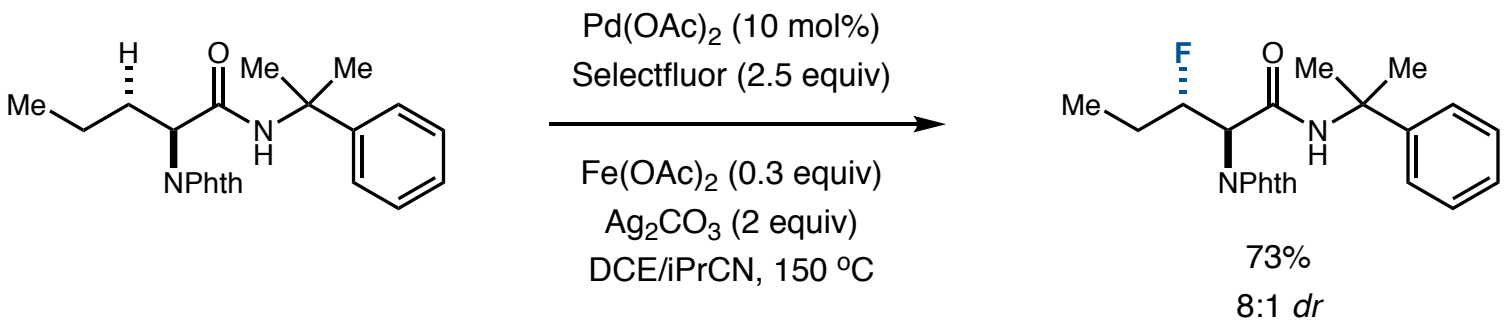
Fluorination of Unactivated C-H Bonds

- Pd-catalyzed fluorination using 2-(pyridin-2-yl)isopropyl (PIP) as protecting and directing group

  - Shi (JACS, 2015)



  - Ge (Org Lett, 2015)



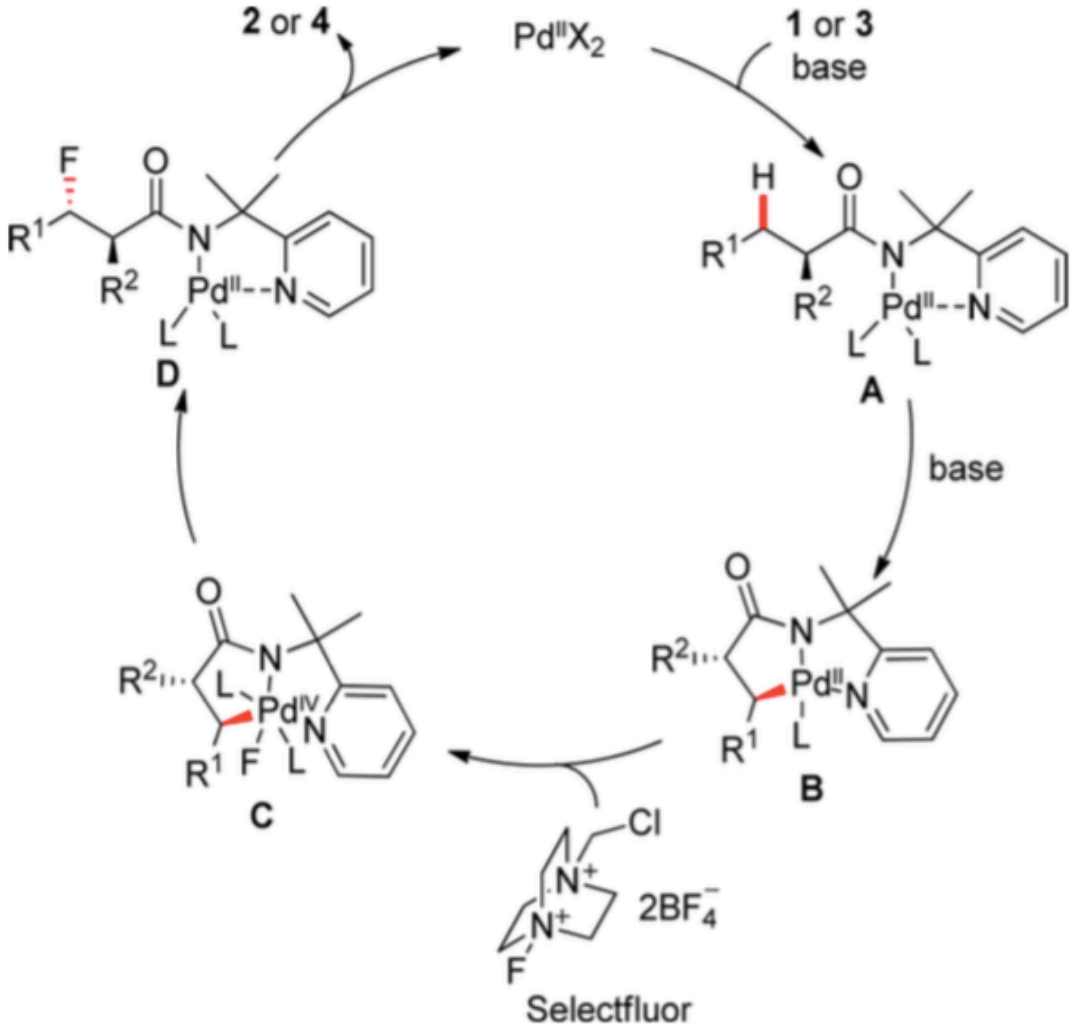
*Z*hang, Q.; Yin, X.-S.; Chen, K.; Zhang, S.-Q.; Shi, B.-F. *J. Am. Chem. Soc.* **2015**, *137*, 8219.

Miao, J.; Yang, K.; Kurek, M.; Ge, H. *Org. Lett.* **2015**, *17*, 3738.

Strategy VI

Fluorination of Unactivated C-H bonds

- Ge's proposed mechanism invokes fluorination of a Pd(II) palladacyclic center

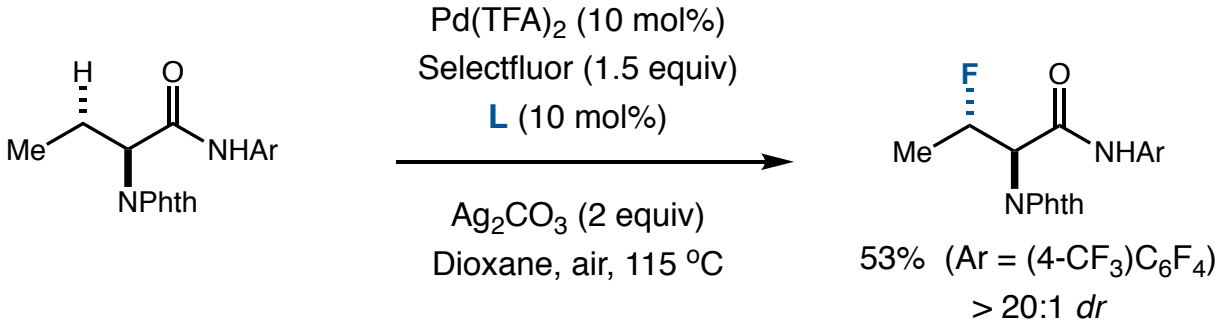


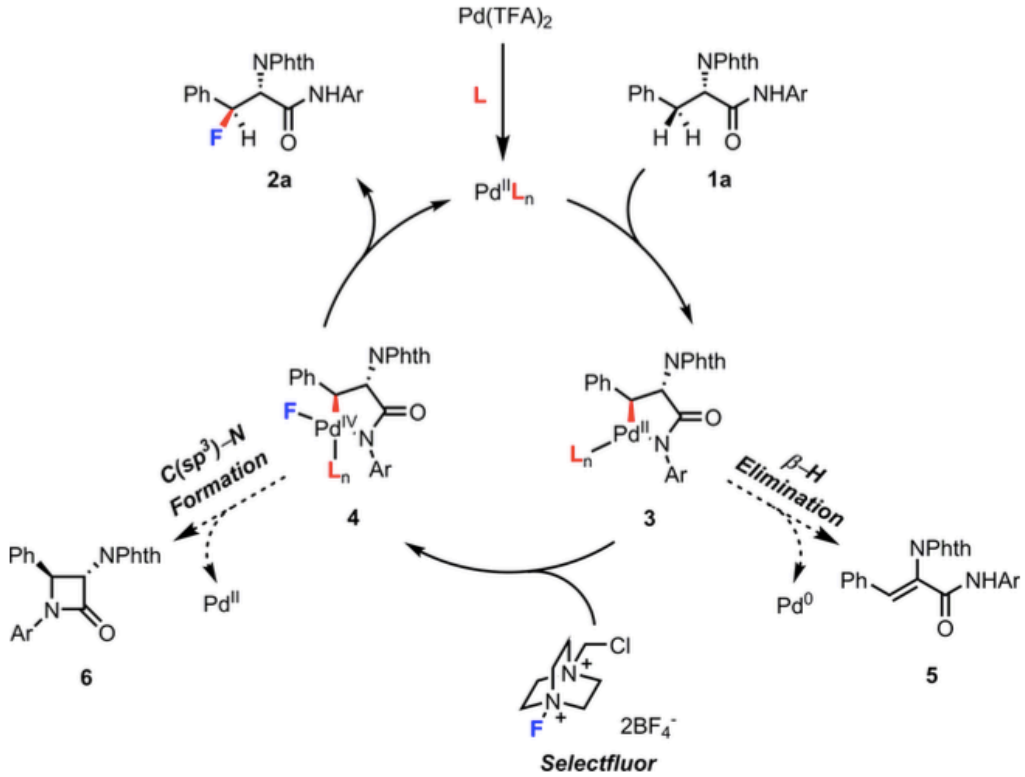
Miao, J.; Yang, K.; Kurek, M.; Ge, H. *Org. Lett.* **2015**, *17*, 3738.

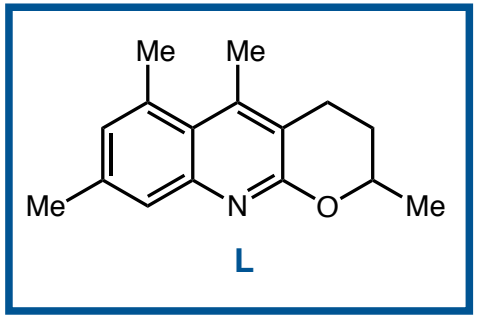
Strategy VI

Fluorination of Unactivated C-H Bonds

- Similar system driven by a Quinoline-based ligand







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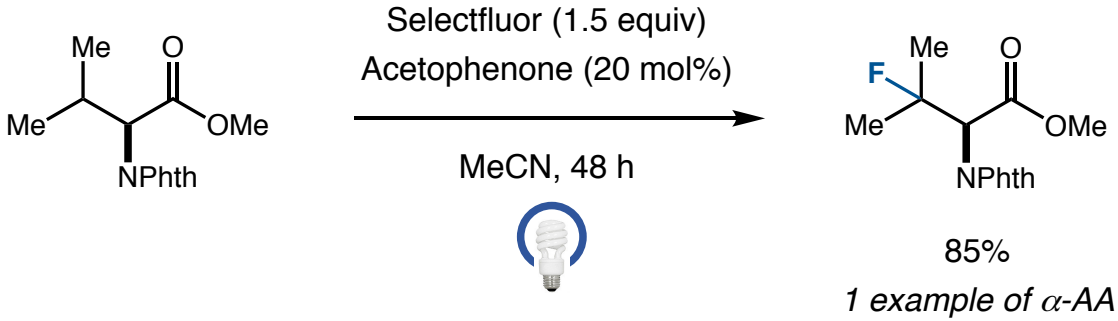
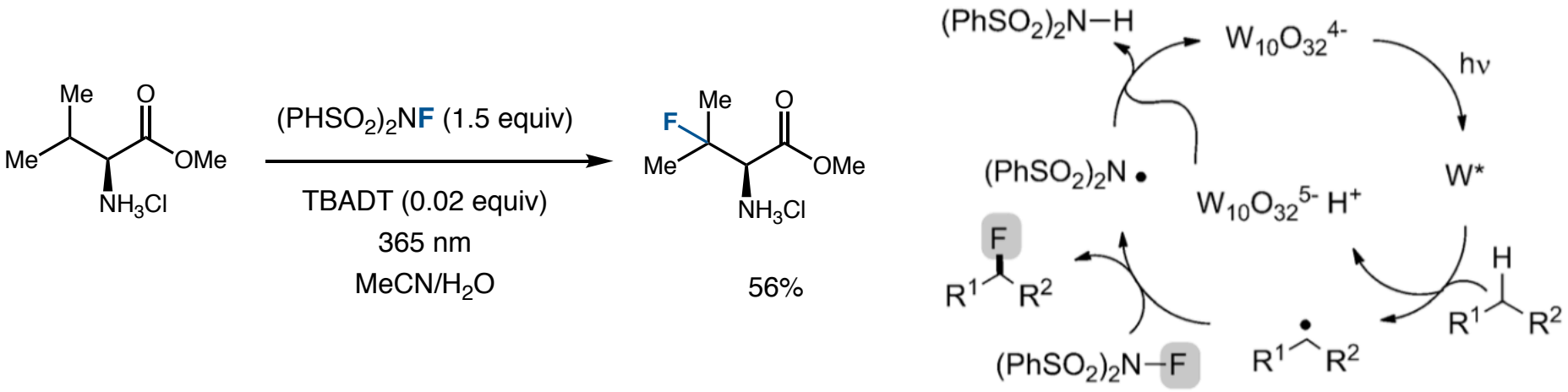
Strategy VI

Fluorination of Unactivated C(sp^3)-H Bonds

- Visible-light promoted fluorination with benzophenone



- Decatungstate-catalyzed C-H fluorination of branched aliphatic with NFSI



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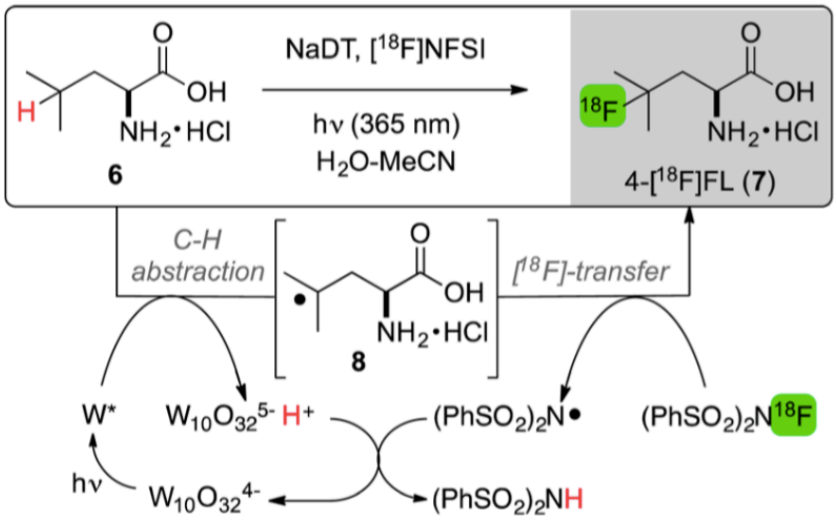
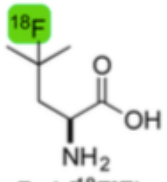
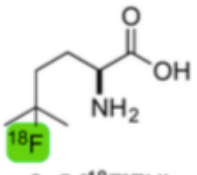
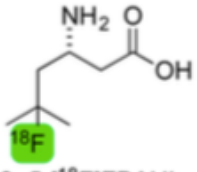
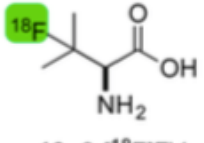
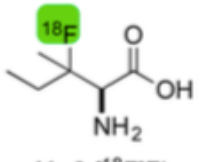
Strategy VI

Fluorination of Unactivated C(sp^3)-H Bonds

- Decatungstate-catalyzed C-H fluorination: suitable system for preparation of ^{18}F-labeled α-AA$_F$



- Preparation/purification of marked NFSI: 10 min
- Reaction time: 40 min
- Purification time: 10 min
- Total process from ^{18}F generation: 60 min
- Product isolated directly as formulation for IV injection

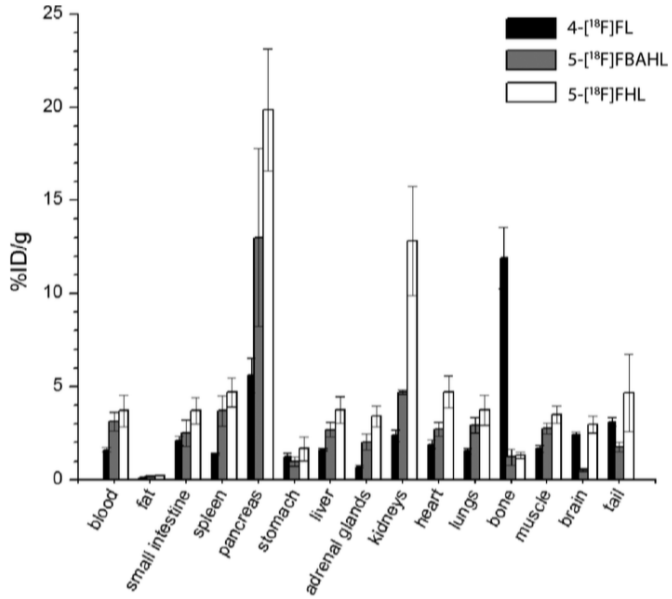
^{18}F-labelled amino acid	 7: 4-[^{18}F]FL	 8: 5-[^{18}F]FHL	 9: 5-[^{18}F]FBAHL	 10: 3-[^{18}F]FV	 11: 3-[^{18}F]FI
radiochemical yield (%)[b,c]	23.3 ±3.3%	27.9 ±3.3%	29.8 ±0.7%	6.4 ±0.4%	<5%[d]

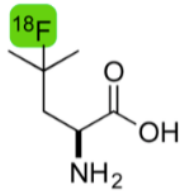
Nodwell, M. B.; Britton, R.; et al. *J. Am. Chem. Soc.* **2017**, *139*, 3595.

In Vivo Biological Investigation

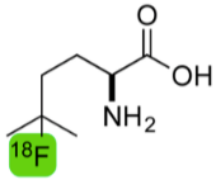
^{18}F Marked α-AA$_F$ Prepared by Fluorination of Unactivated C-H Bonds

- 5-[^{18}F]FHL showed a normal distribution profile in healthy mice after 60 minutes

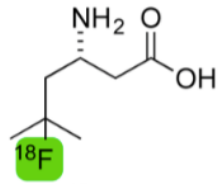




7: 4-[^{18}F]FL



8: 5-[^{18}F]FHL



9: 5-[^{18}F]FBAHL

Nodwell, M. B.; Britton, R.; et al. *J. Am. Chem. Soc.* **2017**, *139*, 3595.

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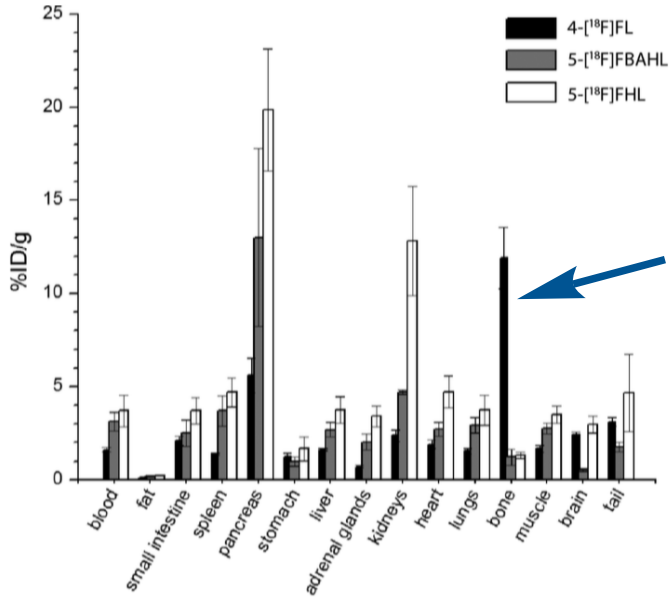
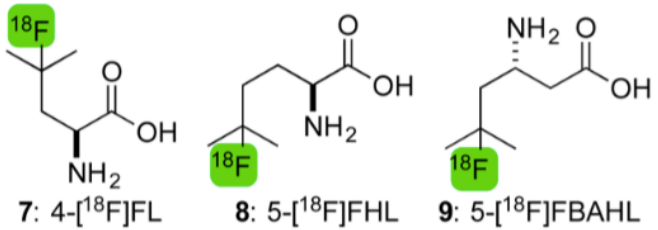
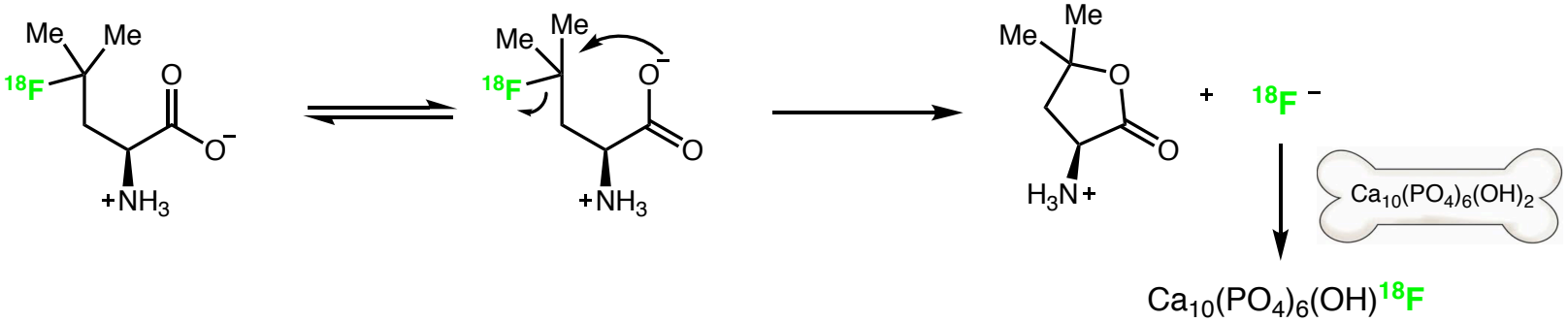




- 4-[^{18}F]FL accumulation in bones reveals *in vivo* production of fluoride by lactonisation

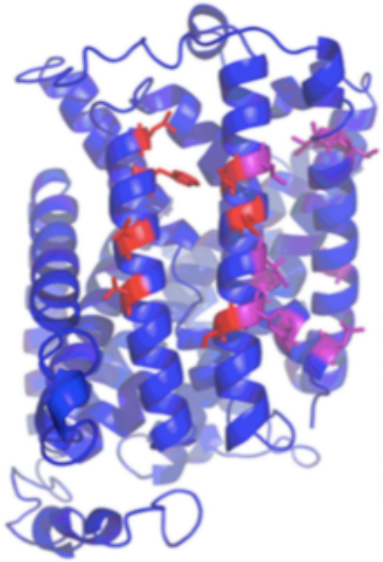


Nodwell, M. B.; Britton, R.; et al. *J. Am. Chem. Soc.* **2017**, *139*, 3595.

In Vivo Biological Investigation

^{18}F Marked α-AA$_F$ Prepared by Fluorination of Unactivated C-H Bonds

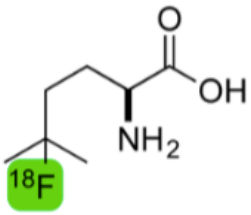
- LAT1 surexpression in tumor can be revealed by 5-[^{18}F]FHL in PET imaging



LAT1

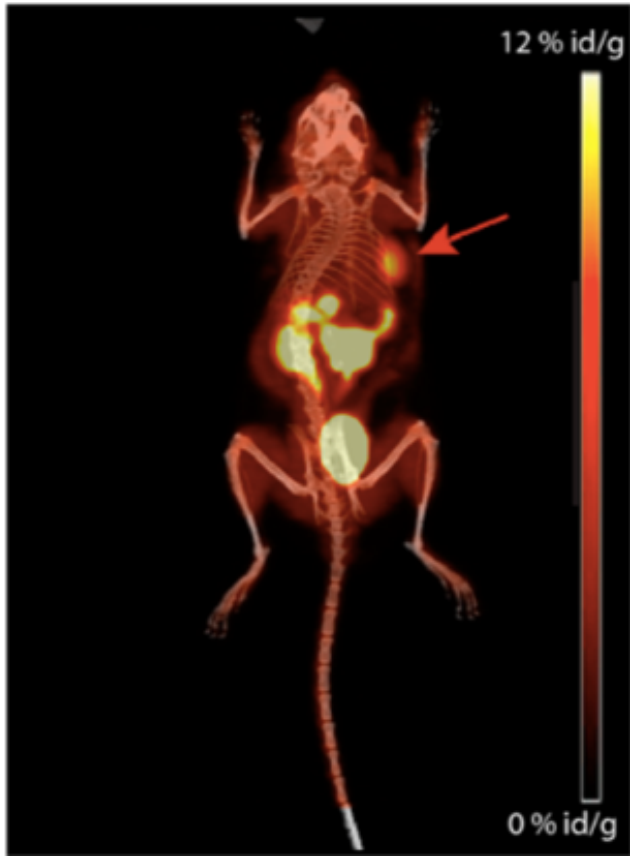
α-AA transport protein

Surexpressed in many cancer cell lines



8: 5-[^{18}F]FHL

60 min



Nodwell, M. B.; Britton, R.; et al. *J. Am. Chem. Soc.* **2017**, *139*, 3595.