New F-18 Labeling Methods

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Scholars Trained in Advanced Radiochemistry Technology

Outline

1. Nucleophilic aromatic substitution

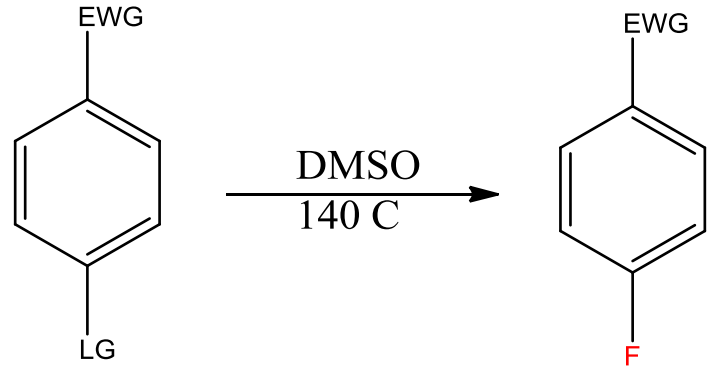
2. Prosthetic group chemistry (labeling peptide and protein)
    1. Random substitution
    2. Site directed

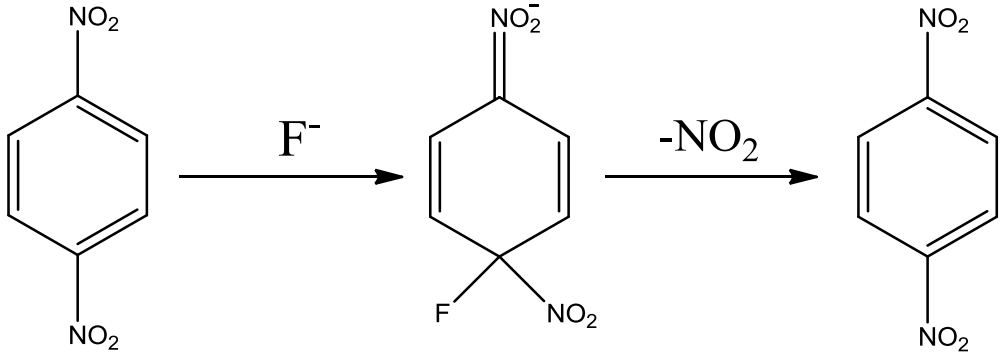
3. New F18-Labeling Methodologies:
    1. Protic solvent
    2. Ionic liquid
    3. Iodonium salts
    4. Transition metal catalysis
    5. Enzyme catalysis
    6. Polymer supported chemistry

NUCLEOPHILIC AROMATIC SUBSTITUTION (S_NAR)

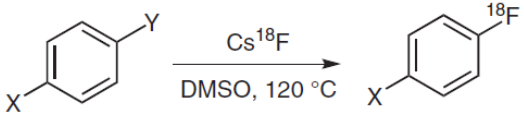
Precursor Requirements

(a) Activating effect: EWG: 3-NO_2 < 4-CH_3CO < 4-CN < 4-NO_2





Effect of activating (X) and leaving groups (Y) on nucleophilic aromatic fluorination



X	Y	k_{rel} (80 °C)	k_{rel} (120 °C)
NO_2	NMe_3ClO_4	400	30000
NO_2	NO_2	40	420
CN	NMe_3ClO_4	16	100
COMe	NMe_3ClO_4	8	33
CN	NO_2	1	1

Meisenheimer complex; delocalization electron onto EWG

(b) Leaving group: I < Br < Cl <F < NO_2 < N^+Me_3

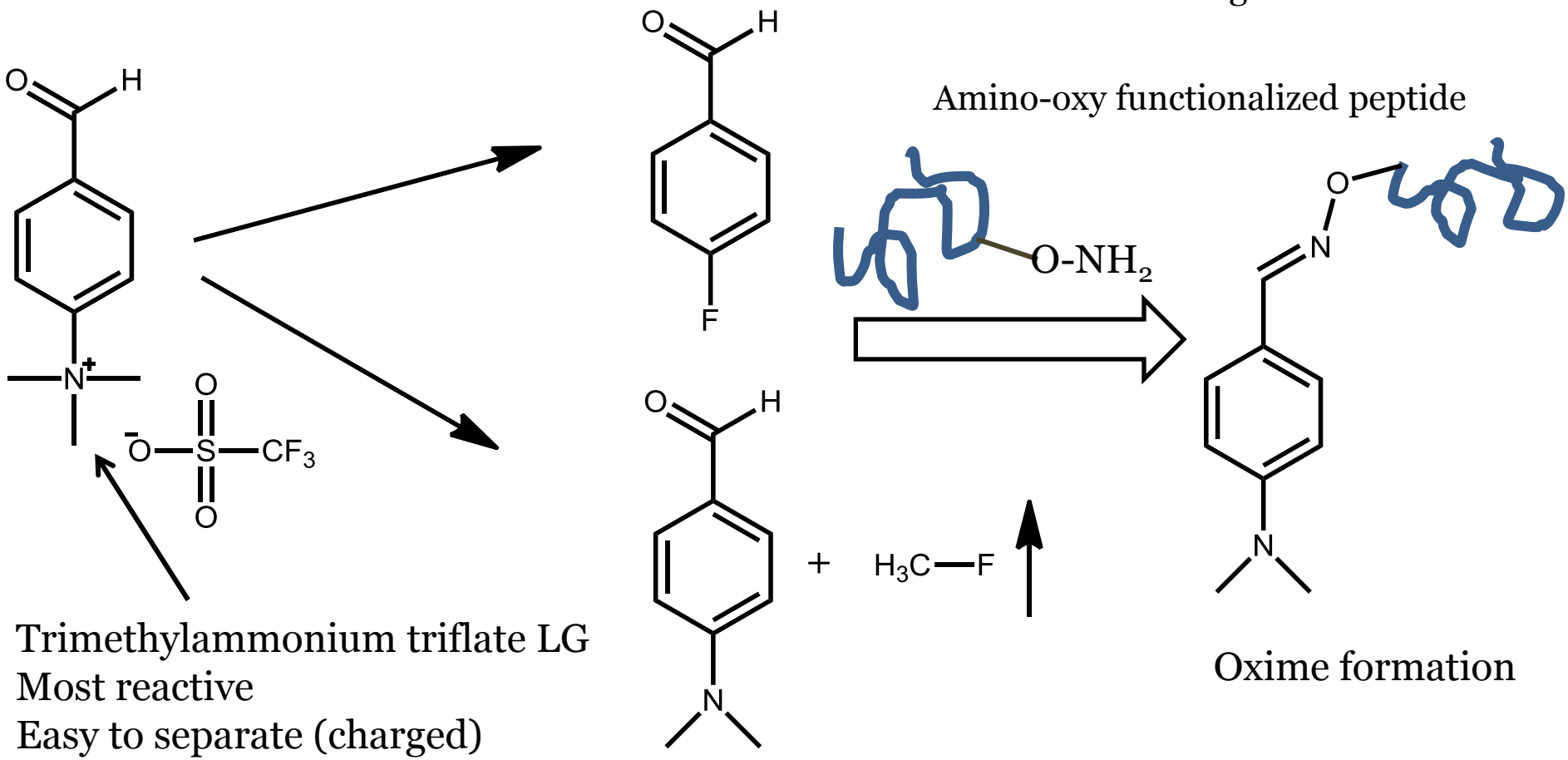
(C-F bond making is RLS. Polar effects favors addition step)

(c) Solvent effect: DMSO > DMAc (N,N,-dimethylacetamide) > sulfolane >> acetonitrile

Side Reactions in S_{NAr}

Side reactions

Fluorobenzaldehyde → site specific peptide conjugation; away from the binding sites
(Prosthetic group)

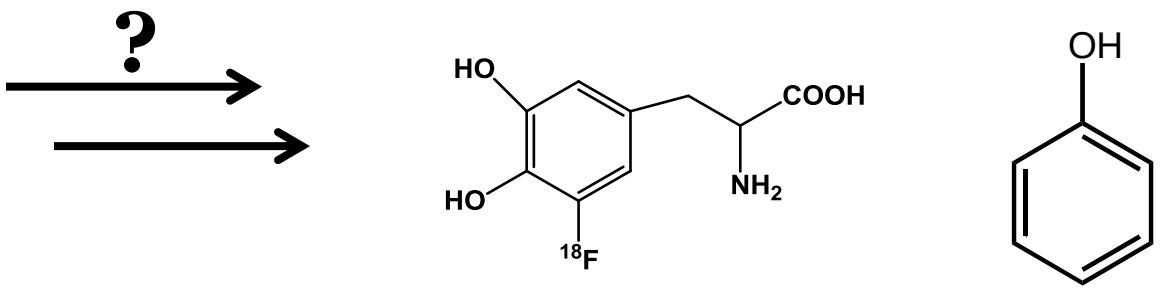


Trimethylammonium triflate LG
Most reactive
Easy to separate (charged)

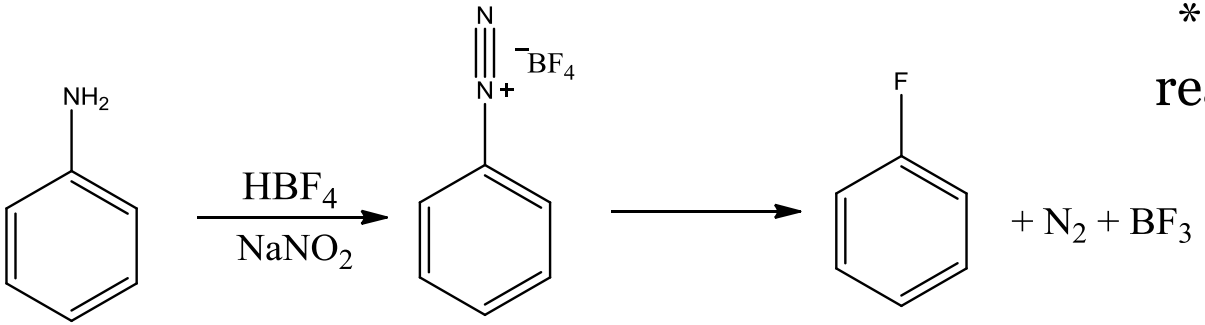
Oxime formation

Li, X. et al. Bioconjugate Chem. 2008, 19, 1684-1688
Solin, O. et al. J Fluorine Chemistry, 2012, 143, 49-56

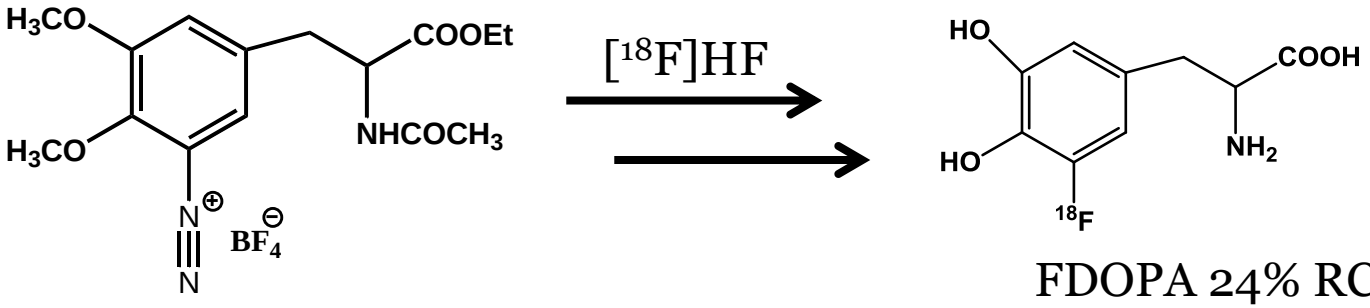
Nucleophilic Aromatic Substitution
of substrate without EWG?



Balz-Schiemann reaction



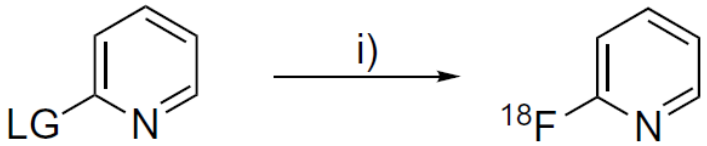
* High temperature, harsh reagent, corrosive, explosive
Low yield ~ 2-15% RCY
Only 1 F is from BF_4 is transferred to arene



FDOPA 24% RCY

[^{18}F]F-Nucleophilic *Heteroaromatic* Substitution

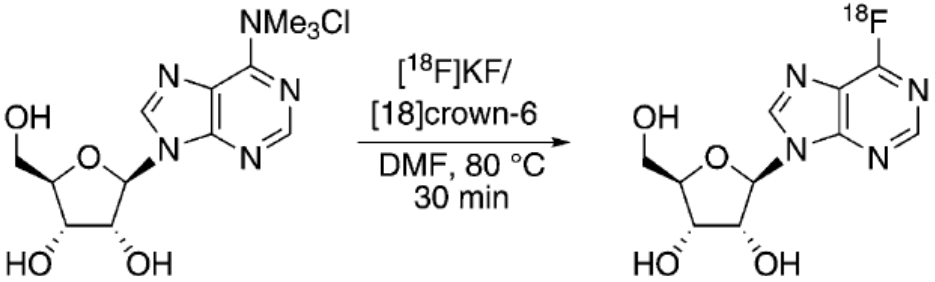
LUMO of pyridine at ortho and para position lower than benzene
No need activating group



Coenen, H.H. 2007. Basic Fluorine-18 Labeling Methods

LG	150 °C RCY (%)	180 °C RCY (%)
I	1	19
Cl	23	57
Br	25	87
NO_2	92	89
$(CH_3)_3N^+CF_3SO_3^-$	90	92

Scheme 46. i) ^{18}F]F$^-$/K_2CO_3/K_{222}, DMSO



6-[^{18}F]fluoro-9-β-D-ribofuranosylpurine
63 % RCY

Irie, T. et al. App Radiat Isot. 1982. 33 445.
Gouverneur, V. et al. Angew Chem Int Ed. 2012, 51, 2-14

TABLE 2. Temperature dependence of the radiochemical yields of ^{18}F-(IV)

Reaction temp. (°C)	Radiochemical yield (%)[1]
room temp	55–66
50–60	68–75
80–90	0.4–1.4

[1] Yields from the reaction for 20 min of 15 μmol of (II) with the solubilized K^{18}F (1.2 μmol) in DMF-CE solution before addition of (II).

Summary Nucleophilic Substitution

1. Preferred method
2. High specific activity of $[18F]F^-$ vs $[18F]F_2$ (1740 Ci/umole vs 0.1 Ci/umole)
3. Easy to handle (liquid vs gas)
4. $[^{18}F]F^-$ Can be transported and distributed to nearby imaging clinicic (Decentralized model of PET probe production)
5. S_N2, leaving group, solvent, phase transfer catalyst and base
6. Side reactions, optimization
7. Activated substrate and good leaving group for S_{NAr}

PEPTIDE AND PROTEIN LABELING

Why label peptide and proteins?

Features

- Target tumor cell receptors
- Hormone receptor in cancerous cell
- Key regulators of cellular function and cell growth
- Peptide as PET traces- quantify apoptosis and angiogenic processes
- Multidendate characteristic→ Higher binding affinity, selectivity

Why label with F18?

- Moderate half-life
- Low β^+ energy (lower radiation, short positron pathlengths (image resolution)
- High % of β^+ decay vs other radiometals (i.e.: Cu^{64} 18%, Ga^{68} 57%, Y^{86} 33%)

S.M. Okarvi. Eur J Nucl Med. 2001, 28, 929

How to label with Fluorine-18?

1. Direct labeling

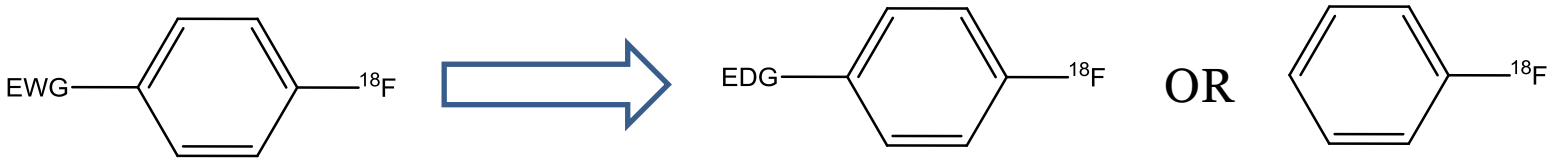
(i) Electrophilic Substitution

Oxidative, low yield, low SA

(ii) Nucleophilic Substitution

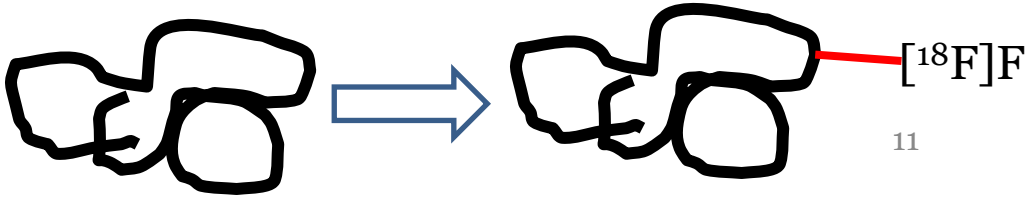
High pH, high temperature, organic solvent

2. Via built-up procedures



3. Via prosthetic groups

[18F]F-Substrates-coupling group + Peptide or protein



F18-Peptides and Proteins

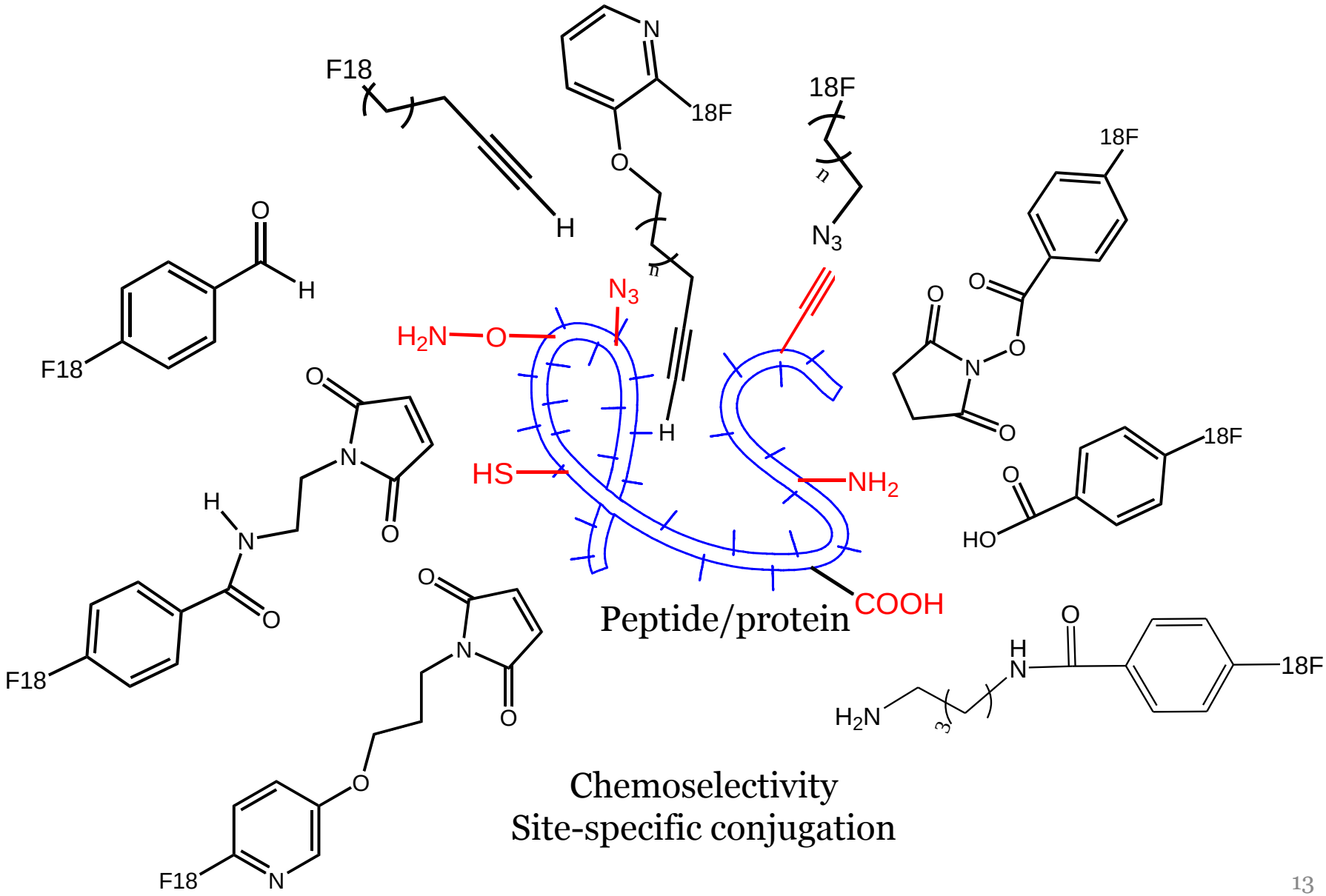
Prosthetic group and labeling

1. Possessed reactive coupling moieties: allow mild labeling conditions suitable for biomolecules
2. Temperatures: for peptide up to 100 C; for protein ~ RT
3. Mild pH : physiological pH for protein; wider range pH for peptide.
4. Does not disrupt binding affinity of peptide/proteins
5. In aqueous/buffer solution or alcoholic solution (peptide only).
6. Formation of desirable product at tracer level concentration
7. Negligible steric impairment upon labeling
8. High specific activity
9. High radiochemical yield

2-types of labeling chemistry (choice of prosthetic group?)

1. Random
2. Site specific **

Commonly used [18F]Prosthetic groups



Chemoselectivity
Site-specific conjugation

Banister et al. Current Radiopharm, 2010, 3, 68-80

Prosthetic group: Succinimydyl-4-fluorobenzoate

RANDOM LABELING AMINE REACTIVE

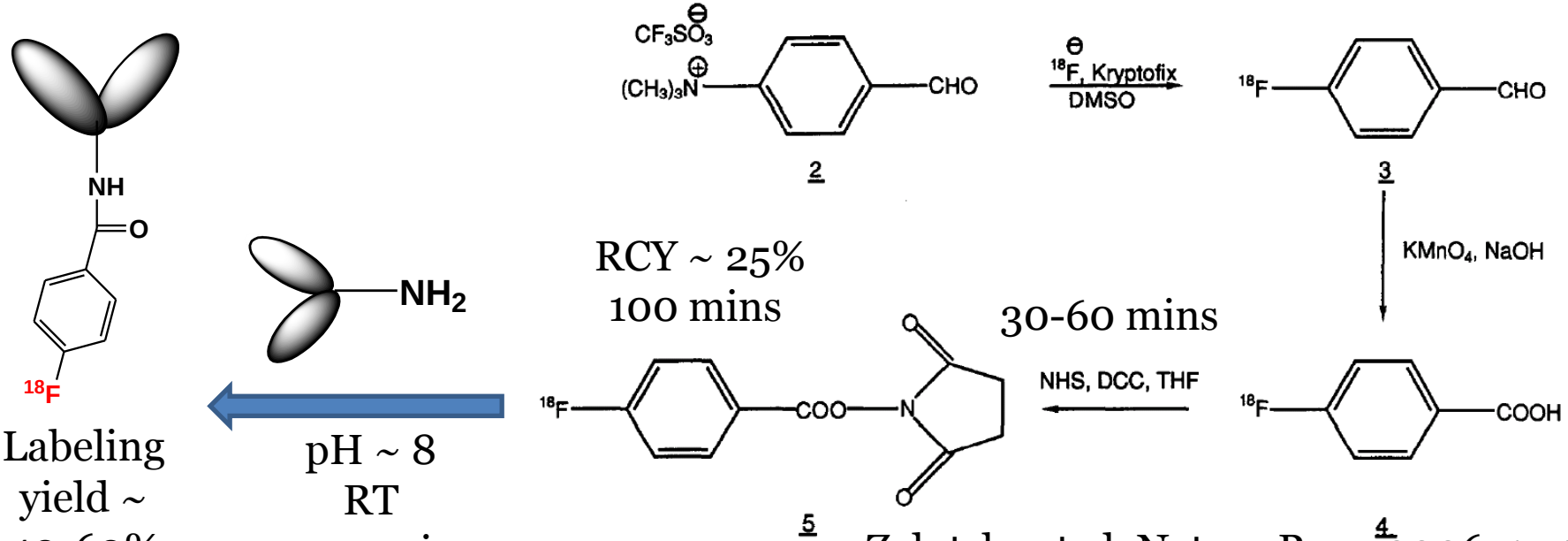
N-Succinimidyl 4-[^{18}F] fluorobenzoate ([^{18}F]SFB)

Labeling Proteins with Fluorine-18 Using *N*-Succinimidyl 4-[^{18}F]Fluorobenzoate

Nucl. Med. Biol. Vol. 19, No. 3, pp. 275–281, 1992

GANESAN VAIDYANATHAN and MICHAEL R. ZALUTSKY*

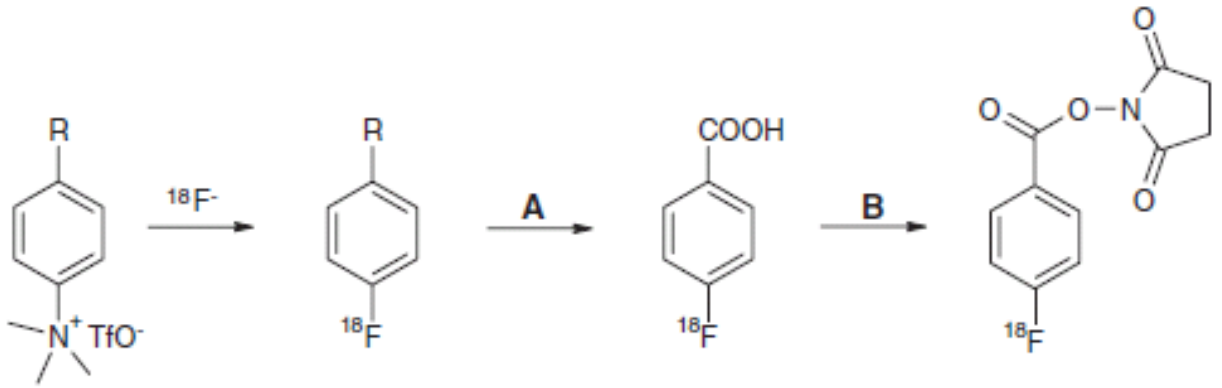
Department of Radiology, Duke University Medical Center, Durham, NC 27710, U.S.A.



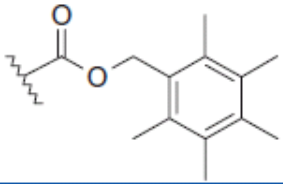
Zalutsky et al. Nature Proc. 2006. 1 , 1656

Improvement in [^{18}F]SFB Synthesis

steps?
pots?
Reaction time?
Yield?
Reagent sensitivity?
Automation?



^{18}F-SFB

R	# pots	A	B	RCY[a]	Reference
-CHO	3	$KMnO_4$/NaOH/HCl	NHS/DCC	25%	Vaidyanathan and Zalutsky[7]
-CHO		$KMnO_4$/NaOH/HCl	DSC[b]	51%	Vaidyanathan and Zalutsky[8,9]
-COOEt	2	NaOH/HCl	TSTU[c]	50–60%	Wester *et al.*[10]
-COOEt	1	Pr_4NOH	HSTU[d]	43.8±4.6%	Tang *et al.*[11]
-COO^{t}Bu	1	TFA	TSTU	44–53%	Wüst *et al.*[12]
		TFA	DSC/DMAP	44%	Azarian *et al.*[13]

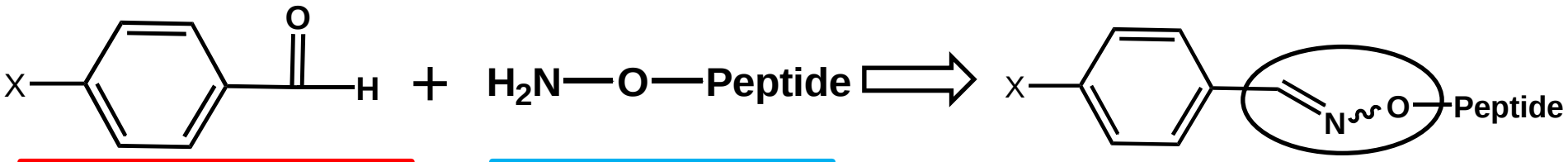
[a]Total radiochemical yield, decay-corrected.
[b]*N,N′*-discuccinimidyl carbonate.
[c]*O*-(*N*-succinimidyl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate.
[d]*O*-(*N*-Succinimidyl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate.

Glaser et al. J Label Compd Radiopharm. 2009, 52, 327

Prosthetic group: Aldehyde functional group

SITE-SPECIFIC LABELING AMINO-OXY REACTIVE

Oxime formation



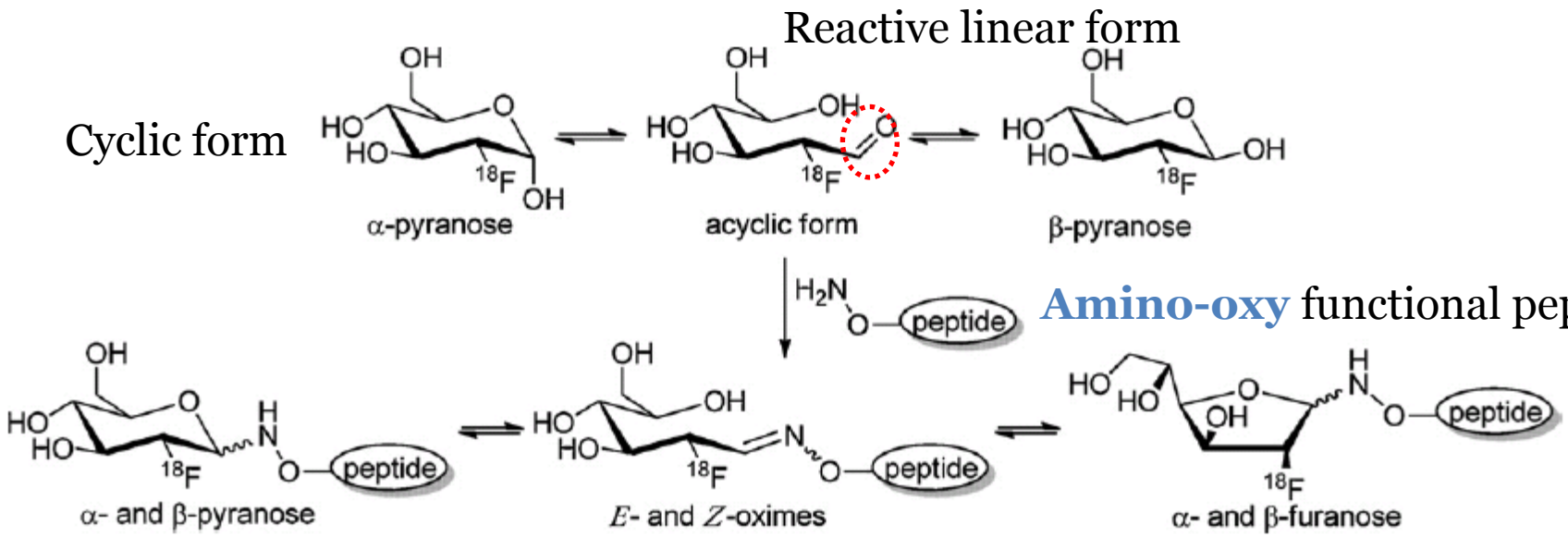
Benzaldehyde funct.

Amino-oxy funct.

Oxime bond

Selective despite the presence of other amino acid functionalities

(1) [^{18}F]FDG as prosthetic group



Scheme 8. Proposed product profile in [^{18}F]FDG conjugations with peptide [30,51].

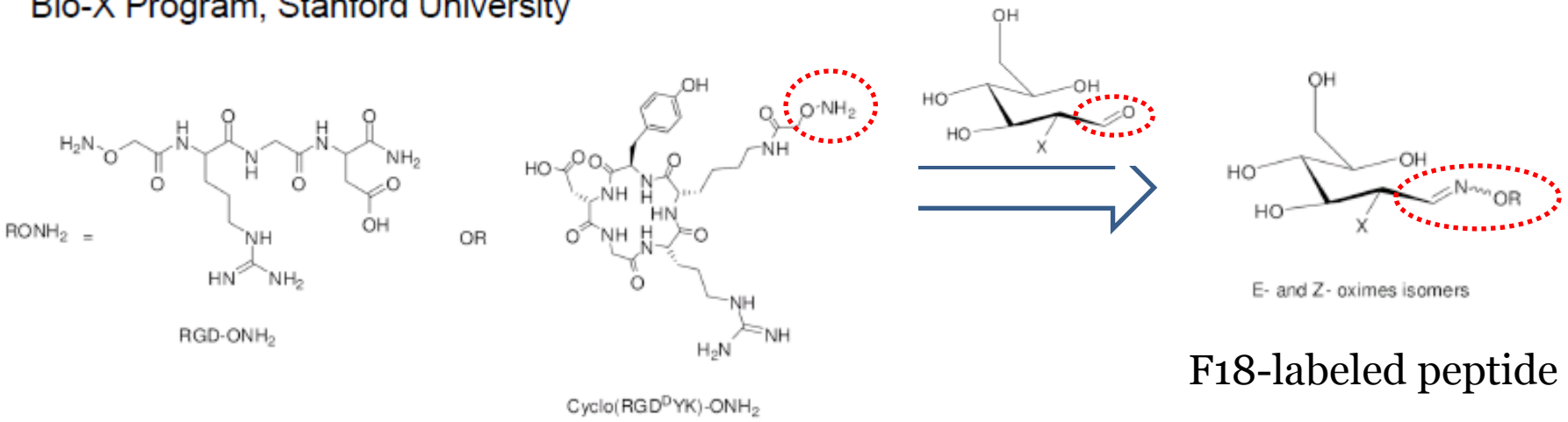
Solin, O. et al. J Fluorine Chemistry, 2012, 143, 49-56

A Novel Method for Direct site-specific Radiolabeling of Peptides Using [^{18}F]FDG

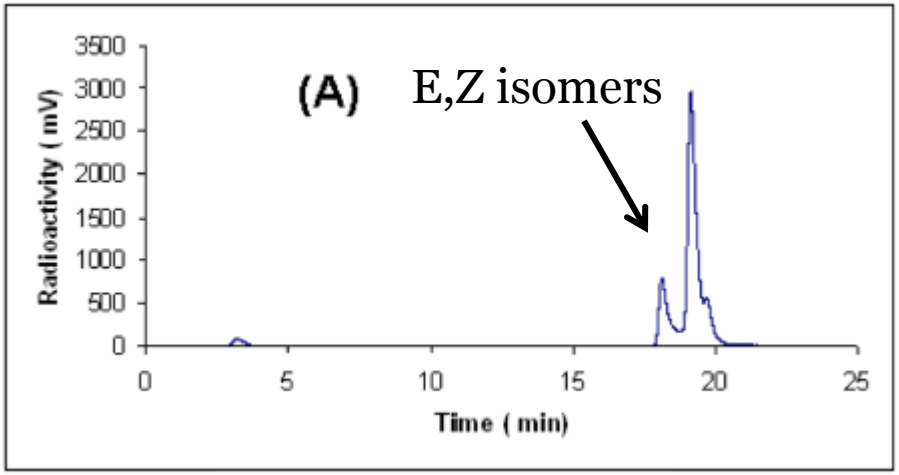
Bioconjug Chem. 2009 March ; 20(3): 432–436.

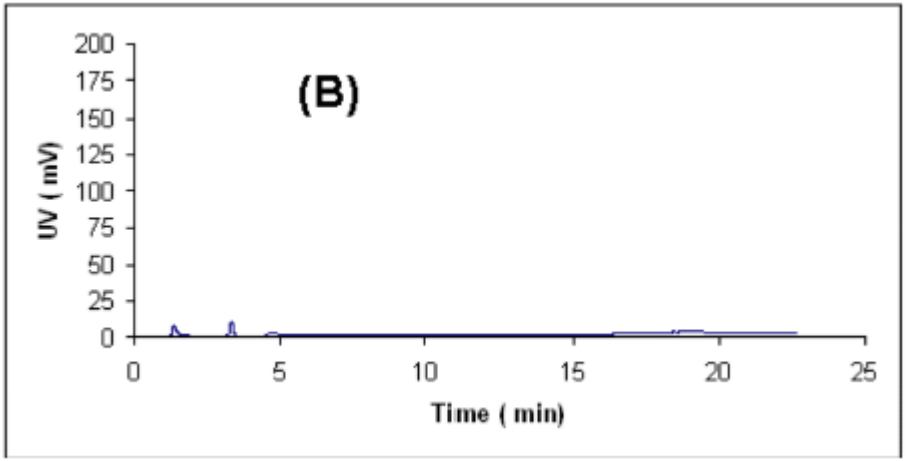
Mohammad Namavari†, **Zhen Cheng**†, **Rong Zhang**‡, **Abhijit De**†, **Jelena Levi**†, **Joshua K. Hoerner**‡, **Shahriar S. Yaghoubi**†, **Faisal A. Syud**‡, and **Sanjiv S. Gambhir***,†

†Molecular Imaging Program at Stanford (MIPS), Departments of Radiology and Bioengineering, Bio-X Program, Stanford University



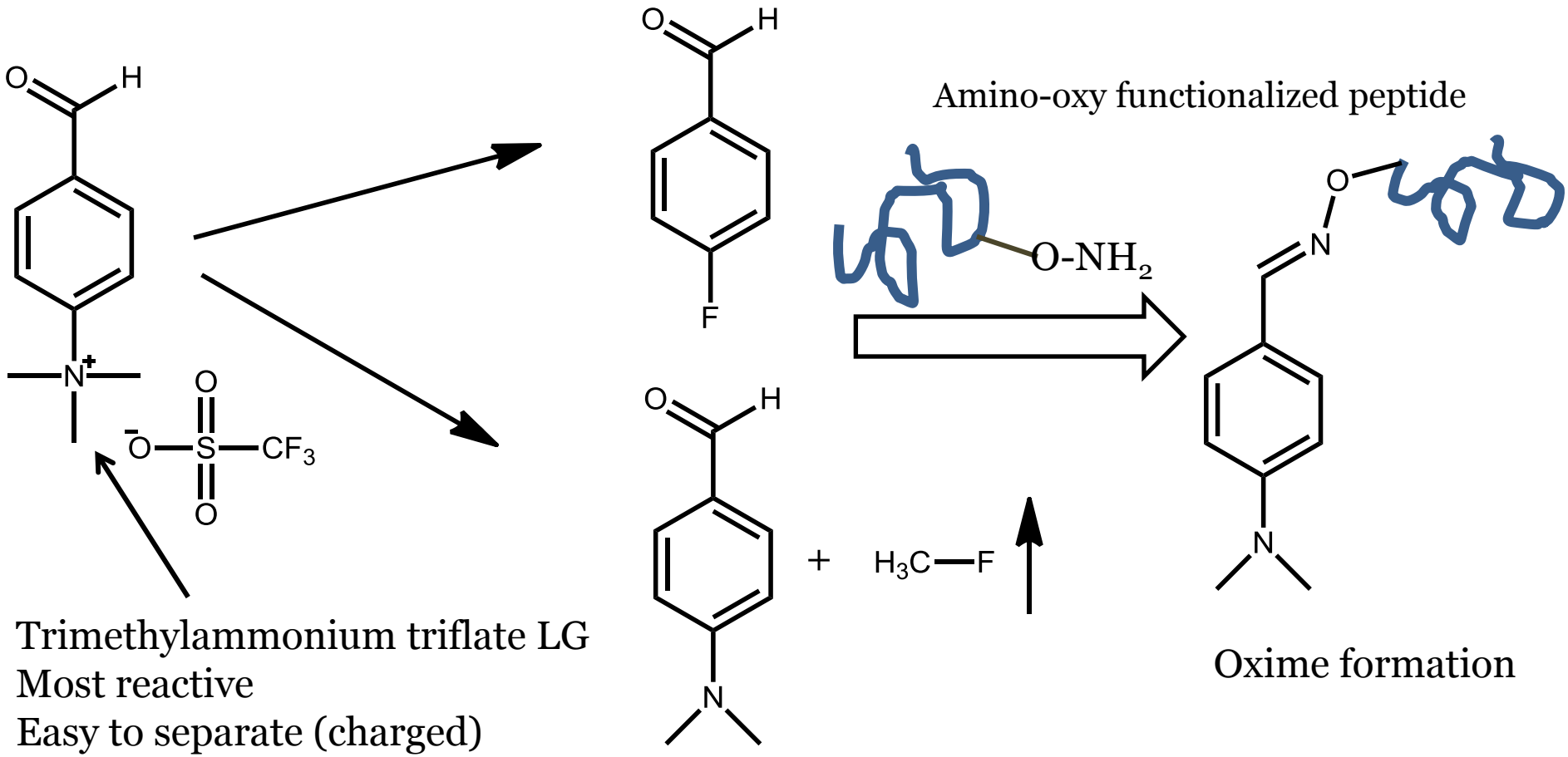
F18-labeled peptide





[^{18}F]Fluorobenzaldehyde

Fluorobenzaldehyde → site specific conjugation conjugation; away from the binding sites, retain immunoreactivity of peptide/protein



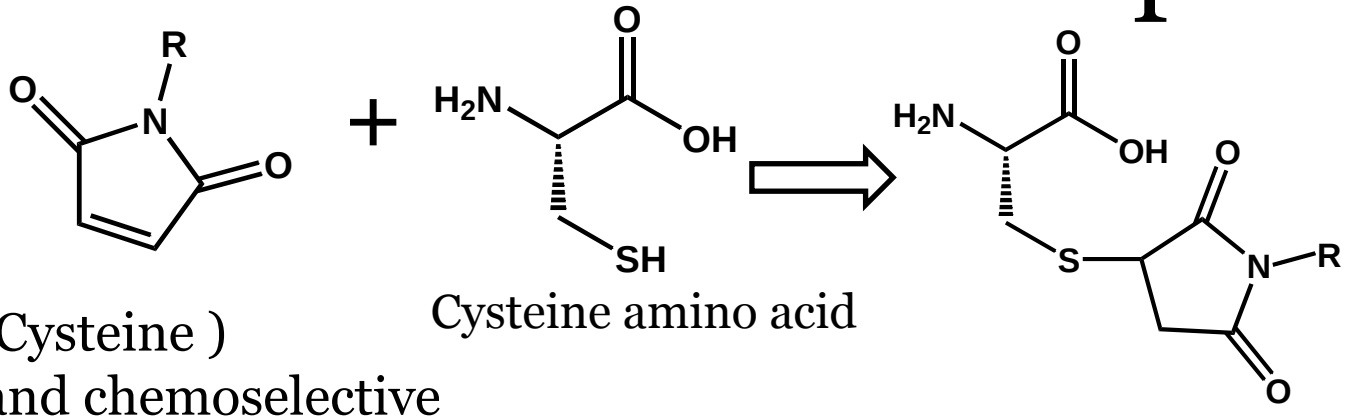
Li, X. et al. Bioconjugate Chem. 2008, 19, 1684-1688
Solin, O. et al. J Fluorine Chemistry, 2012, 143, 49-56

Prosthetic group: Maleimide

SITE SPECIFIC THIOL-REACTIVE

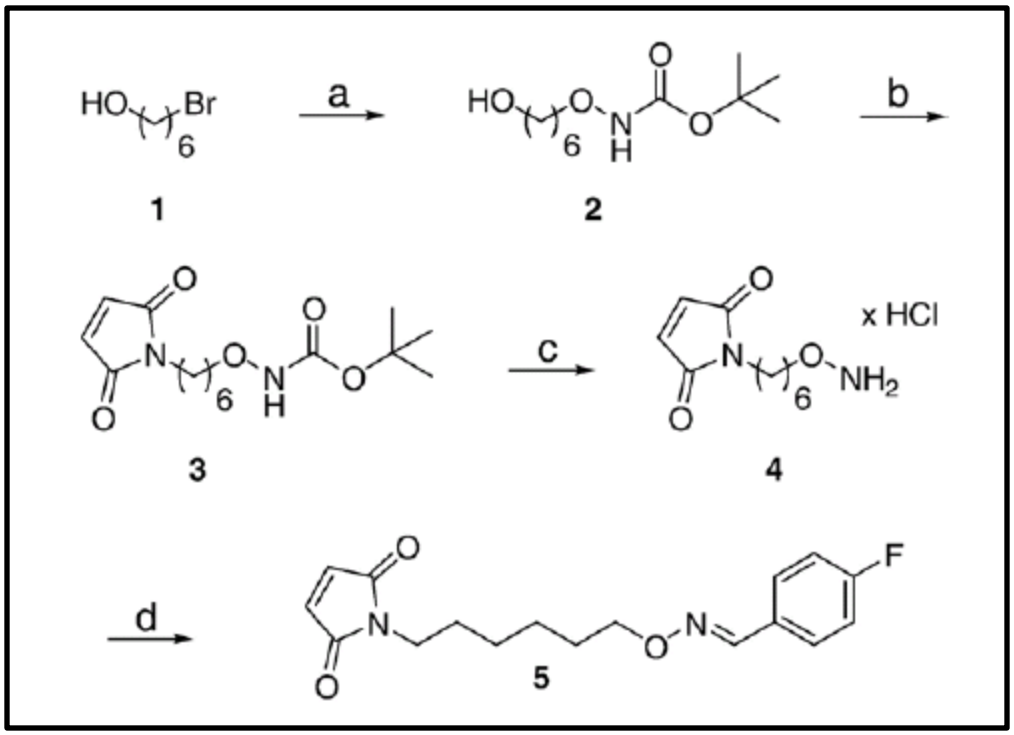
Maleimide Prosthetic Group

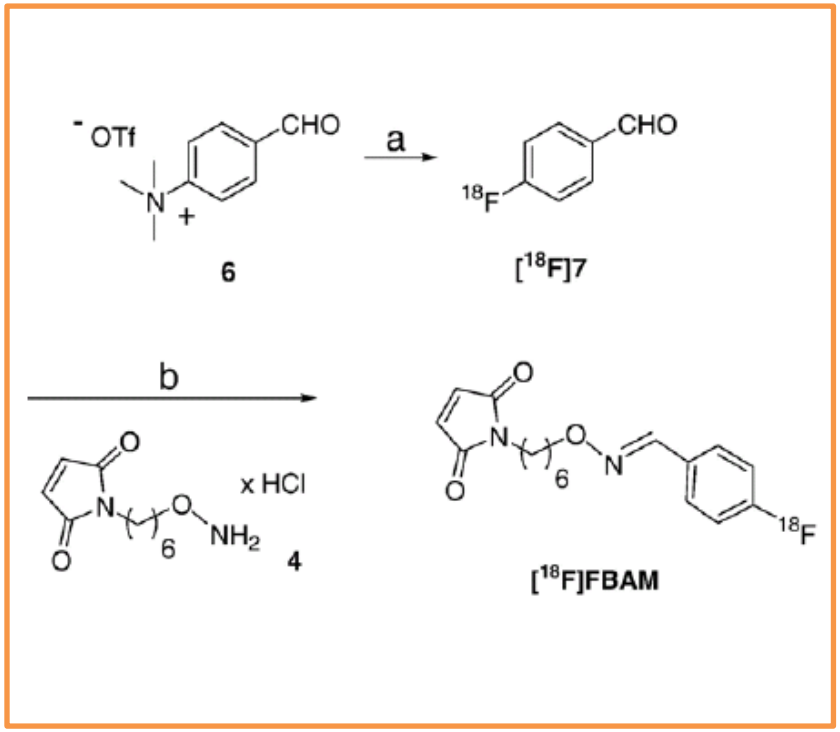
Maleimide thiol reactive functional group



Cysteine amino acid

Thiol reactive (eg.: Cysteine)
Site specific, regio and chemoselective





Shiue et al. JLCR. **1987**, 26, 287
Wuest et al. Nuc Med Biol. **2007**, 34, 5-15

Prosthetic group: Alkyne and azide

SITE SPECIFIC CLICK CHEMISTRY

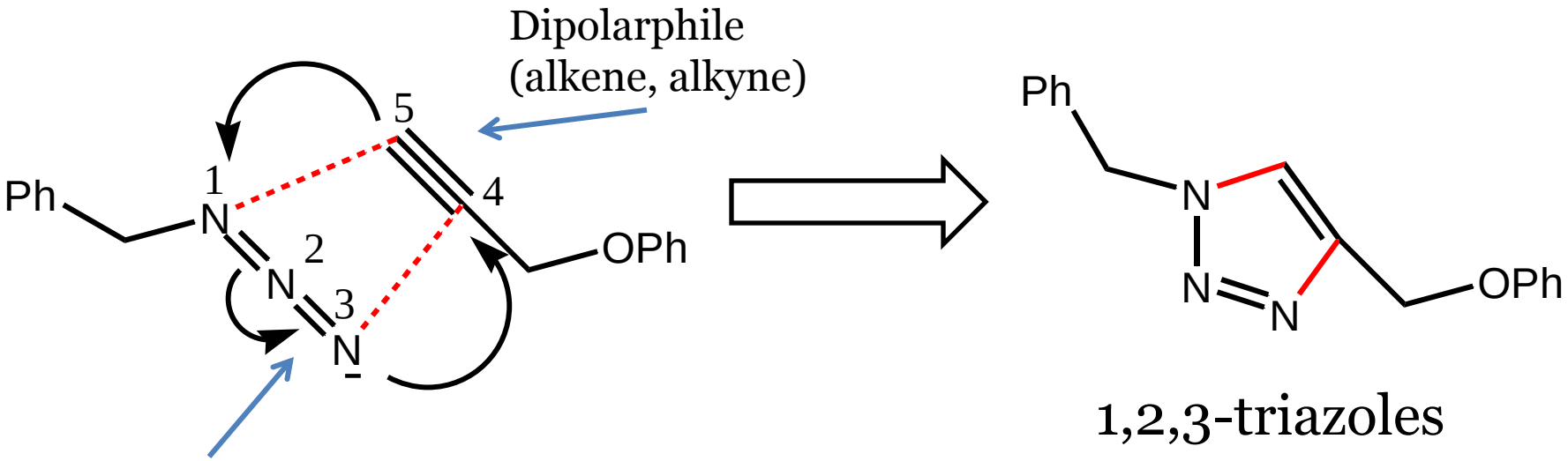
Click Chemistry

1. Mild
2. Large exotherm (driving force)
3. Stereospecific
4. High yielding
5. No side products
6. Final product is stable

Examples of Click Chemistry

- 1,3 dipolar Huisgen cycloaddition
- Thiol-ene Click reaction
- Inversed electron demand Diels Alder
- Nucleophilic substitution on strained rings

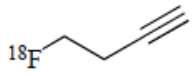
1,3-dipolar Huisgen cycloaddition



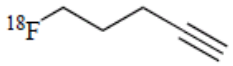
1,3-dipolar Huisgen reaction for peptide labeling

Terminal alkyne and azide → triazole (both precursors and product are stable; not easily cleaved)

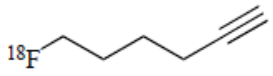
F18-labeled terminal **alkyne** prosthetic group



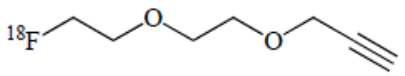
[^{18}F]Fluorobutyne (**1**)
[29]



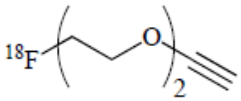
[^{18}F]Fluoropentyne (**2**)
[29, 28]



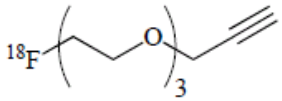
[^{18}F]Fluorohexyne (**3**)
[29, 30]



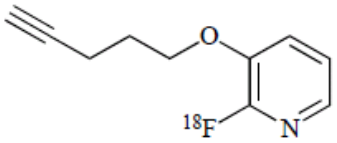
3-(2-(2-[^{18}F]fluoroethoxy)ethoxy)prop-1-yne (**4**)
[31]



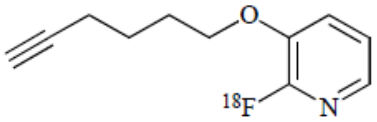
^{18}F-PEG$_2$-alkyne (**5**)
[32]



^{18}F-PEG$_3$ (**6**)
[33]

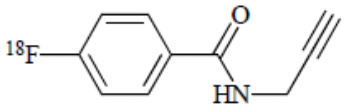


[^{18}F]FPyKYNE (**7**)
[34]

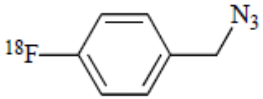


[^{18}F]FPy5yne (**8**)
[35]

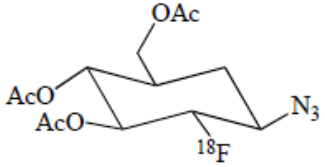
F18-labeled **azide** prosthetic group



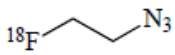
4-[^{18}F]-fluoro-*N*-(prop-2-ynyl)-benzyamide (**9**)
[36]



azidomethyl-[^{18}F]-fluoro-benzene (**10**)
[37]



6-(acetoxymethyl)-4-azido-3-[^{18}F]fluorocyclohexane-1,2-diyl diacetate (**11**)
[38]



[^{18}F]Fluoroethylazide (**12**)
[39-41]

Wangler B. et al. Curr Med Chem. 2010, 17, 1092

NEW LABELING METHODOLOGIES

Ideal F18 Radiochemistry?

Parameter	Current	Ideal (Future?)
Time	40~300 mins	ASAP
Yield (RCY)	1~60%	AHAP
Selectivity	Many side products	Single product
Purification	HPLC	None or cartridge
Reaction requirement	Azeotropic drying	Reaction in aqeuous
Reaction condition	Basic, high temp, organic solvent, high pH	In aqueous, mild temp and pH
Multistep	1-3 steps	1 step and direct

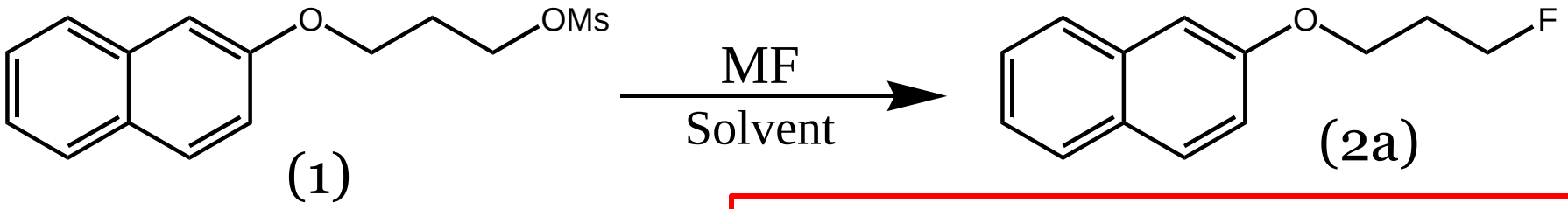
New F18 Labeling Chemistry

Features	New Chemistry
Higher yield and selectivity	Bulky protic solvent
	Diaryliodonium salts precursor Transition metal catalysis
Labeling in aqueous [18F]fluoride ion solution	Al-F
	Arylboronic acid
	Enzymatic fluorination
	Si-F
Direct labeling complex molecules	Si-F
	Transition metal catalysis
HPLC-free	Solid phase radiosynthesis
	Fluorous phase synthesis

Aliphatic substitution: (1) Bulky protic solvent; (2) ionic liquid as solvent

HIGHER YIELD AND SELECTIVITY

(1) Fluorionation in Protic Solvents



(1) (2a)

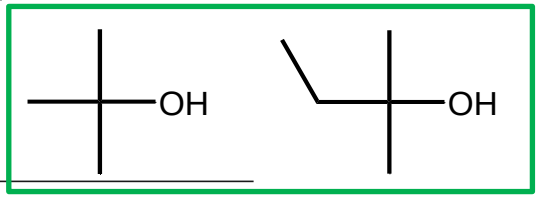
+ alcohol (2b) + alkene (2c) + ether (2d)

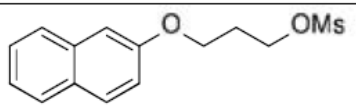
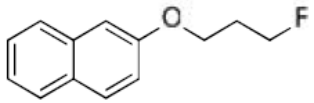
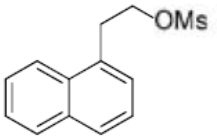
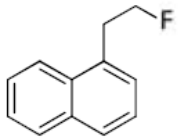
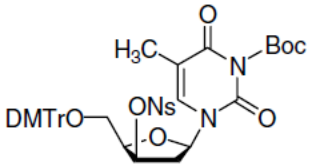
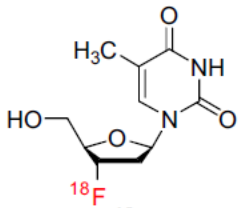
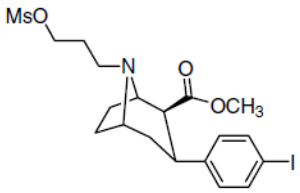
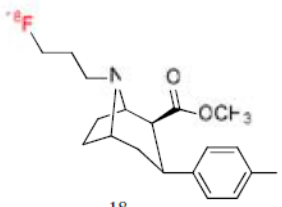
					yield of product[b] (%)				
entry	solvent	MF	temp (°C)	time (h)	1	2a	2b	2c	2d
1	*t*-BuOH	CsF	80	6	trace	92	-	-	7
2	*n*-BuOH	CsF	80	6	4[c]	64	-	-	30
3	CH_3CN	CsF	80	6	91	7[c]	-	trace	-
4	DMF	CsF	80	6	33	48	8[c]	9[c]	-
5	1,4-dioxane	CsF	80	6	94	-	-	-	-
6	benzene	CsF	80	6	97	-	-	-	-
7	*tert*-amyl alcohol	CsF	80	6	-	93	-		5(5[c])
8	*tert*-amyl alcohol	CsF	90	2.5	-	94	-	-	4(5[c])
9	*t*-BuOH	CsBr	80	6	94	4[d]	-	-	trace
10	CH_3CN	CsBr	80	6	68	32[d]	-		-
11	*tert*-amyl alcohol	RbF	90	24	13	76	-		9
12	*tert*-amyl alcohol	KF	90	24	90	trace	-		7[c]

J. AM. CHEM. SOC. 2006, *128*, 16394−16397 Cold reaction, NMR yield

Effect of solvents and examples

Precursor (RX, R-OSO_2R) —CsF, TBAF / Protic solvent→ RF



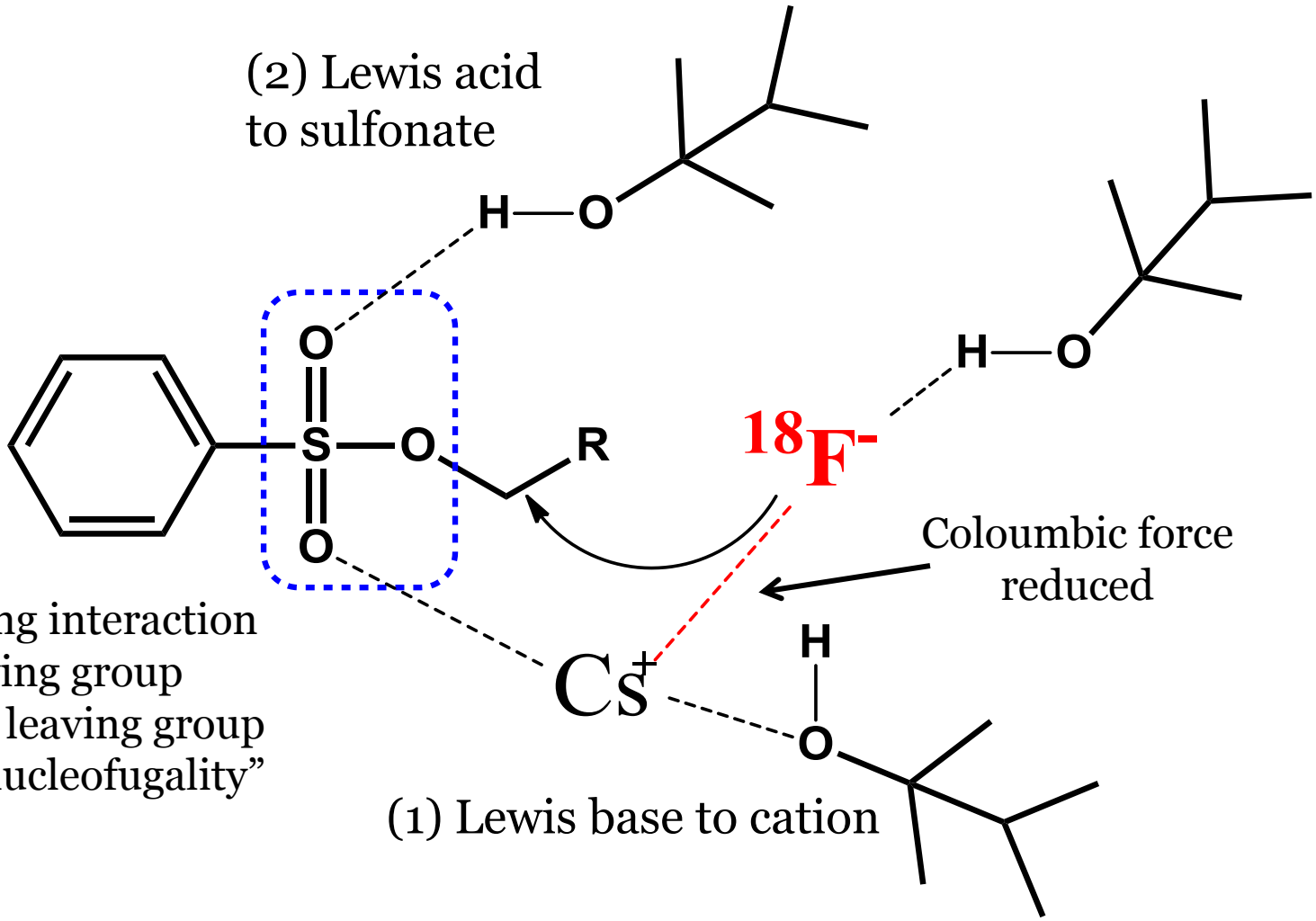
Entry	Precursor	Temp. (C)	Time (min)	Yield (%)	Product
1		80	6	92	
	CsF in *t*-BuOH				
	CsF in CH_3CN	80	6	7	
2		80	1	87	
	TBAF in *t*-BuOH				
	TBAF in in CH_3CN	80	1	33	
5		120	10	65.5±5.4	
	Method in literature	110	10	15.0±5.4	^{18}F-FLT
4		100	20	35.8±5.2	
	Method in literature	90	10	only 1%	^{18}F-FP-CIT

Kim and Chi et al.

J. AM. CHEM. SOC. 2006, *128*, 16394−16397

Proposed Mechanism

How is the fluoride ion more flexible?

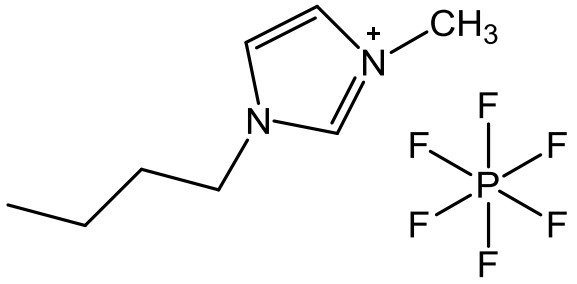


Lee et al. *J Phys Chem A,* **2007**, 111,

(2) Fluorination in Ionic Liquid

Definition:

- Salt in liquid state at room temperature
- Low vapor pressure
- As green solvent;
- ion-pairs– moderate conductivity,
- AQ and organic miscibility→ functionalization, anion
- catalytic effect

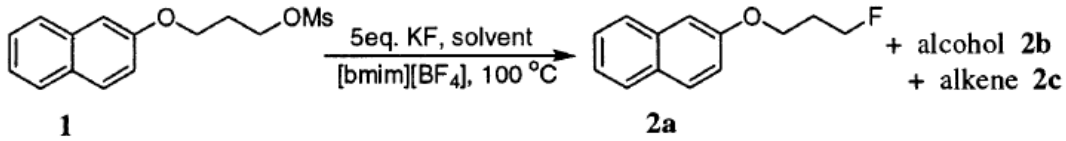


<u>Ionic liq in Nucleophilic fluorination</u>

- Water tolerant < 10%
- Eliminate need for azeotropic drying
- Accelerate reaction rate
- Improve selectivity→ Reduce side products

Controlled exps w/o IL; low yield

Table 1. Fluorinations of Mesylate **1** with KF under Various Reaction Conditions[a]



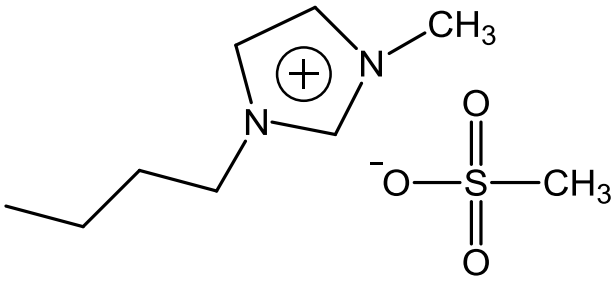
1 **2a**

entry	[bmim][BF_4] mL (equiv)	CH_3CN (mL)	H_2O (μL)	raction time (h)	yield of product (%)[b] 1	2a	2b	2c
1	5	–	0	2	–	85	–	10
2	5	–	90 (5 equiv)	1.5	–	92	–	–
3	3.2	1.6	90	1.5	–	93	trace	–
4	1.6	3.2	90	1.5	–	94	–	–
5	3	1.5	500	1.5	–	88	6	–
6	1	4	90	1.5	–	92	–	–
7	0.57 (3)	4.4	90	3	–	91	trace	–
8	0.19 (1)	4.8	90	6	–	89	trace	–
9	0.1 (0.5)	5	90	12	trace	84	8	trace
10	–	5	0	24	86	trace	–	–
11	18-crown-6 (2)	5	0	24	53	40	–	–

[a] All reactions were carried out on a 1.0 mmol reaction scale of mesylate **1** using 5 mmol of KF at 100 °C. [b] Isolated yield.

Mechanism: Theoretical Modeling

S_N2 mechanism with [bmim][OMs]



"Flexible" fluoride ion

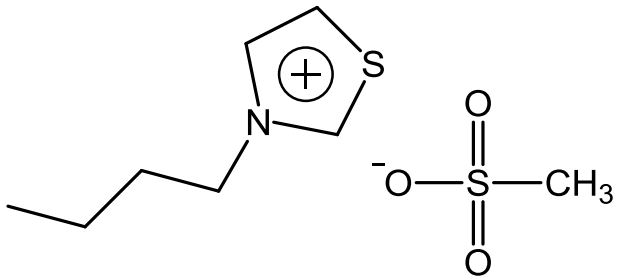
[bmim]+

Substrate

[OMs]-

1.940
1.957
1.684
3.192
3.088
3.329
3.188

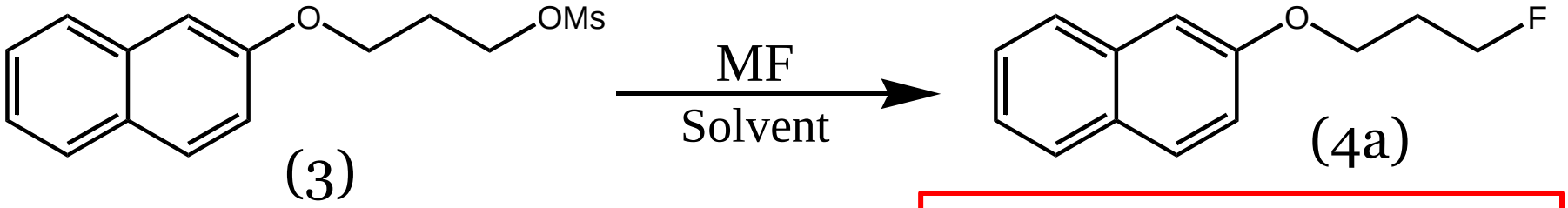
[N-butylthiazolium][Oms]



N/R; Acidic proton

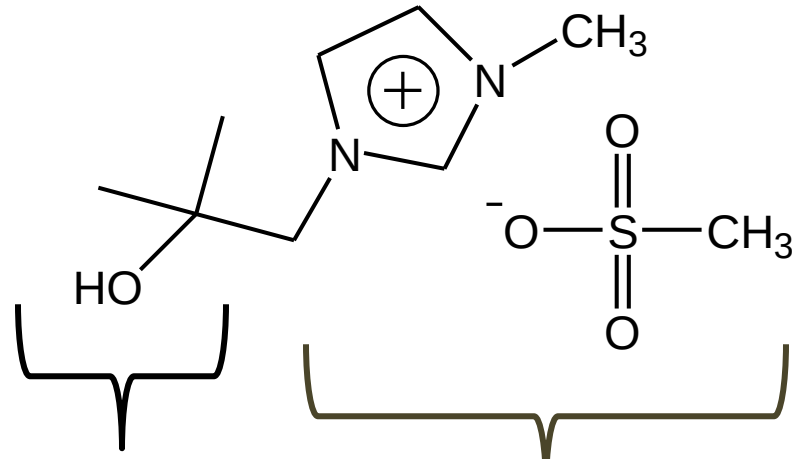
Lee et al. *Org Biomol. Chem.*, **2011**, 9, 418

Protic alcohol and IL in ONE molecule



(3) (4a)

+ alcohol (4b) + alkene (4c)



Protic alcohol
Better Lewis acid

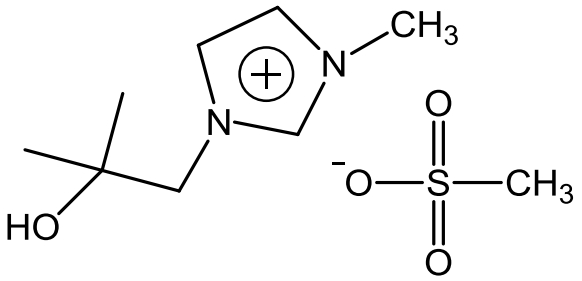
Ionic liquid
Better Lewis base

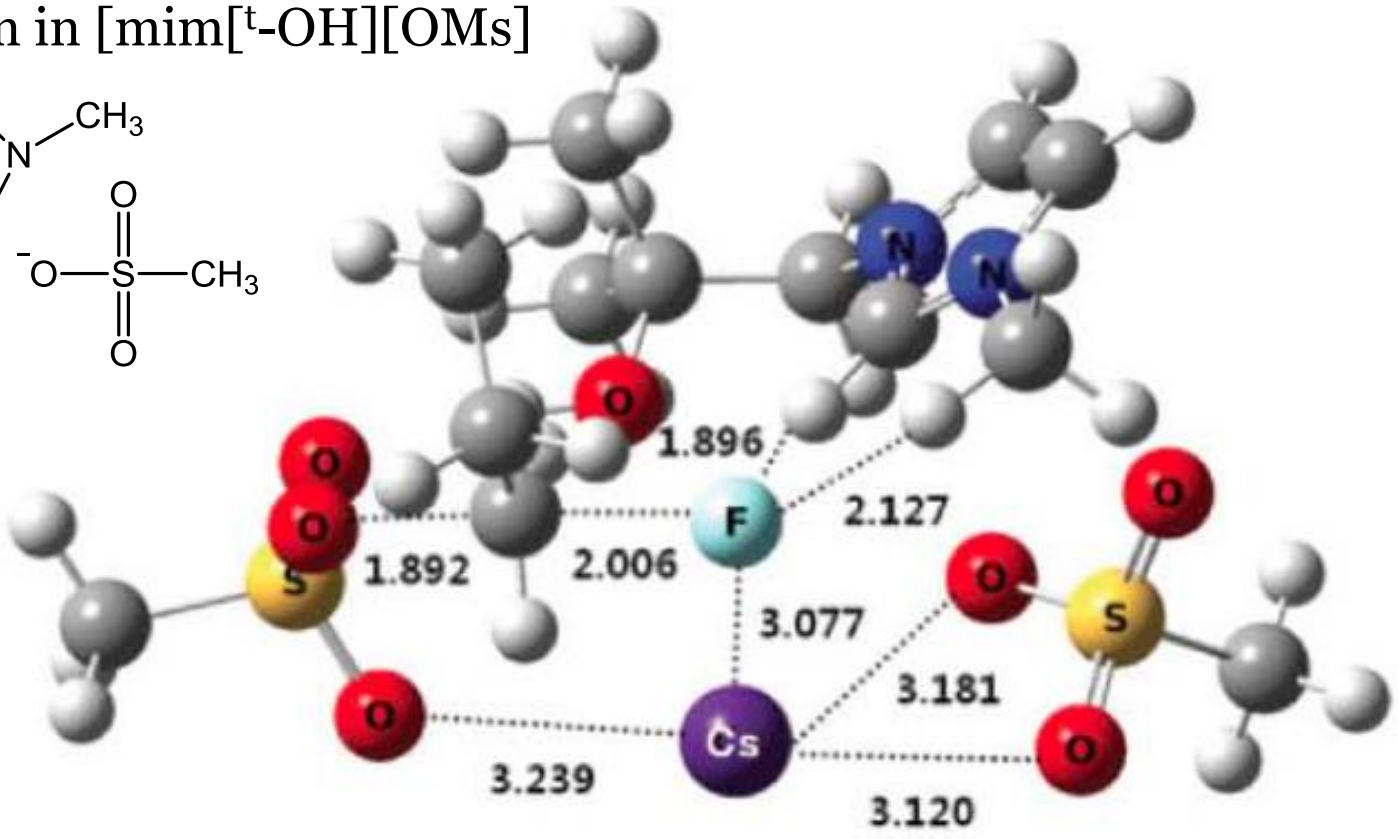
			yield[b] (%)			
entry	imidazolium salt	equiv	3	4a	4b	4c
1	[bmim][OTf]	0.5	80	18	2	–[c]
2	[bmim][BF_4]	0.5	73	24	3	–
3	[bmim][OMs]	0.5	64	32 (30)[d]	4	–
4	**2a**	0.5	–	100 (97)[d]	–	–
5	**2b**	0.5	11	85	3	–
6	**2c**	0.5	28	70	2	–
7	**2d**	0.5	42	56	2	–
8[e]	**2a**	3.0	–	93	7	trace
9[f]	**2a**	3 mL	–	84	10	6
10[g]	*t*-BuOH	0.5	77	23 (22)[d]		–
11[h]	[bmim][OMs]/*t*-BuOH	0.5/0.5	58	40 (37)[d]	2	trace
12[i]	**2a**	0.5	–	83	5	12

Chi et al. *Org Lett*. **2008**, 10, 733

Synergestic Effect of Ionic Liquid and Tertiary Alcohol

S_N2 mechanism in [mim[^{t}-OH][OMs]





Computational modeling of the ionic liquid-alcohol, Cs-F and substrate

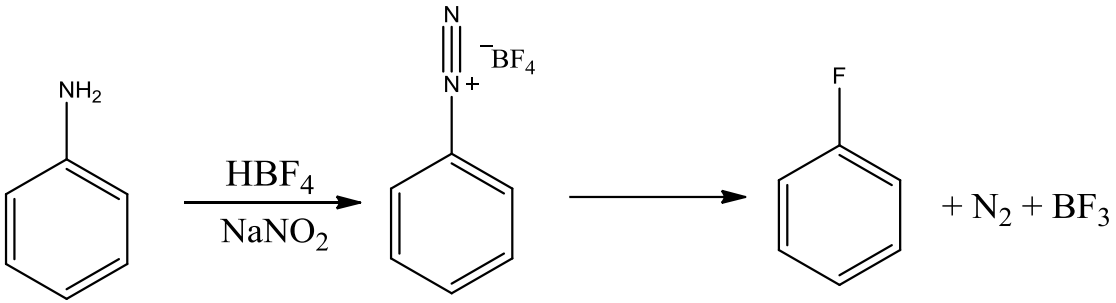
- Lower energetic barrier
- Retard acidic H—F interactions
- Acidic OH interacts with LG
- Retards E2 side reaction

Aromatic substitution: (1) Iodonium salt precursor, (2) Transition metal catalysis

HIGHER YIELD AND SELECTIVITY

Conventional n.c.a. Aromatic Substituion

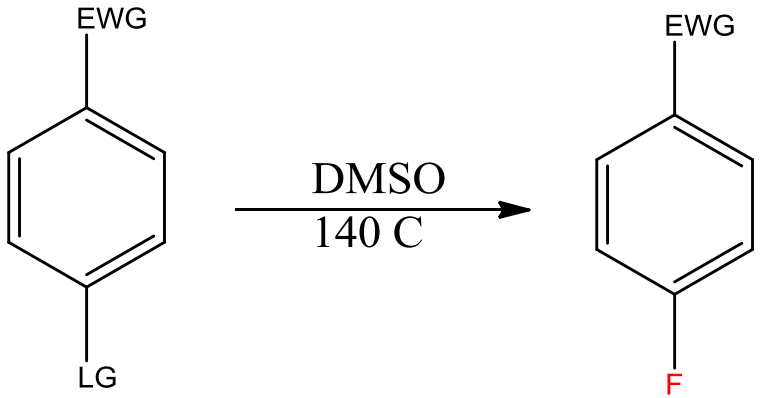
Balz-Schiemann reaction



Limitation

- Harsh condition
- High temperature
- low yield

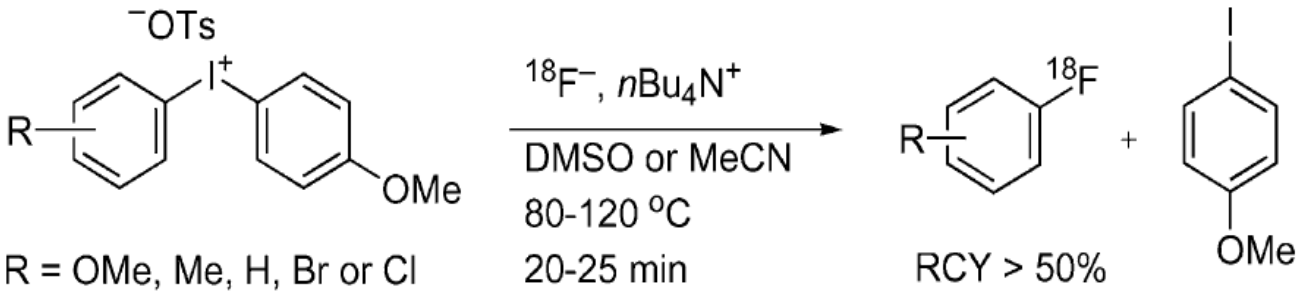
S_{NAr}

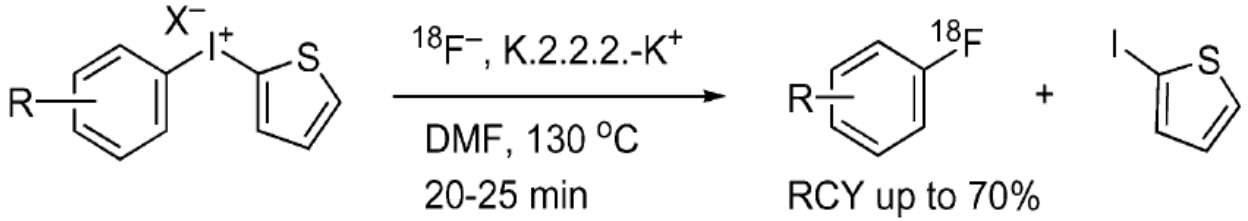


- Electron deficient arenes only
- High temperature
- Low yield

(1) Diaryliodonium Salt Precursor

- Electron rich and deficient arene
- Ortho effect; substitution directed to the least electron-rich ring (chemoselective)





R = H, 4-Me, 2-OMe, 3-OMe, 4-OMe, 4-OBn, 4-I, 4-Br, 4-Cl
X = Br, I, OTs, OTf

** Handling of diaryliodonium salt

Rules

Table 2 [^{18}F]Fluoroarene synthesis

Run	Diaryliodonium salts **5**					Radiochem. yield[a] (%)	Radiochem. loss[b] (%)	Product ratio	
	Cpd.	R-aryl	R′-aryl	A^-	Salt			**3A**	**3B**
1	**a**	C_6H_5	C_6H_5	$CF_3SO_3^-$	KF-APE 2.2.2	80	NM	1.00	—
2	**b**	C_6H_5	4-$CH_3OC_6H_4$	$CF_3SO_3^-$	KF-APE 2.2.2	96	NM	1.00	0
3	**c**	C_6H_5	4-$Bu^tOC_6H_4$	$CF_3SO_3^-$	KF-APE 2.2.2	95	NM	1.00	0
4	**d**	C_6H_5	4-BrC_6H_4	$CF_3SO_3^-$	KF-APE 2.2.2	95	NM	0.30	0.70
5	**e**	C_6H_5	4-IC_6H_4	$CF_3SO_3^-$	KF-APE 2.2.2	94	NM	0.15	0.85
6	**f**	C_6H_5	4-FC_6H_4	$CF_3SO_3^-$	KF-APE 2.2.2	92	NM	0.10	0.90
7	**g**	C_6H_5	2,4,6-$(CH_3)_3C_6H_2$	$CF_3SO_3^-$	KF-APE 2.2.2	96	NM	0	1.00
8	**h**	2-$CH_3C_6H_4$	4-$Bu^tC_6H_4$	$CF_3SO_3^-$	CsF	60	20	0.80	0.20
9	**i**	2-$CH_3C_6H_4$	4-$CH_3C_6H_4$	$CF_3SO_3^-$	CsF	50	26	(1.00)[c]	
10	**j**	2-$CH_3C_6H_4$	4-$CH_3C_6H_4$	$CF_3CO_2^-$	CsF	45	22	(1.00)[c]	
11	**k**	2-$CH_3C_6H_4$	4-$CH_3OC_6H_4$	$CF_3CO_2^-$	CsF	64	9	1.00	0
12	**l**	3-$CH_3C_6H_4$	4-$CH_3OC_6H_4$	$CF_3SO_3^-$	CsF	66	10	1.00	0
13	**m**	2-$CH_3C_6H_4$	2,4,6-$(CH_3)_3C_6H_2$	$CF_3SO_3^-$	CsF	65	11	0.20	0.80
14	**n**	2,4,6-$(CH_3)_3C_6H_2$	2,4,6-$(CH_3)_3C_6H_2$	$CF_3SO_3^-$	CsF	50	20	(1.00)[c]	
15	**o**	2,4,6-$(CH_3)_3C_6H_2$	4-$CH_3OC_6H_4$	$CF_3CO_2^-$	CsF	67	8	1.00	0
16	**p**	3,5-$(CH_3)_2C_6H_3$	4-$CH_3OC_6H_4$	$CF_3SO_3^-$	CsF	45	19	1.00	0
17	**q**	4-$(CH_3)_3CCH_2C_6H_4$	3-$CH_3OC_6H_4$	$CF_3SO_3^-$	CsF	66	9	1.00	0

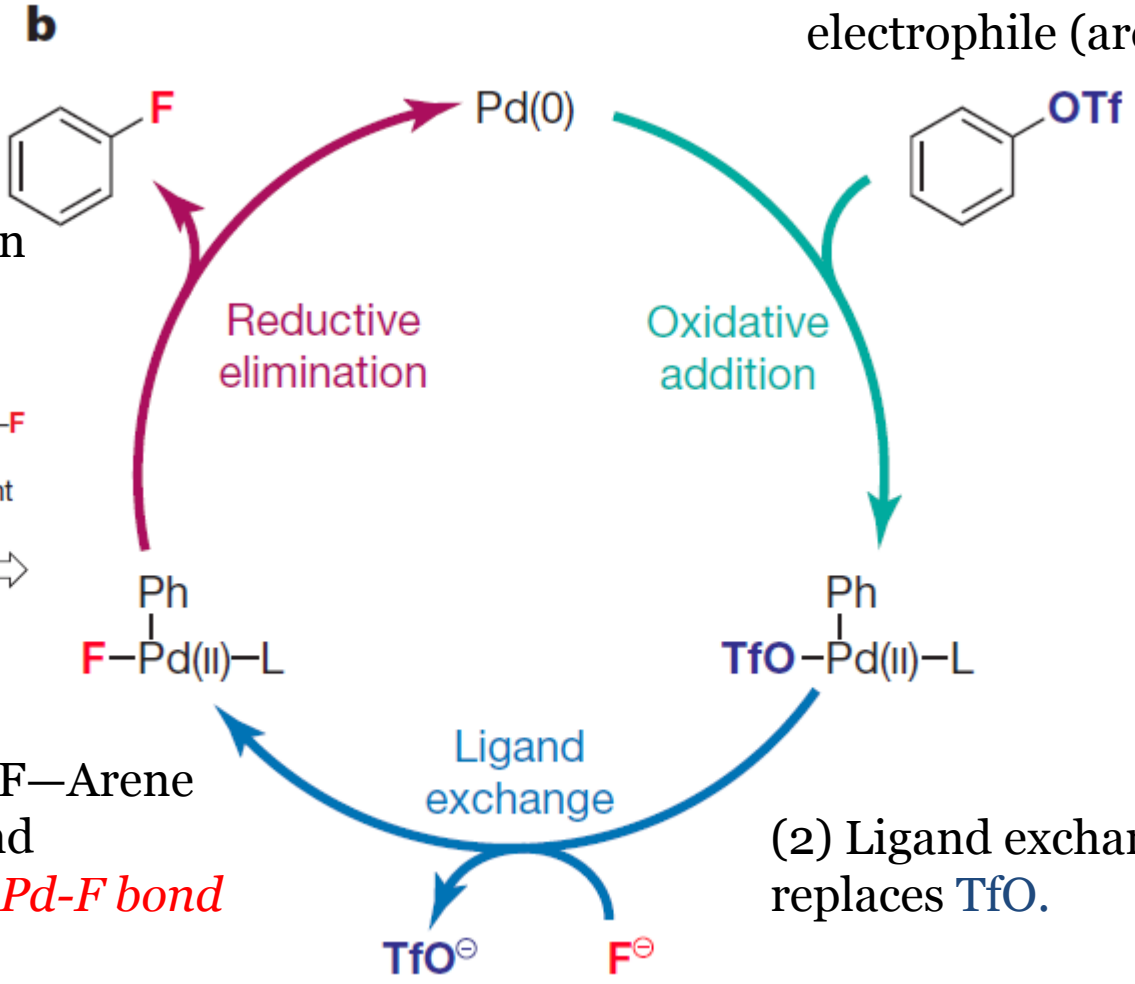
Substitution favored on the ***most*** electron deficient arene. Strong EWG (alkyl, alkoxy, hydroxyl)

Mixtures when arene is moderately activated (eg.: F, Br, I)

N/R when both arenes are substituted

(ii) Transition metal catalysis for C-F

(1) Oxidative addition: metal inserted into σ-bond electrophile (arene)



(4) Reductive elimination
** challenging with C-F

Ease of C–Y reductive elimination

C–C > C–N > C–O > C–F

Early development (since 1960s) Recent development (2000s)

Increasing difficulty in Pd-catalysed C–Y cross-coupling

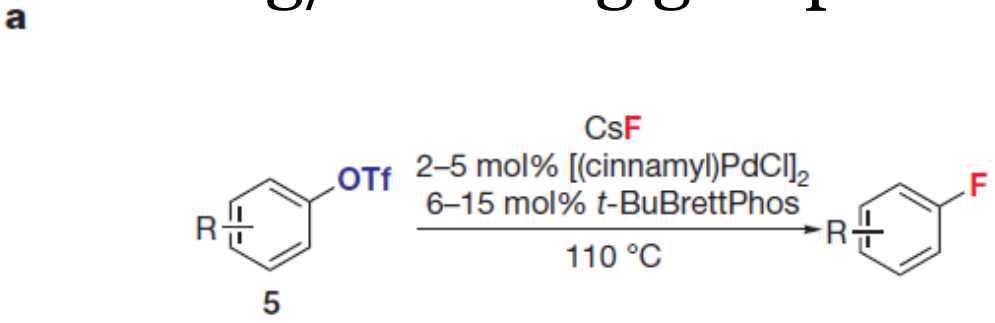
(3) Coupling of F—Arene form new σ-bond
High polarized Pd-F bond

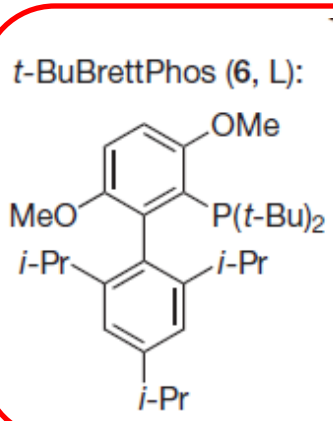
(2) Ligand exchange, F replaces TfO.

Ritter et al. *Synthesis*. **2010**, 11, 1804

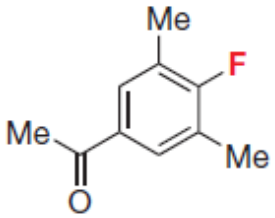
Ritter et al. *Nature*. **2011**, 473, 470

Regioselective aromatic fluorination, without the need of activating/directing group

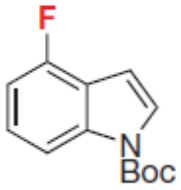




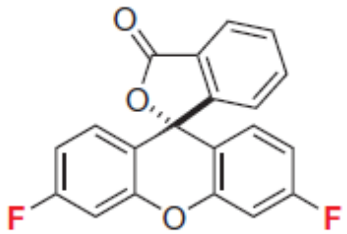
Bulky ligand
Promote reductive elimination of C-F from the arylpalladium (II) complex



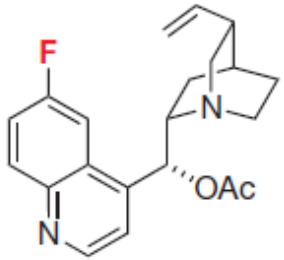
83%



73%



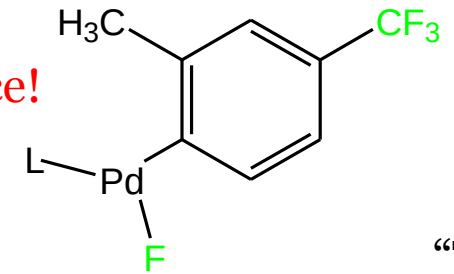
73%

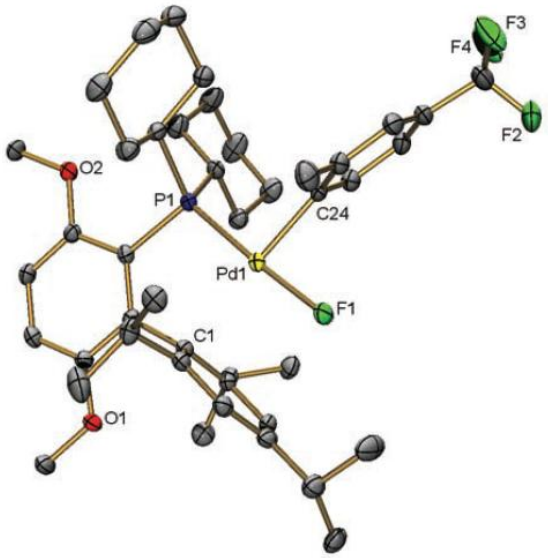


70%

Wide scope of aryl triflates: electron rich and poor arene, hindered substrates, heteroatoms

- Requires stoichiometry fluoride source!





"T-shaped" arylpalladium fluoride complex

Donald A. Watson, *et al.*
Science **325**, 1661 (2009)

Revisit Fluorination Methods

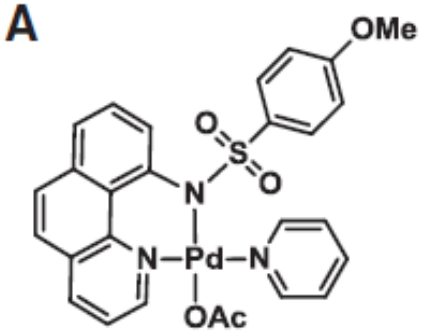
Electrophilic fluorination

- F_2 source- difficult to handle
- Low specific activity; dilution with F19
- Highly reactive towards arenes

Nucleophilic fluorination

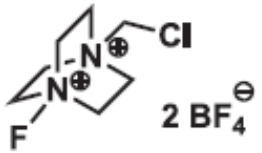
- F^- ion source in H2O, ease of handling
- High specific activity
- Water hydration; need activation
- Not reactive to unactivated arenes

Fluoride Ion Reversal in Polarity

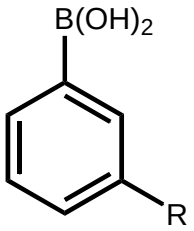


7 = [Pd]–OAc

Electrophilic fluorine source

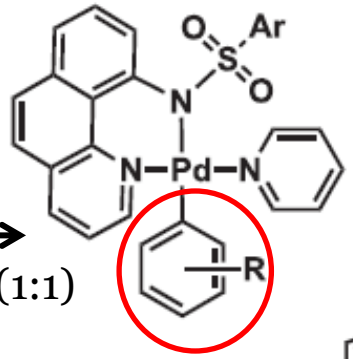


(F-TEDA)

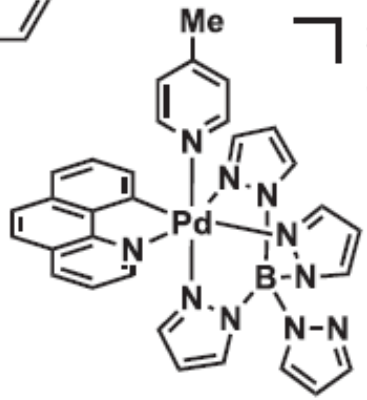


Benzene/MeOH (1:1)
K_2CO_3
23 °C, 10 h

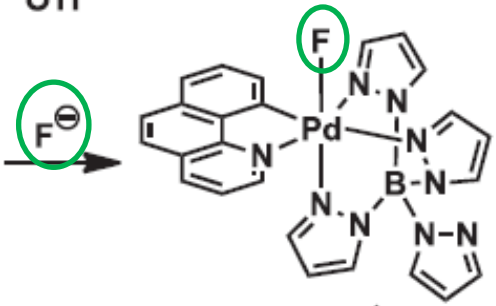
" A"Pd-aryl complex ***electrophilic fluorination***



"B" Pd-F complex



1



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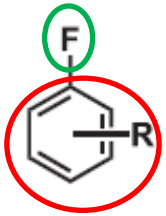
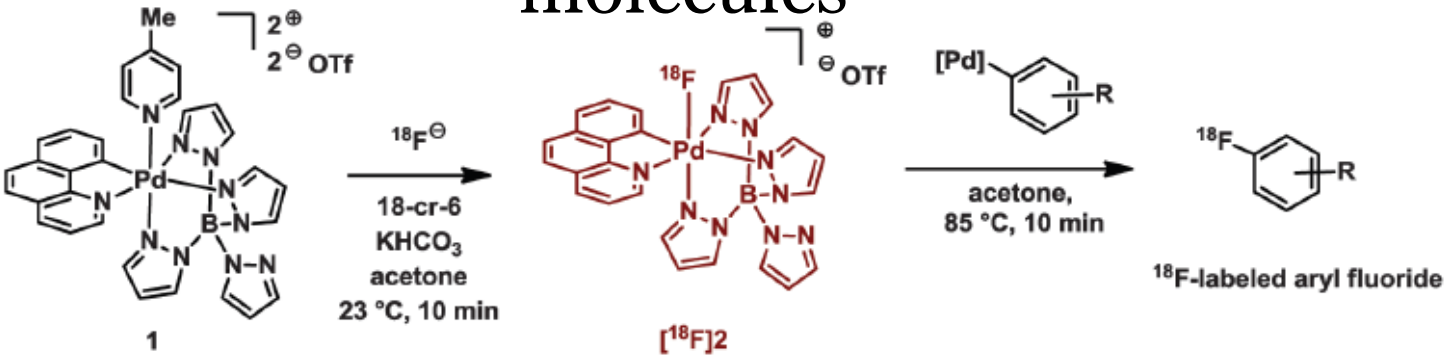
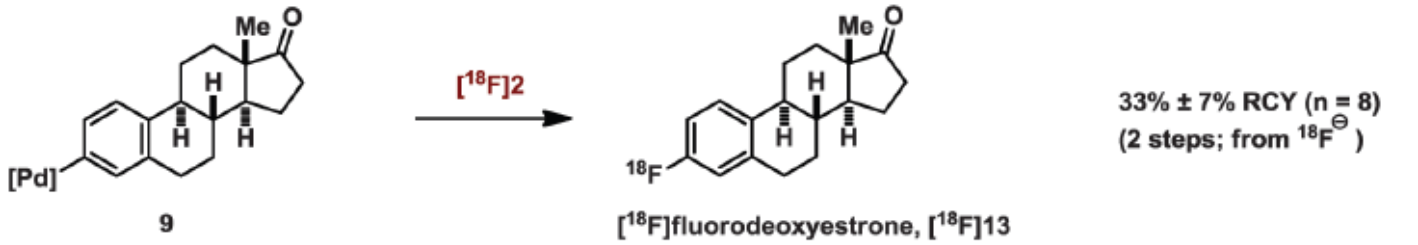


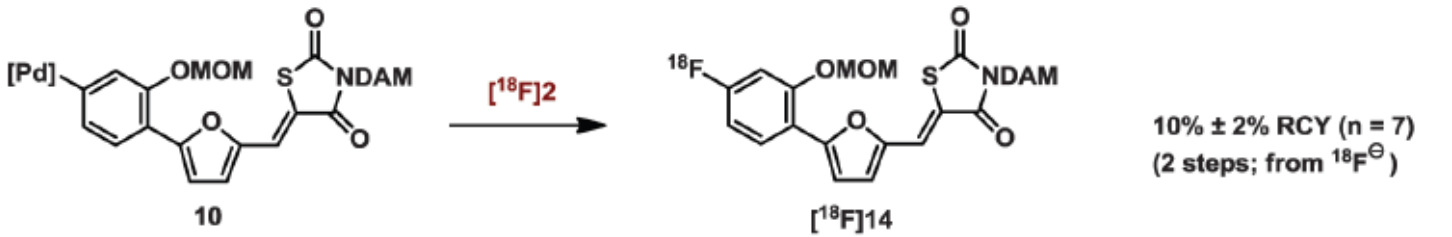
aryl fluoride

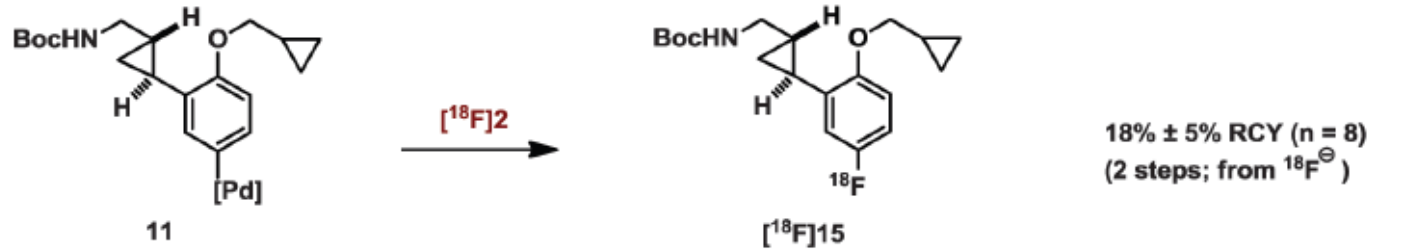
Ritter et al. *Science* **2011**, 334, 639.

Late-stage/Direct radiofluorination of complex molecules









Eliminate azeotropic drying using new F18-prosthetic group chemistry

N.C.A RADIOLABELING IN AQUEOUS SOLUTION

Fluorination in Aqueous media

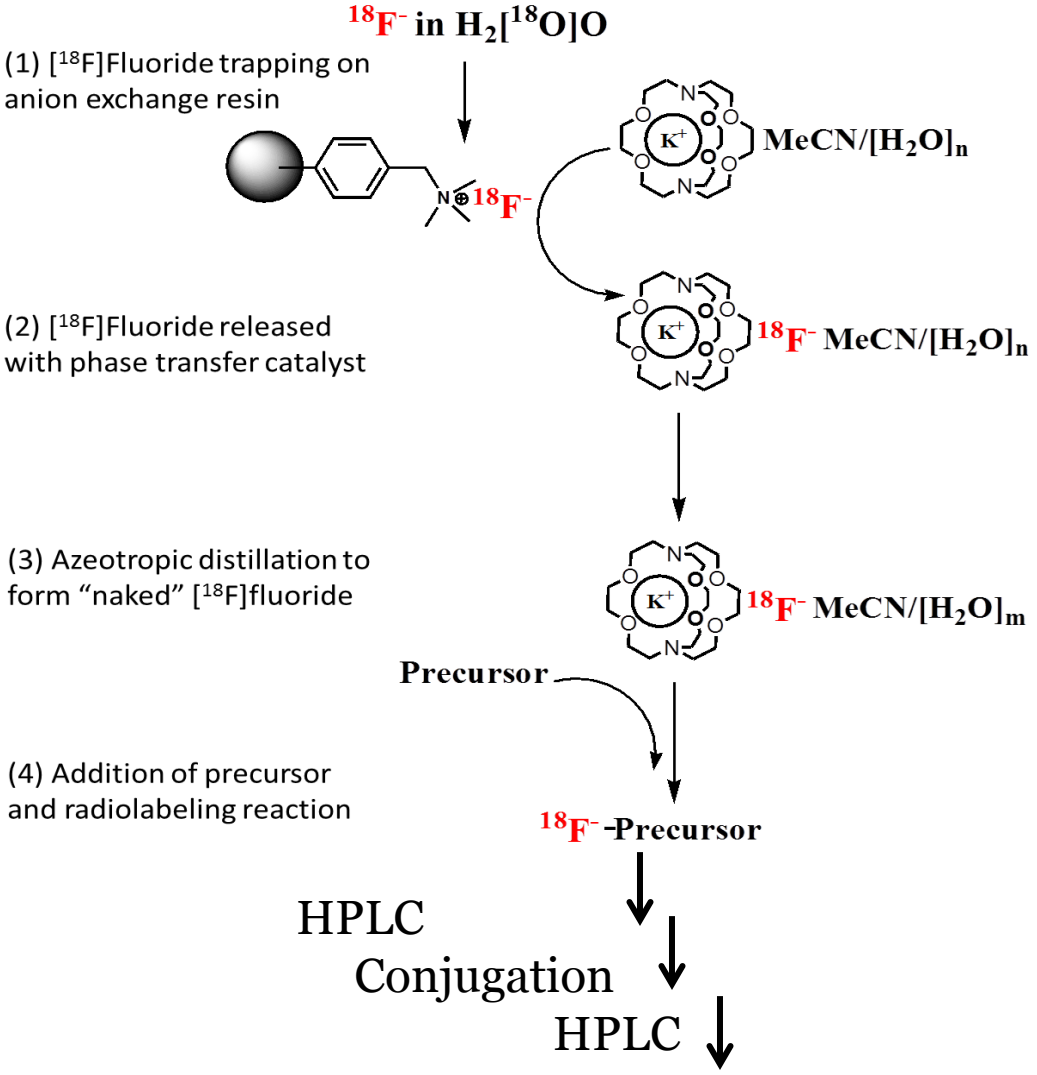
1. Si-F chemistry
2. Al-F chemistry
3. Enzymatic fluorination

Features:

- Preparation of *F18-prosthetic group* directly in aqeuous media.
- Or *direct labeling* with F18 ion in aqueous.
- Eliminate lengthy azeotropic drying steps
- Reduce radiolabeling time

Conventionally

Radiolabeling peptide/protein with n.c.a F18

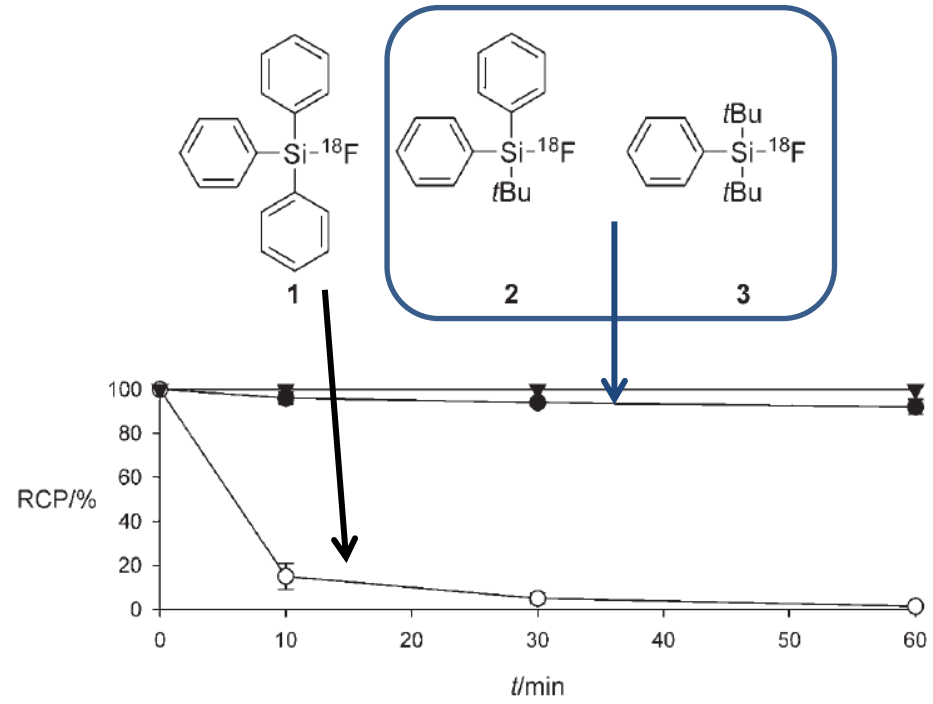


Organofluorosilanes (Si-[^{18}F]F)

Schirrmacher et al. App Sci. 2012. 2, 277

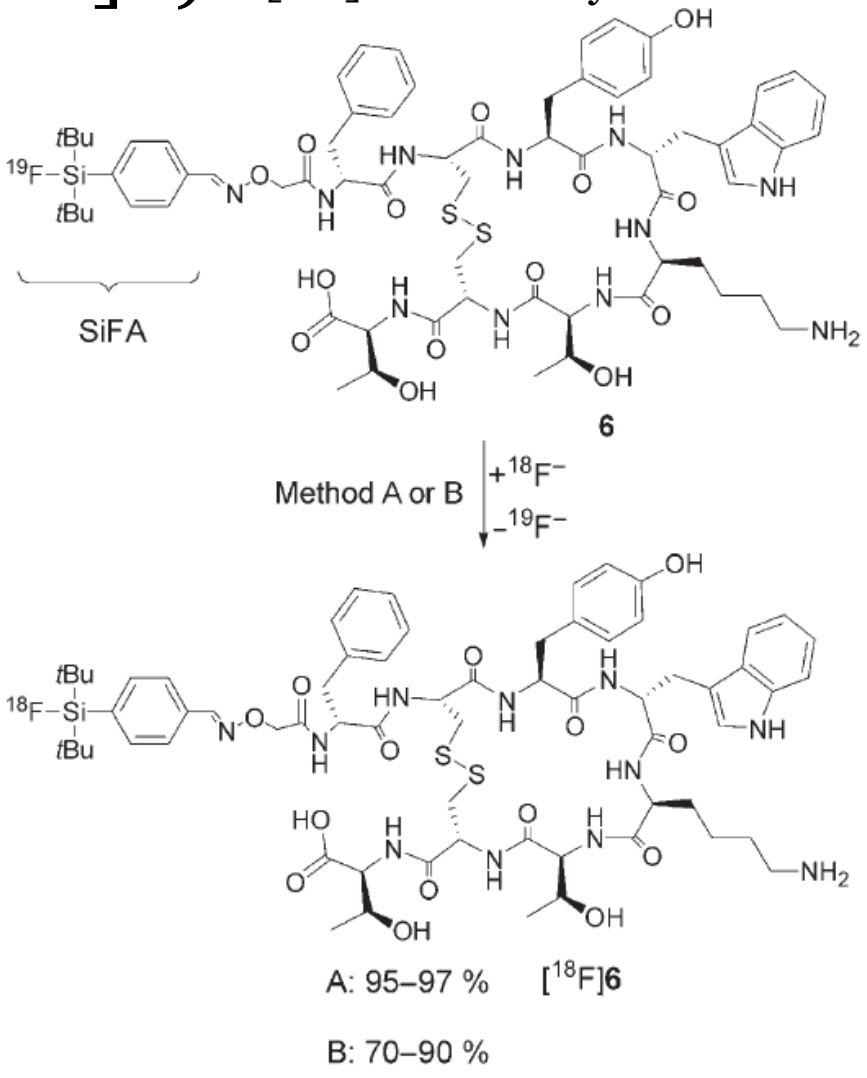
Si-F bond: 153-167 kcal/mole
Mild reaction conditions
Extremely specific
No by-product; cartridge purification
Labeling in directly in [^{18}F]F$^-$/[^{18}O]H_2O

Isotopic exchange; exchange efficiencies depend on substituent on aromatic ring



Stability of R_3Si-F in human

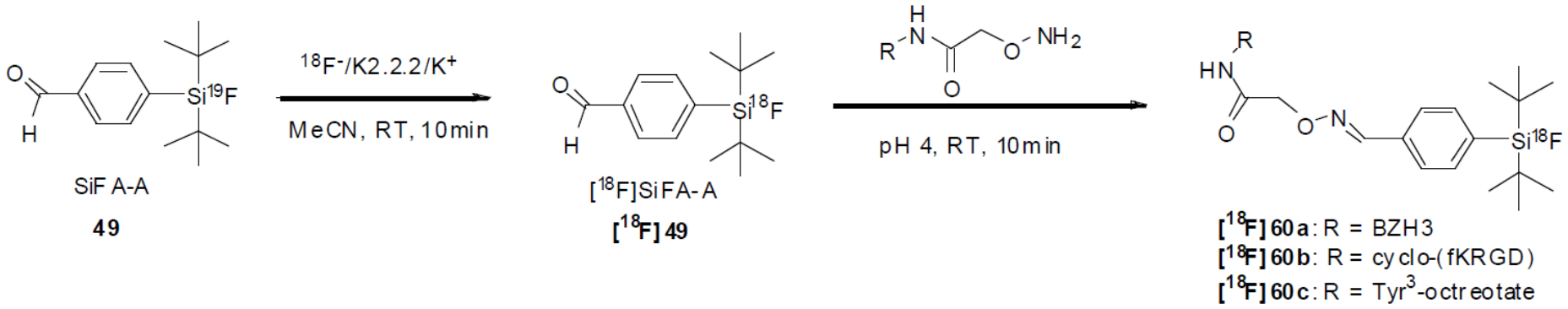
[^{18}F]-labelled Tyr^3-octreotate



Method A: [^{18}F]F$^-$/K_{222}/K^+, MeCN, RT, 10-15 mins
RCY: 95 – 97%
Method B: [^{18}F]F$^-$/[^{18}O]H_2O, MeCN, 95 °C, 30 mins
RCY: 70 – 90%
Specific act ~ 108 mCi/µmole

Organofluorosilanes in Radiochemistry

(i) Isotopic exchange method; 2 steps→ improved specific activities ~ 1 Ci/umole



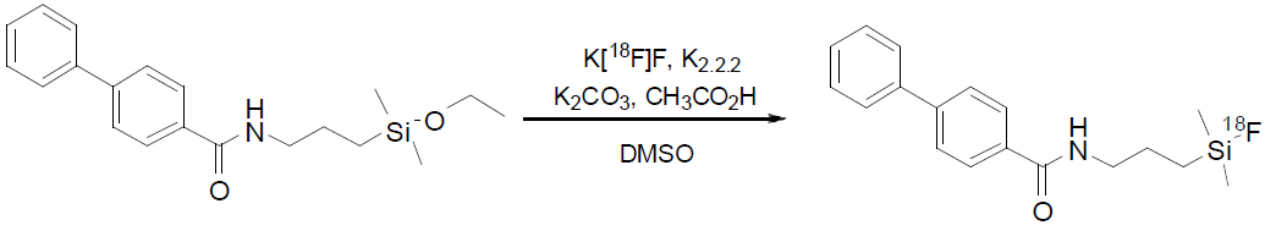
SiF A-A
49

[^{18}F]SiFA-A
[^{18}F] 49

[^{18}F] 60a: R = BZH3
[^{18}F] 60b: R = cyclo-(fKRGD)
[^{18}F] 60c: R = Tyr^3-octreotate

Overall RCY = 12 %

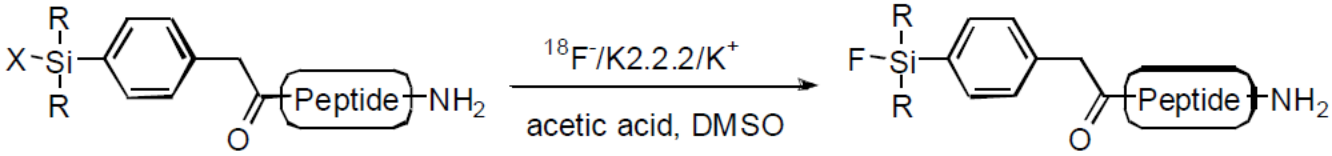
(ii) Organosilane substrate with hydroxyl and ethoxyl leaving group



9a

[^{18}F]10a

F18 incorporation ~ 50%
Overall RCY: 15%



65a: R = *iso*-Propyl, X = OH
65b: R = *tert*-Butyl, X = H

[^{18}F]66a: R = *iso*-Propyl
[^{18}F]66b: R = *tert*-Butyl

F18 incorporation ~ 37%
Overall RCY: 7%
Specific act: 1.6 Ci/µmole

Al-^{18}F Complex

$Al^{3+} + F^- \rightarrow AlF^{2+}$ chelates → AlF^{2+}

*eliminate azeotropic drying

Conventional workflow	The Al-F chemistry
F-18 drying	Al-[^{18}F]F complex
fluorination → prosthetic group	Sep-Pak
HPLC purification	Conjugation
Conjugation	HPLC purification
HPLC purification	

1 – 3 hours

1 hour

$AlCl_3$ hexahydrate + [^{18}F]fluoride/H_2O

↓

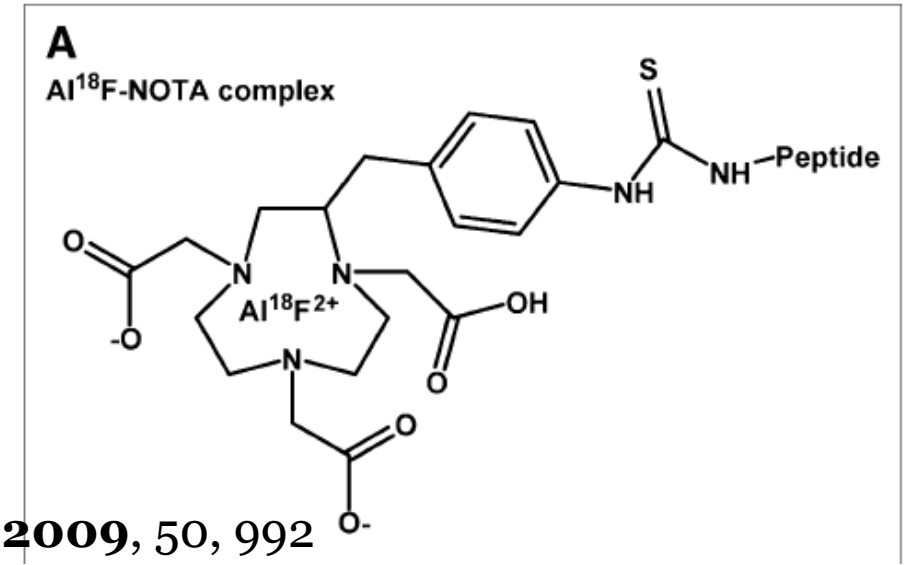
Sep-Pak

↓

$[Al^{18}F]^{2+}$ + NOTA-peptide

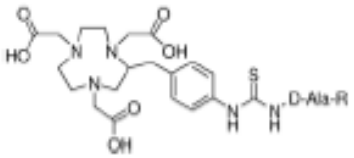
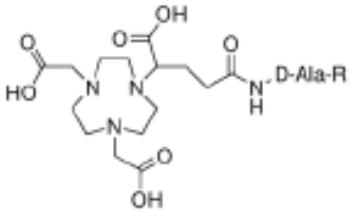
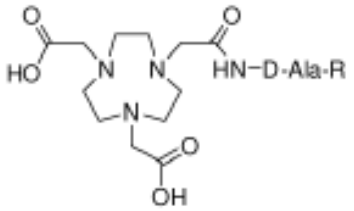
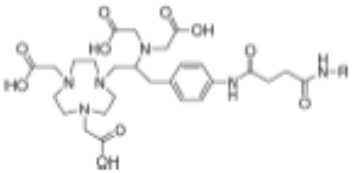
↓

100 °C
15 mins



McBride et al. JNM **2009**, 50, 992

Improved ^{18}F-Labeling with $[Al\text{-}F]^{2+}$ chelate complex

PEPTIDE	Structure	Maximum ^{18}F-Labeling Yield
IMP449		44%
IMP460		5.8%
IMP461		31%
IMP467		87%

Optimization

1. Reduced volume
2. Concentration of chelates (>40 nmoles)
3. Concentration of peptide (>10 nmoles)
4. Labeling time (>5 mins)
5. Labeling temp (>100 C)
6. pH 4~5 (yield 70%)

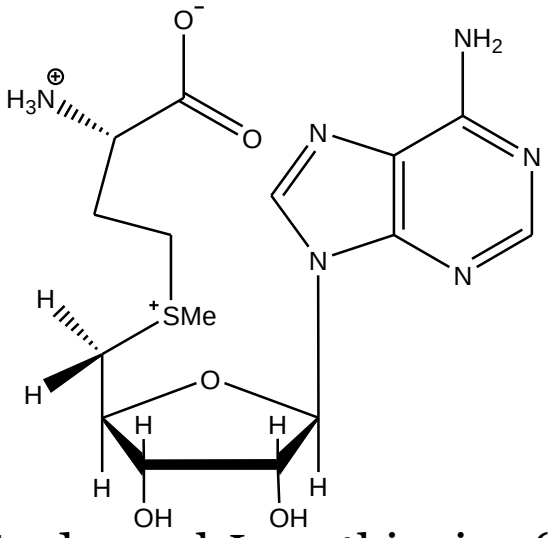
McBride et al. *Bioconj Chem*. **2010**, 21, 1331-1340

Comparison to other methods

Author	Schirrmacher	Hohne	Marik	Glaser	Poethko	Wester/ Mading	Becaud	McBride
Chemistry	Silicon	Silicon	Click	Click	Oxime	Amide	Direct substitution	Al-F
Rx steps	2	1	2	2	2	many	1	1
Process time (min)	40	115-155	30	65	75	60	35	30
Yield (%)	55	13	79	50	40	10	57	51
HPLC-purification steps	1	1	Distillation+ Sep-Pak	1+ distillation	1	2	1	SPE
Specific activity (Ci/umole)	6-18	1.8	>1	high	high	high	2	3

McBride et al. *Bioconj Chem*. **2010**, 21, 1331-1340

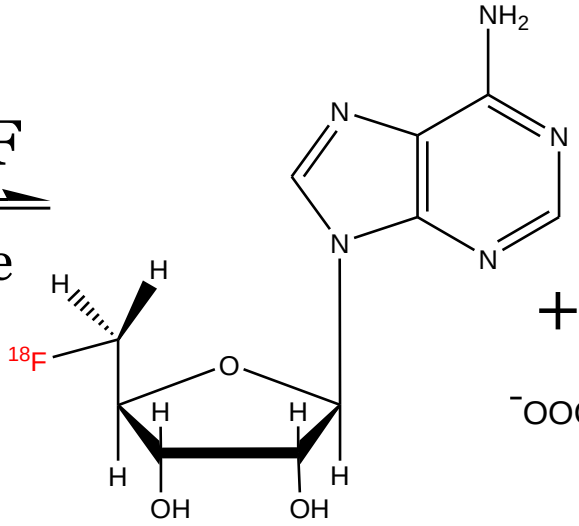
Enzyme Catalyzed C-[^{18}F]F



S-adenosyl-L-methionine (SAM)

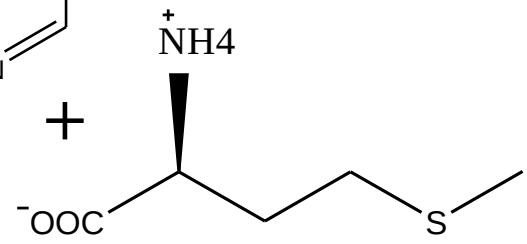
[^{18}F]HF

Fluorinase
40 °C
5 hrs

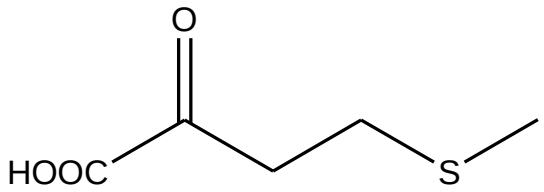


5'-fluoro-5'-deoxyadenosine
(5'-FDA)

L-methionine



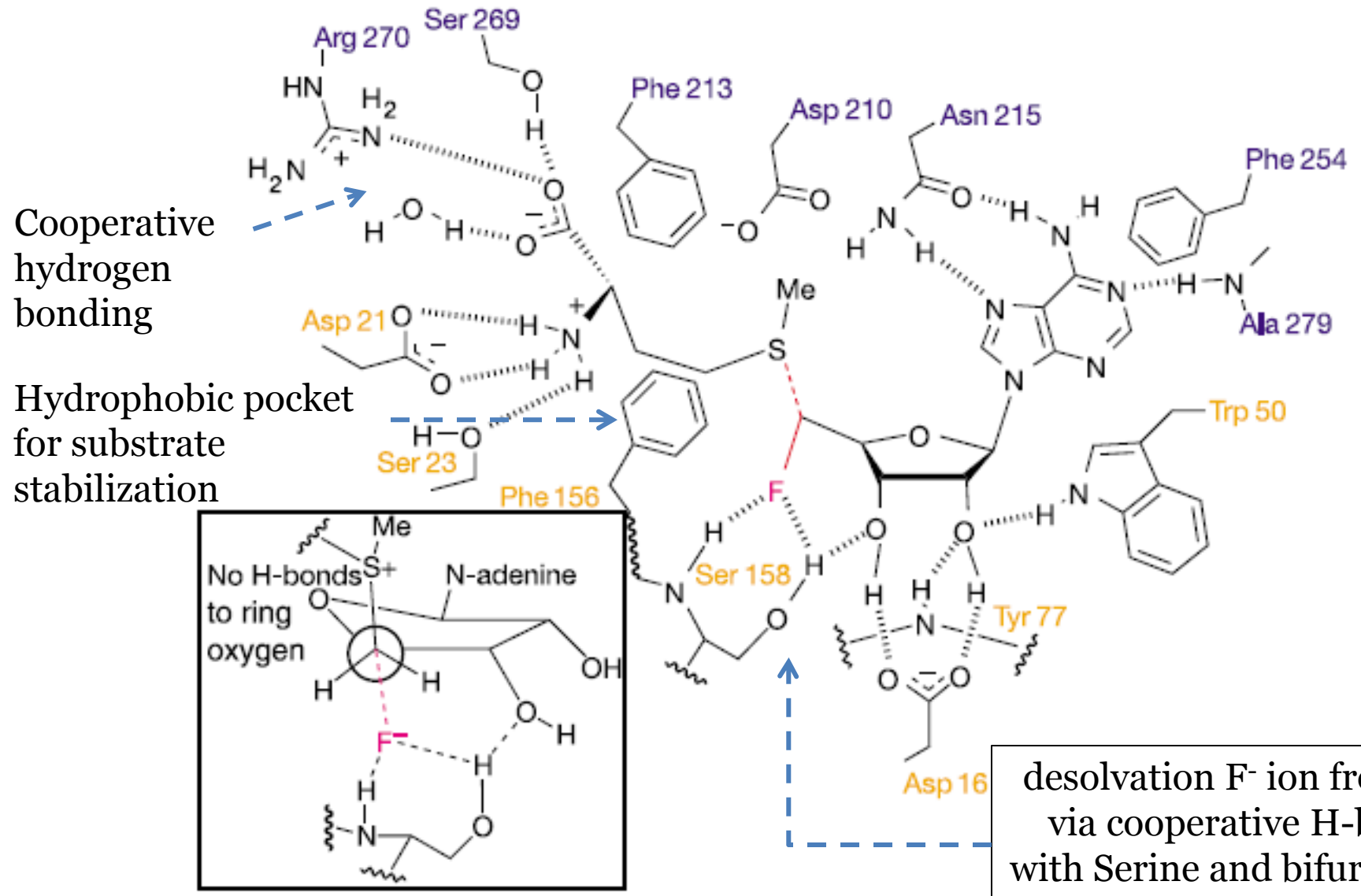
L-amino acid oxidase



Features:
Radiofluorination in aqueous (H_2O)
Eliminate azeotropic drying steps
Eliminate need of phase transfer catalyst

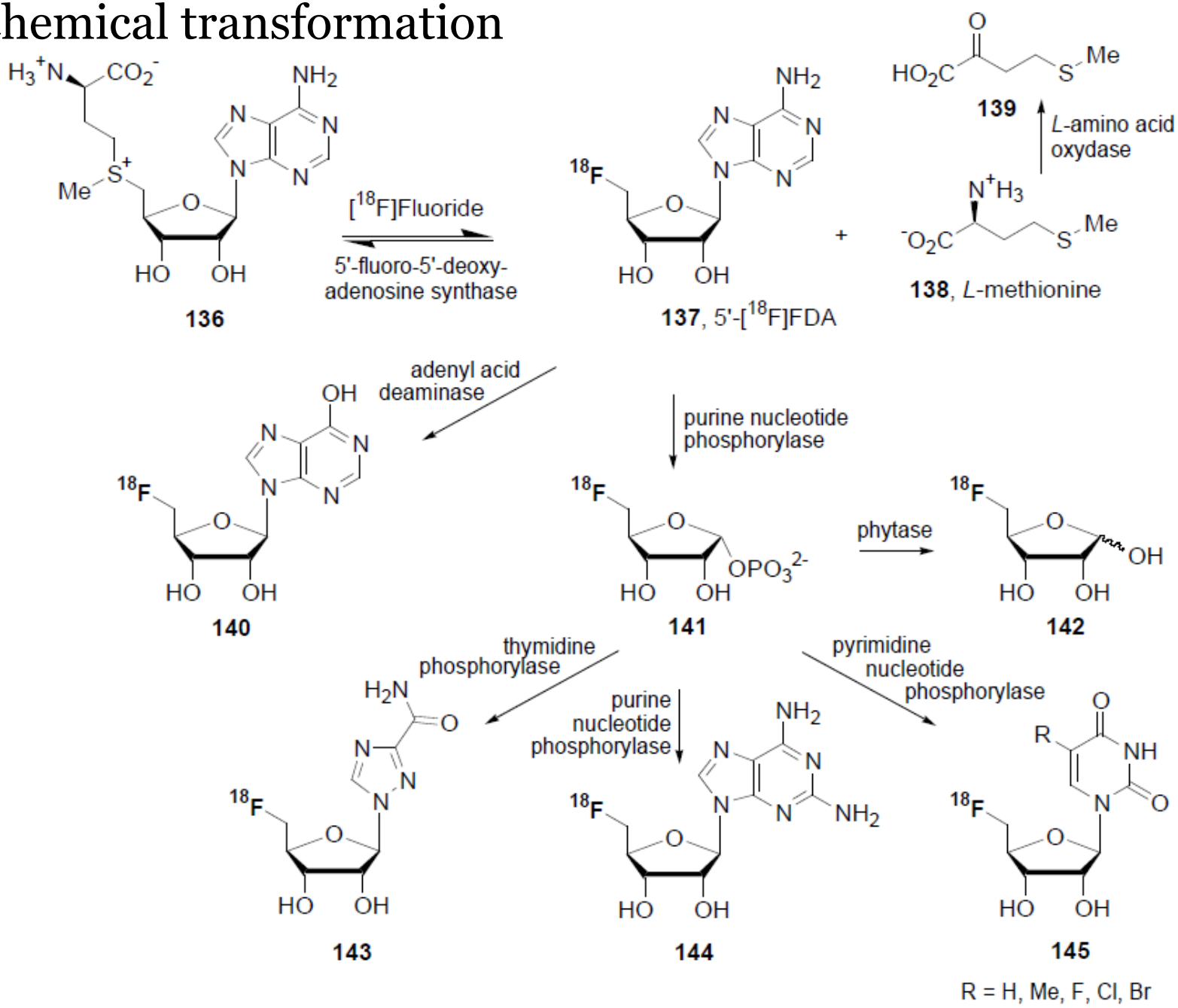
Results:
Without oxidase co-enzyme: 1% RCY 5hr
With oxidase co-enzyme: 95% RCY 1 hr

Mechanism of fluorinase



Representation of enzyme active site

Derivatives of F18-labelled 5'-fluorodeoxyadenosine via biochemical transformation



(1) Polymer supported reagent, (2) Polymer supported precursors, (3) fluorous phase detagging

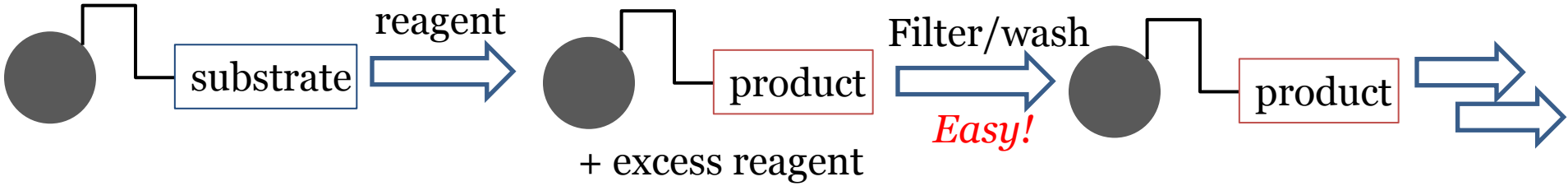
HPLC-FREE APPROACHES

Polymer Supported Radiosynthesis

Advantages:

1. Synthetic intermediate don't have to be isolated
2. Large excess of reagents are use to drive reaction forward, without complicating purification
3. Reagents simply wash away in each step
4. Selective cleavage→ simplify purification
5. Support hazardous reagents→ increase safety
6. Amenable to automation

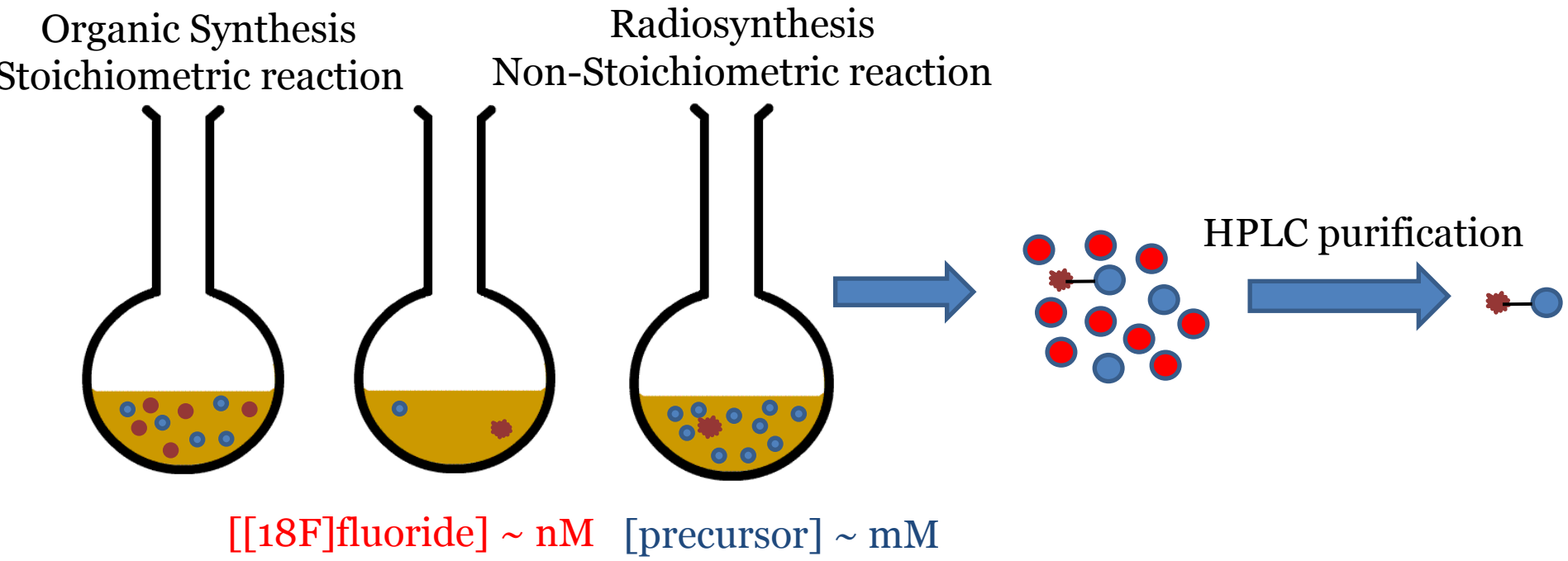
Solid Phase Synthesis



"Conventional" Solution Phase Synthesis

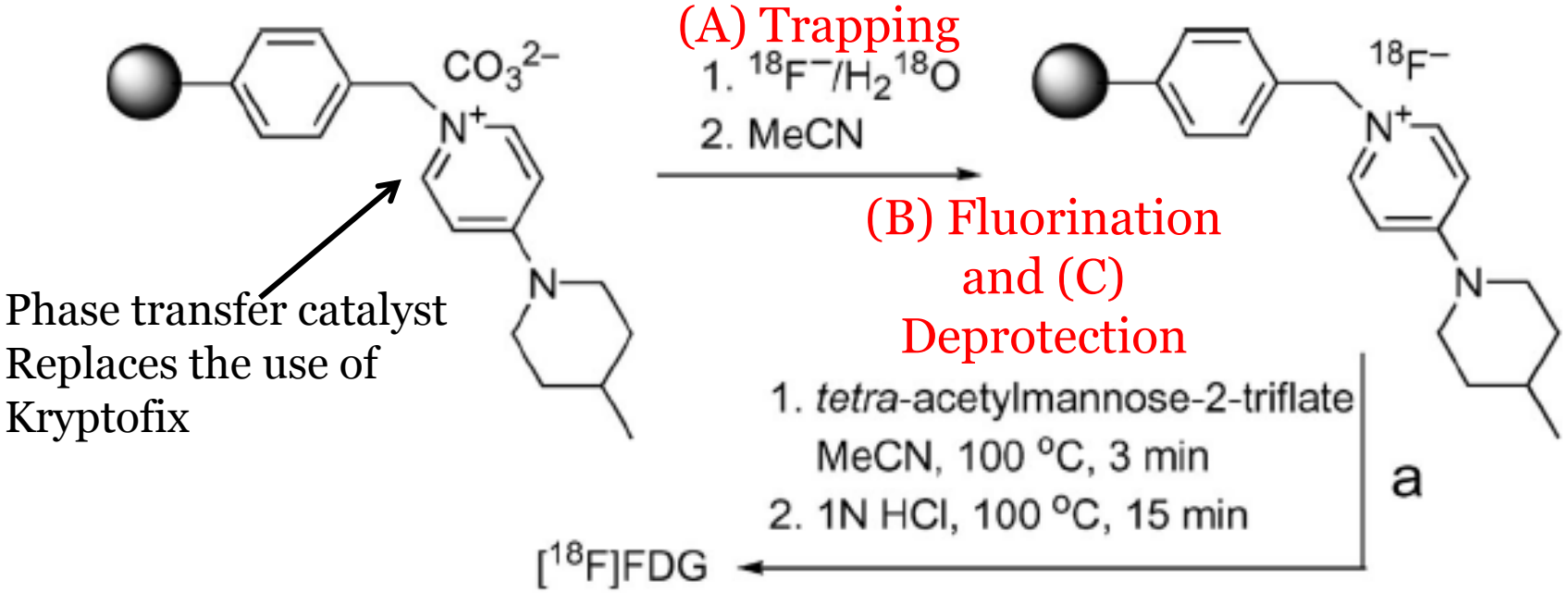
Time consuming and costly

Implication of Non-Stoichiometry Reactions in Radiochemistry



(a) Increases reaction kinetics in a short reaction time
(b) Complicates purification

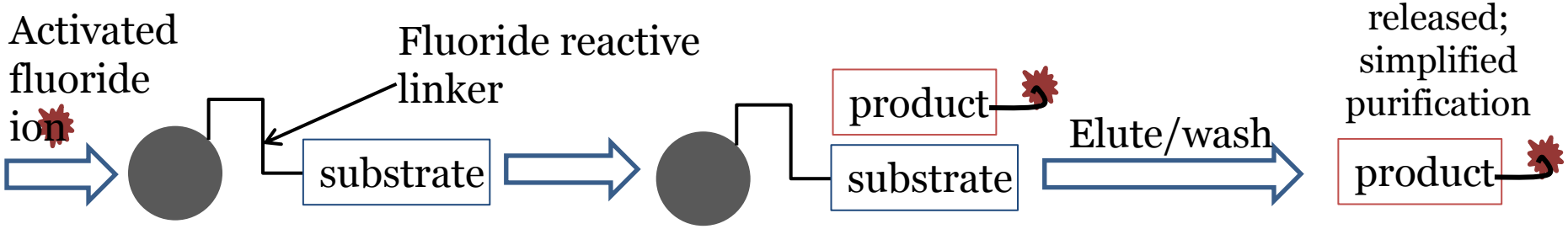
(1) Polymer supported *reagent*



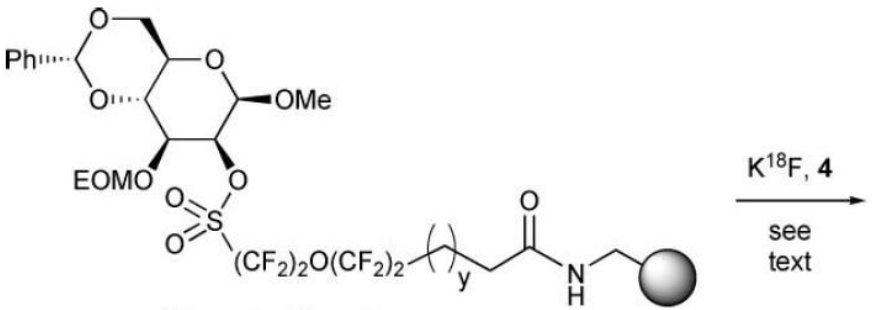
Phase transfer catalyst
Replaces the use of
Kryptofix

Phase transfer catalyst= 4-aminopyridinium salt supported on polymer support; <u>eliminate</u> the need of using the toxic Kryptofix; simplify purification

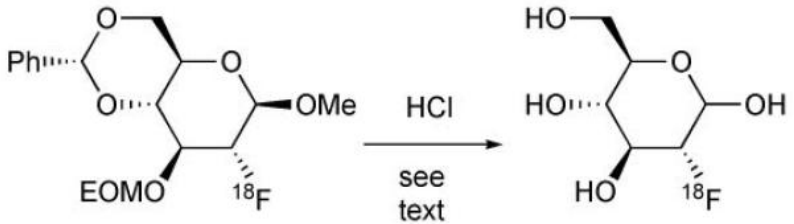
(2) Polymer supported *precursor*



Only fluorinated product is released; simplified purification



33 y = 1; **34** y = 3;
35 y = 9; **36** y = 15

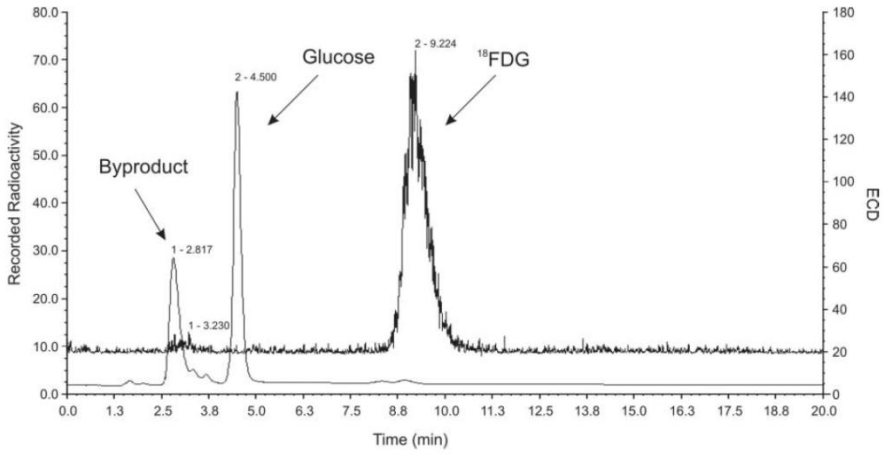


[18F]-18 [18F]-3

Effect of linker chain length

Entry	Linker length (*y*)	Radiochemical yield (%)[a]	[18F]fluoride remaining (%)[a]
1	0	45–50	50–55
2	1	75	25
3	3	92	8
4	9	85–90	10–15
5	15	77	23

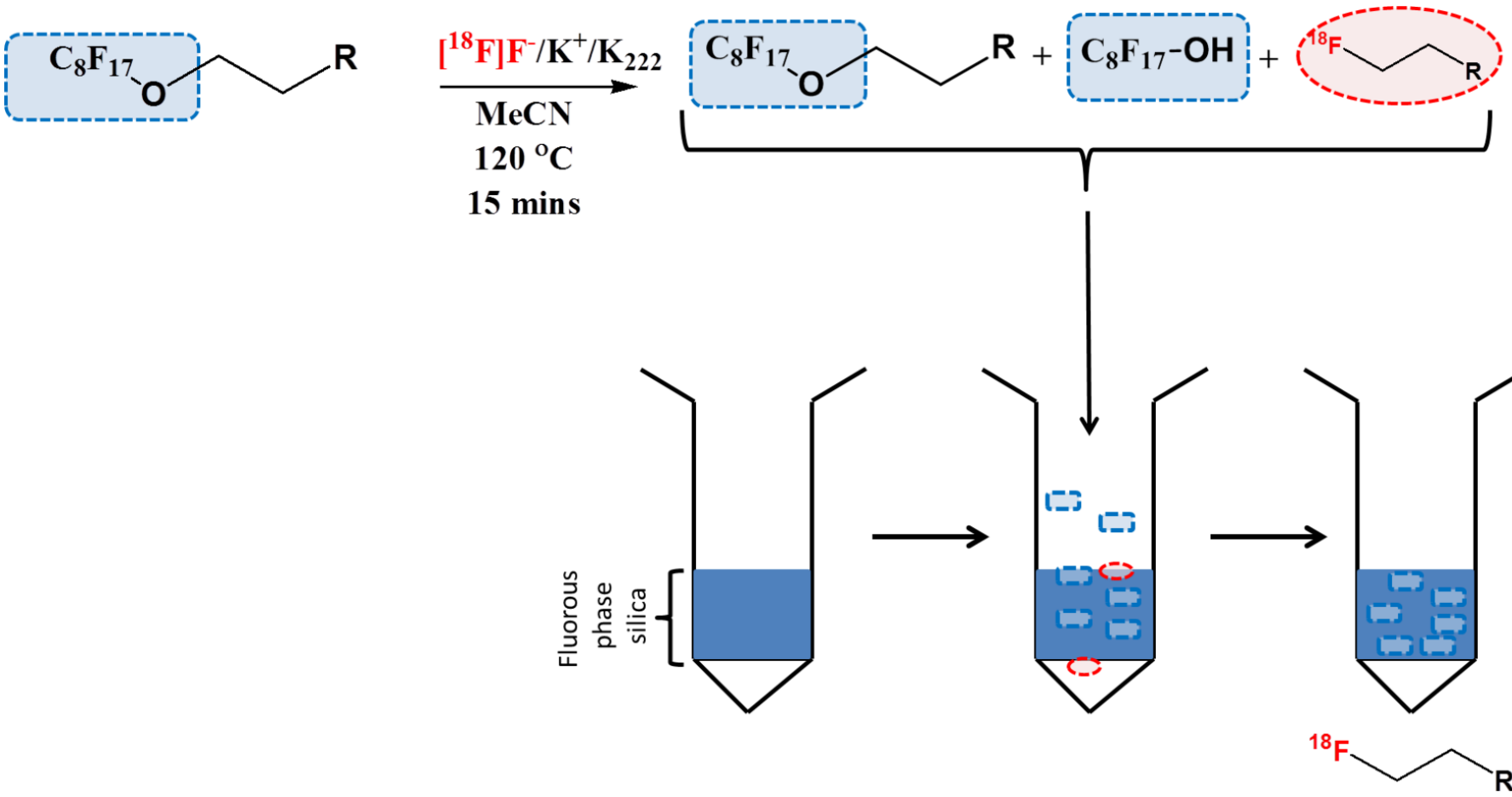
[a] Determined by HPLC with γ-detection (decay corrected).



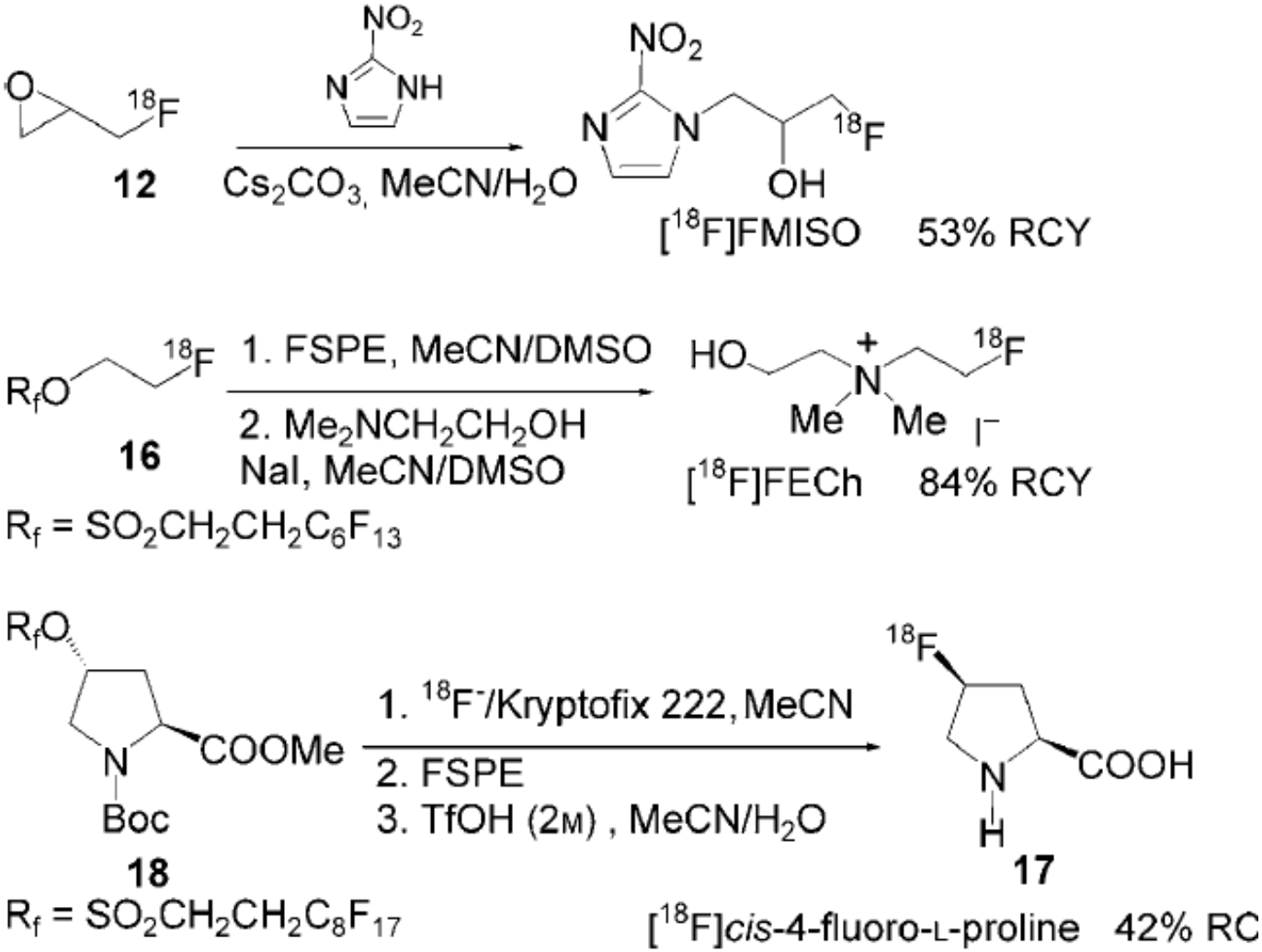
Org. Biomol. Chem., 2009, **7**, 564–575

(3) Fluorous phase detagging

** Solution phase reaction kinetics
** Simplified purification; separation based on fluorous vs non-fluorous compound



Examples of PET probes synthesized via fluorous detagging approach without HPLC purification



** Reduction in specific activities

Selected References

[1] L. Cai, S. Lu, and V. W. Pike, “Chemistry with [18F]Fluoride Ion (Eur. J. Org. Chem. 17/2008),” European Journal of Organic Chemistry, vol. 2008, no. 17, pp. 2843-2843, Jun. 2008.

[2] D. W. Kim, H. J. Jeong, S. T. Lim, and M. H. Sohn, “Recent Trends in the Nucleophilic [18F]-radiolabeling Method with No-carrier-added [18F]fluoride,” Nuclear Medicine and Molecular Imaging, vol. 44, no. 1, pp. 25-32.

[3] T. Furuya, A. S. Kamlet, and T. Ritter, “Catalysis for fluorination and trifluoromethylation,” Nature, vol. 473, no. 7348, pp. 470-477, May 2011.

[4] Linjing Mu, P. A. Schubiger, and S. M. Ametamey, “[18F]Fluorosilicon- and [18F]Fluoroboron-based Biomolecules for PET Imaging,” Current Radiopharmaceuticals, vol. 3, no. 3, pp. 224-242, Jul. 2010.