Production of ^{18}F-Labeled Radiopharmaceuticals

Part 2
(PET Radiochemistry, Lecture #5)

Main Contents of Lecture

- Reactivity of NCA [^{18}F]fluoride
- Radiofluoridation chemistry
  - Aliphatic nucleophilic substitution
  - Aromatic nucleophilic substitution
    - In benzenes
    - In pyridines
- Derived labeling agents
  - Aliphatic
    - One carbon
    - Two carbon
  - Aromatic
- Macromolecule labeling

Reactivity of Fluoride

- In aqueous solution, fluoride ion is highly hydrated [$F^-(H_2O)_n$]
- The water of hydration 'quenches' the negative charge
- Aqueous fluoride is therefore a poor nucleophile
- Removal of all (or nearly all) of the water of hydration creates 'naked' fluoride (F^-), a powerful nucleophile in dipolar aprotic organic media

Nucleophilicity for 'naked' halides: $F^- >> Cl^- > Br^- > I^-$

Note: 'Naked' fluoride is also a powerful base

Solvation Energies of Halide Ions in Water and DMSO

Halide ion	**Water**	**DMSO**
	- ΔH_{sol} (kcal/mol)	
F^-	123	102
Cl^-	89	80.3
Br^-	81	76.1
I^-	72	70.0

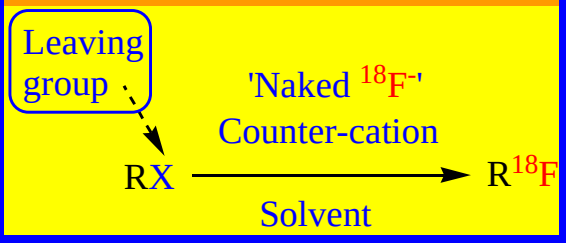
$F^-(H_2O)_n$ Hydrogen bonding

$F^-(Me_2SO)_n$ No hydrogen bonding

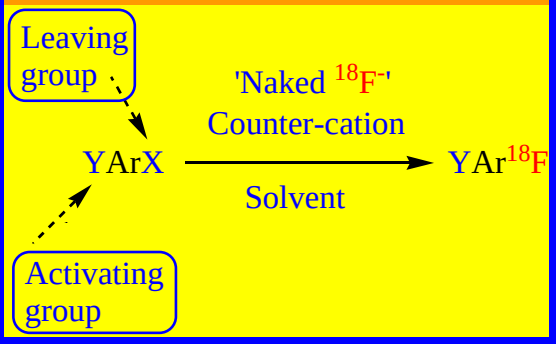
F^- is much more basic in DMSO (pK_a = 15) than in water (pK_a = 3.2)

Nucleophilic Substitution

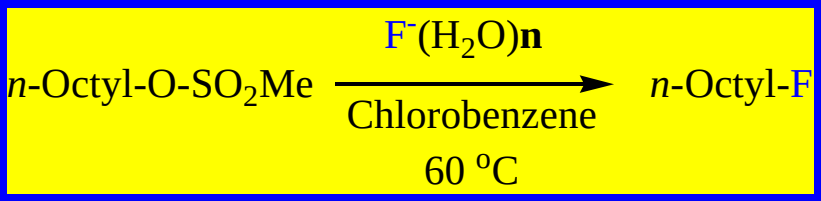
Aliphatic: X is bonded to sp^3 carbon



Aromatic: X is bonded to sp^2 carbon



Influence of Stepwise Desolvation on Nucleophilic Substitution

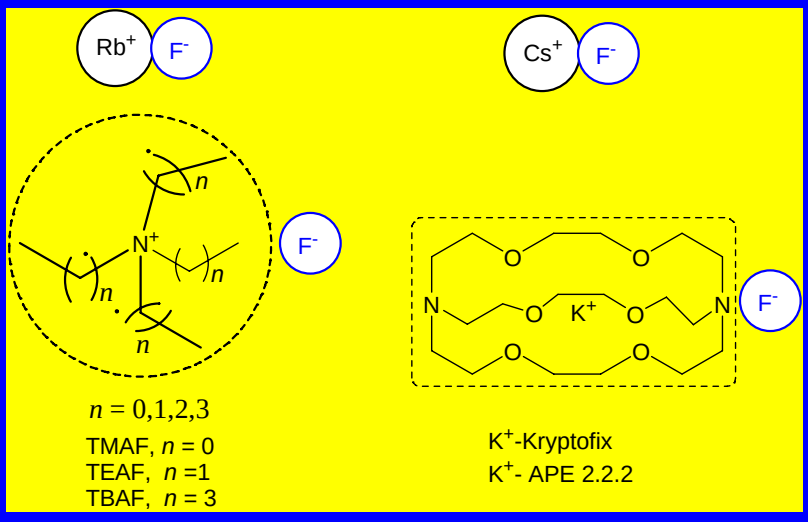


n	Relative rate
8.5	1
6.0	1
4.0	2
3.0	9
2.6	20
1.8	52
1.5	96
0.0	822

Generation of Reactive NCA [^{18}F]Fluoride

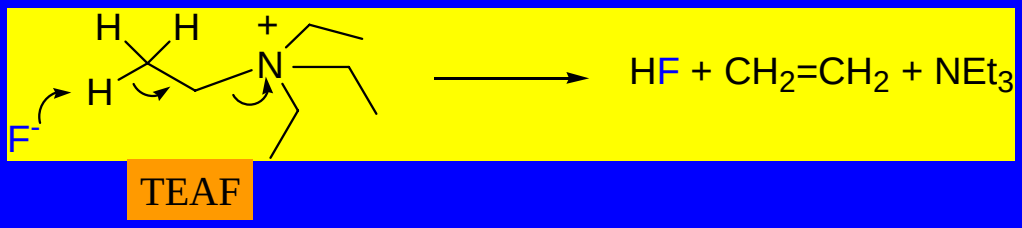
- Several procedures are commonly used for the generation of reactive NCA [^{18}F]fluoride from aqueous cyclotron-produced [^{18}F]fluoride. These share a common strategy: dissolution of the [^{18}F]fluoride with a large counterion in a polar aprotic solvent
- Generally, water is removed by distillation from an added base *e.g.* Rb_2CO_3, $CsCO_3$, $Et_4N^+OH^-$ (TEA^+OH^-), *n*-$Bu_4N^+OH^-$ (TBA^+OH^-), [K-18-crown-6]$^+$-carbonate or [K-APE]$^+$-carbonate
- Final drying of the NCA [^{18}F]fluoride complex may be achieved by repetitive addition of acetonitrile and evaporation of the generated acetonitrile-water azeotrope

Solubilization of 'Naked' Fluoride



Separation of charge and weak solvation promote strong fluoride nucleophilicity (and basicity) in polar aprotic solvents

'Naked' Fluoride as Base



TEAF

- Anhydrous fluoride is a very strong base
- Anhydrous *tetra-n*-alkylammonium fluorides are intrinsically unstable (and difficult to obtain)

Vessel Considerations for Reactions of NCA [^{18}F]Fluoride

Reaction vessels need to be chosen to permit efficient resolubilization after drying of the NCA [^{18}F]fluoride salt

- Vigorous drying tends to adhere the NCA [^{18}F]fluoride to the walls of the vessel, hindering resolubilization for further reaction
- Reactions are often performed in sealed small-volume pyrex, glassy carbon or platinum vessels
- In general glassy carbon and platinum vessels allow efficient resolubilization
- Resolubilization efficiency may depend on solvent and base

Solvent-base System for Reaction

Solvent

The choice of solvent is important for the success of any reaction with NCA [^{18}F]fluoride. Ideally, the solvent should be:

- Stable to base ('naked' fluoride is a strong base)
- Aprotic (to avoid protons, H-bonding)
- Non-nucleophilic (to avoid competitive reactions)
- Polar (for high solvent power)
- High boiling
- Thermally stable

Base

Base must be added to

- Retain NCA [^{18}F]fluoride in solution (prevent protonation to volatile H^{18}F)
- Provide bulk counter anion to bulk cation

Solvents for Aliphatic Nucleophilic Substitution

Solvent		B.pt. ^{o}C
• Dichloromethane		40
• THF		67
• Acetonitrile	Favored	82
• DMF		153
• *o*-Dichlorobenzene		180
• DMSO	Useful for high reaction temperature Prone to decompose below its boiling point Can act as an oxidant	189
• Nitrobenzene		211
• Tetramethylenesulfone (sulfolane)		285

Solvents for Aromatic Nucleophilic Substitution

- Here protophilic solvents favor reaction, particularly DMSO and sulfolane
- In practice DMSO is probably the most useful solvent
- The reactions are generally more difficult than aliphatic substitutions and often require the high temperatures that are possible with DMSO and sulfolane

Choice of Added Base

Criteria for the choice of added base for nucleophilic substitutions with NCA [^{18}F]fluoride are not yet clearly established

• TBA^+OH^-	Potential instability
• $[K\text{-}APE]^+$ carbonate	Versatile
• $[K\text{-}APE]^+$ oxalate	Less basic than carbonate
• Cs_2CO_3	Less effective than those above?
• Rb_2CO_3	Less effective than those above?

Choice of Leaving Group

Aliphatic nucleophilic substitution

Halides:
$I^- > Br^- > Cl^- > F^-$

Sulphonates:
mesyl ($MeSO_3^-$), triflyl ($CF_3SO_3^-$), tosyl (p-$MeC_6H_4SO_3^-$)

Cyclic leaving groups:
cyclic sulphate, cyclic sulphite, cyclic sulphamate

High radiochemical yields can be obtained

Choice of Leaving Group

Aromatic nucleophilic substitution

- Activation is required by at least one electron-withdrawing substituent: *e.g.* NO_2, CN, COR, CONH, CO_2R or CHO *ortho* or *para* to the leaving group, which is generally: NO_2, Br, Cl or N^+Me_3
- High radiochemical yields are obtained on simple substrates, but are more difficult to achieve on structures that are larger and sensitive to base

Other Structural Features Required of Precursor

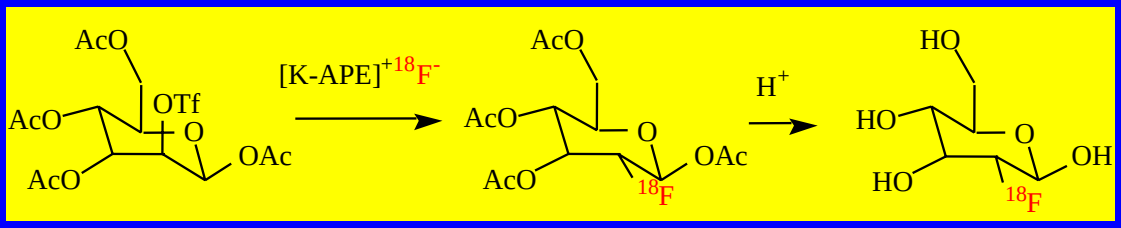
- Generally the precursor (RX or YArX) should not contain structural elements that can reduce the nucleophilicity of 'naked' fluoride:

  No acidic groups (ArOH, CO_2H..)
  No enolizable groups (-CH_2CO-)
  No hydrogen bonding groups (OH, NH)

- The precursor should not contain labile F atoms (a source of carrier)
- The precursor should not be base-sensitive
- The precursor should be stable to storage in the dry state

Preparation of FDG by Aliphatic Nucleophilic Substitution

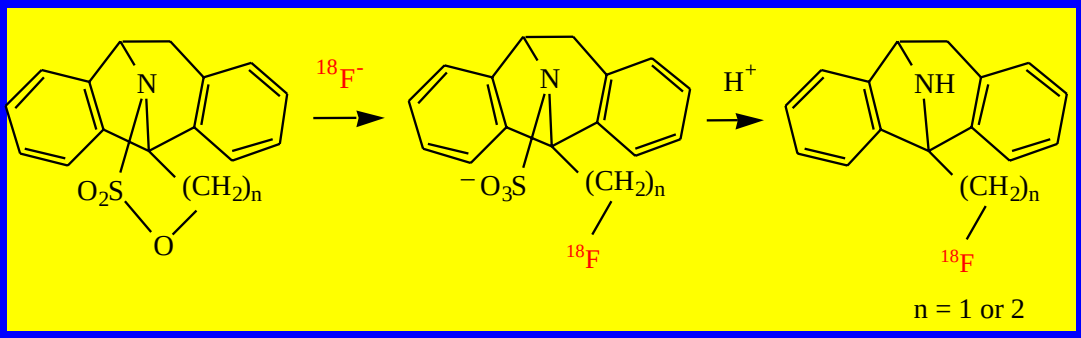
- triflate leaving group



This (and subtle variations) are the most efficient radiosyntheses of FDG known

Preparation of [^{18}F]Fluoroalkyl-MK801s by Aliphatic Nucleophilic Substitution

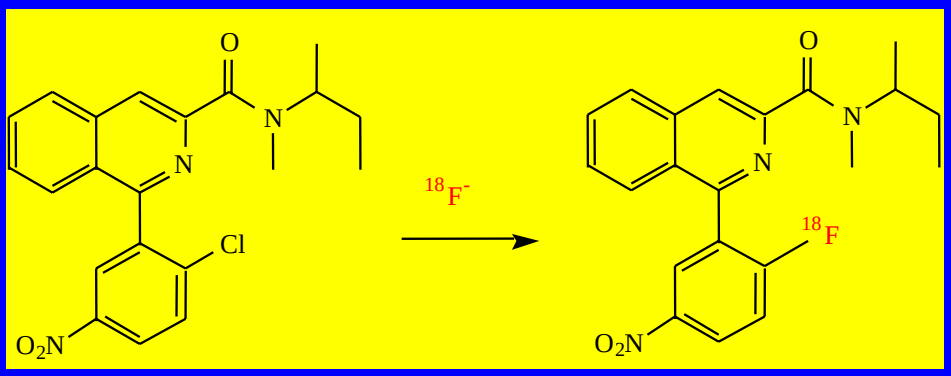
- cyclic sulphamate leaving group



- Cyclic leaving group effectively ‘protects’ amino group
- Good radiochemical yields can be obtained using a variety of counter cations

Preparation of [^{18}F]PK14105 by Aromatic Nucleophilic Substitution

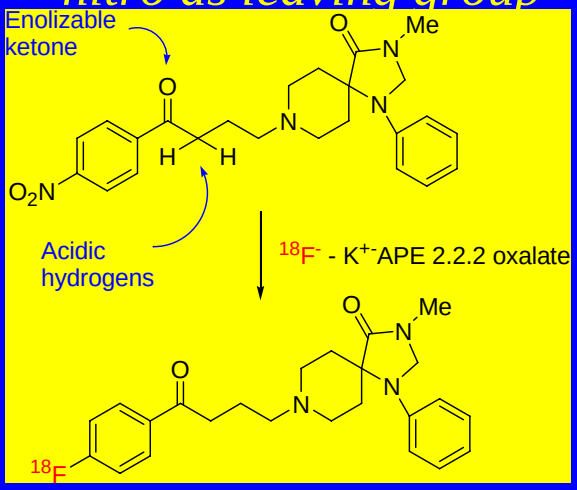
- chloro as leaving group



- The electron-withdrawing *p*-nitro group acts as an activating group
- The product and precursor are difficult to separate

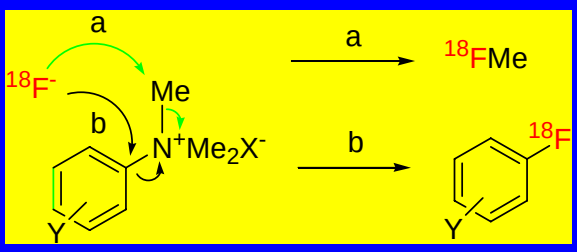
Preparation of [^{18}F]N-Methylspiperone by Aromatic Nucleophilic Substitution

- nitro as leaving group



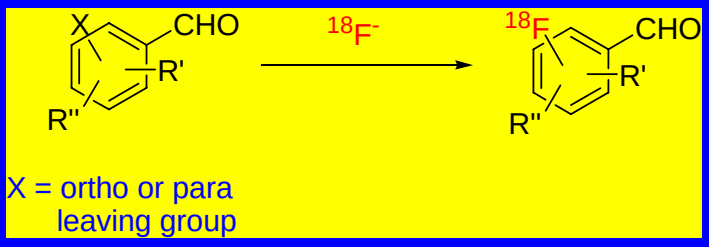
- Stronger base decomposes the precursor giving lower yield
- Enolization of the precursor probably explains erratic yields

Special Considerations for N(Me)$_3$ as Leaving Group



***p*-Y**	**[^{18}F]4-F-ArY (%)**	**[^{18}F]FMe (%)**
F	2	85
Br	12.5	70
CHO	71	1
COOEt	77	2
COMe	90	1.5
CN	87	0
NO_2	88	0

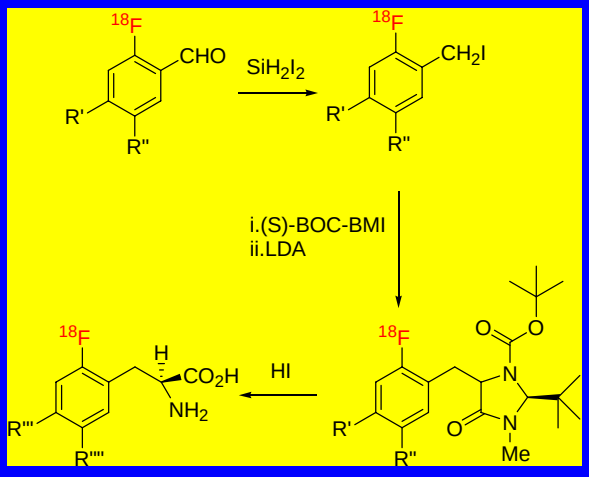
Preparation of o- and p-[^{18}F]Fluorobenzaldehydes



- X can be $N^{+}Me_3$ or NO_2
- R' or R" can be H, Cl, Ome
- Good radiochemical yields can be achieved

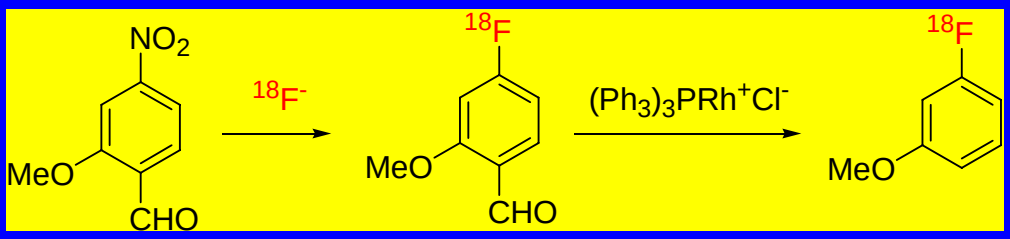
- [^{18}F]Fluorobenzaldehydes are useful labeling agents

[^{18}F]Fluorobenzaldehydes in ^{18}F-Labeled L-Amino Acid Synthesis



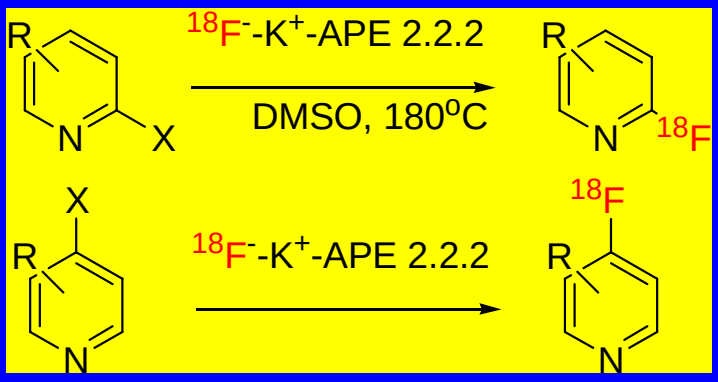
- [^{18}F]L-4-Fluorophenylalanine (R'= R" = H) and FDOPA (R' = R" = OMe; R'"= R"" = OH) have been prepared in 15% radiochemical yield

[^{18}F]Fluoroarenes from [^{18}F]Fluorobenzaldehydes



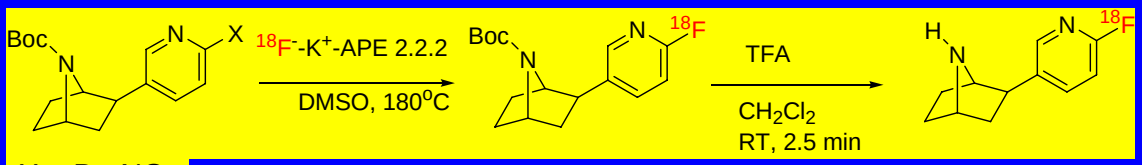
- This constitutes a two-step procedure for introducing NCA [^{18}F]fluoride into non-activated (electron-rich) rings
- Radiochemical yields are 70-80% overall

Radiofluoridation of Pyridines



- X may be Cl, Br, NO_2, N^+Me_3 (R = H, or structure residue)
- Radiochemical yields up to 96% may be obtained with N^+Me_3

Radiofluoridation of an Epibatidine Derivative

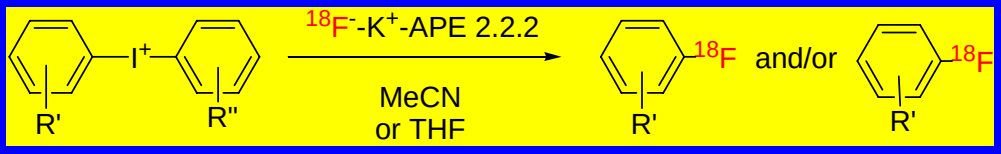


X = Br, NO_2

- Radiochemical yields up to 25% overall can be achieved

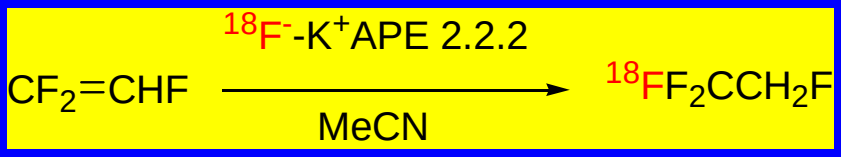
The labeled product is a radioligand for nicotinic acetylcholine receptors

Reactions of [^{18}F]Fluoride with Diaryliodonium Salts



- R' and R" may be electron-donating (*e.g.* Me, alkyl, MeO) or electron-withdrawing
- A bulky group in *ortho* position favors introduction of ^{18}F into the same ring
- Otherwise ^{18}F is introduced into ring bearing electron-withdrawing group(s)
- Application of the method to more complex structures has proved challenging
- This constitutes a one-step procedure for introducing NCA fluorine-18 into electron-rich arenes

Nucleophilic Addition of [^{18}F]Fluoride



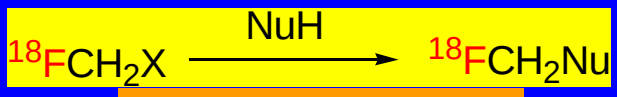
- A rare example!
- The proton must come from the solvent of from APE 2.2.2

The gaseous product has been used as a radiotracer of the *in vivo* disposition and human safety of the aerosol propellant HFA 134a (Klea^{TM})

Aliphatic One Carbon Labeling Agents Produced from [^{18}F]Fluoride

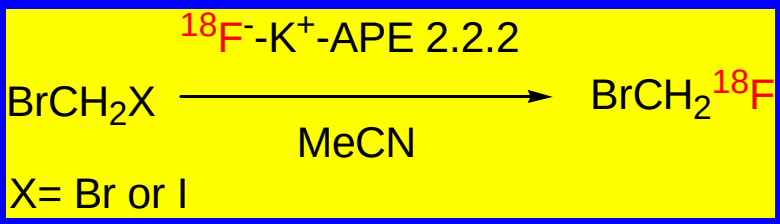
[^{18}F]Fluoromethyl bromide	$^{18}FCH_2Br$
[^{18}F]Fluoromethyl triflate	$^{18}FCH_2OTf$
[^{18}F]Fluoromethyl iodide	$^{18}FCH_2I$

These agents are useful for ^{18}F-fluoromethylation reactions:



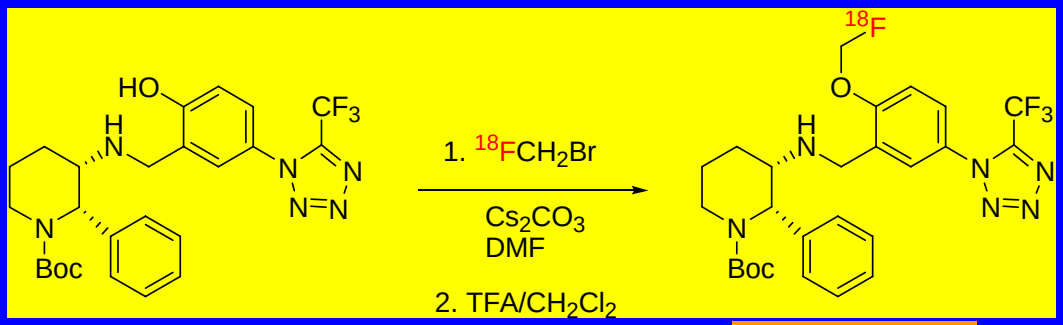
NuH = ArOH, RNH_2…

Preparation of [^{18}F]Fluoromethyl Bromide



- Radiochemical yield is ~ 40% from dibromomethane
- Specific radioactivities as high as 25 Ci/μmol have been achieved
- The product is gaseous and can be trapped in organic solvents for further reaction
- Demonstrated applications include the ^{18}F-fluoromethylation of phenols

Preparation of [^{18}F]SPA-RQ



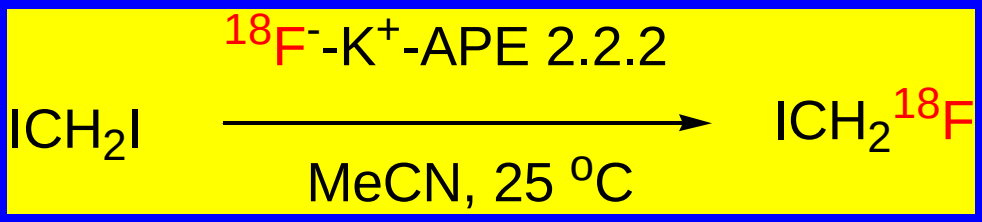
[^{18}F]SPA-RQ

- The fluoromethylation reation proceeds in very high yield
- [^{18}F]SPA-RQ is the first effective radioligand for the NK_1 receptor

Preparation of [^{18}F]Fluoromethyl Triflate

- Radiochemical yield is ~ 47% from [^{18}F]fluoride
- The product is gaseous and can be trapped in organic solvents for further reaction
- The product is more reactive than [^{18}F]fluoromethyl bromide
- Demonstrated application is for ^{18}F-fluoromethylation of a tertiary amine

Preparation of [^{18}F]Fluoromethyl Iodide



- Radiochemical yield: ~ 40% from ^{18}F-fluoride
- Preparation time: 15 min
- The product is gaseous and can be trapped in organic solvents for further reaction
- Demonstrated applications are for ^{18}F-fluoromethylation of amines, carboxylic acids, thiols and phenols

Aliphatic Two Carbon Labeling Agents Produced from [^{18}F]Fluoride

[^{18}F]2-Fluoroethyl bromide	$^{18}FCH_2CH_2Br$
[^{18}F]2-Fluoroethyl iodide	$^{18}FCH_2CH_2I$
[^{18}F]2-Fluoroethyl tosylate	$^{18}FCH_2CH_2OTs$
[^{18}F]2-Fluoroethyl mesylate	$^{18}FCH_2CH_2OMs$
[^{18}F]2-Fluoroethyl triflate	$^{18}FCH_2CH_2OTf$

These agents are useful for ^{18}F-fluoroethylation reactions:

$$^{18}FCH_2CH_2X \xrightarrow{NuH} {}^{18}FCH_2CH_2Nu$$

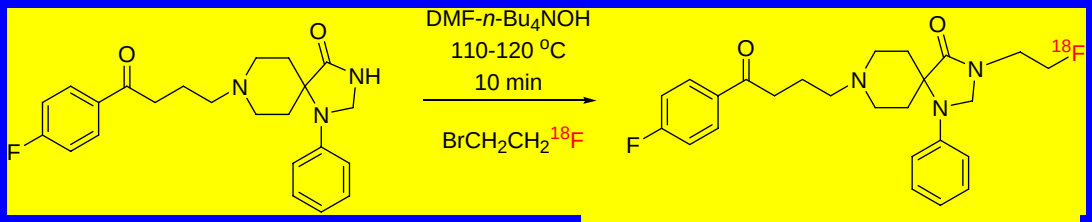
Preparation of [^{18}F]2-Fluoroethyl Bromide

$$BrCH_2CH_2X \xrightarrow{^{18}F^-} BrCH_2CH_2{}^{18}F$$

X = OTs, ONos, OMes, OTs, Br or I

- Radiochemical yields are variable (5-80%), depending on X and report
- X = OTs or OTf appear to give good reliable radiochemical yields (43-80%)
- The labeled product can be distliled out for further reaction

An Application of [^{18}F]2-Fluoroethyl Bromide



[^{18}F]*N*-Fluoroethyl-spiperone

- Radiochemical yield: 56%

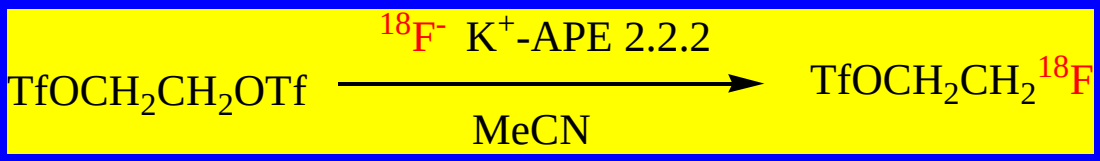
[^{18}F]*N*-Fluoroethyl-spiperone is a D_2 receptor radioligand

Preparation of [^{18}F]2-Fluoroethyl Iodide

$$ICH_2CH_2I \xrightarrow[MeCN]{^{18}F^-\ K^+\text{-APE 2.2.2}} ICH_2CH_2{}^{18}F$$

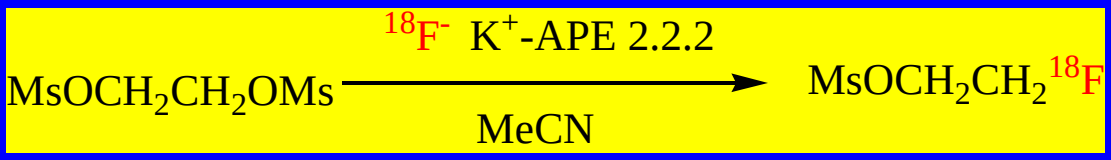
- Preparation time: 10-15 min
- Radiochemical yield: 30-40%
- No applications reported

Preparation of [^{18}F]2-Fluoroethyl Triflate



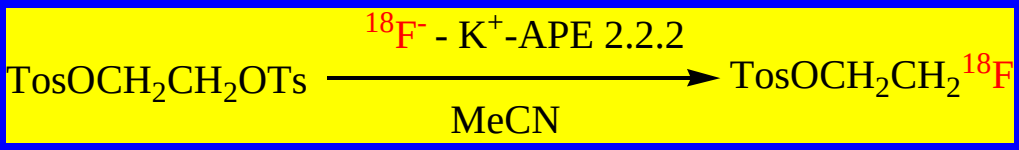
- Preparation time: ~ 10 min
- Radiochemical yield: 36%
- Applied to ^{18}F-fluoroalkylation of amines (*des*-ethyl-raclopride and other benzamides)

Preparation of [^{18}F]2-Fluoroethyl Mesylate



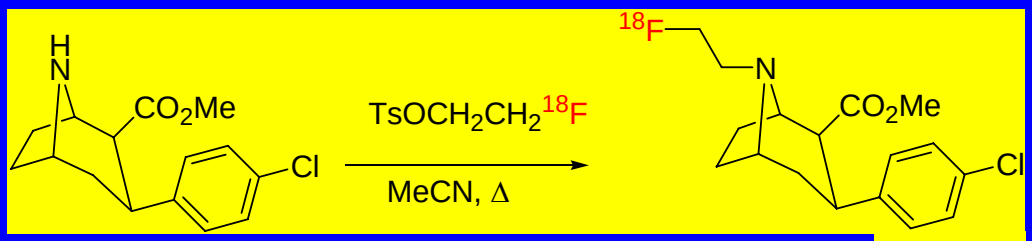
- Preparation time: ~ 10 – 15 min
- Radiochemical yield: 79%
- No applications reported

Preparation of [^{18}F]2-Fluoroethyl Tosylate



- The radiochemical yield can be near quantitative
- The labeled product is usually purified before use but can vbe used *in situ*

Application of [^{18}F]2-Fluoroethyl Tosylate to the Labeling of FECNT



FECNT

- High radiochemical yield can be achieved

FECNT is a dopamine transporter radioligand

Longer Chain Aliphatic Labeling Agents Produced from [^{18}F]Fluoride

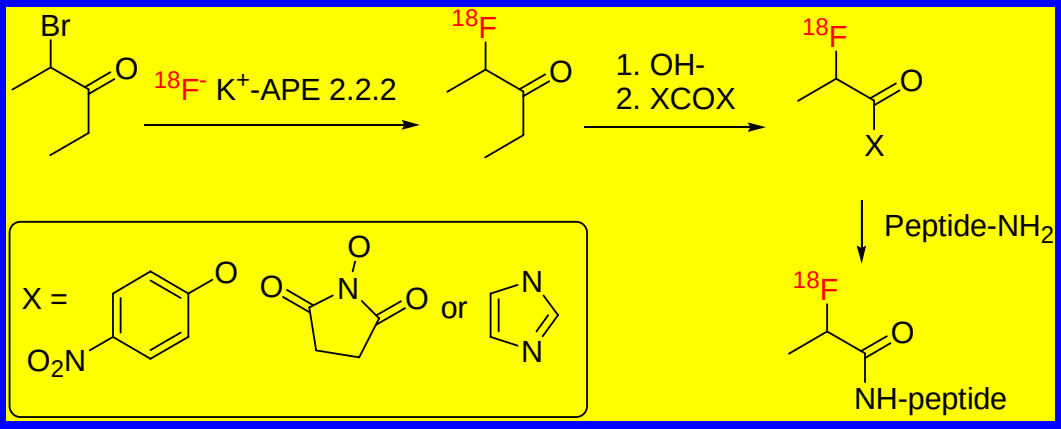
[^{18}F]2-Fluoropropyl bromide	$^{18}FCH_2CH_2CH_2Br$
[^{18}F]2-Fluoropropyl iodide	$^{18}FCH_2CH_2CH_2I$
[^{18}F]2-Fluoropropyl tosylate	$^{18}FCH_2CH_2CH_2OTs$
[^{18}F]2-Fluoropropyl mesylate	$^{18}FCH_2CH_2CH_2OMs$
[^{18}F]2-Fluoropropyl triflate	$^{18}FCH_2CH_2CH_2OTf$

- These are prepared by methods similar to those used to prepare the shorter chain alkylating agents

Labeling of Macromolecules with Fluorine-18

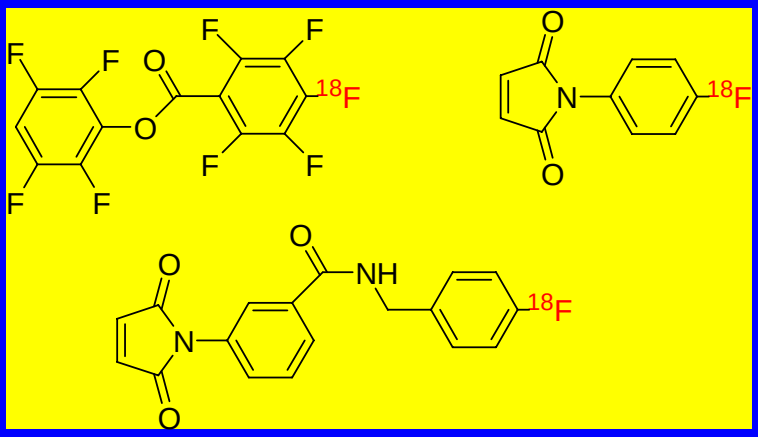
Labeling of proteins
Labeling of oligonucleotides

Protein Labeling



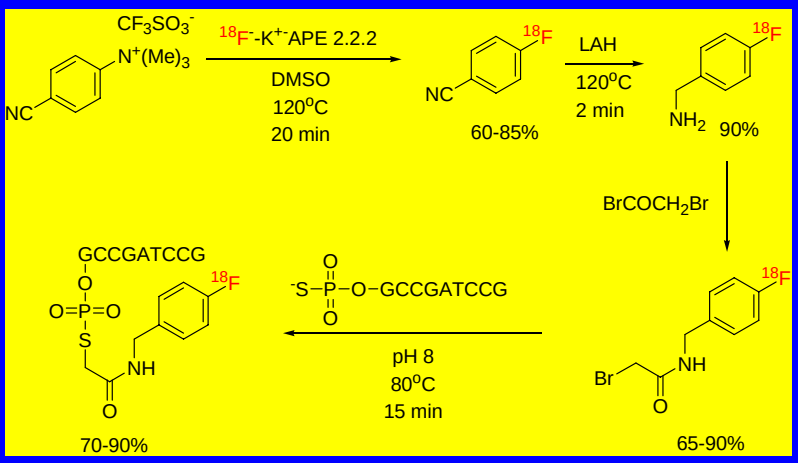
These reactions proceed efficiently

Some other Protein ^{18}F-Labeling Agents



- These are all activated for mild reactions with nucleophiles in peptides/proteins

Oligonucleotide Labeling



- Overall radiochemical yield:: 40%(decay-corrected)
- Preparation time: 220 min

Conclusions

- NCA [^{18}F]fluoride can be produced in high activities (multi-Ci) and high specific radioactivity
- NCA [^{18}F]fluoride can be used efficiently in labeling reactions
- There are still some obstacles to the use of NCA [^{18}F]fluoride for labeling *e.g. in* electron-rich aryl rings, in *m*-orientation to substituents
- [^{18}F]fluoride can be used to prepare a range of useful mainly (electrophilic) labeling agents