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FORSCHUNGSZENTRUM

^{18}F-Labeling Chemistry

1st QUALI-START-UP SCIENCE LECTURES

Johannes Ermert, INM-5

Advantages of tracers labelled with short-lived positron-emitters for *in-vivo* application

^{11}C ($t_{1/2}$=20 min), ^{18}F ($t_{1/2}$=110 min)

molar activity > 10^{11} Bq/µmol

⟹ minute amount of mass applied (<1 µg)

⟹ small radiation doses (<10 mSv)

⟹ quantitative imaging with PET
(high spatial and temporal resolution)

Basic aspects of no-carrier-added radiolabelling

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Radiosyntheses can be classified as

• carrier-free (c.f.)

The absolute lack of a carrier is ideally only achieved when artificial radioelements (e.g. astatine) are used and the presence of longer-lived radioisotopes of the element can be excluded.

• no-carrier-added (n.c.a.)

When performing labelling reactions with cyclotron-produced radioisotopes of natural-occuring elements, traces of stable isotopes of these elements are omnipresent and act as isotopic carriers, provided that they are in the same chemical state. Possible sources of such contaminations are the air, target and reaction vessels, chemicals and solvents.

• carrier-added (c.a.)

Under several circumstances, weighable quantities of the natural-occuring element are added to the system in order to increase the radiochemical yield or even to make certain labelling methods possible.

Properties of fluorine-18

- **97% β^+, E_{max} = 0.635 MeV**
  - smallest positron-energy;
    suitable for PET with high resolution

- **$T_{1/2}$ = 109.7 min**
  - half-life suitable for extended syntheses
    and PET protocols

- **C-F bond**
  - covalent and stable; monovalent chemistry;
  - organic analog-compounds

History

1937 A.H. Snell
First production at cyclotron hys. Rev. 51, 143

1940 J.F. Volker, H.C. Hodge, H.J. Wilson
Absorption on dentine and bones J. Biol. Chem. 123, 543

Production of fluorine-18

Nuclear reactions	$^{18}O(p,n)^{18}F$	$^{16}O(^{3}He,p)^{18}F$	$^{20}Ne(d,\alpha)^{18}F$	$^{18}O(p,n)^{18}F$
Target	$H_2{}^{18}O$ 1)	H_2O	Ne/0.2% F_2 2)	1) $[^{18}O]O_2$ 2) Kr/0.8% F_2 3)
Energy of particle [MeV]	$16 \rightarrow 0$	$36 \rightarrow 0$	$11 \rightarrow 0$	$12 \rightarrow 0$
Chemical form of ^{18}F	$^{18}F^-_{aq}$	$^{18}F^-_{aq}$	$[^{18}F]F_2$	$[^{18}F]F_2$
Target yield [Bq/µAh]	$2.22 \cdot 10^9$	$2.59 \cdot 10^8$	$3.7 – 4.4 \cdot 10^8$	$\approx 1 \cdot 10^8$
Molar activity [Bq/mmol]	$< 3.7 \cdot 10^{15}$	$< 3.7 \cdot 10^{15}$	$3.7 \cdot 10^{10\text{-}11}$	$4.8 \cdot 10^{11}$

1) Ti-Target with Ti-window; 2) passivated Ni-Target; 3) two-step irradiation method

S.M. Qaim, G. Stöcklin. *Radiochim. Acta* **34**, 25 (1983).
M. Guillaume, A. Luxen. *Appl. Radiat. Isot.* **42**, 749 (1991).
E. Hess, G. Blessing, H.H. Coenen, S.M. Qaim. *Appl. Radiat. Isot.* 52, 1431 (2000).

Nucleophilic substitution with $[^{18}F]$fluoride is practically the only labelling method at the no-carrier-added level !

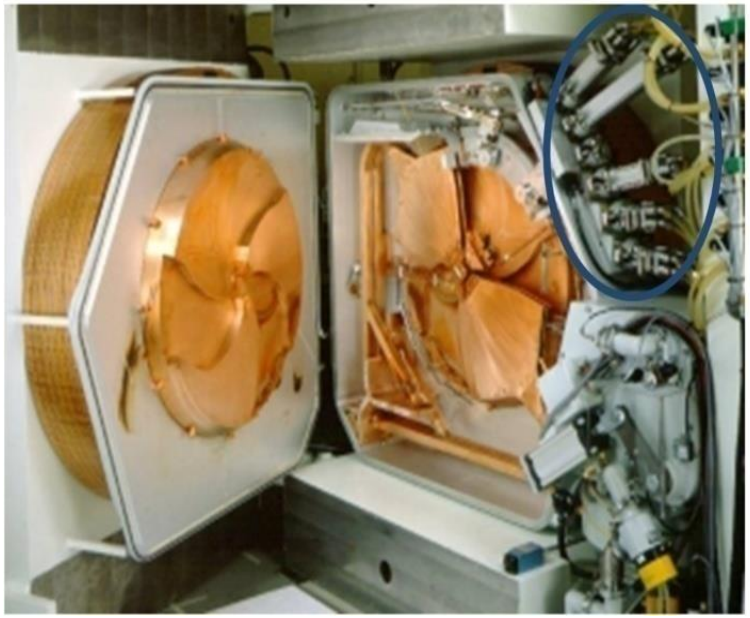
$H_2{}^{18}O$-target for $^{18}F^-_{aq}$ production

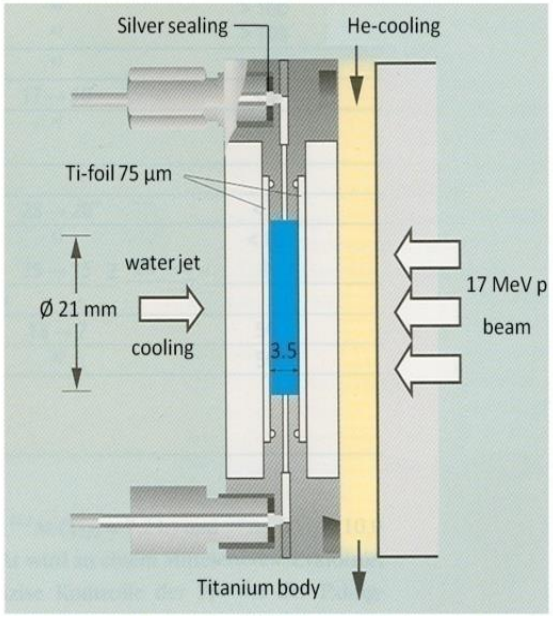
Nuclear reaction: $^{18}O(p,n)^{18}F$

Production yield of $^{18}F^-_{aq}$: 74 GBq (2 Ci)

Recycling of ^{18}O-Wasser: Adsorption of $^{18}F^-$ on anion exchange column (AG 1x8 or QMA)

Desorption with aqueous K_2CO_3 solution





General strategies of n.c.a. ^{18}F-labelling

Direct ^{18}F-labelling or "late-stage labelling"

^{18}F nuclide is introduced "directly" into the complete target molecule of interest. Often protection groups have to be removed or other transformations are required.

Indirect ^{18}F-labelling or "building block Approach"

Built-up synthesis of small molecules or prosthetic groups for macromolecules.
Typically small ^{18}F-labelled aryl groups bear reactive functional groups for transformation reactions. They are used to react with more complex biological molecules which may not be suitable or stable enough to tolerate direct fluorination methods.

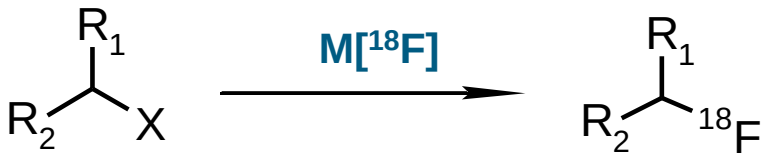
Banister S et al., Current Radiopharm., **3**, 68-80 (2010)
Ermert J and Coenen HH, Current Radiopharm., **3**, 109-126 (2010)
Ermert J and Coenen HH, Current Radiopharm., **3**, 127-160 (2010)
Coenen HH and Ermert J, Current Radiopharm., **3**, 163-173 (2010)
van der Born D et al., Chem. Soc. Rev., **46**, 4709-4773 (2017).

Comparison of electrophilic and nucleophilic fluorine-18

	Electrophilic	Nucleophilic
Nuclear reaction	$^{20}Ne(d,\alpha)^{18}F$ (Ne) $^{18}O(p,n)^{18}F$ ($^{18}O_2$)	$^{18}O(p,n)^{18}F$ ($H_2{}^{18}O$)
Batch of production (E.O.B., 1 h, 15 µA)	10 GBq 30 GBq	50 GBq
Fluorinating agent	$[^{18}F]F_2$	$^{18}F^-$
Theoretical maximum radiochemical yield	50 %	100 %
Mass involved	50-200 µmol	n.c.a.
Specific activity	≤ 0.1 GBq / µmol ≤ 0.5 GBq / µmol	> 50 GBq / µmol

Prerequisites of nucleophilic ^{18}F-substitution

Aliphatic:



R_1 = Alkyl, Aryl

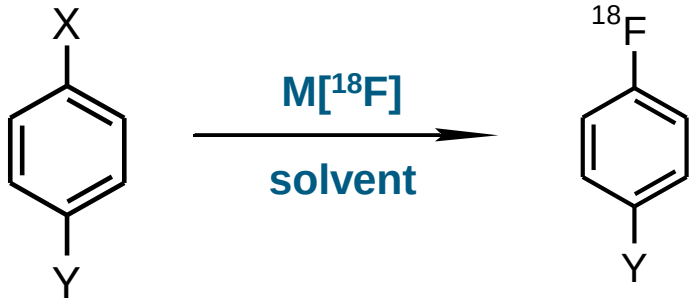
R_2 = Alkyl, Aryl, H

X = Br, I, Tosylate, Mesylate, Triflate

M = Cs, Rb, R_4N, K⊂2.2.2.

Solvent: Acetonitrile, DMF, DMSO, tert.-Butanol

Aromatic:



X = (F), Br, I, NO_2, $(CH_3)_3N^+$

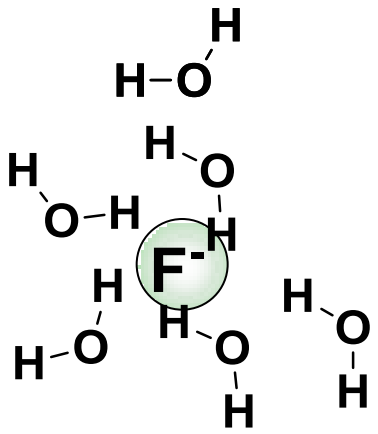
Y = CHO, COOR, COR, CN, NO_2

M = Cs, Rb, R_4N, K⊂2.2.2.

Solvent: DMSO, DMF, DMAA

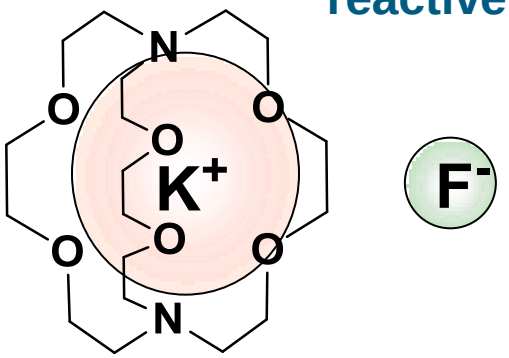
Labelling with n.c.a. [^{18}F]fluoride: Basic principles

non-reactive



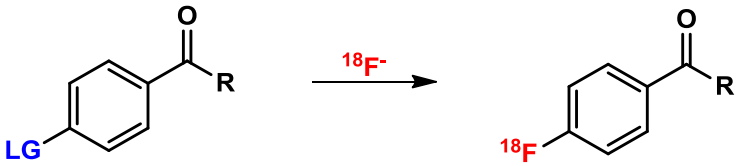
Absolute dryness
→ naked, reactive fluoride for substitution reactions

reactive

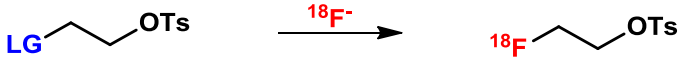


Cryptant Kryptofix®2.2.2.

Aliphatic and aromatic *nucleophilic substitution*

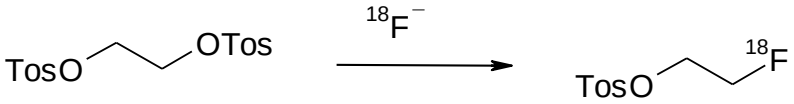


S_NAr ***or***



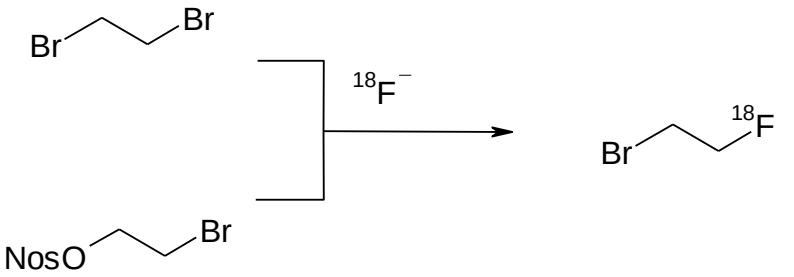
S_N2

^{18}F-labelling by aliphatic prosthetic groups

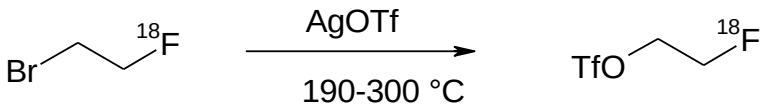


2-[^{18}F]Fluoroethyltosylate

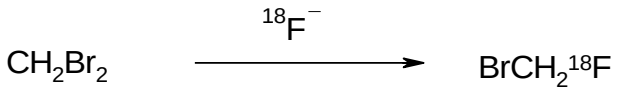
Review: T. Kniess et al. MedChemComm 2015, 6, 1714



1-Bromo-2-[^{18}F]fluoroethane

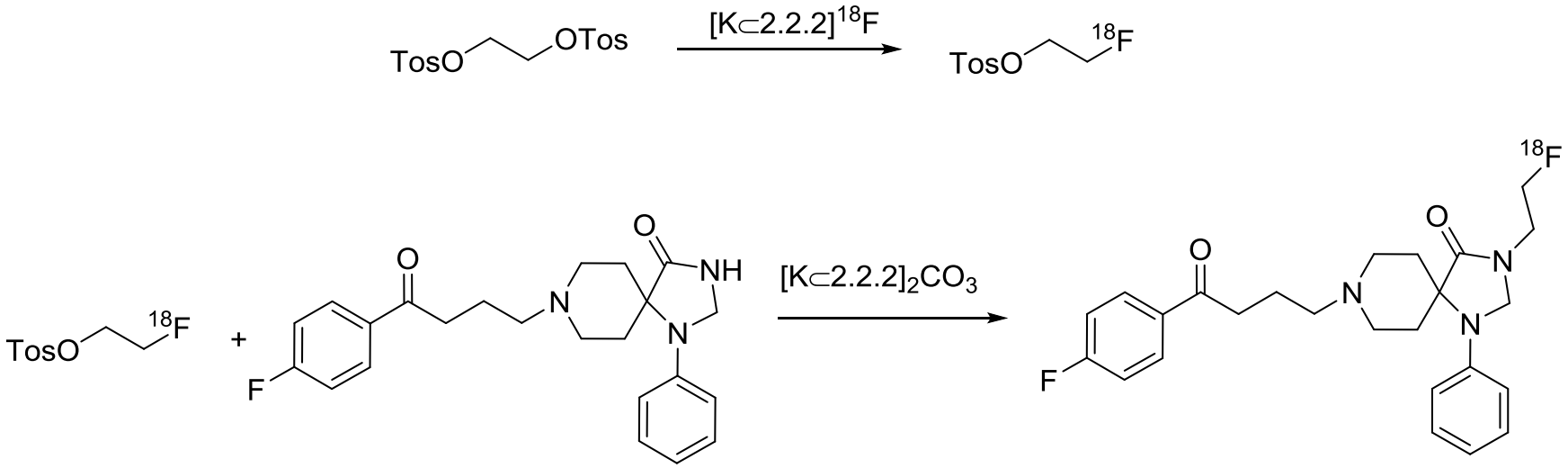


2-[^{18}F]Fluoroethyltriflate



1-Bromo-2-[^{18}F]fluoromethane

N.c.a. ^{18}F-fluoroethylation of Spiperone

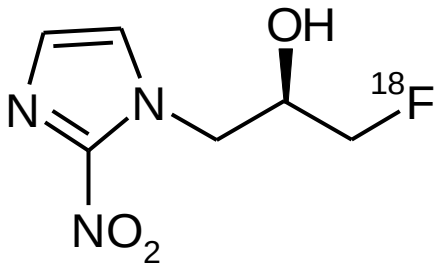


Radiochemical yield: 15 - 20 %

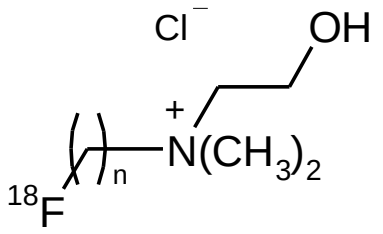
Molar activity: > 37 TBq/ mmol

Block et al. J. Label. Compd. Radiopharm 1986, 23, 1042.

Important ^{18}F-tracers labelled via aliphatic nucleophilic substitution

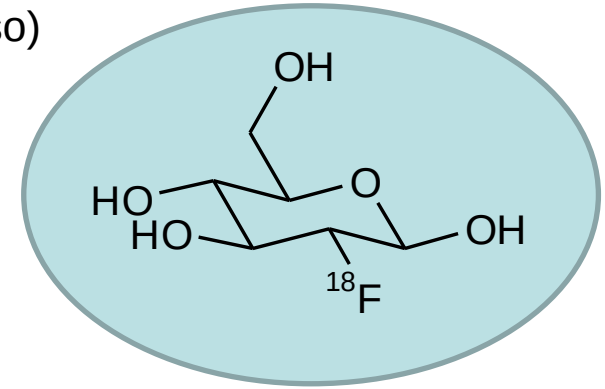


[^{18}F]Fluoromisonidazole (FMiso)
hypoxic cell marker

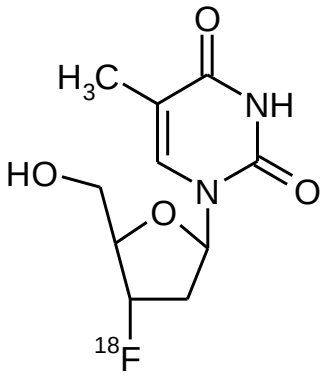


n = 1,2

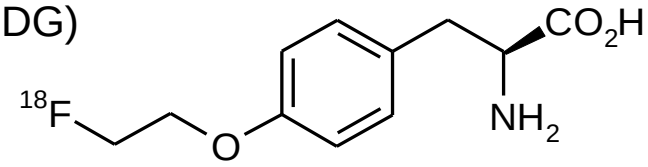
[^{18}F]Fluoro(m)ethylcholine (FCH)
CK marker



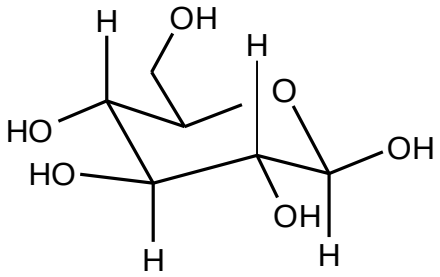
[^{18}F]Fluoro-2-deoxy-D-glucose (FDG)
phosphorylase marker



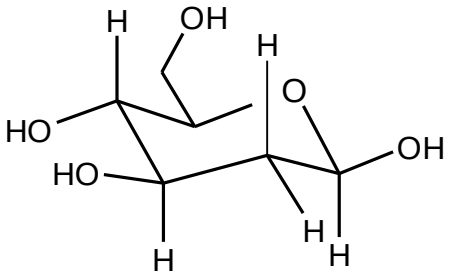
3´-Deoxy-3´-[^{18}F]fluorothymidine (FLT)
proliferation marker



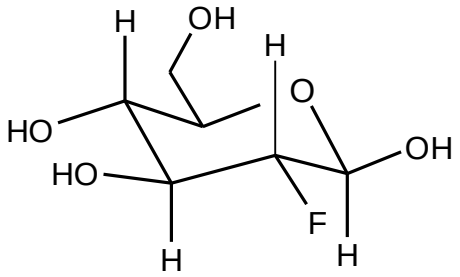
Regional glucose consumption in human brain quantified by PET and [^{18}F]FDG



D-Glucose

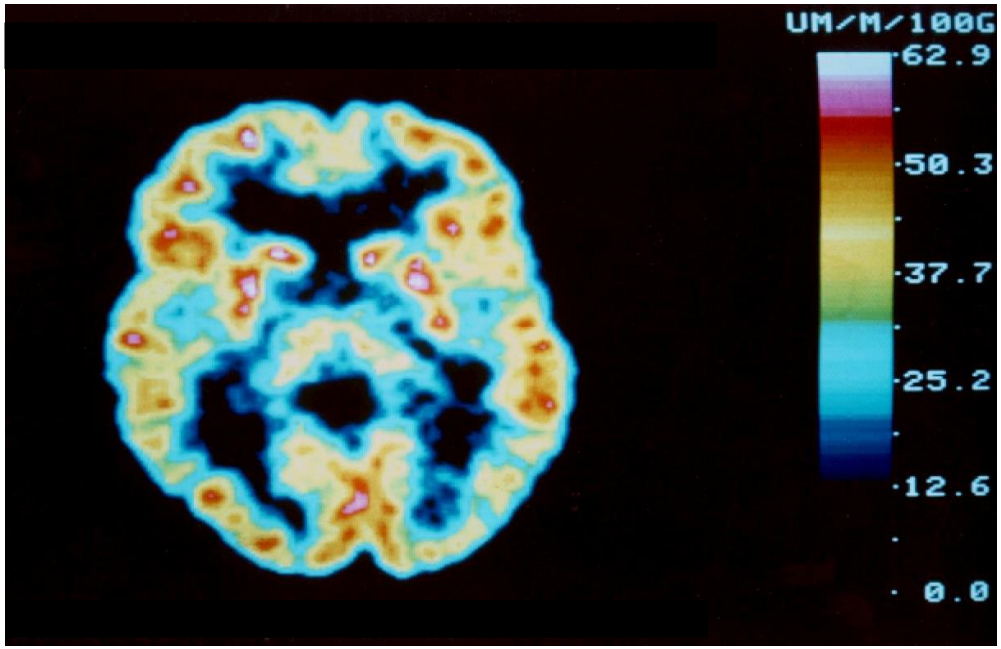


2-Deoxy-D-glucose



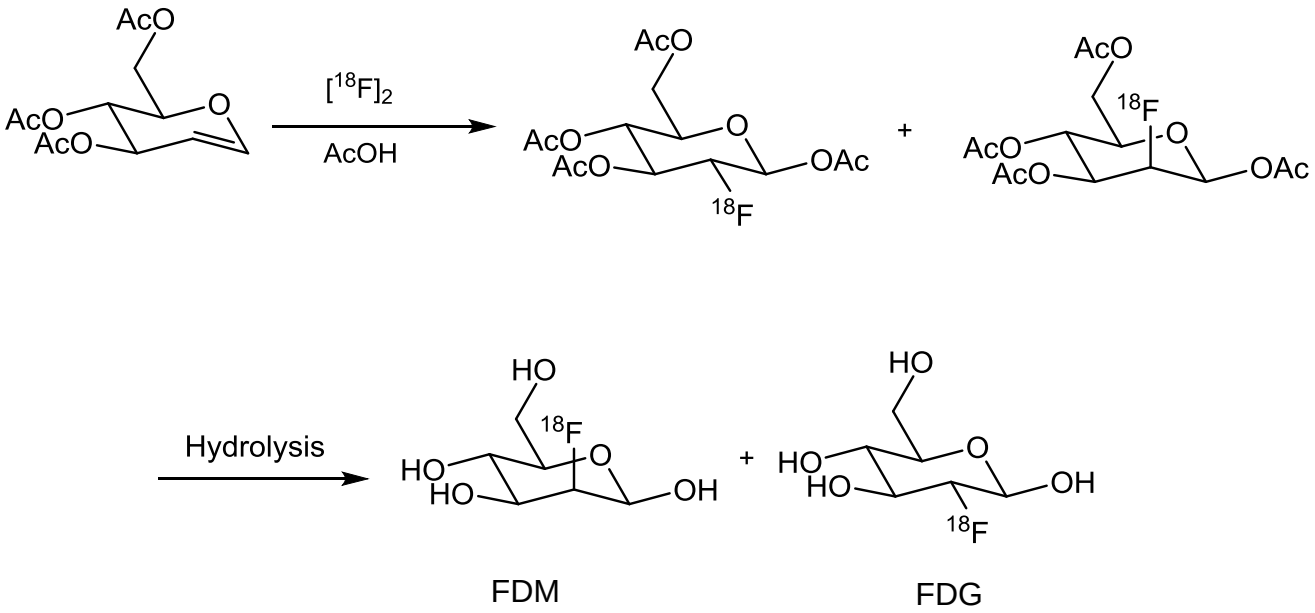
2-Deoxy-2-fluoro-D-glucose

2-Fluoro-2-deoxy-D-glucose (FDG)



Electrophilic Radiosynthesis of [^{18}F]fluoro-2-desoxy-glucose (FDG)

Ido et al. J. Label. Compds Radiopharm. 14, 175-183 (1978)



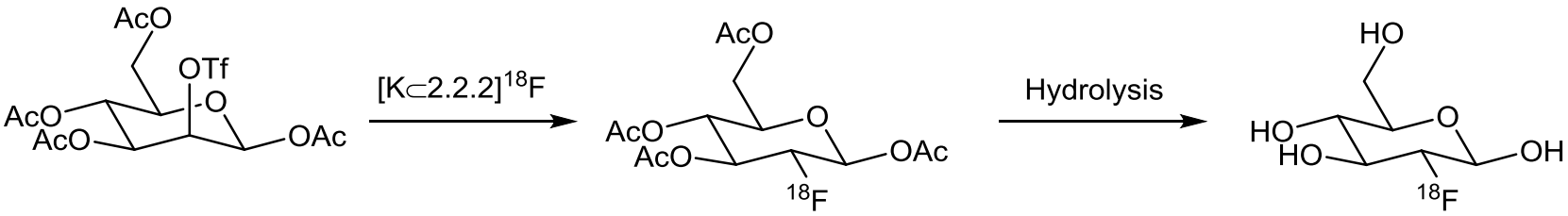
First synthesis by Ido et al. (1978) using [^{18}F]F_2 with tri-O-acetylglucal in Freon

<u>Problems:</u>

- Low yields (10%)
- Low specific activities
- Problem of stereospecificity (FDG:FDM low)

Nucleophilic Radiosynthesis of [^{18}F]fluoro-2-desoxy-glucose (FDG)

Hamacher, K., Coenen, H.H., Stöcklin, G.; J. Nucl. Med. 27, 235 - 238 (1986)



Tf = trifluoromethylsulfonyl
Ac = acetyl

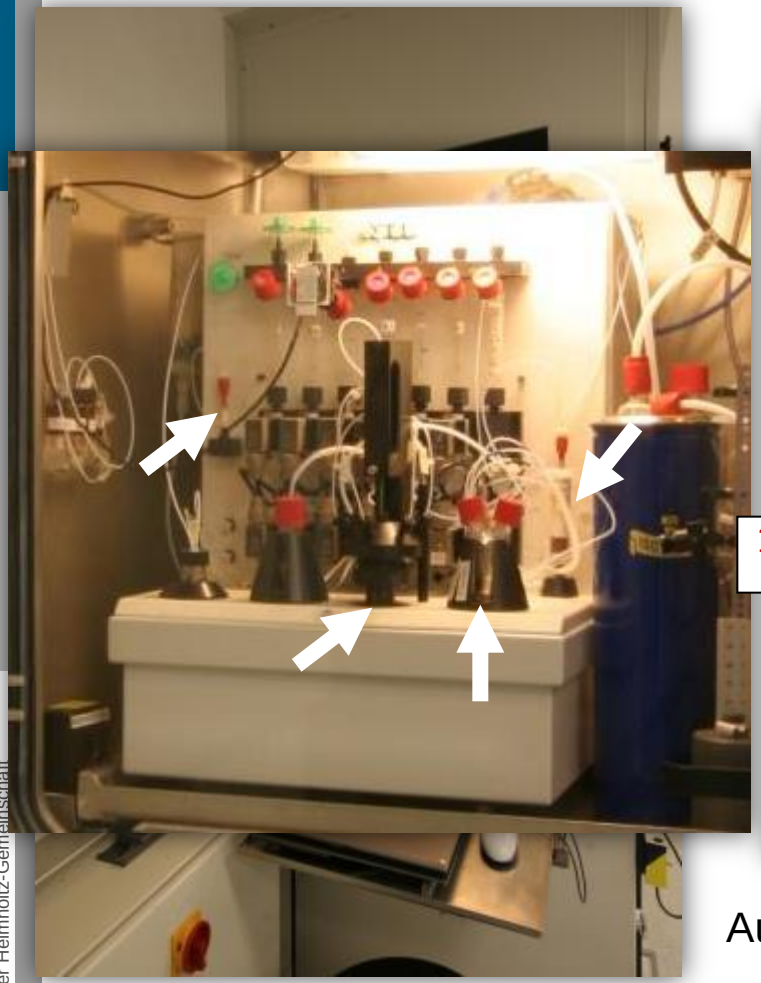
Hydrolysis:

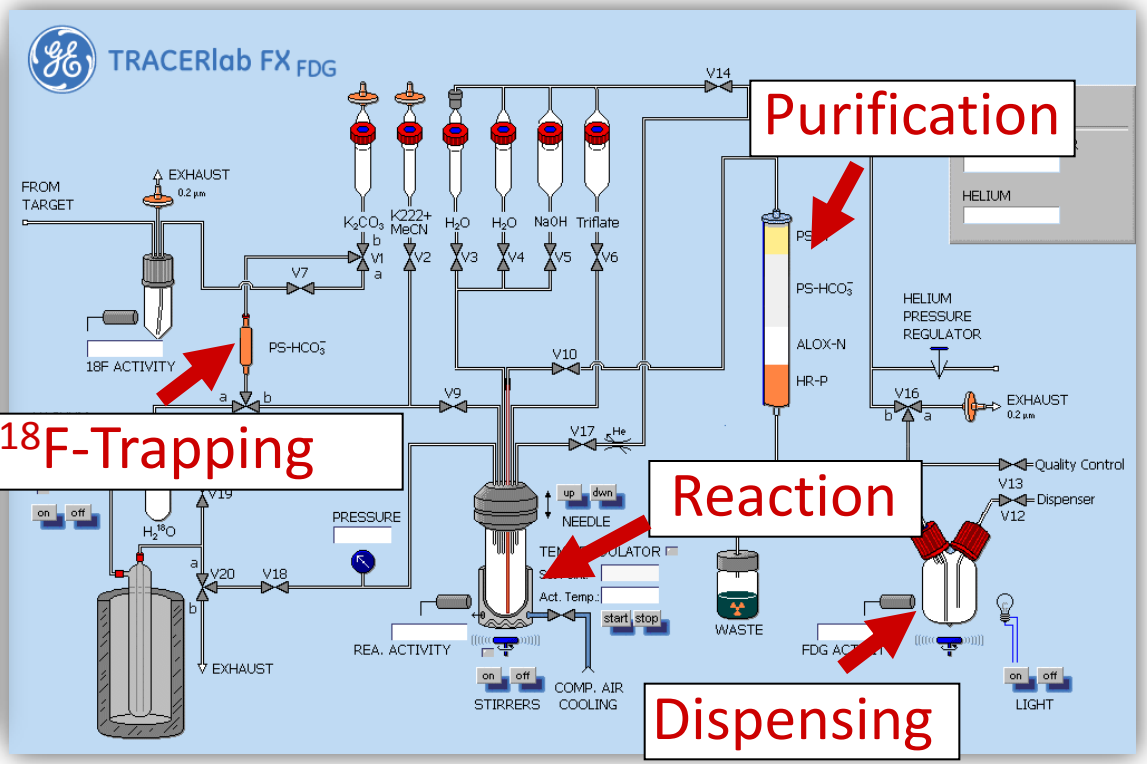
HCl: longer reaction time; side product formation

NaOH: short reaction time, better RCY

F. Füchtner, J. Steinbach, P. Mäding, B. Johannsen, Appl. Rad. Isot. 47, 61(1996)

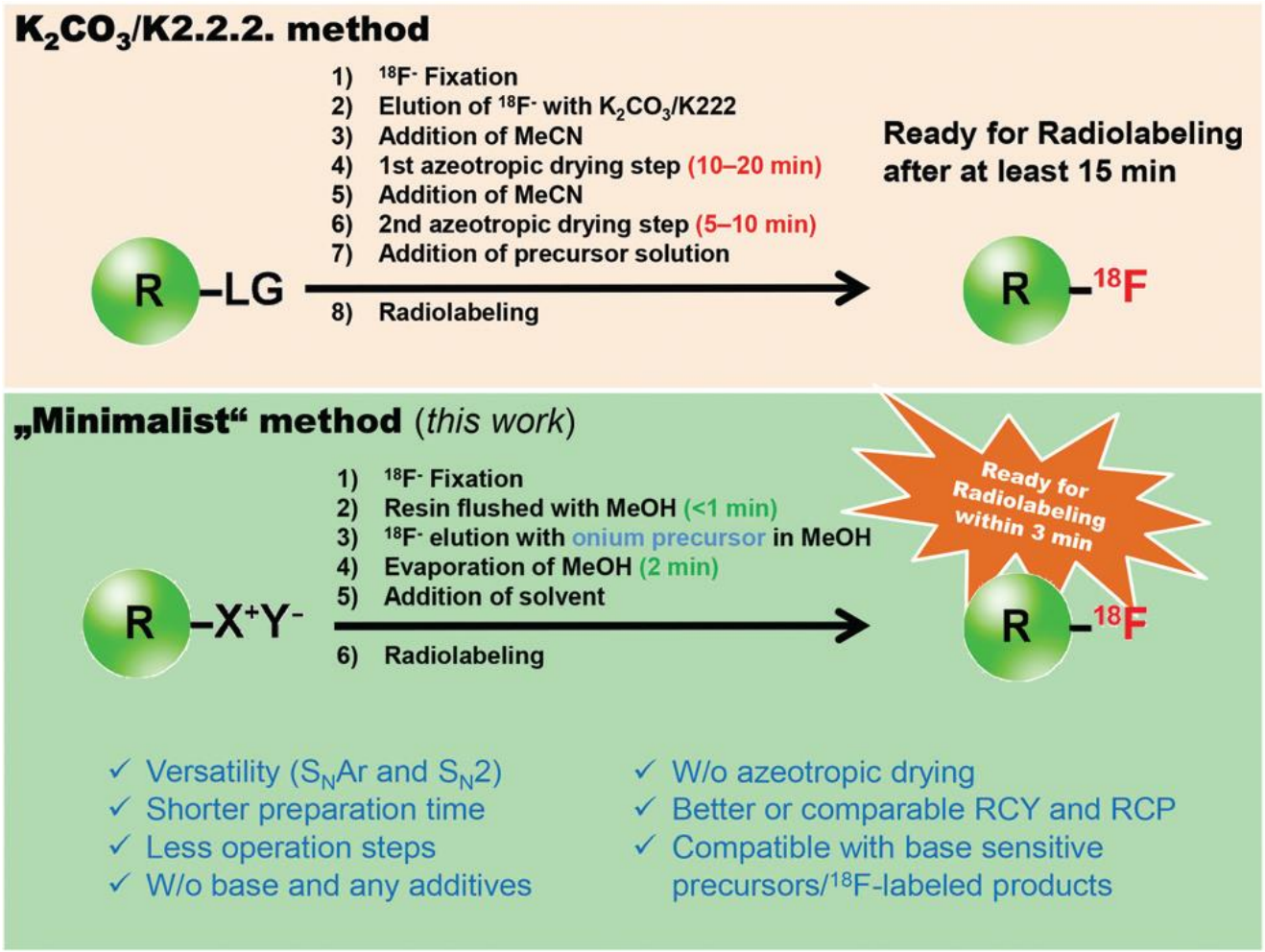
Radiosynthesis in hot cells





Automated and remotely controlled synthesis module

Radiosynthesis in hot cells



R. Richarz, P. Krapf, F. Zarrad, E.A. Urusova, B. Neumaier, B.D. Zlatopolskiy, Org. Biomol. Chem. 12 (2014) 8094-8099.

Present synthesis module for nucleophilic ^{18}F-fluorination

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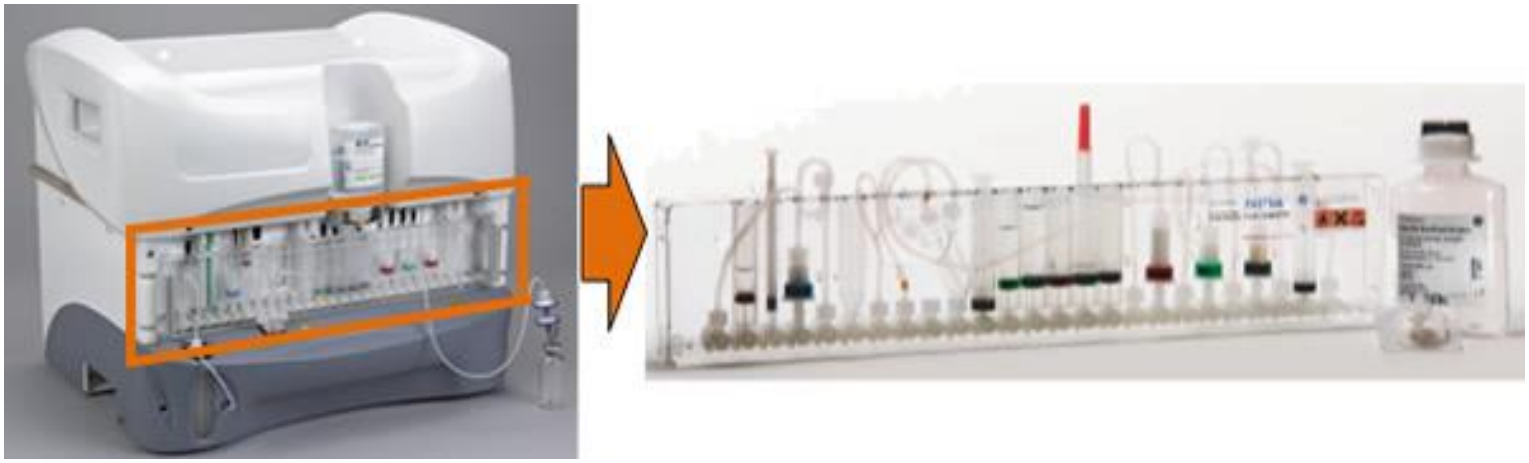
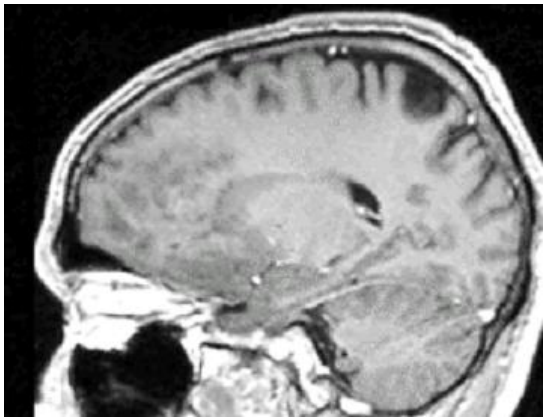
GE FASTlab

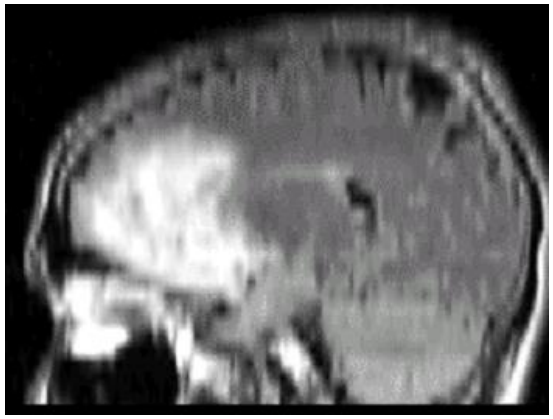


- Access to a variety of PET tracers, non-proprietary and GE proprietary tracers
- Ease of operation
- Facilitation of GMP and regulatory compliance
  - Integrated pharmaceutical grade cassette, preloaded with chemicals and all components
  - 1 fully validated and documented kit
  - ONE consolidated Certificate of Analysis
  - White papers
- Operational efficiency
  - High reliability
  - High/consistent yield

[^{18}F]FET PET in Cerebral Gliomas

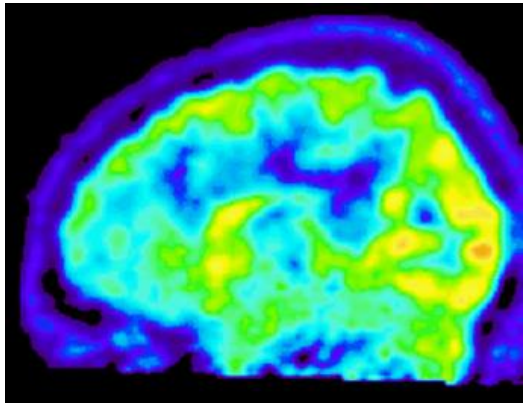


MR (T1 + Gd)

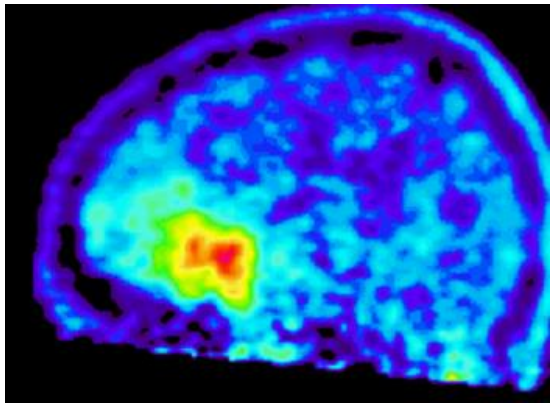


MR (T2, Flair)

MRT



[^{18}F]FDG PET

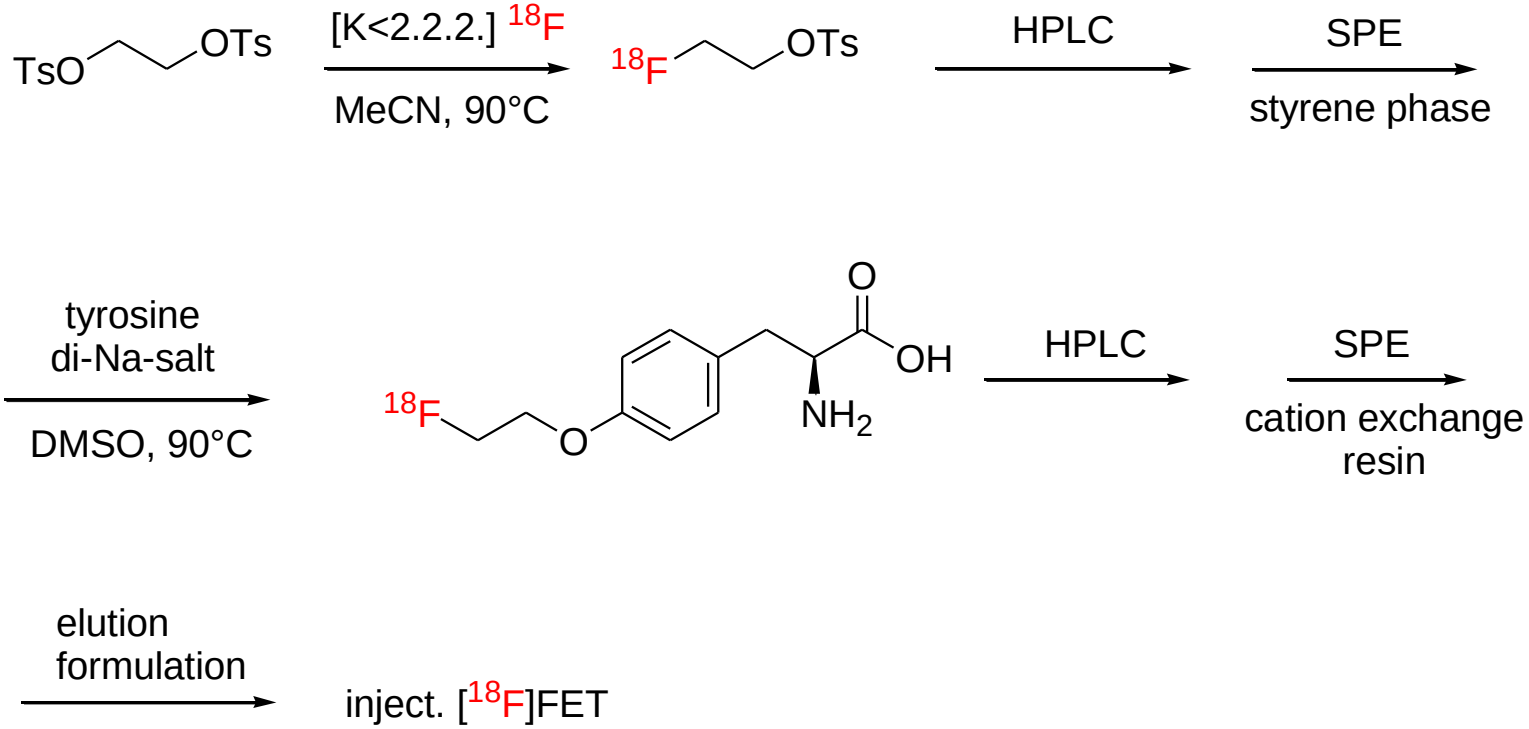


[^{18}F]FET PET

PET

Teaching point:
Biopsy controlled studies proved an excellent delineation of gliomas by FET PET.
FDG PET is not helpful in many cases since uptake in normal brain tissue is high.

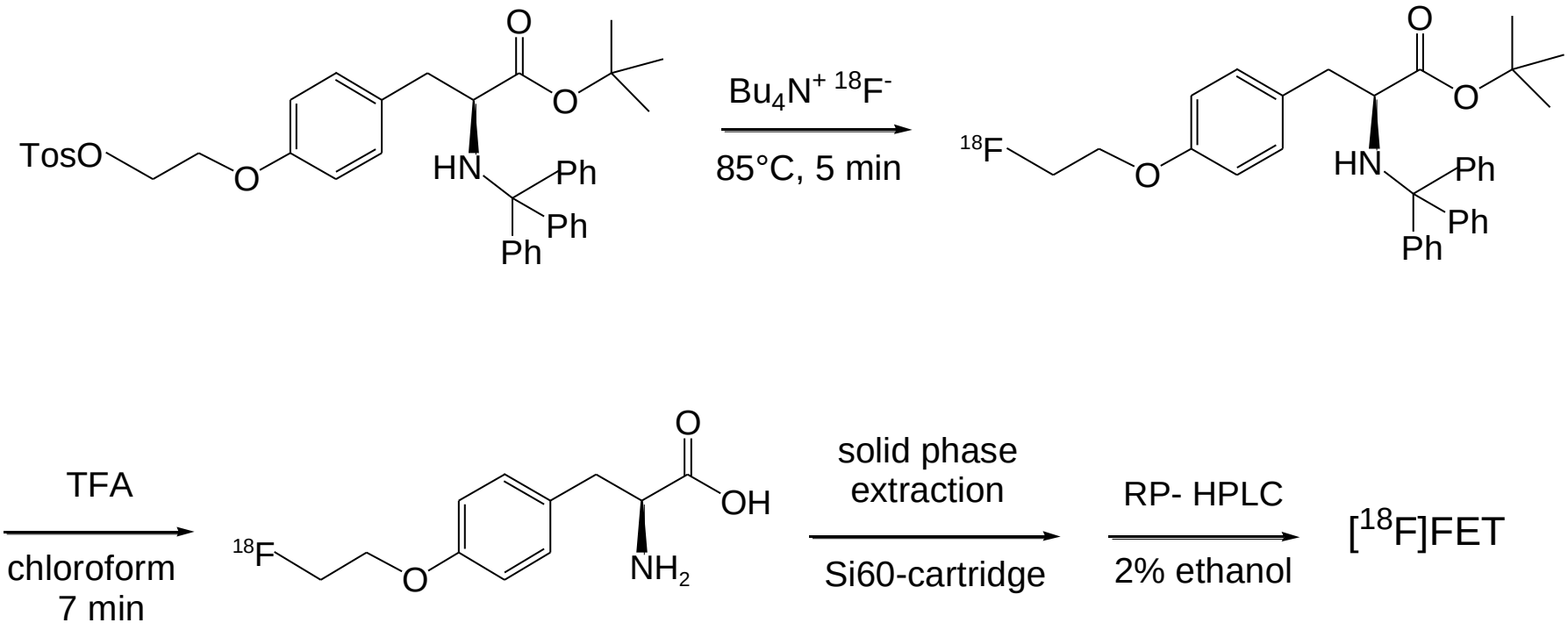
Two-step synthesis of O-(2-[^{18}F]fluoroethyl)-L-tyrosine



RCY: 20…30 %

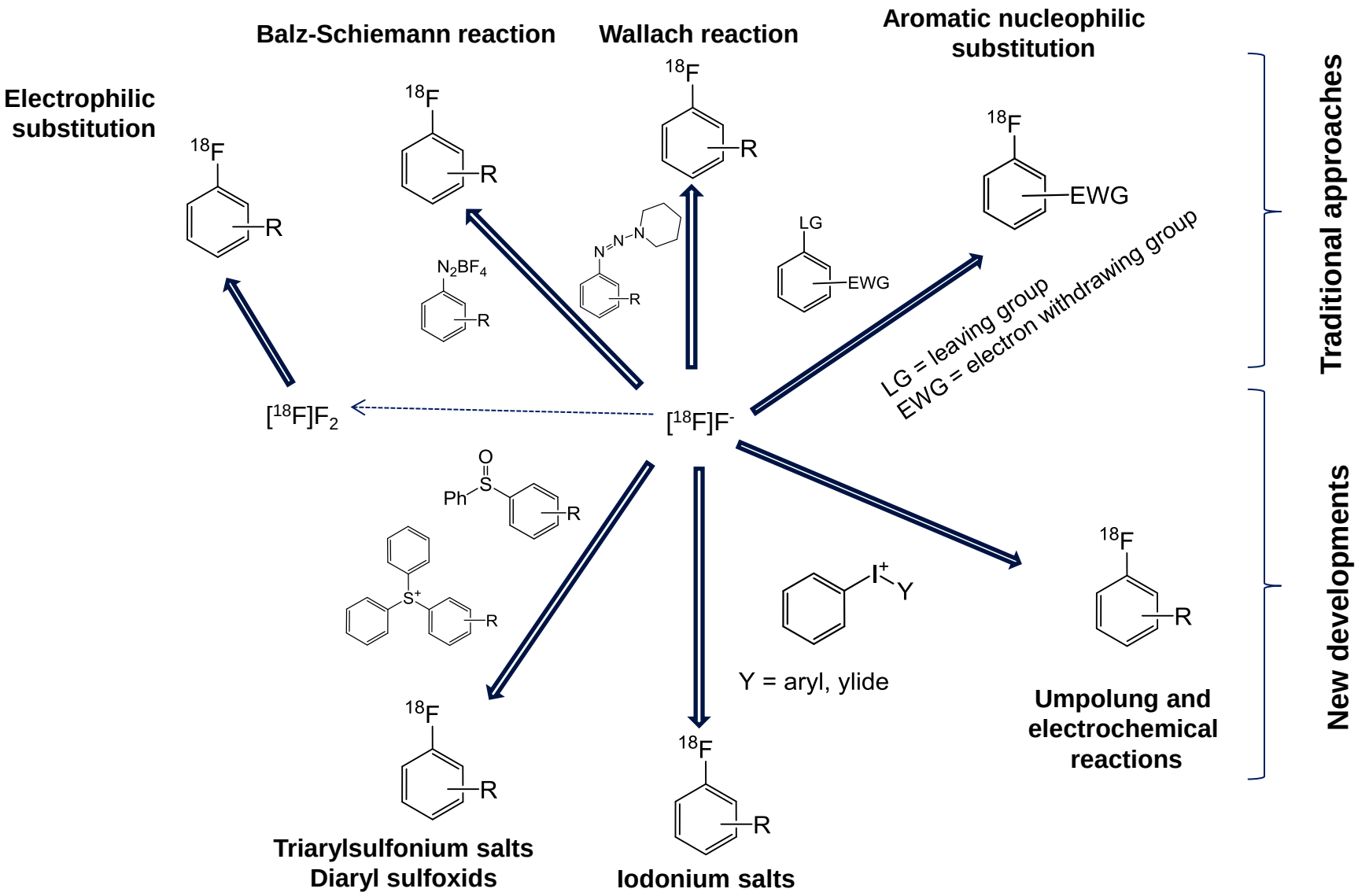
Wester PhD thesis, FZJ 1996

Efficient syntheses of O-(2-[^{18}F]fluoroethyl)-L-tyrosine via nucleophilic fluorination



RCY: 55…60 %

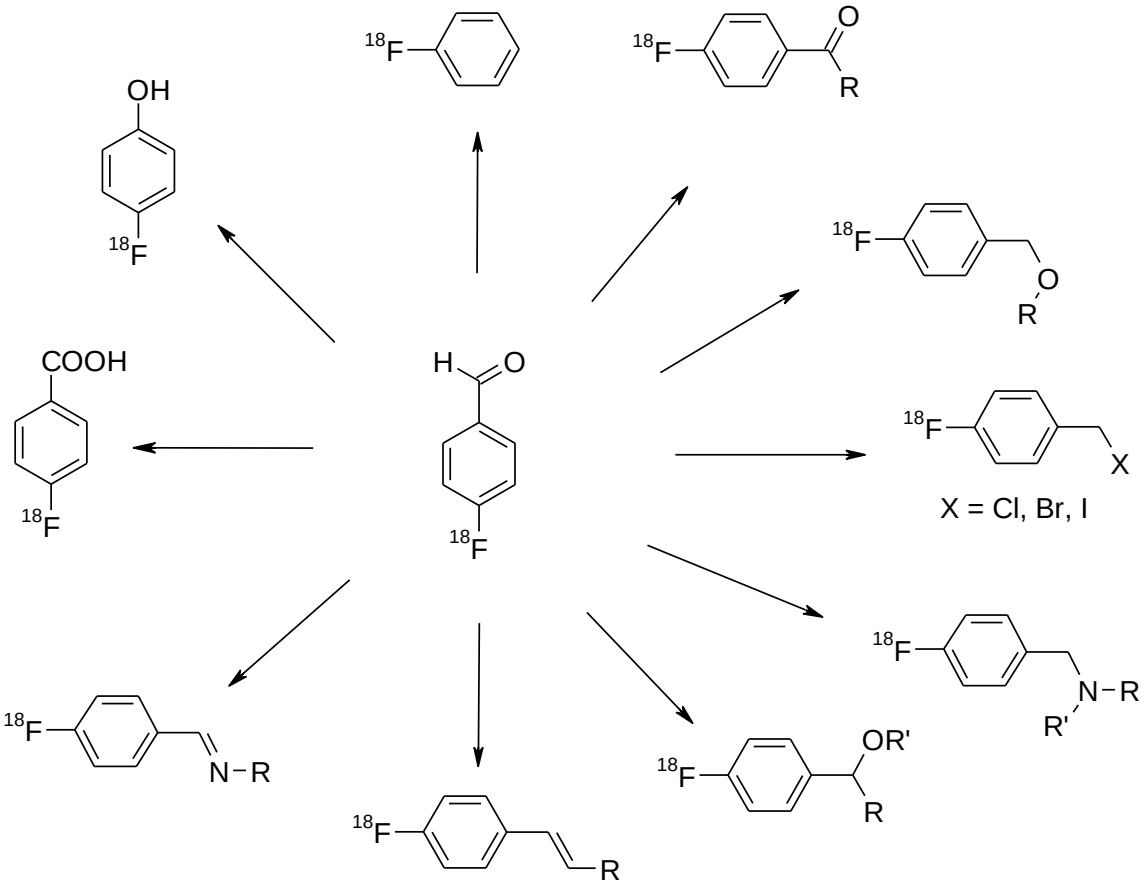
[^{18}F]Fluoride and nucleophilic aromatic substitution



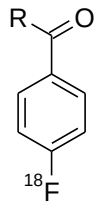
Ermert, BioMed Res. Int., (2014) 812973.

Directly accessible ^{18}F-intermediates (synthons or building blocks) for build-up syntheses

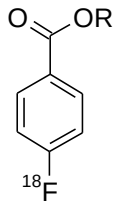
[^{18}F]Fluorobenzaldehyde



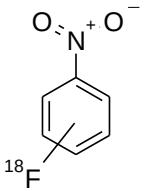
Other intermediates:



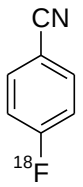
[^{18}F]Fluoroarylketone



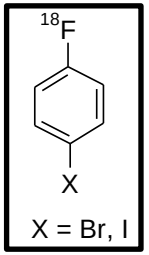
[^{18}F]Fluorobenzoic acid ester



[^{18}F]Fluoronitroarene



[^{18}F]Fluorobenzonitrile



[^{18}F]Fluorohaloarene

Nucleophilic ^{18}F-substitution of arenes via “onium” salts

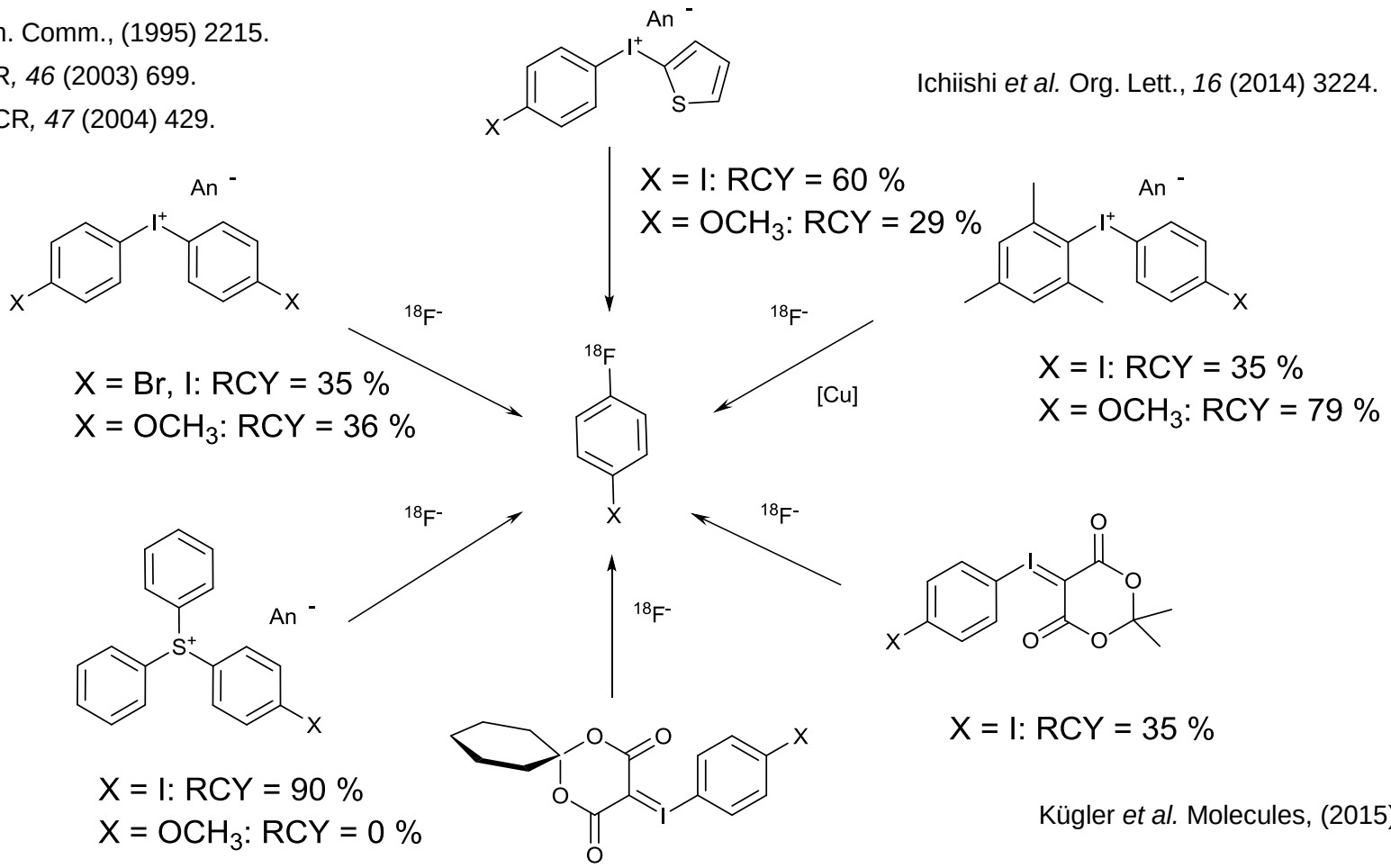
Ross *et al.* JACS, *129* (2007) 8018.

Pike *et al.*, Chem. Comm., (1995) 2215.

Wüst *et al.*, JLCR, *46* (2003) 699.

Ermert *et al.*, JLCR, *47* (2004) 429.

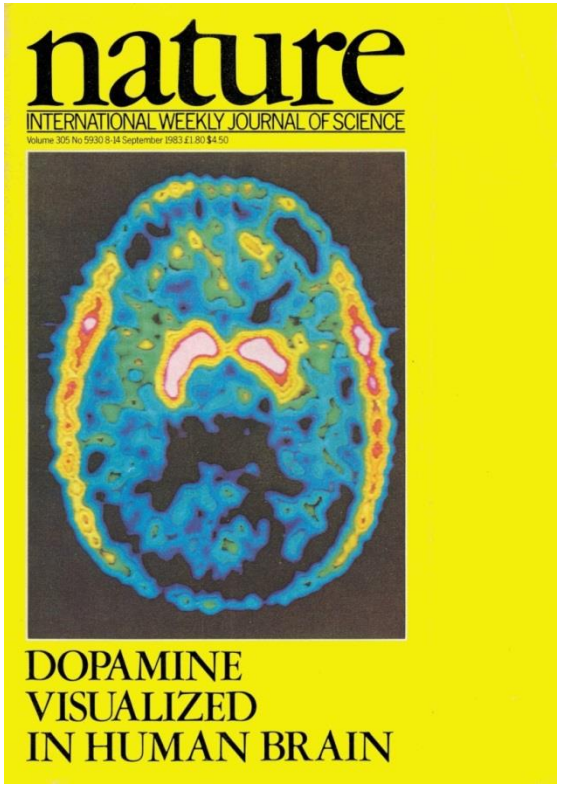
Ichiishi *et al.* Org. Lett., *16* (2014) 3224.

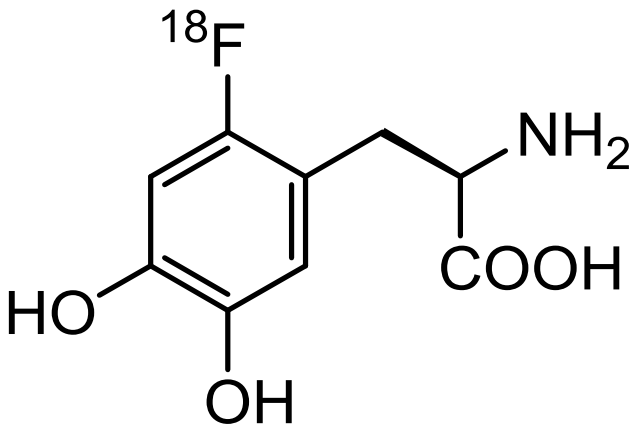


Kügler *et al.* Molecules, (2015) 470.

Rotstein *et al.* Nat. Comm., *5* (2014) 4365.

6-[^{18}F]Fluoro-L-DOPA

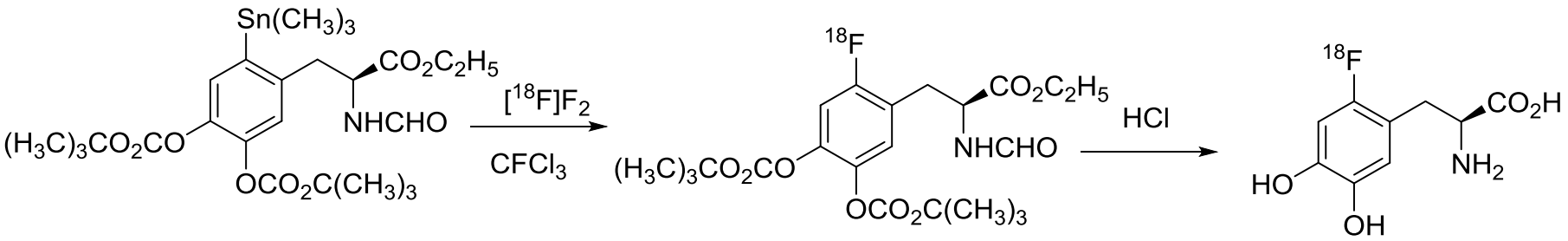




E.S. Garnett, G. Firnau, C. Nahimias Nature **305**, 137-138 (1983)

Derivative of anti-parkinsonism drug L-DOPA
Measurement of decarboxylase activity
to assess presynaptic dopaminergic function

Electrophilic Synthesis of 6-[^{18}F]Fluoro-L-DOPA

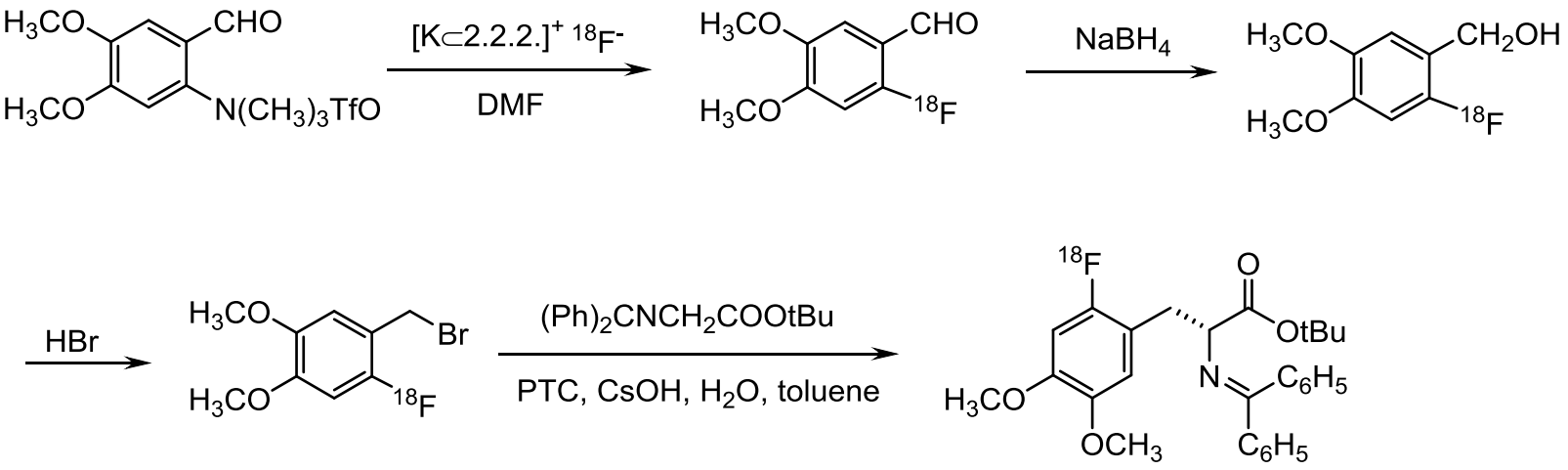


Namavari et al., 1982

de Vries et al., 1999

- 2 step c.a. synthesis
- ee = > 98 %
- process established on commercial synthesizer
- [^{18}F]F_2 target necessary

6-[^{18}F]Fluoro-L-DOPA by build-up synthesis



PTC

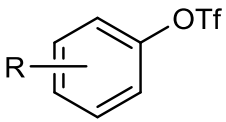
Lemaire *et al*. Eur. J. Org. Chem. (2004) 2899.

Libert *et al.* J. Nucl. Med. *54* (2013) 1154.

- 5 step n.c.a. synthesis
- ee = > 96 %
- process established on commercial synthesizer

Transition metal mediated nucleophilic radiofluorination

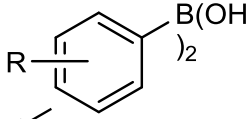
Cardinale *et al.* JLCR, *55* (2012) 450.



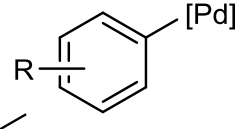
Pd complex
Cs[^{18}F]

- Pd complex commercial available
- carrier necessary

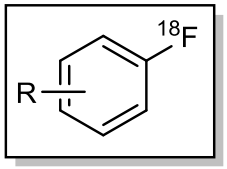
Lee *et al.* Science, *334* (2011) 639.



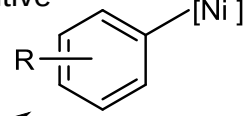
- Pd complex synthesis necessary and difficult



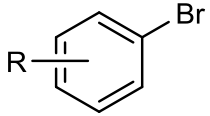
Pd[IV] ^{18}F complex



- Ni complex synthesis necessary
- Oxidant very sensitive



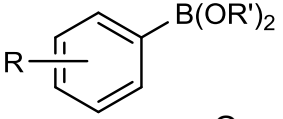
[^{18}F]F$^-$, 18-crown-6
strong oxidant



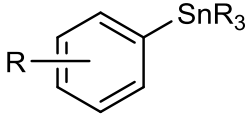
Lee *et al.* JACS, *134* (2012) 17456.
Zlatopolskiy *et al.* ChemistryOpen, *4* (2015) 457.

[^{18}F]F$^-$, K⊂2.2.2.
[Cu(OTf)$_2$(py)$_4$]

Tredwell *et al*. Angew. Chem., Int. Ed., *53* (2014) 7751.

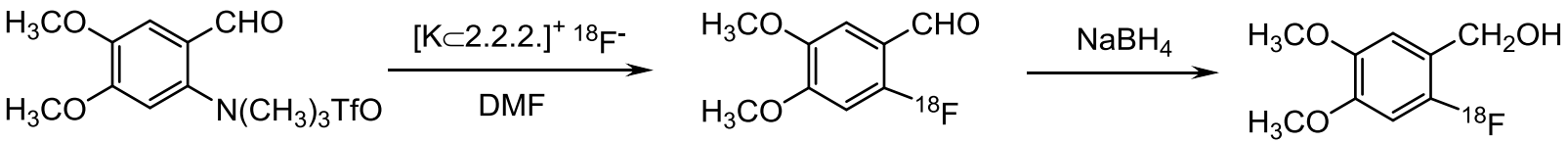


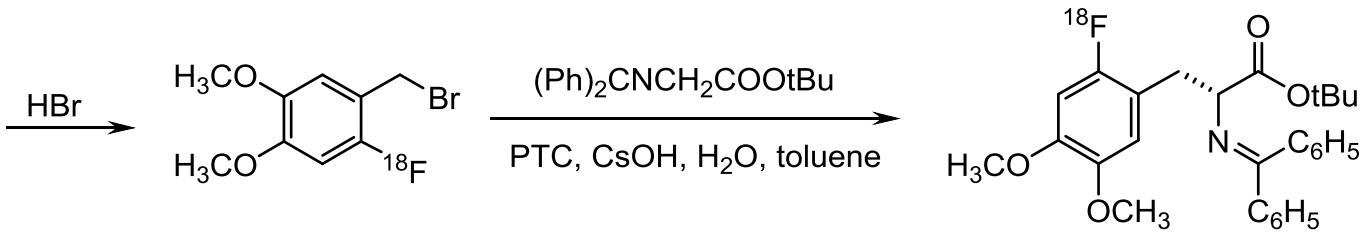
- Cu complex commercially available
- Presently most promising methods

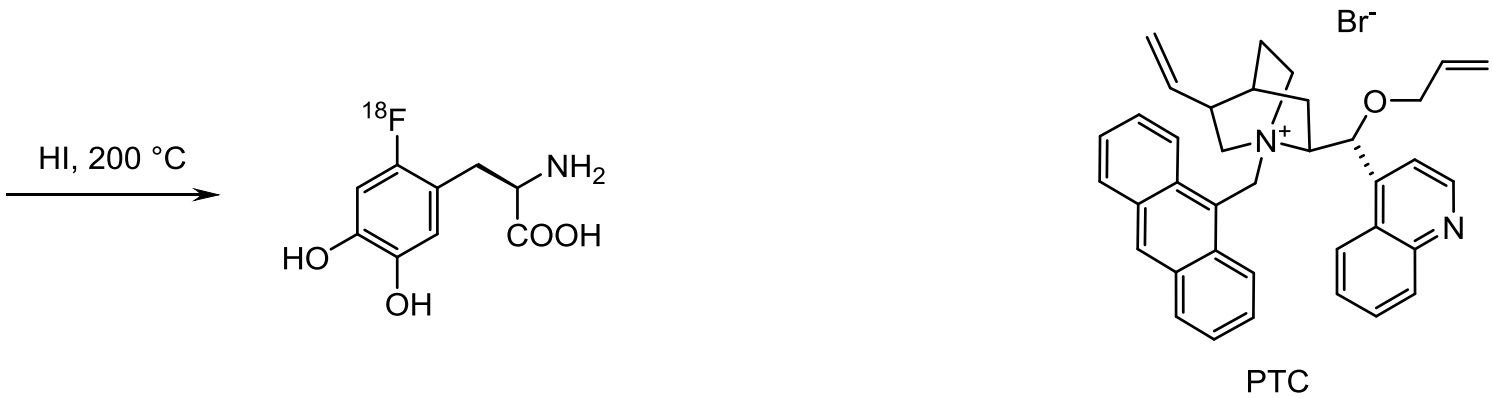


Makaravage *et* al., *Org. Lett.* 18 (2016) 5440.

6-[^{18}F]Fluoro-L-DOPA by build-up synthesis







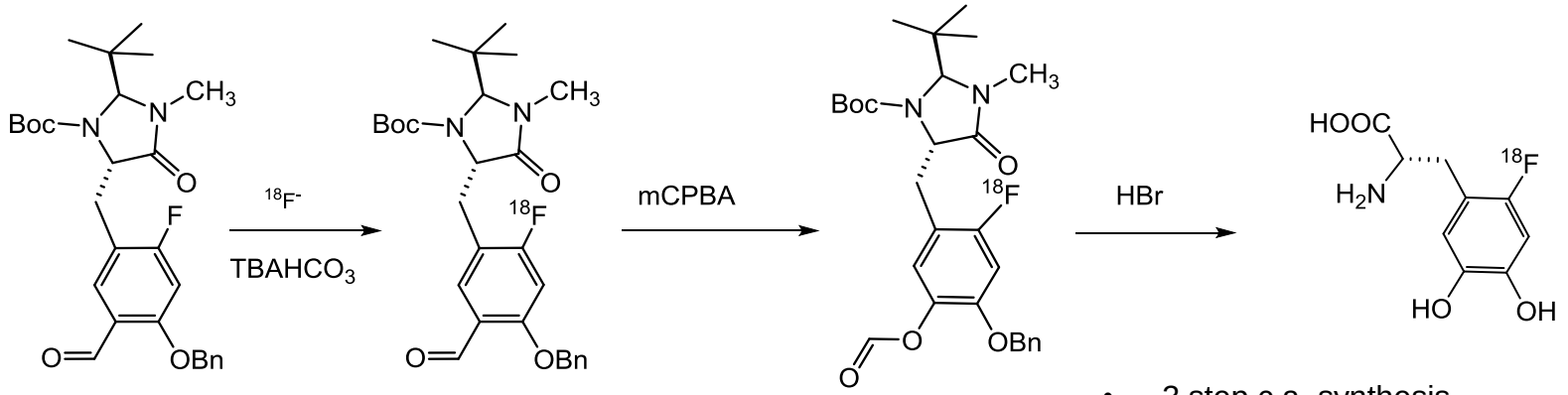
PTC

Lemaire *et al*. Eur. J. Org. Chem. (2004) 2899.

Libert *et al.* J. Nucl. Med. *54* (2013) 1154.

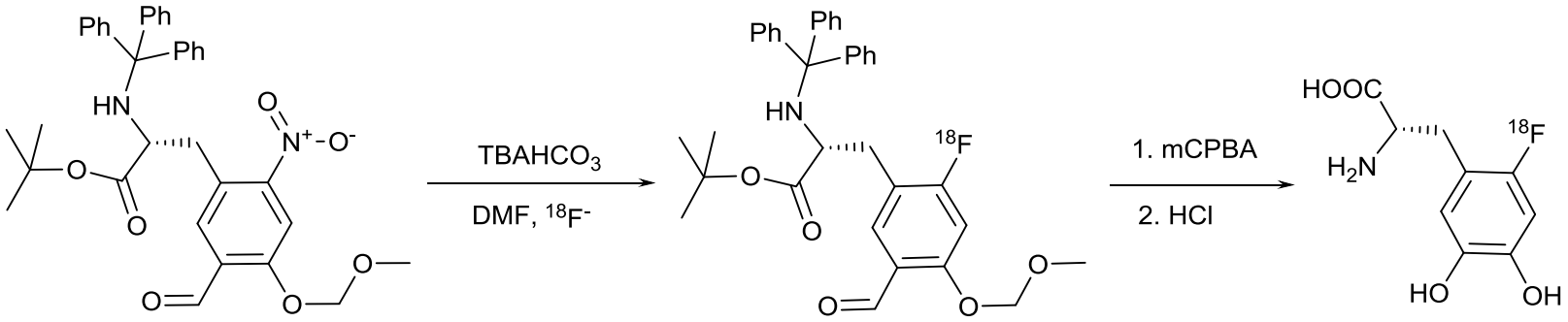
- 5 step n.c.a. synthesis
- ee = > 96 %
- process established on commercial synthesizer

6-[^{18}F]Fluoro-L-DOPA by direct labelling



- 3 step c.a. synthesis
- ee = 92- 96 %
- hydrolysis under harsh conditions

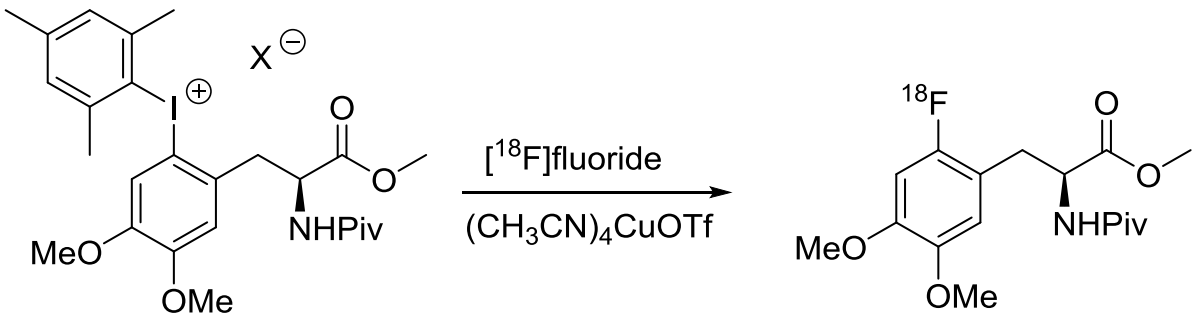
Wagner *et al.* J. Nucl. Med. *50* (2009) 1724.
Castillo *et al.* Org. Biomol. Chem. *9* (2011) 765.



- 3 step n.c.a. synthesis
- ee = > 96 %
- process established on commercial synthesizer

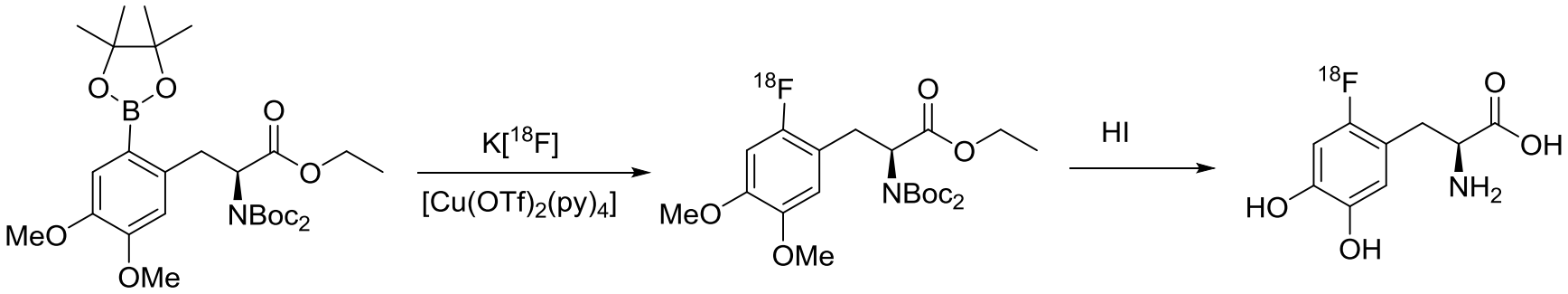
Martin *et al.,* JLCR, *56* (2013) S126.

6-[^{18}F]Fluoro-L-DOPA by copper catalysis



- RCY = 17 % (X = OTos)
- RCY = 31 % (X = BF_4)

Ichiishi *et al*. Org. Lett., *16* (2014) 3224.



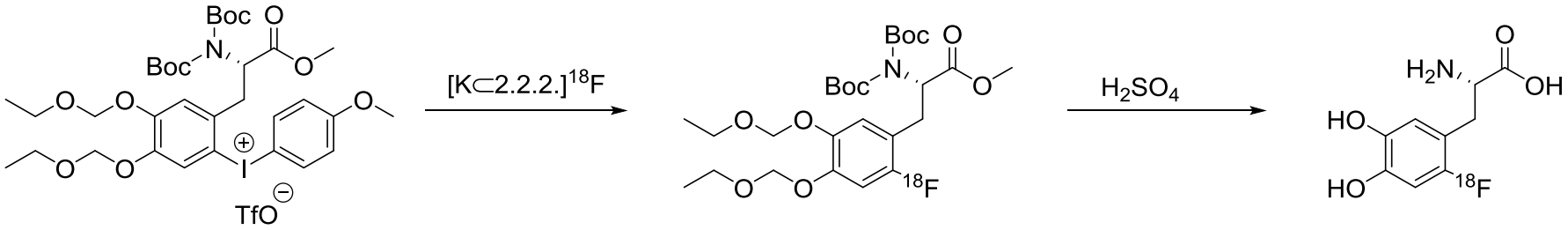
Tredwell *et al.* Angew. Chem., Int. Ed., *53* (2014) 7751.

- 2 step n.c.a. synthesis
- ee = > 98 %
- overall RCY 12%

Comparison of both methods on a preparative scale

Zlatopolskiy *et al.* Chem. - Eur. J., *21* (2015) 5972.

6-[^{18}F]Fluoro-L-DOPA by iodonium salts



Kuik *et al.* J. Nucl. Med., *56* (2015) 106.

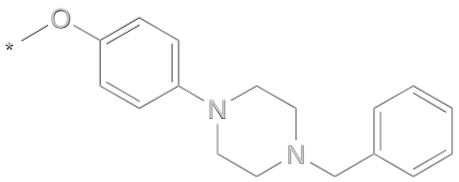
- 2 step n.c.a. synthesis
- ee = > 99 %
- specific activity of 35 GBq/µmol
- overall RCY 14%
- process established on commercial synthesizer

Reviews 6-[^{18}F]fluoro-L-DOPA syntheses:

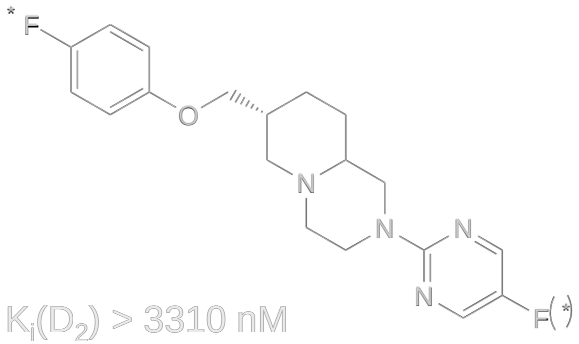
Pretze *et al.* BioMed Res. Int., (2014) 674063.

Edwards and Wirth, JLCR, 58 (2015) 183.

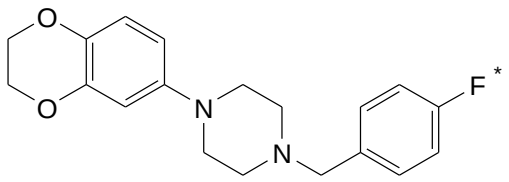
D_4 Ligands - selection II



$K_i(D_2)$ > 5000 nM
$K_i(D_3)$ > 5000 nM
$K_i(D_4)$ = 93 nM clog P 3,37

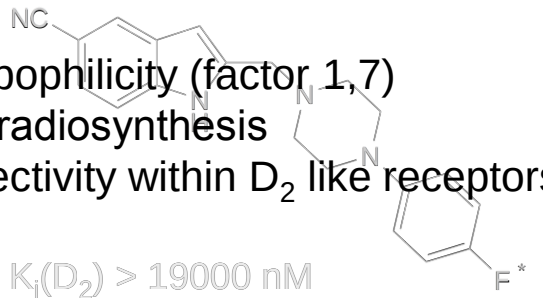


$K_i(D_2)$ > 3310 nM
$K_i(D_4)$ = 3.4 nM clog P 3,28



$K_i(D_2)$ > 5000 nM
$K_i(D_3)$ > 5000 nM
$K_i(D_4)$ = 4 nM clog P 3,27

„FBPBD“



- lowest lipophilicity (factor 1,7)
- „easier“ radiosynthesis
- high selectivity within D_2 like receptors

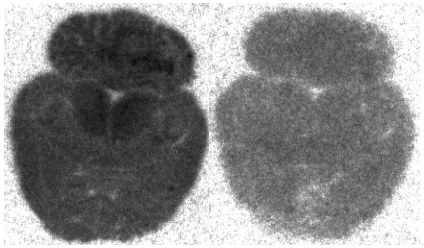
$K_i(D_2)$ > 19000 nM
$K_i(D_3)$ > 15000 nM
$K_i(D_4)$ = 1 nM clog P 3,51

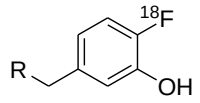
J. Med. Chem. 39 (1996) 1941.
***Bio. Med. Chem.* 9 (2001) 3207.**
Bioorg. Med. Chem. Let. 8 (1999) 725.
J. Med. Chem. 43 (2000) 4563.

clog P: Ø MarvinSketch 5.1.4; ALOGPS 2.1

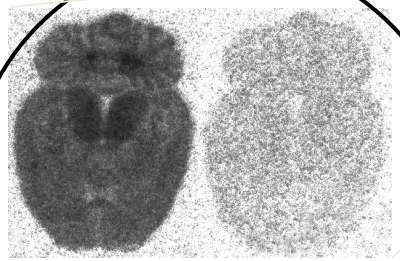
Correlation:
lipophilicity – non-specific binding (rat)

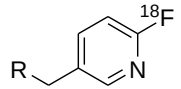
global competition



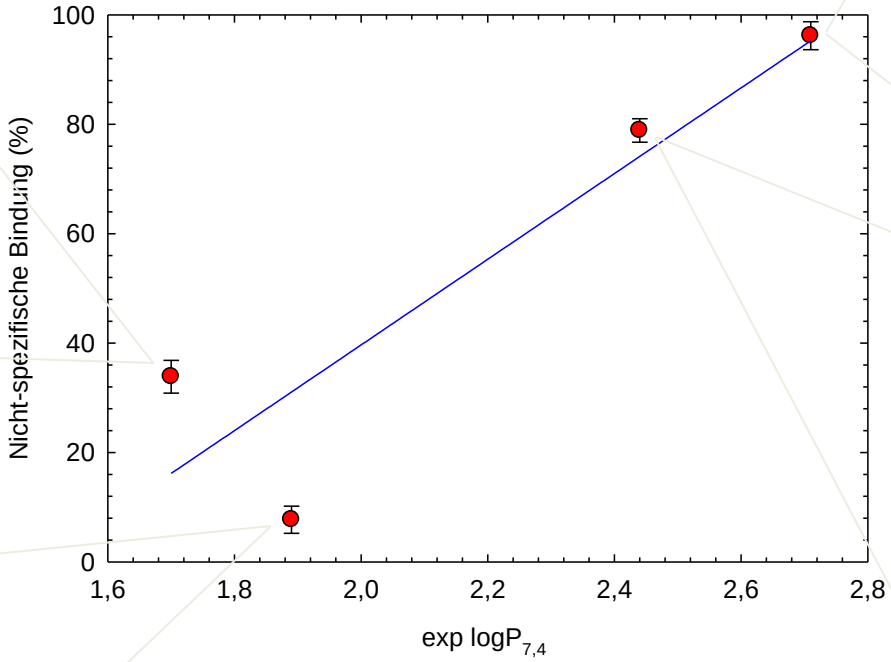


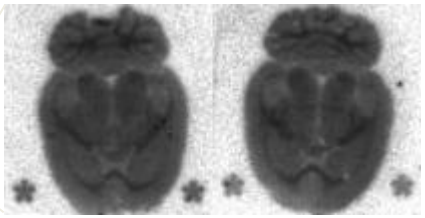
[^{18}F]**1d**

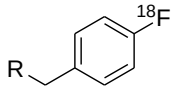




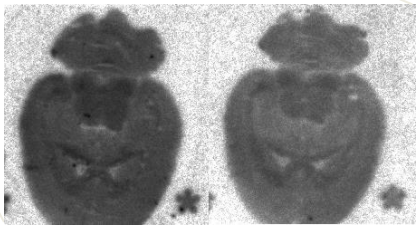
[^{18}F]**1e**

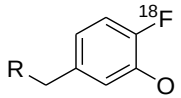






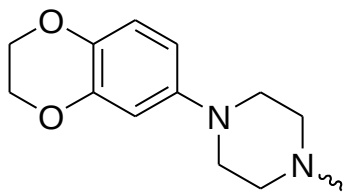
[^{18}F]**1a**



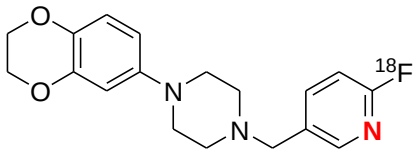


[^{18}F]**1b**

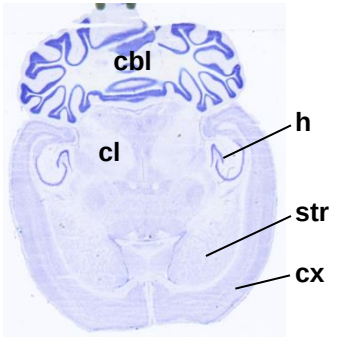
R:

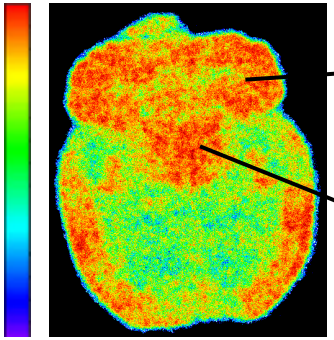


Ex vivo braindistribution of [^{18}F]1e (mouse)

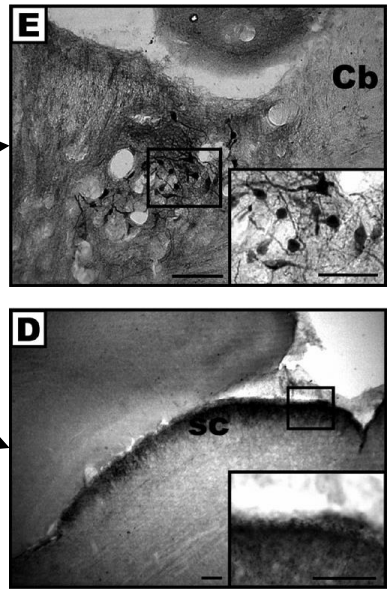


[^{18}F]**1e**





Global binding
15 min p.iVI.



- accumulation in cortex (cx) and hippocampus (h)
- no binding in basal-ganglia (str) → no $D_{2/3}$
- *high accumulation in cerebellum (cbl) and colliculum (cl)*

Combination: Immunohistochemistry / BAC

Eur. J. Neurosci. 24 (2006) 2429.

Kügler F, Sihver W, Ermert et al. *J Med Chem, 2011;54:8343-8352.*

Summary

Labelling reactions for ^{18}F-fluorination generally afford rather harsh conditions => complex molecules are somewhat difficult to label.

Indirect labelling is often unavoidable, especially with complex molecules and macromolecules.

Novel developments with iodonium salts and/or transition metal mediated reactions allow late-stage ^{18}F-fluorination of (electron-rich) aromatic compounds.

Only remotely controlled performable and efficient radiosyntheses allow acceptance in practice and broad application!