Preparation of n.c.a. 6-[^{18}F]fluoro-L-tryptophan using copper-mediated radiofluorination

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Ninth International Conference on Nuclear and Radiochemistry – NRC9
August 31^{h} 2016 | Dominique Schäfer

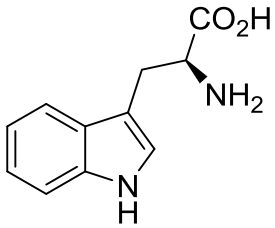
Positron emission tomography for molecular imaging

- Imaging on the molecular level without pharmacodynamic interference
- Quantitation of concentrations and metabolic rates
- Resolution
  - Temporal: seconds to minutes
  - Spatial: 5 mm (standard)

- Fluorine-18 most important positron emitter:
  - half-life: 109.7 min
  - 97% β^+ (3% EC)
  - 0.63 MeV max. positron energy

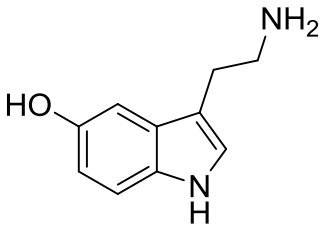
L-Tryptophan

- Essential amino acid
  - Active transport through BBB
  - Upregulated consumption in tumors[1]

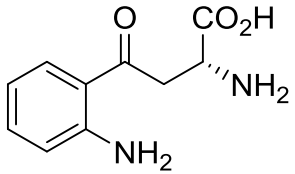


L-Tryptophan

- Involved in
  - Protein synthesis
  - Serotonin synthesis(<5%)
  - Kynurenine pathway(>90%)



Serotonin

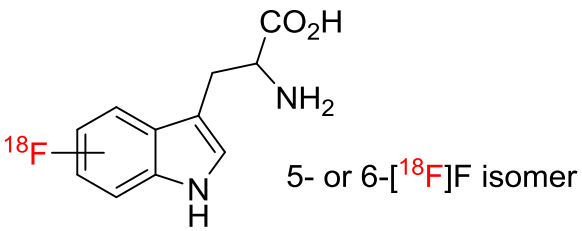


L-Kynurenine

[1]: Optiz et al., *Nature*, **2011**, Vol. 478, *7368*, 197-203.

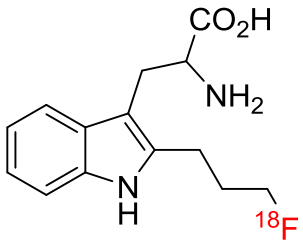
Radiofluorination of tryptophan

- [^{18}F]Fluorotryptophan
  - Only insufficient yields[1] or prosthetic groups[2,3]

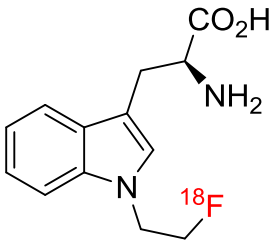


5- or 6-[^{18}F]F isomer

Atkins et al.[1]



Chiotellis et al.[2]



Sun et al.[3]

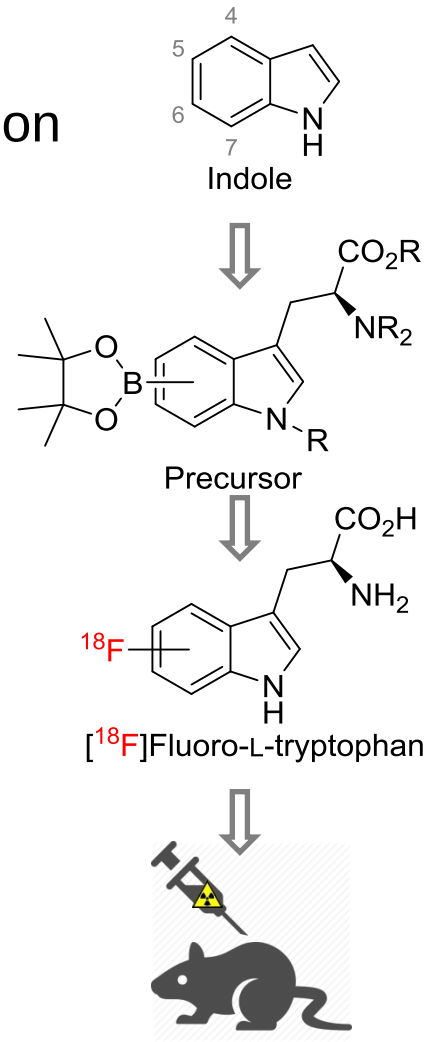
- New approaches of radiofluorination using transition-metals[4-6]

  → Radiofluorination of electron-rich arenes

[1]: Atkins et al., *J. Nucl. Med*. **1971**, Vol. 13, *10*, 713-19.
[2]: Sun et al., *Appl Rad Isot.* **2012**, *70*, 676-80.
[3]: Chiotellis et al., *Eur. J. Med. Chem*. **2013**, *70*, 768-80.
[4]: Lee et al., *Science*,**2011**, *334*, 639-42.
[5]: Lee et al., *J. Am. Chem. Soc.* **2012**, *134,* 17456-58
[6]: Ichiishi et al., *Org. Lett.* **2014**, *16*, 3224-27.

Aim of this work

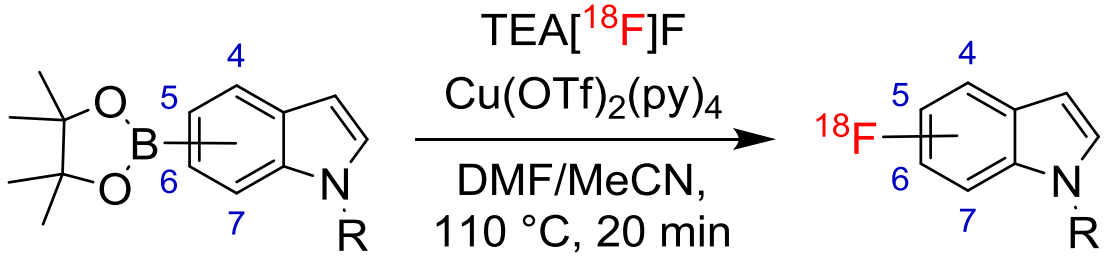
- Determination of the most reactive substitution position of indole model-compound using copper-mediated radiofluorination[1]
- Synthesis of appropriate tryptophan precursor
- Optimization of radiosynthesis
- Transfer to an automated synthesis device
- Preclinical evaluation



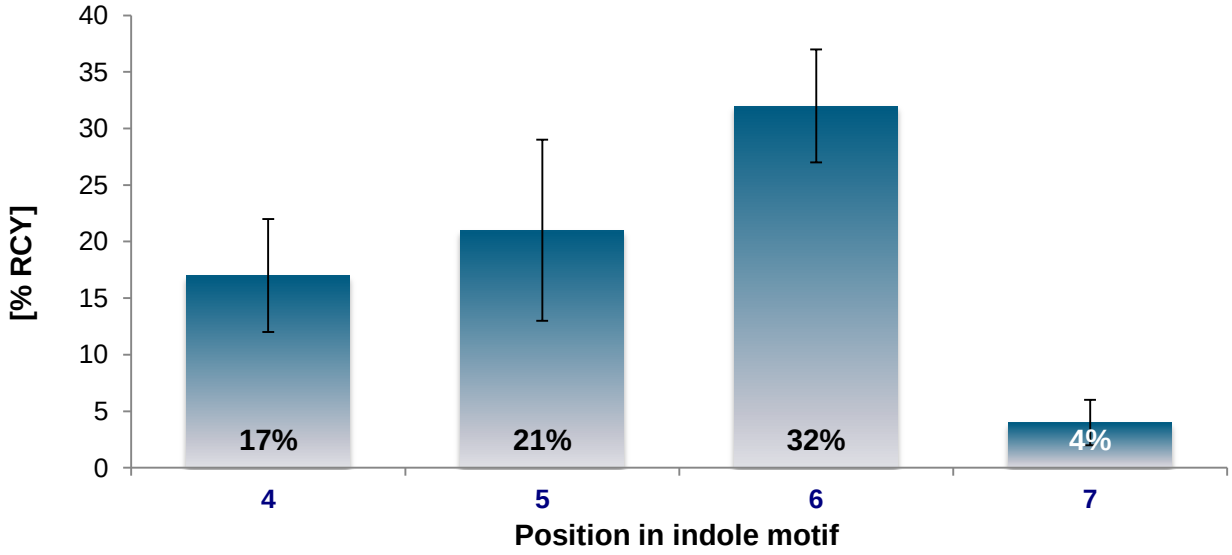
[1]: Tredwell et al., *Angew. Chem.*, **2014**, Vol. 53, *30*, 7751-7751.

Possible labelling positions

Copper-mediated radiofluorination of indolyl pinacolyl boronates



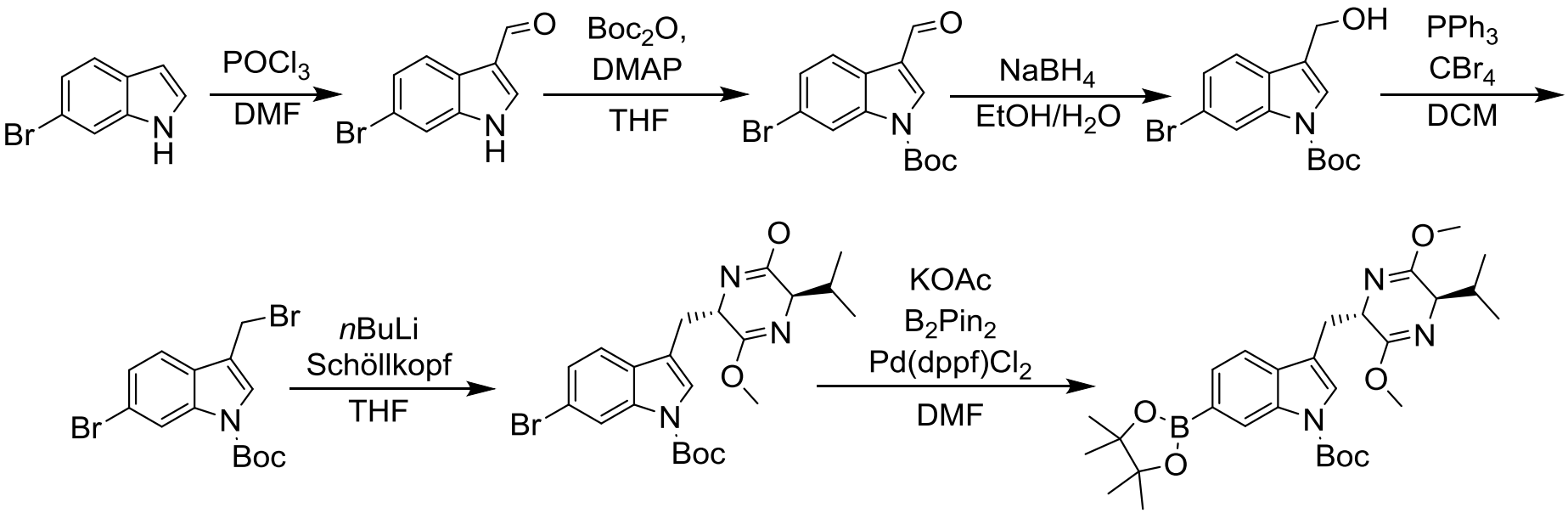
4,5,6: R=Tos
7: R = H



Highest RCY in position-6

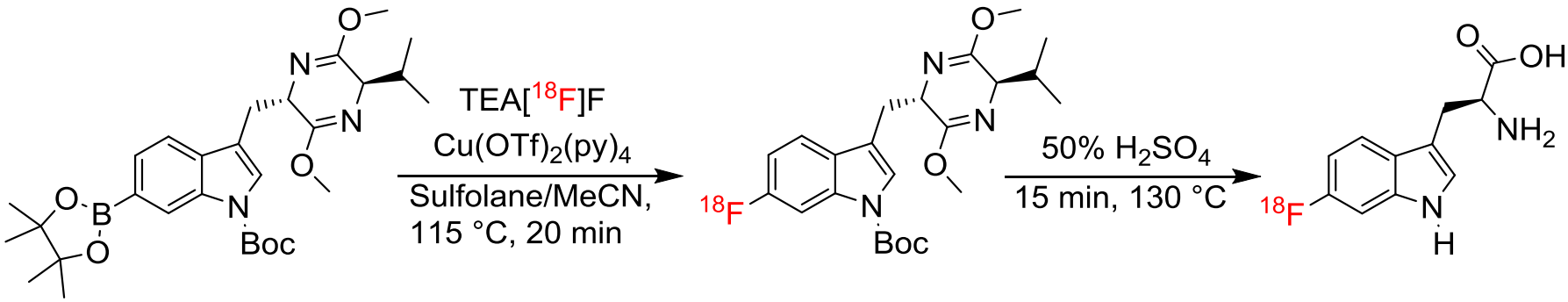
[1]: Tredwell et al., *Angew. Chem.*, **2014**, Vol. 53, *30*, 7751-7751.

Precursor synthesis



Overall yield: 35 %

Radiosynthesis of 6-[^{18}F]fluoro-L-tryptophan



^{18}F-Labelling: 53±10% RCY

Hydrolysis: 43±6% RCY

Radiochemical yield (RCY): 16±4%

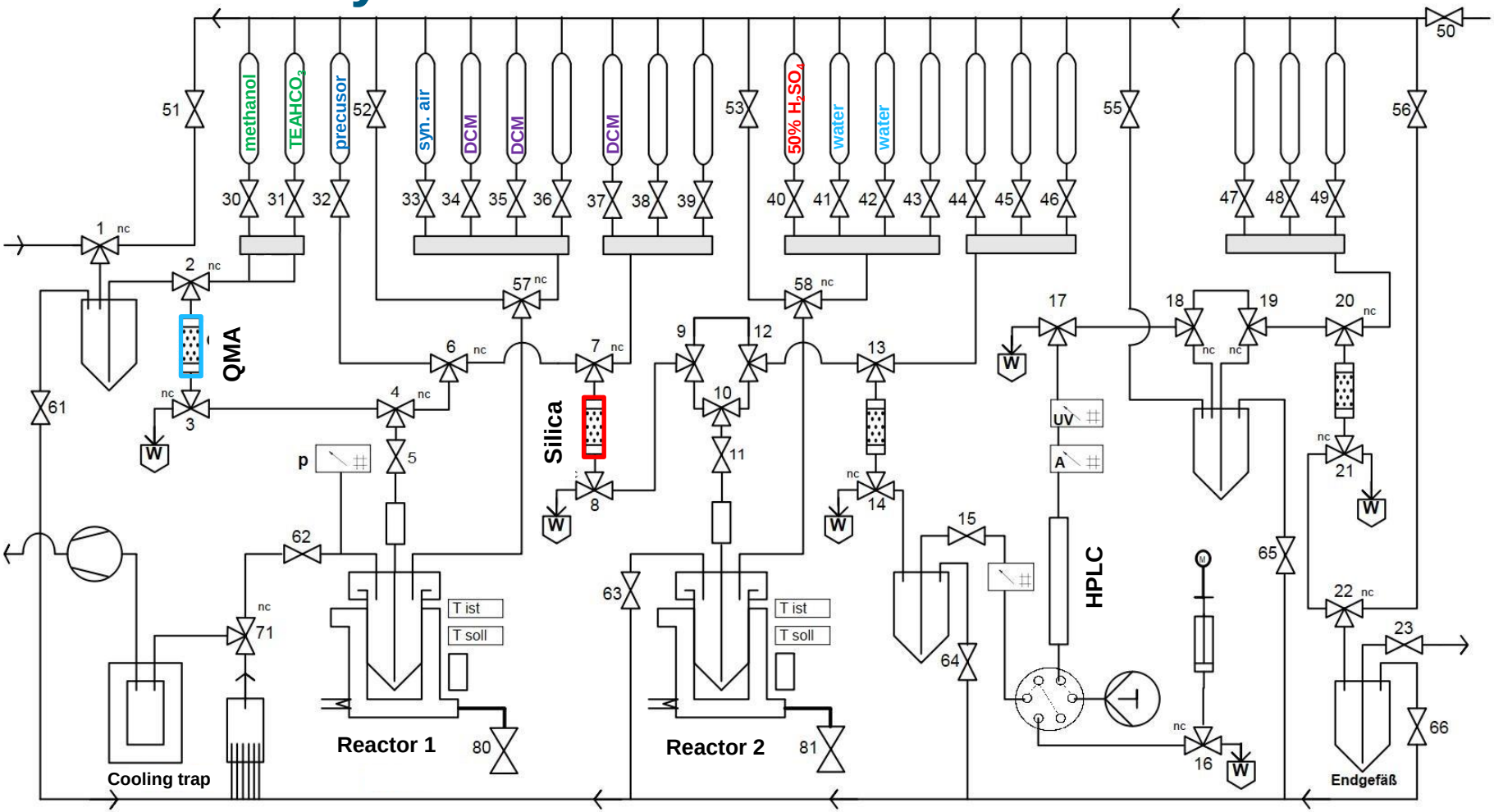
Total synthesis time: 110 min

L-Enantiomeric excess (e.e.): 89 %

Specific activity: 280 GBq/µmol (from 150 MBq 6-[^{18}F]F-Trp)

→Amenable to automation!

Automated synthesis device

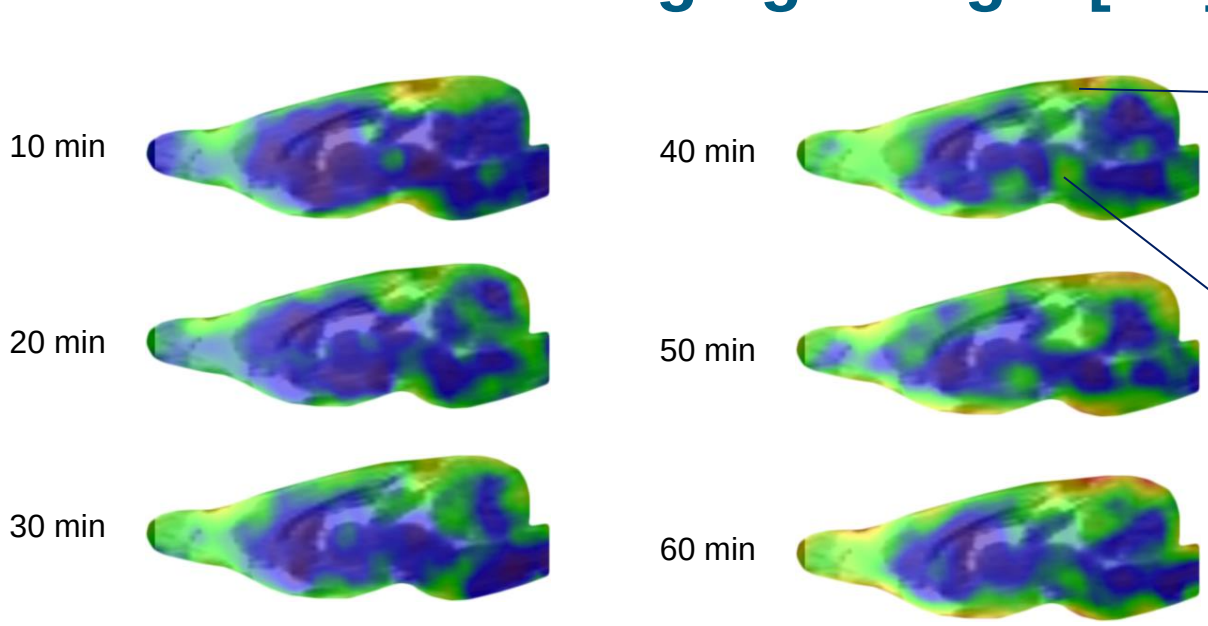


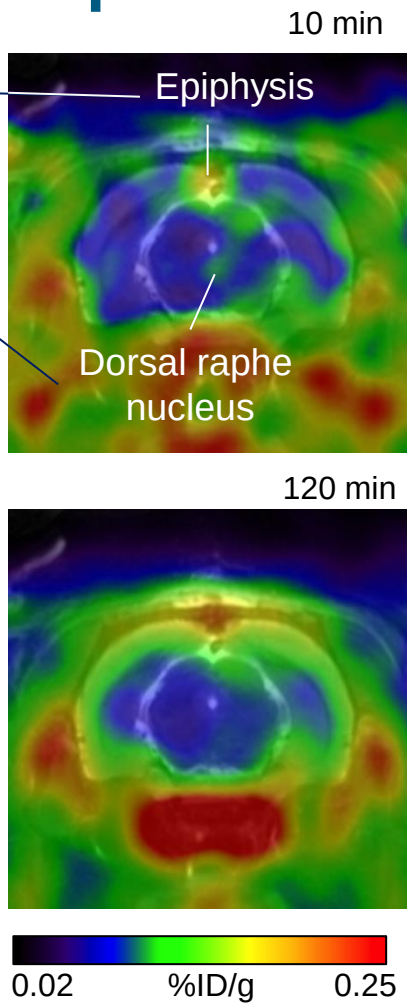
Adaption of reaction parameters

- Amount of precursor and solvents x1.5
- *N*-Methyl-2-pyrrolidone as solvent
- Reaction under synthetic air

- After semi-preparative HPLC: 13±1% RCY
- 1.8 GBq 6-[^{18}F]fluoro-L-tryptophan
  - → in 8 mL of 10% EtOH/H_2O
  - →27 GBq starting activity
  - →100 min after EOB;

Preclinical PET imaging using 6-[^{18}F]F-Trp

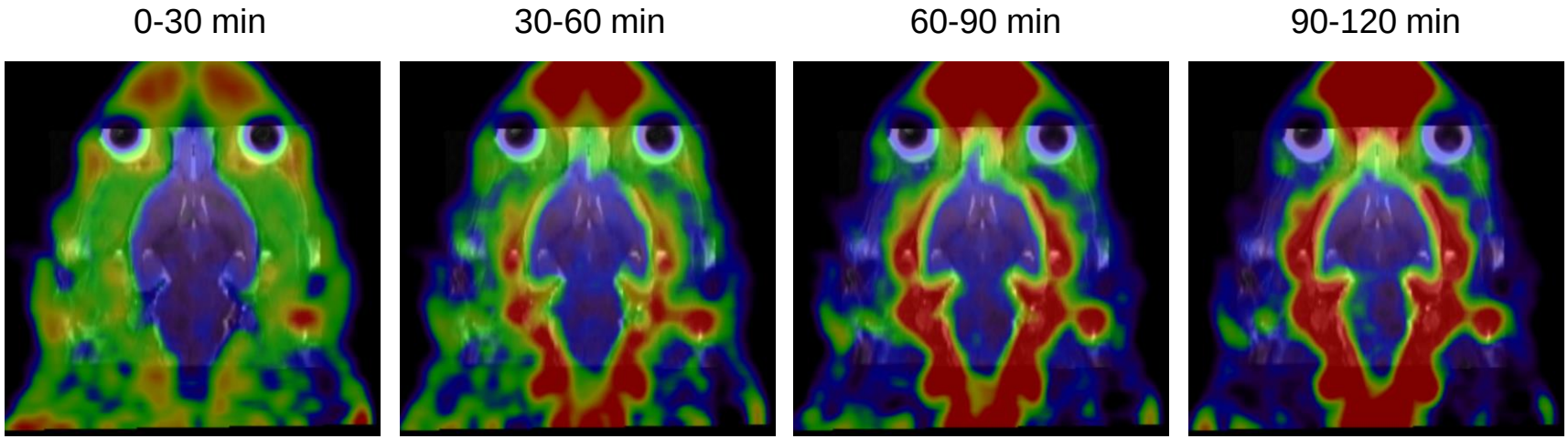


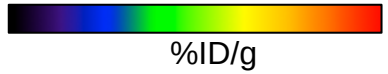


- 75 MBq 6-[^{18}F]F-Trp in 0.5 mL 10% EtOH/H_2O
- Intravenous injection
- 120 min measurement

Images: PD Dr. Heike Endepols, IREMB UK Köln.

Preclinical PET imaging using 6-[^{18}F]F-Trp





- Very low uptake in brain

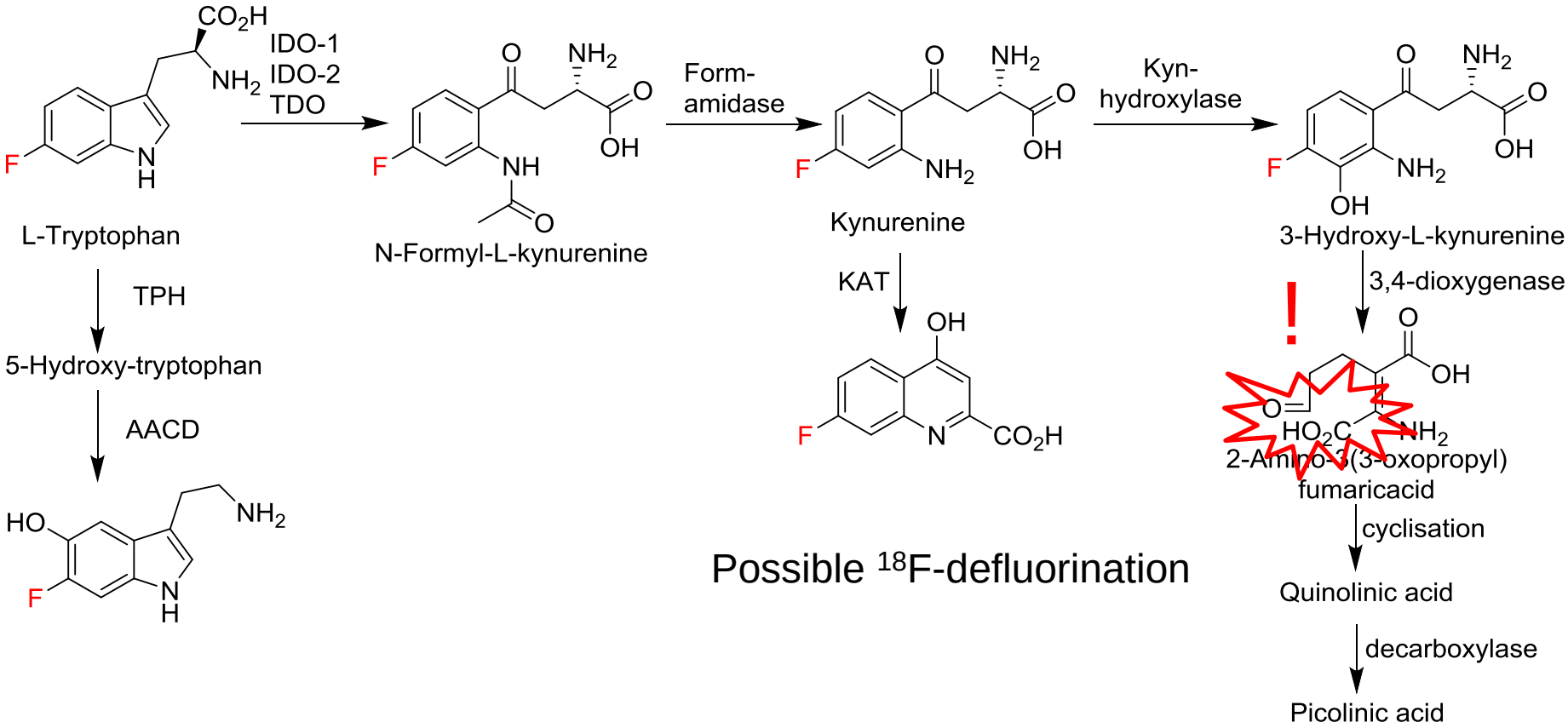
  → Contradiction to literature[1]

- Accumulation in bone after 30-60 min

  → ^{18}F-defluorination of radiotracer

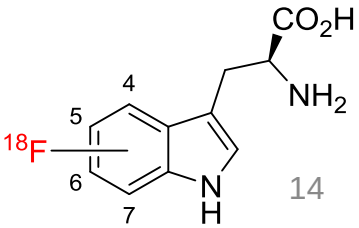
[1]: Chanut et al., *Biochem Pharm.,* **1993**, Vol. 45, *5*, 1049-1057.
Images: PD Dr. Heike Endepols, IREMB UK Köln.

Hypothesis of 6-[^{18}F]F-Trp degradation



Summary & Outlook

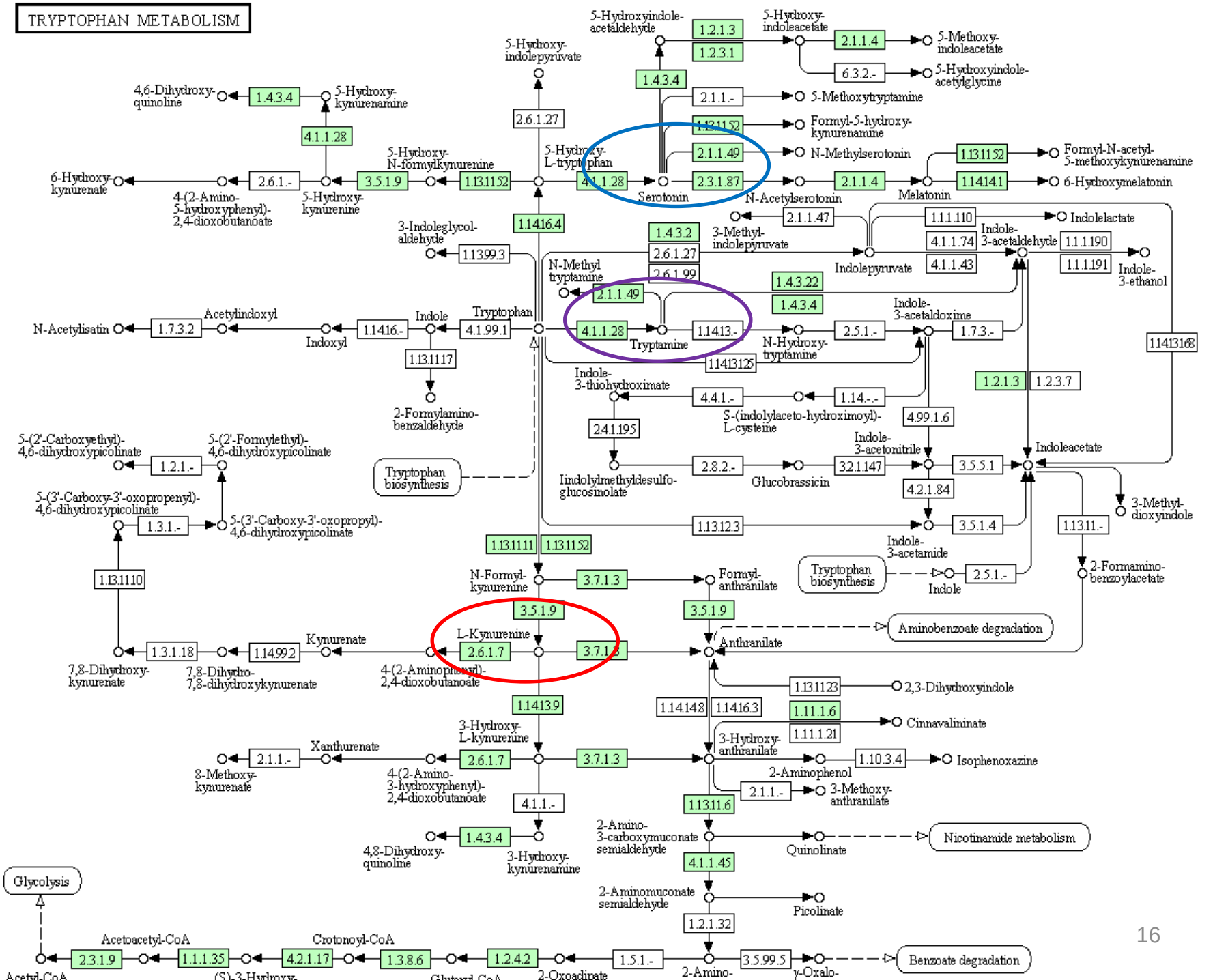
- Highest RCY of model indolyl pinacolyl boronates in 6-position (33±4%RCY)
- 6-Step precursor synthesis, 35% overall yield
- Radiosynthesis: 16±4% RCY of 6-[^{18}F]F-Trp
- Successful transfer of radiosynthesis to an automated device
- Determination of 6-[^{18}F]F-Trp distribution in a normal rat brain
  → PET images reveal *in vivo* defluorination of 6-[^{18}F]F-Trp
- ^{18}F-Fluorination in other substitution positions in tryptophan
  → e.g. position 4, 5 or 7



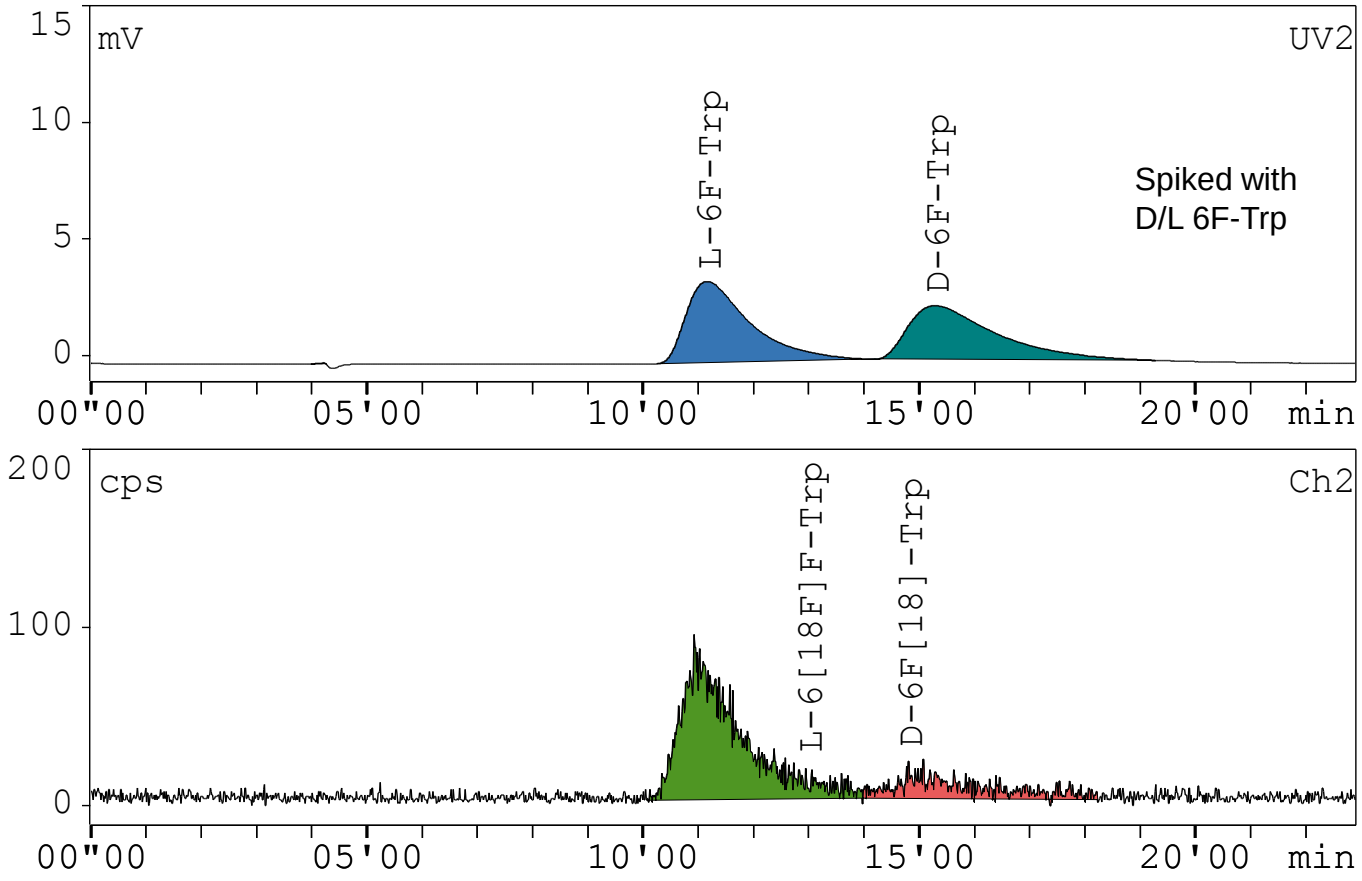
Thank you for your attention!

Acknowledgment

- PD Dr. Heike Endepols (IREMB, UK Köln)
- Dipl. Ing. Stefan Stüsgen (INM-5, Forschungszentrum Jülich)
- Dr. Marcus Holschbach (INM-5, Forschungszentrum Jülich)
- Dr. Dirk Bier (INM-5, Forschungszentrum Jülich)

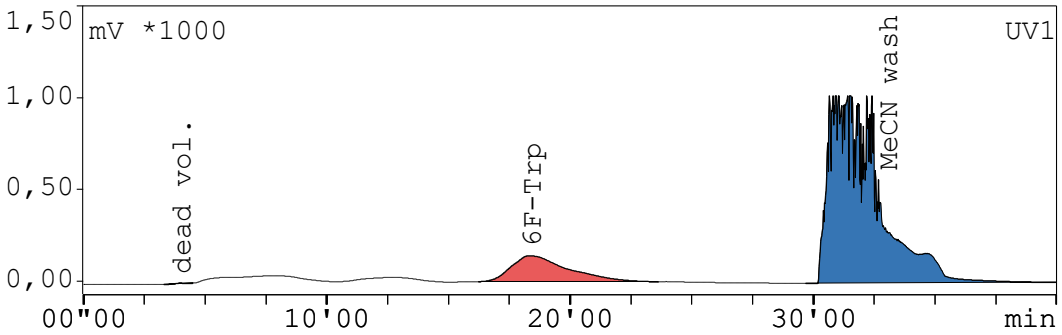


Chiral HPLC



Chirobiotic T, 40% MeOH/H_2O+0.1%TEAA pH 4.1, 0.7 mL/min, 0°C

Hydrolysis of cold 6F-Precursor



Semipep. HydroRP, 10% EtOH/Water, 2.5 mL/min.

→6-Fluorotryptophan m/z: 222.08