New ^{68}Ga-labelled amino acids: Could they be used for tumour targeting?

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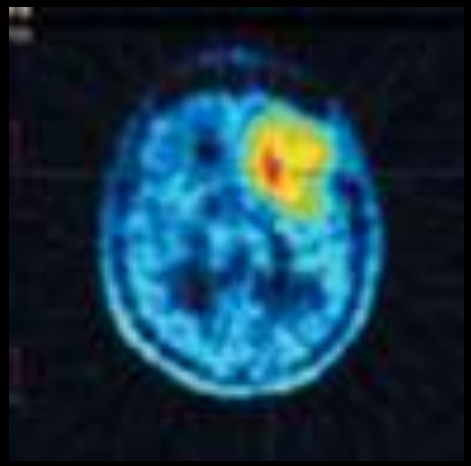
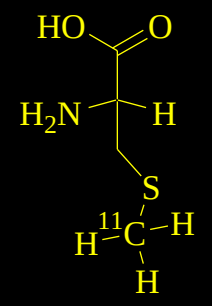
COST BM0607, Krakow

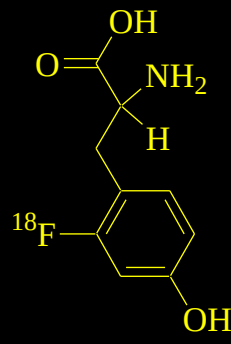
Radioactive labelled amino acids

- Mainly used in (brain) tumour-targeting
- Visualising the protein-synthesis-rate (PSR)
- Particularly suitable for brain tumour imaging, due to the very low amino acid consumption of the brain





L-5-[^{11}C]Methionine (MET)

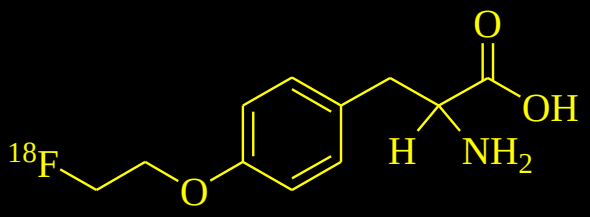


L-2-[^{18}F]Fluorotyrosine

Langström et al., J Nucl Med, 1987; Coenen et al., J Nucl Med, 1989

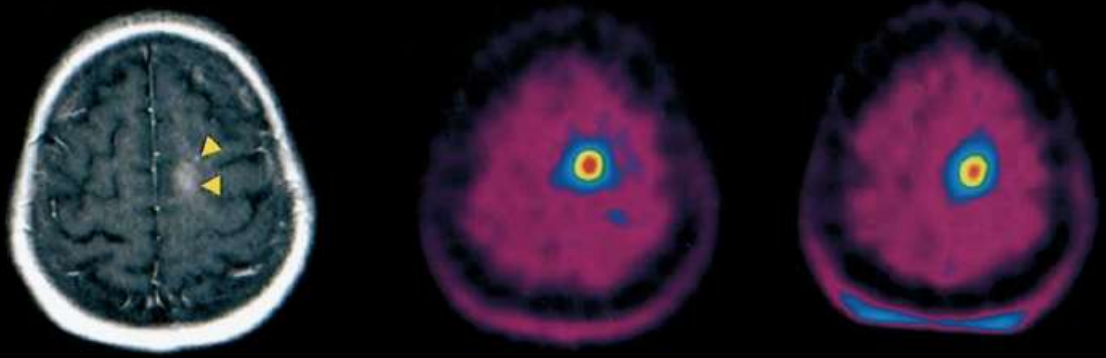
Radioactive labelled amino acids

- Amino acids, which are transported into the cell but not incorporated in proteins (FET), showed similar tumour uptake



O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET)

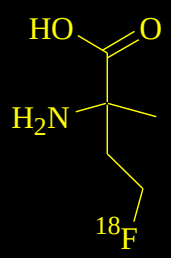
MET FET



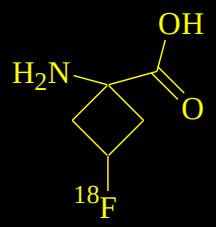
Wester et al., J Nucl Med, 1999, Weber et al., Eur J Nucl Med, 2000

Radioactive labelled amino acids

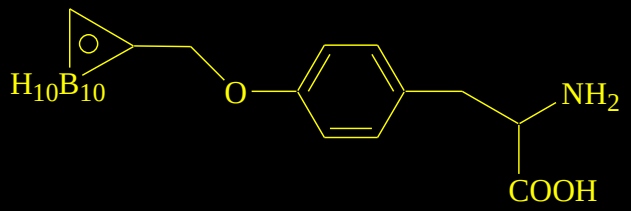
- Artificial amino acids (FMAB, FACBC) were accumulated in tumour cells
- Amino acid transporter allows broad structure variations
- Coupling of a carborane-cluster for BNCT was tolerated



FMAB



FACBC

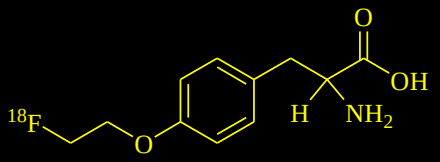


O-(o-Carborane-1-ylmethyl)-tyrosine

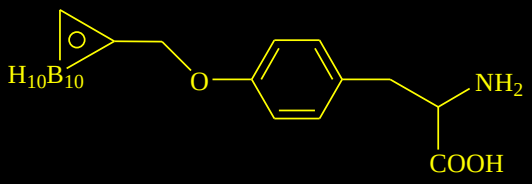
Goodman et al., Nucl. Med. Biol., 2003; Wyzlic et al., Tetrahedron Lett., 1992

A ^{68}Ga-labelled amino acid?

- Perfect availability of ^{68}Ga via the ^{68}Ge/^{68}Ga-generator
- No need of an in-house cyclotron
- Lower costs
- Amino acid transporter tolerates wide structure variations
- Brain tumours often disturb the blood-brain-barrier

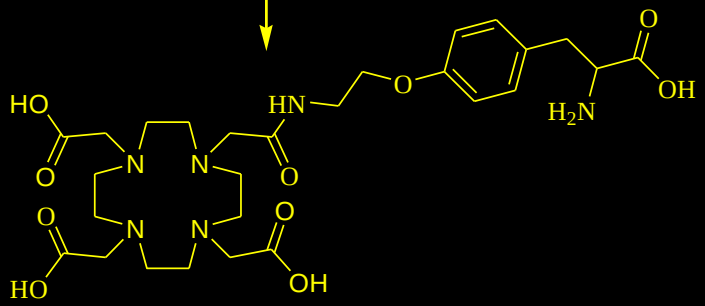


O-(2-[^{18}F]Fluoroethyl)-L-tyrosine (FET)



O-(o-Carborane-1-ylmethyl)-tyrosine

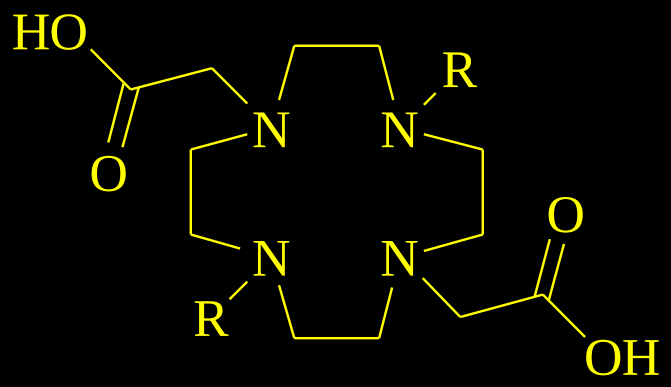
?



DOTA-tyrosine

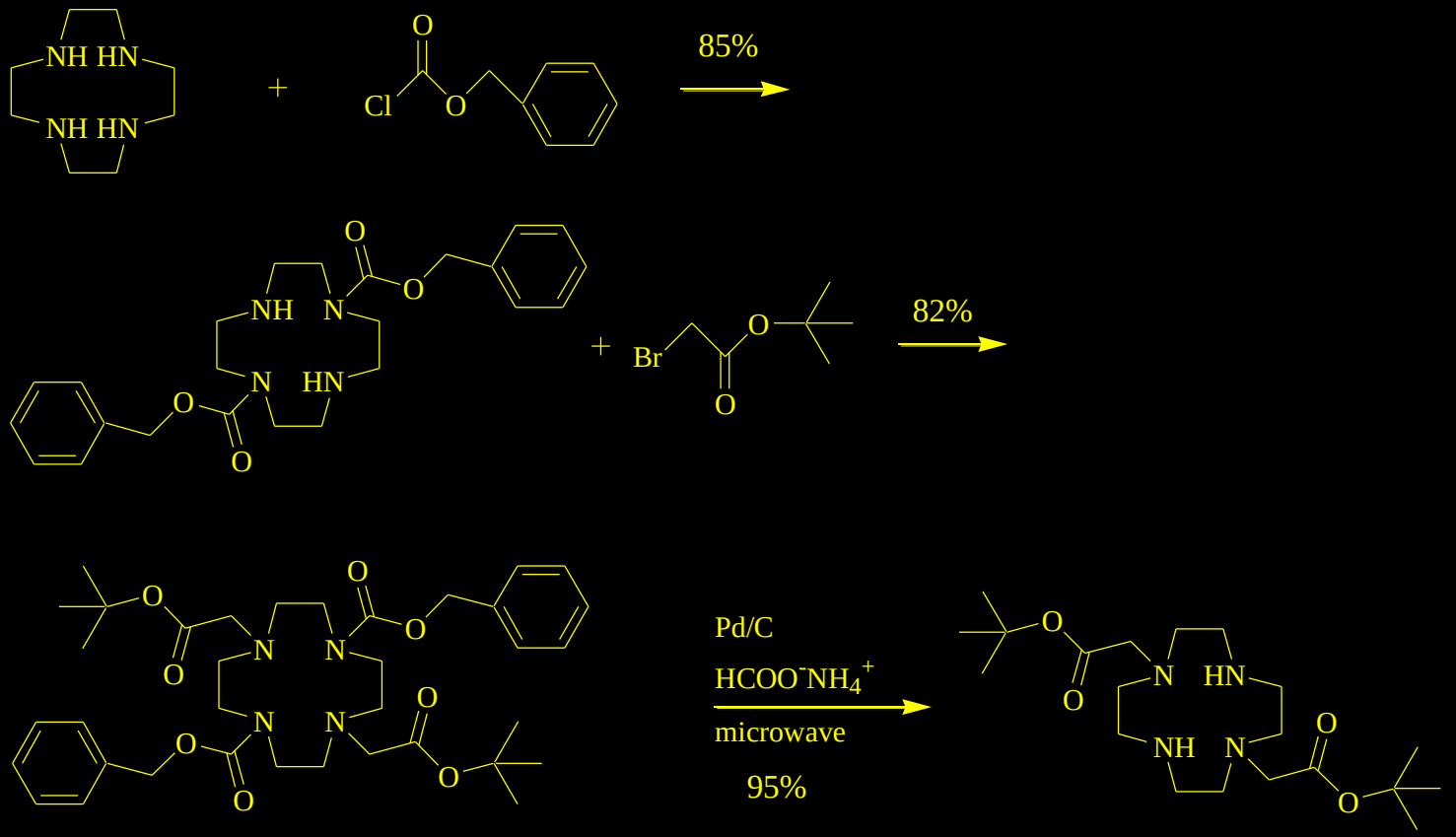
DO2A as bifunctional chelator

- Possibility to couple two amino acids directly to one BFC
- Higher amino acid concentration should increase the tumour affinity



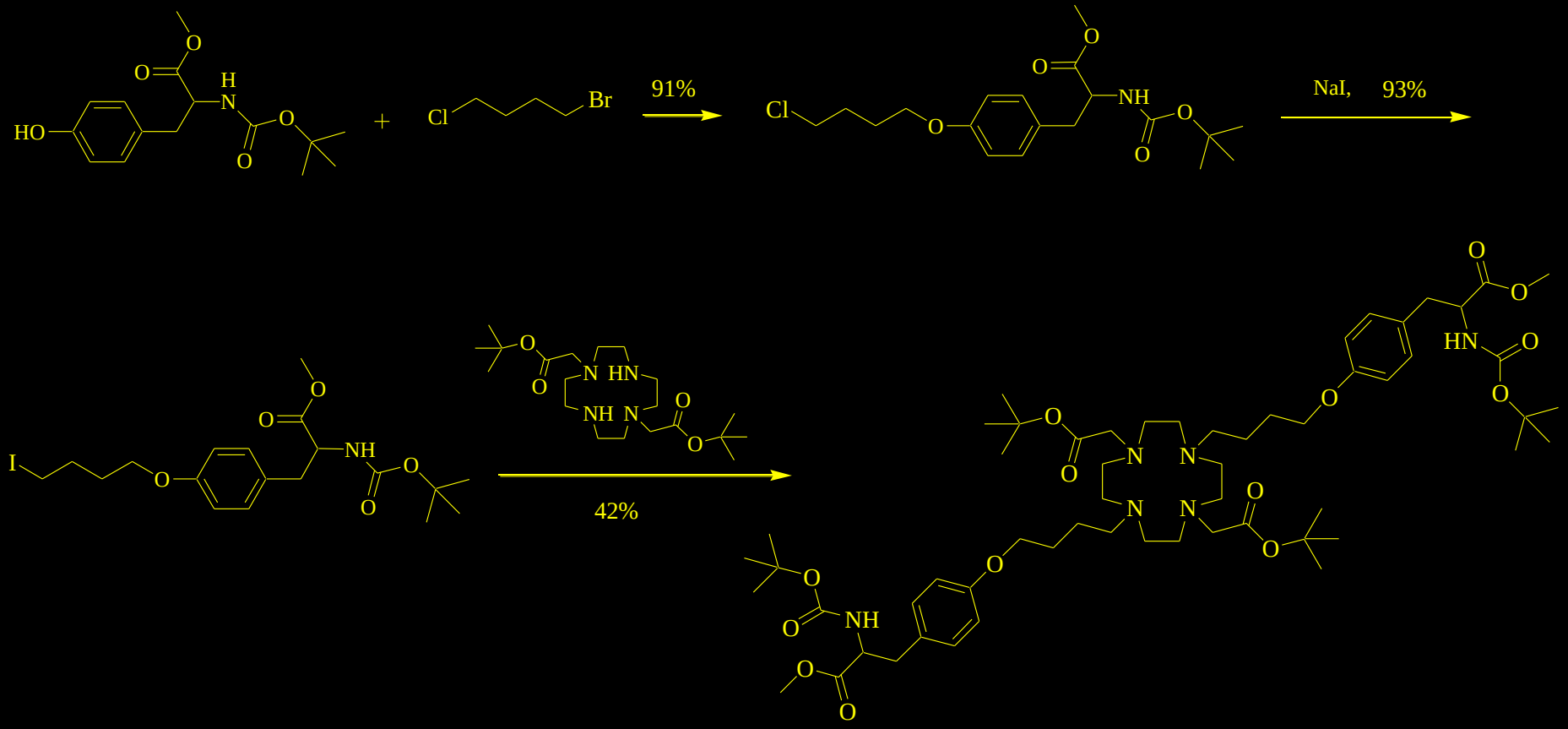
Synthesis of the chelator

DO2A:

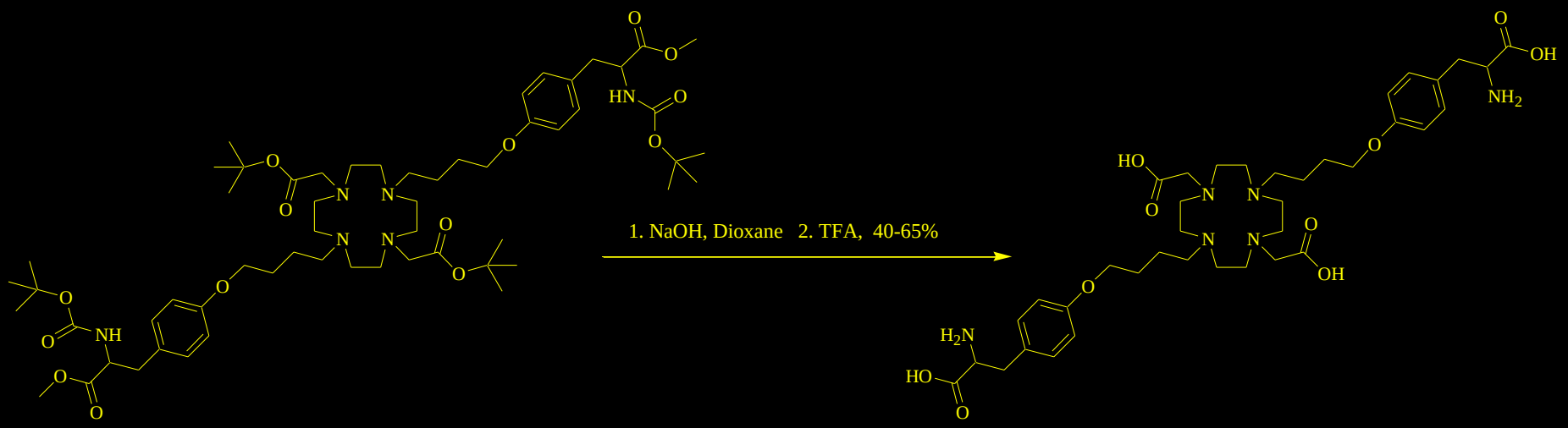


Kovacs, Sherry, J Chem Soc Chem Comm, 1995

Coupling chelator-spacer-tyrosine



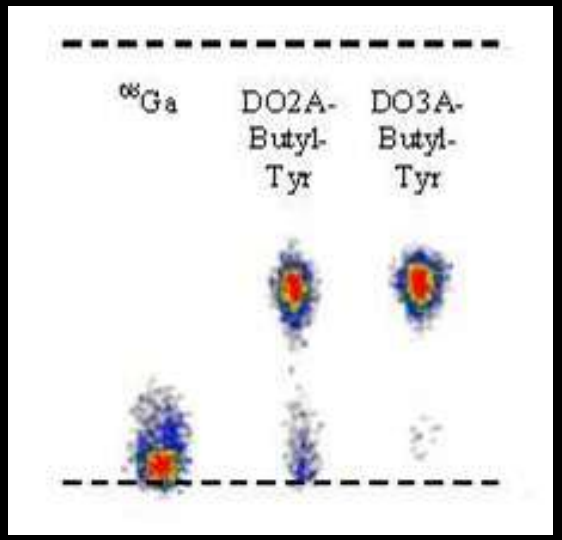
Cleavage of the protective groups



- Cleavage was carried out in two consecutive steps
- Precursors were purified by HPLC
- TFA-salts were removed over a cation-exchanger

^{68}Ga-labelling

- 50 µl generator eluate
- 5 ml millipore water
- 90 C, 15 minutes
- Analysis via radio-TLC
- Yields: DO2A 70%, DO3A > 90%
- Radiochemical purity > 98%
- DTPA-challenge proved complex-stability over two hours

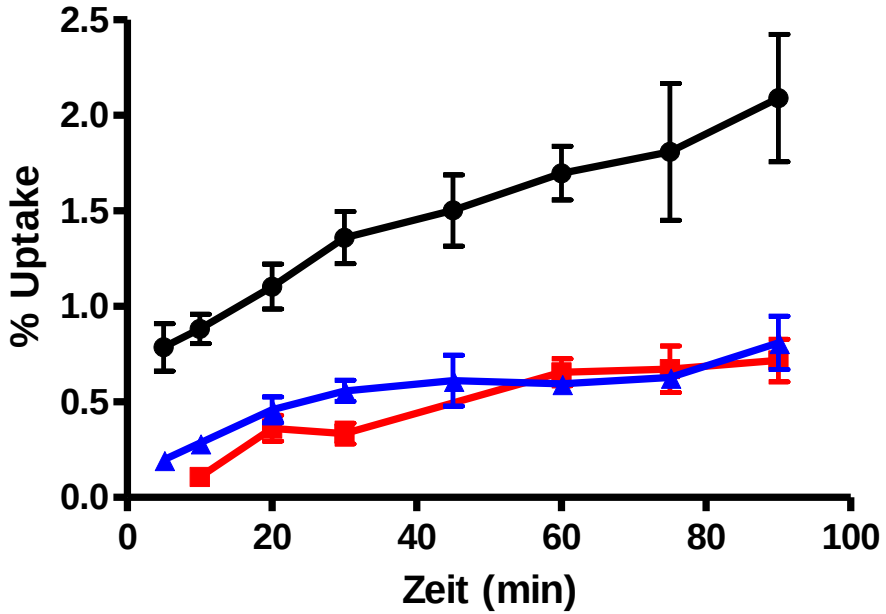


Cell studies

- Established cell assay at Erlangen University
- F-98 glioblastoma cell line
- 4 C
- DO2A-Bu-Tyr
- DO2A for non-specific binding
- Blocking experiments with an amino acid cocktail (TRP, SER, BCH)
- Uptake measured over 90 minutes

Prante et al., Nucl Med Biol, 2007

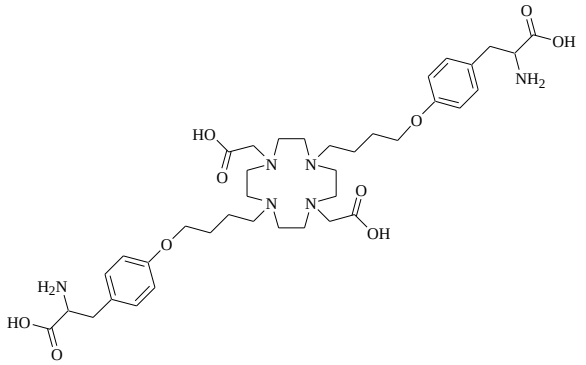
Cell studies



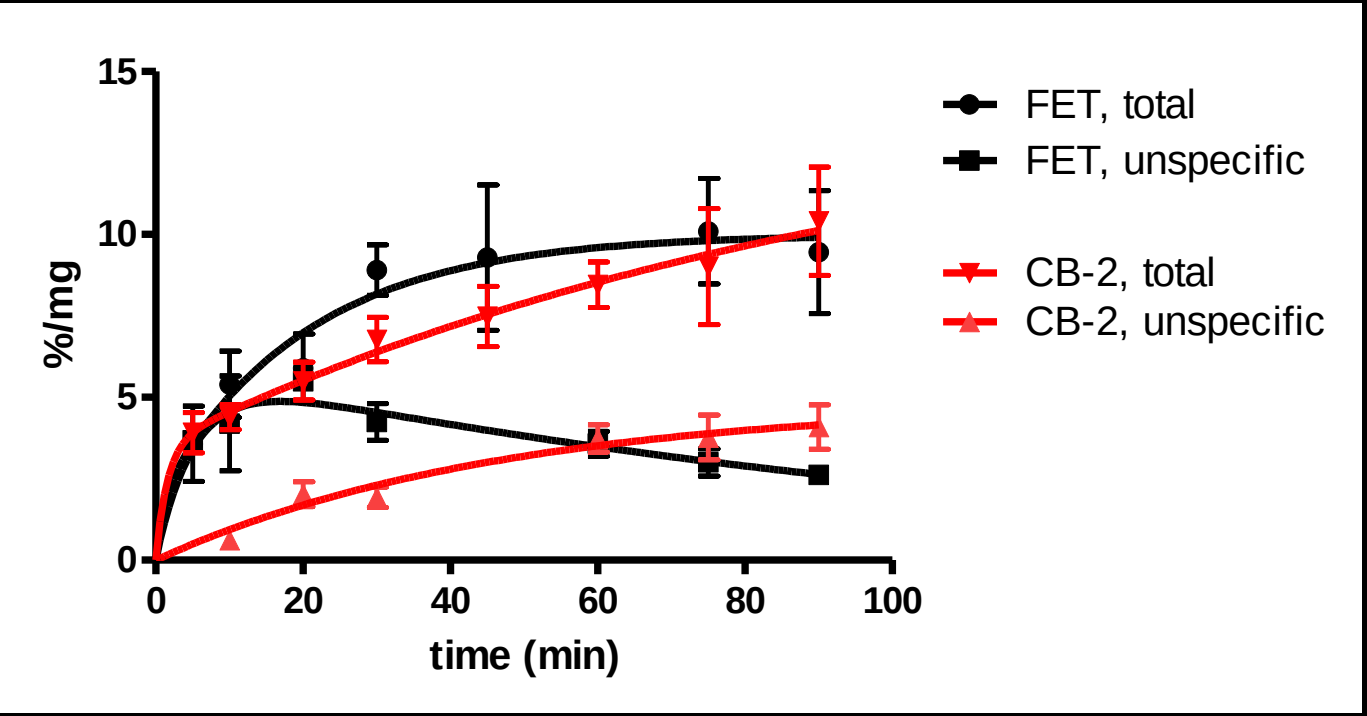
PBS, CB-2, 16.10.07, n=6

PBS-Block, CB-2, 15.+16.10.07, n=6

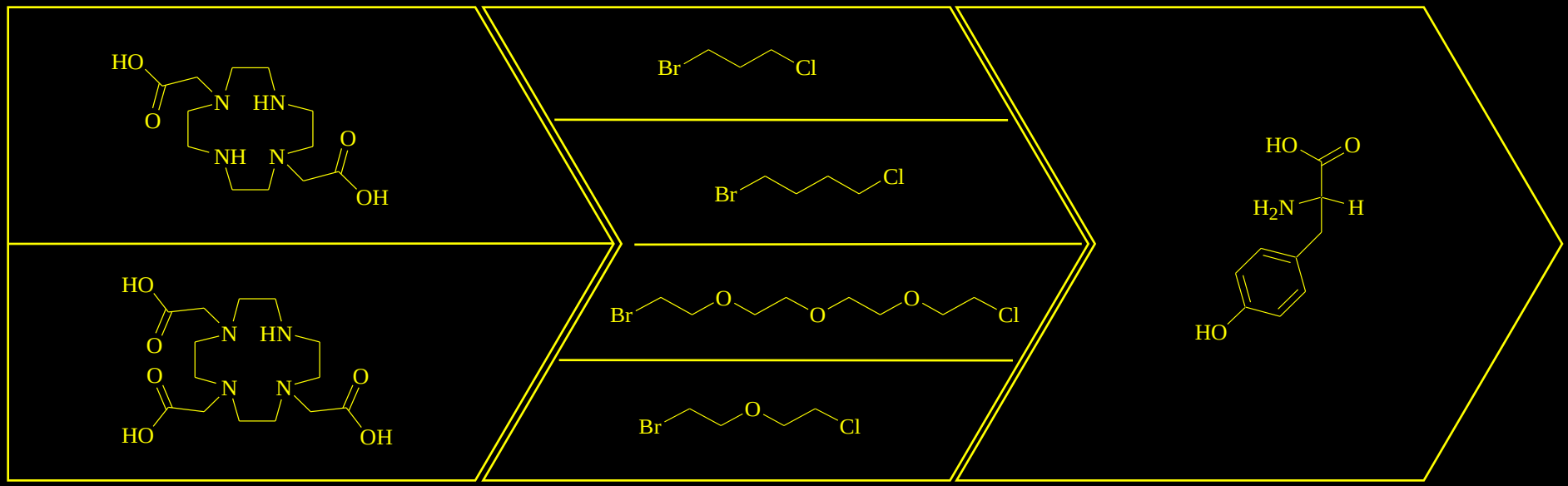
PBS, DO2A, 15.10.07, n=6



Comparison with FET



Concept



- Different Spacers to vary length and lipophilicity
- DO3A-analogues as references
- Analogue synthesis-route and cleavage of protective groups in comparable yields

Conclusion and outlook

- It seems to be possible to visualize tumours with ^{68}Ga-labelled amino acids *in vivo*

- Next steps:

  Using cell and PET-studies to compare DO2A- and DO3A-derivatives

  Using the best compound with glioblastoma-bearing rats to check bbb-crossing and visualizing of brain tumours

Acknowledgement

University of Mainz:

- Radiopharmaceutical group
- Technical staff Nuclear Chemistry
- H. Kolshorn, NMR-facilities

University of Erlangen:

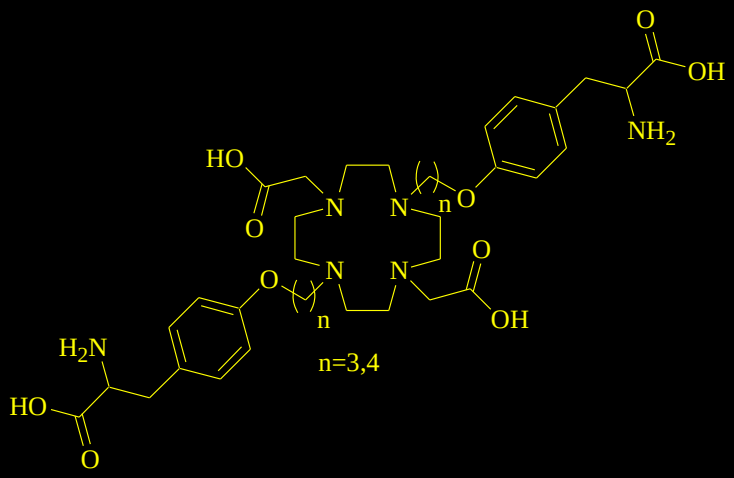
- T. Kuwert and S. Maschauer

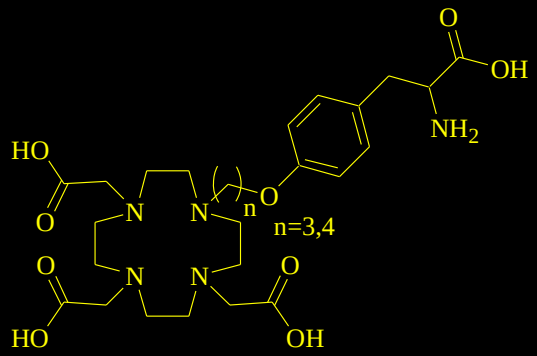
^{68}Ga as PET-nuclide

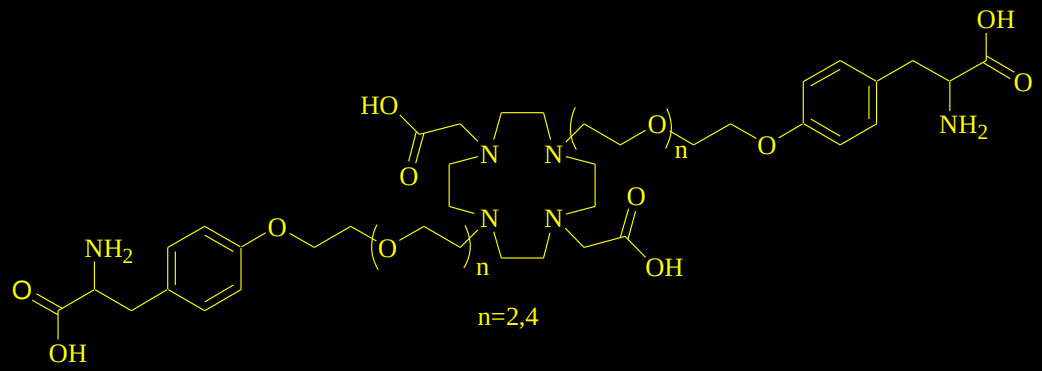
- Availability via ^{68}Ge/^{68}Ga-generator system
- $t_{1/2}$ mother: 271 days
- $t_{1/2}$ daughter: 68 minutes
- 89% β^+-decay
- Easy purification and concentration to provide n.c.a. ^{68}Ga in 400 µl in 5 minutes
- Generator can be used again after 3-4 hours

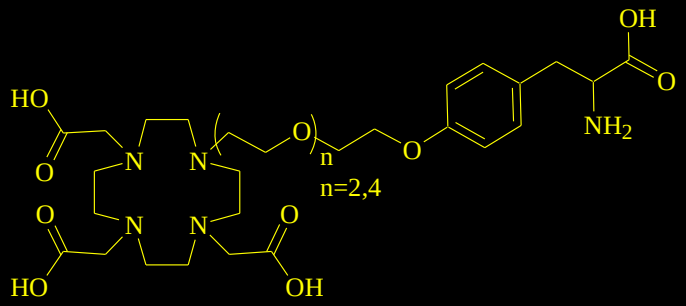
Zhernosekov et al., J. Nucl. Med., 2007

Synthesised precursors



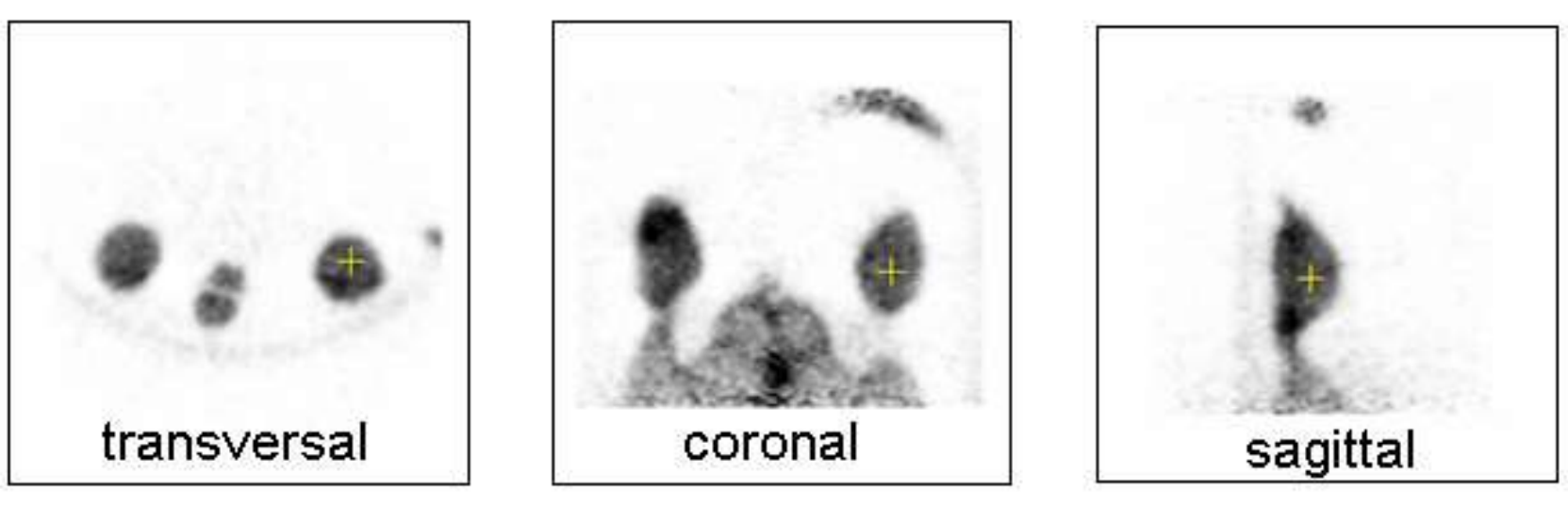


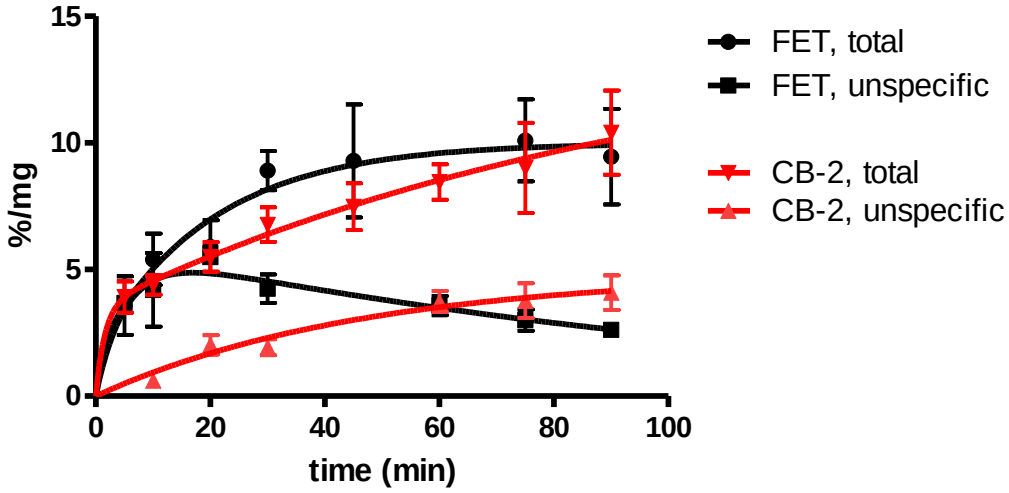




Small Animal PET-study

- Tumour-bearing Kopenhagenrat
- DO2A-Bu-Tyr, 25 MBq in 1 ml 0,9% NaCl-solution
- Measured for 60 minutes




15
10
5
0
%/mg
0
20
40
60
80
100
time (min)
FET, total
FET, unspecific
CB-2, total
CB-2, unspecific

Outline

- Motivation
- Synthesis of the precursors
- ^{68}Ga-labelling
- Preliminary evaluation
- Summary and outlook

Summary

- Easy and high yield chelator synthesis
- Synthesis of eight DO2A/DO3A-tyrosine precursors with different spacers
- ^{68}Ga-labelling in high yields
- DTPA-challenge proved complex-stability
- Cell studies showed a specific uptake in F98-glioblastoma cells