

Synthesis and Analysis of 2- and 4- ^{211}At -Phenylalanine and their Uptake in Human Glioma Cell Cultures

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Aim

- **Synthesis and Verification of the Identity of 2- and 4-²¹¹At-Phenylalanine.**
- **Test of the Uptake of 2- and 4-²¹¹At-Phenylalanine in a Glioma-Cell Line in vitro.**

Methods: Production of Astatine-211

- $^{209}\text{Bi}(\alpha,2n)^{211}\text{At}$ **28.0 MeV, 10 μ A, 15 mC (25 Min)**
(30.5 MeV machine energie)
- **Yield (EOB): 90 - 110MBq (EOB) (79 - 96%)**
- **Dry Distillation: RT - 920°C, 30 Min**
- **Recovery Solvent: 500 μ l Methanol**
- **Yield (actual) : 90 \pm 10 MBq (ca.90 %)**

Methods:

Cu⁺ catalysed nukleophilic Halogen-Exchange

1. 1 mg Iodo-Phenylalanine in 1ml minivial (ca 3 μ Mol)
2. 28 μ l solvent 1 1 ***[CuSO₄ (3,04 mg / 1 ml H₂O)] (ca. 0,5 μ Mol)***
3. 500 μ l solvent 2 ***[1 mg SnSO₄ + 2,5 mg gentisic acid + 5 mg citric acid / 1ml H₂O.]***
4. ²¹¹At in methanol ***[ca. 50 - 90 MBq in 500 μ l]***
5. Reaction-temperature und -time ***[120°C and 60 min]***

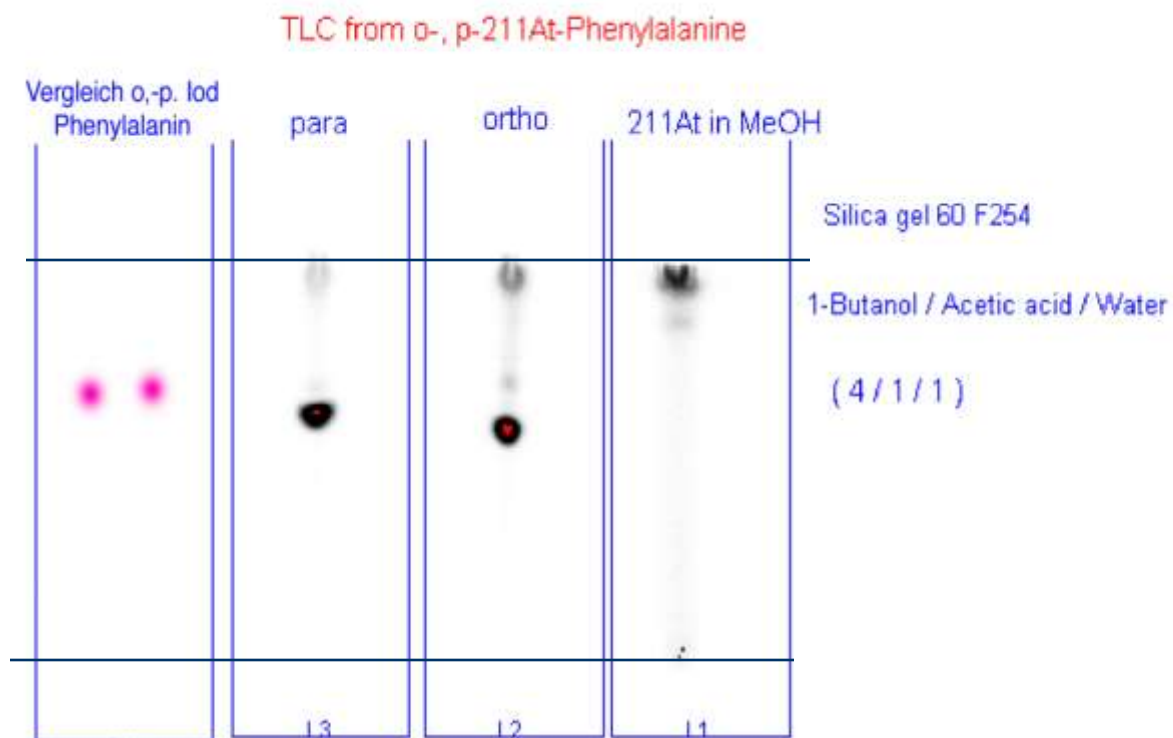
Result: Radiochemical Yield

	Radiochemical Yield
2-²¹¹At-Phenylalanin	75 ± 5 %
4-²¹¹At-Phenylalanin	80 ± 5 %

Analytical Methods: DC und Reverse Phase HPLC

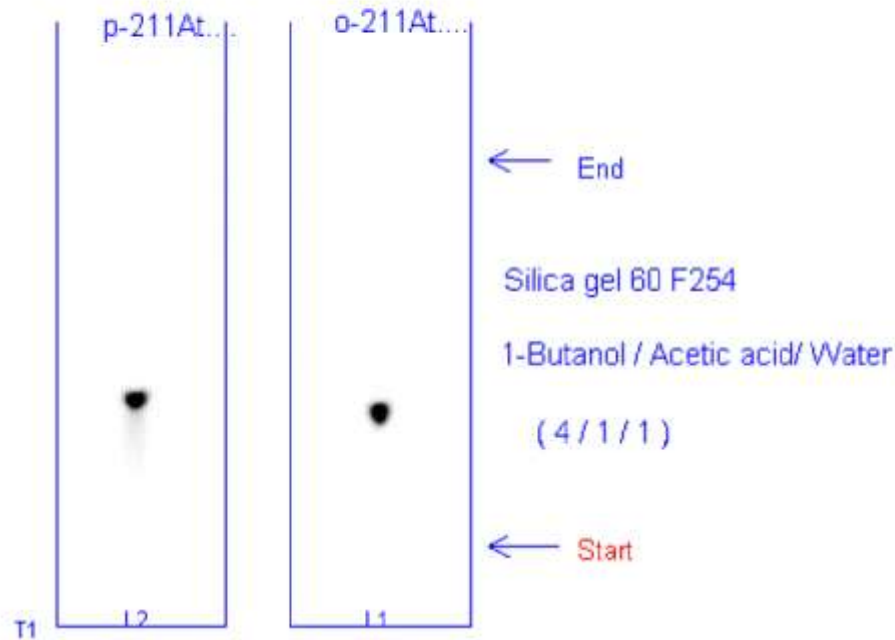
	Sorbent	Eluent	Length or Flow
TLC for 4-I-, 4-At- Phenylalanine	Si-60	Butanol/ Essigsäure/ Wasser 4 / 1 / 1	8 cm
RP-HPLC for 2-, 4- Hal- Phenylalanine <i>Semi-präparative und analytic</i>	RP-18 e, 5 μ , 250*4mm	Phos.buffer pH 2,4/ Acetonitrile (80/20)	1 ml/ Min

Analytical Methods: TLC of the raw product



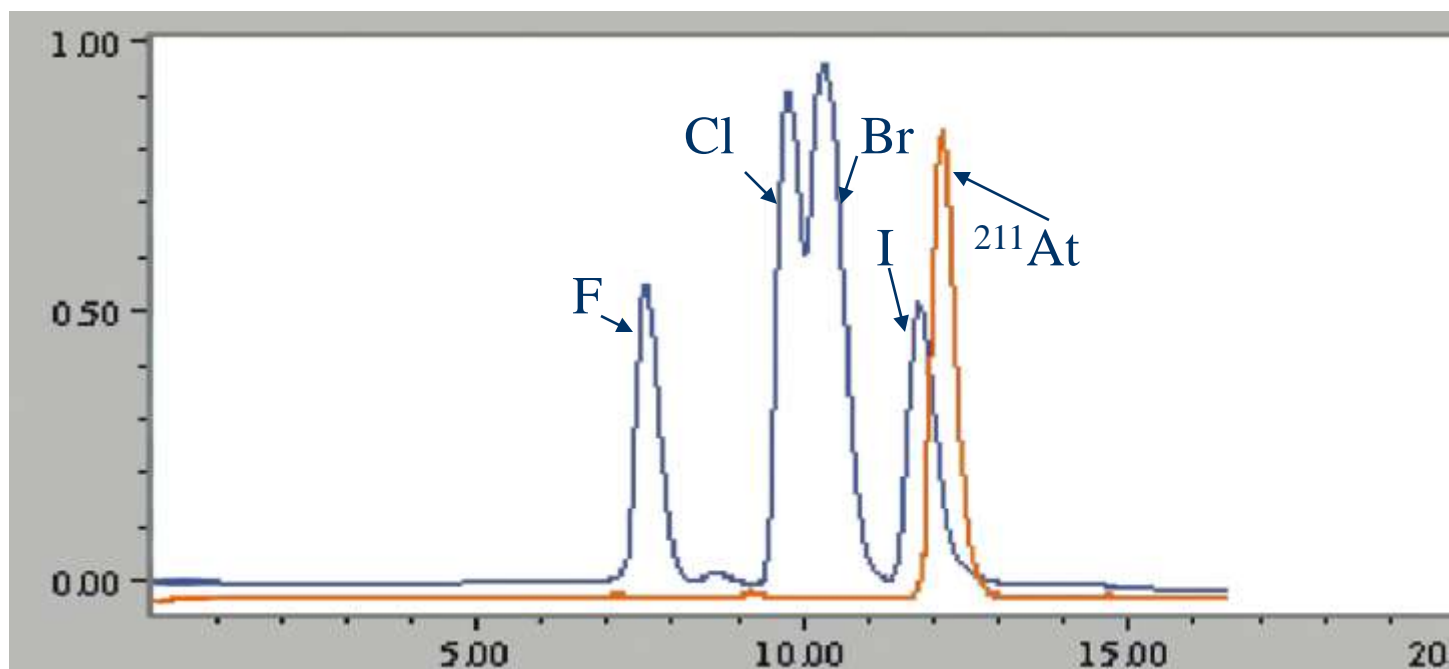
TLC of the purified products

Quality control of o-, p-211At-Phenylalanines after HPLC separation



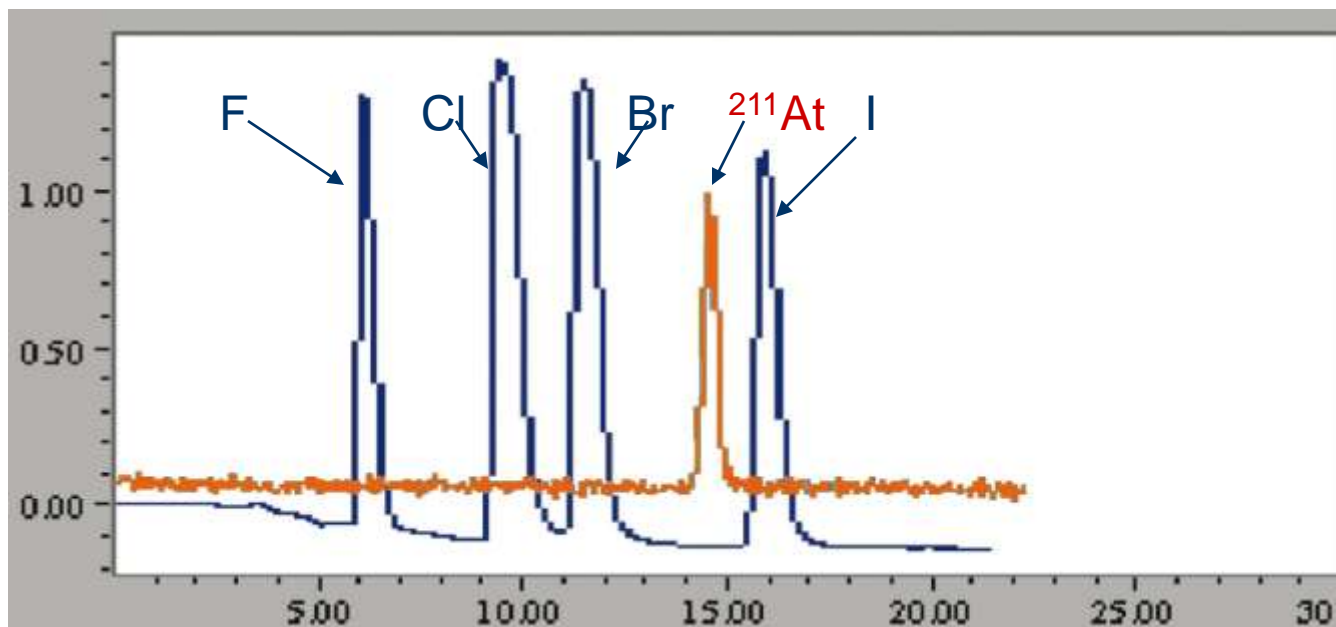
HPLC of 2-Hal-Phenylalanines

Elution Sequenz: 2-F-, 2-Cl-, 2-Br-, 2-I- und 2-²¹¹At- Phenylalanine.



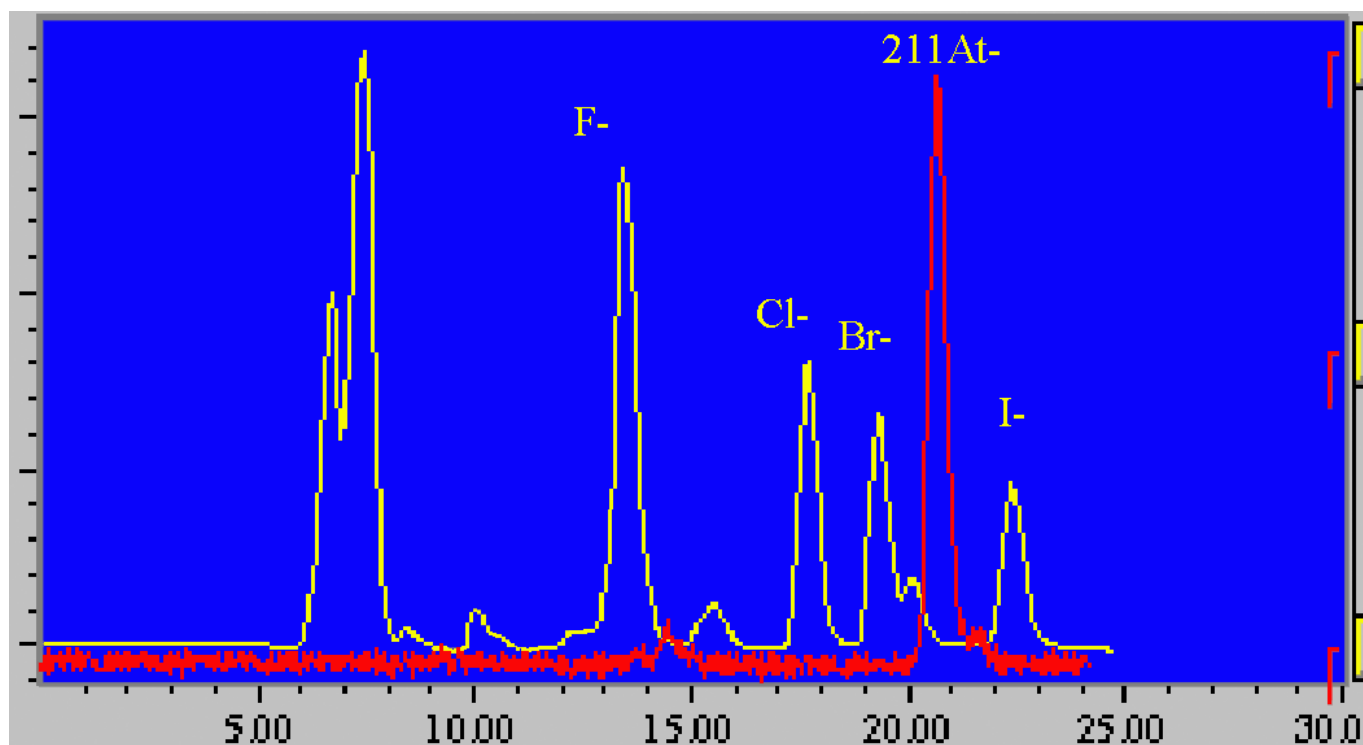
HPLC of 4-Hal-Phenylalanines

Elution Sequenz: 4-F-, 4-Cl-, 4-Br-, 4-I- and 4-²¹¹At-Phenylalanine.



For Comparison: HPLC of 3-Hal-Benzoic Acid Succinimidy-ESTERS

Elution Sequenz: 3-F-, 3-Cl-, 3-Br-, 3-I- and 3-□[²¹¹At]SAB.



Uptake in Glioma Cells

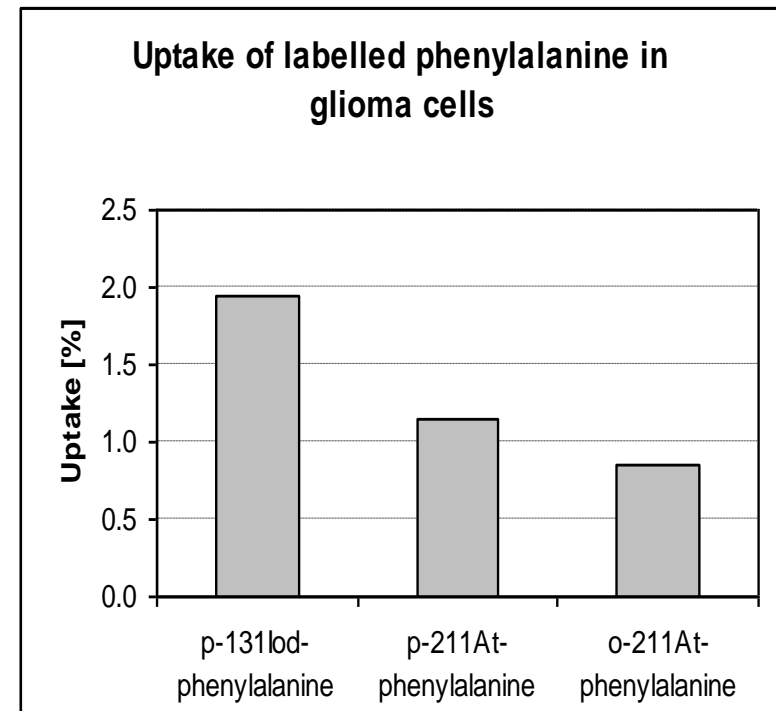
- Kinetic Experiment

The uptake of 2- und 4-²¹¹At-Phe in DBTRG-glioma cells is rapid with a broad maximum between 5 and 15 minutes.

Uptake in Glioma cells

- Binding Experiment

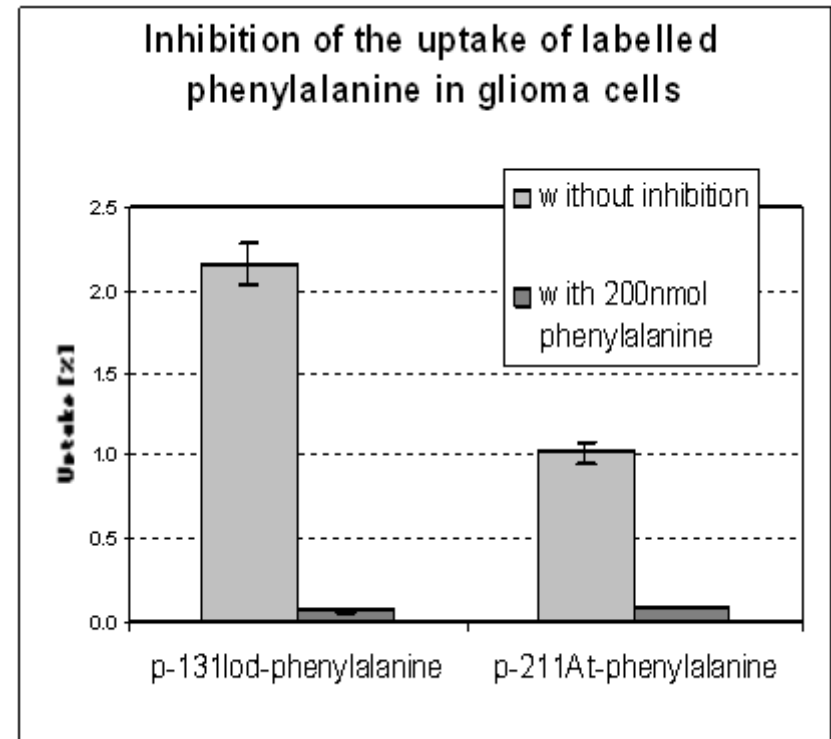
- The max uptake of 2- und 4- ^{211}At -Phe is about half of that found for 4- ^{131}I -Phe.



Uptake in Glioma cells

- Competitive Binding-Experiment

The Inhibition by cold phenylalanine is about 8-10-fold for 4-²¹¹At-Phe, and about 20-fold for 4-¹³¹I-Phe.



Conclusion

- ✓ Nucleophilic Cu^{+} - catalysed halogen exchange is an effective method for the preparation of ^{211}At -labelled Phenylalanines.
- ✓ Despite a smaller uptake as compared to 4-Iod-Phenylalanine, 2- and 4- ^{211}At -Phenylalanine present themselves as substrates for the neutral amino acid transport at glioma cells.
- ✓ We shall test 4- ^{211}At -Phenylalanine for therapeutic applications in a rat-glioma-model.