5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

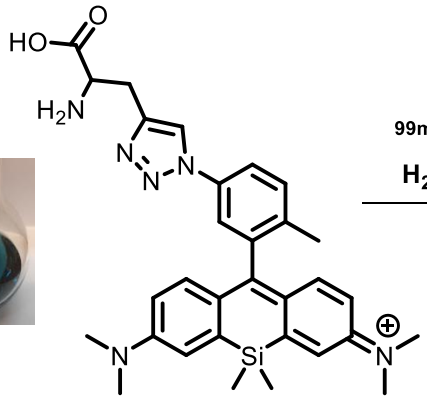
Novel radiolabeled silicon rhodamine dyes for bimodal scintigraphic and optical imaging

Thines Kanagasundaram[1,2]*, Carsten Sven Kramer[1], Eszter Boros[3], Klaus Kopka[1]

[1]German Cancer Research Center, Division of Radiopharmaceutical Chemistry, Heidelberg, Germany; [2]Heidelberg University, Department of Inorganic Chemistry, Heidelberg, Germany; [3]Stony Brook University, Department of Chemistry, New York, United States of America.

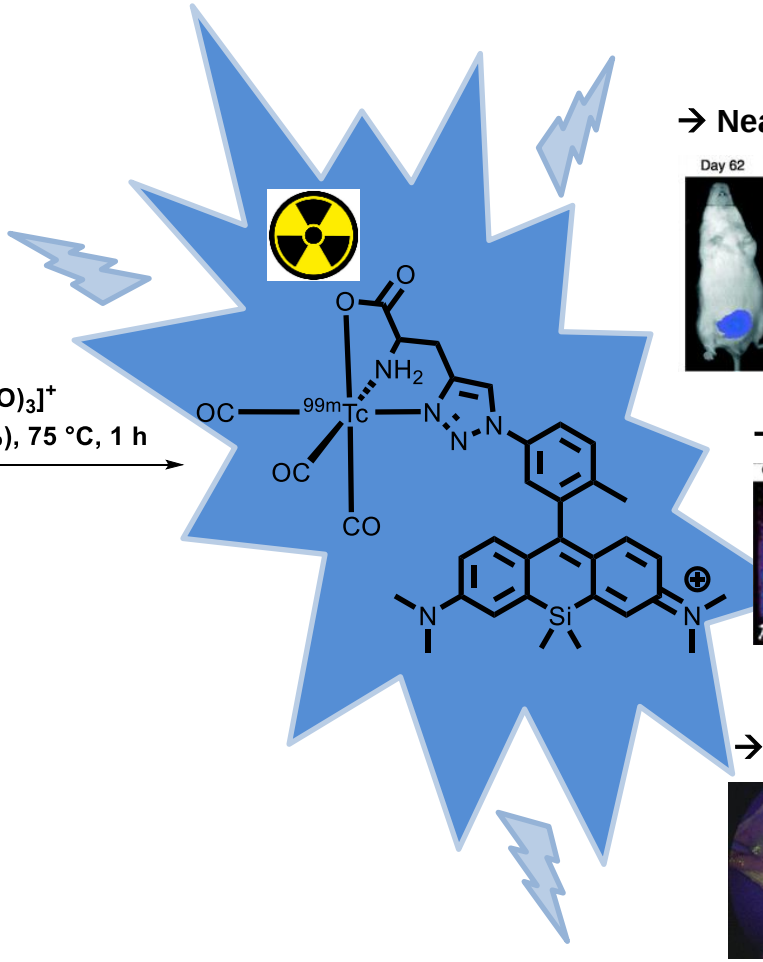
* Corresponding author: t.kanagasundaram@dkfz-heidelberg.de

Novel radiolabeled silicon rhodamine dyes for bimodal scintigraphic and optical imaging

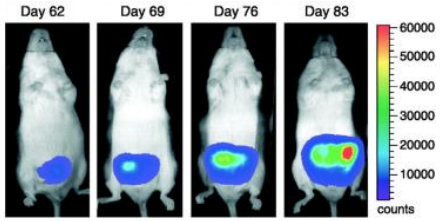


$^{99m}Tc[H_2O)_3(CO)_3]^+$

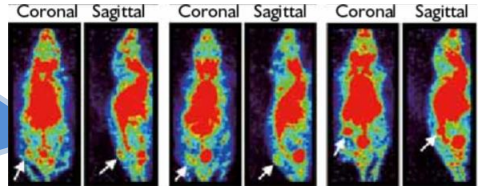
H_2O/EtOH (5%), 75 °C, 1 h



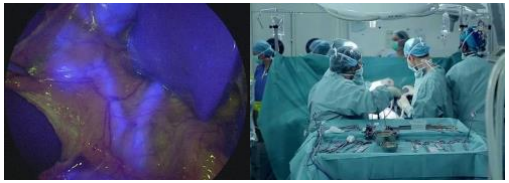
→ Near-infrared optical Imaging



→ SPECT-Imaging



→ Fluorescence-guided surgery



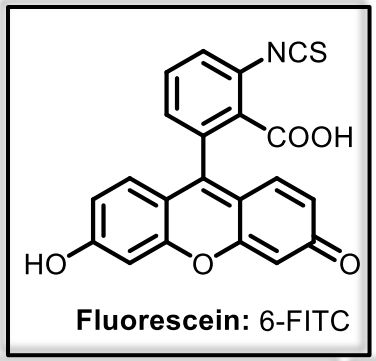
© B. Jang, S. Park, S. H. Kang, J. K. Kim, S. K. Kim, I. H. Kim, Y. Choi, *Quant. Imaging Med. Surg* **2012**, *2*, 1–11.
© S. K. Lyons, E. Lim, A. O. Clermont, J. Dusich, L. Zhu, K. D. Campbell, R. J. Coffee, D. S. Grass, J. Hunter, T. Purchio , D. Jenkins, *Cancer Res .* **2006**, *66*, 4701–4707.

Abstract:
Radiolabeled fluorescent dyes are decisive for bimodal imaging and currently in demand for radio- and optical imaging. This powerful strategy enables the accurate differentiation of healthy and affected tumor tissues. The introduced small molecule based dyes are intended to be used for noninvasive PET and SPECT imaging (prestaging) followed by light-guided R0-tumor-resection.
Herein we describe the synthesis of novel radiolabeled near-infrared emitting silicon rhodamine dyes for optical- and scintigraphic imaging and fluorescence-guided surgery. Additionally the organic dyes are developed for sentinel-lymphnode detection (SNL).
The dyes were synthesized through copper(I)-catalyzed alkyne-azide [3+2]-cycloaddition with alkyne-functionalized biomolecules to receive respective 1,2,3-triazoles for complexing the prominent SPECT-radiometal technetium-99m or to introduce the chelator DOTA for the well-known gallium-68 PET-emitter. The fluorophores were characterized with NMR, mass spectrometry and optical properties were determined. These first-in-class dyes show promising optical properties such as high quantum yields up to 0.45 and high extinction coefficients in the range of charge transfer metal complexes (ca. 120.000 $M^{-1}cm^{-1}$). The subsequent conjugation of these dyes to the SPECT-compatible radiometal technetium-99m with the aim to elucidate their potential as sentinel lymph node detecting agents was successfully investigated and stability tests in aqueous solution were performed. Furthermore, a silicon rhodamine dye was coupled with a prominent biovector (PSMA-617 binding motif) which show high selective tumor enrichment in prostate-cancer cells. The radiolabeled silicon dyes are subject of current and upcoming biological evaluation.
Keywords: Cancer Treatment, Optical Imaging, Molecular Imaging, Si-rhodamines, CuAAC-chemistry

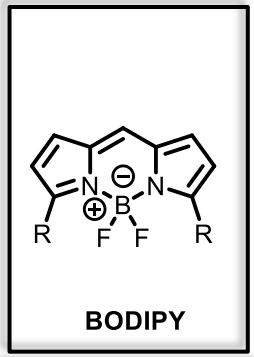
Introduction

Biologically relevant fluorescent dyes:

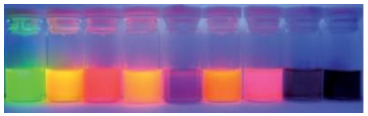
- Fluorescein dyes
- BODIPY dyes
- Phtalocyanine dyes
- Cyanine dyes
- Squaraine dyes

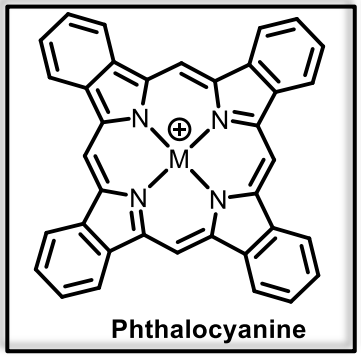


Fluorescein: 6-FITC

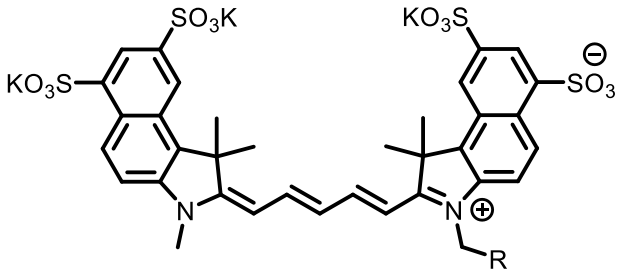


BODIPY

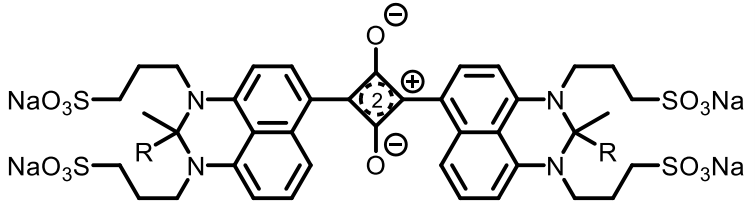




Phthalocyanine



Cyanine: Cy5.5



Squaraine

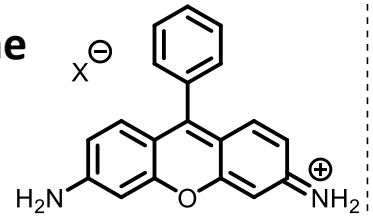
Disadvantages: low solubility, lipophilicity, limited optical properties

→ Requirements for biological applications: optical properties in NIR-region (600–950 nm), large stokes shifts and quantum yields, high chemical and physical stability

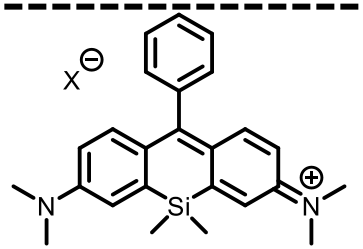
J. O. Escobedo, O. Rusin, S. Lim, R. M. Strongin, *Curr. Opin. Chem. Biol.* **2010**, *14*, 64–70.
V. N. Belov, C. A. Wurm, V. P. Boyarskiy, S. Jakobs, S. W. Hell, *Angew. Chem. Int. Ed.* **2010**, *49*, 3520–3523.
S. Luo, E. Zhang, Y. Su, T. Cheng, C. Shi, *Biomaterials* **2011**, *32*, 7127–7138.
C. Jinping, M. Masatoshi, S. Hiroshi, A. Osuka, *Chem. Eur. J.* **2009**, *15*, 5942–5949.

Introduction

rhodamine

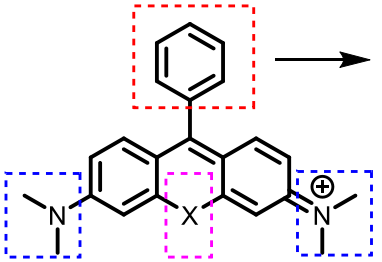


Si-rhodamine



- NIR-fluorophore
- reduced autofluorescence, photobleaching and light scattering effects
- high quantum yields and extinction coefficients
- watersoluble

Influence of rhodamine-derivatization on the optical properties



substitution of benzene moiety:
15 nm redshift

alkylation of N-atom or ring expansion:
up to 120 nm redshift

replacement of position 10 with: Si, Ge, P, S, Se, Te etc.
up to 150 nm redshift (compared to O)

<u>Relevance of near-infrared light</u>

- minimal absorption of NIR light in water and hemoglobin
- high spatial resolution
- low photodamage to cells and tissue

V. N. Belov, C. A. Wurm, V. P. Boyarskiy, S. Jakobs, S. W. Hell, *Angew. Chem. Int. Ed.* **2010**, *49*, 3520–3523.
N. Butkevich, G. Y. Mitronova, S. C. Sidenstein, J. L. Klocke, D. Kamin, D. N. H. Meineke, E. D'Este, P.-T. Krämer, J. G. Danzl, V. N. Belov, S. W. Hell, *Angew. Chem. Int. Ed.* **2016**, *55*, 3290–3294.
T. Ikeno, T. Nagano, K. Hanaoka, *Chem. Asian J.* **2017**, *12*, 1435–1446.

Goal of the Project

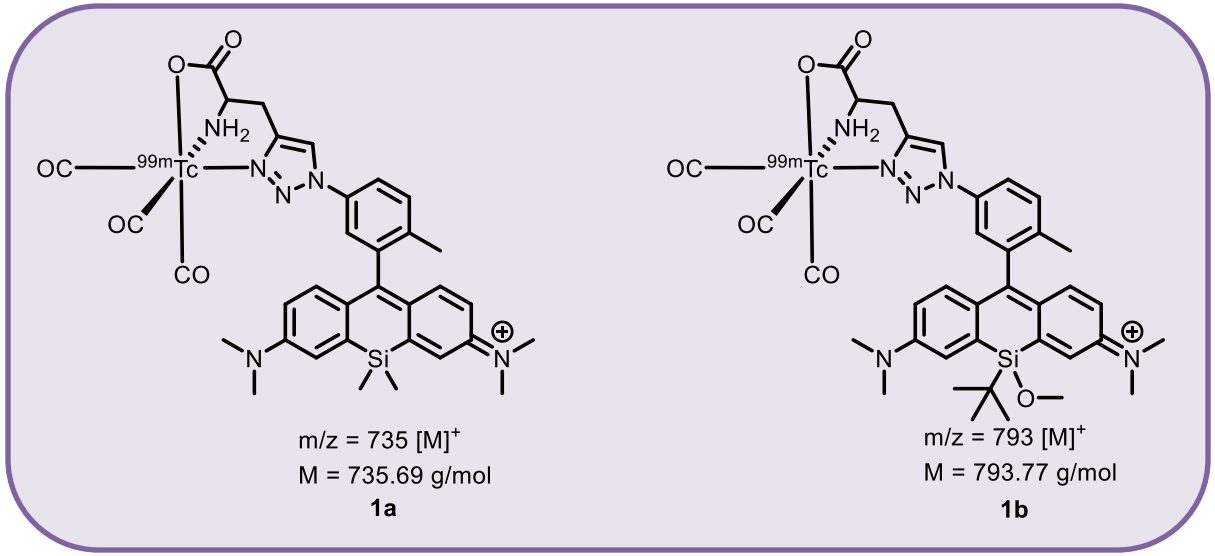
- Synthesis of small molecule based radiotracers for scintigraphic imaging and fluorescence imaging-guided surgery of tumors and their metastases

	Scintigraphic imaging	**Fluorescence imaging**
advantages	whole body imaging high tissue penetration high sensitivity	high spatial resolution no radiation exposure
disadvantages	low spatial resolution radiation exposure time-dependent	lack of tissue penetration

→ Combination of both techniques promises synergistic effects with high tissue penetration and spatial resolution from whole body to subcellular level

Goal of the Project

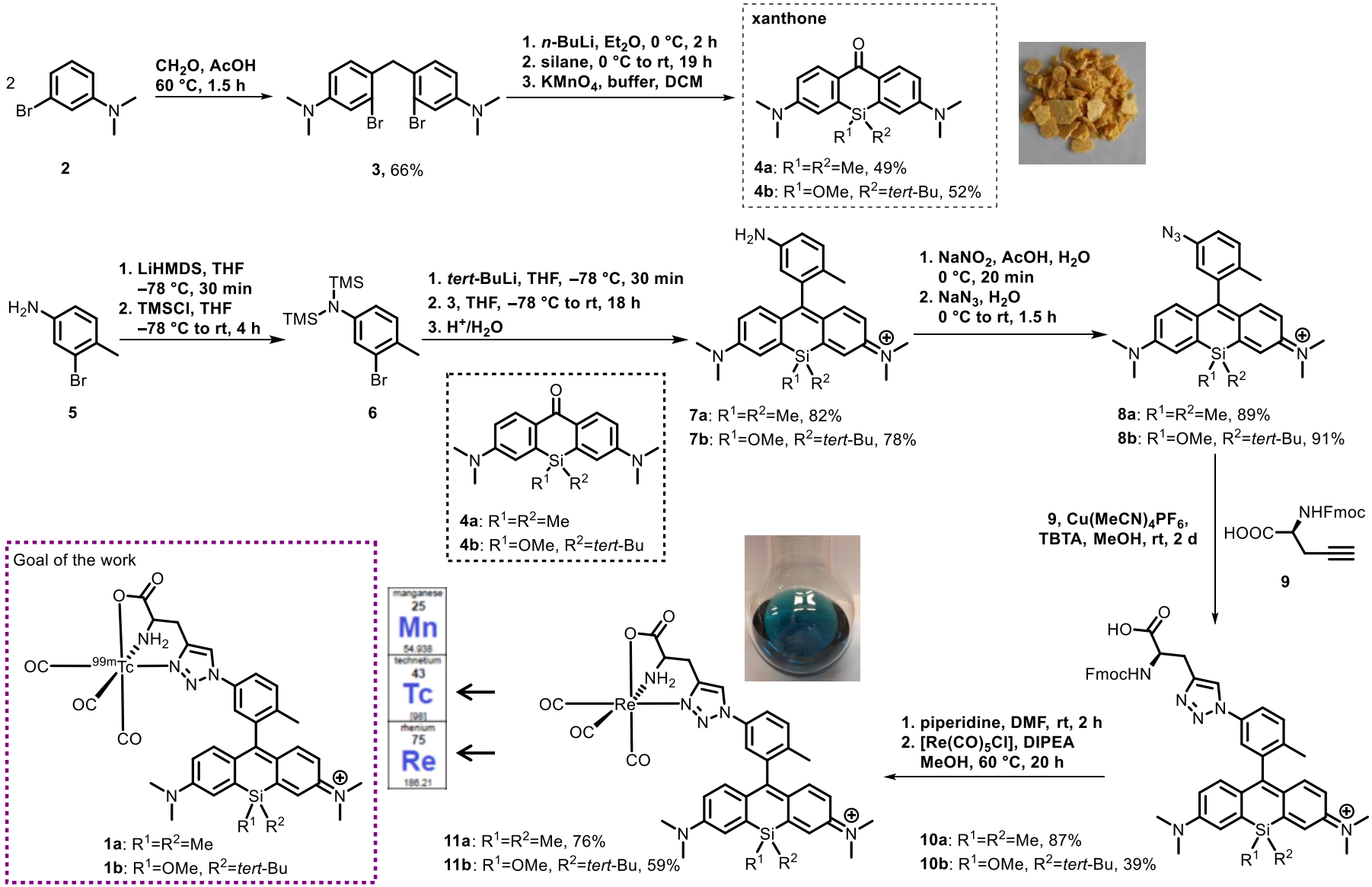
- Synthesis of radiolabeled Si-rhodamines for SPECT-, optical-imaging and fluorescence-guided biopsy for tumor resection
- Radiolabeling with technetium-99m: half-time of 6.01 hours (SPECT-emitter)
- Additional goal: sentinel lymph node detection



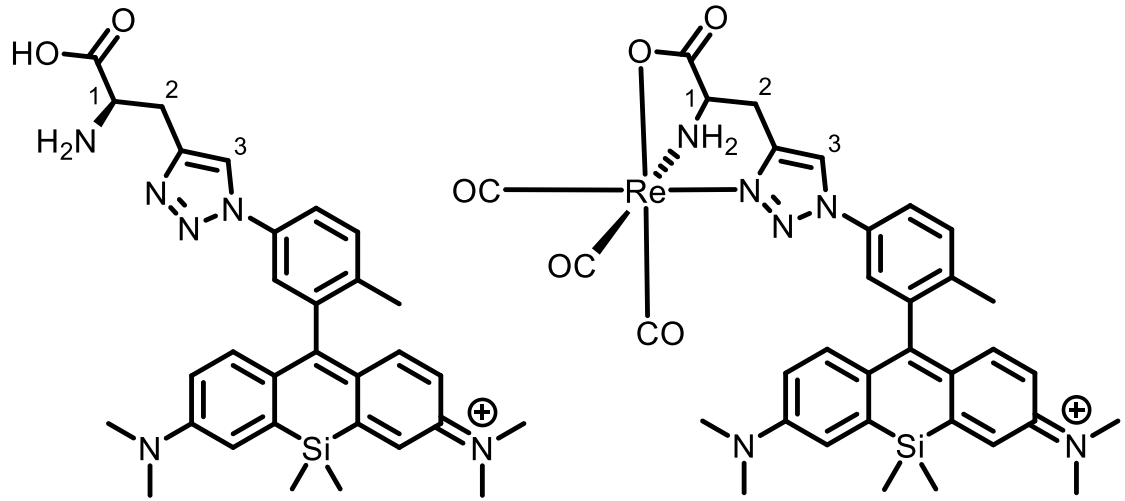
→ Synthesis of the rhenium(I) analogues as cold surrogates for chemical characterization

→ Chemical characterization: NMR, mass analyses, UV/VIS/NIR-properties, stability tests, radiochemical experiments

Results – Chemical Synthesis



Results – ^{1}H-NMR-Data



protons	ligand		Re-complex	
	δ (ppm)	J [Hz]	δ (ppm)	J [Hz]
1	3.93	5.0	4.14	
2	3.41		3.46	
3	8.47		8.63	
NH	-		5.97	10.8, 5.5
NH	-		5.27	11.3

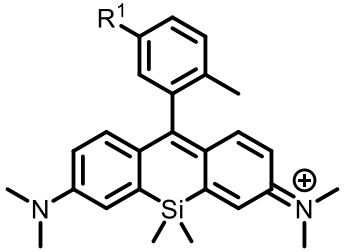
Chemical shifts in methanol-d_4 [400 MHz]

Results – Optical Properties

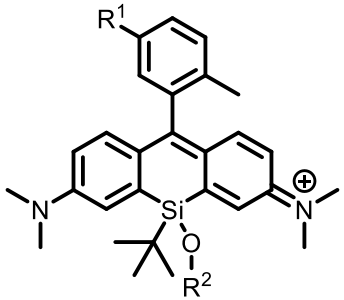
	Compound R^1	Solvent	$\lambda_{abs,\ max}$	λ_{em}	ε_{max}	Quantum Yield
1a	$-NH_2$	MeOH	653 nm	666 nm	91.900 $M^{-1}cm^{-1}$	<0.010
		PBS (pH = 7.4)	651 nm	654 nm	77.300 $M^{-1}cm^{-1}$	<0.010
1b	$-NH_2$	MeOH	664 nm	679 nm	67.750 $M^{-1}cm^{-1}$	0.074
		H_2O/EtOH (5%)	665 nm	680 nm	51.600 $M^{-1}cm^{-1}$	0.052
		PBS (pH = 7.4)	663 nm	678 nm	61.900 $M^{-1}cm^{-1}$	0.026
		MeCN	665 nm	687 nm	120.000 $M^{-1}cm^{-1}$	0.010
1a	$-N_3$	MeOH	651 nm	670 nm	156.500 $M^{-1}cm^{-1}$	0.175
		H_2O/EtOH (5%)	651 nm	668 nm	123.700 $M^{-1}cm^{-1}$	0.103
		PBS (pH = 7.4)	651 nm	668 nm	99.000 $M^{-1}cm^{-1}$	0.116
1b	$-N_3$	MeOH	663 nm	680 nm	73.900 $M^{-1}cm^{-1}$	0.076
		H_2O/EtOH (5%)	663 nm	677 nm	77.200 $M^{-1}cm^{-1}$	0.054
		MeCN	664 nm	685 nm	98.000 $M^{-1}cm^{-1}$	0.450
1a	**1,2,3-triazole**	**MeOH**	**655 nm**	**670 nm**	**79.900 $M^{-1}cm^{-1}$**	**0.042**
		H_2O/EtOH (5%)	**654 nm**	**675 nm**	**73.890 $M^{-1}cm^{-1}$**	**0.098** ←
		PBS (pH = 7.4)	**655 nm**	**669 nm**	**79.900 $M^{-1}cm^{-1}$**	**0.127** ←
1b	**1,2,3-triazole**	**H_2O/EtOH (5%)**	**671 nm**	**693 nm**	**59.970 $M^{-1}cm^{-1}$**	**0.048**
1a	**Re-complex**	**MeOH**	**654 nm**	**671 nm**	**63.900 $M^{-1}cm^{-1}$**	**0.135**
		H_2O/EtOH (5%)	**654 nm**	**674 nm**	**22.100 $M^{-1}cm^{-1}$**	**0.104** ←
		PBS (pH = 7.4)	**651 nm**	**668 nm**	**39.100 $M^{-1}cm^{-1}$**	**0.090** ←
1b	**Re-complex**	**PBS (pH = 7.4)**	**671 nm**	**693 nm**	**33.300 $M^{-1}cm^{-1}$**	**0.052**

*** FDA approved fluorescent dyes**

	5-aminolevulinic acid*	**H_2O**	**405 nm**	**635 nm**	**5000 $M^{-1}cm^{-1}$**	**0.080**
	Indocyanine green (ICG)*	**blood**	**807 nm**	**822 nm**	**121.000 $M^{-1}cm^{-1}$**	**0.001**
	Fluorescein*	**H_2O**	**489 nm**	**515 nm**	**88.000 $M^{-1}cm^{-1}$**	**0.85**



1



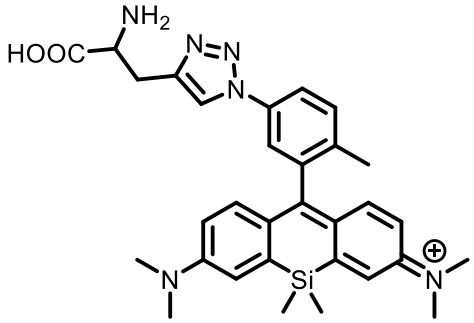
2

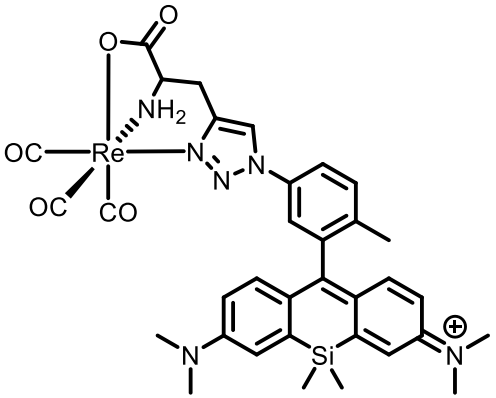
a: R^2 = Me
b: R^2 = H

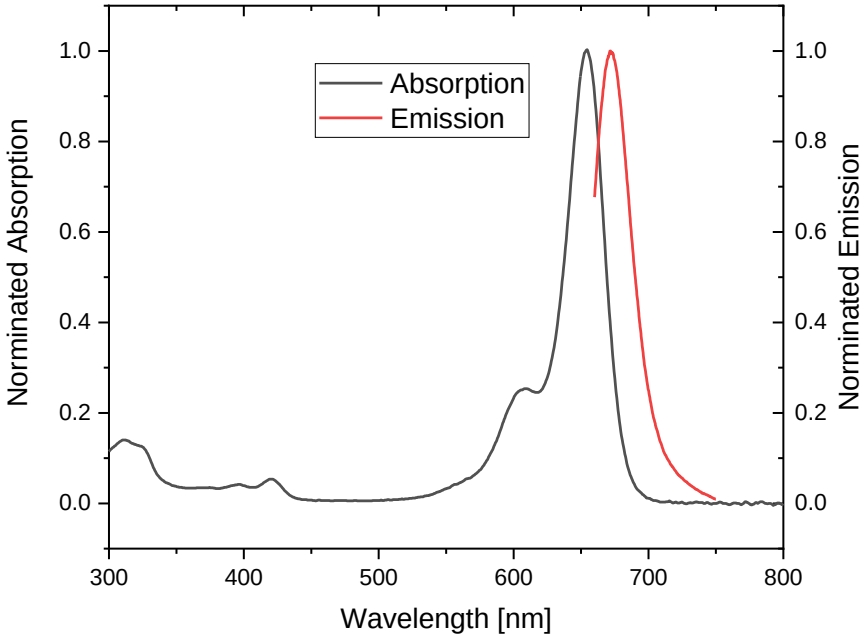
→ No significant impact on quantum yield after complexation with Re(I)-complex

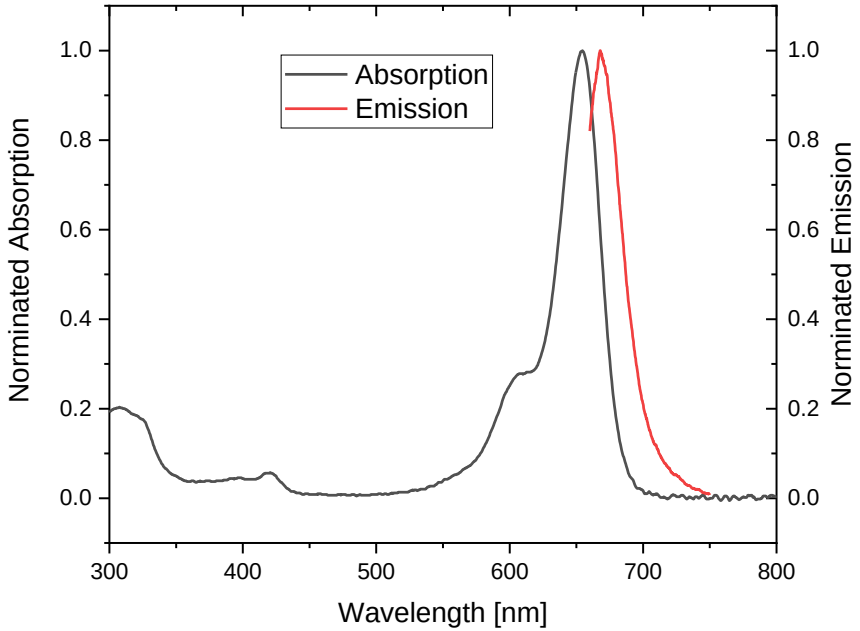
S. Gioux, H. S. Choi, J. V. Frangioni, *Mol. Imaging* **2010**, *9*, 237–255.

Results – Optical Properties – UV/VIS/NIR-spectra

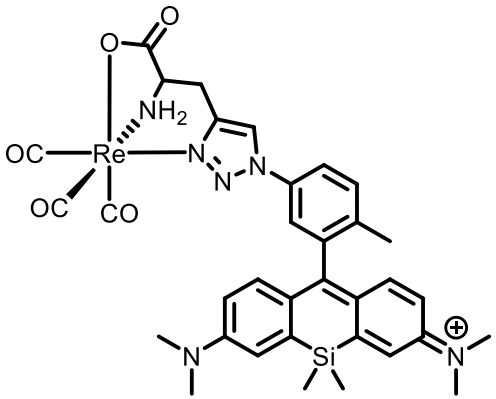




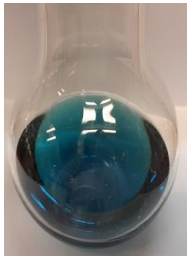


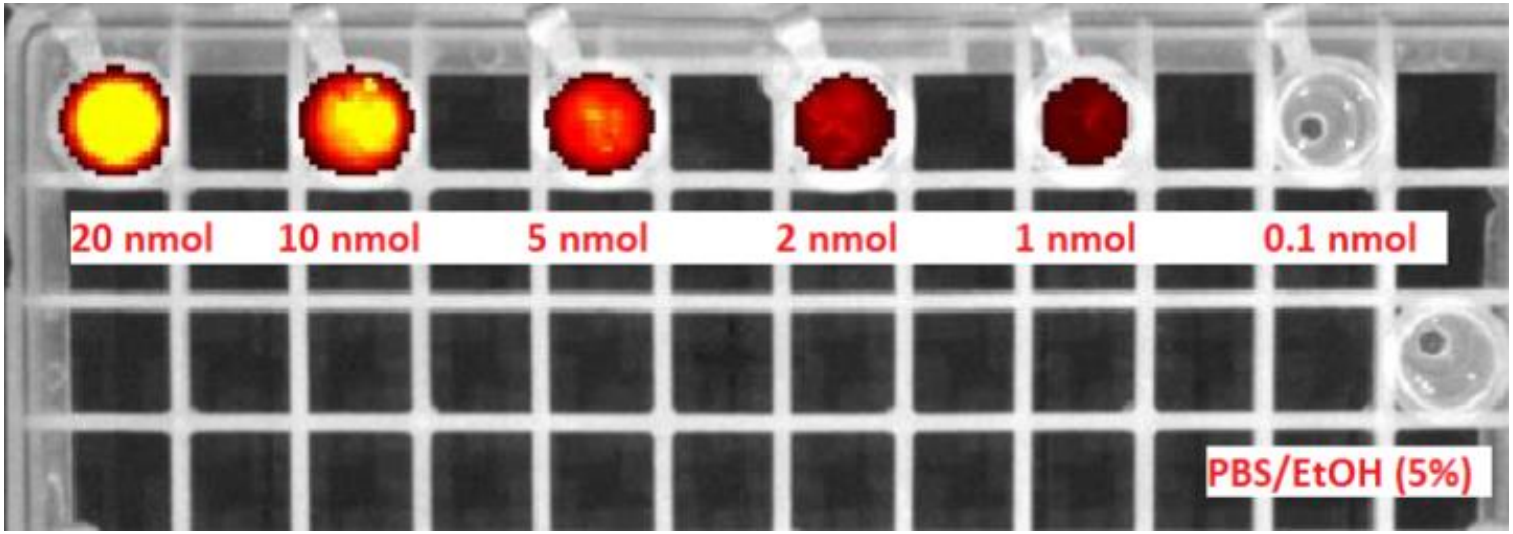


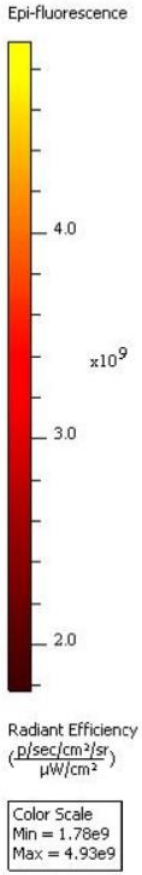
Results – Optical Properties – IVIS-imaging



→ **After 24 h: stable in PBS and histidine/PBS (37 °C)**

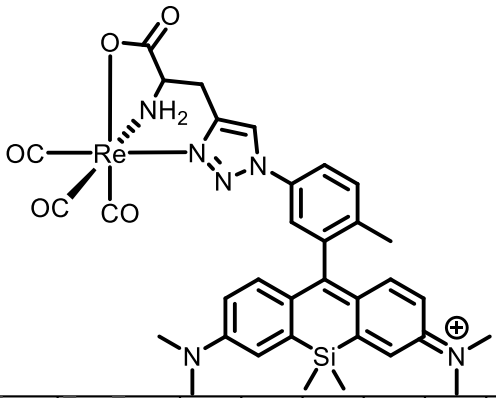


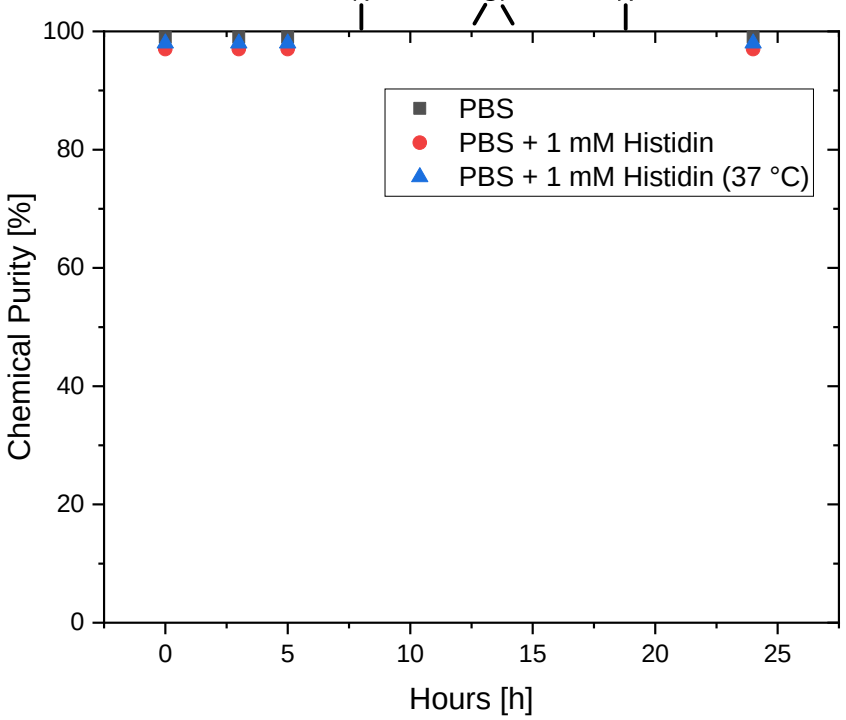


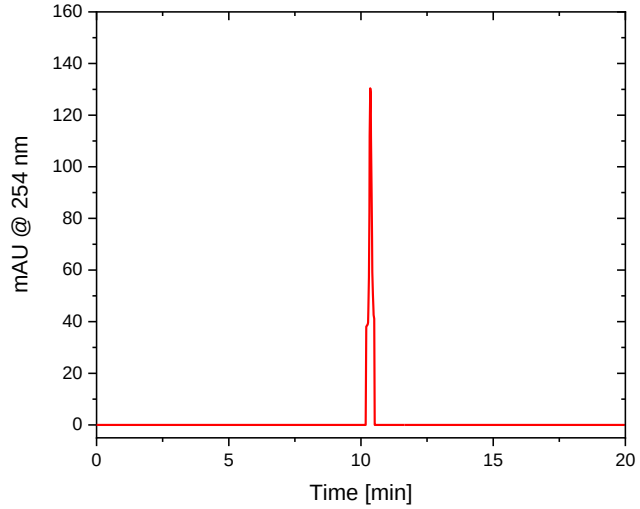


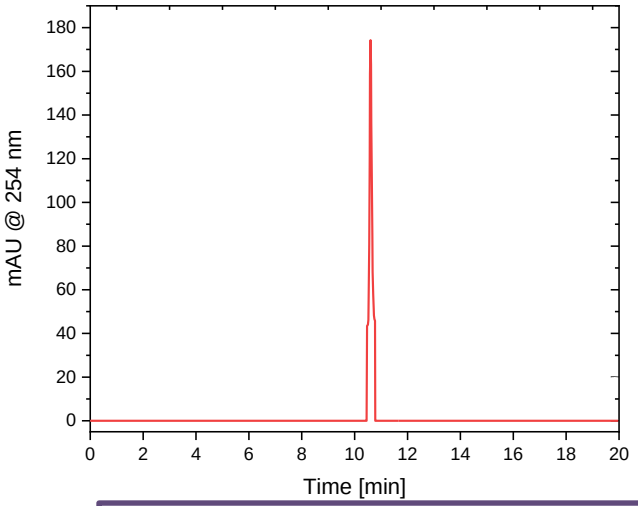
→ For *in vitro* and *in vivo* experiments in optical imaging experiments a maximum of 5 nmol of rhenium complex in an aqueous solution gives suitable and intensive emission signals

Results – Stability Tests in PBS, Histidine and Serum Plasma





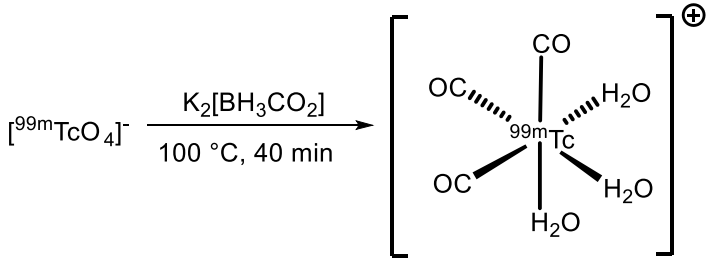


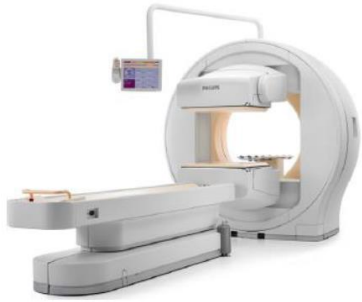


→ serum plasma: still stable after 7 h at 37 °C

high stability in aqueous solution and serum plasma

Results – First Radiolabeling Experiments with $[^{99m}Tc(CO)_3(H_2O)_3]^+$

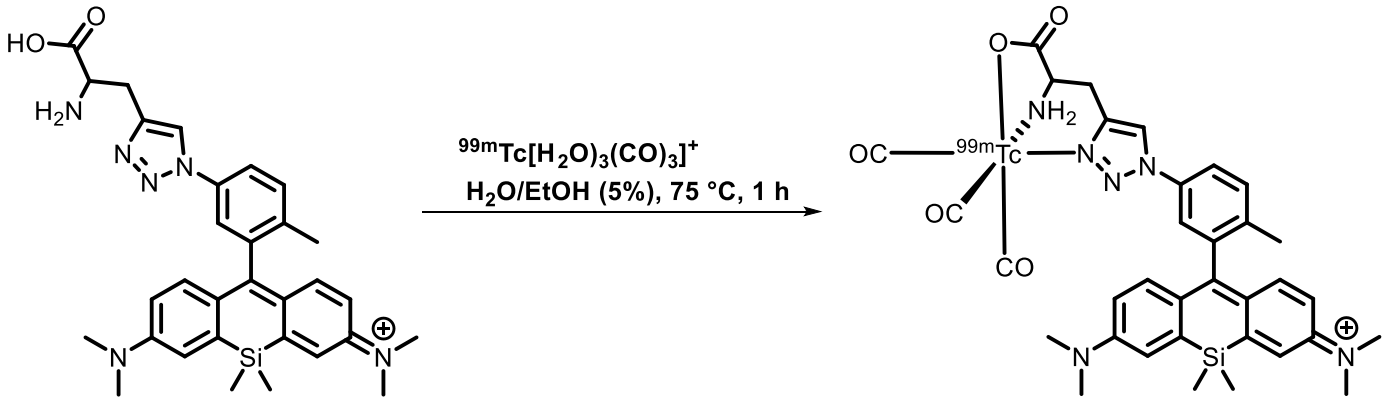




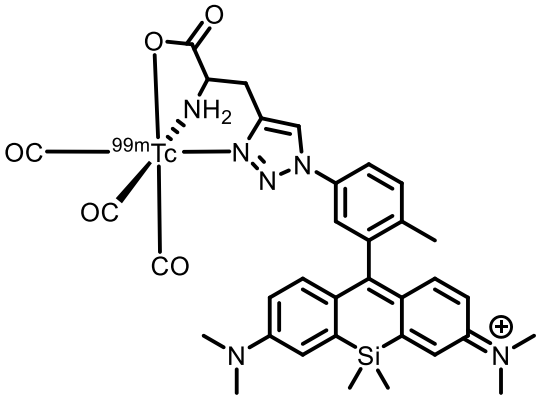
Siemens Healthineers©

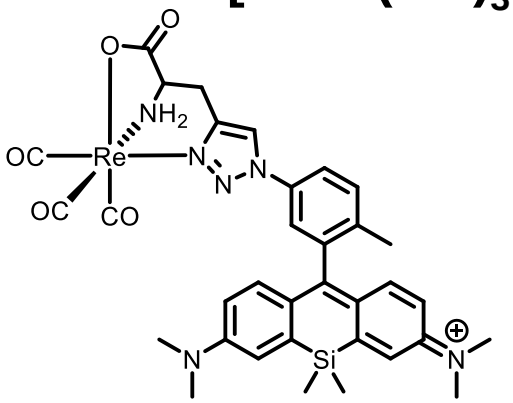
- Synthesis of radioactive technetium-99m complex via IsoLink™
- Properties of technetium-99m:
  - half-time: 6.01 hours
  - energy: 143 keV
- Prominent isotope for SPECT-imaging (single photon emission computed tomography)

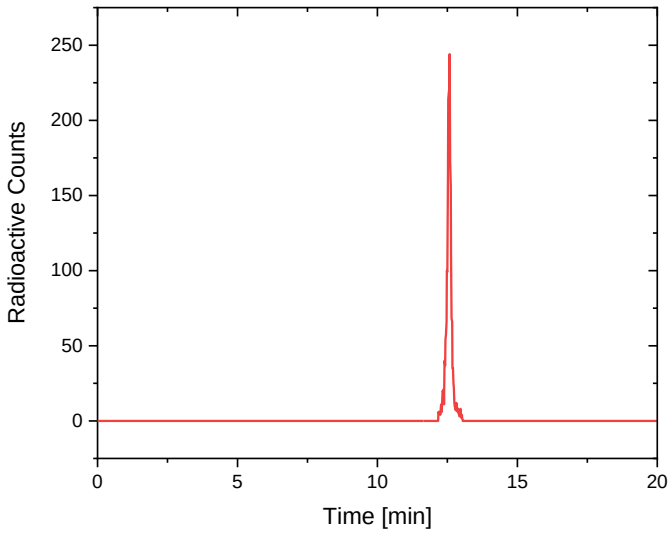
Radiolabeling of fluorescent Si-rhodamine with technetium-99m:

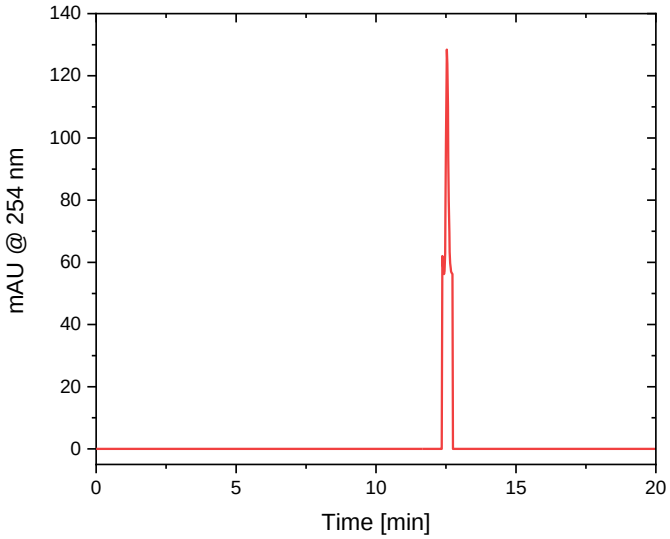


Results – First Radiolabeling Experiments with $[^{99m}Tc(CO)_3(H_2O)_3]^+$







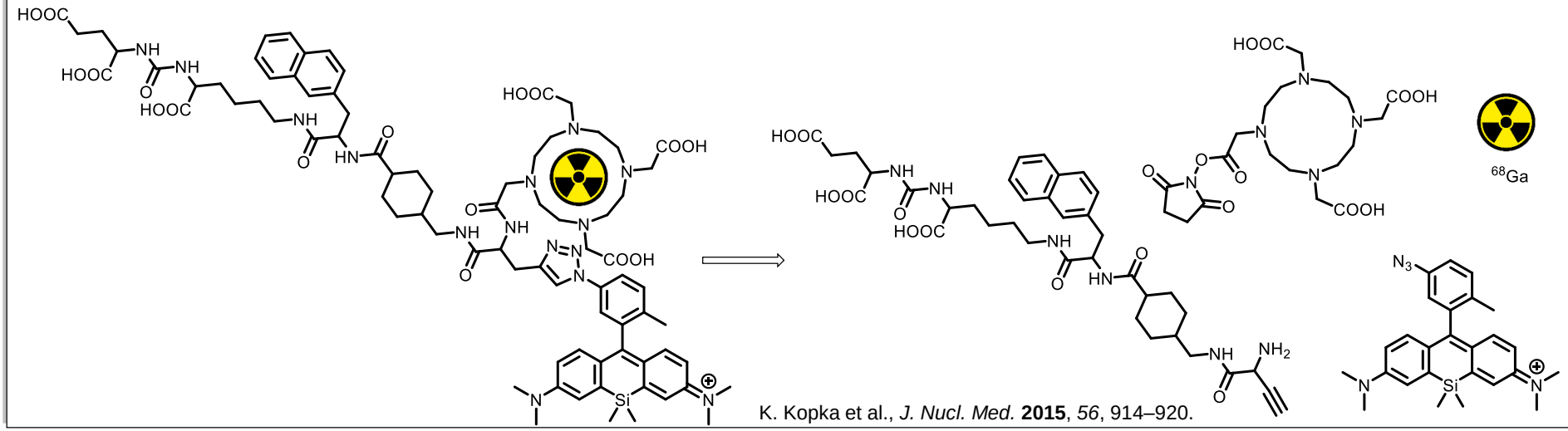


Compound	Retention time	RCY	RCP	Stability
Re-Si-rhodamine	12.5 min			Stable (PBS, PBS/His at 37 °C)
^{99m}Tc-Si-rhodamine	12.6 min	59%	>99%	Stable in PBS after 6 h

Conclusion

- Synthesis of small-molecule based Re- and ^{99m}Tc-complexes and fluorescent dyes for bimodal imaging and sentinel lymph node detection
- Compounds were characterized with NMR, mass spectrometry and optical properties
- Fluorescent dyes show high purity, unique optical properties and high stability in PBS, histidine/PBS and human serum
  → Compared with conventional FDA approved dyes very promising optical properties
- Upcoming research:
  - determination of *in vivo* stabilities
  - cellular and preclinical experiments *in vitro* and *in vivo* to determine intrinsic selectivity
  - introducing biovectors into dye for selective enrichment in tumors

→ Synthesized radiotracer for potential PET- and optical imaging with PSMA-617 binding moiety as target vector with high affinity to prostate cancer tumor cells:



K. Kopka et al., *J. Nucl. Med.* **2015**, *56*, 914–920.

Acknowledgments

City of Heidelberg
© Hotel.de/blog

Division of Radiopharmaceutical Chemistry, DKFZ, Heidelberg, Germany:

Prof. Dr. Klaus Kopka, Dr. Carsten S. Kramer, Martin Schäfer, Yvonne Remde

Stony Brook University, New York, USA: Assist. Prof. Dr. Eszter Boros, Brett Vaughn and the whole group

Institute of Inorganic Chemistry, Heidelberg University, Germany: Prof. Dr. Peter Comba

MPI for Biomedical Research, Heidelberg, Germany: Dr. Alexey Butkevich, Jessica Matthias (Prof. Dr. Dr. Stefan Hell)