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ESR12 Fellow

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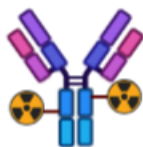
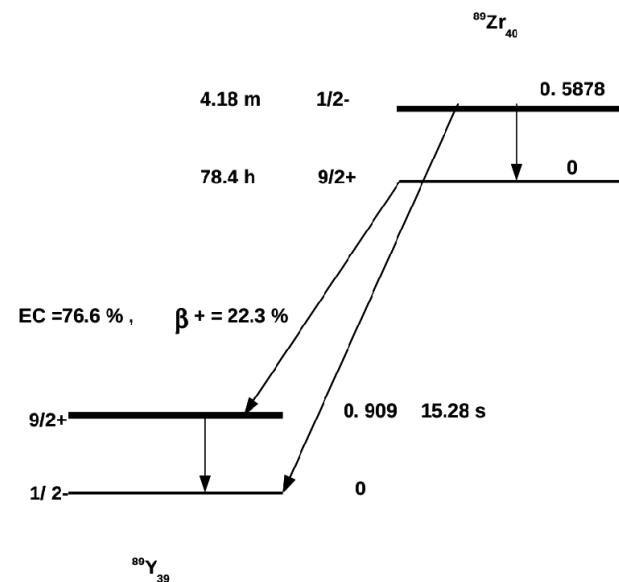
[maria.Vlastara@tagworkspharma.com]

Meeting 2
Edinburgh, 3rd February 2020

Zr-89 characteristics

- Low positron energy 395 keV
- High resolution and quantitative imaging
- Residualizing isotope
- $t_{1/2} = 78.4\text{h}$

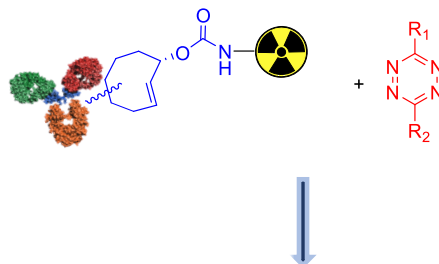
Best nuclide for PET imaging of slow-accumulating biomolecules (**nanoparticle-based molecules, mabs...**)



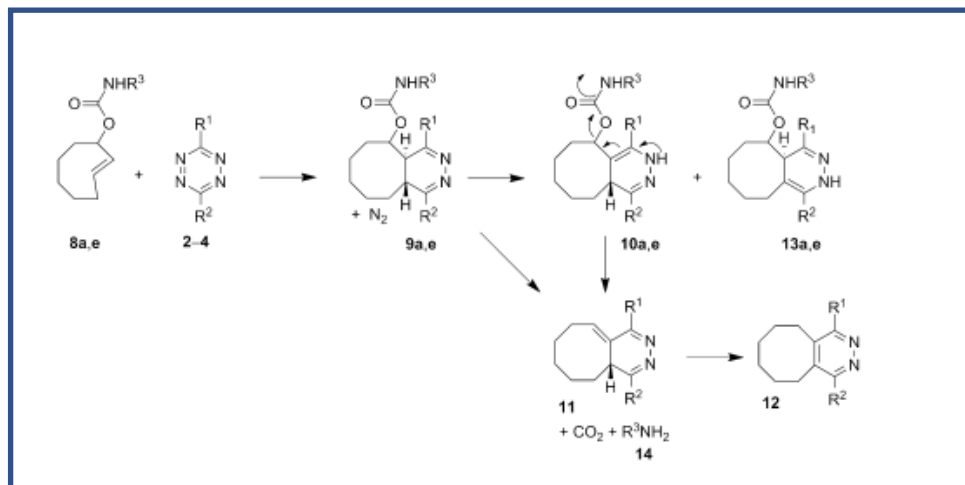
^{89}Zr -trastuzumab uptake after 5d the injection



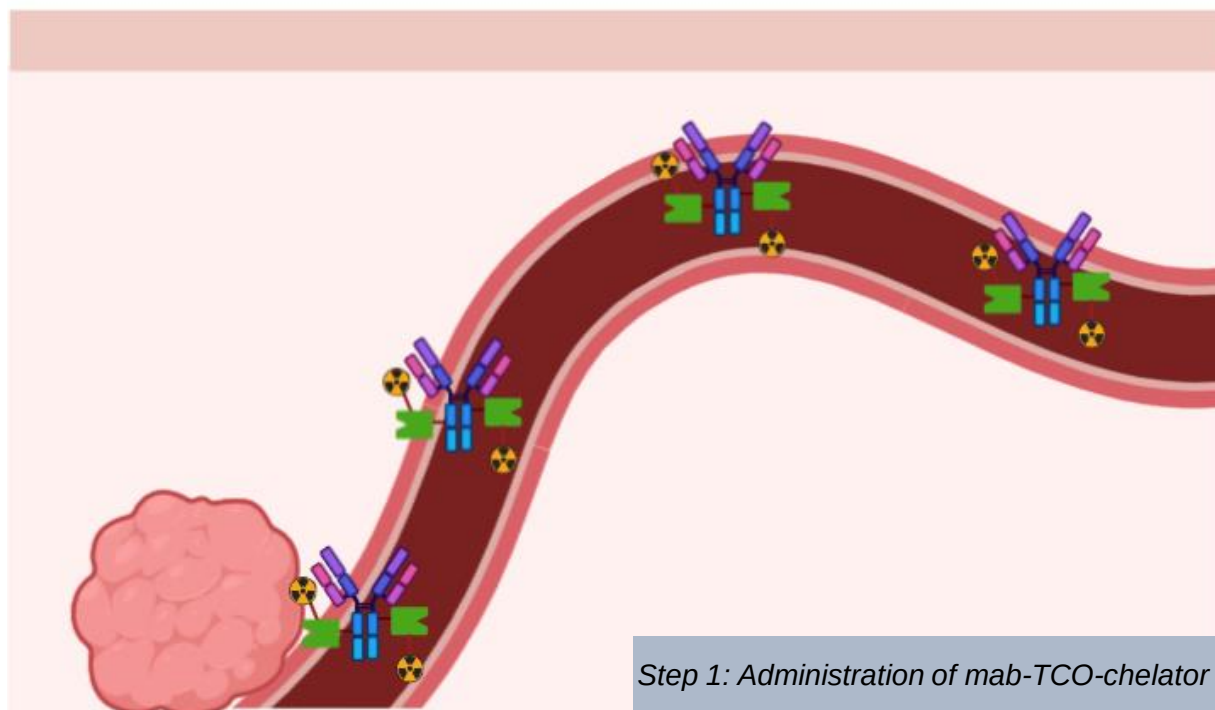
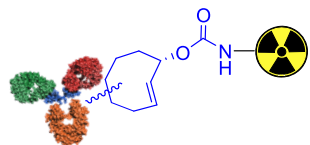
Van de Watering, et al. (2014). Zirconium-89 labeled antibodies: a new tool for molecular imaging in cancer patients. *BioMed research international*, 2014.

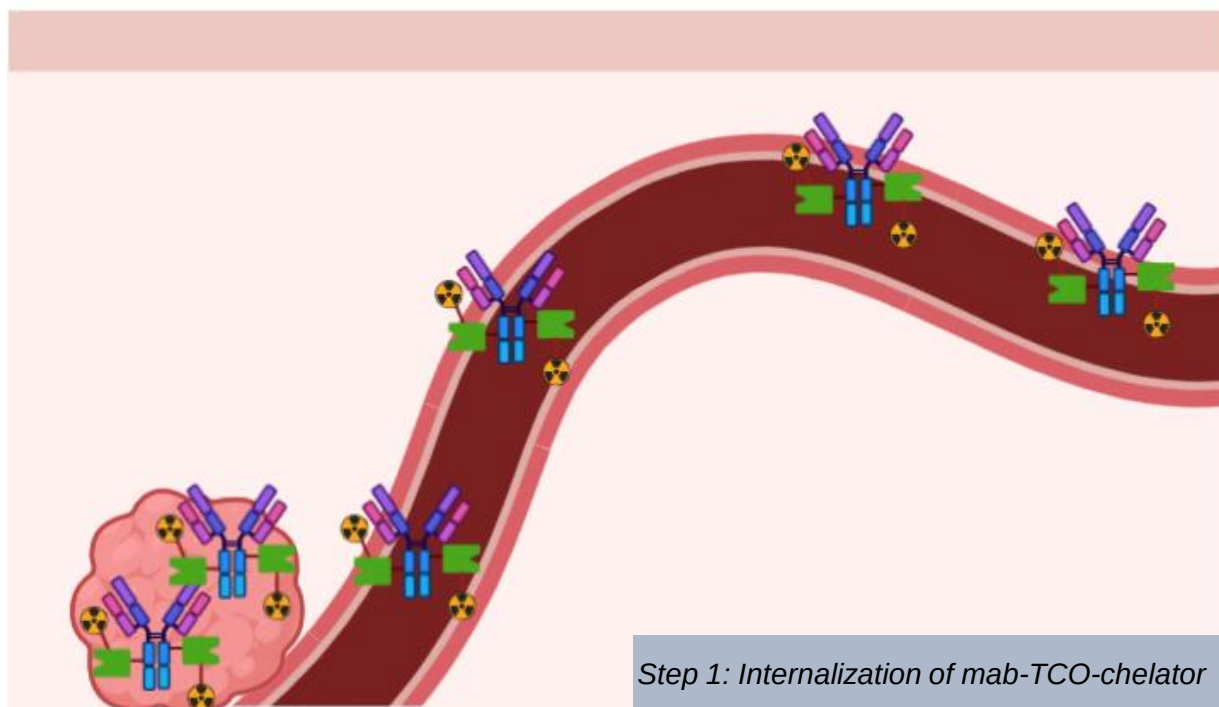
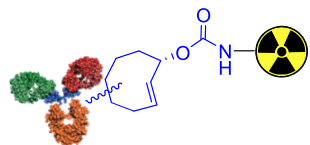


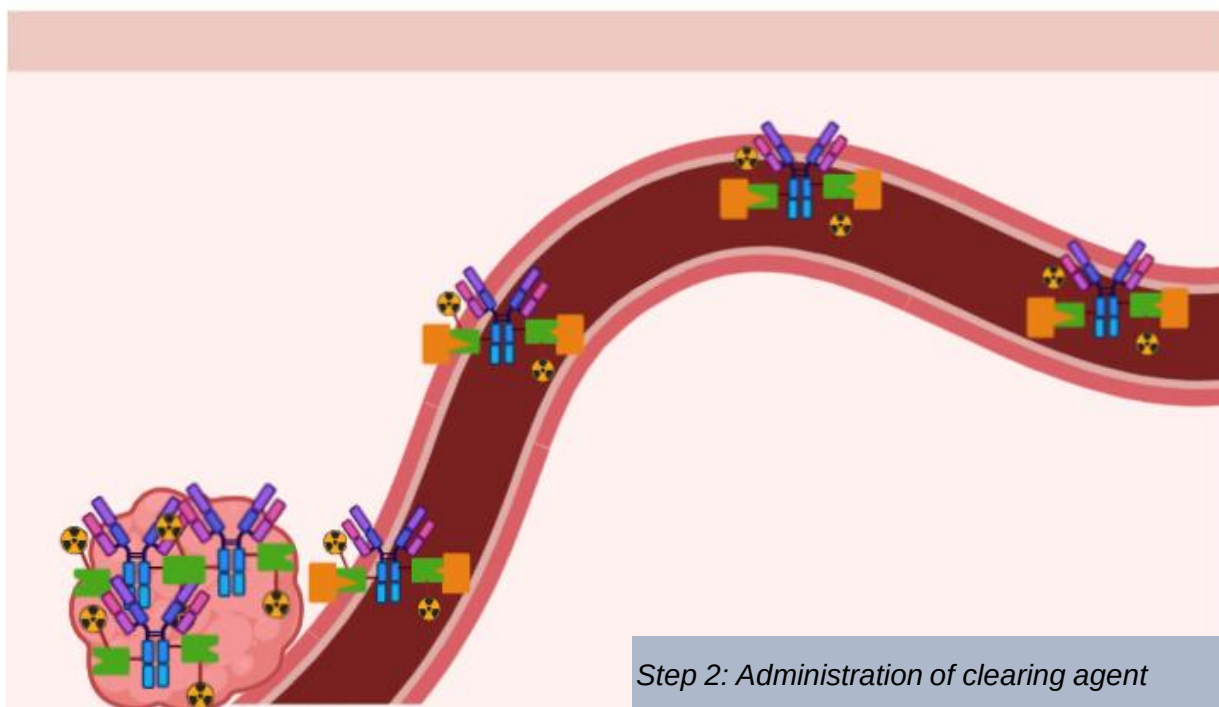
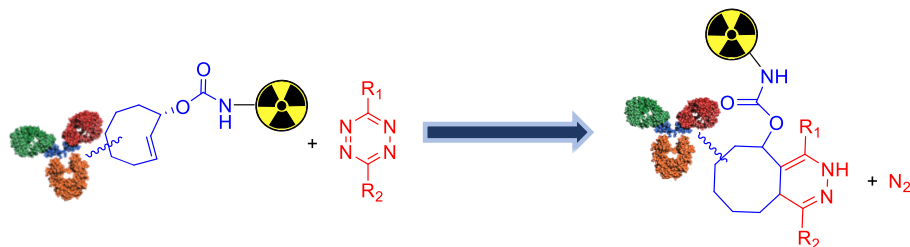
- Bioorthogonal
- Fast kinetics with $k_2 = 1.9 - 2.3 \times 10^6 M^{-1} s^{-1}$
- Applicable in aqueous medium, in vitro, in vivo
- Click-to-release (via an ether, **carbamate**, ester)

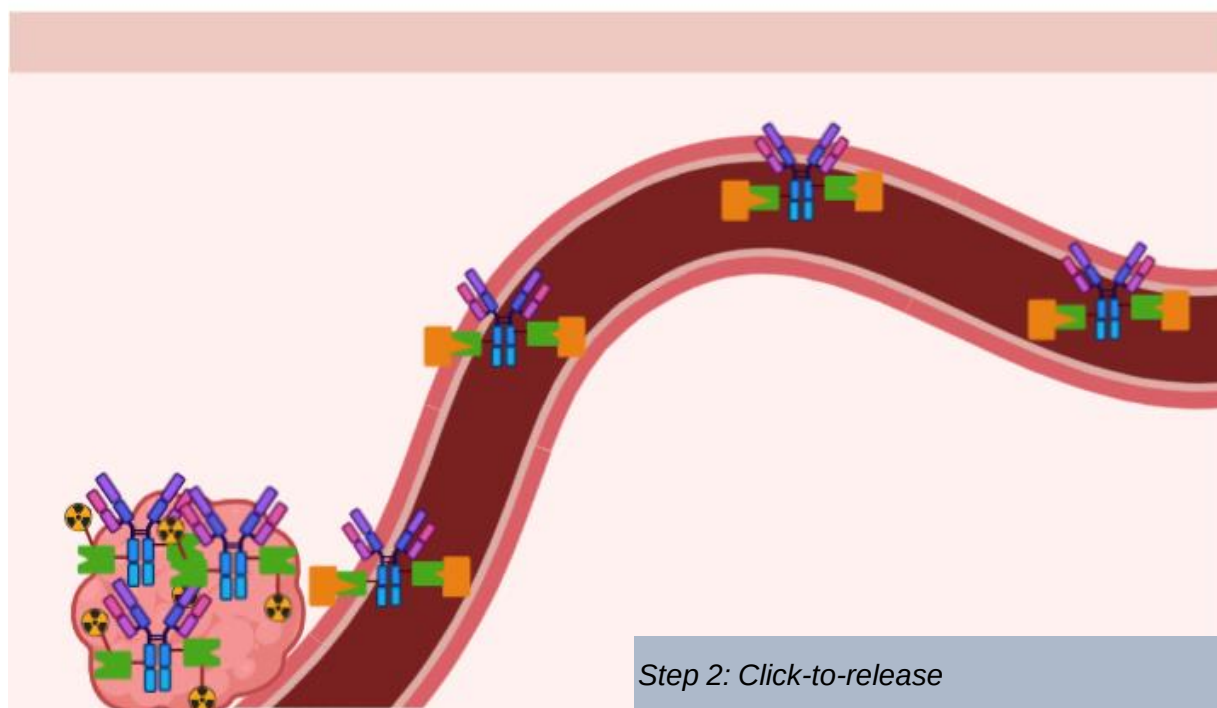
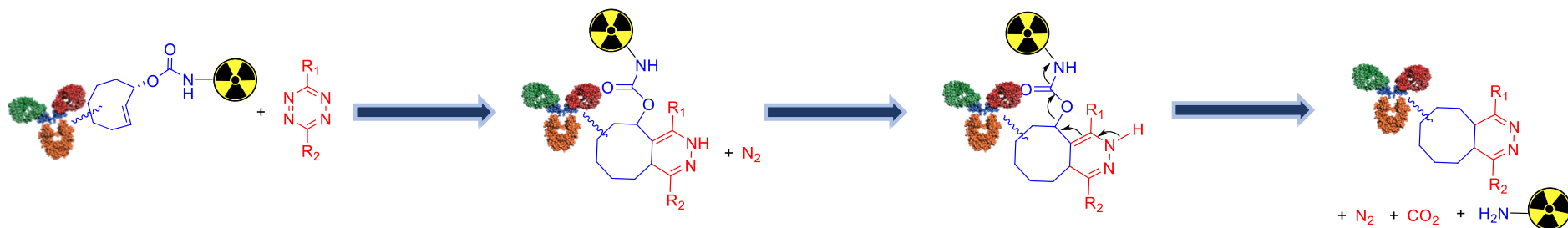


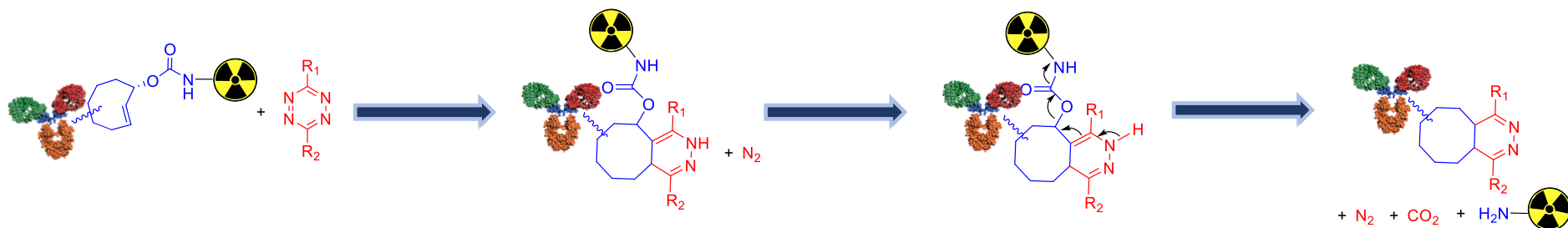
Versteegen, Ron M., et al. "Click to release: instantaneous doxorubicin elimination upon tetrazine ligation." *Angewandte Chem.* 52.52 (2013): 14112-14116
 Rossin, R., & Robillard, M. S. (2014). Pretargeted imaging using bioorthogonal chemistry in mice. *Current opinion in chemical biology*, 21, 161-169.



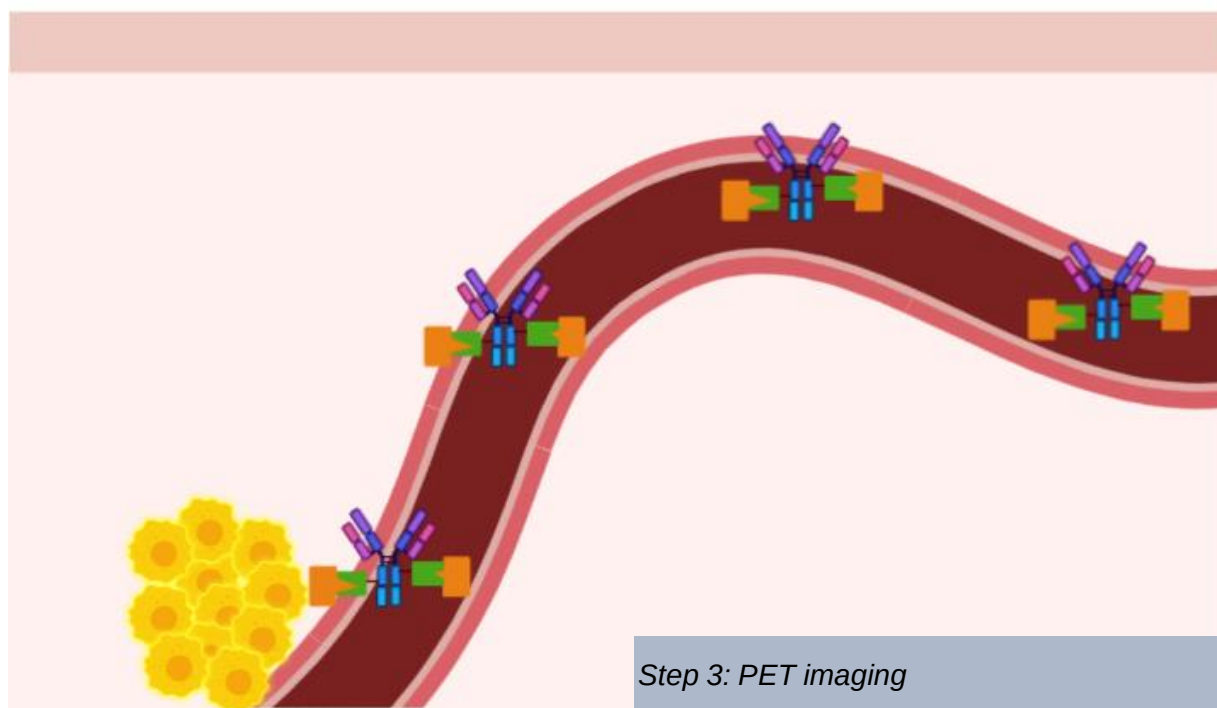


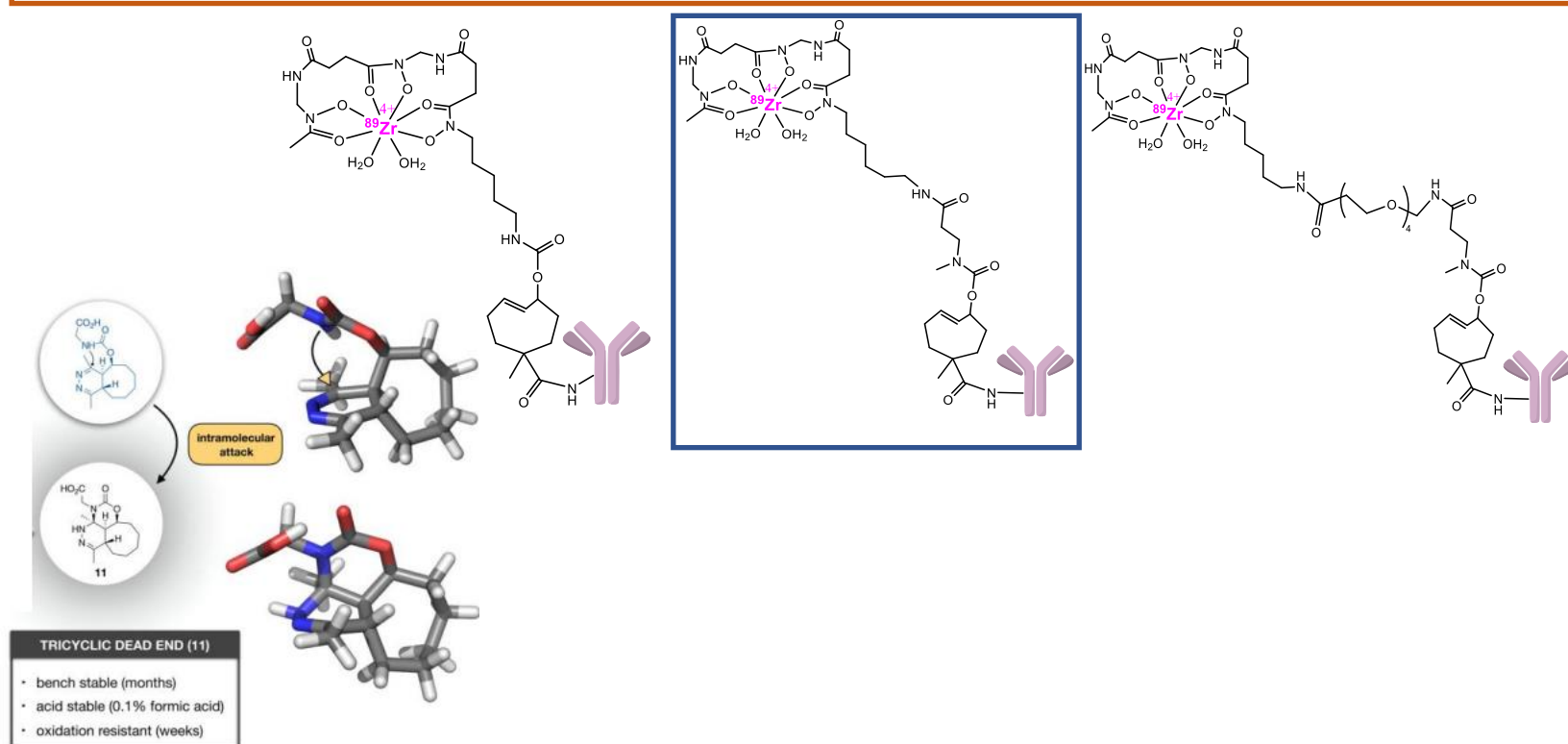
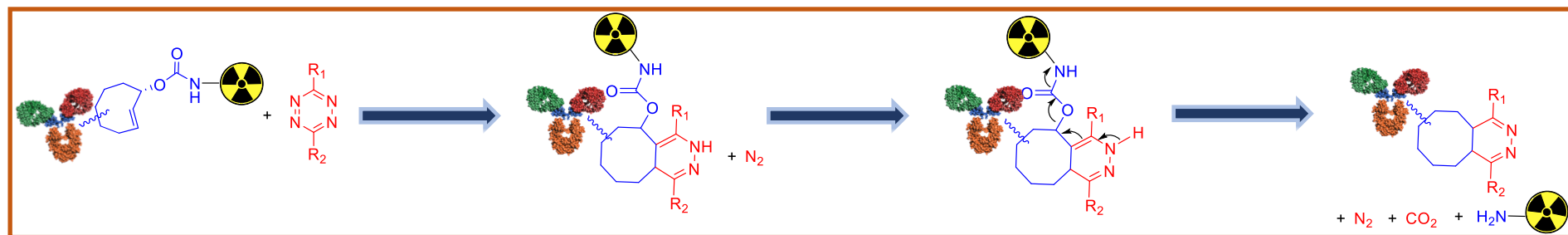




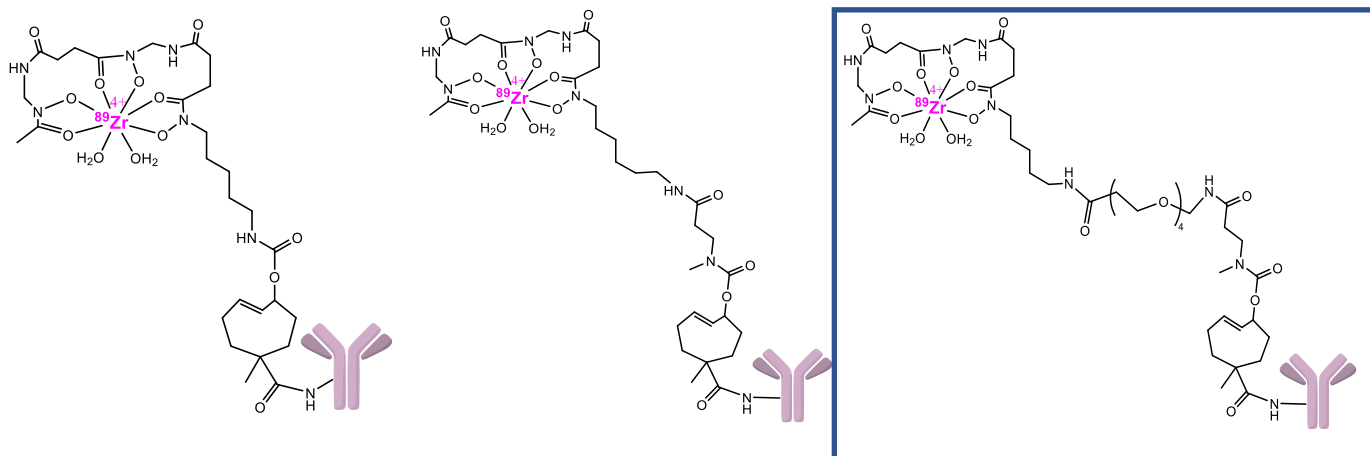
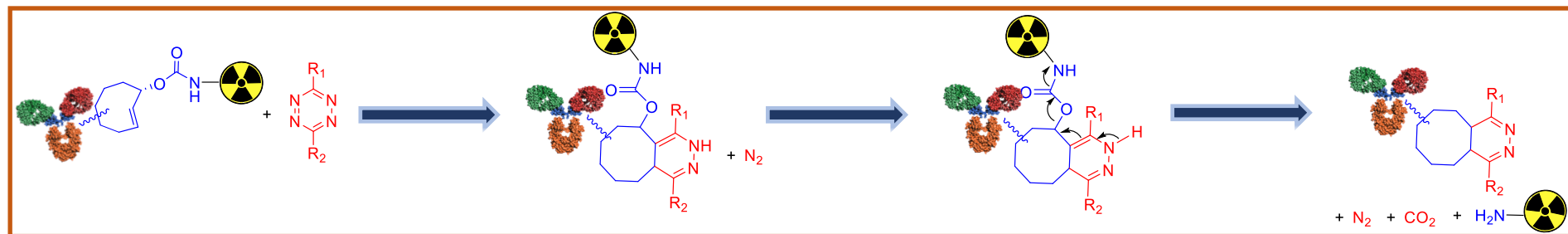


- ✓ Improved tumor-to-blood ratios
- ✓ Quick imaging
- ✓ Decrease dose in case of radiotherapy

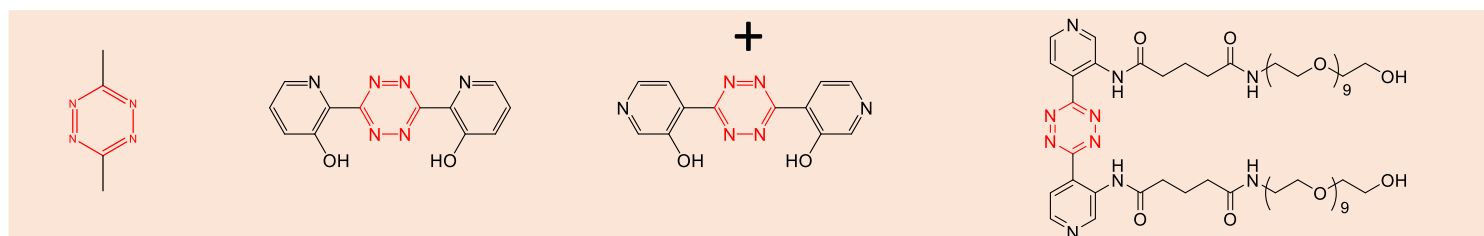
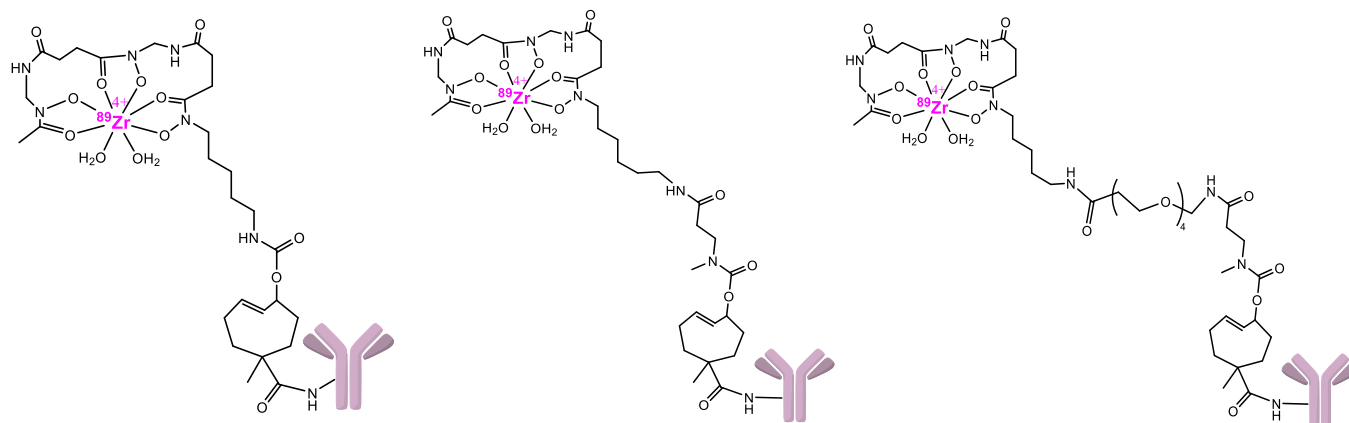
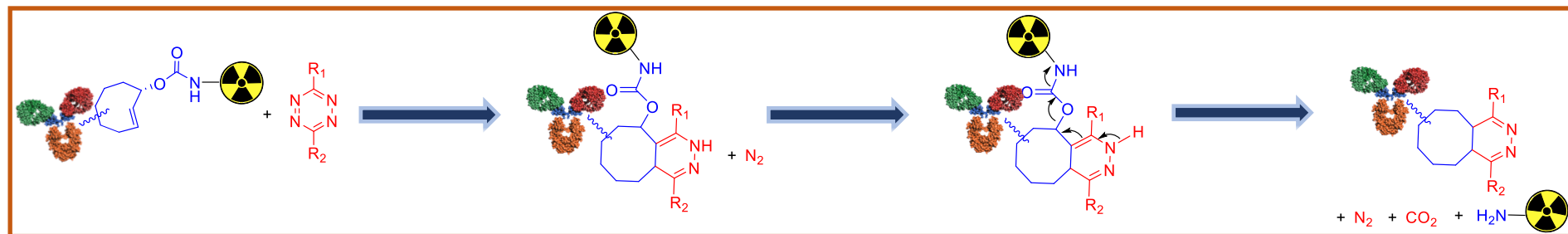




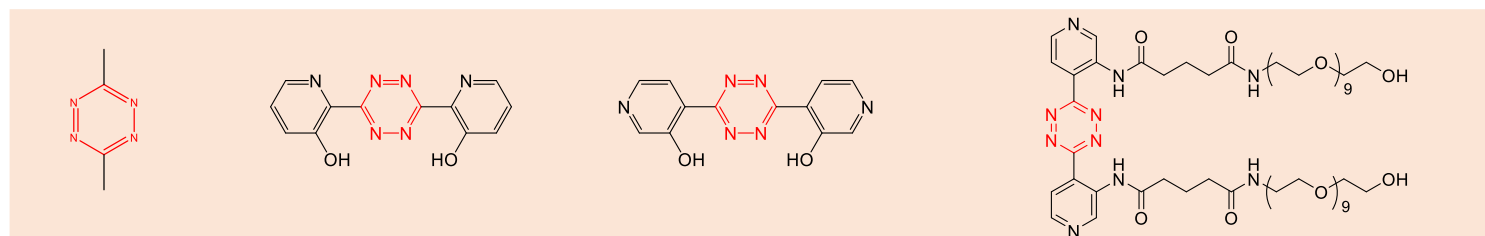
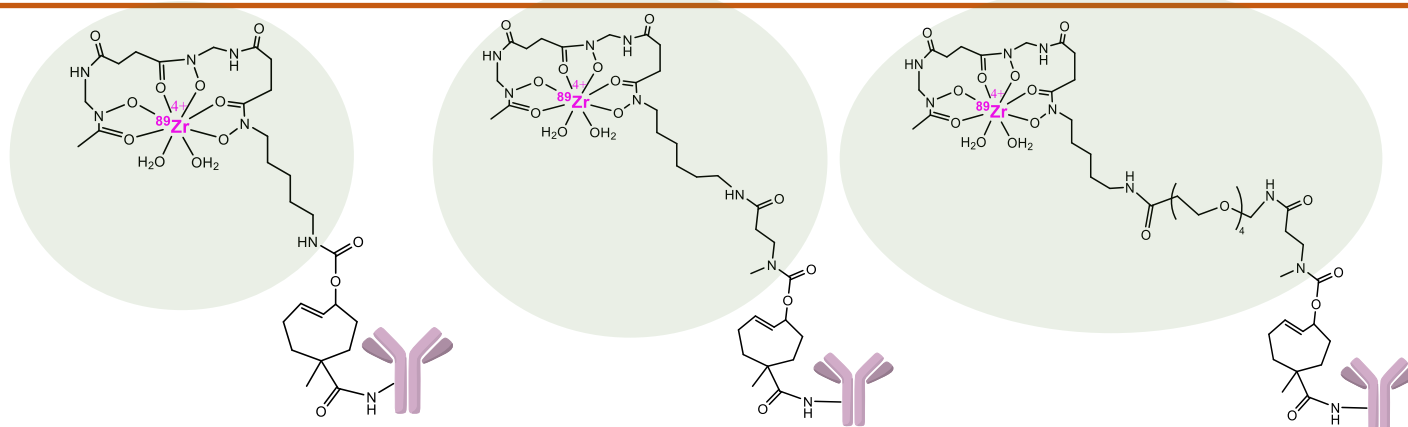
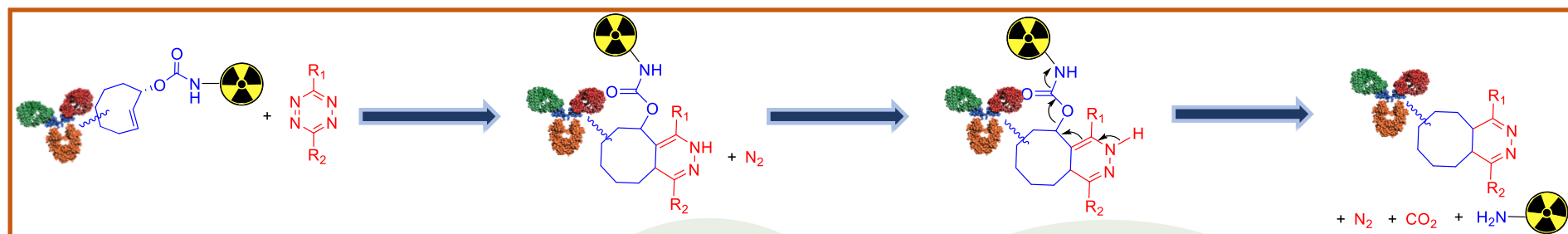
Carlson, J. C., Mikula, H., & Weissleder, R. (2018). Unraveling tetrazine-triggered bioorthogonal elimination enables chemical tools for ultrafast release and universal cleavage. *Journal of the American Chemical Society*, 140(10), 3603-3612.



Avoid steric hindrance from Zr-core

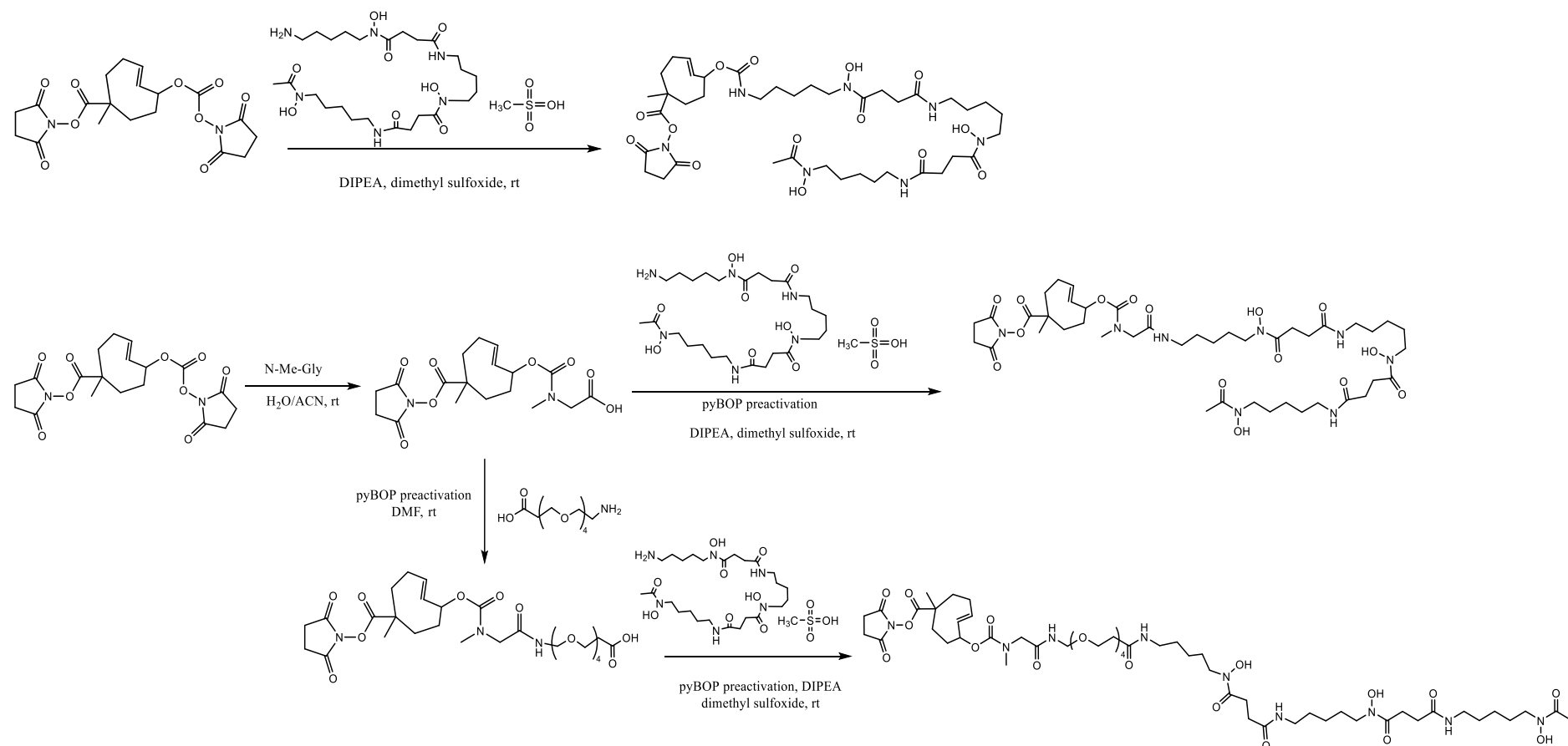


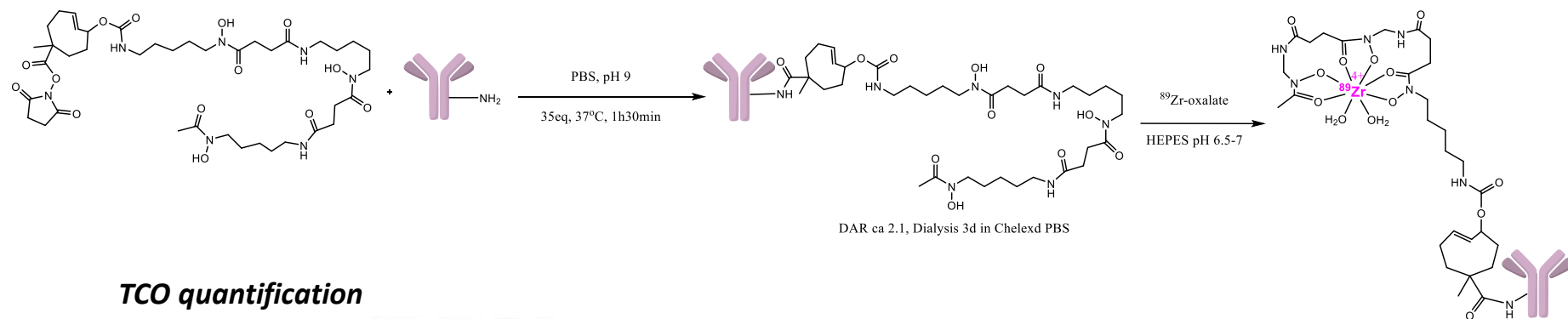
Release



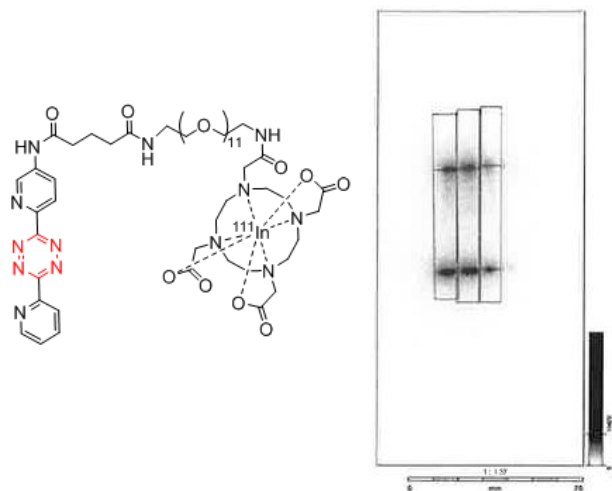
Release

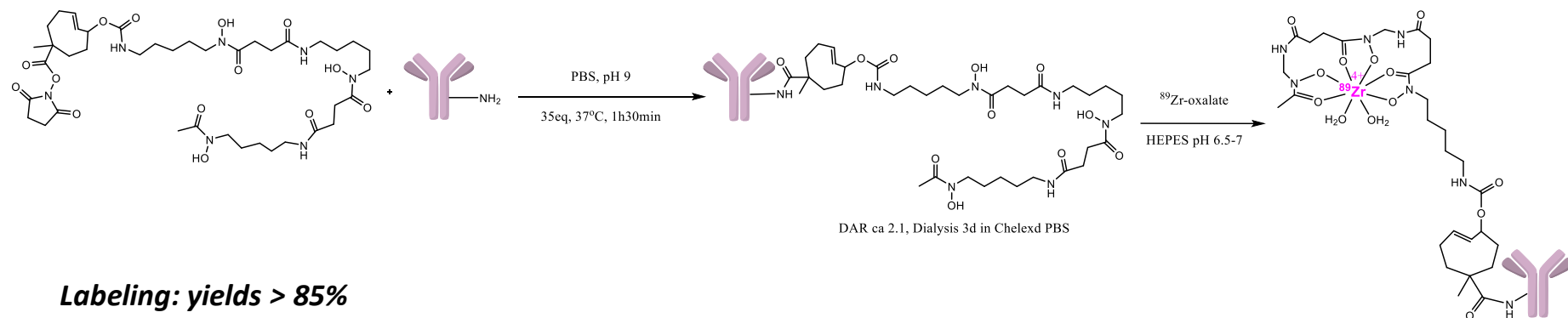
- Synthesis of TCO-chelator





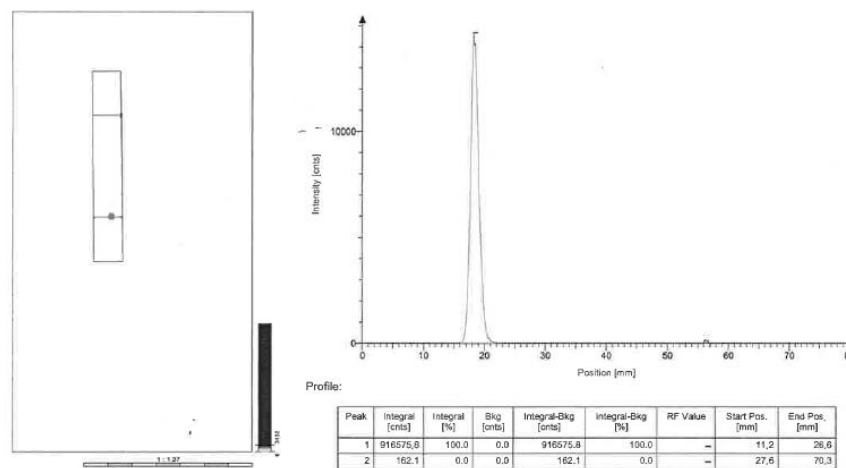
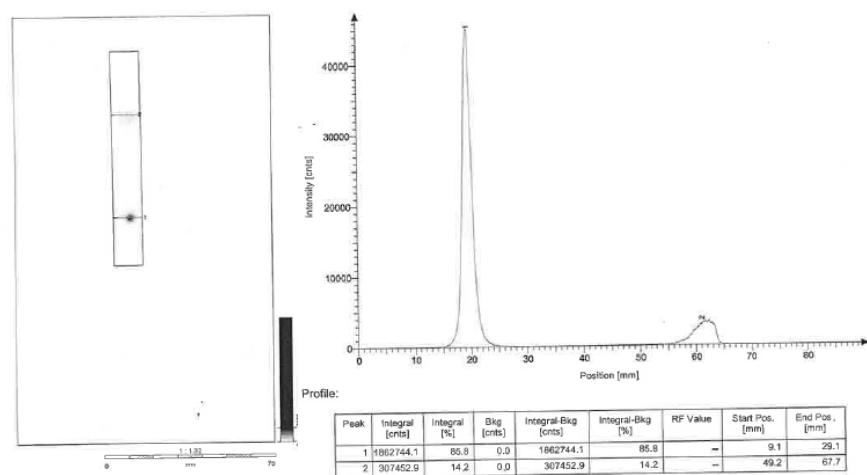
TCO quantification

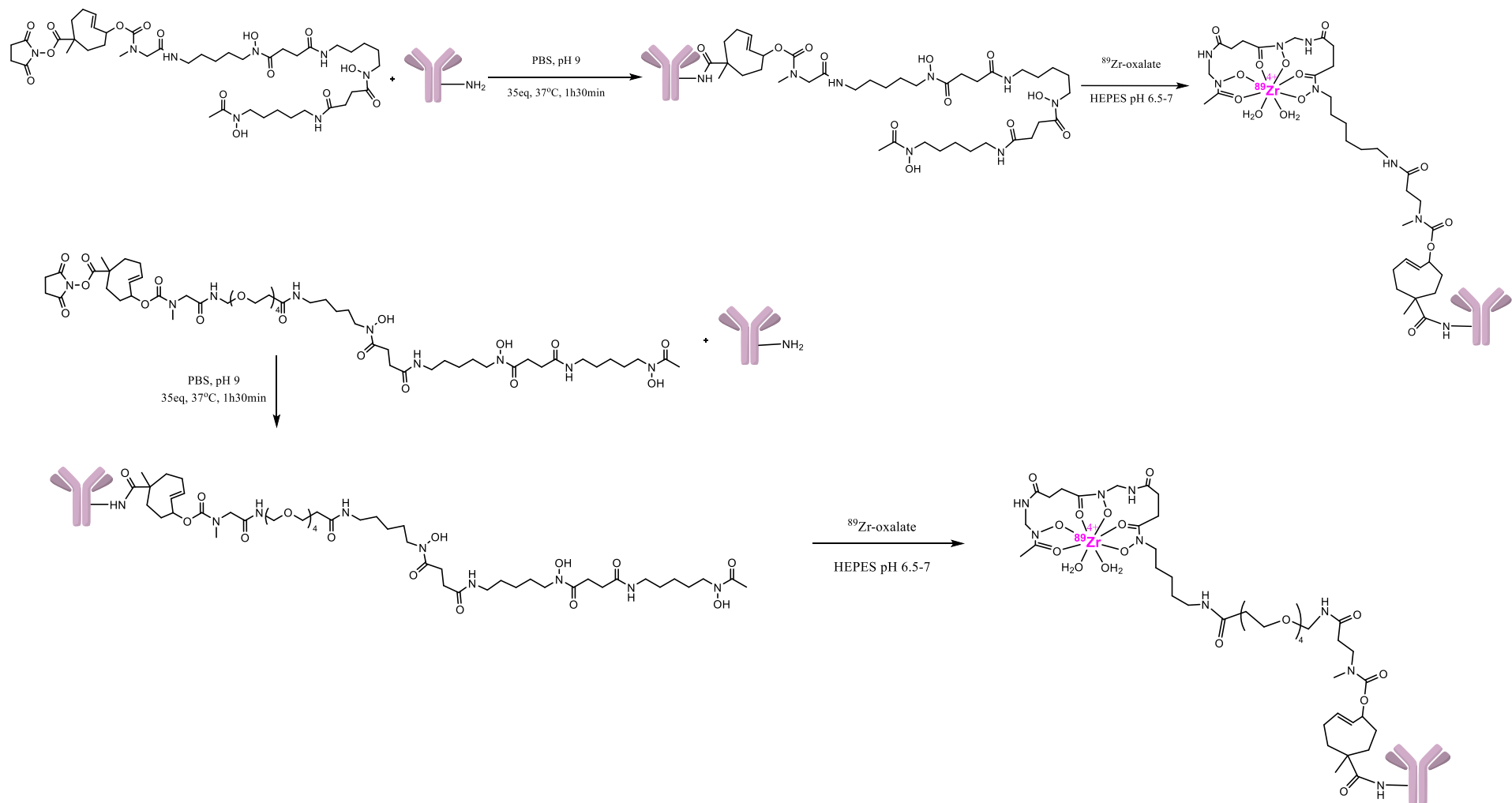




Labeling: yields > 85%

Purification: >99%





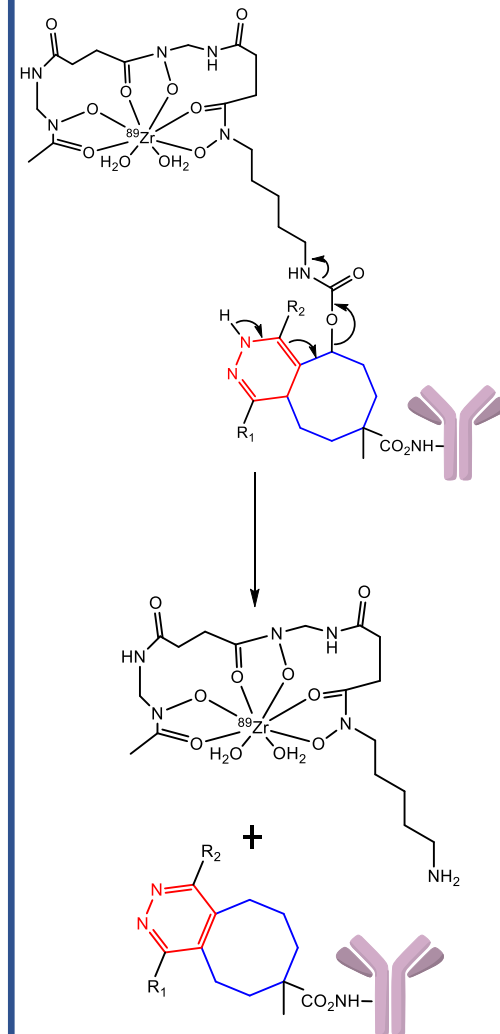
- Release TCO-spacer-chelator

Analysis – Follow the release

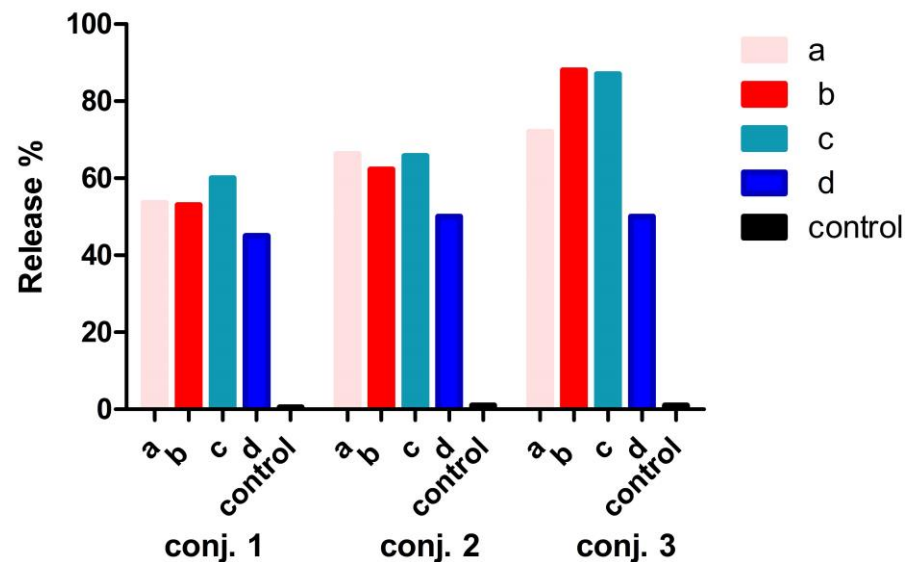
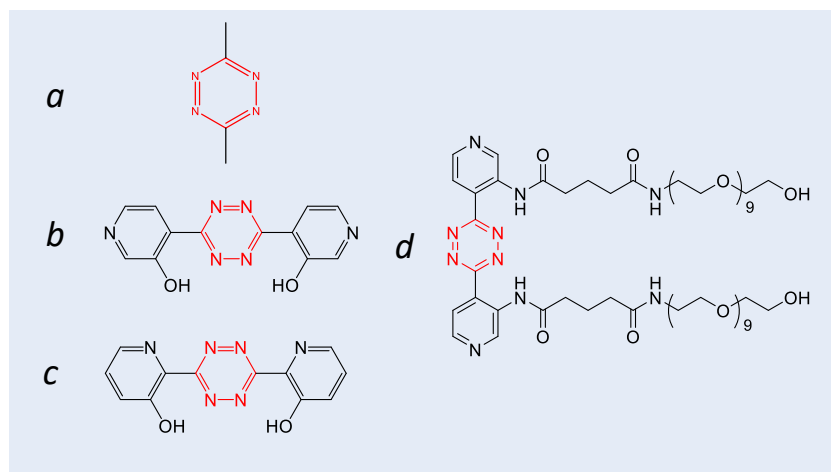
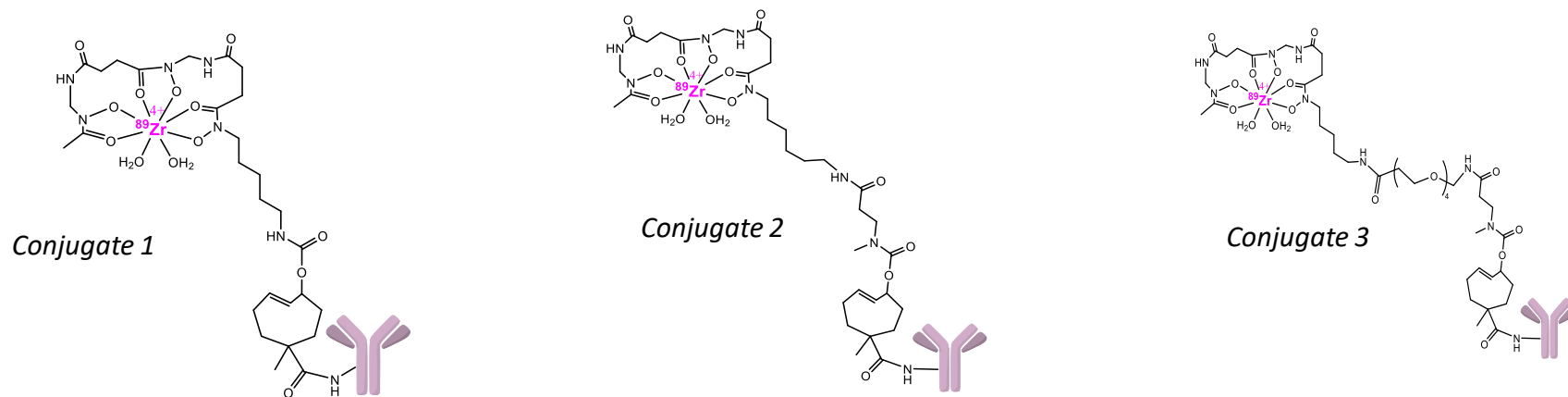
Conditions	Comment
RP-TLC C8	Possible interactions with silica, multiple species (especially with plasma)
SDS-PAGE	Diffusing of releasing species, ^{89}Zr -DFO multiple complexes
Percipitation of mab	20-25% percipitation of ^{89}Zr -DFO
SEC-FPLC with eluent PBS	mab-conjugates retained to the column
*SEC-FPLC with eluent 20mM phosphate	mab-conjugates retained to the column
*SEC-FPLC with eluent 20mM phosphate + 0.1% SDS	mab-conjugates slightly retained to the column
*SEC-FPLC with eluent 0.3% SDS in H_2O	Released species retained to the column
*SEC-FPLC with eluent 0.5% SDS in H_2O	mab-conjugates and released species elute from the column

*column type: BIOSEP SEC s3000

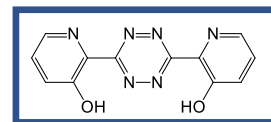
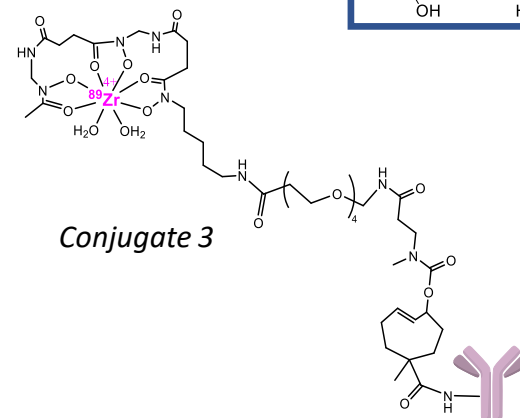
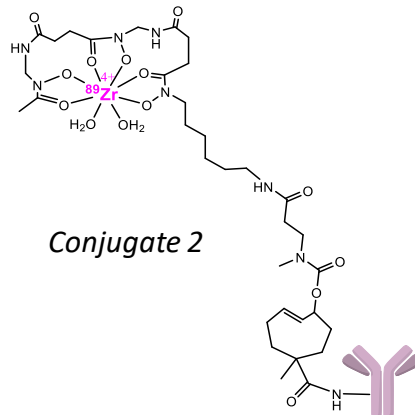
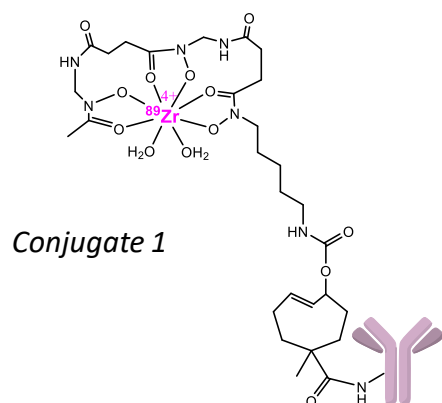
Released products



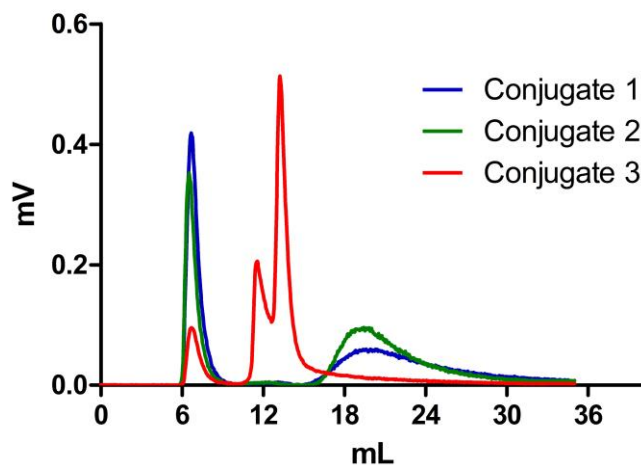
- Release TCO-spacer-chelator



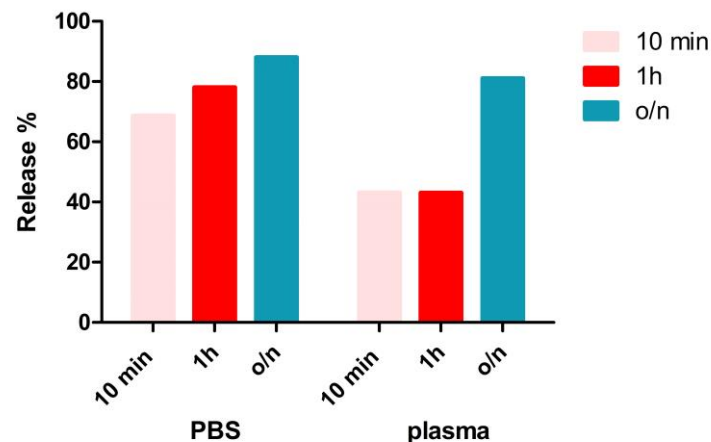
- Combined release in PBS for Tz c



Tetrazine c overnight in PBS



✓ Fractions were collected and checked



Release occurs at 10 min: PBS 69%

Plasma 43%

- Discussion-Further steps
 - Synthesize a conjugate with a spacer between the mab and the TCO
 - *The hydrophobic TCO may be burried within the mab*
- Test in vivo

- Zr-89 labelling
- Antibody conjugations
- Tz/TCO click-chemistry

- Presentation Research Meeting Nuclear Medicine Department, Title: “In vivo click and click-to-release strategies for in vivo catalyst activation”
- Hands-on Session at the Radboud Summer School: Labeling PSMA with In-111, Purification methods, cell internalization assay”
- Teaching sessions in Nuclear Medicine Department, Title: “Radionuclide: ^{89}Zr ”

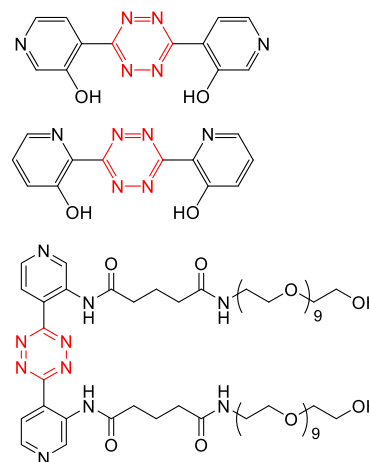
THANK YOU..

- Release TCO-spacer-chelator

Conjugate	Tz	Release o/n % PBS
1	a	53,6
	b	53%
	c	60%
	d	45%
	control	0.5

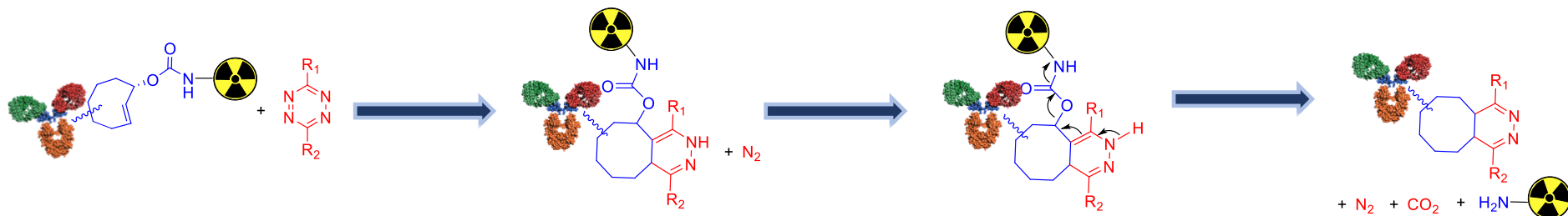
Conjugate	Tz	Release on % PBS
2	a	66,3
	b	62,3
	c	65,8
	d	50
	control	1

Conjugate	Tz	Release on % PBS
3	a	72
	b	88
	c	87
	d	50
	control	1

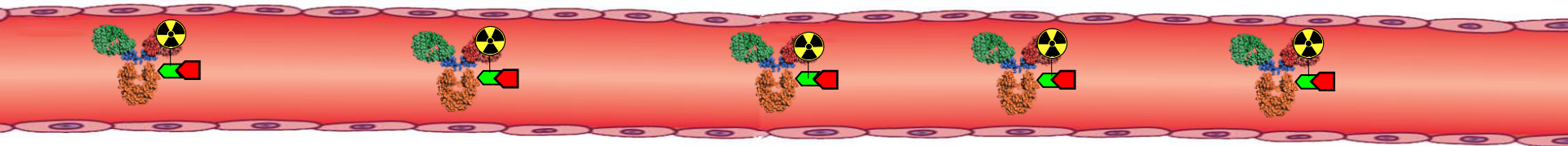


a: bis-Me Tz
b: FJM576
c: FJM565
d: FJM595
control: PBS

Chelate cleavage of radiolabeled catalyst to improve the tumor-blood ratios in imaging

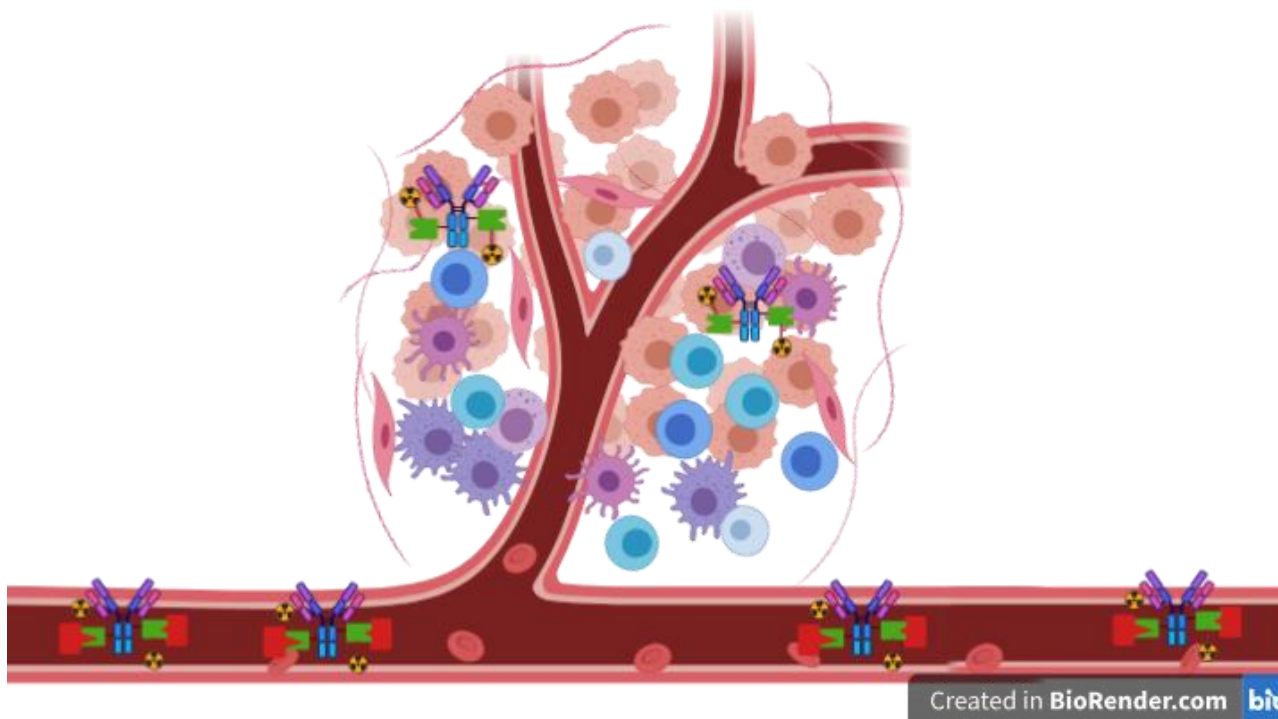
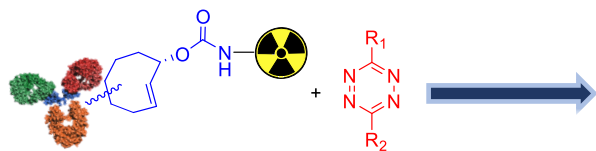


Blood circulation

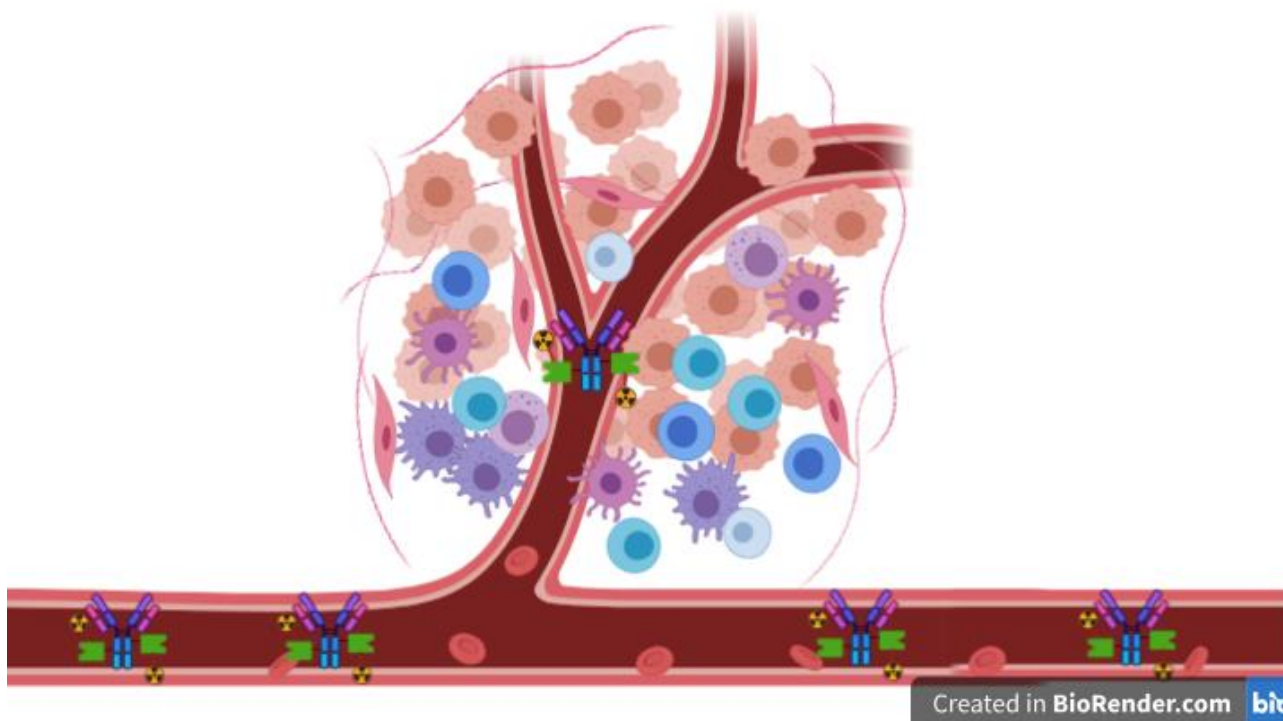
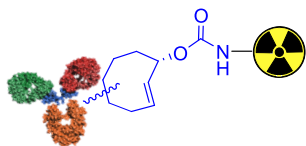


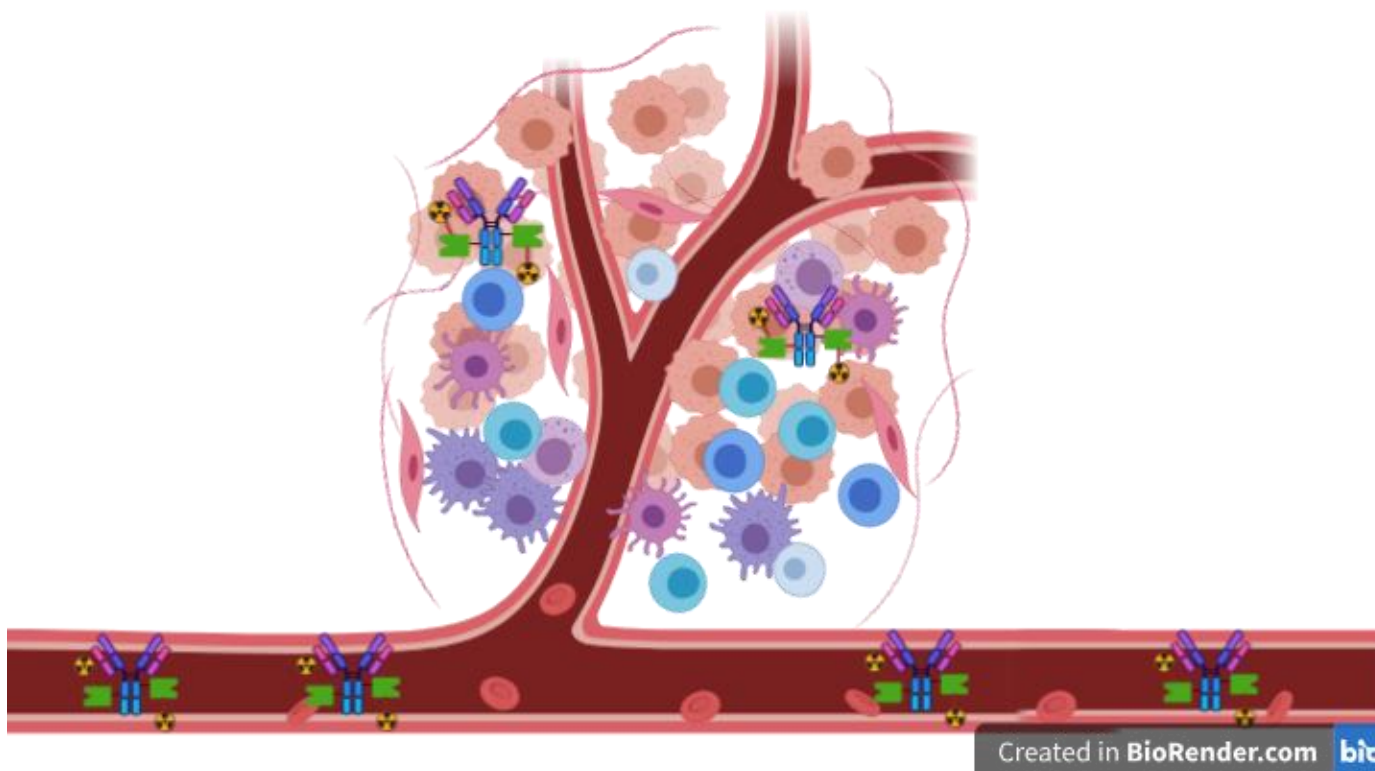
Step 1 Administration of radiolabeled antibody

Step 2 Administration of clearing agent (Tz)



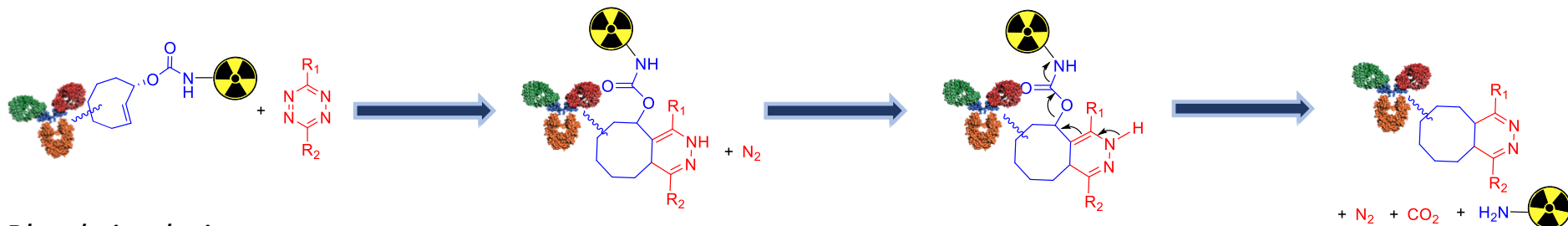
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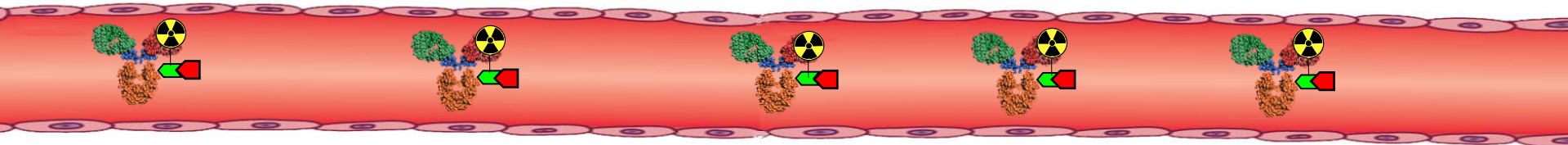


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- Future steps
- Install NCS group to obtain mab-spacer-TCO-chelator



Blood circulation

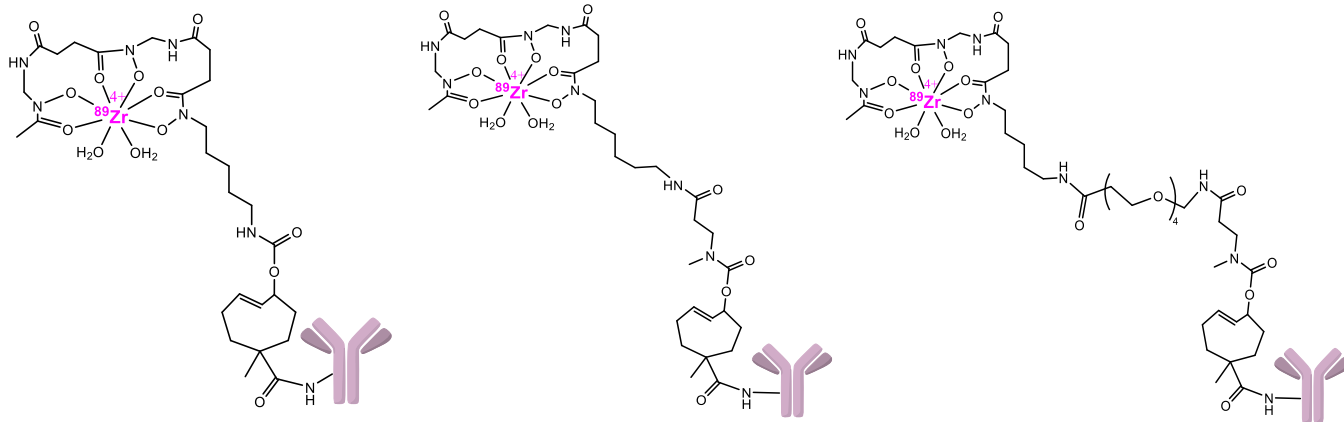


Step 1 Administration of radiolabeled antibody

Step 2 Administration of clearing agent (Tz)

Step 3 *Clearing of probe*

- ✓ Low tumor-blood ratios
- ✓ Improvement of imaging
- ✓ Dosimetry-Radiotherapy



- Synthesis of TCO-chelator
- Conjugation of TCO-chelator to mab
- DAR, Mab concentration
- Labelling, purification
- Reaction with Tz (Release in PBS/plasma)