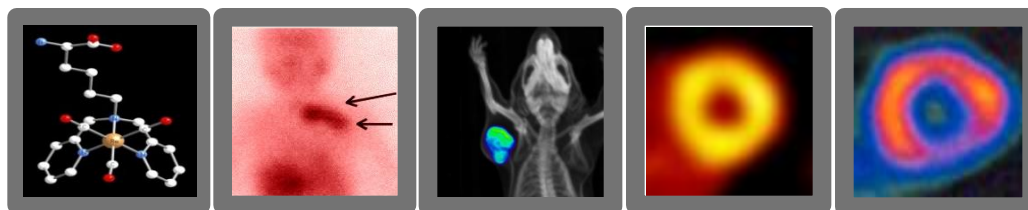


Influence of Functionalized Chelators on Affinity and Pharmacokinetics of $^{99m}\text{Tc}(\text{CO})_3$ Labeled Small Molecules Targeting Prostate Specific Membrane Antigen (PSMA)



Kevin P. Maresca, Shawn M. Hillier, Genliang Lu, John C. Marquis, Craig N. Zimmerman, William C. Eckelman, John L. Joyal, John W. Babich

Abstract # 250

SNM June 2010

molecularinsight
pharmaceuticals

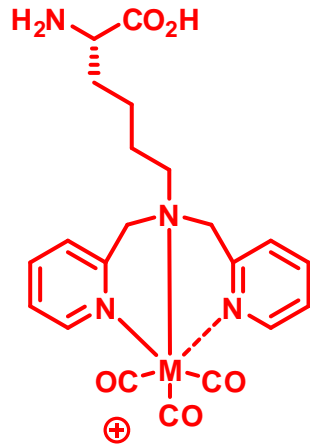
PIONEERS IN MEDICINE. PARTNERS IN CARE.

Single Amino Acid Chelate (SAAC) Technology

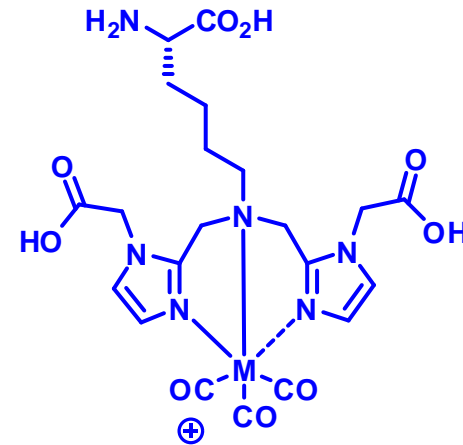
- Novel bifunctional chelators for the $\{M(I)(CO)_3\}^+$ core derivatized from the amino acid lysine
 - Maresca *et al.* *Bioconjugate Chemistry* **2009**, 20(8), 1625-1633. (SAAC)
- SAAC II derivatives utilize polar functionalized imidazoles to enhance the renal clearance and reduce liver/GI accumulation
 - Maresca *et al.* *Bioconjugate Chemistry* **2010**, xxx, 000-000. (SAAC II)
- Peptides and small molecules (SSTR2 peptides / CAIX / PSMA)
- Utilize the SAAC II fragments to explore the chelator effect on ^{99m}Tc -PSMA inhibitors

First versus Second Generation of SAAC complexes

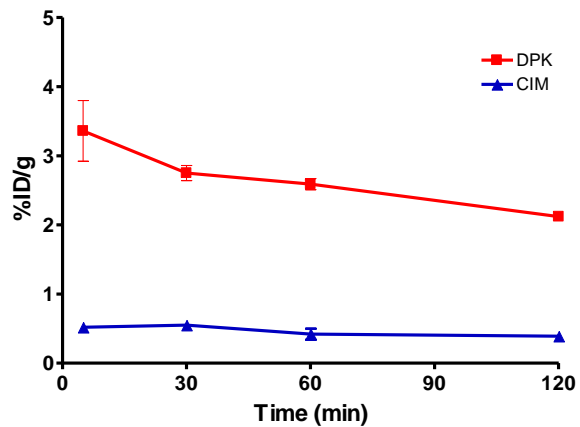
DPK (SAAC)



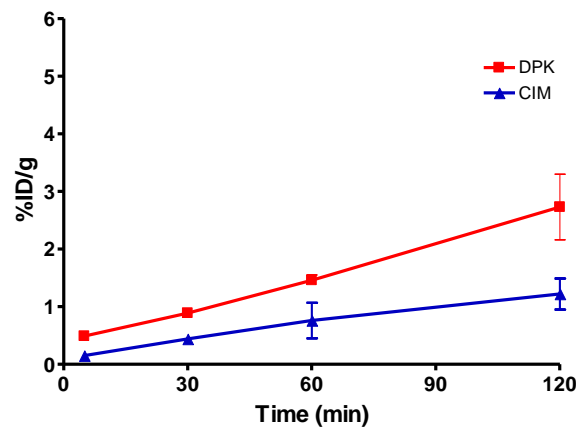
CIM (SAAC II)



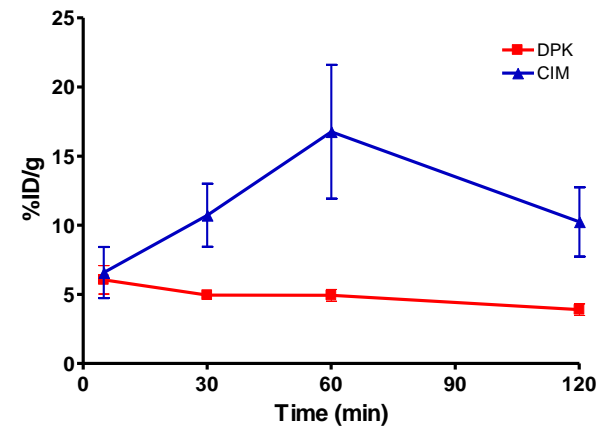
Liver



GI



Kidneys



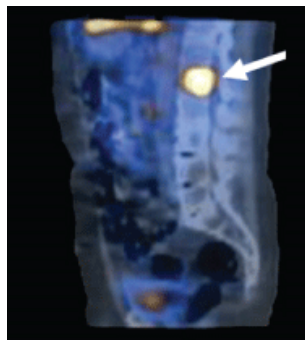
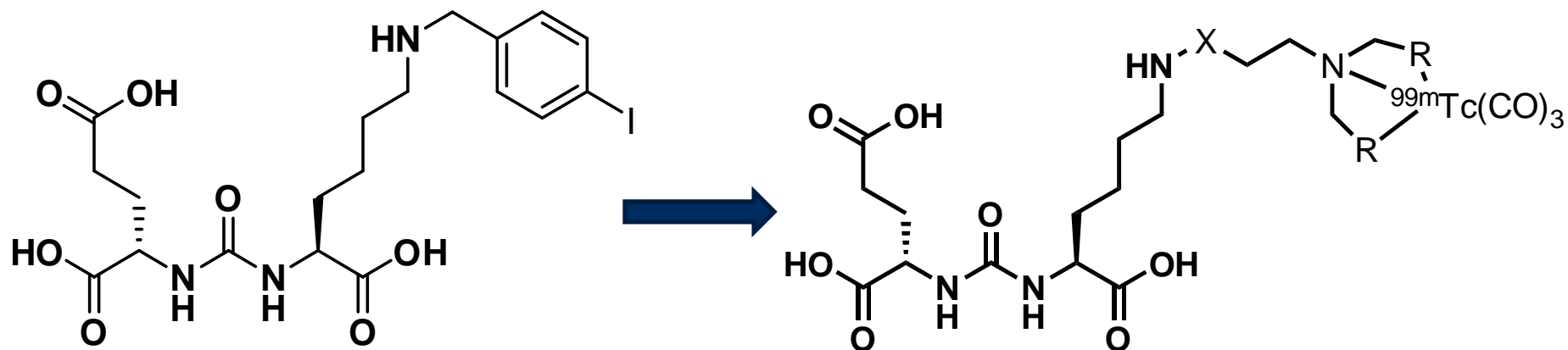
Prostate Cancer and PSMA

- Prostate cancer is the second leading cause of cancer related deaths amongst men in the USA
 - ~200,000 men are diagnosed with prostate cancer each year
- Challenging to detect recurrent disease despite MRI, CT, bone scan, and several PET agents
- PSMA is a transmembrane protein expressed in normal prostate, and expression is increased in prostate cancer

^{99m}Tc -PSMA Inhibitors

- Molecular Insight and others have pursued ^{99m}Tc -PSMA inhibitors
 - Molecular Insight NIH Funding Initiated (**2004** J. Babich 1R43EB004253)
 - Babich *et al.* 229th ACS National Meeting, San Diego, CA, United States, March 13-17, **2005**
 - Frangioni *et al.* *J of Nuc Med.* **2007**, 48(8), 1379-89.
 - Pomper *et al.* *J Med Chem.* **2008**, 51(15), 4504-17.
 - Low *et al.* *Mol Pharm.* **2009**, 6(3), 780-9.
- High affinity complexes reported
- Poor pharmacokinetics has hindered the development of a radiopharmaceutical

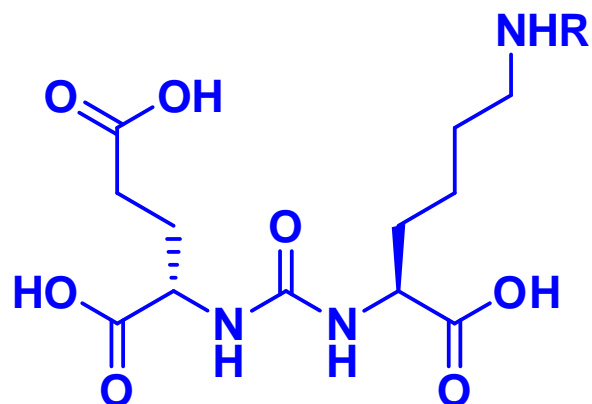
Design of ^{99m}Tc -PSMA Inhibitors



Explore the influence of the chelator on the pharmacokinetics

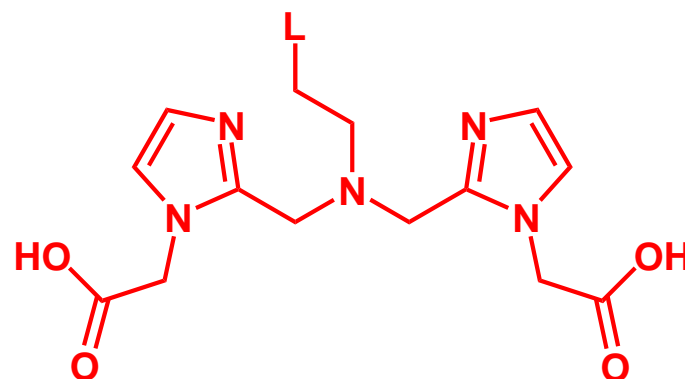
- ^{123}I -Troxef currently in clinical trials for metastatic prostate cancer
- Excellent image quality with detection of both bone and soft tissue lesions
- Potential imaging of primary prostate cancer
- ^{99m}Tc is ideal (less expensive with kit potential)

Combining of SAAC II Derived Chelators with PSMA Inhibitor Ligands



**Glu-Urea-Lys
Building Block**

+



**Di-N-(Carboxymethylimidazole)
CIM Fragment**

Evaluation of Chelators

■ Synthesize Ligands

- Simple alkylation followed by reductive amination and deprotection

■ Radiolabeling

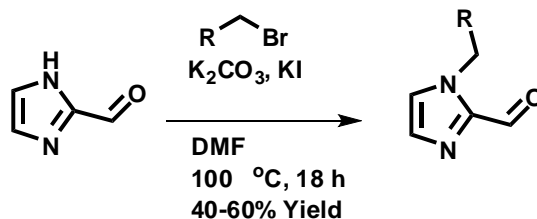
- $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ (Alberto *et al. J. Am. Chem. Soc.* **1998**, 120, 7987-7988.)
- 10^{-4} M ligand concentration, 100 °C, 60 minutes
- HPLC purified
- Evaluated stability

■ Tissue Distribution Studies

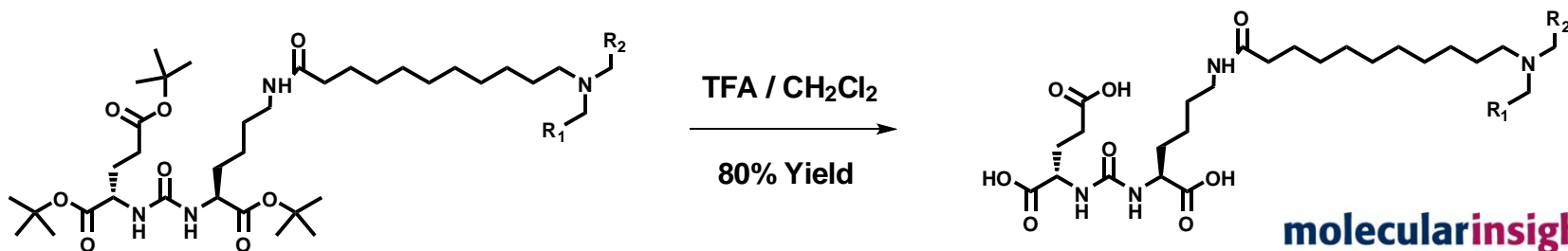
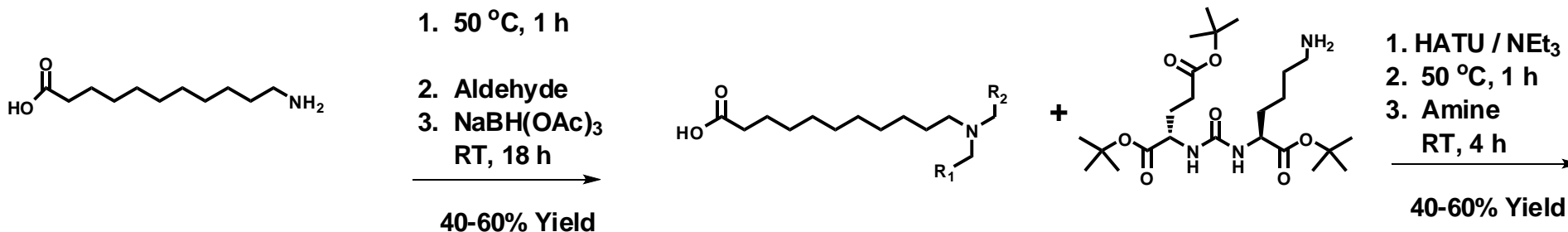
- LNCaP xenograft mice at 60 minutes post injection

Synthesis of Glu-Urea-Lys-C11 Compounds

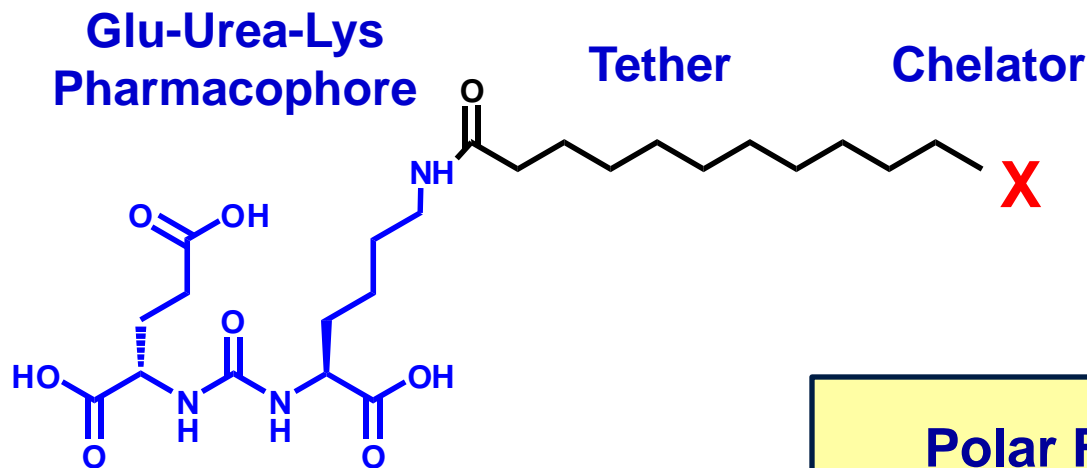
1. Imidazole Derivatization



2. Glu-Urea-Lys-C11 Compound Coupling/Deprotection

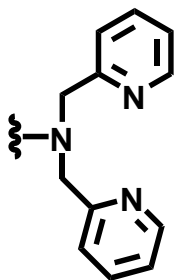


Structures of PSMA Inhibitors with Chelators Derived from a SAAC



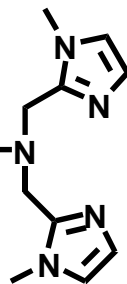
X =

1 =



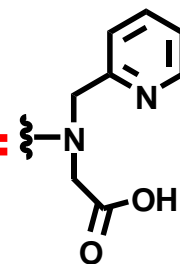
DPA

2 =



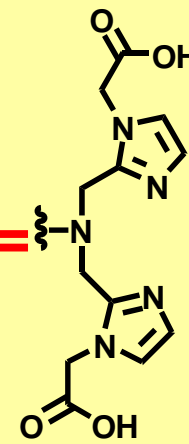
Di(MI)

3 =



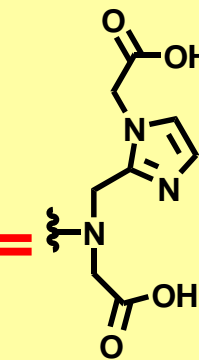
PAMA

4 =



Di(CIM)

5 =

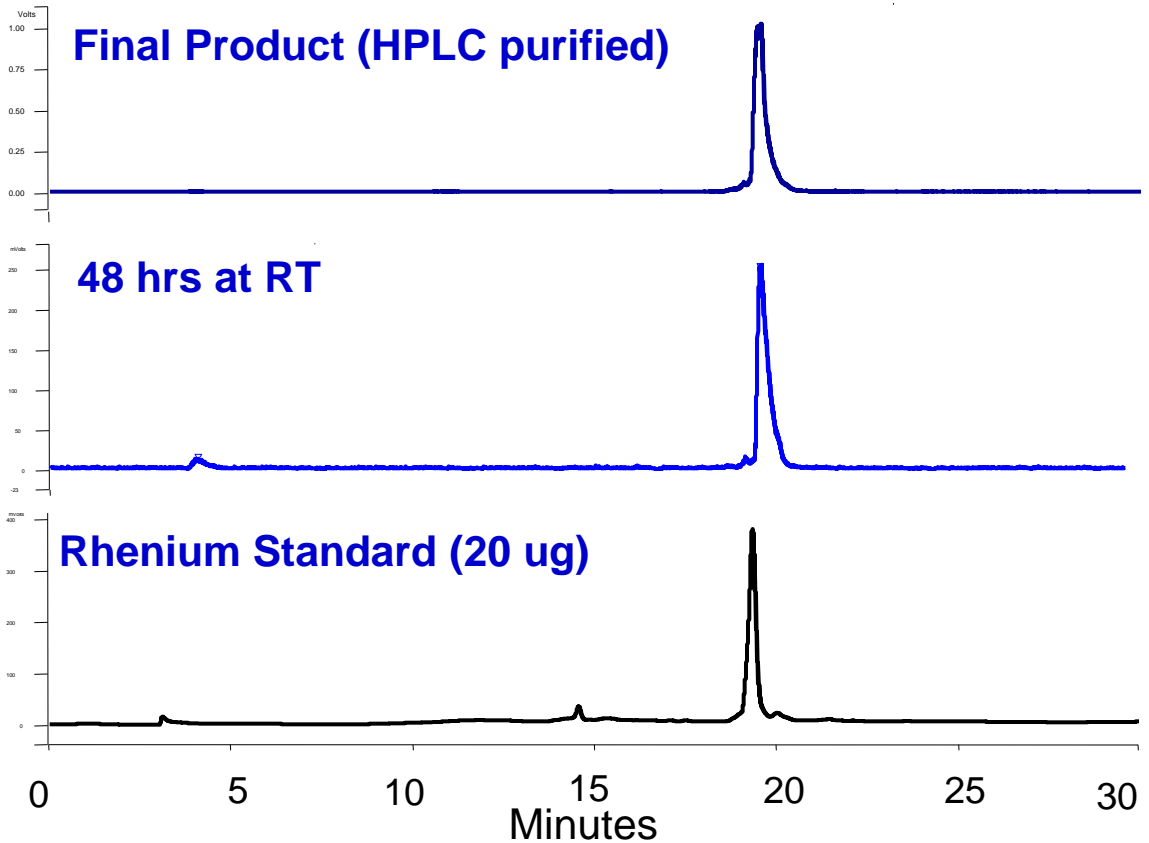


Mono-CIM-Mono-Acid

Polar Functionalized Imidazoles

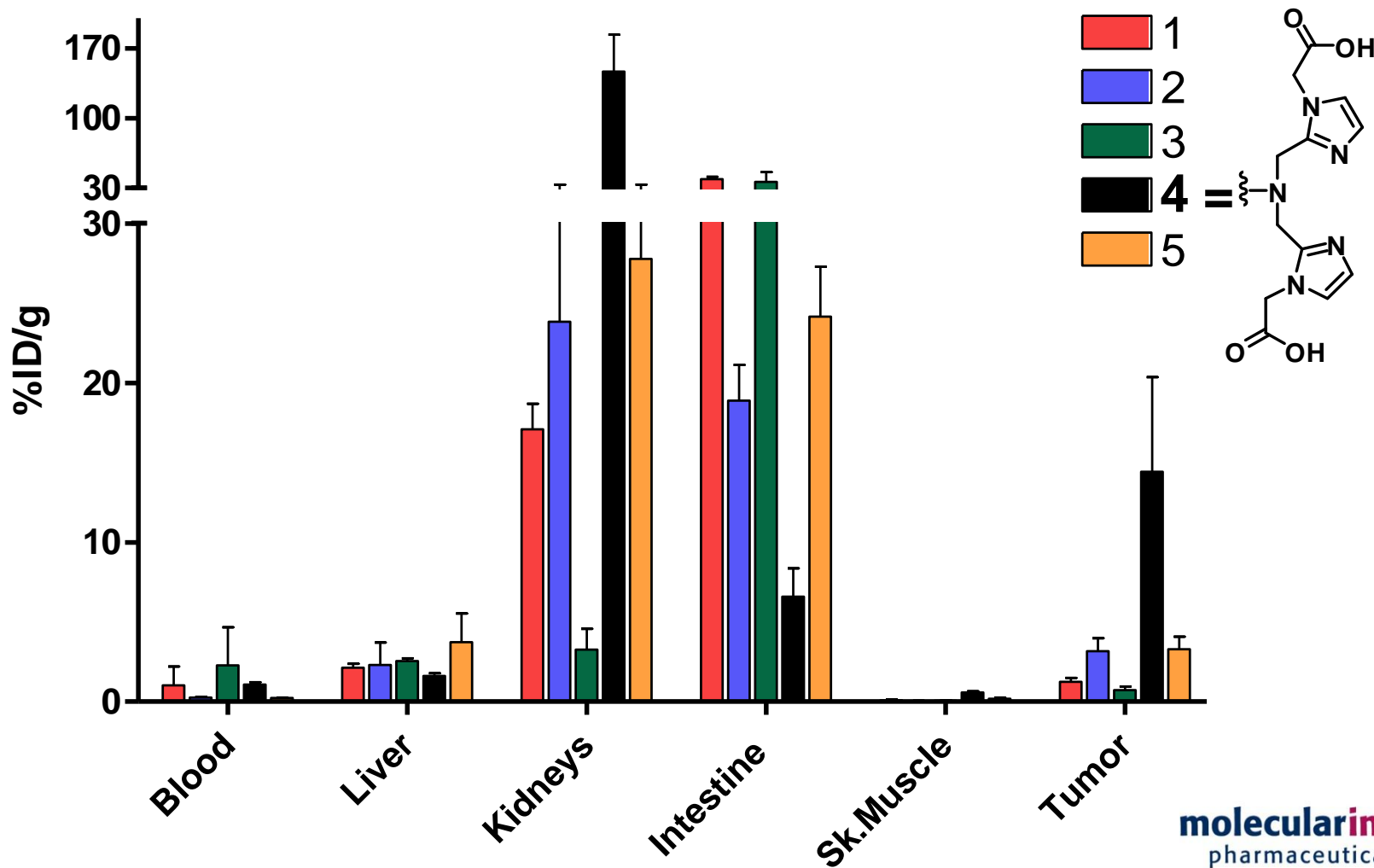
Radiolabeling of Di(*N*-Carboxymethylimidazole) Compound

- Isolink™ kits (Covidien)
- 10^{-4} M, 100 °C, 60 min
- RCY = 80-95%
- RCP = 98%
- Stable > 24 h (pH 7.4 at RT and 37 °C)



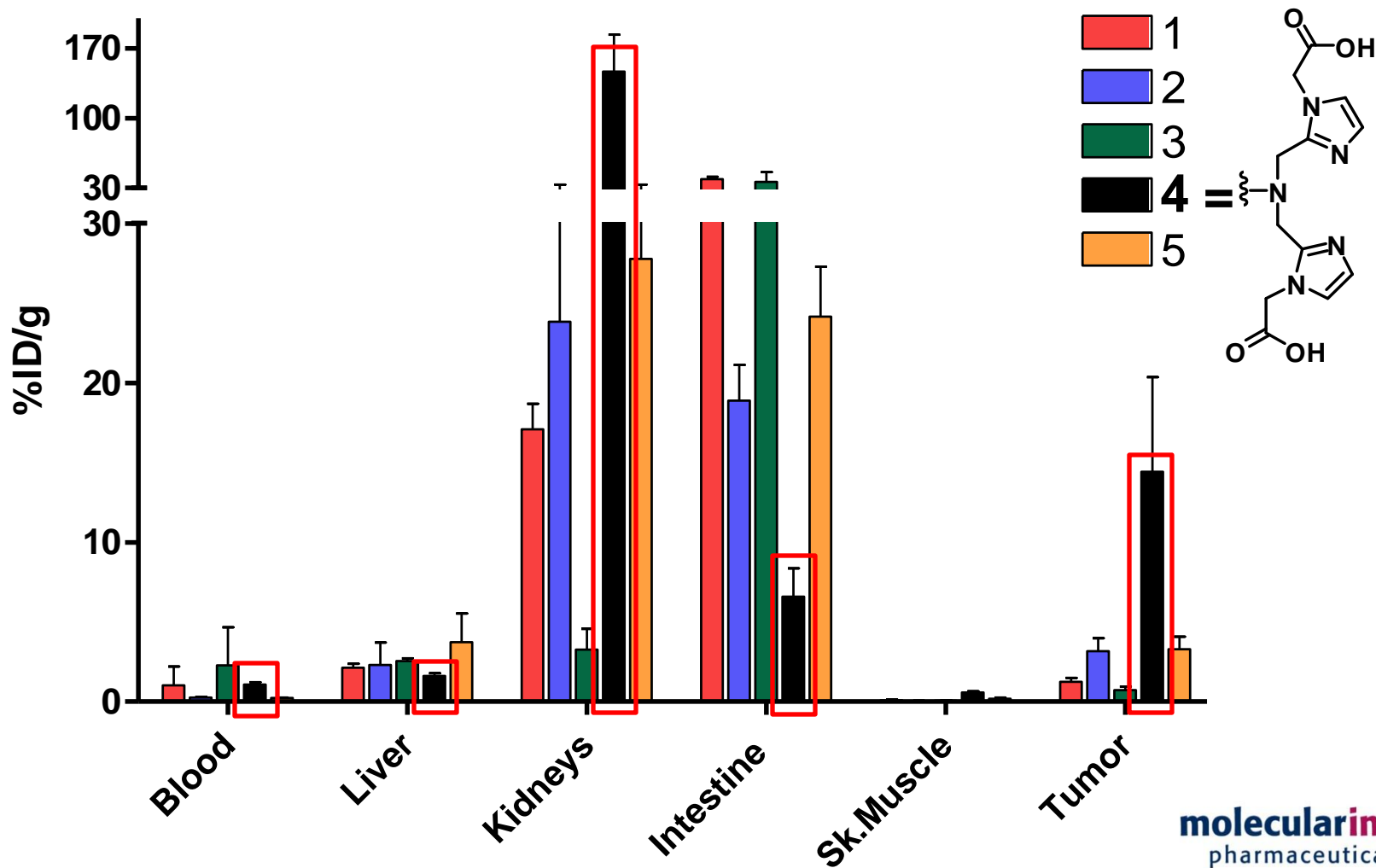
SAAC II Derived Ligands Enhance Renal Clearance and Reduce Liver/GI Accumulation

Tissue distribution of $^{99m}\text{Tc}(\text{CO})_3\text{-Glu-Urea-Lys-C11-}$ complexes in LNCaP xenograft mice at 1 hr



SAAC II Derived Ligands Enhance Renal Clearance and Reduce Liver/GI Accumulation

Tissue distribution of $^{99m}\text{Tc}(\text{CO})_3\text{-Glu-Urea-Lys-C11-}$ complexes in LNCaP xenograft mice at 1 hr



Manufacturing of $^{99m}\text{Tc}(\text{CO})_3\text{-Glu-Urea-Lys-C11-Complexes}$

Rxn

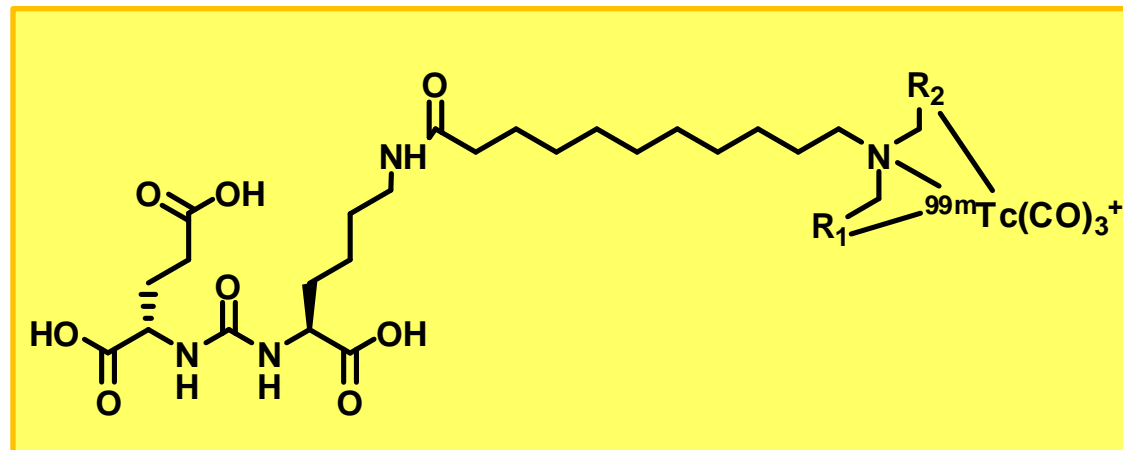
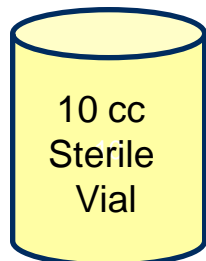
- Mix $^{99m}\text{TcO}_4^-$ / Ligand / Isolink Kit (Saline)
- Heat at 100 °C for 60'

Purify

- PH adjust to 7 with 0.2 mL 1 N HCl
- C18 SP Light (Optional); Elute with Ethanol

Dilute

- Dilute with saline (10% Ethanol / Saline)
- GV filter (0.2 um 33mm syringe filter)



Final Stable Sterile Complex in 1 Hour

Conclusions

- Synthesized a series of Glu-Urea-Lys-C11-complexes with different tridentate chelators of varying polarity and lipophilicity
- Evaluated and optimized radiolabeling
 - Radiolabeled as *tert*-butyl-protected or deprotected compounds
- Employing SAAC II fragments, demonstrated the ability to achieve more favorable pharmacokinetics with the additional benefit of increased affinity
 - Substantially shifted the clearance profiles from hepatobiliary to renal
 - Achieved high tumor uptake (~15% ID/g)

Acknowledgements

- MIP colleagues and collaborators
 - S. Hillier *et al.* presentation # 481 (SAAC-II-Tc-PSMA)
 - J. Marquis *et al.* presentation # 482 (SAAC-II-SSTR2 peptides)
- Covidien for Isolink™ supply
- Funding in part
 - NIH (1R43EB004253 and 1R41A1054080-01)
 - DOE (D2-FG02-99ER62791)
- Thank you for your attention