



Article

Preparation of a Zirconium-89 Labeled Clickable DOTA Complex and Its Antibody Conjugate

Falguni Basuli ^{1,*}, Olga Vasalatiy ¹, Jianfeng Shi ¹, Kelly C. Lane ¹, Freddy E. Escorcía ^{2,3} and Rolf E. Swenson ¹

¹ Chemistry and Synthesis Center, National Heart, Lung and Blood Institute, Bethesda, MD 20892, USA; vasalatiyo@nhlbi.nih.gov (O.V.); jianfeng.shi@nih.gov (J.S.); kclane013@gmail.com (K.C.L.); rolf.swenson@nih.gov (R.E.S.)

² Molecular Imaging Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA; freddy.escorcía@nih.gov

³ Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA

* Correspondence: bhattacharyaf@mail.nih.gov; Tel.: +1-301-827-1107

Abstract: Desferrioxamine B (DFO) is the clinical standard chelator for preparing zirconium-89 labeled antibodies. In the current study, the stabilities of a zirconium-89 labeled panitumumab (PAN; Vectibix[®]) with three different chelators (DFO, DFO*, and DOTA) were compared. PAN is an anti-HER1/EGFR monoclonal antibody approved by the FDA for the treatment of HER1-expressing colorectal cancers and was used as the model antibody for this study. DFO/DFO* conjugates of PAN were directly radiolabeled with zirconium-89 at room temperature to produce [⁸⁹Zr]Zr-DFO/DFO*-PAN conjugates following a well-established procedure. A zirconium-89 labeled DOTA-PAN conjugate was prepared by an indirect radiolabeling method. A cyclooctyne-linked DOTA chelator (BCN-DOTA-GA) was first radiolabeled with zirconium-89 at 90 °C under a two-step basic pH adjustment method followed by conjugation with PAN-tetrazine at 37 °C to produce a labeled conjugate, BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN. High reproducibility of the radiolabeling was observed via this two-step basic pH adjustment. The overall radiochemical yield was 40–50% (n = 12, decay uncorrected) with a radiochemical purity of >95% in 2 h synthesis time. All three conjugates were stable in whole human serum for up to 7 days at 37 °C. The kinetic inertness of the conjugates was assessed against the EDTA challenge. BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN exhibited excellent inertness followed by [⁸⁹Zr]Zr-DFO*-PAN. [⁸⁹Zr]Zr-DFO-PAN displayed the lowest level of inertness.

Keywords: DOTA; BCN-DOTA-GA; PAN; DFO; DFO*; stability



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1. Introduction

Positron emission tomography (PET) is favorable among the clinically established non-invasive imaging techniques due to its unique properties such as excellent sensitivity, spatial resolution, and accurate quantification of small tumor lesions [1–3]. The functional insights acquired via PET imaging enable an earlier diagnosis and clear characterization of the disease state, which is crucial for providing a reliable prognosis and guiding effective therapeutic strategies [1]. Many radiolabeled peptides and protein-based drugs have been developed for diagnosis and therapy [1,4–8]. Gallium-68 and fluorine-18 are commonly used PET radionuclides for the production of low-molecular-weight tracers; however, large-molecular-weight proteins and antibodies require longer-lived radionuclides due to their slower in vivo pharmacokinetics and long blood pool residence time. Among the longer-lived PET isotopes, zirconium-89 ($t_{1/2} = 78.4$ h; $E_{\beta^+}(\text{mean}) = 396$ keV; $E_{\text{max}} = 897$ keV) has been widely used to radiolabel antibodies due to its favorable decay characteristics and the excellent match of the zirconium-89 half-life with the time an antibody needs to reach the target [9–13]. In addition, the half-life of zirconium-89 is suitable to produce a companion diagnostic imaging agent for actinium-225 ($t_{1/2} = 9.9$ days)- and thorium-227

($t_{1/2}$ = 18.7 days) labeled therapeutic radiopharmaceuticals [14,15]. Moreover, zirconium-89 can be produced in large quantities via the proton irradiation of the monoisotopic yttrium (^{89}Y) target on a low-energy medical cyclotron and can be efficiently separated from the target [16].

Typically, when incorporating a radiometal for imaging or targeted radionuclide therapy, it is crucial to utilize an appropriate chelator with accessible Lewis basic donor groups for the effective binding and stabilization of the radiometal ion. Additionally, the chelator should contain a reactive functional group for bioconjugation under mild conditions. A hydroxamate-based natural siderophore chelator, desferrioxamine B (DFO, Figure 1), is the gold standard in preclinical and clinical studies for incorporating zirconium-89 into the biomolecules of interest [10,17–21]. However, several investigations have pointed out that the limited kinetic inertness of $[\text{}^{89}\text{Zr}]\text{Zr-DFO}$ complexes leads to the liberation of free zirconium ($[\text{}^{89}\text{Zr}]\text{Zr}^{4+}$) [22–26]. Free $[\text{}^{89}\text{Zr}]\text{Zr}^{4+}$ accumulates into the bones and joints, which compromises the quality of images, and it increases the radiation dose to hematopoietic bone marrow [24,27–29]. The observed instability of the $[\text{}^{89}\text{Zr}]\text{Zr-DFO}$ complex is partially a consequence of the mismatch between the zirconium ion's coordination number of eight and DFO's hexadentate chelation, which leaves two coordination sites open to interact with other substrates [30–32]. Similarly, DFO may not be a suitable chelator to develop a theranostic (therapeutic and diagnostic) agent for therapeutic radiometals (e.g., actinium-225 or thorium-227), which typically form stable eight-coordinate species as well. This emphasizes the need for superior chelators with enhanced stability, which could potentially reduce the uptake of radionuclides in the bone and streamline the development of theranostic agents.

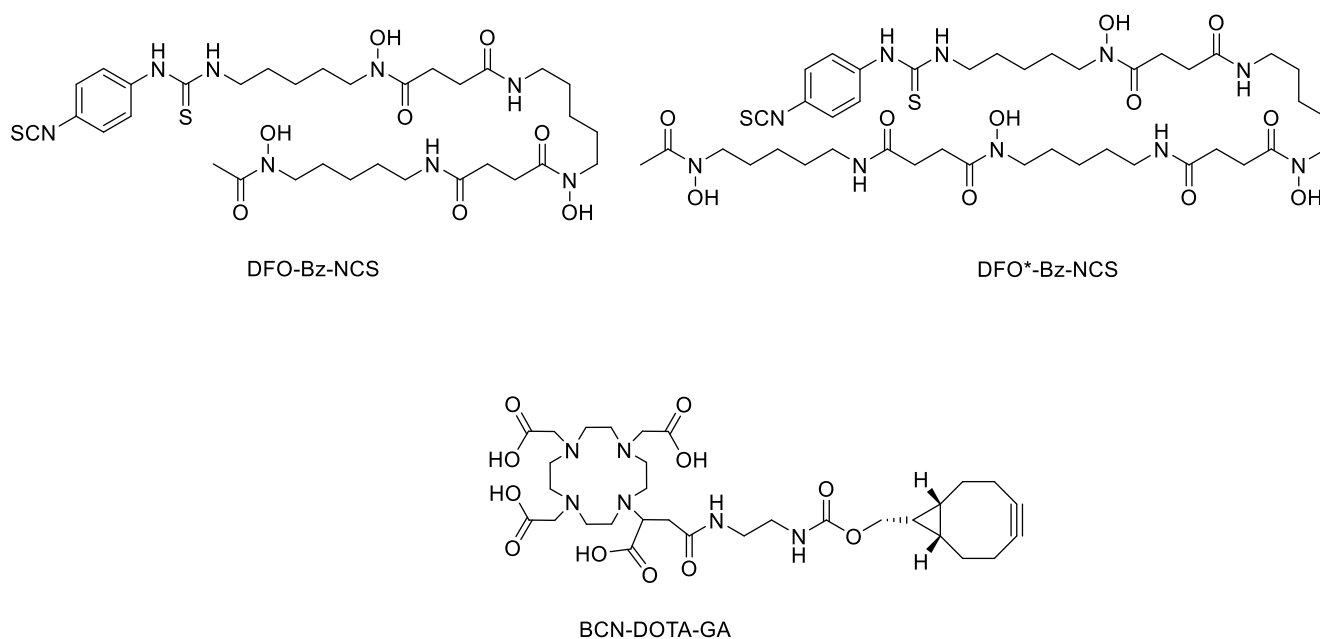


Figure 1. Structures of the target chelators DFO-Bz-NCS, DFO*-Bz-NCS, and BCN-DOTA-GA.

Several new chelators (≥ 8 coordination sites) that can saturate the zirconium ion coordination sphere, such as DFO* (Figure 1), oxoDFO*, HOPO, CTH-36, DFO-Em, DFO-Km, DFO2, DFO2p, etc., have been developed and investigated [24,26,33–41]. Many of these complexes have demonstrated superior *in vivo* stability compared to DFO. Patra et al. developed the first octadentate bifunctional chelating agent, DFO*, for zirconium-89 labeling [24]. Salih et al. designed and synthesized a glutamic acid-based octadentate chelator named DFO-Em [38]. However, the attempted synthesis of the bifunctional version of the DFO-Em via N-hydroxysuccinimide (NHS) ester activation of the glutamic acid side chain for conjugation to biomolecules was unsuccessful. Therefore, they replaced the glutamic acid linker in DFO-Em with lysine to prepare another chelator, DFO-Km [37]. One of the

key highlights of DFO-Em and DFO-Km ligands is in their modular amino acid linker system that can be swapped with other amino acid linkers or chelating moieties, thereby enabling the synthesis of wide varieties of derivatives. Recently, Damerow et al. compared the relative stability of [^{89}Zr]Zr-DFO-, [^{89}Zr]Zr-CTH-36-, [^{89}Zr]Zr-3,4,3-(LI-1,2-HOPO), and [^{89}Zr]Zr-DFO*-c(RGDfK) complexes against challenge with ethylenediaminetetraacetic acid (EDTA) [42]. A rapid trans chelation was observed for the [^{89}Zr]Zr-DFO complex, which indicates a low kinetic inertness of this conjugate. The [^{89}Zr]Zr-CTH-36-c(RGDfK) complex demonstrated unexpected susceptibility to the challenge. However, [^{89}Zr]Zr-3,4,3-(LI-1,2-HOPO) and [^{89}Zr]Zr-DFO*-c(RGDfK) were highly stable.

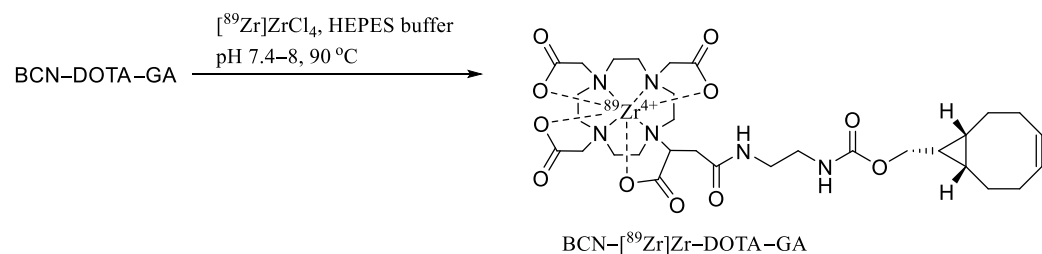
A tetraazamacrocyclic ligand, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), is a well-known chelator in radiopharmaceutical applications as it can readily form stable complexes with numerous radiometals, such as [^{68}Ga]Ga, [^{64}Cu]Cu, [^{90}Y]Y, [^{117}Lu]Lu, [^{225}Ac]Ac, and [^{227}Th]Th [14,15,43–46]. Moreover, the same DOTA-functionalized molecule can be radiolabeled with either diagnostic or therapeutic radiometals to form theranostic radiopharmaceuticals, which will allow for the accurate determination of the radiation dose before treatment as well as excellent monitoring of treatment effectiveness [47,48]. In 2017, Pandya et al. reported the structural characterization of Zr-DOTA using single-crystal X-ray diffraction and the use of DOTA as [^{89}Zr]Zr-chelators [49]. The crystal structure reveals a saturated coordination sphere around the Zr^{4+} ion. Additionally, [^{89}Zr]Zr-DOTA demonstrated remarkable *in vivo* stability. Prive et al. further optimized the reaction conditions to successfully prepare zirconium-89 labeled DOTA-PSMA-617 and DOTAGA-PSMA-I&T and they demonstrated its first clinical application in a human patient [50]. However, Damerow et al. observed no labeling of the DOTA-GA-modified peptide even after applying harsh conditions (99 °C for several hours). Herein, the synthesis of BCN-[^{89}Zr]Zr-DOTA-GA under two-step pH adjustments at basic conditions is presented. This zirconium-89 labeled BCN prosthetic group was used for labeling PAN via a well-known click approach to prepare BCN-[^{89}Zr]Zr-DOTA-GA-PAN and the stability of this complex was compared with [^{89}Zr]DFO/DFO*-PAN conjugates. The BCN-[^{89}Zr]Zr-DOTA-GA-PAN conjugates showed excellent stability in whole human serum and an EDTA challenge study for up to 7 days.

2. Results and Discussion

Although DOTA is commonly used as a chelator to prepare various radiopharmaceuticals with radiometals, its application with zirconium-89 remains relatively unexplored. In 2017, Pandya et al. reported the successful synthesis of zirconium-89 labeled DOTA using [^{89}Zr]ZrCl₄ [49]. Labeling of DOTA with [^{89}Zr]Zr-oxalate (commonly used for zirconium-89 labeling) resulted in poor radiochemical conversion. A quantitative formation of a radiolabeled DOTA complex, [^{89}Zr]Zr-DOTA, was observed by incubating 10 µg of DOTA with an aliquot of [^{89}Zr]ZrCl₄ (1.1 mCi, 40.7 MBq) at 90 °C in HEPES buffer (pH 7.2) for 1 h. To test the feasibility of the preparation of a radiolabeled p-SCN-Bz-DOTA bifunctional chelator, the non-radioactive chelation chemistry was explored first. A non-radioactive zirconium complex of p-SCN-Bz-DOTA was successfully prepared by reacting ZrCl₄ with p-SCN-Bz-DOTA at 80 °C for 2 h in anhydrous dimethyl sulfoxide (DMSO) in the presence of triethylamine [51]. However, under the same condition, the zirconium-89 radiolabeling reaction proceeded without the formation of any desired labeled product. The aqueous basic radiolabeling condition resulted in the decomposition of the chelator.

Therefore, the labeling of another ligand, DOTA-GA, functionalized with BCN (Figure 1, Scheme 1), a cyclooctyne for copper-free click chemistry, as it is stable in aqueous basic conditions, was explored. The radiochemical conversion was first tested with [^{89}Zr]Zr-oxalate and the result monitored by analytical high-performance liquid chromatography (HPLC). HPLC analysis revealed the formation of no new peaks, and the same results have also been reported by other researchers [42]. The radiolabeling of BCN-DOTA-GA was then tested under various conditions (Table 1) using [^{89}Zr]ZrCl₄. The radiochemical conversion (RCC) was monitored by HPLC and radio thin-layer chromatography (TLC). Less than 20%

conversion was observed with 10 µg of chelator and 1 mCi of [⁸⁹Zr]ZrCl₄ at 80 °C for 1 h. No improvement in the RCC was observed by increasing the temperature to 90 °C with the same amount of the chelator and zirconium-89.



Scheme 1. Preparation of BCN-[⁸⁹Zr]Zr-DOTA-GA.

Table 1. Radiolabeling conditions tested to prepare BCN-[⁸⁹Zr]Zr-DOTA-GA.

Entry ^a	Amount of Ligand (µg)	Amount of Zirconium-89 (mCi)	Temperature (°C)	Yield (%) by Radio TLC
1	10	1	80	<20
2	10	1	90	<20
3	30	1	80	95
4	30	1	90	98
5	30	2	90	92
6	30	3	90	83
7	30	4	90	74

^a HEPES buffer (0.5 M), pH 7.2–7.6.

However, an increasing amount of the chelator (30 µg) demonstrated a nearly quantitative RCC (Table 1, entry 4) at 90 °C. A higher amount of zirconium-89 (2–4 mCi) with the same amount of chelator (30 µg) at the same temperature (90 °C) resulted in a decreased RCC (entry 5–7). In order to balance the RCC and the molar activity, 30 µg ligand, 3 mCi zirconium-89, and 90 °C were chosen as the optimal conditions. After incubation for 1 h, an aliquot of the reaction mixture was mixed with EDTA and analyzed by radio TLC and analytical HPLC. A representative radio TLC (developer, 0.1 M EDTA) and HPLC (eluent, acetonitrile in water) are depicted in Figure 2A,B. The major radio TLC peak at 50 mm represents BCN-[⁸⁹Zr]Zr-DOTA-GA. The minor radio TLC peak at 100 mm matches with the radio TLC peak for [⁸⁹Zr]Zr-EDTA (Figure 2C) and the other minor peak at 10 mm could be the hydroxide species of zirconium-89. It is important to mention here that a gradual increase in the pH is necessary for a successful radiolabeling reaction. The initial pH of the reaction mixture was ~7.2 after mixing zirconium-89 and the BCN-DOTA-GA chelator. The reaction mixture was heated at 90 °C for 30 min and the pH was adjusted to ~7.6 followed by heating for an additional 30 min at the same temperature. If the pH of the reaction was adjusted to ~7.6 at the beginning of the reaction, then the formation of a trace amount of BCN-[⁸⁹Zr]Zr-DOTA-GA was observed by TLC analysis (Figure 2D). A major radio TLC peak at 10 mm was observed. This could be due to the hydrolysis of [⁸⁹Zr]ZrCl₄ to inert zirconium hydroxide as reported in the literature [42]. This peak matches with the radio TLC of the [⁸⁹Zr]ZrCl₄ after heating at 90 °C for 30 min (Figure 2E) at pH ~8. The successful radiolabeling with a two-step pH adjustment at the acidic conditions was reported by Privé et al. in 2021 [50]. The PSMA ligand was first incubated at pH 3–4 for 30 min at 95 °C followed by pH adjustment with 0.5 M MES buffer (pH 5.5), and subsequent incubation at pH 4–5 for an additional 30 min at 95 °C. Using the two-step strategy, they radiolabeled two therapeutic ligands (PSMA-617 and PSMA-I&T) with [⁸⁹Zr]ZrCl₄. The two-step method was utilized to increase the molar activity of the tracers for their imaging study. However, the two-step acidic radiolabeling condition did not work for the production of BCN-[⁸⁹Zr]Zr-DOTA-GA in this current study.

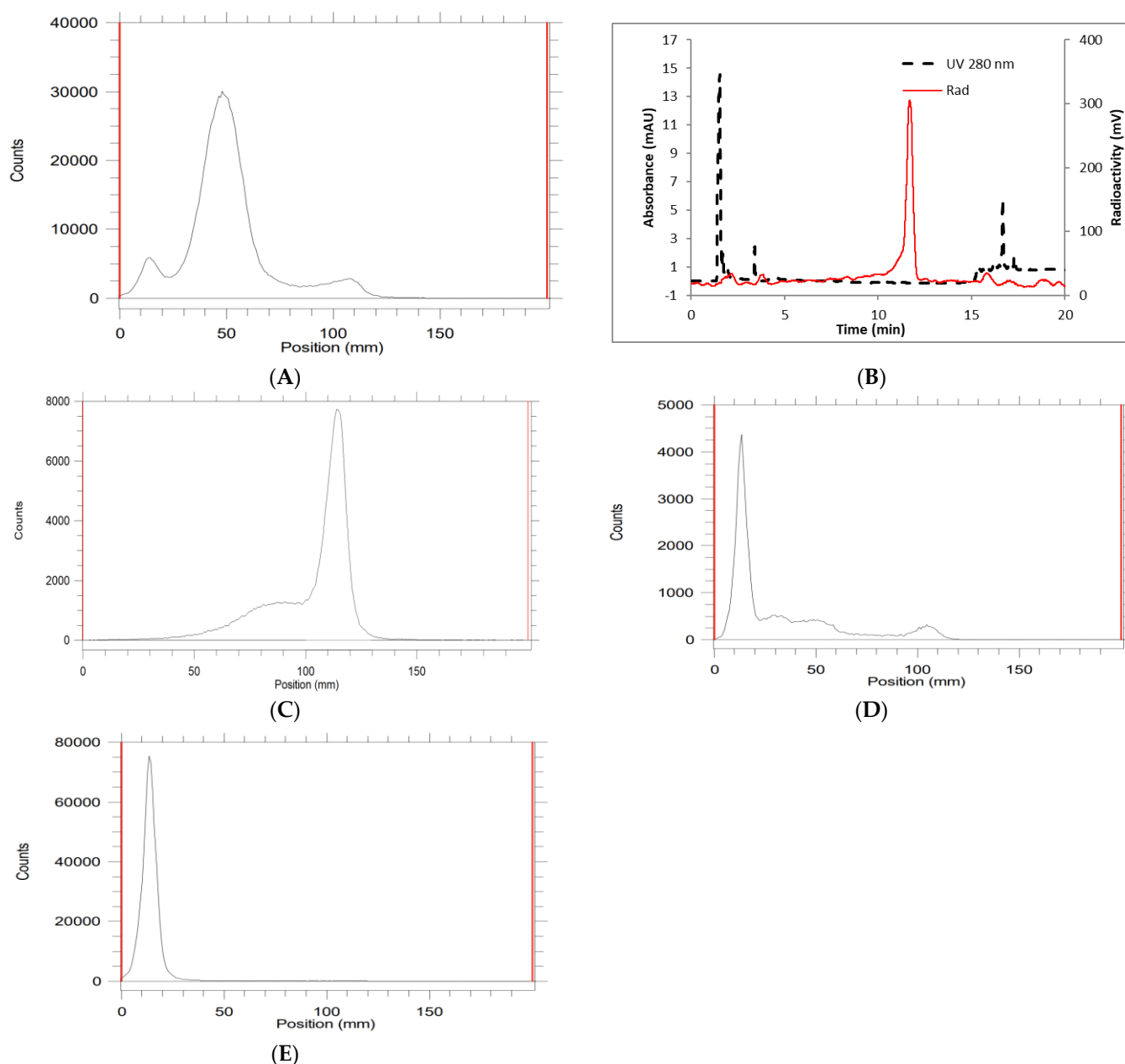
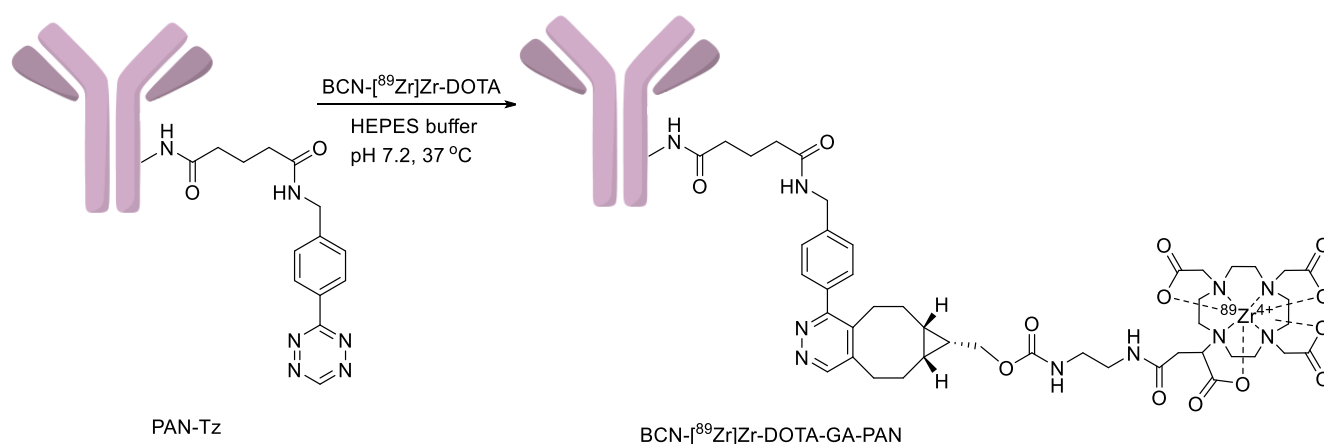


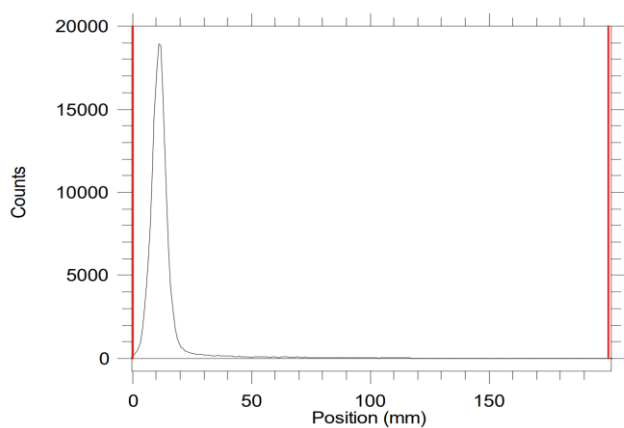
Figure 2. Representative radiochemical conversion to BCN-[⁸⁹Zr]Zr-DOTA-GA in two-step pH adjustment (A) radio TLC; (B) analytical HPLC. Radio TLC of (C); [⁸⁹Zr]Zr-EDTA; (D) BCN-[⁸⁹Zr]Zr-DOTA-GA reaction starting at high pH; (E) [⁸⁹Zr]ZrCl₄ heated at high pH (~8). TLC condition: iTLC-SG, developer, 0.1 M EDTA pH 7; HPLC condition: Agilent XDB C-18 (4.6 × 150 mm, 5 μ); eluent, 0–45% B in 13 min, 45–90% B from 13–14 min, 90% B until 19 min, A = 0.05% TFA in water, B = 0.05% TFA in acetonitrile, flow rate 1 mL/min; black line UV detector, red line radio detector. Retention time = 11.6 min.

The zirconium-89 labeling of the DOTA chelator requires elevated temperatures; therefore, it is not suitable for the direct labeling of heat-sensitive biomolecules like the antibody (PAN). Consequently, PAN radiolabeling must be performed through an indirect labeling approach. This method involves the initial preparation of labeled DOTA (BCN-[⁸⁹Zr]Zr-DOTA-GA) followed by the coupling of this labeled prosthetic group to the antibody functionalized with tetrazine (PAN-Tz) via a copper-free cyclooctyne, tetrazine click reaction. To prepare the (PAN-Tz) conjugate, PAN was reacted with a 5-fold molar excess of the tetrazine NHS-active ester at pH 7.4 and purified by a PD-10 column using

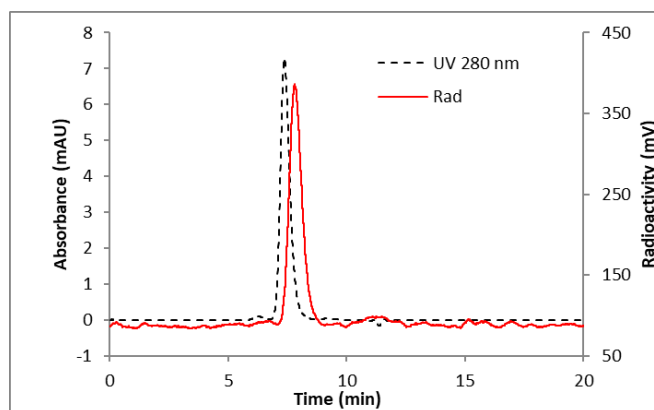
0.5 M HEPES buffer (pH 7.2). The click reaction of BCN- ^{89}Zr -DOTA-GA (Scheme 2) with PAN-Tz was monitored by radio TLC. The complete consumption of BCN- ^{89}Zr -DOTA-GA was observed in 30 min at 37 °C with 1 mg of PAN-Tz. However, a reduced amount of IgG-Tz (0.5 mg) resulted in 70% consumption of BCN- ^{89}Zr -DOTA-GA. The final radiolabeled conjugate, BCN- ^{89}Zr -DOTA-GA-PAN, was purified by a PD-10 column using saline (pH 7.0) as an eluent. The overall radiochemical yields starting from the radiolabeling of BCN-DOTA-GA were 40–50% ($n = 12$, decay uncorrected) in 2 h. The radiochemical purity assessed by TLC and HPLC was >95% (Figure 3). The molar activities were 200–300 Ci/mmol. The conjugate, BCN- ^{89}Zr -DOTA-GA-PAN, was stable (95% intact after 7 d) in whole human serum at 37 °C up to 7 d (Figure 4).



Scheme 2. Preparation of BCN- ^{89}Zr -DOTA-GA-PAN.



(A)



(B)

Figure 3. BCN- ^{89}Zr -DOTA-GA-PAN; (A) radio TLC; (B) HPLC chromatogram of pure conjugates. TLC condition: plate, iTLC-SG, developer, 0.1 M EDTA pH 7; HPLC condition: column, TSKgel SuperSW3000 (4.6 mm ID $\times$ 30 cm, 4 μm); eluent, 0.1 M sodium phosphate, 0.1 M sodium sulfate, 0.05% sodium azide, 10% *iso*-propyl alcohol (pH 6.8), flow rate 0.35 mL/min; black line UV detector, red line radio detector. Retention time = 7.8 min.

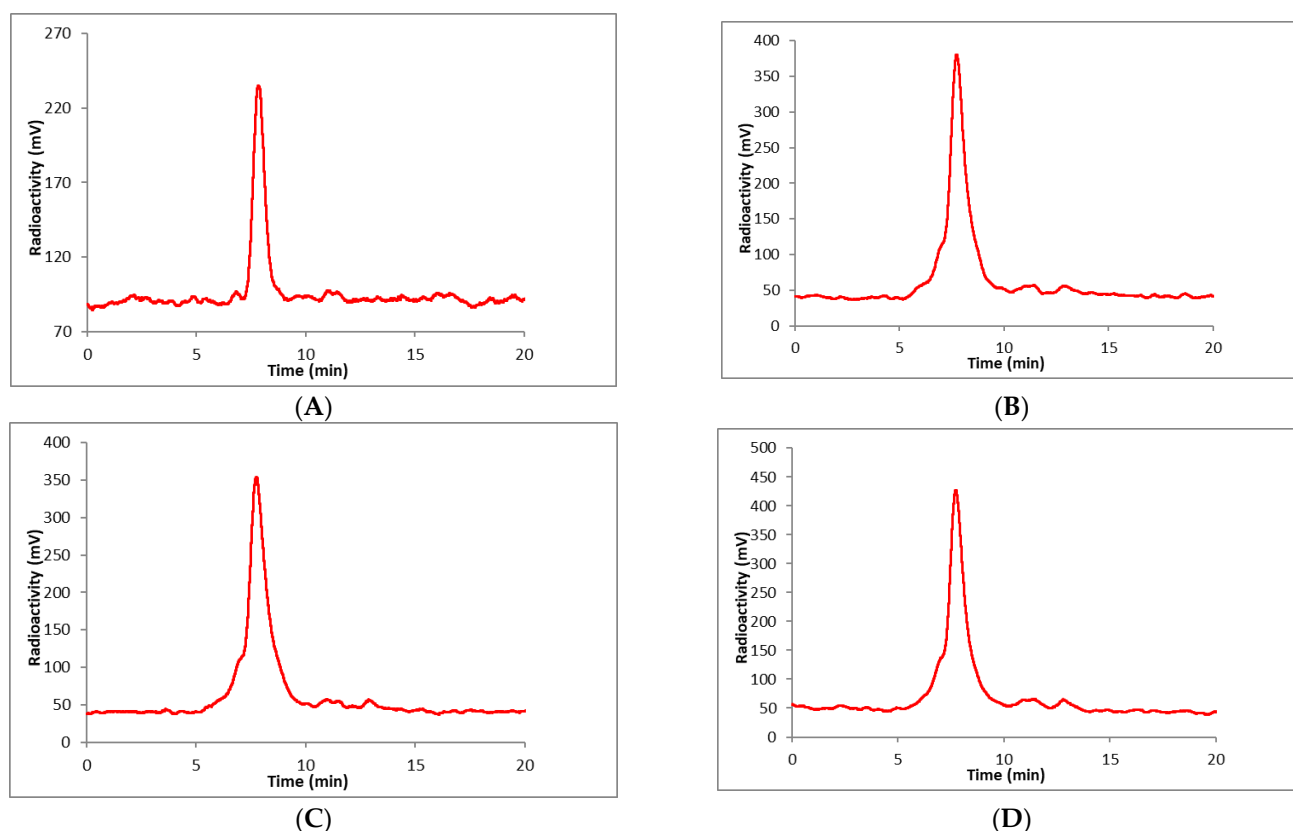


Figure 4. Radio HPLC chromatogram of BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN in whole human serum at 37 °C, (A) 0 min (after mixing with serum); (B) 3 d; (C) 5 d; (D) 7 d. HPLC condition: column, TSKgel SuperSW3000 (4.6 mm ID × 30 cm, 4 μm); eluent, 0.1 M sodium phosphate, 0.1 M sodium sulfate, 0.05% sodium azide, 10% isopropyl alcohol (pH 6.8), flow rate 0.35 mL/min. Retention time = 7.7 min.

To compare the stability of the BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN with DFO and DFO* conjugates, [⁸⁹Zr]Zr-DFO-PAN and [⁸⁹Zr]Zr-DFO*-PAN were prepared. The commercially available p-isothiocyanatobenzyl-desferrioxamine (DFO-Bz-NCS) is the most commonly used bifunctional chelator to prepare DFO conjugates. It is conjugated to primary amines of the antibody via thiourea linkage formation. The DFO*-Bz-NCS is also commercially available. For the current study, DFO-PAN and DFO*-PAN conjugates were prepared and purified following the literature method using a 5-fold molar excess of either DFO-Bz-NCS or DFO*-Bz-NCS [52]. The purities of the conjugates were determined by HPLC using a size-exclusion column (SE-HPLC). The concentrations of the conjugates were measured using the bicinchoninic acid (BCA) assay. The number of chelators per antibody (1.8 ± 0.2) was determined using 25 mM nonradioactive zirconium chloride mixed with a trace amount of zirconium-89 following the literature method [53]. Zirconium-89 labeling of the conjugates was performed according to the literature method to prepare [⁸⁹Zr]Zr-DFO-PAN and [⁸⁹Zr]Zr-DFO*-PAN [54]. The isolated radiochemical yields were in the range of 80–95% ($n = 6$). The molar activities of the conjugates were 1200–2700 Ci/mmol ($n = 6$) with a radiochemical purity >95% as confirmed by SE-HPLC. The stabilities of the conjugates were tested in whole human serum at various time points. Both conjugates were stable (95% intact after 7 d) in whole human serum at 37 °C up to 7 d (Figure 5). Although different linkers (Figure 1) were used for the formation of the radiolabeled PAN-DOTA and PAN-DFO/DFO* conjugates, comparable stabilities were observed in whole human serum at 37 °C. This result indicates that linkers do not have any significant impact on the stability of the labeled PAN conjugates (BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN, [⁸⁹Zr]Zr-DFO-PAN, and [⁸⁹Zr]Zr-DFO*-PAN).

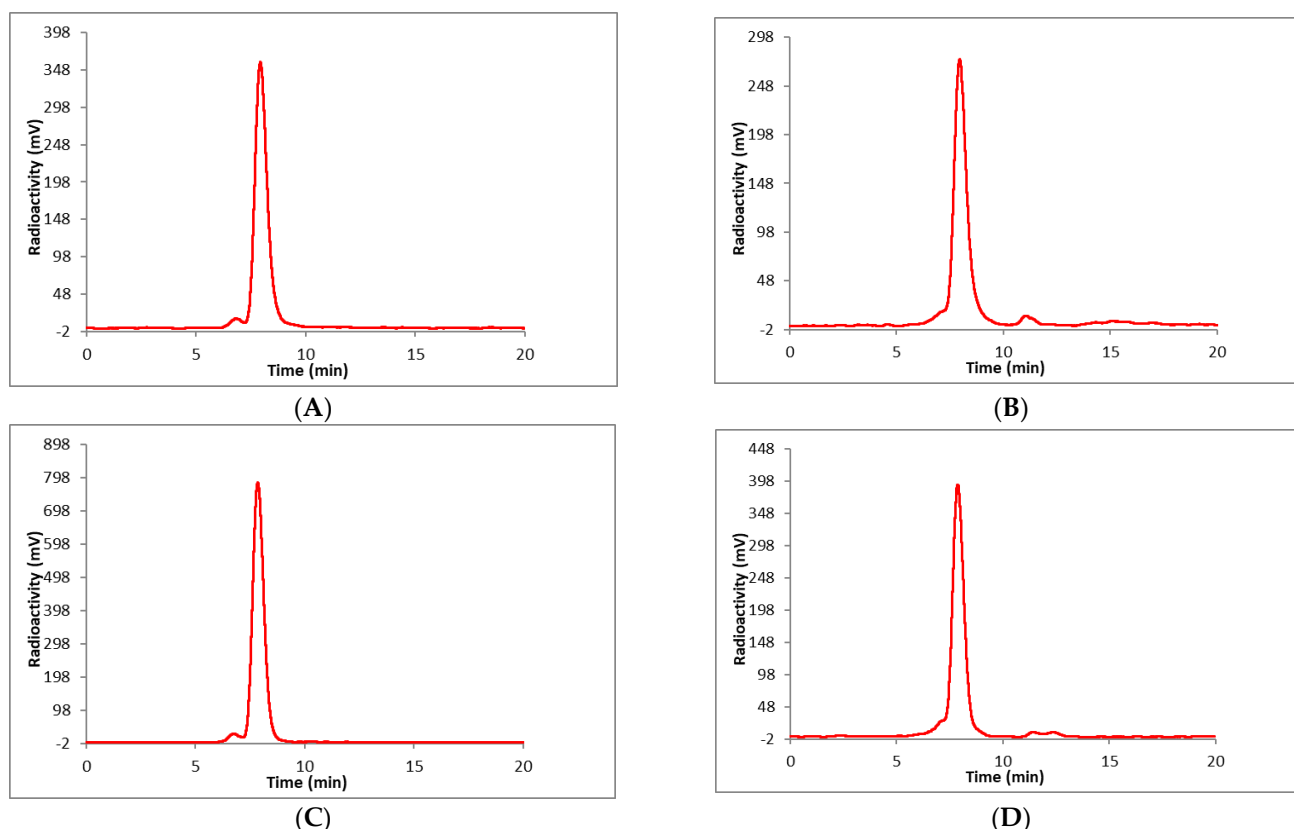


Figure 5. Radio HPLC chromatogram in whole human serum of [^{89}Zr]Zr-DFO-PAN (A) 0 min (after mixing with serum); (B) 7 d and [^{89}Zr]Zr-DFO*; (C) 0 min (after mixing with serum); (D) 7 d. HPLC condition: column, TSKgel SuperSW3000 (4.6 mm ID $\times$ 30 cm, 4 μm); eluent, 0.1 M sodium phosphate, 0.1 M sodium sulfate, 0.05% sodium azide, 10% *isopropyl* alcohol (pH 6.8), flow rate 0.35 mL/min. Retention time = 7.9 min ([^{89}Zr]Zr-DFO-PAN) and 7.8 ([^{89}Zr]Zr-DFO*).

The trans chelation experiments using EDTA as a challenging agent are the standard method for the determination of the kinetic inertness of zirconium-89 labeled conjugates [55]. Therefore, EDTA challenge reactions were performed for all three labeled conjugates with a large excess (1000 equivalents) of EDTA at room temperature and 37 $^{\circ}\text{C}$ up to 7 d (Figure 6). The degree of zirconium-89 trans chelation was determined by SE-HPLC at various time points. As expected and observed by others, rapid trans chelation was observed for the [^{89}Zr]Zr-DFO-PAN conjugates at both temperatures (22% and 10% intact after 7 days at room temperature and 37 $^{\circ}\text{C}$, respectively) [42,49]. Much slower trans chelation was observed for the [^{89}Zr]Zr-DFO*-PAN (64% and 50% intact after 7 days at room temperature and 37 $^{\circ}\text{C}$, respectively). In contrast, the BCN-[^{89}Zr]Zr-DOTA-GA-PAN showed excellent stability against EDTA trans chelation at both temperatures (>94% intact at both temperatures).

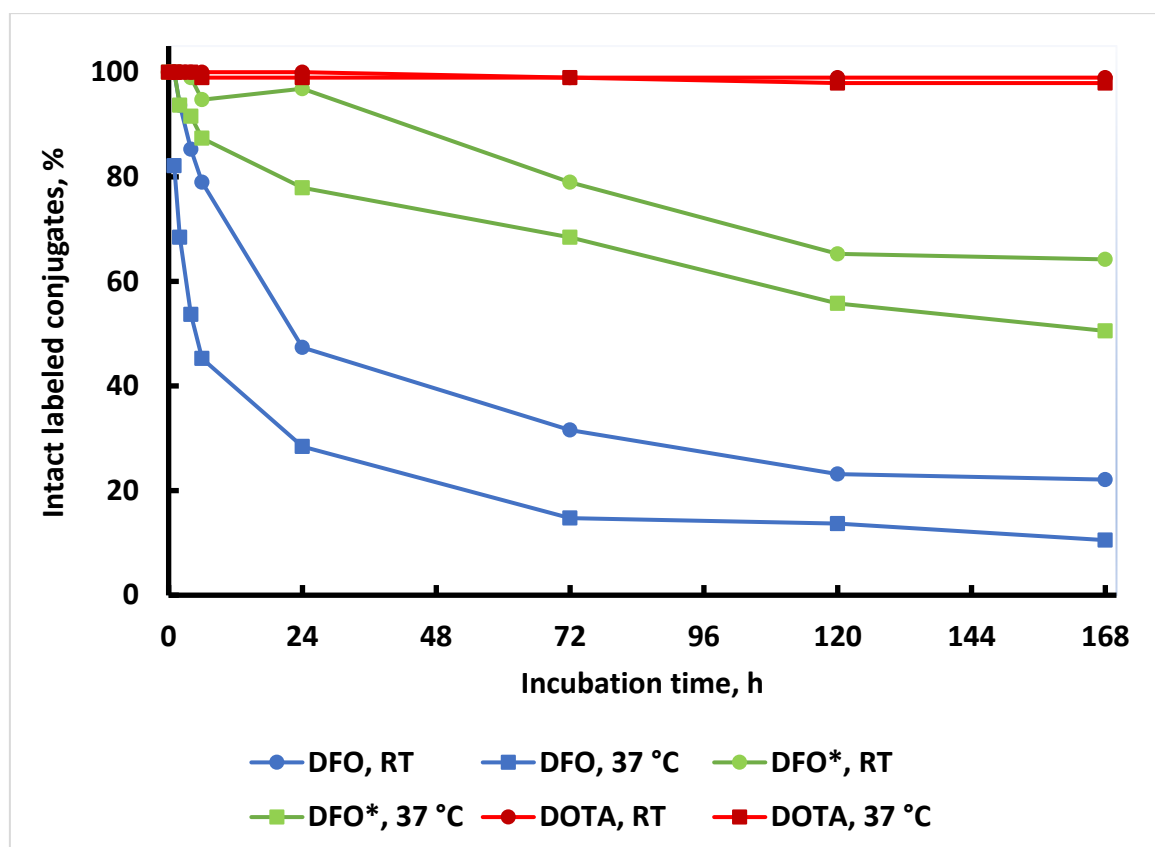


Figure 6. EDTA challenging study at room temperature and 37 °C of three conjugates: BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN (red), [⁸⁹Zr]Zr-DFO-PAN (blue), and [⁸⁹Zr]Zr-DFO*-PAN (green). The challenge reactions at room temperature are depicted as circles and at 37 °C as squares. The radiochemical purity of the conjugates was normalized to 100% at the start of the EDTA challenge.

3. Conclusions

Zirconium-89 labeling of the DOTA chelator was optimized through a two-step basic pH adjustment at 90 °C to prepare BCN-[⁸⁹Zr]Zr-DOTA-GA. Using this labeled prosthetic group antibody conjugate, BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN was successfully prepared via click chemistry with high radiochemical yield and purity. All three conjugates demonstrated excellent stability in whole human serum. BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN maintained its inertness when exposed to a large excess of EDTA. However, a rapid trans chelation was observed for [⁸⁹Zr]Zr-DFO-PAN, with the order of [⁸⁹Zr]Zr-DFO-PAN > [⁸⁹Zr]Zr-DFO*-PAN > BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN. This result suggests that the DFO conjugate exhibits the lowest chemical inertness, while the DOTA conjugate demonstrates the highest.

4. Materials and Methods

The p-isothiocyanatobenzyl-desferrioxamine (DFO-Bz-NCS) was purchased from Macrocytics, Inc. (Plano, TX, USA). BCN-DOTA-GA was purchased from Chematech (France). DFO*-Bz-NCS was obtained from ABX GmbH (Radeberg, Germany). Panitumumab was acquired from NIH pharmacy (Bethesda, MD, USA). Sodium acetate and Tris-HCl were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The lyophilized whole human serum was obtained from MP Biomedicals, LLC (Solon, OH, USA) and dissolved in 2 mL saline. This serum solution was directly used without inactivation for the stability studies. All other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification. PD-10 desalting columns were obtained from GE Healthcare Biosciences (Pittsburgh, PA, USA). [⁸⁹Zr]Zr-oxalate and zirconium-89 chloride were obtained from 3D Imaging (Little Rock, AR, USA). Ana-

lytical high-performance liquid chromatography (HPLC) analyses were performed on an Agilent 1200 Series instrument equipped with a multi-wavelength UV detector connected in series with a Bioscan flow count radio detector. The size-exclusion column, TSKgel SuperSW3000 (SE, 4.6 mm ID $\times$ 30 cm, 4 μ m), was obtained from Tosoh Bioscience LLC. (King of Prussia, PA, USA). The iTLC papers were read in an Eckert & Ziegler TLC scanner (B-AR2000-1, Hopkinton, MA, USA). A bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific) with bovine gamma globulin standard was used to determine the conjugate concentrations.

4.1. Synthesis of DFO-PAN and DFO*-PAN

The DFO and DFO* conjugates of panitumumab (PAN) were prepared according to the literature method [52]. Briefly, PAN (20 mg/mL) was buffer-exchanged into 0.1 M NaHCO₃ (containing 0.9% NaCl, pH 8.9), the concentration was adjusted to 5 mg/mL using NaHCO₃ (containing 0.9% NaCl, pH 8.9), and treated with a 5-fold molar excess of DFO-Bz-NCS or DFO*-Bz-NCS (5 mg/mL in DMSO). The mixture was gently rocked at 37 °C for 75 min before stopping the conjugation reaction with the addition of 1 M Tris (to a final concentration of 12–15 mM). The DFO-PAN and DFO*-PAN conjugates were purified twice by two PD-10 columns (spin protocol) into 0.25 M sodium acetate (pH 5.5).

4.2. Synthesis of [⁸⁹Zr]Zr-DFO-PAN and [⁸⁹Zr]Zr-DFO*-PAN

Zirconium-89 labeling of the conjugates (DFO-PAN and DFO*-PAN) was performed according to the literature method to obtain radiolabeled conjugates ([⁸⁹Zr]Zr-DFO-PAN and [⁸⁹Zr]Zr-DFO*-PAN) [54]. Briefly, a stock solution of [⁸⁹Zr]Zr-oxalate (~440 MBq) was diluted with 1.2 mL of HEPES buffer (0.5 M, pH 7.1–7.3). From this stock solution, ~150 MBq of zirconium-89 was used per radiolabeling reaction. The aliquot of zirconium-89 (~150 MBq, 0.4 mL) was added to 20 μ L of 2,5-dihydroxybenzoic acid (~5 mg/mL, pH adjusted to 7 with 2 M Na₂CO₃ solution). DFO or DFO* conjugate (0.6 mg) was added to the solution. The reaction mixture was further diluted with HEPES buffer (0.5 M, pH 7.1–7.3) to reach the final mixture volume of 1 mL. The reaction mixture was incubated for 1 h at room temperature and challenged with diethylenetriamine pentaacetate, DTPA, (0.1 M, 5 μ L, pH 7) for an additional 10 min. The radiolabeled conjugates were purified by a PD-10 column using 0.9% NaCl (pH 7). The molar activity was determined by HPLC using a size-exclusion column (t_R = 8.0 min). The purity of the conjugate was determined by HPLC and TLC.

4.3. Synthesis of PAN-Tz

Panitumumab (PAN, 20 mg/mL, PBS buffer pH 7.4) was buffer-exchanged to 5 mg/mL and reacted with 5 eq excess of the tetrazine NHS ester (CAS#1244060-64-9, 5 mg/mL in DMSO) at 4 °C overnight. The conjugate was purified with 0.5 M HEPES buffer (pH 7.2) using a PD-10 column. The purity of the conjugate (PAN-Tz) was confirmed by analytical size-exclusion HPLC. The protein concentration was determined by BCA assay. The tetrazine to antibody ratio was 4.5 ± 0.2 , as determined by Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI-MS) using an Acquity UPLC H-Class coupled to a Xevo G2-XS QToF (Waters Corporation, Milford, MA, USA). The sample was de-glycosylated using Rapid PNGase F (New England BioLabs, Ipswich, MA, USA) before analysis and desalted online using an XBridge Protein BEH C4 column (Waters Corporation) with a water/acetonitrile gradient containing 0.1% formic acid. Data processing was performed using UNIFI software version 1.9.4.053 (Waters Corporation) and peaks were assigned based on the unmodified antibody as a reference.

4.4. Synthesis of BCN-[⁸⁹Zr]Zr-DOTA-GA-PAN

HEPES buffer pH 7.2 (0.5 M; 445 μ L) was added to the solution of [⁸⁹Zr]Cl₄ (115 μ L 1N HCl). If the supplied volume of HCl is different, then the volume of HEPES buffer needs to be adjusted accordingly. An aliquot of this solution (130 μ L, ~3 mCi) was added

to the solution of BCN-DOTA-GA (15 μ L, 30 μ g, 43 nmol) in 0.5 M HEPES buffer (pH 7.4). The final pH of the solution was $\sim$ 7.2. The reaction mixture was heated at 90 $^{\circ}$ C for 30 min followed by the addition of 135 μ L 0.5 M HEPES (pH 7.68) and continued heating at the same temperature for an additional 30 min. The formation of the DOTA complex was checked by TLC and HPLC (TLC condition: iTLC-SG, developer, 0.1 M EDTA pH 7; HPLC condition: Agilent XDB C-18 (4.6 $\times$ 150 mm, 5 μ); eluent, 0–45% B in 13 min, 45–90% B in 14 min, 90% B until 19 min, A = 0.05% TFA in water, B = 0.05% TFA in acetonitrile, flow rate 1 mL/min). PAN-Tz conjugate in 0.5 M HEPES buffer (1 mg, 4.8–5.2 mg/mL) was added and the reaction was incubated for 0.5 h at 37 $^{\circ}$ C. The radiolabeled conjugate was purified into 0.9% NaCl (pH 7) by a PD-10 column. The molar activity and the purity of the radiolabeled conjugate were determined by SE-HPLC (condition: TSKgel super3000 4.6 mm $\times$ 30 cm, 4 μ m column; eluent: 0.1 M sodium phosphate, 0.1 M sodium sulfate, 0.05% NaN₃; pH 6.8 + 10%IPA; flow rate: 0.35 mL/min). The stability of the radiolabeled conjugate in whole human serum at 37 $^{\circ}$ C was assessed by SE-HPLC and TLC for up to 7 d.

4.5. Storage and In Vitro Serum Stability

The storage stability of the conjugates was assessed in anticipation of any required storage before biological investigation. Following radiosynthesis, all three labeled conjugates ($[^{89}\text{Zr}]\text{Zr-DFO-PAN}$, $[^{89}\text{Zr}]\text{Zr-DFO}^*\text{-PAN}$, and BCN- $[^{89}\text{Zr}]\text{Zr-DOTA-GA-PAN}$) in saline were stored at 4 $^{\circ}$ C for 24 h and reanalyzed by SE-HPLC to confirm the integrity of the antibody conjugates. All three conjugates were stable at those conditions for at least up to 24 h at 4 $^{\circ}$ C.

To determine in vitro serum stability, whole human serum (500 μ L) was added to a solution of $[^{89}\text{Zr}]\text{Zr-DFO-PAN}$, $[^{89}\text{Zr}]\text{Zr-DFO}^*\text{-PAN}$, or BCN- $[^{89}\text{Zr}]\text{Zr-DOTA-GA-PAN}$ ($\sim$ 500 μ Ci in 500 μ L of saline, pH 7.0) and kept at 37 $^{\circ}$ C for up to 7 d. The radiochemical stability was determined at various time points by directly injecting an aliquot of the solution using the SE HPLC.

4.6. EDTA Challenge Study

To perform the EDTA challenge study, each labeled conjugate, BCN- $[^{89}\text{Zr}]\text{Zr-DOTA-GA-PAN}$, $[^{89}\text{Zr}]\text{Zr-DFO-PAN}$, and $[^{89}\text{Zr}]\text{Zr-DFO}^*\text{-PAN}$ (200 μ Ci, in 250 μ L saline, pH 7.0), was added to a solution of EDTA (1000 eq in HEPES buffer pH 7.0) and incubated at either room temperature or at 37 $^{\circ}$ C with gentle rocking for 7 days. The stabilities of the labeled conjugates were analyzed using SE-HPLC (size-exclusion column) by directly injecting an aliquot of the conjugates at 1 h, 2 h, 4 h, 6 h, 1 d, 3 d, 5 d, and 7 d.

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References

1. Duclos, V.; Iep, A.; Gomez, L.; Goldfarb, L.; Besson, F.L. PET Molecular Imaging: A Holistic Review of Current Practice and Emerging Perspectives for Diagnosis, Therapeutic Evaluation and Prognosis in Clinical Oncology. *Int. J. Mol. Sci.* **2021**, *22*, 4159. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Rowe, S.P.; Pomper, M.G. Molecular imaging in oncology: Current impact and future directions. *CA Cancer J. Clin.* **2022**, *72*, 333–352. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Rong, J.; Haider, A.; Jeppesen, T.E.; Josephson, L.; Liang, S.H. Radiochemistry for positron emission tomography. *Nat. Commun.* **2023**, *14*, 3257. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Davis, R.D.; Drake, C.; Ippisch, R.C.; Moore, M.; Sutcliffe, J.L. Fully automated peptide radiolabeling from [^{18}F]fluoride t. *RSC Adv.* **2019**, *9*, 8638–8649. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Koatale, P.C.; Welling, M.M.; Ndlovu, H.; Kgatle, M.; Sipho Mdanda, S.; Mdlophane, A.; Okem, A.; Takyi-Williams, J.; Sathekge, M.M.; Ebenhan, T. Insights into Peptidoglycan-Targeting Radiotracers for Imaging Bacterial Infections: Updates, Challenges, and Future Perspectives. *ACS Infect Dis.* **2024**, *10*, 270–286. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Jagoda, E.M.; Vasalatiy, O.; Basuli, F.; Opina, A.C.L.; Williams, M.R.; Wong, K.; Lane, K.C.; Adler, S.; Ton, A.T.; Szajek, L.P.; et al. Immuno-PET Imaging of the Programmed Cell Death-1 Ligand (PD-L1) Using a Zirconium-89 Labeled Therapeutic Antibody, Avelumab. *Mol. Imaging* **2019**, *18*, 1536012119829986. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Li, X.-G.; Haaparanta, M.; Solin, O. Oxime formation for fluorine-18 labeling of peptides and proteins for positron emission tomography (PET) imaging: A review. *J. Fluor. Chem.* **2012**, *143*, 49–56. [\[CrossRef\]](#)
8. Olberg, D.E.; Hjelstuen, O.K. Labeling strategies of peptides with ^{18}F for positron emission tomography. *Curr. Top. Med. Chem.* **2010**, *10*, 1669–1679. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Menke-van der Houven van Oordt, C.W.; Gootjes, E.C.; Huisman, M.C.; Vugts, D.J.; Roth, C.; Luik, A.M.; Mulder, E.R.; Schuit, R.C.; Boellaard, R.; Hoekstra, O.S.; et al. ^{89}Zr -cetuximab PET imaging in patients with advanced colorectal cancer. *Oncotarget* **2015**, *6*, 30384–30393. [\[CrossRef\]](#)
10. Van de Watering, F.C.; Rijpkema, M.; Perk, L.; Brinkmann, U.; Oyen, W.J.; Boerman, O.C. Zirconium-89 labeled antibodies: A new tool for molecular imaging in cancer patients. *Biomed. Res. Int.* **2014**, *2014*, 203601. [\[CrossRef\]](#)
11. Fischer, G.; Seibold, U.; Schirmacher, R.; Wängler, B.; Wängler, C. ^{89}Zr , a radiometal nuclide with high potential for molecular imaging with PET: Chemistry, applications and remaining challenges. *Molecules* **2013**, *18*, 6469–6490. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Yoon, J.K.; Park, B.N.; Ryu, E.K.; An, Y.S.; Lee, S.J. Current Perspectives on ^{89}Zr -PET Imaging. *Int. J. Mol. Sci.* **2020**, *21*, 4309. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Poot, A.J.; Adamzek, K.W.A.; Windhorst, A.D.; Vosjan, M.J.W.D.; Kropf, S.; Wester, H.-J.; van Dongen, G.A.M.S.; Vugts, D.J. Fully Automated ^{89}Zr Labeling and Purification of Antibodies. *J. Nucl. Med.* **2019**, *60*, 691–695. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Abou, D.S.; Longtine, M.; Fears, A.; Benabdallah, N.; Unnerstall, R.; Johnston, H.; Shim, K.; Hasson, A.; Zhang, H.; Ulmert, D.; et al. Evaluation of Candidate Theranostics for $^{227}\text{Th}/^{89}\text{Zr}$ Paired Radioimmunotherapy of Lymphoma. *J. Nucl. Med.* **2023**, *64*, 1062–1068. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Kratochwil, C.; Bruchertseifer, F.; Giesel, F.L.; Weis, M.; Verburg, F.A.; Mottaghy, F.; Kopka, K.; Apostolidis, C.; Haberkorn, U.; Morgenstern, A. 225Ac-PSMA-617 for PSMA-Targeted α -Radiation Therapy of Metastatic Castration-Resistant Prostate Cancer. *J. Nucl. Med.* **2016**, *57*, 1941–1944. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Sharifian, M.; Sadeghi, M.; Alirezapour, B.; Yarmohammadi, M.; Ardaneh, K. Modeling and experimental data of zirconium-89 production yield. *Appl. Radiat. Isot.* **2017**, *130*, 206–210. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Van Dongen, G.; Beaino, W.; Windhorst, A.D.; Zwezerijnen, G.J.C.; Oprea-Lager, D.E.; Hendrikse, N.H.; van Kuijk, C.; Boellaard, R.; Huisman, M.C.; Vugts, D.J. The Role of ^{89}Zr -Immuno-PET in Navigating and Derisking the Development of Biopharmaceuticals. *J. Nucl. Med.* **2021**, *62*, 438–445. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Vázquez, S.M.; Endepols, H.; Fischer, T.; Tawadros, S.-G.; Hohberg, M.; Zimmermanns, B.; Dietlein, F.; Neumaier, B.; Drzezga, A.; Dietlein, M.; et al. Translational Development of a Zr-89-Labeled Inhibitor of Prostate-specific Membrane Antigen for PET Imaging in Prostate Cancer. *Mol. Imaging Biol.* **2022**, *24*, 115–125. [\[CrossRef\]](#)
19. Ulaner, G.A.; Hyman, D.M.; Ross, D.S.; Corben, A.; Chandarlapaty, S.; Goldfarb, S.; McArthur, H.; Erinjeri, J.P.; Solomon, S.B.; Kolb, H.; et al. Detection of HER2-Positive Metastases in Patients with HER2-Negative Primary Breast Cancer Using ^{89}Zr -Trastuzumab PET/CT. *J. Nucl. Med.* **2016**, *57*, 1523–1528. [\[CrossRef\]](#)
20. Price, E.W.; Carnazza, K.E.; Carlin, S.D.; Cho, A.; Edwards, K.J.; Sevak, K.K.; Glaser, J.M.; de Stanchina, E.; Janjigian, Y.Y.; Lewis, J.S. ^{89}Zr -DFO-AMG102 Immuno-PET to Determine Local Hepatocyte Growth Factor Protein Levels in Tumors for Enhanced Patient Selection. *J. Nucl. Med.* **2017**, *58*, 1386–1394. [\[CrossRef\]](#)
21. Jauw, Y.W.S.; O'Donoghue, J.A.; Zijlstra, J.M.; Hoekstra, O.S.; Menke-van der Houven van Oordt, C.W.; Morschhauser, F.; Carrasquillo, J.A.; Zweegman, S.; Pandit-Taskar, N.; Lammertsma, A.A.; et al. ^{89}Zr -Immuno-PET: Toward a Noninvasive Clinical Tool to Measure Target Engagement of Therapeutic Antibodies In Vivo. *J. Nucl. Med.* **2019**, *60*, 1825–1832. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Bhatt, N.B.; Pandya, D.N.; Wadas, T.J. Recent Advances in Zirconium-89 Chelator Development. *Molecules* **2018**, *23*, 638. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Jagoda, E.M.; Lang, L.; Bhadrasetty, V.; Histed, S.; Williams, M.; Kramer-Marek, G.; Mena, E.; Rosenblum, L.; Marik, J.; Tinianow, J.N.; et al. Immuno-PET of the hepatocyte growth factor receptor Met using the 1-armed antibody onartuzumab. *J. Nucl. Med.* **2012**, *53*, 1592–1600. [\[CrossRef\]](#) [\[PubMed\]](#)

24. Patra, M.; Bauman, A.; Mari, C.; Fischer, C.A.; Blacque, O.; Häussinger, D.; Gasser, G.; Mindt, T.L. An octadentate bifunctional chelating agent for the development of stable zirconium-89 based molecular imaging probes. *ChemComm* **2014**, *50*, 11523–11525. [[CrossRef](#)] [[PubMed](#)]
25. Sarbisheh, E.K.; Salih, A.K.; Raheem, S.J.; Lewis, J.S.; Price, E.W. A High-Denticity Chelator Based on Desferrioxamine for Enhanced Coordination of Zirconium-89. *Inorg. Chem.* **2020**, *59*, 11715–11727. [[CrossRef](#)] [[PubMed](#)]
26. Deri, M.A.; Ponnala, S.; Zeglis, B.M.; Pohl, G.; Dannenberg, J.J.; Lewis, J.S.; Francesconi, L.C. Alternative chelator for ^{89}Zr radiopharmaceuticals: Radiolabeling and evaluation of 3,4,3-(LI-1,2-HOPO). *J. Med. Chem.* **2014**, *57*, 4849–4860. [[CrossRef](#)] [[PubMed](#)]
27. Abou, D.S.; Ku, T.; Smith-Jones, P.M. In Vivo biodistribution and accumulation of ^{89}Zr in mice. *Nucl. Med. Biol.* **2011**, *38*, 675–681. [[CrossRef](#)] [[PubMed](#)]
28. Summers, K.L.; Sarbisheh, E.K.; Zimmerling, A.; Cotelesage, J.J.H.; Pickering, I.J.; George, G.N.; Price, E.W. Structural Characterization of the Solution Chemistry of Zirconium(IV) Desferrioxamine: A Coordination Sphere Completed by Hydroxides. *Inorg. Chem.* **2020**, *59*, 17443–17452. [[CrossRef](#)]
29. Ulaner, G.A.; Hyman, D.M.; Lyashchenko, S.K.; Lewis, J.S.; Carrasquillo, J.A. ^{89}Zr -Trastuzumab PET/CT for Detection of Human Epidermal Growth Factor Receptor 2-Positive Metastases in Patients With Human Epidermal Growth Factor Receptor 2-Negative Primary Breast Cancer. *Clin. Nucl. Med.* **2017**, *42*, 912–917. [[CrossRef](#)]
30. Racow, E.E.; Kreinbühl, J.J.; Cosby, A.G.; Yang, Y.; Pandey, A.; Boros, E.; Johnson, C.J. General Approach to Direct Measurement of the Hydration State of Coordination Complexes in the Gas Phase: Variable Temperature Mass Spectrometry. *J. Am. Chem. Soc.* **2019**, *141*, 14650–14660. [[CrossRef](#)]
31. Holland, J.P. Predicting the Thermodynamic Stability of Zirconium Radiotracers. *Inorg. Chem.* **2020**, *59*, 2070–2082. [[CrossRef](#)] [[PubMed](#)]
32. Meijs, W.E.; Herscheid, J.D.; Haisma, H.J.; Pinedo, H.M. Evaluation of desferal as a bifunctional chelating agent for labeling antibodies with Zr-89. *Int. J. Rad. Appl. Instrum. A* **1992**, *43*, 1443–1447. [[CrossRef](#)]
33. Chomet, M.; Schreurs, M.; Bolijn, M.J.; Verlaan, M.; Beaino, W.; Brown, K.; Poot, A.J.; Windhorst, A.D.; Gill, H.; Marik, J.; et al. Head-to-head comparison of DFO* and DFO chelators: Selection of the best candidate for clinical ^{89}Zr -immuno-PET. *Eur. J. Nucl. Med. Mol. Imaging* **2021**, *48*, 694–707. [[CrossRef](#)] [[PubMed](#)]
34. Vugts, D.J.; Klaver, C.; Sewing, C.; Poot, A.J.; Adamzek, K.; Huegli, S.; Mari, C.; Visser, G.W.M.; Valverde, I.E.; Gasser, G.; et al. Comparison of the octadentate bifunctional chelator DFO*-pPhe-NCS and the clinically used hexadentate bifunctional chelator DFO-pPhe-NCS for ^{89}Zr -immuno-PET. *Eur. J. Nucl. Med. Mol. Imaging* **2017**, *44*, 286–295. [[CrossRef](#)] [[PubMed](#)]
35. Seibold, U.; Wängler, B.; Wängler, C. Rational Design, Development, and Stability Assessment of a Macrocyclic Four-Hydroxamate-Bearing Bifunctional Chelating Agent for ^{89}Zr . *ChemMedChem* **2017**, *12*, 1555–1571. [[CrossRef](#)]
36. Deri, M.A.; Ponnala, S.; Kozlowski, P.; Burton-Pye, B.P.; Cicek, H.T.; Hu, C.; Lewis, J.S.; Francesconi, L.C. p-SCN-Bn-HOPO: A Superior Bifunctional Chelator for ^{89}Zr ImmunoPET. *Bioconjug Chem.* **2015**, *26*, 2579–2591. [[CrossRef](#)] [[PubMed](#)]
37. Salih, A.K.; Dominguez Garcia, M.; Raheem, S.J.; Ahiahonu, W.K.; Price, E.W. DFO-Km: A Modular Chelator as a New Chemical Tool for the Construction of Zirconium-89-Based Radiopharmaceuticals. *Inorg. Chem.* **2023**, *62*, 20806–20819. [[CrossRef](#)]
38. Salih, A.K.; Raheem, S.J.; Garcia, M.D.; Ahiahonu, W.K.; Price, E.W. Design, Synthesis, and Evaluation of DFO-Em: A Modular Chelator with Octadentate Chelation for Optimal Zirconium-89 Radiochemistry. *Inorg. Chem.* **2022**, *61*, 20964–20976. [[CrossRef](#)]
39. Khozimeh Sarbisheh, E.; Summers, K.L.; Salih, A.K.; Cotelesage, J.J.H.; Zimmerling, A.; Pickering, I.J.; George, G.N.; Price, E.W. Radiochemical, Computational, and Spectroscopic Evaluation of High-Denticity Desferrioxamine Derivatives DFO2 and DFO2p toward an Ideal Zirconium-89 Chelate Platform. *Inorg. Chem.* **2023**, *62*, 2637–2651. [[CrossRef](#)]
40. Richardson-Sanchez, T.; Tieu, W.; Gotsbacher, M.P.; Telfer, T.J.; Codd, R. Exploiting the biosynthetic machinery of *Streptomyces pilosus* to engineer a water-soluble zirconium(IV) chelator. *Org. Biomol. Chem.* **2017**, *15*, 5719–5730. [[CrossRef](#)]
41. Roy, J.; Jagoda, E.M.; Basuli, F.; Vasalatiy, O.; Phelps, T.E.; Wong, K.; Ton, A.T.; Hagemann, U.B.; Cuthbertson, A.S.; Cole, P.E.; et al. In Vitro and In Vivo Comparison of 3,2-HOPO Versus Deferoxamine-Based Chelation of Zirconium-89 to the Antimesothelin Antibody Anetumab. *Cancer Biother. Radiopharm.* **2021**, *36*, 316–325. [[CrossRef](#)] [[PubMed](#)]
42. Damerow, H.; Hübner, R.; Judmann, B.; Schirmacher, R.; Wängler, B.; Fricker, G.; Wängler, C. Side-by-Side Comparison of Five Chelators for ^{89}Zr -Labeling of Biomolecules: Investigation of Chemical/Radiochemical Properties and Complex Stability. *Cancers* **2021**, *13*, 6349. [[CrossRef](#)] [[PubMed](#)]
43. Cheng, M.; Tann, M. Highly variable biodistribution of ^{68}Ga labeled somatostatin analogues ^{68}Ga -DOTA-NOC and ^{68}Ga -DOTA-TATE in neuroendocrine tumors: Clinical implications for somatostatin receptor directed PET/CT. *Hepatobiliary Surg. Nutr.* **2022**, *11*, 654–661. [[CrossRef](#)] [[PubMed](#)]
44. Werner, R.A.; Bluemel, C.; Allen-Auerbach, M.S.; Higuchi, T.; Herrmann, K. ^{68}Ga Gallium- and ^{90}Y Yttrium-/ ^{177}Lu Lutetium: “theranostic twins” for diagnosis and treatment of NETs. *Ann. Nucl. Med.* **2015**, *29*, 1–7. [[CrossRef](#)] [[PubMed](#)]
45. Wong, P.; Li, L.; Chea, J.; Hu, W.; Poku, E.; Ebner, T.; Bowles, N.; Wong, J.Y.C.; Yazaki, P.J.; Sligar, S.; et al. Antibody Targeted PET Imaging of ^{64}Cu -DOTA-Anti-CEA PEGylated Lipid Nanodiscs in CEA Positive Tumors. *Bioconjug Chem.* **2020**, *31*, 743–753. [[CrossRef](#)] [[PubMed](#)]
46. Uccelli, L.; Boschi, A.; Cittanti, C.; Martini, P.; Panareo, S.; Tonini, E.; Nieri, A.; Urso, L.; Caracciolo, M.; Lodi, L.; et al. $^{90}\text{Y}/^{177}\text{Lu}$ -DOTATOC: From Preclinical Studies to Application in Humans. *Pharmaceutics* **2021**, *13*, 1463. [[CrossRef](#)]

47. Werner, R.A.; Weich, A.; Kircher, M.; Solnes, L.B.; Javadi, M.S.; Higuchi, T.; Buck, A.K.; Pomper, M.G.; Rowe, S.P.; Lapa, C. The theranostic promise for Neuroendocrine Tumors in the late 2010s—Where do we stand, where do we go? *Theranostics* **2018**, *8*, 6088–6100. [[CrossRef](#)] [[PubMed](#)]
48. Fani, M.; Peitl, P.K.; Velikyan, I. Current Status of Radiopharmaceuticals for the Theranostics of Neuroendocrine Neoplasms. *Pharmaceuticals* **2017**, *10*, 30. [[CrossRef](#)]
49. Pandya, D.N.; Bhatt, N.; Yuan, H.; Day, C.S.; Ehrmann, B.M.; Wright, M.; Bierbach, U.; Wadas, T.J. Zirconium tetraazamacrocyclic complexes display extraordinary stability and provide a new strategy for zirconium-89-based radiopharmaceutical development. *Chem. Sci.* **2017**, *8*, 2309–2314. [[CrossRef](#)]
50. Privé, B.M.; Derks, Y.H.W.; Rosar, F.; Franssen, G.M.; Peters, S.M.B.; Khreish, F.; Bartholomä, M.; Maus, S.; Gotthardt, M.; Laverman, P.; et al. ⁸⁹Zr-labeled PSMA ligands for pharmacokinetic PET imaging and dosimetry of PSMA-617 and PSMA-I&T: A preclinical evaluation and first in man. *Eur. J. Nucl. Med. Mol. Imaging* **2022**, *49*, 2064–2076. [[CrossRef](#)]
51. Lee, Y.S.; O'Connor, R.D.; Vasalatiy, O. Investigation of Two Zr-p-NO₂Bn-DOTA Isomers via NMR and Quantum Chemical Studies. *Eur. J. Inorg. Chem.* **2023**, *26*, e202300439. [[CrossRef](#)] [[PubMed](#)]
52. Vosjan, M.J.; Perk, L.R.; Visser, G.W.; Budde, M.; Jurek, P.; Kiefer, G.E.; van Dongen, G.A. Conjugation and radiolabeling of monoclonal antibodies with zirconium-89 for PET imaging using the bifunctional chelate p-isothiocyanatobenzyl-desferrioxamine. *Nat. Protoc.* **2010**, *5*, 739–743. [[CrossRef](#)] [[PubMed](#)]
53. Meares, C.F.; McCall, M.J.; Reardan, D.T.; Goodwin, D.A.; Diamanti, C.I.; McTigue, M. Conjugation of antibodies with bifunctional chelating agents: Isothiocyanate and bromoacetamide reagents, methods of analysis, and subsequent addition of metal ions. *Anal. Biochem.* **1984**, *142*, 68–78. [[CrossRef](#)] [[PubMed](#)]
54. Jagoda, E.M.; Basuli, F.; Olkowski, C.; Weiss, I.; Phelps, T.E.; Wong, K.; Ton, A.T.; Lane, K.C.; Adler, S.; Butcher, D.; et al. Immuno-PET Imaging of Siglec-15 Using the Zirconium-89-Labeled Therapeutic Antibody, NC318. *Mol. Imaging* **2023**, *2023*, 3499655. [[CrossRef](#)]
55. Feiner, I.V.J.; Brandt, M.; Cowell, J.; Demuth, T.; Vugts, D.; Gasser, G.; Mindt, T.L. The Race for Hydroxamate-Based Zirconium-89 Chelators. *Cancers* **2021**, *13*, 4466. [[CrossRef](#)]

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