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Synthesis and Evaluation of Diaza-Crown Ether-Backboned Chelator Containing Hydroxamate Groups for Zr-89 Chelation Chemistry

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Abstract

Zirconium-89 (^{89}Zr) has been explored for molecularly targeted positron emission tomography (PET) imaging of various diseases. We synthesized and evaluated a novel chelator (DA-18C6-BHA) for ^{89}Zr. The new chelator is structured on a macrocyclic backbone (1,10-diaza-18-crown-6) and contains hydroxamates as acyclic donor groups. The new chelator ((DA-18C6-BHA) was rapidly labeled with ^{89}Zr under mild conditions. The ^{89}Zr-labeled DA-18C6-BHA complex remained stable in human serum and apotransferrin for 7 days. When challenged with excess EDTA solution, ^{89}Zr-labeled DA-18C6-BHA was shown to hold ^{89}Zr without losing considerable radioactivity to EDTA. The ^{89}Zr-labeled DA-18C6-BHA complex displayed high complex stability in normal mice as evidenced by low bone uptake.

Keywords

Zr-89; Chelator; PET Imaging

Positron-emitting radionuclides used for positron emission tomography (PET) imaging include ^{18}F ($t_{1/2}$ = 1.83 h, $E_{\beta+}$ = 0.63 MeV), ^{64}Cu ($t_{1/2}$ = 12.7 h, $E_{\beta+}$ = 0.66 MeV, E_{γ} = 1.35 MeV), ^{68}Ga ($t_{1/2}$ = 1.12 h, $E_{\beta+}$ = 1.88 MeV, E_{γ} = 0.51 MeV), and ^{89}Zr ($t_{1/2}$ = 78.4 h, $E_{\beta+}$ = 0.90 MeV, E_{γ} = 0.91 MeV).[1–5] Research efforts have been made on discovery of PET imaging agents with high binding affinity and selectivity to specific biomarkers on tumor cells.[1–3] A number of target-specific biomolecules including peptides and antibodies have been labeled with radionuclides for preclinical and clinical evaluations of targeted PET imaging of cancers.[5–7]

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Supporting material. Materials and methods and copies of NMR spectra and TLC chromatograms for assessment of radiolabeling reaction kinetics and serum stability and EDTA challenge and *in vivo* biodistribution data. This material is available free of charge online.

Among the positron-emitting radionuclides in clinical use, a long-lived ^{89}Zr is well suited for PET imaging of molecular targets using an antibody with a long biological half-life.[1,5–8] In particular, ^{89}Zr-based PET tracers have a clinical value in pre- and post-treatment imaging and monitoring tumor response in antibody therapy.[5–6] ^{89}Zr(IV) is a highly charged cation with strong binding with anionic oxygens such as hydroxyl group in bone mineral hydroxylapatite (HA).[1,5,9]

DFO (desferrioxamine) is a hydroxamate-based chelator in clinical use for the treatment of iron overload diseases including β-thalassemia.[10] Given the high binding avidity of the hydroxamates for a hard oxophilic cation, DFO has been also extensively explored for labeling of various biomolecules with ^{89}Zr.[8,11] DFO was shown to rapidly form a 6-coordinate complex with Zr(IV), and ^{89}Zr-DFO complex remained intact in human serum.[12,13] However, DFO-antibody conjugate labeled with ^{89}Zr has a limited *in vivo* stability in mice and displayed high bone uptake.[12,13] The elevated bone uptake of ^{89}Zr-DFO can be rationalized by high affinity of ^{89}Zr(IV) released from the complex for bone mineral, hydroxylapatite (HA). While research efforts have been made to develop improved chelation chemistry that can sequester the bone-seeking ^{89}Zr with high *in vivo* stability, DFO remains the standard chelator for clinical use.[1,12]

In this paper, we report synthesis and evaluation of a novel chelating agent (DA-18C6-BHA) that is built on a macrocyclic backbone (1,10-diaza-18-crown-6) tethered with hydroxamate as an acyclic donor group. The new chelator and DFO were comparatively studied for radiolabeling with ^{89}Zr. The ^{89}Zr-radiolabeled new chelator (^{89}Zr-DA-18C6-BHA) and ^{89}Zr-DFO were comparatively evaluated for complex stability in human serum and apotransferrin and excess EDTA solution and biodistribution profile in normal mice.

The novel chelator (DA-18C6-BHA) contains diaza-18-crown-6 as a macrocyclic backbone and hydroxamates as tetradentate binding moieties. The four oxygens in the macrocyclic backbone are expected to participate in complexation with ^{89}Zr(IV) and contribute to the formation of an eight-coordinate Zr complex. Synthesis of the new chelator (DA-18C6-BHA) includes reaction of compound **2**[14] as a key precursor molecule with 1,10-diaza-18-crown-6 (**3**, Scheme 1). Compound **2** was prepared from *O*-paramethoxybenzyl (PMB)-protected *N*-methyl hydroxylamine (**1**)[15] by a modified procedure of the reported method.[14] Compound **1** was reacted with bromoacetyl bromide in CH_3CN at 0 °C to afford compound **2** in 77% isolated yield. Compound **2** was further reacted with 1,10-diaza-18-crown-6 (**3**) to produce *O*-PMB protected chelating agent **4** in 41% isolated yield. Deprotection of PMB in **4** was accomplished by reaction of **4** with TFA and triethylsilane to produce the desired chelating agent **5** in nearly quantitative yield.

The new chelator (DA-18C6-BHA) and DFO were comparatively evaluated for radiolabeling with ^{89}Zr at room temperature (Supporting Information). The new chelator (DA-18C6-BHA) and DFO rapidly bound to ^{89}Zr and were shown to be effective in binding ^{89}Zr at pH 7 and room temperature. Radiolabeling of DA-18C6-BHA or DFO with ^{89}Zr was nearly complete in 10 min (>98% radiolabeling efficiency). Stability of ^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO in human serum was determined (Table 1 and Supporting Information). Dissociation of ^{89}Zr from ^{89}Zr-DA-18C6-BHA or ^{89}Zr-DFO in human serum (pH 7.0 and

37 °C) was measured over 7 days. The serum stability data suggest that DA-18C6-BHA and DFO remained inert in serum for 7 days. A minimal amount of ^{89}Zr from ^{89}Zr-DA-18C6-BHA or ^{89}Zr-DFO was released to serum (<1% at 168 h time point).

^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO were evaluated for complex stability in the presence of EDTA (Table 2 and Supporting Information). ^{89}Zr-DA-18C6-BHA or ^{89}Zr-DFO was incubated with a 100-fold molar excess of EDTA. ^{89}Zr-DA-18C6-BHA was shown to have high complex stability in EDTA solution, and only a trace amount of ^{89}Zr (<1%) was transchelated to EDTA. In contrast, a significant amount of ^{89}Zr was dissociated from ^{89}Zr-DFO complex at 168 h-post incubation (>12%).

^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO were further evaluated for complex stability in the presence of apotransferrin (Table 3 and Supporting Information). ^{89}Zr-DA-18C6-BHA or ^{89}Zr-DFO was incubated with a 5-fold molar excess of apotransferrin. Both ^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO complex remained stable in apotransferrin solution (PBS, pH 7.0), and no considerable amount of ^{89}Zr was released to the apotransferrin solution over 7 days.

We performed biodistribution studies in CD-1 normal mice (intravenous injection, n = 4) to evaluate *in vivo* stability of ^{89}Zr-DFO and ^{89}Zr-DA-18C6-BHA. The mice were euthanized at 1 h, 4 h, and 24 h. The selected organs (liver, kidney, muscle, bone) and the blood were harvested, wet weighed, and the radioactivity was measured in a γ-counter (Figures 1 and 2 and Supporting Information). Both ^{89}Zr-DA-18C6-BHA (Figure 1) and ^{89}Zr-DFO (Figure 2) showed rapid blood clearance and low uptake in normal organs (<0.44% ID/g) over 24 hours. ^{89}Zr-DA-18C6-BHA exhibited a negligible level of radioactivity in blood at all time points (≤0.04 %ID/g). The bone and muscle uptake of ^{89}Zr- DA-18C6-BHA remained minimal at all time points (≤0.11% ID/g). ^{89}Zr-DA-18C6-BHA exhibited the highest retention in liver (0.44 %ID/g at 4 h). Renal uptake of ^{89}Zr-DA-18C6-BHA was decreased from 0.21 %ID/g (1 h) to 0.12 %ID/g (24 h). ^{89}Zr-DFO showed a minimal uptake in blood, liver, and muscle at 24 hours (0.02 %ID/g, 0.01 %ID/g, and 0.03 %ID/g, respectively). ^{89}Zr-DFO showed relatively higher accumulation of radioactivity in the kidney at all time points (≤0.25 %ID/g) when compared to other organs. ^{89}Zr-DFO displayed minimal bone uptake which was decreased from 0.09 %ID/g (4 h) to 0.04 %ID/g (24 h). The *in vivo* data suggest that both ^{89}Zr-DFO and ^{89}Zr-DA-18C6-BHA presented an excellent biodistribution profile in normal mice as evidenced by low uptake in blood and normal organs including bone.

In summary, the new chelator (DA-18C6-BHA) built on diaza-18-crown-6 containing two hydroxamate donor groups was synthesized and evaluated. The new chelator rapidly bound to ^{89}Zr, and the corresponding ^{89}Zr-labeled DA-18C6-BHA was shown to display high complex stability in human serum and apotransferrin for 7 days. When challenged by excess EDTA solution, ^{89}Zr-DA-18C6-BHA remained inert and was favorably compared to ^{89}Zr-DFO for complex stability. Both ^{89}Zr–DA-18C6-BHA and ^{89}Zr–DFO were shown to be stable in mice and have favorable biodistribution profiles with low bone uptake. The radiolabeling and *in vitro* and *in vivo* complex stability data clearly demonstrate that DA-18C6-BHA is an efficient chelator for ^{89}Zr radiolabeling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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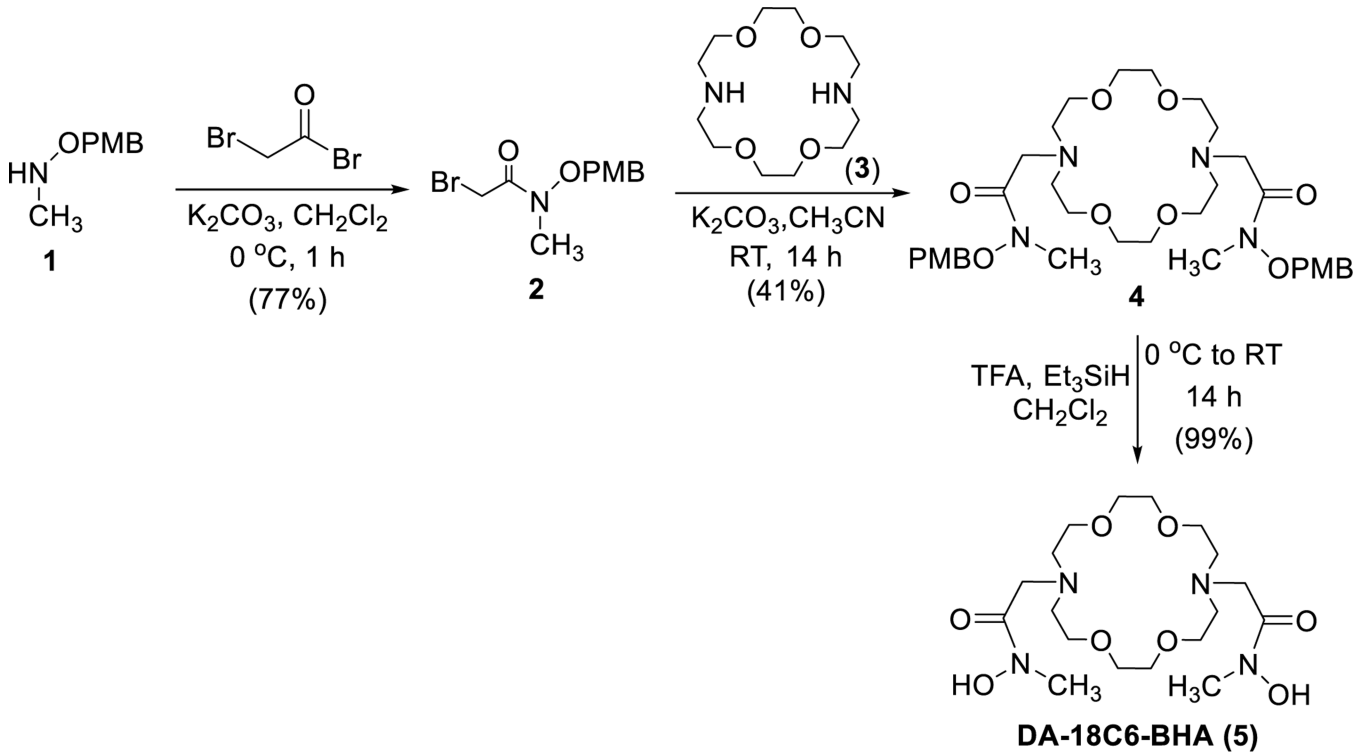

Scheme 1.
Synthesis of chelator DA-18C6-BHA (5) for Zr(IV)-89

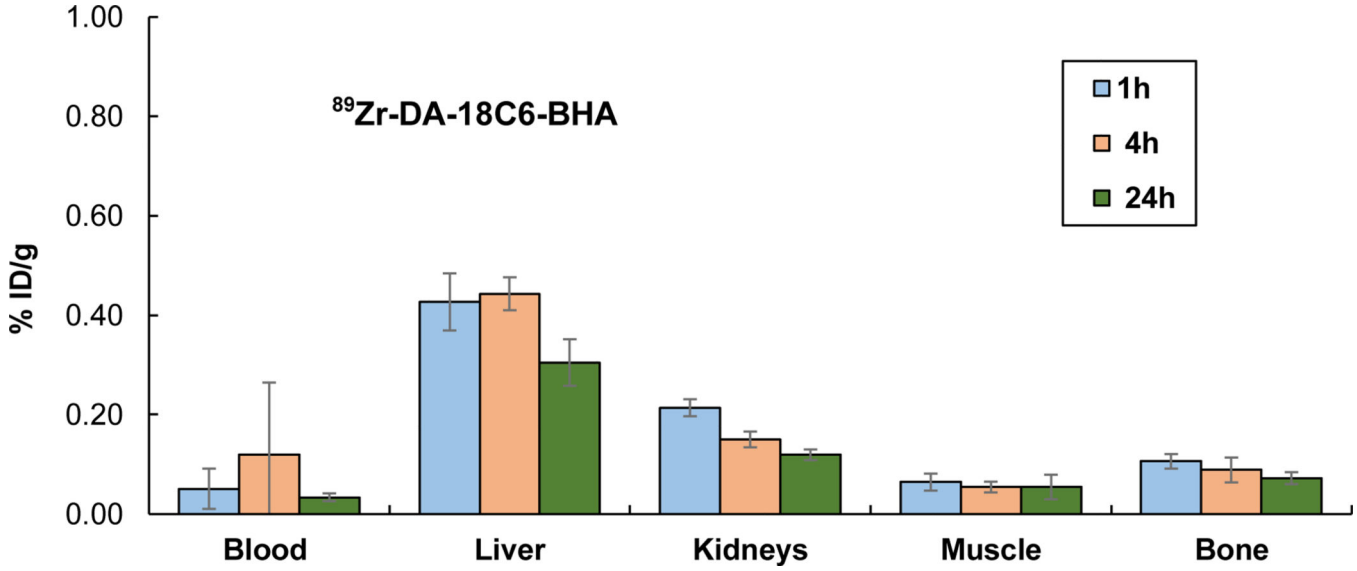

Figure 1.
In Vivo Biodistribution of ^{89}Zr-DA-18C6-BHA in CD-1 mice (n = 4, intravenous injection)

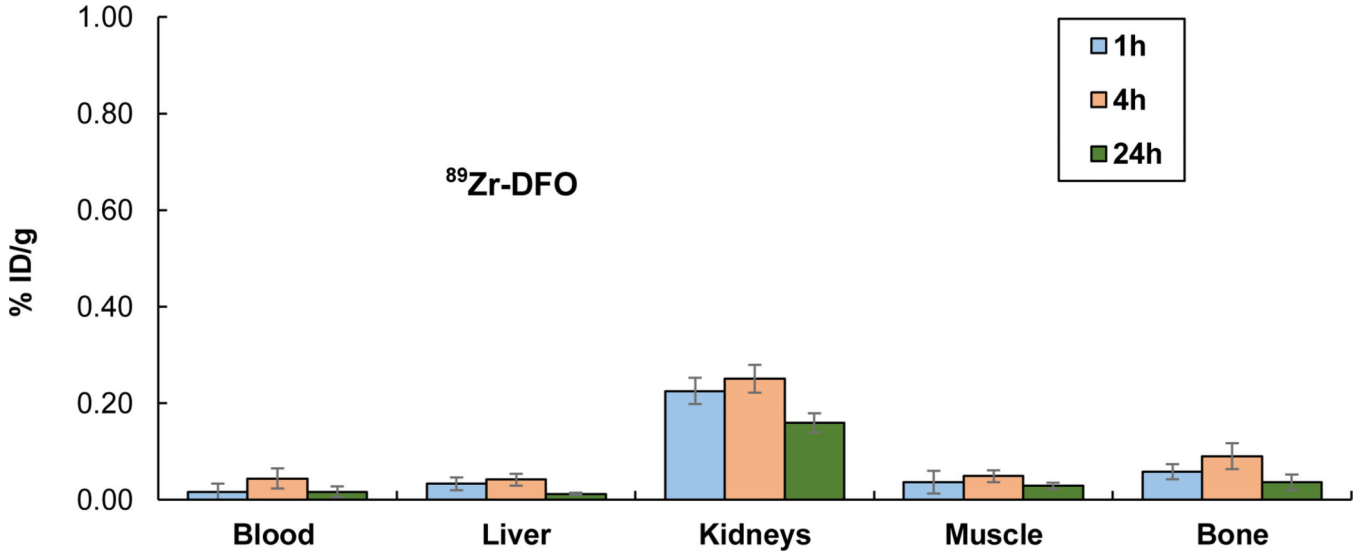

Figure 2.
In Vivo Biodistribution of ^{89}Zr-DFO in CD-1 mice (n = 4, intravenous injection)

Table 1.

Complex Stability of ^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO in human serum (pH 7.0, 37°C, ITLC, duplicate).

	^{89}Zr-Chelator Complex (%)	
Time	**DA-18C6-BHA**	**DFO**
0 h	99.4 ± 0.1	99.5 ± 0.5
24 h	99.1 ± 0.0	99.2 ± 0.1
48 h	98.8 ± 0.7	99.3 ± 0.3
72 h	98.7 ± 0.2	98.7 ± 0.8
168 h	99.0 ± 0.3	99.0 ± 0.5

Table 2.

Complex Stability of ^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO in EDTA solution (pH 7.0, 37°C, ITLC, duplicate).

	^{89}Zr-Chelator Complex (%)	
Time	**DA-18C6-BHA**	**DFO**
0 h	99.3 ± 0.1	99.4 ± 0.1
1 h	99.6 ± 0.1	98.1 ± 0.3
24 h	99.0 ± 0.2	92.5 ± 0.4
48 h	99.0 ± 0.2	89.1 ± 0.2
168 h	99.1 ± 0.3	87.3 ±1.8

Table 3.

Complex Stability of ^{89}Zr-DA-18C6-BHA and ^{89}Zr-DFO in apotransferrin solution (pH 7.0, 37°C, ITLC, duplicate).

	^{89}Zr-Chelator Complex (%)	
Time	**DA-18C6-BHA**	**DFO**
0 h	98.8 ± 0.5	99.0 ± 0.1
24 h	98.3 ± 0.4	98.8 ± 0.2
48 h	98.0 ± 0.2	97.8 ± 0.6
72 h	97.9 ± 0.4	98.0 ± 0.3
168 h	98.8 ± 0.3	98.4 ± 0.4