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Synthesis and evaluation of bifunctional tetrahydroxamate chelators for labeling antibodies with ^{89}Zr for imaging with positron emission tomography

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ABSTRACT

Two novel bifunctional tetrahydroxamate chelators **3** and **4** were synthesized and evaluated for labeling antibodies with ^{89}Zr for positron emission tomography imaging. Compared to previously reported tetrahydroxamate chelators **1** and **2** with an iminodiacetamide backbone, **3** and **4** were based on an extended iminodipropionamide and dipropylenetriamine backbone, respectively. Trastuzumab conjugates of **3** and **4** were efficiently labeled with ^{89}Zr (>95% radiochemical yield). The in vitro plasma stability of ^{89}Zr-**4**-Trastuzumab and especially ^{89}Zr-**3**-Trastuzumab was greatly improved over previously reported ^{89}Zr-**1**-Trastuzumab and ^{89}Zr-**2**-Trastuzumab, but their demetalation remained higher and faster than ^{89}Zr-deferoxamine (DFO)-Trastuzumab. These observations were confirmed by PET imaging and biodistribution in mice, with significant higher bone uptake for ^{89}Zr-**4**-Trastuzumab, followed by ^{89}Zr-**3**-Trastuzumab, and to a lesser extent for ^{89}Zr-DFO-Trastuzumab. Molecular modeling showed that **3** and **4** with an extended backbone could form eight-coordinate Zr-complexes as compared to only seven-coordinate Zr-complexes of **1** and **2**. Our data suggest further elongation of linker length between hydroxamate motifs of this class of chelators is needed to reach a better Zr-coordination configuration and improve in vivo stability.

Positron emission tomography (PET) is a non-invasive molecular imaging modality that uses positron-emitter-tagged molecules to locate the distribution and assess the activity/function of targets of interest. PET is highly sensitive and quantifiable, and is routinely used in the clinic for the diagnosis, prognosis and treatment monitoring of cancer.[1,2] With the increasing number of monoclonal antibodies (mAbs) and antibody-drug conjugates being approved for cancer therapy, there is an urgent need to radiolabel these mAbs for PET imaging. Imaging with radiolabeled mAbs can be used to identify potential responders for treatment, and to monitor the change of target expression level during the treatment. The positron emitter used for radiolabeling has to match the slow pharmacokinetics of mAbs, and provide sufficient signal to obtain high-resolution PET images.

Among the various positron emitters used in the clinic, zirconium-89 (^{89}Zr) is considered the most attractive one for radiolabeling mAbs for PET imaging.[3] This is because its 78.4-h half-life matches well with the slow pharmacokinetics of mAbs. Its low positron energy (E_{max} = 908.97 keV) and high positron yield (99% per decay) provide high-resolution PET images. In addition, ^{89}Zr can be produced easily using medical cyclotrons via proton bombardment on natural yttrium target (100% abundance of ^{89}Y). Moreover, the production of ^{89}Zr using ^{89}Y targets as well as the methodologies for extracting ^{89}Zr from bombarded ^{89}Y target have been well established.[3,4]

Despite the great potential of ^{89}Zr for labeling mAbs for PET imaging, it is actually not trivial to radiolabel mAbs with ^{89}Zr, and have the ^{89}Zr-mAb complexes be stable in vivo for several days without suffering from demetalation. Currently, the derivatives of deferoxamine (DFO, Fig. 1), a bacterial siderophore, are the only

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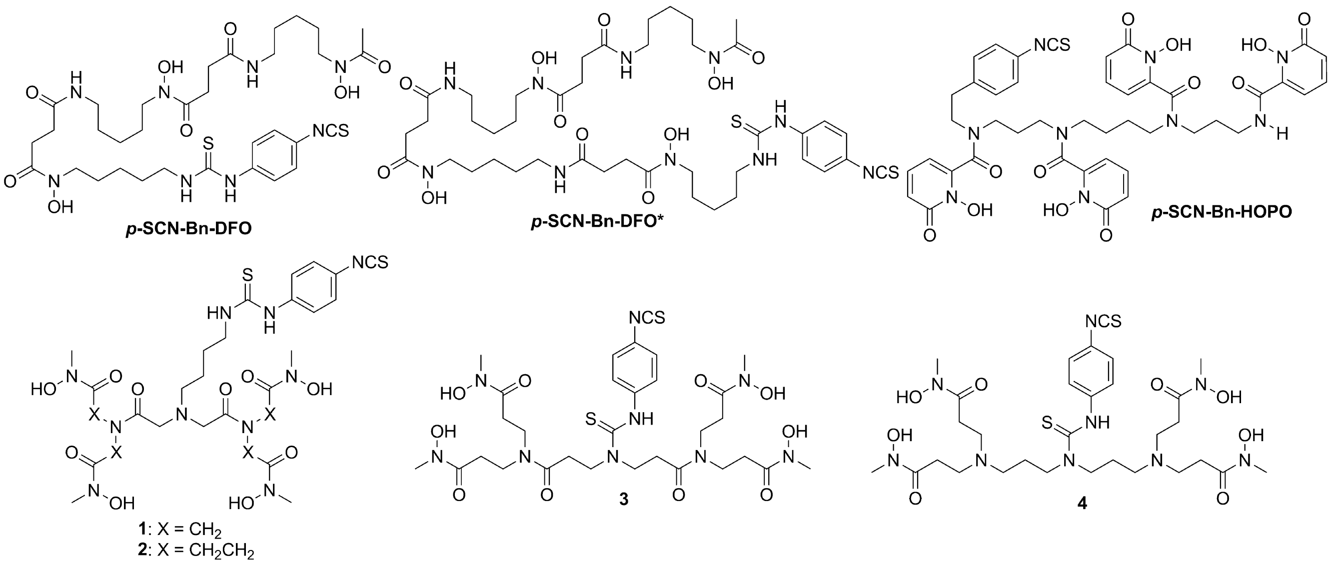

Fig. 1. Chemical structures of reported bifunctional tetrahydroxamate chelators for labeling antibodies with ^{89}Zr.

commercially available bifunctional chelators for labeling mAbs with ^{89}Zr for PET imaging. However, ^{89}Zr-labeled DFO-mAb conjugates are not stable enough in vivo as high bone uptake (up to 15.1%ID/g, 5 days post-injection) resulting from demetalation is constantly observed in preclinical studies in mice.[5] DFO has three hydroxamate groups for metal complexation, and is a hexadentate chelator. The in vivo instability of ^{89}Zr-DFO complex has been suggested to be due to the inability of DFO to provide octadentate complexation required to stably chelate Zr^{4+}.[6,7]

In the past 10 years, several attempts to develop bifunctional octadentate chelators for labeling mAbs with ^{89}Zr have been reported.[8–15] However, only two of these chelators (*p*-SCN-Bn-HOPO and *p*-SCN-Bn-DFO*, Fig. 1) resulted in more stable ^{89}Zr-labeled mAbs when compared with the gold standard *p*-SCN-Bn-DFO.[12,14] The synthesis and evaluation of *p*-SCN-Bn-HOPO was reported by Deri et al.[12] ^{89}Zr-HOPO-Trastuzumab showed much less bone uptake than that of ^{89}Zr-DFO-Trastuzumab in mice at Day 14 post-injection (2.4 ± 0.3%ID/g vs 17.0 ± 4.1%ID/g).[12] The synthesis of the octadentate chelator DFO* was first reported by Mindt's group,[6] and the synthesis and evaluation of its bifunctional derivative *p*-SCN-Bn-DFO* was reported by Vugts et al.[14] Compared to ^{89}Zr-DFO-Trastuzumab, ^{89}Zr-DFO*-Trastuzumab showed much less uptake in bone (knees) in mice at Day 7 post-injection (1.38 ± 0.23 vs 8.20 ± 2.94% ID/g).[14] While promising, neither *p*-SCN-Bn-HOPO nor *p*-SCN-Bn-DFO* are commercially available yet. The reported procedures for the preparation of these two bifunctional chelators are not trivial, and only small scale batch production (few mg) of both chelators has been demonstrated in the literature.[12,14]

Recently, we reported the synthesis and evaluation of two compact and symmetrical bifunctional tetrahydroxamate chelators **1** and **2** (Fig. 1) based on an iminodiacetamide backbone.[15] Both **1** and **2** were facilely synthesized, and their mAbs conjugates could chelate ^{89}Zr in >90% radiochemical yields. However, biodistribution study in mice showed that bone uptake values of ^{89}Zr-**1**-Trastuzumab (19.5 ± 3.59%ID/g) and ^{89}Zr-**2**-Trastuzumab (18.3 ± 2.91% ID/g) were significantly higher than that of ^{89}Zr-DFO-Trastuzumab (7.58 ± 0.36%ID/g) at Day 3 post-injection.[15] The inferior in vivo stability of ^{89}Zr complexes of **1** and **2** was possibly the results of improper coordination arrangement. This could be due to the steric constraints imposed by the 2,2′-iminodiacetamide backbone and/or the shorter arm (for example, $X = CH_2$ for **1**, Fig. 1) of hydroxamate moieties.

To improve the coordination arrangement, we synthesized and evaluated two bifunctional tetrahydroxamate chelators **3** and **4** (Fig. 1). Instead of the iminodiacetamide backbone in **1** and **2**, **3** was designed based on an extended iminodipropionamide backbone. To further increase flexibility, **4** was prepared based on a diisopropylenetriamine backbone. The arm length of the four hydroxamate moieties remained the same at $X = CH_2CH_2$ as in **2** (Fig. 1). To simplify the design and their preparation, the isothiocyanate group on **3** and **4** for mAb conjugation was derived directly from the central amino group without an additional butylene pendant arm shown in **1** and **2**.

Synthesis of the bifunctional tetrahydroxamate chelator **3** with an iminodipropionamide backbone is depicted in Scheme 1. Coupling of di-(2,3,5,6-tetrafluoro)phenyl 3,3′-(*tert*-butoxycarbonylazanediyl)dipropionate **5**[15] and 3,3′-azanediylbis(*N*-(benzyloxy)-*N*-methylpropionamide) **6**[15] in dioxane afforded **7** in 91% yield. The *N*-Boc protecting group of **7** was removed after treatment with trifluoroacetic acid, and the free amine **8** was obtained quantitatively. Subsequent Pd-catalyzed hydrogenation removed all four benzyl protecting groups, and the free tetrahydroxamate ligand **9** was obtained in 99% yield. Treating **9** with excess 1,4-phenylene diisothiocyanate provided the bifunctional chelator **3** in 15% yield.

The procedures for preparation of the bifunctional tetrahydroxamate chelator **4** with a dipropylenetriamine backbone are shown in Scheme 2. Coupling *N*-Boc-3,3′-diaminodipropylamine **10**[16] and *N*-methyl-*N*-(benzyloxy)-2-propenamide **11**[17] via Michael addition afforded **12** in 63% yield. Subsequent removal of *N*-Boc and *O*-benzyl protecting groups with trifluoroacetic acid and Pd-catalyzed hydrogenation, respectively, provided the free amine **13** and the free tetrahydroxamate ligand **14** quantitatively. To add the additional isothiocyanate function group for antibody coupling, **14** was treated with excess 1,4-phenylene diisothiocyanate, and the desired bifunctional tetrahydroxamate chelator **4** was isolated in 24% yield.

The efficiency of new bifunctional tetrahydroxamate chelators **3** and **4** for labeling mAbs with ^{89}Zr was evaluated using Trastuzumab, and compared with DFO. Using a 5:1 chelator-to-Trastuzumab ratio, the conjugation yielded on average 1.24 ± 0.08 of DFO, 1.62 ± 0.20 of chelator **3** and 1.41 ± 0.26 of chelator **4** per