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A solid phase-assisted approach for the facile synthesis of a highly water-soluble zirconium-89 chelator for radiopharmaceutical development

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Nuclear medicine has seen impressive growth in recent years. An important development in this field was through the application of new radionuclides, e.g., ⁸⁹Zr ($t_{1/2}$ = 78.4 h, β^+ 0.396 MeV), the physical properties of which allows the use of antibodies as biological vectors for specific cancer targeting in combination with high resolution imaging by positron emission tomography (PET). The most commonly used chelator for ⁸⁹Zr-based PET imaging is the hexadentate desferrioxamine (DFO) chelator. However, due to the instability of this complex, there has been a strong push towards the development of octadentate chelators. We report an ether derivative, oxoDFO*, resembling the motif of DFO with four hydroxamic acid groups for binding of the radiometal and four ether linkages to increase the water solubility. Very importantly, the synthesis of this chelator follows a solid phase-assisted approach allowing for an attractive synthetic methodology widening the scope for access to DFO-like chelators in highly efficient synthetic sequences.

The radionuclide zirconium-89 (⁸⁹Zr) has gained much interest in recent years for applications in nuclear medicine due to its intrinsic properties.¹⁻⁴ In contrast to other commonly utilised PET radionuclides such as fluorine-18 or gallium-68, the physical half-life of ⁸⁹Zr ($t_{1/2}$ = 78.4 h) matches the biological half-life of most antibodies (usually in the order of days).¹ This enables the antibody coupled zirconium complex to accumulate at the site of the tumor leading to an improvement in signal-to-noise ratios at late time points. Furthermore, good spatial resolution can also be achieved *via* the use of ⁸⁹Zr due to its relatively low positron emission

energy (β^+ 0.396 MeV).² To date, the only chelator for complexation of ^{89}Zr utilised in the clinic is the desferrioxamine (DFO) chelator (Figure 1, **1**). Preclinical evidence indicates sequestration of ^{89}Zr in radiation sensitive bones due to the release of ^{89}Zr from the chelator leading to reduced signal-to-noise ratios while also raising concerns of the use of the chelator in clinical applications.⁵⁻⁷ As a consequence, considerable effort has been placed in the development of new ^{89}Zr chelators with increased *in vivo* stability.

It has been proposed that the instability of the ^{89}Zr -DFO complex is a direct result of the hexadentate nature of DFO, which allows two coordination sites of the metal to be occupied by water molecules. Consequently, in recent years chelators with an increasing denticity have been reported in order to enhance the *in vivo* stability of ^{89}Zr chelators by saturating the ^{89}Zr coordination sphere.⁸⁻¹⁷ This concept led to the development of DFO*, an octadentate ^{89}Zr chelator which has pioneered the way for innovation in the field of ^{89}Zr chelators and which is now commercially available (Figure 1, **2**).⁸ Superior characteristics of the radioimmunoconjugate, ^{89}Zr -DFO*-trastuzumab, were established *in vivo* in comparison to the reference ^{89}Zr -DFO-trastuzumab.⁹ Comparable tumor targeting was observed with less unspecific uptake in bones as well as non-targeted tissue and organs. However, the low water solubility of DFO* could potentially impede protein modification protocols. With this in mind, we have designed an octadentate derivative of DFO*, termed oxoDFO*, which incorporates ether linkages aimed to enhance water solubility (Figure 1, **3**). Binding of ^{89}Zr to the oxoDFO* derivative mimics that of DFO* with four hydroxamic acid groups.

Unaware of work in progress, a structurally similar ether-based compound was recently published by Codd and co-workers utilising a “microbiological-chemical synthetic” approach.¹⁸ However, this approach plagued authors with low yielding reactions (2 mg, 21%) limiting access and wider application of their ether based

Figure 1: Structures of DFO, DFO* and oxoDFO*.

product. In addition to the investigation of the oxoDFO* structure as a potential ^{89}Zr chelator, we had also intended to explore a solid phase-assisted synthetic approach as an attractive alternative of accessing DFO-like structures. To date, synthetic procedures for the access of DFO-like compounds have solely occurred in the solution phase with the exception of the work reported by Seibold *et al.*¹⁹ A solid phase technique was attempted, however, solution phase chemistry proved to be more successful in this case. No solid phase-assisted synthetic methodology has been successfully applied to the synthesis of these types of compounds. The advantages of such an approach include high yielding reactions, simplified multigram scale synthesis, and fewer purification steps. The solid phase methodology allows for a wide scope for the access of similar chelators in the future. Herein, we report the efficient synthesis towards an oxoDFO* chelator capable of conjugation to biomolecules for targeted radiolabelling.

The proposed synthetic route for access to the oxoDFO* consists of two distinct steps; construction of the monomer unit and solid phase-assisted chemistry to build the chelator. Firstly, the monomeric Fmoc-protected ether chains (**7**) were prepared on a multiple gram scale in six steps with an excellent overall yield of 60% (Scheme 1). Step-wise nucleophilic substitution of the bromine atoms of **4** with potassium phthalimide and *tert*-butyl

(benzyloxy)carbamate afforded intermediate **5**. Sequential phthalimide deprotection and Fmoc protection of this analogue yielded the Fmoc derivative **6**. The final step consisted of Boc deprotection followed by consecutive coupling to succinic anhydride which yielded the final monomeric protected hydroxamate unit **7** for solid phase-assisted synthesis of the ^{89}Zr chelator.

Scheme 1: Synthesis of 10-(benzyloxy)-1-(9H-fluoren-9-yl)-3,11-dioxo-2,7-dioxa-4,10-diazatetradecan-14-oic acid (**7**); i) DMF, 80 °C, 48 h, N_2 , 89%; ii) *tert*-butyl (benzyloxy)carbamate, NaI, NaH, DMF, 85 °C, 24 h, N_2 , 79%; iii) N_2H_4 , EtOH, 90 °C, 90 mins; iv) FmocCl, Na_2CO_3 , 1,4-dioxane, H_2O , RT, 3 h, two steps 99%; v) TFA, 20 °C, 2 h; vi) DIPEA, succinic anhydride, DMF, RT, 1 h, N_2 , two steps 88%.

For this purpose, the monomeric unit was anchored to the Fmoc deprotected TentaGel S Ram resin (Scheme 2). After further Fmoc deprotection, of our monomer in this case, elongation of the tethered chain occurred using a HATU coupling cocktail using standard Fmoc solid phase peptide synthesis (SPPS). Fmoc deprotection and sequential monomer coupling was completed four times in total to build our resin-bound protected oxoDFO* analogue, **9**. Fmoc deprotection and cleavage of the benzyl protected oxoDFO* from the resin resulted in **10** in 91% yield used in the next step without further purification. The final oxo chelator, **3**, was obtained after palladium catalyzed hydrogenation in 77% yield.

It has been established within the literature that isothiocyanates (NCS) are able to form stable linkages with monoclonal antibodies through conjugation to the primary amines of Lysine residues.²⁰ As a result, addition of an isothiocyanate functionality to oxoDFO* was explored in order to obtain a bifunctional chelating agent (BFCA) for radiopharmaceutical development. The oxoDFO* analogue, **3**, was coupled to 4-isothiocyanatobenzoic acid to yield the isothiocyanate derivative **11**, oxoDFO*-*p*Phe-NCS (Scheme 2). The isothiocyanate derivative was isolated after preparative HPLC in a high purity ($\geq 95\%$) as determined by LC analysis. Low yields of this reaction could be due to the lack of selectivity between the two terminal groups but could potentially be improved by attempting to couple 4-isothiocyanatobenzoic acid while resin bound. OxoDFO*-*p*Phe-NCS **11**, is ideally suited for coupling to monoclonal antibodies for applications in immuno-PET imaging.

Scheme 2: Solid phase-assisted synthesis of oxoDFO* analogue, **3**, and its NCS derivative oxoDFO*-pPhe-NCS, **11**.; i) Deprotection: 20 vol. % piperidine/DMF ii) Coupling: **7**, HATU, DIPEA/2,6-lutidine/DMF (3.5/3.5/93 : v/v/v) iii) Deprotection: 20 vol. % piperidine/DMF; iv) Cleavage: TFA/TIPS/H₂O (38/1/1 : v/v/v), 91%; v) 10% Pd/C, H₂, 5 bar, RT, 4 h, 77%; vi) 4-isothiocyanatobenzoic acid, HATU, DIPEA, DMF, 12 h, RT, N₂, 15%.

In order to demonstrate the feasibility of radiolabelling, complexation of ^{nat}Zr(IV) to the ligand, oxoDFO*, **3**, was examined. Complexation of ^{nat}Zr(IV) was achieved *via* a previously reported procedure⁸ at RT and neutral pH which are optimal reaction conditions for the radiolabelling of sensitive biomolecules (e.g., mAb). HRMS data supporting the formation of the desired complex ($m/z_{\text{calc.}} = 934.2706$ [M+Na]⁺, $m/z_{\text{obs.}} = 934.2706$ [M+Na]⁺).

The hydrophobicity of oxoDFO*, **3**, oxoDFO*-pPhe-NCS, **11**, and [^{nat}Zr-**3**] were determined *via* the shake flask method with distribution coefficients ($\log D_{7.4}$) of -1.5 ± 0.2 , -1.2 ± 0.1 and -2.1 ± 0.1 respectively. As anticipated, oxoDFO* derivatives exhibited a remarkably increased hydrophilicity in comparison to the corresponding DFO* ($\log D_{7.4} = -0.4397$) analogue. Furthermore, our oxoDFO*analogue, **3**, was highly water soluble in the absence of co-solvents (approx. >50 mg/mL).

Conclusions

Research and development into new chelators for applications in ⁸⁹Zr-PET imaging has undergone remarkable growth in recent years. Recently reported octadentate chelators provide an improvement on the *in vivo* stability of ⁸⁹Zr chelators. Although a set of requirements exist for a potential (radio)metal chelator to make it to market, it is crucial that synthetic targets can be obtained in facile, high yielding synthetic sequences and have the potential to be accessed easily on a multi-gram scale. Our solid phase-assisted approach presents a method which meets these requirements. We have demonstrated this concept through a highly efficient solid phase-assisted synthetic methodology for the preparation of an ether based DFO-like chelator. The octadentate oxoDFO* chelator, **3**, incorporates four ether chains resulting in a highly hydrophilic chelator ($\log D_{7.4} = -1.5 \pm 0.2$). The prepared isothiocyanato BFCA, **11**, maintains its hydrophilicity ($\log D_{7.4} = -1.2 \pm 0.1$) and highlights the potential applications of the oxoDFO* structure for bioconjugations and applications in immuno-PET imaging. The successfully employed solid phase-assisted approach should inspire further the use of this methodology in the development of future DFO-like target structures to ensure highly efficient syntheses for clinical applications of these exciting new class of ⁸⁹Zr chelators. As discussed with Codd and co-workers, the radiolabelling of oxoDFO* with ⁸⁹Zr will be published in due course.

Conflicts of interest

There are no conflicts to declare.

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