Revival of TE2A; a better chelate for Cu(II) ions than TETA?†

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Received (in Cambridge, UK) 8th December 2009, Accepted 25th February 2010
First published as an Advance Article on the web 23rd March 2010
DOI: 10.1039/b925703a

A highly effective synthetic route for TE2A was developed and the ^{64}Cu-labeled TE2A complexes showed higher kinetic inertness and faster clearance than most commonly used TETA analogs.

Interest in the synthesis, structures and properties of metal complexes based on various types of *N*-functionalized polyazamacrocycles has increased tremendously over the last few decades, due to their potential application in the field of medical imaging and therapy (*e.g.* MRI contrast agents, diagnostic radiopharmaceuticals and radioimmunotherapy).[1–3] In particular, cyclen and cyclam backbones containing *N*-acetic acid pendant arms *e.g.* DOTA,[4] DO2A,[5] TETA,[6] cross-bridged-DO2A (CB-DO2A),[7] cross-bridged-TE2A (CB-TE2A)[6,8] and their derivatives have been intensively studied as potential bifunctional chelates (BFCs) for imaging and therapy (Fig. 1). The metal complexes of these BFC agents are found to show different levels of kinetic inertness and thermodynamic stability when used *in vivo*, depending on their ligand structure.[9–12] The overall charge of the metal complexes, which is determined mostly by the number of coordinated acetate pendent arms and the oxidation state of the metal ions, also has a huge effect on the clearance pattern of the complexes and activity uptake in non-target organs.[13]

The relationship between their structure and *in vivo* physical/biological properties is fairly well documented in the case of cyclen-based BFCs. However, very few studies have been carried out on some of their cyclam-based counterparts, in spite of the structural similarity between cyclen and cyclam. To our surprise, we found that although TE2A was first synthesized in 1988,[14] only a few metal complexes of TE2A were further reported by the same group in the early 1990s.[15] No systematic studies on the use of TE2A as a potential BFC have been carried out so far. When considering that the ^{64}Cu–DO2A complex shows faster clearance than its DOTA counterpart[11] and that the TE2A derivative, CB-TE2A, forms extremely stable Cu(II) complexes,[7] this oversight becomes all the more mysterious.

To the best of our knowledge, there is only one report on the synthesis of TE2A in the literature, which was published by Parker *et al.*[14,16] This synthetic procedure suffers from several serious disadvantages, such as the co-formation of various regioisomers (*cis* and two different *trans*) with other substituted by-products of the cyclam, tedious column purification, harsh reaction conditions and, therefore, a very low overall yield from the cyclam (16%). Even though twenty years have passed since the first report of TE2A, the regioselective synthesis of 1,8-*N*,*N*′-difunctionalized cyclams (*e.g.* TE2A) over the unwanted formation of other regioisomers and other substituted products is still a great challenge for the synthetic chemist.

Herein we report a new facile synthetic protocol for TE2A and demonstrates its usefulness as an effective chelate for ^{64}Cu.

First, we protected the cyclam using formaldehyde to give the tricyclic bis-aminal **2** containing two six-membered rings. The alkylation of compound **2** with *t*-butyl bromoacetate afforded the selective dialkylation at the 1,8-position without the formation of any *cis*-disubstituted product. The product **3** having two non-adjacent quaternary nitrogen atoms is insoluble in CH_3CN and was isolated by simple filtration (see Scheme 1).

The cleavage of the two aminal bridges and ester hydrolysis of **3** could be achieved in one step by treating it with 5 equivalent of KOH in an ethanol–water mixture (1 : 1) at 80 °C for 8 h, and the crude product was then run through Dowex ion-exchange resin (1X8, OH form) to afford the hydrochloride salt of TE2A in excellent yield. This new synthetic method for TE2A is very straightforward and very easy to handle. No tedious column purification is needed and the overall yield attained from the cyclam is 86%, which is much higher than the value of 16% achieved in the previously reported syntheses.[16] TE2A (**4**) and the intermediate (**3**) were fully characterized by NMR spectroscopy, elemental analysis and HR-MS.

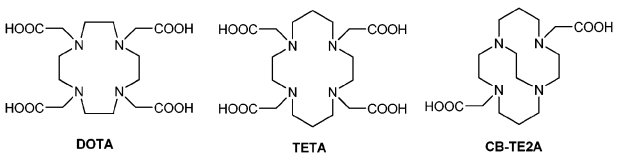

Fig. 1 Some commonly used bifunctional chelators.

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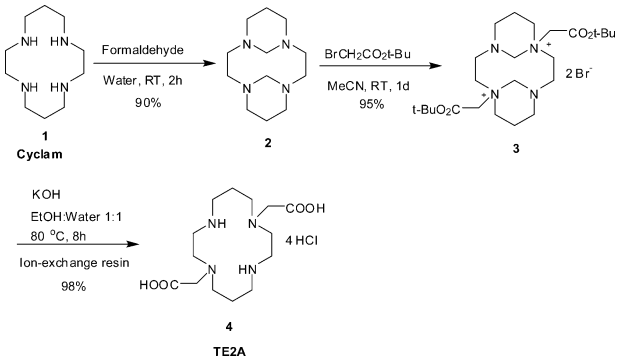
† Electronic supplementary information (ESI) available: Experimental procedures for the synthesis, acidic decomplexation, cyclic voltammetry, radiolabeling and biodistribution studies of TE2A. Half-lives for acid decomplexation and reduction potentials of Cu–DOTA and Cu–CB-TE2A. See DOI: 10.1039/b925703a



Scheme 1 Three-step synthesis of TE2A from cyclam.

The prepared TE2A was complexed with $Cu(ClO_4)_2{\cdot}6H_2O$ to afford its Cu(II) complex, Cu–TE2A, in 89% yield. The Cu–TETA complex was also prepared and characterized according to the literature method for comparison studies. Then, in order to evaluate the kinetic stabilities of the copper(II) complexes, which are known to be more important than their thermodynamic stabilities in determining the *in vivo* stabilities of Cu–tetraazamacrocycle complexes,[17] acidic decomplexation and cyclic voltammetry experiments were performed.[18]

The half-lives for the acid-assisted decomplexation of Cu–TE2A and Cu–TETA under pseudo first-order conditions at high HCl concentrations are summarized in Table 1. In spite of the structural similarity of the two ligands of Cu–TE2A and Cu–TETA, they showed dramatically different kinetic inertnesses to acidic decomplexation. The kinetic inertness of Cu–TE2A in 5 M HCl was at least one-order of magnitude higher than that of Cu–TETA. The half-life of Cu–TE2A in 12 M HCl (90 °C) was only 2.6 min, but this is still more than twice the value of 1.1 min for Cu–TETA. The progress of acidic decomplexation in 5 M HCl at 90 °C was also monitored by repeated injection of a reaction fraction into an HPLC system (Fig. 2a–b). As the integral of the intact Cu(II) complex peak decreased, the peak for free Cu(II) ions gradually increased. Whereas 3% of the Cu–TE2A complex still remained intact after 6 h, all of the Cu–TETA complexes were decomposed within 40 min.

Another important mechanism leading to the loss of the copper(II) ion from chelates in biological systems is the reduction of Cu(II) to Cu(I) and subsequent demetallation. Therefore, the reduction potential of Cu(II) complexes might provide a good guideline for predicting their *in vivo* stabilities.[17,18] Both the Cu–TE2A and Cu–TETA complexes yielded irreversible reduction voltammograms in 0.1 M aqueous sodium acetate solution adjusted to pH 7 (Fig. 2c–d). However, in our experiment, the reduction potential of Cu–TE2A was −1.10 V (*vs.* Ag/AgCl), while that of Cu–TETA was only −0.88 V, which means that Cu–TE2A is more difficult to reduce to the Cu(I) complex than Cu–TETA, by a factor of about 200 mV, and that Cu–TE2A might be more kinetically inert to reduction and subsequent demetallation of the Cu(I) ions under physiological conditions.[19]

This dramatic difference in the kinetic inertness to acidic decomplexation of the two Cu(II) complexes might be due to their different coordination geometries. The X-ray crystal

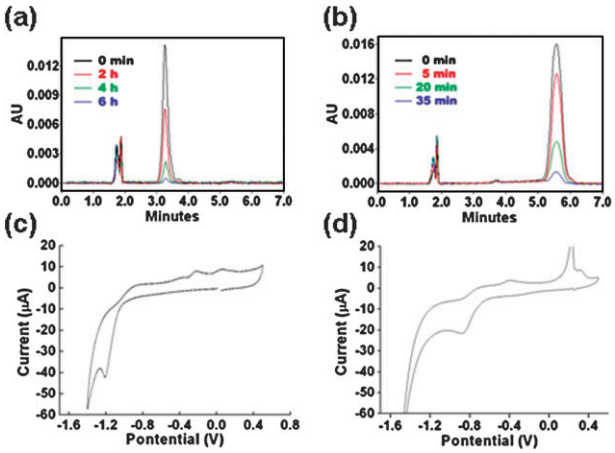


Fig. 2 Time-dependant HPLC chromatograms of Cu–TE2A (a) and Cu–TETA (b) during acidic decomplexation in 5 M HCl at 90 °C. Cyclic voltammograms (scan rate 100 mV s^{-1}, 0.1 M NaOAc, pH 7) of Cu–TE2A (c) and Cu–TETA (d).

structure of the Cu–TE2A complex revealed that the Cu(II) ion is strongly coordinated by the four nitrogen atoms and the two axial positions are occupied by two carboxylate oxygen atoms in the octahedral coordination geometry.[14,15] In this case, a strong N_4 in-plane ligand field is attained, which endows the complex with high kinetic inertness and thermodynamic stability. In contrast, the core coordination sphere of the Cu–TETA complex involves N_2O_2 coordination in the equatorial plane and two ring N_2 atoms in the axial positions, which may be the factor that increases its kinetic lability.[15,20]

Encouraged by the higher *in vitro* kinetic stability of Cu–TE2A, we decided to test the *in vivo* stability of the metal complexes labelled with radioactive copper ions. The ^{64}Cu radionuclide ($t_{1/2}$ = 12.7 h) is one of most widely studied radiometal ions in PET (positron emission tomography) imaging, and also shows good potential in the targeted radiotherapy of cancer, thanks to its attractive decay mode (β^+ 19%, β^- 40%).[21] TE2A was quantitatively radiolabeled with ^{64}Cu by the addition of $^{64}CuCl_2$ to a solution of TE2A in 0.1 M NH_4OAc (pH 6.8), followed by 20 min incubation at 30 °C. Within 5 min, the labeling yield of TE2A reached more than 90%. The radiolabeling yield was measured by radio-TLC and doubly confirmed by a radio-HPLC system.

The radiolabeled ^{64}Cu–TE2A did not show any noticeable degradation at 37 °C for 24 h in serum stability studies. We further carried out side by side comparative biodistribution studies using the ^{64}Cu–labeled TE2A and TETA in order to evaluate the *in vivo* stability and clearance of ^{64}Cu–TE2A (Fig. 3). Currently, TETA is one of the most commonly used ligands for radiolabeling studies of ^{64}Cu, because of the easy radiolabeling and excellent *in vivo* stability of the labeled complex. Although ^{64}Cu-labeled CB-TE2A was recently reported to show superior *in vivo* stability and higher resistance to transmetallation as compared to TETA, the utilization of CB-TE2A as a BFC for the radiolabeling of heat-sensitive biomolecules such as antibodies is limited by the harsh labeling conditions at elevated temperature (95 °C).[6]

The biodistribution data clearly showed that the ^{64}Cu–TE2A complex was excreted from the body faster than

Table 1 Half-lives for acid decomplexation[a] and reduction potentials of copper(II) complexes

Complex	5 M HCl, 50 °C	5 M HCl, 90 °C	12 M HCl, 90 °C	E_{red}/V *vs.* Ag/AgCl
Cu–TETA	4.1(3) h	4.7(4) min	1.1(3) min	−0.88 (irrev)
Cu–TE2A	92.6(2) h	46.2(8) min	2.6(5) min	−1.10 (irrev)

[a] Half-lives are mean values of 2–3 experiments.

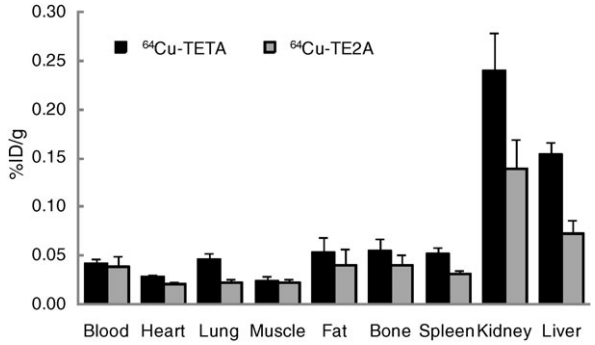


Fig. 3 Biodistribution data of ^{64}Cu-labelled TETA and TE2A at 24 h post-injection (five Sprague-Dawley rats per time point).

its TETA counterpart; the activity uptake of ^{64}Cu–TE2A was lower than that of ^{64}Cu–TETA in all organs at 24 h post-injection (Fig. 3), which suggests that ^{64}Cu–TE2A is more stable under physiological conditions than ^{64}Cu–TETA and that the transchelation of the free copper ion from the ^{64}Cu–TE2A complex is minimized.[13,22] The higher stability and faster clearance pattern of ^{64}Cu–TE2A might be attributed to its core coordination sphere and overall charge. While the overall charge of ^{64}Cu–TE2A is neutral, that of ^{64}Cu–TETA is −2 under physiological conditions because of the presence of two non-coordinated carboxylate groups.

In conclusion, the facile synthesis of TE2A, superior kinetic stability of the Cu–TE2A complex compared to that of Cu–TETA and quantitative labelling with ^{64}Cu at low temperature are expected to lead to the widespread use of TE2A as a new chelator for various copper(II) radionuclides. Furthermore, many new bifunctional chelators could be derived based on the TE2A backbone and utilized to label disease-specific peptides and antibodies. Easy access to TE2A would also allow for the further structural modification of various metal complexes and enable its application in diverse research fields, such as optical imaging probes, MRI reagents, and catalysis, in addition to medical imaging and radiotherapy.

This work was supported by MEST/KOSEF (Nuclear R&D Program, M20806000010808M060010810), a Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2008-331-E00262), and the Brain Korea 21 Project in 2009. The production of ^{64}Cu was supported partially by the QURI project of MOST and KOSEF. The Korea Basic Science Institute (Daegu) is acknowledged for the NMR and MS measurements.

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