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ORIGINAL ARTICLE

A New Synthesis of TE2A—a Potential Bifunctional Chelator for ^{64}Cu

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Abstract
Purpose The development of a new bifunctional chelator, which holds radiometals strongly in living systems, is a prerequisite for the successful application of disease-specific biomolecules to medical diagnosis and therapy. Recently, TE2A was reported to make kinetically more stable Cu(II) complexes than TETA. Herein, we report a new synthetic route to TE2A and explore its potential as a bifunctional chelator.
Methods TE2A was synthesized using the regioselective alkylation of benzyl bromoacetate and successive deprotection of the methylene bridge and benzyl group. Salt-free TE2A was radiolabeled with ^{64}Cu and microPET imaging was performed to follow the clearance pattern of the ^{64}Cu-TE2A complex. TE2A was conjugated with cyclic RGD peptide and the TE2A-c(RGDyK) conjugate was radiolabeled with ^{64}Cu.
Results TE2A was prepared in salt-free form from cyclam in an overall yield of 74%. The microPET images showed that ^{64}Cu-TE2A is excreted rapidly from the body by the kidney and liver. TE2A was successfully conjugated with c(RGDyK) peptide through one carboxylate group and the TE2A-c(RGDyK) conjugate was radiolabeled with ^{64}Cu in 94% yield within 30 min.
Conclusion TE2A can be used by itself as a bifunctional chelator without any further structural modification.

Keywords TE2A · Bifunctional chelator · ^{64}Cu · Conjugation · RGD peptide

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Introduction

The discovery and synthesis of tetraazacycloalkane derivatives have been the subject of growing interest during the past two decades owing to the ability of such macrocycles to coordinate various metal cations [1–4]. Macrocylic structures are extremely favorable for metal complexation. The strong affinity shown by polyamines and their selective binding of certain metals result in their use as metal catalysts [5], the active sites of metalloenzymes [6–8], agents which cleave phosphate esters [9, 10] including DNA [11] and RNA [12], molecular luminescence probes [13], MRI contrast agents [14], and nuclear radiophamaceuticals for diagnosis [15–17] and therapy [18].

Among the various macrocyclic compounds, DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) have been extensively used as bifunctional chelators (BFCs) for complexation with paramagnetic Gd ions and radioactive metal ions such as ^{64}Cu, ^{111}In, and ^{86}Y [19–21]. Generally, DOTA forms more stable complexes with lanthanide metal ions, while TETA forms more kinetically stable complexes with transition metal ions [22–24]. Especially, DOTA is known to have decreased stability with ^{64}Cu compared with TETA [20].

Very recently, we found that TE2A (1,8-*N*,*N*′-bis-(carboxymethyl)-1,4,8,11-tetraazacyclotetradecane) forms more kinetically stable Cu(II) complexes than TETA and reported the great potential of TE2A as a BFC [25]. However, the conjugation of TE2A with the amino group of biomolecules does not seem to be straightforward, because it has only two carboxylate groups and, upon conjugation, one of the two carboxylate pendants would be converted to an amide and its binding ability as a ligand would therefore be compromised. Furthermore, TE2A has two reactive secondary amines, which would compete with the amino group of biomolecules when the carboxylic acid group of TE2A is activated and, therefore, the conjugation reaction between TE2A and biomolecules such as peptides and antibodies would be very complicated, leading to the limited formation of the desired conjugate.

Herein, we report a new synthetic method to make salt-free TE2A and demonstrate that TE2A can be conjugated with small peptides and radiolabeled with ^{64}Cu in high yield, clearly indicating that it can be used by itself as a BFC without any further structural modification.

Materials and methods

General methods and materials

1,4,8,11-Tetraazacyclotetradecane (cyclam) was purchased from CheMatech (Dijon, France); c(RGDyK) peptide was purchased from FutureChem (Seoul, Korea). All other reagents and solvents were purchased from Sigma-Aldrich (St. Louis, Mo., USA) and were used as received. ^{64}Cu was produced at KIRAMS (Seoul, Korea) by the ^{64}Ni(p,n)^{64}Cu nuclear reaction using an MC50 Cyclotron (Scanditronix, Sweden). All ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian Unity Inova 500-MHz instrument. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS700 or Quattro Premier XE mass spectrometer. HPLC was accomplished on a Waters 600 series HPLC system and Zorbax Agilent Prep-C18 column (21.2× 100 mm, 5 μm). The radio-TLC measurements were performed using a Bioscan 200 imaging scanner (Bioscan, Washington, D.C., USA).

The bisaminal compound, 1,4,8,11-tetraazatricyclo [9.3.1.1^{4,8}]-hexadecane (**1**) was prepared by the modification and scale-up of the previously reported method [26].

Optimization of alkylation reaction conditions

A mixed solution of 1,4,8,11-tetraazatricyclo[9.3.1.1^{4,8}]-hexadecane (**1**, 0.325 g, 1.44 mmol) and different numbers of equivalents (two– to fourfold) of benzyl bromoacetate in various solvents (20 ml) was stirred at room temperature for 24 h. The precipitated solid was filtered, washed with CH_3CN (2×10 ml), dried under a vacuum, and weighed to calculate the isolated yield.

Preparation of TE2A

*1,8-N,N′-Bis-(benzyloxycarbonylmethyl)-4,11-diazoniatricyclo[9.3.1.1^{4,8}]hexadecane dibromide (**2**)* Four equivalents of benzyl bromoacetate (10.7 ml, 15.64 g, 68.28 mmol) was added in one portion to a stirred solution of **1** (3.83 g, 17.07 mmol) in CH_3CN (50 ml). The reaction mixture was stirred at room temperature for 24 h. The yellowish-white precipitate formed was then filtered, washed with CH_3CN (2×25 ml) and dried under a vacuum. The crude product was recrystallized in ethanol to give a white solid **2** (10.25 g, 88% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.32–7.41(m, 10H, ArH), 5.16(s, 4H), 3.52(s, 4H), 3.33 (s, 4H), 3.09(br s, 8H), 2.85(br s, 4H), 2.75(t, 4H, J=5 Hz), 1.86(br s, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.20, 135.54, 128.46, 128.21, 128.03, 66.43, 55.97, 54.06, 52.80, 51.27, 47.39, 44.15, 22.15, 18.52. HRMS (ESI): calculated for $C_{30}H_{42}N_4O_4$, 523.3284 [(M+H)$^+$]; found, 523.3281 [(M+H)$^+$].

*1,8-N,N′-Bis-(benzyloxycarbonylmethyl)-1,4,8,11-tetraazacyclotetradecane (**3**)* A 3 M NaOH solution (100 ml) was added to **2** (2.25 g, 3.29 mmol). After stirring for 3 h, the resultant solution was extracted with $CHCl_3$ (3×100 ml). The combined organic phases were washed by brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure to give an oil, which solidified on standing. (1.55 g, 95% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20–7.14(m, 10H, ArH), 4.92(s, 4H), 3.25(s, 4H), 2.71–2.66(m, 12H), 2.48(t, 4H, J=4.2 Hz), 1.70(br s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.36, 135.09, 128.34, 128.12, 127.84, 66.21, 54.70, 54.01, 51.96, 49.12, 46.36, 24.39. HRMS (FAB): calculated for $C_{28}H_{41}N_4O_4$, 497.3128 [(M+H)$^+$]; found, 497.3129 [(M+H)$^+$].

*1,8-N,N′-Bis-(carboxymethyl)-1,4,8,11-tetraazacyclotetradecane, TE2A (**4**)* To a solution of **3** (3.5 g, 7.04 mmol) in anhydrous ethanol (40 ml), 10% Pd/C (1.05 g) was added. The resulting mixture was stirred under H_2 gas at room temperature for 10 h. The reaction mixture was filtered through a celite pad, which was washed with methanol (2× 10 ml). The combined filtrate was evaporated under a vacuum to give an oily residue, which was triturated with Et_2O to provide an off-white solid. (2.19 g, 98% yield). ^{1}H NMR (500 MHz, D_2O): δ 3.48(br s, 2H), 3.0–3.2(m, 10H), 2.80(br s, 6H), 2.67(br s, 2H), 1.84(br s, 4H); ^{13}C NMR (125 MHz, D_2O): δ 179.0, 56.3, 55.7, 48.9, 45.4, 22.8; HRMS (FAB): calculated for $C_{24}H_{28}N_4O_4$, 317.2189 [(M+H)$^+$]; found, 317.2185 [(M+H)$^+$].

Radiolabeling of TE2A with ^{64}Cu

No carrier added $^{64}CuCl_2$ in 0.01 *N* HCl (5 μl, 0.4–0.5 mCi) was added to 100 μl of a 5 mM solution of the ligand TE2A in a buffer solution (0.1 M ammonium acetate) at pH 6.8, followed by 20 min incubation at 30°C. The formation of the complex was verified by radio-TLC using methanol:10% ammonium acetate (1:1 v/v) as the mobile phase on a silica plate. Distilled water was also tried as a solvent instead of 0.1 M ammonium acetate buffer solution.

MicroPET imaging studies

All animal experiments were conducted according to the guidelines of Kyungpook National University. Seven-week-old BALB/c mice (male, ~22 g) were used for the MicroPET imaging study. A Concorde MicroPET R4 Rodent Model scanner (Concorde Microsystems, Tenn., Knoxville, USA) or MicroPET scanning (Inveon Imaging system, Siemens, Erlangen, Germany) was used for the MicroPET imaging study. The mice were scanned in the supine position in bed and anesthetized by 1–2% isoflurane in 100% O_2 for imaging. The mice were injected with 180 μCi of ^{64}Cu-TE2A in 200 μl saline via a tail vein. At 1, 4, and 24 h postinjection, the mice were scanned for 30 min, 1 h and 2 h, respectively. All microPET images were reconstructed by a two-dimesional ordered-subsets expectation maximum (OSEM2D) algorithm, and displayed by using ASIPro VM (6.0.5.0) software (Concorde Microsystems, Tenn., Knoxville, USA). A region of interest (ROI) was placed on kidney and liver in the transaxial microPET images that include the entire organ volume. The radioactivity within the whole organ was summed. The data were calculated in the percentage injected dose per gram (%ID/g) for time-activity curve.

Preparation of TE2A-c(RGDyK) conjugate

TE2A was activated by EDC at pH 5.5 for 30 min at 4°C, with a molar ratio of TE2A:EDC:SNHS=1:0.5:0.6. Typically, TE2A (12 mg, 37.9 μmol), EDC (3.6 mg, 18.9 μmol), and SNHS (4.9 mg, 22.7 μmol) were dissolved in 500 μl water and 0.1 *N* NaOH (200 μl) was added to adjust the pH to 5.5 at 4°C. The reaction mixture was stirred for 30 min at 4°C. Cyclic RGDyK peptide (1.42 mg, 2.3 μmol) was dissolved in water (100 μl) and added to the reaction mixture, and the pH was adjusted to 8.5 with 0.1 *N* NaOH (200 μl). The reaction mixture was incubated overnight at 4°C. The TE2A-RGD conjugate was purified by semi-preparative HPLC (Zorbax Agilent Prep-C18; 21.2 × 100 mm; mobile phase starting from 95% solvent A (0.1% TFA in water) and 5% solvent B(0.1% TFA in acetonitrile) (0–2 min) to 35% solvent A and 65% solvent B at 32 min; flow rate 3 ml/min). The peak containing the TE2A-c(RGDyK) conjugate was collected, lyophilized (0.7 mg) and dissolved in water for use in the subsequent radiolabeling studies. LRMS (FAB): calculated for $C_{41}H_{69}N_{13}O_{12}$, 935.52[$(M+H_2O)^+$]; found, 935.17 [$(M+H_2O)^+$].

^{64}Cu-radiolabeling of TE2A-c(RGDyK) conjugate

TE2A-c(RGDyK) was labeled with ^{64}Cu by the addition of 1–2 mCi of ^{64}Cu to the conjugates in 0.1 *N* NaOAc (pH 5.5 and 8) buffer (2–5 μg TE2A-c(RGDyK) per mCi of ^{64}Cu), followed by 30 min incubation at 50°C. The radiochemical yield was determined by radio-TLC using a silica plate as the stationary phase and 1:1 MeOH:10% NH_4OAc as the developing solvent.

Results

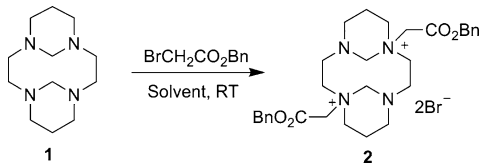
We optimized the conditions for the reaction of benzyl bromoacetate with the bisaminal compound, 1,4,8,11-tetraazatricyclo[9.3.1.1^{4,8}]-hexadecane (**1**). We tested various solvents for the synthesis of the *trans-N,N′*-disubstituted cyclam using benzyl bromoacetate as an alkylating agent. Acetonitrile was found to be the most effective (88%), while the other solvents, THF and $CHCl_3$, gave modest yields of 42% and 46%, respectively (Table 1). We also tested the use of two and four equivalents of benzyl bromoacetate in the reaction and it was observed that four equivalents of benzyl bromoacetate gave a higher yield as well as higher purity, while two equivalents of benzyl bromoacetate gave a mixture of the mono- and di-substituted isomers.

Using the optimized conditions, 1,8-*N,N′*-bis-(benzyloxycarbonylmethyl)-4,11-diazoniatricyclo[9.3.1.1^{4,8}]hexadecane dibromide (**2**) was prepared using four equivalents of benzyl bromoacetate in acetonitrile at room temperature in 88% yield. The methylene bridge of **2** was cleaved by treating it with 3 M NaOH at room temperature to yield 1,8-*N,N′*-bis-(benzyloxycarbonylmethyl)-1,4,8,11-tetraazacyclotetradecane (**3**) in 95% yield. Simple hydrogenation using H_2/(10% Pd/C) in ethanol removed the benzyl group and finally afforded 1,8-*N,N′*-bis-(carboxymethyl)-1,4,8,11-tetraazacyclotetradecane, TE2A (**4**) in 98% yield (Scheme 1). The overall yield of TE2A from the cyclam was 74%.

TE2A was quantitatively radiolabeled with ^{64}Cu by 20 min incubation at 30°C in distilled water or 0.1 M ammonium acetate buffer (Scheme 2).

The labeled ^{64}Cu-TE2A was injected into BALB/c mice and the static microPET images at 1, 4, 24 h postinjection

Table 1 Optimization of reaction conditions



Run	Alkylating agent	Equivalency	Solvents	Yield (%)[a]
1	Benzyl bromoacetate	4	CH_3CN	88%
2	Benzyl bromoacetate	2	CH_3CN	52%
3	Benzyl bromoacetate	4	THF	42%
4	Benzyl bromoacetate	4	$CHCl_3$	46%

[a] Isolated yield

were obtained. The coronal and transverse images of the mice at 4 h after the injection of ^{64}Cu-TE2A are depicted in Fig. 1a. Only clearance organs such as the kidney, liver are clearly recognized, while no other specific uptake in other tissue is observed. Time-activity curve analysis suggests that ^{64}Cu-TE2A is mainly cleared out from body through renal excretion (Fig. 1b).

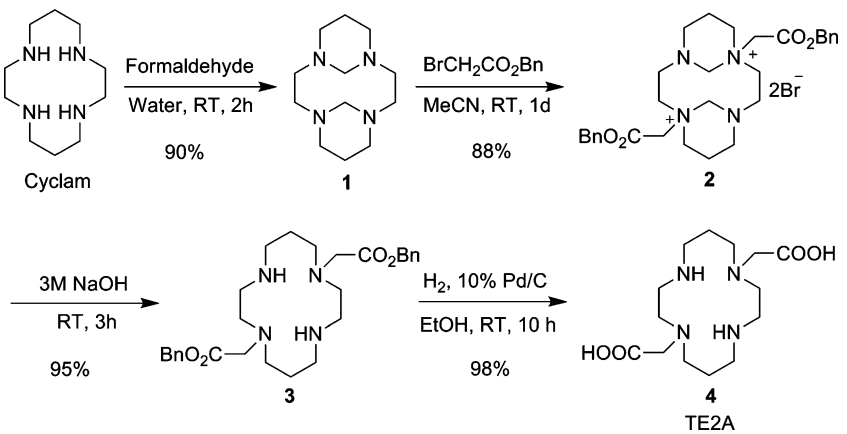
TE2A was conjugated with the cyclic RGD peptide c(RGDyK) using the standard EDC/SNHS conjugation method (Scheme 3). The molecular ion peak of the purified conjugates was clearly detected by mass spectrometry (Fig. 2). The conjugate was radiolabeled with ^{64}Cu in 0.1 M NaOAc buffer solution at different pH values. The labeling yields were 87 and 94% in pH 5.5 and 8 buffer solutions, respectively, within 30 min at 50°C.

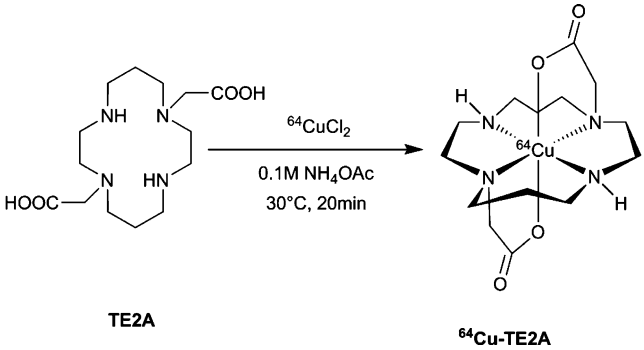
Discussion

We herein report a new alternative regioselective procedure for the synthesis of the *trans-N,N′*-disubstituted cyclam (**3**) which can be easily converted to the potential bifunctional chelator, TE2A (**4**).

TE2A was first synthesized in 1988 by Parker et al. [27, 28] and a few metal complexes using this ligand were further reported in 1992 by the same group [29]. However, to the best of our knowledge, no further papers on TE2A have since been published. Quite recently, we reported a new facile method of synthesizing TE2A [25]. We used a bisaminal compound to make the regioselective *trans*-disubstituted cyclam and were able to prepare a derivative of compound **2** using *t*-butyl bromoacetate in 95% yield, and then deprotected both the *t*-butyl group and methylene

Scheme 1 Four-step synthesis of salt-free TE2A from cyclam





Scheme 2 Radiolabeling of TE2A with ^{64}Cu and the expected coordination structure of ^{64}Cu-TE2A complex

bridge in one step to yield TE2A in the hydrochloride salt form. The overall yield from the cyclam to TE2A was increased to 86% from 16% in the previous method [27, 28].

In the present study, we employed the benzyl-protected bromoacetate instead of the *t*-butyl group. The benzyl ester is widely used in synthetic chemistry as a protecting group of carboxylic acids, because it can be easily removed by hydrogenation, while demonstrating high stability in a basic environment. The introduction of the benzyl bromoacetate group into the bisaminal compound (**1**) was optimized using various solvents and different molar ratios of the two starting compounds (Table 1). The best yield was obtained when four equivalents of benzyl bromoacetate was reacted with the bisaminal in acetonitrile. Even though a high excess of four equivalents of alkylating agent was used, no tri- nor four-substituted side products were found, presumably due to the high steric hindrance and electrostatic repulsion between the potentially close *cis*-located quaternary ammonium salts of the possible side products [26, 30].

The beauty of this new synthetic method lies in the selective deprotection of the methylene bridge of the cyclam compound (**2**) without affecting the benzyl protection group of the carboxylic acid. Because the benzyl ester group of carboxylic acids is robust, even under strongly basic conditions, only the methylene bridge could be removed to give the *trans*-disubstituted compound (**3**) in salt-free form. In the following step, the benzyl group was easily removed by simple hydrogenation to afford TE2A in salt-free form in high yield (Scheme 1). Compared with our recent, novel method of synthesizing TE2A [25], the current one seems to be less effective, because the total number of synthesis steps from the cyclam is four versus three in the previous one and the overall yield is also slightly lower (74 vs 86%). However, as mentioned above, the final compound, TE2A, could be obtained in salt-free form using this method. Therefore, it is not necessary to purify TE2A using an ion-exchange column and, instead, very pure crystalline TE2A can be obtained by simple recrystallization. High purity of chelate is prerequisite for the successful radiolabeling with tracer amount of ^{64}Cu ions. It is also worth noting that the *trans*-disubstituted compound (**3**), which could not be obtained in the previous method, might be used as an invaluable synthetic synthon for many TE2A-based derivatives. Compound **3** has two secondary amines, on which any second alkylating group could be further introduced to prepare tri-substituted TE2A derivatives.

We attempted to radiolabel the salt-free TE2A with ^{64}Cu under several different conditions. First, we found that the salt-free TE2A could be quantitatively radiolabeled with ^{64}Cu simply in water at 30°C. The acidity of TE2A in salt-free form when dissolved in distilled water is around pH 7, while the solution of TE2A in HCl salts showed a very acidic pH (≤ pH 1). However, TE2A also showed a quantitative labeling yield in 0.1 M NH_4OAc buffer solution at pH 6.5–6.8 (Scheme 2).

The microPET studies of ^{64}Cu-TE2A showed the good clearance of the radiolabeled complex from the body of the mice. During the 24-h follow-up studies, only the liver, kidney and bladder were clearly visualized. Except for these clearance organs, no other specific tissue uptake of the activity was recognized, suggesting the high in vivo kinetic stability of the ^{64}Cu-TE2A complexes. Even though we had difficulties in quantifying the remaining activity in kidney and liver at 24 h postinjection because of blurred outline of the organs in microPET images, the time activity curve analysis at 1 and 4 h clearly suggests that ^{64}Cu-TE2A complexes are cleared out mainly via renal route instead of hepatobilliary system. The percent injected dose per gram values in kidney and liver at 24 h (0.052 and 0.057, respectively) calculated from microPET images are comparable with the corresponding data in the biodistribution studies (0.139 and 0.073%ID/g, respectively) [25].

Even though TE2A can be easily radiolabeled with ^{64}Cu and ^{64}Cu-TE2A complexes show high kinetic stability in

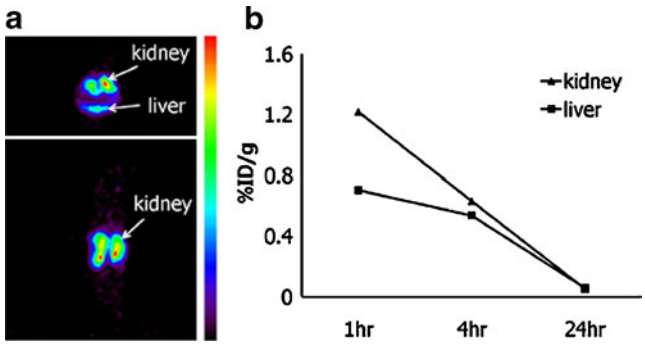
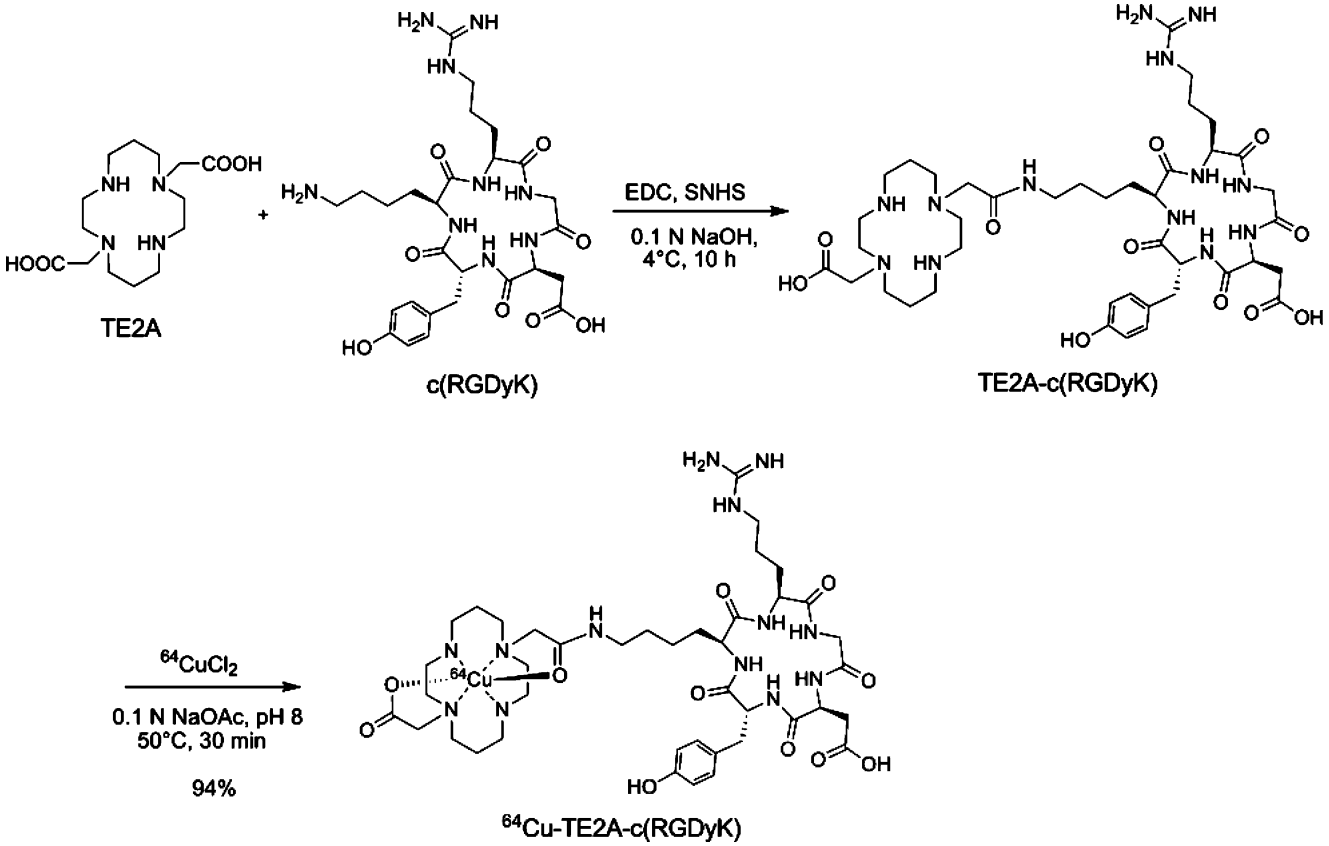


Fig. 1 **a** MicroPET image of ^{64}Cu-TE2A in BALB/c mouse at 4 h postinjection. The transverse (*top*) and coronal (*bottom*) images are shown. **b** Time-activity curves for liver and kidney of BALB/c mouse



Scheme 3 Conjugation of TE2A with c(RGDyK) peptide and radiolabeling of the conjugates with ^{64}Cu

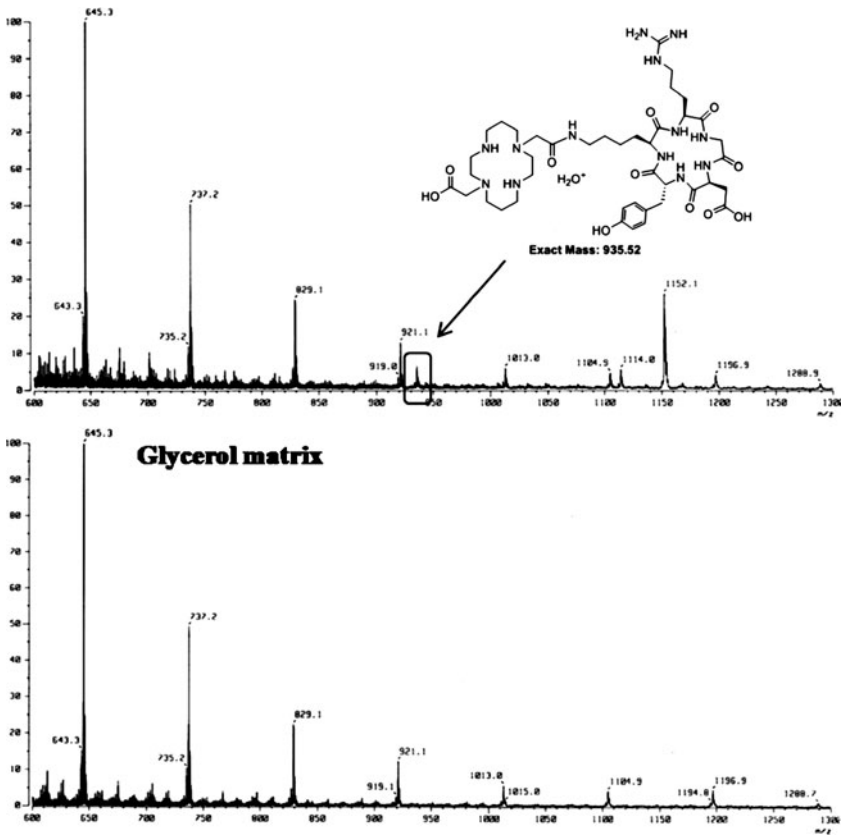


Fig. 2 Mass spectra of TE2A-c(RGDyK) conjugates (*top*) and glycerol matrix (*bottom*)

living systems, if it were unable to be conjugated with biomolecules such as peptides or antibodies, its usefulness as a BFC would be very limited. We chose a cyclic RGD peptide, which has been conjugated with almost all bifunctional chelators [31], for our conjugation trials. The standard EDC/SNHS conjugation methods were employed to conjugate TE2A with c(RGDyK) peptide. After trying various conjugation conditions, we were finally able to conjugate TE2A with c(RGDyK) successfully and isolate the pure TE2A-c(RGDyK) conjugate using HPLC purification. The purified TE2A-c(RGDyK) conjugate was characterized by mass spectrometry (Fig. 2). The TE2A-c(RGDyK) conjugate was detected by mass spectrometry as the water adduct at m/e 935 [32]. However, even though we tried various reaction conditions, the conjugation yield was always low and the HPLC chromatogram of the conjugation mixture always showed many peaks of side products in addition to the desired peak of TE2A-c(RGDyK). This complicated conjugation reaction of TE2A with the peptide seems to originate from the intrinsic reactivity of TE2A toward activated carboxylic acid groups. Not only the amino group of the lysine residue of c(RGDyK) peptide, but also the two secondary amines of TE2A, might attack the activated carboxylate group of TE2A.

The purified TE2A-c(RGDyK) conjugate was subjected to radiolabeling with ^{64}Cu, and more than 90% of the free ^{64}Cu ions were complexed with the conjugates within 30 min at 50°C, even though we did not optimize the labeling conditions (Scheme 3).

At first glance, the coordination geometry of the ^{64}Cu-TE2A-c(RGDyK) complex seems to be very different from that of ^{64}Cu-TE2A, because, during conjugation, one of the two carboxylate groups is converted to an amide group and, therefore, its binding affinity as a ligand for the Cu(II) ion would be significantly decreased, resulting in the breakdown of the kinetically stable octahedral coordination geometry of ^{64}Cu-TE2A (Scheme 2) [27, 29]. However, according to the reported results of the Cu-CB-TE2A (Cu(II) complex of the cross-bridged TE2A) [33], which has two *trans*-disubstituted carboxylate groups just like TE2A, there is very little change in the core coordination geometry around the Cu(II) ions, when one carboxylate group (carboxymethyl) of CB-TE2A was converted to an amide group (acetamido). In the X-ray crystallographic studies of CU(II)-CB-TEAMA, the amide pendant arm was found to be coordinated via the carbonyl oxygen and the distorted octahedral coordination geometry is maintained, as in the Cu-CB-TE2A complex [34]. The biodistribution studies also showed the good in vivo stability of the ^{64}Cu-cross-bridged monoamides, supporting the hypothesis that CB-TE2A is able to be used as a BFC without any structural modifications of the macrocycle backbone [35]. When considering the structural similarity between TE2A and CB-TE2A in terms of the two carboxylate groups located in the *trans*-positions, the carbonyl oxygen of the amide bond of the TE2A-c(RGDyK) conjugate is expected to coordinate to the Cu(II) ion, and the octahedral coordination geometry around the Cu(II) ions would also be maintained in the ^{64}Cu-TE2A-c(RGDyK) complex (Scheme 3).

Even though TE2A was experimentally proven to be conjugated with the small peptide and the conjugate was successfully radiolabeled, new TE2A-based chelates, which can be more easily conjugated with biomolecules while maintaining the kinetic stability of the Cu complex, are under development by our group.

In conclusion, we prepared salt-free TE2A in high yield using the regioselective *trans*-disubstitution of benzyl bromoacetate, followed by two successive deprotection reactions. The salt-free TE2A showed a good radiolabeling yield with ^{64}Cu(II) ions in buffer solution and even in water. TE2A could be conjugated with the cyclic RGD peptide by employing the standard EDC/SNHS conjugation method and the TE2A-c(RGDyK) conjugate was successfully radiolabeled with ^{64}Cu in high yield, which demonstrates that TE2A can be used as a potential bifunctional chelate without any further structural modification. New TE2A-based chelators containing other functional groups for conjugation, in addition to the two carboxylic acid groups in the *trans*-positions, are currently under development.

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