Synthesis of Bifunctional Chelating Agent Derived from Lysine and its Radiolabeling with ^{99m}Tc

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Several types of chelating agents for radiometal ion complexes have been proposed for use as both radiotracers and in radiotherapy, and for paramagnetic metal ion complexes used as MRI contrast-enhancing agents.[1-4] In the past decade, bifunctional chelating agents (BFCAs) have received increasing interest because of their important role in a successful application of bioactive molecule-based metal complexes for the forementioned purposes.[5-6] Functionalized EDTA as a BFCA was first used for evaluating a scintigraphy, but this metal complex revealed a low stability for a *in vivo* condition.[7-9] Other bifunctionalized cyclic chelating agents based on oligoaza macrocycle derivatives such as MeO-DOTA-NCS (α-(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), 2B- DOTA-NCS (2-(*p*-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), TEPPA (1,4,8,11-tetraazacyclotetradecan-1,4,8,11-tetrapropionic acid), and so on were then introduced and are now commercially available.[10-12] The functionalized pendant-arms of oligoaza derivatives can be utilized to conjugate bioactive molecules.[13] and their complexes with metal ions show a better stability than acyclo-chelating agents. However, despite their good properties, forming a metal complex demands an incubation of about 0.5 hr ~ 2 hrs, and, at times, a high temperature. Therefore, we have concentrated on the development of new BFCAs which have a higher affinity and stability to almost all kinds of lanthanides ions using diethylene triamine pentaacetic acid (DTPA). We previously reported on a synthetic method for DTPA derivatives whose original moiety are intact by using cysteine.[14] In line with the DTPA series, another DTPA derivative is introduced for a simple amide coupling with a C-terminal in bioactive peptides or small molecules. Therefore we have designed another DTPA based on a lysine backbone and focused on reducing the steps for a easier preparation of their DTPA derivative in addition to increasing the yield. In this paper, we describe the preparation and radiolabeling of lysine-based DTPA with one of the most ideal (140 keV γ-emission, $t_{1/2}$ = 6 h) and cost-effective radioisotopes for a nuclear imaging, ^{99m}Tc.

Experimental Section

Instruments and materials. The NMR spectra were recorded by Bruker Avance 500 (500 MHz, ^{1}H, KRICT, Daejeon) with a ppm unit ratio versus TMS (δ = 0) as an internal standard. Mass spectra were measured with the Hewlett Packard HP 1100 series LC/MDS (Chungnam National Univ.). Column purifications were performed with CombiFlash® (Teledyn Isco, Inc.) and RediSep® silica column. Sodium pertechnetate ([^{99m}Tc] $NaTcO_4$) was obtained from a ^{99}Mo-^{99m}Tc generator (Sam Young Unitech. Co., LTD.). Radioactivities were measured by using an ionizing chamber (Atomlab 200, Bio-dex) and the radiolabeling yield was determined by an ITLC scanner (Trance maste 200, Berthold). All the chemicals and reagents used in this study were analytical grade and purchased from Aldrich, and used without any further purification.

***N*$_\varepsilon$-(*tert*-Butoxycarbonyl)-L-lysine methylester (1). From a) procedure:** To a suspension of L-lysine (5.5 mmol) in CH_3OH, chlorotrimethylsilane (19.2 mmol) was dropwisely added for 10 min. After stirring for 24 hrs, the reaction mixture was evaporated *in vacuo* to give the crude product as a white powder. Without any further purification, the crude product was dissolved in 2 drops of water, CH_3OH, and triethylamine, solution of di-*tert*-butyl-dicarbonate (6.0 mmol) in CH_2Cl_2 was added for 10 min under -10 °C and stirred overnight. The reaction mixture was reduced the volume until 3 mL and purified with flash column chromatography with gradient condition (CH_2Cl_2/CH_3OH 95:5 then 80:20) to give a product with 86% yield.

Lysine methylester (Intermediate): ^{1}H NMR (CD_3OD) δ 1.58 (m, 2H), 1.73 (p, 2H), 1.98 (m, 2N), 2.99 (t, 2H), 3.89 (s, 3H), 4.11 (t, 1H) (LC/MSD M+1): cald. for 260.17 found 260.9.

N$_\varepsilon$-(*tert*-Butoxycarbonyl)-L-lysine methylester: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.49 (2H), 1.53 (m, 2H), 1.63 (br m, 1H), 1.76 (br m, 1H), 3.11 (d, 2H), 3.51 (s, 1H), 3.72 (s, 3H), 4.62 (t, 1H) (LC/MSD M+1): cald. for 260.17 found 260.7.

From b) procedure: A solution of diazomethane (35 mmol)[15-16] in diethyl ether (30 mmol) was added cautiously portionwise to a stirred solution of *N*$_\varepsilon$-(*tert*-butoxycarbonyl)-L-lysine (4.1 mmol) in CH_3OH under 0 °C. The reaction mixture was maintained under N_2 condition and stirred at room temperature for 4 hrs. Evaporation of the solvent under reduced pressure gave the product with quantitative yield.

N$_\varepsilon$-(*tert*-Butoxycarbonyl)-L-lysine methylester: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.49 (2H), 1.53 (m, 2H), 1.63 (br m, 1H), 1.76 (br m, 1H), 3.11 (d, 2H), 3.51 (s, 1H), 3.72 (s, 3H), 4.62 (t, 1H) (LC/MSD M+1): cald. for 260.17 found 260.7.

***N*$_\varepsilon$-(*tert*-Butoxycarbonyl)-L-Lys(tBu$_4$-DTPA) methylester (2).** To **14** (3.1 mmol) dissolved in CH_3CN and DMF. Two molar phosphate buffer (pH = 8) and **cpd 2** (10.7 mmol) pre-

Table 1. Logarithms of the equilibrium quotients at the 1: 1 metal ion complexes

	Metal ion and Formation constant(Log K, 20 °C)					
	Y^{3-}	Sm^{3-}	Ho^{3+}	Er^{3+}	Lu^{3+}	Dy^{3-}
DTPA (diethylenetriamine pentaacetic acid)	22.13	22.44	22.88	22.83	22.60	22.92
EDTA (ethylenediamine tetraacetic acid)	18.09	17.14	18.62	18.85	19.83	18.30
CDTA (trans-1,2-cyclohexylenedinitrilo tetraacetic acid)	19.85	19.08	20.6	21.38	22.21	20.39

pared as previously reported was added. The resulting mixture was vigorously stirred for 48 hrs at room temperature. Organic layer was extracted with CH_2Cl_2 and repeated three times. The solvent was evaporated to afford a residue as pale yellow oil form. Purification was performed with flash column chromatography with gradient condition (Hexane/Ethylacetate, from 100:0 to 0:100 during the 30 min) to give a pale oil form product with 76 % yield. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.46 (s, 36H), 1.49 ~ 1.6 (mm, 6H), 2.88 (br, 8H), 3.11 (br, 2H), 3.43 (s, 8H), 3.51 (s, 1H), 3.70 (s, 3H), 4.62 (t, 1H) (LC/MSD M+1): cald. for 802.53 found 803.2.

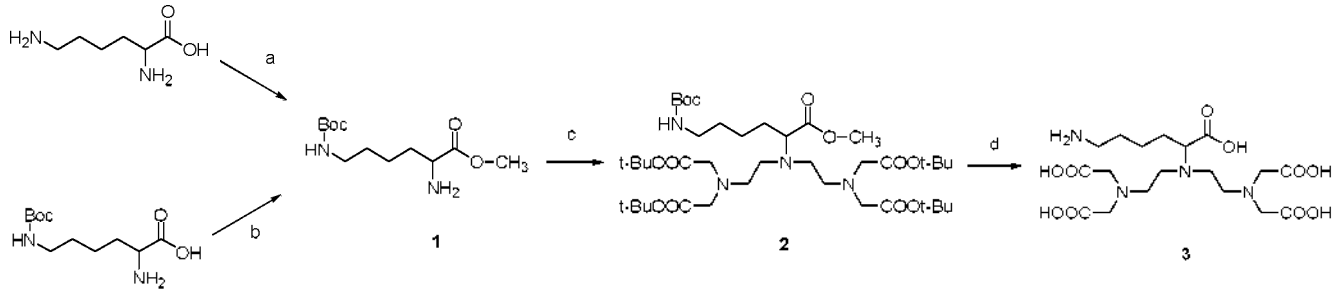
Hydrolysis (3). Hydrolysis was performed with 3 N-HCl at 100 °C for 20 mins. After evaporation, recrystalization was performed by using CH_3OH and diethylether. ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 2H), 1.71 (m, 4H), 1.90 (m, 2H), 3.0 ~ 3.7 (m, 9H), 3.92 (s, 8H) (LC/MSD M-1): cald. for 464.21 found 464.0.

Preparation of the ^{99m}Tc-DTPA complex. After the preparation of 2 mg of $SnCl_2$ dissolved in 0.1 N HCl (1 mL) under inert atmosphere, 0.1 mL of $SnCl_2$ solution was added to another vial containing 0.5 mg DTPA in N_2 purged HCl solution (1 mL, pH = 5) and then 0.2 mL of freshly eluted $^{99m}TcO_4^-$ (3 mCi) from ^{99}Mo-^{99m}Tc generator was added. The reaction mixture was shaken for 10 min. at room temperature and filtered through 0.22 μL membrane filter. ITLC test revealed labeling yield as 98 %.

Results and Discussion

Developing new BFCAs that can coordinate easily with metal ions and last for a long time, is one of our on going projects in oder to introduce radiometal ions to bioactive molecules such as peptides, antibodies, and drugs. DTPAs and oligoaza macrocycles are the most common candidate compounds for developing novel BFCA. We first developed a new BFCA related with DTPA because DTPA forms more stable complexes with heavy metal ions by up to 3 ~ 4 orders of a magnitude compared with EDTA or CDTA (Table 1).[17] In addition to this, DTPA is more effective than oligoaza macrocycle for the reaction kinetics. Namely, it may not need a high temperature and a long incubation time for the formation of the complex.

In order to introduce functional groups to DTPA, we designed BFCAs by using functionalized amine, lysine was used in this paper as the supporting body for the DTPA derivative. The preparation scheme and chemical structures of all the intermediate compounds are depicted in Scheme 1. N_ε-(*tert*-butoxycarbonyl)-L-lysine methylester (**1**) was synthesised from b) procedure with methylation by using diazald in oder to protect against Boc-hydrolysis. Diazomethane was prepared according to F. Arndt's report and used 8-fold excess amounts to provide quantitative methylester without any other side products in a methanol solvent. We also prepared **1** by using a) one-pot procedure including methylation and a N-Boc protection. Carboxyl methylester was performed under a methanol solvolysis with 3.5 eq TMS-Cl and a selective N_ε-Boc protection was achieved using $(Boc)_2O$ (1.1eq) at -10 °C with a triethylamine solvent. The data revealed consistent results checked by NMR, IR and TLC. **2** was prepared by using our previous report but we didn't get satisfactory results for the yield. Thus a small amount DMF as a phase catalyst was used to provide **2** with a 76 % yield. For the labeling, we chose ^{99m}Tc because DTPA shows specific binding efficiency for heavy metals and ^{99m}Tc-DTPAs have also been used in a clinical application such as renal studies for providing both anatomical and functional information, a determination of the GFR, to locally image inflammatory bowel disease and others.[18] ^{99m}Tc-DTPA derivative complex was prepared by simply mixing ^{99m}Tc, $SnCl_2$, and DTPA derivative at room temperature in HCl solution (pH = 5). To determine the amount of $^{99m}TcO_4^-$, the sample was chromatographed on ITLC-SG (Gelman Sciences Ins. USA) using MEK as a mobile phase. Unbounded $^{99m}TcO_4^-$ migrated with the solvent front, whereas $^{99m}TcO_2$ and the labeled material remained at



Scheme 1. Reaction pathway for lysine-based DTPA. Reagent and condition; (a) i) TMS-Cl/CH_3OH/RT, 24 hrs; ii) $(Boc)_2O$/CH_3OH,TEA/ 0 °C, 12 hrs; (b) Diazomethane/CH_3OH/-10 °C, 4 hrs; (c) cpd 1/phosphate buffer pH = 8/RT, 48 hrs; (d) 3 N-HCl/100 °C, 20 mins

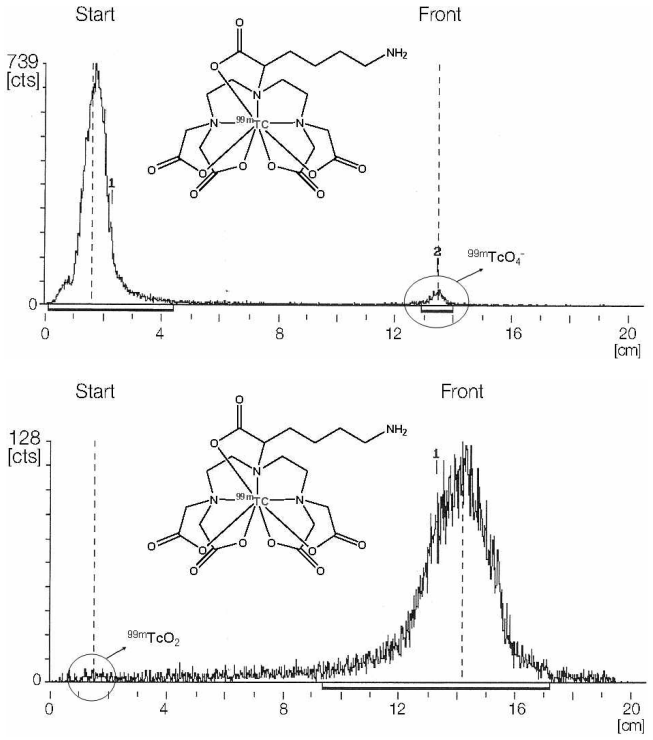


Figure 1. Radiochromatogram of lysine based DTPA complex; A: Bioactive material binding site. Conditions: developing solvent(a) MEK, (b) 0.9% saline, stationary phase (ITLC-SG). starting point (1.5cm). distance from solvent front (about 13 cm).

the origin. The amount of $^{99m}TcO_2$ was determined using saline to develop an ITLC strip. In this system $^{99m}TcO_2$ was retained at the origin, whereas free $^{99m}TcO_4$ and the labeled material moved with the solvent front. The extent of the labeling was calculated as the following equation, the ITLC test revealed a labeling yield of 98 %.

$$\text{Labeling yield} = 100 - \%\ ^{99m}TcO_4 - \%\ ^{99m}TcO_2$$

Conclusion

In summary, we described a simple synthesis of a DTPA derivative as a bifunctional chelating agent (BFCA). The prepared DTPA derivative can be applied for the solid phase synthesis of peptides to develop peptide-based target radionuclide therapeutic agents. Furthermore, bioconjugation with bioactive molecules, such as peptide will be implemented for the development of radioimmunotherapeutics or radioimmunodiagnostics.

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