Transactions of the Korean Nuclear Society Spring Meeting
Jeju, Korea, May 10-11, 2007

Preparation of Lysine-based DTPA as a BFCA for Radioimmunotherapy

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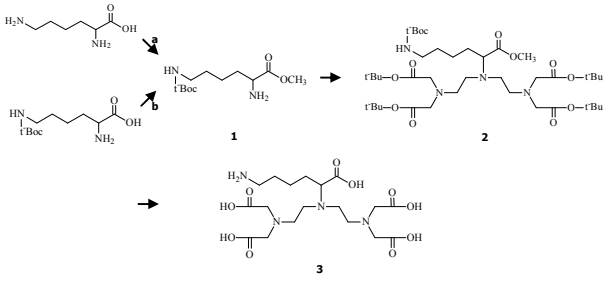
1. Introduction

During the past decade, many bifunctional chelating agents (BFCAs) have been studied, synthesized and used for radiopharmaceuticals especially for labeling of antibodies and regionally selective drugs. General researches for preparation of DTPA derivatives have been performed using cyclic DTPA dianhydride. Because one or two carboxylic groups of DTPA were used to conjugate with biomolecule results to lower chelating power for radionuclides.

Our goal was to synthesize new bifunctional chelating agents for their application in development of new medical imaging and therapeutic agents. To obtain more effective new chelating agents, we focused on the development of DTPA derivatives whose original functional groups are intact. In our study, we described the synthesis of a DTPA analogue derived from lysine.

2. Methods and Results

The preparation scheme and chemical structures of all the intermediate compounds are indicated in scheme 1.



Scheme 1. Preparation of lysine-based DTPA.

2.1.1 N_ε -(tert-butoxycarbonyl)-L-lysine methylester ***(1)*** *from* ***a***

To a suspension of L-lysine (1.0g, 5.5 mmol) in CH_3OH, chlorotrimethylsilane (2.43ml, 19.2 mmol) was dropwisely added for 10min. After stirring for 24hrs, the reaction mixture was evaporated in vacuo to give the crude product (about 1.1 g) as a white powder. Without any further purification, crude product was dissolved in 2 drops of water, CH_3OH(20 ml), and triethylamine (4 ml), the solution of Di-tert-butyl-dicarbonate (1.31, 6.0 mmol) in CH_2Cl_2 (4 ml) was added for 10 min at 10℃ and stirred overnight. The reaction mixture was reduced to 3 ml volume and purified using flash column chromatography with gradient condition (CH_2Cl_2/CH_3OH 95:5) to yield 86% of product **1**.

2.1.2 1 N_ε -(tert-butoxycarbonyl)-L-lysine methylester ***(2)*** *from* ***b***

A solution of diazomethane (35 mmol)[5] in diethyl ether (30 mmol) was added cautiously portionwise to a stirred solution of N_ε-(tert-butoxycarbonyl)-L-lysine (1g, 4.1 mmol) in CH_3OH (10 mL) at 0℃. 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 **1** with quantitative yield.

2.1.3 N_ε -(tert-butoxycarbonyl)-L-lys(tBu4-DTPA) methylester ***(2)***

To **1** (0.8g, 3.1mmol) dissolved in CH_3CN (10 mL) and DMF (5 mL), 2M phosphate buffer (pH=8, 10 mL) and *N,N*-bis[(*tert*-butoxycarbonyl) methyl] -2-bromoethyl-amine (3.78g, 10.7mmol) prepared previous report 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 using flash column chromatography with gradient condition (Hexane/Ethyl acetate, from 100:0 to 0:100 for 30 min) to gave a product as pale oil form with 76 % yield of **2**.

2.1.4 Hydrolysis ***(3)***

Hydrolysis was performed with 3N-HCl at 100℃ for 20 mins. After evaporation, recrystalization was performed by using CH_3OH and diethyl ether.

2.2 Preparation of ^{99m}Tc-DTPA complex

After the preparation of 2 mg of $SnCl_2$ dissolved in 0.1N HCl (1 ml) under inert atmosphere, 0.1 ml of $SnCl_2$ solution was added to another vial containing 0.5 mg of 3 in N_2 purged HCl solution (1ml, 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 gently stirred for 10 min at room temperature and filtered through 0.22 $\mu\ell$ membrane filter. ITLC test revealed labeling yield as 97 %.

Labeling efficiency (%) = 100 - $^{99m}TcO_4^-$ - $^{99m}TcO_2$

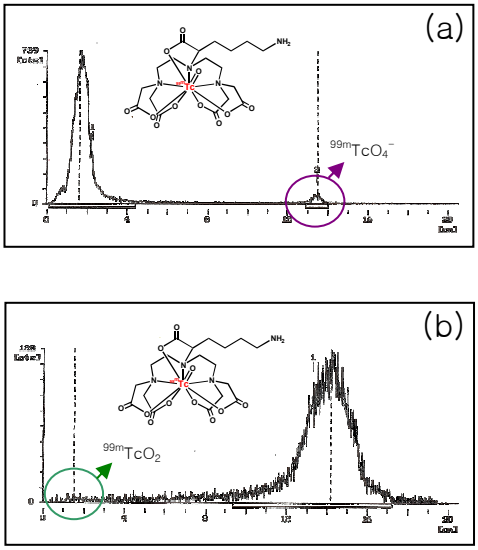


Fig. 1 Radiochromatogram of lysine based DTPA complex; Conditions; developing solvent(a) MEK, (b) 0.9% saline; stationary phase (ITLC-SG)

3. Conclusion

We established the preparation method for lysine based DTPA as a bifunctional chelating agent (BFCA). This established method for the synthesis of DTPA derivative with sustaining chelating power is very useful for conjugation with other molecules for the development of medical imaging agents and therapeutic agents.

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