Preparation of a lysine-based DTPA derivative for the conjugation with biomolecules

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1. Introduction

For radiopharmaceutical development, many bifunctional chelating agents (BFCAs) have been synthesized and applied. Among them, EDTA(Ethylenediamine tetra-acetic acid) and DTPA(Diethylenetriamine penta-acetic acid) have been used for the radiolabeling with lanthanide nuclides.

Until now, many of researches are applying the cyclic DTPA dianhydride to introduce DTPA chelator to bioactive molecules. However, its inherent disadvantage is that DTPA dianhydride produces major side product DTPA bis-molecules, which may cause instability of radionuclide binding *in-vivo*.

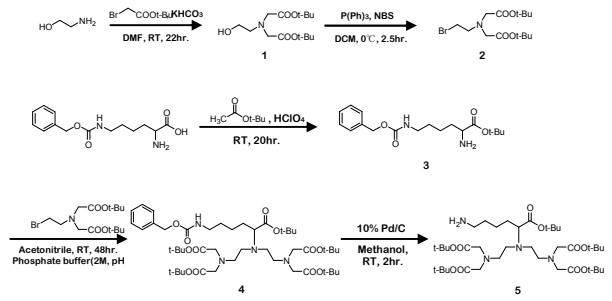
In this study, in order to develop the DTPA based bifunctional chelator for the radiolabeling with radionuclides including Re-188, In-111, Y-90, Ho-166, Lu-177 and so on, we prepare lysine-based DTPA derivative.

We describe, herein, the convenient synthetic method of the lysine based DTPA derivative derived from Nε - Z protected lysine.

2. Methods and Results

All chemicals and reagents used in this experiment were obtained from chemical suppliers (Singma or Fluka Co.) and used without any further purification.

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 N,N-Bis[(tert-butoxycarbonyl)methyl]-2-ethanolamine(1)

To *tert*-butyl bromoacetate(2.2 eq) which was dissolved in DMF under a N_2 condition, K_2CO_3 was added. The reaction mixture was cooled down to 0℃ and ethanolamine(1 eq) was added dropwisely for 5min. The reaction mixtures was continuously stirred at 0℃ for 30min and then left overnight at RT. After an addition of c-$NaHCO_3$ and diethyl ether, the organic layer was separated and washed with c-$NaHCO_3$ and with brine. The solvent was evaporated to give crude 1 as an oil.

2.2 N,N-Bis[(tert-butoxycarbonyl)methyl]-2-bromoethylamine (2)

To crude 1 dissolved in CH_2Cl_2, Ph_3P(1.2 eq) was added, the solution was cooled down to 0℃, and NBS(1.2 eq) was added for 5 min. After 2h, solvent was removed and separated with column chromatography (Ether : n-Hxane=4:6) to obtain 2.

2.3 2-Amino-6-benzyloxycarbonylamino-hexanoic acid tert-butyl ester (3)

$HClO_4$(1.5 eq) was added dropwise to a suspension of H-Lys(z)-OH(1 eq) in *tert*-butyl acetate(25 eq) to afford a clear solution. After 20h, 10% Na_2CO_3 solution was dropped into the reaction solution obtaining precipitation of the unreacted starting material. 10N NaOH solution was added to adjust to pH 10 the aqueous phase then the organic phase was separated, washed with water and dried(Na_2SO_4). Evaporation gave 3 as a colorless oil.

2.4 6-Benzyloxycarbonylamino-2-{bis-[2-(bis-tert-butoxycarbonylmethyl-amino)-ethyl]-amino}-hexanoic acid tert-butyl ester (4)

2M Phosphate buffer(pH 8) was added to a solution of compound 3 and 2(2.4 eq) in CH_3CN, and the mixture was vigorously stirred for 2 h. The two phases were separated and the aqueous phase replaced with fresh 2M phosphate buffer(pH 8). After 48h, the organic layer was separated and concentrated to dryness. The residue was dissolved in CH_2Cl_2, washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography with 3:7 EtOAc/n-hexane to give 4.

2.5 6-Amino-2-{bis-[2-(bis-tert-butoxycarbonylmethyl-amino)-ethyl]-amino}-hexanoic acid tert-butyl ester (5)

10% Pd/C(10%) was added to a solution of 4 in methanol and the suspension was stirred over 2h under a hydrogen atmosphere at RT. The mixture was filtered over celite and evaporated. The residue was chromatography with methanol to give 5.

3. Conclusions

We have established the synthetic method of lysine-based DTPA derivative which has penta *tert*-butyl ester and an amine group.

The prepared DTPA derivative, can be applied for the solid phase synthesis of peptides to develop peptide-based target radionuclide compounds for diagnosis and therapy.

Furthermore, bioconjugation with bioactive molecules, such as peptide will be implemented for the development of radioimmunotherapeutics or radioimmuno-diagnostics.

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