Preparation of a Phenylanine-based DTPA derivative for an efficient 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 [1], 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 phenylanine-based DTPA derivatives which contain eight coordination groups when it react with lanthanides to form a stable complex.

We describe, herein, the synthesis of the DTPA derivative which possesses an amine(NH_2) or an isothiocyanate(N=C=S) functionality in the structure for an easy conjugation with biomolecules.

2. Methods and Results

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

2.1 Preparation of 4-Nitro-phenylalanine-DTPA ester derivative

2.1.1 bromoethyl-iminodiacetate t-Bu_2-ester (2)

To t-butyl bromoacetate(2.2 eq) in DMF under N_2, $KHCO_3$(1.1 eq) was added. The reaction mixture was cooled down to 0℃ and ethanolamine(1 eq) was dropwisely added over 5 min. The reaction mixture was stirred at 0℃ and then continuously stirred overnight at room temperature. After evaporation and extraction using ether and concentrated sodium bicarbonate(c-$NaHCO_3$) solution, the organic layer was washed with brine. The solvent was evaporated to give crude product as an oil form. To the resulting compound dissolved in CH_2Cl_2, Ph_3P(1.2 eq) was added, the solution was cooled to 0℃, and NBS(1.2 eq) was added. After 2 hrs, solvent was removed and separated with column chromatography(Ether: Hexane = 4 : 6) to give **2**.

2.1.2 4-Nitro-phenylalanine methyl ester (3)

4-Nitro-phenylalanine in MeOH was reacted with chlorotrimethylsilane(TMS-Cl) (2 eq) for 2 hrs at 0℃ under nitrogen atmosphere. The reaction mixture was evaporated and re-dissolved in methanol.

The solvent was treated with ethyl acetate to give the precipitate as a white powder which a single spot was monitored by TLC.

2.1.3 4-Nitro-phenylalanine (t-Bu_4-DTPA) methyl ester (4)

To the solution of **3** in CH_3CN and 2 M phosphate buffer(pH 8), the prepared compound **2** (3 eq) was added and stirred for 20hrs.

The CH_3CN layer was isolated and evaporated to afford a residue which partitioned between buffer and ethyl acetate. The organic layer was dried and separated with column chromatography with ethylacetate: hexane(3: 7) to give 4 as pale yellow oil.

2.2 Preparation of 4-isothiocyanate-phenylalanine-DTPA derivative

2.2.1 4-amino-phenylalanine t-Bu$_4$-DTPA methyl ester (5)

To the solution of 4 in ethanol, three equivalent of Tin(II) chloride was added under nitrogen atmosphere. The reaction was monitored by using TLC and ninhydrin test.

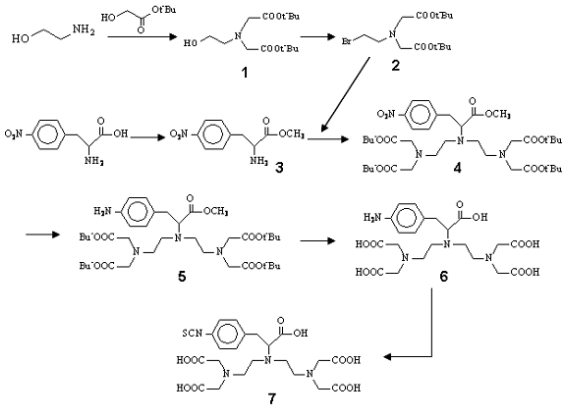
After stirring for 24hr, the solvent was evaporated and purified with column chromatography (dichloromethane : methanol = 20 : 1, rf = 0.2) to give **5**.

2.2.2 4-Amino-phenylalanine-DTPA (6)

To the solution of 5 in methanol, conc-HCl was added and *cooled to 0 ℃* for 1 hr. After the reaction was completed, the solvent was evaporated and recrystallized to afford **6** as a white powder.

2.2.3 4-isothiocyanate-phenylalanine-DTPA (7)

To the solution of 6 in water, 1.3 equivalent of thiophosgene($CSCl_2$) was added and stirred for 2 hrs. The water layer was collected in a vial, and then freeze dried to give the desired product **7**.



Scheme 1. Reaction pathway for DTPA derivatives

3. Conclusion

We have established the synthetic method of phenylalanine-based DTPA derivative which has isothiocyanate(N=C=S) group to be coupled with amine containing ligands to form a stable thiourea bond.

The prepared DTPA isothiocynante derivative can be applied as a bifunctional chelating agent for the conjugation with bioactive molecules such as peptides, antibodies, proteins, etc.

The intermediates, amino-phenylalanine-DTPA derivatives, can be applied for the solid phase synthesis of peptides to develop peptide-based target radionuclide therapeutic agents.

Furthermore, bioconjugation experiments with antibodies will be implemented for the development of radioimmunotherapeutics or radioimmuno-diagnostics by using the prepared isothiocyanate-phenylalanine-DTPA in the near future.

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