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Facile Synthesis of Histidine Derivatives for *fac*-$[M(CO)_3]^+$ precursor [M=^{99m}Tc, ^{188}Re]

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1. Introduction

The technetium-99m(^{99m}Tc) is one of the most widely used radionuclides in nuclear medicine, because of its preferable properties($T_{1/2}$= 6.02 hr, E_γ= 140 keV). More than 85% of diagnostic scans were performed in hospitals worldwide.

A variety of ^{99m}Tc-based radiopharamceuticals have been developed for evaluating organ function and assessing disease status by imaging methods.

The labeling of biologically active molecules with ^{99m}Tc is the most important research area. Although ^{99m}Tc complexes with a +5-oxidation state are commonly available recently, many researches have been focused on the ^{99m}Tc precursor with the +1-oxidation state $[M(CO)_3]^+$ due to its small size and ease in preparation [1].

A variety of researches have revealed that histidine complexes with the *fac*-$[M(CO)_3]$ precursor show a good stability through *in-vivo* and *in-vitro* studies [2].

In this study, we present a synthetic approach for the histidine derivatives as bifunctional chelating agent for the conjugation with biomolecules as well as for a labeling with the fac-$[M(CO)_3]^+$ precursor [M=^{99m}Tc, $^{186/188}$Re].

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. The reaction was monitored by using TLC glass-backed sheets pre-coated with silica-gel G-25 UV_{254}. The NMR spectra were recorded with Bruker Avance 500(KRICT, Daejeon)

2.1 Preparation of histidine derivatives

2.1.1 N_α-Boc-L-histidine methyl ester (1)

To N_α-Boc-L-histidine(1eq) in methanol(MeOH), DMAP(1eq) and DCC(1.1eq) in dichloromethane were added successively. The reaction mixture was stirred for 2 days at room temperature under nitrogen atmosphere. The filtrate was evaporated and purified by column chromatography (MC:MeOH=95:5, v/v) to yield a white powder.

2.1.2 N_τ-bromobuthyl-N_α-Boc-L-histidine methyl ester (2)

NaH (1.5eq) and 1,4-dibromobutane was dropwisely added to the prepared N_α-Boc-L-histidine (1eq) **1** in DMF at -10℃. After 1 hr, the solvent was removed *in vacuo* and purified (MC:MeOH=15:1) to obtain ***2*** as an oil.

2.1.3 N_τ-azidobuthyl-N_α-Boc-L-histidine methyl ester(3)

A mixture of ***2***(1eq) and sodium azide(1.2eq) in DMF was stirred for 24 hr at RT under nitrogen atmosphere. After evaporation and extraction with dichloromethane and water, the organic layer was combined, dried and evaporated. The intermediate was purified (MC:MeOH=15:1) to obtain ***3*** as a colorless oil.

2.1.4 N_τ-aminobuthyl-N_α-Boc-L-histidine methyl ester(4)

Reduction of azide was performed by using triphenylphosphine (1.2eq). The progress of the reaction was monitored by using ninhydrin. After stirring for 24hr, the organic solution was extracted with MeOH/hexane. The alcoholic layer was purified (Butanol:MeOH:NH_4OH=10:1.5:1) to obtain a sticky and colorless oil **4**.

2.1.5 N_τ-aminobuthyl-histidine (5)

To hydrolyze the ***cpd 5***, 0.2 N NaOH(5eq) was dropwisely added to the metanolic solution. After 2 hrs of stirring at RT, the mixture was neutralized with 1 N HCl. The crude product was purified with

Butanol-MeOH-NH_4OH(10:1.5:1) to give a white powder **5**.

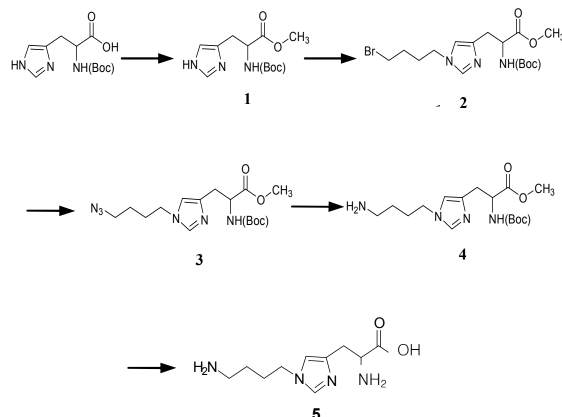

Fig. 1. Synthetic scheme for the synthesis of Histidine derivatives.

2.2 Radiolabeling with fac-[^{99m}Tc(CO)$_3$] precursor

2.2.1 Preparation of fac-[^{99m}Tc(CO)$_3$] precursor

To a vial containing potassium bronocarbonate, sodium teterborate, sodium/potassium titrate and sodium carbonate, one milliliter of the fleshly-eluted sodium pertechnate (Samyoung Unitech) was added and heated at 110℃ for 15 min and analyzed by HPLC.

2.2.2 Preparation of [^{99m}Tc(CO)$_3$]-N$_\tau$-aminobuthyl-histidine

100μl of [^{99m}Tc(H_2O)$_3$(CO)$_3$]$^+$(185MBq) was added to the vial of **5** in a PBS buffer (pH = 7.4, 400μl) to adjust at the molar concentration of 10^{-6}M.

Then the vial was incubated at 75℃ for 30min and analyzed by HPLC. The complex showed a single complex with a high radio-labeling yield (>98%).

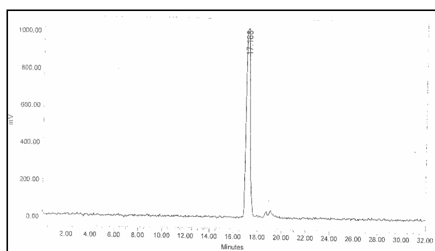

Fig. 2. HPLC chromatogram of [^{99m}Tc(CO)$_3$]-histidine complex

3. Discussion and Conclusion

Despite the good properties for the labeling with the *fac*-[M(CO)$_3$] precursor, it is difficult to introduce a functional group at the Nτ position of histidine[3].

To introduce a functional group at the imidazole ring of histidine for the effective conjugation with biomolecules, we prepared several kinds of histidine derivatives (**2, 3, 4, 5** in Fig. 1).

The functionalization was achieved by the protection of the N_α position of histidine to give a selective alkylation under a strong base condition.

It may infer that the *N*-Boc protecting group causes a steric effect at τ(N-1) thus *N*-α-Boc-histidine undergoes a pure τ(N-3) substitution reaction [4].

The proposed reactions, applied for this study, are helpful in developing a new chelating agent by using histidine.

In conclusion, a synthetic method to modify the imidazole ring of histidine was successfully established. The prepared histidine derivatives can be applied as a bifunctioal chelator for the *fac*-[M(CO)$_3$] $^+$ precursor for a conjugation with biomolecules.

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