Study on radiolabeling of 1,2,3-triazole analogs with *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$ *via* click chemistry

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Abstract Click chemistry was used to study on radiolabeling of 1,2,3-triazole analogs with *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$. CuSO$_4$/L-sodium ascorbate was chosen as the catalyst system, three terminal alkynes were conjugated with two different azides respectively, and then the new prepared *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$ was coordinated to the six triazoles. The results showed that the radiochemical yields (RCY) of the conjugation of *fac*-[^{188}Re(CO)$_3$]$^+$ with six triazoles were over 90%, and the triazoles showed high stability in phosphate-buffered saline and new-born calf serum. The preliminary biological evaluation results showed that the new ^{188}Re-labeling method *via* click chemistry could have general application in labeling bioactive molecules in high radiochemical yield and high specific activity for further SPECT research.

Key words Tricarbonyl Rhenium-188, Stability, Triazole analogs, Radiotherapy, Click chemistry

1 Introduction

The "click chemistry" could be carried out in high yields under mild and tolerable conditions of neutral pH and room temperature in aqueous media within a reasonable reaction time[1,2]. Due to these favorable aspects with click chemistry, the use of this strategy for making ^{18}F-labeled biomolecules has been reported [3-10]. And now it has been a mature method for the labeling of ^{18}F[11,12]. Recently, the organometallic precursor *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$ was shown to be an ideal candidate agent for labeling biomolecules[13] because of the high stability of the three carbonyl groups and the substitution liability of the three water molecules[14,15]. And for *fac*-[^{188}Re (CO)$_3$]$^+$ labeling, many research groups have reported the use of "click to chelate" for compounds labeling or SPECT imaging[16-19].

Our group focuses on the preparation of the organometallic precursor *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$[20] and the labeling method of this organometallic precursor[21]. We have labeled an RGD-containing peptide with *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$[22] and obtained encouraged results. In this paper six triazoles were obtained *via* click chemistry, and the excellent radiochemical yields and stability in phosphate-buffered saline and new-born calf serum have been shown to be an extraordinarily ideal method for *fac*-[^{188}Re (CO)$_3$]$^+$ labeling.

2 Materials and methods

2.1 General

Pyridine-2-methylamine, Bis(pyridin-2-ylmethyl)amine and L-Propargylglycine were purchased from Aldrich Co., Ltd; c(RGDfk)-N$_3$ was synthesized by China Tech Peptide Co., Ltd. ^{188}Re-perrhenate was eluted from ^{188}W/^{188}Re generator (Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai, China) using 0.9% saline; Plus QMA Sep-Pak cartridges were manufactured by Waters Corporation (Massachusetts, USA). All reagents were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai). γ counter

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(SN-697, Shanghai Rihuan Photoelectronic Instrument Co., Ltd., Shanghai, China).

Plus QMA Sep-Pak cartridges were produced by Waters Corporation (Ma, USA); A Dionex P680 pump equipped a PDA-100 ultraviolet detector and a radiometric detector system with a Macherey-Nagel C-18 reversed phase column (5 μm, 150×4.6 mm) were used to perform HPLC; (HPLC method: the flow rate was set at 1 mL/min, with the mobile phase starting from 95% B (0.1% trifluoroacetic acid in water) and 5% A (0.1% trifluoroacetic acid in acetonitrile) to 5% B and 95% A at 30 min. Thin layer chromatography (TLC) analysis was performed using silica gel plates (silica gel 60 GF254, mobile phase: 99% CH_3OH and 1% concentrated HCl) on a Bioscan system AR-2000 with Winscan software of Version 3.09 (Beijing, China).

2.2 Preparation of *fac*-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$

As described in the literature[20], 5 mg $BH_3{\cdot}NH_3$ and 5 mg $K_2[H_3BCO_2]$ were placed in a 10 mL glass vial, to which the mixture of ^{188}Re-perrhenate eluate and concentrated H_3PO_4, flushed with nitrogen for 20 min were injected and then the glass vial was incubated at 75℃ for 15 min. The reaction was ended by cooling in ice bath. In addition, the QMA Sep-Pak cartridge was used to purify the product. The chelating efficiency was determined by TLC.

2.3 Radiolabeling of small organic molecules

100 μL of triazole analog solution (dissolved in methanol, 0.01 mol/L) was mixed with 900 μL of freshly prepared ^{188}Re tricarbonyl complex solution (37 MBq/mL) and incubated at 75℃ for 1 hour. The radiolabeling efficiency was determined by HPLC.

2.4 Radiolabeling of c(RGDfk)-N_3 peptide

100 μL of c(RGDfk)-N_3 peptide (1 mg) solution was mixed with 900 μL of freshly prepared ^{188}Re tricarbonyl complex (37 MBq/mL) and incubated at 75℃ for 30 min. The radiolabeling efficiency was determined by HPLC.

2.5 Octanol–water partition coefficient

Approximately 111 kBq of conjugation compounds in 500 μL of PBS (pH=7.4) were added to 500 μL of octanol in an Eppendorf microcentrifuge tube. The mixture was vigorously vortexed for 1 min at room temperature and centrifuged at 12 500 rpm for 5 min. After centrifugation, 200 μL aliquots of both layers were measured using a γ-counter. The experiment was carried out in triplicate. And the octanol–water partition coefficient (log*P*) was obtained by the following formula:

$$\log P = \log\left(\frac{\text{counts in octanol}}{\text{counts in water}}\right).$$

2.6 Stability *in vitro*

^{188}Re-labeled triazole analogs were mixed with phosphate-buffered saline or new-born calf serum for the stability test. The admixtures were incubated at 37℃ for 24 hours. Stability was determined at various time points (0, 1, 4, 8 and 24 h) by HPLC.

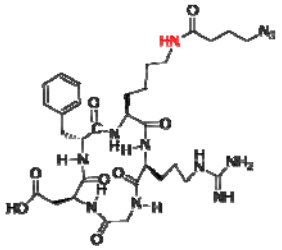

Fig.1 The chemical structure of c(RGDfk)-N_3 peptide.

3 Results and discussion

3.1 Radiolabeling of c(RGDfk)-N_3 peptide and benzyl azides

The chemical structure of c(RGDfk)-N_3 peptide was shown in Fig.1. The radiolabeled efficiencies of the labeled compounds were 93%, 94%, 95%, 95%, 91% and 92% respectively and the retention times (t_R) were 9 min, 19 min, 8 min, 10 min, 14 min and 10 min respectively according to the radio-high-performance liquid chromatography (Fig.2). The shoulder peaks on the main peaks of compound 1 and compound 5 to be exported were determined and the optical isomers were produced.

3.2 Octanol–water partition coefficient

The octanol-water partition coefficients (log*P*) for the six labeled compounds were illustrated in Table 1. The data indicate that the tracers containing c(RGDfk)-N_3 peptide are slightly more hydrophilic than containing benzyl azides.

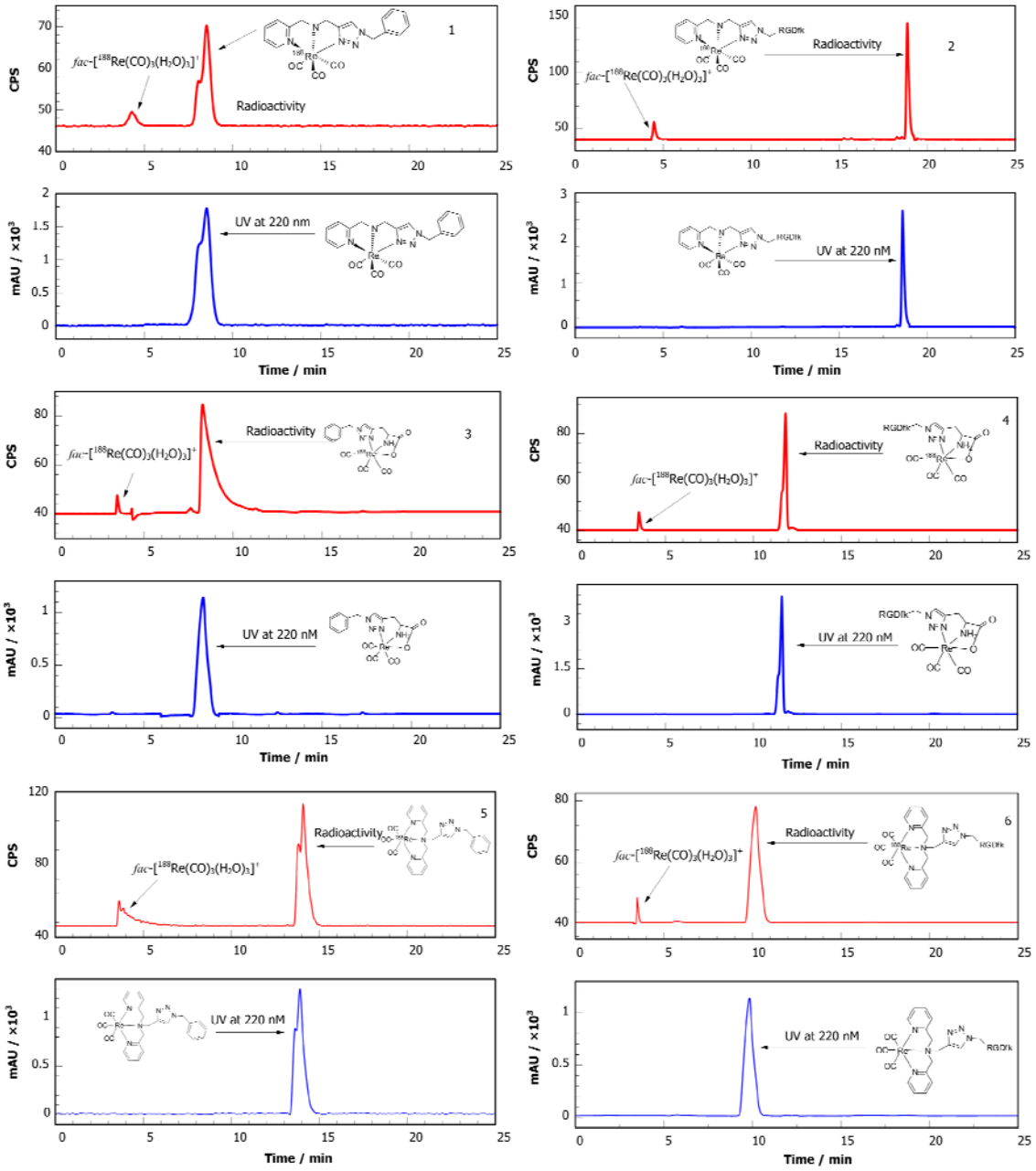

Fig.2 Radio-high-performance liquid chromatography of six triazole compounds, the aboves were the radioactive data, and the belows were the ultraviolet spectrum data of standards. CPS: count per second.

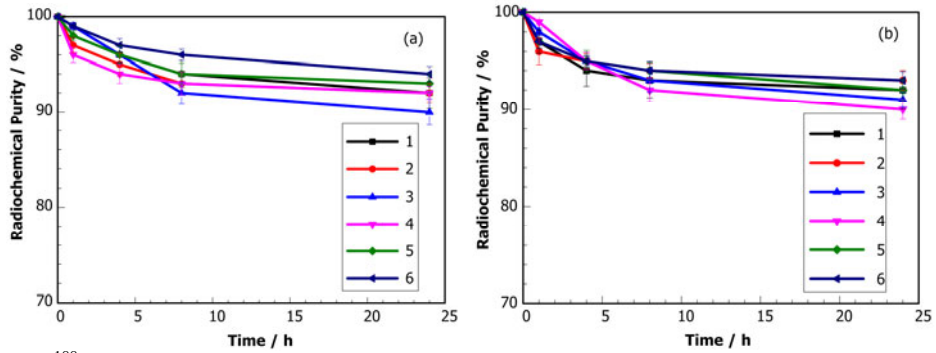

Fig.3 Stability of the ^{188}Re-labeled compounds in the presence of phosphate-buffered saline (**a**) and newborn calf serum (**b**).

3.3 Stability *in vitro*

The stability of the ^{188}Re-labeled compounds at 37℃ in the presence of phosphate-buffered saline or newborn calf serum was monitored by radio-HPLC. After 24 hours incubation, the radiochemical purity was more than 90% in both selected conditions, which was shown in Fig.3.

Table 1 Octanol-water partition coefficients of the labeled compounds

Entries	log*P*
1	0.84±0.02
2	-2.35±0.03
3	0.75±0.04
4	-2.06±0.02
5	0.97±0.05
6	-1.74±0.04

4 Conclusion

Six ^{188}Re-labeled compounds were successfully prepared using a simple click chemistry method. The main role of the click chemistry was to synthesis the bifunctional chelating agents containing triazole rings.

The use of click chemistry in compounds 1, 2, 3 and 4 was "conjugation-chelating" and in compounds 5 and 6 was just "conjugation". The well chemical yields and ideal stability *in vitro* of ^{188}Re-labeled c(RGDfk)-N_3 peptide compounds forebode the further *in vivo* research even the tumor SPECT imaging and radiotherapy.

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