Study on Radiolabeling of 1,2,3-Triazole Analogs with fac-[188Re(CO)3(H2O)3]+ via Click Chemistry (Postprint)

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Abstract

Click chemistry was used to study on radiolabeling of 1,2,3-triazole analogs with fac-[188Re(CO)3(H2O)3]+. CuSO4/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-[188Re(CO)3(H2O)3]+ was coordinated to the six triazoles. The results showed that the radiochemical yields (RCY) of the conjugation of fac-[188Re(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 188Re-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.

Full Text

Study on Radiolabeling of 1,2,3-Triazole Analogs with fac-$[188Re(CO)_3(H_2O)_3]^+$ via Click Chemistry

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Abstract

Click chemistry was employed to investigate the radiolabeling of 1,2,3-triazole analogs with fac-$[188Re(CO)_3(H_2O)_3]^+$. $CuSO_4$/L-sodium ascorbate was selected as the catalyst system, three terminal alkynes were conjugated with two

different azides respectively, and the newly prepared fac-[188Re$(CO)_3(H_2O)_3]^+$ was then coordinated to the six resulting triazoles. The results showed that the radiochemical yields (RCY) of the conjugation of fac-[188Re$(CO)_3]^+$ with the six triazoles exceeded 90%, and the triazoles exhibited high stability in phosphate-buffered saline and newborn calf serum. Preliminary biological evaluation results demonstrated that this new ^{188}Re-labeling method via click chemistry could have general applicability for labeling bioactive molecules with high radiochemical yield and high specific activity for further SPECT research.

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

Introduction

Click chemistry can be carried out with high yields under mild, tolerable conditions of neutral pH and room temperature in aqueous media within a reasonable reaction time [**?**, **?**]. Due to these favorable aspects, the use of this strategy for preparing ^{18}F-labeled biomolecules has been reported extensively [**?**], and it has now become a mature method for ^{18}F labeling [**?**, **?**]. Recently, the organometallic precursor fac-[188Re$(CO)_3(H_2O)_3]^+$ was shown to be an ideal candidate agent for labeling biomolecules [**?**] because of the high stability of the three carbonyl groups and the substitution lability of the three water molecules [**?**, **?**]. For fac-[188Re$(CO)_3]^+$ labeling, many research groups have reported the use of "click to chelate" for compound labeling or SPECT imaging [**?**]. Our group has focused on the preparation of the organometallic precursor fac-[188Re$(CO)_3(H_2O)_3]^+$ [**?**, **?**] and has labeled an RGD-containing peptide with fac-[188Re$(CO)_3(H_2O)_3]^+$ [**?**], obtaining encouraging results. In this paper, six triazoles were obtained via click chemistry and demonstrated excellent radiochemical stability in phosphate-buffered saline and newborn calf serum, proving this to be an extraordinarily ideal method for fac-[188Re$(CO)_3]^+$ labeling.

Experimental

2.1 General Materials and Methods

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 a ^{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). A γ counter (SN-697, Shanghai Rihuan Photoelectronic Instrument Co., Ltd., Shanghai, China) was used for radioactivity measurements.

High-performance liquid chromatography (HPLC) was performed using a Dionex P680 pump equipped with a PDA-100 ultraviolet detector and a ra-

diometric detector system with a Macherey-Nagel C-18 reversed phase column (5 m, 150$\times$4.6mm). $The HPLC method employed a flow rate of 1 mL/min, with the mobile phase starting from 95\_{$ and 1% concentrated HCl) on a Bioscan system AR-2000 with Winscan software Version 3.09 (Beijing, China).

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

As described in the literature [**?**], 5 mg $BH_3 \cdot NH_3$ and 5 mg $K_2[H_3BCO_2]$ were placed in a 10 mL glass vial. To this vial, a mixture of ^{188}Re-perrhenate eluate and concentrated H_3PO_4 was injected after flushing with nitrogen for 20 min. The glass vial was then incubated at 75°C for 15 min, and the reaction was terminated by cooling in an ice bath. The product was purified using a QMA Sep-Pak cartridge, and the chelating efficiency was determined by TLC.

2.3 Radiolabeling of Small Organic Molecules

One hundred microliters 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°C for 1 hour. The radiolabeling efficiency was determined by HPLC.

2.4 Radiolabeling of c(RGDfk)-N$_3$ Peptide

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

2.5 Octanol–Water Partition Coefficient

Approximately 111 kBq of the conjugation compounds in 500 L of PBS (pH=7.4) was added to 500 L 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 L aliquots of both layers were measured using a γ-counter. The experiment was carried out in triplicate, and the octanol–water partition coefficient (logP) was calculated using the following formula: logP = $\log_{10}$(counts in octanol / counts in water).

2.6 In Vitro Stability

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

[Figure 1: see original paper] The chemical structure of c(RGDfk)-N_3 peptide.

Results

3.1 Radiolabeling of c(RGDfk)-N_3 Peptide and Benzyl Azides

The chemical structure of c(RGDfk)-N_3 peptide is shown in Fig. 1. The radiolabeling efficiencies of the six labeled compounds were 93%, 94%, 95%, 95%, 91%, and 92%, respectively, with corresponding retention times (tR) as determined by radio-high-performance liquid chromatography (Fig. 2). The shoulder peaks observed on the main peaks of compound 1 and compound 5 were determined to be optical isomers.

[Figure 2: see original paper] Radio-high-performance liquid chromatography of six triazole compounds. The upper traces show radioactive data, and the lower traces show ultraviolet spectrum data of standards. CPS: counts per second.

3.2 Octanol-Water Partition Coefficient

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

3.3 In Vitro Stability

The stability of the ^{188}Re-labeled compounds at 37°C in the presence of phosphate-buffered saline or newborn calf serum was monitored by radio-HPLC. After 24 hours of incubation, the radiochemical purity remained above 90% under both conditions, as shown in Fig. 3 [Figure 3: see original paper].

[Figure 3: see original paper] Stability of the ^{188}Re-labeled compounds in the presence of phosphate-buffered saline (a) and newborn calf serum (b).

Octanol-water partition coefficients of the labeled compounds

Entries	logP
Compound 1	0.84 ± 0.02
Compound 2	-2.35 ± 0.03
Compound 3	0.75 ± 0.04
Compound 4	-2.06 ± 0.02
Compound 5	0.97 ± 0.05
Compound 6	-1.74 ± 0.04

Entries	logP

Conclusion

^{188}Re-labeled compounds were successfully prepared using a simple click chemistry method. The primary role of click chemistry was to synthesize bifunctional chelating agents containing triazole rings. For compounds 1, 2, 3, and 4, click chemistry served a "conjugation-chelating" function, while for compounds 5 and 6 it served only a "conjugation"function. The high radiochemical yields and ideal in vitro stability of the ^{188}Re-labeled compounds foreshadow their potential for further in vivo research, including tumor SPECT imaging and radiotherapy.

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Note: Figure translations are in progress. See original paper for figures.