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Efficient method for iodine radioisotope labeling of cyclooctyne-containing molecules using strain-promoted copper-free click reaction

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ABSTRACT

Herein we report an efficient method for iodine radioisotope labeling of cyclooctyne-containing molecules using copper-free click reaction. For this study, radioiodination using the tin precursor **2** was carried out at room temperature to give ^{125}I-labeled azide ([^{125}I]**1**) with high radiochemical yield (85%) and excellent radiochemical purity. Dibenzocyclooctyne (DBCO) containing cRGD peptide and gold nanoparticle were labeled with [^{125}I]**1** at 37 °C for 30 min to give triazoles with good radiochemical yields (67–95%). We next carried out tissue biodistribution study of [^{125}I]**1** in normal ICR mice to investigate the level of organ accumulation which needs to be considered for pre-targeted in vivo imaging. Large amount of [^{125}I]**1** distributed rapidly in liver and kidney from bloodstream and underwent rapid renal and hepatobiliary clearance. Moreover [^{125}I]**1** was found to be highly stable (>92%) in mouse serum for 24 h. Therefore [^{125}I]**1** could be used as a potentially useful radiotracer for pre-targeted imaging. Those results clearly indicated that the present radiolabeling method using copper free click reaction would be quite useful for both in vitro and in vivo labeling of DBCO group containing molecules with iodine radioisotopes.

1. Introduction

For several decades, iodine radioisotopes have widely been applied to the radiolabeling of biomolecules for diagnostic and therapeutic purposes.[1] ^{124}I is a useful radioisotope for positron emission tomography (PET) imaging[2] and ^{123}I/^{125}I-labeled molecules are utilized in single photon emission computed tomography (SPECT) imaging.[3–6] ^{131}I has been used for the treatment of diseases such as thyroid cancer.[7,8] Several radiochemical methods for the labeling of biomolecules or small molecules with iodine radioisotopes have been developed.[9–13] Among them, electrophilic aromatic substitution reaction has widely been applied to the radiolabeling of tyrosine-containing peptide or proteins. In many cases, this method offered a simple synthetic procedure and high labeling efficiency. But iodine radioisotope, which was labeled by the above method, was normally found to be unstable in living subjects and such deiodination resulted in high background or false signals.[14–16] Moreover direct radiolabeling using a strong oxidant for the formation of reactive iodo species often cause decreased biological activity of the substrate. Therefore several prosthetic groups have been developed for indirect labeling of iodine radioisotopes. Well-established prosthetic groups include radioiodine labeled *N*-hydroxyl succinimidyl ester[17,18] and maleimide[19] for labeling amino and thiol group, respectively, and benzaldehyde derivative[20] for labeling aminooxy group via oxime formation. These methods have been utilized in the radiolabeling of various biomolecules. But most of them provided randomly labeled products because the reactive groups do not have chemoselectivity. Moreover conventional conjugation methods often required large excesses amount of substrate because reaction rate of the reaction was not fast enough to achieve high conversion yield.

In recent years, copper-free click reaction using strained cyclooctyne is extensively investigated and utilized for the bioorthogonal and biocompatible labeling of a variety of biomolecules, living cells, and animals, which contain artificially introduced azide group.[21–25] Due to excellent specificity and rapid reaction rate of copper-free click reaction, it has also been employed to labeling of radioisotopes for nuclear imaging. Their results have been successfully applied to the efficient syntheses of ^{18}F-labeled small

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Table 1
In vitro radiolabeling results using [^{125}I]**1**

Entry	Amount of substrate **4** (nmol)	Reaction conditions	Time (min)	Radiochemical yield of [^{125}I]**5** (%)
1	20	DMSO (20 μL), rt	15	62
2	20	DMSO (20 μL), rt	30	84
3	20	DMSO/mouse plasma (20 μL), 37 °C	30	>99
4	2	DMSO/mouse plasma (20 μL), 37 °C	30	87
5	0.2	DMSO/mouse plasma (20 μL), 37 °C	30	14

molecules and peptides.[26–30] In addition to a few examples of in vitro radiolabeling application, it has been investigated for in vivo pre-targeted imaging of tumor using ^{18}F-labeled tracers.[31] Furthermore the same reaction was also applied to efficient ^{64}Cu labeling of nanoparticles and cancer targeting peptides for the specific and long-term PET imaging.[32–34] Despite of recent advances in the radiosynthesis, labeling of iodine radioisotopes using copper-free click reaction has not been extensively studied. Therefore we envisioned that use of this conjugation reaction would enable efficient radiolabeling of biomolecules with iodine radioisotopes and also provide a wide range of new imaging or therapeutic agents. Herein we describe a rapid and specific labeling method using ^{125}I-labeled azide, which can be a useful prosthetic group for radiolabeling of dibenzocyclooctyne (DBCO) containing molecules. We further present the biodistribution result of ^{125}I-labeled azide derivative ([^{125}I]**1**, Scheme 1) in normal mice to investigate its potential application of pre-targeted in vivo imaging.

2. Results and discussion

2.1. Radiolabeling of the tin precursor 2 with ^{125}I

Scheme 1 showed synthesis of the compound **2** and radiolabeling procedure. The compound **1** was prepared by using commercially available 11-azido-3,6,9-trioxaundecan-1-amine **3** and 4-iodobenzoic acid (Scheme 1). Palladium catalyzed stannylation of **1** using bis(tributyltin) and tetrakis-(triphenylphosphine)palladium gave the tin precursor **2**. Radiolabeling of **2** was carried out using [^{125}I]NaI and chloramine T as an oxidant at room temperature for 15 min to give the labeling product [^{125}I]**1**. The radiolabeling has been accomplished with up to 150 MBq of radioactivity and the desired product was obtained with an excellent radiochemical yield (85–90%) after preparative HPLC purification. Observed specific radioactivity of the product [^{125}I]**1** was 40.7 GBq/μmol. Analytical HPLC result revealed that the radiochemical purity of the ^{125}I-labeled product was more than 99%. In vitro stability test of [^{125}I]**1** was performed in mouse serum, showing that more than 93% of [^{125}I]**1** were stable for 24 h as obtained by analytical radio HPLC (Fig. S1).

2.2. ^{125}I-Labeling of the DBCO group functionalized substrate 3 and cRGD peptide 8 using [^{125}I]1

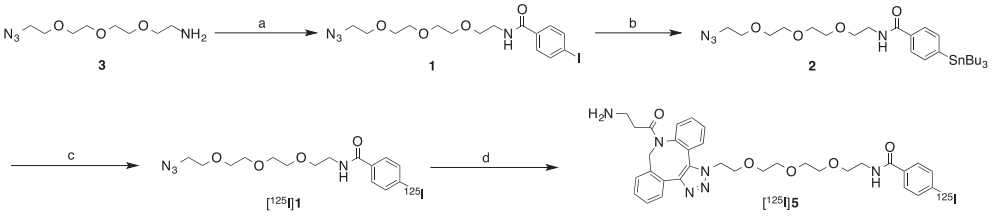
The radiolabeling efficiency of [^{125}I]**1** was determined with a DBCO substrate **4** which can be used as a potentially useful linker for biomolecule conjugation (Scheme 1). The reaction was carried out in DMSO at room temperature and the amount of DBCO substrate **4** was varied from 0.2 nmol to 20 nmol (Table 1). The conversion yields were determined by radio-HPLC integration. A non-radioactive analog **5** was synthesized by using the azide **1** as a reference for HPLC characterization of [^{125}I]**5**. [^{125}I]**1** was reacted with 20 nmol of the substrate to give the product [^{125}I]**5** in 84% radiochemical yield at room temperature within 30 min (entry 2, Table 1). The conjugation was also carried out in plasma/DMSO, which was similar with the biological environment (entries 3–5, Table 1). At 37 °C, 14% of conversion yield was observed with 10 μM (0.2 nmol) of the substrate **4** within 30 min, however the observed radiochemical yield was greatly increased (87%) with 10-fold higher concentration of DBCO substrate.

To apply [^{125}I]**1** as a prosthetic group for the synthesis of radiolabeled tumor targeting peptide, DBCO group conjugated cRGD peptide was prepared as a model substrate (Scheme 2). DBCO-NHS ester **7** was reacted with cRGDyK under basic condition to give the desired product **8** in 80% yield. In the radiolabeling experiment, the peptide **8** (100 μM, 2 nmol) was reacted with [^{125}I]**1** at 37 °C in DMSO. After stirring for 30 min, the observed labeling yield of the labeled product was [^{125}I]**9** was 67% as determined by analytical radio-HPLC. Given that [^{125}I]**1** could be converted to triazoles with modest to high radiochemical yields within 30 min, we concluded that the present method should be appropriate for radiolabeling of DBCO group containing small molecule and peptide.

2.3. ^{125}I-Labeling of DBCO group functionalized gold nanoparticle

We next investigated ^{125}I-labeling of gold nanoparticle using [^{125}I]**1** (Scheme 3). Previous study reported that iodide ion was rapidly absorbed on the surface of gold nanoparticle and thus ^{125}I could be easily labeled with polyethylene glycol modified 13 nm gold nanoparticle.[35] The labeling procedure of this method was quite simple and excellent radiochemical yield was provided. But in vitro stability of ^{125}I-labeled gold nanoparticle which was obtained by the above method was not promising in mouse serum and showed that more than 35% of radioactivity was liberated from gold nanoparticle over 30 h. Dehalogenation of iodine radioisotope in living animal might cause high accumulation of radioactivity in some organs such as thyroid and stomach. Therefore efficient as well as physiologically stable labeling method was necessary for the preparation of radiolabeled nanomaterials.

For this study, DBCO group modified gold nanoparticle was prepared by the following procedure (Scheme 3). Excess amount of thiolated polyethylene glycol (MW 5000) that contains DBCO group was added to the citrated stabilized 13 nm gold



Scheme 1. Reagents and conditions: (a) 4-iodobenzoic acid, HBTU, DIPEA, DMF, rt, 90%; (b) $Pd(Ph_3P)_4$, Sn_2Bu_6, 1,4-dioxane, reflux, 65%; (c) [^{125}I]NaI, chloramine T, EtOH, 85% radiochemical yield; (d) DBCO-amine **4**, (Reaction conditions and results were shown in Table 1).