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Facile and Efficient Postsynthetic Tritium Labeling Method Catalyzed by Pd/C in HTO

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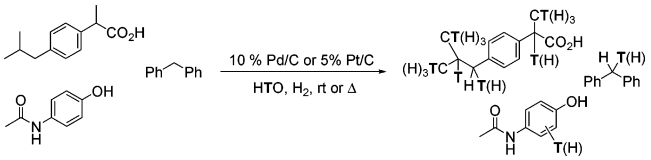

We have developed a facile and efficient tritium labeling method using a Pd/C−HTO−H_2 system. This method can provide multitritium-labeled compounds in highly diluted HTO under T_2 gas-free conditions, and is environmentally benign since purification by silica gel column chromatography is not necessary, which causes a large quantity of radioactive waste such as silica gel and eluent.

Tritium (T) is a most versatile radionuclide and the detection sensitivity of tritium is roughly a million times higher than that of deuterium. Organic compounds labeled with tritiums are widely used in the investigation for life sciences as an important tracer, such as biosynthetic pathways, analysis of drug metabolism, autoradiography, radioassay, and so on.[1] While some tritium-labeled compounds are commercially available, they are relatively expensive and it is quite difficult to obtain a desired compound. There are mainly two types of methods to prepare tritium-labeled organic molecules as follows: H−T exchange methods[2] and reductive synthetic methods.[2,3] The latter reductive methods starting from a reducible precursor are effective for the incorporation of tritium atoms onto specific positions with high specific radioactivity using a stoichiometric amount of tritiated reducing reagents such as $LiBT_4$ or $LiAlT_4$ and transition metal-catalyzed reductive dehalogenation and hydrogenation using T_2 gas. On the other hand, the transition metal-catalyzed H−T exchange replacement by T_2 gas of the hydrogen bound to the carbon of an unlabeled compound can postsynthetically introduce tritiums into various compounds with high specific activity.[2,4] While available carrier free T_2 gas has extremely high specific radioactivity and must be handled in a tracer scale, most of the conventional labeling methods use T_2 gas. Consequently, the development of a facile and efficient tritium labeling method without T_2 gas has been desired. Several H−T exchange reactions in HTO catalyzed by Rh,[7] Pt,[8] Ru,[7b,9] and so on[7a,10] have been reported. Especially, Garnett et al. reported an efficient and pioneering H−T exchange reaction in HTO catalyzed by platinum black prepared by the in situ reduction of PtO_2 with $NaBH_4$.[8] However, the magnitude of the tritium efficiency was widely varied.

We have recently disclosed a chemoselective C−H/C−D exchange reaction on the benzylic carbon in D_2O using Pd/C as a heterogeneous catalyst in the presence of a catalytic amount of H_2 gas at room temperature[5] and multideuterium introduction including the nonactivated carbon under the heating conditions.[6] As an extension of these methods, we planned to apply them to the H−T exchange reaction in HTO. In this paper we describe an efficient and facile H−T exchange reaction catalyzed by heterogeneous Pd/C in highly diluted HTO as a tritium source.

In the deuteration reactions, the use of high-purity D_2O is essential to achieve high deuterium efficiency.[5,6] On the other hand, T_2O with high specific activity is highly

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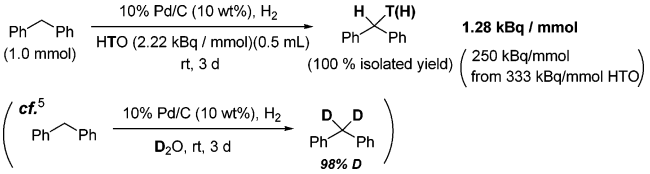
SCHEME 1. Tritium Incorporation into Diphenylmethane with Use of Pd/C−HTO−H_2 at Room Temperature

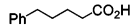
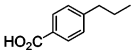


hazardous and labile and is not readily available.[1a] The magnitude of the tritium efficiency was expected to drastically decrease with the highly diluted conditions because it should be affected by the frequency of contacts between the substrate and HTO. However, we expected that the incorporation method of tritium atoms into organic compounds with highly diluted HTO could be suitable for practical use since tritium is an extremely sensitive radioisotope. In an initial attempt, we examined the incorporation of tritiums on the benzylic site using HTO diluted to 2.22 kBq/mmol in the presence of 10 wt % (vs the substrate) of 10% Pd/C and a catalytic amount of H_2 gas at room temperature (Scheme 1).

As a result, tritium was successfully incorporated into diphenylmethane in 1.28 kBq/mmol specific activity.[11] It was surprising that the tritium incorporation was smoothly achieved regardless of the use of highly diluted HTO (2.22 kBq/mmol, ca. 1 ppb of pure T_2O). The specific activity of diphenylmethane was obviously increased to 250 kBq/mmol with an increase in the tritium concentration of HTO (333 kBq/mmol). Although it is not determined where the tritium atoms are incorporated, it is expected that they are selectively incorporated into the benzylic position based upon our H−D exchange reactions[5] (Scheme 1).

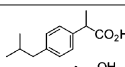
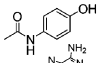
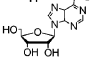
Next, we applied the heating conditions to our tritium labeling reaction using the Pd/C−HTO−H_2 system that would be expected to promote the tritium introduction. The tritiation of 5-phenylvaleric acid proceeded with 1.82 kBq/mmol at 160 °C, which indicates the multitritium incorporation occurs under these conditions since the obtained specific activity of 5-phenylvaleric acid was roughly 3 times higher than that of HTO (0.67 kBq/mmol, Table 1, entry 1).[12] The combination of Pd/C−HTO−H_2 is indispensable for the progress of an efficient H−T exchange reaction since no tritium incorporation was observed in the absence of either Pd/C or H_2 gas (Table 1, entries 2 and 3). Further purifications are not necessary except for the simple removal of the heterogeneous catalyst by filtration and extraction, and the analytically pure products (>98% purity by HPLC analysis) were obtained. This is an important advantage of the presented method because silica gel column chromatography is usually required for purification in the conventional method and silica gel flowed with the tritiated compounds formed radioactive waste. These conditions were also applicable to the labeling reaction of 4-propylbenzoic acid with 5.50 kBq/mmol, which was more than twice the specific activity of HTO (2.22 kBq/mmol).

TABLE 1. Multitritium Incorporation with Use of the Pd/C−HTO−H_2 System at 160 °C[c]

Entry	Substrate	HTO (kBq/mmol)	Product (kBq/mmol)	Yield (%)
1		0.67	1.82	98
2[a]		0.67	0	-
3[b]		0.67	0	-
4		2.22	5.50	87

[a] In the absence of Pd/C. [b] Argon atmosphere [c] Reaction conditions: substrate (0.5 mmol), 10% Pd/C (10 wt %), HTO (2.0 mL).

TABLE 2. Tritium Incorporation with Bioactive Compounds as a Substrate[a]

Entry	Substrate	Catalyst	Temp (°C)	HTO (kBq/mmol)	Product (kBq/mmol)	Yield (%)
1		10% Pd/C	160	0.67	3.42	96
2		5% Pt/C	160	2.22	4.26	72
3		10% Pd/C	110	2.22	0.70	99
4		10% Pd/C	110	2.22	0.62	94

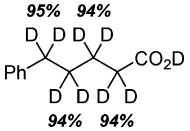
[a] Reaction conditions: substrate (0.5 mmol), 10% Pd/C (10 wt %) or 5% Pt/C (20 wt %), HTO (2.0 mL).

Further application to bioactive compounds was also examined. As a result, all of the substrates such as ibuprofen, acetaminophen, adenosine, and phenylalanine were tritiated (Table 2). Surprisingly, efficient tritium incorporation was achieved on ibuprofen and 3.42 kBq/mmol of specific activity is more than 5 times higher than 0.67 kBq/mmol of HTO. On the other hand, when 5% Pt/C, which was an effective catalyst for the deuteration of aromatic rings,[13] was used in place of 10% Pd/C in 2.22 kBq/mmol of HTO, tritiums were successfully incorporated into the acetaminophen molecule in 4.26 kBq/mmol. The reaction with adenosine and phenylalanine as substrates gave rather lower specific activities (0.70 and 0.62 kBq/mmol, respectively, in 2.22 kBq/mmol of HTO). The reason for the lower efficiency is not clarified, but it may be caused by the lower reaction temperature.[14]

In summary, we have developed a facile and efficient tritiation method catalyzed by Pd/C or Pt/C using HTO as a tritium source in the presence of a catalytic amount of H_2 gas. The method presented here provides a T_2 gas-free, and totally postsynthetic tritium labeling method

(11) Our results indicated that the efficiency of the tritium atom transfer from HTO to substrate is roughly two orders higher than Williams' results.[8d]

(12) In our previous report of the H−D exchange reaction with the Pd/C−D_2O−H_2 system,[6] methylenes of 5-phenylvaleric acid were almost fully deuterated at 160 °C.



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(14) In the case of adenosine or phenylalanine, lower temperature (110 °C) was used for each reaction to avoid cleavage of the glycosyl bond or racemization.

under neutral conditions without requiring the chromatographic purification. The reaction is applicable to various substrates and tolerates a wide range of different functional groups, such as amine, carboxylic acid, amide, and so on. The multitritium incorporation provides tritium-labeled compounds with high specific activities, using HTO possessing low specific activities. Accordingly, the presented reaction possesses the potential to be an efficient, applicable, and environmentally benign tritium labeling method.

Experimental Section

Caution! The compounds containing tritium atoms are hazardous, especially in the case of inhalation. All experiments were conducted with groves in the fume food in the control area for radiation. And care should be taken with the internal pressure of the reaction, which increases to ca. 2.5 atm in the sealed tube at 160 °C.

Typical Procedure for the Tritiation Reactions with Pd/C−HTO−H_2 at Room Temperature. A suspension of diphenylmethane (1.0 mmol) and 10% Pd/C (10 wt % of substrate) in HTO (0.5 mL) was stirred at room temperature in a test tube under H_2 atmosphere for 72 h. The mixture was diluted with ether (20 mL) and filtered with a membrane filter (Millipore, Millex-LH, 0.45 μm). The filtrate was extracted with ether (2 × 20 mL). The combined Etheral layer was washed with H_2O and saturated aq NaCl and dried with $MgSO_4$, then concentrated in vacuo. Measurements of the specific activity of the substrate were made on a liquid scintillation analyzer.

[^{3}H]Diphenylmethane (1). Yield 100% (99.0% pure by HPLC), specific activity 1.28 kBq/mmol (using 2.22 kBq/mmol of HTO).

Typical Procedure for the Tritiation Reactions with Pd/C−HTO−H_2 at 160 °C. A suspension of 3-phenylpropionic acid (0.5 mmol) and 10% Pd/C (10 wt % of substrate) in HTO (2 mL) was stirred at 160 °C in a sealed tube under H_2 atmosphere for 24 h. The mixture was diluted with ether (20 mL) and filtered with a membrane filter (Millipore, Millex-LH, 0.45 μm). The filtrate was extracted with ether (2 × 20 mL). The combined etheral layer was washed with H_2O and saturated aq NaCl and dried with $MgSO_4$, then concentrated in vacuo. Measurements of the specific activity of substrate were made on a liquid scintillation analyzer.

[^{3}H]3-Phenylpropionic acid (2). Yield 98% (99.3% pure by HPLC), specific activity 1.82 kBq/mmol (using 0.67 kBq/mmol of HTO).

Supporting Information Available: Experimental procedures and spectral data for tritiated compounds and the deuteration data of each substrate in Table 2 as a reference of the tritiation reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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