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Research Article

A new practical tritium labelling procedure using sodium borotritide and tetrakis(triphenylphosphine)palladium(0)

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Summary

A simple, mild and versatile new tritium (^{3}H) labelling method on a micro scale using sodium borotritide (NaB^3H_4) and a transition–metal complex catalyst is described. ^{3}H-labelled compounds were prepared effectively by ^{3}H hydrogenolysis of appendant functional groups in target compounds. The appendant functional group such as bromo, iodo or sulfonate in various target compounds can be replaced by tritium (^{3}H) in moderate yields. The new method was established by optimization of the reaction conditions and examination of its applicability using four types of model substrates in tracer runs. Then, various drug candidates and ligands for drug discovery were labelled with tritium on a micro scale. The specific radioactivity of the ^{3}H-labelled compounds used for the studies on receptor binding ranged from 12 to 20 Ci/mmol. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: ^{3}H-labelling procedure; ^{3}H-terprenin; ^{3}H-rosiglitazone; ^{3}H-(+)-S-145; ^{3}H-LSD; ^{3}H-oxycodone

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Introduction

Tritium labelled compounds with high specific radioactivity have been prepared mostly by the catalytic hydrogenation or dehalogenation with gaseous tritium.[1,2] This traditional method needs a sophisticated apparatus and a large amount of tritium gas,[3] so that the method has been commercially performed usually as the custom synthesis at professional institutes. Alternatively, ^{3}H-labelled compounds have been prepared by exchange of the hydrogen in target compounds with tritium using gaseous tritium or tritiated water.[4] During the past two decades, popular reducing reagents such as trialkyl-tin hydride,[5] metal hydride and lithium- or sodium-selectrides have appeared as their tritide forms to close a methodological gap but have seldom been used as tritiation reagents.[6,7] These tritiation-reagents have not been widely used because of their instability, difficult preparation and low chemo-selectivity. Recently, the increasing importance of ^{3}H-labelled compounds with high specific radioactivity for studies on drug discovery has prompted us to search for a new facile ^{3}H-labelling procedure. Taking a high specific radioactive tritium labelling into consideration, we have focused our attention on sodium borotritide ($NaB^{3}H_4$) as a tritiation reagent that is commercially available and easily handled. As for the substrate, halogenated target compounds were chosen as easily obtainable precursors, which can be reduced by one-step reductive tritiation. To the best of our knowledge, hydrogenolysis of halides is quite difficult by reduction with $NaBH_4$ alone. Hydrogenolysis of halides with $NaBH_4$ in combination with a metal salt such as $PdCl_2$, $CuCl_2$, $FeCl_2$, $ZnCl_2$, $NiCl_2$, or $CoCl_2$ has been reported in the literature,[8,9] but there is no applicable reduction process, which can perform the reductive-tritiation in a chemo-selective manner on a micro scale. In the case of ^{3}H-labelling, a limited amount of radioactive $NaB^{3}H_4$ should be used in contrast to hydrogenolysis of halides with an excess of non-radioactive $NaBH_4$ in combination with a metal salt. During the last decade, the reactivity of homogeneous transition organo-metallic complexes and their application to a carbon–halogen bond activation have been intensively investigated. Those organo-metallic complexes were widely used as catalysts in the Suzuki reaction[10,11] and the Heck reaction[12,13] and are widely reported in the chemical literature. These reports provide an insight into the ^{3}H-labelling by hydrogenolysis of halides with $NaB^{3}H_4$ and a transition organo-metallic complex as the catalyst. The literature survey has revealed that a few studies on hydrogenolysis-type

dehalogenation by the combination of $NaBH_4$ and a transition–metal complex catalyst were reported[14,15] but ^3H-labelling using NaB^3H_4 with or without catalyst in hydrogenolysis-type reactions have never appeared.

Selection of catalyst for hydrogenolysis-type tritium labelling

Initially, we searched for a potent reducing system in combination of NaB^3H_4 and a catalyst selected from various transition organo-metal complexes. In the catalyst selection (Entry No. 1–6), 4-bromobiphenyl was used as a model substrate because of easy separation of the product [4-^{3}H]-biphenyl. The organo-palladium complexes such as $Pd(PPh_3)_4$, $Pd(OAc)_2$, $Pd(PhCN)_2Cl_2$ and $Pd(dba)_3$ worked well as the catalyst (Figure 1), (Table 1). The catalyst selection and tracer experiment (Entry No. 1–19) with model substrates were carried out according to general procedure A described in the experimental section.

This hydrogenolysis-type tritium labelling with the combination of NaB^3H_4 and $Pd(PPh_3)_4$ was extended from biphenyl bromide to other biphenyl and biphenyl-alkyl halides and sulfonates using four types of

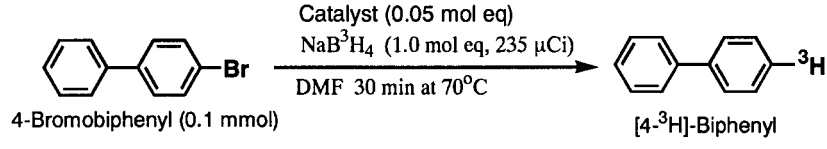

Figure 1. Catalyst selection for ^3H-hydrogenolysis of 4-bromobiphenyl

Table 1. Catalyst selection for the ^3H-hydrogenolysis

Entry no.	Catalyst	Chemical yield (%)	Radio-chemical yield (%)[a]	Total act. (μCi)	Spec. act. (μCi/mmol)
1	$Pd(PPh_3)_4$	56.8	44.9	26.4	465
2	$Pd(OAc)_2$	62.7	62.8	36.9	589
3	$Pd(PhCN)_2Cl_2$	62.2	58.0	34.0	547
4	$Pd(dba)_3$	43.3	46.0	27.0	624
5	$Rh(PPh_3)_3Cl$	36.6	26.6	15.6	426
6	$Ru(PPh_3)_3Cl_2$	6.40	2.88	1.69	264

[a] Calculated theoretically based on 1/4 tritium of NaB^3H_4.

model substrates in order to establish a new ^{3}H-labelling as a versatile method as shown in Figures 2–5 with the corresponding results shown in Tables 2–5.

Results and discussion

In each type of the model substrates, iodides, bromides, trifluoromethanesulfonates (OTf), tosylates (OTs) and mesylates (OMs) can be

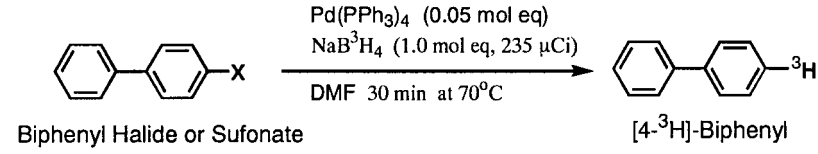

Figure 2. ^{3}H-hydrogenolysis of biphenyl halides and sulfonates

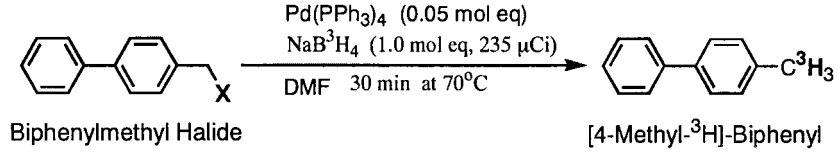

Figure 3. ^{3}H-hydrogenolysis of biphenylmethyl halides

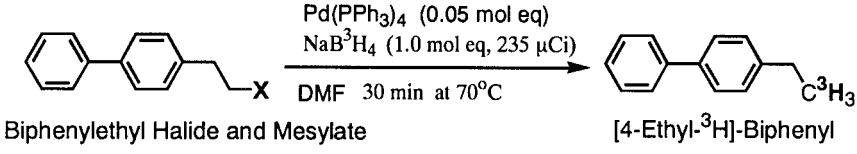

Figure 4. ^{3}H-hydrogenolysis of biphenylethyl halides and mesylate

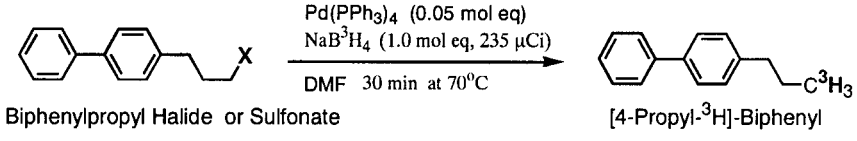

Figure 5. ^{3}H-hydrogenolysis of biphenylpropyl Iodide and sulfonates

Table 2. Results of ^{3}H-hydrogenolysis of biphenyl halides and sulfonates

Entry no.	Appendant group X	Chemical yield (%)	Radiochemical yield (%)[a]	Total act. (μCi)	Spec. act. (μCi/mmol)
7 (1)	Br	56.8	44.9	26.4	465
8	I	70.0	65.0	38.2	546
9	OTf	55.3	50.6	29.7	537
10	OMs and OTs[b]	—	—	—	—
11	Cl and F[c]	—	—	—	—

[a] Calculated theoretically based on 1/4 tritium of NaB^3H_4.
[b] Hydrolysis on aryl-OTs and aryl-OMs led to phenols.
[c] No reaction on aryl chloride and fluoride.

Table 3. Results of ^{3}H-hydrogenolysis of biphenylmethyl halides

Entry no.	Appendant group X	Chemical yield (%)	Radiochemical yield (%)[a]	Total act. (μCi)	Spec. act. (μCi/mmol)
12	Br	48.7	51.1	30.0	616
13	I	48.0	46.0	27.0	563

[a] Calculated theoretically based on 1/4 tritium of NaB^3H_4.

Table 4. Results of ^{3}H-hydrogenolysis of biphenylethyl halides and Mesylate

Entry no.	Appendant group X	Chemical yield (%)	Radiochemical yield (%)[a]	Total act. (μCi)	Spec. act. (μCi/mmol)
14	Br	44.8	57.0	33.5	748
15	I	71.4	61.4	36.1	506
16	OMs	34.2	35.7	21.0	614

[a] Calculated theoretically based on 1/4 tritium of NaB^3H_4.

Table 5. Results of ^{3}H-hydrogenolysis of biphenylpropyl iodide and sulfonates

Entry no.	Appendant group X	Chemical yield (%)	Radiochemical yield (%)[a]	Total act. (μCi)	Spec. act. (μCi/mmol)
17	I	69.4	58.7	34.5	497
18	OMs	41.0	45.4	26.7	651
19	OTs	32.5	38.0	22.3	686

[a] Calculated theoretically based on 1/4 tritium of NaB^3H_4.

replaced by tritium by this labelling procedure, except for the aryl tosylate and the aryl mesylate, which underwent prior hydrolysis leading to phenols. On the other hand, biphenyl chloride and fluoride remained unchanged. The inertness of the chloride and fluoride against

this combination is rather advantageous because the chloro and fluoro groups are often included as indispensable bio-active related moieties in drug candidates and drug-discovery ligands that should be labelled. The tracer runs (Entry No. 1–19, Figures 1–5 and Tables 1–5) and actual tritium labelling runs (Figures 7–11 and Table 6) were carried out according to optimized general procedures A and B, respectively. The examination of the reaction conditions revealed that more harsh reaction conditions such as doubling the catalyst loading, a prolonged reaction time and an elevated temperature had little effect or reduced the yields in the tracer runs. A plausible catalytic reaction mechanism for the tritium labelling of halides or sulfonates is proposed in Figure 6 based on the specific radioactivity of the products and generation of [^{3}H]-diborane gas. A quarter of the tritium of NaB^3H_4 was employed for labelling and three quarters of tritium was exhausted as diborane ($B_2{}^3H_6$). In the same fashion, the hydrogenolysis–type reduction of biphenyl bromide with a combination of $Pd(PPh_3)_4$ and $NaB(OMe)_3H$, instead of $NaBH_4$, was carried out successfully in a cold, micro

Table 6. Tritium labelled compounds with high specific radioactivity

Labelled compounds name	Total act. (mCi)	Specific activity (Ci/mmol)	Radiochem. purity (%)	NaB^3H_4 (Ci/mmol)
[^{3}H]-S-145-Me	61.3	12.1	99.2	58
[^{3}H]-Terprenin	3.57	11.7	98.5	90
[^{3}H]-Rosiglitazone	12.5	18.1	99.5	90
[^{3}H]-LSD	14.0	15.5	96.7	90
[^{3}H]-Oxycodone	6.49	12.0	99.9	90

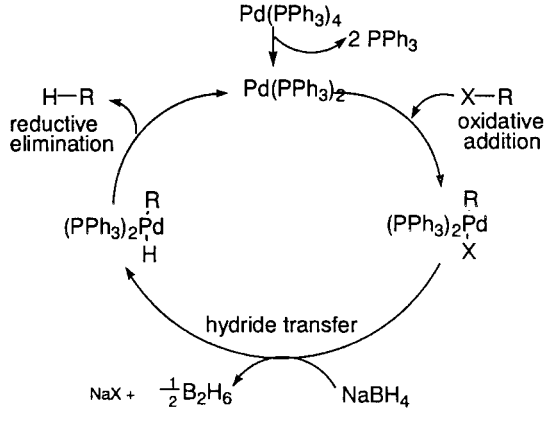

Figure 6. Plausible Mechanism

scale experiment, indicating a potential for tritium labelling with $NaB(OMe)_3{}^3H$, which is not commercially available at present.

Application

In order to demonstrate the simplicity, applicability and efficiency of the new method, it has been applied to the ^3H-labelling of drug candidates

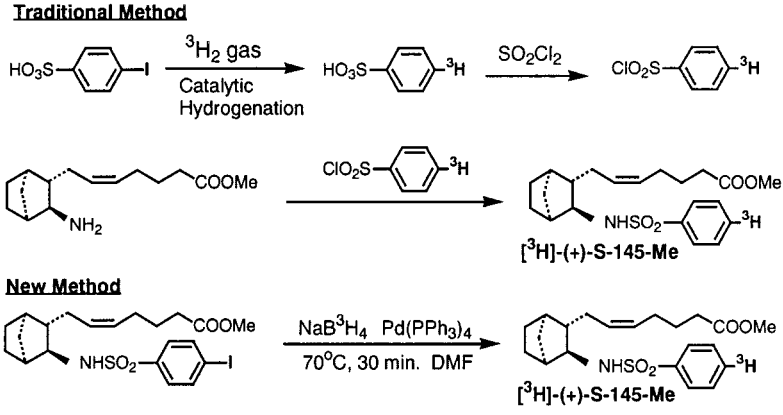

Figure 7. Synthetic scheme for ^3H-labelled S-145-Me

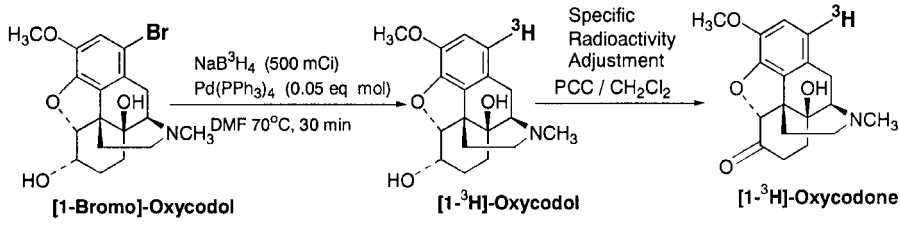

Figure 8. Synthetic scheme for [^{3}H]-labelled oxycodone

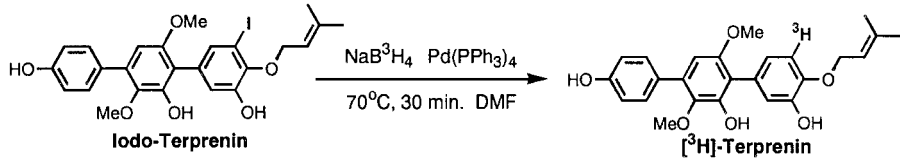

Figure 9. Synthetic scheme for [^{3}H]-labelled terprenin

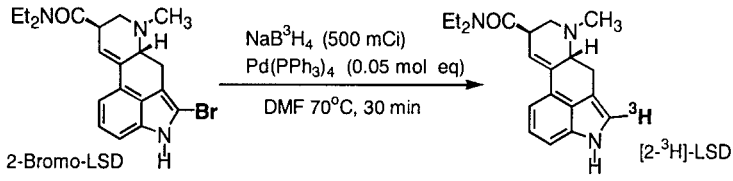

Figure 10. Synthetic scheme for [^{3}H]-labelled lysergide ([^{3}H]-LSD)

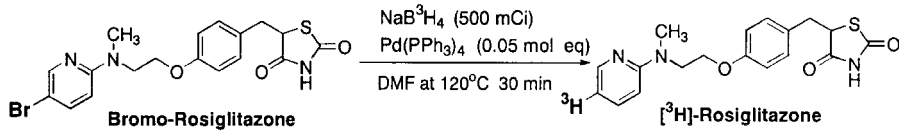

Figure 11. Synthetic scheme for ^{3}H-labelled Rosiglitazone (BRL 49653)

and drug-discovery ligands. It is noteworthy that the ^{3}H-labelled compounds, which could not be obtained by traditional methods because of simultaneous hydrogenation of an indispensable unsaturated bond, were synthesized on a micro scale from the corresponding mono-halo precursors according to general procedure B described in the experimental section. The low-to-moderate yields in tritium incorporation are compensated for by the low cost of the starting borotritide and the high value of tritium labelled product obtained. The results of ^{3}H-labelling of drug candidates and drug-discovery ligands are summarized in Table 6.

Tritium labelling synthesis of S-145-Me (Thromboxane-A_2 receptor antagonist)

As the first application, a selective thromboxane-A_2 receptor antagonist (S-145 methylester) was labelled by the method for the receptor binding studies. The method was easier and more economical as compared with our previous stepwise ^{3}H-labelling, which consisted of three-step process including catalytic ^{3}H-hydrogenation,[16] as shown in the synthetic scheme (Figure 7). The radioactive diborane ($B_2{}^3H_6$) generated in the new methods did not affect the double bond under the labelling conditions.

Tritium labelling synthesis of oxycodone

An analgesic semi-synthetic derivative from opium alkaloids, oxycodone[17] was labelled with tritium in order to facilitate the ADME studies of oxycodone as shown in Figure 8. [1-^{3}H]-oxycodone was synthesized in 2 steps via [1-^{3}H]-6α-oxycodol from 1-bromo-6α-oxycodol by the new ^{3}H-labelling method and following oxidation with PCC.

Tritium labelling synthesis of terprenin

A potent IgE suppressant, Terprenin,[18] was ^{3}H-labelled in one step without damage to the prenyl group, as shown in Figure 9, for the studies on bio-reaction mechanism of the IgE suppressant.

Tritium labelling synthesis of lysergide (LSD)

For drug discovery studies, a narcotic, Lysergide (LSD),[19] regarded as a serotonin ($5HT_{1A}$) antagonist, was labelled with tritium. [^{3}H]-LSD with high specific activity was synthesized from unlabelled LSD via 2-bromo LSD by the new tritium labelling procedure, without damage to the indispensable double bond, as shown in Figure 10.

Tritium labelling synthesis of rosiglitazone

Rosiglitazone (BRL49653),[20] a PPARγ-receptor agonist, was also labelled as a ligand by the new method.

Material

Sodium borotritide (NaB^3H_4:2.35 mCi/mmol)

A mixture of sodium borotritide (NaB^3H_4: 100 mCi, 18.5 mg, 489 μmol, 204 mCi/mmol) and sodium hydride ($NaBH_4$: 1.65 g, 39.4 mmol) was dissolved in anhydrous DMF (50 ml) at 45°C by sonication. After removal of an insoluble substance by filtration under nitrogen, the filtrate was evaporated to dryness in vacuo at 45°C to give a crystalline residue. The residue was triturated with anhydrous THF, and the precipitate was collected by filtration and dried in vacuo at room temperature to a constant weight to give sodium borotritide (NaB^3H_4: 59.6 mCi, 1.06 g, 25.2 mmol, 56.2 μCi/mg, 2.35 mCi/mmol). The specific

radioactivity of the diluted NaB^3H_4 was determined by measuring the radioactivity of [4-hydroxymethyl-^{3}H]-biphenyl obtained by reduction of biphenyl aldehyde.

Experimental

The radiochemical purity of labelled compounds was determined by TLC followed by liquid scintillation counting, using Packard Tricarb 1000 and HPLC with a radioactivity flow monitor, Packard FLO-ONE 525TR. The labelled compounds were identified with the corresponding unlabelled authentic samples by comparison of TLC (Rf value) and HPLC (retention time). TLC was carried out using thin layer plates (Merck silica gel 60_{F254} 0.25 mm). The specific radioactivity of ^{3}H-labelled compounds was determined by means of measurement of both radioactivity and UV absorption. Throughout the synthesis, all of the reactions were carried out under a nitrogen atmosphere in order to protect from moisture and oxygen.

General ^{3}H-labelling procedure A

To a stirred solution of 4-bromobiphenyl (0.1 mmol) and 5%mol of catalyst (0.005 mmol) in anhydrous DMF (1.0 ml) was added a solution of NaB^3H_4 (235 μCi, 4.18 mg, 0.1 mmol, 2.35 mCi/mmol) at room temperature under a nitrogen atmosphere. After stirring at 70°C for 30 min, the mixture was poured into cold water (10 ml) and extracted with ethyl acetate (20 ml). The extract was washed with water (10 ml × 2), dried with sodium sulfate and concentrated below 30°C under reduced pressure to about 0.5 ml. From the concentrate, the pure tritiated compound ([4-^{3}H]-biphenyl) was obtained by appropriate small-scale chromatographic purification and identified by HPLC co-chromatography with authentic cold material and the radiochemical yield was determined by liquid scintillation counting.

General ^{3}H-labelling procedure B [1,2]

A solution of halide (ca. 18 μmol) and $Pd(PPh_3)_4$(1.13 mg, 0.9 μmol) in anhydrous DMF (200 μl) was added onto a solid NaB^3H_4 (500 mCi,

[1] NaB^3H_4 (58 Ci/mmol) was purchased from NEN™ Life Science Products Inc.
[2] NaB^3H_4 (90 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc.

0.336 mg, 8.62 μmol, 58 Ci/mmol) in a single portion at room temperature under a nitrogen atmosphere. After being allowed to stand at 70°C for 30 min, the mixture was poured into cold water (5 ml) and extracted with ethyl acetate (5 ml × 2). The extracts were washed with water (3 ml × 2), dried with sodium sulfate and concentrated below 30°C under reduced pressure to about 0.5 ml. From the concentrate, the pure tritiated compound was obtained by appropriate small-scale chromatographic purification and identified by HPLC co-chromatography with authentic cold material and the radiochemical yield was determined by liquid scintillation counting.

Conclusion

The new, simple and versatile tritium (^{3}H) labelling method aforementioned provides an easy, economical alternative to traditional ^{3}H-labelling. The method (NaB^3H_4-catalyst hydrogenolysis) is applicable to aryl, benzyl, allyl, homo-allyl and alkyl-bromides, iodides and sulfonates, which may be prepared as precursors of target compounds. The method can be applied to obtain ^{3}H-labelled compounds which cannot be prepared by ^{3}H-hydrogenation because of simultaneous reduction of indispensable unsaturated bonds, nitrile and/or nitro groups. The method gave ^{3}H-labelled compounds with sufficient specific activity (10–20 Ci/mmol) for studies on receptor binding and pre-ADME.

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