# Palladium-Catalyzed Base-Selective H–D Exchange Reaction of Nucleosides in Deuterium Oxide

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**Abstract:** We have developed an efficient and extensive deuterium incorporation method using a heterogeneous Pd/C–$D_2O$–$H_2$ system into the base moiety of nucleosides. The results presented here provide a deuterium gas-free, totally catalytic, and post-synthetic deuterium labeling method in $D_2O$ media.

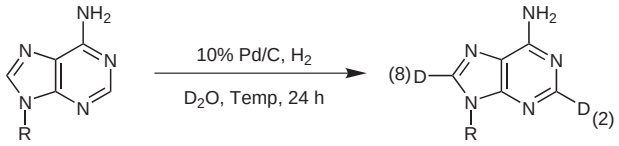
**Key words:** nucleosides, palladium, reaction, H–D exchange, base-selective

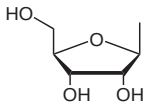
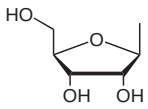
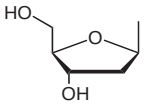
Nucleoside analogs are noteworthy as biologically active targets for the development of potential antiviral and antitumor agents[1] and as synthetic oligonucleotide probes.[2] Deuterium-labeled compounds have a number of important uses in many different branches of science, including analysis of drug metabolism, investigation of reaction mechanisms, kinetics, and so on.[3,4] Deuterium-labeled nucleosides have proven valuable in metabolic studies[5] and structural analyses of DNA.[6] For the preparation of base-selectively deuterium-labeled nucleic acids, the previous methods are chiefly categorized under the following two types: the multi-step synthetic method starting from originally deuterium-labeled small synthons,[7] and the post-synthetic H–D exchange displacement of the hydrogen bound to the carbon of an unlabeled compound by deuterium using a catalytic method.[8] It is apparent that the latter process is highly effective and accessible for the preparation of deuterium-labeled nucleosides. However, such conventional post-synthetic procedures for the incorporation of deuterium into the base-moieties of nucleosides are often limited to activated positions of the molecules,[8a–8c,e,g] leading to low levels of deuterium incorporation,[8e,f] and require a vast amount of the catalyst,[8d,f,g] addition of acidic or basic additives,[8a–8c,e,f] and/or expensive deuterium atmosphere.[8d,f,g,9]

We have recently reported an efficient and chemoselective exchange of deuterium derived from $D_2O$ with hydrogen atoms on a benzylic carbon using Pd/C as a heterogeneous catalyst in the presence of a catalytic amount of hydrogen gas at room temperature.[10] We also found that application of heat could promote the catalyst activity of the Pd/C–$D_2O$–$H_2$ system and lead to a H–D exchange reaction even on non-activated carbon.[11,12] Herein, we describe a distinctly general and selective procedure for the H–D exchange reaction at the base moiety of nucleosides applying the Pd/C–$D_2O$–$H_2$ system with heating conditions.

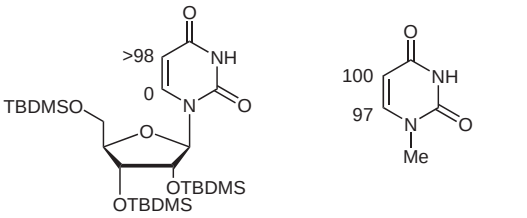
To explore the scope of our method for the H–D exchange of nucleic acids, the reaction of a number of substrates was investigated (Tables 1– 4).

**Table 1** H–D Exchange Reaction of Adenine Derivatives




| Entry | R | Temp (°C) | D content (%)[a] 2-D | 8-D | Yield (%) |
|---|---|---|---|---|---|
| 1 | H | 110 | 96[b] | 96[b] | 99 |
| 2 |  | 110 | 95 | 92 | 99 |
| 3 |  | 160 | 95 | 96 | 98 |
| 4 |  | 160 | 96 | 96 | 81 |

[a] Determined by $^1H$ NMR spectroscopy using 3-trimethylsilyl-1-propanesulfonic acid sodium salt (DSS) as an internal standard.
[b] Indicates the average D content.




**1** (93% isolated yield) **2** (100% isolated yield)

**Figure 1** Deuterium efficiency of **1** and **2** by the H–D exchange reaction at 160 °C for 24 hours.

Typically, the reaction is carried out in 1.0 mL of $D_2O$ using 0.25 mmol of the substrate and 10% Pd/C (10 wt%) at 110–160 °C under a hydrogen atmosphere. The reactions are usually very clean and no chromatographic separation

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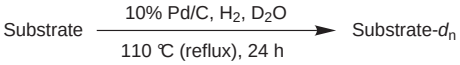
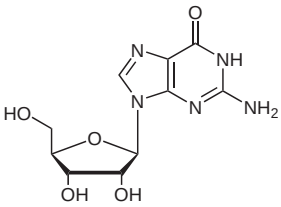
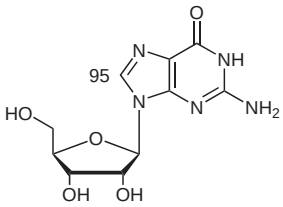
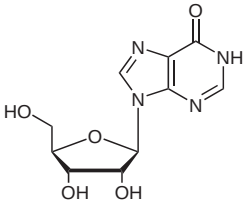
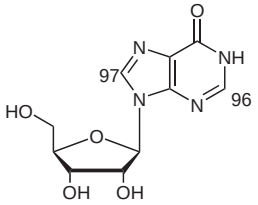
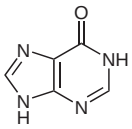
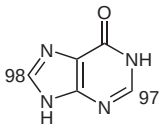
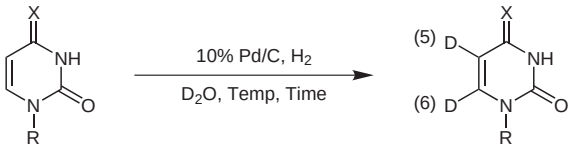
**Table 2** H–D Exchange Reaction of Guanosine, Inosine, and Hypoxanthine

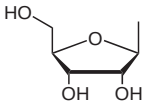
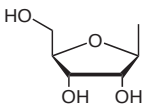
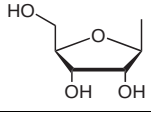



| Entry | Substrate | D content (%)[a] | Yield (%) |
|---|---|---|---|
| 1 |  |  | 99 |
| 2 |  |  | 100 |
| 3 |  |  | 92 |

[a] Determined by $^1$H NMR spectroscopy using DSS as an internal standard.

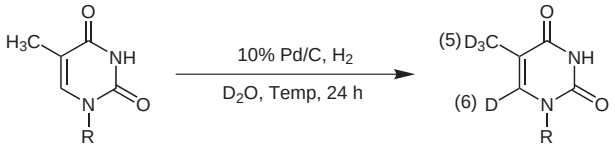
**Table 3** H–D Exchange Reaction of Uracil and Cytosine Derivatives

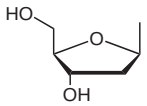



| Entry | X | R | Temp (°C) | Time (h) | D content (%)[a] | | Yield (%) |
|---|---|---|---|---|---|---|---|
| | | | | | 5-D | 6-D | |
| 1[b] | O | H | 140 | 48 | – | – | – |
| 2 | O | H | 160 | 24 | 98 | 97 | 100 |
| 3[b] | O |  | 140 | 48 | – | – | – |
| 4 | O |  | 160 | 24 | 94 | 35 | 100 |
| 5[b] | NH | H | 110 | 24 | – | – | – |
| 6 | NH | H | 160 | 48 | 96 | 96 | 98 |
| 7 | NH |  | 140 | 48 | 93 | 35 | 100 |

[a] Determined by $^1$H NMR spectroscopy using DSS as an internal standard.
[b] Partial hydrogenation of the 5,6-double bond was observed.

**Table 4** H–D Exchange Reaction of Thymine Derivatives




| Entry | R | Temp (°C) | D content (%) | | Yield (%) |
|---|---|---|---|---|---|
| | | | 5-$CD_3$ | 6-D | |
| 1 | H | 110 | 97 | 96 | 89 |
| 2[b] |  | 140 | – | – | – |



[a] Determined by $^1$H NMR spectroscopy using DSS as an internal standard.
[b] Hydrolytic cleavage of deoxyribose was observed.

is required to obtain spectrally pure deuterated products in excellent yields.[13,14] When uracil, uridine, or cytosine was used as the substrate, partial hydrogenation of the 5,6-double bond was observed at a relatively lower temperature (110–140 °C, Table 3, entries 1, 3, and 5). It is noteworthy that this drawback can be overcome by raising the temperature to 160 °C (Table 3, entries 2, 4, and 6). The 5-methyl group of thymine was deuterated entirely, together with the 6-position at 110 °C without partial hydrogenation (Table 4, entry 1). No competitive deuterium incorporation into the sugar moieties was observed in all cases.[15] It should be noted that the exchange reaction using pyrimidine nucleosides, such as uridine and cytidine, led to lower deuterium incorporation at the 6-position (Table 3, entries 4 and 7) although the use of uracil and cytosine, which lack the sugar moiety, gave excellent deuterium efficiency (Table 3, entries 2 and 6). For these reasons, it may be concluded that the steric hindrance arising from the 5′-hydroxy group lowered deuterium incorporation; also no deuterium incorporation into the 6-position of the more hindered 2′,3′,5′-tris-*O*-TBDMS-uridine (**1**) under the reaction conditions confirmed this while the deuteration of 1-methyluracil (**2**) possessing a small methyl substituent at the 1-position gave excellent deuterium efficiencies at both 5- and 6-positions (Figure 1).[12a]

A limitation of this methodology is that thymidine, a deoxy-pyrimidine nucleoside, decomposed with complete hydrolysis at the glycosyl bond (Table 4, entry 2) even though nearly quantitative deuteration efficiency was achieved in 2′-deoxyadenosine without hydrolysis (Table 1, entry 4).

In summary, the present $D_2$ gas-free and selective H–D exchange reaction retains sufficient usefulness in nucleic acid chemistry. It discloses a convenient route to the post-synthetic introduction of deuterium atoms into the base moiety of nucleosides with high deuterium efficiency under neutral reaction conditions. Studies to further elucidate the scope of this incorporation method are currently underway.

## Acknowledgment

The authors thank the Research Foundation of Gifu Pharmaceutical University.

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(13) **Typical Procedure for Deuteration of Adenosine (Table 1, entry 3):** Adenosine (66.8 mg, 0.25 mmol) and 10% Pd/C (6.7 mg, 10 wt% of the substrate, Aldrich) in $D_2O$ (1 mL) was stirred at 160 °C in a sealed tube under a $H_2$ atmosphere for 24 h. After cooling, the reaction mixture was filtered using a membrane filter (Millipore Millex®-LG). The filtered catalyst was washed with boiling water (50 mL) and the combined filtrates were concentrated in vacuo to give adenosine-$d_2$ as a white powder (66.3 mg, 98%). The deuterium content (%) was determined by $^1$H NMR using 3-trimethylsilyl-1-propanesulfonic acid sodium salt (DSS) as an internal standard and confirmed by mass spectroscopy. $[\alpha]_D^{20}$ –55 (*c* 0.38, $H_2O$) [adenosine Lit.[16] $[\alpha]_D^{11}$ –62 (*c* 0.71, $H_2O$)]. $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 8.37 (s, 0.053 H), 8.12 (s, 0.042 H), 7.34–7.30 (br s, 2 H), 5.90 (d, *J* = 6.4 Hz, 1 H), 5.45–5.41 (m, 2 H), 5.20 (d, *J* = 4.9 Hz, 1 H), 4.63 (dd, *J* = 4.9, 6.4 Hz, 1 H), 4.16 (dd, *J* = 3.4, 4.4 Hz, 1 H), 3.99 (dd, *J* = 3.4, 3.4 Hz, 1 H), 3.72–3.67 (m, 1 H), 3.60–3.54 (m, 1 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ = 156.2, 152.3 (small peak), 149.0, 139.9 (small peak), 119.3, 87.9, 85.9, 73.4, 70.6, 61.6. $^2$H NMR (400 MHz, DMSO): δ = 8.02 (br). MS (ES+): *m*/*z* (%) = 269 (3) [M + 2].

(14) **Specific rotations of nucleosides:** Table 1, entry 2 $[a]_D^{19}$ –60 (*c* 0.38, $H_2O$) {adenosine Lit.[16] $[\alpha]_D^{11}$ –62 (c 0.71, $H_2O$)}; Table 1, entry 3 $[\alpha]_D^{20}$ –55 (*c* 0.38, $H_2O$) {adenosine Lit.[16] $[\alpha]_D^{11}$ –62 (*c* 0.71, $H_2O$)}; Table 1, entry 4 $[\alpha]_D^{20}$ –19 (*c* 0.36, $CH_3OH$) {deoxyadenosine $[\alpha]_D^{20}$ –20 (*c* 0.36, $CH_3OH$)}; Table 2, entry 1 $[\alpha]_D^{20}$ –59 (*c* 0.25, 0.02 N NaOH) {guanosine $[\alpha]_D^{20}$ –61 (c 0.30, 0.02 N NaOH)}; Table 2, entry 2 $[\alpha]_D^{21}$ –46 (*c* 0.34, $H_2O$) {inosine Lit.[16] $[\alpha]_D^{18}$ –49 (*c* 0.9, $H_2O$)}; Table 3, entry 4 $[\alpha]_D^{21}$ +5 (*c* 0.27, $H_2O$) {uridine Lit.[16] $[\alpha]_D^{20}$ +4 (*c* 2)}; Table 3, entry 7 $[\alpha]_D^{21}$ +25 (*c* 0.26, $H_2O$) {cytidine Lit.[16] $[\alpha]_D^{25}$ +31 (*c* 0.7, $H_2O$)}; **1** $[\alpha]_D^{21}$ +18 (*c* 0.74, $CH_3Cl$) {2′,3′,5′-tris-*O*-TBDMS-uridine $[\alpha]_D^{22}$ +22 (*c* 0.83, $CH_3Cl$)}.

(15) Although Matsubara et al. recently reported quite interesting H–D exchange reaction of primary alcohols at the α-position using $RuCl_2(PPh_3)_2$ as a catalyst, we observed no competitive deuterium incorporation into the sugar moieties, see: Takahashi, M.; Oshima, K.; Matsubara, S. *Chem. Lett.* **2005**, *34*, 192.

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