Alternative I–D Exchange Reaction on Pyrimidine and Purine Nuclei Mediated by Tributyltin Hydride Using THF-d_8 as a Deuterium Source

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Abstract: A method for the regioselective deuteration of pyrimidine and purine rings mediated by Bu_3SnH using THF-d_8 as a deuterium source on the basis of a radical reaction was developed.

Key words: regioselective deuteration, pyrimidine, purine, THF-d_8, tributyltin hydride

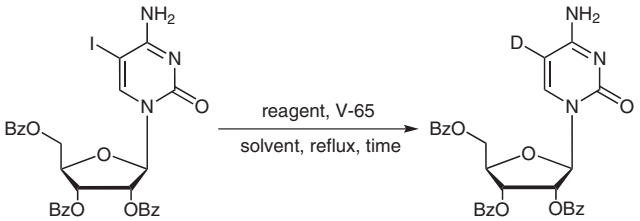
Many organic compounds containing a pyrimidine or purine ring, especially nucleic acid derivatives, have been recognized as biologically important compounds on the basis of their antitumor or virus activities.[1] The development of deuterium-labeling methods of such compounds has been demanded for a wide range of studies involving the metabolism and structural analysis.[2] One of the useful labeling methods so far reported is the radical-mediated iodine–deuterium (I–D) exchange using Bu_3SnD as the D source.[3]

During our research related to nucleic acid chemistry, the I–D exchange reaction at the 5-position of 2′,3′,5′-tri-*O*-benzoyl-5-iodocytidine using Bu_3SnD (98 atm% D) and 2,2′-azobis(2,4-dimethylvaleronitrile) (V-65) was found to proceed with low D incorporation in THF (4% D efficiency, Table 1, entry 1), EtOAc (13% D efficiency), acetone (6% D efficiency), MeOH (22% D efficiency), EtOH (11% D efficiency), or MeCN (19% D efficiency). However, a nearly quantitative incorporation (99% D efficiency) was achieved when using THF-d_8 as the solvent (entry 2). Interestingly, the use of Bu_3SnH instead of Bu_3SnD also worked well for the exchange (96% D efficiency, entry 3). These results indicate that THF-d_8 plays a crucial role in the present deuteration.

A part of the ^{1}H NMR charts of the 5-deuterated product (Table 1, entry 3), 2′,3′,5′-tri-*O*-benzoylcytidine, and the starting 2′,3′,5′-tri-*O*-benzoyl-5-iodocytidine are indicated as (a), (b), and (c) in Figure 1, respectively. A trace peak for the 5-position (0.04 H, 96% D efficiency) and a singlet peak for the 6-position were observed on chart (a). The D introduction into the 5-position was also confirmed by ^{2}H NMR and MS spectra.

A variety of deuterated solvents were examined as the D source for the D incorporation into the cytidine derivatives. As shown in Table 2, the most preferable solvent to achieve a high D content was THF-d_8.[4] Deuterated methanol (CD_3OD) and deuterated ethanol (CH_3CD_2OH) produced higher D contents of 92% and 79%, respectively (entries 3 and 6), compared to the deuterated methanol and ethanols possessing different labeling patterns such as CH_3OD, CD_3CH_2OH, and CH_3CH_2OD (entries 4, 5, and 7).

Table 1 Reaction Using Bu_3SnD or Bu_3SnH in THF or THF-d_8



Entry	Substrate (mg)	Reagent (equiv)	V-65 (equiv)	Solvent (mL)	Time (h)	D content[a] (%)	Yield[b] (%)
1	100	Bu_3SnD (1.7)	0.3	THF (2)	4	4	68
2	20	Bu_3SnD (1.2)	0.2	THF-d_8 (2)	1	99	64
3	20	Bu_3SnH (2.4)	0.4	THF-d_8 (2)	2.5	96	42

[a] Determined by ^{1}H NMR spectroscopy in DMSO-d_8.
[b] Isolated yield.

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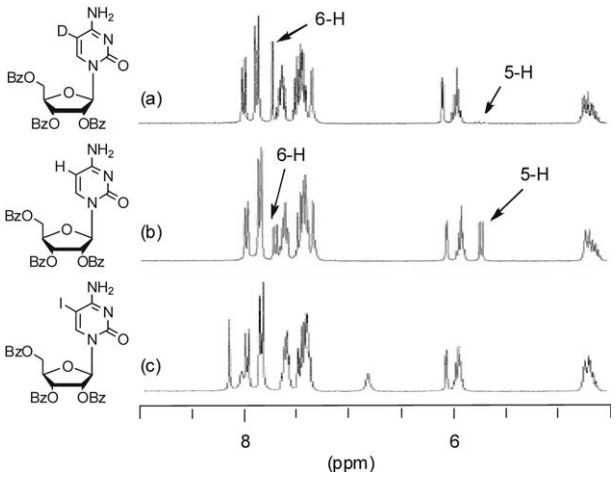
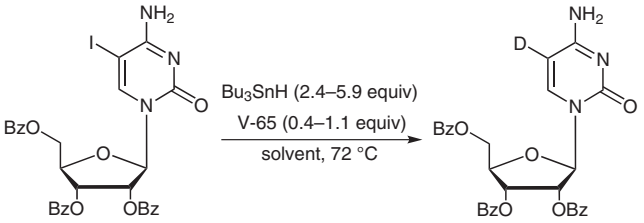


Figure 1 ^{1}H NMR (270 MHz, DMSO-d_8) spectra: (a) 2′,3′,5′-tri-*O*-benzoyl-5-deuterocytidine; (b) 2′,3′,5′-tri-*O*-benzoylcytidine; (c) 2′,3′,5′-tri-*O*-benzoyl-5-iodocytidine

Table 2 D Content on Using Various Solvents



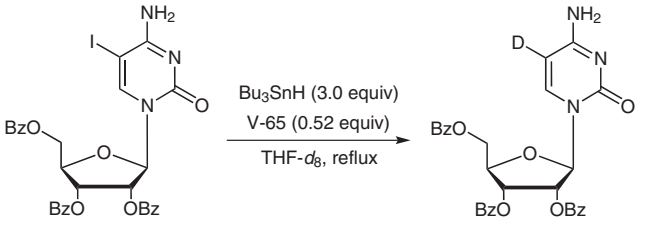
Entry	Solvent (100 v/w)[a]	D content (%)[b]
1	THF-d_8	97
2	acetone-d_6	59
3	CD_3OD	92
4	CH_3OD	33
5	CD_3CH_2OH	7
6	CH_3CD_2OH	79
7	EtOD	32
8	CD_3CN	22
9	EtOAc-d_8	59
10	EtOAc–THF-d_8, 9:1[c]	49
11	MeCN–THF-d_8, 9:1[c]	78
12	THF–THF-d_8, 1:1[c]	9

[a] The solvent (mL) use is expressed as the ratio based on the weight (g) of 2′,3′,5′-tri-*O*-benzoyl-5-iodocytidine.
[b] Determined by ^{1}H NMR spectroscopy in DMSO-d_8 and D_2O.
[c] The ratio of volume to volume.

It appears that the deuterium atoms on the carbon atoms adjacent to the oxygen atoms of solvents are quite important for the efficient deuteration. When the reaction was carried out in $CD_3CO_2C_2D_5$, a moderate D incorporation was achieved (Table 2, entry 9), while a low D efficiency was obtained in CD_3CN (entry 8). We next investigated the transfer of deuterium from THF-d_8 diluted with EtOAc, MeCN, or THF (entries 10–12). The mixed solvent of THF-d_8 and MeCN produced a higher D efficiency (78%) than the mixture of THF-d_8 and EtOAc (49%). These results indicated that the transfer of D from THF-d_8 is more strongly obstructed by the solvent which works better as a hydrogen (deuterium) donor (entries 8 vs. 9 and 10 vs. 11). Moreover, the mixture of THF-d_8 and THF gave only a low D content (9%), that is, a hydrogen atom from THF is more easily incorporated into the cytosine ring by comparison with a deuterium atom from THF-d_8 based upon the deuterium isotope effect.

We also investigated the relation between the use of THF-d_8 and the D efficiency (Table 3). Decreasing the use of THF-d_8 led to lower D contents.

Table 3 The Effect of Solvent Volume on the D Content

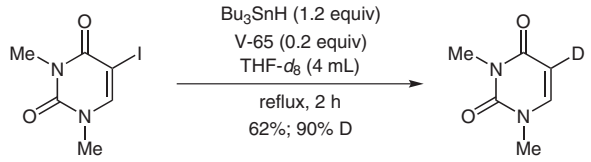


Entry	Use of THF-d_8 (v/w)[a]	D content (%)[b]
1	100	97
2	40	80
3	20	78

[a] The use of THF-d_8 (mL) is expressed as the ratio based on the weight (g) of 2′,3′,5′-tri-*O*-benzoyl-5-iodocytidine.
[b] Determined by ^{1}H NMR spectroscopy in DMSO-d_8 and D_2O.

The I–D exchange reaction described above using Bu_3SnH would proceed by a radical mechanism because the reaction rate significantly decreased by the addition of 6 equivalents of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)[5] as a radical scavenger. Moreover, the lack of the radical initiator (V-65) significantly suppressed the reaction progress.

The reaction of 5-iodo-1,3-dimethyluracil with Bu_3SnH also proceeded with a 90% D efficiency (Scheme 1), indicating that uracil as well as cytidine derivatives could be applicable to the reaction. To specify which deuterium atoms of THF-d_8 are likely to be introduced on the uracil ring during the present deuteration, we compared the proton peak intensities of THF on the ^{1}H NMR charts before and after the reaction. Since a significant increase in the peak intensity corresponding to the protons at the 2- and 5-positions (adjacent to oxygen atom) of THF was observed, it is obvious that the deuterium abstraction mainly took place at the 2- and 5-positions of THF-d_8 (Figure 2).



Scheme 1 Deuteration of 5-iodo-1,3-dimethyluracil with Bu_3SnH and THF-d_8

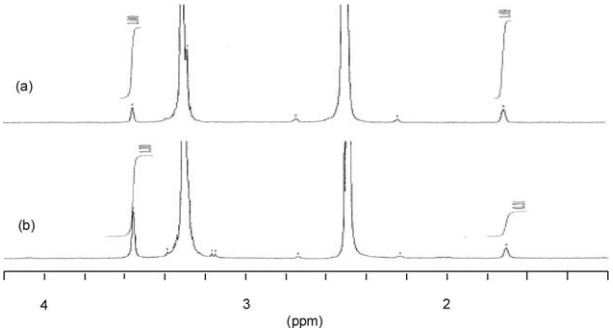


Figure 2 ^{1}H NMR (270 MHz, DMSO-d_6) spectra of residual proton peaks in THF-d_8 before (a) and after (b) the deuteration of 5-iodo-1,3-dimethyluracil using Bu_3SnH and V-65 in THF-d_8

The deuteration of 2′,3′,5′-tri-*O*-benzoyl-5-iodouridine[6] (Table 4, entry 1), 3′,5′-di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5-iodouridine (entry 2), and 2′,3′,5′-tri-*O*-*tert*-butyldimethylsilyl-8-iodoadenosine[7] (entry 3) also provided regioselectively deuterated products in high D efficiencies (90–92%). The present I–D exchange method is applicable to the deuteration of both the pyrimidine and purine nucleosides.

In summary, we have developed a new regioselective introduction method of a deuterium atom into pyrimidine and purine nuclei using THF-d_8 as the D source and Bu_3SnH (not necessary to use Bu_3SnD) as a radical mediator.

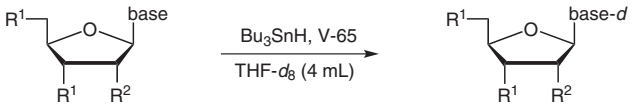
2′,3′,5′-Tri-*O*-benzoyl-5-deuterocytidine (Table 1, entry 3): Typical Procedure for the I–D Exchange Reaction

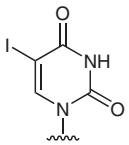
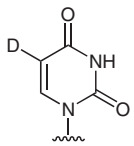
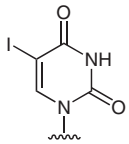
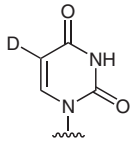
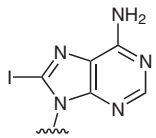
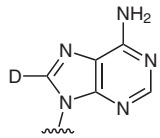
To a solution of 2′,3′,5′-tri-*O*-benzoyl-5-iodocytidine (20 mg, 0.029 mmol) and 2,2′-azobis(2,4-dimethylvaleronitrile) (V-65, 1.5 mg, 0.006 mmol) in THF-d_8 (2 mL) was added Bu_3SnH (9.4 μL, 0.035 mmol), and the mixture was stirred under reflux for 1.5 h. Then, V-65 (1.8 mg, 0.007 mmol) and Bu_3SnH (9.4 μL, 0.035 mmol) were added, and the reaction mixture was stirred under reflux for another 1 h. After cooling to r.t., hexane (8 mL) was added to the reaction mixture. The precipitate was corrected on the filter paper and washed with hexane to give 2′,3′,5′-tri-*O*-benzoyl-5-deuterocytidine (6.8 mg, 42%). ^{1}H NMR (270 MHz, DMSO-d_6): δ = 4.58–4.72 (m, 3 H, 4′-H, 5′-H), 5.74 (d, *J* = 7.3 Hz, 0.04 H, 5-H), 5.89–5.98 (m, 2 H, 2′-H, 3′-H), 6.07 (d, *J* = 3.1 Hz, 1 H, 1′-H), 7.35–8.02 (m, 18 H, 6-H, Bz, NH_2). ^{2}H NMR (61 MHz, DMSO-d_6): δ = 5.77 (br). MS–FAB$^+$: *m/z* = 557 [M + 1].

5-Deutero-1,3-dimethyluracil (Scheme 1)

1,3-Dimethyl-5-iodouracil (40.0 mg, 0.150 mmol), Bu_3SnH (48 μL, 0.178 mmol), V-65 (7.5 mg, 0.030 mmol), and THF-d_8 (4 mL) were

Table 4 Deuteration of Other Pyrimidine and Purine Nucleosides



	Substrate			Substrate-*d*		
Entry	Base	R^1	R^2	Base-*d*	D content (%)[a]	Yield (%)[b]
1[c]		OBz	OBz		90	67
2[d]		OTBDMS	H		92	97
3[e]		OTBDMS	OTBDMS		92	91

[a] Determined by ^{1}H NMR spectroscopy in DMSO-d_8.
[b] Isolated yield.
[c] Bu_3SnH (2.4 equiv), V-65 (0.4 equiv), reflux, 5 h.
[d] Bu_3SnH (1.2 equiv), V-65 (0.2 equiv), reflux, 3 h.
[e] Bu_3SnH (2.4 equiv), V-65 (0.4 equiv), reflux, 5 h.

used. After the completion of the reaction, hexane (16 mL) was added to the reaction mixture, and the precipitate was corrected on the filter paper to give 5-deutero-1,3-dimethyluracil (13.2 mg, 62%). ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.15 (s, 3 H, NCH_3), 3.29 (s, 3 H, NCH_3), 5.66 (d, *J* = 7.9 Hz, 0.10 H, 5-H), 7.67 (t, 1 H, 6-H). ^{2}H NMR (61 MHz, DMSO-d_6): δ = 5.67 (s). MS (EI$^+$): *m/z* = 141 [M$^+$].

2′,3′,5′-Tri-*O*-benzoyl-5-deuterouridine (Table 4, entry 1)

2′,3′,5′-Tri-*O*-benzoyl-5-iodouridine (40.0 mg, 0.059 mmol), Bu_3SnH (37.6 μL, 0.140 mmol), V-65 (6.3 mg, 0.025 mmol), and THF-d_8 (4 mL) were used. Purification by column chromatography on silica gel (hexane → hexane–EtOAc = 2:1) produced the 2′,3′,5′-tri-*O*-benzoyl-5-deuterouridine (22.0 mg, 67%). ^{1}H NMR (270 MHz, DMSO-d_6): δ = 4.60–4.75 (m, 3 H, 4′-H, 5′-H), 5.68 (d, *J* = 8.1 Hz, 0.10 H, 5-H), 5.89–5.96 (m, 2 H, 2′-H, 3′-H), 6.16 (d, *J* = 3.6 Hz, 1 H, 1′-H), 7.42–8.02 (m, 16 H, 6-H, Bz), 11.51 (s, 1 H, NH). MS–FAB$^+$: *m/z* = 558 [M + 1].

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5-deuterouridine (Table 4, entry 2)

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5-iodouridine (40.2 mg, 0.069 mmol), Bu_3SnH (22.3 μL, 0.083 mmol), V-65 (3.5 mg, 0.014 mmol), and THF-d_8 (4 mL) were used. Purification by column chromatography on silica gel (hexane → hexane–EtOAc = 2:1) produced the 3′,5′-di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5-deuterouridine (30.5 mg, 97%). ^{1}H NMR (270 MHz, DMSO-d_6): δ = 0.07 and 0.09 (each as s, 12 H, SiMe), 0.88 and 0.89 (each as s, 18 H, Si*t*-Bu), 2.08–2.26 (m, 2 H, 2′-H), 3.68–3.80 (m, 3 H, 4′-H, 5′-H), 4.26–4.31 (m, 1 H, 3′-H), 5.50 (d, *J* = 8.2 Hz, 0.08 H, 5-H), 6.13 (t, 1 H, 1′-H), 7.70 (t, 1 H, 6-H), 11.32 (s, 1 H, NH). MS–FAB$^+$: *m/z* = 458 [M + 1].

2′,3′,5′-Tri-*O*-*tert*-butyldimethylsilyl-8-deuteroadenosine (Table 4, entry 3)

2′,3′,5′-Tri-*O*-*tert*-butyldimethylsilyl-8-deuteroadenosine (40.1 mg, 0.054 mmol), Bu_3SnH (34.8 μL, 0.129 mmol), V-65 (5.5 mg, 0.022 mmol), and THF-d_8 (4 mL) were used. Purification by column chromatography on silica gel (hexane → hexane–EtOAc = 2:1) produced the 2′,3′,5′-tri-*O*-*tert*-butyldimethylsilyl-8-deuteroadenosine (30.2 mg, 91%). ^{1}H NMR (270 MHz, DMSO-d_6): δ = –0.35, –0.10, 0.09, 0.12, and 0.14 (each as s, 18 H, SiMe), 0.72, 0.90, and 0.93 (each as s, 27 H, Sit-Bu), 3.71–3.79 (m, 1 H, 4′-H), 4.00–4.05 (m, 2 H, 5′-H), 4.33 (br, 1 H, 3′-H), 4.90–4.94 (m, 1 H, 2′-H), 5.94 (d, *J* = 6.3 Hz, 1 H, 1′-H), 7.30 (s, 2 H, NH_2), 8.13 (s, 1 H, 2-H), 8.34 (s, 0.08 H, 8-H). MS–FAB$^+$: *m/z* = 611 [M$^+$].

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