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Highly diastereoselective chemoenzymatic synthesis of (2′*R*)- and (2′*S*)-2′-deoxy[2′-^{2}H]guanosines

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Abstract—In an effort to develop an efficient synthetic method of highly diastereoselective (2′*R*)- and (2′*S*)-2′-deoxy[2′-^{2}H]guanosines, chemoenzymatic conversion was investigated. The synthesis of (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]guanosine was achieved by biological transdeoxyribosylation using (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]uridine, 2,6-diaminopurine, and *Enterobacter aerogenes* AJ-11125, followed by treatment with adenosine deaminase. (2′*S* > 98% de)-2′-Deoxy[2′-^{2}H]guanosine was synthesized from (2′*S* > 98% de)-2′-deoxy[2′-^{2}H]uridine and 2,6-diaminopurine using thymidine phosphorylase and purine nucleoside phosphorylase instead of *E. aerogenes* AJ-11125.

Structural studies concerning biologically functional DNA or RNA are important for delineating mechanisms related to the interaction of genes with proteins or drugs. The ready availability of (2′*R*)- and/or (2′*S*)-2′-deoxy[2′-^{2}H]ribonucleosides with high diastereoselectivity is extremely important in studies concerning the conformational analysis of sugar moieties in DNA by nuclear magnetic resonance (NMR) spectroscopy.[1] Synthetic methods leading to the production of (2′*R*)- and/or (2′*S*)-2′-deoxy[2′-^{2}H]ribonucleosides have been reported. The chemical synthesis of (2′*R*)- and (2′*S*)-2′-deoxy[2′-^{2}H]cytidines from a glycal was reported by Fraser-Reid and co-workers.[2] Robins and co-workers reported the synthesis of (2′*R*)-2′-deoxy[2′-^{2}H]adenosine and -uridine by the reductive deuteration of adenosine and uridine derivatives functionalized with either 2′-chloro[3] or 2′-*O*-phenoxythiocarbonyl (*O*-PTC)[4] with Bu_3Sn^2H/AIBN, which resulted in lower stereoselectivity (2′*R* = 76% de at the highest ratio). The synthesis of highly diastereoselective (2′*R*)- and (2′*S*)-2′-deoxy-[2′-^{2}H]ribonucleosides was accomplished by Chattopadhyaya and co-workers.[5] Although this represented the first report concerning the synthesis of (2′*R*)- and (2′*S*)-2′-deoxy[2′-^{2}H]guanosine derivatives, it leaves much to be desired, in terms of synthesis efficiency.[5] We developed a novel and efficient method for the highly diastereoselective synthesis of (2′*R*)- and (2′*S*)-2′-deoxy[2′-^{2}H]nucleosides. Optimal conditions for the synthesis of (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]uridine, -adenosine, and -thymidine were found using the Bu_3Sn^2H–Et_3B system, with a bromo group at the 2′ position as the leaving group, a 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group for the protection of the hydroxyl groups at the 3′ and 5′ positions of nucleosides as the deuteration substrate, and a reaction temperature of −78 °C.[6] (2′*S* > 98% de)-2′-deoxy[2′-^{2}H]nucleosides were synthesized by application of this method to [2′-^{2}H]arabinonucleosides prepared by reductive deuteration of 2′-ketonucleosides with NaB^2H_4.[7] These highly diastereoselective deuterated compounds were used to investigate intrastrand C2′ hydrogen abstraction induced by photoirradiation of 5-halouracil-containing oligonucleotides using a stereoselective C2′-deuterated deoxyadenosine,[8] and for the investigation of the sugar conformation of DNA decamers using a stereoselective (2′*R*)- or (2′*S*)-deuterium-labeled DNA by proton–proton *J* coupling constants.[1] The aforementioned synthetic methods, however, are not entirely satisfactory in terms of diastereoselectivity

Keywords: (2′*R* > 98% de)-2′-Deoxy[2′-^{2}H]guanosine; (2′*S* > 98% de)-2′-Deoxy[2′-^{2}H]guanosine; Chemoenzymatic conversion.
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and the overall yield of (2′*R*)- and (2′*S*)-2′-deoxy-[2′-^{2}H]guanosines.

We then investigated the development of an efficient and highly diastereoselective synthesis of (2′*R*)- and (2′*S*)-2′-deoxy[2′-^{2}H]guanosines by chemoenzymatic conversion of (2′*R* > 98% de)- and (2′*S* > 98% de)-2′-deoxy[2′-^{2}H]-uridine, respectively. This letter reports a chemoenzymatic synthesis of (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]-guanosine (**1**) and (2′*S* > 98% de)-2′-deoxy[2′-^{2}H]guanosine (**2**).

We studied the effect of leaving groups (*O*-PTC, Br) at the 2′ position and protecting groups (Bz, TIPDS) at the 3′ and 5′ hydroxyl groups of the guanosine derivative, radical initiators (AIBN, AIBN and ultrasound irradiation, Et_3B), reaction temperature, and deuteration reagents [Bu_3Sn^2H, $(Me_3Si)_3Si^2H$] on diastereoselectivity (Scheme 1). The results are shown in Table 1. The deuteration of 3′,5′-di-*O*-Bz-*N*^{2}-isobutyryl-2′-*O*-PTC-guanosine (**3**) using the Bu_3Sn^2H–AIBN system yielded 52% de at 65 °C, 64% de under high-intensity ultrasound irradiation at 12 °C, but the reaction did not proceed at −60 °C (entries 1–3 in Table 1). Although the Bu_3Sn^2H–Et_3B system was employed for 2′-bromo-2′-deoxy-*N*^{2}-isobutyryl-3′,5′-*O*-TIPDS-guanosine (**4**), it could not yield highly diastereoselective (2′*R*)-2′-deoxy[2′-^{2}H]guanosine derivative (**2′*R*-6**) even at −78 °C, which in the case of (2′*R* > 98% de)-2′-deoxyadenosine, -thymidine, and -2′-deoxyuridine provided excellent diastereoselectivity (Scheme 1, entries 4–8 in Table 1, Fig. 1).

Reductive deuteration using the $(Me_3Si)_3Si^2H$–Et_3B system in 2,2,5,5-tetramethyltetrahydrofuran (tetramethyl-THF) was then attempted. Application of this system to 2′-bromo-2′-deoxy-3′,5′-*O*-TIPDS-uridine (**8**) gave (2′*R* > 98% de)-2′-deoxy-3′,5′-*O*-TIPDS-[2′-^{2}H]uridine (**9**) in 89% yield at 0 °C and 87% yield at room temperature.[9] Therefore, it might be expected that the reductive deuteration reaction using the $(Me_3Si)_3Si^2H$–Et_3B system might involve a steric effect, yielding higher diastereoselectivity in comparison with reactions utilizing the

Table 1.

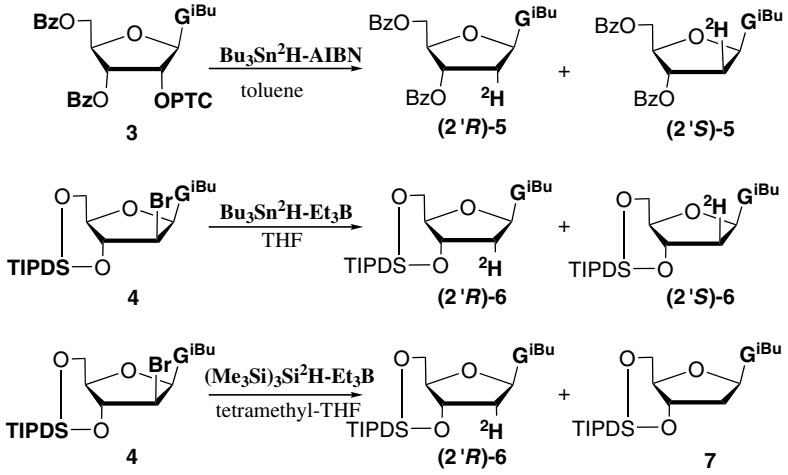
Entry	Substrate	Reagent	Temp (°C)	% de
1	**3**	Bu_3Sn^2H–AIBN	65	52
2	**3**	Bu_3Sn^2H–AIBN +)))[a]	12	64
3	**3**	Bu_3Sn^2H–AIBN +)))[a]	−60	—[b]
4	**4**	Bu_3Sn^2H–Et_3B	−3	56
5	**4**	Bu_3Sn^2H–Et_3B	−25	67
6	**4**	Bu_3Sn^2H–Et_3B	−55	67
7	**4**	Bu_3Sn^2H–Et_3B	−65	72
8	**4**	Bu_3Sn^2H–Et_3B	−78	82
9	**4**	$(Me_3Si)_3Si^2H$–Et_3B	−10	—[c]

[a]))): Ultrasound irradiation.
[b] No reaction.
[c] Non-labeled **7** is included as 59%.

Bu_3Sn^2H–Et_3B system. Based on this expectation, the reductive deuteration of **4** using the $(Me_3Si)_3Si^2H$–Et_3B system was performed. Contrary to expectations, a non-deuterated 2′-deoxyguanosine derivative **7** having a hydrogen atom at the 2′ position resulted in 59% yield, in addition to **(2′*R*)-6**, even at −10 °C (Scheme 1, entry 9 in Table 1, Fig. 1). This was rationalized as follows. The ethyl radical generated in the first stage abstracted the deuterium of $(Me_3Si)_3Si^2H$ to give the tris(trimethylsilyl)silyl (TTMSS) radical, and then the TTMSS radical generated abstracted Br from **4** in the targeted reaction cycle (solid line in Scheme 2). In this step, by competing with Br of **4**, the hydrogen of **4** and/or solvent was drawn out by the TTMSS radical to give rise to $(Me_3Si)_3SiH$ (broken line), because of the steric hindrance of $(Me_3Si)_3Si^2H$ and the larger isobutyrylguanine base compared with other nucleobases (Scheme 2).

In an effort to overcome the aforementioned drawback, a chemoenzymatic synthetic study of (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]guanosine (**1**) from (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]uridine (**10**) was undertaken.

Previously, the synthesis of 9-β-D-arabinosyladenine by a transglycosylation reaction between adenine and 1-β-D-arabinofuranosyluracil was reported by Utagawa and co-workers.[10] Later, Yokozeki and co-workers synthesized 2′-deoxyadenosine and 2′-deoxyguanosine by



Scheme 1. Chemical synthesis of **1**.

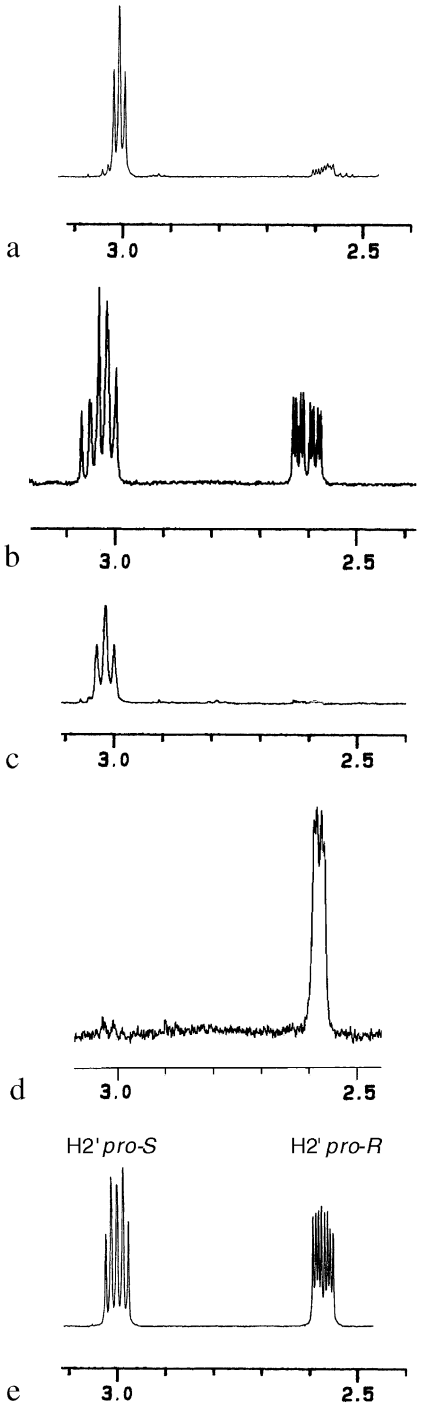
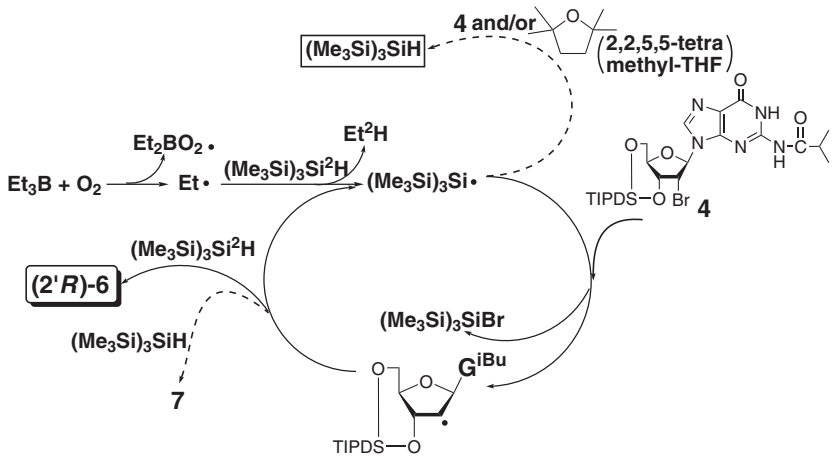


Figure 1. The sugar moiety H2′*pro-R* and H2′*pro-S* region ^{1}H NMR spectral data of the 3′,5′-di-*O*-acetyl-2′-deoxy[2′-^{2}H]guanosine perpetrated are showed. Spectra of **a**–**c** were (2′*R*)-derivatives. The spectrum **a** obtained by the reaction conducted with the Bu_3Sn^2H–Et_3B system, **b**: with the $(Me_3Si)_3Si^2H$–Et_3B system, **c**: by a transglycosylation, the spectrum **d** was (2′*S*)-3′,5′-di-*O*-acetyl-2′-deoxy[2′-^{2}H]guanosine by the preparation of transdeoxyribosylation, and the spectrum **e** was non-labeled 3′,5′-di-*O*-acetyl-2′-deoxyguanosine. The spectra were recorded with a Bruker DPX 400 spectrometer. Chemical shifts were recorded in the δ scale relative to an internal reference of CH_3OH (3.35 ppm).

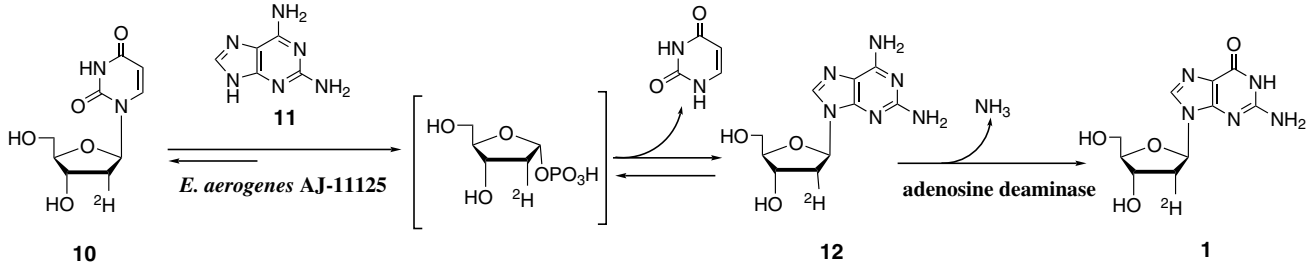
application of this strategy.[11] We investigated the synthesis of **1** from **10** by this method. The reaction solution of a total volume of 20 mL of 50 mM potassium phosphate buffer (KPB) (pH 7.0) contained 100 mM **10**, 150 mM 2,6-diaminopurine (**11**), and 250 mg of wet cells of *Enterobacter aerogenes* AJ-11125 (Ajinomoto culture collection) prepared according to Yokozeki.[11] The reaction solution was incubated at 60 °C with shaking for 1 h and the reaction was stopped by boiling the solution to give {(2*R*)-2-deoxy[2-^{2}H]ribosyl}-2,6-diaminopurine (**12**).

This reaction product **12** was then converted to **1** by adenosine deaminase. After protection of the hydroxyl groups at the 3′ and 5′ positions of **1** with the acetyl groups, 3′,5′-di-*O*-Ac-2′-deoxy[2′-^{2}H]guanosine was obtained by silica gel column chromatography in 43% overall yield from **10** and >98% de, which is the same as that of **10** (Scheme 3). The sugar moiety H2′*pro-R* and H2′*pro-S* region ^{1}H NMR spectral data of the labeled products are shown in Figure 1, together with non-labeled 3′,5′-di-*O*-acetyl-2′-deoxyguanosine for comparison. The spectrum **a** obtained by the reaction conducted with the Bu_3Sn^2H–Et_3B system shows the ratio 2′*R*:2′*S* = 91:9 (82% de), the spectrum **b** by the $(Me_3Si)_3Si^2H$–Et_3B system shows non-deuterated **7** in 59% yield in addition to **(2′*R*)-6**. The spectrum **c** prepared by chemoenzymatic synthesis clearly shows the absence of the 2′*pro-R* proton (Fig. 1).

The synthesis of (2′*S* > 98% de)-2′-deoxy[2′-^{2}H]guanosine (**2**) was then investigated. Chemical conversion of the uridine, adenosine, and ribosylthymine to the corresponding (2′*S*)-2′-deoxy[2′-^{2}H]uridine, -adenosine, and -thymidine was performed by a sequence of reactions involving seven steps.[7] Following reductive deuteration of the intermediate 2′-ketonucleosides with NaB^2H_4, the resulting [2′-^{2}H]arabinonucleosides were converted to 2′-bromo-2′-deoxy[2′-^{2}H]ribonucleosides via the 2′-*O*-Tf-derivatives. The highly diastereoselective reduction of these compounds by the Bu_3SnH–Et_3B system, which was established based on the synthesis of (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]nucleosides, yielded highly diastereoselective (2′*S*)-2′-deoxy[2′-^{2}H]nucleosides. In the case of (2′*S*)-2′-deoxy[2′-^{2}H]guanosine, however, 93% de resulted, with an overall yield of 31%. Both the % de and overall yield of (2′*S*)-2′-deoxy[2′-^{2}H]guanosine were unsatisfactory. Therefore, we studied the synthesis of **2** utilizing a chemoenzymatic approach. The first synthesis of labeled nucleosides utilizing a transglycosylation reaction using thymidine phosphorylase, purine nucleoside phosphorylase, and adenosine deaminase was reported by Jones and co-workers[12] by application of the findings of Krenitsky and co-workers.[13] 2′-Deoxy[7-^{15}N]guanosine was synthesized by a transglycosylation reaction between [7-^{15}N]diaminopurine and thymidine using thymidine phosphorylase and purine nucleoside phosphorylase, followed by deamination with adenosine deaminase. Concerning the sugar moiety of nucleosides, Ono and co-workers[14] reported the transdeoxyribosylation of ^{13}C/^{2}H doubly labeled 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyuridine from ^{13}C/^{2}H doubly labeled thymidine based on the report of Jones. We carried out the synthesis of **2** from (2′*S* > 98% de)-2′-deoxy[2′-^{2}H]uridine (**13**) utiliz-



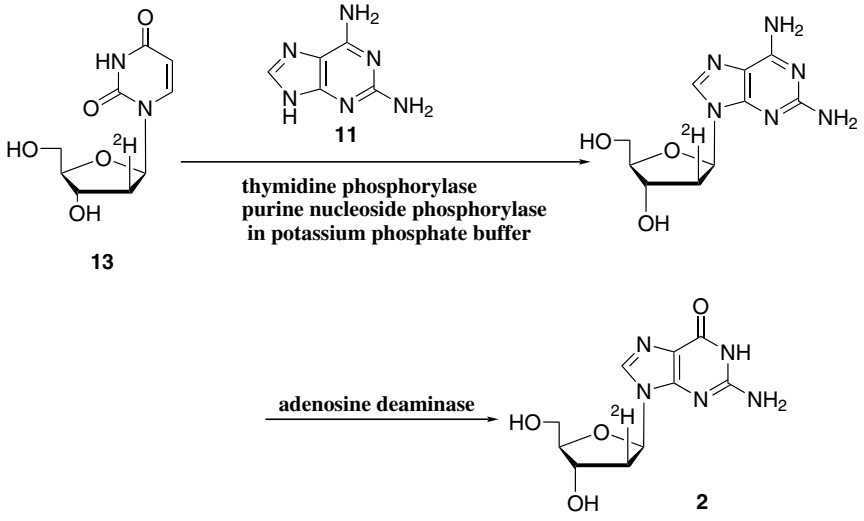
Scheme 2. Reaction mechanism.



Scheme 3. Chemoenzymatic synthesis of **1**.

ing the method of Jones and co-workers.[12] (2′*S* > 98% de)-**13** as starting material was prepared from uridine involving seven steps that included stereoselective reductive deuteration of the resulting 2′-oxouridine derivative with NaB^2H_4 in EtOH–H_2O (2:1)[7] and highly stereoselective $(Me_3Si)_3SiH$–Et_3B reduction of the bromide.[9] The transdeoxyribosylation of **13** and **11** in the presence of thymidine phosphorylase and purine nucleoside phosphorylase in KPB yielded {(2*S*)-2-deoxy[2-^{2}H]ribosyl}-2,6-diaminopurine, which was treated with adenine deaminase to give **2**. The yields were 69% and 41% at 40 °C and 25 °C, respectively (Scheme 4).

It seems that the difference in yield might be related to the solubility of **11**, which is low at 25 °C, the optimal enzymatic temperature. At 40 °C, the reaction pro-



Scheme 4. Enzymatic synthesis of **2**.

ceeded better than at 25 °C due to the higher solubility of **11**, notwithstanding the fact that the enzyme is partially inactivated at the higher temperature. Therefore, a modified reaction was carried out by adding enzyme on two separate occasions during the reaction time. The yield of **2** was improved to 87% following purification using an anion exchange resin. The diastereoselectivity of (2′*S*)-3′,5′-di-*O*-Ac-2′-deoxy[2′-^{2}H]guanosine acetylated **2** was >98% de (Fig. 1d).

In conclusion, an efficient synthesis of (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]guanosine was achieved by a biological transdeoxyribosylation reaction between (2′*R* > 98% de)-2′-deoxy[2′-^{2}H]uridine and 2,6-diaminopurine using *E. aerogenes* AJ-11125, followed by treatment with adenosine deaminase. (2′*S* > 98% de)-2′-Deoxy[2′-^{2}H]-guanosine was efficiently synthesized using thymidine phosphorylase and purine nucleoside phosphorylase instead of *E. aerogenes* AJ-11125.

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