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Synthesis of (5'*S*)-[5'-$^{2}H_{1}$;1',2',3',4',5'-$^{13}C_{5}$]-Thymidine *via* Stereoselective Deuteration of a 5-Oxoribose Derivative

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Abstract: (5'*S*)-[5'-$^{2}H_{1}$;1',2',3',4',5'-$^{13}C_{5}$]-Thymidine has been synthesized by a stereoselective deuteride transfer reaction from (-)- or (+)-[2-$^{2}H_{1}$]-isobornyloxymagnesium bromide to a 5-oxoribose derivative, which can be readily prepared from [$^{13}C_{6}$]-D-glucose. The overall yield from D-glucose to thymidine was 27%. The various nucleosides with a stereoselective ^{2}H-label together with ^{13}C at the C5' position, which have become available by the present method, will be quite useful for stereospecific assignment of the diastereotopic C5' methylene signals, and also for conformational analyses of the O5'-*C5'* bonds in nucleic acid oligomers.

Our recent efforts have been directed toward developing synthetic methods for preparing ribo- and deoxyribonucleosides labeled with stable-isotopes, such as ^{13}C, ^{15}N, and/or ^{2}H, at various positions. The isotopically labeled nucleosides can be incorporated at desired sites of DNA or RNA sequences by automated solid-phase synthesis, and the site-specifically labeled oligomers thus obtained have been successfully used for heteronuclear multidimensional NMR spectroscopy.[1-9] One of the prominent features of the chemical synthetic methods is their extreme flexibility, which allows the preparation of virtually all possible isotopomeric nucleosides. This is in distinct contrast to commonly employing enzymatic methods using isotopically labeled nucleic acid hydrolyzates, which do not even allow the preparation of site-specifically labeled oligomers.[10,11] We have already reported several applications of isotopically labeled nucleosides for NMR studies of DNA oligomers; for example, (2'*R*)- and (2'*S*)-[2'-$^{2}H_{1}$;*ul*-^{13}C,^{15}N]-2'-deoxyadenosines,[6] and (5'*R*)/(5'S)-[5'-$^{2}H_{1}$;1',2',3',4',5'-$^{13}C_{5}$]-thymidine[7,8] were used for the stereospecific assignment of the diastereotopic methylene protons at C2' and C5', respectively. Stereoselectively deuterated (5'*S*)/(5'*R*)-[5'-$^{2}H_{1}$;1',2',3',4',5'-$^{13}C_{5}$]-thymidines, with an approximately 2 : 1 ratio,[12] have been used to develop for the first time a reliable method to determine the β-angle for a labeled nucleotide residue in a DNA dodecamer.[7,8] In this case, highly stereoselective discrimination of the *pro-R* and *pro-S* protons at the C5' was possible, even with this moderate stereoselectivity, since two discrete ^{1}H-^{13}C HSQC cross peaks, with intensities assumed to be nearly proportional to the residual proton concentration, were clearly observed.

However, a careful ^{1}H-^{31}P HSQC study using a DNA dodecamer containing (5'*S*)/(5'*R*)-[5'-$^{2}H_{1}$]-nucleotides, but which lacks ^{13}C labels at the C5' positions, led us to realize that the same level of stereoselectivity may not sufficient to allow for making decisive stereospecific assignments of the C5' methylene protons.[9] The incidental signal overlap between diastereotopic protons can also be another cumbersome problem in some cases when making stereospecific assignments, even with ^{2}H/^{13}C-doubly labeled nucleotides. Versatile methods for highly stereoselective deuteration of the C5' methylene should therefore be explored for ribo- and deoxyribonucleosides. Obviously, few of the existing deuteration methods are economically feasible to apply to the expensive ^{13}C precursors.[13] For example, the method used for (5'*S*)-[5'-$^{2}H_{1}$]-cytidine[14] seems to be unsuitable for preparing ^{13}C/^{2}H-doubly labeled nucleosides. In the course of our investigation into the synthesis of a series of selectively deuterated nucleosides,[15] a 5-oxoribose derivative, which can be readily prepared from D-glucose, was chosen as an appropriate precursor for the present purpose. We report in the following a novel stereoselective deuteration for the 5-oxoribose to (5*S*)-[5-$^{2}H_{1}$]-ribose derivative, which was then used to prepare a ^{2}H/^{13}C-doubly labeled thymidine, as an example.

A [1,2,3,4,5-$^{13}C_{5}$]-5-oxoribose derivative, **2**, was synthesized in a 69.3 % yield from [*ul*-^{13}C]-D-glucose, **1**, (98 ^{13}C atom %; Chlorella Ind. Co. Ltd.).[15] Reduction of **2** with either $NaBD_4$ or $LiAlD_4$ gave [5-$^{2}H_{1}$; 1,2,3,4,5-$^{13}C_{5}$]-ribose derivatives (**3**) in good yields, but with low stereoselectivity.[15] Among the numerous existing stereoselective reduction methods for carbonyl compounds, only a few can be practically used for stereoselective deuteration of the formyl group of **2**. We found that the reduction with (-)-[2-$^{2}H_{1}$]-isobornyloxymagnesium bromide, containing 5-10% (-)-[2-$^{2}H_{1}$]-bornyloxymagnesium bromide,[16] which was prepared from (+)-camphor, $LiAlD_4$,

and n-butylmagnesium bromide, gave only the (*S*)-isotopomer of the monodeuterated ribose derivative, **3**.[17] The reaction was carried out in refluxing benzene for 40 min, in the presence of a 15-fold molar excess of (-)-[2-$^2H_1$]-isobornyloxymagnesium bromide, and the (*S*)-[5-$^2H_1$]-ribose derivative was obtained in an 83% yield, together with a 13% recovery of **2**. In the obtained ribose derivative, no (*R*)-isotopomer was detected, but a small amount (2%, which was equivalent to 1.6% from **2**) of non-deuterated product was present. A large excess of deuterated magnesium reagent was necessary for obtaining the reduced product in higher yields, but was not essential to achieve higher stereoselectivity for the deuteride transfer reaction. In fact, we obtained only the (*S*)-isotopomer of **3** with a 10 molar excess of the magnesium reagent, but the yield was 55%, even after prolonged reaction time. The reaction, carried out at room temperature (15 molar excess, 1 hour, in benzene), gave the ribose derivative in a rather low yield (30%), with a 40% recovery of **2**. It was noted that the ribose derivative in this preparation contained 15% non-deuterated derivative (4.5% from **2**), although again no (*R*)-isotopomer was detected. This substantial increase of non-deuterated derivative was presumably due to the hydride transfer from contaminating n-butylmagnesium bromide, which may exist in the preparation of (-)-[2-$^2H_1$]-isobornyloxymagnesium bromide.[16]

The above results were rather unexpected, since previous reports on the deuteride transfer reactions, which were for achiral substrates such as benzaldehyde or acetaldehyde, have indicated that the reactions were not highly stereoselective.[16] The same reaction between **2** and (+)-[2-$^2H_1$]-isobornyloxymagnesium bromide, which was prepared from (-)-camphor in lieu of (+)-camphor, produced almost identical results, including the stereochemistry of **3**. The result clearly indicates that the chirality of the ligand is not responsible for the origin of the stereoselectivity of the deuteride transfer process. These observations gave us important clues to help deduce the origin of the highly stereoselective deuteride transfer reaction that occurred for the 5-oxoribose derivative **2**. Taking the previous model for the reaction intermediate postulated for simple aldehydes into account, we propose the model shown in Figure 2, in which the magnesium ion coordinates to three oxygen atoms, instead of the two in the previous model, including the endocyclic oxygen in the sugar ring.[18] With this template effect, the deuteride ion will be forced to attack exclusively from the *si*-face of the 5-formyl group, leading to the exclusive production of the 5*S*-isotopomer.

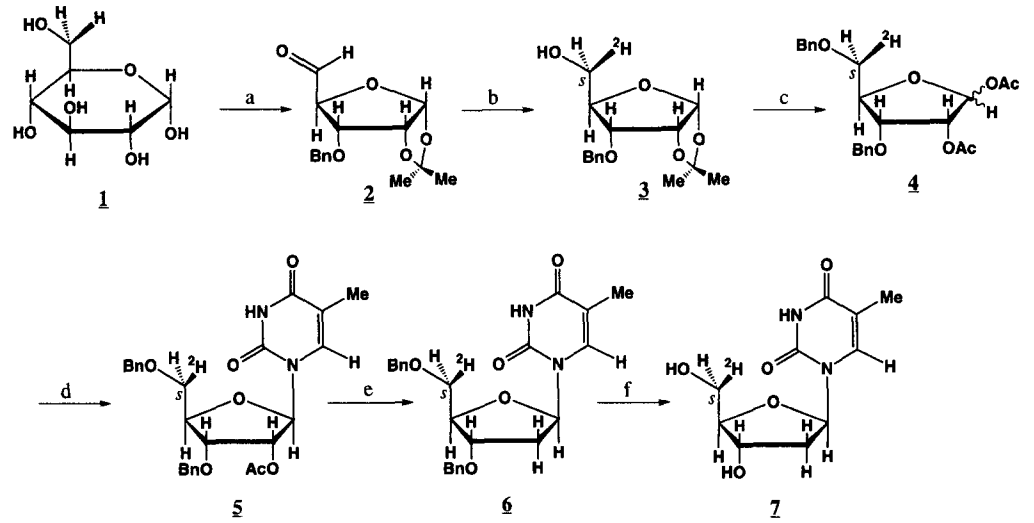

Figure 1. A scheme for the synthesis of (5'*S*)-[1',2',3',4',5'-$^{13}C_5$;5'-$^2H_1$]-thymidine. *a*; see reference 15. *b*; see text. *c*; 1) BnBr, NaH, DMF. 2) 80% aq. AcOH, reflux. 3) Ac_2O, Et_3N, DMAP, CH_3CN, 81% from **3**. *d*; silylated thymine, TMSOTf, dichloroethane, 88%. *e*; 1) 2N NaOH/EtOH, 2)phenyl chlorothionoformate, Py, 3) Bu_3SnH, AIBN, benzene, 86% from **5**. *f*; 10% Pd/C, MeOH, 76%.

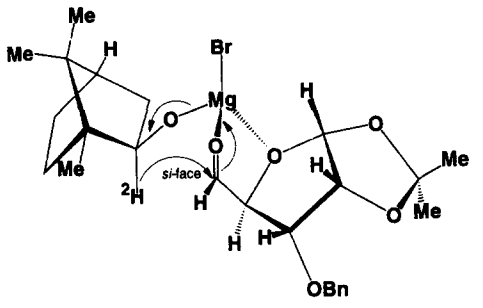

Figure 2. The proposed model for the magnesium template effect leading to the stereoselecive *si*-face deuteride transfer from (-)-[2-^{2}H$_1$]-isobornyloxymagnesium bromide to **2**.

We used benzyl groups for the protection of the 3- and 5-hydroxyl groups in the ribose derivative **4**[19] (Figure 1), rather than the conventional acyl groups, thus making it possible to leave these two protected hydroxyl groups during the subsequent deoxygenation processes of the 2-hydroxy group. This eliminates the cumbersome deprotection and reprotection processes for the deoxygenation of the 2'-hydroxy group of the nucleosides.[20] With these processes, the stereoselectively doubly labeled thymidine, (5'*S*)-[5'-^{2}H$_1$;1',2',3',4',5'-^{13}C$_5$]-thymidine (**7**), was obtained in a 27% overall yield from [ul-^{13}C]-D-glucose. In Figure 3 shows the deoxyribose proton regions of the ^{1}H-NMR spectra of **7**, together with that of authentic thymidine. The ^{1}H-signals attached to the ^{13}C-labeled sites are split into doublets by one bond ^{1}H-^{13}C spin coupling constants (Fig. 3b), which can be eliminated by ^{13}C-irradiation (Fig. 3c). The almost complete loss of the signal due to the *pro-R* proton of the C5' methylene indicates the high deuteration level at this site (Fig. 3b,c). The other ribo- and deoxyribonucleosides, such as adenosine and deoxyadenosine, have been synthesized by this route. Applications of these highly stereoselectively ^{2}H/^{13}C-double labeled nucleosides for NMR studies will be published elsewhere.

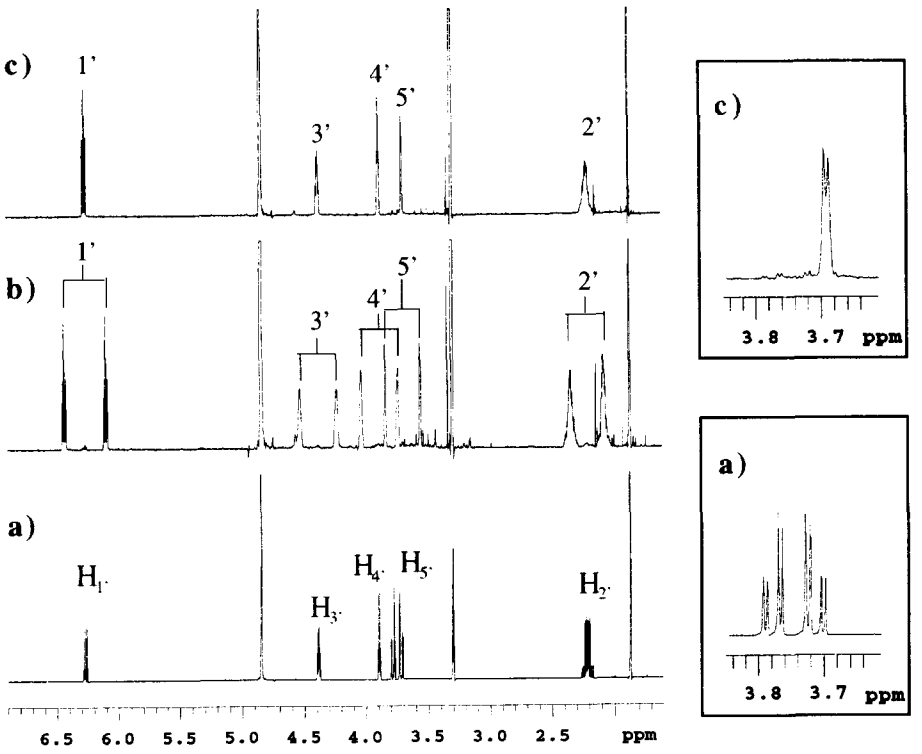

Figure 3. 500 MHz ^{1}H-NMR spectra (ribose region) of thymidine and (5'*S*)-[5'-^{2}H$_1$;1',2',3',4',5'-^{13}C$_5$]-thymidine (**7**) in methanol-D$_4$. a): Thymidine. b): **7** without ^{13}C-irradiation. c): **7** with ^{13}C-irradiation. The expanded 5'-proton regions of a) (*bottom*) and c) (*top*) are shown in boxes at the right.

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17. To a 5 mL benzene solution of **2** (0.50 g, 1.8 mmol) was added (-)-[$^2H_1$]-isobornyloxy bromide, prepared from (-)-[$^2H_1$]-isoborneol (27.2 mmol) and *n*-BuMgBr (26.7 mmol), according to the reported method.[15] The mixture was refluxed for 40 min, and then was cooled to room temperature. HCl (0.1N, 30 mL) was added to the reaction mixture, which was stirred at room temperature for 10 min, and was extracted with $CHCl_3$ (200 mL). The organic layer was washed successively with a sat. $NaHCO_3$ solution, and a sat. NaCl solution, and was then dried over Na_2SO_4 before being concentrated. The residue was chromatographed over a silica gel column (85 g) with 50% AcOEt in *n*-hexane as the eluent. Fractions were combined and concentrated to give **3** (0.41 g, 1.5 mmol, 83% from **2**) and unreacted **2** (65 mg, 13%).
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