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Synthesis of [5'-$^2H_1$]-Nucleosides with Defined (5'*S*)/(5'*R*)-Ratios

Yu Oogo, Akira (Mei) Ono#, Akira Ono, Masatsune Kainosho*

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University,
1-1 Minami-ohsawa, Hachioji, Tokyo, 192-0397 Japan.
Core Research for Evolutional Science and Technology (CREST)
of the Japan Science and Technology Corporation (JST).

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Abstract: A method for preparing 5'-monodeuterated nucleosides with defined (5'*S*)/(5'*R*)-ratios, starting from glucose, is described. The 5-oxopentose derivatives, derived from glucose, were treated with $LiAlD_4$ in the presence of LiI and/or *t*-amylalcohol to give stereoselectively 5-monodeuterated pentose derivatives. Using various reaction conditions, the stereoselectivity of the monodeuterated 5-hydroxymethyl group could be partially controlled, in the range of isotopomeric ratios, (5*S*)/(5*R*), of 4 : 1 to 1 : 7.4. Using appropriate mixtures of the 5-monodeuterated pentoses with different isotopomeric ratios, we could prepare 5'-monodeuterated nucleosides with defined isotopomeric ratios, which will be useful for NMR studies of oligonucleotides.

We have shown that stereoselectively deuterated nucleosides are useful for NMR studies of DNA/RNA oligomers.[1-10] For example, the vicinal P5-H5'/H5" coupling constants, which are essential NMR parameters to determine the β-angles, could be accurately measured using DNA oligomers with $^{13}C/^{2}H$-doubly labeled residue(s).[3,4] Thus far, this is the most accurate and generally applicable method for determining the β-angles in nucleic acid oligomers. Stereospecific assignment of the H5'/H5" signals, which is also essential for precise structural determinations of nucleic acids in solution, could be accomplished by ^{1}H-^{31}P correlation spectroscopy using DNA oligomers labeled with stereoselectively 5'-monodeuterated nucleosides prepared from 2'-deoxynucleosides.[11] Although these 5'-monodeuterated nucleosides were successfully used for the NMR analyses of DNA oligomers with limited sizes,[3-5] this approach has an inherent problem. The different (5'S)/(5'*R*)-ratio for each nucleoside prepared by this method[11] may lead to ambiguities in assigning the residual 5' *pro*-S and *pro*-R protons, based on the relative intensities of the cross peaks in the ^{1}H-^{31}P correlation spectra.[5] The cross peak intensities are substantially affected by the vicinal coupling constants between P5' and H5'/H5", and may not necessarily be correlated with the residual proton concentrations. Most of this ambiguity can be mostly avoided, if one can use $^{13}C/^{2}H$-doubly labeled nucleosides, since the relative intensities of the cross peaks observed in the ^{1}H-^{13}C correlation spectra reflect the isotopomeric ratios more accurately. An alternative, less expensive, way to avoid this problem would be to use a pair of 5'-monodeuterated nucleosides with different, but defined, (5'S)/(5'*R*)-ratios. With these nucleosides in hand, one can estimate the contribution from the different magnetization transfer efficiencies of the 5' *pro*-S and *pro*-R protons.

We have been developing various methods for preparing isotopically labeled nucleosides from labeled glucoses, which are commercially available. In our approach, the labeled glucoses are converted to the ribose derivatives, which are then coupled with the pyrimidine or purine bases to give the various nucleosides.[6-10] As all of the labeled nucleosides prepared by this approach have the identical isotopomeric distribution in their sugar moieties, for example, the (5'*S*)/(5'*R*)-ratios in the 5'-monodeuterated nucleosides, the interpretations of the NMR spectra become straightforward. Note that the (5'S)/(5'*R*)-ratios of the 5'-monodeuterated nucleosides prepared from nucleosides vary substantially with each nucleoside.[11] In this paper, we describe a method to prepare 5'-monodeuterated nucleosides with defined (5'*S*)/(5'*R*)-ratios. As we used glucose as a starting material, the same method can be used to prepare $^{2}H/^{13}C$-doubly labeled nucleosides as well.

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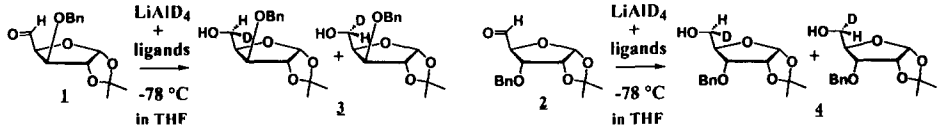
Stereoselectively 5-monodeuterated ribose derivatives can be obtained by the deuteration of the 5-oxoribose derivatives. The previously reported method to prepare the (5*S*)-[5-$^2H_1$]-ribose,[9] however, cannot be adapted to the preparation of (5*R*)-[5-$^2H_1$]-ribose. We have thus investigated a method to control the stereochemistry of the deuteration of 5-oxopentose derivatives, using various ligand and reaction conditions.[5]



Ligands	S : R	Ligands	S : R
1) -----	1 : 1.2	6) -----	1 : 1.6
2) LiI (2 eq.)	1 : 1.2	7) LiI (2 eq.)	1 : 1.6
3) *t*-Amylalcohol	1 : 2.5	8) *t*-Amylalcohol	1.8 : 1
4) *t*-Amylalcohol + LiI (2 eq.)	1 : 4.5	9) *t*-Amylalcohol + LiI (2 eq.)	2.9 : 1
5) *t*-Amylalcohol + LiI (10 eq.)	1 : 7.4	10) *t*-Amylalcohol + LiI (10 eq.)	4 : 1

Figure 1. Deuteration of the 5-oxopentose derivatives with $LiAlD_4$ in the presence of ligands.

Treatment of the 5-oxoxylose derivative 1 and the 5-oxoribose derivative 2, synthesized from glucose,[8,12] with $LiAlD_4$ in THF gave the 5-monodeuterated pentose derivatives. However, the stereoselectivities of the deuteration reactions were rather low (Fig 1, *1* and *6*). The addition of LiI, which was successfully used for the stereoselective reduction of β-alkoxy ketones,[13] did not largely improve the stereoselectivity (Fig. 1, *2,7*). Also, the modification of $LiAlD_4$ with a bulky alcohol, which was effective for the stereoselective reduction of β-dicarbonyl compounds,[14] was not effective (Fig. 1, *3,8*). The stereoselectivity, however, was markedly improved by the simultaneous addition of LiI and *t*-amylalcohol-modified-$LiAlD_4$ (Fig. 1, *4,5,9,10*).[15,16]

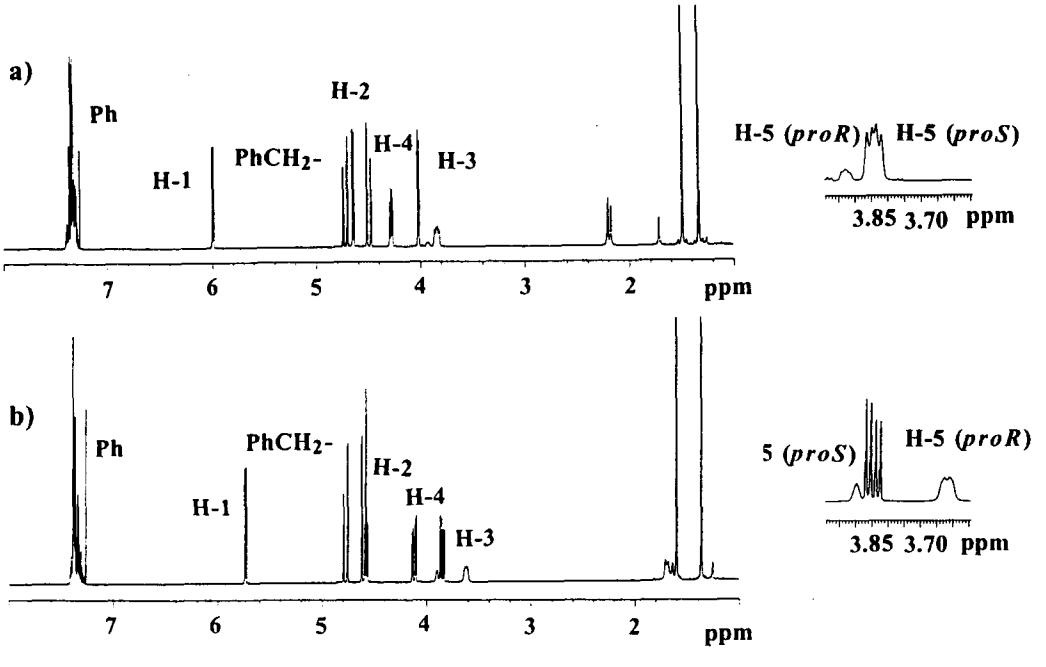


Figure 2. 300 MHz NMR spectra. a) 3 with a 1 : 7.4 (5*S*)/(5*R*)-ratio. b) 4 with a 4 : 1 (5*S*)/(5*R*)-ratio.

Note that the opposite selectivities were observed between the reactions with 1 and 2 (Fig. 1, *5* and *10*). The *R*-isotopomer was preferentially obtained from 1, but the *S*-isotopomer was obtained from 2. The (5*S*)/(5*R*)-ratios varied from 4 : 1 to 1 : 7.4 by changing the combinations of the substrates and the reaction conditions, as shown in the ^{1}H-NMR spectra for 3 and 4 (Fig. 2). Obviously, the stereoselectivity of the deuteration reaction was affected by the cooperatively between the template effect by the coordinated lithium ion and the steric effect by the modification of $LiAlD_4$ with a bulky alcohol. Mori *et al.* hypothesized the stereoselectivity of the reduction of β-alkoxy ketones was improved by the coordination of the lithium ion between a carbonyl oxygen and a β-alkoxy oxygen.[13] The stereoselectivities observed in our study can also be explained by a similar model, as follows: a lithium ion coordinates between the carbonyl and ring oxygen atoms of 2, leading the modified $LiAlD_4$ with a bulky alcohol to attack preferentially the carbonyl group from the less hindered *si*-face. If the lithium ion was coordinated between the carbonyl and the 3-benzyloxy oxygens of 1, rather than the carbonyl and ring oxygens, the modified $LiAlD_4$ would have preferentially attacked the *re*-face.

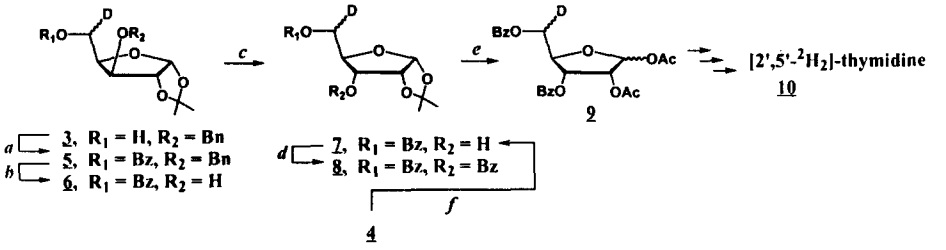


Figure 3. A scheme for the conversion of 3 and 4 to the protected 1-*O*-acetylribose 9. *a*) BzCl, 99%. *b*) Pd/C, H_2, in AcOH, 99%. *c*) (1) PDC, AcOH, molecular sieves 4A, CH_2Cl_2.[17] (2) $NaBH_4$ in MeOH, 98%. *d*) BzCl, 95%. *e*) see reference 18. *f*) see reference 8.

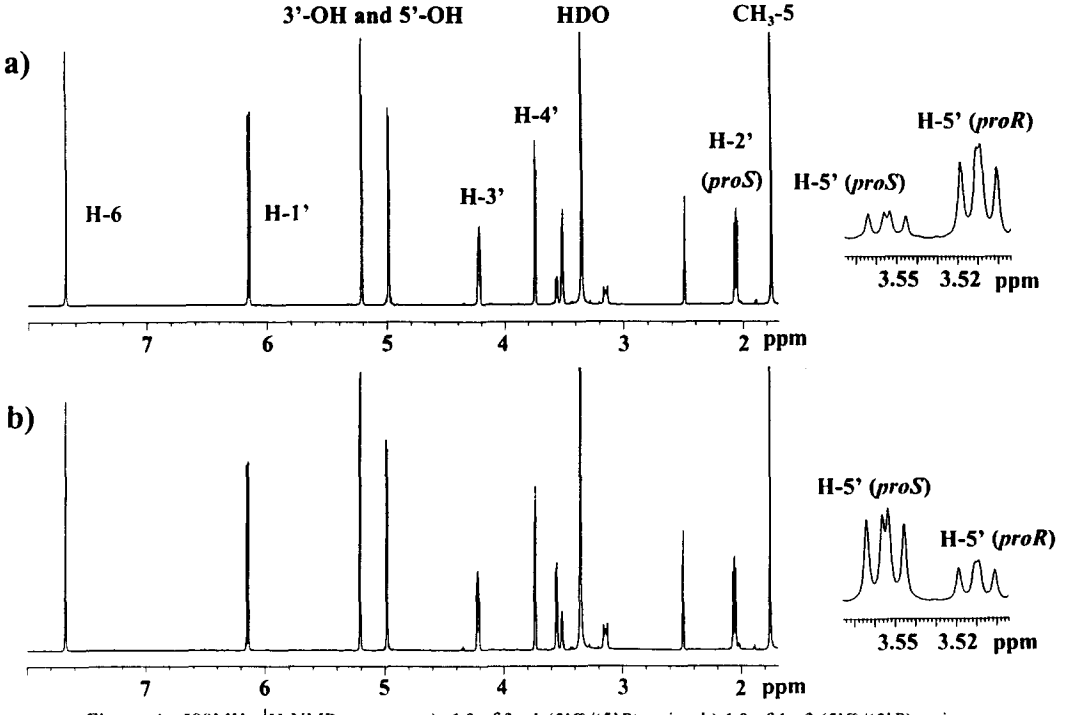


Figure 4. 500MHz ^{1}H-NMR spectra. a) 10 of 3 : 1 (5'*S*)/(5'*R*)-ratio. b) 10 of 1 : 3 (5'*S*)/(5'*R*)-ratio.

Compounds **3** and **4** were then efficiently converted into [2',5'-$^2H_2$]-thymidine **10** (Fig. 3), *via* the common intermediate **7**, by the chemical glycosylation method.[18-20] These procedures made it possible to prepare [5'-$^2H_1$]-nucleosides with defined (5'S)/(5'*R*) ratios. For example, a pair of isotopomeric mixtures of **10**, with 1 : 3 and 3 : 1 (5*S*)/(5*R*) ratios, was successfully prepared by mixing the intermediates, **7**, derived from **3** and **4**, to yield the target ratios. The ratios of **7** were completely maintained in the thymidines **10**, as shown in their ^{1}H-NMR spectra (Fig. 4).

In conclusion, a method for synthesizing 5-monodeuterated riboses with defined (5'*S*)/(5'*R*)-ratios was developed. The isotopomeric ratios can be precisely adjusted for the desired values by mixing various 5-monodeuterated ribose derivatives with different (5*S*)/(5*R*)-ratios. The same method can be adapted for ^{13}C-labeled glucoses, for the preparation of ^{2}H/^{13}C-doubly labeled nucleosides, which will facilitate various new NMR methods for nucleic acid oligomers and their protein complexes.[4,5] The results of the NMR studies will be reported elsewhere.

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15. A typical experiment was as follows: *t*-Amylalcohol (2.9 mL, 26.6 mmol) was added dropwise to a suspension containing $LiAlD_4$ (0.37 g, 8.9 mmol) in dry THF (59 mL), and the mixture was stirred at room temperature for 20 min, and then at - 78 °C for 20 min. A solution containing **1** (1.2 g, 4.4 mmol) and LiI (5.9 g, 44.4 mmol) was stirred at room temperature for 10 min, and then at -78 °C for 50 min. The prepared solution containing the modified $LiAlD_4$ was added to the solution containing **1**, and the reaction mixture was stirred at -78 °C for 2 h. After the reaction mixture was stirred at room temperature for 10 min, 2N NaOH (3 mL) was added, and the reaction mixture was stirred for an additional 20 min. The precipitates were filtered off, and the solution was concentrated *in vacuo*. The residue was partitioned between Et_2O and water. The organic layers were combined, dried over Na_2SO_4, and concentrated. The residue was chromatographed over a silica gel column (Ø 3.0 x 12.5 cm) with 25% AcOEt in *n*-hexane as an eluent. The fractions were combined and concentrated to give **3** (1.1 g, 4.0 mmol, 91%) as an oil.
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