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Systematic Synthesis of Specifically $^{13}C/^{2}H$-Labeled Nucleosides from [*ul*-$^{13}C_6$]-D-Glucose

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Abstract: A systematic approach, which combines previously reported reactions with appropriate modifications, was established for preparing a variety of selectively deuterated nucleosides from glucose. We have developed a systematic method of synthesizing specifically deuterated nucleosides from glucose. Selectively $^{13}C/^{2}H$-doubly labeled nucleosides, such as [1',2',3',4',5'-$^{13}C_5$;3',5'-$^{2}H_2$]- and [1',2',3',4',5'-$^{13}C_5$;4',5'-$^{2}H_2$]-adenosines, have been synthesized starting from [*ul*-$^{13}C_6$]-D-glucose. These labeled nucleosides are very useful for precise conformational analyses of nucleic acids in solution by heteronuclear multidimensional NMR spectroscopy.

It has long been anticipated that heteronuclear multidimensional NMR methods could be just as useful for structural studies of nucleic acids as for proteins. The lack of efficient preparation methods for isotopically labeled nucleic acids has been an obvious obstacle. During the past several years, however, a few methods have been established to prepare isotopically labeled RNA and DNA oligomers.[1] One employs enzymatic reactions using RNA or DNA polymerases to prepare labeled nucleic acid oligomers, which are useful for structural analyses by heteronuclear NMR spectroscopy. The enzymatic methods are efficient to prepare uniformly labeled oligomers or oligomers labeled with specific residue types.[2,3] Alternative methods, using solid-phase chemical synthesis, have also been investigated and have been shown to be useful for the preparation of selectively labeled oligomers, which cannot be synthesized by enzymatic reactions. The most crucial step for either of these two approaches, however, is to establish an efficient preparation method for isotopically labeled nucleosides, prepared either by isolation from the labeled biomass, by microbial fermentation with labeled precursors, or by chemical synthesis. One of the pronounced advantages of the chemical synthesis of labeled nucleosides is its capability of preparing virtually any conceivable isotopomer of a labeled nucleoside.[4-16] Using solid-phase synthesis, one can incorporate selectively labeled nucleosides at any desired site in order to obtain accurate NMR parameters, such as hetero- or homonuclear spin-couplings, which are difficult to obtain from uniformly labeled nucleic acids. These parameters are essential to refine the solution structures of nucleic acids and their protein complexes, since the NOE distance constraints for nucleic acids are not as abundant as those for proteins. We report here a systematic approach, combining previously reported reactions for similar compounds, to prepare virtually any type of nucleic acid with a specifically $^{13}C/^{2}H$-doubly labeled sugar moiety, from various commercially available ^{13}C-labeled glucoses.

We have already reported several applications of chemically synthesized labeled DNA/RNA oligomers for NMR studies. For example, $^{13}C/^{15}N$-labeled (2'*R*)- and (2'*S*)-[*ul*-^{13}C;*ul*-^{15}N;2'-$^{2}H_1$]-2'-deoxyadenosines, which were prepared by a combination of fermentation and chemical synthesis, were incorporated into DNA oligomers. Since

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one of the methylene protons was selectively substituted with a deuteron, the signals for the *pro*-S and the *pro*-R protons were unambiguously assigned.[8] Furthermore, the selective deuteration increased the accuracy of the vicinal coupling constants measured by HCCH-E.COSY.[8] The β-angle, which had been difficult to measure by conventional NMR techniques, was precisely determined using the DNA oligomers containing [1',2',3',4',5'-$^{13}C_5$;5'-$^{2}H_1$]-thymidine, which was a mixture of the (5'*S*)- and (5'*R*)- isotopomers in an approximate ratio of 2 : 1.[9,10] From these studies, we expect that the DNA/RNA oligomers containing $^{13}C/^{2}H$-doubly labeled nucleosides will be useful for precise analyses of the sugar-phosphate backbone structures.

However, no efficient method for synthesizing $^{13}C/^{2}H$-doubly labeled nucleosides has been reported. Recently, Quant *et al.* and Agrofoglio *et al.* described the syntheses of ^{13}C-labeled nucleosides starting from labeled glucose,[17,18] which are the most efficient methods thus far to our knowledge. However, their methods seemed to be difficult to apply to the synthesis of deuterium labeled nucleosides. Chattopadhyaya and co-workers have used DNA/RNA oligomers containing fully deuterated sugars for NMR studies.[19] Very recently, Tolbert and Williamson reported a novel method for preparing RNA oligomers containing 3',4',5'-deuterated nucleosides, using chemical and enzymatic syntheses.[20] However, these methods are not suitable for site-selective deuteration. Thus, we have made efforts to invent a general strategy for preparing properly deuterated nucleoside.[15,16] In this report, we describe a systematic method of introducing the deuteron(s) into the 2, 3, 4, and 5 positions of ribose, and applications to the synthesis of $^{13}C/^{2}H$-doubly labeled nucleosides.

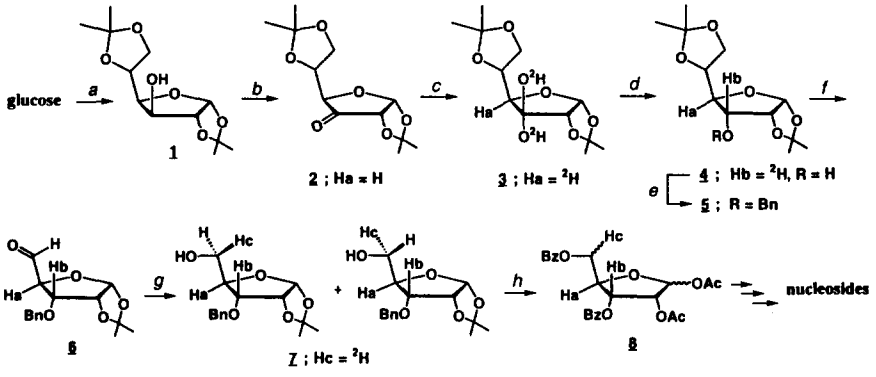

Figure 1. A scheme for the synthesis of multiply deuterated nucleosides. *a*) I_2, acetone,[21] 96%. *b*) PDC, AcOH, molecular sieves 4A, CH_2Cl_2, 88%.[22] *c*) D_2O-pyridine (1 : 4, v/v), reflux, 93%.[23] *d*) $NaBD_4$, 95%.[24] *e*) BnBr, NaH in DMF, r.t., 97% *f*) (1) 80% AcOH, r.t., 16 h. (2) $NaIO_4$, MeOH/H_2O, 89%.[25] *g*) (method A) $NaBD_4$, MeOH/H_2O, r.t., 1 h, 89%. (method B) $LiAlD_4$, THF, 0 °C to r.t., 1 h, 91%.[26] *h*) (1) 80% AcOH solution, reflux, 2 h. (2) Ac_2O, Et_3N, DMAP, CH_3CN, r.t., 2 h, 87%.[27]

Our strategy to synthesize site-selectively deuterated nucleosides is schematically shown in Fig. 1. Glucose was chosen as the starting material, since various ^{13}C-labeled glucoses are commercially available. Deuteration of the 4-position of **2**, a key compound derived from glucose, was accomplished by refluxing **2** in D_2O - pyridine (1 : 4, v/v). After three successive deuteration reactions, the 4-deuterated 3-ketosugar **3** (in a hydrate form) was obtained in 93% yield.[23] The 4-position was selectively substituted with deuterium, and thus deuterium incorporation at the 4-position was higher than 97%, but no deuterium incorporation into the 2-position was observed, according to the NMR analysis (data not shown). Obviously, the enolization with the 2-position was prohibited by the conformational rigidity due to the 1,2-di-*O*-isopropylidene group. Reduction of the 3-keto group of **3** with $NaBD_4$ gave the 3-deuterated allose derivative **4** in 95% yield.[24] Benzylation, followed by selective

deprotection of the 5,6-acetonide in an 80% acetic acid solution, gave 1,2-*O*-isopropylidene-3-*O*-benzyl-D-allose, which was treated with $NaIO_4$ to yield the 5-oxoribose derivative **6** in 86% yield.[25] Treatment of **6** with deuterated reagents, such as $NaBD_4$ and $LiAlD_4$ gave the 5-deuterated ribose derivative **7**,[26] which can be converted to the 1-acetylribose derivative **8**,[27] a key ribose derivative for nucleoside synthesis. The typical overall yield of the synthesis of **8** from glucose, including all deuteration steps, was 50%, which was sufficient for the preparation of $^{13}C/^{2}H$-doubly labeled DNA/RNA oligomers for NMR studies. The procedures for the conversion of **8** to nucleosides[28] and [2'-$^{2}H_1$]-2'-deoxynucleosides[29] have been established. Consequently, by the present strategy, a deuteron can be introduced into any position within the C2', C3', C4' and C5' of a nucleoside moiety.

For instance, [1',2',3',4',5'-$^{13}C_5$;4',5'-$^{2}H_2$]adenosine **9** and [1',2',3',4',5'-$^{13}C_5$;3',5'-$^{2}H_2$]adenosine **10** were synthesized starting from 98% [*ul* $^{13}C_6$]-glucose. Selected regions of the ^{1}H-NMR (500 MHz, MeOH-d_4) spectra of the multiply labeled adenosines are shown in Fig. 2. As a reference, $^{13}C/^{15}N$-uniformly labeled adenosine, prepared by microbial fermentation[30], is shown in Fig. 2a. The ^{1}H-signals attached to the ^{13}C-labeled sites are split into doublets by the large one bond ^{1}H-^{13}C coupling constants. In this particular experiment, a deuteron was non-stereoselectively introduced into the 5'-positions of **9** and **10**, and thus the intensities of the remaining proton signals of the *R* and *S* isotopomers were almost equal (Fig. 2b, 2c). The complete loss of the signals due to the H-4' of **9** and the H-3' of **10** indicated high deuteration levels at these positions. Application studies of these $^{13}C/^{2}H$-doubly labeled nucleosides for DNA/RNA structural analyses will be published elsewhere.

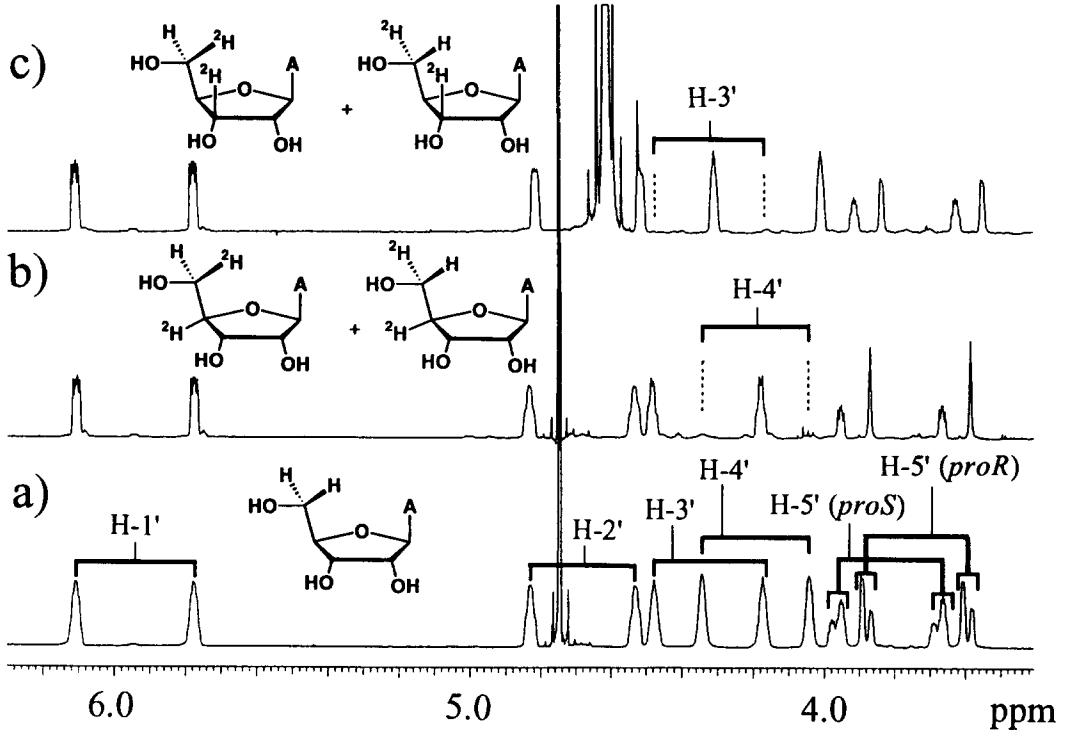

Figure 2. 500 MHz ^{1}H-NMR (MeOH-d_4, 30 °C) spectra. a) $^{13}C/^{15}N$-uniformly labeled adenosine. b) [1',2',3',4',5'-$^{13}C_5$; 4',5'-$^{2}H_2$]-adenosine (**9**). c) [1',2',3',4',5'-$^{13}C_5$; 3',5'-$^{2}H_2$]-adenosine (**10**).

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