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Stereospecific Syntheses of 3′-Deuterated Pyrimidine Nucleosides and Their Site-Specific Incorporation into DNA

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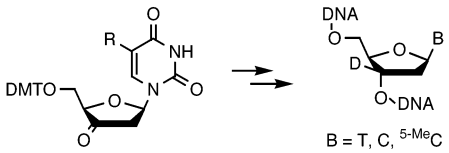
ABSTRACT



2′-Deoxy-3′-deutero pyrimidines have been synthesized in high yields and incorporated into deoxyoligonucleotides using standard phosphoramidite chemistry. A key synthetic step is a stereospecific reduction of 3′-keto nucleosides using sodium triacetoxyborodeuteride to give 3′-deuterated thymidine and 2′-deoxy uridine nucleosides. Conversion of the corresponding phorphoramidites 7a and 7b to 4-triazolo derivatives has, for the first time, enabled incorporation of 2′-deoxy-3′-deutero cytidine and 2′-deoxy-3′-deutero-5-methyl cytidine into oligonucleotides.

The importance of protein interactions with the phosphodiester backbone of DNA is becoming increasingly apparent as more high-resolution structures of protein/DNA complexes reveal numerous direct protein contacts with the sugar−phosphate backbone.[1,2] It is likely that the structurally redundant sugar−phosphate backbone plays a role in sequence-specific recognition and that the local mobility of the backbone is a component of the recognition mechanism. For example, the results from molecular dynamics simulations have suggested that the B_I to B_{II} DNA conformational change can provide an indirect readout mechanism for DNA.[3,4] We are interested in applying solid-state deuterium NMR, whose line shapes and relaxation times give information about the dynamic state of individual torsion angles, to study the ϵ and ζ torsion angles that are strongly correlated to the B_I to B_{II} transition. However, this requires the sequence-specific incorporation of 3′-deutero nucleosides into DNA, which has not been extensively studied.[5]

The most obvious synthetic approach for deuteration in the 3′-position of nucleosides is through reduction of the corresponding 3′-keto nucleosides with a deuterated metal hydride. However, this approach has two drawbacks. First, the 3′-keto nucleosides are not very stable because of their susceptibility to β-elimination of the nucleobase, especially when cytosine is the base.[6−8] Second, the 3′-ketone is preferentially reduced from the α-face of the ketone, yielding 2′-deoxyxylonucleosides. For example, 3′-deutero-5′-*O*-tritylthymidine was prepared in only 9% yield from the corresponding ketone.[7] Another recently reported synthetic strategy is 3′-labeling of a protected 3-oxoribose, followed

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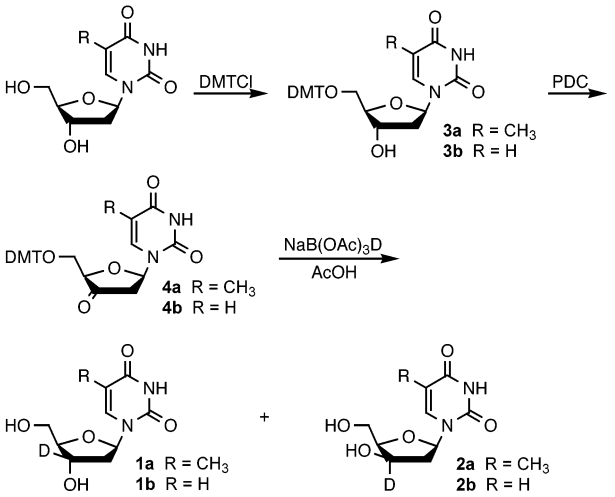
by glycosylation to give 3′-deuteriothymidine in 19% overall yield after 11 synthetic steps.[9]

We describe here the stereospecific synthesis of 2′-deoxy-3′-deutero pyrimidine nucleosides in high yields and their incorporation into DNA. This route is efficient and suitable for large-scale preparation of pyrimidine nucleosides **1a** and **1b** (Scheme 1). The synthetic strategy utilizes the free 5′-

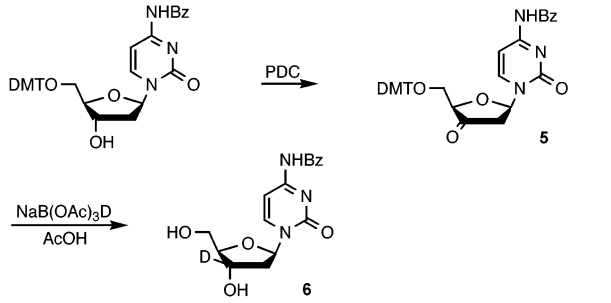
Scheme 1. Syntheses of **1a** and **1b**



hydroxyl groups of 3′-keto thymidine and 2′-deoxy-3′-keto uridine to direct reduction to the β-face of the 3′-ketone through coordination with sodium triacetoxyborodeuteride.[10,11] Selective oxidation of the 3′-hydroxyl group required the use of a 5′-hydroxyl protecting group that could be removed after oxidation without elimination of the nucleobase, because the free 5′-hydroxyl group is required for directing the reducing agent to the β-face of the ketone. Since the reductions are performed under acidic conditions, we chose the acid-labile dimethoxytrityl protecting group.

Oxidation of 5′-dimethoxytrityl thymidine was accomplished with pyridinium dichromate (PDC), resulting in 81% yield of the corresponding 3′-keto thymidine (**4a**).[12,13] Following oxidation, ketone **4a** was deprotected and reduced by treatment with sodium triacetoxyborodeuteride, formed in situ from sodium borodeuteride and acetic acid, in a two-step, one-pot procedure to give **1a** in 85% yield. Figure S1 in Supporting Information illustrates the effect of sodium triacetoxyborodeuteride on the change in stereoselectivity of the reduction, when compared to sodium borodeuteride. The sodium borodeuteride reduction preferentially reduced the ketone from the α-face (Figure S1a),[7] whereas sodium triacetoxyborodeuteride delivered the hydride predominantly

Scheme 2. Synthesis of 3′-Deuterated Cytidine Derivative **6**



from the β-face (Figure S1b) to yield a mixture of 1-(2′-deoxy-3′-deutero-β-D-ribofuranosyl)thymine (**1a**) and 1-(2′-deoxy-3′-deutero-β-D-xylofuranosyl)thymine (**2a**) (95:5 ratio by ^{1}H NMR), readily separated by column chromatography or HPLC. Deuterium incorporation at the 3′-position of the sugar ring was evident by the absence of the signal at 4.27 ppm from its ^{1}H NMR spectrum. The 5′-hydroxyl group of **1a** was subsequently protected as a dimethoxytrityl (DMT) ether and the 3′-hydroxyl group phosphitylated to yield phosphoramidite **7a**, which was incorporated into DNA using standard phosphoramidite chemistry.

The same strategy that was used to prepare 3′-deutero thymidine was applied in the synthesis of 2′-deoxy-3′-deutero cytidine, the synthesis of which has not been reported. Previous attempts to prepare the keto derivative by oxidation of 4-*N*-acetyl-2′-deoxy-5′-*O*-trityl cytidine with CrO_3/pyridine/Ac_2O resulted in β-elimination and formation of 4,5-dihydro-5-trityloxymethylfuran-4-one.[7] In contrast, oxidation of 4-*N*-benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)cytidine[14] with PDC afforded the corresponding keto-derivative **5** in 75% yield, which was characterized by ^{1}H NMR and HRMS. However, the 3′-keto derivative of 2′-deoxy cytidine (**5**) was much more unstable under the conditions of deprotection/reduction than the corresponding thymidine derivative **4a**. This is presumably due to the ease of protonation of cytidine under the acidic conditions, which accelerates β-elimination of the nucleobase. As a result, the expected product **6** was obtained in only 10% yield.

These results led us to investigate an alternative synthetic route, utilizing the known conversion of a thymine base to a methylcytosine through the corresponding triazole derivative (Scheme 3).[15,16] 2′-Deoxy-3′-keto uridine (**4b**) was synthesized by the oxidation of 2′-deoxy-5′-*O*-dimethoxytrityl uridine (**3b**) with PDC in 80% yield. Subsequent treatment with triacetoxyborodeuteride resulted in a mixture of 1-(2′-deoxy-3′-deutero-β-D-ribofuranosyl)uracil (**1b**) and 1-(2′-deoxy-3′-deutero-β-D-xylofuranosyl)uracil (**2b**) (94:6 ratio by ^{1}H NMR). Compound **1b** was isolated in 83% yield and converted to the protected phosphoramidite **7b**, from which

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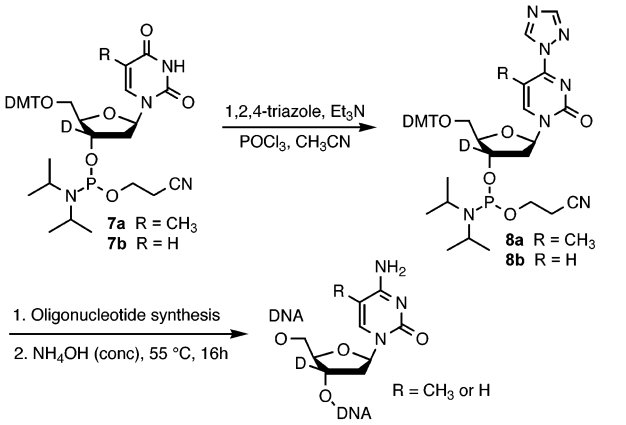
Scheme 3. Incorporation of 3′-Deuterated dC into DNA



the 2′-deoxy-3′-deutero-4-triazolo uridine phosphoramidite **8b** was prepared in quantitative yield. This monomer was incorporated into oligodeoxyribonucleotides using standard automated DNA synthesis protocols. After deprotection and purification of the oligomer, ESI-MS analysis verified the incorporation of deuterium, and enzymatic digestion, followed by analytical RP-HPLC, showed the return of the nucleosides in their expected ratios (see Supporting Information). 2′-Deoxy-3′-deutero-5-methyl cytidine was also incorporated into oligomers using the triazole derivative **8a**, prepared from 2′-deoxy-3′-deutero thymidine (**1a**), to study the effect of 2′-deoxycytidine methylation on the mobility of the sugar by deuterium solid-state NMR (Scheme 3).

In summary, we have shown that stereospecific reduction of 3′-keto nucleosides is a short, high-yielding synthetic route to 3′-deuterated thymidine and 2′-deoxy uridine nucleosides. Conversion of the corresponding phorphoramidites **7a** and **7b** to 4-triazolo derivatives has, for the first time, enabled incorporation of 2′-deoxy-3′-deutero cytidine and 2′-deoxy-3′-deutero-5-methyl cytidine into oligonucleotides. Deuterium solid-state NMR experiments containing these 3′-deuterated nucleotides at specific sites in biologically relevant DNA sequences are underway, and those results will be reported in due course.

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Supporting Information Available: Experimental procedures and full characterization for compounds and ^{1}H, ^{13}C and ^{31}P NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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