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3′-Deoxy-2'-Phosphoramidites of Adenosine and 5-Methyluridine Used for the Solid Phase Synthesis of Unnatural 3'-Deoxy-2'-5"-Oligonucleotides.

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Abstract: Protected phosphoramidites of 3'-deoxyadenosine and 3'-deoxy-5-methyluridine have been synthesized, and used in solid phase synthesis of 3'-deoxyoligonucleotides with the unusual 2'-5" linkage.

The sugar-phosphate backbone of nucleic acids normally consists of adjacent ribose moieties joined via a 3' to 5" phosphodiester linkage. While some 2',5" linked RNAs have been studied,[1] surprisingly little is known about 2',5"-linked DNA except for theoretical discussions of its possible double helix structure.[2] 3′-Deoxyadenosine (cordycepin) is a naturally occuring nucleoside posessing antibacterial activity. 2'-5'' Linked oligoadenylates have been found to be inducers of interferon and potent inhibitors of cell-free protein synthesis.[3] We are interested in the properties of 2',5" linked DNA oligomers[4] and describe herein the synthesis of protected phosphoramidites of 3'-deoxyadenosine and 3'-deoxy-5-methyluridine (which we will call *iso*-thymidine) that we have successfully used for solid phase oligonucleotide synthesis.

2',3'-Anhydroadenosine (**2**) was prepared from adenosine by a literature procedure,[5] the 5′-hydroxyl group protected with 4,4′-dimethyoxytrityl chloride[6a] and the epoxide selectively opened with lithium triethylborohydride to give **3**.[5] Condensation of **3** with phenoxyacetyl chloride[6b] followed by treatment with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite[6c,d] and diisopropylethylamine in THF gave the desired phosphoramidite **4** in 50% yield after purification. Cordycepin (**3** without the protecting group) and other 3'-deoxyribonucleosides have been prepared from commercially available ribonucleosides by various selective protection schemes,[7] but a more general route to these interesting compounds involves the attachment of the desired base to a suitably protected 3′-deoxyribose unit. This strategy has been successfully applied to a

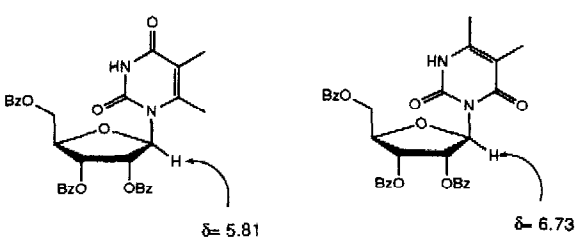
synthesis of cordycepin[8] and should also be applicable to the synthesis of other 3-deoxyribonucleosides possessing natural and unnatural bases.

Commercially available 1,2-isopropylidene-D-xylose was converted to the known anomeric acetate **8**[8] by selective benzoylation of the primary hydroxyl group followed by deoxygenation of the 3-position via tin hydride reduction of the corresponding phenylthionocarbonate.[9] Subsequent treatment with acetic anhydride in acetic acid and catalytic sulfuric acid provided **8**. Conversion to the *iso*-thymidine derivative was then accomplished by a modified Hilbert-Johnson reaction as developed by Vorbruggen.[10] Treatment of **8** with bis-trimethylsilylthymine[11] and tin tetrachloride in acetonitrile gave protected nucleoside **9** in 92% yield as a single anomer. The stereochemistry of the anomeric center is controlled by the well known participation of the 2'-acetate; with such participating groups at 2α the anomeric center is always established as β, while without such a group α/β mixtures are obtained. Cordycepin has been synthesized by an analogous reaction.[8]

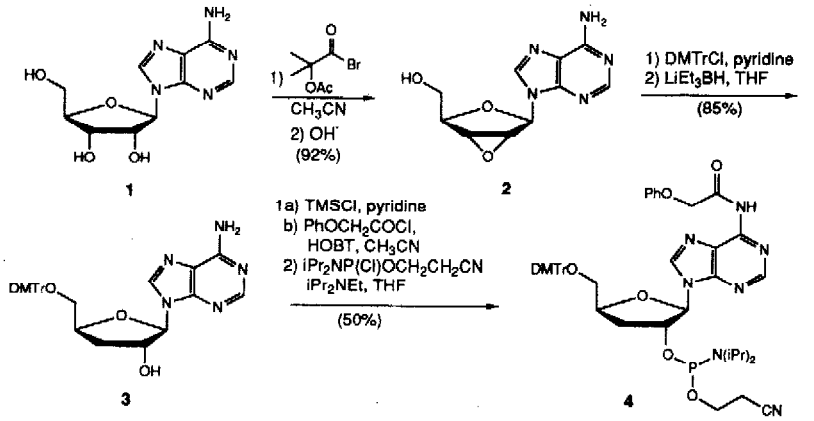
Vorbruggen and others have observed that uracil derivatives unfunctionalized at the 6-position (as in our case) attack exclusively at N-1, but for 6-alkyl-uracil derivatives a mixture of N-1- and N-3-attached nucleosides were formed with the ratio being very sensitive to reaction conditions.[10c] The structures of N-β-1- versus N-β-3-ribonucleosides were determined by the pH dependence in the UV spectra of uracil derivatives, and it was observed that the H-1' proton NMR resonances for the N-β-3-ribofuranosides were significantly deshielded compared with the N-β-1 analogues due to the increased proximity of the pyrimidine carbonyls (figure 1). In our case, the H-1' proton of **9** is at 5.83 ppm, consistent with (cf. the 5.81 ppm in figure 1) the N-β-1 nucleoside.

Completion of the synthesis was acomplished by deprotection of **9** followed by selective protection of the 5'-hydroxyl with 4,4'-dimethoxytritylchloride and 2'-phosphoramidite formation to give **12**.[12] With both desired phosphoramidites in hand, **4** and **12** were then used in solid phase oligonucleotide synthesis. Oligonucleotide synthesis was successfully carried out on an Applied Biosystems Model 381A DNA synthesizer using the manufacturer's unmodified cycles, columns, and reagents except for phosphoramidites **4** and **12**. We have synthesized 16-mers of various sequences containing 2',5'' linkages, including oligomers derived exclusively from **4** and **12**. Analysis was done by denaturing polyacrylamide gel electrophoresis (PAGE). The properties of these interesting compounds are reported elsewhere.[4]

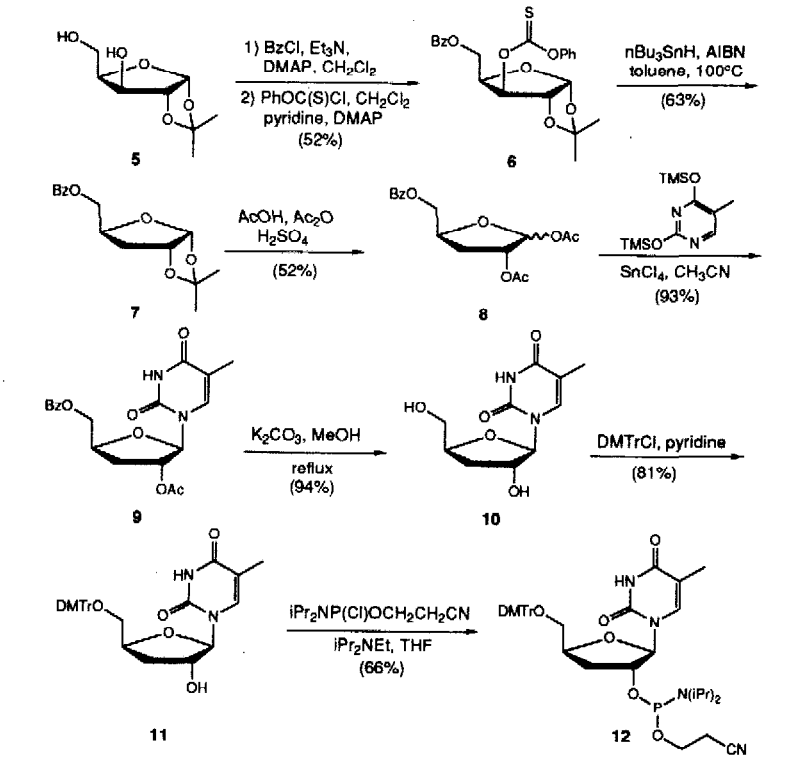
Figure 1.



Scheme 1



Scheme 2



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12. All new compounds have been characterized by high field proton NMR and mass spectroscopy. In addition, phosphoramites **4** and **12** were also characterized by ^{31}P NMR (4: 151.3 and 150.4; 12: 151.9 and 150.5).

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