Conformational and Orientational Switching of Uridine Derivatives by Borates

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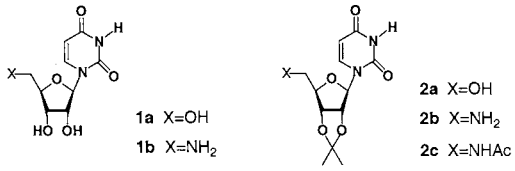
We have demonstrated for the first time that the *syn-anti* orientation of 5'-amino-5'-deoxyuridine (**1b**) can readily be switched by adding borate as an external controlling factor. In borate added phosphate buffer, the *syn/anti* ratio of **1b** dramatically increased with increasing borate concentration. This unique *syn* preference is most probably driven by the cooperative action of cyclic esterification of the **1b**'s 2',3'-*cis*-diol with borate and of hydrogen-bonding formation between 2-carbonyl oxygen and 5'-amino proton.

Recently, nucleoside analogs have received much attention not only as effective agents against HIV and AIDS[1] but also as key compounds for the preparation of antisense RNA and/or antigene nucleic acid model molecules.[2] Thus, a very wide variety of nucleoside analogs have already been synthesized through modifications of native nucleosides in order to enhance affinity and efficiency of recognizing specific sequences of DNA/RNA molecules.[3] This approach may be regarded as internal or intrinsic control of the binding affinity and efficiency through the intramolecular modifications. However, this strategy does not always give satisfactory results and more flexible and versatile methods to control the binding process are needed. In this context, there may be another approach to control the recognition process, which employs some external denaturating factor(s) such as added salts or urea, although their effects are not constructive.

In the recognition process involving DNA/RNA, the base orientation of nucleoside attached to the glycosyl group plays the major role, where the *anti* orientation is essential for the base recognition. Hence, if we can switch the *syn-anti* orientation of nucleoside analogs by an external factor, this can be a versatile, powerful tool to control the nucleic acid recognition. It is well documented that unusual *syn* orientation is induced in some cases by internal factors like substitution.[4,5] Thus, introduction of a bulky substituent at the 6-position of pyrimidine base induces unusual *syn* orientation to the pyrimidine nucleosides,[4] while cyclization of the *cis*-2',3'-diol of uridine enhances the *syn/anti* ratio of the resulting 2',3'-*c*UMP and 2',3'-*O*-isopropylidene uridine (**2a**) through the 2',3'-*O*-cyclic furanose structure.[6] However, in most cases, the *syn* orientation is not favored at all and the switching of the *syn-anti* orientation is believed to be difficult to induce by external factors.

We wish now to propose a novel strategy to control externally the *syn-anti* orientation of the pyrimidine nucleoside, such as uridine (**1a**), which is materialized by the specific interaction of the *cis*-2',3'-diol of sugar moiety with borate added to the bulk solution.[7]

Pyrimidine nucleosides are known to favor the *anti* orientation in solution.[8] One possible strategy to enhance the *syn* orientation is to utilize the cooperative effects of the hydrogen bonding interaction between the 2-carbonyl and 5'-hydroxyl groups and the bridging substitution of the *cis*-2',3'-diol. Unfortunately, the 5'-hydroxyl proton, being lost inevitably in nucleotides or nucleic acid model compounds, is not available in the possible hydrogen bonding interaction with the 2-carbonyl group. We therefore substitute the 5'-hydroxyl group of **1a** with an amino group in this study and discuss the *syn-anti* orientation of the resulting 5'-amino-5'-deoxyuridine (**1b**)[9] in the presence/absence of added borate as an external switching factor.



Scheme 1. Structures of uridine and its derivatives.

In order to examine the effects of both the amino substitution of the furanose's 5'-hydroxyl group and the ketalization of *cis*-2',3'-diol in uridine, we first synthesized 2',3'-*O*-isopropylidene uridine (**2a**)[10] and 2',3'-*O*-isopropylidene 5'-amino-5'-deoxyuridine (**2b**),[11] which were subjected to the circular dichroism (CD) spectral study in phosphate buffer (pH 7.2).[12] As can be seen from Figure 1a, the ketalization of **1a** reduced the CD intensity of **2a** to some extent but did not appreciably affect the CD spectral profile of **2a**, whereas the amino substitution caused drastic changes in the major CD bands of **2b**, affording a almost flattened CD spectrum. The molar ellipticity of the major CD band around 270 nm ($[\theta]_{max}$) is known to reflect both the glycosyl orientation and the puckering of the furanose ring.[13] Since the ketalization fixes the sugar puckering of **2a** and **2b** to a comparable extent, the drastic CD spectral changes observed for **2b** should solely originate from the amino substitution, reflecting a much reduced contribution of the *anti* conformer. Typical $[\theta]_{max}$ values for the *anti* and *syn* orientations are 10000 and 100–1000 deg cm^2 dmol^{-1}, respectively.[14] Although the $[\theta]_{max}$ value obtained for **2a** (6000) is somewhat smaller than that for **1a** (9700), evidently the *anti* orientation is still predominant even in the 2',3'-*O*-isopropylidene derivative **2a**. In sharp contrast to **1a** and **2a**, 2',3'-*O*-isopropylidene 5'-amino-5'-deoxyuridine **2b** gave a much reduced $[\theta]_{max}$ of 1200 under the same conditions. This clearly indicates the predominant *syn* orientation induced probably by the intramolecular hydrogen bonding interaction between the 5'-amino proton and the uracil's 2-carbonyl group. Significantly, 2',3'-*O*-isopropylidene 5'-*N*-acetylamino-5'-deoxyuridine (**2c**) also gave a CD spectrum which is very close in shape and intensity to that of **2b**. The fact that the *N*-acetyl derivative **2c** can also induce the *syn* orientation through the intramolecular hydrogen bond to the 2-carbonyl is crucial for the purpose of the orientation control of DNA/PNA analogs.

These results prompted us to propose a novel strategy to effect the *anti*-to-*syn* switching of the base orientation of nucleosides and possibly nucleic acid model compounds containing 5'-aminouridine by an external factor. As demonstrated above, the 2',3'-*O*-cyclic furanose ring is essential to induce the *syn* orientation in addition to the intramolecular hydrogen bond between the 5'-amino proton and the 2-carbonyl. In this study, we therefore employed the borate ester formation with furanose's 2',3'-diol as a convenient tool to induce the 2',3'-*O*-cyclic furanose ring in conjunction with the intramolecular hydrogen bonding interaction.

CD spectra of uridine **1a** and 5'-aminouridine **1b** were measured in phosphate and borate buffer solutions[15] in order to examine the effects of borate buffer and the amino substitution. As can be seen

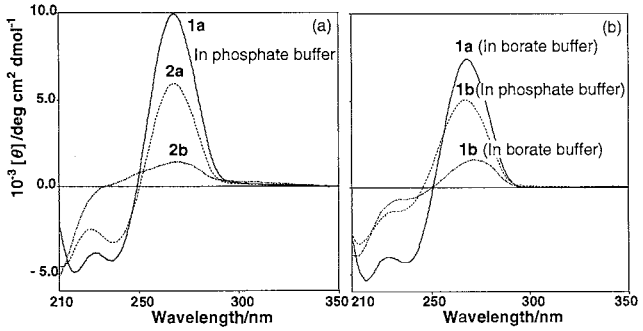


Figure 1. CD Spectra of (a) **1a**, **2a**, and **2b** in phosphate buffer solution (pH 7.2); (b) **1a** and **1b** in phosphate (pH 7.2) and borate buffer solution (pH 7.2).

from the CD spectra shown in Figure 1b, the $[\theta]_{max}$ value of **1a** decreased from 9700 in phosphate buffer to 7400 (76% of the original value) in a borate buffer. This value is similar to that observed for **2a** (6000) in phosphate buffer, and means that the borate added to the solution appreciably induces the *syn* orientation through the ester formation, although the effect is moderate. On the other hand, the 5'-aminouridine **1b** gave a considerably smaller $[\theta]_{max}$ value of 5100 (53% of the original value) than that for **1a** even in a phosphate buffer, owing to the intramolecular hydrogen bonding interaction. Interestingly, the use of a borate buffer led to a further decrease of the $[\theta]_{max}$ value to 1600 (31% of the original value), which is almost comparable to that of **2b** in a phosphate buffer. It is thus demonstrated that the cooperation of the 5'-amino substitution and the borate buffer is quite effective in inducing the *syn* orientation of a uridine derivative.

This unique borate-driven conformational switching from *anti* to *syn* of the base orientation was further confirmed by the NMR measurements of **1b** in both buffer solutions.[16] Figure 2 illustrates the ^{1}H NMR (upper trace in each spectrum pair a and b) and differential NOE spectra (lower trace) of **1b** in phosphate (a) and borate (b) buffers. In the ^{1}H NMR spectrum in borate buffer, both H5 and H6 are at higher field than in the phosphate buffer as was the case with the 2',3'-*O*-isopropylidene derivative **2a**.[6] The spin coupling constants, $J_{2',3'}$ (6.8 Hz) and $J_{1',2'} + J_{3',4'}$ (8.2 Hz) of **1b** in borate buffer are the same as those of 2',3'-*c*UMP.[17] This fact indicates that in borate buffer the sugar puckering of **1b** is quite similar to that of 2',3'-*c*UMP. In the NOE spectra in phosphate buffer, where the uracil's H6 at δ 7.6 is presaturated, the uracil's H5 and/or furanose's H1' at δ 5.8 (26.7%), H2' at δ 4.4 (7.9%), H3' at δ 4.1 (2.7%), and H5' at δ 3.0 (2.0%) gave notable NOE peaks, as shown in Figure 2a. This NOE profile observed entirely agrees with the *anti* orientation of **1b** illustrated in Figure 2 (top right).

In borate buffer, **1b** afforded a completely different NOE spectrum. As shown in Figure 2b, the presaturation of uracil's H6 gave evident NOE signals only with the uracil's H5 (14.5%) and furanose's H1' (12.5%) but no appreciable NOE peaks with the other furanose protons. This unique NOE profile, in addition to the flattened CD peaks discussed above, provides the definitive evidence in support of the predominant contribution of the *syn* orientation in **1b** in borate buffer, which is driven by the borate ester formation as illustrated in Figure 2 (bottom right).

We have demonstrated that the *syn-anti* orientation of 5'-aminonucleoside derivative can readily be switched by adding borate as an external controlling factor. This rather simple, but effective, strategy using borate as an external factor to control base orientation should be applicable to the recognition of DNA/RNA by nucleic acid model compounds containing 5'-aminonucleoside derivative though amide bonding.

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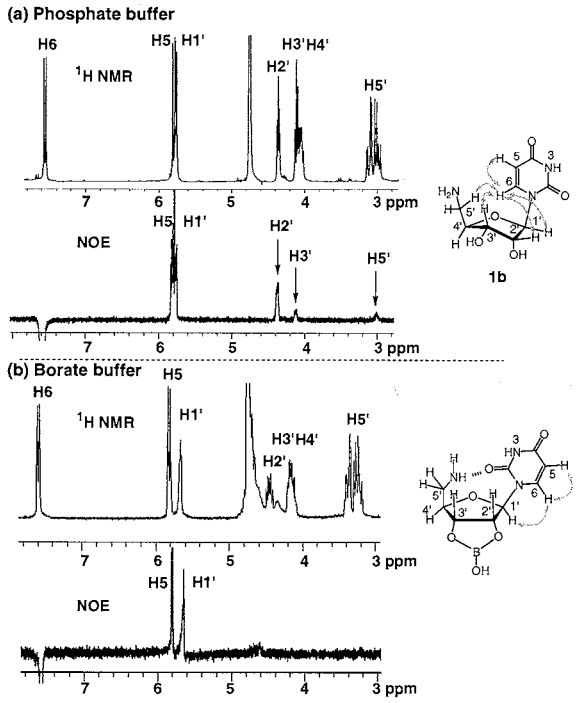

Figure 2. ^{1}H NMR and differential NOE spectra of **1b** obtained with presaturation at H6 (δ 7.6) in (a) phosphate buffer (pH 7.2) and (b) borate buffer (pH 7.2).

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