Synthesis and conformation of 3′-*O*,4′-*C*-methyleneribonucleosides, novel bicyclic nucleoside analogues for 2′,5′-linked oligonucleotide modification

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Novel bicyclic nucleoside analogues 3′-*O*,4′-*C*-methyleneribonucleosides 1 are conveniently prepared starting from uridine; the sugar puckering of 1 is found to be nearly in the *S*-conformation by means of PM3 calculations and ^{1}H NMR studies.

Non-genetic 2′,5′-linked oligonucleotides have been attracting much attention since the syntheses and properties of oligonucleotides with 2′,5′-linkages were reported in 1992.[1–3] Using NMR studies[4] and molecular modeling,[5] it was suggested that oligoribonucleotides containing 2′,5′-linkages (2′,5′-RNA) preferentially adopted the C2′-*endo* conformation (*S*-conformation), while 2′,5′-linked oligodeoxyribonucleotides (2′,5′-DNA) existed mainly with C3′-*endo* sugar puckering (*N*-conformation). Interestingly, these results are the opposite of what is observed with the corresponding 'genetic' 3′,5′-RNA and -DNA. There have been some reports concerning successful application of 2′,5′-linked oligonucleotides to antisense uses.[6–9] On the other hand, 2-5A (2′,5′-oligoadenylate 5′-triphosphate) is known to be formed in interferon-treated cells and to have antiviral activity with the aid of 2-5A dependent ribonuclease (RNase L),[10] and intensive studies on various 2-5A analogues have been reported.[11]

We have designed a bicyclic nucleoside analogue, 3′-*O*,4′-*C*-methyleneribonucleoside **1**,[12] which is a promising precursor for both novel types of therapeutic nucleoside derivatives and 2′,5′-linked oligonucleotide analogues. The sugar moiety in **1** consists of a 2,6-dioxabicyclo[3.2.0]heptane ring system, with the tetrahydrofuran part conformationally restricted by the fused oxetane ring. Here we describe a convenient first synthesis of **1** (B = U and C), and also discuss its conformation.

The synthetic route to the target molecule **1** (B = U) is shown in Scheme 1. A selective toluene-*p*-sulfonylation of the diastereotopic hydroxymethyl groups in 2′,3′-*O*-cyclohexylidene-4′-hydroxymethyluridine[13,14] **2** afforded monotosylate **3** (69%) along with its isomer **4** (12%). The stereochemistry at C4′ in **3** was confirmed by means of NOE measurements. Acidic hydrolysis of **3** gave triol **5** (94%) and then selective protection of the primary hydroxy group in **5** with a 4,4′-dimethoxytrityl (DMTr) group in the usual manner afforded **6** (56%).

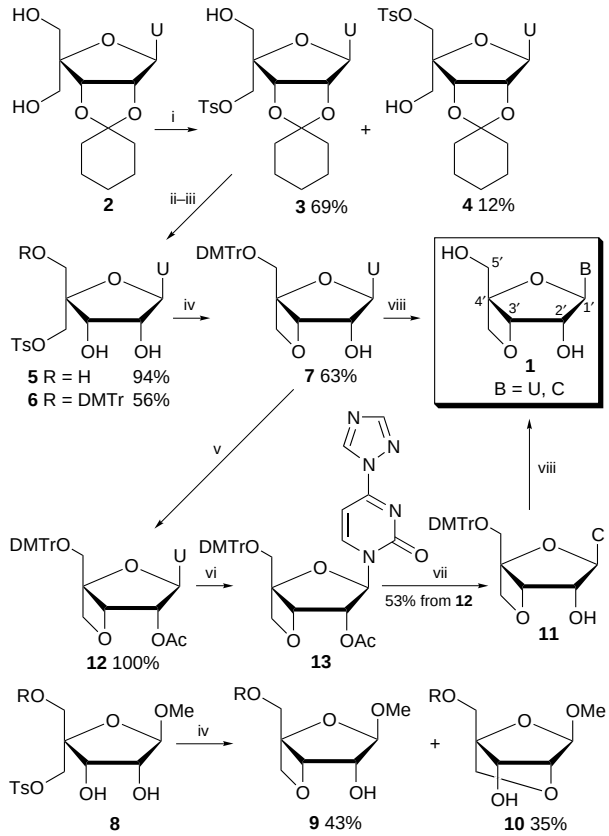
Oxetane ring formation from **6** was accomplished on treatment with a large excess of sodium hexamethyldisilazide in THF at room temperature, yielding the desired product **7**† (63%). The exclusive oxetane ring formation is attributable to the predominant *S*-conformation of the starting material **6**,‡ in which only the 3′-OH is thought to be located near the 4′-methylene carbon centre; the 2′-OH is too far away to attack the 4′-methylene carbon. In fact, 1′-methoxy congener **8**, which should exist mainly in the *N*-conformation due to the anomeric effect,‡ gives a *ca.* 1:1 mixture of 2′-*O*,4′-*C*-methylene derivative **9** and 3′-*O*,4′-*C*-methylene derivative **10** under the same conditions.[15]

Transformation of **7** into the cytidine derivative **11** by exchange of nucleobase was also undertaken as shown in Scheme 1. After 2′-*O*-acetylation of **7**, treatment of **12** with 1,2,4-triazole and 4-chlorophenyl phosphorodichloridate in pyridine[16] gave **13**, which was then subjected to ammonolysis to give **11**† (overall 53% yield from **7**). Acid catalysed detritylation of **7** and **11** gave the corresponding 2′,5′-diol compounds **1** (B = U)† and **1** (B = C)† quantitatively.

In general, the sugar moiety in natural and modified nucleosides exists in an equilibrium between *N*- and *S*-conformations. The conformation of nucleoside analogues in solution is easily obtainable from coupling constants between the ribose ring protons in the ^{1}H NMR spectrum.[17] The simplest equation for calculation of percentage of *S* conformation is shown in eqn. (1). The calculation results for the obtained nucleoside

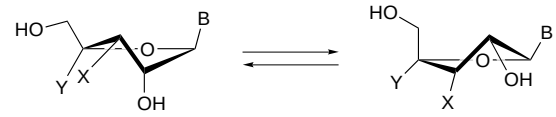
$$S(\%) = 100 \times (J_{1'2'} - 1)/6.9 \qquad (1)$$

analogues are summarized in Table 1, and show that all of the 3′-*O*,4-*C*-methyleneribonucleoside analogues have very large *S* values. A PM3 calculation[18]§ also indicates that an *S*-conformation (C1′-*exo*-C2′-*endo*) is the most stable conformation for the ribose ring in **1** (Fig. 1). It is very interesting and noteworthy that the oxetane ring fused nucleoside deriva-

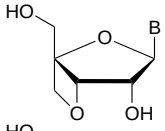
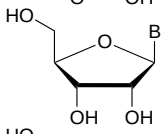
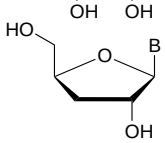


Scheme 1 *Reagents and conditions*: i, TsCl (1.7 equiv.), 110 °C; ii, CF_3CO_2H–H_2O (98:2), room temp.; iii, 4,4′-dimethoxytrityl chloride, DMAP, pyridine, room temp., iv, $(Me_3Si)_2NNa$ (10 equiv.), THF, 20 °C; v, Ac_2O, pyridine, room temp.; vi, 1,2,4-triazole, 4-chlorophenyl phosphorodichloridate, pyridine, room temp.; vii, aqueous NH_3, 1,4-dioxane, room temp.; viii, 3% CCl_3CO_2H in H_2O, CH_2Cl_2, room temp.

Table 1 Conformational analysis of nucleoside analogues using ^{1}H NMR spectroscopy (in CD_3OD)



N-conformation ⇌ *S*-confirmation

Nucleoside analogues		$J_{1'2'}$/Hz	*S* (%)[a]
	B = U	7.5	94
	B = C	7.3	91
	B = U	4.6	52
	B = C	2.8	26
	B = U	1.2[b]	3
	B = C	0[c]	0

[a] Calculated from eqn. (1). [b] Ref. 19 (Measured in [$^2H_6$]DMSO). [c] Ref. 20.

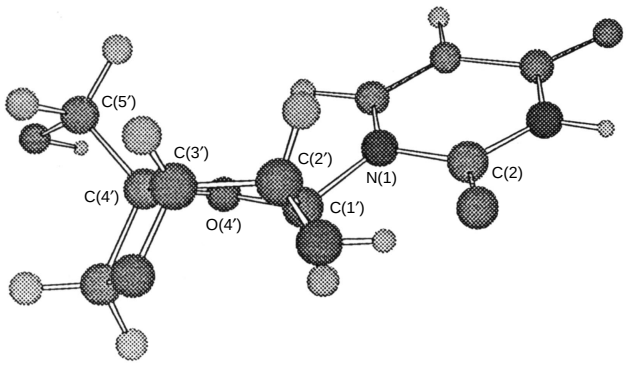


Fig. 1 Computer-generated (PM3) representation of **1** (B = U)

tives **1** exist predominantly in an *S*-conformation, in contrast with the typical monocyclic nucleoside compounds (Table 1).[19,20]

Further chemical modification of **1** and its incorporation into oligonucleotides are now in progress.

Footnotes and References

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† *Selected data* for **7**: mp 120–121 °C (Et_2O–hexane); $[\alpha]_D^{21}$ −37.7 (*c* 1.1, acetone); ^{1}H NMR ([$^2H_6$]acetone): δ 2.92 (1 H, br s), 3.47, 3.51 (2 H, AB, *J* 10), 3.85 (6 H, s), 4.36 (1 H, dd, *J* 4, 4), 4.52, 4.83 (2 H, AB, *J* 8), 5.11 (1 H, d, *J* 4), 5.57 (1 H, d, *J* 8), 6.51 (1 H, d, *J* 8), 6.96 (4 H, d, *J* 9), 7.39–7.41 (7 H, m), 7.52 (2 H, d, *J* 5), 7.71 (1 H, d, *J* 9); *m*/*z* (EI) 558 (M^+). For **11**: mp 139–140 °C (Et_2O–hexane); $[\alpha]_D^{22}$ + 0.60 (*c* 0.34, MeOH); ^{1}H NMR (CD_3OD): δ 3.40, 3.50 (2 H, AB, *J* 10), 3.76 (6 H, s), 4.14 (1 H, dd, *J* 4, 7), 4.48, 4.72 (2 H, AB, *J* 7), 4.98 (1 H, d, *J* 4), 5.79 (1 H, d, *J* 7), 6.56 (1 H, d, *J* 7), 6.85 (4 H, d, *J* 9), 7.21–7.42 (9 H, m), 7.67 (1 H, d, *J* 7); *m*/*z* (FAB) 558 $(M + H)^+$. For **1** (B = U): mp 215–216 °C (AcOEt); $[\alpha]_D^{17}$ −18.2 (*c* 1.2, acetone); ^{1}H NMR (CD_3OD): δ 3.74, 3.82 (2 H, AB, *J* 12), 4.21 (1 H, dd, *J* 5, 8), 4.42, 4.82 (2 H, AB, *J* 8), 5.05 (1 H, d, *J* 5), 5.66 (1 H, d, *J* 9), 6.38 (1 H, d, *J* 8), 7.67 (1 H, d, *J* 9); Calc. for $C_{10}H_{12}N_2O_6 \cdot 1/2H_2O$; C, 45.29; H, 4.94; N, 10.56. Found C, 45.07; H, 4.82; N, 10.15%. For **1** (B = C): mp 224–225 °C (Pr^iOH); $[\alpha]_D^{25}$ +32.6 (*c* 0.49, H_2O); ^{1}H NMR (CD_3OD): δ 3.73, 3.81 (2 H, AB, *J* 12), 4.16 (1 H, dd, *J* 5, 7), 4.52, 4.81 (2 H, AB, *J* 8), 5.04 (1 H, d, *J* 5), 5.94 (1 H, d, *J* 8), 6.41 (1 H, d, *J* 7), 7.68 (1 H, d, *J* 8); Calc. for $C_{10}H_{13}N_3O_5 \cdot 1/3H_2O$; C, 45.98; H, 5.27; N, 16.09. Found 46.04; H, 5.02; N, 15.82%.

‡ $J_{1'2'}$ Values (6.0 Hz for **6** and 0 Hz for **8**) provide the conformational information.

§ The MOPAC93 molecular orbital package (CS MOPAC Pro™, Cambridge Soft Corporation) utilizing the MNDO-PM3 Hamiltonian was used for the semi-empirical MO calculations. Geometry optimization was carried out with the use of the keyword MMOK implemented in MOPAC. All initial structures used for the MO calculation were generated by reference to the X-ray structures of natural nucleosides (ref. 21). Numerical calculations were performed on a Power Macintosh computer.

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