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Synthesis of 2'-*O*,4'-*C*-Methyleneuridine and -cytidine. Novel Bicyclic Nucleosides Having a Fixed $C_{3'}$-*endo* Sugar Puckering

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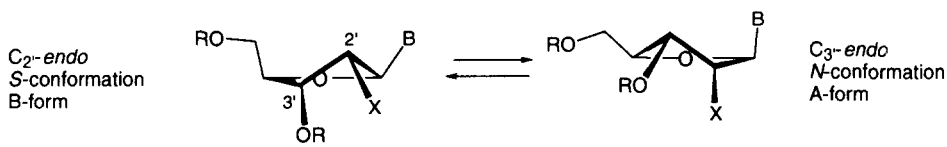
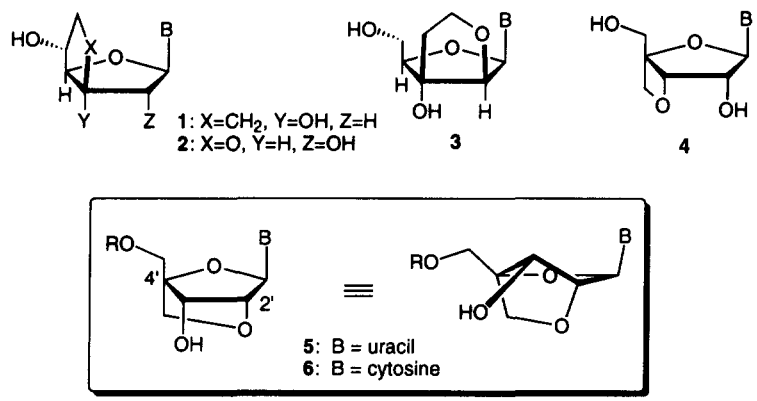
Abstract: 2'-*O*,4'-*C*-Methyleneuridine and -cytidine, novel bicyclic nucleoside analogs having a typical C3'-*endo* sugar puckering, were synthesized starting from uridine *via* a several-step sequence. © 1997 Elsevier Science Ltd.

As a direct way to treat serious diseases such as cancer, viral infections and genetic disorders, antisense (targeting mRNA) and antigene (targeting DNA) strategies for selective regulation of gene expression are attracting the widespread attention of many scientists.[1] Recent studies have been focused on developing various types of backbone-, sugar-, and/or base-modified oligoribo- or oligodeoxyribonucleotides to find more effective antisense and antigene molecules.[2] In particular, many efforts are concentrated on acquisition of the derivatives possessing high binding affinity with complementary RNA and/or DNA strands. The sugar moiety of nucleosides (nucleotides) has two representative preferential conformations, $C_{2'}$-*endo* and $C_{3'}$-*endo* conformations (in other words, *S*- and *N*-conformations). Although each nucleoside (nucleotide) exists in an individual equilibrium mixture between these two conformations, it is well known that the B-form DNA duplex possesses $C_{2'}$-*endo* sugar puckering and the A-form RNA duplex has the $C_{3'}$-*endo*.[3]

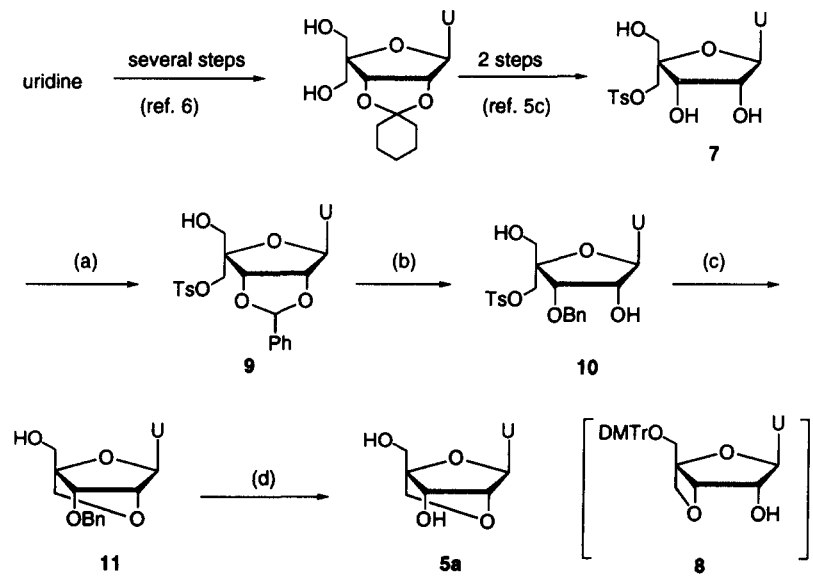


Increasing conformational inflexibility of the sugar moiety in nucleosides (oligonucleotides) is expected to be one of the most promising ways for gaining high binding affinities with complementary single-stranded RNA and/or double-stranded DNA.[4] Some intensive studies concerning restriction of sugar conformation in nucleosides have been reported recently, such as compounds **1**-**4** and others.[5] Although such nucleoside analogs are conformationally restricted to some extent, conformational flexibility of the sugar part still remains because of the *cis*-fused bicyclic ring system of all the analogs. Therefore, to date, strict fixation of the sugar puckering of nucleosides (oligonucleotides) has not been achieved. If a nucleoside analog with a 'fixed' *N*- or *S*-conformation of the sugar moiety is available, it would serve as a potential synthon for many kinds of biological, biophysical, pharmacological, and other investigations. In this communication, we wish to describe the synthesis of 2'-*O*,4'-*C*-methyleneuridine (**5**) and -cytidine (**6**), the novel modified nucleosides with the 'fixed' $C_{3'}$-*endo* sugar puckering.



The starting material, 4'-(*p*-toluenesulfonyloxymethyl)uridine (**7**), was prepared from uridine by a several-step sequence.[5d,6] Because the reaction of 5'-*O*-dimethoxytrityl derivative of **7** under the alkaline conditions was found to give exclusively the oxetane product **8**,[5d] an alternative route should be necessary for the present purpose. In order to protect the C_3-hydroxyl group selectively, compound **7** was initially treated with benzaldehyde in the presence of zinc chloride to give 2',3'-*O*-benzylidene derivative **9** as the sole diastereoisomer.[7] On treatment of **9** with sodium cyanoborohydride and titanium tetrachloride in acetonitrile, an exclusive O_2-C bond cleavage took place smoothly to afford **10**, probably owing to steric hindrance of the C_4-tosyloxymethyl group.[8] On exposure of **10** to sodium hexamethyldisilazide in tetrahydrofuran, the desired compound **11** was produced in a moderate yield. Finally, the usual reductive debenzylation of **11** furnished the target molecule **5a**[9] quantitatively (Scheme 1).

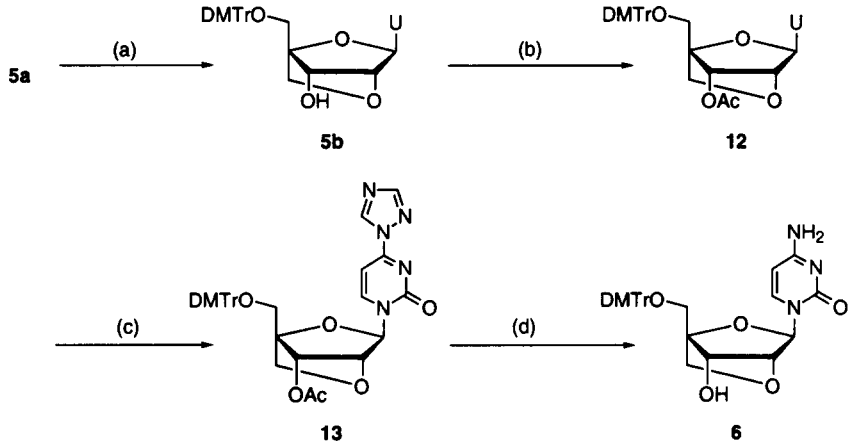
Scheme 1



Reagents and Conditions: (a) PhCHO, $ZnCl_2$, r.t., 80%; (b) $NaBH_3CN$, $TiCl_4$, MeCN, r.t., 75% (c) NaHMDS (3.3 eq.), THF, r.t., 61%; (d) H_2 (1 atm), 10%Pd-C, MeOH, r.t., quant.

Transformation of **5a** into the cytidine congener **6** was achieved as follows. 5'-*O*-Dimethoxytritylation of **5a** to **5b** followed by 3'-*O*-acetylation in the usual manner gave **12**, a pyridine solution of which was treated with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole[10] to afford **13** in 80% yield. A mild ammonolysis of **13** produced **6**[9] in 99% yield (Scheme 2).

Scheme 2



Reagents and Conditions: (a) 4,4'-dimethoxytrityl chloride, DMAP, pyridine, r.t., 94%; (b) Ac_2O, pyridine, r.t., quant.; (c) 4-chlorophenyl phosphorodichloridate, 1,2,4-triazole, pyridine, r.t., 80%; (d) 28%NH_4OH, dioxane, r.t., 99%.

Considering the rigid bicyclo[2.2.1]heptane ring system, it is easily assumed that compounds **5** and **6** have a typical *N*-conformation of the sugar part. Actually, in the ^1H NMR spectra, both **5** and **6** exhibited all singlet signals for $C_{1'}$-, $C_{2'}$- and $C_{3'}$-protons, and an X-ray crystallographic analysis[11] of **5a** showed that the sugar pucker pseudorotation phase (*P*)[12] and χ angel[3] were 17.4° and 195.7°, respectively, characteristic of the typical $C_{3'}$-*endo* form of sugar puckering with *anti*-nucleobase orientation (Fig. 1). These data clearly prove the validity of the above assumption.

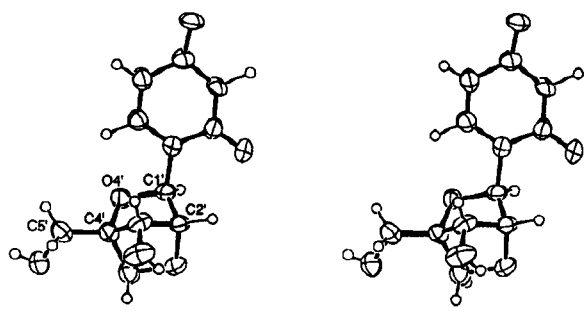


Fig. 1. X-ray Crystal Structure of **5a**

Thus, we successfully demonstrated the synthesis of novel bicyclic nucleosides **5** and **6** with a methylene bridge over the 2'-*O* and 4'-*C* positions in natural ribonucleosides and clearly showed that these compounds had the typical $C_{3'}$-*endo* (*N*-conformation) of sugar puckering, the first example of nucleoside analogs having a rigid *N*-conformation of the sugar moiety with $C_{3'}$- and $C_{5'}$-OH functionality.[13]

Various studies on these bicyclic nucleosides are now in progress.

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7. Although a trace amount of the diastereomer was detected in the crude product, the major isomer was isolated only after a purification process.
8. To our best knowledge, there has been no report concerning selective C-O bond cleavage of 2',3'-*O*-benzylidene ribofuranosyl derivatives.
9. Selected data for representative new compounds. **5a**: mp 239-243 °C (MeOH). ^{1}H NMR (CD_3OD) δ: 3.76, 3.96 (2H, AB, J = 8 Hz), 3.90 (2H, s), 4.04 (1H, s), 4.28 (1H, s), 5.55 (1H, s), 5.69 (1H, d, J = 8 Hz), 7.88 (1H, d, J = 8 Hz). *Anal.* Calcd for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93%. Found: C, 46.74; H, 4.70; N, 10.84%. **6**: mp 157-159 °C (AcOEt-hexane). ^{1}H NMR ($CDCl_3$) δ: 3.44, 3.52 (2H, AB, J = 11 Hz), 3.74 (6H, s), 3.70, 3.88 (2H, AB, J = 8 Hz), 4.24 (1H, s), 4.53 (1H, s), 5.61 (1H, s), 5.74 (1H, d, J = 7 Hz), 6.82 (4H, d, J = 9 Hz), 7.15-7.43 (9H, m), 7.91 (1H, d, J = 7 Hz), 8.16 (2H, s). *Anal.* Calcd for $C_{31}H_{31}N_3O_7 \cdot H_2O$: C, 64.69; H, 5.78; N, 7.30%. Found: C, 64.64; H, 5.77; N, 7.45%.
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11. Atomic coordinate, bond lengths, bond angles, and thermal parameters of **6a** can be obtained on request from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (UK)
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