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1',6'-Methano Carbocyclic Thymidine: Synthesis, X-ray Crystal Structure, and Effect on Nucleic Acid Duplex Stability

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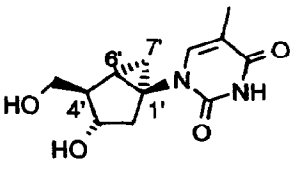
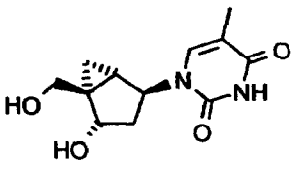
Abstract: *The title compound 1 has been synthesized via bicyclic lactone 3 and amine 9 as the key intermediates. X-ray crystallography reveals the bicyclic skeleton of 1 to adopt a boat-like (3'-exo) conformation. Oligodeoxyribonucleotides incorporating up to 10 building blocks 1 in place of natural thymidine are still capable of binding to complementary DNA or RNA, albeit with lower affinity than the unmodified parent compounds.*

The emergence of antisense oligonucleotides as a novel class of potential therapeutic agents[1] has provided a strong stimulus for the design and synthesis of modified oligonucleotides and oligonucleotide analogs.[1,2] For antisense agents to be effective inhibitors of protein expression *in vivo*, they have to resist the action of (single-stranded) DNA degrading enzymes and bind to their target mRNA sequences with high affinity and in a sequence-specific manner.[1] Mainly in pursuit of compounds that would meet both these requirements various types of backbone-, sugar-, and base-modified oligonucleotides have been prepared in recent years and their resistance to nuclease degradation as well as their binding affinity for complementary nucleic acids have been studied in some detail.[1,2] In this context we have recently described the synthesis of bicyclic thymidine analog **2** (*Fig. 1*) and we have provided preliminary evidence that **2** containing oligonucleotides exhibit increased affinity for complementary RNA (as compared to their unmodified counterparts).[3] In this communication we now wish to report on the synthesis of an isomer of **2**, namely 1',6'-methano carbocyclic thymidine **1** (*Fig. 1*), its X-ray crystal structure, and its effect on DNA/DNA and DNA/RNA duplex stability.[4,5]

Figure 1

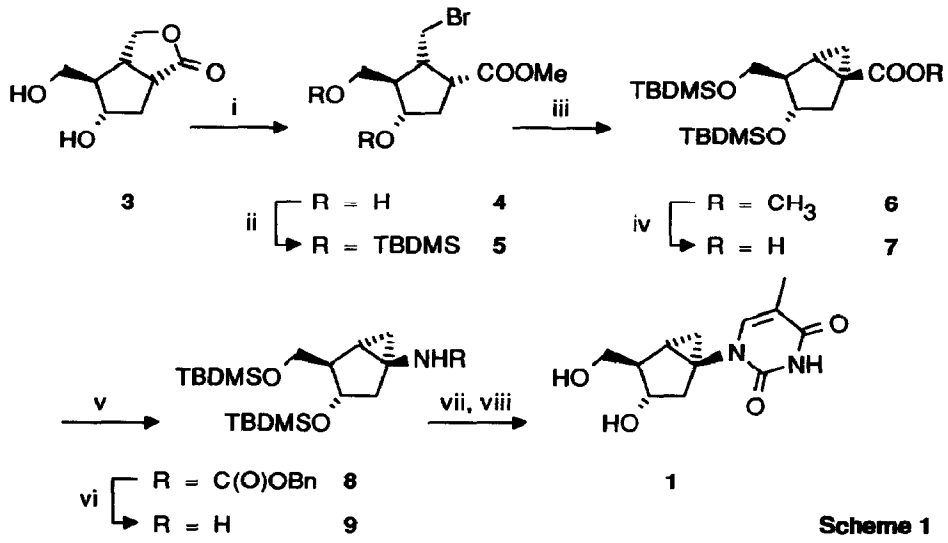


1



2

With the thymine base attached to a bridgehead carbon atom of the bicyclo[3.1.0]hexane system, retrosynthetic analysis of **1** immediately indicated that the heterocyclic ring system would have to be constructed from a suitably OH-protected bicyclic amine such as **9** (*Scheme 1*) in the final steps of the synthesis. As shown in *Scheme 1* our synthesis of **9** (and subsequently **1**) relies on the known homochiral bicyclic lactone **3**[6] as the central intermediate, a compound that has not been previously exploited for the synthesis of carbocyclic nucleosides.[7,8] Opening of the lactone ring of **3** with TMS-Br/MeOH gave a 70% yield of γ-bromo ester **4**, which proved to be a relatively unstable compound and, therefore, immediately after chromatography was converted to its bis-TBDMS ether **5**. This seemingly simple transformation was severely hampered by the pronounced tendency of the γ-bromo ester part of the molecule to reform the 5-membered lactone ring, resulting in varying amounts of the bis-TBDMS ether of **3** as a major by-product of the



Scheme 1

i: TMSBr (10 equiv.), MeOH, $ZnBr_2$ (cat.), 0°, 18h, 70%. ii: N-TBDMS N-methyl acetamide (3 equiv.), DMF, 0° → r.t., 2.5h, 64%. iii: K-OBu^t, t-BuOH, r.t., 30 min, 76%. iv: KOH/EtOH, 80°, 5h, 78%. v: a. DPPA, Et_3N, toluene, 0°, 1h, r.t., 1h; b. 80°, 2h; c. BnOH, 80°, 2h, 100°, 15 min, 85%. vi: H_2, 10% Pd-C, toluene, 84%. vii: $CH_3OCH{=}C(CH_3)C(O)NCO$, CH_2Cl_2, 0° → r.t., 18h, 85%. viii: 0.2N HCl/EtOH-H_2O 9/1, refl., 10h, 80%.

silylation reaction. Among the different methods investigated for the introduction of the TBDMS-groups (TBDMS-Cl/imidazole/DMF; TBDMS-OTf/lutidine/DMF; $CH_3C(O)CH{=}C(OTBDMS)CH_3$/p-TosOH; N-TBDMS N-methyl acetamide (MTBSA)[9]), the use of MTBSA in DMF proved to be the most advantageous in terms of yield as well as cleanliness of the reaction. Gratifyingly, subsequent formation of the three-membered ring proceeded very smoothly and a 76% yield of bicyclic ester **6** was obtained after a 30-minute treatment of **5** with K-OBu^t in t-BuOH at 0°. Saponification of **6** with KOH/EtOH at 80° gave bicyclic acid **7** (78%), which was converted to the benzyloxycarbonyl protected amine **8** by a 3-step one-pot procedure, involving formation of the carboxylic acid azide, *in situ Curtius* rearrangement,[10] and finally quenching of the ensuing isocyanate with benzyl alcohol. Removal of the amino protecting group *via* catalytic hydrogenation then gave bicyclic amine **9** in 71% overall yield (based on **7**). Construction of the heterocyclic base moiety was accomplished by reaction of **9** with β-methoxy α-methacryloyl-isocyanate and subsequent cyclization of the resulting acryloyl-urea under acidic conditions.[11] The latter step was accompanied by loss of the TBDMS-protecting groups and directly provided **1** in 68% yield for the two step sequence **9** → **1**.[12]

Fig. 2 shows an ORTEP drawing of the X-ray crystal structure of **1**.[13] The bicyclic skeleton of the molecule adopts a boat-like (3'-exo) conformation with a puckering amplitude of the cyclopentane ring of 27°. The torsion angle about the C-5' - C-4' - C-3' - O-3' bond is 144° and thus matches the corresponding torsion angle δ of the nucleotide units in canonical B-DNA duplexes (*vide infra*).[14] Contrary to the crystal structures of most pyrimidine nucleosides[14] the heterocyclic base is found in a *syn* orientation with its carbonyl oxygen O^2 being involved in an intramolecular hydrogen bond with the 5'-OH group. The conformations of the bicyclo[3.1.0]hexane system in **1** and **2** (*Fig. 1*) are virtually superimposable,[3] which is likely to reflect a certain intrinsic preference of this type of bicyclic ring system for a boat-like conformation.[15]

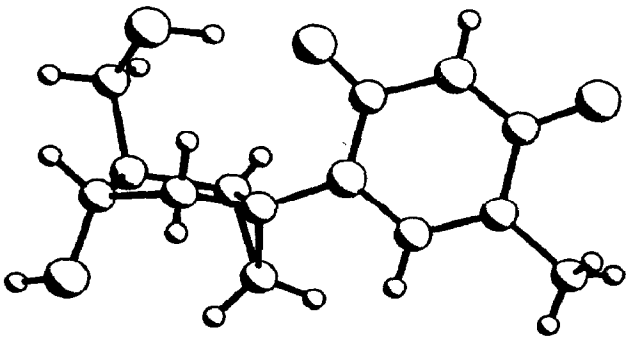


Figure 2: X-ray crystal structure of 1',6'-methano carbocyclic thymidine **1**.

Table 1 summarizes a set of representative data for the hybridization of **1** containing oligodeoxyribonucleotides with complementary RNA and DNA.[16] Substitution of **1** for natural thymidine in the DNA strand of *DNA/RNA* heteroduplexes causes a decrease in melting temperature of 1.0° to 1.9°/modification; this contrasts with the (enhancing) effects of ***2*** on DNA/RNA heteroduplex stability[3] and may reflect the need for the bicyclic skeleton of **1** to adopt a more unfavourable (chair-like) 3'-endo conformation within an A-type duplex structure.[17] As indicated by the more negative ΔT_m-values, duplex destabilization by **1** is even more pronounced for *DNA/DNA* hybrids. In view of the B-DNA like δ-angle of **1** (*vide supra*), which might suggest a favourable preorganization of the modified nucleotide units for B-type duplex formation, this finding appears to be rather surprising and cannot be fully rationalized at this point. It should be noted, however, that similar discrimination in favor of hybridization to complementary RNA rather than DNA has also been observed for oligonucleotides incorporating other types of sugar-modified building blocks.[5,18]

Table 1: Hybridization of **1** containing oligodeoxynucleotides with complementary RNA and DNA

Sequence[a]	ΔT_m/mod. *vs.* RNA[b]	(T_mWT[c])	ΔT_m/mod. *vs.* DNA[b]	(T_mWT[c])
I	- 1.9	52.3	- 4.4	43.0
II	- 1.8	62.3	- 2.3	62.5
III	- 1.1	50.2	- 2.5	54.1

[a]Sequences are: **I**: 5'-TTTT**1**CTCTCTCTCT-3'; **II**: 5'-**1**CCAGG**1**G**1**CCGCA**1**C-3'; **III**: 5'-GCG**tttttttttt**GCG-3'; **1** = 1. [b]Difference in melting temperature (T_m) between the modified DNA/RNA or DNA/DNA duplex and the respective unmodified wild-type (WT) duplex per modification ($\Delta T_m = T_m - T_m(WT)$). T_m's were determined in 10 mM phosphate buffer, pH 7, 100 mM Na^+. [c]T_m of the corresponding wild-type duplex in °C.

A more comprehensive discussion of the hybridization properties of oligonucleotides containing 1',6'-methano carbocyclic nucleotide building blocks (including fully modified mixed sequences) will be the subject of a future report.

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13. The structure was solved by direct methods (SHELXS 86). Full matrix least squares refinements with anisotropic temperature factors for all non-H-atoms converged at an R-factor of 0.068. Out of 32 H-atom 18 could be located in the difference Fourier map; the positions of the remaining ones were calculated. Inclusion of H-atoms in the least squares refinements led a final R-factor of 0.04. The structure shows two molecules A and B per asymmetric unit, which have slightly different conformations. For molecule A (shown in Fig.1), C-7' is located 0.42 Å below the plane formed by the remaining four atoms of the five-membered ring, for molecule B the deflection is 0.38 Å.
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