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4',6'-Methano Carbocyclic Thymidine: A Conformationally Constrained Building Block for Oligonucleotides

Karl-Heinz Altmann*#, Rudolf Kesselring#, Eric Francotte§, and Grety Rihs+

CIBA, Central Research Laboratories#, R-1060.2.34, Pharmaceutical Research Department§, and Physics Department+, CH-4002 Basel, Switzerland.

Abstract: *The synthesis of the title compound 1 has been accomplished in 20 chemical steps starting from D-ribonolactone. X-ray crystallography shows the bicyclic skeleton of 1 to adopt a boat-like ("2'-exo") conformation and preliminary hybridization data indicate that the substitution of 1 for natural thymidine in DNA/RNA heteroduplexes may increase their thermodynamic stability.*

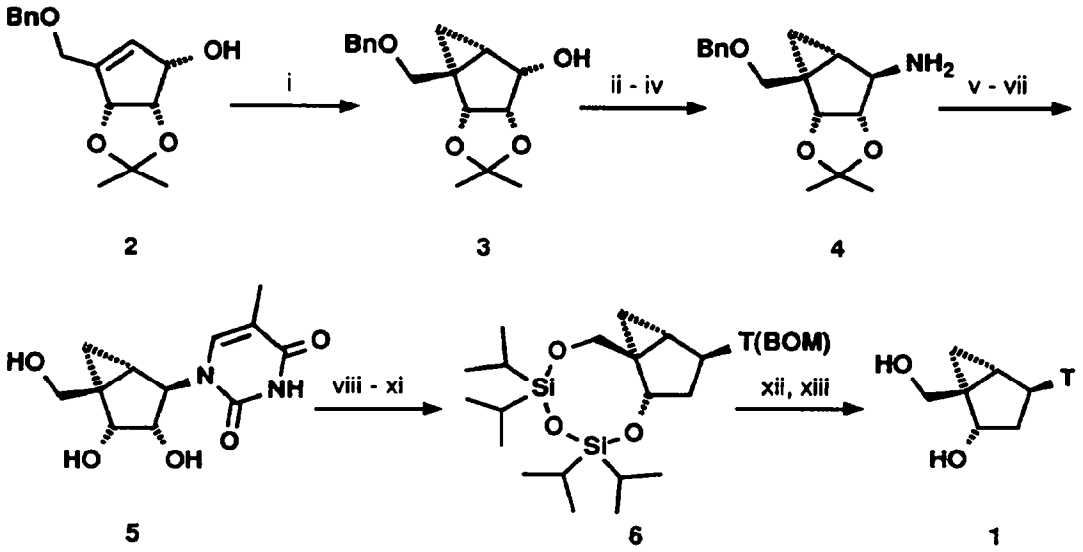
The emergence of antisense oligonucleotides as a potential new class of therapeutic agents has sparked significant interest in the possibilities to increase the thermodynamic stability of DNA/RNA heteroduplexes by the chemical modification of the DNA strand.[1] With RNA/RNA duplexes being generally more stable than the corresponding DNA/RNA or DNA/DNA hybrids, this should in principle be accomplishable by restricting the conformational freedom of the DNA strand in terms of the conformational characteristics of RNA/RNA duplex structures (A-type conformation).[2-4] With respect to modifications of the sugar moiety this would entail stabilization of a *3'-endo* or a closely related conformation with a torsion angle about the C-4' - C-3' bond (corresponding to the torsion angle δ in natural oligonucleotides) of ~ 80°.[2] We felt that bicyclo[3.1.0]hexane derived nucleoside analogs of type I (Fig. 1) could potentially meet this requirement.[5] On the basis of experimental[6] as well as theoretical[6c,7] studies on the preferred conformations of various bicyclo[3.1.0]hexane derivatives the bicyclic skeleton of nucleoside analogs of type I can be expected to adopt a boat-like ("2'-exo") conformation, which would be closely related to the 3'-endo conformation of the sugar moieties in A-type double helices.[2,8]

In this paper we now report on the synthesis of bicyclo[3.1.0]hexane based thymidine analog 1 (Fig. 1, Base = thymine) and its X-ray crystal structure together with some preliminary data on the hybridization properties of 1 containing oligonucleotides.[9]

Figure 1

I

As shown in *Scheme 1* our synthesis of 1 proceeds through the known cyclopentenol 2[10a] as the first key intermediate, which was obtained in 7 steps from D-ribonolactone with an optical



Scheme 1

i: Zn/Cu, CH_2I_2, Et_2O, refl., 18h, 73%; ii: Tos-Cl, Et_3N, CH_2Cl_2, DMAP(cat), r.t., 48h, 77%; iii: NaN_3, DMF, 70°, 18h, 88%; iv: H_2, Lindlar's catalyst, 4h, quant.; v: $CH_3OCH{=}C(CH_3)CONCO$, CH_2Cl_2, - 78° - r.t., 30 min., 95%; vi: 0.2 N HCl EtOH/H_2O 9/1, refl., 20h, 80%; vii: H_2, 10 % Pd-C, AcOEt/MeOH 1/1 (84% ee); viii: TIPSi-Cl_2, imidazole, DMF, 67% (2 steps); ix: BOM-Cl, DBU, CH_3CN, r.t., 1h, 85%; x: $CH_3C_6H_4OC(S)Cl$, DMAP, Et_3N, CH_2Cl_2, r.t., 3h, 40°, 18h, 90%; xi: 1. Bu_3SnH, AIBN, DME, 80°, 3h; 2. Preparative HPLC on *Chiralcel OD*, 250 x 4.6 mm, hexane/isopropanol 95/5, 65% (100% ee); xii: TBAF, THF, r.t., 4h, 99%; xiii: 1. H_2, 10% Pd-C, r.t., 2h; 2. NaOMe, r.t., 20h, 88%.

purity of ~ 50%.[10b] **2** was then converted to the bicyclo[3.1.0]hexane derivative **3** by *Simmons-Smith* cyclopropanation; due to the directing effect of the allylic hydroxyl group,[11] **3** was obtained as a single diastereoisomer in 73% yield (18% of starting **2** recovered). Tosylation of **3** followed by displacement of the tosyloxy group with azide ion and subsequent reduction of the azide moiety via catalytic hydrogenation over Lindlar's catalyst[12] gave partially protected amino triol **4** in 68% overall yield. This compound was elaborated into the bicyclic *ribo-thymidine* analog **5** by reaction with the acyl-isocyanate derived from β-methoxy α-methacrylic acid, acid-catalyzed cyclization of the resulting acryloyl urea,[13] which was also accompanied by the cleavage of the 2',3'-acetonide, and finally removal of the O-5'-benzyl protecting group by catalytic hydrogenation over 10% Pd-C.

According to our synthetic strategy transformation of **5** into the desired *thymidine* analog **1** via radical deoxygenation at C-2' required selective thioacylation of the 2'-OH group with tolyl chlorothioformate.[14] To this end the 3'- and 5'-hydroxyl groups of **5** were selectively protected by means of the TIPSi protecting group,[15] which was followed by reversible blockage of N-3 of the base moiety by reaction with benzyl chloromethyl ether (BOM-Cl) in the presence of 1,8-diazabicyclo[5.4.0]undecene-7 (DBU) in 85% yield.[16] This approach then allowed for the straightforward conversion of the 2'-OH group to the desired thiocarbonate, which was obtained in 90% yield; in contrast, extensive N-acylation was observed upon reaction of TIPSi protected **5** with 1.05 equiv. of tolyl chlorothioformate without base protection.[17] Radical reduction of the thiocarbonate with Bu_3SnH in the presence of AIBN[14] furnished protected thymidine analog **6** (84% ee); optically pure **6** was obtained by preparative HPLC of this partially

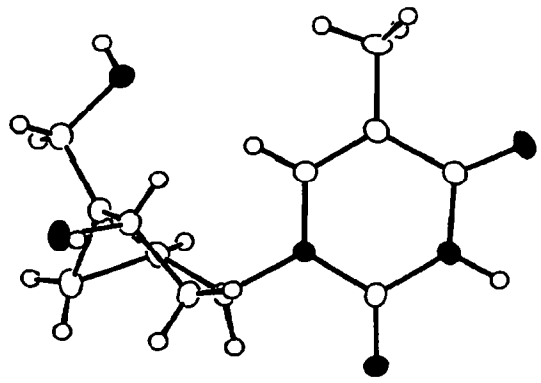


Figure 2: X-ray crystal structure of 4',6'-methano carbocyclic thymidine **1**.

racemic material on a *Chiralcel OD* column in 65% yield. Cleavage of the TIPSi protecting group with TBAF in THF followed by removal of the BOM group by catalytic hydrogenation over 10% Pd-C and subsequent treatment of the ensuing formaldehyde adduct at N-3 with NaOMe then gave enantiomerically pure **1** in 87% yield (based on **6**).[18]

Fig. 2 shows an ORTEP drawing of the X-ray crystal structure of **1**.[19] As predicted the bicyclic skeleton of the molecule adopts a boat-like conformation which is equivalent to a 2'-exo conformation of natural nucleosides. C-2' is deflected from the C-3' - C'4' - C-6' - C-1' plane by 0.44 Å, corresponding to a puckering amplitude of 27°.[2] The torsion angle about the C-4' - C-3' bond is 75° and thus very similar to the value of ~ 80° for the corresponding torsion angle δ in canonical A-type nucleic acid duplexes (*vide supra*).

So far the effect of **1** on DNA/RNA duplex stability has been investigated for two modified oligodeoxynucleotides each incorporating a single modified building block, i.e. 5'-TTT T1C TCT CTC TCT-3' (**A**) and 5'-TTT TTC TCT C1C TCT-3' (**B**).[20] The melting temperatures (T_m's) of the heteroduplexes of **A** and **B** with complementary RNA exceed the T_m of the unmodified wild-type duplex (52.3°) by 0.8° and 2.1°, respectively,[23] thus indicating that in both cases substitution of **1** for natural thymidine does indeed increase the thermodynamic stability of the DNA/RNA heteroduplex. These findings are in line with the general ideas that led to the design of conformationally constrained nucleoside analogs **I**, but additional experiments are required in order to determine the generality of the observed effects.

Acknowledgement

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19. The structure was solved by direct methods (SDP MULTAN 82). Full matrix least squares refinements with anisotropic temperature factors for all non-H-atoms converged at an R-factor of 0.069. All H-atoms could be located in the difference Fourier map. Inclusion of H-atoms in the least squares refinements improved the R-factor to 0.058.
20. For the purpose of oligonucleotide synthesis **1** was converted to the corresponding 5'-O-(4,4'-dimethoxytrityl) 3'-(2-cyanoethyl-N,N-diisopropylamino) phosphite **7**.[21a] Oligonucleotides were synthesized on an ABI 390 DNA synthesizer using standard phosphoramidite chemistry,[21b] except for the couplings of **7**, where reaction times of 10 (**A**) and 20 min (**B**) were employed. The 5'-DMTr-protected products were purified by RP-HPLC and according to capillary electrophoresis (CE) the fully deprotected oligonucleotides were at least 95% pure. Analysis of **A** and **B** by MALDI-TOF MS[22] gave the correct masses. It should be noted, however, that the coupling effiency of **7** was extremely low, which so far has effectively prevented the synthesis of oligonucleotides incorporating two or more bicyclic thymidine residues **1**.
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