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BICYCLO-OLIGONUCLEOTIDES WITH INVERTED CONFIGURATION AT C(5'): SYNTHESIS AND PROPERTIES

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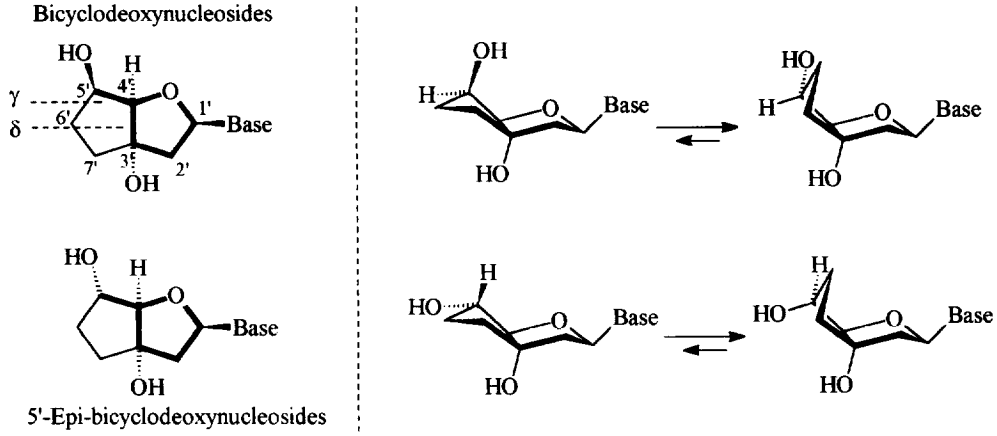
Abstract: 5'-Epi-bicyclothymidine and oligodeoxynucleotides thereof were prepared using solid phase phosphoramidite chemistry. UV-melting curves with complementary DNA and RNA furnish values of ΔT_m/mod. of -2.7° to -6.5°C compared to the natural reference system. The reduction of pairing affinity can directly be correlated with a change of the orientation of the C(4')-C(5') bond in the duplexes.

The design and synthesis of oligonucleotide analogs that form stable duplexes and triplexes with the natural nucleic acids, that are resistant to nucleases, and that readily cross cell membranes is in the focus of antisense and antigene research.[1-3] With this respect, a large number of oligonucleotides with modifications in the carbohydrate backbone were prepared and their biophysical and biological properties investigated.[4] In order to evaluate the potential of increasing pairing efficiency of an oligonucleotide analog to its complementary sequence by structural preorganization of its sugar-phosphate backbone, we recently synthesized a new type of nucleoside (bicyclodeoxynucleosides, *Figure 1*)[5] as well as oligonucleotides thereof (bicyclo-DNA).[6-9] These nucleosides differ from the natural 2'-deoxynucleosides by an additional ethylene bridge spanning the centers C(3') and C(5').

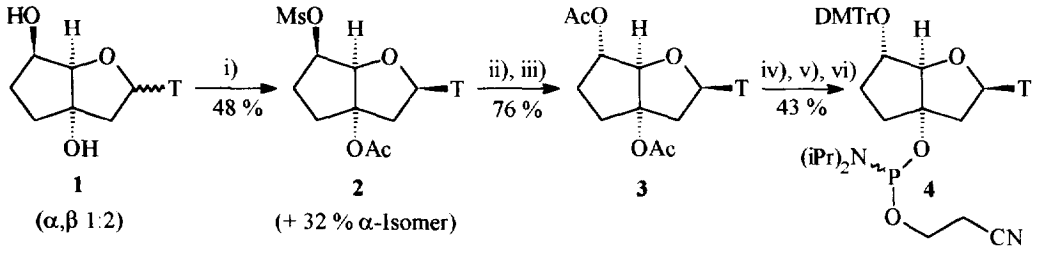
Figure 1



Due to the bicyclic nature of these nucleoside analogs, the bond C(4')-C(5'), being part of the carbocyclic ring, is restricted in its torsional freedom compared to the natural 2'-deoxyribonucleotides. Therefore the geometry of the corresponding backbone torsion angle γ can be controlled by choice of the configuration at the center C(5'), allowing for a specific analysis of the influence of this torsion angle to the complex formation properties of a corresponding oligonucleotide. While in natural DNA of the A- or B-conformation, torsion angle γ occurs uniformly in the synclinal (+*sc*) arrangement,[10] in the bicyclodeoxynucleosides it is uniformly restricted to the anticlinal range (+*ac*) with the hydroxyl group in the preferred pseudoequatorial position as revealed by NMR- and X-ray analysis. A change of configuration at C(5'), as in the 5'-epi-bicyclodeoxynucleosides, therefore would force torsion angle γ to the -*sc* (or -*ac*) conformational range, which is not observed in DNA and not accessible in the bicyclodeoxynucleosides. In this communication we report on the synthesis of the 5'-epi-bicyclothymidine as well as on the pairing properties of oligonucleotides thereof with complementary DNA and RNA.

The synthesis started from the α,β-mixture of bicyclothymidine α,β-**1** (*Scheme 1*), that was obtained as described previously.[5] Selective mesylation of the secondary hydroxyl group in the anomeric mixture α,β-**1** afforded **2** after chromatographic separation of the corresponding α-anomer. Acetylation of the tertiary alcohol **2** followed by inversion of configuration at C(5')[11] by S_N2 displacement of the sulfonate group with CsOAc, yielded the diacetate **3**[12] that was further converted to the dimethoxytrityl protected phosphoramidite **4** in analogy to conditions reported previously[7].

Scheme 1



i) 1.2 eq. MsCl, pyridine, 0°→r.t., 2h, 48% β + 32% α; ii) 3.2 eq. Ac_2O, 0.1 eq. DMAP, pyridine, 0°→r.t., 2h, 88%; iii) CsOAc, DMSO, 80°, 16h, 87%, iv) 0.2M NaOH, THF/MeOH/H_2O 5:4:1, 0°, 30 min., 86%; v) 4 eq. $DMTr^+CF_3SO_3^-$, pyridine, r.t., 8h, 64%; vi) $(iPr_2)NP(Cl)(OCH_2CH_2CN)$, $(iPr)_2NEt$, 0°→r.t., 30 min., 78%.

Structural analysis of the diacetate **3**[12] and the free 5'-epi-bicyclothymidine in solution reveals the same preferred conformation of the bicyclo-skeleton as in the corresponding bicyclodeoxynucleosides (*Figure 1*). This becomes obvious from the ^{1}H-NMR coupling pattern of the resonances of H-C(4') and H-C(5'), showing both very small or 0 Hz coupling constants. As a consequence, the secondary oxo-substituent at C(5') in the epi-series is preferentially axially oriented, thus restricting torsion angle γ to the -*sc*-range.[13]

The oligonucleotides **5-8** (*Table 1*) were prepared on a 1.3 μmol scale by automated solid-phase synthesis on a Pharmacia Gene Assembler Special®. For reasons of synthetic convenience, commercially available, deoxycytidine-loaded controlled pore glass (CPG) was used as the starting unit in the synthesis of **8**. The synthesis cycle only differed from that of natural DNA by a prolonged (14 min.) coupling time and a slightly

extended (60 s.) detritylation time. Average coupling yields (92%) were less efficient as in the corresponding bicyclodeoxynucleoside series.[7] Purification of the crude oligomers was performed by HPLC,[14] and the structural integrity of the isolated oligonucleotides **5-8** was subsequently analyzed by matrix-assisted laser-desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry.[15]

Complementary duplex formation[16] with $d(A_{10})$ and poly(rA) of the oligomers **5-8** was followed by UV-melting curves (10 mM NaH_2PO_4, 1M NaCl, pH 7.0). T_m-values and relative hyperchromicities obtained are listed in *Table 1*.

Table1

		T_m [°C] (Hyperchrom. %) with Complementary nucleotides		ΔT_m/mod	
		$d(A_{10})$	poly(rA)	$d(A_{10})$	poly(rA)
	$d(T_{10})$	31.2° (30%)	35.6° (37%)	---	---
5	5'-(T-T-T-T-T-**T**-T-T-T-T)-3'	26.3° (23%)	30.7° (16%)	-4.9°	-4.9°
6	5'-(T-T-T-**T**-T-**T**-T-T-T-T)-3'	25.8° (14%)	25.5° (19%)	-2.7°	-5.0°
7	5'-(T-T-T-T-**T**-**T**-T-T-T-T)-3'	20.2° (10%)	22.5° (12%)	-5.5°	-6.5°
8	5'-(**T**-**T**-**T**-**T**-**T**-**T**-**T**-**T**-**T**-C)-3'	---	---	---	---
9	5'-(**t**-T-**t**-T-**t**-T-**t**-T)-3'	32.1° (15%)	38.6 (38%)	+0.2°	+0.5°
10	5'-(**t**-**t**-**t**-**t**-**t**-**t**-**t**-**t**-**t**)-3'[7]	27° (27%)	34° (40%)	-0.4°	-0.2°
11	5'-(T-T-T-T-T-C-T-T-T-T)-3'	8.9° (16%)	19.5° (15%)	-22.3°	-16.1°

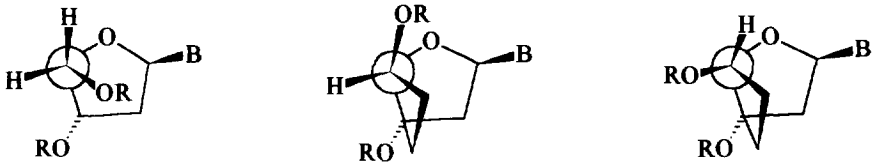
T = 5'-epi-bicyclothymidine; **t** = bicyclothymidine, T and C = natural 2'-deoxyribonucleosides. conc. (base-pair) = 39-45μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0,

Replacement of one thymidine residue by 5'-epi-bicyclothymidine in the center of a decathymidylic acid sequence (**5**) leads to a significant reduction of the pairing efficiency to its DNA or RNA complement compared to the unmodified reference sequence. As expected, substitution with two modified nucleosides as in sequences **6** and **7** further lowers DNA and RNA affinity, and the sequence **8**, consisting of a block of 5'-epi-bicyclothymidines, shows no pairing at all to its complement under the conditions used. In all cases **5-7**, however, the reduction in pairing energy is much less than in a mismatched duplex, where a natural deoxycytidine nucleoside appears opposite to the adenine base in the center of the duplex (sequence **11**).

ΔT_m per modification (ΔT_m/mod.) for sequence **7**, containing two contiguous modified nucleosides is about the same as in the sequence having only one nucleoside modification (**5**) but almost twice as much as in the case of sequence **6**, where the two modified nucleosides are spaced by a natural one. This indicates that strain in the duplex, built up by the structurally rigid 5'-epi-bicyclodeoxynucleotides, can be relieved in part by interrupts of the more flexible natural 2'-deoxynucleosides. The same was observed previously on the sequences **9** and **10**, containing bicyclothymidine (*Table 1*).[7]

The comparison of pairing efficiencies of sequences containing bicyclothymidine (**9, 10**) and 5'-epi-bicyclothymidine (**5-8**) underlines the importance of the geometry of the C(4')-C(5')-bond in complementary DNA-duplex formation. In the case of the homo-oligothymidylate sequences investigated here, a change of torsion angle γ from the normal +*sc* range to the +*ap* range (bicyclodeoxyoligonucleotides, *Figure 3*) leads to a moderate reduction in pairing energy. However, a further change to the -*sc* range (5'-epi-bicyclooligonucleotides, *Figure 3*) dramatically decreases the ability of DNA and RNA duplex formation.

Figure 3



A- and B-DNA
$\gamma = +sc$

Bicyclo-DNA
$\gamma = +ac$

5'-epi-bicyclo-DNA
$\gamma = -sc$

Understanding structure-activity relationships in complementary duplex formation, as in the case presented here, is of importance in the further design of DNA and RNA binding analogs.

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References and Notes

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11) Attempts to invert the configuration at C(5') in **1** directly by a *Mitsunobu* reaction (Mitsunobu, O. *Synthesis*, **1981**, 1-28) using a variety of carboxylic acids as nucleophiles were not successful in our hands.
12) $[\alpha]^{20°}_{D}$ = -32 (c = 0.5, MeOH). ^{1}H-NMR (300MHz, $CDCl_3$): 1.73-2.01 (*m*, 4H, H-C(6'), H-C(7'), H-C(2')); 1.87 (*d, J=1.0*, 3H, Me-C(5)); 2.03, 2.04 (2*s*, 6H, 2 OAc); 2.65-2.78 (*m*, 1H, H-C(6')/H-C(7')); 2.85 (*dd*, 1H, *J* = 5.3, 14.7, H-C(2')); 4.34 (*s*, 1H, H-C(4')); 5.01 (*s(b)*, 1H, H-C(5')); 6.12 (*dd*, 1H, *J* = 5.3, 9.5, H-(C1')); 7.10 (*s*, 1H, H-C(6)); 10.08 (*bs*, 1H, NH). ^{13}C-NMR (75MHz, $CDCl_3$, DEPT): 12.44 (*q*, CH_3-C(5)); 20.96, 21.52 (2*q*, $COCH_3$); 30.02, 35.89 (2*t*, C(6'), C(7')); 42.33 (*t*, C(2')), 76.48 (*d*, C(5')); 85.28 (*d*, C(1')); 91.05 (*d*, C(4')), 92.27 (*s*, C(3')); 111.40 (*s*, C(5)); 134.85 (*d*, C(6)); 150.52 (*s*, C(2)); 164.01 (*s*, C(4)); 170.13, 170.18 (2*s*, $COCH_3$). EI-MS: 352 (M^+).
13) Molecular mechanics calculations on **3** (MacroModel V4.5, Amber force field, H_2O) resulted in an energetic advantage of the conformer with the axial oxo-substituent over that with equatorial orientation (*Figure 1*) by 2.28 kcal/mol.
14) Oligomers **5-8** were purified by ion-exchange HPLC: Nucleogen DEAE 60-7, 125x4.0 mm, *Macherey & Nagel*; A: 20 mM KH_2PO_4 pH 6.0 in MeCN/H_2O 1:4, B: A + 1M KCl, flow 0.8 ml/min; t_R, **5**: 20 min. (20-60%B in 30 min.), **6**: 21 min. (20-60%B in 30 min.), **7**: 16 min. (20-60%B in 30 min.), **8**: 22 min. (50%B isocr.).
15) m/z (monoanion, H^+-form): **5**: calc. 3006.07, found 3005.4; **6**: calc. 3032.10, found: 3029.1, **7**: calc. 3032.10, found 3031.9, **8**: calc. 3530.53, found 3532.0: matrix conditons as described: Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191-3196.
16) Because of the non-existence of melting transitions at 284 nm we can exclude *Hoogsteen* triplex formation in all systems described under the chosen conditions.

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