Bicyclo[3.2.1]-DNA, a new DNA analog with a rigid backbone and flexibly linked bases: pairing properties with complementary DNA

Carl Epple and Christian Leumann

Background: The structural and conformational variety in nucleic acid complexes is largely controlled by the sugar–phosphate backbone. In order to modulate specific features such as strength or selectivity of complex formation by designing nucleotide analogs, a deeper understanding of the relationship between mononucleotide structures and the properties of their oligomers is necessary. One approach involves comparing the properties of DNA analogs displaying well defined modifications in their backbone structure with those of natural DNA and RNA.

Results: We have designed and synthesized a new DNA analog, 'bicyclo[3.2.1]-DNA', which has a rigid phosphodiester backbone that emulates a B-DNA-type conformation, to which the nucleobases are attached via a flexible open-chain linker. A UV-melting curve analysis shows that bicyclo[3.2.1]-DNA forms stable duplexes with complementary DNA, although generally with lower Tm values than pure DNA duplexes. Duplex formation is strictly constrained to antiparallel complementary sequences, and base-mismatch discrimination is slightly enhanced compared to pure DNA duplexes. In addition, bicyclo[3.2.1]-DNA sequences are resistant to a 3′-exonuclease.

Conclusions: The furanose unit present in natural nucleosides is not necessary for a competent and stable phosphodiester-based pairing system, provided that the backbone is conformationally constrained. The information for the preference of antiparallel strand association in B-DNA is not merely a consequence of bases being attached to a specific side of the furanose unit, but is also encoded in the backbone itself. Furthermore, conformational flexibility in the base-pairing region does not lead to a loss of mismatch in base-pair formation.

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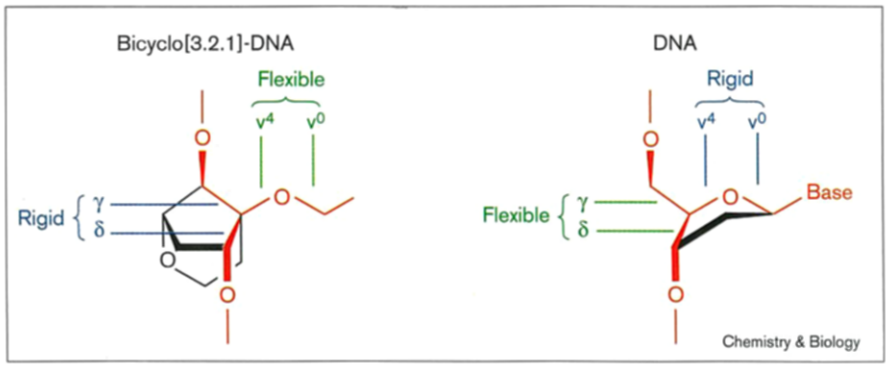
Introduction

Conformational restriction of the sugar–phosphate backbone in synthetic DNA analogs aims at increasing complex stability in complexes with complementary natural single-stranded RNA or double-stranded DNA. This area of research has recently attracted much attention in the context of antisense and antigene research [1–3]. Besides this, oligonucleotide analogs with defined structural alterations in their repeating backbone unit might serve as tools for understanding the supramolecular association and folding of nucleic acids in detail, and thus might lead to a more general correlation of mononucleotide structures with their association behavior at the oligomeric level. Ultimately, this might facilitate design of artificial pairing systems with predefined properties with potential applications not only in biology and medicine, but also in materials science and computer technology [4].

In extending the concept of conformational preorganization of oligonucleotide single strands (for a recent review see [3]) we designed and synthesized the new DNA analog, bicyclo[3.2.1]-DNA (Figure 1). The artificial bicyclic sugar surrogate in the underlying nucleosides of bicyclo[3.2.1]-DNA orients and fixes the backbone torsion angles γ and δ in the range of those observed in B-type DNA duplexes. The bases are attached to the backbone via a flexible methyleneoxy linker. Bicyclo [3.2.1]-DNA therefore constitutes a DNA analog with a backbone expected to emulate a B-form double helix and a base-pairing region that is flexible and no longer part of a ring system, and thus differs conceptually from the known DNA-like pairing systems such as bicyclo-DNA [5,6] and hexitol-DNA (HNA) [7,8]. These latter two systems have rigid backbones and base attachments or, in the case of glycerol-derived nucleic acid analogs [9], have a conformationally flexible backbone and base linkages (Figure 2).

The design of bicyclo[3.2.1]-DNA was originally inspired by observations made during the structural analysis of

Figure 1

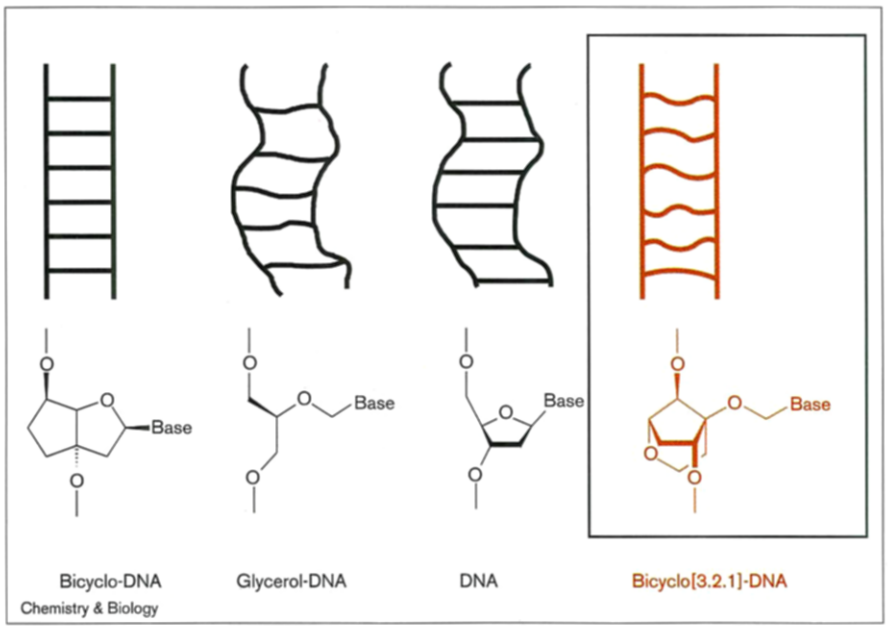


Comparison of the structure of bicyclo[3.2.1]-nucleosides with natural 2′-deoxynucleosides. The relevant backbone torsion angles (γ, δ) that are conformationally fixed in bicyclo[3.2.1]-DNA and relatively flexible in DNA, as well as the relevant (endocyclic) torsion angles (ν^4, ν^0) that are flexible in bicyclo[3.2.1]-DNA and relatively rigid in DNA, are highlighted. The red parts of the structure are common to both nucleic acid types.

homo-DNA, an oligonucleotide analog built from 2′,3′-dideoxyglucopyranosyl nucleosides [10]. From interpretation of a combined multinuclear two-dimensional nuclear magnetic resonance (NMR) structural analysis and molecular dynamics simulation of a homo-DNA duplex, we hypothesized that the high thermodynamic stability observed in homo-DNA duplexes might arise in part from an entropic duplex stabilization due to enhanced dynamic mobility of the base-pairs in the duplex (partially molten structure in the center of the duplex) [11]. In bicyclo[3.2.1]-DNA, backbone rigidity and base-pair flexibility are structurally disentangled, which might allow a specific assessment of the energetic impact of the enhanced dynamic mobility in the base-pairing region. Apart from that, we expected that comparing the selectivity in duplex formation of bicyclo[3.2.1]-DNA (parallel versus antiparallel strand alignment and base-mismatch discrimination) would increase our understanding of the structural role of the furanose ring in DNA and RNA.

Although it is known that the completely flexible, glycerol-based DNA analogs fail to form stable complexes with natural nucleic acids [9,12,13], we report here that bicyclo[3.2.1]-DNA pyrimidine sequences, 10–20 nucleotides in length, are able to form duplexes with complementary DNA. Furthermore, duplex formation is strongly restricted to the antiparallel mode, and a differential (but not absolute) increase in stability with increasing duplex length, compared to pure DNA duplexes, is observed. In addition to this, base-mismatch discrimination in bicyclo[3.2.1]-DNA–DNA duplexes is enhanced relative to DNA–DNA duplexes, surprisingly

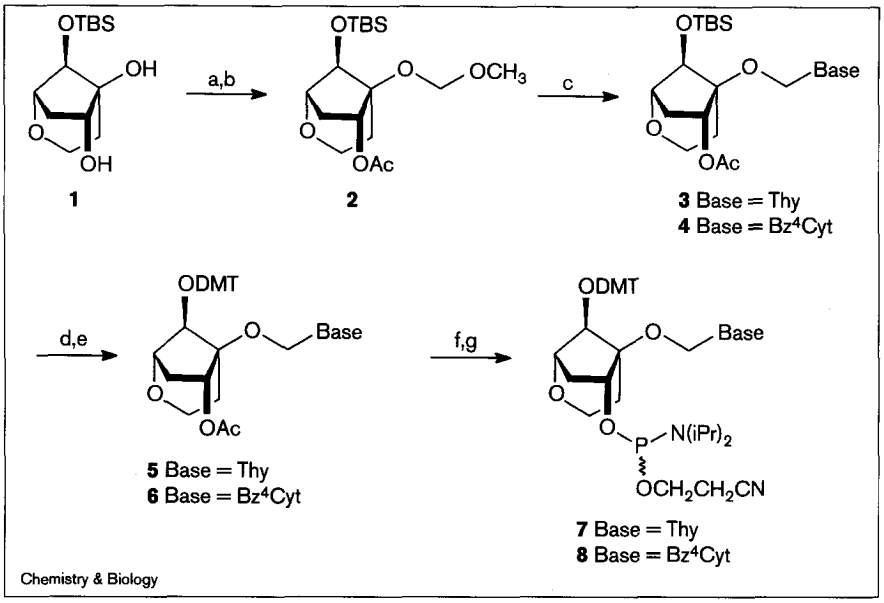
Figure 2



Schematic representation of the relative rigidity or flexibility of backbones and base-pairing regions within duplex structures of DNA and selected analogs. Wavy lines indicate relative structural flexibility and straight lines indicate relative rigidity. The relative flexibility of the DNA backbone is manifest in the occurrence of the different conformational families A-, B-, and Z-DNA.

Figure 3

Synthesis of the bicyclo[3.2.1]-DNA building blocks containing cytosine and thymine bases. **(a)** Ac_2O (20 eq.), pyridine, r.t., 92%; **(b)** NaI (5 eq.), MOM-Cl (5 eq.), $(iPr)_2NEt$ (7 eq.), DME, 90°C, 93%; **(c)** thymine or N^4Bz-cytosine (3 eq.), BSA (6 eq.), TBDMS-triflate (2 eq.), MeCN, 60°C, 91% (**3**), 77% (**4**); **(d)** TBAF (2–3 eq.), THF, r.t.; **(e)** DMT-triflate (3 eq.), pyridine, 60°C, 70% (**5**), 67% (**6**) (yields over two steps); **(f)** NaOH (10 eq.) in THF/MeOH/H_2O 5:4:1, 4°C→ r.t.; **(g)** $(iPr)_2NP(Cl)OCH_2CH_2CN$ (2 eq.), $(iPr)_2NEt$ (4 eq.), THF, r.t., 71% (**7**), 75% (**8**) (yields over two steps).



characterizing the bicyclo[3.2.1]-DNA as a more selective pairing partner.

Results and discussion

Synthesis of bicyclo[3.2.1]-oligonucleotides

The preparation of building blocks **7** and **8** (Figure 3) for use in the automated solid-phase phosphoramidite chemistry for oligonucleotide synthesis started with the common bicyclic sugar surrogate **1** prepared in 10 steps from D-arabinose [14]. Selective acetylation of the secondary hydroxyl group in **1** followed by introduction of the methoxy methyl (MOM) group into the tertiary hydroxyl function yielded **2**, which, under Vorbrüggen conditions [15] using the *in situ* persilylated (protected) bases, could be converted into the nucleoside derivatives **3** and **4**. Selective fluoride-induced removal of the silyl group followed by tritylation gave **5** and **6**, which were then converted into the phosphoramidite building blocks **7** and **8** by standard ester hydrolysis followed by phosphitylation.

Oligonucleotides were synthesized on a commercial DNA synthesizer on a 1.3 µmol scale starting with commercially available natural thymidine-containing solid-support material and using an appropriately modified natural DNA synthesis protocol (see the Materials and methods section). Coupling efficiencies of > 98% were obtained, and these readily allowed for the synthesis of oligomers as long as 20 nucleotides. With this method we prepared the sequences **9–16** depicted in Table 1. All sequences containing bicyclo[3.2.1]-nucleosides were analyzed using matrix-assisted laser-desorption/ionization time of flight (MALDI–TOF) mass spectrometry. As an example, Figure 4 shows the spectrum of the eicosamer **15**. The analysis of duplex formation between oligonucleotide

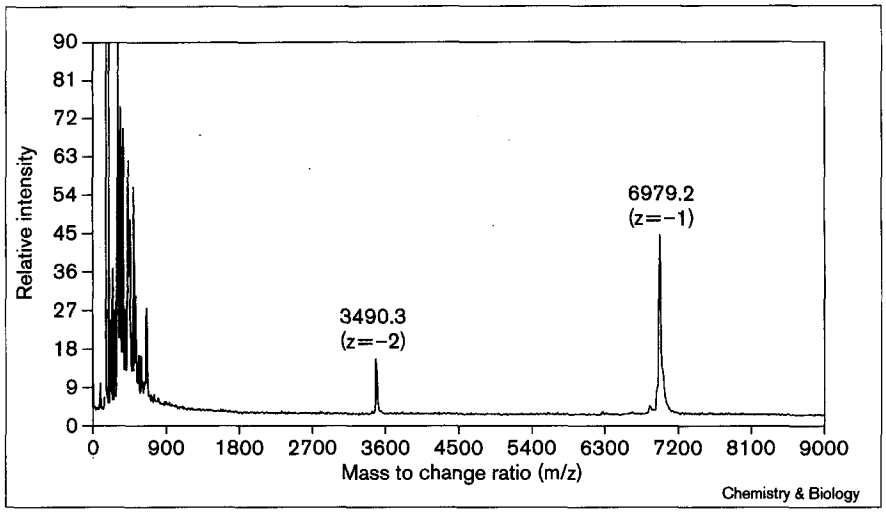
Table 1

Melting temperature of sequences 9–16 with complementary DNA.

	Tm (DNA complement)	ΔTm/mod	MALDI–TOF(calc'd)	MS [M−−H]− (found)
9 5′-(TTTTT t TTTT)	25.5°C	−7.5	3035.1	3033.0
10 5′-(TTT t t t t TTT)	13.3°C	−4.9	3203.3	3201.6
11 5′-(t t t t t t t t t T)	5°C	−3.1	3483.5	3481.1
12 $d(T_{10})$	33°C	0		
13 5′-(t t t t t c t c t c t c t c T)	37.6°C	−1.3	5209.8	5211.6
14 d(TTTTTCTCTCTCTCT)	54.9°C	0		
15 5′-(t c t t c c c t c t c t t t t t c t t T)	48.1°C (~15°C)	−0.7	6981.1	6979.2
16 d(TCTTCCCTCTCTTTTTCTTT)	61.9°C (24.6°C)	0		

T, thymidine; C, 2′-deoxycytidine; t, bicyclo[3.2.1]-thymidine; c, bicyclo[3.2.1]-cytidine. Tm data in parenthesis refer to melting of the corresponding sequence with its parallel-oriented DNA complement. Data were taken from from UV-melting curves (λ = 260 nm in 10 mM Na-cacodylate, pH 7.0, 1M NaCl; oligonucleotide conc.: 4 µM (decamers), 2 µM (all others)), and MALDI–TOF mass spectral data of modified sequences.

Figure 4



MALDI–TOF mass spectrum of the bicyclo[3.2.1]-DNA eicosamer **15**.

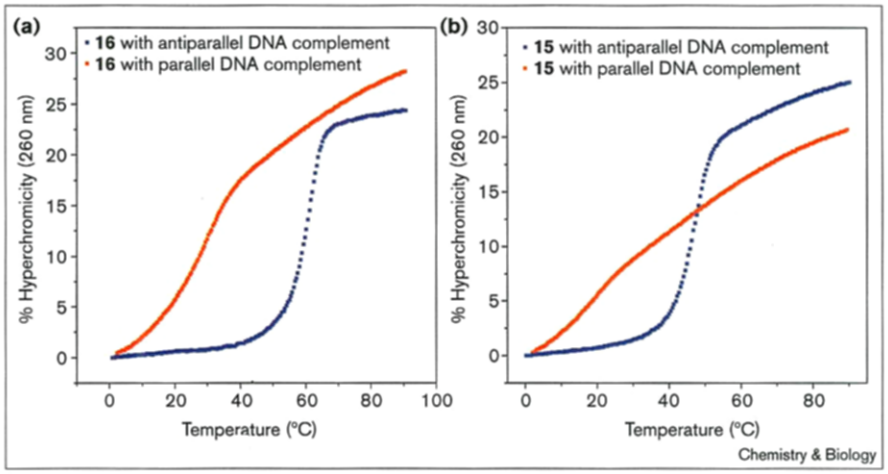
sequences was performed using UV-melting curve analysis (1M NaCl, pH 7.0). The corresponding melting temperatures (Tm) are listed in Table 1. UV-melting curves of the eicosamers **15** and **16** with their parallel and antiparallel oriented DNA complements are depicted in Figure 5.

Pairing properties of bicyclo[3.2.1]-oligonucleotides

In a first series of oligonucleotides, we focused on the pairing properties of the homobasic decamer sequences **9–11**, containing one, four or nine bicyclo[3.2.1]-T residues with their DNA complement d(A_{10}). By comparing the Tm data (Table 1) it becomes obvious that incorporation of one bicyclic T residue (sequence **9**) leads to a decrease of Tm by about 7.5°C relative to its natural equivalent. Consequently, incorporation of four consecutive residues, as in **10**, leads to a reduction in Tm of ~20°C relative to that of the natural duplex d(A_{10})•d(T_{10}). Interestingly enough, the decamer sequence **11**, which, with the exception of the 3′-terminal nucleotide unit, can be regarded as fully modified, still forms a duplex with d(A_{10}) with a Tm of 5°C. From these experiments we can conclude that although the furanose ring is missing in this DNA analog, complementary base-pairing on the level of homo-T decamers is maintained, albeit on an overall lower affinity level than natural DNA. Relative destabilization, with respect to the natural control sequences, is strongly dependent upon the number of modifications and sharply decreases with increasing number of modifications.

In order to further explore base-pairing selectivity and affinity of bicyclo[3.2.1]-DNA we prepared the asymmetric, cytosine- and thymine-containing pyrimidine pentadecamer **13** and eicosamer **15**. Both sequences contain only one natural thymidine unit at the 3′-end of the sequence

Figure 5



Melting curves of equimolar mixtures of **(a)** the natural DNA eicosamer **16** and **(b)** the corresponding bicyclo[3.2.1]-DNA sequence **15** with their antiparallel-oriented (blue) and parallel-oriented (red) DNA complements. Total oligonucleotide concentration was 2 μM in 10 mM sodium cacodylate, 1M NaCl, pH 7.0.

Table 2

Thermodynamic measurements of duplex formation of oligonucleotides 13–16 with their antiparallel DNA complement from UV-melting curves.

	ΔH (kcal mol^{-1})	ΔS (cal K^{-1} mol^{-1})	$\Delta G^{25°C}$ (kcal mol^{-1})	ΔH (calc'd [17]) (kcal mol^{-1})
13	−54.2 ± 0.4	−149.4 ± 1.3	−9.7 ± 0.8	
14	−101.1 ± 0.4	−281.6 ± 1.3	−17.1 ± 0.7	−103.4
15	−94.5 ± 0.8	−268.0 ± 2.4	−14.7 ± 1.6	
16	−150.7 ± 1.2	−420.9 ± 3.5	−25.2 ± 1.8	−152.7

Buffer conditions were as described in Table 1.

and can therefore largely be considered 'fully modified' because terminal nucleoside residues are known to contribute only marginally to duplex stability (as a result of end fraying). Both sequences **13** and **15** efficiently form duplexes with their natural antiparallel DNA complement (Table 1, Figure 5). Interestingly, we note again that the stability of the hybrid duplexes relative to the native DNA duplexes increases with increasing oligonucleotide length, even though the relative ratio of A–T and G–C base-pairs in the duplex is constant for both the 15-mer and 20-mer sequence. Although the average change in Tm per modified residue (ΔTm/mod) in the case of the pentadecamer **13** is −1.3°C, it amounts only to −0.7°C for the eicosamer-**15**-containing duplex, the latter thus approaching the stability of the natural DNA duplex.

Thermodynamics of duplex formation

We have calculated the thermodynamics of duplex formation in the antiparallel pentadecamer and eicosamer series by curve fitting to the experimental melting curves in analogy to previous results [16]. The data obtained are summarized in Table 2. From the calculated free energies of duplex formation ($\Delta G^{25°C}$), we conclude that the hybrid duplexes are less stable than the pure DNA duplexes. Thus, as expected, the Tm values reflect the thermodynamic stability of the complexes. The duplex formation enthalpies (ΔH) are less favorable for the hybrid systems than for pure DNA. The lower enthalpic affinity, however, is in part compensated by a more favorable entropy term in the hybrid systems. The enthalpies measured for the two natural duplexes correlate well with those calculated using tabulated nearest neighbor interaction data [17], thus validating the trends observed. Caution must be applied in interpreting the data in detail however, because with the given duplex lengths (15- and 20-mers), true two-state melting and association behavior is not warranted.

Bicyclo[3.2.1]-DNA prefers the antiparallel strand alignment

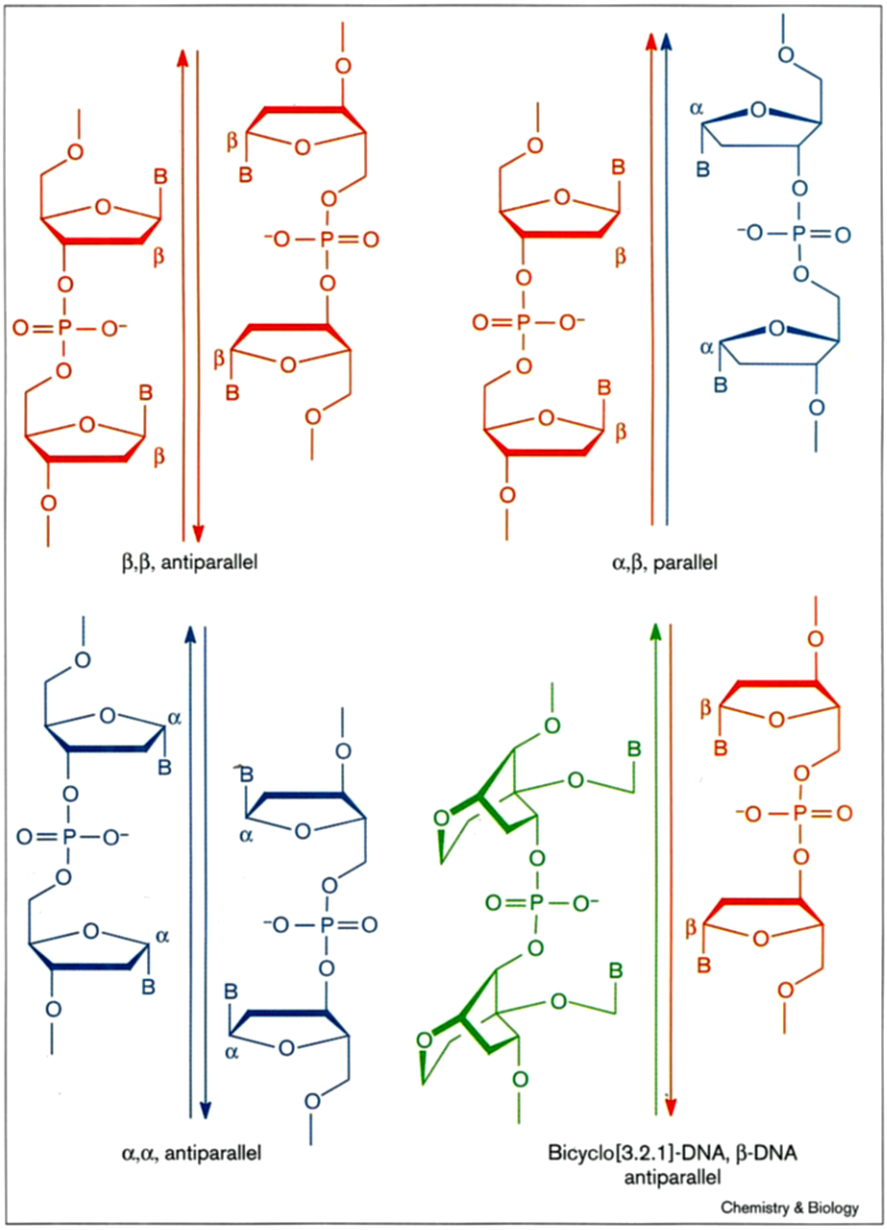
Although there is no obvious restriction on the orientation of the bases in bicyclo[3.2.1]-DNA, the eicosamer **15** strongly prefers its antiparallel DNA complement over the parallel one for duplex formation, and thus behaves as its natural equivalent **16** (Figure 5). Tm values of 48.1°C in the case of antiparallel complementary strands and ~15°C for the parallel complementary strands were found for the hybrid system, whereas 61.9° and 24.6°C, respectively, were found in the natural DNA duplex. We anticipate that melting of the stoichiometric mixtures of parallel complementary strands in both the hybrid and the pure DNA system are not due to the melting of parallel duplexes, but are a result of partially antiparallel paired duplex structures showing up to nine continuous matched base-pairs and 5′-overhanging ends.

The strong preference for antiparallel strand alignment in bicyclo[3.2.1]-DNA–DNA duplexes is not a feature observed in the peptide nucleic acid analog PNA, the only other DNA analog with open chain-linked bases for which this was experimentally investigated. PNA is known to form Watson–Crick duplexes with DNA and RNA in both the parallel and antiparallel orientations [18]. The observed selectivity of bicyclo[3.2.1]-DNA becomes even more interesting if one considers the results obtained with α-DNA, built from α-anomeric deoxyribonucleosides. It is known [19] that the strands in Watson–Crick duplexes of α-DNA are oriented in an antiparallel fashion, as in natural β-DNA, whereas α-DNA–β-DNA hybrid duplexes show parallel aligned strands (Figure 6) [20]. Bicyclo[3.2.1]-DNA, in which both the α- and β-like arrangement of the bases is possible, still strongly prefers antiparallel strand alignment. These data suggest that strand orientation in nucleic-acid duplexes is not only a consequence of a fixed relative orientation of the nucleobase, but also conforms to — and more importantly can be remotely controlled by — the sugar–phosphate backbone. This interpretation, however, needs further support, which should come from a structural analysis of a bicyclo[3.2.1]-DNA–DNA duplex and by complementary base-pairing experiments in the pure unnatural system.

Bicyclo[3.2.1]-DNA discriminates mismatched bases

One of the obvious open questions was whether bicyclo-[3.2.1]-DNA, with its flexible base-pairing region, is able to discriminate base mismatches in the same way as natural DNA. We therefore investigated base-mismatch formation with the 15-mer duplexes indicated in Table 3.

Figure 6



Preferred strand orientations in duplexes containing α-DNA (blue), β-DNA (red) and bicyclo[3.2.1]-DNA (green) strands.

Interestingly, mismatch discrimination is enhanced in all cases in the hybrid duplexes compared to the DNA duplexes, as can be seen from the corresponding ΔTm values (Table 3). Remarkably, the duplex destabilization caused by a C–X base arrangement is more homogeneous with respect to the nature of X. This becomes obvious by comparing the C–T mismatch that lies within the stability of the other mismatches in the hybrid duplex system, but which seems to occupy a energetically privileged position in the pure DNA duplex. This striking base–base interaction selectivity in the hybrid duplexes still awaits a chemical rationalization and definitely needs to be scrutinized by further investigations in both the bicyclo[3.2.1]-DNA–DNA hybrid system as well as in the pure bicyclo system.

Circular dichroism spectra of bicyclo[3.2.1] hybrid-duplexes

In order to evaluate the structure of a bicyclo[3.2.1]-DNA–DNA hybrid we recorded circular dichroism (CD) spectra of the eicosamer duplexes (Figure 7). Comparison of the CD spectra of the natural and the hybrid duplexes reveals in both cases a negative cotton effect, with a minimum around 245 nm, and positive cotton effects with maximum ellipticity around 275 nm in the DNA duplex and 280 nm in the hybrid duplex. This is fully consistent

Table 3

ΔTm values between matched and mismatched duplexes of 13 and 14 with their antiparallel DNA complement.

	d(AGAGAGAXAGAAAAA)			
	X = G	X = T	X = C	X = A
13 5′-(ttttt**c**t**c**t**c**t**c**T)	0°C	−17.4°C	−18.2°C	−19.2°C
14 d(TTTTT**C**T**C**TCTCTCT)	0°C	−12.9°C	−17.4°C	−16.0°C

Experimental conditions were as described in Table 1.

with the hybrid duplex adopting a Watson–Crick base-paired, right-handed double helix. The relative intensities of the negative and positive bands, however, are different with a ratio of ~1:1 for the DNA duplex and ~1:2 for the hybrid duplex. The spectrum of the pure DNA duplex thus complies fully with a B-DNA structure, whereas the hybrid duplex shows elements of an A-form double helix. This is of interest because the overall shape of an A-form helix in this case cannot have its origin directly in the sugar–phosphate backbone (the torsion angle δ cannot adopt values observed in A-DNA), but must be due to the base–linker element, allowing for an A-DNA-like stacking pattern of base pairs.

Resistance against nucleolytic degradation

Not unexpectedly, bicyclo[3.2.1]-DNA seems to be resistant to the action of 3′-exonucleases. Incubation of the pentadecamer **13** with a cocktail of snake venom phosphodiesterase and alkaline phosphatase led, after 6 h of digestion at 37°C, to only one single oligomeric product (determined by high-performance liquid chromatography, HPLC, control) the molecular mass of which (determined using MALDI-TOF MS) is in accord with a tetradecamer structure arising from removal of the 3′-terminal natural thymidine nucleotide unit in **13**. Thus the internucleosidic linkages between bicyclo[3.2.1]-nucleoside residues were not attacked by the phosphodiesterase.

Significance

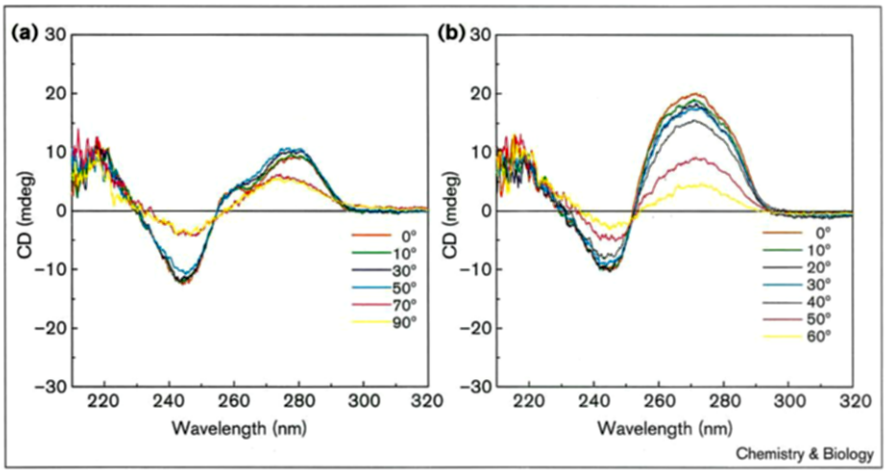
The DNA analog presented here is a promising candidate for obtaining fundamental insights into the structural factors controlling the supramolecular association of DNA. An important issue in this respect is the finding that a backbone structure which is geometrically preorganized in the B-form still leads to antiparallel strand alignment in the duplex, even though the nucleobases, as the central recognition units, are linked in a flexible way to the backbone. This is different from what is known of the peptide nucleic acid analog PNA and suggests that preference for antiparallelism in DNA duplexes goes along with an intrinsic propensity of its backbone for this arrangement. Another remarkable property of bicyclo[3.2.1]-DNA, which needs to be addressed more specifically in the future, is its slightly enhanced selectivity in base-pair formation in spite of the higher structural dynamics in the base-pairing region.

An intriguing problem in the supramolecular chemistry of DNA is the structural rationalization of the subtle, compensatory enthalpy–entropy equilibria responsible for the nonlinearity of thermic duplex stability as a function of duplex length. The observation that bicyclo[3.2.1]-DNA–DNA duplexes become differentially more stable with increasing duplex length, compared to pure DNA, poses the interesting question of whether there exists a sequence length above which bicyclo[3.2.1]-DNA–DNA duplexes are more stable than DNA–DNA duplexes. In this context, the evaluation of purine- and mixed-base-containing bicyclo[3.2.1]-DNA sequences will be of interest.

Because of its enhanced selectivity in DNA binding by similar binding efficiency with oligonucleotides 15–20 bases in length, and because of its high resistance to the action of nucleases, bicyclo[3.2.1]-DNA is also an

Figure 7

CD spectra of duplexes of **(a) 16** and **(b) 15** with the corresponding antiparallel DNA complement at the temperatures indicated. Experimental conditions were as described in Figure 5.



interesting candidate for potential antisense and antigene applications.

Materials and methods

Synthesis of oligomers

Full experimental details for the synthesis of the building blocks and intermediates will be given elsewhere (C.E., C. Roberts, and C.L., unpublished observations). All oligo-bicyclodeoxynucleotides were prepared on solid phase on a DNA synthesizer (Pharmacia LKB Gene Assembler Special) using phosphoramidite methodology. The syntheses were performed on a 1.3 µmol scale in the 'trityl-off' mode. As the starter unit commercially available thymidine-containing CPG (loading capacity: 32–45 µmol/g) was used. Chain assembly was effected using the standard DNA synthesis cycle with the following modifications: use of 10% trichloroacetic acid instead of 3% dichloroacetic acid for detritylation (no change in detritylation time); prolonging the coupling time from 1.5 to 6–10 min. All other parameters were left unchanged. Coupling yields, according to trityl assay, of > 98% were obtained. After standard removal of protecting groups and detachment from the solid support (25% NH_3, r.t, 10 h for oligo-bicyclo[3.2.1]-thymidine sequences; 55°C, 12–16 h for all other sequences), oligomers were purified to homogeneity using DEAE and/or ion-exchange HPLC as described [5], and their sequence integrity was analyzed using MALDI–TOF mass spectrometry [21]. All experimentally determined molecular masses $(M-H)^-$, (Table 1) were found to be within 1.5‰ of the calculated mass.

UV-melting curves

A *Varian Cary 3E* UV/VIS-spectrometer equipped with a temperature controller unit and connected to a *Compaq ProLinea 3/25 zs* personal computer was used. A standard buffer (10 mM Na-cacodylate, 1M NaCl, pH 7.0) was used for all experiments. Temperature gradients of 0.5°/min. were applied and data points were collected in intervals of ~0.3°. %Hyperchrom. (wavelength) = $100 \times ((D(T)-D_o)/D_o)$; D(T) = absorption at temperature T; D_o = lowest absorption in the temperature interval. The transition temperature Tm was determined as described previously [22]. Extinction coefficients of modified oligonucleotides were estimated to be identical to their natural equivalents. Extinction coefficients for natural DNA-sequences were determined as described previously [23].

Thermodynamic data

Thermodynamic data were measured using standard buffer conditions (1M NaCl, 10 mM Na-cacodylate, pH 7.0) by fitting a simulated curve to the experimental UV-melting curve. The simulated curves are based on a bimolecular interacting system obeying the two-state model [24] and were obtained using our fitting function 'alpha' which is built in analogy to that described previously [16]. The fitting process was performed with the program *Origin V 2.9* (Microcal Inc.).

CD spectroscopy

CD spectra were recorded on a *Jasco J-500A* spectropolarimeter with *IF-500 II Interface* connected to an *PC/AT* personal computer. The cell was thermostated by a *Julabo F20* circulating bath. Temperatures were determined directly in the sample solution.

*Enzyme digestion of **13***

In 1 ml digestion buffer (0.15 M NaCl, 10 mM Tris–HCl, pH 7.0) 0.7 $O.D.^{260\ nm}$ of **13** were treated with 6U of alkaline phosphatase (Böhringer Mannheim) and 6mU snake venom phosphodiesterase (*Crotalus durissus*, Böhringer Mannheim). The mixture was incubated at 37°C and left until no more increase in UV absorbance at 260 nm was detectable (6 h).

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References

1. Hunziker, J. & Leumann, C. (1995). Nucleic acid analogues: synthesis and properties. In *Modern Synthetic Methods*. (Ernst, B., Leumann, C. eds.), pp. 331-417, Verlag Helvetica Chimica Acta, Basel.
2. Herdewijn, P. (1996). Targeting RNA with conformationally restricted oligonucleotides. *Liebigs Ann.* 1337-1348.
3. Kool, E.T. (1997). Preorganization of DNA: design principles for improving nucleic acid recognition by synthetic oligonucleotides. *Chem. Rev.* **97,** 1473-1487.
4. Niemeyer, C.M. (1997). DNA as a material for nanotechnology. *Angew. Chem. Int. Ed. Engl.* **36,** 585-587.
5. Bolli, M., Litten, C., Schütz, R. & Leumann, C. (1996). Bicyclo-DNA: a Hoogsteen-selective pairing system. *Chem. Biol.* **3,** 197-206.
6. Bolli, M., Trafelet, H.U. & Leumann, C. (1996). Watson–Crick base-pairing properties of bicyclo-DNA. *Nucleic Acids Res.* **24,** 4660-4667.
7. Van Aerschot, A., Verheggen, I., Hendrix, C. & Herdewijn, P. (1995). 1,5-Anhydrohexitol nucleic acids, a new promising antisense construct. *Angew. Chem. Int. Ed. Engl.* **34,** 1338-1339.
8. Hendrix, C., Rosemeyer, H., Verheggen, I., Seela, F., Van Aerschot, A. & Herdewijn, P. (1997). 1,5,-Anhydrohexitol oligonucleotides: synthesis, base-pairing and recognition by regular oligodeoxyribonucleotides and oligoribonucleotides. *Chem. Eur. J.* **3,** 110-120.
9. Schneider, K.C. & Benner, S.A. (1990). Oligonucleotides containing flexible nucleoside analogues. *J. Am. Chem. Soc.* **112,** 453-455.
10. Hunziker, J., *et al.*, & Eschenmoser, A. (1993). Why pentose and not hexose nucleic acids?, part III, oligo(2',3'-dideoxy-β D-glucopyranosyl) nucleotides (homo-DNA): base-pairing properties. *Helv. Chim. Acta* **76,** 259-352.
11. Otting, G., Billeter, M., Wüthrich, K., Roth, H.-J., Leumann, C. & Eschenmoser, A. (1993). Why pentose and not hexose nucleic acids? Part IV, homo-DNA: ^1H-,^{13}C-,^{31}P- and ^{15}N NMR spectroscopic investigation of ddGlc (A-A-A-A-A-T-T-T-T-T) in aqueous solution. *Helv. Chim. Acta* **76,** 2701-2756.
12. Nielsen, P., Kirpekar, F. & Wengel, J. (1994). Incorporation of (R)- and (S)-3',4'-seco-thymidine into oligodeoxynucleotides: hybridization properties and enzymatic stability. *Nucleic Acids Res.* **22,** 703-710.
13. Peng, L. & Roth, H.-J. (1997). Synthesis and properties of 2'-deoxy-1',2'-seco-D-ribosyl(5'→3')oligonucleotides (=1',2'-Seco-DNA) containing adenine and thymine. *Helv. Chim. Acta* **80,** 1494-1512.
14. Egger, A., Hunziker, J., Rihs, G. & Leumann, C. (1998). Synthesis of bridgehead-functionalized bicyclo[3.2.1]octanes via titanium and tributyltinhydride induced pinakol coupling, synthesis of conformationally rigid glycerol substitutes. *Helv. Chim. Acta* **81,** in press.
15. Vorbrüggen, H. & Bennua, B. (1981). A new simplified nucleoside synthesis. *Chem. Ber.* **114,** 1279-1286.
16. Petersheim, M. & Turner, D. (1983). Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGUp and ACCGGUp. *Biochemistry* **22,** 256-263.
17. Breslauer, K.J., Frank, R., Blöcker, H. & Marky, L.A. (1986). Predicting DNA duplex stability from the base sequence. *Proc. Natl Acad. Sci. USA* **83,** 3746-3750.
18. Egholm, M., *et al.*, & Nielsen, P.E. (1993). PNA hybridizes to complementary oligonucleotides obeying the Watson–Crick hydrogen bonding rules. *Nature* **365,** 566-568.
19. Morvan, F., Rayner, B., Imbach, J.-L., Chang, D.-K. & Lown, J.W. (1987). α-DNA III: Characterization by high field ^1H–NMR, anti-parallel self recognition and conformation of the unnatural hexadeoxyribonucleotides α-d(CATGCG) and α-d(CGCATG). α-oligodeoxynucleotides as potential cellular probes for gene control. *Nucleic Acids Res.* **15,** 4241-4255.
20. Morvan, F., *et al.*, & Lown, J.W. (1987). α-DNA V: parallel annealing, handedness and conformation of the duplex of the unnatural α-hexadeoxyribonucleotide α-d(CATGCG) with its β-complement β-d(GTACG-C) deduced from high field ^1H–NMR. *Nucleic Acids Res.* **15,** 7027-7044.
21. Pieles, U., Zürcher, W., Schär, M. & Moser, H.E. (1993). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a powerful tool for the mass and sequence analysis of natural and modified oligonucleotides. *Nucleic Acids Res.* **21,** 3191-3196.
22. Marky, L.A. & Breslauer, K.J. (1987). Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers* **26,** 1601-1620.
23. Bolli, M., Lubini, P. & Leumann, C. (1995). Nucleic acid analogs with restricted conformational flexibility in the sugar-phosphate backbone ('Bicyclo-DNA', part 5), synthesis, characterization and pairing properties of oligo-D-(bicyclodeoxynucleotides) of the bases adenine and thymine (α-Bicyclo-DNA). *Helv. Chim. Acta* **78,** 2077-2096.
24. Marky, L.A. & Breslauer, K. J. (1987). Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers* **26,** 1601-1620.