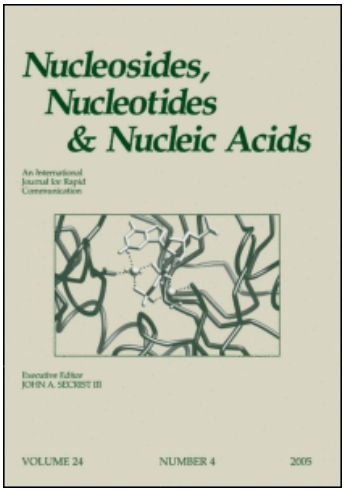

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ENGINEERING DNA TOPOLOGY WITH LOCKED NUCLEOSIDES: A STRUCTURAL STUDY

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▫ *DNA dodecamers modified with nucleotide building blocks based on a bicyclo[3.1.0]hexane system that effectively locks the ribose template into an RNA-like or North (N) conformation were analyzed by various biophysical techniques including high field nuclear magnetic resonance (NMR). Replacement of either one or both of the center thymidines in the Dickerson Drew dodecamer (CGCGAAT*T*CGCG) caused a progressive shift in the bending propensity of the double helix as shown by a newly developed rapid technique that compares the residual dipolar coupling (RDC) values of the modified duplexes with those previously determined for the native DNA.*

INTRODUCTION

The design of novel DNA oligomers that assume predefined structural features is currently a fervent area of research. Synthetic oligodeoxynucleic acids (ODNs) with chemical modifications in either the nucleobase[1] or furanose portion[2] of the nucleotide building blocks have been prepared by several groups and their biophysical properties have been evaluated. Since many of these analogues have been shown to hybridize efficiently with RNA, there is great therapeutic potential for synthetic ODNs in antisense technologies where regulation of specific gene translation may slow or halt the progression of disease. Moreover, binding of ODNs to proteins such as transcription factors often leads to structural adjustments in the

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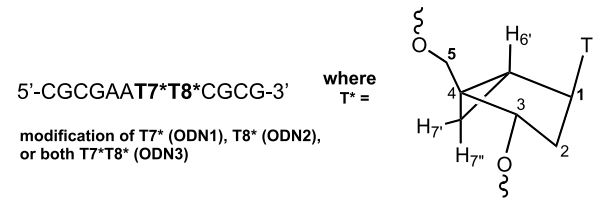

FIGURE 1 Structure of synthetic oligomers and the *N*-locked bicyclo[3.1.0]hexane building block used in this study.

helical parameters that may induce bends or kinks in the duplex. The ability to pre-organize ODNs to specific global folds that mimic these adjustments may lead to surrogates that could perturb protein production at the level of transcription.

A structural parameter that is critical to the overall duplex fold in DNA/RNA is the pucker of the furanose ring. In standard B-DNA, the pucker is 2′-endo or "South" (*S*) whereas A-DNA and RNA are characterized by 3′-endo or "North" (*N*) puckers. During bending of B-DNA, there is an obligatory adjustment of the sugar pucker from *S* toward *N* to relieve torsional and angular strain.* With this as a background, we set out to explore the structural consequences imparted to a standard *S*-type ODN of incorporation of nucleotide analogues designed to "lock" the pseudosugar pucker in the *N* hemisphere.

RESULTS AND DISCUSSION

We studied the Dickerson Drew dodecamer (DDD), which previously has been thoroughly characterized by a wide range of techniques, including X-ray crystallography and NMR, complemented by extensive computational work, and is often used as a model of a standard B-DNA sequence. Our laboratory has prepared "rigid" *N*-type nucleotides based on a bicyclo[3.1.0]hexane template and constructed ODNs modified with these novel building blocks.[4] The synthetic ODN's and the structure of the sugar analogue are shown in Figure 1. Several lines of evidence confirmed the existence of a stable duplex for all modified ODNs. Thermodynamic data obtained by differential scanning calorimetry (DSC) showed that the stability of the duplex was only slightly modified from the native DDD. Melt temperatures (T_m) changed by less than 3°C overall for the double modified ODN**3** (Table 1).

Circular dichroism (CD) spectra run at various temperatures and salt concentrations showed that ODNs **1**–**3** maintained their B-DNA-like character. However, subtle changes were observed that suggested a tendency toward an A-like DNA fingerprint for the monosubstituted oligomers ODN**1** and ODN**2**, with a slightly more pronounced shift toward A-DNA in the disubstituted analogue

*See for example Ref. [3].

TABLE 1 Thermodynamic Data Calculated for ODN's **1**–**3** from DSC Measurements

		$\Delta H_{(cal)}$	$\Delta G_{(37°C)}$
ODN	(°C)	(Kcal/mol)	
1	62.1	−54.8	−10.1
2	62.2	−45.7	−9.3
3	60.3	−51.1	−10.0
DDD	62.9	−52.4	−11.1

DSC measurements were performed in duplicate on a Microcal (Northhampton, MA) VP-DSC calorimeter. Samples were equilibrated at 10°C for 30 min and scanned from 10 to 90°C at a rate of 60°C/h. Thermodynamic values (T_m, $\Delta H_{(cal)}$, $\Delta G_{(37°C)}$) were calculated from ΔC_p vs. temperature plots using the origin software package.

(ODN**3**). The temperature dependence of the one-dimensional NMR spectra mirrored the results of the DSC and CD data: Imino protons for residues G2, G4, G10, T7, and T8 were all observed at 5–25°C and disappeared upon heating to temperatures close to the calculated T_m. Chemical shift changes were also minimal with temperature. The data ruled out the possible presence of bulged or hairpin-type structures in the modified duplexes.

The entire proton systems of all three analogues were assigned by standard 2-dimensional proton NMR experiments (COSY, NOESY, TOCSY) at 500 MHz. The bicyclo[3.1.0]hexane system is a useful marker since the chemical shifts of the cyclopropane protons (H6′, H7′ and H7″ in Figure 1) resonate at high field positions that are only sparsely populated with other proton signals. However, predicting the global fold of ODNs using typical NOE restraint-based modeling is hampered by the paucity of long-range correlations in these linear polymers. This limitation can be addressed by examination of residual dipolar couplings (RDCs) for ODNs in weakly aligned systems. RDC values allow the modeling of long-range correlations since they report on the relative orientation of internuclear vectors with respect to an overall molecular alignment tensor.[5] We have developed a technique to rapidly characterize the bending in the modified ODNs by comparing a selected set of RDCs of ODNs **1**–**3** measured in Pf1 phage with data obtained for the native DDD whose structure has been determined previously to a high degree of accuracy by RDC analysis in the same alignment media.[6] We collected ^{1}H-^{13}C correlation spectra at natural abundance and compared data for the peripheral residues (those whose chemical shifts were not affected by the modifications) to those from the native DDD. The data showed that the number and position of the modified nucleotides in the duplex cause bending of the helical axis to different degrees (ODN**2** < ODN**1** < ODN**3**).[6] This robust and facile method will be applied to various ODNs substituted with the locked nucleotide analogues.

In conclusion, we have evaluated the structures of three modified ODNs by a variety of biophysical methods, including a new procedure for analyzing RDC data at natural abundance to rapidly determine bending of a modified ODN when a high

resolution structure of the unmodified oligomer is available. The full characterization of ODNs **1**–**3** using complete sets of RDCs is currently in progress.

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