Solution Structure of *n*-Type DNA Oligomers Possessing a Covalently Cross-Linked Watson – Crick Base Pair Model

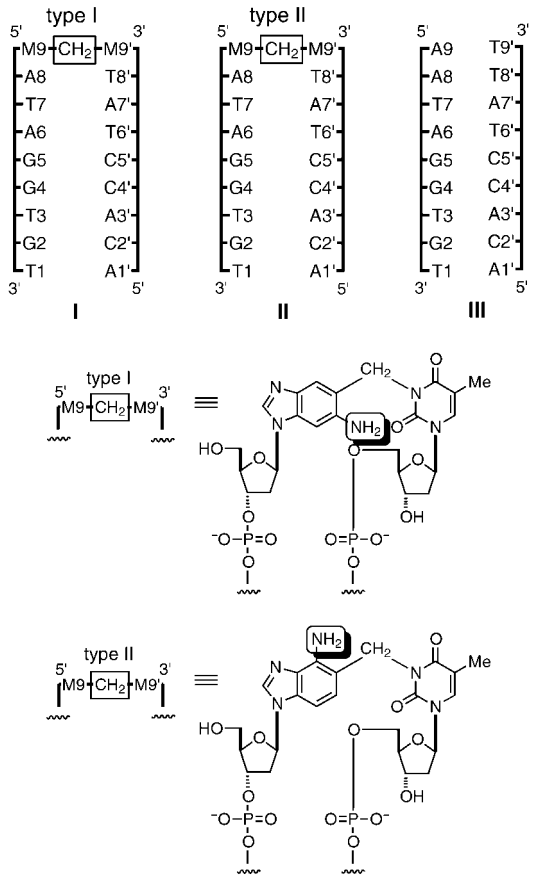
Hong-Yu Li, Yao-Ling Qiu, and Yoshito Kishi*[a]

KEYWORDS:

DNA structures · nucleosides · nucleotides · oligonucleotides · Watson – Crick base pairs

We have recently reported a general synthetic route to DNA oligomers containing a covalently cross-linked Watson – Crick base pair model.[1] In this paper, using *n*-type DNA oligomers as examples, we present experimental results demonstrating that these oligomers indeed exhibit conformational properties close to those of corresponding native DNA duplexes.

For the current studies, we used DNA oligomers **I** and **II**, containing an arbitrarily chosen base sequence.[2] To compare the global conformations of **I** and **II** with that of the corresponding native DNA duplex **III**, we first measured their circular dichroism (CD) spectra (Figure 1).[3] These exhibited the distinct characteristics of B-form DNA.[4] Figure 2 summarizes the temperature-dependent CD spectra, yielding the melting temperature (T_m) curves and thermodynamic parameters (Table 1) for



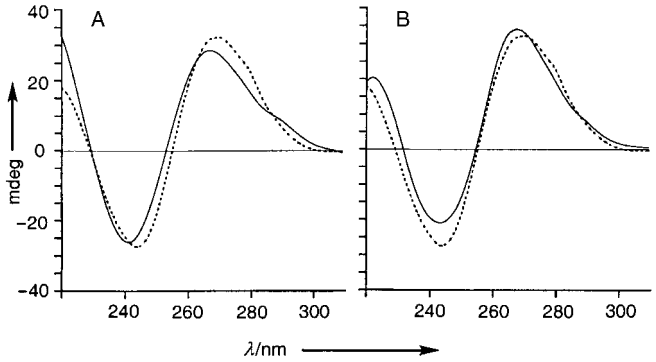


Figure 1. *CD spectra (10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl; DNA concentration: 40 – 80 μM) of **I** and **II**, in reference to **III**. Figures A and B show the CD spectra of **I** and **II** (solid lines), respectively, in reference to **III** (broken line).*

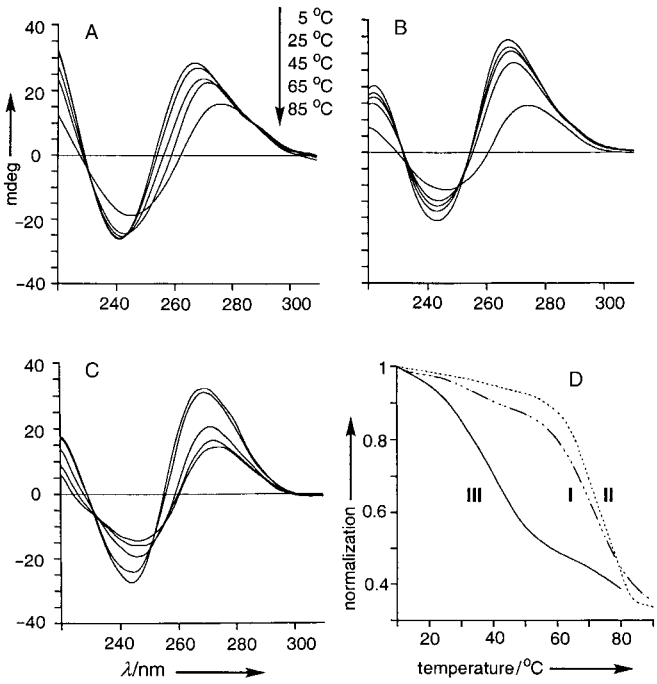


Figure 2. *Temperature-dependent CD spectra and melting curves of **I** – **III** (10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl; DNA concentration: 40 – 80 μM). Figures A – C show the CD spectra of **I** – **III**, respectively, at the temperatures indicated in Figure A. Figure D presents the melting curves of **I** – **III** at 267 nm. No concentration dependency of T_m was detected at 50 versus 150 μM of **I**.*

Table 1. *Thermodynamic parameters of DNA oligomers **I** – **III**.*

	I	II	III
T_m [°C]	71	73	37
$-\Delta H°$ [kcal mol^{-1}]	82.1	83.6	56.9
$-\Delta S°$ [cal mol^{-1} K]	238.7	241.6	171.0
$-\Delta G°$ [kcal mol^{-1}]	11.0	11.6	6.0

[a] *Prof. Dr. Y. Kishi, Dr. H.-Y. Li, Dr. Y.-L. Qiu*
Department of Chemistry and Chemical Biology
Harvard University
Cambridge, MA 02138 (USA)
Fax: (+1) 617-495-5150
E-mail: kishi@chemistry.harvard.edu

I–**III**.[5] The fact that **I**–**III** give clean melting curves suggests a transition from a single preferred conformation into a melted state. Notably, however, the CH_2-bridged oligomers **I** and **II** possess a significantly higher T_m value than the native duplex **III**, that is, $T_m^{\mathbf{I}} - T_m^{\mathbf{III}} = 34\,^{\circ}C$ and $T_m^{\mathbf{II}} - T_m^{\mathbf{III}} = 36\,^{\circ}C$. As expected, the entropy term is an important contributor to the observed, enhanced stability of B-form double helices.[6]

NMR spectroscopic studies provided more detailed information on the preferred conformation of **I** and **II**. Both **I** and **II** gave a well-resolved NMR spectrum (not shown), again suggesting that they exist in one preferred conformation.[7] The NOESY (nuclear Overhauser enhancement spectroscopy) spectra of **I** and **II** are shown in Figure 3. Two sets of NOE chains (solid and broken lines) were identified in each spectrum, confirming the base sequence incorporated in their synthesis. 2′-Deoxyriboses are known to adopt a 2′-*endo* conformation in B-form duplexes or to adopt a 3′-*endo* conformation in A-form duplexes.[7] Therefore, a B-form duplex gives stronger cross-peaks between the base proton and 2′-deoxyribose 2′ protons than those between the base proton and 2′-deoxyribose 1′ and 3′ protons. Indeed, this is the case for **I** and **II** (Figure 4), establishing that their 2′-deoxyriboses adopt the 2′-*endo* conformation characteristic of a B-form duplex.[7]

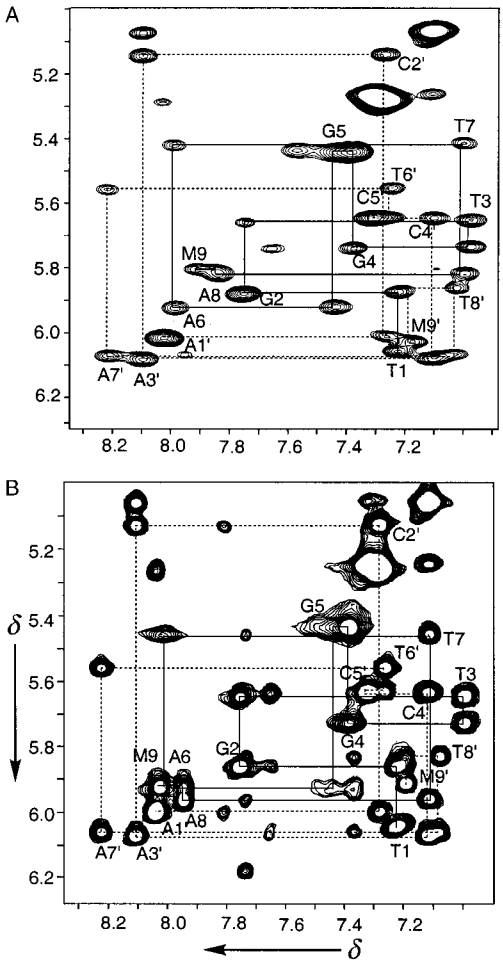


Figure 3. *NOESY NMR spectra (600 MHz; 200 ms; D_2O, 10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl) of* ***I*** *and* ***II****. Figures A and B show the purine-H.8 or pyrimidine-H.6/2′-deoxyribose-H.1′ region of* ***I*** *and* ***II****, respectively. Two NOE chains are shown by solid and broken lines in each figure.*

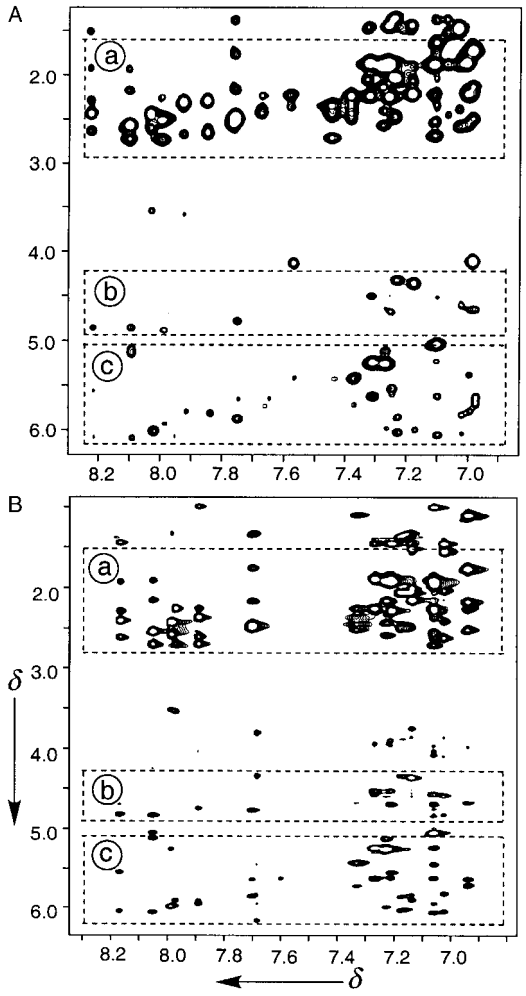


Figure 4. *NOESY NMR spectra (600 MHz; 200 ms; D_2O, 10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl) of* ***I*** *and* ***II****. Figures A and B show the purine-H.8 or pyrimidine-H.6/2′-deoxyribose-H.2′ (area ⓐ), the purine-H.8 or pyrimidine-H.6/2′-deoxyribose-H.3′ (area ⓑ), and the purine-H.8 or pyrimidine-H.6/2′-deoxyribose-H.1′ NOEs (area ⓒ) of* ***I*** *and* ***II****, respectively. In a B-form conformation, the intensity of these intranucleotide NOEs is known to be base-H/2′-H ≫ base-H/1′-,3′-H.*

A series of imino-H/D exchange NMR experiments provided information on the transition from the single preferred conformation into a melted state. The imino proton region in the ^{1}H NMR spectra are shown in Figure 5, with the indicated assignment of eight signals through an NOE experiment.[8] The temperature-dependent studies established the order of the H/D exchange rates for these imino protons to be 1(T1/A1′) > 8(A8/T8′) > 2(G2/C2′) > 7(T7/A7′) > 3(T3/A3′) > 6(A6/T6′) > 4(G4/C4′) ≈ 5(G5/C5′), indicating that the melting event takes place first at the T1/A1′ base pair, second at the A8/T8′ base pair adjacent to the CH_2-bridged base pair, third at the G2/C2′ base pair, and fourth at the T7/A7′ base pair.

  1439-4227/01/02/05 $ 17.50+.50/0 CHEMBIOCHEM **2001**, No. 5

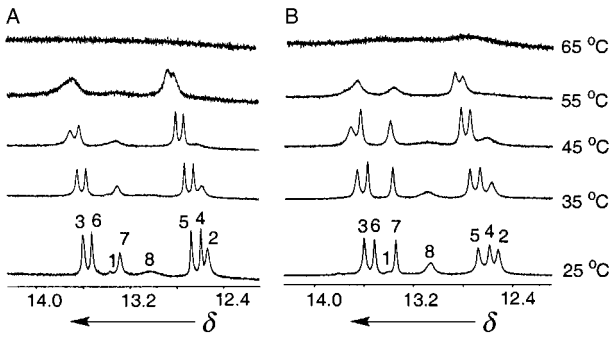


Figure 5. *Temperature-dependent imino proton exchange experiments (500 MHz; H_2O/D_2O (9:1), 10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl) of* ***I*** *(A) and* ***II*** *(B).*

No distinct difference in the conformational properties of **I** and **II** was initially recognized in the outlined experiments. However, subsequent TOCSY (total correlation spectroscopy) NMR experiments gave the first indication that, although they share a preferred conformation in the global sense, **I** and **II** behave slightly differently in the local conformational sense. **II** contains six T bases, including the T portion of the CH_2-bridged base pair, and six cross-peaks between the methyl and the vinyl protons of thymines were correspondingly observed in its TOCSY spectrum (Figure 6 B).[9] On the other hand, although **I** also contains only six T bases, eight cross-peaks were observed in its TOCSY spectrum (Figure 6 A).[7, 10] Considering this information, all of the NMR data were closely reexamined. Figure 7 shows the aromatic regions of the ^{1}H NMR spectra of **I** and **II**. In the spectrum of **I**, two sets of M9, A8, M9′, and T8′ aromatic signals were detected. In the spectrum of **II**, however, no corresponding extra signals were seen. Consistent with these observations, in addition to the NOE chains identified, two NOE chains, that is, M9-A8-T7 and M9′-T8′-A7′, were recognized in the boosted spectrum of **I**, and the cross-peaks T7 and A7′ merge to the main NOE chains originally identified. On the other hand, there were no additional NOE chains detected even in the boosted spectrum of **II**.

Based on these NMR spectroscopic studies, we conclude that **I** and **II** share a preferred global conformation, but they exhibit slightly different local conformations around the CH_2 bridge. **I** adopts two distinct conformations around the CH_2-bridged base pair end, whereas **II** exists in a single preferred conformation

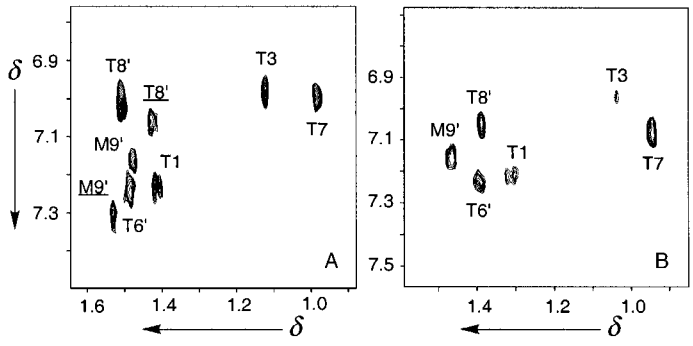


Figure 6. *TOCSY NMR spectrum (600 MHz; 50 ms; D_2O, 10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl) of* ***I*** *(A) and* ***II*** *(B). The cross-peaks marked with T8′ and M9′ are due to the minor conformer.*

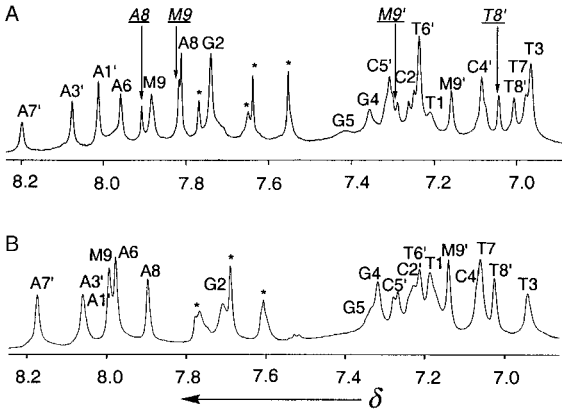


Figure 7. *The aromatic-proton region in the ^{1}H NMR spectrum (600 MHz, D_2O, 10 mM Na_2HPO_4 (pH 6.8), 0.1 M NaCl) of* ***I*** *(A) and* ***II*** *(B), with indication of the assignment of aromatic protons (purine-H.8 or pyrimidine-H.6). The signals marked with A8, M9, M9′, and T8′ are the aromatic protons of the minor conformer. The signals marked with an asterisk are the H.2 protons of adenines. Note that, due to the difference in relaxation time for each proton, the peak intensity does not accurately correspond to the number of protons.*

including the CH_2-bridged base pair end. In the mononucleoside base pair series, we demonstrated that both type I and II base pair models adopt a Watson – Crick base pairing orientation in the solid phase.[11] Additionally, they exist predominantly in one preferred conformation in solution, and the rotational energy barrier along the CH_2 bridge was estimated to be ca. 10 kcal mol^{-1} through variable-temperature NMR studies. However, we have not obtained direct experimental evidence to demonstrate that the dominant preferred solution conformation corresponds to the solid-phase structure. In this context, the NOESY NMR spectrum of **II** is informative. Two sets of internucleotide NOEs were clearly observed on both the M9/A8 and M9′/T8′ sides, which is possible only in a Watson – Crick base pairing orientation (Figure 8).

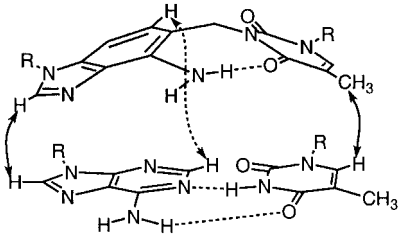


Figure 8. *Internucleotide NOEs observed for the CH_2-bridged base pair and the adjacent base pair of* ***II****.*

It is conceivable that the rotational energy barrier may become higher when the CH_2-bridged Watson – Crick base pair model is incorporated into a DNA duplex such as **I**. Thus, there is a possibility that the two local conformers, most likely corresponding to the Watson – Crick and reverse Watson – Crick base pairings,[12] are isolable. Indeed, two well-separated peaks were observed in the HPLC analysis of **I** (4.6 × 150-mm Vydac C18 column; 2 → 15 % MeCN/10 mM HCO_2NH_4 over 20 min; flow rate: 1 mL min^{-1}; retention time: 10.8 and 11.6 min with an area

integration of 3:2). Two fractions could be separated under these conditions, yielding two compounds without cross-contamination. Interestingly, the two isolated compounds were found to equilibrate at 70 °C, with an energy barrier of approximately 32 – 33 kcal mol^{-1}.[13] On the other hand, oligomer **II** behaved as a single compounds under the same HPLC conditions.

Finally, it is interesting to compare the NMR characteristics of **I** and **II** with those of the corresponding native DNA duplex **III**. There are a large number of NMR studies on DNA/RNA oligomers reported in the literature,[7] and we expect that it should be possible to estimate the structural characteristics of **III** predicted from the literature, and then compare those with the structural characteristics experimentally found for **I** and **II**. Specifically, by using the method developed by Giessner-Prettre and Pullman,[14] we have calculated the chemical shifts expected for the aromatic protons of **III** and compared them with those observed for **I** and **II** (Figure 9).[15, 16] The standard deviation between calculated and observed chemical shifts has been demonstrated to be within $\delta = 0.17$.[17] Figure 9 shows that all of the relevant proton signals of both **I** and **II** are indeed within this standard deviation,[15] supporting again the view that *n*-type DNA oligomers **I** and **II** exhibit conformational properties close to those of the corresponding native duplex DNA **III**.

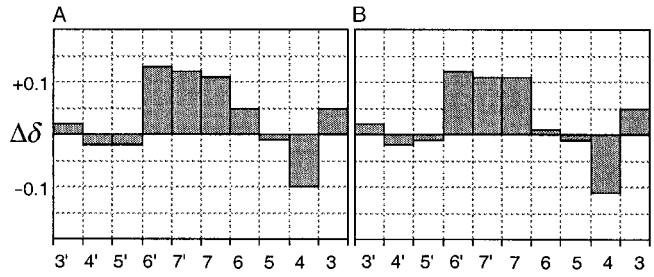


Figure 9. *Difference between the experimentally determined chemical shift values of* ***I*** *(A) or* ***II*** *(B) and the calculated values of* ***III****. The* x *and* y *axes represent the purine-H.8 or pyrimidine-H.6 of a nucleotide (represented only by a number, i.e., 3 = T3) and* $\Delta\delta$ *(*$\Delta\delta = \delta_{exp} - \delta_{calcd}$*), respectively.*

In conclusion, we have shown that the conformational characteristics of *n*-type DNA duplexes **I** and **II** compare well with those of the native DNA duplex. These results suggest that DNA and/or RNA oligomers incorporating a CH_2-bridged base pair may offer unique opportunities to address a variety of chemical and biological questions regarding nucleic acids. With this notion, we are engaged in the conformational study of *n*-type DNA/RNA, RNA/DNA, and RNA/RNA duplexes as well as *n*-type DNA/DNA composed of noncomplementary DNA strands.

Financial support from the National Institutes of Health (NS 12108) is gratefully acknowledged.

[1] H.-Y. Li, Y.-L. Qiu, E. Moyroud, Y. Kishi, *Angew. Chem.* **2001**, *113*, 1519; *Angew. Chem. Int. Ed.* **2001**, *40*, 1471.
[2] The duplex **III** was prepared by mixing 500 μL each of 45 μM solutions of the two corresponding strands purchased from Operon Technologies, California. For the synthesis and characterization of **I** and **II**, see ref. [1].
[3] All the CD experiments were carried out on a JASCO 710 spectrometer in the Verdine group. We thank Prof. G. Verdine for his generosity and help.
[4] V. I. Ivanov, L. E. Minchenkova, A. K. Schyolkina, A. I. Poletayev, *Biopolymers* **1973**, *12*, 89.
[5] a) L. A. Marky, K. J. Breslauer, *Biopolymers* **1987**, *26*, 1601; b) G. E. Plum, K. J. Breslauer in *Comprehensive Natural Products Chemistry, Vol. 7* (Eds.: E. T. Kool, D. H. R. Barton, K. Nakanishi, O. Meth-Cohn), Elsevier, New York, **1999**, pp. 15 – 53.
[6] Breslauer, Glick, and co-workers reported the design, synthesis, and analysis of analogues of d(CGCGAATTCGCG)$_2$ possessing one or two intrahelical disulfide cross-links: S. E. Osborne, J. Volker, S. Y. Stevens, K. J. Breslauser, G. D. Glick, *J. Am. Chem. Soc.* **1996**, *118*, 11 993. Although the cross-linked nucleotide in this work is not a complementary Watson – Crick base pair, the analogue with one disulfide link corresponds to an *n*-type oligomer. The thermodynamic parameters reported for this cross-linked duplex and its corresponding native duplex are: T_m [°C] = 87.8 vs. 70, $-\Delta H^\circ$ [kcal mol^{-1}] = 98.9 vs. 85.4, and $-\Delta S^\circ$ [cal mol^{-1} K] = 274.0 vs. 248.9.
[7] For reviews on NMR spectroscopy of nucleic acids, see for example: a) K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**; b) C. de los Santos in *Comprehensive Natural Products Chemistry, Vol. 7* (Eds.: E. T. Kool, D. H. R. Barton, K. Nakanishi, O. Meth-Cohn), Elsevier, New York, **1999**, pp. 55 – 80; c) G. M. Clore, A. M. Gronenborn, *FEBS Lett.* **1985**, *179*, 187.
[8] D. R. Kearns, D. J. Patel, R. G. Shulman, *Nature* **1971**, *229*, 338.
[9] a) C. Griesinger, G. Otting, K. Wüthrich, R. R. Ernst, *J. Am. Chem. Soc.* **1988**, *110*, 7870; b) H. Wang, E. R. P. Zuiderweg, G. D. Glick, *J. Am. Chem. Soc.* **1995**, *117*, 2981.
[10] There seem to be two cross-peaks for the T1 moiety in both TOCSY spectra, suggesting that the T1 portion of the oligomers may exist in two conformational states.
[11] a) X. Qiao, Y. Kishi, *Angew. Chem.* **1999**, *111*, 977; *Angew. Chem. Int. Ed.* **1999**, *38*, 928; b) Y.-L. Qiu, H.-Y. Li, G. Topalov, Y. Kishi, *Tetrahedron Lett.* **2000**, *41*, 9425.
[12] To confirm that two conformers correspond to the Watson – Crick and reverse Watson – Crick pairings, we examined the pattern of internucleotide NOEs in the NOESY NMR spectrum of **I**. However, the efforts met with some technical difficulties—note that the benzimidazole ring of **I** has a different substitution pattern from **II**.
[13] Because both isomers gradually decomposed at this temperature, we could not accurately measure the $T_{1/2}$ value for the isomerization. By using the time course for the first 30 minutes, its activation energy was estimated to be approximately 32 – 33 kcal mol^{-1}.
[14] C. Giessner-Prettre, B. Pullman, *Q. Rev. Biophys.* **1987**, *20*, 113.
[15] This calculation is not applicable to the terminal two nucleotides.
[16] By using the NOESY experiment, complete assignments of nonexchangeable protons in **I** and **II** have been made.
[17] S. S. Wijmenga, M. Kruithof, C. W. Hilbers, *J. Biomol. NMR* **1997**, *10*, 337.

Received: November 20, 2000
Revised version: January 24, 2001 [Z 185]