J|A|C|S
ARTICLES

Published on Web 02/24/2010

Base−Base Recognition of Nonionic Dinucleotide Analogues in an Apolar Environment Studied by Low-Temperature NMR Spectroscopy

Zhou Xiao† and Klaus Weisz*

Institute of Biochemistry, Ernst-Moritz-Arndt University Greifswald, Felix-Hausdorff-Str. 4, D-17489 Greifswald, Germany

Received December 3, 2009; E-mail: weisz@uni-greifswald.de

Abstract: Two self-complementary dinucleotide analogues $T_{Si}A$ and $A_{Si}T$ with a nonionic diisopropylsilyl-modified backbone were synthesized, and their association in a nonaqueous aprotic environment was studied by NMR spectroscopy. Using a $CDClF_2/CDF_3$ solvent mixture, measurements at temperatures as low as 113 K allowed the observation and structural characterization of individual complexes in the slow exchange regime. The $A_{Si}T$ analogue associates to exclusively form a dinucleotide antiparallel duplex with regular Watson−Crick base pairing, but both A and T nucleosides exhibit a predominant C3′-endo sugar pucker reminiscent of an A-type conformation. In contrast to $A_{Si}T$, the $T_{Si}A$ dinucleotide is found to exhibit significant variability and flexibility. Thus, different secondary structures with weaker hydrogen bonds for all $T_{Si}A$ structures are observed at low temperatures. Although a B-like Watson−Crick antiparallel dinucleotide duplex with a preferred C2′-endo sugar pucker largely predominates at temperatures above 153 K, two additional species, namely a dinucleotide Hoogsteen duplex with a *syn* glycosidic torsion angle of the adenosine nucleoside and a presumably intramolecularly folded structure, are increasingly populated upon further cooling. By adding typical DNA intercalators like anthracene or benz[*c*]acridine derivatives to the $A_{Si}T$ dinucleotide duplex in the aprotic solvent environment, no binding of the polycyclic aromatic molecules can be detected even at lower temperatures. Obviously, van der Waals and stacking interactions are insufficient to compensate for the other unfavorable contributions to the overall free energy of binding, and only in the presence of additional hydrophobic effects in an aqueous environment does binding occur.

Introduction

The reliable storage and readout of genetic information in nucleic acids are based on their specific hydrogen bond mediated base−base recognition. These interactions will generally result in the formation of the well-known DNA double helix with adenine−thymine (AT) and guanine−cytosine (GC) base pairs. Consequently, much emphasis has been placed on the nucleobases as the major determinants of DNA secondary structure, and only a small amount of attention has been paid in the past to the role of the sugar phosphate backbone that carries the nucleic acid bases. However, the design of backbone-modified nucleic acids, e.g., for use in antisense technologies for improved cellular uptake and nuclease resistance,[1] has greatly stimulated interest in backbone-induced structural effects recently. Thus, a regular Watson−Crick duplex was reported for a short $G_{SO2}C$ dinucleotide analogue with a nonionic dimethylenesulfone linkage in the crystal.[2] In contrast, NMR structural studies on a dimeric dimethylenesulfone-linked $U_{SO2}C$ RNA analogue in chloroform have indicated the formation of a parallel duplex with both interstrand and intrastrand hydrogen bonds much different from regular base pairing.[3] Interestingly, the stabilizing hydrogen bond network was found to also include backbone hydroxyl groups in addition to base−base interactions.

Obviously, a string of phosphate negative charges along the chain associated with significant charge repulsion will invariably promote a more extended conformation and suppress the collapse to a coiled structure with largely intramolecular interactions. Yet another widely acknowledged idea assumes a more prominent role of the backbone in the base recognition scheme and a direct impact of the charged phosphodiester on the molecular base−base recognition by directing interstrand interactions toward the Watson−Crick edge of the heterocycles to maximize the separation between the negative backbones of the contacting strands[4] (for an excellent review, see ref 4b). Consequently, much can be learned about the inherent critical properties of the natural DNA backbone for the storage and release of genetic information by looking at DNA analogues with a nonphosphate scaffold. Also, questioning the ability of nucleic acids to still serve as a carrier of genetic information in a nonaqueous environment directly calls for a better understand-

† Present address: Nanyang Technological University, Singapore.

(1) Selected reviews: (a) Nielsen, P. E. *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 167–183. (b) Cook, P. D. In *Antisense Drug Technology: Principles, Strategies, and Applications*; Crooke, S. T., Ed.; Marcel Dekker: New York, 2001; pp 29−56. (c) Micklefield, J. *Curr. Med. Chem.* **2001**, *8*, 1157–1179. (d) Sanghvi, Y. S. In *DNA and Aspects of Molecular Biology*; Kool, E. T., Ed.; Elsevier Science Ltd.: Oxford, 2002; Vol. 7, pp 285−311. (e) Kurreck, J. *Eur. J. Biochem.* **2003**, *270*, 1628–1644.

(2) Roughton, A. L.; Portmann, S.; Benner, S. A.; Egli, M. *J. Am. Chem. Soc.* **1995**, *117*, 7249–7250.

(3) Steinbeck, C.; Richert, C. *J. Am. Chem. Soc.* **1998**, *120*, 11576–11580.

(4) (a) Huang, Z.; Benner, S. A. *J. Org. Chem.* **2002**, *67*, 3996–4013. (b) Benner, S. A.; Hutter, D. *Bioorg. Chem.* **2002**, *30*, 62–80.

10.1021/ja910220s

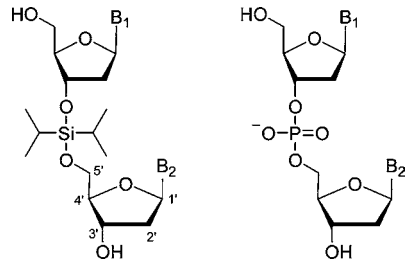


Figure 1. Nonionic triisopropylsilyl backbone with atom numbering of the 2′-deoxyribose (left) and natural anionic phosphodiester backbone (right).

ing of the impact of a nonionic backbone on structure and recognition. A nucleic acid in a more hydrophobic environment will probably rely on a neutral backbone to ensure sufficient solubility. In this case, will the nonpolar backbone still allow for a well-defined recognition code between two strands with only a few mispairings to allow for occasional mutations? Another important issue within this context deals with the binding of ligands under such conditions. Clearly, ligand binding allows for gene regulation, a prerequisite for a controlled expression of genomic sequences. Excluding water molecules and thus hydrophobic effects from the binding event, studies on ligand binding to double-helical DNA in a nonaqueous environment will also yield valuable information on the various contributions that determine the affinity toward duplex DNA under physiological conditions.

To study the structure and recognition of nucleic acids with a nonionic backbone, we here report on NMR structural studies of diisopropylsilyl-linked dinucleotides as model systems for a neutral nucleic acid. A silyl backbone seems to be an attractive phosphodiester surrogate given its high Si−O bond energy, achirality, and lipophilicity and the similarity of silicon in size, bond angles, and bond lengths to phosphorus (Figure 1). In addition, silicon is the eighth most common element in the universe by mass and thus of particular interest when considering constituents of nucleic acid analogues having potentially evolved as carriers of genetic information in an extraterrestrial environment.

With only a limited number of interactions, intermolecular forces between short dinucleotides tend to be weak, and consequently hydrogen-bonded complexes in solution are often in fast exchange on the NMR chemical shift time scale at ambient temperatures preventing the unambiguous characterization of coexisting associates. To circumvent averaging effects and to characterize hydrogen-bonded complexes by liquid-state NMR in detail, NMR measurements have been performed in the past at very low temperatures by employing a deuterated Freon mixture $CDClF_2/CDF_3$ as solvent.[5] Thus, with measurements in the liquid state as low as 113 K individual hydrogen-bonded complexes of free nucleosides in slow exchange could be observed and unambiguously characterized in solution with respect to type and strength of formed hydrogen bonds.[5c,d] The low-temperature NMR experiments on the association of silyl-linked dinucleotides $A_{Si}T$ and $T_{Si}A$ not only provide detailed structural information in terms of base−base recognition and the formation of specific hydrogen bonds but also allow for an assessment of the major forces promoting the binding of ligands to DNA.

Experimental Section

NMR experiments were performed on a Bruker AVANCE 600 spectrometer. Temperatures were adjusted by a Eurotherm variable-temperature unit to an accuracy of ±1.0 °C. The temperatures used for determining equilibrium constants for self-association were calibrated by using a standard solution of 4% CH_3OH in CD_3OD in the range 293−233 K. Low-temperature spectra were recorded with a specially designed low-temperature dual probehead. ^1H NMR chemical shifts in the freonic mixture were referenced relative to $CHClF_2$ ($\delta_H = 7.18$ ppm).

The deuterated freonic mixture $CDClF_2/CDF_3$ was prepared as described[6] and handled on a vacuum line which was also used for the sample preparation. Chemicals were purchased from Sigma-Aldrich, Deisenhofen, Germany. Detailed procedures for the preparation of $A_{Si}T$ and $T_{Si}A$ and their characterization are provided as Supporting Information.

Results and Discussion

Synthesis. An internucleotide diisopropylsilyl linkage was chosen as a neutral phosphodiester analogue based on its favorable stability and reactivity.[7] Also, additional silyl protecting groups at the 5′- and 3′-OH of nucleosides have previously been found to promote solubility in freonic solvents especially at low temperatures.[8] Synthetic methods for silyl-linked oligonucleotides have been reported in the past.[7,9] In a typical procedure, a 5′-protected nucleoside is 3′-silylated with a silylating reagent and a base. Finally, this silyl-linked nucleoside continues to react with a second 3′-protected nucleoside to produce a fully protected 3′,5′-silyl-linked dinucleotide. Problems with this strategy often involve low yields due to the formation of both undesired 3′,3′-symmetrical dimers as byproducts and a significant loss of product upon deprotection and final purification.[9a] To reduce the formation of 3′,3′-coupling products and to allow direct coupling of a 3′-silyl intermediate to an unprotected nucleoside, a hindered base procedure was developed and successfully employed for the preparation of various silyl-linked nucleosides.[9b] For our structural studies, the use of high-purity products was of major concern. In this respect, the best results in our own efforts to synthesize fully protected 5′- and 3′-*O*-di-(*tert*-butyldimethylsilyl) dinucleotides with a diisopropylsilyl linkage were obtained with slightly modified protocols for the mixed thymidine−adenosine ($T_{Si}A$) or adenosine−thymidine ($A_{Si}T$) dinucleotide.

For the $A_{Si}T$ synthesis, a strategy with 5′- and 3′-TBDMS-protected nucleosides was employed to avoid byproducts and thus difficult separations. After *N*-benzoylation, reaction of

(5) (a) Siegel, J. S.; Anet, F. A. L. *J. Org. Chem.* **1988**, *53*, 2629–2630. (b) Golubev, N. S.; Denisov, G. S. *J. Mol. Struct.* **1992**, *270*, 263–276. (c) Weisz, K.; Jähnchen, J.; Limbach, H.-H. *J. Am. Chem. Soc.* **1997**, *119*, 6436–6437. (d) Dunger, A.; Limbach, H.-H.; Weisz, K. *Chem.—Eur. J.* **1998**, *4*, 621–628. (e) Smirnov, S. N.; Benedict, H.; Golubev, N. S.; Denisov, G. S.; Kreevoy, M. M.; Schowen, R. L.; Limbach, H.-H. *Can. J. Chem.* **1999**, *77*, 943–949. (f) Schlund, S.; Mladenovic, M.; Basílio Janke, E. M.; Engels, B.; Weisz, K. *J. Am. Chem. Soc.* **2005**, *127*, 16151–16158. (g) Hupp, T.; Sturm, C.; Basílio Janke, E. M.; Pérez Cabre, M.; Weisz, K.; Engels, B. *J. Phys. Chem. A* **2005**, *109*, 1703–1712.

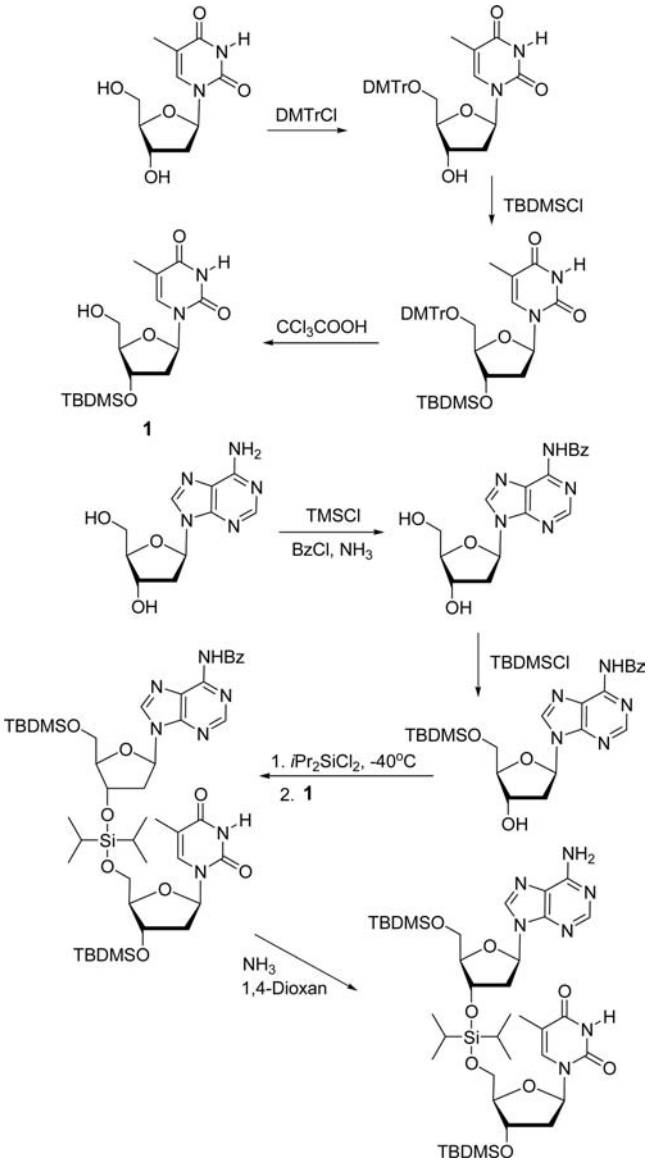
(6) Golubev, N. S.; Smirnov, S. N.; Gindin, V. A.; Denisov, G. S.; Benedict, H.; Limbach, H.-H. *J. Am. Chem. Soc.* **1994**, *116*, 12055–12056.

(7) Cormier, J. F.; Ogilvie, K. K. *Nucleic Acids Res.* **1988**, *16*, 4583–4594.

(8) Basílio Janke, E. M.; Limbach, H.-H.; Weisz, K. *J. Am. Chem. Soc.* **2004**, *126*, 2135–2141.

(9) (a) Ogilvie, K. K.; Cormier, J. F. *Tetrahedron Lett.* **1985**, *26*, 4159–4162. (b) Saha, A. K.; Sardaro, M.; Waychunas, C.; Delecki, D.; Kuntny, R.; Cavanaugh, P.; Yawman, A.; Upson, D. A.; Kruse, L. I. *J. Org. Chem.* **1993**, *58*, 7827–7831.

Scheme 1. Synthesis Scheme of $A_{Si}T$[a]



[a] DMTr = dimethoxytrityl, TMS = trimethylsilyl, Bz = benzoyl, TBDMS = *tert*-butyldimethylsilyl, *i*Pr = isopropyl.

adenosine with *tert*-butyldimethylsilyl chloride yielded the 5′-protected nucleoside with high regioselectivity. On the other hand, 3′-silylation of thymidine was achieved following a transient 5′-tritylation. It was found out that using imidazole in the absence of a hindered base gives better yields and formed byproducts are mostly 3′−3′ adenosine dimers. With this strategy, only the benzoyl group has to be removed after separation of the desired 5′−3′ coupling product from the reaction mixture. The synthesis scheme of $A_{Si}T$ is outlined in Scheme 1.

For the synthesis of $T_{Si}A$, 5′-*O*-*tert*-butyldimethylsilyl thymidine was directly coupled with the nonsilylated N^6-benzoyl-2′-deoxyadenosine in the presence of the hindered base 2,6-di-*tert*-butyl-4-methylpyridine and diisopropyldichlorosilane as the silylating reagent. The latter was found to give better results compared to bis(trifluoromethanesulfonyl)diisopropylsilane upon addition of the amino-protected 2′-deoxyadenosine as the second nucleoside. Following the coupling reaction, some 3′,3′-thymidine dimer can be isolated as a byproduct after column separation. The 5′−3′-dinucleotide was TBDMS-protected at its free 3′-OH, and the benzoyl group was finally removed by treatment with ammonia.

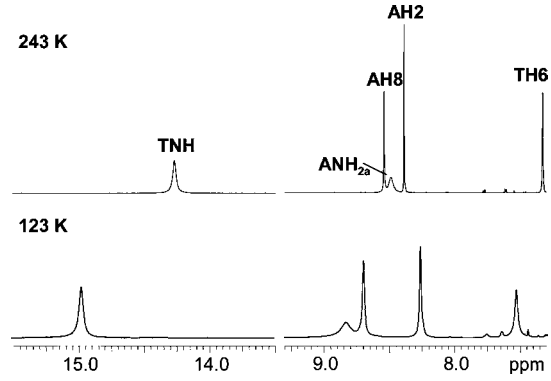


Figure 2. ^{1}H NMR spectra of $A_{Si}T$ in Freon at 243 and 123 K showing base proton spectral regions.

Final HPLC-purified dinucleotides were subjected to a detailed NMR spectroscopic characterization using COSY and NOESY experiments. For an unambiguous confirmation of a 3′−5′ diisopropylsilyl linkage, a gradient-enhanced ^{1}H−^{29}Si HMQC experiment was acquired with a delay optimized for long-range ^{1}H−^{29}Si couplings of 3 Hz.[10] Correlations observed between ^{29}Si within the internucleotide linkage and H3′ of the 5′-terminal nucleoside as well as H5′/H5″ of the 3′-terminal nucleoside confirm the correct structure (see the Supporting Information).

NMR Structural Studies on $A_{Si}T$. To obtain information on low energy structures of potential associates under aprotic solvent conditions, the $A_{Si}T$ dinucleotide was dissolved in a freonic mixture and studied by NMR measurements down to temperatures low enough to reach the slow exchange regime. When the sample was cooled to 123 K, the thymine imino proton signal shifted downfield and all resonances exhibited some gradual line broadening as expected for slower molecular reorientation rates associated with a decreased spectral resolution. However, as seen in Figure 2, with only a single set of proton resonances even under slow exchange conditions at 123 K and with no indication for coalescence over the wide temperature range studied, a single well-defined structure is formed and favored at all temperatures.

The downfield-shifted T imino resonance at 15 ppm and 123 K points to its participation in a hydrogen bond to adenine. In general, assignment to either a Watson−Crick or Hoogsteen geometry can be achieved through the observation of ^{1}H−^{1}H NOE contacts of the imino resonance to either A H2 or A H8 protons, respectively. Because of this critical information, the adenine base protons were unambiguously assigned making use of the ^{1}H−^{13}C ^3J scalar couplings within the purine ring system for coherence transfer experiments.[11,12]

As seen in Figure 3 (bottom), adenine C4 at 150 ppm shows crosspeaks to both H2 and H8 protons in a ^{1}H−^{13}C HMBC experiment establishing an H2−H8 through-bond correlation via the C4 atom. In addition, H8 exhibits another crosspeak to C5 at ∼120 ppm, whereas H2 is correlated through scalar

(10) Hewitt, J. M.; Lenhart, W. C.; Moore, R. N.; Saha, A. K.; Weis, A. L. *Nucleosides Nucleotides* **1992**, *11*, 1661–1666.
(11) Hilbers, C. W.; Wijmenga, S. S. In *Encylcopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; Wiley: Chichester, U.K., 1996; pp 3346−3359.
(12) Van Dongen, M. J. P.; Wijmenga, S. S.; Eritja, R.; Azorin, F.; Hilbers, C. W. *J. Biomol. NMR* **1996**, *8*, 207–212.

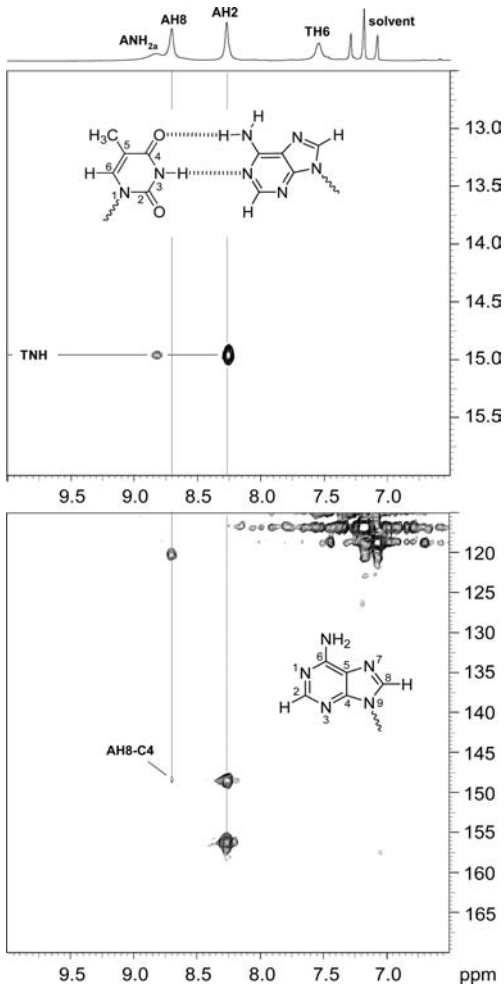


Figure 3. H2 and H8 crosspeak region of a $^{1}H-^{13}C$ HMBC spectrum of $A_{Si}T$ with long-range coupling $^{3}J_{CH}$ optimized to 10 Hz (bottom); 2D NOE spectrum of $A_{Si}T$ with an 80 ms mixing time showing crosspeaks between imino and base protons (top). Both spectra were acquired at 133 K in Freon. Insets show the adenine base (bottom) and an AT Watson−Crick base pair (top) with atom numbering.

coupling with C6 at ~157 ppm. With HMBC spectra taken at different temperatures we also noticed that H2 and H8 exchanged positions with decreasing temperature. Above 243 K, H2 is more downfield shifted but becomes more upfield shifted compared to H8 at lower temperatures.

With A H2 and H8 assigned, an NOE crosspeak found for the T imino to a hydrogen-bonded adenine amino proton as well as a strong contact to A H2 established a Watson−Crick arrangement in a symmetrical, antiparallel dimer (Figure 3, top). The complete assignment of all resonances follows established strategies for nucleosides and oligonucleotides.[13] With only a single species present, it is essentially based on the analysis of a 2D NOE experiment performed at 163 K to take advantage of well-resolved signals (see the Supporting Information). Compared to even lower temperatures, exchange crosspeaks between labile protons can still be observed at 163 K but do not compromise the assignments and the structural analysis based on the nonlabile protons. As expected for a Watson−Crick geometry with antiparallel strands, intranucleotide contacts for T H6 and A H8 with their own sugar protons clearly suggest an *anti* conformation for both nucleosides.[14,15] Also, in line with a helix-type duplex structure, T H6 exhibits additional medium to strong sequential contacts to adenosine H2′, H2″, and H3′ protons (for all proton chemical shifts of $A_{Si}T$, see the Supporting Information).[15]

A semiquantitative evaluation of NOE crosspeak intensities allows us to gain additional information on the dinucleotide duplex conformation. There are several lines of evidence that support the formation of an A-type duplex. These include moderate to strong internucleotide crosspeaks between T H6 and A H3′ and A H2′, a weak sequential crosspeak between T H6 and A H1′, and the presence of another moderately strong intrastrand contact between A H2 and T H1′. Also, a stronger cross peak of A H8 with H3′ compared to H2′/H2″ and a strong cross peak between H2″ and H4′ in the adenine nucleotide indicate a C3′-*endo* sugar pucker.[15]

A sugar conformational analysis was also performed on the basis of measured $^{1}H-^{1}H$ scalar couplings at 243 K (see the Supporting Information). With resolution-enhancing window functions for processing, multiplet structures of deoxyribose proton resonances are well resolved at these higher temperatures and allow for a fairly accurate determination of coupling constants without resorting to crosspeak simulations. Assuming a two-state equilibrium between North (C3′-*endo*) and South (C2′-*endo*) conformers, a coupling constant analysis with the optimized Karplus relationship of Altona et al.[16] indicates a nearly pure C3′-*endo* sugar pucker for adenosine, while the T nucleoside is mixed with ~20−40% of a C2′-*endo* conformation. This again supports an A-type dinucleotide duplex for $A_{Si}T$ under these conditions.

The A-typical conformation of the $A_{Si}T$ dimer contrasts with B-DNA duplexes generally observed in an aqueous environment. It is well-known that DNA adopts a conformation that significantly depends on the presence of water molecules, and X-ray diffraction studies have repeatedly shown that low relative humidity favors A conformations. The observation of an A-type duplex in the present study may thus be taken as the result of lacking hydration. However, a partial impact by the modified silyl backbone on the observed preference of a C3′-*endo* pucker for the deoxyribonucleosides, a major determinant of an A-type duplex structure, cannot be excluded.

The $A_{Si}T$ self-association was further characterized by its equilibrium constant K_a determined by a nonlinear least-squares fit of the thymine imino proton chemical shift measured as a function of dinucleotide concentration in methylene chloride. Thus, an association constant of 135 M^{-1} is obtained at 294 K. A subsequent van't Hoff analysis of $K_a(T)$ determined in a temperature range between 249 and 294 K yields a $\Delta H° = -64 \pm 2.5$ kJ/mol and a $\Delta S° = -178 \pm 9.4$ J/K·mol. Interestingly, $\Delta H°$ for the $A_{Si}T$ association mediated by a total of four hydrogen bonds is approximately three times as large compared to the association enthalpy of a free adenosine and uridine nucleoside under the same solution conditions.[17] On the other hand, with a nearly 5-fold increase in $-\Delta S°$ the entropic penalty

(13) (a) Feigon, J.; Wright, J. M.; Leupin, W.; Denny, W. A.; Kearns, D. R. *J. Am. Chem. Soc.* **1982**, *104*, 5540–5541. (b) Scheek, R. M.; Russo, N.; Boelens, R.; Kaptein, R.; van Boom, J. H. *J. Am. Chem. Soc.* **1983**, *105*, 2914–2916.

(14) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(15) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley-Interscience: New York, 1986; pp 205−214.

(16) (a) De Leeuw, F. A. A. M.; Altona, C. *J. Chem. Soc., Perkin Trans. 2* **1982**, 375–384. (b) Rinkel, L. J.; Altona, C. *J. Biomol. Struct. Dyn.* **1987**, *4*, 621–649.

(17) Xiao, Z.; Weisz, K. *J. Phys. Org. Chem.* **2007**, *20*, 771–777.

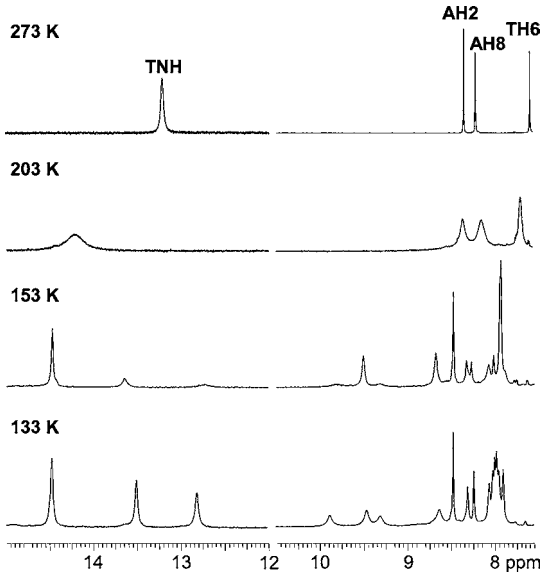


Figure 4. ^{1}H NMR spectra of $T_{Si}A$ in Freon showing base proton spectral regions as a function of temperature.

of forming a base-paired complex has significantly increased for the $A_{Si}T$ duplex when compared to the free AU base pair. Disregarding minor differences between uridine and thymidine nucleosides, these data indicate that in addition to the formation of four Watson−Crick hydrogen bonds stacking interactions considerably contribute to the stability of the dinucleotide duplex in the apolar environment. However, reorganization of the silyl backbone upon duplex formation adds a significant entropic cost to the dinucleotide self-association.

NMR Structural Studies on $T_{si}A$. Upon preparing a $T_{Si}A$ solution in Freon we noticed a lower solubility compared to $A_{Si}T$, yet the poorer solubility did not compromise low-temperature ^{1}H NMR measurements. As shown in Figure 4, $T_{Si}A$ resonances start to considerably broaden at temperatures below 243 K. Interestingly, two more upfield shifted imino signals appear in addition to the major imino resonance and gradually become more intense with decreasing temperature below a clearly noticeable coalescence point for the imino protons at ∼183 K.

Upon closer inspection, three sets of signals can also be found in the base proton region between 10 and 7.5 ppm and H1′ region between 7 and 6 ppm. Apparently, different structures for $T_{Si}A$ are formed and coexist under slow exchange conditions at low temperatures. In the following, the three species are denoted $^{1}T_{Si}A$, $^{2}T_{Si}A$, and $^{3}T_{Si}A$ and refer to structures with their imino signals resonating at increasingly higher field, respectively. Integration of the three imino signals at 133 K results in a relative population of approximately 9:6:5 for structures 1, 2, and 3.

Due to significant signal broadening, a reliable sugar pucker analysis of $T_{Si}A$ based on $^{1}H-^{1}H$ scalar coupling constants required higher temperatures and was performed at 273 K. Therefore, it should mostly reflect the conformation of $^{1}T_{Si}A$. Again, assuming a dynamic equilibrium between the two major N- and S-type sugar conformations, the *J* coupling constants determined for the T and A nucleosides in $T_{Si}A$ indicate a B-like sugar conformation with a population of approximately 90% and 60% C2′-*endo* sugar pucker, respectively. Similar to $A_{Si}T$, the pyrimidine nucleoside seems to have a stronger propensity to adopt an S-type conformation when compared to the purine

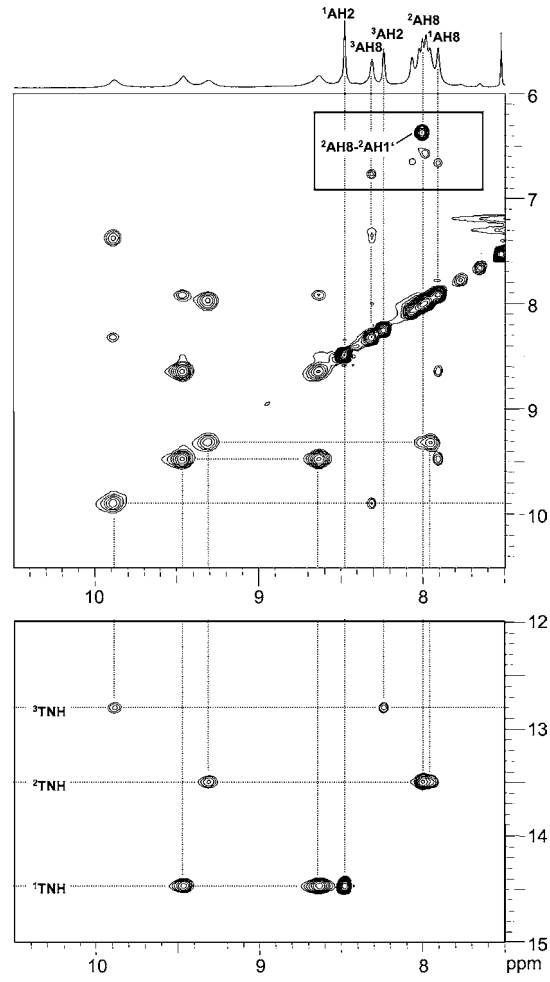


Figure 5. Portion of a 2D NOE spectrum of $T_{Si}A$ in Freon showing regions with amino/H2/H8-amino/H2/H8/H1′ (top) and amino/H2/H8-imino (bottom) crosspeaks. The boxed region encloses base-H1′ contacts. The spectrum was acquired at 133 K with an 80 ms mixing time.

nucleoside. Surprisingly, however, the generally favored S-type sugar pucker for the A and T nucleosides in $T_{Si}A$ stands in striking contrast to the N-type pucker found for $A_{Si}T$ at 243 K.

To unambiguously assign the three imino resonances to corresponding complex structures, a 2D NOE experiment at 133 K was performed. At this temperature, no crosspeaks due to chemical exchange are observed and NOE contacts directly identify protons in close spatial proximity. Regions of the 2D NOE spectrum showing imino to amino/H2/H8 NOE contacts are shown in Figure 5 (bottom). The imino signal for structure $^{1}T_{Si}A$ at 14.48 ppm exhibits three strong crosspeaks in the amino/H2/H8 region at 9.47, 8.64, and 8.48 ppm. The two downfield shifted resonances at 9.47 and 8.64 ppm can easily be assigned to the two adenine amino protons since they are mutually connected by a strong crosspeak as shown in the top spectrum of Figure 5. Assignment of the remaining signal at 8.48 ppm to either H2 or H8 is again crucial for the determination of the hydrogen-bonding pattern in the complex. With only a very weak NOE contact in the H1′ region and no connectivities to any other sugar protons, the signal must be attributed to the H2 proton, thus establishing a symmetric antiparallel Watson−Crick duplex for $^{1}T_{Si}A$. Additional assignments make use of a moderately strong NOE contact between adenine amino protons and adenine H8 at 7.91 ppm and intraresidue NOE connectivities between H8 and its sugar

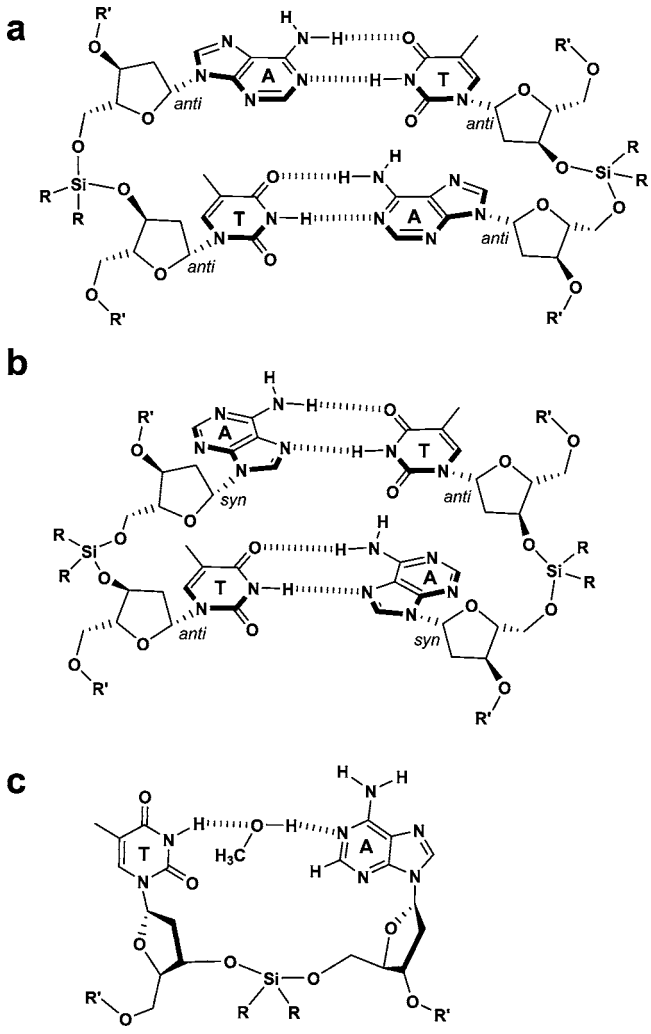


Figure 6. Schematic model for (a) $^{1}T_{Si}A$ antiparallel Watson−Crick duplex, (b) $^{2}T_{Si}A$ antiparallel Hoogsteen duplex, and (c) putative $^{3}T_{Si}A$-folded dinucleotide structure; R = isopropyl, R′ = *tert*-butyldimethylsilyl.

protons. A weaker NOE contact observed between A H8 and A H3′ when compared to A H8 and A H1′ again supports data from the *J* coupling analysis in having a predominant C2′-*endo* sugar conformation.[15] The B-type Watson−Crick dinucleotide duplex structure of $^{1}T_{Si}A$ is schematically shown in Figure 6a (for a full list of chemical shift assignments of $^{1}T_{Si}A$, see the Supporting Information).

Likewise, the T imino proton of structure $^{2}T_{Si}A$ located at 13.51 ppm shows crosspeaks to adenine amino protons at 9.31 and 7.96 ppm mutually connected by a strong NOE contact. Another strong contact to a resonance at ∼8.0 ppm obviously overlaps with one of the imino-amino crosspeaks. Depending on a Watson−Crick or Hoogsteen hydrogen bond geometry, this crosspeak may be attributed to either H2 or H8, respectively. Unfortunately, due to the lower concentration of $T_{Si}A$ in the Freon sample and signal broadening at low temperatures, a $^{1}H-^{13}C$ HMBC experiment as has been successfully applied for $A_{Si}T$ failed to unambiguously distinguish H2 and H8 in this case. Therefore, alternative efforts were directed toward the H1′ region. The signal at 8.0 ppm connected by a crosspeak to the ^{2}T imino exhibits an unusually strong NOE contact to an H1′ anomeric proton without exhibiting noticeable contacts to other sugar protons (Figure 5 top). For an *anti* glycosidic torsion angle, the distance between adenine H8 and H1′ is expected to be ∼3.8 Å. Changing the glycosidic torsion angle to *syn* will considerably shorten the H8−H1′ distance to ∼2.6 Å. On the other hand, adenine H2 is expected to show only a weak or no NOE contact to its own H1′ proton depending on the presence of *anti* and *syn* conformations, respectively. Apparently, the strong NOE contact of the resonance at 8.0 ppm in the H1′ region is only compatible with an H8 proton and a *syn* glycosidic torsion angle suggesting an antiparallel Hoogsteen arrangement for the $^{2}T_{Si}A$ self-associate. Unfortunately, sugar protons of the $^{2}T_{Si}A$ complex could only partially be assigned due to significant signal overlap (see the Supporting Information for a list of chemical shift assignments of $^{2}T_{Si}A$). A schematic model of a Hoogsteen dinucleotide duplex for $^{2}T_{Si}A$ is depicted in Figure 6b.

For the remaining least populated $^{3}T_{Si}A$ structure the rather upfield shifted imino proton again exhibits NOE contacts to adenine amino protons at 9.89 and 7.37 ppm as well as to either A H2 or A H8 at 8.24 ppm. With no prominent NOE contact to any sugar proton, the signal at 8.24 ppm can be attributed to A H2. Accordingly, another resonance at 8.31 ppm exhibiting a crosspeak to the amino proton at 9.89 ppm and also showing a strong crosspeak to an H1′ proton must be assigned to A H8 of $^{3}T_{Si}A$. With H8−H1′ being the strongest crosspeak of this H8 signal in the sugar proton region, a glycosidic torsion angle close to a *syn*-like conformation can be derived for this adenine nucleoside.

Interestingly, the ^{3}T imino proton exhibits another strong NOE contact to a signal at 3.54 ppm as well as a very broad crosspeak to a resonance centered at ∼8.96 ppm and only observable upon further lowering the 2D threshold level (not shown). Additional medium to weak NOE contacts connect the resonance at 3.54 ppm to ^{3}A H2 at 8.24 ppm, to the two ^{3}A amino protons at 9.89 and 7.37 ppm, and to the very broad signal at 8.96 ppm (see the Supporting Information). Also, whereas this signal appears as a sharp singlet at higher temperatures it resolves into a doublet below 200 K before broadening again at very low temperatures. Based on its chemical shift and its resolved coupling at lower temperatures, the signal may be attributed to methyl protons of tightly bound residual methanol within the sample. Consequently, the broad signal observed at 8.96 ppm at 133 K must arise from the methanol OH proton. Given these assignments, the observed NOE contacts for $^{3}T_{Si}A$ can be rationalized by methanol-mediated base pairing with OH of methanol bridging the adenine N1 acceptor and the thymine imino donor (see Figure 6c). Although possible, an alternative methanol-bound symmetric dimer is hardly conceivable. Rather, folding with methanol-mediated intramolecular AT base pairing seems plausible for this additional $T_{Si}A$ structure. Indeed, molecular models suggest that such a bridging function of methanol enables the formation of an otherwise overly strained intramolecular base pair.

The presumed presence of residual methanol and non-nucleosidic protons raises the question of impurities within the sample and their influence on the complex formation of $T_{Si}A$ at low temperatures. With $T_{Si}A$ well characterized and found to be pure by both mass spectrometry and ^{1}H NMR, those impurities must come from the freonic solvent. However, upon codissolving $T_{Si}A$ and $A_{Si}T$ in Freon (vide infra), no change in the respective complex formation of either $T_{Si}A$ or $A_{Si}T$ is observed, indicating that traces of methanol or other impurities cannot be attributed to the different association behavior of $T_{Si}A$ and $A_{Si}T$. Consequently, the $^{3}T_{Si}A$ species may depend on residual CH_3OH but seems to nevertheless be based on an intrinsic tendency of $T_{Si}A$ to intramolecularly fold into compact

structures as often observed for nonionic oligonucleotides in aqueous solution.

It has to be noted that, being less stabilized by stacking interactions, TA steps in nucleic acids are generally found to exhibit more flexibility and are more deformable compared to more rigid AT steps.[18] Obviously, the formation of additional $T_{Si}A$ structures at low temperatures again points to the higher structural variability of this dinucleotide even under nonaqueous apolar solution conditions. Thus, a second symmetric duplex structure with antiparallel strands and AT Hoogsteen pairing is formed through a *syn* glycosidic torsion angle of the adenosine nucleoside. The Hoogsteen dinucleotide duplex is increasingly populated at very low temperatures and is not significantly less populated compared to the regular Watson−Crick duplex at 113 K. Isolated Hoogsteen base pairs have frequently been observed in aqueous solution, such as in the closing loop of DNA hairpins, in circular oligonucleotides, in chemically modified nucleic acid structures, or upon protein binding and intercalation by drugs like echinomycin.[19] Most noticeably, a fully Hoogsteen base-paired antiparallel duplex DNA with adenosines in the *syn* conformation has been found for a d(ATATAT) sequence by single-crystal X-ray crystallography demonstrating its ability to constitute an alternative secondary structure to the classical B-DNA double helix.[20] It might be argued that AT sequences are strongly polymorphic and special packing effects may be responsible for the Hoogsteen structures seen in the crystal. However, an antiparallel Hoogsteen duplex in polyd(AT) sequences was found to be stable in an aqueous solution during molecular dynamics simulations with an energy similar to that of the B-type duplex.[21] Also, a dynamic conversion of the fully Watson−Crick to a partly Hoogsteen base-paired structure was reported as a result of a single-point substitution of the O4′ oxygen by CH_2 at an adenosine sugar residue in a self-complementary DNA double helix.[22] Overall these data suggest that an antiparallel Hoogsteen duplex exhibiting a smaller strand separation is an accessible structure for an AT-alternating sequence and might compete with normal B-DNA for natural nucleic acids and even more so for nonionic nucleic acid analogues with no electrostatic repulsions between the two backbones.

For the homoassociation of the self-complementary $T_{Si}A$ and $A_{Si}T$ dinucleotides, duplexes formed through two AT base pairs are only possible with an antiparallel strand orientation. To also test the potential formation of parallel-stranded structures with the backbone-modified analogues in an aprotic environment, a 1:1 mixture of $A_{Si}T$ and $T_{Si}A$ were dissolved in the freonic solvent and subjected to additional low temperature NMR experiments. In case of heteroassociation, a parallel duplex stabilized by two AT base pairs is expected to form. However, even when the temperature is decreased to 123 K, no additional signals of a heteroassociate are observed for the mixture and spectra are always a mere superposition of the two individual $A_{Si}T$ and $T_{Si}A$ spectra (not shown). Obviously, the favored formation of antiparallel structures as typically found in natural systems persists with the backbone-modified nucleic acids in aprotic solvents, indicating that neither solvation effects nor steric or electrostatic effects of the backbone have a major impact on this widely observed preference. Yet, parallel-stranded duplexes are known to also form under specific conditions and can be stabilized at neutral pH by reverse Watson−Crick A−T base pairs.[23] Alternatively, parallel-stranded structures can also be formed through Hoogsteen A−T base pairing.[24] However, reported duplexes with A−T sequences only form under conditions where a parallel strand orientation is enforced or extensive mismatches in the antiparallel orientation limit competition with a regular antiparallel structure.

Hydrogen Bond Interactions. Comparison of proton chemical shifts in the slow exchange regime at 133 K can be used as a sensitive measure for the relative strength of hydrogen bonds. A more deshielded proton in the hydrogen bridge originates from a displacement of the proton toward the acceptor atom and is associated with a lengthening of the covalent A−H bond with an attenuation of shielding effects by the sigma electron pair and a shortening of the H···B hydrogen bond in the A−H···B hydrogen bridge.[5e,25] With an imino proton chemical shift of 15 ppm in the Watson−Crick duplex, $A_{Si}T$ exhibits the most downfield shifted proton indicating the strongest hydrogen bond interaction among all dinucleotide duplexes. In contrast, the T imino signal of the Watson−Crick duplex formed by the $T_{Si}A$ dinucleotide is shifted upfield by 0.5 ppm compared to the imino proton in $A_{Si}T$. Interestingly, these data again suggest the presence of a more flexible $T_{Si}A$ structure held together by weaker hydrogen bonds when compared to $A_{Si}T$. The $T_{Si}A$ imino resonance of the additional Hoogsteen duplex resonating at 13.5 ppm is further upfield shifted indicating a stronger Watson−Crick NH···N1 hydrogen bond when compared to the corresponding Hoogsteen NH···N7 hydrogen bond. Such a relative strength of AT Watson−Crick and Hoogsteen hydrogen bonds is also suggested by previous scalar coupling analyses for Hoogsteen−Watson−Crick T−A−T triplets in an intramolecular DNA triplex[26] and has also been found as a result of corresponding low temperature NMR studies in Freon for a mixture of free uridine and adenosine nucleosides that have shown the formation of minor amounts of Hoogsteen base pairs in addition to predominant Watson−Crick base pairs.[25] Finally, the most shielded imino proton with a chemical shift of 12.8 ppm assigned to the third structure of $T_{Si}A$ supports its tentative assignment to an intramolecularly folded structure with methanol-mediated AT base pairing.

Interaction of Polycyclic Mutagens with $A_{si}T$ in Aprotic Solvents. Since $A_{Si}T$ has been shown to form a single well-defined Watson−Crick duplex structure in a freonic solvent, it was employed as a potential target for DNA binding ligands. Thus, the polycyclic aromatic hydrocarbon 9-(methylaminom-

(18) (a) Packer, M. J.; Dauncey, M. P.; Hunter, C. A. *J. Mol. Biol.* **2000**, *295*, 71–83. (b) Mack, D. R.; Chiu, T. K.; Dickerson, R. E. *J. Mol. Biol.* **2001**, *312*, 1037–1049.
(19) Mendel, D.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 910–914.
(20) (a) Abrescia, N. G. A.; Thompson, A.; Huynh-Dinh, T.; Subirana, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2806–2811. (b) Abrescia, N. G. A.; González, C.; Gouyette, C.; Subirana, J. A. *Biochemistry* **2004**, *43*, 4092–4100.
(21) Cubero, E.; Abrescia, N. G. A.; Subirana, J. A.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **2003**, *125*, 14603–14612.
(22) Isaksson, J.; Zamaratski, E.; Maltseva, T. V.; Agback, P.; Kumar, A.; Chattopadhyaya, J. *J. Biomol. Struct. Dyn.* **2001**, *18*, 783–806.
(23) (a) van de Sande, J. H.; Ramsing, N. B.; Germann, M. W.; Elhorst, W.; Kalisch, B. W.; v. Kitzing, E.; Pon, R. T.; Clegg, R. C.; Jovin, T. M. *Science* **1988**, *241*, 551–557. (b) Otto, C.; Rippe, K.; Thomas, K.; Ramsing, N. B.; Jovin, T. M. *Biochemistry* **1991**, *30*, 3062–3069. (c) Parvathy, V. R.; Bhaumik, S. R.; Chary, K. V. R.; Govil, G.; Liu, K.; Howard, F. B.; Miles, H. T. *Nucleic Acids Res.* **2002**, *30*, 1500–1511.
(24) Raghunathan, G.; Miles, H. T.; Sasisekharan, V. *Biopolymers* **1994**, *34*, 1573–1581.
(25) Dunger, A.; Limbach, H.-H.; Weisz, K. *J. Am. Chem. Soc.* **2000**, *122*, 10109–10114.
(26) Dingley, A. J.; Masse, J. E.; Peterson, R. D.; Barfield, M.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 6019–6027.

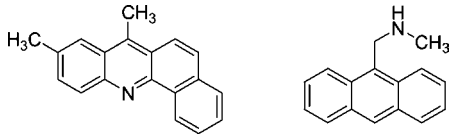


Figure 7. Structures of 7,9-dimethylbenz[*c*]acridine (left) and 9-(methylaminomethyl)anthracene (right).

ethyl)anthracene[27] and the heterocyclic 7,9-dimethylbenz[*c*]acridine[28] known to exert mutagenic effects on DNA by intercalating between nucleic acid base pairs under physiological conditions were selected for binding studies with the backbone-modified dinucleotide (Figure 7). By employing apolar solvent conditions, hydrophobic effects associated with the release of water upon complex formation are eliminated, and any potential interaction between an intercalating ligand and the nucleic acid analogue is expected to mostly rely on enthalpic contributions from aromatic stacking interactions, where dipole−dipole and dispersion energies play a major role.

The two ligands were mixed with $A_{Si}T$ in a 1:1 molar ratio and dissolved in CD_2Cl_2 or Freon. Low temperatures are expected to additionally promote ligand binding in the case of negative binding enthalpies. However, due to the limited solubility of the anthracene derivative, only the benz[*c*]acridine could be measured at temperatures below 200 K in the freonic solvent. Upon decreasing the temperature, no extra set of dinucleotide resonances due to the formation of additional ligand complexes in a slow exchange are observed and there are also no obvious shifts or line broadening effects of DNA resonances in the presence of either compound as anticipated in case of intercalation, terminal stacking, or other nonspecific exterior binding (see the Supporting Information). The absence of any ligand−duplex interaction is further confirmed by additional $^1H-^{29}Si$ HMBC experiments showing no noticeable changes in the ^{29}Si chemical shifts or $^1H-^{29}Si$ coupling constants. Because ligand intercalation should be accompanied by significant distortions of the silyl backbone, intercalation can be safely excluded.

Apparently, interactions between the nonionic ligand and DNA are strongly disfavored under our aprotic solvent conditions. Recently, stacking interactions in a simple binary proflavine−TA base pair model were studied with van der Waals density functional calculations.[29] For the uncharged acridine derivative, a considerable dispersion energy of <-10 kcal/mol was found to promote stacking interactions and intercalation. Because dispersion energies are hardly affected by the transfer from an aqueous to an apolar environment, the lack of additional electrostatic contributions as occurring with protonated (charged) molecules and polyanionic DNA but mostly the lack of hydrophobic contributions associated with a favorable binding entropy must be responsible for the absence of duplex binding by the present ligands under our solution conditions. These observations highlight the crucial role of water release with its positive entropy upon ligand binding to DNA under physiological conditions and cast doubt on whether such molecules may be used as effective modulators of genetic information in a nonaqueous environment.

Conclusions

The study of nonionic nucleic acid analogues in a non-natural aprotic environment allows for a more detailed understanding of the influence of backbone structure, base sequence, and solvent on the hydrogen bond mediated association to form different secondary structures. It also gives some insight into the ability of nucleic acids to serve as carriers of genetic information in various environments. But most importantly, by significantly changing parameters affecting intra- and intermolecular interactions, the reported low-temperature NMR studies provide valuable information on the predominant factors that govern the formation of nucleic acid structures and ligand−DNA interactions and that otherwise are difficult to extract due to their often inextricable influence under physiological conditions. In particular, the present studies highlight the impact of water playing a direct structural role, modulating electrostatic interactions, or being associated with hydrophobic effects. Apparently, a sequence-specific DNA strand association following simple rules is still supported under our nonaqueous experimental conditions at least for short sequences. However, the regular Watson−Crick recognition scheme seems to be less robust upon changing the sequence as already apparent for the $T_{Si}A$ dinucleotide forming additional complexes with a different hydrogen bond pattern. Available thermodynamic data for a number of specific drug−DNA association reactions indicate that hydrophobic transfer processes from aqueous solution into the interior of DNA provides the predominant driving force for drug binding.[30] Consequently, the lack of water is suggested to be a critical determinant of any intercalation process between DNA base pairs essentially preventing the reversible formation of stable DNA−intercalator complexes if not promoted by exceptionally strong dipole−dipole and dispersion forces. Likewise, the DNA affinity of groove binding agents interacting with only a limited number of specific hydrogen bonds or salt bridges is expected to be low upon the removal of hydrophobic effects in nonaqueous environments.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, for financial support (Grant No. WE 1933/10-1).

Supporting Information Available: $^1H-^{29}Si$ HMBC spectrum of $A_{Si}T$; tables with (i) ^1H NMR chemical shifts of $A_{Si}T$ at 163 K, (ii) $^1H-^1H$ scalar coupling constants of $A_{Si}T$ at 243 K, (iii) ^1H NMR chemical shifts of $T_{Si}A$ at 133 K, and (iv) $^1H-^1H$ scalar coupling constants of $T_{Si}A$ at 273 K; NOESY spectrum of $A_{Si}T$ in Freon at 163 K; imino proton chemical shift of $A_{Si}T$ as a function of concentration at 294 K; portion of a NOESY spectrum of $T_{Si}A$ in Freon at 133 K; ^1H NMR spectra of $A_{Si}T$ without and with 7,9-dimethylbenz[*c*]acridine at 253 K; synthesis and characterization of $A_{Si}T$ and $T_{Si}A$. This material is available free of charge via the Internet at http://pubs.acs.org.

JA910220S

(27) Becker, H.-C.; Nordén, B. *J. Am. Chem. Soc.* **1999**, *121*, 11947–11952.
(28) Chan, E. W.; Ball, J. K. *Biochim. Biophys. Acta* **1971**, *238*, 31–45.
(29) Li, S.; Cooper, V. R.; Thonhauser, T.; Lundqvist, B. I.; Langreth, D. C. *J. Phys. Chem. B* **2009**, *113*, 11166–11172.
(30) Chaires, J. B. *Biopolymers* **1998**, *44*, 201–215.