Deoxyadenosine and Thymidine Bases Held Proximal and Distal by Means of a Covalently-Linked Dimensional Analogue of dA·dT: Intramolecular vs Intermolecular Hydrogen Bonding[1]

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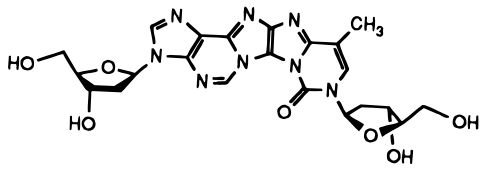
Abstract: Deoxyadenylic acid and deoxythymidilic acid have been attached to a covalently-linked cross section, dA⟩⟨dT, on the same side (proximal) and on opposite sides (distal) by synthetic sequences involving various combined protection/deprotection steps. The structures in aqueous solution buffered at pH 7.0 have been examined by 2D-NOESY NMR spectroscopy. The covalently-linked cross section provides a template that stabilizes dA·dT base pairing in the proximal isomer at 2 °C. In the distal isomer, it contributes to favorable conformations for intermolecular association.

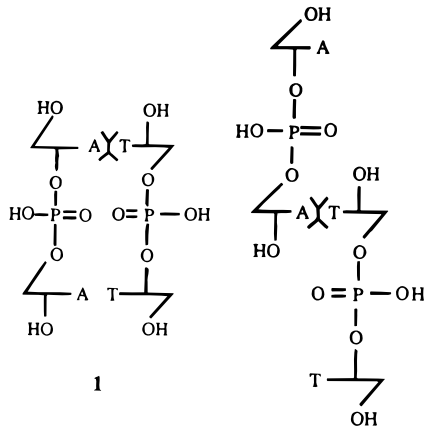
Introduction

The forces that stabilize associations between nucleic acid bases, namely, hydrogen bonding and base stacking, have been well studied,[2] and various cross-linking methods have been utilized to examine structural interactions between bases or strands of bases.[3−14] Hydrogen bonding between imides and adenine or adenosine has been examined and identified in structures consisting of aromatic stacking surfaces rendered water soluble.[15−17] We have now provided water soluble constructs that allow us to determine interactions between deoxyadenosine and deoxythymidine units held in place by the template dA⟩⟨dT that is a dimensional mimic of a fixed dA·dT base pair.[18] The proximal isomer (**1**) locates the dA and



dA⟩⟨dT

dT units so that they can bond intramolecularly, whereas the distal isomer (**1′**) fixes these units where intramolecular bonding is unfavorable but where they are free to associate intermolecularly. In effect, the distal **1′** serves as a control for the intramolecularity of any observed dA−dT interaction in **1**.



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(1) Abbreviations used: A*,N^6-(allyloxycarbonyl)-2′-deoxyadenosine; dA⟩⟨dT, 3,9-bis(2′-deoxy-β-D-ribofuranosyl)-11-methyl-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one; AOC, allyloxycarbonyl; DMAP, 4-(dimethylamino)pyridine; DMT, 4,4′-dimethoxytrityl; $Pd_2(dba)_3$·$CHCl_3$, tris(dibenzylideneacetone)dipalladium(0) chloroform adduct; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; 2D-NOESY, two-dimensional nuclear Overhauser effect spectroscopy.

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Results and Discussion

Synthesis. The starting material for the synthesis of the proximal isomer (**1**) was 3-(3′,5′-di-*O*-acetyl-2′-deoxy-*β*-D-ribofuranosyl)-11-methyl-9-[3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxanediyl)-2′-deoxy-*β*-D-ribofuranosyl]-3*H*-pyrimido[1″,6″:1′,2′]imidazo[4′,5′:4,5]imidazo[2,1-*i*]purin-8(9*H*)-one (**2**),[18] selectively protected on each side of dA⟩dT. The disiloxane protection was removed by stirring in tetrahyrofuran with tetrabutylammonium fluoride at 25 °C for 1.5 h to give **3**, protected on the deoxyadenosine side by *O*-acetyl groups. Compound **3**, as the acetonitrile solvate monohydrate, was determined by X-ray analysis to be very close to coplanar for the atoms constituting the central pentacyclic N-aromatic system (root-mean-square deviation 0.03 Å).[19] The further steps in the synthesis of the bis-triethylammonium salt (**17**) of the desired proximal isomer **1** are outlined in Scheme 1 (see the Experimental Section). We found it advantageous to use a combination of the conventional protection/deprotection reagents employed in the synthesis of oligodeoxyribonucleotides in solution, along with allyl/allyloxycarbonyl protection on phosphite and phosphate and on the 6-NH_2 of deoxyadenosine, respectively.[20] The mild Hayakawa−Noyori methodology, including hydrogenolytic cleavage of these protecting groups,[20] was conserving of structures and quantities throughout. The average yield at each step was 84%, and the overall yield from **3** to **17** was 10%. The structures of the intermediates and product (**17**), once they were determined to be homogeneous, were established by the sequential synthetic procedure, by their low- and/or high-resolution mass spectra, and by ^{31}P NMR, as deemed necessary. The final purification of the proximal example (**1**) for NMR study was achieved by reverse phase HPLC using aqueous sodium phosphate and a methanol gradient. This was followed by desalting on the same HPLC column. The appropriate fractions were pooled, and the sample was dried under reduced pressure and kept frozen.

The synthesis of the distal isomer (**1′**) proceeded from the common intermediate in both syntheses (**11** in Scheme 1 = **2′** in Scheme 2). The yield from **2′** to product **10′** was 21%, comprised of an average yield of 78% for each step. The structures of the intermediates, once determined to be homogeneous, were established by the sequential synthetic procedure and by their mass spectra. The final product (**1′**), first obtained as the disodium salt (**10′**), was purified, desalted, and preserved as for the proximal isomer. The high-resolution mass spectrum confirmed the structure.

This extended exercise in synthesis has further purpose. Thus, the proximal isomer **1** is a potential substrate for blunt-end ligation. The distal isomer **1′**, suitably derivatized, is a possible candidate for self assembly.[21,22] If the latter intention is realized, a stiff, rodlike oligodeoxynucleotide analogue of poly(dA)·poly(dT) would be created, one that offers little opportunity for bending and none for slippage. In the present study, the surface of dA⟩dT provides an undissociable equivalent of dA·dT. The proximal model **1**, which prevents any fraying beyond the terminal dA and dT, thus serves as a limiting case. Fraying of a duplex, wherein the second base pair, dA·dT, is dissociable, has been thoroughly examined in the case of [d(TAGCGCTA)]$_2$.[23]

NMR. The complete formulas of the proximal **1** and distal **1′** isomers are shown in Figure 1, depicted as viewed from the

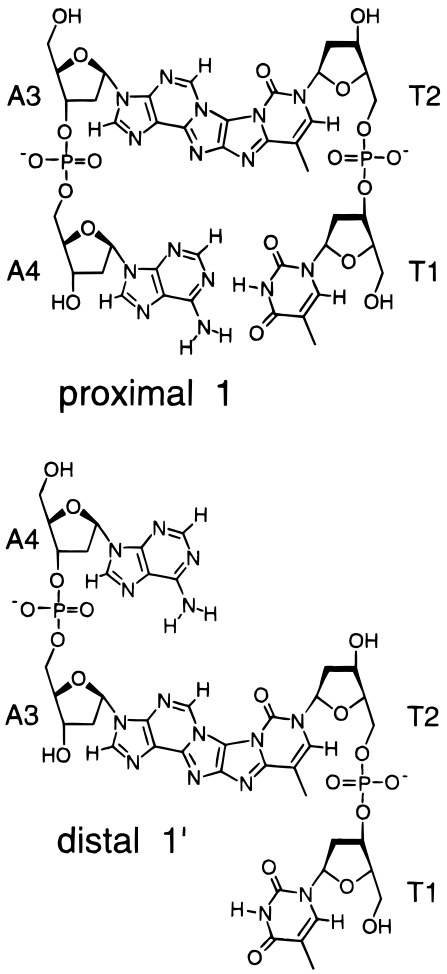

Figure 1. Chemical structures of proximal **1** and distal **1′**. The total structures are given as viewed from the major groove.

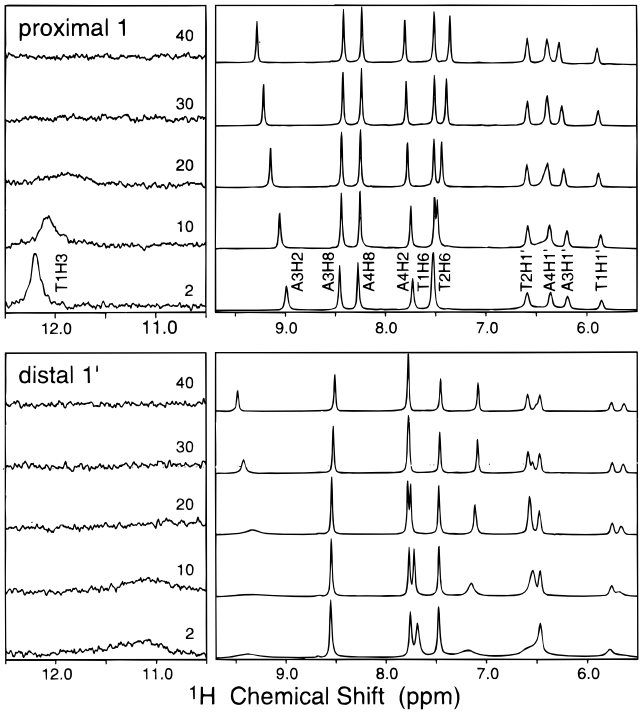

Figure 2. Temperature-dependent 1D-NMR spectra of the proximal-type (**1**) and the distal-type (**1′**) oligonucleotides. The T1H3 imino proton of **1** is clearly detected at 12.20 ppm at 2 °C, whereas that of **1′** is seen as a broad resonance centered at 11.2 ppm.

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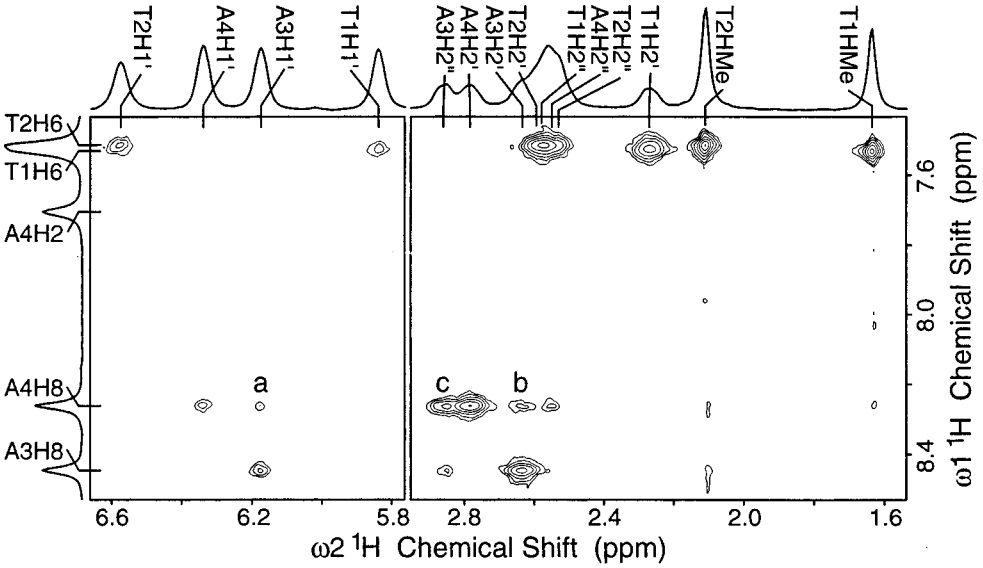

Figure 3. The observed nonexchangeable proton 2D-NOESY spectrum showing the expanded aromatic to H1′ and H2′/H2″ region of the proximal-type **1** at 2 °C. NOE's a, b, and c are from A4H8 to A3H1′, A4H8 to A3H2′, and A4H8 to A3H2″, respectively.

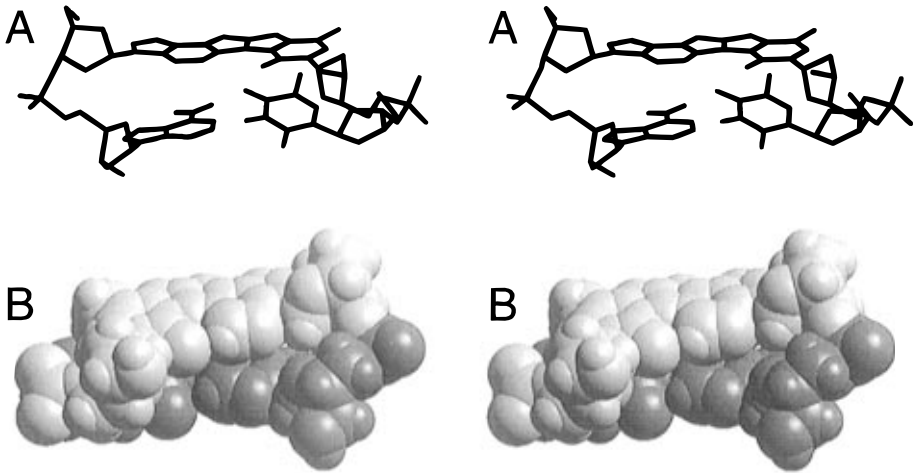

Figure 4. The NOE-restrained refined structure of the proximal-type **1**. The upper stereoscopic view (A) is from the major-groove direction. The lower stereoscopic view (B) is from the minor-groove direction.

major groove of a potential helical structure. The terminal deoxythymidine and deoxyadenosine are designated as T1 and A4, respectively. The pyrimidine and purine moieties in the fused base, dA^dT, are designated as T2 and A3, and the hydrogens therein are numbered as they would be for the separate components. This system of lettering and numbering makes the proton interactions more readily traceable in the discussion of the nuclear magnetic resonance. As background, the proton resonances in the deoxyadenosine portion of dA^dT have been previously assigned as 9.73 for "H2" (actually H5 in the full pentacyclic numbering system) and 8.62 for "H8" (actually H2). The aromatic proton resonance in the thymidine portion has been assigned as 7.74 for "H6" (actually H10), also in DMSO as the solvent.[18] These, in turn, were checked by comparison with assignments in the dA^dU series that were established by long-range heteronuclear $^{1}H/^{13}C$ correlations.[24]

In Figure 2 are compared the temperature-dependent behaviors of the imino proton and aromatic proton resonances from their 1D-NMR spectra. The resonance assignments for proximal **1** have been confirmed by using the nonexchangeable proton 2D-NOESY and TOCSY spectra (data not shown) recorded at 2 °C. The T1H3 imino proton is clearly detected at 12.20 ppm, which indicates that T1 forms a Watson−Crick base pair with A4 and is stacked on the fused base dA^dT, forming a miniduplex at 2 °C. Intramolecular association of the imino proton persists at least up to 20 °C, which is remarkable for such a small miniduplex that is analogous to a pair of dinucleoside monophosphates. On the basis of the sharpened signal at 2 °C for T1H3, it is possible to state qualitatively that equilibration with open forms is not occurring rapidly. The shape of the signals for the temperature range 2−20 °C is the combined result of the hydrogen-bonded ⇄ nonbonded equilibrium and the rates of unbonding and exchange.

Of the aromatic protons, A3H2 shows the greatest chemical shift, 8.95 ppm, at 2 °C in the aqueous buffer. Base stacking at the lowest temperature is indicated also by this proton resonance. As the temperature is raised to 40 °C, the A3H2 signal progresses downfield to 9.28 ppm. The deshielding with rising temperature is consistent with the loss of the ring current effect as the T1−A4 base pair becomes unstacked, i.e., with greater participation of open conformations. There appears to be a crossover of T2H6 and T1H6 from coincidence at about

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7.53 ppm at 2 °C to a difference in chemical shift of ca. 0.16 ppm at 40 °C.

For the distal isomer **1′**, broad resonance was observed, centered at 11.2 ppm, at 2 °C, which indicates that T1H3 may be loosely involved in hydrogen-bonding interaction *inter*molecularly with acceptor molecules (Figure 2). While the A3H2 is at higher field at 2 °C than at 40 °C, the signal at 2 °C is broadened, suggesting a family of partially stacked conformations. Other nonexchangeable resonances also exhibit broad signals that sharpen as the temperature is raised. The differences may be the result of a large change in the spin−spin relaxation times, and the broadening at low temperature is consistent with the existence of different degrees of aggregation.

Conclusive evidence for the duplex structure of proximal **1** is found in the observed internucleotide NOE crosspeaks between A4H8 and (a) A3H1′ (Figure 3), (b) A3H2′, and (c) A3H2″, and between A3H1′ and A4H5′/5″ (data not shown). These NOE crosspeaks are consistent with a right-handed twist to the helix.

The 3D solution structure of proximal **1** was obtained by a combined SPEDREF[25] and NOE-constrained molecular dynamics refinement.[26] In the refined duplex, the T1 base is stacked significantly with the fused base, whereas only the N6 amino group of A4 is stacked above the fused base (Figure 4). The sugar pucker in the refined structure is of the S-type for the T1 and A3 nucleotides, and of the N-type for the T2 and A4 nucleotides. The measured $J_{1',2'}/J_{1',2''}$ coupling constants of the four sugar rings from the PE-COSY spectrum are 7.4/6.5, 7.1/9.5, 8.0/5.5, and 6.0/8.4 Hz, respectively, for T1, T2, A3, and A4 units. Those values are consistent with the sugar puckers seen in the refined structure.

Our structural study shows that the fused base pair dA⟩⟨dT provides an excellent template to stabilize substantially the formation of adjacent dA·dT base pairs. When dA and dT are attached on opposite sides of the template, intermolecular association is detected and the prospect of self-assembly is thus recognized, possibly through appropriate 3′-*O*-phosphoramidite substitution.

Experimental Section

General. The synthesis section can be abbreviated because the reaction conditions for the synthesis of all intermediates are given in the legends to Schemes 1 and 2. The intermediates, which are interrelated sequentially, were characterized by their chemical conversions, by comparative TLC, and, where we considered it necessary, by mass spectrometry and NMR. Many of the intermediates in the sequence were isolated by method A, as follows: The reaction mixture was poured into cold water and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by chromatography over silica gel. Where there were variations from this methodology for individual intermediates, the separate conditions are mentioned. The quantities used ranged from hundreds of milligrams down to tens of milligrams. More specific details of each step can be made available by communication with the authors, but the experimental procedures will be evident to anyone versed in blocking/deblocking sequences.[20,27]

Compound 1

Allyl Phosphorodichloridite, CH_2═$CHCH_2OPCl_2$. This reagent was prepared according to the method described for methoxyphosphorodichloridite.[28] Although the reagent has been prepared and used by the Noyori group,[20,29−31] there have been reports of low yields and of explosions during distillation.[32] Accordingly, we feel that it is important to describe the synthesis of allyl phosphorodichloridite in detail, applying the precautions indicated. *At every stage, the preparation and purification were carried out in a well-ventilated hood behind a protective shield.* In a 100-mL three-necked, round-bottomed flask equipped with an immersion thermometer, dropping funnel, and calcium chloride drying tube was placed 50 g (0.36 mol) of 99.99+% phosphorous trichloride under an argon atmosphere, and the mixture was cooled to approximately −55 °C in dry ice/isopropyl alcohol. The dropping funnel was equipped with a rubber septum through which argon could be purged. The PCl_3 was cooled for 15 min to attain a −55 °C inside temperature. Allyl alcohol (21.11 g, 0.36 mol, 99+%) was added slowly (2 h) through the dropping funnel, during which time the temperature of the reaction mixture was allowed to rise to −30 °C. It was then allowed to rise to 20 °C, and stirring was continued overnight (20 h) under a slow stream of argon. The volume of the reaction mixture decreased by about 10%. The thermometer and drying tube were replaced with stoppers, and the dropping funnel was replaced by a fractionating column. The reaction mixture was heated by an oil bath to a final temperature of 80 °C during fractionation. The first fraction of distillate (4.7 g) was collected at 20 °C and 50 mmHg (discarded), the second (2.5 g) was collected at 35 °C (discarded), and a third fraction (7.0 g) was collected at 40 °C and 15 mmHg, and a final fraction (15 g) at 51−54 °C, giving a total yield of 39%. Distillation was discontinued while the reaction mixture still remained colorless. The residual oil was cooled to −80 °C, and to it was added 30 mL of $CHCl_3$−1-butanol (1:1). The temperature in the flask was allowed to rise to 20 °C, and the flask was left for about 15 h, during which time a bright yellow precipitate settled out. Final (safe) decomposition of the contents of the flask was effected by cooling to −70 °C and addition of 10 mL of 2 M NaOH.

(Allyloxy)bis(diisopropylamino)phosphine, CH_2═$CHCH_2OP[N(i\text{-}Pr)_2]_2$, was prepared from CH_2═$CHCH_2OPCl_2$,[20] bp 115−116 °C/2 mmHg, yield 71%, purity >99% (^{31}P NMR).

Compounds **2** and **3** were synthesized as described previously.[18]

4. After step *b* (see legend for Scheme 1), sequential manipulation A (see General, above), and the use of 5% CH_3OH in $CHCl_3$ containing a trace of Et_3N in chromatography, appropriate fractions were pooled and concentrated. The residual oil was taken up in anhydrous CH_2Cl_2, and addition of hexane furnished a colorless precipitate. Solvent was removed under reduced pressure, and the colorless powder was dried under high vacuum to give **4**, $C_{47}H_{46}N_8O_{11}$, in 92% yield: low-resolution FAB MS *m*/*e* 899.2 (MH^+).

5. After step *c* and method A, the use of 2−4% CH_3OH in $CHCl_3$ in chromatography, pooling of fractions, and concentration, **5**, $C_{53}H_{60}N_8O_{11}Si$, was obtained as a colorless foam: yield 93%; low-resolution FAB MS *m*/*e* 1013.6 (MH^+).

6. After step *d*, the solution was poured into saturated $NaHCO_3$ solution and extracted with ethyl acetate. The ethyl acetate was dried over anhydrous Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The residual oil was purified by chromatography on silica gel using 3−7% CH_3OH in $CHCl_3$. Concentration of appropriate fractions was followed by precipitation of the product with CH_2Cl_2−hexane (1:9). Removal of solvent under vacuum gave compound **6**, $C_{32}H_{42}N_8O_9Si$, as a colorless solid: yield 82%; low-resolution FAB MS *m*/*e* 711.2 (MH^+).

5′-*O*-(*tert*-Butyldimethylsilyl)thymidine 3′-*O*-(Allyl *N*,*N*-diisopropylphosphoramidite), $C_{25}H_{46}N_3O_6PSi$, 7. To a solution of 5′-*O*-(*tert*-butyldimethylsilyl)thymidine (330 mg, 0.92 mmol) in anhydrous CH_3CN (6 mL, dried over CaH_2 prior to use) was added diisopropylamine (0.142 mL, 1.02 mmol), 1*H*-tetrazole (71 mg, 1.02 mmol), and

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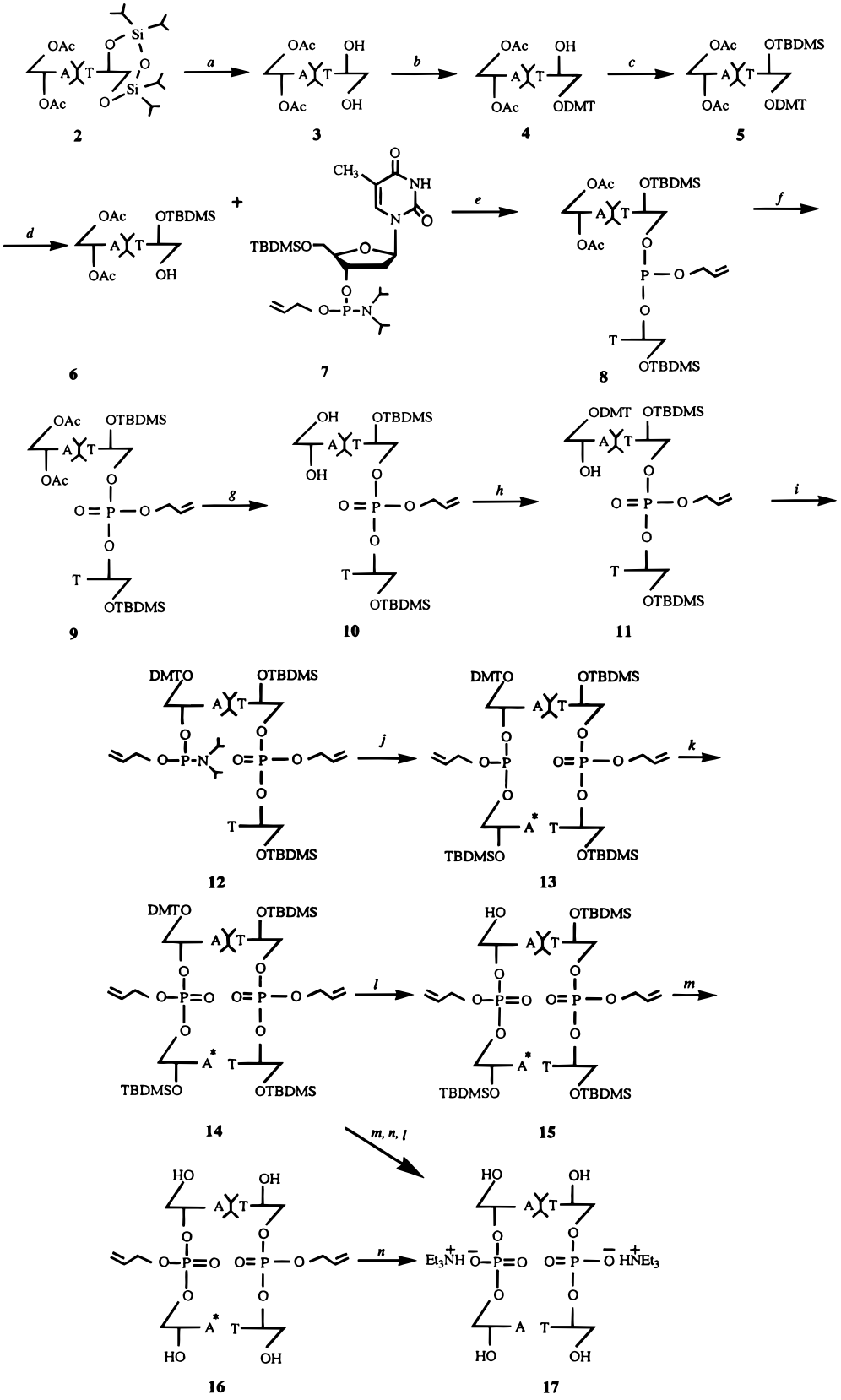
Scheme 1[a]



[a] (*a*) TBAF on silica gel, THF, 1.5 h, 25 °C; (*b*) DMTCl, Py, 20 h, 25 °C; (*c*) TBDMSCl, imidazole, DMF, 4 h, 25 °C; (*d*) $Cl_2CHCOOH$, CH_2Cl_2, 3 min, 25 °C; (*e*) **7**, 1*H*-tetrazole, CH_3CN, 2 h, 25 °C; (*f*) excess *t*-BuOOH, CH_2Cl_2, 3.5 h, 0 °C; (*g*) *t*-$BuNH_2$, CH_3OH, 15 min, 0 °C, then 4 h, 25 °C; (*h*) DMTCl, Py, DMAP, 2.5 h, 25 °C; (*i*) $CH_2{=}CHCH_2OP[N(i\text{-}Pr)_2]_2$, $HN(i\text{-}Pr)_2$, 1*H*-tetrazole, CH_3CN, 2.5 h, 25 °C; (*j*) 3′-*O*-TBDMS-A*, 1*H*-tetrazole, CH_3CN, 1.5 h, 25 °C; (*k*) *t*-BuOOH, CH_2Cl_2, 1 h, 0−25 °C; (*l*) $Cl_2CHCOOH$, CH_2Cl_2, 3 min, 25 °C; (*m*) TBAF on silica gel, THF, 14 h, 25 °C; (*n*) $(C_6H_5)_3P$, THF, $C_4H_9NH_2$, HCOOH, $Pd_2(dba)_3{\cdot}CHCl_3$, 5 h, 25 °C. Note: In terms of double-strand helicity, these formulas are depicted as viewed from the major groove.

(allyloxy)bis(diisopropylamino)phosphine (0.43 mL, 1.4 mmol). The reaction mixture was stirred at 20 °C for 1.5 h, taken up in ethyl acetate (30 mL), washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by chromatography on silica gel (5 × 1.5 in. column) using hexane−ethyl acetate (1:1) as eluent. Removal of solvent under reduced pressure and eventually under high vacuum yielded **7** (95%) as a colorless gum, the purity of which was checked by ^{31}P NMR, giving a chemical shift value identical with that described for the 5′-*O*-dimethoxytrityl derivative.

8. After step *e* and method A, with 5% CH_3OH in $CHCl_3$ as the eluting chromatographic solvent, appropriate fractions were combined and concentrated, and CH_2Cl_2−hexane (1:9) was added to effect precipitation. Removal of solvent and drying for 3 h under high vacuum yielded **8**, $C_{51}H_{73}N_{10}O_{15}PSi_2$, as a light yellow solid: yield 99%; homogeneous by a single spot on TLC.

9. After step *f*, CH_3OH was added before concentration. Purification was effected by chromatography on silica gel, with 5% CH_3OH in $CHCl_3$, and the usual workup to furnish the desired intermediate **9**, $C_{51}H_{73}N_{10}O_{16}PSi_2$: yield 79%; homogeneous single spot on TLC.

10. After step *g*, CH_3OH was removed. Purification was effected on a short silica gel column using 10−15% CH_3OH in $CHCl_3$ as eluent. Appropriate fractions were combined and concentrated, followed by precipitation of the residual oil with CH_2Cl_2−hexane (1:20). Removal of the solvent furnished the deacetylated product **10**, $C_{47}H_{69}N_{10}O_{14}PSi_2$, as a colorless solid: yield 77%; homogeneous by a single spot on TLC.

11. After step *h*, addition of CH_3OH, and concentration under reduced pressure, the residual oil was co-evaporated with anhydrous toluene. This residue was purified by flash chromatography on silica gel using 5−7% CH_3OH in $CHCl_3$ followed by 15% CH_3OH in $CHCl_3$. Appropriate fractions were combined and concentrated to give intermediate **11**, $C_{68}H_{87}N_{10}O_{16}PSi_2$, as a colorless powder: yield 79%; homogeneous by a single spot on TLC.

12. After step *i* and method A, including purification on silica gel using hexane−ethyl acetate−CH_3OH (45:45:10) for elution, appropriate fractions were combined and concentrated to give intermediate **12**, $C_{77}H_{106}N_{11}O_{17}P_2Si_2$, as a colorless foam: yield 82%; homogeneous by single spot on TLC.

13. After step *j* and method A, 5−7% CH_3OH in $CHCl_3$ eluent fractions were concentrated to give **13**, $C_{91}H_{121}N_{15}O_{22}P_2Si_3$, as a colorless solid: yield 83%; low-resolution FAB MS *m*/*e* 1922.5 (MH^+), 1938.1 $(MH + O)^+$.

14. After step *k*, CH_3OH was added and the combined solvent was removed in vacuo. The residue was dissolved in 10% CH_3OH in $CHCl_3$ and chromatographed on a short silica gel column. Appropriate fractions were combined and concentrated, and the residual oil was precipitated with CH_2Cl_2−hexane (1:20). Removal of the solvent in vacuo furnished the fully protected **14**, $C_{91}H_{121}N_{15}O_{23}P_2Si_3$, as a colorless solid: yield 94%; low-resolution FAB MS *m*/*e* 1938.9 (MH^+).

15. The first of the protecting groups was removed by step *l*. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to give **15**, $C_{70}H_{103}N_{15}O_{21}P_2Si_3$ (yield approximately 85%), which was used directly under conditions in step *m* without further purification.

16. After step *m*, the solvent was removed under reduced pressure, and the residue was triturated three times with methanol and filtered through cotton wool. The solvent was removed from the filtrate, and the residue was purified on a short silica gel column, with 20% CH_3OH in $CHCl_3$ as the eluent. Appropriate fractions were combined and concentrated. Addition of CH_3CN gave a colorless precipitate. Solvent was removed under reduced pressure to give **16**, $C_{52}H_{61}N_{15}O_{21}P_2$: yield 59%; homogeneous by a single spot on TLC; low-resolution FAB MS *m*/*e* 1294.4 (MH^+).

17. After step *n*, the residue was taken up in ethyl acetate which was extracted with water several times. The aqueous layer was rotoevaporated for 10 min to remove any dissolved ethyl acetate. It was purified on a Sephadex A-25 column preequilibrated with 0.01 M TEAB buffer using 0.01−0.7 M linear gradient of TEAB. This method gave **17** in extremely low yield. Therefore, an alternative method was used to prepare compound **17**. The sequence of reaction steps after **14** was changed, in which case the bisphosphate was obtained after purification and desalting (see below).

17 (Preferred). After steps *m*, *n*, and *l*, ice water was added to the reaction flask, and dichloromethane was removed under reduced pressure. The aqueous layer was neutralized with 1 M NaOH to pH 7 and purified on a preequilibrated (0.01 M TEAB) column using 0.01−0.7 M TEAB as linear gradient. Appropriate fractions were combined and lyophilized. The residue was dissolved in deionized water, transferred to a smaller flask, and relyophilized to give **17**, $C_{56}H_{79}N_{17}O_{19}P_2$, as a colorless fluffy solid: yield 50% based on **14**; low-resolution FAB MS (negative ion) *m*/*e* 1228.2 ($M - Et_3N^+H - 1 = 1228.6$).

The deblocking sequence, i.e., removal of TBDMS, allyl, AOC, and DMT groups, and subsequent purification of **1** are as described in parallel and in greater detail for **1′** (see below).

Compound 1′

Compound 2′, $C_{68}H_{87}N_{10}O_{16}Si_2P$, was prepared as described above (**11**).

After step *a* (see legend for Scheme 2), the reaction mixture was quenched with water and extracted with ethyl acetate. The extract was dried over anhydrous Na_2SO_4, filtered, and purified by chromatography on silica gel, using 5% CH_3OH in $CHCl_3$ with a trace of Et_3N as eluent. Appropriate fractions were pooled and concentrated to give **3′**, $C_{74}H_{101}N_{10}O_{16}PSi_3$, as a colorless foam: yield 92%; homogeneous by a single spot on TLC.

After step *b*, the reaction mixture was poured into saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, using 7% CH_3OH in $CHCl_3$, and concentration of the appropriate fractions. Solid **4′**, $C_{53}H_{83}N_{10}O_{14}PSi_3$, was obtained by solution of the residue in a minimum amount of CH_2Cl_2 and addition of hexane, followed by solvent removal under reduced pressure: yield 84%; homogeneous by a single spot on TLC.

Compound 5′, N^6-(Allyloxycarbonyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-(allyl *N*,*N*-diisopropylphosphoramidite) was synthesized according to the literature procedure.[20]

Compound 6′, $C_{91}H_{121}N_{15}O_{22}P_2Si_3$, was obtained by reaction of **4′** with **5′** (10% molar excess) in 1*H*-tetrazole and anhydrous CH_3CN after stirring for 3 h at 25 °C. The reaction mixture was diluted with ethyl acetate and washed with brine, and the organic layer was dried over anhydrous Na_2SO_4. After filtration and flash chromatography on silica gel, using 5% CH_3OH in $CHCl_3$ plus a trace of Et_3N as eluent, the appropriate fractions were concentrated to give **6′**: yield 76%; homogeneous by a single spot on TLC. Approximately 12% of unreacted **4** was recovered.

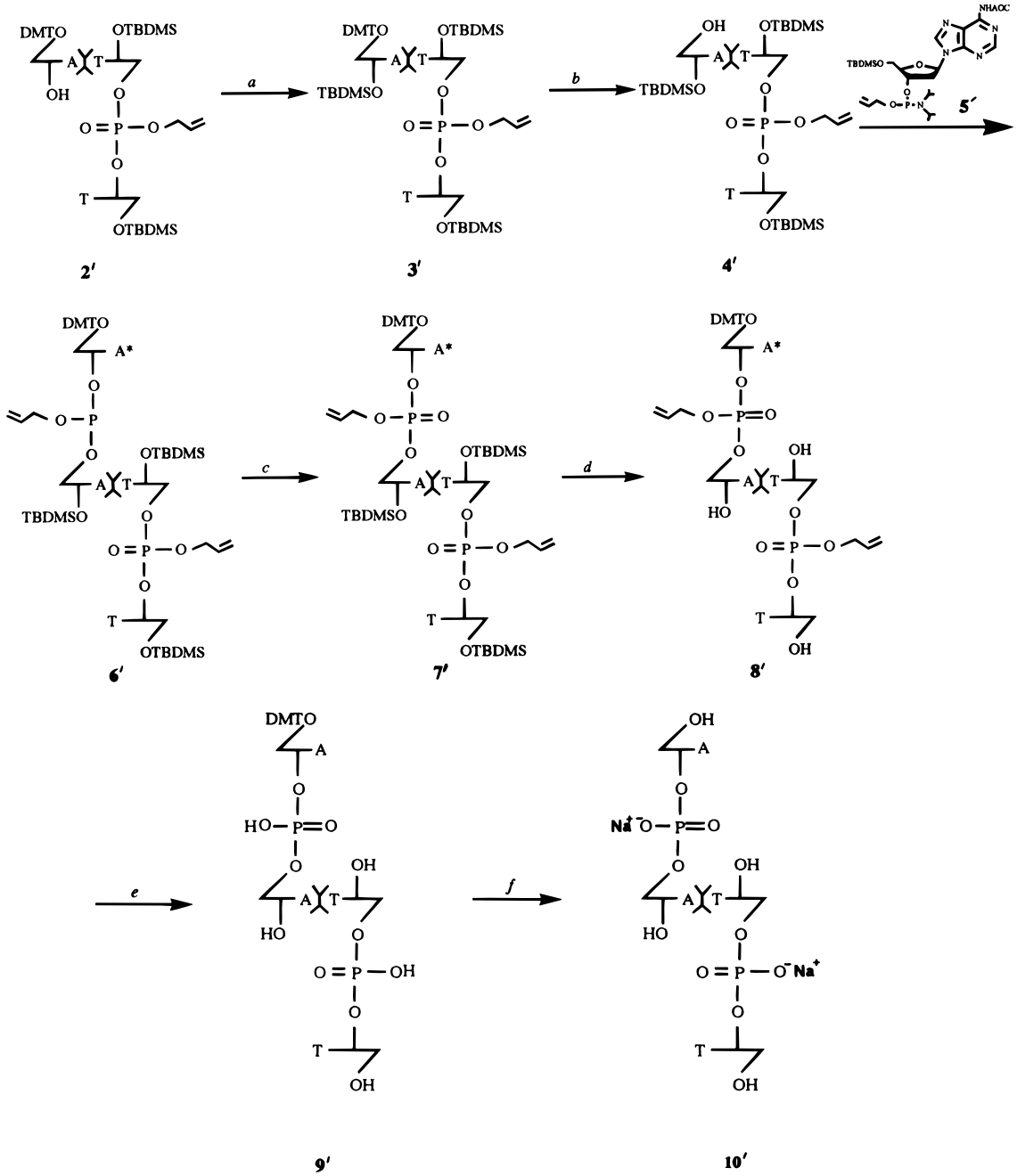
After step *c*, the reaction mixture was quenched with CH_3OH and the solution was concentrated. The residue was purified on a silica gel column, elution with 2−7% CH_3OH in $CHCl_3$. Concentration of the collected fractions gave **7′**, $C_{91}H_{121}N_{15}O_{23}P_2Si_3$, as a light yellow foam: yield 86%; homogeneous by a single spot on TLC; low-resolution FAB MS *m*/*e* 1938.7 (MH^+).

After step *d*, the silica gel was removed by filtration and washed repeatedly with THF and CH_3OH. Purification of the concentrated filtrate and washings was effected by chromatography on a silica gel column, elution with $CHCl_3$ followed by 10−20% CH_3OH in $CHCl_3$. Appropriate fractions were combined and concentrated to give a light yellow gum. Trituration with 1:1 ether−hexane caused the separation of a solid. The supernatant was decanted, and the solid was washed with hexane and dried under high vacuum to give **8′**, $C_{73}H_{79}N_{15}O_{23}P_2$, as an off-white solid: yield 79%; homogeneous by a single spot on TLC; low-resolution FAB MS *m*/*e* 1596.7 (MH^+).

After step *e*, ethyl acetate was added and solvent was removed under vacuum. The residue was azeotroped three times with anhydrous toluene and was finally dried under vacuum for 3 h to give intermediate **9′**, $C_{63}H_{67}N_{15}O_{21}P_2$, which was used directly in the removal of the DMT protecting group.

After step *f*, acetic acid was added and the mixture was stirred for 2 h. Solvent was removed in vacuo. The residue was dissolved, and the pH was quickly adjusted to 7 by addition of 1 M NaOH. The

Scheme 2[a]



[a] (*a*) TBDMSCl, imidazole, anhydrous DMF, 3.5 h, 25 °C; (*b*) $Cl_2CHCOOH$, CH_2Cl_2, 3 min, 25 °C; (*c*) excess *t*-BuOOH, CH_2Cl_2, 25 h, 0 °C; (*d*) TBAF on silica gel, THF, 2.5 h, 25 °C; (*e*) $(C_6H_5)_3P$, THF, $C_4H_9NH_2$, HCOOH, $Pd_2(dba)_3{\cdot}CHCl_3$, 4 h, 25 °C; (*f*) $Cl_2CHCOOH$, CH_2Cl_2, 5 min, 25 °C. Note: In terms of double-strand helicity, these formulas are depicted as viewed from the major groove.

aqueous layer was extracted with ethyl acetate. The combined organic extracts were then extracted with water. The aqueous layers were combined and concentrated to dryness. The solid that separated upon treatment with 90% EtOH was collected, washed with 90% EtOH, and quickly transferred to a vial and dried under high vacuum at 25 °C: **10′**, $C_{42}H_{47}N_{15}O_{19}P_2Na_2$, yield 53% based on **8′**.

Purification and desalting for the NMR study of **1′** was conducted as follows. (This was also done for compound **1**.) We used an ODS 18 Altex reverse-phase HPLC column and a gradient of 20−70% B in 20 min (A = 50 mM sodium phosphate at pH 5.6; B = 95% MeOH), kept 70% B constant for 15 min, and re-equilibrated the column to 20% B in 10 min. The flow rate for preparative separations was 2 mL/min with the UV detector set at 256 nm. The major fractions, 17−20 min, were collected, pooled, lyophilized, and desalted on the same reverse phase column. The column was equilibrated with deionized water (20 column volumes). Samples were dissolved in water and injected into the column. The column was washed with water for 10−15 min at 2 mL/min, followed by a gradient of 0−100% B in 5 min. The product eluted with 100% B after 5−6 min (A = H_2O, B = 95% MeOH). Appropriate fractions were pooled and lyophilized to a solid which was transferred to a small sintered funnel using ether as a solvent. It was then placed in an Eppendorf tube, dried overnight under reduced pressure, and stored in the freezer until use: **1′**, $C_{42}H_{49}N_{15}O_{19}P_2$: UV λ_{max} (H_2O) 328 nm, 260, 236; low-resolution FAB MS *m/e* 1130.6 (MH^+); high-resolution FAB MS *m/e* 1130.2910 ($C_{42}H_{50}N_{15}O_{19}P_2$ requires 1130.2882 amu).

NMR Spectroscopy. Solutions of DNA oligomer were prepared as described earlier.[25] Lyophilized powder (2.0 mg) was dissolved in 0.55 mL of H_2O containing 20 mM phosphate buffer at pH 7.0, resulting in a 3.0 mM duplex solution. NMR spectra were collected on a Varian VXR500 500 MHz spectrometer, and the data were processed with FELIX v1.1 (Hare Research, Woodinville, WA). The nonexchangeable 2D NOE spectra were collected at 2 °C at a mixing time of 0.05 s and a total recycle delay of 6.41 seconds where the average T1 relaxation

Table 1. Chemical Shifts (ppm) for Proximal **1** at 2 °C

	HMe/2	H6/8	H1′	H2′	H2″	H3′	H4′	H5′	H5″	H1/3	H6(a)[a]	H6(b)[a]
T1	1.64	7.53	5.84	2.27	2.59	4.71	4.13	3.78	3.78	12.2		
T2	2.12	7.51	6.58	2.58	2.54	4.74	4.17	4.17	4.25	–		
A3	8.95	8.44	6.18	2.64	2.87	4.93	4.33	3.81	3.81		–	–
A4	7.70	8.26	6.34	2.79	2.55	4.79	4.31	4.21	4.21		na	na

[a] H6(a) are base-pair hydrogen-bonded amino protons, H6(b) are not.

was 1.8 s. The data were collected by the States/TPPI technique[33] with 512 t_1 increments and 2048 t_2 complex points each the average of 24 transients. TOCSY (total correlated spectroscopy) spectra were used together with the NOE spectra to derive the assignment using the standard sequential assignment procedure. The chemical shifts of all assigned resonances are listed in Table 1.

A starting model was built using the atomic coordinates of the crystal structure of dA^dT[19] on which the remaining nucleotides were added with a B-DNA conformation using program MIDAS.[34] The duplex model was energy-minimized by conjugate gradient minimization using X-PLOR.[26] X-PLOR's all atom force field for DNA was used with explicit hydrogen bond potentials. Refinement of the starting model was carried out by the SPEDREF procedures.[25] Minimization of the residual errors was performed by conjugate gradient minimization with the NOE constraints within the program X-PLOR[25] employing 40 cycles of SPEDREF refinement. The isotropic correlation time (τ_c) for each refinement was empirically determined to be 2 ns. Another starting model was built using canonical A-DNA and was similarly refined. A family of converged models was obtained from the refinement with the *R* factor in the range of ∼21%. The refined structures based on the two starting models have a root mean square deviation of 1.1 Å between them, suggesting a reasonable convergence of the refinement process.

Exchangeable proton 1D spectra at 2 °C in 90% H_2O were collected with the 1 not 1 pulse sequence as the read pulse.[35] The excitation offset was set to one-quarter of the spectral bandwidth, which was set to 12 000 Hz. The PE-COSY spectrum was collected following the procedure of Bax and Lerner.[36]

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