strongly polarized than CO, e.g., O_2, such interactions could serve to catalyze rearrangement of the ligands such as proposed in Scheme I.

Conclusions

In this paper it is proposed that the ion–molecule reaction between $Cr(CO)_5^-$ and O_2 to produce $Cr(CO)_3O_2^-$ and $Cr(CO)_3O^-$ occurs by a competitive reaction mechanism. The two mechanisms involve (i) simple ligand exchange to produce the $Cr(CO)_3O_2^-$ anion and (ii) addition of O_2 to $Cr(CO)_5^-$ followed by intramolecular nucleophilic addition of O^- to a CO ligand to form $Cr(CO)_3O^-$ and CO plus CO_2.

A surprisingly large temperature dependence for the rate of reaction of $Cr(CO)_5^-$ with O_2 is observed. The strong temperature dependence of the reaction rate is attributed to formation of a high-energy $[Cr(CO)_5^-]^*$ species. The high-energy $Cr(CO)_5^-$ anion may be formed by thermal decomposition of $Cr(CO)_6$ to give a high spin state of $Cr(CO)_5$, which is subsequently ionized by electron attachment to give $[Cr(CO)_5^-]^*$.

A particularly important result of this study is the proton and electron affinity data obtained for $Cr(CO)_3O^-$ and $Cr(CO)_3O_2^-$.

Although the oxygen ligand in these two ions differs significantly, the fact that both ions have the same proton and electron affinities suggests that the oxygen atom and molecule interact with the $Cr(CO)_3$ moiety in an analogous manner. We propose that in both $Cr(CO)_3O^-$ and $Cr(CO)_3O_2^-$ the Cr atom is best described as a Cr° oxidation state and the oxygen ligand as O^- and O_2^-, respectively. These results suggest that proton and electron affinity data may be extremely useful as diagnostic tools for probing the electronic environment of metal centers in gas-phase organometallic systems. A similar suggestion has been reported by Squires on the interplay between electron and proton affinities of metal atoms and ions.[37]

(37) Squires, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 4385.

A Parallel Right-Handed Duplex of the Hexamer d(TpTpTpTpTpT) with Phosphate Triester Linkages

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Abstract: We show in this work that stable parallel thymine–thymine (T–T) base pairs can be formed in aqueous solution. Initially, this observation was made with 3',5'-di-*O*-acetylthymidine in water which showed an imino resonance at 13.45 ppm in the ¹H NMR spectrum. Using the nucleoside diphosphate d(pTp), the formation of T–T base pairs could only be induced via methylation of the phosphate groups. This leads to the suggestion that intermolecular electrostatic phosphate–phosphate repulsion precludes T–T base pairing for unmodified d(pTp). It is shown that T–T pairing is also manifest on the dinucleotide level, provided that the phosphate groups are methylated. Using the dinucleoside phosphate **1** which was separated in its diastereomeric forms, it was shown that the miniduplex melts at $T_m \approx 30$ °C. Furthermore, it was shown that the duplex of **1** is parallel. From the detailed conformational analysis of the individual diastereomers it follows that the duplex has a right-handed helical sense, since the backbone bonds $C_{4'}-C_{5'}$ and $C_{5'}-O_{5'}$ are preferentially γ^+ and β^t, and the furanoses reside primarily in the south conformation. With the hexamer d(TpTpTpTpTpT), it was shown that T–T pairing also occurs on the hexanucleotide level, after methylation of the phosphate groups. The resulting duplex has a T_m value of approximately 65 °C as was established with UV hyperchromicity and with variable-temperature 500-MHz ¹H NMR. It could be clearly established that the duplex is parallel. Molecular modelling studies on the duplex of phosphate-methylated d(TpTpTpTpTpT) yielded a remarkably slim, parallel structure with about eight residues per turn. The possible relevance of these alternative DNA-like duplexes is briefly mentioned.

Thymine–thymine (T–T) base pairing was recently observed in the crystal structures of 3',5'-di-*O*-acetylthymidine[1] and *cis*-thymidine-3',5'-*N*,*N*-dimethylphosphoramidate.[2] Both crystal structures display an approximate twofold rotational symmetry, which is due to the fact that the T-bases are linked via two virtually identical N_3–H···O_4 hydrogen bonds (Figure 1). Following the convention of Rose et al.,[3] it is easily seen that the α faces of the coupled bases are on the same side; i.e., the T-bases are parallel. Therefore, the 5'→3' vectors run in the same direction on both

sides of the T–T pair. The present work is focused on the formation of T–T base pairs in solution. It was found that parallel T–T pairing readily occurs on, e.g., the mono-, di-, and hexanucleotide level, provided that the backbone phosphate groups are triesterified. In a previous communication,[1a] we already published our preliminary results on the phosphate-methylated hexanucleotide duplex. Various physico-chemical techniques (high-resolution proton NMR, UV hyperchromicity) were used in order to characterize these non-Watson–Crick parallel duplexes.

Results and Discussion

T–T Pairing on the Mononucleotide Level. We first studied T–T pairing using the acetylated nucleoside 3',5'-di-*O*-acetylthymidine[1a] (vide supra) and the nucleoside diphosphate d(pTp). In fact, 3',5'-di-*O*-acetylthymidine provided the first indication that T–T pairing may occur in aqueous solution, since the imino proton NMR signal was found at a remarkably low-field position (13.45 ppm[4]) in comparison with unlinked thymidine bases (11.2

(1) (a) Koole, L. H.; van Genderen, M. H. P.; Frankena, H.; Kocken, H. J. M.; Kanters, J. A.; Buck, H. M. *Proc. K. Ned. Akad. Wet., Ser. B* **1986**, *89*, 51 (communicated by H. M. Buck at the meeting of Nov 25, 1985). (b) Wilson, C. C.; Low, J. N.; Tollin, P.; Wilson, H. R. *Acta Crystallogr., Sect. C: Cryst. Struct.* **1984**, *40*, 1712.

(2) Bentrude, W. G.; Sopchik, A. E.; Setzer, W. N. *Acta Crystallogr., Sect. C: Cryst. Struct.* **1986**, *42*, 584.

(3) Rose, I. A.; Hanson, K. R.; Wilkinson, K. D.; Wimmer, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2439.
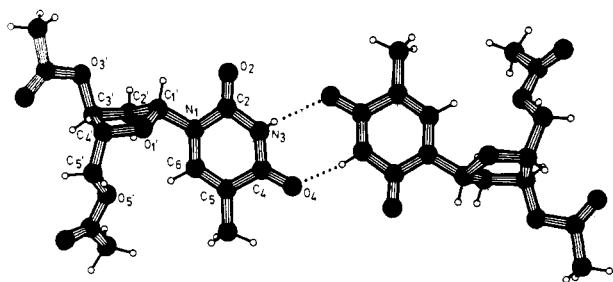
Figure 1. X-ray structure of the 3′,5′-di-*O*-acetylthymidine dimer. The atomic numbering is indicated in the left-side monomer.
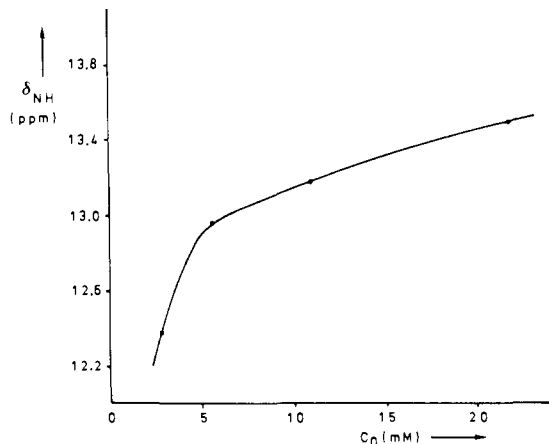

Figure 2. Imino chemical shift as a function of C_0, the primitive concentration of phosphate-methylated d(pTp).

ppm[5]). Using the d(pTp) system, which contains charged phosphomonoester moieties, no indication for T–T pairing was found. This suggests that electrostatic phosphate–phosphate repulsions reduce the propensity for T–T pairing. Indeed, methylation on the phosphate groups with methyl methanesulfonate results in a distinct imino resonance in water at 13.5 ppm,[4,6] thus indicating T–T pairing. For the phosphate-methylated d(pTp), it was also found that the chemical shift of the imino protons is strongly concentration dependent. At higher dilution, broadening and upfield shifting are observed, which is consistent with gradual dissociation of the dimeric structure.

Figure 2 shows the imino chemical shift as a function of C_0, which denotes the primitive concentration of phosphate-methylated d(pTp).[7] From these data, it follows that the duplex dominates to a C_0 value as low as 5 mM, demonstrating that the T–T pairing is very stable. We have used the data in Figure 2 also to establish a lower limit of the formation constant K for the T–T dimer. From the fact that $K = C_0^{-1}$ at the midpoint of the dimerization equilibrium (which is below $C_0 = 5$ mM), it follows that $K > 200$ M^{-1}. As far as we are aware, this is the first self-association constant that refers to mononucleotides in aqueous solution.[8] Using the high-resolution ^{1}H NMR spectra of the dimers of 3′,5′-di-*O*-acetylthymidine and phosphate-methylated d(pTp) in water, it could also be established that the T–T pairing is parallel. The observation of a single, degenerate spectrum for both model systems proves that the hydrogen-bonded nucleotides are symmetry-related and hence linked via two N_3—H···O_4 hydrogen bonds (vide supra). In the case of antiparallel T–T pairing via one N_3—H···O_4 and one N_3—H···O_2 hydrogen bond, far more

Table I. Spectral Data of Both Diastereomers of **1**, as Determined from the Two-Dimensional *J*-Resolved 300-MHz ^{1}H NMR Spectra

	slow fraction[a] $\delta(^{31}$P) 2.04		fast fraction[a] $\delta(^{31}$P) 2.14	
	top	bottom	top	bottom
$J_{H1'H2'}$	8.2	7.2	8.4	7.6
$J_{H1'H2''}$	6.0	6.6	6.0	6.4
$J_{H2'H3'}$	6.2	7.2	6.0	6.4
$J_{H2''H3'}$	2.8	3.6	2.6	3.6
$J_{H3'H4'}$	2.6	2.8	2.4	3.2
$J_{H4'H5'}$	4.0	2.6[b]	4.0	2.6[b]
$J_{H4'H5''}$	4.4	4.0[b]	4.4	4.0[b]
$J_{PH3'}$	5.9		6.4	
$J_{PH5'}$		6.0[b]		6.0[b]
$J_{PH5''}$		5.6[b]		5.6[b]

[a] See ref 11. [b] Obtained by simulation.

complicated ^{1}H NMR spectra would be obtained, since the coupled nucleotides then reside in different magnetic environments.

T–T Pairing on the Dinucleotide Level. T–T base pair formation on the dinucleotide level was studied with the dinucleoside phosphate **1**. This system was synthesized via a slight modification of the common phosphite triester method, devised originally by Caruthers et al.[9] Initially, **1** was obtained as a mixture of the

1
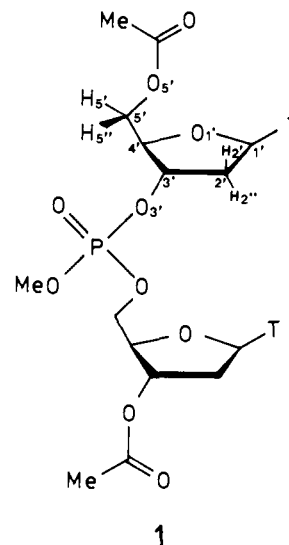
R_P and S_P diastereomers. In water, this mixture corresponds to a two-line ^{31}P NMR spectrum (2.14 and 2.04 ppm), and a highly crowded ^{1}H NMR spectrum. The R_P/S_P mixture was separated by reversed-phase HPLC, according to the procedure of Stec et al.[10] (see Experimental Section). The diastereomer which was eluted first corresponds to the downfield ^{31}P peak at 2.14 ppm, whereas the slower fraction is found upfield at 2.04 ppm.[11] For each of the diastereomers in water, at 20 °C, the imino protons were found at 13.1 ppm in the ^{1}H NMR spectrum,[12] i.e., **1** is also present in the duplex form. We observed that increasing the sample temperature results in substantial broadening and upfield shifting of the imino resonance toward 12.5 ppm, which indicates dissociation (melting) of the duplex structure. From the melting curves (imino chemical shift vs. temperature, not shown) we concluded that the melting temperature (T_m) of **1** is roughly 30 °C. The detailed ^{1}H NMR spectra of the diastereomers of **1** at 20 °C show that the coupled strands give rise to identical spectra;

(4) Measured at 500 MHz on the Bruker WM 500 spectrometer of the Dutch National 500/200 hf NMR Facility at Nijmegen, the Netherlands.

(5) Haasnoot, C. A. G.; den Hartog, J. H. J.; de Rooij, J. F. M.; van Boom, J. H.; Altona, C. *Nature* (*London*) **1979**, *281*, 235.

(6) Exclusive methylation of the phosphate groups was accomplished according to: Rhaese, H.-J.; Freese, E. *Biochim. Biophys. Acta* **1969**, *190*, 418.

(7) The primitive concentration C_0 equals the concentration of the free monomer plus twice the concentration of the duplex.

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

(9) (a) Matteucci, M. D.; Caruthers, M. H. *Tetrahedron Lett.* **1980**, *21*, 719. (b) Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185. (c) Pon, R. T.; Danha, M. J.; Ogilvie, K. K. *Nucleic Acids Res.* **1985**, *13*, 6447.

(10) Stec, W. J.; Zon, G.; Uznanski, B. *J. Chromatogr.* **1985**, *326*, 263.

(11) A tentative R_P/S_P assignment can be made on the basis of the ^{31}P chemical shifts. See, e.g.: Herdering, W.; Kehne, A.; Seela, F. *Helv. Chim. Acta* **1985**, *68*, 2119. This assignment results in S_P configuration for $\delta(^{31}$P) = 2.14 and R_P configuration for $\delta(^{31}$P) = 2.04 ppm.

(12) Measured on a Bruker AC 200 spectrometer at the Eindhoven University of Technology.

Table II. Conformational Characteristics of the 2′-Deoxyribose Ring, and the β and γ Bonds of Both Diastereomers of **1**

| | slow fraction[a] $\delta(^{31}P)$ 2.04 | | fast fraction[a] $\delta(^{31}P)$ 2.14 | |
	top	bottom	top	bottom
x(south)	0.83	0.70	0.84	0.72
$x(\gamma^+)$	0.53	0.70	0.53	0.70
$x(\beta^t)$		0.65		0.65

[a] See ref 11.

i.e., the structure is highly symmetric. Therefore, the spectra are consistent only with a duplex with parallel T–T pairing (vide supra). For the individual diastereomers, it was possible to analyze the conformations of the 2′-deoxyribose ring and, in part, the phosphate backbone in great detail by means of high-resolution ^{1}H NMR. In order to obtain a complete set of vicinal proton-proton coupling constants, we measured a two-dimensional J-resolved ^{1}H NMR spectrum at 300 MHz[13] for both structures. The results of these experiments are summarized in Table I. It is well-known, that the 2′-deoxyribose ring in nucleosides and nucleotides is involved in a rapid two-state conformational equilibrium between a south form ($C_{2'}$-endo/$C_{3'}$-exo twist), and a north form ($C_{2'}$-exo/$C_{3'}$-endo twist).[14] According to Altona[15,16] the population of the south form can be accurately estimated on the basis of $J_{H1'H2''}$ and $J_{H2''H3'}$:

$$x(\text{south}) = \{17.8 - J_{H1'H2''} - J_{H2''H3'}\}/10.9$$

As can be seen in Table II, the south conformation dominates for the two furanose rings in each of the diastereomers. The coupling constants $J_{H4'H5'}$ and $J_{H4'H5''}$ were used to analyze the conformation around the $C_{4'}$–$C_{5'}$ (γ) bonds. The formula[15,16]

$$x(\gamma^+) = \{13.1 - J_{H4'H5'} - J_{H4'H5''}\}/9.7$$

was used for this purpose. Clearly, the γ^+ conformation (in which $O_{5'}$ is located above the 2′-deoxyribose ring) dominates in both the top (5′-O-acetyl) and the bottom (5′-O-phosphate) residues in the diastereomers. The three-bond phosphorus–proton coupling constants $J_{PH5'}$ and $J_{PH5''}$ were used to describe the conformation around the central $C_{5'}$–$O_{5'}$ (β) bond of the R_P and S_P structures. For this, we used the formula[15]

$$x(\beta^t) = \{23.9 - J_{PH5'} - J_{PH5''}\}/18.9$$

From Table II, it appears that both diastereomers have a dominant contribution of β^t (in which the phosphorus is in a trans orientation with respect to $C_{4'}$) to the conformational equilibrium around the central $C_{5'}$–$O_{5'}$ backbone bond. The data in Table II do not show significant conformational differences between the diastereomers. For both structures, the combination of south (2′-deoxyribose), γ^+ ($C_{4'}$–$C_{5'}$), and β^t (central $C_{5'}$–$O_{5'}$) is preferred. It should be mentioned that the same conformation is encountered in standard right-handed B DNA structures, both in solution and in the solid state. In summary, from the overall structural information on the diastereomeric forms of **1**, we conclude that both systems exist as stable symmetric miniduplexes with right-handed parallel phosphate–sugar backbone strands. It should be mentioned that one- and two-dimensional NOE spectroscopy cannot bolster this structural model. The symmetry of the structure implies that any interstrand NOE contact has a stronger and overlapping intrastrand counterpart. Therefore the use of NOEs for interatomic distance estimation is essentially excluded.

T–T Pairing on the Hexanucleotide Level. In order to investigate T–T base-pair formation also on the hexanucleotide level, we used the hexanucleoside pentaphosphate d(TpTpTpTpTpT).[1a] The methylation of the phosphate groups with methyl methane-

sulfonate[6] was essentially complete (99%), as was shown by precipitation of only 1% of the unmethylated starting material with ethanol/water (75:25 v/v). However, since the phosphate methylation is not stereospecific, a multicomponent mixture of diastereomers is obtained, which could not be separated by means of HPLC techniques. Nonetheless, it could be clearly shown that the phosphate-methylated d(TpTpTpTpTpT) is present as a stable duplex in aqueous solution. This conclusion was based on the observation that the imino protons resonate at 13.3 ppm in the ^{1}H NMR spectrum.[4] Furthermore, increasing the sample temperature results in a double helix → coil transition, as was observed with UV hyperchromicity and variable-temperature NMR experiments. Using the UV hyperchromicity technique, we observed a reversible dissociation of the double helix at a T_m of approximately 67 °C, for a substrate concentration of 1.3×10^{-5} M in water.[1a] It was found that the melting behavior is identical in aqueous Tris/EDTA buffer solutions (pH 7.5) of 20 mM and 0.2 M. The fact that T_m is not influenced by the ionic strength of the solution is consistent with the absence of a formal negative charge on the phosphate groups. The neutral character of the methylated substrate also enabled us to study the melting behavior in less polar solvents. The same T_m value of approximately 67 °C was found in a 80:20 (v/v) mixture of ethanol and water. No melting point in the temperature range 10–80 °C was found using the hydrogen-bond disrupting solvent hexamethylphosphoric triamide (HMPT).[17] This means that in the latter case only the single strand is present, which is confirmed by an imino chemical shift of 12.5 ppm in HMPT.[4] In the case of phosphate-methylated d(TpTpTpTpTpT), it was not possible to determine the conformation of the phosphate–sugar backbone from the high-resolution ^{1}H NMR spectrum. Evidently, this is due to the fact that the duplex contains 10 chiral phosphate groups, and therefore exists as a complex diastereomeric mixture with a highly crowded ^{1}H NMR spectrum. However, the subspectra of the imino- and base-methyl protons are well-defined (Figure 3), since these protons are located at an appreciable distance from the chiral phosphate groups. In both subspectra, three peaks in the approximate ratio 1:1:4 are observed. Most likely, the terminal base pairs are associated with the lower peaks, whereas the imino- and base-methyl resonances of the inner base pairs practically coincide at 13.29 and 1.32 ppm, respectively. We have used these simple subspectra in two ways for further characterization of the duplex structure of phosphate-methylated d(TpTpTpTpTpT). First, the chemical shifts of the imino- and base-methyl protons were measured as a function of the sample temperature. The melting curves that were obtained are given in Figure 4. All curves show a melting transition at T_m = 64 °C.[18] Remarkably, the UV hyperchromicity measurements (vide supra) resulted in virtually the same T_m value. This means that the dilution from 10 mM (NMR sample) to 13 μM (UV sample) does not induce a measurable degree of dissociation of the duplex, which reflects the marked stability of the parallel structure. Secondly, the subspectra were used to discriminate between the symmetric, parallel T–T and the asymmetric, antiparallel T–T coupling (Figure 5). The appearance of only two lower peaks in the subspectra is characteristic for a parallel duplex with a twofold symmetry. In this case, each base pair corresponds with exactly one imino- and one base-methyl peak. In contrast, antiparallel T–T base pairing would result in far more complex subspectra, since this structure lacks any symmetry elements (see Figure 5). For instance, the terminal base pairs are expected to correspond with four methyl and four imino resonances. The fact that the phosphate-methylated d(TpTpTpTpTpT) exists as a complex mixture of diastereomers precludes the use of two-dimensional NOE spectroscopy as a reliable tool for structural elucidation. However, we did perform one-dimensional double resonance experiments in which one of the lower base-methyl resonances was

(13) Measured on a Bruker CXP 300 spectrometer at the Eindhoven University of Technology.

(14) Nomenclature in this work follows the recent IUPAC–IUB recommendations. See: *Eur. J. Biochem.* **1983**, *131*, 9.

(15) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413.

(16) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.

(17) (a) Normant, H. *Angew. Chem.* **1967**, *23*, 1029. (b) Normant, H. *Bull. Soc. Chim. Fr.* **1968**, *2*, 791.

(18) The exchange of the imino protons with the solvent is remarkably slow; broadening of the imino proton resonances is only observed above 75 °C. Chemical shifts could be determined accurately for temperatures up to 85 °C.
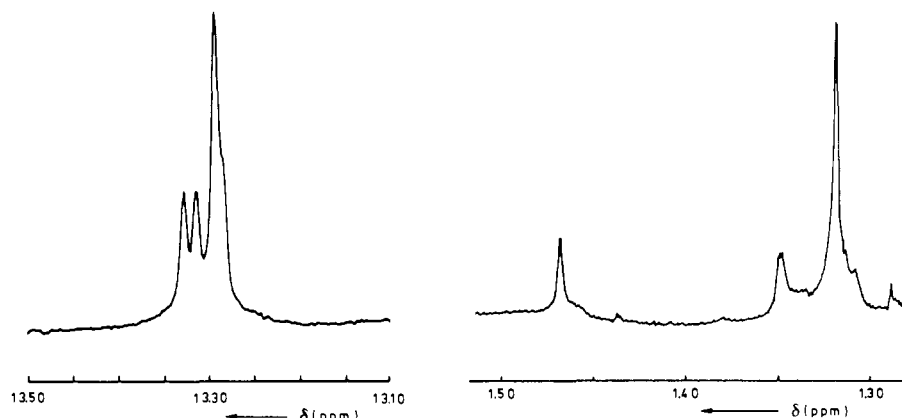
Figure 3. Subspectra from the 500-MHz ^{1}H NMR spectrum of phosphate-methylated d(TpTpTpTpTpT): left, imino resonances; right, base-methyl resonances.
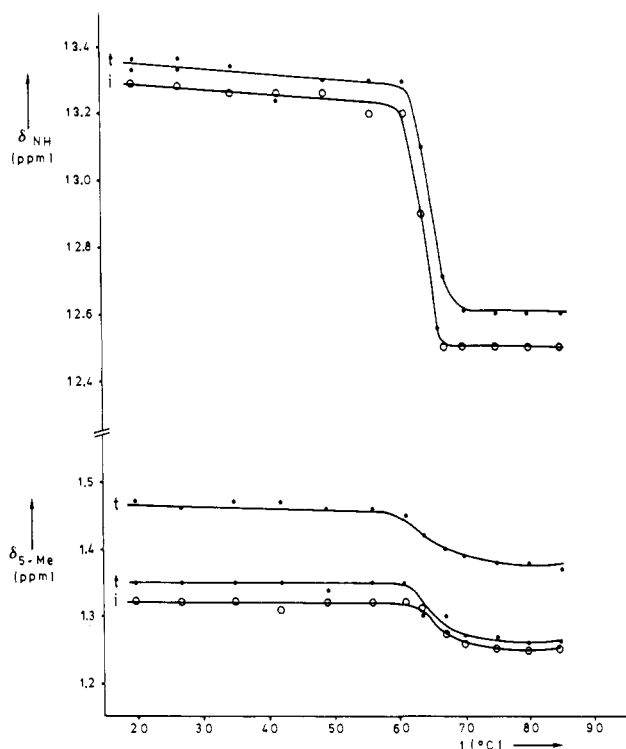

Figure 4. Melting curves of phosphate-methylated d(TpTpTpTpTpT), as obtained with variable-temperature 500-MHz ^{1}H NMR: upper part, imino resonances; lower part, base-methyl resonances. In both curves, the resonances of the inner (i) and terminal (t) base pairs are shown separately. Note that the terminal imino resonances coincide at temperatures higher than 30 °C.

specifically irradiated. No NOE effects in the other base-methyl peaks were observed. These results are consistent with a symmetric parallel structure. In this case, the two methyl groups of the same terminal base pair are simultaneously saturated; i.e., no methyl–methyl contact within the T–T base pair is seen. In the case of an antiparallel arrangement, a methyl–methyl NOE effect within the same pair is expected. It is tempting to raise the question if self-association is restricted to thymidine bases. Interestingly, literature data for the crystal structure of 2'-deoxycytidine (2'-dC) also show the formation of a C–C pair with a parallel arrangement.[19] However, we found for 2'-dC in aqueous solution at 20 °C no low-field resonances of the NH$_2$ protons,[4] which implies that these protons are involved in a rapid exchange with the solvent; i.e., the nucleosides are not coupled. Also on the hexanucleotide level of phosphate-methylated d-

(CpCpCpCpCpC) no NH$_2$ resonances could be detected in the low-field region of the ^{1}H NMR spectrum[4] recorded at 20 and 4 °C. Additionally, no melting transition was observed in a UV hyperchromicity experiment in the temperature range 10–90 °C. It must be concluded that base–base hydrogen bonding in the interior of the parallel duplex is most stable in the case of T-bases, whereas base–solvent bonding in the single strand form is preferred for C-bases. We considered it of interest to synthesize the phosphate-methylated dodecamer d(CpCpCpCpCpCpTpTpTpTpTpT), which represents a combination of phosphate-methylated d(CpCpCpCpCpC) and d(TpTpTpTpTpT). Duplex formation of the dodecamer would result in either a parallel duplex with six T–T and six C–C base pairs, or in a parallel duplex with six T–T base pairs and dangling, nonpaired C-bases. However, since no double helix ⇌ coil transition was found in the temperature range 10–90 °C (based on UV hyperchromicity experiments), it can be concluded that the dodecamer is present in the single-strand form; i.e., the C-bases actually preclude the formation of a parallel duplex via T–T pairing.

Structural Model. A molecular model of the phosphate-triesterified d(TpTpTpTpTpT) was constructed with computer graphics using the structural information provided by the X-ray data of 3',5'-di-O-acetylthymidine, and the detailed NMR data on both diastereomers of the dinucleoside monophosphate 1. Figure 6 shows a top and side view of the proposed structure.[20] We found that the parallel T–T base pairs indeed fit excellently in a right-handed double helix with γ^+ and β^t backbone torsion angles, south conformation of the 2'-deoxyribose rings, and anti conformation of the T-bases. The inherent symmetry results in the formation of two identical grooves, instead of the minor and major grooves that are found in right-handed B DNA. The structure has approximately eight residues per turn, and a rise per base pair of 3.6 Å. The combination of two thymidine bases results in a helix diameter of 15 Å, whereas the purine–pyrimidine base pairs in B DNA correspond with the much greater helix diameter of 21 Å.[8] Interestingly, our structural model also provides a more plausible explanation for the fiber X-ray diffraction pattern of the dinucleoside phosphate d(TpT), as was recently observed by Tollin et al.[21] It was found that this structure crystallizes as a helical structure with about seven units per turn and a rise per base pair of 3.8 Å. The structural model as proposed by Tollin et al. essentially comprises antiparallel T–T base pairs, and a head-to-tail alignment of d(TpT) residues:

$$\text{TpT TpT TpT TpT}$$
$$\text{TpT TpT TpT TpT}$$
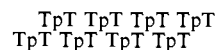
However, it was necessary to invoke unlikely conformational characteristics as a syn orientation of the T-bases[22] and a γ^-

(19) Young, D. W.; Wilson, H. R. *Acta Crystallogr., Sect. B: Struct. Sci.* **1975**, *31*, 961.

(20) Molecular mechanics calculations using the AMBER program confirm this structural model. See: van Genderen, M. H. P.; Koole, L. H.; Aagaard, O. M.; van Lare, C. E. J.; Buck, H. M. *Biopolymers*, in press.

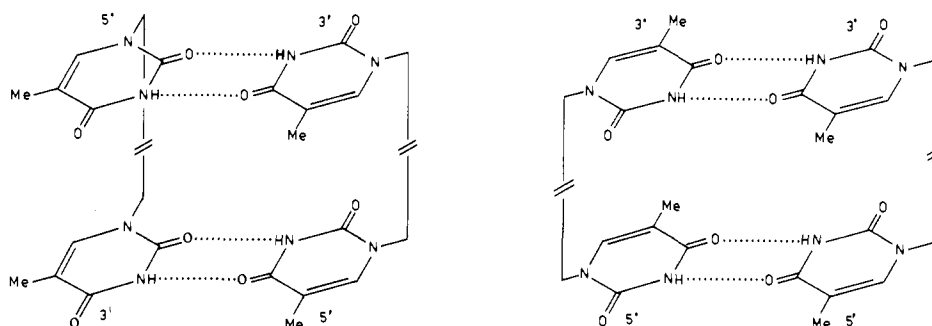(21) Tollin, P.; Walker, R. T.; Wilson, H. R. *Nucleic Acids Res.* **1984**, *12*, 8345.

Figure 5. Schematic representation of the geometries of the terminal base pairs in oligomeric duplex structures with T–T base pairing: left, antiparallel T–T pairing; right, parallel T–T pairing.
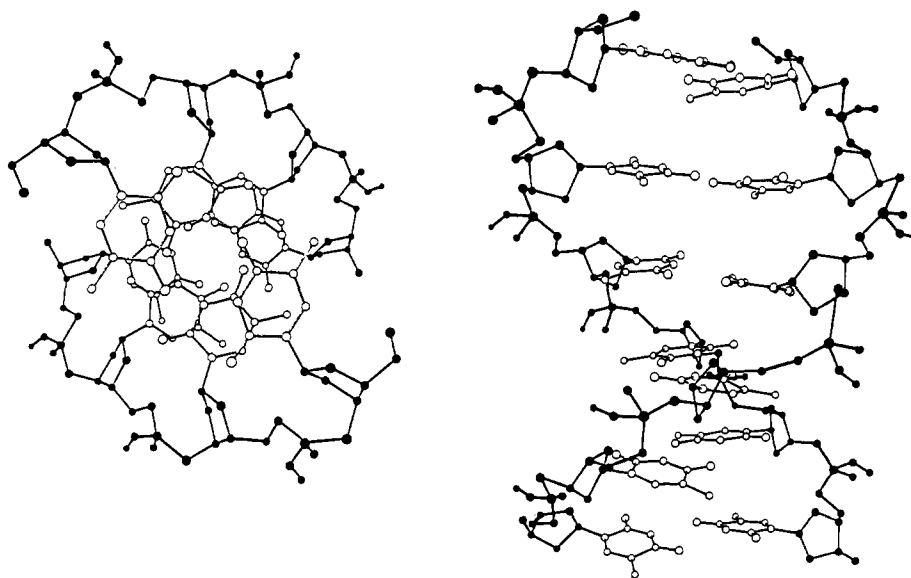

Figure 6. Top and side view of the computer-generated structural model of the parallel duplex of phosphate-methylated d(TpTpTpTpT).

conformation around the $C_{4'}$–$C_{5'}$ bond in order to construct an antiparallel helix. On the basis of our present data, we feel that a head-to-tail structure with parallel T–T pairing gives a more adequate explanation for the fiber X-ray diffraction pattern of d(TpT). The resulting double helical structure has backbone strands that are regularly interrupted. Consequently, phosphate groups are not found in opposite positions, which minimizes electrostatic repulsion. Essentially, this situation parallels the phosphate–methylated system d(TpTpTpTpT) in which the phosphate repulsions are completely eliminated.

Concluding Remarks

It has been shown that the formation of T–T base pairs may occur readily in aqueous solution, provided that interstrand phosphate–phosphate repulsions are eliminated via methylation of the phosphate groups. The resulting duplex structures are parallel and highly symmetric. Detailed conformational analyses of a phosphate-methylated d(TpT) model, for which the R_P and S_P diastereomers were separated, revealed that the backbone conformations are standard; i.e., a close resemblance with respect to right-handed B DNA is seen. The phosphate-methylated hexamer d(TpTpTpTpT) exists as a duplex with an exceptional stability in aqueous solution. Since the duplex is essentially uncharged, the stability of the structure is not influenced by the ionic strength of the medium. However, d(TpTpTpTpT) exists in the single strand form in the hydrogen-bond disrupting solvent HMPT. We feel that the present model systems may be useful as simple artificial probes for phosphate backbone–protein interactions (recognition) in which the negative charges on the

phosphate groups are (partially) neutralized. In this context it is therefore of interest that complex formation between polylysine or polyarginine and DNA leads to an increased stability of the duplex as was reflected in the elevated values of T_m with respect to uncomplexed DNA.[23] Our results now show that neutralization of the phosphate groups may also result in the formation of parallel DNA structures. Finally, it can be concluded that the proposed parallel structures for the phosphate-methylated thymine oligomers differ markedly from familiar DNA systems, and may therefore be considered as a valuable contribution to the design of new DNA-like structures.

Experimental Section

Synthesis. 3′,5′-Di-O-acetylthymidine.[1a] This compound was prepared by adding acetic anhydride (16.5 mmol, 1.55 mL) to a solution of thymidine (8.2 mmol, 2.0 g) in 50 mL of anhydrous pyridine. The solvent was evaporated after 3 h, and the resulting viscous glass was chromatographed on a Woelm silica gel column, using dry 2-butanone as eluent ($R_f = 0.65$). The product was obtained as a white crystalline solid in 41% yield. Anal. Calcd for $C_{14}H_{18}O_7N_2$: C, 51.50; H, 5.50; N, 8.61. Found: C, 51.84; H, 5.62; N, 8.61.

Thymidine 3′,5′-Di(dimethylphosphate).[1a] Thymidine 3′,5′-diphosphate (sodium salt, purchased from P-L Biochemicals) was treated with methyl methanesulfonate according to Rhaese and Freese.[6] Methylation of the phosphate groups was essentially complete, as was shown with ^{31}P NMR spectroscopy. The methylated structure corresponds with two ^{31}P NMR resonances, at 0.4 and 0.6 ppm, whereas two signals, at 3.8 and 4.1 ppm are found for the unmethylated compound.

3′-O-Acetylthymidine. 5′-Tritylthymidine[24] (15.6 mmol, 7.53 g) and acetic anhydride (16 mL) were dissolved in 70 mL of anhydrous pyridine. This mixture was magnetically stirred overnight. After complete removal

(22) A syn conformation for thymine has been observed in the left-handed duplex of d(CGCGATCGCG), in which the C-bases are brominated. See: Feigon, J.; Wang, A. H.-J.; van der Marel, G.; van Boom, J. H.; Rich, A. *Science* **1985**, *230*, 82.

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of the solvent (coevaporation with two 20-mL portions of water), the oily residue was chromatographed on a Woelm silica gel column, using dry 2-butanone as eluent. The yield of 5'-trityl-3'-O-acetylthymidine (R_f = 0.52) was 8.01 g (98%): ¹H NMR (acetone-d_6) δ 1.48 (3 H, s, CH_3 base), 2.06 (3 H, s, CH_3 acetyl), 2.48–2.54 (2 H, m, $H_{2'}/H_{2''}$), 3.46–3.50 (2 H, m, $H_{5'}/H_{5''}$), 4.18 (1 H, m, $H_{4'}$), 5.50 (1 H, m, $H_{3'}$), 6.38 (1 H, dd, $H_{1'}$), 7.24–7.56 (15 H, m, trityl), 7.64 (1 H, s, H_6). A solution of this compound (14.8 mmol, 7.8 g) in 25 mL of a mixture of acetic acid and water (4:1 v/v) was refluxed for 10 min. After complete evaporation of the acetic acid (coevaporation with two 20-mL portions of water), the white residue was chromatographed on a Woelm silica gel column, using dry 2-butanone as eluent. 3'-O-Acetylthymidine (R_f = 0.23) was obtained as a white foam: yield 3.03 g (72%); ¹H NMR (acetone-d_6) δ 1.84 (3 H, s, CH_3 base), 2.09 (3 H, s, CH_3 acetyl), 2.36–2.40 (2 H, m, $H_{2'}/H_{2''}$), 2.88 (1 H, bs, OH), 3.83–3.88 (2 H, m, $H_{5'}/H_{5''}$), 4.09 (1 H, m, $H_{4'}$), 5.35 (1 H, m, $H_{3'}$), 6.32 (1 H, dd, $H_{1'}$), 7.84 (1 H, s, H_6).

3'-(Methoxy-N,N-diisopropylaminophosphino)-5'-tritylthymidine. 5'-Tritylthymidine[24] (14.3 mmol, 6.9 g) was suspended in a mixture of anhydrous chloroform (120 mL) and anhydrous N,N-diisopropylethyl-amine (10 mL). The suspension was magnetically stirred and kept under an atmosphere of dry argon. Chloro(N,N-diisopropylamino)methoxy-phosphoramidite[25] (15.5 mmol, 3.1 mL) was added dropwise over 5 min. After the addition, the reaction mixture was stirred for 2 h. Ethyl acetate (300 mL, prewashed with $NaHCO_3$) was added, and the solution was washed with saturated NaCl solution (4 × 150 mL) and water (1 × 50 mL) and dried over Na_2SO_4. After evaporation of the solvents, a yellow oil was obtained, which was chromatographed on a Woelm silica gel column. The eluent was dichloromethane/hexane/triethylamine (45:45:10). The yield of the desired product(R_f = 0.43) was 5.18 g (58%): ¹H NMR (acetone-d_6) δ 1.16 (12 H, s, CH_3 isopropyl), 1.58 (3 H, s, CH_3 base), 2.52–2.56 (2 H, m, $H_{2'}/H_{2''}$), 3.63–3.68 (2 H, m, $H_{5'}/H_{5''}$), 3.32 and 3.42 (3 H, d, OCH_3, J = 11 Hz), 4.24 (1 H, m, $H_{4'}$), 4.80 (1 H, m, $H_{3'}$), 6.44 (1 H, dd, $H_{1'}$), 7.32–7.60 (15 H, m, trityl), 7.68 (1 H, s, H_6); ³¹P NMR (acetone-d_6) δ 154.6 and 154.1 (intensity ratio 1:1.09).

3',5'-Di-O-acetyldithymidyl-(3'→5')-O-methylphosphate (1). 3'-O-Acetylthymidine (2.46 mmol, 0.70 g) and 3'-(methoxy-N,N-diiso-propylaminophosphino)-5'-tritylthymidine (2.11 mmol, 1.33 g) were dissolved in 15 mL of anhydrous pyridine. 1H-Tetrazole (6.4 mmol, 0.45 g), dissolved in 5 mL of anhydrous pyridine, was added, and the yellowish reaction mixture was stirred at room temperature for 24 h. After complete evaporation of the pyridine (coevaporation with three 10-mL portions of dry dichloromethane), a yellow syrup was obtained. Chromatography on a Woelm silica gel column using dry 2-butanone as eluent afforded 0.73 g (43%) of 3'-O-acetyl-5'-trityldithymidyl-(3'→5')-O-methylphosphite as a slightly colored foam. The two diastereomers were seen separately on the analytical thin layer chromatography plates at R_f = 0.35 and 0.31: ¹H NMR (acetone-d_6) δ 1.51 (6 H, s, CH_3 base), 1.85 (3 H, s, CH_3 acetyl), 2.28–2.42 (4 H, m, $H_{2'}/H_{2''}$), 3.46 (3 H, d, OCH_3, J = 11 Hz), 3.38–3.50 (4 H, m, $H_{5'}/H_{5''}$), 4.10 (2 H, m, $H_{4'}$), 4.55 (1 H, m, $H_{3'}$), 4.65 (1 H, m, $H_{3'}$), 6.35 (1 H, dd, $H_{1'}$), 6.40 (1 H, dd, $H_{1'}$), 7.25–7.52 (15 H, m, trityl), 7.62 (2 H, s, H_6); ³¹P NMR (Me_2SO-d_6) δ 145.7 and 145.2 (intensity ratio 1:0.86). The phosphite was dissolved in 9 mL of dry dioxane, and NO_2 gas was slowly bubbled through. After 10 min, thin layer chromatography indicated complete conversion into the phosphate. The reaction vessel was then sparged with dry nitrogen; after evaporation of the solvent, 20 mL of the detritylation reagent (a mixture of acetic acid and water, 4:1 v/v) was added. This mixture was then refluxed for 10 min, cooled to room temperature, and concentrated. The last traces of acetic acid were removed by coevaporation with two 10-mL portions of water. The remaining yellow oil was chromatographed on a Woelm silica gel column, using a mixture of 2-butanone and tri-ethylamine (95:5 v/v) as the eluent. Concentration of the appropriate fractions (R_f = 0.13) afforded 0.25 g of a yellowish syrup: ³¹P NMR (Me_2SO-d_6) δ 3.9 and 3.7 ppm. Subsequently, the oil was mixed with acetic anhydride (0.8 mL) and stirred for 20 h. The excess of acetic anhydride was thoroughly evaporated, and the residue was carefully chromatographed on a Woelm silica gel column. A mixture of 2-buta-none and triethylamine (95:5 v/v) was used as the eluent. The desired product (0.17 g, 24%), having R_f = 0.38, was obtained as a colorless viscous oil: ¹H NMR (D_2O) δ 1.89 (6 H, s, CH_3 base), 2.15 (6 H, s, CH_3 acetyl), 2.41–2.65 (4 H, m, $H_{2'}/H_{2''}$), 3.80–3.82 (2 H, m, $H_{5'}/H_{5''}$), 3.88

(3 H, d, OCH_3, J = 11 Hz), 4.28–4.51 (4 H, m, $2H_{4'}/H_{5'}/H_{5''}$), 5.07–5.13 (1 H, m, $H_{3'}$), 5.37–5.42 (1 H, m, $H_{3'}$), 6.24–6.35 (2 H, m, $H_{1'}$), 7.57 (1 H, s, H_6), 7.64 (1 H, s, H_6); ³¹P NMR (D_2O) δ 2.14 and 2.04 (intensity ratio 1:1.09).

Chromatographic Separation of the Diastereomers of 1. The separation of the diastereomers of **1** was performed with a Dupont-830 HPLC system which was equipped with a Nucleosil 100-7-C_{18} column (250 × 20 mm), and a Zeiss EM2D UV detector (set at 265 nm). The eluent was prepared from acetonitrile (13%, HPLC grade), glacial acetic acid (1%, aldehyde free), triethylamine (1%, Gold Label grade), and deionized water. The flow rate was 20–25 mL/min. The elution times for the fractions were 38 and 42 min.

Oligonucleotides. The hexamers d(TpTpTpTpTpT)[1a] and d-(CpCpCpCpCpC) were synthesized on a 10-μmol scale with an Applied Biosystems 380A DNA synthesizer following a standard phosphite (OCH₃) triester synthesis protocol. The purity of the material was carefully checked with gel electrophoresis and HPLC. For d-(TpTpTpTpTpT), methylation of the phosphate groups could be performed in a straightforward manner according to the procedure of Rhaese and Freese.[6] The methylation was essentially complete (99%), as was shown by precipitation on only 1% of the unmethylated starting material with ethanol/water (75:25 v/v). In the case of d-(CpCpCpCpCpC), phosphate methylation was accomplished as follows. The product from the synthesizer was dissolved in 1 mL of dry pyridine; benzoyl chloride (10 equiv) was added. After stirring for 24 h, the pyridine was completely removed. The residue was dissolved in 1 mL of water, and methyl methanesulfonate (7 equiv) was added. After standing overnight, the solvent was thoroughly evaporated, and 1.5 mL of a 5% solution of hydrazine in water was added. Base deprotection was complete after stirring at 30 °C for 5 h, as was proven with thin layer chromatography. Finally, the phosphate-methylated substrate was purified by means of short-column chromatography using a 0.2 M Tris/EDTA buffer solution (pH 7.5) as eluent. The dodecamer d-(CpCpCpCpCpCpTpTpTpTpTpT) was synthesized on a 1-μmol scale, also on an Applied Biosystems 380A DNA synthesizer. The purity of this material was checked with gel electrophoresis. Protection of the C-bases, methylation of the phosphate groups, and subsequent deprotection of the C-bases were performed as described above. UV hyper-chromicity experiments on the phosphate-methylated dodecamer were performed without further purification.

Spectroscopy. ¹H NMR spectra were run in the FT mode at 200,[12] 300,[13] or 500⁴ MHz on Bruker NMR spectrometers. Measurements in water refer to a 85:15 mixture of H_2O and D_2O in which deuterium provided the field-frequency lock. The technique as described by Haasnoot et al.[26] was used to suppress the strong H_2O/HDO solvent signal. Proton chemical shifts were referenced against tetramethyl-ammonium chloride (δ 3.18 ppm). In all one-dimensional spectra, appropriate spectral windows (10–15 ppm) were chosen and Fourier transformation was usually performed with 32K data points. The two-dimensional J-resolved spectra were run at 300 MHz with a spectral window of 1600 Hz (8K data points) on the chemical shift (f_2) axis and 30 Hz (128 data points) on the J (f_1) axis. ³¹P NMR spectra were run in the FT mode at 36.4 or 80.9 MHz, also on Bruker NMR spectrometers. Chemical shifts are relative to 85% H_3PO_4; they are designated positive if downfield with respect to the standard. The UV hyper-chromicity measurements were performed on a Perkin-Elmer 124 spectrophotometer, using 10-mm cuvettes and a wavelength of 260 nm.

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