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A parallel right-handed duplex of phosphate-methylated d(CpC) exclusively for the S_P configuration of the phosphate triester group

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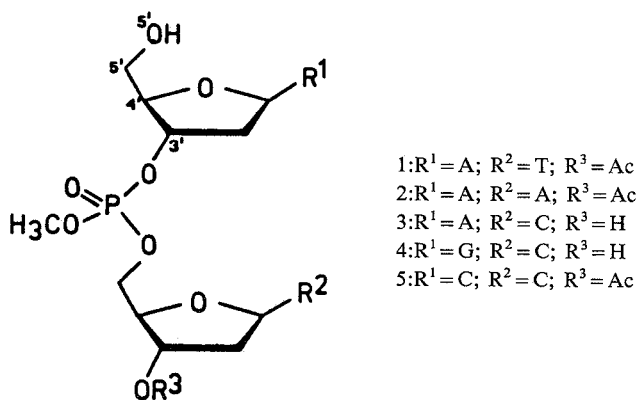
SUMMARY

The phosphate-methylated dinucleotides d(ApT) (1), d(ApA) (2), d(ApC) (3), d(GpC) (4), and d(CpC) (5) have been synthesized, and the S_P and R_P diastereoisomers were separated with reversed phase HPLC. Variable temperature ¹H NMR studies at 300 MHz revealed that 1-4 exist in the single strand form. Surprisingly, a parallel righthanded miniduplex is found for the S_P diastereoisomer of 5, and not for the R_P form of the same compound. The NMR experiments showed a T_m-value of approximately 306 K for the miniduplex of the S_P form of 5, which was corroborated with a UV hyperchromicity experiment. It is proposed that C-C base pairing occurs via two identical N₄-H...N₃ hydrogen bonds as found in the X-ray crystal structure of 2'-deoxycytidine (Young et al., 1975).

Recent investigations in this laboratory have demonstrated that methylation of the phosphate groups in d(T_n) DNA fragments (n = 2-8) results in the formation of a non-Watson & Crick parallel double helix with T-T base pairs (Koole et al., 1986; Koole et al., 1987). The stability of these structures was studied by monitoring the reversible double helix ⇌ coil transition with UV hyperchromicity and variable temperature ¹H NMR. This led to the conclusions that (i), the melting temperature (T_m-value) increases linearly with n, the length of the duplex, and (ii), the ionic strength of the buffer solution does not affect the T_m-value. These observations were explained by the lack of electrostatic phosphate-phosphate repulsion. Our structural model of the parallel double helix was based on the X-ray crystal structure of 3',5'-di-O-acetylthymidine, ¹H NMR experiments on the phosphate-methylated dinucleotide d(TpT), and

on AMBER calculations (van Genderen et al., 1987). This led to a slim parallel duplex with eight base pairs per turn.

We have subsequently been interested in phosphate-methylated DNA oligomers incorporating the bases A, C, and G. Recently, this resulted in a novel general synthesis route for these systems, which utilizes 9-fluorenylmethoxycarbonyl (Fmoc) as temporary NH_2 -protecting group for A, C, and G (Koole et al., 1988). The cleavage of this blocking group can be effectuated under mild basic conditions in such a way that the methylated phosphate groups are preserved. Herein, we wish to report some preliminary results of a conformational study on the phosphate-methylated dinucleotides 1-5, which were obtained as mixtures of R_P and S_P diastereoisomers, which could be separated with reversed phase HPLC (Stec et al., 1985). Phosphorus configurations were assigned on the basis of an analysis of the NOE contacts in the 300 MHz ^1H NMR spectra (Summers et al., 1986). The conformations of the backbone and the 2'-deoxyribose rings were deduced from vicinal proton-proton and proton-phosphorus NMR coupling constants. The results show pronounced preferences for β^t ($\text{C}_{5'}$ - $\text{O}_{5'}$ bond), γ^+ ($\text{C}_{4'}$ - C_5' bond), and $\text{C}_{2'}$ -endo (2'-deoxyribose rings) in all cases. Apparently, the compounds 1-5 adopt a standard B DNA like conformation, irrespective of the configuration at phosphorus.



Base-base stacking and/or duplex formation was studied on the basis of chemical shift vs. temperature profiles of the non-exchangeable base protons in the temperature range 277-348 K. For 1-4, it is found that the S_P and R_P diastereoisomers show virtually identical chemical shift vs. T curves, revealing that the mode and the extent of base-base stacking is not related to the configuration at phosphorus. The absence of a sigmoidal shape indicates that no duplex formation occurs in these compounds. In fact, only the compounds 1 and 4 are capable of duplex formation in a Watson & Crick fashion by a self-complementary arrangement. These are borderline cases, as can be abstracted from our previous work (Moody et al., 1988 a,b).

Surprisingly, the S_P diastereoisomer of 5 (phosphate-methylated d(CpC))

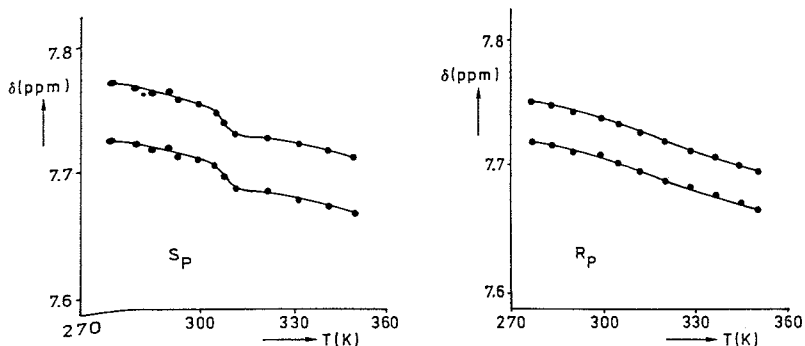


Fig. 1. Chemical shift vs. temperature profiles of the H_6 protons in the S_P and R_P diastereoisomers of phosphate-methylated d(CpC). The upper and lower curves correspond with the 5'- and 3'-residues, respectively (Cheng & Sarma, 1977).

shows a sigmoidal shape for $\delta(H_6)$ vs. T (midpoint 306 K), whereas a continuously decreasing profile was found for its R_P form (figure 1). These observations are clearly reminiscent of exclusive duplex formation with C-C base pairs for the S_P form of 5, and not for the R_P form. Additional evidence was obtained with UV hyperchromicity experiments, which revealed a reversible melting transition with a T_m -value of 304 K for the S_P diastereoisomer, and a continuously increasing UV extinction for the R_P diastereoisomer (figure 2). It should be mentioned that the UV hyperchromicity experiments were performed with 0.01 M Tris/0.01 M $MgCl_2$ buffer solutions (pH 7.5).

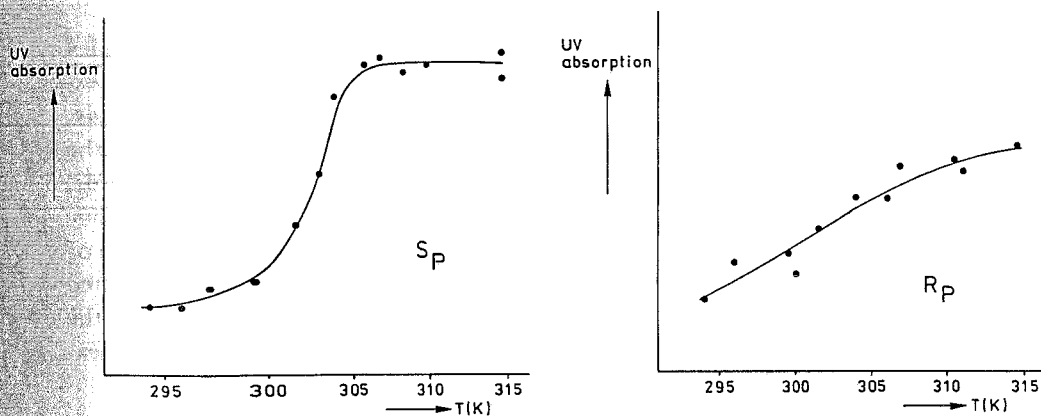
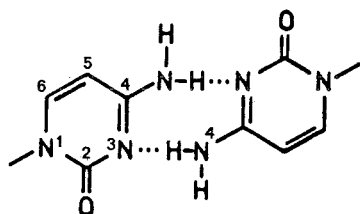
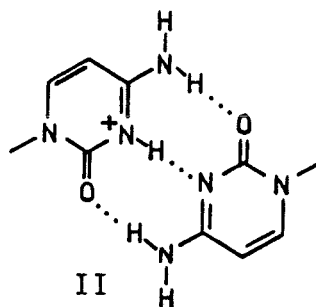


Fig. 2. UV hyperchromicity curve of the S_P and R_P diastereoisomers of phosphate-methylated d(CpC).

In order to arrive at a structural model for the duplex with C-C base pairs, it is important to realize that C-C base pairing is possible only in a parallel manner (Saenger, 1984). Moreover, two modes of parallel C-C base pairing, i.e., I and II, are known. Note that one of the C-bases in II is protonated at N_3 , which results in a third hydrogen bond. Base pairing according to I has been



I



II

found in the X-ray crystal structure of 2'-deoxycytidine (Young et al., 1975). The C⁺-C base pair II has been proposed, based on spectroscopic studies on poly(dC) and poly(C) under acidic conditions (pH 4.5; Adler et al., 1966; Guschlbauer, 1967), and d(CpTpCpTpCpT) at pH 3 (Sarma et al., 1986). However, no direct crystallographic evidence for II has been reported.

We were able to establish that C-C base pairing in the parallel duplex of the S_P form of **5** is conform I, by measuring the T_m-value as a function of the pH of the buffer solution (pH range: 7.5-2.6). These experiments clearly showed that the T_m-value *decreases* when the pH is lowered (for example: T_m = 304 K at pH 7.5 (vide supra), and T_m = 287 K at pH 4). This is consistent with protonation of N₃ (pK_a 4.6; Fasman, 1975), which effectively excludes hydrogen bonding with N₄ of the partner base. Below pH 3.2, an increase of the T_m value was noted, indicating that II then becomes the most important mode for C-C base pairing.

In summary, it has been shown that a novel parallel righthanded duplex structure with C-C base pairs is formed for the S_P diastereoisomer of **5** exclusively. The C-bases are linked via two identical N₄-H...N₃ hydrogen bonds. Model studies suggest that the S_P form accommodates a duplex better than the corresponding R_P one, since the S_P methoxy group is located outwards, whereas in the R_P form a sterically unfavoured inward location of the methoxy group would be present (Van Genderen et al., 1987). The recent observation of Koole et al. that on the hexanucleotide level of phosphate-methylated d(CpCpCpCpCpC) no duplex could be established is in agreement with the presence of a relatively small amount (0.1%) of complete S_P chirality in the hexanucleotide (Koole et al., 1987).

Further experimental and theoretical work is in progress in order to establish the structural properties of the parallel duplex with C-C base pairs.

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