Eur Biophys J (1987) 14: 293–299

European Biophysics Journal

Two-dimensional ^{1}H NMR study of two cyclobutane type photodimers of thymidylyl-(3′ → 5′)-thymidine

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Received February 27, 1986/Accepted in revised form September 6, 1986

Abstract. 2D NMR spectroscopy and *J* coupling constant analysis are applied to resolve the structure of two photoproducts of thymidylyl-(3′ → 5′)-thymidine. These products are cyclobutane type thymine dimers possessing the *cis-syn* (the predominant one) and *trans-syn* geometry. The *cis-syn* is formed in an ANTI-ANTI conformation about the *N*-glycosyl linkages and resembles the normal base-stacked configuration. The glycosidic conformation in solution of the 5′ terminal fragment differs from the crystal in which the less common SYN conformation is observed. In this isomer only the sugar pucker of the 3′ terminal fragment is changed substantially with respect to the dinucleotide. The *trans-syn* isomer is formed in a SYN-ANTI glycosidic conformation. In this isomer the sugar puckers of both deoxyribose rings are affected and a preference for a pure 2′-endo conformation is observed.

Key words: ^{1}H NMR, 2D NOE spectroscopy, thymine photodimer, *cis-syn* dTp[]dT, *trans-syn* dTp[]dT

1. Introduction

It is well known, that UV radiation can induce mutations in DNA, the main effect being on the pyrimidines (Beukers et al. 1960; Wacker et al. 1960; Wulff 1963). The main photoproduct of UV irradiated DNA is the cyclobutane type photodimer of adjacent thymine residues in two isomeric forms. As can be expected on account of the base-stacked configuration of DNA the predominant photoproduct possesses the *cis-syn* geometry (Weinblum and Johns 1966; Blackburn and Davies 1967; Weinblum 1967; Hollis and Wang 1967; Camerman and Camerman 1970). In heat-denatured DNA also the *trans-syn* photoisomer has been observed (Ben-Hur and Ben-Ishai 1968) (*cis-syn* and *trans-syn* refer to the geometry of the cyclobutane junction). Repair enzymes recognize these radiation defects (Wacker 1961; Wulff and Rupert 1962). Little is known, however, about the mechanism of binding of repair enzymes to UV-irradiated DNA. Knowledge about the structure of these modified DNA molecules may therefore be important. Elucidation of the three-dimensional structure of small oligonucleotides containing a thymine photodimer should give valuable information on this point. Small model systems for radiation defects are photoproducts from dTpdT. As reported by Johns et al. (1964) four photoproducts are formed by direct irradiation of dTpdT, while two isomeric forms are present after acetophenone sensitized irradiation (Fig. 1). It has been suggested on steric grounds, that the predominant isomeric product possesses an ANTI-ANTI *cis-syn* geometry and the minor one a SYN-ANTI *trans-syn* geometry (Hruska et al. 1975; Lui and Yang 1978) (ANTI and SYN refer to the conformation about the *N*-glycosyl linkages). A recent X-ray study of a *cis-syn* dTp[]dT dimer (Cadet et al. 1985) has shown that in the crystal the dTp[]- fragment has a SYN glycosidic conformation, while the -[]pdT fragment has the more common ANTI conformation. For the *cis-syn* isomer our results indicate that the conformation in solution is quite different from that in the crystal.

Structural information of oligonucleotides in aqueous solution can be obtained from two-dimensional NMR (Feigon et al. 1982; Scheek et al. 1983; Hare et al. 1983). The two photodimers obtained from an irradiated solution containing acetophenone and dTpdT were separated by RP-HPLC and COSY

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Abbreviations: dTpdT, 2′-deoxythymidylyl-(3′ → 5′)-2′-deoxythymidine; dTp[]dT, cyclobutane type photodimers of dTpdT; dTp- and dTp[]-, their 5′ terminal fragments (fragment A); -pdT and -[]pdT their 3′ terminal fragments (fragment B); RP-HPLC, reversed-phase high-performance liquid chromatography; COSY, two-dimensional correlated spectroscopy; 2D NOE, two-dimensional nuclear Overhauser spectroscopy

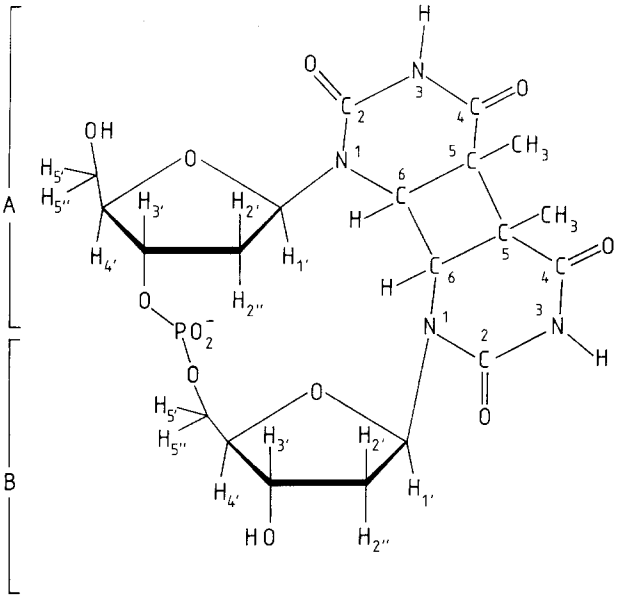

Fig. 1 A and B. Structure of cyclobutane type photodimers of 2′-deoxythymidylyl-(3′ → 5′)-2′-deoxythymidine. **A** and **B** refer to the 5′ and 3′ terminal fragments respectively

and 2D NOE spectra of both photoisomers were recorded. From specific NOE effects and analysis of the J couplings it was possible to define the conformation of both isolated photoproducts.

2. Experimental

The photodimers were produced by irradiation of an aqueous solution of 2 mM dTpdT (Sigma) containing 13 mM acetophenone with a 450 W Xenon lamp (Osram) or with an excimer laser (Lambda Physik EMG 101) at 308 nm. The reaction mixture was kept at room temperature during irradiation by water cooling; nitrogen was bubbled through the solution in order to remove oxygen. Irradiation time was typically 30 min. After removing the solvent by lyophylization the crude reaction mixture was purified by RP-HPLC (Waters gradient system) (Demidov and Potaman 1984). The sample was dissolved in a 0.12 M ammonium acetate in water buffer (solvent system A). This solution was introduced into a Nucleosil 10 C-18 column (Machery-Nagel). Exposed to a linear gradient of 0.10 M ammonium acetate in water-methanol (1:1) (solvent system B) three absorbing compounds were detected at 230 nm. The first two compounds could be identified by ^{1}H NMR as the *cis-syn* and the *trans-syn* isomer of dTpdT, which were formed in a ratio of 6:1. The third originated from the starting compound dTpdT. The buffer system was removed by evaporation of the solvent by means of a Speed-Vac (Savent) and by lyophilization. The samples were lyophilized from ^2H_2O once to remove the residual ^1H_2O.

^{1}H NMR spectra were recorded at 360 MHz of both photodimers dissolved in ^2H_2O at 30 °C using a Bruker HX-360 spectrometer equipped with an ASPECT 2000 computer. Phase-sensitive 2D NOE spectra were obtained as described elsewhere (Scheek et al. 1984). 512 FID's of 2,048 data points each were collected with a spectral width of 5,000 Hz, the carrier on the left side of the spectrum and a mixing period of 450 ms was used. The data were processed on a VAX 11/750 computer with software written in FORTRAN 77. In the t_2 dimension the data points were multiplied with an exponential window function corresponding to 4 Hz line broadening before Fourier transformation. In the t_1 dimension the data points were multiplied by a squared sine bell shifted 90° before Fourier transformation. After two-dimensional Fourier transformation the spectra were phase and baseline corrected in both dimensions (Boelens et al. 1985). The final resolution of the 2D NOE spectrum (512×512) was 5 Hz/point. Absolute value COSY spectra of both stereoisomers were recorded in a similar way. The data points were multiplied by a sine bell function before Fourier transformation in both dimensions.

Pseudorotational analysis of the furanose ring was carried out using a method developed by Haasnoot et al. (1980, 1981). The vicinal spin-spin coupling constants were simulated using an iterative least-squares fit based on a Simplex algorithm.

3. Results and discussion

cis-syn dTp[]dT

The proton spectrum of the predominant photoproduct of dTpdT can be assigned almost completely on the basis of the COSY and 2D NOE spectra presented in Figs. 2 and 3. The distinction between the 3′ and 5′ terminal fragment was made on the basis of the absence of a J coupling between the 5′ and 5″ protons of dTp[]- and ^{31}P (Rance et al. 1985). The 5′ and 5″ protons were pairwise assigned to the specific residues A and B. The other assignments for the ribose and H6 protons all follow from COSY cross peaks and intraresidue NOE's and are in agreement with those given by Hruska et al. (1975) (cf. Table 1).

To assign the methyl resonances cross-sections through the spectrum (Fig. 4) were used. Assignments of these methyl groups follow from the observation of a strong NOE to their own H6 protons and differ from those presented by Hruska (1975). The observation of a 5.9 Hz coupling between the

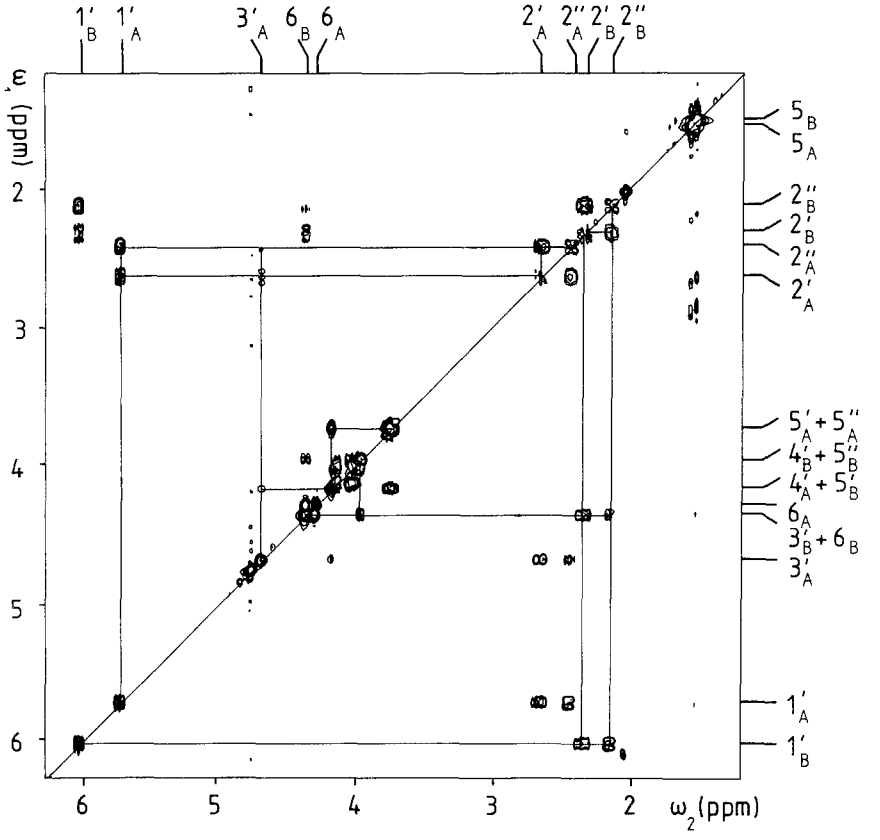

Fig. 2. 360 MHz COSY spectrum of *cis-syn* dTp[]dT in ^2H_2O at 30 °C. In the part above the diagonal the deoxyribose spin system of dTp[]- and below the diagonal the deoxyribose spin system -[]pdT is indicated

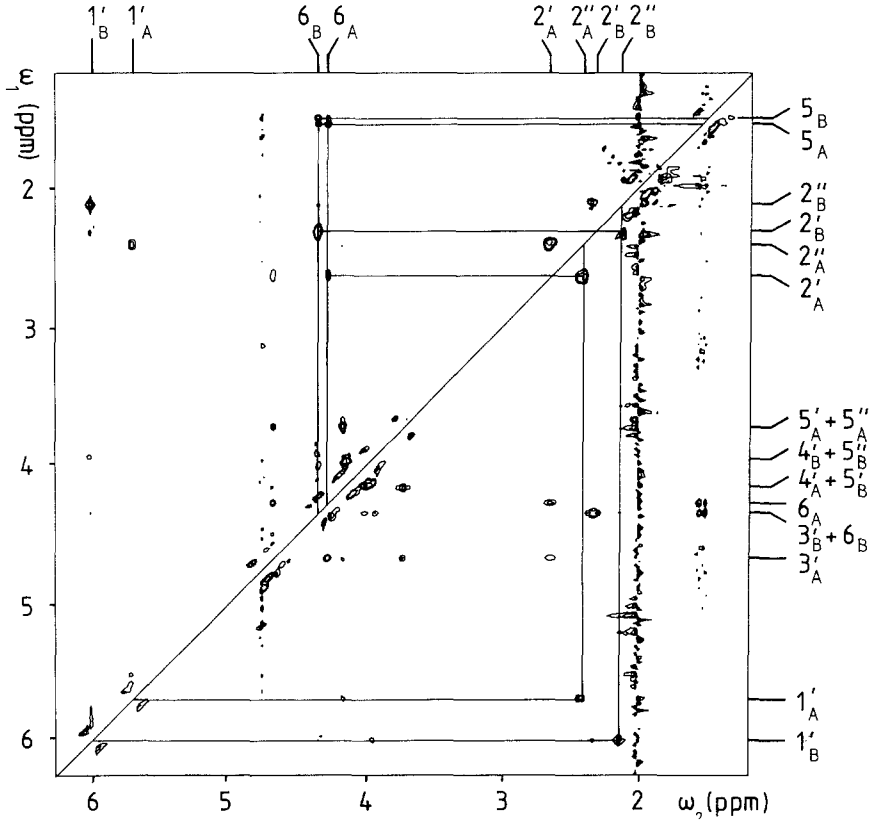

Fig. 3. 360 MHz 2D NOE spectrum of *cis-syn* dTp[]dT in ^2H_2O at 30 °C. Negative contour levels are shown corresponding to the negative sign of the NOE's with respect to the diagonal for a molecule in the fast tumbling limit. In the part above the diagonal NOE cross peaks for the methyl groups 5_A and 5_B and the ribose protons $2'_A$ and $2'_B$ with the H6 (6_A and 6_B) base protons are indicated. Below the diagonal the ribose 1', 2' and 2" NOE's are indicated

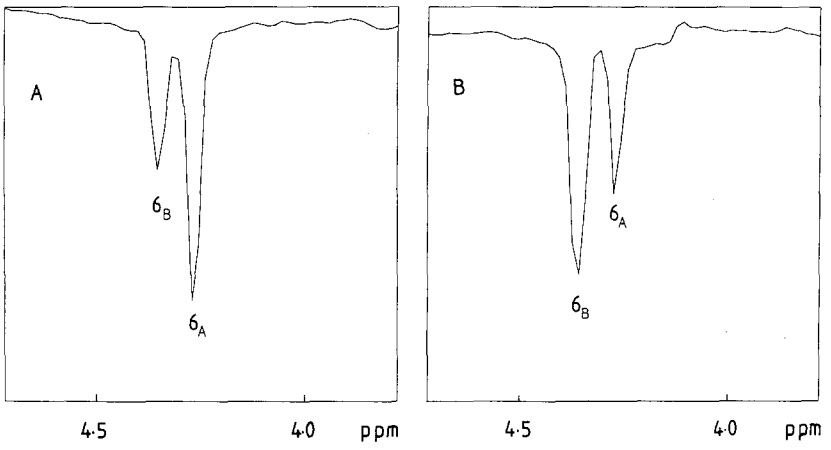

Fig. 4 A and B. Cross-sections through the 2D NOE spectrum of Fig. 3. The region of the H6 base protons are shown at the frequencies of the C-5 methyl groups (**A** and **B**)

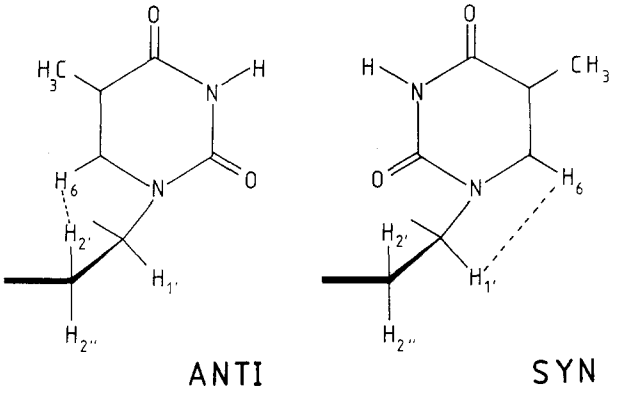

Fig. 5. Short deoxyribose-base proton distances in the case of an ANTI and SYN *N*-glycosidic conformation, which results in relatively strong NOE effects

Table 1. ^{1}H chemical shifts[a] of *cis-syn* dTp[]dT and *trans-syn* dTp[]dT in ppm relative to DDS at 30 °C

	cis-syn dTp[]dT		*trans-syn* dTp[]dT	
	dTp[]-	-[]pdT	dTp[]-	-[]pdT
H1′	5.71	6.01	5.28	5.71
H2′	2.64	2.33	3.30	2.22
H2″	2.42	2.14	2.61	2.02
H3′	4.68	4.27	4.73	4,53
H4′	4.16	3.94	4.14	3.89
H5′[b]	3.75	4.16	3.73	4.17
H5″[b]	3.69	3.94	3.69	4,17
H6	4.28	4.37	4.29	4,22
Me5	1.54	1.50	1.49	1.44

[a] Estimated error ca. 0.002 ppm
[b] The H5′ and H5″ protons were assigned only pairwise

H6 protons indicates a vicinal arrangement of these protons and hence a *syn* geometry. In addition, the comparable size of intra- and interresidue NOE's between the H6 and methyl protons is evidence for a *cis-syn* isomer. The similarity of the two cross-sections shown in Fig. 4 indicates that there is no preferred puckered conformation of the cyclobutane ring-system.

As pointed out (Hruska et al. 1975) there are eight different isomers possible for dTp[]dT. The two *cis-syn* isomers, which can occur, differ with respect to the conformation about the *N*-glycosyl linkages. Inspection of Fig. 3 shows that very pronounced H6-H2′ NOE's are present and that NOE's between H6 and H1′ are weak or absent in both units. As can be seen in Fig. 5 this is characteristic for ANTI conformations for both glycosidic bonds. This is in striking contrast to the conformation in the crystal, where the unusual SYN conformation was found for the dTp[]- unit. Sugar conformations can be determined from an analysis of *J* coupling constants between ribose protons (Altona 1982). An extensive set of coupling constants for dTpdT and *cis-syn* dTp[]dT has been reported by Wood et al. (1974) and Hruska et al. (1975) and was confirmed in the present work. The results of a pseudorotational analysis of the vicinal coupling constants measured in both deoxyribose moieties of dTpdT and the *cis-syn* photodimer are presented in Table 2. If a conformational equilibrium between *S* and *N* type conformers (Altona 1982) is assumed, the system is completely described by four pseudorotational parameters, viz. P_N, ϕ_N, P_S, ϕ_S (P and ϕ represent the phase angle of pseudorotation and the pucker amplitude respectively) and the mole fraction of the *S* conformer X_S. P_N and ϕ_N were kept constant during the iterative process, because the coupling constants are mainly determined by the *S* type conformer. In the case of the A residue similar values for the pseudorotation parameters and mole fraction are found for dTp[]- and dTp-. More important differences are observed between the -pdT and -[]pdT moieties. The pseudorotational phase of the *S* type conformer is shifted from about 180° (i.e. a 2′-endo-3′-exo conformation)

Table 2. Observed and calculated[a] coupling constants (in Hz) for both deoxyribose moieties of dTpdT, *cis-syn* dTp[]dT and *trans-syn* dTp[]dT and pseudorotational parameters[b]

	dTpdT[c]		*cis-syn* dTp[]dT[d]		*trans-syn* dTp[]dT[e]	
	dTp-	-pdT	dTp[]-	-[]pdT	dTp[]-	-[]pdT
$J_{1'2'}$	7.2 (7.5)	6.9 (6.5)	8.5 (8.8)	8.6 (8.5)	10.7 (10.6)	10.7 (10.6)
$J_{1'2''}$	6.1 (6.4)	6.9 (6.6)	5.2 (5.3)	5.6 (5.8)	4.9 (4.9)	4.9 (4.9)
$J_{2'3'}$	6.0 (6.0)	5.8 (5.6)	5.1 (5.2)	7.6 (7.2)	4.9 (4.9)	– –
$J_{2''3'}$	3.8 (4.0)	5.8 (5.4)	3.2 (3.4)	3.5 (3.2)	– –	1.5 (1.6)
$J_{3'4'}$	3.2 (3.3)	4.0 (4.1)	2.6 (2.7)	4.2 (4.5)	– –	– –
rms[f]	0.2	0.3	0.2	0.2	0.1	0.1
P_S	164°	177°	158°	127°	158°	162°
ϕ_S	37°	43°	44°	40°	42°	42°
X_S	68%	55%	78%	77%	100%	100%

[a] Coupling constants calculated according to the optimized parameters are included in parentheses
[b] Optimized pseudorotation parameters (i.e. pseudorotational phase of the ribose ring P and the pucker amplitude ϕ) for S type conformer and the mole fraction of S type present (X_S). The parameters for the N type conformer were constrained to $P_N = 10°$ and $\phi_N = 35°$
[c] Coupling constants were taken from Wood et al. (1974) and were measured at 18°
[d] Coupling constants measured by Hruska et al. (1975) at 18°
[e] This work (measured at 65°); estimated error in J ca. 0.3 Hz
[f] Rms differences between calculated and experimental J-coupling constants given in Hz

observed in -pdT to 127° (i.e. a 1'-exo conformation) in -[]pdT. This corresponds to a dihedral angle of $\delta = 117°$ about the C3'−C4' bond (Haasnoot et al. 1981). This conformation was also found in one of three structures of dTp[]dT calculated by molecular mechanics methods (Rao et al. 1984) ($\delta = 118°$). This thymine dimer structure was generated from a pair of neighbouring thymines in a double helical dodecamer; however, it was not the lowest energy conformation found by these authors. For the dTp[]- fragment we find $\delta = 141°$ (a more normal value for S conformers), which is in agreement with the calculated value of $\delta = 136°$ (Rao et al. 1984).

trans-syn dTp[]dT

The 2D NOE spectrum of the minor photoproduct of irradiated dTpdT is presented in Fig. 6 (COSY spectrum is not shown). The procedure of assignment is the same as outlined before. The assignment of the spectrum confirms the previous one carried out by Lui and Yang (1978) (cf. Table 1). Comparison of the 2D NOE spectra from the two photoproducts (Figs. 3 and 6) shows, that the minor photoproduct can be identified as the *trans-syn* isomer. The argument is as follows: Again a J coupling (7.3 Hz) between the H6 protons indicates a structure of the *syn* family. The cross-section in Fig. 7 shows strong NOE's between the C-5 methyl groups and the H6 base proton residing on the same fragment. In addition small inter-residue NOE's between the C-5 methyl groups and the H6 base proton residing on different fragments are now observed indicating a *trans-syn* isomer.

Hruska et al. (1975) predicted, that the glycosidic bond conformation is SYN for dTp[]- and ANTI for -[]pdT. The other *trans-syn* conformation is supposed to be less favored, because of the repulsion between the C-2 keto oxygen of the -[]pdT fragment and the negatively charged phosphate. Evidence for the conformation about the N-glycosidic bond is provided by NOE analysis (Fig. 5). A strong intra-nucleotide NOE is observed between H6 and H1' of dTp[]-, which is characteristic for a SYN conformation (cf. Fig. 6). A strong intra-nucleotide NOE between H6 and H2' of -[]pdT indicates an ANTI conformation for this unit. This nicely confirms the predictions of Hruska et al. (1975).

J coupling constants of the deoxyribose protons were measured for this photodimer and are given in Table 2. Pseudorotational analysis of these coupling constants shows that both ring systems adopt exclusively an S type conformation. Apparently there is less conformational freedom in the *trans-syn* isomer so that an $S-N$ equilibrium for both ribose rings becomes impossible.

The NMR data of two isomeric photoproducts of dTpdT are consistent with an ANTI-ANTI *cis-syn* isomer as the predominant photoproduct and a SYN-ANTI *trans-syn* isomer as the minor one. In the *cis-syn* isomer only the sugar pucker of the 3' terminal fragment differs substantially from that in dTpdT and is found to be predominantly in a 1'-exo conformation. Apparently the solution structure of this isomer differs considerably from

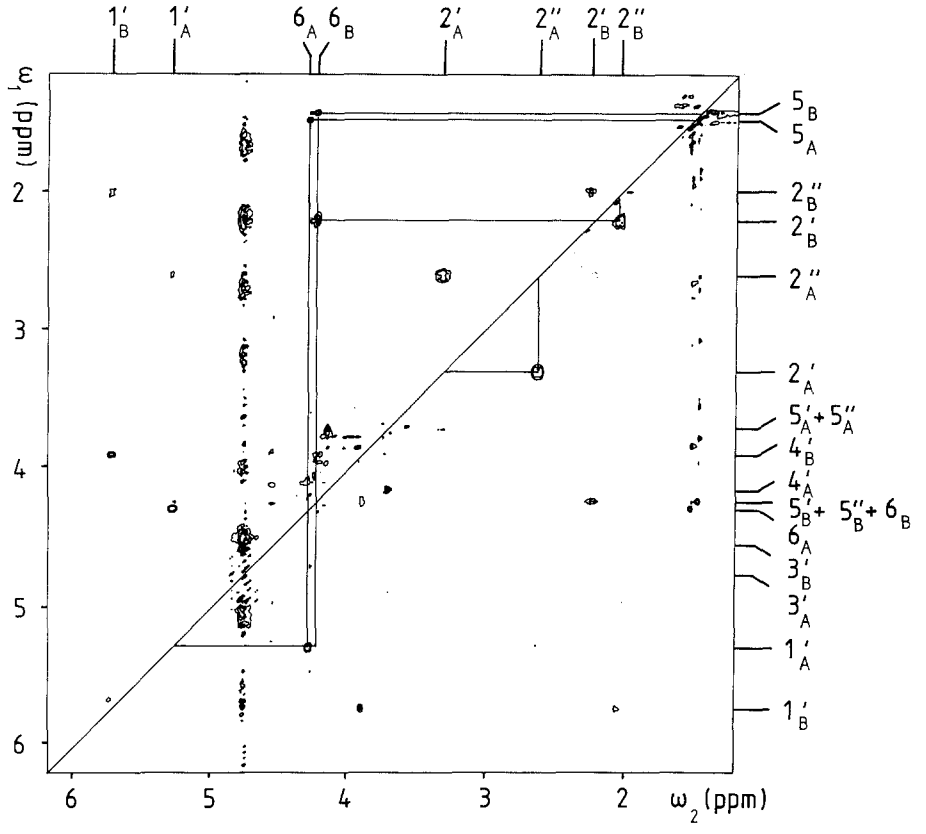

Fig. 6. 360 MHz 2D NOE spectrum of the *trans-syn* dTp[]dT in ^2H_2O at 30 °C. Negative contour levels are shown corresponding to the negative sign of the NOE's with respect to the diagonal for a molecule in the fast tumbling limit. Above the diagonal NOE connectivities are indicated for the methyl groups 5_A and 5_B and the ribose proton $2'_B$ with the H6 base protons (6_A and 6_B). Below the diagonal part NOE connectivities for the H1′ of fragment *A* with both H6 base protons are indicated as well as the connectivities for the 2′ and 2″ ribose protons for both units

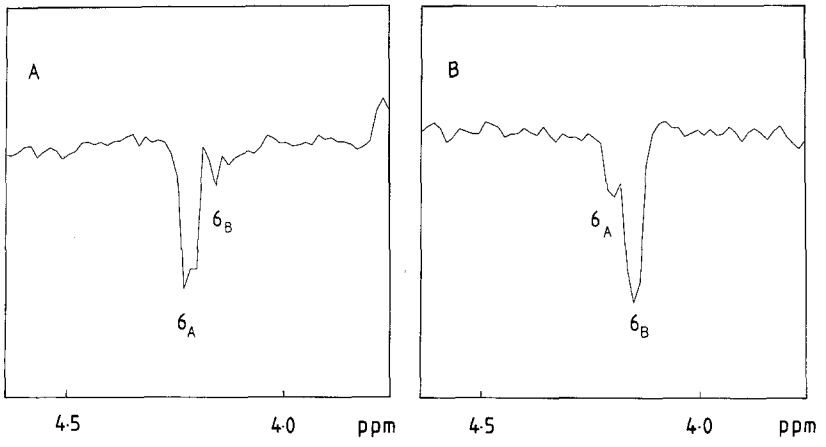

Fig. 7A and B. Cross-sections through the 2D NOE spectrum of Fig. 6. The region of the H6 base protons are shown at the frequencies of the C-5 methyl groups (**A** and **B**)

the crystal structure of a similar *cis-syn* thymine dimer, in which the phosphate group was present as a triester with a cyanoethyl group attached (Cadet et al. 1985). In the latter case a SYN-ANTI glycosidic conformation was found. In the *trans-syn* isomer both sugar puckers differ markedly from the parent dinucleotide and are fixed in a pure 2′-endo conformation. Further investigations on the structure of thymine dimers in oligonucleotides are in progress.

Acknowledgements. This work was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). We thank P. Wietzes and K. Dijkstra for their expert technical assistance.

References

Altona C (1982) Conformational analysis of nucleic acids. Determination of backbone geometry of single-helical RNA and DNA in aqueous solution. Recl Trav Chim Pays-Bas 101:413–433

Ben-Hur E, Ben-Ishai R (1968) *Trans-syn* thymine dimers in ultraviolet-irradiated denaturated-DNA: identification and photoreactivability. Biochim Biophys Acta 166:9–15

Beukers R, Ijlstra J, Berends W (1960) The effect of ultraviolet light on some components of the nucleic acids. Recl Trav Chim 79:101–104

Blackburn GM, Davis RJH (1967) Photochemistry of nucleic acids. III. The structure of DNA-derived thymine photodimer. J Am Chem Soc 89:5941–5945

Boelens R, Scheek RM, Dijkstra K, Kaptein R (1985) Sequential assignment of imino- and amino-protein resonances in

^{1}H NMR spectra of oligonucleotides by two-dimensional NMR spectroscopy. Application to a *lac* operator fragment. J Mag Res 62:378–386

Cadet J, Voituriez L, Hruska FE, Grand A (1985) Crystal structure of the *cis-syn* photodimer of thymidylyl-(3′ → 5′)-thymidine cyanoethyl ester. Biopolymers 24:897–903

Camerman N, Camerman A (1970) Crystal and molecular structure of photodimer *A* of 1,3-dimethylthymine (the isomer in irradiated deoxyribonucleic acid). J Am Chem Soc 92:2523–2527

Demidov VV, Potaman VN (1984) High-performance liquid chromatography of nucleic acid components. I. Thymine dimers in short oligonucleotides. J Chromatogr 285:135–142

Feigon J, Wright JM, Leupin W, Denny WA, Kearns DR (1982) Use of two-dimensional NMR in the study of a double-stranded DNA decamer. J Am Chem Soc 104: 5540–5541

Haasnoot CAG, de Leeuw FAAM, de Leeuw HPM, Altona C (1980) The relationship between proton-proton NMR coupling constants and substituent electronegativities. I. An empirical generalization of the Karplus equation. Tetrahedron 36:2783–2792

Haasnoot CAG, de Leeuw FAAM, de Leeuw HPM, Altona C (1981) The relationship between proton-proton NMR coupling constants and substituent electronegativities. II. Conformational analysis of the sugar ring in nucleosides and nucleotides in solution using a generalized Karplus equation. Org Magn Reson 15:43–52

Hare DR, Wemmer DE, Chou S-H, Drobny G, Reid BR (1983) Assignment of the non-exchangeable proton resonances of d(C-G-C-G-A-A-T-T-C-G-C-G) using two-dimensional nuclear magnetic resonance methods. J Mol Biol 171:319–336

Hollis DP, Wang SY (1967) Structure of homodimers of thymine and dimethylthymine. A nuclear magnetic resonance study. J Org Chem 32:1620–1622

Hruska FE, Wood DJ, Ogilvie KK, Charlton JL (1975) A proton magnetic resonance study of the ultraviolet photoproduct of d(TpT) in aqueous solution. Can J Chem 53:1193–1203

Johns HE, Pearson ML, LeBlanc JC, Helleiner CW (1964) The ultraviolet photochemistry of thymidylyl-(3′ → 5′)-thymidine. J Mol Biol 9:503–524

Lui F-T, Yang NC (1978) Photochemistry of cytosine derivatives. I. Photochemistry of thymidylyl-(3′ → 5′)-deoxycytidine. Biochemistry 17:4865–4876

Rance M, Sørensen OW, Leupin W, Kogler M. Wuthrich K, Ernst RR (1985) Uniform excitation of multiple-quantum coherence. Application to two-dimensional double-quantum spectroscopy. J Magn Res 61:67–80

Rao SN, Keepers JW, Kollman P (1984) The structure of d(CGCGAAT[]TCGCG)*d(CGCGAATTCGCG): the incorporation of a thymine photodimer into a B-DNA helix. Nucleic Acids Res 12:4789–4809

Scheek RM, Russo N, Boelens R, Kaptein R (1983) Sequential resonance assignment in DNA ^{1}H NMR spectra by two-dimensional NOE spectroscopy. J Am Chem Soc 105:2914–2916

Scheek RM, Boelens R, Russo N, van Boom JH, Kaptein R (1984) Sequential resonance assignment in ^{1}H NMR spectra of oligonucleotides by two-dimensional NMR spectroscopy. Biochemistry 23:1371–1376

Wacker A (1961) Strahlenchemische Veränderungen von Pyrimidinen in Vivo und in Vitro. J Chim Phys 58:1041–1045

Wacker A, Dellweg H, Weinblum D (1960) Strahlenchemische Veränderung der Bakterien-Desoxyribonucleinsäure in vivo. Naturwissenschaften 20:477

Weinblum D (1967) Characterization of the properties of isomeric thymine dimers. Biochem Biophys Res Commun 27:384–390

Weinblum D, Johns HE (1966) Isolation and properties of isomeric thymine dimers. Biochim Biophys Acta 114: 450–459

Wood DJ, Hruska FE, Ogilvie KK (1974) Proton magnetic resonance studies of 2′-deoxythymidine, its 3′- and 5′-monophosphates and 2′-deoxythymidylyl-(3′ ← 5′)-2′-deoxythymidine in aqueous solution. Can J Chem 52:3353–3366

Wulff DL (1963) The role of thymine dimer in the photoinactivation of the Bacteriophage T4 ν_1. J Mol Biol 7:431–441

Wulff DL, Rupert CS (1962) Disappearance of thymine photodimer in ultraviolet irradiated DNA upon treatment with a photoreactivating enzyme from Baker's yeast. Biochem Biophys Res Commun 7:237–240