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2'-DEOXY-ß-D-XYLOTHYMIDINYL-(3', 5')-2'-DEOXY-ß-D-XYLOTHYMIDYLATE: STEREOCHEMICAL COURSE OF DINUCLEOSIDE PHOSPHONATE FORMATION AND CONFORMATIONAL PROPERTIES

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ABSTRACT. Coupling of 5'-DMT-protected 1-(2-deoxy-ß-D-xylofuranosyl)thymine 3'-phosphonate with 3'-benzoylated 1-(2-deoxy-ß-D-xylofuranosyl)thymine gave the diastereoisomeric dinucleoside phosphonates **4/5** with the S_P compound in preponderance (de, 29 %). Oxidation of **4/5** (I_2, H_2O) and removal of the protecting groups gave d(xTpxT) which exhibits an inverted CD-spectrum compared to d(TpT) but identical stacking interactions.

The stereoselective formation of dinucleoside monophosphates is an important task for the synthesis of diastereochemically pure oligonucleotides modified at phosphorous. Due to the non-stereoselective condensation in the case of phosphoramidites R_P/S_P, mixtures are formed which cannot be accepted in case of pharmaceutically active compounds e.g. antisense oligonucleotides. Furthermore, spectroscopic and physicochemical characteristics of dinucleoside monophosphates modified at the sugar-phosphate backbone are important for the understanding of the properties of higher molecular weight DNA fragments. X-Ray and NMR-spectroscopic analysis of regular d(pTpT) revealed that both nucleotide units exhibit almost identical conformational parameters so that an extrapolation of the dimer molecular structure to a polynucleotide was reasonable [1].

Recently, we synthesized the first oligo(2'-deoxyxylonucleotide) {d[(xT)$_{12}$-T]} applying phosphonate chemistry on a polymeric support [2]. In order to study the stereochemistry of formation as well as base stacking interactions the synthesis of the model compound d(xTpxT) was then carried out in solution. For the synthesis of 2'-deoxyxylothymidine (**2a**), thymidine (**1**) was converted into 2, 3'-anhydro-5'-O-(4-methoxybenzoyl)thymidine by a one-pot transformation involving a tandem Mitsunobu reaction according to Czernecki and Valery [3]. Treatment of this compound with Dowex 1 x 2 (OH^-) at 50 °C for 3 days gave crystalline **2a** in 65 % yield. After 4,4'-dimethoxytritylation of **2a** at the 5'-hydroxyl (**2b**) [2], the 3'-OH group was either benzoylated using benzoylcyanide [4] and subsequently detritylated to form **2c** or derivatized to the 3'-phosphonate **3** according to the procedure in reference 2.

Compounds **2c** and **3** (0.16 mmol, each) were reacted in the presence of pivaloyl chloride. Flash chromatography (silica gel 60H, 6x15 cm; EtOAc-HOAc, 998:2) afforded a diastereomeric mixture (**4/5**) [5] in 72 % yield as colorless foam. Their structure was confirmed by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra. Comparison of the ^{31}P-NMR signals of **4/5** with those of the protected R_P and S_P isomers of d(TpT) [6] shows almost identical chemical shifts [7]. The slower migrating compound which resonates at lower field [$\delta(^{31}P)$ = 10.76 ppm] was therefore tentatively assigned as the sterically less-hindered S_P diastereoisomer (**5**); the NMR-spectra as well as the quantitative t.l.c.-scanning (HPTLC, silica gel F_{254}; EtOAc-HOAc, 998:2) revealed a significant asymmetric induction during the coupling reaction which is different to the coupling of 2'-deoxy-ß-D-ribo - configured building blocks prepared by phosphonate chemistry. The S_P diastereoisomer was formed in preponderance with a de value of 29 % of **5** (Table). The corresponding diastereoisomeric mixture of fully protected d(TpT) phosphonates exhibited de value of only 10 % of the S_P diastereoisomer.

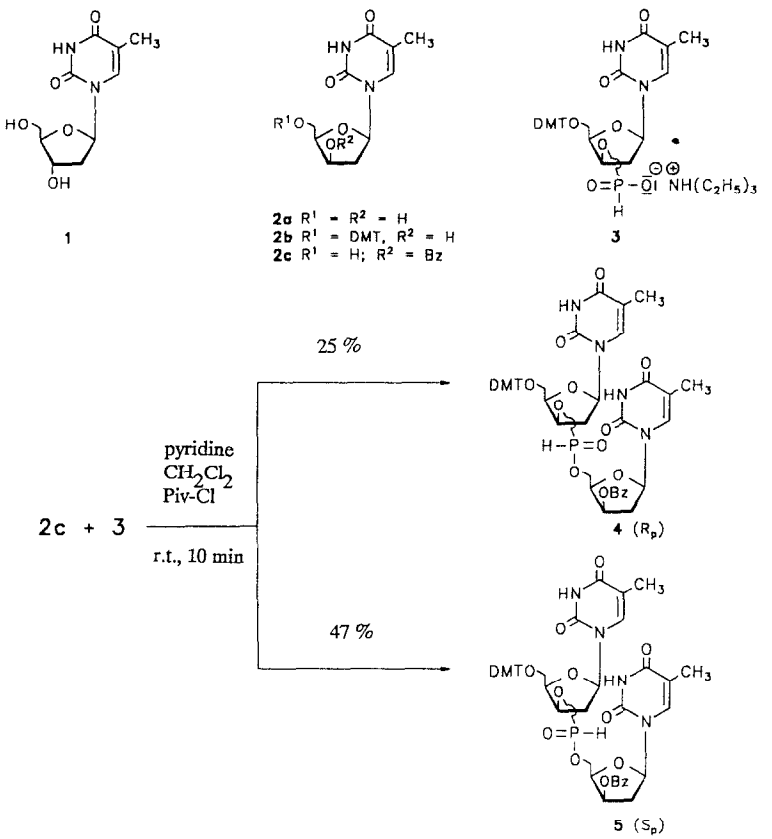


Table. Diastereomeric excess (de [%]) of S_P Compounds of 5'-DMT-3'-Bz protected Dinucleoside Monophosphonates.

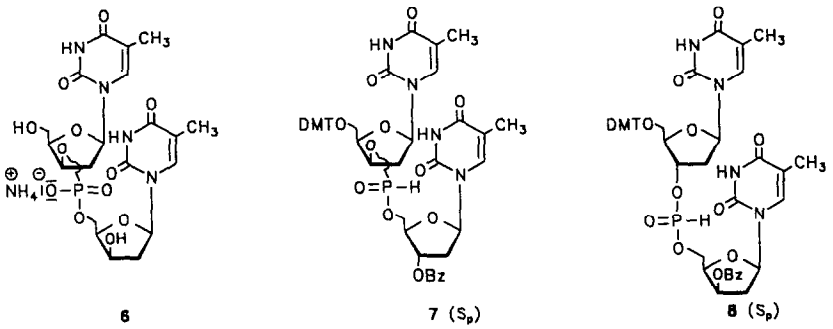
Compound	de [%]	S_P [%]	R_P [%]
5'-DMT-(TpT)-3'-bz	10	55	45
5'-DMT-(xTpT)-3'-bz (**7**)	32	66	34
5'-DMT-(TpxT)-3'-bz (**8**)	35	69	21
5'-DMT-(xTpxT)-3'-bz (**5**)	29	64	36

p: phosphonate internucleotide linkage

This is in line with findings of Stawinsky et al. who also reported a slight asymmetric induction [8]. Coupling of the 3'-phosphonate **3** with 3'-benzoylated thymidine as well as coupling of 5'-DMT-protected thymidine 3'-phosphonate with compound **2c** led to protected d(xTpT) (**7**) and d(TpxT) (**8**), respectively, as diastereomeric mixtures. Interestingly, the S_P-configured diastereoisomer was always formed in preponderance (Table). These findings clearly demonstrate that the asymmetric induction of dinucleoside phosphonate formation shows a de of 29 - 35 % if either one or both of the nucleoside

moieties are in the 3',4'-threo configuration. This result offers the possibility of synthesizing diastereochemically enriched phosphothioates and methylphosphonates, or even pure compounds if the de can be further improved.

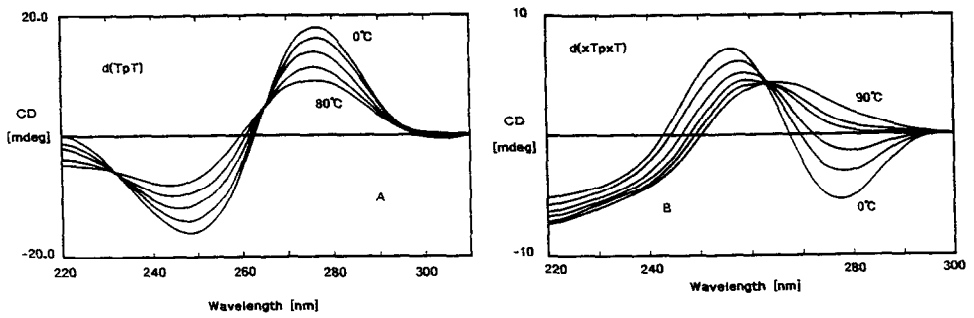
Another interesting question which arose was the conformation of the sugar-phosphate backbone of dimeric xT_d phosphates. For this purpose, the diastereoisomeric phosphonate esters **4/5** (210 mg, 0.22 mmol) were oxidized with I_2/H_2O; excess of iodine was removed by extraction with 1 % aq. Na_2SO_3 [7]. After evaporation of the solvent the residue was triturated (15 min) with 80 % aq HOAc to cleave the 4,4'-dimethoxytrityl group. Subsequent treatment with conc. aq NH_3 for 16 h at r.t. removed the 3'-benzoyl group. Preparative TLC (cellulose plates, (i) EtOAc-acetone-EtOH-H_2O, 18:3:2:2; (ii) 2-PrOH-aq. NH_3-H_2O, 3:1:1) afforded one main zone which was pooled and extracted three times (2-PrOH-aq NH_3-H_2O, 3:1:1) and lyophilized (60% of glassy **6**). Resulting **6** was characterized by ^1H-, ^{13}C-, and ^{31}P-NMR spectra [9].



6 **7** (S_p) **8** (S_p)

Figure 1 displays temperature-dependent CD-spectra of d(TpT) (A) as well as of **6** (B) within a range of 0-90 °C. Until 50 - 60 °C the CD-spectrum of d(xTpxT) exhibits the same general pattern as that of d[$(xT)_{12}$-T] [2] e.g. a negative Cotton effect around 276 nm and a positive one around 255 nm. This means that already the dimer **6** shows the general structural features of the corresponding DNA-fragment [2,10].

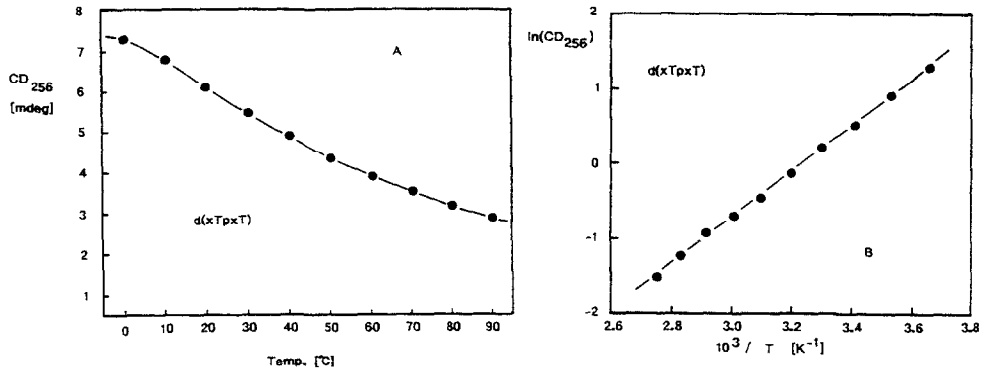
Figure 1. Temperature-dependent CD-spectra of d(TpT) and d(xTpxT)



The appearance of an isodichroic point at 263 nm implies a two-state stack-unstack equilibrium. At 90 °C the CD-spectrum of the sample resembles that of xT_d. The crossing point [θ_0(0 °C) = 267 nm] corresponds with the wavelength of the absorption maximum of the constituent mononucleoside (xT_d, 267 nm), consistent with the exciton theory of identical

interacting chromophores. Comparison of the absolute maximal ellipticity values of d(xTpxT) at a particular temperature with that of regular d(TpT) reveals a strongly different optical transition of the bases [11,12].

Figure 2. A: Ellipticity at 256 nm of d(xTpxT) as a function of temperature; B: van't Hoff plot of the CD melting curve.



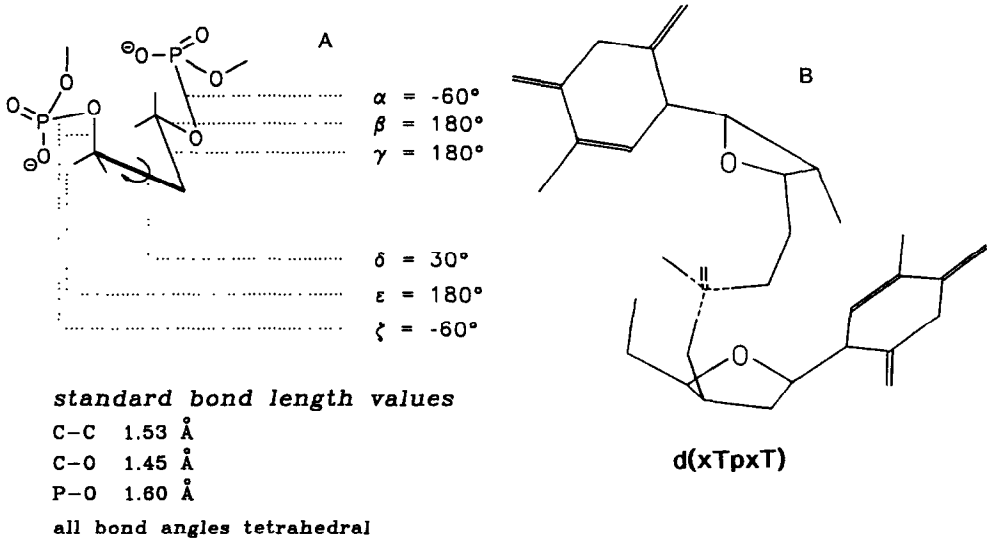
Applying the least-squares analysis of changes in CD-spectra of dinucleotides as a function of temperature in terms of a two-state stack-unstack equilibrium [13] yields the corresponding van't Hoff plot from which a $\Delta H°$-value of - 6.2 kcal/mol and a $\Delta S°$ of -20.0 cal/K mol were calculated. These values are in the range of other dinucleoside monophosphates e.g. d(TpT) [$\Delta H°$ = 6.1 kcal/mol; $\Delta S°$ = -21.0 cal/K mol; according to Figure 1A]. This means that d(TpT) and d(xTpxT) exhibit identical stacking enthalpy values but an altered conformation within the sugar-phosphate backbone - presumably a left-handed helical sense.

In order to substantiate this assumption we applied the conformational analysis of Eschenmoser and Dobler [14] for evaluation of the most preferred structure of a DNA on oligo(2'-deoxyxylonucleotides). This method relies only on three qualitative conformational criteria, e.g. (i) single bonds staggered throughout; (ii) 1,5-repulsion minimized; (iii) phosphodiester conformations according to the anomeric effect. Following these criteria Eschenmoser was able to predict correctly the most preferred and idealized conformation of a "homo"-DNA containing 2',3'-dideoxyglucopyranosylnucleotide building blocks and, moreover, to rationalize the conformations that occur in duplexes of natural A- and B-DNA [14]. The most important outcome of this conformational study was to realize that the helical form of the backbone of DNA duplexes is first and foremost a consequence of the five-memberedness of the sugar ring. The critical structural parameter is the endocyclic torsion angle δ which in a five-membered furanose ring is always greater than 60°, as a consequence of the angle strain which induces flattening of the ring. Arranging the backbone of an oligo(2'-deoxyxylonucleotide) unit under consideration of the three above-mentioned conformational criteria (Figure 3A) the endocyclic torsion angle δ [C(5')-C(4')-C(3')-O(3')] is estimated to be in the range of 20-40° assuming an S-type sugar puckering of the furanose ring. N-type sugar puckering is implausible due to steric repulsion between the heterocyclic base and the 3'-phosphate residue, both placed on the ß-site of the glyconic ring.

Another alteration concerns the exocyclic torsion angle γ [O(5')-C(5')-C(4')-C(3')] which for regular A- and B-DNA ranges around 60°. In case of an oligo(2'-deoxyxylonucleotide) such a value would most probably lead to a 1,5-repulsion between O(5') and O(3'), the extent of which depends on the amplitude of sugar puckering (ν_{max}; the higher the degree of puckering, the lower the 1,5 repulsion). Therefore, it seems reasonable to change γ from $+60°$ to $\pm 180°$.

The roughly estimated δ value [(+20°) - (+40°); sp] leads to a left-handed single-stranded helix with a number n of backbone units per turn of 9 - 12 (n = 360°(δ-60°) and a helical pitch height range of 37.5 - 50.0 Å (H = n · 4.17 Å [14]). These values correspond to those of regular DNA structures.

Figure 3. A: Estimated torsion angles of the sugar-phosphate backbone of d(xTpxT) according to reference 14; B: computer modeled structure of d(xTpxT).



An analogous result (δ = 38°) was obtained from a computer modeling study (Figure 3B; Alchemy II, Tripos Inc., release 1989). Absolute clarification of the secondary structure of a "xylo-DNA" will be procured by an X-ray analysis as well as a detailled NMR-spectroscopic analysis [15] which are underway.

Acknowledgment

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References and Notes

1. N. Camerman, J. K. Fawcett, A. Camerman, *J. Mol. Biol.* **1976**, *107*, 601.
2. H. Rosemeyer, F. Seela, *Helv. Chim. Acta*, **1991**, *74*, 748.
3. S. Czernecki, J.-M. Valery, *Synthesis*, **1991**, 239.
4. M. Krecmerova, F. Seela, 1992, *Nucleosides & Nucleotides*, in press.
5. *(R_P,S_P))-5'-O-[4,4'-Dimethoxytriphenylmethyl)-2'-deoxy-β-D-xylothymidylyl]-(3',5')-(3'-benzoyl)-2'-deoxy-β-D-xylothymidine 3'-Phosphonate (**4/5**).* ^{1}H-NMR (DMSO-d_6): 11.33 (3H, 3 NH); 8.26, 5.38 (d, J = 720 Hz, PH); 7.98 - 6.85 (aromatic H); 6.15 (m, H-1' S_P); 5.54 (m, H-1' R_P); 3.71 (OCH_3); 3.22 (m, H_2-5' R_P); 2.85 (m, H_2-5' S_P); 2.23 (m, H_2-2'); 1.70 (CH_3). ^{13}C-NMR (DMSO-d_6): 165.8 (C=O); 164.8, 164.7, 163.8, 163.7, (4 C-6); 158.2 (C-aromat.); 150.7,

150.5, 150.4, 150.3 (4 C-2); 144.7, 144.6 (DMT); 135.7 - 126.9 (4 C-4, Bz, DMT); 113.3 (C-quart.); 109.3, 109.26, 109.23, 109.1 (4 C-5); 85.9 (DMT); 84.1, 83.4 (2 C-1' R_P); 83.8, 83.1 (2 C-1' S_P); 81.3 - 80.4 (C-4'); 73.0 (C-3'); 58.9 (C-5'); 55.0 (2 OCH_3); 12.3, 12.2 (2 CH_3); ^{31}P-NMR (DMSO-d_6): 10.79 ($^1J(P_{P,H}$ = 720 Hz, $^3J_{P,H}3'$ = 8.9 Hz, S_P); 9.75 ($^1J_{P,H}$ = 720 Hz, $^3J_{P,H}3'$ = 8.6 Hz, R_P).

6. p: 3'-*phosphonate* as internucleotide linkage; p: 3'-*phosphate* as internucleotide linkage.
7. F. Seela, U. Kretschmer, *J. Org. Chem.* **1991**, *56*, 3861.
8. J. Stawinsky, R. Strömberg, M. Thelin, *Nucleosides & Nucleotides*, **1991**, *10*, 511.
9. *2'-Deoxy-β-D-xylothymidynyl-(3',5')-2'-deoxy-β-D-xylothymidylate Ammonium Salt (6).* ^{1}H-NMR (DMSO-d_6): 7.90, 7.78 (2 s, 2 H-6); 6.12 (d, J = 7.5 Hz, 2 H-1'); 4.60, 4.22 (2 m, 2 H-3'); 3.8 (m, 2 H-4', H_2-5'); 3.5 (m, H_2-5'), 1.75, 1.72 (2 s, 2 CH_3). ^{13}C-NMR (DMSO-d_6): 163.9 (C-6); 150.8 (C-2); 137.5, 137.2 (2 C-4); 109.2, 109.0 (2 C-5); 83.6 (C-4'); 83.2, 83.0 (2 C-1'); 82.6 (J = 4.1 Hz, C-4'); 72.2 (J = 5.0 Hz, C-3', 5'-term.); 68.1 (C-3', 3'-term.); 61.1 (J = 4.2 Hz, C-5', 3'-term.); 58.3 (C-5', 5'-term.); 40.2 (2 C-2'); 12.4 (2 CH_3). ^{31}P-NMR (DMSO-d_6): 3.67 (pq, J = 9.5 Hz). UV (H_2O) max, 266 nm (ε 10,900).
10. N. I. Sokolova, N. G. Dolinnaya, N. F. Krynetskaya, Z. A. Shabarova, *Nucleosides & Nucleotides* **1990**, *9*, 515.
11. C. S. M. Olsthoorn, C. A. G. Haasnoot, C. Altona, *Eur. J. Biochem.* **1980**, *106*, 85.
12. J. T. Powell, E. G. Richards, W. B. Gratzer, *Biopolymers* **1972**, *11*, 235.
13. A computer program package for the analysis of changes in CD spectra of dinucleotides as a function of temperature in terms of a two-state stack-unstack equilibrium according to:

$$\ln K = \ln \theta_u - \theta/\theta - \theta_s = \Delta S°/R - \Delta H°/RT$$

(where θ refers to an ellipticity value at a particular wavelength in mdeg; the subscripts u and s denote the stacked and unstacked state; K equilibrium constant) was developed by J. Kehrhahn, Physikalische Chemie, Univ. Osnabrück. The program includes an interactive least-squares fitting of both, the melting curve [θ = f(T)] and the corresponding van't Hoff plot, as well as the plotting of both graphs.
14. A. Eschenmoser, M. Dobler, *Helv. Chim. Acta* **1992**, *75*, 218.
15. T. Löschner, J. Engels, *Nucleic Acids Res.* **1990**, *18*, 5143.