Stereoselective synthesis of P-homochiral oligo(thymidine methanephosphonates)

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ABSTRACT

An approach to the stereoselective synthesis of P-homochiral oligo(thymidine methanephosphonates) is described. Fully protected (Rp)- and (Sp)-diastereomers of HMTTrPMeTAc (**3**) were prepared in the stereospecific reaction of P-chiral nucleotide component 5'-O-monomethoxytritylthymidine 3'-O-[O-(4-nitrophenyl)methanephosphonate] (**1**) and 3'-O-acetylthymidine (**2**) bearing activated 5'-hydroxyl function. Deprotection of the 5'-OH group in **3** and subsequent stepwise reactions of activated 5'-OH oligonucleotide components with (Rp)- or (Sp)- isomers of **1** gave the trinucleotide HMTTrPMeTPMeTAc (**4**) and, subsequently, the tetranucleotide HMTTrPMeTPMeTPMeTAc (**5**) possessing all (Rp)- or all (Sp)- configurations at their internucleotide methanephosphonate P-atoms.

INTRODUCTION

The concept of "hybridization arrest" or "hybridization competition" (1,2,3) relies upon the assumption that the heteroduplex formed between an analogue of ssDNA possessing neutralized negative charges at internucleotide phosphate positions and complementary ssDNA is thermodynamically more stable than analogous natural dsDNA (homoduplex DNA) because of the cancellation of electrostatic repulsion between phosphate moieties. Thus, oligo(nucleoside methanephosphonates) with a base-sequence complementary to viral DNA or RNA should control viral development if introduced into the host-cell, and in this way protect the host-organism from viral invasion. Moreover, oligo(nucleoside methanephosphonates) are resistant to cellular enzymes such as alkyltransferases (contrary to DNA-triesters) (4) and phosphodiesterases, and, what is the most essential for the development of the concept of "third generation antiviral drugs", their high cell uptake is expected due to better penetration of neutral species through cellular membranes. In comparison with DNA triesters, oligo(nucleoside methanephosphonates) possess "molecular volumes" much closer to natural DNA; however, their solubility in physiological media is lower than that of DNA triesters. Results on the in-

hibition of T antigen synthesis in SV40 infected African green monkey kidney cells (without affecting overall cellular protein synthesis) by oligo-(nucleoside methanephosphonate) complementary to the donor splice junction of SV40 large T antigen mRNA are promising. They support the concept of "hybridization competition" as a basis for the rational design of new antiviral drugs(3).

In terms of chemistry, however, oligo(nucleoside methanephosphonates) are P-chiral species and non-stereoselective synthesis leads to a mixture of 2^n diastereomers, where n is the number of internucleotide methanephosphonate functions. The effect of the product inhomogeneity on the hybridization to complementary sequences was shown in the case of oligo(thymidine O-ethyl phosphates)(5). The influence of absolute configuration at phosphorus centre of dinucleoside methanephosphonates(1) and alkyl phosphates(6) on heteroduplex stability was also described in several cases.

Since the synthesis of stereoregular polymers is the perennial challenge to organic chemists, we decided to undertake some efforts to obtain - as a model system - dinucleoside methanephosphonates in a stereoregular manner(7). Low configurational stability of trivalent phosphorus species, which easily undergo P-epimerisation(8), eliminated the approach to stereoregular synthesis *via* nucleoside 3'-O-(methanephosphonamidites) and we have turned our attention towards pentavalent phosphorus species(9).

EXPERIMENTAL

Materials and methods

Column chromatography and TLC were performed on silica gel 230-400 mesh and on silica gel F 254 plates, respectively, both from E.Merck. Reversed phase high performance liquid chromatography (HPLC) was performed with a LDC/Hilton Roy system, using ODS Hypersil 5 μ , 4.6x300 mm column. The following elution conditions were used: gradient from 8% to 40% CH₃CN in 0.1 M TEAB, 1.5% CH₃CN/min. UV spectra were recorded with a UVikon 860 spectrometer (Kontron Instruments AG). ¹H- and ³¹P-NMR spectra were recorded with a Bruker MSL300 spectrometer operating at 300.13 MHz and 121.47 MHz, respectively, with TMS for ¹H and 85% H₃PO₄ for ³¹P as external standards. Positive chemical shift values are assigned for compounds absorbing at lower field than standards. CD spectra were recorded on Dichrograf Mark III (Jobin-Yvon).

(Sp)- and (Rp)-isomers of 5'-O-monomethoxytritylthymidine 3'-O-[O-(4-nitro-phenyl) methanephosphonate] (1).

The title compounds were prepared according to the procedure described earlier (9).

(Sp)- and (Rp)-isomers of 5'-O-monomethoxytritylthymidylyl(3',5')(3'-O-acetylthymidylyl) methanephosphonate (3).

A 2.15 M solution of freshly prepared t-butylmagnesium chloride in dry THF (0.462 ml, 1 mmol) was added under argon to a solution of 3'-O-acetylthymidine (2, 0.284 g, 1 mmol) in anhydrous pyridine (1.0 ml). To the resulting suspension of 5'-hydroxyl activated 2 (25% molar excess), a solution of individual (Rp)- or (Sp)-isomer of 1 (0.535 g, 0.75 mmol) in anhydrous pyridine (2.5 ml) was added. The reaction mixture was stirred for 4 h at room temperature and then left overnight without access to moisture. The reaction was quenched with water (20.0 ml) and the product was extracted with CHCl_3 (2x20 ml). The combined organic fractions were dried over MgSO_4 . Solvents were evaporated and corresponding products (Sp)-3 or (Rp)-3 purified by column silica gel chromatography. The yields and spectral characteristics are presented in Table 1.

(SpSp)- and (RpRp)-isomers of 5'-O-monomethoxytritylthymidylyl (3',5')thymidylyl (3',5')(3'-O-acetylthymidylyl) methanephosphonate (4).

A 2.15 M solution of freshly prepared t-butylmagnesium chloride in dry THF (0.236 ml, 0.511 mmol) was added under dry argon to a solution of 5'-deprotected (Sp)- or (Rp)-isomer of 3 (0.200 g, 0.341 mmol) in dry pyridine (2.0 ml). To the resulting suspension of 5'-hydroxyl activated dinucleotide was added a solution of individual (Rp)- or (Sp)-isomer of 1 (0.304 g, 0.426 mmol; 20% molar excess) in dry pyridine (1.0 ml). Further procedure and product isolation were as described for the synthesis of 3. The yields and spectral characteristics of products 4 are presented in Table 1.

(SpSpSp)- and (RpRpRp)-isomers of 5'-O-monomethoxytritylthymidylyl (3',5')thymidylyl (3',5')thymidylyl (3',5')(3'-O-acetylthymidylyl) methanephosphonate (5).

A 2.15 M solution of freshly prepared t-butylmagnesium chloride in dry THF (0.118 ml, 0.253 mmol) was added under dry argon to a solution of 5'-deprotected (SpSp)- or (RpRp)-isomer of 4 (0.150 g, 0.169 mmol) in dry pyridine (1.7 ml). To the resulting suspension of 5'-hydroxyl activated trinucleotide was added a solution of the (Rp)- or (Sp)-isomer of 1 (0.145 g,

0.203 mmol; 20% molar excess) in dry pyridine (0.75 ml). Further procedure was as described for 4. The yields and spectral characteristics of products 5 are presented in Table 1.

Deprotection of oligonucleotides 3, 4 and 5.

Removal of 5'-O-monomethoxytrityl group. Individual isomers of 3, 4 or 5 (0.4 mmol) were treated with 80% aqueous acetic acid (10 ml) at room temperature⁽¹⁰⁾. The reaction progress was monitored by means of TLC. After 1-3h AcOH was removed by coevaporation with n-BuOH. The oily residue was dissolved in pyridine and dropped into hexane. The precipitate was washed with n-pentane and dried under reduced pressure. The yields of 5'-deprotected oligonucleotides were 95-100%, and they were used for the following coupling reactions without further purification.

Removal of 3'-O-acetyl group. Individual isomers of 5'-deprotected 3, 4 or 5, prepared as above (ca. 1 mg) were treated with 20 μ l of concentrated aqueous ammonia solution (25% w/w) in Eppendorf tubes at room temperature. The reaction progress was monitored by TLC and when completed (ca 0.5 h) the reaction mixture was evaporated. The solid residue was redissolved in 85% CH₃CN-H₂O (v/v) solution and purified by means of HPLC under conditions described above. The retention times of deprotected diastereomers of di-, tri- and tetra(thymidine methanephosphonate) 6, 7 and 8 originating from 3, 4 and 5, respectively, are given in Table 2.

Circular dichroism measurements

The CD spectra of (SpSpSp)-8, (RpRpRp)-8 and d(T)₄ were measured in water solution at concentrations of 0.54 g/l, 0.70 g/l and 0.68 g/l, respectively. The following molar extinction coefficients were used: (SpSpSp)-8, $\epsilon_{265}=8600$ [as for d(TpMeTp)₄TpT-2⁽¹¹⁾], (RpRpRp)-8, $\epsilon_{265}=8300$ [as for (TpMeTp)₄TpT-1⁽¹¹⁾], d(T)₄, $\epsilon_{266}=8700$ [as for d(Tp)₉T⁽¹¹⁾].

RESULTS

P-Homochiral, 5'-O-monomethoxytrityl-3'-O-acetyl tetra(thymidine methanephosphonates), (SpSpSp)-5 and (RpRpRp)-5, were prepared by successive, stepwise stereospecific coupling of a 5'-hydroxyl activated growing oligonucleotide chain with P-chiral (Sp)- or (Rp)-5'-O-monomethoxytritylthymidyl[3'-O-(4-nitrophenyl) methanephosphonate] (1). Synthesis of the fully protected dithymidine methanephosphonates, (Sp)-3 and (Rp)-3, was carried out similarly to the procedure described earlier⁽⁹⁾ but anhydrous pyridine was used as the reaction medium. The use of pyridine resulted in homogeneity of the reaction mixture, owing to better solubility of 5'-activated 2 in

FIGURE 1. Synthesis of individual homochiral diastereomers (SpSpSp) and (RpRpRp) of tetra(thymidine methanephosphonate)

TABLE 1. Characteristics of homochiral diastereomers of di-, tri- and tetra(thymidine methanephosphonate).

Compound	Yield ^a %	TLC ^b R _F	UV ^d		³¹ P-NMR ^e	¹ H-NMR ^e	
			λ max	λ min		δ CH ₃ (ppm)	J FCH ₃ (Hz)
(Sp)- <u>3</u>	60	0.42	254	245	32.72	1.57	17.6
(Rp)- <u>3</u>	66	0.57	255	245	32.14	1.49	17.5
(SpSp)- <u>4</u>	55	0.29	254	243	33.10 32.79	1.58	17.5
(RpRp)- <u>4</u>	55	0.33	254	233	32.52 32.39	1.51 1.42	17.6 17.5
(SpSpSp)- <u>5</u>	40	0.11	254	241	33.35 33.19 32.65	1.59 1.58	17.7 17.5
(RpRpRp)- <u>5</u>	38	0.20	254	234	33.19 32.85 32.74	1.55 1.51 1.45	17.7 17.5 17.4
(SpSpSp)- <u>8</u>	73	0.45 ^c	254	235	35.08 ^f 34.89 34.58	1.67 ^f 1.66 1.655	17.8 17.8 17.8
(RpRpRp)- <u>8</u>	64	0.40 ^c	254	234	34.71 ^f 34.45	1.65 ^f	17.7

a/ Yield of the products after purification

b/ Developing solvent system: CHCl₃-CH₃OH (9:1)c/ Developing solvent system: CH₃CN-H₂O (8:2)d/ In 96% C₂H₅OHe/ In CDCl₃f/ In CD₃OD

pyridine than in the THF/DMF solvent system used previously. The reaction was almost completely stereospecific (not less than 95% as determined by ³¹P-NMR spectroscopy), and only minute amounts of unidentified side products were observed.

Deprotection of the 5'-hydroxyl group of (Sp)-3 and (Rp)-3 was achieved by treatment of the fully protected dinucleotide 3 with 80% aqueous acetic acid for 1-3h(10). Simple repeated precipitation procedure yielded detritylated, chromatographically homogeneous product suitable for the next coupling reaction without further purification. The isolated yield of 5'-deprotected 3 was 95-100%.

Fully protected trinucleotides (SpSp)-4 and (RpRp)-4 were prepared by the

Table 2. HPLC characteristics of deprotected diastereomers of di-, tri- and tetra(thymidine methanephosphonate)

Oligomer	Structure	R _t
(Sp)- <u>5</u>	d(T(PMe)T)	7.23
(Rp)- <u>5</u>	d(T(MeP)T)	6.93
(SpSp)- <u>7</u>	d(T(PMe)T(PMe)T)	9.43
(RpRp)- <u>7</u>	d(T(MeP)T(MeP)T)	9.02
(SpSpSp)- <u>8</u>	d(T(PMe)T(PMe)T(PMe)T)	10.50
(RpRpRp)- <u>8</u>	d(T(MeP)T(MeP)T(MeP)T)	10.26

ODS Hypersil 5 μ , 4.6/300 mm column, gradient from 8% to 40% CH₃CN in 0.1 M TEAB, 1.5% CH₃CN/min.

reaction between 5'-deprotected, 5'-Mg-activated derivative of dinucleotide (Sp)-3 or (Rp)-3 and monomer (Sp)-1 or (Rp)-1, respectively. The general procedure was the same as for synthesis of 3, except that a 50% molar excess of t-butylmagnesium chloride was used for activation of 5'-deprotected dinucleotide 3, as compared to the equimolar amount of Grignard reagent used in the case of 3'-O-acetylthymidine activation. Additionally, an 80% molar excess of monomer 1 (instead of an excess of 5'-activated component as for synthesis of 3) was used. The reaction mixture work-up and purification procedure for fully protected trinucleotides 4 was the same as described for 3. The homochiral, fully protected tetranucleotide (SpSpSp)-5 and (RpRpRp)-5 were obtained in the reaction of homochiral 5'-deprotected, 5'-Mg-activated derivative of trinucleotide (SpSp)-4 or (RpRp)-4 with the (Sp)- or (Rp)-isomer of 1. Removal of the 5'-O-monomethoxytrityl group in 4 was performed as described for 3. The coupling reaction and product isolation were carried out as described for 4.

The fully protected oligomethanephosphonates 3, 4 and 5 were characterized by means of TLC, UV, ¹H- and ³¹P-NMR. The data are given in Table 1. Removal of 5'- and 3'-protecting groups in 3, 4 and 5 yielded deprotected di-, tri- and tetra(thymidine methanephosphonate) 6, 7 and 8, respectively, which were then analysed by means of HPLC. R_t values are given in Table 2. The ³¹P-NMR and ¹H-NMR spectra of PCH₃ groups of diastereomers 8 are shown in Figures 2 and 3, respectively.

The conformations of (SpSpSp)-8 and (RpRpRp)-8 were studied by CD spectroscopy. It was found that the spectra of both all (Sp)- and all (Rp)- isomers

FIGURE 2. ^{31}P -NMR spectra (121.47 MHz) of the individual (SpSpSp) (A) and (RpRpRp) (B) diastereomers of tetramer 8. Chemical shifts are given in ppm (δ) relative to 85% H_3PO_4 .

have the same shape but different molecular ellipticity. The possible relationship between the ellipticity factor and oligomethanephosphonate conformation are discussed below.

DISCUSSION

The method of stereospecific P-chiral internucleotide methanephosphonate linkage formation is based on the substitution of the "good leaving" aryloxy group of P-chiral monomer 1 of defined (Sp) or (Rp) sense of chirality by a base activated 5'-hydroxyl function of a nucleoside or growing oligonucleotide chain. A similar approach has been used previously for the synthesis of diastereomeric 2'-deoxyadenylyl (3',5')2'-deoxyadenylyl S-methylphosphorothioates(12). Our attempt is based on 5'-O-monomethoxytritylthymidine 3'-[O-(4-nitrophenyl) methanephosphonate](1)(9). Diastereomers of this compound can be separated by means of column chromatography. The assignment of the absolute configuration at phosphorus in diastereomers of 1 was described earlier(7).

FIGURE 3. ^1H -NMR spectra (300.13 MHz, PCH_3 region) of individual (SpSpSp) (A) and (RpRpRp) (B) diastereomers of tetramer **3**. Chemical shifts are given in ppm (δ) relative to TMS.

For the activation of the 5'-hydroxyl function according to the procedure originally proposed by Hayakawa (13), *t*-butylmagnesium chloride was chosen because of its selective activation of hydroxyl versus amino functions in base unprotected nucleosides. Reaction of activated 3'-O-acetylthymidine with each individual (Sp)- or (Rp)-diastereomer of **1** appeared to be highly stereospecific and the diastereomeric purity of the crude product **3** exceeds 95%, as assigned by means of ^{31}P -NMR spectroscopy. The presence of traces of the second isomer of **3** may be accounted for by the parallel, slow P-epimerisation of monomer **1** in the course of the coupling reaction. Our working hypothesis is that nucleophilic substitution at phosphorus occurs stereospecifically with inversion of configuration, but unreacted monomer **1** is

FIGURE 4. Circular dichroism spectra of $d(Tp)_3T$ (O---O), $(Sp6p6p)-3$ (O—O) and $(RpRpRp)-3$ (●—●) in H_2O . The molecular ellipticity $[\theta]$, is given per base residue.

simultaneously epimerized by the released 4-nitrophenoxide ion *via* a ligand exchange process. Consistent with this hypothesis is the observation that the diastereomeric purity of dinucleotide 3 increased from ca 80% when a THF/DHF mixture was used as a reaction medium (20 h coupling time)⁽⁹⁾ to

ca. 95% when the reaction was performed in pyridine according to the modified procedure (8 h coupling time).

The absolute configuration at phosphorus in both diastereomeric dinucleotides (Sp)-3 and (Rp)-3 was assigned by means of chemical correlation with monomer 1, the absolute configuration of which was established earlier(7). The finding that the coupling reaction between monomer 1 and activated nucleoside 2 is stereospecific, together with the assumption that nucleophilic substitution at the phosphorus atom of 1 leading to 3 occurs with inversion of configuration, allowed us to assign the absolute configuration at phosphorus in slow- and fast-migrating 3 and completely deprotected diastereomers d(T_{PM}eT) (6) originating from 3, as (Sp) and (Rp), respectively. This assumption was also supported by the fact that the slowly-migrating diastereomer of d(A_{PM}eT) is also of (Sp)-configuration(14). The absolute configuration assignment in dinucleotides 3 is crucial for the discussion of homochirality of tri- and tetramers 4 and 5. On the basis of ³¹P-NMR analysis of crude reaction products we have confirmed that successive coupling reactions leading to the oligonucleotide chain elongation 3→4→5 are also stereospecific. Since there is no reason to assume that stereochemistry of these reactions would be different from that leading to dinucleotide 3, we have assumed that they also occur with inversion of configuration at phosphorus of monomer 1, and we have assigned all (Sp)- or all (Rp)-configurations to diastereomeric tri- and tetramers 4 and 5.

Elongation of the oligonucleotide chain required removing the 5'-O-methoxytrityl group before each subsequent coupling step. This was achieved by treatment of the fully protected oligonucleotides 3, 4 and 5 with 80% aqueous acetic acid, according to the well known literature procedure(10). Successive coupling steps between suitable isomers of 1 and 5'-deprotected oligonucleotides leading to 4 and 5 were performed essentially as described for synthesis of dinucleotides 3. The coupling efficiency was not influenced by changing the ratio of monomer 1 to the 5'-activated component from 0.75:1 for synthesis of 3 to 1.8:1 for synthesis of 4 and 5. This change was adapted because of the lower availability of 5'-OH components (5'-deprotected dinucleotide 3 and trinucleotide 4) for synthesis of 4 and 5 respectively than for synthesis of 3 from 3'-O-acetylthymidine. The observed small decrease in the isolated yields in the series of di-, tri- and tetranucleotide most probably results from losses in the course of isolation steps arising from the decrease of oligomer solubility in polar as well as in apolar solvents. All of the homochiral oligonucleotides 3, 4 and 5 were

characterized by means of TLC (normal phase) and by means of RP-HPLC after deprotection of their 5'- and 3'-hydroxyl functions. An inspection of values of R_f and R_t data included in Table 1 and Table 2, respectively, clearly demonstrate the relationship between absolute configuration at phosphorus and R_f and R_t parameters. In all cases, fully protected homochiral oligonucleotides 3, 4, 5 with all (Rp)-configurations are characterized by higher R_f values (higher mobility on TLC plates) than those with all (Sp)-configurations. It is worth mentioning that similar regularity based on reversed phase HPLC analysis of 5'- and 3'-deprotected oligonucleotides 6, 7 and 8 can also be found. The R_t values for oligonucleotides with all (Rp)-configurations are always lower than those with all (Sp)-configurations at phosphorus of methanephosphonate internucleotide linkages. From the facts discussed above, a hypothesis emerges that a particular absolute configuration at phosphorus generates characteristic features in the oligonucleotide chain conformation which are reflected in chromatographic mobility. Spectroscopic studies (UV, CD, $^1\text{H-NMR}$) described in the literature suggest that diastereomeric di(nucleoside methanephosphonates) have distinct conformations in solution(11,15,16). $^{31}\text{P-}$ and $^1\text{H-NMR}$ data are consistent with this suggestion and show several differences between (Sp)- and (Rp)-isomers of oligomers 3, 4 and 5. The $^{31}\text{P-NMR}$ spectra reveal well separated signals for each phosphorus of the methanephosphonate groups in di- and trinucleotides 3 and 4 and in tetramer 5. The deprotected tetramer 8 originating from 5 gives three signals (each for one phosphorus atom) for isomer (SpSpSp)-8 and two signals in 2:1 ratio for isomer (RpRpRp)-8, Figure 2. On the other hand, POCH_3 protons give well separated doublets in $^1\text{H-NMR}$ spectra only for compounds of the (Rp) series of 3, 4 and 5 (two, four and six signals, respectively). The deprotected tetramer 8 escapes the rule giving relatively well separated signals of three doublets for isomer (SpSpSp)-8 and only one doublet related to three POCH_3 groups (integration data not shown) for isomer (RpRpRp)-8, Figure 3. In the case of the (Sp) series a doublet of POCH_3 protons was observed for dinucleotide 3 but also a doublet (instead of two doublets) of POCH_3 protons was observed for trinucleotide 4, and two doublets with different intensity for tetranucleotide 5. Additionally, for all oligomers POCH_3 resonances of (Rp)-isomers, both in $^{31}\text{P-}$ and in $^1\text{H-NMR}$, occur at slightly higher field than the POCH_3 resonances of (Sp)-isomers. It should be mentioned, however, that in the case of (Sp)- and (Rp)-isomers of d(Ap_{Me} A), whose absolute configurations at phosphorus were

determined by the $^1\text{H-NMR}$ NOE technique, absorption at higher field was assigned to the protons of the POCH_3 group of the (Sp)-isomer(16). Although this may be due to different conformations of $\text{d}(\text{ApMeA})$ and $\text{d}(\text{TpMeT})$ dimers, by analogy to differences in conformations of this oligonucleotides with natural internucleotide bond resulting from differences in base composition(17), more studies are needed for a more detailed discussion of this phenomenon.

Additional and very illuminating evidence of conformational differences, between P-diastereomeric homochiral oligo(thymidine methanephosphonates) 8, is provided by CD spectra. Comparison of CD spectra of tetrathymidylic acid $\text{d}(\text{Tp})_3\text{T}$ and $(\text{RpRpRp})\text{-8}$, recorded under analogous conditions, shows their nearly perfect identity. On the other hand, the CD spectrum of $(\text{SpSpSp})\text{-8}$ has the same shape as that of $(\text{RpRpRp})\text{-8}$ but with molecular ellipticity reduced by 70%. It is especially intriguing that a similar but smaller effect was observed for fast- [(Rp) according to our data, based on comparison of dinucleotide characteristics](7) and slow- isomers of $\text{d}(\text{TpMeTp})_4\text{TpT}$, an oligonucleotide containing alternating methanephosphonate/natural phosphodiester linkages(11). The spectrum of that fast - isomer of $\text{d}(\text{TpMeTp})_4\text{TpT}$ is virtually identical both qualitatively and quantitatively with that of $\text{d}(\text{Tp})_5\text{T}$. The spectrum of the slow- isomer of $\text{d}(\text{TpMeTp})_4\text{TpT}$ is qualitatively the same, but the molecular ellipticity of the peak and trough of this isomer is reduced by 43% with respect to that of the fast - isomer and $\text{d}(\text{Tp})_5\text{T}$. From these similarities two conclusions emerge: first, the absolute configurations at the phosphorus atoms of methanephosphonate groups of individual fast- and slow-isomers of both $\text{d}(\text{TpMe})_3\text{T-8}$ and $\text{d}(\text{TpMeTp})_4\text{TpT}$ are the same, and second, the influence of internucleotide methanephosphonate functions of defined (Rp)- or (Sp)-configuration upon oligonucleotide conformation is much stronger when the internucleotide methanephosphonates are adjacent than when they are separated by a natural phosphate bond.

$^1\text{H-NMR}$ studies of $\text{d}(\text{TpMeT})$ have shown that the sugar puckering and rotamer populations about ψ , ϕ and ϕ' are very similar in both diastereomers (16). By analogy to the assumption that conformational parameters of $\text{d}(\text{TpT})$ and oligo-T are essentially the same(18), the extension of the $\text{d}(\text{TpMeT})$ structure to tetramer 8 seems reasonable. If the assumption that main conformational parameters are the same for both isomers of 8 is true, the apparent differences between conformations of $(\text{SpSpSp})\text{-8}$ and $(\text{RpRpRp})\text{-8}$ could result from differences in solvation of the two isomers, depending on relative

phosphoryl oxygen and methyl group position. Such differences could lead to changes in the N-glycosyl torsion angle as well as to changes in weak intramolecular interactions(11).

Comparison of published CD profiles of fast- and slow- isomers of d(ApMeA) (18), d(ApMeT) (14), d(CpMeG) and d(CpMeC) (19), d(TpMeTp)₄TpT (11) with the CD profiles recorded for d(TpMe)₃T shows that they are qualitatively similar, and that the fast-isomer exhibits a more intense CD pattern than does the other isomer. Unfortunately, if chromatographic mobility and CD criteria are related to the absolute configuration at phosphorus of the methanephosphonate function, they are not always consistent with absolute configuration assignments by instrumental or chemical methods. Thus, the (Sp) absolute configuration has been assigned to the fast-isomers of d(ApMeA) (16) and d(CpMeG) (19) by means of the ¹H-NMR NOE technique and X-ray crystallography, respectively. On the other hand the (Sp)- configuration was established for the slow-isomers of d(TpMeT) (7) and d(ApMeT) (16) by means of chemical correlation and X-ray analysis, respectively. These findings may reflect fundamental differences of molecular structure in solution depending on oligonucleotide base composition (17) or may suggest that reported configurational assignments require further attention.

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This paper is dedicated to Professor F.Cramer on the occasion of his 65th birthday

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