© 1990 Oxford University Press

Diastereomeric dinucleoside-methylphosphonates: determination of configuration with the 2-D NMR ROESY technique

Thomas Löschner and Joachim W.Engels
Institut für Organische Chemie, Niederurseler Hang, D-6000 Frankfurt am Main 50, FRG

Received June 11, 1990; Revised and Accepted August 1, 1990

ABSTRACT

The determination of configuration at phosphorus in diastereomeric dinucleoside-methylphosphonates having the -O-P(=O)(-CH_3)-O- internucleotide linkage with the NOE derived ROESY NMR technique is described for ApT, TpT, ApA, TpA and CpG. For this purpose ROE's from the P-CH_3 group to the protons in the nearest neighbourhood were measured. These ROE's are different within diastereomeric pairs of a dimer enabling us to deduce the individual configuration. The validity of the method is proven in comparison with dimers of known configuration (ApT, TpT). Together with a recently published diastereoselective synthesis method a more homogeneous picture between physical properties and the corresponding configuration is provided. There is an improvement in our knowledge about the stereochemistry of these substances which could not be deduced from the data known before.

INTRODUCTION

Dinucleoside methylphosphonates appeared in the literature more than 10 years ago (1,2). Since that time much effort has been done in the field of synthesis and determination of configuration for these compounds. They have unusual physiological properties and were used in many investigations in form of dimers or oligomers as potential substrates for enzymes or as regulators of gene expression by the antisense approach. For a comprehensive review of this matter see (3,4). For a proper interpretation of the results it is necessary to use the oligomers with a uniform and preferably known configuration at phosphorus being Sp or Rp according to the CIP rules. This is not possible up to now because no general applicable method is known to produce oligonucleoside methylphosphonates with a defined stereochemistry with the exeption of poly dT (5). Furthermore the determination of configuration at phosphorus of even smaller molecules like dimers or derivatives is still troublesome with indirect spectroscopic methods (6,7). It was accomplished only for the ApT dimer via X-ray analysis (8).

The diastereomeric character of this type of molecules should result in different properties within a biological environment. The physical differences are more obvious resulting for example in different solubilities, different curve shapes in CD-spectra or different retention times on RP-HPLC columns for both diastereomers (Table 1).

The ability to form double stranded molecules with complementary oligodeoxynucleotides depends significantly on the stereochemistry of the methylphosphonate unit as shown by Stec for two TpT octamers (9) with either Rp or Sp stereochemistry. Another biological experiment with an oligodeoxynucleotide having one methylphosphonate moiety in the backbone showed different binding and regulatory effects in the lac-repressor/operator system (10) depending on configuration.

These experiments reveal the necessity for a stereospecific synthesis and an applicable method which allows the unambigous determination of configuration in these molecules. Up to now a stereospecific synthetic approach is known for TpT dimers and TpT homopolymers (5,11). Furthermoore a more general approach was published by us for the synthesis of dimers with different nucleosides (17). As far as configuration is concerned only one paper came to our knowledge wherein the configuration of one methylphosphonate unit within an oligodeoxynuclotide octamer was successfully determined by NOE measurements (12) (Table 1). This was possible by stabilizing the conformation within a double strand resulting from a self complementary sequence. We chose dimers for our investigation since they are much easier available by synthesis in the necessary amounts. This in addition allows the comparison of our assignments with other results published so far. The present stereochemical information about nucleoside methylphosphonates is summarized in Table 1 together with two measured physical properties.

With these data extracted from five different investigations (8,11,12,14,15) the physical properties of the diastereomers seem to be complex and unpredictable for the configuration when we started our investigation. Due to a missing rule and unknown configurations the diastereomers of a dimer were named in the literature as isomer 1 and isomer 2 according to their order of elution from a silica gel column. The diastereomers were separated by this way when they were obtained in a fully protected state 7after synthesis by any known method (13,17).

NOE measurements are often hampered by the vanishing cross relaxation rate for correlation times $\tau_c \approx \sqrt{5/2}\omega_0$ characteristic of intermediate size molecules (MW ca. 1000 D). The

measurement of transverse cross relaxation (ROE) does not suffer from this problem (24). The corresponding 2D experiment ROESY (24,25) is therefore used in this publication instead of NOESY.

EXPERIMENTAL

Synthesis and Preassignment

Dinucleoside methylphosphonates were synthesized according to published procedures (16,17). They were purified and separated into the individual diastereomers by silica gel chromatography with EE/MeOH or $CHCl_3$/MeOH gradients. Deprotection of the individual diastereomers was performed as described by Miller (22) with anhydrous ethylendiamine/ethanol at room temperature in 7h followed by chromatography over silica gel with $CHCl_3$/MeOH 9/1. Detritylation was performed with BF_3. Et_2O in CH_2Cl_2/MeOH (18) and final purification was achieved with RP-C_{18} chromatography using 5−10% CH_3CN/H_2O solvent mixtures. The deprotected diastereomers thus obtained were compared with authentic isomers provided by Stec in the TpT (19) and Miller in the ApT (8) case using the different retention times of the diastereomers on the RP-HPLC column. By this way both deprotected ApT and TpT isomers 1 (greater Rf value in silica gel thin layer chromatography in the protected form obtained after synthesis) were coeluted with the corresponding original samples. The shorter retention times observed for isomer 1 were in agreement with the published results (19,20). Therefore the Rp configuration was assigned to each isomer 1 of TpT and ApT.

NMR-Measurements

ROESY-spectra were recorded with a Bruker AM-250 spectrometer equipped with a variable temperature unit. Measurements were done at 27°C after an extensive purge of the sample with N_2. Samples were prepared as 100mM solutions in DMSO or $CDCl_3$(TMS) for the protected dimers and in D_2O or DMSO for the deprotected ones depending on the solubility. The spectra were calibrated at 2.50 ppm (DMSO) and 4.80 ppm (D_2O). The individual assignments of the diastereotopic H5′,H5″-protons in both sugars of a dimer were not possible. If separate signals occured the more highfield shifted ones are designated as H5′ only for consistency.

The ROESY (24,25) spectra were recorded with a mixing sequence producing an average field $\delta B1/2\pi$ = 2.1 kHz of 190 ms duration effected by a train of 14° pulses separated by appropriate delays (27). The transmitter was set to ≈7.5 ppm to avoid any coherent transfer via J-couplings. 700 experiments with 16 transient each were recorded with a relaxation delay of 5 sec. The spectra are presented in phase sensitive mode. Distances were measured on the assumtion of similar correlation times for the reference signal (H2′/H2″) and the unknown ones with a dependence of the intensity on the distance being $I \approx \tau_c/r^6$

Polynomial Baseline correction (BRUKER ABS-command) and integration with offset correction (26) were performed the latter by a program kindly provided by Dr. U. Anders.

RESULTS

It was shown by the work of Bower (12) that different NOE's of the P-CH_3 group were detectable depending on the configuration at phosphorus. With these data absolute configurations were assigned using stereomodels of both isomers. The Rp configuration was assigned for the isomer with no NOE from the P-CH_3 group whereas the Sp configuration was assigned with the aid of a NOE to the H3′-proton of the nucleoside in the 5′ direction of the oligonucleotide (Table 1). If this finding is transfered to simple dimers the same observation should be possible. Having the higher flexibility of a dimer in mind the relevant NOE's could be less different or unobservable. Indeed, prior work in this direction was unsuccesful for ApT and TpT (19,20) because no NOE signals were measurable. Only for ApA an NOE from P-CH_3 to the H3′-Ap proton was observed for isomer 1 (14) which results in the assignment of the Sp configuration for this isomer being in contradiction to the other assignments done later on (Table 1). We failed too when the measurements were done by the normal NOE method. Using the ROE technique with the advantages described above distinguishable ROE's for each isomer were measurable which allow the assignment of the absolute configuration at phosphorus.

For a better understanding of the results a short introduction into the different stereochemical environments of the P-CH_3 groups in both diastereomers is necessary and helpful (Figure 1).

With the assumption of a nearly undistorted internucleoside methylphosphonate linkage as proposed by Kan (14) having both nucleoside bases in a stacking conformation and the acceptance of a greater rotational freedom around the P-O and O-C bonds, the following predictions are reasonable. In the case of the Sp isomer (Fig. 1, left) one ROE should be measurable from the P-CH_3 group to the H3′-Xp proton (X = any 3′OH esterified nucleoside) as indicated by the arrow and which was still found in the oligomeric case investigated by Bower (12). Within the

Table 1: Summary of data known so far concerning the RP-HPLC and CD behaviour of isomer 1 relativ to isomer 2 and the corresponding configuration of isomer 1 determined by NMR or X-ray analysis. [1]= elution from a RP-HPLC column; [2]= curve shape; [3]= configuration determined within an oligodesoxynucleotide (12).

Dimer	HPLC[1]	CD[2]	NMR (NOE)	X-ray structure
ApT 1	faster	flater	Rp (Oligo[3])	Rp (from Sp of ApT 2)
TpT 1	faster	flater	---	Rp (after derivation)
TpA 1	faster	flater	---	---
ApA 1	faster	flater	Sp (Dimer)	---
CpG 1	faster	flater	---	Sp
GpA	faster	---	Rp (Oligo[3])	---
TpC	faster	---	Rp (Oligo[3])	---

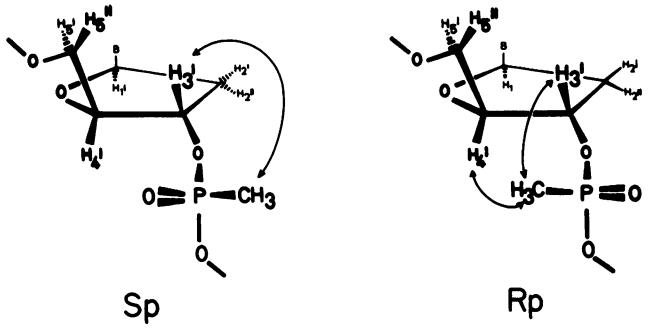

Figure 1 Stereochemical environment of the P-CH_3 group in connection with different stereochemistry of Sp or Rp configurated dinucleoside methylphosphonates.

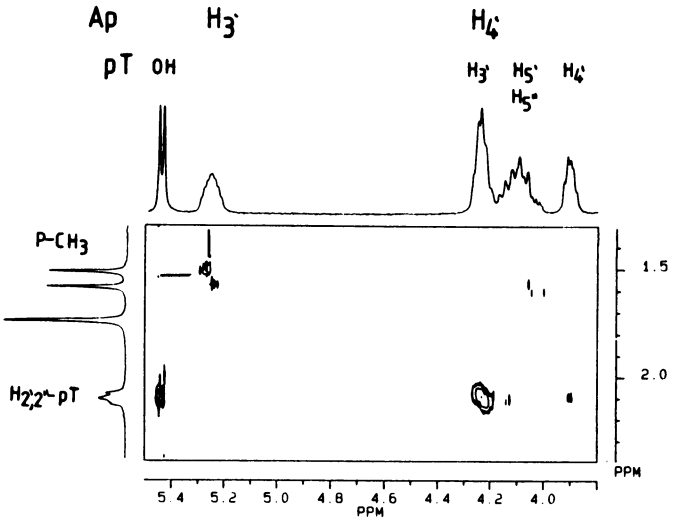

Figure 2 Part of the ROESY spectra of ApT isomer 2 in DMSO

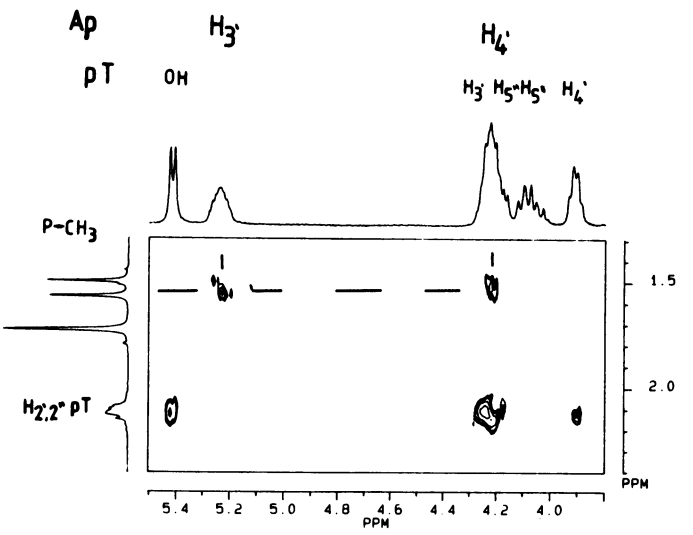

Figure 3 Part of the ROESY spectra of ApT isomer 1 in DMSO

Rp isomer (right) the assumption of two small ROE's to the H3'- and H4'-Xp protons instead of none (12) seems to be possible because of the mentioned rotational freedom. This is indicated in the picture by two arrows.

Example 1 – ApT

The ApT dimer is the test case for the NMR investigation because the configuration is known for sure by X-ray analysis revealing the Sp configuration for isomer 2 (8). The corresponding 2D NMR spectrum obtained with the ROESY method is shown in part in Fig. 2. In this case the dimer carries the 5'-monomethoxytrityl protecting group. Only the part of the 2D spectrum with the ROE's of the P-CH_3 group and the H2',2''-pT protons is shown. Obviously an ROE from the H3'-Ap proton to the P-CH_3 group is visible. This is in agreement with the prediction from the stereomodel for the Sp configuration (Figure 1, left) and the X-ray structure. The ROESY cross peaks have due to the coupling between phosphorus and the methyl protons as well as the sugar ring protons a tilted multiplet structure where the splitting in ω_1 is the *P-CH*$_3$ coupling and in ω_2 is the *P*-O-C-*H3'* coupling.

In comparison to this result Figure 3 depicts the identical part of the ROESY spectrum of isomer 1 having the Rp configuration. Because of some protons overlapping in the ^{1}H-NMR spectrum the assignment of the relevant cross peaks is a little bit more difficult. A comprehensive analysis performed with the whole 2D spectrum showed that the two crosspeaks observed in this case were associated with ROE's from the P-CH_3 group to the H3'- and H4'-Ap protons confirming the prediction of Figure 1 (right) for the Rp configurated isomers.

A special problem in these assignments are the crosspeaks of the H5-protons in the pT part or within the other 5'-OH connected sugars (pY) respectively. These protons are equally separated by 5 bonds from the P-CH_3 protons as it is the case for the H3'-Xp proton. This is schematically shown in Fig. 4. The observation of these ROE's are not predictable and without any correlation to a special H5-proton because we did not assign the diastereotopic protons. Because of the broad signals of the H5-protons in the ^{1}H-NMR spectra no visible intensity of possible cross peaks in the 2D spectra were finally detected. Only in the case of an overlap of both protons a cross peak was visible

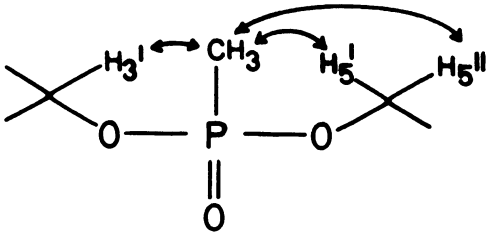

Figure 4 Environment of the P-CH_3 group in view of the directly connected protons.

sometimes. Fortunately a total overlap of both H5-protons with the diagnostic H4'-Xp proton never occured thus avoiding any possible misinterpretations.

The crosspeaks of the P-CH_3 group were integrated and the results are shown in Table 2. The distances measured by this method are within the range of the interproton distances for the sugar protons (23). The H4'-Ap/P-CH_3 distance is significantly longer in the case of isomer 2 as expected from the model.

Example 2 – TpT

The TpT is another dimer with known configuration of the individual diastereomers. From the arguments given (11,19) and the aid of a X-ray structure of a methylphosphonate derivative (21) the Rp configuration was determined for isomer 1. As seen from the prediction in the stereomodel for the Rp isomer (Fig. 1, right) and the investigation of ApT 1 with the Rp configuration two ROE's should be observable when TpT isomer 1 is studied. The final result is shown in Figure 5 which demonstrates the expectations. The H3'-Tp and H4'-Tp ROE's to the P-CH_3 group were present. Additionally both H5-pT protons while overlapping showed an ROE. This ROESY spectrum was recorded with the fully protected TpT dimer having a trityl group at the 5'-OH and a benzoyl group at the 3'-OH. With the protecting groups the overall flexibility of the dimer with the two small pyrimidine bases is reduced and distinct ROE's were measurable. When the deprotected TpT dimers were studied in D_2O to many signals were observed preventing any proper

Table 2: Selected distances in pm within the investigated dimers determined by integration of the ROE's and calibration with the internal H2'/H2'' distance (180 pm) with a calculated error of ≈ 10 pm (--- Not measurable due to overlay, — Not measurable within the sensitivity of the experiment).

Dimer	ApT 1	ApT 2	ApA 1	ApA 2	TpA 1	TpA 2
H3'-Xp/P-CH_3	300	287	331	271	298	290
H4'-Xp/P-CH_3	291	340	312	370	---	340
H4'-pY/P-CH_3	—	—	316	---	---	—

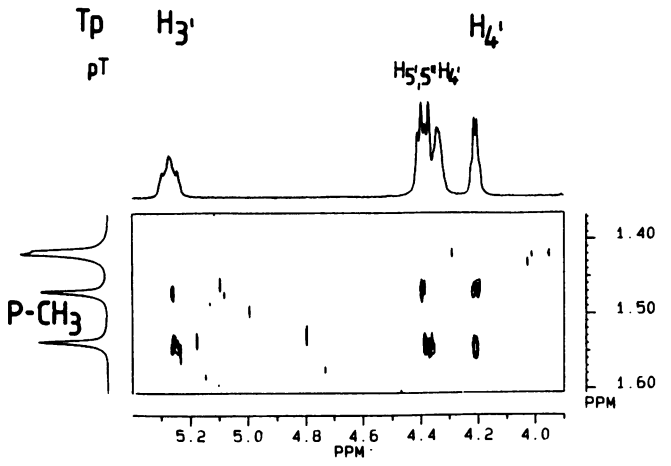

Figure 5 Part of the ROESY spectra of TpT isomer 1 in $CDCl_3$

configurational assignments (Data not shown). This result does not exclude the investigation of deprotected dimers at all as shown in the ApA case with two larger purine bases and clean results (Example 3).

As expected for the Sp configurated TpT isomer 2 no H4'-Tp ROE was observed beside the H3'-Tp ROE (Data not shown). These results support the former assignments and demonstrate the usefulness of the method.

Example 3 – ApA

With these promising results achieved in Example 1 and 2 we started the investigation of dimers with unknown or at least speculative configurations. The ApA dimer is especially interesting in this case since the Sp configuration was proposed by Kan for isomer 1 as a result of their NOE measurements (14). This is opposite to the results of ApT and TpT. If this assignment is correct no uniformity will exist between physical properties and the corresponding configuration (Table 1). The fully deprotected diastereomers were investigated in D_2O in this case because no overlap of the crucial H4'-Ap proton with other protons occured in the ^{1}H-NMR spectrum. The solubility of both isomers in D_2O is high enough to achieve the necessary concentration and it was the same solvent used by Kan for their NOE investigation. The significantly more highfield shifted H4'-Ap resonance in our ApA isomer 2 relative to isomer 1 established the correct relationship to the so called ApA isomer 2 of Kan using the published spectra (14).

The most interesting effects occured for isomer 1 as shown in Figure 6. Four distinct ROE's of the P-CH_3 unit were observed. The H3'-Ap and H4'-Ap ROE's are in accordance with the results obtained for ApT 1 (Fig. 3) and TpT 1 (Fig. 5).

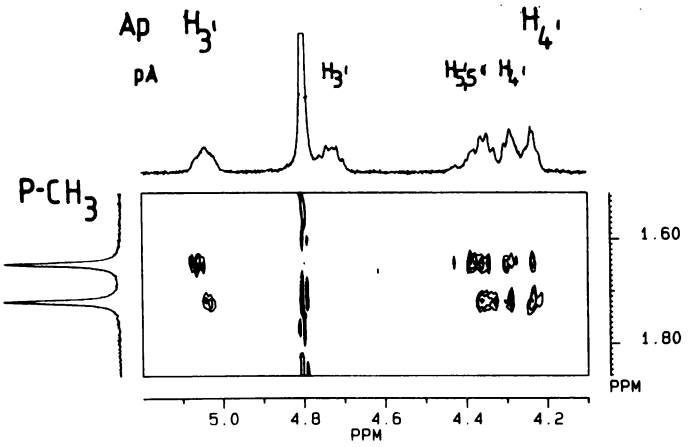

Figure 6 Part of the ROESY spectra of ApA isomer 1 in D_2O

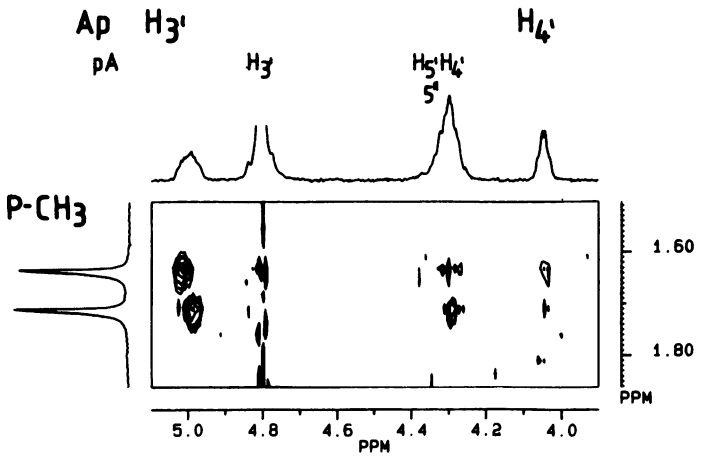

Figure 7 Part of the ROESY spectra of ApA isomer 2 in D_2O

Therefore the Rp-configuration is proposed for isomer 1 being in contrast to the previous assignment (14). Another crosspeak results from the two overlaping H5-pA protons of the diester unit. This is possible but did not allow further conclusive interpretations as discussed in Example 1. The fourth crosspeak resulting from the H4'-pA proton is a new one not observed prior in the ApT and TpT case. It is remarkable at this point that the same ROE occurred in the TpA (Example 4) and CpG case. Therefore it appears that this signal is linked to a 5'-OH bounded purine nucleoside. As far as configuration at phosphorus is concerned this H4'-pA ROE to the P-CH_3 group is possible based upon the Rp configuration and a more stretched conformation of the dimer. In this case the P-CH_3 group is surrounded by the H3'-, H4'-Ap and H4'-pA protons (Table 2). The three ROE's with nearly equal distances to the P-CH_3 are very unfavourable for the Sp configuration (as seen by ball and stick models).

More informations are available from the analysis of the ROESY spectrum of ApA isomer 2. The diagnostic part of this spectrum is shown in Figure 7. In contrast to Figure 6 the intensive H3'-Ap ROE to the P-CH_3 group is conspicuous in connection with the infinitely small H4'-Ap ROE. This is in accordance with the corresponding results for ApT 2 (Fig. 2) and the stereomodel (Fig. 1, left). The Sp configuration is conclusively assigned for this ApA isomer 2. Unfortunately both

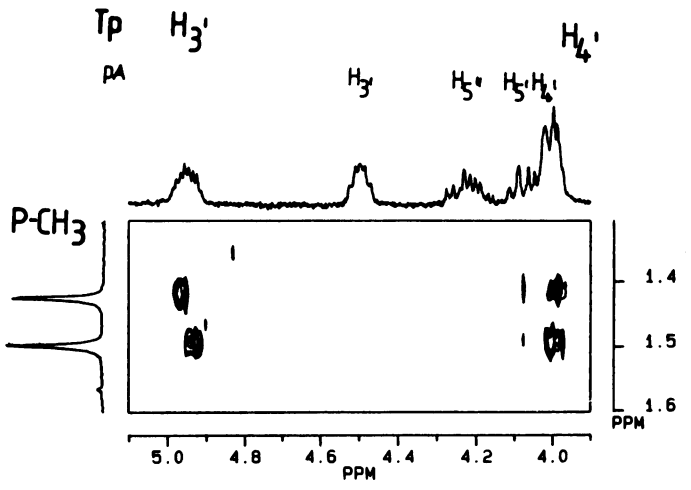

Figure 8 Part of the ROESY spectra of TpA isomer 1 in DMSO

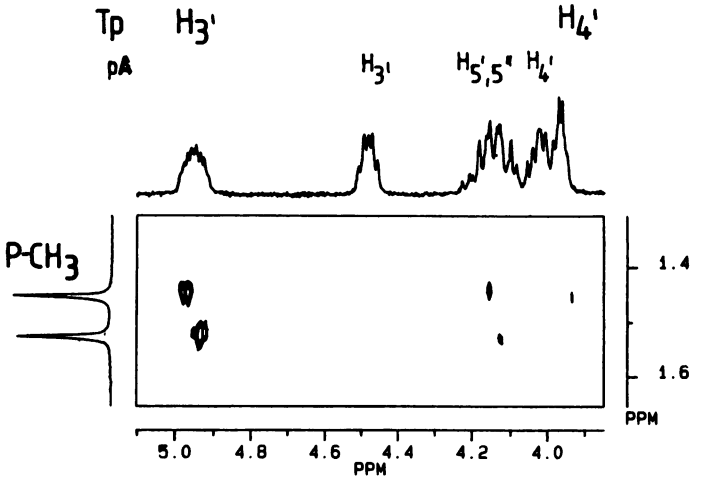

Figure 9 Part of the ROESY spectra of TpA isomer 2 in DMSO

H5'- and H4'-pA protons are superimposed allowing no precise correlation of this small crosspeak.

The quantification of the crosspeak intensities into pm are summarized in Table 2. These values indicate the equal distances in isomer 1 which are between 310 and 330pm. In isomer 2 the H3'-Ap/P-CH_3 distance is 60pm shorter wheras the H4'-Ap distance is lengthened by the same value. This was the best and unequivocal result within all dimers studied.

Example 4–TpA

For this dimer to our knowledge no configurational assignments has been done so far. To assign the configuration within our system for sure both distereomers were investigated in the protected as well as in the deprotected form. The spectra presented here were derived from the deprotected ones. The results of the protected isomers are in the same direction and therefore not shown additionally. Figure 8 displays the relevant part of the ROESY spectrum of the deprotected TpA isomer 1 measured in DMSO because of the low solubility of this dimer in D_2O.

In this Figure 8 the H3'-Tp ROE to the P-CH_3 group is clearly visible. Furthermore the H4'-Tp and the H4'-pA proton showed an ROE to the P-CH_3 group in the molecule. These signals are not well resolved because both resonances are closely together. This is the same result as observed for ApA 1 (Fig. 6). From the H3'- and H4'-Ap crosspeaks the Rp configuration is assigned for this TpA isomer 1 based upon the same arguments outlined in Example 1, 2 and 3. The additional H4'-pA crosspeak supports this assignment as discussed in Example 3 for ApA 1. Further informations were available from the ROESY spectrum of TpA isomer 2 which is depicted with its most important part in Figure 9. In contrast to isomer 1 both H5',''-pA resonances are now superimposed and the H4'-Tp/pA signals are better resolved. The absence of any H4'-proton crosspeak to the P-CH_3 group is obvious whereas the H3'-Tp ROE is still conserved. By reasons given in Example 1 for ApT isomer 2 the Sp configuration was assigned for this TpA isomer 2 with the missing H4'-Tp/P-CH_3 ROE. The integration data are given only for isomer 2 because of the difficulties with the closely related H4'-signals in isomer 1. The values for isomer 2 are comparable with the distances obtained for the other dimers (Table 2).

DISCUSSION

A special comment is necessary for the CpG dimer (Table 1). This molecule was described in 1986 (15). Since that time the promised details about the synthesis and/or the X-ray structure(s) of the so called CpG isomer 1 mentioned therein with the Sp configuration are not published to our knowledge up to now. If we deal with the data given in (15) everything is in agreement with the other dimers as far as isomeric designation and HPLC/CD properties are concerned. Only the opposite configuration is maintained. Therefore we investigated this dimer too. We found the same ROE signals for CpG 1 as for ApA 1 or TpA 1. On the other hand only a very small H4'-Cp/P-CH_3 ROE was observed in CpG isomer 2 (data not shown). From our discussion in Examples 3 and 4 we are convinced that CpG isomer 1 (fast elution from a RP-column) is Rp configurated and makes sense in connection with the physical behaviour. The HPLC/CD similarities with ApT 1 and ApA 1 were particularly mentioned (15) but amazingly no comment to the known and opposite stereochemistry of ApT was given.

Note: While preparing this paper with our opposite results we were informed by the author of the contradictory CpG paper (15) that the assignment with which we have dealt during our investigation was incorrect due to a change by mistake (28). In a positive sense this correction demonstrates the validity and usefulness of our method which is now strongly supported after all.

With the data presented in the foregoing text the contradictory assignments of the diastereomers known so far (Table 1) have to be corrected in some terms. More uniformity between the isomers 1 or 2 and the corresponding configurations is now established. For all dimers investigated the Sp configuration is determined for each isomer 2 of a given dinucleoside methylphosphonate. With these results the formerly proposed Sp configuration for ApA 1 has to be corrected into the Rp ones. This compound is now in agreement with some of its physical properties (CD,HPLC) and with the ApT and TpT diastereomers whose configurations are known for sure and which were confirmed by our NMR measurements. The configurations of the newly determind TpA isomers are in line with the other dimers.

Further evidence for the correct assignment is obtained from the diastereoselective synthesis which results in a considerable excess in the case of isomer 1 for TpT, CpT, CpA and TpA dimers (17). With the known Rp configuration of TpT isomer 1 and the plausible assumption of an identical induction mechanism during the synthesis of the dimers the Rp configuration of TpA isomer 1 is expected in this case too. The configuration of the CpT and CpA diastereomers is defined in the same way. From the diastereoselective synthesis of each isomer 1 (17) the Rp configuration is conclusive for these two dimers. These findings support independantly the assignment of Bower for TpC (Table 1) which is comparable with TpT or CpT. It further demonstrates that the result for ApT is not unique. This dimer is a connecting link because the configuration was determined in the dimer state as well as being incorporated into an oligomer (Table 1). The two independant investigations (8, 12) revealed the same Rp configuration in connection with the faster eluting isomer 1. Therefore the oligomeric bounded TpC and GpA dimers could be as well considered as dimers possessing the same relation between their properties and their configuration as in a oligomer.

Further arguments are available from comparison of the ^{1}H- and ^{31}P-NMR spectra of the fully protected 5'-O-tritylated, 3'-O,N-benzoylated dimers. In all dimers mentioned a highfield shift in the ^{31}P resonance of isomer 1 relative to isomer 2 was observed in $CDCl_3$. The same is true for the ^{1}H resonances of the P-CH_3 group (data not shown). These spectral characteristics were also observed for the GpT dimer which is not mentioned yet. Therefore this dimer is also incorporated in our considerations with the same conclusions. This means Rp configuration at phosphorus for isomer 1. With this relation the assignment of the closely related GpA dimer described above is positively supported.

At last and mentioned here for completeness a longer retention time of each isomer 2 was found if the diastereomers of a dimer were compared on a RP-column.

SUMMARY

If all these facts presented are taken together the same configurational assignment for *all* isomers 1 (Rp) or 2 (Sp) of the dinucleoside methylphosphonates mentioned in Table 1 is conclusive and extended by CpT, CpA and GpT. We believe that the uniformity observed could be extrapolated to the rest of the dimers which are theoretically possible by combination of the 4 deoxynucleosides.

ACKNOWLEDGMENTS

We thank Dr. P.S. Miller (The Johns Hopkins University) for providing an authentic sample of ApT 2 and Dr. W.J. Stec (Polish Academy of Sciences) for providing samples of both TpT isomers for comparison with our products. We thank Dr. G. Zimmermann for recording the ROESY spectra and the introduction into the analysis software. The helpful comments of Dr. C. Griesinger concerning the ROESY technique are gratefully acknowledged.

REFERENCES

1. Agarwal,K., Riftina,F. (1979) *Nucleic Acids Research* **6**, 3009–3023.
2. Miller,P.S., Yano,J., Yano,E., Carroll,C., Jayaraman,K., Ts'o,P.O.P. (1979) *Biochemistry* **18**, 5134–5142.
3. Zon,G. (1988) *Pharmaceutical Research* **5**, 539–549.
4. Stein,C.A., Cohen,J.S. (1988) *Cancer Research* **48**, 2659–2668.
5. Lesnikowski,Z.J., Jaworska,M., Stec,W.J. (1988) *Nucleic Acids Research* 11675–11689.
6. Sopchik,A.E., Cairns,S.M., Bentrude,W.G. (1989) *Tetrahedron Letters* **30**, 1221–1224.
7. Bajwa,G.S., Bentrude,W.G. (1980) *Tetrahedron Letters* **21**, 4683–4686.
8. Chacko,K.K., Lindner,K., Saenger,W., Miller,P.S. (1983) *Nucleic Acids Research* **11**, 2801–2814.
9. Stec,W.J. Presented at the conference 'Recognition Studies in Nucleic Acids', Sheffield (UK) 1989 and *Nucleic Acids Research 18*, 2109–2114 (1990).
10. Noble,S.A., Fisher,E.F., Caruthers,M.H. (1984) *Nucleic Acids Research* **12**, 3387–3404.
11. Lesnikowski,Z.J., Wolkanin,P.J., Stec,W.J. (1987) *Tetrahedron Letters* **28**, 5535–5538.
12. Bower,M., Summers,M.F., Powell,C., Shinozuka,K., Regan,J.B., Zon,G., Wilson,W.D. (1987) *Nucleic Acids Research* **15**, 4915–4930.
13. Katti,S.B., Agarwal,K. (1986) *Tetrahedron Letters* 27, 5327–5330.
14. Kan,L.S., Cheng,D.M., Miller,P.S., Yano,J., Ts'o,P.O.P. (1980) *Biochemistry* **19**, 2122–2132.
15. Callahan,L., Han,F., Watt,W., Duchamp,D., Kezdy,F.J., Agarwal,K. (1986) *Proc. Natl. Acad. Sci.* **83** 1617–1621.
16. Engels,J., Jäger,A. (1982) *Angew. Chem. Suppl.*, 2010–2015.
17. Löschner,T., Engels,J. (1989) *Tetrahedron Letters* **30**, 5587–5590
18. Engels,J. (1979) *Angew. Chem.* **91**, 155–156
19. Lesnikowski,Z.J., Wolkanin,P.J., Stec,W.J. (1987) In Bruzik,K.S. und Stec,W.J. (Eds), Biophosphates and Their Analouges-Synthesis, Structure, Metabolism and Activity. Elsevier Publishers, Amsterdam, 189–194.
20. Stec,W.J., Zon,G., Egan,W., Byrd,R.A., Phillips,L.R., Gallo,K.A. (1985) *J. Org. Chem.* **50**, 3908–3913.
21. Bentrude,W.G., Sopchik,A.E., Bajwa,G.S., Setzer,W.N., Sheldrick,W.S. (1986) *Acta Cryst.* **C42**, 1027–1029.
22. Miller,P.S., Reddy,M.P., Murakami,A., Blake,K.R., Lin,S., Agris,C. H. (1986) *Biochemistry* **25**, 5092–5097.
23. Ven,F.J.M., Hilbers,C.W. (1988) *Eur. J. Biochem.* **178**, 1–38.
24. Bothner-By,A.A., Stephens,R.L., Lee,J., Warren,C.D., Jeanloz,R.W. (1984) *J. Am. Chem. Soc.* **106**, 811–813.
25. Bax,A., Davis,D.G. (1985) *J. Magn. Reson.* **63**, 207–213.
26. Griesinger,C., Ernst,R.R. (1987) *J. Magn. Reson.* **75**, 261–271.
27. Kessler,H., Griesinger,C., Kerssebaum,R., Wagner,K., Ernst,R.R. (1987) *J. Am. Chem.* Soc. **109**, 607–609.
28. Han,F., Watt,W., Duchamp,D.J., Callahan,L., Kezdy,F.J., Agarwal,K., (1990) *Nucleic Acids Research* **18**, 2759–2767.