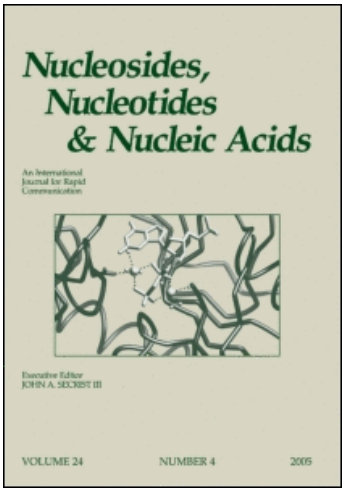


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# Assignment of Absolute Configuration at Phosphorus in Dithymidylyl(3',5')phosphormorpholidates and -Phosphormorpholidothioates

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# ASSIGNMENT OF ABSOLUTE CONFIGURATION AT PHOSPHORUS IN DITHYMIDYLYL(3′,5′)PHOSPHORMORPHOLIDATES AND -PHOSPHORMORPHOLIDOTHIOATES

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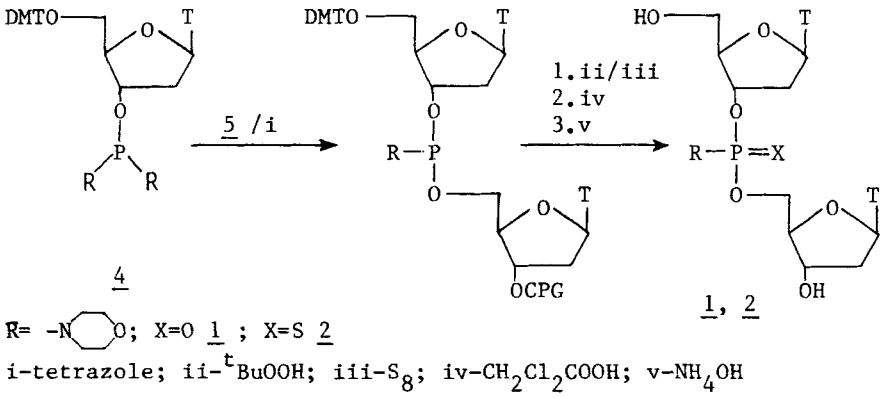
ABSTRACT
Diastereoisomers of dithymidylyl(3′,5′)phosphormorpholidate (1) and morpholidothioate 2 were isolated. Analysis of products of stereospecific reactions allowed us to correlate absolute configuration with RP HPLC elution order - "fast"-eluted isomers are Sp, while "slow"-eluted are Rp isomers.



Froehler[1] has shown that hydrolysis of the P-N bond at internucleotide phosphormorpholidate moiety occurs smoothly on treatment of the oligo(nucleotide morpholidate) with formic acid. Since acid-catalysed solvolysis of P-N bond occurs with inversion of configuration at phosphorus,[2-4] we undertook some experiments to assign the absolute configuration at phosphorus in both diastereoisomers of dithymidylyl (3′,5′) phosphormorpholidate[5] (1) via correlational analysis including the preparation of diastereoisomeric dithymidylyl (3′,5′) phosphormorpholidothioates (2) and their stereospecific conversion into dithymidylyl (3′,5′) phosphorothioates (TpsT-3) of known absolute configuration at phosphorus[6,7], and independently, via stereospecific conversion of 2 into 1. Diastereoisomers of 2 were obtained by condensation of 5′-DMT thymidine-3′-O-phosphodimorpholidite 4 with thymidine 3′-bound to a solid support (5), as described previously[8]. Sulphurization of the solid support-bound dithymidylyl(3′,5′)phosphormorpholidite was achieved by treatment with saturated solution of sulphur in anhydrous 2,6-lutidine for 0.5 h[9]. After detritylation, dithymidylyl(3′,5′) phosphormorpholidothioate (2) was released from the solid support according to standard procedures[10]. Purification and separation of diastereoisomers was achieved by means of RP HPLC[11]. Each diastereoisomer has been treated with 85% formic acid for 15 min at 95°C (0.5 OD units in 50 µl of acid).




4

R= -N O; X=O 1 ; X=S 2

1, 2

i-tetrazole; ii-$^{t}$BuOOH; iii-$S_8$; iv-$CH_2Cl_2COOH$; v-$NH_4OH$

FIG. 1

The products of solvolysis of "fast"2 contained, according to HPLC analysis, the mixture of TpT(23%),(Rp) 3 (25%), and "fast"1 (52%), while that one obtained from solvolysis of "slow"1 contained TpT, (Sp)3, and "slow"(1) (the ratio of respective peak integrations was roughly the same). Each product was identified by coinjection with authentic samples obtained by independent methods. These results allow us to assign the absolute configuration at phosphorus in both diastereoisomers of 2. Because "slow"-(Sp)3 was obtained from "slow"-2 in a stereoinvertive process, the absolute configuration of "slow"-2 must be Rp and, on the contrary, that of "fast"-2 must be Sp. According to previously established procedures for oxidation of phosphorothioates with hydrogen peroxide[12] or butylene 1,2-oxide[13], known to proceed with retention of configuration, we performed reactions of diastereoisomers of 2 with both aforementioned reagents and found, that they led stereoselectively, with retention of configuration to 1. Reference samples of 1 were synthesized independently by oxidation of the above described solid support-bound dithymidylyl(3',5')phosphormorpholidite[8] with *tert*-butyl hydroperoxide in benzene (10% v/v). All subsequent steps were identical as for 2 giving "fast" and "slow" diastereoisomers with retention time 21.9 and 23.3 min, respectively. All appropriate coinjections on HPLC indicated that "fast"-2 was converted into "fast"-1, while "slow"-2 gave "slow"-1 in these oxidations, respectively. These results allow us to assign the absolute configuration at phosphorus in both diastereoisomers of 1, as depicted in the Fig.2:

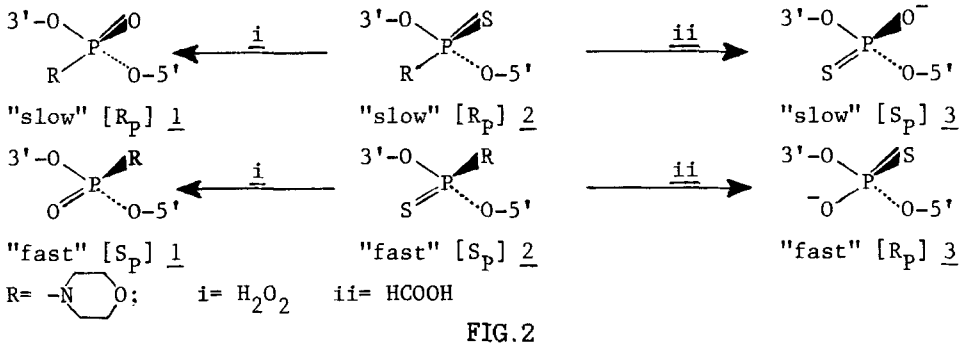



FIG.2

The assignment of absolute configuration at phosphorus atom in both diastereoisomers of 1 complements the work of Shimidzu et. al.[5], who suggested the absolute configuration for the 5'-dimethoxytrityl derivatives of 1 on the basis of NMR. However, these Authors indicated that this assignment required confirmation by X-Ray analysis[14].

To the best of our knowledge, this assignment of absolute configuration at phosphorus in internucleotide phosphoramidates has only one earlier precedent in the case of dithymidyl phosphoranilidates[6]. Because of increasing interest in application of oligonucleotide phosphoramidates as antiviral agents[15], all information concerning the structure and absolute configuration at phosphorus in these compounds may support an improved understanding of the activity and mode of action of anti-sense DNA oligonucleotide phosphoramidates. As pointed out by Marcus-Secura[16], oligonucleotide analogues possessing predetermined absolute configuration at P-chiral centers should have increased potential as gene regulatory substances.

The methodology presented in this communication can be used in principle for the assignment of absolute configuration at phosphorus in other dinucleoside phosphoramidates, including derivatives of secondary as well as primary amines.

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10. Treatment with 25% aqueous ammonia for 2 hours at ambient temperature to cleave from the solid support. No further deprotection was required, thus lyophylized sample was ready for HPLC.
11. RP HPLC chromatography was performed on Spherisorb ODS-2 (LDC/Milton Roy) column (4.6mm x 25cm) using 0.1M TEAB buffer starting from 5% $CH_3CN$/TEAB. Analytical data are summarized in the table.

| Compound | Hplc gradient[a] | Retention time | $^{31}P$ NMR $\delta$[b] | Configuration |
|---|---|---|---|---|
| 1 "fast" | 0.35%/min | 22.1 min | -1.5ppm | Sp |
| 1 "slow" | 0.35%/min | 23.4 min | -0.2ppm | Rp |
| 2 "fast" | 0.22%/min | 45.5 min | 74.22ppm | Sp |
| 2 "slow" | 0.22%/min | 46.0 min | 74.24ppm | Rp |
| 3 "fast" | 0.35%/min | 16.8 min | 56.1ppm | Rp |
| 3 "slow" | 0.35%/min | 17.8 min | 55.9ppm | Sp |

[a]Linear gradient of acetonitrile in 0.1M TEAB buffer (1.5 ml/min).
[b]Samples dissolved in $D_2O$ containing 30% of acetonitrile.

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14. Note that isomers I and II described by Shimidzu et. al.[5] change their "elution order" upon detritylation. This phenomenon, well recognized for many other DNA-analogs[17], explains full compatibility of our results with those described by Shimidzu, since the deprotection of 5'-DMT group from 5'-"fast"-1 gives 5'-OH-"slow"-1, and deprotection of 5'-DMT-"slow" -1 gives 5'-OH-"fast"-1.
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