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ONE POT R_P-DIASTEREOSELECTIVE SYNTHESIS OF DINUCLEOSIDE METHYLPHOSPHONATES USING METHYLDICHLOROPHOSPHINE

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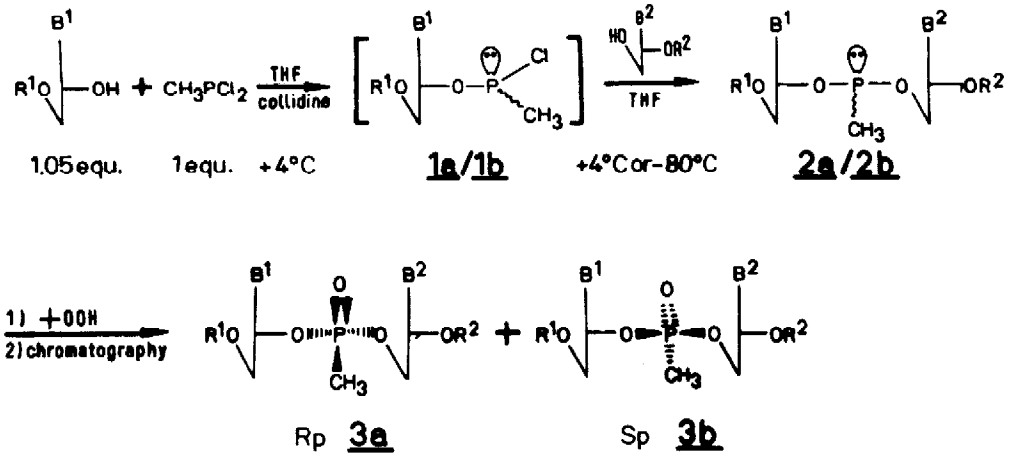
Summary: Using CH_3PCl_2 and suitably protected nucleosides at low temperature remarkable diastereoselectivity is observed in the synthesis of dinucleoside methylphosphonates.

Oligodeoxynucleoside methylphosphonates (ODN-MePhos.) are attractive nucleotide analogues for use as antisense oligonucleotides [1]. They are missing the negative charge on phosphorus which facilitates their penetration into cells and based on electrostatic arguments they should hybridize stronger with complementary DNA and RNA. Their unnatural linkage also proved to be more resistant to degrading enzymes . On the other hand a distinct disadvantage of these ODN-MePhos. is the occurence of two diastereomers at each phosphorus atom resulting in 2^n diastereomers within an ODN containing n methyl substituted phosphorus atoms. Recently it was shown that the all R_P configurated thymidine octamer binds stronger to poly-A than the normal phosphate anologue. The S_P octamer did not bind at all [2]. Until now neither any diastereoselective synthesis at all nor one for the favoured Rp-isomer is described, except for the TpT case [3].

Towards this goal we developed a synthetic procedure which favorably leads to the R_P-isomer of a dinucleoside methylphosphonate under certain conditions. The general method is outlined in Scheme 1. (For experimental conditions see ref. [4]; abbreviations see Table 1).



Scheme 1

The results presented are based on our earlier work where we developed new methods for the synthesis of dinucleoside methylphosphonates. Here we observed an unequal diastereomeric ratio only for TpT [4]. At the time we were unable to assign the absolute configuration on phosphorus. Now we have investigated this reaction with the other bases in more detail concerning the diastereomeric excess and the resulting stereochemistry.
The diastereomeric ratios obtained after work up and analysis of the precipitated product by $^{31}P/^{1}H$-NMR spectroscopy and RP-HPLC are summarized in Table 1.

Table 1: Temperature and base dependent induction in the synthesis of dinucleoside methylphosphonates in THF

R^1	Tr	Tr	Tr	Tr	Tr	Tr	Tr	MMTr	MMTr	MMTr
B^1	T	T	T	T	C^{Bz}	C^{Bz}	C^{Bz}	A^{Bz}	A^{Bz}	$G^{Ibu/NPE}$
B^2	T	T	T	A^{Bz}	T	A^{Bz}	G^{Ibu}	T	A^{Bz}	T
R^2	Bz	TBDMS	TBDPS	Bz	Bz	Bz	TBDMS	Bz	Bz	Bz
4°	2/1	2/1	---	2/1	---	---	1/1	2/1	2/1	----
R_P/S_P -80°	6/1	---	6/1	4/1	8/1	8/1	2/1	2/1	2/1	1/1

(A = adenine, G = guanine, C = cytosine, T = thymine, TBDMS = tert.-butyldimethylsilyl, TBDPS = tert.-butyldiphenylsilyl, Bz = benzoyl, Tr = trityl, MMTr = monomethoxytrityl)

For identification and assignment the individual diastereomers were isolated after silicagel- and RP-chromatography. The Rp-isomer is *always* the one with the higher R_f-value on a silica thin layer plate using $CHCl_3$/MeOH mixtures as eluent and with the smaller ^{31}P-NMR shift (Fig. 1, 3a) relatively to the S_P-isomer.
The diastereomeric excess depends on the temperature and the nucleosides used. This reveals the kinetic and stereochemical aspects of the reaction. The induction is highest at low temperatures (-80°C) and with small bases (T,C) in the first and second step leading to 79% de. at best. The diastereoselectivity is less with purine bases (A,G) in the first step even at low temperatures. Deoxyguanosine is worst showing only a small induction in all cases.
The mechanistic aspects of this reaction are not fully understood yet. Reactions at trivalent phosphorus are complicated due to pseudorotations within a trigonal-bipyramidal transition state resulting from the attack of free nucleophiles. Furthermore the diastereomeric intermediates 1a/1b and 2a/2b (Scheme 1) are not isolatable because of their sensitivity to air and moisture. Therefore the intermediates were investigated in situ by ^{31}P-NMR (Fig.1). The signals observed indicate that 1a/1b exists, even at -80°, as a 1/1 mixture of diastereomers being in rapid equilibrium with one another. We found that the induction observed arises in the second step when the more stable phosphinic diesters 2a/2b are formed from 1a/1b after

adding the second nucleoside. The final oxidation proceeds with retention of configuration and does not change the absolute stereochemistry [5].

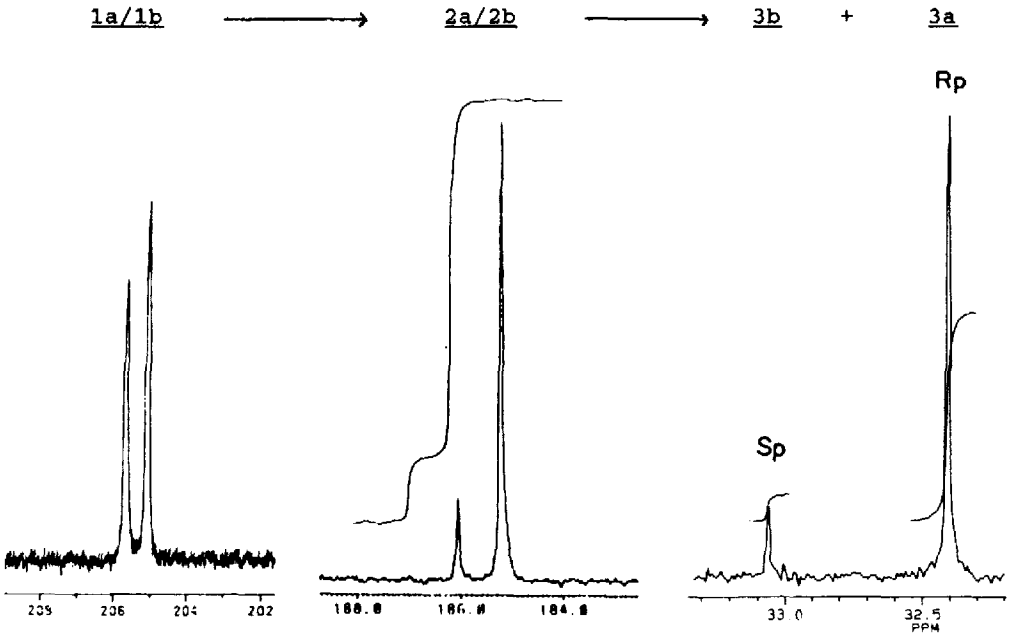


Fig. 1 Diastereomeric intermediates 1a/1b and 2a/2b and the final dimers 3a/3b in the synthesis of a TpT dimer measured at -80°C by ^{31}P-NMR at 121 MHz in THF with 85% H_3PO_4 as external standard.

The stereochemical aspects were supported by the following experiment where HO-T-OBz with the free primary 5`-alcohol group reacted first with CH_3PCl_2. Adding Tr-T-OH at -80° and oxidation of the intermediates 2a/2b results in a 1/1 mixture instead of 6/1. This shows the importance of the stereochemical more demanding secondary alcohol in 1a/1b for the induction. The stereochemistry is independant of changes in the alcohol protecting groups investigated so far. Even the large TBDPS group for the protection of the 3'-alcohol does not diminish the induction (Table 1).

The stereochemistry of the individual dimers was determined by 2D-NMR using the NOE derived ROESY technique [6]. We investigated the environment of the methyl groups within the two diastereomers of a dimer pair. Due to different distances to the neighbouring H3' and H4'-protons of the 5'-nucleoside-part we could assign the stereochemistry of the diastereomers (results of this investigation will be published elsewhere). The results were compared with literature data for the TpT dimers [7] and the X-ray structure for one ApT dimer [8]. This ApT isomer was additionally provided by P.S. Miller as an authentic sample for comparison with our

corresponding dimer. Our results are in full agreement with these "standards" and allow us to prove the usefulness of our method.

With this ROESY NMR method the stereochemistry from the dimers of TpT, TpA, ApT and ApA were determined so far. CpT and CpA were assigned corresponding to the induction of TpT and TpA. CpG is not known for sure so far because of differences with literature data [9], but is under investigation.

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