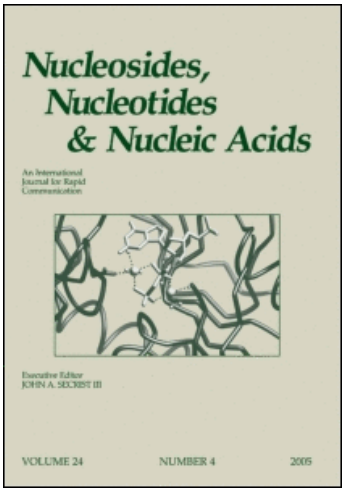

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The reaction of Tetrazole with Phosphoramidites as a Model for the Nucleotide Coupling Step

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THE REACTION OF TETRAZOLE WITH PHOSPHORAMIDITES AS A MODEL FOR THE NUCLEOTIDE COUPLING STEP

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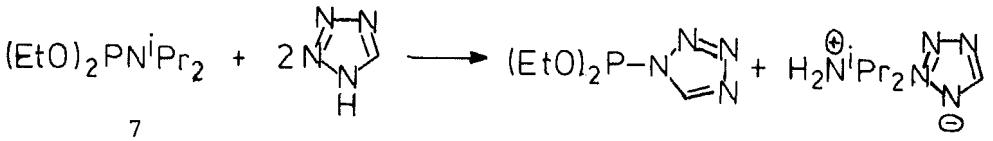
Abstract. The mechanism of the tetrazole-activated coupling step in the synthesis of oligonucleotides via phosphoramidites is studied with the help of model reactions.

The essential step in the synthesis of deoxyoligonucleotides using phosphoramidite monomers is the tetrazole-activated coupling reaction of the phosphoramidite with the free 5'-hydroxyl-function of an immobilized oligonucleotide[1,2]. Although this activation process is widely used in deoxy- and ribooligonucleotide synthesis, its mechanism still remains to be fully clarified.

The main question is, whether tetrazole, a weak acid, serves only as a proton donor yielding as primary product a protonated nucleoside-phosphoramidite[3], or whether it will attack as a nucleophile giving rise to the formation of a "tetrazolide" intermediate[4,5]. Arguments in favour of the existence of a tetrazolophosphane intermediate have come mainly from ^{31}P-NMR spectroscopy. Thus, the appearance of a new signal at 126 ppm on treatment of a nucleoside-phosphoramidite with an excess of tetrazole has been tentatively assigned to the formation of such a species[6,7], but no direct structural proof for the nature of the reactive intermediate was given yet.

Since the high reactivity of the intermediate makes its isolation impossible, we decided to study a model reaction



7

equ. 1

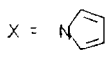
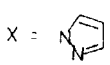
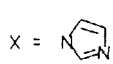
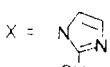
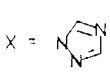
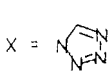
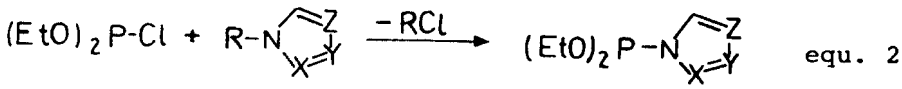
$(EtO)_2P-X$	No.	solvent	$\delta^{31}P$
X =  1	1	DMSO	130.0
X =  2	2	$CDCl_3$	126.6
X =  3	3	$CDCl_3$	129.6
		CD_3CN	125.6
X =  4	4	$CDCl_3$	128.5
X =  5	5	$CDCl_3$	127.9
		CD_3CN	123.9
X =  6	6	$CDCl_3$	130.8
		CD_3CN	126.6

Chart 1: Diethoxy-N-azolyl-phosphorus compounds 1-6 and their $\delta^{31}P$ values (relative to external 5% aquous H_3PO_4)

simulating the phosphoramidite coupling step. For this purpose the conversion of diethoxydiisopropylamino-phosphane 7 with different equivalents of tetrazole (equ. 1) was followed by ^{31}P-NMR and compared to the analogous conversion of a nucleoside-phosphoramidite.

The intermediate of equ. 1, N-tetrazolyl-diethoxyphosphane 6, was prepared and characterized as an authentic substance. A series of N-azolyl-phosphorus compounds (CHART 1) were prepared in order to compare their spectroscopic properties with those of the observed intermediate in the model reaction as well as in oligonucleotide chemistry.

Compounds 2-5 were obtained by the reaction of diethoxychlorophosphane with the respective N-trimethylsilyazoles in benzene[8], while 1 was synthesized from the potassium salt of pyrrole[9]. 6 resulted from the reaction of 8 with sodium tetrazolide.



equ. 2

8 1a - 6a 1 - 6

1a: R = K; 2a - 5a: R = $SiMe_3$; 6a: R = Na

Compounds 1-6 were characterized by ^{1}H, ^{13}C and ^{31}P-NMR spectroscopy and elemental analysis. The ^{31}P-resonance signals of compounds 1-6 are between 123.9 and 130.8 ppm, which is comparable to the shift for the intermediate in nucleoside phosphoramidite activation.

It is remarkable that in the latter case only one signal, which is unusually broad, is observed in spite of the diastereomeric starting material. This is explained by an epimerization at phosphorus due to an exchange with the excess tetrazole of the solution[10]. In accordance with this hypothesis is our observation of broad ^{31}P-NMR signals for the azolophosphanes 1-6 with half band widths of about 30 Hz, if we use deuterochloroform as solvent. An intermolecular exchange of the azolyl group at phosphorus could be responsible for this effect.

The comparison of the ^{31}P-NMR spectra of the N-azolylphosphanes to the spectra obtained in the course of nucleoside phosphoramidite activation suggested that the intermediate observed in both cases should be a tetrazolophosphane. If this suggestion was correct, the model reaction of diethoxy-diisopropyl-aminophosphane 7 with tetrazole according to equ. 1 should give a product which is identical with the tetrazolophosphane 6 obtained previously. This was, in fact, the case: After the reaction of 7 with two equivalents of tetrazole in acetonitrile, diisopropylammoniumtetrazolide could be crystallized and 6 was obtained after distillation in 52% yield.

To decide whether the acidic strength of tetrazole also plays a role or whether the mechanism is just that of a substitution, we activated 7 with different less acidic azoles

like 1,2,4-triazole (pK_a=10.26), imidazole (pK_a=14.10) and also acetic acid (pK_a=4.76), which has the same pK_a-value as tetrazole. The formation of the corresponding azolophosphanes or of the anhydride in the case of acetic acid were followed by ^{31}P-NMR spectroscopy. These experiments demonstrated that two equivalents were enough for a complete formation of the tetrazolophosphane or anhydride, whereas in the case of triazole or imidazole only 30% and 10% yields were obtained.

The results of our studies, which for the first time are based on well characterized azolophosphanes prove the formation of a tetrazolophosphane in the activation process of diethoxydiisopropyl-aminophosphane 7 with tetrazole and are strong evidence for the formation of an analogous intermediate in nucleoside phosphoramidite activation in accordance with the mechanism previously described by O. Dahl[11]. Our NMR-investigations prompt us to suggest a mechanism for the formation of the tetrazolophosphane which consists of two steps: A quick protonation of 7 is followed by a slow formation of the tetrazolophosphane as rate limiting step. The reaction is reversible: After addition of diisopropylamine the starting material is completely recovered. At -30°C the rate of formation of the tetrazolophosphane is very slow. The last step, the formation of the phosphite triester, is irreversible, since the triester cannot be activated any more.

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