

The Effectivity of 1*H*-Triazoles and -Tetrazoles as Activators in Acid-Catalyzed Phosphoramidite Alcoholysis

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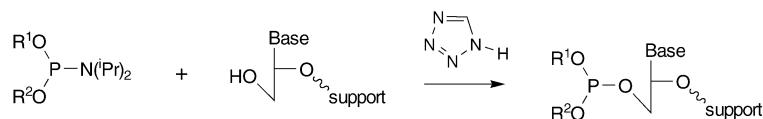
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The efficiency of 5-nitro-1,2,4-1*H*-triazole (**3**), 5-(methylthio)-1*H*-tetrazole (**4**), and 5-(4-nitrophenyl)-1*H*-tetrazole (**5**) as activators in phosphoramidite alcoholysis has been studied relative to 1*H*-tetrazole (**6**). Reactions of these azoles with diisopropyl (diisopropylamido)phosphite (**1a**) were followed in THF, and the rates were found to increase with increasing acidity of the azoles. The *Brønsted* α value of 0.7 determined for this dependence is in agreement with data published earlier.

Introduction. – Phosphoramidite alcoholysis is a reaction used in the coupling step in automated oligonucleotide synthesis: a nucleoside 3'-phosphoramidite (**1b**) is allowed to react repeatedly with the 5'-OH function of the growing, support-bound oligonucleotide chain (*Scheme 1*) [1][2].

Scheme 1. Oligonucleotide Synthesis by the Phosphoramidite Approach



1a $R^1 = R^2 = i\text{Pr}$

1b $R^1 = \text{nucleosid-3'-yl}$
 $R^2 = \text{NC(CH}_2)_2$

An acidic activator, commonly 1*H*-tetrazole (**2**), is used to accelerate the reaction. During the last few years, demand for improvement of the already successful method has increased, together with its application to large-scale synthesis of oligonucleotides. Research on the mechanism of the reaction has been carried out, and new catalysts have been studied as alternatives to the presently applied 1*H*-tetrazole [3]. Among these candidates were more-acidic azole derivatives such as 5-(ethylthio)-1*H*-tetrazole [4] and 5-(4-nitrophenyl)-1*H*-tetrazole [5].

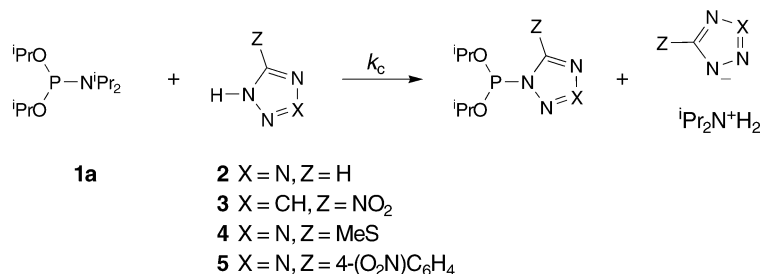
This work is aimed at quantifying the relationship between the acidity of azoles and their catalytic efficiency.

The tetrazole-promoted reactions of diisopropyl (diisopropylamido)phosphite (**1a**) are known to be practically instant in MeCN [6]. In THF, however, the reaction kinetics are slow and can be followed by ³¹P-NMR. The reaction is initiated by displacement of the diisopropylamido group of **1a** by the azole activator. The tetrazolide obtained is

then rapidly converted to a phosphite triester. In fact, the tetrazole-activated alcoholysis is almost as fast as the reaction of **1a** with tetrazole [7].

Results and Discussion. – In the present work, the reaction rates of **1a** (0.1M) with 5-nitro-1,2,4-*H*-triazole (**3**), 5-(methylthio)-1*H*-tetrazole (**4**), and 5-(4-nitrophenyl)-1*H*-tetrazole (**5**) were determined in THF at various activator concentrations (0.08–0.5M).

Scheme 2



The initial reaction rate was determined by fitting an exponential curve to the observed time-dependent concentration of **1a** (Fig. 1). The catalytic rate constant, k_c , was calculated for each azole according to Eqn. 1.

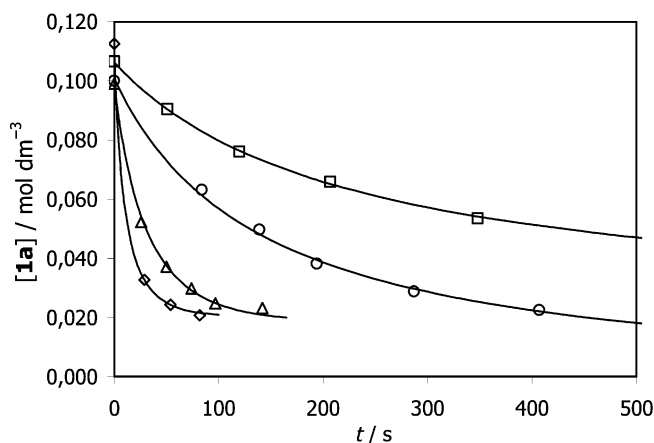


Fig. 1. Time-dependent concentrations of **1a** during its reaction with **3** (0.19 M; □), **2** (0.17 M; ○), **4** (0.18 M; △), and **5** (0.19 M; ◇). Concentrations of 1*H*-tetrazole (**2**) were taken from [7] and normalized to fit those of the other compounds.

$$v_0 = \lim_{t \rightarrow 0} \left(\frac{d[\mathbf{1a}]}{dt} \right) = k_c [\mathbf{1a}]_0 [\text{azole}]_0^n \quad (1)$$

The reaction order in azole concentration was invariably found to be higher than unity, the nonlinear character increasing together with the $\text{p}K_a$ value of the activator

(Table and Fig. 2). However, for all the activators, a reasonable fit ($r > 0.97$) was obtained when using a second-order dependence of the rate on the azole concentration. This allowed us to quantitatively compare the efficiency of **3–5** with that of 1*H*-tetrazole (**2**) studied earlier [7].

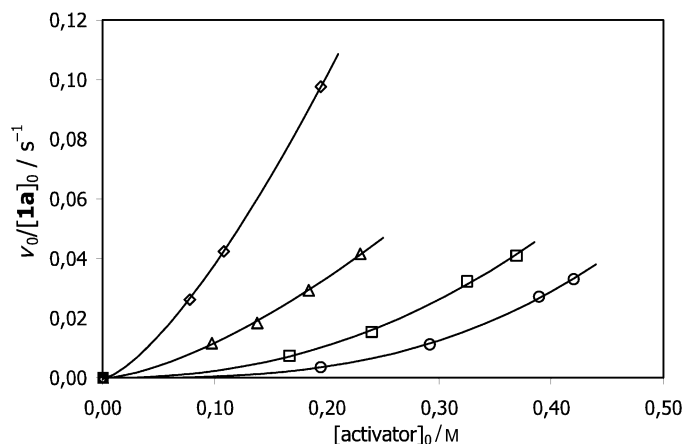


Fig. 2. Nonlinear dependence of $v_0/[1a]$ and activator concentration in the reactions of **1a** with **3** ($\circ$), **6** ($\square$), **4** ($\triangle$), and **5** ($\diamond$), respectively

Table. Reaction of Azole Activators with **1a**

Compound	pK_a	n (best fit)	$k_c/M^{-3} s^{-1}$
5-(4-Nitrophenyl)-1 <i>H</i> -tetrazole (5)	3.66	1.4	2.71
5-(Methylthio)-1 <i>H</i> -tetrazole (4)	4.15	1.5	0.76
1 <i>H</i> -Tetrazole (2)	4.87	2.2	0.31
5-Nitro-1,2,4-1 <i>H</i> -triazole (3)	5.19	2.9	0.19

When the calculated k_c values were plotted logarithmically against the respective pK_a values in THF [8], a Brønsted α value of 0.7 was obtained (Fig. 3). This is in agreement with the reaction being acid-catalyzed: for the $H_4N^+Cl^-$ -catalyzed reaction, α values of 0.05–0.65 have been determined in neat alcohol [9], while ammonium trifluoromethanesulfonate, methanesulfonate, and trifluoroacetate gave rise to values of 0.6–0.9 in MeCN [6].

According to kinetic studies, 1*H*-tetrazole and other acidic azoles also act as nucleophilic activators in the phosphoramidite alcoholysis that proceeds *via* a reactive azolidite intermediate [7]. The reaction rate has been observed to depend on the nucleophilicity of the activator. For ammonium salts in MeCN, a β_{nuc} value of 0.2 has been reported, given the pK_a of an activator is used as a measure of its nucleophilicity [6]. This means that more-acidic azoles (better acid catalysts) are inferior to less-acidic ones regarding the nucleophilic contribution of the activation. Consequently, the α value of 0.7 determined in this work may actually refer to an even more complete proton transfer in the transition state, but the rate-accelerating effect of the increased acidity of the activator is partially cancelled by the lower nucleophilicity of its conjugate base.

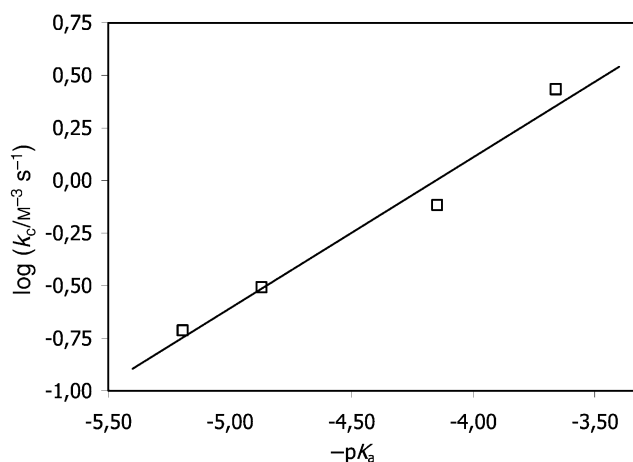


Fig. 3. Brønsted plot for the reaction of **1a** with the activators **3–6** performed in THF at 20°

Experimental Part

Diisopropyl (diisopropylamido)phosphite (**1a**) was prepared as described in [7]. THF was dried over CaH_2 . The azole activators **3–6** were used as purchased, and their THF solns. were dried over molecular sieves (4 Å). In the kinetic studies, the reagents were mixed in a septum-sealed NMR tube, and the reactions were followed by ^{31}P -NMR spectroscopy (202 MHz) as described in [7]. The level of the hydrolysis reaction was kept lower than 7.5% in all kinetic runs.

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