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# SUBSTITUTED 5-PHENYLTETRAZOLES: IMPROVED ACTIVATORS OF DEOXYNUCLEOSIDE PHOSPHORAMIDITES IN DEOXYOLIGONUCLEOTIDE SYNTHESIS.

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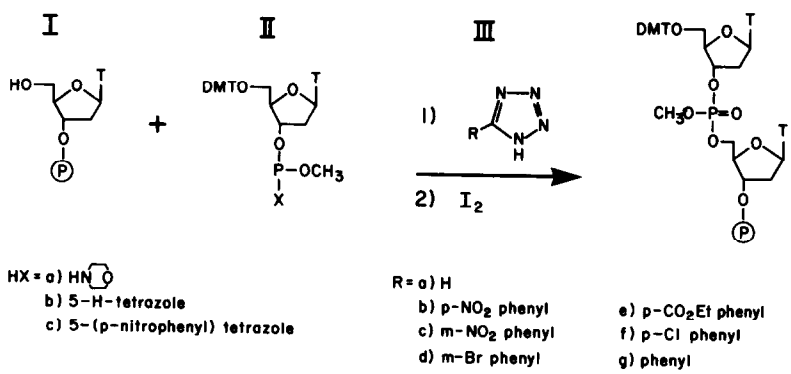
Abstract: 5-(p-nitrophenyl)tetrazole, a tetrazole of enhanced acidity relative to 5-H-tetrazole, is a superior activator of the N-morpholinophosphoramidite in the synthesis of deoxyoligonucleotides.

The synthesis of deoxyoligonucleotides using phosphoramidites has proven to be an efficient and valuable method.[1] 5-H-tetrazole (IIIa, Scheme 1) has been used as an activator of the 5'-dimethoxytrityl nucleoside phosphoramidite (II) for coupling to the 5'-hydroxyl of a polymer bound oligonucleotide (I).[2] Activation of II is presumed to involve protonation of the phosphoramidite by the tetrazole (III).[2] The work presented here evaluates the use of tetrazoles of enhanced acidity, namely substituted 5-phenyltetrazoles (III b-g) as improved activators in the phosphoramidite procedure.

Substituted 5-phenyltetrazoles (III b-g) are readily prepared from the corresponding substituted benzonitrile, sodium azide and ammonium chloride in anhydrous N,N-dimethylformamide (DMF). Reactions were performed as previously reported[3], with the exception that the inorganic salts were thoroughly dried in an Abderhalden tube for 12 hours at 65°C prior to use. Progress of the reaction was monitored by thin layer chromatography (tlc) on silica gel (Merck Silica gel 60 $F_{254}$) using acetic acid/methanol/chloroform (1:1:8) as the eluant.

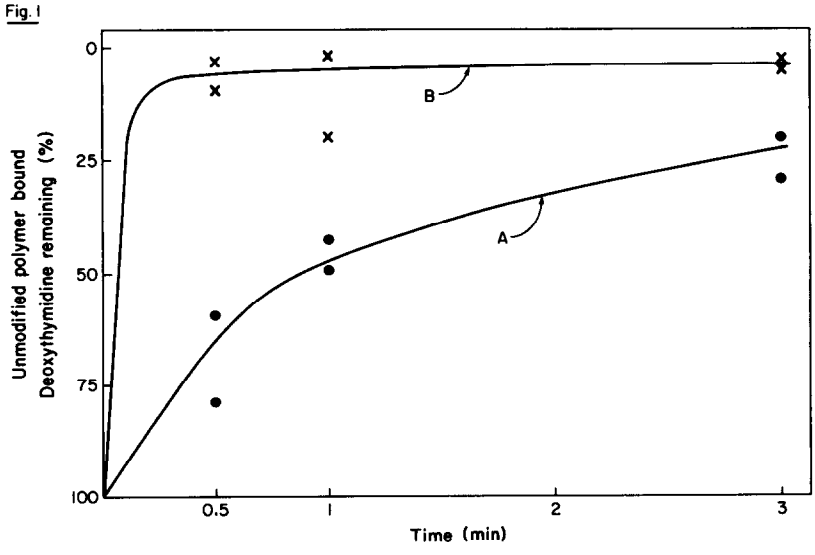
**Scheme I**



Kinetics of phosphoramidite activation and coupling was examined for IIIa-e.[4] Initial experiments, similar to the procedure described below, indicate the order of reactivity to be III b>c>d>e>a. The morpholino-amidite (IIa)[5] was used throughout this study as a result of ease of preparation in high purity and excellent stability both as a solid and in an anhydrous acetonitrile ($CH_3CN$) solution.

To assess the difference in rate of activation between IIIa and IIIb, the kinetics of amidite coupling to the polymer support (I) was determined. Silica gel was derivatized with 5'-dimethoxytrityl-3'-succinyl deoxythymidine to a loading of 25 μmoles/gram by the published procedure.[6] To 10 mg of derivatized silica gel was added the tetrazole (1 ml of 0.1M solution in anhydrous $CH_3CN$, maximum solubility of IIIb is 0.12 M) followed by addition of 5'-dimethoxytrityl deoxythymidine morpholino-amidite (1 ml of 0.05M, IIa). Reactions were quenched at 0.5, 1 and 3 minutes with 0.1M $I_2$ in tetrahydrofuran/2,6-lutidine/water (95:4:1). The assumption of pseudo-first order kinetics with respect to amidite was tested by quenching the solution phase of the final time point in 1-butanol, then triethylamine (TEA). $^{31}P$ nuclear magnetic resonance (NMR) of these aliquots showed the presence of trialkyl phosphite (-139 ppm relative to 5 percent phosphoric acid in $CH_3CN$) in 60-70 percent abundance. Following deblocking with 0.1M p-toluene sulfonic acid/$CH_3CN$ and demethylation with thiophenol/TEA/dioxane (1:1:2) the product was cleaved from the silica gel with concentrated ammonia and lyophilized. High performance liquid chromatography (HPLC) analysis was performed on reverse phase $C_8$ resin (Alltech, 10 micron) using 12 percent aqueous methanol/0.05M triethyl ammonium acetate as the eluant. The graph in Fig. 1 clearly demonstrates the greatly enhanced rate of reaction when IIIb is used as the activator.

Fig. 1




A) 5-H-tetrazole (IIIa) B) 5-p-$NO_2$ phenyl tetrazole (IIIb)

The apparent $pK_a$ values of substituted 5-phenyltetrazoles have been investigated and reported.[7,8] In 50 percent aqueous ethanol a value of 3.7 has been observed for 5-(p-nitrophenyl)tetrazole (IIIb)[7] and 4.8 for 5-H-tetrazole (IIIa) in water.[9] The correlation seen between acidity and reactivity has led us to attempt the preparation of more acidic substituted 5-phenyltetrazoles. Unfortunately, preparation of the tetrazole derivatives of pentafluorobenzonitrile and 3,5-dinitrobenzonitrile, under reaction conditions similar to those referenced above, lead to a mixture of products. Although not confirmed, these products may arise from nucleophilic aromatic substitution.[10,11]

Detritylation is a concern when an acidic activator is used. The rate of detritylation of 5'-dimethoxytrityl deoxythymidine by IIIa and IIIb was examined by preparing equimolar solutions in $CH_3CN$ and monitoring the solutions by tlc. No significant detritylation was observed (less then 10 percent) after 24 hours in either case.

The tetrazoylamidite IIb has been shown to be an active phosphitylating agent.[12,13] Tetrazoylamidites (IIb or IIc) are thus candidates for the reactive species during nucleotide coupling. The $^{31}P$ NMR specta of a solution containing nucleoside morpholinoamidite (IIa) and tetrazole (IIIa or IIIb) in anhydrous $CH_3CN$ show no amidite signals other than the parent IIa (-144ppm). Assuming that morpholine (IIa) and tetrazole (IIb,c) amidites do not exhibit coincidental $^{31}P$ NMR chemical shifts the active coupling species is present in low concentration. This NMR observation and the relationship between tetrazole acidity and activity suggest that protonation of amidite (IIa) is rate determining in the phosphitylation reaction (Scheme 1).

Increased rate of coupling is advantageous when preparing deoxyoligonucleotides with a flow-through column synthesizer. The automated synthesis of deoxyoligomers, accomplished on a SAM-Biosearch machine, has been achieved with the use of IIIb as an activator. Experimental details will be reported at a later date.

The relative rates of activation by several tetrazoles has been reported for the deoxynucleoside N-morpholinophosphoramidites. The results presented herein demonstrate that tetrazoles of enhanced acidity lead to enhanced rates of activation. 5-(p-nitrophenyl) tetrazole (IIIb) is a superior activator and shows potential when used in a flow-through column automated synthesizer. The activator IIIb has been used as the activator of the deoxynucleoside N-morpholinophosphoramidites in the rapid synthesis of deoxyoligonucleotides.

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