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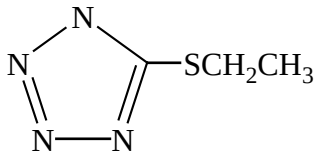
Technical Bulletin

5-Ethylthio-1H-tetrazole as an Activator in Oligonucleotide Synthesis



Formula: $C_3H_6N_4S$

M.W.: 130.17

Introduction

Traditionally the activation step of oligonucleotide synthesis has been performed with a 0.45 to 0.5M solution of 1-H-tetrazole in acetonitrile, show below in figure 1. When the concentration of 1-H-tetrazole exceeds 0.35M, problems with the solution tend to arise. The most common issue has been the crystallization of tetrazole when the solution is stored at a temperature below 18°C or when solution volume on a synthesizer is running low. Recent research suggests that 5-ethylthio-1H-tetrazole (ETT) is a more effective activator that does not suffer from the problems associated with 1-H-tetrazole (1-4).

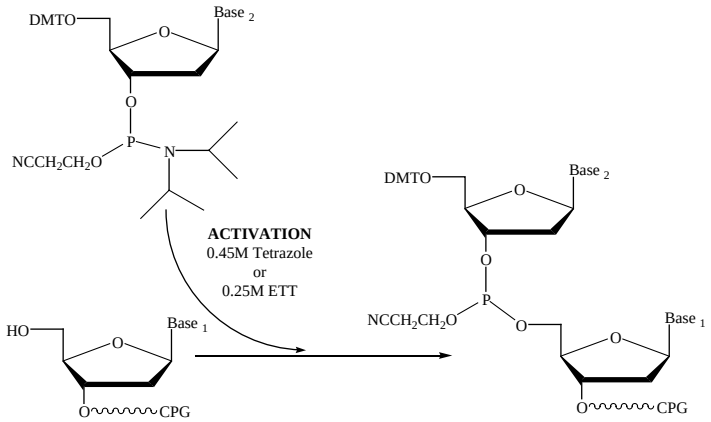


Figure 1: The activation and coupling steps of oligonucleotide synthesis

The Effect on RNA Oligo Synthesis

When synthesizing an RNA oligonucleotide, most of the 2' hydroxyl blocking chemistries are hampered by bulky groups. These bulky groups add a steric hindrance component, which further reduces the already expected low yield. For phosphoramidites that have a low coupling efficiency,

e.g. dyes and reporters, increasing the coupling time generally increases the overall coupling, but this is not true with RNA phosphoramidites. Varying coupling times and monomer concentration can lead to the formation of unwanted side products.

Wincott and coworkers (1) have demonstrated that ETT is a highly effective activator solution for RNA synthesis. In their system, total RNA yield increased several percent when the ETT stock solution was 0.25M and the coupling time reduced by half. Other groups have reported similar findings over a range of 0.25 to 0.75M (2,3). There are several explanations for this effective activation. The most obvious is the slightly increased acidity of the solution due to the presence of the ethylthio group. This slight drop in pH is advantageous in phosphite triester chemistry and leads to a more complete activation. The presence of the ethylthio groups also enhances the compound solubility, thus overcoming solubility issues seen with 1-H-tetrazole.

The Effect on DNA Oligo Synthesis

The effect of ETT on the synthesis of DNA oligonucleotides has also shown increased activation and overall yield. Show below in figure 2, there are comparable yields from the synthesis of a 32mer DNA oligo when using 0.45M 1-H-tetrazole or 0.25M ETT as an activator. It is important to note that these results were obtained by simply replacing the standard activator with ETT, suggesting that activation and coupling times need not be modified.

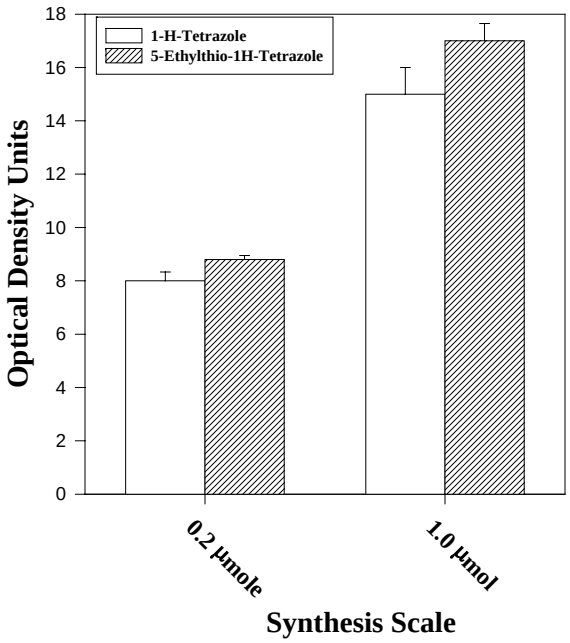


Figure 2: All oligonucleotides were synthesized on an Expedite 8909 automated DNA synthesizer. ETT activator was substituted for standard activator without change in the synthesis cycle. Data shown represents yield after HPLC of the post synthetic product.

While the data above demonstrates that during routine oligo synthesis, ETT is a fine activator, the question of special synthetic needs still remains. With the gaining interest in nucleic acid based therapeutics, the need for large scale production of oligonucleotides has risen. Generally, large scale synthesis is a costly prospect, due to the large amount of synthesis materials required. Wright et al. (4) investigated large scale oligo synthesis using a 0.25M ETT solution as the activator. In their system, they were able to demonstrate an increase in finished product, even when the phosphoramidite molar excess was decreased. Similar to standard synthesis scales, ETT can readily substitute in large scale systems.

Discussion

For many years, scientists in the field of DNA synthesis have attempted to move away from the use of 1-H-tetrazole as an activator. Concern over standard tetrazole activator came from the high concentrations required in the activation step and tetrazole crystals falling out of solution at cooler temperatures. With a final concentration of 0.25M, ETT offers relief from these concerns.

Wright et al. (4) noted that increased activation and coupling efficacy occurred even when the molar excess of phosphoramidites is reduced. This observation gives the nucleic acid chemist a new tool to produce high quality oligos and provides hope for the diagnostics and pharmaceutical manufacturers. With the ever growing concern over cost control in industry, significant savings can occur by reducing phosphoramidite consumption without the loss of needed finished product.

References

1) Wincott, F. et al., Nucleic Acids Res., 1995, 23, 2677.
2) Sproat, B. et al., Nucleoside Nucleotide, 1995, 14, 255.
3) Tsou, D. et al., Nucleoside Nucleotide, 1995, 14, 1481.
4) Wright, P. et al., Tet Lett, 1993, 34, 3373.

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