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ENHANCED COUPLING EFFICIENCY USING 4-DIMETHYLAMINOPYRIDINE (DMAP) AND EITHER TETRAZOLE, 5-(o-NITROPHENYL)TETRAZOLE, OR 5-(p-NITROPHENYL)TETRAZOLE IN THE SOLID PHASE SYNTHESIS OF OLIGORIBONUCLEOTIDES BY THE PHOSPHORAMIDITE PROCEDURE.

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Abstract

5-(p-nitrophenyl)tetrazole/DMAP mixtures are more effective phosphoramidite activators than either tetrazole, 5-(o-nitrophenyl)tetrazole or 5-(p-nitrophenyl)tetrazole for solid phase oligonucleotide synthesis.

Introduction

Now that solid phase synthesis of oligodeoxyribonucleotides by the phosphoramidite or phosphite triester methods has become well established[1] attention is turning towards the synthesis of oligoribonucleotides[2-4]. However, oligoribonucleotide synthesis is much more difficult for two reasons. Firstly, the 2'-hydroxyl group must be adequately protected with a protecting group such as the t-butyldimethylsilyl group[5]. Secondly, the efficiency of internucleotide bond formation is less than with 2'-deoxyribonucleoside derivatives[2-3].

To overcome this latter problem we have undertaken an investigation into the optimization of coupling conditions. Recently, a quantitative study[6] has shown that the ideal phosphate protecting group and amino substituent for tetrazole activated condensation are, respectively, the methyl and N,N-diisopropylamino groups. However, substituted tetrazole derivatives such as 5-(p-nitrophenyl)tetrazole[7] may also be employed as activating reagents to produce the reactive species **3** (Scheme 1). In this study we wish to report a comparative study of the efficiency of tetrazole, **2a**, 5-(o-nitrophenyl)tetrazole[8], **2b**, and 5-(p-nitrophenyl)tetrazole[9], **2c**, in the presence and absence of DMAP[10], in the synthesis of oligoribonucleotides.

To test these activating reagents, the homooligomer $(Up)_8U$ was prepared from phosphoramidite **1**[2] using an Applied Biosystems 380A DNA synthesizer. Pierce long chain alkylamine controlled pore glass, derivatized as previously described[11] (20 mg, 1.0 micromoles of bound nucleoside), was used as

Scheme I

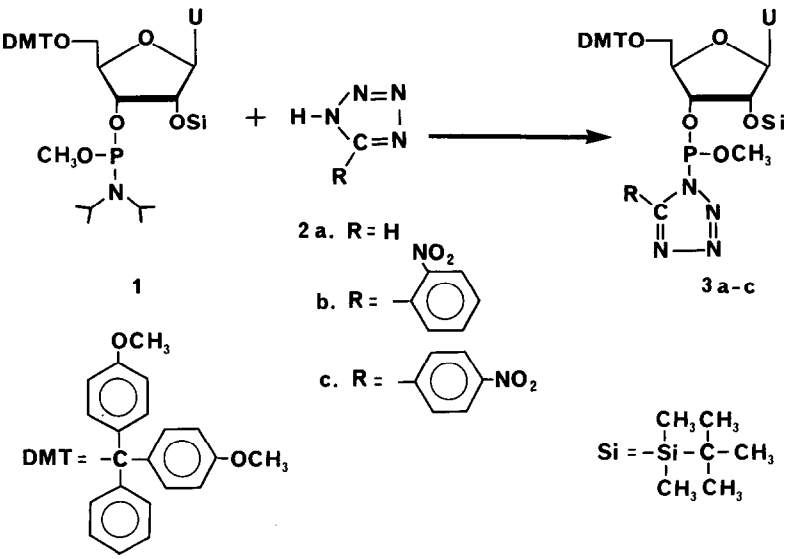

TABLE 1. Coupling Yields Obtained in the Synthesis of $(Up)_8U$ Using **2a-c** in the Presence or Absence of DMAP.

Entry	Activator	[DMAP]	[1]	Coupling Time	Aver. Yield
1,	0.50M **2a**	0	0.1M	3 min	90.0%
2,	0.50M **2a**	0	0.1M	15 min	92.8%
3,	0.50M **2a**	0	0.2M	3 min	94.3%
4,	0.50M **2a**	0	0.2M	15 min	96.2%
5,	0.50M **2a**	0.10M	0.1M	3 min	93.6%
6,	0.50M **2a**	0.10M	0.1M	15 min	93.5%
7,	0.40M **2b**	0	0.1M	15 min	88.0%
8,	0.40M **2b**	0.10M	0.1M	15 min	90.0%
9,	0.12M **2c**	0	0.1M	3 min	91.9%
10,	0.12M **2c**	0	0.1M	15 min	92.1%
11,	0.12M **2c**	0.01M	0.1M	3 min	96.4%
12,	0.12M **2c**	0.01M	0.1M	15 min	94.5%

Note: The concentrations given are the concentrations of the solutions before they were mixed together (1:1) during the coupling reaction.

the insoluble support. The synthesis cycle was similar to the "pulsed" cycles used for oligodeoxyribonucleotide synthesis from cyanoethyl phosphoramidites except that the length of the coupling reaction was extended, as shown in Table 1. A total of only eight to nine equivalents of phosphoramidite reagent were employed in each synthesis cycle. This low ratio of phosphoramidite to bound nucleotide minimized consumption of monomers and allowed the differences between tetrazole derivatives to be more easily detected. After synthesis, the products were deprotected[2], purified by polyacrylamide electrophoresis, and characterized by degradation with snake venom phosphodiesterase.

The coupling yields were determined by spectrophotometric quantitation of the orange dimethoxytrityl cation released during detritylation[11]. The average yield for each synthesis was then calculated for the second through eighth coupling cycle and the results are shown in Table 1.

The results with tetrazole show that under conditions normal for oligodeoxyribonucleotide synthesis (entry #1), derivative **3a** gives yields of only 90%. These yields may be increased somewhat by either increasing the coupling time (entry #2) or the concentration of phosphoramidite reagent (entry #3). This latter approach is undesirable, however, because of the increase in reagent **1** required. When DMAP was added to the tetrazole solution the coupling efficiency improved by 3.6% to give an average yield of 93.6% (entry #5).

The results with 5-(o-nitrophenyl)tetrazole were less satisfactory. The average coupling yield without DMAP was 88% and addition of DMAP only increased the yields to 90%. The reduced coupling efficiency observed with this reagent was most probably due to reduced nucleophilicity caused by steric hindrance from the ortho nitro substituent.

The best coupling results were obtained with 5-(p-nitrophenyl)tetrazole. Average yields of 92% were obtained for both the three and fifteen minute long coupling experiments. This reagent has been previously demonstrated to be a much better activating reagent than tetrazole for both morpholino-[7] and diisopropylaminophosphoramidite[4] derivatives and it is likely that reaction was complete in less than three minutes. Because the addition of DMAP to acetonitrile solutions of 5-(p-nitrophenyl)tetrazole markedly reduces the solubility of the material, a low concentration of DMAP (0.01M) was required in these experiments. However, this catalytic amount still improved the average coupling yield from 91.9% to 96.4%, an increase of 4.5%.

The enhanced reactivity observed with all three of the above tetrazole reagents in the presence of the nucleophilic catalyst[12] DMAP, is in agreement with the mechanism proposed by Dahl et al.[7] in which nucleophilic attack by tetrazole on a protonated phosphoramidite is required. Although the amount of improvement obtained with DMAP is modest for each cycle, the overall effect after many cycles results in significantly higher overall yields.

The above experiments have indicated that optimal results are obtained when 5-(p-nitrophenyl)tetrazole/DMAP solutions are used as the phosphoramidite activator. Average yields as high as 96.4% may be obtained with only an eight to nine-fold excess of phosphoramidite reagent. An increase in reagent excess to twenty to thirty-fold, as is commonly used in the synthesis of oligodeoxyribonucleotides[11], should improve results even further. The results of this study are currently being used to develop solid phase methods for the synthesis of milligram quantities of oligoribonucleotides. The enhanced yields possible with 5-(p-nitrophenyl)tetrazole/DMAP solutions will also be of value in the synthesis of very long oligodeoxyribonucleotide sequences.

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