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# EFFICIENT SYNTHESIS OF ANTISENSE OLIGODEOXYRIBONUCLEOTIDE PHOSPHOROTHIOATES[1]

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**Abstract:** Efficient and economical synthesis of antisense oligodeoxyribonucleotide phosphorothioates utilizing 2 equivalents of phosphoramidite synthon is reported.

Modulation of gene expression by antisense oligonucleotides requires the development of modified oligonucleotides which have increased nuclease resistance, bind to complimentary nucleic acid targets effectively, and also are taken up by cells.[2-10] Among the modifications reported to date, uniformly modified oligodeoxyribonucleotide phosphorothioates have been the first class of compounds to reach the clinic. Both animal data[11] and clinical findings[12] demonstrate the therapeutic potential of oligonucleotide drugs. It is thus of prime importance to develop low-cost, scaleable oligodeoxyribonucleotide phosphorothioate manufacturing technologies. Typically in a solid supported synthesis of oligonucleotides utilizing the phosphoramidite approach, several equivalents of monomeric synthon have been used, presumably to drive the reaction to completion. Given that large scale synthesis of oligonucleotides is needed (100 to 500 gm) for the clinical evaluation of these potential therapeutic agents, the waste of expensive monomeric synthon could be a significant development cost. Consequently, the development of a synthesis using fewer equivalents of synthon would be desirable. We report herein our results on efficient and economical large scale synthesis of oligodeoxyribonucleotide phosphorothioates at reduced amidite excess.

Oligodeoxyribonucleotide phosphorothioate S-d(GCG-TTT-GCT-CTT-CTT-CTT-GCG) (1) and S-d(GCC-CAA-GCT-GGC-ATC-CGT-CA) (2) were chosen as examples. The former sequence, targeted to therapy of CMV retinitis is in Phase I/II clinical trials and the latter for suppression of ICAM expression, is in preclinical trials. The syntheses of both sequences were performed using 11 gm batches of controlled-pore glass (CPG; 480 μmole/synthesis) on a Milligen 8800 automated synthesizer using a modified cycle to include sulfurization using

Beaucage reagent.[13,14] Standard commercially available phosphoramidite synthons were used for syntheses. Table 1 shows conditions used for the synthesis of sequences (1) and (2). Table 2 shows the amount of each synthon used and the results of coupling for both sequences. As shown, synthesis using only two equivalents of each amidite has produced excellent average coupling efficiency (ACE) of 99.1 and 99.3 for sequences (1) and (2) respectively. It was observed that better results in terms of full length content were obtained (based on PAGE densitometry analysis of crude product) using double coupling of amidite synthon instead of single coupling with the same two total equivalents of monomer. Also, better results were obtained when detritylation was performed using flow through technique instead of sparging the reactor.

**Table 1. Conditions for synthesis of sequences (1) and (2).**

| Reagent | Condition Utilized |
|---|---|
| Std. dA phosphoramidite | 0.1 M solution in $CH_3CN$ |
| Std. dC phosphoramidite | 0.1 M solution in $CH_3CN$ |
| Std. dG phosphoramidite | 0.1 M solution in $CH_3CN$ |
| Std. T phosphoramidite | 0.1 M solution in $CH_3CN$ |
| Tetrazole | 0.45 M solution in $CH_3CN$ / 20 fold excess |
| 3H-1,2-Benzodithiol-3-one 1,1-dioxide | 0.05 M solution in $CH_3CN$ / 6 fold excess |
| Deblock Solution | 2.5% $CCl_2CO_2H$ |
| Cap A | $Ac_2O$ / THF (1:9 v/v) |
| Cap B | Py / N-methylimidazole / THF (1:1:8 v/v) |

**Table 2. Equivalents of amidite synthon used and the yields of oligos.**

| Sequence | Total Equivalents of Synthon Used | | | | Average Coupling Efficiency* |
|---|---|---|---|---|---|
| | dA | dG | dC | T | |
| (1) | 2 | 2 | 2 | 2 | 99.1 % |
| (2) | 2 | 2 | 2 | 2 | 99.3 % |

*Based on usual spectrophotometric quantitation utilizing double coupling of amidite synthon and detritylation being perfomed using flow through technique.

Polyacrylamide gel electrophoreses analyses of the crude synthesized oligomers are shown in Fig 1.