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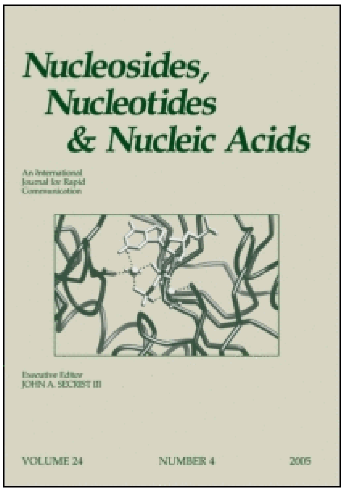

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Oligodeoxyribonucleotide Phosphorothioates: Substantial Reduction of (N-1)-Mer Content Through the Use of Blockmer Phosphoramidite Synthons

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OLIGODEOXYRIBONUCLEOTIDE PHOSPHOROTHIOATES: SUBSTANTIAL REDUCTION OF (N-1)-MER CONTENT THROUGH THE USE OF BLOCKMER PHOSPHORAMIDITE SYNTHONS

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Sequence-specific modulation of gene expression for the treatment of diseases has come to reality. Multiple examples of oligodeoxyribonucleotide phosphorothioates, in which one nonbridging oxygen atom of the internucleotide phosphate group of DNA is replaced by a sulfur atom are currently in advanced clinical trials. Recent advances in phosphoramidite coupling chemistry and solid phase synthesis methodology, together with current state of the art large-scale synthesizers, allow complete assembly of a 20-mer deoxyribonucleotide phosphorothioate at 150 mmole scale in just 8 h. Very high average coupling efficiencies (>98.5%) have been achieved at these scales with only 1.75-fold molar amidite excess.

Coupling of an activated phosphoramidite monomer to the 5'-hydroxyl of the growing chain on a solid support followed by stepwise sulfurization of the trialkylphosphite linkage is the current preferred method for the synthesis of first generation drugs. Problems commonly encountered in this method of automated synthesis include formation of deletion sequences (internal and terminal deletionmers) which have one or more nucleotides absent. Less than quantitative coupling efficiency, incomplete capping or removal of 5'-dimethoxytrityl group, reagent quality as well as work-up protocols could contribute to the formation of these process related impurities. In addition low levels of phosphodiester linkages are also present in the phosphorothioate (PS) oligonucleotide product. These could occur due to side reactions during sulfurization step or by other mechanisms. Reversed phase HPLC purification of crude oligomer takes care of DMT off capped failure sequences. However, the resolution of DMT-on full length from DMT-on failures is rather poor. In addition, PO-contining oligomers are incapable of being separated from the full length PS oligomer. Thus, there is a need to identify an alternate approach to synthesize high-quality antisense drugs.

Assuming that coupling and sulfurization inefficiencies are the main causes, a key to reduce these process-related oligonucleotides could be the use of a blockmer coupling strategy. Recently, we have demonstrated that greater than 70% reduction in (n-1)-mer content and ca. 50% reduction of phosphodiester linkages could be achieved by using dimers in some model sequences[1]. This prompted us to investigate the effect of using trimeric building blocks on the quality of synthesized oligonucleotides. Protected trinucleotides have been reported in literature for use in the controlled codon-by-codon construction of combinatorial libraries of structural genes.[2-5] However no one has used these blockmers to address the issue of improving the quality of synthesized oligonucleotides for antisense applications. Encouraged by our positive results on the use of dimers in model compounds, we decided to investigate the use of trimers in the synthesis of a 20-mer oligodeoxyribonucleotide phosphorothioate (ISIS 2503; 5'-TCC-G-TCA-TC-GC-TCC-TCA-GG-G). The required dimer and trimer synthons were synthesized as reported earlier but with a slight modification.

Oligomerization: Synthesis of the 20-mer oligodeoxyribonucleotide phosphorothioate 5'-TCC-G-TCA-TC-GC-TCC-TCA-GG-G (ISIS 2503) using monomer amidites and blockmer synthons was performed on 1 μmole scale using an ABI 394 DNA/RNA synthesizer. The CPG-bound DMT-on oligomer was treated with 30% ammonium hydroxide for 1 h at room temperature, followed by incubating the solution at 60°C for 18 h. The incubated solution was cooled, concentrated and purified by reversed phase HPLC on a C18 column using standard gradient conditions. The oligonucleotides were analysed by capillary gel electrophoresis. Based on CGE, the amount of (n-1)-mer in monomer based synthesis and trimer based synthesis are 4.2% and 0.6% respectively and represents a substantial reduction in the (n-1)-mer content of the synthesized oligonucleotide. This result clearly demonstrates that most of the (n-1)-mer population is indeed formed during the chain elongation reactions and that only a small portion of it is due to other factors. In summary, we have shown experimentally that use of trimer and dimer building blocks in the solid phase synthesis of oligodeoxyribonucleotide phosphorothioates leads to substantial reduction of (n-1)-mer content leading to high quality of purified active pharmaceutical ingredients.

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