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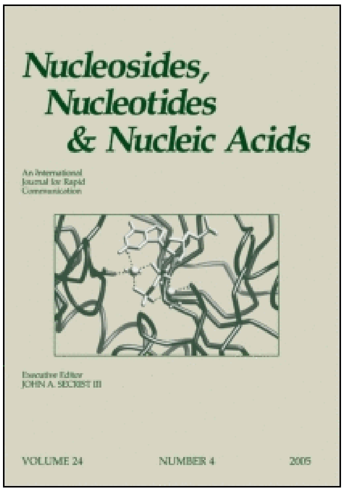

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A New, Efficient Exocyclic Amine Protection Scheme for 2-Aminoadenosine and Derivatives for Incorporation into Oligonucleotides

Bruce S. Ross[a]; Robert H. Springer[a]; Ramesh Bharadwaj[a]; Alice M. Symons[a]; Muthiah Manoharan[a]
[a] Isis Pharmaceuticals, Carlsbad, CA

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A NEW, EFFICIENT EXOCYCLIC AMINE PROTECTION SCHEME FOR 2-AMINOADENOSINE AND DERIVATIVES FOR INCORPORATION INTO OLIGONUCLEOTIDES

Bruce S. Ross*, Robert H. Springer, Ramesh Bharadwaj,
Alice M. Symons and Muthiah Manoharan
Isis Pharmaceuticals, 2292 Faraday Avenue, Carlsbad, CA 92008

Abstract: We introduce a novel approach to incorporate 2-aminoadenosine into oligonucleotides in which 2-fluoroadenosine and its derivatives are used to prepare the monomers and, under normal oligonucleotide deprotection conditions, is converted to the desired 2-aminoadenosine and derivatives.

Several groups have reported that substituting 2-aminoadenosine in place of adenosine in oligonucleotides increases their binding affinity to RNA and DNA without loss of specificity. This presumably is due to the extra hydrogen bond possible from the N-2 to the 0-2 of uridine/thymidine[1] The reported magnitude of this effect varies in the literature, but our own experiments suggests a value of about 1 °C per modification. Despite this incentive, we have not used 2-aminoadenosine analogs routinely for antisense applications because we have found the monomers difficult to prepare. In particular, the protecting groups on the exocyclic amines have been problematic due to their very different electronic properties. In general, the N-2 protecting groups deprotect too slowly under standard conditions, necessitating the use of labile groups or harsher deprotection reagents and conditions; the N-6 protecting groups deprotect too easily during monomer

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and oligo synthesis. Uniform protecting groups such as phenoxyacetyl are not optimal for both positions and combinations of groups add to the complexity of the synthesis. N-6 can be left unprotected, but the yield of the phosphitylation reaction on the monomer is less than desired.[2]

Our solution is to synthesize 2-fluoroadenosine derivatives and after oligomerization, the fluoride can be easily displaced with ammonia under the standard oligonucleotide deprotection conditions. The diazotization/fluorination reaction on the nucleoside as reported by Krolikiewicz and Vorbrüggen[3] is efficient (80% to 90%) and the products and their dimethoxytrityl derivatives tend to be crystalline. It is not necessary to protect the N-6 to afford normal phosphitylation yields.

We have demonstrated the utility of this approach with 2'-deoxy-, 2'-*O*-methyl-, 2'-*O*-(2-methoxyethyl)- and ribo-2-aminoadenosine. Oligomerization and deprotection were done without any modifications of standard conditions. The products were analyzed by CGE, ^{19}F- and ^{1}H-NMR and ES-MS and found to be consistent with the structure. Melting temperature studies against DNA and RNA complement confirmed a value of about 1 °C per modification increase. This enhancement was independent of the number of residues and of the nature of 2'- substitution. Furthermore, the fluoride can also be displaced with other amines and conjugated amines as reported for 2-fluoroinosine.[4,5]

In summary, we now have an efficient way to make 2-aminoadenosine containing oligonucleotides, which will enable routine biological screening in cells and in animals.

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