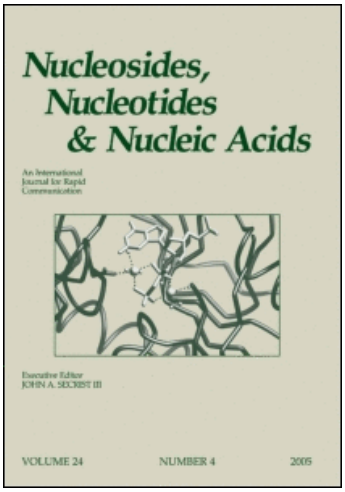

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Fully Protected Nucleoside- Folic Acid Conjugated Phosphoramidites and Their Incorporation into Antisense Oligonuleotides

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To cite this Article Bhat, Balkrishen , Balow, Guity , Guzaev, Andrei , Cook, P. Dan and Manoharan, Muthiah(1999) 'Synthesis of Fully Protected Nucleoside- Folic Acid Conjugated Phosphoramidites and Their Incorporation into Antisense Oligonuleotides', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1471 — 1472
To link to this Article: DOI: 10.1080/07328319908044756
URL: http://dx.doi.org/10.1080/07328319908044756

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1471–1472 (1999)

SYNTHESIS OF FULLY PROTECTED NUCLEOSIDE- FOLIC ACID CONJUGATED PHOSPHORAMIDITES AND THEIR INCORPORATION INTO ANTISENSE OLIGONULEOTIDES

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ABSTRACT : A novel synthesis of the nucleoside-folic acid conjugates has been accomplished. This approach allowed us to synthesize several analogs, which were converted to phosphoramidites and successfully incorporated into therapeutically active antisense oligonucleotides.

Modified oligonucleotides are currently used in new therapeutic approaches such as antisense [1], ribozymes, antigene and gene therapy. One important issue in utilizing these highly target specific macromolecules as drugs is their ability to penetrate cell walls. A second issue is improving the extent of their oral absorption for the ease of administration. One way of overcoming these problems is through receptor mediated endocytosis, in which the drug molecule, once linked to a ligand, is internalized by receptor bearing cells. In this context, folic acid has been demonstrated as a ligand to internalize liposomes, small and larger proteins, toxins and other biologically significant molecules exploiting the folic acid receptor present on certain cancerous and normal cell surfaces [2].

Several groups have recently tried to conjugate folic to oligonucleotides[2-4], but the methods are difficult to scale up and lack regioselectivity. Our approach allows both regiospecificity and the potential to scale up the chemistry needed for *in vivo* studies.

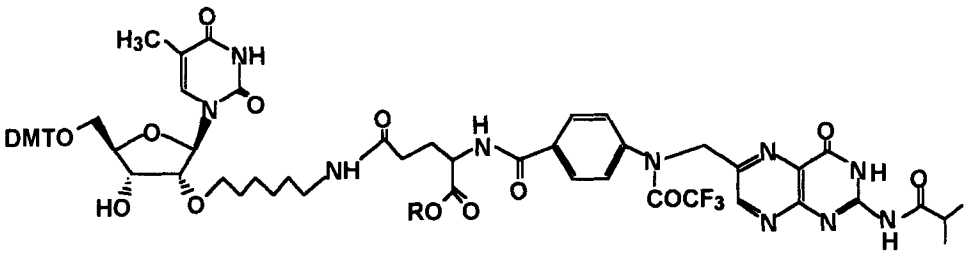

Fig. 1

We wish to report a novel synthesis of folic acid conjugated nucleoside building blocks in which one can control the regiochemistry of conjugation. The synthesis was achieved by the reaction of fully protected pteroic acid with appropriately substituted nucleoside-glutamic acid building blocks, to obtain after chromatographic purification the desired fully protected nucleoside-folic acid conjugate, which was highly soluble in most organic solvents. The structures of all intermediates and the final compound were confirmed by ^{1}H NMR ^{13}C NMR and mass spectroscopy.

The nucleoside folic acid analogs were incorporated into oligonucleotides using the normal phosphoramidite chemistry. These monomer building blocks coupled well and after the proper deprotection and the purification, folic acid oligonucleotide conjugates were obtained in good yields. By using this synthetic approach, we were able to synthesize both α as well as γ- folic acid conjugated oligonuleotides. To our knowledge, this is the first report of the use of phosphoramidite chemistry to incorporate regioselective folic acid nucleoside monomers into oligonuleotides.

Acknowledgments

The authors wish to thank Dr. Bruce Ross for providing several starting materials, Mr. Patrick Wheeler for NMR analysis, Drs. Lendell Cummins and Hans Gaus for mass spectral analysis.

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