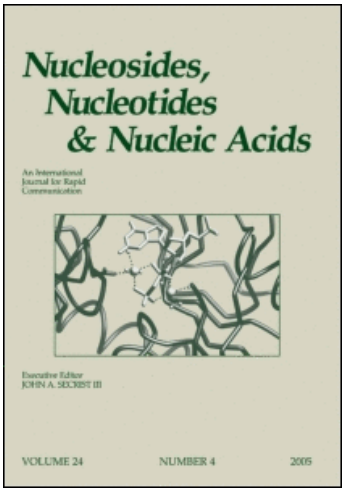

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2'- and 3'- Cholesterol-Conjugated Adenosine and Cytosine Nucleoside Building Blocks: Synthesis of Lipidic Nucleic Acids

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2'- AND 3'- CHOLESTEROL-CONJUGATED ADENOSINE AND CYTOSINE NUCLEOSIDE BUILDING BLOCKS: SYNTHESIS OF LIPIDIC NUCLEIC ACIDS

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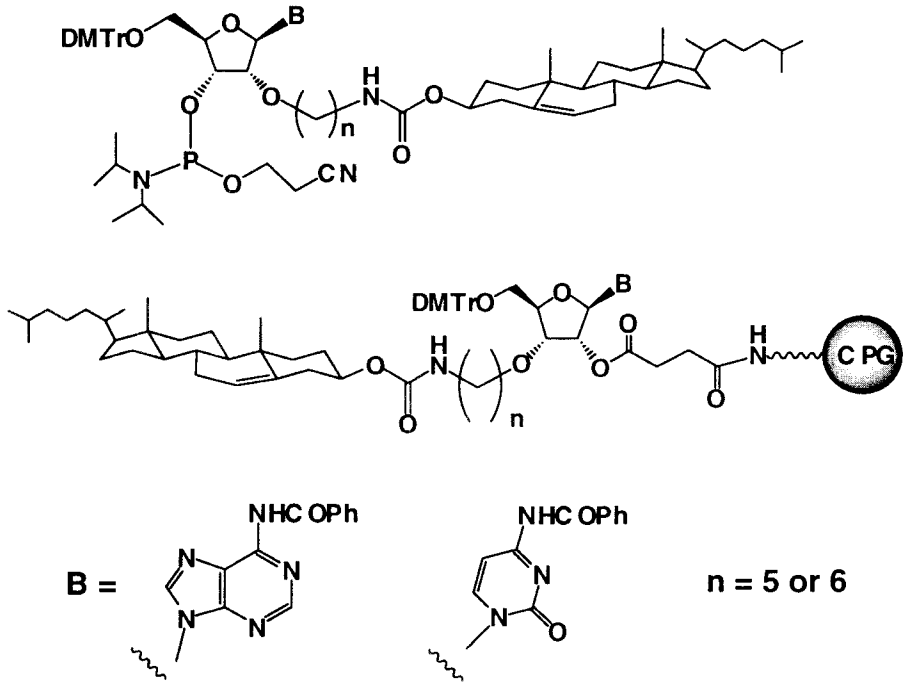
Recently our laboratory introduced[1] chemistries to synthesize 2'- and 3'- cholesterol-uridine conjugates which were incorporated into several antisense oligonucleotides. We have now extended this chemistry to other nucleosides (adenosine and cytosine) and synthesized antisense oligonucleotide conjugates for several disease targets. Synthesis of these cholesterol nucleosides was carried out by condensing cholesterol chloroformate with 2'-*O*-alkylamine or 3'-*O*-alkylamine of the appropriate nucleoside. The 2'-*O*-alkylamines were derived from direct alkylation procedure.

Unprotected adenosine or cytosine was alkylated with *N*-(5-bromopentyl)phthalimide in DMF and sodium hydride to yield a 2'-*O*- and 3'-*O*- alkylation mixture. The 2'-isomer predominates in this mixture. The mixture was then *N*-6- or *N*-4 benzoyl protected using Jones' transient protection and then the 5'-sugar hydroxyl was protected by treatment with dimethoxytrityl chloride/pyridine . The resultant mixture was conveniently separated into the 2'-*O*- and 3'-*O*-isomers on a silica gel column. The major 2'-*O*- isomer was treated with hydrazine in refluxing methanol to yield the free amine which was then condensed with cholesteryl chloroformate to yield the cholesterol derivatized nucleoside. Similarly the 3'-phthalimido isomer yielded 3'-*O*-alkylamine

which was further derivatized to the cholesterol-nucleoside conjugate. The 2'-isomer was phosphitylated to yield the phosphoramidite. The 3'-modified nucloside was succinylated and condensed with long-chain alkylamino CPG.



The 3'-cholesterol C containing CPG was incorporated into Ha-*ras* Oligonucleotide 2570 : named as ISIS 13748. This compound is being evaluated to determine the effect of cholesterol conjugation on RNase H activity. A short oligo (ISIS 13747: GTACA*) was synthesized from 3'-cholesterol A containing CPG to make sure the CPG coupled well during the oligonucleotide synthesis and confirmed by ES/MS and NMR analysis. The 3'-cholesterol A containing CPG was incorporated into mouse C-*raf* kinase antisense oligonucleotide (ISIS-11061) derivatives:

ISIS-13746: (11061-Cholesterol): ATG CAT TCT GCC CCC AAG GA*

ISIS-13245: ATG CAG TCT TCC ACC ACG GA* (4 mismatch control for 13746; the underlined sites are the mismatch positions compared to 13746)

Large scale synthesis and purification of these two oligonucleotides have been completed in 100mg scale. The in vitro and in vivo analysis of these oligonucleotides are in progress.

The entire procedure was repeated for *N*-(6-bromopentyl)phthalimide with both ribonucleosides. All these nucleoside compounds were charecterized by appropriate physical methods (^{13}C and ^{1}H NMR).

Syntheses of guanosine-cholesterol nucleoside derivatives are in progress.

References

[1]Manoharan, M.; Tivel, K. L.; Cook, P. D. *Tetrahedron Lett.* **1995,** *36*, 3651-3654.

2 Manoharan, M.; Guinosso, C. J.; Cook, P. D. *Tetrahedron Lett.* **1991**, *32*, 7171-7175.

3 Broom, A.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 1145-1146.