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Muthiah Manoharan^a; Gopal Inamati^a; Kathleen L. Tivel^a; Patrick Wheeler^a; Kim Stecker^a; P. Dan Cook^a

^a Isis Pharmaceuticals, Carlsbad, CA, USA

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**2'- AND 3'- BIOTIN CONJUGATED NUCLEOSIDE BUILDING BLOCKS:
SYNTHESIS OF BIOTINYLATED OLIGONUCLEOTIDES**

**Muthiah Manoharan, Gopal Inamati, Kathleen L. Tivel,
Patrick Wheeler, Kim Stecker and P. Dan Cook**

Isis Pharmaceuticals, 2292 Faraday Ave., Carlsbad, CA 92008 USA

The vitamin biotin plays a significant role in biological assays based on its unusually high affinity [$K_D=10^{-15}M$] to streptavidin and avidin. This assay can be used for monitoring cellular trafficking of antisense oligonucleotides using biotin conjugation. In addition to the above diagnostic application, biotin conjugation to macromolecules could be used as a vitamin-mediated delivery system for macromolecules into cells. Complexation of avidin to biotin-oligonucleotides (phosphodiester or PNA) have been used to enhance the uptake of oligonucleotides¹. Appropriate placement of biotin in oligonucleotides could also provide increased nuclease resistance.

By conjugating 2'-*O*-(6-aminohexyl)-5'-*O*-dimethoxytrityl-5-methyluridine and 3'-*O*-(6-aminohexyl)-5'-*O*-dimethoxytrityl-5-methyluridine, which were synthesized similar to the corresponding uridine analogs² to *O*-nitrophenyl ester of N¹-(4-*t*-butyl benzoyl)-D-(+)-biotin, we synthesized two biotin conjugate nucleoside building blocks. The 2'-biotin conjugate was further converted to a phosphoramidite (I) and the 3'- conjugate was subsequently converted to the CPG derivative (II).

Using these two building blocks several oligonucleotides having one or more biotin molecules were synthesized either with a uniform phosphodiester or phosphorothioate backbone. The oligonucleotides fell into three categories: (a) short oligonucleotides for NMR structural

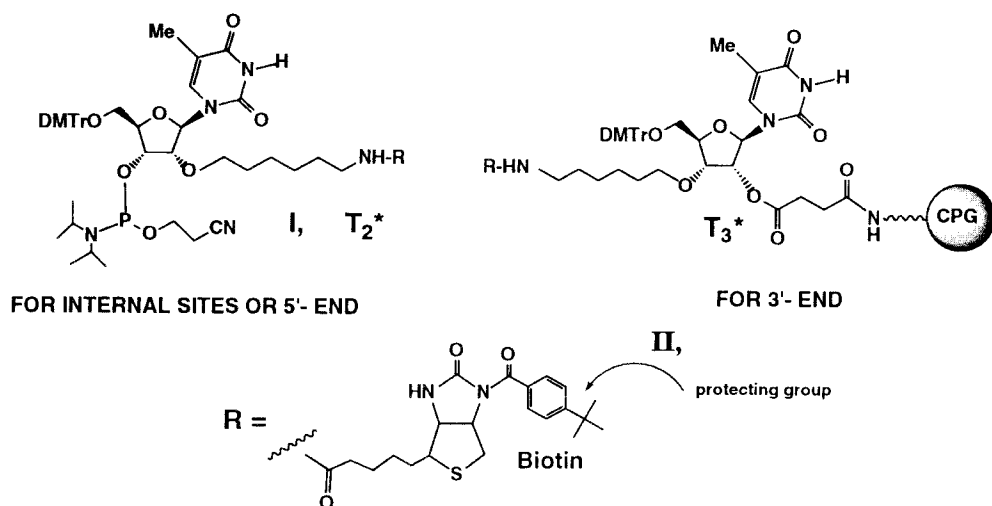


Figure 1. Nucleoside Synthons to synthesize Biotin Oligonucleotides

Murine ICAM-1 Oligonucleotides for *in vivo* nuclease and PK/pharmacological studies

ISIS 13242: T₂'GC ATC CCC CAG GCC ACC AT (P=O) 5'-BIOTIN

ISIS 13243: T₂'GC ATC CCC CAG GCC ACC AT (P=S) 5'-BIOTIN

ISIS 13245: TGC ATC CCC CAG GCC ACC AT₃' (P=O) 3'-BIOTIN

ISIS 13244: TGC ATC CCC CAG GCC ACC AT₃' (P=S) 3'-BIOTIN

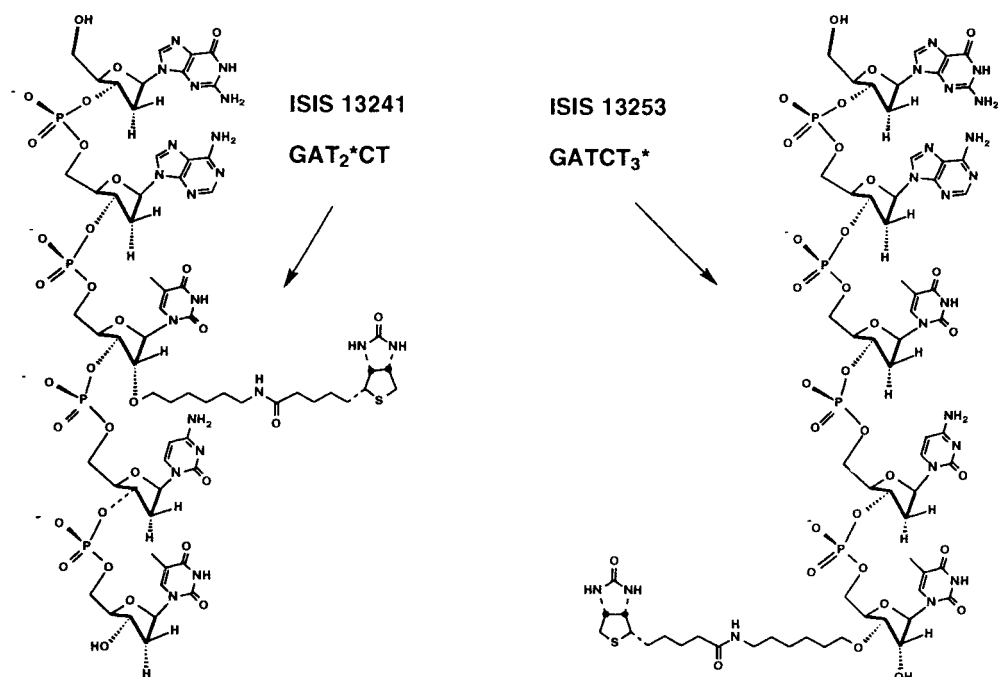
ISIS 13247: T₂'GC ATC CCC CAG GCC ACC AT₃' (P=O) Both ends

ISIS 13246: T₂'GC ATC CCC CAG GCC ACC AT₃' (P=S) Both ends

Figure 2. ICAM-1 Oligonucleotides containing Biotin Residues

studies; (b) ICAM-1 antisense oligonucleotides to evaluate antisense properties; and (c) conjugates to follow the uptake of antisense molecules.

Using the above synthons ICAM-1 oligonucleotides were synthesized and they are being used for nuclease and pharmacokinetic(PK) studies (Figure 2). The compounds are also being evaluated for inhibition of ICAM-1 expression.



	13253	13241
T (°C)	J (1'-2')	
52	4.94	7.14
45	4.95	7.14
37	4.67	7.00
28	4.67	7.00
~%S	48	70

Proton NMR spectra of the compounds 13253 and 13241 indicate that the 3'-substituted compound maintains a much larger proportion of N-type sugar conformation than does the 2'-substituted compound. Assignments were taken from 2-D TOCSY spectra.

The position of substitutions was determined from NOESY spectra. The conformational analysis was carried out from J (1'-2') coupling constants.

References

- ¹ Pardridge, W. M.; Boado, R. J.; Kang, Y.-S. *Proc. Natl. Acad. Sci.* **1995**, *92*, 5592-5596.
- ² Manoharan, M.; Tivel, K.L.; Andrade, L. K.; Cook, P. D. *Tetrahedron Lett.* **1995**, *36*, 3647-3650.