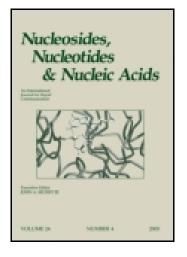
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Stabilization of RNA Bulges by Oligonucleotides Containing 2'-Naphthylmethyl-2'-deoxytubercidine

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Stabilization of RNA Bulges by Oligonucleotides Containing 2'-Naphthylmethyl-2'-deoxytubercidine

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ABSTRACT

A novel nucleoside analogue, 2'-naphthylmethyl-2'-deoxytubercidine, is synthesized and incorporated in oligonucleotides that stabilize bulges in partially complementary RNA.

Key Words: Oligonucleotides; RNA recognition; Bulges; Nucleic acids.

In our research towards development of oligonucleotide based artificial nucleases we are targeting RNA bulges. However, since bulge-containing structures are inherently less stable than fully complementary duplexes,^[1] introduction of potential bulge stabilizing modifications is an important issue. To our knowledge no oligonucleotide modifications have been designed for stabilization of RNA bulges.

In a first approach we concentrated on the direct introduction of an aromatic substituent on the 2'-position using a carbon-carbon bond forming strategy. Molecular modelling suggested that incorporation of a 1-naphtylmethylgroup in the 2'-position could reduce the destabilizing effect of the occurrence of a bulge and

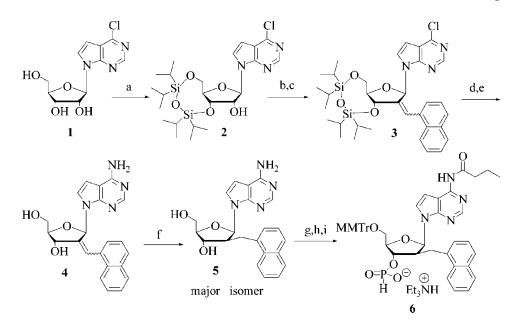
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Scheme 1. Synthesis of the 2'-(1-naphthyl)methyl substituted H-phosphonate building block: a) TIPDSCl₂, pyridine, 95%; b) CrO₃, Ac₂O, pyridine, CH₂Cl₂, 86%; c) naphthylmethyltriphenylphosphonium chloride, nBuLi (1.75 eq), THF, rt, 73%; d) NH₃(l), dioxane, 4 days, 80°C; e) Bu₄N⁺F⁻, THF, 80% over 2 steps; f) H₂, Pd/C, CH₃OH, 5 h, 82%; g) i. (CH₃)₃SiCl, pyridine, ii. butyric anhydride, iii. H₂O, 60%; h) MMT⁺BF₄⁻, Li₂CO₃, 2,6-lutidine, 0°C to rt, 93%; i) i. Imidazole, PCl₃, Et₃N, CH₂Cl₂, -20° C to -78° C, 80% ii. H₂O.

increase specificity for bulged out RNA-target sequences. The synthetic route is outlined in Scheme 1. 6-Cl-Tubercidine 1 (2) was protected with the Markiewicz reagent^[3], oxidized and reacted with the phosphorylide derived from naphtylmethylchloride. This gave an isomeric E/Z mixture in good yield. It was then necessary to convert the 6-Cl-substituent to 6-NH₂. The conditions developed by Seela and coworkers,^[2] involving heating with methanolic ammonia to 50°C during 24 h, led in this case to the predominant formation of MeO-substituted base. However, treatment of **3**, dissolved in dioxane, with liquid ammonia followed by heating to 80°C in a pressure vessel for 4 days led to the desired compound that was subsequently desilylated using TBAF in THF yielding **4** as an isomeric E/Z mixture, which upon hydrogenation gave **5**. Base protection followed by reaction with monomethoxytrityl tetrafluoroborate/LiCO₃/2,6-lutidine^[4] and phosphonylation^[5a] gave **6** which was used in synthesis of both oligodeoxyribo- and oligoribonucleotides by the H-phosphonate approach.^[5b]

UV-melting studies were then performed of complexes between oligos containing **5**, and RNA or DNA fragments that upon binding formed 1-3 nt bulged out regions.^[6]

Incorporation of 2'-deoxy-2'- β -(1-naphthyl) methyltubercidine in either an oligodeoxyribonucleotide or all-2'-O-methyloligoribonucleotide has little effect on the T_m values for complexes with DNA-complements that form small bulges. However, when the target sequence is an RNA-fragment that in the complex forms

Stabilization of RNA Bulges

a small bulge, the naphthylmethyltubercidine modified oligonucleotides give stabilisations of up to 4°C.^[6] This kind of stabilisation is only found with the RNA complements and not with the DNA targets. Thus, the naphthylmethyl tubercidine containing oligonucleotides specifically stabilize bulged RNA.

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