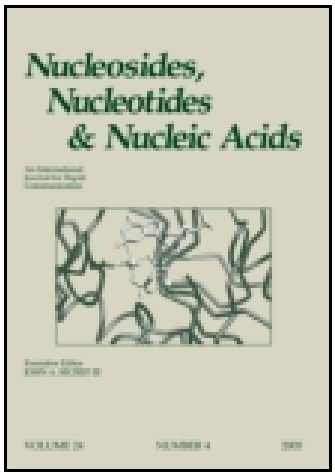

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Stabilization of RNA Bulges by Oligonucleotides Containing 2′-Naphthylmethyl-2′-deoxytubercidine

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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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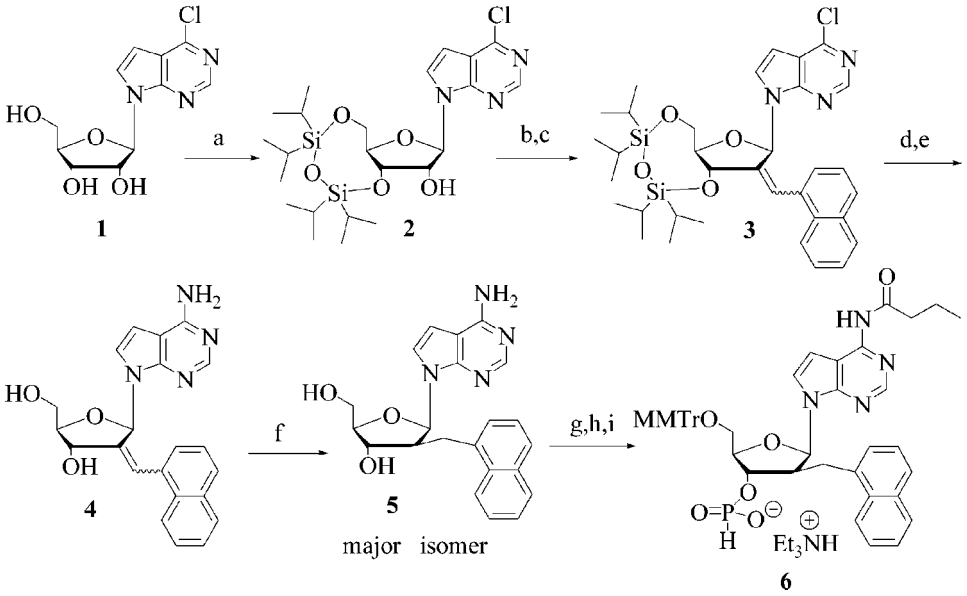

Scheme 1. Synthesis of the 2′-(1-naphthyl)methyl substituted H-phosphonate building block: a) $TIPDSCl_2$, pyridine, 95%; b) CrO_3, Ac_2O, pyridine, CH_2Cl_2, 86%; c) naphthylmethyltriphenylphosphonium chloride, nBuLi (1.75 eq), THF, rt, 73%; d) NH_3(l), dioxane, 4 days, 80°C; e) $Bu_4N^+F^-$, THF, 80% over 2 steps; f) H_2, Pd/C, CH_3OH, 5 h, 82%; g) i. $(CH_3)_3SiCl$, pyridine, ii. butyric anhydride, iii. H_2O, 60%; h) $MMT^+BF_4^-$, Li_2CO_3, 2,6-lutidine, 0°C to rt, 93%; i) i. Imidazole, PCl_3, Et_3N, CH_2Cl_2, −20°C to −78°C, 80% ii. H_2O.

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-NH_2. 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/$LiCO_3$/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

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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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