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Modified Oligonucleotides with a 5'-5' Interbase Semi-rigid Junction for Alternate Strand Triplex Formation

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Abstract: *Solid-phase synthesis of 5'-5'-linked oligonucleotides with opposite polarities tethered via nucleic bases has been performed using a modified dinucleoside bearing an H-phosphonate group at the 3'-position of one nucleoside and a dimethoxytrityl group at the 3'-position of the second nucleoside. This system is aimed at forming a base tetrad at the junction in order to provide better stabilization. The linker used between the two 5'-terminal bases in the same plane involves a triple bond in order to rigidify the junction. The two oligonucleotide chains are either made of natural nucleosides or one of them is built with N3'→P5' phosphoramidates.* © 1998 Published by Elsevier Science Ltd. All rights reserved.

Ever since its initial discovery, the triple-helix structure[1], because of possible applications in biotechnology, diagnostics, and therapeutics, has been the area of intensive studies[2-4]. Applications of the oligonucleotide directed triple-helix formation are however limited because oligopurine tracts on the DNA target are required. Work carried out in several laboratories has shown that the repertoire of the triplex forming sequence can potentially be expanded to adjacent blocks of purines and pyrimidines by allowing the third strand to hybridize with purines on alternate strands by crossing the major groove. This has been achieved by using either oligonucleotides with constant backbone polarity employing a combination of purine and pyrimidine sequences[5, 6] or two oligopyrimidine third strands linked by their 3'- or 5'-ends in order to fulfill the strand orientation requirements[7-13]. The design of alternate third strand oligonucleotides requires linkers crossing the major groove adapted to each type of junction. Several linkers such as 1,3-propane diol or its oligomers[7], 1,2-dideoxy-D-ribose[8], have been used to tether the terminal phosphates of both pyrimidine third strands while xylose residues[9] have been used to link their terminal 2'-deoxyribose units. Alternate third strand oligonucleotides involving only a bridging phosphate[10] between the two 3'-ends of oligopyrimidine strands has also been reported. We have previously reported 3'-3' and 5'-5' alternate third strands involving base-to-base linkages, via a flexible linker[11-13]. Among the different systems, pronounced increases in stability have been reported only with 3'-3' junctions[8-10, 13]. Junctions remain to be optimized to increase the triplex stability together with the participation of the bases close to the junction in the selectivity of hybridization. We now describe the preparation of a new model of the 5'-5' linked oligonucleotide in which an additional base is present at the 5'-end of one pyrimidine strand in order to form a base tetrad at the junction with the aim of providing better stabilization (Figure 1). The linker used between the two 5'-terminal bases in the same plane involves a triple-bond in order to rigidify the junction and the two oligonucleotide chains are either made of natural nucleosides or one of them is built with N3'→P5' phosphoramidates (Figure 2).

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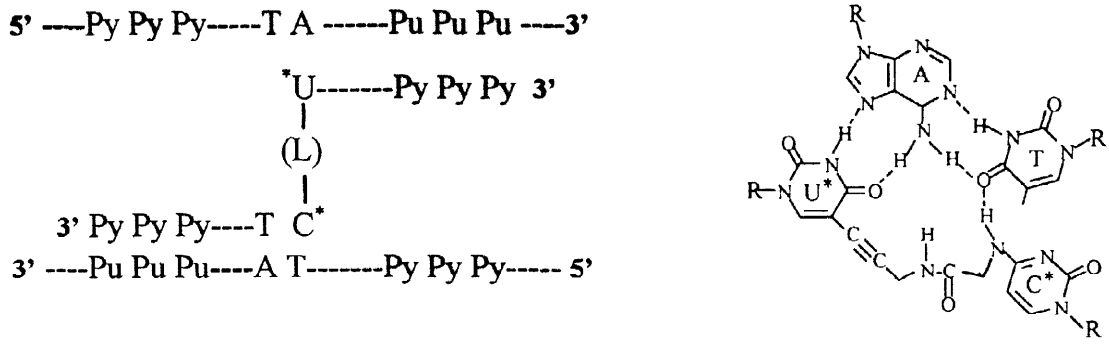

Figure 1: Model for alternate strand triplex formation with a 5'-5'interbase semi-rigid junction

This was achieved using a modified bridged dinucleoside with a dimethoxytrityl group at the 3'-position of one nucleoside and an H-phosphonate group at the 3'-position of the second. This allows the addition of the modified dinucleoside at the 5'-position of the first oligonucleotide assembled with regular 3'-phosphoramidites, bound to the support, while the second oligonucleotide is assembled in the opposite orientation starting from the 3'- position of the second nucleoside and using 5'-phosphoramidites of either natural or 3'-amino-2', 3'-dideoxynucleosides. The key step of the synthesis of 5'-5' interbase linked oligonucleotides described in Figure 2 is the preparation of the H-phosphonate dinucleoside **6**. Since the 5'-hydroxyl groups on both nucleosides are not involved in the oligonucleotide synthesis, in order to avoid the problem of protective group selectivity, we chose to replace them by chlorine atoms which are neither very reactive nor bulky. The new junction reported involves the fixation of the linker via triple-bond formation at the 5-position of 5'-chloro-2',5'-dideoxyuridine and at the amino function of 5'-chloro-2',5'-dideoxycytidine. The synthesis proceeds as follows. First, the 5'-hydroxyl group of 2'-deoxyuridine (Rf= 0.10) is replaced with a chlorine atom to give 5'-chloro-2',5'-dideoxyuridine (Rf= 0.27)[14, 15]. After verification of its stability under the conditions required for oligonucleotide deprotection, the chlorinated compound is tritylated at its 3'-position to give the protected monomer **1** (Rf= 0.76) and then activated with phosphorous oxychloride (instead of phenyldichlorophosphate) in the presence of 3-nitro-1,2,4-triazole (instead of 1,2,4-triazole) to give the nitrotriazolide derivative (Rf= 0.88)[16]. The latter is then reacted with glycine methylester (5 eq) at 50°C in CH_3CN to afford the nucleoside **2** (Rf= 0.56) which is then hydrolyzed to give the carboxylic nucleoside (Rf=0.05). The coupling reaction between the latter and propargylamine in the presence of DCCI affords the nucleoside involving the linker ending with a triple-bond **3** (Rf= 0.50). Compound **3** was also obtained by treatment of compound **2** with propargylamine in CH_3CN for 5 days at 50°C. Compound **3** reacts via a Pd catalyzed reaction[17] with 5-iodo-5'-chloro-2',5'-dideoxyuridine **4** (Rf= 0.38), (obtained by replacement of the 5'-hydroxyl group of 5-iodo-2'-deoxyuridine (Rf= 0.17) with a chlorine atom[15]), to give the dinucleoside **5** (Rf= 0.31)[18]. The H-phosphonate derivative **6** (Rf= 0) was obtained by treatment of **5** with 2-chloro-5,6 benzo 1,3,2-dioxaphosphorin-4-one[19]. The chain assembly was carried out on a Pharmacia Gene Assembler at the μmole scale as follows. The first oligonucleotide was assembled with regular 3'-phosphoramidites in the 3'-5' direction and the modified dinucleoside **6** was coupled manually in the presence of pivaloyl chloride. The H-phosphonate group was then transformed into a 2-cyanoethylphosphotriester group following a procedure adapted from the literature[20]. After acetylation of unreacted hydroxyl groups the synthesis was completed with the assembly of the second oligonucleotide in the opposite direction using 5'-phosphoramidite derivatives of either natural nucleosides with 10 equiv of monomer per cycle and a coupling time of 2.5 min or 3'-amino-2',3'-dideoxynucleosides with 15 equiv. of monomer (obtained following a procedure adapted from the literature[21]) per cycle and a coupling time of 5

min. The obtained coupling yields with 5'-phosphoramidites of natural nucleosides are similar to those obtained with 3'-phosphoramidites, while those obtained with 5'-phosphoramidites of 3'-amino- 2', 3'-dideoxynucleosides are lower in accordance with reported data[21].

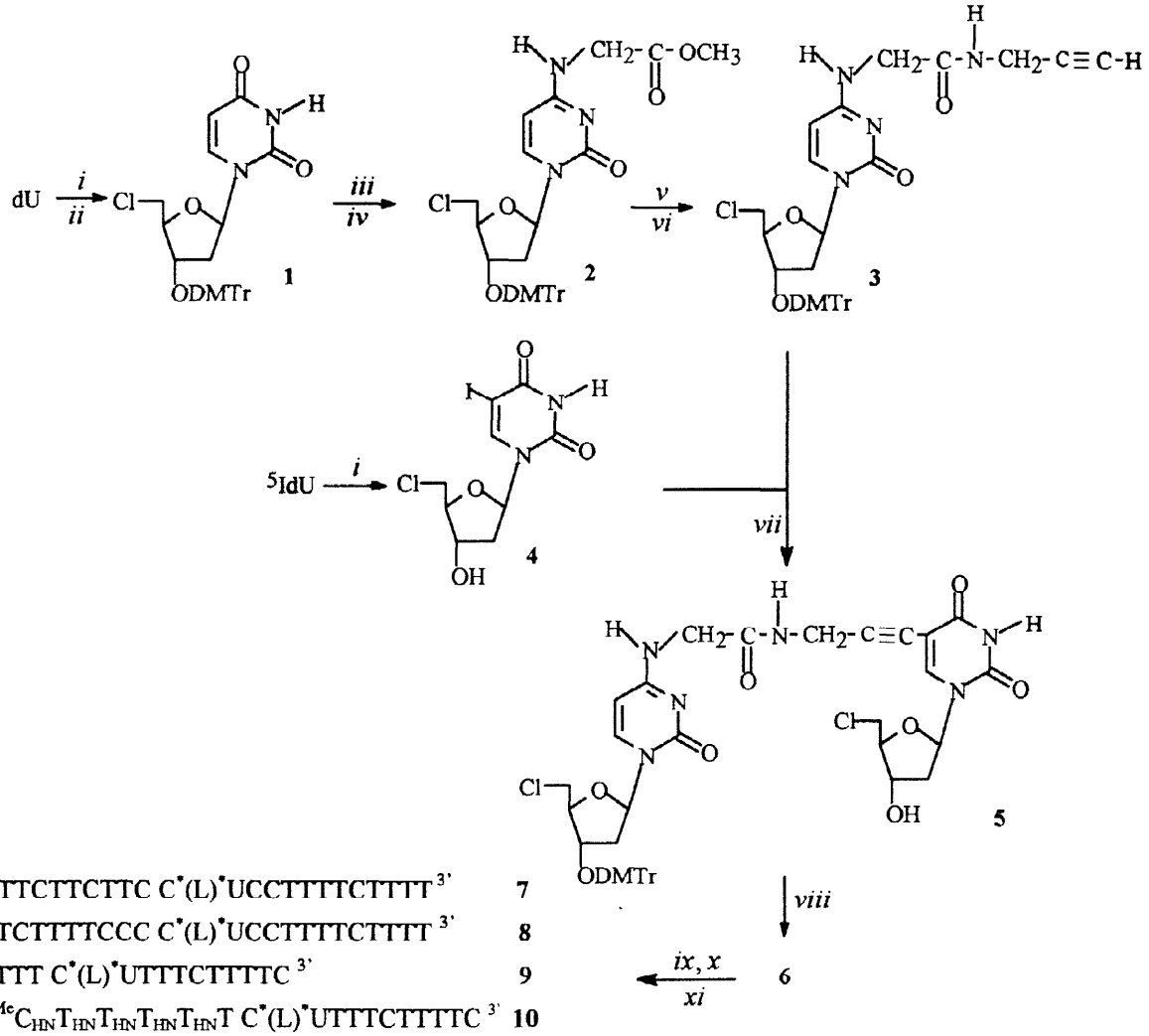

Figure 2: dU = 2'-deoxyuridine; DMTr = dimethoxytrityl; L = -$CH_2C(O)$-N(H)-CH_2-C≡C-; (i) triphenyl-phosphine, CCl_4, DMF; (ii) DMTrCl, pyridine; (iii) phosphorous oxychloride, 3-nitro-1,2,4-triazole, TEA, CH_3CN; (iv) H_2N-$CH_2C(O)$-OCH_3/HCl, DBU, CH_3CN; (v) TEA, MeOH, H_2O; (vi) H_2N-CH_2-C≡C-H, DCCI, pyridine; (vii) tetrakis-(triphenylphosphine)palladium (0), CuI, TEA, DMF; (viii) 2-chloro-5,6 benzo-1,3,2-dioxaphosphorin-4-one, CH_2Cl_2, pyridine, aq. triethyl-ammonium bicarbonate buffer pH 8.5; (ix) coupling of the H-phosphonate derivative of the dimer with the 5'-hydroxyl group of the first oligonucleotide bound to the support and transformation of H-phosphonate into 2-cyanoethylphosphotriester group; (x) assembly of the second oligonucleotide with 5'-phosphoramidites; (xi) H^+, concentrated ammonia.

After removal of the dimethoxytrityl group and deprotection by concentrated ammonia, modified oligonucleotides **7-10** were purified on a DEAE ion exchange column (100 mm x 10 mm) from Millipore, using a linear gradient of NaCl in tris/HCl 0.025M, pH 8, buffer containing 10% CH_3CN. After desalting, the purity of compounds **7-10** was confirmed by reversed-phase analysis on a Nucleosil 100-5 C18 column (125 x 4 mm.) from Macherey-Nagel using a linear gradient of CH_3CN (1% per min) in 0.1M aqueous triethylammonium acetate buffer, pH 7, with a flow rate of 1ml/min (Rt_7 = 25 min 53 sec, Rt_8 = 27 min 28

sec, Rt_9 = 25 min 22 sec, Rt_{10} = 27 min 14 sec). Nucleic base composition of oligonucleotides **7-9** after nuclease degradation by snake venom phosphodiesterase and alkaline phosphatase was ascertained by reversed-phase analysis[22] using a Waters 600E System Controller equipped with a Waters 990 Photodiode Array Detector. In the case of modified oligonucleotide **10** the presence of the 3'-amino-2',3'-dideoxy-nucleosides was verified[22] after additional acidic [23] and phosphatase alcaline treatments.

Studies on the hybridization properties of these modified oligonucleotides are currently in progress in collaboration with an another group and will be published elsewhere.

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