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5'-5' Tethered Oligonucleotides via Nucleic Bases: A Potential New Set of compounds for Alternate Strand Triple-Helix Formation

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Abstract: *The solid-phase synthesis of 5'-5'-linked oligonucleotides tethered via nucleic bases and with opposite polarities has been performed using a modified dinucleoside bearing a phosphoramidite group at the 3'-position of one nucleoside and a dimethoxytrityl group at the 3'-position of the second nucleoside.*

Since the discovery of the triple-helix structure in synthetic polyribonucleic acids[1], studies towards the design of triple-helices stable under physiological conditions have become the area of intense research[2,3,4]. However the requirement for oligopurine tracts in the target double-helix remains a limitation. Recent work 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 pair with purines on alternate strands by cross-overs in the major groove. This has been achieved using either natural oligodeoxyribonucleotides and two classes of DNA triple-helices (Py) x (Pu) .(Py) and (Pu) x (Pu) .(Py)[5,6] or only one set of triplets (Py) x (Pu). (Py) and a modified internucleotide (5'-5')-ends or (3'-3')-ends junction[7-10] between both pyrimidine sequences in order to meet the relative polarity requirement between the third strand and the purine containing strand of the duplex. Until now, only the 3'-3' switch had given nearly satisfactory results[8-10]. The design of the 5'-5'-linked oligomers is difficult because the distance between the 5'-ends of the third strands is much longer than that between the 3'-ends[8]. In order to obtain a good cooperative binding between third-strand oligonucleotide segments, the synthesis of symmetrical 3'-3'- linked oligonucleotides tethered via nucleic bases using a modified support was recently carried out[11]. These compounds, involving a short linker, are able to form stable triple-helices[12]. We now report the preparation of symmetrical and asymmetrical 5'-5'-linked oligonucleotides through nucleic bases using a short linker (Figures 1&2).

This was achieved using a modified bridged dinucleoside with a phosphoramidite group at the 3'-position of one nucleoside and a dimethoxytrityl group at the 3'-position of the second. This allows the addition of the dimer at the 5'- position of the first oligonucleotide assembled with regular 3'-phosphoramidites while the second oligonucleotide is assembled in the opposite orientation starting from the 3'-position of the second nucleoside and using 5'-phosphoramidites.

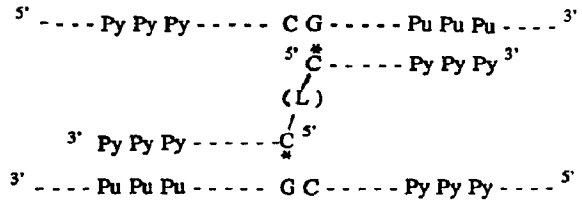

Figure 1: Model for alternate strand triple-helix formation with 5'-5' bridged oligonucleotides

The key step of the synthesis of 5'-5' bridged oligonucleotides described in the following scheme is the preparation of the phosphoramidite dimer **4c.**

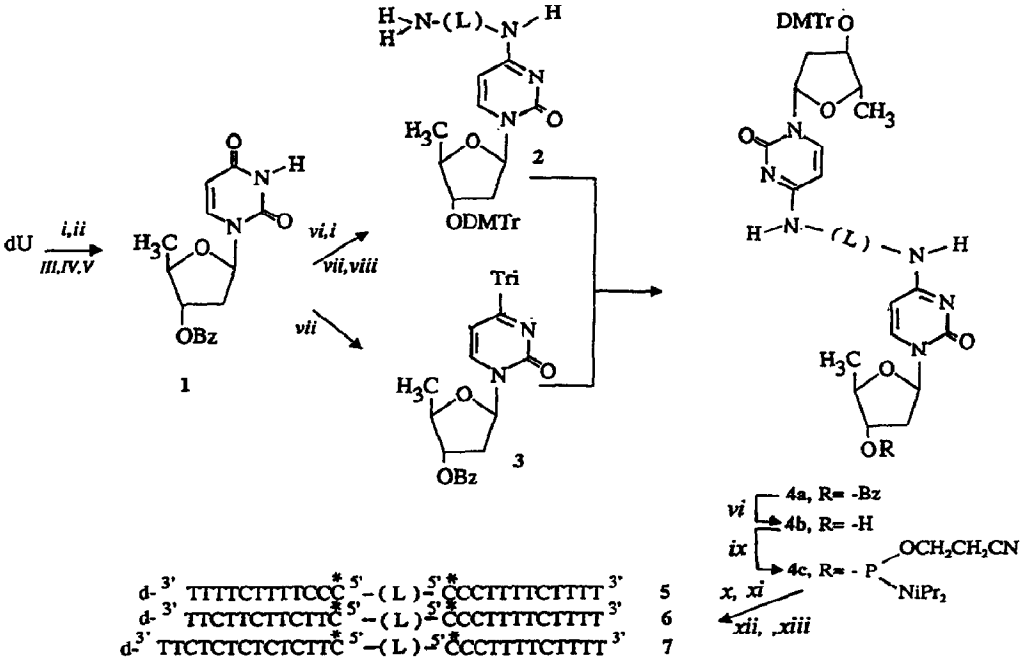

Figure2 : DMTr= dimethoxytrityl; Bz= benzoyl; L= $CH_2CH_2OCH_2CH_2OCH_2CH_2$; Tri= triazolyl; (i) DMTrCl, pyridine; (ii) BzCl, pyridine; (iii) H^+; (iv) PhOSCl/pyridine; (v) nBu_3SnH, AIBN,toluene; (vi) OH^-; (vii) phosphorus oxychloride, 1,3,4 triazole, CH_3CN; (viii) $H_2NCH_2CH_2OCH_2CH_2OCH_2CH_2NH_2$,CH_3CN; (ix) 2-cyanoethyl-N,N-diisopropylamidochloro-phosphite, diisopropylethylamine; (x) coupling of the phosphoramidite derivative of the dimer 4c with the 5'-hydroxyl group of the first oligonucleotide (assembled with regular 3'-phosphoramidite) bound to the support; (xi) assembly of the second oligonucleotide with 5'-phosphoramidites; (xii) concentrated ammonia; (xiii) H^+.

In order to avoid the problem of protective group selectivity, we chose to remove the 5- hydroxyl groups on both nucleosides since they are not involved in the oligonucleotide synthesis. Starting from the deoxyuridine (dU) after suitable protection of the 5' and 3'- hydroxyl functions by the dimethoxytrityl and benzoyl groups, respectively, followed by deprotection and deoxygenation[13,14] at the 5'-position the 2',5'-dideoxy-3'-benzoyl-uridine **1** was obtained. For the first part of the compound **1**, the benzoyl group was replaced by the dimethoxytrityl group, then the C^4 position was activated [15] with phosphorus oxychloride (instead of phenyldichlorophosphate) in the presence of triazole to give the triazolide derivative which was treated with 2,2'- (ethylenedioxy)diethylamine (10 eq.) to afford the monomer **2**. The second part of compound **1** was also activated as the triazolide derivative **3** which was then condensed with the amino group of **2** in CH_3CN to afford the dimer **4a**. Debenzoylation of **4a** followed by phosphitylation of the 3'-hydroxyl function of the obtained dimer **4b**[16] with 2-cyanoethyl-N,N-diisopropylamidochlorophosphite in the presence of diisopropylethylamine gave the phosphoramidite **4c**. The chain assembly was carried out on a Pharmacia Gene Assembler at the µmole scale using phosphoramide chemistry. The first oligonucleotide was assembled with regular 3'-phosphoramidites in the 3'-5' direction, then the dimer **4c** was added and the synthesis was completed with the assembly of the second oligonucleotide in the opposite direction using nucleoside 5'-phosphoramidites[7] with 10 equiv. of monomer per cycle and a coupling time of 2,5 min.. In these conditions the obtained coupling yields are similar to those obtained with 3'-phosphoramidites (97%). The coupling of the dimer **4c** was performed by using 0,1M solution in CH_3CN and a duration of 5 min. After deprotection by concentrated ammonia and acid treatments, compounds **5**, **6** and **7** 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 **5**, **6** and **7** was confirmed by reversed-phase analysis on a Lichrospher 100RP(5µm.) column (125x4mm.) using a linear gradient of CH_3CN (0 to 40% in 20 min.) in 0,1M aqueous triethylammonium acetate buffer, pH 7, with a flow rate of 1ml/min. (Rt_5=14min. 52sec., Rt_6=15min. 2sec., Rt_7=15min.). Nucleic base composition was ascertained after nuclease degradation by endonuclease P1 from *Penicilium citrinum* and alkaline posphatase by reversed-phase analysis using a Waters 600E System Contoller equipped with a Waters 990 Photodiode Array Detector[17].

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

Acknowledgements

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16. An aliquot of compound **4b** has been detritylated to afford the dihydroxy dimer ddC*(L)dd*C which has been analyzed by NMR.
    1H-NMR(DMSO) δ: 7.77 (2H, m, 2 NH); δ: 7.47 (2H, d, $2H_6$); δ: 6.08 (2H, t, J = 7Hz , $2H_{1'}$); δ: 5.80 (2H, d, J = 7 Hz, $2H_5$); δ: 5.20 (2H, d, J = 4.5 Hz, OH) ; δ: 3.88 (2H, m, $2H_{4'}$); δ; 3.80 (2H, m, $2H_{3'}$); δ: 3.5 (8H, m, $CH_2OCH_2CH_2OCH_2$) ; δ: 3.43 (4H, m, H-N-CH_2); δ: 2.15-1.90 (4H, m, $H_{2'}H_{2''}$); δ: 1.25 (6H,d, $2CH_3$)
17. After nuclease degradation of compounds **5**,**6** and **7** the base composition analysis were performed on a Lichrospher 100RP18(5μm) column (125x4 mm.) using a linear gradient of CH_3CN in 0,1M aqueous triethylammonium acetate buffer, pH7, with a flow rate of 1ml/min., 0% CH_3CN for 5min. then 0 to 20% CH_3CN in 20 min and then 20 to 50% CH_3CN in 15 min. (Rt_{dC}=5min. 14sec., Rt_{dT}=14 min. 42 sec. and $Rt_{ddC^*(L)ddC^*}$=36min. 11sec.).

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