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Oligo-β- and -α-Deoxyribonucleotides Involving 2-Aminopurine and Guanine for Triple-Helix Formation

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Abstract: *Purine oligo-β-deoxyribonucleotides and unnatural nuclease-resistant α-anomer derivatives were constructed with 2-aminopurine (amP) and guanine (G) in order to recognize purine sequences in double-helical DNA via isomorphous base triplets amP.AT and G.GC.*

Recent research has shown that pyrimidine oligodeoxyribonucleotides can recognize the major groove of a polypurine-polypyrimidine DNA double helix[1,2]. Thymine (T) and protonated cytosine (C^+) form Hoogsteen hydrogen bonds with Watson-Crick AT and GC base pairs, respectively (Figure 1). Pyrimidine oligo-β-deoxyribonucleotides involving thymine and cytosine have been shown to bind in a parallel orientation with respect to the purine-containing strand, whereas the unnatural nuclease-resistant oligo-α-deoxypyrimidines bind only when they are synthesized in an antiparallel orientation[3]. The potential for therapeutic application in which gene expression is repressed by triple-helix formation has been explored[4,5]. The success of this approach depends on the stability of the triplex under physiological conditions. Previous results have shown that substitution of 5-methylcytosine for cytosine and attachment of an intercalating agent at the end of the third strand increases the stability of the complexes[6]. However, protonation of the *N*-3 of cytosine was still required so that when the triplex contained contiguous C^+. GC triplets, its stability decreased markedly with increasing pH.

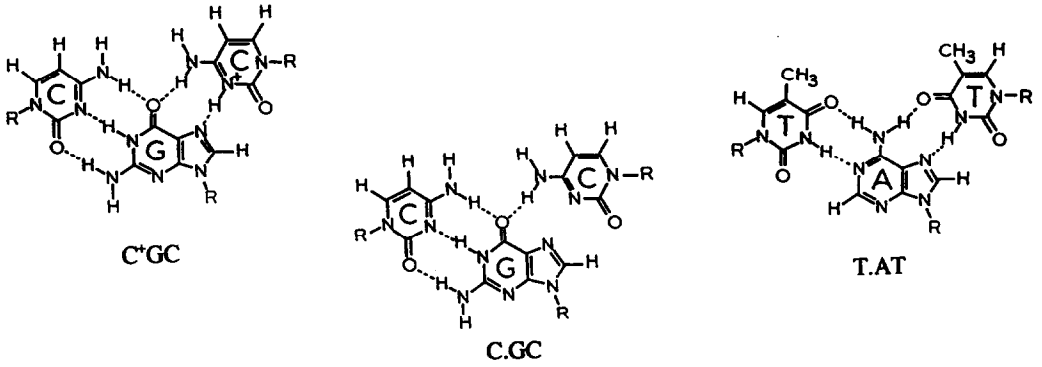

Figure 1: Base triplets formed by Watson-Crick GC and AT base pairs with protonated C(C^+), C and T.

To circumvent this limitation, oligonucleotides involving the following nucleoside couples were constructed: (dT, dG)[4], (dA, dG)[4], [dT, 2'-*O*-methylpseudoisocytidine (Cm)][7], (dT, 6-methyl-8-oxo-deoxyadenosine)[8], [dT, 1-(2'-deoxy-β-D-ribofuranosyl)-3-methyl-5-amino-1H-pyrazolo(4,3-d)pyrimidine-7-one][9]. Among the base-triplet couples used to form the triple helix, only the following are isomorphous: (T.AT, C^+. GC) and (T.AT, Cm.GC). In order to obtain purine.purine pyrimidine isomorphous base triplets, the synthesis of purine oligo-β- and -α-deoxyribonucleotides involving 2-aminopurine (amP) and guanine (G) was carried out to form Hoogsteen hydrogen bonds at neutral pH with Watson-Crick AT and GC base pairs, respectively (Figure 2).

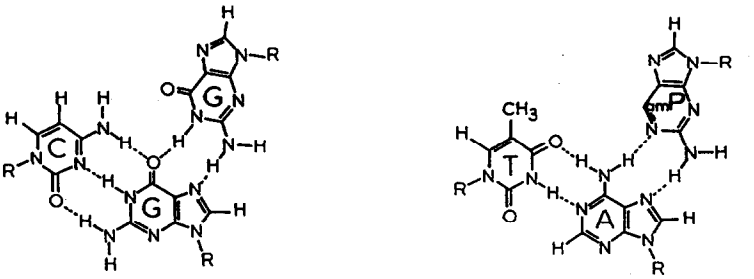

Figure 2: Isomorphous base triplets G.GC and amP.AT.

The synthesis of purine oligo-β- and -α-deoxyribonucleotides (5β, 5α) was performed following the scheme described in Figure 3. This consisted first in the preparation of the 3',5'-di-*O*-(4-nitrobenzoyl)-β-thymidine **1** as a glycosyl donor followed by a transglycosylation procedure[10]. To obtain the protected nucleosides 3β and 3α, bis(trimethylsilyl)acetamide (BSA) (4.1 g, 20 mmol) was added to a mixture of 3',5'-di-*O*-(4-nitrobenzoyl)-β-thymidine **1** (2.6 g, 4.81 mmol) and 2-benzoylamidopurine **2** (3 g, 12.5 mmol) in acetonitrile (30 ml). The mixture was heated at 70 °C for 15 min under stirring to afford a clear solution to which trimethylsilyltrifluoromethane sulfonate (TMSTf) (1.39 g, 6.26 mmol) was added, and stirring was continued for 7 h at 70 °C. After the usual workup and flash chromatography on a silica gel column using chloroform containing methanol as eluent (1 and 2%), 1.6 g (50% yield) of a mixture of 3β and 3α was obtained. The latter was then separated as pure anomers 3β and 3α (isomeric ratio, 1:1) using preparative TLC (silica gel plates with ethyl acetate/toluene, 70:30, v/v). 3α, R_f= 0.37, ^{1}H-N.m.r. 300 MHz ($CDCl_3$) δ: 6.10 ppm (dd, 1 H, $J_{1',2'}$ = 7, $J_{1',2''}$ = 4 Hz, H-1'), $[\alpha]_{546}^{25}$ = -45 (c 0.99, DMF). 3β: R_f= 0.28, ^{1}H-N.m.r. 300 MHz ($CDCl_3$) δ: 6.10 ppm (t, 1 H, J = 7 Hz, H-1'), $[\alpha]_{546}^{25}$ = +21 (c 1.01, DMF). To obtain the phosphoramidite 4β and 4α, the 5'- and 3'-hydroxyls of the protected nucleosides 3β and 3α were selectively deblocked by treatment with 0.05 N NaOH in CH_2Cl_2/CH_3OH (95:5, v/v) at 0 °C (5 min for 3β and 10 min for 3α), then reacted successively with dimethoxytrityl chloride (DMTrCl) in pyridine and (2-cyanoethyl)diisopropylamidochlorophosphite in acetonitrile in the presence of diisopropylethylamine. After the usual workup, flash chromatography on silica gel and precipitation from cold hexane, compounds 4β and 4α were obtained as a white powder (~80% yield). TLC (ethyl acetate/NEt_3, 90:10, v/v) 4β R_f= 0.72 and 0.63; 4α R_f= 0.73 and 0.68 (mixture of diastereomers).

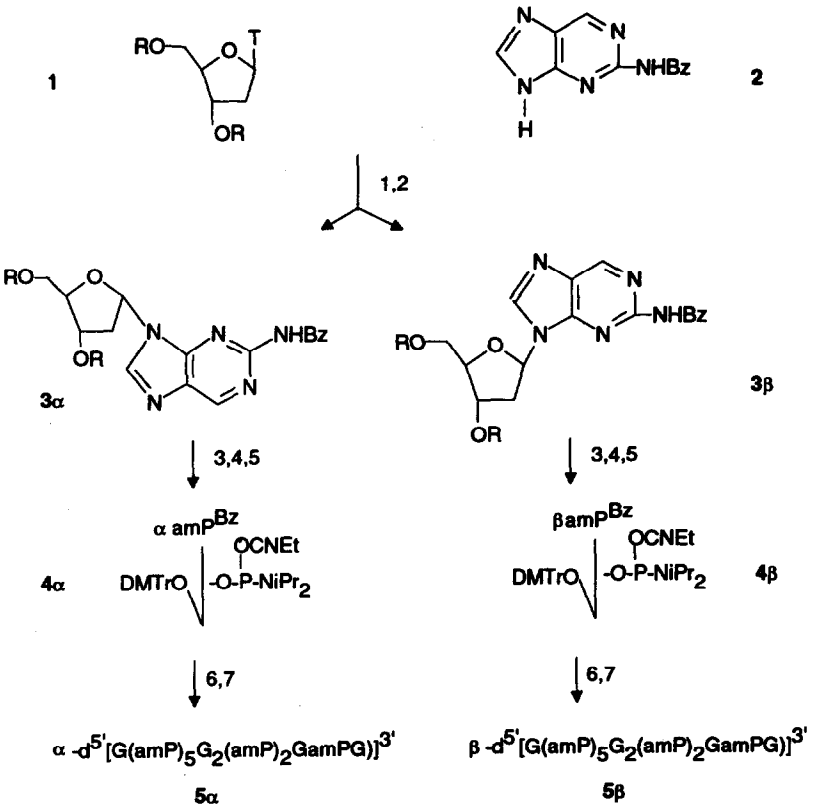

Figure 3: Bz = benzoyl; R = 4-nitrobenzoyl; CNEt = 2-cyanoethyl; iPr = isopropyl; DMTr = dimethoxytrityl; 1 = bis(trimethylsilyl)acetamide, trimethylsilyltrifluoromethane sulfonate, CH_3CN; 2 = separation of the α- and β-anomers; 3 = NaOH, CH_3OH; 4 = dimethoxytrityl chloride, pyridine; 5 = (2-cyanoethyl)diisopropylamidochlorophosphite, diisopropylethylamine; 6 = assembly of the oligonucleotide chain; 7 = concentrated ammonia and acetic acid.

Using the phosphoramidite 4α and the 5'-dimethoxytrityl-*N*-palmitoyl-α-D-deoxyguanosine-[(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite][10], the assembly was carried out on the α-d-G immobilized on Fractosil[11]. The oligo-β-D-deoxyribonucleotide chain was likewise constructed via the phosphoramidite 4β, the commercial phosphoramidite and CPG support. After the unblocking step by concentrated ammonia (30 h at 30 °C) and acetic acid treatments, the purine trideca-α-deoxyribonucleotide 5α and β-deoxyribonucleotide 5β were purified by anion exchange FPLC and reverse phase HPLC. Reverse phase analysis of the purified trideca-α-deoxyribonucleotide 5α on a C_{18} column using a photodiode array detector (Figure 4) gave a homogeneous peak with an absorption spectrum exhibiting two expected characteristics, maxima at 250 nm and 300 nm. It should be noted that a slight red shift of these absorption maxima was observed when the 2-aminopurine was incorporated in an oligomer. The same observation was made with the oligo-β-deoxyribonucleotide 5β (results not shown).

Studies of the interaction of these oligomers with a fragment of double helical DNA involving the polypurine sequence GAAAAAGGAAGAG are currently under way.

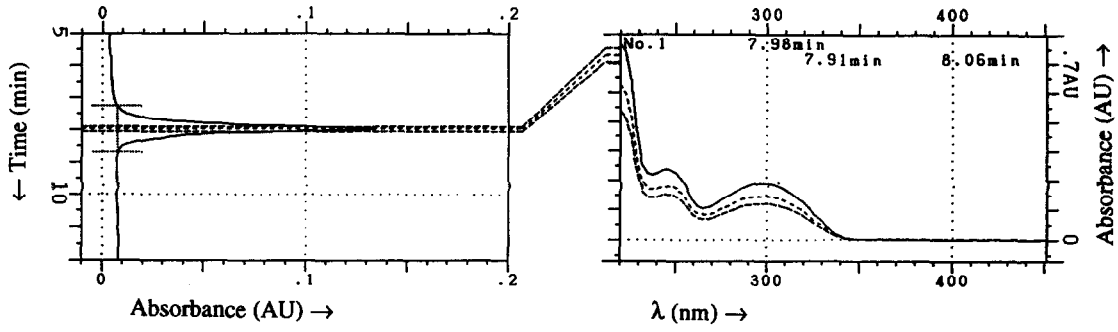

Figure 4: Reverse phase chromatography analysis of α-$d^{5'}[G(amP)_5G_2(amP)_2GamPG]^{3'}$ 5α on a lichrospher 100 RP 18 (5 µm) column (125 mm x 4 mm) using a linear gradient of CH_3CN (0 - 60% volume for 30 min) in 0.1M aqueous triethyl ammonium acetate, pH 7, with a flow rate of 1 ml/min and detection at 260 nm using a photodiode array detector (left). The absorption spectrum shows the homogeneity of the peak (right).

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