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Oligonucleotides Tethered via Nucleic Bases. A Potential New Set of Compounds for Alternate Strand Triple-Helix Formation

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Key words : bridged oligodeoxyribonucleotides, opposite polarities, triple-helix

Abstract : *The solid-phase preparation of oligonucleotides tethered via nucleic bases and with opposite polarities has been performed starting from a bridged dimer bound to a support.*

The early discovery of the triple-helix structure in synthetic polyribonucleic acids[1] followed by most recent work demonstrating the ability of sequence specific triple-helix formation by oligodeoxy[2,3] and oligoribonucleotides[4] has shown the potential usefulness of this structure in many applications including therapeutics. Therefore studies towards the design of triple-helices stable under the physiological conditions have become an area of intense research. In triple-helix the third strand which lies in the major-groove of the target DNA duplex makes hydrogen bonds with purines of the duplex via specific base triplets. However one significant limitation to the practical application of triple-helix is its requirement for oligopurine tracts in the target double-helix, and several approaches have been described to overcome this limitation. Among them, the targeting of sequences made up of both purines and pyrimidines remains a challenge. Recent work has shown that the repertoire of the triplex forming sequences can potentially be expanded to adjacent blocks of pyrimidines and purines by allowing the third strand to pair with purines on alternate strands by cross-overs in the major groove. This can be achieved by the combination of triplet specificities and required strand orientations in order to maintain the relative polarity betwen the third strand and the purine-containing strand of the duplex which would reverse with each switch. A few approaches have been described using either natural oligodeoxyribonucleotides (ODN) and two classes of DNA triple-helices (Py)x(Pu).(Py) and (Pu)x(Pu).(Py)[5,6] or with only one set of triplets (Py)x(Pu).(Py) and a modified internucleotide junction (5'-5' or 3'-3')[7-10] between both pyrimidine sequences. However these oligomers form triple-helices with low stability. Thus in the case of the unmodified oligonucleotides the oligonucleotide backbone must adopt a distorted conformation in order to be able to hybridize with each purine sequence on the two strands. In the latter case the oligonucleotides are tethered through their terminal phosphates via a linker parameters of which are difficult to determine. In addition the synthesis of these compounds involving two oligonucleotides with opposite polarities need the use of both 5' and 3' phosphoramidites[7-10]. In order to increase the degree of cooperativity we report here a new system in which both oligomers are linked through their terminal nucleic bases via a short arm (Figure 1). Furthermore this new system allows the use of the classical 3'-phosphoramidites starting from a modified support derivatized with a bridged dimer.

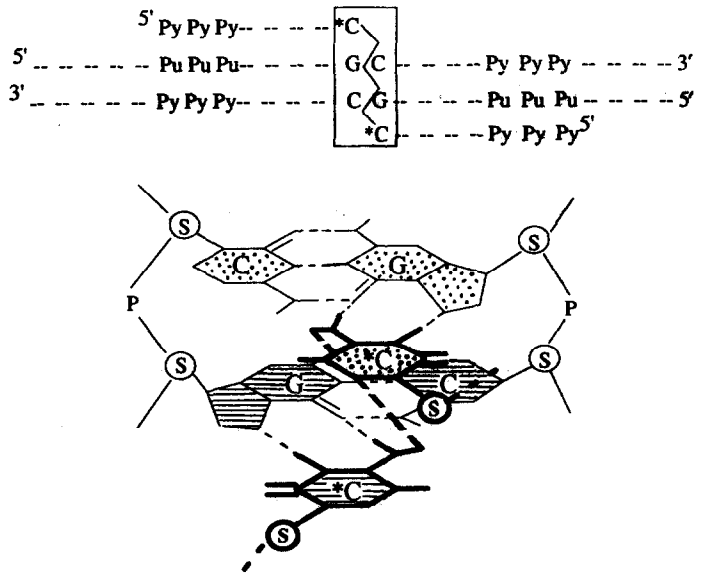
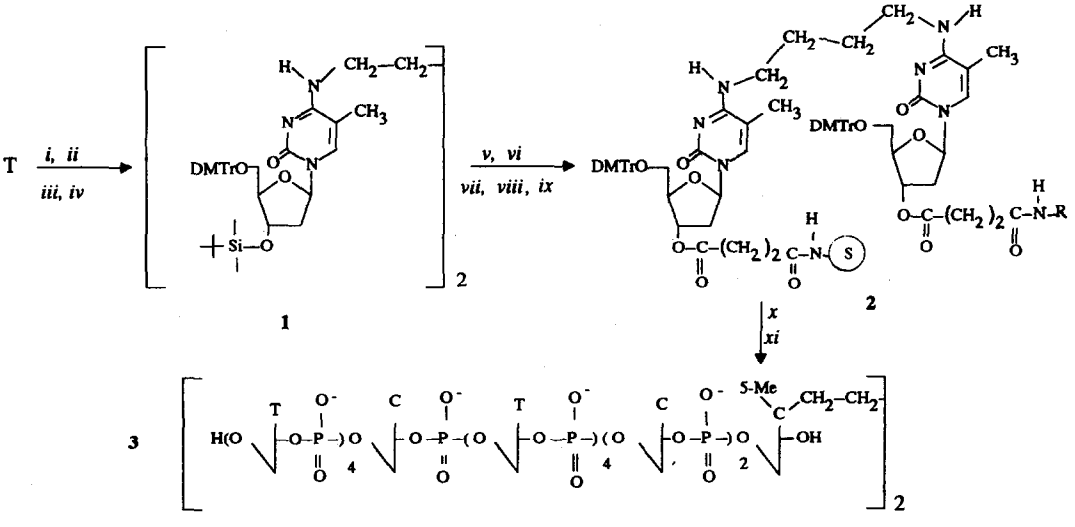


Figure 1 : Model for alternate strand triple-helix formation via the new bridged oligomer

The key step of this synthesis described in the following scheme is the preparation of the modified derivatized support **2**



DMTr = dimethoxytrityl; ┼Si┼ = tert-butyldimethylsilyl; Ⓢ = LCAA-CPG = long-chain alkylamine-controlled pore glass; R=CH_3-CH_2CH_2 or Ⓢ ; (i) DMTrCl, pyridine; (ii) ┼SiCl, imidazole pyridine; (iii) phosphorus oxychloride, triazole, pyridine; (iv) 1,4-diaminobutane, CH_3CN; (v) tetrabutylammonium fluoride; (vi) succinic anhydride, 4-dimethylaminopyridine, pyridine; (vii) = p-nitrophenol, pyridine, dicyclohexylcarbodiimide, dioxane, (viii). LCAA-CPG, NEt_3, DMF ; (ix) propylamine; (x) elongation of the oligodeoxyribonucleotide chain; (xi) concentrated ammonia.

The dimer **1** was synthesized starting from thymidine T using classical methods described for nucleic base modification[11-14]. Protection of the 5'-and 3'-hydroxyl functions of thymidine by the dimethoxytrityl and *tert*-butyldimethylsilyl groups, respectively, followed by activation of the C^4 position on thymine by treatment with phosphorus oxychloride in the presence of triazole gave the triazolide derivative which was treated with 1,4-diaminobutane (0.5 equiv) to afford the expected fully protected dimer **1**[15]. After deprotection of the 3'-hydroxyl function, the dimer was immobilized on LCCA-CPG 500 using the classical procedure[16] to give the modified support **2** with a loading of 15 µmol/g. To ensure the full capping of the unreacted activated ester an additionnal treatment with propylamine was performed. The assembly of the two symmetrical sequences was carried out on a 0.5 µmole scale on the support **2** using the classical phosphoramidite chemistry (with 10 equiv of monomer per cycle). The coupling efficiency was similar to that obtained for the synthesis on the LCAA commercially available derivatized support (> 96 % cycle). After deprotection by concentrated ammonia treatment, compound **3** was purified on a DEAE ion exchange column (100 mm x 10 mm) from Millipore using a linear gradient of NaCl in Tris/HCl 0.025 M, pH8, buffer containing 10% CH_3CN. After desalting the purity of the compound was confirmed by reversed- phase analysis (Figure 2) using a Waters 600E (system controller) equipped with a Waters 990 photodiode Array Detector and nucleic base composition was ascertained after nuclease degradation (Figure 2).

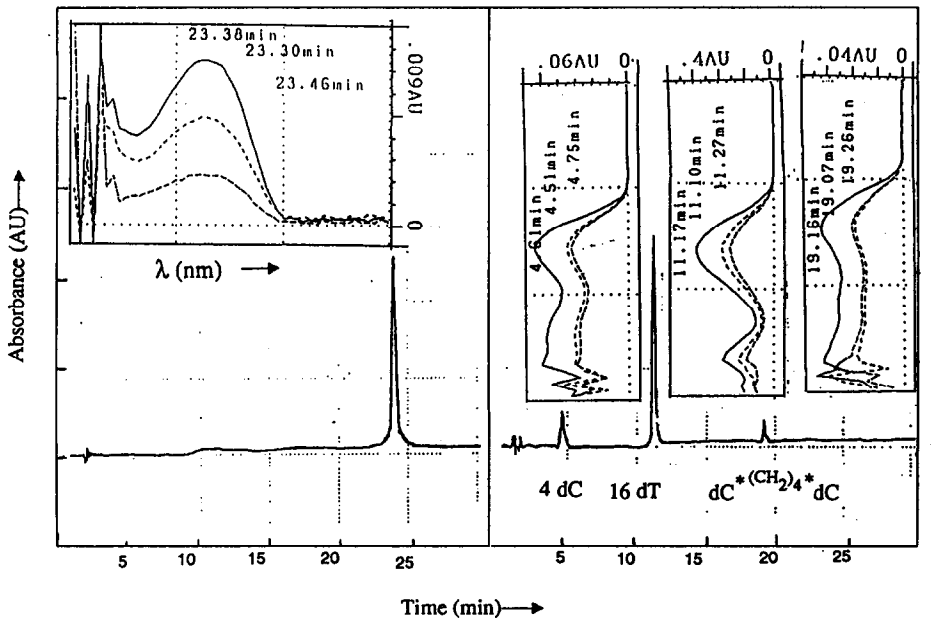


Figure 2 : Reversed-phase analysis on a Lichrospher 100 RP18 (5µm) column (125 x 4 mm) using a linear gradient of CH_3CN in 0.1 M aqueous triethylammonium acetate buffer, pH 7, with a flow rate of 1ml/min [0% CH_3CN for 5 min, then 0 to 30% CH_3CN in 30 min). **3** : d[5'$T_4CT_4C_2$ $\overset{*}{C}(CH_2)_2$-]$_2$ (left) and the base composition analysis of **3** after nuclease degradation with P1 from *Penicilium citrinum* and A.P. alkaline phosphatase (right). The insets show the corresponding absorption spectra recorded between λ=200 nm and λ=350 nm.

The solid phase preparation of oligonucleotides tethered *via* nucleic bases with opposite polarities has been performed starting from the bridged dimer bound to a support. The chain elongation has been carried out using nucleotide-3'-phosphoramidites. Symmetrical sequences were first prepared in order to determine the better size for the linker by hybridization studies. These experiments currently in progress in collaboration with another team will be published elsewhere. The preparation of asymmetrical sequences will be undertaken by replacing one dimethoxytrityl group of the tethered dimer by another protective group which can be selectively removed after the assembly of the first oligomer.

Acknowledgments

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References and Notes

1. Felsenfeld, G.; Davies, D.R.; Rich, A.; *J. Am. Chem. Soc.* **1951**, *79*, 2023-2024.
2. Moser, H.; Dervan, P.B.; *Science* **1987**, *238*, 645-650.
3. Le Doan, T.; Perrouault, L.; Praseuth, D.; Habhoub, N.; Decout, J.L.; Thuong, N.T.; Lhomme, J.; Hélène, C.; *Nucleic Acids Res.* **1987**, *15*, 7749-7760.
4. Roberts, R.W.; Crothers, D.M.; *Science* **1992**, *258*, 1463-1466.
5. Beal, P.A. and Dervan, P.B.; *J. Am. Chem. Soc.* **1992**, *114*, 4976-4982.
6. Jayasena, S.; Johnston, B.H.; *Nucleic Acids Res.* **1992**, *20*, 5279-5288.
7. Horne, D.A.; Dervan, P.B.; *J. Am. Chem. Soc.* **1990**, *112*, 2435-2437.
8. Ono, A.; Chen, C.N.; Kan, L.S.; *Biochemistry* **1991**, *30*, 9914-9921.
9. Froehler, B.C.; Terhorst, T.; Shaw, J.P.; Mc Curdy, S.N.; *Biochemistry* **1992**, *31*, 1603-1609.
10. Mc Curdy, S.; Moulds, C.; Froehler B.; *Nucleosides Nucleotides* **1991**, *10*, 287-290.
11. Sung, W.L.; *Nucleic Acid Res.* **1981**, *9*, 6139-6151.
12. Sung, W.L.; *J. Org. Chem.* **1982**, *47*, 3623-3628.
13. Horn, T. ; Urdea, M.; *Nucleic Acids Res.* **1989**, *17*, 6959-6962.
14. Maggio, A.F.; Lucas, M.; Barascut, J.L.; Pompon, A.; Imbach, J.L.; *Nouveau Journal de Chimie* **1986**, *10*, 643-649.
15. An aliquot of compound **1** has been fully deprotected and analyzed by NMR.
^{1}H-NMR (D_2O) δ : 7.48 (2H, s, $2H_6$) ; δ : 6.26 (2H, t, J = 6,68 Hz, $2H_{1'}$), δ : 4.47 (2H, m, H_3) ; δ : 4.06 (2H, m, $2H_{4'}$); δ : 3.85 (4H, m, $4H_{5',5''}$); δ : 3.45 (4H, m, N-$\underline{CH_2}$-CH_2) ; δ : 2,36 (4H, m, $4H_{2',2''}$); δ : 1.76 (4H, m, N- CH_2-$\underline{CH_2}$-); δ : 1.88 (6H,s, CH_3).
16. Atkinson, T.; Smith, M. in "Oligonucleotide Synthesis A Practical Approach" , Gait, M.J. Ed, IRL Press, Oxford, **1984**.

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