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SYNTHESES AND PROPERTIES OF OLIGOTHYMIDYLATE ANALOGS CONTAINING STEREOREGULATED PHOSPHOROTHIOATE AND PHOSPHODIESTER LINKAGES IN AN ALTERNATING MANNER

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Abstract : Syntheses of decadeoxythymidylates containing stereoregulated phosphorothioate and phosphodiester linkages in an alternating manner starting from preseparated diastereochemically pure phosphorothioate dimers are described. Hybridization of these modified oligonucleotides with the complementary sequence has been investigated.

The potential therapeutic use of modified oligonucleotides as artificial gene control agents faces several problems[1] including : i) the specificity of their binding to the target nucleic sequence under physiological conditions; ii) their uptake by intact cells; iii) their resistance to nuclease activity. To overcome these difficulties the phosphodiester backbone of the oligodeoxyribonucleotide can be replaced by phosphonate[2] , phosphotriester[3-4] or phosphorothioate backbones.[5] However, these modifications introduce chirality at the phosphorus atom. The reversible association of oligonucleotides with complementary sequences is of basic importance in biotechnology and studies of modified oligonucleotides have been conducted with the objective of gaining a further understanding and control of hybridization.[6-8] In order to control the stereochemistry at a modified P center, we have separated the two diastereoisomers at the level of the modified dinucleoside and used these dimers to synthesize decathymidylates with stereoregulated phosphorothioate and phosphodiester linkages in an alternating manner as described in the Scheme I.

The condensation of thymidine-3'-phosphonate **1**[9] with the 3'-silylated 2'-deoxythymidine derivative **2**[10] (1 equiv.) in presence of pivaloyl chloride (1 equiv.) in anhydrous pyridine led to a mixture of diastereomers in quantitative yield and in a 1:1 ratio. The mixture was separated (silica gel column, EtOAC:petether:AcOH,79.95:20:0.05,v/v/v).Transformation of each isomer into the corresponding phosphorothioates **3a** and **3b** was performed by treatment with sulphur in pyridine carbon disulphide (1:1,v/v). In order to assign the absolute configuration of the dinucleoside phosphorothioates, a small amount of each isomer was deprotected by i) desilylation (1M Bu_4NF in THF) and ii) detritylation (80% AcOH) to provide

4a and **4b** (Scheme I). These isomers were characterized by HPLC and enzymatic studies. The S_p isomer **4a** was hydrolysed by P1 nuclease exclusively[11] while the R_p isomer **4b** remains undigested. In addition, the Sp isomer has a greater retention time (20.49 min.) than the R_p isomer (20.18 min.)[12] on reverse phase HPLC Nucleosil C18 (10μm)250 mm X 4.6 mm column from Altech using a CH_3CN gradient (0% to 25% in 25 min.) in triethylammonium acetate buffer 0.03M, pH 7.0 with a flow rate of 1.0 mL/min. ^{31}P NMR spectroscopy in $CDCl_3$ with H_3PO_4 as an external standard has revealed that the S_p diastereomer **3a** is found at higher field (58.14 ppm) than the $R_p$diastereomer **3b** (59.13 ppm). The compounds **3a** and **3b** were desilylated and converted into their respective H-phosphonate derivatives **5a** and **5b** using 2-chloro-4-H-1,3,2-benzodioxa-phosphorin-4-one.[13] These H-phosphonates were used to synthesize decathymidylate analogs by block condensation on solid support using the syringe method[14] (Scheme I). Iodine may cause some desulphurization during conversion of H-phosphonate linkage into phosphodiester linkage.[15] In order to avoid the use of iodine, the H-phosphonate linkage was converted into the corresponding methoxy derivative and at the end of the synthesis the methyl group from the methoxy was deprotected (Scheme I). Hybridization was assayed by changes in A_{260}as a function of temperature[in Tris.HCl buffer, 10 mM,pH 7.0 and 1.0 M NaCl (Ratio 1:1)]. Duplexes formed between $d(T_ST_S)_5$with poly dA were found to have a much lower Tm value (20°C) than the duplexes of $d(T_pT_p)_5$ with poly dA(35°C) .The melting temperature (Tm) of duplex formed between (I)$dT_p(T_ST_p)_4T(R_p)$,(II)$dT_p(T_ST_p)_4T(S_p)$and(III)$dT_p(T_ST_p)_4T(R_p+S_p)$ with poly dA are found to be 32.5°C, 36.5 $^{\circ}$C and 35°C ,respectively., In the case of sequence (I), destabilization may be presumed from the steric interference by the sulphur atom that is orienting into the major groove of the B-form helix, causing only local deformation. The value of such type of compounds as research tool will continue to grow in the years to come in control of gene expression by oligonucleotides.

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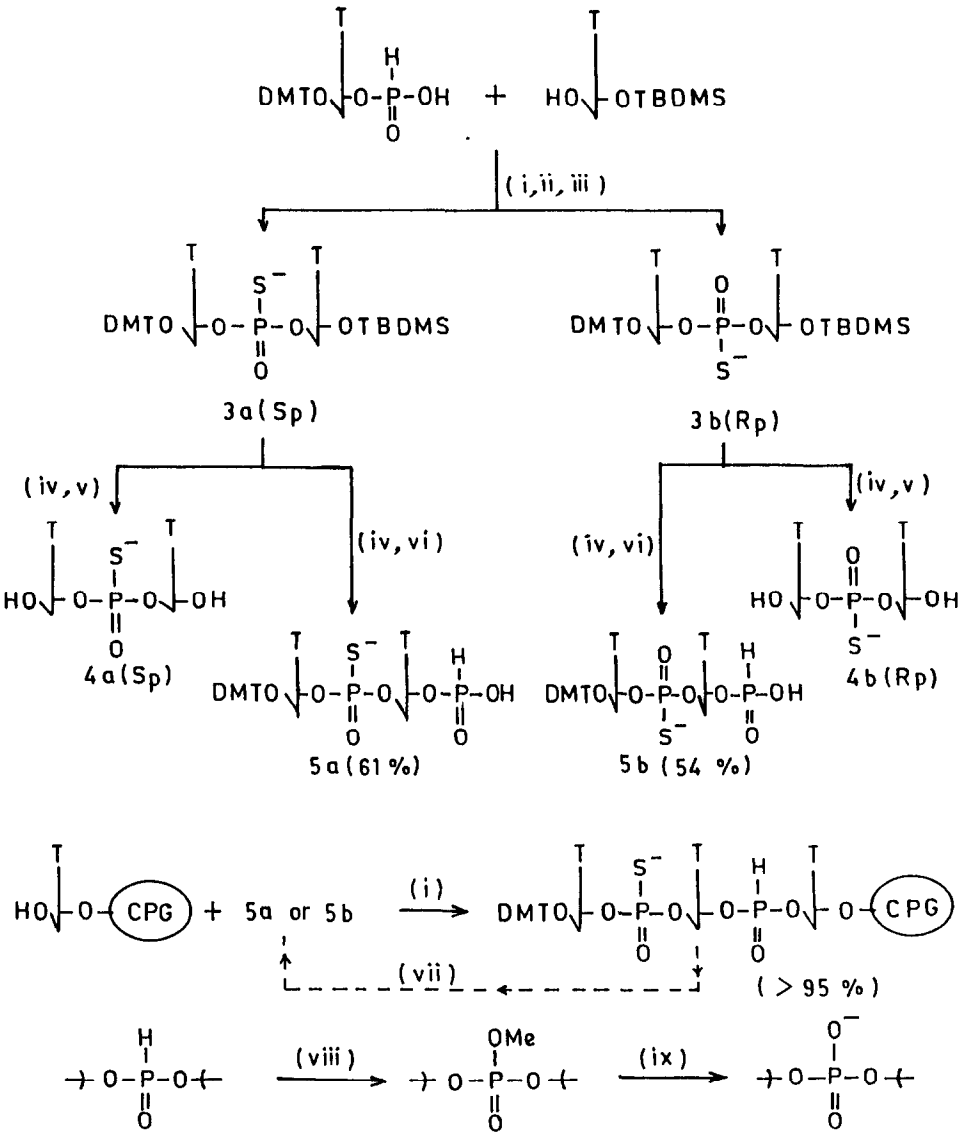

Scheme I : DMT :dimethoxytrityl; TBDMS :tert-butyldimethylsilyl; i :$(CH_3)_3CCOCl$; ii : separation of diastereoisomers; iii : S_8 in pyridine-CS_2 (1:1,v/v); iv :Bu_4NF in THF; v : 80% AcOH; vi :2-chloro--4H-1,3,2-benzodioxaphosphorin-4-one; vii : 3% DCA in CH_2Cl_2,chain elongation ; viii : CCl_4-Et_3N-CH_3OH(9:1:1,v/v/v); ix : thiophenol-dioxane-Et_3N(1:1:1,v/v/v).

References :

1) Helene, C.; Thoung,N.T. *in Molecular Mechanism of Carcinogenic and Antitumor Activity* Chagas, C.; Pullman,B.,Eds, **1987**, pp 205-222.

2) Miller,P.S.;Dreon,N.; Pulford,S.M.;Mc Parland,K.B. *J.Biol.Chem.* **1980**, *255*, 9659-9665.

3) Pless,P.C.; Ts'O,P.O.P.*Biochemistry* **1977**, ,*16*, 1239-1250.

4) Asseline,U.; Barbier,C.; Thuong,N.T. *Phosphorus Sulphur Relat. Elem.* **1986**, *26*,63-75.

5) Stec,W.; Zon,G.; Egan,V.; Stec,B. *J.Am.Chem.Soc.* **1984**, *104*, 6077 -6079.

6) Letsinger,R.L.; Singman,C.N.; Histand,G.; Salunkhe M.M.*J.Am.Chem. Soc.* **1988**, *110* , 4470-4471.

7) Salunkhe,M.M.; Wu,T.; Letsinger,R.L.*J.Am.Chem.Soc.***1992**,*114*, 8768-8772.

8) Letsinger,R.L.; Chaturvedi,S.K.; Farooqui,F.; Salunkhe,M.*J.Am.Chem. Soc.* **1993** ,*115*,7535-7536.

9) Garegg,P.;Regberg,T.;Stawinski,J.;Stromberg,R.*Chemica Scripta* **1985**, *26*, 59-62.

10) Kore,A.R.; Patil S.V.; Salunkhe,M.M. *Synth.Commun.***1993**,*23*, 2599-2603.

11) Seela,F.; Kretschmer,U.*J.Chem.Soc.,Chem.Commun*, **1990**,1154-1159.

12) Cosstick,R.; Ecksstein,F. *Biochemistry*, **1985**, *24* , 3630-3638.

13) Marugg,J.E.; Tromp,M.; Kuyl-Yeheskiely,E.; Van der Marel,G.A.; van Boom,J.H. *Tetrahedron Lett.*, **1986**, *23*, 2661-2664.

14) Tanaka,T.; Letsinger,R.L. *Nucl.Acids Res.***1982**,*10*,3249-3260.

15) Connolly,B.; Potter,B.Eckstein,F.; Pingoud.A.; Grotjahn,L. *Biochemistry* **1984**, *23*, 3443-3453.

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