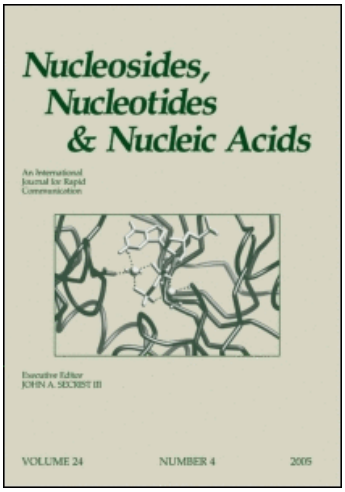

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CONFORMATIONAL RIGIDITY INTRODUCED BY 2′,5′-PHOSPHODIESTER LINKS IN DNA

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ABSTRACT

Conformational properties of 2′,5′-linked 3′-deoxyribonucleotides have been compared with their natural isomer using CD spectroscopy. It is inferred from the salt induced titration curves that the 2′,5′-linked-3′deoxyribonucleotides have rigid phosphodiester backbone.

INTRODUCTION

The transcription and the replication of nucleic acids are the central reactions responsible for the transmission of hereditary information in all living organisms. Biological systems use RNA- and DNA polymerases to condense the mononucleotide units to form the polynucleotides having exclusively 3′,5′-phosphodiester linkages[1]. In contrast to this a non-enzymatic polymerization of activated ribonucleotides, either in the presence/absence of a template[2,3] or in the presence of divalent cations[4], is known to result in the production of oligonucleotides containing predominantly the unnatural 2′,5′-phosphodiester linkages. The preponderance of 2′,5′- over 3′,5′- linked isomers in these reactions was thought to be due to the greater nucleophilic

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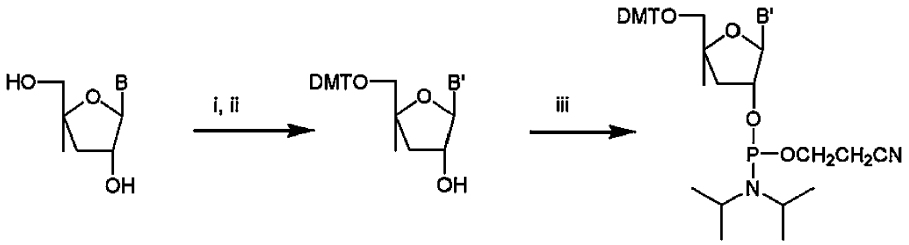
reactivity of the 2′-OH group. If 2′,5′- phosphodiester linkages are easier to form then a basic question arises 'Why nature has selected 3′,5′ phosphodiester linkages in nucleic acids and not 2′,5′-phosphodiester linkage?' Earlier studies done by Usher and McHale[5] had shown that a dodecanucleotide containing one 2′,5′-phosphodiester linkage and its natural isomer hydrolyzed at a comparable rate in the absence of any template. However, the presence of a template caused a 7 fold increase in the rate of hydrolysis of the oligomer containing 2′,5′-bond and a 5 fold decrease in the rate of hydrolysis of the natural oligomer. Dhingra and Sarma[6] with the help of NMR studies have shown that in 2′,5′-linked dimers, the intrinsic molecular stereodynamics are such that in order to attain base-base interactions, the magnitude of the glycosyl torsion angle changes so much that they can't support helical structures. Whereas, the geometries of 3′,5′-linkages allow the formation of helical configurations for RNA. Their findings were further supported by X-ray studies done by Parthasarathy et al[7]. Subsequently other experimental[8] and theoretical[9,10] studies led to contradictory predictions about the possibility of forming helices with 2′,5′-linked ribonucleotides. If the slower rate of hydrolysis for 3′,5′-linkages and/or the inability of 2′,5′-linked nucleotides to form helix are the reasons for the natural preference for 3′,5′-linkages in RNA, then what are the reasons underlying the preponderance of 3′,5′-phosphodiester linkages in DNA? In order to know the details of the intrinsic properties of 3′,5′-linkages, which the 2′,5′-phosphodiester linkages lack, a few reports have been published in the recent past[11–24]. Major efforts have gone into the comparison of their melting curves, their thermodynamic stability and base stacking properties. However, a limited attention[25] has been paid to unravel their conformational properties. In this article we describe a comparative study of the isomeric linkages of oligodeoxyribonucleotides by Circular Dichroism (CD) spectroscopy to assess their conformational flexibility. Towards this purpose we have synthesized 3′,5′-linked DNA decamers 5 and 6 and their corresponding 2′,5′-linked DNA decamers 7 and 8 respectively. The present data enabled us to compare conformational properties of 3′,5′ versus 2′,5′-linked DNAs. We have also calculated the thermodynamic parameters of these decamers by Tm studies. Significance of the conformational and thermodynamic properties of 3′,5′-viz.-a-viz. 2′,5′ DNA that led to the selection of the former linkage in the biological system is also described.

RESULTS AND DISCUSSIONS

The phosphoramidite synthons were obtained from the corresponding 3′-deoxynucleosides[26] as shown in Scheme1. Subsequently oligomer synthesis was carried out (0.2 mmol) on DNA synthesizer using the appropriate phosphoramidite monomers and the DMT-off protocol. At the end of synthesis oligomers were deblocked from the solid support and the remaining



i) TMSCl/C_5H_5N/Acylchloride, ii) DMT-Cl/C_5H_5N, iii) $[(i\text{-}Pr)_2N]_2$ $POCH_2CH_2CN$/Tetrazole/CH_3CN

Scheme 1.

protecting groups were removed by the concentrated ammonia treatment. Completely deblocked decamers were purified to homogeneity by the reversed phase (C-18) HPLC before using for Tm and CD studies.

1a, B = C 2a, B′ = C^{Bz} 3a, B′ = C^{Bz}
1b, B = G 2b, B′ = G^{Ibu} 3b, B′ = G^{Ibu}

Decamer 5:	CGCGCGCGCG	3′,5′-linked
Decamer 6:	GCGCGCGCGC	3′,5′-linked
Decamer 7:	CGCGCGCGCG	2′,5′-linked
Decamer 8:	GCGCGCGCGC	2′,5′-linked

Tm Studies

UV absorbance versus temperature profiles of self-complementary 2′,5′-linked decamers **7** and **8** exhibited sigmoidal curves, similar to their corresponding isomeric 3′,5′-linked decamers 5 and 6 at 0.15 M NaCl concentration, indicative of the association between 2′,5′-linked deoxyribonucleotides. It was also evident that the helix to coil transition in 2′,5′-linked decamers **7** and **8** was not that sharp as observed in the case of 3′,5′ linked decamers **5** and **6**. The thermodynamic parameter viz., enthalpy (ΔH°), entropy (ΔS°) and free energy (ΔG°) for a transition were derived from the absorbance melting curves. This was possible by assuming a two state (all or none) model[27], which assumes that single strands are in equilibrium with only one base paired native structure and there are no partially base paired intermediates in the melting process. This approximation is most appropriate for short (< 12 base pairs) complexes[28]. Thermodynamic properties of the four decamers are summarized in Table 1. It can be easily inferred from the data that values of each decamer vary with the strand concentration. Although, the change is very less, due to a small difference in strand concentrations, it supports a bimolecular association in all decamers. Further, the Tm values of 2′,5′-linked decamers **7** and **8** is 20–30 °C lower as compared to their 3′,5′ linked isomers **5** and **6**. The average values of ΔH°

Table 1. Thermodynamic Parameters of Decamers **5**–**8**

Decamers	Strand Conc. (μM)	Tm (°C)	$-\Delta H^\circ$ (Kcal M^{-1})	$-\Delta S^\circ$ (Cal M^{-1} K^{-1})	ΔG° (Kcal M^{-1})
	11.39	72.61	98.51	145.84	55.1
3′,5′ $[CG]_5$	8.98	71.79	90.92	134.86	50.7
	6.47	71.29	110.40	163.14	61.7
	8.20	70.40	87.83	130.53	48.9
3′,5′ $[GC]_5$	6.34	69.81	96.97	143.54	54.2
	8.74	69.32	90.66	134.69	50.5
	8.33	49.84	34.17	55.68	17.57
2′,5′ $[CG]_5$	6.71	48.70	35.68	57.42	18.56
	4.71	46.78	35.15	56.24	18.39
	11.99	60.35	37.23	59.26	19.56
2′,5′ $[GC]_5$	9.24	59.92	45.03	70.23	24.09
	6.89	58.54	39.93	62.45	21.32

and ΔG° for decamers **5** and **6** reported in the Table 1 were found to be in good agreement with the theoretical values, calculated on the basis of nearest-neighbour thermodynamic data reported in the literature[29]. It is also clear that the ΔH° and ΔG° values obtained for 2′,5′-linked decamers are approximately half that of the corresponding natural decamers **5** and **6**.

Our results support the previous observations for 2′,5′-DNA by Jung and Switzer[16] that the 2′,5′-linked oligonucleotides do associate in a bimolecular fashion but with a less thermodynamic stability as compared to the natural DNA. On the other hand, since this bimolecular association is observed in 150 mM salt concentration, our findings are contradictory to the results reported recently by Breslauer et al.[15] who have shown that 2′,5′-deoxyribonucleotide strands do not associate at low to intermediate sodium ion concentration, instead they form a triplex, that too only at high salt concentration. This difference could be due to the difference in length and composition of the oligomer sequences. The oligomers employed in the present study are palindromic having alternating dC and dG sequences with inherently high affinity for duplex formation. Whereas, the sequence reported by Breslauer[15] are homopolymeric (dA_{16}/dT_{16}) with a higher propensity for triplex formation, because of the favourable Hoogsteen-base pairs, suggesting thereby that 2′,5′-linked oligomers like their 3′,5′-isomers exhibit sequence dependent structural variation.

CD Studies

As expected, the CD spectrum of decamer **5** in 1 M SSc buffer (Fig. 1A) showed a positive band at 275 nm and a negative band at 248 nm,

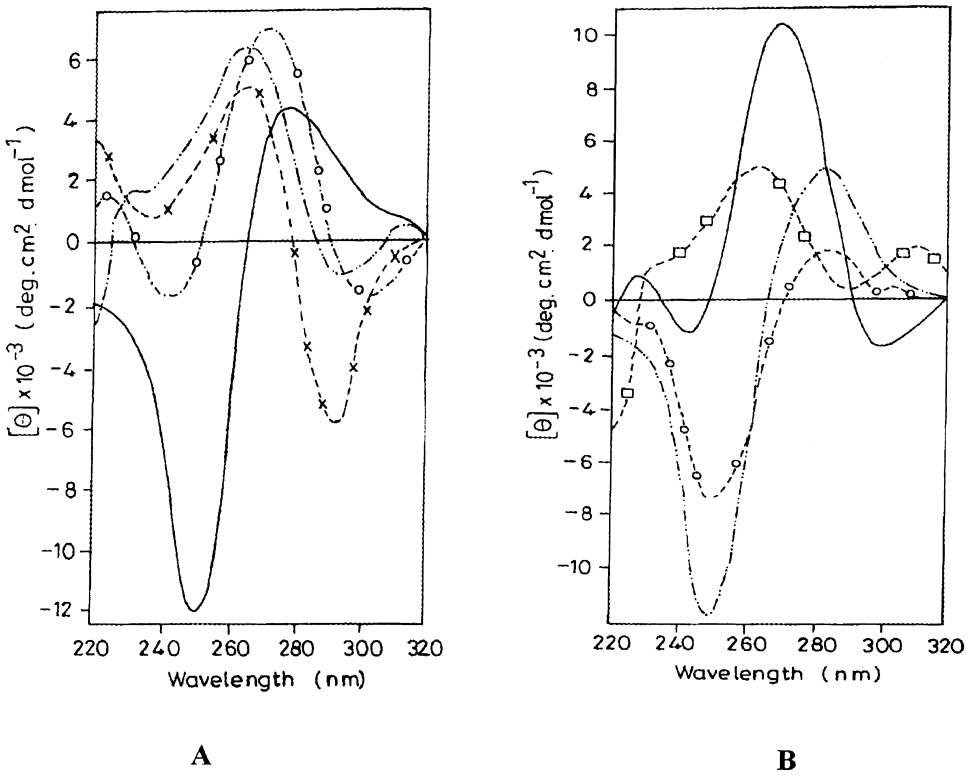


Figure 1. A). CD profiles of 3′,5′(C-G)$_5$ in 1 M NaCl (—), 4 M NaCl (-- × --- × --) and 2′,5′(C-G)$_5$ in water (--o---o--), 4 M NaCl (·—·—·—). B). CD profiles of 3′,5′(G-C)$_5$ in 1 M NaCl (—·—·—), 4M NaCl (--o---o--) and 2′,5′(G-C)$_5$ in water (—), 4 M NaCl (–□–□–).

characteristic of B form of DNA. On titrating with NaCl solution, the CD spectra showed marked changes; the intensity of the 275 nm band progressively decreased and ultimately it was replaced by an intense negative band centered at 290 nm accompanied by a new positive band at 264 nm at 4 M salt concentration. The crossover point was shifted from 264 to 277 nm. Thus, the final spectra had features of the left-handed Z-DNA[30]. As already known[31] that in the process of B to Z transition, the sugar puckering of dG residue changes from C2′-endo to C3′-endo and the glycosyl bond changes to *syn* position whereas the dC residue remains unchanged. As a consequence, the distance between the phosphate group attached to the C5′ and C3′ ribose positions is shortened, leading to an increased charge-charge repulsion between the adjacent phosphate residues under normal conditions, this repulsion acts as a barrier in favour of B-DNA. However, in the presence of excess NaCl, the Na^+ ions shield the negative charges and thus stabilize the Z-form.

The CD spectra of decamer 6 in 1 M SSc buffer (Fig. 1B) also showed a standard B DNA like pattern a positive band at 282 nm and a negative band

at 250 nm. By increasing the salt concentration, the intensity of the positive and negative bands was decreased with concomitant shift in the crossover point towards longer wavelength.

However, the pattern of the CD spectra remained unchanged even at 4.0 M salt concentration. These observations are consistent with earlier findings that the synthetic d[GC]n sequences (where n = 3,4,5) do not assume the left handed conformation even at high ionic strength. A systematic study carried out by Quadrifoglio et al.[32] have shown that at least a hexadecamer or octadecamer d[GC] sequence is required to observe salt induced left-handed conformation. On the contrary, even a short d[CG]n sequence (where n = 3) can undergo B-Z conformation change.

The 2′,5′-linked decamers **7** and **8**, however, showed the CD pattern which resemble with that of A form DNA. In 1 M SSc buffer, the CD pattern of decamer 7 showed a negative band at 295 nm and a broad positive band centered at 264 nm (with a shoulder at 232 nm). The crossover point was observed at 286 nm (data not shown). Assuming that this could be a transition stage between B-A (or B-Z) conformational change, the CD pattern of decamer 7 was checked in H_2O and change in the CD profile was monitored by titration with increasing salt concentration. In H_2O the CD pattern showed two negative bands at 301 and 242 nm, and two positive bands at 271 and 223 nm. The crossover points were observed at 291, 251 and 232 nm. On increasing the salt concentration, the intensities of negative bands were decreased. A positive band at 310 nm in 4 M salt concentration ultimately replaced the 301 nm negative band, whereas the 242 nm negative band disappeared. A shoulder accompanied the main positive band at 270 nm. However, the overall CD pattern remains the same (A-DNA like) all throughout the titration, from H_2O to 4.0 M salt concentration (Fig. 1A). Similar behaviour in the CD pattern of decamer 8 was observed under identical conditions used above in the case of decamer 7 (Fig. 1B). The above results indicate that the 3′,5′-linked oligodeoxyribonucleotides are conformationally flexible and exhibit polymorphism depending on changes in the environment. Whereas the 2′,5′-linked nucleotides exist in A-DNA like structure which is conformationally rigid.

CONCLUSIONS

The results of Tm and CD studies, in aggregate, indicate significant differences in the properties of complexes formed by the association of strands with identical base sequences but different phosphodiester linkages. It is apparent from the Tm studies that 2′,5′-linked oligo-3′-deoxyribonucleotides do form duplex structures likewise their natural 3′,5′-isomers. However, relative to its component single strands, the 2′,5′-duplexes are thermodynamically much less stable than the corresponding 3′,5′-duplexes.

Inspection of the thermodynamic data reveal that the enthalpy required to disrupt a 2′,5′-duplex to its component strands is considerably less than that required to disrupt the corresponding 3′,5′-duplex. With the assumption that the final single-stranded states are energetically equivalent, these data indicate that the forces that stabilize the 2′,5′-duplex are 50–75 Kcal/mole less enthalpic than the forces that stabilize the corresponding 3′,5′-duplex. This is also clear from the free energy change values that relative to their component single strands, the 2′,5′-duplex is stabilized by only 17–24 Kcal/mole of duplex compared with 48–61 Kcal/mole for the 3′,5′-duplex. This dramatic differential thermodynamic stability ($\Delta G^\circ = 31$–37 Kcal/mole at 25 °C) probably reflects better base stacking and pairing interaction when the nucleotide are connected via 3′,5′-phosphodiester linkages rather than via 2′,5′-linkages. Furthermore, the CD studies reveal that the two isomeric duplexes are conformationally nonequivalent. The duplexes containing 3′,5′-phosphodiester linkages exist in B-DNA like structure which is flexible (as it shows B-Z transition on increasing salt concentration), whereas, the duplex formed by 2′,5′-linked nucleotides exist in A-DNA-like structure which is conformationally rigid. Since the context dependent conformational flexibility of oligonucleotides is necessary for various biochemical functions, rather rigid or unstacked conformation of the 2′,5′-DNA could be a negative selective pressure in addition to the weak duplex forming ability. The structural information deduced here for the formation of 2′,5′-duplexes suggest that synthetic oligodeoxyribonucleotides containing all 2′,5′-linkages as well as chimeric linkages (2′,5′/3′,5′) could serve as ideal probes for DNA-protein interaction studies. Such chimeric oligonucleotides have already been used for the targeted destruction of RNA in the antisense oligonucleotide technology[33].

EXPERIMENTAL

All reactions were performed in freshly prepared solutions and dry solvents. Protected 2′-deoxyribonucleosidephosphoramidites and Controlled Pore Glass (CPG) loaded 2′-deoxyribonucleosides were purchased from Cruachem, Scotland. Succinic anhydride, 2,2′-Dithiobis-(5-nitropyridine), 2-Cyanoethyl-N,N,N′,N′-tetraisopropylphosphorodi-amidite and Long chain alkylamine-CPG (mesh 80–120, pore size 500 A°) were obtained from Sigma Chemical Company, USA. 4-Dimethylaminopyridine, 1H-Tetrazole, 4,4′-Dimethoxytritylchloride (DMT-Cl), Isobutyric anhydride and Triphenylphosphine were supplied by Fluka, Switzerland.

3′-Deoxyribonucleotides were synthesized by using published procedure[26]. Protected 3′-deoxyriboncleoside-phosphoramidites and CPG loaded 3′-deoxyribonucleosides were prepared by following procedure. Phosphitylation reactions were carried out in dry argon atmosphere. The Thin layer

chromatography (TLC) was performed on readymade plates (MERCK DC-Alufolien Kieselgel 60 F^{254}, 0.2 mm). Synthesis of decamers were carried out on Pharmacia Gene Assembler Plus using the manufacturers' recommendations for phosphoramidite chemistry. Deblocked decamers were purified on Waters HPLC system using reversed phase column (MERCK 50983-Lichrosopher 100 RP-18, 5 μm, 250 × 4). All UV measurements were carried out on Perkin Elmer (Lambda 15) and Gilford (ResponseTM) UV/VIS spectrophotometer. NMR spectra were recorded on Bruker WM-400 MHz spectrometer. The FAB mass spectra were recorded on Jeol SX-102/DA-6000 double focusing spectrometer.

For enzyme studies, TDW and the buffers were freshly prepared and sterilized before use. Phosphodiesterase (Crotallus durissus) was purchased from Boehringer Mannheim, Germany and alkaline phosphatase from Sigma, USA.

General Method of N-Acylation

To a stirred suspension of predried 3′-deoxynucleoside (1a/b, 10 mmol) in dry pyridine (50 ml) was added freshly distilled TMS-Cl (6.4 ml, 50 mmol) dropwise at 5 °C and the mixture was stirred at room temperature. After 20 min, the acylating reagent* (30 mmol) was added dropwise, under cool condition. The stirring was continued for another 2 h at room temperature. After completion of reaction, the excess of acylating reagent was quenched with chilled MeOH (5 ml). The aqueous NH_3 solution (10 ml) was added dropwise followed by stirring at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in a solution of 1:9 MeOH:$CHCl_3$ (25 ml). The salt thus precipitated was filtered and washed with 1:9 MeOH:$CHCl_3$ (10 ml). Combined organic layer was concentrated. The product was purified by silica gel column chromatography using a linear gradient of 0–10% MeOH in $CHCl_3$. Appropriate fractions were pooled and concentrated to give the product as a white solid (TLC in 10% MeOH in $CHCl_3$).

N^4-Benzoyl-3′-deoxyribocytidine. Yield 82%; R_f 0.58 (), PMR ($CDCl_3 + DMSOd_6$)δ: 9.9 (brs, 1H, N-H); 8.7 (d, 1H, $J_{5-6} = 8$ Hz, H-6); 8.0 (d, 2H, J = 7.8 Hz, Ar-H); 7.56–7.49 (m, 3H, Ar-H); 7.48 (d, $J_{5-6} = 8$ Hz, H-5); 5.83 (s, 1H, H-1′); 5.31 (d, 1H, $J_{2'OH\text{-}2'H} = 4.5$ Hz, 2′-OH); 4.85 (t, 1H, $J_{5'OH\text{-}5'H} = 6$ Hz, 5′-OH); 4.6 (m, 1H, H-4′); 4.4 (s, 1H, H-2′); 4.06 and 3.7 (2 m, 2H, H_a-5′ and H_b-5′); 2.08 and 1.88 (2 m, 2H, H_β-3′ and H_α-3′), FABMS (m/z): 332 $[M + H]^+$.

*Benzoyl chloride was used for the protection of 3′-Deoxyribocytidine, and Isobutyric anhydride for the protection of 3′-Deoxyriboguanosine.

N^2-Isobutyryl-3′-deoxyriboguanosine. Yield 76%, R_f 0.23, PMR ($CDCl_3 + DMSOd_6$)δ: 11.5 (brs, 1H, N-H); 8.55 (s, 1H, H-8); 7.8 (s, 1H, N-H); 6.18 (s, 1H, H-1′); 5.52 (d, 1H, $J_{2'OH\text{-}2'H} = 4.5$ Hz, 2′-OH); 4.94 (t, 1H, $J_{5'OH\text{-}5'H} = 5.8$ Hz, 5′-OH); 4.58 (m, 1H, H-2′); 4.48 (m, 1H, H-4′); 3.97 and 3.65 (2 m, 2H, H_a-5′ and H_b-5′), 2.2 and 1.9 (2 m, 2H, H_β-3′ and H_α-3′), 1.2 (d, 6H, J = 7.6 Hz, CH_3), FABMS (m/z): 338 $[M+H]^+$.

General Method of Dimethoxytritylation

A mixture of N-Acyl-3′-deoxyribonucleoside (10 mmol) was co-evaporated with dry pyridine (2 × 15 ml). The dried residue was dissolved in dry pyridine (70 ml). To this was added DMT-Cl (3.7 g, 11 mmol) at 5 °C and the mixture was stirred at room temperature for 2–4 h. After completion of reaction, the mixture was concentrated and the residue was dissolved in $CHCl_3$ (100 ml). The organic layer was washed successively with aqueous $NaHCO_3$, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated. The product was purified by silica gel column chromatography using a linear gradient of 0–10% MeOH in $CHCl_3$ containing 1% Triethylamine (TEA). Appropriate fractions were pooled and concentrated. The residue was re-dissolved in fresh $CHCl_3$ (10 ml) and precipitated in dry hexane (250 ml). The precipitate was filtered and dried to give the desired products in excellent yields (TLC in 4% MeOH in $CHCl_3$).

N^4-Benzoyl-5′-O-dimethoxytrityl-3′-deoxyribocytidine (2a). Yield 85%, R_f 0.51 (), PMR ($CDCl_3$)δ: 7.9 (d, 1H, $J_{6,5} = 7.8$ Hz, H-6); 7.43–7.24 (m, 14H, Ar-H); 6.88 (m, 5H, Ar-H and H-5); 5.78 (s, 1H, H-1′); 4.72 (brs, 1H, H-4′); 4.53 (brs, 1H, H-2′); 3.8 (s, 6H, OCH_3); 3.53 (dd, 1H, $J_{5'-4'} = 4$ Hz; $J_{5'-5'} = 11$ Hz, H_a-5′); 3.3 (dd, 1H, $J_{5'-4'} = 3.9$ Hz, $J_{5'-5'} = 11.2$ Hz, H_b-5′), 2.25 and 2.1 (2 m, 2H, H_β-3′ and H_α-3′); FABMS(m/z): 632 $[M\text{-}H]^-$.

N^2-Isobutyryl-5′-O-dimethoxytrityl-3′-deoxyriboguanosine (2b). Yield 82%, R_f 0.62, PMR ($CDCl_3$)δ: 9.05 (brs, 1H, N-H); 8.15 (s, 1H, H-8); 7.2–7.45 (m, 9H, Ar-H); 6.82 (m, 4H, Ar-H); 6.15 (s, 1H, H-1′); 4.72 (brs, 1H, 2′-OH); 4.57 (s, 1H, H-2′); 4.45 (s, 1H, H-4′); 3.8 (s, 6H, O-CH_3); 3.4 and 3.3 (2 m, 2H, H_a-5′ and H_b-5′); 2.13 (m, 2H, H_β-3′ and H_α-3′); 1.28 (d, 6H, J = 7.4 Hz, CH_3), FABMS (m/z):638 $[M\text{-}H]^-$.

Synthesis of N-Acyl-5′-O-dimethoxytrityl-3′-deoxyribonucleoside-2′-(2-cyanoethyl)-N,N-diisopropyl-phosphoramidites (3a/b). Predried N-Acyl-5′-O-dimethoxytrityl-3′-deoxyribonucloside (3a/b, 0.5 mmol) was dissolved in dry Acetonitrile (5.0 ml). To this was added 2-cyanoethyl-N,N,N′,N′-tetra-isopropyl-phosphorodiamidite (0.32 ml, 1 mmol) followed by 0.5 M solution of 1-H Tetrazole in dry Acetonitrile (1.0 ml), dropwise with stirring.

The mixture was stirred at room temperature for additional 2 h. It was diluted with a solution of 2% TEA in $CHCl_3$ (50 ml) and washed successively with aqueous $NaHCO_3$, H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in $CHCl_3$ and precipitated in hexane. The precipitate was centrifuged and dried in a vacuum desiccator, TLC [TEA/EtOAc/DCM (1:2:7)]. 3a: Yield 80%, R_f 0.67, ^{31}P NMR ($CDCl_3$)δ: 148.8, 150.5 and 3b: Yield 79%, R_f, 0.65, ^{31}P NMR ($CDCl_3$)δ: 150, 151.3.

Synthesis of N-Acyl-5′-O-dimethoxytrityl-3′-deoxyribonucleoside-2′-O-succinates. A mixture of N-Acyl-5′-O-dimethoxytrityl-3′-deoxyribonucleo-side (0.3 mmol) and DMAP (0.15 mmol) was co-evaporated with dry pyridine (2 × 2 ml) and dissolved in dry DCM (10 ml). To this was added dry TEA (0.5 ml, 3.6 mmol) and succinic anhydride (0.15 g, 1.5 mmol) at 5 °C. The reaction mixture was stirred at room temperature for 6 h. After completion of reaction, the mixture was diluted with 1% TEA/$CHCl_3$ (50 ml) and washed successively with aqueous citric acid, aqueous $NaHCO_3$, H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in $CHCl_3$ and precipitated in hexane. The precipitate was centrifuged and dried to give the desired product as a white solid (TLC in 5% MeOH in $CHCl_3$).

N^4-Benzoyl-5′-O-dimethoxytrityl-3′-deoxyribocytidine-2′-O-succinate. Yield 93%, R_f 0.33 PMR ($CDCl_3$)δ: 8.0 (d, 1H, $J_{6,5} = 8$ Hz, H-6); 7.44–7.2 (m, 13H, Ar-H); 6.82 (m, 5H, Ar-H and H-5), 5.92 (s, 1H, H-1′), 5.4 (s, 1H, H-4′), 4.38 (brs, 1H, H-2′), 3.75 (s, 6H, O-CH_3); 3.56 and 3.28 (2 m, 2H, H_a-5′ and H_b-5′), 2.46–2.3 (m, 6H, -CH_2CH_2-, H_β-3′ and H_α-3′), FABMS (m/z): 732 $[M-H]^-$.

N^2-Isobutyryl-5′-O-dimethoxytrityl-3′-deoxyriboguanosine-2′-O-succinate. Yield 95%, R_f 0.38, PMR ($CDCl_3$)δ: 8.23 (s, 1H, H-8); 7.5–7.12 (m, 9H, Ar-H); 6.75 (m, 4H, Ar-H); 5.8 (s, 1H, H-1′); 4.6 (s, 1H, H-2′); 3.88 (m, 1H, H-4′); 3.78 (s, 6H, O-CH_3); 3.56 and 3.4 (2 m, 2H, H_a-5′ and H_b-5′); 2.8–2.4 (m, 6H, -CH_2CH_2-, H_β-3′ and H_α-3′); 1.06 (s, 6H, CH_3), FABMS (m/z): 738 $[M-H]^-$.

Loading of protected-3′-deoxyribonucleoside on lcaa-CPG. To a stirred solution of N-Acyl-5′-O-dimethoxytrityl-2′-O-succinate (0.2 mmol), DMAP (49 mg, 0.4 mmol) and 2,2′-Dithiobis-(5-nitropyridine) (62 mg, 0.2 mmol) in acetonitrile:1,2-dichloroethane (3:1, 4 ml), was added Triphenylphosphene (52.5 mg, 0.2 mmol) and the mixture was stirred for 5 min to get a clear solution. To this was added lcaa-CPG (1 g, 0.1 mmol of free amino groups) and the suspension was agitated for 45 min at room temperature. It was filtered, washed successively with 1,2-Dichloroethane, acetonitrile and 1%

TEA/ether (10 ml of each) and dried in a vacuum desiccator. The residual amino groups on the support were capped by treating with a mixture of Ac_2O: TEA: N-Methylimidazole: Dichloromethane (DCM) (1:1:0.3:6, 10 ml) at room temperature for 30 min. The derivatized CPG was filtered again and washed with DCM and ether (10 ml of each) followed by drying in a vacuum desiccator. N^2-Isobutyryl-5′-O-dimethoxytrityl-3′-deoxyribo-guanosine loaded-CPG and N^4-Benzoyl-5′-O-dimethoxytrityl-3′-deoxyribo-cytidine loaded-CPG were obtained with a loading capacity of 36.0 and 39.57 μmol/g of CPG respectively.

Synthesis and Purification of Decamers 5–8

The decamers **5–8** were synthesized on DNA synthesizer employing the suitably protected appropriate phosphoramidite monomers and DMT-off protocol recommended by the manufacture (0.2 μmol scale). Completely deblocked oligomers were purified to homogeneity by the reversed phase HPLC using a linear gradient of 10–30% B in A (30 min), flow = 1 ml/min [Where A = 0.1 M triethylammoniumacetate, pH = 7.2 and B = acetonitrile]. Appropriate fractions containing the major peak were pooled and concentrated on a Speedvac by co-evaporation with MeOH to give 6–8 O. D. units of each of the purified decamers. The purified decamers were subjected to analytical HPLC wherein, only one peak was observed in all the cases suggesting the homogeneity of the decamers. The retention time of the decamers **5–8** was found to be 12.16, 12.5, 5.63 and 9.5 min respectively.

Confirmation of Composition

Each of the purified decamer (0.3 A_{260}) was dissolved separately in a mixture of 1 M Tris (pH 8.5, 25 μL), 1 M $MgCl_2$ (25 μL) and H_2O (175 μL) in an eppendorf tube. It was mixed vortexed, heated at 75 °C and then cooled suddenly to 0 °C. The SVPD (0.015units dissolved in 25 μL 0.1 M Tris HCl) was then added and the mixture was incubated at 37 °C. After 2 h, alkaline phosphatase (0.6 units dissolved in 1 μL 0.1 M Tris HCl) was added and the mixture was again incubated at 37 °C for 1.5 h. HPLC profile of the crude reaction mixture was checked with a linear gradient of 0–15% B in A (20 min), flow = 1 ml/min. Disappearance of the peaks corresponding to their respective decamers confirmed the complete hydrolysis. Also, the peaks of equal intensities, corresponding to 2′-dC and 2′-dG were obtained in the HPLC pattern of decamers **5** and **6**. Similarly, the HPLC profiles of decamers **7** and **8** showed the peaks of equal intensities corresponding to 3′-dC and 3′-dG. Retention time (in min) for 2′-dC, 2′-dG, 3′-dC and 3′-dG was

found to be 7.42, 11.68, 9.27 and 13.61 respectively, in the above gradient system.

Tm Studies

Solutions of decamers **5–8** were prepared in 165 mM SSc buffer (150 mM NaCl and 15 mM sodium citrate) adjusted to pH 7.1. Strand concentrations (C_t) were determined from the high temperature absorbance at 260 nm. The UV melting curves at three different concentrations of each decamer were recorded at 260 nm with an automated reference compensator that allowed melting curves to be obtained on three separate samples simultaneously. Four cuvettes (1 cm pathlength, 400 μL capacity, sealed with teflon stoppers, three cuvettes containing samples and the forth one for the reference solvent) were placed in a thermistor-controlled thermoelectric cell holder, circulated by water at a flow rate of 100 ml/min for the control of heating rate. Absorbance data were collected over the temperature range from 20°–90° C at a heating rate of 1 °C/min. After each melting experiment, the samples were cooled to the starting temperature (in 15 min) and the final absorbance values were compared with the initial absorbance before starting the experiment. Less than1% error was found between the absorbance values indicating that no evaporation of the solvent or hydrolysis of the sample occurred during the experiment. The absorbance data thus obtained were used to calculate the Tm and the thermodynamic parameters (ΔH^0, $\Delta S^0$and ΔG^0) using a software programme developed in our laboratory. Using this programme the absorbance values at each temperature (in ° K) were entered to draw A *Vs* T graph. Upon selecting the lower and upper base lines, the "f" vs "1/T" plot and the Tm value (in ° K) were obtained. This was followed by entering the values of n (molecularity, in this case 2) and the strand concentration (C_t) to get Vant Hoff's plot and the values of ΔH^0, ΔS^0, and ΔG^0 were calculated.

CD Studies

All CD spectra were recorded on JASCO J-500 spectropolarimeter equipped with a DP-500N data processor. The base line of the instrument was properly adjusted. An average of two scans was recorded at a sensitivity of 1.0 $m^0 cm^{-1}$ and a scan speed of 50 nm/min. Quartz cells of 1.0 mm path length was used. Solutions of decamers ($\sim$25 μM) were prepared in 1.1 M SSc buffer (1 M NaCl + 100 mM sodium citrate) adjusted at pH 7.2. Titrations were carried out at 20 °C by adding aliquots of 5 M aqueous NaCl solution. The spectra recorded were corrected by multiplying with an appropriate factor so that the resulting spectra correspond to that of same concentration as the original one.

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