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Oligonucleotides with (*N*-thymin-1-ylacetyl)-1-arylserinol backbone: chiral acyclic analogs with restricted conformational flexibility

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Dedicated to the memory of the late Dr V. N. Gogte

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Abstract—All four *threo*/*erythro* stereoisomers of 2(*R*/*S*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*/*S*)-aryl-1,3-propanediol were synthesized from 2(*R*/*S*)-amino-1(*R*/*S*)-aryl-1,3-propanediol in 45–50% overall yield. The inversion of the C1 hydroxyl group in (1*S*, 2*S*), **4a**, and (1*R*, 2*R*), **4d**, was accomplished under Mitsunobu conditions to get (1*R*, 2*S*), **4c**, and (1*S*, 2*R*), **4e** isomers, respectively. Compounds **4a–f** were individually converted into their respective amidite synthons **5a–f**. All these stereoisomers were individually incorporated into oligonucleotides (ODNs) at pre-determined positions and various biophysical studies of their hybrids with complementary DNA were carried out. All the four stereoisomers when present at 3′/5′ terminal positions in the ODNs were almost equally efficient in their binding capacity as the natural oligomers, with (1*S*, 2*S*) being marginally favored over other stereoisomers. The incorporation of these chiral acyclic nucleosides also protected the ODN against enzymatic degradation. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

The hybridization properties of oligonucleotides via Watson–Crick or Hoogsteen base pairing are of fundamental importance for their applications in molecular biology and biotechnology.[1] In addition to the base fidelity, other necessary physico-chemical requirements for employing oligonucleotides (ODNs) as chemotherapeutic antisense/antigene agents are enzymatic resistance, solubility in aqueous media and ability to penetrate the cell membrane.[2–4] The naturally occurring phosphodiester–sugar backbone of DNA largely provides the aqueous solubility but is susceptible to digestion by intracellular enzymes and hence, these ODNs have too short a half-life to be useful as therapeutic agents. The replacement of the sugar ring with open chain structures as in acyclic ODNs endows enzymatic stability but at the cost of their specific binding to the target sites.[5–8] Studies on open chain structures were almost discouraged till the advent of peptide nucleic acids (PNA).[9] The rigid achiral polyamide structure of PNA does not resemble the sugar phosphate backbone in any way but still binds avidly to the natural DNA/RNA sequences with very high sequence specificity. The tight binding of PNA:DNA complexes was attributed to the absence of charge repulsion and an entropic gain due to favorable pre-organization of PNA.[10] Although PNA show tight complexation with DNA at salt concentration much lower than cellular salt concentrations, their poor aqueous solubility and low cell uptake has opened scope for further chemical modifications. The progress in this direction gave way to the hybrid molecules like PHONA,[11] ether linked ODNs,[12] AANA,[13] PPNA,[14] chimeric PNA:DNA sequences[15a] and positively charged chiral PNA oligomers[15b,c] to include advantages of both the structures like superior enzymatic stabilities, aqueous solubility and specific binding ability. The unfavorable entropic factor in acyclic analogs for duplex formation, seems to be adequately compensated by the additional rigid amide linkage to the nucleobase, restricting the degrees of freedom as in 2-deoxy-D-ribose sugar, assuming that the enthalpic contribution of molecular recognition remains unaffected. Additional favorable conformational restrictions[16] to natural DNA as in bicyclo-DNA,[17] or 2′-substituted 2′-deoxy-D-ribose containing DNA are known to further stabilize the DNA duplexes.

Encouraged by these reports, we thought of imparting backbone rigidity into serinol derived acyclic DNA analogs by the introduction of substituents in the acyclic backbone. This would give an opportunity to study the effect of a second chiral center in the backbone, with an intrinsic synthetic advantage of dealing with one primary and one secondary hydroxyl function in a precursor instead of two primary hydroxyl groups as in most other acyclic backbone modifications. Our choice was 2-amino-1-aryl-1,3-propanediol, a synthetic precursor for the broad spectrum antibiotic

Keywords: oligonucleotides; acyclic backbone; antisense/antigene therapeutics.

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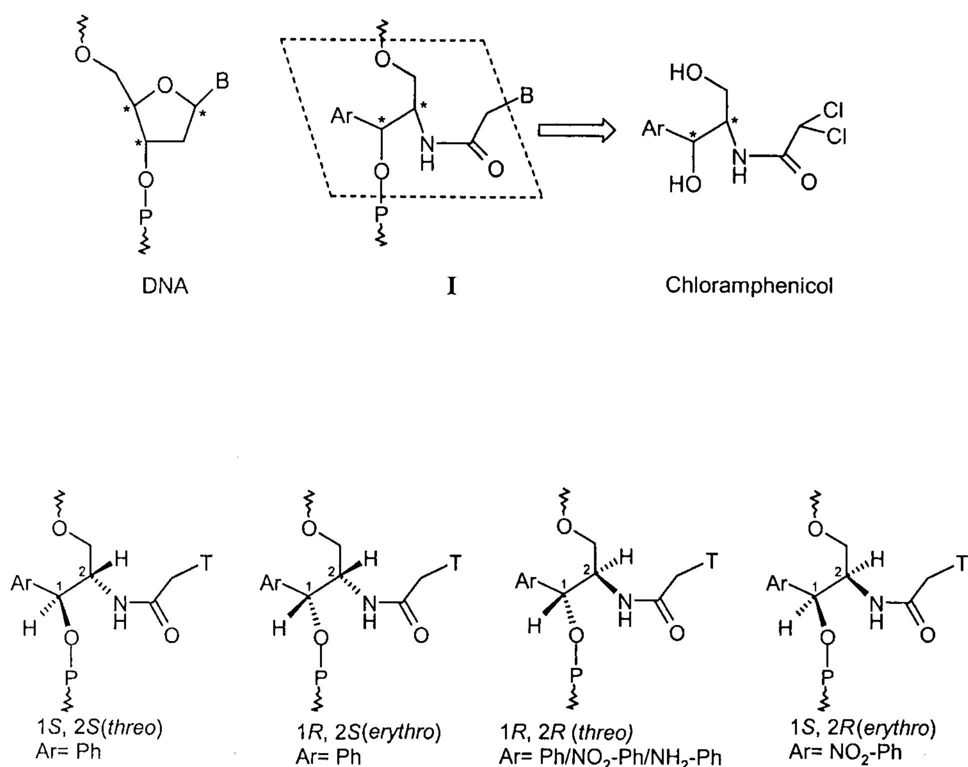
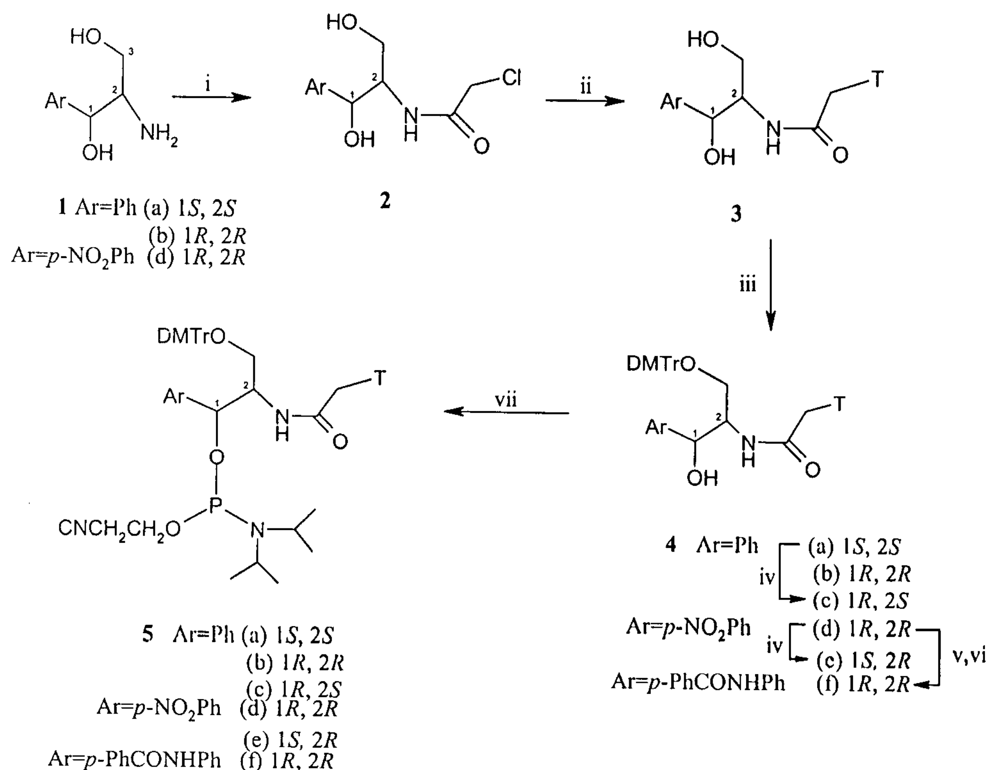
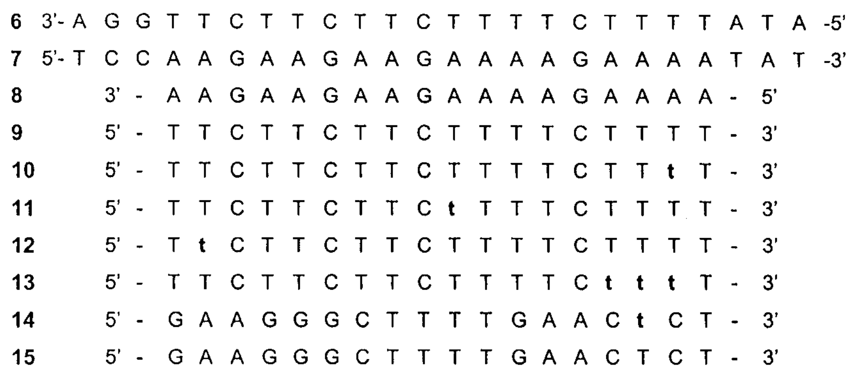


Figure 1. Stereostructures of modified nucleosides.

chloramphenicol and the preliminary results were reported in a communication.[18] In this paper we present the detailed synthesis of all four *threo*/*erythro* stereoisomers of 2(*R*/*S*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*/*S*)-aryl-1,3-propanediol (Fig. 1), their incorporation into DNA and the biophysical studies of their hybrids with complementary DNA. The phenyl substituent in this backbone may restrict the conformational mobility of the acyclic chain, and also introduces



Scheme 1. *Reagents*: (i) $ClCH_2$, (90–95%); (ii) Thymine, anhy. K_2CO_3, DMF, (70–80%); (iii) DMTrCl, Pyridine, (80–85%); (iv) a) PhCOOH, PPh_3, DIAD, dry THF, (80–85%); b) $MeOH/NH_3$, (90%); (vii) $\{[(CH_3)_2CH]_2N\}_2POCH_2CH_2CN$, Tetrazole, EDC (80%).



Scheme 2. Oligonucleotide sequences.

a second chiral center equivalent to the C3′ stereogenic center of deoxyribose sugar, leading to four possible stereoisomers. This would provide a very good model for understanding the importance of the *threo* D-natural cyclic sugar ring based DNA backbone compared to the acyclic analogs with two chiral centers equivalent to C4′ and C3′ of the sugar and also the base attachment through a rigid amide bond instead of C1′. Additionally, depending on its stereochemistry, the phenyl substituent at the 3′ equivalent center of 2-deoxy-D-ribose may be suitably inclined to show favorable stacking interactions as in the case of aromatic PNA.[19] The effect of substituents on the phenyl ring to determine the electronic effects, incorporation of these monomeric thymine units in duplex/triplex forming ODNs at predetermined positions and their biophysical evaluation including enzymatic stability are all reported.

1.1. Synthesis of 3-*O*-dimethoxytrityl-1-*O*-benzoyl-2(*R*/*S*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*/*S*)-aryl-1,3-propanediol and their phosphoramidites

The optically active acyclic nucleoside analogs **5a–f** bearing thymine were prepared from 2-amino-1-aryl-1,3-propanediol **1** (aryl=phenyl or *p*-nitrophenyl)[20] as shown in Scheme 1. The first step involved the reaction of 2(*S*/*R*)-amino-1(*S*/*R*)-aryl-1,3-propanediol with chloroacetyl chloride in the presence of aqueous Na_2CO_3 and dioxane to obtain the *N*-chloroacetyl derivative **2** in more than 80% yield. This was then alkylated with the nucleobase, thymine, in the presence of anhydrous K_2CO_3 to get the thyminyl diol **3**, the primary hydroxyl function of which was directly protected by the 4,4′-dimethoxytrityl group.[21] The pure product **4**, obtained after column chromatography, was characterized by NMR (^{1}H, ^{13}C) spectra, which were identical for both (1*R*, 2*R*) and (1*S*, 2*S*) stereoisomers. The inversion of the C1 hydroxyl in (1*S*, 2*S*) **4a** and (1*R*, 2*R*) **4d** was accomplished under Mitsunobu conditions, using benzoic acid as a nucleophile followed by hydrolysis to get (1*R*, 2*S*) **4c** and (1*S*, 2*R*) **4e**, respectively. The *p*-nitro substituted phenyl ring in **4d** was hydrogenated using 10% Pd–C in methanol/pyridine/water (1:0.5:1, v/v/v) to generate the corresponding *p*-aminophenyl derivative in quantitative yield. The *p*-amino group was then protected by benzoylation using benzoyl chloride in pyridine to give **4f** in 90% yield. All new compounds were characterized for structural purity by ^{1}H, ^{13}C NMR, optical rotation and mass spectrometry. Compounds **4a–f** were individually phosphitylated at the secondary hydroxyl group by 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite reagent in the presence of tetrazole to afford the respective amidite synthons **5a–f**.[22] These were individually characterized by ^{31}P NMR spectroscopy, which showed two signals between 148.7 and 150.9 ppm.

Table 1. UV T_m (°C) of duplexes of ODN **8** with modified ODNs **9–13**[a,b]

	Control	1*S*, 2*S*[c]				1*R*, 2*R*[c]		1*R*, 2*R*[d]		1*S*, 2*R*[d]	
Entry	1	2	3	4	5	6	7	8	9	10	11
ODN	**9**	**10**	**11**	**12**	**13**	**12**	**13**	**10**	**13**	**10**	**13**
T_m	58	58	49	59	55	58	54	56	53	57	53

[a] All values ±0.5°.
[b] **10** one acyclic unit at 3′-end of the sequence, **11** one acyclic unit at the center of the sequence, **12** one acyclic unit at the 5′-end of the sequence, **13** three acyclic units at the 3′-end of the sequence.
[c] Ar=Ph.
[d] Ar=*p*-NO_2—Ph.

1.2. Synthesis and characterization of ODNs containing chiral acyclic backbone

The unmodified ODNs were synthesized using standard β-cyanoethyl phosphoramidite monomers. The phosphoramidites of 2(*R*/*S*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*/*S*)-aryl-1,3-propanediol (**5a–f**) were incorporated at various desired sites in ODNs (Scheme 2) using solid phase (CPG resin) synthesis on an automated DNA synthesizer. The modified ODNs were synthesized by a slight modification in the standard procedure[23] with the coupling time of the modified monomers increased to 15 min, to ensure completion of the reaction. The coupling efficiencies of the modified amidites were similar (>99%) to those of the normal amidites. After the completion of the synthesis, the ODNs were cleaved from the resin and all the base and phosphate protecting groups were simultaneously hydrolyzed by aqueous ammonia treatment to yield the completely deprotected ODNs. The modified ODNs were purified by polyacrylamide gel electrophoresis using 20% PAGE and their purity was ascertained by reverse phase HPLC. The retention of modification in ODNs was confirmed by MALDI-TOF mass spectral results for **10**.

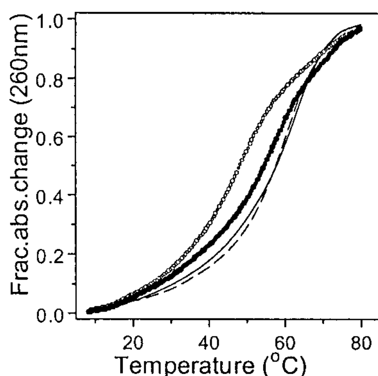


Figure 2. UV melting profiles for duplexes containing 1*S*, 2*S* (Ar=Ph) isomer (at pH 7.1). **8**:**9** (——); **8**:**10** (– – –), **8**:**11** (○○○) and **8**:**12** (●●●).

(Calculated mass for molecular formula $C_{182}H_{234}N_{41}O_{120}P_{17}$ average 5442.65, observed 5444.)

1.3. UV melting studies

1.3.1. Duplex melting studies

The various ODN duplexes were constituted from the unmodified ODN **9** and the modified ODNs **10–13**, using the common complementary unmodified 18-mer **8**. Table 1 summarizes the T_m data obtained from UV melting experiments of control (unmodified) and modified duplexes. It is seen that the incorporation of *threo* (1*S*, 2*S*) isomer at single sites at the 3′-end (Table 1, entry 2) or 5′-end (Table 1, entry 4, Fig. 2) has no effect on duplex T_m as compared to the control (Table 1, entry 1). Increasing the number of modifications to three at the 3′-end (Table 1, entry 5) slightly destabilized the duplex (ΔT_m/mod=−1°C). In contrast, even a single modification at the center of the duplex (Table 1, entry 3) caused a large destabilization (ΔT_m=−9°C) (Fig. 2). In the case of ODNs containing the (1*R*, 2*R*) isomer, the duplex T_m showed a similar trend, i.e. the 5′-end modification was as good as the control (Table 1, entry 7). Three modifications at the 3′-end (Table 1, entry 6) caused a slight destabilization (ΔT_m/mod=−1°C).

The incorporation of one unit of the *erythro* (1*S*, 2*R*) isomer at the 3′-end showed T_m (Table 1, entry 10) as good as that of the control, whereas three modifications with this stereoisomer (Table 1, entry 11) showed a slight destabilization (ΔT_m/mod=−1.5°C), similar to that observed with duplexes containing other ODNs (entries 6–11). Changing the substitution at the *para* position of the phenyl ring in the backbone (Ar=*p*-nitrophenyl) also had a negligible effect upon duplex formation with either a single end modification (ΔT_m/mod=−1°C) or even three modified monomeric units (ΔT_m/mod=−2°C) in the ODNs (Table 1, entries 8, 9). The UV melting profiles of duplexes comprising of **8**:**9**, **8**:**10**, **8**:**11** and **8**:**12** are shown in Fig. 2.

1.3.2. UV melting studies for triplexes

Triplexes were individually constituted from the unmodified 24-mer duplex **7**:**6** and individual ODNs **9–13** as third strand. Since triplex stabilities are sensitive to pH,[24] values

Table 2. UV T_m of triplexes from duplex **6**:**7** and modified ODNs **9–13**[a,b]

	Control	1*S*, 2*S*[c]				1*R*, 2*R*[c]				1*R*, 2*R*[d]		1*S*, 2*R*[d]		1*R*, 2*R*[e]	1*R*, 2*S*[e]
Entry	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ODN	**9**	**10**	**11**	**12**	**13**	**10**	**11**	**12**	**13**	**10**	**13**	**10**	**13**	**10**	**10**
pH 5.8	57	57	40	54	44	54	36	56	40	53	41	54	42	53	55
pH 7.1	39	35	17	31	22	34	17	31	22	33	23	35	22	33	33

[a] All values ±0.5°.
[b] **10** one acyclic unit at 3′-end of the sequence, **11** one acyclic unit at the center of the sequence, **12** one acyclic unit at the 5′-end of the sequence, **13** three acyclic units at the 3′-end of the sequence.
[c] Ar=Ph.
[d] Ar=*p*-NO_2—Ph.
[e] Ar=*p*-NH_2—Ph.

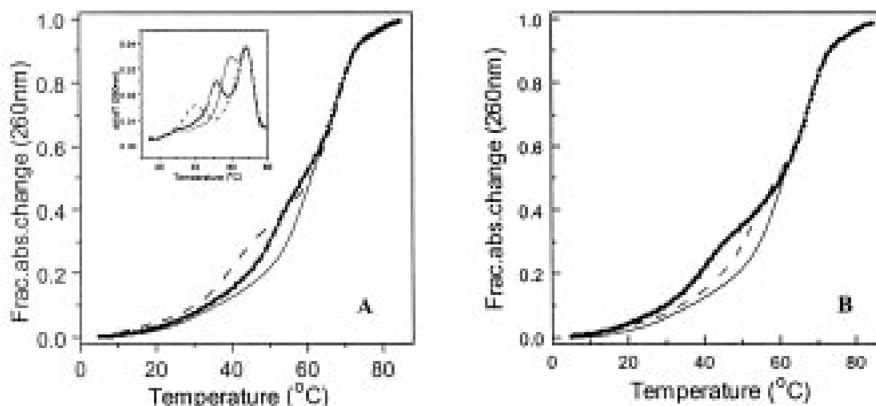


Figure 3. UV melting profiles for triplexes (at pH 5.8) containing (A) 1*R*, 2*R* (Ar=Ph—NO_2) and *inset*—first derivatives. (B) 1*S*, 2*R* (Ar=Ph—NO_2) **9*7**:**6** (——); **10*7**:**6** (– – –); **13*7**:**6** (●●●).

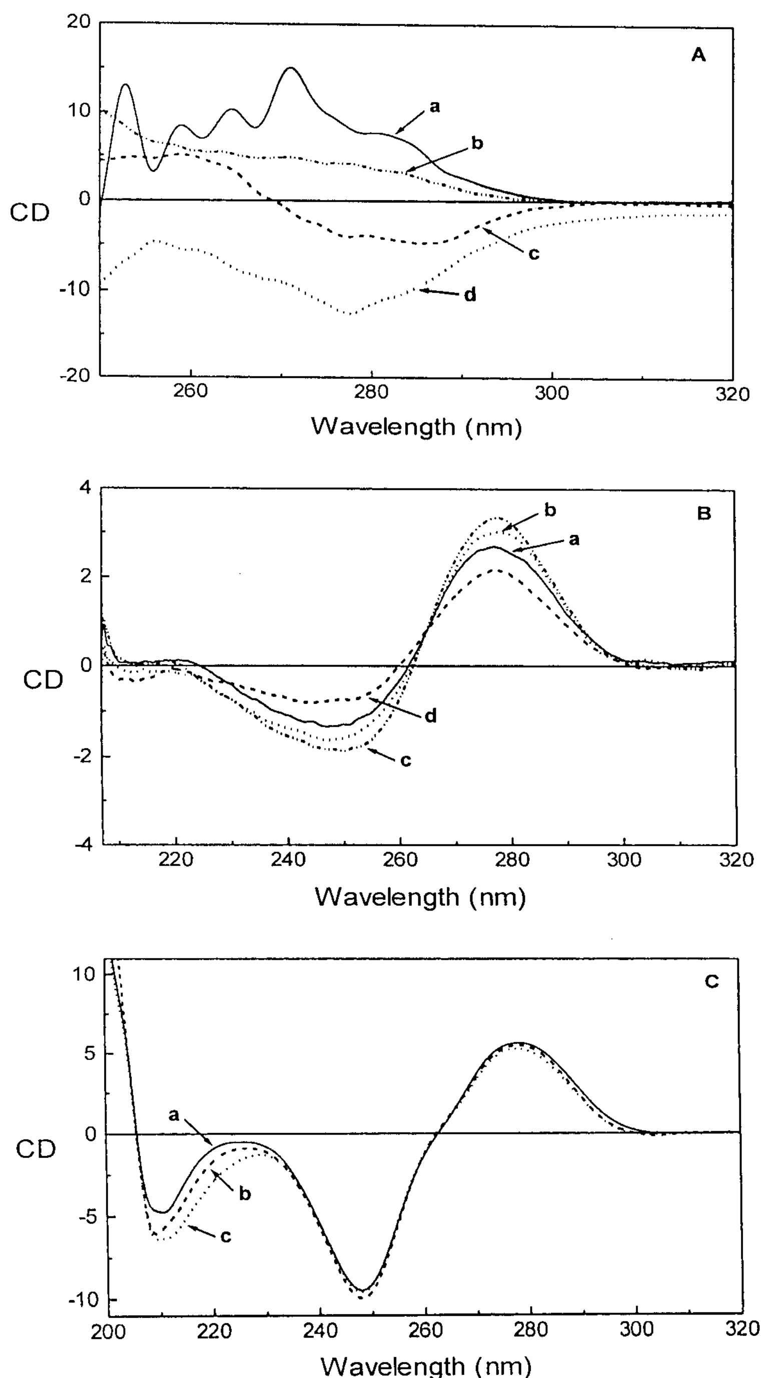


Figure 4. CD spectra of (A) monomers: (a) 1*S*, 2*S* (Ar=Ph); (b) 1*R*, 2*S* (Ar=Ph); (c) 1*R*, 2*R* (Ar=*p*-NO_2—Ph); (d) 1*S*, 2*R* (Ar=*p*-NO_2—Ph); (B) oligonucleotides: (a) **9**; (b) **10**; (c) **11**; (d) **13**; (C) triplexes: (a) **9*7**:**6**; (b) **10*7**:**6**; (c) **13*7**:**6**.

of UV T_m were measured at two different pH values, namely 5.8 and 7.1. All the triplexes showed characteristic biphasic sigmoidal transitions with the transition in the lower temperature range corresponding to the dissociation of the third strand and that in the higher temperature range arising from duplex denaturation. Accurate T_m values were determined from the first derivative curves. The results of the T_m data obtained from the UV melting experiments of various triplex forming ODNs containing chiral acyclic analogs (**4a–f**) are summarized in Table 2.

At pH 5.8, ODNs containing both the *threo* (1*R*, 2*R*) and (1*S*, 2*S*) enantiomers showed triplex formation, with (1*R*, 2*R*) *modifications being slightly less stable than* (1*S*, 2*S*) *modifications*. The triplex stability was also a function of the position of the modification in the sequence. For both, (1*R*, 2*R*) isomer **4b** and (1*S*, 2*S*) isomer **4a**, the most stable triplexes were observed when incorporation of the modified units was at the 3′-end (Table 2, entries 2, 6), followed by a slightly lower stability for the 5′-end modification (Table 2, entries 4, 8). Increasing the number of modifications to three at the 3′-terminus also effected more destabilization (Table 2, entries 5, 9) with a net magnitude of almost −4°C per substitution. The most detrimental effect was observed in

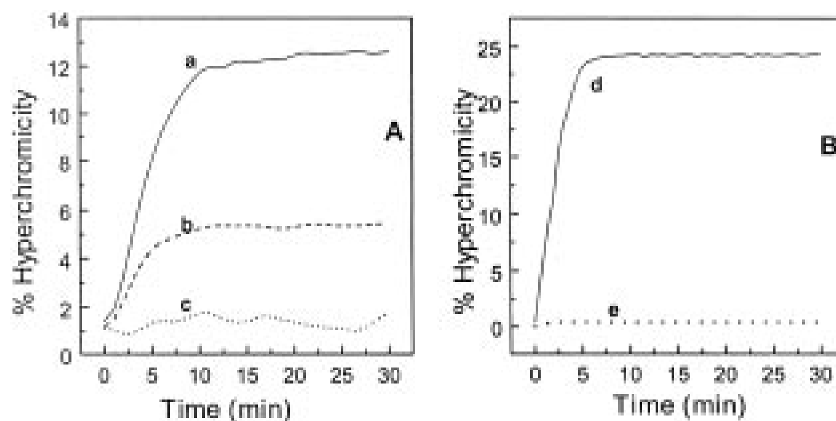


Figure 5. Time course of snake venom phosphodiesterase digestion of oligonucleotides: (a) **9**; (b) **11** (1*S*, 2*S*; Ar=Ph); (c) **10** (1*S*, 2*S*; Ar=Ph); (d) **15**; (e) **14** (1*R*, 2*R*; Ar=*p*-NO_2—Ph).

the case where the modification site is in the center of the sequence (Table 2, entries 3, 7). In general, the stabilization followed the order: control>3′-end>5′-end>three modifications at 3′-end>central modification.

In the case of ODNs containing *p*-nitro-substituted monomers (1*R*, 2*R*) **4d** (Table 2), at pH 5.8 a single modification at the 3′-end (Table 2, entry 10) destabilized the triplex (ΔT_m/mod=−4.5°C), whereas three modifications at the 3′-end caused a significant destabilization of −5°C per modification (Table 2, entry 11) (Fig. 3(A)). The triplexes with *p*-aminophenyl substituted analogs (1*R*, 2*R*) **4f** showed values of T_m similar (Table 2, entry 14) to those of their *p*-nitrophenyl analogs.

In the *erythro* geometry, as in the case of the (1*S*, 2*R*) isomer (Ar=*p*-NO_2—Ph), a single modification at the 3′-end gives a melting temperature identical to that of the (1*R*, 2*R*) isomer (Table 2, entry 12) at pH 5.8. Trisubstitutions with the same isomer at the 3′-end also shows no change in the melting profile (Fig. 3(B)). The trend remains the same even at pH 7.1 (Table 2, entry 13). The other *erythro* isomer, (1*R*, 2*S*), obtained by the inversion of the C1 stereocenter of (1*S*, 2*S*) isomer **4c** when Ar is phenyl, also exhibited no major changes in the binding properties of the triplex at either pH 5.8 or 7.1 (Table 2, entry 15).

The overall melting pattern was similar but less pronounced at pH 7.1. In the case of the control as well as the modified third strand, the values of T_m at this pH decreased by 18–23°C, as expected for C containing sequences.[24] The differences in values of T_m among different stereoisomers are more pronounced at lower pH, 5.8, than at neutral pH 7.1. All the complexes formed with the modified backbone show a linear increase in T_m with increasing salt concentration similar to the control triplexes.

Table 3. Rates of enzymatic digestion of different ODNs

ODN	$T_{1/2}$ (min)	% H[a]
9	4	11
11	3	5.5
10[b]	>120	0
13[b]	>120	0
14	3	24
15	>120	0

[a] % H=[((A_{260} after degradation)−(A_{260} before addition of SVPDE))/(A_{260} before addition of SVPDE)]×100.
[b] Modified oligonucleotides containing isomers 1*S*, 2*S*; 1*R*, 2*R*; 1*R*, 2*S* (Ar=Ph), 1*R*, 2*R*; 1*S*, 2*R* (Ar=*p*-NO_2—Ph).

1.4. CD spectroscopy

The CD spectra of acyclic nucleosides (**4a**, **4c**, **4d** and **4e**) recorded in $CHCl_3$ are shown in Fig. 4(A). Compound **4a** with (1*S*, 2*S*) stereochemistry showed a broad positive band at 270 nm (curve a), whereas the isomer with (1*R*, 2*R*) stereochemistry **4d** showed a broad negative band (curve c) at the same wavelength indicating the mirror image stereochemistry to that of the (1*S*, 2*S*) isomer. The inversion of the (1*S*, 2*S*) isomer at the C1 center of **4a** generating the (1*R*, 2*S*) isomer **4c** retained the broad positive band at 275 nm (curve b). In a similar way, the spectral pattern of (1*S*, 2*R*) as in **4e** showed a negative band at 285 nm (curve d), as that of the (1*R*, 2*R*) isomer. The CD spectra of single stranded ODNs containing different chiral acyclic analogs **4a–4f** are similar to that of the control single strand ODN. A selected set of such CD spectra containing the (1*S*, 2*S*) isomer **4a**, at different positions in ODNs **10**, **11** and **13** along with control **9** are shown in Fig. 4(B). The basic spectral profiles of all the ODNs are similar, irrespective of the position (at the 3′-end or in the center), or the number of modifications (single or three modifications in an ODN). Fig. 4(C) shows the CD spectra of triplexes constituted from the duplex (**7**:**6**) and the modified third strands containing the chiral acyclic backbone **4a** at different positions along with the CD spectrum of the control triplex. A characteristic negative band at around 210 nm, along with a positive band at 275 nm and negative band around 250 nm in all cases indicated successful triplex formation[25] and no differences were seen among the triplexes of control and modified ODNs.

1.5. Enzymatic stability of the oligomers

The stability of ODNs containing the acyclic nucleosides at different positions towards snake venom phosphodiesterase (SVPDE) was studied by following the increase in absorbance at 260 nm (hyperchromicity) after addition of SVPDE.[7,26] Fig. 5 shows the time-dependent hydrolysis of

modified ODNs **10** and **11** in comparison with that of the unmodified oligomer **9**. Since the hyperchromicity upon digestion of a polypyrimidine sequence is less compared to that of polypurines which have a better stacking, it was decided to study the enzymatic degradation of a mixed base sequence **14** as a typical example along with that of unmodified control **15**.

The results of the enzymatic stability studies are shown in Table 3. It is seen that the unmodified ODN **9** is completely digested in 8 min, whereas the ODNs with a single acyclic analog substitution towards the 3′-end, **10**, is stable to enzymatic degradation even after 2 h. An oligomer with an acyclic nucleoside in the central position of ODN, **11**, shows a hyperchromicity of about 50% of that observed for the unmodified ODN. The ODN with three consecutive substitutions at the 3′-end, **13**, is also stable to enzyme digestion. These results can be represented graphically as shown in Fig. 5. Thus, all the different isomers of chiral acyclic nucleosides protected the ODNs against enzymatic degradation.

2. Discussion

Most of the ODNs reported so far with acyclic units in the backbone show a significant decrease in the stability of the complexes formed between the modified ODNs and natural DNA or RNA.[5–7] The reason attributed to this destabilization is that the conformational freedom upon duplex formation from rigid ODNs favorably overrules the entropy loss resulting from hybridization of the ODN bearing flexible acyclic analogs with complementary unmodified ODNs. The well-documented chloramphenicol precursor *threo* 2(*R*)-amino-1(*R*)-aryl-1,3-propanediol and its enantiomer 2(*S*)-amino-1(*S*)-aryl-1,3-propanediol were very easily converted into the nucleoside analogs **4a–f** in excellent yields. The C1 center in both the isomers was easily inverted under Mitsunobu conditions to get the set of the *erythro* pair, i.e. 2(*R*)-amino-1(*S*)-aryl-1,3-propanediol and 2(*S*)-amino-1(*R*)-aryl-1,3-propanediol. The C2 center in this analog is equivalent to the C4′ center, whereas the C1 center is treated as analogous to the C3′ center of deoxyribonucleosides. Both these centers, C4′ and C3′ [*erythro* (3′*S*, 4′*R*)], play a very important role in dictating the stability of natural nucleosides when present in a five-membered furanose sugar ring structure. The substitution on the furanose ring also affects the sugar ring puckering, which in turn directs the helix conformation and thus, stability of the resulting duplexes. The acyclic backbone carries a bulky aryl substituent at C1, a position equivalent to C3′ in the furanose ring. Any structural perturbation in the duplex/triplex due to the incorporation of acyclic backbone **4a–f** was expected to be less pronounced at the 3′/5′ end positions of the sequences. This is clearly demonstrated by the UV melting studies (Tables 1 and 2). The complexes of modified ODNs **10** and **12** with target ss/ds DNA were as stable as those formed by the control ODN **9**. The effect of the acyclic backbone in ODNs on complex formation is expected to be more evident in the central position of the ODNs and is clearly reflected in the stability of the duplexes/triplexes formed comprising modified ODNs with target sequences. Even the presence of three consecutive acyclo analogs is tolerated better at the end of sequences, as compared to just one at the center of modified ODN. The stereochemical preference for any one of the four isomers is not entirely evident from the UV melting studies, though the (1*S*, 2*S*) isomer **4a** shows better stability for both duplex and triplex formation. Aryl substitution at the C1 center was expected to provide additional stabilization through stacking interactions. The sequence **13**, having three consecutive modified monomers, was thus synthesized. The complexes formed with this sequence in all the cases showed some destabilization both, for duplexes ($\Delta T_m = -1–2$°C/mod) and triplexes ($\Delta T_m = -4–5$°C/mod). A marginal preference for the (1*S*, 2*S*) stereoisomer was again observed. The conformational freedom in the open chain analog may entropically disfavor the aryl ring stacking interactions and hence any additional stability due to multiple incorporation of the modified monomer is not seen. The (1*S*, 2*S*) **4a** *threo* isomer thus showed overall better binding efficiency in open chain structure although the geometry of the corresponding C3′ and C4′ centers is (3*S*, 4*R*) *erythro* in the natural furanose ring.

The (1*S*, 2*R*) isomer **4e**, having the *erythro* geometry as in deoxyribose sugar did not show significantly different binding from the other isomers, **4a–d** in its binding properties. Thus, the presence of *erythro* geometry and a second equivalent chiral center at C1 does not favorably assist complex formation. The base attachment through an amide linkage in the acyclic backbone does provide some structural rigidity, but may not be as favorable as when attached directly to the pentofuranose ring in the β orientation as in natural nucleosides. Different substitutions on the phenyl ring such as, *p*-nitro or amino functions caused negligible change in the stability of the complexes and hence showed the acceptance of different substitutions in the 3′/5′ ends of the backbone. Such a finding is of potential use, as the amino substitution can be used as a handle for the attachment of useful linkers or fluorophores.

The CD spectral studies indicated that even though the monomer spectral profiles of (1*S*, 2*S*) and (1*R*, 2*R*) isomers (**4a** and **4b**) are opposite to each other, upon incorporation into ODNs, the basic spectral structure is similar to that of the control ODN. This result reiterates that the intra-strand base stacking in the acyclic backbone is similar to that in natural ODNs. The CD spectra of triplexes containing the modified ODNs showed a pattern similar to that of the unmodified triplex, with the characteristic negative band at 210 nm. This further confirmed the overall similarity in the structure of both, modified and unmodified complexes. The overall CD results suggest that the incorporation of different isomers does not drastically change the basic single strand conformation of ODNs. This may be the reason for the insensitivity of the overall binding patterns in duplexes and triplexes to stereochemistry, since, upon incorporation, all stereoisomers adopt a similar conformation to that of the natural nucleosides.

Water plays an important role in the formation of a hydrogen-bonding network in the major and minor grooves of the DNA duplex. Any hydrophobic substitution in this groove or the addition of salt displaces some of the water molecules to the bulk. This phenomenon of displacement of water

molecules from the ordered hydration network to the disordered surroundings gives an entropic advantage and thereby stabilizes the complex. The phenyl substitution in the described acyclic backbone may cause similar hydrophobic desolvation locally in the major groove.[27] Although the acetamide nucleobase linker and the phenyl rings in the acyclic backbone are hydrophobic in nature, the backbone is composed of negatively charged phosphate groups as internucleoside linkages. The aqueous solubility of the oligomers therefore was found to be as good as natural oligomers.

An important requirement to be met by ODNs as potential therapeutic agents in the antisense or antigene strategy, is their stability against degradation by nucleases present within the cell and in serum. The main nuclease activity present in fetal calf serum is that of 3′-exonuclease.[28] The oligomers containing chiral acyclic nucleosides **I**, with different isomers at their 3′-end, show extremely high stability towards SVPDE. From the observed results of enzymatic digestion experiments, it can be concluded that SVPDE is not able to cleave the phosphodiester linkage between a natural nucleoside and acyclic nucleoside **I**. As a result, ODNs with modification at the 3′-end did not show any hyperchromicity upon enzymatic hydrolysis, whereas, an unmodified ODN is completely broken down within 8 min. An oligomer with an acyclic nucleoside in the center showed a hyperchromicity about 50% of that observed for the unmodified ODN resulting most probably from sequential degradation of a 19-mer ODN to a 10-mer after which it is stabilized by the presence of the acyclic nucleoside at its 3′-end.

3. Conclusion

The present study demonstrates that the incorporation of acyclic nucleosides derived from precursors of chloramphenicol with two chiral centers within the normal DNA backbone, leads to the formation of stable DNA duplexes and triplexes when present at the 3′/5′ ends of the ODNs. Different substitutions on the phenyl ring only slightly decrease the stability of the complexes, thereby proving the versatility of this backbone for fluorescent labelling. The structural adaptation of all the four stereoisomers with C1/C2 stereogenic centers allows the oligomers to form duplexes/triplexes with target sequences with almost equal efficiency as that of the natural oligomer, the diastereomer (1*S*, 2*S*) being marginally favored over other stereoisomers. The important finding is that the incorporation of this chiral acyclic nucleoside at the 3′-end of the ODN protected the ODN against enzymatic degradation, without compromising the stability of the complexes.

The presently observed hybridization properties of the modified ODNs along with the exhibited enzymatic stability have potential use in the design of chimeric backbone based second generation antisense/antigene therapeutic agents.[29] A novel class of nucleoside analogs (β-lactam nucleoside chimeras) has been reported as an example of potential dual action drugs.[30] The presently used modification is a close substructure of the active pharmacophore chloramphenicol and the hybrid molecules (monomers and oligomers) as designed here could be further examples of this class of molecules. This is, to the best of our knowledge, the first example of an acyclic nucleoside analog with two chiral centers forming stable duplexes and triplexes.

4. Experimental

All the chemicals used were of laboratory or analytical grades. All the solvents used were purified according to the literature procedures. TLC was performed on Merck pre-coated 60 F_{254} plates and the spots were rendered visible by UV light and/or as dark spots after spraying with perchloric acid in ethanol (60%) followed by charring. Column chromatography was carried out for purification of compounds on Loba silica gel (100–200 mesh). ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Bruker ACF 200 spectrometer fitted with an Aspect 3000 computer. All chemical shifts are referred to internal TMS unless otherwise mentioned and chemical shifts are expressed in δ scale (ppm). ^{31}P NMR spectra were recorded at 81 MHz with 85% H_3PO_4 as external reference. Optical rotations were measured on a JASCO DIP-181 polarimeter.

4.1. General procedure for *N*-acetylation

The starting amine (1 mmol) was taken in dioxane (50 ml) containing 10% aq. Na_2CO_3 (50 ml, pH~8.5). The mixture was cooled in an ice-bath and 2-chloroacetyl chloride was added in two portions (2.5 mmol each). After 5–10 min, the pH was adjusted to ~8.5 by the addition of aq. Na_2CO_3. The reaction mixture was concentrated to half its volume and extracted with ethyl acetate (80 ml×3). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography to obtain a thick colorless liquid which solidified after keeping.

4.1.1. 2(*S*)-(*N*-Chloroacetyl)-amino-1(*S*)-phenyl-1,3-propanediol, 2a. Mp 151°C; ν_{max}(Nujol) 3386, 1653, 1537 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3+D_2O$) δ 7.35 (brs, 5H, Ph), 5.05 (d, 1H, *J*=4.0 Hz, PhCH), 4.1 (m, 1H, C2—*H*), 4.0 (d, 1H, *J*=15.1 Hz, CH_2Cl), 3.89 (d, 1H, *J*=15.7 Hz, CH_2Cl), 3.82 (d, 2H, *J*=5.0 Hz, CH_2OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.0 (CO), 140.8 (Ar), 128.2, 127.6, 125.6 (Ar), 72.0 (C-1), 62.1 (C-3), 56.6 (C-3), 42.3 (CH_2Cl). [Found: C, 54.41; H, 5.34; N, 5.75. $C_{11}H_{14}NO_3Cl$ requires C, 54.7; H, 5.74; N 5.8.] $[\alpha]_D^{20}$=+36.0 (*c*=0.2, MeOH).

4.1.2. 2(*R*)-(*N*-Chloroacetyl)-amino-1(*R*)-phenyl-1,3-propanediol, 2b. Mp 150°C; ν_{max}(Nujol) 3380, 1652, 1537 cm^{-1}; ^{1}H NMR (200 MHz) δ 7.4 (bs, 5H, Ph), 5.05 (d, 1H, *J*=4.8 Hz, PhCH), 4.2 (m, 1H, C2—H), 4.0 (d, 1H, *J*=15.1 Hz, CH_2Cl), 3.89 (d, 1H, *J*=15.7 Hz, CH_2Cl), 3.7 (d, 2H, *J*=5.0 Hz, CH_2OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.0 (CO), 140.9 (Ar), 128.4, 127.9, 125.4 (Ar), 72.2 (C-1), 62.4 (C-3), 56.3 (C-3), 42.2 (CH_2Cl). [Found: C, 54.41; H, 5.34; N, 5.75. $C_{11}H_{14}NO_3Cl$ requires C, 54.7; H, 5.74; N 5.8.] $[\alpha]_D^{20}$=−37.0 (*c*=0.2, MeOH).

4.1.3. 2(*R*)-(*N*-Chloroacetyl)-amino-1(*R*)-*p*-nitrophenyl-1,3-propanediol, 2c. Mp 96°C; ν_{max}(Nujol) 3420, 1700, 1510 cm^{-1}; ^{1}H NMR (200 MHz) δ 7.5, 8.05 (dd, 4H, *J*=10.8 Hz, Ar), 7.2 (d, 1H, *J*=6.5, CHAr), 5.34 (d, 1H,

J=6 Hz, C2—H), 5.15 (Br, 1H, OH), 4.5 (Br, 1H, OH), 4.1 (m, 1H), 3.85 (d, 1H, J=16.2 Hz, CH_2Cl), 3.7 (d, 1H, J=16.2 Hz, CH_2Cl), 3.65 (m, 2H, CH_2OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.4 (CO), 148.9, 146.9 (Ar), 126.5, 122.9 (Ar), 71.02 (C-1), 61.98 (C-3), 55.95 (C-2), 42.09 (CH_2Cl). [Found: C, 45.65; H, 4.85; N, 9.59. $C_{11}H_{13}N_2O_5Cl$ requires C, 45.7; H, 4.5; N, 9.7.] $[\alpha]_D^{20}=-10.4$ (c=0.3, MeOH).

4.2. General procedure for the preparation of *N*-thymin-1-ylacetylamino-1-aryl-1,3-propanediol

The *N*-chloroacetyl compound (1 mmol) was taken in dry DMF (25 ml). Thymine (1 mmol) and anhydrous K_2CO_3 (1.1 mmol) were added and the reaction mixture stirred overnight at room temperature or 55°C. After completion of the reaction as checked by TLC, the solvent was completely removed to get the crude product. Purification by column chromatography gave the compounds **3a–c** as gums which slowly solidified upon keeping. In some cases the crude material could be directly used for the protection of the primary hydroxyl group without purification as thymine nucleobase impurity could not be completely separated.

4.2.1. 2(*S*)-(*N*-Thymin-1-ylacetyl)-amino-1(*S*)-phenyl-1,3-propanediol, 3a. Mp 213°C; ν_{max}(Nujol) 3440, 1695, 1510 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6) δ 11.1 (s, 1H, NH), 7.3 (m, 5H, Ph), 5.0 (d, 1H, J=3.5 Hz, PhCH), 4.3 (2×d, 2H, J=15.9 Hz, CH_2—T), 4.1 (m, 1H, C-1 H), 3.65 (m, 2H, CH_2OH), 1.85 (s, 3H, T—CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.0, 158.1, 164.3 (CO), 154.8–125.6, 113.4 (Ar), 111.5 (C-5), 73.1 (C-1), 62.1 (C-3), 56.1 (C-2), 49.3 (CH_2 T), 12.6 (T—CH_3). [Found: C, 57.4; H, 5.67. $C_{16}H_{19}N_3O_5$ requires C, 57.65; H, 5.70.] FAB MS: (M+H) 334, (M+Na), 356. α_D=+25.0 (c=0.4, MeOH).

4.2.2. 2(*R*)-(*N*-Thymin-1-ylacetyl)-amino-1(*R*)-*p*-nitrophenyl-1,3-propanediol, 3c. Mp 257°C; ν_{max}(Nujol) 3441, 1700, 1510 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6) δ 11.2 (s, 1H, NH), 7.65, 8.15 (2×d, 5H, J=9.6 Hz, Ar, T—C6), 5.85 (d, 1H, J=6.0 Hz), 5.75 (brs, 1H, OH), 5.05 (brs, 1H, OH), 4.25 (dd, 2H, J=16.1 Hz, CH_2T), 3.45 (m, 2H, CH_2OH), 1.95 (s, 3H, T—CH_3) ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.1, 163.5, 150.2 (CO), 140.5, 126.4, 121.8 (Ar), 107.8 (C-5), 69.2 (C-1), 60.02 (C-3), 55.6 (C-2), 48.48 (CH_2T), 10.87 (T—CH_3). FAB MS: (M+H) 378, (M+Na) 401. α_D=+51.2 (c=0.3, MeOH).

4.3. General procedure for dimethoxytrityl protection

The starting material (1 mmol) was co-evaporated twice with dry pyridine (5 ml) and taken in dry pyridine (10 ml). 4,4′-Dimethoxytrityl chloride (1.2 mmol) was added in two batches in the interval of 0.5–1 h. The reaction mixture was stirred at ambient temperature and monitored for completion by TLC. After completion of the reaction, the solvent was completely removed and the pure product in the form of a foam was obtained after column chromatography using DCM/methanol as the eluting system.

4.3.1. 3-*O*-Dimethoxytrityl-2(*S*)-(*N*-thymin-1-ylacetyl)-amino-1(*S*)-phenyl-1,3-propanediol, 4a. ν_{max}(Nujol) 1704, 1688, 1660, 1525, 1505, 1110 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.74 (s, 1H, NH), 6.75–7.5 (m, 19H, Ar, T—C6), 5.05 (d, 1H, J=5.4 Hz), 4.2 (m, 1H), 4.1 (d, 1H, J=16.2 Hz), 3.9 (d, 1H, J=16.2 Hz), 3.8 (s, 6H), 3.36 (m, 2H), 1.86 (d, 3H, J=1.5 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 110.4 (C-5), 86.4 (CPh[OMePh]$_2$), 73.3 (C-1), 62.7 (C-3), 55.1 (C-2), 54.8 (OCH_3), 49.6 (CH_2—T), 11.7 (T—CH_3). FAB MS: (M+Na) 658.

4.3.2. 3-*O*-Dimethoxytrityl-2(*R*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*)-phenyl-1,3-propanediol, 4b. ν_{max}(Nujol) 1700, 1686, 1660, 1515, 1505, 1110 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.74 (s, 1H), 6.85–7.5 (m, 19H), 5.05 (d, 1H, J=5.4 Hz), 4.25 (m, 1H), 4.1 (d, 1H, J=16.2 Hz), 3.9 (d, 1H, J=16.2 Hz), 3.8 (s, 6H), 3.5 (m, 2H), 1.9 (d, 3H, J=1.4 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 110.8 (C-5), 86.4 (CPh[OMePh]$_2$), 73.1 (C-1), 63.5 (C-3), 55.5 (C-2), 55.2 (OCH_3), 50.5 (CH_2—T), 12.2 (T—CH_3). FAB MS: (M+Na) 658.

4.3.3. 3-*O*-Dimethoxytrityl-2(*R*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*)-*p*-nitrophenyl-1,3 propanediol, 4d. ^1H NMR (200 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.65 (s, 1H), 6.85–8.05 (m, 18H, Arom), 5.05 (d, 1H, J=5.8 Hz), 4.25 (m, 1H), 4.3 (d, 1H, J=17.6 Hz), 4.05 (d, 1H, J=17.6 Hz), 3.8 (s, 6H), 3.35 (m, 2H), 1.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 75.476 MHz) δ 110.8 (C-5), 86.2 (CPh[OMePh]$_2$), 71.6 (C-1), 62.4 (C-3), 55.5 (C-2), 54.7 (OCH_3), 49.8 (CH_2—T), 11.5 (T—CH_3). FAB MS: (M+H) 680, (M+Na) 703.

4.4. General procedure for Mitsunobu reaction and hydrolysis of resulting benzoyl ester

The starting compound **4** (1 mmol) was taken in dry THF (10 ml), to which, triphenylphosphine (1.5 mmol) and benzoic acid (1.5 mmol) were added. The reaction mixture was stirred for a few min in an ice-bath, DIAD (1.5 mmol) was added and the reaction was stirred at ambient temperature for a few hours and monitored by TLC. After completion of the reaction, the solvent was completely removed. The pure product was obtained after column chromatography using 20–70% ethyl acetate with petroleum ether containing 0.5% pyridine as eluent. The compound was taken in methanol (AR, 5 ml), to which, methanol saturated with ammonia (5 ml) was added. The reaction mixture was left tightly closed at room temperature overnight. The solvent was completely removed and the pure products were obtained as foams using column chromatography in good yields.

4.4.1. 3-*O*-Dimethoxytrityl-2(*S*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*)-phenyl-1,3-propanediol, 4c. ^1H NMR (200 MHz, $CDCl_3$) δ 6.75–7.4 (m, 19H), 5.85 (d, 1H, J=5.4 Hz), 4.2 (2×d, 2H, J=16.2 Hz), 4.05 (m, 1H), 3.75 (s, 6H), 3.36 (m, 2H), 1.85 (d, 3H, J=1.5 Hz). ^{13}C NMR ($CDCl_3$, 50 Hz) δ 110.7 (C-5), 86.5 (CPh[OMePh]$_2$), 74.5 (C-1), 61.5 (C-3), 55.0 (C-2), 54.7 (OCH_3), 50.1 (CH_2—T), 12.0 (T—CH_3). FAB MS: (M+Na) 658.

4.4.2. 3-*O*-Dimethoxytrityl-2(*R*)-(*N*-thymin-1-ylacetyl)-amino-1(*S*)-*p*-nitrophenyl-1,3-propanediol, 4e. ^1H NMR (200 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.65 (s, 1H), 6.80–8.05 (m, 18H), 5.05 (d, 1H, J=5.8 Hz), 4.3 (m, 1H), 4.15 (2×d,

2H, J=16.8 Hz), 3.75 (s, 6H), 3.25 (m, 2H), 1.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 110.8 (C-5), 86.7 ($CPh[OMePh]_2$), 73.7 (C-1), 61.1 (C-3), 55.5 (C-2), 55.0 (OCH_3), 50.4 (CH_2—T), 12.0 (T—CH_3). FAB MS: (M+H) 680, (M+Na) 703.

4.4.3. 3-*O*-Dimethoxytrityl-2(*R*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*)-*p*-aminophenyl-1,3-propanediol, 4f. To a solution of 3-*O*-dimethoxytrityl-2(*R*)-(*N*-thymin-1-ylacetyl)-amino-1(*R*)-*p*-nitrophenyl-1,3-propanediol **4c** (0.15 g, 0.22 mmol) in pyridine/methanol (6 ml, 1:1) was added 10% Pd/C (0.02 g). The reaction mixture was hydrogenated under 40 psi hydrogen pressure for 4 h. The reaction mixture was filtered and the filtrate was concentrated to get product **4f** (0.14 g, yield 98%), which was used for further reaction without purification.

^{1}H NMR (200 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.65 (s, 1H), 6.85–8.05 (m, 18H), 5.05 (d, 1H, J=5.8 Hz), 4.25 (m, 1H), 4.18 (2×d, 2H, J=17.6 Hz), 3.8 (s, 6H), 3.35 (m, 2H), 1.85 (s, 3H).

4.5. General procedure for amidite preparation

The starting material **4** (0.5 mmol) and tetrazole (0.5 mmol) were co-evaporated with dry dichloroethane (5–10 ml) twice and re-dissolved in dry dichloroethane (5 ml). 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.6 mmol) was added and the reaction mixture was stirred at ambient temperature for 3 h. It was then diluted with dichloromethane and washed with 10% aqueous sodium bicarbonate solution. The organic phase was dried over anhydrous Na_2SO_4 and concentrated to a foam. The residue was dissolved in minimum amount of dichloromethane and loaded on a column of dry silica gel packed in dry DCM. The product **5** was eluted using a mixture of ethyl acetate/dichloromethane/TEA (1:1:0.1). The compound was obtained as a mixture of diastereomers. ^{31}P NMR (81 MHz, $CDCl_3$): δ **5a** 1*S*, 2*S* (Ar=Ph), 149.7, 149.3; **5b** 1*R*, 2*R* (Ar=Ph), 150.3, 149.6; **5c** 1*R*, 2*R* (Ar=*p*-NO_2—Ph), 151.2, 150.1; **5d** 1*R*, 2*S* (Ar=Ph), 149.7, 148.7; **5e** 1*S*, 2*R* (Ar=*p*-NO_2—Ph), 150.9, 149.3; **5f** 1*R*, 2*R* (Ar=*p*-NH_2—Ph), 150.6, 149.5.

4.6. Oligonucleotide synthesis and purification

N-Protected standard nucleoside phosphoramidites (A, T, G and C) and nucleoside derivatized controlled pore glass supports were purchased from Cruachem, UK. The DNA synthesis was carried out on Pharmacia LKB-Gene Assembler Plus. The crude ODNs were cleaved from the support, deprotected using aqueous ammonia at 55°C for 16 h and desalted using NAP-10 gel filtration columns. The desalted ODNs were purified by 20% PAGE under denaturing conditions. The purity of the ODNs were rechecked by reverse phase HPLC using the buffer systems A: 5% CH_3CN in 0.1M triethylammmoniumacetate pH 7.0 (TEAA) and B: 30% CH_3CN in 0.1M TEAA using a gradient A to B of 1.5% min^{-1} at a flow rate of 1.5 ml min^{-1}. The absorption spectrum of each peak was scanned in the range of 200–600 nm using a diode array detector. **10** (calculated mass for molecular formula $C_{182}H_{234}N_{41}O_{120}P_{17}$ average 5442.65, observed 5444).

4.7. UV melting experiments

Duplex and triplex melting experiments were carried out in the buffer 100mM sodium cacodylate containing 20mM $MgCl_2$ and 1M NaCl at the pH mentioned in each case. Appropriate ODNs, each at a strand concentration of 1 μM based on UV absorbance at 260 nm calculated using molar extinction coefficients of dA=15.4, dC=7.3, dG=11.7, T=8.8 cm^2 μmol^{-1} were mixed and heated at 80°C for 3 min, cooled to room temperature followed by overnight storage at 4°C. The A_{260} at various temperatures were recorded using a Perkin–Elmer lambda 15 UV/VIS spectrophotometer, fitted with a water jacketed 5-cell holder and a Julabo temperature programmer with a heating rate of 0.5°C min^{-1} over a range of 5–80°C. The spectrophotometer chamber was flushed with dry nitrogen gas to prevent moisture condensation at temperatures below 15°C. The melting temperatures T_m were determined from the midpoints of the transitions in the plots of fraction absorbance change versus temperature and were further confirmed by differential (dA/dT versus T) curves. The T_m values are accurate to ±0.5°C over the reported values and are the average of three sets of experiments.

4.8. Circular dichroic spectral studies

Circular dichroism spectra were recorded on a JASCO J-715 spectropolarimeter attached to a Julabo water circulator for maintaining the temperature. The samples were scanned in the range of 320–200 nm at a scan speed of 200 nm min^{-1}, band width 1.0 nm, sensitivity 10 mdeg, resolution 0.1 nm and response factor 2 s. Each spectrum was recorded as an accumulation of five scans using a 10 mm cell. The samples were made in a similar manner to that for UV melting experiments by taking 1 μM of each appropriate strand. The cell was thermostated using a Julabo water circulator at 10°C for all measurements.

4.9. Enzymatic stability of oligonucleotides

A solution of the ODNs (0.2–0.3 OD) in 2.0 ml of the pH 8.6 buffer (0.1M Tris–HCl; 0.1M NaCl; 14mM $MgCl_2$) was digested with 1.2 units SVPDE (Sigma) (34 ml of a solution of the enzyme in the following buffer: 5mM Tris–HCl; pH 7.5; 50% glycerol (v/v)) at 25°C. During digestion, the increase in UV absorbance at 260 nm was followed. The absorption versus time curve of the digestion was plotted from which the hyperchromicity and half-life of the oligomer were evaluated.

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References

1. (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584. (b) Cook, P. D. *Anticancer Drug Res.* **1991**, *6*, 585. (c) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923–1927. (d) Bennett, C. F. In *Applied Antisense Oligonucleotide Technology*, Stein, C. A., Craig, A. M., Eds.; Wiley-Liss: New York, 1998; pp 129–146.
2. Crooke, S. T. In *Burgers Medicinal Chemistry and Drug Discovery*, Wolff, M. E., Ed.; John Wiley: New York, 1995.
3. Sun, J.-S.; Garestier, T.; Helene, C. *Curr. Opinion Struct. Biol.* **1996**, *6*, 327.
4. Crooke, S. T. *Chem. Ind.* **1996**, 90–93.
5. (a) Oglivie, K. K.; Nguyen-Ba, N.; Gillen, M. F.; Radatus, B. K.; Cheriyan, U. O.; Hanna, H. R.; Smith, K. O.; Galloway, K. S. *Can. J. Chem.* **1984**, *62*, 241–252. (b) Hakimelahi, G. H.; Zarrinehzad, M.; Jarrahpour, A. A.; Sharghi, H. *Helv. Chim. Acta* **1987**, *70*, 219–231.
6. (a) Schneider, K. C.; Benner, S. A. *J. Am. Chem. Soc.* **1990**, *112*, 453–455. (b) Azymah, M.; Chavis, C.; Lucas, M.; Morvan, F.; Imbach, J.-L. *Nucleosides Nucleotides* **1992**, *11*, 1241–1255.
7. (a) Augustyns, K.; Van Aerschot, A.; Van Schepdael, A.; Urbanke, C.; Herdewijn, P. *Nucleic Acids Res.* **1991**, *19*, 2587–2593. (b) Vandendriessche, F.; Snoeck, R.; Janssen, G.; Hoogmartens, J.; Van Aerschot, A.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1992**, *35*, 1458.
8. (a) Nielsen, P.; Kiepeker, F.; Wengel, J. *Nucleic Acids Res.* **1994**, *22*, 703–710. (b) Nielsen, P.; Dreioe, L. H.; Wengel, J. *Bioorg. Med. Chem. Lett.* **1995**, *3*, 19–28.
9. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1499. (b) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895–1897.
10. Nielsen, P. E. In *Peptide Nucleic Acids, Protocols and Applications*, Nielsen, P. E., Egholm, M., Eds.; Horizon Scientific Press: Norfolk, 1999.
11. Peyman, A.; Uhlmann, E.; Wagner, K.; Augustin, S.; Breipohl, G.; Will, D. W.; Schaffer, A.; Wallmeier, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2636–2638.
12. Merle, Y.; Bonneil, E.; Merle, L.; Sagi, J.; Szemzo, A. *Int. J. Biol. Macromol.* **1995**, *17*, 239.
13. (a) Ramasamy, K. S.; Seifert, W. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1799–1804. (b) Benheida, R.; Devys, M.; Fourrey, J.-L.; Lecubin, F.; Sun, J.-S. *Tetrahedron Lett.* **1998**, *39*, 6167–6170.
14. (a) Van der Laan, A. C.; Stromberg, R.; Van Boom, J. H.; Kuyl-Yeheskiely, E.; Effimov, V. A.; Chakhmakhcheva, O. G. *Tetrahedron Lett.* **1996**, *37*, 7857–7860. (b) Kehler, J.; Henriksen, U.; Vejbjerg, H.; Dahl, O. *Bioorg. Med. Chem.* **1998**, *6*, 315–322.
15. (a) Uhlmann, E.; Peymann, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2796–2823. (b) Sforza, S.; Corradini, R.; Ghirardi, S.; Dossena, A.; Marchelli, R. *Eur. J. Org. Chem.* **2000**, 2905–2913. (c) D'Costa, M.; Kumar, V.; Ganesh, K. N. *Organic Lett.* **1999**, *1*, 1513–1516.
16. Herdewijn, P. *Leibigs Ann.* **1996**, *9*, 1337–1348.
17. Bolli, M.; Lubini, P.; Leumann, C. *Helv. Chim. Acta* **1995**, *78*, 2077–2096.
18. Rana, V. S.; Kumar, V. A.; Ganesh, K. N. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2837–2840.
19. Tsantrizos, Y. S.; Lunetta, J. F.; Boyd, M.; Fader, L. D.; Wilson, N.-C. *J. Org. Chem.* **1997**, *62*, 5451–5457.
20. Boehringer, C. F.; Soehne, G.m.b.H. Brit. Patent 741,711, 1955; *Chem. Abstr.* **1957**, *51*, 5830h.
21. Jones, R. A. In *Oligonucleotide Synthesis: A Practical Approach*, Gait, M. J., Ed.; IRL Press: Oxford, 1984; pp 27.
22. Sinha, N. D.; Biernat, J.; McManus, J.; Koster, H. *Nucleic Acids Res.* **1984**, *12*, 4539–4557.
23. *Pharmacia LKB Gene Assembler Plus User's Manual.*
24. Ganesh, K. N.; Kumar, V. A.; Barawkar, D. A. In *Supramolecular Control of Structure and Reactivity*, Hamilton, A. D., Ed.; John Wiley: New York, 1996; Vol. 3, pp 267–315.
25. (a) Rana, V. S.; Ganesh, K. N. *Nucleic Acids Res.* **2000**, *28*, 1162–1169. (b) Manzini, G.; Xodo, L. E.; Gasparotto, D. *J. Mol. Biol.* **1990**, *213*, 833–843.
26. Xiang, G.; McLaughlin, L. W. *Tetrahedron* **1998**, *54*, 375–392.
27. Rajeev, K. G.; Jadhav, V. R.; Ganesh, K. N. *Nucleic Acids Res.* **1997**, *25*, 4187–4193.
28. Shaw, J.-P.; Kent, K.; Bird, J.; Fishback, J.; Froehler, B. *Nucleic Acids Res.* **1991**, *19*, 747–750.
29. Agrawal, S. *Trends Biotechnol.* **1996**, *14*, 376.
30. Domling, A.; Starnecker, M.; Ugi, I. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2238–2239.