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OLIGONUCLEOTIDES WITH (N-THYMIN-1-YLACETYL) 1-PHENYLSERINOL IN BACKBONE: CHIRAL ACYCLIC ANALOGUES THAT FORM DNA TRIPLEXES

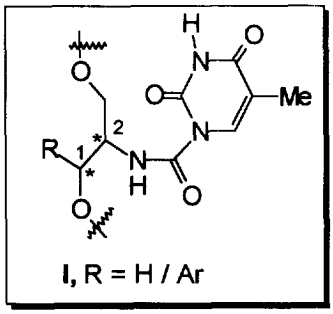
Vipul S. Rana, Vaijayanti A. Kumar* and Krishna N. Ganesh*

Organic Chemistry Synthesis Division, National Chemical Laboratory, Pune 411008, India.

Abstract: The synthesis of oligonucleotides containing chiral acyclic 2(R/S)-(N-thymin-1-ylacetyl)-amino-1(R/S)-phenyl-1,3-propanediol unit in the backbone (I, R=Ar) is described. When used as third strand of a triplex with complementary natural duplex, the modified oligonucleotides form stable triplexes with triplex <=> duplex transition Tm dependent on the number, position and stereochemistry of modification. © 1997 Elsevier Science Ltd.

Oligonucleotides and their analogs are finding increasing applications in bioorganic chemistry and molecular biology, in particular, to study the mode of action of nucleic acid binding drugs including antisense/antigene agents[1-4] and as diagnostic agents.[5,6] This has necessitated chemical modifications of nucleic acids at sugar,[7] phosphate[8] or nucleobase[9,10] residues, aimed at enhancing cell permeability and imparting nuclease resistance, without compromising on their hybridizing abilities via Watson-Crick (duplex) or Hoogsteen (triplex) base pairing. The replacement of 2-deoxy-2-ribose moiety of nucleoside by an acyclic chain has led to several analogues which show resistance to enzymes but the general finding is that these acyclic ODN analogues do not form duplexes of acceptable stability with natural oligonucleotides,[11-13] with the exception of peptide like acyclic backbone (PNA).[14] The loss in conformational freedom upon duplex formation from rigid natural oligonucleotides seems to favourably overrule the entropy loss resulting from hybridization of ODN of flexible acyclic analogues with complementary unmodified ODN. This has discouraged development of ODNs containing acyclic units on backbone.[15]

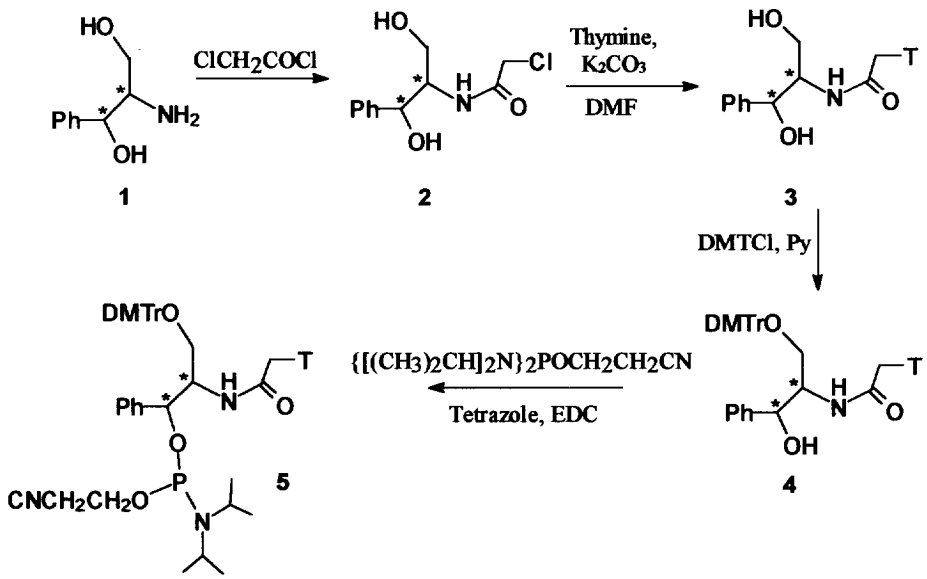
Recently, Ramasamy et al[16] reported the use of novel L-serinyl acyclic analogues (**I, R = H**) in which the internucleotide phosphate bond connects the 4'-equivalent position of the serinol monomer to the 5'-carbon of adjacent monomer. These upon incorporation at 3' end of oligonucleotides remarkably increased resistance to 3'-exonucleases but disfavoured duplex formation with natural component. The evaluation of corresponding oligonucleotides containing D-serinyl analogues or the effect of such modified ODNs on triplex stability have not been reported.



I, R = H / Ar

In our quest for modified oligonucleotides with superior duplex and triplex stabilities,[17-19] we thought of imparting backbone rigidity into serinol derived acyclic analogues through introduction of substituents in the acyclic backbone. A similar approach has been used to improve the stability of duplexes by introduction of additional substituents on the carbohydrate moiety.[20] An immediate choice was 2-amino-1-phenyl-1,3-propanediol **1**, a synthetic precursor for the broad spectrum antibiotic chloramphenicol. The phenyl substituent may not only restrict the conformational mobility of the acyclic chain, but also introduces a second chiral center in serinol, leading to 4 possible stereoisomers. The standard nucleobases can be linked to the 2-amino group through the acetyl chain as in PNA.[19] Here we describe the synthesis of suitably protected phosphoramidite blocks **5** corresponding to 1*R*,2*R* and 1*S*,2*S* configurations, site specific incorporation of these into oligonucleotides and their physicochemical evaluation for duplex and triplex properties.

Scheme 1



The 1*R*,2*R* and 1*S*,2*S* stereoisomers of 2-amino-1-phenyl-1,3-propanediol **1**[21] were transformed to the desired target phosphoramidite monomers **5** in four steps (Scheme 1) involving (i) treatment with chloroacetyl chloride / aqueous sodium bicarbonate to get N - chloroacetyl derivative[19] **2** (ii) N-alkylation of nucleobase thymine with **2** in presence of K_2CO_3 to obtain **3**, (iii) transformation of **3** into the corresponding 3-O- DMT compound[22] **4** and (iv) phosphoramidation of 1-hydroxy group using standard protocol[23] to yield the desired target amidite monomers **5**. All compounds were unambiguously characterized for structural purity by ^1H and ^{13}C

NMR and optical rotation.[24] The oligonucleotides **6-13** were synthesised by phosphoramidite chemistry on solid phase (CPG resin) using Pharmacia GA synthesiser. For sequences **10-13**, the modified amidite monomer **5** was employed for coupling in place of standard amidites at positions indicated and these were as efficient (> 99%) as the normal ones. The modified oligonucleotides **10-13** were purified by polyacrylamide gel electrophoresis (PAGE). The purity of oligonucleotides **6-13** were ascertained by reverse phase HPLC.

6	3'-	A	G	G	T	T	C	T	T	C	T	T	C	T	T	T	T	C	T	T	T	A	T	A -5'
7	5'-	T	C	C	A	A	G	A	A	G	A	A	G	A	A	A	A	G	A	A	A	A	T	A T -3'
8			3'	-	A	A	G	A	A	G	A	A	G	A	A	A	A	G	A	A	A	A	-	5'
9			5'	-	T	T	C	T	T	C	T	T	C	T	T	T	T	C	T	T	T	T	-	3'
10			5'	-	T	T	C	T	T	C	T	T	C	T	T	T	T	C	T	T	t	T	-	3'
11			5'	-	T	T	C	T	T	C	T	T	C	t	T	T	T	C	T	T	T	T	-	3'
12			5'	-	T	t	C	T	T	C	T	T	C	T	T	T	T	C	T	T	T	T	-	3'
13			5'	-	T	T	C	T	T	C	T	T	C	T	T	T	T	C	t	t	t	T	-	3'

t = modified monomer.

Duplex and Triplex melting studies

Oligonucleotide duplexes were constituted from unmodified **9** and modified **10-13** using the common complementary unmodified 18 mer **8**. The triplexes were individually constituted from the unmodified 24 mer duplex **7:6** and individual oligonucleotides **9-13** as third strand. The stability of duplexes and triplexes were measured by UV melting experiments by following UV absorbance change at 260 nm in the temperature range 5-80°C. Monophasic sigmoidal transitions are indicative of duplex formation while biphasic curves are characteristic of triplexes. The Tm values were confirmed by distinct peaks in the first derivative plots.

Table-1 indicates Tm data obtained for control (unmodified) and modified duplexes and Table-2 shows Tm of triplexes. As seen from these results, the inclusion of chiral, acyclic moiety into the DNA backbone does not hinder the formation of duplexes and triplexes. Incorporation of *SS* isomer **5** (1*S*,2*S*) in single sites at 3' (entry 2) or 5' (entry 4) ends have almost no effect on duplex Tm as compared to the control (entry 1). Increasing the number of modifications to three at 3'-end (entry 5) slightly destabilised the duplex (ΔTm = -3°C). In contrast, even a single modification at the center of the duplex (entry 3) effected a large destabilization (ΔTm = -9°C). Similar results were seen for modifications incorporating *RR* isomer **5** (entry 6,7).

In case of triplexes, Tms were measured at two different pHs (Table 2). In case of control as well as the modified third strand, Tms at pH 5.8 were higher by 18-20°C compared to those at pH 7.1, as expected for C containing sequences.[10] The oligonucleotides containing either *RR* or *SS* modifications in the third strand at 3' ends **10** were more stable than those at 5'-end **12** at either pH condition. In all modifications except one (**10**, *SS*, pH 5.8), triplexes were destabilised by 3-8° compared to the control triplex **9*7:6**. Increasing the number of

modifications to 3 towards 3'-end (13) also increased the destabilization but the most destabilised triplex at both pHs was the one derived from modification in center (11, ΔTm = -14°C). In general, at both pHs, the stabilization followed the order, control > 3' >5' > 3'x > M and *RR* modifications were less stable than *SS* modifications although both isomers showed triplex formation. The *SS* modified ODN 10 was as good as the control 9 in forming the triplex at pH 5.8 and at pH 7.1, it was less stable than control by only 4°. A significant outcome of triplex Tm data is that ODNs having the acyclic chloramphenicol analogue within the normal DNA backbone, can still form stable DNA triplexes unlike many ODNs with other acyclic analogues and sensitivity of

Table 1: UV-Tm (°C) data for duplexes*

		1*S*,2*S*				1*R*,2*R*	
Entry→	1	2	3	4	5	6	7
Duplex	**8:9**	**8:10**	**8:11**	**8:12**	**8:13**	**8:11**	**8:12**
Tm°C	58	58	49	59	55	48	58

Table 2: UV-Tm (°C) data for triplexes from common duplex 7:6*

		1*S*,2*S*				1*R*,2*R*			
pH	**Control(9)**	**10**	**11**	**12**	**13**	**10**	**11**	**12**	**13**
5.8	57	57	40	54	44	54	36	53	40
7.1	39	35	17	31	22	34	17	31	22

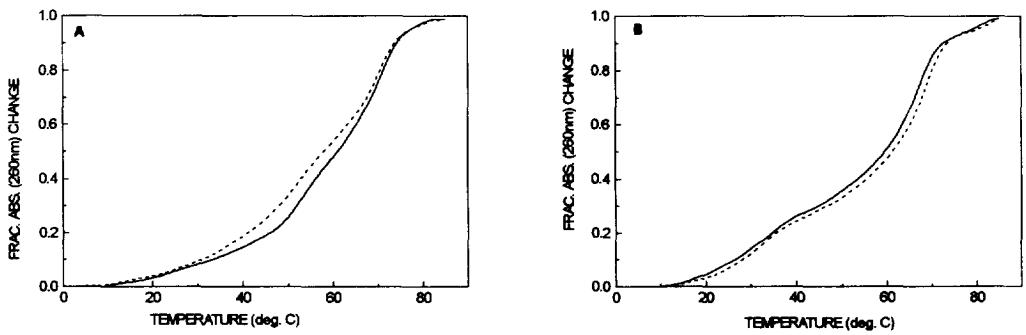


Figure 1: UV melting profiles[25] for triplexes **12** (1*R*, 2*R*)***7:6** (- - -) and **12** (1*S*, 2*S*)***7:6** (—) at **(A)** pH 5.8 and **(B)** pH 7.1.

Tm to stereochemistry point to the importance of correct geometry in the backbone of the third strand. The differences among *RR* and *SS* isomers are more at lower pH 5.8 than at neutral pH 7.1. The triplex Tm increased linearly with salt concentration (from 400mM NaCl to 1M NaCl) at pH 5.8 as well as at pH 7.1.[26]

In summary, this paper demonstrates that DNA duplex and triplex formations are not hampered by insertion of acyclic 2-amino-1-phenyl-1,3-propanediol into the regular 2-deoxyribose-phosphate backbone of DNA and ODNs with *SS* modifications are better than those with *RR*. To our knowledge, this is perhaps the first report demonstrating the formation of stable triple helices involving chiral acyclic moieties in the backbone of third strand. The specific effect of stereochemistry of the chiral carbons on the stability of triplexes suggests the possibility of further fine tuning through *SR/RS* combinations. The presence of phenyl substituent in the acyclic backbone perhaps enforces conformational rigidity on the acyclic backbone and may cause hydrophobic desolvation locally in the major groove. Further, different substitutions on phenyl ring can be explored to engineer better set of analogues. Since an equivalent serinol substitution in oligonucleotides has already been shown to increase the resistance to 3'-exonucleases, the presently observed superior hybridization properties may be of potential use to design chimeric backbone based second generation antisense/antigene therapeutic agents.[27] A novel class of nucleoside analogues (β-lactam-nucleoside chimera) have been recently reported as examples of potential dual action drugs.[28] The presently used modification is a close substructure of active pharmacophore chloramphenicol and the hybrid molecules (monomers and oligomers) as designed here could be further examples of this class of molecules.

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References and Notes:

1. (a) Uhlmann, E.; Peyman, A. *Chem. Rev.***1990**, *90*, 543. (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.
2. Crooke, S. T. in *Burgers Medicinal Chemistry and Drug Discovery*, Ed. Wolff, M. E., John Wiley & Sons, **1995.**
3. Sun, J-S.; Gavestier, T.; Helene, C. *Curr. Opinion Stru. Biol.*, **1996**, *6*, 327
4. Matteucci, M. D.; Wagner, R.W. *Nature* **1996**, *384*, 20.
5. (a) Kricca, L.J. in *Non isotopic DNA probe Techniques*, **1992**, Academic Press Inc., San Diego. (b) Abramowitz, S. *Trends in Biotech.*, **1996**, *14*, 397.
6. Jadhav, V. R.; Barawkar, D.A.; Natu, A. A.; Ganesh, K. N. *Nucleosides & Nucleotides*, **1997**, *16*, 107.
7. Sanghvi, Y. S.; Cook, P. D. *Carbohydrate Modification in Antisense Research*, **1994**, ACS Symposium Series 580, American Chemical Society, Washington D.C.
8. (a) Mesmaeker, A. D.; Altmann, K.-H.; Waldner, A.; Wendeborn, S. *Curr. Opinion Str. Biol.* **1995**, *5*, 343. (b) Nielsen, P.E. *Ann. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 167.

9. Sanghvi, Y.S. in Antisense Research and Applications, Ed. Crooke, S.T. and Lebleu, B. 1993, CRC Press, 273-289.
10. Ganesh, K. N.; Kumar, V.A.; Barawkar, D.A. in *Supramolecular Control of Structure & Reactivity*, Ed. Hamilton, A.D. **1996**, John Wiley & Sons, 263-327.
11. Schneider, K. C.; Benner, S. A. *J. Am. Chem. Soc.* **1990**, *112*, 453.
12. Vendendriessche, F.; Augustyns, K.; Aerschot, A. V.; Busson, R.; Hoogmartens, J.; Herdewijn, P. *Tetrahedron*, **1993**, *49*, 7223.
13. Nielsen, P.; Dreioe, L. H.; Wengel, J. *BioMed. Chem.*, **1995**, *3*, 19.
14. (a) Egholm, M.; Nielsen, P.E.; Buchardt, O. *Bioconj. Chem.* **1994**, *5*, 3. (b) Hyrup, B.; Egholm, M.; Nielsen, P. E. *BioMed. Chem.* **1996**, *4*, 5.
15. Herdewijn, P. *Liebigs Ann.* **1996**, 1337.
16. Ramasamy, K. S.; Seifert, W. *BioMed. Chem. Lett.* **1996**, *6*, 1799.
17. Rajeev, K.G.; Sanjayan, G. J.; Ganesh, K. N. *J. Org. Chem.* **1997**, *62*, 0000.
18. Barawkar D. A.; Rajeev, K. G.; Kumar, V. A.; Ganesh, K. N. *Nucleic Acids Res.* **1996**, *24*, 1229.
19. Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. *Tetrahedron* **1996**, *52*, 15017.
20. Kawasaki, A. M.; Casper, M. D.; Freier, E. A.; Lesnik, M. C. Z.; Gummins, L. L.; Gonzalez, C.; Cook, P.D. *J. Med. Chem.* **1993**, *36*, 831.
21. Boehringer, C. F. Soehne G.m.b.h. *Brit. P.* 741,711; **1955**; *Chem. Abst.*, **1957**, *51*, 5830h.
22. Jones, R.A. In *Oligonucleotide Synthesis: a practical approach*, Gait, M.J., Ed. IRL Press, 1984, 27.
23. Sinha, N. D.; Biernat, J.; McManus, J.; Koster, H. *Nucleic Acids Res.* **1984**, *12*, 4539.
24. Selected data: All the enantiomers 1*R*, 2*R* and 1*S*, 2*S* showed superimposable ^{1}H and ^{13}C NMR signals.
Compound **2** (1*S*, 2*S*), ^{1}H NMR, ($CDCl_3 + D_2O$), δ, 7.35 (brs, 5H, ArH), 5.05 (d, 1H, J = 4.0 Hz, C1-H), 4.1 (m, 1H, C2-H), 3.95 (dd, 2H, J = 19 Hz, $COCH_2Cl$), 3.82 (d, 2H, J = 5.0 Hz, C3-H), ^{13}C NMR, ($CDCl_3$), δ, 167.06 (CO), 140.8 (Ar), 128.2, 127.6, 125.6 (Ar), 72.05 (C-1), 62.13 (C-3), 56.65 (C-2), 42.3 (CH_2Cl). $[\alpha]_D^{20}$ = +36.0 (c = 0.2, MeOH). $[\alpha]_D^{20}$ for **2** (1*R*, 2*R*) = -37.0 (c = 0.2, MeOH).
Compound **4** (1*S*, 2*S*), ^{1}H NMR ($CDCl_3$), δ, 8.74 (s, 1H, *T*-NH), 6.75-7.5 (m, 19H, DMT + Ar + *T*-H6), 5.05 (d, 1H, J = 5.4 Hz, C1-H), 4.2 (m, 1H, C2-H), 4.18 (dd, 2H, J = 16.2 Hz, *T*-CH_2), 3.8 (s, 6H, 2 x OCH_3), 3.36 (ddd, 2H, C3-H), 1.86 (d, J=1.5 Hz, *T*-CH_3), ^{13}C NMR ($CDCl_3$), δ, 110.4 (C5), 86.0 (DMT-C), 72.3 (C-1), 62.7 (C-3), 55.1 (C-2), 54.8 (DMT-OCH_3), 49.6 (*T*- CH_2), 11.7 (*T*-CH_3).
25. All the duplexes and triplexes were individually constituted by mixing equimolar amounts of the appropriate two strands for duplex and three strands for triplex, heating at 80°C for 5 min. followed by slow cooling in buffer, 100 mM sodium cacodylate containing 20 mM $MgCl_2$ and 1M NaCl at pH 5.8. Accurate Tm values were determined from first derivative curves.
26. Triplex Tms of *RR* isomer of **10*7:6** were 29°C (400mM NaCl), 31°C (600 mM NaCl), 32°C (800 mM NaCl) and 33°C (1M NaCl) at pH 7.1 and were 51°C (400mM NaCl), 52°C (600 mM NaCl), 53°C (800 mM NaCl) and 54°C (1M NaCl) at pH 5.8. The *SS* isomer showed a similar pattern.
27. Agarwal, S. *Trends in Biotech.*, **1996**, *14*, 376.
28. Domling, A.; Starnecker, M.; Ugi, I. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2238.
29. *NCL Communication Number* : 6402.

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