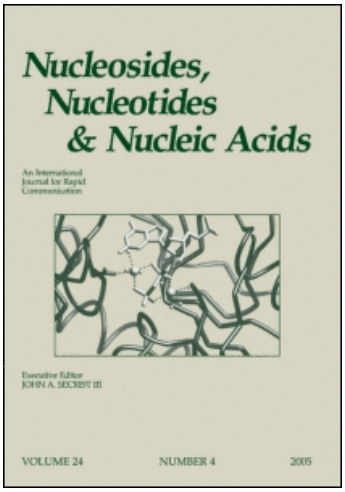

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Serge P. Parel[a]; Judith Marfurt[a]; Christian J. Leumann[a]
[a] Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

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DNA TRIPLE-HELIX FORMATION AT PYRIMIDINE-PURINE INVERSION SITES

Serge P. Parel, Judith Marfurt, and Christian J. Leumann*

Department of Chemistry and Biochemistry, University of Bern,
CH-3012 Bern, Switzerland

ABSTRACT

A systematic investigation of a series of triplex forming oligonucleotides (TFOs) containing α-, and β- thymidine, α- and β-N7-hypoxanthine, and α- and β- N7 and N9 aminopurine nucleosides, designed to bind to T-A inversion sites in DNA target sequences was performed. Data obtained from gel mobility assays indicate that T-A recognition in the antiparallel triple-helical binding motif is possible if the nucleoside αN9-aminopurine is used opposite to the inversion site in the TFO.

Despite more than a decade of intense research efforts directed to the removal of the homopurine/homopyrimidine sequence requirement for stable DNA triple-helix formation by third strand oligonucleotides, a universally applicable solution to the problem is still elusive (1, 2). A number of third-strand design options have been investigated in the past. In order to recognize DNA duplexes containing purine/pyrimidine block sequence intervals, triplex forming oligonucleotides (TFO) binding via a combination of the purine and the pyrimidine motif (3, 4), as well as TFO's with 3′-3′- or base-base junctions were studied (5–10). Furthermore, TFO's containing extended nucleobases spanning a complete Watson-Crick base-pair in the major groove (11, 12), and base-modified TFO's selectively recognizing pyrimidine bases (13, 14) were investigated with some success. In a different approach TFO's using the α-, and β-configuration of nucleosides as a structural switch to cross the major groove and target purine bases at either strand were

*Corresponding author.

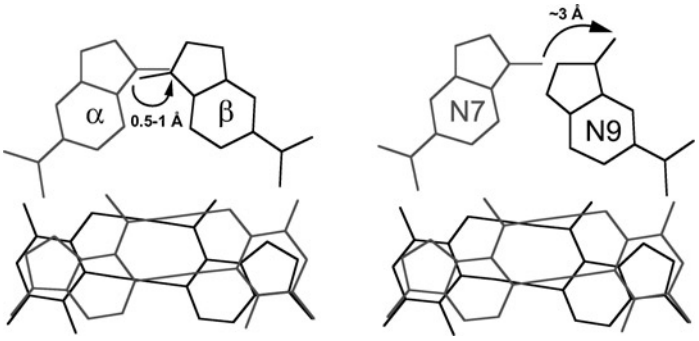

Figure 1. Schematic picture of a α, β-switch model (left) and a N7/N9 switch model (right) for alternate strand purine recognition by TFO's, here demonstrated for the case of A-T,T-A recognition by 2-aminopurine nucleosides (see text). Distances are from molecular modeling and indicate movements of C(1′) atoms upon superposition of two consecutive base-pairs.

introduced (15,16). In all of these cases either the affinity of the corresponding TFO's to their target, or the selectivity in base-pair recognition, or both did not fulfill the required criteria for a broader application in DNA based therapy and diagnostics.

Over the recent years we investigated two different switch models for targeting purine bases on both strands of a Watson-Crick duplex by TFO's. One of them is the α, β-switch model, which was independently proposed by *Hélène* and *Behr* (see above) while the other corresponds to a N7/N9 switch of third strand purine bases (Fig. 1).

In early experiments we explored the α, β-switch model on the example of alternate strand adenine recognition by TFO's containing α- and β-thymidine. However, we found that oligonucleotides containing only α- or alternating α- and β-thymidine units undergo self-pairing (17), and thus evade DNA duplex recognition. The same switch model was subsequently applied to N7-hypoxanthine nucleosides, mostly for the reasons of the longer distance between the nucleosidic bond and the functional groups necessary for establishing base-base interactions (permitting larger distances in spanning the major groove) and the larger surface of the base, enhancing intrastrand stacking in the TFO, relative to the thymine switch model (18). For this reason we prepared the three TFOs **1**–**3** depicted in Figure 2 and determined their affinity for the corresponding DNA targets by UV-melting curve analysis and by gel shift experiments.

We recognized that in neither case affinity of one of the TFOs **1**–**3** to a given target was observed, thus ruling out the α, β-switch model as a succesful triplex binding motif in this case.

Possible reasons for this failure might be unfavorable structural distorsions in the phosphodiester backbone and/or missing intrastrand stacking at the α, β- and β, α-steps in the TFO. However, we were able to show by DNase I footprint

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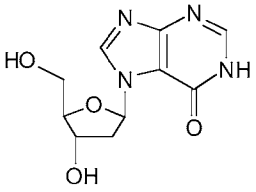
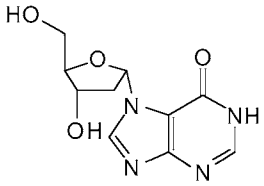


β7H



α7H

TFOs	DNA target sequences	Tm [a]
1 d(α7H)$_{10}$T	d(A$_{10}$C$_4$T$_{10}$)	<0¡C
	d(G$_{10}$T$_4$C$_{10}$)	<0¡C
2 d(β7H)$_{10}$T	d(A$_{10}$C$_4$T$_{10}$)	<0¡C
	d(G$_{10}$T$_4$C$_{10}$)	<0¡C
3 d(α7H,β7H)$_5$T	poly(dA-dT)	<0¡C
	poly(dG-dC)	<0¡C

a) c=4.3 μM, 10 mM NaH_2PO_4, 1M NaCl, pH7.0; only third strand melting data

Figure 2. α, β-switch- as well as pure α- and β- configured TFOs containing N7-hypoxanthine deoxynucleosides show no binding to corresponding DNA hairpin or polymer duplexes as determined by UV-melting curves and (in the case of hairpin DNA target duplexes) by gel mobility assays.

experiments that both the α- and β-hypoxanthine nucleosides, as single substitutions within a TFO designed to bind in the parallel mode, recognize selectively a G-C base-pair with affinities similar to that of protonated 5-methyl cytosine at pH 7.0 (19, 20).

In the search for new nucleoside analogs according to Figure 1 we also evaluated oligonucleotides containing the four aminopurine isomers α7-, α9-, β7- and β9-aminopurine (AP) (21). Molecular modeling suggested that the α, β-switch in the N7- derivatives, suitable to recognize alternate strand adenine bases, brings only minor distorsions with respect to the relative dislocation of the glycosidic bond for consecutive α- and β-nucleosides in the TFO-DNA complex (Fig. 1, left)

With purine bases in general, and with AP specifically, alternate strand adenine recognition seems also possible without a change of anomeric configuration, just by using the point of attachment of the base to the sugar as the structural switch (N7/N9-switch, Fig. 1, right). Molecular modeling suggested in this case a dislocation of the C(1′)-atoms of two consecutive N7/N9 units in a TFO-DNA complex to be ca. 3 Å. It therefore had to be shown by experiment whether the backbone distorsion arising from this partial matched situation still permits triplex formation.

In first experiments we investigated whether pure homobasic AP-oligonucleotides can recognize contigouos adenine bases on one strand in a DNA duplex. For the case of the base α7AP we were able to show by UV-melting curve analysis at high (1M NaCl) and low (0.15M NaCl) salt, and by gel shift experiments that the oligomer d(α7AP)$_{14}$T does not bind to its target DNA d(GCTA$_{15}$TCG): d(CGAT$_{15}$AGC) under these conditions (Fig. 3, only gel shift exp. shown). Thus no new binding motif emerges with this isomer of AP. In the same context the properties of homo-oligonucleotides of the other three AP-isomers still need to be explored.

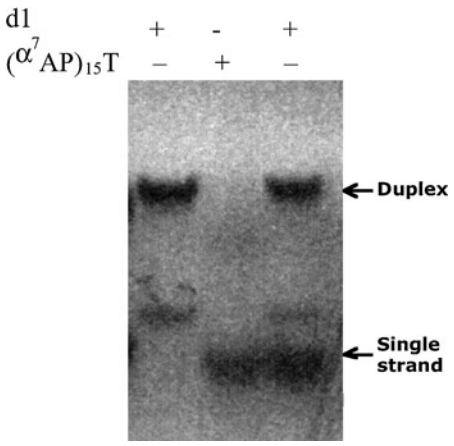


Figure 3. UV-shadowing of a 20% (19:1) non-denaturing polyacrylamide gel (TBE-buffer, 4°C): d1 = d(GCTA$_{10}$TCG):d(CGAT$_{10}$AGC), single strand = d(a7AP)$_{14}$T; left lane, d1 only, middle lane: single strand only; right lane: 1:1 stoichiometric mixture of d1 and single strand.

Table 1. DNA Target Sequences and TFOs Used in the Gel Mobility Assays

TFO	DNA Target
d2	5′-GCTAGGAGAAGG-**A**-GGAGGTCG-3′
	3′-CGATCCTCTTCC-**T**-CCTCCAGC-5′
d3	5′-GCTAGGAGAAGG-**T**-GGAGGTCG-3′
	3′-CGATCCTCTTCC-**A**-CCTCCAGC-5′
R	5′-GGTGG-**T**-GGTTGTGGT-3′
s1	5′-GGTGG-$^{\alpha}$**7AP**-GGTTGTGGT-3′
s2	5′-GGTGG-$^{\beta}$**7AP**-GGTTGTGGT-3′
s3	5′-GGTGG-$^{\alpha}$**9AP**-GGTTGTGGT-3′
s4	5′-GGTGG-$^{\beta}$**9AP**-GGTTGTGGT-3′

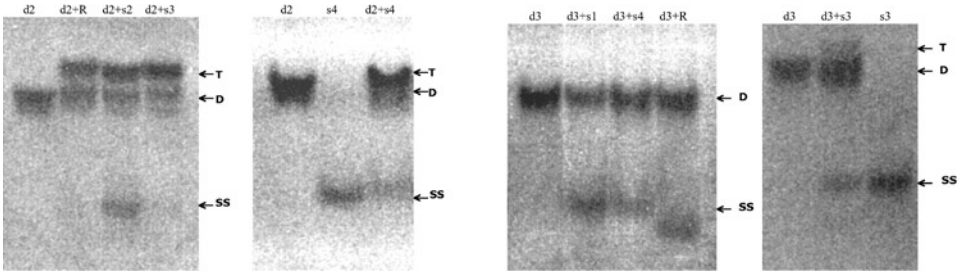


Figure 4. Gel mobility assays (20% non-denaturing polyacrylamide gel, 19:1, TBE buffer, 4°C, UV-shadowing) of the duplex target and TFO sequences shown in Table 1.

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Table 2. Summary of Qualitative Data for Triplex Formation from Gel Shift Assays of s1-s4 to the DNA Targets d2 and d3

	TFO				
Duplex Target	**R** (T)	**s1** ($^{\alpha}$7AP)	**s2** ($^{\beta}$7AP)	**s3** ($^{\alpha}$9AP)	**s4** ($^{\beta}$9AP)
d2	+	$-^{a}$	+	+	(+)
d3	−	−	*nd*	(+)	−

a) determined by UV-melting curve; + strong, (+) moderate, − no binding, n.d. not dtermined.

The picture changes if the four isomers α- and β-7AP, and α- and β-9AP are introduced into oligonucleotides designed to bind in the antiparallel triplex (purine) binding motif. For this we arbitrarily chose a part of the HER2 promoter sequence as the DNA target (**d2**). This sequence was chosen due to the availability of triplex binding data in the antiparallel motif (22, 23). A variant of it in which one of the central A-T base-pairs was mutated into a T-A base-pair served as the T-A inversion sequence (**d3**), and a series of corresponding TFOs (**s1**-**s4**) containing each of the four AP-isomers opposite to the mutation position were investigated (Table 1). Screening for affinity of each of the TFOs **s1**-**s4** to the corresponding targets was performed by gel mobility assays, as previously described. Examples of the gels as well as qualitative binding data derived thereof, are displayed in Figure 4 and Table 2, respectively.

From these data it appears that only **s3**, carrying a α9AP-unit is capable of recognizing the T-A inversion in duplex **d3**. On the other hand, **s2**, **s3** and **s4** all bind to the parent A-T containing duplex **d2**. The affinity of the α9AP containing TFO (**s4**) for the parent duplex **d2** and that of the α9AP containing TFO (**s3**) to the duplex with T-A base-pair inversion (**d3**) can be rationalized by invoking base contacts via the exo-amino function and N3 (and not N1) of AP to adenine (Fig. 5). However, other arrangements arising from the *syn*-conformation of the nucleoside, or from rare tautomeric forms or protonated forms of AP can not be excluded at this point.

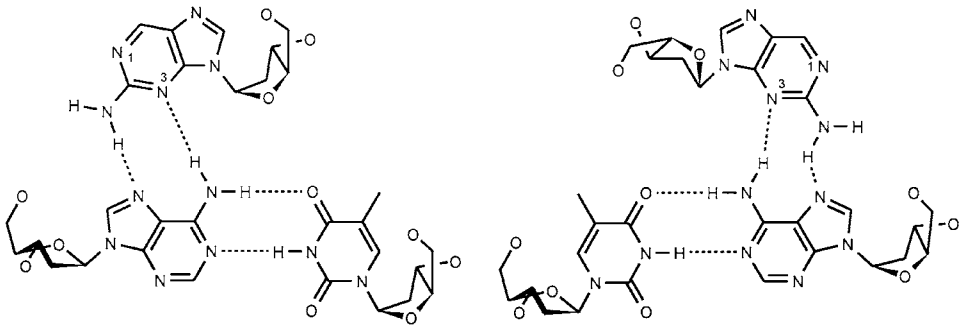

Figure 5. Putative base-triples occuring in the complexes of s4·d2 and s3·d3.

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In summary we have investigated a series of different nucleoside analogs, designed to bind according to the switch models proposed. Of these, only the N9-aminopurine series shows signs to be a successful example of such an α, β-switch if used in conjunction with the antiparallel triplex binding motif. These results, however, are only of qualitative nature, and more experiments as e.g. footprint experiments are necessary to determine association constants and base-pairing selectivity of the new nucleoside analogs. Work in this direction is currently underway.

An interesting fact is that both β7AP and α9AP seem to act as substitutes for the base thymine (or adenine) in the antiparallel triple-helical binding motif. Due to the inherent fluorescence properties of the N9 aminopurine nucleosides, especially the latter analogue may prove useful as a fluorescent probe, e.g. for diagnostic purposes.

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REFERENCES

1. Fox, K. R. *Curr. Med. Chem.* **2000**, *7*, 17–37.
2. Neidle, S. *Anti-Cancer Drug Design* **1997**, *12*, 433–442.
3. Sun, J. S.; De Bizemont, T.; Duval-Valentin, G.; Montenay-Garestier, T.; Hélène, C. *C. R. Acad. Sci. Paris* **1991**, *t. 313, Série III,* 585–590.
4. Beal, P. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 4976–4982.
5. Horne, D. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2435–2437.
6. Ono, A.; Chen, C.-N.; Kan, L.-S. *Biochemistry* **1991**, *30*, 9914–9921.
7. De Bizemont, T.; Duval-Valentin, G.; Sun, J.-S.; Bisagni, E.; Garestier, T.; Hélène, C. *Nucleic Acids Res.* **1996**, *24*, 1136–1143.
8. Zhou, B.-W.; Marchand, C.; Asseline, U.; Thuong, N. T.; Sun, J.-S.; Garestier, T.; Hélène, C. *Bioconjugate Chem.* **1995**, *6*, 516–523.
9. Asseline, U.; Thuong, N. T. *Tetrahedron Lett.* **1994**, *35*, 5221–5224.
10. Asseline, U.; Thuong, N. T. *Tetrahedron Lett.* **1993**, *34*, 4173–4176.
11. Lehmann, T. E.; Greenberg, W. A.; Liberles, D. A.; Wada, C. K.; Dervan, P. B. *Helv. Chim. Acta* **1997**, *80*, 2002–2022.
12. Huang, C.-Y.; Cushman, C. D.; Miller, P. S. *J. Org. Chem.* **1993**, *58*, 5048–5049.
13. Stilz, H.-U.; Dervan, P. B. *Biochemistry* **1993**, *32*, 2177–2185.
14. Gowers, D.M.; Fox, K. R. *Nucleic Acids Res.* **1999**, *27*, 1569–1577.
15. Thuong, N.T.; Hélène, C. *Angew. Chem.* **1993**, *105*, 697–723.
16. Behr, J.-P.; Doronina, S. O. *Chem. Soc. Rev.* **1997**, 63–71.
17. Neidlein, U.; Leumann, C. *Tetrahedron Lett.* **1992**, *33*, 8057–8060.
18. Marfurt, J. "Herstellung und Paarungseigenschaften von Oligonucleotiden enthaltend unnatürliche N7-verknüpfte Hypoxanthin-Nukleoside (N7-Inosin-Derivate)", Ph.D. thesis, University of Bern 1997.

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19. Marfurt, J.; Hunziker, J.; Leumann, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3021–3024.
20. Marfurt, J.; Parel, S. P.; Leumann, C. J. *Nucleic Acids Res.* **1997**, *25*, 1875–1882.
21. Parel, S. P., "2-Aminopurine nucleotides in DNA-triplex formation: Selective recognition of a T-A base-pair in the antiparallel recognition motif. Ph.D. thesis, University of Bern, 2000.
22. Krawczyk, S. H.; Milligan, J. F.; Wadwani, S.; Moulds, C.; Froehler, B. C.; Matteucci, M. D. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3761–3764.
23. Milligan, J. F.; Krawczyk, S. H.; Wadwani, S.; Matteucci, M. D. *Nucleic Acids Res.* **1993**, *21*, 327–333.

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