ORGANIC LETTERS

1999
Vol. 1, No. 10
1527−1530

Promiscuous Watson–Crick Cross-Pairing within the Family of Pentopyranosyl (4′→2′) Oligonucleotides[1]

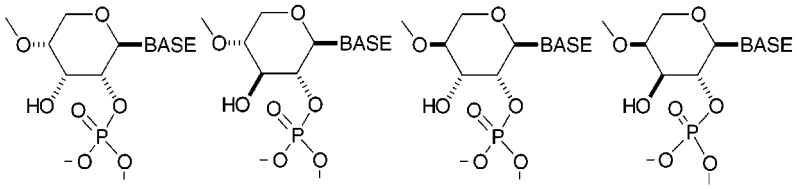
Oliver Jungmann, Harald Wippo, Michael Stanek, Hoan K. Huynh, Ramanarayanan Krishnamurthy, and Albert Eschenmoser*

The Skaggs Institute for Chemical Biology at The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule, Universitätstrasse 16, CH-8092 Zürich, Switzerland

rkrishna@scripps.edu

Received July 23, 1999

ABSTRACT



The D-β-ribo, D-β-xylo, L-α-lyxo, and L-α-arabino members of the pentopyranosyl (4′→2′) oligonucleotide family show efficient intersystem cross-pairing among each other. This family of configurationally isomeric and conformationally well-defined pairing systems offers an opportunity to study structural factors that determine cross-communication between informational oligonucleotide systems of different backbone structure.

Pentopyranosyl (4′→2′) oligonucleotides constitute a family of isomeric nucleic acid systems which differ from natural nucleic acids in that they contain aldopentose building blocks in the pyranose (instead of furanose) form and have the phosphodiester junction between sugar carbons 2′ and 4′ (instead of 3′ and 5′) (Figure 1). In the context of a systematic study,[3] we had synthesized the D-β-ribo-, D-β-xylo-, L-α-lyxo-, and the D-α-arabinopyranosyl oligonucleotides, studied their base-pairing properties in comparison to RNA, and found that all four members show stronger Watson−Crick base-pairing than RNA itself,[4] the α-arabinopyranosyl (4′→2′) system even being one of the strongest oligonucleotide pairing systems encountered thus far.[5] We had also observed examples of intersystem cross-pairing within the family of pentopyranosyl oligonucleotides.[4] Here we report results of a systematic cross-pairing study which, inter alia, also includes the so far missing L series of the α-arabinopyranosyl system.[6]

Table 1 summarizes duplex melting temperatures (T_m values in °C) of *intra*system pairing and *inter*system cross-pairing of the nonselfcomplementary base sequences A_8 + T_8, A_{12} + T_{12}, 4′-TATTTTAA-2′ + 4′-TTAAAATA-2′ and 4′-ATTCAGCG-2′ + 4′-CGCTGAAT-2′, as determined by

* To whom correspondence should be addressed at the Scripps Research Institute. Fax: ++1-858-784-9573.

(1) Chemistry of α-Aminonitriles. 26. Part 24: ref 2. Part 25: ref 4.

(2) Micura, R.; Kudick, R.; Pitsch, S.; Eschenmoser, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 680.

(3) Eschenmoser, A. *Verh. Ges. Dtsch. Naturforsch. Aerzte* **1990**, *116*, 135. Eschenmoser, A.; Löwenthal, E. *Chem. Soc. Rev.* **1992**, *21*, 1.

(4) Beier, M.; Reck, F.; Wagner, T.; Krishnamurthy, R.; Eschenmoser, A. *Science* **1999**, *283*, 699. See also references on the chemistry of pyranosyl−RNA cited therein.

(5) For probably the strongest oligonucleotide base-pairing system known ("LNA") see: Koshkin, A. A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V. K.; Singh, S. K.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 13252.

(6) A summary on the synthesis of pentopyranosyl (4′→2′) oligonucleotides was given in ref 4 and in: Pitsch, S.; Wendeborn, S.; Jaun, B.; Eschenmoser, A. *Helv. Chim. Acta* **1993**, *76*, 2161. A full account, to be published in *Helv. Chim. Acta*, is in preparation.

10.1021/ol990183y CCC: $18.00
Published on Web 10/22/1999

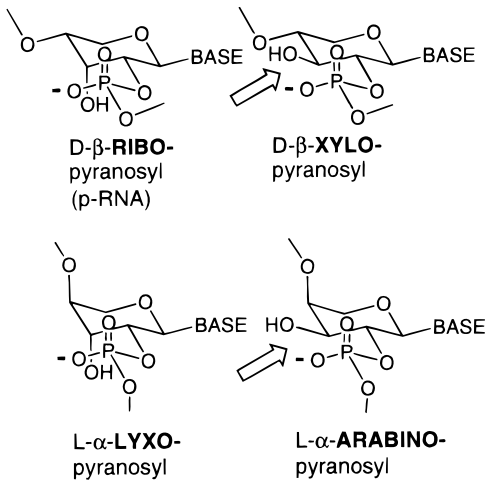


Figure 1. Idealized base-pairing conformations of the four pentopyranosyl (4′→2′) oligonucleotide systems investigated. For the conformational analysis of such systems see refs 6 and 16. The arrow symbol ⟹ points to severe steric interaction expected to induce a major deviation from the idealized pairing conformation in the actual duplexes. For an NMR structure analysis in the ribopyranosyl series see ref 9.

Table 1. T_m Values of Duplexes Formed by Cross-Pairing in the Family of Pentopyranosyl (4′→2′) Oligonucleotides[a]

		pr	px	pl	pa	r	
SELF-PAIRING		- / (5) / 15 / 6	- / 7 / (10) / •	15 / 20 / 23 / -	(5) / 8 / 48 / (5)	- / - / - / (10)	I / II / III / IV
- / - / 22 / 30	pr	46 / 68 / 46 / 68	46 / 70 / 46 / •	31 / 52 / 30 / 57	45 / 68 / 45 / 70		I / II / III / IV
- / - / 23 / •	px	45 / 70 / 46 / •	47 / 73 / 44 / •	27 / 48 / 26 / •	43 / 70 / 46 / •		I / II / III / IV
(5) / 16 / 20 / 10	pl	43 / 66 / 40 / 55	42 / 63 / 37 / •	51 / 74 / 46 / 62	67 / (89) / 61 / 73		I / II / III / IV
27 / 52 / 49 / 19	pa	59 / 83 / 54 / 67	55 / 83 / 53 / •	68 / (90) / 62 / 74	79 / (95) / 75 / (88)		I / II / III / IV
- / - / - / (10)	RNA r					23 / 54 / 17 / 52	I / II / III / IV

I 4′-AAAAAAAA-2′ / 2′-TTTTTTTT-4′
II 4′-AAAAAAAAAAAA-2′ / 2′-TTTTTTTTTTTT-4′
III 4′-TTAAAATA-2′ / 2′-AATTTTAT-4′
IV 4′-ATTCAGCG-2′ / 2′-TAAGTCGC-4′

[a] Conditions: $c = 5 + 5$ μM, 1.0 M NaCl, 0.01 M NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0; error of T_m determination estimated ±0.5 °C. T_m of pa(T_{12})•pa(A_{12}) is ca. 95 °C measured in 0.15 M NaCl; some of the data are taken from ref 4. Legend: pr = D-β-ribopyranosyl, px = D-β-xylopyranosyl, pl = L-α-lyxopyranosyl, pa = L-α-arabinopyranosyl, and r = D-β-ribofuranosyl (5′→3′) (RNA). The color of the symbols relates to oligonucleotide sequences of the same color in the formulas of duplexes I−IV given at the bottom of the table. T_m values in black refer to *inter*system cross-pairing and those in shaded diagonal to *intra*system cross-pairing; T_m values in color refer to *self*-pairing of corresponding strands. Symbols: (-) no pairing observed; (•) not investigated; numbers in brackets refer to borderline observations.

temperature-dependent UV spectroscopy under standard conditions (ca. $5 + 5$ μM, 1.0 M NaCl, 0.01 M NaH_2PO_4, pH 7.0). Figure 2 shows representative examples of T_m

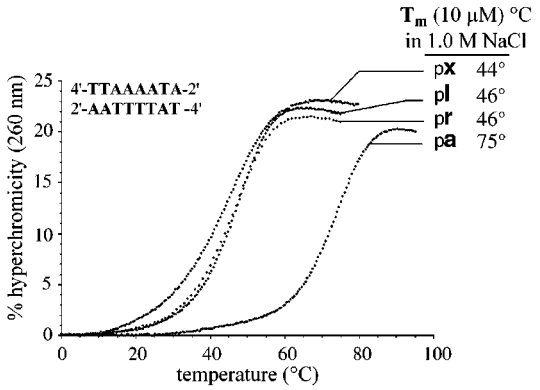


Figure 2. UV T_m curves (heating) of intrasystem-pairing duplexes (4′-TTAAAATA-2′) + (4′-TATTTTAA-2′) of the four pentopyranosyl (4′→2′) oligonucleotide systems. For conditions see the footnote of Table 1. Curves obtained on cooling are essentially identical with curves obtained on heating. T_m values are derived from maxima of the first-derivative curve (software Kaleidagraph). For method and interpretation see ref 7.

curves, all showing no hysteresis. *Intra*system pairing duplexes (T_m values in Table 1 in shaded diagonal) were also characterized by temperature-dependent CD spectroscopy (Figure 3) and by thermodynamic data[7] (Table 2). For

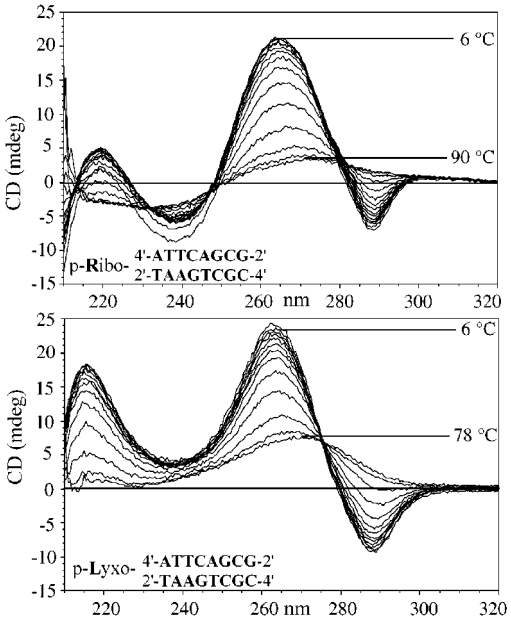


Figure 3. Temperature-dependent CD curves of a duplex formation in D-β-ribopyranosyl (4′→2′) and L-α-lyxopyranosyl (4′→2′) series. Temperature ranges are 6−90 and 6−78 °C, respectively. For conditions see the footnote of Table 1.

Table 2. T_m and Thermodynamic Data of Pentopyranosyl (4′→2′) Oligonucleotide Duplexes[a]

duplex	oligo-nucleotide system	T_m °C (10 μM) 0.15 M NaCl	$\Delta G^{25\,°C}$ kcal/mol	ΔH kcal/mol	$T\Delta S^{25\,°C}$ kcal/mol
-A_{12} T_{12}-	p**r**	60.8	-15.4	-69.0	-53.6
	p**x**	63.0	-17.0	-82.9	-65.9
	p**l**	68.0	-19.8	-97.1	-77.3
	p**a**	95			
	RNA	34.9	-10.1	-76.4	-66.3
-TTAAAATA AATTTTAT-	p**r**	38.8	-9.8	-49.6	-39.8
	p**x**	33.3	-8.7	-39.9	-30.4
	p**l**	41.8	-10.6	-55.5	-44.9
	p**a**	64.2	-15.3	-66.1	-50.8
	RNA	12.1	-5.2	-53.5	-48.3
-ATTCAGCG TAAGTCGC-	p**r**	61.4	-13.9	-57.5	-43.6
	p**x**				
	p**l**	57.6	-13.0	-54.9	-41.9
	p**a**	80.5	-17.6	-63.0	-45.4
	RNA	46.4	-11.2	-52.8	-41.5

[a] Determined at 0.15 M NaCl; for buffer conditions see caption of Table 1. Thermodynamic data from plots of T_m^{-1} versus Ln (c);[7] experimental error estimated in ΔH values ±5%. For data of other duplexes see ref 4.

combinations where *inter*system cross-pairing competes with comparably strong self-pairing of one or both partner strands, *inter*system duplex formation was confirmed by measurement[8] of mixing curves (Figure 4).

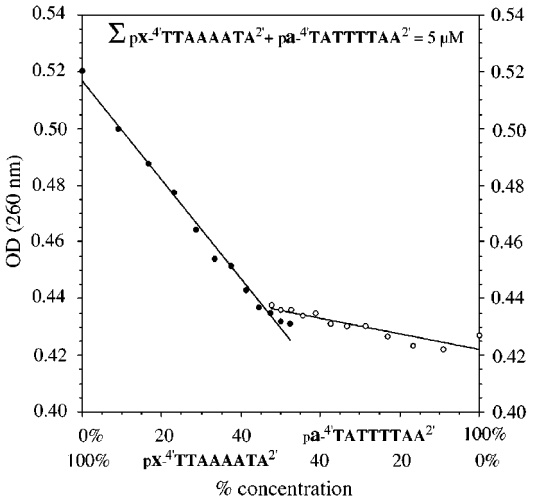


Figure 4. Mixing curve[8] for the pairing between D-β-xylopyranosyl-(4′-TTAAAATA-2′)- and L-α-arabinopyranosyl-(4′-TATTTTAA-2′) (c = 5 μM in 1 M NaCl, 0.01 M NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0; T = 20 °C).

The data demonstrate that *inter*system cross-pairing within the (4′→2′) pentopyranosyl family occurs irrespective of whether the partner strands contain homobasic sequences, irregular adenine−thymine sequences, or sequences that include cytosine and guanine. This behavior points to the capability of all members of the (4′→2′) pentopyranosyl family to adopt a common type of duplex structure, which we expect to correspond to the weakly twisted ladder structure deduced for the pyranosyl−RNA duplex (4′-CGAATTCG-2′) by NMR structure analysis.[9] The overall consistency within the cross-pairing data demands that the pairing mode in all four pentopyranosyl systems must be Watson−Crick, the mode that has been shown to operate in the ribopyranosyl series.[9]

The thermal stabilities of the intersystem cross-pairing duplexes reflect the configurational and conformational differences of their respective partner strands. T_m values are similar to those of the corresponding *intra*system pairing duplexes, provided that both partner systems have the *equatorial* 4′-phosphodiester conformation. When this conformation is axial, T_m's are lower; they are lower still when the conformation is axial in one partner and equatorial in the other. In such combinations, *inter*system cross-pairings between sequences A_n and T_n (n = 8 and 12) show a remarkably regular dependence of the T_m values on constitution: duplexes show consistently lower thermal stabilities when the all-pyrimidine strand (as opposed to the all-purine strand) has the equatorial 4′-phosphodiester conformation (Table 3). A reason for this may be sought in the following:

Table 3. Regularities Observed for the Stabilities of Cross-Pairing Duplexes An·Tn (n = 8, 12) (Excerpts from Table 1)[a]

An • Tn	T_m (10 μM) °C in 1.0 M NaCl		4'-conformation in all-pyrimidine strand
	n = 8	n = 12	
p**r** • p**x**	45	70	e equatorial
p**x** • p**r**	46	70	e
p**l** • p**a**	68	90	a axial
p**a** • p**l**	67	89	a
p**r** • p**l**	**43**	**66**	**a**
p**l** • p**r**	31	52	e
p**x** • p**l**	**42**	**63**	**a**
p**l** • p**x**	27	48	e
p**r** • p**a**	**59**	**83**	**a**
p**a** • p**r**	45	68	e
p**x** • p**a**	**55**	**83**	**a**
p**a** • p**x**	43	70	e

[a] Duplexes show consistently higher thermal stabilities when the all-pyrimidine strand (as opposed to the all-purine strand) has the axial 4′-phosphodiester conformation.

paired pentopyranosyl (4′→2′) strands with an equatorial 4′-phosphodiester conformation have a larger backbone inclination[2,9] than strands in which this conformation is axial (see

(7) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601.
(8) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman: San Francisco, CA, 1980; Part III (The Behavior of Biological Macromolecules), pp 1135−1139.
(9) Schlönvogt, I.; Pitsch, S.; Lesueur, C.; Eschenmoser, A.; Jaun, B.; Wolf, R. M. *Helv. Chim. Acta* **1996**, *79*, 2316.

Figure 1 and ref 2). In *inter*system cross-pairing, backbones with a larger (p-RNA-like) inclination can adapt to partner backbones with a smaller inclination by changing the nucleosidic torsion angles toward less negative values, and vice versa.[10] Such an adaption can be expected to be easier for purine strands than for pyrimidine strands, since pyrimidines are known to be sterically more constrained than purines with respect to variation of nucleosidic torsion angles.[11]

Although the base sequences chosen for our cross-pairing studies were specifically intended to be nonselfcomplementary, some of them show remarkably strong self-pairing. In the α-arabinopyranosyl system, the all-pyrimidine sequences pa(T_8) and pa(T_{12}) undergo T−T self-pairing (T_m = 27 and 52 °C, respectively, under standard conditions), in marked contrast to the corresponding all-adenine oligomers, which show hardly any self-association (Table 1).[12] Furthermore, the mixed A,T sequences of duplex type III (Table 1) show self-pairing not only in the notorious arabino series but in all four pentopyranosyl systems.[13] This behavior is believed to be a consequence of interstrand base-stacking in the pentopyranosyl oligonucleotide series[2] and demonstrates that an oligonucleotide system can become overly tolerant toward nucleobase mismatches when its level of base-pairing strength is much higher than that of the natural nucleic acids.

Our findings on the pairing of isomeric pentopyranosyl (4′→2′) oligonucleotides point to the possibility that a large number of chimeric oligonucleotide systems with backbones containing a random distribution of the four different pentopyranosyl backbone units would share the cross-pairing potential[14] of the pentopyranosyl family. An entire family of oligonucleotide systems may thus cross-communicate by base-pairing between oligonucleotide strands whose constitutional diversity is based not only on variation of base sequence but also on a library of backbone chimeras. This is an aspect that deserves attention in the etiological context.[15]

The members of the pentopyranosyl (4′→2′) oligonucleotide family do not cross-pair with RNA or DNA; their ability to recognize complementary base sequences is orthogonal to that of the natural nucleic acids. Thus far, we have encountered two families of oligonucleotide systems with pairing capabilities that are mutually orthogonal as well as orthogonal to those of the natural nucleic acids; the other family is homo-DNA and some of its relatives.[2,16] Orthogonality of base-pairing capability, specifically with respect to DNA, could prove to be a valuable property of artificial oligonucleotide systems, for example, in the context of synthetic nanochemistry.[17]

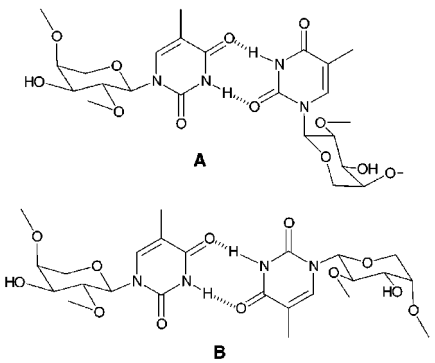
Acknowledgment. This work was supported by the Skaggs Research Foundation. O.J. thanks the Deutsche Forschungsgemeinschaft for fellowship support, and M.S. acknowledges an Erwin Schrödinger Postdoctoral Fellowship from the Fonds zur Förderung der Wissenschaftlichen Forschung (Wien, Austria).

OL990183Y

(10) Changing the nucleosidic torsion angle χ from the (idealized) value of −120° (C1′−H in plane with nucleobase in anti configuration) toward −60° (C1′−C2′ in plane with nucleobase) induces a corresponding reorientation of the base-pair axes at the cost of increasing steric repulsion between C2′(H) and the nucleobase, an interaction thought to be more repulsive than when χ changes from −120° toward −180°. On the other hand, matters are complicated because a repulsion between the nucleobase and the C5′ methylene group of the neighboring downstream sugar is to be expected when χ values change toward −180°, provided the 4′-phosphodiester conformation is equatorial, but not when it is axial.

(11) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 69−78.

(12) The constitution of this thymine−thymine pairing is presumed to correspond to mode A shown below, demanding an antiparallel strand orientation. The alternative mode B would require parallel strand orientation, which can be excluded for pairing systems whose backbone and base-pair axes are strongly inclined relative to each other, such as those of pentopyranosyl (4′→2′) oligonucleotides (see Groebke et al., ref 16). The observed T_m values are concentration dependent (47 °C at 2 μM, 52 °C at 10 μM in 1.0 M NaCl) and, therefore, do not refer to intramolecular hairpin formation. Thymine−thymine self-pairing has been observed before e.g. in d(αT)$_{20}$ strands (Neidlein, U.; Leumann, C. *Tetrahedron Lett.* **1992**, *33*, 8057 and references cited therein). For an interpretation of the weakness of purine−purine self-pairing in the pentopyranosyl series see ref 6.



A

B

(13) Thermodynamic data for the self-pairing duplex 4′-TATTTTAA-2′ in the pl and (in parentheses) pa series: ΔG = −4.6 (−9.4) kcal/mol, ΔH = −66.6 (−53.2) kcal/mol, $T\Delta S$ = −62.0 (−43.8) kcal/mol, determined from T_m concentration dependence.[7]

(14) We have tested two examples: 4′-(plT-prT-plT-prT-plA-prA-plA-prA)-2′, T_m = 52 °C (c = 19 μM in 1.0 M NaCl) and 43 °C (c = 10 μM in 0.15 M NaCl) respectively; 4′-(prT-plT-prT-plT-prA-plA-prA-plA)-2′, T_m = 42 °C (c = 10 μM in 0.15 M NaCl). For comparison: pr(T_4A_4), T_m = 40 °C; pl(T_4A_4), T_m = 47 °C (c = 10 μM in 0.15 M NaCl).[4]

(15) See also the recent investigations on RNA-PNA chimeras: Koppitz, M.; Nielsen, P. E.; Orgel, L. E. *J. Am. Chem. Soc.* **1998**, *120*, 4563.

(16) Eschenmoser, A.; Dobler, M. *Helv. Chim. Acta* **1992**, *75*, 218. Hunziker, J.; Roth, H.-J.; Böhringer, M.; Giger, A.; Diederichsen, U.; Göbel, M.; Krishnan, R.; Jaun, B.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1993**, *76*, 259. Groebke, K.; Hunziker, J.; Fraser, W.; Peng, L.; Diederichsen, U.; Zimmermann, K.; Holzner, A.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1998**, *81*, 375.

(17) See e.g.: Seeman, N. C. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3220.