ORGANIC LETTERS

1999
Vol. 1, No. 10
1531−1534

L-α-Lyxopyranosyl (4′→3′) Oligonucleotides: A Base-Pairing System Containing a Shortened Backbone[1]

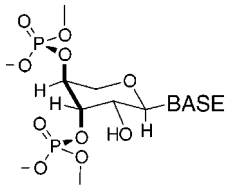
Folkert Reck, Harald Wippo, René Kudick, Martin Bolli, Griet Ceulemans, Ramanarayanan Krishnamurthy, and Albert Eschenmoser*

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 L-α-lyxopyranosyl (4′→3′) oligonucleotide system shows cooperative base-pairing in spite of containing only five instead of the usual six covalent bonds per repetitive backbone unit. In contrast, corresponding D-β-ribofuranosyl (4′→3′) oligonucleotides do not show adenine–thymine pairing under comparable conditions. The difference in pairing behavior relates to the conformation of the two systems' vicinal 3′,4′-phosphodiester substituents, which is diaxial in the lyxopyranosyl system and 3′-axial-4′-equatorial in the ribopyranosyl system.

The backbones of almost all oligonucleotidic nucleic acid analogues that have become known in this decade contain the same number of six covalent bonds per repetitive backbone unit as the natural nucleic acids.[3] Important exceptions are the (5′→2′) isomers of RNA[4] and DNA,[5,6] where this number is seven. Although base-pairing strength in these latter systems is distinctly lower than in their natural counterparts, they still have the properties of informational base-pairing systems.

In the context of our studies toward a chemical etiology of nucleic acid structure[7,8] it seems relevant to the general aim of the project to establish whether, in the family of pentopyranosyl oligonucleotide systems, phosphodiester junctions other than those between positions 2′ and 4′ (corresponding to six bonds per backbone unit) can be compatible with informational base pairing.[9] It would not be surprising if, for example, pentopyranosyl (4′→3′) oli-

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

(1) Chemistry of α-Aminonitriles. 27. Part 26: Reference 2.

(2) Jungmann, O.; Wippo, H.; Stanek, M.; Huynh, H. K.; Krishnamurthy, R.; Eschenmoser, A. *Org. Lett.* **1999**, 1, 1527.

(3) For recent reviews of anti-sense oligonucleotide research see e.g., Hunziker, J.; Leumann, C. In *Modern Synthetic Methods*; Ernst, B., Leumann, C., Eds.; Verlag Helvetica Chimica Acta: Basel, Switzerland, 1995; Vol. 7, p 331. Hyrup, B.; Nielsen, P. E. *Bioorg. Chem. Med. Chem. Lett.* **1996**, *4*, 5. Herdewijn, P. *Liebigs Ann. Chem.* **1996**, 1337. For exceptions from the "six-bonds rule" among oligoamide nucleic acid analogues see e.g.: Diederichsen, U.; Schmitt, H. W. *Eur. J. Org. Chem.* **1998**, *827*, 7. Diederichsen, U. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1886.

(4) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685.

(5) Dougherty, J. P.; Rizo, C. J.; Breslow, R. *J. Am. Chem. Soc.* **1992**, *114*, 6254. Sheppard, T. L.; Breslow, R. *J. Am. Chem. Soc.* **1996**, *118*, 9810.

(6) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.* **1992**, *114*, 6255. Prakash, T. P.; Jung, K.-E.; Switzer, C. *Chem. Commun.* **1996**, 1793.

(7) Eschenmoser, A. 40 Years of DNA Double Helix. *Proceedings of the R. A. Welch Foundation 37th Conference on Chemical Research*; R. A. Welch Foundation: Houston, TX, 1993; p 201.

(8) Beier, M.; Reck, F.; Wagner, T.; Krishnamurthy, R.; Eschenmoser, A. *Science* **1999**, *283*, 699.

10.1021/ol990184q CCC: $18.00
Published on Web 10/22/1999

gonucleotide systems behaved differently from the corresponding (4′→2′) systems and lacked the capability of cooperative base pairing, since they contain only five, instead of the usual six, bonds per backbone unit. Here we report that L-α-lyxopyranosyl (4′→3′) oligonucleotides—in marked contrast to corresponding D-β-ribopyranosyl (4′→3′) oligonucleotides—do show cooperative base-pairing. The lyxopyranosyl (4′→3′) system is, as far as we are aware, the first phosphodiester base-pairing system with a "shortened" backbone[3] (Figure 1).

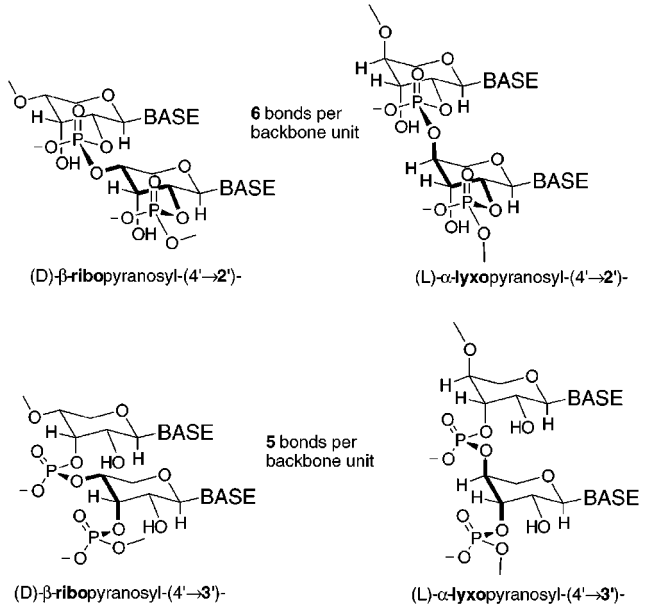


Figure 1. Idealized pairing conformations of D-β-ribopyranosyl (4′→2′) and L-α-lyxopyranosyl (4′→2′) strands and of the corresponding (4′→3′) isomers.

The preparation of base sequences containing a (4′→3′) phosphodiester junction in the D-β-ribopyranosyl and L-α-lyxopyranosyl series (Figure 1) follows a pattern analogous to that of the previously summarized synthesis of base sequences in the (4′→2′) pentopyranosyl series, using benzoyl protection for the sugar's extra hydroxyl group.[8,10,11] The required building blocks **1a,b** and **2a**−**d** containing a free 3′-hydroxyl and a benzoyl-protected 2′-hydroxyl group (Figure 2) were prepared in both series from intermediates previously used for the synthesis of (4′→2′) oligomers.[12] Characterization of base sequences was of the same standard as previously adopted and described;[8,10,11] specifically, all

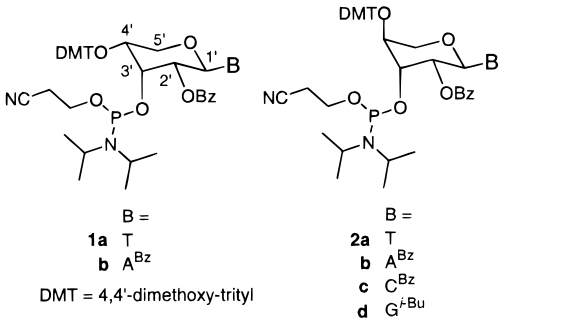


Figure 2. Intermediates used for the solid-support synthesis of D-β-ribopyranosyl (**1a**,**b**) and L-α-lyxopyranosyl (4′→3′) oligonucleotides (**2a**−**d**).[12]

oligomers were over 95% pure according to HPLC and had the expected molecular weight according to the MALDI-TOF spectrum.[13]

Table 1 summarizes duplex stabilities in terms of T_m values and thermodynamic data for duplexes of selected base sequences of L-α-lyxopyranosyl (4′→3′) oligonucleotides;

Table 1. T_m Values of Duplexes of the L-α-Lyxopyranosyl (4′→3′) Oligonucleotide Series (in °C, c = 10 μM, 1.0 M NaCl, in 0.01 M Tris·HCl or 0.01 M NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0) in Comparison to Corresponding Values of the D-β-Ribopyranosyl (4′→3′), D-β-Ribopyranosyl (4′→2′), and L-α-Lyxopyranosyl (4′→2′) Series[a]

	T_m (10 μM) ° C 1.0 M NaCl				(4'→3')pl 0.15 M NaCl		
duplexes	pr 4'→2'	pr 4'→3'	pl 4'→2'	pl 4'→3'	ΔG 25 °C	ΔH	TΔS 25 °C
$A_8 + T_8$	45.5	< 0	51.0	41.2	-10.0	-42.1	-32.1**
$A_{12} + T_{12}$	67.6		74.0	67.0	-14.0	-51.8	-37.8**
T_4A_4	40*	< 0	49.6	17.5			
A_4T_4	27*	< 0	41.1	< 5			
$(TA)_4$	40*		43.2	30.1	-6.3	-33.9	-27.6
$(AT)_4$	38*		44.7	26.6	-6.1	-32.5	-26.4
-TATTTTAA ATAAAATT-	45.9		46.3	12.6			
$(CG)_3$	65*			45.1	-9.5	-42.5	-33.0
$(GC)_3$	62*			35.0	-8.0	-34.3	-26.3
-ATTCAGCG TAAGTCGC-	67.6		61.9	39.0	-8.4	-24.9	-16.5

[a] Thermodynamic data are of L-α-lyxopyranosyl (4′→3′) series (1.0 M NaCl, 0.01 M NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0), determined from plots of T_m^{-1} versus Ln (c);[19] experimental error estimated in ΔH values ±5%. Legend: (*) in 0.15 M NaCl; (**) in 1.0 M NaCl.

corresponding T_m data of the D-β-ribopyranosyl (4′→3′), D-β-ribopyranosyl (4′→2′), and L-α-lyxopyranosyl (4′→2′) series[8] are given for comparison. Base pairing in the (4′→3′) lyxopyranosyl series is additionally documented by T_m curves

(9) For a study on the six- versus seven-bond question in a series of nucleic acid analogues that do not contain phosphodiester internucleotide linkages, see: Stork, G.; Zhang, C.; Gryaznov, S.; Schulz, R. *Tetrahedron Lett.* **1995**, *36*, 6387. See also De Mesmaeker, A.; Waldner, A.; Wendeborn, S.; Wolf, R. M. *Pure Appl. Chem.* **1997**, *69*, 437.

(10) Pitsch, S.; Krishnamurthy, R.; Bolli, M.; Wendeborn, S.; Holzner, A.; Minton, M.; Leseur, C.; Schlönvogt, I.; Jaun, B.; Eschenmoser, A. *Helv. Chim. Acta* **1995**, *78*, 1621. Bolli, M.; Micura, R.; Pitsch, S.; Eschenmoser, A. *Helv. Chim. Acta* **1997**, *80*, 1901.

(11) Pitsch, S.; Wendeborn, S.; Jaun, B.; Eschenmoser, A. *Helv. Chim. Acta* **1993**, *76*, 2161.

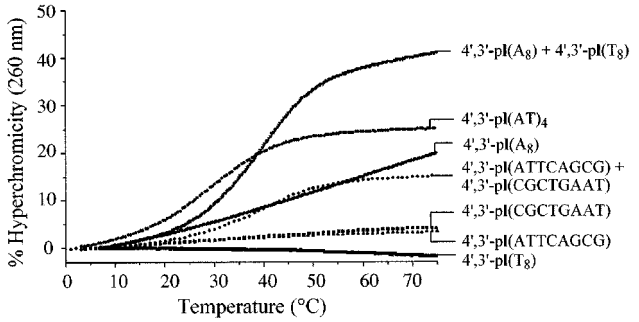


Figure 3. UV T_m curves for selected duplexes in the L-α-lyxopyranosyl (4′→3′) oligonucleotide series. Curves of (non-self-complementary) single strands are also shown. For conditions see the footnote of Table 1.

(Figure 3), CD spectra (Figure 4), and a mixing curve[14] (Figure 5) of selected examples. The pairing is generally weaker than in the isomeric (4′→2′) system and, above all,

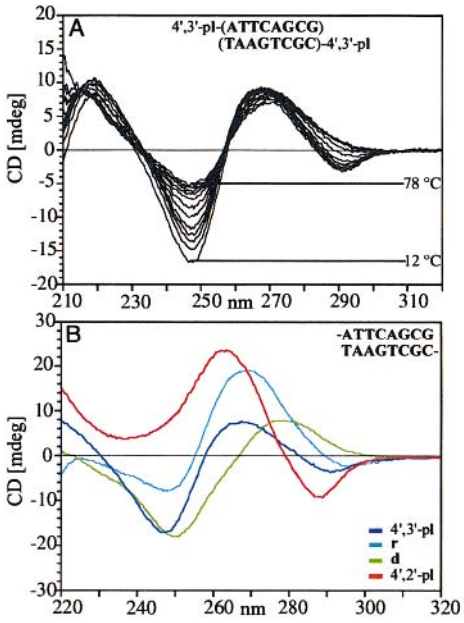


Figure 4. (A) Temperature-dependent CD curves of a duplex in the L-α-lyxopyranosyl (4′→3′) oligonucleotide series (temperature range 12−78 °C, intervals 6 °C). For conditions see the footnote of Table 1. (B) Comparison of the CD spectra (T = 12 °C) of duplexes derived from the sequences −ATTCAGCG and −CGCTGAAT (written in the 4′→3′, 4′→2′, or 5′→3′ direction) in L-α-lyxopyranosyl (4′→3′), L-α-lyxopyranosyl (4′→2′), RNA, and DNA series. For conditions see the footnote of Table 1.

more base-sequence dependent. The observed pattern points to a partial obstruction of pairing, presumably due to a less than optimal fit between the partner strands. It is also probable that, depending on base sequence, more than a single pairing mode are involved.[15] A special aspect of the

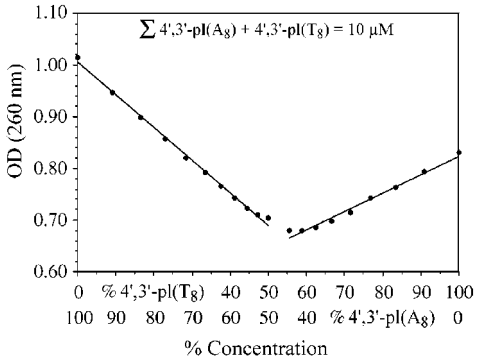


Figure 5. Mixing curve[14] for the pairing between A_8 and T_8 in the L-α-lyxopyranosyl (4′→3′) series (c = 10 μM in 1 M NaCl, 0.01 M Tris·HCl, pH 7.0; T = 20 °C).

behavior of the (4′→3′) lyxopyranosyl system is the ability of some of its sequences to undergo intersystem cross-pairing with DNA and RNA, a property shown by none of the pentopyranosyl (4′→2′) systems investigated thus far. In this cross-pairing (Table 2) we observe a drastic difference in

Table 2. T_m Values of Cross-Pairing Duplexes Formed between L-α-Lyxopyranosyl (4′→3′) Oligonucleotide Sequences and Complementary DNA and RNA Sequences in the Ratio 1:1[a]

duplexes	T_m (10 μM)
4',3'-pl(A_8) + d(T_8)	42°
d(A_8) + 4',3'-pl(T_8)	—
4',3'-pl(A_{12}) + d(T_{12})	63.4°
d(A_{12}) + 4',3'-pl(T_{12})	17.2°
4',3'-pl(A_8) + r(U_8)	21°
r(A_8) + 4',3'-pl(T_8)	—
4',3'-pl(A_{12}) + r(T_{12})	62.1°
r(A_{12}) + 4',3'-pl(T_{12})	—
4',3'-pl(TTAAAATA) + d(TATTTTAA)	< 5°
d(") + 4',3'-pl(")	—
4',3'-pl(ATTCAGCG) + d(CGCTGAAT)	28.6°
d(") + 4',3'-pl(")	26.4°
d(") + d(")	36.3°
4',3'-pl(") + r(")	42.0°
r(") + 4',3'-pl(")	36.5°
r(") + r(")	52.0°

[a] For conditions see the footnote of Table 1. The symbol — denotes that no T_m was observed.

the stability of duplexes An·Tn, depending on which of the partner strands contain the pyrimidine and which the purine bases. This is reminiscent of the analogous, yet less pronounced, phenomenon encountered in the intersystem cross-pairing within the pentopyranosyl (4′→2′) family; there

we conjectured that the phenomenon is related to backbone adjustments via strand-specific changes of nucleosidic torsion angles.[2] The mixing curve for the 4′,3′-pl$(A)_8$·d$(T)_8$ cross-pairing (Figure 6) indicates the formation of a (1:1) duplex

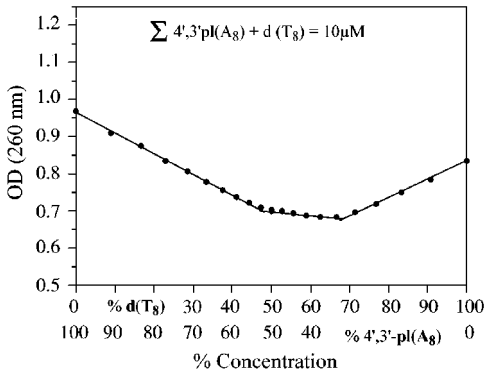


Figure 6. Mixing curve[14] for the intersystem cross-pairing between 3′,4′-pl(A_8) and d(T_8) (for conditions see the caption for Figure 3; T = 15 °C). Note the formation of a duplex 3′,4′-pl(A_8)·d(T_8) as well as of a triplex 3′,4′-pl(A_8)·2d(T_8).

as well as of a (1:2) triplex under standard conditions. *Inter*system cross-pairing with DNA and RNA involving the antiparallel complementary sequences −ATTCAGCG and −CGCTGAAT shows unexceptional behavior; its strength is comparable to that of corresponding *intra*system pairings (Table 1, Figure 4).

Since none of the members of the pentopyranosyl (4′→2′) oligonucleotide family show intersystem cross-pairing with DNA or RNA, the behavior of the L-α-lyxopyranosyl (4′→3′) system toward these natural systems is striking. One of the general prerequisites of a system to undergo cross-pairing with DNA or RNA could be that the backbone inclination[16] must be small, as is the case in B-DNA.[17] The inclination in the lyxopyranosyl (4′→3′) system is supposed to be smaller than that in all the members of the pentopyranosyl (4′→2′) family, as the conformational presentation given in Figure 1 suggests.[16] On the other hand, the fact that the α-lyxopyranosyl (4′→3′) system behaves as a base-pairing system, whereas the β-ribopyranosyl (4′→3′) system does not,[18] may relate to the diaxial arrangement of the vicinal phosphodiester functions in the lyxo system. The two phosphodiester substituents in this conformation are as far apart from each other as they possibly can be while being bound to two adjacent carbons. This may be responsible for the violation of the rule that oligonucleotide base-pairing systems should have (at least) six covalent bonds per backbone unit. The case calls for a corresponding loosening of the constitutional constraints for backbone design in oligonucleotide chemistry. In our own work, the findings about the D-β-lyxopyranosyl (4′→3′) oligonucleotide system led us to extend the experimental screening of base-pairing behavior of potentially natural nucleic acid alternatives to the uncharted territory of *tetro*furanosyl (3′→2′) oligonucleotides.

Acknowledgment. This work was supported by the Skaggs Research Foundation.

OL990184Q

(12) The 3′-hydroxy derivatives **1a**,**b** are intermediates already used in the synthesis of D-β-ribopyranosyl sequences.[11] The 3′-hydroxy precursors of **2a**−**d** are prepared by regioselective 2′-hydroxy monobenzoylation (2 mol equiv of benzoyl chloride, 5 mol equiv of pyridine, CH_2Cl_2, 0 °C, 30 min) of the 4′-DMT-2′,3′-dihydroxy derivatives which are obtained by alkaline hydrolysis (**2b**, THF/H_2O (2:1), 15% aqueous NaOH, 0 °C, 2 h; **2a**, K_2CO_3, MeOH, 4 °C, 4 h) of the 3′-benzoyl-2′-hydroxy derivatives that are intermediates of L-α-lyxopyranosyl (4′→2′) sequences.[8] In the **2c**,**d** series, the corresponding 3′-acetyl (instead of 3′-benzoyl) derivatives were hydrolyzed. For full experimental details on the preparation and characterization of oligonucleotide sequences in the D-β-ribopyranosyl (4′→3′) and L-α-lyxopyranosyl (4′→3′) series see a forthcoming paper by: Wippo, H.; Reck, F.; Kudick, R.; Bolli, M.; Ramaseshan, M.; Ceulemans, G.; Krishnamurthy, R.; Eschenmoser, A. *Helv. Chim. Acta*, in preparation.

(13) Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191.

(14) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman: San Francisco, 1980; Part III (The Behavior of Biological Macromolecules), pp 1135−1139.

(15) Pairing in the duplexes A_n·T_n (n = 8, 12) may well occur in the Hoogsteen or reverse-Hoogsteen mode as opposed to duplexes containing G and C bases, in which pairing is presumably in the Watson−Crick mode. A determination would require the preparation of these duplexes in quantities sufficient for NMR analysis. For the properties of a nucleic acid analogue that pairs in the Hoogsteen mode see: Bolli, M.; Litten, J. C.; Schütz, R.; Leumann, C. J. *Chem. Biol*. **1996**, *3*, 197.

(16) Micura, R.; Kudick, R.; Pitsch, S.; Eschenmoser, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 680. A paper that will give a definition of the term "backbone inclination" in terms of an algorithm for the derivation of numerical values from X-ray structure data of oligonucleotide duplexes is in preparation together with M. Egli (Northwestern University).

(17) In the B-type conformation of double-stranded DNA the (local) backbone axis is approximately orthogonal to the (local) base-pair axis.

(18) Observations about the nonpairing of the β-ribopyranosyl (4′→3′) system refer to adenine−thymine pairing of the sequences $A_8 + T_8$, A_4T_4, and T_4A_4; experimental details will be published in *Helv. Chim. Acta*.[12]

(19) Marky, L. A.; Breslauer, R. J. *Biopolymers* **1987**, *26*, 1601.