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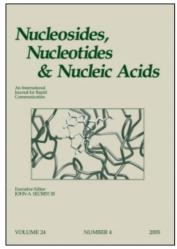
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Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713597286

α-LNA (α-D-Configured Locked Nucleic Acid)

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Online publication date: 09 August 2003

To cite this Article Christensen, Nanna K., Petersen, Michael , Vester, Birte and Nielsen, Poul(2003) ' α -LNA (α -D-Configured Locked Nucleic Acid)', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1143 — 1145

To link to this Article: DOI: 10.1081/NCN-120022821 URL: http://dx.doi.org/10.1081/NCN-120022821

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS Vol. 22, Nos. 5–8, pp. 1143–1145, 2003

α-LNA (α-D-Configured Locked Nucleic Acid)

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ABSTRACT

Two pyrimidine α -LNA nucleoside monomers have been synthesised and incorporated into α -configured oligonucleotides. A fully modified mixed α -LNA sequence displays unprecedented parallel stranded hybridisation with complementary RNA and a remarkable selectivity for RNA over DNA. Modelling shows α -LNA: RNA to form an extended duplex with a very broad major groove.

Key Words: Locked nucleic acid; Parallel duplexes; Selective RNA-recognition.

LNA (Locked Nucleic Acid) is a nucleic acid analogue that displays unprecedented recognition of both single stranded DNA and RNA in all-modified LNA-oligomers as well as in LNA/DNA and LNA/RNA-mixmers. [1,2] The LNA monomers are bicyclo[2,2,1] nucleosides locked in an N-type conformation. [1,2] α -DNA, i.e., α -D-configured oligodeoxynucleotides, forms stable parallel stranded duplexes with complementary DNA and RNA. [3] Conformationally restricted analogues of

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DOI: 10.1081/NCN-120022821 Copyright © 2003 by Marcel Dekker, Inc.

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270 Madison Avenue, New York, New York 10016

1525-7770 (Print); 1532-2335 (Online)

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Table 1. Buffer: Na₂HPO₄ (10 mM), NaCl (100 mM), EDTA (0,1 mM), pH 7.0; 1.5 μM of each strand. All detected T_M's were enhanced 3-6°C in a high salt buffer (NaCl, 750 mM). Boldface letters $\sim \alpha$ -LNA.

$T_{ m m}$ ($\Delta T_{ m m}$	per modification)/°C	Parallel DNA	ap DNA	Parallel RNA	ap RNA	Mismatch RNA (A to C)
α-DNA α-DNA/ α-LNA	5'-\alpha-\text{"CT"}C\text{"CTT"}CTTT 5'-\alpha-\text{"CTT"}C\text{"CTT}\text{"CTTT} 5'-\alpha-\text{"CTT}C\text{"CTTT}	30.5 (-12.5)		32.0 26.0 (-6.0) 22.0 (-1.7)	< 10 < 10 < 10	
mixmers α-LNA	5'-α- ^m CT ^m C ^m CTT ^m CTTT	< 10	< 10	61.5 (+2.9)	< 10	47.0

 α -DNA that contains bicyclic α -configured nucleosides have been introduced.^[4,5] However, these have been restricted towards S-type conformations^[4,5] which are also the conformations preferred by α -DNA. Subsequently, α -LNA is the first α -DNA analogue to be restricted in an N-type conformation due to the locked bicyclic carbohydrate skeleton.^[6,7]

The successful synthesis of the α-LNA thymine monomer was accomplished from diacetone-α-D-allose through standard steps ending with a Vorbrüggen nucleobase coupling reaction, a ring-closing reaction and, subsequently, separation of the α - and (β -) LNA monomers. [6,7] The former was DMT-protected and converted by standard steps into two different phosphoramidites of either the thymine or the N-4benzoyl-5-methylcytosine α -LNA monomers. [7] These as well as the two corresponding α-DNA phosphoramidites were used as building blocks for oligonucleotide sequences (Table 1).[7]

In α -DNA/ α -LNA mixmers, the exchange of one or six α -DNA monomers with α-LNA monomers results in strongly decreased affinity against both DNA and RNA when compared to the unmodified α-DNA sequence. On the other hand, a fully modified mixed α-LNA sequence shows no recognition of DNA but a very strong recognition of RNA. This strong preference for RNA as well as for parallel over antiparallel duplex formation was confirmed by the use of a gel shift analysis. MD-simulations were performed assuming Watson-Crick base pairing (as no change in T_M was detected when lowering pH from 7 to 5) and suggested the α-LNA/RNA duplex to be an extended duplex with a major groove width of 20 A and the nucleobases of the α -LNA monomers in syn-positions. Other duplex parameters were close to normal A- or B-type duplexes.

ACKNOWLEDGMENTS

The Nucleic Acid Center is funded by the Danish National Research Foundation for the studies on nucleic acid chemical biology.



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