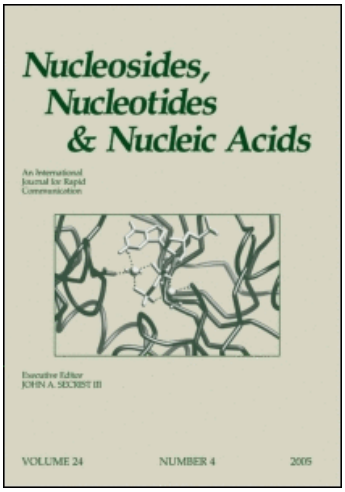

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LNA (LOCKED NUCLEIC ACID) AND THE DIASTEREOISOMERIC α-L-LNA: CONFORMATIONAL TUNING AND HIGH-AFFINITY RECOGNITION OF DNA/RNA TARGETS

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ABSTRACT

The remarkable binding properties of LNA (Locked Nucleic Acid) and α-L-LNA (the α-L-*ribo* configured diastereoisomer of LNA) are summarized, and hybridization results for LNA/2′-*O*-Me-RNA chimera and LNAs with a "dangling" nucleotide are introduced. In addition, results from NMR investigations on the furanose conformations of the individual nucleotide monomers in different duplexes are presented. All these data are discussed with focus on the importance of conformational steering of unmodified nucleotides in partly modified LNA and α-L-LNA sequences in relation to the unprecedented binding properties of LNA and α-L-LNA.

INTRODUCTION

LNA (Locked Nucleic Acid) has been defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomers

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(LNA monomers, Fig. 1) locked in a C3′-*endo*/^3E *north*(*N*)-type furanose conformation (1–4). Unprecedented hybridization properties have been demonstrated for LNAs of various compositions, *e.g*. LNAs consisting of LNA and DNA monomers (1–6), LNAs consisting of LNA and RNA monomers (6,7), phosphorothioate-LNA

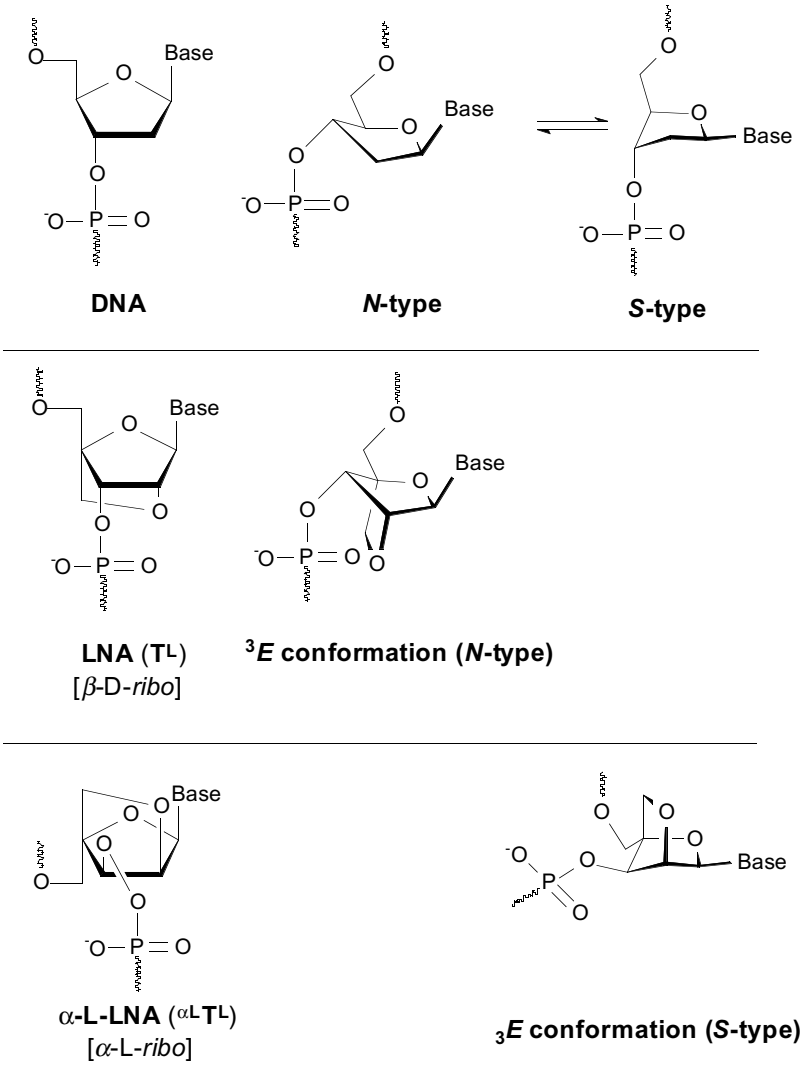

Figure 1. The structures of nucleotide monomers of DNA, LNA (monomer T^L : Base = thymin- 1-yl) and α-L-LNA (monomer $^{\alpha L}T^L$: Base = thymin-1-yl). The conformational equilibrium between *N*-type and *S*-type conformers (*e.g*. the C3′-*endo*/^3E *north(N)*-type and C3′-*exo*/$_3E$ *south(S)*-type conformations shown) of an unmodified DNA monomer is indicated. Also shown are the locked *N*-type (C3′-*endo*/^3E) and *S*-type (C3′-*exo*/$_3E$; see also ref. 15) furanose conformations of an LNA and an α-L-LNA monomer, respectively.

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Table 1. Melting Temperatures (T_m values) for Five Different LNAs (1,2,6,7)

Entry	LNA	DNA Target		RNA Target	
		T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1	5′-(**G**^{L}**T**^{L}**G**^{L}**A**^{L}**T**^{L}**A**^{L}**T**^{L}**G**$^{L\ Me}$**C**^{L})	64	+4.0	74	+5.1
2	5′-d(G**T**^{L}GA**T**^{L}A**T**^{L}GC)	44	+5.3	50	+7.3
3	5′-r(G**T**^{L}GA**T**^{L}A**T**^{L}GC)	55	+9.3	63	+8.3
4	5′-d(GTGA**T**^{L}ATGC)	35	+8.0	–	–
5	5′-r(GUGA**T**^{L}AUGC)	38	+11.0	–	–

A medium salt buffer was used: 100 mM sodium chloride, 10 mM sodium phosphate, pH 7.0; ΔT_m values denote calculated changes in T_m value *per modified monomer introduced* compared with the corresponding unmodified reference duplexes; d(sequence) denotes an oligodeoxyribonucleotide; r(sequence) denotes an oligoribonucleotide; A, C, G, T and U denote the standard DNA/RNA monomers; **A**^{L}, ^{Me}**C**^{L} (5-methylcytosine base), **G**^{L} and **T**^{L} denote LNA monomers.

(8), 2′-amino-LNA (9), and fully modified LNA (2,5,10). In Table 1, T_m values for selected LNAs are shown documenting the significantly increased binding affinities obtained towards both DNA and RNA targets compared with the corresponding unmodified reference duplexes (ΔT_m values = +4.0 to +11.0°C, Table 1). LNA hybridization follows the Watson-Crick base-pairing scheme with generally improved selectivities (2,5,10).

More recently, we have studied diastereoisomeric forms of LNA, *e.g.* xylo-LNA (β-D-*xylo* configured LNA) and α-L-LNA (α-L-*ribo* configured LNA; defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl nucleotide monomers, Fig. 1) (11–14). Interestingly, α-L-LNA has shown appealing hybridization properties despite its unnatural configuration. Thus, towards RNA complements partly modified α-L-LNAs display T_m values approaching those of parent LNA (ΔT_m values $\sim$ +5 °C, Table 2). The furanose ring of an α-L-LNA monomer is locked in a C3′-*exo*/$_3$*E south(S)*-type conformation (15), and molecular modeling has shown a close proximity in space between three key atoms in relation to nucleic acid structure, namely N1, O3′ and C5′ (13).

The structural effects resulting from the incorporation of LNA and α-L-LNA monomers into oligodeoxyribonucleotides thus forming partly modified LNA and α-L-LNA sequences, respectively, have been studied by NMR spectroscopy and

Table 2. Melting Temperatures (T_m values) for Two α-L-LNAs (13,14) ($^{\alpha L}$**A**^{L} and $^{\alpha L}$**T**^{L} denote α-L-LNA monomers)

Entry	α-L-LNA	DNA Target		RNA Target	
		T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1	5′-d(G($^{\alpha L}$**T**^{L})GA($^{\alpha L}$**T**^{L})A($^{\alpha L}$**T**^{L})CC)	37	+2.7	45	+5.7
2	5′-d(GC($^{\alpha L}$**A**^{L})T($^{\alpha L}$**A**^{L})TC($^{\alpha L}$**A**^{L})C)	37	+2.7	42	+4.7

See the caption below Table 1 for conditions and abbreviations used.

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are discussed herein. In addition, data from hybridization studies with LNA/2′-*O*-Me-RNA and α-L-LNA/2′-*O*-Me-RNA chimera as well as LNAs with a "dangling" nucleotide are introduced and discussed.

RESULTS AND DISCUSSION

2D NMR has been used to study the furanose conformations of 9-mer oligodeoxynucleotides containing three LNA or α-L-LNA monomers (Table 3). The results for LNA:DNA and LNA:RNA duplexes have been published (16,17). The furanose ring of all DNA monomers for the reference DNA:DNA duplex (Table 3, column 1) can be described as being in an *S*-type conformation (18). The cross peak patterns in the DQF COSY spectra of the DNA:DNA reference duplex clearly indicated predominantly *S*-type conformation for all DNA monomers even though a precise value of the % *S*-type conformation could not be obtained in all cases due to spectral overlap. The data obtained for the LNA:DNA duplex (Table 3, column 2) reveal that the furanose ring of unmodified DNA monomers in the LNA strand are tuned towards an *N*-type conformation by neighboring LNA nucleotides. A similar conformational steering effect was not observed for the corresponding α-L-LNA:DNA duplex (Table 3, column 3) as the furanose rings of all central DNA monomers are almost exclusively populating an *S*-type conformation (19). Similar but more pronounced results were observed for the duplexes towards the RNA complement (20). Thus, compared to the DNA:RNA reference duplex (Table 3, column 4), the furanose rings of all central DNA monomers of the LNA strand of the LNA:RNA duplex (Table 3 column 5) are strongly shifted towards

Table 3. Conformations of Nucleotide Monomers in 9-mer Duplexes (DNA:DNA, LNA:DNA and α-L-LNA:DNA)

	% *S*-type Furanose Conformation					
	DNA Complement			RNA Complement		
Sequence	X = T	X = $\mathbf{T}^{L}$	X = $^{\alpha L}\mathbf{T}^{L}$	X = T	X = $\mathbf{T}^{L}$	X = $^{\alpha L}\mathbf{T}^{L}$
5-dC	71	–	92	36	53	75
X	–	n/a	n/a	84	n/a	n/a
dG	75	–	97	77	<10	62
dA	–	55	97	62	<10	77
X	–	n/a	n/a	69	n/a	n/a
dA	–	9	96	73	<10	66
X	84	n/a	n/a	80	n/a	n/a
dG	74	–	38	85	<10	49
dC	85	65	61	64	30	57

The percentage of *S*-type conformation as obtained by NMR experiments are shown. Due to their locked furanose conformations, the LNA/α-L-LNA monomers were not analyzed ("n/a"); Due to spectral overlap some of the furanose conformations could not be determined ("–"). See the captions below Schemes 1 and 2 for conditions and abbreviations used.

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an *N*-type conformation, whereas no conformational tuning is observed for the α-L-LNA:RNA duplex (Table 3, column 6). These results point to an RNA-like structure of LNA and to a more DNA-like structure of α-L-LNA. This correlates with their locked furanose conformations although the unnatural configurations of an α-L-LNA monomer make a simple comparison with the standard DNA/RNA monomers irrelevant, at least when based solely on furanose ring conformations. It is important to note that LNA as an RNA mimic induces RNA-like structure not only at the monomeric level but also with respect to overall duplex structure, *i.e* induction of A-form characteristics of duplexes.

The results and conclusions described above are supported by binding studies with LNA/2′-*O*-Me-RNA and α-L-LNA/2′-*O*-Me-RNA chimera. The T_m values for duplexes involving the DNA reference, the fully modified LNA and the fully modified 2′-*O*-Me-RNA are depicted in Table 4, entries 1a, 1b and 1c, respectively. The unique properties of LNA are emphasized by the high-affinity recognition of both the DNA and RNA complement (Table 4, entry 1b). 2′-*O*-Me-RNA is a known RNA mimic (21) and a significantly increased binding affinity towards the RNA target, but not the DNA target, is observed (Table 4, entry 1c compared to entry 1a). Subsequently, the effect of introducing three modified monomers in the 2′-*O*-Me-RNA strand was examined and the results compared with the fully modified 2′-*O*-Me-RNA (Table 4, entry 1c). The introduction of three DNA monomers (Table 4, entry 2a) results in significantly reduced binding affinities whereas three α-L-LNA monomers (Table 4, entry 2c) has a marginal positive effect. However, the introduction of three LNA monomers (Table 4, entry 2b) has a strong positive effect with significantly increased T_m values towards both the DNA (+20°C) and the RNA (+15°C) target. The incorporation of LNA monomers therefore appears as a very convenient and efficient way of increasing the binding affinity of 2′-*O*-alkylated RNA strands. This may not only be important in

Table 4. Melting Temperatures (T_m values) Obtained for LNA/2′-*O*-Me-RNA and α-L-LNA/2′-*O*-Me-RNA Chimera

Entry	Sequence	DNA Target T_m/°C	RNA Target T_m/°C
1	5′-(GTGATATGC)		
	a) all-DNA	28	28
	b) all-LNA	64	74
	c) all-2′-*O*-Me-RNA	33	49
2	5′-(G^{OMe}-$\underline{X}$-G^{OMe}-A^{OMe}-$\underline{X}$-A^{OMe}-$\underline{X}$-G^{OMe}-C^{OMe})		
	a) $\underline{X}$ = T	22	34
	b) $\underline{X}$ = $\mathbf{T}^L$	53	64
	c) $\underline{X}$ = $^{\alpha L}\mathbf{T}^L$	38	52

A^{OMe}, C^{OMe}, G^{OMe} and U^{OMe} monomers were used in the fully modified 2′-*O*-Me-RNA sequence and denote 2′-*O*-Me-RNA monomers. See the captions below Schemes 1 and 2 for conditions and abbreviations used.

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relation to possible diagnostic or therapeutic applications, but also indicate that LNA monomers strengthen the *N*-type conformational preorganization of the furanose rings of 2′-*O*-alkyl RNA monomers. Due to the different furanose conformation, a similar effect is not seen for the α-L-LNA monomers.

To further study the apparent conformational tuning of partly modified LNA, the sequences displayed in Table 5 having "dangling" nucleotides were synthesized. It has been reported that a "dangling" nucleotide increases the thermal stability of DNA:DNA duplexes due to favorable stacking interactions (22). Analogously, increased T_m values of duplexes towards both DNA and RNA targets were seen upon the introduction of a single "dangling" nucleotide in the 9-mer DNA strand at either the 3′-end or the 5′-end (Table 5, entries 2 and 3 compared with entry 1; $\Delta T_m = +1$ to $+4^\circ$C). It appears that the effect is more pronounced when the "dangling" nucleotide is positioned at the 3′-end and when RNA is used as complement. Somewhat surprising in light of the unprecedented affinity-enhancing effect resulting from the introduction of an LNA monomer in the center of a DNA strand (*e.g.* Table 1, entry 4), no significant *additional* stabilizing effect was observed when an LNA monomer was incorporated as "dangling" nucleotide (Table 5, entries 4 and 5). However, when the additional $\mathbf{T}^L$ monomer was incorporated in the 3′-end, ΔT_m values of +4 °C were observed towards not only the RNA but also the DNA complement, whereas no stabilizing effect resulted from incorporation of the "dangling" LNA monomer in the 5′-end. As depicted in entries 6–10, similar relative results were obtained for the 9-mer partly modified LNA sequence containing three LNA monomers and the corresponding 10-mers with one "dangling" DNA or LNA nucleotide. From this study it can be extracted that a "dangling" LNA nucleotide, relative to a "dangling" DNA nucleotide, has a stabilizing effect when positioned at the 3′-end and a destabilizing effect when positioned at the 5′-end, at

Table 5. Melting Temperatures (T_m values) Obtained for DNA and LNA Sequences with a "Dangling" Nucleotide at the 3′-end or 5′-end

Entry	Sequence	DNA Target T_m/°C	RNA Target T_m/°C
1	5′-d(GTGATATGC)	32	30
2	5′-d(GTGATATGC<u>T</u>)	34	34
3	5′-d(<u>T</u>GTGATATGC)	33	31
4	5′-d(GTGATATGC<u>**T**</u>^{L})	36	34
5	5′-d(<u>**T**</u>^{L}GTGATATGC)	32	29
6	5′-d(G**T**^{L}GA**T**^{L}A**T**^{L}GC)	46	54
7	5′-d(G**T**^{L}GA**T**^{L}A**T**^{L}GC<u>T</u>)	48	57
8	5′-d(<u>T</u>G**T**^{L}GA**T**^{L}A**T**^{L}GC)	47	56
9	5′-d(G**T**^{L}GA**T**^{L}A**T**^{L}GC<u>**T**</u>^{L})	50	56
10	5′-d(<u>**T**</u>^{L}G**T**^{L}GA**T**^{L}A**T**^{L}GC)	45	56

The "dangling" nucleotide is underlined. The following buffer was used: 140 mM sodium chloride, 10 mM sodium phosphate, pH 7.0. See the caption below Scheme 1 for abbreviations used.

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least when DNA is used as complement. When designing LNA sequences in order to obtain increased binding affinity these results appear useful, *e.g.* should it be taken into consideration that centrally positioned LNA monomers display the most pronounced affinity-enhancing effect.

CONCLUSION

The remarkable binding properties of partly modified LNA can at least in part be explained by conformational tuning of the furanose rings of the unmodified nucleotide monomers towards an *N*-type conformation. This is shown by NMR experiments and supported by thermal melting studies. The situation is different for the stereoisomeric α-L-LNA for which no significant conformational tuning of the unmodified nucleotide monomers in partly modified sequences is observed. Interestingly, as the furanose ring of an α-L-LNA monomer is locked in a DNA-like $_3E$ (*S*-type) conformation, the very strong recognition of RNA using α-L-LNA questions the dogma that *N*-type preorganization is a prerequisite for superior RNA binding.

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REFERENCES

1. Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455.
2. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.
3. Wengel, J. *Acc. Chem. Res.* **1999**, *32*, 301.
4. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401.
5. Koshkin, A. A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V. K.; Singh, S. K.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 13252.
6. Kværnø, L.; Kumar, R.; Dahl, B. M.; Olsen, C. E.; Wengel, J. *J. Org. Chem.* **2000**, *65*, 5167.
7. Singh, S. K.; Wengel, J. *Chem. Commun.* **1998**, 1247.
8. Kumar, R.; Singh, S. K.; Koshkin, A. A.; Rajwanshi, V. K.; Meldgaard, M.; Wengel, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2219.
9. Singh, S. K.; Kumar, R.; Wengel, J. *J. Org. Chem.* **1998**, *63*, 10035.
10. Ørum, H.; Jakobsen, M. H.; Koch, T.; Vuust, J.; Borre, M. B. *Clin. Chem.* **1999**, *45*, 1898.

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11. Rajwanshi, V. K.; Håkansson, A. E.; Dahl, B. M.; Wengel, J. *Chem. Commun.* **1999**, 1395.
12. Rajwanshi, V. K.; Håkansson, A. E.; Kumar, R.; Wengel, J. *Chem. Commun.* **1999**, 2073.
13. Rajwanshi, V. K.; Håkansson, A. E.; Sørensen, M. D.; Pitsch, S.; Singh, S. K.; Kumar, R.; Nielsen, P.; Wengel, J. *Angew. Chem. Int. Ed.* **2000**, *39*, 1656.
14. Håkansson, A. E.; Bryld, T.; Wengel, J. *Manuscript in preparation*.
15. The furanose conformation of an α-L-LNA monomer could alternatively be assigned as *N*-type because of its L-configuration. However, to facilitate a direct comparison with the conformation of the natural DNA/RNA monomers and the parent LNA monomers, the C3'-*exo*/$_3E$ furanose conformation is considered herein as equivalent to an *S*-type conformation.
16. Petersen, M.; Nielsen, C. B.; Nielsen, K. E.; Jensen, G. A.; Bondensgaard, K.; Singh, S. K.; Rajwanshi, V. K.; Koshkin, A. A.; Dahl, B. M.; Wengel, J.; Jacobsen, J. P. *J. Mol. Recognit.* **2000**, *13*, 44.
17. Bondensgaard, K.; Petersen, M.; Singh, S. K.; Rajwanshi, V. K.; Kumar, R.; Wengel, J.; Jacobsen, J. P. *Chem. Eur. J.* **2000**, *6*, 2687.
18. Jensen, G. A.; Jacobsen, J. P. *Submitted*.
19. Jensen, G. A.; Wengel, J.; Jacobsen, J. P. *Manuscript in preparation*.
20. Nielsen, K. E.; Wengel, J.; Jacobsen, J. P. *Manuscript in preparation*.
21. Blommers, M. J. J.; Pieles, U.; De Mesmaeker, A. *Nucleic Acids Res.* **1994**, *22*, 4187.
22. Guckian, K. M.; Schweitzer, B. A.; Ren R. X.-F.; Sheils, C. J.; Paris, P. L.; Tahmassebi, D. C.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 8182.

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