[13] a) Naphthalene: C. P. Brock, J. D. Dunitz, *Acta Cryst. Sect. B* **1982**, *38*, 2218–2228; b) biphenyl: J. Trotter, *Acta Cryst.* **1961**, *14*, 1135–1139; c) anthracene: C. P. Brock, J. D. Dunitz, *Acta Cryst. Sect. B* **1990**, *46*, 795–806.

[14] Weight percent of guest loss by TGA (obs/calcd): **1**: 48.6/48.6, **2**: 51.8/51.7, **3**: 52.5/54.0.

[15] A full hemisphere of data was collected on a Siemens SMART diffractometer with $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). Data were corrected for Lorentz polarization, absorption, and decay using SADABS. Structures were solved by direct methods (SHELXS) and refined with full-matrix least squares based on $|F^2|$ (SHELX-97-2). Hydrogen atoms were placed in calculated positions using a riding model. X-ray data for **1** ($C_{21}H_{21}N_3O_3S$): $0.42 \times 0.36 \times 0.21$ mm, monoclinic, $P2_1/n$ (no. 14), $a = 7.6376(1)$, $b = 22.0442(4)$, $c = 11.8162(1)$ Å, $\beta = 91.611(1)^\circ$, $Z = 4$, $V = 1988.65(5)$ Å^3, $\rho_{calcd} = 1.321$ g cm^{-1}, $\mu = 0.190$ mm^{-1}, $1.8 < 2\theta < 24.0^\circ$, $T = 173$ K, $R_1 = 0.0351$ and $wR_2 = 0.0819$ for 2621 ($I > 2\sigma[I]$) of 3107 unique reflections and 253 parameters. X-ray data for **2** ($C_{25}H_{25}N_3O_3S$): $0.49 \times 0.22 \times 0.22$ mm, monoclinic, $P2_1/n$ (no. 14), $a = 7.6612(6)$, $b = 26.306(2)$, $c = 11.4887(9)$ Å, $\beta = 90.458(2)^\circ$, $Z = 4$, $V = 2315.3(3)$ Å^3, $\rho_{calcd} = 1.284$ g cm^{-1}, $\mu = 0.171$ mm^{-1}, $1.5 < 2\theta < 25.0^\circ$, $T = 173$ K, $R_1 = 0.0359$ and $wR_2 = 0.0920$ for 3086 ($I > 2\sigma[I]$) of 4084 unique reflections and 289 parameters. X-ray data for **3** ($C_{29}H_{30}N_3O_3S$): $0.24 \times 0.09 \times 0.03$ mm, monoclinic, $P2_1/n$ (no. 14), $a = 7.559(5)$, $b = 11.96(1)$, $c = 26.85(2)$ Å, $\beta = 94.17(8)^\circ$, $Z = 4$, $V = 2422(3)$ Å^3, $\rho_{calcd} = 1.36$ g cm^{-1}, $\mu = 0.171$ mm^{-1}, $1.9 < 2\theta < 24.0^\circ$, $T = 173$ K, $R_1 = 0.2791$ and $wR_2 = 0.5839$ for 2704 ($I > 2\sigma[I]$) of 3801 unique reflections and 186 parameters. The highly mosaic nature of all samples made accurate integration of the reflection intensities difficult and precluded the possibility of a more accurate single-crystal structure determination for compound **3**. We surmise that the high mosaicity can be attributed to the larger lattice mismatch (8%), compared to **1** and **2**, between the host structure and the native anthracene structure. X-ray powder diffraction of the bulk material (see the Supporting Information) reveals a unit cell identical to that determined from the single-crystal data and, based upon extensive studies of closely related compounds, confirms the structural assignment of the brick architecture for **3**. Importantly, the metric parameters with which this manuscript is principally concerned (i.e., b_1 and b_2) can be obtained from the lattice constants. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-136727 (**1**), -136728 (**2**), and -136729 (**3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[16] Unique interplanar dihedral angles in **1**: 51°, 56°; **2**: 57°, 67°, 56°, 66°; **3**: 47°, 54°. A subtle difference between the inclusion compounds and the pure guest structures is the nonparallelism between face-to-face arenes in **1**–**3** (**1**: 14°; **2**: 5°; **3**: 10°).

[17] K. Biradha, K. V. Domasevitch, B. Moulton, C. Seward, M. J. Zaworotko, *Chem. Commun.* **1999**, 1327–1328.

[18] Packing fraction calculations performed with Molecular Simulations Inc. Cerius^2 (v. 3.5) software using a probe radius of 0.5 Å.

The Eight Stereoisomers of LNA (Locked Nucleic Acid): A Remarkable Family of Strong RNA Binding Molecules**

Vivek K. Rajwanshi, Anders E. Håkansson, Mads D. Sørensen, Stefan Pitsch, Sanjay K. Singh, Ravindra Kumar, Poul Nielsen, and Jesper Wengel*

For more than a decade, chemists have searched the chemical space in order to obtain nucleic acid analogues with certain desirable properties, such as increased stability towards nucleolytic degradation and increased binding affinity (and specificity) towards complementary natural nucleic acid targets.[1] A strong impetus for this research has been, and continues to be, the therapeutic promises of the antisense strategy.[2] Unprecedented thermal affinities of duplexes involving the nucleic acid analogue "LNA" (Locked Nucleic Acid, **T$^{\mathbf{L}}$**, β-D-ribo isomer, Figure 1) have recently been reported by us[3, 4] and others.[5] The furanose ring of LNA, being part of a dioxabicyclo[2.2.1]heptane skeleton, is efficiently locked in a C-3′-*endo* (N-type) conformation and we have initiated a program which focuses on the synthesis and properties of the likewise conformationally locked configurational isomers of LNA. Recently we have published the syntheses of the two first diastereoisomeric forms of LNA, namely "xylo-LNA" (**xT$^{\mathbf{L}}$**, β-D-xylo isomer) and "α-L-LNA" ($^{\alpha\mathbf{L}}$**T$^{\mathbf{L}}$**, α-L-ribo isomer, Figure 1).[6, 7]

In this report, the RNA binding of all eight possible stereoisomers of LNA is evaluated. Synthesis of "α-L-xylo-LNA" ($^{\alpha\mathbf{L}}$**xT$^{\mathbf{L}}$**, α-L-xylo isomer,[7] Figure 1) has been accomplished and the remaining four stereoisomers (Figure 1), all enantiomers of the four synthesized diastereoisomers, are indirectly evaluated by hybridization studies of the four synthesized stereoisomers towards enantiomeric RNA targets (*ent*-RNA, also known as L-RNA or mirror-image RNA).[8]

In Table 1 the results from hybridization studies towards RNA complements for homo-thymine derivatives are shown. Whereas the remarkable binding affinities of fully modified[7] LNA, α-L-LNA, and xylo-LNA have been reported earlier,[3,6b] it can be seen that fully modified α-L-xylo-LNA [5′-($^{\alpha\mathbf{L}}$**xT$^{\mathbf{L}}$**)$_9$T] is unable to hybridize towards both RNA and *ent*-RNA.[9] The reason for this is presently unclear but the

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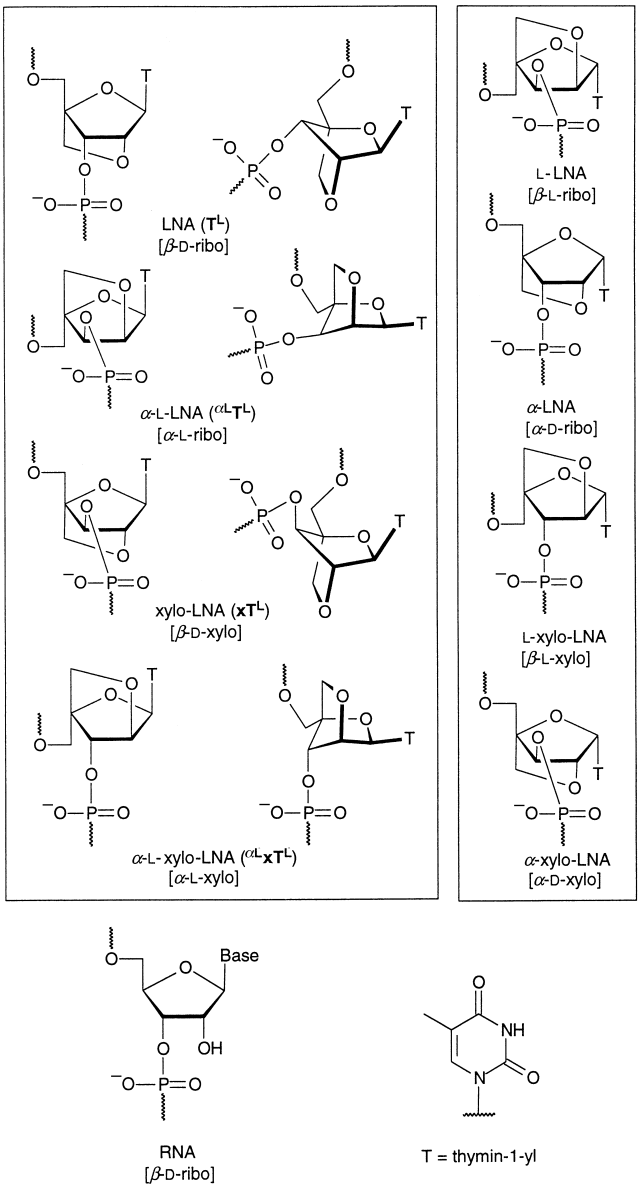

Figure 1. The four synthesized diastereoisomeric LNAs are shown to the left together with their locked furanose conformations, as obtained from molecular modeling studies. To the right, the structures of the indirectly evaluated remaining four LNA stereoisomers are depicted. Also shown are the structure of RNA and the thymine base (T). The configurations are indicated in the square brackets.

unnatural configuration may in this case prevent a suitable positioning of the thymine base for hybridization. Interestingly, the formation of very stable complexes with *ent*-RNA was detected for LNA, α-L-LNA, and xylo-LNA as indicated by the T_m values of 52 °C, 40 °C, and 39 °C, respectively.

Table 1. Binding studies of homo-thymine diastereoisomeric LNAs towards RNA (rA_{14}), singly mis-matched RNA (5′-$r(A_6CA_7)$), enantiomeric RNA (*ent*-rA_{14}), and singly mismatched enantiomeric RNA (*ent*-5′-$r(A_6CA_7)$) as shown by melting temperatures (T_m values [°C]).[a]

Sequence	rA_{14}	5′-$r(A_6CA_7)$	*ent*-rA_{14}	*ent*-5′-$r(A_6CA_7)$
T_{10}	18[b]	no T_m[c]	no T_m[c]	no T_m[c]
5′-$(\mathbf{T^L})_9$T	71[b]	61	52	51
5′-$(^{\alpha L}\mathbf{T^L})_9$T	66[d]	49	40	no T_m[c]
5′-$(\mathbf{xT^L})_9$T	57[d]	47	39	36
5′-$(^{\alpha L}\mathbf{xT^L})_9$T	no T_m[c]	no T_m[c]	no T_m[c]	no T_m[c]

[a] T_m values obtained from the maxima of the first derivatives of the melting curves (absorbance at 260 nm (A_{260}) versus temperature; gradient 0.5 °C min^{-1}); recorded in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM ethylenediaminetetraacetate (EDTA), pH 7.0); 1.5 mM concentrations of the two complementary strands assuming identical extinction coefficients for modified and unmodified thymine (oligo)nucleotides. [b] See reference [3a]. [c] No cooperative transition above 10 °C. [d] See reference [6b].

Experiments towards the singly mismatched RNA target [5′-$r(A_6CA_7)$] showed the hybridization of LNA, α-L-LNA, and xylo-LNA towards RNA to be selective (changes in T_m values of −10 °C, −17 °C, and −10 °C, respectively). Using the singly mismatched *ent*-RNA target [*ent*-5′-rA_6CA_7], LNA and xylo-LNA displayed only slightly reduced T_m values compared to the fully matched situation. However, the selectivity of α-L-LNA:*ent*-RNA hybridization appears significant. Taken together, the melting results shown in Table 1 are quite remarkable, as illustrated by the ΔT_m values (change in T_m value per modification) ranging from +2.3 to +5.9 °C for the six hybridizing LNAs. The decisive factor for binding towards the RNA and *ent*-RNA targets for the LNAs shown in Table 1 is anticipated to be the nine consecutive LNA-type monomers. However, the fact that all sequences contain a 3′-end natural thymidine monomer could have a minor influence on the T_m data, as this monomer interacts differently with the RNA and the ent-RNA targets. It is noteworthy that only a very few of the large number of oligonucleotide analogues so far synthesized, such as the phosphodiester analogues HNA (hexitol nucleic acid)[10] and tricyclo-DNA,[11] have displayed binding affinities towards complementary RNA comparable with those obtained with the six stereoisomeric LNAs.

Especially fascinating is the fact that the binding affinity of α-L-LNA towards RNA is comparable to that of the parent LNA. This prompted us to further study the hybridization properties of a 9-mer mixed sequence α-L-LNA, containing three α-L-LNA thymine monomers ($^{\alpha L}\mathbf{T^L}$) and six unmodified deoxynucleotide monomers (Table 2, entry 3) in comparison with the corresponding DNA (Table 2, entry 1) and LNA (Table 2, entry 2). From the results shown in entry 3 it can be extracted that the binding affinity of the α-L-LNA is excellent

Table 2. Binding studies of mixed sequence 9-mer DNA, LNA, and α-L-LNA as shown by melting temperatures (T_m values [°C]).[a]

Entry		5′-d(G**X**GA**X**A**X**GC):3′-d(CACT**N**TACG)				5′-d(G**X**GA**X**A**X**GC):3′-r(CACU**N**UACG)	
		N = A	C	T	G	A	C
1	**X** = T	28/28[b]	11/13[b]	12/15[b]	19/20[b]	28/29[b]	10/no T_m[b]
2	**X** = $\mathbf{T^L}$	44[c]	23[c]	27[c]	30[c]	50[c]	33[c]
3	**X** = $^{\alpha L}\mathbf{T^L}$	37	19	19	28	45	23

[a] For experimental details, see footnote [a] of Table 1. [b] These T_m values varied between two experimental series; the first value relates to the data in entry 2; the second value relates to the data in entry 3. [c] See reference [3b].

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towards both complementary DNA ($\Delta T_m = +3.0\,^\circ C$) and RNA ($\Delta T_m = +5.3\,^\circ C$), though lower than that of LNA ($\Delta T_m = +5.3\,^\circ C$ towards DNA and $\Delta T_m = +7.3\,^\circ C$ towards RNA). The binding affinities obtained towards the singly mismatched sequences prove the excellent binding specificity of α-L-LNA towards both RNA and DNA targets (Table 2, entry 3 relative to entries 1 and 2).

CD spectra were recorded in order to evaluate the overall conformation of the duplexes involving LNA, xylo-LNA and α-L-LNA. In Figures 2 and 3, the CD spectra of these diastereoisomeric LNAs hybridized towards complementary RNA (rA_{14}) and *ent*-RNA (*ent*-rA_{14}), respectively, are shown. From the CD spectra in Figure 2 it can be seen that the

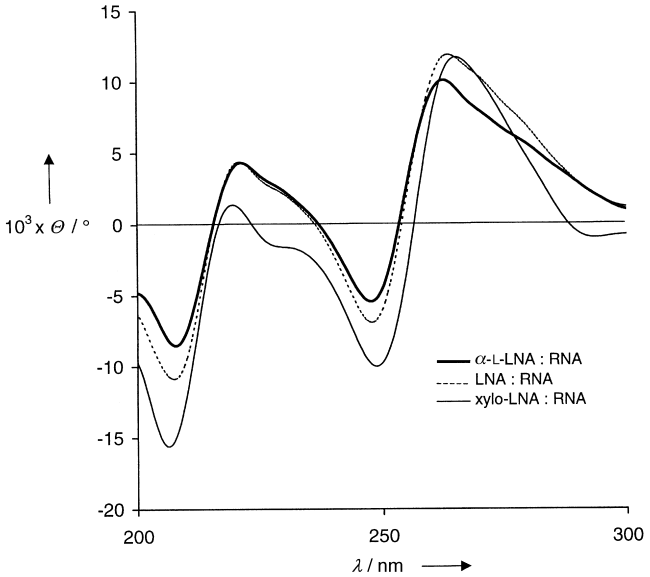

Figure 2. CD spectra recorded at 20 °C of α-L-LNA:RNA [5′-($^{\alpha L}\mathbf{T}^{L}$)$_9$-T:$rA_{14}$], LNA:RNA [5′-($\mathbf{T}^{L}$)$_9$T:$rA_{14}$], and xylo-LNA:RNA [5′-($\mathbf{xT}^{L}$)$_9$T:rA_{14}] duplexes. Conditions otherwise as described in footnote [a] of Table 1.

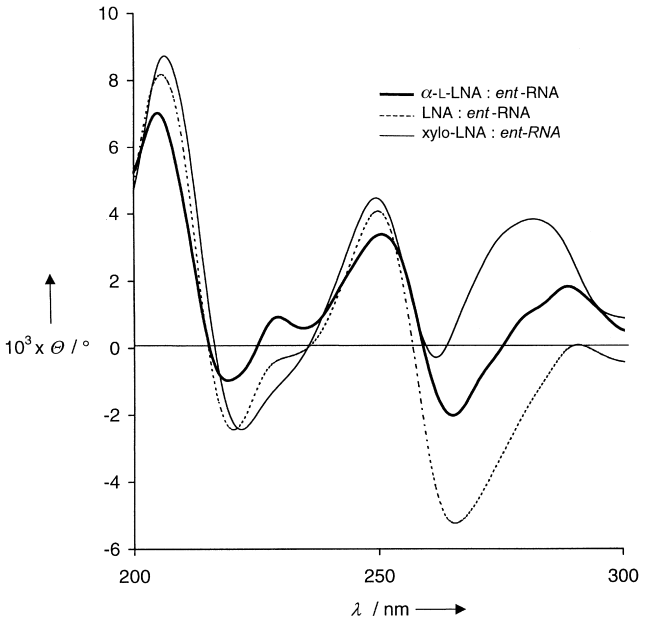

Figure 3. CD spectra recorded at 20 °C of α-L-LNA:*ent*-RNA [5′-($^{\alpha L}\mathbf{T}^{L}$)$_9$-T:*ent*-$rA_{14}$], LNA:*ent*-RNA [5′-($\mathbf{T}^{L}$)$_9$T:*ent*-$rA_{14}$], and xylo-LNA:*ent*-RNA [5′-($\mathbf{xT}^{L}$)$_9$T:*ent*-rA_{14}] duplexes. Conditions otherwise as described in footnote [a] of Table 1.

conformations of the LNA:RNA and α-L-LNA:RNA duplexes are strikingly similar whereas the xylo-LNA:RNA duplex displays somewhat deviating characteristics (for example, a reduced ellipticity value at 220 nm). The CD spectra of the LNA:RNA and α-L-LNA:RNA duplexes closely resemble the CD spectrum of a reference A-type DNA:RNA (T_{14}:rA_{14}) duplex.[12] This strongly suggests that LNA and α-L-LNA bind with complementary RNA in the antiparallel duplex mode which was likewise indicated by the hybridization data shown in Table 2. Figure 3 demonstrates a structural similarity, especially between the LNA:*ent*-RNA and α-L-LNA:*ent*-RNA duplexes and, for example, a characteristic positive ellipticity values above 240 nm for the xylo-LNA:*ent*-RNA duplex. These CD spectra reveal that α-L-LNA, like LNA,[3c] can be regarded as an RNA mimic.

Both the T_m experiments and the CD spectra point to a structural similarity between an LNA monomer and an α-L-LNA monomer, such as $\mathbf{T}^{L}$ and $^{\alpha L}\mathbf{T}^{L}$ (Figure 1), having an identical constitution but different configuration at three out of the four asymmetric carbon atoms. This is further illustrated in Figure 4 which displays energy-minimized structures of the corresponding thymine nucleosides aligned to show the close three-dimensional fit between three key atoms in relation to nucleic acid structure, namely N-1, O-3′ and C-5′.[13]

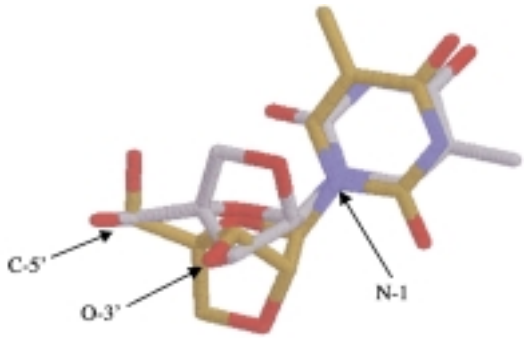

Figure 4. Energy-minimized structures of the LNA thymine nucleoside (yellow) and the α-L-LNA thymine nucleoside (gray) (HyperChem program, Polak-Ribiere algorithm).

Remarkably increased binding affinities towards RNA, compared to the DNA reference, have been documented for six out of the eight stereoisomeric LNAs which contain conformationally locked furanose moieties. This indicates that the potential for efficient hybridization is not restricted to the natural β-D-ribofuranosyl nucleic acids (RNA) and that the position of the conformational equilibria for the different potentially natural pentofuranose stereoisomers could have played a role in nature's choice[14] of the β-ribofuranosyl isomer.[15] The remarkable binding affinities and specificities obtained for LNA and α-L-LNA, both in a fully and in a partly modified context, establish these molecules as unique nucleic acid mimics.

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Metal Incorporation into and Dimerization of M_3E_4 Clusters (M = Mo, W; E = S, Se) in Supramolecular Assemblies with Cucurbituril: A Molecular Model of Intercalation**

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Incorporation of transition and post transition elements M′ into trinuclear M_3^{IV} incomplete cuboidal clusters $[M_3E_4(H_2O)_9]^{4+}$ (M = Mo, W; E = S, Se), first reported in 1986,[1] has developed into a field of considerable research interest.[2, 3] The most fascinating aspect is that the zero oxidation state heteroatom can in many cases be used as the source of M′. Thus with $[Mo_3S_4(H_2O)_9]^{4+}$, direct incorporation of Hg, Ga, In, Tl, Sn, Pb, Sb, Bi, Fe, Co, Ni, Cu, and Pd has been achieved.[3] Characterization has, however, remained a serious problem, and in many cases, in particular with the highly charged double cubes (e.g. M′ = Hg, In, Sn, Sb), crystallization has only been possible as the pts^- salt of the aqua ion $[Mo_6M'S_8(H_2O)_{18}](pts)_8 \cdot x\,H_2O$ ($pts^- = p$-toluenesulfonate).[2, 4, 5] This may also be regarded as a supramolecular approach, since the pts^- ion is responsible for hydrogen bonding which holds the structure together.

Here we report rational syntheses of examples of a potentially rich and versatile class of supramolecular structures based on cucurbituril adducts. These can be prepared with the trinuclear clusters and heterometal-containing double cubes obtained as derivatives of $[M_3E_4(H_2O)_9]^{4+}$. Both the trinuclear and double cube products have a molecular C_3 axis. Cucurbituril ($C_{36}H_{36}N_{24}O_{12}$) is a macrocyclic cavitand with D_{6h} symmetry, having two identical carbonyl-fringed portals.[6] We have identified as potential hydrogen-bond donors six coordinated water molecules, *cis* to the unique μ_3-E group capping the triangle of Mo or W atoms, as complementary to six cucurbituril portal oxygen acceptor atoms. Crystallization should therefore be facilitated by the formation of hydrogen-bonded supramolecular aggregates. Coordination of cucurbituril to Na^+, Rb^+, and Cs^+ has been reported.[7] Also, aliphatic and aromatic ammonium ions show high affinity towards cucurbituril.[8]

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