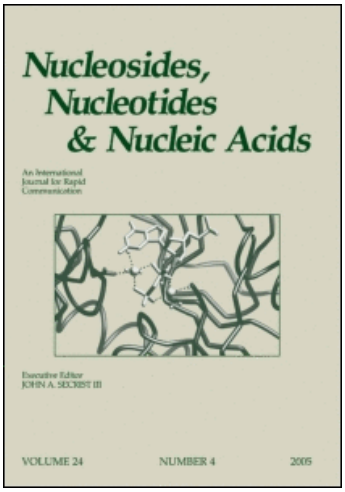

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Two Carbocyclic Locked Nucleic Acid Analogues Give Structural Information about the Role of Hydration in A-Type Duplexes

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TWO CARBOCYCLIC LOCKED NUCLEIC ACID ANALOGUES GIVE STRUCTURAL INFORMATION ABOUT THE ROLE OF HYDRATION IN A-TYPE DUPLEXES

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□ *Two locked nucleic acid (LNA) analogues with three-carbon 2′-4′ linkages, saturated or unsaturated, are synthesized using a ring-closing metathesis based strategy. Strongly stabilized duplexes with complementary RNA and slightly destabilized duplexes with complementary DNA are observed. CD-spectroscopy indicates a less pronounced shift toward A-type duplexes compared to LNA. These results combining a strong N-type conformation with the absence of a 2′-oxygen demonstrate a stronger importance of minor groove hydration in an intermediate duplex type than in an A-type duplex.*

Keywords Locked nucleic acid; RNA recognition; duplex hydration

Locked nucleic acid (LNA)[1–3] and its analogue ENA[4] are among the most intriguing nucleic acid analogues due to their unprecedented affinity for complementary RNA and DNA sequences. The LNA and ENA monomers (Figure 1) are both locked in the N-type conformation. A single incorporation of an LNA monomer in an oligodeoxynucleotide (ODN) leads to an increase in the thermal stability of 3–8°C of a DNA:RNA duplex and 3–5°C of a DNA:DNA duplex.[2] This behavior is related to the ability of LNA-monomers to conformationally steer their neighboring 2′-deoxynucleotides into an N-type conformation and hereby to induce A-type or A-type like duplex conformation.[3]

ENA demonstrates almost the same stabilisation of duplexes as LNA,[4] whereas other analogues in which the 4′-2′ linkage has another constitution (i.e., a "COC" or an even longer "COCO" or "CCCO") demonstrates somewhat less pronounced stabilization of a DNA:RNA duplex and a neutral or even destabilizing effect on DNA:DNA duplexes.[4,5] The 4′-2′-linkage with three carbon atoms, however, has been only briefly mentioned in a review[6]

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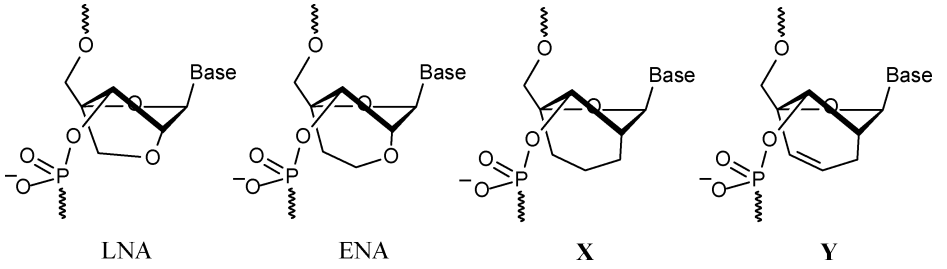
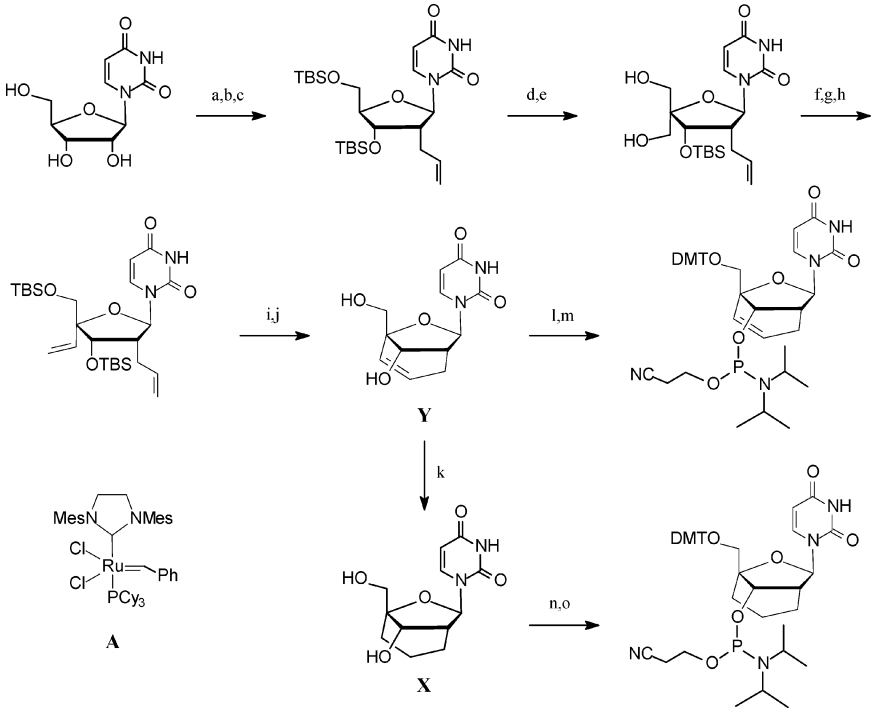


FIGURE 1 LNA and ENA and two new carbocyclic analogues.

and reported to give a destabilized DNA:RNA duplex which is a surprising result compared to the results of the other analogues. We, therefore, decided to synthesise this analogue, **X**, by a ring-closing metathesis based strategy giving access also to the unsaturated analogue **Y** (Figure 1).

The synthesis started from uridine following a linear approach (Scheme 1) and took advantage of the reported selective 5′,3′-silylation[7]



SCHEME 1 a) TBSCl, DABCO, $AgNO_3$, THF, 90%, ref. 7; b) PhOCSCl, DMAP, CH_3CN, 79%; c) $AllylSnBn_3$, AIBN, toluene, 65%, ref. 8; d) aq. AcOH, 75%; e) i) Dess-Martin periodinane, CH_2Cl_2; ii) H_2CO, MeOH, aq. NaOH, dioxane then $NaBH_4$, 52%; f) i) BzCl, pyridine, CH_3CN, 65%; ii) TBSCl, Im., DMF; iii) NaOMe, MeOH, 71%; g) Dess-Martin periodinane, CH_2Cl_2, 93%; h) Ph_3PCH_3Br, BuLi, THF, 95%; i) **A**, CH_2Cl_2, 96%; j) TBAF, THF, 98%; k) H_2, PtO_2, MeOH, 100%; l) DMTCl, pyridine, CH_3CN, 78%; m) $NC(CH_2)_2OPClN(iPr)_2$, DIPEA, CH_2Cl_2 63%; n) DMTCl, Et_3N, CH_2Cl_2, 78%; o) $NC(CH_2)_2OPClN(iPr)_2$, DIPEA, CH_2Cl_2 57%.

TABLE 1 Hybridization data.

	T_m (ΔT_m per modification)/ °C	
ODN sequence	Complementary DNA	Complementary RNA
5′-dGTGATATGC-3′	30.0	27.5
5′-dGTGA**X**ATGC-3′	29.0 (−1.0)	32.0 (+4.5)
5′-dG**X**GA**X**A**X**GC-3′	27.0 (−1.0)	38.0 (+3.5)
5′-dGTGA**Y**ATGC-3′	29.0 (−1.0)	31.5 (+4.0)
5′-dG**Y**GA**Y**A**Y**GC-3′	23.0 (−2.3)	34.5 (+2.3)

Buffer: 5 mM Na_2HPO_4, 10 mM NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0; 1.5 μM of each strand.

and 2′-allylation,[8] protecting group manipulations, a 4′-vinylation and a very efficient ring-closing metathesis using Grubbs second generation catalyst **A** to give the analogue **Y**. Hydrogenation gave **X** and both analogues were converted into DMT-protected phosphoramidites and incorporated into ODN′s (Table 1)

The ODNs were mixed with complementary DNA and RNA sequences and the stability of the duplexes were determined. Significant increases in stability of the DNA:RNA duplexes were observed. These are only slightly smaller than the increases found for LNA and ENA. On the other hand, slight decreases in stability of the DNA:DNA duplexes were observed. This is different to the data obtained with LNA and ENA for which increased stability was found also for DNA:DNA sequences. CD-spectra (not shown) demonstrate that the shift toward A-type (for DNA:RNA) or A-type like duplexes (for DNA:DNA) is smaller for the duplexes containing **X** or **Y** than for comparable LNA sequences. In other words, the conformational steering on neighbouring 2′-deoxynucleotides is smaller for **X** and **Y** than it is for LNA (and probably also ENA) nucleotides. This might be due to the lack of a 2′-oxygen atom in the former hampering the hydration pattern in the minor groove of the duplexes. Apparently, this hydration pattern is more important in intermediate type DNA:DNA duplexes with a narrow minor groove than in the compact A-type DNA:RNA duplexes with a broader minor groove.

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