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LNA stereoisomers: *xylo*-LNA (β-D-*xylo* configured locked nucleic acid) and α-L-LNA (α-L-ribo configured locked nucleic acid)

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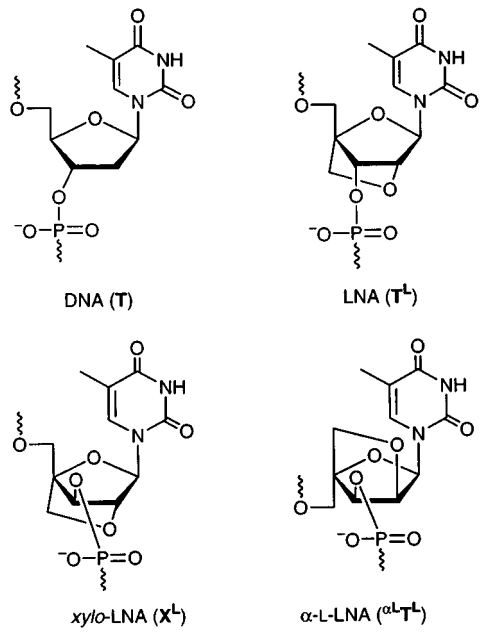
Synthesis of xylo-LNA containing one 2′-*O*,4′-*C*-methylene-β-D-xylofuranosyl thymine nucleotide monomer and α-L-LNAs containing one or four 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl thymine nucleotide monomer(s) has been accomplished using phosphoramidite chemistry with pyridine hydrochloride as activator; oligothymidylate α-L-LNA displays strongly enhanced affinity towards complementary RNA.

In a series of papers, Seela *et al.* have synthesized and studied xylo-DNA containing one or more 2′-deoxy-β-D-xylofuranosyl nucleotide(s).[1–4] Compared with the corresponding natural 2′-deoxy-β-D-ribofuranosyl oligonucleotide reference (**T**, thymine

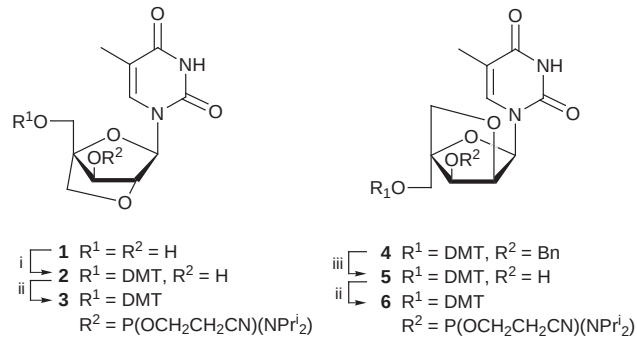


derivatives are shown for all the four different monomers), *xylo*-DNA generally displays decreased thermal affinity towards complementary single stranded DNA.[1–4] We[5–9] and others[10] have recently reported unprecedented thermal stabilities of duplexes involving LNA (Locked Nucleic Acid, $\mathbf{T^L}$)† and complementary single stranded DNA or RNA. Here the first stereoisomers of LNA are introduced, namely *xylo*-LNA ($\mathbf{X^L}$) containing one or more 2′-*O*,4′-*C*-methylene-β-D-xylofuranosyl nucleotide monomer(s), and α-L-LNA containing one or more 2'-*O*,4'-*C*-methylene-α-L-ribofuranosyl nucleotide monomer(s) ($^{\alpha L}T^L$).

Conversion of the thymine *xylo*-LNA nucleoside **1**[11] and the α-L-LNA nucleoside **4**,[12] which both were obtained from D-glucose, into the phosphoramidite building blocks **3** and **6**, respectively, was performed as depicted in Scheme 1. Nucleoside **1** was reacted first with 1.5 equiv. of 4,4′-dimethoxytrityl chloride (DMTCl) in anhydrous pyridine (25 h, room temperature) and then with an additional 1.0 equiv. (21 h, room temperature) to afford the 5′-*O*-DMT-protected *xylo*-LNA nucleoside **2**‡ in 50% yield after column chromatographic separation from the 3′-*O*-DMT isomer (isolated in 29% yield). Interactions (sterical interference and/or hydrogen bonding) between the three substituents at the α-face of the furanose ring are possible explanations for the unusually low reactivity, and low selectivity, towards dimethoxytritylation of the primary hydroxy group of nucleoside **1**. Subsequent phosphitylation of the 3′-hydroxy group afforded in 51% yield the phosphoramidite derivative **3**.§ The 5′-*O*-DMT-protected derivative **5**[12]¶ was obtained in 80% yield by debenzylation of nucleoside **4** and subsequently converted into the phosphoramidite **6** in low yield.‖

The oligomers were synthesized on an automated DNA synthesizer by use of the phosphoramidite approach.[13] Seela *et al.* have reported that the use of 3′-*O*-phosphoramidites in their syntheses of *xylo*-DNA required a ten-fold extension of the standard coupling time. Therefore they applied phosphonate chemistry for oligomerization of 5′-*O*-DMT protected 2′-deoxy-β-D-xylofuranosyl monomers.[3] Analogously, the coupling yield of amidite **3** was only 15% after 10 min coupling time using standard concentrations and 1*H*-tetrazole as activator (sequence 5′-$\mathbf{X^L}T_6$; coupling yields determined spectrophotometrically by the release of the 4,4′-dimethoxytrityl group after each coupling step). This inefficient coupling of **3**, which strongly contrasts with the nearly quantitative coupling of the parent diastereoisomeric LNA amidite,[5–7] is probably caused by steric hindrance during the reaction between the 5′-hydroxy group of 5′-OH-T_6 and the amidite **3**, the latter having three sterically demanding groups oriented towards the α-face of the furanose ring. In an attempt to improve the coupling yield of amidite **3**, syntheses of 5′-$\mathbf{X^L}T_6$ using different activators[14–16] and coupling times were performed (Table 1). These experiments showed that the use of pyridine hydrochloride as activator may indeed allow efficient coupling of sterically very hindered phosphoramidite building blocks. This is an important result as research in this area moves towards bicyclic,[9] tricyclic,[17] functionalized[9] and branched[18] oligonucleotides.

We then turned to the synthesis of the *xylo*-LNA and α-L-LNA sequences **8**–**10** shown in Table 2 using the optimized conditions (pyridine hydrochloride; 10 min coupling time)



Scheme 1 *Reagents and conditions*: i, DMTCl, anhydrous pyridine (50%); ii, Pr^i_2NEt, 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite, anhydrous CH_2Cl_2 (**3**: 51%; **6**: 7%‖); iii, ammonium formate, Pd/C, MeOH (80%).

Table 1 Model syntheses of 5′-$\mathbf{X^L}T_6$

Activator	*t*/min	Yield[a,b] (%)
1*H*-Tetrazole	10 min	15
1*H*-Tetrazole	30 min	31
4,5-Dicyanoimidazole[c]	30 min	71
Pyridine hydrochloride[d]	10 min	> 99

[a] Refers to the coupling yield for amidite **3**. [b] The coupling yield of the unmodified ß-cyanoethyl T-amidite was > 99%. [c] Ref. 14. [d] Ref. 15,16.

Table 2 *Xylo*-LNA (**8**) and α-L-LNAs (**9** and **10**) synthesized; T_m values measured[a]

Sequence	dA_{14} Complement T_m/°C (ΔT_m/°C)	rA_{14} Complement T_m/°C (ΔT_m/°C)	Mass found $[M - H]^-$	Mass calc. $[M - H]^-$
7 5′-T_{14}	32	28		
8 5′-$T_7\mathbf{X^L}T_6$	19 (−13)	24 (−4)	4221.2	4223.8
9 5′-$T_7(^{\alpha L}\mathbf{T^L})T_6$	32 (±0)	33 (+5)	4225.4	4223.8
10 5′-$T_5(^{\alpha L}\mathbf{T^L})_4T_5$	36 (+1)	46 (+4.5)	4309.0	4307.8

[a] Melting temperatures (T_m values) obtained from the maxima of the first derivatives of the melting curves (A_{260} *vs.* temperature) recorded in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 mM concentrations of the two complementary strands (assuming identical extinction coefficients for all modified and unmodified thymine nucleotides). Also shown are changes in T_m value *per modification* (ΔT_m) compared with the reference values obtained for **7**.

affording coupling yields of ~99% for amidite **3** and 97–99% for amidite **6**.** Contrary to amidite **6**, consecutive incorporation of amidite **3** resulted in reduced coupling yields (85–97%; sequences not shown). The oligomers **8**–**10** were synthesized in the DMT-off mode and directly ethanol-precipitated after cleavage from the solid support yielding products with > 90% purity as judged from capillary gel electrophoresis.

The results from preliminary binding studies in medium salt buffer are shown in Table 2. The thermal stability of complexes formed between *xylo*-LNA **8**, containing a single $\mathbf{X^L}$ monomer, and complementary single stranded DNA (dA_{14}) and RNA (rA_{14}) was significantly reduced (ΔT_m/mod = −13 and −4 °C, respectively) when compared with the unmodified T_{14} reference **7**. Contrary to this, the binding affinity of α-L-LNAs **9** and **10** was unchanged or slightly improved towards complementary DNA, and strongly increased towards complementary RNA (ΔT_m/mod = +4.5 and +5 °C). The latter results compare closely with our results obtained earlier for the corresponding LNA oligothymidylate sequences.[5] Modeling studies on monomers $\mathbf{X^L}$ and $^{\alpha L}\mathbf{T^L}$ clearly show their furanose conformations to be very different. Thus, whereas $\mathbf{X^L}$ is locked in a 3′-*endo* (N-type) conformation, $^{\alpha L}\mathbf{T^L}$ is locked in a 3′-*exo* (S-type) conformation. The preliminary binding data indicate that the inverted configuration at C-3′ (compared with *e.g.* LNA and DNA) in *xylo*-LNA monomer $\mathbf{X^L}$ causes an unfavorable local disruption of the regular duplex structures. However, the 2′-*exo* conformation of monomer $^{\alpha L}\mathbf{T^L}$ allows the formation of very stable hetero duplexes despite the configuration being inverted at both C-3′ and C-4′. In fact, superimposition of models of the LNA monomer $\mathbf{T^L}$ and the α-L-LNA monomer $^{\alpha L}\mathbf{T^L}$ reveals the possibility of a very close three-dimensional positioning of the thymine moieties and of the C-5′- and C-3′-oxygen atoms for the two monomers existing in locked 3′-*endo* and 3′-*exo* conformations, respectively.

The first LNA stereoisomers, *xylo*-LNA and α-L-LNA, have been synthesized using phosphoramidite chemistry on an automated DNA synthesizer applying extended coupling times and pyridine hydrochloride as activator. This synthetic method should be generally applicable for coupling of sterically hindered phosphoramidite building blocks. α-L-LNA containing one and four α-L-LNA monomers recognized complementary RNA with remarkably increased affinity. Currently, the binding properties of a variety of *xylo*-LNA and α-L-LNA oligomers are being studied in full detail.

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Notes and references

† We have defined LNA as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-ß-D-ribofuranosyl nucleotide monomer(s).

‡ *Selected data* for **2**: $\delta_H(C_5D_5N)$ 13.12 (1H, br s, NH), 8.07 (1H, d, *J* 1.2, H-6), 7.84–7.00 (13H, m, DMT), 6.26 (1H, s, H-1′), 4.93 (1H, d, *J* 2.2, H-2′), 4.61 (1H, m, H-3′), 4.38 (1H, d, *J* 8.0, H-5″a), 4.30 (1H, d, *J* 8.0, H-5″b), 4.20 (1H, d, *J* 10.0, H-5′a), 3.87 (1H, d, *J* 10.0, H-5′b), 3.72 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 1.97 (3H, s, CH_3); *m/z* (FAB) 573 $[M + H]^+$ (found: C, 66.6; H, 5.7; N, 4.7; $C_{32}H_{32}N_2O_8.0.25H_2O$ requires C, 66.6; H, 5.7; N, 4.9%). This compound was acetylated on an analytical scale (Ac_2O, anhydrous pyridine) yielding a mono-acetate for which the signal for the H-3′ proton was found (^1H–^1H COSY NMR analysis) at δ 5.47 (compared with δ 4.61 for **2**) proving compound **2** as being the 5′-*O*-DMT derivative.

§ *Selected data* for **3**: $\delta_P(Me_3CN)$ 154.0, 151.8.

¶ *Selected data* for **5**: $\delta_H[(CD_3)_2SO]$ 11.39 (1H, br s, NH), 7.62 (1H, d, *J* 1.0, H-6), 7.44–6.89 (13H, m, DMT), 5.97 (1H, s, H-1′), 5.94 (1H, d, *J* 4.3, HO-3′), 4.44 (1H, d, *J* 4.3, H-3′), 4.23 (1H, s, H-2′), 4.13 (1H, d, *J* 8.4, H-5″a), 3.92 (1H, d, *J* 8.4, H-5″b), 3.74 (6H, s, OCH_3), 3.31 (2H, m, H-5′), 1.86 (3H, s, CH_3); *m/z* (FAB) 573 $[M + H]^+$.

‖ *Selected data* for **6**: δ_P(MeCN) 149.9, 149.3; *m/z* (FAB) 773 $[M + H]^+$. This phosphitylation has not yet been optimized and the yield was only 7% because of the need for repeated column chromatographic purification.

** All oligomers were prepared on a Biosearch 8750 DNA Synthesizer on CPG solid supports using the standard conditions of the synthesizer. However, the couplings of amidites **3** and **6** were performed with the changes described in the text and after premixing the amidite with the activator (1*H*-tetrazole 0.45 M; 4,5-dicyanoimidazole and pyridine hydrochloride 0.50 M) in anhydrous MeCN in a syringe followed by direct injection of this mixture into the column reactor during the coupling time applied. The 5′-*O*-DMT group was removed on the synthesizer immediately after completion of the sequences. Subsequent treatment with concentrated ammonia [32% (w/w), 12 h, 55 °C] and ethanol-precipitation afforded the product oligomers.

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