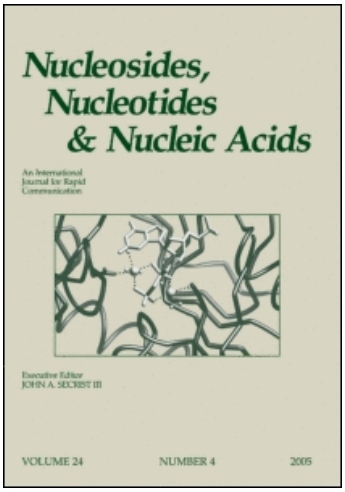

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SYNTHESIS AND EVALUATION OF α-LNA

Nanna K. Christensen[a]; Jakob K. Dalskov[a]; Paul Nielsen[a]
[a] Department of Chemistry, University of Southern Denmark, Odense M, Denmark

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SYNTHESIS AND EVALUATION OF α-LNA

Nanna K. Christensen, Jakob K. Dalskov, and Poul Nielsen*

Department of Chemistry, University of Southern Denmark,
5230 Odense M, Denmark

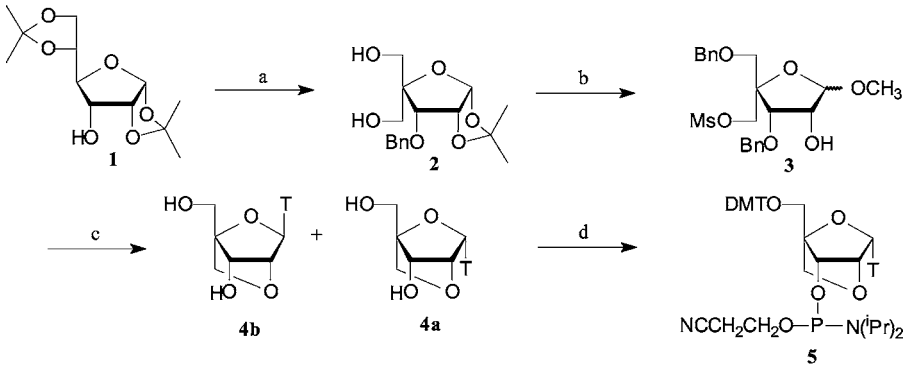
ABSTRACT

Three different synthetic routes to the α-configured LNA thymine monomer starting from D-allose or D-arabinose were investigated. The introduction of one or four α-LNA monomers into α-DNA had a destabilizing effect on the duplexes. However, a fully modified α-LNA sequence displayed strong recognition of complementary RNA, but no transition with DNA.

LNA (locked nucleic acid), is defined as oligonucleotides containing the LNA monomer, which is locked in a C-3′-endo conformation (1). Due to the unprecedented thermal affinities towards complementary RNA, LNA is a perfect candidate for antisense therapeutics and diagnostic purposes. Oligodeoxynucleotides with α-configuration (α-DNA) are known to hybridize to complementary nucleic acids in a parallel manner (2). The α-anomer of LNA, α-LNA, would be the first analogue of α-DNA (3) to be restricted in a C-3′-endo conformation. Therefore, it should potentially display an unprecedented parallel recognition of complementary nucleic acids (4).

For the synthesis of α-LNA several starting materials were considered. The synthesis required an appropriate carbohydrate available in the furanose form, with suitable protecting groups and with the configuration at C-3 defined in a D-glycero configuration. From these requirements the D-allose derivative **1** was chosen as a starting material. Thus, the same initial steps as in the original synthesis of β-LNA (1) were used including a selective benzylation of the diol **2** (Scheme 1). Coupling of thymine to the methyl furanoside **3** produced an anomeric mixture (α/β 1.3:1)

*Corresponding author.



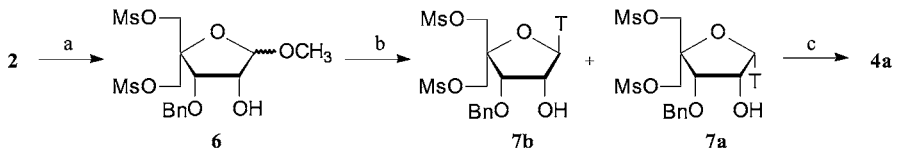
Scheme 1. a) ref. 5 (~80%, four steps); b) ref. 6 (50%, three steps); c) i. Thymine, BSA, TMS-Cl, CH_3CN, then TMS-Tf, ii. TBAF, THF, iii. NaH, DMF (57%, three steps), iv. H_2, $Pd(OH)_2$-C, EtOH (98%); d) i. DMTCl, $AgNO_3$, Pyridine, THF (80%), ii. $EtN(^iPr)_2$, $NC(CH_2)_2OP(Cl)N(^iPr)_2$, CH_2Cl_2 (79%). T = thymin-1-yl.

in a reasonable yield using the best of several tested conditions. The two anomeric LNA monomers **4a** and **4b** were tediously separated and the phosphoramidite **5** synthesized and used in automated DNA synthesis (4).

From the same starting material a simpler synthetic route has been approached. Thus, coupling of thymine to the dimesylated methyl furanoside **6** gave the anomers **7a** and **7b** (α/β 1:1) which were easily separated but obtained in a very low yield (Scheme 2).

In an earlier attempt, a bicyclic phenyl thioglycoside was synthesized from **3** affording a general bicyclic glycoside donor (6). However, a coupling reaction afforded **4a** in low yields and the strategy shown in Scheme 1 was superior to this method.

Finally, a synthetic strategy starting from D-arabinose **8** was approached (Scheme 3). Conversion to the protected analogue **10** was followed by a convenient transformation to the diol **11**. The transformation of the exact enantiomer of **11** to the α-L-LNA T-monomer in **8** steps has recently been reported (7), and since no chiral reagents were used, the same reactions should apply to **11**. The nucleobase coupling was performed on a peracylated sugar revealing only the α-nucleoside in a high yield (7).



Scheme 2. a) MsCl, pyridine (90%), ii. 20% HCl/MeOH (95%); b) i. Thymine, BSA, TMS-Cl, CH_3CN, then TMS-Tf, ii. TBAF, THF (24%); c) i. NaOH, EtOH, H_2O, ii. H_2, $Pd(OH)_2$-C, EtOH (42%, two steps).

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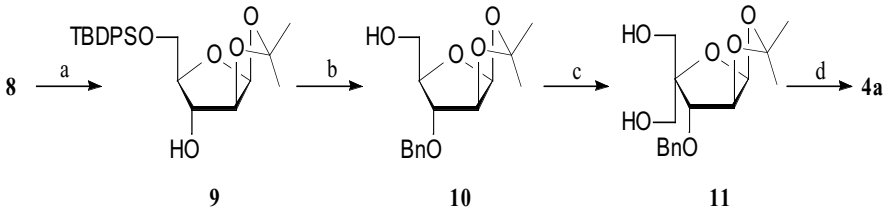

Scheme 3. a) ref. 8 (∼50%, three steps); b) ref. 9 (62%, two steps); c) i. $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2 (95%), ii. CH_2O, NaOH, H_2O, THF; d) ref. 7 (32% overall expected (7), eight steps).

The phosphoramidite **5** was used in combination with unmodified phosphoramidites in the synthesis of oligonucleotides **12**–**19**. As expected, the introduction of one α-LNA monomer into DNA (**13**) results in a very strong decrease in affinity. In α-DNA, the exchange of one or four α-nucleotides with the α-LNA monomer (**15** and **16**) also results in strongly decreased affinity against both DNA and especially RNA. This indicates that the α-LNA monomer do not influence the conformation of the neighbouring α-nucleotides as suggested for the β-LNA monomers incorporated in DNA (10). The fully modified α-LNA sequence **19** shows no transition with complementary DNA. However, a strong recognition of RNA is observed. This duplex is confirmed by the fact that a clear melting transition with a decrease in T_m of 8°C is observed against the mismatched complementary RNA-sequence. With the present sequence it is not possible to determine if the α-LNA prefers a parallel recognition as expected.

In conclusion, three different synthetic pathways have been explored. The first (Scheme 1) gives the best yields but includes an inconvenient separation of

Table 1. Hybridization Data of α-LNA Sequences

	Sequence	dA_{14} Complement $T_m/°C^a$	dA_{14} Complement $\Delta T_m/°C^b$	rA_{14} Complement $T_m/°C^a$	rA_{14} Complement $\Delta T_m/°C^b$	rA_6CA_7 Comp. $T_m/°C^a$
12	5′-T_{14}	33.0		30.0		
13	5′-T_6 $\alpha\mathbf{T}^{L}T_7$	21.5	−11.5	22.0	−8.0	
14	5′-αT_{14}	32.0		43.0		
15	5′-$\alpha T_7\mathbf{T}^{L}T_6$	25.5	−6.5	35.0	−8.0	
16	5′-$\alpha T_5\mathbf{T}^{L}{}_4T_5$	26.0	−1.5	24.5	−4.6	
17	5′-T_{10}	22.0		20.0		
18	5′-αT_{10}	18.0		33.5		22.0
19	5′-$\alpha\mathbf{T}^{L}{}_{10}$	no T_m[c]		45.0	+1.2[d]; +2.5[e]	37.0

[a] Melting temperatures (T_m) obtained in a buffer containing 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.5 μM concentrations of each strand assuming identical extinction coefficients for all thymine nucleotides.
[b] ΔT_m/modification compared with the reference strands **12** and **14**.
[c] No clear cooperative transition was seen.
[d] Compared with **18**.
[e] Compared with **17**. $\mathbf{T}^{L}$ = the α-LNA T-monomer.

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anomers. The last synthesis (Scheme 3) presents a longer route, but the target compound is obtained as the only major product. In the present oligothymidylate sequence, α-LNA displays the highest affinity towards RNA of any α-D-configured oligonucleotide analogue. The preferred direction of recognition as well as the RNA-selectivity indicated for **19** is under further investigation in our laboratory.

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