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## SYNTHESIS AND EVALUATION OF $\alpha$ -LNA

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### ABSTRACT

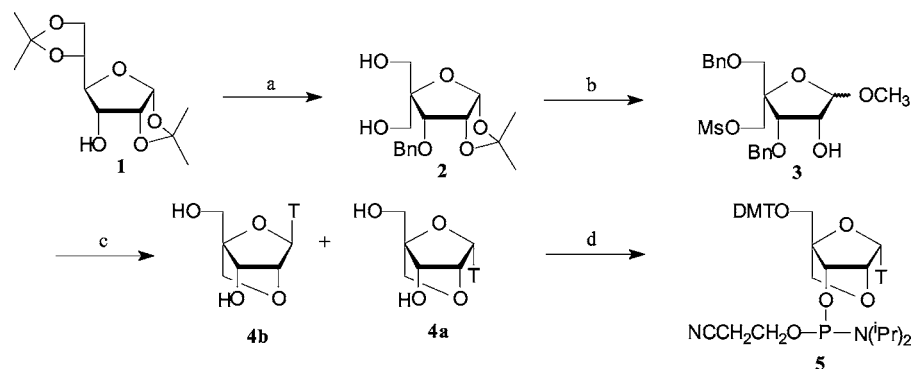
Three different synthetic routes to the  $\alpha$ -configured LNA thymine monomer starting from D-allose or D-arabinose were investigated. The introduction of one or four  $\alpha$ -LNA monomers into  $\alpha$ -DNA had a destabilizing effect on the duplexes. However, a fully modified  $\alpha$ -LNA sequence 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  $\alpha$ -configuration ( $\alpha$ -DNA) are known to hybridize to complementary nucleic acids in a parallel manner (2). The  $\alpha$ -anomer of LNA,  $\alpha$ -LNA, would be the first analogue of  $\alpha$ -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  $\alpha$ -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  $\beta$ -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 ( $\alpha/\beta$  1.3:1)

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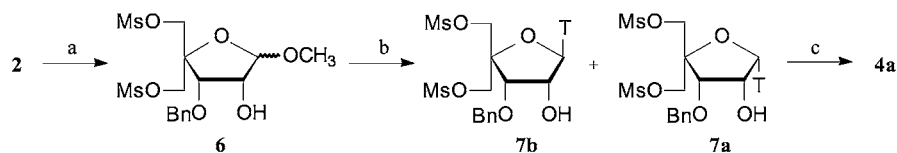
**Scheme 1.** a) ref. 5 (~80%, four steps); b) ref. 6 (50%, three steps); c) i. Thymine, BSA, TMS-Cl, CH<sub>3</sub>CN, then TMS-Tf, ii. TBAF, THF, iii. NaH, DMF (57%, three steps), iv. H<sub>2</sub>, Pd(OH)<sub>2</sub>-C, EtOH (98%); d) i. DMTCl, AgNO<sub>3</sub>, Pyridine, THF (80%), ii. EtN(<sup>i</sup>Pr)<sub>2</sub>, NC(CH<sub>2</sub>)<sub>2</sub>OP(Cl)N(<sup>i</sup>Pr)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> (79%). T = thymine-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** ( $\alpha/\beta$  1:1) which were easily separated but obtained in a very low yield (Scheme 2).

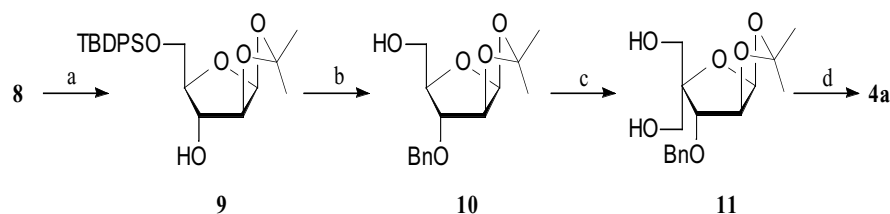
In an earlier attempt, a bicyclic phenyl thioglycoside was synthesized from **3** affording a general bicyclic glycoside donor (6). However, a coupling reaction afforded **4a** in low yields and the strategy shown in Scheme 1 was superior to this method.

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  $\alpha$ -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  $\alpha$ -nucleoside in a high yield (7).



**Scheme 2.** a) MsCl, pyridine (90%), ii. 20% HCl/MeOH (95%); b) i. Thymine, BSA, TMS-Cl, CH<sub>3</sub>CN, then TMS-Tf, ii. TBAF, THF (24%); c) i. NaOH, EtOH, H<sub>2</sub>O, ii. H<sub>2</sub>, Pd(OH)<sub>2</sub>-C, EtOH (42%, two steps).





**Scheme 3.** a) ref. 8 (~50%, three steps); b) ref. 9 (62%, two steps); c) i. (COCl)<sub>2</sub>, DMSO, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub> (95%), ii. CH<sub>2</sub>O, NaOH, H<sub>2</sub>O, 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  $\alpha$ -LNA monomer into DNA (**13**) results in a very strong decrease in affinity. In  $\alpha$ -DNA, the exchange of one or four  $\alpha$ -nucleotides with the  $\alpha$ -LNA monomer (**15** and **16**) also results in strongly decreased affinity against both DNA and especially RNA. This indicates that the  $\alpha$ -LNA monomer do not influence the conformation of the neighbouring  $\alpha$ -nucleotides as suggested for the  $\beta$ -LNA monomers incorporated in DNA (**10**). The fully modified  $\alpha$ -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  $\alpha$ -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  $\alpha$ -LNA Sequences

Sequence	dA <sub>14</sub> Complement		rA <sub>14</sub> Complement		rA <sub>6</sub> CA <sub>7</sub> Comp. $T_m/^\circ C^a$
	$T_m/^\circ C^a$	$\Delta T_m/^\circ C^b$	$T_m/^\circ C^a$	$\Delta T_m/^\circ C^b$	
<b>12</b> 5'-T <sub>14</sub>	33.0		30.0		
<b>13</b> 5'-T <sub>6</sub> $\alpha$ T <sup>L</sup> -T <sub>7</sub>	21.5	-11.5	22.0	-8.0	
<b>14</b> 5'- $\alpha$ T <sub>14</sub>	32.0		43.0		
<b>15</b> 5'- $\alpha$ T <sub>7</sub> T <sup>L</sup> -T <sub>6</sub>	25.5	-6.5	35.0	-8.0	
<b>16</b> 5'- $\alpha$ T <sub>5</sub> T <sup>L</sup> -T <sub>4</sub> T <sub>5</sub>	26.0	-1.5	24.5	-4.6	
<b>17</b> 5'-T <sub>10</sub>	22.0		20.0		
<b>18</b> 5'- $\alpha$ T <sub>10</sub>	18.0		33.5		22.0
<b>19</b> 5'- $\alpha$ T <sup>L</sup> -T <sub>10</sub>	no $T_m^c$		45.0	+1.2 <sup>d</sup> ; +2.5 <sup>e</sup>	37.0

<sup>a</sup>Melting temperatures ( $T_m$ ) obtained in a buffer containing 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.5  $\mu$ M concentrations of each strand assuming identical extinction coefficients for all thymine nucleotides.

<sup>b</sup> $\Delta T_m$ /modification compared with the reference strands **12** and **14**.

<sup>c</sup>No clear cooperative transition was seen.

<sup>d</sup>Compared with **18**.

<sup>e</sup>Compared with **17**. T<sup>L</sup> = the  $\alpha$ -LNA T-monomer.



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,  $\alpha$ -LNA displays the highest affinity towards RNA of any  $\alpha$ -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.

### ACKNOWLEDGMENT

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