

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

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

Nanna K. Christensen<sup>a</sup>; Jakob K. Dalskov<sup>a</sup>; Paul Nielsen<sup>a</sup>

<sup>a</sup> Department of Chemistry, University of Southern Denmark, Odense M, Denmark

Online publication date: 31 March 2001

**To cite this Article** Christensen, Nanna K. , Dalskov, Jakob K. and Nielsen, Paul(2001) 'SYNTHESIS AND EVALUATION OF  $\alpha$ -LNA', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 825 — 828

**To link to this Article:** DOI: 10.1081/NCN-100002438

**URL:** <http://dx.doi.org/10.1081/NCN-100002438>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

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

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

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

### ABSTRACT

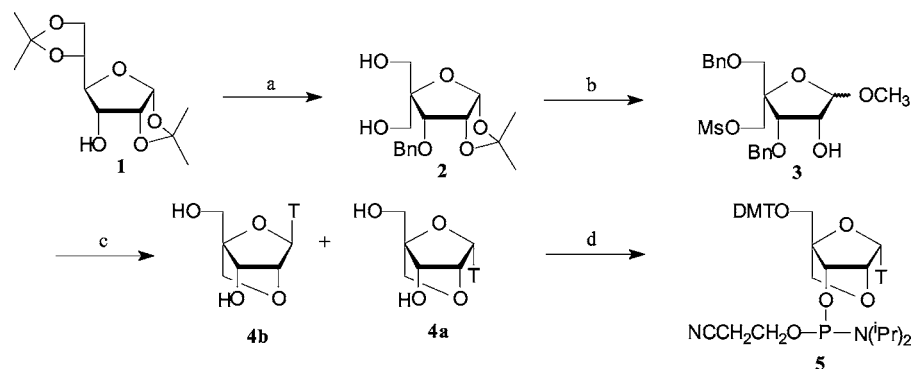
Three different synthetic routes to the  $\alpha$ -configured LNA thymine monomer starting from D-allose or D-arabinose were investigated. The introduction of one or four  $\alpha$ -LNA monomers into  $\alpha$ -DNA had a destabilizing effect on the duplexes. However, a fully modified  $\alpha$ -LNA sequence 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)

---

\*Corresponding author.



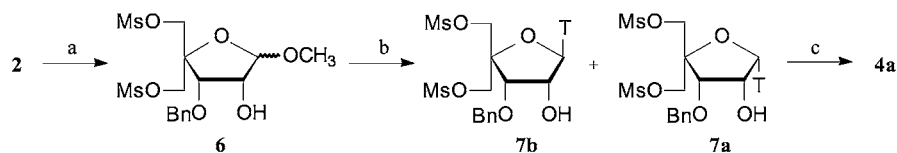
**Scheme 1.** a) ref. 5 (~80%, four steps); b) ref. 6 (50%, three steps); c) i. Thymine, BSA, TMS-Cl, CH<sub>3</sub>CN, then TMS-Tf, ii. TBAF, THF, iii. NaH, DMF (57%, three steps), iv. H<sub>2</sub>, Pd(OH)<sub>2</sub>-C, EtOH (98%); d) i. DMTCl, AgNO<sub>3</sub>, Pyridine, THF (80%), ii. EtN(<sup>i</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).

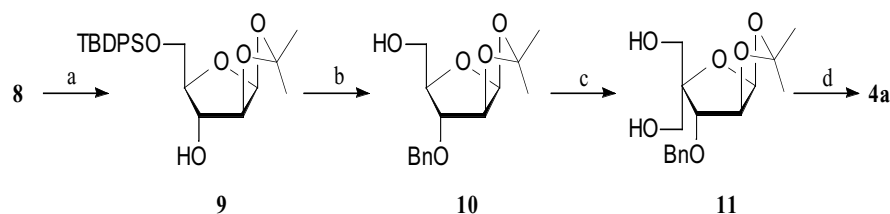
In an earlier attempt, a bicyclic phenyl thioglycoside was synthesized from **3** affording a general bicyclic glycoside donor (6). However, a coupling reaction afforded **4a** in low yields and the strategy shown in Scheme 1 was superior to this method.

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

The Danish Natural Science Research Council is thanked for financial support.

### REFERENCES

1. Koshkin A. A.; Singh S. K.; Nielsen P.; Rajwanshi V. K.; Kumar R.; Meldgaard M.; Olsen C. E.; Wengel J. *Tetrahedron*, **1998**, 3607–3630.
2. (a) Morvan F.; Rayner B.; Imbach J.-L.; Chang D.-K.; Lown J. *Nucleic Acids Res.*, **1986**, *14*, 5019–5035. (b) Lancelot G.; Guesnet J.-L.; Vovelle F. *Biochemistry*, **1989**, *28*, 7871–7878.
3. Other examples of analogues of  $\alpha$ -DNA: (a) Bolli, M.; Lubini, P.; Leumann, C. *Helv. Chim. Acta*, **1995**, *78*, 2077–2096; (b) Laurant, A.; Naval, M.; Debart, F.; Vasseur, J.-J.; Rayner, B. *Nucleic Acid Res.*, **1999**, *27*, 4151–4159.
4. Parts of the present work has been described in: Nielsen P.; Dalskov J. K. *Chem. Comm.*, **2000**, 1179–1180.
5. Youssefyeh R. D.; Verheyden J. P. H.; Moffat J. G. *J. Org. Chem.*, **1979**, *44*, 1301–1309.
6. Nielsen P.; Wengel J. *Chem. Comm.*, **1998**, 2645–2646.
7. Håkansson A. E.; Koshkin A. A.; Sørensen M. D.; Wengel J. *J. Org. Chem.*, **2000**, *65*, 5161–5166.
8. Vasquez-Tato M. P.; Seijas J. A.; Fleet G. W. J.; Mathews C. J.; Hemmings P. R.; Brown D. *Tetrahedron*, **1995**, 959–974.
9. Pakulski Z.; Zamojski A. *Tetrahedron*, **1995**, 871–908.
10. Petersen M.; Nielsen C. B.; Nielsen K. E.; Jensen G. A.; Bondensgaard K.; Singh S. K.; Rajwanshi V. K.; Koshkin A. A.; Dahl B. M.; Wengel J.; Jacobsen J. P. *J. Mol. Recognit.*, **2000**, *13*, 44–53.



## **Request Permission or Order Reprints Instantly!**

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

**[Order now!](#)**

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081NCN100002438>