Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2285–2290

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# *Xylo*-Configured Oligonucleotides (XNA, Xylo Nucleic Acid): Synthesis of Conformationally Restricted Derivatives and Hybridization Towards DNA and RNA Complements

Nicolai E. Poopeiko,[a] Martin Juhl,[b] Birte Vester,[c] Mads D. Sørensen[b] and Jesper Wengel[a,*]

[a]*Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*
[b]*Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*
[c]*Nucleic Acid Center, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*

Received 6 February 2003; revised 2 April 2003; accepted 24 April 2003

**Abstract**—*Xylo*-Configured oligonucleotides (XNA) containing a novel conformationally restricted 2′-deoxy-2′-fluoro-β-D-xylofuranosyl nucleotide monomer, a novel conformationally locked 2′-amino-2′-deoxy-2′-*N*,4′-*C*-methylene-β-D-xylofuranosyl nucleotide monomer, and a known 2′-deoxy-β-D-xylofuranosyl nucleotide monomer (XNA monomers) have been synthesized and their hybridization towards DNA and RNA complements studied. Thermal denaturation studies of nine-mer mixed-base sequences composed of a mixture of XNA monomers and DNA monomers revealed preferential hybridization towards RNA complements relative to DNA complements. For 14-mer homo-thymine XNAs containing thirteen XNA monomers, stable complexes towards single-stranded DNA and RNA were formed at pH 7. Gel-shift experiments revealed these complexes to involve at least two XNA strands per DNA or RNA target strand.


## Introduction

In the design of antisense oligonucleotides several aspects have to be considered, for example, resistance towards degradation by nucleases and efficient hybridization towards RNA targets.[1–4] In general, the most efficient RNA binding has been obtained with antisense oligonucleotides structurally mimicking RNA, for example, containing furanose rings restricted or locked in an *N*-type (*north* type, C3′-*endo* type) conformation.[1–4] This has convincingly been demonstrated for bicyclic oligonucleotides, for example, LNA (locked nucleic acid),[3,5–8] and by the introduction of electronegative groups at the 2′-position of the furanose ring.[1–4,9–11]

In a comprehensive study[12–16] of oligonucleotides containing *xylo*-configured 2′-deoxynucleotides ('xylo-DNA'; monomer **A**, Fig. 1), Seela et al. showed that 2′-deoxyxylonucleosides predominantly adopt an *N*-type furanose conformation.[16] The hybridization properties of xylo-DNA have been intensively studied towards DNA complements and towards xylo-DNA complements, but not towards RNA complements.[12–17] Incorporation of a single xylo-DNA monomer into a DNA

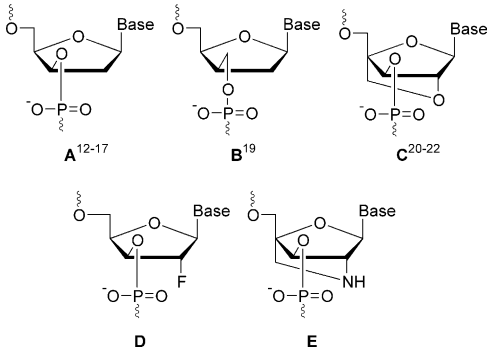



**Figure 1.** Structures of XNA monomers.

*Corresponding author. Tel.: +45-6650-2510; fax: +45-6615-8780; e-mail: jwe@chem.sdu.dk

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00441-4

strand significantly reduced the binding affinity towards the complementary DNA target.[12–16,18] An almost fully modified homo-thymine xylo-DNA formed a complex with complementary DNA of comparable thermal stability as the reference DNA:DNA duplex,[12,17] and fully modified self-complementary xylo-DNA formed a duplex of similar stability to that formed by the corresponding DNA.[16] An almost fully modified homo-thymine xylo-DNA was shown to form a complex with complementary RNA of similar thermal stability as the reference duplex.[17] Methylene-extended xylo-DNA (monomer **B**, Fig. 1) has been synthesized and was found to induce limited, but significant, destabilization when hybridized towards DNA complements.[19] Notably, monomers **A** and **B** were both found to induce protection against 3′-exonucleolytic degradation.[12,19] Whereas the introduction of a few xylo-LNA monomers (monomer **C**, Fig. 1) into a homo-thymine DNA strand had a very negative influence on the hybridization properties, an almost fully modified homo-thymine xylo-LNA displayed high-affinity recognition of both complementary DNA and RNA.[20-22] Due to the methylene linkage between the O2′ and the C4′ atoms of xylo-LNA monomers these are effectively locked in an *N*-type furanose conformation.

In order to further study and understand the influence of preorganization of *xylo*-configured monomers (XNA-monomers[23]) on the hybridization towards RNA and DNA complements, we herein describe synthesis, binding properties and binding modes of various XNAs,[23] i.e., xylo-DNA[12–17] (monomer **A**, Fig. 1), the novel conformationally restricted 2′-fluoro-xylo-DNA (monomer **D**, Fig. 1), and the novel conformationally locked 2′-amino-xylo-LNA (monomer **E**, Fig. 1). We speculated that the introduction of an electronegative 2′-fluoro substituent at the 2′-position of xylo-DNA monomers could increase the population of the *N*-type furanose conformation, and that the presence of the secondary amine functionality in 2′-amino-xylo-LNA would be of potential interest as a conjugation site and as a protonation site.

## Results and Discussion

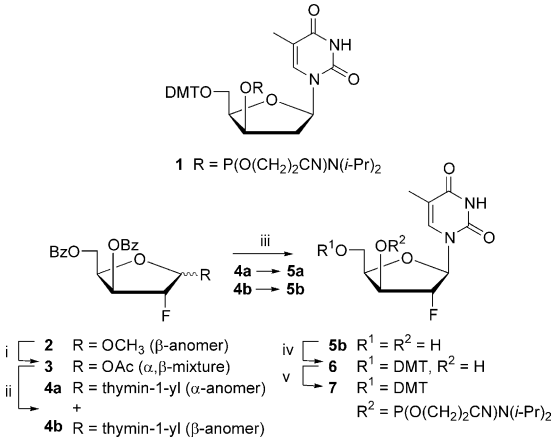
### Synthesis of XNA monomers

Phosphoramidite monomers were used on an automated DNA synthesizer for the synthesis of all XNAs studied (**ON2**–**ON7** and **ON9**–**ON15**; Table 1). The known xylo-DNA phosphoramidite building block **1** (Scheme 1) was prepared from thymidine essentially as described[12,24] and used for incorporation of monomer $^{d}$**xT** (Fig. 1, monomer **A**, Base = thymin-1-yl).

Synthesis of 1-(2-deoxy-2-fluoro-β-D-xylofuranosyl)-thymine (**5b**) and the corresponding phosphoramidite building block **7** was performed as depicted in Scheme 1. Methyl 2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-β-D-xylofuranoside (**2**) was obtained from D-xylose as described[25] and was then by acetolysis converted into 1-*O*-acetyl furanose **3** in excellent yield. Condensation of compound **3** with silylated thymine using TMS-triflate as catalyst afforded the protected anomeric nucleosides which were separated by column chromatography to give the α-anomer **4a** and the β-anomer **4b** in yields of 24 and 49% respectively. Nucleosides **4a** and **4b** were deprotected using saturated methanolic ammonia to afford the α-anomer **5a** and the β-anomer **5b**[26] in yields of 75% and 83%, respectively. The site of attachment of fluorine at C2(′) was confirmed by the large geminal coupling constants $^{2}J_{H2(′),F}$ of ca. 48-51 Hz for compounds **2**–**5** and the large geminal coupling constants $^{1}J_{C2(′),F}$ of ca. 184–191 Hz ($^{13}$C NMR).[27,28] The anomeric configurations of **5a** and **5b** were assigned by $^{1}$H NMR. Thus, firm evidence for the β-configuration of **5b** was obtained from the appearance of the signal of H1′ (δ 6.02) as a doublet with a large coupling constant ($^{3}J_{H1′,F} = 21.4$ Hz). In contrast, the signal for H1′ in the spectrum of **5a** appeared as doublet of doublets with a larger coupling constant between the H1′ and H2′ protons ($^{3}J_{H1′,H2′} = 3.3$ Hz). The lack of a significant coupling between protons H1′ and H2′ in the case of **5b** is characteristic of a *trans*-1′,2′-configuration in furanose derivatives,[29] and hence of β-configuration of compound **5b**. It should be noted that in the spectrum of **5a**, the signal for H4′ is shifted ~0.4 ppm down field in comparison with the corresponding signal in the spectrum of **5b** which further supports the anomeric assignments.[30]

Using standard transformations in satisfactory yields, nucleoside **5b** was, via the DMT (4,4′-dimethoxytrityl) protected nucleoside **6**, converted into the desired phosphoramidite derivative **7**[31] (Scheme 1) which was used for incorporation of monomer $^{F}$**xT** (Fig. 1, monomer **D**, Base = thymin-1-yl) into XNAs (Table 1).

Synthesis of 1-(2-amino-2-deoxy-2-*N*,4-*C*-methylene-2-*N*-trifluoroacetyl-β-D-xylofuranosyl)thymine (**19**) and the corresponding 2′-amino-xylo-LNA phosphoramidite building block **21** starting from nucleoside **8** was carried out in thirteen steps in an overall yield of 5.2% (Scheme 2). The novel nucleoside **8** was prepared from 3-*O*-benzyl-1,2-di-*O*-acetyl-4-*C*-mesyloxymethyl-5-*O*-mesyl-L-*threo*-




**Scheme 1.** Reagents and conditions: (i) AcOH/$Ac_2O$ (3.8/1, v/v), concd $H_2SO_4$, 75 min, 97%; (ii) persilylated thymine, TMS–triflate, 1,2-dichloroethane, reflux, 3 h; (iii) satd. $NH_3$ in MeOH, 20 h; (iv) DMTCl, pyridine, 20 h, 90%; (v) $NCCH_2CH_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, $CH_2Cl_2$, 30 min, 81%.

**Table 1.** XNAs synthesized and hybridization data[a]

***ON1***: 5′-d(GTGATATGC)

**ON2**: 5′-d(GTGA$\underline{^{d}xT}$ATGC)

**ON3**: 5′-d(GTGA$\underline{^{F}xT}$ATGC)

**ON4**: 5′-d(GTGA$\underline{^{NH}xT^{L}}$ATGC)

**ON5**: 5′-d(G$\underline{^{d}xT}$GA$\underline{^{d}xT}$A$\underline{^{d}xT}$GC)

**ON6**: 5′-d(G$\underline{^{NH}xT^{L}}$GA$\underline{^{NH}xT^{L}}$A$\underline{^{NH}xT^{L}}$GC)

**ON7**: 5′-d(G$\underline{^{\alpha L}T^{L}}$GA$\underline{^{NH}xT^{L}}$A$\underline{^{\alpha L}T^{L}}$GC)

***ON8***: 5′-$T_{14}$

**ON9**: 5′-$T_7\underline{^{d}xT}T_6$

**ON10**: 5′-$T_7\underline{^{F}xT}T_6$

**ON11**: 5′-$T_7\underline{^{NH}xT^{L}}T_6$

**ON12**: 5′-$(\underline{^{d}xT})_{13}T$

**ON13**: 5′-$(\underline{^{F}xT})_{13}T$

**ON14**: 5′-$(\underline{^{d}xT})_2(\underline{^{F}xT})(\underline{^{d}xT})_3(\underline{^{F}xT})(\underline{^{d}xT})_3(\underline{^{F}xT})(\underline{^{d}xT})_2T$

**ON15**: 5′-$(\underline{^{d}xT})_3[(\underline{^{d}xT})(\underline{^{NH}xT^{L}})]_4(\underline{^{d}xT})_2T$

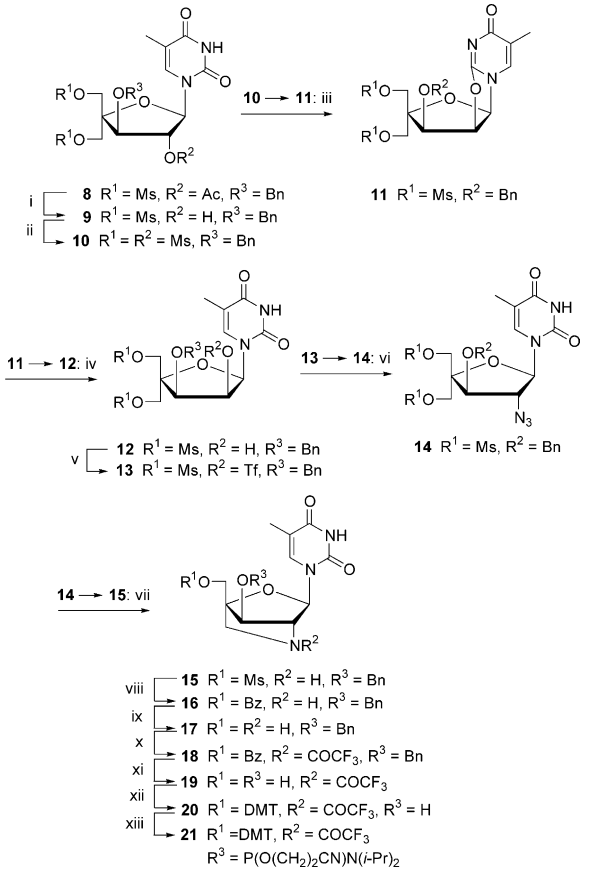
Hybridization data- $\Delta T_m$ values relative to reference ON1/ON8

| | Complementary DNA | Complementary RNA |
|---|---|---|
| ***ON1***[b] | 28 °C/30 °C/31 °C[b] | 26 °C/27 °C/29 °C[b] |
| **XNA** | **Δ $T_m$ values**[b] | **Δ $T_m$ values**[b] |
| **ON2** | −6 °C | −1 °C |
| **ON3** | −5 °C | +1 °C |
| **ON4** | −3 °C | +1 °C |
| **ON5** | No $T_m$ | No $T_m$ |
| **ON6** | No $T_m$ | −10 °C |
| **ON7** | −3 °C | +8 °C |
| ***ON8***[b] | 30 °C/33 °C [b] | 29 °C/30 °C[b] |
| **XNA** | **Δ $T_m$ values**[b] | **Δ $T_m$ values**[b] |
| **ON9** | −10 °C | −4 °C |
| **ON10** | −10 °C | −4 °C |
| **ON11** | −7 °C | −3 °C |
| **ON12** | +/−0 °C | +9 °C |
| **ON13** | +3 °C | +7 °C |
| **ON14** | +1 °C | +9 °C |
| **ON15** | +9 °C | +16 °C |

[a]Melting temperatures ($T_m$ values) were obtained from the maxima of the first derivatives of the melting curves ($A_{260}$ versus 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 oligonucleotides). A, C, G and T are DNA monomers, $\underline{^{d}xT}$ = thymin-1-yl xylo-DNA monomer, $\underline{^{F}xT}$ = thymin-1-yl 2′-fluoro-xylo-DNA monomer, $\underline{^{NH}xT^{L}}$ = thymin-1-yl 2′-amino-xylo-LNA monomer, $\underline{^{\alpha L}T^{L}}$ = thymin-1-yl α-L-LNA monomer; 'no $T_m$' indicates the absence of a cooperative transition above 5 °C.

[b]Shown are changes in $T_m$ values ($\Delta T_m$ values) compared with the reference $T_m$ values obtained for the reference oligonucleotides **ON1** and **ON8** (reference $T_m$ values from different experimental series are given). All transitions were significant and monophasic, and no transitions were detected in experiments conducted without complementary strands.

pentofuranose[32] by condensation with silylated thymine using TMS–triflate as catalyst (90% yield). Deacetylation of **8** using saturated ammonia in MeOH produced nucleoside **9** in an excellent yield of 93%. In order to obtain the (2′*R*)-2′-azido configured nucleoside **14** a double inversion strategy was applied. The first inversion was carried out by mesylation of **9** in 86% yield to give nucleoside **10** which by treatment with DBU efficiently was converted into the 2,2′-anhydro nucleoside **11** in 89% yield. Opening of intermediate **11** with retention of configuration at C2′ by refluxing in a mixture of aqueous sulfuric acid (0.4 M) and acetone (1/1, v/v) resulted in near quantitative formation of the *erythro*-configurated nucleoside **12**. Triflylation of **12** proved troublesome and the optimal result was achieved with 1.1 equiv of $Tf_2O$, 10 equiv of pyridine and 4 equiv of DMAP in dichloromethane. However, triflate **13** could not be separated from an unidentified byproduct, and this mixture was reacted with sodium azide in DMF for 18 h furnishing C2′-inverted 2′-azido-2′-deoxynucleoside **14** in 65% yield (from **12**). The reduction of the azido group was carried out using a modified Staudinger reaction with trimethylphosphine in a mixture of THF and aqueous NaOH affording the bicyclic nucleoside **15** in 81% yield. Demesylation of **15** was carried out by a substitution reaction using sodium benzoate and 15-crown-5 in DMF at 120 °C under high dilution conditions. Benzoate **16** could not be separated from 15-crown-5 and therefore the mixture was subjected to debenzoylation affording nucleoside **17** in 48% yield (two steps). Protection of the secondary amino group went smoothly using ethyl trifluoroacetate and DMAP,




**Scheme 2.** Reagents and conditions: (i) satd. $NH_3$ in MeOH, rt (93%); (ii) MsCl, pyridine, rt (86%); (iii) DBU, $CH_3CN$ (89%); (iv) 0.4 M aq $H_2SO_4$/acetone (1/1, v/v), reflux (98%); (v) $Tf_2O$, pyridine, DMAP, $CH_2Cl_2$, 0 °C; (vi) $NaN_3$, DMF, rt (65%, 2 steps); (vii) $P(CH_3)_3$, 2 M aq NaOH, THF, rt (81%); (viii) BzONa, 15-crown-5, DMF, 120 °C; (ix) satd. $NH_3$ in MeOH, rt (48%, 2 steps); (x) $CF_3CO_2Et$, DMAP, rt (93%); (xi) 10% Pd/C, $H_2$, rt (96%); (xii) DMTCl, pyridine, rt (94%); (xiii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, $CH_2Cl_2$, rt (35%).

producing **18** in 93% yield, which was followed by a high-yielding debenzylation reaction affording diol **19**.[33] The results of an NOE experiment of diol **19** support the configuration assigned to the prepared nucleosides (mutual NOE effects between H1′ and the H5″ protons (1%/1%), between H3′ and the H5″ protons (2%/1%) and between the H6 proton of the thymine moiety and H5′ (3%/<1%) were observed). Standard 5′-*O*-DMT protection (94% yield of **20**) followed by 3′-*O*-phosphitylation furnished the desired phosphoramidite building block **21**[34] (35% yield) suitable for incorporation of 2′-amino-xylo-LNA monomer $\underline{^{NH}\mathbf{xT}^{L}}$ (Fig. 1, monomer **E**, Base = thymin-1-yl) into XNAs (Table 1).

Conformational restriction of 2′-deoxy-2′-fluoro monomer $\underline{^{F}\mathbf{xT}}$ relative to 2′-deoxy monomer $\underline{^{d}\mathbf{xT}}$ was verified by comparing $^{1}$H NMR data for the corresponding 5′-*O*-DMT protected nucleosides. Thus, whereas the signal of the H1′ proton for 1-(2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl)thymine appears as a double doublet ($^{3}J_{H1',H2'} = 2.7$ and 6.2 Hz),[12] the signal of the H1′ proton of 1-(2-deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl)thymine (**6**) appears as a doublet ($^{3}J_{H1',F} = 21.9$ Hz) indicating strong *N*-type conformational restriction[35,36] of the furanose ring of **6**, and accordingly also $\underline{^{F}\mathbf{xT}}$. For the 2′-amino-xylo-LNA nucleosides (including monomer $\underline{^{NH}\mathbf{xT}^{L}}$) having the bicyclo[2.2.1]heptane constitution, a locked *N*-type furanose conformation was validated by the appearance of the signal for H1′ in the $^{1}$H NMR spectrum of nucleoside **19**[33] as a singlet as has been reported also for other LNA derivatives.[5–7]

## Synthesis of XNA oligomers

The reference DNA strands **ON1** and **ON8** and the XNAs **ON2**–**ON7** and **ON9**–**ON15** (Table 1) were prepared on an automated DNA synthesizer using the phosphoramidite approach (0.2 μmol scale, CPG solid supports).[37] The *xylo*-configured phosphoramidites **1**, **7** and **21**, the known α-L-LNA thymine amidite[21,38] (used for incorporation of monomer $\underline{^{\alpha L}\mathbf{T}^{L}}$ into **ON7**) and commercially available DNA phosphoramidites were applied. The coupling yields were >99% for DNA phosphoramidites (using 2 min coupling time and 1*H*-tetrazole as catalyst), ~99% for phosphoramidite **1**, 89–96% for phosphoramidite **7**, and 99% for phosphoramidite **21**. Extended coupling time (10–30 min) and a modified procedure with pyridine hydrochloride as catalyst[21] were used for the three *xylo*-configured phosphoramidites **1**, **7** and **21**. The satisfactory coupling yields obtained for these *xylo*-configured phosphoramidites are noteworthy as low coupling yields for *xylo*-configured phosphoramidites led Seela et al. to use the alternative *H*-phosphonate method for automated synthesis of xylo-DNA.[12–14,16] After standard deprotection and cleavage from the solid support using 32% aqueous ammonia (16 h, 55 °C), the oligomers were prepared for use by desalting. The purity of all oligomers (>80%) was verified by capillary gel electrophoresis and the composition of representative oligomers by MALDI-MS analysis.[39]

## Hybridization properties of XNA towards DNA complements

The strongly destabilizing effect of incorporating a few isolated xylo-DNA monomers into a DNA strand with regard to hybridization towards DNA complements is confirmed in the present study [monomer $\underline{^{d}\mathbf{xT}}$;[12–16] **ON2** and **ON5** (relative to **ON1**) and **ON9** (relative to **ON8**)]. Likewise, incorporation of one or three 2′-fluoro-xylo-DNA monomers ($\underline{^{F}\mathbf{xT}}$) or 2′-amino-xylo-LNA monomers ($\underline{^{NH}\mathbf{xT}^{L}}$) leads to decreased affinity towards DNA with a tendency towards less detrimental effect for the conformationally locked 2′-amino-xylo-LNA monomer. The (almost) fully modified homo-thymine XNAs **ON12**–**ON15** display $T_m$ values in the same range as that of the reference **ON8** with a moderate affinity-enhancing effect of the 2′-amino-xylo-LNA monomer $\underline{^{NH}\mathbf{xT}^{L}}$. Notably, it is possible to combine different XNA monomers and obtain satisfactory results as shown for the chimeric XNAs **ON14** and **ON15**.

## Hybridization properties of XNA towards RNA complements

Direct comparison between the $T_m$ values obtained towards the DNA and RNA complements clearly demonstrates the RNA selective hybridization of XNA. Remarkably, taking the different configurations of the DNA and XNA monomers into consideration, the stereoirregular XNAs **ON2**–**ON4** display unchanged RNA-binding relative to reference **ON1**.

Incorporation of three XNA monomers (**ON5** and **ON6**) abolishes, or significantly reduces, the affinity towards RNA, but by combining α-L-LNA, DNA and 2′-amino-xylo-LNA monomers (**ON7**), high-affinity recognition of RNA is achieved. Notably, by combining these three differently configured monomers a positive effect only on RNA binding affinity is induced (compare **ON4** and **ON7**). The preferential RNA hybridization of XNA is substantiated by the significantly increased thermal stabilities obtained with the (almost) fully modified homo-thymine XNAs **ON12**–**ON15** with the highest $T_m$ value achieved for **ON15** containing 2′-amino-xylo-LNA monomers. The $\Delta T_m$ value obtained for **ON12** (+9 °C) is higher than the $\Delta T_m$ value reported for an analogous homo-thymine XNA [($\underline{^{d}\mathbf{xT}}$)$_{12}$T] (+3 °C).[17] The use of slightly different hybridization conditions is a likely explanation for this difference.

## Hybridization characteristics of XNA

The thermal denaturation studies show that conformational restriction of XNA monomers leads to improved binding affinity as most clearly observed for XNAs **ON4**, **ON11** and **ON15** containing the locked 2′-amino-xylo-LNA type monomer $\underline{^{NH}\mathbf{xT}^{L}}$ (compare with XNAs **ON2**, **ON9** and **ON12**, respectively, containing xylo-DNA monomer $\underline{^{d}\mathbf{xT}}$). Importantly, different monomeric nucleotides, including different XNA type monomers, can be combined allowing fine-tuning of the hybridization properties of XNA (see **ON7**, **ON14** and **ON15**). In melting experiments with one mis-match centrally positioned

in the complementary DNA/RNA, XNA-mediated hybridization (**ON2**, **ON3**, **ON4** and **ON7**) was found to follow the Watson–Crick base pairing rules (data not shown).

For the mixed-sequence 9-mer XNAs containing a mixture of XNA and DNA monomers, hybridization is assumed to take place via duplex formation. However, as triplex formation between homo-pyrimidine XNA (xylo-DNA; mixed T/C sequences) and single-stranded DNA complements has earlier been demonstrated,[40] we decided to investigate the binding mode of XNAs **ON12**, **ON13** and **ON15** towards single-stranded DNA ($dA_{14}$) and RNA ($rA_{14}$) complements (Fig. 2).[41]

As expected, the complex formed between **ON8** and $dA_{14}$ is a duplex (Fig. 2, upper right panel; **ON8** and $rA_{14}$ are not shown because of co-migration of single strands and complex). In accordance with earlier studies,[40] the gel mobilities shown in Figure 2 reveal that two XNA strands per $dA_{14}$ target strand are involved in complex formation both for **ON12** and **ON15** (and also for **ON13**, not shown). It should be noted that analogous hybridization behavior has been demonstrated for other nucleic acid analogues, for example, peptide nucleic acid.[42]

With the RNA target $rA_{14}$ the results indicate the formation of a mixture of complexes, including one or several with XNA:$rA_{14}$ ratio(s) $\geq 2$. Further preliminary experiments[43] indeed indicate changes in the distribution among complexes during incubation for several h. Thus, whereas complexes of higher-order structure apparently are involved, we anticipate that hybridization between **ON12**, **ON13** and **ON15** and $rA_{14}$, as with $dA_{14}$, initially involves triplex formation.

## Conclusion

*Xylo*-Configured oligonucleotides (XNA) containing a novel conformationally restricted 2′-fluoro-xylo-DNA monomer, a novel conformationally locked 2′-amino-xylo-LNA and a known xylo-DNA monomer have been synthesized. Thermal denaturation studies revealed preferential hybridization towards RNA complements for 9-mer mixed-base XNAs and 14-mer homo-pyrimidine XNAs, composed of a mixture of XNA and DNA monomers (and α-L-LNA monomers for **ON7**). Stable complexes towards single-stranded DNA and RNA targets were formed at pH 7 for 14-mer homo-thymine XNAs containing 13 XNA monomers. Gel-shift experiments revealed these complexes to involve at least two XNA strands per target strand. The binding affinity of XNA can be tuned by combining different types of nucleotide monomers and conformational restriction of XNA monomers clearly leads to improved binding. We are currently exploring the binding mode of homo-pyrimidine XNA and optimizing the hybridization properties of XNA.

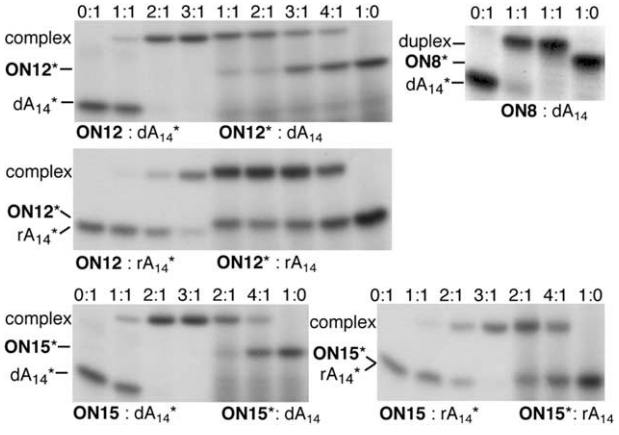



**Figure 2.** Non denaturing gel electrophoresis of hybridization complexes. '*' Indicates the 5′-$^{32}$P labelled oligo visible on the gel autoradiogram. The ratios on the top of the individual lanes are the ratios of the two strands (indicated at the bottom of each gel).[41]

## Acknowledgements

The Danish National Research Foundation and the Danish Research Agency are thanked for financial support. Ms. Britta M. Dahl, University of Copenhagen, is thanked for oligonucleotide synthesis and Dr. Michael Meldgaard, Exiqon A/S, for MALDI-MS analyses.

## References and Notes

1. Herdewijn, P. *Liebigs Ann*. **1996**, 1337.
2. Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res*. **1997**, *25*, 4429.
3. Wengel, J. *Acc. Chem. Res*. **1999**, *32*, 301.
4. Kværnø, L.; Wengel, J. *Chem. Commun*. **2001**, 1419.
5. Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun*. **1998**, 455.
6. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.
7. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett*. **1998**, *39*, 5401.
8. Braasch, D. A.; Corey, D. R. *Chem. Biol*. **2000**, *55*, 1.
9. Guschlbauer, W.; Jankowski, K. *Nucleic Acid Res*. **1980**, *8*, 1421.
10. Olson, W. K. *J. Am. Chem. Soc*. **1982**, *104*, 278.
11. Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzales, C.; Cook, P. D. *J. Med. Chem*. **1993**, *36*, 831.
12. Rosemeyer, H.; Seela, F. *Helv. Chim. Acta* **1991**, *74*, 748.
13. Rosemeyer, H.; Krecmerova, M.; Seela, F. *Helv. Chim. Acta* **1991**, *74*, 2054.
14. Seela, F.; Wörner, K.; Rosemeyer, H. *Helv. Chim. Acta* **1994**, *77*, 883.
15. Schöppe, A.; Hinz, H.-J.; Rosemeyer, H.; Seela, F. *Eur. J. Biochem*. **1996**, *239*, 33.
16. Seela, F.; Heckel, M.; Rosemeyer, H. *Helv. Chim. Acta* **1996**, *79*, 1451.
17. Alekseev, Y. I.; Gottikh, M. B.; Romanova, E. A.; Shabarova, Z. A. *Mol. Biol*. **1996**, *30*, 206.
18. Changes in duplex stabilities ($\Delta T_m$ values) are discussed relative to the corresponding reference duplex formed by hybridization between two unmodified (DNA or RNA) complementary strands.
19. Svendsen, M. L.; Wengel, J.; Dahl, O.; Kirpekar, F.; Roepstorff, P. *Tetrahedron* **1993**, *49*, 11341.

20. Rajwanshi, V. K.; Kumar, R.; Kofod-Hansen, M.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1407.
21. Rajwanshi, V. K.; Håkansson, A. E.; Dahl, B. M.; Wengel, J. *Chem. Commun*. **1999**, 1395.
22. Rajwanshi, V. K.; Håkansson, A. E.; Kumar, R.; Wengel, J. *Chem. Commun*. **1999**, 2073.
23. The term 'XNA' is defined herein as an oligonucleotide containing one or more *xylo*-configured nucleotide monomers ('XNA-monomer(s)').
24. Kong, J. P.; Kim, S. K.; Moon, B. J.; Kim, S. J.; Kim, H. B. *Nucleosides Nucleotides* **2001**, *20*, 1751. We used sodium hydroxide instead of lithium hydroxide during reaction on the 3′-*O*-mesylated intermediate.
25. Wright, J. A.; Wilson, D. P.; Fox, J. J. *J. Med. Chem*. **1970**, *13*, 269.
26. 1-(2-Deoxy-2-fluoro-β-D-xylofuranosyl)thymine (**5b**): $^{1}$H NMR (300 MHz, $CD_3OD$) δ 7.71 (s, 1H, H-6), 6.02 (d, 1H, $J$ = 21.4 Hz, H-1′), 4.95 (d, 1H, $J$ = 49.4 Hz, H-2′), 4.35 (dd, 1H, $J$ = 3.3 and 10.4 Hz, H-3′), 4.26–4.21 (m, 1H, H-4′), 4.00–3.86 (m, 2H, H-5′), 1.86 (br s, 3H, $CH_3$); $^{13}$C NMR (75.5 MHz, $CD_3OD$; selected signals) δ 138.5, 111.0, 100.7 ($J$ = 184.9), 90.4 ($J$ = 38.4), 85.0, 73.8 ($J$ = 25.8), 60.7, 12.6.
27. Michalik, M.; Hein, M.; Michael, F. *Carbohydr. Res*. **2000**, *327*, 185.
28. Kowollik, G.; Langen, P. *Z. Chem*. **1975**, *15*, 147.
29. Lemieux, R. U.; Lineback, D. R. *Ann. Rev. Biochem*. **1963**, *32*, 155.
30. Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balzarini, J.; De Clercq, E. *J. Med. Chem*. **1991**, *34*, 2195 and references cited therein.
31. 1-(3-*O*-((2-Cyanoethoxy)(*N*,*N*-diisopropylamino)phosphino)-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-xylofuranosyl)thymine (**7**): $^{31}$P NMR (300 MHz, $CH_3CN$) δ 153.8 and 151.2.
32. Sørensen, M. D.; Kværnø, L.; Bryld, T.; Håkansson, A. E.; Verbeure, B.; Gaubert, G.; Herdewijn, P.; Wengel, J. *J. Am. Chem. Soc*. **2002**, *124*, 2164.
33. 1-(2-Amino-2-deoxy-2-*N*,4-*C*-methylene-2-*N*-trifluoroacetyl-β-D-xylofuranosyl)thymine (**19**) [major rotamer (rotamer ratio 2:3)]: $^{1}$H NMR (400 MHz, ($(CD_3)_2SO$) δ 11.25 (bs, 1H, NH), 7.53 (d, $J$ = 1.3 Hz, 1H, H-6), 5.98 (bs, 1H, 3′-OH), 5.59 (s, 1H, H-1′), 5.14 (t, $J$ = 5.7 Hz, 1H, 5′-OH), 4.65 (br s, 1H, H-2′), 4.18 (t, $J$ = 2.4 Hz, 1H, H-3′), 3.92–3.84 (m, 2H, H-5′), 3.68 (d, $J$ = 11.9 Hz, 1H, H-5″), 3.42 (d, $J$ = 11.9, 1H, H-5″), 1.76 (s, 3H, $CH_3$); $^{13}$C NMR (100.6 MHz, ($(CD_3)_2SO$)) δ 164.10, 154.18 (q, $J$ = 37.4 Hz), 150.02, 136.78, 115.75 (q, $J$ = 287.3 Hz), 106.13, 89.22, 87.88, 72.30, 60.82, 57.12, 53.19, 12.50.
34. 1-(2-Amino-3-*O*-((2-cyanoethoxy)(*N*,*N*-diisopropylamino)-phosphino)-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-*N*,4-*C*-methylene-2-*N*-trifluoroacetyl-β-D-xylofuranosyl)thymine (**21**): $^{31}$P NMR (400 MHz, $CH_3CN$) δ 154.9, 154.2, 152.5, 152.3.
35. Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc*. **1972**, *94*, 8205.
36. Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc*. **1973**, *95*, 2333.
37. Caruthers, M. H. *Acc. Chem. Res*. **1991**, *51*, 2155.
38. Håkansson, A. E.; Koshkin, A. A.; Sørensen, M. D.; Wengel, J. *J. Org. Chem*. **2000**, *65*, 5161.
39. MALDI-MS *m*/*z* ($[M-H]^-$; found/calcd) **ON4**, 2776/2778; **ON6**, 2833/2833; **ON7**, 2834/2834; **ON9**, 4191/4197; **ON10**, 4210/4215; **ON11**, 4220/4221; **ON12**, 4191/4197; **ON14**, 4247/4251; **ON15**, 4300/4302.
40. Ivanov, S. A.; Alekseev, Y. I.; Gottikh, M. B. *Mol. Biol*. **2002**, *36*, 131.
41. Hybridization was performed in 100 mM NaCl, 10 mM KCl, 20 mM Tris–HCl (pH 7.5) by heating to 65 °C, slowly cooling after 2 min to 37 °C followed by incubation at 5 °C for 1–2 h. The resulting complexes were then analyzed on 20% acrylamide gels run at approximately 14 °C.
42. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc*. **1992**, *114*, 1895.
43. Poopeiko, N. E.; Juhl, M.; Vester, B.; Wengel, J. Manuscript in preparation.