Pure Appl. Chem., Vol. 77, No. 1, pp. 319–326, 2005.
DOI: 10.1351/pac200577010319

Novel nucleic acid architectures involving locked nucleic acid (LNA) and pyrene residues: Results from an Indo-Danish collaboration*

B. Ravindra Babu[1], Raunak[1,2], Mads D. Sørensen[3], Patrick J. Hrdlicka[1], Smriti Trikha[1,2], Ashok K. Prasad[2], Virinder S. Parmar[2], and Jesper Wengel[1,‡]

[1]Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark; [2]Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi 110 007, India; [3]Department of Chemistry, University of Copenhagen, DK-2100 Copenhagen, Denmark

Abstract: We report herein our results for locked nucleic acid (LNA)-type oligonucleotides containing pyrene residues. Pyrene has a large hydrophobic and planar surface area and is therefore a potential intercalating unit; furthermore, it is interesting as a fluorescent tag when covalently bound to DNA. Synthesis and hybridization of conformationally locked universal base surrogates are described together with efficient interstrand communication as shown by the formation of pyrene excimer bands for duplexes containing 2'-*N*-(pyren-1-yl)methyl-2'-amino-LNA monomers positioned in a zipper-like manner within a DNA duplex.

INTRODUCTION

Universal bases and locked furanose conformations

An ideal universal base analog forms isoenergetic base pairs with each of the natural DNA bases (nucleotides). Such bases have attracted much attention owing to their potential utility in the design of oligonucleotide primers for degenerate polymerase chain reaction (PCR) or as hybridization probes when the identity of a base in the target sequence is unknown. Many of the known universal base analogs are non-hydrogen bonding, hydrophobic, and planar aromatic moieties that stabilize duplex DNA by stacking interactions [1]. Promising universal bases with a 2-deoxy-β-D-ribofuranosyl moiety have been reported, e.g., 3-nitropyrrole [2], 5-nitroindole [3], isocarbostyril [4], 8-aza-7-deazaadenine [5], and pyrene derivatives [6], but generally significant duplex destabilization has been observed. Thus, while incorporation of one of these monomers into a DNA strand induces little variation in the duplex melting temperature (T_m value; used as a measure for duplex stability) when placed opposite the four natural DNA bases, decreases in the T_m value of between 4–10 °C per universal nucleotide monomer, compared to the T_m value of the corresponding fully complementary reference DNA:DNA duplex, are typical [1–7].

While the T_m value variations exhibited by oligonucleotides containing a pyrenyl DNA monomer (Fig. 1, **Py**) [6,8] toward the four natural bases in a complementary DNA strand are small, the average T_m values were moderately depressed (–4.5 to –6.8 °C) compared to the unmodified DNA:DNA duplex. We envisioned that the binding affinity for universal hybridization could be improved by enforcing a locked furanose conformation of the nucleotide containing pyrene as aglycon. Therefore, we decided to

*Paper based on a presentation at the 24th International Symposium on the Chemistry of Natural Products and the 4th International Congress on Biodiversity, held jointly in Delhi, India, 26–31 January 2004. Other presentations are published in this issue, pp. 1–344.

‡Corresponding author

synthesize the locked nucleic acid (LNA) and α-L-LNA derivatives $\mathbf{Py^L}$ and $\mathbf{^{\alpha L}Py^L}$, respectively. LNA [9–13] (Fig. 1) is defined as an oligonucleotide containing one or more 2'-*O*,4'-*C*-methylene-*β*-D-ribofuranosyl nucleotide monomer(s) [10], and is characterized by very high binding affinity and efficient Watson–Crick discrimination when hybridized with single-stranded DNA or RNA targets [9–15]. Also, α-L-LNA-containing oligonucleotides display very efficient recognition of both DNA and RNA targets [16]. Whereas $\mathbf{Py^L}$ is based on the 2'-*O*,4'-*C*-methylene-*β*-D-ribofuranosyl moiety known to be preorganized in a locked C3'-*endo* (*N*-type) RNA-like furanose conformation, its diastereoisomer $\mathbf{^{\alpha L}Py^L}$ is based on the 2'-*O*,4'-*C*-methylene-*α*-L-ribofuranosyl moiety (also preorganized in a locked C3'-*endo* furanose conformation). α-L-LNA-containing oligonucleotides have been shown to structurally mimic DNA by CD and NMR studies of duplexes, and α-L-LNA/DNA mixmer strands hybridize to DNA with overall duplex geometry of the B-type and with preserved Watson–Crick base pairing [17]. Therefore, the pyrenyl α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ should furnish information on the effect of the pyrenyl aglycon when locked in a DNA-like conformation, and allow direct comparison with $\mathbf{Py^L}$ locked in an RNA-like conformation. In addition, the corresponding phenyl analogs $\mathbf{Ph^L}$ and $\mathbf{^{\alpha L}Ph^L}$ were also synthesized (Fig. 1).

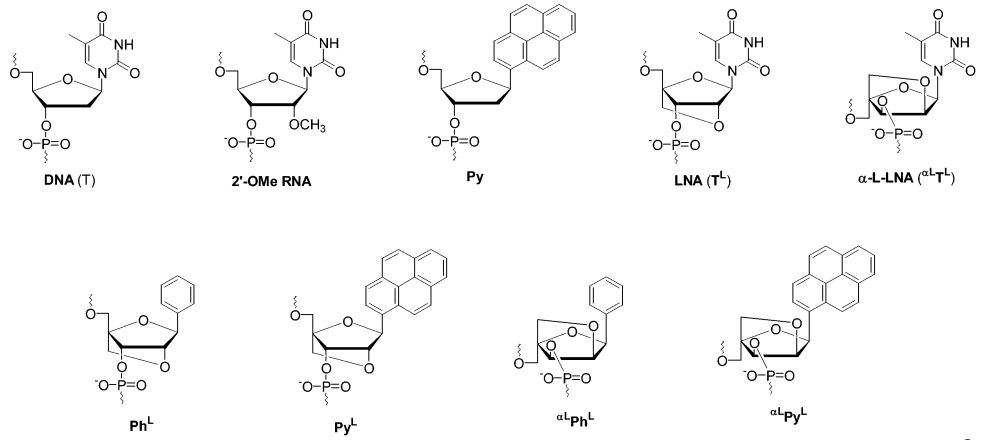
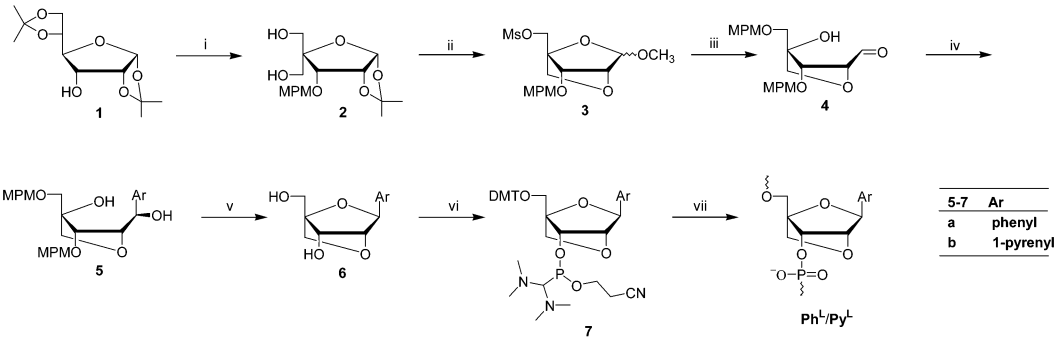


Fig. 1 Structures of nucleotide monomers: DNA (T), 2'-OMe-RNA, pyrenyl DNA monomer (Py), LNA (T^L), α-L-LNA ($^{\alpha L}T^L$), LNA-type phenyl (Ph^L) and pyrenyl (Py^L) monomers, α-L-LNA-type phenyl ($^{\alpha L}Ph^L$) and pyrenyl ($^{\alpha L}Py^L$) monomers. The short notations shown are used in Table 1. For DNA, 2'-OMe-RNA, LNA and α-L-LNA, the thymine monomers are shown.

RESULTS AND DISCUSSION

Synthesis of the LNA-type phosphoramidite building blocks 7a and 7b [18,19]

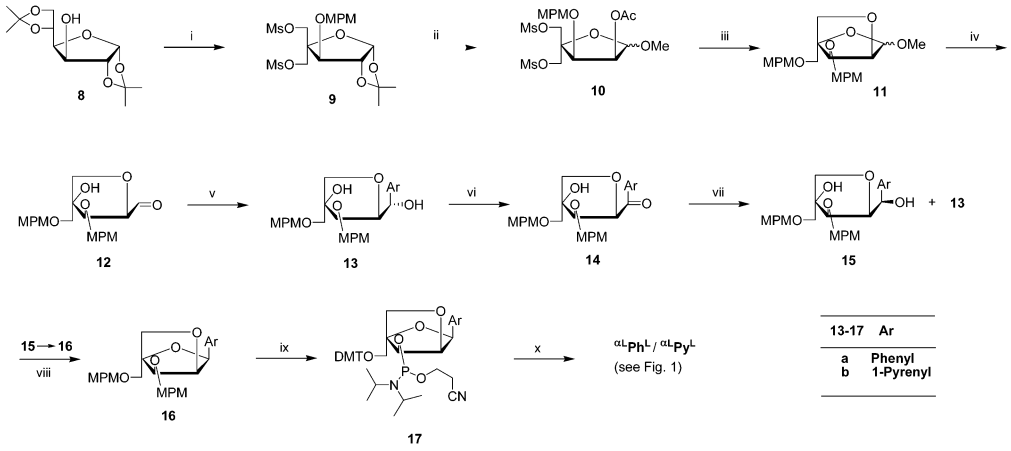
Synthesis of the phosphoramidite building blocks **7a** and **7b** suitable for incorporation of the LNA-type aryl *C*-glycosides $\mathbf{Ph^L}$ and $\mathbf{Py^L}$ is shown in Scheme 1. Commercially available 1,2:5,6-di-*O*-isopropylidene-*α*-D-allofuranose was converted into aldehyde **4** in 26 % overall yield. Nucleophilic addition of phenyl and 1-pyrenyl Grignard reagents to aldehyde **4** yielded diastereoselectively **5a** and **5b**, respectively [18,19], in analogy to similar syntheses of other *C*-glycosides of LNA [20–22]. The diols **5a** and **5b** were cyclized under Mitsunobu conditions (TMAD, PBu_3) to afford the bicyclic β-configured *C*-nucleoside derivatives, which, upon oxidative removal of the *p*-methoxybenzyl protection groups with DDQ, furnished the diols **6a** and **6b**, respectively. Selective 5'-*O*-dimethoxytritylation followed by 3'-*O*-phosphitylation afforded the phosphoramidite building blocks **7a** and **7b**. The configurations of compounds **5**–**7** were assigned based on ^{1}H NMR spectroscopy, including NOE experiments. In addition, a single-crystal X-ray diffraction structure of the phenyl analog **6a** verified the assigned constitution and relative configuration as well as the locked *N*-type furanose conformation [19].



Scheme 1 (i) (a) *p*-Methoxybenzyl chloride, NaH, THF; (b) 80 % aq. AcOH; (c) $NaIO_4$, H_2O, THF; (d) HCHO, aq. NaOH (2 M), dioxane (77 %); (ii) (a) MsCl, pyridine; (b) H_2O–HCl–CH_3OH (1:1.5:8.5, v/v/v); (c) NaH, DMF (69 %); (iii) (a) KOAc, dioxane, 18-crown-6; (b) sat. methanolic ammonia; (c) *p*-methoxybenzyl chloride, NaH, THF; (d) 80 % aq. AcOH (49 %); (iv) ArMgBr, THF (**5a**: 88 %; **5b**: 89 %); (v) (a) TMAD, Bu_3P, C_6H_6; (b) DDQ, CH_2Cl_2, H_2O (**6a**: 51 %; **6b**: 59 %); (vi) (a) DMTCl, pyridine; (b) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (**7a**: 47 %; **7b**: 42 %); (vii) DNA synthesizer. MPM = *p*-methoxybenzyl, TMAD = *N*,*N*,*N*',*N*'-tetramethylazodicarboxamide.

Synthesis of the α-L-LNA-type phosphoramidite building blocks 17a and 17b [23]

The above protocol for synthesis of *C*-aryl nucleosides of LNA (Scheme 1) inspired the formulation of a general approach toward synthesis of phosphoramidite building blocks **17a** and **17b** suitable for incorporation of the α-L-LNA-type aryl *C*-glycosides $^{\alpha L}\mathbf{Ph}^{\mathbf{L}}$ and $^{\alpha L}\mathbf{Py}^{\mathbf{L}}$ (Scheme 2). 1,2:5,6-Di-*O*-isopropylidene-α-D-glucose (**8**) was transformed into the di-*O*-mesylated furanoside **9** in 61 % overall yield. Methanolysis of **9** followed by epimerization at C2 afforded 2-*O*-acetyl derivative **10**. Deacetylation of **10**, subsequent intramolecular cyclization, and then nucleophilic displacement of the



Scheme 2 (i) (a) *p*-Methoxybenzyl chloride, NaH, THF, *n*-$Bu_4N^+I^-$; (b) 60 % aq. AcOH; (c) $NaIO_4$, H_2O, THF; (d) HCHO, aq. NaOH (2 M), dioxane; (e) MsCl, pyridine (61 %); (ii) (a) H_2O–HCl–CH_3OH (1:1.5:8.5, v/v/v); (b) Tf_2O, pyridine; (c) KOAc, 18-crown-6-ether, toluene (66 %); (iii) (a) sat. methanolic ammonia; (b) NaH, DMF; (c) KOAc, 18-crown-6-ether, dioxane; (d) sat. methanolic ammonia; (e) *p*-methoxybenzyl chloride, NaH, THF, *n*-$Bu_4N^+I^-$ (47 %); (iv) 70 % aq AcOH (83 %); (v) ArMgBr, THF (**13a**: 74 %; **13b**: 71 %); (vi) Dess–Martin periodinane, CH_2Cl_2 (**14a**: 76 %); PDC, 3 Å molecular sieves, CH_2Cl_2 (**14b**); (vii) $NaBH_4$, THF, H_2O (**15a**: 22 %; **15b**: 16 % [from **13b**]); (viii) TMAD, Bu_3P, benzene (**16a**: 97 %; **16b**: 88 %); (ix) (a) DDQ, CH_2Cl_2, H_2O; (b) DMTCl, pyridine; (c) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (**17a**: 32 %; **17b**: 12 %); (x) DNA synthesizer. MPM = *p*-methoxybenzyl, TMAD = *N*,*N*,*N*',*N*'-tetramethylazodicarboxamide.

remaining mesylate with an acetate followed by methanolysis and then protection of the primary hydroxy group with a *p*-methoxybenzyl moiety furnished the 2,5-dioxabicyclo[2.2.1]heptane derivative **11**. The bicyclic compound **11** was converted in 83 % yield to the monocyclic aldehyde **12** (enantiomer of aldehyde **4**, Scheme 1). As expected, reaction of the aldehyde **12** with phenyl or pyrenyl Grignard reagents, therefore, exclusively yielded the *S*-epimers **13a** and **13b**, respectively. For synthesis of the desired 2'-*O*,4'-*C*-methylene-α-L-ribofuranosyl nucleosides, epimerization at C1' (nucleoside numbering) was required, and oxidation of the furanoses **13a** and **13b** followed by reduction with sodium borohydride afforded the desired *R*-epimers **15a** and **15b**, respectively. The diols **15a** and **15b** were each efficiently cyclized under Mitsunobu conditions to afford the bicyclic α-L-configured *C*-aryl nucleosides **16a** and **16b**. Oxidative removal of the *p*-methoxybenzyl protection groups followed by 5'-*O*-dimethoxytritylation of the primary hydroxyl groups and subsequent phosphitylation using standard conditions furnished the desired phosphoramidite building blocks **17a** and **17b** (Scheme 2) [23].

Synthesis and thermal denaturation studies of oligonucleotides containing LNA monomers Ph^L and Py^L and α-L-LNA monomers $^{\alpha L}Ph^L$ and $^{\alpha L}Py^L$

All oligonucleotides **ON2**–**ON9** (Table 1) were prepared in 0.2 μmol scale using the phosphoramidite approach on a Biosearch 8750 DNA synthesizer. The stepwise coupling efficiencies of phosphoramidites **7a**, **17a**, and **17b** (10 min coupling time) and of phosphoramidite **7b** (20 min coupling time) were >96 % and of unmodified deoxynucleoside and 2'-*O*-methylribonucleoside phosphoramidites >99 % (with standard coupling time) using 1*H*-tetrazole as activator (pyridine hydrochloride was used as activator when coupling **17b**). After standard deprotection and cleavage from the solid support using 32 % aqueous ammonia (12 h, 55 °C), the oligomers were purified by precipitation from ethanol. The composition of oligomers **ON2**, **ON3**, and **ON5**-**ON9** was verified by MALDI-MS analysis and their purity (>80 %) by capillary gel electrophoresis [19,23].

Table 1 Thermal denaturation experiments [T_m values (°C) shown] for **ON1**–**ON9** toward DNA complements with each of the four natural bases in the central position[a].

DNA target:	3'-d(CACT**Y**TACG)	**Y:**	**A**	**C**	**G**	**T**
ON1	5'-d(GTGATATGC)		28[b]/27[c]/36[d]	11[b]	12[b]	19[b]
ON2	5'-d(GTGA**Ph^L**ATGC)		12[b]	5[b]	6[b]	7[b]
ON3	5'-d(GTGA**Py^L**ATGC)		18[b]	17[b]	18[b]	19[b]
ON4	5'-[2'-OMe(GTGATATGC)]		35[b]	14[b]	19[b]	21[b]
ON5	5'-[2'-OMe(G**T^L**GA**Py^L**A**T^L**GC)]		39[b]	38[b]	37[b]	40[b]
ON6	5'-(GTGA$^{\alpha L}$**Ph^L**ATGC)		<5[c]/12[d]	<5[c,d]	<5[c,d]	<5[c]/12[d]
ON7	5'-(GTGA$^{\alpha L}$**Py^L**ATGC)		21[c]/30[d]	22[c]/31[d]	27[c]/34[d]	23[c]/33[d]
ON8	5'-(G$^{\alpha L}$**T^L**GA$^{\alpha L}$**Ph^L**A$^{\alpha L}$**T^L**GC)		<5[c]/14[d]	<5[c,d]	<5[c,d]	<5[c]/13[d]
ON9	5'-(G$^{\alpha L}$**T^L**GA$^{\alpha L}$**Py^L**A$^{\alpha L}$**T^L**GC)		24[c]/33[d]	23[c]/33[d]	31[c]/36[d]	26[c]/34[d]

[a]Melting temperatures (T_m values/°C) measured as the maximum of the first derivative of the melting curve (A_{260} vs. temperature) recorded in medium salt buffer [b,c](10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) or in high salt buffer[d] (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5[b] or 1.0[c,d] μM concentrations of the two strands; A = adenin-9-yl monomer, C = cytosin-1-yl monomer, G = guanin-9-yl monomer, T = thymin-1-yl monomer; see Fig. 1 for structures of **T^L**, **$^{\alpha L}$T^L**, **Ph^L**, **$^{\alpha L}$Ph^L**, **Py^L**, **$^{\alpha L}$Py^L**, and 2'-OMe-RNA monomers; DNA sequences are shown as d(sequence) and 2'-OMe-RNA sequences as 2'-OMe(sequence).

The hybridization of the oligonucleotides **ON1**–**ON9** (Table 1) toward four 9-mer DNA targets with the central base being each of four natural bases was studied by thermal denaturation experiments (determination of T_m values). Compared to the DNA reference **ON1**, introduction of the phenyl LNA monomer **Ph^L** (**ON2**) resulted in reduced thermal stability of the duplexes, and universal hybridization was not achieved due to preferential binding to the target DNA with the central adenine monomer. The

pyrene LNA monomer $\mathbf{Py^L}$ (**ON3**) displayed more encouraging properties. Firstly, the binding affinity toward all four components was increased compared to **ON2**. Secondly, universal hybridization was accomplished as shown by the four T_m values all being within 17–19 °C. With respect to universal hybridization, $\mathbf{Py^L}$ thus parallels the pyrene DNA derivative **Py**, but the decrease in thermal stability compared to the **ON1**:DNA reference duplex was more pronounced for $\mathbf{Py^L}$ (ΔT_m ~ –10 °C) than reported for **Py** (ΔT_m ~ –5 °C, **Py** incorporated into a 12-mer DNA sequence) [6].

To study the effect of the $\mathbf{Py^L}$ in duplexes containing RNA-type oligonucleotides, we synthesized **ON4** and **ON5**, out of which **ON4** was the reference RNA-mimicking oligonucleotide composed entirely of 2'-OMe-RNA monomers. As improved binding affinity is considered important for universal hybridization probes [1], we constructed **ON5** as a mixture of six 2'-OMe-RNA monomers, one central pyrenyl LNA monomer $\mathbf{Py^L}$, and two affinity-enhancing LNA thymine monomers $\mathbf{T^L}$. Indeed, the 2-OMe-RNA/LNA chimera **ON5** displayed universal hybridization behavior as revealed by the measured T_m values (37–40 °C). It is important to note that all four T_m values obtained for **ON5** are higher than the T_m values obtained for the two fully complementary reference duplexes **ON1**:DNA (T_m = 28 °C) and **ON4**:DNA (T_m = 35 °C). These results demonstrate that the pyrene LNA monomer $\mathbf{Py^L}$ displays universal hybridization behaviour both in a DNA context (**ON3**) and in an RNA-like context (**ON5**), in the latter case with satisfactory binding affinities induced by the presence of affinity-enhancing monomers [19].

The hybridization of the oligonucleotides **ON6**–**ON9** containing $\mathbf{^{\alpha L}Ph^L}$/$\mathbf{^{\alpha L}Py^L}$ towards DNA targets was studied at medium and high salt concentrations. Introduction of the phenyl α-L-LNA monomer $\mathbf{^{\alpha L}Ph^L}$ (**ON6**) reduced the thermal stability of the resulting duplexes very significantly, even when combined with two thymine α-L-LNA monomers (monomers $\mathbf{^{\alpha L}T^L}$, **ON8**) known to be affinity-enhancing in this sequence context [24]. Interestingly, incorporation of one pyrenyl α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ (**ON7**) induced T_m values being only moderately reduced relative to those of the DNA reference **ON1** (under both medium and high salt conditions). A weak positive effect on the T_m values was in this case induced by the introduction of two thymine α-L-LNA monomers (**ON9**). It is apparent from the studies on **ON7** and **ON9** that the pyrenyl α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ displays a subtle preference for "pairing" with a guanine monomer (most pronounced under medium salt conditions). Fluorescence studies suggested that the pyrene moiety of the pyrenyl α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ interacts with base stacking, possibly by intercalation [23].

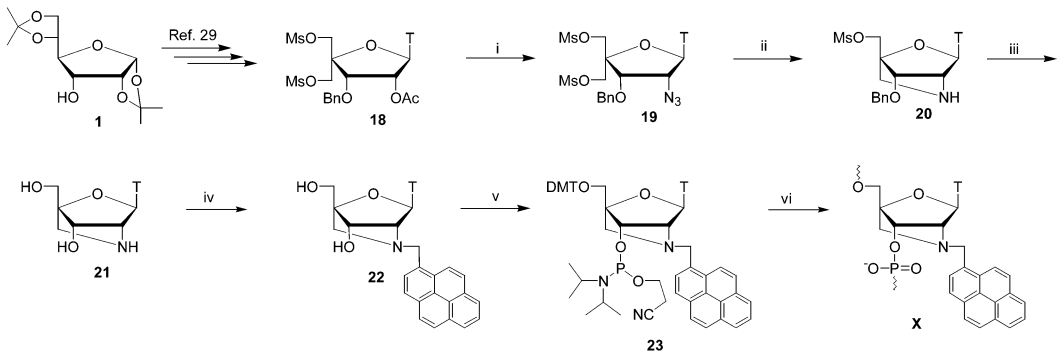
Comparison of the pyrenyl LNA monomer $\mathbf{Py^L}$ (**ON3**) and the pyrenyl α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ (**ON7**) reveals several interesting differences. Under medium salt conditions, the affinity was determined to be significantly lower for $\mathbf{Py^L}$, but the hybridization with the four DNA target strands is more universal. However, the performance of the pyrenyl α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ as a universal base is improved under high salt conditions [T_m values = 30–34 °C (**ON7**) and 33–36 °C (**ON9**)].

We have shown that universal hybridization is possible with a conformationally restricted RNA-like monomer exemplified by the pyrene LNA monomer $\mathbf{Py^L}$, both in a DNA context (**ON3**) and in an RNA context (**ON5**). Universal hybridization can also be achieved with conformationally locked DNA-mimicking monomers as exemplified by the pyrenyl-α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$ (**ON7** and **ON9**). Importantly, we have shown that the general problem of decreased affinity of universal hybridization probes can be solved by combining the pyrene LNA monomer $\mathbf{Py^L}$ with affinity-increasing monomers (2'-OMe-RNA and LNA monomers), or by the use of the pyrenyl-α-L-LNA monomer $\mathbf{^{\alpha L}Py^L}$, preferably in combination with α-L-LNA monomers.

2'-*N*-(Pyren-1-yl)methyl-2'-amino-LNA: Synthesis and interstrand communication

The 2'-nitrogen atom of 2'-amino-LNA monomers is very suitable for functionalization of high-affinity oligonucleotides [25–28]. In the context of oligonucleotide labeling, we became interested in the attachment of pyrenyl moieties at the brim of the minor groove in nucleic acid duplexes using a short

linker. The synthesis of the phosphoramidite building block **23** suitable for incorporation of the 2'-*N*-(pyren-1-yl)methyl-2'-amino-LNA monomer **X** is shown in Scheme 3.



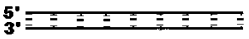
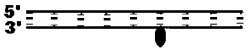
Scheme 3 (i) (a) Half-saturated methanolic ammonia; (b) MsCl, pyridine; (c) DBU, CH_3CN; (d) 0.1 M aq. H_2SO_4, acetone; (e) Tf_2O, DMAP, pyridine, CH_2Cl_2; (f) NaN_3, DMF (76 %); (ii) PMe_3 (1 M, THF), aq. NaOH (2 M), THF (93 %); (iii) (a) NaOBz, DMF, 100 °C; (b) saturated methanolic ammonia; (c) H_2, $Pd(OH)_2$/C, EtOH (95 %); (iv) pyrene-1-carbaldehyde, AcOH, $NaCNBH_3$, MeOH (94 %); (v) (a) DMTCl, pyridine; (b) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (59 %); (vi) DNA synthesizer.

Nucleoside **18** was obtained from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**1**) following the published procedure [29]. Deacetylation followed by double inversion at C2' afforded the azide **19** in 76 % yield. In situ reduction of the azide under Staudinger conditions and subsequent intramolecular cyclization furnished the bicyclic nucleoside **20**. Nucleophilic replacement of the mesyloxy group at C5' with a benzoyloxy group, followed by debenzoylation in methanolic ammonia and reductive debenzylation at C3' gave the desired 2'-amino-LNA nucleoside **21**. Reductive N-alkylation of **21** by reaction with pyrene-1-carbaldehyde furnished 2'-*N*-(pyren-1-yl)methyl derivative **22**. The phosphoramidite building block **23** was obtained by standard 5'-*O*-dimethoxytritylation followed by 3'-*O*-phosphitylation [28].

Incorporation of a single 2'-*N*-(pyren-1-yl)methyl-2'-amino-LNA monomer **X** into a mixed sequence 9-mer did not significantly affect the thermal stability against complementary DNA (Table 2, T_m values for **ON1**:**ON4** and **ON1**:**ON5** relative to **ON1**:**ON2**), indicating that the bulky pyrenylmethyl moiety can be accommodated in the minor groove of the studied duplexes [30]. Upon excitation at 340 nm, the steady-state fluorescence emission spectra of the singly modified duplexes exhibited structured monomer bands at λ_{max} ~378 nm and λ_{max} ~398 nm, and only very low levels of emission at λ = 430–530 nm, the characteristic pyrene excimer band region (Table 2, **ON1**:**ON4** and **ON1**:**ON5**). Insertion of two **X** monomers in a "1 + 1 downstream zipper" (Table 2, **ON3**:**ON5**), was accompanied by significantly increased thermal stability (relative to **ON1:ON5**), and the appearance of a distinct band corresponding to excimer fluorescence in the fluorescence emission spectrum. Insertion of two **X** monomers in a "1 + 1 upstream zipper" (**ON3**:**ON4**) gave no significant increase in thermal stability and no excimer band formation indicating a directional preference for interstrand communication (Table 2). These results reveal a stabilizing effect due to stacking (interstrand communication) of the pyrenyl moieties in the downstream zipper motif, which was confirmed by molecular modeling [30]. Thus, a reliable molecular communication system based on interstrand pyrene excimer formation between 2'-*N*-(pyren-1-yl)methyl-2'-amino-LNA monomers has been introduced.

Table 2 2'-*N*-(pyren-1-ylmethyl)-2'-amino-LNA and reference oligonucleotides, thermal denaturation studies, excimer band formation, and schematic illustration of duplexes.

ON1 5'-GTG ATA TGC
ON2 5'-GCA TAT CAC
ON3 5'-GTG A**X**A TGC
ON4 5'-GCA **X**AT CAC
ON5 5'-GCA TA**X** CAC

Duplex	T_m	Excimer	Schematic illustration
ON1+**ON2**	28 °C	-	
ON1+**ON4**	29 °C	-	
ON1+**ON5**	27 °C	-	
ON3+**ON5**	35 °C	+	
ON3+**ON4**	30 °C	-	

Melting temperatures (T_m values) and steady-state fluorescence emission spectra (19 ± 0.1 °C) were measured in medium salt buffer (see caption of Table 1 for details). A, C, G, and T denote DNA monomers. The "dark drops" denote pyrenylmethyl moieties (monomer **X**) [30].

CONCLUSION

Nondiscriminating universal hybridization with tunable binding affinity has been accomplished with a conformationally restricted RNA-mimicking LNA monomer containing pyren-1-yl as aglycon, both in a DNA context and in an RNA context, and also with the corresponding DNA-mimicking pyrenyl-α-L-LNA monomer in a DNA context [19,23]. Furthermore, a reliable molecular communication system based on interstrand pyrene excimer formation between 2'-*N*-(pyren-1-yl)methyl-2'-amino-LNA monomers has been introduced [30] that appears superior to other similar systems [31]. This accomplishment underlines the use of functionalized 2'-amino-LNA monomers for oligonucleotide-based Ångström-scale chemical engineering [32].

REFERENCES

1. D. Loakes. *Nucleic Acids Res.* **29**, 2437 (2001) and references therein.
2. R. Nichols, P. C. Andrews, P. Zhang, D. E. Bergstrom. *Nature* **369**, 492 (1994).
3. D. Loakes and D. M. Brown. *Nucleic Acids Res.* **22**, 4039 (1994).
4. M. Berger, Y. Wu, A. K. Ogawa, D. L. McMinn, P. G. Schultz, F. E. Romesberg. *Nucleic Acids Res.* **28**, 2911 (2000).
5. F. Seela and H. Debelak. *Nucleic Acids Res.* **28**, 3224 (2000).

6. T. J. Matray and E. T. Kool. *J. Am. Chem. Soc.* **120**, 6191 (1998).
7. J. S. Oliver, K. A. Parker, J. W. Suggs. *Org. Lett.* **3**, 1977 (2001).
8. E. T. Kool, J. C. Morales, K. M. Guckian. *Angew. Chem., Int. Ed.* **39**, 990 (2000).
9. M. Petersen and J. Wengel. *Trends Biotechnol*. **21**, 74 (2003).
10. S. K. Singh, P. Nielsen, A. A. Koshkin, J. Wengel. *Chem. Commun.* 455 (1998).
11. A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen, J. Wengel. *Tetrahedron* **54**, 3607 (1998).
12. S. Obika, D. Nanbu, Y. Hari, J. Andoh, K. Morio, T. Doi, T. Imanishi. *Tetrahedron Lett.* **39**, 5401 (1998).
13. J. Wengel, A. Koshkin, S. K. Singh, P. Nielsen, M. Meldgaard, V. K. Rajwanshi, R. Kumar, J. Skouv, C. B. Nielsen, J. P. Jacobsen, N. Jacobsen, C. E. Olsen. *Nucleosides Nucleotides* **18**, 1365 (1999).
14. J. Wengel. *Acc. Chem. Res.* **32**, 301 (1999).
15. Wengel, M. Petersen, K. E. Nielsen, G. A. Jensen, A. E. Håkansson, R. Kumar, M. D. Sørensen, V. K. Rajwanshi, T. Bryld, J. P. Jacobsen. *Nucleosides Nucleotides Nucleic Acids* **20**, 389 (2001).
16. M. D. Sørensen, L. Kværnø, T. Bryld, A. E. Håkansson, B. Verbeure, G. Gaubert, P. Herdewijn, J. Wengel. *J. Am. Chem. Soc.* **124**, 2164 (2002).
17. K. M. E. Nielsen, M. Petersen, A. E. Håkansson, J. Wengel, J. P. Jacobsen. *Chem. Eur. J.* **8**, 3001 (2002).
18. B. R. Babu and J. Wengel. *Chem. Commun.* 2114 (2001).
19. B. R. Babu, A. K. Prasad, S. Trikha, N. Thorup, V. S. Parmar, J. Wengel. *J. Chem. Soc., Perkin Trans 1* 2509 (2002).
20. S. Obika, Y. Hari, K. Morio, T. Imanishi. *Tetrahedron Lett*. **41**, 215 (2000).
21. S. Obika, Y. Hari, K. Morio, T. Imanishi. *Tetrahedron Lett*. **41**, 221 (2000).
22. Y. Hari, S. Obika, M. Sakaki, K. Morio, Y. Yamagata, T. Imanishi. *Tetrahedron* **58**, 3051 (2002).
23. Raunak, B. R. Babu, M. D. Sørensen, V. S. Parmar, N. H. Harrit, J. Wengel. *Org. Biomol. Chem*. **2**, 80 (2004).
24. V. K. Rajwanshi, A. E. Håkansson, M. D. Sørensen, S. Pitsch, S. K. Singh, R. Kumar, P. Nielsen, J. Wengel. *Angew. Chem., Int. Ed.* **39**, 1656 (2000).
25. S. K. Singh, R. Kumar, J. Wengel. *J. Org. Chem.* **63**, 6078 (1998).
26. S. K. Singh, R. Kumar, J. Wengel. *J. Org. Chem.* **63**, 10035 (1998).
27. C. Rosenbohm, S. M. Christensen, M. D. Sørensen, D. S. Pedersen, L. Larsen, J. Wengel, T. Koch. *Org. Biomol. Chem*. **1**, 655 (2003).
28. M. D. Sørensen, M. Pedersen, J. Wengel. *Chem. Commun*. 2130 (2003).
29. A. A. Koshkin, J. Fensholdt, H. M. Pfundheller, C. Lomholt. *J. Org. Chem*. **66**, 8504 (2001).
30. P. J. Hrdlicka, B. R. Babu, M. D. Sørensen, J. Wengel. *Chem. Commun.* 1478 (2004).
31. N. N. Dioubankova, A. D. Malakhov, D. A. Stetsenko, M. J. Gait, P. E. Volynsky, R. G. Efremov, V. A. Korshum. *ChemBioChem* **4**, 841 (2003).
32. J. Wengel. *Org. Biomol. Chem.* **2**, 277 (2004).