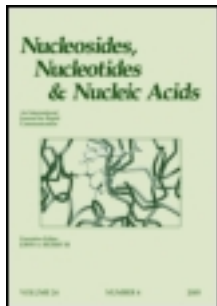


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### N2'-Functionalized 2'-Amino- $\alpha$ -L-LNA Adenine Derivatives—Efficient Targeting of Single Stranded DNA

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## N2'-FUNCTIONALIZED 2'-AMINO- $\alpha$ -L-LNA ADENINE DERIVATIVES—EFFICIENT TARGETING OF SINGLE STRANDED DNA

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□ *The synthesis of two pyrene-functionalized 2'-amino- $\alpha$ -L-LNA adenine building blocks is outlined and initial results from thermal denaturation studies are presented.*

**Keywords** Conformationally restricted nucleosides; antisense; antigene

### INTRODUCTION

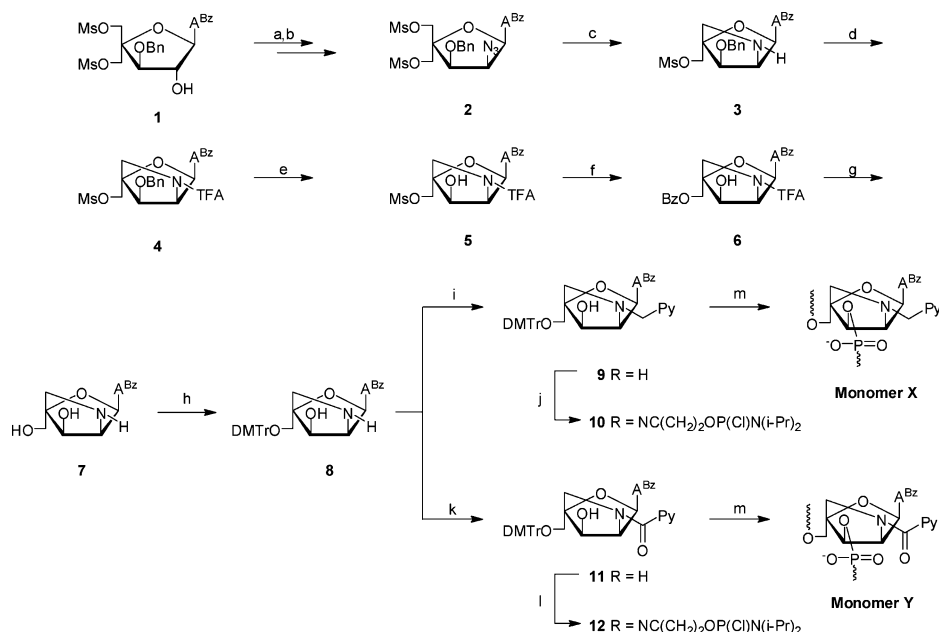
Motivated by the potential of LNA<sup>[1]</sup> and  $\alpha$ -L-LNA<sup>[2]</sup> within nucleic acid based therapeutics and diagnostics, we have recently explored the corresponding 2'-amino analogs, which allow extensive chemical derivatization of nucleic acids.<sup>[3–5]</sup> Incorporation of pyrene-functionalized 2'-amino- $\alpha$ -L-LNA thymine monomers into short DNA strands, results in dramatically increased thermal affinity towards DNA complements relative to unmodified reference strands.<sup>[4]</sup> This property has been used to develop a novel strategy for targeting of double-stranded DNA.<sup>[4]</sup> Due to the challenging synthetic route towards these building blocks,<sup>[5]</sup> we set out to synthesize the corresponding 2'-amino- $\alpha$ -L-LNA adenine derivatives described herein.

### RESULTS AND DISCUSSION

The developed synthetic route to the desired pyrene-functionalized 2'-amino- $\alpha$ -L-LNA adenine phosphoramidites **10** and **12** initiates from the

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**SCHEME 1** Reagents and conditions: a)  $(\text{CF}_3\text{SO}_2)_2\text{O}$ , an. pyridine, an.  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ ; b)  $\text{NaN}_3$ , 15-crown-5, an. DMF,  $40^\circ\text{C}$ , 89% (over 2 steps); c) 2 M aq.  $\text{NaOH}$ ,  $\text{PMe}_3$ , THF, rt, 85%; d)  $(\text{CF}_3\text{CO})_2\text{O}$ , an. pyridine, an.  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ , 62%; e)  $\text{BCl}_3$ , an.  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$  to rt, 87%; f)  $\text{NaOBz}$ , 15-crown-5, an. DMF,  $90^\circ\text{C}$ , 83%; g) 2 M aq.  $\text{NaOH}$ , 1,4-dioxane/ $\text{H}_2\text{O}$ ,  $0^\circ\text{C}$ , 60%; h)  $\text{DMTrCl}$ , an. pyridine,  $0^\circ\text{C}$  to rt, 34%; i)  $\text{PyCHO}$ ,  $\text{NaBH}(\text{OAc})_3$ , 1,2-DCE, rt, 68%; j)  $\text{NC}(\text{CH}_2)_2\text{OP}(\text{Cl})\text{N}(\text{i-Pr})_2$ , DIPEA, 1,2-DCE, rt, 51%; k)  $\text{PyCOOH}$ ,  $\text{EDC}\cdot\text{HCl}$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 64%; l)  $\text{NC}(\text{CH}_2)_2\text{OP}(\text{Cl})\text{N}(\text{i-Pr})_2$ , DIPEA, 1,2-DCE, rt, 67%; m) DNA synthesizer.  $\text{A}^{\text{Bz}}$ : 6*N*-benzoyladenine-9-yl; Py: pyren-1-yl.

known adenine derivative **1** (Scheme 1).<sup>[2]</sup> O2'-Triflation of alcohol **1** followed by nucleophilic substitution affords azido nucleoside **2**, which upon subjecting to conditions favoring a tandem Staudinger/intramolecular nucleophilic substitutions furnishes known bicyclic nucleoside **3**<sup>[6]</sup> in excellent yield. After a subsequent series of protecting group manipulations, amino alcohol **8** was obtained, which upon chemoselective N2'-functionalization via reductive amination or by EDC mediated coupling afforded nucleosides **9** and **11**, respectively. After standard phosphitylation the desired amidites **10** and **12** was obtained as suitable building blocks for incorporation into oligodeoxyribonucleotides (ONs) via automated DNA synthesis.

ONs containing a single incorporation of monomer **X** or **Y**, exhibit greatly increased thermal affinities towards complementary DNA (up to  $+11.0^\circ\text{C}$ ) while lower increases are observed toward RNA complements (Table 1). Single nucleotide mismatches opposite of the site of incorporation are discriminated well (footnote Table 1). Full details of this study will be presented elsewhere.

**TABLE 1** Thermal denaturation temperatures ( $T_m$  values) of pyrene-functionalized 2'-amino- $\alpha$ -L-LNA towards DNA/RNA complements<sup>a</sup>

		$T_m$ ( $\Delta T_m$ )/°C	
		DNA	RNA
<b>ON1</b>	5'-GCA <b>TXT</b> ACG	34.0 (+6.0)	24.0 (−0.5)
<b>ON2</b>	5'-GCA <b>TYT</b> ACG	39.0 (+11.0)	27.0 (+2.5)
<b>ON3</b>	5'-GCA <b>TAT</b> ACG	28.0	24.5

<sup>a</sup>  $T_m$  values/°C ( $\Delta T_m$  = change in  $T_m$  value calculated relative to DNA:DNA or DNA:RNA reference duplex) recorded in medium salt buffer ([Na<sup>+</sup>] = 110 mM, [Cl<sup>−</sup>] = 100 mM, pH 7.0 (NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>)), using 1.0  $\mu$ M concentrations of each strand.  $T_m$  values (°C) of **ON1** and **ON2** towards DNA strands containing a single mismatch in the central position A/C/G: 18.5/27.0/19.5 and 18.0/27.0/22.0, respectively.  $T_m$  values (°C) of **ON1** and **ON2** towards RNA strands containing a single mismatch in the central position A/C/G: 13.5/16.0/14.0 and 16.5/16.0/13.5, respectively.

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