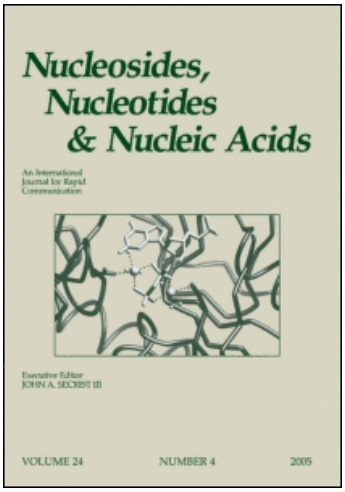


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

N2'-Functionalized 2'-Amino-α-L-LNA Adenine Derivatives—Efficient Targeting of Single Stranded DNA

Nicolai K. Andersen[a]; Jesper Wengel[a]; Patrick J. Hrdlicka[ab]
[a] Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark [b] Department of Chemistry, University of Idaho, Moscow, Idaho, USA

To cite this Article Andersen, Nicolai K. , Wengel, Jesper and Hrdlicka, Patrick J.(2007) 'N2'-Functionalized 2'-Amino-α-L-LNA Adenine Derivatives—Efficient Targeting of Single Stranded DNA', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1415 — 1417
To link to this Article: DOI: 10.1080/15257770701539153
URL: http://dx.doi.org/10.1080/15257770701539153

Nucleosides, Nucleotides, and Nucleic Acids, 26:1415–1417, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701539153

Taylor & Francis
Taylor & Francis Group

N2′-FUNCTIONALIZED 2′-AMINO-α-L-LNA ADENINE DERIVATIVES—EFFICIENT TARGETING OF SINGLE STRANDED DNA

Nicolai K. Andersen and Jesper Wengel □ *Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark*

Patrick J. Hrdlicka □ *Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark; Department of Chemistry, University of Idaho, Moscow, Idaho, USA*

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

Keywords Conformationally restricted nucleosides; antisense; antigene

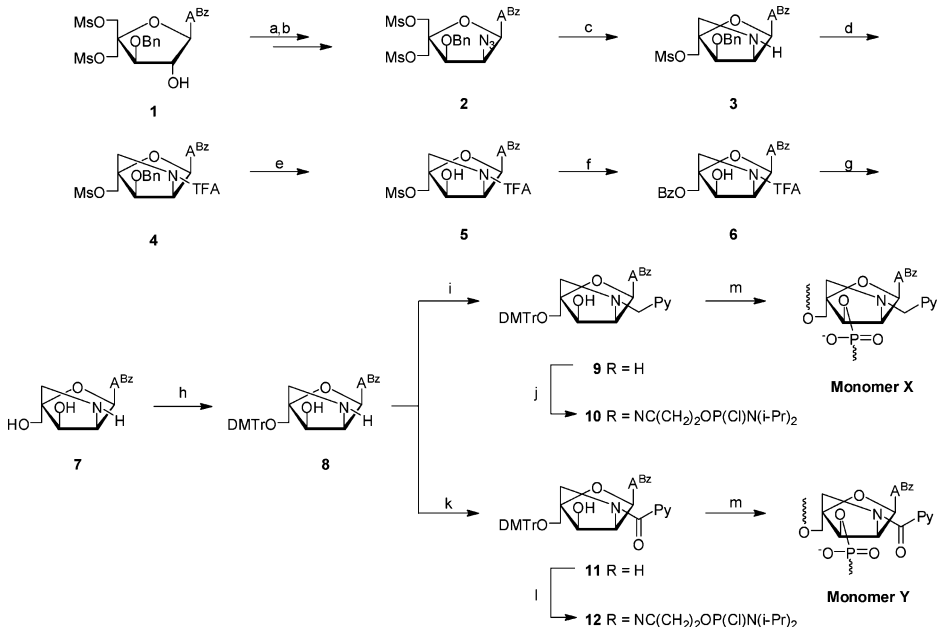
INTRODUCTION

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

RESULTS AND DISCUSSION

The developed synthetic route to the desired pyrene-functionalized 2′-amino-α-L-LNA adenine phosphoramidites **10** and **12** initiates from the

The Danish National Research Foundation is thanked for financial support.
Address correspondence to Patrick J. Hrdlicka, Department of Chemistry, University of Idaho, Moscow, ID 83843-2343. E-mail: Hrdlicka@uidaho.edu



SCHEME 1 Reagents and conditions: a) $(CF_3SO_2)_2O$, an. pyridine, an. CH_2Cl_2, −78°C; b) NaN_3, 15-crown-5, an. DMF, 40°C, 89% (over 2 steps); c) 2 M aq. NaOH, PMe_3, THF, rt, 85%; d) $(CF_3CO)_2O$, an. pyridine, an. CH_2Cl_2, 0°C, 62%; e) BCl_3, an. CH_2Cl_2, −78°C to rt, 87%; f) NaOBz, 15-crown-5, an. DMF, 90°C, 83%; g) 2 M aq. NaOH, 1,4-dioxane/H_2O, 0°C, 60%; h) DMTrCl, an. pyridine, 0°C to rt, 34%; i) PyCHO, $NaBH(OAc)_3$, 1,2-DCE, rt, 68%; j) $NC(CH_2)_2OP(Cl)N(iPr)_2$, DIPEA, 1,2-DCE, rt, 51%; k) PyCOOH, EDC·HCl, CH_2Cl_2, rt, 64%; l) $NC(CH_2)_2OP(Cl)N(iPr)_2$, DIPEA, 1,2-DCE, rt, 67%; m) DNA synthesizer. A^{Bz}: 6*N*-benzoyladenine-9-yl; Py: pyren-1-yl.

known adenine derivative **1** (Scheme 1).[2] O2′-Triflation of alcohol **1** followed by nucleophilic substitution affords azido nucleoside **2**, which upon subjection to conditions favoring a tandem Staudinger/intramolecular nucleophilic substitutions furnishes known bicyclic nucleoside **3**[6] 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°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-α-L-LNA towards DNA/RNA complements[a]

		T_m (ΔT_m)/°C	
		DNA	RNA
ON1	5′-GCA T**X**T ACG	34.0 (+6.0)	24.0 (−0.5)
ON2	5′-GCA T**Y**T ACG	39.0 (+11.0)	27.0 (+2.5)
ON3	5′-GCA TAT ACG	28.0	24.5

[a] T_m values/°C (ΔT_m = change in T_m value calculated relative to DNA:DNA or DNA:RNA reference duplex) recorded in medium salt buffer ($[Na^+]$ = 110 mM, $[Cl^-]$ = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), using 1.0 μM 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.

REFERENCES

1. Petersen, M.; Wengel, J. LNA: A versatile tool for therapeutics and genomics. *Trends Biotechnol.* **2003**, 21, 74–81.
2. Sørensen, M.D.; Kværnø, L.; Bryld, T.; Håkansson, A.E.; Verbeure, B.; Gaubert, G.; Herdewijn, P.; Wengel, J. α-L-*ribo*-Configured locked nucleic acid (α-L-LNA): Synthesis and properties. *J. Am. Chem. Soc.* **2002**, 124, 2164–2176.
3. Hrdlicka, P.J.; Babu, B.R.; Sørensen, M.D.; Harrit, N.; Wengel, J. Multilabeled pyrene-functionalized 2′-amino-LNA probes for nucleic acid detection in homogenous fluorescence assays. *J. Am. Chem. Soc.* **2005**, 127, 13293–13299 and references cited herein.
4. Hrdlicka, P.J.; Kumar, T.S.; Wengel, J. Targeting of mixed sequence double-stranded DNA using pyrene-functionalized 2′-amino-α-L-LNA. *Chem. Comm.* 4279–4281.
5. Kumar, T.S.; Madsen, A.S.; Wengel, J.; Hrdlicka, P.J. Synthesis and hybridization studies of 2′-amino-α-L-LNA and a tetracyclic 'locked LNA.' *J. Org. Chem.* **2006**, 71, 4188–4201.
6. Sørensen, M.D.; Wengel, J.; Koch, T., Christensen, S.M.; Rosenbohm, C.; Pedersen, D.S. Synthesis of locked nucleic acid derivatives. Patent application WO03095467.