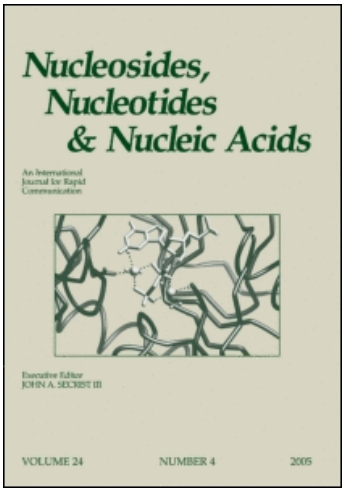



**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Synthesis and Biophysical Studies of N2'-Functionalized 2'-Amino-α-L-LNA

T. Santhosh Kumar[a]; Andreas S. Madsen[a]; Jesper Wengel[a]; Patrick J. Hrdlicka[b]
[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** Kumar, T. Santhosh , Madsen, Andreas S. , Wengel, Jesper and Hrdlicka, Patrick J.(2007) 'Synthesis and Biophysical Studies of N2'-Functionalized 2'-Amino-α-L-LNA', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1403 — 1405
**To link to this Article: DOI:** 10.1080/15257770701538841
**URL:** http://dx.doi.org/10.1080/15257770701538841

*Nucleosides, Nucleotides, and Nucleic Acids*, 26:1403–1405, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701538841

Taylor & Francis
Taylor & Francis Group

# SYNTHESIS AND BIOPHYSICAL STUDIES OF N2′-FUNCTIONALIZED 2′-AMINO-α-L-LNA

**T. Santhosh Kumar, Andreas S. Madsen, and Jesper Wengel** □ *Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark*

**Patrick J. Hrdlicka** □ *Department of Chemistry, University of Idaho, Moscow, Idaho, USA*



□ *A synthetic route towards a selected set of N-acylated and N-alkylated derivatives of 2′-amino-α-L-LNA phosphoramidite building blocks has been developed. Biophysical studies suggest that the 2-oxo-5-azabicyclo[2.2.1]heptane skeleton of 2′-amino-α-L-LNA allows precise positioning of intercalators in the core of nucleic acid duplexes.*

**Keywords** 2′-Amino-α-L-LNA; pyrene; oligonucleotides; intercalation

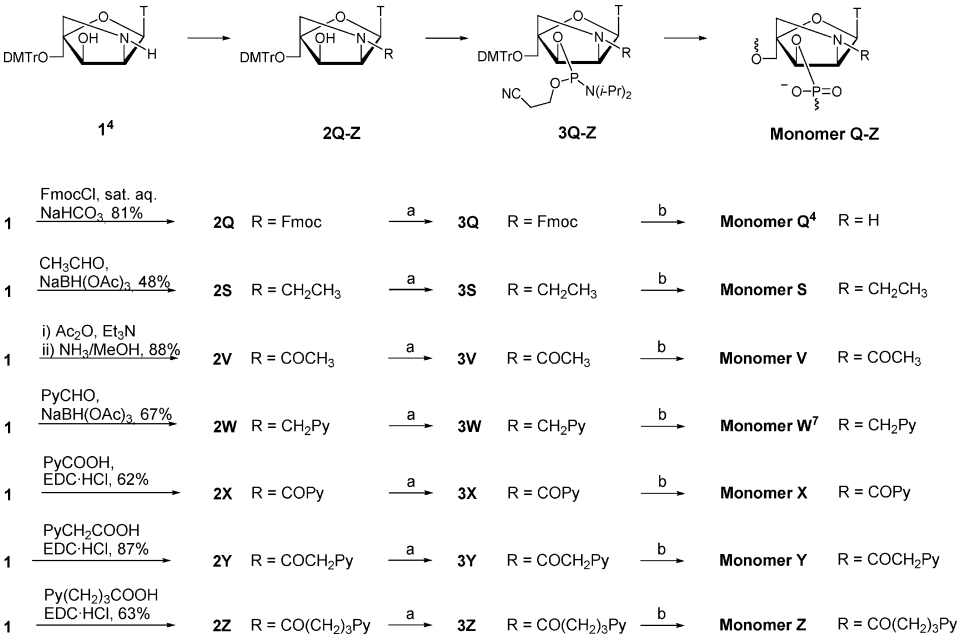
## INTRODUCTION

The high affinity hybridization of LNA,[1] 2′-amino-LNA,[2] α-L-LNA,[3] and 2′-α-L-amino-LNA[4] toward complementary DNA/RNA complements are well established. As an extension of our recent efforts to use N2′-functionalized 2′-amino-LNA monomers as building blocks in nucleic acid based diagnostics and therapeutics,[5] we have developed an interest in N2′-functionalized 2′-amino-α-L-LNA building blocks. Among these, double stranded 2′-*N*-(pyren-1-yl)methyl-2′-amino-α-L-LNA have been shown to target double-stranded DNA.[6] Herein, we present the synthesis and biophysical studies of N2′-functionalized 2′-amino-α-L-LNA.

## RESULTS AND DISCUSSION

The synthesis of a selected set of N2′-functionalized 2′-amino-α-L-LNA phosphoramidites is conveniently achieved in two steps from key

We greatly appreciate funding from The Danish National Research Foundation.
Address correspondence to T. Santhosh Kumar, Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, 5230 Odense M, Denmark. E-mail: skt@chem.sdu.dk




**SCHEME 1** Reagents and conditions: a) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, 56% for **3Q**, 63% for **3S**, 90% for **3V**, 88% for **3W**, 60% for **3X**, 75% for **3Y** and 36% for **3Z;** b) DNA synthesizer; DMTr = 4,4′-dimethoxytrityl, Fmoc = 9′-fluorenylmethoxycarbonyl, Py = pyren-1-yl.



intermediate **1** by chemoselective carbamoylation (monomer **Q**) or EDC-mediated N-acylation (monomers **X-Z**), reductive amination (monomers **S** and **W**), or peracylation followed by selective deacylation (monomer **V**, Scheme 1). Further details on the synthesis and incorporation of N2′-functionalized 2′-amino-α-L-LNA phosphoramidites **3Q-Z** into short oligodeoxyribonucleotides (ONs) will be presented elsewhere.

Incorporation of a single pyrene functionalized 2′-amino-α-L-LNA monomer (**W-Z**) results in dramatic increases in duplex stability with DNA complements of up to +19.5°C and significantly smaller increases in duplex stability with RNA complements. A single incorporation of non-functionalized 2′-amino-α-L-LNA monomer **Q** results in comparably more modest increases in duplex stability with DNA/RNA complements (Table 1).[4] Surprisingly, single incorporations of ethyl or acetyl substituted 2′-amino-α-L-LNA monomers (**S** or **V**) into ONs result in greatly decreased thermal affinities towards its DNA/RNA complements. The observed DNA selectivity (Table 1), limited mismatch discrimination, molecular modeling studies and hybridization induced bathocromic shifts of pyrene absorption maxima (data not shown), suggest that the 2-oxo-5-azabicyclo[2.2.1]heptane skeleton of these monomers positions the pyrene moiety suitably for intercalation upon hybridization.

**TABLE 1** Thermal denaturation temperatures of duplexes formed by 5′-d(GCA**B** AT CAC)[a] and DNA/RNA complements[b]

| | [$T_m$ ($\Delta T_m$/mod)/°C] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **B** = Q | **B** = S | **B** = V | **B** = W | **B** = X | **B** = Y | **B** = Z |
| **DNA** | 31.0 (+2.5) | 22.5 (−6.0) | 19.0 (−9.5) | 44.0 (+15.5) | 48.0 (+19.5) | 45.0 (+16.5) | 35.0 (+6.5) |
| **RNA** | 29.0 (+4.5) | 21.5 (−3.0) | 20.5 (−4.0) | 32.0 (+7.5) | 36.0 (+11.5) | 36.5 (+12.0) | 31.0 (+6.5) |

[a] $T_m$ values of unmodified duplex (where **B** = **T**) toward its complementary DNA and RNA are 28.5°C and 24.5°C, respectively.

[b] Thermal denaturation temperatures recorded in medium salt buffer ($[Na^+] = 110$ mM, $[Cl^-] = 100$ mM, pH 7.0 (adjusted with 10 mM $NaH_2PO_4$/5 mM $Na_2HPO_4$)), using 1.0 $\mu$M concentrations of the two complementary strands. See synthetic scheme for structure of monomers.

## REFERENCES


1. Wengel, J. Synthesis of 3′-*C*- and 4′-*C*-branched oligodeoxynucleotides and the development of locked nucleic acid (LNA). *Accounts Chem. Res.* **1999**, 32, 301–310.
2. Singh, S.K.; Kumar, R.; Wengel, J. Synthesis of 2′-amino-LNA: A novel conformationally restricted high-affinity oligonucleotide analogue with a handle. *J. Org. Chem.* **1998**, 63, 10035–10039.
3. 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.
4. Kumar, T.S.; Madsen, A.S.; Wengel, J.; Hrdlicka, P.J. Synthesis and hybridization studies of 2′-amino-α-L-LNA and tetracyclic "Locked LNA." *J. Org. Chem.* **2006**, 71, 4188–4201.
5. 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 homogeneous fluorescence assays. *J. Am. Chem. Soc.* **2005**, 127, 13293–13299 and references cited therein.
6. 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.* **2005**, 4279−4281.