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

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□ *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

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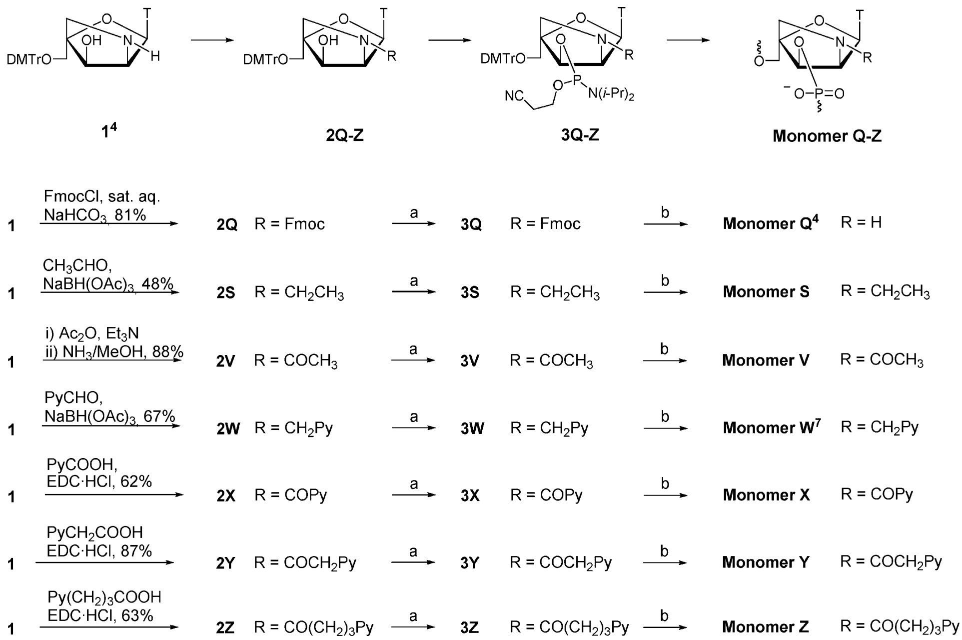

SCHEME 1 Reagents and conditions: a) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, 56% for **3Q**, 63% for **3S**, 90% for **3V**, 88% for **3W**, 60% for **3X**, 75% for **3Y** and 36% for **3Z;** b) DNA synthesizer; DMTr = 4,4′-dimethoxytrityl, Fmoc = 9′-fluorenylmethoxycarbonyl, Py = pyren-1-yl.

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 (ΔT_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 μM 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.

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