Parallel RNA-strand recognition by 2′-amino-β-L-LNA

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ARTICLE INFO

ABSTRACT

A short synthetic route to the first β-L-*ribo* configured locked nucleic acid (LNA), that is, 2′-amino-β-L-LNA thymine phosphoramidite **6**, has been developed from bicyclic nucleoside **1**. Incorporation of 2′-amino-β-L-LNA thymine monomers into α-DNA strands results in probes forming stable duplexes with complementary RNA in parallel orientation.

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Oligonucleotides (ONs) are widely explored as modulators of gene expression within the antisense regime to develop powerful research tools and therapeutics. Chemical modification of ONs is necessary to protect them adequately from enzymatic degradation by nucleases and to facilitate strong binding to complementary nucleic acid targets.[1] The use of ONs modified with conformationally restricted nucleotide building blocks has been a particularly successful approach toward this end.[2] LNA[3,4] (locked nucleic acid, β-D-*ribo* configuration), arguably the most promising member from this class of building blocks, exhibits greatly increased thermal affinity toward complementary antiparallel (ap) DNA/RNA and markedly improved stability toward nucleases.[5]

Similarly, α-DNA[6,7] (α-D-*ribo* configuration, Fig. 1) has been explored as a potential building block within the antisense strategy as it is highly resistant toward enzymatic degradation[8–10] and forms stable duplexes with complementary DNA and RNA. The two strands are aligned in parallel orientation to form a right handed helix held together by Watson–Crick base pairing.[11,12] More recently, analogs of α-DNA with C5-propynyl pyrimidines,[13,14] modified backbones including cationic phosphoramidate backbones[14–18] or conformationally restricted sugar moieties,[19–21] have been explored in order to optimize recognition of target oligonucleotide strands in parallel orientation.
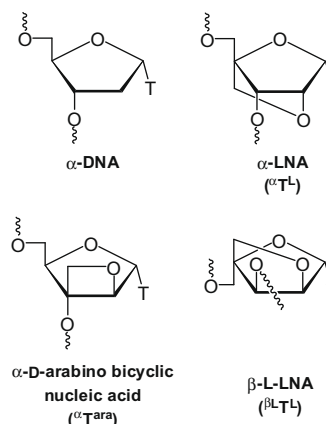
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Figure 1. Building blocks utilized for parallel strand recognition.

As an example of the latter category, fully modified oligothymidylate,[21a] mixed pyrimidine,[21b] or mixed sequence[21c] α-LNA (α-D-*ribo* configuration, Fig. 1) exhibit markedly higher thermal affinity toward complementary parallel (p) RNA than α-DNA, while not leading to duplex formation with complementary pDNA, apDNA, or apRNA.[21a–c] However, α-LNA/α-DNA-mixmers, that is, α-DNA strands with interspersed incorporations of α-LNA monomers, exhibit lower affinity toward pDNA/pRNA than unmodified α-DNA.[21b] The functional incompatibility between these mono-

mers has been attributed to the inability of α-DNA building blocks to adopt N-type[22] conformations imposed by α-LNA monomers.[21b] In contrast, mixmers between [3.2.0]bicyclic monomer ᵅT^ara (*E*-type,[22] Fig. 1) and α-DNA, display slightly higher affinity toward pRNA than unmodified α-DNA, but decreased affinity toward pDNA.[21d] Recent hybridization studies with fully modified 'α-LNA' and 'β-L-LNA' (β-L-*ribo* configuration, Fig. 1) studied in the mirror image world (i.e., α-L-LNA and β-D-LNA, respectively, against L-DNA/L-RNA targets), have suggested β-L-LNA as a possible structural and functional mimic of α-DNA.[21c] Thus, 'β-L-LNA' was found to form stable duplexes with both pRNA and pDNA but not with apRNA and apDNA. While the furanose conformation of β-L-LNA by definition is N-type due to its L-stereochemistry,[22] the furanose atoms overlay poorly with those of α-LNA (N-type). Studies evaluating β-L-LNA/α-DNA mixmers as probes for parallel strand recognition of nucleic acid targets have been precluded as the synthesis of β-L-LNA nucleotides has not been realized until now.

Herein, we have taken advantage of the recent synthetic availability of β-L-*ribo* configured LNA nucleosides obtained as prominent byproducts during our synthesis of 2′-amino-α-L-LNA monomers[23] and report: (a) the first synthesis of a β-L-*ribo* configured LNA phosphoramidite, (b) the automated solid-phase synthesis of fully modified 2′-amino-β-L-LNA and mixmers with DNA and α-DNA monomers, and (c) results from thermal denaturation experiments of complexes between these ONs and p/ap DNA/RNA targets.

The synthesis of 2′-amino-β-L-LNA phosphoramidite building block **6** initiates from β-L-*ribo* configured bicyclic nucleoside **1** (Scheme 1).[23] Nucleophilic displacement of the O5′-mesylate group of nucleoside **1** with a benzoate, and subsequent cleavage thereof using saturated methanolic ammonia afforded amino alcohol **2** (53% yield, two steps). Protection of the secondary amino group of **2** as a trifluoroacetamide furnished nucleoside **3** in 89% yield, which upon debenzylation using hydrogen and 20% Pd(OH)₂/C in ethyl acetate gave diol **4** in 70% yield. Subsequent O5′-protection as the 4,4′-dimethoxytrityl (DMTr) ether afforded nucleoside **5** in 70% yield, which upon O3′-phosphitylation (56%

yield) concluded this short sequence of protecting group manipulations to provide the desired 2′-amino-β-L-LNA phosporamidite building block **6** (Scheme 1). The identity of all reported compounds was fully ascertained by NMR (¹H, ¹³C, COSY, and/or HETCOR) and MALDI-HRMS, while purity was verified by 1D NMR.[24]

Several observations directly or indirectly support the suggested β-L-*ribo* configuration of bicyclic phosphoramidite **6**: (a) key ¹H NMR Nuclear Overhauser Enhancements (NOE) between H6 and H2′/H3′, and between H1′ and H5″ in starting material **1** have previously been identified and discussed,[23] (b) β-L-*ribo* configured nucleoside **2** exhibits specific rotation of identical magnitude but opposite sign relative to the known enantiomer[25] (Table S1),[24] and (c) ¹H/¹³C NMR data of β-L-nucleosides **2** and **4**–**6** are identical to those of the corresponding enantiomers.[24–26]

Automated synthesis of ONs was performed on a 0.2 μmol scale using universal CPG solid supports. 2′-Amino-β-L-LNA monomer **X** was incorporated into DNA and α-DNA strands, and was moreover oligomerized to a fully modified ON. The corresponding phosphoramidite building blocks for the incorporation of α-DNA thymine and 5-methylcytosine monomers were synthesized as previously described.[27,28] Standard procedures were applied for ON synthesis except for extended coupling time (25 min) and the use of 1*H*-tetrazole during incorporation of 2′-amino-β-L-LNA phosphoramidite building block **6**. The stepwise coupling yield was >95% for α-DNA phosphoramidites and ∼99% for the phosphoramidite building block **6**. Following standard workup and purification protocols,[24] the composition and purity (>80%) of all modified ONs were verified by MALDI-MS (Table S4) and ion-exchange HPLC, respectively.[24] The hybridization properties of these ONs with DNA/RNA targets in parallel/antiparallel orientation were studied by thermal denaturation measurements (A_{260} vs T) using a neutral medium salt phosphate buffer.

First, monomer **X** was incorporated once or thrice into mixed sequence 9-mer DNA strands (**ON1** and **ON2**). Singly modified **ON1** formed a significantly destabilized duplex with complementary apRNA, while transitions were not even observed with the other mixtures, attesting detrimental effects of monomer **X** on du-
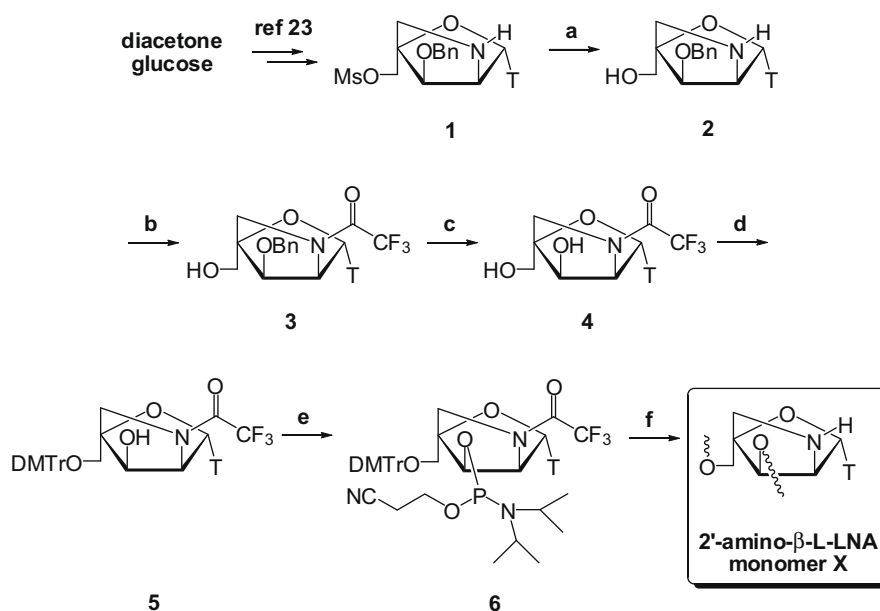
Scheme 1. Reagents and conditions: (a) (i) NaOBz, 15-crown-5, DMF, Δ; (ii) satd NH₃/MeOH, rt, 53% over two steps; (b) (CF₃CO)₂O, pyridine, CH₂Cl₂, 0 °C to rt, 89%; (c) H₂, Pd(OH)₂/C, EtOAc, rt, 70%; (d) DMTrCl, DMAP, pyridine, rt, 70%; (e) NC(CH₂)₂OP(Cl)N(*i*-Pr)₂, *N,N*-diisopropylethylamine, CH₂Cl₂, rt, 56%; (f) DNA synthesizer; T = thymin-1-yl; DMTr = 4,4′-dimethoxytrityl.