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Synthesis, nucleic acid hybridization properties and molecular modelling studies of conformationally restricted 3′-*O*,4′-*C*-methylene-linked α-L-ribonucleotides

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Abstract—Nucleotides with conformationally restricted carbohydrate rings such as locked nucleic acid (LNA), α-L-LNA or 2′,5′-linked 3′-*O*,4′-*C*-methyleribonucleotides exhibit significant potential as building blocks for antigene and antisense strategies. 2′,5′-Linked α-L-*ribo* configured monomer **X** (termed α-L-ONA) was designed as a potential structural mimic of α-L-LNA. The corresponding phosphoramidite building block of monomer **X** was obtained in five steps (10% overall yield) from the easily obtainable thymine derivative **1**. Incorporation of monomer **X** into oligodeoxyribonucleotides (ONs) results in dramatically decreased thermal stabilities with DNA/RNA complements (ΔT_m/mod = −11.5 to −17.0 °C) compared to unmodified reference ONs. Less pronounced decreases (ΔT_m/mod = −4.5 to −8.5 °C) are observed when monomer **X** is incorporated into triplex forming ONs and targeted against double-stranded DNA (parallel orientation, pyrimidine motif). This biophysical data, together with modelling studies, suggest that 2′,5′-linked α-L-ONA is a poor structural mimic of α-L-LNA.

Keywords: LNA; 2′,5′-Linked nucleic acids; Conformationally restricted nucleosides; Oligonucleotides; Oxetane

1. Introduction

The antigene and antisense strategies, that is, targeting of double-stranded DNA and single-stranded RNA with exogenous probes, respectively, continue to be highly desirable approaches to achieve specific control of gene expression.[1] During the past two decades, substantial efforts have been directed towards developing modified oligonucleotide probes with improved antigene or antisense characteristics such as increased target affinity and mismatch discrimination and enhanced stability towards enzymatic degradation.[2,3] A particularly successful approach to realize this has been to modify oligodeoxyribonucleotides (ONs) with nucleotides that have conformationally restricted carbohydrate rings.[4–7]

The flexible furanose ring of unmodified nucleosides adopts many different conformations in solution, although they tend to cluster in two regions that are classified as belonging to either the North (*N*) or South (*S*) part of the pseudorotational cycle.[8] The dioxabicyclo-[2.2.1]heptane skeletons of LNA (locked nucleic acid, β-D-*ribo* configuration, Fig. 1)[9–13] and its diastereomer α-L-LNA (α-L-*ribo* configuration, Fig. 1)[14–16] efficiently lock the furanose ring in an *N*-type conformation. (The furanose ring of α-L-LNA is, according to definitions, conformationally restricted in an *N*-type conformation as a result of the L-configuration; however, α-L-LNA does not overlay onto a typical *N*-type framework but rather overlays an *S*-type framework.) A single incorporation of an LNA or α-L-LNA building block into ONs induces, depending on sequence length and composition, large increases in thermal stability towards complementary DNA or RNA of up to +10 °C relative to unmodified ONs.[9–16] NMR studies have suggested that the increases in thermal stability are related to the

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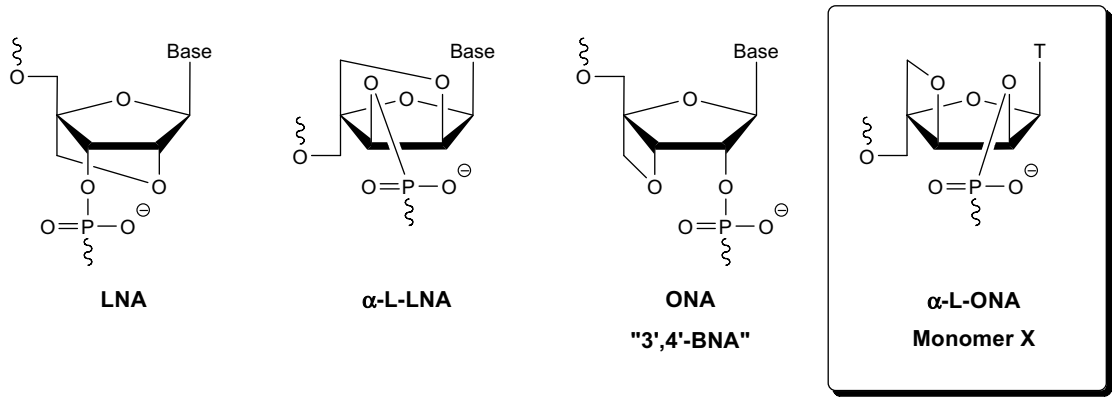

Figure 1. Structures of LNA,[9–13] α-L-LNA[14–16] and ONA (previously termed 3′,4′-BNA)[38–40] along with the novel α-L-ONA (3′-*O*,4′-*C*-methylene-linked α-L-ribonucleotide, monomer **X**) reported herein. T = Thymin-1-yl.

predisposition of LNA to behave as an A-type (RNA) mimic with regard to helical structure[17,18] whereas α-L-LNA behaves as an B-type (DNA) mimic.[19,20] The potential of LNA and α-L-LNA as triplex forming oligonucleotides (TFOs) is obvious, as a single incorporation of LNA[21–24] or α-L-LNA[25] nucleotides into triplex forming ONs generally induces very prominent increases in thermal stability of up to +10.0 and +6.0 °C, respectively.

2′,5′-Linked nucleic acids (2′,5′-NAs)[26–28] have been suggested as potential antisense and antigene probes due to the following properties: (a) 2′,5′-NAs form duplexes with normal RNA (i.e., 3′,5′-linked RNA) albeit they bind with lower affinity than isosequential DNA, (b) 2′,5′-NAs display a remarkable preference for complexation with RNA complements, (c) homopyrimidine chimeras of 2′,5′-linked DNA and normal DNA exhibit significantly increased thermal affinity towards double-stranded DNA compared to an unmodified ON and (d) 2′,5′-NAs exhibit high resistance to nuclease digestion.[29–35]

2′,5′- and 3′,5′-NAs have been suggested to exhibit an interesting inverse relationship between nucleotide geometry and phosphodiester linkage (Fig. 2), that is, *S*-type 2′,5′-NAs adopt a compact backbone (intrastrand distance between two phosphorous atoms is below 6 Å) similar to that of *N*-type 3′,5′-NAs, whereas *N*-type 2′,5′-NAs adopt an extended backbone (P–P distance above 7 Å) similar to that of *S*-type 3′,5′-NAs.[36,37] The preference for complexation with RNA complements and prominent stabilization of triplexes with 2′,5′-NAs has therefore, and in the light of the properties of *N*-type nucleosides such as LNA, been suggested to arise from a tendency of 2′,5′-NAs to adopt compact *S*-type furanose conformations.[35]

	β-D-*ribo*	
	N-type	*S*-type
3′,5′-linked	Compact	Extended
2′,5′-linked	Extended	Compact

Figure 2. Nucleotide conformation of β-D-*ribo* configured nucleotides having *N*- and *S*-type sugar puckering, emphasizing the inverse relationship between intrastrand P–P distance (compact vs extended) and the phosphodiester linkage (3′,5′ vs 2′,5′).

The synthesis of bicyclic 2′,5′-linked nucleosides in which the furanose ring is conformationally biased towards a compact *S*-type conformation, has recently been reported.[38–41] Incorporation of 3′-*O*,4′-*C*-methyleribonucleotides (Fig. 1, herein termed ONA for 'oxetane nucleic acid') into ONs results in significantly decreased thermal affinities towards DNA complements while the affinity towards RNA complements is virtually unchanged compared to unmodified ONs.[38] The potential of ONA as TFOs has been evaluated in a single study but, arguably due to suboptimal sequence design, ONA was found to destabilize triplexes,[40] and a more thorough evaluation of ONA as TFOs is therefore awaited.

The inverse relationship of nucleotide geometry and phosphodiester linkage between 2′,5′- and 3′,5′-NAs (Fig. 2) suggests that the 2′,5′-linked ONA mimics the structure of LNA and that a 2′,5′-linked bicyclic nucleoside with α-L-*ribo* configuration such as monomer **X** (Fig. 1) would behave as a structural mimic of α-L-LNA, which is known to adopt an extended backbone.[19,20] The incorporation of a 2′,5′-linked nucleoside with inverted stereochemistry at the C2′-, C3′- and C4′-positions (relative to normal RNA) into ONs has not been reported and we therefore set out to synthesize monomer **X** and to evaluate this missing member of the bicyclic nucleoside family.

2. Results and discussion

Phosphoramidite **6** was identified as a suitable building block for incorporation of monomer **X** using automated DNA synthesis. Retrosynthetic analysis revealed bicyclic