ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 4007–4009

www.rsc.org/chemcomm

COMMUNICATION

Probing the furanose conformation in the 2′–5′strand of *iso*DNA : RNA duplexes by freezing the nucleoside conformations†

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Received 6th December 2010, Accepted 4th February 2011
DOI: 10.1039/c0cc05402j

Sugar conformations in the *iso*DNA strand of *iso*DNA : RNA duplexes are preferred S-type locked/frozen in contrast to N-type locked conformations preferred in DNA : RNA duplexes.

2′-5′-Phosphodiester-linked RNA occurs naturally (Fig. 1).[1] It could have been a primary substitute to the natural 3′,5′-linkage as it is the major product of many non-template, non-enzymatic oligomerizations of nucleotide monomers depending on conditions,[2] but it is not used to encode the genetic information. In 1991, Damha *et al*. synthesized the 2′,5′-linked RNA (*iso*RNA Fig. 1) that was found to exhibit self-pairing as well as pairing with RNA,[3] but its complexation with complementary DNA was not observed. The duplexes of *iso*RNA with complementary RNA (*iso*RNA : RNA) showed very similar CD patterns as the natural 3′,5′-DNA : RNA duplexes, from which it was deduced that the overall structures of these complexes would be comparable *i.e*., compact A-form duplex structure.[4] NMR studies in solution for self-pairing *iso*RNA duplexes suggested a similar structure in which the ribose sugars assumed C2′-*endo* (S- type) puckering for the *iso*RNA strands.[5] From the similarity found in the CD spectra of *iso*RNA : RNA and the self-pairing *iso*RNA duplexes, along with the NMR studies for the latter duplex, it was deduced that the 2′,5′-RNA strand in the *iso*RNA : RNA duplex might also assume the structural features of a natural DNA strand and the sugar residues would adopt S-type *i.e*., C2′-*endo* conformations. Molecular modelling studies reported by Yathindra *et al*.[5] also have suggested that to arrive at the compact A-type overall structure of the *iso*RNA : RNA duplex, the S-type or C2′-*endo* geometry of repeating nucleotides is stereochemically favoured in the *iso*RNA strand of the duplex. Very similar results were found independently by Breslow[6] and Switzer[7] for the 2′,5′-linked 3′-deoxyribonucleic acids (*iso*DNA) as well. CD spectroscopy,[7a] and modeling studies[8] predicted that the sugar conformations in the *iso*DNA strand of *iso*DNA : RNA duplexes would have predominantly S-type sugar conformations. Contrary to this, the NMR studies on single-stranded 2′,5′-RNA[9a] and 2′,5′-d(G_4C_4)[9b] implied an extended structure in which the sugar conformations were N-type. The C3′-*endo* or N-type sugar pucker has also been observed for single-stranded 2′,5′-RNA in crystal structures.[10] Thus, the structural requirements of the *iso*RNA/*iso*DNA : RNA duplexes (predominant S-type geometry of the 2′,5′-strand in *iso*RNA/*iso*DNA : RNA duplex) do not match with the sugar conformations at the single stranded 2′,5′-oligomer level. In the single-stranded 2′,5′-oligomer, the stereoelectronic (*gauche* and anomeric) effects of the 2′-hydroxy group, would accentuate the preference for 3′-*endo* (N-type) sugar conformations in the absence of the 3′-OH group. While modelling the *iso*DNA : RNA duplex, it was therefore assumed that the RNA strand would impose its structure[11] on the *iso*DNA strand, and the individual nucleosides in the isomeric DNA strand would be required to assume S-type *i.e*., C2′-*endo* conformation while binding to the complementary RNA strand to give rise to a stable duplex. Thus, it was suggested that the *iso*-linked DNA would be under conformational and topological constraints in the duplex.[8]

Considering these predictions, we hereby report the study of the effects of the incorporation of monomers which bear either locked or frozen S-type/N-type sugar conformations. Such studies are not previously reported for the *iso*DNA : RNA duplexes, although the literature is abundant with the locked or frozen DNA analogues in DNA : RNA duplexes that have

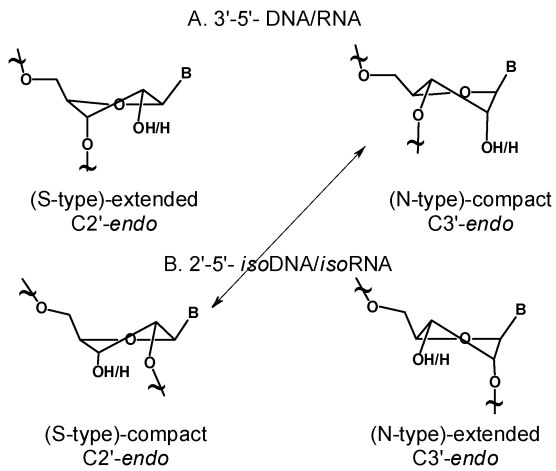

Fig. 1 3′-5′-DNA/RNA and 2′-5′-*iso*DNA/*iso*RNA in compact and extended conformations.

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† Electronic supplementary information (ESI) available: ^{1}H, ^{13}C, ^{31}P NMR, mass spectral data for compounds **II–IV**, HPLC profiles and mass spectra for the modified oligomers 2–9, and melting curves for the complexes with RNA. See DOI: 10.1039/c0cc05402j

an entropic advantage.[12,13] We envisaged that applying such structural locks on the *iso*DNA strand should allow us to understand the structural preferences of the sugars in the isomerically-linked strand of the chimeric duplexes and thereby provide experimental proof for the proposed structural preferences. The specific RNA binding of *iso*DNA, supplemented by favoured geometrical features for complex formation and enzymatic stability, would have potential applications in antisense therapeutics.

According to the proposed model,[11] the induced S-type conformations in the *iso*DNA strand obtained by either chemically locking or freezing the structure conducive for RNA binding should positively enhance the strength of binding to RNA and *vice-versa* for N-type conformations. Such experimental work is often important and necessary, as the predicted 2′/3′-*endo* conformational preferences of nucleosides using MD simulations sometimes do not agree with the experimental observations.[14]

We compared the sugar conformations of 3′-deoxy uridine, the S-type/N-type locked systems and also of the 3′-ribofluoro/xylofluoro nucleosides (Fig. 2, $R_1 = R_2 = H$, and ESI†). 3′-Deoxyuridine was shown to be in 97% N-type,[15] *i.e.*, C3′-*endo* sugar pucker which is consistent with the *O*4′-C1′-C2′-*O*2′ *gauche* effect as well as the anomeric effect which brings the nucleobase in pseudoaxial orientation.[10] The 3′-*O*-4′-*C*-methylene-linked uridine ($\mathbf{U^S}$)[15] (C2′-*endo* locked) and the xylo-3′-*O*-5′-*C*-methylene-linked uridine ($\mathbf{U^N}$)[16] (C3′-*endo* locked) would lock the monomer in S-type and N-type conformations, respectively (Fig. 2, **I** & **II**, $R_1 = R_2 = H$, and ESI†). In compound **II**, the flexible 5-membered ring probably allows some amount of S-type character. The monomeric conformations of 3′-deoxy-3′-ribofluoro uridine ($\mathbf{^rU^F}$)[17] **III**, and 3′-deoxy-3′-xylofluoro uridine ($\mathbf{^XU^F}$)[18] **IV**, were found to be almost frozen in S-type and N-type conformations, respectively (ESI†). The %S for each chosen unit was calculated from the H1′–H2′ NMR coupling constants as earlier reported (ESI†).[15] Compounds **I–Ib** were synthesized by using the reported procedure.[15] Transformation of **II–IV** ($R_1 = R_2 =$ H, Fig. 2) to the DMT derivatives **IIa–IVa** (R_1 = DMT, $R_2 =$ H, Fig. 2) and subsequent conversion to 2′-*O*-phosphoramidite derivatives, yielded the desired monomeric building blocks (Fig. 2, **IIb–IVb**) for incorporation into *iso*DNA oligomers. We chose to synthesize a biologically relevant sequence used for miRNA downregulation.[19] The 2′-5′-linked *iso*DNA sequence **DNA1** was synthesized using commercially available monomeric building blocks and standard phosphoramidite chemistry on solid supports using an automated Bioautomation DNA Synthesizer. Incorporation of the modified units (**Ib–IVb**) at the center and towards the 2′-end could be effectively achieved to get the modified oligomers. The oligomers (**DNA1–DNA9**) listed in Table 1 were cleaved from the solid support, purified by HPLC and characterized by MALDI-TOF mass spectrometry (ESI†).

The thermal stabilities (melting temperatures, T_ms) of *iso*DNA : DNA and *iso*DNA : RNA duplexes containing N-type locked, S-type locked, ribo-fluoro and xylo-fluoro modifications were determined and compared with the unmodified duplex to study their hybridization properties. The results of UV-T_m studies of complexes with complementary RNA are summarized in Table 1. The unmodified **DNA1** and modified 2′,5′-linked oligomers (**DNA2–DNA9**) were found to bind selectively only to complementary RNA, exhibiting sharp monophasic melting transitions, while no transitions were observed for the complexes with complementary DNA. The complexes formed with oligomers containing an increasing number of N-type locked monomer (**DNA4**, **DNA5**)

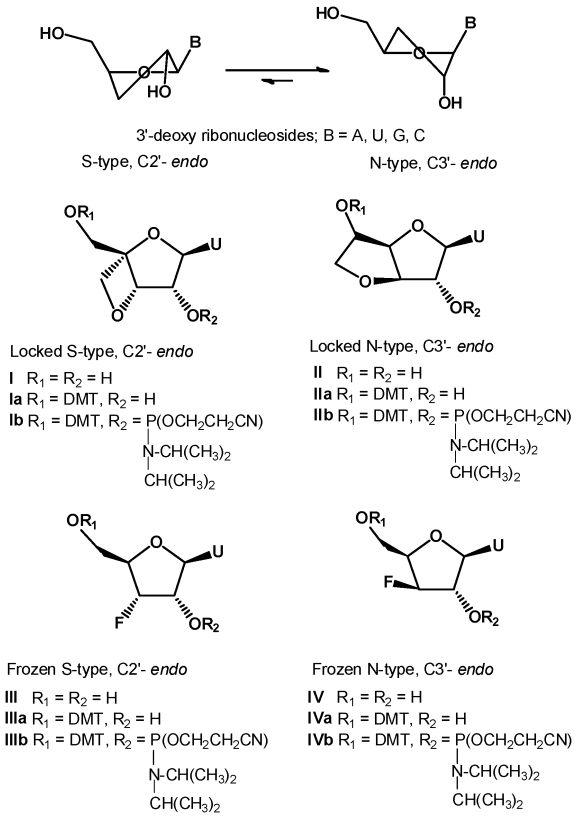

Fig. 2 3′-Deoxyribo nucleosides and proposed locked/frozen 3′-*endo* and 2′-*endo* nucleoside analogues (**I–IV**).

Table 1 UV-T_m (°C) values of *iso*DNA : RNA duplexes[a]

No	Sequences (5′ → 2′)	T_m °C	ΔT_m °C
DNA 1	DNA 1 CACCATTGTCACACTCCA	50.5	—
DNA 2	DNA 2 CACCATTGTCACAC$\mathbf{U^S}$CCA	51.0	+0.5
DNA 3	DNA 3 CACCATTG$\mathbf{U^S}$CACAC$\mathbf{U^S}$CCA	52.8	+2.3
DNA 4	DNA 4 CACCATTGTCACAC$\mathbf{U^N}$CCA	49.4	−1.1
DNA 5	DNA 5 CACCATTG$\mathbf{U^N}$CACAC$\mathbf{U^N}$CCA	42.8	−7.7
DNA 6	DNA 6 CACCATTGTCACAC$\mathbf{^XU^F}$CCA	45.7	−4.8
DNA 7	DNA 7 CACCATTG$\mathbf{^XU^F}$CACAC$\mathbf{^XU^F}$CCA	44.5	−6.0
DNA 8	DNA 8 CACCATTGTCACAC$\mathbf{^rU^F}$CCA	50.5	0.0
DNA 9	DNA 9 CACCATTG$\mathbf{^rU^F}$CACAC$\mathbf{^rU^F}$CCA	51.7	+1.2

[a] Melting temperatures (T_ms) were obtained from the maxima of the first derivatives of the melting curves (A_{260nm} *versus* temperature), measured in buffer containing 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4, using 1 μM concentration of each of the two complementary strands. Each experiment was repeated at least thrice and the values are accurate to ±0.5 °C. Complementary RNA sequence = 5′-UGGAGUGUGACAAUGGUG-3′. T_m of DNA 5′-CACCATTGTCACACTCCA-3′ with complementary RNA = 59.0 °C. S-locked uridine ($\mathbf{U^S}$), N-locked uridine ($\mathbf{U^N}$), 3′-ribo-3′-fluoro uridine ($\mathbf{^rU^F}$), 3′-xylo-3′-fluoro uridine ($\mathbf{^XU^F}$).

with complementary RNA were considerably destabilized compared to the control 2′,5′-sequence ($\Delta T_m \approx -1.1$ to -7.7 °C). In contrast, the oligomers containing the S-type locked monomers (**DNA2**, **DNA3**) effected modest stabilization of the complex with RNA ($\Delta T_m \approx +0.5$ to $+2.3$ °C). Oligomers bearing locked S-type units thus formed more stable complexes with RNA compared to the unmodified complex. Apparently, the S-type locked conformation at the modified site would be in compliance with the predicted S-type conformations of the *iso*DNA strand in the *iso*DNA : RNA duplex and therefore could stabilize the duplex. The imparted stability due to pre-organization in this geometry was not found to be as large as in the case of 3′,5′-LNA : RNA duplexes ($\Delta T_m \approx +4$ °C/mod).[13a] This could be because the S-type conformations would bring the nucleobases in pseudoequatorial position in which, the stacking and hydrogen bonding interactions are not as strong as in the N-type sugar geometry,[10] when the nucleobase assumes pseudoaxial orientation, as in LNA : RNA duplexes. The significant destabilization of the *iso*DNA : RNA complex by locking the sugar conformation in N-type geometry was also in compliance with the predicted geometry of the *iso*DNA : RNA duplex. The 3′-deoxy-3′-xylofluoro uridine, found to be in ≈ 100% N-type geometry, caused destabilization of the duplex (Table 1, $\Delta T_m \approx -4.8$ to -6.0 °C) but the oligomer with the 3′-deoxy-3′-ribofluoro uridine modifications in which the sugar geometry is S-type, showed similar melting behaviour as unmodified duplex ($\Delta T_m \approx +1.0$ °C). These results indicate that N-type to S-type conformational change that the 3′-deoxyuridine presumably undergoes while in duplex state is to some extent resisted by 3′-deoxy-3′-xylofluoro riboside due to the favourable *O*4′-C4′-C3′-F3′ *gauche* effect in N-type sugar geometry, considering comparable steric interactions between fluorine and hydrogen atoms[15] (Fig. 3). For the 3′-deoxy-3′-ribofluoro derivative the preferred sugar pucker would be S-type again due to dominating *O*4′-C4′-C3′-F3′ *gauche* effect. The frozen conformation could not increase the stability of the duplexes when present in the oligomer probably due to its inability to further strengthen the stacking and hydrogen bonding interactions in S-type geometry of the sugar when the base orientation becomes pseudoequatorial.

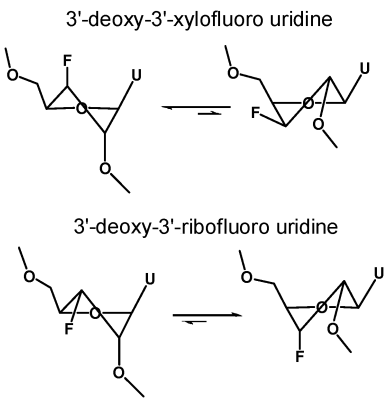

Fig. 3 *O*4′-C4′-C3′-F3′ *gauche* effect in 3′-deoxy-3′-xylofluoro and 3′-deoxy-3′-ribofluoro sugars.

Stabilization of *iso*DNA : RNA duplexes by S-locked/frozen monomer units and destabilization of the same by N-locked/frozen monomers provides a proof, for the first time, for the prediction that in stable *iso*DNA : RNA duplexes, the DNA strand would prefer to assume S-type geometry. As the oligomers bind only to the RNA targets and chemical modifications are known to make them compatible with biological environments,[15] this work opens up an entirely new paradigm of oligonucleotides for development as antisense therapeutics.

This work was supported by CSIR, New Delhi (NWP0036) and in part by Wellcome Trust, UK. NE got her fellowship from UGC, New Delhi.

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