A novel class of conformationally restricted oligonucleotide analogues: synthesis of 2′,3′-bridged monomers and RNA-selective hybridisation

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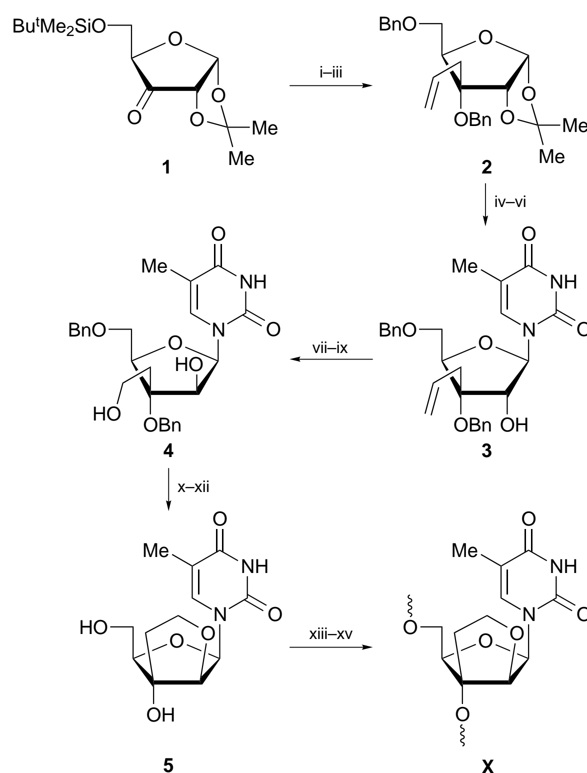
A novel 2′,3′-bicyclic nucleoside 5 has been synthesised and incorporated into oligonucleotide analogues resulting in strong and selective binding to an RNA complement.

Research towards modified oligonucleotides able to hybridise with ssRNA (antisense or ribozyme approach) as a novel class of therapeutic agents currently attracts much attention.[1] The potential of enhancing duplex stability using conformationally restricted oligonucleotide analogues has been investigated, as recently described in a review by Herdewijn.[2] Bicyclo nucleosides with an additional 3′,5′-ethylene bridge,[3] bicyclic carbocyclic nucleosides with a 1′,6′- or 4′,6′-methano bridge,[4] 2′,3′-riboacetal dimers,[5] 3′,6′-anhydrogluco-2′,5′-formacetal trimers[6] and conformationally restricted 1,5-anhydro-2,3-dideoxy-d-*arabino*-hexitol nucleosides[7] have been synthesised and incorporated into oligodeoxynucleotides in order to obtain an entropic advantage through the rigidity of the structures, thereby increasing duplex stability. Here we report on the synthesis and the promising melting results for 2′,3′-bridged bicyclic pentofuranose oligonucleotides. This work was stimulated by molecular modelling studies indicating the possibility of a preferential 3′-*endo* conformation of the monomers as required for RNA-selective binding.

The bicyclic nucleoside **5** was synthesised in twelve steps from the known ulose **1**[8] in an overall yield of 10% using a strategy (see Scheme 1) where the additional alkyl chain was introduced before, and the cyclisation accomplished after, the nucleoside coupling step.‡ Whereas Grignard additions on 3′-keto nucleosides afforded *xylo*-configured isomers as major products,[9] the presence of the 1,2-*O*-isopropylidene group in ulose **1** directs the addition of Grignard reagents to proceed from the β-face of the furanose. Thus, the first step was a stereoselective Grignard addition of an allyl group at the 3-*C*-position generating, after desilylation and concomitant benzylation, the furanoside **2** with the preferred *ribo* configuration in 59% yield. An allyl group was preferred to a vinyl group as this Grignard addition was more successful in our hands. The required inversion of the configuration at the 2-carbon could most conveniently be performed, using an anhydro-approach,[10] after the nucleoside coupling step. Using standard methods, furanose **2** was deprotected and acetylated, whereafter the 2′-*O*-unprotected nucleoside **3** was obtained in 79% yield (from **2**) using the silyl Hilbert–Johnson/Birkofer method as modified by Vorbrüggen and co-workers[11] followed by deacetylation. Mesylation and treatment with aqueous base gave a 2-*O*,2′-*O*-anhydro intermediate which was directly hydrolysed affording an *arabino*-configured nucleoside. The double bond was oxidatively cleaved and 3′-*C*-hydroxyethyl nucleoside **4** was obtained in 32% yield (from **3**) after reduction. Selective tosylation of the primary hydroxy group, cyclisation using strong base and debenzylation afforded the nucleoside **5** in 68% yield (from **4**).§ The new bicyclic nucleoside **5** was incorporated into oligonucleotides as monomer **X** (see Scheme 1) after 4,4′-dimethoxytrityl(DMT)-protection of the 5′-hydroxy group and conversion to the corresponding phosphoramidite building block.

Six modified oligonucleotides were synthesised[12] and evaluated towards both complementary single-stranded DNA and RNA.¶ As seen from Table 1, incorporation of one to four modified bicyclic nucleotides **X** into a 14-mer destabilises duplexes with the DNA complement dA_{14} by 2–3 °C per modification, irrespective of **X** being incorporated in a row or alternating with unmodified nucleotides. Analogously, a lowering of the melting temperature by approximately 3 °C per modification was observed for duplexes with the RNA complement rA_{14} when incorporating monomer **X** once or several times alternating with unmodified nucleotides.

More encouraging results were obtained towards the RNA complement when incorporating the bicyclic nucleoside **X** in a row of four (5′-$T_5X_4T_5$-3′), as a minor increase in melting temperature compared to the unmodified control was observed. Following this trend, an increase in the melting temperature of



Scheme 1 *Reagents and conditions*: i, allylMgBr, Et_2O, THF; ii, Bu_4NF, THF, 69% for two steps; iii, BnBr, NaH, DMF, 86%; iv, 80% AcOH, then Ac_2O, pyridine, 97%; v, thymine, *N*,*O*-bis(trimethylsilyl)acetamide, MeCN, $Me_3SiOSO_2CF_3$, 86%; vi, MeONa, MeOH, 95%; vii, $MeSO_2Cl$, pyridine, 89%; viii, NaOH, EtOH, H_2O, 74%; ix, $NaIO_4$, cat. OsO_4, THF, H_2O, then $NaBH_4$, THF, H_2O, 49%; x, *p*-$MeC_6H_4SO_2Cl$, pyridine; xi, NaH, DMF, 83% for two steps; xii, H_2, $Pd(OH)_2$–C, EtOH, 82%; xiii, DMTCl, pyridine, 97%; xiv, $NC(CH_2)_2OP(Cl)N(Pr^i)_2$, $EtN(Pr^i)_2$, CH_2Cl_2, 88%; xv, DNA synthesiser

12.5 °C was observed for the duplex between the (almost) fully modified oligonucleotide 5′-$\mathbf{X}_{13}$T-3′ and complementary RNA. These results indicate that these new 2′,3′-bicyclo oligonucleotides ('2′,3′-BcNAs') form a duplex structure with complementary RNA which is thermally significantly more stable than the corresponding unmodified DNA–RNA duplex. The structure of the duplex 2′,3′-BcNA–RNA is probably different from that of wild type DNA–RNA as indicated by the destabilising effect of one or two bicyclic nucleosides in an otherwise unmodified DNA strand. The shapes of the circular dichroism spectra, known to be diagnostic of a particular duplex structure, recorded for $T_{14}:rA_{14}$ and $\mathbf{X}_{13}T:rA_{14}$ are, however, similar. It is thus clear that conclusions about the structural basis for the observed increase in thermal stability must await results from other sequences and from extensive molecular modelling and NMR studies.

Degradation by nucleases signifies a serious limitation to *in vivo* application of both natural and many modified oligonucleotides. We have therefore evaluated the novel 2′,3′-BcNA ($\mathbf{X}_{13}$T) against snake venom phosphodiesterase (a 3′-exonuclease) using a method described earlier.[13] Whereas the unmodified control (T_{14}) is fully degraded within 10 min, $\mathbf{X}_{13}$T (or $\mathbf{X}_{13}$) remains intact for the 60 min monitored. Thus, although other nucleases need to be evaluated, this result suggests that 2′,3′-BcNAs may be effectively protected against nucleolytic degradation.

In conclusion, a new bicyclic thymidine analogue **5** has been synthesised and used as a monomer to construct a novel class of conformationally restricted 2′,3′-bicyclo oligonucleotides ('2′,3′-BcNAs'). Melting experiments revealed excellent thermal stability of a 2′,3′-BcNA–RNA duplex, which, together with the observed stability towards 3′-*exo* nucleolytic degradation, suggests 2′,3′-BcNAs to be interesting candidates as antisense molecules.

Table 1 Melting experiments of modified oligonucleotides[a]

Oligonucleotide	Complimentary ssDNA (dA_{14}) T_m/°C	ΔT_m/°C	Complimentary ssRNA (rA_{14}) T_m/°C	ΔT_m/°C
5′-T_{14}-3′	34.5		29.5	
5′-$T_7\mathbf{X}T_6$-3′	32.0	−2.5	26.5	−3.0
5′-$T_6\mathbf{X}_2T_6$-3′	30.0	−4.5	28.0	−1.5
5′-$T_6\mathbf{X}T\mathbf{X}T_5$-3′	29.5	−5.0	23.0	−6.5
5′-$T_5\mathbf{X}_4T_5$-3′	23.0	−11.5	30.5	+1.0
5′-$T_3(T\mathbf{X})_4T_3$-3′	23.0	−11.5	16.5	−13.0
5′-$\mathbf{X}_{13}T$-3′	< 10	< −24	42.0	+12.5

[a] Measured at 260 nm in medium salt buffer: 1 mm EDTA, 10 mm Na_3PO_4, 140 mm NaCl, pH 7.2; concentration of each strand: 1.0 μm; T = thymidine monomer; dA = 2′-deoxyadenosine monomer; rA = adenosine monomer; **X** = monomer derived from nucleoside **5**; T_m = melting temperature determined as the local maximum of the first derivative of the absorbance *vs*. temperature curve; ΔT_m = change in T_m compared to unmodified controls.

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Footnotes

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‡ Full characterisation of all intermediates will be published as part of a forthcoming publication.

§ The structure of nucleoside **5** was verified from MS, ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY and NOE experiments. *Selected data* for **5**: ^{1}H NMR (250 MHz; [$^2H_6$]DMSO): δ 11.3 (1 H, br s, NH), 7.36 (1 H, d, *J* 1.1, 6-H), 5.80 (1 H, d, *J* 4.3, 1′-H), 5.61 (1 H, s, OH), 4.86 (1 H, m, 5′-Ha), 3.89 (1 H, d, *J* 4.2, 2′-H), 3.85 (1 H, m, 2″-H), 3.83–3.64 (3 H, m, 4′-H, 5′-Hb, 2″-H), 2.14 (1 H, m, 1″-H), 1.81 (1 H, m, 1″-H), 1.78 (3 H, d, *J* 1.0, Me); ^{13}C NMR (62.9 MHz; CD_3OD): δ 166.7 (C-4), 152.2 (C-2), 139.7 (C-6), 110.1 (C-5), 89.4, 89.1, 85.5, 85.2 (C-1′, C-2′, C-3′, C-4′), 71.4 (C-2″), 61.6 (C-5′), 37.0 (C-1″), 12.7 (Me) (Found: C, 47.4; H, 5.7; N, 9.0; $C_{12}H_{16}N_2O_6{\cdot}H_2O$ requires C, 47.7; H, 6.0; N, 9.3%). Mutual NOE contacts between H-1′ and H-2′ and between H-1′ and H-4′, as well as the lack of contacts between H-5′ and H-1′ or H-2′ and between H-2′ and H-6, confirm the assigned β-*arabino* configuration.

¶ The oligonucleotides were synthesised on a Pharmacia Gene Assembler in 0.2 μmol scale using commercial 2-cyanoethyl phosphoramidites (2 min coupling time, *ca*. 99% stepwise yield) and the amidite derived from nucleoside **5** (2 × 12 min coupling time, *ca*. 95% stepwise yield). Purification of 5′-*O*-DMT-ON oligonucleotides was accomplished using disposable reversed-phase chromatography cartridges (Cruachem Inc.), which includes 5′-*O*-detritylation. The composition of the oligonucleotides were verified by MALDI-MS and the purity by capillary gel electrophoresis. Minor peaks (< 5%) originating from $\mathbf{X}_{10}$T, $\mathbf{X}_{11}$T and $\mathbf{X}_{12}$T were detected when running $\mathbf{X}_{13}$T.

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