LNA (locked nucleic acid) and analogs as triplex-forming oligonucleotides

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The triplex-forming abilities of some conformationally restricted nucleotide analogs are disclosed and compared herein. 2′-Amino-LNA monomers proved to be less stabilising to triplexes than LNA monomers when incorporated into a triplex-forming third strand. N2′-functionalisation of 2′-amino-LNA monomers with a glycyl unit induced the formation of exceptionally stable triplexes. Nucleotide analogs containing a C2′,C3′-oxymethylene linker (*E*-type furanose conformation) or a C2′,C4′-propylene linker (*N*-type furanose conformation) had no significant effect on triplex stability proving that conformational restriction *per se* is insufficient to stabilise triplexes.

Introduction

Antigene technology offers the potential of modulating gene expression by molecular interactions at the level of DNA. To achieve this, double stranded DNA (dsDNA) must be sequence-specifically targeted, and one approach is to use triplex-forming oligonucleotides (TFOs).[1] A TFO is an oligonucleotide (ON) that binds to dsDNA in the major groove of the duplex thereby forming a triplex. Polypyrimidine TFOs are the subject of this investigation and they are able to bind to a stretch of polypurine dsDNA in a parallel orientation forming Hoogsteen hydrogen bonds. The requirement of a polypurine target sequence limits the number of available TFO target sites. Furthermore, DNA cytosine monomers must be protonated in order to efficiently recognise guanine nucleotides within dsDNA which makes the formation and stability of these triplexes highly dependent on an acidic medium (*e.g.* pH <6). The main challenge of this research area is therefore to develop TFOs that have sufficiently high affinity to dsDNA at physiological conditions.

Many oligonucleotide modifications have been tested for their ability to form stable triplexes with dsDNA.[1] One promising approach is known as the dual recognition strategy. In this approach, the nucleobase or the sugar part of one or more nucleotide monomers is linked to a unit that contains one or more amines that are sufficiently basic to be at least partly protonated under physiological conditions.[1a,2,3] These protonated amines can potentially make favourable electrostatic interactions with *e.g.* the negatively charged oxygens of the phosphate backbone of the dsDNA target duplex. Particularly noteworthy are studies involving 2′-*O*-(2-aminoethyl)-RNA modified ONs.[3] A TFO containing four incorporations of a 2′-*O*-(2-aminoethyl)-RNA monomer furnished a triplex that was more thermally stable than the unmodified reference by 3.5 °C per modification. Distance dependence was observed in that the equivalent TFO with four incorporations of a 2′-*O*-(3-aminopropyl)-RNA monomer showed only 2.1 °C stabilisation per modification.[3a]

Another very successful approach has been to conformationally restrict the sugar part of one or more of the nucleotides of a TFO into a C3′-*endo* (*N*-type) conformation,[4,5] with LNA (locked nucleic acid)[6] monomers being a prime example (Fig. 1, monomer **B**). LNA monomers have previously been shown to significantly enhance triplex stabilities,[7] although TFOs composed entirely of LNA monomers do not form triplexes.[7b] O2′,O4′-ethylene-linked nucleic acid (ENA) induces slightly lower thermal stabilities of triplexes than LNA, but fully modified ENA-TFOs are able to form stable triplexes even at pH 7.2.[4] This deviating behavior of LNA and ENA can be explained by their different sugar puckering amplitudes.[8]

The amino analog of LNA, 2′-amino-LNA (Fig. 1, monomer **D**), has been shown to stabilise duplexes to the same extent as LNA.[9] However, the triplex-forming ability of TFOs containing 2′-amino-LNA monomers has so far not been studied. One advantage of 2′-amino-LNA monomers could arise as a consequence of protonation of the N2′-atom. However, as a pK_a value for the corresponding acid of 6.17 has been reported,[10] this effect is unlikely to be significant at physiological conditions. However, in addition to effects related to the conformationally locked furanose moiety of a 2′-amino-LNA monomer, its secondary amino group can function as a chemical handle onto which various molecular units can be attached.[11,12] Thus, N2′-functionalisation could introduce a unit containing another amino group which, in case of its protonation under physiological conditions, would combine the effects of conformational restriction and a dual recognition strategy. Therefore we included the N2′-glycyl 2′-amino-LNA monomer (Fig. 1, monomer **F**) in this study. In addition, we wanted to investigate what the effect would be of having other amino groups in various distances from the 2′-amino-LNA scaffold. Thus, monomers **H**, **I** and **J** (Fig. 1) were examined for triplex-forming ability.

Monomers containing large planar aromatic groups have been reported to stabilise a triplex by intercalation.[13] As pyrene is known to be an efficient intercalator of dsDNA, we conjugated pyrene to

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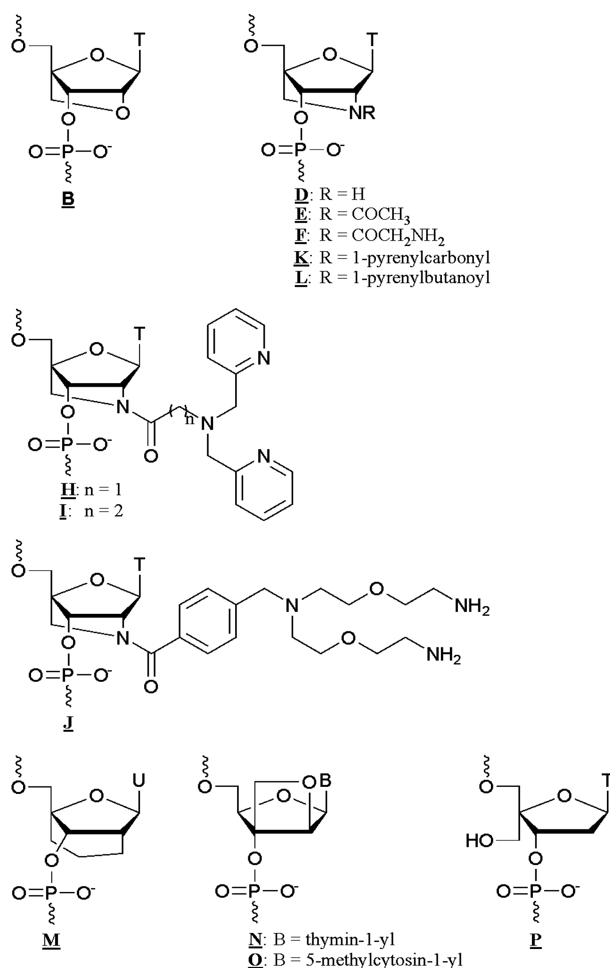

Fig. 1 Structures of the modifications tested.

the N2′-atom of a 2′-amino-LNA monomer (Fig. 1, monomers **K** and **L**) and studied the effect of these monomers on triplex stability.

Furthermore, we wanted to investigate the effects of other conformationally restricted nucleotide monomers as constituents of TFOs. To study the effect of the O2′-atom in LNA and ENA, we included the C2′,C4′-propylene-linked monomer **M** (Fig. 1). This monomer is like LNA and ENA locked in an *N*-type furanose conformation. In studies on duplex stabilities, this monomer was found to destabilise duplexes with DNA complements but to strongly stabilise duplexes towards RNA complements.[14] Conformational restriction into an O4′-*endo* (*E*-type) furanose conformation was studied by inclusion of the 2′,3′-bicyclo arabino nucleic acid monomers **N** and **O** (Fig. 1).[15] We judged these monomers to be of interest as the nucleotide monomers of some TFOs of completely unmodified triplexes have been found to adopt southeast-type furanose conformations.[1c] For comparative purposes we also included a flexible non conformationally restricted C4′-hydroxymethyl-DNA monomer in this study (Fig. 1, monomer **P**). DNA strands containing this monomer have shown binding towards single stranded DNA and RNA of similar stability as unmodified DNA,[16] and the additional hydroxy group of this monomer could induce a different hydration pattern of a triplex.

Results and discussion

The modifications tested are shown in Fig. 1. The dsDNA target for the TFOs contains a 16 nucleotide-long stretch of purines known as the HIV-1 polypurine tract (Fig. 2).[17] TFOs having the modified monomers in different positions were synthesised using the phosphoramidite approach (Table 1) on an automated DNA synthesizer (see Experimental for details). The synthesis of the phosphoramidites of monomers **B**,[6] **D**,[9] **I**,[18] **M**,[14] **N**[15a,15c]§ and **P**[16] have been published in the literature. Synthesis of the phosphoramidites of monomers **E**,[19] **F**,[19] **H**,[19] **J**,[20] **K**[19] and **L**[19] will be published elsewhere.

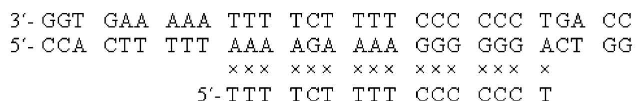

Fig. 2 Sequence of the target duplex and the unmodified TFO reference. "x" denotes Hoogsteen hydrogen bonds.

Table 1 shows the thermal stabilities (melting temperatures; T_m values) recorded for the triplex-to-duplex transitions in buffers adjusted to pH 6.0 and pH 7.0. The difference in melting temperature per modified monomer relative to the unmodified DNA **TFO-1** is also shown. The unmodified triplex melts at 26.5 °C at pH 6.0 whereas no triplex-to-duplex transition was observed above 5 °C in the pH 7.0 buffer. The target DNA duplex melts at 69 °C.

As reported earlier,[7] LNA-modified TFOs (**TFO-2** and **TFO-3**) show increased thermal stabilities relative to **TFO-1** with ΔT_m/modification of 10.0 and 7.0 °C at pH 6.0 for one and three modifications, respectively. Also at pH 7.0, triplex-to-duplex transitions were observed. However, although triplexes containing LNA monomers in the third strands are stable, the stabilities at neutral pH still need to be improved. To gain further insight into the properties of LNA TFOs we investigated the effect of the parent 2′-amino-LNA modification (monomer **D**). As seen in Table 1, **TFO-4** and **TFO-5** containing 2′-amino-LNA monomer **D** produce triplexes that are somewhat less stable than their LNA counterparts. The fact that TFOs containing LNA or 2′-amino-LNA monomers display more stable triplexes than unmodified TFOs is most certainly a result of their locked *N*-type furanose conformation. At pH 6.0, but not pH 7.0, the N2′-atom of 2′-amino-LNA is likely to be protonated assuming that the pK_a value determined for the monomer[10] is valid also in a TFO, but this protonation seems not to positively influence triplex stability. This suggests that geometrical constraints prevent the formation of stabilising ion-pair like interactions between the protonated N2′-atom and, *e.g.*, phosphate groups on the dsDNA scaffold, and that the cations present in the buffer limit the effect of a lesser overall negative charge of the TFO.

The triplex-forming abilities of TFOs containing N2′-functionalised 2′-amino-LNA monomers were investigated. The 2′-*N*-acetyl 2′-amino-LNA monomer **E** (**TFO-6** and **TFO-7**) induced increased triplex stability in all cases tested and furnished results similar to the LNA-modified **TFO-2** and **TFO-3**.

§ Monomer **O** was introduced using the 4-(triazol-1-yl) phosphoramidite prepared from the thymine phosphoramidite[15a,c] using a literature method.[23]

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Table 1 Melting temperatures of triplexes containing modified monomers

Modification	TFO sequence		pH 6.0		pH 7.0
			T_m[a]	ΔTm/mod[b]	T_m[a]
None	TTT TCT TTT CCC CCC T	**TFO-1**	26.5	—	n.t.[c]
LNA	TTT TCT TTB CCC CCC T	**TFO-2**	36.5	10.0	9.5
LNA	TTB TCB TTB CCC CCC T	**TFO-3**	47.5	7.0	23.0
2′-Amino-LNA	TTT TCT TTD CCC CCC T	**TFO-4**	35.5	9.0	n.t.[c]
2′-Amino-LNA	TTD TCD TTD CCC CCC T	**TFO-5**	45.5	6.3	16.0
N2′-Acetyl-2′-amino-LNA	TTT TCT TTE CCC CCC T	**TFO-6**	36.5	10.0	11.5
N2′-Acetyl-2′-amino-LNA	TTE TCE TTE CCC CCC T	**TFO-7**	45.5	6.3	24.0
N2′-Glycyl-2′-amino-LNA	TTT TCT TTF CCC CCC T	**TFO-8**	38.0	11.5	13.0
N2′-Glycyl-2′-amino-LNA	TTF TCF TTF CCC CCC T	**TFO-9**	51.5	8.3	28.0
N2′-Bis(pyridylmethyl)glycyl-2′-amino-LNA	TTT TCT TTH CCC CCC T	**TFO-10**	40.5	14.0	n.t.[c]
N2′-Bis(pyridylmethyl)alanyl-2′-amino-LNA	TTT TCT TTI CCC CCC T	**TFO-11**	37.5	11.0	n.t.[c]
N2′-Bis[(aminoethoxy)ethyl]amino-2′-amino-LNA	TTT TCT TTJ CCC CCC T	**TFO-12**	34.0	7.5	n.t.[c]
N2′-Bis[(aminoethoxy)ethyl]amino-2′-amino-LNA	TTJ TCJ TTJ CCC CCC T	**TFO-13**	43.5	5.7	n.t.[c]
N2′-Pyrenylcarbonyl-2′-amino-LNA	TTT TCT TTK CCC CCC T	**TFO-14**	29.0	2.5	n.t.[c]
N2′-Pyrenylcarbonyl-2′-amino-LNA	TTK TCK TTK CCC CCC T	**TFO-15**	n.t.[c]	<−7.2	n.t.[c]
N2′-Pyrenylbutanoyl-2′-amino-LNA	TTT TCT TTL CCC CCC T	**TFO-16**	25.5	−1.0	n.t.[c]
N2′-Pyrenylbutanoyl-2′-amino-LNA	TTL TCL TTL CCC CCC T	**TFO-17**	n.t.[c]	<−7.2	n.t.[c]
2′,4′-Propylene-"LNA"	TTT TCM TTT CCC CCC T	**TFO-18**	26.0	−0.5	n.d.[d]
2′,4′-Propylene-"LNA"	TTM TCM TTM CCC CCC T	**TFO-19**	30.5	1.3	n.d.[d]
2′,4′-Propylene-"LNA"	TTT TCM MMM CCC CCC T	**TFO-20**	29.0	0.6	n.d.[d]
2′,3′-Oxymethylene-arabino	TTT TCN TTT CCC CCC T	**TFO-21**	25.5	−1.0	n.d.[d]
2′,3′-Oxymethylene-arabino	TTN TCN TTN CCC CCC T	**TFO-22**	25.5	−0.3	n.d.[d]
2′,3′-Oxymethylene-arabino	NTT NCT NTT OCC OCC T	**TFO-23**	27.5	0.2	n.d.[d]
2′,3′-Oxymethylene-arabino	NTN TOT NTN COC OCO T	**TFO-24**	24.5	−0.3	n.d.[d]
2′,3′-Oxymethylene-arabino	NNN NON NNN OOO OOO T	**TFO-25**	29.0	0.2	n.d.[d]
C4′-Hydroxymethyl-DNA	TTT TCP TTT CCC CCC T	**TFO-26**	27.5	1.0	n.t.[c]
C4′-Hydroxymethyl-DNA	TTP TCP TTP CCC CCC T	**TFO-27**	27.5	0.3	n.t.[c]
C4′-Hydroxymethyl-DNA	TTT TCT PPP CCC CCC T	**TFO-28**	28.0	0.5	n.t.[c]

[a] Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} *vs.* temperature) in a buffer containing 10 mM sodium cacodylate, 150 mM NaCl and 10 mM $MgCl_2$. The concentration of the duplex was 1 µM and the concentration of the TFO was 1.5 µM. [b] Difference in melting temperature per modification compared with the reference **TFO1**. [c] n.t. = no transition observed above 5 °C. [d] n.d. = not determined.

Interestingly, stability is gained in comparison to TFOs containing the unfunctionalised 2′-amino-LNA monomer **D**. With respect to duplex-forming capability we have observed that N2′-acyl functionalised 2′-amino-LNAs display higher thermal stabilities than N2′-alkyl functionalised 2′-amino-LNAs.[11] It is possible that the N2′-carbonyl structural unit allows more favourable hydration which could lead to duplex and triplex stabilisation.

TFO-8 and **TFO-9** containing N2′-glycyl functionalised 2′-amino-LNA monomer **F** formed very stable triplexes displaying T_m values even higher than those induced by LNA monomer **B**. As the distal amino group of monomer **F** is expected to be protonated both at pH 6.0 and pH 7.0, this points to the possibility of triplex-stabilising electrostatic interactions similar to those reported for TFOs containing 2′-*O*-(2-aminoethyl)-RNA monomers.[3] Thus, the most stable triplexes at pH 7.0 of this study were observed with three incorporations of monomer **F** giving triplexes that were at least 23 °C more stable than the unmodified reference at pH 7.0.

Previous studies of a TFO almost fully modified with 2′-*O*-(2-aminoethyl)-RNA monomers suggested its triplex stabilising effect to be caused by an increased association rate constant.[3a] The stabilising effect of incorporating the conformationally restricted LNA monomer into a TFO has been shown to arise from a significantly decreased dissociation rate constant at pH 6.8.[7c] It is possible that the exceptional stability of triplexes containing monomer **F** is the result of combining conformational and electrostatic effects leading potentially to both increased association rates and decreased dissociation rates.

Investigation of protonated aromatic amines as potential triplex stabilisers led us to include monomers **H** (**TFO-10**) and **I**[18] (**TFO-11**) that contain N2′-linked bis(2-pyridylmethyl)amino moieties. Both these monomers induced the formation of very stable triplexes at pH 6.0, and **TFO-10** showed the highest increase in T_m value per modification of the entire study. Increasing the linker length by one methylene group (from a glycine to a β-alanine derived linker) resulted in a slightly less stable triplex at pH 6.0 (**TFO-11**). This underlines the importance of linker length for optimally positioning a protonated amine for favourable electrostatic interactions. Extension of the linker by one methylene group was observed to reduce triplex stability in the corresponding, but not similar, 2′-*O*-(2-aminoethyl)-RNA system (extension to 2′-*O*-(2-aminopropyl)-RNA).[3a] Although exceptionally stable triplexes were formed at pH 6.0 no triplex-to-duplex transition was observed above 5 °C at pH 7.0 for the TFOs containing monomers **H** and **I**. The bis(2-pyridylmethyl)amino moieties of these monomers are known to chelate metal ions and we have reported strong effects on duplex stabilities of these monomers upon addition of different divalent metal ions.[18] We cannot rule out that metal chelation plays a role for the triplex results reported herein. Unfortunately it was not possible to obtain TFOs with three incorporations of monomers **H** or **I**.

To evaluate if several protonated amino functionalities in one monomer could give even more stable triplexes, we incorporated monomer **J** into **TFO-12** and **TFO-13**. Triplexes with these TFOs displayed lower T_m values than the corresponding TFOs

containing the parent 2′-amino-LNA monomer **D** at pH 6.0. At pH 7.0, no triplex transition was observed demonstrating that protonated amino functionalities have to be linked appropriately to a TFO in order to induce a stabilising effect.

Conjugation of pyrene to 2′-amino-LNA using a short carbonyl linker[11] (monomer **K**) or a flexible butanoyl linker (monomer **L**) allowed us to attempt to combine intercalation and conformational restriction. **TFO-14** and **TFO-16** having one incorporation of monomers **K** and **L**, respectively, formed triplexes of only approximately the same stability as the unmodified reference **TFO-1** at pH 6.0. No triplex-to-duplex transition was observed for **TFO-15** and **TFO-17**, each containing three modifications. This shows that the pyrene units, possibly for steric reasons, have a detrimental effect on triplex stability and that they are not intercalating into the dsDNA target duplex.

The triplexes containing the monomer **M** (**TFO-18**–**TFO-20**) were of comparable stability as that formed by the unmodified reference **TFO-1**. The C2′,C4′-propylene linkage of monomer **M** restricts the sugar to an *N*-type furanose conformation.[14] Based on this, it could be expected that this monomer could give rise to very stable triplexes as do LNA,[7] 2′-amino-LNA and ENA[4] monomers. Therefore, the absence of the O2′-atom in monomer **M** is the likely reason why this LNA-type monomer does not produce very stable triplexes. It should be noted that the fact that monomer **M** contains a uracil and not a thymine nucleobase cannot explain the lack of triplex-stabilising effect. These results, together with those obtained for monomer **D**, reveal that the presence of an O2′-atom is important for triplex stability, likely by promoting favourable triplex hydration.

Monomers **N** and **O** are *arabino*-configured nucleotides having a C2′,C3′-oxymethylene linker. These have previously by NMR been shown to adopt an *E*-type conformation.[15b] As nucleotides with this restricted conformation have not yet been investigated within TFOs, both the thymine and 5-methylcytosine derivatives were included in order to study also a fully modified sequence. Monomers **N** and **O** were incorporated into TFOs in different numbers (**TFO-21**–**TFO-25**). All the corresponding triplexes displayed thermal stabilities similar to that of the unmodified triplex. Hence, the C2′,C3′-oxymethylene linker has no effect on triplex stability at pH 6.0. The exchange of the nucleobase cytosine for 5-methylcytosine is expected to give a small stabilising effect[21] which indicates that the effect of restricting the furanose ring in an *E*-type conformation might even be slightly destabilising. It is noteworthy that the triplex formed by the fully modified **TFO-25**¶ was not destabilised relative to the one formed by the unmodified reference **TFO-1**. Thus, a TFO composed entirely of nucleotides restricted into an *E*-type furanose conformation can form a stable triplex.

Finally, we determined that the C4′-hydroxymethyl modification[16] (monomer **P**) is neutral or slightly beneficial with respect to triplex stability (**TFO-26**–**TFO-28**). This modification can be considered as a monocyclic variant of LNA monomers by having, relative to DNA monomers, an additional carbon–oxygen unit for interaction with water molecules. Our results indicate that restriction of monomers into an *N*-type furanose conformation is more important than presence of additional oxygen atoms, *i.e.*, hydration *per se*, for increased triplex stability.

¶ For synthetic reasons, the nucleotide at the 3′-end was unmodified.

Conclusions

This comparative study has underlined that restriction of the furanose ring of nucleotide monomers of a triplex-forming oligonucleotide (TFO) into an *N*-type conformation is a preferred approach to stabilise triplexes. The importance of a 2′-heteroatom of these conformationally restricted monomers has been demonstrated, but also that an -O-CH_2- (LNA) or an -N(COR)-CH_2-(N2′-acylated 2′-amino-LNA) linkage between the C2′ and C4′ carbon atoms is more stabilising than an -NH-CH_2- linkage. Among the acyl groups attached to the N2′-atom of 2′-amino-LNA monomers, a glycyl group induces very strong stabilisation. We have, in addition, shown that the incorporation of *arabino*-configured monomers restricted into an *E*-type furanose conformation into a TFO has no effect on triplex stability. The continued search for triplex-stabilising nucleotide modifications is important to realise the promises of the antigene approach for gene silencing. With the results reported herein, important advances have been achieved, and further structural and biophysical studies are ongoing to further improve design and performance of triplex-forming oligonucleotides.

Experimental

Synthesis of oligomers

Unmodified oligodeoxynucleotides (dsDNA target and **TFO-1**) were purchased from TAG Copenhagen A/S. Modified ONs were obtained by automated DNA synthesis using an Applied Biosystems Expedite 8909 Nucleic Acid Synthesis System. Syntheses were carried out on a 0.2 μmol synthesis scale. Modified monomers (see Fig. 1) were incorporated into oligodeoxynucleotides using the corresponding phosphoramidites§ with tetrazole as an activator to give **TFO-2**–**TFO-28**. The amino functionalities of the N2′-linked units of the phosphoramidite derivatives of monomers **D**, **F** and **J**, were protected as trifluoroacetyl amides and the free hydroxy group of the phosphoramidite of monomer **P** as its benzoyl ester. The following step-wise coupling times were used (coupling yield): monomer **B**: 15 minutes (99%); monomer **D**: 15 minutes (99%); monomer **E**: 10 minutes (88%); monomer **F**: 15 minutes (98%); monomer **H**: 25 minutes (98%); monomer **I**: 25 minutes (98%); monomer **J**: 20 minutes (97%); monomer **K**: 16 minutes (87%); monomer **L**: 16 minutes (95%); monomer **M**: 15 minutes (94%); monomer **N**: 20 minutes (96%); monomer **O**: 20 minutes (96%); monomer **P**: 20 minutes (97%). All oligomers were deprotected by standard conditions (12 h, 55 °C) using concentrated aqueous ammonia. Oligomers containing monomer **J** were flushed with a solution of Et_3N and MeCN (1 : 1) for 2 h followed by washing with MeCN before deprotection with aqueous ammonia.[22] The oligomers were synthesised in the DMT-ON mode and were purified by reverse phase HPLC using a 7.8 × 150 mm Xterra MS C18 10 μm column on a Waters 600 system. The purity of the oligomers after standard detritylation and desalting was confirmed as above 80% by anion-exchange HPLC using a 4 × 250 mm analytical DNAPac PA-100 column on a LaChrom system. Masses of the oligomers were measured by MALDI-TOF-MS recorded on an Applied Biosystems Voyager-DE STR spectrometer. Measured masses of the oligomers (calculated masses for MH^+): **TFO-2**: 4729 (4729);

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TFO-3: 4787 (4785); **TFO-4**: 4730 (4728); **TFO-5**: 4783 (4782); **TFO-6**: 4768 (4770); **TFO-7**: 4907 (4908); **TFO-8**: 4784 (4785); **TFO-9**: 4956 (4954); **TFO-10**: 4966 (4968); **TFO-11**: 4979 (4982); **TFO-12**: 5034 (5034); **TFO-13**: 5704 (5703); **TFO-14**: 4956 (4957); **TFO-15**: 5466 (5467); **TFO-16**: 5001 (4999); **TFO-17**: 5591 (5593); **TFO-18**: 4728 (4726); **TFO-19**: 4778 (4778); **TFO-20**: 4804 (4804); **TFO-21**: 4730 (4728); **TFO-22**: 4786 (4784); **TFO-23**: 4870 (4868); **TFO-24**: 4981 (4980); **TFO-25**: 5218 (5218); **TFO-26**: 4727 (4730); **TFO-27**: 4789 (4790); **TFO-28**: 4790 (4790).

Thermal denaturation studies

T_m values were determined on a Perkin Elmer Lambda 35 UV–VIS spectrometer equipped with a PTP-6 Peltier temperature controller. Quartz optical cells with a path length of 1.0 cm were used. The concentrations of oligomers were calculated using the following extinction coefficients (OD_{260}/μmol): G, 12.01; A, 15.20; T, 8.40; C, 7.05; pyrene, 20.4. The oligomers (1.0 μM of each duplex strand and 1.5 μM of the TFO) were thoroughly mixed, denatured by heating, and subsequently cooled to 4 °C. The measuring interval was 5–90 °C. Thermal denaturation temperatures (T_m values) were determined as the maximum of the first derivative of the thermal denaturation curve (A_{260} *vs.* temperature) recorded in a buffer containing 10 mM sodium cacodylate, 150 mM NaCl and 10 mM $MgCl_2$. The pH was adjusted to either 6.0 or 7.0. A temperature ramp of 0.5 °C min^{-1} was used. Reported thermal denaturation temperatures are an average of at least two independent measurements within ±1.0 °C.

Notes and references

1 (*a*) S. Buchini and C. J. Leumann, *Curr. Opin. Chem. Biol.*, 2003, **7**, 717; (*b*) K. Fox, *Curr. Med. Chem.*, 2000, **7**, 17; (*c*) J. Robles, A. Grandas, E. Pedroso, F. J. Luque, R. Eritja and M. Orozco, *Curr. Org. Chem.*, 2002, **6**, 1333.

2 (*a*) D. A. Barawkar, V. A. Kumar and K. N. Ganesh, *Biochem. Biophys. Res. Commun.*, 1994, **205**, 1665; (*b*) D. A. Barawkar, K. G. Rajeev, V. A. Kumar and K. N. Ganesh, *Nucleic Acids Res.*, 1996, **24**, 1229; (*c*) C.-H. Tung, K. J. Breslauer and S. Stein, *Bioconjugate Chem.*, 1996, **7**, 529; (*d*) N. Atsumi, Y. Ueno, M. Kanazaki, S. Shuto and A. Matsuda, *Bioorg. Med. Chem.*, 2002, **10**, 2933; (*e*) A. Mayer, A. Häberli and C. J. Leumann, *Org. Biomol. Chem.*, 2005, **3**, 1653; (*f*) M. Sollogoub, R. A. J. Darby, B. Cuenoud, T. Brown and K. R. Fox, *Biochemistry*, 2002, **41**, 7224.

3 (*a*) B. Cuenod, F. Casset, D. Hüsken, F. Natt, R. M. Wolf, K.-H. Altmann, P. Martin and H. E. Moser, *Angew. Chem., Int. Ed.*, 1998, **37**, 1288; (*b*) M. J. J. Blommers, F. Natt, W. Jahnke and B. Cuenod, *Biochemistry*, 1998, **37**, 17714; (*c*) T. Carlomagno, M. J. J. Blommers, J. Meiler, B. Cuenod and C. Griesinger, *J. Am. Chem. Soc.*, 2001, **123**, 7364; (*d*) S. Buchini and C. J. Leumann, *Eur. J. Org. Chem.*, 2006, 3152.

4 (*a*) M. Koizumi, K. Morita, M. Daigo, S. Tsutsumi, K. Abe, S. Obika and T. Imanishi, *Nucleic Acids Res.*, 2003, **31**, 3267; (*b*) K. Morita, C. Hasegawa, M. Kaneko, S. Tsutsumi, J. Sone, T. Ishikawa, T. Imanishi and M. Koizumi, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 73.

5 (*a*) A. A. Koshkin, *Tetrahedron*, 2006, **62**, 5962; (*b*) N. Kumar, K. E. Nielsen, S. Maiti and M. Petersen, *J. Am. Chem. Soc.*, 2006, **128**, 14.

6 (*a*) S. K. Singh, P. Nielsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455; (*b*) A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen and J. Wengel, *Tetrahedron*, 1998, **54**, 3607; (*c*) S. Obika, D. Nanbu, Y. Hari, J.-i. Andoh, K.-i. Morio, T. Doi and T. Imanishi, *Tetrahedron Lett.*, 1998, **39**, 5401; (*d*) M. Petersen and J. Wengel, *Trends Biotechnol.*, 2003, **21**, 74.

7 (*a*) S. Obika, Y. Hari, T. Sugimoto, M. Sekiguchi and T. Imanishi, *Tetrahedron Lett.*, 2000, **41**, 8923; (*b*) S. Obika, T. Uneda, T. Sugimoto, D. Nanbu, T. Minami, T. Doi and T. Imanishi, *Bioorg. Med. Chem.*, 2001, **9**, 1001; (*c*) H. Torigoe, Y. Hari, M. Sekiguchi, S. Obika and T. Imanishi, *J. Biol. Chem.*, 2001, **276**, 2354; (*d*) B.-W. Sun, B. R. Babu, M. D. Sørensen, K. Zakrzewska, J. Wengel and J.-S. Sun, *Biochemistry*, 2004, **43**, 4160.

8 J. J. Sørensen, J. T. Nielsen and M. Petersen, *Nucleic Acids Res.*, 2004, **32**, 6078.

9 (*a*) S. K. Singh, R. Kumar and J. Wengel, *J. Org. Chem.*, 1998, **63**, 10035; (*b*) C. Rosenbohm, S. M. Christensen, M. D. Sørensen, D. S. Pedersen, L.-E. Larsen, J. Wengel and T. Koch, *Org. Biomol. Chem.*, 2003, **1**, 655.

10 O. P. Varghese, J. Barman, W. Pathmasiri, O. Plashkevych, D. Honcharenko and J. Chattopadhyaya, *J. Am. Chem. Soc.*, 2006, **128**, 15173.

11 M. D. Sørensen, M. Petersen and J. Wengel, *Chem. Commun.*, 2003, 2130.

12 P. J. Hrdlicka, B. R. Babu, M. D. Sørensen, N. Harrit and J. Wengel, *J. Am. Chem. Soc.*, 2005, **127**, 13293.

13 V. V. Filichev and E. B. Pedersen, *J. Am. Chem. Soc.*, 2005, **127**, 14849.

14 N. Albæk, M. Petersen and P. Nielsen, *J. Org. Chem.*, 2006, **71**, 7731.

15 (*a*) N. K. Christensen, M. Petersen, P. Nielsen, J. P. Jacobsen, C. E. Olsen and J. Wengel, *J. Am. Chem. Soc.*, 1998, **120**, 5458; (*b*) H. V. Tømmerholt, N. K. Christensen, P. Nielsen, J. Wengel, P. C. Stein, J. P. Jacobsen and M. Petersen, *Org. Biomol. Chem.*, 2003, **1**, 1790; (*c*) P. K. Sharma and P. Nielsen, *J. Org. Chem.*, 2004, **69**, 5742.

16 (*a*) J. Fensholdt, H. Thrane and J. Wengel, *Tetrahedron Lett.*, 1995, **36**, 2535; (*b*) H. Thrane, J. Fensholdt, M. Regner and J. Wengel, *Tetrahedron*, 1995, **51**, 10389.

17 M. Faria, C. D. Wood, L. Perrouault, J. S. Nelson, A. Winter, M. R. H. White, C. Hélène and C. Giovannangeli, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 3862.

18 B. R. Babu, P. J. Hrdlicka, C. J. McKenzie and J. Wengel, *Chem. Commun.*, 2005, 1705.

19 B. R. Babu, manuscript in preparation.

20 T. Umemoto and J. Wengel, manuscript in preparation.

21 (*a*) T. J. Povsic and P. B. Dervan, *J. Am. Chem. Soc.*, 1989, **111**, 3059; (*b*) L. E. Xodo, G. Manzini, F. Quadrifoglio, G. A. van der Marel and J. H. van Boom, *Nucleic Acids Res.*, 1991, **19**, 5625; (*c*) S. F. Singleton and P. B. Dervan, *Biochemistry*, 1992, **31**, 10995.

22 (*a*) D. C. Capaldi, H. Gaus, A. H. Krotz, J. Arnold, R. L. Carty, M. N. Moore, A. N. Scozzari, K. Lowery, D. L. Cole and V. T. Ravikumar, *Org. Process Res. Dev.*, 2003, **7**, 832; (*b*) R. K. Kumar, A. P. Guzaev, C. Rentel and V. T. Ravikumar, *Tetrahedron*, 2006, **62**, 4528.

23 S. A. Ivanov, E. M. Volkov, T. S. Oretskaya and S. Müller, *Tetrahedron*, 2004, **60**, 9273.

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