COMMUNICATION

ChemComm
www.rsc.org/chemcomm

DNA-selective hybridization and dual strand invasion of short double-stranded DNA using pyren-1-ylcarbonyl-functionalized 4′-*C*-piperazinomethyl-DNA†

Torsten Bryld, Torben Højland and Jesper Wengel*

Nucleic Acid Center‡, Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark. E-mail: jwe@chem.sdu.dk; Fax: +45 6615 8780; Tel: +45 65502510

Received (in Cambridge, UK) 17th February 2004, Accepted 18th March 2004
First published as an Advance Article on the web 13th April 2004

Incorporation of a novel pyren-1-ylcarbonyl-functionalized 4′-*C*-piperazinomethyl-DNA monomer into oligodeoxynucleotides leads to increased thermal stability of duplexes with DNA complements but reduced thermal stability of duplexes with RNA complements. This DNA-selective hybridization§ is explored for recognition of double-stranded DNA by a novel dual strand invasion approach.

Functionalized oligonucleotides are being studied in our laboratory for Ångström-scale chemical engineering[1–3] towards applications within nucleic acid nanotechnology.[3,4] 4′-*C*-Piperazinomethyl-DNA monomers (Fig. 1) are interesting building blocks in this context as the distal nitrogen atom allows attachment of a range of functionalities facing the minor groove of nucleic acid duplexes. To explore this opportunity we have synthesized oligodeoxynucleotides (ONs) containing the 4′-*C*-(*N*-pyren-1-ylcarbonyl)piperazinomethyl thymidine monomer **Z** and evaluated its effect on nucleic acid hybridization in a direct comparison with the corresponding 4′-*C*-piperazinomethyl monomer **X** (Fig. 1, T = thymin-1-yl).

The phosphoramidite building blocks **1** and **2** (for description of synthesis of **1** and **2**, see ESI) were used to synthesize **ON2**–**ON7** and **ON9**–**ON11** (Table 1) on an automated DNA synthesizer. The coupling yields of **1** and **2** were 98% and 90% (10 min coupling time, 1*H*-tetrazole as activator), respectively, and ~99% for unmodified DNA phosphoramidites (2 min coupling time, 1*H*-tetrazole as activator). Phosphoramidite **2** was used for synthesis of **ON7** and **ON9**–**ON11** and phosphoramidite **1** for synthesis of **ON2** and **ON4**. In addition, **1** was also used for synthesis of **ON3** and **ON5** employing an on-column conjugation approach involving selective removal of the Fmoc groups and subsequent reaction with 1-pyrenecarboxylic acid and HBTU in DMF following essentially a procedure published for synthesis of 5′-end conjugated ONs.[5,6] The composition of the synthesized ONs was verified by MALDI-MS¶ and the purity (> 80%) by capillary gel electrophoresis.

The hybridization properties of the modified ONs were determined in a medium salt buffer and compared with those of the reference DNA strands **ON1** and **ON8** (Table 1). Incorporation of one, two or three 4′-*C*-piperazinomethyl monomer(s) **X** (**ON2**, **ON4** and **ON6**) induced a small increase in the thermal denaturation temperature (T_m value) with ΔT_m values (change in T_m value *per modification*) of approximately +2 °C towards the DNA complement and virtually no change in the T_m value towards the RNA target. These data are similar to those obtained for the corresponding 4′-*C*-(*N*-methyl)piperazinomethyl monomer[1] thus confirming the applicability of the C4′ position for conjugation of ONs.[7–11]

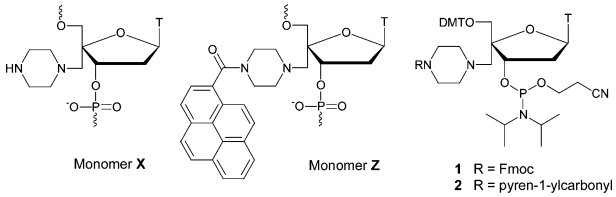

Fig. 1 Structures of monomers **X** and **Z** and phosphoramidites **1** and **2**.

The tendency towards DNA-selective hybridization§ observed for ONs containing monomer **X**, and the fact that RNA monomers derivatized at the O2′ position with pyrene-containing moieties[12–14] and pyrene-containing pseudo-nucleotides (intercalating nucleic acids)[15] are among the very rare ON modifications that induce DNA-selectivity, prompted us to investigate the pyren-1-ylcarbonyl-functionalized 4′-*C*-piperazinomethyl-DNA monomer **Z**. As seen in Table 1, incorporation of a single **Z** monomer induces a remarkable stabilization of duplexes formed with DNA complements (**ON3** and **ON9**, ΔT_m values of +7.0 and +9.0 °C) but a significant destabilization of the duplexes formed with RNA complements (ΔT_m values of −8.0 and −4.0 °C). Incorporation of two **Z** monomers (**ON5**, **ON10** and **ON11**) substantiates this trend, although the increase in thermal stability per modification towards DNA is significantly lower than that observed for the examples with incorporation of only one **Z** monomer. Notably, incorporation of three **Z** monomers (**ON7**) leads to a small increase in the thermal stability of the duplex with DNA but no hybridization (above 10 °C) with the RNA complement. These results strongly suggest the ability to engineer the relative thermal stability of short DNA:DNA and DNA:RNA duplexes by incorporation of one or a few 4′-*C*-(pyren-1-ylcarbonyl)piperazinomethyl monomer(s). The generality of this concept needs to be further studied, but it is encouraging that it is operational when monomer **Z** is neighboured both by two purine nucleotides (**ON3**) and by two pyrimidine nucleotides (**ON9**). For comparison, the effect of one 2′-*O*-(pyrenylmethyl)-RNA monomer was reported to be strongly sequence dependent.[12]

Table 1 ONs synthesized and thermal denaturation studies[a]

		DNA target	MM-DNA	RNA target
		T_m (ΔT_m)/°C	T_m/°C	T_m (ΔT_m)/°C
ON1	5′-GTGATATGC	29 (ref)	13/18/18	28 (ref)
ON2	5′-GTGA**X**ATGC	31 (+2.0)	14/23/18	28 (±0)
ON3	5′-GTGA**Z**ATGC	36 (+7.0)	19/29/23	20 (−8.0)
ON4	5′-GCA**X**A**X**CAC	34 (+2.5)		26 (−1.0)
ON5	5′-GCA**Z**A**Z**CAC	37 (+4.0)		20 (−4.0)
ON6	5′-G**X**GA**X**A**X**GC	34 (+1.7)		26 (−0.7)
ON7	5′-G**Z**GA**Z**A**Z**GC	31 (+0.7)		< 10 (< −6.0)
ON8	5′-GTGTTTTGC	32 (ref)		32 (ref)
ON9	5′-GTGT**Z**TTGC	41 (+9.0)	19/25/24	28 (−4.0)
ON10	5′-GTG**Z**T**Z**TGC	34 (+1.0)		21 (−5.5)
ON11	5′-G**Z**GTTT**Z**GC	34 (+1.0)		20 (−6.0)

[a] Thermal denaturation temperatures [T_m values (ΔT_m = change in T_m value *per modification* calculated relative to the T_m value of reference **ON1** or **ON8**)] measured as the maximum of the first derivative of the melting curve (A_{260} *vs.* temperature; 10 °C to 80 °C with an increase of 1 °C/min) recorded in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, pH 7.0) using *ca.* 1 μM concentrations of the two complementary strands. A, C, G and T are standard DNA monomers. In the column "MM-DNA" are listed T_m values recorded for mis-matched DNA target strands containing a single mis-matched nucleotide; the values are listed in the following order (indicating the mis-matched nucleotide in the central position of the target DNA): C/G/T.

† Electronic supplementary information (ESI) available: short description of synthesis of the phosphoramidites **1** and **2** and procedure used to record fluorescence emission spectra. See http://www.rsc.org/suppdata/cc/b4/b402414a/

‡ A research center funded by the Danish National Research Foundation for studies on nucleic acid chemical biology.

DOI: 10.1039/b402414a

The ability to discriminate mis-matched complements as efficiently as the reference **ON1** was confirmed for the singly-modified **ON2**, **ON3** and **ON9** (Table 1).

Steady-state fluorescence emission spectra of **ON3** and **ON5** hybridized to DNA and RNA revealed typical pyrene monomer fluorescence emission bands between 380 and 410 nm (data not shown). A significant difference in pyrene monomer fluorescence when hybridized with DNA or RNA was not observed. This suggests that the DNA-selective hybridization induced by monomer **Z** is due to favorable interaction of the pyrene units with the DNA:DNA duplex within the minor groove rather than intercalation of the pyrene units, a reasoning which is similar to that proposed for the 2′-*O*-(pyrenylmethyl)-RNA monomer.[12] Molecular modelling and NMR experiments are ongoing to investigate the molecular basis for the hybridization characteristics of 4′-*C*-piperazinomethyl-DNA.

We decided to explore the DNA-selectivity induced by monomer **Z** for targeting double-stranded DNA (dsDNA). Currently, sequence-specific recognition of dsDNA by oligonucleotide analogues is hampered by target sequence limitations and the requirement of unnatural salt concentrations, although progress has been accomplished using triplex-forming oligonucleotides,[16] strand invading PNA,[17,18] LNA[19,20] and pseudo-complementary DNA[21,22] or PNA.[23] In the latter approach, nucleobase analogues are used that sterically prevent cross-hybridization while allowing recognition of natural DNA. The absence of cross-hybridization between **ON7**, containing three **Z** monomers, and its RNA complement, suggests the use of **ON7**+RNA as a reagent mixture for recognition of the two complementary segments of a dsDNA duplex (Fig. 2).

We used the duplex **ON1**:DNA as a dsDNA target model and the changes in fluorescence emission of **ON7** upon hybridization to monitor the processes in solution (Fig. 2; **ON7** was present in *ca.* 2/3 molar ratio to the target). An excimer band at 430–520 nm is seen in the fluorescence emission spectrum of single-stranded **ON7**, which can be explained by the flexibility of the single stranded **ON7** allowing the pyrene units to form pyrene–pyrene pairs. No excimer band is observed for the mixture of **ON7** and DNA indicating formation of a rigid duplex structure. However, for the mixture of **ON7** and RNA, an excimer band is evident and the fluorescence emission spectrum resembles the spectrum of **ON7** alone. This corroborates the inability of **ON7** and RNA to form a duplex above 10 °C at the applied conditions. The strand invasion experiments were performed at 10 °C under medium salt conditions (110 mM Na^+). First **ON7** was added to the preformed target duplex **ON1**:DNA. The strong excimer band observed throughout the experiment (24 h) revealed the absence of significant duplex invasion. However, addition of a mixture of **ON7** and RNA to the preformed duplex **ON1**:DNA induced efficient duplex invasion as shown by the absence of an excimer band in the spectrum recorded of this mixture.‖ The importance of addition also of the RNA strand strongly indicates this strand to partake in duplex formation with the **ON1** strand of the original DNA duplex during strand invasion, and the process to involve dual strand invasion (Fig. 2).

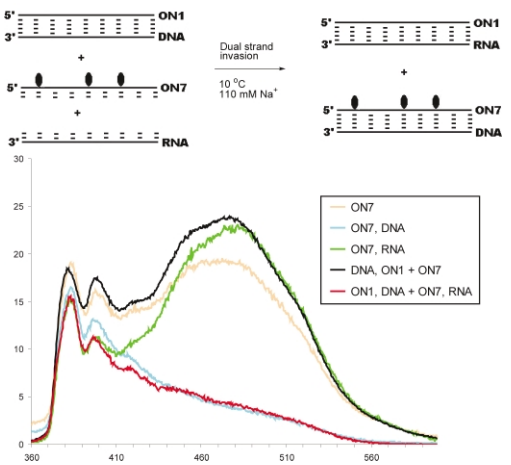

Fig. 2 Dual strand invasion of dsDNA (**ON1**:DNA duplex). Fluorescence emission spectra of **ON7**,DNA (15 min), **ON7**,RNA (15 min), **ON7** alone (15 min), **ON1**,DNA after addition of **ON7** (24 h), and **ON1**,DNA after addition of **ON7**,RNA (60 min, see schematic drawing); in parentheses is shown for each spectrum the elapsed time after mixing at which the spectrum was recorded. **ON7** was used in *ca.* 0.15 μM concentration (~2/3 molar ratio to the target); see Table 1 for buffer used.

The above example is the first of dual invasion of a mixed-sequence DNA duplex not employing the concept of pseudo-complementary nucleobases.[21–23] The key to this novel approach is the DNA-selectivity induced by incorporation of the 4′-*C*-(pyren-1-ylcarbonyl)piperazinomethyl monomer **Z**. That medium salt conditions and a preformed dsDNA target were applied are encouraging towards fulfilling the goal of developing a general method for sequence-specific molecular recognition of mixed-sequence dsDNA. However, studies involving longer and more biologically relevant double-stranded DNA target segments are needed to establish the scope and limitations of this novel dual strand invasion strategy.

We thank the Danish National Research Foundation for financial support, Ms Britta M. Dahl for ON synthesis and Dr Michael Meldgaard, Exiqon A/S, for MALDI-MS analysis.

Notes and references

§ By "DNA-selective hybridization" is understood the formation of duplexes with DNA complements that are significantly more thermally stable than duplexes formed with RNA complements.

¶ MALDI-MZ: *m/z* ([M − H]$^-$ found/calcd.) 2852/2853 (**ON2**), 3122/3125 (**ON3**), 2879/2878 (**ON4**), 3336/3332 (**ON5**), 3048/3048 (**ON6**), 3730/3731 (**ON7**), 3061/3059 (**ON9**), 3387/3388 (**ON10**), 3387/3387 (**ON11**).

‖ In Fig. 2 is shown the spectrum recorded 60 min after addition of **ON7** and RNA; this spectrum was identical to that recorded 24 h after the addition, whereas gradually decreasing excimer band intensity was observed in the spectra recorded during the first minutes after the addition.

1 M. Raunkjær, T. Bryld and J. Wengel, *Chem. Commun.*, 2003, 1604.
2 M. D. Sørensen, M. Petersen and J. Wengel, *Chem. Commun.*, 2003, 2130.
3 J. Wengel, *Org. Biomol. Chem.*, 2004, **2**, 277.
4 N. C. Seeman, *Chem. Biol.*, 2003, **10**, 1151.
5 A. A. Mokhir, C. N. Tetzlaff, S. Herzberger, A. Mosbacher and C. Richert, *J. Comb. Chem.*, 2001, **3**, 374.
6 T. Bryld, T. Højland and J. Wengel, manuscript in preparation.
7 H. Thrane, J. Fensholdt, M. Regner and J. Wengel, *Tetrahedron*, 1995, **51**, 10389.
8 H. Maag, B. Schmidt and S. J. Rose, *Tetrahedron Lett.*, 1994, **35**, 6449.
9 G. Wang and W. E. Seifert, *Tetrahedron Lett.*, 1996, **37**, 6515.
10 Y. Ueno, K. Tomino, I. Sugimoto and A. Matsuda, *Tetrahedron*, 2000, **56**, 7903.
11 I. Detmer, D. Summerer and A. Marx, *Eur. J. Org. Chem.*, 2003, 1837.
12 K. Yamana, R. Iwase, S. Furutani, H. Tsuchida, H. Zako, T. Yamaoka and A. Murakami, *Nucleic Acids Res.*, 1999, **27**, 2387.
13 K. Yamana, H. Zako, K. Asazuma, R. Iwase, H. Nakano and A. Murakami, *Angew. Chem., Int. Ed.*, 2001, **40**, 1104.
14 V. A. Korshun, D. A. Stetsenko and M. J. Gait, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1092.
15 U. B. Christensen and E. B. Pedersen, *Nucleic Acids Res.*, 2002, **30**, 4918.
16 S. Buchini and C. J. Leumann, *Curr. Opin. Chem. Biol.*, 2003, **7**, 717.
17 P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science*, 1991, **254**, 1497.
18 V. V. Demidov, *Expert Rev. Mol. Diagn.*, 2001, **1**, 343.
19 E. L. Chernolovskaya, A. A. Koshkin and V. V. Vlassov, *Nucleosides Nucleotides Nucleic Acids*, 2001, **20**, 847.
20 K. M. L. Hertoghs, J. H. Ellis and I. R. Catchpole, *Nucleic Acids Res.*, 2003, **31**, 5817.
21 I. V. Kutyavin, R. L. Rhinehart, E. A. Lukhtanov, V. V. Gorn, R. B. Meyer, Jr. and H. B. Gamper, Jr., *Biochemistry*, 1996, **35**, 11170.
22 J. Woo, R. B. Meyer, Jr. and H. B. Gamper, *Nucleic Acids Res.*, 1996, **24**, 2470.
23 J. Lohse, O. Dahl and P. E. Nielsen, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 11804.