COMMUNICATION

ChemComm
www.rsc.org/chemcomm

N-Methylpiperazinocarbonyl-2′,3′-BcNA and 4′-*C*-(*N*-methylpiperazino)methyl-DNA: introduction of basic functionalities facing the major groove and the minor groove of a DNA:DNA duplex

Michael Raunkjær, Torsten Bryld and Jesper Wengel*

Nucleic Acid Center†, Department of Chemistry, University of Southern Denmark, DK-5230 Odense M, Denmark

Received (in Cambridge, UK) 14th March 2003, Accepted 6th May 2003
First published as an Advance Article on the web 4th June 2003

Piperazino-functionalized 2′,3′-BcNA and 4′-*C*-hydroxymethyl-DNA are appropriate molecular architectures for the introduction of basic functionalities facing the major groove and the minor groove of nucleic acid duplexes, respectively. 4′-*C*-(*N*-Methylpiperazino)methyl-DNA monomers induce significantly increased thermal stability of a DNA:DNA duplex.

Conjugation of various molecular entities to oligonucleotides (ONs) has been performed in order to improve the therapeutic potential of antisense ONs.[1] Attachment of alkylamines, assumed to be protonated under physiological conditions, is attractive as a strategy for increasing the binding affinity towards the negatively charged target strands. ONs conjugated with alkylamines at the phosphate,[2] base[3–6] and sugar[7–10] moieties have been prepared and their binding towards complementary ONs studied. We have over recent years studied the effect of incorporating a number of *C*-hydroxymethylated monomers into ONs, including 2′-*O*,3′-*C*-((2″-*C*-hydroxymethyl)ethylene)-linked bicyclic nucleotides (2″-*C*-hydroxymethyl-2′,3′-BcNA nucleotides)[11] and 4′-*C*-hydroxymethyl nucleotides[12,13] (Fig. 1). In this communication, we present the results of our studies on incorporation of *N*-methylpiperazinocarbonyl-2′,3′-BcNA and 4′-*C*-(*N*-methylpiperazino)methyl-DNA nucleotide monomers **X** and **Y** (Scheme 1 and Table 1) into ONs with the purpose of evaluating the effect on hybridization of introducing the basic *N*-methylpiperazino group.

Synthesis of the necessary phosphoramidite derivatives **6** and **10** is depicted in Scheme 1. For synthetic convenience, an amide linkage was chosen for attaching the *N*-methylpiperazino group in the 2′,3′-BcNA series and an amine linkage in the 4′-*C*-hydroxymethyl series. Oxidation of 2′-*O*,3′-*C*-((2″*R*)-2″-*C*-(hydroxymethyl)ethylene)-linked bicyclic nucleotide **1**[11] by the free radical 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and iodobenzene diacetate (BAIB) afforded the carboxylic acid **2** which was converted into amide **3** by reaction with *N*-methylpiperazine in anhydrous THF using 1,1′-carbonyldiimidazole (CDI) as coupling agent. Debenzylation of amide **3** by hydrogenolysis with H_2 and 20% $Pd(OH)_2$/C as catalyst in acetone gave the nucleoside diol **4**. Selective protection of the primary hydroxy group of **4** by reaction with 4,4′-dimethoxytrityl chloride (DMTCl) and DMAP in anhydrous pyridine afforded 5′-*O*-DMT derivative **5**.‡ Subsequent standard phosphitylation of the 3′-hydroxy group of **5** afforded the phosphoramidite **6**§ in an overall yield of 37% (from **1**). The 4′-*C*-hydroxymethyl group of the known nucleoside **7**[12] was converted into the 4′-*C*-(*N*-methylpiperazino)methyl group by reacting with triflouromethanesulfonic anhydride in anhydrous pyridine to give *in situ* the triflate which was treated with 10 equivalents of *N*-methylpiperazine in anhydrous THF to give nucleoside **8**. The *tert*-butyldimethylsilyl group was removed to give nucleoside **9**¶ which was phosphitylated at the 3′-hydroxy group to furnish the phosphoramidite **10**‖ in an overall yield of 27% (from **7**).

The reference **ON1** and the functionalized oligomers **ON2**–**ON5** (Table 1) were synthesized in 0.2 μmol scale on an automated DNA synthesizer using standard DNA amidites,

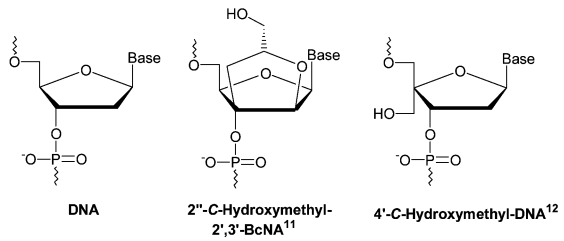
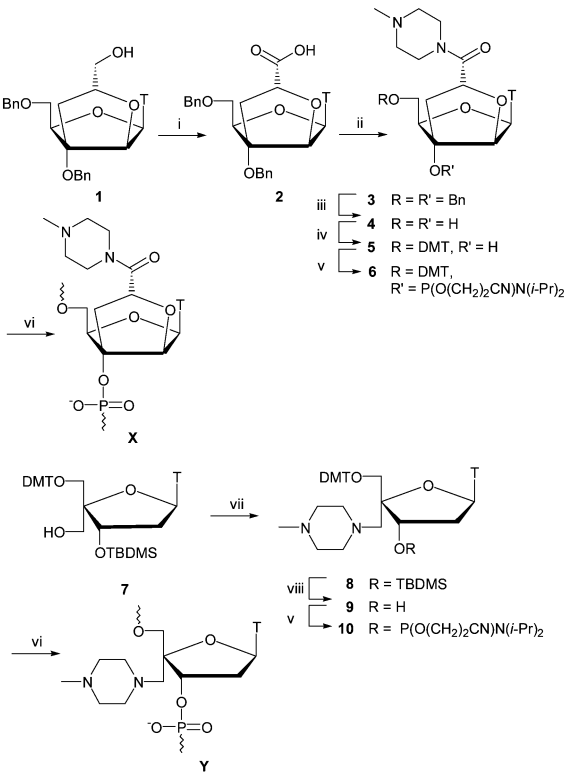


Fig. 1



Scheme 1 *Reagents and conditions*: i) TEMPO, BAIB, CH_2Cl_2, H_2O, 5 °C, 82%; ii) *N*-methylpiperazine, CDI, anhydrous THF, r.t., 85%; iii) H_2, 20% $Pd(OH)_2$/C, acetone, r.t., 90%; iv) DMTCl, DMAP, anhydrous pyridine, r.t., 80%; v) 2-cyanoethyl *N*,*N*-diisopropylphosphoramido-chloridite, DIPEA, CH_2Cl_2, r.t., 75% (for **7**), 63% (for **10**); vi) DNA synthesizer; vii) a) Tf_2O, anhydrous pyridine, CH_2Cl_2, b) *N*-methylpiperazine, anhydrous THF (54%); viii) TBAF, THF (77%). T = thymin-1-yl, DMT = 4,4′-dimethoxytrityl.

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

DOI: 10.1039/b302963h

amidite **6** (~98% step-wise coupling yield, 10 min coupling time using pyridine hydrochloride as activator and *tert*-butyl hydroperoxide for oxidation**[14]), and amidite **10** (>90% coupling yield, 10 min coupling time using 1*H*-tetrazole as activator and standard iodine oxidation). Satisfactory purities (>80 %) of **ON2**–**ON5** were verified by capillary gel electrophoresis and the compositions by MALDI-MS analysis.††

The hybridization properties of **ON2**–**ON5** were evaluated by thermal denaturation studies (Table 1). Incorporation of *N*-methylpiperazinocarbonyl-2′,3′-BcNA monomer **X** induced decreased T_m values in the medium salt buffer towards both the DNA and the RNA complement (data for **ON2** and **ON3** relative to **ON1**). Comparison with T_m values reported for the corresponding 2″-*C*-hydroxymethyl-2′,3′-BcNA modification[11] reveals a weak destabilizing effect of exchanging the hydroxymethyl group with the piperazinocarbonyl group. Interestingly, the hybridization properties of **ON2** and **ON3** relative to reference **ON1** were improved when performing the experiments in the low salt buffer. These data indicate that the basic piperazino group of monomer **X** is at least partly protonated at pH 7.0 leading to a favorable effect of partial charge neutralization at reduced salt concentrations.

Towards the DNA complement in the medium salt buffer, **ON4** and **ON5** containing 4′-*C*-(*N*-methylpiperazino)methyl-DNA monomer **Y** displayed significantly increased T_m values relative to reference **ON1**. The T_m values towards the RNA complement were as for the reference. An additional affinity-enhancing effect was seen when changing to the low salt buffer. In comparison with the corresponding 4′-*C*-hydroxymethyl-DNA monomer,[12] similar 4′-*C*-aminoalkyl-DNA monomers,[8–10] and a biotinylated 4′-*C*-alkyl monomer,[15] 4′-*C*-*N*-(methylpiperazino)methyl monomer **Y** displays the most significant affinity-enhancing effect towards a DNA complement (while still obeying the Watson–Crick base pairing rules).‡‡ It is noteworthy that **ON5**, especially at low salt conditions, displays a clear preference of hybridizing towards DNA rather than RNA.

NMR studies[16] and molecular modelling have shown the 2′-*O*,3′-*C*-ethylene linkage of 2′,3′-BcNA type monomers to face the major groove and the 4′-*C*-alkyl substitutent of 4′-*C*-alkyl-DNA type monomers to face the minor groove. Thus, we have herein shown that orientation of the basic piperazino moieties of monomers **X** and **Y** towards the major and minor groove of DNA:DNA duplexes is compatible with satisfactory hybridization properties. We are currently studying the versatility of piperazino-functionalized monomers for further conjugation and the generality of high-affinity DNA recognition by oligonucleotides containing 4′-*C*-(*N*-methylpiperazino)methyl monomer **Y**.

Table 1 ONs synthesized and thermal denaturation studies[a]

	110 mM Na^+		40 mM Na^+	
	DNA target	RNA target	DNA target	RNA target
Sequence	$T_m(\Delta T_m)$/°C	$T_m(\Delta T_m)$/°C	$T_m(\Delta T_m)$/°C	$T_m(\Delta T_m)$/°C
5′-d(GTGATATGC) (**ON1**)	29	26[b]/28[c]	22	19[b]/20[c]
5′d(GTGA**X**ATGC) (**ON2**)	27 (−2)	24[b] (−2)	22 (±0)	18[b] (-1)
5′-d(G**X**GA**X**A**X**GC) (**ON3**)	19 (−10)	21[b] (−5)	18 (−4)	19[b] (±0)
5′-d(GTGA**Y**ATGC) (**ON4**)	33 (+4)	28[c] (±0)	26 (+4)	21[c] (+1)
5′-d(G**Y**GA**Y**A**Y**GC) (**ON5**)	35 (+6)	29[c] (+1)	31 (+9)	22[c] (+2)

[a] Melting temperatures [T_m values (ΔT_m values are calculated relative to the T_m value of reference **ON1**)] 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^{-1}) recorded in medium salt buffer ["110 mM Na^+"] (10 mM sodium phosphate, 100 mM sodium chloride, pH 7.0) and low salt buffer ["40 mM Na^+"] (10 mM sodium phosphate, 30 mM sodium chloride, pH 7.0) using 1 μM concentrations of the two complementary strands. [b] These values were determined in a single experimental series. [c] These values were determined a second experimental series.

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

Notes and references

‡ ^{13}C NMR data of (1*S*,3*R*,5*R*,6*R*,8*R*)-6-((4,4′-dimethoxytrityl)oxymethyl)-5-hydroxy-3-((*N*-methylpiperazino)carbonyl)-8-(thymin-1-yl)-2,7-dioxa-bicyclo[3.3.0]octane (**5**): δ ($(CD_3)_2SO$) 166.1, 163.6, 158.1, 150.0, 144.7, 137.4, 135.4, 135.2, 129.7, 127.8, 127.7, 126.7, 113.2, 107.5, 87.9, 86.6, 85.7, 82.1, 80.2, 77.9, 62.0, 55.0, 54.9, 54.0, 45.4, 44.8, 41.4, 37.3, 12.1.
§ ^{31}P NMR data of amidite **6**: δ ($(CD_3)_2SO$) 142.9, 142.6.
¶ ^{13}C NMR data of 5′-*O*-(4,4′-dimethoxytrityl)-4′-*C*-((*N*-methylpiperazino-)methyl)thymidine (**9**): δ ($(CD_3)_2SO$) 163.6, 158.1, 150.3, 144.8, 135.7, 135.4, 135.3, 129.8, 129.8, 127.9, 127.7, 126.7, 113.2, 109.5, 88.2, 86.0, 83.3, 72.1, 65.2, 58.7, 55.0, 54.0, 45.8, 11.7.
‖ ^{31}P NMR data of amidite **10**: δ ($(CD_3)_2SO$) 154.8, 154.6.
** The use of standard iodine oxidation when coupling phosphoramidites of tertiary alcohols should be avoided due to the risk of backbone cleavage and concomitant low coupling yields (ref. 14).
†† MALDI-MS: *m*/*z* ([M–H]$^-$ found/calcd.) 2921/2922 (**ON2**); 3256/3258 (**ON3**); 2863/2866 (**ON4**); 3089/3090 (**ON5**).
‡‡ T_m values recorded in medium salt buffer for **ON4** towards DNA targets with centrally positioned mis-matched nucleotides: 19 °C, 21 °C and no transition (centrally positioned C, G and T nucleotides, respectively).

1 M. Manoharan, *Antisense Nucleic Acid Drug Devel.*, 2002, **12**, 103.
2 R. L. Letsinger, C. N. Singman, G. Histand and M. Salunkhe, *J. Am. Chem. Soc.*, 1988, **110**, 4470.
3 K. S. Ramasamy, M. Zounes, C. Gonzalez, S. M. Freier, E. A. Lesnik, L. L. Cummins, R. H. Griffey, B. P. Monia and P. D. Cook, *Tetrahedron Lett.*, 1994, **35**, 215.
4 T. P. Prakash, D. A. Barawkar, V. Kumar and K. N. Ganesh, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1733.
5 K.-Y. Lin and M. D. Matteucci, *J. Am. Chem. Soc.*, 1998, **120**, 8531.
6 A. M. Soto, B. I. Kankia, P. Dande, B. Gold and L. A. Marky, *Nucleic Acids Res.*, 2002, **30**, 3171.
7 R. H. Griffey, B. P. Monia, L. L. Cummins, S. Freier, M. J. Greig, C. J. Guinosso, E. Lesnik, S. M. Manalili, V. Mohan, S. Owens, B. R. Ross, H. Sasmor, E. Wancewicz, K. Weiler, P. D. Wheeler and P. D. Cook, *J. Med. Chem.*, 1996, **39**, 5100.
8 G. Wang and W. E. Seifert, *Tetrahedron Lett.*, 1996, **37**, 6515.
9 M. Kanazaki, Y. Ueno, S. Shuto and A. Matsuda, *J. Am. Chem. Soc.*, 2000, **122**, 2422.
10 H. M. Pfundheller, T. Bryld, C. E. Olsen and J. Wengel, *Helv. Chim. Acta*, 2000, **83**, 128.
11 M. Raunkjær, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans 1*, 1999, 2543.
12 H. Thrane, J. Fensholdt, M. Regner and J. Wengel, *Tetrahedron*, 1995, **51**, 10389.
13 K. D. Nielsen, F. Kirpekar, P. Roepstorff and J. Wengel, *Bioorg. Med. Chem.*, 1995, **3**, 1493.
14 C. Scheuer-Larsen, B. M. Dahl, J. Wengel and O. Dahl, *Tetrahedron Lett.*, 1998, **39**, 8361.
15 H. Magg, B. Schmidt and S. J. Rose, *Tetrahedron Lett.*, 1994, **35**, 6449.
16 L. B. Jørgensen, P. Nielsen, J. Wengel and J. P. Jacobsen, *J. Biomol. Struc. Dyn.*, 2000, **18**, 45.