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Thermal Stability Studies of the Non Canonical Base Pair between 5-Methyl-isocytosine and Isohypoxanthine

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Abstract: The hybridization properties of 5-methyl-isocytosine (^{5Me}isoC) and isohypoxanthine (isoI) have been investigated by using ultraviolet melting techniques. These two bases were incorporated into heptadecanucleotides using the phosphoramidite chemistry. The base pair ^{5Me}isoC/isoI was found to be isoenergetic with the A/T base pair. A small destabilization was observed when isoI was located opposite T or G and can be interpreted by a pairing mode between these bases and isoI in its lactam form.

INTRODUCTION

Among many theoretical base pairing schemes differing from those encountered in canonical nucleic acids, the base pair between isocytosine (isoC) and isoguanine (isoG) postulated by A. Rich[1] is the only one that permits the use of N-nucleosides.[2] S.A. Benner and co-workers demonstrated the possibility to incorporate enzymatically such a modified base pair into DNA and RNA.[2-4] This base pair was found to be less specific than natural base pairs, because of the tautomerism of the purine member, isoG, whose equilibrium between the lactim and the lactam forms led it to pair not only with isoC but also with T. Moreover, the pyrimidine nucleoside 2'-deoxyisocytidine is subject to depyrimidination in certain synthetic conditions and to a partial oxidative deamination when it is inserted into a synthetic oligodeoxyribonucleotide.

The present paper reports on a new non canonical base pair (Figure 1) derived from the isoC/isoG scheme.[5] The chemical faisability and the structural stability of the base pairing scheme between 5-methyl-isocytosine (^{5Me}isoC) and isohypoxanthine (isoI) were investigated. The use of ^{5Me}isoC has already been reported as an improvement on the pyrimidine partner of the isoC/isoG pair.[6,7] A simple access to 2'-deoxyisoinosine (disoI) from isoI by an enzymatic transglycosylation was previously described.[8] A chemical route is also possible.[8,9]

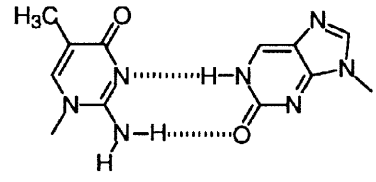

Figure 1

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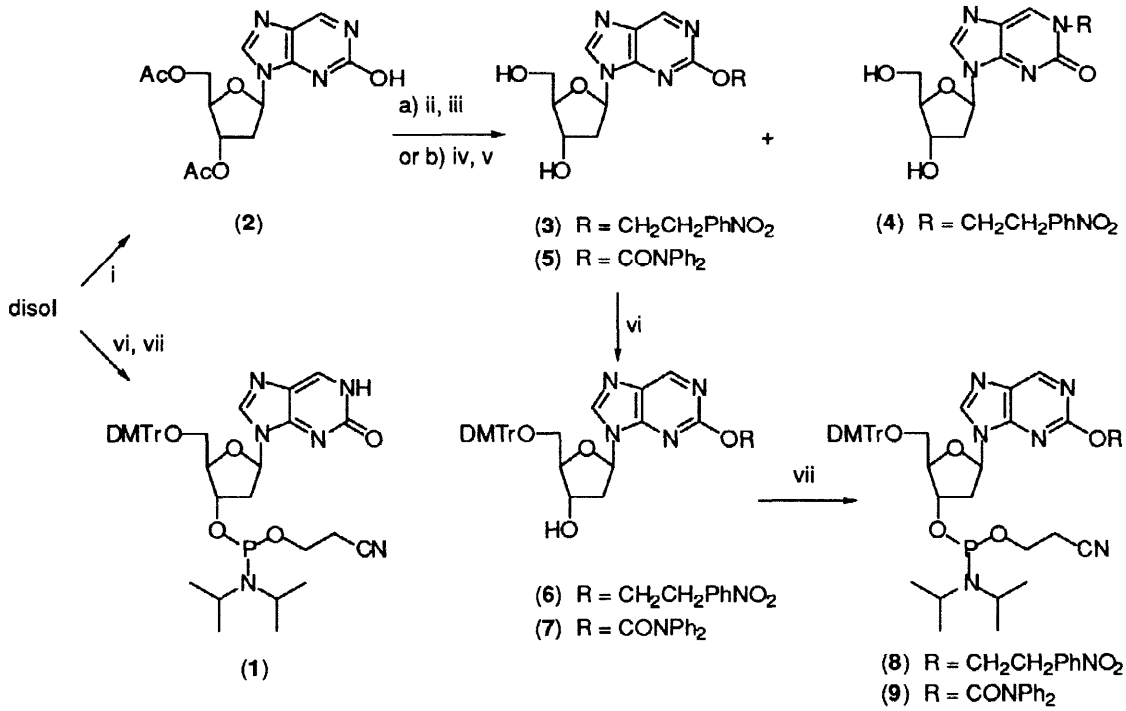
RESULTS AND DISCUSSION

Synthesis

Oligodeoxynucleotides containing isoI residue can be synthesized using either the phosphoramidite **1** (Scheme 1)[8] or the H-phosphonate derivative.[9,10] The preparation of the phosphoramidite building block **1** was tedious : the 5'-*O*-dimethoxytritylation of disoI proceeded with an unsatisfactory yield and the phosphitylation gave a mixture of 3'-*O*-phosphoramidite and 3'-*O*,O^2-diphosphoramidite. In order to achieve the large scale synthesis of a suitable phosphoramidite building block, two different protecting groups were tested for the exocyclic oxygen on carbon 2. The *p*-nitrophenylethyl group was introduced according to the Mitsunobu reaction (Scheme 1), by treating the 3'-*O*,5'-*O*-diacetylated disoI (**2**) with triphenylphosphine, *p*-nitrophenylethanol and diethylazodicarboxylate (1.5 eq. of each) in THF or dioxane. After the removal of acetyl groups by treatment with methanol/ammonia, two compounds were isolated in yields varying with respect to the solvent used : compounds **3** and **4** were isolated in 60 and 13% yields in THF, versus 52% and 15%, respectively, in dioxane. Such a dependence towards the solvent has been reported for the protection of 2-pyridone.[11] The alkylation site for compounds **3** and **4** was assigned based on the analysis of their ^{1}H and ^{13}C-NMR spectra as the *O*- and *N*-alkylated nucleosides, respectively. The alkylated nitrogen (N^1 or N^3) of compound **4** was determined by means of two dimentional ^{1}H,^{13}C-COLOC-NMR spectroscopy using the parameters described by others [12].



Scheme 1 : Synthesis of 3'-phosphoramidites derivatives of disoI, without O^2-protection (**1**) and after O^2-protection by either the *p*-NPE group (**8**) or the DPC group (**9**). Reagents: i: Ac_2O/pyridine; ii: Ph_3P, DEAD, *p*-NO_2-$C_6H_4(CH_2)_2OH$; iii: NH_4OH/methanol; iv: DPC-Cl/pyridine; v: 2N NaOH/EtOH/pyridine; vi: DMTrCl/pyridine; vii: $(iPr_2N)_2PO(CH_2)_2CN$/diisopropylammonium tetrazolide/CH_3CN.

The diphenylcarbamoyl group was introduced by treating **2** with DPC-Cl/DIEA in pyridine (85% yield) followed by mild alkaline treatment to afford **5** in 70% yield from **2** (Scheme 1). The *O*-NPE (**3**) and *O*-DPC (**5**) nucleosides were converted into their 5'-DMT derivatives (**6** and **7** in 57% yield), then into their 3'-*O*-phosphoramidites (**8** and **9** in 60% and 85% yields, respectively) according to standard protocols.[13] Both these protecting groups were stable enough under the acidic conditions used in DNA synthesis. The diphenycarbamoyl group was retained because of a higher phosphitylation yield and an easier deprotection mode after completion of the DNA synthesis (cleavage occuring at the same step than all the protection of natural bases). The use of DPC protecting group for disoI has been reported by Seela.[14]

Phosphoramidite **9** was incorporated into oligodeoxynucleotides at a predetermined site on an Expedite DNA synthesizer (Millipore). Stepwise elongation was carried out according to standard protocols, except for the modified phosphoramidites (concentration of 0.15M in CH3CN and coupling time of 10 min.). The coupling yield of **9** was quite similar to that of the commercial phosphoramidites, as determinated by spectroscopic evaluation of the released trityl cation (the average yield per step is 85-89%). At the end of elongation, the resin was cleaved by 33% aqueous ammonia treatment, and the resulting solution was heated for 8 h at 55 °C. Crude oligodeoxynucleotides were purified by reverse phase HPLC. Oligomers containing isoI were isolated in 10-22% overall yield depending on the sequence. Purified oligomers were characterized by electrospray ionization mass spectrometry. Base composition was checked by reverse phase HPLC analysis of the enzymatic digest (phosphodiesterase and alkaline phosphatase). Sequences containing d^{5Me}isoC were synthesized using the commercially available phosphoramidite described by Thomas Horn.[7]

Thermal denaturation studies

In order to assess the stability of duplexes containing the new base pair, thermal melting experiments were conducted on complementary heptadecanucleotides containing at the central position ^{5Me}isoC/isoI and all possible combinations with natural bases. In all cases, sharp melting profiles were observed indicating cooperativity and reversibility of the denaturation/association process. Typical melting curves are illustrated in Figure 2. The melting temperatures (Tm) and the thermodynamic data for helix-coil transitions (ΔH, ΔS, ΔG) were calculated according to litterature.[15,16]

5'- ACTTGGCCXCCATTTTG -3'
3'- TGAACCGGYGGTAAAAC -5'

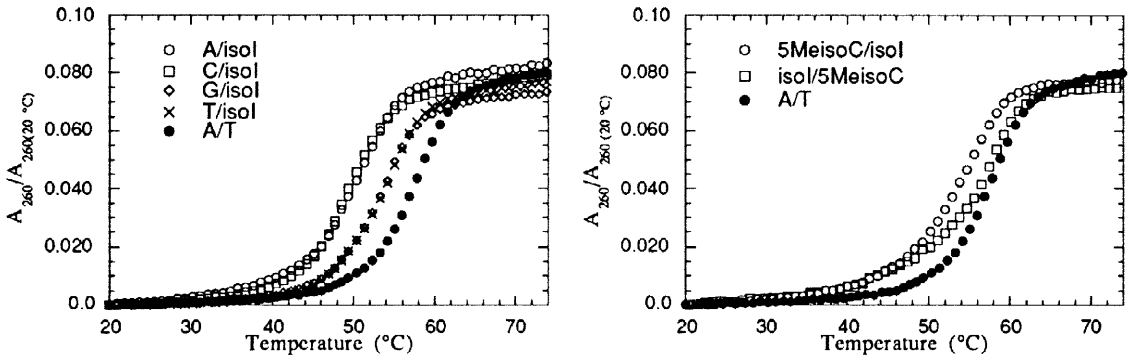


Figure 2 : Normalized melting profiles for duplexes containing isoI or ^{5Me}isoC. Melting transitions were measured at 260 nm in 100 mM NaCl, 10 mM sodium cacodylate buffer (pH 7.0) at 1 μM concentration of each strand.

The heteroduplexes containing either the isoI/^{5Me}isoC pair (entry 15, Tm = 57.0 °C) or the canonical A/T and T/A pairs (entries 1 and 2, Tm = 58.0 °C and 57.0 °C) are equally stable, thus indicating the stability of the postulated pairing scheme between ^{5Me}isoC and isoI in its lactam form (Figure 1). An obvious context effect can be inferred from the different Tm observed for the ^{5Me}isoC/isoI pair (entry 14, Tm = 55.0 °C). All other combinations (N/isoI or N/^{5Me}isoC) are lower melting than the isoI/^{5Me}isoC pair: the Tms are 6.5-8.0 °C lower for C and A opposite isoI, while they are only 3.0-3.5 °C lower for T and G opposite isoI.

Table 1: Melting Temperatures and Thermodynamic Data.

entry	X/Y	$-\Delta H$ (kJ/mol)	$-\Delta S$ (kJ/mol.K)	$-\Delta G_{298}$ (kJ/mol)	Tm (°C)
1	A/T	494.5	1.38	84.0	58.0
2	T/A	491.6	1.37	82.3	57.0
3	A/isoI	408.7	1.15	66.9	50.5
4	C/isoI	334.0	0.92	58.9	49.0
5	G/isoI	469.4	1.32	75.4	53.5
6	T/isoI	465.9	1.31	76.0	54.0
7	isoI/T	440.4	1.23	73.7	54.0
8	isoI/isoI	456.1	1.29	70.6	50.5
9	A/^{5Me}isoC	440.4	1.23	73.7	54.0
10	C/^{5Me}isoC	488.7	1.38	75.3	52.0
11	G/^{5Me}isoC	471.6	1.31	79.1	56.0
12	T/^{5Me}isoC	446.3	1.28	65.4	47.0
13	^{5Me}isoC/^{5Me}isoC	436.0	1.24	66.9	49.0
14	^{5Me}isoC/isoI	468.0	1.31	77.2	55.0
15	isoI/^{5Me}isoC	503.7	1.41	83.6	57.0

Recently Seela et al. examined the duplex stability of modified $(dA)_{12}/(dT)_{12}$ oligomers.[17] When one or two isoI residues were located in the middle opposite the standard nucleosides (A, C, G, T), destabilization was observed. The authors concluded that isoI doesn't form favored hydrogen bonded structures with the four conventional bases within an antiparallel duplex. Different pairing modes can be proposed to explain the data presented here (significantly less decreased Tm for isoI/T and isoI/G, entries 5-6) : Watson-Crick base pairs with isoI in its lactim form (Figure 3, a and c) or non Watson-Crick base pairs with isoI in its lactam form (Figure 3, b and d). Since the Tm value observed for isoI/T is below the value observed for the A/T pair, the isoI/T pair presumably adopts a wobble conformation, which implies the lactam form of isoI. To account for the stability of the isoI/G pair, two pairing schemes deriving from those proposed by Kamiya et al.[18] for the isoG/G pair may be invoked (Figure 3, c and d).

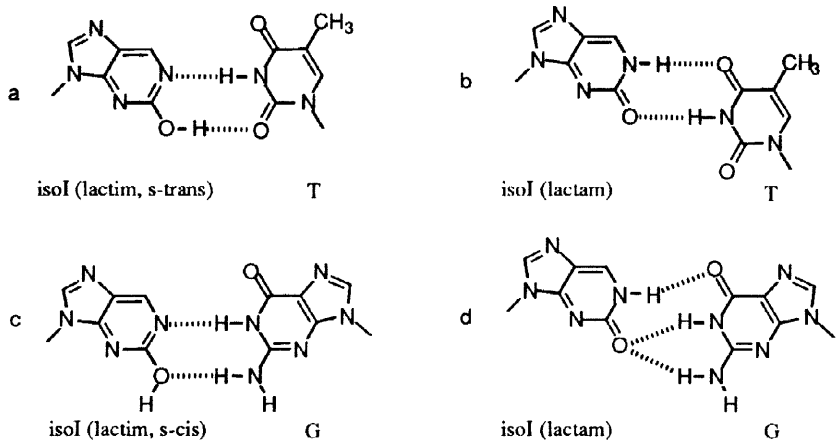


Figure 3 : Hydrogen bonding schemes of base pairs formed between isoI and T or G.

Examination of thermodynamic data shows that the most favorable ΔH and ΔG terms are found for the heteroduplexes with the smallest destabilization. ΔG is more favorable for the heteroduplex containing the isoI/^{5Me}isoC pair than for the heteroduplex containing the ^{5Me}isoC/isoI pair. This is consistent with the respective melting temperatures, thus indicating that the context effect is due to stacking.

CONCLUSION

We have described the synthesis of a suitable phosphoramidite building block of isoI involving the protection of the exocyclic oxygen on C2. Heptadecamers containing either isoI or ^{5Me}isoC at the central position were prepared. From the melting data, the isoI/^{5Me}isoC base pair appears thermodynamically as stable as the canonical A/T pair. This is consistent with the base pairing scheme initially postulated involving two hydrogen bonds, isoI being predominantly under the lactam form. The stability of the isoI/T and isoI/G pairs can be accounted for by hydrogen bonding schemes involving also the lactam form of isoI. These results are in agreement with the observations regarding the tautomeric equilibrium of isoI[17] and isoG[19,20] establishing that the predominant form of these purines in polar solvents such as water is the lactam one.

In summary, an ambiguous base pairing caused by the N(1)-H ⇔ O(2)-H tautomerism is observed for isoI, making it a potent mutagen tool. This point, as the key point of specificity, remain to be assessed and will be the subject of a separate paper.

EXPERIMENTAL

General materials and methods. NMR spectra were recorded using a Bruker AC300P spectrometer. Chemical shifts are given in ppm (δ) relative to residual solvent peak for ^{1}H and ^{13}C spectra and to 85% H_3PO_4 as an external standard for ^{31}P spectra. Thin layer chromatography was performed on Merck silica gel 60F-254 aluminium-backed plates (0.2 mm) and vizualization was performed by UV illumination and by staining with *p*-anisaldehyde/EtOH/sulfuric acid. Column chromatography was performed with Merck silica gel 60 (70-230 or 230-400 mesh). Reverse phase HPLC was performed on a Nucleosil 5C18 column (Macherey, 10x250 mm) using a linear gradient of acetonitrile in 10 mM triethylammonium acetate buffer (TEAA) pH 7.0 as eluent over 20 min. with a flow rate of 5.5 mL/min. (prep.) or 1.0 mL/min. (anal.). Phosphoramidites were purchased

from Eurogentech. Electrospray mass spectra were recorded in the positive-ion or negative-ion mode on a Perkin Elmer Sciex API 365 spectrometrer. FAB mass spectra were recorded in the positive mode using a 3-nitrobenzyl alcohol as matrix and are reported as m/z, relative intensity, assignement.

Mitsunobu reaction. Diethylazodicarboxylate (0.33 mL, 1.65 mmol) was slowly added at 4°C to a mixture of compound **2** (0.37 g, 1.11 mmol), triphenylphosphine (0.43 g, 1.65 mmol) and *p*-nitrophenylethanol (0.76 g, 1.65 mmol) in dry dioxan or THF (22 mL). After 30 min., the solution was reduced to a small volume (5 mL) and CH_2Cl_2 (150 mL) was added. The solution was washed in turn with 5% $NaHCO_3$ (150 mL), then with water (150 mL). The dried organic layer was concentrated under reduced pressure and purified by silica gel column chromatography eluted with CH_2Cl_2/MeOH. Two compounds were isolated that were still contaminated with triphenylphosphine. The mixture was treated with 33% aqueous NH_4OH in MeOH for 30 min. The solvent was removed and the residue was purified by silica gel column chromatography eluted with CH_2Cl_2/MeOH to give **3** (60% using THF, 52% using dioxan in 2 steps) and **4** (13% using THF, 15% using dioxan in 2 steps).

O^2-p-nitrophenylethyl-2'-deoxyisoinosine (3). R_f (CH_2Cl_2/MeOH:90/10): 0.15; UV (MeOH): λ_{max} 210 nm ($31x10^3$), 278 nm ($17.9x10^3$); ^{1}H-NMR (DMSO-d6): 2.31 and 2.74 (2m, 2x1H, H2' and H2"); 3.24 (t, J=6.4 Hz, 2H, $OCH_2C\underline{H_2}$); 3.56 (m, 2H, H5' and H5"); 3.87 (m, 1H, H4'); 4.43 (m, 1H, H3'); 4.63 (t, J=6.4 Hz, 2H, OCH_2); 4.96 (t, J=5.5 Hz, 1H, 5'OH); 5.37 (d, J=4.1 Hz, 1H, 3'OH); 6.36 (t, J=6.8 Hz, 1H, H1'); 7.63 (d, J=8.6 Hz, 2H, Hmeta NPE); 8.24 (d, J=8.5 Hz, 2H, Hortho NPE); 8.59 (s, 1H, H8); 8.91 (s, 1H, H6); ^{13}C-NMR (DMSO-d6): 34.42 ($OCH_2\underline{C}H_2$); 39.32 (C2'); 61.68 (C5'); 67.14 ($O\underline{C}H_2CH_2$); 70.75 (C3'); 83.30 (C1'); 87.96 (C4'); 123.51 (Cmeta NPE); 130.36 (Cortho NPE); 130.45 (C5); 144.3 (C6); 146.31 and 147.10 (C1 and Cpara NPE); 149.79 (C8); 152.77 (C2); 160.81 (C4); Anal. Calcd. for $C_{18}H_{21}N_5O_6$ + 1/2 H_2O: C, 52.68; H, 4.91; N, 17.07; Found: C, 52.58; H, 4.99; N, 17.55; MS (ES): 402.1 $(M+H)^+$.

N^1-p-nitrophenylethyl-2'-deoxyisoinosine (4). R_f (CH_2Cl_2/MeOH:90/10): 0.10; ^{1}H-NMR (DMSO-d6): 2.25 and 2.60 (2m, 2x1H, H2' and H2"); 3.15 (t, J=7.2 Hz, 2H, $NCH_2C\underline{H_2}$); 3.55 (m, 2H, H5' and H5"); 3.85 (m, 1H, H4'); 4.25 (t, J=7.2 Hz, 2H, $NC\underline{H_2}CH_2$); 4.40 (m, 1H, H3'); 5.05 (m, 1H, 5'OH); 5.35 (d, J=3.5 Hz, 1H, 3'OH); 6.15 (t, J=6.8 Hz, 1H, H1'); 7.55 (d, J=8.5 Hz, 2H, Hortho NPE); 8.20 (d, J=8.5 Hz, 2H, Hmeta NPE); 8.40 (s, 1H, H8); 8.70 (s, 1H, H6); ^{13}C-NMR (DMSO-d6): 34.25 ($NCH_2\underline{C}H_2$); 39.15 (C2'); 51.97 ($N\underline{C}H_2CH_2$); 61.80 (C5'); 70.88 (C3'); 83.06 (C1'); 88.03 (C4'); 123.26 (C5); 123.80 (Cmeta NPE); 130.48 (Cortho NPE); 141.76 (C6); 146.17 (C8); 146.47 and 146.52 (C1 and Cpara NPE); 154.9 (C2); 158.52 (C4); MS (ES): 402.24 $(M+H)^+$.

O^2-diphenylcarbamoyl-2'-deoxyisoinosine (5). To compound **2** (0.53 g, 1.57 mmol) in anhydrous pyridine (10 mL) was added diisopropylethylamine (0.33 mL, 1.9 mmol) followed by diphenylcarbamoyl chloride (0.54 g, 2.35 mmol). After 20 min., CH_2Cl_2 (80 mL) was added and the mixture was poured into saturated $NaHCO_3$. The organic layer was washed with water, dried and concentrated under reduced pressure. The resulting foam was dissolved in a mixture of pyridine/ethanol (5/2 mL), cooled to 4°C, and 2N NaOH (5 mL) was added. After 10 min., the mixture was neutralized by adding 50W Dowex (pyridinium form). The solution was filtered and the filtrate was concentrated under reduced pressure. Purification by silica gel column chromatography eluted with CH_2Cl_2/MeOH gave **5** (0.47 g, 70% in 2 steps). R_f (CH_2Cl_2/CH_3OH:90/10): 0.49; ^{1}H-NMR (DMSO-d6): 2.36 and 2.73 (2m, 2x1H, H2' and H2"); 3.56 (m, 1H, H5'); 3.61 (m, 1H, H5"); 3.90 (m, 1H, H4'); 4.45 (m, 1H, H3'); 4.99 (t, J=5.5 Hz, 1H, 5'OH); 5.39 (d, J=4.3 Hz, 1H, 3'OH); 6.43

(t, J=6.6 Hz, 1H, H1'); 7,32 (m, 2H, Hpara Ph); 7,47 (m, 8H, Hortho and meta Ph); 8,84 (s, 1H, H8); 9,14 (s, 1H, H6); ^{13}C-NMR (DMSO-d6): 39.49 (C2'); 61.70 (C5'); 70.77 (C3'); 83.73 (C1'); 88.32 (C4'); 127.0 and 129.62 (Ph); 133.10 (C5); 141.96 (Ph); 146.31 (C6); 150.07 (C8); 151.61 (C2); 152.89 (C4); 155.67 ($CONPh_2$). MS (ES): 448.0 $(M+H)^+$.

5'-O-(4,4'-dimethoxytrityl)-O^2-p-nitrophenylethyl-2'-deoxyisoinosine (6). Compound **3** (0.24 g, 0.62 mmol) was dried by coevaporations with dry pyridine (2x2 mL) and then dissolved in dry pyridine (15 mL). 4,4'-Dimethoxytrityl chloride (0.30 g, 0.77 mmol) was added and the reaction was allowed to stir at room temperature. After 6 h, additionnal DMTrCl (0.35 mmol) was added and the mixture was stirred overnight. Methanol (5 mL) was then added to the mixture and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2, washed in turn with aqueous $NaHCO_3$, water and dried (Na_2SO_4). The product was then purified by silica gel column chromatography eluted with CH_2Cl_2/MeOH. To the resulting foam dissolved in CH_2Cl_2 was added vigorously petroleum ether. The precipitated product was filtered off and dried *in vacuo* to give compound **6** as a white powder (0.25 g, 57%) and unreacted **3** (30%). R*f* (CH_2Cl_2/MeOH: 90/10): 0.29; ^{1}H-NMR (DMSO-d6): 2.37 and 2.92 (2m, 2x1H, H2' and H2"); 3.15 (m, 4H, $OCH_2C\underline{H}_2$, H5' and H5"); 3.70 (2s, 6H, OCH_3); 4.00 (m, 1H, H4'); 4.50 (m, 3H, $OC\underline{H}_2CH_2$ and H3'); 5.40 (d, J=4.5 Hz, 1H, 3'OH); 6.40 (t, J=6.2 Hz, 1H, H1'); 6.70 and 6.75 (2 d, J=8.6 Hz, 2x2H, H Arom. DMT); 7.15 (m, 7H, H Arom. DMT); 7.25 (m, 2H, H Arom. DMT); 7.55 (d, J=8.6 Hz, 2H, Hortho NPE); 8.15 (d, J=8.5 Hz, 2H, Hmeta NPE); 8.50 (s, 1H, H8); 8.93 (s, 1H, H6); ^{13}C-NMR: 34.13 ($OCH_2\underline{C}H_2$); 38.10 (C2'); 54.82 (OCH_3); 64.10 (C5'); 66.78 ($O\underline{C}H_2$); 70.44 (C3'); 83.35 (C1'); 85.19 (Cq DMT); 85.83 (C4'); 112.83 (C3 and C5 DMT); 123.30 (Cmeta NPE); 126.45-129.53 (C DMT); 130.05 (Cortho NPE); 130.47 (C5); 135.35 (C1 DMT); 144.41 (C6); 144.76 (C1' DMT); 146.08 and 146.74 (C1 and Cpara NPE); 149.72 (C8); 152.36 (C2); 157.78 (C4 DMT); 160.52 (C4); MS (FAB): 704.3 (23.1) $(M+H)^+$, 303.1 (100) $(DMT)^+$.

5'-O-(4,4'-dimethoxytrityl)-O^2-diphenylcarbamoyl-2'-deoxyisoinosine (7). Compound **5** (0.76 g, 1.7 mmol) was treated with 4,4'-dimethoxytrityl chloride (2.0 mmol) as described for **6** to give **7** as a slighty yellow powder (0.72 g, 57%) and unreacted **5** (30%). R*f* (CH_2Cl_2/MeOH:90/10): 0.57; ^{1}H-NMR (DMSO-d6): 2.45 and 2.85 (2m, 2x1H, H2' and H2"); 3.15 (m, 2H, H5' and H5"); 3.70 (s, 6H, OCH_3); 4.00 (m, 1H, H4'); 4.48 (m, 1H, H3'); 5.44 (d, J = 4.7 Hz, 1H, 3'OH); 6.44 (t, J = 6 Hz, 1H, H1'); 6.72 and 6.76 (each d, J = 9 Hz, 2x2H, H Arom. DMT); 7.17 (m, 7H, H Arom. DMT); 7.30 (m, 4H, H Arom. DMT and Hpara Ph); 7.43 (m, 8H, Hortho and meta Ph); 8.73 (s, 1H, H8); 9.13 (s, 1H, H6). ^{13}C-NMR (DMSO-d6): 38.60 (C2'); 54.88 (OCH_3); 63.89 (C5'); 70.24 (C3'); 83.13 (C1'); 85.30 (Cq DMT); 85.95 (C4'); 112.93 (C3 and C5 DMT); 126.48-129.57 (Ph and DMT); 132.77 (C5); 135.34 (C1 DMT); 141.65 (Ph); 144.32 (C1' DMT); 145.92 (C6); 149.78 (C8); 151.28 (C2); 152.55 (C4); 155.47 ($CONPh_2$); 157.84 (C4 DMT); MS (ES): 748.6 $(M-H)^-$.

5'-O-(4,4'-dimethoxytrityl)-O^2-p-nitrophenylethyl-2'-deoxyisoinosine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (8). Compound **6** (0.20 g, 0.29 mmol) and diisopropylammonium tetrazolide (0.025 g, 0.15 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphoramidite (0.11 g, 0.32 mmol) was added under argon at room temperature. After 3 h, CH_2Cl_2 was added and the solution was washed in turn with 2% Na_2CO_3, with sat. NaCl solution, then dried and concentrated to dryness. The resulting foam was purified by flash chromatography (CH_2Cl_2 /EtOAc/Et_3N:45/45/10). The resulting foam was dissolved in CH_2Cl_2 and added under vigourous stirring into cooled pentane (-70°C) to give **8** (0.16 g, 60%). R*f* (CH_2Cl_2/EtOAc/Et_3N:45/45/10): 0.77 and 0.69; ^{1}H-NMR

(DMSO-d6): 1.10 (m, 12H, CH_3 of iPr); 2.55-2.95 (m, 4H, H2', H2" and CH_2CN); 3.40-3.80 (m, 12H, OCH_2, OCH_3, H5', H5" and $OCH_2\underline{CH_2}$); 4.10 (m, 1H, H4'); 4.50 (m, 2H, $OC\underline{H_2}CH_2$); 4.80 (m, 1H, H3'); 6.43 (dd, J=5.8 Hz and J=12.2 Hz, 1H, H1'); 6.70 (m, 4H, H Arom. DMT); 7.15 (m, 7H, H Arom. DMT); 7.30 (m, 2H, H Arom. DMT); 7.53 (dd, J=3.1 Hz and J=7.0 Hz, 2H, Hortho NPE); 8.15 (d, J=8.6 Hz, 2H, Hmeta NPE); 8.53 and 8.54 (2s, 1H, H8, diast. R and S); 8.941 and 8.945 (2s, 1H, H6, diast. R and S); ^{31}P-NMR (DMSO-d6): 151.11 and 150.24 (diast. R and S); ^{31}P-NMR ($CDCl_3$): 147.05.

5'-O-(4,4'-dimethoxytrityl)-O^2-diphenylcarbamoyl-2'-deoxyisoinosine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (9). Compound **7** (0.30 g, 0.40 mmol) was phophitylated, worked-up as described for **8**. The resulting foam was purified by flash chromatography (CH_2Cl_2/EtOAc/Et_3N:45/45/5) to give **9** as a white foam (0.36 g, 95%). R*f* (CH_2Cl_2/EtOAc/Et_3N:45/45/10): 0.77 and 0.62; ^{1}H-NMR ($CDCl_3$): 1.10-1.35 (m, 12H, CH_3 of iPr); 2.50-2.75 (m, 4H, H2', H2" and CH_2CN) ; 3.25-4.00 (m, 12H, OCH_3, 2xCH iPr, H5', H5", OCH_2); 4.20-4.30 (m, 1H, H4'); 4.75 (m, 1H, H3'); 6.50 (dd, J=6.3 Hz, J=11.4 Hz, 1H, H1'); 6.75 (d, 4H, H Arom. DMT); 7.25 (m, 9H, H Arom. DMT and Ph); 7.40 (m, 10H, H Arom. DMT and Ph); 8.25 and 8.30 (2s, 1H, H8, diast. R and S); 9.00 (2s, 1H, H6, diast. R and S); ^{31}P-NMR ($CDCl_3$): 147.15; MS (FAB): 950.8 (3.1) $(M+H)^+$, 303.2 (100) $(DMT)^+$.

Oligonucleotides synthesis. Oligonucleotides were synthesized by the phosphoramidite method on an automated DNA synthesizer (Expedite Millipore) on the 1-μmol-scale. The modified phosphoramidite building block **9** was dissolved in dry acetonitrile at a concentration of 0.15M. Oligonucleotides were cleaved from the support with concentrated ammonia at room temperature for 4x15 min and then deprotected by heating the ammonia solution at 55°C for 5-8 h. DMT-on oligomers were purified by reverse phase HPLC with a linear gradient of acetonitrile in TEAA over 20 min. After treatment with 80% AcOH, oligomers were further purified by HPLC. The yield was about 10-22% (20-40 OD_{260} units/oligonucleotide). Heptadecanucleotides containing modified nucleosides were analyzed by positive-ion ES mass spectroscopy. Observed MW for 5'-ACTTGGCCXCCATTTTG-3' when X=^{5Me}isoC: 5126.57±0.25 (calcd 5126.38) and when X=isoI: 5137.44±0.52 (calcd 5137.37); observed MW for 5'-CAAAAGGYGGCCAAGT-3' when Y=^{5Me}isoC: 5242.54±0.61 (calcd 5242.49) and when Y=isoI: 5253.49±0.85 (calcd 5253.47).

Composition analysis. Oligomers containing isoI (1 OD_{260}) were digested with snake venom phosphodiesterase (22.5 mU) and alkaline phosphatase (1 U) in 100 mM Tris-HCl (pH 9) buffer (0.4 mL) at 37 °C for 3 h. The digest solutions were passed through a sep-pak cartridge and then analyzed by reverse phase HPLC. The peaks were detected by a Waters 990 photodiode Array Detector. The relative composition of each oligomer was inferred by dividing the integrated peak area at 254 nm of each nucleoside by its extinction coefficient.

Thermal denaturation studies. Freshly stock solutions were prepared by dissolving each oligonucleotide in water and the concentration was determined by UV spectroscopy at 260 nm. The extinction coefficient of oligonucleotide at 25°C was taken as the sum of the mononucleotides in the strand, ^{5Me}isoC was assimilated to C and isoI to an abasic site. The oligomers were mixed together each at a final concentration of 1 μM in 10 mM sodium cacodylate, 100 mM sodium chloride at pH 7.1 and allowed to incubate at 90°C for 15 min. Absorbance versus temperature was recorded on a Kontron Uvikon 941 spectrometer, the temperature control was done with a Huber PD415 temperature programmer connected to a refrigerated ethylene glycol-water bath. The heating rate was 0.15°C/min. Absorbance measurements were taken at 45 s intervals. Cooling profiles were identical to melting profiles.

Thermodynamic analysis. Thermodynamic data were calculated from one melting profile. The van't Hoff transition enthalpy was calculated from the differential melting curves using the equation ΔH_{VH}(J/mol) = $-18.28/(1/T_{max}-1/T_2)$, where T_{max} is the temperature (in Kelvin) at the maximum and T_2 is the temperature at the upper half-height of the differential melting curves. For T_{max} and T_2 calculations, data from the UV melting measurements were exported into KaleidaGraph (Synergy Software). As a compromise, $\partial\alpha/\partial(1/T)$ where α is the fraction of strands bound in a helix, was substituted with $\partial A/\partial T$ (A = absorbance) in the data process. The free energy at T = Tmax where $\alpha = 0.414$ is calculated from the equations $\Delta G_{Tmax} = - RT_{max}\ln K_{Tmax}$ and $K_{Tmax} = 2\alpha(1-\alpha)^2 C_T$ with $C_T = 2\times10^{-6}$ M. The entropy of transition is calculated from $\Delta S = (\Delta H-\Delta G)/T_{max}$. The value of ΔG at 298 K was calculated using standard thermodynamic expressions. The melting temperature Tm at $\alpha = 0.5$ can be calculated from T_{max} using $Tm = T_{max}(1-T_{max}/\Delta H)$.

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