Supplementary Information

Formation of new base pairs between inosine and 5-methyl-2-thiocytidine derivatives

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General Remarks

$^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded at 270, 68 and 109 MHz, respectively. The chemical shifts were measured from tetramethylsilane for $^1$H NMR spectra and CDCl$_3$ (77 ppm) for $^{13}$C NMR spectra and 85% phosphoric acid (0 ppm) for $^{31}$P NMR spectra. Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd., and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Anion-exchange HPLC was done on a Waters Alliance system with a Waters 3D UV detector and a Gen-PakTM FAX column (Waters, 4.6 x 100 mm). A linear gradient (10-60%) of Solvent I (1 M NaCl in 25 mM phosphate buffer (pH 6.0)) in solvent II (25 mM phosphate buffer (pH 6.0)) was used at 50 °C at a flow rate of 1.0 mL/min for 45 min. ESI mass was performed by use of Mariner™ (PerSeptive Biosystems Inc.). MALDI-TOF mass was performed by use of Bruker Daltonics [Matrix: 3-hydroxypicolinic acid (100mg/ml) in H$_2$O-diammoniumhydrogen citrate (100 mg/ml) in H$_2$O (10:1, v/v)]. Highly cross-linked polystyrene was purchased from ABI.

Synthesis of DNA probes 2-4 and RNA probe 2.

The synthesis of probes was carried out on nucleoside-loaded CPG resins (1 µmol scale) in ABI 392 DNA synthesizer. (Activator: 0.25 M solution of 5-benzylthio-1H-tetrazole, Oxidizer: 0.02 M solution of I$_2$) The fully protected oligomer after chain elongation was deprotected and released from the resin by treatment with a 28% ammonia solution at room temperature for 8 h. The crude mixture was purified by Sep-Pak C18 cartridge and anion-exchange HPLC.

DNA probe 2: d[GCCm$^5$s$^2$CGTGAC], Yield 10%, MALDI-TOF Mass (M+H) calcd for $[C_{87}H_{112}N_{34}O_{51}P_{8}S + H]^+$ 2729, found 2733

DNA probe 4: d[TTTC$^5$s$^2$Ts$^2$Tm$^5$s$^2$Cs$^2$Ts$^2$TCTT], Yield 70%, MALDI-TOF Mass (M+H) calcd for $[C_{118}H_{156}N_{27}O_{24}P_{11}S_{5} + H]^+$ 3637, found 3639
RNA probe 2: 2'-MeO-[GCCm5s2CGTGAC], Yield 41%, MALDI-TOF Mass (M+H) calcd for [C95H128N34O60P8S + H]^+ 2986, found 2986

**Figure S1.** Anion exchange HPLC profiles of purified oligomers obtained in synthesis of a) DNA probe 2, b) RNA probe 2, and d) DNA probe 4.

**T_m Measurement**

An appropriate oligonucleotide (2 µM) and its complementary 2 µM DNA or RNA probes were dissolved in a buffer consisting of 150 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA adjusted to pH 7.0. The solution was kept at 80 °C for 10 min for complete dissociation of the duplex to single strands, cooled at the rate of -1.0 °C/min, and kept at 5 °C for 10 min. After that, the melting temperatures (T_m) were determined at 260 nm using a UV spectrometer (Pharma Spec UV-1700™, Shimadzu) by increasing the temperature at the rate of 1.0 °C/min.
Figure S2. Melting curves of the duplexes forming between DNA probe 1 and RNA targets 1-3.

Figure S3. Melting curves of the duplexes forming between DNA probe 2 and RNA targets 1-3.
Figure S4. Melting curves of the duplexes forming between RNA probe 1 and RNA targets 1-3.

Figure S5. Melting curves of the duplexes forming between RNA probe 2 and RNA targets 1-3.