Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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# 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 Aliance 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$^{TM}$ (PerSeptive Biosystems Inc.). MALDI-TOF mass was performed by use of Bruker Daltonics [Matrix: 3-hydoroxypicolinic acid (100mg/ml) in $H_2O$-diammoniumhydrogen citrate (100 mg/ml) in $H_2O$ (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 $[C_{87}H_{112}N_{34}O_{51}P_8S + H]^+$ 2729, found 2733

DNA probe 4: d[TTTCs$^2$Ts$^2$Tm$^5$s$^2$Cs$^2$Ts$^2$TCTT], Yield 70%, MALDI-TOF Mass (M+H) calcd for $[C_{118}H_{156}N_{27}O_{74}P_{11}S_5 + H]^+$ 3637, found 3639

RNA probe 2: 2'-MeO-[GCCm$^5$s$^2$CGTGAC], Yield 41%, MALDI-TOF Mass (M+H) calcd for $[C_{95}H_{128}N_{34}O_{60}P_8S + H]^+$ 2986, found 2986

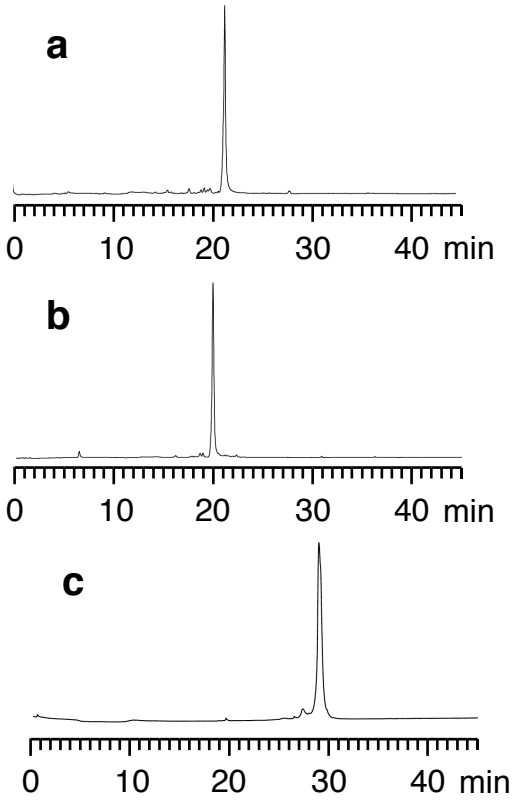


**Figure S1.** Anion exchange HPLC profiles of purified oligomers obtained in synthesis of a) DNA probe 2, b) RNA probe 2, and d) DNA probe 4.

### $T_m$ 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.

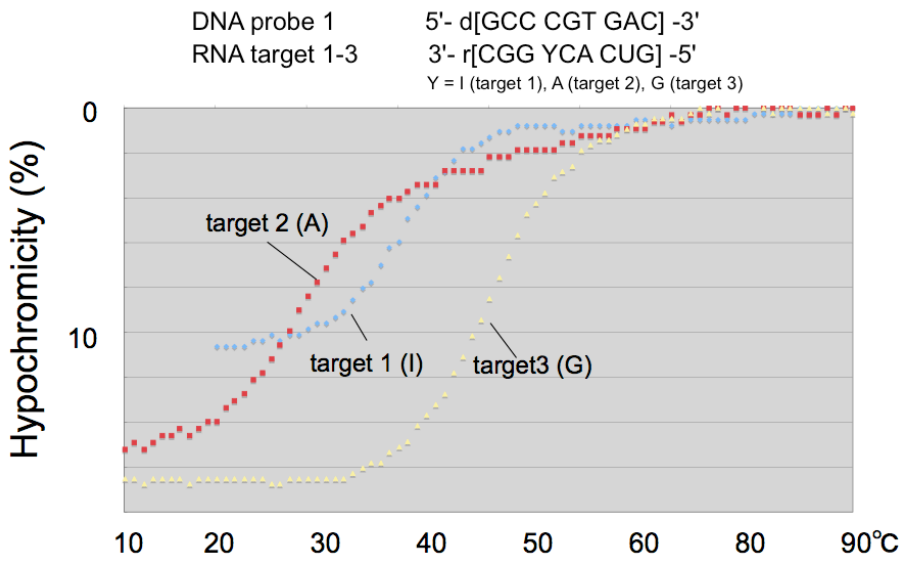


**Figure S2**. Melting curves of the duplexes forming between DNA probe 1 and RNA targets 1-3.

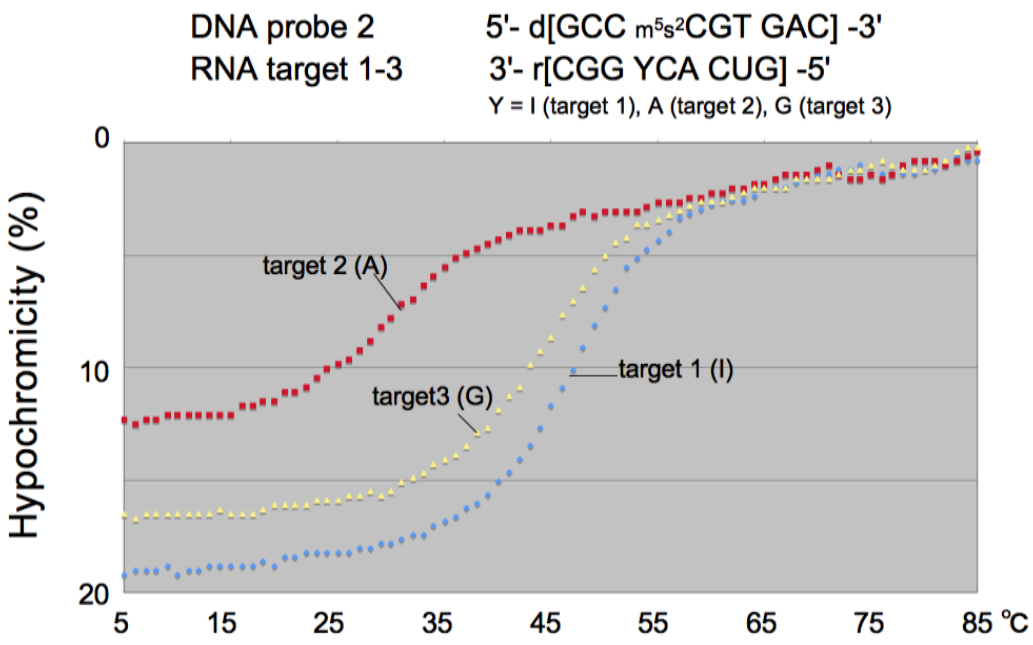


**Figure S3.** Melting curves of the duplexes forming between DNA probe 2 and RNA targets 1-3.

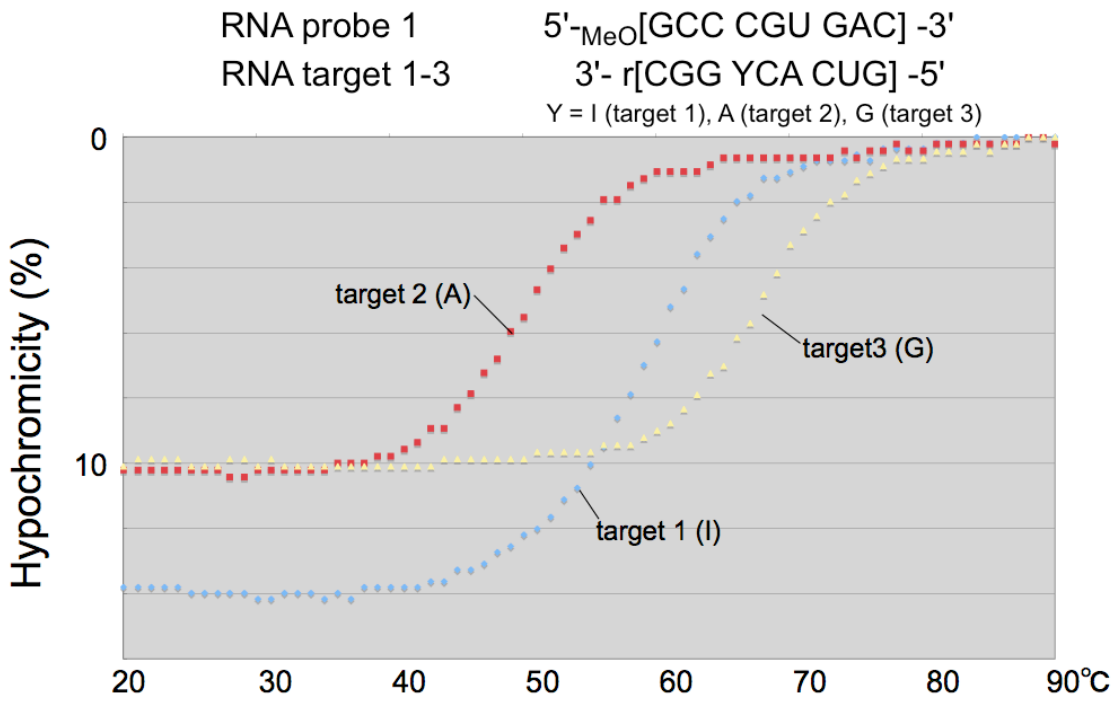


**Figure S4.** Melting curves of the duplexes forming between RNA probe 1 and RNA targets 1-3.

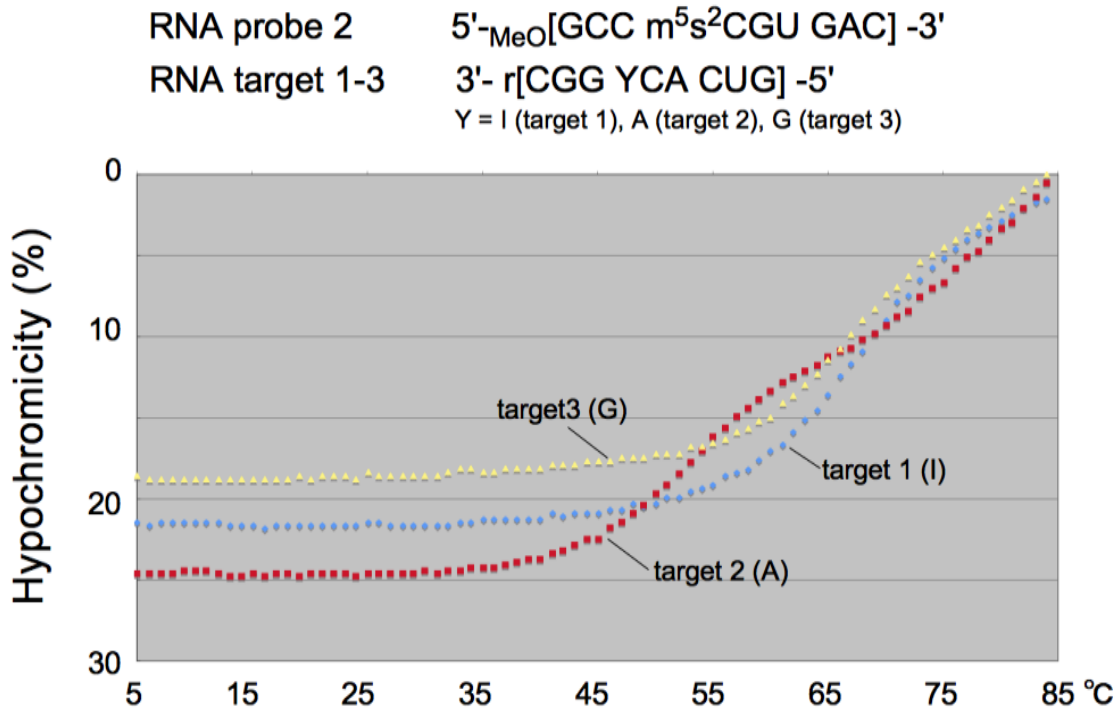


**Figure S5.** Melting curves of the duplexes forming between RNA probe 2 and RNA targets 1-3.