Supporting Information

N-Methyl substituted 2',4'-BNA^{NC}: a highly nuclease-resistance nucleic acid analogue with high-affinity RNA selective hybridization

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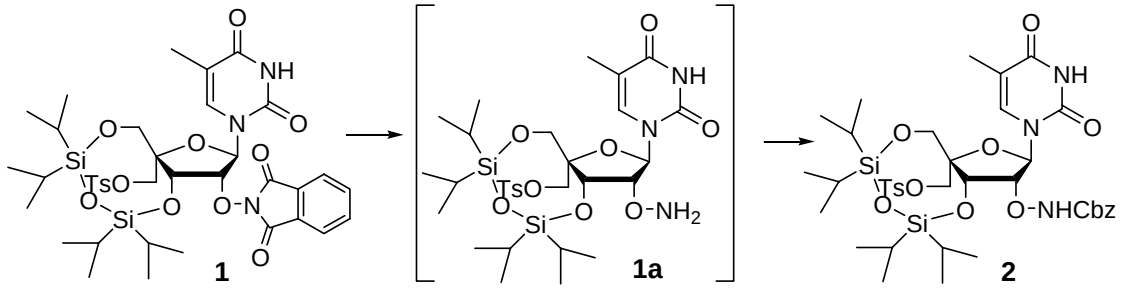
1. General Methods:

Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under an N_2 atmosphere. Dichloromethane, THF and pyridine were distilled from CaH_2. Dry MeCN was used in the form in which it was purchased. ^{1}H NMR (300 or 270 MHz), ^{13}C NMR (75 or 67 MHz) and ^{31}P NMR spectra (202 MHz) were recorded on JEOL EX-270, JEOL-AL-300 and JEOL GX-500 spectrometers, respectively. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane for ^{1}H or $CHCl_3$ (δ = 77.0) for ^{13}C NMR spectra. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. Optical rotations were recorded on a JASCO DIP-370 instrument. Mass spectra were measured on JEOL JMS-600 or JMS-700 mass spectrometers. For column chromatography, silica gel FL 100D was used.

2. Synthesis of 2',4'-BNA^{NC}[N-Me] thymine monomer and phosphoroamidite:

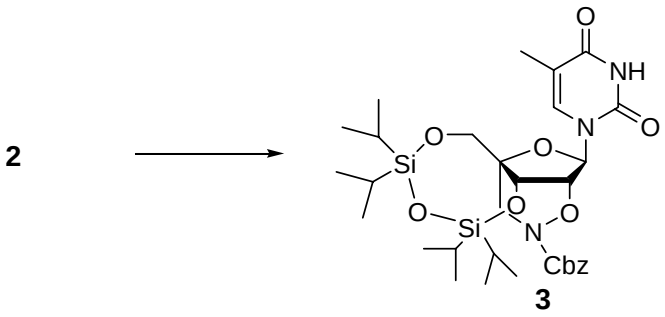
2'-*O*-(*N*-Benzyloxycarbonylamino)-5-methyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4'-(*p*-toluenesulphonyloxymethyl)uridine (2).



Hydrazine monohydrate (0.12 ml, 2.38 mmol, 1.25 mmol) was added to a stirred solution of **1**[1] (1.16 g, 1.40 mmol) in ethanol (35 ml) and the mixture was stirred at room temperature for 10 min. The mixture was filtered to separate the precipitate, and the filtrate was concentrated to remove ethanol. The concentrated mixture was then diluted with EtOAc, washed with water and brine, dried over Na_2SO_4 and concentrated again to give crude amine **1a**[1] (0.93 g), which was employed for the next reaction without further purification.

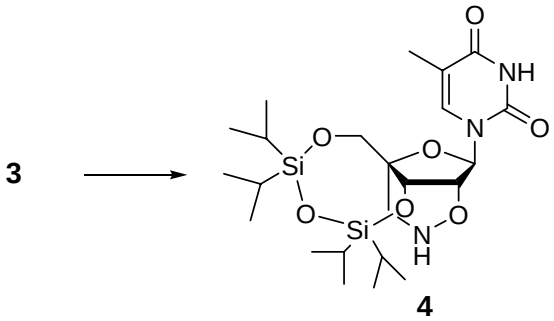
To a solution of the crude **1a** (0.93 g) in CH_2Cl_2 (15 ml) were added an aqueous solution of $NaHCO_3$ (4.0 ml, 4.2 mmol) and benzyl chloroformate (0.30 ml, 2.1 mmol) under ice cooling, and the mixture was stirred at the same temperature for 1 h. The reaction mixture was then poured into saturated $NaHCO_3$ solution and extracted with EtOAc. The organic phase was washed with water and brine, dried over Na_2SO_4 and concentrated. The resultant residue was purified by column chromatography (hexane/ EtOAc = 1/4) to give **2** (0.92 g, 94% from **1**) as a white powder: Mp 82-84 °C; $[\alpha]_D^{25}$ = -15.3 (*c* = 0.94, $CHCl_3$); IR (KBr): 3198, 3065, 2950, 2868, 1703, 1600, 1462, 1365, 1245, 1177 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94-1.10 (m, 28H), 1.86 (s, 3H), 2.43 (s, 3H), 3.82 (d, *J* = 12 Hz, 1H), 3.87 (d, *J* = 12 Hz, 1H), 4.25 (d, *J* = 11 Hz, 1H), 4.54 (d, *J* = 11 Hz, 1H), 4.72 (dd, *J* = 1, 6 Hz, 1H), 5.01 (d, *J* = 6 Hz, 1H), 5.16 (d, *J* = 12 Hz, 1H), 5.18 (d, *J* = 12 Hz, 1H), 5.39 (d, *J* = 1 Hz, 1H), 6.96 (br s, 1H), 7.31 (d, *J* = 8 Hz, 2H), 7.34 (s, 5H), 7.80 (d, *J* = 8 Hz, 2H), 8.02 (s, 1H), 8.69 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.2, 12.3, 12.5, 12.9, 13.1, 17.0, 17.0, 17.1, 17.1, 17.1, 17.3, 17.4, 21.6, 64.1, 67.7, 68.2, 74.3, 84.5, 87.2, 92.2, 110.5, 127.9, 128.1, 128.3, 128.4, 129.6, 132.8, 135.2, 139.3, 144.4, 149.3, 156.7, 163.9; MS (FAB) *m/z* 834 ($M+H^+$), 856 ($M+Na^+$). *Anal*. Calcd for $C_{38}H_{55}N_3O_{12}SSi_2$: C, 54.72; H, 6.65; N, 5.04. Found: C, 54.62; H, 6.58; N, 4.93.

2'-*O*,4'-*C*-(*N*-Benzyloxycarbonylaminomethylene)-5-methyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3).



A solution of **2** (3.81 g, 4.57 mmol) in THF (15 ml) was added dropwise at 0 °C to a stirred suspension of NaH (60% in oil, 0.55 g, 13.7 mmol) in THF (25 ml) and the mixture was stirred, initially at 0 °C for 1 h and then at room temperature, for an additional 5 h. The reaction mixture was neutralized with saturated oxalic acid solution and extracted with EtOAc. The organic phase was washed with water and brine, dried (Na_2SO_4) and concentrated. The concentrate was purified by column chromatography ($CHCl_3$ 100% to $CHCl_3$/ EtOAc = 99/1) to afford 2.87 g (57%) of **3** as a white powder: Mp 98-100 °C; $[\alpha]_D^{25}$ = -14.0 (*c* = 0.78, $CHCl_3$); IR (KBr): 2946, 2869, 1698, 1464, 1361, 1271, 1218, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99-1.12 (m, 28H), 1.91 (s, 3H), 3.63 (d, *J* = 11 Hz, 1H), 3.69 (d, *J* = 12 Hz, 1H), 3.85 (d, *J* = 11 Hz, 1H), 4.11 (d, *J* = 12 Hz, 1H), 4.11 (d, *J* = 3 Hz, 1H), 4.55 (d, *J* = 3 Hz, 1H), 5.21 (d, *J* = 12 Hz, 1H), 5.28 (d, *J* = 12 Hz, 1H), 5.97 (s, 1H), 7.23-7.45 (m, 5H), 7.59 (d, *J* = 1 Hz, 1H), 8.07 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.1, 12.7, 12.7, 12.7, 13.3, 16.8, 16.9, 17.0, 17.1, 17.2, 17.4, 46.4, 59.5, 64.2, 68.1, 82.0, 82.5, 85.5, 110.4, 128.2, 123.3, 128.5, 134.4, 135.6, 149.5, 155.0, 164.0; MS (FAB) *m/z* 662 ($M+H^+$). *Anal.* Calcd for $C_{31}H_{47}N_3O_9Si_2 \cdot 1/9\ H_2O$: C, 56.08; H, 7.17; N, 6.33. Found: C, 55.79; H, 7.12; N, 6.23.

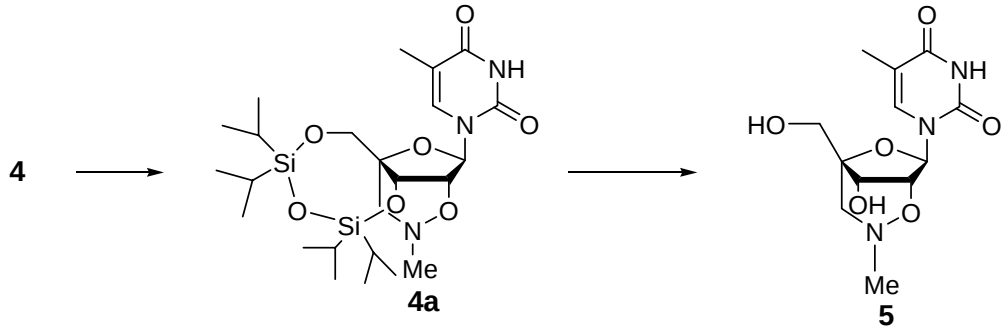
2'-*O*,4'-*C*-Aminomethylene-5-methyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (4).



Boron trichloride (1 M in hexane, 5.29 ml, 5.29 mmol) was added at 0 °C to a stirred solution of **3** (0.35 g, 0.53 mmol) in CH_2Cl_2 (10 ml) and the whole was stirred at 0 °C for 1 h. After addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with EtOAc. The organic phase was

washed with water and brine, dried over Na_2SO_4, and concentrated. The concentrate was purified by column chromatography ($CHCl_3$/MeOH =50/1) to afford **4** (0.27 g, 96%) as a white powder: Mp 113-115 °C; $[\alpha]_D^{26}$ = -6.1 (*c* = 0.93, $CHCl_3$); IR (KBr): 3479, 3262, 3071, 2946, 2868, 1698, 1464, 1388, 1363, 1272, 1229, 1165, 1143 cm^{-1}; ^1H NMR ($CHCl_3$) δ 0.94-1.13 (m, 28H), 1.93 (d, *J* = 1 Hz, 3H), 2.53 (d, *J* = 13 Hz, 1H), 3.67 (d, *J* = 13 Hz, 1H), 3.68 (d, *J* = 13 Hz, 1H), 4.06 (d, *J* = 13 Hz, 1H), 4.10 (d, *J* = 3 Hz, 1H), 4.35 (d, *J* = 3 Hz, 1H), 6.16 (s, 1H), 7.73 (d, *J* = 1 Hz, 1H), 8.31 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.2, 12.8, 13.0, 13.4, 16.9, 17.0, 17.1,17.1, 17.1, 17.3, 17.3, 17.5, 48.6, 60.0, 64.4, 81.0, 82.5, 85.4, 110.3, 134.3, 150.0, 164.2; MS (FAB) *m/z* 528 ($M+H^+$). *Anal.* Calcd for $C_{239}H_{41}N_3O_7Si_2 \cdot 1/10H_2O$: C, 52.17; H, 7.84; N, 7.93. Found: C, 52.02; H, 7.78; N, 7.89.

5-Methyl-2'-*O*,4'-*C*-(*N*-methylaminomethylene)uridine (5).



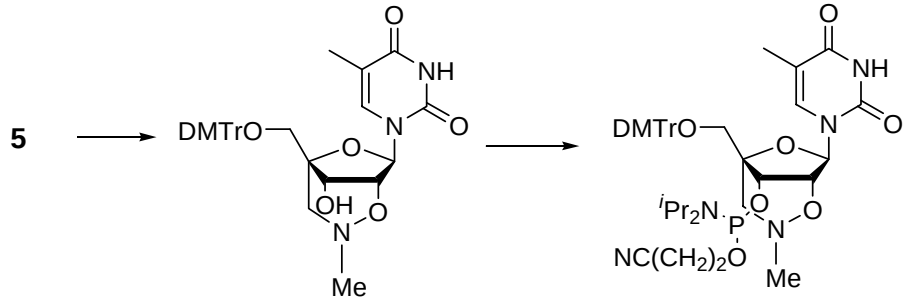
4a **5**

A 20% solution of formalin (0.06 ml, 0.40 mmol) was added at room temperature to a stirred solution of **3** (0.19 g, 0.36 mmol) in methanolic solution of pyridinium *p*-toluenesulfonate (1 M, 3.6 ml, 3.6 mmol) and the mixture was stirred for 10 min. The mixture was then cooled to 0 °C and sodium cyanoborohydrate (45 mg, 0.72 mmol) was added to the reaction mixture. After stirring at 0 °C for 1 h, the reaction mixture was diluted with EtOAc, washed periodically with water, saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4 and concentrated. The resultant residue was purified by column chromatography (hexane/ EtOAc = 2/1) to give compound **4a** (-5-methyl-2'-*O*,4'-*C*-(*N*-methylaminomethylene)-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine) (0.19 g, quant.) as a white powder: Mp 180-181 °C; $[\alpha]_D^{22}$ = -14.3 (*c* = 0.91, $CHCl_3$); IR (KBr): 3172, 2945, 2868, 1697, 1464, 1388, 1267, 1228, 1165 cm^{-1}; ^1H NMR ($CHCl_3$) δ 0.99-1.12 (m, 28H), 1.92 (d, *J* = 1 Hz, 1H), 2.60 (d, *J* = 11 Hz, 1H), 2.75 (s, 3H), 2.91 (d, *J* = 11 Hz, 1H), 3.67 (d, *J* = 13 Hz, 1H), 3.95 (d, *J* = 3 Hz, 1H), 4.04 (d, *J* = 13 Hz, 1H), 4.33 (d, *J* = 3 Hz, 1H), 6.24 (s, 1H), 7.72 (d, *J* = 1 Hz, 1H), 8.52 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.4, 12.8, 12.9, 13.0, 13.5, 17.1, 17.2, 17.3, 17.3, 17.5, 17.5, 45.6, 57.6, 60.1, 64.3, 80.6, 83.8, 86.0, 109.9, 134.9, 150.0, 164.1; MS (FAB) *m/z* 542 ($M+H^+$). *Anal.* Calcd for $C_{24}H_{43}N_3O_7Si_2$: C, 53.20; H, 8.00; N, 7.76. Found: C, 53.42; H, 7.976; N, 7.78.

Tetrabutylammonium fluoride (1M in THF, 0.17 ml, 0.17 mmol) was added at room temperature

to **4a** (46 mg, 0.085 mmol) in THF (2 ml). After stirring the mixture at room temperature for 5 min, the reaction mixture was concentrated and chromatographed on silica gel (EtOAc /MeOH = 15/1) to afford **5** (25 mg, quant.) as a powder: Mp 254-255 °C; $[\alpha]_D^{23}$ = -19.9 (*c* = 0.92, pyridine); IR (KBr): 3811, 3331, 3066, 2888, 1696, 1470, 1387, 1267, 1229 cm^{-1}; ^1H NMR (pyridine-d_5) δ 1.87 (s, 3H), 2.79 (s, 3H), 2.94 (d, *J* = 11 Hz, 1H), 3.33 (d, *J* = 11 Hz, 1H), 4.23 (s, 2H), 4.72 (d, *J* = 2 Hz, 1H), 4.87 (d, *J* = 2 Hz, 1H), 6.98 (s, 1H), 8.42 (s, 1H); ^{13}C NMR (pyridine-d_5) δ 13.1, 45.8, 58.4, 60.7, 64.7, 82.4, 84.8, 86.4, 109.4, 136.0, 151.6, 165.2; MS (FAB) *m/z* 299 ($M+H^+$). *Anal.* Calcd for $C_{12}H_{17}N_3O_6 \cdot 1/6H_2O$: C, 47.68; H, 5.78; N, 13.90. Found: C, 47.39; H, 5.69; N, 13.74.

3'-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-5-methyl-2'-*O*, 4'-*C*-(*N*-methylaminomethylene)uridine (6).



Next, 4,4'-dimethoxytrityl chloride (0.22 g. 0.64 mmol) was added at room temperature to a stirred solution of **5** (0.16 g, 0.54 mmol) in pyridine (10 ml) and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into saturated $NaHCO_3$ (aq.) solution and extracted with EtOAc. The organic phase was washed with water and brine, dried (Na_2SO_4) and concentrated. The concentrate was purified by column chromatography (1% triethylamine in hexane/ EtOAc = 1/2 to EtOAc /MeOH = 30/1) to afford 0.30 g (93%) of **5a** (5'-*O*-(4,4'-dimethoxytrityl)-5-methyl- 2'-*O*,4'-*C*-(*N*-methylaminomethylene)uridine) as a white powder: Mp 133-134 °C; $[\alpha]_D^{19}$ = -21.3 (*c* = 0.73, $CHCl_3$); IR (KBr): 3183, 3066, 2930, 2837, 1690, 1607, 1509, 1463, 1300, 1253, 1177 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (d, *J* = 1 Hz, 3H), 2.72 (s, 3H), 2.78 (s, 2H) 3.33 (d, *J* = 11 Hz, 1H), 3.35 (d, *J* = 11 Hz, 1H), 3.79 (s, 6H), 4.24 (m, 1H), 4.36 (d, *J* = 3 Hz, 1H), 6.35 (s, 1H), 6.82-6.86 (m, 4H), 7.20-7.47 (m, 9H) 7.76 (d, *J* = 1 Hz, 1H) 8.75 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 12,3, 45.6, 55.2, 58.5, 61.5, 65.4, 81.0 , 83.3, 85.5, 86.6, 110.3, 113.2, 127.0, 127.9,129.9, 129.9, 1135.0, 135.2 , 144.1, 149.7, 158.5, 158.5, 163.8; MS (FAB) *m/z* 601 ($M+H^+$), 624 ($M+Na^+$). HRMS (FAB) Calcd for $C_{33}H_{35}N_3O_8Na$ ($M+H^+$): 624.2322. Found: 624.2299.

Subsequently, 4,5-dicyanoimidazole (40 mg. 0.34 mmol) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (0.13 ml, 0.42 mmol) were added to a stirred solution

of **5a** (0.17 g, 0.28 mmol) in acetonitrile (6 ml) at room temperature, and the mixture was stirred at room temperature for 4 h. After addition of saturated $NaHCO_3$ (aq.) solution, the reaction mixture was extracted with EtOAc. The organic phase was washed sequentially with saturated $NaHCO_3$ solution, water and brine, and dried over Na_2SO_4 and concentrated. Purification by column chromatography (1% triethylamine in hexane/ EtOAc = 1/1) afforded a white mass, which was further purified by precipitation by pouring a concentrated EtOAc solution of the mass to excess hexane. The precipitate was a white powder composed of an enantiomeric mixture of **6** (0.20 g, 88%): Mp 106-108 °C; ^{31}P NMR (acetone-d_6) δ 148.66, 149.79; MS (FAB) *m/z* 802 ($M+H^+$). HRMS (FAB) Calcd for $C_{42}H_{53}N_5O_9P$ ($M+H^+$): 802.3581. Found: 802.3559.

3. Synthesis, purification and characterization of 2',4'-BNA^{NC}[N-Me]- and 2',4'-BNA (LNA)-modified oligonucleotides:

Synthesis of 0.2 μmol scale of the natural (**15**), 2,4'-BNA^{NC} (N-Me) modified (**7** to **10**) and 2',4'-BNA modified (**11** to **14**) oligonucleotides was performed using the Expedite (TM) 8909 Nucleic Acid Synthesis System according to a standard phosphoroamidite protocol with 1-H tetrazole as the activator. The coupling time of 2',4'-BNA^{NC} monomers was 5 minutes and the coupling yield was 96% to 99%. The solid supported oligonucleotides were then treated with concentrated ammonium hydroxide solution at room temperature for 1.5 h and then at 55 °C for 16 h. The ammonia solutions of the oligonucleotides were concentrated, and the crude oligonucleotides were initially purified by NENSORB^{TM} PREP cartridge and then further purified via reverse-phase HPLC with a Wako Wakopack ^{R} WS-DNA (10 mm X 250 mm) column using 8-32% MeCN in 0.1 M triethylammonium acetate buffer (pH 7.0). The oligonucleotides were analyzed for purity by HPLC and characterized by MALDI-TOF mass spectroscopy.

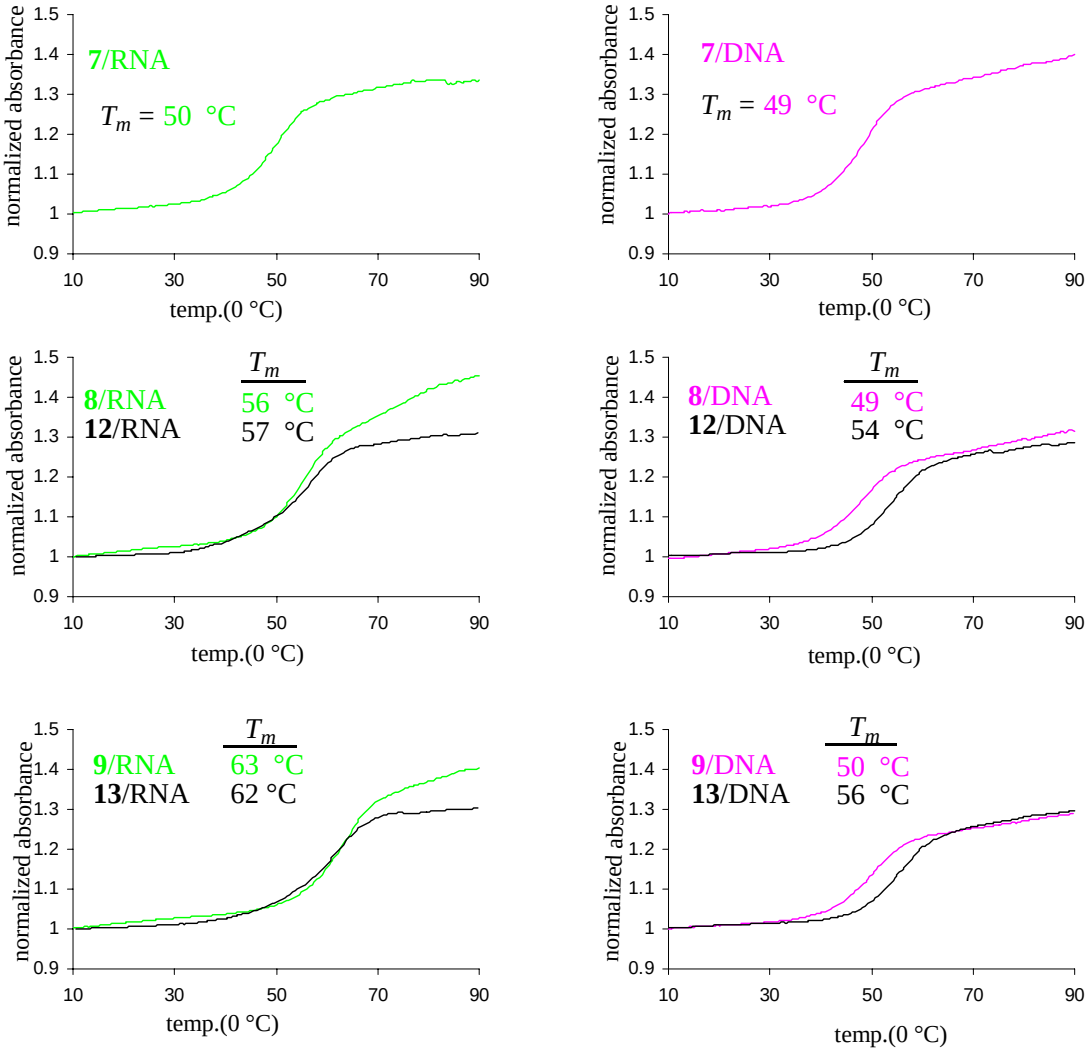
MALDI-TOF-MS data $[M-H]^-$ for the TFOs:

Oligonucleotides (5'd.....3')[a]	Calcd $(M-H)^-$	Found $(M-H)^-$
GCGTTTTTTGCT	3632.4	3632.6
GCGTT**T**TTTGCT	3689.5	3688.5
GCGTT**T**T**T**TGCT	3746.5	3746.9
GCG**T**T**T**T**T**TGCT	3803.6	3804.9
TTTTTTTT**T**T	3036.1	3036.4
GCGTT**t**TTTGCT	3660.4	3660.8
GCGTT**t**T**t**TGCT	3788.4	3688.7
GCG**t**T**t**T**t**TGCT	3716.4	3716.9
TTTTTTTT**t**T	3007.0	3007.1

[a] **T** = 2',4'-BNA^{NC}[N-Me]-modified and **t** = 2',4'-BNA (LNA)-modified oligonucleotides, respectively.

4. UV melting experiments:

UV melting experiments were carried out on a Beckman DU-650 spectrometer equipped with a T_m analysis accessory. Equimolecular amounts of the target RNA/DNA strand and oligonucleotide were dissolved in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl to give final strands concentration of 4 μM and annealed by heating the samples at 90 °C for 5 minutes followed by slow cooling to room temperature. Then the samples were stored at 4 °C for 1 h. The melting profile was recorded at 260 nm from 10 to 90 °C at a scan rate of 0.5 °C /min. The T_m was calculated as the temperature of the half-dissociation of the formed duplexes, determined by the first derivative of the melting curve.



5. Reference:

1. S. M. A. Rahman, S. Seki, S. Obika, S. Haitani, K. Miyashita and T. Imanishi, *Angew. Chem. Int. Ed.* 2007, **46**, 4306.