Supporting Information

# Synthesis, RNA selective hybridization and high nuclease resistance of an oligonucleotide containing novel bridged nucleic acid with cyclic urea structure

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#### **1. General Methods**

Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N<sub>2</sub> atmosphere. Dichloromethane, DMF, THF, MeCN, triethylamine and pyridine were distilled from CaH<sub>2</sub>. <sup>1</sup>H-NMR (500, 300 and 270 MHz), <sup>13</sup>C-NMR (126, 75.5 and 67.8 MHz) and <sup>31</sup>P-NMR (202 MHz) were recorded on JEOL JEOL JNM-LA-500, JEOL JNM-AL-300 or JNM-EX-270 spectrometers. Chemical shift are reported in parts per million downfield from internal tetramethylsilane (0.00 ppm) and residual solvent for <sup>1</sup>H-NMR or chloroform-*d*<sub>1</sub> (77.0 ppm), methanol- *d*<sub>4</sub> (49.0 ppm) and DMSO-*d*<sub>6</sub> (39.7 ppm) for <sup>13</sup>C-NMR. IR spectra were recorded on a JASCO FT/IR-200 or JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO DIP-370 instrument. Mass spectra were measured on JEOL JMS-600, JMS-700 or JMS-D300 mass spectrometers. For column chromatography, silica gel PSQ-100B, FL-100D or Cosmosil 75C<sub>18</sub>-OPN was used. For flash column chromatography, silica gel FL-60D was used.

#### 2. Synthesis of new compounds

### **Compound 2**



Under a N<sub>2</sub> atmosphere, trifluoromethanesulfornyl chloride (11.8 mL, 110 mmol) was added to a stirred solution of compound **1** (17.3 g, 37.0 mmol) and DMAP (18.0 g, 147 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) at 0 °C and the mixture was stirred for 20 min. After addition of saturated aqueous NaHCO<sub>3</sub> to the reaction mixture, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Aqueous 1 N NaOH (89 mL) was poured into a THF solution (250 mL) of the residue, the mixture was stirred at room temperature for 5 h. After concentration of the reaction mixture under reduced pressure, saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with AcOEt. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Under a N<sub>2</sub> atmosphere, pyridine (15.0 mL, 180 mmol) and trifluoromethanesulfonic anhydride (12.4 mL, 74 mmol) were added to the dry CH<sub>2</sub>Cl<sub>2</sub> solution (200 mL) of the residue and stirred at room temperature for 2.5 h. H<sub>2</sub>O was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/AcOEt = 1/1) to afford compound 2 (9.9 g, 37%) as a white amorphous solid;  $[\alpha]_D^{24}$  +74.4 (c 1.00, CHCl<sub>3</sub>). IR (KBr): 3646, 3200, 1704, 1455, 1417 cm<sup>-1</sup>. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ: 1.87 (3 H, d, *J* = 1 Hz), 3.44 (1 H, d, *J* = 10 Hz), 3.68 (1 H, d, *J* = 10 Hz), 3.45 (1 H, d, *J* = 12 Hz), 4.52 (1 H, d, J = 12 Hz), 4.54 (1 H, d, J = 12 Hz), 4.56 (1 H, s), 4.62 (1 H, d, J = 11 Hz), 4.75 (1 H, d, J = 11 Hz), 4.80 (1 H, d, J = 12 Hz), 5.43 (1 H, dd, J = 2, 4 Hz), 6.27 (1 H, brs), 7.12 (1 H, d, J = 1 Hz), 7.16-7.40 (10 H, m), 9.34 (1 H, brs). <sup>13</sup>C-NMR (126 MHz,  $CDCl_3$ )  $\delta$ : 12.3, 30.2, 34.1, 68.3, 73.6, 82.5, 83.8, 85.4, 85.7, 111.4, 118.1 (q, J = 319Hz, CF<sub>3</sub>), 118.5 (q, J = 320 Hz, CF<sub>3</sub>), 125.4, 127.7, 127.7, 128.1, 128.2, 128.3, 128.4, 128.5, 128.5, 128.6, 128.7, 128.8, 135.3, 136.5, 150.1, 163.6. MS (FAB) m/z: 733  $(M+H^+)$ . High-resolution MS (FAB): Calcd for  $C_{27}H_{27}F_6N_2O_{11}S_2$  (M+H<sup>+</sup>): 733.0960. Found: 733.0962. Anal. Calcd for C<sub>27</sub>H<sub>26</sub>F<sub>6</sub>N<sub>2</sub>O<sub>11</sub>S<sub>2</sub>: C, 44.26; H, 3.58; N, 3.82. Found: C, 44.56; H, 3.63; N, 3.81.

### **Compound 3**



Under a N<sub>2</sub> atmosphere, sodium azide (3.38 g, 52.0 mmol) was added to a solution of the compound **2** (9.51 g, 13.0 mmol) in DMF (75 mL) and stirred at room temperature for 10 h. After concentration of the organic layer under reduced pressure, saturated aqueous NaHCO<sub>3</sub> was added and the resulting mixture was extracted with AcOEt. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (hexane/AcOEt = 1/1) to afford compound **3** (13.3 g, 100%) as a yellow amorphous solid; Mp. 42–45 °C.  $[α]_D^{24}$  -6.10 (c 0.75, CHCl<sub>3</sub>). IR (KBr): 3174, 3065, 3033, 2928, 2871, 2107, 1693, 1455, 1268, 1097 cm<sup>-1</sup>. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ: 1.54 (3 H, s), 3.27, 3.71 (2 H, AB, *J* = 13 Hz), 3.44, 3.65 (2 H, AB, *J* = 11 Hz), 4.02 (1 H, dd, *J* = 6, 6 Hz), 4.27 (1 H, d, *J* = 6 Hz), 4.42, 4.48 (2 H, AB, *J* = 12 Hz), 4.48, 4.76 (2 H, AB, *J* = 11 Hz), 6.04 (1 H, d, *J* = 6 Hz), 7.20-7.38 (11 H, m), 9.38 (1 H, s). <sup>13</sup>C-NMR (75.5 MHz, CDCl<sub>3</sub>) δ: 12.1, 52.7, 64.8, 71.5, 73.8, 74.7, 79.0, 86.7, 87.1, 111.5, 127.7, 128.2, 128.3, 128.5, 128.6, 128.7, 135.2, 136.5, 136.8, 150.3, 163.5. MS (FAB) *m/z*: 519 (M+H<sup>+</sup>). High-resolution MS (FAB): Calcd for C<sub>25</sub>H<sub>26</sub>O<sub>5</sub>N<sub>8</sub> (M+H<sup>+</sup>): 519.2104. Found: 519.2088.

### **Compound 4**



Under a N<sub>2</sub> atmosphere, trimethylphosphine (1 M in THF, 13.1 mL, 13.1 mmol) and H<sub>2</sub>O (3 mL) were added to a solution of compound 3 (1.36 g, 2.62 mmol) in THF (10 mL) and stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure, and the residue was briefly purified by flash silica gel column chromatography (chloroform/methanol = 4/1) to afford the residue. Under a N<sub>2</sub> atmosphere, triethylamine (1.8 mL, 13.1 mmol) and 4-nitrophenyl chloroformate (528 mg, 2.62 mmol) were added to the DMF solution (104 mL) of the residue and the mixture was stirred at room temperature for 1 h. After concentration of the reaction mixture under reduced pressure, H<sub>2</sub>O was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (chloroform/methanol = 50/1, 10/1 and 4/1) to afford compound 4 (780 mg, 60%) as a white powder; Mp. 192–195 °C (CHCl<sub>3</sub>).  $[\alpha]_{D}^{23}$  +79.5 (c 1.00, CHCl<sub>3</sub>). IR (KBr): 3030, 1695, 1454, 1276, 1076 cm<sup>-1</sup>. <sup>1</sup>H-NMR (270 MHz, CD<sub>3</sub>OD)  $\delta$ : 1.45 (3 H, d, J = 1 Hz), 2.70, 3.29 (2 H, AB, J = 15 Hz), 3.49, 3.70 (2 H, AB, J = 12 Hz), 3.74 (1 H, d, J = 7 Hz), 4.24 (1 H, d, J = 7 Hz), 4.35, 4.61 (2 H, AB, J = 13 Hz), 4.42, 4.49 (2 H, AB, J = 13 Hz), 5.87 (1 H, s), 7.10–7.30 (10 H, m), 7.73 (1 H, d, J = 1 Hz). <sup>13</sup>C-NMR (75.5 MHz, DMSO- $d_6$ )  $\delta$ : 12.2, 43.5, 57.2, 70.5, 71.3, 73.4, 73.7, 86.0, 90.6, 109.5, 128.3, 128.3, 128.4, 128.5, 128.9, 129.1, 136.0, 138.9, 139.0, 151.2, 161.5, 164.6. MS (FAB) m/z: 493

(M+H<sup>+</sup>). Anal. Calcd for  $C_{26}H_{28}O_6N_4 \cdot H_2O$ : C, 61.16; H, 5.92; N, 10.97. Found: C, 60.97; H, 5.84; N, 10.70.

### **Compound 5**



Under a H<sub>2</sub> atmosphere, a mixture of 20% Pd(OH)<sub>2</sub>/C (1.18 g) and compound **4** (660 mg, 1.34 mmol) in dry THF (13 mL) was stirred at room temperature for 18 h. The reaction mixture was filtered and concentrated to afford compound **5** (392 mg, 94%) as a white powder; Mp. 205–207 °C (CH<sub>3</sub>OH).  $[\alpha]_D^{24}$  +93.6 (c 0.80, H<sub>2</sub>O). IR (KBr): 3343, 1705, 1517, 1479, 1427cm<sup>-1</sup>. <sup>1</sup>H-NMR (270 MHz, D<sub>2</sub>O)  $\delta$ : 1.83 (3 H, s), 2.96, 3.30 (2 H, AB, J = 14 Hz), 3.72, 3.81 (2 H, AB, J = 13 Hz), 3.84 (2 H, d, J = 7 Hz), 4.35 (1 H, d, J = 7 Hz), 5.87 (1 H, s), 7.85 (1 H, s). <sup>13</sup>C-NMR (67.8 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 12.6, 41.8, 59.0, 61.2, 65.3, 86.2, 88.7, 108.6, 135.6, 150.2, 160.6, 163.8. MS (FAB) *m/z*: 313 (M+H<sup>+</sup>). High-resolution MS (FAB): Calcd for C<sub>12</sub>H<sub>16</sub>O<sub>6</sub>N<sub>4</sub> (M+H<sup>+</sup>): 313.1148. Found: 313.1154.

### **Compound 5'**



Under a N<sub>2</sub> atmosphere, 4,4'-dimethoxytrityl chloride (326 mg, 0.96 mmol) was added to a stirred solution of compound **5** (100 mg, 0.32 mmol) in dry pyridine (2 mL) and the mixture was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography (AcOEt/methanol = 8/1) to afford compound **5'** (149 mg, 76%) as a white powder; Mp. 187–189 °C (pyridine).  $[\alpha]_D^{26}$  +23.1 (c 0.80, CH<sub>2</sub>Cl<sub>2</sub>). IR (KBr): 3334, 2525, 2074, 1694, 1506, 1251, 1116, 1072, 1036 cm<sup>-1</sup>. <sup>1</sup>H-NMR (270 MHz, CD<sub>3</sub>OD)  $\delta$ : 1.20 (3 H, d, *J* = 1 Hz), 2.61, 3.16 (2 H, AB, *J* = 15 Hz), 3.21 (2 H, s), 3.65 (6 H, s), 4.52 (1 H, d, J = 7 Hz ), 5.81 (1 H, s), 6.70–7.40 (13 H, m), 7.69 (1 H, d, J = 1 Hz). <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD)  $\delta$ : 12.4, 43.6, 55.7, 60.8, 64.4, 67.6, 87.3, 88.0, 91.3, 110.7, 114.2, 128.0, 128.9, 129.3, 131.3, 136.3, 136.6, 136.7, 145.7, 151.8, 160.1, 160.2, 163.7, 166.3. MS (FAB) *m*/*z*: 615 (M+H<sup>+</sup>). *Anal*. Calcd for C<sub>33</sub>H<sub>34</sub>O<sub>8</sub>N<sub>4</sub>·3/2H<sub>2</sub>O: C, 61.76; H, 5.81; N, 8.73. Found: C, 62.01; H, 5.66; N, 8.72.

### **Compound 6**



Under a N<sub>2</sub> atmosphere, 4,5-dicyanoimidazole (0.25 M in acetonitrile, 1.37 mL, 0.343 mmol) and 2-cyanoethyl-*N*,*N*,*N'*,*N'*-tetraisopropylphosphordiamidite (0.15 mL, 0.46 mmol) were added to a stirred solution of compound **5'** (141 mg, 0.229 mmol) in dry THF (2 mL) at room temperature and the mixture was stirred at room temperature for 1 h. After addition of H<sub>2</sub>O, the resulting mixture was concentrated under reduced pressure and the residue was purified by flash silica gel column chromatography (acetonitrile/AcOEt = 1/3 and 3/1) and then further purified by precipitation from AcOEt/hexane to afford compound **6** (84 mg, 45%) as a diastereomeric mixture; Mp. 142–145 °C (CH<sub>3</sub>CN). <sup>31</sup>P-NMR (202 MHz, acetone-*d*<sub>6</sub>)  $\delta$ : 149.2, 150.8. MS (FAB) *m/z*: 837 (M+Na<sup>+</sup>). High-resolution MS (FAB): Calcd for C<sub>42</sub>H<sub>51</sub>O<sub>9</sub>N<sub>6</sub>PNa (M+Na<sup>+</sup>): 837.3353. Found: 837.3351.

# 3. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of new compounds

### <sup>1</sup>H-NMR Spectra of Compound 2



# <sup>13</sup>C-NMR Spectra of Compound 2



## <sup>1</sup>H-NMR Spectra of Compound **3**



# <sup>13</sup>C-NMR Spectra of Compound **3**



![](_page_10_Figure_0.jpeg)

## <sup>1</sup>H-NMR Spectra of Compound 4

# <sup>13</sup>C-NMR Spectra of Compound 4

![](_page_11_Figure_2.jpeg)

![](_page_12_Figure_0.jpeg)

<sup>1</sup>H-NMR Spectra of Compound **5** 

#### Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2010

![](_page_13_Figure_0.jpeg)

![](_page_13_Figure_1.jpeg)

![](_page_14_Figure_0.jpeg)

## <sup>1</sup>H-NMR Spectra of Compound **5'**

Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2010

# <sup>13</sup>C-NMR Spectra of Compound **5'**

![](_page_15_Figure_2.jpeg)

![](_page_16_Figure_0.jpeg)

# <sup>31</sup>P-NMR Spectra of Compound **6**

### 4. Synthesis, purification and characterization of oligonucleotides (Table S1)

Synthesis of 0.2 µmol scale of oligonucleotides 7-11 containing 2',4'-BNA with cyclic urea structure 5 was performed using the Expedite (TM) 8909 Nucleic Acid Synthesis System according to а standard phosphoramidite protocol with 0.5 Μ 5-ethylthio-1*H*-tetrazole as the activator. The coupling time of 6 was prolonged to 40 minutes and the capping time was 60 seconds. 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 8-12 h. The resulting solutions was evaporated under reduced pressure, and the obtained crude oligonucleotides were initially purified by Sep-Pak Plus C<sub>18</sub> Environmental Cartridge and then further purified by reverse-phase HPLC with a Waters XTerra MS C<sub>18</sub> (10 mm X 50 mm) column with a linear gradient of MeCN (3-4% over 40 min for 7, 2.5-3% over 40 min for 8, 2.5-4% over 40 min for 9, 2-3% over 45 min for 10, 3-5% over 30 min for 11) in 0.1 M triethylammonium acetate (pH 7.0). The oligonucleotides were analyzed for purity by HPLC and characterized by MALDI-TOF mass spectroscopy.

Ologonucleotides <sup>[a]</sup>	Yield (%)	Calcd $[M-H]^-$	Found $[M-H]^-$
5'-d(GCGTTXTTTGCT)-3' (7)	8	3702.4	3702.2
5'-d(GCGTTXTXTGCT)-3' (8)	3	3772.4	3772.6
5'-d(GCGXTXTXTGCT)-3' (9)	11	3842.4	3842.6
5'-d(GCGXXXXXXGCT)-3' (10)	2	4052.6	4051.7
5'-d(TTTTTTTT <b>X</b> T)-3' (11)	15	3050.0	3049.6

Table S1 Yields and MALDI-TOF-MS data [M-H]<sup>-</sup> for the oligonucleotides

[a]  $\mathbf{X}$  = the bridged nucleotide with cyclic urea structure.

# 5. MALDI- TOF-MS data of oligonucleotides

## Oligonucleotide 7

![](_page_18_Figure_3.jpeg)

![](_page_19_Figure_2.jpeg)

![](_page_20_Figure_2.jpeg)

![](_page_21_Figure_2.jpeg)

![](_page_22_Figure_2.jpeg)

#### 6. UV melting experiments and melting profiles (Figures S1 and S2)

UV melting experiments were carried out on SHIMADZU UV-1650PC spectrometer equipped with a  $T_m$  analysis accessory. Equimolecular amounts of the target RNA or DNA strand and oligonucleotide were dissolved in buffer A (10 mM sodium phosphate at pH 7.2 containing 100 mM NaCl) to give final strands concentration of 4  $\mu$ M and annealed by heating the samples at 95 °C followed by slow cooling to room temperature. The melting profile was recorded at 260 nm from 5 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.

![](_page_23_Figure_3.jpeg)

**Fig. S1** UV melting curves for the duplexes formed by oligonucleotides containing 2',4'-BNA with cyclic urea structure and the target RNA strand, 5'-r(AGCAAAAAACGC)-3'.

![](_page_24_Figure_1.jpeg)

**Fig. S2** UV melting curves for the duplexes formed by oligonucleotides containing 2',4'-BNA with cyclic urea structure and the target DNA strand, 5'-d(AGCAAAAAACGC)-3'.

### 7. CD spectroscopic analyses and CD profiles (Figures S3 and S4)

CD spectra at 4 °C were recorded in buffer A (see UV melting experiments) on a JASCO J-720W spectropolarimeter. Cell path length was 0.1 cm. The duplex nucleic acid concentration used was 4  $\mu$ M. The samples were prepared in the same manner as described for the UV melting experiments.

![](_page_25_Figure_3.jpeg)

Fig. S3 CD spectra for the duplex involving oligonucleotides 7-12 and their complementary RNA, 5'-r(AGCAAAAAACGC)-3'. X = 2',4'-BNA with cyclic urea structure.

![](_page_26_Figure_1.jpeg)

Fig. S4 CD spectra for the duplex involving oligonucleotides 7-12 and their complementary DNA, 5'-d(AGCAAAAAACGC)-3'.  $\mathbf{X} = 2',4'$ -BNA with cyclic urea structure.

![](_page_27_Figure_1.jpeg)

Compound 5	2',4'-BNA <sup>COC</sup>	PrNA
$v_0$ : -3.06°	-6.12°	-1.00°
$v_1$ : -22.11°	-18.69°	-21.74°
v <sub>2</sub> : 36.51°	34.28°	34.68°
v <sub>3</sub> : -39.19°	-38.70°	-36.03°
v <sub>4</sub> : 26.98°	28.38°	23.39°
v <sub>max</sub> : 39.59°	38.59°	36.89°
<i>P</i> : 22.75°	27.32°	19.92°
C2' ••• C1'':		
3.040 Å	3.054 Å	3.139 Å

**Fig. S5** Energy-minimized structures, endocyclic sugar torsion angles  $v_0-v_4$ , maximum torsion angle  $v_{max}$ , pseudorotation phase angle *P* and distance between C2' and C1'' of compound **5**, 2',4'-BNA<sup>COC</sup> and PrNA. Theoretical calculation was carried out using HF/6-31G\* basis set (Spartan '06, Wavefunction, Inc). *P* and  $v_{max}$  are calculated as follows: tan  $P = (v_4 + v_1 - v_3 - v_0)/(2 \cdot v_2 \cdot (\sin 36^\circ + \sin 72^\circ)); v_{max} = v_2 / \cos P$ .

The  $v_{max}$  values of urea-type BNA (compound **5**), BNA<sup>COC</sup> and PrNA are calculated to be 39.59°, 38.59° and 36.89°, respectively.  $T_m$  values (with RNA complement) of each oligonucleotide are in the same ascending order; urea-type BNA (1–2.5 °C/mod)  $\geq$ 2',4'-BNA<sup>COC</sup> (1–2.5 °C/mod) [1] > PrNA (-0.5°C/mod) [2]. Since  $v_{max}$  is the maximum degree of pucker, the high value of  $v_{max}$  could indicate the trigonal planer urea function in **5** constrains the sugar in a more rigid conformation than in 2',4'-BNA<sup>COC</sup> and PrNA. Equally, the distance between C2' and C1" of urea-type BNA was found to be shorter than those of 2',4'-BNA<sup>COC</sup> by 0.014 angstrom and PrNA by 0.099 angstrom, respectively, which may also support the conformational rigidity of urea-type BNA. This structural feature of BNA monomers would be one important factor to explain the differences in RNA affinity of the BNA oligonucleotides.

Y. Hari, S. Obika, R. Onishi, K. Eguchi, T. Osaki, H. Oishi and T. Imanishi, *Bioorg. Med. Chem.*, 2006, 14, 1029.

<sup>[2]</sup> K. Morita, M. Takagi, C. Hasegawa, M. Kaneko, S. Tsutsumi, J. Sone, T. Ishikawa, T. Imanishi and M. Koizumi, *Bioorg. Med. Chem.*, 2003, 11, 2211.