A 4-[(3R,4R)-dihydroxypyrrolidino]pyrimidin-2-one nucleobase for a CG base pair in triplex DNA

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Contents

1. HPLC and MALDI-TOF-Mass spectra for TFOs 2a-p Page S2 – S17
2. Synthesis of TFO 2o from a monomer bearing the modified nucleobase Page S18 - S21
3. $^1$H, $^{13}$C and $^{31}$P spectra for new compounds Page S22 – S26
TFO-2a

HPLC

Column: Waters XBridge® MS C$_{18}$ 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2b

HPLC

Column: Waters XBridge® MS C$_{18}$ 2.5 μm, 4.6 x 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2c

HPLC

Column: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2d

HPLC

Column: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50ºC.

MALDI-TOF-Mass
TFO-2e

HPLC

Column: Waters XBridge® MS C_{18} 2.5 \mu m, 4.6 \times 50 \text{ mm}.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2f

HPLC
Column: Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2g

HPLC

Column: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2h

HPLC

Column: Waters XBridge® MS C₁₈ 2.5 µm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2i
HPLC
Column: Waters XBridge® MS C18 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2j

HPLC

Column: Waters XBridge® MS C<sub>18</sub> 2.5 µm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
TFO-2k

HPLC

Column: Waters XBridge® MS C\textsubscript{18} 2.5 μm, 4.6 \times 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50ºC.

MALDI-TOF-Mass
TFO-2l
HPLC
Column: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
**TFO-2m**

**HPLC**

Column: Waters XBridge® MS C18 2.5 μm, 4.6 x 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

**MALDI-TOF-Mass**
TFO-2n

**HPLC**

Column: Waters XBridge® MS C$_{18}$ 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

![HPLC Chromatogram](image)

**MALDI-TOF-Mass**
**TFO-2o**

**HPLC**

Column: Waters XBridge® MS C\textsubscript{18} 2.5 μm, 4.6 × 50 mm.

Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate: 1.0 mL/min.

Column temp.: 50°C.

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**MALDI-TOF-Mass**

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TFO-2p
HPLC
Column: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50ºC.

MALDI-TOF-Mass
Scheme S1. Synthesis of TFO 2o. \( \text{C} = 2'\text{-deoxy-5'-methylcytidine. Reagent and conditions: (i) 2,4,6-triisopropylphenylsulfonyl chloride (TIPS-Cl), Et}_3\text{N, DMAP, CH}_2\text{Cl}_2, \text{rt, 2 h, 93%; (ii) (35,45)-dihydroxyproline, Et}_3\text{N, CH}_2\text{Cl}_2-\text{MeOH, rt, 6 h, 85%; (iii) Ac}_2\text{O, DMAP, pyridine, rt, 2 h, quant.; (iv) TBAF, AcOH, THF, rt, 24 h, quant.; (v) }i\text{-Pr}_2\text{NP(Cl)OCH}_2\text{CH}_2\text{CN, }i\text{-Pr}_2\text{NEt, CH}_2\text{Cl}_2, 0^\circ\text{C, 3 h, 90%; (vi) oligonucleotide synthesis.}}

TFO 2o was synthesized as shown in Scheme S1. The oligonucleotide synthesis was achieved by using the common phosphoramidite protocol. The synthesis of S6 from S1\(^1\) was carried out as below.

**General:** Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N\(_2\) atmosphere. \(^1\)H NMR (400.00 MHz), \(^{13}\)C NMR (100.53 MHz) and \(^{31}\)P NMR (161.84 MHz) were recorded on JEOL JNM-ECS-400 spectrometers. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane (0.00 ppm) for \(^1\)H NMR, CDCl\(_3\) (77.00 ppm) for \(^{13}\)C NMR, or external H\(_3\)PO\(_4\) (0.00 ppm) for \(^{31}\)P NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO P-2200 instrument. For column chromatography, Fuji Silysia PSQ-100B, FL-60D and FL-100D was used as silica gel.

3'-\text{O-tert-Butyldimethylsilyl-2'-deoxy-5'-O-dimethoxytrityl-4-O-(2,4,6-triisopropylphenyl)sulfonyluridine (S2):} Under a N\(_2\) atmosphere, 2,4,6-triisopropylbenzenesulfonyl chloride (423 mg, 1.40 mmol) was added to a solution of compound S1\(^1\) (750 mg, 1.16 mmol), DMAP (14 mg, 0.11 mmol) and Et\(_3\)N (0.81 mL, 5.82 mmol) in anhydrous CH\(_2\)Cl\(_2\) (7.0 mL) and the resulting mixture was stirred at room temperature for 2 h. After addition of saturated aqueous NaHCO\(_3\) solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuo. The residue was purified by column chromatography (\(n\)-hexane/AcOEt =
5/1) to give compound S2 (991 mg, 93%) as a white amorphous powder. Mp 73 − 74°C. [α]D30 +91.3 (c 1.00, CHCl3). IR νmax (KBr): 2957, 1684, 1542, 1509, 1362, 1281, 1253, 1181, 1108, 1036 cm⁻¹. 1H NMR (CDCl3): δ −0.07 (3H, s), −0.01 (3H, s), 0.79 (9H, s), 1.26 (12H, d, J = 6.9 Hz), 1.31 (6H, d, J = 6.9 Hz), 2.23 (1H, ddd, J = 4.1, 6.4 and 13.6 Hz), 2.50 (1H, ddd, J = 6.4, 6.4 and 13.6 Hz), 2.91 (1H, sept, J = 6.9 Hz), 3.32 (1H, dd, J = 2.8 and 11.0 Hz), 3.55 (1H, dd, J = 2.8 and 11.0 Hz), 3.81 (6H, s), 3.92 (1H, ddd, J = 2.8, 2.8 and 6.4 Hz), 4.25 (2H, sept, J = 6.9 Hz), 5.69 (1H, d, J = 7.3 Hz), 6.08 (1H, dd, J = 4.1 and 6.4 Hz), 6.82-6.85 (4H, m), 7.20-7.35 (11H, m), 8.50 (1H, d, J = 7.3 Hz). 13C NMR (CDCl3): δ −5.08, −4.64, 17.82, 23.43, 23.44, 24.35, 24.62, 25.59, 29.62, 34.22, 41.90, 55.21, 61.23, 69.70, 86.58, 86.86, 87.29, 94.53, 113.20, 113.23, 124.01, 127.19, 127.94, 128.14, 130.06, 130.08, 130.62, 134.95, 135.05, 143.99, 146.06, 151.17, 153.80, 154.42, 158.71, 166.92. MS (FAB): m/z 933 (M+Na+). HRMS (FAB): Calcd for C51H66N2NaO9SSi (M+Na+): 933.4156. Found: 933.4153.

1-[3-O-tert-Butyldimethylsilyl-2-deoxy-5-O-dimethoxytrityl-β-D-ribosyl]-4-[3(3R,4R)-dihydroxy pyrrolidino]pyrimidin-2-one (S3): Under a N2 atmosphere, a solution of (3R,4R)-dihydroxypyrrolidine2 (339 mg, 3.29 mmol) in MeOH (1.0 mL) was added to a solution of compound S2 (3.00 g, 3.29 mmol) and Et3N (2.3 mL, 16.5 mmol) in CH2Cl2 (50 mL) and the resulting mixture was stirred at room temperature for 6 h. After addition of water, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was purified by column chromatography (CH2Cl2/MeOH = 20/1) to give compound S3 (2.0 g, 85%) as a white amorphous powder. Mp 114–115°C. [α]D24 −12.2 (c 1.0, CHCl3). IR νmax (KBr): 3339, 2931, 2857, 1638, 1506, 1462, 1293, 1253, 1177, 1106, 1036 cm⁻¹. 1H NMR (CDCl3): δ −0.06 (3H, s), 0.00 (3H, s), 0.80 (9H, s), 2.16-2.19 (1H, m), 2.40 (1H, ddd, J = 6.0, 6.0 and 13.1 Hz), 3.27-3.33 (2H, m), 3.54-3.56 (1H, m), 3.66-3.67 (1H, m), 3.77 (6H, s), 3.77-3.82 (1H, m), 3.89-3.90 (1H, m), 4.00-4.10 (1H, m), 4.40-4.50 (3H, m), 5.33-5.38 (2H, m), 5.59 (1H, brs), 6.23 (1H, t, J = 6.0 Hz), 6.83-6.86 (4H, m), 7.22–7.32 (7H, m), 7.41 (2H, d, J = 7.3 Hz), 8.07 (1H, d, J = 7.3 Hz). 13C NMR (CDCl3): δ −5.1, −4.7, 17.7, 25.5, 41.8, 52.4, 52.6, 55.1, 61.5, 69.8, 74.3, 74.8, 85.6, 85.7, 86.6, 93.2, 113.0, 113.1, 126.9, 127.8, 128.1, 130.1, 130.4, 144.3, 156.0, 158.5, 161.6. MS (FAB): m/z 730 (M+H⁺). HRMS (FAB): Calcd for C40H52N3O8Si (M+H⁺): 730.3518. Found: 730.3548.

1-[3-O-tert-Butyldimethylsilyl-2-deoxy-5-O-dimethoxytrityl-β-D-ribosyl]-4-[3(3R,4R)-dipropoxy pyrrolidino]pyrimidin-2-one (S4): Under a N2 atmosphere, Ac2O (77 μL, 0.82 mmol) was added to a solution of compound S3 (200 mg, 0.27 mmol), DMAP (3.3 mg, 0.027 mmol) in pyridine (20 mL) and the resulting mixture was stirred at room temperature for 2 h. After addition of water, the reaction mixture was diluted with Et2O, washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was purified by column chromatography (CH2Cl2/MeOH = 20/1) to give compound S4 (2.0 g, 85%) as a white amorphous powder. Mp 114–115°C. [α]D24 −12.2 (c 1.0, CHCl3). IR νmax (KBr): 3339, 2931, 2857, 1638, 1506, 1462, 1293, 1253, 1177, 1106, 1036 cm⁻¹. 1H NMR (CDCl3): δ −0.06 (3H, s), 0.00 (3H, s), 0.80 (9H, s), 2.16-2.19 (1H, m), 2.40 (1H, ddd, J = 6.0, 6.0 and 13.1 Hz), 3.27-3.33 (2H, m), 3.54-3.56 (1H, m), 3.66-3.67 (1H, m), 3.77 (6H, s), 3.77-3.82 (1H, m), 3.89-3.90 (1H, m), 4.00-4.10 (1H, m), 4.40-4.50 (3H, m), 5.33-5.38 (2H, m), 5.59 (1H, brs), 6.23 (1H, t, J = 6.0 Hz), 6.83-6.86 (4H, m), 7.22–7.32 (7H, m), 7.41 (2H, d, J = 7.3 Hz), 8.07 (1H, d, J = 7.3 Hz). 13C NMR (CDCl3): δ −5.1, −4.7, 17.7, 25.5, 41.8, 52.4, 52.6, 55.1, 61.5, 69.8, 74.3, 74.8, 85.6, 85.7, 86.6, 93.2, 113.0, 113.1, 126.9, 127.8, 128.1, 130.1, 130.4, 144.3, 156.0, 158.5, 161.6. MS (FAB): m/z 730 (M+H⁺). HRMS (FAB): Calcd for C40H52N3O8Si (M+H⁺): 730.3518. Found: 730.3548.
concentrated in vacuo. The residue was purified by column chromatography (n-hexane/AcOEt = 1/3 to 1/5) to give compound S4 (232 mg, quant.) as a white amorphous powder.

Mp 88–91°C. [α]D24 +16.2 (c 1.0, CHCl3). IR νmax (KBr): 2931, 2857, 1745, 1656, 1506, 1462, 1369, 1283, 1248, 1178, 1107, 1068, 1035. 1H NMR (CDCl3): δ –0.07 (3H, s), –0.01 (3H, s), 0.80 (9H, s), 2.05 (3H, s), 2.11 (3H, s), 2.22 (1H, ddd, J = 3.7, 6.9 and 13.7 Hz), 2.47 (1H, ddd, J = 6.9, 6.9 and 13.7 Hz), 3.33 (1H, dd, J = 2.8 and 10.6 Hz), 3.38 (1H, d, J = 11.9 Hz), 3.57 (1H, dd, J = 2.8 and 10.6 Hz), 3.69-3.73 (1H, m), 3.80 (6H, s), 3.87-4.03 (3H, m), 4.48 (1H, q, J = 6.9 Hz), 5.21-5.25 (3H, m), 6.29 (1H, dd, J = 3.7 and 6.9 Hz), 6.83-6.86 (4H, m), 7.25-7.32 (7H, m), 7.40 (2H, d, J = 6.9 Hz), 8.18 (1H, d, J = 7.3 Hz). 13C NMR (CDCl3): δ –5.1, –4.7, 17.8, 20.7, 20.8, 25.6, 42.0, 50.6, 50.7, 55.1, 61.5, 69.8, 73.6, 74.6, 85.8, 86.6, 91.7, 113.1, 113.1, 127.0, 127.8, 128.2, 130.1, 130.2, 135.2, 135.3, 141.3, 144.2, 155.3, 158.5, 161.8, 169.3, 169.6. MS (FAB) m/z 814 (M+H +). HRMS (FAB): Calcd for C44H56N3O10Si (M+H+): 814.3729. Found: 814.3763.

1-[2-Deoxy-5-O-dimethoxytrityl-β-D-ribo-5-y]-4-[(3R,4R)-diacetoxypyrrolidino]pyrimidin-2-one (S5): Under a N2 atmosphere, TBAF (1.0 M in THF, 0.81 mL, 0.81 mmol) and AcOH (8.1 uL) were added to a solution of compound S4 (550 mg, 0.68 mmol) in THF (30 mL) at 0°C and the resulting mixture was stirred at room temperature for 24 h. After addition of saturated aqueous NaHCO3 solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was purified by column chromatography (AcOEt/MeOH = 20/1) to give compound S5 (476 mg, quant.) as a white amorphous powder.

Mp 101–103°C. [α]D25 +14.2 (c 1.0, CHCl3). IR νmax (KBr): 3311, 2999, 2838, 1744, 1639, 1505, 1460, 1370, 1290, 1245, 1178, 1036 cm−1. 1H NMR (CDCl3): δ 2.04 (3H, s), 2.09 (3H, s), 2.19 (1H, ddd, J = 6.0, 6.0 and 12.5 Hz), 2.67 (1H, ddd, J = 6.0, 6.0 and 12.5 Hz), 3.38-3.48 (3H, m), 3.72-3.98 (4H, m), 3.78 (6H, s), 4.11-4.14 (1H, m), 4.57 (1H, brs), 5.24 (2H, brs), 5.31 (1H, d, J = 7.8 Hz), 6.34 (1H, t, J = 6.0 Hz), 6.83 (4H, d, J = 9.2 Hz), 7.20–7.31 (7H, m), 7.40 (2H, d, J = 7.3 Hz), 7.99 (1H, d, J = 7.8 Hz). 13C NMR (CDCl3): δ 20.7, 20.8, 42.1, 50.7, 50.8, 55.1, 62.9, 71.0, 73.6, 74.6, 85.9, 86.2, 86.6, 92.0, 113.1, 126.8, 127.8, 128.1, 130.1, 135.4, 135.6, 141.3, 144.4, 155.5, 158.5, 161.7, 169.4, 169.6. MS (FAB) m/z 700 (M+H+). HRMS (FAB): Calcd for C38H42N3O10 (M+H+): 700.2865. Found: 700.2906.

1-[3-O-[2-Cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-O-dimethoxytrityl-β-D-ribo-5-y]-4-[(3R,4R)-diacetoxypyrrolidino]pyrimidin-2-one (S6): Under a N2 atmosphere, i-Pr2NP(Cl)OCH2CH2CN (0.14 ml, 0.64 mmol) was added to a solution of S5 (300 mg, 0.43 mmol) and i-Pr2NEt (0.37 mL, 2.2 mmol) in anhydrous CH2Cl2 (20 mL) at 0°C and the resulting mixture was stirred at room temperature for 3 h. After addition of saturated aqueous NaHCO3 solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was purified by column chromatography (AcOEt/MeOH = 20/1) to give compound S5 (476 mg, quant.) as a white amorphous powder.

Mp 101–103°C. [α]D25 +14.2 (c 1.0, CHCl3). IR νmax (KBr): 3311, 2999, 2932, 2838, 1744, 1639, 1505, 1460, 1370, 1290, 1245, 1178, 1036 cm−1. 1H NMR (CDCl3): δ 2.04 (3H, s), 2.09 (3H, s), 2.19 (1H, ddd, J = 6.0, 6.0 and 12.5 Hz), 2.67 (1H, ddd, J = 6.0, 6.0 and 12.5 Hz), 3.38-3.48 (3H, m), 3.72-3.98 (4H, m), 3.78 (6H, s), 4.11-4.14 (1H, m), 4.57 (1H, brs), 5.24 (2H, brs), 5.31 (1H, d, J = 7.8 Hz), 6.34 (1H, t, J = 6.0 Hz), 6.83 (4H, d, J = 9.2 Hz), 7.20–7.31 (7H, m), 7.40 (2H, d, J = 7.3 Hz), 7.99 (1H, d, J = 7.8 Hz). 13C NMR (CDCl3): δ 20.7, 20.8, 42.1, 50.7, 50.8, 55.1, 62.9, 71.0, 73.6, 74.6, 85.9, 86.2, 86.6, 92.0, 113.1, 126.8, 127.8, 128.1, 130.1, 135.4, 135.6, 141.3, 144.4, 155.5, 158.5, 161.7, 169.4, 169.6. MS (FAB) m/z 700 (M+H+). HRMS (FAB): Calcd for C38H42N3O10 (M+H+): 700.2865. Found: 700.2906.
2.21-2.31 (1H, m), 2.41 (1H, t, J = 6.4 Hz), 2.57-2.69 (2H, m), 3.34-3.40 (2H, m), 3.49-3.61 (4H, m), 3.69-4.12 (5H, m), 3.80 (3H, s), 3.80 (3H, s), 4.57-4.68 (1H, m), 5.22-5.25 (3H, m), 6.33 (0.5H, dd, J = 5.0 and 6.4 Hz), 6.37 (0.5H, t, J = 6.0 Hz), 6.82-6.86 (4H, m), 7.24–7.33 (7H, m), 7.39–7.42 (2H, m), 7.97 (0.5H, d, J = 7.8 Hz), 8.07 (0.5H, d, J = 7.3 Hz). 31P NMR (CDCl3): δ 148.51, 149.09. MS (FAB) m/z 900 (M+H+). HRMS (FAB): Calcd for C_{47}H_{59}N_{5}O_{11}P (M+H+): 900.3943. Found: 900.3990.

References


Compound S2
Compound S3
Compound S4
Compound S5

$^{1}H$-NMR (CDCl$_3$)

$^{13}C$-NMR (CDCl$_3$)
Compound S6 (diastereoisomers on the basis of chirality at the phosphorus)