Electronic Supplementary Information

2’,4’-BNA bearing a chiral guanidinopyrrolidine-containing nucleobase with potent ability to recognize the CG base pair in parallel-motif DNA triplex

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Contents

1. Synthesis Page S2 – S9
2. HPLC charts and MALDI-TOF-Mass spectra for TFOs Page S10– S22
3. $^1$H, $^{13}$C and $^{31}$P spectra for the new compounds Page S23– S35
**General:** Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N₂ atmosphere. H NMR (400.00 MHz), ¹³C NMR (100.53 MHz), and ³¹P 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 H NMR, CD₃OD (49.00 ppm) or CDCl₃ (77.00 ppm) for ¹³C NMR, or external H₃PO₄ (0.00 ppm) for ³¹P NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO P-2200 instrument. Mass spectra were measured on a JEOL JMS-600, JEOL JMS-700, Bruker Daltonics Autoflex II TOF/TOF or JEOL JMS-S3000 mass spectrometer. For silica gel column chromatography, Fuji Silysia PSQ-100B, FL-60D and FL-100D were used. For amine silica gel column chromatography, Fuji Silysia DM-1020 was used.

**Scheme S1.** Synthesis of guanidinopyrrolidines. *Reagent and conditions:* (i) (3S)-(+)-1-benzyl-3-aminopyrrolidine, (BocNH)₂CS [¹], DIPEA, EDCI, CH₂Cl₂, rt, 8 h, 88%; (ii) (1) TFA, CH₂Cl₂, rt, 3 h; (2) 20% Pd(OH)₂-C, MeOH, rt, 12 h, 86%; (iii) (3R)-(−)-1-benzyl-3-aminopyrrolidine, (BocNH)₂CS, DIPEA, EDCI, CH₂Cl₂, rt, 3 h, 91%; (iv) (1) TFA, CH₂Cl₂, rt, 1 h; (2) 20% Pd(OH)₂-C, MeOH, rt, 8 h, 80%; (v) (3S)-(+)1-benzyl-3-(methylamino)pyrrolidine, (BocNH)₂CS, DIPEA, EDCI, CH₂Cl₂, rt, 24 h, 51%; (vi) (1) TFA, CH₂Cl₂, rt, 3 h; (2) 20% Pd(OH)₂-C, MeOH, rt, 5 h, quant.; (vii) (3R,4R)-1-benzyl-3,4-diaminopyrrolidine, (BocNH)₂CS, DIPEA, EDCI, CH₂Cl₂, rt, 3 h, 61%; (viii) (1) TFA, CH₂Cl₂, rt, 3 h; (2) 20% Pd(OH)₂-C, MeOH, rt, 10 h, 80%.

All guanidine derivatives used in this study were synthesized in Scheme S1.

**(3S)-1-Benzyl-3-{N,N'-bis-[2-tert-butoxy]carbonyl}guanidino}pyrrolidine (S1):** Under a N₂ atmosphere, EDCI (104 mg, 0.543 mmol) was added to a solution of (3S)-(+)1-benzyl-3-aminopyrrolidine (63.8 mg, 0.362 mmol), N,N'-bis-(2-tert-butoxy)carbonyl-thiourea [(BocNH)₂CS] (63.8 mg, 0.362 mmol) and DIPEA (0.189 mL, 1.09 mmol) in anhydrous CH₂Cl₂ (20 mL) and the resulting mixture was stirred at room temperature for 8 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 silica gel column chromatography (n-hexane/AcOEt = 5/1) to give compound S1 (134 mg, 88%) as a white amorphous powder.

Mp 95–96°C. [α]D22 +4.02 (c 1.0, CHCl3). IR νmax (KBr): 3326, 3114, 2978, 2792, 1721, 1638, 1612, 1564, 1415, 1327, 1158, 1056, 1027 cm−1. 1H NMR (CDCl3) δ 1.49 (9 H, s), 1.51 (9 H, s), 1.65–1.74 (1 H, m), 2.24–2.34 (2 H, m), 2.59 (2 H, d, J = 4.6 Hz), 2.81–2.88 (1 H, m), 3.60 (1 H, AB, J = 12.8 Hz), 3.64 (1 H, AB, J = 12.8 Hz), 4.68 (1 H, ddt, J = 4.6, 8.5 and 8.5 Hz), 7.21–7.35 (5 H, m), 8.67 (1 H, d, J = 8.3 Hz), 11.5 (1 H, s). 13C NMR (CDCl3): δ 27.97, 28.20, 32.42, 49.63, 52.31, 59.58, 60.30, 78.98, 82.78, 126.80, 128.11, 128.44, 138.91, 152.98, 155.24, 163.53. HRMS (MALDI): Calcd for C22H44N4O4Na (M+Na+): 441.2472. Found: 441.2474. Anal. Calcd for C22H34N4O4: C, 63.13; H, 8.19; N, 13.39. Found: C, 62.74; H, 7.97; N, 13.24.

(3S)-3-Guanidinopyrrolidine-TFA (S2): TFA (20 mL) was added to a solution of S1 (500 mg, 1.19 mmol) in CH2Cl2 (20 mL) and the resulting mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated in vacuo, the crude product was dissolved in MeOH (10 mL). Under a H2 atmosphere, the solution was added to a solution of 20% Pd(OH)2-C (300 mg) in MeOH (10 mL) and the resulting mixture was stirred at room temperature for 12 h. After the reaction mixture was filtered, the solution was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CH2Cl2/MeOH = 1/1) to give compound S2 (247 mg, 86%) as a white amorphous powder.

Mp 138–140°C. [α]D23 −9.40 (c 1.0, MeOH). IR νmax (KBr): 3339, 1675, 1523, 1428, 1344, 1201, 1133, 1034 cm−1. 1H NMR (CD3OD) δ 2.05–2.13 (1 H, m), 2.39 (1 H, dddd, J = 6.4, 7.3, 7.3 and 14.4 Hz), 3.28–3.33 (1 H, m), 3.38–3.52 (2 H, m), 3.58 (1 H, dd, J = 12.4 Hz), 4.34–4.39 (1 H, m). 13C NMR (CDCl3) δ 31.69, 45.24, 50.90, 51.91, 118.08 (q, J = 292 Hz, TFA), 158.44, 163.39 (q, J = 34.5 Hz, TFA). MS (FAB) m/z 129 (M+H+). HRMS (FAB): Calcd for C8H13N4 (M+H+): 129.1135. Found: 129.1142.

(3R)-1-Benzyl-3-[(N,N’-bis-[(2-tert-butoxy)carbonyl]guanidino]pyrrolidine (S3): Under a N2 atmosphere, EDCI (104 mg, 0.543 mmol) was added to a solution of (3R)-(−)-1-benzyl-3-aminopyrrolidine (63.8 mg, 0.362 mmol), (BocNH)2CS (63.8 mg, 0.362 mmol) and DIPEA (0.189 mL, 1.09 mmol) in anhydrous CH2Cl2 (20 mL) and the resulting mixture was stirred at room temperature for 8 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 silica gel column chromatography (n-hexane/AcOEt = 5/1) to give compound S3 (134 mg, 88%) as a white amorphous powder.

Mp 96–97°C. [α]D25 −4.49 (c 1.0, CHCl3). IR νmax (KBr): 3326, 3114, 2978, 2792, 1721, 1638, 1612, 1563, 1415, 1327, 1158, 1056, 1027 cm−1. 1H NMR (CDCl3) δ 1.49 (9 H, s), 1.51 (9 H, s), 1.65–1.74 (1 H, m), 2.24–2.36 (2 H, m), 2.60 (2 H, d, J = 4.6 Hz), 2.82–2.88 (1 H, m), 3.60 (1 H, AB, J = 12.9 Hz), 3.64 (1 H, AB, J = 12.9 Hz), 4.68 (1 H, ddt, J = 4.6, 6.5 and 6.5 Hz), 7.22–7.36 (5 H, m), 8.67 (1 H, d, J = 8.2 Hz), 11.5 (1 H, s). 13C NMR (CDCl3) δ 28.07, 28.30, 32.52, 49.71,
MeOH (10 mL).

(3R)-3-Guanidinylpyrrolidine-TFA (S4): TFA (10 mL) was added to a solution of S3 (200 mg, 0.478 mmol) in CH$_2$Cl$_2$ (10 mL) and the resulting mixture was stirred at room temperature for 1 h. After the reaction mixture was concentrated in vacuo, the crude product was dissolved in MeOH (10 mL). Under a H$_2$ atmosphere, the solution was added to a solution of 20% Pd(OH)$_2$-C (400 mg) in MeOH (10 mL) and the resulting mixture was stirred at room temperature for 8 h. After the reaction mixture was filtered, the solution was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CH$_2$Cl$_2$/MeOH = 1/1) to give compound S4 (88.2 mg, 80%) as a white amorphous powder.

Mp 138–140°C. $\left[\alpha\right]_D^{24}$ -9.37 (c 1.0, MeOH). IR $\nu_{\max}$ (KBr): 3345, 1675, 1523, 1428, 1344, 1201, 1133, 1034 cm$^{-1}$. $^1$H NMR (CD$_2$OD) $\delta$ 1.69–1.77 (1 H, m), 2.16 (1 H, dddd, $J$ = 7.8, 7.8, 7.8 and 15.6 Hz), 2.77 (1 H, dd, $J$ = 4.1 and 11.9 Hz), 2.87–2.93 (1 H, m), 2.99–3.05 (1 H, m), 3.11 (1 H, dd, $J$ = 6.4 and 11.9 Hz), 3.98–4.03 (1 H, m). $^{13}$C NMR (CDCl$_3$) $\delta$ 33.73, 46.05, 53.41, 53.61, 118.13 (q, $J$ = 292 Hz, TFA), 158.33, 163.29 (q, $J$ = 34.5 Hz, TFA). MS (FAB) m/z 129 (M+H$^+$). HRMS (FAB): Calcd for C$_3$H$_{13}$N$_4$ (M+H$^+$): 129.1135. Found: 129.1140.

(3S)-1-Benzyl-3-{N,N’-bis-[2-tert-butoxy carbonyl]-1-methylguanidinob}pyrrolidine (S5): Under a N$_2$ atmosphere, EDCI (4.03 g, 21.0 mmol) was added to a solution of (3S)-(+)1-benzyl-3-(methylamino)pyrrolidine (2.0 g, 10.5 mmol), (BocNH)$_2$CS (5.81 g, 21.0 mmol) and DIPEA (5.49 mL, 31.5 mmol) in anhydrous CH$_2$Cl$_2$ (50 mL) and the resulting mixture was stirred at room temperature for 24 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 silica gel column chromatography (n-hexane/AcOEt = 2/1) to give compound S5 (2.30 g, 51%) as colorless syrup.

$\left[\alpha\right]_D^{25}$ -25.5 (c 1.0, CHCl$_3$). IR $\nu_{\max}$ (KBr): 3156, 2977, 2931, 2792, 1747, 1632, 1604, 1496, 1451, 1393, 1295, 1234, 1144, 1082 cm$^{-1}$. $^1$H NMR (CDCl$_3$) $\delta$ 1.38–1.48 (19 H, m), 1.82–1.91 (1 H, m), 2.17–2.29 (2 H, m), 2.46 (1 H, dd, $J$ = 8.3 and 11.0 Hz), 2.79–2.82 (1 H, m), 2.91–2.95 (1 H, m), 3.07 (3 H, s), 3.48 (1 H, AB, $J$ = 12.8 Hz), 3.66 (1 H, AB, $J$ = 12.8 Hz), 7.22–7.33 (5H, m), 10.1 (1 H, brs). $^{13}$C NMR (CDCl$_3$) $\delta$ 28.09, 29.01, 32.52, 53.62, 57.44, 59.94, 79.10, 81.59, 126.96, 128.23, 128.43, 138.77, 150.67, 155.36, 162.58. HRMS (MALDI): Calcd for C$_{23}$H$_{36}$N$_4$O$_4$Na (M+Na$^+$): 455.2629. Found: 455.2633.

(3S)-3-(1-Methylguanidino)pyrrolidine-TFA (S6): TFA (10 mL) was added to a solution of S5 (1.0 g, 2.31 mmol) in CH$_2$Cl$_2$ (10 mL) and the resulting mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated in vacuo, the crude product was dissolved in MeOH (10 mL). Under a H$_2$ atmosphere, the solution was added to a solution of 20% Pd(OH)$_2$-C
(1.0 g) in MeOH (30 mL) and the resulting mixture was stirred at room temperature for 5 h. After the reaction mixture was filtered, the solution was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CH₂Cl₂/MeOH = 1/1) to give compound S6 (610 mg, quant.) as a white amorphous powder.

Mp 180–181°C. [α]D²⁵ = −2.07 (c 0.5, MeOH). IR νmax (KBr): 3346, 3156, 1670, 1625, 1469, 1435, 1205, 1133 cm⁻¹. ¹H NMR (CDCl₃) δ 1.94 (1 H, dddd, J = 5.5, 6.4, 6.4 and 12.2 Hz), 2.21 (1 H, dddd, J = 5.5, 6.4, 6.4 and 12.2 Hz), 2.39 (3 H, s), 2.36 (1 H, dd, J = 4.6 and 10.6 Hz), 3.34–3.48 (2 H, m), 3.51–3.60 (2 H, m). ¹³C NMR (CDCl₃) δ 31.19, 34.21, 46.42, 52.64, 59.81, 118.02 (q, J = 292 Hz, TFA), 156.26, 162.83 (q, J = 34.5 Hz, TFA). MS (FAB) m/z 143 (M+H⁺). HRMS (FAB): Calcd for C₅H₁₃N₄ (M+H⁺): 143.1291. Found: 143.1293.

(3R,4R)-1-Benzyl-3,4-di{N,N'-bis-[2-tert-butoxy]carbonyl]guanidino}pyrrolidine (S7): Under a N₂ atmosphere, EDCI (3.75 g, 19.6 mmol) was added to a solution of (3R,4R)-1-benzyl-3,4-diaminopyrrolidine (1.10 g, 5.75 mmol), (BocNH)₂CS (3.60 g, 13.0 mmol) and DIPEA (6.81 mL, 39.1 mmol) in anhydrous CH₂Cl₂ (100 mL) and the resulting mixture was stirred at room temperature for 8 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 silica gel column chromatography (n-hexane/AcOEt = 10/1) to give compound S7 (2.37 g, 61%) as a white amorphous powder.

Mp 93–95°C. [α]D²⁵ = −15.7 (c 1.0, CHCl₃). IR νmax (KBr): 3321, 3126, 2979, 2976, 1722, 1613, 1413, 1335, 1154, 1056 cm⁻¹. ¹H NMR (CDCl₃) δ 1.47 (18 H, s), 1.50 (18 H, s), 2.44 (2 H, dd, J = 4.6 and 9.6 Hz), 3.07 (2 H, dd, J = 6.4 and 9.6 Hz), 3.59 (1 H, AB, J = 13.3 Hz), 3.64 (1 H, AB, J = 13.3 Hz), 4.51–4.57 (2 H, m), 7.22–7.34 (5 H, m), 8.67 (2 H, d, J = 7.3 Hz), 11.4 (2 H, s). ¹³C NMR (CDCl₃) δ 28.00, 28.22, 56.21, 59.17, 59.21, 78.93, 82.90, 126.96, 128.18, 128.41, 138.38, 152.88, 155.69, 163.42. MS (FAB) m/z 676 (M+H⁺). HRMS (FAB): Calcd for C₃₅H₃₄N₈O₈ (M+H⁺): 676.4028. Found: 676.4022.

(3R,4R)-3,4-Diguanidinylpyrrolidine-TFA (S8): TFA (30 mL) was added to a solution of S7 (1.00 g, 1.48 mmol) in CH₂Cl₂ (60 mL) and the resulting mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated in vacuo, the crude product was dissolved in MeOH (50 mL). Under a H₂ atmosphere, the solution was added to a solution of 20% Pd(OH)₂-C (500 mg) in MeOH (50 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was filtrated, the solution was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (MeOH) to give compound S8 (489 mg, 80%) as a white amorphous powder.

Mp 89–91°C. [α]D³⁰ = −9.74 (c 1.0, MeOH). IR νmax (KBr): 3366, 3175, 1675, 1433, 1202, 1136 cm⁻¹. ¹H NMR (CDCl₃) δ 2.79 (2 H, dd, J = 6.0 and 6.0 Hz), 3.35 (2 H, dd, J = 6.0 and 11.0 Hz), 3.98 (2 H, dd, J = 6.0 and 11.0 Hz). ¹³C NMR (CDCl₃) δ 51.49, 58.77, 117.92 (q, J = 293 Hz, TFA), 158.39, 163.17 (q, J = 34.5 Hz, TFA). HRMS (MALDI): Calcd for C₆H₁₅N₇Na (M+Na⁺): 208.1281.
Found: 208.1280.

**Synthesis of TFOs by using the PEM method:** TFOs were synthesized on a 0.2-μmol scale on an automated DNA synthesizer (GeneDesign nS-8) using the common phosphoramidite protocol (Synthesis mode: DMTr-ON). The CPG resin-supported oligonucleotides were treated with 10% aqueous guanidinopyrrolidines solution at room temperature for 2 h for conversion of the triazolylated nucleobase into the desired guanidinopyrrolidine-containing nucleobases. Then, additional treatment with 28% aqueous NH₃ solution at room temperature for 5–6 h resulted in complete removal of the acetyl groups of the 5-methylcytosine bases and the complete cleavage of oligonucleotides from the CPG resin. After the two solutions were combined, the solvent was removed in vacuo. The crude TFOs obtained were purified with Nap™-10 columns (GE Healthcare) for removal of the excess amount of pyrrolidines and then treated with Sep-Pak® Plus C18 cartridges (Waters) followed by reversed-phase HPLC (Waters XBridge® MS C₁₈ 2.5 μm, 10 × 50 mm). The composition of the TFOs was confirmed by MALDI-TOF-MS analysis (Table S1).

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<th>Sequence of TFOs</th>
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Scheme S2. Synthesis of TFO containing GP^B. C = 2'-deoxy-S-methylcytidine. Reagent and conditions: (i) 2,4,6-trisopropylphenylsulfonyl chloride (TPS-Cl), Et$_3$N, DMAP, CH$_2$Cl$_2$, rt, 12 h, 70%; (ii) (3S)-(−)-3-(trifluoracetamido)pyrrolidine hydrochloride, Et$_3$N, CH$_2$Cl$_2$, rt, 3 h, 90%; (iii) 1N NaOHaq., THF, rt, 2 h, 91%; (iv) N,N'-bis-[2-(cyanoethoxy)carbonyl]-S-methylisothiourea$^3$, DMF, rt, 15 h, 85%; (v) i-Pr$_2$NP(Cl)OCH$_2$CH$_2$CN, i-Pr$_2$NEt, CH$_2$Cl$_2$, 0°C, 3 h, 83%; (vi) oligonucleotide synthesis.

TFO containing GP^B was synthesized as shown in Scheme S2. The oligonucleotide was synthesized using the common phosphoramidite protocol. The synthesis of S14 from S9$^4$ was carried out as described below.

1-(3-O-Acetyl-5-O-dimethoxetyl-2-O,4-C-methylene-β-D-ribofuranosyl)-4-O-(2,4,6-trisopropylphenyl)sulfonylthymine (S10): Under a N$_2$ atmosphere, 2,4,6-trisopropylbenzenesulfonyl chloride (197 mg, 0.651 mmol) was added to a solution of compound S9$^4$ (200 mg, 0.325 mmol), DMAP (3.97 mg, 0.0325 mmol) and Et$_3$N (0.136 mL, 0.976 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) and the resulting mixture was stirred at room temperature for 12 h. After addition of saturated aqueous NaHCO$_3$ solution, the reaction mixture was diluted with CH$_2$Cl$_2$, washed with water and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (n-hexane/AcOEt = 10/1) to give compound S10 (200 mg, 70%) as a white amorphous powder.

Mp 99–101°C. [α]$_D^{23}$ +64.8 (c 1.0, CHCl$_3$). IR $\nu_{\text{max}}$ (KBr): 2959, 1751, 1683, 1605, 1510, 1379, 1252, 1175, 1071 cm$^{-1}$. $^1$H NMR (CDCl$_3$) $\delta$ 1.26 (6 H, d, $J$ = 6.8 Hz), 1.29 (6 H, d, $J$ = 6.8 Hz), 1.33 (6 H, d, $J$ = 6.8 Hz), 1.80 (3 H, s), 2.00 (3 H, s), 2.91 (1 H, sept, $J$ = 6.8 Hz), 3.31 (1 H, AB, $J$ = 11.0 Hz), 3.55 (1 H, AB, $J$ = 11.0 Hz), 3.80–3.84 (8 H, m), 4.32 (2 H, sept, $J$ = 6.8 Hz), 4.68 (1 H, s), 5.08 (1 H, s), 5.65 (1 H, s), 6.82–6.85 (4 H, m), 7.21–7.42 (9 H, m), 8.01 (1 H, s). $^{13}$C NMR (CDCl$_3$) $\delta$ 12.40, 20.64, 23.40, 23.46, 24.38, 24.53, 29.59, 55.21, 57.53, 70.30, 72.11, 77.22, 86.79, 87.14, 88.23, 104.34, 113.26, 113.30, 124.04, 127.16, 127.99, 128.08, 129.98, 130.02, 130.63, 134.98, 135.02, 142.04, 143.98, 151.21, 153.25, 154.41, 158.71, 166.83, 169.26. MS (FAB) m/z 881 (M+H$^+$). HRMS (FAB): Calcd for C$_{49}$H$_{57}$N$_2$O$_{11}$S (M+H$^+$): 881.3678. Found: 881.3691.
1-(3-O-Acetyl-5-O-dimethoxytrityl-2-O,4-C-methylene-β-d-ribofuranosyl)-4-[(3S)-3-(trifluoroacetamido)pyrrolidino]-5-methylpyrimidin-2-one (S11): Under a N₂ atmosphere, (3S)-(−)-3-(trifluoroacetamido)pyrrolidine hydrochloride (54.6 mg, 0.250 mmol) was added to a solution of compound S10 (200 mg, 0.227 mmol) and Et₃N (94.9 µL, 0.681 mmol) in CH₂Cl₂ (10 mL) 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 CH₂Cl₂, washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt) to give compound S11 (158 mg, 90%) as a white amorphous powder.

Mp 141–143°C. [α]D³⁰ +4.01 (c 1.0, CHCl₃). IR νmax (KBr): 3201, 2956, 1748, 1714, 1653, 1611, 1505, 1471, 1302, 1249, 1078, 1050 cm⁻¹. ¹H NMR (CDCl₃) δ 1.97 (3 H, s), 1.97 (3 H, s), 2.17–2.22 (2 H, m), 3.35 (1 H, AB, J = 11.0 Hz), 3.52 (1 H, AB, J = 11.0 Hz), 3.76–4.01 (11 H, m), 4.28–4.32 (1 H, m), 4.60–4.64 (2 H, m), 5.05 (1 H, s), 5.68 (1 H, s), 6.80–6.82 (4 H, m), 7.18–7.32 (7 H, m), 7.42–7.44 (2 H, m), 7.53 (1 H, s), 7.93 (1 H, brs). ¹³C NMR (CDCl₃) δ 17.90, 20.51, 31.39, 47.04, 48.55, 53.29, 55.01, 57.84, 70.65, 72.13, 77.70, 86.48, 86.56, 87.53, 102.57, 113.05, 113.08, 115.88 (q, J = 288 Hz), 126.89, 127.81, 129.88, 134.92, 135.03, 137.85, 144.11, 154.63, 157.71 (q, J = 137 Hz), 158.48, 162.42, 169.33. MS (FAB) m/z 779 (M+H⁺). Anal. Calcd for C₄₀H₄₁F₃N₄O₇: C, 61.69; H, 5.31; N, 7.19. Found: C, 61.67; H, 5.36; N, 6.91.

1-(5-O-Dimethoxytrityl-2-O,4-C-methylene-β-d-ribofuranosyl)-4-[(3S)-3-aminopyrrolidino]-5-methylpyrimidin-2-one (S12): 1N aqueous NaOH (5 mL) was added to a solution of compound S11 (200 mg, 0.257 mmol) in THF (5 mL) and the resulting mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated in vacuo, was dissolved in CH₂Cl₂. The solution was washed with saturated aqueous Na₂CO₃, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt /MeOH = 1/3) to give compound S12 (150 mg, 91%) as a white amorphous powder.

Mp 153–156°C. [α]D²⁴ +20.8 (c 1.0, CHCl₃). IR νmax (KBr): 3280, 2949, 1650, 1607, 1505, 1467, 1302, 1251, 1177, 1050 cm⁻¹. ¹H NMR (CDCl₃) δ 1.66–1.74 (1 H, m), 1.96–2.07 (4 H, m), 3.44–3.53 (3 H, m), 3.60 (1 H, dddd, J = 4.6, 4.6, 5.0 and 5.0 Hz), 3.70–3.96 (11 H, m), 4.23 (1 H, s), 4.49 (1 H, s), 5.66 (1 H, s), 6.84 (4 H, dd, J = 2.8 and 8.9 Hz), 7.20–7.37 (7 H, m), 7.48 (2 H, d, J = 7.8 Hz), 7.55 (1 H, s). ¹³C NMR (CDCl₃) δ 18.35, 33.95, 47.70, 50.44, 55.43, 57.70, 59.12, 70.14, 71.96, 79.65, 86.57, 87.65, 87.93, 102.25, 113.41, 127.12, 128.13, 128.33, 130.32, 130.36, 139.09, 144.91, 155.08, 158.74, 163.18. MS (FAB) m/z 641 (M+H⁺). HRMS (FAB): Calcd for C₃₇H₄₁N₄O₇ (M+H⁺): 641.2970. Found: 641.2969.

4-[(3S)-3-{N,N'-Bis-[2-cyanoethoxy]carbonyl]guanidinyl}pyrrolidino]-1-(5-O-dimethoxytrityl-2-O,4-C-methylene-β-d-ribofuranosyl)-5-methylpyrimidin-2-one (S13): Under a N₂ atmosphere, N,N'-bis-[2-cyanoethoxy]carbonyl]-S-methylisothiourea³ was added to a solution of compound S12 (100 mg, 0.156 mmol) in DMF (2 mL) and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo. The residue was purified by
silica gel column chromatography (AcOEt to AcOEt/MeOH = 10/1) to give compound S13 (116 mg, 91%) as a white amorphous powder.

Mp 193–195°C. [α]_D^30 +9.18 (c 1.0, CHCl₃). IR ν max (KBr): 3330, 3185, 2959, 2252, 1743, 1648, 1505, 1469, 1294, 1254, 1209, 1085, 1052 cm⁻¹. ¹H NMR (CDCl₃) δ 1.97–2.07 (4 H, m), 2.22–2.28 (1 H, m), 2.75 (2 H, t, J = 6.0 Hz), 2.77 (2 H, t, J = 6.0 Hz), 3.45 (1 H, AB, J = 11.0 Hz), 3.51 (1 H, AB, J = 11.0 Hz), 3.59 (1 H, d, J = 5.0 Hz), 3.72–3.88 (11 H, m), 3.96–4.03 (1 H, m), 4.24 (1 H, d, J = 5.0 Hz), 4.30 (2 H, t, J = 6.4 Hz), 4.37 (2 H, t, J = 6.4 Hz), 4.47 (1 H, s), 4.63–4.70 (1 H, m), 6.83 (4 H, dd, J = 2.3 and 8.7 Hz), 7.20–7.31 (3 H, m), 7.36 (4 H, dd, J = 2.3 and 8.7 Hz), 7.48 (2 H, d, J = 7.3 Hz), 7.60 (1 H, s), 8.45 (1 H, d, J = 6.8 Hz), 11.65 (1 H, s). ¹³C NMR (CDCl₃) δ 17.86, 19.56, 30.67, 47.05, 50.18, 54.26, 55.13, 58.63, 59.72, 60.80, 70.03, 71.56, 79.21, 86.32, 87.25, 87.66, 101.98, 113.10, 116.24, 116.97, 126.88, 127.84, 127.98, 130.01, 135.36, 135.42, 139.25, 144.55, 152.84, 154.64, 155.41, 158.43, 162.61, 163.14. MS (FAB) m/z 877 (M+H⁺). HRMS (FAB): Calcd for C₄₅H₄₉N₉O₁₁ (M+H⁺): 877.3515. Found: 877.3521.

4-{(3S)-3-[(N,N’)-Bis-[(2-cyanoethoxy)carbonyl]guanidinyl]pyrrolidino}-1-{3-O-[(disopropylamino)phosphino]-5-O-dimethoxytrityl-2-O,4-C-methylene-β-d-ribofuranosyl}-5-methylpyrimidin-2-one (S14): Under a N₂ atmosphere, i-Pr₂NP(Cl)OCH₂CH₂CN (0.458 mL, 2.05 mmol) was added to a solution of S13 (1.50 g, 1.71 mmol) and i-Pr₂NEt (0.894 mL, 5.13 mmol) in anhydrous CH₂Cl₂ (20 mL) at 0°C and the resulting mixture was stirred at 0°C for 3 h. After addition of saturated aqueous NaHCO₃ solution, the reaction mixture was diluted with CH₂Cl₂, washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH₂Cl₂/MeOH = 20/1) to give compound S14 (1.53 g, 83%) as a white amorphous powder.

Mp 107–109°C. ¹H NMR (CDCl₃) δ 0.96–1.28 (12 H, m), 1.87 (1.8 H, s), 2.05 (1.2 H, s), 2.00–2.08 (1 H, m), 2.23–2.32 (1 H, m), 2.38 (1.2 H, t, J = 6.4 Hz), 2.55 (0.8 H, t, J = 6.4 Hz), 2.77 (2 H, t, J = 6.4 Hz), 2.79 (2 H, t, J = 6.4 Hz), 3.35–4.12 (23 H, m), 4.65–4.72 (2 H, m), 5.76 (1 H, s), 6.82–6.88 (4 H, m), 7.22–7.38 (7 H, m), 7.46–7.49 (2 H, m), 7.65 (0.4 H, s), 7.67 (0.6 H, s), 8.45 (1 H, d, J = 6.9 Hz), 11.7 (1 H, s). ³¹P NMR (CDCl₃) δ 148.6, 149.1. MS (FAB) m/z 1077 (M+H⁺). HRMS (FAB): Calcd for C₅₄H₆₆N₁₀O₁₂ (M+H⁺): 1077.4594. Found: 1077.4592.

References
**TFO: 5’-TTTTTCGPTCTCTCT-3’**

**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.

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**MALDI-TOF-Mass**
TFO: 5’-TTTTCTGPTCTCCTC-3’

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

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TFO: 5’-TTTTTCTmGPTCTCTCTCT-3’

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.
**TFO: 5’-TTTTTCTdiGPTCTCTCT-3’**

**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.

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**MALDI-TOF-Mass**

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*Electronic Supplementary Material (ESI) for Chemical Communications*
**TFO: 5'-TTTTTCTGETCTCTCT-3'**

**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.
**TFO: 5'-TTTTTCTGP^OMe^TCTCTCT-3'**

**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: 5’-TTTTCTGPTCTCTCT-3’**

**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**

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Electronic Supplementary Material (ESI) for Chemical Communications
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**TFO: 5’-TTTTTTCGP^B^TCTCTCTCT-3’**

**HPLC**

Column: Waters XBridge® MS C\(_{18}\) 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.: 50°C.

**MALDI-TOF-Mass**
**TFO: 5'-TTTTCTGPTCTCTCT-3'**

**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: 5’-TTTTTTCGP<sup>B</sup>CTTCTC-3’**

**HPLC**

Column: Waters XBridge<sup>®</sup> 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: 5’-TTTTTGP<sup>B</sup>TGP<sup>B</sup>TGP<sup>B</sup>TCTCT-3’

HPLC

Column: Waters XBridge<sup>®</sup> 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

Electronic Supplementary Material (ESI) for Chemical Communications
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**TFO: 5'-TTTTGPP-TTGPB-TTGPB-TTCT-3’**

**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.

![HPLC Chromatogram](image)

**MALDI-TOF-Mass**

![MALDI-TOF Mass Spectrum](image)
TFO: 5’-TTTGP^B-TCTGP^B-TCTGP^B-TCT-3’

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
Compound S1
Compound S2
Compound S3
Compound S4
Compound S5
Compound S6
Compound S7
Compound S8

S30
Compound S10
Compound S11
Compound S12
Compound S13
Compound S14