Electronic Supplementary Information

Synthesis of oligonucleotides containing N,N-disubstituted 3-deazacytosine nucleobases by post-elongation modification and their triplex-forming ability with double-stranded DNA

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Contents

1. Synthesis of new secondary amines Page S1–S6
2. Representative HPLC charts of crude TFOs before HPLC purification Page S7
3. Representative UV-melting curves of triplexes Page S7
4. \(^1\text{H}, \text{\(^{13}\text{C}\) and \(^{31}\text{P}\) spectra for the new compounds} Page S8–S24
5. HPLC charts and MALDI-TOF-Mass spectra for TFOs Page S25–S35
**General:** Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N₂ atmosphere. ¹H NMR (400 MHz) and ¹³C NMR (101 MHz) were recorded on JEOL JNM-ECS-400 spectrometers. Chemical shifts are reported in parts per million downfield from an internal standard [tetramethylsilane (0.00 ppm) for ¹H NMR, or CD₃OD (49.00 ppm) or CDCl₃ (77.00 ppm) for ¹³C NMR]. IR spectra were recorded on a JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO P-2200 instrument. Mass spectra were measured on a JEOL JMS-700 mass spectrometer. For silica gel flash column chromatography, Fuji Silysia PSQ-100B, FL-100D was used. For amine silica gel column chromatography, Fuji Silysia DM-1020 was used.

**Synthesis of secondary amines:** All new secondary amines S1-S4 used in this study were synthesized in Scheme S1.

![Scheme S1. Synthesis of guanidinomethylpyrrolidines. Reagents and conditions:](image)

**Scheme S1.** Synthesis of guanidinomethylpyrrolidines. Reagents and conditions: (i) NaN₃, DMF, 60 °C, 10 h, 88% (S6), 95% (S11); (ii) n-Bu₃P, THF–H₂O, room temperature, 10 h, quant. (S7), quant. (S12); (iii) (BocNH)₂CS, DIPEA, EDCI–HCl, CH₂Cl₂, room temperature, 5–13 h, 71% (S8), 66% (S13), 64% (S15), 89% (S17); (iv) TFA, CH₂Cl₂, room temperature, 2–10 h; (v) H₂, 20% Pd(OH)₂-C, MeOH, room temperature, 10–13 h, 97% (S1), 79% (S2), 75% (S3), 87% (S4); (vi) MsCl, Et₃N, DMAP, CH₂Cl₂, room temperature, 3 h, quant.

**(3S)-3-Azidomethyl-1-[([R]-1-phenylethyl)pyrrolidine (S6):** Under a N₂ atmosphere, NaN₃ (459 mg, 7.06 mmol) was added to a solution of compound S5 (1.0 g, 3.53 mmol) in anhydrous DMF (50 mL) and the resulting mixture was stirred at 60 °C for 10 h. After addition of saturated aqueous NaHCO₃ solution, the reaction mixture was extracted with Et₂O. The organic extracts were washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (n-hexane/AcOEt = 5/1) to give compound S6 (715 mg, 88%) as a yellow syrup.

[α]D²⁸ +38.3 (c 1.0, CHCl₃). IR ν_max (KBr) 3061, 3028, 2971, 2930, 2872, 2784, 2095, 1492, 1452, 1368, 1280, 1150 cm⁻¹. ¹H NMR (CDCl₃) δ 1.36 (3H, d, J = 6.4 Hz), 1.41–1.53 (1H, m), 1.95–2.04...
(1H, m), 2.26 (1H, dd, J = 6.4 and 13.2 Hz), 2.32–2.43 (2H, m), 2.55–2.67 (2H, m), 3.17 (2H, q, J = 6.4 Hz), 3.25 (2H, d, J = 7.3 Hz), 7.20–7.33 (5H, m). 13C NMR (CDCl3) δ 23.02, 28.12, 37.15, 52.23, 55.64, 56.52, 65.47, 126.85, 127.04, 128.24, 145.39. MS (FAB) m/z 231 (M+H+). HRMS (FAB): Calcd for C13H19N4 (M+H+), 231.1604; found, 231.1610.

(3R)-3-Aminomethyl-1-[(R)-1-phenylethyl]pyrrolidine (S7): n-Bu3P (1.52 mL, 6.08 mmol) was added to a solution of compound S6 (700 mg, 3.04 mmol) in THF (30 mL) and H2O (6 mL), and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was concentrated in vacuo, the residue was purified by amine silica gel column chromatography (CH2Cl2/MeOH = 100/1 to 20/1) to give compound S7 (639 mg, quant.) as a yellow syrup. [α]D25 +53.2 (c 1.0, CHCl3). IR νmax (KBr) 3335, 2969, 2785, 2596, 2158, 1750, 1491, 1452, 1372, 1309, 1219, 1148 cm⁻¹. 1H NMR (CDCl3) δ 1.08 (2H, brs), 1.37 (3H, d, J = 6.4 Hz), 1.38–1.46 (1H, m), 1.95–2.04 (1H, m), 1.93–2.03 (1H, m), 2.08–2.22 (2H, m), 2.34 (1H, ddd, J = 6.4, 8.3 and 13.6 Hz), 2.59–2.69 (3H, m), 2.75 (1H, ddd, J = 6.4, 8.3 and 13.6 Hz), 3.16 (2H, q, J = 6.4 Hz), 7.19–7.33 (5H, m). 13C NMR (CDCl3) δ 23.09, 28.28, 40.84, 47.03, 52.50, 57.35, 65.86, 126.73, 127.09, 128.18, 145.60. MS (FAB) m/z 205 (M+H+). HRMS (FAB): Calcd for C13H21N2 (M+H+), 205.1699; found, 205.1709.

(3R)-3-[N,N’-bis(tert-butoxycarbonyl)guanidinomethyl]-1-[(R)-1-phenylethyl]pyrrolidine (S8): Under a N2 atmosphere, EDCI•HCl (141 mg, 0.734 mmol) was added to a solution of compound S7 (100 mg, 0.489 mmol), (BocNH)2CS2 (135 mg, 0.489 mmol), and DIPEA (0.256 mL, 1.47 mmol) in anhydrous CH2Cl2 (10 mL); the resulting mixture was stirred at room temperature for 8 h. After addition of saturated aqueous NaHCO3 solution, the reaction mixture was extracted with CH2Cl2. The organic extracts were washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (n-hexane/AcOEt = 1/1) to give compound S8 (155 mg, 71%) as a colorless syrup. [α]D30 +10.3 (c 1.0, CHCl3). IR νmax (KBr) 3330, 3280, 3129, 2975, 2931, 2876, 2783, 1795, 1722, 1639, 1415, 1366, 1318, 1133, 1056, 1027 cm⁻¹. 1H NMR (CDCl3) δ 1.37 (3H, d, J = 6.4 Hz), 1.50 (18H, s), 1.92–2.01 (1H, m), 2.37–2.53 (5H, m), 3.15 (1H, q, J = 6.4 Hz), 3.37 (1H, ddd, J = 7.0, 7.0 and 13.3 Hz), 3.47 (1H, ddd, J = 7.0, 7.0 and 13.3 Hz), 7.20–7.38 (5H, m), 8.51 (1H, s), 11.5 (1H, s). 13C NMR (CDCl3) δ 23.42, 28.26, 28.42, 28.58, 36.31, 46.13, 52.98, 56.99, 65.86, 79.45, 83.14, 127.05, 127.51, 128.48, 145.91, 149.00, 153.34, 156.73, 163.92. MS (FAB) m/z 447 (M+H+). HRMS (FAB): Calcd for C24H39N4O4 (M+H+), 447.2966; found, 447.2970.

(3R)-3-Guanidinomethylpyrrolidine, TFA salt (S1): TFA (5 mL) was added to a solution of S8 (1.0 g, 2.42 mmol) in CH2Cl2 (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was concentrated in vacuo, the crude product was dissolved in MeOH (5 mL). Under a H2 atmosphere, the solution was added to a solution of 20% Pd(OH)2-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction...
mixture was filtered, the filtrate was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CHCl₃/MeOH = 1/1) to give compound S1 (600 mg, 97%) as a yellow syrup. 

\([\alpha]_D^{24} -6.22 \ (c 1.0, \text{MeOH})\). IR \(\nu_{\text{max}}\) (KBr) 3141, 2152, 1679, 1511, 1436, 1202, 1139 cm\(^{-1}\). ¹H NMR (CD₃OD) \(\delta\) 1.44 (1H, dddd, \(J = 5.0, 6.9, 6.9\) and 14.7 Hz), 1.98 (1H, dddd, \(J = 5.0, 6.9, 6.9\) and 14.7 Hz), 2.37 (1H, ddd, \(J = 6.9, 6.9\) and 14.7 Hz), 2.55 (1H, dd, \(J = 7.3\) and 11.4 Hz), 2.82–2.97 (2H, m), 3.02 (1H, dd, \(J = 7.3\) and 11.4 Hz), 3.15 (2H, d, \(J = 7.3\) Hz). ¹³C NMR (CD₃OD) \(\delta\) 30.85, 39.89, 45.78, 46.92, 50.93, 118.16 (q, \(J = 293\) Hz), 158.84, 163.21 (q, \(J = 34.5\) Hz). MS (FAB) \(m/\text{z}\) 143 (M+H\(^{+}\)).

HRMS (FAB): Calcd for C₆H₁₅N₄ (M+H\(^{+}\)), 143.1291; found, 143.1299.

(3R)-3-Methanesulfonyloxymethyl-1-[(R)-1-phenylethyl]pyrrolidine (S10): Under a N\(_2\) atmosphere, MsCl (0.566 mL, 7.31 mmol) was added to a solution of compound S9 (1.0 g, 4.87 mmol), DMAP (59.5 mg, 0.487 mmol), and Et\(_3\)N (2.04 mL, 14.6 mmol) in anhydrous CH\(_2\)Cl\(_2\) (40 mL) at 0 °C; the resulting mixture was stirred at room temperature for 3 h. After addition of saturated aqueous NaHCO₃ solution, the reaction mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (n-hexane/AcOEt = 1/1) to give compound S10 (1.43 mg, quant.) as a yellow syrup.

\([\alpha]_D^{25} +40.9 \ (c 1.0, \text{CHCl}_3)\). IR \(\nu_{\text{max}}\) (KBr) 3616, 3027, 2971, 2936, 2876, 2789, 1492, 1453, 1355, 1283, 1174 cm\(^{-1}\). ¹H NMR (CDCl\(_3\)) \(\delta\) 1.37 (3H, d, \(J = 6.4\) Hz), 1.46–1.54 (1H, m), 1.93–2.02 (1H, m), 2.28 (1H, dd, \(J = 4.5\) and 9.0 Hz), 2.36 (1H, ddd, \(J = 8.7, 8.7\) and 8.7 Hz), 2.51–2.63 (2H, m), 2.71 (1H, ddd, \(J = 5.0, 8.7\) and 8.7 Hz), 2.94 (3H, s), 3.20 (1 H, q, \(J = 6.4\) Hz), 4.07–4.14 (2H, m), 7.20–7.30 (5H, m). ¹³C NMR (CDCl\(_3\)) \(\delta\) 23.06, 26.74, 36.50, 37.15, 51.84, 55.35, 65.19, 72.66, 126.92, 126.97, 145.29. MS (FAB) \(m/\text{z}\) 284 (M+H\(^{+}\)). HRMS (FAB): Calcd for C\(_{14}\)H\(_{22}\)NO\(_3\)S (M+H\(^{+}\)), 284.1315; found, 284.1317.

(3R)-3-Azidomethyl-1-[(R)-1-phenylethyl]pyrrolidine (S11): Under a N\(_2\) atmosphere, NaN\(_3\) (642 mg, 9.88 mmol) was added to a solution of compound S10 (1.4 g, 4.94 mmol) in anhydrous DMF (30 mL) and the resulting mixture was stirred at 60 °C for 10 h. After addition of saturated aqueous NaHCO\(_3\) solution, the reaction mixture was extracted with Et\(_2\)O. The organic extracts were washed with water and brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (n-hexane/AcOEt = 1/1) to give compound S11 (1.08 g, 95%) as a yellow syrup.

\([\alpha]_D^{21} +59.3 \ (c 1.0, \text{CHCl}_3)\). IR \(\nu_{\text{max}}\) (KBr) 3061, 3027, 2970, 2931, 2872, 2785, 2095, 1491, 1451, 1367, 1280, 1151 cm\(^{-1}\). ¹H NMR (CDCl\(_3\)) \(\delta\) 1.35 (3H, d, \(J = 6.4\) Hz), 1.41–1.49 (1H, m), 1.90–1.99 (1H, m), 2.18 (1H, dd, \(J = 5.5\) and 9.6 Hz), 2.59 (1H, ddd, 5.5, 9.2 and 9.2 Hz), 2.68 (1H, dd, 7.8 and 9.6 Hz), 3.14–3.25 (3H, m), 7.18–7.31 (5H, m). ¹³C NMR (CDCl\(_3\)) \(\delta\) 22.32, 28.74, 36.90, 52.73, 56.77, 56.44, 65.33, 126.17, 126.85, 128.16, 145.32. MS (FAB) \(m/\text{z}\) 231 (M+H\(^{+}\)). HRMS (FAB): Calcd for C\(_{13}\)H\(_{19}\)N\(_4\) (M+H\(^{+}\)), 231.1604; found, 231.1609.
(3S)-3-Aminomethyl-1-[\((R)\)-1-phenylethyl]pyrrolidine (S12): n-Bu₃P (2.17 mL, 8.68 mmol) was added to a solution of compound S11 (1.0 g, 4.34 mmol) in THF (60 mL) and H₂O (12 mL), and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was concentrated in vacuo, the residue was purified by amine silica gel column chromatography (CH₂Cl₂/MeOH = 50/1) to give compound S12 (887 mg, quant.) as a yellow syrup.

\[\alpha\]D₂₃ +54.1 (c 1.0, CHCl₃). IR \(\nu_{\text{max}}\) (KBr) 3277, 2970, 2783, 2602, 2151, 1570, 1490, 1453, 1373, 1310, 1220, 1147 cm⁻¹. ¹H NMR (CDCl₃) \(\delta\) 1.09 (1H, brs), 1.37 (3H, d, \(J = 6.4\) Hz), 1.38–1.46 (1H, m), 1.88–1.97 (1H, m), 2.11 (1H, dd, \(J = 6.0\) and 8.7 Hz), 2.14–2.25 (1H, m), 2.41 (1H, ddd, \(J = 6.0\), 8.7 and 8.7 Hz), 2.52 (1H, ddd, \(J = 6.0\), 8.7 and 8.7 Hz), 2.64 (1H, dd, \(J = 1.9\) and 6.8 Hz), 2.64 (1H, dd, \(J = 1.9\) and 6.8 Hz), 2.79 (1H, dd, \(J = 7.8\) and 8.7 Hz), 3.16 (2H, q, \(J = 6.4\) Hz), 7.19–7.33 (5H, m).

¹³C NMR (CDCl₃) \(\delta\) 23.44, 28.59, 40.91, 47.42, 52.83, 57.44, 66.07, 126.95, 127.26, 128.44, 145.92. MS (FAB) \(m/z\) 205 (M+H⁺). HRMS (FAB): Calcd for C₁₃H₂₁N₂ (M+H⁺), 205.1699; found, 205.1707.

(3S)-3-[\(N,N'\)-bis(tert-butoxycarbonyl)guanidinomethyl]-1-[\((R)\)-1-phenylethyl]pyrrolidine (S13): Under a N₂ atmosphere, EDCI•HCl (3.66 g, 19.1 mmol) was added to a solution of compound S12 (3.0 g, 14.7 mmol), (BocNH)₂CS (4.06 g, 14.7 mmol), and DIPEA (7.67 mL, 44.0 mmol) in anhydrous CH₂Cl₂ (30 mL); the resulting mixture was stirred at room temperature for 5 h. After addition of saturated aqueous NaHCO₃ solution, the reaction mixture was extracted with CH₂Cl₂. The organic extracts were washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (\(n\)-hexane/AcOEt = 5/1 to 2/1) to give compound S13 (4.02 g, 66%) as a colorless syrup.

\[\alpha\]D₃₁ –13.7 (c 1.0, CHCl₃). IR \(\nu_{\text{max}}\) (KBr) 3330, 3288, 3128, 2975, 2932, 2877, 2785, 1795, 1722, 1639, 1415, 1366, 1314, 1134, 1055, 1027 cm⁻¹. ¹H NMR (CDCl₃) \(\delta\) 1.38 (3H, d, \(J = 6.4\) Hz), 1.44–1.58 (19 H, m), 1.98 (1H, dddd, \(J = 5.0\), 5.0, 9.6 and 19.2 Hz), 2.22 (1H, dd, \(J = 5.0\) and 9.6 Hz), 2.31–2.50 (2H, m), 2.61 (1H, dd, \(J = 7.8\) and 9.2 Hz), 2.75 (1H, ddd, \(J = 5.0\), 5.0 and 9.6 Hz), 3.16 (1H, q, \(J = 6.4\) Hz), 3.32 (1H, ddd, \(J = 5.0\), 6.0 and 13.4 Hz), 3.46 (1H, ddd, \(J = 6.0\), 6.0 and 13.4 Hz), 7.19–7.38 (5H, m), 8.62 (1H, s), 11.5 (1H, s). ¹³C NMR (CDCl₃) \(\delta\) 23.44, 28.59, 40.91, 47.42, 52.83, 57.44, 66.07, 126.95, 127.26, 128.44, 145.92. MS (FAB) \(m/z\) 447 (M+H⁺). HRMS (FAB): Calcd for C₂₄H₃₉N₄O₄ (M+H⁺), 447.2966; found, 447.2972.

(3S)-3-Guanidinomethylpyrrolidine, TFA salt (S2): TFA (5 mL) was added to a solution of compound S13 (800 mg, 1.93 mmol) in CH₂Cl₂ (5 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 (5 mL). Under a H₂ atmosphere, the solution was added to a solution of 20% Pd(OH)₂-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was filtered, the filtrate was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CHCl₃/MeOH = 1/1 to 1/5) to give compound S4.
S2 (390 mg, 79%) as a yellow syrup.

\([\alpha]_D^{24} +6.63 \text{ (c 1.0, MeOH). IR } v_{\text{max}} \text{ (KBr) 3158, 2494, 1681, 1511, 1430, 1201, 1136 cm}^{-1}. H\text{ NMR (CD}_3\text{OD) } \delta 1.40–1.49 \text{ (1H, m), 1.94–2.02 (1H, m), 2.36 (1H, ddd, } J = 7.8, 7.8 \text{ and } 14.8 Hz), 2.54 (1H, dd, } J = 6.0 \text{ and 7.8 Hz), 2.83–2.97 (2H, m), 3.02 (1H, ddd, } J = 7.8 \text{ and 7.8 Hz), 3.15 (2H, d, } J = 7.8 \text{ Hz). C NMR (CD}_3\text{OD) } \delta 30.88, 39.93, 45.77, 46.95, 50.96, 118.16 \text{ (q, } J = 293 Hz), 158.86, 163.21 \text{ (q, } J = 34.5 Hz). MS (FAB) m/z 143 (M+H\textsuperscript{+}). HRMS (FAB): Calcd for C\textsubscript{6}H\textsubscript{15}N\textsubscript{4} (M+H\textsuperscript{+}), 143.1291; found, 143.1298.

(2R)-1-Benzoxycarbonyl-2-[N,N’-bis(tert-butoxycarbonyl)guanidinomethyl]pyrrolidine (S15):
Under a N\textsubscript{2} atmosphere, EDCI\textcenterdot HCl (1.06 g, 5.55 mmol) was added to a solution of commercially available compound 14 (1.0 g, 4.27 mmol), (BocNH\textsubscript{2})CS (1.18 g, 4.27 mmol), and DIPEA (2.23 mL, 12.8 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (30 mL); the resulting mixture was stirred at room temperature for 5 h. After addition of saturated aqueous NaHCO\textsubscript{3} solution, the reaction mixture was extracted with CH\textsubscript{2}Cl\textsubscript{2}, washed with water and brine, dried over Na\textsubscript{2}SO\textsubscript{4}, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (n-hexane/AcOEt = 5/1 to 3/1) to give compound S15 (1.30 g, 64%) as a yellow syrup.

\([\alpha]_D^{27} +37.4 \text{ (c 0.5, CHCl}_3). IR v_{\text{max}} \text{ (KBr) 3327, 3287, 3136, 2936, 2887, 1706, 1639, 1575, 1450, 1413, 1369, 1329, 1137, 1056 cm}^{-1}. H\text{ NMR (CDCl}_3, \text{ as a mixture of atropisomers) } \delta 1.47 (6H, s), 1.50 (12H, s), 1.78–2.04 (4H, m), 3.40–3.70 (4H, m), 4.07–4.12 (1H, m), 5.08–5.30 (2H, m), 7.28–7.38 (5H, m), 8.51 (0.5H, s), 8.59 (0.5H, s), 11.49 (1H, s). C NMR (CDCl}_3, \text{ as a mixture of atropisomers) } \delta 22.98, 23.81, 27.94, 28.21, 28.70, 29.47, 43.70, 44.15, 46.61, 46.88, 56.11, 56.96, 66.68, 66.77, 79.01, 79.09, 82.78, 82.97, 127.70, 127.78, 127.86, 128.33, 136.79, 152.87, 152.99, 154.98, 155.30, 156.43, 156.51, 163.50. MS (FAB) m/z 477 (M+H\textsuperscript{+}). HRMS (FAB): Calcd for C\textsubscript{24}H\textsubscript{37}N\textsubscript{4}O\textsubscript{7} (M+H\textsuperscript{+}), 477.2708; found, 477.2717.

(2R)-2-Guanidinomethylpyrrolidine, TFA salt (S3): TFA (2 mL) was added to a solution of compound S15 (1.3 g, 2.73 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (2 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 (5 mL). Under a H\textsubscript{2} atmosphere, the solution was added to a solution of 20% Pd(OH\textsubscript{2})\textcenterdot C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was filtered, the filtrate was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CHCl\textsubscript{3}/MeOH = 2/1 to 1/5) to give compound S3 (526 mg, 75%) as a yellow syrup.

\([\alpha]_D^{24} –0.34 \text{ (c 1.0, MeOH). IR } v_{\text{max}} \text{ (KBr) 3143, 1676, 1523, 1420, 1200, 1137 cm}^{-1}. H\text{ NMR (CD}_3\text{OD) } \delta 1.43 (1H, dddd, } J = 5.0, 6.8, 6.8 \text{ and 13.6 Hz), 1.67-1.84 (2H, m), 1.91–2.00 (1H, m), 2.84 (1H, ddd, } J = 6.8, 6.8 \text{ and 13.6 Hz), 2.93 (1H, ddd, } J = 5.0, 6.8 \text{ and 13.6 Hz), 3.12 (1H, dd, } J = 6.8 \text{ and 13.6 Hz), 3.20 (1H, dd, } J = 5.0 \text{ and 13.6 Hz). C NMR (CD}_3\text{OD) } \delta 26.74, 29.93, 47.06, 47.72, 58.83, 118.19 \text{ (q, } J = 292 Hz), 159.71, 163.18 \text{ (q, } J = 34.5 Hz). MS (FAB) m/z 143 (M+H\textsuperscript{+}). HRMS (FAB): Calcd for C\textsubscript{6}H\textsubscript{15}N\textsubscript{4} (M+H\textsuperscript{+}), 143.1291; found, 143.1288.

S5
(2S)-1-Benzoxycarbonyl-2-\([N,N']\text{-bis(tert-butoxycarbonyl)guanidinomethyl]}\text{pyrrolidine (S17):}

Under a N\(_2\) atmosphere, EDCI•HCl (1.23 g, 6.41 mmol) was added to a solution of commercially available compound S16 (1.0 g, 4.27 mmol), (BocNH)_2CS (1.18 g, 4.27 mmol), and DIPEA (2.23 mL, 12.8 mmol) in anhydrous CH\(_2\)Cl\(_2\) (30 mL); the resulting mixture was stirred at room temperature for 13 h. After addition of saturated aqueous NaHCO\(_3\) solution, the reaction mixture was extracted with CH\(_2\)Cl\(_2\). The organic extracts were washed with water and brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (n-hexane/AcOEt = 10/1 to 5/1) to give compound S17 (1.81 g, 89%) as a yellow syrup.

\([\alpha]_D^{31} -39.4 \ (c \ 1.0, \ CHCl_3).\) IR \(\nu_{\text{max}}\) (KBr) 3328, 3288, 3127, 2935, 2887, 1707, 1639, 1576, 1447, 1412, 1367, 1327, 1138, 1056 cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), as a mixture of atropisomers) \(\delta\) 1.47 (6H, s), 1.50 (12H, s), 1.77-2.04 (4H, m), 3.40–3.71 (4H, m), 4.07–4.12 (1H, m), 5.08–5.30 (2H, m), 7.28–7.38 (5H, m), 8.51 (0.5H, s), 8.59 (0.5H, s), 11.49 (1H, s). \(^13\)C NMR (CDCl\(_3\), as a mixture of atropisomers) \(\delta\) 22.75, 23.58, 27.71, 27.98, 28.47, 29.24, 43.49, 43.92, 46.37, 46.64, 55.84, 56.71, 66.43, 66.51, 78.71, 78.79, 82.49, 82.68, 127.45, 127.55, 127.60, 128.11, 136.55, 152.62, 152.73, 154.70, 155.05, 156.20, 156.27, 163.27. MS (FAB) \(m/z\) 477 (M+H\(^+\)). HRMS (FAB): Calcd for C\(_{24}\)H\(_{37}\)N\(_4\)O\(_7\) (M+H\(^+\)), 477.2708; found, 477.2707.

(2S)-2-Guanidinomethylpyrrolidine, TFA salt (S4): TFA (5 mL) was added to a solution of compound S17 (900 mg, 1.89 mmol) in CH\(_2\)Cl\(_2\) (5 mL) and the resulting mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated in vacuo, the crude product was dissolved in MeOH (5 mL). Under a H\(_2\) atmosphere, the solution was added to a solution of 20% Pd(OH)\(_2\)-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 13 h. After the reaction mixture was filtered, the filtrate was concentrated in vacuo. The residue was purified by amine silica gel column chromatography (CHCl\(_3\)/MeOH = 1/1) to give compound S4 (421 mg, 87%) as a yellow syrup.

\([\alpha]_D^{25\,+} +1.12 \ (c \ 1.0, \ MeOH).\) IR \(\nu_{\text{max}}\) (KBr) 3143, 1680, 1517, 1426, 1202, 1137 cm\(^{-1}\). \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 1.34 (1H, dddd, \(J = 5.0, 6.8, 6.8\) and 13.6 Hz), 1.59–1.77 (2H, m), 1.81–1.90 (1H, m), 2.74 (1H, ddd, \(J = 6.8, 6.8\) and 13.6 Hz), 2.81 (1H, ddd, \(J = 5.0, 6.8\) and 13.6 Hz), 3.03 (1H, dd, \(J = 6.8\) and 13.6 Hz), 3.10 (1H, dd, \(J = 5.0\) and 13.6 Hz). \(^13\)C NMR (CD\(_3\)OD) \(\delta\) 26.75, 29.93, 47.06, 47.73, 58.85, 119.06 (q, \(J = 292\) Hz), 159.73, 163.19 (q, \(J = 34.5\) Hz). MS (FAB) \(m/z\) 143 (M+H\(^+\)). HRMS (FAB): Calcd for C\(_6\)H\(_{15}\)N\(_4\) (M+H\(^+\)), 143.1291; found, 143.1298.

References
Fig. S1. Representative HPLC charts of crude TFOs before HPLC purification.

Fig. S2. Representative UV-melting curves of triplexes. Conditions: 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM MgCl₂. The concentration of each oligonucleotide used was 1.89 µM.
Compound 4

$\text{H NMR (CDCl}_3\text{)}$

$\text{C NMR (CDCl}_3\text{)}$

S9
Compound 5

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

$^{13}C$ NMR (CDCl$_3$)
Compound 1
Compound S6
Compound S7

1H NMR (CDCl3)

13C NMR (CDCl3)
Compound S1

H NMR (CD3OD)

13C NMR (CD3OD)
Compound S10
Compound S11
Compound S12
Compound S13
Compound S2
Compound S15
Compound S3

1H NMR (CD3OD)

13C NMR (CDCl3)

S22
Compound S17
Compound S4
TFO 7a

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 7b

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 7c

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 7d

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 7e

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 7f**

**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**
TFO 7g

HPLC
Column: Waters XBridge\textsuperscript® MS C\textsubscript{18} 2.5 \(\mu\)m, 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 7h

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 7i

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 7j

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 7k**

**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)