SUPPORTING INFORMATION

**Design and synthesis of unsymmetric macrocyclic hexaoxazole compounds with ability to induce distinct G-quadruplex topologies in telomeric DNA**

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1. Synthesis of 6, 8, D, 10, 11

Synthesis of 6

To a solution of carboxylic acid $A^{1}$ (300 mg, 0.504 mmol) in THF-$H_2$O (1:1, 20 mL) was added NMM (0.22 mL, 2.02 mmol), DMT-MM (207 mg, 0.756 mmol) and serine methylester $B^{2}$ (78.4 mg, 0.504 mmol), and the mixture was stirred at room temperature. After being stirred for 3 h, to the reaction mixture was added $H_2$O, and the organic layer was extracted with CHCl$_3$, washed with 1.2 N HCl, dried over MgSO$_4$, and filtered. The filtrates were concentrated in vacuo to give $\beta$-hydroxy amide, which was used without further purification. To a solution of the $\beta$-hydroxyl amine in CH$_2$Cl$_2$ (15 mL), was added Na$_2$CO$_3$ (134 mg, 1.26 mmol) and DAST (61 $\mu$L, 0.46 mmol) at 0°C under argon atmosphere. The reaction mixture was stirred for 10 min, quenched with NaHCO$_3$ aq to give a solution of oxazoline, which was used without further purification. To a solution of the crude oxazoline in CH$_2$Cl$_2$ (15 mL), was added BrCCl$_3$ (0.4 mL, 4.2 mmol) and DBU (0.7 mL, 4.2 mmol). The mixture was stirred at 0°C under argon atmosphere. After being stirred for 24 h, to the reaction mixture was added $H_2$O and the organic layer was extracted with CHCl$_3$, washed with 1.2 N HCl, dried over MgSO$_4$, and filtered. The filtrates were concentrated in vacuo. The residue was purified by silica gel column to give 6 (202 mg, 68%, 3 steps). $[\alpha]_D^{25} = -16.3$ (c 1.6, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.45 (s, 1H), 8.42 (s, 1H), 8.33 (s, 1H), 8.31 (s, 1H), 7.35 (br, 5H), 5.56 (d, $J = 9.0$Hz, 1H), 5.12 (s, 2H), 5.05 (br, 1H), 4.55 (br, 1H), 3.95 (s, 3H), 3.09 (br, 2H), 2.04-1.90 (m, 3H), 1.64-1.41 (m, 10H), 1.27-1.21 (m, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 165.7, 163.7, 161.4, 156.9, 156.2, 155.4, 144.0, 139.6, 136.1, 134.5, 130.9, 129.8, 128.6, 128.2, 67.3, 52.4, 49.5, 29.7, 28.5, 22.5 ppm; HRMS (ESI, M+Na) calcd for $C_{32}H_{36}N_6O_{10}$Na 685.2197, found 685.2234.
To a solution of 4 \(^2\) (125 mg, 189 µmol) in THF-H\(_2\)O (3:1, 4 mL), was added LiOH-H\(_2\)O (11.9 mg, 284 µmol) at room temperature. The mixture was stirred for 30 min, quenched with 3N HCl to give a solution of carboxylic acid 5, which was used without further purification. To a solution of 6 (100 mg, 189 µmol) in MeOH (10 mL), was added 10% Pd/C (10 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 2 h. The reaction mixture was filtered through a pad of Celite, and eluted with CHCl\(_3\)-MeOH (9:1). The filtrates were concentrated in vacuo to give amine 7, which was used without further purification. To a solution of carboxylic acid 5 in THF-H\(_2\)O (1:1, 10 mL) was added NMM (83 µL, 756 µmol), DMT-MM (77.7 mg, 284 µmol) and amine 7, and the mixture was stirred at room temperature. After being stirred for 4 h, to the reaction mixture was added H\(_2\)O, and the organic layer was extracted with CHCl\(_3\), washed with 1.2 N HCl, dried over MgSO\(_4\), and filtered. The filtrates were concentrated in vacuo to give 8 (153 mg, 80%, 3 steps). \([\alpha]_0^{25} = +5.1 (c 2.6, \text{CHCl}_3)\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.46-8.14 (m, 6H), 7.55 (d, \(J = 9.0\) Hz, 1H), 7.35 (s, 5H), 5.59 (br, 1H), 5.48 (dd, \(J = 9.0, 15.6\) Hz, 2H), 5.12 (s, 2H), 4.58 (br, 2H), 3.94 (s, 3H), 3.10 (br, 6H), 2.05-1.40 (m, 28H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 165.7, 164.7, 159.9, 156.2, 155.6, 154.6, 143.9, 141.8, 139.6, 136.8, 136.1, 134.4, 131.0, 129.8, 128.6, 128.2, 79.3, 67.3, 52.4, 46.9, 40.3, 40.0, 33.6, 29.7, 28.5, 22.8, 22.5 ppm; HRMS (ESI, M+Na) calcd for \(\text{C}_{49}\text{H}_{56}\text{N}_{10}\text{O}_{15}\text{Na}\) 1047.3706, found 1047.3824.
Synthesis of D

To a solution of carboxylic acid A (2.93 g, 5.04 mmol) in THF-H$_2$O (1:1, 30 mL) was added NMM (1.1 mL, 10.1 mmol), DMT-MM (2.07 g, 7.56 mmol) and amine C (1.6 g, 5.04 mmol), and the mixture was stirred at room temperature. After being stirred for 8 h, to the reaction mixture was added H$_2$O, and the organic layer was extracted with CHCl$_3$, washed with 1.2 N HCl, dried over MgSO$_4$, and filtered. The filtrates were concentrated in vacuo to give β-hydroxy amide, which was used without further purification. To a solution of the β-hydroxy amine in DMF (15 mL) was added TIPSCI (2.2 mL, 10.1 mmol) and imidazole (687 mg, 10.1 mmol), and the mixture was stirred at 0 °C under argon atmosphere. After being stirred for 24 h, to the reaction mixture was added H$_2$O, and the organic layer was extracted with CHCl$_3$, washed with NH$_4$Cl, dried over MgSO$_4$, and filtered. The filtrates were concentrated in vacuo to give D (1.04 g, 58%, 2steps). [α]$^25_D$ = +8.0 (c 1.8, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 8.31-8.28 (m, 3H), 8.20 (m, 1H), 7.87 (d, J = 9.0 Hz, 2H), 7.34 (br, 5H), 5.54-5.48 (m, 2H), 5.12 (s, 2H), 4.55 (br, 2H), 4.31-4.26 (m, 1H), 4.16-4.11 (m, 1H), 3.90 (s, 3H), 3.08 (br, 2H), 2.06-1.92 (m, 3H), 1.50-1.40 (m, 11H), 1.03-0.99 (m, 21); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 165.7, 163.1, 161.6, 160.0, 156.2, 154.5, 144.2, 141.6, 139.6, 139.1, 136.8, 136.2, 133.6, 131.1, 129.8, 128.6, 128.2, 79.2, 67.2, 64.8, 52.2, 49.4, 40.0, 33.6, 29.7, 28.5, 22.5, 17.9, 11.9 ppm; HRMS (ESI, M+Na) calcd for C$_{44}$H$_{59}$N$_7$O$_{12}$SiNa 928.3890, found 928.3889.
Synthesis of 10

To a solution of D (1.04 g, 1.15 mmol) in MeOH (15 mL) was added 10% Pd/C (100 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 2 h. To the reaction mixture was filtered through a pad of Celite and eluted with CHCl₃-MeOH (9:1). The filtrates were concentrated in vacuo to give amine, which was used without further purification. To a solution of carboxylic acid E² was added NMM (0.3 mL, 2.30 mmol), DMT-MM (474 mg, 1.73 mmol) and amine, and the mixture was stirred at room temperature for 6 h. To the reaction mixture was added H₂O, and the organic layer was extracted with CHCl₃, washed with 1.2 N HCl, dried over MgSO₄, and filtered. The filtrates were concentrated in vacuo to give 10 (813 mg, 61%, 2 steps). [α]₂⁵<sup>D</sup> = -9.9 (c 1.6, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 8.33-8.15 (m, 5H), 7.81 (d, J = 6.0 Hz, 1H), 7.33 (s, 5H), 5.53-5.40 (m, 3H), 5.12 (s, 2H), 4.62 (br, 1H), 4.18 (dd, J = 3.3, 9.0 Hz, 4H), 3.9 (s, 3H), 3.09 (br, 1H), 1.52-1.46 (m, 30H), 0.87-0.83 (m, 25H); ¹³C NMR (75 MHz, CDCl₃) δ 165.1, 164.2, 163.1, 161.5, 160.3, 156.2, 156.1, 154.4, 144.2, 141.7, 139.8, 139.2, 139.2, 136.7, 136.1, 135.4, 133.5, 131.0, 129.8, 128.5, 79.1, 67.2, 64.7, 52.2, 49.4, 47.0, 40.0, 33.4, 29.5, 28.4, 22.5, 17.8, 11.8 ppm; HRMS (ESI, M+Na) calcd for C₅₈H₇₀N₁₀O₁₆SiNa 1223.5410, found 1223.5421.
Synthesis of 11

To a solution of 10 (1.54 g, 1.33 mmol) in MeOH (40 mL), was added 10% Pd/C (154 mg) at room temperature and mixture was stirred under hydrogen atmosphere for 4 h. The reaction mixture was filtered through a pad of Celite and eluted with CHCl₃-MeOH (9:1). The filtrates were concentrated in vacuo and the residue was purified by silica gel column to give amine, which was used without further purification. To a solution of amine in THF-H₂O (3:1, 20 mL), was added LiOH·H₂O (84.0 mg, 2.00 mmol) at room temperature for 2 h. To the reaction mixture was added 3N HCl, and volatiles were removed under vacuum conditions to give a carboxylic acid, which was used without further purification. To a solution of the crude carboxylic acid in dry DMF was added ¹Pr₂NET (1.0 mL, 5.32 mmol), DMAP (16.2 mg, 1.33 mmol) and BOPCl (511 mg, 2.01 mmol). The mixture was stirred at room temperature under argon atmosphere. After being stirred for 24 h, to the resulting mixture was added H₂O and the organic layer was extracted with CHCl₃, washed with 1.2 N HCl, dried over MgSO₄, and filtered. The filtrates were concentrated in vacuo. The residue was purified by silica gel column to give 11 (241 mg, 25%, 3 steps). [α]ᵡ⁰³⁵ = -50.3 (c 1.1, CHCl₃); ¹H NMR (300 MHz, DMSO-d₆) δ 9.18 (d, J = 9.0 Hz, 2H), 9.02-8.60 (m, 6H), 7.95 (d, J = 9.0 Hz, 1H), 6.83 (br, 2H), 5.38-5.31 (m, 3H), 4.10-3.99 (m, 4H), 2.91 (br, 4H), 2.10-1.98 (m, 8H), 1.44-1.35 (m, 22H), 1.03-0.73 (m, 18H); ¹³C NMR (75 MHz, CDCl₃) δ 166.1, 163.3, 162.3, 160.7, 159.9, 159.6, 156.6, 156.1, 154.7, 142.1, 141.9, 141.4, 139.4, 138.5, 136.7, 135.9, 130.7, 129.3, 79.3, 65.1, 49.8, 47.0, 45.2, 40.4, 33.9, 33.0, 22.9, 17.9, 11.8 ppm; HRMS (ESI, M+Na) calcd for C₄₉H₇₀N₁₀O₁₃SiNa 1057.4781, found 1057.4791.

Reference
$^{1}$H NMR (300 MHz, CDCl$_3$)
$^{13}$C NMR of 6

$^{13}$C NMR (75 MHz, CDCl$_3$)
$^1$H NMR of 8
$^{13}$C NMR of 8

CDCl$_3$
$^1$H NMR of macrocyclic bisamide 9

$^1$H NMR (400 MHz, CDCl$_3$)

CDCl$_3$
$^{13}$C NMR of macrocyclic bisamide 9
$^{1}H$ NMR of 4,2-L2H2-6OTD (2)
$^{13}$C NMR of 4,2-L2H2-6OTD (2)
$^1$H NMR of D

$^1$H NMR (300 MHz, CDCl$_3$)
$^{13}$C NMR of D
$^1$H NMR of 10

![NMR Spectrum](image)
$^{13}$C NMR of 10
$^1$H NMR of 11
$^{13}$C NMR (75 MHz, CDCl$_3$)
$^1$H NMR of macrocyclic bisamide 12
$^{13}$C NMR of macrocyclic bisamide 12

[Diagram of the $^{13}$C NMR spectrum of macrocyclic bisamide 12 in CDCl$_3$.]

CDCl$_3$
$^1$H NMR of 5,1-L2H2-GOTD (3)
$^{13}$C NMR of 5,1-L2H2-6OTD (3)
2. FRET melting assay of G4 forming DNA sequences

Table S1  ΔT_m (°C) values in FRET melting assay of G4 forming DNA sequences in the presence of ligands 1-3.

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<th>Ligands</th>
<th>ΔT_m values (°C)</th>
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<tr>
<td></td>
<td>bcl-2</td>
</tr>
<tr>
<td>1 (control)</td>
<td>14.9 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>6.5 ± 0.1</td>
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</tbody>
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3. CD titration experiments

Fig. S1  CD titration spectra of ss-telo24 (10 µM) in 50 mM Tris-HCl buffer (without ion, pH 7.6) with 1, 2 and 3 (0–5 eq.). a) 3,3-L2H2-6OTD (1), b) 4,2-L2H2-6OTD (2), c) 5,1-L2H2-6OTD (3).
Fig. S2 CD titration spectra of ss-telo24 (10 µM) in 50 mM Tris-HCl buffer (100 mM KCl, pH 7.6) with 1, 2 and 3 (0–5 eq.). a) 3,3-L2H2-6OTD (1), b) 4,2-L2H2-6OTD (2), c) 5,1-L2H2-6OTD (3).
Fig. S3 CD titration spectra of ss-telo24 (10 µM) in 50 mM Tris-HCl buffer (100 mM NaCl, pH 7.6) with 1, 2 and 3 (0–5 eq.). a) 3,3-L2H2-6OTD (1), b) 4,2-L2H2-6OTD (2), c) 5,1-L2H2-6OTD (3).
5. FRET melting curves

Fig. S4 FRET melting curves of 0.4 µM Flu-ss-telo24 with 2.0 µM 1, 2 and 3. a) 3,3-L2H2-6OTD (1), b) 4,2-L2H2-6OTD (2), c) 5,1-L2H2-6OTD (3).