Phenanthroline polyazamacrocycles as G-quadruplex DNA binders

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Synthesis

[16]phenN₄: Yield 92%. m.p. 208–209°C. ¹H NMR (600 MHz, D₂O): δ (ppm) 1.45 (m, 4H, NCH₂CH₂CH₂), 1.81 (m, 4H, NCH₂CH₂CH₂), 3.23 (m, 4H, NCH₂CH₂CH₂), 4.66 (s, 4H, phenCH₂N), 7.78 (d, 2H, phen), 7.99 (s, 2H, phen) and 8.53 (d, 2H, phen); ¹³C NMR (150 MHz, D₂O): δ (ppm) 25.46 (NCH₂CH₂CH₂), 25.65 (NCH₂CH₂CH₂), 47.59 (NCH₂CH₂CH₂), 51.64 (phenCH₂N), 123.02, 127.07, 129.10, 138.88, 144.12 and 151.37 (phen). HRMS-ESI m/z calcd for C₂₀H₂₅N₄ [L+H]⁺ 321.2074, found 321.2128.

[32]phen₂N₄: Yield 70%. m.p. 201–202°C. ¹H NMR (600 MHz, D₂O): δ (ppm) 1.37 (m, 8H, NCH₂CH₂CH₂), 1.73 (m, 8H, NCH₂CH₂CH₂), 3.16 (m, 8H, NCH₂CH₂CH₂), 4.61 (s, 8H, phenCH₂N), 7.73 (d, 4H, phen), 7.88 (s, 4H, phen) and 8.45 (d, 4H, phen); ¹³C NMR (150 MHz, D₂O): δ (ppm) 25.39 (NCH₂CH₂CH₂), 25.58 (NCH₂CH₂CH₂), 47.68 (NCH₂CH₂CH₂), 51.22 (phenCH₂N), 123.31, 127.21, 129.01, 139.49, 144.12 and 151.12 (phen)). HRMS-ESI m/z calced for C₄₀H₄₉N₈ [L+H]⁺ 641.4075, found 641.4088.

[30]phen₂N₆: Yield 40%. m.p. 224–225°C. ¹H NMR (600 MHz, D₂O): δ (ppm) 3.34 (t, 8H, NCH₂CH₂N), 3.53 (t, 8H, NCH₂CH₂N), 4.65 (s, 8H, phenCH₂N), 7.46 (d, 4H, phen), 7.81 (s, 4H, phen), 8.18 (d, 4H, phen); ¹³C NMR (150 MHz, D₂O): δ (ppm) 43.81 (NCH₂CH₂N), 45.45 (NCH₂CH₂N), 51.18 (phenCH₂N), 67.83 (dioxane), 122.15, 127.33, 128.84, 139.07, 143.44, 151.35 (phen). HRMS-ESI m/z calced for C₃₆H₄₃N₁₀ [L+H]⁺ 615.3667, found 615.3708.

[34]phen₂N₆: Yield: 50%; m.p. 227–228 °C; ¹H NMR (600 MHz, D₂O, 25 °C): δ=2.31 (m, 8H; NCH₂CH₂CH₂N), 3.27 (t, 16H; NCH₂CH₂CH₂N), 3.43 (t, 16H; NCH₂CH₂CH₂N), 4.71 (s, 8H; phenCH₂N), 7.82 (d, 4H; phen), 8.01 (s, 4H; phen), 8.56 ppm (d, 4H; phen); ¹³C NMR (150 MHz, D₂O): δ (ppm) 44.26 (NCH₂CH₂CH₂N), 44.52 (NCH₂CH₂CH₂N), 44.72 (NCH₂CH₂CH₂N), 48.99 (methanol), 52.08 (phenCH₂N), 67.97 (dioxane), 123.43, 127.26, 129.32, 139.32, 144.32, 151.09 ppm (phen). HRMS-ESI m/z calced for C₄₀H₅₁N₁₀ [L+H]⁺ 671.4293, found 671.4341.
**Fig. S1** FRET-melting stabilization by [16]phenN₄ and [32]phen₂N₄ (1 μM) of the labelled F21T sequence (0.2 μM) in buffer 10 mM KCl + 90 mM LiCl and in the presence of increasing concentrations of competitor ds26 (0, 15 and 50 equivalents).
**Fig. S2** CD titration spectra of (A) [16]phenN₄, (B) [30]phen₂N₆, (C) [32]phen₂N₄ and (D) [34]phen₂N₆ with c-MYC G4 in 100 mM LiCl. Some of the intermediate spectra were removed for better clarity.
Fig. S3 CD titration spectra of (A) [16]phenN₄, (B) [30]phen₂N₆, (C) [32]phen₂N₄ and (D) [34]phen₂N₆ with 22AG G4 in 10 mM KCl + 90 mM LiCl. Some of the intermediate spectra were removed for better clarity.
Fig. S4 CD titration spectra of (A) [16]phenN₄, (B) [30]phen₂N₆, (C) [32]phen₂N₄ and (D) [34]phen₂N₆ with 22AG G₄ in 100 mM NaCl. Some of the intermediate spectra were removed for better clarity.
Fig S5 Fluorescence titrations of the ligand [16]phenN₄ with increasing concentration of (A) c-MYC and (B) 22AG G₄s [5 μM in 100 mM KCl, pH 7.2, concentrations 0–25 μM]. Insets: Stern-Volmer plots obtained by plotting F₀/F as a function of DNA concentration.

Fig S6 Fluorescence titrations of the ligand [30]phen₂N₆ with increasing concentration of (A) c-MYC and (B) 22AG G₄s [5 μM in 100 mM KCl, pH 7.2, concentrations 0–25 μM]. Insets: Stern-Volmer plots obtained by plotting F₀/F as a function of DNA concentration.

Fig S7 Fluorescence titrations of the ligand [34]phen₂N₆ with increasing concentration of (A) c-MYC and (B) 22AG G₄s [5 μM in 100 mM KCl, pH 7.2, concentrations 0–25 μM]. Insets: Stern-Volmer plots obtained by plotting F₀/F as a function of DNA concentration.
Fig. S8 Job plots resulting from the method of continuous variation analysis for (A) ligand [16]phenN₄ with c-MYC G₄, (B) ligand [16]phenN₄ with 22AG G₄, (C) [32]phen₂N₄ with c-MYC G₄ and (D) [32]phen₂N₄ with 22AG G₄.
Fig. S9 - $^1$H NMR spectra of the ligands (A) [16]phenN₄, (B) [32]phen₂N₄ (C) [30]phen₂N₆ and (D) [34]phen₂N₆ (protons d are underneath the water signal and could not be assigned).
Fig. S10. $^{13}$C NMR spectra of the ligands (A) [16]phenN$_4$, (B) [32]phen$_2$N$_4$ (C) [30]phen$_2$N$_6$ and (D) [34]phen$_2$N$_6$. 