Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Electronic supporting Information

## Acid-base properties of functionalised tripodal polyamines and their interaction with nucleotides and nucleic acids

Alejandra Sornosa-Ten,[a] M. Teresa Albelda,[a,*] Juan C. Frías,[a] Enrique García-España,[a,*] José M. Llinares,[b] Ana Budimir,[c] and Ivo Piantanida.[d,*]

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**Table S1** Logarithms of the stability constants for the interaction of monophosphate nucleotides ($MP^{2-} \equiv A$) with tripodal polyamine **7** determined in 0.15 mol·dm$^{-3}$ NaCl at 298.0 ±0.1 K

| Reaction | AMP | CMP | Reaction | GMP | TMP | UMP |
|---|---|---|---|---|---|---|
| $A + HL \leftrightarrows HAL$ | 3.22 (1) | 2.44 (1) | $H_{-1}A + HL \leftrightarrows AL$ | 3.66 (3) | 3.30 (1) | 4.40 (1) |
| $A + H_2L \leftrightarrows H_2AL$ | 3.23 (1) | 2.21 (1) | $H_{-1}A + H_2L \leftrightarrows HAL$ | 3.44 (4) | 3.72 (1) | 5.03 (1) |
| $A + H_3L \leftrightarrows H_3AL$ | 3.57 (1) | 2.41 (2) | $H_{-1}A + H_3L \leftrightarrows H_2AL$ | - | - | - |
| $A + H_4L \leftrightarrows H_4AL$ | 3.80 (1) | 2.80 (1) | $H_{-1}A + H_4L \leftrightarrows H_3AL$ | - | - | - |
| $A + H_5L \leftrightarrows H_5AL$ | 3.90 (1) | 2.86 (1) | $H_{-1}A + H_5L \leftrightarrows H_4AL$ | - | - | - |
| $A + H_6L \leftrightarrows H_6AL$ | 4.40 (1) | 3.52 (1) | $H_{-1}A + H_6L \leftrightarrows H_5AL$ | - | - | - |
| $HA + L \leftrightarrows HAL$ | - | - | $A + L \leftrightarrows AL$ | 3.80 (3) | 2.98 (1) | 4.81 (1) |
| $HA + HL \leftrightarrows H_2AL$ | - | - | $A + HL \leftrightarrows HAL$ | 3.31 (4) | 3.14 (1) | 4.67 (1) |
| $HA + H_2L \leftrightarrows H_3AL$ | - | - | $A + H_2L \leftrightarrows H_2AL$ | 3.27 (4) | 2.92 (1) | 4.32 (1) |
| $HA + H_3L \leftrightarrows H_4AL$ | - | - | $A + H_3L \leftrightarrows H_3AL$ | 3.16 (3) | 3.25 (1) | 4.34 (1) |
| $HA + H_4L \leftrightarrows H_5AL$ | - | - | $A + H_4L \leftrightarrows H_4AL$ | 3.40 (3) | 3.43 (1) | 4.41 (1) |
| $HA + H_5L \leftrightarrows H_6AL$ | 4.61 (1) | - | $A + H_5L \leftrightarrows H_5AL$ | 3.46 (2) | 3.56 (1) | 4.40 (1) |
| $HA + H_6L \leftrightarrows H_7AL$ | 3.72 (1) | 1.87 (3) | $A + H_6L \leftrightarrows H_6AL$ | 3.90 (2) | 3.93 (1) | 4.67 (1) |
| $HA + H_7L \leftrightarrows H_8AL$ | 3.55 (1) | - | $A + H_7L \leftrightarrows H_7AL$ | - | - | |
| $H_2A + H_5L \leftrightarrows H_7AL$ | 3.55 (1) | - | $HA + H_5L \leftrightarrows H_6AL$ | - | 3.93 (3) | 4.81 (1) |
| | | | $HA + H_6L \leftrightarrows H_7AL$ | 2.94 (3) | 2.89 (2) | 3.69 (1) |
| | | | $HA + H_7L \leftrightarrows H_8AL$ | | | 3.41 (1) |

[a] Charges omitted. [b] Numbers in parentheses are standard deviations in the last significant figure.

**Table S2**: Logarithms of the protonation constants of nucleotide monophosphates ($MP^{2-} \equiv A$) determined in NaCl 0.15 mol·dm$^{-3}$ at 298.0 ± 0.1 K.

| Reaction | AMP | CMP | GMP | TMP | UMP |
|---|---|---|---|---|---|
| $H_{-1}A + H \leftrightarrows A$[a] | - | - | 9.65(2) | 10.10(3) | 9.88(4) |
| $A + H \leftrightarrows AH$ | 6.06(1)[b] | 5.99(1) | 6.03(4) | 6.27(2) | 6.14(4) |
| $H + HA \leftrightarrows H_2A$ | 3.90(1) | 3.68(2) | - | - | - |

[a] Charges omitted. [b] Numbers in parentheses are standard deviations in the last significant figure.

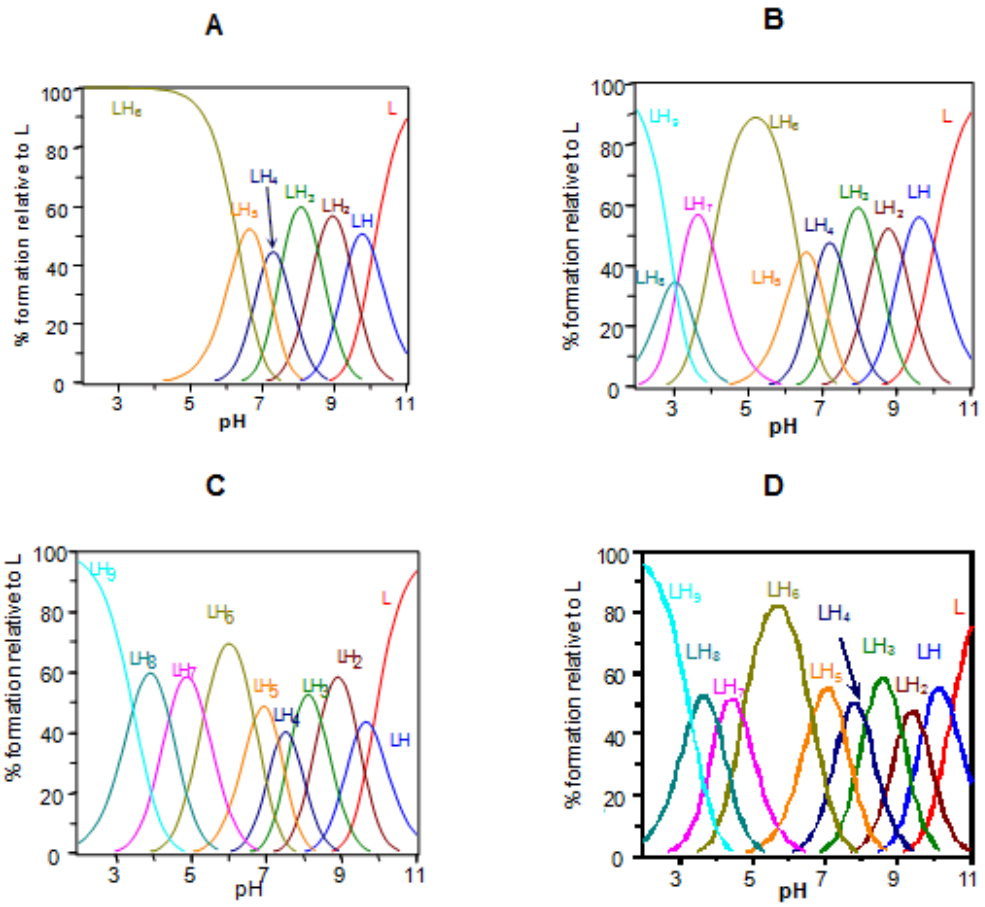


**Figure S1**: Distribution diagrams for the protonation of synthesized ligands **4**-**7** (A = **4**; B = **5**; C = **6**; D = **7**)

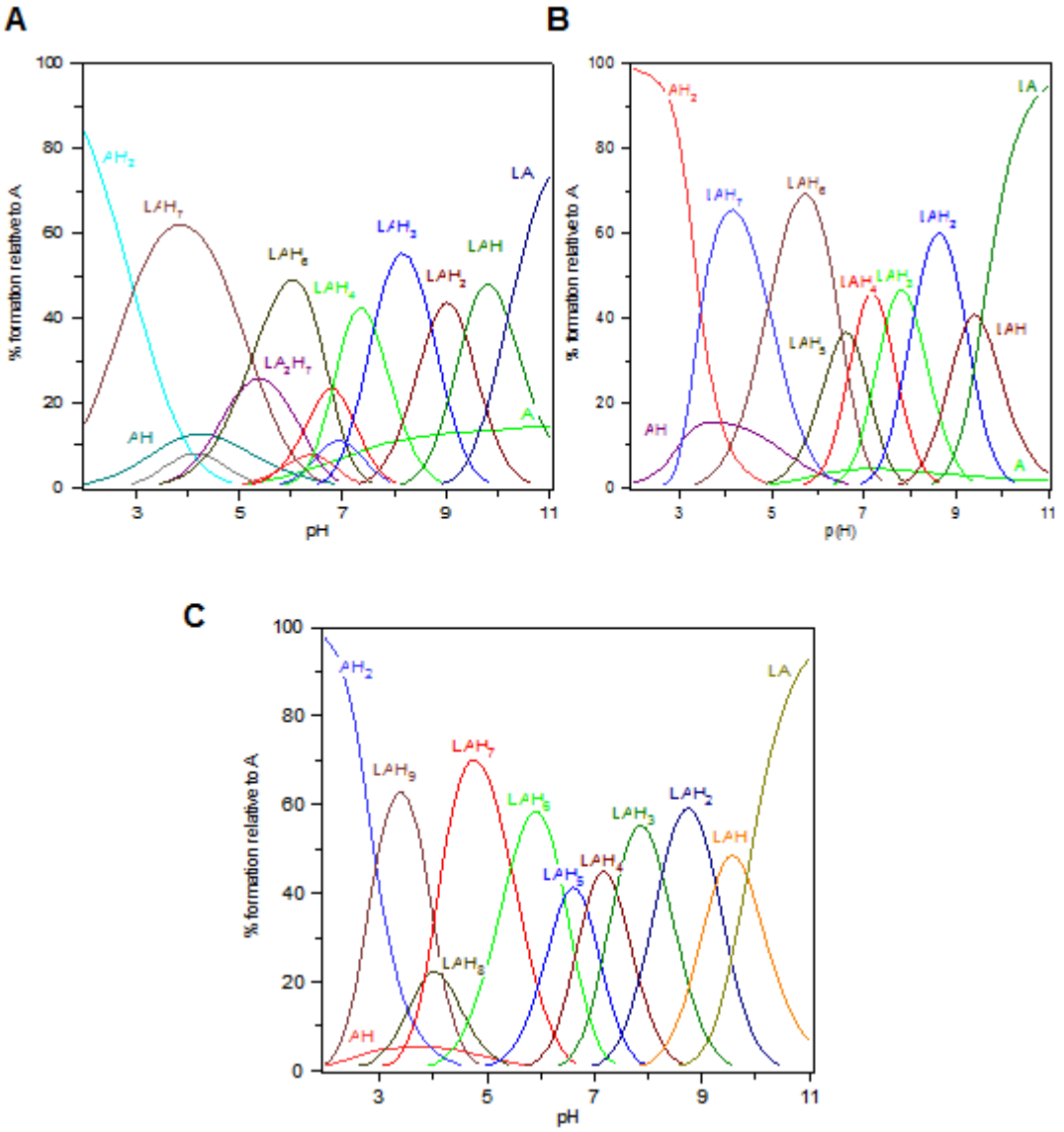


**Figure S2:** Distribution diagrams for the systems a) AMP-**4**, b) AMP-**5**, c) AMP-**6**. (A = AMP). [L] = [A] = $10^{-3}$ M.

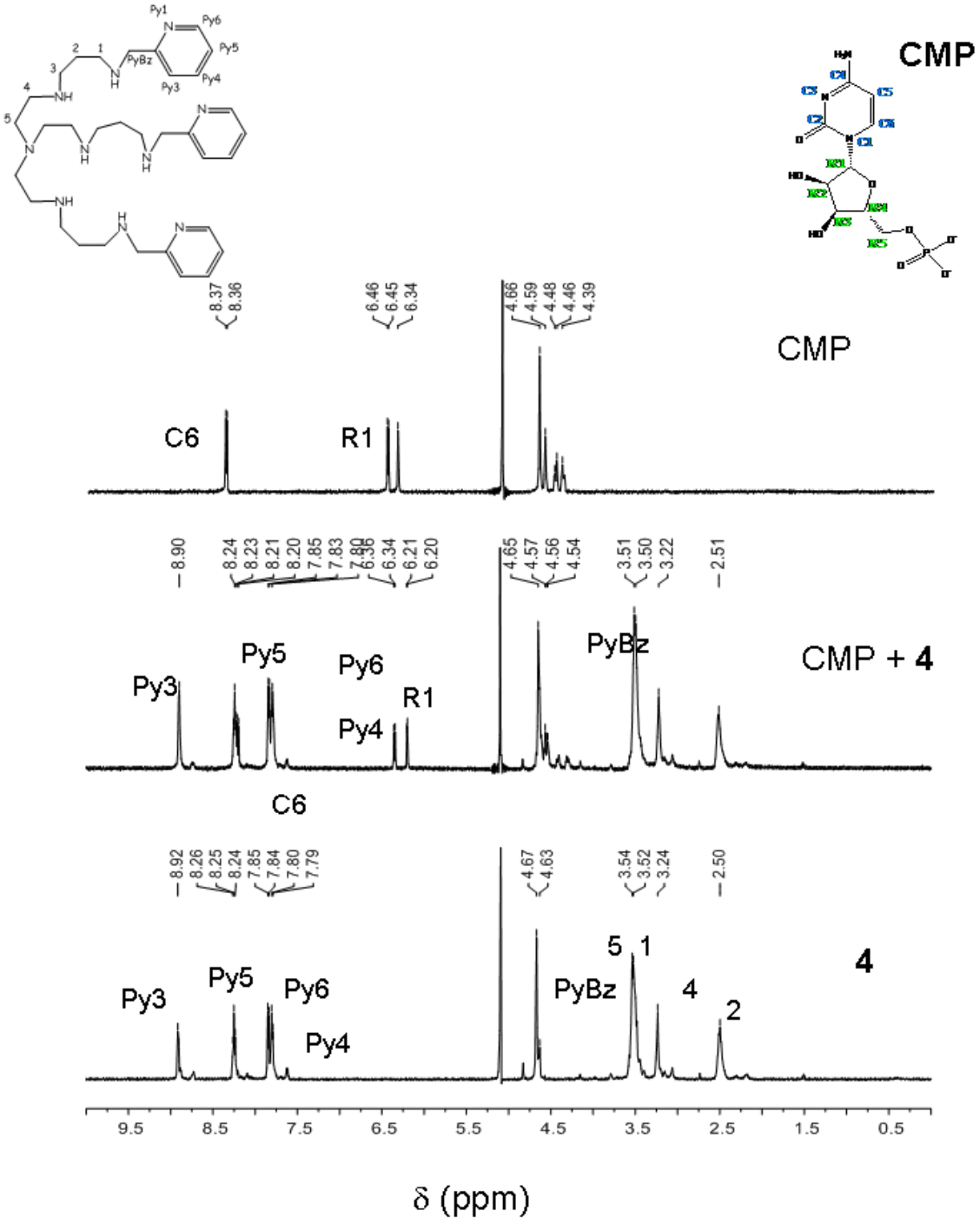


**Figure S3:** $^{1}$H NMR spectra of the mononucleotide CMP, the system CMP + **4** and ligand **4** in $D_2O$ at pD ≈ 6.5.

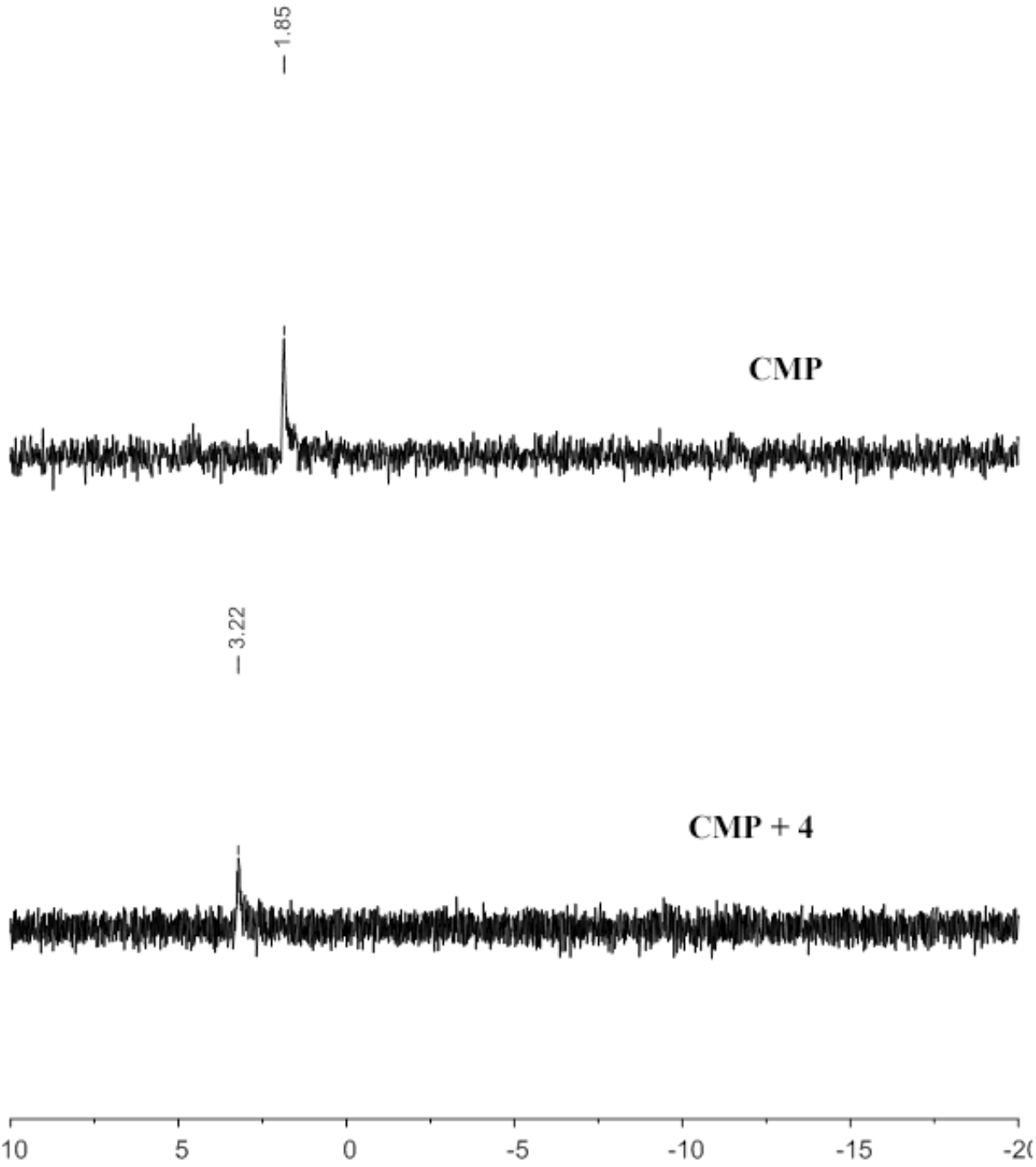


**Figure S4:** $^{31}$P NMR spectra of the mononucleotide CMP and the system CMP + **4** in $D_2O$ at pD ≈ 6.5.

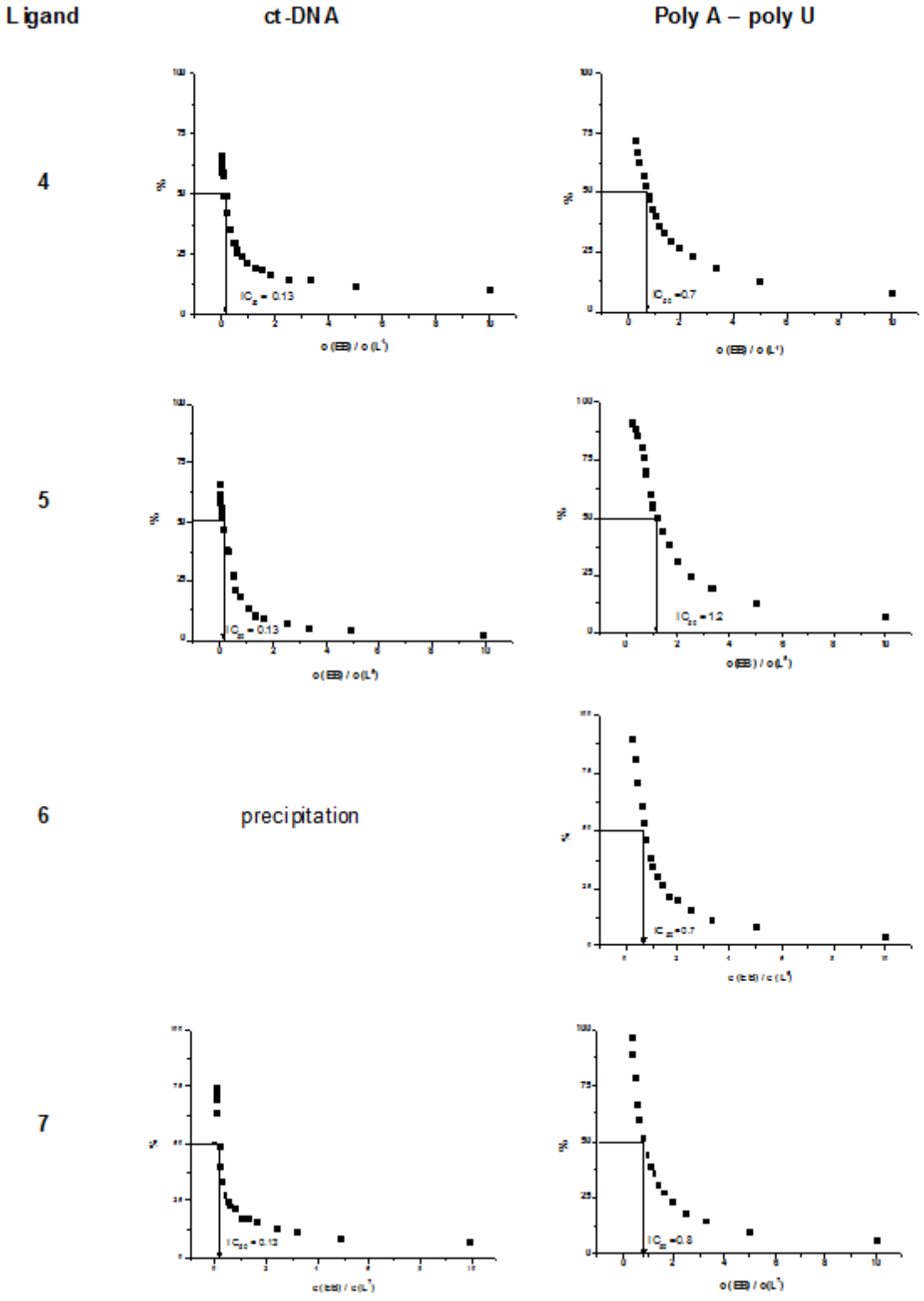


**Figure S5** Ethidium bromide (**EB**) displacement assay: to polynucleotide solution ($c$ = 5 × $10^{-5}$ mol $dm^{-3}$) **EB** was added ($c$ = 5 × $10^{-6}$ mol·$dm^{-3}$; ***r*** ([**EB**]/[polynucleotide] = 0.1), and quenching of the **EB**/polynucleotide complex fluorescence emission ($\lambda_{ex}$ = 520 nm, $\lambda_{em}$ = 601 nm) was monitored as function of $c$(**EB**)/$c$(compound). The given $IC_{50}$ values present the ratio $c$(**EB**)/$c$(compound) at which 50% of **EB** is displaced from the polynucleotide ($IC_{50}$ = [Int(**EB**/polynucleotide) – Int($\mathbf{EB}_{free}$)]/2, where Int(**EB**/polynucleotide) is fluorescence intensity of **EB**/ polynucleotide complex and Int($\mathbf{EB}_{free}$) is fluorescence intensity of the free **EB** before polynucleotide is added).

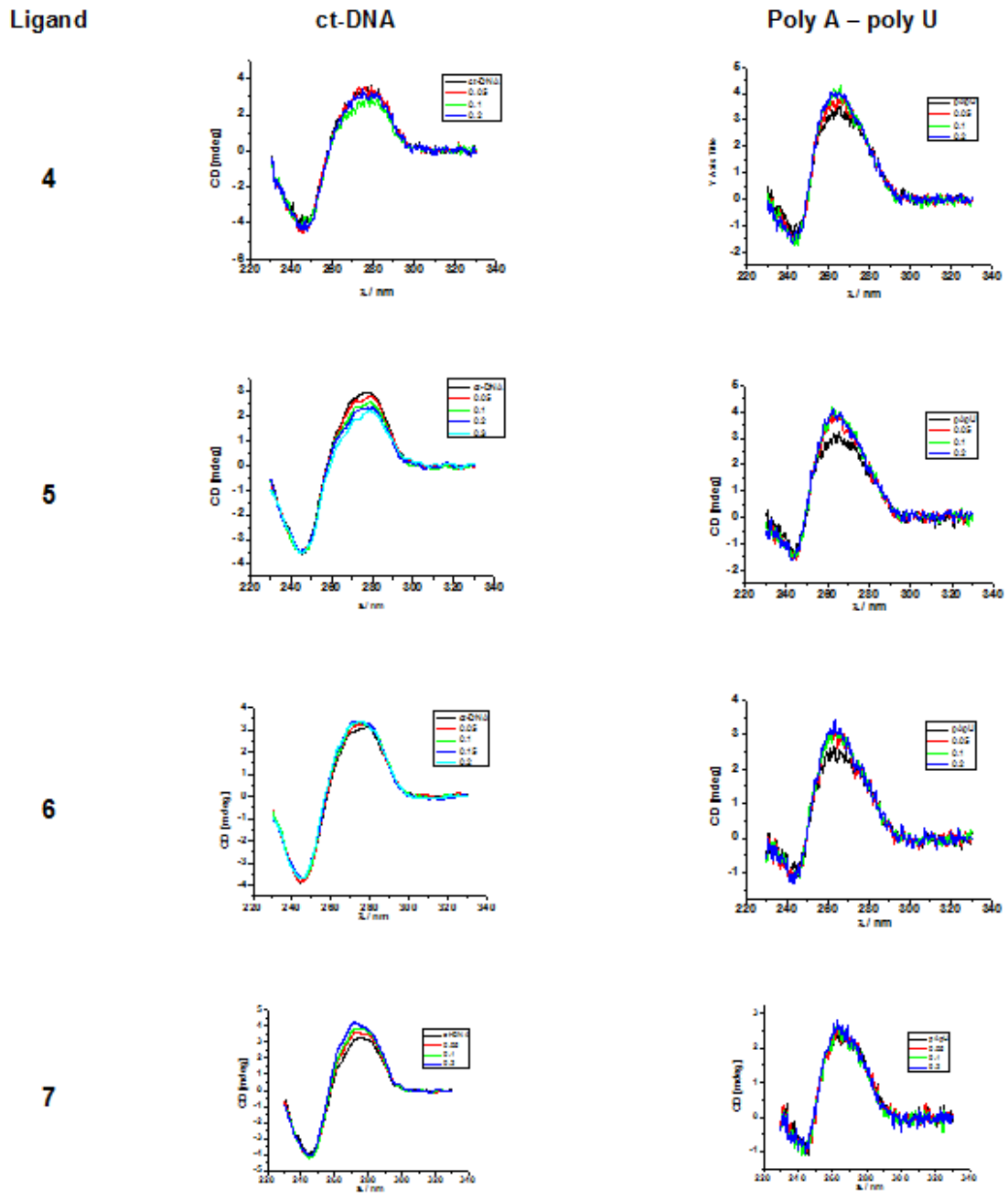


**Figure S6** CD titration of polynucleotides ($c$ = 3.0 × $10^{-5}$ mol·$dm^{-3}$) with **4-7** at molar ratios ***r*** = [compound]/[polynucleotide] (pH = 5.0, citrate buffer, $I$ = 0.05 mol·$dm^{-3}$).

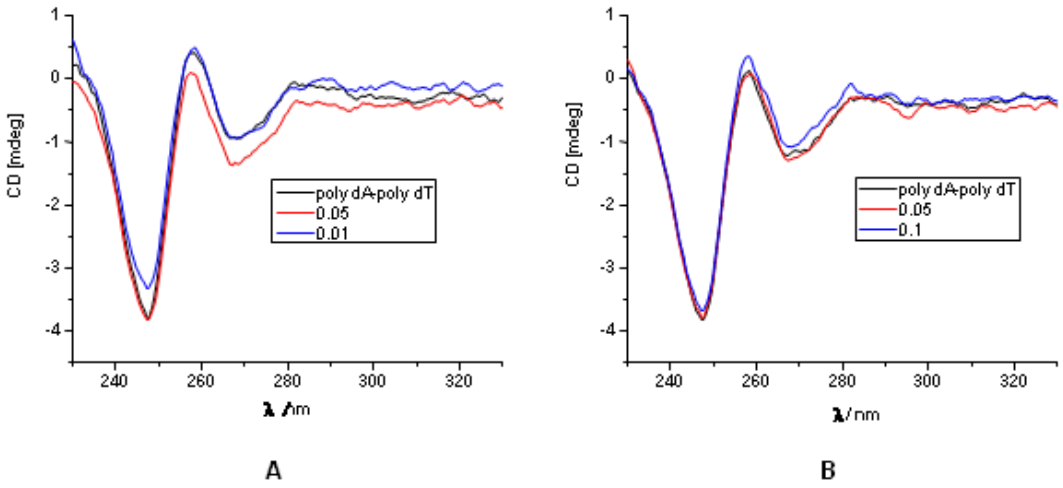


**Figure S7** CD titration of poly dA – poly dT (c = $3.0 \times 10^{-5}$ mol·dm$^{-3}$) with **4** (A) and **7** (B) at different molar ratios r = [compound]/[polynucleotide] (pH = 5.0, citrate buffer, $I$ = 0.05 mol·dm$^{-3}$).

# Synthetic Data

### *General procedure*

Precursor polyamine **1** (3.20 mmol) and the corresponding carbaldehyde (11.3 mmol) were stirred for 1.5 h in 150 mL of dry EtOH. $NaBH_4$ (21.10 mmol) was then added and the resulting solution stirred for 2 h at room temperature. The solvent was removed at reduced pressure. The resulting residue was treated with water and dichloromethane. The organic phase was removed at reduced pressure and the resulting residue was dissolved in ethanol and precipitated as its hydrochloride salt of trisubstituted receptor.

**Tris(8-(3'-pyridyl)-3,7-diazaoctyl)amine (5)**

*Yield* = 28%. *UV* : $\varepsilon_{max}^{259} = 1.60\ (8) \times 10^4\ M^{-1}\ cm^{-1}$.

*Anal. Calcd for* $C_{33}H_{54}N_{10}\cdot 7HCl$: C, 47.01%, H, 7.29%, N, 16.62%. Found: C, 47.0%, H, 7.7%, N, 16.3%.

$^1H$ *NMR* ($D_2O$), *δ(ppm):* 2.19-2.29 (2H, m,), 2.96 (2H, t, *J* = 6 Hz,), 3.23-3.37 (6H, m,), 4.57 (2H, s, H8), 8.12 (1H, ddd, $J_1$ = 8 Hz, $J_2$ = 2 Hz, $J_3$ = 5 Hz), 8.68(1H, d, *J* = 8 Hz), 8.89 (1H, d, *J* = 5 Hz,), 8.97 (1H, d, *J* = 2 Hz,).

$^{13}C$ *NMR* ($D_2O$), *δ(ppm):* 23.9, 44.8, 45.0, 45.2, 49.1, 60.0, 128.0, 142.5, 145.1, 149.2

**Tris(8-(4'-pyridyl)-3,7-diazaoctyl)amine (6)**

*Yield* = 27%). *UV*: $\varepsilon_{max}^{259} = 1.51\ (7) \times 10^4\ M^{-1}\ cm^{-1}$.

*Anal. Calcd for* $C_{33}H_{54}N_{10}\cdot 9HCl\cdot 4H_2O$: C, 39.99%, H, 6.60%, N, 14.13%. Found: C, 40.0%, H, 6.9%, N, 14.1%.

$^1H$ *NMR* ($D_2O$), *δ(ppm):* 2.15-2.31 (2H, m), 2.94 (2H, t, *J* = 6 Hz), 3.16-3.38 (6H, m), 4.55 (2H, s), 7.95 (2H, d, *J* = 7 Hz), 8.79 (2H, d, *J* = 7 Hz).

$^{13}C$ *NMR* ($D_2O$), *δ(ppm):* 23.0, 45.0, 45.2, 45.6, 49.3, 49.8, 127.9, 142.4.

**Tris(8-(4'-imidazolyl)-3,7-diazaoctyl)amine** (**7**)

*Yield* = 31%.

*Anal. Calcd for* $C_{27}H_{51}N_{13}\cdot 7HCl\cdot 3H_2O$: C, 37.40%, H, 7.43%, N, 21.00%. Found: C, 37.5%, H, 7.7%, N, 21.3%.

$^1H$ *NMR* ($D_2O$), *δ(ppm):* 2.14-2.24 (2H, m, H5), 2.92-2.95 (2H, m, H1), 3.15-3.29 (6H, m, H2, H4 and H6), 4.31 (2H, s, H8), 7.44 (1H, s, H5'), 8.04 (1H, s, H2').

$^{13}C$ *NMR* ($D_2O$), *δ(ppm):* 22.6 (C5), 43.0 (C8), 43.6 (C6), 44.6 (C4), 44.9 (C2), 48.8 (C1), 119.0 (C5'), 136.7 (C2').