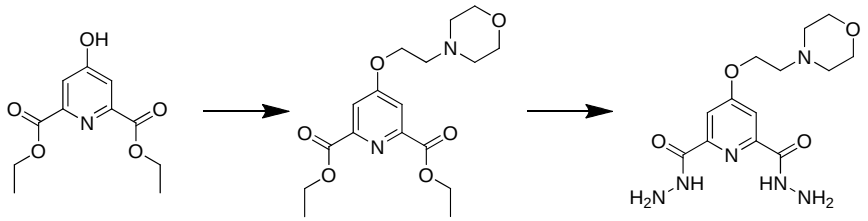
ELECTRONIC SUPPLEMENTARY INFORMATION

Experimental details

General remarks: All commercially available chemicals were reagent grade and used without further purification. NMR spectra were acquired on a Bruker Avance 300 spectrometer (^{1}H: 300 MHz, ^{13}C: 75 MHz) at 25 °C; chemical shifts are given in ppm (δ) values. Multiplicities of ^{13}C NMR signals were determined from DEPT-135 experiments. Elemental microanalysis of all novel compounds was performed by the *Service de Microanalyse*, CNRS–ICSN, Gif-sur-Yvette, France and by the *Service Chromato–Masse–Microanalyse*, BioCIS (UMR 8076), Châtenay-Malabry, France. The purity of final compounds was assessed by LC/MS analysis (Waters Alliance 2695 equipped with a Phenomenex Luna C18(2) 1.6 µm column and a photodiode array detector; eluent A: water with 0.1% formic acid, eluent B: MeCN with 0.1% formic acid, gradient elution with 0 to 20% of eluent B). Mass spectra (MS, ESI in the positive-ion mode) were recorded with a Waters ZQ instrument (cone voltage: 30 V).

Reagents: Synthesis of **L1**–**L3**, **A1**, **A2**, as well as symmetric bis(acylhydrazones) (**A1-L1-A1**, **A2-L1-A2**, **A1-L2-A1**, **A2-L2-A2**, **A1-L3-A1**, **A2-L3-A2**) was described elsewhere.[1] Synthesis of **L4**, **A3**, **A2-L4-A2** and **A5-L1-A5**, as well as hybrids **5a** and **5b**, is described below. PhenDC3 was a generous gift of Dr. Marie-Paule Teulade-Fichou (Institut Curie).

6-Formyl-1-methylquinolinium iodide (A3): A solution of quinoline-6-aldehyde (0.39 g, 2.50 mmol) and iodomethane (1.56 mL, 3.55 g, 25.0 mmol) in acetone (25 mL) was stirred at 60 °C for 18 h and then cooled to room temperature. The precipitated solid was filtered, washed twice with acetone, once with ether, and dried, to give **A3** (89%) as a red solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.31 (s, 1H), 9.64 (d, J = 5.4 Hz, 1H), 9.48 (d, J = 8.4 Hz, 1H), 9.08 (d, J = 1.4 Hz, 1H), 8.69 (d, J = 9.1 Hz, 1H), 8.61 (dd, J = 9.1, 1.7 Hz, 1H), 8.30 (dd, J = 8.4, 5.8 Hz, 1H), 4.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 191.9 (C_q), 152.3 (CH), 148.5 (CH), 140.7 (C_q), 135.7 (C_q), 134.0 (CH), 132.4 (CH), 129.1 (C_q), 123.2 (CH), 120.6 (CH), 45.8 (CH_3); MS (ESI$^+$): m/z (%) = 190.2 (11) [M + H_2O]$^+$, 172.2 (100) [M]$^+$.



6 7 **L4**

Diethyl 4-(2-morpholinoethoxy)pyridine-2,6-dicarboxylate (7): Diethyl chelidamate **6** (2.00 g, 8.36 mmol) was dissolved in dry acetonitrile (130 mL), and 4-(2-chloroethyl)morpholine hydrochloride (2.02 g, 10.9 mmol) and K_2CO_3 (3.47 g, 25.1 mmol) were added. The resulting mixture was maintained under reflux for 18 h and then cooled, filtered, and evaporated to dryness. The residue was partitioned between DCM (50 mL) and H_2O (50 mL), and the aqueous layer was extracted with DCM (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, filtered through SiO_2 and the solvent was removed under vacuum, to give **7** (3.50 g, 79%) as a yellow viscous liquid that was used in next step without purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 2H), 4.48 (q, J = 7.2 Hz, 4H), 4.28 (t, J = 5.5 Hz, 2H), 3.76–3.71 (m, 4H), 2.86 (t, J = 5.5 Hz, 2H), 2.63–

2.54 (m, 4H), 1.46 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.3 (C_q), 164.1 (C_q), 149.7 (C_q), 114.1 (CH), 66.4 (CH_2), 66.1 (CH_2), 61.6 (CH_2), 56.6 (CH_2), 53.5 (CH_2), 14.1 (CH_3); MS (ESI$^+$): m/z (%) = 114.0 (100) [4-vinylmorpholin-4-ium]$^+$; 353.3 (32) [M + H]$^+$.

4-(2-Morpholinoethoxy)pyridine-2,6-dicarbohydrazide (L4): A solution of compound **7** (1.25 g, 3.50 mmol) and hydrazine hydrate (1.40 mL, 1.40 g, 28.4 mmol) in methanol (100 mL) was heated under reflux for 18 h and then cooled to room temperature. The precipitate was filtered, washed twice with methanol, once with ether, and dried, to give the bis(acylhydrazide) **L4** as a white solid (0.840 g, 73%) which was sufficiently pure and employed without further purification; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.58 (s, 2H), 7.59 (s, 2H), 4.61 (s, 4H), 4.31 (t, J = 5.5 Hz, 2H), 3.64–3.50 (m, 4H), 2.72 (t, J = 5.4 Hz, 2H), 2.48 (s, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.9 (C_q), 161.7 (C_q), 150.5 (C_q), 109.7 (CH), 66.3 (CH_2), 66.2 (CH_2), 56.6 (CH_2), 53.6 (CH_2); MS (ESI$^+$): m/z (%) = 114.2 (100) [4-vinylmorpholin-4-ium]$^+$; 325.3 (12) [M + H]$^+$.

***N′*^{2},*N′*^{6}-Bis[(1-methylquinolinium-4-yl)methylene]-4-(2-morpholinoethoxy)pyridine-2,6-dicarbohydrazide iodide (A2-L4-A2):** 4-[2-(Morpholin-4-yl)ethoxy]pyridine-2,6-dicarbohydrazide **L4** (162 mg, 0.5 mmol) and 4-formyl-1-methylquinolinium iodide **A2** (329 mg, 1.1 mmol) were mixed in DMF (2 ml) and the reaction mixture was stirred for 2 hours at 100 °C, then cooled and filtered. The collected product was recrystallized from MeCN/H_2O (1:1 v/v) and dried in vacuo over P_2O_5, to give A2-L4-A2 (154 mg, 41%) as a red solid; m.p. (decomp.) 212 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.95 (s, 2H), 9.80 (s, 2H), 9.53 (d, J = 6.2 Hz, 2H), 9.06 (d, J = 8.4 Hz, 2H), 8.61 (d, J = 8.9 Hz, 2H), 8.56 (d, J = 6.1 Hz, 2H), 8.43–8.31 (m, 2H), 8.26–8.14 (m, 2H), 7.95 (s, 2H), 4.68 (s, 6H), 4.48 (s, 2H), 3.61 (s, 4H), 2.81 (s, 2H), 2.53 (s, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.6 (C_q), 159.9 (C_q), 149.6 (C_q), 149.4 (CH), 147.0 (C_q), 143.4 (CH), 139.0 (C_q), 135.3 (CH), 130.4 (CH), 126.3 (C_q), 125.9 (CH), 120.0 (CH), 118.9 (CH), 112.6 (CH), 66.9 (CH_2), 66.2 (CH_2), 56.6 (CH_2), 53.5 (CH_2), 45.7 (CH_3); MS (ESI$^+$): m/z (%) = 631.4 (15) [M − H]$^+$, 316.3 [M]$^{2+}$, 169.2 (100) [4-cyano-1-methylquinolinium]$^+$; anal. calcd. for $C_{35}H_{36}I_2N_8O_4$ × 0.7 H_2O (899.13): C 46.75, H 4.19, N 12.46; found: C 46.74, H 3.98, N 12.46.

N′^{2},N′^{6}-Bis[4-(4-methylpiperazin-1-yl)benzylidene]pyridine-2,6-dicarbohydrazide (A5-L1-A5): A mixture of pyridine-2,6-dicarbohydrazide **L1** (108 mg, 0.550 mmol) and 4-(4-methylpiperazino)benzaldehyde **A5** (283 mg, 1.38 mmol) in *i*-PrOH (4.0 mL) was heated for 18 h at 100 °C and then cooled to room tempetature. The solvent was removed in vacuo, and the product was recrystallized from THF/Et_2O, to give **A5-L1-A5** (218 mg, 69%) as a yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.15 (s, 2H), 8.63 (s, 2H), 8.39–8.19 (m, 3H), 7.66 (d, J = 8.7 Hz, 4H), 7.03 (d, J = 8.8 Hz, 4H), 3.30–3.22 (m, 8H), 2.45 (s, 8H), 2.22 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.1 (C_q), 152.4 (C_q), 150.6 (CH), 148.5 (C_q), 139.9 (CH), 128.6 (CH), 125.2 (CH), 123.7 (C_q), 114.5 (CH), 54.4 (CH_2), 47.1 (CH_2), 45.8 (CH_3); MS (ESI$^+$): m/z (%) = 568.6 (14) [M + H]$^+$; 284.9 (100) [M + 2 H]$^{2+}$; anal. calcd. for $C_{31}H_{37}N_9O_2$ × 1.2 H_2O (589.3): C 63.18, H 6.74, N 21.39; found: C 63.18, H 6.51, N 21.25.

*General procedure of synthesis of amides **2a–b**:* In a 250 mL round-bottom flask, 4-chloropyridine-2,6-dicarboxylic acid (543 mg, 3.00 mmol) was dissolved in a 9:1 (v/v) DCM / DMF mixture (30 mL). A corresponding amine (476 mg, 3.30 mmol), HOBt (53 mg, 0.4 mmol), and EDCI × HCl (719 mg, 8.99 mmol) were added and the mixture was stirred at RT overnight. The completion of the reaction was checked by TLC. When the spot of starting material disappeared, the solution was washed with aqueous $NaHCO_3$ solution, water, aqueous HCl (0.05 M), water, brine, and concentrated in vacuo. EtOAc was added to the resulting viscous liquid and desired product was collected by filtration.

Methyl 6-[(quinolin-6-yl)carbamoyl]pyridine-2-carboxylate (2a): Yield: 645 mg (70%). Pale solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.70 (s, 1H), 8.83 (d, J = 2.8 Hz, 1H), 8.58 (d, J = 1.7 Hz, 1H), 8.44–8.31 (m, 2H), 8.31–8.21 (m, 2H), 8.14 (dd, J = 9.1, 2.1 Hz, 1H), 8.03 (d, J = 9.1 Hz, 1H), 7.52 (dd, J = 8.3, 4.2 Hz, 1H), 3.98 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.7 (C_q), 162.5 (C_q), 150.4 (C_q), 149.5 (CH), 146.7 (CH), 145.1 (C_q), 139.7 (CH), 135.9 (CH), 135.7 (CH), 129.5 (CH), 128.2 (CH), 127.6 (CH), 125.8 (CH), 124.2 (CH), 121.9 (CH), 116.6 (CH), 52.8 (CH_3); MS (ESI$^+$): m/z (%) = 308.3 (100) [M + H]$^+$.

Methyl 6-{[4-(4-methylpiperazin-1-yl)phenyl]carbamoyl}pyridine-2-carboxylate (2b): Yield 776 mg (73%). Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.98 (s, 1H), 8.48 (dd, J = 7.8, 0.9 Hz, 1H), 8.25 (dd, J = 7.8, 0.9 Hz, 1H), 8.13–8.00 (m, 1H), 7.71 (d, J = 8.9 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 4.04 (s, 3H), 3.32–3.10 (m, 4H), 2.71–2.48 (m, 4H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.1 (C_q), 161.0 (C_q), 150.6 (C_q), 148.5 (C_q), 146.5 (C_q), 138.9 (CH), 130.1 (C_q), 127.4 (CH), 125.6 (CH), 121.4 (CH), 116.6 (CH), 55.2 (CH_2), 53.1 (CH_3), 49.5 (CH_2), 46.3 (CH_3); MS (ESI$^+$): m/z (%) = 355.4 (100) [M + H]$^+$.

6-[6-(methoxycarbonyl)pyridine-2-amido]-1-methylquinolin-1-ium iodide (3a). Compound **2a** (386 mg, 1.26 mmol) was dissolved in $CHCl_3$ (25 mL). Iodomethane (1.79 mL, 4.07 g, 28.7 mmol) was added and the mixture was stirred in sealed tube at 61 °C for 18 h. The precipitate was filtered, washed twice with acetone and dried in vacuo, to give **3a** (500 mg, 89%) as a yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.17 (s, 1H), 9.39 (d, J = 5.6 Hz, 1H), 9.27 (d, J = 8.4 Hz, 1H), 9.07 (d, J = 2.0 Hz, 1H), 8.67–8.53 (m, 2H), 8.44–8.36 (m, 1H), 8.36–8.29 (m, 2H), 8.13 (dd, J = 8.4, 5.8 Hz, 1H), 4.63 (s, 3H), 3.99 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.7 (C_q), 163.7 (C_q), 150.1 (C_q), 148.5 (CH), 146.9 (C_q), 146.4 (CH), 139.9 (CH), 139.0 (C_q), 135.3 (C_q), 130.1 (C_q), 129.6 (CH), 128.0 (CH), 126.2 (CH), 122.4 (CH), 120.1 (CH), 117.7 (CH), 52.9 (CH_3), 45.3 (CH_3); MS (ESI$^+$): m/z (%) = 322.3 (100) [M + H]$^+$.

*General procedure of synthesis of acylhydrazides **4a–b***: Hydrazine hydrate (0.45 mL, 463 mg, 50.1 mmol) was added to a solution of **3a** or **3b** (0.42 mmol) in MeOH (10 mL). After stirring for 3 h at room temperature the resulting precipitate was filtered, washed with MeOH, acetone and dried in vacuo.

6-[6-(Hydrazinecarbonyl)pyridine-2-amido]-1-methylquinolin-1-ium iodide (4a): Yield 170 mg (90%). Pale solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.43 (s, 1H), 10.79 (s, 1H), 9.41 (d, J = 5.9 Hz, 1H), 9.31 (d, J = 8.3 Hz, 1H), 9.04 (s, 1H), 8.63 (s, 2H), 8.40 (dd, J = 6.5, 2.2 Hz, 1H), 8.36–8.22 (m, 2H), 8.16 (dd, J = 8.2, 5.7 Hz, 1H), 4.76 (s, 2H), 4.65 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.6 (C_q), 162.1 (C_q), 148.7 (C_q), 148.6 (CH), 147.8 (C_q), 146.4 (CH), 140.0 (CH), 138.8 (C_q), 135.4 (C_q), 130.1 (C_q), 129.8 (CH), 125.2 (CH), 125.0 (CH), 122.4 (CH), 120.1 (CH), 118.3 (CH), 45.3 (CH_3); MS (ESI$^+$): m/z (%) = 322.3 (100) [M + H]$^+$.

6-(Hydrazinecarbonyl)-*N*-[4-(4-methylpiperazin-1-yl)phenyl]pyridine-2-carboxamide (4b): Yield 141 mg (95%). Yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.78 (s, 1H), 10.75 (s, 1H), 8.33–8.13 (m, 3H), 7.65 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 9.1 Hz, 2H), 4.69 (s, 2H), 3.15–3.06 (m, 4H), 2.48–2.41 (m, 4H), 2.22 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.2 (C_q), 161.2 (C_q), 149.0 (C_q), 148.5 (C_q), 148.0 (C_q), 139.6 (CH), 129.6 (C_q), 124.4 (CH), 124.3 (CH), 122.4 (CH), 115.4 (CH), 54.6 (CH_2), 48.3 (CH_2), 45.8 (CH_3); MS (ESI$^+$): m/z (%) = 355.4 (100) [M + H]$^+$.

*General procedure for the synthesis of compounds **5a** and **5b**:* A mixture of acylhydrazide **4a** or **4b** (0.25 mmol), aldehyde **A5** or **A2**, respectively (0.75 mmol), and a catalytic amount of AcOH (10 µL)

in *n*-PrOH (4 ml) was heated at reflux for 18 h. The precipitate was collected by filtration, washed with MeOH, acetone, and recrystallized from MeCN / H_2O (1:1 v/v).

1-Methyl-6-(6-{*N*'-[4-(4-methylpiperazin-1-yl)benzylidene]hydrazinecarbonyl}pyridine-2-amido)quinolin-1-ium iodide (5a): Yield 40 mg (22%). Orange solid; m.p. (decomp.) 172 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.30 (s, 1H), 11.49 (s, 1H), 9.41 (d, *J* = 5.8 Hz, 1H), 9.33 (d, *J* = 8.3 Hz, 1H), 9.15 (s, 1H), 8.75–8.59 (m, 3H), 8.50–8.30 (m, 3H), 8.16 (dd, *J* = 8.4, 5.9 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 4.65 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.6 (C_q), 159.0 (C_q), 152.2 (CH), 150.7 (CH), 148.7 (C_q), 148.6 (C_q), 148.0 (C_q), 146.5 (CH), 140.2 (CH), 138.9 (C_q), 135.9 (C_q), 130.1 (C_q), 129.8 (CH), 128.6 (CH), 125.9 (CH), 125.5 (CH), 123.8 (C_q), 122.4 (CH), 120.2 (CH), 117.8 (CH), 114.6 (CH), 54.2 (CH_2), 46.8 (CH_2), 45.4 (CH_3), 45.3 (CH_3); MS (ESI$^+$): *m*/*z* (%) = 508.5 (15) [*M* + H]$^+$, 254.9 (100) [*M*]$^{2+}$; anal. calcd. for $C_{29}H_{30}IN_7O_2$ × 2 H_2O × 0.5 CH_3COOH (701.6): C 51.36, H 5.17, N 13.98; found: C 51.26, H 4.89, N 13.72.

***N'*^{2}-[(1-Methylquinolinium-4-yl)methylene]-6-{[4-(4-methylpiperazin-1-yl)phenyl]carbamoyl}pyridine-2-carbohydrazide iodide (5b):** Yield 78 mg (49%). Orange solid; m.p. (decomp.) 270 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.16 (s, 1H), 10.75 (s, 1H), 9.58 (s, 1H), 9.53 (d, *J* = 5.7 Hz, 1H), 9.04 (d, *J* = 8.4 Hz, 1H), 8.60 (d, *J* = 8.8 Hz, 1H), 8.55 (d, *J* = 5.9 Hz, 1H), 8.45 (d, *J* = 7.1 Hz, 2H), 8.41 – 8.31 (m, 2H), 8.21 (dd, *J* = 7.5, 7.2 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 4.67 (s, 3H), 3.90–3.00 (m, 4H), 2.85 (s, 3H), 2.67–2.32 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.0 (C_q), 160.5 (C_q), 149.4 (CH), 149.2 (C_q), 147.4 (C_q), 147.1 (C_q), 146.6 (C_q), 143.3 (CH), 140.3 (CH), 139.0 (C_q), 135.2 (CH), 130.6 (C_q), 130.5 (CH), 126.2 (C_q), 126.1 (CH), 125.9 (CH), 125.8 (CH), 122.2 (CH), 120.0 (CH), 119.4 (CH), 116.2 (CH), 52.6 (CH_2), 46.1 (CH_2), 45.7 (CH_3), 42.5 (CH_3); MS (ESI$^+$): *m*/*z* (%) = 508.4 (100) [*M* + H]$^+$, 254.8 (20) [*M*]$^{2+}$; anal. calcd. for $C_{29}H_{30}IN_7O_2$ × HI × H_2O × 0.3 CH_3COOH (799.4): C 44.47, H 4.31, N 12.26; found: C 44.66, H 4.43, N 12.18.

Oligonucleotides: DNA oligonucleotides used in this work are listed in Table S1. HPLC-purified oligonucleotides were purchased from Eurogentec. For DCC pull-down experiments, 5′-biotinylated oligonucleotides were dissolved in **K1 buffer** (10 mM $LiAsO_2Me_2$, 1 mM KCl, 99 mM LiCl, pH 7.2) at a concentration of 100 µM and annealed (5 min at 95 °C, followed by slow cooling to room temperature). Oligonucleotide solutions were stored at –20 °C when not in use. For fluorescence melting experiments, dual-labelled oligonucleotides were dissolved in deionized water at a concentration of 100 µM, diluted and annealed in the appropriate buffer.

Capturing protocol for extraction of ligands from DCLs: Stock solutions of the building blocks were prepared in DMSO/H_2O (1:1 v/v) at a concentration of 2 mM. Stock solutions of the catalysts **1a–d** were prepared in the **DCC buffer** (100 mM NH_4OAc, 1.5 mM KCl, pH 6.4) at a concentration of 25 mM. DCLs were prepared by mixing the stock solutions of the building blocks (cf. Table S2) with the DCC buffer. The solutions were supplemented with the catalyst (final concentration: 10 mM) and biotinylated DNA oligonucleotides pre-folded in K1 buffer (5 µL, final concentration: 5 µM) or an equal volume of the K1 buffer, to a final library volume of 100 µL. The libraries were left to equilibrate for 24 h without stirring at ambient temperature. Then, NaOH solution (2 M, 1 µL) was added to stop the acylhydrazone exchange. In parallel, 4 × 100 µL of streptavidin-coated magnetic beads (Dynabeads MyOne Streptavidin T1, 10 mg mL^{-1} suspension, ThermoFisher) were washed with the DCC buffer (2 × 200 µL), biotin solution (2 mM, 200 µL), DCC buffer (200 µL), decanted, and the reaction mixtures were added to the beads. The solutions were incubated for 20 min at ambient temperature without stirring. The supernatant was discarded, and the beads were washed with 3 ×

200 µL of the DCC buffer. Formamide (100 µL) was added and mixed with the beads. Samples were heated at 50 °C for 30 minutes, decanted and the supernatant was subjected to HPLC analysis (injection volume: 5 µL). Column: Atlantis T3, 3 µm, 3 × 100 mm (Waters), eluent A: H_2O + 0.01% TFA; eluent B: ACN + 0.01% TFA; flow rate: 0.8 mL min^{-1}; detection wavelength: 310 nm. Elution methods were developed as to optimize the separation of DCL components, with a typical gradient as following: 0 min, 5% B; 20 min, 13% B; 25 min, 30% B; 26 min, 80% B; 30 min, 80% B (linear curves).

Fluorimetric titrations were performed as described elsewhere.[2] 5′-Cy5-labelled oligonucleotides were dissolved at a concentration of 1 µM in a 10 mM $LiAsO_2Me_2$, 100 mM KCl buffer, pH 7.2, annealed at 95 °C for 5 min, slowly cooled to room temperature, and then further diluted with the **titration buffer** (10 mM $LiAsO_2Me_2$, 100 mM KCl, 0.5 w/v % CHAPS, 0.05 v/v % Triton X-100, pH 7.2) to a final concentration of 2.22 nM. Ten serial dilutions (1:1) of ligands in H_2O containing 0.1 vol.% DMSO were prepared starting from two ligand solutions with initial concentrations of 100 µM and 18 µM, respectively, resulting in a total of 22 solutions with different ligand concentrations and two no-ligand controls. The oligonucleotide solution (90 µL) and ligand solutions (10 µL) were transferred into 96-well black, flat-bottom polystyrene non-binding (NBS) microplates (Corning) (final oligonucleotide concentration: 2 nM, final ligand concentration: from 10 µM to 1.8 nM). After incubation for 2 h at room temperature, fluorescence intensity was measured on a CLARIOstar Plus microplate reader (BMG) using 590BP50 (excitation), LP639 (dichroic) and 675BP50 (emission) filters and an integration time of 0.5 s per well. The fluorescence intensity was normalized by dividing the raw value by the mean intensity of no-ligand wells. The experiments were performed in triplicate. The titration curves (Fig. S3) were fitted to a 1:1 binding model using Equation (1) in OriginPro 2020b (OriginLab Corporation, Northampton, MA, USA).

$$F = F_0 - (F_0 - F_b) \times \frac{A + \frac{x}{c} + 1 - \sqrt{(A + \frac{x}{c} + 1)^2 - 4 \times \frac{x}{c}}}{2}, \quad (1)$$

where F_0 and F_b are fluorescence intensity in the absence and in the presence of saturating concentration of the ligand, respectively; x is a concentration of the ligand and $A = \frac{K_d}{c}$, where K_d is the dissociation constant and c is the concentration of labelled oligonucleotide (2 nM).

Electrospray mass spectrometry. Native ESI-MS spectra of DNA and DNA–ligand complexes were obtained using an Exactive ESI–Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). ESI spray voltage and capillary voltage were 3.50 kV and –3.5 kV, respectively. Capillary temperature was set to 275 °C. The syringe injection rate was 200 µL/h. The presented spectra (Figs S4–S5) result from 3-min accumulations (1 scan per 1.1 s). The concentrations of the initial stocks solutions of DNA (~1 mM) were measured by UV absorbance at 260 nm on a Uvikon XS. 50 µM solutions of DNA were annealed in buffer that contained 100 mM trimethylammonium acetate (TMAA, Ultra for HPLC, Fluka analytical) and 1 mM KCl (>99.999%, Sigma) by heating at 85 °C for 10 minutes and slowly cooling to room temperature. Analyzed samples contained 5 µM of G4-DNA, 0 or 5 µM of ligand, and 1 µM of the oligonucleotide 5′-d(TTTTTT)-3′ (dT_6, internal control) in a 100 mM TMMA, 1 mM KCl buffer, and were incubated 2 h before the injection.

Charge state 6− was examined because it contained fewer nonspecific potassium adducts. We assume that the response factors of free DNA, DNA+L and DNA+2L complexes are equal. This was supported by the fact that total DNA peak areas compared to the signal of dT_6 did not change significantly without or with ligand. Based on this assumption, the concentration of each DNA form is determined from the mass balance equation and from the peak areas:

$$[DNA]_{free} = [DNA]_{tot} \cdot \frac{A(DNA)}{A(DNA)+A(DNA+L)+A(DNA+2L)} \quad (2)$$

$$[DNA + L] = [DNA]_{tot} \cdot \frac{A(DNA+L)}{A(DNA)+A(DNA+L)+A(DNA+2L)} \quad (3)$$

$$[DNA + 2L] = [DNA]_{tot} \cdot \frac{A(DNA+2L)}{A(DNA)+A(DNA+L)+A(DNA+2L)} \quad (4)$$

The concentration of free ligand was determined through the mass balance equation:

$$[L]_{free} = [L]_{tot} - [DNA + L] - 2[DNA + 2L] \quad (5)$$

Apparent consecutive dissociation constants (K_{D1} and K_{D2}) were calculated using Equations (6–7):

$$K_{D1} = \frac{[\mathrm{DNA}]_{free} \times [\mathrm{L}]_{free}}{[\mathrm{DNA+L}]} \quad (6)$$

$$K_{D1} = \frac{[\mathrm{DNA+L}] \times [\mathrm{L}]_{free}}{[\mathrm{DNA+2L}]} \quad (7)$$

Fluorescence melting experiments were performed as described elsewhere using a real-time qPCR machine (9700HT, Applied BioSystems).[3] The assay was performed with double-labelled oligonucleotides (5′-FAM, 3′-TAMRA), which were annealed prior to the experiments (95 °C, 5 min) in corresponding buffer (*Pu24T* and *myc22*: 10 mM $LiAsO_2Me_2$, 1 mM KCl, 99 mM LiCl, pH 7.2; *25TAG*: 10 mM $LiAsO_2Me_2$, 100 mM KCl, pH 7.2; *22CTA*: 10 mM $LiAsO_2Me_2$, 10 mM KCl, 90 mM LiCl, pH 7.2) at a concentration of 0.23 µM. Unlabelled double-stranded DNA competitor (ds26, 5′-d(CAATCGGATCGAATTCGATCCGATTG)-3′) was annealed in the same buffer at a strand concentration of 210 µM. Thermal denaturation runs were performed in 96-well plates using a heating ramp of 0.5 °C per minute from 25 to 95 °C; the fluorescence intensity was monitored in the FAM channel. Each well contained 0.2 µM of double-labelled G4, 1 µM of tested compound, and 0, 3 or 10 µM of ds26 competitor, in a total volume of 25 µL of buffer. The denaturation temperatures (T_m) were determined from the maxima of first-derivative plots of FAM emission intensity vs. temperature, and ligand-induced T_m shifts (ΔT_m) were calculated as a difference of mean denaturation temperatures in the presence and in the absence of ligands, from three independent experiments.

Table S1. DNA oligonucleotides used in this work.

Acronym	Sequence (5′ → 3′)	Structure	PDB	Ref.	Use			
					DCC[a]	FM[b]	FT[c]	MS[d]
Pu24T	TGAGGGTGGTGAGGGTGGGGAAGG	G4 (snap-back parallel)	2MGN	[4,5]	X	X	X	X
myc22	TGAGGGTGGGTAGGGTGGGTAA	G4 (parallel)	1XAV	[6]		X	X	
25TAG	TAGGGTTAGGGTTAGGGTTAGGGTT	G4 (hybrid, *Type 2*)	2JSL	[7]		X	X	X
22CTA	AGGGCTAGGGCTAGGGCTAGGG	G4 (anti-parallel)	2KM3	[8]		X	X	
hp2	GTTATATCT-HEG-TGATATAAC[e]	hairpin			X	X	X	
dT_{22}	TTTTTTTTTTTTTTTTTTTTTT	single-stranded			X		X	
dT_6	TTTTTT	single-stranded						X[f]
ds26	CAATCGGATCGAATTCGATCCGATTG	self-complementary duplex				X[g]		

[a] DCC pull-down experiments: the oligonucleotides were 5′-modified with biotin. [b] Fluorescence melting experiments: oligonucleotides were labelled with 5′-FAM and 3′-TAMRA. [c] Isothermal fluorimetric titrations: oligonucleotides were labeled with 5′-Cy5. [d] Native ESI mass spectrometry. [e] HEG = hexa(ethylene glycol) linker. [f] Internal reference. [g] Unlabeled *ds26* was used as a competitor in fluorescence melting experiments.[9]

Table S2. Compositions of DCLs 1–3 and conversion rate of the corresponding reactants.

Reactant	DCL1		DCL2		DCL3	
	c (μM)	*conv. (%)*[a]	*c* (μM)	*conv. (%)*	*c* (μM)	*conv. (%)*
A1	120	*81*				
A2	120	*61*	60	*87*	80	*83*
A3			60	*94*	80	*97*
A4			60	*≈100*		
A5			60	*34*	80	*41*
L1	40	*97*	120	*91*	60	*95*
L2	40	*88*				
L3	40	*≈100*				
L4					60	*97*
Total acylhydrazone	240		240		240	
Catalyst **1b**	10 mM		10 mM		10 mM	
DNA[b]	5		5		5	

[a] Conversion, calculated as per relative peak area of the reactants before and after addition of the catalyst and incubation for 24 h (in the absence of DNA). [b] Except for “blank” libraries and samples treated with beads only.

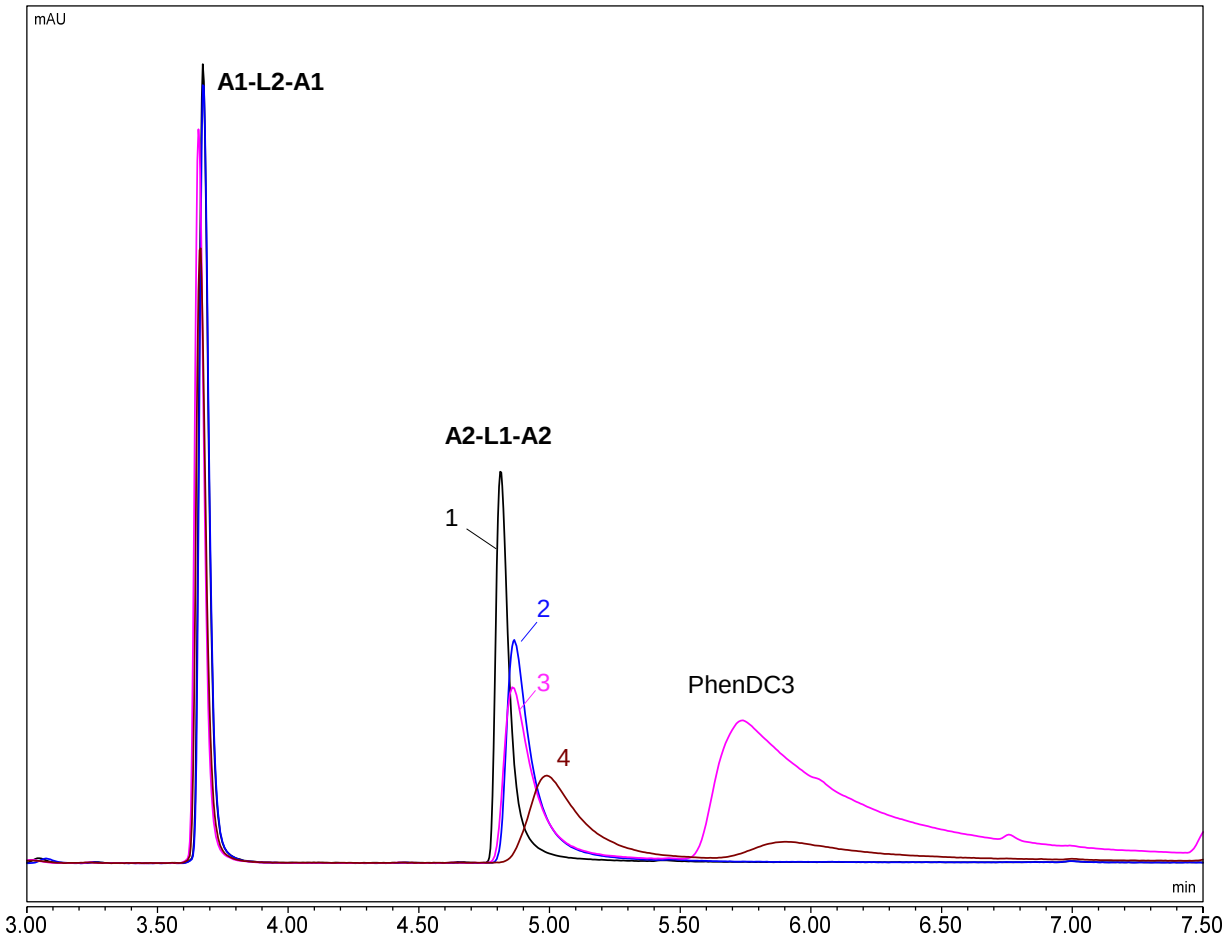


Fig. S1 HPLC profiles of mixtures of **A1-L2-A1** and **A2-L1-A2** (25 µM each in 100 mM NH_4OAc, 1.5 mM KCl buffer, pH 6): (1) in the absence of G4-DNA; (2) in the presence of *Pu24T* (50 µM); (3) same as (2) followed the addition of PhenDC3 (125 µM); (4) same as (2) followed addition of equal volume of formamide prior to injection. Samples were incubated for 1 h at room temperature prior to analysis. The injection volume was 5 µL in (1–3) and 10 µL in (4), to compensate for the addition of formamide.

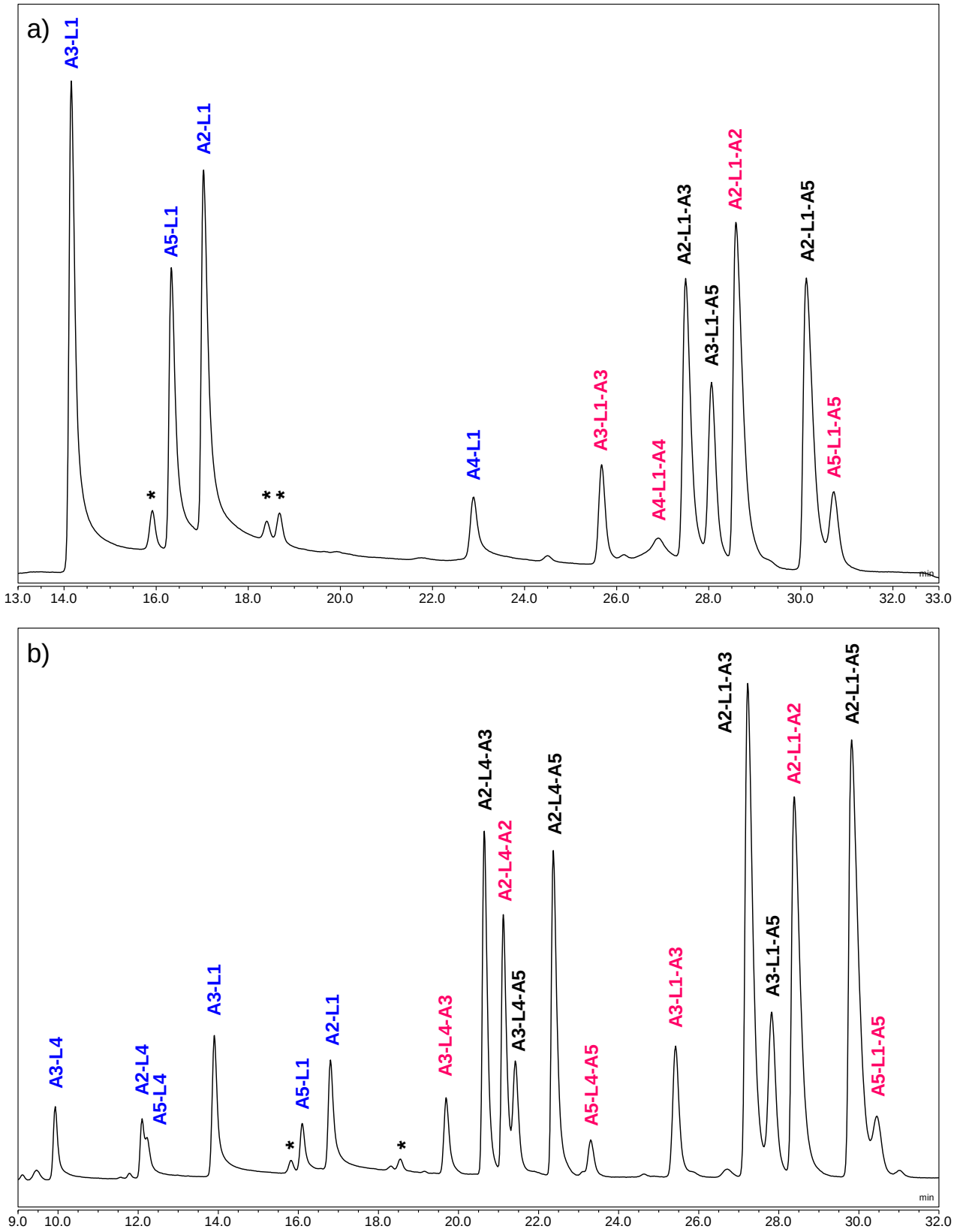
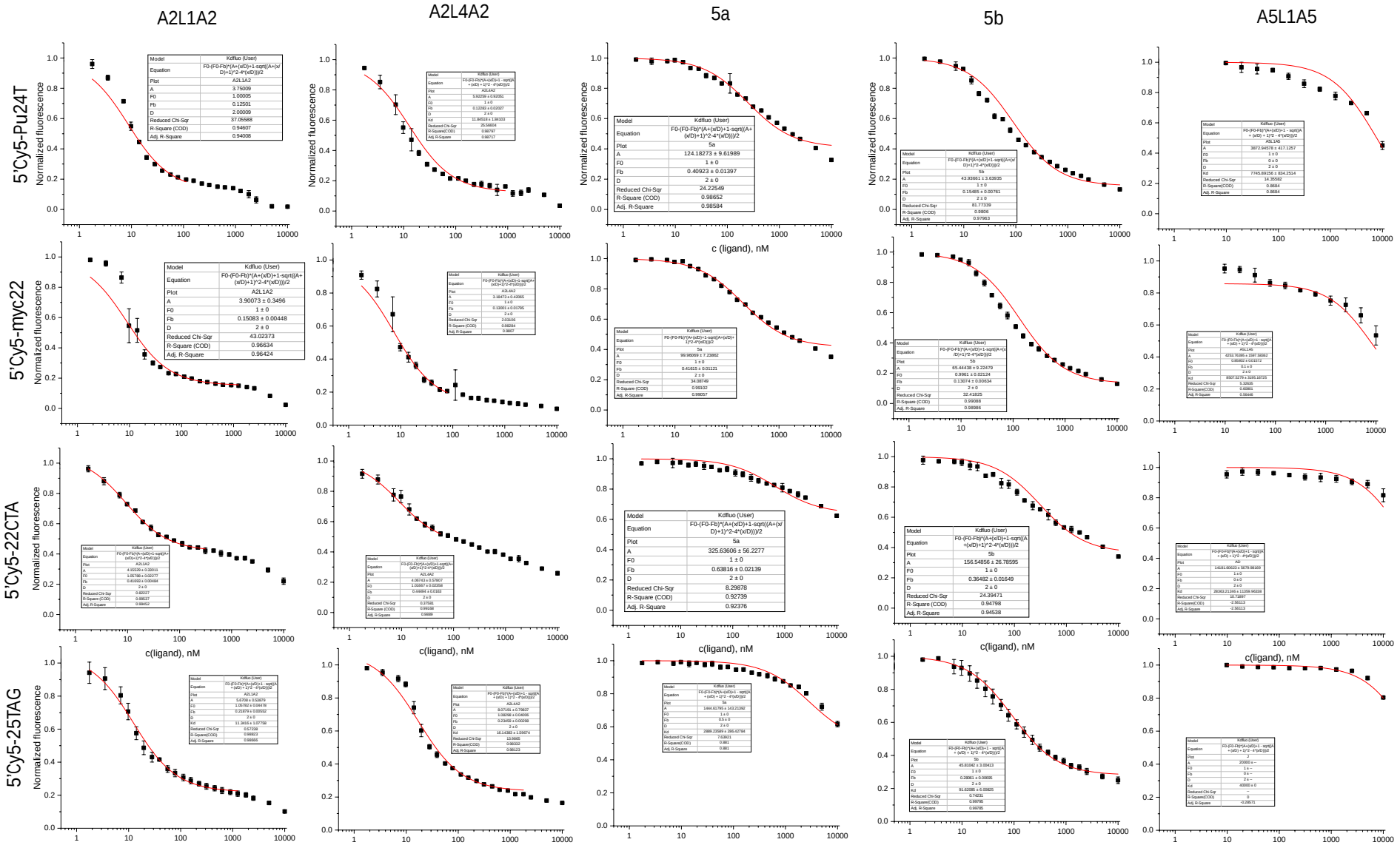


Fig. S2 HPLC profiles of untemplated a) DCL2 and b) DCL3. DCL2: *c*(**A2**) = *c*(**A3**) = *c*(**A4**) = *c*(**A5**) = 60 µM, *c*(**L1**) = 120 µM, *c*(**1b**) = 10 mM. DCL3: *c*(**A2**) = *c*(**A3**) = *c*(**A5**) = 80 µM, *c*(**L1**) = *c*(**L4**) = 60 µM, *c*(**1b**) = 10 mM. Reactions were performed in 100 mM NH_4OAc, 1 mM KCl buffer, pH 6.0. Peaks labelled with asterisks could not be assigned.


A2L1A2
A2L4A2
5a
5b
A5L1A5
5'Cy5-Pu24T
5'Cy5-myc22
5'Cy5-22CTA
5'Cy5-25TAG
Normalized fluorescence
c (ligand), nM
c(ligand), nM

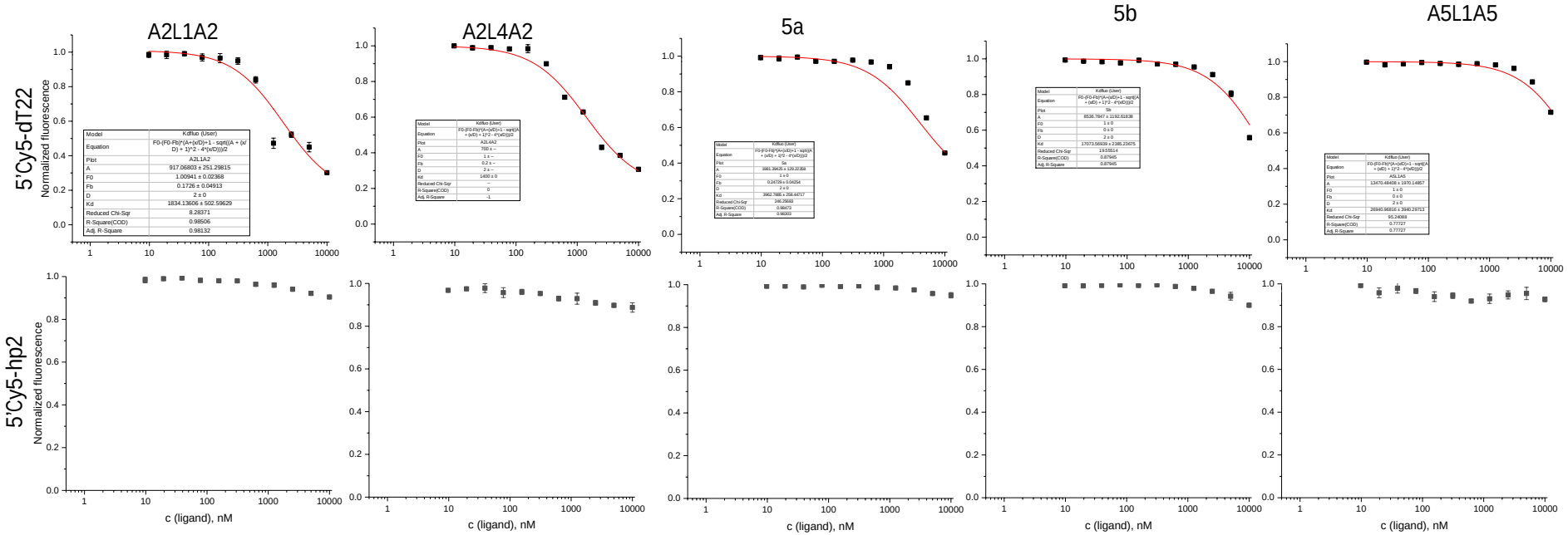


Fig. S3 Normalized changes of fluorescence intensity (λ_{ex} = 590^{BP50} nm, λ_{em} = 675^{BP50} nm) upon titrations of ligands (final concentration: 1.8 nM to 10 µM) to 5′-Cy5-labelled oligonucleotides (*c* = 2 nM in 10 mM $LiAsO_2Me_2$, 100 mM KCl, 0.5 w/v % CHAPS, 0.05 v/v % Triton X-100, pH 7.2). Data are means ± s.d. from three independent experiments. Red lines represent fits of experimental data to a 1:1 binding model (Eq. 1).

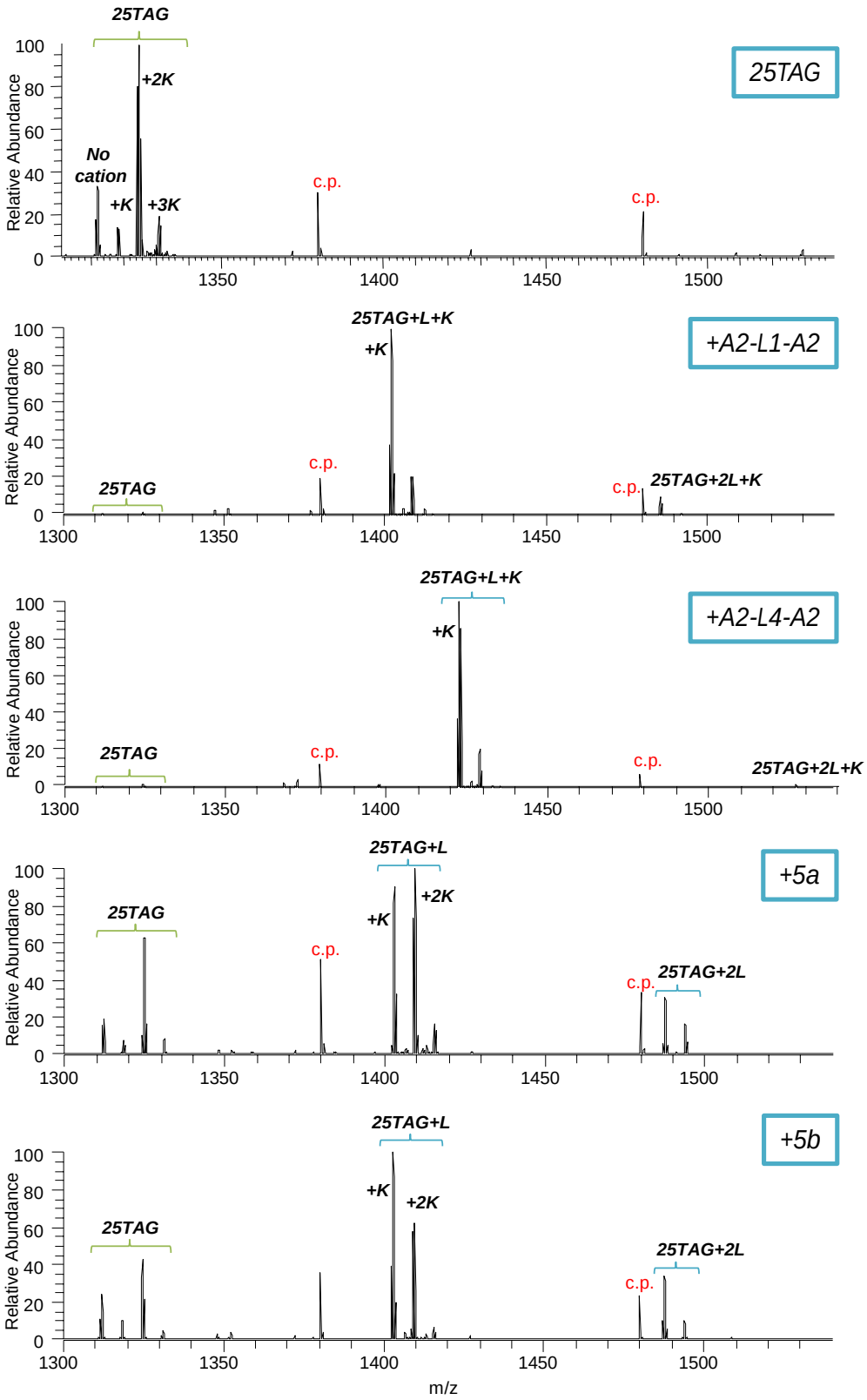


Fig. S4 ESI-MS spectra of *25TAG* (5 µM) in the absence (top) and in the presence of equimolar concentrations of ligands (L) **A2-L1-A2**, **A2-L4-A2**, **5a**, and **5b**, in 100 mM TMAA + 1 mM KCl buffer. c.p. = calibration peak (dT_6).

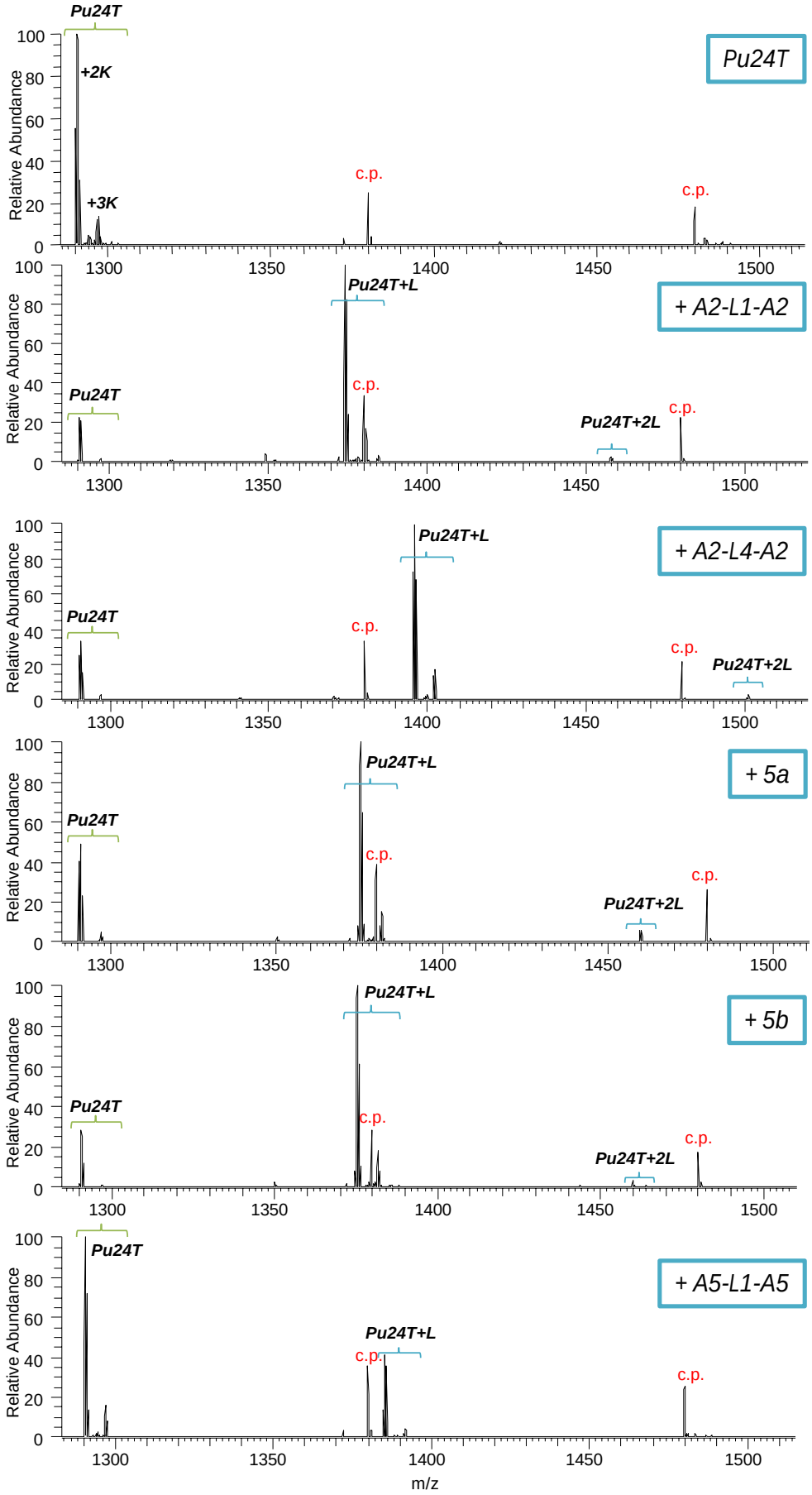


Fig. S5 ESI-MS spectra of *Pu24T* (5 μM) in the absence (top) and in the presence of equimolar concentrations of ligands (L) **A2-L1-A2**, **A2-L4-A2**, **5a**, **5b**, and **A5-L1-A5**, in 100 mM TMAA + 1 mM KCl buffer. c.p. = calibration peak (dT_6).

Table S3. Apparent dissociation constants (K_d / nM) determined from mass-spectra of G4-DNA–ligand complexes.

Ligand	*Pu24T*	*25TAG*
A2-L1-A2	85	n.d.[a]
A2-L4-A2	220	< 7.0
5a	470	330
5b	240	120
A5-L1-A5	9000	not measured

[a] The K_d value could not be determined since the peak of unbound G4-DNA was undetectable.

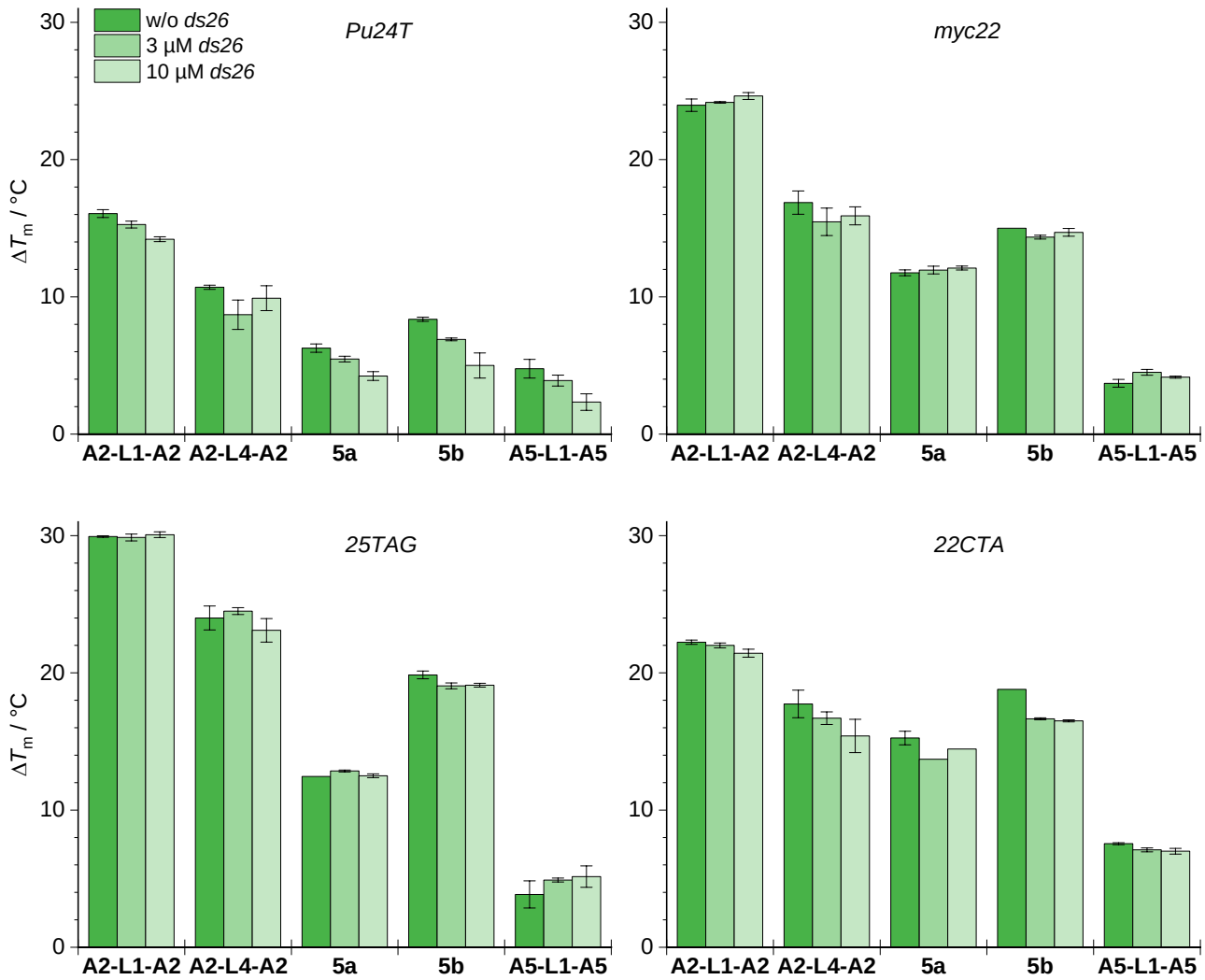


Fig. S6 Effect of unlabelled double-stranded competitor (*ds26*) on the ligand-induced stabilization (ΔT_m, °C) of G4-DNA (*Pu24T*, *myc22*, *25TAG*, *22CTA*) observed in fluorescence-melting experiments. Data are mean ± s.d. from three measurements.

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