Electronic Supplementary Information for Dalton Transactions

Electronic Supplementary Information

Experimental:

1,10-phenanthroline-4-carboxaldehyde[1]. In a flask containing a solution obtained by dissolving SeO_2 (0.85 g 7.6 mmoles) in 15 mL of dioxane/water (96:4) and heated to refluxing, a solution of 4-methyl-1,10-phenanthroline (0.70 g, 3.6 mmoles) in 67 mL of dioxane:water (96:4) was added dropwise during 1 hour. The solution was refluxed for 3 hours and the resulting suspension was filtered through celite while still warm. The volume of the solution was reduced to 30 mL under diminished pressure. After standing overnight, the solid was collected by filtration to yield 850 mg (79%) of product as a yellow powder. ^{1}H NMR (DMSO-d_6) δ 10.69 (s, 1H), 9.42 (d, J = 4.4 Hz, 1H), 9.16 (dd, J = 1.6, 4.3 Hz, 1H), 8.96 (d, J = 9.2 Hz, 1H), 8.54 (dd, J = 1.6,8.0 Hz, 1H), 8.23 (d, J = 4.4 Hz, 1H), 8.19 (d, J = 9.2 Hz, 1H), 7.83 dd, J = 4.3, 8.0 Hz,, 1H).

1. S. H. Bossmann, N. D. Ghatlia, M. F. Ottaviani, C. Turro, H. Durr and N. J. Turro, *Synthesis*, 1996, **11**, 1313.

(E)-N^1-((1,10-phenanthrolin-4-yl)methylene)-N^2,N^2-dimethylethane-1,2-diamine. The 1,10-phenanthroline-4-carboxaldehyde (234 mg, 1.12 mmoles) was dissolved in 4 mL of toluene. Activated molecular sieves were introduced and then *N,N*-dimethylethylenediamine (400 mg, 4.54 mmoles). The solution was stirred in a stoppered flask overnight. The suspension that was obtained was filtered, the solvent was removed under diminished pressure and then on a vacuum pump to remove the excess of *N,N*-dimethylethylenediamine to afford 260 mg (83%) of product as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 9.25 (d, J = 4.6 Hz, 1H), 9.21 (dd, J = 1.7, 4.3 Hz, 1H), 9.04 (s, 1H), 8.71 (d, J = 9.2 Hz, 1H), 8.26 (dd, J = 1,7, 8.0 Hz, 1H), 7.96 (d, J = 4.6 Hz, 1H), 7.87 (dd, J = 4.3, 8.0 Hz,1H), 7.65 (dd, J = 8.0 and 4.3 Hz, 1H), 3.94 (t, J = 6.8 Hz, 2H), 2.74 (t, J = 6.8 Hz, 2H), 2.36 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 159.0, 150.4,150.0, 146.9, 146.2, 139.1, 135.8, 128.0, 128.2, 127.3, 126.2, 123.2, 122.4, 60.9, 59.8, 45.8.

(E)-N^1-((1.10-phenanthrolin-4-yl)methylene)-N^2,N^2-dimethylethane-1,2-diamine. The $NaBH_4$ (53 mg, 1.40 mmoles) was added to a cold solution obtained by dissolving the imine (120 mg, 0.43 mmole) in methanol (2 mL). The solution was allowed to warm to room temperature and then was refluxed for 5 minutes. Water (4 mL) was added and the solution was extracted with $CHCl_3$. The organic phase was dried over $MgSO_4$ and the solvent removed at diminished pressure to afford 112 mg (93%) of the product as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 9.20 (dd, J = 1,6, 4.2 Hz, 1H), 9.14 (d, J = 4.5 Hz, 1H), 8.25 (dd, J = 1.6, 8.0 Hz, 1H), 8.13 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 4.5 Hz, 1H), 7.63 (dd, J = 4.4, 8.0 Hz, 1H), 4.35 (s, 2H), 2.82 (t, J = 5.8 Hz, 2H), 2.49 (t, J = 5.9 Hz, 2H), 2.23 (s, 12H). ^{13}C NMR ($CDCl_3$): 150.2, 150.0, 146.4, 146.2, 145.8, 135.7, 128.1, 127.2, 126.2, 122.9, 122.1, 58.9. 50.5, 47.1, 45.5 (one carbon missing)

N^1-((1.10-phenanthrolin-4-yl)methylene)-N^2,N^2-dimethylethane-1,2-diamine hydrochloride salt (K34). The crude amine (112 mg) was dissolved in acetone, acidified

with 10% aqueous HCl and acetone was added to precipitate the hydrated hydrochloride salt as an off white powder. ^{1}H NMR (DMSO-d_6) δ 9.36 (d, J = 4.8 Hz, 1H), 9.30 (dd, J = 4.9, 1.4 Hz, 1H), 9.09 (d, J = 8.2 Hz, 1H), 8.57 (d, J = 9.2 Hz, 1H), 8.40 (s, 1H), 8.37 (t, J = 4.6 Hz, 1H), 8.22 (dd, J = 8.2 , 4.9 Hz, 1H), 5.01 (s, 2H), 3.68 (d, J = 5.3 Hz, 2H), 3.65 (d, J = 5.3 Hz, 2H), 2.89 (s, 6H).^{13}C NMR (D_2O) δ 150.3, 145.8, 144.0, 139.1, 138.8, 136.7, 129.5, 127.6, 126.8, 125.9, 125.2, 124.2, 52.5, 47.2, 43.4, 42.4 (dioxane as internal standard, 66.50).
This salt on treatment with aqueous KOH and extraction into $CDCl_3$ followed by ^{1}H NMR analysis indicated reconversion to the free base.

1,10-Phenanthroline-2,9-dicarboxaldehyde. The SeO_2 (4.73 g, 42.5 mmoles) was dissolved in dioxane (150 mL) and water (3 mL), the flask placed in an oil bath and heated to reflux. The 2,9-dimethyl-1,10-phenanthroline (4.35 g, 20 mmoles) was dissolved in dioxane (100 mL), placed in a dropping funnel and quickly added to the refluxing mixture over a period of 7 minutes. The mixture was refluxed for 0.5 h and then decanted from the Se into a large flask. The product immediately separated and the mixture was cooled in an ice bath to about 10^{o}C to afford a light brown solid (5.2 g). The crude material was heated in chloroform (150 mL) and some insoluble material was removed by filtration. Concentration of the filtrate led to the dialdehyde (3.50 g, 71%) as a pale tan solid quite suitable for use in the next stap. Beautiful pink colored needles could be obtained by crystallization from acetonitrile, mp 261-261^{o}C (dec). ^{1}H NMR (DMSO-d_6) δ 10.36 (s, 2H), 8.78 (d, J = 8.0 Hz, 2H), 8.31 (d, J = 8.0 Hz, 2H), 8.28 (s, 2H).

(N^1,N^1,E,N^1,N^1E)-N^1,N^1-(1,10-phenanthroline-2,9-diyl)bis(methan-1-yl-1-ylidene)bis(N^2,N^2-dimethylethane-1,2-diamine The 1,10-phenanthroline-2,9-dicarboxaldehyde (1.07 g, 4.53 mmoles) was placed in toluene (20 ml, previously dried over molecular sieves) and 8-12 molecular sieves were added to the suspension. The N,N-dimethylethylenediamine (1.60 g, 18.2 mmoles) was added and within 5 min a yellow-orange solution developed. The mixture was stirred at rt for 24 h and the sieves were removed by filtration to afford a yellow-orange filtrate. The toluene and excess diamine were removed via rotary evaporation to yield an orange oil. This material readily crystallized from pentane or pentane:chloroform mixtures (25:2) to afford beautiful crystals (0.91 g, 53%), mp 69-71^{o}C (additional material could be obtained from the filtrate). ^{1}H NMR ($CDCl_3$) δ 8.91 (s, 2H), 8.44 (d, J = 8.3 Hz, 2H), 8.30 (d, J = 8.3 Hz, 2H), 7.86 (s, 2H), 3.91 (t, J = 6.90 Hz, 4H), 2.71 (t, J = 6.90, 4H), 2.35 (s, 12H). ^{13}C NMR ($CDCl_3$) δ 163.8, 154.0, 145.4, 136.7, 129.6, 127.2, 120.4, 60,0, 59.4, 45.8. Calcd.for $C_{22}H_{28}N_6$: C, 70.18, H, 7.50, N, 22.32. Found: C, 70.03, H, 7.51, N, 22.25.

N^1,N^1-(1,10-phenanthroline-2,9-diyl)bis(methylene)bis(N^2,N^2-dimethylethane-1,2-diamine. To a solution of the bis-imine (300 mg, 0.80 mmol) in 6 mL of MeOH was added $NaBH_4$ (150 mg, 3.9 mmol) at 0 °C. The solution was allowed to warm to room temperature and then was refluxed for 15 minutes. Water was added (12 mL) and the mixture was extracted with $CHCl_3$ (3 x 20 mL). The extract was dried over $MgSO_4$, the solvent was removed by roto evaporation and then under vacuum to afford as a viscous yellow oil. ^{1}H-NMR ($CDCl_3$): δ 8.21 (d, J = 8.2 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H), 7.74

(s, 2H), 4.35 (s, 4H), 2.85 (t, J = 6.2 Hz 4H), 2.51 (t, J = 6.2 Hz, 4H), 2.26 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 160.9, 145.3, 136.5,127.7,125.8, 122.0, 60.0, 59.2, 45.7, 45.6.

N^1,N^1-(1,10-phenanthroline-2,9-diyl)bis(methylene)bis(N^2,N^2-dimethylethane-1,2-diamine hydrochloride (K35). The hydrated hydrochloride salt was obtained by dissolving the product in acetone, followed by acidification with aqueous hydrochloric acid and then addition of acetone to afford the salt (115 mg, quantitatively) as an off-white powder. ^1H NMR (DMSO-d_6): δ 8.64 (d, J = 8.2 Hz, 2H), 8.11 (s, 2H), 7.92 (d, J = 8.3 Hz, 2H), 4.79 (s, 4H), 3.76-3.73 (m, 8H), 2.89 (s, 12H). ^1H NMR (D_2O): δ 8.54 (d, J = 8.3 Hz, 2H), 7.94 (s, 2H), 7.83 (d, J = 8.3 Hz, 2H), 4.87 (s, 4H), 3.60 m, 8H), 2.86 (s, 12 H)^{13}C NMR (D_2O) δ 150.7, 142.3, 141.0, 130.4, 128.3, 125.1, 53.2, 51.6, 44.2, 42.8 (one drop dioxane as internal standard 67.2).
Treatment of a small amount of the salt with aqueous KOH, extraction of the free base in $CDCl_3$, drying over Na_2SO_4 and ^1H NMR analysis indicated complete conversion to the free base.

Ligands and metal ions solutions:
Stock solutions of K34 (4 mM), K35 (4 mM), were prepared in deionized water; Phen (7 mM) in DMSO. They were diluted to the appropriate concentration in the working buffer prior to use.
$CuSO_4$ (1 M) and $NiCl_2$ (0.8 M) were dissolved in deionized water and metal ion concentrations were determined by ICP (Optima 3000 DV Perkin Elmer).

UV Titrations:
Spectrophotometric titrations were performed at 25°C in 10 mM Tris-HCl, 50 mM KCl, pH 7.5. with a Perkin-Elmer Lambda 20 apparatus. Complex formation was followed recording ligand spectra upond incrementa additions od metal ion solutions. To determine complex stoichiometry, different amounts of metal ions and ligands solutions were mixed to reach a final constant total concentration (0.3 mM). The ligand extinction coefficient was calculated at each point of the titration (at 272 and 278 nm for K34 and K35 respectively) and was plotted against the metal ion molar fraction.

Telomerase Activity Assay (TRAP assay):
An aliquot of $5x10^6$ JR8 cells in exponential phase of growth was pelleted and lysed for 30 min on ice using 100 µl of 0.5% CHAPS, 1 mM EGTA, 25% 2-mercaptoethanol, 1.74% PMSF and 10% w/v glycerol. The lysate was centrifuged at 13000 rpm for 30 min at 4 °C and the supernatant collected, stored at –80 °C, and used as the telomerase source. Telomerase activity was assayed using a modified telomere repeat amplification protocol (TRAP) assay[2]. Briefly, an appropriate primer TS (5'-AATCCGTCGAGCAGAGTT-3'; Biosense) have been 5'-labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. After enzyme inactivation (85 °C for 5 min), a 50 µl TRAP reaction mix (50 µM of dNTPs, 0.2 µg of labelled TS, 0.1 µg of return primer ACX, 500 ng of protein extract, 2 U *Taq* polymerase) was prepared in the presence/absence of increasing drug concentration in 20 mM Tris-HCl pH 8.3, 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.05% v/v Tween- 20. Then, telomerase elongation step has been performed (30 min at 30 °C) followed by a PCR amplification step (30 cycles of: 30 s at 37 °C and 30 s at 58 °C). The reaction

products were loaded onto a 10% polyacrylamide gel (19:1) in TBE 0.5X. Gels were transferred to Whatman 3MM paper, dried under vacuum at 80 °C and read using a phosphorimager 840 (Amersham).

2. Kim, N. W.; Wu, F. Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Res.* **1997**, *25*, 2595-2597.

Fluorescence melting studies:
Melting experiments were performed in a Roche LightCycler, using an excitation source at 488 nm and recording the fluorescence emission at 520 nm. Target DNAs were HTS (5'-AGGGTTAGGGTTAGGGTTAGGGT-3', labeled with Dabcyl at 5' end and Fluorescin at 3' end), dsHTS (HTS annealed to its not labelled complementary strand) and ds-random (Fluorescin 5' labelled 5'-GTGAGATACCGACAGAAG-3' annealed with its complementary strand labeled with Dabcyl at 3' end). They were provided by Biosense. Mixtures (20 μL containing 0.25 μM DNA and variable concentrations of tested derivatives in 50 mM potassium buffer (10 mM LiOH; 50 mM KCl pH 7.4 with H_3PO_4) were first denatured by heating to 95 °C for 5 min and then cooled to 30 °C at a rate of 0.5 °C min^{-1}. Then temperature was slowly increased (0.2 °C/min) up to 90 °C and again lowered at the same rate to 30°C. Recordings were taken during both these melting and annealing reactions to check for hysteresis.
T_m values were determined from the first derivatives of the melting profiles using the Roche LightCycler software. Each curve was repeated at least three times and errors were ± 0.4°C.

Circular dichroism measurements:
Circular dichroism spectra from 230 to 350 nm were recorded using 10 mm path length cells on a Jasco J 810 spectropolarimeter equipped with a NESLAB temperature controller and interfaced to a PC 100 in 10 mM Tris-HCl, 50 mM KCl pH 7.4. The used sequence was d(AGGGTTAGGGTTAGGGTTAGGGT). Before data acquisition, DNA solutions (4 μM) were heated at 95°C for 5 min and left to cool at room temperature o.n.. The reported spectrum of each sample represents the average of 3 scans recorded with 1-nm step resolution. Observed ellipticities were converted to mean residue ellipticity [θ] = deg x cm^2 x $dmol^{-1}$ (Mol. Ellip.).

Mass spectrometry:
All mass spectra were obtained using a Mariner™ mass spectrometer, Applied Biosystems (Foster City, CA). Analytical solutions were prepared in water/methanol (10%-90%) and contained 100 μM ligand and different stoichiometric amounts of metal ions. An Harvard model 11 syringe pump (Holliston, MA) set at flow rate of 20 μl/min was used to infuse the sample solutions. The ESI source was operated in positive ion mode with an electrospray voltage of 4.5 kV. Spectra were acquired over the *m/z* range 100-4000 by summing 100 scans.

Phen + 1 eq $CuSO_4$ (m/z) 181.0644 (M) (8%), 199.5156 (M+Cu+2DMSO)(28), 287.9981 (M+Cu+2Na) (47), 211.5244 (2M+Cu) (17)
Phen + 2 eq $CuSO_4$ (m/z) 181.0644 (M) (9%), 199.5156 (M+Cu+2Na) (16), 287.9981 (M+Cu+2Na)(38), 211.5244 (2M+Cu) (37)
Phen + 3 eq $CuSO_4$ (m/z) 181.0644 (M) (14), 287.9981 (M+Cu+2Na) (16), 211.5244 (2M+Cu) (30), 298.0731 (3M+Cu) (21), 381.5610 (3M+Cu+NaCl) (19)
Phen + 1 eq $NiCl_2$ (m/z) 197.0093 (M+Ni+2DMSO) (52), 209.0355 (2M+Ni) (48)
Phen + 2 eq $NiCl_2$ (m/z) 209.0355 (2M+Ni) (63), 299.0618 (3M+Ni) (37)
Phen + 3 eq $NiCl_2$ (m/z) 299.0618 (3M+Ni) (100)
K34 + 1 eq $CuSO_4$ (m/z) 172.5593 (M+Cu) (33%), 189.5369 (M+Cu+Cl) (42), 194.5527 (M+Cu+2Na) (25)
K34 + 2 eq $CuSO_4$ (m/z) 172.5593 (M+Cu) (33%), 189.5369 (M+Cu+Cl) (42), 194.5527 (M+Cu+2Na) (25)
K34 + 3 eq $CuSO_4$ (m/z) 172.5593 (M+Cu) (33%), 189.5369 (M+Cu+Cl) (42), 194.5527 (M+Cu+2Na) (25)
K34 + 1 eq $NiCl_2$ (m/z) 187.0466 (M+Ni+Cl) (67%), 192.0512 (M+Ni+2Na) (33)
K34 + 2 eq $NiCl_2$ (m/z) 187.0466 (M+Ni+Cl) (45%), 192.0512 (M+Ni+2Na) (22), 309.1489 (2M+Ni) (9), 327.1293 (2M+Ni+Cl) (24)
K34 + 3 eq $NiCl_2$ (m/z) 187.0466 (M+Ni+Cl) (13%), 192.0512 (M+Ni+2Na) (7), 309.1489 (2M+Ni) (21), 327.1293 (2M+Ni+Cl) (57), 449.2253 (3M+Ni) (2)
K35 + 1 eq $CuSO_4$ (m/z) 356.1040 (M+Cu-C5H13N) (34%), 385.1349 (M+Cu-C3H8N) (46), 478.1697 (M+Cu+Cl) (20)
K35 + 2 eq $CuSO_4$ (m/z) 356.1040 (M+Cu-C5H13N) (31%), 385.1349 (M+Cu-C3H8N) (45), 478.1697 (M+Cu+Cl) (24)
K35 + 3 eq $CuSO_4$ (m/z) 356.1040 (M+Cu-C5H13N) (34%), 385.1349 (M+Cu-C3H8N) (44), 478.1697 (M+Cu+Cl) (22)
K35 + 1 eq $NiCl_2$ (m/z) 191.1405 (M) (66%), 219.0989 (M+Ni) (34)
K35 + 2 eq $NiCl_2$ (m/z) 191.1405 (M) (29%), 219.0989 (M+Ni) (71)
K35 + 3 eq $NiCl_2$ (m/z) 191.1405 (M) (14%), 219.0989 (M+Ni) (86)

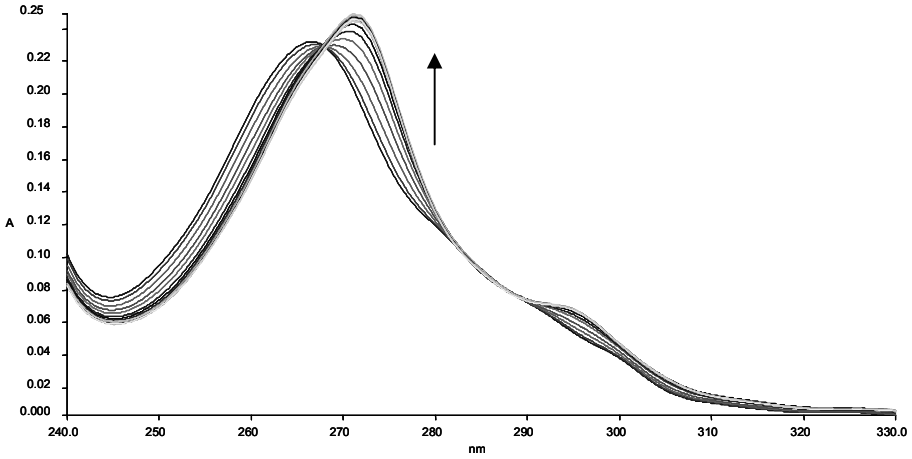

Fig. S1 : Titration of K34 (17 μM) with Ni(II) (0-30mM) monitored in 10 mM Tris, 50 mM KCl, pH 7.5. Arrow indicates the effects of incrementing metal ion concentration.

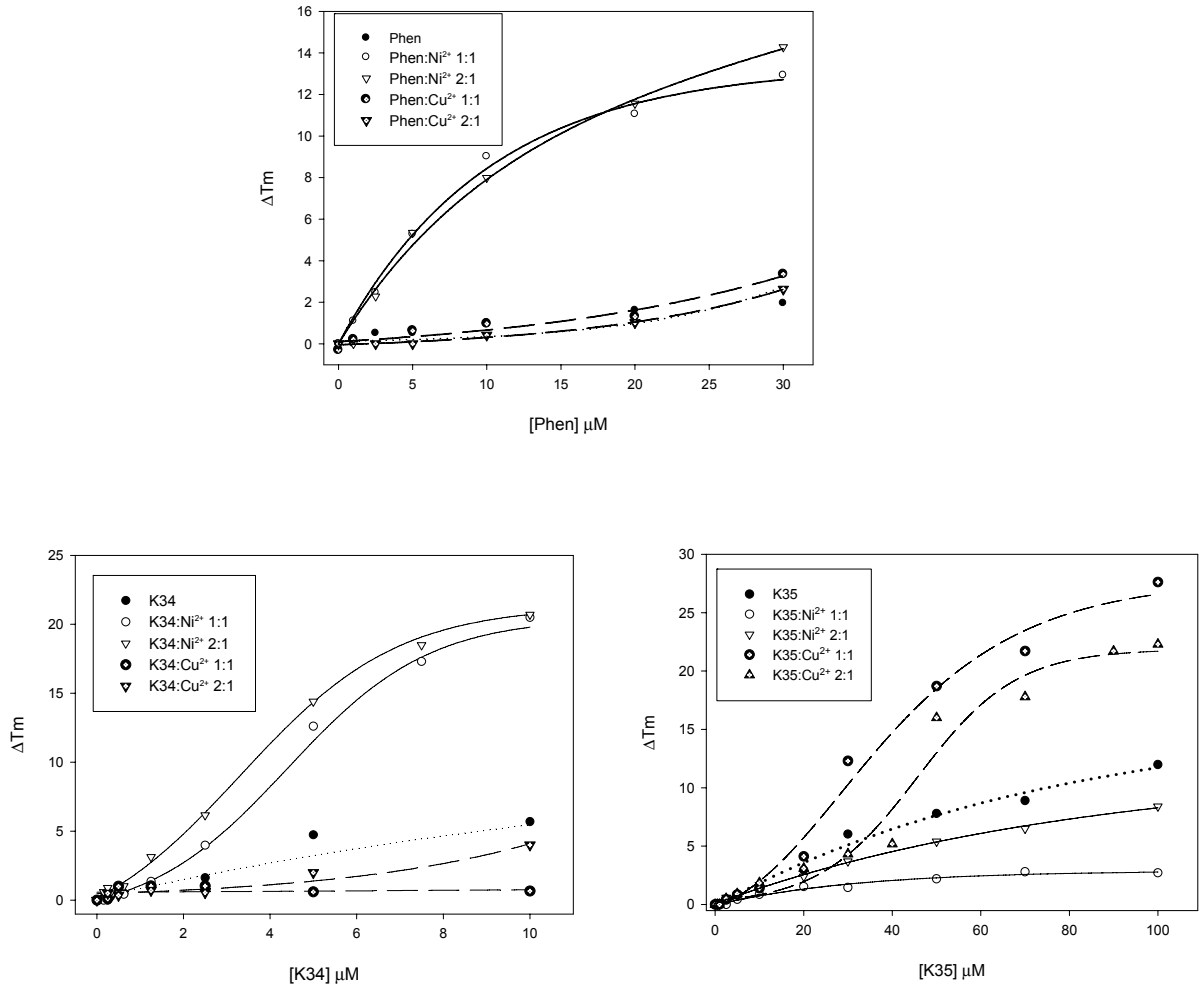

Fig. S2 : Variation of HTS (0.25 µM) melting temperature (ΔTm) produced by increasing concentrations of tested ligands (5 µM) in the presence/absence of Cu^{2+} or Ni^{2+} at different stoichiometric ratio. Conditions: 50 mM potassium buffer, pH 7.4. Heating rate 0.2°C/min. Error ± 0.4°C.

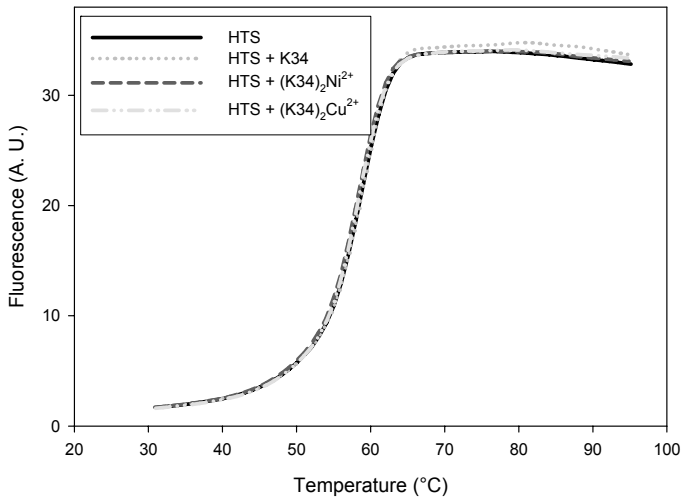

Fig. S3 : Melting profiles of a double stranded DNA (ds-random, 0.25 μM) recorded in the presence/absence of K34 (5 μM) in the presence/absence of 0.5 equivalents of Cu^{2+} or Ni^{2+}. Conditions: 50 mM potassium buffer, pH 7.4. Heating rate 0.2°C/min.

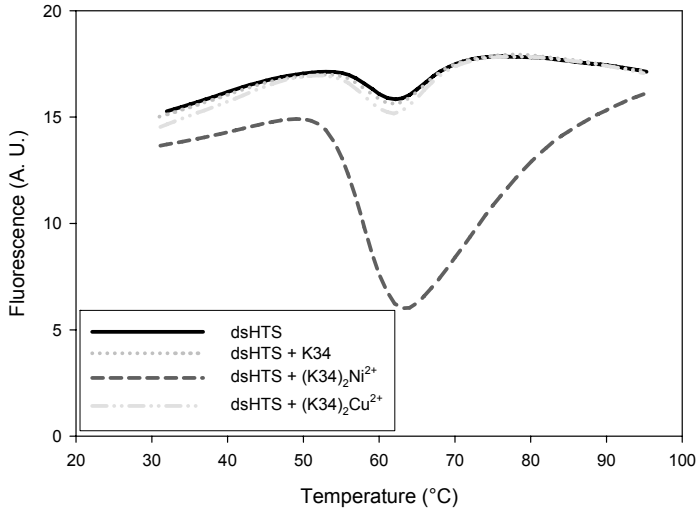

Fig. S4 : Melting profiles of HTS annealead to its complementary strand (dsHTS, 0.25 μM) recorded in the presence/absence of K34 (5 μM) in the presence/absence of 0.5 equivalents of Cu^{2+} or Ni^{2+}. Conditions: 50 mM potassium buffer, pH 7.4. Heating rate 0.2°C/min.

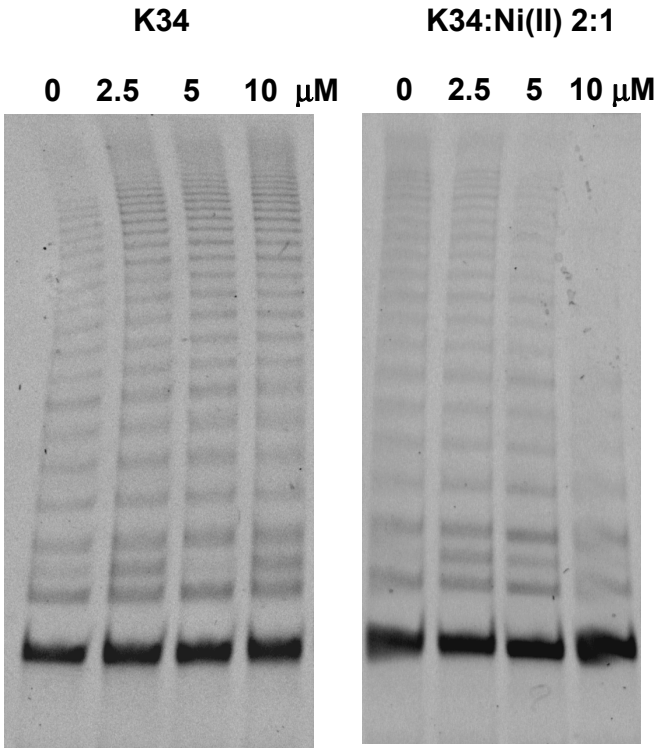

Fig. S5 : Trap assay performed with increasing concentrations of K34 in the presence/absence of 0.5 equivalents of Ni^{2+}. The cell extract used was obtained from JR8 cells.