Asymmetric triplex metallohelices with high and selective activity against cancer cells

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Computational Work

Starting points for geometry optimisations were taken from crystallographic data that was available, and where unavailable the starting structures were created from existing crystallographic fragments. Monometallic structures were first optimised using B3LYP-D3(BJ)\(^1\) functional and the 6-31g basis set, with convergence criteria of 0.0001 a.u. as implemented in the Firefly quantum chemistry package,\(^2\) which is partially based on the GAMESS(US) source code.\(^3\) Bimetallic systems were optimised using ligand field molecular mechanics (LFMM)\(^4\) as implemented in the DommiMOE program,\(^5\) before being annealed at 500 K for 1 ns prior to re-optimisation. Single point energies of all structures was performed using the B3LYP-D3(BJ) functional and the def2-TZVP basis set as implemented in the Firefly quantum chemistry package.\(^2\)

Equilibrium selectivities were determined from the difference between the statistically corrected energies of each isomer at 298 K as outlined in ref 6 (pp. 217-220); \(\text{RTln}(K)\) (\(K\) is the number of ways a single isomer can be formed) was subtracted from the isomer energies to obtain the statistically corrected isomer energies \(A\). These were converted to isomer proportions \(P\), where \(P = \exp(-A/\text{RT})\).

Synthesis

All solvents and chemicals purchased from commercial sources (Sigma-Aldrich, Acros, Fisher Scientific or Alfa Aesar) were used without further purification unless otherwise stated. Sodium hydride dispersions in mineral oil were placed in a Schlenk vessel under an inert atmosphere and washed three times with diethyl ether to remove the oil, then dried and stored under argon in an MBraun dry box. Where appropriate, reactions were carried out under argon using a dual manifold argon/vacuum line and standard Schlenk techniques or in an MBraun dry box. Necessary solvents were dried by heating to reflux for 3 d under dinitrogen over the appropriate drying agents (potassium for tetrahydrofuran, sodium/potassium alloy for diethyl ether, and calcium hydride for acetonitrile and pyridine) and degassed before use. Tetrahydrofuran and diethyl ether were additionally pre-dried over sodium wire. Dried solvents were stored in glass ampoules under argon. All glassware and cannulae were stored in an oven at > 375 K.
Deuterated solvents were purchased from Sigma-Aldrich and Cambridge Isotope Laboratories. NMR spectra were recorded on Bruker Spectrospin 300/400/500 MHz spectrometers – see page 26 and manuscript for spectra. Routine NMR assignments were confirmed by \(^1\)H-\(^1\)H (COSY) and \(^{13}\)C-\(^1\)H (HMBC) correlation experiments where necessary. The spectra were internally referenced using the residual protio solvent (CDCl\(_3\), CD\(_3\)CN etc.) resonance relative to tetramethylsilane (\(\delta = 0\) ppm). ESI mass spectra were recorded on an Agilent Technologies 1260 Infinity spectrometer or a Bruker Daltonics MicroTOF spectrometer. Infra-Red spectra were measured using a Bruker Alpha-P FTIR spectrometer. Elemental analyses were performed by Medac Ltd. Chobham, Surrey GU24, 8JB, UK or Warwick Analytical Service, Coventry, CV4 7EZ.

Optical rotation measurements were performed on a Perkin Elmer Polarimeter 341 by Warwick Analytical Services, Coventry, UK. In all cases the following parameters were used: solvent methanol, temperature 20°C, pathlength 100 mm, wavelength 589 nm.

Thermogravimetric analysis (DSC1-1600 scanning calorimeter) was used to determine the amount of water of crystallisation present in the chloride salts of iron (II) triplex metalllohelices. See page 28 for plots and further details.

We are grateful to the National Crystallographic Service\(^7\) for recording the data for compounds \(R_{c}\Delta_{Fe,HHT}-[Zn_{2}L_{3a}]ClO_{4}\) and \(R_{c}\Delta_{Fe,HHT}-[Zn_{2}L_{3b}]ClO_{4}\). Structural data for these compounds was recorded in house using a Siemens SMART CCD single crystal diffractometer using MoK\(\alpha\) (\(\lambda = 0.71073\) Å) or CuK\(\alpha\) (\(\lambda = 1.54184\) Å) radiation source. All structures were solved by direct methods using SHELX (TREF)\(^8\)\(^9\) with additional light atoms found by Fourier methods. Crystal refinement was performed using SHELX97.\(^9\)

The compounds 5-(chloromethyl)-2,2'-bipyridine,\(^10\)\(^11\) 5-hydroxypicolinaldehyde,\(^12\) phenylglycinol\(^13\) and Pyrazine-2-carboxaldehyde\(^14\) were synthesised by known methods.

**5-methoxypicolinaldehyde**

![Methoxypicolinaldehyde](image)
5-hydroxypicolinaldehyde (2.0 g, 1 eq.), methyl iodide (2.3 g, 1 ml, 1 eq.), and potassium carbonate (2.69 g, 1.2 eq.) were dissolved in acetonitrile (50 ml) and heated at reflux (85°C) for 18 h, allowed to cool to ambient temperature and filtered through a silica plug. Solvents were removed under reduced pressure and the crude material was dissolved in dichloromethane (20 ml), filtered and solvents were removed under reduced pressure to give the desired compound as white solid.

Yield = 1.2 g, 72%.

$^1$H NMR (300 MHz, 298 K, CDCl$_3$) $\delta$H 9.92 (1H, s, CHO), 8.36 (1H, d, $^4$J$_{HH}$ = 3.0 Hz), 7.90 (1H, d, $^3$J$_{HH}$ = 8.5 Hz), 7.24 (1H, dd, $^3$J$_{HH}$ = 8.5 Hz, $^4$J$_{HH}$ = 2.5 Hz, Py), 3.89 (3H, s, OCH$_3$).

$^{13}$C($^1$H) NMR (75 MHz, 298 K, CDCl$_3$) $\delta$C 192.1 (CHO), 159.1, 146.4, 138.7, 123.5, 120.0 (Py), 56.0 (OCH$_3$).


IR $\nu$ cm$^{-1}$ 3099 w, 3052 w, 3033 w, 2984 w, 2947 w, 2831 w, 1692 s, 1572 s, 1489 m, 1452 m, 1380 w, 1308 m, 1261 m, 1222 m, 1117 m, 1007 s, 898 m, 852 s, 750 m, 734 m.

Elemental Analysis found (Calculated for C$_7$H$_7$NO$_2$) % C 61.00 (61.31), H 4.98 (5.14), N 10.00 (10.21).

5-(benzyloxy)picinaldehyde

5-hydroxypicolinaldehyde (2.0 g, 1 eq.), benzyl bromide (0.73 g, 0.5 ml, 1.05 eq.), and potassium carbonate (2.69 g, 1.2 eq.) were dissolved in acetonitrile (50 ml) and heated at reflux (85°C) for 18 h, allowed to cool to ambient temperature and filtered through a silica plug. Solvents were removed under reduced pressure and the crude material was dissolved in dichloromethane (20 ml), filtered and solvents were removed under reduced pressure to give the desired compound as yellow solid.

Yield = 0.67 g, 77%.
(S)-2-(2,2'-bipyridin-5-ylmethoxy)-1-phenylethanamine

\[
\text{\textit{S}-Phenylglycinol (1.00 g, 1 eq.) was dissolved in dry tetrahydrofuran (20 ml) and was added dropwise to a stirred suspension of sodium hydride (0.36 g, 2 eq.) in dry tetrahydrofuran (10 ml). The solution was stirred for 1 h at ambient temperature before a solution of 5-(bromomethyl)-2,2'-bipyridine (1.82 g, 1 eq.) in dry tetrahydrofuran (20 ml) was added dropwise. The solution was stirred for a further 1 h at ambient temperature. The reaction mixture was then heated to reflux (65°C) for 2 h, allowed to cool the ambient temperature, and quenched with the addition of brine (40 ml). The dichloromethane extracts (4 x 60 ml) were dried over sodium sulphate and the solvent was removed to leave a dark yellow oil. This crude product was purified by Kügelrohr distillation (excess phenylglycinol removed at 120°C under high vacuum) to give a yellow oil that solidifies on standing.}
\]

Yield 1.25 g, 56 %.

\[
\text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3\textsuperscript{)} \delta \text{H 8.61-8.59 (1H, m), 8.56 (H, d, J_{HH} 2.0 Hz), 8.33-8.29 (2H, m), 7.76-7.67 (2H, m), 7.33-7.16 (6H, m, Ar), 4.54 (2H, s, CH}_2\textsuperscript{)}\text{, 4.19 (1H, dd, J_{HH} 9.0 Hz,}
\]

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$^{4}J_{HH} = 4.0$ Hz, CH), 3.58 (1H, dd, $^{3}J_{HH} = 9.0$ Hz, $^{4}J_{HH} = 4.0$ Hz), 3.44 (1H, t, $^{3}J_{HH} = 8.5$ Hz, CH$_2$).

$^{13}$C($^{1}$H) NMR (75 MHz, CDCl$_3$) δ ppm 156.0, 155.7, 149.3, 148.6, 142.3, 137.0, 136.4, 133.7, 128.5, 127.6, 126.9, 123.8, 121.1, 120.9 (Ar), 76.9 (CH$_2$), 70.7 (CH$_2$), 55.6 (CH).

MS (ESI) 306 [M+H]$^+$. 

IR $\nu$ cm$^{-1}$ 3361 w, 3296 w, 3055 w, 3027 w, 2902 w, 2860 w, 1589 w, 1574 w, 1558 w, 1490 w, 1459 m, 1434 w, 1414 w, 1387 w, 1349 w, 1257 m, 1098 m, 1038 w, 1022 m, 991 w, 936 w.

Elemental Analysis found (Calculated for C$_{19}$H$_{19}$N$_3$O) % C 74.75 (74.73), H 6.13 (6.27), N 13.67 (13.76).

Optical Rotation $+15.41^\circ$ (1.65 g /100 ml MeOH).

The $R$ enantiomer was synthesised in an identical manner but starting from $R$-Phenylglycinol.

Yield 1.34 g, 60 %.

Optical Rotation -14.04$^\circ$ (1.64 g /100 ml MeOH).

5-(2,2'-bipyridin-5-ylmethoxy)picolinaldehyde

5-(hydroxy)-picolinaldehyde (2.0 g, 1.0 eq.) was dissolved in acetonitrile (50 ml). Potassium carbonate (2.65 g, 1.2 eq.) followed by 5-(chloromethyl)-2,2'-bipyridine (3.32 g, 1.0 eq.) were added and the solution was stirred at reflux (80 °C) for 16 h. The reaction mixture was filtered through a silica plug and the solvent was removed under reduced pressure. The crude product was taken up in dichloromethane (50 ml), filtered and the solvent was removed under reduced pressure to give the desired 5-(2,2'-bipyridin-5-ylmethoxy) picolinaldehyde, a pale brown solid.

Yield 3.67 g, 79%.
$^1$H NMR (400 MHz, 298 K, CDCl$_3$) δ$_H$ 9.94 (1H, s, CHO), 8.69 (1H, d, $^3$J$_{HH}$ = 2.0 Hz), 8.62 (1H, d, $^3$J$_{HH}$ = 4.5 Hz), 8.47 (1H, d, $^3$J$_{HH}$ = 2.5 Hz), 8.40 (1H, $^3$J$_{HH}$ = 8.0 Hz), 8.34 (1H, d, $^3$J$_{HH}$ = 8.0 Hz), 7.91 (1H, d, $^3$J$_{HH}$ = 8.5 Hz), 7.84 (1H, dd, $^3$J$_{HH}$ = 8.5 Hz, $^4$J$_{HH}$ = 2.5 Hz), 7.77 (1H, td, $^3$J$_{HH}$ = 8.0 Hz, $^4$J$_{HH}$ = 2.0 Hz), 7.34 (1H, dd, $^3$J$_{HH}$ = 8.0 Hz, $^4$J$_{HH}$ = 3.0 Hz), 7.27 (1H, m, Py), 5.21 (2H, s, CH$_2$).

$^{13}$C ($^1$H) NMR (75 MHz, 298 K, CDCl$_3$) δ$_C$ 192.1 (CHO), 149.4, 148.6, 139.0, 138.1, 137.2, 1366, 131.9, 130.9, 124.3, 124.2, 123.5, 122.7, 121.4, 121.2 (Py), 68.1 (CH$_2$).


IR $\nu$ cm$^{-1}$ 2811 w, 1701 s, 1567 s, 1455 m, 1204 s, 788 s, 736 s, 590 s.

Elemental Analysis Found (calculated for C$_{17}$H$_{13}$N$_3$O$_2$) % C 69.82 (70.09), H 4.66 (4.50), N 14.01 (14.42).

**General procedure for the synthesis of complexes HHT-[Zn$_2$L$_n$$^a$][ClO$_4$]$_4$ (n = 2a-c, 3a-e).**

Zn(ClO$_4$)$_2$$\cdot$6H$_2$O (2 eq.) was add to a stirred solution of the either the desired $R$-chiral amine (3 eq.) and 5-(2,2’-bipyridin-5-ylmethoxy)picolinaldehyde (3 eq.) or the desired substituted aldehyde (3 eq.) and (R)-2-(2,2’-bipyridin-5-ylmethoxy)-1-phenylethanamine (3 eq.) in acetonitrile (20 ml) at ambient temperature and stirred for 18 h. The resulting pale yellow solution yielded the desired product as a white or yellow crystalline solid on the addition of ethyl acetate.

$R$,$\Lambda$$_{Zn}$,HHT-[Zn$_2$L$_3$$^a$][ClO$_4$]$_4$$\cdot$4H$_2$O

Yield 0.11 g, 43%.

$^1$H NMR (400 MHz, 298 K, CD$_3$CN) δ$_H$ 8.99 (1H, s), 8.91 (1H, s, CHN), 8.51-8.44 (4H, m), 8.33 (1H, t, $^3$J$_{HH}$ = 7.0 Hz) 8.22-8.19 (2H, m, Ar), 8.16-8.03 (7H, CHN / Ar), 7.97 (2H, d,
\[ ^3J_{HH} = 9.0 \text{ Hz}, \] 7.91-7.86 (2H, m), 7.84-7.76 (3H, m), 7.71-7.67 (3H, m), 7.60-7.55 (3H, m), 7.50 (1H, t, \[ ^3J_{HH} = 6.0 \text{ Hz} \]), 7.20 (1H, t, \[ ^3J_{HH} = 7.0 \text{ Hz} \]), 7.06 (3H, t, \[ ^3J_{HH} = 7.5 \text{ Hz} \]), 6.97 (1H, t, \[ ^3J_{HH} = 7.5 \text{ Hz} \]), 6.87 (2H, t, \[ ^3J_{HH} = 6.0 \text{ Hz} \]), 6.78 (2H, d, \[ ^3J_{HH} = 7.5 \text{ Hz} \]), 6.69 (2H, t, \[ ^3J_{HH} = 7.5 \text{ Hz} \]), 6.57-6.53 (3H, m), 6.20 (4H, q, \[ ^3J_{HH} = 7.5 \text{ Hz, Ar} \]), 5.68 (1H, q, \[ ^3J_{HH} = 7.5 \text{ Hz, CH,} \)), 5.19-5.06 (7H, m, CH / CH\2), 5.01 (1H, d, \[ ^3J_{HH} = 9.5 \text{ Hz, CH\2} \]), 1.81 (3H, d, \[ ^3J_{HH} = 6.5 \text{ Hz} \]), 1.65 (3H, d, \[ ^3J_{HH} = 6.5 \text{ Hz, CH\3} \]), 1.45 (3H, d, \[ ^3J_{HH} = 6.5 \text{ Hz, CH\3} \]).

\[^{13}C\{^1H\} \text{ NMR (126 MHz, 298 K, CD\3CN)} \delta_C 162.0, 161.3, 160.2 (CHN), 159.6, 159.6, 159.0, 150.1, 149.7, 149.3, 149.2, 149.1, 149.0, 149.0, 149.0, 148.3, 148.0, 143.3, 142.3, 142.0, 142.0, 141.9, 141.8, 141.3, 140.6, 140.5, 140.4, 139.2, 139.1, 138.3, 135.7, 135.0, 134.7, 132.3, 132.1, 132.0, 129.2, 129.0, 128.8, 128.7, 128.5, 128.3, 128.0, 127.8, 127.4, 126.1, 125.7, 125.6, 124.45, 124.3, 124.0, 123.9, 123.87, 123.7, 123.6, 123.4 (Ar), 68.6, 68.5, 68.5 (CH), 65.2, 64.8, 64.5 (CH\2), 22.3, 22.3, 21.7 (CH\3).

MS (ESI) m/z 324 [Zn\textsubscript{2}L\textsubscript{3}]\textsuperscript{4+}

IR ν cm\textsuperscript{-1} 3120 w, 1571 m, 1224 w, 1083 s, 791 w, 702 w, 621 m, 412 w.

Elemental Analysis found (Calculated for C\textsubscript{73}H\textsubscript{66}Cl\textsubscript{4}N\textsubscript{12}O\textsubscript{19}Zn\textsubscript{2}.4H\textsubscript{2}O) % C 49.39 (50.49), H 3.64 (4.18), N 9.18 (9.42).

\[ R_{c,A,Zn,HHT-\left[Zn\textsubscript{2}L\textsubscript{2b}\right][ClO\textsubscript{4}]_{4.4H\textsubscript{2}O} \]

Yield 0.09 g, 40%.

\[^1H \text{ NMR (400 MHz, 298 K, CD\3CN)} \delta_H 9.01 (1H, s), 8.92 (1H, s, CHN), 8.53 (3H, t, \[ ^3J_{HH} = 8.5 \text{ Hz} \]), 8.47 (1H, d, \[ ^3J_{HH} = 5.0 \text{ Hz} \]), 8.37 (1H, t, \[ ^3J_{HH} = 8.5 \text{ Hz} \]), 8.26 (1H, d, \[ ^3J_{HH} = 7.5 \text{ Hz, Ar} \]), 8.22-8.00 (12H, m, Ar/CHN), 7.92 (1H, m), 7.87-7.80 (3H, m), 7.76-7.67 (4H, m), 7.60 (1H, s), 7.55 (1H, m), 7.34 (1H, d, \[ ^3J_{HH} = 7.5 \text{ Hz} \]), 7.06 (1H, d, \[ ^4J_{HH} = 3.5 \text{ Hz} \]), 6.98 (1H, d,
$^3J_{HH} = 7.5 \text{ Hz}$, $6.87 \ (1 \text{H, d, } ^4J_{HH} = 3.5 \text{ Hz})$, $6.71 \ (2 \text{H, d, } ^3J_{HH} = 7.5 \text{ Hz})$, $6.62 \ (1 \text{H, d, } ^3J_{HH} = 8.5 \text{ Hz})$, $6.54 \ (1 \text{H, d, } ^4J_{HH} = 3.5 \text{ Hz})$, $6.27-6.25 \ (2 \text{H, m})$, $6.20-6.10 \ (5 \text{H, m, Ar})$, $5.63 \ (1 \text{H, q, } ^3J_{HH} = 6.5 \text{ Hz, CH})$, $5.26 \ (1 \text{H, d, } ^3J_{HH} = 10.0 \text{ Hz, CH}_2)$, $5.19-5.16 \ (4 \text{H, m, CH}_2/\text{CH})$, $5.11 \ (2 \text{H, d, } ^3J_{HH} = 10.0 \text{ Hz})$, $5.06 \ (1 \text{H, d, } ^3J_{HH} = 10.0 \text{ Hz, CH}_2)$, $3.85 \ (3 \text{H, s})$, $3.77 \ (3 \text{H, s})$, $3.68 \ (3 \text{H, s})$, $1.81 \ (3 \text{H, d, } ^3J_{HH} = 6.5 \text{ Hz})$, $1.69 \ (3 \text{H, d, } ^3J_{HH} = 6.5 \text{ Hz})$, $1.51 \ (3 \text{H, d, } ^3J_{HH} = 6.5 \text{ Hz, CH}_3)$.

$^{13}C\{^1H\} \text{ NMR (126 MHz, 298 K, CD}_3\text{CN)} \delta_C \ 162.1, 161.6, 160.6 \ (\text{CHN}), \ 160.3, 159.8, 159.7, 159.4, 150.8, 150.4, 150.2, 150.0, 149.3, 149.0, 148.8, 144.0, 143.1, 142.7, 142.6, 141.3, 141.0, 139.9, 139.0, 136.6, 135.9, 135.5, 133.8, 133.2, 133.0, 132.9, 132.8, 132.4, 131.3, 129.8, 129.7, 129.3, 128.8, 128.5, 128.2, 127.8, 125.0, 125.0, 124.6, 124.4, 124.3, 124.1, 124.0, 118.5, 115.5, 115.1, 115.0, 114.7 \ (\text{Ar})$, $69.4, 69.2(\text{CH}_2)$, $65.4 \ (\text{CH})$, $65.2 \ (\text{CH}_2)$, $65.05, 64.7 \ (\text{CH})$, $56.3, 56.2, 56.1 \ (\text{OCH}_3)$, $23.0, 23.0, 22.4 \ (\text{CH}_3)$.

MS (ESI) m/z 425 [L+H$^+$], 501 [Zn$_2$L$_3$][ClO$_4$]$^{3+}$, 801 [Zn$_3$L$_3$][ClO$_4$]$^{2+}$

IR v cm$^{-1}$ 2900.7 br, w, 1573 w, 1513 m, 1316 w, 1249 w, 1091 s, 835 w, 622 m, 416 w.

Elemental Analysis Found (Calculated for C$_{78}$H$_{72}$Cl$_4$N$_{12}$O$_{22}$Zn$_2$.4H$_2$O) % C 49.23 (49.99), H 3.73 (4.03) N 8.36 (8.97).

$R_{cm, Zn, HHT-}[Zn$_2$L$_{2e}$][ClO$_4$]$_4$.4H$_2$O

Yield 0.13 g, 47%.

$^1H \text{ NMR (500 MHz, 298 K, CD}_3\text{CN)} \delta_H \ 9.07 \ (1 \text{H, s})$, $8.98 \ (1 \text{H, s, CHN})$, $8.53-8.47 \ (4 \text{H, m})$, $8.35 \ (1 \text{H, t, } ^3J_{HH} = 6.5 \text{ Hz})$, $8.22 \ (3 \text{H, d, } ^3J_{HH} = 8.0 \text{ Hz})$, $8.16 \ (4 \text{H, t, } ^3J_{HH} = 8.5 \text{ Hz})$, $8.12 \ (2 \text{H, t, } ^3J_{HH} = 7.5 \text{ Hz})$, $8.06 \ (2 \text{H, d, } ^3J_{HH} = 8.0 \text{ Hz})$, $7.98-7.96 \ (3 \text{H, m})$, $7.92-7.89 \ (4 \text{H, m})$, $7.86-7.76 \ (4 \text{H, m})$, $7.70 \ (1 \text{H, d, } ^3J_{HH} = 5.5 \text{ Hz})$, $7.66 \ (2 \text{H, d, } ^3J_{HH} = 9.0 \text{ Hz})$, $7.55-7.51 \ (4 \text{H, m})$, $7.37 \ (2 \text{H, d, } ^3J_{HH} = 8.5 \text{ Hz})$, $7.07-7.03 \ (2 \text{H, m})$, $6.90 \ (1 \text{H, s})$, $6.58 \ (1 \text{H, s})$, $6.52 \ (3 \text{H, dd, } ^3J_{HH} = 8.0 \text{ Hz, } ^4J_{HH} = 4.0 \text{ Hz, Ar})$, $5.85 \ (1 \text{H, q, } ^3J_{HH} = 6.0 \text{ Hz, CH})$, $5.39-5.34 \ (2 \text{H, m})$, $5.30-5.25 \ (2 \text{H, m, CH}_2)$, $5.20 \ (1 \text{H, d, } ^3J_{HH} = 9.0 \text{ Hz, CH})$, $5.11 \ (2 \text{H, t, } ^3J_{HH} = 10.0 \text{ Hz, CH}_2)$, $5.04 \ (1 \text{H, CH}),$
d, $^3J_{HH} =$ 9.0 Hz), 1.87 (3H, t, $^3J_{HH} =$ 6.5 Hz), 1.73 (3H, t, $^3J_{HH} =$ 6.5 Hz), 1.47 (3H, t, $^3J_{HH} =$ 6.5 Hz, CH₃).

$^{13}$C$\text{^{1}H}$ NMR (126 MHz, 298 K, CD₃CN) δC 164.1, 163.3, 162.4 (CHN), 160.8, 160.4, 159.9, 150.7, 150.5, 150.3, 150.1, 149.7, 149.4, 149.3, 149.2, 149.0, 148.8, 148.5, 148.4, 148.0, 147.7, 144.0, 143.0, 142.9, 142.7, 140.9, 140.8, 140.7, 130.0, 139.5, 136.8, 136.2, 135.3, 133.4, 133.3, 132.8, 129.2, 128.7, 127.9, 127.7, 127.6, 125.0, 125.0, 124.9, 124.5, 124.3, 124.2, 124.1, 124.1, 124.0, 118.3 (Ar), 69.3, 69.2, 69.1 (CH), 65.1, 64.6, 64.5 (CH₂), 22.6, 22.5, 21.9 (CH₃).

MS (ESI) m/z 361 [Zn₂L₃]$^{4+}$.

IR ν cm⁻¹: 3450 br, w, 2986 w, 1571 w, 1349 w, 1316 w, 1082 m, 622 m.

Elemental Analysis Found (Calculated for C$_{75}$H$_{63}$Cl$_{4}$N$_{15}$O$_{25}$Zn$_{2}$·4H$_{2}$O) % C 46.02 (46.94), H 3.22 (3.73), 10.55 (10.95).

$^{1}$H NMR (300 MHz, 298 K, CD₃CN) δH 9.34(1H, s), 9.32(1H, s, CHN), 9.22 (1H, s), 9.17 (1H, s, Ar), 9.12 (1H, s, CHN), 8.86-7.37 (31H, m), 7.16,-6.83 (7H, m), 6.68 (2H, t, $^3J_{HH} =$ 8.0 Hz), 6.53 (2H, t, $^3J_{HH} =$ 8.0 Hz), 6.07 (2H, d, $^3J_{HH} =$ 7.0 Hz, Ar), 5.47 (1H, dd, $^3J_{HH} =$ 11.5 Hz $^4J_{HH} =$ 3.5 Hz, CH), 5.22- 5.09 (3H, m, CH₂), 4.96 (1H, dd, $^3J_{HH} =$ 11.5 Hz $^4J_{HH} =$ 2.5 Hz) 4.85 (1H, dd, $^3J_{HH} =$ 10.5 Hz $^4J_{HH} =$ 3.0 Hz, CH), 4.56-4.41 (3H, m, CH₂), 4.29 (1H, t $^3J_{HH} =$ 11.0 Hz), 4.17 (1H, t, $^3J_{HH} =$ 10.5 Hz), 4.09 (1H, t, $^3J_{HH} =$ 11.0 Hz, CH₂), 3.60 (1H, dd, $^3J_{HH} =$ 10.5 $^4J_{HH} =$ 3.5 Hz), 3.52-3.42 (2H, m, CH₂).

$^{13}$C$\text{^{1}H}$ NMR (126 MHz, 298 K, CD₃CN) δC 164.7, 164.6, 163.9 (CHN), 151.3, 151.2, 150.4, 150.1, 149.9, 149.9, 149.6, 149.5, 149.5, 149.4, 149.1, 149.0, 148.9, 148.7, 148.6, 147.8, 147.8, 147.6, 142.0, 143.7, 143.6, 142.9, 142.8, 142.8, 142.6, 142.5, 142.8, 138.4,
138.0, 137.8, 135.3, 134.6, 134.1, 131.9, 131.5, 130.9, 130.8, 130.6, 130.1, 129.8, 129.7, 129.6, 129.5, 129.3, 128.3, 128.3, 128.1, 127.8, 127.1, 127.0, 124.6, 124.3, 124.2, 124.1, 123.6, 123.4 (Ar), 70.6, 70.5, 70.4, 70.1, 70.0, 69.8 (CH₂), 69.5, 69.4, 68.4 (CH).


IR ν cm⁻¹ 3066 w, 1728 w, 1647 w, 1599 m, 1570 w, 1495 w, 1475 m, 1440 m, 1406 w, 1317 w, 1246 w, 1078 s, 1015 m, 931 m, 841 w.

Elemental Analysis found (Calculated for C₇₅H₆₆Cl₄Zn₂N₁₂O₁₉.₄H₂O) % C 50.13 (52.62), H 3.80 (3.89), N 9.11 (9.81).

Crystallography

$R_C, Δ_{Zn}, HHT- [Zn₂L₃][ClO₄]₂$, $M = 1920.69$, monoclinic, space group C2 (no. 5), $a = 36.032(8)$ Å, $b = 13.162(3)$ Å, $c = 18.767(4)$ Å, $β = 99.699(7)$°, $V = 8773(3)$ Å³, $Z = 4$, $T = 100.15$ K, $μ(CuKα) = 2.478$ mm⁻¹, $Dcalc = 1.454$ g/mm³, 45035 reflections measured (4.776 ≤ 2θ ≤ 133.182), 15293 unique ($R_{int} = 0.0651$) which were used in all calculations. The final $R_1$ was 0.0733 (I > 2σ(I)) and $wR_2 = 0.2066$ (all data). GooF 1.050, Flack 0.045(8).

The asymmetric unit contains one ethyl acetate (C101-C105), one half occupied acetonitrile (N200-C202) and two half occupied methanol (N300-C301 and N00-C401) molecules. No hydrogens were located on the latter methanols but were included in the formula. The hydrogen atoms on the hydroxyls were placed at calculate positions. Perchlorates were located in five postions. ClO₄(Cl40) lies on the ‘c’ cell face and was modelled at half occupancy with a part-1 instruction. ClO₄(Cl50) was modelled at half occupancy. Many DFIX, DANG and SIMU restraints were used to give the partially occupied counterions, solvents and some atoms of the ligands chemically sensible bond lengths, angles and thermal parameters. The B alerts in from CifCheck and the reallively large R value for arise from the weak data for this large structure.
$RC_3\Delta Zn, HHT-\left[Zn_2L_3\right][\text{ClO}_4]_4\cdot 3\text{H}_2\text{O}$

Yield 0.70 g, 71%.

$^1$H NMR (400 MHz, 298 K, CD$_3$CN) $\delta$H 9.25 (1H, d, $^4$J$_{HH} = 1.5$ Hz, Ar), 9.23 (1H, s, HCN), 9.19 (1H, d, $^4$J$_{HH} = 1.5$ Hz, Py), 9.14 (1H, s), 8.77 (1H, s, HCN), 8.60-7.28 (31H, m) 7.22-6.95 (6H, m), 6.92 (1H, t, $^3$J$_{HH} = 8.0$ Hz), 6.72 (2H, t, $^3$J$_{HH} = 7.5$ Hz), 6.58 (2H, t, $^3$J$_{HH} = 8.0$ Hz), 6.12 (2H, d, $^3$J$_{HH} = 8.0$ Hz), 6.00 (2H, d, $^3$J$_{HH} = 8.0$ Hz, Ar), 5.50 (1H, dd, $^3$J$_{HH} = 11.5$ Hz, $^4$J$_{HH} = 3.5$ Hz, CH), 5.26-5.11 (3H, m, CH$_2$), 4.98 (1H, dd, $^3$J$_{HH} = 12.0$ Hz, $^4$J$_{HH} = 3.2$ Hz), 4.85 (1H, dd, $^3$J$_{HH} = 11.0$ Hz, $^4$J$_{HH} = 3.5$ Hz, CH), 4.60-4.44 (3H, m, CH$_2$), 4.31 (1H, t, $^3$J$_{HH} = 11.0$ Hz), 4.23 (1H, t, $^3$J$_{HH} = 11.0$ Hz), 4.05 (1H, t $^3$J$_{HH} = 11.0$ Hz), 3.66 (1H, dd, $^3$J$_{HH} = 10.5$ Hz, $^4$J$_{HH} = 3.5$ Hz), 3.56 (1H, dd, $^3$J$_{HH} = 11.0$ Hz, $^4$J$_{HH} = 3.5$ Hz), 3.49 (1H, dd, $^3$J$_{HH} = 11.0$ Hz, $^4$J$_{HH} = 3.5$ Hz, CH$_2$) 2.39 (3H, bs, OH).

$^{13}$C{$^1$H} NMR (126 MHz, CD$_3$CN) $\delta$C 163.6, 163.4, 162.8 (CHN), 159.6, 159.2, 158.8, 151.3, 151.1, 150.3, 150.1, 140.0, 149.9, 149.5, 149.1, 149.0, 149.0, 148.7, 148.6, 143.9, 143.6, 143.6, 142.8, 142.4, 142.2, 140.3, 140.2, 138.7, 138.7, 138.5, 138.3, 138.0, 137.7, 135.9, 135.0, 134.6, 133.1, 132.8, 132.0, 129.7, 129.5, 129.5, 129.4, 128.3, 128.2, 128.0, 127.8, 127.1, 127.0, 126.7, 124.6, 124.3, 124.2, 124.1, 123.6, 123.4 (Ar), 70.6, 70.6, 70.0 (CH$_2$), 69.9 (CH), 69.8(CH$_2$), 69.7 (CH), 69.6, 69.4(CH$_2$), 67.9 (CH).

MS (ESI) m/z 780 [Zn$_2$L$_3$][ClO$_4$]$_2$.$^2$.$^+$.  

IR v cm$^{-1}$ 3166 br, 1644 w, 1592 w, 1572 m, 1495 w, 1475 m, 1439 m, 1405 w, 1317 w, 1280 w, 1248 w, 1212 w, 1076 s, 1040 s, 929 m, 840 m.

Elemental Analysis found (Calculated for C$_{75}$H$_{66}$Cl$_4$Zn$_2$N$_{12}$O$_{22}$$\cdot$3H$_2$O) % C 49.37 (49.66), H 3.78 (4.00), N 8.83 (9.27).
**$R_{C,\Delta Zn, HHT-}\left[Zn_2L^3\right][ClO_4]_4.4H_2O$**

Yield 0.81 g, 81%.

$^1$H NMR (400 MHz, 298 K, CD$_3$CN) $\delta$H 9.26 (1H, s), 9.25 (1H, s CHN), 9.19 (1H, s), 9.17 (1H, s, Ar), 8.81 (1H, s, CHN), 8.57-7.32 (31H, m), 7.19 (1H, t, $^3$J$_{HH} = 7.0$ Hz), 7.15-7.07 (3H, m), 7.03 (1H, t, $^3$J$_{HH} = 7.5$ Hz), 6.99 (2H, d, $^3$J$_{HH} = 7.5$ Hz) 6.92 (1H, t, $^3$J$_{HH} = 7.5$ Hz), 6.72 (2H, t, $^3$J$_{HH} = 7.5$ Hz), 6.58 (2H, t, 8.0 Hz), 6.12 (2H, d, $^3$J$_{HH} = 7.5$ Hz), 6.00 (2H, d, $^3$J$_{HH} = 7.5$ Hz, Ar), 5.49 (1H, dd, $^3$J$_{HH} = 11.5$ Hz $^4$J$_{HH} = 3.5$ Hz, CH), 5.28-5.12 (3H, m, CH$_2$), 4.98 (1H, dd, $^3$J$_{HH} = 11.5$ Hz $^4$J$_{HH} = 2.5$ Hz), 4.84 (1H, dd, $^3$J$_{HH} = 10.5$ Hz $^4$J$_{HH} = 3.0$ Hz, CH), 4.59-4.46(3H, m, CH$_2$), 4.32 (1H, t, $^3$J$_{HH} = 11.0$ Hz), 4.19 (1H, t, $^3$J$_{HH} = 11.0$ Hz), 4.13 (1H, t, $^3$J$_{HH} = 11.1$ Hz, CH$_2$), 3.92 (3H, s), 3.89 (3H, s), 3.79 (3H, s, OCH$_3$), 3.67 (1H, dd, $^3$J$_{HH} = 10.0$ Hz $^4$J$_{HH} = 4.0$ Hz), 3.55 (1H, dd, $^3$J$_{HH} = 11.0$ Hz $^4$J$_{HH} = 3.0$ Hz), 3.49 (1H, dd, $^3$J$_{HH} = 11.0$ Hz, $^4$J$_{HH} = 4.0$ Hz, CH$_2$).

$^{13}$C {$^1$H} NMR (126 MHz, 298 K, CD$_3$CN) $\delta$C 163.8, 163.5, 163.0 (CHN), 161.5, 161.2, 160.8, 151.3, 151.1, 150.3, 150.1, 140.0, 149.9, 149.5, 149.1, 149.1, 149.0, 148.8, 148.6, 143.9, 143.6, 143.6, 142.8, 142.4, 142.2, 140.5, 140.5, 139.4, 139.3, 139.1, 138.4, 138.0, 137.7, 135.9, 134.9, 134.5, 133.1, 132.8, 132.1, 129.8, 129.7, 129.6, 129.5, 129.5, 129.4, 128.3, 128.2, 128.0, 127.9, 127.1, 127.0, 124.6, 124.3, 124.2, 124.2, 124.1, 123.8, 123.6, 123.5 (Ar), 70.6, 70.6, 70.00 (CH$_3$), 70.00 (CH), 69.9 (CH$_2$), 69.8 (CH), 69.6, 69.5 (CH$_2$), 66.0 (CH), 57.6, 57.5, 57.3 (CH$_3$).


IR $\nu$ cm$^{-1}$ 3251 br, 1643 w, 1601 w, 1571 m, 1495 w, 1475 w, 1440 w, 1405 w, 1316 m, 1280 w, 1225 w, 1176 w 1075 s, 1014 s, 932 m, 843 m.
Elemental Analysis found (Calculated for C\textsubscript{78}H\textsubscript{72}Cl\textsubscript{4}Zn\textsubscript{2}N\textsubscript{12}O\textsubscript{22}·4H\textsubscript{2}O) % C 49.66 (49.94), H 3.93 (4.30), N 8.70 (8.97).

\( R_{C,\Delta Zn,HHT} - [Zn\textsubscript{2}L\textsubscript{3}]^{3+}[ClO\textsubscript{4}]_{4}4\text{H}_2\text{O} \)

Yield 0.83 g, 73 %.

\(^{1}\)H NMR (500 MHz, 298 K, CD\textsubscript{3}CN) \( \delta \)H 9.22 (1H, s, HCN), 9.18 (1H, d, \(^4\)J\textsubscript{HH} = 1.5 Hz), 9.10 (1H, d, \(^4\)J\textsubscript{HH} = 1.5 Hz, Ar), 9.07 (1H, s), 8.73 (1H, s, HCN), 8.49-8.43 (2H, m), 8.33 (1H, d, \(^4\)J\textsubscript{HH} = 1.5 Hz), 8.33-7.17 (53H, m), 6.68 (2H, t, \(^3\)J\textsubscript{HH} = 7.5 Hz), 6.54 (2H, t, \(^3\)J\textsubscript{HH} = 7.5 Hz), 6.07 (2H, d, \(^3\)J\textsubscript{HH} = 7.5 Hz), 5.93 (2H, d, \(^3\)J\textsubscript{HH} = 7.5 Hz, Ar), 5.40 (1H, dd, \(^3\)J\textsubscript{HH} = 10.5 Hz, \(^4\)J\textsubscript{HH} = 3.5 Hz, CH), 5.24-5.09 (9H, m, CH\textsubscript{2}), 4.91 (1H, dd, \(^3\)J\textsubscript{HH} = 11.0 Hz, \(^4\)J\textsubscript{HH} = 3.0 Hz), 4.77 (1H, dd, \(^3\)J\textsubscript{HH} = 10.0 \(^4\)J\textsubscript{HH} = 4.0 Hz, CH), 4.55-4.44 (3H, m), 4.29 (1H, t, \(^3\)J\textsubscript{HH} = 11.0 Hz), 4.15 (1H, t, \(^3\)J\textsubscript{HH} = 11.0 Hz), 4.07 (1H, t, \(^3\)J\textsubscript{HH} = 11.0 Hz), 3.56 (1H, dd, \(^3\)J\textsubscript{HH} = 10.4 \(^4\)J\textsubscript{HH} = 3.5 Hz), 3.49 (1H, dd, \(^3\)J\textsubscript{HH} = 11.5 Hz, \(^4\)J\textsubscript{HH} = 3.0 Hz), 3.43 (1H, dd, \(^3\)J\textsubscript{HH} = 11.0 Hz, \(^4\)J\textsubscript{HH} = 3.5 Hz, CH\textsubscript{2}).

\(^{13}\)C\textsubscript{\textsuperscript{1}\textsubscript{H}} NMR (126 MHz, 298 K, CD\textsubscript{3}CN) \( \delta \)C 163.7, 163.5, 162.9 (CHN), 160.3, 160.0, 159.5, 159.0, 151.3, 151.0, 150.3, 150.0, 149.9, 149.8, 149.4, 149.1, 149.0, 149.0, 148.7, 148.5, 143.9, 143.6, 143.5, 142.8, 142.4, 142.2, 140.7, 140.7, 140.6, 140.4, 139.2, 139.1, 138.6, 138.3, 138.0, 137.7, 136.3, 136.1, 136.1, 135.7, 134.9, 134.5, 133.0, 132. 8, 131.9, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.4, 129.0, 128.7, 128.7, 128.7, 128.3, 128.2, 128.0, 127.7, 127.5, 127.7, 127.0, 126.4, 125.9, 125.4, 124.7, 124.4, 124.2, 124.2, 123.6, 123.5 (Ar), 71.9, 71.8, 71.7, 70.6, 70.5 (CH\textsubscript{2}), 70.0 (CH), 69.9, 69.8 (CH\textsubscript{2}), 69.8 (CH), 69.5, 69.4 (CH\textsubscript{2}), 68.0 (CH).

MS (ESI) m/z 408 [Zn\textsubscript{2}L\textsubscript{3}]^{4+}, 577 [Zn\textsubscript{2}L\textsubscript{3}][ClO\textsubscript{4}]^{3+}, 915 [Zn\textsubscript{2}L\textsubscript{3}][ClO\textsubscript{4}]^{2+}. 
IR ν cm⁻¹ 3510 br, 3059 br, 2933 w, 2873 w, 1605 m, 1517 w, 1496 w, 1468 m, 1442 m, 1419 w, 1408 w, 1364 w, 1319 w, 1176 m, 1070 s, 959 w, 939 w, 932 m.

Elemental Analysis found (Calculated for C₉₆H₈₄Cl₄Zn₂N₂₁₂O₂₂·₄H₂O) % C 54.74 (54.84), H 3.99 (4.41), N 7.89 (7.99).

**R₉₅ΔZn,HHT-[Zn₂L₃e][ClO₄]₄·₄H₂O**

Yield 0.68 g, 71 %.

¹H NMR (500 MHz, 298 K, CD₃CN) δH 9.51 (1H, s), 9.44 (2H, s, Ar), 9.38 (1H, s, CHN), 9.15 (1H, s), 9.10, (1H, s, Ar), 9.03 (1H, s, CHN), 8.9 4(1H, d, 3JHH = 3.0 Hz), 8.84 (2H, t, 3JHH = 3.0 Hz, Ar), 8.70 (1H, s, CHN), 8.54 (1H, d, 3JHH = 8.0 Hz), 8.49 (1H, d, 3JHH = 8.5 Hz), 8.40 (1H, s), 8.30-7.76 (17H, m), 7.58-7.48 (5H, m), 7.19 (1H, t, 3JHH = 7.5 Hz), 7.09 (2H, t, 3JHH = 7.5 Hz), 7.02 (1H, t, 3JHH = 7.5 Hz), 6.98 (2H, d, 3JHH = 7.5 Hz), 6.91 (1H, t, 3JHH = 7.5 Hz), 6.72 (2H, t, 3JHH = 7.5 Hz), 6.58 (2H, t, 3JHH = 7.5 Hz), 6.12 (2H, d, 3JHH = 7.5 Hz), 6.00 (2H, d, 3JHH = 7.5 Hz, Ar), 5.49 (1H, dd, 3JHH = 11.0 Hz, 4JHH = 3.0 Hz, CH), 5.27-5.15 (3H, m, CH₂), 5.01 (1H, dd, 3JHH = 11.0 Hz, 4JHH = 3.0 Hz), 4.89 (1H, dd, 3JHH = 11.0 Hz, 4JHH = 3.0 Hz, CH), 4.60-4.49 (3H, m, CH₂), 4.33 (1H, t, 3JHH = 11.5 Hz), 4.21 (1H, t, 3JHH = 11.5 Hz), 4.14 (1H, t, 3JHH = 11.5 Hz), 3.68 (1H, dd, 3JHH = 11.0 Hz, 4JHH = 3.0 Hz), 3.56 (1H, dd, 3JHH = 11.0 Hz, 4JHH = 3.0 Hz, CH₂).

¹³C{¹H} NMR (126 MHz, 298 K, CD₃CN) δC 163.7, 163.0, 162.6 (CHN), 152.7, 152.6, 152.1, 151.9, 151.7, 151.3, 150.3, 150.9, 150.4, 150.00, 149.8, 149.3, 149.2, 149.1, 149.0, 148.4, 144.3, 144.0, 143.8, 143.7, 143.5, 143.4, 143.0, 142.7, 142.1, 141.9, 141.7, 138.5, 138.1, 138.1, 134.9, 133.9, 133.8, 130.2, 130.0, 130.0, 129.9, 129.9, 129.8, 129.6, 128.6, 128.4, 128.3, 128.2, 127.9, 127.2, 127.1, 124.6, 124.5, 124.3, 124.2, 123.8, 123.7 (Ar), 71.1, 70.7 (CH), 70.6, 69.8, 69.7, 69.5, 69.5 (CH₂), 69.1 (CH).
MS (ESI) m/z 395 [Zn₃L₃]^{4+}, 471 [Zn₃L₃][ClO₄]^{2+}.

IR ν cm⁻¹ 3521 br, 1644 w, 1603 w, 1495 w, 1475 m, 1441 m, 1406 w, 1318 w, 1249 w, 1177 w, 1078 s, 931 m, 844 m.

Elemental Analysis found (Calculated for C₇₂H₆₃Cl₄Zn₂N₁₅O₁₉·4H₂O) % C 48.26 (48.39), H 3.66 (4.00), N 11.65 (11.76).

**General synthesis of HHT-[Fe₂Lⁿ]Cl₄ (where n =2a-c, 3a-c).**

Anhydrous iron(II) chloride (2 eq.) was added to a stirred solution of the either the desired $R_C$-chiral amine (3 eq.) and 5-(2,2'-bipyridin-5-ylmethoxy)picolinaldehyde (3 eq.) or the desired substituted aldehyde (3 eq.) and (R)-2-(2,2'-bipyridin-5-ylmethoxy)-1-phenylethanamine (3 eq.) in methanol (20 ml) at ambient temperature to give a purple solution that was then heated to reflux (65°C) for 24 – 48 h. The reaction was allowed to cool to ambient temperature, filtered through a celite plug and the solvents were removed *in vacuo* to give the desired dark purple solid. Note that the presence of water of crystallisation in these compounds was confirmed by NMR and IR spectroscopy and the absence of other solvents was confirmed also by NMR. The hydration number was then determined by thermogravimetric analysis (page 28) and the relevant mass loss was correlated with microanalytical data.

**$R_C$,ΛFe₆,HHT-[Fe₂L²a]Cl₄·11H₂O**

Yield 0.31 g, 96%.

$^{13}$C {¹H} NMR (126 MHz, 298 K, MeOH) δC 171.1, 170.8, 170.3 (CHN), 161.1, 160.9, 160.8, 160.5, 160.3, 160.0, 159.4, 158.7, 158.4, 157.2, 156.6, 155.1, 153.9, 153.8, 153.7, 142.1, 141.4, 140.8, 140.7, 140.5, 140.5, 139.7, 139.75, 139.6, 136.7, 136.6, 136.6, 133.5, 132.7,
132.5, 130.3, 130.2, 129.9, 129.8, 129.6, 129.1, 128.9, 128.7, 128.6, 128.5, 127.6, 126.1, 126.0, 125.3, 125.0, 124.9, 124.7 (Ar), 70.5, 70.5, 69.6, 69.6, 69.5, 69.3 (CH/CH₂), 26.4, 25.6, 25.2 (CH₃).

MS (ESI) m/z 323 [Fe₂L₃]⁺.

IR ν cm⁻¹ 3358 br, s, 3001 w, 1555 m, 1467 w, 1233 m, 759 w, 701 m.

Elemental Analysis Found (calculated for C₇₅H₆₆Cl₄Fe₂N₁₂O₃.11H₂O) % C 54.70 (55.09), H 4.76 (5.42), N 9.67 (10.28).

$S_{c,3\Delta}{\text{Fe,HHT-[Fe}_{2}L^{2a}_{3}]}\text{Cl}_{4.11}\text{H}_{2}\text{O}$

Data as for R-enantiomer

Yield 0.32 g, 96%.

Elemental Analysis Found (calculated for C₇₅H₆₆Cl₄Fe₂N₁₂O₃.11H₂O) % C 55.02 (55.09), H 4.99 (5.42), N 10.04 (10.28).

$R_{c,\Lambda}{\text{Fe,HHT-[Fe}_{2}L^{2b}_{3}]}\text{Cl}_{4.14}\text{H}_{2}\text{O}$

Yield 0.33 g, 94%.

$^{13}$C{¹H} NMR (126 MHz, 298 K, MeOH) δC 170.6, 170.9, 169.7 (CHN), 161.8, 161.1, 161.0, 160.9, 160.6, 160.5, 160.4, 160.2, 160.0, 159.4, 158.6, 158.3, 157.2, 156.6, 155.0, 154.0, 153.9, 153.8, 140.5, 139.7, 139.6, 136.7, 136.6, 133.3, 133.1, 132.6, 132.5, 131.3, 129.8,
129.1, 129.0, 128.5, 127.4, 127.4, 125.9, 125.3, 125.0, 124.9, 124.5, 115.6, 115.4, 115.2, 114.9 (Ar), 70.5, 70.0, 69.6, 69.5, 69.0, 68.7 (CH/CH2), 56.0, 55.9, 55.9, (OCH3), 26.3, 25.5, 25.1 (CH3).

MS (ESI) m/z 346 [Fe2L3]4+

IR ν cm⁻¹ 3359 br s, 2968 m, 1607 w, 1510 m, 1301 m, 1235 s, 1023 m, 834 m.

Elemental Analysis Found (calculated for C78H72Cl4Fe2N12O6.14H2O) % C 51.97 (52.65), H 5.33 (5.67), N 9.29 (9.45).

\( S_c\Delta Fe,HHT-[Fe_2L^{2b}_3]Cl_4.14H_2O \)

Data as for R-enantiomer

Yield 0.32 g, 96%.

Elemental Analysis Found (calculated for C78H72Cl4Fe2N12O6.14H2O) % C 52.83 (52.65), H 5.48 (5.67), N 9.43 (9.45).

\( R_c\Lambda Fe,HHT-[Fe_2L^{2c}_3]Cl_4.13H_2O \)

Yield 0.36 g, 99%.

MS (ESI) m/z 357 [Fe2L3]4+

IR ν cm⁻¹: 3352 br s, 3035 m, 1602 m, 1561 m, 1216 m, 1344 s, 1232 s, 1011 m, 853 m, 788 w, 753 w.
Elemental Analysis Found (calculated for $C_{75}H_{63}Cl_4Fe_2N_{15}O_9.13H_2O$) % C 48.88 (49.88), H 4.31 (4.97), N 10.65 (11.63).

$S_{c5}A_{Fe,HHT}$-$[Fe_2L_3^{c5}]Cl_4.13H_2O$

Data as for $R$-enantiomer

Yield 0.33 g, 0.021 mmol, 90%

Elemental Analysis Found (calculated for $C_{75}H_{63}Cl_4Fe_2N_{15}O_9.13H_2O$) % C 50.15 (49.88), H 4.35 (4.97), N 10.89 (11.63).

$R_{c5}A_{Fe,HHT}$-$[Fe_2L_3^{3a}]Cl_4.9H_2O$

Yield 0.68 g, 87%.

$^{13}$C ($^1$H) NMR (126 MHz, 298 K, MeOH) $\delta_C$ 174.7, 174.6, 174.0 (CHN), 161.0, 160.4, 160.3, 160.0, 159.9, 159.9, 159.5, 159.3, 158.9, 158.7, 156.4, 155.7, 155.4, 154.8, 154.4, 154.2, 161.0, 141.6, 141.4, 140.5, 140.0, 139.8, 138.8, 138.6, 138.3, 135.3, 133.8, 133.5, 132.7, 132.0, 131.4, 130.6, 130.3, 130.2, 130.1, 129.9, 129.7, 129.6, 129.2, 129.0, 128.9, 128.7, 127.0, 125.8, 125.1, 124.2, 123.6 (Ar), 74.5, 74.4, 72.6 (CH), 70.9, 70.8, 70.5, 69.9 (CH$_2$).

MS (ESI) m/z 323 $[Fe_2L_3]^{4+}$.

IR $\nu$ cm$^{-1}$ 3331 br, 3023 w, 1604 m, 1493 m, 1438 m, 1403 w, 1360 w, 1318 w, 1242 m, 1102 m, 1073 s, 1010 w, 936 m, 866 w, 836 w.

Elemental analysis found (calculated for $C_{75}H_{66}Cl_4Fe_2N_{12}O_3\cdot9H_2O$) % C 56.52 (56.33), H 5.31 (5.92), N 10.68 (10.51)
$S_c\Delta \{\text{Fe}, \text{HHT}-[\text{Fe}_2\text{L}^{3a}]\text{Cl}_4\cdot 9\text{H}_2\text{O}$

Data as for $R$-enantiomer

Yield 0.64 g, 82%.

MS (ESI) m/z 323.7 $[\text{Fe}_2\text{L}_3]^{4+}$.

Elemental analysis found (calculated for $C_{75}H_{66}Cl_4Fe_2N_{12}O_3\cdot 9\text{H}_2\text{O}$) % C 56.02 (56.33), H 5.22 (5.29), N 10.34 (10.51).

$R_c\Delta \{\text{Fe}, \text{HHT}-[\text{Fe}_2\text{L}^{3b}]\text{Cl}_4\cdot 9\text{H}_2\text{O}$

Yield 0.59 g 74%.

MS (ESI) m/z 335.7 $[\text{Fe}_2\text{L}_3]^{4+}$

IR $\nu$ cm$^{-1}$ 3313 br, 2922 br, 1552 s, 1490 m, 1466 s, 1439 s, 1403 w, 1362 w, 1283 s, 1221 s, 1108 m, 1074 s, 1026 m, 936 m, 837 m.

Elemental analysis found (calculated for $C_{75}H_{76}Cl_4Fe_2N_{12}O_{11}\cdot 9\text{H}_2\text{O}$) % C 54.88 (54.96), H 4.78 (5.14), N 10.22 (10.02).

$S_c\Delta \{\text{Fe}, \text{HHT}-[\text{Fe}_2\text{L}^{3b}]\text{Cl}_4\cdot 9\text{H}_2\text{O}$

Data as for $R$-enantiomer

Yield 0.64 g 80%.
$R_{c,\Delta Fe,HHT-[Fe_2L^{3c}]Cl_4}9H_2O$

Yield 0.64 g, 76%.

$^{13}$C\{$^1$H\} NMR (126 MHz, 298 K, MeOH) $\delta_C$ 172.5, 172.4, 171.8 (CHN), 161.2, 160.5, 160.4, 160.3, 160.2, 160.1, 160.0, 159.7, 159.1, 158.8, 156.4, 155.4, 155.0, 154.4, 152.8, 152.5, 152.5, 145.2, 144.6, 143.7, 141.4, 141.3, 141.1, 140.4, 140.0, 139.7, 138.8, 138.5, 138.3, 136.0, 134.3, 134.1, 133.4, 132.7, 132.3, 130.3, 130.2, 130.1, 130.0, 129.8, 128.9, 128.8, 128.5, 128.4, 126.9, 125.4, 125.0, 124.7, 124.0, 123.5, 122.4, 122.2, 122.1, 120.0 (Ar), 73.8, 73.7, 71.7 (CH), 70.6, 70.5, 70.3, 70.2, 70.0, 69.8 (CH$_2$), 57.1, 57.0 (CH$_3$).

MS (ESI) m/z 346 [Fe$_2$L$_3$]$^{4+}$.

IR $\nu$ cm$^{-1}$ 3379 br, 2932 br, 1591 m, 1557 s, 1493 m 1466 m, 1435 m, 1439 m, 1403 m, 1363 m, 1304 m, 1277 m, 1237 s, 1185 w, 1136 w, 1110 m, 1074 s, 1008 m, 962 w, 937 m, 824 m.

Elemental analysis found (calculated for C$_{78}$H$_{72}$Cl$_4$Fe$_2$N$_{12}$O$_6$·9H$_2$O) % C 55.52 (55.46), H 5.19 (5.37), N 10.17 (9.95).

$S_{c,\Lambda Fe,HHT-[Fe_2L^{3c}]Cl_4}8H_2O$

Data as for $R$-enantiomer

Yield 0.67 g, 80%.

Elemental analysis found (calculated for C$_{78}$H$_{72}$Cl$_4$Fe$_2$N$_{12}$O$_6$·8H$_2$O) % C 55.7 (55.46), H 5.24 (5.37), N 9.92 (9.95).
**Rc,ΔFe,HHT-[Fe2L3d]Cl4.7H2O**

Yield 0.71 g, 74%.

$^{13}$C{$^{1}$H} NMR (126 MHz, 298 K, MeOH) δC 172.6, 172.5, 171.7 (CHN), 161.1, 160.3, 159.9, 159.6, 159.4, 159.2, 159.0, 158.8, 158.6, 156.3, 155.3, 154.8, 154.2, 153.1, 152.9, 152.7, 144.0, 143.8, 143.4, 141.3, 141.1, 140.3, 139.9, 139.7, 138.8, 138.5, 138.2, 136.4, 136.1, 136.0, 135.9, 134.2, 134.0, 133.2, 132.7, 132.0, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 128.8, 128.7, 128.6, 128.5, 126.8, 125.5, 125.0, 124.8, 124.7, 124.6, 124.3, 124.0, 123.5 (Ar), 73.8, 73.7 (CH), 72.2, 72.0, 71.9 (CH$_2$), 71.7(CH), 70.6, 70.5, 70.3, 69.9, 69.7 (CH$_2$).

Ms (ESI) m/z 403 [Fe$_2$L$_3$]$^{4+}$

IR $\nu$ cm$^{-1}$ 3294 br, 3027 br, 2932 br, 1591 m 1556 s, 1599 m, 1467 m, 1402 w, 1372 w, 1275 m, 1229 s, 1109 w, 1075 s, 1003 m, 938 w, 842 m.

Elemental analysis found (calculated for C$_{96}$H$_{84}$Cl$_4$Fe$_2$N$_{12}$O$_6$·7H$_2$O) % C 61.79 (61.29), H 5.04 (5.25), N 9.02 (8.93).

**S$_c$A$_{Fe,HHT}$-[Fe$_2$L$_3$]$^{3d}$Cl$_4$.7H$_2$O**

Data as for $R$-enantiomer

Yield 0.73 g, 76%.

MS (ESI) m/z 403.3 [Fe$_2$L$_3$]$^{4+}$.

Elemental analysis found (calculated for C$_{96}$H$_{84}$Cl$_4$Fe$_2$N$_{12}$O$_6$·7H$_2$O) % C 61.18 (61.29), H 5.17 (5.25), N 9.02 (8.93).
Supplementary Information

$R_{c_{5}}\Delta_{Fe,HHT}\text{-}[Fe_{2}L^{3e}]Cl_{4}\cdot10H_{2}O$

Yield 0.6 g, 75 %

$^{13}C\{^1H\}$ NMR (126 MHz, 298 K, MeOH) $\delta_{C}$ 173.8, 173.3, 172.5 (CHN), 160.5, 159.6, 159.6, 159.2, 158.9, 158.8, 158.7, 157.6, 157.0, 156.3, 156.1, 155.6, 154.9, 154.4, 152.0, 151.3, 151.0, 150.7, 150.2, 149.9, 148.9, 148.3, 142.2, 142.1, 141.9, 140.9, 140.8, 140.5, 140.1, 139.3, 139.2, 138.5, 134.8, 133.4, 133.3, 130.7, 130.5, 130.5, 130.3, 130.2, 130.2, 130.1, 129.2, 128.6, 128.4, 127.0, 125.9, 125.4, 125.3, 125.0, 124.3, 124.0 (Ar), 74.9, 74.7, 73.1 (CH), 70.7, 70.6, 70.4, 70.2, 70.1, 69.9 (CH$_2$).

MS (ESI) m/z 324.4 $[Fe_{2}L_{3}]^{4+}$

IR $\nu$ cm$^{-1}$ 3364 br, 3062 w, 1603 m, 1572 w, 1494 w, 1464 m, 1464 m, 1440 w, 1404 w, 1366 m, 1318 w, 1288 w, 1241 w, 1172 s, 1142 w, 1109 m, 1073 s, 1007 m, 937 m, 840 m.

Elemental analysis found (calculated for C$_{72}$H$_{63}$Cl$_{4}$Fe$_{2}$N$_{15}$O$_{3}$·10H$_{2}$O) % C 53.61 (53.38), H 5.03 (5.16), N 12.49 (12.97).

$S_{c_{5}}\Delta_{Fe,HHT}\text{-}[Fe_{2}L^{3e}]Cl_{4}\cdot10H_{2}O$

Data as for $R$-enantiomer

Yield 0.67 g, 83 %.

MS (ESI) m/z 324 $[Fe_{2}L_{3}]^{4+}$.

Elemental analysis found (calculated for C$_{72}$H$_{63}$Cl$_{4}$Fe$_{2}$N$_{15}$O$_{3}$·10H$_{2}$O) % C 53.55 (53.38), H 5.05 (5.16), N 12.92 (12.97).
Hannon Cylinder ([Fe2(C25H20N4)3]Cl4.4H2O)

To our knowledge, while an indication of the method of synthesis of this compound has appeared, a synthetic protocol and characterising data have not previously been reported.

4,4-Methylenedianiline (3.0 eq.) and 2-pyridinecarboxaldehyde (6.0 eq.) were dissolved in methanol and heated to reflux (70°C) until a white solid precipitated (typically 30 min). At this point iron(II) chloride tetrahydrate (2.0 eq.) was slowly added to the mixture and the purple solution was heated for a further 18 h. The solution was allowed to cool to ambient temperature, the solvent was removed under reduced pressure and the crude solid was dried in vacuo at ambient temperature for 18 h. The resulting purple powder was suspended in dichloromethane (50 ml) then collected by filtration, washed with dichloromethane (10 ml) and dried in vacuo at ambient temperature for 18 h.

Yield = 3.64 g, 2.5 mmol, 76%.

\(^1\)H NMR (300 MHz, 298 K MeOD) \(\delta\) 9.17 (6H, s, CHN), 8.71 (6H, d, \(^3\)J\(_{HH}\) = 7.5 Hz), 8.49 (6H, t, \(^3\)J\(_{HH}\) = 7.5 Hz), 7.86 (6H, t, \(^3\)J\(_{HH}\) = 7.5 Hz), 7.46 (6H, d, \(^3\)J\(_{HH}\) = 5.0 Hz), 7.05 (12H, br s), 5.61 (12H, br s, Ar), 4.07 (6H, s, CH₂).

\(^{13}\)C\(^{1}\)H NMR (75 MHz, 298 K, MeOD) \(\delta\)C 176.7 (CHN), 160.0, 156.8, 150.7, 143.1, 141.0, 132.5, 131.5, 131.1, 122.7 (Ar), 39.7 (CH₂).

MS (ESI) m/z 310 [Fe₂L₃]⁴⁺ 425 [Fe₂L₃][Cl]³⁺

IR \(\nu\) cm\(^{-1}\) 3327.65 br, 2996.34 br, 2897.07 w, 1581.47 m, 1499.58 w, 1468.25 w, 1434.96 w, 1352.02 w, 1294.29 w, 1256.73 w, 1236.20 m, 1202.36, 1154.91 w, 1107.37 w, 1045.39 w, 1015.62 w, 862.11 w, 815.31 w.

Thermogravimetric analysis (25 to 400°C, 10 °C/min, dinitrogen flow): mass loss of 5% observed 70-120°C was shown by mass spectrometry to be principally water. Calculated for C₇₅H₆₀Cl₄Fe₂N₁₂.4H₂O, 4.9%.

Elemental analysis found (Calculated for C₇₅H₆₀Cl₄Fe₂N₁₂.4H₂O) % C 61.71 (61.91), H 4.60 (4.71), N 11.35 (11.55).
NMR Spectra

Figure S1. $^1$H (500 MHz) and $^{13}$C{$^1$H} (126 MHz) NMR spectra of $R_c$,$\Lambda_{Zn,HHT}$\-[Zn$_2$L$^{2a}$]ClO$_4$$_4$ in $d^3$-acetonitrile at 298 K with key assignments. Residual protic solvent $\delta_H$ 1.95, $\delta_C$ 117, water $\delta_H$ 2.19. * indicates the presence of the HHH-isomer.
Figure S2. $^{13}$C-$^1$H (126 MHz) NMR spectrum of $R_c, \Lambda_{Fe,HHT}$-[Fe$_2$L$_2$$^a$$_3$][ClO$_4$]$_4$ in $d^4$-methanol at 298 K with key assignments. Residual solvent $\delta_C$ 49.00 ($\delta_C$ 52.35 free amine).

Figure S3. $^{13}$C-$^1$H (126 MHz) NMR spectrum of $R_c, \Delta_{Fe,HHT}$-[Fe$_2$L$_3$$^a$$_3$][ClO$_4$]$_4$ in $d^4$-methanol at 298 K with key assignments.
Thermogravimetric Analysis

Thermogravimetric analysis was used to determine the amount of water of crystallisation present in the chloride salts of iron (II) triplex metallohelices. An accurately weighed 40 µl aluminium crucible was heated from 25 to 400°C at 10 °C/min under dinitrogen in a DSC1-1600 scanning calorimeter. The mass lost was plotted against temperature (see below) and the % mass loss from 70-120°C (loss of water) was calculated.
Figure S4. Thermogravimetric analysis of triplex metallohelices, indicating mass lost due to water of crystallisation and thermal decomposition.
Absorbance Spectroscopy

Circular Dichroism

Spectra were measured on a Jasco J-815 spectrometer, calibrated conventionally using 0.060% ACS a holmium filter. Measurements were collected using a 1 cm path-length quartz cuvette. The parameters used were; bandwidth 1 nm, response time 1 sec, wavelength scan range 200 – 750 nm, data pitch 0.2 nm, scanning speed 100 nm/min and accumulation 4.

Figure S5. CD spectra of the pairs of enantiomers of triplex metallohelices (0.03 mM in water); each enantiomer shows an equal and opposite spectrum to its pair.
Stability in Aqueous Media

Visible absorbance spectra for stability studies were recorded using a Carey IE spectrometer. Measurements were collected in a 1 cm path-length polystyrene cuvette and the standard parameters used were bandwidth 1 nm, response time 1 sec, wavelength scan range 350 – 800 nm, data pitch 0.2 nm, scanning speed 200 nm/min and accumulation 1. The intensity of the MLCT band (500-600 nm) of a 0.03 mM solution of each compound was measured over time in RPMI cell culture medium at 37°C and 0.2 M hydrochloric acid at 20°C.

Table S1. % reduction of MLCT band (in 500-600 nm region) for triplex metallohelices – 0.03 mM 24 h in 0.2 M hydrochloric acid, pH 1.0 at 20°C and 96 h in RPMI cell culture medium at 37°C.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>pH 1.0 (HCl) at 20°C</th>
<th>RPMI at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decomposition t½/h (esd)</td>
<td>% of complex remaining after 24 h</td>
</tr>
<tr>
<td>Hannon Cylinder</td>
<td>1.39 (9.5E-3)</td>
<td>0.0†</td>
</tr>
<tr>
<td>Rc,ΔFe,HHT-[Fe2L3a]Cl4</td>
<td>10.26 (0.07)</td>
<td>20</td>
</tr>
<tr>
<td>Rc,ΔFe,HHT-[Fe2L3b]Cl4</td>
<td>11.53 (0.14)</td>
<td>24</td>
</tr>
<tr>
<td>Rc,ΔFe,HHT-[Fe2L3c]Cl4</td>
<td>2.01 (0.26)</td>
<td>0.0†</td>
</tr>
<tr>
<td>Rc,ΔFe,HHT-[Fe2L3d]Cl4</td>
<td>#</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Rc,ΔFe,HHT-[Fe2L3e]Cl4</td>
<td>#</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Sc,ΔFe,HHT-[Fe2L3f]Cl4</td>
<td>#</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Rc,ΔFe,HHT-[Fe2L3g]Cl4</td>
<td>#</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Sc,ΔFe,HHT-[Fe2L3h]Cl4</td>
<td>#</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

# one half-life not reached over period of weeks
* did not show measurable decrease in MLCT band absorbance over the time period measured
† complex completely hydrolysed after 1.5 h
‡ complex completely hydrolysed after 12 h

DNA Denaturation

The denaturation temperature (Tm) of DNA indicates its thermal stability and is defined as the temperature at which half of the DNA strands have unwound from the double-helical a random coil state.15 This was measured to be 68.3 ± 0.5°C for linear calf thymus (ct-) DNA via absorbance spectroscopy (260 nm). The ability of triplex metallohelices to interact with linear ct-DNA was investigated by observing the affect they had on its denaturation temperature, along with the flexicate complex Δ7a from reference 16 and the Hannon Cylinder. ct-DNA (0.5 mg/ml, 7.5×10⁻⁵ per base) was mixed with each complex (7.5 µM) in buffered conditions (10mM Tris, 1 mM EDTA at pH 7.0) to give 10 base: 1 complex. The
absorbance at 260 nm as a function of temperature (every 1°C, 25-90°C) was measured in a 1 cm masked quartz cuvette at a rate of 0.4 °C min⁻¹ and run in triplicate. $T_m$ was calculated from the first derivative of a Boltzmann sigmoidal fit of the plot of absorbance at 260 nm against temperature for each complex.

Figure S6. Untreated ct-DNA ($7.5 \times 10^{-5}$ per base in 10mM Tris, 1 mM EDTA at pH 7.0) denaturation temperature ($T_m$) and treated with the flexicate complex $\Delta 7a$ from ref 16, Hannon Cylinder [Fe₂(C₂₃H₂₀N₄)₃]Cl₄.4H₂O, HHT-[Fe₂$L^{2a}$]Cl₄ and HHT-[Fe₂$L^{3a}$]Cl₄ (7.5 µM)

Anticancer Experiments

Chemosensitivity (MTT assay)

MDA-MB-468 (human breast adenocarcinoma) and HCT116 p53++ (human colon carcinoma) cells were incubated in 96-well plates at a cell concentration of $2.0 \times 10^4$ cells/ml. The cells were used when between 50 and 80% confluent in the stock flasks. Complete cell media containing RPMI-1640, supplemented with 10% foetal calf serum, sodium pyruvate (1 mM) and 1-glutamine (2 mM), was used to prepare the desired cell concentration and reference wells. Plates containing cells were incubated for 24 h at 37°C in an atmosphere of 5% CO₂, prior to drug exposure. All compounds were dissolved in complete RPMI-1640 cell media to give an initial concentration of 100 mM and diluted further with cell media to obtain concentrations ranging from 100 µM – 5 nM. Cell media (200 µl) was added to the reference cells and differing concentrations of drug solution (200 µl) were added to the remaining wells. The plates were incubated for a further 96 h at 37°C in an atmosphere of 5% CO₂. 3-(4,5-Dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/ml, 20µl per well) was added to each well and incubated for a further 4 h at 37°C in an
atmosphere of 5% CO₂. Upon completion all solutions were removed from the wells and dimethyl sulfoxide (150 µl) was added to each well to dissolve the purple formazan crystals. A Thermo Scientific Multiskan EX microplate photometer was used to measure the absorbance at 540 nm. Lanes containing 100% cell media and untreated cells were used as a blank and 100% cell survival respectively. Cell survival was determined as the absorbance of treated cells minus the blank cell media, divided by the absorbance of the untreated control; this value was expressed as a percentage. The IC₅₀ values were determined from a plot of percentage cell survival against drug concentration (µM). All assays were conducted in triplicate and the mean IC₅₀ ± standard deviation was determined.

**FACS Assays (H2AX and Cell Cycle)**¹⁷

**Cell Preparation**

HCT116 p53++ cells (5 × 10⁵ cells/flask, 10 ml complete RPMI medium) were incubated for 18 h at 37°C in 5% CO₂, then treated with 10 µM triplex metallohelix (10 ml in complete RPMI medium for 24 h). The supernatant, containing any dead cells, was collected and the cells were harvested by trypsinisation. This single cell suspension in trypsin was added to the supernatant and centrifuged at 1500 rpm (300 g) for 5 min. The cells were washed twice with PBS (phosphate buffered saline), re-suspended in ice-cold methanol in PBS (90:10) and incubated in ice for 30 min, then stored at -20°C until required for analysis.

**H2AX Assay**

The pre-treated cells were washed twice in incubation buffer (PBS containing 0.5 mM BSA) then re-suspended in 100 µl incubation buffer for 10 min at room temperature. 2 µl of primary rabbit anti-human phosphor Histone H2AX (Ser 139) antibody (1:50 final dilution) was added and incubated at RT and incubated for a further 1 h. This was then washed twice with incubation buffer, re-suspended in 100 µl incubation buffer containing Alexa Fluor conjugated anti-rabbit IgG secondary antibody (1:1000 final dilution) and incubated in the absence of light at room temperature for 30 min then the cells were stored at 0°C until analysis using the FACS. The H2AX expression assay was repeated in triplicate with each compound and the mean expression ± standard deviation was determined.
Table S2. $\gamma$-H2AX expression of cells treated with each compound, alongside untreated control cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\gamma$-H2AX /foci per cell (esd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>26.01 (6.15)</td>
</tr>
<tr>
<td>$R_{c}\Delta_{Fe},HHT-\left[Fe_2L^{2a}\right]Cl_4$</td>
<td>23.35 (3.64)</td>
</tr>
<tr>
<td>$R_{c}\Delta_{Fe},HHT-\left[Fe_2L^{3a}\right]Cl_4$</td>
<td>31.11 (6.48)</td>
</tr>
</tbody>
</table>

Cell Cycle Assay

300 $\mu$l PBS containing propidium iodide (40 $\mu$g/ml) and RNAse A (200$\mu$g/ml) was added to the pre-treated cells and they were incubated in the absence of light at room temperature for 30 min. 200$\mu$l ice-cold PBS was added (final volume of 600 $\mu$l) and the cells were placed on ice until analysis using the FACS. The cell cycle assay was repeated four times with each compound and the mean % cells in each phase ± standard deviation was determined. Red fluorescence was observed at 488nm excitation by flow cytometry and data analysed using WinMDI2.9 and Cylchred software. As can be seen in table S3, treatment with either compound both increased the population of cell in the sub G1 and G2/M phases and decreased the population of the S phase of the cell cycle a statistically significant amount when compared to the untreated control cells.

Table S3. Percentages of cells in each phase of the cell cycle after treatment with each compound, compared to an untreated control.

<table>
<thead>
<tr>
<th></th>
<th>% Sub G1 (esd)</th>
<th>% G1 (esd)</th>
<th>% S (esd)</th>
<th>% G2/M (esd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>3.90 (1.44)</td>
<td>44.41 (3.79)</td>
<td>28.98 (4.80)</td>
<td>20.20 (2.24)</td>
</tr>
<tr>
<td>$R_{c}\Delta_{Fe},HHT-\left[Fe_2L^{2a}\right]Cl_4$</td>
<td>12.65 (3.48)</td>
<td>42.90 (9.15)</td>
<td>13.02 (2.90)</td>
<td>26.89 (3.28)</td>
</tr>
<tr>
<td>$R_{c}\Delta_{Fe},HHT-\left[Fe_2L^{3a}\right]Cl_4$</td>
<td>29.01 (5.10)</td>
<td>4.27 (1.58)</td>
<td>8.91 (2.47)</td>
<td>45.99 (5.55)</td>
</tr>
</tbody>
</table>
**Single Cell Gel Electrophoresis Assay**

The induction of single strand breaks (SSB) and cross linking in HCT116 p53++ cells was determined via single cell gel electrophoresis. Cells were seeded at $3 \times 10^5$ cells in 6 well plates in complete RPMI-1640 medium and incubated for 18 h at 37°C in an atmosphere of 5% CO$_2$. Following treatment with each compound (10 $\mu$M in complete RPMI-1640 medium for 24 h) the cells were washed twice with Hanks balanced salt solution (HBSS), harvested by trypsinisation and embedded in 0.5% low-melting point agarose and transferred to agarose coated glass slides. These slides were immersed in freshly prepared ice-cold lysis buffer (2.5 M NaCl, 100 mM Na$_2$EDTA, 10 mM Trisma base, 1% sodium hydroxide, pH 10.0) 1% Triton X-100 and 10% dimethyl sulfoxide. The slides were then submerged in electrophoresis buffer (300 mM sodium hydroxide, 1 mM Na$_2$EDTA, pH > 13.0) for 30 min in a horizontal gel electrophoresis, and then subjected to electrophoresis at 0.6 V cm$^{-1}$ for 25 min. Following electrophoresis, the slides were neutralised (3 × drop wise addition of 0.4 M Trisma buffer, pH 7.5) rinsed with water and fixed with 100% ice-cold ethanol and dried in air for 18 h. To detect cross-links drug treated cells were further treated with 100 $\mu$M of hydrogen peroxide for 20 minutes prior to gel electrophoresis. Immediately before analysis the slides were stained with SYBR$^\text{TM}$ Gold solution (Molecular probes Inc.) and viewed with an epifluorescent microscope (Nikon Eclipse E800, Japan). The tail moment was measured on 50 randomly selected cells using Comet assay III software (Perceptive Instruments, UK) and each assay was performed in triplicate. As can be seen in table S4, treatment with all of the compounds tested did not cause a statistically significant level of either single strand breaks or cross links in HCT116 p53++ cells.

**Table S4.** Average tail moments of comets from cells treated with each compound, alongside untreated control cells and those treated with hydrogen peroxide only.

<table>
<thead>
<tr>
<th></th>
<th>SSB tail moment (esd)</th>
<th>x-link tail moment (esd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>3.51 (1.30)</td>
<td>1.87 (0.39)</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>36.04 (6.77)</td>
<td>36.44 (4.02)</td>
</tr>
<tr>
<td>R$_c$,$\Delta$Fe,$HHT$-[Fe$<em>2$L$</em>{2a}$]Cl$_4$</td>
<td>2.44 (0.55)</td>
<td>34.62 (5.45)</td>
</tr>
<tr>
<td>S$_c$,$\Lambda$Fe,$HHT$-[Fe$<em>2$L$</em>{2a}$]Cl$_4$</td>
<td>2.26 (1.50)</td>
<td>-</td>
</tr>
<tr>
<td>R$_c$,$\Delta$Fe,$HHT$-[Fe$<em>2$L$</em>{3a}$]Cl$_4$</td>
<td>2.26 (0.42)</td>
<td>32.02 (0.76)</td>
</tr>
<tr>
<td>S$_c$,$\Lambda$Fe,$HHT$-[Fe$<em>2$L$</em>{3a}$]Cl$_4$</td>
<td>1.38 (0.51)</td>
<td>-</td>
</tr>
</tbody>
</table>
Antimicrobial Experiments

Preparation of Bacterial Stocks

Verified stocks of MRSA [USA300] and E. coli [TOP10] were grown to $10^8$ cfu/ml (cfu = colony forming units) respectively in sterile Luria-Bertani (LB) medium, as measured by OD$_{600}$ (optical density measured at wavelength: 600 nm) and confirmed by hemocytometer measurement. These were used to diluted of $10^6$ cfu/ml in sterile Mueller-Hinton (MH) broth (15% w/v glycerol) and flash-frozen in liquid N$_2$ to store use.

Minimum Inhibitory Concentration

In order to assess the potency of the triplex metallohelices minimum inhibitory concentrations (MICs) were established using the standardised macrobroth dilution method in cation-adjusted MH broth. 200µl aliquots (128 µg/ml triplex metallohelix in sterilized MH broth, diluted $2^n$ µg/ml × 5) were added to 96-well plates in duplicate. This was inoculated with each bacterial strain (bacterial density of $10^3$ cfu/ml, ~200 cells per well) and sealed. After mixing at 720 strokes/min for 10 seconds, growth was monitored over 20 h at 37 °C by recording OD$_{600}$ every 10 mins with an iEMS 96-well plate reader. The lowest concentration to inhibit growth across each repeat is classified as the MIC. Positive (medium and untreated bacteria) and negative (medium only) controls were run with each plate. The antimicrobial properties of our recently reported flexicate systems 16 were reproduced as a positive control alongside ampicillin (Table S5).

Minimum Bactericidal Concentration

10 µl of the bacteria/compound mix (128 µg/ml) was recovered from the MIC 96-well plate and diluted 1:20 in sterile phosphate buffered saline, spread onto a sterile LB/agar plate and incubated for 18 h at 37 °C (in duplicate). The MBC was determined to be the lowest concentration at which this dilution/plating experiment showed no visible growth across both plates to within a 5% error.
Table S5. Recorded MIC and MBC values for triplex metallohelices and Ampicillin against Methicillin-Resistant Staphylococcus aureus (USA300) [MRSA] and Escherichia coli (TOP10) [E. Coli].

<table>
<thead>
<tr>
<th></th>
<th>MRSA (USA300)</th>
<th>E. Coli (TOP10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC/µg ml⁻¹</td>
<td>MBC/µg ml⁻¹</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&lt;2</td>
<td>-</td>
</tr>
<tr>
<td>flexicate Δ7a (ref 16)</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>flexicate Δ7a (ref 16)</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>$R_{c}$,$\Lambda_{Fe},HHT-[Fe_{2}L^{2a}]Cl_{4}$</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>$S_{c}$,$\Lambda_{Fe},HHT-[Fe_{2}L^{2a}]Cl_{4}$</td>
<td>&gt;128</td>
<td>&gt;128</td>
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<tr>
<td>$R_{c}$,$\Lambda_{Fe},HHT-[Fe_{2}L^{2a}]Cl_{4}$</td>
<td>&gt;128</td>
<td>&gt;128</td>
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<tr>
<td>$S_{c}$,$\Lambda_{Fe},HHT-[Fe_{2}L^{2a}]Cl_{4}$</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

References

21. V. Lorian, Antibiotics in Laboratory Medicine, Williams & Wilkins, 1991.