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Asymmetric triplex metallohelices with high and selective activity against cancer cells

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An antiparallel strand arrangement in water-soluble helicates creates an amphipathic functional topology akin to that of host-defence peptides. High and selective cancer cell line toxicity is exhibited, causing dramatic changes in the cell cycle without DNA damage, and remarkably there is no significant toxicity to MRSA and E. coli.
Abstract

Small cationic amphiphilic α-helical peptides are emerging as agents for the treatment of cancer and infection, but they are costly and display unfavourable pharmacokinetics. Helical coordination complexes may offer a three-dimensional scaffold for the synthesis of mimetic architectures; however, the high symmetry and modest functionality of current systems offer little scope to tailor the structure to interact with specific biolomolecular targets, or to create libraries for phenotypic screens. Here we report the highly stereoselective asymmetric self-assembly of very stable, functionalised metallohelices. Their anti-parallel Head-to-Head-to-Tail ‘triplex’ strand arrangement creates an amphipathic functional topology akin to that of the active sub-units of e.g. host-defence peptides and p53. The metallohelices display high, structure-dependent toxicity to the human colon carcinoma cell-line HCT116 p53++ causing dramatic changes in the cell cycle without DNA damage. They have lower toxicity to human breast adenocarcinoma cells (MDA-MB-468), and most remarkably they show no significant toxicity to the bacteria MRSA and E. coli.

150 words
The active regions of many disease-resisting proteins – such as the human antimicrobial host-defence peptide cathelicidin LL-37\textsuperscript{1} and p53\textsuperscript{2} which prevents mutations in the genome – are based around a cationic amphipathic $\alpha$-helix. Correspondingly, these small units have been investigated as clinical antibiotic and anticancer compounds.\textsuperscript{3-6} Significant progress has also been made in the development of synthetic derivatives with greater structural integrity, such as stapled peptides,\textsuperscript{7,8} and peptidic foldamers based on unnatural building blocks.\textsuperscript{9-11} In addition, several non-peptide synthetic scaffolds have been put forward\textsuperscript{12,13} in an effort to provide synthetically tractable systems while still presenting key recognition features of the $\alpha$-helix.

**Fig 1 | Metallohelix architectures.** Metal helicates are usually based on rigid ditopic bidentate ligands AB–BA giving – at octahedral centres – $D_3$-symmetric enantiomers $[M_2(AB–BA)_3]$ (a), while the use of directional ligands AB–CD leads to mixtures of $C_3$-symmetric Head-to-Head-to-Head (b) and $C_1$-symmetric Head-to-Head-to-Tail “triplix” architectures (c). A highly stereoselective approach to this latter architecture has now been designed and realised. Computational investigations showed that $\pi$-stacking phenomena in stereogenic units of type (d) based on L\textsuperscript{1} were modulated by introducing bpy units as in (e). A series of L\textsuperscript{1}-bpy (i.e. AB–CD) ditopic ligands such as L\textsuperscript{2a} and L\textsuperscript{3a} (f) were subsequently used to make a library of triplex metallohelices.

Certain self-assembling multimetallic coordination complexes, known as helicates\textsuperscript{14} [Fig 1(a)], resemble $\alpha$-helices in terms of their diameter and charge, but since they are synthesised using symmetric rigid ligands AB–BA they have much higher symmetry. When directional ligands AB–CD are employed, Head-to-Head-to-Head [HHH, Fig. 1(b)] and Head-to-Head-to-Tail [HHT, (c)] constitutions are introduced, and a total of four pairs of enantiomers $[M_2(AB–CD)_3]$ are possible. Correspondingly, these architectures are observed in mixtures of stereo- and/or optical isomers.\textsuperscript{15-22} Also, most helicates contain little if any external functionality, are incompatible with water, and are not readily available in enantiomerically pure form.\textsuperscript{23} For example, a prototypical $[M_2(AB–BA)_3]$ system\textsuperscript{24} for which several biological studies have been reported\textsuperscript{25-27} is based on few analogues, requires chromatographic resolution or lengthy syntheses, and undergoes significant hydrolysis on
experimental timescales (*vide infra*). Thus, although helicates are appealing as a potential framework for peptidomimetic chemistry, they cannot currently provide us with anything approaching the exquisite functionality and topography present in natural α-helix systems.

Recently we showed that by linking two diastereomerically pure tris-chelate metal complex units28–31 via the groups X [Fig 1(d)] we could access water-compatible, stereochemically inert, functionalised helices we called flexicates.32,33 These compounds are highly symmetrical, with the general structure of Fig1(a). We previously established that the very high stereoselection (Δ v. Λ) in the monometallics arises largely from steric effects, while the arene π-interactions shown are principally responsible for fac/mer selectivity and the unusual stability of flexicates in water.28 Since the mer configurations cannot be present in a helicate-like structure in any event we reasoned that π-stacking effects might be exploited to deliver stereoselection for the desirable, asymmetric HHT isomers of [M₂(AB–CD),] while still retaining control over helicity. Here we report a highly stereoselective self-assembly of diverse, stable, functionalised, metallohelices with anti-parallel ‘triplex’ arrangement of strands. These metallohelices have amphipathic topology and very high, structure-dependent, selective biological activity. As such we believe that these triplex metallohelices represent a new and versatile α-helix mimetic structure type.

**Results**

**Design, synthesis and characterisation**

We considered a directional ditopic ligand design AB–CD where AB– is provided by a derivative of L₁ [Fig 1], and –CD comes from an achiral bidentate; here we chose 2,2'-bipyridine (bpy) because it is expected to form strong intramolecular π-stacks with co-ligands L₁ but not with itself, and also since a large number of derivatives are known. The feasibility of a diastereoselective HHT isomer synthesis using putative new helicands such as L₂a and L₃a was assessed first by computing the relative energies of individual metal units expected in that architecture (Table 1, entries 1-4).

For [FeL₁²(bpy)]²⁺ six isomers are possible, but only the Δ- and Λ-cis,cis diastereomers have the appropriate connectivity to be present in the HHT structure. Of these, the Δ diastereomer [depicted in Fig 1(e)] was found to be the more stable by 9.62 kcal mol⁻¹ due to a strong steric clash involving methyl groups in the Λ isomers. Additionally, both isomers feature one parallel-offset π-stacking interaction34 between a metal-coordinated pyridine ring and the neighbouring L₁ ligand, and a second interaction between L₁ and bpy. Grimme has noted that the magnitude of such interactions increases with the number of rings involved.35
For the doubly bpy-substituted unit \([\text{FeL}^1(\text{bpy})_2]^{2+}\) the two possible isomers are \(C_1\) symmetric diastereomers. Both contain a parallel-offset \(\pi\)-stacking interaction between the phenyl ring and an adjacent bpy ligand, but steric again dictate that the \(\Delta\) enantiomer is the lower in energy, this time by 2.58 kcal mol\(^{-1}\) (entry 4). We conclude that the presence of one or two \(\text{L}^1\)-type ligands is sufficient to deliver stereocontrol at a single metal centre, and thus that a HHT structure [Fig 1(c)] in this series is likely to be homochiral, \textit{i.e.} have the same absolute configuration at both metal centres.

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<th>Entry</th>
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<th>Population/(%) (298 K)</th>
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\(\text{Table 1} \) Calculated energies (DFT) of selected isomers for monometallic units and metallohelices. In the stereochemical descriptors, subscripts \(\alpha\) and \(\beta\) refer to the pyridineimine-rich and bpy-rich metal centres respectively. The equilibrium selectivities were determined the statistically corrected energies of each isomer at 298 K.

The potential effects of helication and the thermodynamic selectivity \(\text{HHT} \text{vs} \text{HHH}\) were then investigated for both \(\text{Zn(II)}\) and \(\text{Fe(II)}\) with \(\text{L}^{2a}\) and \(\text{L}^{3a}\): ligands for which reasonable synthetic routes were envisaged and a large number of derivatives are possible (\textit{vide infra}). For the particular carbon-centred configurations used here, the homochiral \(\Delta,\Delta\) HHT isomers were found to be the lowest energy by between 0.95 and 3.05 Kcal mol\(^{-1}\) (Table 1, entries 5, 9, 13, 17), followed by the corresponding HHH. The effect of helication is also apparent; the homochiral HHH isomers are more stable than the corresponding mesocate-like structures (entry 6 \& 8 etc). Following a correction for the fact that each HHT structure can be formed in three ligand permutations compared with only one for
each HHH, the estimated equilibrium selectivities for the asymmetric helicate are excellent, particularly for \( L^3 \).

Fig 2 | Synthesis and characterisation of triplex metallohelices. (a) Self-assembly from versatile components of a wide range of functionalised helices in which the strands are arranged head-head-tail; (b) \(^1\)H NMR spectrum and key assignments for \( R_{C', \Delta Zn, HHT-}[ZnL^3a][ClO_4]_4 \). The small singlet at 8.76 ppm may be due to the three spectroscopically equivalent imine protons in the \( C_3 \)-symmetric HHH isomer. On this assumption the diastereomeric purity is >98\%o; (c) space-filling model of the cationic unit in \( R_{C', \Delta Zn, HHT-} \).
The new compounds 2 and 3 were combined with a selection of metal sources, plus aldehydes or phenethylamines as shown in Fig 2 in one-pot reactions to give the two ranges of homochiral helicates shown. By analogy with the bi-directional motif of triplex DNA, we refer to these as *triplex* metallohelices. The compounds were characterised by NMR spectroscopy, mass spectrometry, UV/vis, circular dichroism, IR, thermogravimetry and microanalysis. For example, mixing three equivalents each of amine R-3 and 2-pyridinecarboxaldehyde with two equivalents of 

\[ \text{Zn(ClO}_4)_2 \cdot 6\text{H}_2\text{O} \] 

led to the clean formation of \( R_c\Delta_{\text{Zn}}\text{HHT-}[\text{Zn}_3\text{L}_{3\text{b}}]_3[\text{ClO}_4]_4 \). The \(^1\)H NMR spectrum of this complex [Fig 1(b)] indicates the presence of three spectroscopically unique ligand environments. Of particular interest is that two of the bpy atoms \( H^b \) are observed at unusually low field (ca 9.2 ppm). We propose that this results from the close proximity (ca 2.5 Å) of these protons to the ether oxygen atom of an adjacent ligand as observed in the computed structure and as indicated in Figure 1(a). No such interaction is observed for the remaining \( H^b \), the resonance being observed further up field at 8.41 ppm. Similarly, two sets of phenyl ring protons \( H^d \) and \( H^e \) (6.65-5.85 ppm) experience strong through-space shielding from the bpy unit of an adjacent ligand. These observations indicate that the solution structure corresponds very closely to that computed above. Overall, the equilibrium selectivity for the isomer shown is exceptionally good (>98%) and consistent with calculation (99.8% Table 1).

In the case of \( R_c\Delta_{\text{Zn}}\text{HHT-}[\text{Zn}_3\text{L}_{3\text{b}}]_3[\text{ClO}_4]_4 \) very small needle like crystals were obtained by slow vapour diffusion of ethyl acetate into a solution of the compound in acetonitrile/methanol. An X-ray diffraction study confirmed the homochiral (\( \Delta_{\text{Zn}} \)) HHT structure depicted in Figure 2(a), (c) and (d). The single “conventional” phenyl/pyridine \( \pi \)-stack has a centroid–centroid distance of ca 3.60 Å, within the range we have reported previously. The two \( \pi \)-stacks between phenyl and 2,2’-bipyridine rings may be described by two such centroid-centroid distances: a shorter at 3.60-3.69 Å and a longer of 4.41-4.60 Å. The rings are close to parallel (ca 5°) with interplanar distances of ca 3.38 Å, significantly shorter than those computed by Grimme for similar scenarios (ca 3.5 Å). This may be due to the greatly increased polarity caused by coordination of the biaryl to the metal. The two cross-strand bpy-H→O interactions noted above are also observed (ca 2.5 Å). For the overall structure, and unlike conventional helicate systems, the triplex assembly leads to a slightly flattened structure [Fig 2(b),(c)] and pronounced asymmetry of functional group placement; a basal region contains the three hydroxyl groups and the structure narrows to a hydrophobic ridge comprising five \( \pi \)-stacked rings.

Water soluble triplex metallohelices were obtained using the above self-assembly reactions and iron(II) chloride. \(^1\)H-NMR spectra were broadened in comparison with the Zn(II) systems but
well resolved and fully assigned $^{13}$C spectra were obtained. Microanalysis, thermogravimetric analysis and mass spectra were also consistent with the proposed formulation. The CD spectra of the enantiomers in water were equal and opposite (see ESI), and the compounds were found to be remarkably stable. At pH 7 and 20 °C, no significant hydrolysis was detected over weeks for $R_{c}$-$\Lambda_{Fe}$-HHT-$[Fe_{2}L^{2a}]$Cl$_{4}$ and $R_{c}$-$\Lambda_{Fe}$-HHT-$[Fe_{2}L^{3a}]$Cl$_{4}$, compared to Hannon’s cylinder.$^{36}$ $[Fe_{2}(C_{25}H_{20}N_{4})_{3}]Cl_{4}$ which in our hands decomposed far more rapidly. In strong acid (pH 1) the hydrolysis kinetics are cleanly first order and $R_{c}$-$\Lambda_{Fe}$-HHT-$[Fe_{2}L^{2a}]$Cl$_{4}$ was recorded to have $t_{1/2}$ of ca 10.3 h, compared to 1.4 h for $[Fe_{2}(C_{25}H_{20}N_{4})_{3}]Cl_{4}$. Remarkably $R_{c}$-$\Lambda_{Fe}$-HHT-$[Fe_{2}L^{3a}]$Cl$_{4}$ is so stable to hydrolysis even at pH 1 that a half-life could not be recorded over 10 d. Furthermore, the compounds have excellent stability in the phosphate-rich RPMI-1640 cell growth medium over 96 h; for the same $L^{2a}$ and $L^{3a}$ compounds, 30% and 0% decay were observed, whereas $[Fe_{2}(C_{25}H_{20}N_{4})_{3}]Cl_{4}$ decomposed by ca 90%.

**Anticancer activity and mechanistic studies**

The sixteen new water-soluble Fe(II) triplex systems were screened, along with cisplatin for comparison, for their activity against cancer cell lines MDA-MB-468 (human breast adenocarcinoma) and HCT116 p53$^{++}$ (human colon carcinoma with wild type p53). The latter was chosen following a recent report that FK-16 – a short amphipathic $\alpha$-helix derived from the human host-defence peptide LL-37 – induced apoptosis in a closely related cell-line.$^{37}$ The resulting range of IC$_{50}$ values indicated great selectivity, covering ca two orders of magnitude (0.5 – 70 $\mu$M) [Figs 3(a), (b)]. Notably, and in agreement with the stability studies above, no decolouration of the drug solutions was detected during the 96 h experiments.

In MDA-MB-468 the $L^{2}$ compounds have moderate toxicity and do not display enantiomeric selectivity; the nitroarene enantiomers ($L^{2n}$) alone had similar activity to cisplatin. In contrast, all $\Delta_{Fe}$-$L^{3}$ compounds are more active than their $\Lambda_{Fe}$ enantiomers. Hydroxyl ($L^{3b}$) or methoxy ($L^{3x}$) functionality at position X reduces the observed activity ca 4-fold compared with the parent compound bearing $L^{3a}$, whereas the benzyloxy ($L^{3d}$) and pyrazine ($L^{3e}$) compounds had similar activity. The same eight compounds as a group showed substantially higher activity against HCT116 p53$^{++}$, with several examples in the nanomolar range. $\Delta_{Fe}$-$L^{2}$ compounds are more active than the $\Lambda_{Fe}$ enantiomers. Enantiomers of $L^{3a}$ and benzyloxy ($L^{3d}$) compounds are up to seven times more potent than cisplatin while the others, noticeably the hydroxyl ($L^{3b}$) are much less so.
Clinical anticancer treatments commonly include the use of DNA-binding or modifying drugs such as cisplatin. Such “alkylators” and DNA cleavage agents cause chemically irreversible reactions leading, in the absence of repair, to cell death.\textsuperscript{38,39} While the metal ions here are intended to be structure-forming rather than reactive, and since some binding to naked calf thymus (ct-) DNA was detected by DNA melting ($\Delta T_{m}$ = -3 - 13 °C), we sought to exclude the possibility of DNA damage. In a single cell gel electrophoresis assay, the absence of a ‘comet’ tail for HCT116 p53$^{++}$ cells that had been treated with $\text{RC}_{2a} \Lambda_{\text{Fe}}[\text{HHT-FeL}^{2\alpha}]{\text{Cl}}_4$ or $\text{RC}_{2a} \Lambda_{\text{Fe}}[\text{HHT-FeL}^{3\alpha}]{\text{Cl}}_4$ indicates the absence of breaks (both single and double strand) and alkali labile sites. In a related test, triplexes also failed to retard the formation of comets in cells with deliberately strand-damaged DNA, consistent with the absence of cross linking activity. In addition, these compounds did not significantly alter the production of $\gamma$-H2AX – a histone regarded as a universal marker for DNA damage.\textsuperscript{40}
The accumulation of cancer cells in particular phases of the cell cycle can point to mode of action.\textsuperscript{41} Following incubation with triplex metallohelices\textsuperscript{10} µM, 24 h) and staining, HCT116 p53\textsuperscript{++} cells were analysed by fluorescence-activated cell sorting (FACS).\textsuperscript{41} Compared with control untreated cells, compound $R_{C,\Delta Fe,HHT-[Fe_2L^2a]}$Cl\textsubscript{4} caused a dramatic increase in the proportion of cells in the G\textsubscript{2}/M phase (from ca 20 to 46\% of cells) and sub G\textsubscript{1} phase (ca 4 to 30\%) and a corresponding reduction in G\textsubscript{1} phase (ca 44 to 4\%) and S phase (ca 29 to 9\%). Cells in the sub G\textsubscript{1} phase are considered apoptotic\textsuperscript{42,43} and this data indicates that triplex helicates induce programmed cell death in HCT116 p53\textsuperscript{++} colon carcinoma cells. $R_{C,\Delta Fe,HHT-[Fe_2L^2a]}$Cl\textsubscript{4} showed a similar but less extreme effect.

Given the high potency and substantial effects on the cancer cell cycle, we were very surprised to discover that enantiomers of the parent triplex compounds showed no signs of toxicity (bactericidal or inhibitory action) towards Methicillin-Resistant \textit{Staphylococcus aureus} (MRSA - USA300) or \textit{Escherichia coli} (TOP10) at up to the highest measured concentration of 128 µg/ml i.e. two orders of magnitude higher than the recorded IC\textsubscript{50} values against the cancer cell lines.

\textbf{Discussion}

A strategy for the selective formation of anti-parallel ‘triplex’ metallohelices has been successfully established though molecular modelling, synthetic and structural studies. Ultimately, while absolute configuration at the metal centres was achieved by conventional diastereoselection, the far greater challenge of selectivity for the target asymmetric HHT structure was achieved by maximisation of π-stacking and other secondary interactions between ligands. Specifically, the relatively strong phenyl-bpy π-stacks and in some cases bifurcated C-H…O/N interactions are present only in the triplex architectures. This indicates how other new systems can be designed. These secondary interactions contribute also to chemical stability such that rates of hydrolysis in biologically-relevant media are exceptionally low. In addition, the modular self-assembly has allowed us to produce what we believe to be the largest and most diverse library of metallohelices to date. The potential recognition elements are arranged by the nature of the helix fold, and thereby via selection of molecular sub-component, a number of functional architectures can be made. We believe that this takes us a significant step towards α-helix-peptide-like candidates for biochemical targets and phenotypic screens.

To this end, and as with the peptidic α-helices they are designed to emulate, triplex metallohelices have been shown to display potent, selective and structure-dependent toxicity to certain cancer cell-lines and – remarkably – no measured toxicity to example Gram positive and Gram negative bacteria. This is particularly striking given that our earlier symmetrical flexicate compounds inhibit bacterial growth at low concentrations.\textsuperscript{32} A subtle biomimetic mechanism rather than broad-
spectrum cytotoxicity is thus indicated, and this is corroborated by the observation of major changes to the cell cycle without detected DNA damage. In future work we will establish whether an extrinsic or intrinsic pathway for apoptosis has been activated: do triplexes interact with membrane proteins or enter the cell and interfere with key internal pathways?

The remarkable selectivity and stability, the unprecedented scope for structural variation, and the potent and selective activity suggest that triplex metallohelices may represent a new area of chemical space for the synthesis of novel pharmaceuticals.

**Methods**

Full details of computations, synthesis, characterisation and microbiological experiments are given in the Supplementary Information. Outlines of key procedures are detailed below.

**Molecular mechanics and DFT calculations**

Monometallic structures were first optimised using B3LYP-D3(BJ)\(^{44}\) functional and the 6-31g(d) basis set, with convergence criteria of 0.0001 a.u. as implemented in the Firefly quantum chemistry package,\(^{45}\) which is partially based on the GAMESS(US) source code.\(^{46}\) Bimetallic systems were optimised using ligand field molecular mechanics (LFMM)\(^{47}\) as implemented in the DommiMOE program,\(^{48}\) before being annealed at 500 K for 1 ns prior to re-optimisation. Single point energies were determined using the B3LYP-D3(BJ)\(^{44}\) functional and the def2-TZVP basis set.\(^{45}\)

**Synthesis of water soluble triplex metallohelices**

Anhydrous iron(II) chloride (2 eq.) was added to a stirred solution of the either the desired 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 mixture was allowed to cool to ambient temperature, filtered through a celite plug and the solvents were removed in vacuo to give a dark purple solid (>95% yield). Water of crystallisation in these compounds was confirmed by NMR and IR spectroscopy. The hydration number was then determined by thermogravimetric analysis and the relevant mass loss was correlated with microanalytical data. NMR, IR and MS data were consistent with the proposed formulations. CD spectra of enantiomers in water were equal and opposite.

**Stability in aqueous media**

Visible absorbance spectra for stability studies were recorded using a Carey IE spectrometer. 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 also in 0.2 M hydrochloric acid at 20°C so that comparative \(t_{1/2}\) values for hydrolysis could be obtained.
**Chemosensitivity (MTT assay)**

Cells were incubated in 96-well plates at a cell concentration of $2.0 \times 10^4$ cells/ml in RPMI-1640, supplemented with 10% foetal calf serum, sodium pyruvate (1 mM) and l-glutamine (2 mM). Plates were incubated for 24 h at 37°C in an atmosphere of 5% CO$_2$, prior to drug exposure, then incubated for 96 h. 3-(4,5-Dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/ml) was added to each well and incubated for a further 4 h. The IC$_{50}$ values were determined from a plot of percentage cell survival against drug concentration (μM). All assays were conducted in triplicate and the mean IC$_{50}$ ± standard deviation was determined.

**γ-H2AX assay**

In triplicate, and against untreated control cells, drug-treated HCT116 wild type p53 cells were washed twice in incubation buffer (PBS containing BSA) then re-suspended. Primary rabbit anti-human phosphor Histone H2AX (Ser 139) antibody (1:50 final dilution) was added and incubated at RT for 1 h before treatment with Alexa Fluor conjugated anti-rabbit IgG secondary antibody (1:1000 final dilution) and incubation for 30 min before fluorescence activated cell sorting (FACS).

**Cell cycle assay**

300 µl PBS containing Propidium Iodide (40 µg/ml) and RNAse A (200µg/ml) was added to drug-treated cells before incubation for 30 min and FACS analysis. The 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.

**Single cell gel electrophoresis (comet) assays**

Cells were seeded in complete RPMI-1640 medium and incubated, then treated with drug (10 µM, 24 h), washed, harvested and embedded in agarose. Following horizontal gel electrophoresis (25 min) the slides were stained with SYBR™ 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). Each assay was performed in triplicate.

**Antimicrobial Experiments**

The standardised macrobroth dilution method in cation-adjusted Mueller-Hinton broth was used. Bacterial growth was monitored over 20 h at 37 °C 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 were reproduced as a positive control, alongside ampicillin.
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Author Contributions

ADF performed the computational work under the direction of DJF; ADF and RAK synthesised and characterised the compounds following early studies by SEH, and developed and conducted stability studies; RAK and QMAA performed the biological work under the direction of RMP; PG developed a synthesis of [Fe₂(C₂₅H₂₀N₄)₃]Cl₄ and performed stability studies; GJC solved and refined the X-ray crystal data; DHS conducted the antimicrobial experiments under the direction of DIR; PS conceived and directed the project, interpreted the data and wrote the paper.
Figure Legends & Tables

Figure 1 | Metallohelix architectures.

Figure 2 | Synthesis and characterisation of triplex metallohelices.

Figure 3 | Exposure of cancer cells to triplex metallohelices.

Table 1 | Calculated energies (DFT) of selected isomers for monometallic units and metallohelices.
References


