Supplementary Information

Accompanying

"Double Strand DNA Cleavage with a Binuclear Iron Complex"

by

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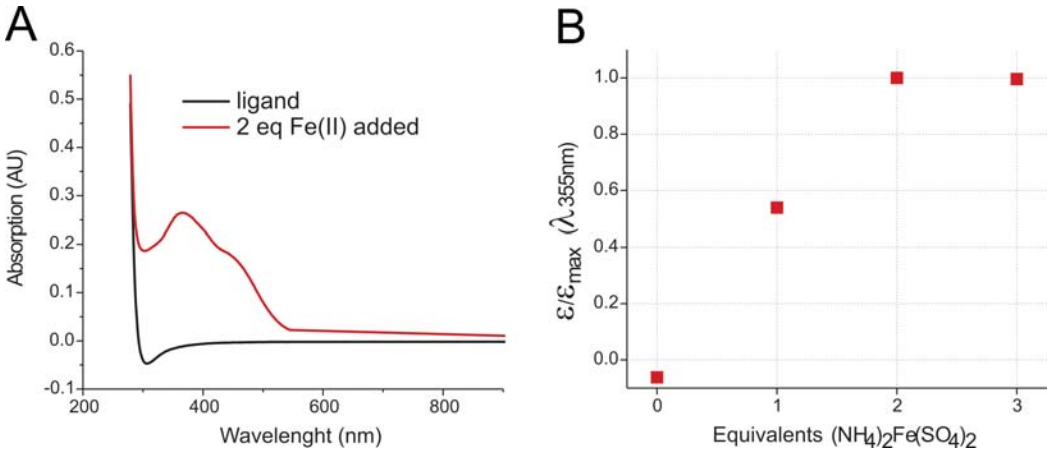


Fig. S1 (a) UV/Vis spectrum of the ligand **3c** (black) and after addition of 2 equivalents of $(NH_4)_2Fe(SO_4)_2$ (red). (b) Job plot of the $\varepsilon/\varepsilon_{max}$ at 355 nm against the amount of equivalents of $(NH_4)_2Fe(SO_4)_2$.

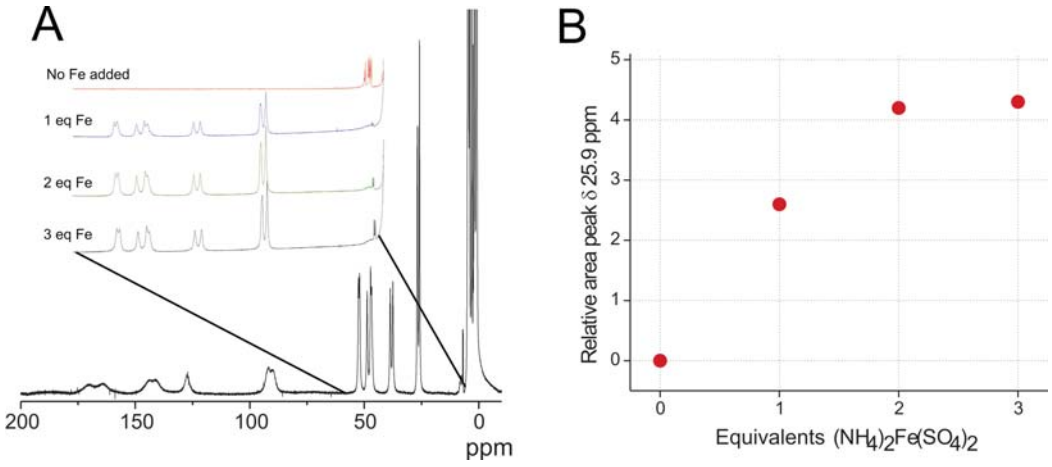


Fig. S2 (a) ^{1}H NMR spectrum of **3c** in D_2O after addition of 2 equivalents of $(NH_4)_2Fe(SO_4)_2$. Insert: Titration of $(NH_4)_2Fe(SO_4)_2$ to **3c** followed by ^{1}H NMR. (b) Job plot of the relative area of the peak at δ 25.9 ppm compared to the acetone peak against the amount of equivalents of $(NH_4)_2Fe(SO_4)_2$.

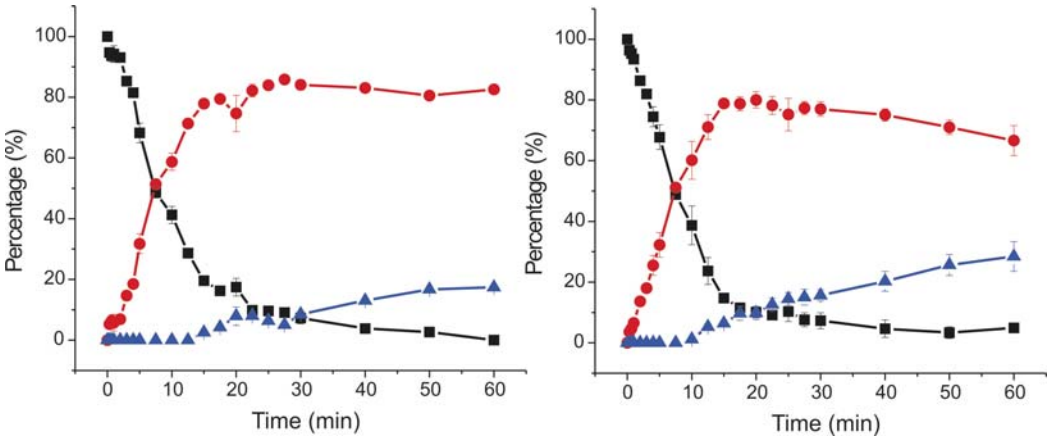
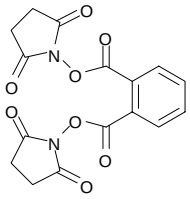


Fig. S3 Time profile of the cleavage of supercoiled (form I, ■) DNA to nicked (form II, ●) and linear (form III, ▲) with **3a** (left) and **3b** (right). Error bars represent the root mean square (r.m.s.) based on three runs. A correction factor of 1.31 was used to compensate for the reduced ethidium bromide uptake capacity of supercoiled DNA. (*vide infra*)

Experimental and Synthetic Procedures

General synthetic experimental remarks: reagents were purchased from Aldrich, Acros, Merck or Fluka and were used as provided. All solvents were reagent grade and were dried and distilled before use according to standard procedures. Synthetic reactions were performed under a nitrogen atmosphere using common Schlenk techniques. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200, a Varian VXR 300 or a Varian Mercury Plus 400 spectrometer at ambient temperature. Chemical shift values are denoted in δ values (ppm) relative to residual solvent peaks unless stated otherwise ($CDCl_3$, ^{1}H δ = 7.24, ^{13}C δ = 77.0; CD_3OD, ^{1}H δ = 3.30, ^{13}C δ = 49.0). Electrospray ionization mass spectra (MS-ESI^{+}) were recorded on a Triple Quadrupole LC/MS/MS Mass spectrometer (API 3000, Perkin-Elmer Sciex Instruments). Chemical ionization mass spectra (MS-CI^{+}) were recorded on an AEI MS-902. UV/Vis spectra were recorded on a Hewlett Packard 8453 diode array spectrophotometer at ambient temperature. Melting points were recorded on a Büchi B-545 melting point apparatus.

1-[(2-{[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}benzoyl)oxy]-2,5-pyrrolidinedione (5a)



To a cooled (0°C) solution of *N*-hydroxysuccinimide (1.27 g; 11.0 mmol) in THF (30 mL) was added triethyl amine (1.11 g; 1.52 mL; 11.0 mmol) and freshly distilled *o*-phthaloyl dichloride (1.02 g; 0.73 mL; 5.00 mmol). The resulting white suspension was stirred for 2 hours at room temperature. The solvent was removed and the residue was taken up in dichloromethane (100 mL), washed with water (3 × 50 mL) and dried over Na_2SO_4. Filtration and evaporation of the solvent yielded an off-white solid, which was recrystallized from isopropyl alcohol to yield a white solid (1.40 g; 3.90 mmol; 78 %). Mp 161.9-162.0°C.

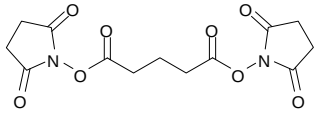
^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01-8.06 (m, AA'BB', 2H), 7.71-7.78 (m, AA'BB', 2H), 2.85 (s, 8H).

^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 168.7, 161.5, 133.2, 130.9, 127.3 25.7.

MS (CI^+) *m/z*: 378.1 $[M + NH_4^+]^+$ (100%).

Anal. calcd (%) for $C_{16}H_{12}N_2O_8$: C 53.34, H 3.36, N 7.78; found: C 53.0, H 3.34, N 7.68.

1-({5-[(2,5-dioxo-1-pyrrolidinyl)oxy]-5-oxopentanoyl}oxy)-2,5-pyrrolidinedione (5b)



Following the procedure as for **5a**, starting from freshly distilled glutaryl dichloride (0.845 g; 0.64 mL; 5.00 mmol). A white solid (1.61 g; 4.93 mmol; 99 %) was obtained after recrystallization from isopropyl alcohol. Mp 129.1-130.7°C.

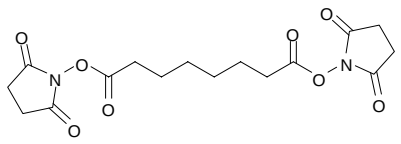
^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.81 (s, 8H), 2.77 (t, *J* = 7.3 Hz, 4H) 2.16 (quintet, *J* = 7.3 Hz, 2H).

^{13}C NMR (50.3 MHz, $CDCl_3$) δ 169.0, 167.7, 29.5, 25.5, 19.5.

MS (CI^+) *m/z*: 344.1 $[M + NH_4^+]^+$ (100%).

Anal. calcd (%) for $C_{13}H_{14}N_2O_8$: C 47.86, H 4.33, N 8.59; found: C 47.8, H 4.30, N 8.45.

1-({8-[(2,5-dioxo-1-pyrrolidinyl)oxy]-8-oxooctanoyl}oxy)-2,5-pyrrolidinedione (5c)



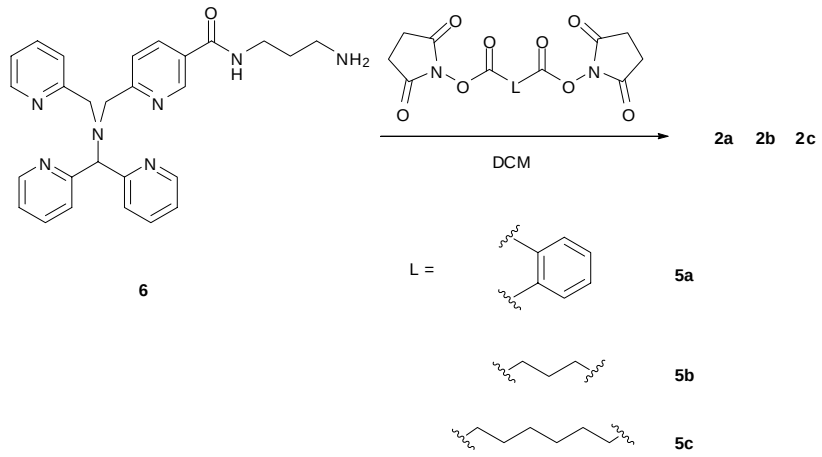
A solution of suberic acid (1.19 g; 6.84 mmol), DCC (1.73 g; 15.0 mmol) and *N*-hydroxysuccinimide (3.10 g; 15.0 mmol) in THF (250 mL) was stirred overnight. The white suspension was filtered and the filtrate was concentrated. Dichloromethane (150 mL) was added and the solution was washed with water (2 × 50 mL) and sat. $NaHCO_3$ (50 mL). After drying (Na_2SO_4) and filtration, the solvent was evaporated. Recrystallization from isopropyl alcohol afforded a white solid (1.99 g; 5.40 mmol; 79 %). Mp 166.2-167.0°C.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.80 (s, 4H), 2.58 (t, 7.3 Hz, 4H), 1.74 (m, 4H), 1.43 (m, 4H).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ 169.1, 168.5, 30.7, 28.1, 25.6, 24.2.

MS (CI^+) *m/z*: 386.2 $[M + NH_4^+]^+$ (100%).

Anal. calcd (%) for $C_{16}H_{20}N_2O_8$: C 52.17, H 5.47, N 7.61: found: C 52.3, H 5.54, 7.50.

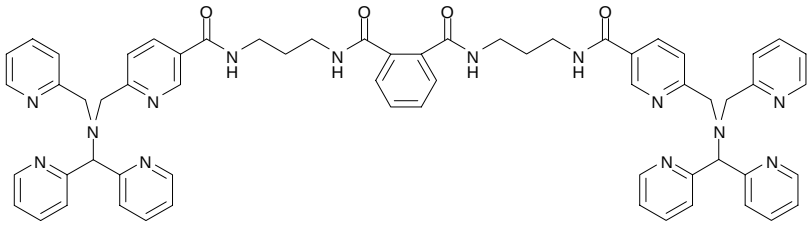


Scheme S1 Synthesis of ligands **2a-c**.

Synthesis of ligands 2a-c, general procedure

The synthesis of ligands **2a-c** is outlined in Scheme S1. Two equivalents of the propane amine substituted N4Py **6**[1] were added to a solution (~ 0.1 mM in CH_2Cl_2) of one equivalent of *N*-hydroxysuccinimide activated diacid **5a**, **5b** or **5c**. The mixture was stirred overnight at room temperature. The mixture was washed with water (3 × 10 mL) and dried over Na_2SO_4. After filtration the solution was concentrated and the product was precipitated with Et_2O. Further purification was achieved by size exclusion chromatography over a Sephadex LH20 column with MeOH as the mobile phase. After evaporation of the MeOH, the oily product was taken up in a minimum amount of CH_2Cl_2 and the product was precipitated with Et_2O.

***N*[1],*N*[2]-bis(3-{[(6-{[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl}-3-pyridinyl)carbonyl]amino}propyl)phthalamide (2a)**



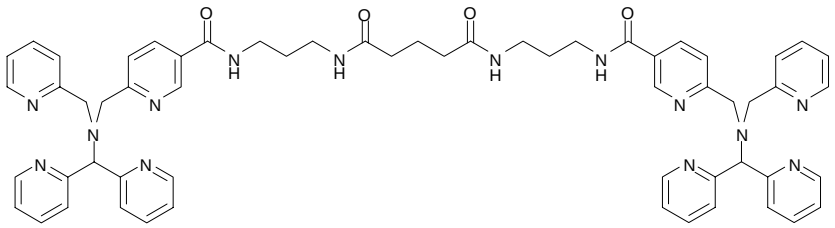
Starting from **5a** (39.3 mg; 0.109 mmol) and **6** (0.102 g; 0.218 mmol), **2a** was obtained as a pale light brown solid (0.101 g; 95 μmol; 94%).

^{1}H-NMR (400 MHz, CD_3OD): δ 8.78 (d, J = 2.2 Hz, 2H), 8.45 (m, 4H), 8.35 (m, 2H), 8.04 (dd, J = 8.1, 2.2 Hz, 2H), 7.79-7.64 (m, 14H), 7.55 (m, 2H), 7.49 (m, 2H), 7.25 (m, 4H), 7.18 (m, 2H), 5.33 (s, 2H), 3.99 (s, 4H), 3.95 (s, 4H), 3.44 (m, 8H), 1.85 (m, 4H).

^{13}C-NMR (50.3 MHz, CD_3OD): δ 171.8, 167.7, 163.8, 160.7, 160.3, 150.0, 149.5, 148.7, 138.5, 138.4, 137.2, 136.9, 131.3, 129.9, 128.8, 125.6, 124.8, 124.1, 124.0, 123.7, 74.2, 58.7, 58.3, 38.3, 38.2, 29.8.

MS (ESI$^+$) *m/z*: 1065.5 ([M + H$^+$]$^+$, 895.6 [M – Py-CH_2-Py + H$^+$]$^+$, 533.5 [M + 2H$^+$]$^{2+}$, 356.1 [M + 3H$^+$]$^{3+}$.

N^1,N^5-bis(3-{[(6-{[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl}-3-pyridinyl)carbonyl]amino}propyl)pentanediamide (2b)



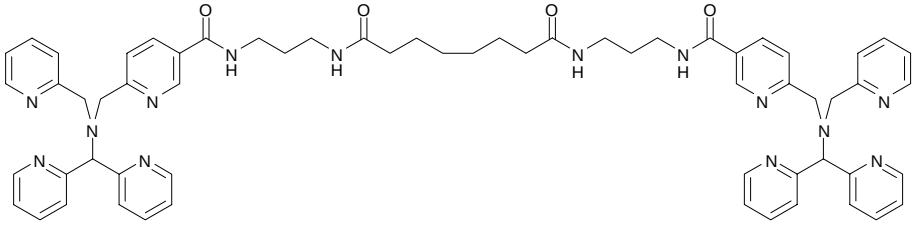
Starting from **5b** (33 mg; 0.102 mmol) and **6** (95 mg; 0.203 mmol), **2b** was obtained as a pale light brown solid (92 mg; 89 μmol; 87%).

^{1}H-NMR (300 MHz, CD_3OD): δ 8.79 (d, J = 1.8 Hz, 2H), 8.46 (dd, J = 4.8, 0.7 Hz, 4H), 8.35 (dd, J = 4.8, 0.7 Hz, 2H), 8.08 (dd, J = 8.1, 2.2 Hz, 2H), 7.67-7.80 (m, 14H), 7.26 (m, 4H), 7.19 (m, 2H), 5.34 (s, 2H), 4.01 (s, 4H), 3.96 (s, 4H), 3.40 (dd, J = 6.6 7.0, 4H), 3.25 (t, J = 6.6 Hz, 4H), 2.24 (t, J = 7.3 Hz, 4H), 1.91 (m, 2H), 1.78 (m, 4H).

^{13}C-NMR (50.3 MHz, CD_3OD): δ 175.5, 167.8, 163.8, 160.8, 160.3, 150.0, 149.5, 148.5, 138.5, 138.4, 136.9, 130.0, 125.6, 124.8, 124.2, 124.0, 123.7, 74.4, 58.8, 58.3, 38.3, 37.7, 36.2, 30.2, 23.1

MS (ESI$^+$) *m/z* 1031.7 [M + H$^+$]$^+$, 861.6 [M – Py-CH_2-Py + H$^+$]$^+$, 516.6 [M + 2H$^+$]$^{2+}$, 344.7 [M + 3H$^+$]$^{3+}$.

N^1,N^8-bis(3-{[(6-{[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl}-3-pyridinyl)carbonyl]amino}propyl)octanediamide (2c)



Starting from **5c** (0.206 g; 0.558 mmol) and **6** (0.522 g; 1.12 mmol), **2c** was obtained as a pale light brown solid (0.338 g; 0.315 mmol; 56%).

^{1}H-NMR (300 MHz, CD_3OD): δ 8.79 (d, J = 2.2, 2H), 8.45 (d, J = 5.1, Hz, 4H), 8.34 (d, J = 4.8 Hz 2H), 8.08 (dd, J = 8.1, 2.2 Hz, 2H), 7.80-7.65 (m, 14H), 7.26 (m, 4H), 7.19 (m, 2H), 5.35 (s, 2H), 4.02 (s, 4H), 3.97 (s, 4H), 3.39 (dd, J = 7.0, 6.6 Hz, 4H) 3.24 (dd, J = 7.0, 6.6 Hz, 4H), 2.18 (t, 7.3 Hz, 4 H), 1.77 (m, 4H) , 1.60 (m, 4H), 1.33 (m , 4H)

^{13}C-NMR (75.5 MHz, CD_3OD): δ 176.3, 167.8, 163.9, 160.8, 160.3, 149.9, 149.5, 148.6, 138.4, 138.3, 136.8, 129.9, 125.6, 124.8, 124.2, 124.0, 123.6, 74.4, 58.8, 58.4, 38.3, 37.8, 37.1, 30.2, 29.9, 26.8.

MS (ESI^+) *m/z*: 1095.6 $[M + Na^+]^+$, 1073.7 $[M + H^+]^+$, 925.6 $[M - Py\text{-}CH_2\text{-}Py + Na^+]^+$, 903.5 $[M - Py\text{-}CH_2\text{-}Py + H^+]^+$, 559.5 $[M + 2Na^+]^{2+}$, 548.6 $[M + H^+ + Na^+]^{2+}$, 537.5 $[M + 2H^+]^{2+}$.

DNA cleavage experiments, general remarks

pUC18 plasmid DNA, isolated from *E. Coli* XL1 Blue, was purified using QIAGEN maxi kits. Restriction enzymes and buffers were purchased from New England Biolabs (NEB). Agarose used for the agarose slab gels was purchased from Invitrogen. A solution of number IV dye,[2] consisting of 0.08 % bromophenol blue and 40% sucrose (6 × conc.) was added to the samples prior to electrophoresis. All gels were run on 1.2 % agrose slab gels for 90 min. at 70 V. Gels were stained in a ethidium bromide bath (1.0 μg/mL) for 45 minutes.[3] Pictures of the agarose slab gels were taken with a Appligience High Performance CCD camera. The bands on the film were quantified using the software program Scion Image 4.0.3.2.

Determination of the correction factor for supercoiled pUC18 plasmid DNA[4]

A general stock solution (consisting of pUC18 plasmid DNA, restriction buffer 3 (NEB) and water) was divided in 60 identical samples (0.1 μg pUC18 plasmid DNA per sample). PstI restriction enzyme (6 units; 60 fold excess) or an identical volume of water were added to the samples to a final volume of 50 μL. The samples were incubated for 2 hr at 37°C, followed by heating at 80°C for 20 minutes. After cooling to room temperature 10 μL loading buffer was added. 15 μL of each sample was loaded onto an agarose gel. After running and sequential staining of the gel in an ethidium bromide bath, the intensities of the bands of both form I and form III were compared (Fig. S4). From the relative difference in intensity between supercoiled and linear forms, a correction factor of 1.31 (+/- 0.04) was determined.

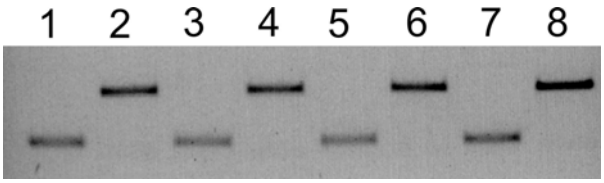


Fig. S4 Agarose gel slab after running and staining; Odd lanes: supercoiled pUC18 plasmid DNA, no restriction enzyme added; even lanes: linearized DNA by restriction digest of supercoiled pUC18 plasmid DNA.

pUC18 plasmid DNA cleavage studies

A solution of **3a-c** or **4** (*in situ* prepared by addition of 2.0 equiv. (1.0 equiv. in the case of **4**) of $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ to the ligand in H_2O) and a solution of dithiothreitol (DTT) were added simultaneously to a buffered (Tris-HCl, 10 mM, pH = 8.0) solution of supercoiled or nicked (*vide infra*) pUC18 plasmid DNA (0.1 μg/μL; 300 μM in base pairs) to give a final concentration of 1.0 μM (in iron(II)) and 1.0 mM reductant. The mixture with a final volume of 50 μl was incubated at 37°C. At indicated times (0.33, 0.66, 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 40, 50 and 60 min) a sample (2 μL) was taken from the reaction, diluted in 15 μL water and 3 μL loading buffer and immediately frozen in liquid nitrogen.

Isolation of nicked DNA

To a solution of supercoiled pUC18 plasmid DNA (206 μg) in water and restriction buffer 3 (NEB) was added N.BstI (250 units; 1.2 fold excess). The mixture was

incubated at 55°C for 4 hrs, followed by heating to 80°C for 20 min. After cooling to room temperature, the mixture was added to a premixed solution of buffers 1, 2 and 3 of a QIAGEN maxi-kit (SDS removed via centrifugation). The mixture was loaded on a column (QIAGEN maxi kit) and the manufacturer's protocol was followed. The DNA was isolated and the concentration was determined by measurement of A_{260}. Gel electrophoresis showed a complete conversion of supercoiled to nicked pUC18 plasmid DNA (Fig. S5). Yield 61 μg (30%).

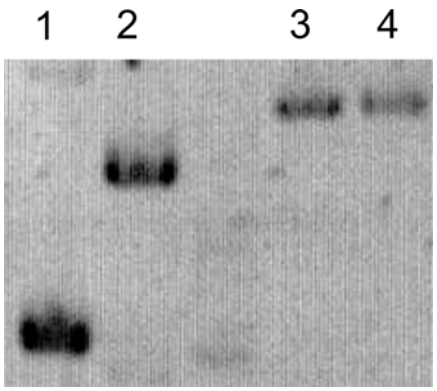


Fig. S5 Gel electrophoresis of isolated nicked DNA. Lane 1, supercoiled pUC18 plasmid DNA; lane 2, pUC18 plasmid digested with PstI (linear DNA); lanes 3-4, Samples of the isolated nicked DNA.

Statistical analysis

The amount of linear DNA is related to the number of double strand breaks (*m*) per molecule by the first term of a Poisson distribution:

$$f(III) = m \times e^{-m}$$

where *f(III)* is the fraction of linear (form III) DNA. The average number of single strand cuts (*n*), can be calculated from the remaining fraction supercoiled (form I) DNA, *f(I)*, by:

$$f(I) = e^{-(n+m)}$$

Errors in both *n* and *m* were calculated using the minimum and maximum values obtained for forms I and III.

Titration of $(NH_4)_2Fe(SO)_4$ to ligand 3c

UV/Vis: To a solution of **3c** in H_2O was added 0, 1, 2 or 3 equivalents of $(NH_4)_2Fe(SO)_4$. The relative extinction coefficient ($\varepsilon/\varepsilon_{max}$) was plotted against the amount of equivalents of $(NH_4)_2Fe(SO)_4$ (Fig. S1b).

^{1}H NMR: A solution of **3c** in D_2O (with acetone as internal standard[5]) was titrated with $(NH_4)_2Fe(SO)_4$ (Fig. S2a). The relative area of the peak at δ 25.9 ppm with respect to the acetone peak was plotted against the amount of equivalents of $(NH_4)_2Fe(SO)_4$ (Fig. S2b).

References and notes

[1] Roelfes, G.; Branum, M. E.; Wang, L.; Que, Jr., L.; Feringa, B. L. *J. Am. Chem. Soc.* **2000**, *122*, 11517-11518.

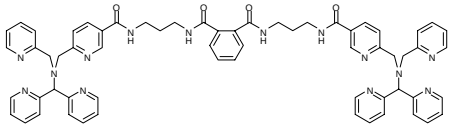
[2] Sambrook, J.; Russell, D. W. *Molecular Cloning: a Laboratory Manual, Third Edition*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor (NY): 2001.

[3] Bauer, W.; Vinograd, J. *J. Mol. Biol.* **1968**, *33*, 141-171.

[4] Shubsda, M. F.; Goodisman, J.; Dabrowiak, J. C. *J. Biochem. Biophys. Methods* **1997**, *34*, 73-79.

[5] The solvent residual peak of D_2O tends to shift after the addition of the $(NH_4)_2Fe(SO)_4$. Therefore, the spectra were referenced with respect to the acetone peak (δ 2.22 ppm).

N^1,N^2-bis(3-{[(6-{[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl}-3-pyridinyl)carbonyl]amino}propyl)phthalamide (2a)



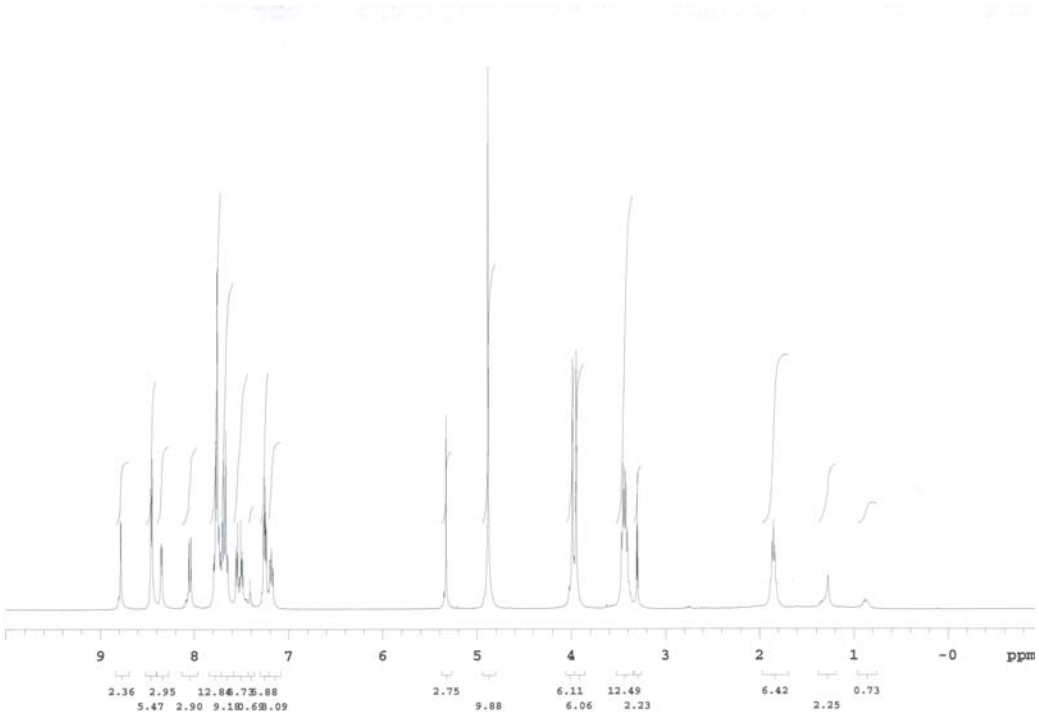


Fig. S7 ^{1}H NMR **2a**

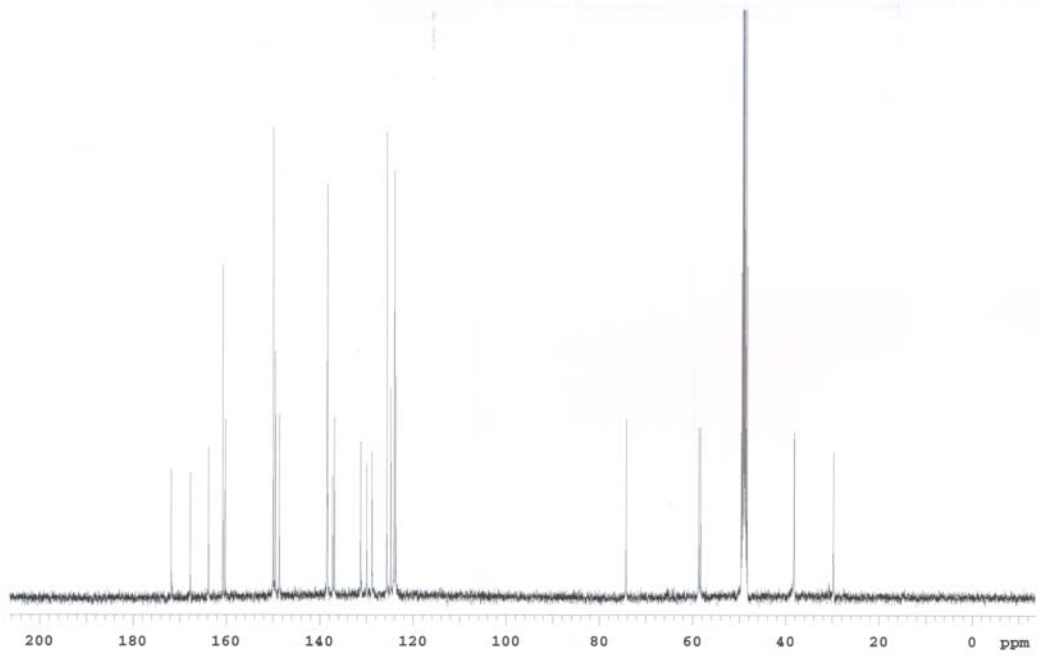
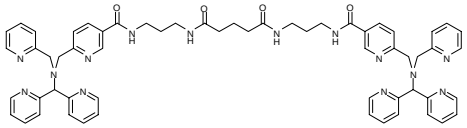


Fig. S8 ^{13}C NMR **2a**

N^1,N^5-bis(3-{[(6-{[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl}-3-pyridinyl)carbonyl]amino}propyl)pentanediamide (2b)



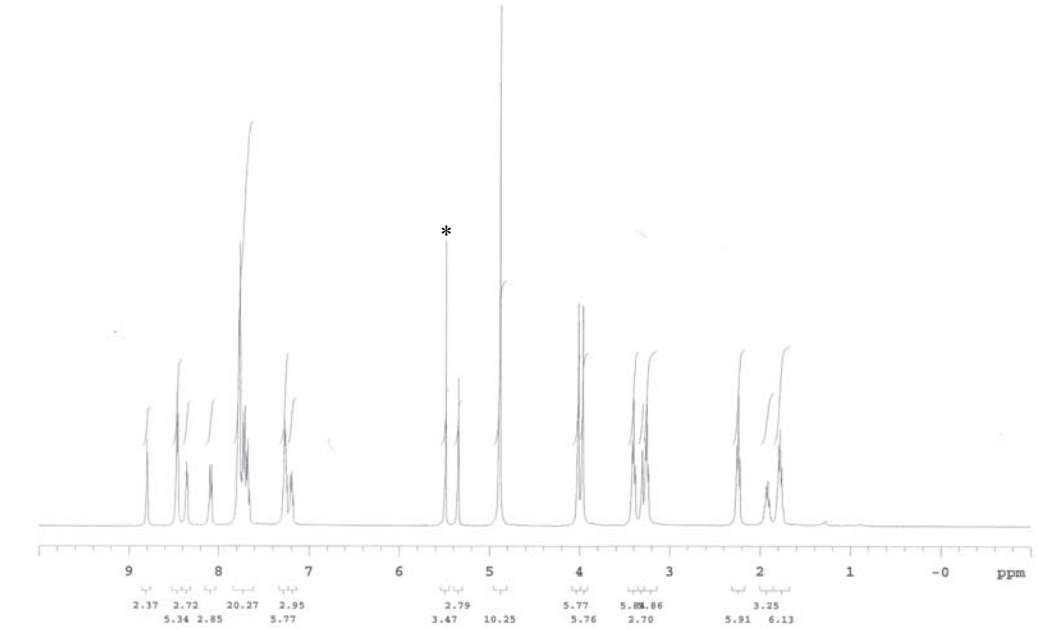


Fig. S9 ^{1}H NMR **2b**

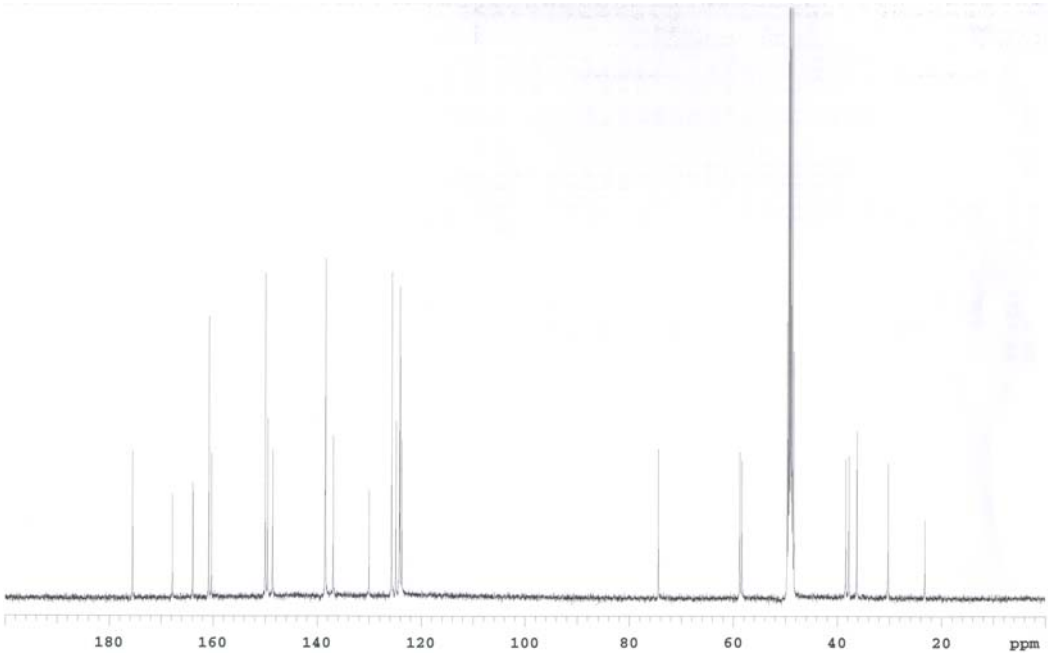
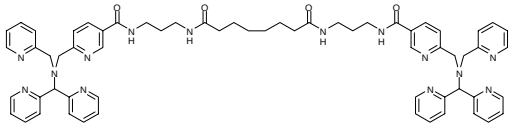


Fig. S10 ^{13}C NMR **2b**

N^1,N^8-bis(3-{[(6-{[[di(2-pyridinyl)methyl](2-pyridinylmethyl)amino]methyl}-3-pyridinyl)carbonyl]amino}propyl)octanediamide (2c)



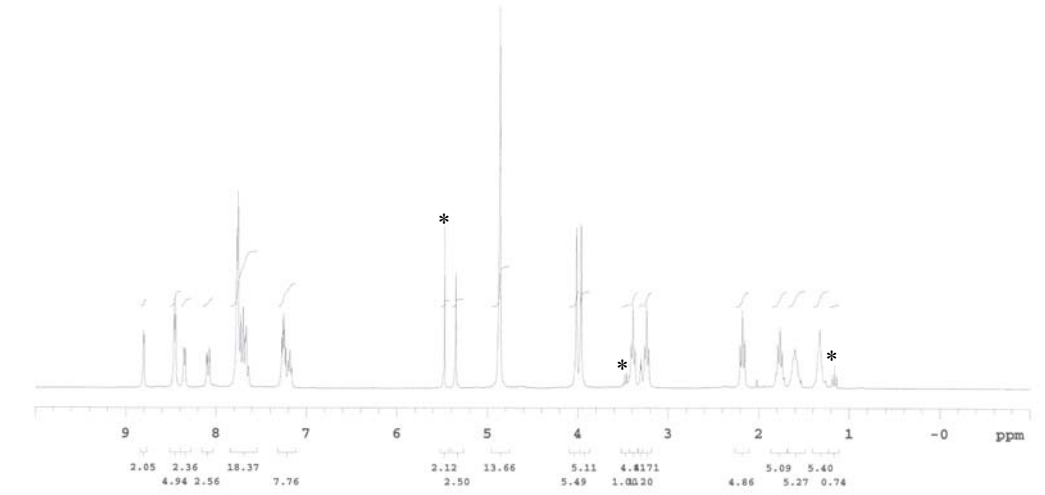


Fig. S11 ^{1}H NMR **2c**

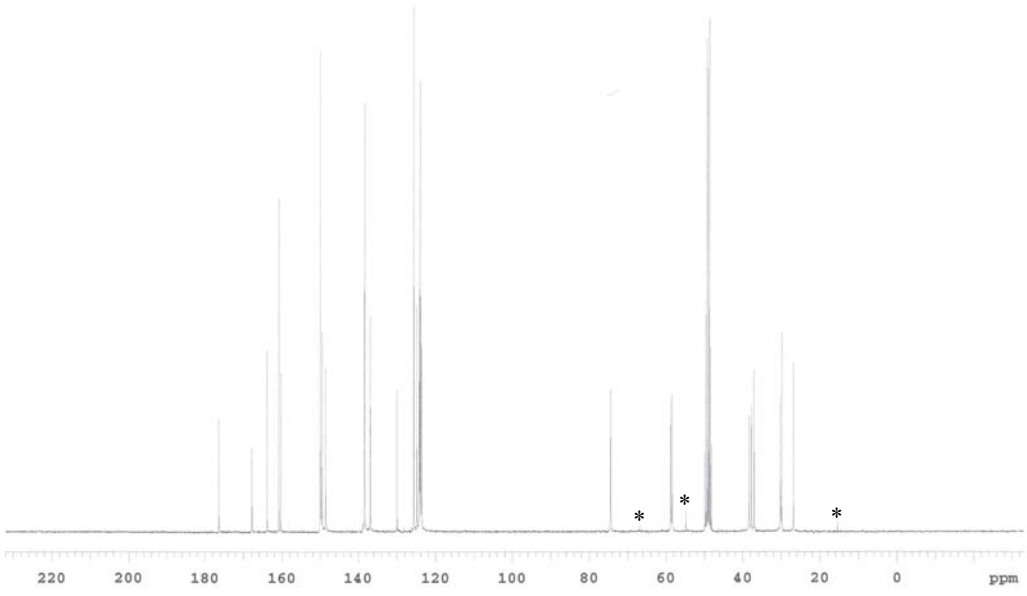


Fig. S12 ^{13}C NMR **2c**

* Residual solvent peaks (DCM/Et_2O) from precipitation.