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Induction of B- to Z-DNA transition by copper and zinc complexes with C(15) substituted macrocyclic pentaaza ligands†

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The new macrocyclic ligand 15-fluoro-15-methyl-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**2**) was synthesised and its crystal structure determined together with the ones of the known analogues of **2**, 15-fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**1**) and 15,15-difluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**3**). The binding behaviour of all three ligands to copper and zinc was studied in the solid state. They can bind to the metal centre by either triple coordination (N3) with all secondary amines or after double deprotonation of the two amides with all five nitrogen atoms (N5). The N5 coordination mode is favoured by the presence of one or two fluorine substituents at the C(15) position and by a high pH in the case of aqueous solutions. Circular dichroism titrations of poly d(GC) with the metal complexes showed that only **4** and **5**, that is the copper complexes of **1** and **2**, induced a complete B- to Z-DNA transition. The degree of cooperativity of the transition was found to be 3.4 and 7.3 for **4** and **5** respectively. As a possible hypothesis to explain this difference, the additional methyl group in **5** compared with **4** may be involved in a hydrophobic interaction with the DNA. Ligand **2**, the copper complex **6** of the bis fluoro substituted ligand **3**, and the zinc complex **7** of ligand **1** did not induce any change in the direction of Z-DNA. In the case of **6**, the CD spectrum of the DNA actually showed no change at all, indicating that the complex was even not interacting with the B form of DNA. Therefore it is assumed that the bis fluoro substitution is causing the complex to be in the neutral N5 coordination mode at the experimental conditions of pH 7. The electrostatic contribution together with the shielding effect of the ligand might explain the absence of any interaction with the DNA.

Introduction

DNA is a flexible and dynamic molecule that can assume a variety of interconverting forms. Each form has its own biological role in the regulation of the life of the cell. Selective stabilisation of one of these forms can help to discover and better understand their biological roles. Genetic information is provided by the DNA in at least two different ways. First, the sequence of its nucleotides determines the primary structure of the proteins. Second, DNA can regulate gene expression through its shape.[1]

Since the left-handed Z-DNA is distinguished from the other DNA conformations by its own very characteristic geometry,[2] it can be recognised due to its geometrical features and to a less extent by the sequences of the nucleobases. Aliphatic, including naturally occurring, polyamines are able to induce the formation of Z-DNA.[3,4] There have been several reports about synthetic, rigid organic compounds which favor the formation of Z-DNA.[5,6] Some transition metals such as copper or nickel are also known to induce Z-DNA,[7–9] but this process is strongly inhibited in the presence of only 10 mM sodium chloride.[10,11] Copper ions were found to bind to N(7) of guanine when crystals of a hexamer in the Z-DNA form were soaked with $CuCl_2$.[12] Furthermore the complex of copper with diethylenediamine but not copper chloride on its own was shown to induce Z-DNA.[13] Rokita and co-workers synthesised the small complex NiHFGN (Fig. 1) and showed that it promotes the conversion of poly d(GC) from the B to the Z form in a molar ratio complex : nucleotides = 1 : 3.[14] The basic core of HFGN (1,4,7,10,13-pentaazacyclohexadecan-14,16-dione) had been introduced by Kimura and co-workers when studying the corresponding copper and nickel complexes intended to inhibit superoxide dismutase and work as an oxygen activator respectively.[15,16]

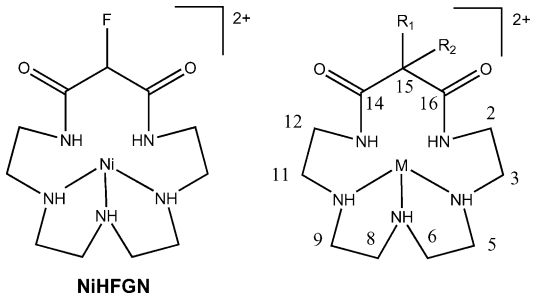

Fig. 1 Left: complex NiHFGN synthesised by Rokita and co-workers.[14] Right: structure of the complexes studied (see Table 1).

Table 1 Numbering scheme of the complexes studied

M	$R_1 = F, R_2 = H$	$R_1 = F, R_2 = Me$	$R_1 = F, R_2 = F$
—	**1**	**2**	**3**
Cu	**4**	**5**	**6**
Zn	**7**	—	**8**

We investigated how different metals on the one hand and various substituents at the C(15) position of 1,4,7,10,13-pentaazacyclohexadecan-14,16-dione on the other hand influence the ability of the resulting metal complexes to promote the transition from B- to Z-DNA relative to the original complex NiHFGN. The substitutents were chosen to have very different electronic but similar sterical properties.

Experimental

Chemicals and methods

Chemicals were purchased from Aldrich or Fluka and used without further purification. Diethyl fluoromalonate was purchased from Apollo Scientific Limited, and diethyl difluoromalonate from ABCR. Tetraethylene pentamine was isolated from tetraethylene pentamine pentahydrochloride in 96% yield.[17]

† Electronic supplementary information (ESI) available: ORTEP plots and crystallographic information tables for **1**·2HCl, **2**·2HCl, and **3**. CD spectrum of **2** added to poly d(GC). See http://www.rsc.org/suppdata/dt/b5/b500317b/

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Table 2 Crystallographic data of **4**, **10**, **7**, and **5**

	4	**10**	**7**	**5**
Empirical formula	$C_{15}H_{30}CuFN_5O_7$	$C_{11}H_{22}CuFN_5O_3$	$C_{15}H_{30}FN_5O_7Zn$	$C_{16}H_{32}CuFN_5O_{27}$
M/g mol^{-1}	474.98	354.88	476.81	489.01
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$I2/a$	$P2_1/n$	$I2/a$	$P2_1/n$
a/Å	15.0078(6)	8.7165(5)	15.2026(8)	10.2427(5)
b/Å	9.2569(5)	11.8274(8)	9.1232(4)	13.4133(10)
c/Å	29.9742(12)	14.3976(8)	30.4076(14)	16.2893(8)
α/°	90	90	90	90
β/°	100.063(5)	101.960(6)	100.810(6)	100.645(6)
γ/°	90	90	90	90
V/Å^3	4100.1(3)	1452.08(15)	4142.6(3)	2199.4(2)
Z	8	4	8	4
μ/mm^{-1}	1.121	1.534	1.241	1.048
Reflections collected	23587	26374	43906	23320
Independent reflections [R_{int}]	4937 [0.0796]	3978 [0.0977]	6266 [0.0547]	5317 [0.0760]
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0511$ $wR_2 = 0.1286$	$R_1 = 0.0528$ $wR_2 = 0.1358$	$R_1 = 0.0455$ $wR_2 = 0.1258$	$R_1 = 0.0697$ $wR_2 = 0.2052$

15-Fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**1**) was synthesised according to the literature.[18] All reactions were performed under a nitrogen atmosphere. The reactions were monitored by HPLC or thin layer chromatography (TLC). TLC was carried out on 0.25 mm Merck silica gel aluminium plates (60 F_{254}) or aluminium oxide pre-coated plastic sheets (alox N/UV_{254}) using UV light or the Schlittler staining solution[19] as the visualising agent.

Column chromatography was performed on silica gel (particle size 0.040–0.063 mm) or aluminium oxide (basic, 0.05–0.15 mm, pH 9.5 ± 0.5). Ion exchange chromatography was performed on DOWEX 2 X8 20–50 or AMBERLYST A26-OH.

Electrospray ionisation (ESI) mass spectra were recorded on a Merck Hitachi M-8000 spectrometer. Electron impact (EI) and fast atom bombardment (FAB) mass spectra were recorded on a Finnigan MAT mass spectrometer model 8230 equipped with an ION Tech ionisator (Teddington England). In the case of FAB-MS the samples were dissolved in ethylene glycol and *para*-nitrobenzyl alcohol (*p*-NBA) was used as a matrix.

Gas chromatography-mass spectra (GC-MS) were recorded on a Varian-Chrompack CP-3800 gas chromatographer equipped with a Saturn 2000 electron impact mass spectrometer (EI-MS). The samples were carried by He through a CP-SIL 8 CB-MS column (30 m × 0.25 mm). UV/vis spectra were measured on a Varian Cary 50 spectrometer. IR spectra were recorded on a Perkin Elmer BX FT-IR spectrometer using samples in KBr pellets. NMR spectra were determined with the help of a Varian Gemini 200 or 300 MHz and a Bruker DRX 500 MHz spectrometer. The chemical shifts are relative to residual solvent protons as reference. Circular dichroism measurements were performed using a Jasco J-810 spectropolarimeter equipped with a Jasco PFD-4255 Peltier temperature controller. Elemental analyses were carried out on a Leco CHNS-932 elemental analyser.

Crystallographic data were collected on a Stoe IPDS diffractometer at 183(2) K using a graphite-monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). Suitable crystals were covered with Paratone N oil, mounted on top of a glass fibre and immediately transferred to the diffractometer. Eight thousand reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit cell parameter refinement with the program CELL.[20] Data were collected for Lorentz and polarisation effects as well as for absorption (numerical). Structures were solved with direct methods using SHELXS-97[21] or SIR97[22] and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[23] Crystallographic data is presented in Table 2. ORTEP plots were drawn with the program ORTEP-3 for Windows[24] at a probability of 50%; non-acidic hydrogen atoms were omitted for clarity.

CCDC reference numbers 260290–260295 and 265759.

See http://www.rsc.org/suppdata/dt/b5/b500317b/ for crystallographic data in CIF format.

Syntheses of mononucleating ligands and complexes

2-Fluoro-2-methyl-diethyl malonic ester[25] (9). Diethyl fluoromalonate (4.5 ml, 22.5 mmol) was added dropwise to a suspension of NaH (2.7 g, 112.5 mmol) in 60 ml of dry THF. The mixture was stirred at r.t. until the gas evolution ceased. Iodomethane (1.43 ml, 22.9 mmol) was added dropwise to the mixture, which was stirred at r.t. for 12 h. The suspension was then filtrated and the solvent was removed under vacuum. The residue was dissolved in water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on silica gel. The elution was started with hexane 100% and then changed to 5 : 1 hexane : CH_2Cl_2 to obtain a colourless viscous oil. Yield 2.87 g (66.5%). $R_f = 0.1$ (100% hexane). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.28$ (t, $J = 7.2$ Hz, 6 H, CH_3), 1.75 (d, $J = 23.7$ Hz, 3 H, $CFCH_3$), 4.25 (q, $J = 7.2$ Hz, 4 H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 13.76$ (CH_3), 20.48 (d, $J = 23$ Hz, $CFCH_3$), 62.50 (CH_2), 92.30 (d, $J = 194$ Hz, CF), 166.90 (d, $J = 25$ Hz, C=O). MS (GC-MS): R.T. = 9.14 min; m/z (%) = 193 (100) $[M]^+$.

15-Fluoro-15-methyl-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (2). A solution of 2-fluoro-2-methyl-diethyl malonic ester (**9**) (908 mg, 4.73 mmol) in 2 ml of dry ethanol was added to a solution of 0.89 g of tetraethylenepentamine (4.7 mmol) dissolved in 62 ml of dry ethanol. The solution was refluxed for 2 days. The solvent was then removed under vacuum and the crude residue was recrystallised from 20 : 1 CH_3CN : MeOH as a white solid. Since the product was partially a hydrochloride salt, it was purified by ion-exchange chromatography (AMBERLYST A-26 OH). The AMBERLYST was washed with bidistilled water and with MeOH, and was then activated with 1 M NaOH. The product was recovered by using water as eluent. Yield 177 mg (42%). $R_f = 0.17$ (80% CH_2Cl_2, 18% MeOH, 2% conc. ammonia). ^{1}H NMR (300 MHz, D_2O): $\delta = 1.62$ (d, $J = 23.4$ Hz, 3 H, CH_3), 2.53–2.64 (m, 12 H, CH_2), 3.05–3.65 (m, 4 H, CH_2NHCO). ^{13}C NMR (75 MHz, D_2O): $\delta = 18.99$ (d, $J = 23.1$ Hz, CH_3), 36.47 (CH_2), 44.52 (CH_2), 44.58 (CH_2), 44.67 (CH_2), 93.40 (d, $J =$ 196 Hz, CF), 167.79 (d, $J = 22.7$ Hz, C=O). MS (ESI): m/z (%) = 290 (100) $[M + H]^+$, 272 (42) $[M - F + 2H]^+$. IR (KBr): 1691 cm^{-1} νC=O.

15,15-Difluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione[26] (3). Diethyl difluoromalonate (1 ml, 5.924 mmol) was added to a solution of 1.12 g of tetraethylenepentamine (5.920 mmol) in 110 ml of dry ethanol. The solution was refluxed for 24 h. The solvent was then removed under vacuum and the

crude residue was recrystallised from 10 : 1 CH_3CN : MeOH to give a white solid. Yield 692.5 mg (40%). R_f = 0.76 (80% CH_2Cl_2, 18% MeOH, 2% conc. ammonia; on alox). ^{1}H NMR (500 MHz, MeOH-d_4): δ = 2.69 (s, 8 H, H(5,6,8,9)), 2.77–2.79 (m, 4 H, H(3,11)), 3.45–3.47 (m, 4 H, H(2,12)). ^{13}C NMR (125 MHz, MeOH-d_4): δ = 39.26 (C(2,12)), 48.69 (C(5,9)), 49.50 (C(6,8)), 49.76 (C(3,11)), 110.33 (t, J = 261 Hz, C(15)), 164.47 (t, J = 24.4 Hz, C(14,16)). MS (ESI): m/z (%) = 293 (100) $[M]^+$, 274 (48) $[M - F]^+$. EA calcd for $C_{11}H_{21}F_2O_2N_5$: C, 45.02; H, 7.21; N, 23.88; found: C, 45.81; H, 7.10; N, 23.84. IR (KBr): 1695 cm^{-1} νC=O.

Cu(II) complex of 15-fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (4). 15-Fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**1**) (50 mg, 0.1818 mmol) was added to a solution of 36.3 mg of copper acetate monohydrate (0.1818 mmol) in 15 ml of dry ethanol. The solution was stirred at r.t. for 3 h. The solvent was removed under vacuum to yield a blue product. Violet crystals were obtained from vapour diffusion of hexane into an ethanolic solution of the complex. Yield 82 mg (quantitative). MS (FAB): m/z (%) = 336 (100) $[M]^+$. EA calcd for $C_{15}H_{28}CuFN_5O_6{\cdot}2H_2O$: C, 36.57; H, 6.55; N, 14.22; found: C, 36.63; H, 6.32; N, 14.40. IR (KBr): 1696 cm^{-1} νC=O$_{ligand}$, 1570 cm^{-1} νC=O$_{acetate}$ (blue complex). IR (KBr): 1618 cm^{-1} νC=O$_{ligand}$ (violet complex). UV-Vis (10^{-3} M, EtOH): λ_{max} = 580 nm (ε = 107 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, bi-distilled water, pH = 5): λ_{max} = 606 nm (ε = 135 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, 1 mM sodium cacodylate buffer, pH = 7): λ_{max} = 585 nm (ε = 136 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, water adjusted with 0.1 M NaOH to a pH of 9.5): λ_{max} = 577 nm (ε = 136 cm^{-1} M^{-1}).

Cu(II) complex of 15-fluoro-15-methyl-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (5). 15-Fluoro-15-methyl-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione·1.5HCl (**2**) (50 mg, 0.145 mmol) was added to a green solution of 31.82 mg of copper acetate monohydrate (0.145 mmol) in 18 ml of dry ethanol. The dark blue solution was stirred at r.t. for 3 h. The ethanol was removed under vacuum to yield a blue residue, which was passed through an ion-exchange column (DOWEX2 X8 20–50, previously equilibrated with a saturated solution of ammonium acetate) with water. Yield 65 mg (95%). MS (ESI): m/z (%) = 350 (100) $[M - 2$ acetate$]^+$. IR (KBr): 1692 cm^{-1} νC=O$_{ligand}$, 1564 cm^{-1} νC=O$_{acetate}$. UV-Vis (10^{-3} M, bi-distilled water, pH = 5): λ_{max} = 614 nm (ε = 92 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, 1 mM sodium cacodylate buffer, pH = 7): λ_{max} = 606 nm (ε = 89 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, water adjusted with 0.1 M NaOH to a pH of 9.5): λ_{max} = 565 nm (ε = 120 cm^{-1} M^{-1}).

Cu(II) complex of 15,15-difluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (6). $CuCl_2{\cdot}H_2O$ (29 mg, 0.171 mmol) was dissolved in 4 ml of absolute ethanol. Silver triflate (87.6 mg, 0.341 mmol) was added to the green solution, which became light blue while a precipitate of AgCl formed. The mixture was stirred for 30 min and was then filtrated. 15,15-Difluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**3**) (50 mg, 0.17 mmol) was added to the solution. The colour immediately turned dark blue. The solution was stirred at r.t. for 3 h and the solvent removed under vacuum to give a glassy blue product. Yield 111 mg (quantitative). MS (FAB): m/z (%) = 338 (29%) $[M - F + H]^+$, 356 (100) $[M]^+$. EA calcd for $C_{13}H_{21}CuF_8N_5O_8S_2$: C, 23.83; H, 3.23; N, 10.69; found: C, 23.91; H, 3.57; N, 10.46. IR (KBr): 1718 cm^{-1} νC=O$_{ligand}$, 1256 cm^{-1} νS=O$_{triflate}$, 1031 cm^{-1} νS–O$_{triflate}$, 639 cm^{-1} δO–S–O$_{triflate}$. UV-Vis (10^{-3} M, EtOH): λ_{max} = 604 nm (ε = 122 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, bi-distilled water, pH = 5): λ_{max} = 596 nm (ε = 84 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, 1 mM sodium cacodylate, buffer pH = 7): λ_{max} = 587 nm (ε = 102 cm^{-1} M^{-1}). UV-Vis (10^{-3} M, water adjusted with 0.1 M NaOH to a pH of 9.5): λ_{max} = 577 nm (ε = 104 cm^{-1} M^{-1}).

Zn(II) complex of 15-fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (7). 15-Fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**1**) (50 mg, 0.182 mmol) was added to a solution of 39.9 mg of zinc acetate·2H_2O (0.182 mmol) in 15 ml of dry ethanol. The solution was refluxed for 12 h and the solvent removed under vacuum to obtain a white product. Yield 83 mg (quantitative). ^{1}H NMR (500 MHz, MeOH-d_4): δ = 1.96 (s, 6 H, CH_3), 2.40–3.57 (m, 16 H, CH_2). EA calcd for $C_{15}H_{28}FN_5O_6Zn{\cdot}2H_2O$: C, 36.49; H, 6.53; N, 14.19; found: C, 36.37; H, 6.52; N, 14.16. IR (KBr): 1700 cm^{-1} νC=O$_{ligand}$, 1576 cm^{-1} νC=O$_{acetate}$.

Zn(II) complex of 15,15-difluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (8). 15,15-Difluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**3**) (50 mg, 0.171 mmol) was added to a solution of 37.4 mg of zinc acetate·2H_2O (0.171 mmol) in 10 ml of dry ethanol. The solution was refluxed for 3 h and the solvent removed under vacuum to obtain a white product. Yield 81 mg (quantitative). MS (FAB): m/z (%) = 356 (100) $[M]^+$. ^{1}H NMR (200 MHz, MeOH-d_4): δ = 1.95 (s, 6 H, CH_3), 2.49–3.45 (m, 16 H, CH_2). IR (KBr): 1715 cm^{-1} νC=O$_{ligand}$, 1597 cm^{-1} νC=O$_{acetate}$.

CD titration of poly d(GC) with 1, 4, 5, 6, 7 and Cu(OAc)$_2$

A 0.1 mM poly d(GC) solution in 1 mM sodium cacodylate buffer (pH = 7) was titrated with various aliquots of 1 mM solutions of the metal complexes **4**, **5**, **6**, **7**, the ligand **1**, or of $Cu(OAc)_2$ in the same buffer. The samples were warmed to 60 °C for 5 min and then cooled down to 25 °C for the CD measurements. The CD spectra were smoothed by adjacent averaging.

Results and discussion

The new ligand **2** was synthesised analogously to the known ligands **1** [18] and **3** [26] in a yield of 42%. The crystal structures of **1**·2HCl, **2**·2HCl, and **3** were determined and are shown in the ESI.† The presence of the hydrochlorides in **2** originates from HCl abstraction of the solvent used (dichloromethane) to grow the crystals. In the case of **3** the use of only partially neutralised pentahydrochloride salt of tetraethylene pentamine, following the published procedure,[17] caused the presence of the HCl molecules.

Complexes of ligand 1

The reaction of ligand **1** with either one equivalent of copper(II) or zinc(II) acetate at r.t. in ethanol gave the blue copper(II) complex **4** or the colourless zinc(II) complex **7**, respectively. The blue Cu compound **4** has an amide carbonyl IR vibration at 1696 cm^{-1} (in KBr) and an UV-Vis absorption maximum dependent on the solvent and on the pH of the solution (Table 3). It can also be seen that the λ_{max} of absorbance of complex **4** moves to lower values in more alkaline solutions, which corresponds to the formation of the penta-nitrogen coordinated complex (Fig. 2).

This change in the coordination mode can also be seen from the colour of the solution of the complex, which changes from blue (at pH = 5, tri-amino coordination mode) to violet (at pH = 9.5, penta-nitrogen coordination mode). Two different crystal types were obtained depending on the solvent system used to grow them. The blue crystals of **4** were obtained by vapour diffusion of pentane in CH_2Cl_2. The copper(II) centre is in a distorted trigonal bipyramidal conformation (Fig. 3). The three amine nitrogen atoms coordinate meridionally, while the

Table 3 Dependency of the λ_{max} on solvent and pH for complexes **4**, **5**, and **6**

Complex	Ethanol	pH = 5	pH = 7	pH = 9.5
4	580	606	585	577
5	—	614	606	565
6	604	596	587	577

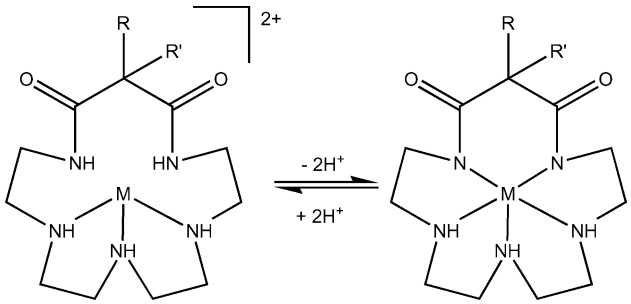

Fig. 2 Equilibrium between the tri-amino and the bis deprotonated penta-nitrogen coordinated metal complexes.

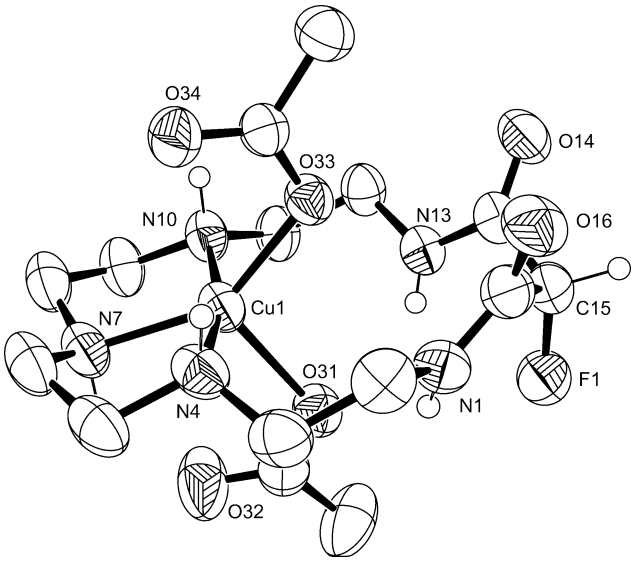

Fig. 3 ORTEP plot of the blue complex **4**, solvent molecule omitted.

remaining positions are occupied by two acetate groups. One acetate group is asymmetrically coordinated to the copper, since the oxygen atom O(34) is only weakly bonded to the metal with an O–Cu distance of 2.877(3) Å. The other acetate is mono-coordinated *via* O(31) to the metal centre, its second carbonyl oxygen O(32) forming a weak hydrogen bond to N(7) (2.943 (6) Å). The fluorine atom is in an axial position relative to the macrocyclic ring.

By vapour diffusion of hexane into a solution of ethanol, we obtained the violet crystals of **10** in which the copper(II) centre is coordinated to the five nitrogen atoms of the ligand in a distorted square pyramidal conformation due to previous deprotonation of the amide nitrogen atoms (Fig. 4). The overall geometry, with the exception of the additional fluorine atom present in **10**, closely resembles the analogue double deprotonated Ni complex LM in Fig. 2 (M = Ni^{2+}, $R_1 = R_2 = H$).[27] The geometrical similarity is not restricted to the molecular level: both complexes crystallised in the same space group, with the unit cells of the two crystal structures being almost identical. Obviously, the extra fluorine atom in **10** does not disrupt the crystal packing compared with Kimura's structure. The complex **10** shows an amide carbonyl IR vibration at 1618 cm^{-1} (in KBr) compared with 1696 cm^{-1} in the case of **4**. This is a clear sign that the metal ion in **10** is coordinated to the three secondary amines and to the two deprotonated amide nitrogen atoms of the ligand. This shift of the IR frequencies of the amide bonds has also been observed in the analogue nickel complexes by Rokita *et al.*[28]

Comparing the carbonyl bond lengths of the two different copper crystals **4** and **10**, one can notice that they are longer in the purple complex **10** (1.267(4) Å) than in the blue crystals **4** (1.227(4) Å). On the other hand, the corresponding C(16/14)–N(1/13) and C(15/15)–C(14/16) bond lengths are the same in both complexes. Therefore the negative charge is only delocalised onto the oxygen atoms, which indicates a stronger single bond

Table 4 Selected bond lengths of complexes **4**, **5**, **10**, and **7**

	4 (M = Cu)	**5** (M = Cu)	**10** (M = Cu)	**7** (M = Zn)
M(1)–N(1)			1.960(3)	
M(1)–N(4)	2.077(3)	2.072(5)	2.058(3)	2.185(2)
M(1)–N(7)	2.030(3)	2.026(4)	2.077(3)	2.0876(19)
M(1)–N(10)	2.063(3)	2.071(5)	2.030(3)	2.201(2)
M(1)–N(13)			2.063(3)	
M(1)–O(31)	2.158(3)	2.199(4)		2.0042(16)
M(1)–O(33)	1.995(2)	1.966(3)		2.0103(15)

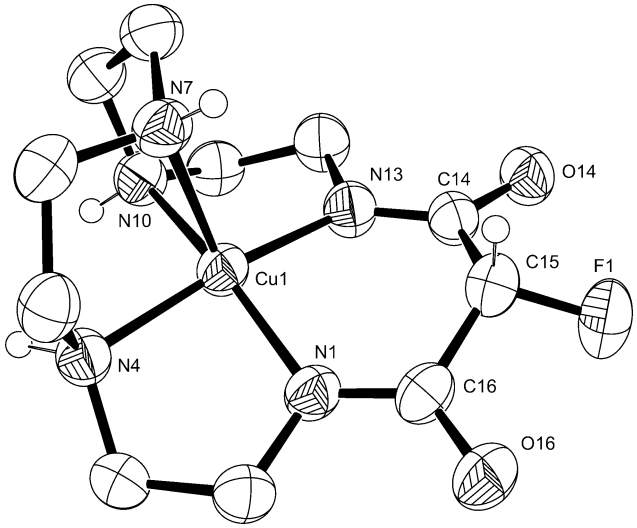

Fig. 4 ORTEP plot of the violet complex **10**, solvent molecule omitted.

character of the carbonyl bonds. An enolate is not formed, though this would be doubly stabilised by the adjacent carbonyl groups. The pyramidalisation of C(15), the bond lengths of C(15)–C(14) as well as C(15)–C(16) in **4** compared with **10**, and the existence of the experimentally found (but not refined) hydrogen atom on C(15) exclude this possibility.

The colourless Zn(II) complex **7** shows an IR amide bond absorption at 1700 cm^{-1}; again, this indicates that the metal ion is not coordinated to the amide nitrogen atoms of the ligand. In the crystal obtained from vapour diffusion of hexane into a solution of chloroform, the Zn(II) centre is coordinated to the three amine nitrogen atoms of the ligand and to the two acetate molecules in a distorted trigonal bipyramidal mode (Fig. 5). Each acetate group acts as a mono-dentate ligand, which is very similar to the case of the copper complex **4**. The fluorine atom is axial relative to the plane of the azamacrocycle.

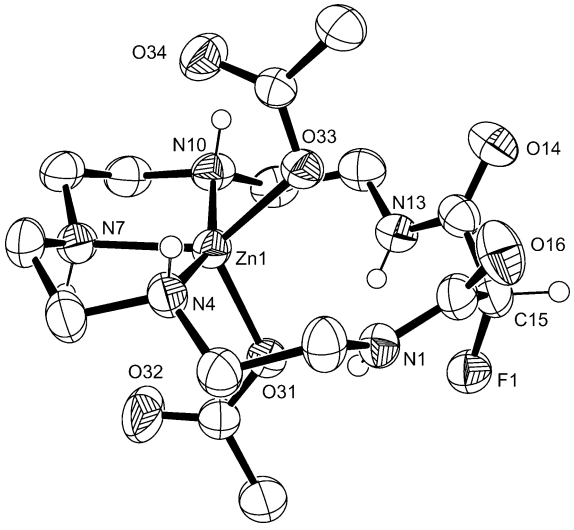

Fig. 5 ORTEP plot of the Zn complex **7** without solvent molecule.

Complexes of ligand 2

The blue copper(II) complex **5** was synthesised by reacting **2**·2HCl with Cu(II)acetate and the chloride was exchanged by acetate ions. As observed for **4**, the UV-Vis absorption maximum of the complex is dependent on the pH of the solution (Table 3). The amide carbonyl IR vibration is at 1692 cm^{-1}.

The X-ray structure of the blue crystals of **5**, obtained by vapour diffusion of pentane into a solution of dichloromethane, shows that the Cu(II) centre is coordinated to the three amine nitrogen atoms of the ligand and to the two acetate molecules in a distorted square pyramidal mode (Fig. 6). Each acetate group contributes as a mono-dentate ligand. A water molecule is linked through a hydrogen bond to oxygen O(34). There are furthermore two weak hydrogen bonds between N(3) and oxygen O(32) as well as between O(34) and N(4).

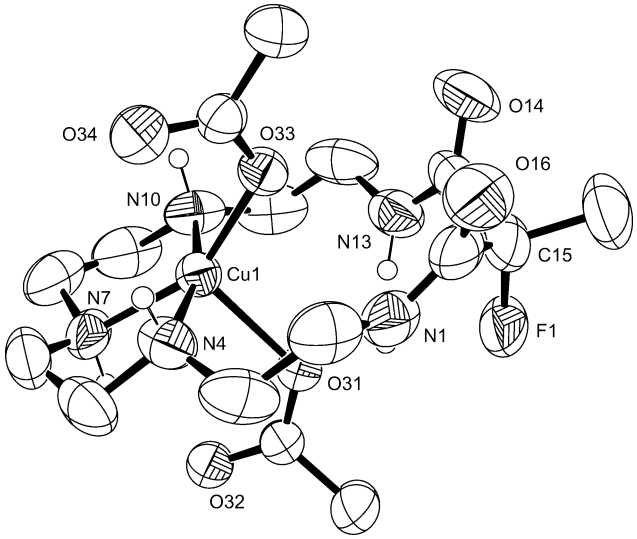

Fig. 6 ORTEP plot of the blue complex **5** without solvent molecule.

Complexes of ligand 3

Ligand **3** was reacted with either Cu(II)triflate or Zn(II)acetate in ethanol at r.t. to form the complexes **6** and **8**, respectively. The blue copper(II) complex **6** was synthesised by reacting the ligand **3** with Cu(II)triflate, which had been previously obtained from $CuCl_2$ and silver triflate. The complex has a UV-Vis absorption maximum dependent on the solvent and on the pH of the solution (Table 3), and an amide carbonyl IR absorption at 1718 cm^{-1}. It was not possible to grow crystals of the complex.

Table 3 shows that the λ_{max} of absorbance of complex **6** moves to lower values as the pH of the solution increases, which corresponds to the formation of the penta-nitrogen coordinated complex. In this case the difference of λ_{max} of absorbance between the solution at pH = 5 and 9 is smaller than in the case of complex **4**. The second fluorine atom, with its electron withdrawing action, enhances the acidity of the amide hydrogen atoms. Once in aqueous solution, these hydrogen atoms are easily removed, leading to the penta-nitrogen coordination of the metal centre.

Induction of Z-DNA by copper acetate, ligand 2, and complexes 4, 5, 6, and 7

The capability of the complexes **4**, **5**, **6**, and **7** to induce the B- to Z-DNA transition was studied. Aliquots (2–5 μl) of a 1 mM solution of each complex were added to an approximate 0.1 mM solution of poly d(GC) in 1 mM sodium cacodylate buffer at pH = 7. The samples were warmed to 60 °C to induce a temporary and partial melting of the DNA. This ensured the completion of the otherwise slow B- to Z-DNA transition.[29] Before measuring the spectra, the samples were cooled down to 25 °C for 5 min.

A titration of poly d(GC) with **2** was carried out to determine if the ligand itself was able to induce B- to Z-DNA transition. The CD spectrum of the poly d(GC) after the addition of the product did not change indicating that the DNA was still in the B-form (Fig. S4).†

The interaction of $Cu(OAc)_2$ with poly d(GC) was studied as a control as to whether copper ions on their own can promote the B- to Z-DNA transition. The CD spectrum showed that even after the addition of 0.6 equivalents of copper *versus* DNA phosphate groups the transition to Z-DNA had not yet reached its midpoint (Fig. 7). Interestingly, Zacharias *et al.* mentioned that copper(II) chloride was not able to induce the B- to Z-DNA transition at all.[30] This might be another case where chloride anions inhibit the transition metal driven induction of Z-DNA.[31] The interaction of complex **7** with poly d(GC) is also shown in Fig. 7. There is a change exclusively in the positive band of the spectrum while the negative band is untouched. Thus the zinc complex **7** binds to the B-DNA, but does not promote the transition from B- to Z-DNA.

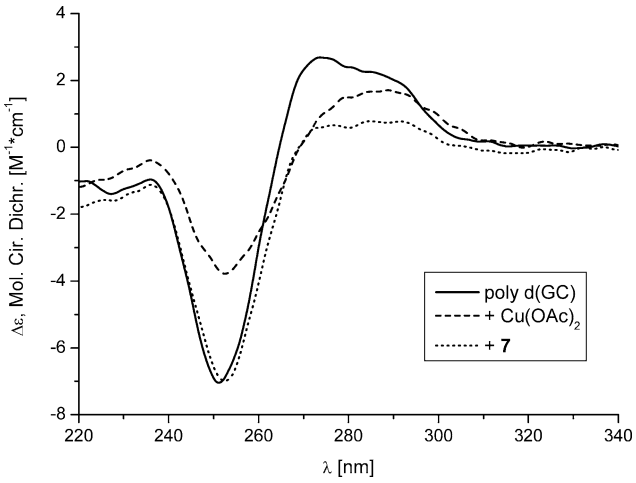

Fig. 7 CD spectra of poly d(GC) before and after the addition of 0.6 equivalents of $Cu(OAc)_2$ or 0.57 equivalents of **7**.

The copper complexes **4**, **5**, and **6** behave in different ways (Fig. 8). In the case of **6**, because of the strong influence of two fluorine substituents on the acidity of the amide hydrogen atoms, the equilibrium in Fig. 2 is shifted to the right side in favour of the penta-nitrogen coordinated copper. The neutral complex is thus not able to interact with the DNA and to promote its transition from B- to Z-form.

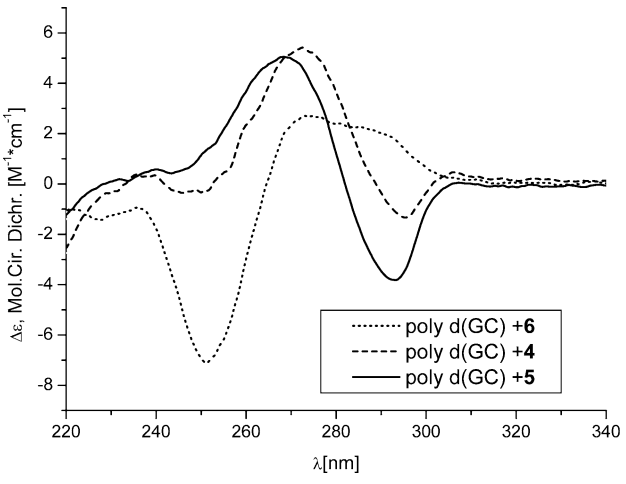

Fig. 8 CD spectra of poly d(GC) after the addition of 0.66 equivalents of **4**, 0.64 equivalents of **5**, and 0.67 equivalents of **6**.

The other two copper complexes **4** and **5** have an amazingly different effect on poly d(GC). Looking at the CD signal at a wavelength of 255 nm, one can easily follow the change from the negative signal of B-DNA to the positive one of Z-DNA. The two titrations of poly d(GC) with **4** and **5** are shown in Figs. 9 and 10, respectively. In the case of complex **4** the midpoint is reached after the addition of 0.37 equivalents of the complex *versus* DNA phosphates and the transition from B- to Z-DNA occurs over a wide concentration range of added **4**. In the case of **5**, the transition starts suddenly after the addition of 0.35 equivalents and the midpoint is achieved after the addition of 0.45 equivalents of the complex *versus* DNA phosphates. Figs. 9 and 10 additionally show the simulation of the experimental curves using the Hill equation for an equilibrium between two states:[32,33]

$$y = y_\infty + \frac{y_0 - y_\infty}{1 + \left(\frac{x}{K}\right)^n}$$

where y is the spectroscopic response, x is the concentration of the added reagent, y_0 is the response when $x = 0$, y_∞ is the maximal response when $x = \infty$, K is the concentration of x at the midpoint, and n is the degree of cooperativity that determines the slope of the curve. The fitting was achieved by varying y_0, y_∞, and K to minimise the least square deviations of $y_{\text{experimental}}$ *versus* $y_{\text{calculated}}$. For the complexes **4** and **5** the cooperativity (n) was found to be 3.4 and 7.3, respectively. It seems that the introduction of the neither sterically demanding nor electronically very influential methyl group has an unexpectedly large influence upon the cooperativity of the transition. Lacking a molecular structure of the DNA to copper adducts, we can only assume that the additional methyl group is involved in a hydrophobic interaction with the DNA.

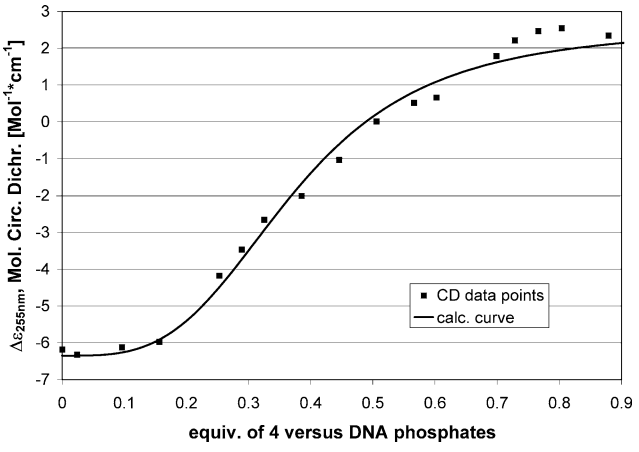

Fig. 9 Titration of poly d(GC) with **4** monitored by CD at 255 nm.

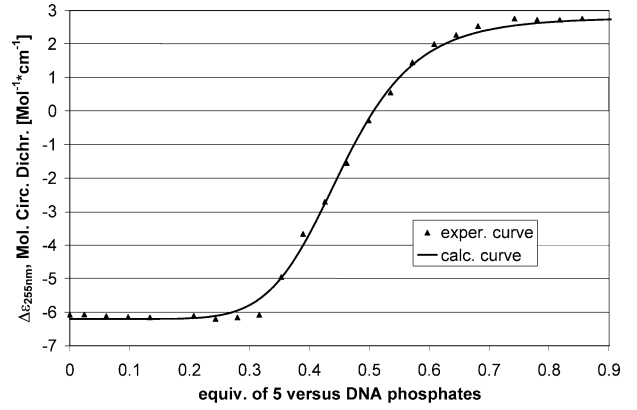

Fig. 10 Titration of poly d(GC) with **5** monitored by CD at 255 nm.

Conclusions

The new macrocyclic trisaza bisamide ligand **2** was synthesised analogously to the known ligands **1**[18] and **3**[26] in a yield of 42%. The used ligands can either bind to the metal centre by triple nitrogen coordination (N3) with the secondary amines[17] or after double deprotonation of the two amides with all five nitrogen atoms (N5). Here, the overall charge of the complex is zero and the ligand occupies five out of six coordination sites of a distorted octahedron.[27] The N5 coordination mode is favoured if one or two fluorine substituents at the C(15) position are present and if the pH is high in the case of aqueous solutions. When 15-fluoro-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione (**1**) was reacted with nickel acetate, all three secondary amines plus one oxygen of an amide group were found to coordinate to the nickel in the crystal structure.[26,28] Such an NNNO mode was never observed during our studies. All our crystal structures of complexes in the N3 mode for LH_2M^{2+} rather resemble the overall geometry of the nickel acetate complex with 1,4,7,10,13-pentaazacyclohexadecan-14,16-dione.[17]

Circular dichroism titrations of poly d(GC) with the metal complexes showed the ability of the systems studied to promote the B- to Z-DNA transition. Ligand **2**, the copper complex **6** with the bis fluoro substituted ligand **3** and the zinc complex **7** were not able to induce any change in the direction of Z-DNA. In the case of **6**, the CD spectrum of the DNA actually showed no change at all, indicating that the complex was not even interacting with the B form of DNA. Therefore we assume that the bis fluoro substitution causes the complex at the experimental conditions of pH 7 to be in the neutral LM form. The electrostatics together with the shielding effect of the ligand might explain the absence of any interaction with the DNA. Copper acetate generated a partial transition. This is remarkable since copper chloride was inactive in generating Z-DNA,[30] most likely due to chloride inhibition.[31] Both complexes **4** and **5** induced Z-DNA with a midpoints of 0.37 and 0.45 equivalents of the complex *versus* DNA phosphates, respectively. The cooperativities of **4** and **5** were found to be 3.4 and 7.3, respectively. A possible hypothesis to explain this remarkable difference might be that the additional methyl group of **5** is involved in a hydrophobic interaction with the DNA.

In the future the amount of metal complex needed for the B- to Z-DNA transition should be reduced. The use of dinuclear metal complexes might help to achieve this goal. We are currently exploring this hypothesis.

Acknowledgements

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References

1 A. Herbert and A. Rich, *J. Biol. Chem.*, 1996, **271**, 11595.
2 A. Rich, A. Nordheim and A. H.-J. Wang, *Annu. Rev. Biochem.*, 1984, **53**, 791.
3 M. Behe and G. Felsenfeld, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 1619.
4 R. V. Gessner, C. A. Frederick, G. J. Quigley, A. Rich and A. H.-J. Wang, *J. Biol. Chem.*, 1989, **264**, 7921.
5 X. Qu, J. O. Trent, I. Fokt, W. Priebe and J. B. Chaires, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 12032.
6 Y. Xu, Y. X. Zhang, H. Sugiyama, T. Umano, H. Osuga and K. Tanaka, *J. Am. Chem. Soc.*, 2004, **126**, 6566.
7 G. V. Fazakerley, *Nucleic Acids Res.*, 1984, **12**, 3643.
8 S. M. Middendorf and H. Diebler, *Ber. Bunsen-Ges. Phys. Chem.*, 1996, **100**, 641.
9 T. Schoenknecht and H. Diebler, *J. Inorg. Biochem.*, 1993, **50**, 283.

10 J. H. van de Sande, L. P. McIntosh and T. M. Jovin, *EMBO J.*, 1982, **1**, 777.
11 B. Spingler, P. Antoni, B. Wild and G. Viola, manuscript in preparation.
12 B. H. Geierstanger, T. F. Kagawa, S.-L. Chen, G. J. Quigley and P. S. Ho, *J. Biol. Chem.*, 1991, **266**, 20185.
13 A. Woisard, G. V. Fazakerley and W. Guschlbauer, *J. Biomol. Struct. Dyn.*, 1985, **2**, 1205.
14 H.-C. Shih, N. Tang, C. J. Burrows and S. E. Rokita, *J. Am. Chem. Soc.*, 1998, **120**, 3284.
15 E. Kimura, A. Sakonaka and M. Nakamoto, *Biochim. Biophys. Acta*, 1981, **678**, 172.
16 E. Kimura, A. Sakonaka, R. Machida and M. Kodama, *J. Am. Chem. Soc.*, 1982, **104**, 4255.
17 Z. Zhang, J. L. Petersen and A. M. Stolzenberg, *Inorg. Chem.*, 1996, **35**, 4649.
18 C.-C. Cheng, S. E. Rokita and C. J. Burrows, *Angew. Chem., Int. Ed.*, 1993, **32**, 277.
19 E. Schlittler and J. Hohl, *Helv. Chim. Acta*, 1952, **35**, 29.
20 *STOE-IPDS Software package*, Darmstadt, Germany, 1998.
21 G. M. Sheldrick, *Acta Crystallogr. Sect. A: Fundam. Crystallogr.*, 1990, **A46**, 467.
22 A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori and R. Spagna, *J. Appl. Crystallogr.*, 1999, **32**, 115.
23 *SHELXL97-2: Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, 1997.
24 L. J. Farrugia, *J. Appl. Crystallogr.*, 1997, **30**, 565.
25 N. Ishikawa, A. Takaoka and M. K. Ibrahim, *J. Fluorine Chem.*, 1984, **25**, 203.
26 C.-C. Cheng, PhD Dissertation, State University of New York, Stony Brook, 1993.
27 R. Machida, E. Kimura and Y. Kushi, *Inorg. Chem.*, 1986, **25**, 3461.
28 C.-C. Cheng, J. Gulia, S. E. Rokita and C. J. Burrows, *J. Mol. Cat. A: Chem.*, 1996, **113**, 379.
29 H.S. Basu, H. C. A. Schwietert, B. G. Feuerstein and L. J. Marton, *Biochem. J.*, 1990, **269**, 329.
30 W. Zacharias, J. E. Larson, J. Klysik, S. M. Stirdivant and R. D. Wells, *J. Biol. Chem.*, 1982, **257**, 2775.
31 B. Spingler, *Inorg. Chem.*, 2005, **44**, 831.
32 C.-W. Chen, R. H. Knop and J. S. Cohen, *Biochemistry*, 1983, **22**, 5468.
33 A. De Lean, P. J. Munson and D. Rodbard, *Am. J. Physiol.*, 1978, **235**, E97.