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Dimeric yttrium(III) and neodymium(III) macrocyclic complexes: potential catalysts for hydrolysis of double-stranded DNA

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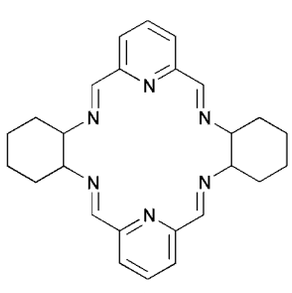
Dimeric complexes of macrocycle L, $[\{ML(OH)(H_2O)\}_2](NO_3)_4$ where M = Y and Nd, have been prepared *via* template synthesis. X-Ray structural analysis of $[\{YL(OH)(H_2O)\}_2]^{4+}$ showed each yttrium atom to be coordinated to six nitrogens in the macrocycle, two hydroxide bridging units and one water molecule; the two macrocycles within the dimer have virtually identical conformations. Both dimeric complexes have the remarkable ability to degrade dsDNA, while the corresponding monomers have no activity. Cleavage likely involves random attack at single strands, in which closed circular plasmid DNA is initially converted to a nicked DNA intermediate before final cleavage.

Introduction

The increasing interest in using lanthanide ions or complexes as artificial restriction enzymes for cleaving DNA has prompted us to investigate the application of lanthanide macrocyclic complexes in this area. Studies on the catalytic hydrolysis of RNA by lanthanide(III) macrocyclic complexes, for example macrocycles of hexadentate Schiff bases [1] and tetraamine macrocycles with four hydroxymethyl pendant arms,[2] have been reported and yet relatively few examples of DNA hydrolysis can be found in the literature. The sites of cleavage catalysed by lanthanides on both DNA and RNA are at the phosphodiester linkages. However cleavage of DNA phosphodiester linkages is relatively more difficult than that of RNA and so far the complex $[Ce_2(OH)_4]^{4+}$ has been reported to be the most effective catalyst for DNA hydrolysis.[3] Recently, dilanthanide, La^{III} and Ce^{IV}, complexes of polyaminocarboxylates have been shown to cleave double-strand supercoiled plasmid DNA successfully at 55 °C within a 3 hour period.[4] There is no example in the literature reporting the application of kinetically stable macrocyclic complexes as catalysts for double-stranded DNA hydrolysis.

In this study, two dimeric Y^{III} and Nd^{III} complexes of an 18-membered hexaaza macrocycle (L) have been prepared and characterised. Their efficiency as catalysts for double-stranded DNA hydrolysis is examined along with that of the corresponding monomeric macrocyclic complexes.



L

Results and discussion

In our previous studies,[5] we have successfully used template synthesis to prepare the monomeric metal complexes of L, $[ML(H_2O)_3]Cl_3$ for M = Y and Gd; $[ML(NO_3)_2]NO_3$ for M = Dy. In this study, the use of yttrium(III) nitrate and neodymium(III) nitrate instead of the chloride salts in the template synthesis, under the same experimental conditions, produced the dimeric complexes $[\{ML(OH)(H_2O)\}_2]^{4+}$ M = Y and Nd. (The synthesis of the macrocyclic ligand (L) has, as reported in our previous studies,[5] resulted in the corporation of two 1,2-diaminocyclohexane groups with the same chirality.) Elemental analysis of both compounds and the magnetic moment of the Nd^{III} complex indicated that dimeric compounds were formed. Microanalysis of the nitrogen content indicated four nitrate counter-ions were present requiring the presence of a hydroxyl group to counter-balance the positive charge on each of the metal ions.

However the results obtained by mass spectrometry did not give the parent ion peaks for the dimeric species $[\{ML(OH)(H_2O)\}_2](NO_3)_4$. Only species of the type $[ML(NO_3)_2]^+$ and $[ML(NO_3)_2OH]^+$ were detected and their fragmentation patterns were similar to those of the monomeric compounds.[5]

It was rather difficult to present more concrete evidence to support the existence of the dimer $[\{NdL(OH)(H_2O)\}_2]^{4+}$ compared to the Y(III) dimer. The paramagnetic properties of the Nd(III) complex have hindered the use of NMR to characterise the complex thoroughly and attempts to grow suitable crystals for solid state structural characterisation proved unsuccessful. Electronic spectroscopic studies on the Nd(III) dimer at 25 mmol dm^{-3} showed that the hypersensitive bands in the electronic spectrum of $[\{NdL(OH)(H_2O)\}_2]^{4+}$ were similar to $[Nd(H_2O)_9]^{3+}$ indicating that the electrons responsible for the absorption bands are not directly influenced by the macrocycle donor atoms nor the hydroxyl groups.[6]

NMR spectroscopy

NMR spectroscopy of the diamagnetic Y(III) complex has proved to be a useful tool to confirm the existence of the dimer. The NMR spectra of the monomer $[YL(H_2O)_3]^{3+}$ and the dimer $[\{YL(OH)(H_2O)\}_2]^{4+}$ in D_2O were different. The monomer $[YL(H_2O)_3]^{3+}$ was found to have one set of signals (more evident in the ^{13}C NMR spectrum) and the $[\{YL(OH)(H_2O)\}_2]^{4+}$ showed two inequivalent sets of signals both in the ^1H- and ^{13}C-NMR spectra (Table 1). The inequivalence in the dimer may either arise within the individual L units in the dimer or from differences between the two monomeric units, $[YL(OH)(H_2O)]$, in the dimeric species. The asymmetry observed in the dimer is likely to arise from the steric constraint and the conformational change of the macrocycle in accommodating the bridging hydroxyl units.

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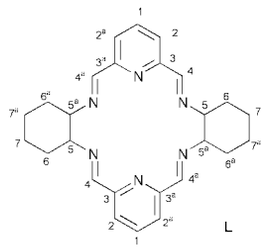
Table 1 Proton and carbon-13 NMR data for complexes $[YL(H_2O)_3]^{3+}$ and $[\{YL(OH)(H_2O)\}_2]^{4+}$ in D_2O



Assignment	δ_H [b]		δ_C [b]	
	$[YL(H_2O)_3]^{3+}$	$[\{YL(OH)(H_2O)\}_2]^{4+}$	$[YL(H_2O)_3]^{3+}$	$[\{YL(OH)(H_2O)\}_2]^{4+}$
1	8.63 (t)	8.45 (t)	145.92	144.74
2	8.31 (d)	8.21 (d)	132.51	132.43
2[a]	—	8.06 (d)	—	131.25
3	—	—	154.59	155.00
3[a]	—	—	—	153.21
4	9.12 (s)	8.94 (s)	165.81	163.52
4[a]	—	8.57 (s)	—	163.43
5	3.98 (m)	3.23 (m)	70.69	70.74
5[a]	—	[c]	—	68.48
6	1.9, 2.6 (m)	[c]	32.91	32.26
6[a]	—	[c]	—	31.27
7,7a	1.7, 2.2 (m)	[c]	26.90	26.16
$J(H^1–H^2)$/Hz	7.7	7.6		
$J(H^1–H^{2a})$/Hz	—	7.7		

[a] Signals from the asymmetric unit of $[\{YL(OH)(H_2O)\}_2]^{3+}$. [b] Values in ppm relative to the sodium salt of 3-trimethylsilylpropionic-2,2,3,3-d_4 acid. [c] Broad multiplets, complete assignment cannot be achieved.

Table 2 Selected mean bond lengths (Å) and angles (°) for $[\{YL(OH)(H_2O)\}_2](NO_3)_4\cdot 6H_2O$

Y(1)–Y(2)	3.709(3)	Y(1)–N(2B), Y(2)–N(2C)	2.578(13)
Y(1)–O(1), Y(2)–O(1)	2.235(9)	Y(1)–N(2A), Y(2)–N(2D)	2.701(14)
Y(1)–O(1W), Y(2)–O(2W)	2.339(15)	Y(1)–N(1E), Y(2)–N(1F)	2.609(14)
N(2B)–Y(1)–N(2BA)	172.2(9)	N(2C)–Y(2)–N(2CA)	171.2(9)
N(2B)–Y(1)–N(2AA)	60.4(5)	N(2C)–Y(2)–N(2DA)	60.0(5)
N(2B)–Y(1)–N(1E)	61.7(5)	N(2C)–Y(2)–N(1F)	61.8(5)
N(2A)–Y(1)–N(1E)	60.4(5)	N(1D)–Y(2)–N(2F)	61.1(5)
N(2A)–Y(1)–N(2AA)	148.1(9)	N(2D)–Y(2)–N(2DA)	148.2(10)
N(1E)–Y(1)–N(1EA)	150.5(10)	N(1F)–Y(2)–N(1FA)	152.1(10)
O(1)–Y(1)–O(1A)	67.9(5)	O(1A)–Y(2)–O(1)	67.9(5)
O(1)–Y(1)–O(1W)	146.1(2)	O(1)–Y(2)–O(2W)	146.1(2)
O(1W)–Y(1)–Y(2)	180	O(2W)–Y(2)–Y(1)	180
N(2B)–Y(1)–Y(2)	86.1(4)	N(2C)–Y(2)–Y(1)	85.6(4)
N(2A)–Y(1)–Y(2)	106.0(5)	N(2D)–Y(2)–Y(1)	105.9(5)
N(1E)–Y(1)–Y(2)	104.7(5)	N(1F)–Y(2)–Y(1)	104.0(5)
Y(1)–O(1)–Y(2)	112.1(4)		

Symmetry transformations used to generate equivalent atoms (indicated by extra final A): $-x, -y, z$.

Crystal structure

Despite the poor diffraction by the only crystals that could be obtained, the single crystal X-ray structure analysis confirms the dimeric formulation $[\{YL(OH)(H_2O)_2\}_2](NO_3)_4\cdot 6H_2O$ and establishes that the molecule consists of two independent macrocyclic units connected by two hydroxyl ligands coordinated to the central Y atoms with the overall structure illustrated in Fig. 1(a) and 1(b). The two macrocycles are rotated from an eclipsed configuration as can be seen in Fig. 1(b) so that the N_{py}–Y(1)–Y(2)–N_{py} torsion angle is 83(1)°.

Mean values of selected bond lengths and angles are listed in Table 2. Each yttrium atom has nine-coordination with six nitrogen donors forming an 'equatorial' set {Y–N range 2.578(13)–2.701(14) Å} with a water molecule in one axial position {mean Y–OH_2 2.339(15) Å} and two symmetrically bridging hydroxyl groups on the other side {mean Y–OH 2.235(9) Å}. The dimeric unit has crystallographic C_2 symmetry with the two water ligands and the two yttrium atoms lying on a two-fold axis, so that the two halves *within* each macrocyclic ring are related by symmetry. Within the dimer the two macrocycles have virtually identical conformations. Two opposite nitrogen donors in each macrocycle are in an approximately linear arrangement relative to the metal atoms, N(2B)–Y(1)–N(2BA) 172.2(9)° in the first, and N(2C)–Y(2)–N(2CA) 171.2(9)° in the second; each macrocyclic ring 'folds' about this line away from the other ring and towards the axial water ligand. The nitrogen donor atoms in the two halves of each folded donor set are coplanar to within 0.04 Å; the dihedral angle between the best planes through N(2B)–N(1E)–N(2A)–N(2BA) and N(2B)–N(1EA)–N(2AA)–N(2BA) in the first macrocycle is 44(1)° and the equivalent angle in the second macrocycle between N(2C)–N(1F)–N(2D)–N(2CA) and N(2C)–N(1FA)–N(2DA)–N(2CA) has the same value. This asymmetric folding of each macrocyclic ring precludes any further equivalence, the imine nitrogen atoms about which folding occurs, N(2B) and N(2C), are in virtually identical environments, but this is markedly different from that of the other

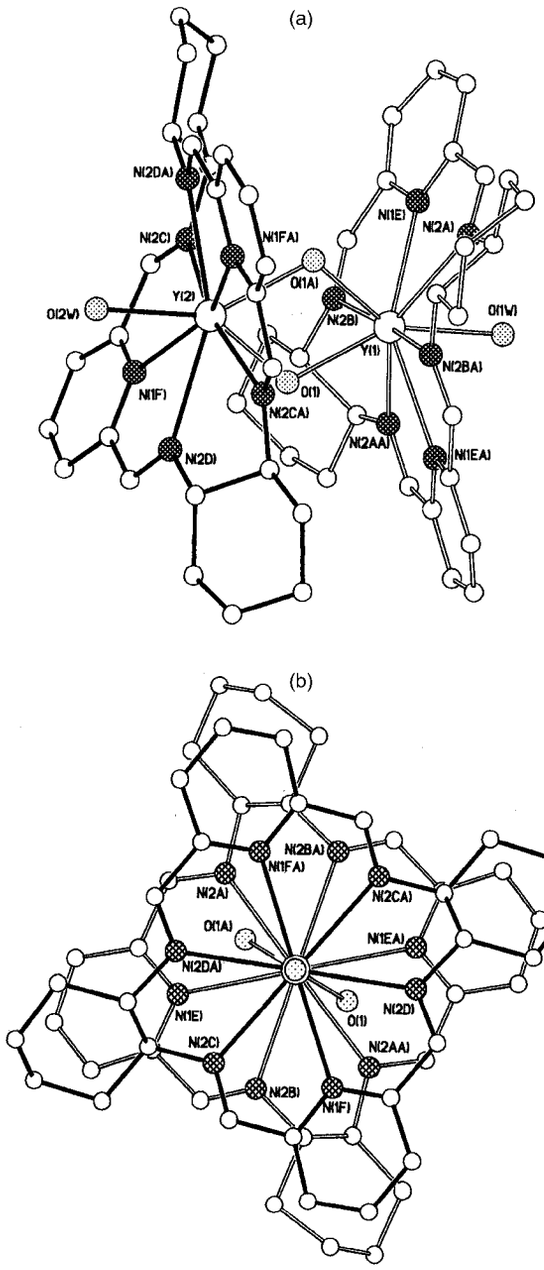


Fig. 1 The structure of the dimeric tetracation $[\{YL(OH)(H_2O)_2\}_2]^{4+}$ in the hydrated nitrate salt. (a) A view showing the overall structure and folding of the macrocycles about N(2B)–Y(1)–B(2BA) and N(2C)–Y(2)–N(2C). The complex has a crystallographic C_2 axis running through the two yttrium atoms and the terminal water ligands O(1W) and O(2W); a second letter, A, denotes the equivalent position $-x, -y, z$. (b) A view of the dimeric cation down the crystallographic C_2 axis showing the staggered conformation of the macrocyclic ligands.

imine atoms N(2A) and N(2C) where there is no folding of the donor set. Therefore the observed solid state structure is consistent with the two sets of signals observed in the ^{13}C NMR spectrum for atoms C(2)–C(7) arising from conformational differences within the two inequivalent macrocyclic ligands.

Hydrolysis of double-stranded DNA

We assessed the ability of the complexes to hydrolyse double-stranded DNA. A representative experiment is shown in Fig. 2. We found that all monomeric complexes tried, of Gd^{3+}, Y^{3+} and Dy^{3+}, had no measurable ability to cleave dsDNA but the dimeric complexes, of Y^{3+} and Nd^{3+}, showed marked cleavage activity. We found that each dimer cleaved 90–95% of the supercoiled, closed, circular form of plasmid DNA but were slightly less active towards the more conformationally relaxed

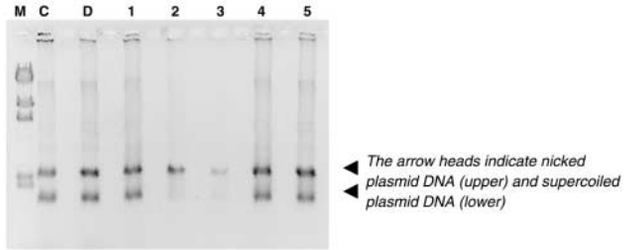


Fig. 2 Digestion of double-stranded DNA by dimeric, but not monomeric, lanthanide complexes. pSP72 plasmid DNA (Promega Ltd) was incubated with 0.1 mM lanthanide complex under the conditions described by Barnum and Que.[4] **M**: molecular weight markers; **C**: undigested plasmid DNA; **D**: plasmid incubated with DMSO only; **1**: $[GdL(H_2O)_3]^{3+}$; **2**: $[\{YL(OH)(H_2O)\}_2]^{4+}$; **3**: $[\{NdL(OH)(H_2O)\}_2]^{4+}$; **4**: $[YL(H_2O)_3]^{3+}$; **5**: $[DyL(H_2O)_3]^{3+}$. The photograph is of DNA separated on an agarose gel, stained with ethidium bromide, viewed under UV light. A negative image is shown for clarity.

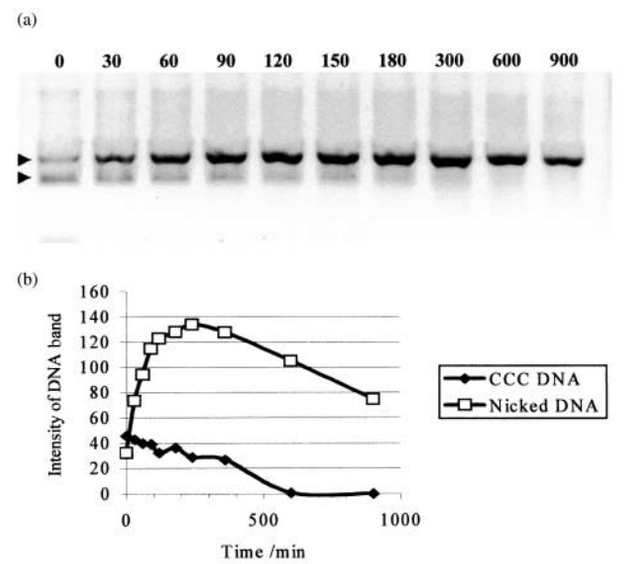


Fig. 3 Time course of cleavage of plasmid DNA by $[\{YL(OH)(H_2O)\}_2]^{4+}$ illustrating the relative changes in closed circular and nicked DNA with time. (a) Time course of degradation of plasmid DNA by $[\{YL(OH)(H_2O)\}_2]^{4+}$. Time points in minutes; upper arrowhead indicates nicked, and lower arrow supercoiled, plasmid DNA. (b) Analysis of image in (a). Closed circular DNA (CCC DNA) and nicked DNA bands were quantified and plotted against time.

nicked DNA (60% and 85% DNA cleaved for the Y^{3+} and Nd^{3+} dimers respectively, mean of three measurements). We could not observe any band corresponding to linearised plasmid and to investigate the mechanism of cleavage further we carried out kinetic analyses. A representative experiment is shown in Fig. 3 in which cleavage by the Y^{3+} dimer is followed over time. The bands of closed circular plasmid and nicked plasmid DNA were quantified and a plot of the variation in concentration of these two forms with time is shown in Fig. 3b. The data indicate that the intact, closed, circular plasmid is attacked by random cleavage of single strands to generate the conformationally relaxed nicked form of plasmid, which initially increases in concentration. When all the closed circular plasmid has been nicked, the nicked DNA then gradually disappears, presumably through further random attacks. These findings extend the observations of Branum and Que[4] with other dilanthanide complexes although the mechanism by which cleavage occurs is distinct from the polyaminocarboxylate complexes.

Conclusions

These findings expand the range of new metal complexes which are capable of hydrolysing double-stranded DNA which may

therefore have the potential for therapeutic applications in genetically based disease.

Experimental

Materials

All reagents and solvents were generally of GPR grade, obtained from Aldrich Chemical Company and used without further purification. 2,6-Diformylpyridine was prepared in 89% yield by following a literature method.[7]

Physical measurements

Elemental analyses (C, H, N) were performed by the Scientific Analysis and Consultancy Service at the University of North London using a Carlo Erba 1106 Microanalyser. IR spectra were recorded on a Digilab FTS-40 infrared spectrometer as KBr microdisks. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AM 250 FT-NMR spectrometer. Magnetic susceptibility measurements were carried out on a Johnson Matthey Chemicals magnetic susceptibility balance. Corrections for the diamagnetism of the complexes were estimated from Pascals constant.[8] LSI mass spectra were obtained using a Vacuum Generator (VG) Kratos Profile Double Focusing mass spectrometer, equipped with a Cs fast ion gun operating at 10 kV.

Synthesis

Dimeric nitrate complexes of yttrium(III) and neodymium(III) were prepared by following the same procedure for synthesizing the monomeric complex reported previously.[5] A solution of (±)-*trans*-1,2-diaminocyclohexane (15.0 mmol) in methanol (10 cm^3) was added dropwise over 1/2 h to a solution of metal nitrate (7.5 mmol) and 2,6-pyridinedialdehyde (15.0 mmol) in methanol (100 cm^3) and heated for 4 h. The precipitate formed was filtered hot under suction, washed with cold methanol (2 × 10 cm^3) and diethyl ether (5 cm^3) and dried *in vacuo* over calcium chloride. For $[\{YL(OH)(H_2O)\}_2](NO_3)_4$, yield 66%. Found: C, 45.5; H, 4.8; N, 16.9. $C_{52}H_{66}N_{16}O_{16}Y_2$ requires C, 46.2; H, 4.9; N, 16.6%. IR (cm^{-1}): ν(C=N)$_{imine}$ 1651w, ν(C=N)$_{py}$ 1594w. LSI (*m*/*z*): 640 $[YL(NO_3)_2 + H]^+$, 577 $[YL(NO_3)_2]^+$. For $[\{NdL(OH)(H_2O)\}_2](NO_3)_4$, yield 68%. Found: C, 42.1; H, 4.4; N, 15.7. $C_{52}H_{66}N_{16}O_{16}Nd_2$ requires C, 42.7; H, 4.5; N, 15.3%. IR (cm^{-1}): ν(C=N)$_{imine}$ 1649w, ν(C=N)$_{py}$ 1593m. LSI (*m*/*z*): 711 $[NdL(NO_3)_2OH]^+$, 694 $[NdL(NO_3)_2]^+$, 649 $[NdL(NO_3)OH]^+$, 632 $[NdL(NO_3)]^+$; μ_{eff} 5.2 μ_B (288 K).

X-Ray crystallography

Crystals of $[\{YL(OH)(H_2O)_2\}_2](NO_3)_4\cdot 6H_2O$ diffracted relatively poorly at high angle. Data were collected with a Phillips *PW*1100 diffractometer in the θ-range 3–25° using Mo-K$_\alpha$ radiation by the method described previously.[9] The data were corrected for Lorentz-polarisation effects and for absorption using ψ-scans. The structure was solved by the Patterson method and full-matrix refinement was on F^2 with 2517 independent data (Table 3);[10] one nitrate counter-ion was severely disordered accounting for the poor diffraction by the crystals. Due to the resultant shortage of data, chemically equivalent bonding distances in the two halves of the dimeric molecule were constrained to be equal within an e.s.d. of 0.01 Å for bonds to yttrium and 0.02 Å for other bonds, and within the nitrate anions to within 0.03 Å. All hydrogen atoms (except those of the OH and H_2O species) were included at calculated positions, and anisotropic displacement parameters were assigned to the metal atoms and full occupancy nitrogen and oxygen atoms.

CCDC reference number 162531.

See http://www.rsc.org/suppdata/dt/b1/b102533n/ for crystallographic data in CIF or other electronic format.

Table 3 Crystal data and structure refinement parameters for $[\{YL(OH)(H_2O)\}_2](NO_3)_4\cdot 6H_2O$

Molecular formula	$C_{52}H_{78}N_{16}O_{22}Y_2$
M	1457.12
Crystal system	Orthorhombic
Space group	*Aba*2
a/Å	16.677(3)
b/Å	20.114(4)
c/Å	18.724(4)
U/Å^3	6281(1)
Z	4
D_c/Mg m^{-3}	1.541
μ/mm^{-1}	1.928
T/K	293(2)
λ/Å	0.71069
F(000)	3024
θ range/°	3.17 to 24.99
hkl ranges	0 to 19, 0 to 23, 0 to 22
Independent reflections	2517
Data/restraints/parameters	2517/105/312
Goodness-of-fit on F^2	1.093
Final *R* indices [$I > 2\sigma(I)$]	$R1 = 0.0693$, $wR2 = 0.1658$
R indices (all data)	$R1 = 0.0794$, $wR2 = 0.1754$
Absolute structure parameter	0.00(4)
Largest difference peak and hole/e Å^{-3}	0.928 and −0.341

DNA cleavage

pSP72 plasmid DNA (Promega) was prepared by standard methods[11] and incubated with metal complex under the conditions described by Branum and Que.[4] Complexes were prepared as stock solutions of 10 mM in DMSO. 1.0 mM of complex was incubated with 2.5 μg of DNA in a total volume of 50 μl in 5 mM Tris-HCl buffer pH 7.6 at 55 °C for 3 h, or for the indicated times. Samples were analysed for DNA hydrolysis by agarose electrophoresis followed by staining of DNA bands with ethidium bromide.[11] Images of gels taken under UV light were collected and analysed with UVItech imaging equipment and software (UVItech Ltd, Cambridge, UK).

Acknowledgements

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