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Synthesis and phosphate ester cleavage properties of copper(II) complexes of guanidinium-bridged *bis*(1,4,7-triazacyclononane) ligands

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Dedicated to Prof. Martin Bennett on the occasion of his 80th birthday and for his outstanding contributions to the field of Inorganic Chemistry.

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ABSTRACT

The synthesis and characterization of three new *bis*(1,4,7-triazacyclononane) ligands, **L^1**, **L^2** and **L^3**, featuring a bridging guanidinium group between two macrocyclic units, is reported. The corresponding binuclear copper(II) complexes have been studied as agents to accelerate the cleavage of P–O bonds within two model phosphodiesters, namely *bis*(*p*-nitrophenyl)phosphate (BNPP) and 2-hydroxypropyl-*p*-nitrophenylphosphate (HPNPP). The results of a comparative study of cleavage rates, using the mononuclear copper(II)–tacn complexes bearing single alkylguanidinium groups as a reference, revealed that the binuclear copper(II) complexes are generally less effective cleavage agents, which may be related to a tendency to form hydrolytically inactive hydroxo-bridged species at near-neutral pH and above. However, at pH 7, these complexes produced 4–18-fold increases in the rate of BNPP hydrolysis compared to the parent complex, $[Cu(tacn)(OH_2)_2]^{2+}$. Likewise, at pH 6, the complexes cleaved HPNP 5–130-fold faster than $[Cu(tacn)(OH_2)_2]^{2+}$, suggesting some degree of cooperative interplay between the two proximal copper(II) centers and the protonated guanidinium bridging group in promoting phosphodiester hydrolysis under these conditions.

1. Introduction

Metal complexes that mimic natural metallonucleases are of significant interest in the medicinal and biotechnology areas [1–19]. Substitutionally labile copper(II) complexes have been extensively explored from this view point because they can allow rapid binding of the substrate and release of products [1,2,5,7–9,14,16,20–24]. Notably, copper(II) can bind to phosphate esters, which is necessary to activate phosphodiester bonds (as found in DNA and RNA) towards nucleophilic attack. The Cu(II) center also lowers the pK_a of coordinated water, providing a metal-bound hydroxide at near neutral pH, that can act as a nucleophile to attack the substrate, making such complexes promising catalysts for phosphodiester hydrolysis [1,2,5,7–10,14,16,21–25].

In the pursuit of cleavage agents with improved activity, recent research has focused on multinuclear metal complexes which incorporate the types of functionalities found in the active site of the natural enzyme that complement the hydrolytic action of the metal ion(s). For example, the idea of combining multiple metal centers with positively charged guanidinium group(s) in artificial metallo-nucleases arises from nature [1,2,23,24,26,27]. An excellent example is provided by the well-studied enzyme alkaline phosphatase (AP), which exploits two zinc centers, in conjunction with key serine and arginine residues, to facilitate the rapid cleavage of phosphate monoesters. The positively charged guanidinium group present in the side chain of an arginine amino acid residue is postulated to assist with substrate activation and transition state stabilization, whilst the serine residue provides the initial attacking nucleophile, leading to the formation of a phosphoseryl intermediate during the AP catalytic cycle.

Previously, we have investigated the copper(II) complexes of tacn-guanidinium derivatives as nuclease mimics (tacn = 1,4,7-triazacyclononane) [28–30]. From these exploratory studies, evidence for cooperativity between the copper(II) centers and guanidinium pendants was obtained. Comparative studies of the copper(II) complexes with appended guanidinium groups *versus* the unsubstituted derivative revealed significant rate enhancement in the cleavage of a diverse range of phosphodiester substrates [28–30]. Continued interest in the development of more efficient synthetic metallo-nucleases prompted us to explore the combined effect of using two metal ions and a guanidinium group on cleavage activity. Herein, we report the synthesis and characterization of three new *bis*(tacn) ligands featuring a bridging guanidine group (**L^1**–**L^3**, see Fig. 1), together with an examination of

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the ability of the corresponding copper(II) complexes to hydrolytically cleave phosphate esters under near-physiological conditions, using two model phosphodiesters, *bis*(*p*-nitrophenyl)phosphate (BNPP) and 2-hydroxypropyl-*p*-nitrophenylphosphate (HPNPP).

2. Experimental

2.1. Materials and chemicals

Chemicals and solvents were of reagent or analytical grade, and were used as received, unless otherwise indicated. Distilled H_2O and HPLC grade chloroform were used throughout. THF was dried over 4 Å molecular sieves and then freshly distilled from Na/benzophenone prior to use. 1,4-*bis*(*tert*-Butoxycarbonyl)-1,4,7-triazacyclononane [31], 1-(2-aminoethyl)-4,7-bis(tert-butoxycarbonyl)-1,4,7-triazacyclononane, 1-(3-aminopropyl)-4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane, 1-(4-aminobutyl)-4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane [29] and sodium salt of 2-hydroxypropyl-*p*-nitrophenylphosphate [32,33] (NaHPNPP) were prepared according to the literature procedures.

2.2. Instrumentation and methods

Infrared spectra were recorded as KBr disks using a Bruker Equinox FTIR spectrometer fitted with an ATR platform at 4.0 cm^{-1} resolution. *Microanalyses* were performed by Campbell Microanalytical Service, Otago, New Zealand. *^{1}H NMR and ^{13}C NMR spectra* were recorded at 25 °C in D_2O or $CDCl_3$ (as listed) on a Bruker AC200, AM300 or DX400 spectrometer. Chemical shifts were recorded on the δ scale in parts per million (ppm). The chemical shifts, δ, were calibrated using either tetramethylsilane (TMS) or signals due to the residual protons of deuterated solvents. The abbreviations used to describe the resonances for ^{1}H NMR spectra are: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), br t (broad triplet). *Low resolution electrospray ionization mass spectra (ESI-MS)* were measured on a Micromass Platform *II* Quadrupole Mass Spectrometer fitted with an electrospray source. The capillary voltage was at 3.5 eV and the cone voltage at 35 V. *Thin Layer Chromatography (TLC)* was performed using silica gel 60 F-254 (Merck) plates with detection of species present by UV irradiation or $KMnO_4$ oxidation. *UV–Vis spectra* were recorded in 1 cm quartz cuvettes using Varian Cary Bio 300 or 5G spectrophotometers.

2.3. Syntheses

2.3.1. N-ethoxycarbonyl-N′-(2-(4,7-bis(tert-butoxycarbonyl))-1,4,7-triazacyclononan-1-yl)ethyl-thiourea (**1**)

Ethoxycarbonyl isothiocyanate (0.900 g, 6.85 mmol) was added dropwise to a stirred solution of 1-(2-aminoethyl)-4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (2.32 g, 6.23 mmol) in dichloromethane (DCM) (20 mL). The resulting solution was stirred overnight (O/N) at room temperature (RT). The solvent was removed under reduced pressure and the crude product purified by silica gel chromatography using 2% MeOH/$CHCl_3$ as the eluent. The product (R_f = 0.25) was isolated as a yellow oil. Yield: 2.46 g (78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, 3H, J = 7.2 Hz, ethyl CH_3), 1.42 (s, 18H, ^{t}Bu CH_3), 2.69 (m, 4H, tacn CH_2), 2.79 (m, 2H, ethyl CH_2), 3.24 (m, 4H, tacn CH_2), 3.43 (m, 4H, tacn CH_2), 3.66 (m, 2H, ethyl CH_2), 4.17 (q, 2H, J = 7.2 Hz, ethyl CH_2), 8.25 (br s, 1H, N*H*), 9.76 (br t, 1H, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (ethyl CH_3), 28.1 (^{t}Bu CH_3), 43.4 (ethyl CH_2), 48.8, 49.6 (tacn CH_2), 49.9, 50.7 (tacn CH_2), 53.8 (ethyl CH_2), 54.6, 55.0 (tacn CH_2), 62.1 (ethyl CH_2), 79.1 (quaternary ^{t}Bu *C*), 152.4 (*C*=*O*), 155.1 (*C*=*O*), 178.9 (*C*=*S*). IR (neat), ν(cm^{-1}): 3289s (ν_{N-H}), 2977s (ν_{C-H}), 1715s ($\nu_{C=O}$), 1694s ($\nu_{C=O}$), 1682s ($\nu_{C=O}$), 1456s, 1247s (ν_{C-O}), 1155s, 1035s ($\nu_{C=S}$), 988m, 918m, 859m. ESI-MS (*m*/*z*): 504.3 (100%) $[M+H]^+$.

2.3.2. N-ethoxycarbonyl-N′-(3-(4,7-bis(tert-butoxycarbonyl))-1,4,7-triazacyclononan-1-yl)propyl-thiourea (**2**)

Compound **2** was prepared in an identical manner to **1** by reacting ethoxycarbonyl isothiocyanate (0.590 g, 4.49 mmol) with 1-(3-aminopropyl)-4,7-*bis*(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (1.58 g, 4.09 mmol) in DCM (20 mL). Work-up and purification as per **1** yielded the product (R_f = 0.30) as a yellow oil. Yield: 1.76 g (83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, 3H, J = 7.2 Hz, ethyl CH_3), 1.42 (s, 18H, ^{t}Bu CH_3), 1.70 (m, 2H, propyl CH_2), 2.45–2.56 (m, 6H, tacn CH_2 and propyl CH_2), 3.21 (m, 4H, tacn CH_2), 3.40 (m, 4H, tacn CH_2), 3.60 (m, 2H, propyl CH_2), 4.13 (q, 2H, J = 7.2 Hz, ethyl CH_2), 8.35 (br s, 1H, N*H*), 9.70 (br t, 1H, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0 (ethyl CH_3), 26.4 (propyl CH_2), 28.3 (^{t}Bu CH_3), 43.2 (propyl CH_2), 48.9, 50.1 (tacn CH_2), 50.4, 50.7 (tacn CH_2), 53.2 (propyl CH_2), 53.6, 54.0 (tacn CH_2), 62.1 (ethyl CH_2), 79.2 (quaternary ^{t}Bu *C*), 152.5 (*C*=*O*), 155.3 (*C*=*O*), 178.9 (*C*=*S*). IR (neat), ν(cm^{-1}): 3292s (ν_{N-H}), 2977s (ν_{C-H}), 1715s ($\nu_{C=O}$), 1694s ($\nu_{C=O}$), 1682s ($\nu_{C=O}$), 1456s, 1246s (ν_{C-O}), 1154s, 1037s ($\nu_{C=S}$), 991m, 914s, 859m. ESI-MS (*m*/*z*): 518.3 (100%) $[M+H]^+$.

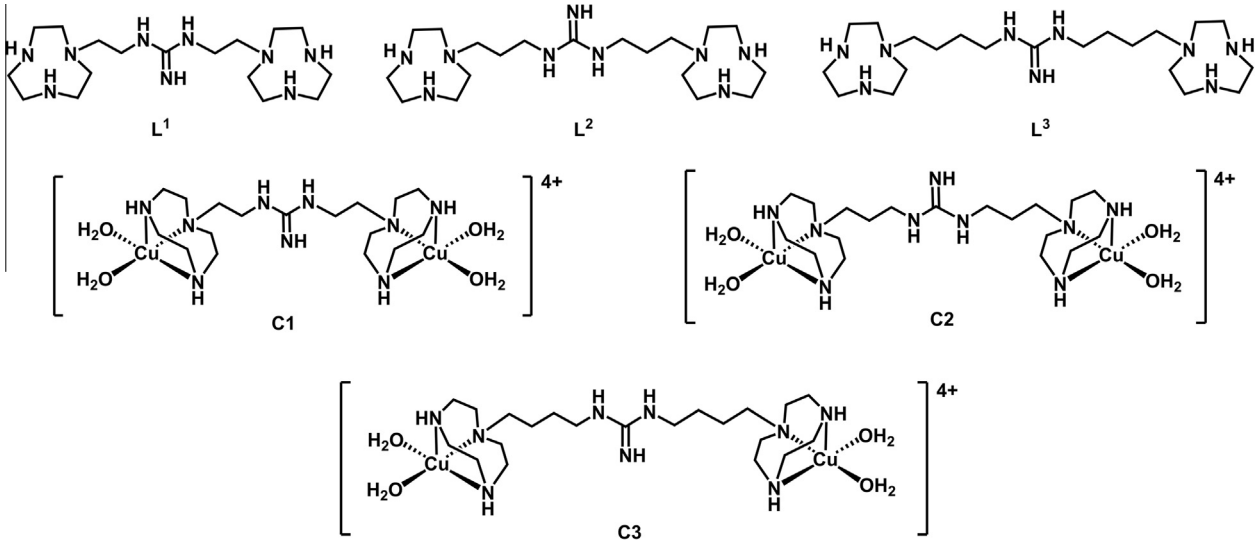

Fig. 1. Ligands **L^1**–**L^3** (isolated as heptahydrochloride salts) and their Cu(II) complexes, **C1**–**C3**, developed in this study.