



# Impact of the substitution pattern of xylyl-bridged 1,4-bis(1,4,7-triazacyclononane) ligands on the crystal structures of their zinc(II) complexes and their ability to bind to N-terminally tagged proteins<sup>☆</sup>

Campbell J. Coghlan<sup>a,b,1</sup>, Eva M. Campi<sup>a,b</sup>, Stuart R. Batten<sup>b</sup>, W. Roy Jackson<sup>a,b</sup>, Milton T.W. Hearn<sup>a,b,\*</sup>

<sup>a</sup> Centre for Green Chemistry, Monash University, Victoria 3800, Australia

<sup>b</sup> Department of Chemistry, Monash University, Victoria 3800, Australia

## ARTICLE INFO

### Article history:

Received 17 November 2017

Accepted 12 January 2018

### Keywords:

Zinc(II) aza-macrocyclic complexes

Crystal structures

Xylynyl linkers

IMAC separations

Bis(tacn) ligands

## ABSTRACT

Reactions of *ortho*-, *para*- and substituted *meta*-xylynyl bridged bis(tacn) ligands with two or three molar equivalents of Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O at pH 5.0 in the presence of NaClO<sub>4</sub> or NH<sub>4</sub>PF<sub>6</sub> resulted in the formation of a range of crystalline zinc complexes. In the presence of perchlorate counter ions the L<sup>px</sup> and L<sup>phx</sup> ligands gave rise to dinuclear Zn<sup>2+</sup> coordination complexes [Zn<sub>2</sub>Cl<sub>2</sub>(L<sup>px</sup>)(H<sub>2</sub>O)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub>(H<sub>2</sub>O) and [Zn<sub>2</sub>Cl<sub>2</sub>(L<sup>phx</sup>)(H<sub>2</sub>O)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> respectively, which formed infinite 1D polymeric chains. In contrast, in the presence of the hexafluorophosphate counter ion, the L<sup>nix</sup> ligand formed a unique tetranuclear Zn<sup>2+</sup> complex, [Zn<sub>4</sub>Cl<sub>4</sub>(L<sup>nix</sup>)(H<sub>2</sub>O)<sub>3</sub>](Cl)(PF<sub>6</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>4</sub>, the L<sup>max</sup> ligand formed an octanuclear complex [Zn<sub>8</sub>Cl<sub>4</sub>(L<sup>max</sup>)<sub>4</sub>(H<sub>2</sub>O)<sub>10</sub>](PF<sub>6</sub>)<sub>6,7</sub>(NO<sub>3</sub>)<sub>1,3</sub>(H<sub>2</sub>O)<sub>3,3</sub>, whilst the L<sup>ox</sup> ligand formed a mononuclear sandwich structure, [Zn(L<sup>ox</sup>)](PF<sub>6</sub>)<sub>2</sub>(H<sub>2</sub>O). The protonated metal-free L<sup>px</sup> ligand crystallised as [H<sub>4</sub>(L<sup>px</sup>)](NO<sub>3</sub>)<sub>2</sub>(PF<sub>6</sub>)(H<sub>2</sub>O)<sub>2</sub> with the stacked ligand cations forming a hydrogen-bonded network. These structural attributes provide an explanation for the significant differences in binding behaviour of recombinant N-terminally tagged proteins with the corresponding Zn<sup>2+</sup>-azamacrocyclic complexes, compared to the analogous Cu<sup>2+</sup>- or Ni<sup>2+</sup>-azamacrocyclic complexes, when immobilised onto support materials and used as immobilised metal affinity chromatographic (IMAC) adsorbents.

© 2018 Elsevier Ltd. All rights reserved.

## 1. Introduction

Over the past two decades, the physicochemical properties of a variety of xylynyl bridged bis(tacn) metal complexes have been investigated. These macrocyclic complexes have been based on derivatised *ortho*-, *meta*- and *para*-xylenes [1–10], on complexes generated from the functionalisation of tri- or tetra-methylbenzenes [11–13] or on complexes derived from a *meta*-xylene core

but with additional functionalisation of the aromatic ring [14–18]. These ligands and their metal complexes have been used in a variety of applications, ranging from modelling of enzyme active sites [13,19], phosphate ester hydrolysis as models for DNA or RNA cleavage [2,10,14,15,20], and chemical oxidation reactions as catalysis [21,22]. Graham and co-workers have also examined [3,5] the binding behaviour of several Cu<sup>2+</sup>-xylynyl bridged bis(tacn) complexes with a selection of small peptides and small globular proteins.

Recently, research effort has focused on the potential of these ligands in immobilised metal affinity chromatography (IMAC) as a platform technology for protein purification [23–28]. IMAC involves the immobilisation of a mono- to tetra-dentate chelating ligand onto a solid support material followed by coordination of a suitable metal ion to this immobilised ligand. The metal ion then interacts with specific metal binding sites presented as accessible motifs within the folded structure of the protein. Often today, these motifs are introduced as unique peptide ‘tags’ by genetic engineering procedures and attached at the N- or C-terminus of the protein of interest. This approach enables the ready removal

**Abbreviations:** Tacn, 1,4,7-triazacyclononane; L<sup>ox</sup>, 1,2-bis(1,4,7-triazacyclonon-1-yl-methyl)benzene; L<sup>px</sup>, 1,4-bis(1,4,7-triazacyclonon-1-ylmethyl)benzene; L<sup>phx</sup>, 3,5-bis(1,4,7-triazacyclonon-1-ylmethyl)-1,1'-biphenyl; L<sup>max</sup>, 3,5-bis(1,4,7-triazacyclonon-1-ylmethyl)-benzoic acid; L<sup>nix</sup>, 3,5-bis(1,4,7-triazacyclonon-1-ylmethyl)-1-nitrobenzene.

<sup>☆</sup> This article is dedicated to our colleague and good friend, Professor Leone Spiccia, whose untimely death in December 2016 cut short a brilliant career in inorganic and materials chemistry.

\* Corresponding author at: Centre for Green Chemistry, Monash University, Victoria 3800, Australia.

E-mail address: [milton.hearn@monash.edu](mailto:milton.hearn@monash.edu) (M.T.W. Hearn).

<sup>1</sup> Current address: School of Chemistry and Physics, Centre for Advanced Nanomaterials, The University of Adelaide, Adelaide 5005, Australia.

of unrelated proteins and most fermentation/cell culture contaminants in a single step with subsequent elution of the target protein selectively [23–28].

Previous studies have predominantly examined the various coordination modes and protein binding behaviour of several of these xylenyl bridged bis(tacn) ligands and related alkyl bridged bis(tacn) ligands with  $\text{Cu}^{2+}$  [2–4,6,7,9–11,13,14,17,18,24–28] and  $\text{Ni}^{2+}$  [4,7,8,11,12,17,24–28] ions. Much less attention has been given to the coordination status and protein binding behaviour of xylenyl bridged bis(tacn) ligands complexed with  $\text{Zn}^{2+}$  ions [1,10,14,28]. This circumstance can be contrasted to the strong interest in the dinuclear zinc complexes of other classes of bis(tacn) ligands, such as the 2-hydroxypropyl-linked analogues, which show cooperativity between the metal ions in the cleavage of phosphate esters and RNA [2,10,29–31]. Surprisingly, in some earlier exploratory studies major differences in selectivity and capture efficiency have been noted with wild type or N-terminally tagged recombinant proteins with different immobilised xylenyl bridged ligands coordinated with  $\text{Zn}^{2+}$  ions compared to the corresponding immobilised  $\text{Cu}^{2+}$ - or  $\text{Ni}^{2+}$ -complexes [24–28]. Hitherto, a rational structural explanation for these differences in binding behaviour has been lacking. The studies described in this paper were designed to address this issue, namely to allow a comparison of the crystal structures of a range of xylenyl bridged bis(tacn) ligands (Fig. 1) when complexed with  $\text{Zn}^{2+}$  ions, and to build upon the insights gained earlier with the corresponding  $\text{Cu}^{2+}$ - and  $\text{Ni}^{2+}$ -complexes of similar bridged bis(tacn) ligands containing a functionalised aromatic ring as part of the linker. The objective was to uncover a structural basis for the observed differences in binding characteristics of recombinant N-terminally tagged proteins with these bis(tacn)  $\text{Zn}^{2+}$  complexes when immobilised as IMAC adsorbents.

## 2. Results & discussion

### 2.1. Preparation of ligands

The  $\text{L}^{\text{PX}}$  and  $\text{L}^{\text{OX}}$  ligands were prepared based on methods reported by Zompa and co-workers [34] for the synthesis of alkylene-bridged bis(azamacrocycles). The reaction of 2 equivalents of 1,4,7-triazatricyclo[5.2.1.0<sup>4,10</sup>]decane (tacn orthoamide) with  $\alpha,\alpha'$ -dibromo-*p*-xylene or  $\alpha,\alpha'$ -dibromo-*o*-xylene respectively, followed by hydrolytic workup and treatment with conc. HCl, gave the  $\text{L}^{\text{PX}}$  and  $\text{L}^{\text{OX}}$  ligands as their hydrochloride salts ( $\text{L}\cdot\text{6HCl}$ ) in ca 80% yield. The  $\text{L}^{\text{PHX}}$ ,  $\text{L}^{\text{NIX}}$  and  $\text{L}^{\text{CAX}}$  ligands were prepared in ca. 85% yield using similar methods with appropriately substituted  $\alpha,\alpha'$ -dibromo-*m*-xylene [17]. The products were analysed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy and electrospray ionisation mass spectroscopy (ESI-MS) and, following crystallisation, were of high purity, e.g., ~97–99%.

### 2.2. Preparation and characterisation of metal complexes

The  $\text{6HCl}$  salts of the different bis(tacn) ligands were dissolved in water containing 2 molar equivalents of  $\text{Zn}(\text{NO}_3)_2\cdot\text{6H}_2\text{O}$  with adjustment to pH 5.0. The counter ion,  $\text{PF}_6^-$ , as solid ammonium hexafluorophosphate, or the counter ion,  $\text{ClO}_4^-$ , as solid sodium perchlorate, was then added. These solutions were left to evaporate for several days. Filtration afforded crystals of the  $\text{Zn}^{2+}$ -complexes suitable for X-ray structure determination. Chromatographic analysis of these solutions and subsequently elemental analysis of the recovered crystals of the  $\text{Zn}^{2+}$ -complexes were consistent with formation of the desired products in excellent yields. As observed with other bis(tacn) ligand–metal complexes, the presence of varying amounts of adventitiously bound water, which proved resistant

to removal, resulted in the lower carbon values than theoretically expected upon elemental analysis of the recovered crystalline products.

### 2.3. The structure of $[\text{Zn}(\text{L}^{\text{OX}})](\text{PF}_6)_2(\text{H}_2\text{O})$ (1)

Structural data for the  $[\text{ZnL}^{\text{OX}}](\text{PF}_6)_2(\text{H}_2\text{O})$  complex **1** was compared to the corresponding perchlorate counter-ion complex to allow the effects of different counter ions on the crystal structure of the complex to be examined. To this end, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for the  $[\text{ZnL}^{\text{OX}}](\text{PF}_6)_2(\text{H}_2\text{O})$  complex **1** were obtained and found to be consistent with data [2] for the zinc complex of the same ligand but with a perchlorate counter-ion present. Although the  $\text{PF}_6^-$  salt, **1**, crystallised in a different space group (triclinic *P*-1 compared [1] to monoclinic *P*2<sub>1</sub>/*c* for the  $\text{ClO}_4^-$  salt), the X-ray crystallographic structures of both  $\text{Zn}^{2+}$ - $\text{L}^{\text{OX}}$  complexes were found to be similar. Both tacn rings of the  $\text{L}^{\text{OX}}$  ligand bind to single  $\text{Zn}^{2+}$  ion ( $\text{M}-\text{N} = 2.147(3)-2.301(4)$  Å), giving a sandwich structure (Fig. 2) with the  $\text{Zn}^{2+}$  ion residing in a constrained octahedral geometry due to Jahn–Teller distortion. The *cis* angles relating to the individual tacn rings ranged from 77.97(14)° to 82.02(14)°, while those involving nitrogen atoms from different tacn rings ranged from 87.37(16)° to 115.95(14)°. The *trans* angles ranged between 160.87(14)° and 166.38(13)°. In contrast, when 3 molar equivalents of  $\text{Ni}(\text{NO}_3)_2$  were similarly employed with the same  $\text{L}^{\text{OX}}$  ligand, a mixture of the analogous mononuclear sandwich structure and an extended binuclear structure (both as their perchlorate salts) was obtained. Moreover, studies by Graham et al. have found [4] that the corresponding mononuclear  $\text{Cu}^{2+}$ -complex was formed when a limiting amount of  $\text{Cu}(\text{NO}_3)_2$  (1 molar equivalent) was used, but the extended binuclear  $\text{Cu}^{2+}$ -complex was generated when 2 or 3 molar equivalents of  $\text{Cu}(\text{NO}_3)_2$  were employed. Collectively, these studies have confirmed that under pH and buffer conditions favourable for protein stability under IMAC conditions, the immobilised  $\text{L}^{\text{OX}}$  ligand can adopt an extended structure with two  $\text{Cu}^{2+}$  or  $\text{Ni}^{2+}$  ions bound, but only a sandwich structure is present for the corresponding  $\text{Zn}^{2+}$ - $\text{L}^{\text{OX}}$  complex.

### 2.4. The structure of $[\text{H}_4\text{L}^{\text{PX}}](\text{NO}_3)_3(\text{PF}_6)(\text{H}_2\text{O})_2$ (2)

Crystals of the protonated metal-free  $\text{L}^{\text{PX}}$  ligand **2** were obtained from the reaction of  $\text{L}^{\text{PX}}$  with  $\text{Zn}(\text{NO}_3)_2$ , using  $\text{PF}_6^-$  as a counter ion. The asymmetric unit contained halves of two unique  $\text{H}_4\text{L}^{\text{PX}}$  cations, three nitrate anions, one hexafluorophosphate anion, and two intercalated water molecules. Both  $\text{H}_4\text{L}^{\text{PX}}$  ligand cations lie across inversion centres which relate the two halves of each  $\text{L}^{\text{PX}}$  ligand. Each ligand carries a +4 charge, since each secondary amine has two hydrogen atoms attached. In contrast, the  $\text{L}^{\text{PX}}$  ligand when coordinated to a zinc metal ion has only a single hydrogen on each secondary amine, and the complexed ligand therefore has no overall charge. This +4 charge of the protonated metal-free  $\text{L}^{\text{PX}}$  ligand is balanced by three  $\text{NO}_3^-$  anions and a  $\text{PF}_6^-$  anion.

The two crystallographically distinct ligands are orientated in different directions and stack in an alternating fashion along the *x* axis, crossing at the central benzene rings, for which the closest C...C contact is 3.510 Å (Fig. 3). Furthermore, there is extensive hydrogen bonding throughout. Each secondary amine proton is hydrogen bonded to either a nitrate or a water oxygen atom; there are also weaker C–H...O interactions between one of the aliphatic tacn protons and a nitrate oxygen. The intercalated water molecules also make hydrogen bonds to the nitrate anions and to the  $\text{PF}_6^-$  anions. The  $\text{PF}_6^-$  anions are also hydrogen bonded to the water molecules and to an aliphatic proton from a tacn ring. The hydrogen bonding interactions connect the components into complicated sheets, as shown in Fig. 4. These sheets are then interconnected by the  $\text{PF}_6^-$  anions and the backbone of one of the

Download English Version:

<https://daneshyari.com/en/article/7763089>

Download Persian Version:

<https://daneshyari.com/article/7763089>

[Daneshyari.com](https://daneshyari.com)