Citation for published version:

Betts, HM, Pascu, SI, Buchard, A, Bonnitcha, PD & Dilworth, JR 2014, 'One-pot synthesis, characterisation and kinetic stability of novel side-bridged pentaazamacrocyclic copper(ii) complexes', *RSC Advances*, vol. 4, no. 25, pp. 12964-12970. <https://doi.org/10.1039/c3ra47450j>

DOI:

[10.1039/c3ra47450j](https://doi.org/10.1039/c3ra47450j)

Publication date:

2014

Document Version

Peer reviewed version

[Link to publication](#)

University of Bath

Alternative formats

If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

COMMUNICATION

1

One-pot synthesis, characterisation and kinetic stability of novel side-bridged pentaazamacrocyclic copper(II) complexes

Helen M. Betts,* Sofia I. Pascu,* Antoine Buchard, Paul D. Bonnitcha and Jonathan R. Dilworth*

This work focuses on a new, one-step facile, synthesis method for a new family of side-bridged copper(II) complexes based on 15-membered pentaazamacrocycles and their detailed spectroscopic and structural characterisation. Synchrotron X-ray crystal structure backed up by DFT computational modeling and further

electrochemical and aqueous stability studies have been carried out and the results seem to demonstrate that these complexes have relatively reasonable stability kinetically and thermodynamically and compare well with the state-of-the-art in copper complexes available for bifunctional chelator scaffolds.

Please check this proof carefully. Our staff will not read it in detail after you have returned it. Translation errors between word-processor files and typesetting systems can occur so the whole proof needs to be read. Please pay particular attention to: tabulated material; equations; numerical data; figures and graphics; and references. If you have not already indicated the corresponding author(s) please mark their name(s) with an asterisk. Please e-mail a list of corrections or the PDF with electronic notes attached - do not change the text within the PDF file or send a revised manuscript. Corrections at this stage should be minor and not involve extensive changes. All corrections must be sent at the same time.

Please bear in mind that minor layout improvements, e.g. in line breaking, table widths and graphic placement, are routinely applied to the final version.

We will publish articles on the web as soon as possible after receiving your corrections; **no late corrections will be made.**

Please return your **final** corrections, where possible within **48 hours** of receipt by e-mail to: advances@rsc.org

1	Queries for the attention of the authors	1																					
	Journal: RSC Advances																						
5	Paper: c3ra47450j	5																					
	Title: One-pot synthesis, characterisation and kinetic stability of novel side-bridged pentaazamacrocyclic copper(II) complexes																						
10	Editor's queries are marked like this... 1 , and for your convenience line numbers are inserted like this... 5	10																					
	Please ensure that all queries are answered when returning your proof corrections so that publication of your article is not delayed.																						
15	<table border="1"> <tr> <th>Query Reference</th><th>Query</th><th>Remarks</th></tr> <tr> <td>1</td><td>For your information: You can cite this article before you receive notification of the page numbers by using the following format: (authors), RSC Adv., (year), DOI: 10.1039/c3ra47450j.</td><td></td></tr> <tr> <td>2</td><td>Please check that the inserted CCDC number is correct.</td><td></td></tr> <tr> <td>3</td><td>Please carefully check the spelling of all author names. This is important for the correct indexing and future citation of your article. No late corrections can be made.</td><td></td></tr> <tr> <td>4</td><td>A citation to Fig. 6 has been added here, please check that the placement of this citation is suitable.</td><td></td></tr> <tr> <td>5</td><td>A citation to Table 2 has been added here, please check that the placement of this citation is suitable.</td><td></td></tr> <tr> <td>6</td><td>Please check that the GA text fits within the allocated space indicated on the front page of the proof. If the entry does not fit between the two horizontal lines, then please trim the text and/or the title.</td><td></td></tr> </table>	Query Reference	Query	Remarks	1	For your information: You can cite this article before you receive notification of the page numbers by using the following format: (authors), RSC Adv., (year), DOI: 10.1039/c3ra47450j.		2	Please check that the inserted CCDC number is correct.		3	Please carefully check the spelling of all author names. This is important for the correct indexing and future citation of your article. No late corrections can be made.		4	A citation to Fig. 6 has been added here, please check that the placement of this citation is suitable.		5	A citation to Table 2 has been added here, please check that the placement of this citation is suitable.		6	Please check that the GA text fits within the allocated space indicated on the front page of the proof. If the entry does not fit between the two horizontal lines, then please trim the text and/or the title.		15
Query Reference	Query	Remarks																					
1	For your information: You can cite this article before you receive notification of the page numbers by using the following format: (authors), RSC Adv., (year), DOI: 10.1039/c3ra47450j.																						
2	Please check that the inserted CCDC number is correct.																						
3	Please carefully check the spelling of all author names. This is important for the correct indexing and future citation of your article. No late corrections can be made.																						
4	A citation to Fig. 6 has been added here, please check that the placement of this citation is suitable.																						
5	A citation to Table 2 has been added here, please check that the placement of this citation is suitable.																						
6	Please check that the GA text fits within the allocated space indicated on the front page of the proof. If the entry does not fit between the two horizontal lines, then please trim the text and/or the title.																						
20		20																					
25		25																					
30		30																					
35		35																					
40		40																					
45		45																					
50		50																					
55		55																					

COMMUNICATION

One-pot synthesis, characterisation and kinetic stability of novel side-bridged pentaazamacrocyclic copper(II) complexes†

Cite this: DOI: 10.1039/c3ra47450j

Received 10th December 2013
Accepted 18th February 2014Helen M. Betts,^{‡*a} Sofia I. Pascu,^{*b} Antoine Buchard,^b Paul D. Bonnitcha^a and Jonathan R. Dilworth^{*a}

DOI: 10.1039/c3ra47450j

www.rsc.org/advances

Single-step synthetic protocols are reported for novel macrocyclic copper(II) complexes incorporating a piperazine unit in the ligand backbone. Synchrotron X-ray structure determination confirmed the copper(II) coordination geometry in the solid state as distorted square pyramidal with chloride occupying the axial position above the N4 ligand plane. DFT calculations, electrochemical and UV/vis investigations in biological media all confirmed that compounds in this class have high stability, and compare well in both thermodynamic and kinetic terms with the ubiquitous Cu(II) cyclam compounds, and are therefore of relevance for future use as synthetic scaffolds for the binding, transport and tracing of copper ions *in vitro*.

Introduction

Radiopharmaceuticals are compounds that are labelled with a radioactive isotope, and designed to accumulate specifically in a target tissue *in vivo*. Once localised in their target tissue, emissions from decay of the radioisotope allow non-invasive molecular imaging, or delivery of a therapeutic radiation dose. Copper complexes are attractive candidates for development as radiopharmaceuticals for a number of reasons.^{1,2} Several copper radioisotopes are available which decay by various modes, with half-lives ranging from a few minutes to several days. The choice of radioisotope, copper-60 (β^+ , EC, $t_{1/2} = 0.38$ h), copper-62 (β^+ , EC, $t_{1/2} = 0.16$ h) copper-64 (β^+ , EC, β^- , $t_{1/2} = 12.7$ h) or copper-67 (β^- , $t_{1/2} = 62$ h), is determined based on the desired application and isotope availability.³ The coordination chemistry of copper is well studied, its chemistry limited to two main oxidation states, and radiolabelling is typically achieved by simple heating of a

pro-ligand in a buffered aqueous solution of the radioisotope. New ligands designed to deliver radiocopper specifically to diseased tissue are therefore of great interest for both imaging and therapeutic applications. The most widely used approach for development of copper radiopharmaceuticals is based on a bifunctional chelate (BFC).⁴ A BFC contains a ligand set for coordination of the copper ion and a biological targeting group, joined by a covalent linker (Fig. 1).

Tetraaza macrocycles have been extensively explored as ligands for copper radiopharmaceuticals.⁵ Copper(II) complexes of cross-bridged cyclam derivatives based on **2** have significantly higher kinetic stability *in vivo* than complexes of cyclam, **1**, and recent research effort has been focussed on developing bifunctional chelates of these complexes^{1,6,7} (Fig. 2). Side-bridged analogues containing a piperazine unit in the macrocycle backbone, such as Et-cyclam **5**, have also been reported.⁸ In acid dissociation studies, copper complexes $[\text{Cu5}][\text{ClO}_4]_2$ and $[\text{Cu1}][\text{ClO}_4]_2$ are of similar stability, but the experimental conditions used in these studies were not directly relevant to those experienced by BFCs *in vivo*.⁹ Despite the extensive reports in the literature of bridged 14-membered copper(II) macrocycles and their bifunctional chelates, their 15-membered analogues have not been studied to such extent.

The 15-membered tetraaza macrocycle **6** and its copper(II) complex are key examples of 15-membered macrocycles which have a tethering group for conjugation to a biologically active molecule.¹⁰ The considerable advantage of **6** over the more popular 14-membered derivatives is that it can be prepared in good yield in a simple, one-step procedure from commercially available tetramines, formaldehyde and nitromethane. The aim of our work was to develop this chemistry as a facile route to

Fig. 1 Schematic representation of a bifunctional chelator.

^aUniversity of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK. Tel: +44 (0)1865 272689^bUniversity of Bath, Chemistry Department, Bath, BA2 7AY, UK. E-mail: s.pascu@bath.ac.uk

† Electronic supplementary information (ESI) available. CCDC 975683. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ra47450j

‡ Present address: Nottingham University Hospitals NHS Trust, PET/CT Centre, Nottingham City Hospital, Nottingham, NG5 1PB, UK

Fig. 2 Chemical structures of ethylene strapped macrocyclic ligands.

Fig. 3 ORTEP representation (where displacement ellipsoids are drawn at the 40% probability level) of the molecular structure of **7b** resulting from synchrotron X-ray diffraction measurements. The perchlorate counter-ion has been omitted for clarity.

prepare novel side-bridged macrocycles, and examine the suitability of the resulting copper(II) complexes as potential radio-pharmaceuticals. This article describes the synthesis, characterisation and stability assessment of novel 15-membered copper(II) macrocycles **7a** and **7b** (Fig. 2).

Results and discussion

Copper(II) macrocycle synthesis

The novel complexes **7a** and **7b** were prepared in a one-step method using primary amines to complete the cyclisation step.¹¹ The side-bridged tetramine bis(3-aminopropyl)piperazine was reacted with formaldehyde and either benzylamine or allylamine in the presence of copper(II) nitrate (Scheme 1).

The deep blue reaction mixtures were heated at reflux for 24 h, and then cooled to room temperature. It proved impossible to isolate a pure perchlorate salt directly from the reaction mixture, so the reaction solution was purified on a Sephadex-SP C25 column, eluted with sodium chloride solution. After addition of aqueous sodium perchlorate to the major band eluted from the column, solutions were stored at 4 °C. After 14 days, 7-benzyl-1,5,7,9,13-penta-aza-(1,13-ethano)-cyclopentadecane

Scheme 1 Synthesis of macrocyclic complexes **7a** and **7b**.

chlorocopper(II) perchlorate **7a** and 7-allyl-1,5,7,9,13-penta-aza-(1,13-ethano)-cyclopentadecane chloro copper(II) perchlorate **7b**, were isolated as fine blue needles obtained in 68% and 59% yield, respectively. Elemental analysis confirmed the purity of both complexes. The UV/vis spectra of **7a** and **7b** showed two main bands at 285 nm and 571 nm. The weak d-d transition observed at 571 nm was shifted relative to that reported in the literature for $[\text{CuI}][\text{ClO}_4]_2$ and $[\text{Cu5}][\text{ClO}_4]_2$, consistent with a coordinated axial ligand for the new complexes.^{12,13}

Crystals of **7b** suitable for X-ray analysis were isolated by slow diffusion of Et_2O into a solution of the complex in MeCN. Data was collected using radiation from a synchrotron source (Daresbury, UK). The molecular structure is shown in Fig. 4, and selected bond lengths and angles are given in Table 1.

The copper(II) ion is coordinated in distorted square based pyramidal geometry. The bonds between the copper ion and piperazine nitrogen atoms (N3) and (N4) are slightly longer than the copper to secondary amine bonds (N1) and (N2). The ethylene bridging unit causes the piperazine fragment bite angle to be 72.25°, which is notably more acute than the other N-Cu-N angles as a result of the steric restriction. In the previously reported crystal structures of $[\text{Cu4}][\text{ClO}_4]_2$ and $[\text{Cu5}][\text{ClO}_4]_2$, the analogous angles are 74.00° and 77.71° respectively, illustrating the restraint imposed by the piperazine side-bridge.⁹ In the structure of **7b**, a chloride counter-ion is coordinated to the copper ion in an axial site, with a long bond of

Fig. 4 Cyclic voltammograms for compound **7a** in 0.1 M NaCl at scan rates 25, 150 and 300 mV s^{-1} .

Table 1 Significant molecular parameters for the X-ray structure of **7b**

Bond	Bond length Å	Bond	Angle °
Cu(1)–N(1)	2.048	N(1)–Cu(1)–N(2)	91.08
Cu(1)–N(2)	2.043	N(2)–Cu(1)–N(4)	95.45
Cu(1)–N(3)	2.091	N(4)–Cu(1)–N(3)	72.25
Cu(1)–N(4)	2.112	N(3)–Cu(1)–N(1)	94.62
Cu(1)–Cl(3)	2.462		

2.462 Å. A non-coordinating perchlorate counter ion is also present (not shown in the figure). In the crystal structure of [Cu5][ClO₄]₂, the copper ion is found in a distorted square planar environment with deviation of 0.06 Å towards the perchlorate counter-ion, from the plane defined by the nitrogen atoms.⁹ A similar distortion of 0.11 Å was observed in the crystal structure of non-cyclic structure [Cu4][ClO₄]₂.⁹ The distortion of the copper(II) from the plane towards the chloride in **7b** is more pronounced, and is 0.346 Å. The complex [Cu1][ClO₄]₂, without the piperazine bridge, shows the metal perfectly in the plane of the nitrogen atoms.¹³ Literature reports show a tendency for the reinforced systems to deviate from square planar coordination to a square based pyramidal geometry, and this is also found for **7b**.^{9,10}

Electrochemistry

The reduction of copper(II) to copper(I) and ligand dissociation is considered the main pathway for loss of copper from macrocyclic BFCs *in vivo*.^{2,5} It is therefore undesirable for prospective radiopharmaceuticals of copper(II) macrocycles to be reduced at biologically accessible potentials. To assess the reduction of the novel 15-membered copper(II) macrocycles, cyclic voltammetry of **7a** was examined. Experiments were conducted in 0.1 M NaCl solution, using a glassy carbon working electrode, platinum wire auxiliary electrode and Ag/AgCl aqueous reference electrode. Potentials are quoted relative to the saturated calomel electrode (SCE). The cyclic voltammograms of **7a** recorded with scan rates of 25, 150 and 300 mV s^{−1} are displayed in Fig. 4. The copper(II)–copper(I) reduction couple for **7a** was completely irreversible at all scan rates tested (5–350 mV s^{−1}). With a scan rate of 150 mV s^{−1}, *E*_p was −0.82 V vs. SCE, which appears to be outside the range of biologically accessible potentials.¹⁴ However displacement of chloride with a neutral ligand would give a dicationic complex likely to reduce at more positive potentials. The negative shift of reduction potential with increased scan rate is consistent with either an electrochemically irreversible process or reduction followed by a rapid chemical reaction. Similar irreversible reductions occur in the case of [Cu(II)TETA] (*E*_p = −0.96 V vs. SCE).¹⁵ The electrochemical information alone is not sufficient to predict *in vivo* behaviour of the complexes, but the redox profile suggests that **7a** would not be easily reduced under biological conditions.

Kinetic stability tests by UV-vis spectroscopy

The kinetic stability of **7a** was studied under conditions relevant to biomedical applications.¹⁶ The copper(II) complexes of cyclam **1**, and the non-macrocyclic ligands 1,4-bis(3-aminopropyl)-piperazine **4**, and 1,2-bis(3-aminopropyl) ethylenediamine **3**,

were also prepared to compare their stability with that of **7a**. The aqueous stability of **7a** was first compared with [Cu1]Cl₂, by monitoring the UV/vis spectra of the complexes over time. In H₂O alone, no changes to either spectrum were observed after 48 h. The stability of **7a** and [Cu1]Cl₂ to a biologically relevant redox challenge was tested by addition of cysteine to the complexes in phosphate buffer at pH 7.

After 20 h incubation in phosphate buffer at room temperature, 92% of the initial absorbance at 571 nm remained for **7a**. After 20 h incubation after addition of cysteine, 91% of compound **7a** remained intact.

These results were almost identical to those observed for [Cu1]Cl₂ under the same conditions, which showed 94% remaining after 20 h in buffer and 93% remaining after 20 h with cysteine. Both of these complexes are therefore kinetically stable under these conditions. The non-macrocyclic complexes [Cu3][ClO₄]₂ and [Cu4][ClO₄]₂ were instantly decolourised on addition of cysteine, indicating reduction to copper(I), and ligand loss. Human serum stability tests were then performed. Solutions of **7a** and [Cu1]Cl₂ were incubated at 37 °C in human serum, and monitored by UV/vis spectroscopy over 24 h. The

Fig. 5 (a) Change in the UV/vis spectrum of **7a** after addition to human serum. (b) Percentage decrease in peak absorbance over time for **7a** and [Cu1]Cl₂.

Fig. 6 GaussView representation of the molecular structure of **7a** cation only (without inclusion of a counter anion) resulting from DFT optimisation using the protocol uM06/6-31+G(d,p)/SCRF = (cpcm, solvent = methanol)/temperature = (337.85). The hydrogen atoms have been omitted for clarity.

overlaid spectra for **7a** at several time points are displayed in Fig. 5a, and a comparison of **7a** with $[\text{CuI}]\text{Cl}_2$ is displayed in Fig. 5b. After 3 h in human serum 95% of **7a** remained intact compared with 97% of $[\text{CuI}]\text{Cl}_2$ but after 24 h 77% of **7a** remained intact, compared with 94% for $[\text{CuI}]\text{Cl}_2$. Solutions of compounds $[\text{Cu3}][\text{ClO}_4]_2$ and $[\text{Cu4}][\text{ClO}_4]_2$ became colourless almost instantly upon addition to human serum, indicating that the copper was rapidly stripped from the ligand and bound to the serum proteins (Fig. 6).

Thermodynamic stability estimated by DFT calculations in solution

Thermodynamics of the coordination reactions between copper(II) chloride dihydrate and ligands 7-benzyl-1,5,7,9,13-penta-aza-(1,13-ethano)-cyclopentadecane or 1,4,8,11-tetraazacyclotetradecane (cyclam) were examined using DFT calculations.

Scheme 2 DFT calculated reaction schemes and corresponding relevant T_d parameters at the formation of the **7a** cation in solution.

Table 2 Significant molecular parameters for the DFT optimised structure of **7a**. (The counter anion was excluded for clarity and to reduce computational time)

Bond	Bond length Å	Bond	Angle °
Cu(1)–N(1)	2.070	N(1)–Cu(1)–N(2)	90.70
Cu(1)–N(2)	2.083	N(2)–Cu(1)–N(4)	94.56
Cu(1)–N(3)	2.107	N(4)–Cu(1)–N(3)	71.76
Cu(1)–N(4)	2.107	N(3)–Cu(1)–N(1)	94.62
Cu(1)–Cl(1)	2.434		

Full coordinates for all stationary points, together with computed free Gibbs energy and vibrational frequency data, DFT calculated reaction schemes and corresponding relevant T_d parameters at the formation of the **7a** cation in solution are included in Scheme 2. Both variants likely to form from solution, *e.g.* with and without inclusion of a chloride ion in the copper coordination sphere were modelled, as shown in Scheme 2(a) and (b), respectively. These were found to compare well in terms of T_d stability with the copper(II) cyclam dichloride $[\text{CuI}]\text{Cl}_2$ (**1c**), with **7a** being the most thermodynamically stable in the series (Table 2).

Conclusions

New side-bridged copper(II) complexes of 15-membered pentaa-macrocycles have been prepared in a single-step reaction. The novel complexes were fully characterised, including an X-ray crystal structure of the *N*-allyl derivative. Electrochemical and aqueous stability studies suggested that these complexes are reduced outside of a biologically accessible window, and have good kinetic stability. Their thermodynamic stability, evaluated by DFT calculations in solution, rendered them potential candidates for kinetic evaluation in biological media and pointed out to the fact that these compounds compare well with the established copper analogues based on the cyclam motif commonly used in radiochemistry work with ^{67}Cu and ^{64}Cu . They were found to be of comparable kinetic stability, albeit marginally less kinetically stable, than the known 14-membered cyclam analogues in aqueous environments.^{5–9} Therefore, these compounds, although representing useful alternatives to the known systems as synthetic scaffolds for future development work towards BFCs for radiopharmaceutical applications, their close similarity to known systems did not justify in full extensive radiolabelling development work with $^{64}\text{Cu(II)}$ at present. Radiolabelling studies are in progress, encouraged by their thermodynamic and kinetic stability, particularly with the view to screen their availability to binding other metals of relevance to PET imaging, such as Ga-68 and evaluate their potential for tagging with targeting biomolecules. Those findings will be reported elsewhere.

Experimental section

General procedure for macrocycle formation

Copper(II) nitrate trihydrate (5 mmol) was added to bis(amino-propyl)piperazine (1.0 mL, 5.0 mmol) in MeOH (60 mL), and

formaldehyde (0.82 mL, 10 mmol) was added. The desired amine component (5.5 mmol) was added, and the reaction stirred at reflux for 24 h. The reaction was cooled to room temperature, and analysed by MS.

Copper(II) cyclam dichloride [Cu1]Cl₂

Cyclam (0.30 g, 1.5 mmol) was dissolved in EtOH (40 mL) and [Cu(II)Cl₂·2H₂O] (0.26 g, 1.5 mmol) was added and the reaction stirred at reflux for 4 h. The reaction was filtered and filtrate evaporated to dryness. The residue was recrystallised from *n*-PrOH, yielding purple crystals (0.43 g, 86.6%).

Bis(aminopropyl) ethylene diamine copper(II) diperchlorate [Cu3][ClO₄]₂

Bis(aminopropyl)ethylene diamine (0.92 mL, 5.0 mmol), was dissolved in EtOH (20 mL) and a solution of [Cu(II)(ClO₄)₂·6H₂O] (1.85 g, 5 mmol) in H₂O (5 mL) was added. The reaction stirred for 30 min at room temperature, and was chilled in the fridge for 1 h. The purple precipitate was filtered and rinsed with EtOH (1.95 g, 89.7%). MS (ES⁺) *m/z* 236.1074 (calc. for C₈H₂₁N₄Cu 236.1062) [M - H]⁺.

Bis(aminopropyl)piperazine copper(II) diperchlorate [Cu4][ClO₄]₂

Bis(aminopropyl)piperazine (1.03 mL, 5 mmol) was dissolved in H₂O (15 mL) and [Cu(II)(ClO₄)₂·6H₂O] (1.84 g, 5 mmol) was added. The reaction stirred at room temperature for 1 h. The solution was stored at room temperature for 4 days, after which the purple crystals were filtered, and washed with ice cold H₂O. MS *m/z* 362.09 (calc. for C₁₀H₂₄CuN₄ClO₄ 362.08).

7-Benzyl-1,5,7,9,13-penta-aza-(1,13-ethano)-cyclopentadecane chloro copper(II) perchlorate (7a)

Bis(aminopropyl)piperazine (1.0 mL, 5.0 mmol) was dissolved in MeOH (60 mL) and [Cu(II)(NO₃)₂·3H₂O] (1.20 g, 5.0 mmol) was added. Formaldehyde (37% in H₂O), (1.66 mL, 20 mmol) and benzylamine (0.60 mL, 5.5 mmol) were added. The reaction stirred at reflux for 24 h. The reaction was cooled, filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in H₂O (5 mL) and loaded onto a Sephadex-SP C25 column (35 × 200 mm), washed with H₂O (50 mL) and eluted with NaCl (aq., 0.1 M). The major band was concentrated under reduced pressure. MeOH (20 mL) was added, and the mixture was filtered. Saturated NaClO₄ (aq.) was added to the filtrate, and the solution was stored at 4 °C for 7 days. Fine blue crystals were formed, which were filtered and rinsed with ice-cold H₂O (2 mL) (1.79 g, 67.7%). Elemental analysis (calc. + 2H₂O) C 40.88 (40.32), H 6.45 (6.59), N 12.42 (12.37). UV/vis λ_{max} (H₂O) 571 nm (ε = 259.7 mol⁻¹ dm³ cm⁻¹). IR ν (cm⁻¹) 3424, 3205, 2870, 1471, 1416, 1287, 1221, 1097, 1026, 968, 925, 842, 788, 746, 701, 622. MS (ES⁺) *m/z* 429.1737 (calc. for C₁₉H₃₃N₅ClCu 429.1720).

7-Allyl-1,5,7,9,13-penta-aza-(1,13-ethano)-cyclopentadecane chloro copper(II) perchlorate (7b)

[Cu(II)(NO₃)₂·3H₂O] (0.60 g, 2.5 mmol) was dissolved in MeOH (40 mL) and bis(3-aminopropyl)piperazine (0.50 mL, 2.5 mmol) was added. Formaldehyde (37% in H₂O, 0.83 mL, 10 mmol) and allylamine (0.23 mL, 2.75 mmol) were added. The mixture was refluxed for 24 h. The reaction mixture was evaporated to dryness, and product redissolved in H₂O, and loaded onto a Sephadex-SP C25 column (35 × 200 mm). Elution with NaCl (aq., 0.1 M) yielded 3 bands, and the product was found in the major band, which was the first eluted. The solution was evaporated, MeOH was added and the mixture was filtered. Excess saturated NaClO₄ (aq.) was added, and the mixture cooled to 4 °C for 7 days, after which blue needles formed. The product was filtered, rinsed with ice-cold H₂O (2 mL) and dried in air (0.71 g, 59.2%). Elemental analysis (calc.) C 37.58 (37.54), H 6.47 (6.51), N 14.46 (14.59). UV/vis λ_{max} (H₂O) 571 nm (ε = 269.7 mol⁻¹ dm³ cm⁻¹). IR ν (cm⁻¹) 3442, 3194, 2870, 2360, 1470, 1419, 1293, 1093, 1027, 968, 922, 787, 623. MS (ES⁺) *m/z* 343.1801 (calc. for C₁₅H₃₀N₅Cu 343.1797) [M - H]⁺.

Kinetic stability tests

The copper macrocycles were prepared as 0.015 M stock solutions in DMSO. Dilutions were performed as described below. Solutions were prepared in quartz cuvettes (3 mL) for direct monitoring by UV/vis at desired time-points. The cuvettes were removed from the instrument between recordings.

Phosphate buffer stability

The stock solution (0.1 mL) was added to phosphate buffer (pH = 7.0, 2.9 mL), and stored at room temperature between recordings.

Cysteine challenge

The stock solution (0.1 mL) was added to phosphate buffer (pH = 7.0, 1.9 mL), and cysteine solution (1.0 mL, 2 mM in phosphate buffer), (10–20 equivalents of cysteine). The solutions were stored at room temperature.

Human serum binding

The stock solution was prepared at 0.015 M in H₂O. The stock (0.2 mL) was added to human serum (from human male AB plasma, Sigma-Aldrich) (2.8 mL) and incubated in a water bath at 37 °C between recordings.

Synchrotron X-ray diffraction studies

Blue-coloured, single crystals were grown by the slow diffusion of diethyl ether vapor into an acetonitrile solution (*ca.* 1 mg mL^{−1}) of **7b**. A single crystal of **7b** was selected under inert atmosphere, encased in perfluoro-polyether oil, and mounted on the end of a glass fiber. The fiber, secured on a goniometer head was then placed under a stream of cold nitrogen maintained at 150 K.

Crystals were small and weakly diffracting, so a synchrotron radiation source was used to collect diffraction data for this compound (at 150 K). Data were collected at Station 9.8, Daresbury SRS, UK, using a Bruker SMART CCD diffractometer. The structure was solved by direct methods using the program SIR92.¹⁷ The refinement and graphical calculations were performed using the CRYSTALS¹⁸ software package. The structure was refined by full-matrix least-squares procedure on *F*. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were located in Fourier maps and their positions adjusted geometrically (after each cycle of refinement) with isotropic thermal parameters.

Selected crystallographic data

Moiety formula [C₁₅ H₃₁ Cl Cu N₅]⁺, [ClO₄][−] *M* = 477.88, *Z* = 8, monoclinic, space group *P*2₁/*n*, *a* = 11.9964(8) Å; *b* = 15.8250(11) Å, *c* = 21.4986(15) Å; beta = 91.7800(10)°, *U* = 4079.4(5) Å³, *T* = 150(2) K, *μ* = 1.364 mm^{−1}. Of 38 420 reflections measured, 11 021 were independent (*R*_{int} = 0.061); final *R* = 0.0437 (4626 reflections with *I* > 3σ(*I*) and *wR* = 0.0497). Fig. 3 contains the ORTEP diagram (40% probability) and Table 1 lists significant molecular parameters.

DFT computational details

Full coordinates for all stationary points, together with computed free Gibbs energy and vibrational frequency data, are also available *via* the corresponding Gaussian 09 output files, stored in the digital repository (DOI: 10.6084/m9.figshare.907517). All calculations employed the following protocol: uM06/6-31++G(d,p)/SCRF = (cpcm, solvent = methanol)/temperature = (337.85). The M06 functional is one of the hybrid meta-generalized gradient-approximations functionals (hybrid meta-GGAs), developed by the group of Prof. Donald Truhlar at the University of Minnesota. M06 includes 27% of Hartree–Fock exchange, has been parametrised including both transition metals and non-metals, and is recommended for application in transition metal thermochemistry and non-covalent interactions.¹⁹ The calculations were all carried out using a temperature of 337.85 K and the self-consistent-reaction-cavity continuum solvation model to account for the coordination reactions conditions (methanol reflux). Implementation of the self-consistent-reaction-cavity continuum solvation model has recently been enhanced to allow efficient evaluation of first and second energy derivatives, following an earlier suggestion of the method.^{20,21} All open shell systems were treated with the unrestricted approach. The split-valence double ζ with polarization and diffuse functions 6-31++G(d,p) basis set was selected to account for potential ions and nonbonding interactions, while allowing the models to scale up to the maximum size of 59 atoms, and an associated maximum of 677 basis functions.

All geometries were fully optimized without any symmetry or geometry constraints. The nature of all the stationary points as minima was verified by calculations of the vibrational frequency spectrum, and characterised by no imaginary mode. Free energies were calculated within the harmonic approximation for vibrational frequencies. Only the most stable conformational isomers are reported for all intermediates. All calculations were performed using the Gaussian09 suite of codes.²²

Acknowledgements

H.M.B. and J.R.D. thank GSK for funding and Dr Jason Holland (Oxford and Harvard) for helpful discussions and collaborations. S.I.P. thanks the Royal Society for her URF. The authors thank Dr John E. Warren for collaboration on synchrotron crystallography. We thank the EPSRC National service for mass spectrometry at Swansea and the EPSRC NCS Service at Southampton for assistance with synchrotron crystallography investigations. The authors would also like to acknowledge the use of the EPSRC UK National Service for Computational Chemistry Software (NSCCS) at Imperial College London, as well as the High Performance Computing (HPC) facilities (Aquila cluster) at the University of Bath, in carrying out this work.

Notes and references

- 1 M. Shokeen and C. J. Anderson, *Acc. Chem. Res.*, 2009, **42**(7), 832–841.
- 2 P. S. Donnelly, *Dalton Trans.*, 2011, **40**, 999–1010.

- 3 P. J. Blower, J. S. Lewis and J. Zweit, *Nucl. Med. Biol.*, 1996, **23**, 957–980.
- 4 S. Liu, *Adv. Drug Delivery Rev.*, 2008, **60**, 1347–1370.
- 5 T. J. Wadas, E. H. Wong, G. R. Weisman and C. J. Anderson, *Chem. Rev.*, 2010, **110**(5), 2858–2902.
- 6 X. Sun, M. Wuest, G. R. Weisman, E. H. Wong, D. P. Reed, C. A. Boswell, R. Motekaitis, A. E. Martell, M. J. Welch and C. J. Anderson, *J. Med. Chem.*, 2002, **45**, 469–477.
- 7 C. J. Anderson, T. J. Wadas, E. H. Wong and G. R. Weisman, *Q. J. Nucl. Med. Mol. Imag.*, 2008, **52**, 185–192.
- 8 K. P. Wainwright, *Inorg. Chem.*, 1980, **19**, 1396–1398.
- 9 M. Boiocchi, M. Bonizzoni, L. Fabbrizzi, F. Foti, M. Licchelli, A. Poggi, A. Taglietti and M. Zema, *Chem.–Eur. J.*, 2004, **10**, 3209–3216.
- 10 L. M. Englehardt, G. A. Lawrance, T. M. Manning and A. H. White, *Aust. J. Chem.*, 1989, **42**, 1859–1867.
- 11 F. Arjmand, S. Parveen, M. Chauhan, S. Parveen and S. Tabassum, *Transition Met. Chem.*, 2006, **31**(2), 237–245.
- 12 R. Kowallick, M. Neuburger, M. Zehnder and T. A. Kaden, *Helv. Chim. Acta*, 1997, **80**, 948–959.
- 13 P. A. Tasker and L. Sklar, *J. Cryst. Mol. Struct.*, 1975, **5**, 329–344.
- 14 A. Dey, F. E. Jenney, Jr, M. W. W. Adams, E. Babini, Y. Takahashi, K. Fukuyama, K. O. Hodgson, B. Hedman and E. I. Solomon, *Science*, 2007, **318**, 1464–1468.
- 15 K. S. Woodin, K. J. Heroux, C. A. Boswell, E. H. Wong, G. R. Weisman, W. Niu, S. A. Tomellini, C. J. Anderson, L. N. Zakharov and A. L. Rheingold, *Eur. J. Inorg. Chem.*, 2005, 4829–4833.
- 16 P. J. Barnard, S. R. Bayly, J. P. Holland, J. R. Dilworth and P. A. Waghorn, *Q. J. Nucl. Med. Mol. Imag.*, 2008, **52**, 235–244.
- 17 A. Altomare, G. Casciarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori and M. Camalli, *J. Appl. Crystallogr.*, 1994, **27**, 435.
- 18 D. J. Watkin, C. K. Prout, J. R. Carruthers, P. W. Betteridge and R. I. Cooper, *CRYSTALS Issue 11*, Chemical Crystallography Laboratory, Oxford, UK, 2001.
- 19 Y. Zhao and D. Truhlar, *Theor. Chem. Acc.*, 2008, **120**, 215–241.
- 20 G. Scalmani and M. J. Frisch, *J. Chem. Phys.*, 2010, **132**, 114110–114115.
- 21 D. M. York and M. Karplus, *J. Phys. Chem. A*, 1999, **103**, 11060–11079.
- 22 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, Revision A.1*, Gaussian, Inc., Wallingford CT, 2009.