A Click Fluorophore Sensor that Can Distinguish Cu^{II} and Hg^{II} via Selective Anion-Induced Demetallation

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Abstract: A cyclam-based fluorescent sensor featuring a novel triazole pendant arm has been synthesised using click chemistry. The sensor is highly responsive to both Cu^{II} and Hg^{II} in neutral aqueous solution and displays excellent selectivity in the presence of various competing metal ions in 50-fold excess. The addition of specific anions such as I⁻ and S₂O₃²⁻ causes a complete revival of fluorescence only in the case of Hg^{II}, providing a simple and effective method for distinguishing solutions containing Cu^{II}, Hg^{II} or a mixture of both ions, even in doped seawater samples. X-ray crystal structures of both the Hg^{II} sensor complex and a model Cu^{II} complex show that pendant triazole coordination occurs

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through the central nitrogen atom (N2), providing to the best of our knowledge the first reported examples of this unusual coordination mode in macrocycles. Fluorescence, mass spectrometry and ¹H NMR experiments reveal that the mechanism of anion-induced fluorescence revival involves either displacement of pendant coordination or complete removal of the Hg^{II} from the macrocycle, depending on the anion.



at the metal in the presence of avidin signalled by changes in the EPR spectrum. $^{\left[10\right] }$

We now outline the synthesis of a cyclam-based fluorescent sensor **3** featuring a novel triazole pendant, linked to the macrocycle via the N1 position rather than C4. The rationale behind the design of this ligand was to alter the ring size of the coordinating pendant arm relative to **1** as well as changing the fluorophore, thereby modifying the selectivity and fluorescent response to metal ion binding. The result is a system that coordinates via N2 and responds selectively to Cu^{II} and Hg^{II} (in stark contrast to the zinc sensor **1**). Detection of Cu^{II} is important as it is both an essential biological trace element and an environmental pollutant,^[11] whilst Hg^{II} is well known for its high toxicity even at low concentrations.^[12]

Whilst numerous cyclam-based fluorescent sensors have been developed in recent years, many have only been studied in organic solvents or organic/aqueous mixtures, limiting their practical application.^[13] Another common challenge when designing sensors is poor selectivity for specific metal ions, including the inability to distinguish between Cu^{II} and Hg^{II} due to their similar fluorescence quenching properties.^[13d,e,14] Our new sensor is able to operate in neutral aqueous solution and in the presence of excess competing

Introduction

Chemical sensing is an active area of research driven by the vast potential for industrial, environmental and biological application.^[1] A range of chemosensors have been reported in the literature for the detection of many different chemical species including metal ions, anions and organic molecules.^[2] Despite their apparent structural diversity, the majority of these sensors are comprised of two main components: a domain which binds the target molecule, and a reporter which signals the binding event through an observable change.^[3] Given this modular design, click chemistry provides a convenient synthetic method for uniting the two components.^[4] The copper(I)-catalysed azide-alkyne cycloaddition reaction is particularly suited to the synthesis of metal ion sensors as the resultant 1,2,3-triazole acts as both a covalent linker and a potential coordination site for metal ions.^[5] The vast majority of chelating triazoles coordinate through the side nitrogen atom (N3),^[5a] with DFT calculations showing that N3 has a greater electron density than the central nitrogen atom (N2).^[6] In the Cambridge Structural Database, there are very few reports of click-generated triazoles which exhibit exclusive N2 coordination.^[7]

Previous work involving our group has used N-monofunctionalised cyclam ligands with 1,2,3-triazole pendant arms as sensors (see below). Compound **1** is a selective Zn^{II} sensor, signalling metal coordination with a six-fold increase in fluorescence emission of the pendant naphthalimide.^[8] The development of heterogeneous sol–gel sensors based on **1** has also been explored.^[9] An alternative approach has involved compound **2**, with the pendant biotin molecule acting as a binding site for avidin, and the loss of triazole coordination

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metal ions. The sensor can be used to distinguish Cu^{II} and Hg^{II} effectively, even when both are present as a mixture, through the addition of specific anions. This sensor-andanion system represents a new method for detecting these metals simultaneously and independently.

Results and Discussion

Synthesis: Model compound **7** was prepared to test the feasibility of the synthetic route and investigate the coordination properties of the novel triazole pendant (Scheme 1). Initial attempts to alkylate tri-Boc-protected cyclam **4** by refluxing with stoichiometric toluenesulfonic acid-2-azidoethyl ester proved low yielding (29%), with the majority of starting materials being reclaimed, consistent with previous observations that **4** is a poor nucleophile for unstabilised primary electrophiles.^[15] Using the tosylate in excess (ca. 16 equiv) afforded the alkylated cyclam **5** in an excellent 89% yield, with the unreacted azide easily reclaimed by chromatography. Reaction of **5** with phenylacetylene under standard click conditions yielded the triazole **6** in good yield, and this was deprotected using trifluoroacetic acid to give the model compound **7** in near quantitative yield.



To investigate the coordination properties of **7**, the Cu^{II} complex **8** was prepared by treatment with copper(II) perchlorate, and X-ray diffraction quality crystals were grown by slow diffusion of diethyl ether into an acetonitrile solution of **8**. As expected, the pendant triazole adopts a different coordination mode to previously reported azamacrocyclic pendant triazoles.^[8-10] In complex **8**, the triazole is coordinated to the



Figure 1. ORTEP representation of the single-crystal X-ray structure of **8** with 50% thermal ellipsoids.^[16] The non-coordinated perchlorate anion has been deleted for clarity. The pendant triazole is coordinating through the central nitrogen atom (N2) to form a six-membered metallocycle. The cyclam ring is in the expected *trans*-III configuration.

metal centre via N2 rather than N3, forming a six-membered metallocycle (Figure 1).

Sensor **3** was synthesised via an analogous route in excellent yield (Scheme 2). The Cu^{II} and Hg^{II} complexes **10a** and **10b** were then prepared by adding the corresponding metal perchlorate salt.

X-ray diffraction quality crystals of **10b** were grown by slow diffusion of ether into a nitromethane solution of **10b**, with the resulting structure indicating coordination via N2 also occurs in the case of Hg^{II} (Figure 2).



Scheme 2. i) 7-Ethynyl-4-methyl-2*H*-chromen-2-one, $CuSO_4 \cdot 5H_2O$ (5 mol%), Na ascorbate (10 mol%), *t*BuOH/H₂O 1:1, 16 h, 86%; ii) TFA (20%) in CH₂Cl₂, RT, 6 h, 97%; iii) a) Cu(ClO₄)₂·6H₂O, EtOH, reflux, 1 h, 72%; b) Hg(ClO₄)₂·3H₂O, MeOH, RT, 16 h, 58%.

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Figure 2. ORTEP representation of the single-crystal X-ray structure of **10b** with 50% thermal ellipsoids.^[16] The anions and nitronate solvent molecules have been deleted for clarity. The pendant is triazole coordinating through the central nitrogen atom (N2). The cyclam ring is in the *trans*-I configuration.

Metal-ion sensing: To assess the metal-ion sensing ability of compound **3** in aqueous solution, a wide range of metal ions were screened, of which only Cu^{II} and Hg^{II} caused a significant change in fluorescence emission (Figure 3). In contrast to the previously reported Zn^{II} sensor **1** which exhibits an increase in fluorescence due to inhibition of photoinduced electron transfer (PET), Cu^{II} and Hg^{II} trigger a quenching response with sensor **3** which can be attributed to well-known paramagnetic and heavy metal effects.^[17] Interesting-



Figure 3. Fluorescence emission spectra of **3** (10 μ M) in HEPES buffer (10 mM, pH 7.4) ca. 5 min after the addition of various metal ions (1 equiv). Only Cu^{II} and Hg^{II} trigger a significant response.

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ly, sensor 3 is only able to operate by a fluoresence quenching mechanism because it appears to be less prone to intramolecular PET than sensor 1, although further investigations are currently being carried out to determine whether the modified pendant connectivity or the fluorophore itself is responsible for the different sensing and fluorescence properties.

Fluorescence titrations of sensor **3** with Cu^{II} and Hg^{II} confirm the expected 1:1 binding stoichiometry, with association constants of $2.2(\pm 0.3) \times 10^8$ and $7.1(\pm 0.8) \times 10^7 \text{ M}^{-1}$ estimated for Cu^{II} and Hg^{II}, respectively, using a non-linear curve fitting analysis (see Supporting Information).^[18] The detection limits were calculated to be 3.5×10^{-7} and 4.3×10^{-7} M, respectively.^[19] Screening the response of **3** to Cu^{II} and Hg^{II} at different pH indicates optimum performance at neutral pH (Figure 4). Presumably, the coordination of metal ions at low pH is less favoured due to protonation of the amine groups in the cyclam ring. At high pH, the lone pairs of the non-protonated amine groups act to quench the fluorescence of the coumarin fluorophore by PET,^[20] thus attenuating the full quenching response normally seen upon addition of Cu^{II} or Hg^{II}.



Figure 4. Fluorescence emission of ligand 3 (\diamond) at different pH, and ca. 5 min after adding 1 equiv of Cu^{II} or Hg^{II}. Maximal response to Cu^{II} (**n**) and Hg^{II} (\diamond) occurs around neutral pH.

To investigate the effectiveness of sensor 3 in the presence of competing metal ions, 50 equivalents of various metal ions were added to a 10 µM solution of 3, followed after about 5 min by the addition of one equivalent of Cu^{II} or Hg^{II} (Figure 5). In almost all cases, the quenching response was completely unaffected by the competing metal ions, a welcome result given the ability of cyclam to bind a range of transition-metal ions.^[21] In competition experiments between Cu^{II} and Hg^{II}, neither ion was able to displace the other from the ligand, which can be attributed to their similar binding constants and the kinetic macrocyclic effect.^[21] Notably, a 50-fold excess of Zn^{II} caused some quenching of fluorescence which was not affected by the subsequent addition of Cu^{II} or Hg^{II}, suggesting that a reasonably stable complex forms with Zn^{II}. However, this quenching response was significantly slower (equilibration over ca. 1 h) than the response to Cu^{II} and Hg^{II}. In a modified competition experiment, premixed solutions containing Zn^{II} (1 and 50 equiv)

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Figure 5. Investigating the selectivity of ligand **3** in the presence of competitive metal ions. Metal ions (50 equiv) were added to **3** (10 μ M) in HEPES buffer (10 mM, pH 7.4), followed after ca. 5 min by addition of either Cu^{II} or Hg^{II} (1 equiv). In all cases except Zn^{II}, the excess competing ions do not significantly affect the response to Cu^{II} or Hg^{II}.

and either Cu^{II} or Hg^{II} (1 equiv) were added to a solution of ligand **3** (Figure 6). The response to Hg^{II} was not significantly affected by the presence of even 50 equivalents of Zn^{II} , whilst the Cu^{II} response tolerated one equivalent of Zn^{II} , but was affected slightly by a 50-fold excess.



Figure 6. Modified competition experiments to further investigate the interference by Zn^{II} . Pre-mixed solutions containing Zn^{II} (1 or 50 equiv) with either Cu^{II} or Hg^{II} (1 equiv) were added to a solution of **3** (10 μ M) in HEPES buffer (10 mM, pH 7.4) and the fluorescence was measured after ca. 5 min. The slow response of **3** to Zn^{II} allows Cu^{II} and Hg^{II} to be detected in the presence of Zn^{II} .

Effect of coordinating anions: The tolerance of this system to a range of competitive anions was also screened by adding a large excess (150 equiv) of various sodium salts to the Cu^{II} and Hg^{II} complexes of **3** (Figure 7). Whilst none of the anions tested had any effect on the fluorescence emission of the Cu^{II} complex (see Supporting Information), addition of Cl⁻, Br⁻, I⁻, SCN⁻ and S₂O₃²⁻ caused some revival of fluorescence emission for the Hg^{II} complex. In particular, the quenching response of Hg^{II} was nullified by less than five equivalents of I⁻ and S₂O₃²⁻.

Fluorescence, mass spectrometry and NMR experiments were carried out to determine whether the anion-induced fluorescence revival was due to anion displacement of the pendant group or complete removal of Hg^{II} from the macrocycle (Scheme 3).^[22] It was reasoned that Cu^{II} should bind to the cyclam macrocycle if complete removal of HgII was occurring. The addition of one equivalent of Cu^{II} after anion addition triggered the full quenching response in all cases except Cl⁻ (Figure 8). This result suggests demercuration of the complex, although this does not rule out the possibility of lowered binding affinity for Hg^{II} due to displacement of



Figure 7. Fluorescence emission spectra upon addition of anions (150 equiv) after adding Hg^{II} (1 equiv) to **3** in HEPES buffer (10 mm, pH 7.4). The original fluorescence spectra of ligand **3** and Hg^{II} complex **10b** are also shown. Cl⁻, Br⁻, I⁻, SCN⁻ and S₂O₃²⁻ cause some revival of fluorescence emission.



Scheme 3. Two possible mechanisms for anion-induced fluorescence revival: a) displacement of pendant coordination or b) complete removal of Hg^{II} from the cyclam macrocycle.

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Figure 8. Fluorescence revival upon addition of anions (5 equiv of I⁻ and $S_2O_3^{2-}$, 150 equiv of Br⁻, Cl⁻, SCN⁻) to the Hg^{II} complex **10b** in HEPES buffer (10 mM, pH 7.4), then subsequent addition of Cu^{II} (1 equiv). In all cases except Cl⁻, Cu^{II} is able to cause significant fluorescence quenching, indicating that Hg^{II} is completely removed from the macrocycle.

pendant coordination, with Hg^{II} retained inside the macrocycle until Cu^{II} is introduced. The observed formation of precipitates with Br⁻, I⁻ and SCN⁻ in more concentrated solutions is also consistent with macrocycle demercuration, as the corresponding inorganic Hg^{II} salts are highly insoluble in aqueous solution.^[23] No precipitate was observed for S₂O₃²⁻, as Hg^{II} thiosulfate complexes are water-soluble.^[24]

Mass spectrometry (ESI+) experiments on solutions containing Hg^{II} complex **10b** and each of the anions were consistent with the fluorescence results (see Supporting Information). The mass spectra of complex **10b** mixed with Br⁻, I⁻, SCN⁻ and S₂O₃⁻ do not show a peak for the intact Hg^{II} complex, and instead have one dominant peak that corresponds to the free ligand **3** (m/z 454 [M+H]⁺). In contrast, the mass spectrum of complex **10b** without additional anions shows only a peak for the intact complex (m/z 754, [M^{2+} +ClO₄⁻]⁺), and no free ligand signal. The mass spectrum for the sample containing **10b** and Cl⁻ shows both free ligand **3** and complex **10b**, indicating that Cl⁻ does not completely remove Hg^{II} from the macrocycle.

¹H NMR spectra of the anion-treated mixtures were also obtained. Comparing the spectra of free ligand 3 and Hg^{II} complex 10b, a downfield shift is apparent in all the peaks due to the deshielding effect of Hg^{II} (Figure 9). This shift is most pronounced in the peaks corresponding to the hydrogens of the cyclam ring and the triazole, due to their proximity to the metal centre. When Cl- is added, minimal changes are observed for the cyclam peaks, whilst the dye and triazole peaks shift upfield, consistent with retention of the metal in the macrocycle and movement of the pendant away from the metal centre. In contrast, when $S_2O_3^{2-}$ is added, all the peaks shift upfield and the spectrum reverts back to one similar to the uncomplexed ligand. Hence, it can be concluded that Cl- displaces pendant ligand coordination, whereas Br⁻, I⁻, SCN⁻ and S₂O₃²⁻ strip Hg^{II} from the cyclam ring.

Distinguishing between Cu^{II} and Hg^{II} : The different responses of the Cu^{II} and Hg^{II} complexes **10a** and **10b** to anions



Figure 9. ¹H NMR spectra in D₂O of a) ligand **3**; b) complex **10b**; c) complex **10b** and Cl⁻; d) complex **10b** and S₂O₃²⁻. Cyclam ring CH₂ peaks are underlined. Pendant triazole/dye peaks are labelled with asterisks. Comparing b) and c), the upfield shift in pendant peaks indicates displacement of triazole coordination. Comparing b) and d), the upfield shift in the cyclam peaks indicates removal of Hg^{II} from the macrocycle.

can be utilised as a simple method of distinguishing between solutions containing each metal ion and a mixture of both ions (Figure 10). Fluorescence quenching indicates the presence of either Cu^{II} or Hg^{II} ; no fluorescence change upon subsequent addition of thiosulfate is indicative of Cu^{II} , whilst a large increase is indicative of Hg^{II} . If both Cu^{II} and Hg^{II} are present in solution, the initial fluorescence quenching response lies between those of Cu^{II} and Hg^{II} individually, and the addition of thiosulfate causes a drop in fluorescence due to substitution of Cu^{II} for Hg^{II} in the macrocycle.

To test the efficacy of the sensor in a real world context, the quenching experiments were then repeated using Cu^{II}



Metal Ions Added

Figure 10. Fluorescence emission of **3** in HEPES buffer (10 mm, pH 7.4) upon addition of Cu^{II} , Hg^{II} or a mixture of both (1 equiv of each metal ion), followed by $S_2O_3^{2-}$ (5 equiv). No change is observed with Cu^{II} only, whilst an increase in fluorescence is seen for Hg^{II} , and a decrease in fluorescence for the mixture of Cu^{II} and Hg^{II} . The effect of varying the stoichiometry of the mixture is detailed in the Supporting Information.

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and Hg^{II} doped seawater samples from Darling Harbour in Sydney, Australia. Despite the wide range of cations and anions present in seawater, the sensor was still effective for detecting Cu^{II} and Hg^{II} , exhibiting similar fluorescence changes to the experiments conducted in distilled water (see Supporting Information). The only significant difference was a reduced amount of quenching in response to Hg^{II} , attributed to pendant displacement by the high concentration of Cl^- in seawater.

Conclusion

Click chemistry has been used to synthesise a fluorescent sensor which responds to both Cu^{II} and Hg^{II} . This sensor has desirable properties for practical applications, including selectivity for detecting Cu^{II} and Hg^{II} in the presence of excess competing metal ions, as well as optimum functionality in neutral aqueous solution and even seawater. Furthermore, we have studied the responses of the Cu^{II} and Hg^{II} complexes to the addition of competing anions, and utilised their different responses to distinguish Cu^{II} from Hg^{II} . The Hg^{II} complex and Cu^{II} model complex are the first two reported examples of N2 triazole coordination in macrocycles.

The contrasting selectivity of **3** for Cu^{II} and Hg^{II} compared to the previously reported Zn^{II} sensor **1** presumably hinges on the different length of the pendant arm (there are three bridging atoms between the tertiary amine and triazole ligands in **3** vs two atoms in **1**) and the different electronics of triazole coordination through N2 and N3. Further work is being carried out to determine whether the difference in pendant dye, triazole coordination mode or other factors are responsible for the different fluorescence characteristics (quenching vs PET turn-on) of sensors **3** and **1**. This work will enhance our understanding of the photophysical processes behind the fluorescence responses, and allow the design of new azamacrocyclic sensors with novel fluorescent properties.

Experimental Section

General information

All reagents were purchased from Sigma–Aldrich and used without further purification. Toluenesulfonic acid-2-azidoethyl ester,^[25] 7-ethynyl-4methyl-2*H*-chromen-2-one^[26] and tri-Boc-protected cyclam $4^{[27]}$ were prepared according to literature procedures.

Fluorescence emission spectra were recorded at 25 °C on a Varian Cary Eclipse Fluorimeter with excitation at 327 nm. Unless otherwise specified, emission was recorded at 389 nm. Quantum yields were calculated using L-tryptophan as a standard reference (Φ =0.13 in H₂O, pH 6).^[28] Fluorescence experiments were carried out by adding a small (2–10 µL) amount of the relevant ion (2–100 mM) in HEPES buffer (10 mM, pH 7.4) to a stock solution of ligand **3** (2 mL, 10 µM). For pH studies, a stock solution of ligand **3** was made in HEPES buffer (10 mM) and the pH adjusted with HClO₄ or NaOH prior to addition of metal ions. Binding constants were obtained by titrating metal ion solutions (0.5–100 mM) containing ligand **3** (10 µM) into a stock solution of ligand **3** (2 mL, 10 µM) in order

to maintain a constant ligand concentration, and fitting the titration curves to a 1:1 binding model using a non-linear curve fitting analysis.^[18] Details of the X-ray crystallographic studies are given in the Supporting Information. CCDC 789681 (8), 789855 (10b) contain the supplementary crystallographic data for this paper. These data can be obtained free of

charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif ¹H NMR peaks are assigned according to the scheme defined in Support-

ing Information (Section 1). For metal complexes which exhibit a complex mixture of isotopes, the mass of the most abundant species for each ion fragment has been re-

ported; M^{2+} is used to denote the divalent metal-ligand cation.

Synthetic procedures

SAFETY NOTE: Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of material should be prepared and these should be handled with caution.

11-(2-Azidoethyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic

acid tri-*tert*-butyl ester (5): Toluenesulfonic acid-2-azidoethyl ester (1.26 g, 5.22 mmol) and Na₂CO₃ (2.02 g, 19.1 mmol) were added to a solution of 1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester **4** (413 mg, 0.825 mmol) in acetonitrile (25 mL). After refluxing for 72 h, the reaction mixture was filtered and concentrated in vacuo. Purification by column chromatography (2% to 5% MeOH in CH₂Cl₂) gave azide **5** as a pale yellow oil (413 mg, 89%). ¹H NMR (200 MHz, CDCl₃): δ = 3.47–3.18 (m, 14H, CH₂BocNCH₂, H⁶), 2.69–2.55 (m, 4H, H⁶, H^e), 2.45 (t, 2H, J = 5.7 Hz, H^b), 1.86 (qn, 2H, J = 7.4 Hz, H^d), 1.68 (qn, 2H, J = 7.4 Hz, H^a), 1.44 ppm (s, 27H, C(CH₃)₃); ¹³C NMR (50 MHz, CDCl₃): δ = 155.2, 155.0, 79.0, 54.0, 53.7, 51.9, 48.7, 48.2–46.1 (several peaks), 45.4, 28.1, 26.4 ppm; IR (ATR, CDCl₃): ν =2973, 2097, 1689, 1463, 1412, 1391, 1247, 1163 cm⁻¹; HRMS (ESI): m/z: calcd for C₂₇H₅₁N₇O₆Na: 592.3793, found 592.3785 [*M*+Na]⁺.

11-(2-(4-Phenyl-1H-1,2,3-triazol-1-yl)ethyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-tert-butyl ester (6): Compound 5 (376 mg, 0.660 mmol) and phenylacetylene (74 µL, 0.68 mmol) were dissolved in tBuOH/H2O 1:1 (10 mL), and CuSO4·5H2O (5 mol%, 8 mg dissolved in 1 mL H₂O) and sodium ascorbate (10 mol%, 13 mg dissolved in 1 mL H2O) were added. After stirring at RT for 16 h, 5% NaHCO3 solution (5 mL) was added and the reaction mixture was extracted with CH₂Cl₂ (4×40 mL). The organic extracts were dried over MgSO4, concentrated in vacuo and purified by flash chromatography (ethyl acetate/hexane 1:1) to give triazole 6 as a white foam (335 mg, 76%). M.p. 71-77 °C; ¹H NMR (200 MHz, CDCl₃): $\delta = 7.89-7.79$ (m, 2H, ArH), 7.81 (s, 1H, H^t), 7.48–7.30 (m, 3H, ArH), 4.41 (t, 2H, J = 6.5 Hz, H^f), 3.38–3.08 (m, 12H, $CH_2BocNCH_2$), 2.98 (t, 2H, J = 6.4 Hz, H^e), 2.67 (t, 2H, J =5.1 Hz, H^c), 2.51 (t, 2H, J = 5.6 Hz, H^b), 1.83–1.60 (m, 4H, H^a, H^d), 1.46 (s, 18H, $C(CH_3)_3$), 1.44 ppm (s, 9H, $C(CH_3)_3$); ¹³C NMR (50 MHz, $CDCl_3$): $\delta = 155.3, 155.2, 155.1, 147.1, 130.4, 128.5, 127.8, 125.2, 120.2,$ 79.3, 79.2, 54.7, 53.5, 52.0, 48.3-46.0 (several peaks), 45.5, 28.5, 28.2, 26.4 ppm; IR (ATR, CDCl₃): v =2976, 2935, 1689, 1466, 1413, 1391, 1246, 1163 cm⁻¹; HRMS (ESI): m/z: calcd for C₃₅H₅₈N₇O₆: 672.4443, found 672.4435 [M+H]+.

1-(2-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)-1,4,8,11-tetraazacyclotetrade-

cane (7): Ester **6** (335 mg, 0.499 mmol) was dissolved in CH₂Cl₂ (12 mL) and trifluoroacetic acid (3 mL) was added dropwise. After stirring at RT for 6 h, the reaction mixture was concentrated under reduced pressure. The oily residue was dissolved in H₂O (5 mL) and 10% aqueous Na₂CO₃ solution was added until effervescence ceased. The solution was extracted with chloroform (5×40 mL), the extracts dried over Na₂SO₄ and concentrated in vacuo to give the free amine **7** as a white solid (335 mg, 99%). M.p. 86–89 °C; ¹H NMR (200 MHz, CDCl₃): δ = 8.10 (s, 11H, H^t), 7.85 (dd, 2H, *J* = 8.2, 1.4 Hz, ArH), 7.48–7.29 (m, 3H, ArH), 4.56 (t, 2H, *J* = 6.2 Hz, H^f), 2.93 (t, 2H, *J* = 6.2 Hz, H^e), 2.78–2.66 (m, 6H, CH₂NHCH₂), 2.58–2.36 (m, 8H, CH₂NHCH₂, H^c), 2.31 (t, 2H, *J* = 5.5 Hz, H^b), 1.92 (brs, 3H, NH), 1.76–1.61 ppm (m, 4H, H^a, H^d); ¹³C NMR (50 MHz, CDCl₃): δ = 146.9, 130.6, 128.7, 127.9, 125.4, 121.5, 54.0, 52.9, 51.0, 50.5, 48.3, 47.9, 47.4, 47.1, 47.0, 46.5, 28.0, 25.9 ppm; IR

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(ATR, CDCl₃): ν =3500–2700 (br), 2816, 1687, 1465, 1131 cm⁻¹; HRMS (ESI): m/z: calcd for C₂₀H₃₄N₇: 372.2870, found 372.2870 [M+H]⁺.

[Cu(7)](ClO₄)₂ (8): To a refluxing solution of **7** (40.0 mg, 0.108 mmol) in EtOH (3 mL), a solution of Cu(ClO₄)₂6 H₂O (40.5 mg, 0.109 mmol) in EtOH (1 mL) was added dropwise. After refluxing for 1 h, the reaction mixture was cooled on ice and the solvent was decanted. The solid residue was triturated with ice-cold EtOH (3×2 mL) and diethyl ether (3× 3 mL), then dried under a flow of nitrogen to give the complex **8** as a purple powder (54.2 mg, 74%). Purple block-shaped crystals suitable for X-ray diffraction were obtained by slow diffusion of diethyl ether into an acetonitrile solution of the complex. M.p. 184–187°C; IR (ATR, acetone): $\nu = 3239$, 2880, 1703, 1458, 1363, 1089, 772, 697, 623 cm⁻¹; LRMS (ESI): m/z (%): 547.3 (100) $[M^{2+}+TFA^{-}]^+$, 533.3 (80) $[M^{2+}+ClO_4^{-}]^+$, 433.1 (88) $[M^{2+}-H^+]^+$; elemental analysis calcd (%) for C₂₀H₃₃Cl₂N₇O₈Cu: C 37.89, H 5.25, N 15.47; found: C 37.54, H 5.30, N 15.29.

11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl)-11-(2-(4-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl (2-(4-Methyl-2-oxo-2H-chromen-7-yl))ethyl (2-(4-Methyl-2-0x))ethyl (2-(4-Met

1.4.8.11-tetraazacvclotetradecane-1.4.8-tricarboxvlic acid tri-*tert*-butvl ester (9): Compound 9 was prepared in a similar manner to compound 5 using 4 (583 mg, 1.02 mmol) and 7-ethynyl-4-methyl-2H-chromen-2-one (189 mg, 1.03 mmol). Purification by flash chromatography (ethyl acetate/hexane 3:2) gave triazole 9 as a pale yellow foam (660 mg, 86%). M.p. 93–101 °C; ¹H NMR (200 MHz, CDCl₃): $\delta = 7.96$ (s, 1 H, H^t), 7.88 (dd, 1 H, J = 8.3, 1.6 Hz, H^x), 7.74 (d, 1 H, J = 1.4 Hz, H^z), 7.66 (d, 1 H, J = 8.3 Hz, H^y), 6.30 (d, 1H, J = 1.2 Hz, COCH), 4.44 (t, 2H, J =6.5 Hz, H^f), 3.40–3.12 (m, 12H, $CH_2BocNCH_2$), 3.01 (t, 2H, J = 6.6 Hz, H^e), 2.69 (t, 2H, J = 5.4 Hz, H^c), 2.54 (t, 2H, J = 5.6 Hz, H^b), 2.46 (d, $3H, J = 1.1 Hz, CH_3$, 1.86–1.61 (m, 4H, H^a, H^d), 1.46 (s, 18H, C(CH₃)₃), 1.44 ppm (s, 9H, C(CH₃)₃); ¹³C NMR (50 MHz, CDCl₃): $\delta = 160.0$, 155.1, 155.0, 154.9, 153.4, 151.8, 145.2, 133.9, 124.8, 121.5, 120.9, 118.9, 114.1, 112.7, 79.1, 79.0, 54.5, 53.3, 51.8, 48.3-45.0 (several peaks), 28.4, 28.0, 26.2, 18.0 ppm; IR (ATR, CDCl₃): v=2975, 2933, 1736, 1688, 1621, 1464, 1414, 1390, 1242, 1161 cm⁻¹; HRMS (ESI): m/z: calcd for C₃₉H₅₉N₇O₈Na: 776.4317, found 776.4323 [M+Na]⁺.

1-(2-(4-(4-Methyl-2-oxo-2*H*-chromen-7-yl)-1*H*-1,2,3-triazol-1-yl)ethyl)-

1,4,8,11-tetraazacyclotetradecane (3): Compound **3** was prepared in a similar manner to compound **7** using **9** (736 mg, 0.976 mmol), trifluoroacetic acid (4 mL) and CH₂Cl₂ (16 mL) to give the free amine **3** as a yellow solid (430 mg, 97%). M.p. 76–81 °C; ¹H NMR (200 MHz, CDCl₃): $\delta = 8.28$ (s, 1H, H⁺), 7.87 (dd, 1H, J = 8.2, 1.6 Hz, H^x), 7.77 (d, 1H, J = 1.6 Hz, H²), 7.66 (d, 1H, J = 8.2 Hz, H³), 6.30 (d, 1H, J = 1.1 Hz, COCH), 4.59 (t, 2H, J = 6.3 Hz, H⁴), 2.97 (t, 2H, J = 6.3 Hz, H^e), 2.82–2.16 (m, 19H, CH₂NHCH₂, H^b, H^c CH₃), 1.80–1.64 ppm (m, 4H, H^a, H^d); ¹³C NMR (50 MHz, CDCl₃): $\delta = 160.0$, 153.3, 151.8, 144.9, 133.9, 124.8, 122.1, 120.8, 118.8, 114.1, 112.6, 54.1, 52.4, 51.2, 49.9, 47.9, 47.6, 47.3, 46.9, 46.7, 27.8, 25.7, 18.1 ppm; IR (ATR, CDCl₃): $\nu = 3279$, 3135, 2920, 2815, 1718, 1620, 1463, 1389, 1240, 1158 cm⁻¹; HRMS (ESI): m/z: calcd for C₂₄H₃₆N₇O₂: 454.2925, found 454.2926 [*M*+H]⁺; quantum yield: $\Phi = 0.10$ (H₂O, pH 7).

[Cu(3)](ClO₄)₂ (10a): Complex **10a** was prepared in a similar manner to **8** using **3** (41.4 mg, 0.0912 mmol) and Cu(ClO₄)₂6H₂O (34.0 mg, 0.0918 mmol) to give the complex **10a** as a purple powder (46.9 mg, 72%). M.p. 220–222°C; IR (ATR, acetone): v = 3233, 2885, 1704, 1621, 1456, 1365, 1226, 1089, 942, 623 cm⁻¹; LRMS (ESI): m/z (%): 615.1 (100) $[M^{2+}+ClO_4^{-}]^+$, 517.1 (84) $[M^{2+}-H^+]^+$; elemental analysis calcd (%) for C₂₄H₃₅Cl₂N₇O₁₀Cu·H₂O: C 39.27, H 5.08, N 13.36; found: C 39.23, H 4.95, N 13.07; quantum yield: $\Phi = 0.0046$ (H₂O, pH 7).

[Hg(3)](ClO₄)₂ (10b): To a solution of **3** (19.3 mg, 0.0426 mmol) in MeOH (3 mL), a solution of Hg(ClO₄)₂·3H₂O (23.9 mg, 0.0527 mmol) in MeOH (1 mL) was added dropwise. After stirring for 16 h, the solvent was decanted and the solid residue was triturated with ice-cold EtOH (3×2 mL) and diethyl ether (3×3 mL). The solid was dried under a flow of nitrogen to give the complex **10b** as a white powder (21.2 mg, 58%). Colourless rod-shaped crystals suitable for X-ray diffraction were obtained by slow diffusion of diethyl ether into a nitromethane solution of the complex. M.p. 206 °C (decomp); ¹H NMR (300 MHz, CD₃CN): δ = 8.62 (s, 1H, H¹), 7.89 (s, 1H, H^x), 7.85 (m, 2H, H^y, H^z), 6.34 (d, 1H, *J* = 1.4 Hz, COC*H*), 4.83–4.64 (m, 2H, H^f), 4.15 (m, 1H, H^e), 4.03 (m, 1H,

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H^e), 2.97 (t, 2H, J = 6.3 Hz, H^e), 3.56–2.58 (m, 16H, CH₂NHCH₂, H^b, H^e), 2.47 (d, 3H, J = 1.1 Hz, CH₃), 1.94–1.51 ppm (m, 4H, H^a, H^d); ¹³C NMR (75 MHz, CD₃CN): $\delta = 161.1$, 154.9, 153.8, 148.2, 133.3, 127.2, 126.7, 122.5, 121.5, 116.0, 114.5, 59.4, 55.8, 55.6, 54.8, 54.7, 52.8, 49.6, 48.5, 47.2, 46.5, 29.0, 26.4, 18.6 ppm; IR (ATR, acetone): $\nu = 3260$, 2891, 1705, 1621, 1453, 1366, 1088, 942, 623 cm⁻¹; LRMS (ESI): m/z: 753.9 (100) $[M^{2+} + CIO_4^{-}]^+$, 346.9 (72) $[M^{2+} + HCI]^{2+}$, 326.5 (67) $[M^{2+}]$; elemental analysis calcd (%) for C₂₄H₃₅Cl₂N₇O₁₀Hg: C 33.79, H 4.14, N 11.49; found: C 33.64, H 3.96, N 11.21; quantum yield: Φ = 0.031 (H₂O, pH 7).

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