CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200802425

Responsive Metal Complexes: A Click-Based "Allosteric Scorpionate" Complex Permits the Detection of a Biological Recognition Event by EPR/ENDOR Spectroscopy

Emiliano Tamanini,[a] Stephen E. J. Rigby,[a] Majid Motevalli,[a] Matthew H. Todd,*[b] and Michael Watkinson*[a]

Abstract: Chemical sensing is a mature field, and many effective sensors for small anions and cations have been devised. Metal complexes have been used widely for this purpose, but there are fewer reports of their use in the detection of organic and biological analytes. To date metal complexes have been used in sensing via the direct displacement of a pre-existing ligand by an analyte, or by an adventitious complementarity between the complex and analyte. These strategies do not permit a general approach to the sensing of biological molecules with metal complexes because of the demands to engineer molecular recognition into the complex architecture. We describe a fundamentally new approach to this field—the "allosteric scorpionate" metal complex. The binding partner of a biological analyte is attached to a scorpionate ligand on a metal complex, remote from the metal centre. Binding of the analyte causes a change in the primary coordination sphere at the metal, thereby revealing the presence of the biological molecule. We show that azamacrocyclic complexes with a triazole scorpion ligand may be easily assembled with the [3+2] Huisgens 'click' cycloaddition. We demonstrate the synthesis of a biotin-functionalised cyclam derivative using this methodology. This, and our previously communicated zinc sensor, are to the best of our knowledge the first examples of a triazole being employed as a scorpion ligand on an azamacrocycle. Coordination by the triazole to the metal is perturbed by the binding of avidin to the pendant ligand. This event can be sensitively detected with EPR spectroscopy, and the details of the coordination change probed with ENDOR spectroscopy, confirming the loss of the axial triazole nitrogen donor upon binding to avidin. This represents the first metal complex where remote, 'allosteric' coordination of an analyte has been shown to cause a change in the primary coordination sphere of the metal. Since the synthesis is modular and straightforward, other biological ligands may easily be introduced, and the associated binding events may be probed.

Keywords: azamacrocycles · click chemistry · copper · EPR spectroscopy · sensors

Introduction

Metal complexes have been used extensively as drugs and imaging agents but there is an increasing awareness that their full potential in medicine and biology has not yet been exploited since metal complexes are typically non-selective in their interactions with biological molecules.[1] Whilst direct changes to a metal's coordination environment as a result of analyte binding has been used as a sensing strategy for many years,[2] for example through the displacement of a coordinating dye (Strategy I, Figure 1),[3] the use of metal complexes in the sensing of organic and biological analytes has been much less explored.[4] Hamachi and co-workers used a zinc(II) complex for the sensing of phosphorylated peptides that detected the phosphate moiety rather than a

[a] Dr. E. Tamanini, Dr. S. E. J. Rigby, M. Motevalli, Dr. M. Watkinson
School of Biological and Chemical Sciences
Queen Mary, University of London
Mile End Road, London E1 4NS (UK)
Fax: (+44) 20-7882-7427
E-mail: m.watkinson@qmul.ac.uk

[b] Dr. M. H. Todd
School of Chemistry
The University of Sydney
NSW 2006 (Australia)
Fax: (+61) 2-9351-3329
E-mail: m.todd@chem.usyd.edu.au

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200802425.

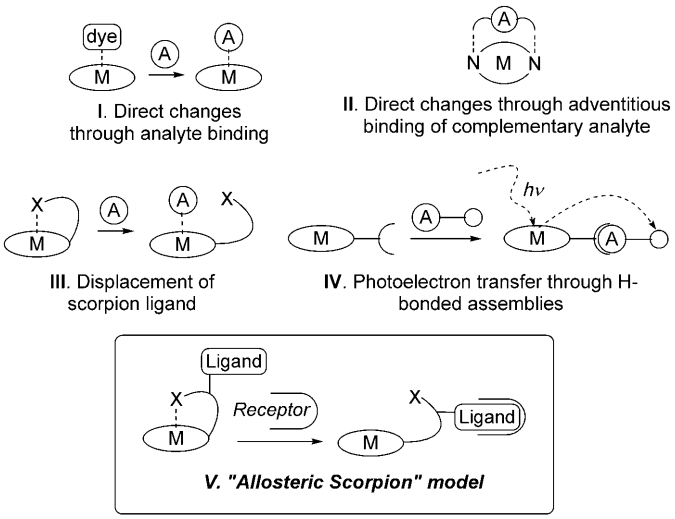


Figure 1. Sensing strategies for organic/biological analytes involving metal complexes. The mechanism demonstrated here is the "allosteric scorpion" model.

direct detection of the biological molecule itself.[5] Alternatively, there can be an adventitious recognition event between complex and analyte (Strategy II, Figure 1): several (largely *N*-containing) natural products such as flavins and amino acids have also been shown to coordinate effectively to the metal centre of azamacrocyclic complexes[6] and direct coordination of amino acid side chains to zinc(II) azamacrocycles is involved in the anti-HIV activity of a xylyl bis-cyclam.[7] The detection of nucleobases by this method is well-known,[8] but Kimura and Aoki have also demonstrated the detection of nucleobases by the displacement of a metal-bound scorpion ligand (Strategy III, Figure 1).[9] In the case of a scorpionate coumarin ligand, sensing of anions was possible in aqueous solution.[10] It is also possible to detect biological analytes with metal complexes by photoelectron transfer between the metal centre and an appropriately derivatised analyte brought together by hydrogen bonding (Strategy IV, Figure 1),[11] and sensing has also been well-explored using electron transfer between antenna molecules attached to macrocyclic lanthanide complexes.[12] Metal complexes hold great promise for the detection of diverse organic and biological analytes—they may be assembled through a metal templating effect,[13] and Hayashi and coworkers have shown, through the use of reconstituted myoglobin, that protein binding can alter the photophysical properties of an encapsulated zinc–porphyrin complex.[14]

However, in all of the chemical sensing strategies described above that rely on a change in the coordination environment of the metal complex for analyte detection there is a de facto requirement for direct binding of the analyte to the metal centre. Selectivity in these systems therefore also requires either the construction of a complex that is complementary to the analyte (i.e. conversion of the complex to a host) or adventitious complementarity between the macrocycle and the analyte.

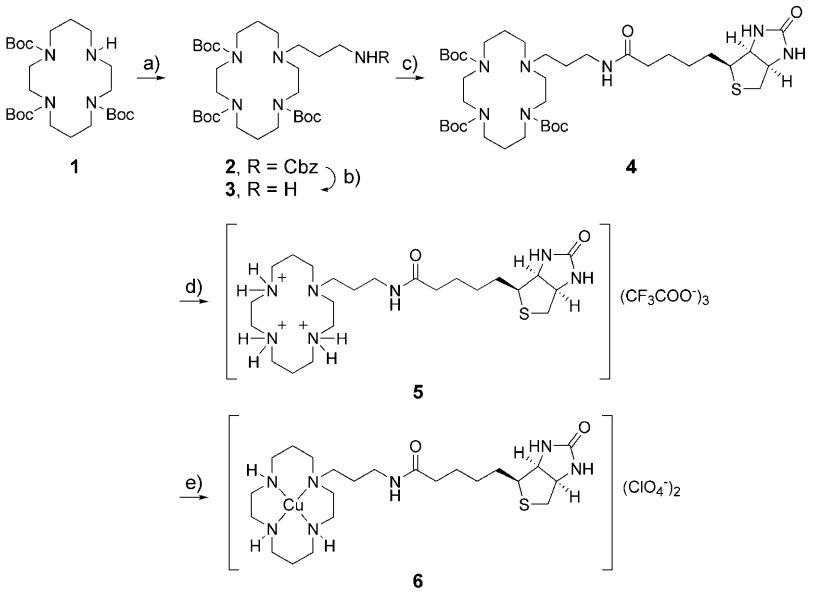
A new approach would be the generation of complexes where selective binding of an analyte to a remote, pendant site alters the primary coordination sphere and thereby converts the metal centre into an active catalytic site or one that emits a detectable signal that can be spectroscopically probed to elicit information on the nature and strength of the interaction (Strategy V, Figure 1). This may be thought of as an 'allosteric scorpion' approach. Specificity between ligand and receptor is not reliant on the structure of the metal complex itself, which acts as a reporter. We present here the first demonstration of such a sensing strategy.

Azamacrocycles, such as cyclam, are the perfect scaffold for this strategy since they exhibit robust coordination chemistry (including a range of scorpion complexes),[15] which has resulted in their application in a diverse number of biological and medicinal areas.[16] It has previously been reported that the stereochemistry of five-coordinate copper(II) complexes with pentadentate nitrogen donors is very susceptible to changes in the local environment about the metal centre.[17] Given this and the weak binding of the axial ligand in Cu^{II} complexes due to the Jahn–Teller effect we selected this basic system for a proof of concept study of the allosteric scorpion model. The lability of pendant arm scorpionate ligands has been known for some time through observation of changes in the metal's coordination sphere.[15d] Recently the lability of primary amine scorpionate donors in aqueous solution has been shown to be essentially independent of the metal ion in complexes of Co^{II}, Ni^{II} and Cu^{II}.[18]

Given this wealth of basic work, there are surprisingly few applications of scorpion complexes in sensing. Those that have appeared rely on either acid labilisation of the scorpion ligand resulting in molecular devices capable of monitoring changes in pH,[19] or on competitive substitution of the labile ligand by an anion[2b] or nucleobase[10] as described above. To the best of our knowledge no reports have appeared using scorpion complexes in the *general* sensing of biological molecules, and there have been no reports of complexes built to function on an allosteric scorpion model. We hypothesised that a strong binding interaction with a biological analyte might be able to perturb the primary coordination sphere of the metal by remote binding of the scorpion arm. In our design the presence of the biological analyte is relayed to a change in the metal's primary coordination by selective binding to the sidearm. This offers significant potential for the sensing of biological molecules, due to the selectivity in the interaction with the biological target of interest, and the generality of the design.

We report here a proof of concept study of this allosteric scorpion design, and use EPR and ENDOR spectroscopy to detect a biological recognition event, namely the binding of biotin by the protein avidin at biologically relevant concentrations of analyte. We further show the nature of the change in the coordination chemistry of the metal complex by ENDOR spectroscopy, which is due to the programmed change in the coordination mode of a scorpionate ligand in line with the design.

The interaction between biotin and avidin represents the strongest known interaction in Nature resulting in it finding a wide array of applications in biology and chemistry.[20] Only rarely however, has the system been employed to probe changes at a metal centre, although it has been shown that biotinylated ruthenium and iron complexes show changes by luminescence spectroscopy[21] and cyclic voltammetry[22] upon avidin binding. An obvious method to probe changes in the coordination environment of a metal is UV/Vis spectroscopy; however, we elected to use EPR spectroscopy since it is inherently sensitive and responsive to subtle changes in the coordination sphere of a metal, and can operate in the micromolar range[23] required in biological samples, precluding the need for high concentrations of the analyte, in this case avidin. Further, it was anticipated that fine details of any changes in the coordination chemistry of the complex upon avidin binding could be elucidated with ENDOR spectroscopy.



Scheme 1. Synthesis of cyclam–biotin complex **6**. a) $OHC(CH_2)_2NHCbz$, $NaBH(OAc)_3$; b) Pd/C, H_2; c) biotin, HATU, DIPEA, DMAP; d) TFA (20%) in CH_2Cl_2; e) $Cu(ClO_4)_2{\cdot}6H_2O$, pH 8.

Significant levels of asymmetric induction have been attained in catalytic reactions as a result of the avidin–biotin interaction affecting the secondary coordination sphere of a metal, suggesting that binding of the large proteinaceous analyte might be sufficient to perturb the local environment about the metal centre to allow detection of the binding event.[24] It was therefore essential that we prepared a simple, first-generation biotinylated metal complex and examined whether any changes in the metal centre could be observed by EPR spectroscopy upon avidin binding.

Results and Discussion

A copper(II) complex of a cross-bridged cyclam analogue functionalised with biotin has previously been prepared, but binding to (strept)avidin was not demonstrated.[25] We prepared the related, novel biotinylated copper(II) complex **6** (Scheme 1) and unequivocally demonstrated its binding to avidin using standard titration methods (see the Supporting Information).

While there was clearly binding between **6** and avidin, as evidenced by the standard titrimetric assay, there were no changes in the EPR spectrum of the complex upon addition of avidin. Figure 2a and b are both consistent with the presence of square-planar Cu^{II}. The relatively small g anisotropy, $g_\perp = 2.06$ and $g_{||} = 2.19$, and the $A_{||}^{Cu}$ value of 198 G, giving rise to the 'overshoot' feature at high field, are typical of planar N_4 complexes. Importantly, the difference spectrum (Figure 2c) clearly shows the spectra to be essentially identi-

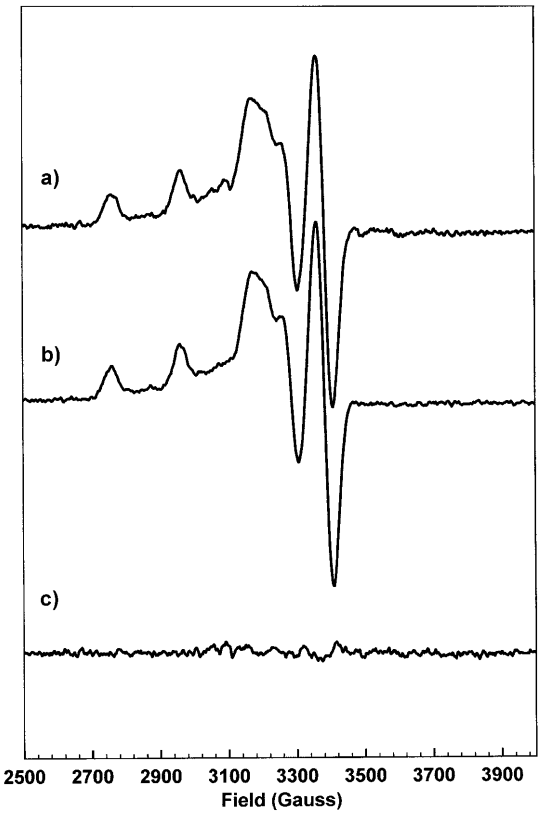


Figure 2. X-band EPR spectra at 20 K of a 100 μM aqueous solution of a) compound **6**, b) compound **6** plus one equivalent of avidin c) the difference spectrum b) minus spectrum a).

cal. As the unpaired electron of the Cu^{II} ion resides principally in the $d_{x^2-y^2}$ orbital, changes in coordination in this plane will have the greatest influence on its EPR spectrum. Given that this plane is occupied by the nitrogen ligands of the macrocycle, it appears that binding to avidin can only induce perturbations of the secondary coordination sphere which is too distant from the $d_{x^2-y^2}$ orbital to influence the EPR spectrum and suggested as anticipated that a change in the primary coordination sphere was required for transduction of the binding event.

We were attracted by the broad utility of the Sharpless/Huisgens Cu^{I}-catalyzed [3+2] click cycloaddition of alkynes and azides to generate the 'scorpion' ligand.[26] The reaction has been recently used in the incorporation of biomolecules into radiolabelled complexes[27] and in lanthanide-containing dendrimers in which the resulting triazole acts as a non-coordinating photosensitizer.[28] We hypothesised that the triazole could be perfectly situated to act as a chelating ligand at the weakly coordinating axial position and we have demonstrated this principle by developing a highly selective sensor for zinc using this approach.[29] This sensor and the present report are to the best of our knowledge the first examples of a triazole being used as a scorpionate ligand on an azamacrocycle. Hence to test the robustness of the coordination of the triazole we prepared model complex **10** (Scheme 2), and were delighted to find that single-crystal X-ray crystallography revealed that the expected coordination geometry was adopted with the triazole occupying the apical site and the *trans* I (*R*,*S*,*R*,*S*) (++++) conformation being adopted (Figure 3).

Bond lengths about the copper(II) centre are typical of related complexes (Cu1–N1 2.051, Cu1–-N2 2.002, Cu1–N3 2.005, Cu1–N4 2.025, Cu1–N5 2.323 Å). Given that we wished to use the perturbation of the scorpion-like interaction as a means of detecting the nature of the interaction of the proteinaceous ligand with the complex we were keen to investigate whether the addition of large quantities of competing anions affected the coordination sphere of the complex. We therefore monitored the effect of the addition of large excesses (ca. 200 equiv) of a number of anionic ligands on **10** (NaF, NaCl, KBr, KI, NaOH, NaOAc, K_2SO_4, $NaHCO_3$, Na_2CO_3, NaH_2PO_4, KH_2PO_4, Na_3PO_4 and $(CO_2H)_2$). In only two cases did we observe significant changes in the UV/Vis spectra (for the phosphate and hydroxide anions, Figure 4), due to the adversely high pH of these solutions (also see Supporting Information). These measurements clearly demonstrate that the interaction between the triazole and the copper(II) centre is relatively robust and that perturbations in the primary coordination sphere of complexes of this type are not likely to occur in biological samples as a result of simple anion exchange for the triazole, unlike in closely related cyclen systems in which the pendant arm donors were labile in the presence of a number of anions at neutral pH.[10a,11] We were therefore encouraged that the 'click'-generated system had potential in the detection of protein binding.

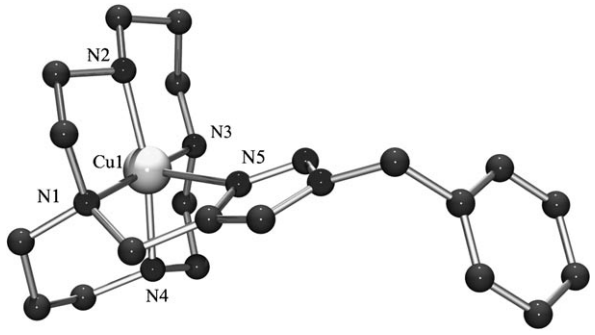


Figure 3. Single-crystal X-ray structure of **10** (cation) showing the scorpion role of the triazole moiety.[30]

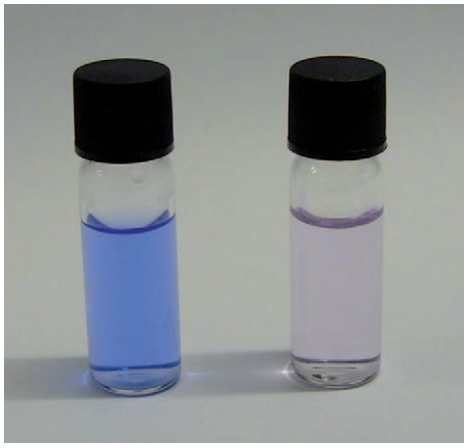
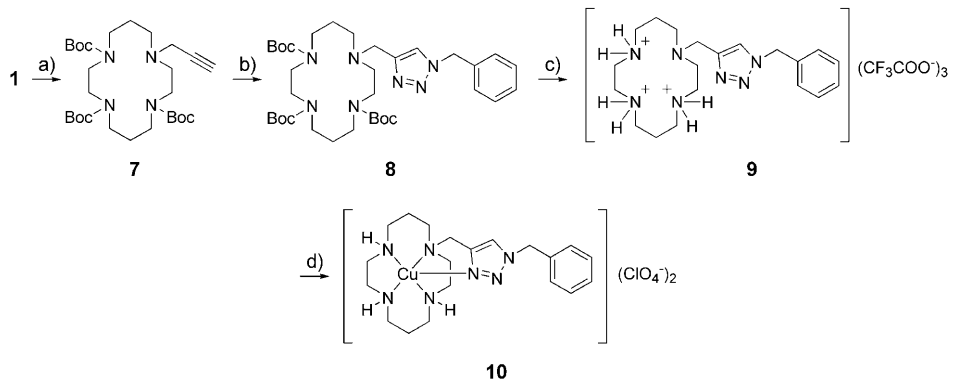
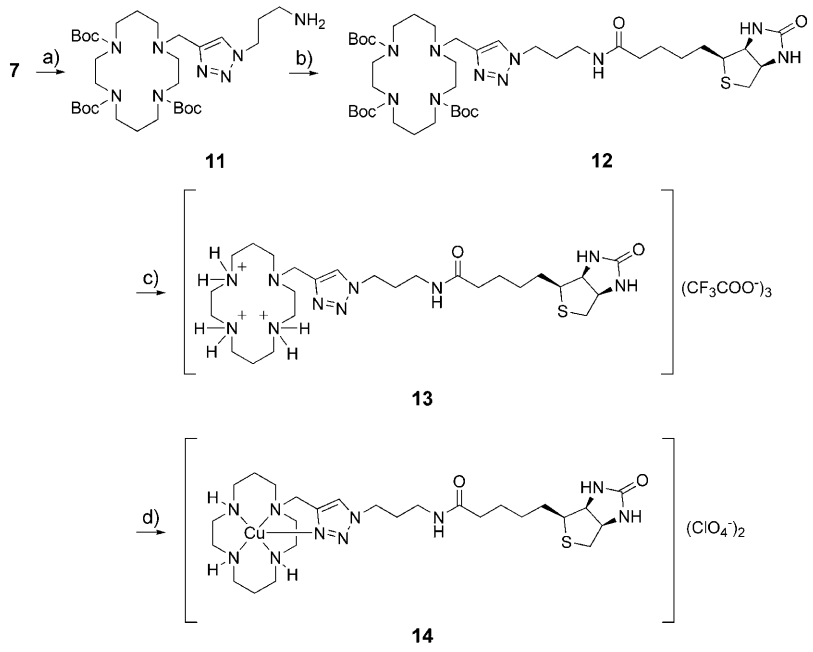


Figure 4. Visual detection of the perturbation of the coordination sphere of **10** (left hand vial) in the presence of hydroxide (right hand vial).



Scheme 2. Synthesis of model cyclam-click complex **10**. a) Propargyl bromide, Na_2CO_3; b) benzyl azide, $CuSO_4$, Na ascorbate; c) TFA (20%) in CH_2Cl_2; d) $Cu(ClO_4)_2{\cdot}6H_2O$, pH 8.



Scheme 3. Synthesis of cyclam-click-biotin conjugate **14**. a) 3-Azidopropylamine, $CuSO_4$, Na ascorbate; b) biotin, HATU, DIPEA, DMAP; c) TFA (20%) in CH_2Cl_2; d) $Cu(ClO_4)_2 \cdot 6H_2O$, pH 8.

To validate our allosteric scorpion concept we prepared complex **14** (Scheme 3). The click cycloaddition allows the incorporation of biotin onto azamacrocycles more easily than traditional synthetic approaches.[31]

The binding between **14** and avidin was shown to be essentially identical to that between avidin and **6** (see the Supporting Information). The EPR spectrum of **14** at biologically relevant concentrations in frozen solution (Figure 5a) was clearly different to that of **6** and now indicated that a mixture of two coordination environments was present, namely a five-coordinate copper(II) species resulting from coordination of the triazole and a square-planar copper(II) species that is analogous to **6**. Two overlapping contributions to the EPR spectrum are evident. One is quite similar to the spectrum of **6** having $g_\perp = 2.06$ and $g_{||} = 2.19$ but with a smaller $A_{||}^{Cu}$ of 186 G. The second contributing spectrum (the $g_{||}$ features of which are marked with * in Figure 5a) exhibits $g_{||} = 2.25$ and $A_{||}^{Cu} = 176$ G, while maintaining the $g_\perp = 2.06$. These parameters are indicative of a five-coordinate Cu^{II} ion in a square geometry (effectively a 'square-based pyramid').[32] Therefore in frozen solution (and presumably in liquid solution) **14** exists as an equilibrium mixture of four- and five-coordinate species. Upon addition of avidin, subtle but significant changes to the EPR spectrum are observed, (Figure 5b) and the $g_{||} = 2.25$ contribution is greatly reduced in intensity, while the $g_\perp = 2.19$ contribution shows a relative increase in intensity. This suggests an interconversion between the two forms of **14** observed on binding to avidin and is consistent with the conversion of the five-coordinate copper(II) to square-planar copper(II) as envisioned in the allosteric scorpion design. This is clearly displayed in the difference spectrum (Figure 5c). Although the signal to noise ratio is relatively poor as a consequence of the biologically relevant concentration of **14** used, clear negative features in the $g_{||}$ region of the difference spectrum are observed which are marked with arrows and are consistent with a significant reduction in the concentration of the five-coordinate species on binding to avidin, although the feature at 3058 G is poorly resolved due to line width. In addition an apparently first derivative line at 2948 G (marked 1 in the difference spectrum) results from changes in intensity of the spectra arising from both four- and five-coordinate species. Even more obvious and significant deviations are observed in the $g_\perp$ region of the difference spectrum reflecting the increased magnitude and shift in this signal upon avidin binding. This clearly shows that the binding of avidin is able to perturb the primary coordination sphere of the metal and that this process can be detected spectroscopically.

Although these EPR measurements provide qualitative evidence that the coordination sphere of the copper(II)

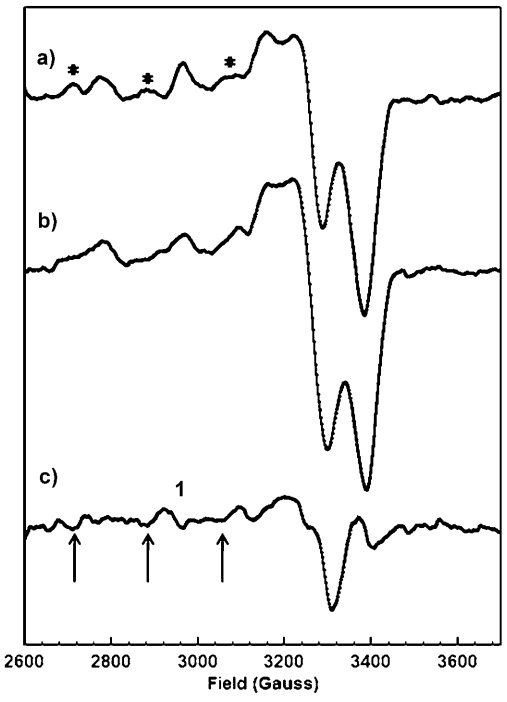


Figure 5. X-band EPR spectra of a 100 μM aqueous solution at 20 K of a) compound **14**, b) compound **14** plus one equivalent of avidin, c) difference spectrum b−a.

centre in **14** changes upon binding of avidin which is entirely consistent with the labilisation of the scorpionate triazole, we sought to probe changes to the primary coordination sphere of the metal ion in frozen solution in more detail using ENDOR (electron nuclear double resonance) spectroscopy. Figure 6 shows the X-band Davies FID-detected

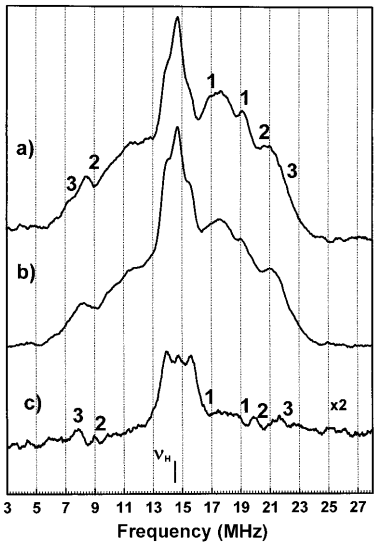


Figure 6. X-band Davies FID-detected ENDOR spectra of frozen solutions of 300 μM aqueous solutions of a) compound **14**, b) compound **14** plus avidin and c) the difference spectrum a−b.

ENDOR spectra of **14** recorded in frozen aqueous solutions at 20 K in the absence and presence of avidin together with the difference spectrum.[33] The spectrum in the absence of avidin (Figure 6a) clearly shows the presence of strong ^{14}N hyperfine coupling of 37.2 MHz, shown as 1, which is significantly enhanced when compared to that of the avidin-bound compound (Figure 6b). There are also additional ^{1}H hyperfine couplings of 13.3 MHz and 11.2 MHz marked as 2 and 3. The magnitude of the ^{14}N coupling is typical of nitrogen directly coordinated to the paramagnetic metal ion, while the ^{1}H hyperfine couplings are suggestive of protons attached to a metal ion ligating group, presumably the methylene protons linking the triazole to the cyclam framework which will be inequivalent when the triazole is coordinated to the copper centre. While the additional features in the spectrum are not quantitative, they unequivocally show that in a portion of **14** the copper(II) ion is five-coordinate and that this is converted to a four-coordinate species, through the loss of a nitrogen ligand, on binding to avidin. This is entirely consistent with our proposed allosteric scorpion scheme shown in Figure 1.

Conclusions

We have shown it is possible to detect the binding of a biological molecule to a metal complex that operates on an allosteric scorpion model, where remote binding is relayed to the metal centre through a change in the metal's primary coordination environment. We have developed a macrocycle with a scorpionate triazole ligand for this purpose, derived from a click cycloaddition. The ease of this synthesis, and the wide range of macrocycles and biological moieties that may be coupled, make the approach appealingly modular. The allosteric scorpion approach is also general, in that the binding event is not dependent on the structure of the metal complex, and complementarity can exploit inherent binding capabilities of any receptor and ligand. Since our design has been shown to sense a biological molecule at biologically relevant concentrations we suggest this design could have wide application to target diverse biomedical applications that would benefit from selective, responsive metal complexes. We assume that a steric interaction between the biotinylated ligand and the bulky analyte avidin forces dissociation of the triazole ligand from the metal centre, but the exact mechanism of this key change is currently being investigated in our laboratories.

Experimental Section

NMR spectra for all novel compounds, titration curves for avidin–HABA with complexes **6** and **14**, and UV/Vis spectroscopic changes for complex **10** in response to addition of anions may be found in the Supporting Information.

11-(3-Benzyloxycarbonylaminopropyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (2): *N*-Cbz-3-Aminopropanal (benzyl 3-oxopropylcarbamate)[34] (305 mg, 1.47 mmol) in THF (5 mL) was added to a solution of tri-Boc cyclam (**1**)[19a] (882 mg, 1.76 mmol) in THF (15 mL). $NaBH(OAc)_3$ (934 mg, 4.41 mmol) was added and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with aqueous $NaHCO_3$ (5%) and water. The organic phase was dried over $MgSO_4$, filtered, concentrated and purified by flash chromatography on silica gel (EtOAc 100%) to give **2** as a white solid (815 mg, 80% yield). m.p. 44–47°C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 7.36$–7.28 (m, 5H), 5.56 (bs, 1H), 5.07 (s, 2H), 3.38–3.14 (m, 14H), 2.57–2.47 (m, 2H), 2.46–2.32 (m, 4H), 1.88–1.54 (m, 6H), 1.44 (s, 1H), 1.42 ppm (s, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 156.4$ (C), 155.6 (C), 155.3 (C), 136.8 (C), 128.3 (CH), 127.9 (CH), 127.8 (CH), 79.3 (3 × C), 66.1 (CH_2), 53.4 (CH_2), 52.7 (CH_2), 51.5 (CH_2), 47.7 (CH_2), 47.3 (3 × CH_2), 46.7 (CH_2), 45.9 (CH_2), 39.6 (CH_2), 28.4 (9 × CH_3), 26.6 ppm (2 × CH_2); IR (CH_2Cl_2): $\tilde{\nu} = 3055$, 2985, 1710, 1685 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{36}H_{62}N_5O_8$) 692.4593, found 692.4599.

11-(3-Aminopropyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (3): Pd/C (10 mol%, 121 mg) was added to **2** (780 mg, 1.13 mmol) dissolved in MeOH (30 mL), and the resulting mixture was stirred under H_2 (1 atm.) for 16 h at room temperature. The crude product was filtered through a short plug of Celite and the solvent was removed in vacuo to give **3** as a white solid in quantitative yield. The product was used without further purification: m.p. 47–49°C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 3.46$–3.12 (m, 12H), 2.82–2.30 (m, 10H), 1.94–1.54 (m, 6H), 1.44 (s, 18H), 1.43 ppm (s, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 155.7$ (2 × C), 155.5 (C), 79.5 (3 × C), 53.4 (CH_2), 52.9 (CH_2), 51.4 (CH_2), 48.2 (2 × CH_2), 47.4 (m, 4 × CH_2), 45.8 (CH_2), 40.2 (CH_2), 28.5 (9 × CH_3), 26.6 ppm (m, 2 × CH_2); IR (CH_2Cl_2): $\tilde{\nu} = 3055$, 2985, 1686 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{28}H_{56}N_5O_6$) 558.4225, found 558.4228.

11-{3-[5-(2-Oxohexahydrothieno[3,4-*d*]imidazol-4-yl)pentanoylamino]-propyl}-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (4): Biotin (374 mg, 1.67 mmol), HATU (634 mg, 1.67 mmol),

DIPEA (580 μL, 3.33 mmol) and DMAP (13 mg, 10 mol%) were pre-activated in DMF (10 mL) for 10 min at room temperature. Then **3** (620 mg, 1.11 mmol) was added and the yellow solution stirred for 24 h at room temperature. The solvent was evaporated in vacuo and the crude was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 90:10 rising to 85:15) to give **4** as a pale yellow solid (450 mg, 52% yield): m.p. 109–111 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 6.24$ (br, 1H, NH), 5.49 (br, 1H, NH), 4.53–4.42 (m, 1H), 4.34–4.23 (m, 1H), 3.46–3.04 (m, 15H), 2.96–2.82 (m, 1H), 2.71 (d, $J = 12.8$ Hz, 1H), 2.58–2.47 (m, 2H), 2.46–2.24 (m, 4H), 2.24–1.98 (m, 4H), 1.92–1.48 (m, 10H), 1.43 ppm (s, 27H); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 173.5$ (C), 164.1 (C), 155.6 (m, 3 × C), 79.8 (m, 3 × C), 61.9 (CH), 60.3 (CH), 55.8 (CH), 51.8 (m, 3 × CH_2), 48.1–45.5 (m, 7 × CH_2), 40.6 (CH_2), 37.5 (CH_2), 36.0 (CH_2), 28.6 (9 × CH_3), 28.4 (CH_2), 28.1 (CH_2), 26.5 (m, 2 × CH_2), 25.8 ppm (CH_2); IR (CH_2Cl_2): $\tilde{\nu} = 1685$, 1465, 1265 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{38}H_{70}N_7O_8S$) 784.5001, found 784.5000.

11-{3-[5-(2-Oxohexahydrothieno[3,4-*d*]imidazol-4-yl)pentanoylamino]-propyl}-1,4,8,11-tetraazacyclotetradecanetrihydrotrifluoroacetate (5): Compound **4** (219 mg, 0.28 mmol) was dissolved in TFA (20% in CH_2Cl_2, 10 mL) and the resulting solution was stirred at room temperature overnight. The solvent was removed in vacuo to give **5** as a pale yellow solid (229 mg, quantitative): m.p. 41–43 °C; ^{1}H NMR (270 MHz; D_2O): $\delta = 4.61$ (dd, $J = 7.9$, 4.7 Hz, 1H), 4.41 (dd, $J = 7.9$, 4.4 Hz, 1H), 3.66–3.16 (m, 20H), 3.10 (m, 1H), 3.00 (dd, $J = 12.8$, 4.7 Hz, 1H), 2.77 (d, $J = 12.8$ Hz, 1H), 2.28 (t, $J = 6.9$ Hz, 2H), 2.18–1.98 (m, 4H), 1.97–1.79 (m, 2H), 1.78–1.28 ppm (m, 6H); ^{13}C NMR (100 MHz, D_2O): $\delta = 177.6$ (C), 165.7 (C), 163.3 (q, C, TFA), 116.7 (q, C, TFA), 62.4 (CH), 60.6 (CH), 55.8 (CH), 51.9 (CH_2), 49.8 (CH_2), 47.4 (CH_2), 45.2–42.0 (m, 4 × CH_2), 40.7 (2 × CH_2), 40.0 (CH_2), 36.6 (CH_2), 35.7 (CH_2), 28.3 (CH_2), 28.0 (CH_2), 25.4 (CH_2), 23.9 (CH_2), 20.9 (CH_2), 19.7 ppm (CH_2); IR (CH_2Cl_2): $\tilde{\nu} =$ 3294 (br), 3055, 2985, 1681 (br) cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{23}H_{46}N_7O_2S$) 484.3428, found 484.3430.

Complex 6: Compound **5** (200 mg, 0.097 mmol) was dissolved in MeOH/H_2O (1:1, 6 mL) and the pH of the solution was adjusted to 8 by dropwise addition of 2N NaOH. A solution of $CuSO_4 \cdot 6H_2O$ (36 mg, 0.097 mmol) in MeOH (1 mL) was added dropwise and the dark purple solution was stirred overnight at room temperature. The solvent was removed in vacuo, the residue dissolved in MeOH (10 mL) and filtered through a plug of Celite. MeOH was removed in vacuo to give a dark purple powder which was recrystallized from hot EtOH/Et_2O to give complex **6** as dark purple crystals (55 mg, 75% yield). IR (CH_2Cl_2): $\tilde{\nu} =$ 1681, 1107, 735 cm^{-1}; HRMS (ES) calcd for $[M+TFA]^+$, ($C_{25}H_{45}O_4N_7CuF_3S$) 659.2496, found 659.2492.

11-Prop-2-ynyl-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (7): To a solution of **1** (250 mg, 0.50 mmol) in CH_3CN (15 mL) were added Na_2CO_3 (212 mg, 1 mmol) and propargyl bromide (67 μL, 0.60 mmol). The mixture was heated at reflux (85 °C) overnight. The insoluble salts were removed by filtration and the solvent removed in vacuo. The crude material was purified by flash column chromatography on silica gel to give **7** as a white solid (211 mg, 78% yield): m.p. 47–49 °C; ^{1}H MNR ($CDCl_3$, 270 MHz): $\delta = 3.48$–3.15 (m, 14H), 2.71–2.58 (m, 2H), 2.49 (t, $J = 5.4$ Hz, 2H), 2.14 (s, 1H), 1.98–1.77 (m, 2H), 1.76–1.60 (m, 2H), 1.44 ppm (s, 27H); ^{13}C NMR ($CDCl_3$; 67.5 MHz): $\delta = 155.8$ (C), 155.5 (2 × C), 79.6 (2 × C), 79.5 (C), 77.9 (C), 73.2 (CH), 53.0 (CH_2), 51.9 (CH_2), 50.7 (CH_2), 48.0 (CH_2), 47.5 (CH_2), 46.9 (CH_2), 46.7 (CH_2), 44.8 (CH_2), 41.9 (CH_2), 28.5 (9 × CH_3), 25.5 ppm (2 × CH_2); IR (CH_2Cl_2): $\tilde{\nu} = 3301$, 2135, 1685 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{28}H_{51}N_4O_6$) 539.3803, found. 539.3800.

11-(1-Benzyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (8): Benzyl azide (130 μL, 1.02 mmol) was added to a solution of **7** (500 mg, 0.929 mmol) in H_2O/*t*BuOH (1:1, 20 mL) under nitrogen. $CuSO_4 \cdot 5H_2O$ (5 mol%, 12 mg dissolved in H_2O (1 mL)) and sodium ascorbate (10 mol%, 18 mg dissolved in H_2O (1 mL)) were added. The cloudy solution was stirred under nitrogen overnight at room temperature. 5% $NaHCO_3$ (5 mL) was added to the solution and the product was extracted with CH_2Cl_2 (3 × 40 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The crude material was purified by passage through a short plug of silica gel (EtOAc/*n*-hexane 6:4 to 8:2) to give **8** as a white solid (552 mg, 89% yield): m.p. 52–54 °C; ^{1}H MNR ($CDCl_3$, 270 MHz): $\delta = 7.36$–7.12 (m, 6H), 5.44 (s, 2H), 3.68 (s, 2H), 3.36–3.06 (m, 12H), 2.58–2.42 (m, 2H), 2.42–2.22 (m, 2H), 1.88–1.70 (m, 2H), 1.70–1.52 (m, 2H), 1.38 (s, 18H), 1.34 ppm (s, 9H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): $\delta = 155.5$, 155.3, 143.9, 134.6, 128.9, 128.5, 127.8, 122.2, 79.3, 53.8, 52.4, 50.7, 48.6, 47.3, 46.6, 45.2, 28.3, 28.2, 26.5 ppm; IR (CH_2Cl_2): $\tilde{\nu} = 3055$, 2985, 1685 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{35}H_{58}N_7O_6$) 672.4443, found 672.4449.

11-(1-Benzyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1,4,8,11-tetraazacyclotetradecanetrihydrotrifluoroacetate (9): Compound **8** (200 mg, 0.298 mmol) was deprotected with 20% TFA in DCM (5 mL) for 3 h at room temperature. The solvent was removed in vacuo to give **9** as a colourless glue (209 mg, 98% yield); ^{1}H MNR (D_2O; 270 MHz): $\delta = 8.08$ (s, 1H), 7.46–7.28 (m, 5H), 5.56 (s, 2H), 4.14 (s, 2H), 3.54–3.32 (m, 6H), 3.32–3.12 (m, 8H), 2.99 (pseudo t, $J = 6.8$ Hz, 2H), 2.18–1.96 ppm (m, 4H); ^{13}C NMR (D_2O; 67.5 MHz): $\delta = 162.5$ (q, TFA), 138.1, 134.4, 129.2, 128.9, 128.2, 126.8, 116.3 (q, TFA), 54.2, 48.9, 47.9, 45.9, 42.1, 41.8, 41.6, 39.2, 39.1, 38.4, 19.4, 19.2 ppm; IR (CH_2Cl_2): $\tilde{\nu} =$ 3500–2100 (br), 3433, 2854, 2530, 1774, 1670 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{20}H_{34}N_7$) 372.2870, found 372.2870.

Complex 10: Compound **9** (212 mg, 0.298 mmol) was dissolved in water (3 mL) and 2 N NaOH was added dropwise to adjust the pH to 8. A solution of $CuSO_4$ $6H_2O$ (110 mg, 0.298 mmol) in MeOH (3 mL) was added dropwise and the dark blue solution was stirred overnight at room temperature. The solvent was evaporated, the residue dissolved in MeOH (10 mL) and filtered through a plug of Celite. MeOH was evaporated in vacuo to give **10** as a dark purple powder (159 mg, 84% yield). Dark purple crystals, suitable for X-ray crystallography, were obtained by slow diffusion of toluene into a solution of complex **10** in acetonitrile: IR (CH_3CN): $\tilde{\nu} = 1685$, 1635, 1103, 1037, 748 cm^{-1}; HRMS (ES) calcd for $[M+ClO_4]^+$, ($C_{20}H_{33}O_4N_7ClCu$) 533.1573, found 533.1579.

3-Azido-propylamine (15):[35] Sodium azide (221 mg, 3.4 mmol) was added to a solution of 1-bromo-3-aminopropane hydrobromide (438 mg, 2.0 mmol) in water (10 mL), and the mixture was stirred at 80 °C overnight. The reaction mixture was cooled in an ice bath and diethyl ether (20 mL) and NaOH (60 mg) were added, keeping the temperature below 10 °C. After separation of the organic phase, the aqueous phase was extracted with more Et_2O (2 × 20 mL). The organic phases were collected, dried over $MgSO_4$ and filtered. The solvent was carefully removed under reduced pressure (water bath temperature ca. 30 °C). The product (**15**) was obtained as a colourless liquid (130 mg, ca. 55% yield). Because of the low boiling point of **15** (ca. 48–50 °C at 15 mm Hg) the solvent was not completely removed and a small amount of Et_2O was still present, observed in the ^{1}H NMR spectrum. ^{1}H MNR ($CDCl_3$; 270 MHz): $\delta = 3.34$ (t, $J = 6.7$ Hz, 2H), 2.77 (t, $J = 6.8$ Hz, 2H), 1.70 (q, $J = 6.7$ Hz, 2H), 1.47 ppm (bs, 2H, NH_2).

11-[1-(3-Amino-propyl)-1*H*-[1,2,3]triazol-4-ylmethyl]-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (11): To a solution of **7** (200 mg, 0.372 mmol) and **15** (55 mg, 0.557 mmol) in H_2O/*t*BuOH (1:1, 3 mL), $CuSO_4 \cdot 5H_2O$ (5 mol%, 4.6 mg dissolved in H_2O (0.5 mL)) and sodium ascorbate (10 mol%, 7.4 mg, dissolved in H_2O (0.5 mL)) were added under nitrogen. The yellow solution was stirred at room temperature overnight. Water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed with 5% aqueous $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated to give **11** as a pale yellow solid (214 mg, 90% yield) which was used without further purification in the next step. An analytical sample was purified through a short silica column ($CHCl_3$/MeOH, 8:2). ^{1}H NMR ($CDCl_3$; 400 MHz): $\delta = 7.48$ (s, 1H), 4.54–4.38 (m, 2H), 3.74–3.60 (m, 2H), 3.40–3.14 (m, 14H), 3.85 (bs, 2H, NH_2), 2.58–2.46 (m, 2H), 2.38–2.26 (m, 2H), 2.12–1.92 (m, 2H), 1.88–1.72 (m, 2H), 1.68–1.56 (m, 2H), 1.36 ppm (s, 27H); ^{13}C NMR ($CDCl_3$; 67.5 MHz): $\delta = 155.5$, 155.3, 143.1, 122.4, 79.2, 52.7, 50.9, 48.4, 47.3, 46.6, 45.1, 38.0, 33.0, 28.2, 26.4 ppm; IR (CH_2Cl_2): $\tilde{\nu} = 3455$, 2337, 1685 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{31}H_{59}N_8O_6$) 639.4552, found. 639.4553.

11-(1-{2-[5-(2-Oxohexahydrothieno[3,4-*d*]imidazol-4-yl)pentanoylamino]-propyl}-1*H*-[1,2,3]triazol-4-ylmethyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylic acid tri-*tert*-butyl ester (12): Biotin (57 mg,

0.235 mmol), HATU (72 mg, 0.235 mmol), DIPEA (54 μL, 0.312 mmol) and DMAP (10 mol%) were added to a solution of **11** (100 mg, 0.156 mmol) in DMF (3 mL). The solution was stirred overnight at room temperature. The solvent was then removed in vacuo and the crude was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 8:2) to give **11** as a pale yellow solid (94 mg, 70% yield): m.p. 95–97 °C; ^{1}H NMR ($CDCl_3$; 270 MHz): $\delta = 7.53$ (s, 1H), 6.89 (bs, 1H, NH), 6.39 (bs, 1H, NH), 5.56 (bs, 1H, NH), 4.58–4.56 (m, 2H), 4.38 (t, $J = 6.4$ Hz, 2H), 3.44–3.24 (m, 1H), 3.82–3.62 (m, 2H), 3.44–3.02 (m, 15H), 2.90 (dd, $J = 12.8$, 4.5 Hz, 1H), 2.72 (d, $J = 12.8$ Hz, 1H), 2.68–2.52 (m, 2H), 2.50–2.34 (m, 2H), 2.26–2.02 (m, 4H), 1.94–1.54 (m, 10H), 1.44 ppm (s, 27H); ^{13}C NMR ($CDCl_3$; 67.5 MHz): $\delta = 173.9$ (C), 164.4 (C), 155.9 (2 × C), 155.6 (2 × C), 143.4 (C), 123.1 (CH), 79.7 (3 × C), 61.8 (CH), 60.3 (CH), 55.9 (CH), 51.9 (CH_2), 51.1 (CH_2), 50.0–46.0 (m, 7 × CH_2), 45.5 (CH_2), 40.7 (CH_2), 36.3 (CH_2), 35.9 (CH_2), 30.1 (CH_2), 28.5 (9 × CH_3), 28.3 (CH_2), 28.0 (CH_2), 26.5 (CH_2), 25.7 ppm (CH_2); IR (CH_2Cl_2): $\tilde{\nu} = 3305$, 2098, 1678 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{41}H_{73}N_{10}O_8S$) 887.5148, found. 887.5142.

11-(1-{2-[5-(2-Oxohexahydrothieno[3,4-*d*]imidazol-4-yl)pentanoylamino]-propyl}-1*H*-[1,2,3]triazol-4-ylmethyl)-1,4,8,11-tetraazacyclotetradecanetrihydrotrifluoroacetate (13): Compound **12** (81 mg, 0.0937 mmol) was dissolved in TFA (20% in DCM, 5 mL). The resulting solution was stirred at room temperature overnight and the solvent was removed in vacuo to give **13** as a pale yellow semi-solid (84 mg, quantitative yield): ^{1}H NMR (D_2O; 270 MHz): $\delta = 8.09$ (s, 1H), 4.60–4.36 (3 m, total 4H), 4.19 (s, 2H), 3.70–3.14 (m, 18H), 3.13–2.88 (m, 2H), 2.73 (d, $J = 12.8$ Hz, 1H), 2.34–2.00 (m, 8H), 1.82–1.28 ppm (m, 6H); ^{13}C NMR ($CDCl_3$; 67.5 MHz): $\delta =$ 176.9, 165.3, 162.4 (q, TFA), 137.1, 127.4, 116.0 (q, TFA), 62.2, 60.4, 55.4, 48.5, 48.4, 45.3, 41.6, 41.3, 39.7, 38.5, 38.4, 38.3, 38.0, 37.8, 36.1, 35.5, 28.8, 28.0, 27.7, 25.2, 18.8 ppm; IR (CH_2Cl_2): $\tilde{\nu} = 1766$, 1666 cm^{-1}; HRMS (ES) calcd for $[M+H]^+$, ($C_{26}H_{49}N_{10}O_2S$) 565.3755, found. 565.3753.

Complex 14: Compound **13** (87 mg, 0.096 mmol) was dissolved in MeOH/H_2O (1:1, 6 mL) and the pH of the solution was adjusted to 8 by dropwise addition of 2N NaOH. A solution of $CuSO_4{\cdot}6H_2O$ (35 mg, 0.096 mmol) in MeOH (1 mL) was added dropwise and the dark blue solution was stirred overnight at room temperature. The solvent was evaporated, the residue dissolved in MeOH (10 mL) and filtered through a plug of Celite. MeOH was evaporated in vacuo to give **14** as dark blue crystals (63 mg, 79% yield). IR (CH_2Cl_2): $\tilde{\nu} = 1693$, 1103, 1037, 752, 725 cm^{-1}; HRMS (ES) calcd for $[M+ClO_4]^+$, ($C_{26}H_{48}O_6N_{10}ClCuS$) 726.2458, found 726.2465.

HABA assays of complexes 6 and 14:[21] The binding of the copper(II)–biotin complexes was assessed by HABA assays. Typically, to a mixture of HABA (300.0 μM) and avidin (7.6 μM) in 50 mM HEPES buffer pH 7.0 (3 mL) were added 10 μL aliquots of the copper(II)–biotin complex (1 mM). The formation of the copper–avidin adduct was indicated by a decrease of the absorbance at 500 nm due to the displacement of HABA from the avidin. By plotting $-\Delta A_{500\ nm}$ versus [Cu]:[avidin], the binding of the copper(II)–biotin complex to avidin was determined.

Experimental details of EPR spectral analysis: EPR spectra were obtained at X-band using a Bruker ELEXSYS E500 spectrometer, equipped with an Oxford Instruments ESR900 liquid helium cryostat. EPR spectra for blanks (complexes alone) were recorded by using 250 μL of 100 μM solutions of complexes in HEPES buffer. For 1:1 mixtures (complexes-avidin) samples were again 250 μL, 100 μM in complexes and 100 μM in avidin.

Acknowledgements

We thank the EPSRC (GR/T17014/01) for funding through the Life Sciences Initiative. We are also grateful to the EPSRC for the provision of the National Mass Spectrometry Service (University of Wales, Swansea) and the X-ray crystallography service (University of Southampton). We thank Professor Lisa Hall (Cambridge) for stimulating discussions.

[1] T. W. Hambley, *Science* **2007**, *318*, 1392–1393.
[2] a) P. D. Beer, S. R. Bayly, *Top. Curr. Chem.* **2005**, *255*, 125–162; b) R. Martínez-Máñez, F. Sancenón, *Chem. Rev.* **2003**, *103*, 4419–4476; c) P. D. Beer, P. A. Gale, *Angew. Chem.* **2001**, *113*, 502–532; *Angew. Chem. Int. Ed.* **2001**, *40*, 486–516; d) P. V. Bernhardt, E. G. Moore, *Aust. J. Chem.* **2003**, *56*, 239–258; e) *Coord. Chem. Rev.* **2000**, *205*, Issue 1; f) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566.
[3] a) L. Fabbrizzi, A. Leone, A. Taglietti, *Angew. Chem.* **2001**, *113*, 3156–3159; *Angew. Chem. Int. Ed.* **2001**, *40*, 3066–3069; b) S. L. Tobey, E. V. Anslyn, *Org. Lett.* **2003**, *5*, 2029–2031; c) M. S. Han, D. H. Kim, *Angew. Chem.* **2002**, *114*, 3963–3965; *Angew. Chem. Int. Ed.* **2002**, *41*, 3809–3811; d) H. Aït-Haddou, S. L. Wiskur, V. M. Lynch, E. V. Anslyn, *J. Am. Chem. Soc.* **2001**, *123*, 11296–11297.
[4] For example, Lanthanide–cyclodextrin conjugates for the sensing of small aromatic molecules: C. M. Rudzinski, W. K. Hartmann, D. G. Nocera, *Coord. Chem. Rev.* **1998**, *171*, 115–123.
[5] a) A. Ojida, Y. Mito-oka, K. Sada, I. Hamachi, *J. Am. Chem. Soc.* **2004**, *126*, 2454–2463; b) A. Ojida, Y. Miyahara, T. Kohira, I. Hamachi, *Biopolymers* **2004**, *76*, 177–184; c) a hybrid biosensor was recently reported that employs a mixture of phosphoprotein binding domains and metal complex-based chemosensor: T. Anai, E. Nakata, Y. Koshi, A. Ojida, I. Hamachi, *J. Am. Chem. Soc.* **2007**, *129*, 6232–6239; for a similar principle applied to the detection of phosphorylated natural products: d) H.-W. Rhee, C.-R. Lee, S.-H. Cho, M.-R. Song, M. Cashel, H. E. Choy, Y.-J. Seok, J.-I. Hong, *J. Am. Chem. Soc.* **2008**, *130*, 784–785; e) S. Aoki, M. Zulkefeli, M. Shiro, M. Kohsako, K. Takeda, E. Kimura, *J. Am. Chem. Soc.* **2005**, *127*, 9129–9139; sensing of phosphorylated membranes: f) K. M. DiVittorio, W. M. Leevy, E. J. O'Neil, J. R. Johnson, S. Vakulenko, J. D. Morris, K. D. Rosek, N. Serazin, S. Hilkert, S. Hurley, M. Marquez, B. D. Smith, *ChemBioChem* **2008**, *9*, 286–293.
[6] a) J. Geduhn, T. Walenzyk, B. König, *Curr. Org. Synth.* **2007**, *4*, 390–412; b) R. Reichenbach-Klinke, M. Kruppa, B. König, *J. Am. Chem. Soc.* **2002**, *124*, 12999–13007; c) C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, P. Fornasari, C. Giorgi, B. Valtancoli, *Eur. J. Inorg. Chem.* **2003**, 1974–1983; d) R. S. Dickins, S. Aime, A. S. Batsanov, A. Beeby, M. Botta, J. I. Bruce, J. A. K. Howard, C. S. Love, D. Parker, R. D. Peacock, H. Puschmann, *J. Am. Chem. Soc.* **2002**, *124*, 12697–12705.
[7] a) X. Liang, J. A. Parkinson, M. Weishaupl, R. O. Gould, S. J. Paisey, H.-s. Park, T. M. Hunter, C. A. Blindauer, S. Parsons, P. J. Sadler, *J. Am. Chem. Soc.* **2002**, *124*, 9105–9112.
[8] a) C. J. Burrows, S. E. Rokita, *Acc. Chem. Res.* **1994**, *27*, 295–301; b) D. R. McMillin, K. M. McNett, *Chem. Rev.* **1998**, *98*, 1201–1219; c) T. Ito, S. Thyagarajan, K. D. Karlin, S. E. Rokita, *Chem. Commun.* **2005**, 4812–4814.
[9] a) S. Aoki, D. Kagata, M. Shiro, K. Takeda, E. Kimura, *J. Am. Chem. Soc.* **2004**, *126*, 13377–13390; b) E. Kimura, H. Kitamura, K. Ohtani, T. Koike, *J. Am. Chem. Soc.* **2000**, *122*, 4668–4677; c) E. Kimura, T. Koike, *Chem. Commun.* **1998**, 1495–1500; d) E. Kikuta, M. Murata, N. Katsube, T. Koike, E. Kimura, *J. Am. Chem. Soc.* **1999**, *121*, 5426–5436; e) M. Shionoya, T. Ikeda, E. Kimura, M. Shiro, *J. Am. Chem. Soc.* **1994**, *116*, 3848–3859. See also f) B. König, M. Pelka, M. Subat, I. Dix, P. G. Jones, *Eur. J. Org. Chem.* **2001**, 1943–1949.
[10] S. Mizukami, T. Nagano, Y. Urano, A. Odani, K. Kikuchi, *J. Am. Chem. Soc.* **2002**, *124*, 3920–3925.
[11] a) A. Harriman, Y. Kubo, J. L. Sessler, *J. Am. Chem. Soc.* **1992**, *114*, 388–390; b) C. Turro, C. K. Chang, G. E. Leroi, R. I. Cukier, D. G. Nocera, *J. Am. Chem. Soc.* **1992**, *114*, 4013–4015; c) T. H. Ghaddar, E. W. Castner, S. S. Isied, *J. Am. Chem. Soc.* **2000**, *122*, 1233–1234; d) J. L. Sessler, C. M. Lawrence, J. Jayawickramarajah, *Chem. Soc. Rev.* **2007**, *36*, 314–325; e) A. Satake, Y. Kobuke, *Tetrahedron* **2005**, *61*, 13–41; f) M. D. Ward, *Chem. Soc. Rev.* **1997**, *26*, 365–375.
[12] T. Gunnlaugsson, J. P. Leonard, *Chem. Commun.* **2005**, 3114–3131.
[13] B. Linton, A. D. Hamilton, *Chem. Rev.* **1997**, *97*, 1669–1680.

[14] a) T. Hayashi, T. Takimura, H. Ogoshi, *J. Am. Chem. Soc.* **1995**, *117*, 11606–11607; b) V. Heleg-Shabtai, T. Gabriel, I. Willner, *J. Am. Chem. Soc.* **1999**, *121*, 3220–3221; c) T. Hayashi, Y. Hisaeda, *Acc. Chem. Res.* **2002**, *35*, 35–43.

[15] a) *The Chemistry of Macrocyclic Ligand Complexes* (Ed.: L. F. Lindoy), Cambridge University Press, Cambridge **1989**; b) P. V. Bernhardt, G. A. Lawrance, *Coord. Chem. Rev.* **1990**, *104*, 297–343; c) K. P. Wainwright, *Adv. Inorg. Chem.* **2001**, *52*, 293–334; d) T. A. Kaden, *Top. Curr. Chem.* **1984**, *122*, 157–179; e) M. Meyer, V. Dahaoui-Gindrey, C. Lecomte, R. Guilard, *Coord. Chem. Rev.* **1998**, *178–180*, 1313–1405; f) K. P. Wainwright, *Adv. Inorg. Chem.* **2001**, *52*, 293–334; g) P. Comba, S. M. Luther, O. Maas, H. Pritzkow, A. Vielfort, *Inorg. Chem.* **2001**, *40*, 2335–2345; h) L. Fabbrizzi, M. Licchelli, P. Pallavicini, L. Parodi, *Angew. Chem.* **1998**, *110*, 838–841; *Angew. Chem. Int. Ed.* **1998**, *37*, 800–802; i) S. El Ghachtouli, C. Cadiou, I. Dechamps-Olivier, F. Chuburu, M. Aplincourt, T. Roisnel, *Eur. J. Inorg. Chem.* **2006**, 3472–3482.

[16] a) X. Liang, P. J. Sadler, *Chem. Soc. Rev.* **2004**, *33*, 246–266; b) F. Liang, S. Wan, Z. Li, X. Xiong, L. Yang, X. Zhou, C. Wu, *Curr. Med. Chem.* **2006**, *13*, 711–727; c) A. Aoki, E. Kimura, *Chem. Rev.* **2004**, *104*, 769–788; d) D. Parker in *Crown Compounds: Towards Future Applications*, VCH, Weinheim, **1992**, pp. 51–67; e) T. J. Norman, D. Parker, L. Royle, A. Harrison, P. Antoniw, D. J. King, *J. Chem. Soc. Chem. Commun.* **1995**, 1877–1878.

[17] G. A. McLachlan, G. D. Fallon, R. L. Martin, L. Spiccia, *Inorg. Chem.* **1995**, *34*, 254–261.

[18] L. Siegfried, T. A. Kaden, *Dalton Trans.* **2005**, 3079–3082.

[19] a) L. Fabbrizzi, F. Foti, M. Licchelli, P. M. Maccarini, D. Sacchi, M. Zema, *Chem. Eur. J.* **2002**, *8*, 4965–4972; b) C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, L. Borsari, C. Giorgi, B. Valtancoli, C. Lodeiro, J. C. Lima, A. J. Parola, F. Pina, *Dalton Trans.* **2004**, 591–597; c) J. Otsuki, T. Akasak, K. Araki, *Coord. Chem. Rev.* **2008**, *252*, 32–56.

[20] M. Wilchek, E. A. Bayer, *Methods Enzymol.* **1990**, *184*, 5–13. For a recent example, see: S. Burazerovic, J. Gradinaru, J. Pierron, T. R. Ward, *Angew. Chem.* **2007**, *119*, 5606–5610; *Angew. Chem. Int. Ed.* **2007**, *46*, 5510–5514.

[21] K. K.-W. Lo, W.-K. Hui, *Inorg. Chem.* **2005**, *44*, 1992–2002.

[22] A. L. Eckermann, K. D. Barker, M. R. Hartings, M. A. Ratner, T. J. Meade, *J. Am. Chem. Soc.* **2005**, *127*, 11880–11881.

[23] *Electron Paramagnetic Resonance: Elementary Theory and Practical Applications, Appendix F* (Eds.: J. A. Weill, J. R. Bolton), Wiley, New York, **2007**, p. 548.

[24] a) M. Skander, C. Malan, A. Ivanova, T. R. Ward, *Chem. Commun.* **2005**, 4815–4817; b) G. Klein, N. Humbert, J. Gradinaru, A. Ivanova, F. Gilardoni, U. E. Rusbandi, T. R. Ward, *Angew. Chem.* **2005**, *117*, 7942–7945; *Angew. Chem. Int. Ed.* **2005**, *44*, 7764–7767; c) M. Creus, A. Pordea, T. Rossel, A. Sardo, C. Letondor, A. Ivanova, I. LeTrong, R. E. Stenkamp, T. R. Ward, *Angew. Chem.* **2008**, *120*, 1422–1426; *Angew. Chem. Int. Ed.* **2008**, *47*, 1400–1404.

[25] E. A. Lewis, R. W. Boyle, S. J. Archibald, *Chem. Commun.* **2004**, 2212–2213.

[26] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 3563–3565; *Angew. Chem. Int. Ed.* **2001**, *40*, 3455–3457; b) J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249–1262.

[27] T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwe, R. Schibli, *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.

[28] P. Antoni, M. Malkoch, G. Vamvounis, D. Nyström, A. Nyström, M. Lindgren, A. Hult, *J. Mater. Chem.* **2008**, *18*, 2545–2554.

[29] E. Tamanini, A. Katewa, L. Sedger, M. H. Todd, M. Watkinson, *Inorg. Chem.* **2009**, *48*, 319–324.

[30] CCDC-645442 (**10**) contains 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

[31] S. Krivickas, E. Tamanini, M. H. Todd, M. Watkinson, *J. Org. Chem.* **2007**, *72*, 8280–8289.

[32] a) F. E. Mabbs, D. Collison, *Electron Paramagnetic Resonance of d Transition Metal Compounds (Studies in Inorganic Chemistry Series)*, Elsevier, Amsterdam, **1992**, pp. 394–408; b) E. V. Rybak-Akimova, A. Y. Nazarenko, L. Chen, P. W. Krieger, A. M. Herrera, V. V. Tarasov, P. D. Robinson, *Inorg. Chim. Acta* **2001**, *324*, 1–15.

[33] a) E. R. Davies, *Phys. Lett.* **1974**, *47A*, 1–2; b) A. Schweiger, G. Jeschke, *Principles of Pulse Electron Paramagnetic Resonance*, Oxford University Press, Northamptonshire, **2001**, pp. 360–367.

[34] J.-G. Delcros, S. Tomasi, S. Carrington, B. Martin, J. Renault, I. S. Blagbrough, P. Uriac, *J. Med. Chem.* **2002**, *45*, 5098–5111.

[35] B. Carboni, A. Benalil, M. Vaultier, *J. Org. Chem.* **1993**, *58*, 3736–3741.

Received: November 21, 2008
Published online: February 16, 2009

