Computer-guided design of a Cu(II) receptor and sensor

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The computer program CAVEAT was used to design a linker structure to connect four imidazole 4-carboxamide groups in a distorted square planar arrangement optimal for binding Cu(II) ions. The resulting macrocyclic structure was prepared by the synthesis of a protected monomer followed by amide coupling reactions to form the cyclic tetramer. Binding to Cu(II) ion was evaluated using the inherent fluorescence of the receptor and its quenching upon binding to Cu(II) ion. Much higher concentrations of Zn(II), Co(II), and Ni(II) ions had little or no effect on the fluorescence and did not significantly compete with the fluorescence response to Cu(II) ions.

Introduction

The design and synthesis of specific ligands and receptors for metal ions is a field of longstanding and continuing interest. This work is of importance for a variety of reasons including the development of sensors for the detection and quantitation of metal ions in biological systems and environmental samples.[1–4] Success with a Ca^{2+} sensor and its application to studies of cell calcium demonstrates the value of sensors for biologically important metal ions.[5] Metal receptors have also been designed as models or mimics for metalloproteins.[6] Metal ion receptors have been based on a variety of structural motifs including synthetic acyclic and macrocyclic polydentate ligands as well as synthetic peptides.[7–12] The Cu^{2+} ion is somewhat unique in its preference for distorted square planar coordination and this geometry is observed in a number of interesting Cu^{2+} binding proteins.[13,14] This preference offers the potential for development of selective receptors for Cu^{2+} based on ligand groups organized in a distorted square planar arrangement.[15] A number of Cu^{2+} sensors have been recently developed based on both new and old Cu^{2+}-binding motifs.[10,11,16–18]

Recent structural and computational analysis have illustrated that most complexes between metal ions and multidentate ligands are far from optimal when one considers all of the bond angles and dihedral angles, in addition to the more obvious issues of coordination geometry and bond lengths about the metal ion.[15,19] While metal-binding proteins might be expected to achieve the ideal metal ligand structure, if this has been the direction of evolutionary pressure, more sophisticated design approaches are clearly needed for the design of more optimal man-made multidentate ligands that approximate the ideal metal coordination environments. Recently computer-based design approaches to this objective have begun to be pursued.[20] We have recently begun a research program in the application of the computer program CAVEAT to the design of receptors for molecule and ion recognition.[21–23] CAVEAT searches electronic databases of 3-dimensional chemical structures to identify structures having bonds matching a defined pair of vectors.[24,25] Reported here is the computer-assisted design of a Cu^{2+} receptor, using CAVEAT to identify a linker structure to connect individual ligand groups in the orientation that matches a computational model of the complex with unlinked ligands. Also reported is the synthesis of the receptor based on this design as well as initial studies of Cu^{2+} affinity and selectivity and the utility of this receptor as a fluorescence-based Cu^{2+} sensor.

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Results and discussion

Design

The design of the Cu(II) receptor was based on the premise that a distorted square planar arrangement of imidazole ligands could exhibit selectivity for Cu^{2+} over other metal ions, and that CAVEAT could be used to design a connector moiety to organize the imidazole groups into such an arrangement. The design process is illustrated in Fig. 1. The complex of Cu^{2+} with four modified imidazole amide moieties **1**, analogous to type 2 Cu(II) centers in proteins[13] was optimized using PM3 calculations. The initial distorted square planar structure was not precisely symmetrical, with the near planar N–Cu–N angles ranging from 94.45–95.38°. The structure was re-optimized in PM3 with the "in plane" N–Cu–N angles constrained at 95°. The resulting highly symmetrical structure **2** had Cu–N bond lengths of 1.93 Å, in fair agreement with the average distance of 1.98 Å observed in Cu–imidazole complexes.[26] The symmetry assures that one appropriately designed linker structure can be used for linking each consecutive imidazole pair in a tetrameric structure. To design the linker structure, the amide *N*-methyl and imidazole *N*-methyl bonds of adjacent ligands of **2** (represented by the bonds in bold in the upper right portion of structure **1**) were used to define the vectors for CAVEAT. A CAVEAT search was conducted of the TRIAD and ILLIAD databases, both being collections of computer-generated structures developed specifically for use with CAVEAT.[24] Several possible structures to serve as linkers between ligand moieties were thereby identified. Of these, structure **3** from ILLIAD was chosen for development, in which the C–H_a and C–H_b bonds match the vectors defined by

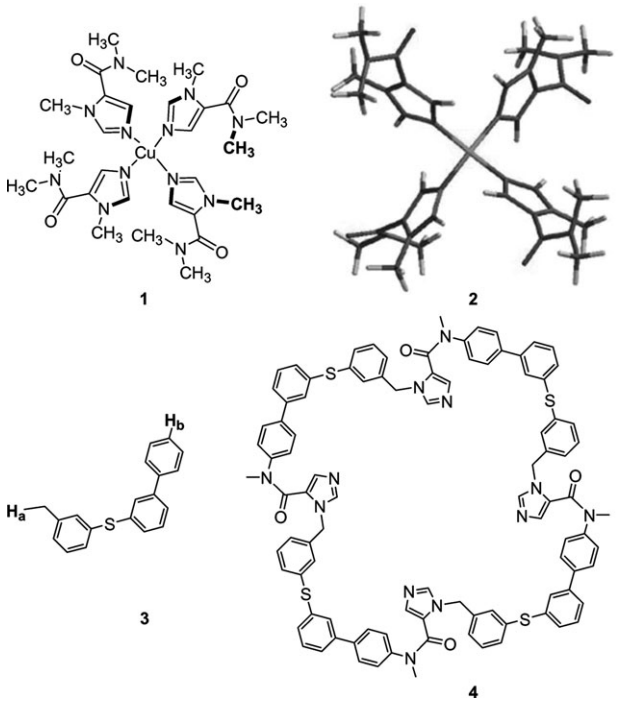
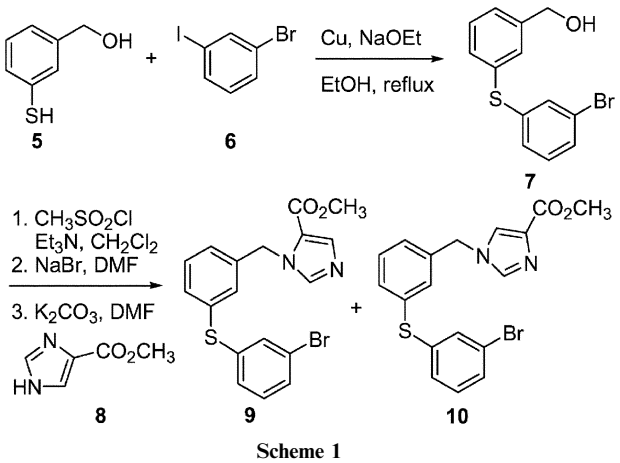


Fig. 1 Structures in the design of the macrocyclic Cu(II) receptor **4**.

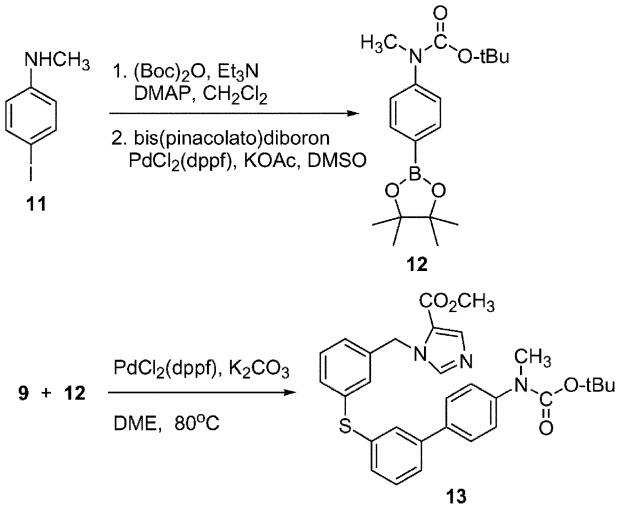
the imidazole–methyl and amide–methyl C–N bonds, respectively. Incorporation of this linker structure between consecutive monomeric groups in the copper complex **1** gives the macrocyclic structure **4**. Computational modeling of the Cu^{2+} complex of **4** further supported this design, including the absence of significant strain introduced at the points of connection between linker and ligand. The receptor has a fair degree of flexibility, but modeling indicates it is sufficiently constrained that tetrahedral coordination to a metal ion would impart significant strain relative to the designed distorted square planar coordination.

Synthesis

The preparation of the macrocyclic tetramer **4** began with synthesis of a protected monomer unit. The initial steps in the synthesis are shown in Scheme 1. The thioether **7** was prepared by copper-mediated coupling of 3-mercaptobenzyl alcohol **5**[27] with 3-bromoiodobenzene **6**.[28] Conversion of the benzylic alcohol to the bromide *via* the mesylate followed by reaction with methyl-4-imidazolecarboxylate **8** and base formed the desired intermediate **9** along with the isomer **10** resulting from alkylation of the wrong imidazole nitrogen. The isomers were readily separated by column chromatography. The assignment of isomers was confirmed by synthesis of **9** by *N*-alkylation of a glycine ester with the mesylate derived from **7** followed by formation of the imidazole ring using Gold's salt, a known route for the synthesis of imidazole derivatives.[29] However, this reaction gave a low yield and was difficult to reproduce so the synthetic route of Scheme 1 was preferred. The remainder of the synthesis of the protected monomer is shown in Scheme 2. *t*-Boc protection of *p*-iodo-*N*-methylaniline **11**[30] followed by conversion of the iodide to the boronic ester by the Miyaura



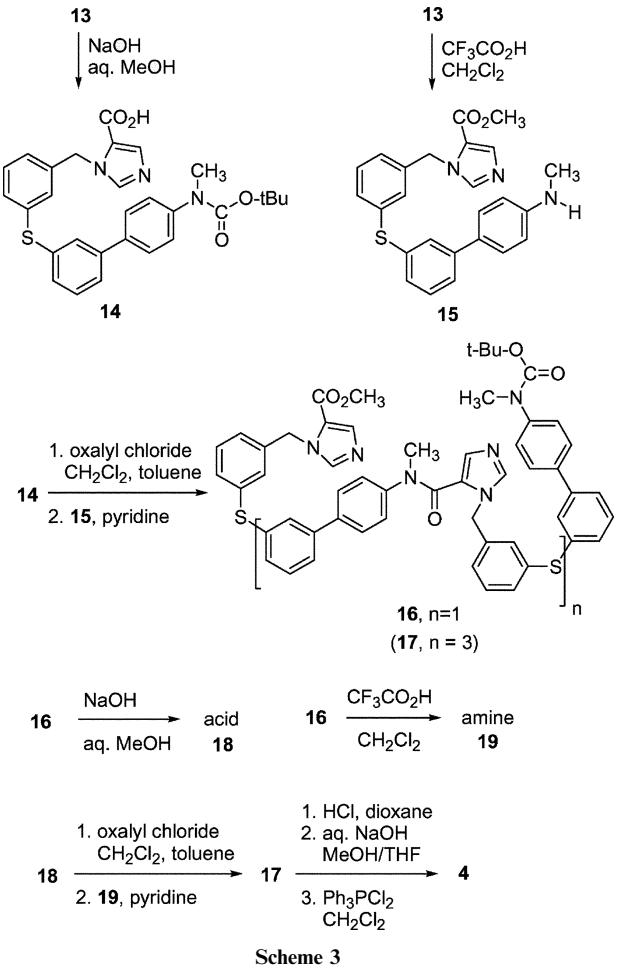
Scheme 1



Scheme 2

method formed **12**.[31] Suzuki coupling of **9** with **12** formed the protected monomer unit **13**.[32,33]

The monomer **13** was converted to the cyclic tetramer **4** as shown in Scheme 3. Separate samples of **13** were deprotected to form the free carboxylic acid **14** and the free amine **15**. **14** was converted to the acid chloride followed by reaction with **15** to form the protected dimer **16**.[34,35] Separate samples of **16** were deprotected to the free acid **18** and the free amine **19** as with the monomer **13**. The dimer units were again coupled *via* the acid chloride to form the protected tetramer **17**. The amine was deprotected with HCl to avoid introduction of trifluoroacetic acid, which was difficult to separate from the deprotected tetramer and when present caused problems in the coupling step. The ester was hydrolyzed with sodium hydroxide as in the previous deprotections. Attempts to form the cyclic tetramer *via* the acid chloride and using several other coupling agents were unsuccessful. Cyclization was successfully accomplished using dichlorotriphenylphosphorane in dilute (0.4 mM) solution at reflux for three days to form the desired macrocycle **4**. Formation of the macrocycle resulted in a marked simplification of the ^{1}H NMR spectrum as expected for this highly symmetrical product. Most notably, the four closely spaced singlets between 3.26 and 3.33 for the four *N*-methyl groups of the protected linear tetramer **17** collapsed



Scheme 3

to a single peak at 3.26 integrating to 12 hydrogens in **4**. Another interesting observation was the shifting of H-4 of the imidazole ring to about 6.4 ppm in the imidazole 5-carboxamide group, apparently due to substantial loss of conjugation of the amide carbonyl with the imidazole ring due to twisting caused by steric effects of the tertiary amide. A slightly smaller shift was reported though not discussed for a morpholine amide of imidazole-5-carboxylic acid.[36] As predicted from this analysis **13**, **16**, **17**, and **4** exhibited the diagnostic 0, 1, 3, and 4 upfield shifted imidazole protons respectively, further supporting the structure of **4**.

Binding studies

The macrocycle **4** was studied as a receptor for Cu^{2+} and other metal ions using fluorescence. The integral phenyl biphenyl thioether moiety of the receptor provided a good fluorophore for observation of Cu(II)-induced fluorescence quenching. **4** is insoluble in water, so these experiments were conducted in 1 : 1 methanol : tetrahydrofuran. **4** exhibited a maximum absorbance at 290 nm and an emission maximum at 357 nm, which were used as the excitation and emission wavelengths in binding experiments. Fig. 2 shows the fluorescence emission spectra for **4** with increasing concentration of $CuCl_2$. As expected, the paramagnetic Cu(II) ion substantially quenched the fluorescence of the receptor, with slightly more

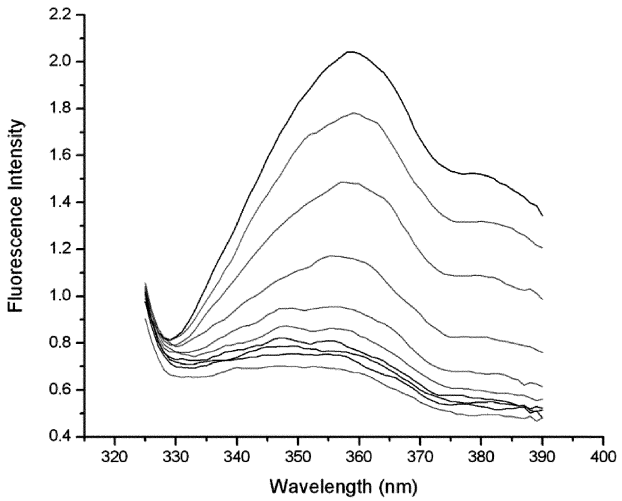


Fig. 2 Decreasing fluorescence of **4** (initial conc. 32.6 μM) with increasing [$CuCl_2$] (0, 7.7, 15.3, 23.7, 32.1, 40.4, 48.7, 56.9, 65.0, and 86.9 μM).

than 50% quenching observed at saturation. A plot of fluorescence intensity *vs.* $CuCl_2$ concentration gave a dissociation constant for the Cu^{2+}–**4** complex of 3.2×10^{-6} M. This binding constant in organic solvent is influenced by the counter ion, as the equivalent titration with copper(II) triflate gave a linear decrease in fluorescence with increasing copper salt until one equivalent was reached after which the fluorescence was unchanged, with insufficient curvature in the fluorescence intensity *vs.* [Cu^{2+}] plot to accurately deduce a binding constant. The titration was also performed with Zn(II) triflate, Ni(II) chloride and Co(II) chloride. Zn^{2+} and Ni^{2+} showed no quenching or other change in the fluorescence spectrum at concentrations up to 2.2×10^{-4} and 4.5×10^{-4} M, respectively, while Co^{2+} gave about 5% quenching at a concentration of 2.8×10^{-4} M. As binding of these metal ions could not be directly evaluated using fluorescence, selectivity for Cu(II) ions was assessed by competition. Stepwise addition of Cu(II) chloride to each of the solutions of **4** containing Ni^{2+}, Co^{2+}, or Zn^{2+} resulted in a fluorescence quenching curve that was indistinguishable from the titrations with no other metal salt present, with significant quenching observed even at the first concentration of 7.7×10^{-6} M Cu^{2+}. This indicates that Ni^{2+}, Co^{2+}, and Zn^{2+} ions do not compete significantly with binding of Cu^{2+} and that the selectivity for Cu^{2+} is at least 100-fold *vs.* each of the other three metals. The selectivity *vs.* Ni^{2+} is noteworthy as Ni^{2+} exhibits similar or even greater affinity than Cu^{2+} toward some simple ligands such as bipyridine, and selectivity *vs.* Ni^{2+} has been an issue with some Cu^{2+} sensors.[10,37] The aqueous insolubility of **4** limits the more complete affinity and selectivity analysis of this initial design.

Conclusion

A novel computer-guided approach was demonstrated for designing a multidentate ligand for optimal positioning of individual ligand groups for metal ion binding. This approach

may be valuable in designing selective metal ion receptors that with incorporation of appropriate solubility and fluorescence signaling properties may be valuable as metal ion sensors. This work further demonstrates the broad utility of the computer program CAVEAT in molecular design.

Experimental

General

PM3 calculations were performed using PC Spartan Pro from Wavefunction, Inc. Chemicals were obtained from commercial suppliers and used without further purification. Column chromatography was performed on silica gel 60 (40–63 μm). NMR spectra were obtained on a Varian Gemini-2300 instrument.

Synthesis

[3-(3-Bromo-phenylsulfanyl)-phenyl]-methanol (7). To a solution of **5**[27] (4.21 g, 0.030 mol) and sodium ethoxide (0.090 mol) in ethanol (45 mL) was added copper powder (0.2 g, 3.13 mmol) and 3-iodobromobenzene **6** (3.84 ml, 8.51 g, 0.03 mol). The mixture was stirred at reflux for 36 h under nitrogen. After cooling to room temperature, the solution was filtered and concentrated to dryness. The residue was dissolved in ethyl acetate (100 mL) and washed with water (3 × 30 mL), dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (silica gel, hexanes : ethyl acetate 8 : 1 to 4 : 1) to give **7** (5.05 g, 17 mmol, 43%) as a brown solid. δ_H (300 MHz; $CDCl_3$; Me_4Si) 4.67 (2H, s, CH_2O), 7.14–7.43 (8H, m, aromatic).

.Methanesulfonic acid 3-(3-bromo-phenylsulfanyl)-benzyl ester. To a solution of **7** (6.67 g, 22.6 mmol) and Et_3N (6.3 ml, 4.57 g, 45.2 mmol) in dichloromethane (80 mL) was added dropwise a solution of methanesulfonyl chloride (2.63 ml, 3.89 g, 34.0 mmol) in dichloromethane (20 mL) at 0 °C over 30 min under nitrogen. The mixture was stirred at 0 °C for 3 h and the reaction was terminated by the addition of ice-cold water (30 mL). The organic layer was washed with ice-cold water (2 × 20 mL), dried ($MgSO_4$) and concentrated to give the product (8.32 g, 22.3 mmol, 99%) as a yellow solid, which was used directly in the next step. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.94 (3H, s, $MeSO_2$), 5.19 (2H, s, CH_2O), 7.17–7.44 (8H, m, aromatic).

[3-(3-Bromo-phenylsulfanyl)-benzyl]-bromide. To a solution of methanesulfonic acid 3-(3-bromo-phenylsulfanyl)-benzyl ester (1.2 g, 3.22 mmol) in DMF (20 mL) was added sodium bromide (0.40 g, 3.89 mmol). The solution was stirred for 4 h at room temperature under nitrogen. Then the solution was diluted with ethyl acetate (80 mL) and water (40 mL). The organic layer was washed with water (2 × 30 mL), dried ($MgSO_4$) and concentrated to give the product (1.14 g, 3.18 mmol, 99%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 4.44 (2H, s, CH_2Br) 7.16-7.45 (8H, m, aromatic).

3-[3-(3-Bromo-phenylsulfanyl)-benzyl]-3*H*-imidazole-4-carboxylic acid methyl ester (9). To a solution of [3-(3-bromo-phenylsulfanyl)-benzyl]-bromide (1.56 g, 4.36 mmol) in DMF (30 mL) was added methyl-4-imidazolecarboxylate **8** (0.6 g, 4.76 mmol) and K_2CO_3 (3.01 g, 21.8 mmol). The solution was stirred at room temperature overnight under nitrogen. The solution was diluted with ethyl acetate (100 mL), washed with water (3 × 30 mL), dried ($MgSO_4$), and concentrated. The residue was purified by the column chromatography (silica gel, hexanes : ethyl acetate 4 : 1 to 2 : 3) to give the pure product **9** (0.8 g, 2.0 mmol, 46%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.80 (3H, s, OMe), 5.49 (2H, s, CH_2), 7.05–7.40 (8H, m, aromatic), 7.66 (1H, s, imidazole), 7.76 (1H, s, imidazole). δ_C (75.6 MHz; $CDCl_3$; Me_4Si) 49.34, 51.41, 122.19, 122.89, 126.24, 129.01, 129.75, 129.82, 130.04, 130.88, 132.91, 135.39, 137.64, 137.73, 137.99, 142.06, 160.35.

(4-Iodophenyl)-methyl-carbamic acid *tert*-butyl ester. To a solution of (4-iodophenyl)-methylamine **11**[25] (0.99 g, 4.25 mmol) in dichloromethane (25 mL) was added triethylamine (1.2 ml, 0.86 g, 8.5 mmol), DMAP (0.052 g, 0.43 mmol) and di-*tert*-butyl dicarbonate (1.40 g, 6.4 mmol). The solution was stirred overnight at reflux under nitrogen. The solution was diluted with dichloromethane (40 mL), washed with aq. HCl (1 N, 2 × 20 mL), dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel (hexanes : ethyl acetate 8 : 1) to give (4-iodophenyl)-methyl-carbamic acid *tert*-butyl ester (1.18 g, 3.54 mmol, 83%) as a dark oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.44 (9H, s, $OCMe_3$), 3.23 (3H, s, NMe), 7.00 (2H, d, *J* 8.7, H2,6), 7.62 (2H, d, *J* 8.7, H3,5). δ_C (75.6 MHz; $CDCl_3$; Me_4Si) 28.19, 36.96, 77.42, 89.34, 127.19, 137.44, 143.48, 154.19.

Methyl-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl]-carbamic acid *tert*-butyl ester (12). To a mixture of bis(pinacolato)diboron (0.34 g, 1.34 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.029 g, 35.5 μmol) and potassium acetate (0.35 g, 3.57 mmol) was added a solution of (4-iodophenyl)-methyl-carbamic acid *tert*-butyl ester (0.4 g, 1.2 mmol) in DMSO (6 mL) under nitrogen. The solution was stirred at 80 °C for 3.5 h under nitrogen. After cooling to room temperature, the solution was diluted with ethyl acetate (40 mL), washed with water (4 × 20 mL), dried ($MgSO_4$) and concentrated. The resulting residue was purified by column chromatography on silica gel (hexanes : ethyl acetate 8 : 1) to give **12** (0.25 g, 7.51 mmol, 63%) as a yellow solid. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.34 (12H, s, CMe_2), 1.44 (9H, s, $OCMe_3$), 3.27 (3H, s, NMe), 7.24 (2H, d, *J* 8.7, H2,6), 7.76 (2H, d, *J* 8.7, H3,5).

3-{3-[4′-(*tert*-Butoxycarbonyl-methyl-amino)-biphenyl-3-yl-sulfanyl]-benzyl}-3*H*-imidazole-4-carboxylic acid methyl ester (13). To a mixture of **12** (0.86 g, 2.58 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.113 g, mmol) and K_2CO_3 (1.37 g, 6.21 mmol) was added a solution of **9** (0.8 g, 1.98 mmol) in dimethoxyethane (15 mL) under nitrogen. The solution was stirred at 80 °C for 24 h under nitrogen. After cooling to room temperature, the solution was concentrated to dryness. Ethyl acetate (80 mL) was added and the solution was washed with water (2 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated. The resulting residue was purified by column chromatography (silica gel, hexanes : ethyl acetate 4 : 1 to 2 : 3) to give **13** (0.66 g, 1.25 mmol, 63%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.47 (9H, s, $OCMe_3$), 3.28 (3H, s, NMe), 3.76 (3H, s, CO_2Me), 5.46 (2H, s, CH_2), 6.95–7.50 (12H, m,

aromatic), 7.60 (1H, s, imidazole), 7.74 (1H, s, imidazole). δ_C (75.6 MHz; $CDCl_3$; Me_4Si) 24.86, 28.15, 36.86, 83.28, 83.56, 124.13, 134.97, 146.32, 154.26.

3-{3-[4′-(*tert*-Butoxycarbonyl-methyl-amino)-biphenyl-3-ylsulfanyl]-benzyl}-3*H*-imidazole-4-carboxylic acid 14. To a solution of **13** (0.91 g, 1.72 mmol) in methanol (18 mL) was added a solution of NaOH (0.8 g, 0.02 mol) in water (2 mL). The solution was stirred at room temperature for 24 h. The solution was concentrated to dryness and water (20 mL) was added. Aq. HCl (1 N) was added dropwise until the pH reached 5. The mixture was extracted with dichloromethane (3 × 60 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give **14** (0.82 g, 1.60 mmol, 93%) as an orange oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.47 (9H, s, $OCMe_3$), 3.27 (3H, s, NMe), 5.50 (2H, s, CH_2), 7.00–7.50 (12H, m, aromatic), 7.74 (1H, s, imidazole), 7.78 (1H, s, imidazole).

3-[3-(4′-Methylamino-biphenyl-3-ylsulfanyl)-benzyl]-3*H*-imidazole-4-carboxylic acid methyl ester (15). To a solution of **13** (0.92 g, 1.74 mmol) in dichloromethane (6 mL) was added dropwise trifluoroacetic acid (1.25 mL, 0.85 g, 7.46 mmol). The solution was stirred for 2 h at room temperature. The reaction mixture was added dropwise to an aq. saturated $NaHCO_3$ solution (40 mL). The solution was extracted with dichloromethane (70 mL). The organic layer was washed with saturated aq. $NaHCO_3$ (2 × 30 mL), and brine (20 mL), dried ($MgSO_4$) and concentrated to give **15** (0.74 g, 1.72 mmol, 99%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.86 (3H, s, NMe), 3.76 (3H, s, CO_2Me), 5.45 (2H, s, CH_2), 6.66 (2H, d, *J* 8.4, aromatic *ortho* to N), 6.90–7.54 (10H, m, aromatic), 7.60 (1H, s, imidazole), 7.74 (1H, s, imidazole).

3-(3-{4′-[(3-{3-[4′-(*tert*-Butoxycarbonyl-methyl-amino)-biphenyl-3-ylsulfanyl]-benzyl}-3*H*-imidazole-4-carbonyl)-methyl-amino]-biphenyl-3-ylsulfanyl}-benzyl)-3*H*-imidazole-4-carboxylic acid methyl ester (16). To a solution of **14** (0.82 g, 1.60 mmol) in dichloromethane (10 mL) and toluene (10 mL) with 1 drop of dimethylformamide at 0 °C under nitrogen was added dropwise oxalyl chloride (1.2 mL, 0.83 g, 6.5 mmol). The solution was stirred for 1 h at 0 °C under nitrogen and the reaction mixture was concentrated to dryness. Anhydrous dichloromethane (5 mL) was added and the resulting solution was added dropwise to a solution of **15** (0.74 g, 1.72 mmol) in pyridine (5 mL) at 0 °C under nitrogen. The reaction mixture was allowed to warm to room temperature over 0.5 h and was stirred overnight at room temperature under nitrogen. The mixture was then concentrated to dryness. The resulting residue was dissolved in dichloromethane (60 mL), washed with aqueous saturated $NaHCO_3$ (2 × 20 mL), brine (20 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography on silica gel (hexanes : ethyl acetate 1 : 4 to ethyl acetate : methanol 18 : 1) to give **16** (0.94 g, 1.0 mmol, 63%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.45 (9H, s, $OCMe_3$), 3.25 (3H, s, NMe), 3.31 (3H, s, NMe), 3.76 (3H, s, CO_2Me), 5.45 (2H, s, CH_2), 5.49 (2H, s, CH_2), 6.36 (1H, s, imidazole), 6.87 (2H, d, *J* 8.1, aromatic *ortho* to amide N), 6.90–7.74 (25H, m, aromatic, 3H from imidazole).

3-(3-{4′-[(3-{3-[4′-(*tert*-Butoxycarbonyl-methyl-amino)-biphenyl-3-ylsulfanyl]-benzyl}-3*H*-imidazole-4-carbonyl)-methyl-amino]-biphenyl-3-ylsulfanyl}-benzyl)-3*H*-imidazole-4-carboxylic acid (18). The procedure for the synthesis of **14** was followed to give **18** (94%). δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.47 (9H, s, $OCMe_3$), 3.26 (3H, s, NMe), 3.34 (3H, s, NMe), 5.47 (2H, s, CH_2), 5.52 (2H, s, CH_2), 6.13 (1H, s, imidazole), 6.70–7.75 (27H, m, aromatic, 3H from imidazole).

3-{3-[4′-(Methyl-{3-[3-(4′-methylamino-biphenyl-3-ylsulfanyl)-benzyl]-3*H*-imidazole-4-carbonyl}-amino)-biphenyl-3-ylsulfanyl]-benzyl}-3*H*-imidazole-4-carboxylic acid methyl ester (19). The procedure for the synthesis of **15** was followed to give **19** (99%). δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.82 (3H, s, amine NMe), 3.32 (3H, s, amide NMe), 3.77 (3H, s, CO_2Me), 5.45 (2H, s, CH_2), 5.49 (2H, s, CH_2), 6.39 (1H, s, imidazole), 6.60 (2H, d, *J* 8.7, aromatic *ortho* to amine N), 6.87 (2H, d, *J* 8.7, aromatic *ortho* to amide N), 6.90–7.80 (23H, m, aromatic, 3H from imidazole).

Linear tetramer (17). The procedure for the synthesis of **16** was followed, but the product was purified by column chromatography on silica gel (ethyl acetate : methanol : Et_3N, 19 : 1 : 1) to give **17** (60%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.46 (9H, s, $OCMe_3$), 3.24 (3H, s, NMe), 3.28 (3H, s, NMe), 3.29 (3H, s, NMe), 3.31 (3H, s, NMe), 3.77 (3H, s, CO_2Me). 5.44 (2H, s, CH_2), 5.49 (6H, s, 3 unresolved CH_2), 6.36 (2H, s, unresolved imidazole H), 6.38 (1H, s, imidazole) 6.86–6.91 (6H, m, aromatic *ortho* to amide N), 6.94–7.74 (47H, m, aromatic, 7H from imidazole). δ_C (75.6 MHz; $CDCl_3$; Me_4Si) 28.24, 37.08, 37.81, 37.84, 37.86, 45.27, 49.50, 49.72, 51.38, 80.38, 122.24, 125.00, 125.46, 125.65, 126.05, 126.19, 126.23, 126.28, 127.02, 127.27, 128.12, 128.84, 129.35, 129.39, 129.56, 129.62, 129.68, 129.72, 129.86, 129.93, 130.08, 130.12, 130.17, 130.20, 130.51, 130.54, 130.72, 134.22, 135.27, 135.36, 135.42, 135.46, 136.77, 136.80, 136.83, 136.92, 137.35, 137.81, 137.86, 139.44, 139.49, 139.60, 140.82, 140.86, 141.65, 142.05, 143.33, 143.49, 154.49, 160.35, 160.65.

Cyclic tetramer (4). To a solution of **17** (0.124 g, 72.0 μmol) in dioxane (2 mL) was added dropwise a solution of HCl in dioxane (4 M, 2 mL). The solution was stirred overnight at room temperature. The reaction was quenched by the addition of triethylamine (2 mL). The resulting mixture was concentrated to dryness and then dissolved in dichloromethane (40 mL). The solution was washed with saturated $NaHCO_3$ (2 × 20 mL), dried ($MgSO_4$) and concentrated to give the free amine of **17** (0.117 g, 72.1 μmol, 99%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.83 (3H, s, amine NMe), 3.29–3.33 (9H, 3s, amide NMe), 3.77 (3H, s, OMe), 5.47 (8H, 4s, CH_2), 6.36 (3H, 3s, imidazole), 6.60 (2H, d, *J* 8.7, aromatic *ortho* to NHMe), 6.82–6.91 (6H, m, aromatic *ortho* to amide NMe), 6.92–7.80 (45H, m, aromatic). The amine (0.117 g, 72.1 μmol) was dissolved in methanol (5 mL) and THF (8 mL), and a solution of NaOH (52.6 mg, 1.32 mmol) in water (1 mL) was added. The solution was stirred for 2 days at room temperature. The mixture was concentrated to dryness and dissolved in water (15 mL). An aq. solution of HCl (1 N) was added dropwise until the pH reached 6. The mixture was extracted with dichloromethane (3 × 50 mL). The combined organic

layers were dried ($MgSO_4$) and concentrated to give the fully deprotected linear tetramer (0.1 g, 62.2 μmol, 86%) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.82 (3H, s, amine NMe), 3.28–3.33 (9H, 3s, amide NMe), 5.44–5.50 (8H, 4s, CH_2), 6.26 (1H, s, imidazole), 6.36 (2H, s, imidazole), 6.60 (2H, d, *J* 8.1), 6.61–7.80 (51 H, m, aromatic). A portion of this product (0.0475 g, 29.5 μmol) was dissolved in dichloromethane (60 mL) and triphenylphosphine dichloride (0.06 g, 18.0 mmol) was added. The solution was stirred for 3 days at reflux under nitrogen. After cooling to room temperature, the solution was washed with saturated aq. $NaHCO_3$ (20 mL), dried ($MgSO_4$) and concentrated. The resulting residue was purified by column chromatography (ethyl acetate : methanol : Et_3N, 18 : 1 : 1) to give **4** (0.031 g, 19.5 μmol, 66% for the final step) as a brown oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.26 (12H, s, NMe), 5.47 (8H, s, CH_2), 6.36 (4H, s, imidazole), 6.79 (8H, d, *J* 8.7, aromatic *ortho* to N), 7.00–7.60 (44H, m, aromatic). *m*/*z* (ES) 1589.46 (MH^+. $C_{96}H_{77}N_{12}O_4S_4$ requires 1589.51).

Fluorescence titrations

The fluorescence titrations were performed using an Aminco-Bowman Series2 Spectrometer at 20 °C. The emission spectrum was scanned from 325–390 nm with excitation at 290 nm. Spectra were obtained for the solution of **4** (3.26×10^{-5} M, 3.1 mL) in 1 : 1 methanol : tetrahydrofuran and after two successive additions of 9 μL and six additions of 10 μL of a 2.65×10^{-3} M $CuCl_2$ solution and of 10 μL of a 7.04×10^{-3} M $CuCl_2$ solution, all in 1 : 1 methanol : tetrahydrofuran. For data fitting, the fluorescence intensity at 357 nm was used. $NiCl_2$ solution (17.4 mM) was added in six 5 μL portions followed by five 10 μL portions to a solution of **4** (3.0 mL, 32.6 μM) in 1 : 1 methanol : tetrahydrofuran. $CoCl_2$ titrations were performed by addition to a solution of **4** (2.09×10^{-5} M, 2.9 mL) of 1.04×10^{-3} M $CoCl_2$ solution (6×20 μL, 4×40 μL) followed by 1.04×10^{-2} M $CoCl_2$ solution (2×10 μL, 2×20 μL). Zinc triflate titrations were performed by addition to a solution of **4** (2.09×10^{-5} M, 3.0 mL) of 8.6×10^{-3} M $Zn(OTf)_2$ solution (6×5 μL, 3×10 μL, 20 μL).

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