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Copper, Nickel, and Zinc Cyclam–Amino Acid and Cyclam–Peptide Complexes May Be Synthesized with "Click" Chemistry and Are Noncytotoxic

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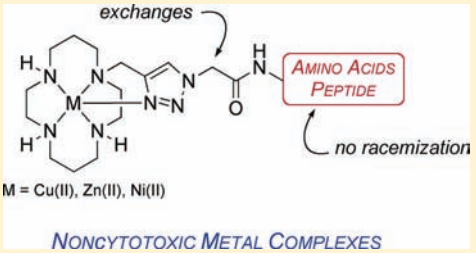
Supporting Information

ABSTRACT: We describe the synthesis of cyclam metal complexes derivatized with amino acids or a tripeptide using a copper(I)-catalyzed Huisgen "click" reaction. The linker triazole formed during the synthesis plays an active coordinating role in the complexes. The reaction conditions do not racemize the amino acid stereocenters. However, a methylene group adjacent to the triazole is susceptible to H/D exchange under ambient conditions, an observation which has potentially important implications for structures involving stereocenters adjacent to triazoles in click-derived structures. The successful incorporation of several amino acids is described, including reactive tryptophan and cysteine side chains. All complexes are formed rapidly upon introduction of the relevant metal salt, including synthetically convenient cases where trifluoroacetate salts of cyclam derivatives are used directly in the metalation. None of the metal complexes displayed any cytotoxicity to mammalian cells, suggesting that the attachment of such complexes to amino acids and peptides does not induce toxicity, further supporting their potential suitability for labeling/imaging studies. One Cu(II)–cyclam–triazole–cysteine disulfide complex displayed moderate activity against MCF-10A breast nontumorigenic epithelial cells.



INTRODUCTION

The combination of metal complexes and amino acids or peptides holds significant promise for a variety of biological and medical applications.[1−7] There have been a few recent examples exploiting the copper(I)-catalyzed azide–alkyne Huisgen cycloaddition (a "click" reaction) to unite peptides and metal complexes.[8−17] The advantages of such an approach are the compatibility of the reagents with biologically relevant motifs and the potential role the resultant triazole can play in the coordination chemistry of the final complex. Peptides frequently engage in highly specific interactions with biological molecules, trafficking metal complexes that may be labeled with a metal ion that may be monitored (e.g., via magnetic resonance imaging or positron emission tomography). For metal complexes to be used in this way, it is usually desirable for the complex to be inert, and for the peptide to act simply as a targeting agent.[18−22] To date, there are few examples in which the peptide is anything more than this;[23] it would greatly broaden the scope of potential therapeutic applications if the metal complex could be *chemically active* (as opposed to radioactive) and taken to its site of action by the peptide.

We have shown that a click-derived metal complex, containing a pendant biotin motif, undergoes a change in primary coordination geometry upon binding to the cognate biomolecule avidin.[24] This result implies that the act of biomolecule/ligand binding could trigger activation of a metal complex *in vivo*. To explore this striking possibility requires, as a first step, the synthesis of a broader range of derivatized metal complexes containing the pendant triazole and biological ligands. We here report the synthesis of amino-acid- and peptide-derived cyclam complexes *via* click chemistry. Our results indicate that such conjugates may be assembled easily and in high yields and that this approach offers an attractive complement to more traditional methods of conjugating metal complexes to peptides that rely on the formation of single covalent bonds (creating, for example, amides,[22] oximes,[16] or maleimides[25]), coordination events that join peptide and ligand moieties[26] or coordination events of metals into suitably functionalized amino acid residues.[27]

EXPERIMENTAL SECTION

General Materials. All reactions except solid phase peptide synthesis were carried out in ordinary glassware. Solid phase peptide synthesis was performed in 10 mL polypropylene syringes with filters, purchased from Torviq. All reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, Merck, Mimotopes, GL Biochem, or Ajax Finechem. Wang resin and HMBA-AM resin were purchased from

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Novabiochem. Reagents were used as received unless otherwise specified. Hexane and ethyl acetate were distilled before use; dichloromethane and methanol were distilled over calcium hydride.

Instrumentation and Methods. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 300 K on a Bruker AVANCE 200 spectrometer (^{1}H at 200.13 MHz and ^{13}C at 50.32 MHz), a Bruker AVANCE 300 spectrometer (^{1}H at 300.13 MHz and ^{13}C at 75.47 MHz), or a Bruker DRX 400 spectrometer (^{1}H at 400.13 MHz and ^{13}C at 100.61 MHz). ^{1}H and ^{13}C NMR spectra are referenced to solvent signals or (for some ^{1}H NMR spectra) tetramethylsilane. ^{1}H NMR signals are reported with chemical shift values δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad), relative integral, coupling constants J (Hz), and assignments. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer. Ultraviolet−visible spectra were recorded on a Cary 4000 or Cary 1E UV−visible spectrophotometer. Low-resolution and high-resolution mass spectra were recorded on a Finnigan LCQ mass spectrometer and a Bruker 7T Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer, respectively. Ionization of samples was carried out using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Optical rotation α was measured on a PerkinElmer 341 polarimeter with a sodium lamp in a semimicro fused silica polarimeter cell (length, 100 mm; capacity, 3.0 mL) at 20 °C unless otherwise stated. Melting points were determined on an OptiMelt 100 automated melting point apparatus. Elemental analyses were carried out by the Campbell Microanalytical Laboratory (University of Otago, New Zealand) on a Carlo Erba EA 1108 elemental analyzer. Analytic reverse phase high-performance liquid chromatography (RP-HPLC) was carried out on a Waters 2695 separations module with a Waters 2996 photodiode array detector and an Alliance series column heater. A Waters SunFire C18 column (5 μm, 2.1 × 150 mm) was used at 30 °C at a flow rate of 0.2 mL/min. Preparative RP-HPLC was carried out on a Waters 600 controller with a Waters 600 pump and a 2998 photodiode array detector. A Waters SunFire C18 OBD column (5 μm, 19 × 150 mm) was used at a flow rate of 7 mL/min. Mobile phases of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) in different ratios were used in both analytic and preparative HPLC. Enantioselective normal phase HPLC was performed on a Waters 510 HPLC pump with a 2487 dual λ absorbance detector and a Waters 410 differential refractometer. A Daicel 19325 CHIRALPAK AD-H analytic column (5 μm, 4.6 × 250 mm) was eluted with 10% isopropanol/hexane at a flow rate of 0.5 mL/min. Data acquired from both analytic and enantioselective HPLC were processed using Waters Empower 2 software. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} precoated aluminum plates (0.2 mm) and visualized with UV irradiation (254 nm), with ninhydrin or vanillin staining. Flash column chromatography was carried out using Merck silica gel 60 (SiO_2, 0.040−0.063 mm).

General Synthetic Procedure A: The Cu^I-Catalyzed Huisgen 1,3-Dipolar Cycloaddition of Azides and Alkynes.[24,28] Alkyne (1.00 equiv) and azide (1.00 equiv) were dissolved in H_2O/*t*-BuOH (1:1, 50 mM in alkyne or azide). A brown cloudy solution of $CuSO_4{\cdot}5H_2O$ (0.05 equiv, 5 mol %) and sodium ascorbate (0.10 equiv, 10 mol %) in H_2O (25 mM in copper) was added. The reaction mixture was stirred at room temperature for 12 h, quenched with 5% $NaHCO_3$ solution (100 mL/mol copper), and extracted with DCM (3×). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure and the residue purified by flash column chromatography (silica gel, EtOAc unless otherwise stated) to give the desired triazole.

General Synthetic Procedure B: TFA-Mediated Boc Removal. Boc-protected amine (1.0 equiv) was dissolved in a mixture of TFA/DCM/H_2O (90:5:5, 5 mM). The reaction mixture was stirred at room temperature for 6 h and concentrated under reduced pressure to give the desired trifluoroacetate.

General Synthetic Procedure C: Basification of Trifluoroacetates.

(i) Trifluoroacetate was dissolved in H_2O (0.5 mL), basified with 5 M KOH or 2 M NaOH to pH 11−12, and extracted with $CHCl_3$ (3×). The combined organic extracts were washed with H_2O (0.5 mL) and concentrated under reduced pressure to give the desired *N*-functionalized cyclam.

(ii) Basification of trifluoroacetates was achieved by employing the above procedure C(i), but using saturated Na_2CO_3 in place of 5 M KOH or 2 M NaOH.

General Synthetic Procedure D: Metal Complexation.[29]

(i) A solution of $M(ClO_4)_2{\cdot}6H_2O$ (M = Cu, Ni, Zn; 1.0 equiv) in EtOH (0.1 M) was added dropwise to a solution of *N*-functionalized cyclam (1.0 equiv) in EtOH (0.1 M) at room temperature. The reaction mixture was heated at reflux for 1 h and cooled in an ice bath, and the solvent was decanted. The remaining solid residue was washed with ice-cold EtOH (3×) and diethyl ether (3×) and dried *in vacuo* to give the desired metal complex.

(ii) Metal complexation was achieved by employing the above procedure D(i) but using *N*-functionalized cyclam trifluoroacetate in place of *N*-functionalized cyclam and an extended reaction time (3 h).

(iii) Metal complexation was achieved by employing the above procedure D(i) but using *N*-functionalized cyclam trifluoroacetate in place of *N*-functionalized cyclam, an extended reaction time (6 h), and a centrifuge to isolate the metal complex from the suspension.

CAUTION! *Perchlorate salts of metal complexes with organic ligands are potentially explosive and should be handled with care. Only small amounts of material should be prepared.*

Tri-*tert*-butyl-11-((1-(2-(benzylamino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (5). Propargyl-tri-Boc-cyclam (**4**, 516 mg, 0.958 mmol) and 2-azido-*N*-benzylacetamide (**3**, 183 mg, 0.962 mmol) were reacted using general synthetic procedure A to give **5** as a white foam (588 mg, 84%). R_F (EtOAc): 0.42. IR: ν_{max}/cm^{-1} 3310, 2973, 2930, 1682, 1549, 1455, 1411, 1364, 1241, 1156, 772, 698. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, $C(CH_3)_3$), 1.46 (s, 18H, 2 × $C(CH_3)_3$), 1.60−1.75 (m, 2H, $CH_2CH_2CH_2$), 1.87 (br s, 2H, $CH_2CH_2CH_2$), 2.42 (m, 2H, $CH_2N(CH_2$-triazole$)CH_2$), 2.60 (m, 2H, $CH_2N(CH_2$-triazole$)CH_2$), 3.20−3.45 (br m, 12H, 3 × CH_2C-(Boc)CH_2), 3.78 (s, 2H, NCH_2-triazole), 4.43 (d, 2H, J 5.7, $CONHCH_2Ph$), 5.08 (s, 2H, triazole-CH_2CONH), 6.96 (br s, 1H, CONH), 7.20−7.33 (m, 5H, Ph-H), 7.64 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.6, 28.6, 43.7, 45.5, 46.9, 47.2, 47.5, 51.3, 52.9, 79.7, 124.5, 127.7, 127.8, 128.8, 137.5, 144.1, 155.6, 155.9, 165.2. MS (ESI): m/z 729.1 ([M + H]$^+$, 100%), 751.1 ([M + Na]$^+$, 48%), 1163.8 (36%), 1457.1 ([2M + H]$^+$, 20%), 1479.2 ([2M + Na]$^+$, 62%), 1496.2 ([2M+K]$^+$, 14%). HRMS (ESI): 751.44886 ([M + Na]$^+$). Calcd for $C_{37}H_{60}N_8O_7Na$ ([M + Na]$^+$): 751.44772.

11-((1-(2-(Benzylamino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-11-aza-1,4,8-triazoniacyclotetradecane-1,4,8-triium-2,2,2-trifluoroacetate (6). Compound **5** (148 mg, 0.203 mmol) was deprotected using general synthetic procedure B to give **6** as a colorless glue (156 mg, 100%). IR: ν_{max}/cm^{-1} 3296, 3032, 2852, 1778, 1666, 1562, 1454, 1430, 1128, 1059, 1028, 837, 798, 721, 702. ^{1}H NMR (300 MHz, D_2O): δ 2.02−2.15 (m, 4H, 2 × $CH_2CH_2CH_2$), 3.04−3.49 (m, 16H, 2 × $CH_2CH_2CH_2$ and 2 × NCH_2CH_2N), 4.21 (s, 2H, NCH_2-triazole), 4.34 (s, 2H, $CONHCH_2Ph$), 5.23 (s, 2H, triazole-CH_2CONH), 7.22−7.34 (m, 5H, Ph-H), 8.07 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, D_2O): δ 20.0, 20.3, 39.2, 40.2, 42.5, 43.0, 43.6, 47.2, 47.9, 50.0, 52.5, 110.8, 114.7, 118.6, 122.4, 127.7, 127.9, 128.1, 129.2, 137.8, 139.9, 162.3, 162.7, 163.2, 163.7, 167.6. MS (ESI) m/z 429.1 ([M − 3TFA + H]$^+$, 100%). HRMS (ESI): 429.30815 ([M − 3TFA + H]$^+$). Calcd for $C_{22}H_{37}N_8O$ ([M − 3TFA + H]$^+$): 429.30848.

2-(4-((1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-benzylacetamide (7). Compound **6** (156 mg, 0.202 mmol) was subjected to general synthetic procedure C(i) to give **7** as a colorless glue (87 mg, 100%). IR: ν_{max}/cm^{-1} 3277, 2928,

2885, 2815, 1674, 1556, 1454, 724, 697. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.52 (m, 2H, $CH_2CH_2CH_2$), 1.73 (m, 2H, $CH_2CH_2CH_2$), 2.43–2.65 (br m, 16H, 2 × $CH_2CH_2CH_2$ and 2 × NCH_2CH_2N), 2.81 (br s, 3H, 3 × CH_2NHCH_2), 3.64 (s, 2H, NCH_2-triazole), 4.29 (s, 2H, $CONHCH_2Ph$), 4.87 (s, 2H, triazole-CH_2CONH), 7.10–7.25 (m, 5H, Ph-H), 7.77 (s, 1H, triazole-H), 8.11 (br s, 1H, CONH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1, 28.7, 43.6, 47.2, 47.7, 48.0, 48.7, 49.5, 49.8, 51.2, 52.6, 53.9, 54.8, 124.4, 127.4, 127.6, 128.6, 137.8, 145.6, 165.5. MS (ESI): m/z 429.1 ($[M + H]^+$, 100%). HRMS (ESI): 429.30812 ($[M + H]^+$). Calcd for $C_{22}H_{37}N_8O$ ($[M + H]^+$): 429.30848.

[Cu(7)](ClO$_4$)$_2$ Complex (8). Compound **7** (69 mg, 0.16 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (60 mg, 0.16 mmol) were reacted according to general synthetic procedure D(i) to give **8** as a purple powder (96 mg, 86%). m.p.: 104–109 °C. UV–vis (CH_3OH): λ_{max}/nm 574, ε 135. IR: ν_{max}/cm^{-1} 3594, 3368, 3238, 2941, 2882, 1677, 1543, 1454, 1429, 1365, 1232, 1065, 622. HRMS (ESI): 590.17822, 591.18199, 592.17598, 593.17960, 594.17376, 595.17728 ($[M - ClO_4]^+$). Calcd for $C_{22}H_{36}ClCuN_8O_5$ ($[M - ClO_4]^+$) 590.17877, 591.18213, 592.17684, 593.18029, 594.17402, 595.17737. Anal. Calcd for $C_{22}H_{36}Cl_2CuN_8O_9$: C, 38.24; H, 5.25; N, 16.22. Found: C, 37.98; H, 5.30; N, 16.44.

[Cu(7)](PF$_6$)$_2$ Complex (9). To a solution of compound **8** (96 mg, 0.14 mmol) in CH_3OH (5 mL) was added dropwise excess saturated KPF_6 aqueous solution. The mixture was allowed to evaporate slowly to give **9** as a dark blue crystal (72 mg, 66%). m.p.: 232–235 °C. UV–vis (CH_3OH): λ_{max}/nm 597, ε 228. IR: ν_{max}/cm^{-1} 3427, 3278, 2939, 2885, 1681, 1542, 1455, 1430, 1366, 1230, 1098, 1066, 1040, 835, 558. HRMS (ESI): 636.19564 ($[M - PF_6]^+$). Calcd for $C_{22}H_{36}CuF_6N_8OP$ ($[M - PF_6]^+$): 636.19444. Anal. Calcd for $C_{22}H_{36}CuF_{12}N_8OP_2{\cdot}0.5KPF_6$: C, 30.23; H, 4.15; N, 12.82. Found: C, 30.45; H, 4.10; N, 12.82.

CCDC 840171 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

[Ni(7)](ClO$_4$)$_2$ Complex. Compound **7** (152 mg, 0.355 mmol) and $Ni(ClO_4)_2{\cdot}6H_2O$ (130 mg, 0.355 mmol) were reacted according to general synthetic procedure D(i) to give the $[Ni(7)](ClO_4)_2$ complex as a pink powder (151 mg, 62%). m.p.: 162–167 °C. UV–vis (CH_3OH): λ_{max}/nm 524, ε 13. IR: ν_{max}/cm^{-1} 3366, 3269, 2930, 2875, 1678, 1548, 1455, 1432, 1364, 1247, 1228, 1076, 622. HRMS (ESI): 585.18417, 586.18777, 587.18018, 588.18369, 589.17691, 590.17996, 591.17744 ($[M - ClO_4]^+$). Calcd for $C_{22}H_{36}ClN_8NiO_5$ ($[M - ClO_4]^+$): 585.18452, 586.18788, 587.18014, 588.18334, 589.17702, 590.18036, 591.17458. Anal. Calcd for $C_{22}H_{36}Cl_2N_8NiO_9$: C, 38.51; H, 5.29; N, 16.33. Found: C, 38.44; H, 5.51; N, 15.98.

[Zn(7)](ClO$_4$)$_2$ Complex. Compound **7** (89 mg, 0.21 mmol) and $Zn(ClO_4)_2{\cdot}6H_2O$ (78 mg, 0.21 mmol) were reacted according to general synthetic procedure D(i) to give the $[Zn(7)](ClO_4)_2$ complex as a white powder (102 mg, 71%). m.p.: 124–129 °C. IR: ν_{max}/cm^{-1} 3528, 3370, 3267, 2935, 2880, 1669, 1552, 1455, 1429, 1358, 1295, 1247, 1078, 623. HRMS (ESI): 591.17815, 592.18152, 593.17476, 594.17787, 595.17333, 596.17610, 597.17136, 598.17449 ($[M - ClO_4]^+$). Calcd for $C_{22}H_{36}ClN_8O_5Zn$ ($[M - ClO_4]^+$): 591.17832, 592.18168, 593.17523, 594.17852, 595.17393, 596.17732, 597.17108, 598.17443. Anal. Calcd for $C_{22}H_{36}Cl_2N_8O_9Zn$: C, 38.14; H, 5.24; N, 16.17. Found: C, 37.80; H, 5.30; N, 15.92.

(*S*)-Tri-*tert*-butyl-11-((1-(2-((1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-2-oxo-ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (10i). Propargyl-tri-Boc cyclam **4** (1.08 g, 2.00 mmol) and (*S*)-methyl 2-(2-azidoacetamido)-3-phenylpropanoate (**SI-4**, 0.56 g, 2.14 mmol) were reacted using general synthetic procedure A to give **10i** as a white foam (1.47 g, 92%). R_F (EtOAc): 0.44. $[\alpha]_D^{20}$: +18.0 (*c* 0.60, $CHCl_3$). IR: ν_{max}/cm^{-1} 3303, 2976, 2933, 1746, 1682, 1538, 1463, 1412, 1365, 1244, 1158, 732, 700. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H, $C(CH_3)_3$), 1.46 (s, 18H, 2 × $C(CH_3)_3$), 1.65–1.80 (m, 2H, $CH_2CH_2CH_2$), 1.90 (m, 2H, $CH_2CH_2CH_2$), 2.44 (m, 2H, CH_2N-(CH_2-triazole)CH_2), 2.62 (m, 2H $CH_2N(CH_2$-triazole)CH_2), 3.04 (dd, 1H, *J* 14.1 and 6.6, $CHHPh$), 3.13 (dd, 1H, *J* 13.8 and 5.7, $CHHPh$), 3.34 (m, 12H, 3 × $CH_2C(Boc)CH_2$), 3.71 (s, 3H, $COOCH_3$), 3.81 (s, 2H, NCH_2-triazole), 4.83 (dd, 1H, *J* 13.8 and 6.3, $CONHCH$), 5.02 (s, 2H, triazole-CH_2CONH), 6.52 (br s, 1H, CONH), 7.01–7.04 (m, 2H, Ph-H), 7.20–7.30 (m, 3H, Ph-H), 7.54 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.7, 28.6, 37.7, 45.5, 46.9, 47.2, 47.6, 48.6, 51.2, 52.6, 52.8, 53.5, 79.7, 124.3, 127.4, 128.8, 129.2, 135.4, 144.2, 155.6, 155.9, 164.9, 171.3. MS (ESI): m/z 801.1 ($[M + H]^+$, 100%), 823.3 ($[M + Na]^+$, 69%), 1623.3 ($[2M + Na]^+$, 23%). HRMS (ESI): 801.48798 ($[M + H]^+$). Calcd for $C_{40}H_{65}N_8O_9$ ($[M + H]^+$): 801.48690.

(*S*)-Tri-*tert*-butyl-11-((1-(2-((1-methoxy-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (10ii). Propargyl-tri-Boc cyclam **4** (1.355 g, 2.515 mmol) and (*S*)-methyl 2-(2-azidoacetamido)-3-methylbutanoate (**SI-5**, 0.539 g, 2.516 mmol) were reacted using general synthetic procedure A to give **10ii** as a white foam (1.696 g, 90%). R_F (EtOAc): 0.24. $[\alpha]_D^{20}$: +5.2 (*c* 1.0, $CHCl_3$). IR: ν_{max}/cm^{-1} 3318, 2974, 2933, 1744, 1677, 1542, 1465, 1412, 1365, 1243, 1152. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (d, 3H, *J* 6.9, CH_3CHCH_3), 0.90 (d, 3H, *J* 6.9, CH_3CHCH_3), 1.44 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.73 (m, 2H, $CH_2CH_2CH_2$), 1.91 (m, 2H, $CH_2CH_2CH_2$), 2.12–2.20 (m, 1H, $CH(CH_3)_2$), 2.46 (m, 2H, $CH_2N(CH_2$-triazole)CH_2), 2.64 (m, 2H, $CH_2N(CH_2$-triazole)-CH_2), 3.35 (m, 12H, 3 × $CH_2C(Boc)CH_2$), 3.73 (s, 3H, $COOCH_3$), 3.85 (s, 2H, NCH_2-triazole), 4.52 (dd, 1H, *J* 8.7 and 5.1, $CONHCH$), 5.14 (s, 2H, triazole-CH_2CONH), 6.84 (br s, 1H, CONH), 7.67 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8, 18.9, 26.6, 28.5, 31.1, 45.4, 46.8, 47.1, 47.5, 51.1, 52.3, 52.7, 57.6 79.6, 124.4, 143.9, 155.6, 155.7, 155.8, 165.3, 171.8. MS (ESI): m/z 753.6 ($[M + H]^+$, 100%), 775.6 ($[M + Na]^+$, 34%). HRMS (ESI): 753.48753 ($[M + H]^+$). Calcd for $C_{36}H_{65}N_8O_9$ ($[M + H]^+$) 753.48690.

(*S*)-Tri-*tert*-butyl-11-((1-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (10iii). Propargyl-tri-Boc cyclam **4** (1.077 g, 1.999 mmol) and (*S*)-methyl 2-(2-azidoacetamido)-3-(1*H*-indol-3-yl)propanoate (**SI-6**, 0.602 g, 1.998 mmol) were reacted using general synthetic procedure A to give **10iii** as a white foam (1.510 g, 90%). R_F (EtOAc): 0.44. $[\alpha]_D^{20}$: +14.4 (*c* 1.0, $CHCl_3$). IR: ν_{max}/cm^{-1} 3311, 2975, 2934, 1744, 1671, 1540, 1462, 1415, 1365, 1242, 1158, 734. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 27H, 3 × $C(CH_3)_3$), 1.60–1.75 (m, 2H, $CH_2CH_2CH_2$), 1.90 (br s, 2H, $CH_2CH_2CH_2$), 2.22 (br s, 2H, $CH_2N(CH_2$-triazole)CH_2), 2.51 (br s, 2H, $CH_2N(CH_2$-triazole)CH_2), 3.21–3.50 (br m, 14H, 3 × $CH_2C(Boc)CH_2$ and CH_2-indole), 3.70 (m, 5H, $COOCH_3$ and NCH_2-triazole), 4.85–5.09 (m, 3H, $CONHCH$ and triazole-CH_2CONH), 6.48 (br s, 1H, CONH), 6.83 (br s, 2H, indole-H and triazole-H), 7.05–7.18 (m, 2H, indole-H), 7.34 (d, 1H, *J* 7.8, indole-H), 7.48 (d, 1H, *J* 7.8, indole-H), 9.77 (br s, 1H, indole-NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.9, 28.6, 45.5, 47.0, 47.4, 50.3, 52.2, 52.6, 53.1 79.7, 108.7, 111.7, 118.3, 119.4, 121.9, 123.2, 124.0, 127.7, 136.4, 143.2, 155.7, 156.1, 165.2, 171.6. MS (ESI): m/z 840.0 ($[M + H]^+$, 100%), 862.0 ($[M + Na]^+$, 20%). HRMS (ESI): 840.49721 ($[M + H]^+$). Calcd for $C_{42}H_{66}N_9O_9$ ($[M + H]^+$): 840.49780.

(*S*)-Methyl-2-(2-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)-methyl)-1*H*-1,2,3-triazol-1-yl)-acetamido)-3-phenylpropanoate (12i). Compound **10i** (160 mg, 0.200 mmol) was deprotected using general synthetic procedure B, followed by basification using general synthetic procedure C(ii) to give **12i** as a colorless glue (94 mg, 94%). $[\alpha]_D^{20}$: +12.5 (*c* 1.0, CH_3OH). IR: ν_{max}/cm^{-1} 3282, 3192, 3030, 2948, 2817, 1744, 1683, 1553, 1454, 730, 700. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60–1.75 (m, 2H, $CH_2CH_2CH_2$), 1.85 (m, 2H, $CH_2CH_2CH_2$), 2.50–2.65 (m, 4H, $CH_2N(CH_2$-triazole)CH_2), 2.65–2.95 (m, 12H, 3 × CH_2NHCH_2), 3.02–3.19 (m, 2H, CH_2Ph), 3.65 (s, 3H, $COOCH_3$), 3.72 (s, 2H, NCH_2-triazole), 4.42 (br s, 3H, 3 × NH), 4.70–4.76 (m, 1H, $CONHCH$), 5.11 (ABq, 2H, *J* 16.1, $\Delta\nu$ 12.9, triazole-CH_2CONH), 7.13–7.28 (m, 5H, Ph-H), 7.71 (s, 1H, triazole-H), 8.12 (br s, 1H, CONH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.3, 27.1, 37.3, 46.7, 46.8, 47.5, 48.1, 48.8, 49.1, 50.4, 52.2, 52.3, 53.5, 53.7,

53.9, 124.2, 126.8, 128.4, 129.1, 136.2, 144.7, 165.5, 171.5. MS (ESI): m/z 501.4 ([M + H]$^+$, 100%), 513.4 (18%). HRMS (ESI): 501.32926 ([M + H]$^+$). Calcd for $C_{25}H_{41}N_8O_3$ ([M + H]$^+$): 501.32961.

(*S*)-Methyl-2-(2-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)-methyl)-1*H*-1,2,3-triazol-1-yl)-acetamido)-3-methylbutanoate (12ii). Compound **10ii** (150 mg, 0.199 mmol) was deprotected using general synthetic procedure B, followed by basification using general synthetic procedure C(ii) to give **12ii** as a colorless glue (65 mg, 72%). $[\alpha]_D^{20}$: −18.0 (c 0.82, CH_3OH). IR: ν_{max}/cm^{-1} 3262, 3212, 3040, 2953, 2932, 2818, 1742, 1683, 1548, 1463, 1435, 1373, 1336, 1270, 1206, 1148, 1051, 729. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, 3H, J 6.9, CH_3CHCH_3), 0.92 (d, 3H, J 7.2, CH_3CHCH_3), 1.70 (m, 2H, $CH_2CH_2CH_2$), 1.87 (m, 2H, $CH_2CH_2CH_2$), 2.10−2.21 (m, 1H, $CH(CH_3)_2$), 2.56 (m, 4H, $CH_2N(CH_2$-triazole$)CH_2$), 2.65−2.85 (br m, 12H, 3 × CH_2NHCH_2), 3.17 (br s, 3H, 3 × NH), 3.72 (s, 3H, $COOCH_3$), 3.82 (s, 2H, NCH_2-triazole), 4.48 (d, 1H, J 4.5, CONHCH), 5.17 (s, 2H, triazole-CH_2CONH), 7.70 (br s, 1H, CONH), 7.87 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8, 18.8, 25.8, 28.4, 30.7, 46.9, 47.0, 47.7, 48.5, 49.2, 49.3, 50.7, 52.0, 52.3, 52.7, 54.2, 57.6, 124.2, 144.5, 165.5, 171.7. MS (ESI): m/z 453.3 ([M + H]$^+$, 100%), 509.2 (40%). HRMS (ESI): 453.32927 ([M + H]$^+$). Calcd for $C_{21}H_{41}N_8O_3$ ([M + H]$^+$): 453.32961.

(*S*)-Methyl-2-(2-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)-methyl)-1*H*-1,2,3-triazol-1-yl)-acetamido)-3-(1*H*-indol-3-yl)-propanoate (12iii). Compound **10iii** (168 mg, 0.200 mmol) was deprotected in a mixture of TFA/EDT/H_2O (20 mL, 95:2.5:2.5) at room temperature for 1.5 h. The reaction solution was concentrated under reduced pressure to give a brown glue, which was dissolved in distilled water (50 mL), washed with $CHCl_3$ (6 × 10 mL), and concentrated under reduced pressure. The residue was dissolved in distilled water (15 mL) and purified by preparative RP-HPLC (gradient 0% to 25% B over 45 min). The combined fractions were concentrated under reduced pressure, dissolved in distilled water (1.5 mL), taken to pH 11−12 with saturated Na_2CO_3, and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with distilled water (1 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a colorless glue, which was dissolved in MeOH (3 mL) and filtered with a cotton-packed pipet. The filtrate was concentrated under reduced pressure and *in vacuo* to give **12iii** as a colorless glue (66 mg, 61%). $[\alpha]_D^{20}$: +19.8 (c 1.0, CH_3CN). IR: ν_{max}/cm^{-1} 3263, 3050, 2926, 2820, 1740, 1678, 1548, 1448, 1345, 1214, 1113, 1053, 1012, 818, 738. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72 (br s, 2H, $CH_2CH_2CH_2$), 1.93 (br s, 2H, $CH_2CH_2CH_2$), 2.45−2.95 (br m, 16H, $CH_2N(CH_2$-triazole$)CH_2$ and 3 × CH_2NHCH_2), 3.18−3.32 (m, 2H, CH_2-indole), 3.56−3.73 (m, 2H, NCH_2-triazole), 3.66 (s, 3H, $COOCH_3$), 3.79 (br s, 3H, 3 × NH), 4.76 (br s, 1H, CONHCH), 4.93 (ABq, 2H, J 16.2, $\Delta\nu$ 39.4, triazole-CH_2CONH), 6.69 (s, 1H, indole-H), 6.99−7.12 (m, 3H, CONH and indole-H), 7.25 (d, 1H, J 7.8, indole-H), 7.42 (d, 1H, J 7.8, indole-H), 7.46 (s, 1H, triazole-H), 11.27 (br s, 1H, indole-NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.7, 27.1, 28.2, 47.0, 47.3, 48.1, 48.4, 49.4, 49.9, 51.2, 52.5, 52.6, 52.8, 54.1, 54.5, 108.1, 111.6, 118.2, 119.0, 121.6, 124.0, 127.3, 136.5, 145.6, 165.3, 171.8. MS (ESI): m/z 540.3 ([M + H]$^+$, 100%), 270.5 ([M + 2H]$^{2+}$,60%). HRMS (ESI): 540.34067 ([M + H]$^+$). Calcd for $C_{27}H_{42}N_9O_3$ ([M + H]$^+$): 540.34051.

[Cu(12i)](ClO$_4$)$_2$ Complex (13i). Compound **12i** (94.0 mg, 0.188 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (69.6 mg, 0.188 mmol) were reacted according to general synthetic procedure D(i) to give **13i** as a purple powder (107.7 mg, 75%). m.p.: 126−131 °C. UV−vis (CH_3OH): λ_{max}/nm 559, ε 135. IR: ν_{max}/cm^{-1} 3528, 3354, 3244, 2954, 2931, 2885, 1734, 1679, 1639, 1543, 1454, 1227, 1063, 751, 705, 623. HRMS (ESI): 662.19932, 663.20309, 664.19705, 665.20115, 666.19600, 667.19967 ([M − ClO_4]$^+$). Calcd for $C_{25}H_{40}ClCuN_8O_7$ ([M − ClO_4]$^+$): 662.19990, 663.20326, 664.19798, 665.20142, 666.19515, 667.19850. Anal. Calcd for $C_{25}H_{40}Cl_2CuN_8O_{11}{\cdot}H_2O$: C, 38.44; H, 5.42; N, 14.35. Found: C, 38.64; H, 5.37; N, 14.26.

[Cu(12ii)](ClO$_4$)$_2$ Complex (13ii). Compound **12ii** (65.0 mg, 0.144 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (53.2 mg, 0.144 mmol) were reacted according to general synthetic procedure D(i) to give **13ii** as a purple powder (59.8 mg, 58%). m.p.: 128−133 °C. UV−vis (CH_3OH): λ_{max}/nm 563, ε 127. IR: ν_{max}/cm^{-1} 3565, 3352, 3240, 2962, 2881, 1737, 1686, 1541, 1456, 1436, 1366, 1226, 1061, 892, 819, 752, 622, 531. HRMS (ESI): 614.19977, 615.20312, 616.19751, 617.20108, 618.19524, 619.19815 ([M − ClO_4]$^+$). Calcd for $C_{21}H_{40}ClCuN_8O_7$ ([M − ClO_4]$^+$): 614.19990, 615.20326, 616.19797, 617.20142, 618.19515, 619.19850. Anal. Calcd for $C_{21}H_{40}Cl_2CuN_8O_{11}{\cdot}H_2O$: C, 34.41; H, 5.77; N, 15.29. Found: C, 34.63; H, 5.60; N, 14.94.

[Cu(12iii)](ClO$_4$)$_2$ Complex (13iii). Compound **12iii** (65.4 mg, 0.121 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (44.9 mg, 0.121 mmol) were reacted according to general synthetic procedure D(i) to give **13iii** as a purple powder (69.3 mg, 71%). m.p.: 166−171 °C. UV−vis (CH_3CN): λ_{max}/nm 556, ε 139. IR: ν_{max}/cm^{-1} 3570, 3359, 3240, 2954, 2883, 1736, 1698, 1540, 1456, 1434, 1363, 1228, 1060, 752, 621, 531. HRMS (ESI): 701.21037, 702.21370, 703.20854, 704.21145, 705.20660, 706.20879 ([M − ClO_4]$^+$). Calcd for $C_{27}H_{41}ClCuN_9O_7$ ([M − ClO_4]$^+$): 701.21080, 702.21416, 703.20888, 704.21231, 705.20605, 706.20940. Anal. Calcd for $C_{27}H_{41}Cl_2CuN_9O_{11}{\cdot}H_2O$: C, 39.54; H, 5.28; N, 15.37. Found: C, 39.52; H, 5.15; N, 15.32.

[Ni(12i)](ClO$_4$)$_2$ Complex (14i). Compound **12i** (101 mg, 0.202 mmol) and $Ni(ClO_4)_2{\cdot}6H_2O$ (74 mg, 0.202 mmol) were reacted according to general synthetic procedure D(i) to give **14i** as a pink powder (77 mg, 50%). m.p.: 226−231 °C. $[\alpha]_D^{20}$: +5.5 (c 1.0, CH_3OH). UV−vis (CH_3OH): λ_{max}/nm 516, ε 17. IR: ν_{max}/cm^{-1} 3502, 3336, 3270, 2954, 2877, 1741, 1696, 1541, 1455, 1437, 1365, 1225, 1077, 961, 926, 747, 704, 621, 516. HRMS (ESI): 657.20591, 658.20932, 659.20241, 660.20524, 661.19809, 662.20235, 663.19848 ([M − ClO_4]$^+$). Calcd for $C_{25}H_{40}ClN_8NiO_7$ ([M − ClO_4]$^+$): 657.20565, 658.20901, 659.20126, 660.20448, 661.19815, 662.20149, 663.19571. Anal. Calcd for $C_{25}H_{40}Cl_2N_8NiO_{11}$: C, 39.60; H, 5.32; N, 14.78. Found: C, 39.29; H, 5.58, N; 14.42.

[Ni(12ii)](ClO$_4$)$_2$ Complex (14ii). Compound **12ii** (79.6 mg, 0.176 mmol) and $Ni(ClO_4)_2{\cdot}6H_2O$ (64.3 mg, 0.176 mmol) were reacted according to general synthetic procedure D(i) to give **14ii** as a pink powder (30.0 mg, 24%). m.p.: 271−273 °C. $[\alpha]_D^{20}$: −12.7 (c 1.0, CH_3OH). UV−vis (CH_3OH): λ_{max}/nm 515, ε 12. IR: ν_{max}/cm^{-1} 3527, 3353, 3271, 2965, 2935, 2878, 1737, 1682, 1543, 1459, 1437, 1368, 1226, 1063, 960, 927, 824, 621. HRMS (ESI): 609.20579, 610.20882, 611.20160, 612.20506, 613.19789, 614.20062, 615.19668 ([M − ClO_4]$^+$). Calcd for $C_{21}H_{40}ClN_8NiO_7$ ([M − ClO_4]$^+$): 609.20565, 610.20901, 611.20127, 612.20447, 613.19815, 614.20149, 615.19571. Anal. Calcd for $C_{21}H_{40}Cl_2N_8NiO_{11}$: C, 35.52; H, 5.68; N, 15.78. Found: C, 35.80; H, 5.78; N, 15.77.

CCDC 840172 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

[Ni(12iii)](ClO$_4$)$_2$ Complex (14iii). Compound **12iii** (69.3 mg, 0.128 mmol) and $Ni(ClO_4)_2{\cdot}6H_2O$ (47.0 mg, 0.129 mmol) were reacted according to general synthetic procedure D(i) to give **14iii** as a pink powder (70.6 mg, 69%). m.p.: 198−203 °C. $[\alpha]_D^{20}$: +14.2 (c 1.0, CH_3CN). UV−vis (CH_3CN): λ_{max}/nm 516, ε 11. IR: ν_{max}/cm^{-1} 3530, 3367, 3271, 2957, 2928, 2879, 1738, 1686, 1543, 1458, 1438, 1366, 1227, 1099, 1081, 961, 928, 752, 624. HRMS (ESI): 696.21573, 697.21952, 698.21178, 699.21530, 700.20943, 701.21250, 702.20858, 703.21104 ([M − ClO_4]$^+$). Calcd for $C_{27}H_{41}ClN_9NiO_7$ ([M − ClO_4]$^+$): 696.21655, 697.21991, 698.21216, 699.21538, 700.20905, 701.21239, 702.20661, 703.20996. Anal. Calcd for $C_{27}H_{41}Cl_2N_9NiO_{11}{\cdot}0.5H_2O$: C, 40.22; H, 5.25; N, 15.63. Found: C, 40.51; H, 5.43; N, 15.39.

[Zn(12i)](ClO$_4$)$_2$ Complex (15i). Compound **12i** (95 mg, 0.19 mmol) and $Zn(ClO_4)_2{\cdot}6H_2O$ (71 mg, 0.19 mmol) were reacted according to general synthetic procedure D(i) to give **15i** as a white powder (99 mg, 68%). m.p.: 122−127 °C. $[\alpha]_D^{20}$: +6.6 (c 1.0, CH_3OH). IR: ν_{max}/cm^{-1} 3516, 3356, 3272, 2955, 2935, 2880, 1737, 1686, 1543, 1455, 1368, 1228, 1060, 949, 929, 749, 704, 621. HRMS (ESI): 663.19990, 664.20316, 665.19689, 666.19926, 667.19498, 668.19759, 669.19318, 670.19653 ([M − ClO_4]$^+$). Calcd for $C_{25}H_{40}ClN_8O_7Zn$ ([M − ClO_4]$^+$): 663.19945, 664.20281, 665.19636, 666.19965, 667.19507, 668.19845, 669.19221, 670.19556.

Anal. Calcd for $C_{25}H_{40}Cl_2N_8O_{11}Zn{\cdot}0.5H_2O$: C, 38.80; H, 5.34; N, 14.48. Found: C, 38.67; H, 5.37; N, 14.25.

[Zn(12ii)](ClO$_4$)$_2$ Complex (15ii). Compound **12ii** (66.2 mg, 0.146 mmol) and $Zn(ClO_4)_2{\cdot}6H_2O$ (54.5 mg, 0.146 mmol) were reacted according to general synthetic procedure D(i) to give **15ii** as a white powder (57.0 mg, 54%). m.p.: 116−121 °C. $[\alpha]_D^{20}$: −18.8 (*c* 1.0, CH_3OH). IR: ν_{max}/cm^{-1} 3570, 3358, 3241, 3163, 2961, 2937, 2881, 1736, 1684, 1544, 1457, 1436, 1272, 1245, 1081, 624. HRMS (ESI): 615.20026, 616.20365, 617.19696, 618.19962, 619.19530, 620.19783, 621.19359, 622.19789 ([M − ClO_4]$^+$). Calcd for $C_{21}H_{40}ClN_8O_7Zn$ ([M − ClO_4]$^+$): 615.19945, 616.20281, 617.19636, 618.19965, 619.19506, 620.19848, 621.19221, 622.19556. Anal. Calcd for $C_{21}H_{40}Cl_2N_8O_{11}Zn{\cdot}H_2O$: C, 34.32; H, 5.76; N, 15.25. Found: C, 34.64; H, 5.71; N, 15.07.

[Zn(12iii)](ClO$_4$)$_2$ Complex (15iii). Compound **12iii** (47.3 mg, 0.0876 mmol) and $Zn(ClO_4)_2{\cdot}6H_2O$ (32.6 mg, 0.0875 mmol) were reacted according to general synthetic procedure D(i) to give **15iii** as a white powder (52.6 mg, 75%). m.p.: 167−172 °C. $[\alpha]_D^{20}$: +14.3 (*c* 0.7, CH_3CN). IR: ν_{max}/cm^{-1} 3568, 3368, 3272, 3241, 2953, 2878, 1739, 1690, 1544, 1457, 1434, 1358, 1229, 1084, 754, 624. HRMS (ESI): 702.21178, 703.21522, 704.20889, 705.21178, 706.20759, 707.21034, 708.20629, 709.20994 ([M − ClO_4]$^+$). Calcd for $C_{27}H_{41}ClN_9O_7Zn$ ([M − ClO_4]$^+$): 702.21035, 703.21370, 704.20726, 705.21055, 706.20597, 707.20935, 708.20311, 709.20645. Anal. Calcd for $C_{27}H_{41}Cl_2N_9O_{11}Zn{\cdot}0.5H_2O$: C, 39.89; H, 5.21; N, 15.51. Found: C, 39.86; H, 5.15; N, 15.31.

Hexa-*tert*-butyl-11,11′-((1,1′-((((2*R*,2′*R*)-disulfanediylbis(1-ethoxy-1-oxopropane-3,2-diyl))bis(azanediyl))bis(2-oxoethane-2,1-diyl))bis(1*H*-1,2,3-triazole-4,1-diyl))bis(methylene))bis-(1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate) (18). Propargyl-tri-Boc cyclam **4** (252 mg, 0.468 mmol) and (2*R*,2′*R*)-diethyl 3,3′-disulfanediylbis(2-(2-azidoacetamido)propanoate) (**17**, 108 mg, 0.234 mmol) were reacted using general synthetic procedure A. The residue was purified by flash column chromatography (silica gel, EtOAc ramping to EtOAc/MeOH = 95:5) to give **18** as a white foam (239 mg, 66%). R_F (EtOAc/MeOH, 9:1): 0.52. $[\alpha]_D^{20}$: +4.2 (*c* 0.8, $CHCl_3$). IR: ν_{max}/cm^{-1} 3301, 2975, 2934, 1742, 1676, 1540, 1469, 1414, 1367, 1241, 1157. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, 6H, *J* 7.2, 2 × CH_2CH_3), 1.44 (s, 18H, 2 × $C(CH_3)_3$), 1.46 (s, 36H, 4 × $C(CH_3)_3$), 1.72 (m, 4H, 2 × $CH_2CH_2CH_2$), 1.91 (m, 4H, 2 × $CH_2CH_2CH_2$), 2.45 (m, 4H, 2 × $CH_2C(CH_2$-triazole$)CH_2$), 2.64 (m, 4H, 2 × $CH_2C(CH_2$-triazole$)CH_2$), 3.05−3.25 (m, 4H, CH_2SSCH_2), 3.35 (m, 24H, 6 × $CH_2C(Boc)CH_2$), 3.84 (s, 4H, 2 × NCH_2-triazole), 4.20 (q, 4H, *J* 7.2, 2 × CH_2CH_3), 4.78−4.85 (m, 2H, 2 × NHCH), 5.23 (ABq, 4H, *J* 16.5, $\Delta\nu$ 34.7, 2 × triazole-CH_2CONH), 7.73 (br s, 4H, 2 × CONH and 2 × triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 26.5, 28.5, 40.1, 45.3, 46.8, 47.0, 47.4, 48.0, 50.9, 51.7, 52.5, 62.2, 79.7, 124.7, 143.5, 155.6, 155.9, 165.7, 169.6. MS (ESI): *m*/*z* 1508.6 ([M − S + Na]$^+$, 47%), 1540.7 ([M + H]$^+$, 100%), 1562.7 ([M + Na]$^+$, 43%). HRMS (ESI): 1539.86001 ([M + H]$^+$). Calcd for $C_{70}H_{123}N_{16}O_{18}S_2$ ([M + H]$^+$): 1539.86372.

(*R*)-Tri-*tert*-butyl-11-((1-(2-((1-ethoxy-3-((4-methoxybenzyl)-thio)-1-oxopropan-2-yl)amino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (19). Propargyl-tri-Boc cyclam **4** (73 mg, 0.14 mmol) and (*R*)-ethyl 2-(2-azidoacetamido)-3-(4-methoxybenzylthio)propanoate (**16**, 48 mg, 0.14 mmol) were reacted using general synthetic procedure A to give **19** as a white foam (99 mg, 82%). R_F (EtOAc): 0.55. $[\alpha]_D^{20}$: −3.5 (*c* 1.0, $CHCl_3$). IR: ν_{max}/cm^{-1} 3297, 2975, 2933, 1742, 1682, 1610, 1537, 1511, 1464, 1412, 1365, 1242, 1157. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H, *J* 7.2, $COOCH_2CH_3$), 1.44 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.72 (m, 2H, $CH_2CH_2CH_2$), 1.90 (m, 2H, $CH_2CH_2CH_2$), 2.45 (m, 2H, $CH_2C(CH_2$-triazole$)CH_2$), 2.63 (m, 2H, 1 $CH_2C(CH_2$-triazole$)CH_2$), 2.77−2.92 (m, 2H, $CHCH_2S$), 3.34 (m, 12H, 3 × $CH_2C(Boc)CH_2$), 3.64 (s, 2H, SCH_2Ph), 3.79 (s, 3H, OCH_3), 3.83 (s, 2H, NCH_2-triazole), 4.18 (q, 2H, *J* 7.2, $COOCH_2CH_3$), 4.71−4.78 (m, 1H, CONHCH), 5.10 (s, 2H, triazole-CH_2CONH), 6.84 (br d, 2H, *J* 8.4, Ph-H), 6.94 (br s, 1H, CONH), 7.19 (d, 2H, *J* 8.7, Ph-H), 7.65 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 26.6, 28.5, 33.0, 35.9, 45.3, 46.8, 47.0, 47.5, 51.0, 51.9, 52.5, 55.3, 62.0, 79.6, 114.0, 124.3, 129.4, 130.1, 144.0, 155.5, 155.8, 158.8, 165.2, 170.1. MS (ESI): *m*/*z* 891.3 ([M + H]$^+$, 19%), 913.5 ([M + Na]$^+$, 100%). HRMS (ESI): 891.50002 ([M + H]$^+$). Calcd for $C_{43}H_{71}N_8O_{10}S$ ([M + H]$^+$): 891.50084.

(*R*)-Ethyl-2-(2-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)-methyl)-1*H*-1,2,3-triazol-1-yl)acetamido)-3-((4-methoxybenzyl)thio)propanoate (20). Compound **19** (446 mg, 0.500 mmol) was deprotected using general synthetic procedure B, followed by preparative RP-HPLC purification (gradient 10% to 35% B over 45 min) and basification using general synthetic procedure C(ii) to give **20** as a colorless glue (182 mg, 62%). $[\alpha]_D^{20}$: −18.5 (*c* 1.0, CH_3CN). IR: ν_{max}/cm^{-1} 3255, 2931, 2822, 1741, 1688, 1610, 1553, 1512, 1463, 1371, 1341, 1301, 1245, 1214, 1179, 1113, 1031, 915, 830, 732. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, 3H, *J* 7.2, $COOCH_2CH_3$), 1.75 (m, 2H, $CH_2CH_2CH_2$), 1.88 (m, 2H, $CH_2CH_2CH_2$), 2.50−3.00 (br m, 18H, $CH_2N(CH_2$-triazole$)CH_2$ and 3 × CH_2NHCH_2 and $CHCH_2S$), 3.66 (s, 2H, SCH_2Ph), 3.77 (s, 2H, NCH_2-triazole), 3.79 (s, 3H, $PhOCH_3$), 3.98 (br s, 3H, 3 × NH), 4.17 (q, 2H, *J* 7.2, $COOCH_2CH_3$), 4.67−4.72 (m, 1H, CONHCH), 5.13 (s, 2H, triazole-CH_2CONH), 6.84 (d, 2H, *J* 8.4, Ph-H), 7.20 (d, 2H, *J* 8.7, Ph-H), 7.54 (br s, 1H, CONH), 7.83 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 25.8, 27.7, 32.9, 35.9, 47.1, 47.4, 47.7, 48.5, 49.1, 49.4, 50.7, 52.2, 52.8, 53.7, 54.1, 55.4, 61.9, 114.1, 124.3, 129.6, 130.2, 145.3, 158.9, 165.6, 170.3. HRMS (ESI): 591.34389 ([M + H]$^+$). Calcd for $C_{28}H_{47}N_8O_4S$ ([M + H]$^+$) 591.34355.

(*R*)-11-((1-(2-((1-Ethoxy-3-mercapto-1-oxopropan-2-yl)-amino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-11-aza-1,4,8-triazoniacyclotetradecane-1,4,8-triium-2,2,2-trifluoroacetate (21). To a degassed solution of compound **19** (356 mg, 0.399 mmol) in TFA (40 mL) was added anisole (2.2 mL, 20.2 mmol). The reaction mixture was heated at reflux under Ar for 1.5 h, concentrated under reduced pressure, and azeotroped with toluene. The crude residue was dissolved in distilled water (40 mL) and washed with ethyl acetate (2 × 20 mL). The combined organic layers were extracted with water (2 × 20 mL) and pooled aqueous phases back-washed with ethyl acetate (20 mL). The combined aqueous extracts were purified by preparative RP-HPLC (gradient 0% to 25% B over 45 min) to give **21** as a colorless glue (254 mg, 78%). $[\alpha]_D^{20}$: +0.6 (*c* 1.0, CH_3CN). IR: ν_{max}/cm^{-1} 2996, 2844, 2362, 1739, 1677, 1554, 1464, 1430, 1378, 1335, 1199, 1138, 1062, 838, 801, 721. ^{1}H NMR (300 MHz, D_2O): δ 1.04 (t, 3H, *J* 7.2, $COOCH_2CH_3$), 2.00 (m, 4H, 2 × $CH_2CH_2CH_2$), 2.75−2.80 (m, 2H), 3.10−3.25 (m, 8H), 3.35−3.50 (m, 8H) (total 18H, $CH_2N(CH_2$-triazole$)CH_2$ and 3 × CH_2NHCH_2 and $CHCH_2S$), 4.01 (q, 2H, *J* 7.2, $COOCH_2CH_3$), 4.36 (s, 2H, NCH_2-triazole), 4.49 (t, 1H, *J* 5.7, CONHCH), 5.20 (s, 2H, triazole-CH_2CONH), 8.06 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, D_2O): δ 13.5, 19.0, 25.3, 38.3, 38.6, 41.5, 41.9, 45.6, 48.9, 49.1, 52.3, 55.5, 63.4, 110.5, 114.4, 118.2, 122.1, 129.0, 137.5, 161.8, 162.3, 162.7, 163.2, 167.7, 171.5. MS (ESI): *m*/*z* 471.3 ([M − 3TFA + H]$^+$, 96%), 236.2 ([M − 3TFA + 2H]$^{2+}$, 100%). HRMS (ESI): 471.28576 ([M − 3TFA + H]$^+$). Calcd for $C_{20}H_{39}N_8O_3S$ ([M − 3TFA + H]$^+$): 471.28603.

[Cu(20)](ClO$_4$)$_2$ Complex (22). Compound **20** (77.5 mg, 0.131 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (48.6 mg, 0.131 mmol) were reacted according to general synthetic procedure D(i) to give **22** as a purple powder (81.4 mg, 73%). m.p.: 102−107 °C. UV−vis (CH_3CN): λ_{max}/nm 560, ε 123. IR: ν_{max}/cm^{-1} 3570, 3336, 3237, 3158, 2939, 2884, 1737, 1700, 1610, 1539, 1513, 1460, 1430, 1364, 1301, 1239, 1184, 1064, 1027, 891, 833, 744, 676, 620, 530. HRMS (ESI): 752.21426, 753.22046, 754.21230, 755.21773, 756.21284, 757.21608, 758.21757 ([M − ClO_4]$^+$). Calcd for $C_{28}H_{46}ClCuN_8O_8S$ ([M − ClO_4]$^+$): 752.21384, 753.21720, 754.21189, 755.21534, 756.20906, 757.21243, 758.21579. Anal. Calcd for $C_{28}H_{46}Cl_2CuN_8O_{12}S{\cdot}H_2O$: C, 38.60; H, 5.55; N, 12.86. Found: C, 38.52; H, 5.45; N, 12.88.

[Ni(20)](ClO$_4$)$_2$ Complex (23). Compound **20** (102 mg, 0.172 mmol) and $Ni(ClO_4)_2{\cdot}6H_2O$ (62.8 mg, 0.172 mmol) were reacted according to general synthetic procedure D(i) to give **23** as a pink powder (73.8 mg, 51%). m.p.: 65−70 °C. $[\alpha]_D^{20}$: −13.5 (*c* 1.0, CH_3CN). UV−vis (CH_3CN): λ_{max}/nm 531, ε 9. IR: ν_{max}/cm^{-1} 3525, 3269, 2965, 2929, 2877, 1740, 1701, 1611, 1540, 1513, 1458, 1363, 1304, 1233, 1184, 1072, 962, 926, 837, 744, 675, 620, 527. HRMS

(ESI): 747.22020, 748.22474, 749.21679, 750.22061, 751.21606, 752.21830, 753.21712, 754.21937 ([M − ClO_4]$^+$). Calcd for $C_{28}H_{46}ClN_8NiO_8S$ ([M − ClO_4]$^+$): 747.21958, 748.22294, 749.21519, 750.21842, 751.21208, 752.21543, 753.20964, 754.21299. Anal. Calcd for $C_{28}H_{46}Cl_2N_8NiO_{12}S$: C, 39.64; H, 5.47; N, 13.21. Found: C, 39.68; H, 5.62; N, 13.21.

[Zn(20)]$(ClO_4)_2$ Complex (24). Compound **20** (35.2 mg, 0.0596 mmol) and $Zn(ClO_4)_2·6H_2O$ (22.2 mg, 0.0596 mmol) were reacted according to general synthetic procedure D(i) to give **24** as a white powder (41.4 mg, 81%). m.p.: 72−77 °C. $[\alpha]_D^{20}$: −14.0 (*c* 1.0, CH_3CN). IR: ν_{max}/cm^{-1} 3546, 3340, 3250, 2936, 2882, 1737, 1694, 1610, 1541, 1513, 1458, 1367, 1300, 1244, 1183, 1087, 952, 835, 623. HRMS (ESI): 753.21438, 754.21919, 755.21090, 756.21429, 757.20933, 758.21372, 759.21117, 760.21498 ([M − ClO_4]$^+$). Calcd for $C_{28}H_{46}ClN_8O_8SZn$ ([M − ClO_4]$^+$) 753.21338, 754.21674, 755.21028, 756.21359, 757.20898, 758.21238, 759.20613, 760.20949. Anal. Calcd for $C_{28}H_{46}Cl_2N_8O_{12}SZn·0.5H_2O$: C, 38.92; H, 5.48; N, 12.97. Found: C, 38.95; H, 5.45; N, 12.92.

[Cu_2((2*R*,2′*R*)-diethyl-3,3′-disulfanediylbis(2-(2-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)-methyl)-1*H*-1,2,3-triazol-1-yl)-acetamido)propanoate)]$(ClO_4)_4$ Complex (25). Compound **21** (69.5 mg, 0.0855 mmol) and $Cu(ClO_4)_2·6H_2O$ (31.7 mg, 0.0856 mmol) were reacted according to general synthetic procedure D(ii) to give **25** as a purple powder (52.0 mg, 83%). m.p.: 202−207 °C. UV−vis (CH_3CN): λ_{max}/nm 567, ε 182. IR: ν_{max}/cm^{-1} 3588, 3536, 3244, 2940, 2885, 1735, 1692, 1542, 1461, 1368, 1300, 1229, 1091, 623. HRMS (ESI): 631.14824, 631.64972, 632.14663, 632.64801, 633.14600, 633.64711, 634.14562, 634.64619, 635.14497 ([M − $2ClO_4$]$^{2+}$). Calcd for $C_{40}H_{74}Cl_2Cu_2N_{16}O_{14}S_2$ ([M − $2ClO_4$]$^{2+}$): 631.14850, 631.65018, 632.14753, 632.64924, 633.14612, 633.64779, 634.14520, 634.64688, 635.14374. Anal. Calcd for $C_{40}H_{74}Cl_4Cu_2N_{16}O_{22}S_2·4H_2O$: C, 31.27; H, 5.38; N, 14.59. Found: C, 31.22; H, 5.11; N, 14.66.

[Ni(21−3TFA)]$(ClO_4)_2$ Complex (26). Compound **21** (134 mg, 0.165 mmol) and $Ni(ClO_4)_2·6H_2O$ (60.4 mg, 0.165 mmol) were reacted according to general synthetic procedure D(ii) to give **26** as a pink powder (41.0 mg, 34%). m.p.: 150−155 °C. $[\alpha]_D^{20}$: −2.2 (*c* 1.0, CH_3CN). UV−vis (CH_3CN): λ_{max}/nm 518, ε 8. IR: ν_{max}/cm^{-1} 3472, 3271, 2936, 2877, 1737, 1688, 1541, 1461, 1431, 1368, 1308, 1223, 1080, 962, 927, 852, 826, 623. HRMS (ESI): 627.16098, 628.16482, 629.15686, 630.16042, 631.15353, 632.15659, 633.15175 ([M − ClO_4]$^+$). Calcd for $C_{20}H_{38}ClN_8NiO_7S$ ([M − ClO_4]$^+$) 627.16207, 628.16543, 629.15769, 630.16089, 631.15457, 632.15791, 633.15213, 634.15548. Anal. Calcd for $C_{20}H_{38}Cl_2N_8NiO_{11}S·0.5H_2O$: C, 32.58; H, 5.33; N, 15.20. Found: C, 32.51; H, 5.37; N, 15.02.

[Zn(21−3TFA)]$(ClO_4)_2$ Complex (27). Compound **21** (105.8 mg, 0.130 mmol) and $Zn(ClO_4)_2·6H_2O$ (48.5 mg, 0.130 mmol) were reacted according to general synthetic procedure D(ii) to give **27** as a white powder (59.9 mg, 63%). m.p.: 109−114 °C. $[\alpha]_D^{20}$: −2.6 (*c* 1.0, CH_3CN). IR: ν_{max}/cm^{-1} 3574, 3266, 2937, 2881, 1736, 1694, 1540, 1457, 1428, 1367, 1302, 1224, 1082, 951, 879, 827, 623. HRMS (ESI): 633.15390, 634.15766, 635.15024, 636.15331, 637.14900, 638.15167, 639.14659, 640.15012 ([M − ClO_4]$^+$). Calcd for $C_{20}H_{38}ClN_8O_7SZn$ ([M − ClO_4]$^+$): 633.15587, 634.15923, 635.15276, 636.15607, 637.15146, 638.15490, 639.14862, 640.15197. Anal. Calcd for $C_{20}H_{38}Cl_2N_8O_{11}SZn$: C, 32.69; H, 5.21; N, 15.25, $C_{20}H_{38}Cl_2N_8O_{11}SZn·0.5H_2O$: C, 32.29; H, 5.28; N, 15.06. Found: C, 32.34; H, 5.14; N, 15.07.

(*S*)-Methyl-2-((*S*)-2-((*S*)-2-(2-azidoacetamido)-3-methylbutanamido)-3-methylbutanamido)-3-methylbutanoate (31). To a solution of azide-capped trivaline **30** (159 mg, 0.399 mmol) in anhydrous methanol (5 mL) was added thionyl chloride (30 μL, 0.413 mmol) dropwise. The reaction mixture was heated at reflux for 6 h and purified by preparative HPLC (gradient 0% to 50% B over 45 min) to give **31** as a white solid (83 mg, 50%). R_F (CH_2Cl_2/CH_3OH = 95:5): 0.30. m.p.: 207−209 °C. $[\alpha]_D^{20}$: −41.0 (*c* 1.0, DMSO). IR: ν_{max}/cm^{-1} 3281, 3077, 2962, 2102, 1736, 1668, 1632, 1542, 1432, 1382, 1286, 1204, 1143, 1090, 993, 931, 903, 847, 799, 694, 589, 559. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 0.78−0.88 (m, 18H, 3 × $CH(CH_3)_2$), 1.89−1.98 (m, 2H, 2 × $CH(CH_3)_2$), 1.98−2.07 (m, 1H, $CH(CH_3)_2$), 3.60 (s, 3H, $COOCH_3$), 3.87 (ABq, 2H, *J* 15.6, $\Delta\nu$ 11.9, N_3CH_2), 4.14 (t, 1H, *J* 7.2, CONHC*H*), 4.23 (t, 1H, *J* 8.0, CONHC*H*), 4.30 (t, 1H, *J* 7.6, CONHC*H*), 8.00 (d, 1H, *J* 8.8, CONH), 8.13 (d, 1H, *J* 6.8, CONH), 8.15 (d, 1H, *J* 8.4, CONH). ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 18.0, 18.1, 18.3, 18.9, 19.0, 19.1, 29.7, 30.4, 30.7, 50.5, 51.5, 57.3, 57.6, 167.2, 170.5, 171.2, 171.7. MS (ESI): *m*/*z* 413.0 ([M + H]$^+$, 100%), 435.1 ([M + Na]$^+$, 17%), 824.8 ([2M + H]$^+$, 62%), 846.9 ([2M + Na]$^+$, 36%). HRMS (ESI): 435.23244 ([M + Na]$^+$). Calcd for $C_{18}H_{32}N_6NaO_5$ ([M + Na]$^+$): 435.23264. Anal. Calcd for $C_{18}H_{32}N_6O_5$: C, 52.41; H, 7.82; N, 20.37. Found: C, 52.34; H, 8.00; N, 20.44.

Tri-*tert*-butyl-11-((1-((4*S*,7*S*,10*S*)-4,7,10-triisopropyl-3,6,9,12-tetraoxo-2-oxa-5,8,11-triazatri-decan-13-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (32). Propargyl-tri-Boc cyclam **4** (52 mg, 0.096 mmol) and compound **31** (40 mg, 0.097 mmol) were reacted using general synthetic procedure A to give **32** as a white foam (74 mg, 81%). R_F (EtOAc): 0.41. $[\alpha]_D^{20}$: −19.5 (*c* 1.0, $CHCl_3$). IR: ν_{max}/cm^{-1} 3280, 3077, 2969, 2934, 1741, 1681, 1641, 1546, 1466, 1413, 1367, 1239, 1158, 915, 729. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82−0.95 (m, 18H, 3 × $CH(CH_3)_2$), 1.46 (s, 27H, 3 × $C(CH_3)_3$), 1.71 (m, 2H, $CH_2CH_2CH_2$), 1.94 (m, 4H, $CH_2CH_2CH_2$ and 2 × $CH(CH_3)_2$), 2.11 (m, 1H, $CH(CH_3)_2$), 2.43 (m, 2H, $CH_2N(CH_2$-triazole)CH_2), 2.62 (m, 2H, $CH_2N(CH_2$-triazole)CH_2), 3.34 (m, 12H, 3 × CH_2C(Boc)-CH_2), 3.75 (s, 3H, $COOCH_3$), 3.81 (s, 2H, NCH_2-triazole), 4.58−4.67 (m, 3H, 3 × NHC*H*CO), 5.06 (br d, 1 H, *J* 15.6, triazole-CH_2CONH), 5.39 (br d, 1H, *J* 15.6, triazole-CH_2CONH), 7.84 (br s, 2H, CONH and triazole-H), 8.06 (br s, 1H, CONH), 8.37 (br s, 1H, CONH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.9, 18.7, 19.0, 19.1, 26.5, 28.6, 29.7, 30.8, 31.0, 32.4, 45.2, 46.9, 48.1, 50.8, 52.3, 57.3, 58.5, 58.8, 79.5, 124.6, 143.1, 155.6, 155.9, 165.7, 171.3, 172.0, 172.7. MS (ESI): *m*/*z* 952.0 ([M + H]$^+$, 56%), 973.8 ([M + Na]$^+$, 100%). HRMS (ESI): 951.62273 ([M + H]$^+$). Calcd for $C_{46}H_{83}N_{10}O_{11}$ ([M + H]$^+$): 951.62373.

11-((1-((4*S*,7*S*,10*S*)-4,7,10-Triisopropyl-3,6,9,12-tetraoxo-2-oxa-5,8,11-triazatridecan-13-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-11-aza-1,4,8-triazoniacyclotetradecane-1,4,8-triium-2,2,2-trifluoroacetate (33). Compound **32** (290 mg, 0.305 mmol) was deprotected using general synthetic procedure B, followed by preparative HPLC purification (gradient 0% to 30% B over 45 min), to give **33** as a colorless glue (259 mg, 86%). $[\alpha]_D^{20}$: −26.5 (*c* 1.0, CH_3CN). IR: ν_{max}/cm^{-1} 3286, 3078, 2966, 2874, 1739, 1676, 1647, 1550, 1464, 1434, 1397, 1200, 1133, 832, 801, 721. ^{1}H NMR (400 MHz, D_2O): δ 0.83−0.91 (m, 18H, 3 × $CH(CH_3)_2$), 1.79 (br s, 2H, $CH_2CH_2CH_2$), 1.88−2.02 (m, 4H, $CH_2CH_2CH_2$ and 2 × $CH(CH_3)_2$), 2.07−2.14 (m, 1H, $CH(CH_3)_2$), 2.70−3.21 (m, 16H, $CH_2N(CH_2$-triazole)CH_2 and 3 × CH_2NHCH_2), 3.67 (s, 3H, $COOCH_3$), 3.75 (br s, 2H, NCH_2-triazole), 4.08 (d, 2H, *J* 8.0, 2 × NHC*H*CO), 4.18 (d, 1H, *J* 6.0, NHC*H*CO), 5.27 (ABq, 2H, *J* 16.8, $\Delta\nu$ 21.3, triazole-CH_2CONH)), 7.89 (s, 1H, triazole-H). ^{13}C NMR (75 MHz, D_2O): δ 17.8, 18.1, 18.4, 18.6, 18.7, 22.8, 24.6, 30.3, 30.5, 30.6, 44.0, 45.4, 46.6, 46.8, 47.3, 48.7, 52.1, 52.7, 53.0, 54.3, 59.0, 59.7, 60.2, 111.0, 114.8, 118.7, 122.6, 126.3, 144.8, 162.6, 163.1, 163.5, 164.0, 168.2, 173.5, 173.8, 174.2. MS (ESI): *m*/*z* 326.3 ([M − 3TFA +2H]$^{2+}$, 79%), 651.4 ([M − 3TFA + H]$^+$, 100%). HRMS (ESI): 651.46559 ([M − 3TFA + H]$^+$). Calcd for $C_{31}H_{59}N_{10}O_5$ ([M − 3TFA + H]$^+$): 651.46644

[Cu(33-3TFA)]$(ClO_4)_2$ Complex (34). Compound **33** (84.0 mg, 0.0846 mmol) and $Cu(ClO_4)_2·6H_2O$ (31.3 mg, 0.0845 mmol) were reacted according to general synthetic procedure D(iii) to give **34** as a purple powder (63.0 mg, 82%). m.p.: 198−203 °C. UV−vis (CH_3CN): λ_{max}/nm 572, ε 183. IR: ν_{max}/cm^{-1} 3591, 3339, 3298, 3234, 2964, 2881, 1733, 1694, 1647, 1541, 1463, 1363, 1222, 1068, 622. HRMS (ESI): 812.33537, 813.33973, 814.33310, 815.33743, 816.33107, 817.33528, 818.33885 ([M − ClO_4]$^+$). Calcd for $C_{31}H_{58}ClCuN_{10}O_9$ ([M − ClO_4]$^+$): 812.33673, 813.34001, 814.33501, 815.33821, 816.33215, 817.33534, 818.33867. Anal. Calcd for $C_{31}H_{58}Cl_2CuN_{10}O_{13}·H_2O$: C, 39.98; H, 6.49; N, 15.04. Found: C, 39.90; H, 6.47; N, 14.94.

[Ni(33-3TFA)](ClO$_4$)$_2$ Complex (35). Compound **33** (137 mg, 0.138 mmol) and Ni(ClO$_4$)$_2$·6H$_2$O (50.5 mg, 0.138 mmol) were reacted according to general synthetic procedure D(iii) to give **35** as a pink powder (96 mg, 77%). m.p.: 218−220 °C. $[\alpha]_D^{20}$: −12.0 (*c* 1.0, CH$_3$CN). UV−vis (CH$_3$CN): λ_{max}/nm 502, ε 9. IR: ν_{max}/cm^{-1} 3505, 3272, 3084, 2965, 2928, 2878, 1736, 1645, 1542, 1462, 1369, 1312, 1220, 1080, 623. HRMS (ESI): 807.34370, 808.34715, 809.34073, 810.34438, 811.33663, 812.34097, 813.33370 ([M − ClO$_4$]$^+$). Calcd for $C_{31}H_{58}ClN_{10}NiO_9$ ([M − ClO$_4$]$^+$): 807.34248, 808.34576, 809.33834, 810.34131, 811.33518, 812.33833, 813.33258. Anal. Calcd for $C_{31}H_{58}Cl_2N_{10}NiO_{13}$: C, 40.99; H, 6.44; N, 15.42. Found: C, 40.70; H, 6.58; N, 15.18.

[Zn(33-3TFA)](ClO$_4$)$_2$ Complex (36). Compound **33** (122 mg, 0.123 mmol) and Zn(ClO$_4$)$_2$·6H$_2$O (45.8 mg, 0.123 mmol) were reacted according to general synthetic procedure D(iii) to give **36** as a white powder (86 mg, 76%). m.p.: 174−176 °C. $[\alpha]_D^{20}$: −23.0 (*c* 1.0, CH$_3$CN). IR: ν_{max}/cm^{-1} 3531, 3276, 3083, 2965, 2935, 2880, 1735, 1649, 1543, 1463, 1369, 1274, 1220, 1088, 624. HRMS (ESI): 813.33666, 814.34013, 815.33439, 816.33744, 817.33358, 818.33592, 819.33214, 820.33369, 821.33396 ([M − ClO$_4$]$^+$). Calcd for $C_{31}H_{58}ClN_{10}O_9Zn$ ([M − ClO$_4$]$^+$): 813.33627, 814.33958, 815.33335, 816.33646, 817.33206, 818.33527, 819.32914, 820.33239, 821.33572. Anal. Calcd for $C_{31}H_{58}Cl_2N_{10}O_{13}Zn$·1.5H$_2$O: C, 39.52; H, 6.53; N, 14.87. Found: C, 39.51; H, 6.55; N, 14.84.

Cell Culture. Breast cancer cell lines MDA-MB-231 and BT-474 were cultured in phenol red free DMEM/F12 (1:1; Invitrogen) supplemented with 10% heat inactivated fetal bovine serum (FBS, Invitrogen). The nonmalignant breast cell line MCF-10A was cultured in phenol red free DMEM/F12 (1:1) supplemented with 5% horse serum (Invitrogen), 20 ng/mL Epidermal Growth Factor (EGF) (Peprotech), 500 ng/mL hydrocortisone (Sigma-Aldrich), 0.01 mg/mL bovine insulin (Sigma-Aldrich), and 100 ng/mL cholera toxin (Sigma-Aldrich). Pancreatic cancer cell lines BxPC-3, PANC-1, and SU.86.86 were cultured in RPMI-1640 media (Invitrogen) supplemented with HEPES (25 mM; Invitrogen) and 10% FBS. Prostate cancer cell lines LNCaP and PC-3 were maintained in RPMI-1640 supplemented with 10% FBS, and the control RWPE-1 cells were maintained in Keratinocyte Serum Free Media (KSFM, Gibco) supplemented with 20 mg/mL bovine pituitary extract (BPE) and 2 ng/mL epidermal growth factor EGF. All cancer cell lines were purchased from the ATCC and maintained under standard cell culture conditions (5% CO$_2$, 37 °C, 95% humidity). The normal human embryonic kidney cell line, HEK-293, was grown in DMEM plus 2 mM Glutamax (Invitrogen) supplemented with 10% FBS and incubated under standard cell culture conditions (as above).

Cell Viability Assay. Cells were seeded at appropriate densities according to their respective growth rates in 384-well black/clear tissue culture treated plates (Falcon, BD Biosciences) in 45 μL of culture medium using a multidrop liquid handler (Thermo Scientific) and were allowed to adhere overnight under standard cell culture conditions (5% CO$_2$, 37 °C, 95% humidity). Compounds at 10 mM concentrations were diluted 25-fold in sterile MilliQ water, yielding 400 μM compound stocks with a DMSO (Sigma-Aldrich) level of 4%; these solutions were further diluted with 4% DMSO to give 200, 100, 50, and 25 μM concentrations. Compound was added to each well to give final screening concentrations of 40 μM, 20 μM, 10 μM, and 1 μM at 0.4% DMSO for the respective cancer cell lines or 40 μM, 20 μM, 10 μM, 5 μM, and 2.5 μM for the HEK-293 cell line. Intermediate and final plate dilutions were freshly prepared on the day of every screen using the Bravo (Agilent) robotic liquid handling system. Appropriate positive (>10% DMSO or 20 μM Puromycin) and negative (0.4% DMSO) controls were included for each plate, in addition to a serial dose response of a reference compound (Puromycin (Sigma Aldrich)) for determination of IC$_{50}$. The plates were incubated for 72 h (37 °C, 5% CO$_2$ and 95% humidity). For the cancer cell lines, Resazurin (Sigma-Aldrich) was added to a final concentration of 600 μM per well and incubated for 4 h before a measurement of fluorescent intensity was recorded on a Perkin-Elmer EnVision at 530/595 nm. For the HEK-293 cell line, the supernatant was removed after incubation, and 40 μL of 10% Presto Blue substrate (Sigma Aldrich) in DMEM plus 2 mM Glutamax was added to each well. The plates were incubated for a further 3 h and measured on the Perkin-Elmer EnVision at 530/595 nm. The percent inhibition of growth was calculated in relation to the maximum and minimum inhibition of fluorescence caused by >10% DMSO or 20 μM Puromycin (100% inhibition) and 0.4% DMSO (no inhibition). All experiments were performed in triplicate, n = 3.

RESULTS AND DISCUSSION

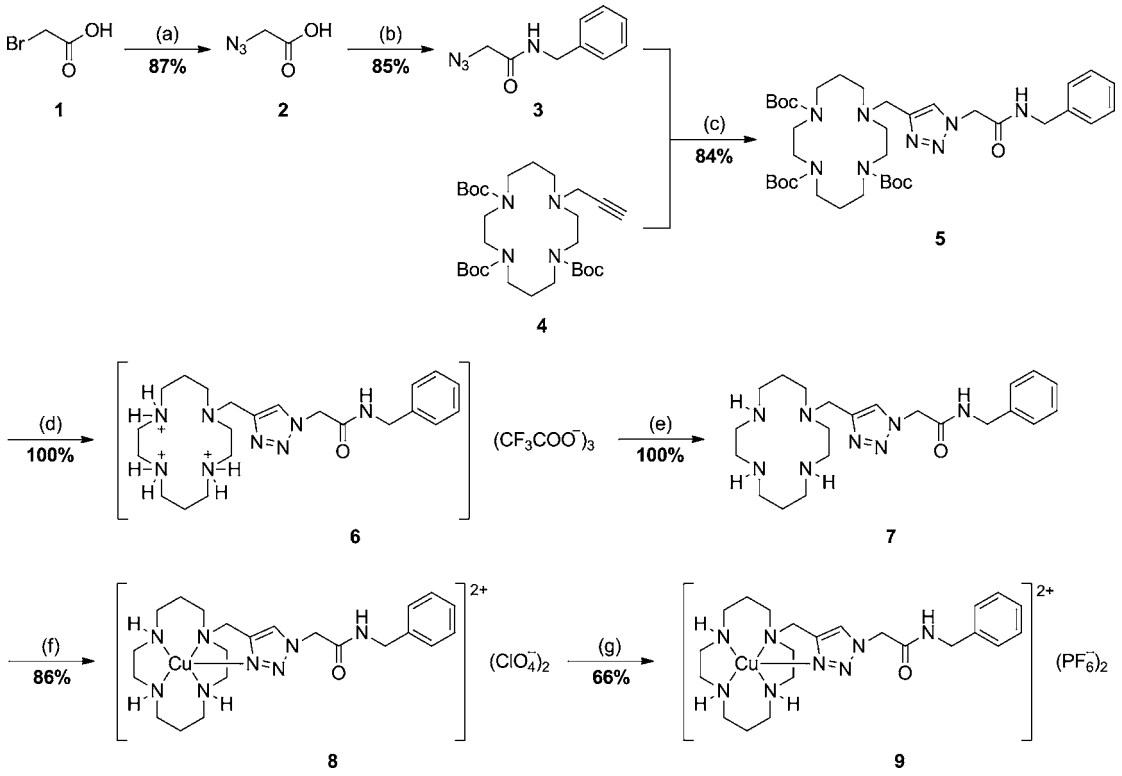
The synthesis of cyclam scorpionand conjugates bearing pendant amino acids requires a synthetic route and conditions that are compatible with amide bonds. To evaluate the viability of our proposed approach, a model compound (**7**, Scheme 1) was first synthesized with a pendant *N*-benzylamide substituent. Azidoacetic acid (**2**)[30,31] and propargyl-tri-Boc cyclam (**4**)[32,33] were synthesized according to literature procedures. Amide coupling of benzylamine to the acid was followed by copper(I)-catalyzed cycloaddition to give the triazole (**5**) in 84% yield.

Preparation of azamacrocycles containing pendant groups using a Boc strategy requires the eventual removal of the Boc groups and basification to recover the free amine ligand. Removal of the Boc groups from **5** with 20% trifluoroacetic acid in DCM[24,34] frequently led to the formation of *tert*-butylated products; use of water or TIS (at 5%, either individually or in combination) as additives suppressed this. Removal of TIS could be effected by dissolution of the residue in water and washing with hexane, but in general the purity of the products and the stability of the isolated samples were optimal when water was the only additive, i.e., when the removal of the Boc groups was effected with 90:5:5 TFA/DCM/H$_2$O in which the typical protected substrate concentration was 5 mM (concentrations of 20−50 mM gave poorer results). While we have no direct evidence here of the formation of salts *via* combination with three (rather than four) equivalents of trifluoroacetic acid, we do have experimental evidence of this stoichiometry for related compounds that will be reported in due course; three equivalents have been presumed previously by us[24] and others.[35] Isolation of the free amine ligand **7** was achieved by basification with 5 M KOH or 2 M NaOH[24] and extraction with CHCl$_3$ in quantitative yield and >98% purity (determined by analytical HPLC) without further purification.

The click reaction generates an aromatic triazole ring that alters the acidity of the surrounding protons. The ^{1}H NMR spectrum of **7** showed H/D exchange at the methylene position derived from the azidoacetic acid (Figure 1). A decrease in the intensity of this signal (at 5.19 ppm) was clear after a few hours at room temperature and was essentially complete after one week in deuterated methanol. In contrast, no such exchange was visible for compound **3**, containing protons in a similar chemical environment but in the absence of the triazole. This increased acidity has important implications for the biological evaluation of related click-derived compounds in which this position adjacent to the triazole contains stereochemical information.[36,37]

The stoichiometry of complexation between Cu(II) and free amine ligand **7** was determined. A Job's plot[38] of absorbance at 570 nm vs the mole fraction of the ligand unequivocally verified the stoichiometric ratio of **7**/Cu(ClO$_4$)$_2$ to be 1:1 (Figure 2a), consistent with the results of a UV−visible spectrophotometric titration (Figure 2b). The absorbance at 570 nm in the titration spectra rose with the addition of Cu(ClO$_4$)$_2$ and reached the maximum value upon addition of one equivalent of metal salt.

Scheme 1. Synthesis of Model Compound 7 and Its Copper Complexes[a]



[a]*Reagents and conditions:* (a) NaN_3, H_2O, rt, O/N. (b) Benzylamine, EDC·HCl, HOBt, DIPEA, DCM, rt, O/N. (c) $CuSO_4·5H_2O$, sodium ascorbate, H_2O/*t*-BuOH, rt, O/N. (d) TFA/DCM/H_2O (90:5:5), 6 h. (e) 5 M KOH or 2 M NaOH. (f) $Cu(ClO_4)_2·6H_2O$, EtOH, reflux, 1 h. (g) KPF_6, MeOH/H_2O.

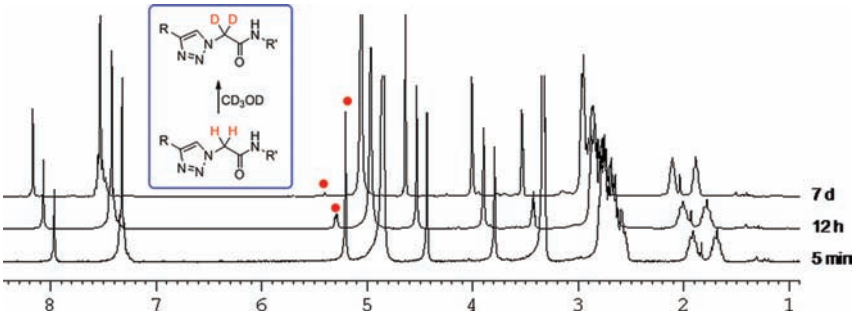


Figure 1. Hydrogen/deuterium exchange in CD_3OD at the methylene center between triazole and amide in **7**.

Bulk reaction of **7** with $Cu(ClO_4)_2$ in EtOH gave an isolable sample of **8**. Data obtained for this compound included intense infrared absorption bands at 1065 and 622 cm^{-1}, indicating the presence of perchlorate anion and a cluster of peaks with the correct isotope pattern at m/z 590−596 ($[\mathbf{8}\text{-}ClO_4]^+$) in the high resolution mass spectrum; these results were further supported by elemental analysis data. Attempts were made to crystallize **8** using standard techniques (slow evaporation, slow cooling, vapor diffusion, liquid−liquid diffusion), but these failed, leading us to investigate counterion metathesis. Saturated aqueous solutions of potassium hexafluorophosphate or chloride were added to methanolic solutions of **8**, followed by slow evaporation, giving in the former case dark blue crystals suitable for X-ray crystallography (Figure 3). [Formula, $C_{22}H_{36}CuF_{12}N_8OP_2$; M, 782.07; monoclinic; space group $P2_1/n$ (#14); a = 9.4510(10), b = 21.342(2), c = 15.342(2) Å; β = 95.2370(10); V = 3081.6(6) $Å^3$; D_c = 1.686 g cm^{-3}; Z = 4; crystal size 0.43 × 0.19 × 0.17 mm; color dark blue; habit prism; temperature 150(2) K; λ(Mo Ka) 0.71073 Å; μ(Mo Ka) 0.919 mm^{-1}; $T(\text{SADABS})_{min,max}$ = 0.776, 0.859; $2\theta_{max}$ 56.6; *hkl* range −12 +12, −27 +27, −20 +20; N, 29752; N_{ind}, 7428(R_{merge} = 0.0229); N_{obs}, 5989(I > 2σ(I)); $N_{var.}$, 457; residuals* $R1(F)$, 0.0465; $wR2(F^2)$, 0.1323; GoF(all), 1.030; $\Delta\rho_{min,max}$ = −0.649, 0.875 e^- $Å^{-3}$. *R1 = $\sum||F_o| - |F_c||/\sum|F_o|$ for $F_o > 2\sigma(F_o)$; wR2 = $(\sum w(F_o^2 - F_c^2)^2/\sum(wF_c^2)^2)^{1/2}$ all reflections $w = 1/[\sigma^2(F_o^2) + (0.0641P)^2 + 4.7151P]$ where $P = (F_o^2 + 2F_c^2)/3$.]

The complexation of ligand **7** with $Ni(ClO_4)_2$ or $Zn(ClO_4)_2$ yielded the corresponding complexes as pink and white powders, respectively. The binding mode between ligand **7** and $Zn(ClO_4)_2$ was investigated by ^1H NMR spectroscopy (data not shown). ^1H NMR titration of ligand **7** with sequential addition of up to two equivalents of $Zn(ClO_4)_2$ in CD_3OD showed that the main proton signals became more and more

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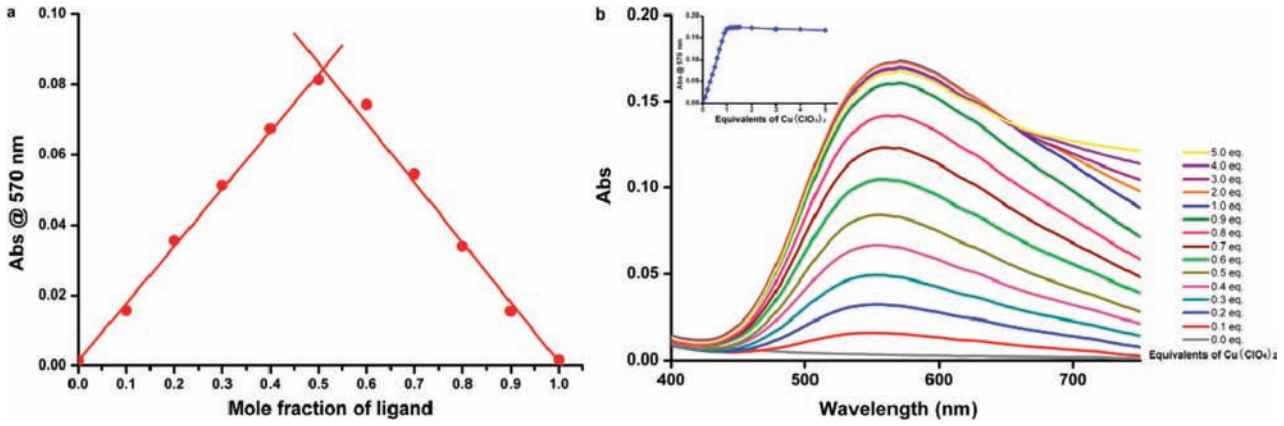

Figure 2. (a) Job's plot showing the 1:1 stoichiometry of the binding between ligand **7** and $Cu(ClO_4)_2$. (b) UV−vis spectrophotometric titration of ligand **7** (1.6 μM) with $Cu(ClO_4)_2$ (160 μM) in MeOH at room temperature (inset: absorbance at 570 nm vs equivalents of $Cu(ClO_4)_2$ added).

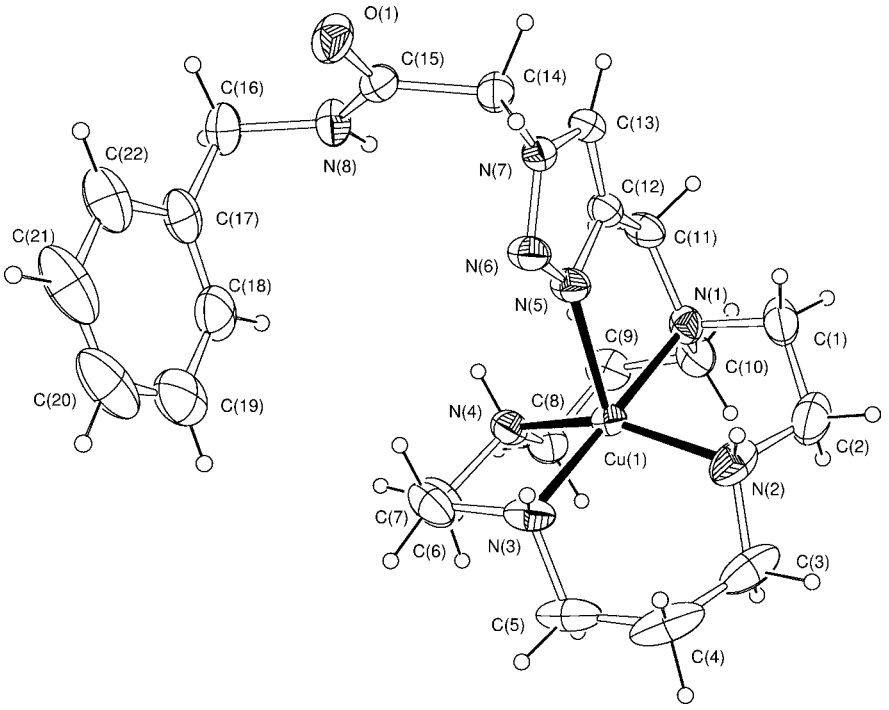

Figure 3. An ORTEP plot of the cation **9** at the 50% probability level. The hexafluorophosphate anions are omitted for clarity. Selected bond lengths (Å) and angles (deg): Cu(1)−N(1), 2.051(2); Cu(1)−N(2), 2.017(3); Cu(1)−N(3), 1.992(3); Cu(1)−N(4), 2.032(2); Cu(1)−N(5), 2.203(2); N(1)−Cu(1)−N(2), 87.77(12); N(1)−Cu(1)−N(3), 179.06(10); N(1)−Cu(1)−N(4), 93.41(10); N(1)−Cu(1)−N(5), 79.60(9); N(2)−Cu(1)−N(3), 92.61(13); N(2)−Cu(1)−N(4), 153.71(11); N(2)−Cu(1)−N(5), 103.63(10); N(3)−Cu(1)−N(4), 86.64(11); N(3)−Cu(1)−N(5), 99.47(9); N(4)−Cu(1)−N(5), 102.41(10). Bond lengths are typical for this kind of complex.[24]

complex and underwent downfield shifts upon addition of up to one equivalent of $Zn(ClO_4)_2$, and further addition of $Zn(ClO_4)_2$ did not result in any appreciable changes, indicating 1:1 stoichiometry of the binding between ligand **7** and $Zn(ClO_4)_2$, as for the copper salt. All of the well-defined signals in the ^{1}H NMR spectrum of ligand **7** were split into unresolvable multiplets in the corresponding spectrum of $[Zn(7)](ClO_4)_2$, except the benzyl group signals, suggesting that all four nitrogen donors on cyclam and a nitrogen atom on the triazole ring are involved in the chelation of ligand **7** with $Zn(ClO_4)_2$. The singlet arising from the triazole H in ligand **7** changed to three separate peaks in the spectrum of purified $[Zn(7)](ClO_4)_2$ (Supporting Information), suggesting the presence of three different diastereomers of the metal complex.[39] The ^{13}C NMR spectrum of the $[Zn(7)](ClO_4)_2$ complex also gave a multiplication of signals vs the free ligand, particularly in the aliphatic regions 20−30 and 40−65 ppm, implying that the metal chelation increases the rigidity of the ligand so the carbons in $[Zn(7)](ClO_4)_2$ become magnetically inequivalent (particularly those in the macrocyclic ring).

The synthetic route to the model compound described above was adapted to the synthesis of metal-cyclam complexes functionalized with the amino acids phenylalanine, valine, and tryptophan (Scheme 2).

Successful deployment of the click reaction in these syntheses requires that the click reaction conditions do not compromise the stereochemical integrity of the amino acids. To verify this, (*S*)-**10i** and *rac*-**10i** were synthesized from (*S*)-phenylalanine methyl ester and the racemate, respectively, and analyzed by enantioselective HPLC. The chromatograms

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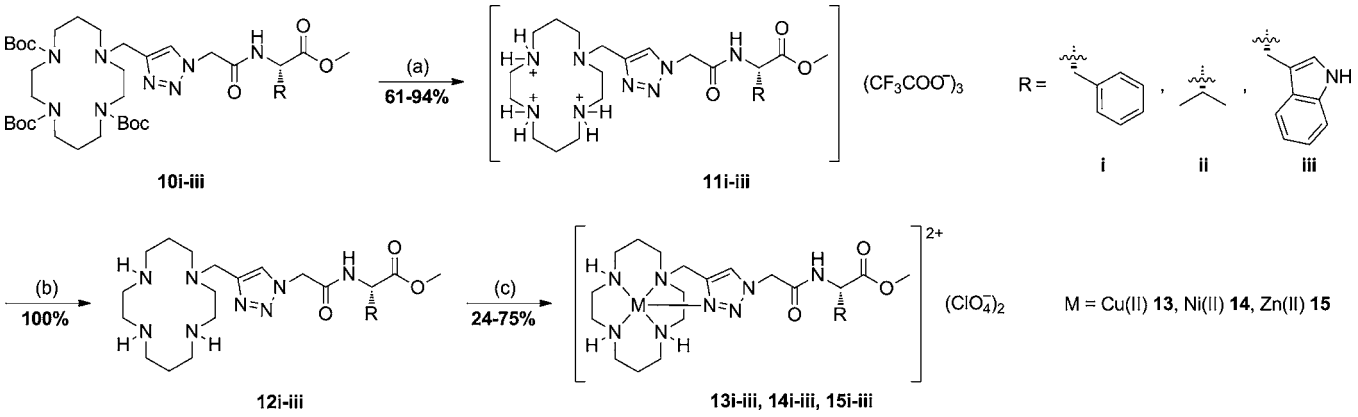
Scheme 2. Synthesis of Metal–Cyclam Complexes Functionalized with Amino Acids[a]



[a]*Reagents and conditions:* (a) **10i** and **10ii**: TFA/DCM/H_2O (90:5:5), rt, 6 h. **10iii**: TFA/EDT/H_2O (90:5:5), rt, 2 h, followed by RP-HPLC purification. (b) Saturated Na_2CO_3, pH 11–12. (c) $M(ClO_4)_2 \cdot 6H_2O$, EtOH, reflux, 1 h.

(Supporting Information) showed a single signal for (*S*)-**10i**, two signals of equal area for *rac*-**10i**, and two signals in a 3:1 ratio for the coinjection of a 1:1 mixture of (*S*)-**10i** and *rac*-**10i**, which unambiguously demonstrated that the conditions employed in this step do not lead to any measurable epimerization.

Removal of Boc groups from **10i** and **10ii** was achieved using TFA/DCM/H_2O (90:5:5) to afford the water-soluble salts **11i** and **11ii** in good to excellent yields and high purities, whereas application of this deprotection procedure to **10iii** failed to give pure trifluoroacetate salt **11iii**. Signals in the mass spectrum of the crude product from this latter reaction (m/z = 596.5 [MH + 56]$^+$, 652.5 [MH + (56 × 2)]$^+$, and 708.7 [MH + (56 × 3)]$^+$) and the appearance of new peaks in the analytical HPLC trace corresponding to compounds less polar than the expected product suggested the attachment of between one and three *tert*-butyl groups to the ligand. This indicated that the water included as a scavenger in the deprotection mixture was not an effective quencher for the *tert*-butyl cation in the presence of an indole nucleophile. Substitution of 5% DCM with 5% triisopropylsilane (TIS) in the deprotection cocktail resulted in reduction of the indole ring (indicated by a signal at m/z = 542.5 ([MH + 2]$^+$) in the mass spectrum), a side reaction consistent with previous observations that triethylsilane/TFA can reduce the indole ring of tryptophan.[40] Replacement of 5% DCM with 5% 1,2-ethanedithiol (EDT) gave satisfactory deprotection. Prior to purification by preparative chromatography, the malodorous EDT could be easily removed by dissolution of the residue in water and washing with chloroform; EDT removal was assayed by monitoring the organic washes with $PdCl_2$ reagent (0.5% aq. $PdCl_2$ containing a few drops of conc. HCl)[41] since sulfur-containing compounds give a yellow color when stained with this reagent.

Basification of **11i–iii** to give the corresponding free amine depended greatly on the base used. The addition of sodium hydroxide (2 M) or sodium hydrogen carbonate (saturated) solutions, both of which had been used in previous studies,[24,34] resulted in either cleavage of the methyl ester (indicated by a signal at m/z [MH − 14]$^+$ in the mass spectrum) or incomplete removal of trifluoroacetate counterions from the ligands, respectively. Successful isolation of the pure amine ligands was achieved by basification to pH 11–12 with saturated aqueous sodium carbonate solution and extraction with chloroform.

Cysteine was chosen as an amino acid that presents more challenging synthetic issues, arising from the nucleophilicity and coordinating ability of the thiol side chain and the tendency of this group to form disulfides under oxidative conditions. *para*-Methoxybenzyl-protected cysteine was coupled with azidoacetic acid to give **16** (Scheme 3), and the corresponding disulfide (**17**) was made by esterification of cystine followed by amide bond formation with azidoacetic acid. Both of these azides underwent the Cu(I)-catalyzed cycloaddition smoothly to yield protected cyclam conjugates in good yields. The route employing the disulfide **18** was not carried further because it was found that the cyclam in the protected derivative **19** could be deprotected orthogonally to or along with the PMB-protected thiol, to give **20** or **21**, respectively. Ligand **21** was isolated as its trifluoroacetate salt because attempts to isolate the free ligand led to the formation of a byproduct presumed to be the disulfide.

Initial attempts to synthesize metal complexes using **12i–iii** in methanol led to difficulties in bulk purification since the starting materials (free amine ligands and metal perchlorates) and the resultant metal complexes are all methanol-soluble. This problem was solved by using ethanol instead since it was found that the copper(II) and zinc(II) complexes are ethanol-insoluble, allowing them to be isolated as precipitates in good yields. The nickel(II) complexes are slightly soluble in ethanol and were therefore obtained in slightly lower yields. Insertion of metal salts into the PMB-protected cysteine derivative **20** gave the expected complexes, as did the complexation of thiol **21** with Ni(II) and Zn(II). In the case of Cu(II), however, reaction of one equivalent of the copper salt with **21** gave 83% of the Cu(II)–disulfide complex **25**, a result which implies the involvement of atmospheric oxygen (the reaction of **21** with Cu(II) was conducted at reflux and open to the air).[42] The synthesis of complexes **25–27** was achieved directly from the TFA salt **21** and required an extended reaction time. The purity of all of the metal complexes was confirmed by elemental analysis.

Of these complexes, only compound **14ii** gave crystals suitable for X-ray diffraction, grown *via* dissolution of the complex in acetonitrile and forced evaporation under a stream of nitrogen (Figure 4). [Formula, $C_{21}H_{40}Cl_2N_8NiO_{11}$; *M*, 710.22; monoclinic; space group, $P2_1$ (#4); a = 10.9527(5), b = 8.6360(4), c = 16.0493(6) Å; β = 95.490(2); V = 1511.10(11) Å^3; D_c = 1.561 g cm^{-3}; Z = 2; crystal size, 0.275 × 0.077 × 0.060 mm;

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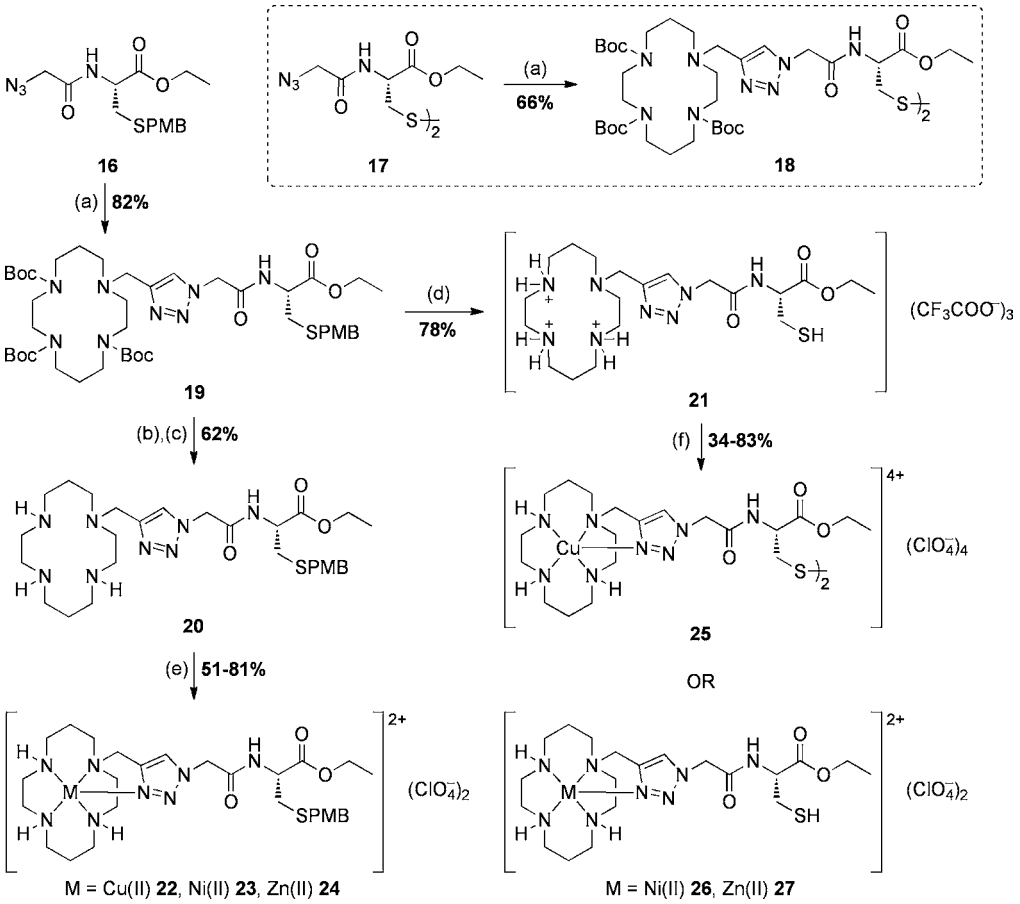
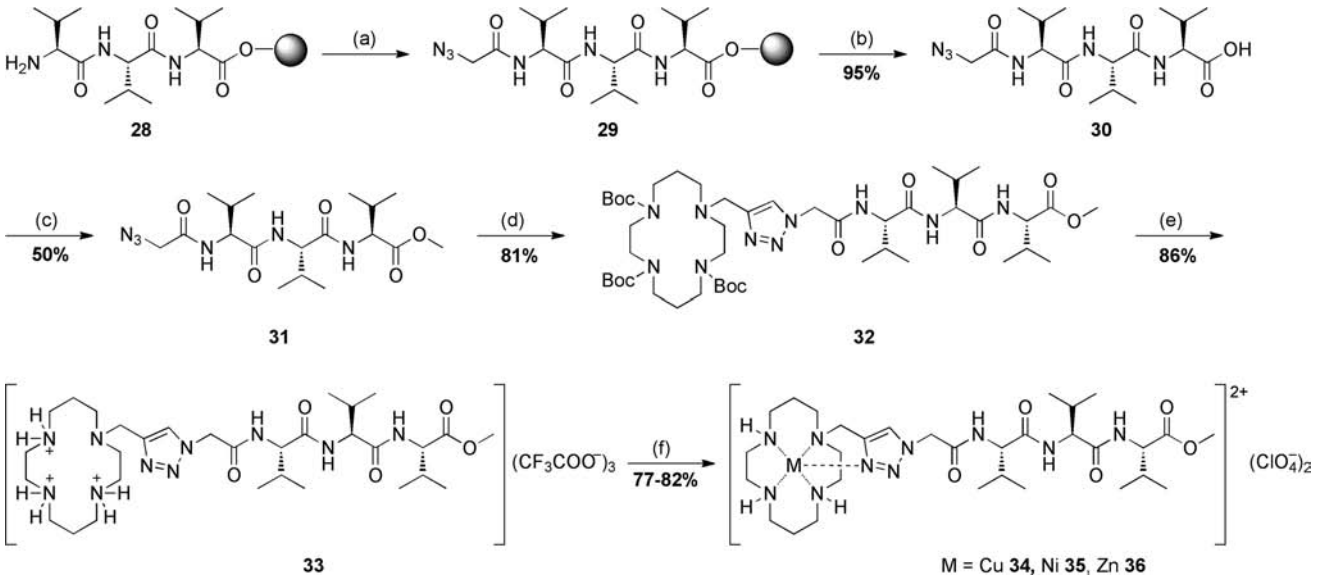
Scheme 3. Synthesis of Metal–Cyclam Complexes Functionalized with Cysteine[a]



[a]*Reagents and conditions:* (a) **4**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*-BuOH, rt, O/N. (b) TFA/DCM/H_2O (90:5:5), rt, 6 h, followed by RP-HPLC purification. (c) Saturated Na_2CO_3, pH 11-12. (d) TFA, anisole, Ar, reflux, 1.5 h, followed by RP-HPLC purification. (e) $M(ClO_4)_2 \cdot 6H_2O$, EtOH, reflux, 1 h. (f) as for (e) except for 3 h reaction time.

Scheme 4. Synthesis of Metal–Cyclam Complexes Functionalized with a Tripeptide[a]



[a]*Reagents and conditions*: (a) **2**-azidoacetic acid, PyBOP, NMM, DMF. (b) TFA/TIS/H_2O (90:5:5), rt, 2 h. (c) $SOCl_2$, MeOH, reflux, 6 h. (d) **4**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*-BuOH, rt, O/N. (e) TFA/DCM/H_2O (90:5:5), rt, 6 h; (f) $M(ClO_4)_2 \cdot 6H_2O$ (M = Cu, Ni, Zn), EtOH, reflux, 6 h.

color pink; habit rod; temperature 150(2) K; λ(Mo Kα), 0.71069 Å; μ(MoKα), 0.888 mm^{-1}; T(SADABS)$_{min,max}$ = 0.850, 1.000; $2\theta_{max}$, 67.3; *hkl* range −16 +17, −10 +13, −25 +24; N, 37588; N_{ind}, 9963(R_{merge} 0.0281); N_{obs}, 8282(I > 2σ(I)); N_{var}, 380;

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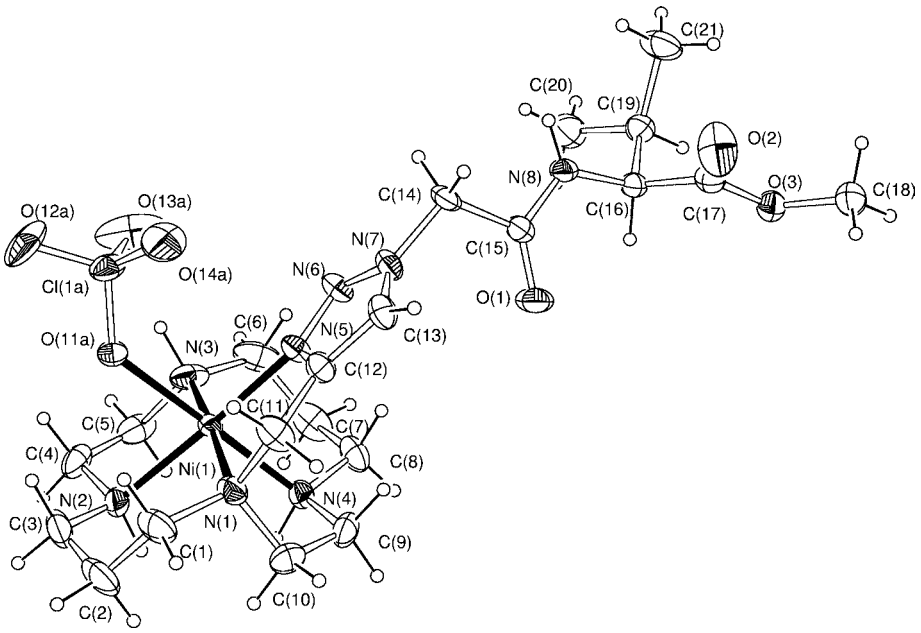

Figure 4. An ORTEP plot of the cation **14ii** at the 50% probability level. The noncoordinated perchlorate anion and minor disordered component of the coordinated perchlorate anion are omitted for clarity. Selected bond lengths (Å) and angles (deg): Ni(1)−N(1), 2.159(2); Ni(1)−N(2), 2.101(2); Ni(1)−N(3), 2.079(2); Ni(1)−N(4), 2.0881(19); Ni(1)−N(5), 2.0691(19); Ni(1)−O(11A), 2.2501(16); Ni(1)−O(11B), 2.1150(14); N(1)−Ni(1)−N(2), 91.55(8); N(1)−Ni(1)−N(3), 174.80(8); N(1)−Ni(1)−N(4), 85.39(8); N(1)−Ni(1)−N(5), 81.18(7); N(1)−Ni(1)−O(11A), 89.01(8); N(1)−Ni(1)−O(11B), 101.32(9); N(2)−Ni(1)−N(3), 84.64(9); N(2)−Ni(1)−N(4), 97.19(7); N(2)−Ni(1)−N(5), 170.12(8); N(2)−Ni(1)−O(11A), 83.94(7); N(2)−Ni(1)−O(11B), 88.55(9); N(3)−Ni(1)−N(4), 91.57(8); N(3)−Ni(1)−N(5), 103.02(8); N(3)−Ni(1)−O(11A), 94.10(8); N(3)−Ni(1)−O(11B), 82.16(9); N(4)−Ni(1)−N(5), 88.92(8); N(4)−Ni(1)−O(11A), 174.30(7); N(4)−Ni(1)−O(11B), 171.09(9); N(5)−Ni(1)−O(11A), 89.23(7); N(5)−Ni(1)−O(11B), 86.36(9); O(11A)−Ni(1)−O(11B), 13.25(8). The bond length between metal center and N donor (2.069 Å) is longer than that seen in a comparable complex (2.044 Å) where there is less steric crowding around the metal[43] but shorter than comparable azamacrocyclic complexes based on scorpionand pyridine donors (2.107 Å).[44].

residuals* $R1(F)$, 0.0407; $wR2(F^2)$, 0.1145; GoF(all), 1.056; $\Delta\rho_{min,max}$, −0.674, 0.903 e$^-$ Å^{-3}. *R1 = $\sum||F_o| - |F_c||/\sum|F_o|$ for $F_o > 2\sigma(F_o)$; wR2 = $(\sum w(F_o^2 - F_c^2)^2/\sum(wF_c^2)^2)^{1/2}$; all reflections $w = 1/[\sigma^2(F_o^2) + (0.0674P)^2 + 0.0154P]$ where $P = (F_o^2 + 2F_c^2)/3$.]

To extend the approach, we utilized these procedures to construct metal−cyclam complexes with an oligopeptide arm. First, an azide-capped tripeptide **30** (Scheme 4) was assembled *via* Fmoc solid phase peptide synthesis (Supporting Information).

The tripeptide was cleaved from the solid support after the synthesis of **29**, rather than continuing the synthesis on the solid phase. While it is possible to use solid supports with linkers designed to generate esters of cleaved materials,[45] initial experiments with methanolysis of **29** anchored instead to HMBA resin gave complex mixtures of products, and these results were inferior to those obtained by solution phase esterification of tripeptide acid **30**. The desired azide-capped tripeptide **30** was released from the resin in excellent overall yield and with >95% purity by analytical HPLC. Esterification of **30** was effected in MeOH in the presence of thionyl chloride, followed by click reaction and acidolytic deprotection to give the corresponding trifluoroacetate salt **33**, which was not basified to recover the free amine ligand but *directly complexed with metal perchlorates* using an extended reaction time (six hours) to afford trivaline-functionalized metal-cyclam complexes **34**−**36**. The complexes could be isolated with centrifugation, a convenient protocol that we expect will be applicable to a wide range of cyclam−peptide metal complexes.

Azamacrocyclic complexes bearing triazole-linked dyes are bioavailable,[34,46] as are azamacrocyclic complexes bearing single amino acids.[47] The metabolic stability of metal-bearing ligands is naturally dependent on the specific coordination environment employed.[48] However, attachment of metal complexes to a peptide can have dramatic effects on the cellular fate of that peptide.[49,50] In this context, the biological activity of the present compounds was assayed against normal human embryonic kidney cells (HEK-293) in triplicate in the range 2.5−40 μM for 3 days. Cell death/viability was measured with Presto Blue, indicating that none of the metal complexes exhibited any cytotoxicity at the doses tested (Supporting Information). The complexes were also assayed against pancreatic (BxPC-3, PANC-1 and SU.86.86), breast (BT-474, MDA-MB-231, MCF-10A (control)), and prostate (LNCaP, PC-3 and RWPE-1 (control)) cancer cell lines. Essentially all compounds were also found to be nontoxic in these cases. The single exception was the disulfide copper complex **25**, which showed moderate activity against MCF-10A breast nontumorigenic epithelial cells.

The results described in this paper suggest the following: (1) Azamacrocyclic complexes bearing amino acid or peptide-based groups may be easily synthesized using click methods, and such an approach offers an attractive alternative to more traditional conjugation techniques. (2) Metal insertion into these conjugates is rapid and proceeds with well-defined stoichiometries. (3) The syntheses do not lead to epimerization of the amino acid stereocenters, but care must be taken if complexes involve stereocenters between triazole rings and adjacent carbonyl groups. (4) A range of amino acids and a tripeptide have been shown to be compatible with the chemistry, and it is expected that longer polypeptides and other amino acids will be similarly compatible. (5) The ligands and their complexes are

nontoxic to a range of cell types, and concentrations up to 40 μM, suggesting that labeled complexes of this general design could be useful tools for imaging and therapeutic applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental descriptions of the syntheses of known compounds and all solid phase procedures. Scans of ^{1}H and ^{13}C NMR spectra for all novel compounds, including Zn(II) complexes. HPLC data for epimerization experiments. Crystallographic information for compounds **9** and **14ii**. HRMS for all amino acid and peptide metal complexes compared to simulated spectra. Bioactivity data from compound screens. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Mewis, R. E.; Archibald, S. J. *Coord. Chem. Rev.* **2010**, *254*, 1686–1712.
(2) Metzler-Nolte, N. In *Topics in Organometallic Chemistry*; Jaouen, G., Metzler-Nolte, N., Eds.; Springer: Berlin, 2010; Vol. *32*, pp 195–217.
(3) Dirscherl, G.; König, B. *Eur. J. Org. Chem.* **2008**, *2008*, 597–634.
(4) Heinze, K.; Beckmann, M.; Hempel, K. *Chem.—Eur. J.* **2008**, *14*, 9468–9480.
(5) Reichert, D. E.; Lewis, J. S.; Anderson, C. J. *Coord. Chem. Rev.* **1999**, *184*, 3–66.
(6) Kremer, C.; Torres, J.; Dominguez, S.; Mederos, A. *Coord. Chem. Rev.* **2005**, *249*, 567–590.
(7) Wu, W.; Lei, P.; Liu, Q.; Hu, J.; Gunn, A. P.; Chen, M.; Rui, Y.; Su, X.; Xie, Z.; Zhao, Y.-F.; Bush, A. I.; Li, Y. *J. Biol. Chem.* **2008**, *283*, 31657–31664.
(8) Lau, Y. H.; Rutledge, P. J.; Watkinson, M.; Todd, M. H. *Chem. Soc. Rev.* **2011**, *40*, 2848–2866.
(9) Struthers, H.; Mindt, T. L.; Schibli, R. *Dalton Trans.* **2010**, *39*, 675–696.
(10) Schmidt, F.; Rosnizeck, I. C.; Spoerner, M.; Kalbitzer, H. R.; König, B. *Inorg. Chim. Acta* **2011**, *365*, 38–48.
(11) Lebedev, A. Y.; Holland, J. P.; Lewis, J. S. *Chem. Commun.* **2010**, *46*, 1706–1708.
(12) Martin, M. E.; Parameswarappa, S. G.; ODorisio, M. S.; Pigge, F. C.; Schultz, M. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4805–4807.
(13) Bogdan, N. D.; Matache, M.; Meier, V. M.; Dobrotă, C.; Dumitru, I.; Roiban, G. D.; Funeriu, D. P. *Chem.—Eur. J.* **2010**, *16*, 2170–2180.
(14) Pfeiffer, H.; Rojas, A.; Niesel, J.; Schatzschneider, U. *Dalton Trans.* **2009**, 4292–4298.
(15) Köster, S. D.; Dittrich, J.; Gasser, G.; Hüsken, N.; Henao Castañeda, I. C.; Jios, J. L.; Della Védova, C. O.; Metzler-Nolte, N. *Organometallics* **2008**, *27*, 6326–6332.
(16) Knör, S.; Modlinger, A.; Poethko, T.; Schottelius, M.; Wester, H.-J.; Kessler, H. *Chem.—Eur. J.* **2007**, *13*, 6082–6090.
(17) Mindt, T. L.; Struthers, H.; Brans, L.; Anguelov, T.; Schweinsberg, C.; Maes, V.; Tourwé, D.; Schibli, R. *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.
(18) Wadas, T. J.; Wong, E. H.; Weisman, G. R.; Anderson, C. J. *Chem. Rev.* **2010**, *110*, 2858–2902.
(19) Tweedle, M. F. *Acc. Chem. Res.* **2009**, *42*, 958–968.
(20) Major, J. L.; Meade, T. J. *Acc. Chem. Res.* **2009**, *42*, 893–903.
(21) Liu, S. *Adv. Drug Delivery Rev.* **2008**, *60*, 1347–1370.
(22) Cowley, A. R.; Dilworth, J. R.; Donnelly, P. S.; Heslop, J. M.; Ratcliffe, S. J. *Dalton Trans.* **2007**, 209–217.
(23) Mizukami, S.; Takikawa, R.; Sugihara, F.; Shirakawa, M.; Kikuchi, K. *Angew. Chem., Int. Ed.* **2009**, *48*, 3641–3643.
(24) Tamanini, E.; Rigby, S. E. J.; Motevalli, M.; Todd, M. H.; Watkinson, M. *Chem.—Eur. J.* **2009**, *15*, 3720–3728.
(25) Lattuada, L.; Gabellini, M. *Synth. Commun.* **2005**, *35*, 2409–2413.
(26) Kunstler, J.-U.; Seidel, G.; Pietzsch, H.-J. *Appl. Radiat. Isot.* **2010**, *68*, 1728–1733.
(27) Bowen, M. L.; Orvig, C. *Chem. Commun.* **2008**, 5077–5091.
(28) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(29) Lau, Y. H.; Price, J. R.; Todd, M. H.; Rutledge, P. J. *Chem.—Eur. J.* **2011**, *17*, 2850–2858.
(30) Cai, J.; Li, X.; Yue, X.; Taylor, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16324–16325.
(31) Banaszynski, L. A.; Liu, C. W.; Wandless, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 4715–4721.
(32) Fabbrizzi, L.; Foti, F.; Licchelli, M.; Maccarini, P. M.; Sacchi, D.; Zema, M. *Chem.—Eur. J.* **2002**, *8*, 4965–4972.
(33) Lo, A. T. S.; Salam, N. K.; Hibbs, D. E.; Rutledge, P. J.; Todd, M. H. *PLoS One* **2011**, *6*, e17446.
(34) Tamanini, E.; Katewa, A.; Sedger, L. M.; Todd, M. H.; Watkinson, M. *Inorg. Chem.* **2009**, *48*, 319–324.
(35) Ritter, S. C.; Eiblmaier, M.; Michlova, V.; König, B. *Tetrahedron* **2005**, *61*, 5241–5251.
(36) Wood, W. J. L.; Patterson, A. W.; Tsuruoka, H.; Jain, R. K.; Ellman, J. A. *J. Am. Chem. Soc.* **2005**, *127*, 15521–15527.
(37) Ko, E.; Liu, J.; Perez, L. M.; Lu, G.; Schaefer, A.; Burgess, K. *J. Am. Chem. Soc.* **2010**, *133*, 462–477.
(38) MacCarthy, P. *Anal. Chem.* **1978**, *50*, 2165–2165.
(39) Lindoy, L. F. *The Chemistry of Macrocyclic Ligand Complexes*; Cambridge University Press: Cambridge, U. K., 1989.
(40) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. *Tetrahedron Lett.* **1989**, *30*, 2739–2742.
(41) Armarego, W. L. F.; Chai, C. L. L. *The Purification of Laboratory Chemicals*, 6th ed.; Butterworth-Heinemann: Burlington, MA, 2009.
(42) Ohta, T.; Tachiyama, T.; Yoshizawa, K.; Yamabe, T.; Uchida, T.; Kitagawa, T. *Inorg. Chem.* **2000**, *39*, 4358–4369.
(43) Zheng, Z.-B.; Wu, R.-T.; Li, J.-K.; Sun, Y.-F. *J. Mol. Struct.* **2008**, *928*, 78–84.
(44) Ghachtouli, S. E.; Cadiou, C.; Déchamps-Olivier, I.; Chuburu, F.; Aplincourt, M.; Patinec, V.; Baccon, M. L.; Handel, H.; Roisnel, T. *New J. Chem.* **2006**, *30*, 392–398.
(45) Dirscherl, G.; Knape, R.; Hanson, P.; König, B. *Tetrahedron* **2007**, *63*, 4918–4928.
(46) Jobe, K.; Brennan, C. H.; Motevalli, M.; Goldup, S. M.; Watkinson, M. *Chem. Commun.* **2011**, *47*, 6036–6038.
(47) Lattuada, L.; Dematti o, S.; Vincenzi, V.; Cabella, C.; Visigalli, M.; Aime, S.; Crich, S. G.; Gianolio, E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4111–4114.
(48) Boswell, C. A.; Sun, X.; Niu, W.; Weisman, G. R.; Wong, E. H.; Rheingold, A. L.; Anderson, C. J. *J. Med. Chem.* **2004**, *47*, 1465–1474.
(49) Neundorf, I.; Hoyer, J.; Splith, K.; Rennert, R.; Peindy Ndongo, H. W.; Schatzschneider, U. *Chem. Commun.* **2008**, 5604–5606.
(50) Noor, F.; Kinscherf, R.; Bonaterra, G. A.; Walczak, S.; Wölfl, S.; Metzler-Nolte, N. *ChemBioChem* **2009**, *10*, 493–502.