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A new high-capacity metal ion-complexing gel containing cyclen

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We describe a new polymeric hydrogel that binds divalent metal ions with large binding constants and capacity. A new acrylate monomer with pendant cyclen, tri-*tert*-butyl-10-(2-(2-(2-(methacryloyloxy)ethoxy)ethoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate, was polymerised by RAFT to a roughly spherical 5-9 unit oligomer bearing a carboxylate-functionalised end-group. This was de-protected and coupled to an amine dendrimer then crosslinked into a nylon-type hydrogel capable of absorbing more than 10 wt% copper ions.

Introduction

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Metallopolymers may possess diverse kinetic, thermodynamic, physical, chemical and structural properties which derive from the presence of the metal ions.^{1, 2} Polyazamacrocycles as ligands are of particular interest and amongst these, cyclens (1,4,7,10tetraazacyclododecane) are very efficient in the complexing of metal ions³⁻¹⁰ and have a very low toxicity.¹¹ These macrocycles tend to fold around the metal ions resulting in octahedral or trigonal bipyramidal coordination.^{12, 13} Polyazamacrocycles typically give complexes of higher thermodynamic and kinetic stability than the corresponding open chain ligands as a result of the chelate effect.^{14, 15} Metal-cyclen complexes contain up to two other metal coordination sites which can bind to anions such as phosphates, amino acids, DNA and peptides, which opens up possibilities to target specific biomolecules^{3, 16-19} and, when functionalised with fluorescent ligands, have been used for sensing anions.^{12, 20-23}

ligands

Brimble^{a,b} and David E. Williams^{a,b}*

Whilst there has been a wealth of literature published regarding the synthesis of functional azamacrocycle analogues for coordination chemistry, to date there have been relatively few attempts to incorporate them into polymers designed to coordinate metals⁷ and these have been directed at attaching the ligand post-polymerisation. Meyerhoff and co-workers detail the synthesis of a Cu²⁺-cyclen complex covalently attached to crosslinked poly(hydroxyethyl methacrylate) polymers (PHEMA).⁵ Subsequent reports^{24, 25} improved upon the synthesis of derivatised polyurethane to contain nitric oxide-generating Cu²⁺-cyclens pendent to the polymer backbone. More recently, Huang *et al.*⁸ also described a method of cyclen functionalisation yielding high molecular weight (M_w = 11,400 g mol⁻¹) polycyclens.

Here we report the synthesis of a novel monomer: tri-tert-

butyl 10-(2-(2-(2-(methacryloyloxy)ethoxy)ethoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (1, Fig. 1) via the acrylate 2 (Scheme 1), its polymerisation to Bocprotected oligomers (**3-Boc, 4-Boc, 5-Boc**), deprotection to give cyclen-appended oligomers (**3, 4, 5**, Fig. 1), characterisation of these oligomers and of their metal- and anion-dependent aggregation, and cross-linking of **5** into a metal ion-complexing hydrogel. We suggest that the hydrogel comprises crosslinked, roughly spherical assemblies with the pendant cyclens dangling free on the periphery.



Fig 1. Chemical structures of Boc-protected, cyclen-containing monomer 1, RAFT polymers **3-Boc** and **5-Boc**, and uncontrolled polymer **4-Boc**.

Experimental

Instrumentation

¹H NMR and ¹³C NMR spectra were recorded at room temperature on a Bruker AC 400 MHz spectrometer using CD₃OD-d₄ or CDCl₃ as solvent, unless otherwise stated. Chemical shifts (δ) are reported relative to the residual proton peak of the solvent used. Mass spectrometry experiments were carried out on a micrOTOF-Q instrument using electro-spray ionisation (ESI) with a positive ion polarity. GPC analysis was performed on a Viscotek VE2001 GPC Max module with a Viscotek TDA 305 detector and all samples were filtered prior to analysis (0.22 µm membrane filter). Polystyrene standards, 95 kDa and 235 kDa, were used as calibration standards for the GPC. THF was used as eluent with a flow rate of 1.0 mL min⁻¹. An estimated dn/dC value of 0.185 cm³ g⁻¹ was used for all the analyses of all GPC data. The theoretical number-average molar mass (M_{n,theo}) was calculated using the following equation M_{n,theo} = M_{CTA} + M_{monomer} ([Monomer]/[CTA] x fraction conversion), where M_{CTA} and M_{monomer} are the molar masses of RAFT agent and monomer, respectively. The contribution of the molar mass of the chains

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Electronic Supplementary Information (ESI) available: NMR, FTIR spectra; GPC analysis; X-ray diffractometry; scanning electron microscopy and energy-dispersive X-ray analysis; dynamic light scattering results; uv-vis and EPR spectroscopy; images. See DOI: 10.1039/x0xx00000x

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initiated by AIBN was neglected.²⁶ Basic molecular modelling was carried out using Avogadro²⁷ software using the following settings - force field: Ghemical, number of steps: 1000, algorithm: steepest descent and convergence: 1 x 10⁻⁶. Infrared data (v_{max}) was collected using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Micro analysis was performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. DLS measurements were performed on Malvern Zetasizer nano (ZPS) and all solutions were filtered through a Millipore membrane with a pore size of 0.22 μ m prior to analysis. All measurements were averaged from >50 individual sample readings. UV-visible spectroscopy was performed using a Shimadzu UV-1700 PharmaSpec; all measurements were performed at room temperature in plastic cuvettes (1 cm) and were corrected for background absorption. The transmittance (T) of the solution was measured at a specified wavelength and the turbidity of the solution was then calculated as (100-T)/100. Solid state UV-vis measurements of copper-complexed crosslinked gel networks were recorded on an Ocean Optics S2000 spectrophotometer using diffuse white light as reference. The reflectance probe was focussed to ca. 1 mm diameter with custom optics. Spectra were recorded using 100 ms integration time for an average of 10 spectra with no boxcar averaging. Light source was an Ocean Optics DH-2000-BAL and spectra were analysed using 00Base32 (Ocean Optics) software. Melting points were recorded in air on an electrothermal melting point apparatus and are uncorrected. SEM images were obtained by placing solid samples on copper conducting tape. The samples were then coated with platinum using a Quorum Q150RS sputter coater (20 mA for 5 mins). Images were then recorded using a Philips XL30S FEG (FEG = Field Emission Gun) SEM with a SiLi (lithium drifted) EDAX-EDS detector with a super ultra-thin window. Accelerating voltages of 5 kV were used. XRD analysis was performed on a Bruker D2 PHASER (Cu- K_{α}). Samples were placed on a single crystal stage and analysed in the solid state without any further sample preparation. EPR spectra were recorded of the powder samples in Suprasil quartz tubes, using a JEOL JES-FA200 spectrometer under identical conditions; magnetic centre field: 325.919 mT (as calibrated by using TEMPOL standard), power: 1 mW, microwave frequency: 9108.897 MHz, modulation frequency: 100 kHz, modulation width: 0.35 mT, amplitude: 20, scan time: 2 min, time constant: 0.03 s. G-values are taken as the point in which the EPR signal crosses the x-axis. The swelling ratios for the crosslinked dendrimer gels were determined by immersing the crosslinked polymer in deionised water for 30 mins at room temperature and results were calculated using the following equation: mass swelling ratio = mass of swollen polymer/mass of dry polymer.

Materials

Silica gel flash chromatography was performed using Davisil silica gel (LC60A 40-63 micron) and alumina chromatography was performed using an ECP activated basic (Brockmann grade 1, 58 angstroms) stationary phase. All solvents and reagents were of technical or analytical grade and were used as received unless otherwise stated. 2.2'-Azobis(isobutyronitrile) (AIBN)

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Synthesis of 2-(2-hydroxyethoxy)ethyl methacrylate

A two-neck round bottom flask equipped with a Dean-Stark apparatus and a condenser was charged with *p*-toluenesulfonic acid monohydrate (380 mg, 2 mmol), toluene (130 mL), diethylene glycol (7.67 mL, 80 mmol) and 4-methoxyphenol (5 mg, 0.2 mol%) as inhibitor. The mixture was heated at reflux for 1 h to remove water. Methacrylic acid (1.7 mL, 20 mmol) was then added and the reaction mixture was stirred at reflux for 1.5 h. The solvent was removed under vacuum and the residue purified by flash chromatography on silica gel was (Hexane/EtOAc: 3/2 to 100% EtOAc) to afford the title compound as a colourless oil (2.79 g, 80%). A few crystals of 4methoxyphenol were added for long-term storage. IR Umax (neat, cm⁻¹): 3428, 2953, 2928, 2876, 1713, 1636, 1453, 1319, 1297, 1167, 1127, 1067, 1039, 942, 888, 815, 654. ¹H NMR (400 MHz, CDCl₃) δ_H (ppm): 6.11 (s, 1H), 5.66 (s, 1H), 4.30 (t, 2H), 3.75-3.70 (m, 4H), 3.59 (t, 2H), 2.32 (br s, 1H), 1.93 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ_c (ppm): 167.3, 136.0, 125.8, 72.3, 69.1, 63.7, 61.6, 18.2. HRMS (ESI) *m/z* calculated for C₈H₁₄NaO₄ [M+Na]⁺ 197.0784, found 197.0788.

Synthesis of 2-(2-(2-bromoacetoxy)ethoxy)ethyl methacrylate 2

To a solution of 2-(2-hydroxyethoxy)ethyl methacrylate containing inhibitor (1.63 g, 9.1 mmol) in chloroform (30 mL), potassium carbonate (1.87 g, 13.5 mmol) was added at 0 °C under a nitrogen environment. Next, 2-bromoacetyl bromide (800 μ L, 9.0 mmol) was added dropwise with stirring at 0 $^{\circ}$ C under nitrogen. The reaction was allowed to warm to room temperature and stirred for 15 h. The reaction mixture was then filtered and the filtrate was evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (Hexane:EtOAc, 4:1 v/v) to afford the pure product as a colourless oil (2.40 g, 90%). IR v_{max} (neat, cm⁻¹): 2959, 2932, 2883, 1738, 1714, 1636, 1453, 1318, 1284, 1165, 1126, 1036, 947, 815, 660. 1 H NMR (400 MHz, CDCl₃) δ_{H} (ppm): 6.13 (s, 1H), 5.58 (s, 1H), 4.34-4.29 (m, 4H), 3.86 (s, 2H), 3.76-3.73 (m, 4H), 1.95 (dd, J₁ = 1.2 Hz, J₂ = 1.3 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) $\delta_{\rm C}$ (ppm): 167.1, 167.0, 135.9, 125.6, 69.0, 68.5, 65.0, 63.5, 25.6, 18.1. HRMS (ESI) m/z calculated for C₁₀H₁₅⁷⁹BrNaO₅ [M+Na]⁺ 316.9995, found 317.0005 and *m/z* calculated for $C_{10}H_{15}^{81}BrNaO_5$ [M+Na]⁺ 318.9975, found 318.9987.

Synthesis of tri-tert-butyl-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (Boc-cyclen)

Boc-cyclen was prepared as described by Kimura *et al.*⁹ A solution of di-*tert*-butyl dicarbonate (1.83 mL, 8 mmol) in chloroform (10 mL, passed through basic alumina) was added slowly over 3 h (syringe pump) to a solution of cyclen (517 mg, 3 mmol) and triethylamine (1.13 mL, 8 mmol) in chloroform (40 mL) at room temperature. The reaction mixture was stirred for 24 h at room temperature and the solvent was evaporated under reduced pressure. The crude product was purified by

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silica gel column chromatography (hexane:EtOAc, 2:3 v/v) to afford the pure *Boc-cyclen* as a white solid (1.25 g, 88%). ¹H NMR (400 MHz, CDCl₃) δ_H (ppm): 3.59 (m, 4H), 3.34-3.24 (m, 8H), 2.80 (m, 4H), 1.44 (s, 9H), 1.42 (s, 18H).

Synthesis of tri-tert-butyl 10-(2-(2-(methacryloyloxy)ethoxy)ethoxy)-2-oxoethyl)-1,4,7,10- tetraazacyclododecane-1,4,7-tricarboxylate 1

A solution of 2-(2-(2-bromoacetoxy)ethoxy)ethyl methacrylate 2 (325 mg, 1.1 mmol) in acetonitrile (5 mL) was added to a mixture of Boc-cyclen (473 mg, 1.0 mmol) and K₂CO₃ (166 mg, 1.2 mmol) in acetonitrile (28 mL) at room temperature. After the reaction mixture was stirred for 22 h at 70 °C, the solution was allowed to cool to room temperature and dichloromethane was added to the reaction which was then washed with water and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (CH₂Cl₂:MeOH, 95:5 v/v) to afford the title compound 1 as a colourless gum (458 mg, 67%). IR u_{max} (neat, cm⁻¹): 2976, 1681, 1413, 1365, 1247, 1155, 732. ^1H NMR (400 MHz, CDCl₃) δ_{H} (ppm): 6.12 (s, 1H), 5.60 (s, 1H), 4.28 (t, 2H), 4.23 (t, 2H), 3.73-3.68 (m, 4H), 3.53-3.46 (m, 7H), 3.39 (m, 2H), 3.32 (m, 5H), 2.94-2.91 (m, 4H), 1.94 (dd, J₁ = 1.3 Hz, J₂ = 1.1 Hz, 3H), 1.45 (s, 9H), 1.43 (s, 18H). ¹³C NMR (100 MHz, CDCl₃) $\delta_{\rm C}$ (ppm): 171.1, 170.5, 167.3, 155.7, 155.3, 136.1, 125.8, 79.7, 79.5, 79.3, 69.1, 69.0, 63.7, 63.2, 54.9, 53.5, 51.0, 50.0, 47.7, 47.4, 47.1, 28.7, 28.5, 18.3. HRMS (ESI) m/z calculated for C₃₃H₅₈N₄NaO₁₁ [M+Na]⁺ 709.3994, found 709.3984.

RAFT polymerization to form Boc-protected polymer 3-Boc

2-Cyano-2-propyl benzodithioate (0.66 mg, 0.003 mmol), monomer 1 (100 mg, 0.146 mmol), AIBN (0.16 mg, 0.001 mmol) and DMF (~1 mL) were added to a Schlenk tube. The mixture was degassed by bubbling with N_2 for 40 mins and the vessel was evacuated and backfilled with N₂ (3 times) and left stirring overnight at 80 °C. The polymerization was quenched in liquid N_2 and a small amount of the crude solution was dissolved in CDCI_3 to confirm the polymer formation by ${}^1\mathsf{H}\,\mathsf{NMR}.$ The solvent was then evaporated under high vacuum. To purify the polymer, the residue was dissolved in a small amount of CHCl₃ and precipitated into a relatively large volume of cold hexane (~100 mL). The suspension was centrifuged at 1500 rpm for 20 mins and the purification process was repeated three times to yield the pure off-white coloured polymer 3-Boc (60 mg, 60% based on monomer weight conversion). GPC: M_w (daltons) = 3,684; M_w/M_n = 2.304. IR *u*_{max} (neat, cm⁻¹): 3457, 2975, 1731, 1678, 1456, 1391, 1414, 1364, 1247, 1156, 1039, 978, 887, 859, 752, 665. ¹H NMR (400 MHz, CDCl₃) δ_H (ppm): 4.31-3.90 (m), 3.64 (m), 3.53-3.29 (m), 2.90-2.88 (m), 2.00-1.78 (m), 1.49-1.39 (m), 1.12-0.81 (m). M.pt (°C): 165-172.

Uncontrolled polymerization to form Boc-protected polymer 4-Boc

Monomer 1 (87 mg, 0.127 mmol), AIBN (1.9 mg, 0.011 mmol) and DMF (~1 mL) were added to a Schlenk tube. The mixture was degassed by bubbling with N_2 for 40 mins. The vessel was

evacuated and backfilled with N₂ (3 times) and definition overnight at 80 °C. The polymerization was defined in N₂ and a small amount of the crude solution was dissolved in CDCl₃ to confirm the polymer formation by ¹H NMR. The solvent was then evaporated under high vacuum. To purify the polymer, the residue was dissolved in a small amount of CHCl₃ and precipitated into a relatively large volume of cold hexane (~100 mL). The suspension was centrifuged at 1500 rpm for 20 mins and the purification process was repeated three times to

mins and the purification process was repeated three times to yield the pure off-white coloured *polymer* **4-Boc** (35 mg, 52% based on monomer weight conversion). GPC: M_w (daltons) = 4,463; M_w/M_n = 2.413. IR ν_{max} (neat, cm⁻¹): 3452, 2974, 1731, 1682, 1459, 1415, 1345, 1248, 1158, 979, 859, 772. ¹H NMR (400 MHz, CDCl₃) δ_H (ppm): 4.35-4.02 (m), 3.58 (m), 3.65-3.20 (m), 3.11-2.81 (m), 2.05-1.72 (m), 1.63-1.38 (m), 1.17-0.66 (m). M.pt (°C): 160-169.

RAFT polymerization to form Boc-protected polymer 5-Boc

was set for to RAFT ratio Monomer 6:1. 3-(((Benzylsulfanyl)thiocarbonyl)sulfanyl)propionic acid (22 mg, 0.08 mmol), monomer 1 (332 mg, 0.48 mmol), AIBN (4.3 mg, 0.026 mmol) and DMF (2 mL) were added to a Schlenk tube. The mixture was degassed by bubbling with N₂ for 40 mins and the vessel was evacuated and backfilled with N₂ (3 times) and left stirring for 22 h at 80 °C. The polymerization was quenched in liquid N2 and a small amount of the crude solution was dissolved in CDCl₃ to confirm the polymer formation by ¹H NMR. The solvent was then evaporated under high vacuum. To purify the polymer, the residue was dissolved in a small amount of CHCl₃ and precipitated into a relatively large volume of cold hexane (~100 mL). The suspension was centrifuged at 1500 rpm for 20 mins and the purification process was repeated three times to yield the pure light yellow coloured polymer 5-Boc (173 mg, 52% based on monomer weight conversion). GPC: Mw (daltons) = 6,491; M_w/M_n = 1.592. IR v_{max} (neat, cm⁻¹): 3332, 2944, 2832, 1448, 1417, 1114, 1023, 839, 770. 1 H NMR (400 MHz, CDCl₃) δ_H (ppm): 4.23-4.09 (m), 3.74 (m), 3.54-3.32 (m), 2.91 (m), 1.72-1.64 (m), 1.48-1.42 (m), 1.28-0.97 (m). M.pt (°C): 159-170.

General procedure for the Boc-deprotection to form oligomers 3, 4, 5

A solution of TFA:CH₂Cl₂ (1:2 v/v, 1.5 mL) was added to the protected oligomer (20 mg) and was left stirring at room temperature for 4 h. The solution was then evaporated to dryness and co-evaporated with CH₂Cl₂:MeOH (15 mL, 2:1 v/v, 2 times), washed with Et₂O and hexane, and finally left to dry on high vacuum to yield the desired deprotected oligomers as yellow gums. Boc group deprotection of all oligomers was confirmed by the absence of Boc protons at δ_H ~1.5 ppm in all ¹H NMR spectra. The consequential estimated molecular weights of the oligomers were then calculated to be equal to (Daltons): **3**; 2,169, **4**; 2,645, **5**; 3,791.

General procedure for the metalation of oligomers 3, 4, 5

Oligomer (16 mg) was initially dissolved in methanol (2 mL). To this a solution of metal-containing compound (dissolved in methanol, or other suitable solvent, 0.5 mL) was titrated in with

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stirring, allowing 5 mins equilibration time between each titration. The quantity of metal ions titrated was dependent on the number of cyclen moieties available for complexation. For example; a solution containing ZnCl₂ (5 mg) titrated into a solution containing 3 (16 mg) is equal to 1 molar equivalent. The resulting precipitate, if any, was then centrifuged (7000 rpm for 15 mins) and washed with methanol (3 times) in order to remove the metal ions that were adsorbed rather than actually complexed by the cyclen moiety. The metallopolymers were then dried under high vacuum ready for further analysis. 3-**CuCl₂**: IR v_{max} (neat, cm⁻¹): 3426, 3217, 2921, 2362, 1722, 1638, 1449, 1377, 1187, 1127, 1074, 963, 859, 747. Elemental analysis (%); C:38.02, H:6.05, N:7.92, Cu:14.2. M.pt (°C): 187-213. 4-CuCl-2: IR U_{max} (neat, cm⁻¹): 3420, 3217, 2947, 2361, 1721, 1636, 1456, 1378, 1186, 1127, 1074, 964, 859, 747. Elemental analysis (%); C:31.79, H:5.51, N:6.67, Cu:12.8. M.pt (°C): 195-216.

Synthesis of 5-PAMAM (G0)

The terminal carboxyl group of polymer **5** (53 mg, 0.014 mmol) was activated with EDC·HCl (8 mg, 0.042) and NHS (8 mg, 0.07) by stirring in methanol for 30 mins at room temperature. The PAMAM (G0) dendrimer (7.2 mg, 0.014 mmol, 20% wt. in methanol) was added to the reaction dropwise and the solution was left stirring at room temperature for 20 h. The reaction mixture was then concentrated and the conjugate was purified by precipitating into Et₂O (50 mL, 3 times) to leave **5-PAMAM** (**G0**) as a light brown solid (46 mg, 79%). IR v_{max} (neat, cm⁻¹): 3378, 2948, 2036, 1639, 1550, 1439, 1365, 1251, 1200, 1778, 1129, 835, 800, 721. ¹H NMR (400 MHz, CD₃OD-d₄) δ_H (ppm): 7.66-7.53 (m, 5H), 4.24 (m), 4.08 (m), 3.70-3.60 (m), 3.56-3.47 (m), 3.29 (m), 3.24 (m), 3.01 (m), 2.92 (m), 2.79 (m), 2.49-2.41 (m), 1.95-1.80 (m), 1.23 (m), 1.07-0.95 (m), 0.90-0.83 (m).

Synthesis of crosslinked-5-PAMAM (G0)

5-PAMAM (G0) (57 mg, 0.01 mmol) and PAMAM (G0) dendrimer (11 mg, 0.02 mmol, 20% wt. in methanol) were dissolved in deionized water (2 mL). Sebacoyl chloride (12 mg, 0.05 mmol) dissolved in hexane (2 mL) was slowly added to the aqueous layer without mixing. Tweezers were used to remove the crosslinked network that had formed at the interface between the two immiscible liquids. The polymer was then washed thoroughly with a saturated solution of NaHCO₃ and methanol to remove any free dendrimer monomer units and free oligomer to leave an off-white coloured solid product *crosslinked-5-PAMAM (G0)* (11 mg). Mass swelling ratio: 11.2. IR u_{max} (neat, cm⁻¹): 3293, 2927, 2850, 1811, 1736, 1585, 1413, 1315, 1254, 1205, 1126, 876, 722, 678. M.pt (°C): 201-214.

Synthesis of crosslinked-PAMAM (G0)

PAMAM (G0) dendrimer (100 mg, 0.194 mmol, 20% wt. in methanol) was dissolved in deionized water (3.5 mL). Sebacoyl chloride (232 mg, 0.97 mmol) dissolved in hexane (4 mL) was slowly added to the aqueous layer without mixing. Polymerization occurred at the interface between the 2 immiscible liquids and tweezers were used to collect the fibres. The crosslinked polymer was then washed thoroughly with a saturated solution of NaHCO₃ and methanol to remove any free

dendritic monomers to leave a white coloured solid product (187 mg). Mass swelling ratio: 8.5. IR ν_{mg} : (hear, cm²), 2932, 2851, 2609, 1739, 1689, 1561, 1465, 1430, 1409, 1352, 1325, 1299, 1254, 1239, 1187, 1048, 927, 754, 724, 678. M.pt (°C): 198-207.

General procedure for copper metallation of crosslinked-5-PAMAM (G0) and crosslinked-PAMAM (G0) dendrimers

To an Eppendorf tube containing CuCl₂ (5 mg, 0.037 mmol) dissolved in methanol (0.5 mL), *crosslinked-5-PAMAM (GO)* or *crosslinked-PAMAM (GO)* (10 mg) was added. The solution was then left standing at room temperature for 30 mins. Excess Cu²⁺ was removed from the dendrimer gel networks by washing with methanol (4 times) and centrifuging (7000 rpm for 15 mins) to isolate the product. The crosslinked networks were then dried under high vacuum to yield the green and light blue coloured products, respectively. *Cu-crosslinked-5-PAMAM (GO)*: Copper (%): 10.92. *Cu-crosslinked-PAMAM (GO)*: Copper (%): 1.93.

Results and discussion

The syntheses of the monomer 1 and polymer 3 are outlined in Scheme 1 and full details are given in the experimental section. Polymerisation was either by reversible addition-fragmentation chain transfer (RAFT) using 2-cyano-2-propyl benzodithioate or 3-(((benzylsulfanyl)thiocarbonyl)sulfanyl)propionic acid to give 3-Boc or 5-Boc, respectively, or by an uncontrolled free-radical with 2,2'-azobis(2-methylpolymerisation initiated propionitrile), AIBN, to give 4-Boc. The RAFT agents were chosen due to their compatibility with methacrylate monomers,²⁶ and for **5-Boc**, the presence of the terminal carboxyl group suitable for post-polymerisation functionalisation. The RAFT synthesized homo-polymers 3-Boc and 5-Boc were achieved using 1 equivalent of chain transfer agent and 0.3 equivalents of AIBN initiator in N₂-purged DMF.

The uncontrolled polymerisation to form **4-Boc** was achieved using 9 mol% AIBN initiator in DMF. All polymers were purified by precipitation in Et₂O three times and their monomer weight conversions recorded (52-60%, Table 1). The products **3-Boc**, **4-Boc** and **5-Boc** were characterised by ¹H NMR spectroscopy (supporting information) and gel-permeation chromatography to determine the weight average molecular weight (M_w) and polydispersity index (M_w/M_n).

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Table 1. Characteristics of polymers 3-Boc, 4-Boc and 5-Boc.

Polymer	method	Monomer conversion	M _{w,exp} ^c	M _w /M _n ^c	Average n	
		(weight %)	(Daltons)			DOI: 10.1039/C6RA006040
3-Boc	RAFT ^a	60	3,684	2.3	5	
4-Boc	uncontrolled	52	4,463	2.4	6	
5-Boc	RAFT^b	52	6,491	1.6	9	

a RAFT agent = 2-cyano-2-propyl benzodithioate

^b RAFT agent = 3-(((benzylsulfanyl)thiocarbonyl)sulfanyl)propionic acid

^c Weight average molecular weight (M_w) and polydispersity index (M_w/M_n) determined by gel-permeation chromatography analysis.

Table 2. F	Precipitation u	pon addition of	metal salts to oligomer	3 in solution in methanol
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Salt	NaCl	CaCl₂	CoCl₂	CuCl ₂	ZnCl₂	Zn(OTf) ₂	Zn(NO ₃) ₂	ZnSO₄	FeCl₃
Precipitate	No	No	Yes	Yes	Yes	No	Yes	Yes	No
Molar equivalents ^a			0.9	1.2	0.8		b	b	

^a Molar equivalents of metal salt at which precipitation commenced.

^b Data not recorded.



Scheme 1. The synthesis of the polymers **3-Boc** and **3**. a) Boc_2O (3 equiv.), Et_3N (3 equiv.), chloroform, RT, 24 h (88%); b) 2-(2-(2-bromoacetoxy)ethoxy)ethyl methacrylate **2**, K_2CO_3 (1.2 equiv.), acetonitrile, 70°C, 22 h (67%); c) 2-cyano-2-propyl benzodithioate (0.02 equiv.), AIBN (0.0067 equiv.), DMF, 80°C, 16 h (60% monomer conversion); d) trifluoroacetic acid (TFA):CH₂Cl₂ (1:2 v/v), RT, 4 h (quant.); e) metal ion solution (ZnCl₂, CoCl₂, or CuCl₂) in MeOH (3 equiv. versus cyclen moiety), RT.

The molecular weights obtained (Table 1) were 5-10 times lower than the targets (based on the relative concentrations of monomer and chain-transfer agent) and there was little difference between the results of RAFT and uncontrolled freeradical polymerisation, which both resulted in oligomers limited to 5-9 units in size. This may arise from steric interferences by the bulky Boc-protected cyclen groups reducing the rate of chain propagation, rendering the termination and chaintransfer processes significantly more important, leading to lower molecular weight polymers.²⁸ It must also be noted that the contribution from the AIBN initiator is not taken into account when calculating the theoretical M_w of the polymers. Given the relatively large 3:1 [CTA]/[initiator] mol ratio, for the RAFT synthesized polymers, the final polymers will have approx. 75% of chains with CTA-termini and approx. 25% with initiatortermini.²⁹ In addition, the polydispersity values ranged from 1.6

2.4 which is higher than expected for controlled polymerization reactions.

After Boc-deprotection by treatment with TFA, the addition of metal salts to 3 in methanol solution formed precipitating aggregates only when the cation was capable of forming a cyclen complex and the anion was capable of complexing to the metal (Table 2). Thus, for example, ZnCl₂ resulted in a precipitate whereas NaCl and Zn(OTf)₂ did not. The oligomers bear no net charge. Coordination of a metal ion to the neutral cyclen ligand will produce a local positive charge and the presence of complexing anions bridging between two metal cyclen complexes on adjacent oligomers provides a driving force for aggregation. Addition of Zn(OTf)₂ did not produce a precipitate, but subsequent addition of ZnCl₂ to the same solution did result in aggregation, supporting this proposal. Structurally characterised examples of molecular Zn2+ complexes of cyclic, neutral polyamines linked by 1, 2 or 3 bridging Cl⁻ ions are numerous.³⁰⁻³⁷

The progression of metal salt-induced aggregation could be conveniently followed by dynamic light scattering and turbidity measurements (Fig. 2). The number-average particle size increased rapidly, from around 5 nm in the absence of metal, as the amount of added metal salt approached molar equivalence with the total amount of cyclen ligands present. The solution was essentially transparent for mole ratios of metal salt to cyclen ligand units < 0.8. Then turbidity rose rapidly with further increases in added salt amount.

The X-ray powder diffraction patterns given in the supplementary information show that the materials are not crystalline. However, some indication of the size scale of objects in the solid oligomers and in the metal-containing precipitates could be ascertained by treating these as X-ray scattering patterns. Fig. 3 shows the normalised and background-corrected scattering intensity where $q = 4\pi \sin\theta/\lambda$ denotes the scattering vector; θ is half of the scattering angle with respect to the incident beam and λ is the X-ray wavelength; l(q) is the scattered intensity. For amorphous or nanocrystalline materials the maxima in q^{-1} correspond to minima in the pair distribution

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function and hence indicate size and distance scales $x \sim q^{-1}_{max} / 1.2.^{38}$ The Boc-protected oligomer **3-Boc** has a characteristic size scale ~ 0.4 nm. The deprotected oligomer **3** is unsurprisingly smaller, ~ 0.35 nm and has a second size scale ~ 0.2 nm. The metallated polymer **3-ZnCl**₂ is expanded to ~ 0.45 nm and has other scales ~ 0.32 nm and 0.18 nm.



Fig. 2. Dynamic light scattering analysis (measured at 400 nm) of turbidity monitoring the self-assembly of the **3-ZnCl**₂ metallopolymer aggregates upon the dropwise addition of a solution of ZnCl₂ (in methanol) to polymer **3** in methanol.



Fig. 3. X-ray powder scattering intensity for the oligomer and metallated precipitate (chloride salt) against the reciprocal of the scattering vector, linear background subtracted and normalised to the maximum intensity. 1: polymer **3**; 2: Bocprotected polymer **3-Boc**; 3: precipitate of **3** with ZnCl₂.

These results can be understood in terms of the steric constraints imposed by the pendant cyclen. A simple molecular model of the deprotected molecule shows that oligomers of length ~ 4-7 units will tend to fold into spheres, with the pendant cyclens extending away to minimise the steric constraints (Fig. 4). These models indicate a diameter for these spherical objects of ~0.3 nm, and a distance scale separating the pendant cyclens ~0.1 - 0.2 nm, broadly consistent with the X-ray

scattering result. This steric constraint would explain why both RAFT and uncontrolled polymerisation Dead to the source of similar size. The metal would then be complexed on the outside. A complexing anion could then bridge between oligomers, leading to the observed aggregation, which is evidently not a simple electrostatic effect, but dependent on the presence of both a cyclen-complexed metal and a metal-complexed anion. That the aggregation attendant upon complexation is essentially stoichiometric with the total amount of cyclen ligand present indicates that all the pendant ligands are indeed accessible, as would be expected for this proposed geometry. The size scales in X-ray scattering for the metallated polymer would correspond to the overall diameter of the proposed ring of metal ions - which have high scattering density relative to the polymer - their average spacing and the diameter of the oligomer core.



Fig. 4. Energy-minimised molecular model of a 5-unit oligomer **3.** Cyclen ligands and the benzodithioate end-group are marked. Molecular modelling was performed using Avogadro²⁷ software - force field: Ghemical, number of steps: 1000, algorithm: steepest descent and convergence: 1×10^{-6} .

 Cu^{2+} ions have a very high binding affinity for cyclen (log K = 24.8)^{5, 11} when compared to other 2⁺ metal ions such as Zn²⁺ (log K = 16.2).¹¹ Several examples of Cu²⁺-complexed cyclens have been reported and X-ray structure analysis has shown that copper is typically coordinated in a square pyramidal geometry with the four macrocyclic nitrogens^{12-15, 39, 40} and that a chloride ion may occupy the remaining apical position.^{10, 13, 18} Titrations of the Boc-deprotected polymers 3 and 4 with CuCl₂ showed visible precipitate formation. The metallopolymer precipitates were collected by centrifugation, washed with methanol and dried. The quantities of Cu2+ in 3-CuCl2 and 4-CuCl2 are very similar ranging from 12.4-14.2 % copper by weight. The UV-Vis spectra of $3-CuCl_2$ and $4-CuCl_2$ (measured in water) showed broad absorbances at λ_{max} ~ 610 nm (supplementary information). This compares with the Cu(cyclen)Cl₂ d-d transition reported at λ_{max} = 594 nm in water^{41} and quite different to an aqueous solution of CuCl₂ which exhibits no absorption in this region of the visible spectrum. Aqueous CuCl₂ has a charge transfer transition at 257 nm which shifts to 264 nm for 3-CuCl₂ and 4-CuCl₂. EPR measurements on 3-CuCl₂ and 4-CuCl₂ (supplementary information) showed broad

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absorptions at g = 2.10977 and 2.11016, respectively, similar to other 5-coordinate Cu²⁺-cyclen complexes reported in the literature.^{14, 42, 43} Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) gave Cu²⁺ to Cl⁻ ratios of 1:2.0 and 1:1.8 for **3-CuCl₂** and **4-CuCl₂**, respectively. All data is consistent with the coordination of Cu²⁺ ions to the cyclen ligands in the two copper metallopolymers.

High-capacity metal ion-complexing gel containing cyclen ligands

RAFT polymerisation of monomer 1 with 3-(((benzylsulfanyl)thiocarbonyl)sulfanyl)propionic acid leaves the end-group functionalised oligomer 5-Boc (approx. 75% given the 3:1 ratio of RAFT agent to AIBN). Following Bocdeprotection, the carboxylated oligomer 5 could be crosslinked as in Scheme 2 (detailed procedure provided in experimental section). A PAMAM (G0) dendrimer was successfully attached to 5 using EDC-NHS coupling to form 5-PAMAM (G0), as confirmed by ¹H NMR spectrum. The FTIR spectrum (supplementary information) of 5-PAMAM (G0) displayed characteristic PAMAM vibrations at 3378 cm⁻¹ (N-H stretch of primary amines); 1639 cm⁻¹ (-C=O stretch); 1550 cm⁻¹, 1439 cm⁻ ¹, 1365 cm⁻¹ (N-H bending of N substituted amide).⁴⁴ In particular however, the amide (-C=O stretching vibration) at 1639 cm⁻¹ is noticeably stronger for 5-PAMAM (G0), when compared with the unconjugated PAMAM (G0) dendrimer, suggesting a higher concentration of amide groups.

This dendrimer-functionalised oligomer and PAMAM (G0) (1:2 mol ratio) were dissolved in water and sebacoyl chloride dissolved in hexane was slowly added without mixing. The crosslinked hydrogel formed at the interface of the two immiscible liquids and the crosslinked-**5**-PAMAM (G0) network material was removed with tweezers. For comparison, a crosslinked-PAMAM (G0) dendrimer without the cyclen oligomer was also prepared.

The mass swelling ratios of the two gels in water were 11.2 and 8.5 for crosslinked-5-PAMAM (G0) and crosslinked-PAMAM (G0), respectively. It is likely that the oligomers retain their globular structure in the crosslinked hydrogel, which would tend to promote a more nanoporous, water-permeable material. The ability of both crosslinked gels to complex copper was compared by suspending them in methanol solutions containing CuCl₂ (0.04 mmol). Immediately, the crosslinked-5-PAMAM (G0) network aggregated and settled toward the bottom of the vessel, while the crosslinked-PAMAM (G0) network remained dispersed throughout the solution. The suspensions were then left sitting at room temperature for 30 min before being centrifuged and washed with methanol (4 times) and then dried under high vacuum to yield the green (Cucrosslinked-5-PAMAM (G0)) and light blue (Cu-crosslinked-PAMAM (G0)) products, respectively (Fig. 5). The cyclen-bearing Cu-crosslinked-5-PAMAM (G0)) gel contained 11 wt% Cu and the unmodified Cu-crosslinked-PAMAM (G0) gel contained 2 wt% Cu. For the former, the mass fraction of copper is only slightly smaller than that observed for the oligomer precipitates (13.5±0.5 wt% Cu for 3-CuCl₂) implying that the mole ratio of ARTICLE

copper to cyclen units is likely to be 1:1. The cyclen is a more effective complexing agent for copper than the certian and the construction of the crosslinked-PAMAM (G0) gel. Furthermore, in the crosslinked-PAMAM (G0)) gel there would be a need for structural mobility to achieve coordination by a significant number of ligand donors around a copper atom. In contrast, the cyclen oligomer crosslinked-**5**-PAMAM (G0) features complete cyclen ligands. As Cu²⁺ has a very high binding affinity for cyclen moieties (log K = 24.8)^{5, 11} these factors could explain the significantly larger uptake of copper by the cyclen crosslinked-**5**-PAMAM (G0) oligomer gel than by the simple amine gel.



Scheme 2: Preparation of crosslinked-**5**-PAMAM (G0) dendrimer-functionalised oligomer.



Fig. 5. Copper-loaded (left) Cu-crosslinked-PAMAM (G0) and (right) Cu-crosslinked-**5**-PAMAM (G0).

Conclusion

In conclusion, the synthesis of acrylates bearing bulky saturated polyamine macrocyles as side-chains is a straightforward way to make nanostructures comprising a network of small globules

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with pendant complexing 'hairs'. These will be of interest to explore further as materials for efficient and selective extraction of metals.

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Supplementary information

¹H NMR, ¹³C NMR, FTIR, GPC, DLS, XRD, UV-visible, EPR and SEM-EDS results relating to this article can be found in the accompanying supplementary information document.

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"Hairy" nano-scale objects cross-linked into high-capacity metal-binding hydrogel

