Poly(*para*-phenylene ethynylene)s functionalized with Gd(III) chelates as potential MRI contrast agents

Katelyn M. Atkins, Francisco M. Martínez, Ali Nazemi, Timothy J. Scholl, and Elizabeth R. Gillies

Abstract: A poly(*para*-phenylene ethynylene) with water-solubilizing groups and Gd(III) chelates conjugated to the polymer backbone was designed and synthesized. Pre- and post-polymerization functionalization approaches were explored and the pre-polymerization approach for the introduction of the Gd(III) chelate was found to be more successful. The UV–vis absorption and fluorescence emission properties of the protected polymers were characterized and were found to be consistent with the results expected for this class of polymers. Removal of the protecting groups followed by chelation of Gd(III) led to a water-dispersible polymer. Relaxivity measurements were performed on this polymer with the aim of evaluating its potential as a new MRI contrast agent, and an r_1 of 1.37 L mmol^{-1} s^{-1} at 310 K and 20 MHz was determined. These results, along with dynamic light scattering analyses, suggested that the polymers formed micrometre-sized assemblies in aqueous solution. Although the relaxivity was relatively modest, these results provide important insights into the assembly properties of this new class of polymers and into the design criteria for future agents.

Key words: poly(*para*-phenylene ethynylene), magnetic resonance imaging, contrast agent.

Résumé : On a développé et synthétisé un poly(*para*-phénylèneéthylène) portant des groupes solubles dans l'eau et des chélates de Gd(III) conjugués au squelette du polymère. On a exploré des approches de fonctionnalisations avant et après les polymérisations et on a trouvé que l'approche prépolymérisation est meilleure pour l'introduction du chélate de Gd(III). On a caractérisé les propriétés d'absorption UV–visible et d'émission de fluorescence des polymères protégés et on a trouvé qu'ils sont en accord avec les résultats attendus pour cette classe de polymères. L'enlèvement des groupes protecteurs, suivi par la chélation du Gd(III) conduit à l'obtention d'un polymère qui peut être dispersé dans l'eau. On a effectué des mesures de relaxation sur ce polymère dans le but d'évaluer son potentiel comme un nouvel agent de contraste en imagerie de résonance magnétique (IRM) et on a déterminé que sa valeur de r_1 est égale à 1,37 L mmol^{-1} s^{-1}, à 310 K et à 20 MHz. Ces résultats, avec les analyses de dispersion dynamique de la lumière, suggèrent qu'en solution aqueuse les polymères forment des assemblées de la taille du micron. Même si la relaxation est relativement faible, ces résultats fournissent connaissances importantes dans les propriétés d'assemblage de cette nouvelle classe de polymère et dans les critères de conceptualisation des futurs agents.

Mots-clés : poly(*para*-phénylèneéthylène), imagerie de résonance magnétique (IRM), agent de contraste.

[Traduit par la Rédaction]

Introduction

Over the past few decades, magnetic resonance imaging (MRI) has emerged as a powerful noninvasive diagnostic tool in medicine.[1–3] It provides excellent spatial resolution and soft tissue contrast for conveying anatomical structure. In cases where the inherent soft tissue contrast is insufficient to distinguish diseased tissues from normal tissues, contrast agents are routinely used.[2,3] These agents are typically stable chelates of the lanthanide ion Gd(III). While these small-molecule agents have enabled significant advancements in MRI, they possess several limitations.

First, the relaxivities of the commercially available agents are only a few percent of the theoretically predicted values.[4] The low sensitivity of these agents imparts a requirement for gram-scale doses to obtain the required contrast. This raises concerns regarding the toxicity of these agents, particularly in patients with kidney disease.[5] Furthermore, there is increasing interest in the development of contrast agents tar-

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geted to specific diseases via binding to receptors in the target tissues. Considering a stoichiometric 1:1 binding of the agent to the target, the target must be present at a concentration of approximately 125 μmol L^{-1}.[3] Unfortunately, many targets are not present at this relatively high concentration in vivo.

To address these limitations, significant efforts have been aimed at enhancing the relaxivities of Gd(III)-based contrast agents. It has been predicted by Solomon–Bloembergen–Morgan theory that increases in the rotational correlation times of the current agents can result in significantly increased relaxivities.[6,7] As macromolecules have slower tumbling rates in solution, much research has involved the conjugation of Gd(III) chelates such as diethylenetriaminepentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to macromolecules. Early efforts involving conventional linear macromolecules led to relatively disappointing results.[8–14] However, the conjugation of these chelates to macromolecules such as proteins[15,16] and dendrimers[17–25] provided much greater enhancements in relaxivity. These results highlight the importance of macromolecular structure and architecture, as flexible backbones allow the Gd(III) chelates to tumble in solution in a manner similar to the small molecules, while those structures possessing well-defined and rigidified conformations provide longer rotational correlation times and thus higher relaxivities.

With the importance of molecular rigidity in mind, our group was interested in investigating poly(*para*-phenylene ethynylene) (PPE) as a backbone for the conjugation of Gd(III) chelates. This polymer backbone, comprising alternating phenyl and alkyne moieties, exists in an elongated linear conformation, with backbone conjugation leading to high levels of fluorescence.[26–31] This fluorescence and its sensitivity to its environment have been exploited for the development and commercialization of sensors.[29–31] More recently, water-soluble analogues of these polymers have been developed and have been investigated as sensors for DNA,[32–34] proteins,[35] and bacteria.[36,37] However, to the best of our knowledge PPEs have never been investigated as MRI contrast agents. We proposed that the rigidified, elongated conformation of PPE would lead to significant enhancements in relaxivity of conjugated Gd(III) chelates. Furthermore, the fluorescence may enable the optical detection of these agents in cells and tissues, thus facilitating biological studies of the agents and potentially providing a dual modality imaging probe, perhaps even with sensor capabilities. Described here is the synthesis of a new PPE with conjugated Gd(III) chelates and the characterization of its optical properties and relaxivity.

Results and discussion

Polymer design

The structure of the target copolymer **1** is shown in Fig. 1. A modified DTPA[38] was selected as the Gd(III) chelate in this polymer, as the extra carboxylate incorporated in the aspartic acid linker allows 8 chelation sites of the ligand to be preserved for binding to the metal ion. Direct use of one of the carboxylates of DTPA for conjugation to the polymer backbone would lead to decreased complex stability, thus increasing the chances of transmetallation by Zn(II), a process that would potentially release toxic free Gd(III) in vivo.[39] In addition, although the conjugation of the modified DTPA chelate to the phenols of the polymer backbone would be expected to provide the highest relaxivity, this conjugation was not efficient in preliminary work. Therefore, an ethyl spacer was incorporated between the polymer backbone and the chelate to introduce a more nucleophilic amine group for the conjugation to the acid-functionalized DTPA.

Although the chelates were expected to impart some aqueous solubility to the PPEs, water-solubilizing oligo(ethylene glycol) moieties were also incorporated in the design. PPEs, like other conjugated polymers having a rigid and hydrophobic backbone, have inherently very poor water solubility that results in aggregation and subsequent fluorescence quenching.[40–42] Carboxylic acid terminated oligo(ethylene glycol) moieties were chosen because they have been previously demonstrated by Wosnick et al. to impart aqueous solubility to PPEs.[37] Indeed, preliminary work in our laboratory demonstrated that neutral tri(ethylene glycol) monomethyl ether derivatives were insufficient to impart aqueous solubility.

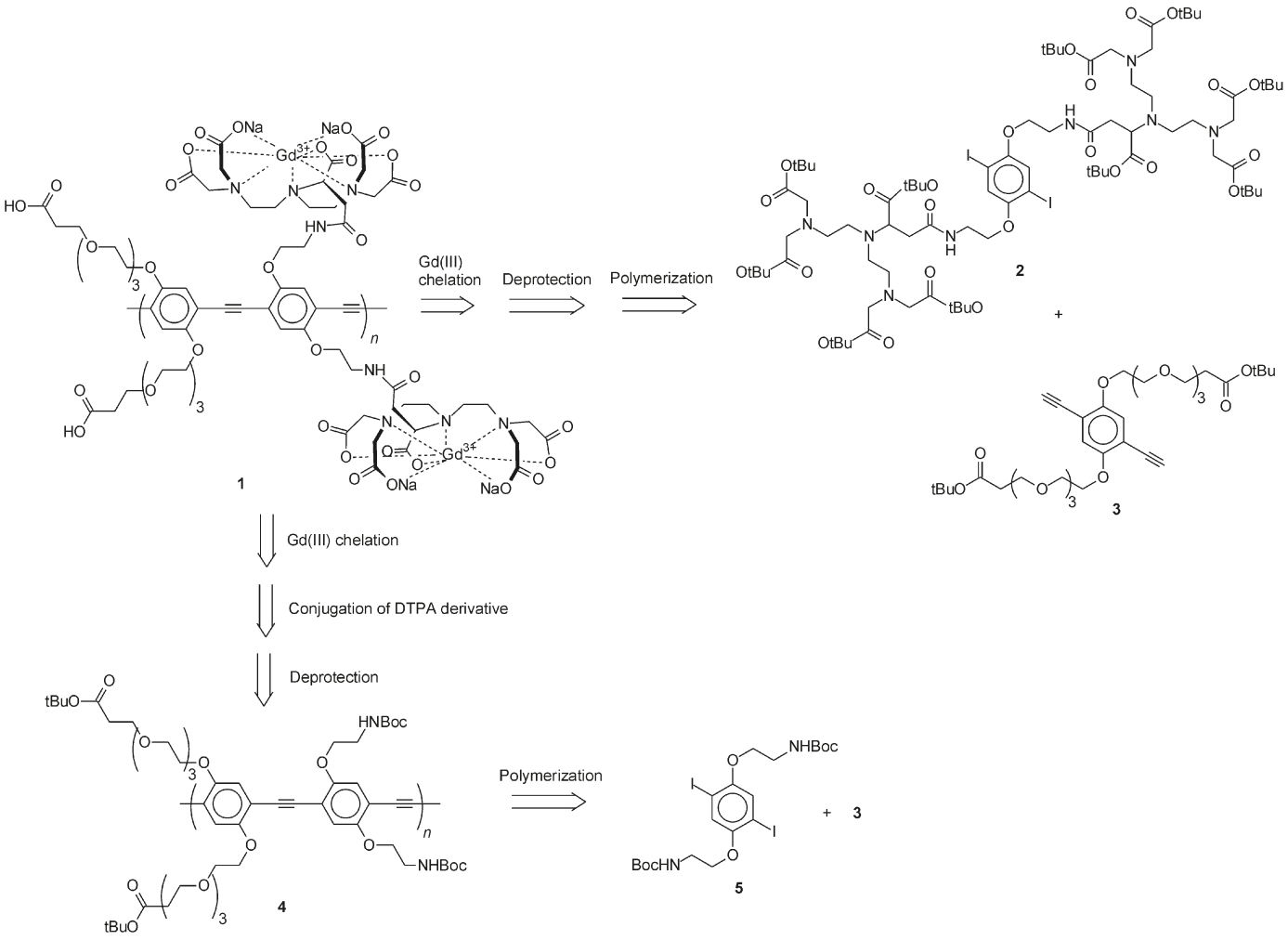
PPEs are typically prepared from diiodophenylene and dialkyne monomers via Sonogashira–Hagihara copolymerizations.[43,44] Based on this strategy, the proposed design incorporated the modified DTPA chelates into the diiodophenylene monomer **2** and the tetra(ethylene glycol) moieties into the dialkyne monomer **3** (Fig. 1). In addition, it was of interest to explore the postpolymerization functionalization of the PPE backbone as an alternative means of preparing the target polymers. This would potentially provide a versatile synthetic method that would allow easy incorporation of targeting ligands in future generations of the agent. Therefore, polymer **4** was also designed as a target, which would be prepared from monomers **3** and **5**.

Monomer syntheses

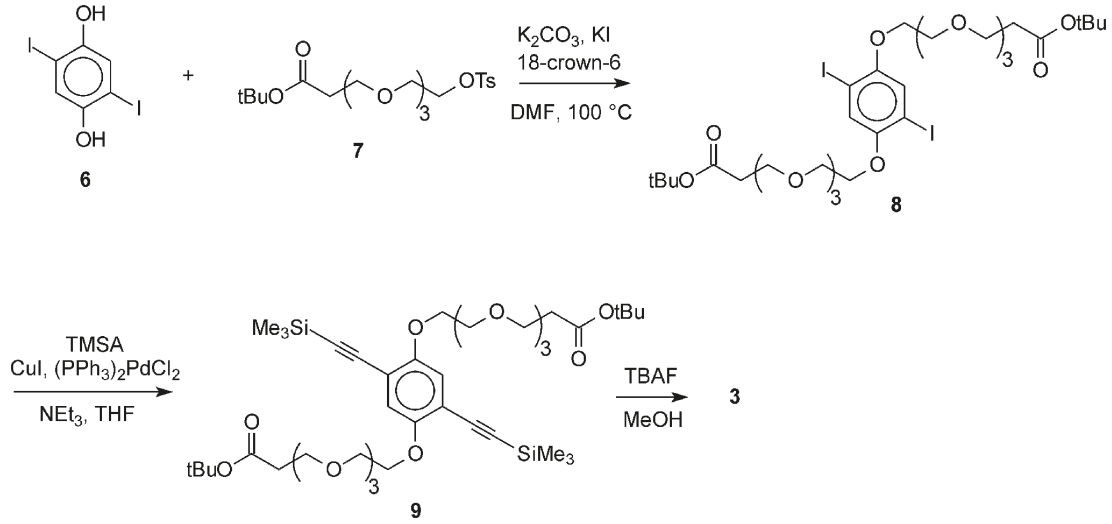
The synthesis of the dialkyne monomer **3** was performed following a protocol similar to that reported by Wosnick et al. for slightly different oligo(ethylene glycol) lengths (di(ethylene glycol) and penta(ethylene glycol)).[37] As shown in Scheme 1, 2,5-diiodo-1,4-hydroquinone (**6**)[30] was reacted with the tosylate **7**,[45] providing **8**. The alkyne moieties were then introduced by a Sonogashira reaction with trimethylsilylacetylene (TMSA) in the presence of catalytic copper(I) iodide and bis(triphenylphosphine)palladium(II) chloride. Finally, the trimethylsilyl protecting groups were removed by treatment of **9** with tetrabutylammonium fluoride (TBAF), providing **3**.

The synthesis of monomer **2** began with the reaction of the tosylate **10**[46] with 2,5-diiodo-1,4-hydroquinone to give the diazide **11** (Scheme 2). The azides were then reduced to amines under Staudinger conditions, providing **12**. Finally, the amines were conjugated to the previously reported DTPA derivative **13**[38] using dicyclohexylcarbodiimide (DCC) in the presence of 4-(dimethylamino)pyridine (DMAP) and 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) to provide monomer **2**. For the preparation of monomer **5**, *tert*-butyl carbamate (Boc) protected 2-bromoethylamine (**14**)[47] was reacted with 2,5-diiodo-1,4-hydroquinone as shown in Scheme 3.

Fig. 1. Design of two alternative routes to a PPE with Gd(III) chelates and water-solubilizing side chains.



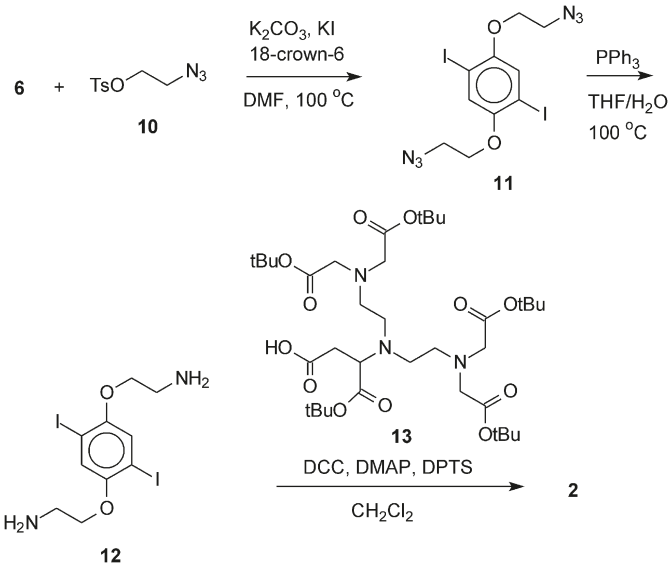
Scheme 1. Synthesis of monomer **3**.



Polymer syntheses and characterization

Monomers **2** and **3** were copolymerized under Sonogashira–Hagihara conditions[43,44] to provide the protected polymer **15**, which was purified by dialysis in *N*,*N*-dimethylformamide (DMF) (Scheme 4). Using size exclusion chromatography (SEC) (Fig. 2), **15** was found to have a weight-average molecular weight (M_w) of 31 300 g mol^{-1} and a polydispersity index (PDI) of 2.6 as determined by a conventional calibration relative to polystyrene standards. Following determination of the refractive index increment (d*n*/d*c*) for

Scheme 2. Synthesis of monomer **2**.



Scheme 3. Synthesis of monomer **5**.

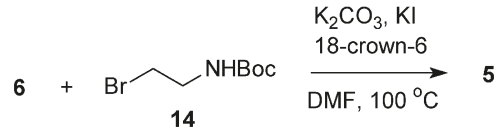
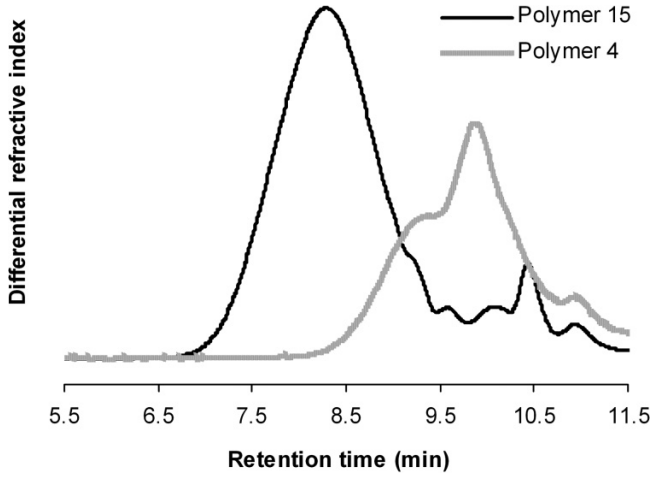


Fig. 2. Size exclusion chromatography traces for polymers **15** and **4**.



the polymer, its absolute molecular weight was also determined by multi-angle light scattering (MALS). This measurement provided an M_w of 40 500 g mol^{-1} and a PDI of 1.9. The values obtained by the conventional calibration and light scattering were therefore in relatively good agreement.

Monomers **3** and **5** were polymerized under the same conditions described above, leading to polymer **4**, which was also purified by dialysis in DMF. This polymer was found by SEC (Fig. 2) to have an M_w of 2640 g mol^{-1} and a PDI of 2.4. The lower M_w of this polymer in comparison with **15** can be attributed at least in part to the lower average monomer molecular weight but was also likely due to a lower degree of polymerization for unknown reasons.

As shown in Fig. 3, both polymers **15** and **4** exhibited red-shifted absorption maxima (λ_{max}) at 430 nm and 406 nm, respectively, relative to that of the dialkyne monomer **3** at 327 nm. This bathochromic shift can be attributed to the significantly increased conjugation of the polymers relative to the monomer and is typical of PPEs.[26,29] Concomitantly, the emission maxima also shifted to 465 nm and 460 nm for polymers **15** and **4**, respectively, relative to the monomer's emission λ_{max} of 372 nm (Fig. 4). These emission maxima are similar to those of other previously reported PPEs.[37,48]

Polymer deprotection and Gd(III) chelation

The *tert*-butyl ester groups on both the tetra(ethylene glycol) and DTPA derivatives of polymer **15** were easily removed by treatment of the polymer with 1:1 trifluoroacetic acid (TFA):CH_2Cl_2 to provide polymer **16** (Scheme 5). The resulting polymer was then dissolved in pure water and the pH was adjusted to 7.4 prior to the introduction of Gd(III) in the form of $GdCl_3{\cdot}6H_2O$, giving the target polymer **1**. Following purification by extensive dialysis, the Gd(III) content was measured by inductively coupled plasma mass spectrometry. Based on these measurements, Gd(III) was successfully introduced into each chelation site and no extra Gd(III) was detected.

Polymer **4** could also be fully deprotected by treatment with 1:1 TFA/CH_2Cl_2 to give **17** (Scheme 6). The next step towards the target was the introduction of the Gd(III) chelates by reaction of **17** with an *N*-hydroxysuccinimidyl ester derivative of **13** or the commercially available *p*-isothiocyanatobenzyl derivative of DTPA. Unfortunately, although **17** was soluble in organic solvents such as DMF and $CHCl_3$ when the amines were present as their TFA salts, all attempts to neutralize or basify the solution or to dissolve the polymer in aqueous buffer for the conjugation led to rapid polymer precipitation. The use of other polar solvents such as dimethylsulfoxide or methanol did not result in enhanced solubility, so this polymer could not be further functionalized.

Relaxivity measurements

The longitudinal relaxivity (r_1) of polymer **1** in 100 mmol L^{-1} pH 7.4 phosphate buffer was measured at 298 and 310 K between 0.01 and 35 MHz using a field cycling relaxometer. The results are shown in Fig. 5. When calculated on a per Gd(III) ion basis, the results corresponded to an r_1 of 1.37 ± 0.03 L mmol^{-1} s^{-1} at 310 K and 20 MHz. This result was unexpectedly low relative to the value of 4.1 L mmol^{-1} s^{-1} measured for Gd-DTPA under the same conditions.[15] In addition, although **1** appeared "soluble" upon first inspection, it was noted that after prolonged standing some material settled from solution, resulting in even lower relaxivities. This material could be redispersed by mixing, resulting in recovery of the relaxivity to the original value. This behaviour, along with a complete lack of polymer fluorescence, suggested that the polymer may have assembled into micrometre-sized particles in solution.

The suspension of polymer **1** was investigated by dynamic light scattering. As shown in Fig. 6, micrometre-sized particles were indeed detected. Optical microscopy was also

Scheme 4. Synthesis of polymer **15**.

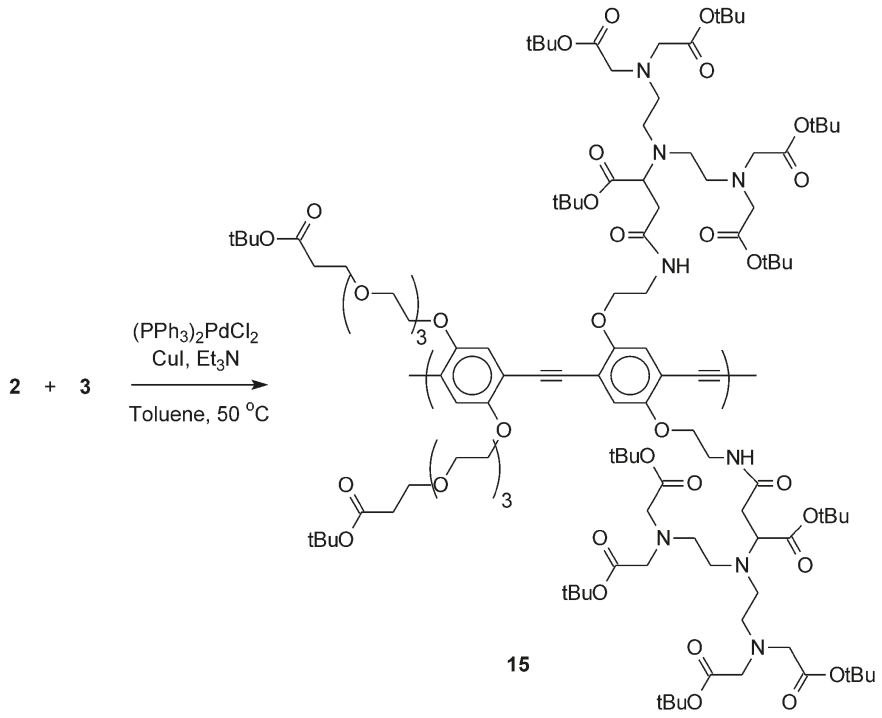


Fig. 3. UV–vis absorption spectra of polymers **15** and **4** and monomer **3** (in CH_3OH).

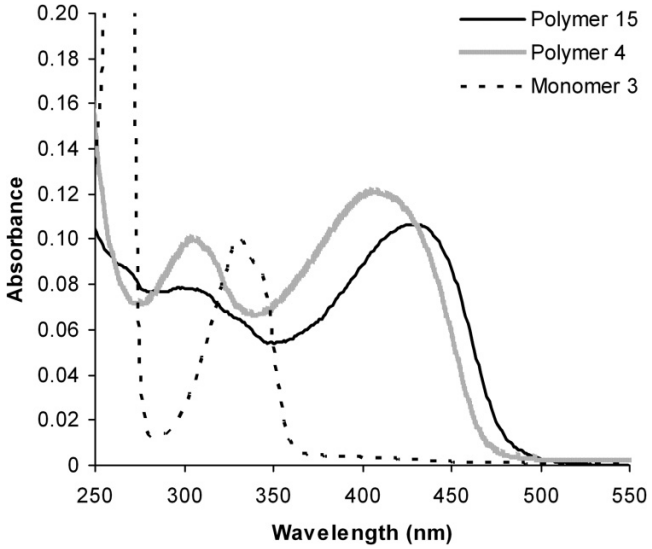
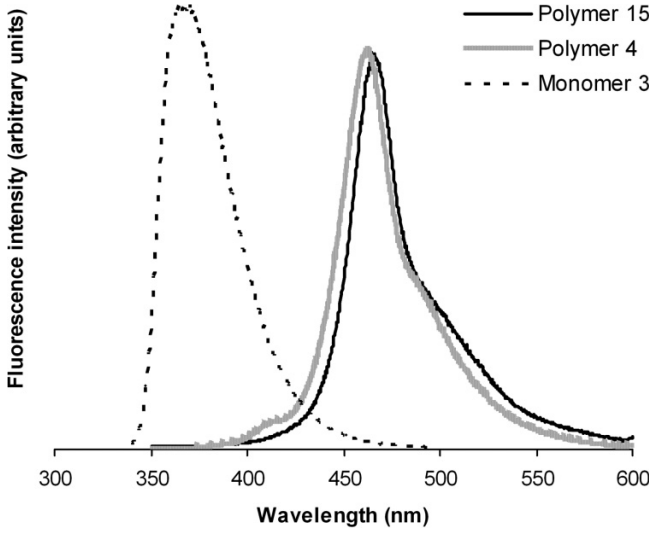


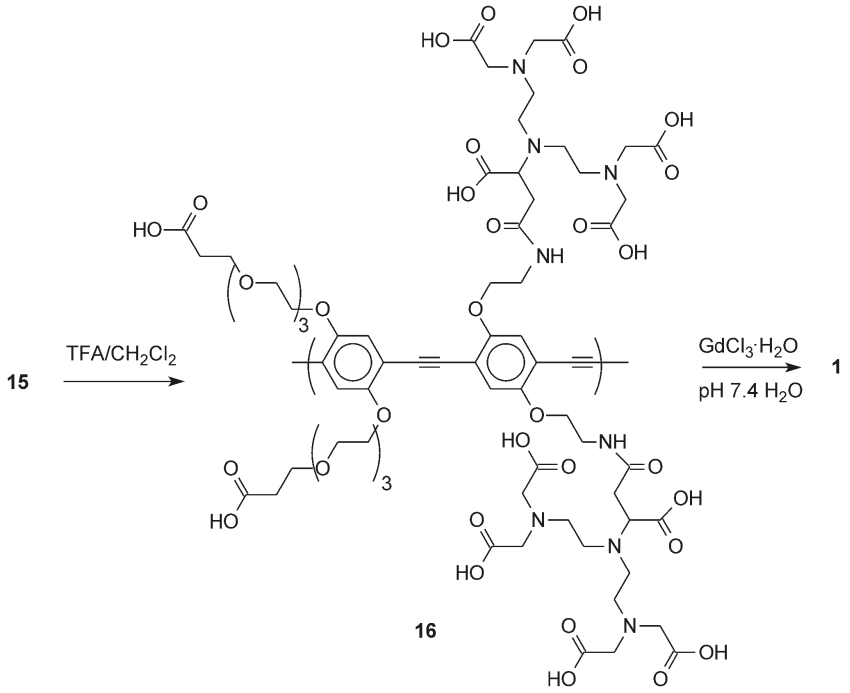
Fig. 4. Fluorescence emission spectra of polymers **15** and **4** and monomer **3** (in CH_3OH).



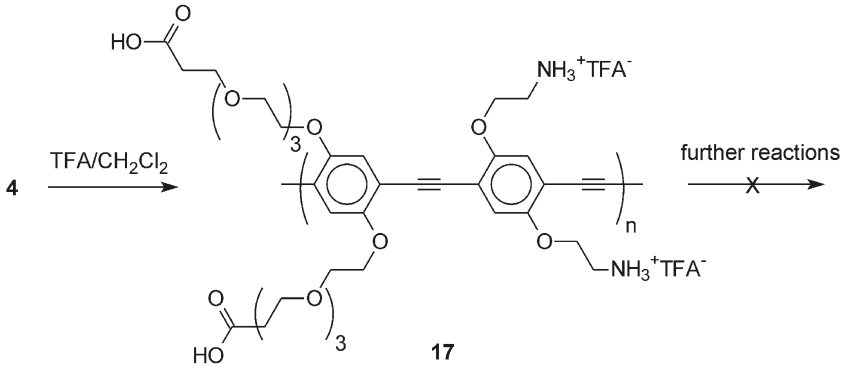
used to verify the size and revealed that the particles were solid and approximately spherical (Fig. 7). By extensive centrifugation it was possible to remove essentially all of the polymer from the suspension. Combined, these results suggest that the relaxivity exhibited by polymer **1** arose primarily through the interaction of water molecules with the Gd(III) ions at the surface of the particles, while the Gd(III) ions on the interior would have very poor access to water, a property that is known to substantially decrease the relaxivities of Gd(III) agents.[49–51] As the r_1 value was calculated based on the total concentration of Gd(III) ions in the sample, it is likely that the relaxivities of the complexes on or near the particle surface were much higher than 1.37 L mmol^{-1} s^{-1}, while those on the interior were much lower. For example, based on a mean particle size of 1.5 μm and assuming that water can freely penetrate the particle to a depth of 10 nm, the relaxivity on a per Gd(III) ion basis would be approximately 35 L mmol^{-1} s^{-1}, much higher than that for Gd-DTPA. However, this estimation is clearly very dependent on the value used for the depth of water penetration and the result ranges from 115 L mmol^{-1} s^{-1} for a penetration depth of 3 nm to 3.9 L mmol^{-1} s^{-1}, very close to the relaxivity of Gd-DTPA, if a penetration depth of 100 nm is assumed (see Supplementary data).

Overall, the significant aggregation of polymer **1** was surprising given the solubilizing carboxylic acid terminated

Scheme 5. Synthesis of the target polymer **1**.



Scheme 6. Deprotection of polymer **4**.



tetra(ethylene glycol) units, the inherently water-soluble Gd(III) complexes, and the previous reports of water-soluble PPEs bearing similar densities of charged moieties.[32,34,37] Considering the multiple competing effects that the incorporation of the polymeric complexes into the micrometre-sized aggregates would have on the relaxivity, it is not possible to confirm definitively that increased relaxivity would result from the rigidified polymer backbone. Thus, the unexpected assembly of polymer **1** introduces a new form of Gd(III) where each particle carries many Gd(III) ions along conjugated polymer backbones. However, to further investigate the effects of the PPE backbone on relaxivity and to exploit its fluorescence properties it will be necessary to design polymers that do not aggregate in water.

Conclusions

In summary, a PPE with conjugated water-solubilizing groups and DTPA derivatives was designed with the aim of obtaining Gd(III) complexes with high relaxivity due to the rigidity of the polymer backbone. Two synthetic routes to the target were explored, one involving the polymerization of a DTPA-functionalized monomer and the other involving a postpolymerization modification. While the postpolymerization modification was not successful owing to poor polymer solubility, the former route successfully led to a polymer having two Gd(III) complexes on alternating phenyl rings. Unexpectedly, this polymer assembled into microparticles in aqueous solution with a relaxivity of 1.37 L $mmol^{-1}$ s^{-1} on a per Gd(III) ion basis. This relaxivity is likely an average of Gd(III) complexes on the microparticle surface with high relaxivities and complexes on the particle interior with low relaxivities due to poor water accessibility.

Experimental

General procedures and materials

All chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. Anhydrous *N*,*N*-dimethylformamide (DMF), tetrahydrofuran

Fig. 5. Longitudinal relaxivity (r_1) of polymer **1** in pH 7.4 buffer as a function of field strength and temperature.

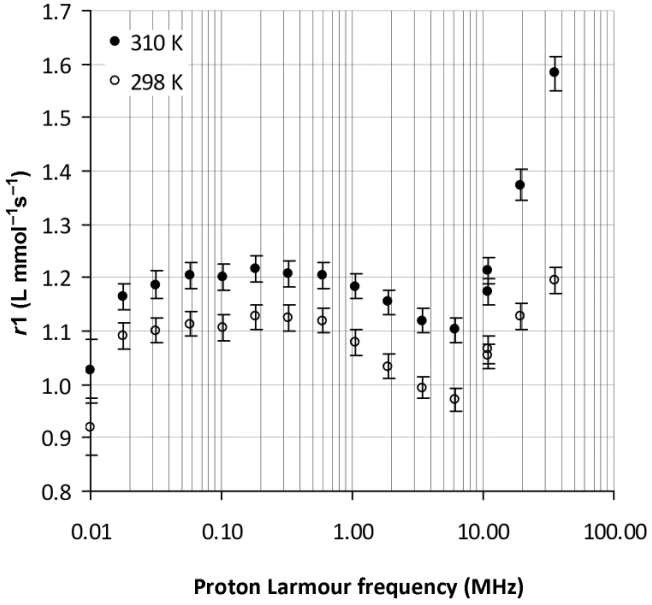
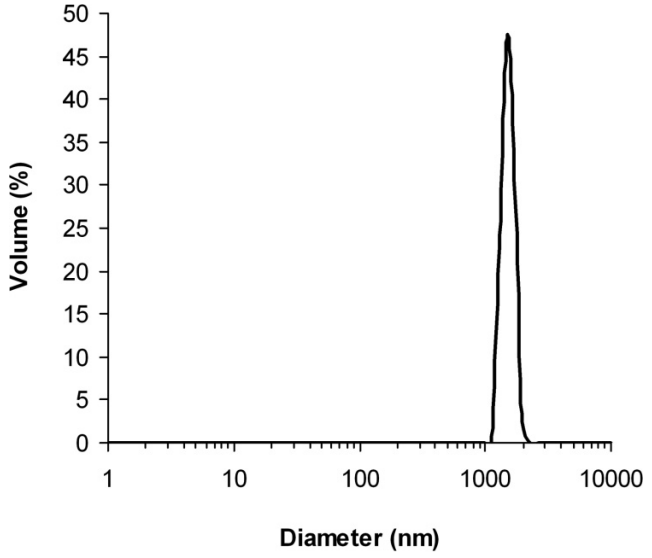
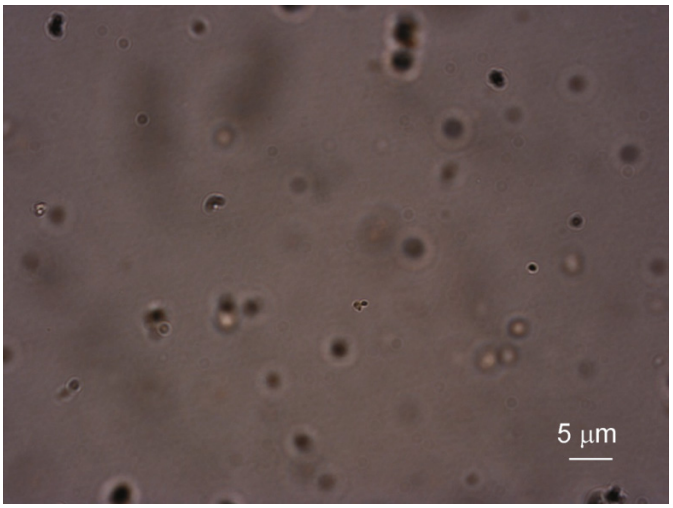


Fig. 6. Size distribution of aggregates formed by polymer **1** in pH 7.4 phosphate buffer, as measured by dynamic light scattering.



(THF), and toluene were obtained from a solvent purification system. CH_2Cl_2 and NEt_3 were distilled over CaH_2. Ultrapure water was obtained from a Barnstead EASYpure II system. Column chromatography was performed using silica gel (0.063–0.200 mm particle size, 70–230 mesh). ^{1}H NMR spectra were obtained at 400 MHz and ^{13}C NMR data were obtained at 100 MHz. Chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26, 77.2) or D_2O (δ 4.75). High-resolution mass spectrometry (HRMS) was performed using a Micromass LCT (electrospray time-of-flight (ES+)) mass spectrometer or a Finnigan MAT 8200 mass spectrometer in time-of-flight ES+ mode. UV–vis absorption spectroscopy was performed on a Varian Cary 300 Bio UV–vis spectrophotometer. Emission spectra were obtaining using a Photon Technology International QM-4 SE spectrofluorometer. Size exclusion chromatography (SEC) was performed in THF using a Waters 515 HPLC pump, Wyatt Optilabrex RI and mini-DAWN-TREOS detectors, and a ResiPore (300 mm × 7.5 mm) column from Polymer Laboratories. Column calibration was performed using polystyrene standards from Polymer Laboratories. Dynamic light scattering was performed on a Zetasizer Nano ZS instrument from Malvern Instruments. Optical microscopy was performed using an Olympus model IX71S8F-3 microscope equipped with a 20× (NA 0.50) objective. Dialyses were performed using Spectra/Por regenerated cellulose membranes with a molecular weight cutoff of 3500 Da. The inductively coupled plasma mass spectrometry (ICP-MS) analysis was performed at the Environmental Analytical Laboratories of the Saskatchewan Research Council. Relaxation rate measurements were performed on a Stelar Spinmaster FFC2000 1T C/DC relaxometer. The concentration of the polymer was 0.17 mg mL^{-1} in a 100 mmol L^{-1} pH 7.4 phosphate buffer solution.

Fig. 7. Optical microscopy image of assemblies formed by polymer **1**.



Synthesis of compound 8

To a flame-dried flask were added 2,5-diiodo-1,4-hydroquinone (0.64 g, 1.8 mmol, 1.0 equiv.), the tosylate **7** (1.9 g, 4.4 mmol, 2.5 equiv.), potassium iodide (0.15 g, 0.88 mmol, 0.50 equiv.), potassium carbonate (1.5 g, 11 mmol, 6.0 equiv.), and 18-crown-6 (0.92 g, 3.5 mmol, 2.0 equiv.). DMF (30 mL) was added and the resulting solution was heated to 100 °C for 48 h under an argon atmosphere. The solvent was removed in vacuo and the resulting residue was dissolved in a small amount of CH_2Cl_2, concentrated to dryness on a small amount of silica gel, and purified by column chromatography on silica gel (20:80 ethyl acetate – hexanes eluent) to yield compound **8** (0.71 g, 46%). IR (cm^{-1}, thin film from CH_2Cl_2): 3088, 2967, 2866, 1726. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.44 (s, 18 H), 2.49 (t, J = 6.63 Hz, 4 H), 3.57–3.80 (m, 20 H), 3.83–3.91 (m, 4 H), 4.04–4.12 (m, 4 H), 7.23 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.8, 153.1, 123.4, 86.4, 80.4, 71.1, 70.7, 70.5, 70.4, 70.2, 69.6, 66.9, 36.24, 28.1. HRMS calcd. for $[M]^+$ ($C_{32}H_{52}I_2O_{12}$): 882.1548; found: 882.1546.

Synthesis of compound 9

Compound **8** (0.69 g, 0.78 mmol, 1.0 equiv.) was added to a flame-dried flask and put under argon atmosphere. Dry THF (6 mL) was added, followed by trimethylsilylacetylene (0.76 g, 7.8 mmol, 10 equiv.) and triethylamine (5.4 mL, 39 mmol, 50 equiv.). Copper(I) iodide (4.5 mg, 23 μmol, 0.029 equiv.) and bis(triphenylphosphine)palladium(II) chloride (11 mg, 16 μmol, 0.020 equiv.) were added to the solution under a flow of argon. The mixture was stirred at room temperature for 48 h, and then the solvent was removed in vacuo. The resulting residue was purified by column chromatography (10:90 ethyl acetate – hexanes to 40:60 ethyl acetate – hexanes eluent gradient) to afford compound **9** (0.54 g, 96%). IR (cm^{-1}, thin film from CH_2Cl_2): 3010, 2954, 2867, 2151, 1729, 1495. ^1H NMR (400 MHz, $CDCl_3$, δ): 0.17 (s, 18 H), 1.37 (s, 18 H), 2.42 (t, J = 6.64 Hz, 4 H), 3.46–3.75 (m, 20 H), 3.80 (t, J = 4.88 Hz, 4 H), 4.05 (t, J = 4.88 Hz, 4 H), 6.85 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.8, 153.8, 117.7, 114.1, 100.8, 100.3, 80.4, 71.1, 70.7, 70.5, 70.3, 69.6, 69.4, 66.8, 36.2, 28.0, –0.1. HRMS calcd. for $[M + Na]^+$ ($C_{42}H_{70}O_{12}NaSi_2$): 845.4304; found: 845.4329.

Synthesis of monomer 3

To a solution of compound **9** (0.51 g, 0.71 mmol, 1.0 equiv.) in methanol (4 mL) was added 1 mol L^{-1} tetrabutylammonium fluoride in THF (1.8 mL, 2.5 equiv.). The resulting solution was stirred for 12 h, and then the solvent was removed in vacuo and the resulting residue was purified by column chromatography (50:50 ethyl acetate – hexanes to 80:20 ethyl acetate – hexanes eluent gradient) to afford monomer **3** (0.36 g, 87%). UV–vis absorption (CH_3OH) λ_{max}: 327 nm. Emission λ_{max}: 372 nm. IR (cm^{-1}, thin film from CH_2Cl_2): 3242, 3010, 2972, 2866, 2092, 1727, 1495. ^1H NMR (400 MHz, $CDCl_3$, δ): 1.42 (s, 18 H), 2.48 (t, J = 6.64 Hz, 4 H), 3.34 (s, 2 H), 3.51–3.77 (m, 20 H), 3.84 (t, J = 5.08 Hz, 4 H), 4.12 (t, J = 4.88 Hz, 4 H), 6.97 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 170.9, 154.0, 118.2, 113.5, 82.8, 80.4, 79.5, 71.0, 70.6, 70.5, 70.4, 69.6, 69.5, 66.8, 36.2, 28.1. HRMS calcd. for $[M]^+$ ($C_{36}H_{54}O_{12}$): 678.3615; found: 678.3605.

Synthesis of compound 11

To a flame-dried flask were added 2,5-diiodo-1,4-hydroquinone (3.3 g, 9.0 mmol, 1.0 equiv.), the tosylate **10** (5.1 g, 23 mmol, 2.5 equiv.), potassium iodide (0.60 g, 3.6 mmol, 0.40 equiv.), potassium carbonate (5.0 g, 36 mmol, 4.0 equiv.), and 18-crown-6 (2.4 g, 9.2 mmol, 1.0 equiv.). DMF (30 mL) was added and the resulting solution was heated at 100 °C for 48 h under an argon atmosphere. The solvent was removed in vacuo and the resulting residue was dissolved in a small amount of dichloromethane, concentrated to dryness on a small amount of silica gel, and purified by column chromatography (10:90 ethyl acetate – hexanes to 30:70 ethyl acetate – hexanes eluent gradient) to yield compound **11** (1.3 g, 32%). IR (cm^{-1}, KBr pellet): 3043, 2920, 2867, 2106, 1692. ^1H NMR (400 MHz, $CDCl_3$, δ): 3.65 (t, J = 5.08 Hz, 4 H), 4.11 (t, J = 5.08 Hz, 4 H), 7.23 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 153.1, 123.7, 85.9, 67.8, 49.2. HRMS calcd. for $[M]^+$ ($C_{10}H_{12}I_2N_6O_2$): 499.8955; found: 499.8964.

Synthesis of compound 12

The diazide **11** (0.25 g, 0.58 mmol, 1.0 equiv.) and triphenylphosphine (0.76 g, 2.9 mmol, 5.0 equiv.) were dissolved in THF (10 mL). Water (5 mL) was added and the resulting mixture was heated at 60 °C for 2 h. The solvent was then removed in vacuo and the residue was purified by column chromatography (90:10 dichloromethane–methanol to 100% methanol eluent gradient) to yield compound **12** (0.19 g, 88%). IR (cm^{-1}, KBr pellet): 3360, 3038, 2925, 2869, 1693, 1526. ^1H NMR (400 MHz, $CDCl_3$, δ): 1.51 (br s, 4 H), 3.10 (t, J = 4.88 Hz, 4 H), 3.99 (t, J = 4.88 Hz, 4 H), 7.21 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 152.8, 123.5, 86.3, 69.8, 45.3. HRMS calcd. for $[M]^+$ ($C_{10}H_{14}I_2N_2O_2$): 447.9145; found: 447.9158.

Synthesis of monomer 2

To a flame-dried flask were added the diamine **12** (0.038 g, 0.10 mmol, 1.0 equiv.), DTPA derivative **13** (0.18 g, 0.25 mmol, 2.5 equiv.), DMAP (0.012 g, 0.10 mmol, 1.0 equiv.), and DPTS (0.029 g, 0.10 mmol, 1.0 equiv.). The flask was placed under nitrogen and dry CH_2Cl_2 (5 mL) was added. Once the reagents had dissolved, DCC (0.12 g, 0.60 mmol, 6.0 equiv.) was added and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was then filtered through cotton to remove the dicyclohexylurea by-product and the solvent was removed in vacuo. The resulting material was purified by column chromatography (20:80 ethyl acetate – cyclohexane eluent) to afford compound **2** (0.074 g, 40%). IR (cm^{-1}, thin film from CH_2Cl_2): 3310, 3060, 2974, 2925, 1729, 1663, 1535, 1455. ^1H NMR (400 MHz, $CDCl_3$, δ): 1.36–1.56 (m, 90 H), 2.55 (br s, 2 H), 2.72 (br s, 2 H), 2.80 (br s, 16 H), 3.38–3.47 (m, 16 H), 3.61–3.81 (m, 4 H), 3.88 (br s, 2 H), 3.94–4.14 (m, 4 H), 7.23 (s, 2 H), 7.83 (br s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 178.4, 171.2, 170.4, 152.8, 123.2, 86.3, 81.5, 81.0, 68.9, 60.9, 55.8, 53.1, 50.1, 41.9, 29.6, 28.21, 28.17. HRMS calcd. for $[M + H]^+$ ($C_{82}H_{141}I_2N_8O_{24}$): 1875.8148; found: 1875.8074.

Synthesis of monomer 5

To a flame-dried flask was added 2,5-diiodo-1,4-hydroquinone (0.56 g, 1.5 mmol, 1.0 equiv.), bromide **14** (0.86 g, 3.8 mmol, 2.5 equiv.), potassium iodide (0.13 g, 0.76 mmol, 0.50 equiv.), potassium carbonate (1.3 g, 9.2 mmol, 6.0 equiv.), and 18-crown-6 (0.81 g, 3.1 mmol, 2.0 equiv.). DMF (30 mL) was added and the resulting solution was heated at 100 °C for 24 h under an argon atmosphere. The solvent was removed in vacuo and the resulting residue was dissolved in a small amount of dichloromethane, concentrated to dryness on a small amount of silica gel, and purified by column chromatography on silica gel (10:90 ethyl acetate – hexanes eluent) to yield compound **5** (0.39 g, 40%). IR (cm^{-1}, thin film from CH_2Cl_2): 3356, 3052, 2974, 1688, 1532. ^1H NMR (400 MHz, $CDCl_3$, δ): 1.47 (s, 18 H), 3.56 (dt, J = 5.08, 5.07 Hz, 4 H), 4.01 (t, J = 5.07 Hz, 4 H), 5.08 (br s, 2 H), 7.19 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 164.6, 152.77, 123.2, 86.5, 71.5, 69.9, 40.8, 28.42. HRMS calcd. for $[M]^+$ ($C_{20}H_{30}I_2N_2O_6$): 648.0193; found: 648.0176.

Synthesis of polymer 15

Monomer **2** (61 mg, 33 μmol, 1.0 equiv.) and monomer **3** (19 mg, 33 μmol, 1.0 equiv.) were added to a flame-dried two-neck round-bottom flask. The monomers were put under a nitrogen atmosphere and dry toluene (2 mL) was added, followed by dry NEt_3 (0.23 mL, 1.6 mmol). To this solution were added bis(triphenylphosphine)palladium(II) chloride (1.0 mg, 1.4 μmol, 0.040 equiv.) and copper(I) iodide (1.0 mg, 5.2 μmol, 0.16 equiv.). The reaction mixture was heated at 50 °C for 48 h. The solvent was removed in vacuo and the resulting residue was dissolved in DMF (2 mL). This solution was then dialyzed against DMF for 24 h to yield polymer **27** (71 mg, quantitative yield). UV–vis absorption (CH_3OH) λ_{max}: 430 nm. Emission λ_{max}: 465 nm. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.23 (s, 18 H), 1.42 (s, 90 H), 2.29–2.46 (m, 10 H), 2.69–2.94 (m, 16 H), 3.06–4.15 (m, 52 H), 7.00 (br s, 2 H), 7.42 (br s, 2 H). SEC: M_w (conventional calibration) = 31 300 g mol^{-1}, PDI = 2.6; M_w (MALS; dn/dc = 0.138) = 40 500 g mol^{-1}, PDI = 1.9.

Synthesis of polymer 4

Monomer **3** (0.37 g, 0.63 mmol, 1.0 equiv.) and monomer **5** (0.41 g, 0.63 mmol, 1.0 equiv.) were added to a flame-dried two-neck round-bottom flask. The monomers were put under a nitrogen atmosphere and dry toluene (5 mL) was added, followed by dry NEt_3 (4.4 mL, 32 mmol). To this solution were added bis(triphenylphosphine)palladium(II) chloride (8.0 mg, 13 μmol, 0.020 equiv.) and copper(I) iodide (4.0 mg, 19 μmol, 0.030 equiv.). The reaction mixture was heated at 50 °C for 48 h. The solvent was then removed in vacuo and the resulting residue was dissolved in DMF (2 mL). This solution was then dialyzed against DMF for 24 h to yield polymer **4** (0.29 g, 47%). UV–vis absorption (CH_3OH) λ_{max}: 406 nm. Emission λ_{max}: 460 nm. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.43 (br s, 36 H), 2.48 (t, J = 6.63 Hz, 4 H), 3.52–4.29 (m, 36 H), 7.06–7.09 (m, 4H). SEC: M_n = 1100 g mol^{-1}, PDI = 2.42.

Deprotection of polymer 15 and complexation of Gd(III)

In a flame-dried flask under a nitrogen atmosphere, polymer **15** (70 mg, 32 μmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (1 mL) and trifluoroacetic acid (TFA) (1 mL) was added. The resulting solution was stirred for 2 h at room temperature and then the solvent was removed in vacuo and the resulting residue was dissolved in ultrapure water (2 mL). The solution was dialyzed against ultrapure water for 24 h. The resulting solution was then lyophilized to yield the deprotected polymer **16** (0.034 g, 71%). ^{1}H NMR (400 MHz, D_2O, δ; note that aromatic peaks were not observed, likely because of solubility limitations and aggregation): 2.30–2.42 (m, 8 H), 2.63–4.02 (m, 70 H). Polymer **16** (10 mg, 7.0 μmol, 1.0 equiv.) was then redissolved in ultrapure water (5 mL) and combined with a solution of gadolinium chloride hexahydrate (1.0 mg, 28 μmol, 4.0 equiv.) in 2 mL of ultrapure water and the pH was adjusted to 7.4 using 0.1 mol L^{-1} NaOH. The resulting solution was stirred for 12 h at room temperature and then the solvent was reduced in vacuo to a volume of 2 mL. This solution was then dialyzed against ultrapure water for 24 h. The solvent was removed using a lyophilizer to yield polymer **1** (12 mg, quantitative yield.). ^{1}H NMR could not be obtained because of the paramagnetic Gd. ICP-MS: mass of polymer analyzed: 0.2 mg; mass of Gd(III) expected: 35 μg; mass of Gd(III) found: 34.8 μg.

Deprotection of polymer 4

In a flame-dried flask under a nitrogen atmosphere, polymer **4** (0.28 g, 0.29 mmol, 1.0 equiv.) was dissolved in dichloromethane (1 mL) and TFA (1 mL) was added. The resulting solution was stirred for 2 h at room temperature and then the solvent was removed in vacuo and the resulting residue was dissolved in DMF (2 mL). The solution was then dialyzed against DMF for 24 h to yield the deprotected polymer **17** (0.118 g, 62%). ^{1}H NMR (400 MHz, D_2O, δ) 2.44 (br s, 4 H), 3.10–4.33 (m, 36 H), 7.01 (br s, 4 H).

Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca).

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