

Contrast Agents for Magnetic Resonance Imaging Synthesized with Ring-Opening Metathesis Polymerization

Matthew J. Allen, Ronald T. Raines, and Laura L. Kiessling*

Contribution from the Departments of Chemistry and Biochemistry, University of Wisconsin–Madison, Madison, WI 53706

E-mail: kiessling@chem.wisc.edu

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Experimental Procedures

Commercial chemicals were of reagent grade or better and used without further purification unless otherwise noted. Dichloromethane and diisopropylethylamine were distilled from calcium hydride; tetrahydrofuran (THF) was distilled from sodium/benzophenone, and methanol was distilled from magnesium.

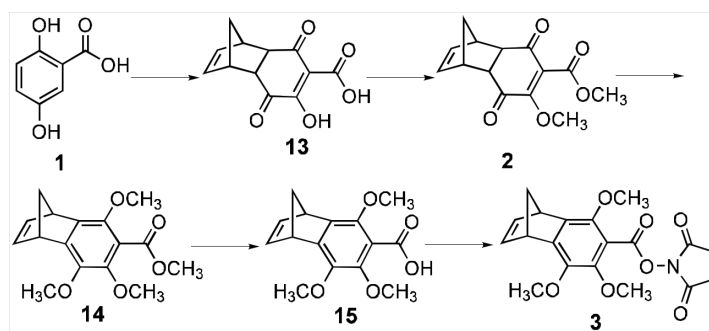
Flash chromatography was performed using silica gel 60, 230-450 mesh (Sorbent Technologies). Analytical thin-layer chromatography (TLC) was carried out on EM Science TLC plates precoated with silica gel 60 F₂₅₄ (250- μ m layer thickness). TLC visualization was accomplished using a UV lamp and/or charring with potassium permanganate stain (3 g KMnO₄, 20 g K₂CO₃, 5 mL 5% (w/v) aqueous NaOH, 300 mL H₂O). MilliQ water and PD-10 columns (Amersham Biosciences) were used for polymer purification.

¹H NMR spectra were obtained using a Bruker AC-300 (300 MHz) or Varian UNITY-500 (500 MHz) spectrometer, and ¹³C NMR spectra were obtained using a Bruker AC-300 (75 MHz) spectrometer. Chemical shifts are reported relative to residual solvent signals (CDCl₃: ¹H: δ 7.27, ¹³C: δ 77.23; CD₃OD: ¹H: δ 3.31, ¹³C: δ 49.15; DMSO-*d*₆: ¹H: δ 2.50, ¹³C: δ 39.51; D₂O: ¹H: δ 4.79, ¹³C: δ 39.51 – internal DMSO-*d*₆ standard). ¹H NMR data are assumed to be first order with apparent doublets and triplets reported as d and t, respectively. Multiplets are reported as m, and resonances that appear broad are designated as bs. High-resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT. Liquid chromatography and mass spectrometry (LC-MS) analysis was performed on a Shimadzu LC-MS containing a C18 column (Supelco Discovery, 2.1 X 150 mm) equilibrated with 0.4% (v/v) formic acid.

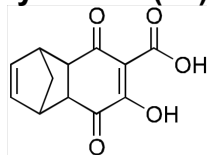
Polydispersity index (PDI) values were obtained using a Beckman Coulter high-performance liquid chromatography system, two Polymer Laboratories PLgel 5 μ m MIXED-D 300 \times 7.5 mm columns in series, Polymer laboratories EasiCal Polystyrene Standards (PS-1), and Cirrus GPC offline GPC/SEC Software Version 1.2. Elemental analyses and Gd solution concentration determinations were performed at Desert Analytics Laboratory, Tucson, AZ.

The longitudinal water proton relaxation rate at 60 MHz was measured by using a Bruker mq60 NMR Analyzer (Bruker Canada, Milton, Ont. Canada) operating at 1.5 T, by means of the standard inversion-recovery technique (20 data points, 8 scans each). A typical 90^o-pulse length was 6.16 μ s, and the reproducibility of the *T*₁ data was \pm 0.3%. Temperature was maintained at 22 $^{\circ}$ C with a Haake G cooling circulator.

3-Hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylic acid ethyl ester (**4**),¹ 10-methoxydec-9-en-2-one,² and (H₂IMes)(3-Br-py)₂(Cl)₂Ru=CHPh³ were synthesized following previously described procedures.

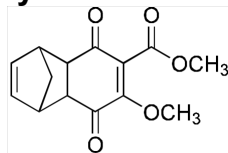


7-Hydroxy-5,8-dioxo-1,4,4 α ,5,8,8 α -hexahydro-1,4-methanonaphthalene-6-carboxylic acid (**13**):

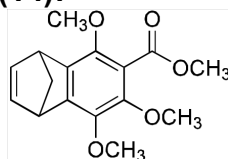


Modifications to a previously described procedure were used.⁴ A suspension of 2,5-dihydroxybenzoic acid (40.0 g, 0.263 mol) and ammonium cerium(IV) sulfate (315 g, 0.528 mol) in carbon tetrachloride (3 L) was mechanically stirred rapidly in the dark for 45 min. The suspension was filtered, and freshly cracked cyclopentadiene (50 mL, 0.61 mmol) was added to the filtrate, and the mixture was allowed to stir. The solution color immediately changed from dark to light yellow. After 10 min, the solvent was removed under reduced pressure. The resulting orange solid was washed with hexanes, dissolved in dichloromethane, and concentrated three times to yield 2.79 g (4.5%) of **13** as an orange solid. Formation of **13** only occurred with new bottles of carbon tetrachloride; distilled carbon tetrachloride did not yield the desired product. ¹H NMR (300 MHz, CDCl₃): δ = 1.55-1.69 (m, 2H, CH₂), 3.32-3.36 (m, 1H, (CH)₂-CH-CH₂), 3.53-3.57 (m, 1H, (CH)₂-CH-CH₂), 3.63-3.68 (m, 2H, (CH)₂-CH-C(O)), 6.10-6.17 (m, 2H, HC=CH), 14.19 (bs, 1H, OH), 15.12 (bs, 1H, C(O)OH); ¹³C NMR (75 MHz, CDCl₃): δ = 47.0 ((CH)₂-CH-CH₂), 47.6 ((CH)₂-CH-CH₂), 49.5 (CH₂), 49.6 ((CH)₂-CH-C(O)), 50.1 ((CH)₂-CH-C(O)), 107.3 ((C(O))₂-C=C), 135.5 (HC=CH), 135.9 (HC=CH), 173.4, 175.5, 193.2, 199.2; ESI-MS calcd for C₁₂H₁₀O₅ [M - H]⁻: 233.0450, found 233.0457.

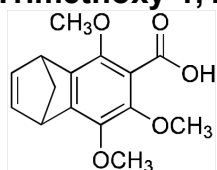
7-Methoxy-5,8-dioxo-1,4,4 α ,5,8,8 α -hexahydro-1,4-methanonaphthalene-6-carboxylic acid methyl ester (**2**):



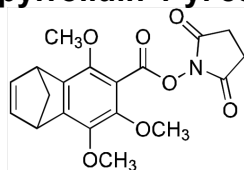
To a cooled (0 °C), stirred mixture of aqueous 50% KOH (100 mL) and diethyl ether (200 mL) was added nitrosomethyl urea (17.6 g, 171 mmol). When the ether layer turned yellow, it was decanted into an Erlenmeyer flask containing KOH pellets at 0 °C. The ether layer was then decanted into an ether solution (200 mL) of **13** (2.00 g, 8.54 mmol) at 0 °C. After 30 min, the reaction mixture was warmed to ambient temperature and stirred for 1 h. Purification was performed using silica gel chromatography (dichloromethane) to yield 1.89 g (85%) of **2** as a sticky yellow solid. ¹H NMR (300 MHz, CDCl₃): δ = 1.41-1.57 (m, 2H, CH₂), 3.21-3.31 (m, 2H, (CH)₂-CH-CH₂), 3.51-3.54 (m, 2H, (CH)₂-CH-C(O)), 3.83 (s, 3H, CO-CH₃), 3.96 (s, 3H, C(O)O-CH₃), 6.07-6.18 (m, 2H, HC=CH); ¹³C NMR (75 MHz, CDCl₃): δ = 48.8 ((CH)₂-CH-CH₂), 49.0 ((CH)₂-CH-CH₂), 49.0 (CH₂), 49.2 ((CH)₂-CH-C(O)), 49.4 ((CH)₂-CH-C(O)), 52.9 (CO-CH₃), 59.8 (C(O)O-CH₃), 135.0 (HC=CH), 136.1 (HC=CH), 158.9, 164.7, 194.8, 195.4; ESI-MS calcd for C₁₄H₁₄O₅ [M + H]⁺: 263.0919, found 263.0929.

5,7,8-Trimethoxy-1,4-dihydro-1,4-methanonaphthalene-6-carboxylic acid methyl ester (14):

To a refluxing solution of **2** (2.50 g, 9.53 mmol) in acetone (60 mL) was added dimethyl sulfate (9.0 mL, 95 mmol). A 10% methanolic KOH solution was added dropwise until the color of the solution stopped changing from purple to tan. The reaction mixture was heated at reflux for 1 h, at which point a 2 N HCl solution was added until the mixture turned clear. The reaction mixture was extracted four times with dichloromethane, dried over sodium sulfate, and concentrated. Purification was performed using silica gel chromatography (1:4 ethyl acetate/hexanes) to yield 1.86 g (67%) of **14** as a yellow oil. ^1H NMR (300 MHz, CDCl_3): δ = 2.15-2.23 (m, 2H, CH_2), 3.83 (s, 3H, O- CH_3), 3.83 (s, 3H, O- CH_3), 3.85 (s, 3H, O- CH_3), 3.90 (s, 3H, $\text{CO}_2\text{-CH}_3$), 4.16-4.18 (m, 2H, CH-CH(C)-CH_2), 6.76-6.82 (m, 2H, HC=CH); ^{13}C NMR (75 MHz, CDCl_3): δ = 47.6 (CH-CH(C)-CH_2), 48.0 (CH-CH(C)-CH_2), 52.5 ($\text{CO}_2\text{-CH}_3$), 61.6 (O- CH_3), 61.9 (O- CH_3), 62.2 (O- CH_3), 68.5 (CH_2), 119.2, 137.2, 142.5 (HC=CH), 142.8 (HC=CH), 144.0, 146.6, 146.8, 147.9, 167.0; TLC: R_f = 0.67 (2:1 ethyl acetate/hexanes); ESI-MS calcd for $\text{C}_{16}\text{H}_{18}\text{O}_5$ [$\text{M} + \text{Na}$] $^+$: 313.1052, found 313.1037.

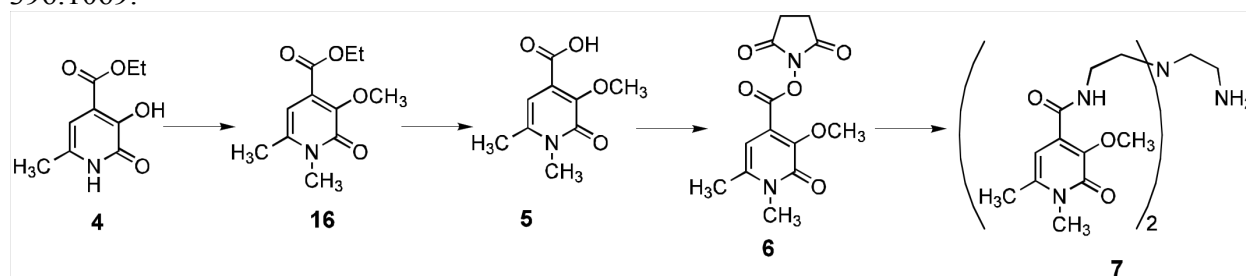
5,7,8-Trimethoxy-1,4-dihydro-1,4-methanonaphthalene-6-carboxylic acid (15):

To a solution of **14** (0.591 g, 2.05 mmol) in methanol (50 mL) was added a 4 N NaOH solution (50 mL), and the resulting reaction mixture was heated at reflux for 5 h. Methanol was removed under reduced pressure and a 3 N HCl solution was added to achieve pH 1. The reaction mixture was extracted three times with ethyl acetate, dried over sodium sulfate, and concentrated under reduced pressure to yield 0.567 g of **15** as a brown oil in nearly quantitative yield. ^1H NMR (300 MHz, CDCl_3): δ = 2.18-2.27 (m, 2H, CH_2), 3.86 (s, 3H, O- CH_3), 3.89 (s, 3H, O- CH_3), 3.92 (s, 3H, O- CH_3), 4.19-4.22 (m, 2H, CH-CH(C)-CH_2), 6.78-6.86 (m, 2H, HC=CH); ^{13}C NMR (75 MHz, CDCl_3): δ = 47.6 (CH-CH(C)-CH_2), 47.9 (CH-CH(C)-CH_2), 61.6 (CH_3), 62.1 (CH_3), 62.5 (CH_3), 68.5 (CH_2), 117.9, 137.8, 142.4 (HC=CH), 142.9 (HC=CH), 144.2, 147.2, 147.6, 148.6, 170.6; ESI-MS calcd for $\text{C}_{15}\text{H}_{16}\text{O}_5$ [$\text{M} - \text{H}$] $^-$: 275.0920, found 275.0910.

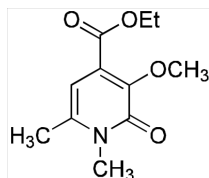
5,7,8-Trimethoxy-1,4-dihydro-1,4-methanonaphthalene-6-carboxylic acid 2,5-dioxypyrrolidin-1-yl ester (3):

To a solution of **15** (0.972 g, 3.52 mmol) in dichloromethane (80 mL) was added *N*-hydroxysuccinimide (0.547 g, 4.75 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide

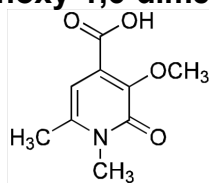
hydrochloride (0.890 g, 4.64 mmol), and the resulting reaction mixture was stirred under dinitrogen for 15 h. The reaction mixture was diluted with dichloromethane (100 mL), washed twice with a 0.01 N HCl solution (50 mL), dried over sodium sulfate, and concentrated under reduced pressure. Purification was performed using silica gel chromatography (dichloromethane → 2:1 ethyl acetate/hexanes) to yield 0.898 g (68%) of **3** as a colorless sticky solid. ^1H NMR (300 MHz, CDCl_3): δ = 2.19-2.27 (m, 2H, CH- CH_2 -CH), 2.89 (bs, 4H, CH_2 - CH_2), 3.86 (s, 3H, O- CH_3), 3.92 (s, 3H, O- CH_3), 3.95 (s, 3H, O- CH_3), 4.19-4.23 (m, 2H, CH-CH(C)- CH_2), 6.78-6.85 (m, 2H, HC=CH); ^{13}C NMR (75 MHz, CDCl_3): δ = 25.9 (CH_2 - CH_2), 47.7 (CH-CH(C)- CH_2), 47.9 (CH-CH(C)- CH_2), 61.7 (O- CH_3), 62.3 (O- CH_3), 62.6 (O- CH_3), 68.6 (CH- CH_2 -CH), 137.7, 142.4 (CH=CH), 143.0 (CH=CH), 144.2, 147.9, 149.6, 149.9, 161.4, 169.1; TLC: R_f = 0.42 (2:1 ethyl acetate/hexanes); ESI-MS calcd for $\text{C}_{19}\text{H}_{19}\text{NO}_7$ [$\text{M} + \text{Na}$] $^+$: 396.1059, found 396.1069.



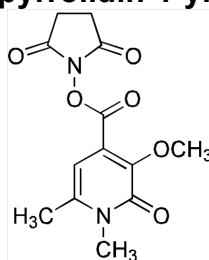
3-Methoxy-1,6-dimethyl-2-oxo-1,2-dihydropyridine-4-carboxylic acid ethyl ester (16):



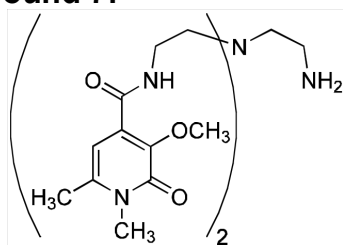
To a solution of **4** (8.69 g, 44.1 mmol) in dimethylformamide (440 mL) was added potassium carbonate (12.7 g, 91.6 mmol) and methyl iodide (27.5 mL, 441 mmol), and the resulting reaction mixture was stirred for 15 h. The reaction was concentrated under reduced pressure and diluted with dichloromethane (200 mL) and water (200 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (50 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification was performed using silica gel chromatography (2:1 ethyl acetate/hexanes) to yield 7.89 g (80%) of **16** as a tan solid. ^1H NMR (300 MHz, CDCl_3): δ = 1.36 (t, 3H, J = 7.1 Hz, CH_2 - CH_3), 2.32 (s, 3H, C- CH_3), 3.52 (s, 3H, N- CH_3), 3.94 (s, 3H, O- CH_3), 4.33 (q, 2H, J = 7.1 Hz, CH_2), 6.16 (s, 1H, CH); ^{13}C NMR (75 MHz, CDCl_3): δ = 14.3 (CH_2 - CH_3), 20.7 (C- CH_3), 31.9 (N- CH_3), 60.4 (O- CH_3), 61.7 (CH_2), 103.8 (CH), 129.5, 140.3, 146.4, 160.7, 165.4; TLC: R_f = 0.20 (2:1 ethyl acetate/hexanes); ESI-MS calcd for $\text{C}_{11}\text{H}_{15}\text{NO}_4$ [$\text{M} + \text{H}$] $^+$: 226.1079, found 226.1072.

3-Methoxy-1,6-dimethyl-2-oxo-1,2-dihydropyridine-4-carboxylic acid (5):

To a solution of **16** (7.51 g, 33.3 mmol) in methanol (350 mL) was added a 4 M sodium hydroxide solution (350 mL). The resulting solution was heated at reflux, and after 2 h, methanol was removed under reduced pressure. An aqueous 3 N HCl solution was used to adjust the resulting solution to pH 1. The solution was extracted with ethyl acetate; the organic extracts were dried over sodium sulfate, and solvent was removed under reduced pressure to yield 6.44 g (98%) of **5** as a tan solid. ^1H NMR (300 MHz, CD_3OD): δ = 2.40 (s, 3H, C- CH_3), 3.58 (s, 3H, N- CH_3), 3.86 (s, 3H, O- CH_3), 6.36 (s, 1H, CH); ^{13}C NMR (75 MHz, CD_3OD): δ = 20.6 (C- CH_3), 32.6 (N- CH_3), 61.0 (O- CH_3), 105.9 (CH), 132.8, 143.4, 146.6, 162.6, 168.2; ESI-MS calcd for $\text{C}_9\text{H}_{11}\text{NO}_4$ [M - H] $^-$: 196.0610, found 196.0615.

3-Methoxy-1,6-dimethyl-2-oxo-1,2-dihydropyridine-4-carboxylic acid 2,5-dioxopyrrolidin-1-yl ester (6):

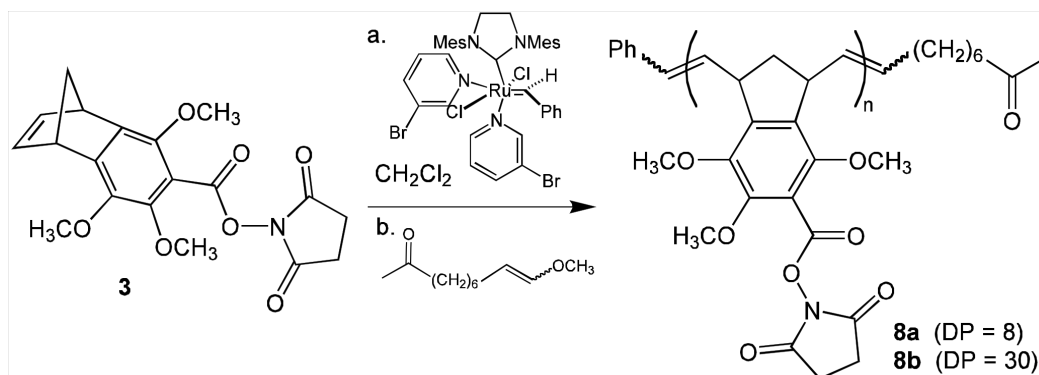
To a solution of **5** (5.36 g, 27.2 mmol) in dichloromethane (500 mL) was added *N*-hydroxysuccinimide (4.23 g, 36.7 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (6.89 g, 35.9 mmol), and the resulting reaction mixture was stirred under dinitrogen for 15 h. The reaction mixture was washed twice with a 0.01 N HCl solution (150 mL), dried over sodium sulfate, and concentrated under reduced pressure. Purification was performed using silica gel chromatography (1:49 methanol/dichloromethane) to yield 7.83 g (98%) of **6** as a tan solid. ^1H NMR (300 MHz, CDCl_3): δ = 2.31 (s, 3H, C- CH_3), 4.08 (s, 4H, CH_2), 3.50 (s, 3H, N- CH_3), 3.98 (s, 3H, O- CH_3), 6.29 (s, 1H, CH); ^{13}C NMR (75 MHz, CDCl_3): δ = 20.6 (C- CH_3), 25.8 (CH_2), 32.0 (N- CH_3), 60.8 (O- CH_3), 103.0 (CH), 122.8, 140.7, 148.8, 159.9, 160.3, 169.0; TLC: R_f = 0.33 (1:19 methanol/dichloromethane); ESI-MS calcd for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_6$ [M + H] $^+$: 295.0930, found 295.0931.

Compound 7:

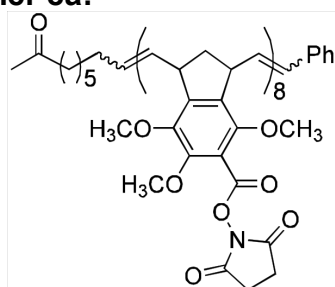
Tritylchloride resin (2.0 g, 3.2 mmol) was swelled in tetrahydrofuran (20 mL) for 15 min at which point tris(2-aminoethyl)amine (4.98 mL, 32.0 mmol) was added, and the resulting

solution was mixed for 20 h. The resin was drained and washed four times with 20 mL of 17:2:1 dichloromethane/methanol/diisopropylethylamine, and three times with 20 mL of dichloromethane. To the washed resin was added dichloromethane (20 mL), diisopropylethylamine (5.57 mL, 32.0 mmol), and **6** (3.77 g, 12.8 mmol), and the resulting solution was stirred for 20 h. The resin was drained and washed three times with 20 mL of dichloromethane and dried under reduced pressure. A solution of 38:1:1 trifluoroacetic acid/triisopropylsilane/water (24 mL) was added to the resin; after 2 h, the resin was filtered and rinsed with trifluoroacetic acid (5 mL). The combined trifluoroacetic acid-containing filtrate was reduced in volume to 2 mL. This solution was added dropwise to diethyl ether (400 mL) at 0 °C, and the resulting precipitate was collected. Purification was performed using basic alumina chromatography (1:9 → 3:7 methanol/dichloromethane) to yield 1.0 g (63%) of **7** as a white solid. ¹H NMR (300 MHz, CD₃OD): δ = 2.39 (s, 6H, C-CH₃), 2.62-2.78 (m, 8H, H₂N-CH₂ and N-(CH₂)₃), 3.48 (t, 4H, *J* = 6.2 Hz, C(O)NH-CH₂), 3.56 (s, 6H, N-CH₃), 3.88 (s, 6H, O-CH₃), 6.40 (s, 2H, CH); ¹³C NMR (75 MHz, CD₃OD): δ = 20.7 (C-CH₃), 32.6 (N-CH₃), 39.1 (C(O)NH-CH₂), 40.3 (H₂N-CH₂), 54.5 (N-(CH₂)₃), 57.7 (N-(CH₂)₃), 60.9 (O-CH₃), 105.7 (CH), 133.3, 143.3, 145.5, 161.9, 166.4; TLC: R_f = 0.21 (1:19 methanol/dichloromethane on basic alumina plates); ESI-MS calcd for C₂₄H₃₆N₆O₆ [M + H]⁺: 505.2775, found 505.2794.

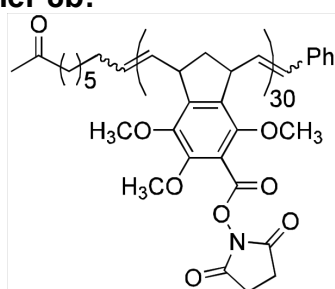
General Procedure for Polymerization Reactions:



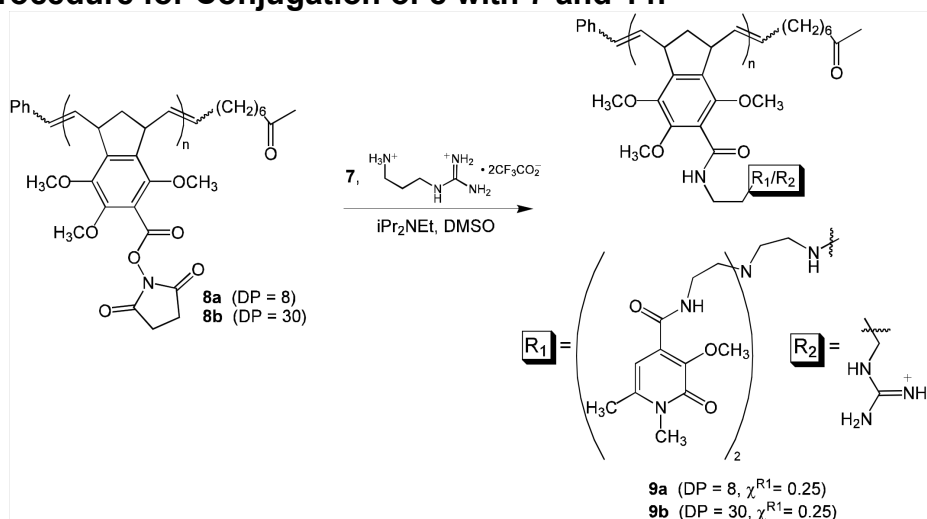
Polymerizations were carried out under an argon atmosphere in scintillation vials. Solutions of (H₂IMes)(3-Br-py)₂(Cl)₂Ru=CHPh (5.35 mM) and **3** (0.134 M) in degassed dichloromethane were cooled to -20 °C. The solutions were combined in the desired monomer to initiator ratio, and degassed dichloromethane was added to bring the final concentration of **3** to 38.3 mM. The reactions were allowed to warm slowly to ambient temperature. After consumption of **3**, as determined by TLC, 10-methoxydec-9-en-2-one (20 μL, 0.10 mmol) was added, and the reaction mixture was allowed to stir for 15 h. The reaction mixtures were added dropwise to a 30-fold volume excess of diethyl ether. The resulting white solid was collected, and residual solvent was removed under reduced pressure.

Polymer 8a:

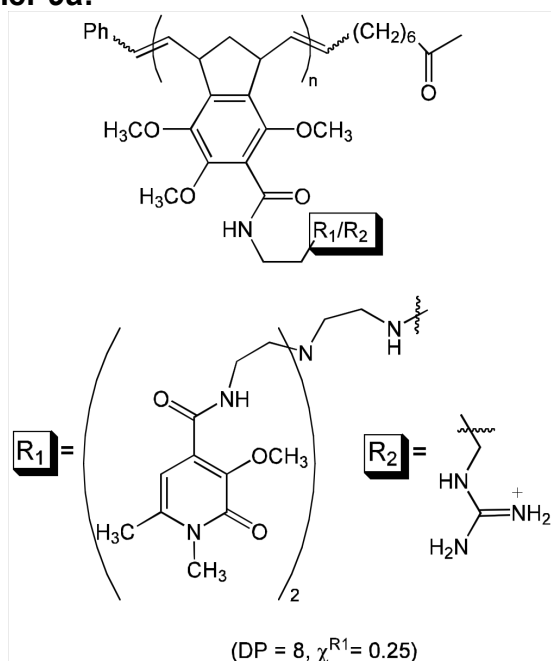
Yield = 76.3 mg (71%) black solid. $^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ = 1.53 (bs), 2.50 (bs), 2.86 (bs, $\text{C(=O)-CH}_2\text{-CH}_2\text{-C(=O)}$), 3.73 (bs, O-CH_3), 4.24 (bs), 5.52 (bs, 18H, HC=CH), 7.14 (bs, 5H, C_6H_5); PDI 1.58; Calculated MW 3217; M_w 2788; M_n 1760.

Polymer 8b:

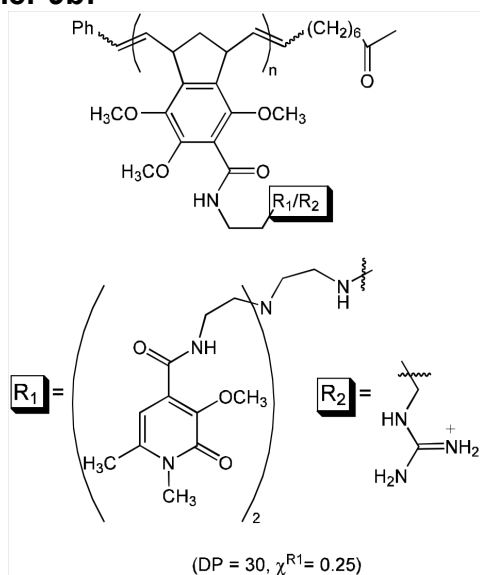
Yield = 165 mg (94%) off-white solid. $^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ = 1.07 (bs), 2.84 (bs, $\text{C(=O)-CH}_2\text{-CH}_2\text{-C(=O)}$), 3.64 (bs, O-CH_3), 5.31 (bs, 63H, HC=CH), 7.00 (bs, 5H, C_6H_5); PDI 1.21; Calculated MW 11431; M_w 5004; M_n 4122.

General Procedure for Conjugation of 8 with 7 and 14:

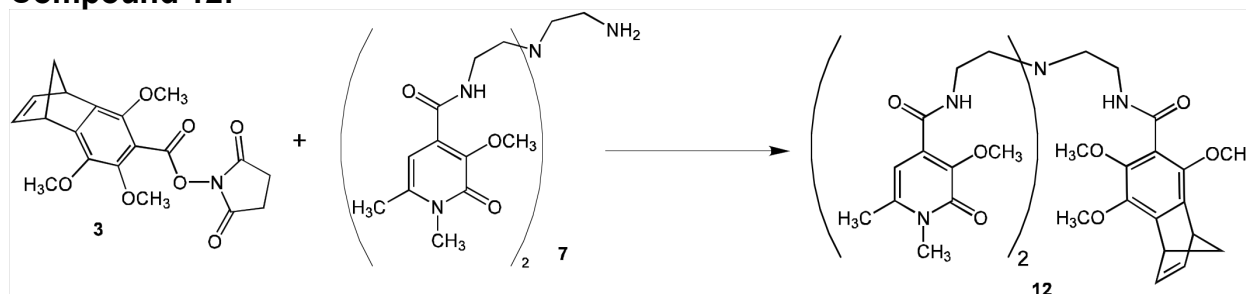
To a solution of **8** (5 mg) in dimethylsulfoxide (200 μL) and diisopropylethylamine (10 equivalents per monomer unit) was added **7** (0.25 equivalents per monomer unit) and *N*-(3-aminopropyl)guanidine bis-trifluoroacetic acid salt (0.75 equivalents per monomer unit). After 15 h, the entire reaction mixture was passed through a PD-10 column. Fractions containing polymer were collected, and solvent was removed under reduced pressure.

Polymer 9a:

Yield = 12.4 mg (70%) brown solid. $^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$): $\delta = 1.18$ (bs), 1.68 (bs), 2.31 (bs), 2.65 (bs), 3.20 (bs), 3.43 (bs), 3.68 (bs), 4.12 (bs), 5.42 (bs, 17H, $\text{HC}=\text{CH}$), 6.22 (bs, 2.9H, $\text{C}-\text{CH}=\text{C}-\text{CH}_3$), 7.20 (bs), 7.69 (bs), 8.31 (bs).

Polymer 9b:

Yield = 8.37 mg (38%) brown glass. $^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$): $\delta = 1.18$ (bs), 1.68 (bs), 2.31 (bs), 2.65 (bs), 3.20 (bs), 3.43 (bs), 3.68 (bs), 4.12 (bs), 5.42 (bs, 62H, $\text{HC}=\text{CH}$), 6.22 (bs, 7.6H, $\text{C}-\text{CH}=\text{C}-\text{CH}_3$), 7.20 (bs), 7.69 (bs), 8.31 (bs).

Compound 12:

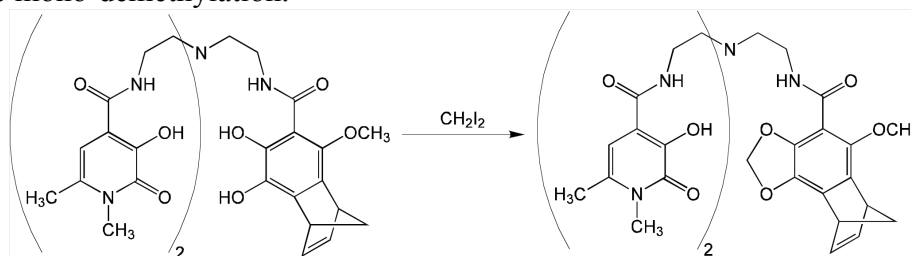
A solution of **7** (0.304 g, 0.603 mmol), **3** (0.150 g, 0.402 mmol), and diisopropylethylamine (0.350 mL, 2.01 mmol) in dichloromethane (10 mL) was stirred under dinitrogen for 4 h, at which point solvent was removed under reduced pressure. Purification was performed using silica gel chromatography (1:19 methanol/dichloromethane) to yield 0.202 g (66%) of **12** as a white solid. ^1H NMR (300 MHz, CDCl_3): δ = 2.10-2.20 (m, 2H, CH-CH₂-CH), 2.33 (s, 6H, C-CH₃), 2.79-2.86 (m, 6H, N-(CH₂)₃), 3.49-3.55 (m, 12H, C(O)NH-CH₂ and N-CH₃), 3.80 (s, 3H, O-CH₃), 3.81 (s, 3H, O-CH₃), 3.82 (s, 3H, O-CH₃), 3.94 (s, 6H, O=C-C(=C)-O-CH₃), 4.11-4.14 (m, 2H, CH-CH(C)-CH₂), 6.27-6.31 (t, 1H, J = 5.6 Hz, (CH₃-O-C)₂-C-C(=O)-NH), 6.55 (s, 2H, CH₃-C=CH-C), 6.73-6.78 (m, 2H, CH=CH), 8.16-8.20 (t, 2H, J = 5.2 Hz, CH-C-C(=O)-NH); ^{13}C NMR (75 MHz, CDCl_3): δ = 20.8 (C-CH₃), 31.9 (N-CH₃), 38.2 (C(O)NH-CH₂), 47.6 (CH-CH(C)-CH₂), 47.9 (CH-CH(C)-CH₂), 53.7 (N-(CH₂)₃), 53.8 (N-(CH₂)₃), 60.0 (O=C-C(=C)-O-CH₃), 61.6 (O-CH₃), 62.1 (O-CH₃), 62.4 (O-CH₃), 68.4 (CH-CH₂-CH), 104.5 (CH₃-C=CH-C), 122.3, 129.3, 137.4, 140.2, 142.4 (CH=CH), 142.9 (CH=CH), 144.2, 145.1, 145.7, 146.9, 147.7, 160.2, 163.9, 166.2; TLC: R_f = 0.26 (2:23 methanol/dichloromethane); ESI-MS calcd for C₃₉H₅₀N₆O₁₀ [M + H]⁺: 763.3667, found 763.3648.

General Procedure for Deprotection of Methyl Ethers and Metallation:

To a solution of methyl ether-protected chelate (1 eq. of **9a**, **9b**, or **12**) in anhydrous dimethylsulfoxide (DMSO) under argon was added a solution of sodium ethanethiolate (2.5 eq. per OMe) in anhydrous DMSO to make a final solution of between 0.4 and 26 mM chelate and between 44 and 328 mM sodium ethanethiolate. The resulting solution was heated to 142 °C for 1.5 h at which point a four-fold volume excess of water was added to quench excess sodium ethanethiolate. The reaction mixture was concentrated to dryness under reduced pressure, and LC-MS of the product resulting from **12** showed one major product with a mass corresponding to four of five methyl ethers removed.

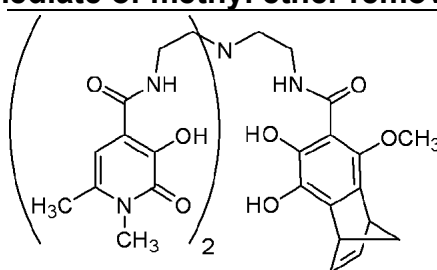
To ensure that the remaining methoxy group was not in a position to interfere with metal chelation, a portion of the intermediate (0.80 mg, 1.2 μmol) was dissolved in anhydrous DMSO (11.4 μL) under argon. To the resulting solution was added CH₂I₂ (1.4 μmol , 0.11 μL) and sodium carbonate (2.5 μmol , 0.27 mg), and the reaction was heated to 55 °C for 8 h. The mixture was cooled to ambient temperature, and 2 mL of water was added. LC/MS indicated that there was one major product with mass ([M+H]⁺ = 721.4) corresponding to the formation of a methylene bridge between the two ortho hydroxyl groups. This result indicates that the remaining methoxy group was not in a position to interfere with metal chelation. Additional support for the position of the remaining methoxy group is found in the work of Feutrill and Mirrington.⁵ They treated a series of anisole compounds with sodium ethanethiolate and found

that *ortho*-methoxy groups underwent complete demethylation while *para*-methoxy groups yielded selective mono-demethylation.



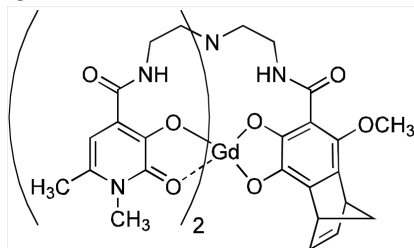
The intermediate was dissolved in water in a concentration between 0.1 and 6.5 mM, and five drops of DMSO were added. One equivalent of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ per chelating group was then added, and the pH of the resulting solution was adjusted to 7 using 0.1 N NaOH and 0.1 N HCl solutions. The reaction mixture was heated to 80 °C for 1 h, at which point the pH was readjusted to 7. The reaction mixture was allowed to sit at ambient temperature for 15 h. The pH was brought to 10 using a 0.1 N NaOH solution to precipitate any unchelated gadolinium as $\text{Gd}(\text{OH})_3$. $\text{Gd}(\text{OH})_3$ was removed by filtration through a 0.45 μm syringe filter. The pH of the filtered solution was brought to 7 using a 0.1 N HCl solution, and the resulting solution was directly used for T_1 acquisition. After measurement of T_1 , solutions were analyzed for Gd concentration, and the T_1 and Gd concentration data were used to calculate per Gd relaxivity. Per Gd relaxivity was multiplied by the number of chelates per polymer (as determined by NMR spectroscopy of polymers **9**) to determine molecular relaxivity values.

Intermediate of methyl ether removal from **12**:

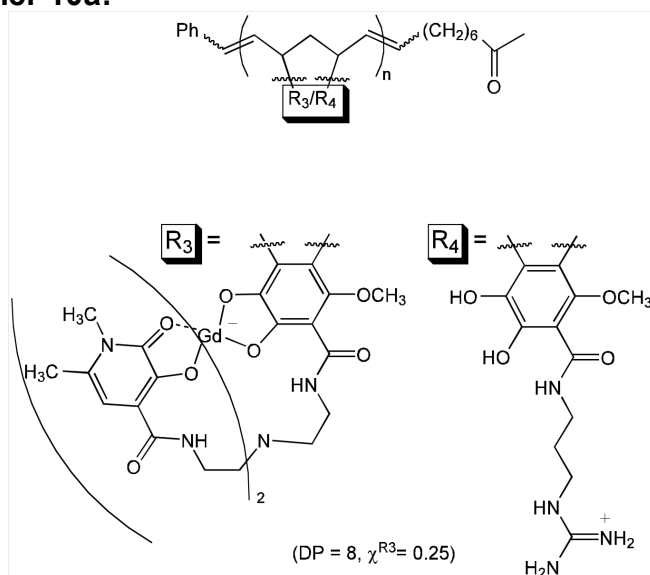


ESI-MS calcd for $\text{C}_{35}\text{H}_{42}\text{N}_6\text{O}_{10}$ $[\text{M} + \text{H}]^+$: 707.3, found 707.4.

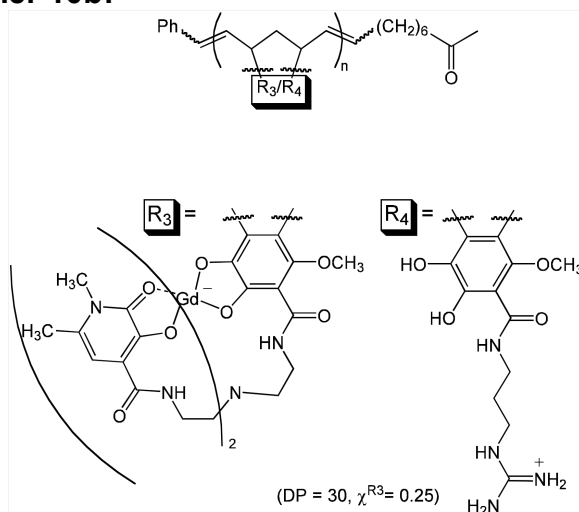
Complex **11**:



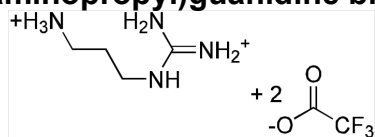
ESI-MS calcd for $\text{C}_{35}\text{H}_{38}\text{GdN}_6\text{O}_{10}$ $[\text{M} - \text{CH}_3 + \text{Na} + 2\text{H}]^+$: Gd isotope pattern centered at 870.2, found Gd isotope pattern centered at 870.1. Maximum Gd concentrations ranged from 0.0054% to 0.0176%. Relaxivity (r_1): $10.5 \pm 0.8 \text{ mM}^{-1}\text{s}^{-1}$ (error is given as the standard deviation).

Polymer 10a:

$\chi^{R3} = 0.25$, $\chi^{R4} = 0.75$. Maximum Gd concentrations ranged from 0.0040% to 0.0046%. Ionic relaxivity (r_1): $10.1 \pm 0.5 \text{ mM}^{-1} \text{ s}^{-1}$; Molecular relaxivity (r_1): $18.8 \pm 0.9 \text{ mM}^{-1} \text{ s}^{-1}$ (errors are given as standard deviations).

Polymer 10b:

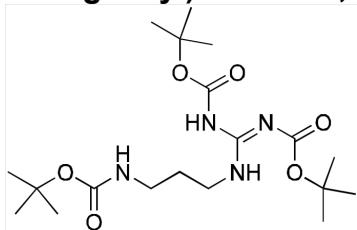
$\chi^{R3} = 0.25$, $\chi^{R4} = 0.75$. Maximum Gd concentrations ranged from 0.0005% to 0.0048%. Ionic relaxivity (r_1): $14.8 \pm 0.2 \text{ mM}^{-1} \text{ s}^{-1}$; Molecular relaxivity (r_1): $111.0 \pm 1.5 \text{ mM}^{-1} \text{ s}^{-1}$ (errors are given as standard deviations).

***N*-(3-aminopropyl)guanidine bis-trifluoroacetic acid salt:**

N-(bis-Boc-guanyl)-*N'*-Boc-1,3-diaminopropane (1.00 g, 2.40 mmol) was dissolved in 20 mL of 95% trifluoroacetic acid (TFA), 2.5% water, and 2.5% triisopropylsilane (v/v/v). After 14 h, the volume was reduced to a sticky residue under a stream of air. The residue was washed

with diethyl ether, dissolved in water, and freeze dried to yield 0.568 g (69%) of the desired salt as an extremely viscous, colorless oil. ^1H NMR (300 MHz, D_2O): $\delta = 1.96\text{--}2.06$ (m, 2H, $\text{CH}_2\text{-CH}_2\text{-CH}_2$), 3.11 (t, 2H, $J = 8.0$ Hz, $\text{N-CH}_2\text{-CH}_2$), 3.35 (t, 2H, $J = 7.0$ Hz, $\text{N-CH}_2\text{-CH}_2$); ^{13}C NMR (75 MHz, D_2O): $\delta = 27.6$ ($\text{CH}_2\text{-CH}_2\text{-CH}_2$), 38.3 (N-CH_2), 39.7 (N-CH_2), 158.3 (C-(N)_3); ESI-MS calcd for $\text{C}_4\text{H}_{12}\text{N}_4$ [$2\text{M} + \text{H}$] $^+$: 233.3, found 233.1; Anal. Calcd for $\text{C}_8\text{H}_{14}\text{F}_6\text{N}_4\text{O}_4$: C, 27.91; H, 4.10; F, 33.12; N, 16.28. Found: C, 27.87; H, 4.24; F, 31.17; N, 15.55.

***N*-(bis-Boc-guanyl)-*N'*-Boc-1,3-diaminopropane:**



To a solution of *N*-Boc-1,3-diaminopropane (5.00 mL, 28.6 mmol) in dimethylformamide (DMF) (100 mL) was added diisopropylethylamine (DIEA) (3.72 mL, 21.4 mmol) and bis-Boc-guanylpiperazine (3.31 g, 10.7 mmol). The reaction mixture was stirred for 14 h, at which time DMF was removed under reduced pressure. Water (20 mL) was added, and the mixture was extracted with dichloromethane. The organic layer was dried over magnesium sulfate, and solvent was removed under reduced pressure. Purification was performed using silica gel chromatography (5:1 \rightarrow 2:1 hexanes/ethyl acetate) to yield 3.07 g (69%) of the desired product as a white solid. ^1H NMR (300 MHz, CDCl_3): $\delta = 1.43$ (s, 9H, CH_3), 1.49 (s, 18H, CH_3), 1.64–1.72 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-CH}_2$), 3.10–3.17 (m, 2H, $\text{NH-CH}_2\text{-CH}_2$), 3.44–3.50 (m, 2H, $\text{NH-CH}_2\text{-CH}_2$), 5.60 (s, 1H, NH), 8.34 (t, 1H, $J = 5.6$ Hz, NH), 11.41 (s, 1H, NH); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 28.3$ (CH_3), 28.5 (CH_3), 28.7 (CH_3), 30.4 ($\text{CH}_2\text{-CH}_2\text{-CH}_2$), 37.3 ($\text{NH-CH}_2\text{-CH}_2$), 37.8 ($\text{NH-CH}_2\text{-CH}_2$), 79.0 ($\text{C}(\text{CH}_3)_3$), 79.4 ($\text{C}(\text{CH}_3)_3$), 83.4 ($\text{C}(\text{CH}_3)_3$), 153.4, 156.4, 156.9, 163.5; TLC: $R_f = 0.36$ (2:1 hexanes/ethyl acetate); ESI-MS calcd for $\text{C}_{19}\text{H}_{36}\text{N}_4\text{O}_6$ [$\text{M} + \text{H}$] $^+$: 417.2713, found 417.2693.

Estimation of Rotational Correlation Time

Values of the rotational correlation time (τ_R) were estimated using the Debye-Stokes equation shown below and data acquired for other linear Gd^{III} containing polymers.^{6,7} Assuming that the microviscosity and density of the polymers are the same, the ratio of molecular radii can be expressed by the ratio of the molecular weights. While the Debye-Stokes equation provides estimation for spherical molecules, the ROMP-derived polymers and polymers in references 6 and 7 are both linear and the ratio of their molecular weights is used only as an approximation.

$$\tau_R = \frac{4\pi\eta r_{\text{eff}}^3}{3k_B T}$$

Debye-Stokes equation (η = microviscosity, r_{eff} = molecular radius, k_B = the Boltzmann constant, T = temperature)

The table below shows average molecular

weight (MW), τ_R , and per Gd relaxivity for two linear polymers from the literature (τ_g , global motion correlation time, was used to estimate τ_R for comparison to ROMP-derived polymers **10a** and **10b** because of the rigid connection to the polymer backbone). The τ_R data for ROMP-derived polymers **10a** and **10b** were estimated by plotting τ_R vs. MW for the published polymers, and using the resulting slope with the molecular weights of polymers **10a** and **10b**. The ratio of τ_R to relaxivity was then examined, and the relaxivity values in parentheses for **10a** and **10b** would be expected for linear polymers with their molecular weights and estimated τ_R values. These estimates match up very well with the actual measurements indicating that there is relaxivity increase due to increase in τ_R . Thus, the observed increase is proportional to what is seen in linear polymers of Gd^{III} diethylenetriaminepentaacetic acid (DTPA).

Polymer	MW (Da)	τ_R (10^{-12} s)	Per Gd relaxivity ($\text{mM}^{-1}\text{s}^{-1}$)
[DTPA-BA(CH ₂) ₁₀] _x	10300	2900	15.4
[DTPA-BA(CH ₂) ₁₂] _x	15700	4400	19.6
10a	4260	1200	10.1 (10.7)
10b	14200	4000	14.8 (18.5)

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