Journal of Materials Chemistry B

RSCPublishing

ARTICLE

Tailored biological retention and efficient clearance of pegylated ultra-small MnO nanoparticles as positive MRI contrast agents for molecular imaging

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

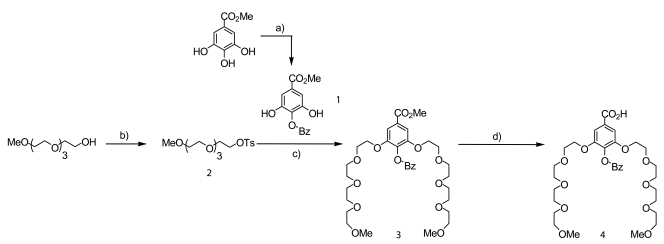
P. Chevallier[a], A. Walter[b], A. Garofalo[b], I. Veksler[a], J. Lagueux[c], S. Bégin-Colin[b], D. Felder-Flesch[b], M.-A. Fortin[a]*

[a] Axe médecine régénératrice, Centre de recherche du Centre hospitalier universitaire de Québec (CR-CHUQ),10 rue de l'Espinay, Québec, G1L 3L5, Canada, Département de génie des mines, de la métallurgie et des matériaux and Centre de recherche sur les matériaux avancés (CERMA), Université Laval, Québec, G1V 0A6, Canada.
[b] Institut de Physique et Chimie des Matériaux de Strasbourg (IPCMS), UMR 7504 CNRS-Université de Strasbourg, 23, rue du Loess, BP 43, 67034 Strasbourg Cedex 2, France.
[c] Axe médecine régénératrice, Centre de recherche du Centre hospitalier universitaire de Québec (CR-CHUQ), 10 rue de l'Espinay, Québec, G1L 3L5, Canada.
*Corresponding author: marc-andre.fortin@gmn.ulaval.ca, Fax: (+1) 418-656-5343; Tel: (+1) 418-656-8682

Supporting Information

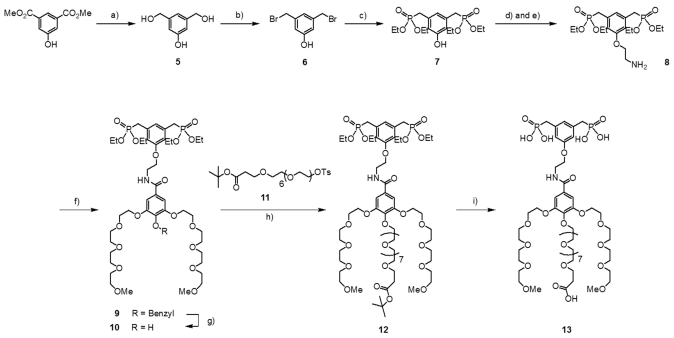
Section 1: Dendron synthesis

para-benzylated methyl gallate **1** and tosylated tetraethyleneglycol monomethyl ether **2** were obtained from commercially available methyl gallate and tetraethyleneoxide monomethyl ether respectively following previously described one-step-procedures.[1] A Williamson coupling between **1** and **2** in the presence of potassium carbonate and potassium iodide, allowed the preparation of ester **3** which was further saponified in basic conditions to afford carboxylic acid **4** in an overal 68 % yield (**Scheme S1**).



Scheme S1. a) Benzyl bromide, $KHCO_3$, KI, DMF, 30°C, 4 days; b) TsCl, NaOH, THF/H2O, RT, 24h; c) K_2CO_3, KI, acetone, reflux, 24h; d) NaOH, MeOH/H_2O, reflux, 2h.

Then, the bisphosphonic tweezer **7** was obtained through a three-step (reduction, bromation, phosphorylation) sequence and with 58% overall yield, starting from commercially available Dimethyl 5-hydroxyisophtalate (**Scheme S2**). Amine **8** was then easily prepared from **7** by a Williamson etherification with Boc-2-bromoethylamine followed by an acidic treatment with trifluoroacetic acid (54% overall yield). Peptidic coupling between **8** and **4** in the presence of BOP and *N,N*-diisopropylethylamine afforded benzylated compound **9** in 87% yield which was further deprotected by hydrogenation to obtain corresponding phenol **10**. Tosylated oligoethyleneglycol **11** reacted with **10** through a Williamson reaction to produced ethyl phosphonate 12 in 90% yield, bearing a long oligoethyleneglycol chain in *para*-position with respect to the phosphonic tweezer. Treatment of **12** with a large excess of trimethylsilyl bromide not only allowed obtaining the phosphonic acid function at the focal point, but also converting the terminal ester into its carboxylic acid counterpart **13** (94%). Compound **13** is referred to as phosphonate dendron (PDn) in the following text. ^1H, ^{13}C, ^{31}P NMR and MALDI data are provided in Section 1 of the Supporting info (SI).



Scheme S2. a) $LiAlH_4$ 1M in THF, THF, reflux, 3h; b) HBr in acetic acid 30%, Acetic acid, RT, 24h; c) $P(OEt)_3$, 160°C, 3h; d) Boc-2-bromoethylamine, K_2CO_3, KI, acetone, reflux, 16h; e) TFA, CH_2Cl_2, 0°C at RT, overnight; f) compound 4, BOP, DIPEA, CH_2Cl_2, RT, 24h; g) Pd/C 10%, H_2, EtOH, RT, overnight ; h) K_2CO_3, KI, acetone, reflux, 16h; i) TMSBr, CH2Cl2, RT, overnight.

Compound **1**: A solution of methyl gallate (20.0 g, 108.6 mmol), benzyl bromide (14.2 mL, 119.0 mmol, 1.1 eq.), $KHCO_3$ (32.4 g, 324.0 mmol, 3.0 eq.) and KI (0.1 g, 0.60 mmol) in DMF (100 mL) was stirred during 4 days at 30°C. The reaction mixture was poured into 1L of water and sulfuric acid was added until neutral pH was reached. The aqueous layer was then extracted 3 times with 150 mL of dichloromethane. The combined organic layers were washed three times with 50 mL of brine, dried over $MgSO_4$ and filtered. The solvent was removed and the residue was purified by column chromatography (SiO_2, dichloromethane/methanol (98/2)) to provide yellow oil. The obtained residue was filtered and washed with petroleum ether to obtain **1** as a white solid in 70% yield.

Compound **2**: A solution of *para*-toluenesulfonyl chloride (22.3 g, 105 mmol) in THF (35 mL) was added dropwise to a solution of tetraethyleneglycol monomethyl ether (20.0 g, 96 mmol) and NaOH (6.7 g, 166 mmol) in a mixture of THF/H_2O (135 mL/45 mL) kept at 0°C. After 1 hour stirring at 0°C, the reaction was allowed to warm at room temperature and further stirred for 20 additional hours. The solution was then poured into 200 mL of brine and the volatiles were evaporated. The resulting mixture was extracted several times with dichloromethane and the combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The solvent was evaporated and the residue (oil) was purified by column chromatography (SiO_2, dichloromethane/methanol (98/2)). **2** was obtained as pale yellow oil in 94% yield.

Compound **3**: A solution of **1** (9.2 g, 33.4 mmol), **2** (26.9 g, 74.3 mmol, 2.2 eq.), K_2CO_3 (28.0 g, 200 mmol, 6.0 eq.) and KI (0.6 g, 3.3 mmol, 0.1 eq.) in acetone (600 mL) was stirred 30 hours at 65°C. The reaction mixture was then filtered over Celite and the solvent was evaporated. The resulting crude product was diluted in dichloromethane (200 mL) and washed twice with an aqueous saturated solution of $NaHCO_3$ and with brine. After drying over $MgSO_4$, filtration and evaporation of the solvent, the crude product was purified by column chromatography (SiO_2, dichloromethane/methanol 98/2 to 95/5) to afford **3** as a colourless oil in 75% yield.

Compound **4**: Sodium hydroxyde (5.1 g, 127.0 mmol, 10 eq.) was added to a solution of compound **3** (8.3 g, 12.7 mmol) in a mixture of methanol/water 4/1 (150 mL). The reaction mixture was stirred 2h at 85°C, then concentrated *in vacuo* and hydrolyzed (200 mL). The pH was adjusted to 3 with HCl 12N and the aqueous solution was extracted with dichloromethane (3 x 100 mL). The combined organic phases were washed with brine and water, dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography (SiO_2, dichloromethane/methanol (95/5)) afforded **4** as a colourless oil in 90% yield.

Compound **5**: $LiAlH_4$ 0.5 M in THF (36.0 mmol, 1.8 eq.) was added dropwise to a solution of dimethyl 5-hydroxyisophtalate (4.20 g, 20.0 mmol) in anhydrous THF (21 mL) kept at 0°C. Then, after 3 hours reflux, the mixture was cooled to room temperature and acidified with 30 mL H_2O/H_2SO_4 10%. The THF was evaporated and the resulting aqueous phase was extracted several times (at least 6 times, TLC control) with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered and evaporated to afford **5** as a white solid in 94% yield.

Compound **6**: HBr 30% in acetic acid (36.0 mmol, 1.8 eq.) was added dropwise to a solution of **5** (2.00 g, 13.0 mmol) in acetic acid (21 mL) kept at 0°C. The mixture was stirred 24 h at room temperature, and then distilled water was added (80 mL). A white precipitate formed and the mixture was stirred 10 minutes more. The resulting aqueous phase was extracted 3 times with dichloromethane (3 x 200 mL) and the organic layer was washed twice with distilled water (2 x 120 mL), twice with a saturated solution of sodium hydrogenocarbonate (2 x 120 mL) and brine (80 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness to afford **6** as a white solid in 96% yield.

Compound **7**: A solution of **6** (2.24 g, 8.0 mmol) in $P(OEt)_3$ (4.0 eq. 5.0 mL), was stirred 2 hours at 160°C. The excess of $P(OEt)_3$ was evaporated under reduced pressure at 70°C. The crude product was purified by column chromatography (SiO_2, dichloromethane/methanol 95/5) to afford **7** as a white solid in 95% yield.

Compound **8**: To a solution of **7** (1.5 g, 3.8 mmol) in acetone (40 mL) were added (2-bromo-ethyl)carbamic acid tert-butyl ester (1.1 g, 4.95 mmol, 1.3 eq.), K_2CO_3 (2.1 g, 15.2 mmol, 4 eq.) and KI (0.1 g, 0.4 mmol, 0.1 eq.). The mixture was stirred 48h at 65°C, filtered over Celite and evaporated under reduced pressure. The resulting crude product was diluted in dichloromethane (100 mL) and washed twice with an aqueous saturated solution of $NaHCO_3$ and with brine. After drying over $MgSO_4$, filtration and evaporation of the solvent, the crude product was purified by column chromatography (SiO_2, dichloromethane/methanol 98/2 to 95/5) to afford (Boc-amino) derivative as white solid (76%). This compound (1.2 g, 2.2 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) at 0°C and trifluoroacetic acid was added dropwise 2 mL (22.0 mmol, 10.0 eq.). The reaction mixture was stirred overnight at room temperature, and then the volatiles were evaporated. The crude product was dissolved in dichloromethane (20 mL) and was washed with NaOH 1N (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness to afford **8** as a white solid in 88% yield which was used without further purification.

Compound **9**: BOP (1.2 g, 2.68 mmol, 1.3 eq.) was added under argon to a solution of **4** (1.45 g, 2.05 mmol, 1.0 eq.)

in distilled dichloromethane (30 mL). After 5 min. stirring, **8** (0.9 g, 2.05 mmol 1.0 eq.) and *N,N*-diisopropylethylamine (1.0 mL, 6.8 mmol, 3 eq.) were added and the reaction mixture was stirred overnight at room temperature. 50 mL of dichloromethane were added and the organic layer was washed with a solution of sodium hydroxyde 1N (2 x 30 mL), HCl 1N (2 x 30 mL), brine (2 x 30 mL) and water (1 x 30 mL), dried over MgSO4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, dichloromethane/methanol 98/2 to 95/5) to afford **9** as colorless oil in 87% yield.

Compound **10**: Pd/C 10% (0.5 eq.) was added to a solution of **9** (2 g, 1.9 mmol) in ethanol absolute (20 mL). The mixture was stirred under an hydrogen atmosphere at room temperature for 16 h. The product was filtered through a plug of Celite before being concentrated and purified by column chromatography (SiO_2, dichloromethane/methanol (98/2 to 90/10) to afford **10** as colorless oil in 87% yield.

Compound **11:** NEt_3 (840 mL, 6.0 mmol, 3.0 eq.) and para-toluenesulfonyl chloride (570 mg, 3.0 mmol, 1.5 eq.) were added sequentially to a solution of Hydroxy-dPEG™$_8$-t-butyl ester (1.00 g, 2.0 mmol) in 20 mL CH_2Cl_2 kept at 0°C. After 40 h stirring at room temperature, the reaction mixture was diluted with CH_2Cl_2 (70 mL), washed with brine, dried over MgSO4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, ethyl acetate/methanol 95/5 to 90/10) to afford **11** as a colorless oil in 70% yield.

Compound **12**: K_2CO_3 (0.13 g, 0.93 mmol, 3 eq.) and KI (18 mg, 0.11 mmol, 0.3 eq.) were added to an equimolar solution of phenol **10** (0.3 g, 0.31 mmol) and **11** (0.20 g, 0.31 mmol) in 10 mL acetone. The reaction mixture was stirred at 60°C during 24 h. After filtration over Celite, the solvent was evaporated and the residue was diluted in dichloromethane (50 mL). The organic layer was washed twice with a saturated solution of $NaHCO_3$, then with brine, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, dichloromethane/methanol (98/2 to 90/10) to afford **12** as a colourless oil in 90% yield.

Compound **13**: TMSBr (0.55 mL, 3 mmol, 30 eq.) was added dropwise to a solution of ethyl phosphonate **12** (0.2 g, 0.14 mmol) in 5 mL distilled dichloromethane kept at 0°C. After stirring overnight at room temperature, the volatiles were evaporated, methanol was added to the crude product and evaporated several times. The phosphonic acid **13** was obtained as orange oil in 94% yield without further purification.

Section 2: Dendron characterisation: ^{1}H, ^{13}C, ^{31}P NMR and MALDI data for compounds 1 – 13:

Compound **1**: ^{1}H NMR (300 MHz, CD_3OD-*d*) δ 7.52 (d, J = 7.5 Hz, 2H, Ar^2-2,6-*H*), 7.31 (m, 3H, Ar^2-3,4,5-*H*), 7.13 (s, 2H, Ar^1-2,6-*H*), 5.18 (s, 2H, Ar^2OCH_2), 3.83 (s, 3H, $COOCH_3$); ^{13}C NMR (75 MHz, CD_3OD-*d*) δ 167.1, 150.5, 138.2, 137.2, 128.5, 128.0, 127.8, 125.0, 108.8, 73.8, 51.2.

Compound **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 1.5 Hz, 2H, Ar-2,6-*H*), 7.28 (d, J = 1.5 Hz, 2H, Ar-3,5-*H*), 4.11-4.08 (m, 2H, $ArSO_2OCH_2$), 3.64-3.47 (m, 14H, OCH_2CH_2O), 3.31 (s, 3H, OCH_3), 2.39 (s, 3H, $ArCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.9, 133.2, 130.0, 72.1, 70.9, 70.7, 70.6, 69.5, 68.8, 59.1, 28.1, 21.8.

Compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 7.7 Hz, 2H, Ar^2-2,6-*H*), 7.28 (m, 5H, Ar^2-3,4,5-*H* and Ar^1-2,6-*H*), 5.12 (s, 2H, Ar^2OCH_2), 4.20-4.17 (t, J = 4.8 Hz, 4H, Ar^1OCH_2), 3.90 (s, 3H, $COOCH_3$), 3.88-3.85 (t, J = 4.8 Hz, 4H, OCH_2CH_2O), 3.74-3.69 (m, 4H, OCH_2CH_2O), 3.67-3.60 (m, 16H, OCH_2CH_2O), 3.54-3.50 (m, 4H, OCH_2CH_2O), 3.35 (s, 6H, $OCH_2CH_2OCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 152.5, 142.2, 138.2, 128.2, 128.0, 127.8, 125.3, 109.1, 74.8, 72.3, 71.2, 71.0, 70.9, 70.8, 70.0, 69.2, 59.3, 52.5. MALDI: calculated for $C_{33}H_{50}NaO_{13}$: 677.33, obtained: 677.03.

Compound **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 7.8 Hz, 2H, Ar^2-2,6-*H*), 7.38 (s, 2H, Ar^1-2,6-*H*), 7.35-7.28 (m, 3H, Ar^2-3,4,5-*H*), 5.13 (s, 2H, Ar^2OCH_2), 4.20-4.16 (t, J = 4.8 Hz, 4H, Ar^1OCH_2), 3.87-3.82 (t, J = 4.8 Hz, 4H, OCH_2CH_2O), 3.74-3.69 (m, 4H, OCH_2CH_2O), 3.67-3.61 (m, 16H, OCH_2CH_2O), 3.54-3.50 (m, 4H, OCH_2CH_2O), 3.37 (s, 6H, $OCH_2CH_2OCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 152.8, 138.2, 142.4, 128.2, 128.0, 127.8, 125.3, 109.2, 74.8, 72.3, 71.2, 71.0, 70.9, 70.8, 70.0, 69.2, 52.5. MALDI: calculated for $C_{32}H_{48}O_{13}$: 640.31, obtained: 640.24; calculated for $C_{29}H_{48}NaO_{13}$: 627.30, obtained: 627.13; calculated for $C_{29}H_{48}KO_{13}$: 643.27, obtained: 643.09.

Compound **5**: ^{1}H NMR (300 MHz, CD_3OD-*d*) δ 6.82 (s, 1H, Ar-4-*H*), 6.71 (s, 2H, Ar-2,6-*H*), 4.52 (d, J = 5.8 Hz, 4H, $ArCH_2OH$); ^{13}C NMR (75 MHz, CD_3OD-*d*) δ 157.2, 143.7, 115.1, 111.6, 63.0.

Compound **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (t, J = 1.3 Hz, 1H, Ar-4-*H*), 6.04 (d, J = 1.3 Hz, 2H, Ar-2,6-*H*), 5.38 (br s, 1H, O*H*), 4.40 (s, 4H, $ArCH_2Br$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.8, 140.0, 122.2, 116.2, 32.7.

Compound **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (bs, 2H, Ar-2,6-*H*), 6.62 (bs, 1H, Ar-4-*H*), 3.99 (m, 8H, $PO(OCH_2CH_3)_2$), 3.49 (d, J = 21.9 Hz, 4H, $ArCH_2P$), 1.23 (t, J = 7.1 Hz, 12H, $PO(OCH_2CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.9, 132.6 (J = 10.6 Hz), 122.4 (J = 6.7 Hz), 115.8, 62.5 (J = 6.6 Hz), 33.6 (J = 138.8 Hz), 16.5 (J = 5.2 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 26.72. MALDI: calculated for $C_{16}H_{29}O_7P_2$: 395.138, obtained: 394.963.

Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (m, 3H, Ar-2,4,6-*H*), 5.25 (br s, 2H, $OCH_2CH_2NH_2$), 4.03-3.92 (m, 10H, $PO(OCH_2CH_3)_2$ and OCH_2CH_2NH), 3.10 (d, J = 21.7 Hz, 4H, $ArCH_2P$), 3.02 (m, 2H, OCH_2CH_2NH), 1.25 (t, J = 7.1 Hz, 12H, $PO(OCH_2CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.0 (J = 2.8 Hz), 133.1 (J = 6.0 Hz), 123.8 (J = 6.8 Hz), 114.5 (J = 5.0 Hz), 70.0, 62.1 (J = 7.0 Hz), 41.5, 33.5 (J = 138.2 Hz), 16.5 (J = 2.7 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 26.24. MALDI: calculated for $C_{18}H_{34}NO_7P_2$: 438.17, obtained: 438.18; calculated for $C_{18}H_{34}NaO_7P_2$: 460.17, obtained: 460.16.

Compound **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 7.7 Hz, 2H, Ar^3-2,6-*H*), 7.35-7.28 (m, 3H, Ar^3-3,4,5-*H*), 7.07 (s, 2H, Ar^2-2,6-*H*), 6.88 (t, J = 5.7 Hz, 1H, OCH_2CH_2NH), 6.85-6.78 (m, 3H, Ar^1-2,4,6-*H*), 5.07 (s, 2H, Ar^3OCH_2), 4.20-4.17 (t, J = 4.8 Hz, 4H, Ar^2OCH_2), 4.15-4.11 (t, J = 5.0 Hz, 2H, OCH_2CH_2NH), 4.08-3.96 (m, 8H, $PO(OCH_2CH_3)_2$), 3.88-3.78 (m, 6H, OCH_2CH_2NH and OCH_2CH_2O), 3.71-3.68 (m, 4H, OCH_2CH_2O), 3.65-3.58 (m, 16H, OCH_2CH_2O), 3.55-3.49 (m,

4H, OCH_2CH_2O), 3.35 (s, 6H, $OCH_2CH_2OCH_3$), 3.08 (d, J = 21.5 Hz, 4H, Ar^1CH_2P), 1.25 (t, J = 7.0 Hz, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 158.6 (J = 2.8 Hz), 152.8, 141.0, 137.8, 133.1 (J = 6.0 Hz), 129.6, 128.2, 128.0, 127.8, 124.0 (J = 6.8 Hz), 114.6 (J = 4.8 Hz), 107.0, 74.9, 72.0, 70.8, 70.7, 70.6, 69.8, 69.1, 66.8, 62.1 (J = 3.4 Hz), 58.9, 53.2, 39.5, 36.8 (J = 3.9 Hz), 33.5 (J = 138.3 Hz), 16.5 (J = 2.7 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 26.08. MALDI: calculated for $C_{50}H_{79}NaNO_{19}P_2$: 1082.87, obtained: 1082.51.

Compound **10**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (s, 2H, Ar^2-2,6-*H*), 6.85-6.78 (m, 3H, Ar^1-2,4,6-*H*), 6.65 (m, 1H, OCH_2CH_2NH), 4.27-4.21 (t, J = 4.7 Hz, 4H, Ar^2OCH_2), 4.15-4.10 (t, J = 5.0 Hz, 2H, OCH_2CH_2NH), 4.08-3.98 (m, 8H, $PO(OCH_2CH_3)_2$), 3.88-3.78 (m, 6H, OCH_2CH_2NH and OCH_2CH_2O), 3.75-3.60 (m, 20H, OCH_2CH_2O), 3.56-3.51 (m, 4H, OCH_2CH_2O), 3.35 (s, 6H, $OCH_2CH_2OCH_3$), 3.09 (d, J = 22.0 Hz, 4H, Ar^1CH_2P), 1.26 (t, J = 7.1 Hz, 12H, $PO(OCH_2CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.3, 158.7 (J = 2.8 Hz), 146.8, 141.0, 133.1 (J = 6.0 Hz), 124.0, 114.6, 108.4, 72.0, 70.8, 70.7, 70.6, 69.8, 69.1, 66.8, 62.1 (J = 3.4 Hz), 58.9, 39.5, 33.4 (J = 138.1 Hz), 16.4 (J = 2.7 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 26.10. MALDI: calculated for $C_{43}H_{74}NO_{19}P_2$: 970.43, obtained: 970.44; calculated for $C_{43}H_{73}NaNO_{19}P_2$: 992.43, obtained: 992.44.

Compound **11:** ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.44 (s, 9H), 2.45 (s, 3H), 2.50 (t, 2H, $_3J$= 6.6 Hz), 3.58-3.73 (m, 32H), 4.16 (t, 2H, $_3J$= 4.9 Hz), 7.34 (2H, AA' part of an AA'BB' system), 7.81 (2H, BB' part of an AA'BB' system). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 21.14, 27.65, 35.84, 66.40, 68.15, 69.00, 70.08, 79.78, 127.46, 129.47, 132.74, 144.32, 170.20. MALDI: calculated for C10H20LiO5: 227.15, obtained: 227.08; calculated for C26H44LiO12S: 587.27, obtained: 587.13.

Compound **12**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.10 (s, 2H, Ar^2-2,6-*H*), 6.87 (t, J = 5.1 Hz, 1H, $Ar^1OCH_2CH_2NH$), 6.80 (t, 1H, J = 2.0 Hz, Ar^1-2-*H*), 6.76 (q, 2H, J = 2.0 Hz, Ar^1-4,6-*H*), 4.22-4.15 (m, 6H, $Ar^2OCH_2CH_2O$), 4.12 (t, 2H, J = 5.1 Hz, $Ar^1OCH_2CH_2NH$), 4.05-3.95 (m, 8H, J = 7.0 Hz, $PO(OCH_2CH_3)_2$), 3.85-3.75 (m, 8H, $Ar^1OCH_2CH_2NH$ and OCH_2CH_2O), 3.70-3.50 (m, 54H, OCH_2CH_2O), 3.33 (s, 6H, $OCH_2CH_2OCH_3$), 3.07 (d, J = 21.7 Hz, 4H, Ar^1CH_2P), 2.48 (t, 2H, J = 6.6 Hz, $Ar^2OCH_2CH_2COOC(CH_3)_3$), 1.42 (s, 9H, $Ar^2OCH_2CH_2COOC(CH_3)_3$), 1.22 (t, J = 7.0 Hz, 12H, $PO(OCH_2CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 167.1, 157.5, 152.4, 141.6, 133.2 (J = 6.0 Hz), 129.4, 124.1, 114.6 (J = 5.0 Hz), 107.3, 80.4, 72.2, 71.9, 70.7, 70.6, 70.5, 70.55, 70.4, 70.3, 69.7, 69.1, 66.8, 66.6, 62.1 (J = 7.0 Hz), 58.9, 39.6, 36.1, 33.8 (J = 137.8 Hz), 27.9, 16.4 (J = 6.0 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 26.06. MALDI: calculated for $C_{66}H_{117}NaNO_{29}P_2$: 1472.72, obtained: 1472.65.

Compound **13**: ^1H NMR (300 MHz, CD_3OD) δ 7.28 (s, 2H, Ar^2-2,6-*H*), 6.92-6.86 (m, 3H, Ar^1-2,4,6-*H*), 4.35-4.20 (m, 8H, $Ar^2OCH_2CH_2O$ and $Ar^1OCH_2CH_2NH$), 3.97 (t, J = 4.5 Hz, 4H, $Ar^2OCH_2CH_2O$), 3.88 (m, 2H, OCH_2CH_2O, 3.80-3.53 (m, 56H, OCH_2CH_2O and $Ar^1OCH_2CH_2NH$), 3.38 (s, 6H, $OCH_2CH_2OCH_3$), 3.18 (d, J = 21.8 Hz, 4H, Ar^1CH_2P), 2.62 (t, 2H, J = 6.0 Hz, $Ar^2OCH_2CH_2COOH$); ^{13}C NMR (75 MHz, CD_3OD) δ 172.2, 168.1, 158.8, 152.3, 141.0, 133.8 (J = 6.0 Hz), 128.8, 123.7, 114.3, 106.4, 72.2, 71.7, 70.4, 70.25, 70.15, 70.1, 70.0, 69.95, 69.4, 68.8, 66.3, 66.1, 60.8, 57.8, 50.8, 39.6, 34.6, 33.6 (J = 134.5 Hz); ^{31}P NMR (81 MHz, CD_3OD) δ 25.19. MALDI: calculated for $C_{54}H_{94}NO_{29}P_2$: 1282.53, obtained: 1282.46; calculated for $C_{54}H_{93}NaNO_{29}P_2$: 1304.53, obtained: 1304.45.

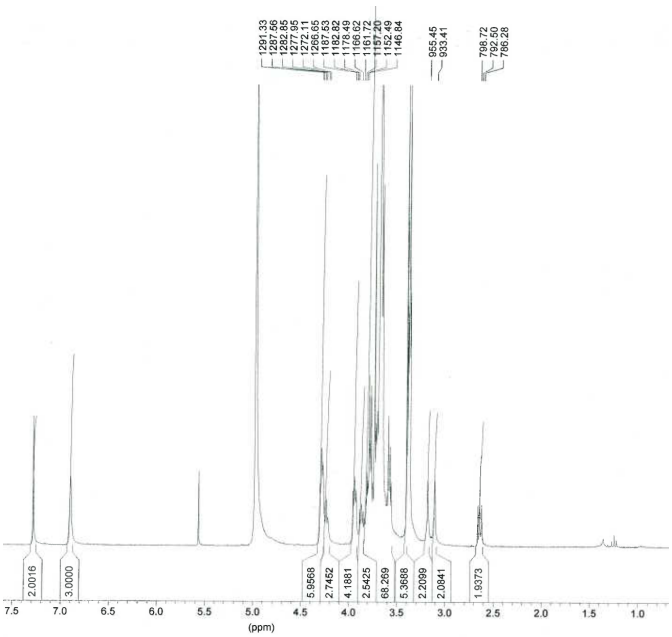


Figure S1. ^1H NMR spectrum (300 MHz, CD_3OD) of bis-phosphonate dendrons (PDn).

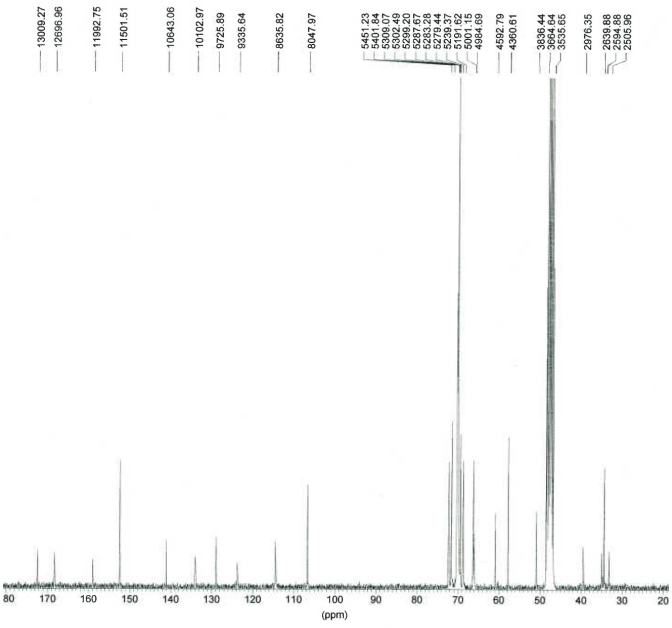


Figure S2. ^{13}C NMR spectrum (300 MHz, CD_3OD) of bis-phosphonate dendrons (PDn).

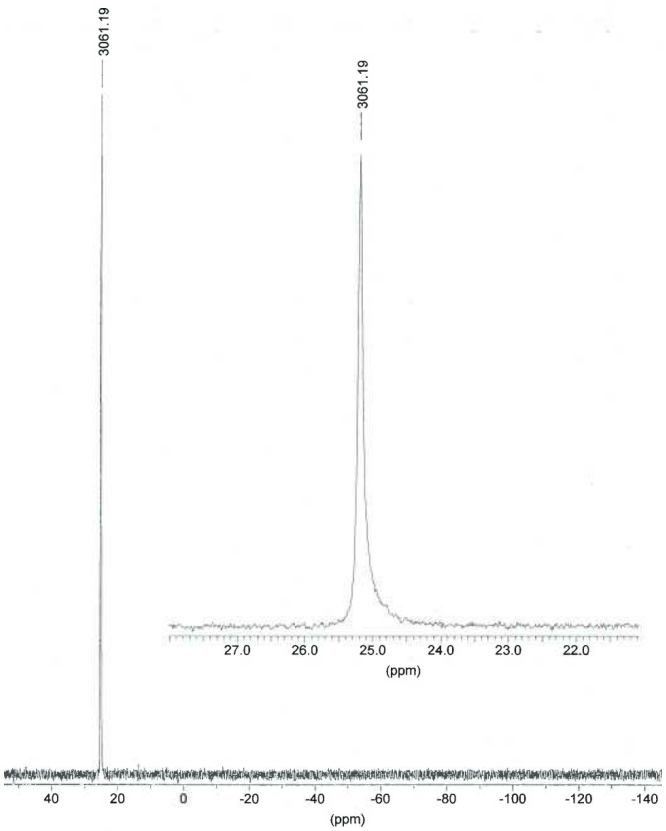


Figure S3. ^{31}P NMR spectrum (300 MHz, CD_3OD) of bis-phosphonate dendrons (PDn).

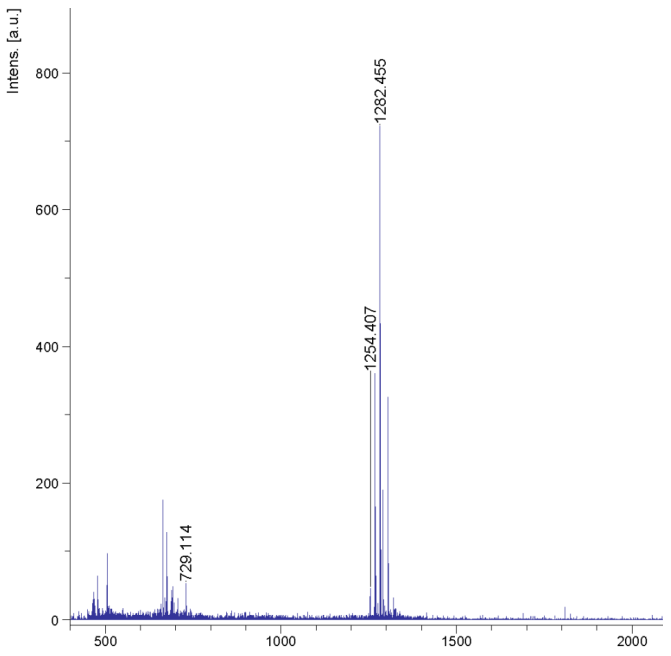


Figure S4. Mass spectrum of bis-phosphonate dendrons (PDn).

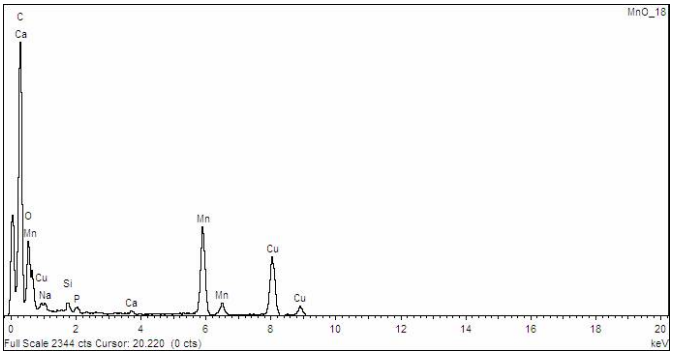


Figure S5. EDS spectrum of MnO@PDns prepared for the relaxometric studies (after lyophilisation, colloidal suspensions in deionised water, filtration and dispersion on a carbon-coated copper grid). Calcium, sodium, and silica are due to background contamination in the HRTEM column.

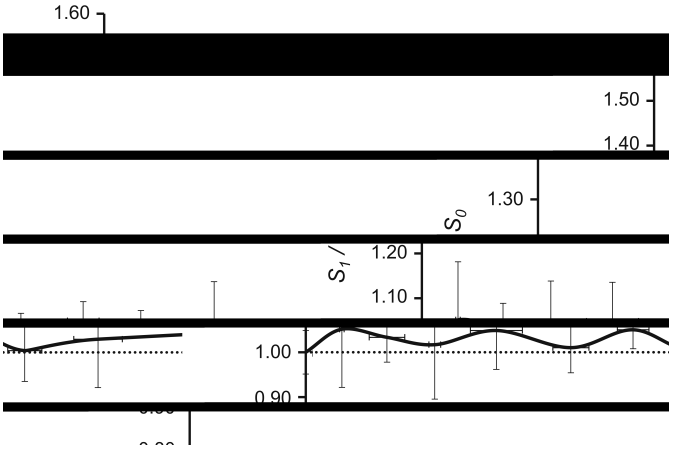


Figure S6. MRI signal (SI) follow-up studies after injection of MnO@PDn, in the liver: no measurable contrast enhancement was found in T_{1w} imaging.

Table S1: Endogenous (control) manganese levels in untreated mice fed on normal diet (data from the literature*)

Organs	(From 1) µg Mn /g organ	(From 2) µg Mn / g organ	(From 3) µg Mn / g organ	Mean* µg Mn / g organ
heart	0.4		0.18	0.3
lungs	0.18		0.18	0.2
liver	1	1.3	2.10	1.5
stomach			4.27	4.3
kidneys	1.35	1.6	0.94	1.3
intestine			3.36	3.4
spleen	0.4		0.45	0.4

* 1) Dubick MA, Keen CL. Tissue trace elements and lung superoxide dismutase activity in mice exposed to ozone. Toxicology letters. 1983;17:355-60 ; 2) Takehara Y, Sakahara H, Masunaga H, Isogai S, Kodaira N, Sugiyama M, et al. Assessment of a potential tumor-seeking manganese metalloporphyrin contrast agent in a mouse model. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine. 2002;47:549-53 ; 3) Ling GN,

Kolebic T, Damadian R. Low paramagnetic-ion content in cancer cells: its significance in cancer detection by magnetic resonance imaging. Physiological chemistry and physics and medical NMR. 1990;22:1-14.

