Supplementary Material (ESI) for Dalton Transactions

Electronic Supplementary Material

to

A new bifunctional Gd^{III} complex of enhanced efficacy for MR-Molecular Imaging applications.

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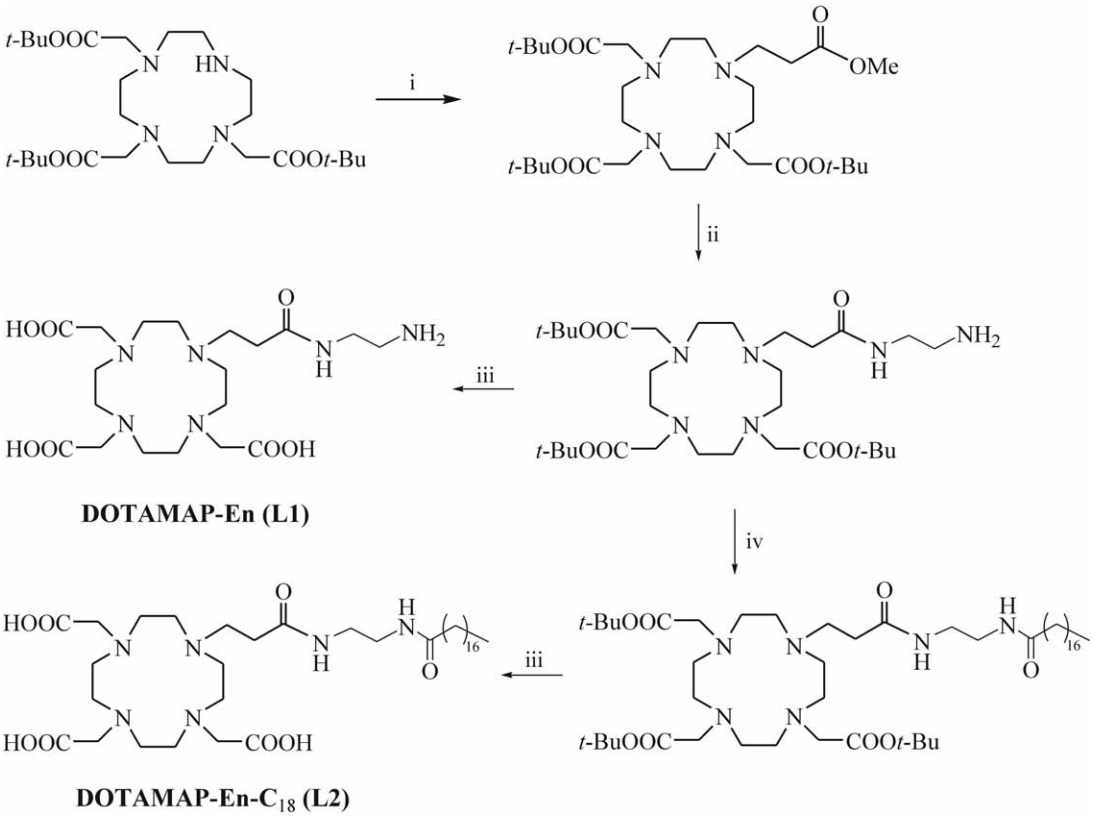
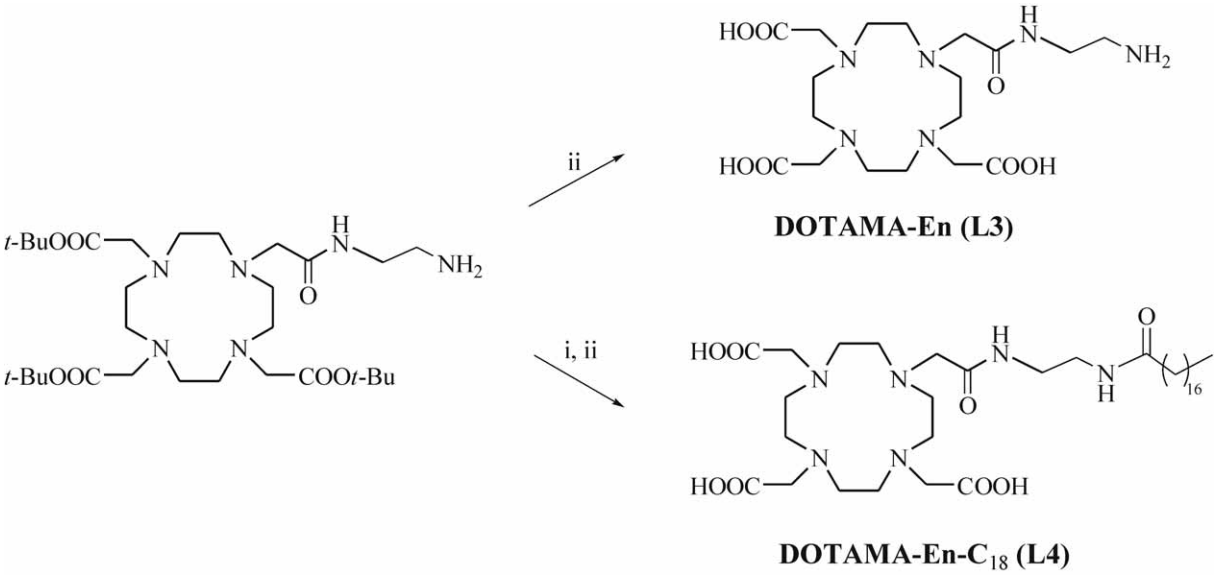
Synthesis and characterization of ligands **L1** , **L2**, **L3** and **L4**.

All chemicals were purchased from Sigma-Aldrich Co. and were used without purification unless otherwise stated. NMR spectra were recorded on a on a JEOL Eclipse Plus 400 (operating at 9.4 Tesla). ESI mass spectra were recorded on a Waters Micromass ZQ.

DO3A(O*t*Bu)$_3$, DOTAMA(O*t*Bu)$_3$En and DOTAMA-En (**L3**) were prepared following the reported procedures.[1, 2]



Scheme S1. Synthesis of ligands **L1** and **L2**. i: methylacrylate, DIPEA, reflux, 4h; ii: ethylenediamine, 60°C, 24h; iii: TFA, CH_2Cl_2 1:1 v:v., RT, 16h; iv: $C_{17}COCl$, CH_2Cl_2, RT, 2h.



Scheme S2. Synthesis of ligands **L3** and **L4**. i: $C_{17}COCl$, CH_2Cl_2, RT, 2h; ii: TFA, CH_2Cl_2 1:1 v:v., RT, 16h.

1-methoxycarbonylethyl-4,7,10-tris-(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane DOTAMAP(O*t*Bu)$_3$ (1): DO3A(O*t*Bu)$_3$ (514mg; 1.0mmol) and DIPEA (0.52mL; 3.0 mmol) were dissolved in methylacrylate (3mL). The mixture was stirred and heated to reflux for 12h, the solution was then evaporated *in vacuo* and the residue was ridissolved in diethyl ether (10mL). The precipitate was filtered off and washed with diethyl ether; the filtrate and the washings were combinated and evaporated *in vacuo* to give the crude product, that was purified by column cromatography (ACN/NH_3 30% : 98/2) obtaining a colourless oil (385mg; 64%). ^{1}H NMR ($CDCl_3$) 400MHz δ= 3.53 (OC*H*$_3$, s, 3H), 3.22-2.92 (NC*H*$_2$CO, m, 6H), 2.47 (NC*H*$_2$CH$_2$, t, 2H, *J* = 7.0Hz), 2.32 (NCH$_2$C*H*$_2$, t, 2H, *J* = 7.0Hz), 2.70-2.15 (NC*H*$_{2ring}$, bs, 16H), 1.34 (C*H*$_3$, s, 18H), 1.32 (C*H*$_3$)$_3$, s, 9H). ^{13}C NMR ($CDCl_3$) 100MHz δ= 173.2, 172.7, 172.6 (*C*O), 82.2, 82.1 (*C*(CH$_3$)), 56.6-55.7 (b, NCH$_2$CO), 52.3-52.0 (b, NCH$_2$ *ring*), 49.8 (OCH$_3$), 30.6 (CH$_2$CH$_2$CO), 28.2-27.9 (*C*H$_3$). ESI-MS (*m/z*): found 601.60 (M+H$^+$) (calc. for $C_{30}H_{56}N_4O_8$: 601.42)

1-(6-amino-4-aza-3-oxohexyl)-4,7,10-tris-(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane DOTAMAP(O*t*Bu)$_3$-En (2): 1 (385mg; 0.64mmol) was dissolved in ethylenediamine (86μL; 1.28mmol). The mixture was stirred and heated at 60°C for one day. The solution was then concentrated *in vacuo*, obtaining a colourless oil (402mg; 100%). ^{1}H NMR ($CDCl_3$) 400MHz δ= 9.89 (N*H*CO, m, 1H), 3.91-3.42 (NC*H*$_2$CO, m, 6H), 3.28 (NHC*H*$_2$, t, 2H, *J* = 5.5Hz), 2.82 (C*H*$_2$NH$_2$, t, 2H, *J* = 5.5 Hz), 2.71-2.20 (NC*H*$_{2ring}$ + C*H*$_2$C*H*$_2$CO, bs, 20H), 1.44 (C*H*$_3$, s, 18H), 1.40 (C*H*$_3$, s, 9H). ^{13}C NMR ($CDCl_3$) 100MHz δ= 177.3, 173.4, 173.3, 171.9 (*C*O), 82.2, 82.0 (*C*), 56.0, 55.8 (*C*H$_2$CO), 52.6-52.0 (NCH$_{2ring}$, b), 50.1 (N*C*H$_2$CH$_2$), 41.6 (*C*H$_2$NH), 41.2 (*C*H$_2$NH$_2$), 32.7 (CH$_2$*C*H$_2$CO), 28.2, 28.0 (*C*H$_3$). ESI-MS (*m/z*): found 629.61 (M+H$^+$) (calc. for $C_{31}H_{60}N_6O_7$: 629.46).

1-(4,6-diaza-3,8-dioxopentacosanyl)-4,7,10-tris-(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane - DOTAMAP(O*t*Bu)$_3$-En-C$_{18}$ (3) & 1-(3,5-diaza-2,7-dioxotetracosanyl)-4,7,10-tris-(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane - DOTAMA(O*t*Bu)$_3$-En-C$_{18}$ (4): A solution of stearoyl chloride (100mg; 0.33mmmol) in dry CH_2Cl_2 (5mL) was added dropwise in 20min, under N_2 atmosphere, to a ice-bath cooled mixture of **2** (205mg; 0.325mmol) or DOTAMA(O*t*Bu)$_3$-en (200mg; 0.325mmol) and K_2CO_3 (287mg; 2.08mmol) in dry CH_2Cl_2 (3mL). The ice-bath was removed after 15min and the suspension was stirred for 2h. The inorganic salts were removed by filtration and the filtrate evaporated *in vacuo*. The crude product was purified on silica gel chromatography CH_2Cl_2/MeOH 95/5 obtaining pure **3** (213mg; 73% yield) or **4** (209mg; 73% yield). **3**: ^{1}H NMR ($CDCl_3$) 400 MHz δ= 8.84 (N*H*CO, m, 1H), 8.36 (N*H*CO, m, 1H), 3.65 (C*H*$_2$CO, s, 6H), 3.52 (NC*H*$_2$CH$_2$, m, 2H), 3.48-2.25 (NC*H*$_{2ring}$, bs, 16H), 3.26 (NHC*H*$_2$, m, 2H), 2.73 (NH$_2$C*H*$_2$, m, 2H), 2.53 (NCH$_2$C*H*$_2$, m, 2H), 2.29 (COC*H*$_2$, t, 2H, *J* = 7.4Hz), 1.60 (COCH$_2$C*H*$_2$, quintet, 2H, *J* = 7.4Hz), 1.45 (C*H*$_3$, s, 18H), 1.44 (C*H*$_3$, s, 9H), 1.24 (C*H*$_{2chain}$, s, 28H), 0.86 (C*H*$_3$, t, 3H, *J*= 7.0Hz). ^{13}C NMR ($CDCl_3$) 100 MHz δ= 174.5, 173.3, 173.0, 172.9

(*C*O), 82.5, 82.3 (*C*), 56.5, 56.2 (*C*H_2CO), 55.8 (N*C*H2CH2), 52.3-49.9 (N*C*H_{2ring}, b), 49.0, 45.6 (NH*C*H_2), 32.0 (CO*C*H_2CC), 29.8, 29.4, 28.3, 28.1 (*C*H_{2chain}), 30.8 (NCH$_2$*C*H$_2$), 28.2, 28.0 (*C*H_3), 22.8 (*C*H_2CH$_3$), 14.2 (*C*H_3). ESI-MS (*m/z*): found 896.02 ($M+H^+$) (calc. for $C_{49}H_{94}N_6O_8$: 895.31).

4: ^{1}H NMR ($CDCl_3$) 400 MHz δ= 8.73 (N*H*CO, bs, 1H), 8.15 (N*H*CO, bs, 1H), 3.4-3.2 (NC*H*$_2$CO, bs, 8H), 3.0-2.3 (NC*H*$_{2ring}$, bs, 16H), 2.42 (CO*C*H_2, t, 2H, *J* = 7.4Hz), 2.22 (NHC*H*$_2$, t, 2H, *J* = 7.7Hz), 2.13 (NHC*H*$_2$, t, 2H, *J* = 7.7Hz), 1.53 (COCH$_2$C*H*$_2$, quintet, 2H, *J* = 7.4Hz), 1.37 (C*H*$_3$, s, 18H), 1.36 (C*H*$_3$, s, 9H), 1.17 (*C*H$_{2chain}$, s, 28H), 0.79 (C*H*$_3$, t, 3H, *J* = 6.6Hz). ^{13}C NMR ($CDCl_3$) 100 MHz δ= 174.5, 172.4, 171.7 (*C*O), 81.9 (*C*), 56.2, 55.8, 55.7 (*C*H$_2$CO), 53.6-47.2 (N*C*H$_{2ring}$, b), 39.4, 39.1 (NH*C*H$_2$), 36.5 (CO*C*H$_2$), 31.9, 29.7, 29.5, 26.0 (*C*H$_{2chain}$), 28.1, 28.0 (*C*H$_3$), 22.7 (*C*H$_2$CH$_3$), 14.1 (*C*H$_3$). ESI-MS (*m/z*): found 881.28 ($M+H^+$) and 903.29 ($M+Na^+$) (calc. for $C_{48}H_{93}N_6O_8$: 881.70).

1-(6-amino-4-aza-3-oxoesyl)-4,7,10-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAMAP-En, L1) & 1-(4,6-diaza-3,8-dioxopentacosanyl)-4,7,10-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAMAP-En-C_{18}, L2) & 1-(3,5-diaza-2,7-dioxotetracosanyl)-4,7,10-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAMA-En-C_{18}, L4): 2 (or **3**, or **4**) (0.2 mmol) was dissolved in a mixture of CH_2Cl_2 and trifluoroacetic acid (1:1 *v:v*, 2mL) and stirred at room temperature overnight. The solution was then evaporated *in vacuo* and the product was precipitated with excess diethyl ether, isolated by centrifugation, washed thoroughly with diethyl ether and dried *in vacuo* obtaining pure desired product as amorphous white solid (quantitative yield).

L1: ^{1}H NMR (CD_3OD) 400 MHz δ= 4.15 (NC*H*$_2$CO, s, 2H), 3.71 (NC*H*$_2$CO, s, 2H), 3.68 (NC*H*$_2$CO, s, 2H), 3.64-3.16 (NC*H*$_{2ring}$, bs, 16H), 3.53 (NC*H*$_2$CH$_2$, m, 2H), 3.43 (NHC*H*$_2$, m, 2H), 3.11 (C*H*$_2$NH$_2$, m, 2H), 2.84 (NCH$_2$C*H*$_2$, m, 2H). ^{13}C NMR (CD_3OD) 100 MHz δ= 173.1, 168.5(*C*O), 55.0, 52.7 (N*C*H$_2$CO), 50.7-48.5 (N*C*H$_{2ring}$, b), 50.1 (N*C*H$_2$CH$_2$), 40.0

(NH*C*H$_2$), 37.3 (*C*H$_2$NH$_2$), 27.5 (NCH$_2$*C*H$_2$CO). ESI-MS (*m/z*): found 461.63 (M+H$^+$) (calc. for $C_{19}H_{36}N_6O_7$: 461.27).

L2: ^{1}H NMR (D_2O) 400 MHz δ= 4.07 (NC*H$_2$*CO, s, 2H), 3.68 (NC*H$_2$*CO, s, 2H), 3.64 (NC*H$_2$*CO, s, 2H), 3.58-3.16 (NC*H$_{2ring}$*, bs, 16H), 3.52 (NC*H$_2$*CH$_2$, m, 2H), 3.34 (NHC*H$_2$*, m, 2H), 3.20 (NHC*H$_2$*, m, 2H), 2.81 (NCH$_2$C*H$_2$*, m, 2H), 2.68(COC*H$_2$*, t, 2H, *J* = 7.4Hz), 1.58 (COCH$_2$C*H$_2$*, quintet, 2H, *J* = 7.4Hz), 1.28 (C*H$_2$ chain*, s, 28H), 0.87 (C*H$_3$*, t, 3H, *J* = 7.0 Hz). ^{13}C NMR (D_2O) 100 MHz δ= 175.9, 171.3 (*C*O), 54.1 (N*C*H$_2$CO), 51.1 (N*C*H$_2$CH$_2$), 50.8-49.3 (N*C*H$_{2ring}$, b), 39.0, 38.5 (NH*C*H$_2$), 36.2 (CO*C*H$_2$), 32.2, 30.3, 30.1, 29.8, 29.6 (*C*H$_{2chain}$), 26.0 (NCH$_2$*C*H$_2$), 22.8 (*C*H$_2$CH$_3$), 14.3 (*C*H$_3$). ESI-MS (*m/z*): found 727.80 (M+H+) (calc. for $C_{37}H_{70}N_6O_8$: 727.53).

L4: ^{1}H NMR (D_2O) 400 MHz δ= 4.0-3.56 (NC*H$_2$*CO, bs, 8H), 3.56-3.0 (NC*H$_{2ring}$*+ NHC*H$_2$*C*H$_2$*, bs, 20H), 2.24 (COC*H$_2$*, t, 2H, *J* = 7.4Hz), 1.58 (COCH$_2$C*H$_2$*, quintet, 2H, *J* = 7.4Hz), 1.29 (C*H$_{2chain}$*, s, 28H), 0.88 (C*H$_3$*, t, 3H, *J* = 6.6Hz). ^{13}C NMR (D_2O) 100 MHz δ= 176.0, 172.4, 169.5 (*C*O), 55.3-47.5 (N*C*H$_2$CO+N*C*H$_{2ring}$, b), 39.9, 38.7 (NH*C*H$_2$), 36.3, 32.3, 30.8, 30.4, 26.0 (*C*H$_{2chain}$), 22.9 (*C*H$_2$CH$_3$), 14.1 (*C*H$_3$). ESI-MS (*m/z*): found 713.17 (M+H$^+$) (calc. for $C_{36}H_{69}N_6O_8$: 713.52).

References:

1. Nycomed Imaging A.S., Polyazacycloalkane Compounds, Patent WO96/28433, 1996.
2. A. Barge, L. Tei, D. Upadhyaya, F. Fedeli, L. Beltrami, R. Stefanıa, S. Aime and G. Cravotto, *Org. Biomol. Chem.*, 2008, **6**, 1176–1184.

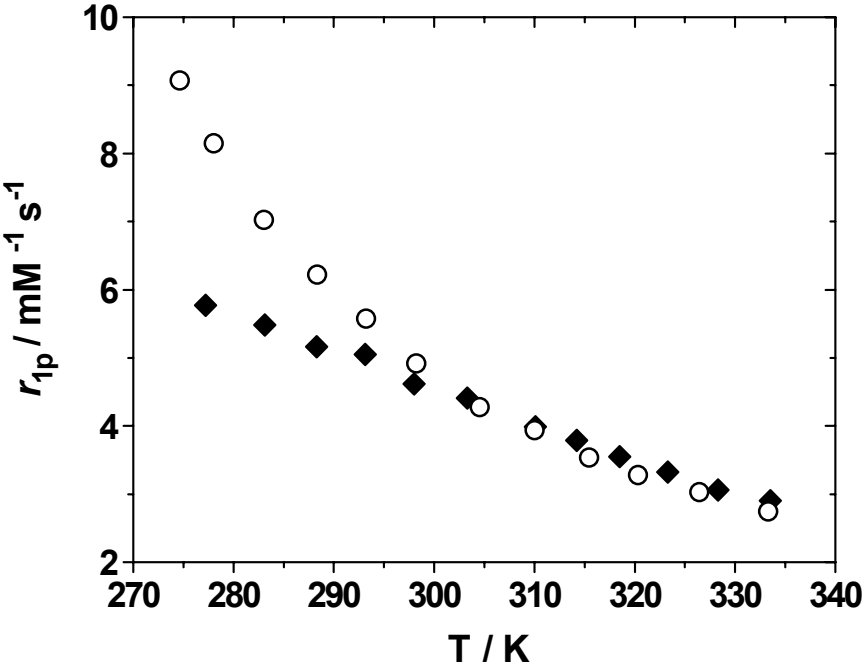


Figure S1. Temperature dependence of the proton relaxivity for Gd**L1** (open circles) and Gd**L3** (diamonds) at 30 MHz.

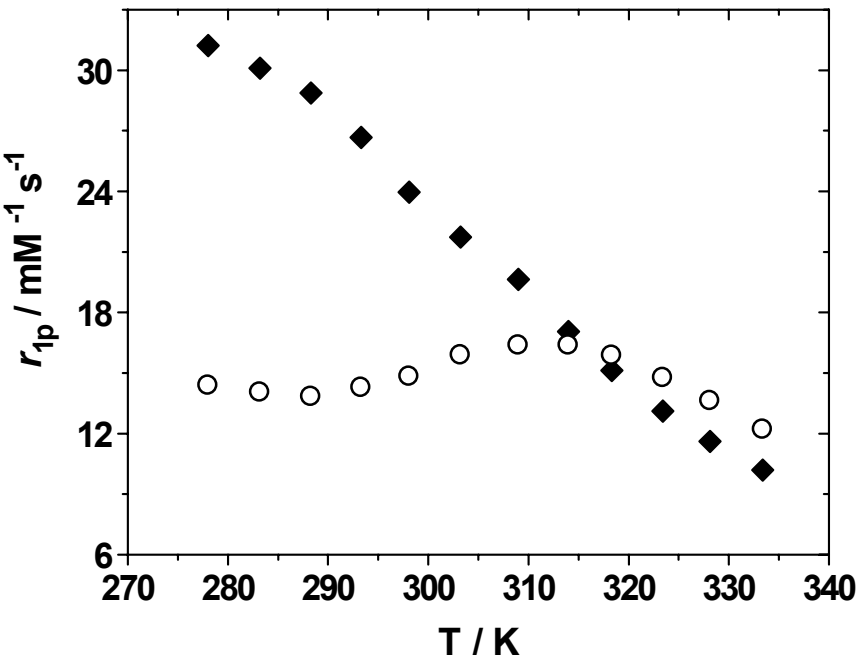


Figure S2. Temperature dependence of the proton relaxivity for Gd**L2** (diamonds) and Gd**L4** (open circles) at 30 MHz.