Magnetic resonance and optical imaging probes for NMDA receptors on the cell surface of neurons: synthesis and evaluation in cellulo

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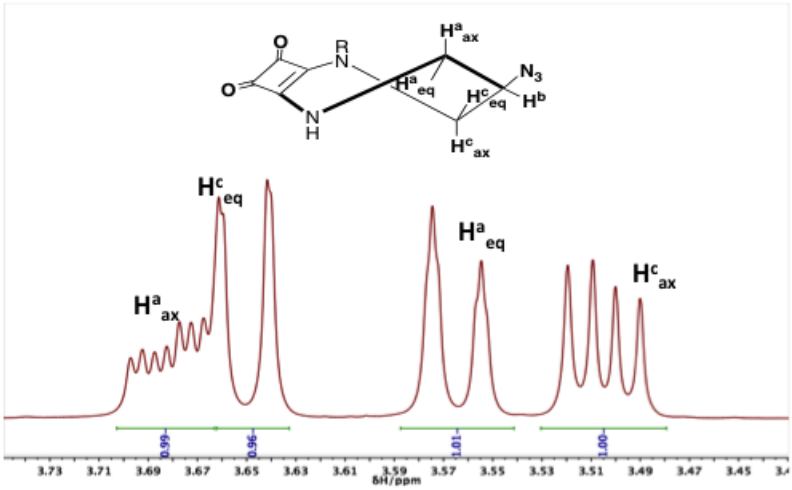
ESI Figure 1 Partial ^{1}H NMR spectrum of the bicyclic azide, **15** (295 K, $CDCl_3$, 700 MHz), showing the twist-chair conformation of the ring that is likely to be preferentially populated.



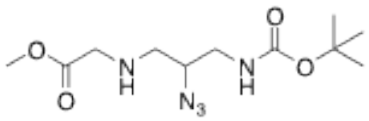
Heterocyclic seven-membered rings can adopt several low-energy conformations, of which the twist-chair is normally lowest in energy, with bulky substituents in the six-position preferring the equatorial site. [i] With this information in mind, the conformation in Figure 1 is proposed. The axial proton, H^a, is coupled to the equatorial proton, H^a_{eq}, J = 14 Hz. It is also coupled to H^b with a 3-bond coupling of 7 Hz, and finally to the NH proton with a 3-bond coupling of 4 Hz. This splitting pattern for H^a_{ax} generates a ddd, observed at around 3.68 ppm. Using 2D ^{1}H-^{13}C HSQC NMR, it is then possible to identify H^a_{eq} as the doublet at 3.55 ppm. A coupling constant of 14 Hz is consistent with the two-bond coupling to H^a_{ax}. There also

appears to be some fine splitting associated with the peak at 3.55 ppm and this reflects a small coupling to either H^b or the NH proton, due to the twisted nature of the 7-membered ring. Since H^c_{ax} is almost eclipsing H^b, it becomes apparent that the doublet of doublets at 3.49 ppm corresponds to H^c_{ax}. The splitting pattern arises due to the coupling with H^c_{eq} and H^b. Finally, with H^c_{eq} close to 90° with respect to H^b, no real coupling is observed between these two resonances, reflected in the very fine splitting in the H^c_{eq} resonance at 3.64 ppm.

(i) D. Parker and B. P. Waldron, *Org. Biomol. Chem*. 2013, **11**, 2827.

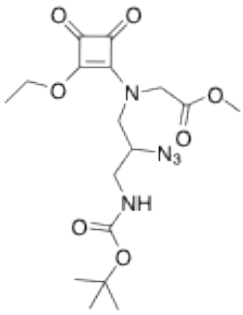
Ligand and Complex synthesis

Methyl -2-((azido-3-((*tert*-butoxycarbonyl)amino)propyl)amino)acetate, 5



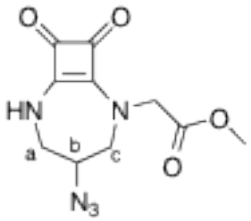
A solution of *tert*-butyl (3-amino-2-azidopropyl)carbamate (395 mg, 1.84 mmol), sodium carbonate (293 mg, 2.76 mmol) and methyl bromoacetate (275 μL, 2.91 mmol) in anhydrous ethanol (8 mL) was boiled under reflux for 8 hours until no further reaction was observed by TLC. The mixture was concentrated under reduced pressure before being partitioned between DCM/H_2O (1:1, 30 mL). The organic portion was separated and the aqueous extracted with dichloromethane (3 x 25 mL). The combined organic fractions were dried over $MgSO_4$, filtered and concentrated, allowing the crude residue to be purified by column chromatography (DCM/MeOH, 100% to 90:10 using 1% increments R_f = 0.41) to give a colourless oil (178 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (9H, s, C(C**H**$_3$)$_3$), 1.85 (1H, br, N**H**), 2.63 (1H, dd, J = 12, 7, C**H**H), 2.76 (1H, dd, J = 12, 5, CH**H**), 3.13 (1H, dt, J = 14, 7, C**H**H), 3.31 (1H, dt, J = 14, 5, CH**H**), 3.39 (2H, s, C**H**$_2$), 3.57-3.65 (1H, m, C**H**), 3.68 (3H, s, C**H**$_3$) 5.09 (1H, br, N**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.4 (C(**C**H$_3$)$_3$), 42.4 (**C**H$_2$), 50.5, 50.6 (**C**H$_2$), 51.9 (**C**H$_3$), 62.0 (**C**N$_3$), 79.7 (**C**(CH$_3$)$_3$), 156.0, 172.7 (**C**O). MS (ES$^+$) *m/z* 288.2 [M+H]$^+$; $C_{11}H_{22}N_5O_4$ requires 288.1672; found 288.1670. [In some preparations, transesterification from solvent meant that the ethyl ester was also present $C_{12}H_{24}N_5O_4$ requires 302.2; found 302.2. methyl:ethyl ester ratio by ^{1}H NMR 3.1:1; this was not deleterious as the ester group was hydrolysed in a later step].

Methyl-2-((azido-3-((*tert*-butoxycarbonyl)amino)propyl)(2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)acetate, 9



To a solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (45 μL, 0.31 mmol) in anhydrous ethanol (0.5 mL), was added a solution of methyl 2-((azido-3-((*tert*-butoxycarbonyl)amino)propyl)amino)acetate (88 mg, 0.31 mmol) in anhydrous ethanol (2.5 mL) over 30 mins. The resulting solution was stirred under argon at room temperature and the progress of the reaction followed by TLC. When no further reaction was observed by TLC, the solution was concentrated under reduced pressure, and the crude residue purified by column chromatography (DCM/MeOH, 100% to 95:5 using 0.5% increments; R_f = 0.58) to give a colourless oil, exisiting as a pair of rotamers (89 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (9H, s, C(C**H**$_3$)$_3$), 1.43 (3H, t, J = 7, OCH$_2$C**H**$_3$), 3.10-3.43 (3H, m, C**H**$_2$), 3.50-3.70 (1H, m, C**H**$_2$), 3.77 (3H, s, C**H**$_3$), 3.79-3.91 (1H, m, C**H**), 4.14-4.28 (1H, m, C**H**$_2$), 4.44-4.56 (1H, m, C**H**$_2$), 4.73 (2H, q, J = 7, OC**H**$_2$CH$_3$), 5.13 (1H, br, N**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 15.8 (OCH$_2$**C**H$_3$), 28.4 (C(**C**H$_3$)$_3$), 42.0, 51.9, 52.0 (**C**H$_2$), 52.9 (**C**H$_3$), 61.2 (**C**N$_3$), 70.3 (O**C**H$_2$CH$_3$), 80.1 (**C**(CH$_3$)$_3$), 155.9 (**C**O), 168.8 (**C**=**C**) , 173.2 (**C**O), 177.8 (**C**=**C**), 183.2, 188.3 (**C**O). MS (ES$^+$) *m/z* 434.0 [M+Na]$^+$; $C_{17}H_{25}N_5O_7Na$ requires 434.1652; found 434.1649. [The ethyl ester was also present in some preparations: $C_{18}H_{27}N_5O_4Na$ requires 448.1; found 448.1].

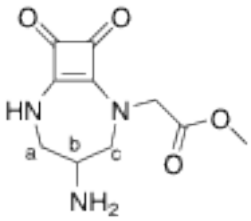
Methyl-2-(4-azido-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetate, 13



To a solution of methyl 2-((azido-3-((*tert*-butoxycarbonyl)amino)propyl)(2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)acetate (89 mg, 0.22 mmol) in anhydrous dichloromethane (1 mL) was added TFA (1 mL), and the resulting solution stirred at room temperature for 1 hour. Reaction was complete after this period, as indicated by TLC, and the solvents were removed under reduced pressure. The residue was re-dissolved in dichloromethane and again reduced

under reduced pressure. This process was repeated 5 times to ensure complete removal of excess TFA. The TFA salt was dissolved in anhydrous ethanol (3 mL) to which a solution of triethylamine (120 μL, 0.868 mmol) in anhydrous ethanol (1.5 mL) was added over a 20 minute period. The resulting solution was heated under reflux for 18 hours, until no further reaction was observed by TLC. At this point, the solvent was removed under reduced pressure and the crude residue purified by column chromatography (DCM/MeOH, 100% to 90:10 using 1% increments; R_f = 0.42) to give a colourless oil (46 mg, 80%). ^{1}H NMR (700 MHz, DMSO-d_6) δ 3.44-3.49 (1H, d, J = 14, **H**^{a}(eq)), 3.56-3.61 (2H, m, **H**$^{a/c}$ (ax)), 3.63 (1H, d, J = 14, **H**^{c} (eq)), 3.69 (3H, s, C**H**$_3$), 4.34 (1H, m, CH), 4.40 (1H, d, J = 17, C**H**$_2$CO$_2$Me), 4.67 (1H, d, J = 17, C**H**$_2$CO$_2$Me), 8.69 (1H, br, N**H**). ^{13}C NMR (176 MHz, DMSO-d_6) δ 47.7 (**C**^{a}), 50.8 (**C**H$_2$CO$_2$Me), 52.2 (**C**H$_3$), 55.7 (**C**^{c}), 58.8 (**C**N$_3$), 167.9, 168.5 (**C**=**C**), 169.0, 181.3, 181.4 (**C**O). MS (ES$^+$) *m/z* 266.1 [M+H]$^+$; $C_{10}H_{12}N_5O_4$ requires 266.0889; found 266.0902. [The ethyl ester was also present in some preparations: $C_{11}H_{14}N_5O_4$ requires 280.1; found 280.1; methyl:ethyl ester ratio by ^{1}H NMR 1.1:1].

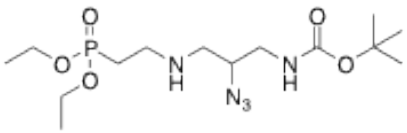
Methyl-2-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetate, 17



To a solution of methyl 2-(4-azido-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetate (43 mg, 0.15 mmol) in anhydrous THF (3.5 mL) was added H_2O (200 μL) and PPh_3 (60 mg, 0.23 mmol). The suspension was stirred under argon at 60 °C and over time, became a clear solution. After stirring for 16 hours, the solvent was removed under reduced pressure and the crude residue partitioned between DCM and H_2O (10 mL, 1:1). The aqueous layer was separated and washed twice with DCM (10 mL), before lyophilisation yielded a white solid (34 mg, 92%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.06-2.48 (2H, br, N**H**$_2$), 3.11-3.18 (2H, m, **H**$^{a/c}$), 3.23-3.28 (1H, m, H^b), 3.32-3.40 (2H, m, **H**$^{a'/c'}$), 3.69 (3H, s, C**H**$_3$), 4.48 (1H, d, J = 17, C**H**$_2$CO$_2$Me), 4.54 (1H, d, J = 17, C**H**$_2$CO$_2$Me), 8.58 (1H, br, N**H**). ^{13}C NMR (151 MHz, DMSO-d_6) δ 51.2 (**C**H$_2$CO$_2$Me), 51.6 (**C**H), 51.9 (**C**H$_2$), 52.2 (**C**H$_3$) 58.8 (**C**H$_2$), 167.5, 168.2, (**C**=**C**), 169.4, 181.3, 181.6 (**C**O). MS (ES$^+$) *m/z* 240.0 [M+H]$^+$; $C_{10}H_{14}N_3O_4$ requires 240.0984; found 240.0979; m.p. > 250 °C.

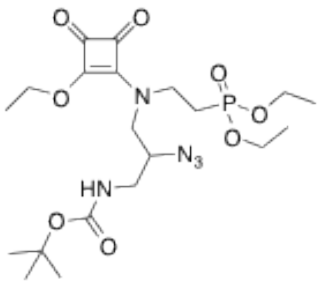
[The ethyl ester was also present in some preparations: $C_{11}H_{16}N_3O_4$ requires 254.1140; found 254.1140; methyl:ethyl ester ratio by ^{1}H NMR 1.1:1].

***tert*-Butyl (2-azido-3-((2-(diethoxyphosphoryl)ethyl)amino)propyl)carbamate, 7**



A solution of *tert*-butyl (3-amino-2-azidopropyl)carbamate (257 mg, 1.20 mmol), sodium carbonate (191 mg, 1.80 mmol) and diethyl (2-bromoethyl)phosphonate (345 μL, 1.90 mmol) in anhydrous ethanol (5 mL) was boiled under reflux for 16 hours until no further reaction was observed by TLC. The mixture was concentrated under reduced pressure, before being partitioned between DCM/H_2O (1:1, 30 mL). The organic portion was separated and the aqueous extracted with dichloromethane (3 x 25 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated, allowing the crude residue to be purified by column chromatography (DCM/MeOH, 100% to 90:10 using 1% increments; R_f = 0.48) to give a colourless oil (147 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (6H, t, J = 7, $P(OCH_2C\mathbf{H}_3)_2$), 1.39 (9H, s, $C(C\mathbf{H}_3)_3$), 1.72 (1H, br, N**H**), 1.92 (2H, dt, $^2J_{H\text{-}P}$ = 18, $J_{H\text{-}H}$ = 7, $PC\mathbf{H}_2CH_2$), 2.62 (1H, dd, J = 12, 6, C**H**H), 2.71 (1H, dd, J = 12, 6, CH**H**), 2.78-2.94 (2H, m, $PCH_2C\mathbf{H}_2$), 3.13 (1H, dt, J = 14, 7, C**H**H), 3.29 (1H, dt, J = 14, 7, CH**H**), 3.56-3.67 (1H, m, C**H**), 4.03-4.16 (4H, qd, $^3J_{H\text{-}H}$ = 7, $^3J_{H\text{-}P}$ = 3, $P(OC\mathbf{H}_2CH_3)_2$), 5.14 (1H, br, N**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 16.5 (d, ^3J = 6, $P(OCH_2\mathbf{C}H_3)_2$), 26.7 (d, ^1J = 140, $NHCH_2\mathbf{C}H_2P$), 28.4 ($C(\mathbf{C}H_3)_3$), 42.47 ($\mathbf{C}H_2$), 43.4 (d, ^2J = 4, $NH\mathbf{C}H_2CH_2P$), 50.4 ($\mathbf{C}H_2$), 61.7 (d, ^2J = 7, $P(O\mathbf{C}H_2CH_3)_2$), 61.8 ($\mathbf{C}N_3$), 79.7 ($\mathbf{C}(CH_3)_3$), 156.0 (**C**O). ^{31}P NMR ($CDCl_3$, 162 MHz) δ 31.29. MS (ES^+) *m/z* 380.2 $[M+H]^+$; $C_{14}H_{31}N_5O_5P$ requires 380.2063; found 380.2054.

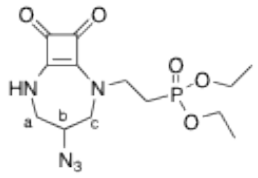
***tert*-Butyl-2-azido-3-((2-(diethoxyphosphoryl)ethyl)(2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)propyl)carbamate, 11**



To a solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (47 μL, 0.32 mmol) in anhydrous ethanol (1 mL), was added a solution of *tert*-butyl (2-azido-3-((2-(diethoxyphosphoryl)ethyl)amino)propyl)carbamate (147 mg, 0.32 mmol) in anhydrous

ethanol (2.5 mL) over 30 mins. The resulting solution was stirred under argon at room temperature and the progress of the reaction followed by TLC. After 48 hours, the solution was concentrated under reduced pressure and the crude residue purified by column chromatography (DCM/MeOH, 100% to 97:3 using 0.5% increments; R_f = 0.26) to give a colourless oil, observed as a pair of rotamers (133 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (6H, t, J = 7, $P(OCH_2C\mathbf{H}_3)_2$), 1.35 (9H, s, $C(C\mathbf{H}_3)_3$), 1.38 (3H, t, J = 7, $OCH_2C\mathbf{H}_3$), 1.98-2.14 (2H, m, $PC\mathbf{H}_2CH_2$), 3.05-3.39 (2H, m, $C\mathbf{H}_2$), 3.45-3.96 (5H, m, $C\mathbf{H}_2C\mathbf{H}$ + $PCH_2C\mathbf{H}_2$), 3.98-4.10 (4H, m, $P(OC\mathbf{H}_2CH_3)_2$), 4.69 (2H, q, J = 7, $OC\mathbf{H}_2CH_3$), 5.29 (1H, br, N**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 15.7 ($OCH_2\mathbf{C}H_3$), 16.3 (d, ^{3}J = 6, $P(OCH_2\mathbf{C}H_3)_2$), 26.0 (d, ^{1}J = 140, $NCH_2\mathbf{C}H_2P$), 28.3 ($C(\mathbf{C}H_3)_3$), 42.1 ($\mathbf{C}H_2$), 45.2 ($N\mathbf{C}H_2CH_2P$), 50.9 ($\mathbf{C}H_2$), 60.96 ($\mathbf{C}N_3$), 62.0 (d, ^{2}J = 7, $P(O\mathbf{C}H_2CH_3)_2$), 70.0 ($O\mathbf{C}H_2CH_3$), 79.8 ($\mathbf{C}(CH_3)_3$), 155.9 (**C**O), 172.4, 177.2 (**C**=**C**), 182.8, 188.2 (**C**O). ^{31}P NMR ($CDCl_3$, 162 MHz) δ 27.38. MS (ES^+) *m/z* 526.1 $[M+Na]^+$; $C_{20}H_{34}N_5O_8PNa$ requires 526.2043; found 526.2027.

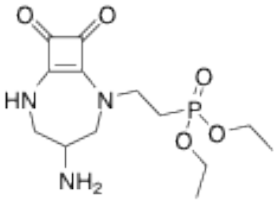
Diethyl-2-(4-azido-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl)phosphonate, 15



To a solution of *tert*-butyl (2-azido-3-((2-(diethoxyphosphoryl)ethyl)(2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)propyl)carbamate (133 mg, 0.26 mmol) in anhydrous dichloromethane (1 mL) was added TFA (1 mL), and the resulting solution stirred at room temperature for 1 hour. Reaction was observed to be complete after this period, as indicated by TLC, and the solvents were removed under reduced pressure. The residue was re-dissolved in dichloromethane and the volume again reduced. This process was repeated 5 times to ensure complete removal of excess TFA. The TFA salt was dissolved in anhydrous ethanol (3 mL) to which a solution of triethylamine (145 μL, 1.04 mmol) in anhydrous ethanol (2 mL) was added over a 20 minute period. The resulting solution was heated to reflux for 18 hours, until no further reaction was observed by TLC. At this point, the solvent was removed under reduced pressure and the crude residue purified by column chromatography (DCM/MeOH, 100% to 90:10 using 1% increments; R_f = 0.21) to give a colourless oil (77 mg, 83%). ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.30 (6H, 2 x t, J = 7, $P(OCH_2C\mathbf{H}_3)_2$), 2.12-2.23 (2H, m, $PC\mathbf{H}_2CH_2$), 3.49 (1H, dd, J = 14, 7, H^c(axial)), 3.55 (1H, d, J = 14, $H^{c/a}$(eq)), 3.64 (1H, d, J = 14, $H^{c/a}$(eq)), 3.67 (1H, ddd, J = 14, 7, 4, H^a(axial)), 3.86-4.12 (7H, m, C**H** + $NC\mathbf{H}_2CH_2P$ + $P(OC\mathbf{H}_2CH_3)_2$), 8.18 (1H, br, s, NH). ^{13}C NMR (176 MHz, $CDCl_3$) δ 16.5 (d, ^{3}J = 6,

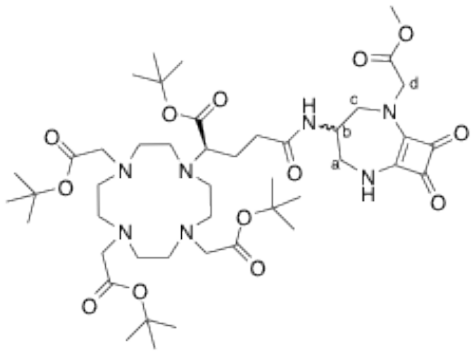
P(OCH_2CH_3)$_2$), 25.9 (d, ^1J = 140, NCH_2CH_2P), 45.9 (NCH_2CH_2P), 48.7, 56.0 (CH_2), 59.4 (CN_3), 62.2 (dd, ^2J = 7, P(OCH_2CH_3)$_2$), 167.7, 168.1 (**C**=**C**), 180.8, 182.4 (**C**O). ^{31}P NMR ($CDCl_3$, 243 MHz) δ 26.78. MS (ES^+) *m/z* 358.1 $[M+H]^+$; $C_{13}H_{21}N_5O_5P$ requires 358.1280; found 358.1269.

Diethyl-2-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl)phosphonate, 19



To a solution of diethyl (2-(4-azido-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl)phosphonate (71 mg, 0.2 mmol) in anhydrous THF (4 mL) was added H_2O (100 μL) and PPh_3 (78 mg, 0.3 mmol). The suspension was stirred under argon at 60 °C and over time, became a clear solution. After stirring for 16 hours, the solvent was removed under reduced pressure and the crude residue partitioned between DCM and H_2O (10 mL, 1:1). The aqueous layer was separated and washed twice with DCM (10 mL), before lyophilisation yielded a white solid (65 mg, 99%), m.p. >250°C. ^{1}H NMR (400 MHz, MeOD) δ 1.34 (6H, t, J = 7 P(OCH_2CH_3)$_2$), 2.31 (2H, dt, J_{H-P} = 18, J_{H-H} = 7, PCH_2CH_2), 3.48-3.66 (5H, m, C**H** + CH_2), 3.98-4.07 (2H, m, NCH_2CH_2P), 4.09-4.19 (4H, qd, $^3J_{H-H}$ = 7, $^3J_{H-P}$ = 3, P(OCH_2CH_3)$_2$). ^{13}C NMR (101 MHz, MeOD) δ 16.7 (d, ^3J = 6, P(OCH_2CH_3)$_2$), 25.5 (d, ^1J = 140, NCH_2CH_2P), 46.4 (NCH_2CH_2P), 51.1 (**C**H), 52.3 (CH_2), 58.0 (CH_2), 63.6 (d, ^2J = 7, P(OCH_2CH_3)$_2$), 169.2, 169.6 (**C**=**C**), 182.7, 182.9 (**C**O). ^{31}P NMR (162 MHz, MeOD) δ 30.97. MS (ES^+) *m/z* 332.1 $[M+H]^+$; $C_{13}H_{23}N_3O_5P$ requires 332.1375; found 332.1362.

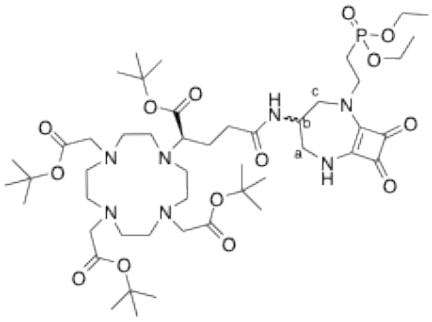
[Conjugate 1]



(*R*)-5-*tert*-Butoxy-5-oxo-4-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]pentanoic acid (108 mg, 0.16 mmol), EDC (36 mg, 0.19 mmol)

and HOBt (25 mg, 0.19 mmol) were dissolved in anhydrous DMF (2 mL) and stirred at room temperature under an atmosphere of argon for 20 minutes. After this period, a pre-stirred solution of methyl 2-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetate (37 mg, 0.16 mmol) and NMM (34 μL, 0.31 mmol) in anhydrous DMF (2 mL) was added dropwise and the resulting solution stirred at room temperature until complete consumption of the starting materials was indicated by ESI-MS. After this period, the solvent was removed under reduced pressure and the crude oil taken up into EtOAc (20 mL). $NaHCO_3$ (20 mL) was added, the layers separated and the aqueous washed with EtOAc (3 x 40 mL). The combined organic portions were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (DCM/MeOH, 100% to 90:10 in 1% increments; R_f =0.39) to yield a plae brown viscous oil. This product was characterized as a pair of diastereoisomers (33 mg, 26%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.41-1.43 (36H, overlapping s, C(C**H**$_3$)$_3$), 1.77-1.89 (1H, m), 1.94-2.13 (4H, m), 2.17-2.38 (4H, m), 2.43-2.69 (7H, m), 2.70-2.86 (4H, m), 2.88-3.08 (3H, m), 3.26-3.58 (6H, m), 3.69 (3H, s, C**H**$_3$), 3.81-3.97 (2H, m, **H**^{e}), 4.31-4.52 (2H, C**H**$_2^d$), 4.57-4.70 (1H, m, C**H**NH), 7.66 (1H, br, N**H**), 8.59 (1H, br, N**H**). ^{13}C NMR (151 MHz, $CDCl_3$) δ 27.9, 28.0 (overlapping C(**C**H$_3$)$_3$), 35.4, 44.5, 47.2 (**C**^{d}), 48.5, 48.7, 49.8, 50.1 (C**H**NH), 52.4, 52.6, 52.8 (**C**H$_3$), 55.6, 55.9, 60.8 (**C**H), 82.0, 82.1, 82.2, 82.6 (**C**(CH$_3$)$_3$), 168.1, 168.6 (**C**=**C**), 171.9, 172.6, 172.8, 172.9, 173.6, 175.3, 182.3, 182.3 (**C**O). MS (ES$^+$) *m/z* 922.4 [M+H]$^+$; $C_{45}H_{76}N_7O_{13}$ requires 922.5501; found 922.5526. [The ethyl ester was also present in one prepration: $C_{46}H_{78}N_7O_{13}$ requires 936.5658; found 936.5686].

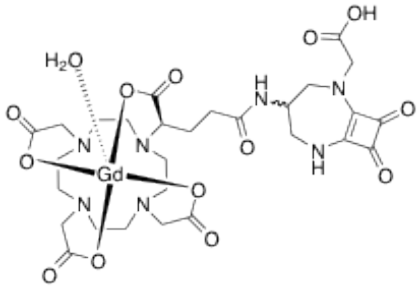
[Conjugate 3]



(*R*)-5-*tert*-Butoxy-5-oxo-4-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]pentanoic acid (138 mg, 0.20 mmol), EDC (45 mg, 0.24 mmol) and HOBt (32 mg, 0.24 mmol) were dissolved in anhydrous DMF (3 mL) and stirred at room temperature under an atmosphere of argon for 20 minutes. After this period, a pre-stirred solution of diethyl (2-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl)phosphonate (65 mg, 0.20 mmol) and NMM (43 μL, 0.39 mmol) in anhydrous DMF (2 mL) was added dropwise and the resulting solution stirred at room temperature until

complete consumption of the starting materials was revealed by ESI-MS. After this period, the solvent was removed under reduced pressure and the crude oil taken up into EtOAc (30 mL). $NaHCO_3$ (30 mL) was added, the layers separated and the aqueous washed with EtOAc (3 x 50 mL). The combined organic portions were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (DCM/MeOH, 100% to 90:10 in 1% increments; R_f =0.41) to yield a pale brown viscous oil. This product was characterized as a pair of diastereoisomers (42 mg, 21%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.28 (6H, t, J = 7, $P(OCH_2C\mathbf{H}_3)_2$), 1.39, 1.40, 1.41, 1.42 (36H, s, $C(C\mathbf{H}_3)_3$), 1.77-2.08 (5H, m), 2.12 (2H, m, $PC\mathbf{H}_2CH_2$), 2.16-2.25 (3H, m), 2.35 (1H, m), 2.45-2.56 (4H, m), 2.57-2.66 (3H, m), 2.66-2.87 (6H, m), 2.90-2.99 (2H, m), 3.28-3.34 (2H, m), 3.39-3.50 (4H, m, $\mathbf{H}^c$, $\mathbf{H}^a$, $C\mathbf{H}CO_2{}^t$Bu) 3.71-3.78 (1H, m, $\mathbf{H}^c$), 3.81-3.89 (2H, m, $PCH_2C\mathbf{H}_2$), 4.01-4.12 (4H, m, $P(OC\mathbf{H}_2CH_3)_2$), 4.14-4.20 (1H, m, $\mathbf{H}^b$), 7.64 (1H, br, N**H**), 7.84 (1H, br, N**H**). ^{13}C NMR (151 MHz, $CDCl_3$) δ 16.5 (d, ^{3}J = 6, $P(OCH_2\mathbf{C}H_3)_2$), 25.6 (d, ^{1}J = 140, $NCH_2\mathbf{C}H_2P$), 27.9, 28.0 (overlapping $C(\mathbf{C}H_3)_3$), 35.1, 44.5, 47.1, 48.1, 48.6 ($\mathbf{C}H_2$), 49.7 ($\mathbf{C}^b$), 50.0 (d, J = 16, $PCH_2\mathbf{C}H_2$), 52.6, 52.7, 52.8, 55.6 ($\mathbf{C}H_2$), 55.8 ($\mathbf{C}^a$), 56.3 ($\mathbf{C}^c$), 60.7 (**C**H), 62.0 (d, ^{2}J = 7, $P(O\mathbf{C}H_2CH_3)_2$), 81.9, 82.0, 82.1, 82.6 ($\mathbf{C}(CH_3)_3$), 167.6, 167.8 (**C**=**C**), 172.6, 172.8, 172.9, 173.4, 175.3, 181.3, 182.0 (**C**O). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.78. MS (ES^+) *m/z* 1014.1 $[M+H]^+$; $C_{48}H_{85}N_7O_{14}P$ requires 1014.589; found 1014.593.

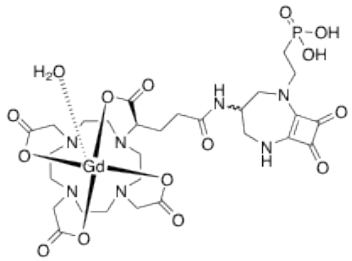
[Gd.L^1]



The **[Conjugate 1]** (10 mg, 0.01 mmol) was dissolved in MeOH (200 μL) with stirring. To this solution was added NaOH (0.5 mg) dissolved in H_2O (2.5 M) and the resulting solution stirred at room temperature overnight. Complete removal of the methyl/ethyl ester groups was verified by ESI-MS at which point the solvent was removed under reduced pressure. The residue was completely dried under high vacuum before being suspended in DCM (1 mL), to which trifluoroacetic acid (1 mL) was added. The resulting yellow solution was stirred at room temperature overnight. Complete removal of the *tert*-butyl ester groups was verified by ESI-MS, at which point excess solvent was removed under reduced pressure. The residue was repeatedly re-dissolved in DCM (2 mL) and the solvent removed under reduced pressure to remove excess TFA. This process yielded **L^1** as a pale-brown solid. MS (ES^+) *m/z* 684.7

$[M+H]^+$. The residue, **L[1]**, as its protonated salt, (6.8 mg, 0.01 mmol) was dissolved in H_2O (0.5 mL) and the pH adjusted to about 5.5 by the addition of NaOH (0.1 M). $GdCl_3.6H_2O$ (4.8 mg, 0.013 mmol) was added as a solution in H_2O (0.5 mL) and the reaction mixture stirred at 60 °C overnight. The pH of the solution was periodically checked and maintained between 5-6 with the addition of NaOH/HCl (0.1 M). Upon completion of complexation, excess gadolinium was removed by the addition of Chelex-100™ with stirring. The Chelex trap was filtered and the complex eluted with excess H_2O. Removal of the water by lyophilisation gave [Gd.L[1]] as a white solid, which was purified by RP-HPLC. HR-MS (ES^+) $C_{28}H_{39}{}^{154}GdN_7O_{13}$ requires 835.1815 $[M+2H]^+$; found 835.1820. r_{1p} = 4.91 $mM^{-1}s^{-1}$ (60 MHz, 310K). RP-HPLC: t_R = 7.1 mins [2-30% MeOH in H_2O over 10 mins].

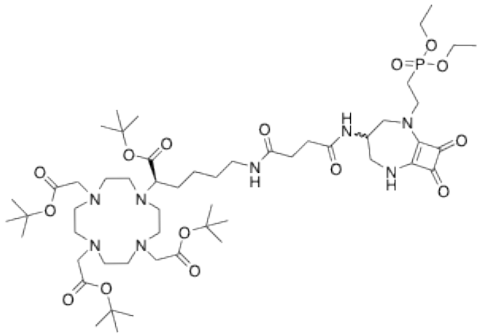
[Gd.L[3]]



The **[Conjugate 3]** (10 mg, 9 μmol) was dissolved in DCM (1 mL) with stirring. To this solution was added trifluoroacetic acid (1 mL) and the resulting solution stirred at room temperature for overnight. Hydrolysis of the *tert*-butyl ester groups was verified by ESI-MS, at which point the excess solvent was removed under reduced pressure. The residue was repeatedly re-dissolved in DCM (2 mL) and the solvent removed under reduced pressure to remove excess TFA. This process yielded the phosphonate ethyl ester as a light-brown solid. This residue was dissolved in DMF (1 mL) to which bromotrimethylsilane (13 μL, 0.08 mmol) was added dropwise. The resulting mixture was heated to 60 °C overnight until complete hydrolysis occurred as indicated by ESI-MS. The solvent was removed under reduced pressure, before the residue redissolved in H_2O. The pH was adjusted to 6 and the aqueous phase washed with DCM (3 x 3 mL) and diethyl ether (3 x 3 mL). The aqueous solvent was then removed by lyophilisation to give the protonated salt of **L[3]** as a light brown solid. MS (ES^+) *m/z* 734.9 $[M+H]^+$. The salt of **L[3]** (7.2 mg, 9 μmol) was dissolved in H_2O (0.5 mL) and the pH adjusted to 5.5. $GdCl_3.6H_2O$ (4.4 mg, 12 μmol) was added as a solution in H_2O (0.5 mL) and the reaction mixture stirred at 60 °C overnight. The pH of the solution was periodically checked and maintained between 5 and 6 with the addition of NaOH/HCl (0.1 M). Upon completion of complexation, excess gadolinium was removed by the addition of Chelex-100™ with stirring. The Chelex trap was filtered and the complex eluted with excess H_2O. Removal of the water by lyophilisation gave a white solid which was purified by RP-HPLC. HR-MS (ES^+) $C_{28}H_{42}{}^{157}GdN_7O_{14}P$ requires 888.1767 $[M+2H]^+$; found 888.1763. r_{1p} =

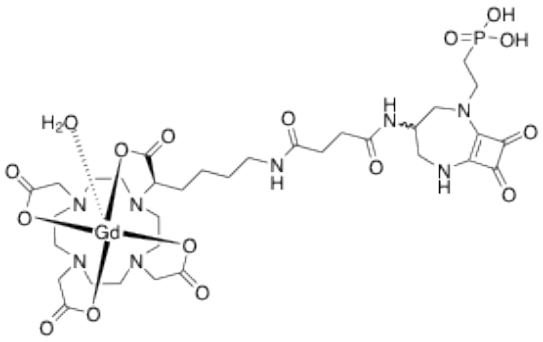
5.76 $mM^{-1}s^{-1}$ (60 MHz, 310K). RP-HPLC: t_R = 7.3 mins [2-30% MeOH in H_2O over 10 mins].

[Conjugate 5]



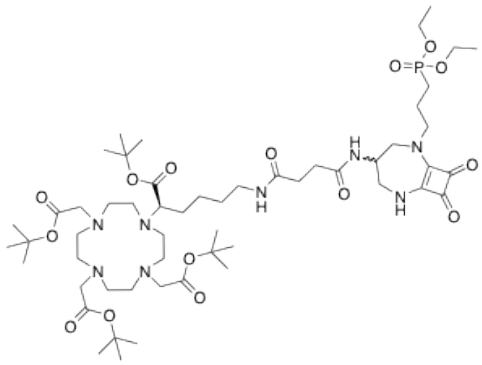
(*R*)-4-((6-*Tert*-butoxy)-6-oxo-5-(4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)hexyl)amino)-4-oxobutanoic acid (168 mg, 0.21 mmol), EDC (48 mg, 0.25 mmol) and HOBt (34 mg, 0.25 mmol) were dissolved in anhydrous DMF (3 mL) and stirred at room temperature under an atmosphere of argon for 20 minutes. After this period, a pre-stirred solution of diethyl (2-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl)phosphonate (70 mg, 0.21 mmol) and NMM (46 μL, 0.42 mmol) in anhydrous DMF (1.0 mL) was added dropwise and the resulting solution stirred at room temperature until complete consumption of the starting materials was revealed by ESI-MS. After this period, the solvent was removed under reduced pressure and the crude oil taken up into EtOAc (30 mL). $NaHCO_3$ (30 mL) was added, the layers separated and the aqueous layer washed with EtOAc (3 x 30 mL). The combined organic portions were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (DCM/MeOH, 100% to 85:15 in 1% increments; R_f =0.22) to yield a pale brown viscous oil. This product was characterized as a pair of diastereoisomers (52 mg, 22%). ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.30 (6H, dt, J = 7, 3, $P(OCH_2C\mathbf{H}_3)_2$), 1.42, 1.42, 1.43 (36H, s, $C(C\mathbf{H}_3)_3$), 1.49-1.69 (6H, m), 1.96-2.06 (2H, m), 2.11-2.18 (2H, m, $PC\mathbf{H}_2CH_2$), 2.19-2.28 (2H, m), 2.47-2.64 (9H, m), 2.71-2.86 (4H, m), 2.93-3.08 (5H, m), 3.11-3.25 (4H, m), 3.30 (1H, d, J = 14, $\mathbf{H}^{c/a}$ (eq)), 3.35 (2H, dd, J = 14, 7, $\mathbf{H}^{c}$ (axial)), 3.42-3.48 (2H, m), 3.74 (1H, ddd, J = 14, 7, 4, $\mathbf{H}^{a}$ (axial)), 3.78-3.82 (1H, m, $\mathbf{H}^{c/a}$ (eq)), 3.86-3.94 (2H, m, $PCH_2C\mathbf{H}_2$), 4.04-4.14 (4H, m, $P(OC\mathbf{H}_2CH_3)_2$), 4.15-4.19 (1H, m, $\mathbf{H}^{b}$), 7.46 (1H, br, N**H**), 7.96 (1H, br, N**H**), 8.67 (1H, br, N**H**). ^{13}C NMR (176 MHz, $CDCl_3$) δ 16.5 (d, ^{3}J = 6, $P(OCH_2\mathbf{C}H_3)_2$), 25.0 ($\mathbf{C}H_2$), 25.5 (d, ^{1}J = 140, $NCH_2\mathbf{C}H_2P$), 26.7 ($\mathbf{C}H_2$), 27.9, 28.0, 28.0 (overlapping $C(\mathbf{C}H_3)_3$), 29.4, 33.0, 38.8, 44.7, 45.7, 47.3 ($\mathbf{C}H_2$), 48.2 (d, J = 16, $PCH_2\mathbf{C}H_2$), 48.5 ($\mathbf{C}H_2$), 49.5 ($\mathbf{C}^{b}$), 50.2, 52.7, 52.8, 55.7 ($\mathbf{C}H_2$), 55.9 ($\mathbf{C}^{a}$), 56.2 ($\mathbf{C}^{c}$), 61.3 (**C**H), 62.1 (d, ^{2}J = 7, $P(O\mathbf{C}H_2CH_3)_2$), 82.0, 82.0, 82.0, 82.1, 82.1, 82.1 ($\mathbf{C}(CH_3)_3$), 167.8, 167.9 (**C**=**C**), 172.7, 172.9, 173.0, 173.3, 173.3, 175.6, 181.5, 182.0 (**C**O). ^{31}P NMR (283 MHz, $CDCl_3$) δ 27.02. MS (ES^+) *m/z* 1113.0 $[M+H]^+$; $C_{53}H_{94}N_8O_{15}P$ requires 1113.658; found 1113.653.

[Gd.L^5]



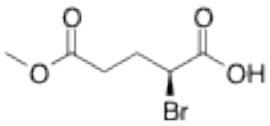
The **[Conjugate 5]** (15 mg, 0.013 mmol) was dissolved in DCM (1 mL) with stirring. To this solution was added trifluoroacetic acid (1 mL) and the resulting solution stirred at room temperature for overnight. Hydrolysis of the *tert*-butyl ester groups was verified by ESI-MS, at which point the excess solvent was removed under reduced pressure. The residue was repeatedly re-dissolved in DCM (2 mL) and the solvent removed under reduced pressure to remove excess TFA. This process yielded the phosphonate ethyl ester as a light-brown solid. This residue was dissolved in DMF (1 mL) to which bromotrimethylsilane (14 μL, 0.104 mmol) was added. The resulting mixture was heated to 60 °C overnight until complete hydrolysis of the phosphonate ethyl ester groups was verified by ESI-MS. The solvent was removed under reduced pressure and the residue re-dissolved in H_2O. The pH was adjusted to 6 and the aqueous phase washed with DCM (3 x 3 mL) and diethyl ether (3 x 3 mL). The aqueous solvent was then removed by lyophilisation to give the protonated salt of **L^5** as a light brown solid. MS (ES$^+$) *m/z* 833.7 $[M+H]^+$. The salt of **L^5** (10.8 mg, 0.013 mmol) was dissolved in H_2O (1.0 mL) and the pH adjusted to 6.0. $GdCl_3.6H_2O$ (5.79 mg, 0.016 mmol) was added as a solution in H_2O (0.5 mL) and the reaction mixture stirred at 60 °C overnight. The pH of the solution was periodically checked and maintained between 5 and 6 with the addition of NaOH/HCl (0.1 M). Upon completion of complexation, excess gadolinium was removed by the addition of Chelex-100™ with stirring. The Chelex trap was filtered and the complex eluted with excess H_2O. Removal of the water by lyophilisation gave a white solid residue that was purified by RP-HPLC. HR-MS (ES$^+$) $C_{33}H_{51}{}^{154}GdN_8O_{15}P$ requires 984.2420 $[M+2H]^+$; found 984.2433. r_{1p} = 4.97 mM^{-1} s^{-1} (60 MHz, 310K). RP-HPLC: t_R = 7.8 mins [2-30% MeOH in H_2O over 10 mins].

[Conjugate 6]



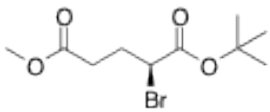
(*R*)-4-((6-*Tert*-butoxy)-6-oxo-5-(4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)hexyl)amino)-4-oxobutanoic acid (144 mg, 0.18 mmol), EDC (41 mg, 0.22 mmol) and HOBt (29 mg, 0.22 mmol) were dissolved in anhydrous DMF (2 mL) and stirred at room temperature under an atmosphere of argon for 20 minutes. After this period, a pre-stirred solution of diethyl (3-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)propyl)phosphonate (62 mg, 0.18 mmol) and NMM (40 μL, 0.36 mmol) in anhydrous DMF (1.5 mL) was added dropwise and the resulting solution stirred at room temperature until complete consumption of the starting materials was revealed by ESI-MS. After this period, the solvent was removed under reduced pressure and the crude oil taken up into EtOAc (30 mL). $NaHCO_3$ (30 mL) was added, the layers separated and the aqueous washed with EtOAc (3 x 30 mL). The combined organic portions were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (DCM/MeOH, 100% to 85:15 in 1% increments; R_f =0.35) to yield a pale brown viscous oil. This product was characterized as a pair of diastereoisomers (60 mg, 22%). ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.27 (6H, dt, J = 7, 3, $P(OCH_2C\mathbf{H}_3)_2$), 1.41, 1.41, 1.42 (36H, s, $C(C\mathbf{H}_3)_3$), 1.47-1.69 (6H, m), 1.69-1.77 (2H, m, $PC\mathbf{H}_2CH_2CH_2$), 1.82-1.89 (2H, m, $PCH_2C\mathbf{H}_2CH_2$), 2.04-2.10 (2H, m) 2.21-2.26 (2H, m), 2.46-2.64 (9H, m), 2.71-2.85 (4H, m), 2.91-3.07 (5H, m), 3.10-3.25 (4H, m), 3.29 (1H, d, J = 14, $\mathbf{H}^{c/a}$ (eq)), 3.34 (2H, dd, J = 14, 7, $\mathbf{H}^{c}$ (axial)), 3.37-3.43 (2H, m), 3.47 (1H, ddd, J = 14, 7, 4, $\mathbf{H}^{a}$ (axial)), 3.70-3.74 (1H, m, $\mathbf{H}^{c/a}$ (eq)), 3.75-3.81 (2H, m, $PCH_2CH_2C\mathbf{H}_2$), 4.01-4.08 (4H, m, $P(OC\mathbf{H}_2CH_3)_2$), 4.15-4.19 (1H, m, $\mathbf{H}^{b}$), 7.56 (1H, br, N**H**), 7.99 (1H, br, N**H**), 8.64 (1H, br, N**H**). ^{13}C NMR (176 MHz, $CDCl_3$) δ 16.5 (d, ^{3}J = 6, $P(OCH_2\mathbf{C}H_3)_2$), 22.8 (d, ^{1}J = 140, $P\mathbf{C}H_2CH_2CH_2$), 24.9 ($\mathbf{C}H_2$), , 26.7 ($\mathbf{C}H_2$), 27.9, 27.9, 28.0, 28.0 (overlapping $C(\mathbf{C}H_3)_3$), 29.4, 33.0, 38.8, 44.7, 45.7, 47.3 ($\mathbf{C}H_2$), 48.5 (d, J = 16, $PCH_2\mathbf{C}H_2CH_2$), 48.6 ($\mathbf{C}H_2$), 49.7 ($\mathbf{C}^{b}$), 50.2 ($\mathbf{C}H_2$), 51.5 (d, ^{3}J = 16, $PCH_2CH_2\mathbf{C}H_2$), 52.7, 52.8, 55.6 ($\mathbf{C}H_2$), 55.9 ($\mathbf{C}^{a}$), 55.9 ($\mathbf{C}^{c}$), 61.3 (**C**H), 61.8 (d, ^{2}J = 7, $P(O\mathbf{C}H_2CH_3)_2$), 82.0, 82.0, 82.0, 82.1, 82.1, 82.1 ($\mathbf{C}(CH_3)_3$), 167.6, 168.1 (**C**=**C**), 172.7, 172.9, 173.0, 173.2, 173.3, 175.5, 181.5, 181.8 (**C**O). ^{31}P NMR (283 MHz, $CDCl_3$) δ 30.91. MS (ES^+) *m/z* 1127.3 $[M+H]^+$; $C_{54}H_{96}N_8O_{15}P$ requires 1127.673; found 1127.673.

(*S*)-2-Bromo-pentanedioic acid 5-methyl ester



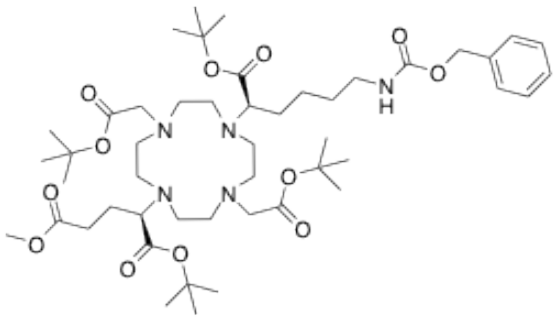
A solution of $NaNO_2$ (6.11 g, 88.5 mmol) in H_2O (50 ml) was added dropwise over 30 min to a stirred solution of (*S*)-glutamic acid 5-methyl ester (7.50 g, 46.5 mmol) and NaBr (13.2 g, 128.1 mmol) in 1 M HBr (276 mL), cooled at –5 ^oC. After 10 h, conc. H_2SO_4 (5 ml) was slowly added to the reaction mixture, which was then extracted with diethyl ether (3 x 300 ml). The combined organic extracts were washed with brine (200 ml), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude material was purified by column chromatography on silica (*n*-hexane/EtOAc, 100% to 80:20 utilizing 1 % increments; R_f = 0.20) to yield a yellow oil (4.35 g, 42 %). ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (1H, m, C**H**$_2$CHBr), 2.42 (1H, m, C**H**$_2$CHBr), 2.60 (2H, m, C**H**$_2$CH$_2$CHBr), 3.69 (3H, s, C**H**$_3$), 4.41 (1H, dd, J = 9, 5, C**H**). ^{13}C NMR (176 MHz, $CDCl_3$) δ 29.7 (**C**H$_2$CH$_2$CHBr), 31.6 (**C**H$_2$CHBr), 44.2 (**C**H), 52.0 (**C**H$_3$), 172.1, 173.4 (**C**O). MS (ES^+) *m/z* 246.8 $[M + Na]^+$.

(*S*)-2-Bromo-pentanedioic acid 5-methyl ester 1-*tert*-butyl ester



A solution of (*S*)-2-bromo-pentanedioic acid 5-methyl ester (2.94 g, 13.1 mmol) in *tert*-butyl acetate (47 ml) and $HClO_4$ in H_2O (70 %, 0.34 mmol) was stirred at room temperature, for 16 h. H_2O (35 ml) was added to the reaction mixture, and the organic phase separated. The organic phase was washed with H_2O (25 ml), followed by 5 % Na_2CO_3 (25 ml). The solvent was removed under reduced pressure to yield a pale yellow oil (2.58 g, 70 %). ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s, C(C**H**$_3$)$_3$), 2.23 (1H, m, C**H**$_2$CHBr), 2.33 (1H, m, C**H**$_2$CHBr), 2.50 (2H, m, C**H**$_2$CH$_2$CHBr), 3.69 (3H, s, C**H**$_3$), 4.23 (1H, dd, J = 9, 5, C**H**). ^{13}C NMR (76 MHz $CDCl_3$) δ 27.9 (C(**C**H$_3$)$_3$), 29.9 (**C**H$_2$CH), 31.5 (**C**H$_2$CH$_2$), 46.8 (**C**H), 52.0 (**C**H$_3$), 82.8 (**C**(CH$_3$)$_3$), 168.4, 172.7 (**C**O). MS (ES^+) *m/z* 303.5 $[M+H]^+$; $C_{10}H_{17}O_4{}^{79}BrNa$ requires 303.0208; found 303.0223.

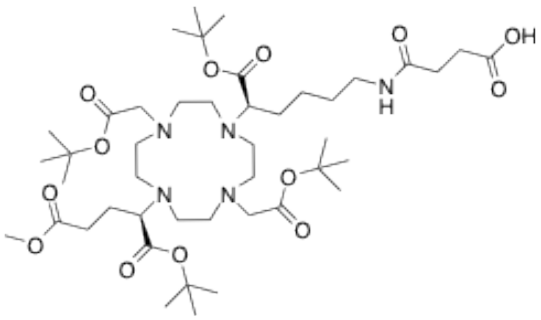
(*R*)-1-*tert*-Butyl 5-methyl 2-(7-((*R*)-6-(((benzyloxy)carbonyl)amino)-1-(*tert*-butoxy)-1-oxohexan-2-yl)-4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)pentandioate, 23



(*R*)-Di-*tert*-butyl 2,2'-(4-(6-(((benzyloxy)carbonyl)amino)-1-(*tert*-butoxy)-1-oxohexan-2-yl)1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate [a] (1.35 g, 1.87 mmol), K_2CO_3 (310 mg, 2.24 mmol) and (*S*)-2-Bromo-pentanedioic acid 5-methyl ester 1-*tert*-butyl ester (631 mg, 2.24 mmol) were stirred as a solution in acetonitrile (20 mL) at 60 °C for 48 hours. Completion of reaction was verified by ESI-MS, at which point the mixture was cooled to room temperature, filtered and the filtrate concentrated under reduced pressure. The crude orange residue was then purified by column chromatography (DCM/MeOH, 100% DCM to 93:7 utilising 1% increments; R_f = 0.41) to give a dark yellow oil (571 mg, 33%). ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.36 (9H, s, C(C**H**$_3$)$_3$), 1.37 (18H, s, C(C**H**$_3$)$_3$), 1.38 (9H, s, C(C**H**$_3$)$_3$), 1.43 – 1.50 (4H, m, C**H**$_2$), 1.54–1.63 (2H, m, C**H**$_2$), 1.64-1.78 (2H, m, C**H**$_2$), 1.84-1.93 (2H, m, C**H**$_2$), 2.38-2.52 (2H, br. m, C**H**$_2$), 2.52-2.61 (4H, br. m), 2.62-2.86 (12H, m, C**H**$_2$), 3.06-3.19 (6H, m), 3.57 (3H, s, C**H**$_3$), 5.00 (2H, s, OC**H**$_2$Ph), 5.17 (1H, s, N**H**), 7.21 – 7.31 (5H, m, Ar-**H**). ^{13}C NMR (176 MHz, $CDCl_3$) δ 27.7, 27.8 (**C**H$_2$), 28.2, 28.3, 28.3 (C(**C**H$_3$)$_3$), 30.4, 32.1, 40.5, 40.7, 44.1, 44.5, 47.1, 47.2, 48.7, 48.8 (**C**H$_2$), 51.4 (CH$_2$), 52.9 (**C**H$_3$), 55.8, 56.0, 59.7 (**C**H$_2$ ring), 61.1 (**C**H$_2$CO$_2$^{t}Bu), 63.2, 63.4 (**C**HCO$_2$^{t}Bu), 66.3 (O**C**H$_2$Ph), 81.8, 81.9, 82.0, 82.5 (**C**(CH$_3$)$_3$), 127.8, 127.9, 128.0, 128.4 (Ar-**C**), 172.8, 172.9, 173.3, 174.0, 174.6, 175.1 (**C**O). MS (ES$^+$) *m/z* 920.8 [M+H]$^+$; $C_{48}H_{82}N_5O_{12}$ requires 920.5782; found 920.5801.

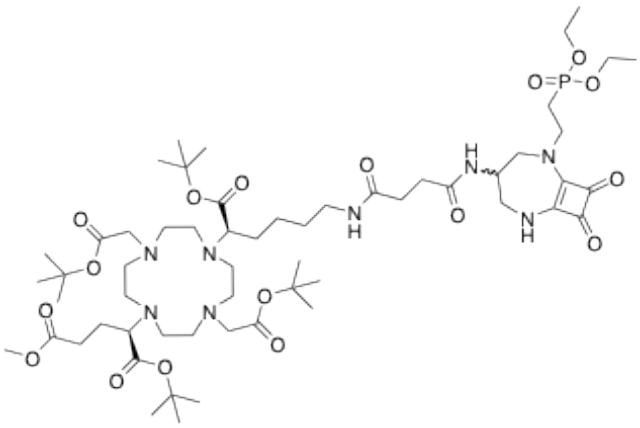
(a) S. M. Vibhute, J. Engelmann, T. Verbic, M. E. Maier, N. K. Logothetis and G. Angelovski, *Org. Biomol. Chem.*, 2013, **11**, 1294-1305.

4-(((*R*)-5-(4,10-Bis(2-(*tert*-butoxy)-2-oxoethyl)-7-((*R*)-1-(*tert*-butoxy)-5-methoxy-1,5-dioxopentan-2-yl)-1,4,7,10-tetraazacyclododecan-1-yl)-6-(*tert*-butoxy)-6-oxohexyl)amino)-4-oxobutanoic acid, 24



(*R*)-1-*tert*-Butyl 5-methyl 2-(7-((*R*)-6-(((benzyloxy)carbonyl)amino)-1-(*tert*-butoxy)-1-oxohexan-2-yl)-4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)pentandioate (279 mg, 0.30 mmol) was dissolved in absolute ethanol (10 mL) to which $Pd(OH)_2/C$ (10%) was added. The mixture was agitated in a Parr hydrogenation apparatus (40 psi) overnight. The catalyst was filtered and the ethanol removed under reduced pressure to yield the free amine. The amine was then dissolved in anhydrous DMF (3.5 mL) and diisopropylethylamine (98 μL, 0.56) was added. To this solution was added succinic anhydride (28 mg, 0.28 mmol) and the resulting mixture stirred at room temperature for 16 hours until complete reaction as verified by ESI-MS. The solvent was removed under reduced pressure and the crude residue that remained purified by column chromatography (DCM/MeOH, 100% to 87:13 in 1% increments; R_f =0.43) to yield a pale brown viscous oil (160 mg, 60% over 2 steps). ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.42 (9H, s, $C(C\mathbf{H}_3)_3$), 1.44 (18H, s, $C(C\mathbf{H}_3)_3$), 1.49 (9H, s, $C(C\mathbf{H}_3)_3$), 1.51-1.68 (6H, m), 2.06-2.66 (16H, m), 2.67-3.08 (10H, m), 3.14-3.37 (6H, m), 3.62 (3H, s, $C\mathbf{H}_3$), 7.75 (1H, br, N**H**), 8.15 (1H, br, O**H**). ^{13}C NMR (176 MHz, $CDCl_3$) δ 27.9, 27.9 ($\mathbf{C}H_2$), 28.0, 28.0, 28.2, 28.3 ($C(\mathbf{C}H_3)_3$), 29.1, 30.7, 32.3, 39.1, 38.8, 44.2, 46.9, 47.3, 48.7, 50.6, 51.7 ($\mathbf{C}H_2$), 51.8 ($\mathbf{C}H_3$), 52.7, 52.8, 56.0, 56.1 ($\mathbf{C}H_2$), 56.4 ($\mathbf{C}H_2CO_2{}^t$Bu), 59.8, 61.2 ($\mathbf{C}HCO_2{}^t$Bu), 82.1, 82.2, 82.7, 83.6 ($\mathbf{C}(CH_3)_3$), 172.9, 173.1, 173.5, 174.0, 174.8, 174.9, 175.4 (**C**O). MS (ES^+) *m/z* 886.7 $[M+H]^+$; $C_{44}H_{80}N_5O_{13}$ requires 886.5753; found 886.5742.

(2*R*)-1-*tert*-Butyl 5-methyl 2-(4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-7-((2*R*)-1-(*tert*-butoxy)-6-(4-((2-(2-(diethoxyphosphoryl)ethyl)-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-4-yl)amino)-4-oxobutanamido)-1-oxohexan-2-yl)-1,4,7,10-tetraazacyclododecan-1-yl)pentanedioate, 25



4-(((*R*)-5-(4,10-Bis(2-(*tert*-butoxy)-2-oxoethyl)-7-((*R*)-1-(*tert*-butoxy)-5-methoxy-1,5-dioxopentan-2-yl)-1,4,7,10-tetraazacyclododecan-1-yl)-6-(*tert*-butoxy)-6-oxohexyl)amino)-4-oxobutanoic acid (157 mg, 0.18 mmol), EDC (41 mg, 0.22 mmol) and HOBt (29 mg, 0.22 mmol) were dissolved in anhydrous DMF (2 mL) and stirred at room temperature under an atmosphere of argon for 20 minutes. After this period, a pre-stirred solution of diethyl (2-(4-amino-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl)phosphonate (59 mg, 0.18 mmol) and NMM (41 μL, 0.36 mmol) in anhydrous DMF (1.0 mL) was added dropwise and the resulting solution stirred at room temperature until complete consumption of the starting materials was revealed by ESI-MS. After this period, the solvent was removed under reduced pressure and the crude oil taken up into EtOAc (30 mL). $NaHCO_3$ (30 mL) was added, the layers separated and the aqueous washed with EtOAc (3 x 30 mL). The combined organic portions were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (DCM/MeOH, 100% to 85:15 in 1% increments; R_f =0.25) to yield a yellow viscous oil (50 mg, 23%). ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.32 (6H, dt, J = 7, 3, $P(OCH_2C\mathbf{H}_3)_2$), 1.43 (36H, s, $C(C\mathbf{H}_3)_3$), 1.44-1.46 (4H, m), 1.56-1.66 (2H, m), 1.75-1.84 (2H, m), 1.90-1.98 (2H, m), 2.12-2.19 (2H, m, $PC\mathbf{H}_2CH_2$), 2.42-2.51 (2H, m), 2.54-2.65 (7H, m), 2.66-2.74 (5H, m), 1.76-2.87 (8H, m), 3.10-3.13 (1H, m), 3.15-3.19 (3H, m), 3.21-3.24 (3H, m), 3.33 (1H, d, J = 14, $\mathbf{H}^{c/a}$ (eq)), 3.50 (1H, d, J = 14, $\mathbf{H}^{c}$ (axial)), 3.64 (3H, s, $C\mathbf{H}_3$), 3.69-3.79 (2H, m, $PCH_2C\mathbf{H}_2$), 3.90 (1H, dd, J = 14, 7, $\mathbf{H}^{a}$ (axial)), 4.05-4.14 (4H, m, $P(OC\mathbf{H}_2CH_3)_2$), 4.27 (1H, m, $\mathbf{H}^{b}$). ^{13}C NMR (176 MHz, $CDCl_3$) δ 16.5 (d, ^{3}J = 6, $P(OCH_2\mathbf{C}H_3)_2$), 24.0 ($\mathbf{C}H_2$), 25.0 (d, ^{1}J = 140, $NCH_2\mathbf{C}H_2P$), 25.2, 27.9, 27.9, 28.3 ($\mathbf{C}H_2$), 28.3, 28.4, 28.5 (overlapping $C(\mathbf{C}H_3)_3$), 30.6, 31.0, 31.4, 31.8, 39.5, 45.2, 48.7 ($\mathbf{C}H_2$), 49.7 (d, J = 16, $PCH_2\mathbf{C}H_2$), 51.5 ($\mathbf{C}^{b}$), 52.9, 53.1, 53.1($\mathbf{C}H_2$), 55.5 ($\mathbf{C}^{a}$), 56.1 ($\mathbf{C}^{c}$), 62.2 (d, ^{2}J = 7, $P(O\mathbf{C}H_2CH_3)_2$), 62.5 ($\mathbf{C}$H), 63.2 ($\mathbf{C}H_2CO_2{}^t$Bu), 64.5 ($\mathbf{C}$H), 80.8, 80.8, 80.8, 81.0 ($\mathbf{C}(CH_3)_3$), 168.0, 168.2 ($\mathbf{C}$=$\mathbf{C}$), 171.1, 172.2, 172.5, 173.0, 173.5, 174.4, 177.2, 181.2, 182.2 ($\mathbf{C}$O). ^{31}P NMR (283 MHz, $CDCl_3$) δ 27.26. MS (ES^+) *m/z* 1199.4 $[M+H]^+$; $C_{57}H_{100}N_8O_{17}P$ requires 1199.694; found 1199.693.

ESI Figure 2 Analytical RP-HPLC traces of the most promising contrast agents, **[Gd.L^5]** (*upper*) and **[Gd.L^7]** (*lower*).