ESI OBC

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

Neil Sim\textsuperscript{a}, Robert Pal\textsuperscript{a}, David Parker, \textsuperscript{**} Jorn Engelmann \textsuperscript{b}, Anurag Mishra\textsuperscript{a,c} and Sven Gottschalk\textsuperscript{b,c}

\textsuperscript{a} Department of Chemistry, Durham University, South Road, Durham DH1 3LE, UK
\textsuperscript{b} High Field MR Centre, Max Planck Institute for Biological Cybernetics, Spemannstrasse 41, Tuebingen, D-72076, Germany.
\textsuperscript{c} Institute for Biological and Medical Imaging, Helmholtz Center, Munich, Neuherberg, D-85764, Germany.

ESI Figure 1 Partial \textsuperscript{1}H NMR spectrum of the bicyclic azide, 15 (295 K, CDCl\textsubscript{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. \textsuperscript{i} With this information in mind, the conformation in Figure 1 is proposed. The axial proton, H\textsubscript{a}, is coupled to the equatorial proton, H\textsubscript{eq}, J = 14 Hz. It is also coupled to H\textsubscript{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\textsubscript{ax} generates a ddd, observed at around 3.68 ppm. Using 2D \textsuperscript{1}H-\textsuperscript{13}C HSQC NMR, it is then possible to identify H\textsubscript{eq} as the doublet at 3.55 ppm. A coupling constant of 14 Hz is consistent with the two-bond coupling to H\textsubscript{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\textsuperscript{b} or the NH proton, due to the twisted nature of the 7-membered ring. Since H\textsubscript{cax} is almost eclipsing H\textsuperscript{b}, it becomes apparent that the doublet of doublets at 3.49 ppm corresponds to H\textsubscript{cax}. The splitting pattern arises due to the coupling with H\textsubscript{ceq} and H\textsuperscript{b}. Finally, with H\textsubscript{ceq} close to 90° with respect to H\textsuperscript{b}, no real coupling is observed between these two resonances, reflected in the very fine splitting in the H\textsubscript{ceq} resonance at 3.64 ppm.


**Ligand and Complex synthesis**

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

![Methyl-2-((azido-3-((tert-butoxycarbonyl)amino)propyl)amino)acetate, 5](image)

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\textsubscript{2}O (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\textsubscript{4}, filtered and concentrated, allowing the crude residue to be purified by column chromatography (DCM/MeOH, 100% to 90:10 using 1% increments R\textsubscript{f} = 0.41) to give a colourless oil (178 mg, 34%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 1.39 (9H, s, C(CH\textsubscript{3})\textsubscript{3}), 1.85 (1H, br, NH), 2.63 (1H, dd, J = 12, 7, CHH), 2.76 (1H, dd, J = 12, 5, CHH), 3.13 (1H, dt, J = 14, 7, CHH), 3.31 (1H, dt, J = 14, 5, CHH), 3.39 (2H, s, CH\textsubscript{2}), 3.57-3.65 (1H, m, CH), 3.68 (3H, s, CH\textsubscript{3}), 5.09 (1H, br, NH). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 28.4 (C(CH\textsubscript{3})\textsubscript{3}), 42.4 (CH\textsubscript{2}), 50.5, 50.6 (CH\textsubscript{2}), 51.9 (CH\textsubscript{3}), 62.0 (CN\textsubscript{3}), 79.7 (C(CH\textsubscript{3}))\textsubscript{3}), 156.0, 172.7 (CO). MS (ES\textsuperscript{+}) m/z 288.2 [M+H]\textsuperscript{+}; C\textsubscript{11}H\textsubscript{22}N\textsubscript{5}O\textsubscript{4} requires 288.1672; found 288.1670. [In some preparations, transesterification from solvent meant that the ethyl ester was also present C\textsubscript{12}H\textsubscript{24}N\textsubscript{5}O\textsubscript{4} requires 302.2; found 302.2. methyl:ethyl ester ratio by \textsuperscript{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, existing as a pair of rotamers (89 mg, 70%).

\[ \text{1H NMR (400 MHz, CDCl}_3) \delta 1.41 \ (9H, s, C(CH}_3)_3), \ 1.43 \ (3H, t, J = 7, OCH}_2CH}_3), \ 3.10-3.43 \ (3H, m, CH), \ 3.77 \ (3H, s, CH}_3), \ 3.79-3.91 \ (1H, m, CH), \ 4.14-4.28 \ (1H, m, CH}_2), \ 4.44-4.56 \ (1H, m, CH}_2), \ 4.73 \ (2H, q, J = 7, OCH}_2CH}_2), \ 5.13 \ (1H, br, NH). \]

\[ \text{13C NMR (101 MHz, CDCl}_3) \delta 15.8 \ (OCH}_2CCH}_3), \ 28.4 \ (C(CH}_3)_3), \ 42.0, \ 51.9, \ 52.0 \ (CH}_2), \ 52.9 \ (CH}_3), \ 61.2 \ (CN}_3), \ 70.3 \ (OCH}_2CH}_2), \ 80.1 \ (C(CH}_3)_3), \ 155.9 \ (CO), \ 168.8 \ (C=CO), \ 173.2 \ (CO), \ 177.8 \ (C=C), \ 183.2, \ 188.3 \ (CO). \]

\[ \text{MS (ES}^+ \text{) m/z 434.0 [M+Na]}; \text{C}_{17}H_{25}N_5O_7Na \text{ requires 434.1652; found 434.1649. } \]

The ethyl ester was also present in some preparations: \( \text{C}_{18}H_{27}N_5O_4Na \text{ 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%). 

\[ \text{1H NMR (700 MHz, DMSO-}d_6\text{)} \delta 3.44-3.49 (1H, d, J = 14, H^a(eq)), 3.56-3.61 (2H, m, H^{a/c}(ax)), 3.63 (1H, d, J = 14, H^c(eq)), 3.69 (3H, s, C\text{H}_3), 4.34 (1H, m, CH), 4.40 (1H, d, J = 17, CH_2CO_2Me), 4.67 (1H, d, J = 17, CH_2CO_2Me), 8.69 (1H, br, NH). \]

\[ \text{13C NMR (176 MHz, DMSO-}d_6\text{)} \delta 47.7 (C^a), 50.8 (CH_2CO_2Me), 52.2 (CH_3), 55.7 (C^c), 58.8 (CN_3), 167.9, 168.5 (C=C), 169.0, 181.3, 181.4 (CO). MS (ES\textsuperscript{+}) m/z 266.1 [M+H]\textsuperscript{+}; C_{10}H_{12}N_5O_4 requires 266.0889; found 266.0902. \]

[Methyl-2-(4-azido-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\textsubscript{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%). \[ \text{1H NMR (600 MHz, DMSO-}d_6\text{)} \delta 2.06-2.48 (2H, br, NH\textsubscript{2}), 3.11-3.18 (2H, m, H^b), 3.22-3.28 (1H, m, H^a), 3.32-3.40 (2H, m, H^{a/c}), 3.69 (3H, s, CH\textsubscript{3}), 4.48 (1H, d, J = 17, CH\textsubscript{2}CO\textsubscript{2}Me), 4.54 (1H, d, J = 17, CH\textsubscript{2}CO\textsubscript{2}Me), 8.58 (1H, br, NH). \]

\[ \text{13C NMR (151 MHz, DMSO-}d_6\text{)} \delta 51.2 (CH\textsubscript{2}CO\textsubscript{2}Me), 51.6 (CH), 51.9 (CH\textsubscript{2}), 52.2 (CH\textsubscript{3}) 58.8 (CH\textsubscript{2}), 167.5, 168.2, (C=C), 169.4, 181.3, 181.6 (CO). MS (ES\textsuperscript{+}) m/z 240.0 [M+H]\textsuperscript{+}; C_{10}H_{14}N_3O_4 requires 240.0984; found 240.0979; m.p. > 250 °C. \]

[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 \textsuperscript{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$_2$O (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$_3$) $\delta$ 1.28 (6H, t, J = 7, P(OCH$_2$CH$_3$)$_2$), 1.39 (9H, s, C(CH$_3$)$_3$), 1.72 (1H, br, NH), 1.92 (2H, dt, $^3$J$_{H-P}$ = 18, $J_{H-H}$ = 7, PCH$_2$CH$_2$), 2.62 (1H, dd, J = 12, 6, CHH), 2.71 (1H, dd, J = 12, 6, CHH), 2.78-2.94 (2H, m, PCH$_2$CH$_2$), 3.13 (1H, dt, J = 14, 7, CHH), 3.29 (1H, dt, J = 14, 7, CHH), 3.78-3.94 (2H, m, P(OCH$_2$CH$_3$)$_2$), 4.03-4.16 (4H, qd, $^3$J$_{H-H}$ = 7, $^3$J$_{H-P}$ = 3, P(OCH$_2$CH$_3$)$_2$), 5.14 (1H, br, NH). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 16.5 (d, $^1$J = 6, P(OCH$_2$CH$_3$)$_2$), 26.7 (d, $^1$J = 140, NHCH$_2$CH$_2$P), 28.4 (C(CH$_3$)$_3$), 42.47 (CH$_2$), 43.4 (d, $^1$J = 4, NHCH$_2$CH$_2$P), 50.4 (CH$_2$), 61.7 (d, $^3$J = 7, P(OCH$_2$CH$_3$)$_2$), 61.8 (CN$_3$), 79.7 (C(CH$_3$)$_3$), 156.0 (CO). $^{31}$P NMR (CDCl$_3$, 162 MHz) $\delta$ 31.29. MS (ES$^+$) m/z 380.2 [M+H]$^+$; C$_{14}$H$_{31}$N$_5$O$_5$P 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\(_3\)) \( \delta \)

\[
1.25 (6H, t, J = 7, P(OCH_2CH_3)_2), 1.35 (9H, s, C(CH_3)_3), 1.38 (3H, t, J = 7, OCH_2C(CH_3)_3), 1.98-2.14 (2H, m, PCH_2CH_2), 3.05-3.39 (2H, m, CH_2), 3.45-3.96 (5H, m, CH_2CH + PCH_2CH_2), 3.98-4.10 (4H, m, P(OCH_2CH_3)_2), 4.69 (2H, q, J = 7, OCH_2CH_3), 5.29 (1H, br, NH).
\]

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \)

\[
15.7 (OCH_2C(CH_3)_3), 16.3 (d, \(^3\)J = 6, P(OCH_2CH_3)_2), 26.0 (d, \(^1\)J = 140, NCH_2CH_2P), 28.3 (C(CH_3)_3), 42.1 (CH_2), 45.2 (NCH_2CH_2P), 50.9 (CH_2), 60.96 (CN), 62.0 (d, \(^2\)J = 7, P(OCH_2CH_3)_2), 70.0 (OCH_2CH_3), 79.8 (C(CH_3)_3), 155.9 (CO), 172.4, 177.2 (C=O), 182.8, 188.2 (CO). \(^{31}\)P NMR (CDCl\(_3\), 162 MHz) \( \delta \)

27.38. MS (ES\(^+\)) \( m/z \)

526.1 [M+Na]\(^+\); C\(_{20}\)H\(_{34}\)N\(_5\)O\(_8\)PNa 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)ethylphosphonate, 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 \( \mu \)L, 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\(_3\)) \( \delta \)

\[
1.30 (6H, 2 x t, J = 7, P(OCH_2CH_3)_2), 2.12-2.23 (2H, m, PCH_2CH_2), 3.49 (1H, dd, J = 14, 7, H^a(axial)), 3.55 (1H, d, J = 14, H^a(eq)), 3.64 (1H, d, J = 14, H^\beta(eq)), 3.67 (1H, ddd, J = 14, 7, 4, H^a(eq)), 3.86-4.12 (7H, m, CH + NCH_2CH_2P + P(OCH_2CH_3)_2), 8.18 (1H, br, s, NH).
\]

\(^{13}\)C NMR (176 MHz, CDCl\(_3\)) \( \delta \)

16.5 (d, \(^3\)J = 6,
ESI OBC Sim, Parker and Pal et al

P(OCH₂CH₃)₂, 25.9 (d, J = 140, NCH₂CH₂P), 45.9 (NCH₂CH₂P), 48.7, 56.0 (CH₂), 59.4 (CN₃), 62.2 (dd, J = 7, P(OCH₂CH₃)₂), 167.7, 168.1 (C=C), 180.8, 182.4 (CO). ³¹P NMR (CDCl₃, 243 MHz) δ 26.78. MS (ES⁺) m/z 358.1 [M+H]+; C₁₃H₂₁N₅O₅P 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 H₂O (100 µL) and PPh₃ (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 H₂O (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. ¹H NMR (400 MHz, MeOD) δ 1.34 (6H, t, J = 7 P(OCH₂CH₃)₂), 2.31 (2H, dt, Jₜ₋₋₉ = 18, Jₚ₋₋₉ = 7, PCH₂CH₂), 3.48-3.66 (5H, m, CH + CH₂), 3.98-4.07 (2H, m, NC₂H₂P), 4.09-4.19 (4H, qd, Jₚ₋₋₃ = 3, P(OCH₂CH₃)₂). ¹³C NMR (101 MHz, MeOD) δ 16.7 (d, J = 6, P(OCH₂CH₃)₂), 25.5 (d, J = 140, NCH₂CH₂P), 46.4 (NCH₂CH₂P), 51.1 (CH), 52.3 (CH₂), 58.0 (CH₂), 63.6 (d, J = 7, P(OCH₂CH₃)₂), 169.2, 169.6 (C=C), 182.7, 182.9 (CO). ³¹P NMR (162 MHz, MeOD) δ 30.97. MS (ES⁺) m/z 332.1 [M+H]+; C₁₃H₂₃N₅O₅P 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). NaHCO₃ (20 mL) was added, the layers separated and the aqueous washed with EtOAc (3 x 40 mL). The combined organic portions were dried over MgSO₄, 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; Rf =0.39) to yield a pale brown viscous oil. This product was characterized as a pair of diastereoisomers (33 mg, 26%).

**1H NMR (600 MHz, CDCl₃)** δ 1.41-1.43 (36H, overlapping s, C(CH₃)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, CH₃), 3.81-3.97 (2H, m, H), 4.31-4.52 (2H, CH₂), 4.57-4.70 (1H, m, CHNH), 7.66 (1H, br, NH), 8.59 (1H, br, NH).

**13C NMR (151 MHz, CDCl₃)** δ 27.9, 28.0 (overlapping C(CH₃)₃), 35.4, 44.5, 47.2 (C₄), 48.5, 48.7, 49.8, 50.1 (CHNH), 52.4, 52.6, 52.8 (CH₃), 55.6, 55.9, 60.8 (CH), 82.0, 82.1, 82.2, 82.6 (C(CH₃)₃), 168.1, 168.6 (C=O), 171.9, 172.6, 172.8, 172.9, 173.6, 175.3, 182.3, 182.3 (CO). MS (ES⁺) m/z 922.4 [M+H]⁺; C₄₅H₇₆N₇O₁₃ requires 922.5501; found 922.5526. [The ethyl ester was also present in one preparation: C₄₆H₇₈N₇O₁₃ 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$_3$) $\delta$ 1.28 (6H, t, J = 7, P(OCH$_2$CH$_3$)$_2$), 1.39, 1.40, 1.41, 1.42 (36H, s, C(CH$_3$)$_3$), 1.77-2.08 (5H, m), 2.12 (2H, m, PCH$_2$CH$_3$), 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, H$_c$, H$_a$, C$_H$CO$_2$tBu) 3.71-3.78 (1H, m, H$_c$), 3.81-3.89 (2H, m, PCH$_2$C$_H$2), 4.01-4.12 (4H, m, P(OCH$_2$CH$_3$)$_2$), 4.14-4.20 (1H, m, H$^p$), 7.64 (1H, br, NH), 7.84 (1H, br, NH).

$^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 16.5 (d, $^3$J = 6, P(OCH$_2$CH$_3$)$_2$), 25.6 (d, $^1$J = 140, NCH$_2$CH$_2$P), 27.9, 28.0 (overlapping C(CH$_3$)$_3$), 35.1, 44.5, 47.1, 48.1, 48.6 (CH$_2$), 49.7 (C$^b$), 50.0 (d, J = 16, PCH$_2$CH$_3$), 52.6, 52.7, 52.8, 55.6 (CH$_2$), 55.8 (C$^a$), 56.3 (C$^c$), 60.7 (CH), 62.0 (d, $^3$J = 7, P(OCH$_2$CH$_3$)$_2$), 81.9, 82.0, 82.1, 82.6 (C(CH$_3$)$_3$), 167.6, 167.8 (C=O), 172.6, 172.8, 172.9, 173.4, 175.3, 181.3, 182.0 (CO).

$^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$ 26.78. MS (ES$^+$) m/z 1014.1 [M+H$^+$]; C$_{48}$H$_{85}$N$_7$O$_{14}$P requires 1014.589; found 1014.593.

[Conjugate 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$_2$O (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, L1, as its protonated salt, (6.8 mg, 0.01 mmol) was dissolved in H2O (0.5 mL) and the pH adjusted to about 5.5 by the addition of NaOH (0.1 M). GdCl3.6H2O (4.8 mg, 0.013 mmol) was added as a solution in H2O (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 H2O. Removal of the water by lyophilisation gave [Gd.L1] as a white solid, which was purified by RP-HPLC. HR-MS (ES+) \( \text{C}_{28}\text{H}_{42}^{157}\text{GdN}_7\text{O}_{14} \) requires 888.1767 [M+2H]+; found 888.1763. 

\( r_{lp} = 4.91 \text{ mM}^{-1}\text{s}^{-1} \) (60 MHz, 310K). RP-HPLC: \( t_R = 7.1 \text{ mins} \) [2-30% MeOH in H2O over 10 mins].

[\text{Gd.L2}]

The [Conjugate 3] (10 mg, 9 \( \mu \)mol) 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 \( \mu \)L, 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 H2O. 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 L3 as a light brown solid. MS (ES+) \( m/z \) 734.9 [M+H]+. The salt of L3 (7.2 mg, 9 \( \mu \)mol) was dissolved in H2O (0.5 mL) and the pH adjusted to 5.5. GdCl3.6H2O (4.4 mg, 12 \( \mu \)mol) was added as a solution in H2O (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 H2O. Removal of the water by lyophilisation gave a white solid which was purified by RP-HPLC. HR-MS (ES+) \( \text{C}_{28}\text{H}_{42}^{157}\text{GdN}_7\text{O}_{14}\text{P} \) requires 888.1767 [M+2H]+; found 888.1763. 

\( r_{lp} = \)
5.76 mM⁻¹s⁻¹ (60 MHz, 310K). RP-HPLC: \( t_R = 7.3 \) mins [2-30% MeOH in H₂O over 10 mins].

[Conjugate 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 H₂O. 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 L⁵ as a light brown solid. MS (ES⁺) m/z 833.7 [M+H]⁺. The salt of L⁵ (10.8 mg, 0.013 mmol) was dissolved in H₂O (1.0 mL) and the pH adjusted to 6.0. GdCl₃·6H₂O (5.79 mg, 0.016 mmol) was added as a solution in H₂O (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 H₂O. Removal of the water by lyophilisation gave a white solid residue that was purified by RP-HPLC. HR-MS (ES⁺) C₃3H₅₁¹⁵⁴GdN₉O₁₅P requires 984.2420 [M+2H]⁺; found 984.2433. r₁p = 4.97 mM⁻¹ s⁻¹ (60 MHz, 310K). RP-HPLC: tᵣ = 7.8 mins [2-30% MeOH in H₂O 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). NaHCO₃ (30 mL) was added, the layers separated and the aqueous washed with EtOAc (3 x 30 mL). The combined organic portions were dried over MgSO₄, 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%).

\[ { }^1 H \text{ NMR (700 MHz, CDCl}_3 \text{)} \delta 1.27 (6H, dt, J = 7, 3, \text{P(OCH}_2\text{C}_3\text{H}_3)_2), 1.41, 1.41, 1.42 (36H, s, \text{C(CH}_3)_3), 1.47-1.69 (6H, m), 1.69-1.77 (2H, m, \text{PCH}_2\text{CH}_2\text{CH}_2), 1.82-1.89 (2H, m, \text{PCH}_2\text{CH}_2\text{CH}_2), 2.04-2.10 (2H, m) 2.21-2.26 (2H, m), 2.46-2.64 (9H, m) 2.71-2.85 (4H, m), 2.91-3.07 (5H, m), 3.10-3.25 (4H, m), 3.29 (1H, d, J = 14, \text{H}_c/\text{a (eq)}), 3.34 (2H, dd, J = 14, 7, \text{H}_c (axial)), 3.37-3.43 (2H, m), 3.47 (1H, ddd, J = 14, 7, 4, \text{H}_a (axial)), 3.70-3.74 (1H, m, \text{H}_c/\text{a (eq)}), 3.75-3.81 (2H, m, \text{PCH}_2\text{CH}_2\text{CH}_2), 4.01-4.08 (4H, m, \text{P(OCH}_2\text{C}_3\text{H}_3)_2), 4.15-4.19 (1H, m, \text{H}_b), 7.56 (1H, br, \text{NH}), 7.99 (1H, br, \text{NH}), 8.64 (1H, br, \text{NH}). \] 

\[ { }^{13} C \text{ NMR (176 MHz, CDCl}_3 \text{)} \delta 16.5 (d, ^3J = 6, \text{P(OCH}_2\text{C}_3\text{H}_3)_2), 22.8 (d, ^1J = 140, \text{PCH}_2\text{CH}_2\text{CH}_2), 24.9 (\text{CH}_2), 26.7 (\text{CH}_2), 27.9, 27.9, 28.0, 28.0 (overlapping \text{C(CH}_3)_3), 29.4, 33.0, 38.8, 44.7, 45.7, 47.3 (\text{CH}_2), 48.5 (d, \text{J = 16, PCH}_2\text{CH}_2\text{CH}_2), 48.6 (\text{CH}_2), 49.7 (\text{C}=\text{O}), 50.2 (\text{CH}_2), 51.5 (d, ^3J = 16, \text{PCH}_2\text{CH}_2\text{CH}_2), 52.7, 52.8, 55.6 (\text{CH}_2), 55.9 (\text{C}=\text{O}), 55.9 (\text{C}), 61.3 (\text{CH}), 61.8 (d, ^2J = 7, \text{P(OCH}_2\text{C}_3\text{H}_3)_2), 82.0, 82.0, 82.1, 82.1, 82.1 (\text{C(CH}_3)_3), 167.6, 168.1 (\text{C}=\text{C}), 172.7, 172.9, 173.0, 173.2, 173.3, 175.5, 181.5, 181.8 (\text{CO}). \] 

\[ { }^{31} P \text{ NMR (283 MHz, CDCl}_3 \text{)} \delta 30.91. \text{ MS (ES}^+ \text{) m/z 1127.3 [M+H]}; \text{C}_{56}\text{H}_{90}\text{N}_8\text{O}_{16}\text{P requires 1127.673; found 1127.673.} \]
(S)-2-Bromo-pentanedioic acid 5-methyl ester

A solution of NaNO₂ (6.11 g, 88.5 mmol) in H₂O (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 °C. After 10 h, conc. H₂SO₄ (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 Na₂SO₄, 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; Rf = 0.20) to yield a yellow oil (4.35 g, 42 %).

1H NMR (400 MHz, CDCl₃) δ 2.30 (1H, m, C₆H₂CHBr), 2.42 (1H, m, C₆H₂CHBr), 2.60 (2H, m, C₆H₂CH₂CHBr), 3.69 (3H, s, C₃H₃), 4.41 (1H, dd, J = 9, 5, C₆H).

13C NMR (176 MHz, CDCl₃) δ 29.7 (C₆H₂CH₂CHBr), 31.6 (C₆H₂CHBr), 44.2 (CH), 52.0 (C₃H₃), 172.1, 173.4 (CO). MS (ES⁺) m/z 246.8 [M + Na]⁺.

(5)-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 HClO₄ in H₂O (70 %, 0.34 mmol) was stirred at room temperature, for 16 h. H₂O (35 ml) was added to the reaction mixture, and the organic phase separated. The organic phase was washed with H₂O (25 ml), followed by 5 % Na₂CO₃ (25 ml). The solvent was removed under reduced pressure to yield a pale yellow oil (2.58 g, 70 %). 1H NMR (400 MHz, CDCl₃) δ 1.47 (9H, s, C(CH₃)₃), 2.23 (1H, m, CH₂CHBr), 2.33 (1H, m, CH₂CHBr), 2.50 (2H, m, CH₂CH₂CHBr), 3.69 (3H, s, CH₃), 4.23 (1H, dd, J = 9, 5, CH). 13C NMR (76 MHz CDCl₃) δ 27.9 (C(CH₃)₃), 29.9 (CH₂CH), 31.5 (CH₂CH₂), 46.8 (CH), 52.0 (CH₃), 82.8 (C(CH₃)₃), 168.4, 172.7 (CO). MS (ES⁺) m/z 303.5 [M+H]+; C₁₀H₁₇O₄BrNa 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-\textit{tert}-butyl 2,2’-(4-(6-(((benzyloxy)carbonyl)amino)-1-(\textit{tert}-butoxy)-1-oxohexan-2-yl)1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate $^{a}$ (1.35 g, 1.87 mmol), K$_2$CO$_3$ (310 mg, 2.24 mmol) and (S)-2-Bromo-pentanedioic acid 5-methyl ester 1-\textit{tert}-butyl ester (631 mg, 2.24 mmol) were stirred as a solution in acetonitrile (20 mL) at 60 $^{\circ}$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$_3$) $\delta$ 1.36 (9H, s, C(CH$_3$)$_3$), 1.37 (18H, s, C(CH$_3$)$_3$), 1.38 (9H, s, C(CH$_3$)$_3$), 1.43 – 1.50 (4H, m, CH$_2$), 1.54–1.63 (2H, m, CH$_2$), 1.64-1.78 (2H, m, CH$_2$), 1.84-1.93 (2H, m, CH$_2$), 2.38-2.52 (2H, br. m, CH$_2$), 2.52-2.61 (4H, br. m), 2.62-2.86 (12H, m, Ar-H). $^{13}$C NMR (176 MHz, CDCl$_3$) $\delta$ 27.7, 27.8 (CH$_2$), 28.2, 28.3, 28.3 (C(CH$_3$)$_3$), 30.4, 32.1, 40.5, 40.7, 44.1, 44.5, 47.1, 47.2, 48.7, 48.8 (CH$_2$), 51.4 (CH$_2$), 52.9 (CH$_2$), 55.8, 56.0, 59.7 (CH$_2$ ring), 61.1 (CH$_2$CO$_2$Bu), 63.2, 63.4 (CHCO$_2$Bu), 66.3 (OCH$_2$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 (CO). MS (ES$^+$) m/z 920.8 [M+H]$^+$; C$_{48}$H$_{82}$N$_5$O$_{12}$ requires 920.5782; found 920.5801.


4-(((R)-5-(4,10-Bis(2-(\textit{tert}-butoxy)-2-oxoethyl)-7-((R)-1-(\textit{tert}-butoxy)-5-methoxy-1,5-dioxopentan-2-yl)-1,4,7,10-tetraazacyclododecan-1-yl)-6-(\textit{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(CH$_3$)$_3$), 1.44 (18H, s, C(CH$_3$)$_3$), 1.49 (9H, s, C(CH$_3$)$_3$), 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, CH$_3$), 7.75 (1H, br, NH), 8.15 (1H, br, OH). $^{13}$C NMR (176 MHz, CDCl$_3$) δ 27.9, 27.9 (CH$_2$), 28.0, 28.0, 28.2, 28.3 (C(CH$_3$)$_3$), 29.1, 30.7, 32.3, 39.1, 38.8, 44.2, 46.9, 47.3, 48.7, 50.6, 51.7 (CH$_2$), 51.8 (CH$_3$), 52.7, 52.8, 56.0, 56.1 (CH$_2$), 56.4 (CH$_2$CO$_2$Bu), 59.8, 61.2 (CHCO$_2$Bu), 82.1, 82.2, 82.7, 83.6 (C(CH$_3$)$_3$), 172.9, 173.1, 173.5, 174.0, 174.8, 174.9, 175.4 (CO). MS (ES$^+$) m/z 886.7 [M+H]$^+$; C$_{44}$H$_{80}$N$_5$O$_{13}$ requires 886.5753; found 886.5742.

(2R)-1-tert-Butyl 5-methyl 2-(4,10-bis(2-(tert-butoxy)-2-oxoethyl)-7-((2R)-1-(tert-butoxy)-6-(4-((2-(diethoxyphosphoryl)ethyl)-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-4-yl)amino)-4-oxobutanimido)-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$_3$) $\delta$ 1.32 (6H, dt, J = 7, 3, P(OCH$_2$CH$_3$)$_2$), 1.43 (36H, s, C(CH$_3$)$_3$), 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, PCH$_2$CH$_2$), 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, H$_c$ (eq)), 3.50 (1H, d, J = 14, H$_c$ (axial)), 3.64 (3H, s, CH$_3$), 3.69-3.79 (2H, m, PCH$_2$CH$_3$), 3.90 (1H, dd, J = 14, 7, H$_b$ (axial)), 4.05-4.14 (4H, m, P(OCH$_2$CH$_3$)$_2$), 4.27 (1H, m, H$^a$). $^{13}$C NMR (176 MHz, CDCl$_3$) $\delta$ 16.5 (d, $^3$J = 6, P(OCH$_2$CH$_3$)$_2$), 24.0 (CH$_2$), 25.0 (d, $^1$J = 140, NCH$_2$CH$_2$P), 25.2, 27.9, 27.9, 28.3 (CH$_2$), 28.3, 28.4, 28.5 (overlapping C(CH$_3$)$_3$), 30.6, 31.0, 31.4, 31.8, 39.5, 45.2, 48.7 (CH$_2$), 49.7 (d, J = 16, PCH$_2$CH$_2$), 51.5 (C$^b$), 52.9, 53.1, 53.1(CH$_2$), 55.5 (C$^b$), 56.1 (C$^b$), 62.2 (d, $^3$J = 7, P(OCH$_2$CH$_3$)$_2$), 62.5 (CH), 63.2 (CH$_2$CO$_2$Bu), 64.5 (CH), 80.8, 80.8, 80.8, 81.0 (C(CH$_3$)$_3$), 168.0, 168.2 (C=O), 171.1, 172.2, 172.5, 173.0, 173.5, 174.4, 177.2, 181.2, 182.2 (CO). $^{31}$P NMR (283 MHz, CDCl$_3$) $\delta$ 27.26. MS (ES$^+$) m/z 1199.4 [M+H]$^+$; C$_{57}$H$_{109}$N$_8$O$_{17}$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).