Supplementary Information

Responsive Imaging Probes for Metabotropic Glutamate Receptors

Anurag Mishra,*[a] Sven Gottschalk,[b] Jörn Engelmann,[b] David Parker*[a]

[a,*] Dr. A. Mishra, Prof. D. Parker

Department of Chemistry

Durham University

South Road, Durham, DH1 3LE, England.

Fax: (+44-191-3342051)

E-mail: anurag.mishra@durham.ac.uk, david.parker@durham.ac.uk

[b] Dr. S. Gottshalk, Dr. J. Engelmann

High Field MR Center

Max Planck Institute for Biological Cybernetics

Spemannstrasse 41,Tuebingen, 72076, Germany.

Table of Contents

Materials and Methods

General Experimental

All solvents and reagents were purchased at analytical grade from their respective commercial suppliers (Aldrich, Fluka, Merck, Strem and VWR) and were used without further purification unless otherwise stated. All air or water-sensitive chemicals were stored under an inert atmosphere. Water refers to high purity water obtained from the "PuriteSTILL Plus" purification system, with conductivity of 0.04 μS cm^{-1}.

^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on a Varian VXR 400 spectrometer at room temperature. Spectra were recorded in commercially available deuterated solvents. All chemical shifts are given in ppm with coupling constants in Hz.

Electron Spray Ionisation-Low Resolution Mass Spectroscopy (ESI-LRMS in positive and negative ion mode) were carried out on a Waters Micromass LCT instrument and accurate masses recorded on a Thermo Finnigan LQT instrument.

Analytical reverse phase HPLC was carried out on a Perkin Elmer system at 295 K using a 150 x 4.66 mm 4 μ Phenomenex Synergi Fusion-RP 80i column using method: 95% solvent A (H_2O, 0.1% HCOOH) and 5% solvent B (MeCN, 0.1% HCOOH) isochratic for 2 min, 5% B to 100% solvent B in 13 min and then running isochratic for 1 min and then back to 5% solvent B in the next 2 min.

Inductively coupled plasma mass spectrometry (ICP-MS) for the evaluation of the Gd^{3+} concentration was performed by Dr. Chris J. Ottley, Earth science, Durham University, Durham. Total cell bound Gd^{3+} concentrations of various **[Gd.L^2]** treated cells were obtained after accounting for the sample-volume change (i.e. 500μL cell volume + 500μL of 60% HNO_3).

Relaxivity measurements were carried out at 310K, 60 MHz (1.4 T) on a Bruker Minispec mq60 instrument. The mean value of three independent measurements was recorded and averaged. The relaxivities of the compounds were calculated as the slope of the function shown in equation (1),

$$1/T_{1,obs} = 1/T_{1,d} + r_1 \times [GdL_n] \quad (1)$$

where $T_{1,obs}$ is the measured T_1, $T_{1,d}$ is the diamagnetic contribution of the solvent (calculated to be 4000 ms) and [GdL^n] is the concentration in mM of the appropriate Gd^{3+} complex (n = 1 - 8). Error for all relaxivity values was less then 0.1 $mM^{-1}s^{-1}$.

The apparent binding constant for the interaction of the Gd^{3+} complex with Human Serum Albumin (HSA) was calculated using equation (2):

$$[X] = \frac{\frac{(R-R_0)/(R_1-R_0)}{K} + [GdL]*(R-R_0)/(R_1-R_0) - [GdL]*\left((R-R_0)/(R_1-R_0)\right)^2}{1-(R-R_0)/(R_1-R_0)} \quad (2)$$

$$K = [GdX]/[X_f]\,[Gd_f] \quad (3)$$

where: [X]: the total concentration of HSA in the solution; [Gd.L]: the total concentration of the complex; K: the binding constant; R: relaxation rate of a given concentration of X; R_0: the initial relaxation rate; R_1: final relaxation rate; [Gd.X]: the concentration of the HSA-coordinated complex; [X_f]: the concentration of free HSA in the mixture; [Gd_f]: the concentration of the free complex.

Emission spectra were measured on a ISA Joblin-Yvon Spex Fluorolog-3 luminescence spectrometer (using DataMax v2.20 software) and 385nm cut-off filter was used. All samples were contained in quartz cuvettes with a path length of 1 cm and polished base. Human glutamate $mGlu_{5A}$ receptor (HmGluR$_5$) was purchased from Perkin Elmer, Germany.

Luminescent titrations were carried out by normalising the emission spectra with the absorption spectra in each point, in order to account for the decrease in the sample concentration caused by addition of [Glu] stock solution, where appropriate. All measurements were carried out in buffer (50 mM TRIS pH=7.4, 2.5 mM Ca^{2+}, 10 mM Mg^{2+}, 10% sucrose, 298 K).

Quantum yield of **[Gd.L^8]** was measured according to a previously reported protocol.[1]

Synthesis.

General synthesis of compounds 11/15/20/25/32/39/44/49.

A solution of **10/14/19/24/31/38/43/48** (1 equiv.), **4** or **7** (1 equiv.), NMM (2 equiv.) and HOBt (1.1 equiv.) in anhydrous DMF (5 mL) was stirred at 0-5˚C for 15 min and then *N'*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide [EDC] (1.1 equiv.) was added. The reaction mixtures were stirred for 12 h at room temperature. The completion of reaction was verified by TLC. The solution was poured into water (40 mL) and extracted with EtOAc (3x50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate evaporated under reduced pressure.

[4,7-Bis-butoxycarbonylmethyl-10-(1-*tert*-butoxy-5-(2-(3-((6-methylpyridin-2-yl)ethynyl)phenoxy)ethylamino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (11). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.2) to give **11** as a light yellow gum (110 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 18H, -C*H*$_3$), 1.42 (s, 9H, -C*H*$_3$), 1.44 (s, 9H, -C*H*$_3$), 1.97 - 2.12 (m, 8H, -CH-C*H*$_2$-C*H*$_2$-CONH and C*H*$_2$ of ring), 2.13 -2.23 (m, 2H, C*H*$_2$ of ring), 2.44 -2.82 (br. m, 10H, C*H*$_2$ of ring), 2.54 (s, 3H, -C*H*$_3$), 2.83 -2.97 (m, 3H, NC*H*CO and NC*H*$_2$CO), 3.24 - 3.54 (m, 4H, NC*H*$_2$CO), 3.56 - 3.67 (m, 2H, C*H*$_2$NHCO), 4.06 (t, *J*=6.0, 2H, -C*H*$_2$O), 6.94 (m, 1H, *H*$_{Ar}$), 7.07 - 7.15 (m, 3H, *H*$_{Ar}$), 7.19 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.34 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.56 (t, *J*=7.5,

1H, H_{Py}), 8.16 (s, 1H, N*H*). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 21.5, 24.4, 27.83, 27.88, 27.9, 35.0, 38.6, 47.0, 48.5, 52.6, 53.4, 55.7, 55.8, 60.5, 66.4, 81.9, 82.0, 82.2, 88.4, 89.0, 116.0, 117.9, 122.6, 123.1, 124.5, 124.5, 129.3, 136.5, 142.5, 158.6, 158.8, 172.5, 172.8, 173.6, 174.5. ESI-LRMS (+) calcd $C_{51}H_{79}N_6O_{10}$: *m/z* 935.5 $[M+H]^+$, found 935.6 $[M+H]^+$. ESI-HRMS (+) calcd $C_{51}H_{78}NaN_6O_{10}$: *m/z* 957.5677 $[M+Na]^+$, found 957.5766 $[M+Na]^+$.

[4,7-Bis-butoxycarbonylmethyl-10-(1-*tert*-butoxy-1,5-dioxo-5-(2-(3-(thiazol-4-ylethynyl)phenoxy)ethylamino)pentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (15). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.2) to give **15** as a dark yellow gum (120 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 18H, -C*H*$_3$), 1.42 (s, 9H, -C*H*$_3$), 1.44 (s, 9H, -C*H*$_3$), 1.95 - 2.17 (m, 4H, -CH-C*H*$_2$-C*H*$_2$-CONH), 2.13 -2.37 (br. m, 8H, C*H*$_2$ of ring), 2.46 -2.58 (m, 4H, C*H*$_2$ of ring), 2.66 -2.83 (m, 4H, C*H*$_2$ of ring), 2.84 -2.96 (m, 3H, NC*H*CO and NC*H*$_2$CO), 3.25 - 3.58 (m, 4H, NC*H*$_2$CO), 3.55 - 3.68 (m, 2H, C*H*$_2$NHCO), 4.07 (t, *J*=6.0, 2H, -C*H*$_2$O), 6.95 (d, *J*=8.0, 1H, H_{Ar}), 7.08 (d, *J*=8.0, 1H, H_{Ar}), 7.11 (s, 1H, H_{Ar}), 7.20 (t, *J*=8.0, 1H, H_{Ar}), 7.60 (s, 1H, C=C*H*-S), 8.11 (s, 1H, N*H*), 8.80 (s, 1H, S-C*H*=N). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 21.3, 27.8, 27.9, 29.2, 31.7, 35.0, 44.3, 47.0, 48.5, 53.8, 55.5, 55.8, 66.4, 69.5, 81.9, 82.0, 82.2, 82.7, 89.6, 115.9, 117.7, 122.3, 123.1, 124.2, 129.4, 138.5, 152.5, 158.6, 172.5, 172.8, 173.5, 173.9. ESI-LRMS (+) calcd $C_{48}H_{75}N_6O_{10}S$: *m/z* 927.5 $[M+H]^+$, found 927.5 $[M+H]^+$. ESI-HRMS (+) calcd $C_{48}H_{75}N_6O_{10}S$: *m/z* 927.5265 $[M+H]^+$, found 927.5249 $[M+H]^+$.

[4,7-Bis-butoxycarbonylmethyl-10-(1-*tert*-butoxy-5-(2-(3-((2-methylthiazol-4-yl)ethynyl)phenoxy)ethylamino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (20). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.15) to give **20** as a yellow gum (65 mg, 48%). ^{1}H NMR (400

MHz, $CDCl_3$): δ 1.43 (s, 18H, -C*H*$_3$), 1.44 (s, 9H, -C*H*$_3$), 1.45 (s, 9H, -C*H*$_3$), 1.95 - 2.11 (m, 4H, -CH-C*H*$_2$-C*H*$_2$-CONH), 2.13 -2.38 (m, 4H, C*H*$_2$ of ring), 2.39 -2.71 (br. m, 8H, C*H*$_2$ of ring), 2.72 (s, 3H, -C*H*$_3$), 2.74 -2.89 (m, 4H, C*H*$_2$ of ring), 2.90 -3.02 (m, 3H, NC*H*CO and NC*H*$_2$CO), 3.24 – 3.54 (m, 4H, NC*H*$_2$CO), 3.55 - 3.68 (m, 2H, C*H*$_2$NHCO), 4.04 (t, *J*=6.0, 2H, -C*H*$_2$O), 6.90 (m, 1H, *H*$_{Ar}$), 7.04 (s, 1H, N*H*), 7.09 (d, *J*=8.0, 1H, *H*$_{Ar}$), 7.19 (t, *J*=8.0, 1H, *H*$_{Ar}$), 7.31 (d, *J*=8.0, 1H, *H*$_{Ar}$), 7.38 (s, 1H, C=C*H*-S). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 19.1, 26.9, 27.7, 27.8, 27.9, 35.0, 38.6, 52.6, 55.4, 55.6, 55.7, 60.3, 60.3, 66.4, 71.7, 81.8, 81.9, 82.1, 83.0, 88.7, 115.7, 117.4, 124.2, 125.7, 129.3, 136.8, 143.2, 158.4, 165.5, 172.5, 172.7, 172.8, 173.3. ESI-LRMS (+) calcd $C_{49}H_{77}N_6O_{10}S$: *m/z* 941.5 $[M+H]^+$, found 941.5 $[M+H]^+$. ESI-HRMS (+) calcd $C_{49}H_{77}N_6O_{10}S$: *m/z* 941.5422 $[M+H]^+$, found 941.5422 $[M+H]^+$.

[4,7-Bis-butoxycarbonylmethyl-10-(1-*tert*-butoxy-5-(2-(5-((2-methylthiazol-4-yl)ethynyl)pyridin-3-yloxy)ethylamino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (25). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.15) to give **25** as a yellow gum (48 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (s, 18H, -C*H*$_3$), 1.26 (s, 9H, -C*H*$_3$), 1.27 (s, 9H, -C*H*$_3$), 1.78 - 1.93 (m, 4H, -CH-C*H*$_2$-C*H*$_2$-CONH), 1.94 -2.08 (m, 4H, C*H*$_2$ of ring), 2.29 - 2.54 (br. m, 8H, C*H*$_2$ of ring), 2.55 (s, 3H, -C*H*$_3$), 2.56 - 2.64 (m, 4H, C*H*$_2$ of ring), 2.65 - 2.83 (m, 3H, NC*H*CO and NC*H*$_2$CO), 3.07 - 3.38 (m, 4H, NC*H*$_2$CO), 3.40 - 3.51 (m, 2H, C*H*$_2$NHCO), 3.92 (t, *J*=6.0, 2H, -C*H*$_2$O), 7.50 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.62 (d, *J*=8.0, 1H, C=C*H*-S), 8.04 (d, *J*=3.0, 1H, *H*$_{Py}$) 8.13 (d, *J*=3.0, 1H, *H*$_{Py}$), 8.16 (s, 1H, N*H*). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 19.2, 25.6, 27.7, 27.8, 27.8, 35.2, 38.4, 52.5, 53.4, 55.4, 55.7, 59.9, 60.5, 66.8, 68.2, 81.8, 81.9, 82.0, 82.2, 86.3, 123.1, 123.2, 136.2, 138.1, 143.0, 144.2, 154.4, 165.7, 172.4, 172.6, 172.8, 173.6. ESI-LRMS (+) calcd

$C_{48}H_{76}N_7O_{10}S$: *m/z* 942.5 $[M+H]^+$, found 942.5 $[M+H]^+$. ESI-HRMS (+) calcd $C_{48}H_{76}N_7O_{10}S$: *m/z* 942.5374 $[M+H]^+$, found 942.5375 $[M+H]^+$.

Di-*tert*-butyl-2,2'-(4-(1-*tert*-butoxy-5-(2-(6-methyl-2-(6-methylpyridin-2-ylcarbamoyl)nicotinamido)ethylamino)-1,5-dioxopentan-2-yl)-10-(2-methoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (32). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.10) to give **32** as a yellow gum (72 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (s, 9H, -C*H*$_3$), 1.40 (s, 18H, -C*H*$_3$), 1.96 - 2.10 (m, 2H, -C*H*$_2$-CONH), 2.15 -2.29 (m, 2H, -CH-C*H*$_2$), 2.36 (s, 3H, -C*H*$_3$), 2.39 -2.48 (m, 2H, C*H*$_2$ of ring), 2.51 (s, 3H, -C*H*$_3$), 2.54 - 2.98 (br. m, 14H, C*H*$_2$ of ring), 3.02 - 3.12 (m, 3H, NC*H*$_2$CO, NC*H*CO), 3.32 - 3.37 (m, 2H, NC*H*$_2$CO), 3.40 (s, 2H, NC*H*$_2$CO), 3.45 - 3.57 (m, 2H, -NH- C*H*$_2$-), 3.57-3.70 (m, 5H, -NH- C*H*$_2$- and -C*H*$_3$), 6.85 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.19 (m, 1H, *H*$_{Py}$), 7.23 (s, 1H, N*H*), 7.59 (t, *J*=8.0, 1H, *H*$_{Py}$), 7.84 (d, *J*=7.0, 1H, *H*$_{Py}$), 8.12 (s, 1H, N*H*), 8.14 (s, 1H, N*H*), 8.16 (d, *J*=7.0, 1H, *H*$_{Py}$). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 22.6, 23.3, 24.0, 27.8, 27.9, 34.8, 39.4, 39.5, 51.9, 52.8, 53.5, 54.6, 55.8, 60.3, 67.6, 82.1, 82.2, 111.9, 119.2, 127.6, 129.9, 137.2, 139.0, 151.3, 156.3, 158.6, 162.6, 167.1, 172.8, 173.0, 173.5, 173.6, 173.6. ESI-LRMS (+) calcd $C_{48}H_{76}N_9O_{11}$: *m/z* 954.5 $[M+H]^+$, found 954.6 $[M+H]^+$. ESI-HRMS (+) calcd $C_{48}H_{76}N_9O_{11}$: *m/z* 954.5664 $[M+H]^+$, found 954.5668 $[M+H]^+$.

4,7-Bis-butoxycarbonylmethyl-10-(1-*tert*-butoxy-5-(2-(2-cyano-4-(6-methylpyridin-2-ylcarbamoyl)phenoxy)ethylamino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (39). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.12) to give **39** as a yellow gum (82 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 18H, -C*H*$_3$), 1.45 (s, 9H, -C*H*$_3$), 1.48 (s, 9H, -C*H*$_3$), 2.16 - 2.33 (m, 4H, -CH-C*H*$_2$-C*H*$_2$-CONH), 2.46 (s, 3H, -C*H*$_3$), 2.50 -2.62 (m, 4H, C*H*$_2$ of ring), 2.63 - 3.05 (br. m,

12H, C*H*$_2$ of ring), 3.29 - 3.39 (m, 2H, NC*H*$_2$CO), 3.40 - 3.51 (m, 2H, NC*H*$_2$CO), 3.52 - 3.59 (m, 1H, NC*H*CO), 3.63 - 3.74 (m, 4H, NC*H*$_2$CO and C*H*$_2$NHCO), 4.36 (t, *J*=6.0, 2H, -C*H*$_2$O), 6.93 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.31 (d, *J*=8.0, 1H, *H*$_{Ar}$), 7.53 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.67 (t, *J*=8.0, 1H, *H*$_{Py}$), 8.19 - 8.22 (m, 2H, *H*$_{Ar}$), 8.58 (s, 2H, N*H*). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 20.7, 21.9, 26.83, 26.86, 27.8, 34.2,36.8, 46.0, 47.1, 47.4, 51.6, 54.5, 54.8, 66.4, 69.5, 81.0, 81.1, 81.3, 101.2, 111.1, 111.9, 114.6, 118.6, 125.7, 132.9, 133.0, 138.4, 150.2, 155.4, 162.0, 163.2, 171.5, 171.8, 172.7, 174.4. ESI-LRMS (+) calcd $C_{51}H_{79}N_8O_{11}$: *m/z* 979.5 $[M+H]^+$, found 979.8 $[M+H]^+$. ESI-HRMS (+) calcd $C_{51}H_{79}N_8O_{11}$: *m/z* 979.5868 $[M+H]^+$, found 979.5847 $[M+H]^+$.

[4,7-Bis-butoxycarbonylmethyl-10(1-*tert*-butoxy-5-(2-(4-(6-(6-methylpyridin-2-ylcarbamoyl)pyridin-2-yl)phenoxy)ethylamino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (44). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.15) to give **44** as a transparent gum (88 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 18H, -C*H*$_3$), 1.45 (s, 9H, -C*H*$_3$), 1.47 (s, 9H, -C*H*$_3$), 1.83 - 1.99 (m, 2H, -CH-C*H*$_2$-), 2.01 - 2.07 (m, 2H, -C*H*$_2$-CONH), 2.15 -2.34 (br. m, 6 H, C*H*$_2$ of ring), 2.54 (s, 3H, -C*H*$_3$), 2.56 - 2.85 (br. m, 10H, C*H*$_2$ of ring), 2.86 - 2.98 (br. m, 3H, NC*H*$_2$CO and NC*H*CO), 3.43 - 3.58 (m, 2H, NC*H*$_2$CO), 3.61 - 3.72 (m, 2H, NC*H*$_2$CO), 3.63 - 3.74 (m, 2H, C*H*$_2$NHCO), 4.23 (t, *J*=6.0, 2H, -C*H*$_2$O), 6.94 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.12 (m, 2H, *H*$_{Ar}$), 7.44 (t, *J*=8.0, 1H, *H*$_{Py}$), 7.66 (t, *J*=8.0 Hz, 1H, *H*$_{Py}$), 7.84 - 7.96 (m, 2H, *H*$_{Ar}$), 8.05 (d, *J*=8.0, 2H, *H*$_{Py}$), 8.15 (s, 1H, N*H*), 8.25 (d, *J*=8.0 Hz, 1H, *H*$_{Py}$), 8.52 (br. s., 1H, N*H*). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 24.1, 25.8, 27.8, 27.91, 27.93, 36.6, 38.6, 47.0, 48.3, 48.5, 52.7, 55.5, 55.8, 60.6, 66.4, 82.0, 82.1, 82.2, 110.9, 115.1, 119.3, 127.5, 128.3, 128.9, 131.7, 138.2, 138.5, 149.6, 153.2, 157.2, 160.4, 162.9, 172.4, 172.7, 172.8, 173.7. ESI-LRMS (+) calcd $C_{55}H_{83}N_8O_{11}$:

m/z 1031.6 $[M+H]^+$, found 1031.4 $[M+H]^+$. ESI-HRMS (+) calcd $C_{55}H_{83}N_8O_{11}$: *m/z* 1031.6175 $[M+H]^+$, found 1031.6188 $[M+H]^+$.

[4,7-Bis-butoxycarbonylmethyl-10-(1-*tert*-butoxy-5-(2-(4-(6-(6-methylpyridin-2-ylcarbamoyl)pyridin-3-yl)phenoxy)ethylamino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (49). The residue was purified by column chromatography (silica gel, 10% MeOH in CH_2Cl_2, R_f = 0.15) to give **49** as a transparent gum (82 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 18H, -C*H*$_3$), 1.48 (s, 9H, -C*H*$_3$), 1.52 (s, 9H, -C*H*$_3$), 1.90 - 2.03 (m, 2H, -CH-C*H*$_2$-), 2.05 - 2.13 (m, 2H, -C*H*$_2$-CONH),2.15 -2.33 (br. m, 6 H, C*H*$_2$ of ring), 2.54 (s, 3H, -C*H*$_3$), 2.55 - 2.88 (br. m, 10H, C*H*$_2$ of ring), 2.89 - 3.01 (br. m, 3H, NC*H*$_2$CO and NC*H*CO), 3.32 - 3.44 (m, 2H, NC*H*$_2$CO), 3.47 - 3.60 (m, 2H, NC*H*$_2$CO), 3.63 - 3.74 (m, 2H, C*H*$_2$NHCO), 4.23 (t, *J*=6.0, 2H, -C*H*$_2$O); 6.95 (d, *J*=8.0, 1H, *H*$_{Py}$), 7.14 (d, *J*=8.0, 2H, *H*$_{Ar}$), 7.57 (d, *J*=8.0, 2H, *H*$_{Ar}$), 7.67 (t, *J*=8.0, 1H, *H*$_{Py}$), 8.04 (dd, *J*=8.0, 2.5 Hz, 1H, *H*$_{Py}$), 8.28 (dd, *J*=8.0, 2.5, 1H, *H*$_{Py}$), 8.28 (s, 1H, -N*H*), 8.82 (d, *J*=8.0, *H*$_{Py}$), 9.00 (br. s, 1H, -N*H*), 10.49 (s, 1H, *H*$_{Py}$). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 24.1, 24.4, 27.91, 27.92, 27.93, 36.7, 38.3, 44.4, 45.7, 46.7, 47.7, 55.5, 55.8, 60.8, 62.6, 81.9, 82.1, 82.2, 110.7, 115.7, 119.2, 122.4, 128.2, 131.3, 134.8, 138.4, 139.4, 146.2, 147.1, 150.6, 157.1, 159.9, 160.9, 172.4, 172.6, 172.8, 173.8. ESI-LRMS (+) calcd $C_{55}H_{83}N_8O_{11}$: *m/z* 1031.6 $[M+H]^+$, found 1031.1 $[M+H]^+$. ESI-HRMS (+) calcd $C_{55}H_{83}N_8O_{11}$: *m/z* 1031.6175 $[M+H]^+$, found 1031.6151 $[M+H]^+$.

General Synthesis of Ligands L_1 - L_8

Compound **25** was dissolved in THF:MeOH:water (3:2:1, 6 mL) and stirred at 0-5°C for 15 min and then LiOH (1.5 equiv.) was added. The reaction mixture was stirred for 2 h at room temperature. The progress of the reaction was monitored by TLC. After completion, the intermediate was dissolved in TFA:CH_2Cl_2 (9:1 mL) and stirred overnight to get $\mathbf{L_5}$. Other

compounds **11/15/20/32/39/44/49** (1 equiv.) were dissolved in TFA:CH_2Cl_2 (9:1 mL) and stirred overnight. The solvent was removed by evaporation and dried under reduced pressure.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(3-((6-methylpyridin-2-yl)ethynyl)phenoxy)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^1). L^1 was obtained as an off-white sticky solid (74 mg, 89%). ^{1}H NMR (400 MHz, D_2O): δ 1.60 - 1.83 (m, 2H, -CH-C*H*$_2$-), 2.21 - 2.38 (m, 2H, -C*H*$_2$-CONH), 2.40 (s, 3H, -C*H*$_3$), 2.50 - 3.20 (br. m, 14H, C*H*$_2$ of ring), 3.22 - 3.48 (br. m, 5H, C*H*$_2$ of ring, NC*H*CO, NC*H*$_2$CO), 3.50 – 4.15 (br. m, 8H, NC*H*$_2$CO, CONH-C*H*$_2$-C*H*$_2$-O), 6.73 (m, 1H, H_{Ar}), 6.79 (m, 1H, H_{Ar}), 6.90 (d, *J*=8.5, 1H, H_{Ar}), 7.02 (t, *J*=7.0, 1H, H_{Ar}), 7.38 (d, *J*=8.5, 1H, H_{Py}), 7.47 (d, *J*=8.5, 1H, H_{Py}), 7.97 (t, *J*=7.0 , 1H, H_{Py}). ESI-LRMS (+) calcd $C_{35}H_{47}N_6O_{10}$: *m/z* 711.4 $[M+H]^+$, found 711.5 $[M+H]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-oxo-4-(2-(3-(thiazol-4-ylethynyl)phenoxy)ethylamino)butyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^2). L^2 as a yellow sticky solid was obtained (62 mg, 82%). ^{1}H NMR (400 MHz, D_2O): δ 1.63 - 2.06 (m, 2H, -CH-C*H*$_2$-), 2.24 - 2.60 (m, 2H, -C*H*$_2$-CONH),2.61 -3.40 (br. m, 14H, C*H*$_2$ of ring), 3.41 - 3.87 (br. m, 8H, C*H*$_2$ of ring, NC*H*$_2$CO), 3.88 - 4.35 (br. m, 5H, NC*H*CO and -O-C*H*$_2$C*H*$_2$NHCO), 6.56 - 7.04 (m, 3H, H_{Ar}), 7.13 (s, 1H, H_{Ar}), 7.51 (s, 1H, C=C*H*-S), 8.86 (s, 1H, S-C*H*=N). ESI-LRMS (+) calcd $C_{32}H_{42}N_6O_{10}S$: *m/z* 703.2 $[M+H]^+$, found 703.2 $[M+H]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(3-((2-methylthiazol-4-yl)ethynyl)phenoxy)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^3). L^3 as a yellow sticky solid was obtained (42 mg, 85%). ^{1}H NMR (400 MHz, D_2O): δ 1.61 - 1.99 (m, 2H, -CH-C*H*$_2$-), 2.23 - 2.52 (m, 2H, -C*H*$_2$-CONH), 2.70 (s, 3H, -C*H*$_3$), 2.73 -3.29 (br. m, 16H, C*H*$_2$ of ring), 3.30 - 3.58 (br. m, 7H, NC*H*$_2$CO, NC*H*CO), 3.71 - 4.08 (br. m, 4H, -O-C*H*$_2$C*H*$_2$NHCO), 6.90 (d, *J*=8.0 Hz, 1H, H_{Ar}), 6.98 (s, 1H, H_{Ar}), 7.20 (t, *J*=8.0 Hz, 1H, H_{Ar}), 7.43

(d, *J*=8.0Hz, 1H, H_{Ar}), 7.53 (s, 1H, C=C*H*-S). ESI-LRMS (+) calcd $C_{33}H_{45}N_6O_{10}S$: *m/z* 717.2 $[M+H]^+$, found 717.4 $[M+H]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(5-((2-methylthiazol-4-yl)ethynyl)pyridin-3-yloxy)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^4). L^4 as a yellow sticky solid was obtained (29 mg, 80%). ^{1}H NMR (400 MHz, MeOD): δ 1.68 - 2.05 (br. m., 2H, -CH-CH_2-), 2.35 - 2.49 (m, 2H, -CH_2-CONH), 2.59 (s, 3H, -CH_3), 2.61 -3.35 (br. m, 20H, CH_2 of ring, NCH_2CO), 3.36 - 3.61 (br. m, 5H, NCH_2CO, NC*H*CO, -CH_2NHCO), 3.96 - 4.11 (m, 2H, -O-CH_2), 7.49 (s, 1H, H_{Py}), 7.62 (s, 1H, C=C*H*-S), 8.19 (s, 1H, H_{Py}), 8.22 (s, 1H, H_{Py}). ESI-LRMS (+) calcd $C_{32}H_{44}N_7O_{10}S$: *m/z* 718.2 $[M+H]^+$, found 718.4 $[M+H]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(6-methyl-2-(6-methylpyridin-2-ylcarbamoyl)nicotinamido)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^5). L^5 as a yellow sticky solid was obtained (50 mg, 86%). ^{1}H NMR (400 MHz, D_2O): δ 1.71 - 2.04 (m, 2H, -CH-CH_2-), 2.30 - 2.53 (m, 2H, -CH_2-CONH), 2.74 (s, 6H, -CH_3), 2.79 -3.66 (br. m, 19H, CH_2 of ring, NCH_2CO, NC*H*CO), 3.69 - 3.93 (br. m, 8H, NCH_2CO, NC*H*CO, -O-CH_2CH_2NHCO), 6.64 - 6.75 (m, 1H, H_{Py}), 7.48 (t, *J*=8.0 Hz, 1H, H_{Py}), 7.80 (d, *J*=8.0 Hz, 2H, H_{Py}), 8.09 (d, *J*=8.0 Hz, 1H, H_{Py}). ESI-LRMS (+) calcd $C_{35}H_{50}N_9O_{11}$: *m/z* 794.3 $[M+Na]^+$, found 794.7 $[M+Na]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(2-cyano-5-(6-methylpyridin-2-ylcarbamoyl)phenoxy)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^6). L^6 as a yellow sticky solid was obtained (57 mg, 90%). ^{1}H NMR (400 MHz, D_2O): δ 1.85 - 2.02 (m, 2H, -CH-CH_2-), 2.46 - 2.56 (m, 2H, -CH_2-CONH), 2.67 (s, 6H, -CH_3), 2.79 -3.40 (br. m, 19H, CH_2 of ring, NCH_2CO, NC*H*CO), 3.47 - 3.71 (br. m, 4H, NCH_2CO), 4.16 - 4.30 (m, 4H, -O-CH_2CH_2NHCO),7.14 (d, *J*=9.0, 1H, H_{Py}), 7.23 (d, *J*=9.0, 1H, H_{Ar}), 7.43 (d, *J*=8.0 Hz,

1H, H_{Ar}), 7.52 (t, *J*=8.0, 1H, H_{Py}), 7.59 (s, 1H, H_{Ar}), 8.15 (d, *J*=9.0, 1H, H_{Py}). ESI-LRMS (+) calcd $C_{35}H_{46}N_8O_{11}$: *m/z* 755.3 $[M+H]^+$, found 755.4 $[M+H]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(4-(6-(6-methylpyridin-2-ylcarbamoyl)pyridin-2-yl)phenoxy)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^7). L^7 as a yellow sticky solid was obtained (60 mg, 88%). ^{1}H NMR (400 MHz, D_2O): δ 1.76 – 1.95 (m, 2H, -CH-CH_2-), 2.34 (s, 6H, -CH_3), 2.38 - 2.54 (m, 2H, -CH_2-CONH), 2.66 -3.25 (br. m, 16H, CH_2 of ring), 3.29 - 3.51 (br. m, 7H, NCH_2CO, NC*H*CO), 3.73 – 3.90 (m, 4H, -O-CH_2CH_2NHCO), 6.45 - 6.67 (m, 3H, H_{Ar}, H_{Py}), 7.03 - 7.19 (m, 2H, H_{Py}), 7.28 - 7.39 (m, 1H, H_{Py}), 7.47 - 7.56 (m, 1H, H_{Py}), 7.70 (d, *J*=7.0, 1H, H_{Ar}), 7.64 (d, *J*=7.0, 1H, H_{Ar}), 7.95 (t, *J*=8.0 Hz, 1H, H_{Py}). ESI-LRMS (+) calcd $C_{39}H_{51}N_8O_{11}$: *m/z* 807.3 $[M+H]^+$, found 807.4 $[M+H]^+$.

[4,7-Bis-carboxymethyl-10-(1-carboxy-4-(2-(4-(6-(6-methylpyridin-2-ylcarbamoyl)pyridin-3-yl)phenoxy)ethylamino)-4-oxobutyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L^8). L^8 as a yellow sticky solid was obtained (57 mg, 90%). ^{1}H NMR (400 MHz, D_2O): δ 1.78 - 2.06 (m, 2H, -CH-CH_2-), 2.33 - 2.60 (br. m., 2H, -CH_2-CONH), 2.72 -3.33 (br. m, 12H, CH_2 of ring), 3.34 - 3.57 (br. m, 7H, , CH_2 of ring, NCH_2CO, NC*H*CO), 3.63 - 4.05 (br. m., 8H, NCH_2CO, -O-CH_2CH_2NHCO), 6.66 (d, *J*=8.0, 2H, H_{Ar}), 7.20 (d, *J*=8.0, 1H, H_{Py}), 7.34 (d, *J*=8.0, 2H, H_{Ar}), 7.54 (d, *J*=8.0, 1H, H_{Py}), 7.67 (d, *J*=8.0, 1H, H_{Py}), 7.79 (d, *J*=8.0, 1H, H_{Py}), 8.03 (t, *J*=7.5, 1H, H_{Py}), 8.48 (d, *J*=8.0 Hz, 1H, H_{Py}). ESI-LRMS (+) calcd $C_{39}H_{51}N_8O_{11}$: *m/z* 807.3 $[M+H]^+$, found 807.4 $[M+H]^+$.

General preparation of gadolinium complexes of L^1 - L^8. Gadolinium complexes of L^1 - L^8 were prepared from corresponding solutions of the ligands (1 eq) and solutions of $GdCl_3.6H_2O$ (1.1 eq). The reaction mixture was stirred at 60° C for 20 h. The pH was periodically checked and adjusted to 6.0 using solutions of NaOH (1 M) and HCl (1 N) as needed. After completion,

the reaction mixture was cooled down and passed through Chelex-100 to trap free Gd^{3+} ions, and the Gd^{3+}-loaded complexes were eluted. The fractions were dialyzed (500 M.Wt cutoff; Spectra/Pro® biotech cellulose ester dialysis membrane, Spectrum Laboratories) and lyophilized to obtain off-white solids. The absence of free Gd^{3+} was checked with xylenol orange indicator. These complexes were characterized by ESI-HRMS in positive mode and the appropriate isotope pattern distribution for Gd^{3+} were recorded. The purity of each complex was analysed by analytical reverse phase HPLC.

[Gd.L^1] ESI-HRMS (+): calcd $C_{35}H_{42}{}^{155}GdNa_2N_6O_{10}$: m/z 907.1985 $[M+2Na]^+$; found 907.1974 $[M+2Na]^+$, t_R = 9.5 min, r_{1p} = 4.77 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^2] ESI-HRMS (+): calcd $C_{32}H_{38}{}^{155}GdNa_2N_6O_{10}S$: m/z 899.1392 $[M+2Na]^+$; found 899.1370 $[M+2Na]^+$, t_R = 11.5 min, r_{1p} = 5.05 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^3] ESI-HRMS (+): calcd $C_{33}H_{40}{}^{155}GdNa_2N_6O_{10}S$: m/z 913.1542 $[M+2Na]^+$; found 913.1540 $[M+2Na]^+$, Anal Calcd for $C_{33}H_{40}{}^{155}GdNa_2N_6O_{10}S(0.5H_2O)$; C, 45.60; H, 4.63; N: 9.66%. Found C, 45.10; H, 4.68; N, 9.57%, t_R = 9.9 min, r_{1p} = 4.71 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^4]. ESI-HRMS (+): calcd $C_{33}H_{39}{}^{155}GdNa_2N_7O_{10}S$: m/z 914.1495 $[M+2Na]^+$; found 914.1491 $[M+2Na]^+$, t_R = 9.7 min, r_{1p} = 5.11 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^5]. ESI-LRMS (+): calcd $C_{35}H_{45}{}^{155}GdN_9O_{11}S$: m/z 923.2 $[M+H]^+$; found 923.6 $[M+H]^+$, t_R = 11.9 min, r_{1p} = 5.45 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^6]. ESI-HRMS (+): calcd $C_{35}H_{42}{}^{155}GdN_8O_{11}$: m/z 905.2199 $[M+H]^+$; found 905.2175 $[M+H]^+$, t_R = 13.6 min, r_{1p} = 5.75 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^7]. ESI-HRMS (+): calcd $C_{39}H_{46}{}^{155}GdN_8O_{11}$: m/z 957.2512 $[M+H]^+$; found 957.2548 $[M+H]^+$, t_R = 11.3 min, r_{1p} = 5.43 $mM^{-1}s^{-1}$ (60 MHz, 310K).

[Gd.L^{8}]. ESI-HRMS (+):$C_{39}H_{46}{}^{155}GdN_8O_{11}$: m/z 957.2512 $[M+H]^+$; found 957.2557 $[M+H]^+$, Anal Calcd for $C_{39}H_{46}{}^{155}GdN_8O_{11}(0.5H_2O)$ C, 48.80; H, 4.83; N: 11.70%. Found C, 48.30; H, 4.85; N, 11.60%, t_R = 11.3 min, r_{1p} = 5.60 $mM^{-1}s^{-1}$ (60 MHz, 310K).

Preparation of primary rat astrocytes. Astroglia-rich primary cultures were prepared from the brains of 1- to 2-days-old Wistar rats as described elsewhere[2]. Briefly, after decapitation cerebral cortices were dissected and placed in sterile Dulbecco's Modified Eagle Medium (DMEM) containing 1 mM sodium pyruvate, 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin and supplemented with 15% foetal bovine serum (FBS) on ice (all from Biochrom AG, Germany). After careful removal of major blood vessels and the meninges, the remaining tissue was dissociated by trituration with a 22G needle. The suspension was centrifuged (1500g, 5 min., RT), vortexed for 2 minutes directly afterwards at high speed, then filtered through an 80 µm sterile nylon sieve and centrifuged again. The pellet was re-suspended in DMEM/15%FBS and passed through a 10 µm sterile nylon sieve without applying any pressure. The filtered cell suspension was then diluted with DMEM/15% FBS (according to 10mL per rat brain) and placed into culture dishes or well plates. The medium was changed after 24 hours and the cells were then maintained in DMEM/15% FBS for one week, after which the medium was changed to DMEM/10% FBS. The complete medium was changed three times a week and the cells reached confluence in poly-D-lysine coated 96 well plates after 10 days and in uncoated 10cm-dishes after 3-4 weeks of culture.

Immunofluorescence. Astroglia rich cultures were grown on special surface-modified glass chamber slides (Thermo Scientific Nunc, Denmark, Roskilde) until they reached confluence. Cells were fixed for 10 minutes at room temperature with 4% paraformaldehyde (Roti®-Histofix 4%, Roth, Germany) in phosphate buffered saline (PBS, Biochrom AG, Germany). For glial

fibrillary acidic protein (GFAP)-staining, membranes of fixed cells were permeabilized for 10 min with 0.25% (v/v) Triton X-100 in PBS, this step was omitted for mGluR5-staining. Unspecific antibody binding sites were blocked by incubating for 30 minutes with 10% (v/v) goat-serum in PBST (i.e. 0.05% (v/v) TWEEN-20 in PBS). Fixed cells were then incubated for 2 hours with the respective primary antibody (diluted in 1% bovine serum albumin (BSA, Roth, Germany) in PBST) in a humidified chamber at RT. After washing, cells were incubated with secondary antibody in 1% BSA in PBST in the humidified chamber in the dark. Afterwards cellular DNA was stained with 10µg/ml Hoechst 33342 (Merck, Germany) for 15 min and slides were mounted overnight with Mowiol 4-88 (Roth, Germany) mounting medium containing 50 mg/mL of the anti-bleaching reagent 1,4-diaza-bicyclo[2.2.2]octane (DABCO, Roth, Germany). Microscopic images were taken on a Zeiss Axiovert 200 M microscope (Germany) using appropriate fluorescence filters and a Zeiss Plan APOCHROMAT 63x/1.4 oil differential interference contrast (DIC) objective. Volocity Acquisition and Visualization software (Perkin Elmer, USA, MA) was used for image capture. The used antibodies were as follows, dilutions are given in parenthesis, and all antibodies were obtained from Abcam (UK, Cambridge): primary antibodies- rabbit polyclonal to mGluR5 (1:100), rabbit polyclonal to GFAP (1:1000); secondary antibody- goat polyclonal to rabbit IgG – H&L conjugated to Cy3 (1:1000).

Cytotoxicity assay. The toxicity of the compounds was investigated with a XTT-based (2,3-bis[2-methoxy-4-nitro5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) colorimetric proliferation assay, combined with staining of the DNA with Hoechst 33342 to simultaneously assess the cell number. Primary rat astrocytes grown in 24 well or 96 well plates to confluency were incubated with various concentrations of compounds under normal culture conditions for 24 hours. As positive control for cell death served an incubation with the apoptosis inducing calcium

ionophore A23187 (Serva Electrophoresis, Germany). After treatment, the medium was removed and the cells were incubated for 20 minutes at 37°C, 10% CO_2 with freshly prepared XTT-solution (2.5mg/15mL containing 1.66 µg/mL phenazine methosulfate in DMEM without phenol red). Absorbance of the developing formazan-dye was measured in a plate reader at 450nm, corrected for background at 690nm. Afterwards, the XTT-solution was exchanged with Hoechst 33342 (16.2 µM) in Hanks' Balanced Salt Solution (HBSS, Biochrom AG, Germany) (100 µL/well) and the cells were incubated for 30 minutes at 37°C, 10% CO_2. After washing with and adding of HBSS (each time 200 µL/well), fluorescence was measured (λ_{exc} 355 nm; λ_{em} 460 nm).

Measurement of cellular relaxation rate at 3T. Primary rat astrocytes were grown in 10cm culture dishes. Cells were trypsinized with Trypsin/EDTA 0.05/0.02% (w/v) (Biochrom AG, Germany), centrifuged and re-suspended to 1 x 10^7 cells/ 500 µL HBSS in 750 µL Eppendorf tubes. A maximum of 20 µL of highly concentrated stock solutions of the compounds were added to obtain up to 200 µM and cells were then incubated for 45 min. at 37°C, 10% CO_2. Afterwards, cells were centrifuged (300×g, 5 min., RT) and the supernatant was kept for MR-measurements. Cells were washed once with HBSS, centrifuged again (300×g, 5 min., RT) and re-suspended carefully in 500 µL of HBSS. In some experiments the wash-solution was also kept for MR-measurements. Cells were allowed to settle down before making MR-measurements. MR imaging of the cell pellets and supernatants, data analysis and determination of the cellular relaxation rate $R_{1,cell}$ was performed from the measured T_1 values according to the literature [3].

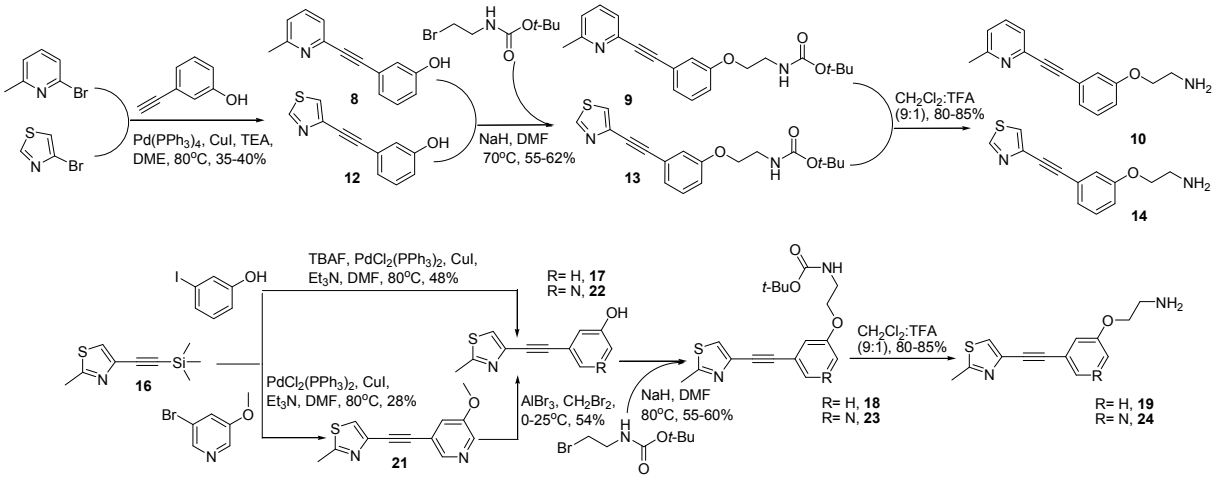
Briefly, MR measurements were performed on a clinical 3T (128 MHz, 21°C) human MR scanner (MAGNETOM Tim Trio, Siemens Healthcare, Germany). Longitudinal relaxation times (T_1) were measured using an inversion recovery sequence to obtain images from a 1 mm thick slice through the samples. The inversion time (Ti) was varied from 23 ms to 3000 ms in about 12

steps. Images were read out with a turbo spin echo technique, acquiring 5 echoes per scan. The repetition time (TR) was 10.000 ms to ensure complete relaxation. A matrix of 256 x 256 voxels was used over a field-of-view of 110 x 110 mm^2. Six averages per Ti were possible within 18 min.

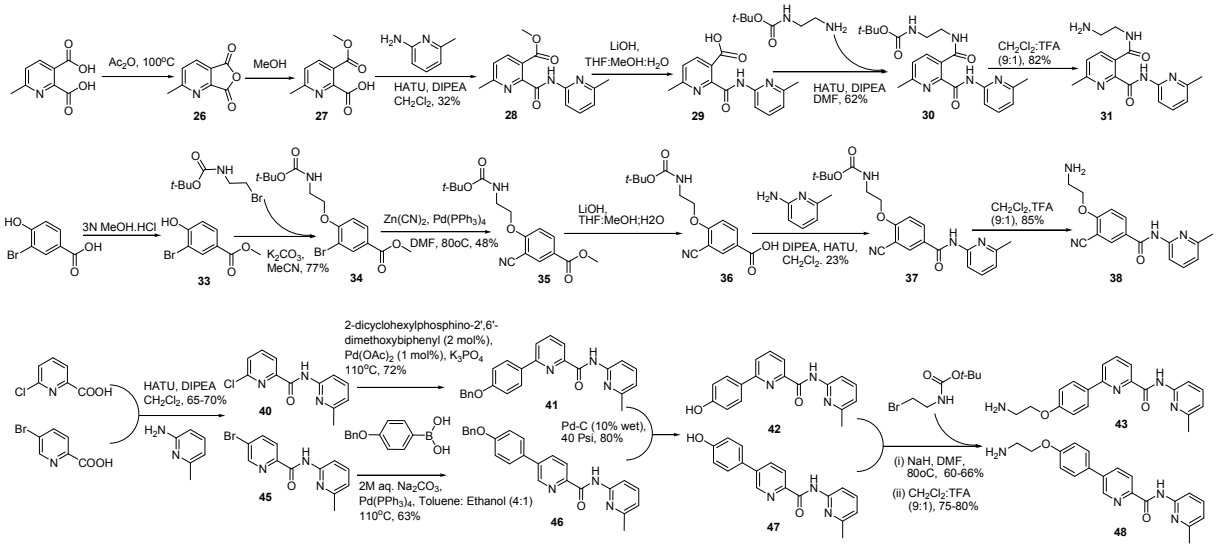
Fitting to relaxivity curves was done using self-written routines in MATLAB 6.5 R13 (The Mathworks Inc., USA). T_1 relaxation data with varying t = TR were fitted to $S=S_0(1-a \times exp(-t/T_1))$. Nonlinear least-squares fitting of three parameters S_0 (initial signal at t=0), T_1 and a was done for each voxel with the Gauss-Newton method (MATLAB function nlinfit). For each fitted parameter, the 95% confidence intervals were calculated (MATLAB functions nlparci, nlpreci) and used as an error estimate of the fitted relaxation times T_1 and S_0. The fit procedure resulted in parameter maps of T_1, S_0 and corresponding error maps σ_{T1}, σ_{S0}.

Circular image-regions in the tubes were defined as Regions Of Interest (ROIs), and the means and distribution width of the relaxation times of voxels in these regions were calculated. An iterative Gaussian fit was used to determine mean and standard deviation (SD) of a distribution with outliers correction. For this purpose a distribution histogram was first fitted to a Gaussian to estimate mean and SD. The tails of the distribution were then discarded by using a threshold of three SDs. A repeated fit proved to be robust and converged to the 'true' Gaussian mean and width of the distribution barring the outliers, observed as a result of the non-linear fit of noisy voxels. The processing of the relaxation data thus resulted in specific $R_1 = 1/T_1$ values for each tube sample including the standard deviation in the selected ROI ensemble. The ensemble error matched closely the errors of a single-voxel fit, which showed that no further systematic errors were introduced by the image encoding.

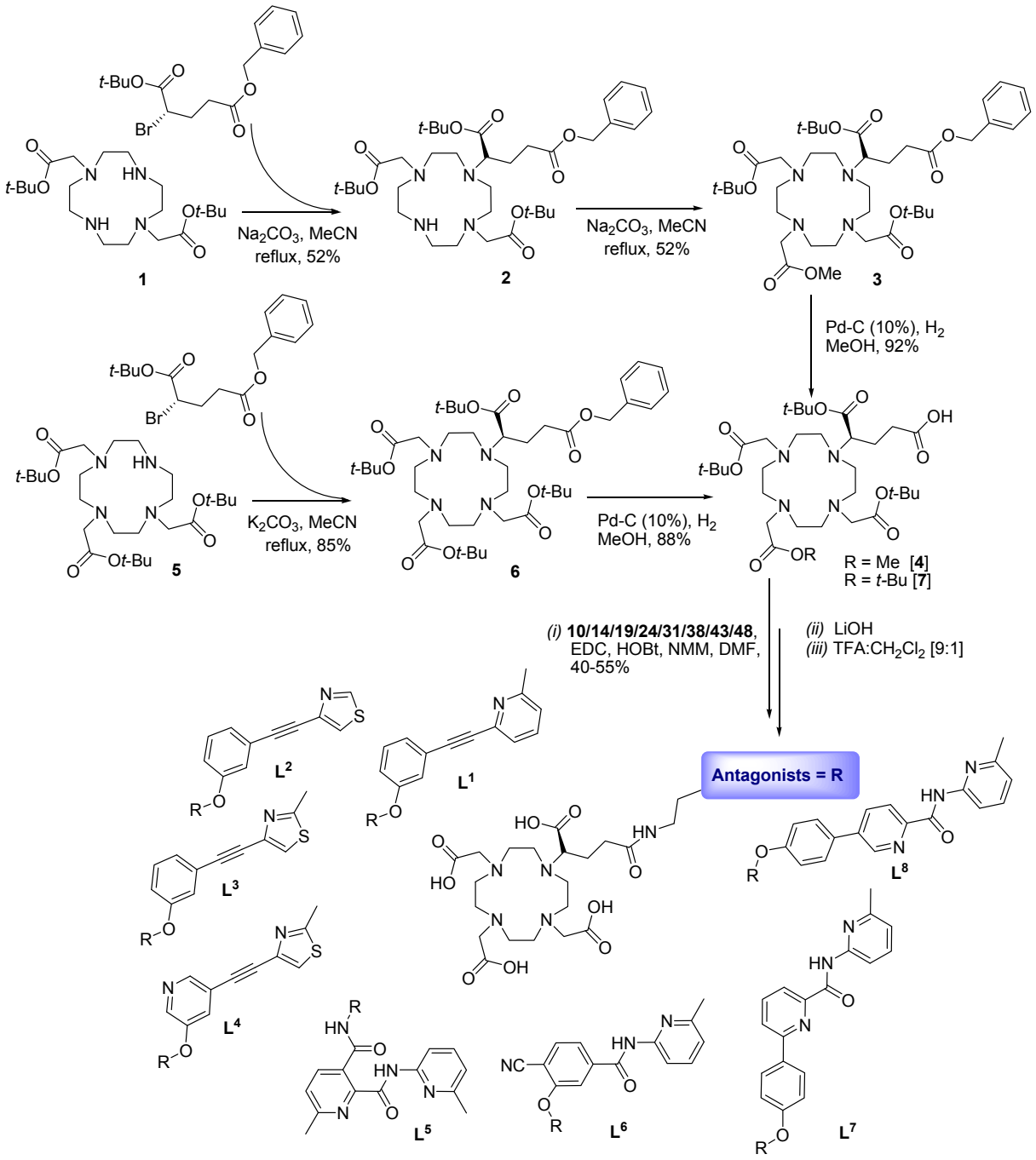
Calcium Fluorescence Assay. Primary rat astroglia were grown in poly-D-lysine-coated 96 well plates (Greiner Bio-One, Germany) for 10-15 days. Glutamate induced changes in cytosolic calcium content were measured according to the literature [4] adapted to sequential well-reading in a fluorescence plate reader (FLUOstar OPTIMA, BMG Labtech, Germany). In short, medium was changed to glutamine-free medium 24 hours prior to measurement. Cells were loaded for 30 minutes at 37°C, 10% CO_2 with 2.5 µM fluo-4 AM (Invitrogen, Germany) in measurement buffer (i.e. HBSS supplemented with 2.5 g/L glucose, 20 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and 2.5 mM probenecid, pH 7.6). After washing cells in measurement buffer (100 µL/well) were incubated for 15 minutes at 37°C in the plate reader with or without compounds present. Glutamate was then injected into the first well and the change in fluo-4 fluorescence (λ_{exc} 500 nm; λ_{em} 520 nm) was recorded over one minute. This was repeated for all wells. In total, nine different glutamate concentrations were used in triplicate. The concentration of the agonist glutamate that gives a response half way between bottom and top of the fitting curve (i.e. EC_{50}) was calculated for each condition using data normalized to the maximum obtainable change in calcium and using nonlinear regression analysis with GraphPad Prism 5.02 for Windows (GraphPad Software, USA).



Scheme S1. Synthesis of alkyne-based amine precursors.



Scheme S2. Synthesis of heterobiaryl/dipyridyl amide-based amine precursors.



Scheme S3. Synthesis of the final ligands, L^1 to L^8

Contrast Agents	Relaxivity ($mM^{-1} s^{-1}$)
[Gd.L^1]	4.77
[Gd.L^2]	5.11
[Gd.L^3]	4.71
[Gd.L^4]	5.05
[Gd.L^5]	5.45
[Gd.L^6]	5.75
[Gd.L^7]	5.43
[Gd.L^8]	5.60

Table S1. Comparative relaxivities of [**Gd.L$^{1-8}$**] (1.4 T, 0.1 M PBS pH 7.4, 310 K)

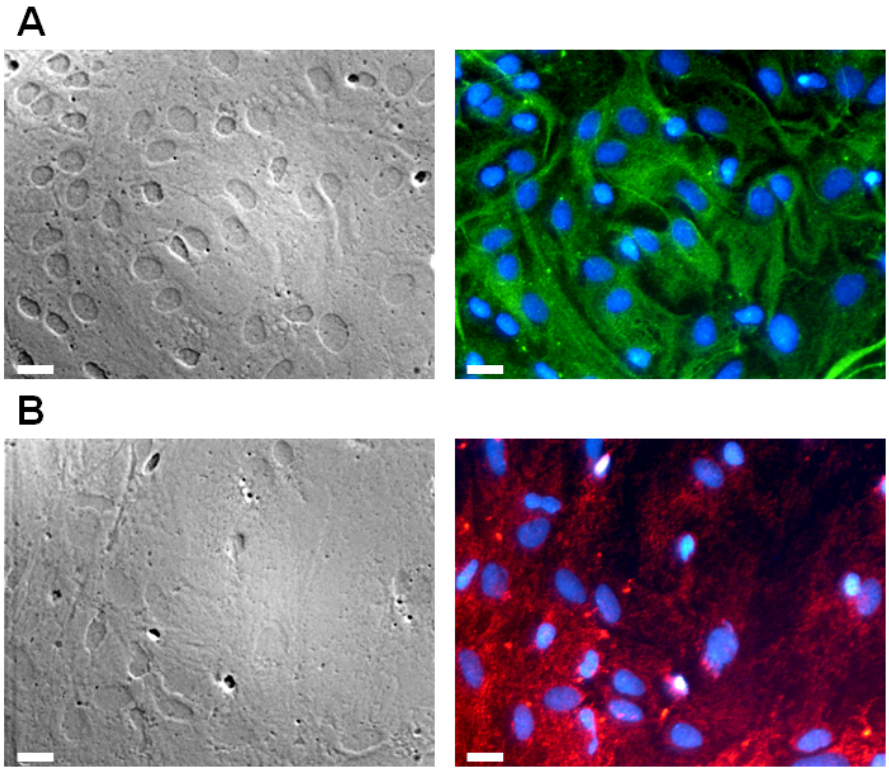


Figure S1. Immunofluorescence assay

Immunostaining of astrocytes for GFAP and mGluR5. Primary rat astrocytes were grown for 3 weeks in surface-modified glass chamber slides. *(A)* GFAP (green, FITC) and *(B)* mGluR5 (red, Cy 3^{TM}) were visualized after staining with specific antibodies. For GFAP-immuno-staining the cell membrane was permeabilized with 0.25% (v/v) Triton X-100 in PBS. Cell nuclei were counterstained with Hoechst 33342 (blue). Cell morphology was examined by differential interference contrast microscopy (left panels). Scale bar 20 µm.

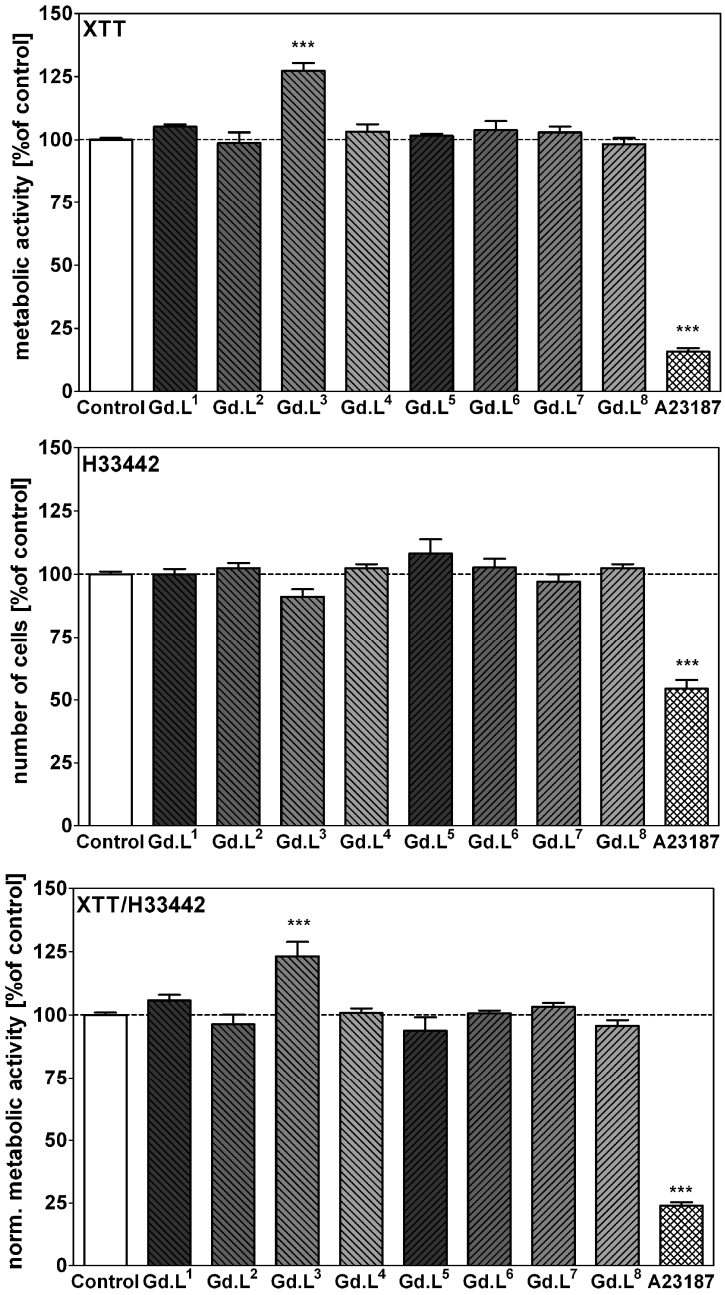


Figure S2. Cytotoxicity of [**Gd.L$^{1-8}$**].

Metabolic acitvity (XTT) as well as the cell number (H33342) were analyzed after treatment of primary astrocytes with 100µM of compounds [**Gd.L$^{1-8}$**] for 24 hours. The calcium ionophore A23187 (10µM, 24h) served as a positive control for cell death. Metabolic activity was further normalized to cell number

(XTT/H33342). All data are given as percent of control (untreated cells). Values are means ± SEM of up to three experiments with 4-6 replicates each. ***P<0.001 vs. untreated cells (control). ANOVA with Bonferroni‘s multiple comparison post test.

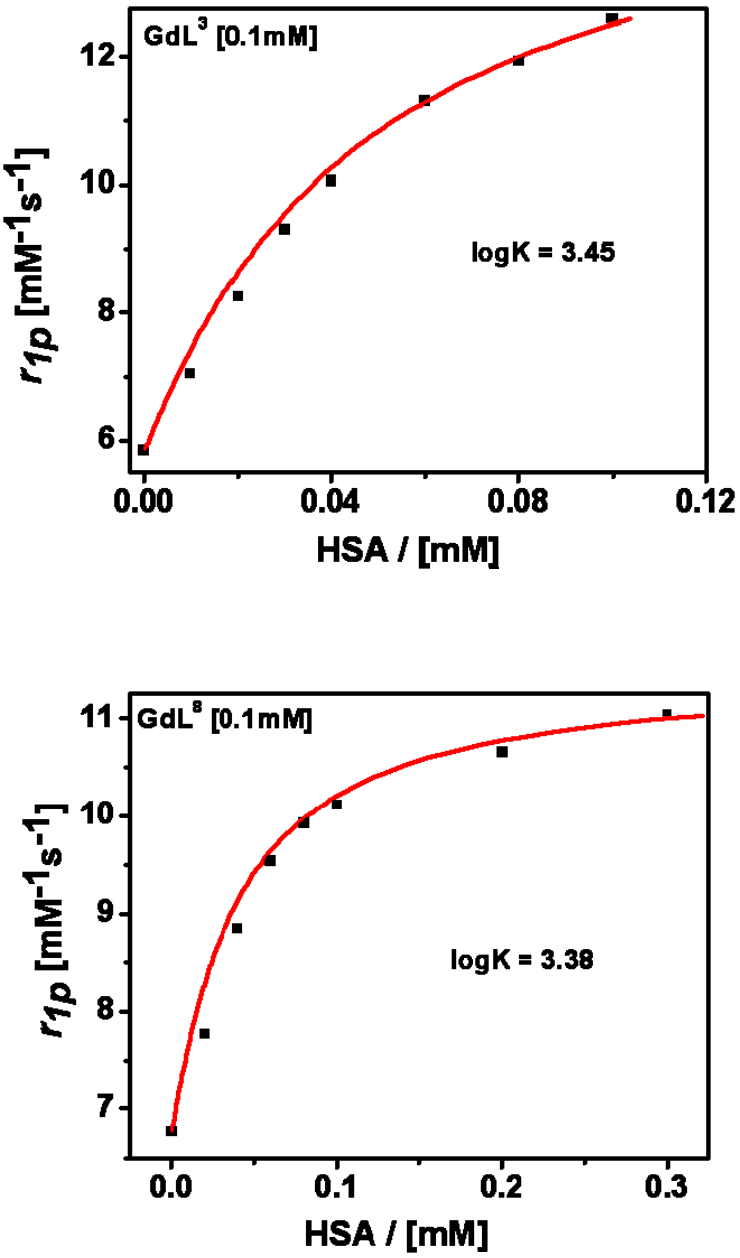
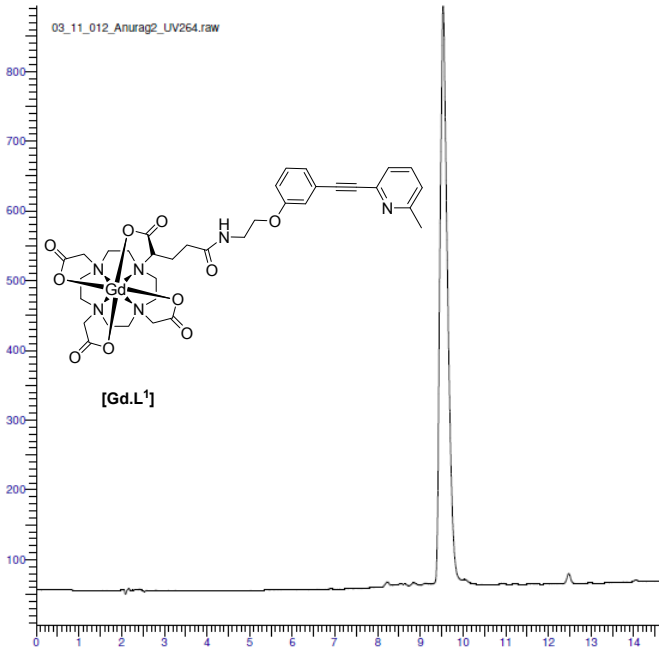
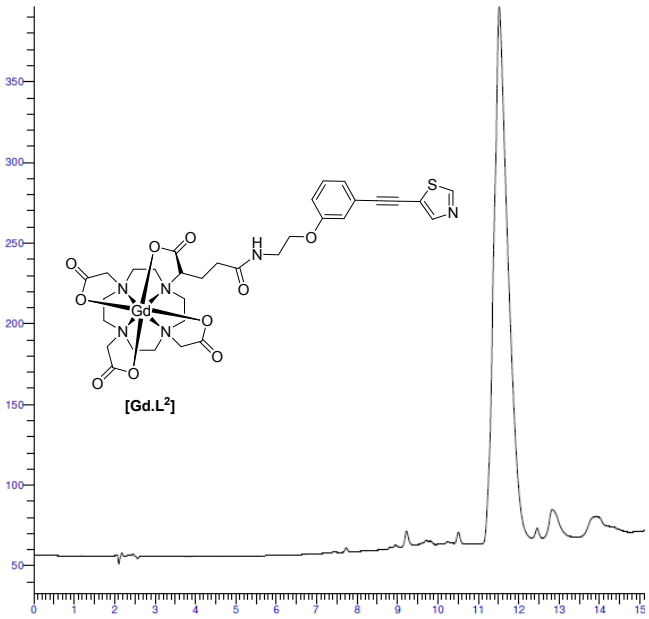
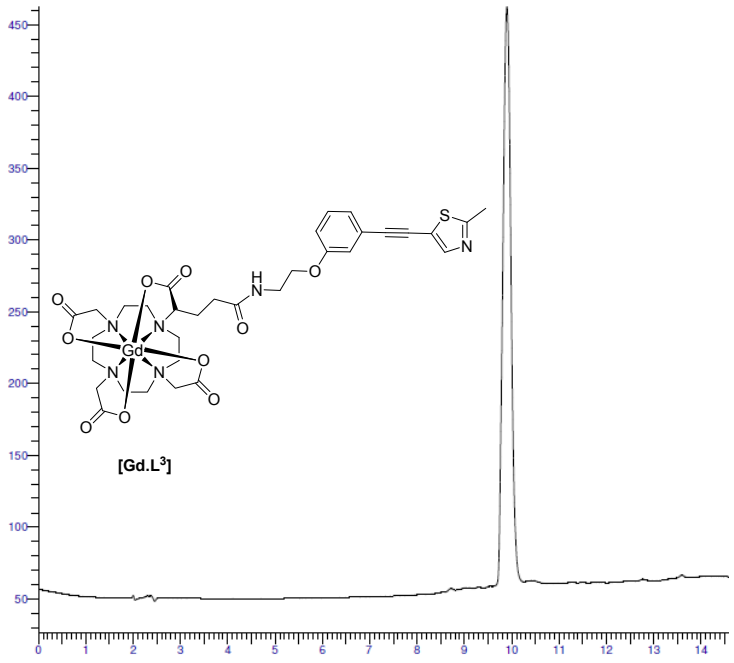


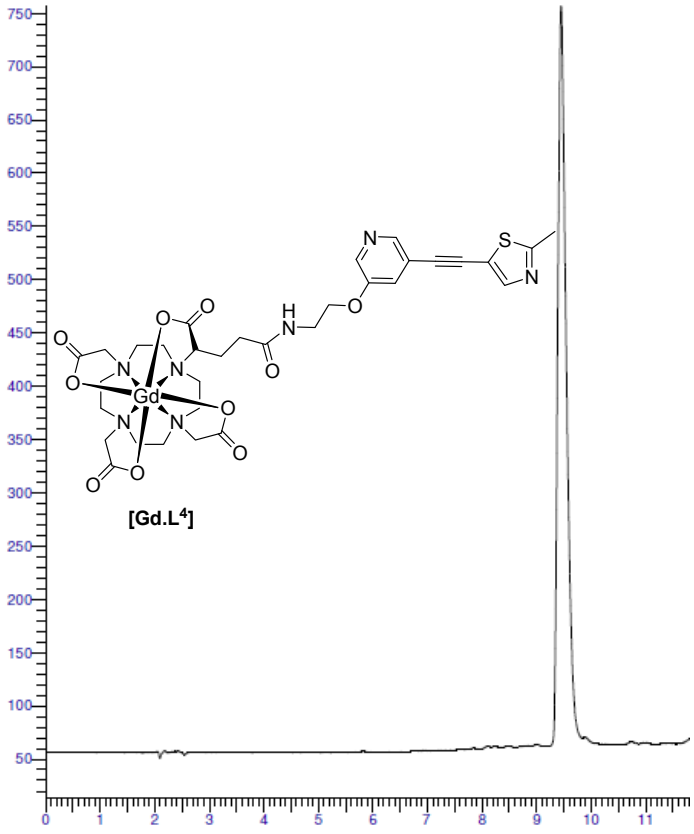
Figure S3. Binding constant determination of **[Gd.L$^{3 \text{ and } 8}$ vs HSA]** (50 mM TRIS pH 7.4, 2.5 mM Ca^{2+}, 10 mM Mg^{2+}, 10% sucrose, 310 K), showing the fit(line) to the data derived by iterative least-squares fitting assuming an 1:1 binding ratio.

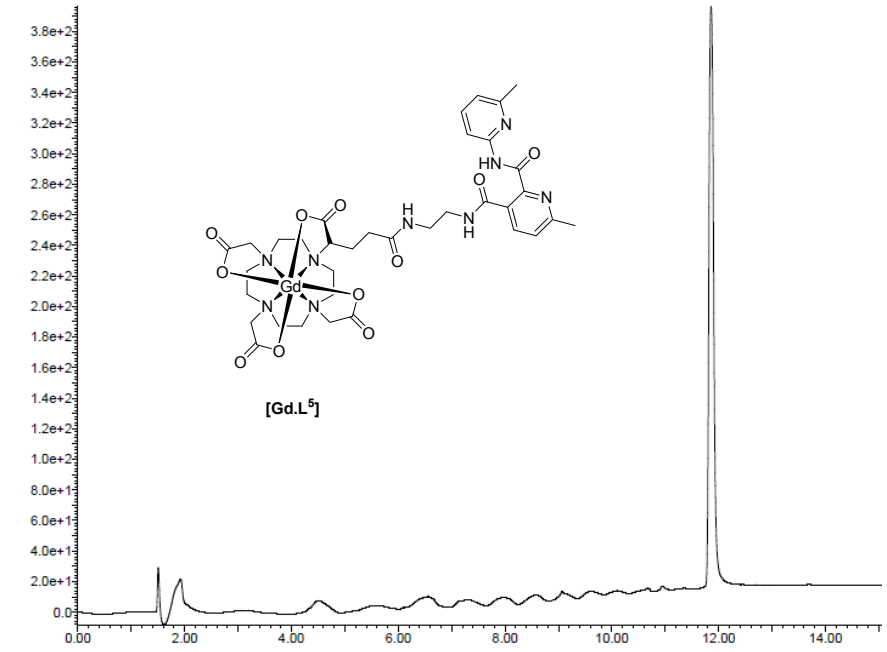
HPLC Chromatograms of [Gd.L^1] – [Gd.L^8]

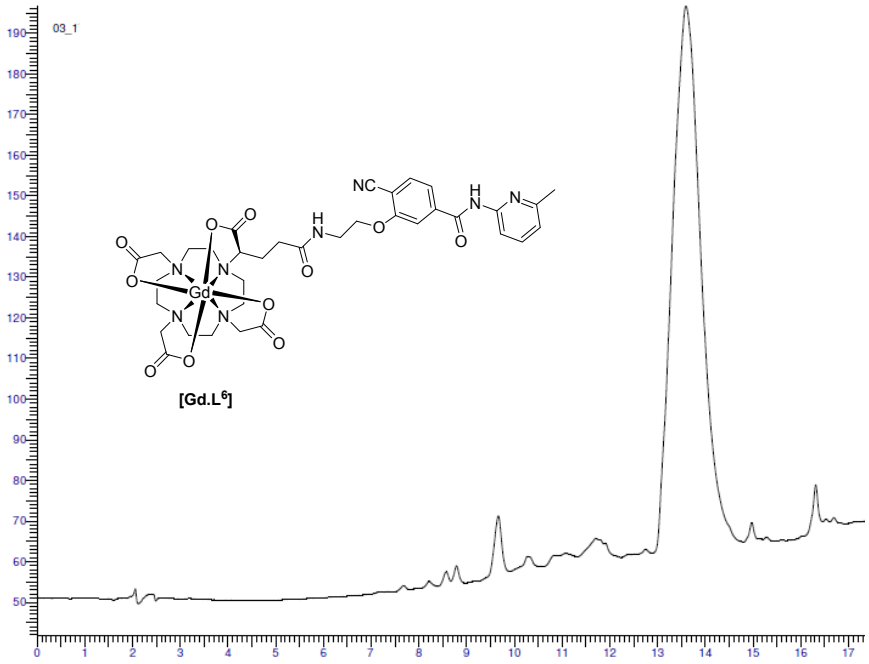


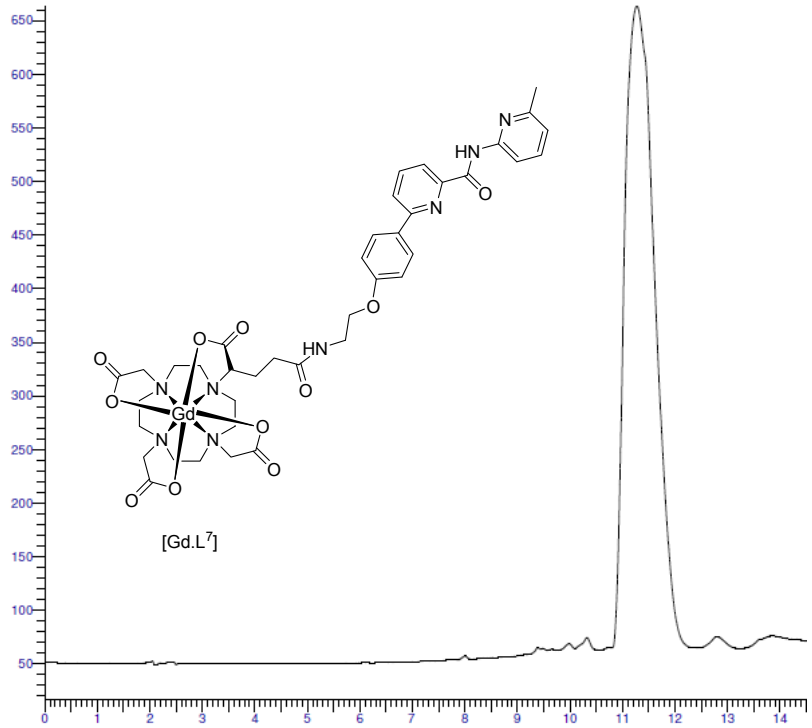




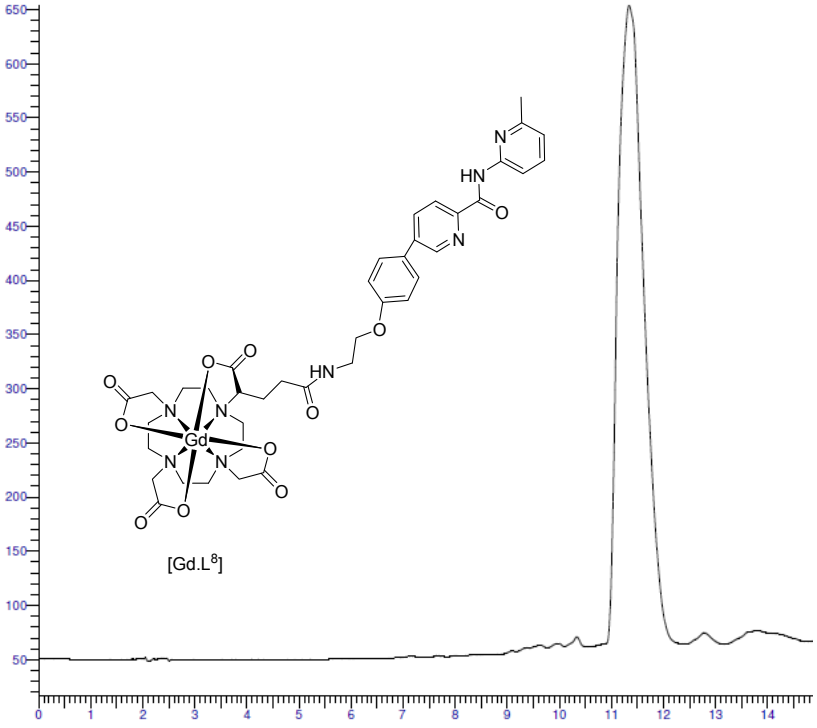



[Gd.L5]


03_1
[Gd.L6]



[Gd.L^{7}]



[Gd.L^{8}]

References

[1] L. Porres, A. Holland, L. O. Palsson, A. P. Monkman, C. Kemp, A. Beeby, *J Fluoresc* **2006**, *16*, 267.

[2] S. Gottschalk, C. Zwingmann, *J Neurochem* **2009**, *109 Suppl 1*, 258.

[3] W. Su, R. Mishra, J. Pfeuffer, K. H. Wiesmuller, K. Ugurbil, J. Engelmann, *Contrast Media Mol Imaging* **2007**, *2*, 42.

[4] K.B. Hansen, H. Bräuner-Osborne, *Methods Mol Biol* 2009, *552*, 269