Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Selective Delivery of Remarkably High Levels of Gadolinium to Tumour Cells Using an Arsonium Salt

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Experimental

General

All reactions were performed under a dry nitrogen atmosphere using conventional Schlenk techniques.[1]

Materials and Methods

Distilled water was used for all experiments requiring water. PhMe was dried over sodium wire and freshly distilled prior to use, according to the procedure by Armarego and Chai.[2] Et_2O was dried over sodium wire and freshly distilled. All other solvents were used without further purification.

All precursor chemicals were purchased from Sigma Aldrich (US).

Preparative HPLC Method

Preparative HPLC was performed on a Waters 600 HPLC system with a Waters 486 tunable absorbance UV/vis detector (λ = 254 nm) and a Sunfire C18 preparative column (19 × 150 mm, 5 μm pore size). Flow rate 7 mL/min. HPLC was performed under gradient flow conditions, starting with 100% Solvent A (Milli-Q water with 0.1% trifluoroacetic acid) and 0% Solvent B (Liquid chromatography-grade acetonitrile with 0.1% trifluoroacetic acid) and moving to 0% Solvent A and 100% Solvent B over 45 min.

Analytical HPLC Method

Analytical HPLC was performed on a Waters 2965 separation module HPLC system with a Waters 2996 photodiode array (PDA) detector (λ = 300 to 200 nm) and a Sunfire C18 analytical column (2.1 × 150 mm, 5 μm pore size). Flow rate 0.2 mL/min. HPLC was performed under gradient flow conditions, starting with 100% Solvent A (Milli-Q water with 0.1% trifluoroacetic acid) and 0%

Solvent B (liquid chromatography-grade acetonitrile with 0.1% trifluoroacetic acid) and moving to 0% Solvent A and 100% Solvent B over 45 min.

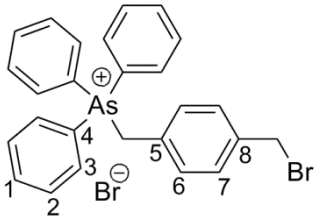
Instrumentation

All ^{1}H, $^{1}H\{^{31}P\}$, $^{13}C\{^{1}H\}$, $^{19}F\{^{1}H\}$ and $^{31}P\{^{1}H\}$ NMR spectra were recorded at 300 K on a Bruker Avance300 spectrometer (^{1}H at 300 MHz, ^{13}C at 75 MHz, ^{19}F at 282 MHz, ^{31}P at 121 MHz). All NMR signals (δ) are reported in ppm. ^{1}H, $^{1}H\{^{31}P\}$ and $^{13}C\{^{1}H\}$ NMR spectra were referenced according to their solvent residual peaks. $^{31}P\{^{1}H\}$ NMR spectra were referenced to external $P(OMe)_3$ at 140.85 ppm. IR spectra were recorded on a Bruker FT-IR Tensor 27 with a KBr background. Melting points were recorded on an MPA161 Digital Melting Point Apparatus and are uncorrected. Low resolution ESI-MS were recorded on a Finnigan LCQ mass spectrometer. High resolution ESI-FTICR-MS data were recorded on a Bruker 7.0T mass spectrometer.

Syntheses

DO3A-*t*Bu$_3$·HBr was prepared as described by Moore.[3] The Gd(III)-triphenylphosphonium complex **2** was prepared as described by Morrison *et al*.[4]

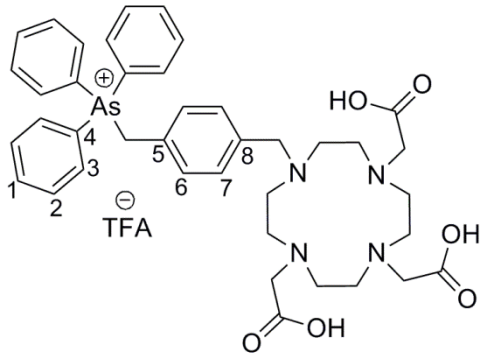
(4-(Bromomethyl)benzyl)triphenylarsonium bromide (3)



Triphenylarsine (0.938 g, 3.06 mmol) and α,α'-dibromo-*p*-xylene (0.889 g, 3.34 mmol) was stirred in nitromethane (8 mL) at 60°C for 1 h. The mixture was then stirred at RT for a further 19 h. The product was filtered off, washed with acetone (10 mL) and dried *in vacuo* to yield **3** as a colourless solid. Yield 1.28 g (73.4%). ^{1}H NMR ($CDCl_3$): δ 7.68-7.61 (m, 15H, H1-H3), 7.27 (d, 2H, $^{3}J_{HH}$ =

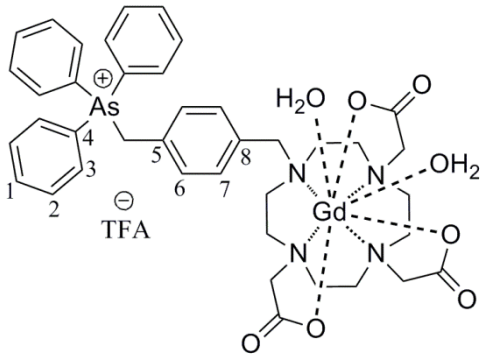
7.45 Hz, H6), 7.15 (d, 2H, $^{3}J_{HH}$ = 6.55 Hz, H7), 5.56 (s, 2H, $AsCH_2$), 4.39 (s, 2H, $BrCH_2$). $^{13}C\{^{1}H\}$ NMR: δ 139.5 (s, H8), 134.1 (s, H1), 132.9 (s, H3), 131.1 (s, H6), 130.5 (s, H2), 129.6 (s, H7), 128.5 (s, H5), 31.6 (s, Br-CH_2), 30.8 (s, As-CH_2). ESI-MS (MeOH): *m/z* = 488.87 ($[M - Br]^+$).

Triphenyl(4-((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)benzyl)arsonium trifluoroacetate (4)



Compound **3** (0.58 g, 1.0 mmol), DO3A-$^{t}Bu_3$·HBr (0.6 g, 1.0 mmol) and Na_2CO_3 (0.11 g, 1.0 mmol) was stirred at reflux in acetonitrile (20 mL) for 19 h. The white precipitate was filtered off and the filtrate was reduced *in vacuo* to yield the ^{t}Bu-protected ligand as an off-white solid. The protected ligand was dissolved in 20 mL of 50% trifluoroacetic acid/CH_2Cl_2 mixture and stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the crude residue was extracted with $CHCl_3$ (3 × 50 mL). The aqueous layer was reduced *in vacuo* to yield a colourless solid. Reverse-phase preparative HPLC was used to purify the product. Yield 0.44 g (52.5%). T_R = 18.5 min. ^{1}H NMR ($CDCl_3$): δ 7.86-7.10 (m, 19H, Ph, H1-H3, H6, H7), 3.45 (s, 2H, $AsCH_2$), 2.35-2.13 (br m, 24H, CH_2). $^{19}F\{^{1}H\}$ NMR (D_2O): δ -75.90 (s). ESI-FTICR-MS for $[M - CF_3CO_2]^+$: Calculated *m/z*. 755.27843; Found 755.27819.

2,2',2''-(10-(4-((Triphenylarsonio)methyl)benzyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetatogadolinium(III) trifluoroacetate (1)



The arsonium ligand **4** (99 mg, 0.114 mmol) was stirred with a suspension of gadolinium(III) oxide (40 mg, 0.110 mmol; 1 eq) in water (10 mL) at 60°C for 16 h. Excess unreacted and insoluble gadolinium(III) oxide was then removed by means of centrifugation and the filtrate was reduced *in vacuo* to yield a colourless solid. HPLC fractions were collected at 18 min to afford the desired product. Yield 94 mg (80.7%). T_R = 18 min. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1681 (C=O, TFA), 1593 (C=O, DO3A). M.p. > 260°C (dec.). ESI-FT-ICR-MS for $[M - CF_3CO_2]^+$: Calculated *m/z* 910.18037; Found 910.17989.

Computational Methods

DFT calculations were carried out with the Q-Chem package,[5] using either Spartan'18,[6] which interfaces with Q-Chem 5.1,[7] or directly using Q-Chem 5.3.[8] Gas-phase optimised geometries of $\mathbf{5}^+$ and $\mathbf{6}^+$ were calculated using the B3LYP hybrid functional[9,10] together with Grimme's DFT-D3 empirical dispersion corrections,[11] and the def2-SV(P) basis set.[12] Frequency calculations confirmed the structures were local minima (absence of imaginary frequencies). Wavefunctions for further analysis were generated through single point calculations using B3LYP with the def2-TZVPD[12] basis set. All DFT calculations employed an unpruned EML (75,302) quadrature formula. Generation and analysis of electrostatic potential maps was carried out with Spartan'18, while electrostatic potential isosurfaces (Fig. 2, main manuscript) were generated and illustrated using AIMAll.[13] NPA charges

were calculated within Q-Chem 5.3, which interfaces with NBO 5.0.[14] IEF-PCM calculations[15–17] were carried out with Q-Chem 5.3 using unscaled Bondi van der Waals radii and 302 Lebedev grid points on all atoms.

Biological Methods

General

Two different cell lines were used for the biological studies reported in this work. The first cell line was T98G (human glioblastoma multiforme). The second cell line used was SVG p12 (human glial cells). Both cell lines were grown as monolayers in Eagle's minimal essential medium (EMEM) supplemented with foetal bovine serum (FBS) (10% *v/v*), L-glutamine (2.5 mM), and an antibiotic antimycotic solution (AA) which contained penicillin (10,000 units/mL), streptomycin (10 mg/mL) and amphotericin B (25 μg/mL). Incubation for all biological studies was performed at 37°C in a humidified 5% CO_2 atmosphere. Cells were grown to >80% confluency before they were harvested. Cell counting was performed on a Countess® Automated Cell Counter from Life Technologies. Centrifugation was performed at 2000 rpm for 3 min.

Cytotoxicity Assays

Cytotoxicity assays were performed for Gd(III) complexes **1** and **2** using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[18] T98G cells were harvested with trypsin (0.1% *v/v*) from a 75-cm^2 flask and pelleted by means of centrifugation. The cells were resuspended in complete EMEM and counted using a hemocytometer (Weber). The cells were seeded with complete EMEM into a 96-well plate such that each well contained 100 μL and 1×10^4 cells. The plate was incubated overnight to allow the cells to adhere to the wells. The plate was then dosed with the selected Gd(III) complex by means of serial dilutions with maximum concentration (C_{max}) 4 mM (N = 4). Each plate included a vehicle control, V_C, (MQ water and EMEM), a positive control, C_{pos}, (cells and EMEM) and a negative control, C_{neg}, (Gd(III) complex and EMEM). The dosed plate was

incubated for 72 h. The MTT solution in PBS (1.7 mg/mL) was added to each well (30 μL) and a further 4 hours of incubation followed. The solution was removed from each well before DMSO (150 μL) was added to dissolve the MTT-formazan crystals. Cell viability was assessed by measuring the absorbance at 600 nm using a Victor3V microplate reader (PerkinElmer). Absorbance measurements were normalised to the C_{pos} wells such that the level of MTT was expressed as % viability according to these wells. A plot of log concentration *versus* % viability was generated by using GraphPad Prism®. IC_{50} values were determined as the concentration of compound required to cause a 50% decrease in cell viability. The standard errors of the IC_{50} values were also reported.

Cell Uptake Studies

Three concentrations of complexes **1** and **2** (10 μL, 100 μL and 1000 μL) were assessed for their *in vitro* uptake by the T98G and SVG p12 cell lines. Three separate solutions of each Gd(III) complex were made up to the necessary concentrations using MQ water. Three individual repeats of each concentration as well as control experiments were conducted on each cell line.

T98G cells were cultured in 25-cm^3 flasks for 3 days to reach confluence. For the dosed flasks, the medium was replaced with dosed medium and then the flasks were incubated for a further 48 h. For the control flasks, the medium was replaced by fresh medium with the appropriate volume of MQ water to afford a vehicle control and incubated for a further 48 h. Medium was then removed and the cells were washed with PBS to remove cell debris. The cells were harvested with trypsin (0.1% *v/v*) and then pelleted and resuspended in PBS (1 mL) twice. A 100 μL aliquot was taken out and set aside for protein analysis. Another 100 μL aliquot was isolated for cell counting. The remaining 0.8 mL cell suspension was centrifuged to afford a cell pellet which was analysed for Gd content by means of ICP-MS.

The above procedure was repeated for the Gd uptake study using the SVG p12 cell line.

The cell pellets were digested in HNO_3 (0.5 mL, 69%) at 65% in a water bath for 18 h. The digest was diluted to 10 mL with HCl (0.1 M), and then measured for Gd by means of ICP-MS. ICP-MS was run on a PerkinElmer ELAN 6100 Inductively Coupled Plasma Emission Mass Spectrometer (ICP-MS) at the Solid State and Elemental Analysis Unit (UNSW Analytical Centre) by Ms Dorothy Yu. Metal uptake concentrations are reported in ng Gd/mg protein and Gd atoms/cell.

Protein Analysis

The protein content of cell solutions was determined using a bicinchoninic acid (BCA) protein assay, which has been described previously.[19] This assay involves the reduction of Cu(II) to Cu(I) by proteins and the subsequent chelation of Cu(I) by two molecules of bicinchoninic acid which results in a colour change that can be measured and compared to a protein standard curve. To perform the assay, the cells were lysed by means of three snap freeze-thaw cycles which released the intracellular contents. The solution was analysed for protein content by taking repeated 25 μL samples (N = 3) and depositing them into a 96-well plate format. A 1 mg/mL bovine serum albumin (BSA) protein standard (200, 400, 800 and 1000 μg/mL, made up to volume with MQ water) was also deposited into the 96-well plate. A freshly prepared solution of BCA and $CuSO_4 \cdot 5H_2O$ (50:1, 200 μL) was added to each well and the plate was incubated at 37°C for 30 min. Absorbance was then measured at 600 nm using a victor3V microplate reader (PerkinElmer). The protein standard curve was fitted using linear regression which allowed for the determination of the protein content in unknown samples. Microsoft Office Excel® 2007 was used for all calculations and graphs.

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