

Supporting Information

Gadolinium-based MRI Contrast Agent for the Detection of Tyrosinase

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Experimental Section

General Methods

Unless otherwise noted, reagents were obtained from Sigma-Aldrich or Fisher Scientific, and used as received. 1,4,7,10-Tetraazacyclododecane-1,4,7-tris(*t*-butyl acetate) was obtained from Macrocyclics. Mushroom Tyrosinase (Sigma cat T 3824) was procured from Aldrich. 3,4-Dihydroxybenzyl Alcohol was purchased from TCI America. Dry solvents were obtained from Aldrich as anhydrous Sure-Seal bottles. Phosphate buffer (pH 7.4) was purchased from Gibco by Life Technology, and filtered before use. The concentration of tyrosinase stock solution (15–20 mg/ml) was calculated by measuring absorbance at 280 nm in pH 7.4 PBS ($E_{1\%}^{1\text{cm}} = 24.9 \pm 0.3$).¹ **Tyr-GBCA 1** (50 mM) stock solution was freshly prepared in DMSO before use. Artificial cerebral spinal fluid (119 mM NaCl, 26.2 mM NaHCO₃, 2.5 mM KCl, 1 mM NaH₂PO₄, 1.3 mM MgCl₂, 10 mM glucose, 2.5 mM CaCl₂) was made by reported procedure.² NMR spectra were obtained on a Varian NMR system, operating at 400 MHz and 500 MHz NMR, and the spectra were analyzed using the Bruker Topspin 3.6.2. NMR processing software. Absorbance spectroscopy was performed on a spectrophotometer (NanoDrop™ 2000, Thermo Scientific™). Purities of compounds were monitored by reverse phase column (PRP-1 Reversed-Phase HPLC Column, 4.1 x 250 mm, 10 μm) via HPLC (Agilent Technology 1260 Infinity II).

Magnetic Resonance Experiment

T_1 relaxation time was measured with a 1.4 T Bruker minispec mq60 NMR analyzer (60 MHz, Bruker Inc., Billerica, MA) at 37°C. The gain was set to 55 db, and T_1 delays of 5-7000 ms were used. Of three separate T_1 measurements, the last two were averaged to stabilize the temperature effect. Longitudinal relaxation times (T_1) were measured in four samples (0.1, 0.2, 0.4, 0.5 mM) that contained various concentrations of **Tyr-GBCA 1**. Relaxivity was obtained by fitting the data to the equation: $1/T_1 = r_1[\text{Tyr-GBCA 1}] + a$.

Electrospray Ionization Experiment

Samples were diluted either 1:10 or 1:20 with 50% methanol or acetonitrile/0.1% formic acid.

MS data was acquired on an LTQ Orbitrap XL (Thermo Fisher Scientific, San Jose, CA) by direct infusion using the integrated syringe pump at a rate of 5 μ L/min. The sample was ionized by electrospray ionization using a Heated Electrospray source with the following settings: Spray voltage (kV) 3.5; Sheath gas (L/min): 2; Capillary Temperature ($^{\circ}$ C): 275; Capillary voltage (V): 35; Tube lens (V): 95; Resolution in the FTICR MS: 7.5-30K.

3-((tert-butyldimethylsilyloxy)benzyl (2-bromoethyl)carbamate (5): Compound **3** (1 g, 4.19 mmol) and DMAP (102 mg, 0.84 mmol) in 30 ml CH_2Cl_2 were subjected to 1.36 g (8.39 mmol) 1,1'-carbonyl-diimidazole. After 1 h, the solution was washed with water, sat. NaHCO_3 , and brine. The organic layer was dried with MgSO_4 and concentrated in vacuo to yield 1.3 g of the clear liquid **4**. The crude dissolved in 20 ml anhydrous CH_2Cl_2 under N_2 and cooled to 0 $^{\circ}$ C. 0.48 ml (4.27 mmol) of MeOTf was added over 10 min. After 30 min, the reaction was diluted to 50 ml with Et_2O and cooled to -20 $^{\circ}$ C to precipitate methylated product. The white solid was collected by filtration, washed with Et_2O and dried in vacuo. The activated compound was dissolved in 20 ml CH_2Cl_2 and 1.20 g (10.85 mmol) of 2-bromoethylamine hydrobromide was added. The slurry was brought to 0 $^{\circ}$ C and 0.80 ml (5.86 mmol) of TEA was added. The reaction was stirred for 2 h and then washed with water and brine. The organic layer was dried (MgSO_4), concentrated in vacuo and purified by silica gel chromatography (silica, 20% EtOAc /Hexane) to give 1.2 g (75 %) **5** as a clear liquid. ^1H NMR (500 MHz, CDCl_3) δ 7.21 (t, 1H, $J=7.8$ Hz), 6.95-6.93 (d, 1H, $J=7.6$ Hz), 6.83 (s, 1H), 6.80-6.79 (d, 1H, $J=8.05$ Hz), 5.17 (br s, 1H, NH), 5.06 (s, 2H), 3.63-3.60 (m, 2H), 3.49-3.47 (t, 2H, $J=5.5$ Hz), 0.99 (s, 9H), 0.20(s, 6H); ^{13}C NMR (125 MHz, CDCl_3) δ 156.22, 155.75, 137.84, 129.51, 120.89, 119.75, 119.69,66.61, 42.76, 32.19, 25.68, 18.16, -4.39; ESI-MS m/z: $[\text{M}+\text{Na}]^+$ 410.07.

tri-tert-butyl 2,2',2''-(10-(2-(((3-hydroxybenzyl)oxy)carbonyl)amino)ethyl)-1,4,7,10-tetraazacyclodecane-1,4,7-triyl)triacetate (6):

To the mixture of 1,4,7,10-tetraazacyclododecane-1,4,7-tris(*tert*-butyl acetate) (1.0 g, 1.94 mmol) and K_2CO_3 (805 mg, 5.82 mmol) in 40 mL of acetonitrile, compound **5** (830 mg, 2.14 mmol) in 20 mL of acetonitrile was added dropwise. The resulting solution was refluxed for 8 hours. After the reaction was complete, the K_2CO_3 was filtered, and the solution was concentrated in vacuo.

Polar impurities were purified by column chromatography (silica, 5% MeOH/CH₂Cl₂). Resulting white solid mixture 610 mg was directly used for the next step.

2,2',2''-(10-(2-(((3-hydroxybenzyl)oxy)carbonyl)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acetic acid (7):

The crude compound **6** (610 mg) was stirred in 4 mL of trifluoroacetic acid and 4 mL of dichloromethane at room temperature. Overnight, then the solvent was removed in vacuo. The dried liquid mixture was treated with 20 mL of Et₂O, resulting in a white solid precipitate. The solid was filtered and purified by reverse phase silica gel column chromatography with 5% acetonitrile/ water. The fractions were collected, and the product was monitored by absorbance spectrum and HPLC. Compound **8** was obtained (348 mg, two steps yield 33%) as white solid. ¹H NMR (500 MHz, D₂O) 7.15(t, 1H), 6.80(s, 1H), 6.80-6.78(d, 1H), 6.74-6.73(d, 1H), 5.02(s, 2H), 3.68(br s, 6H), 3.44(br s, 2H), 3.23(br s, 18H) ¹³C NMR (106 MHz, D₂O) 162.91, 162.56, 157.87, 155.82, 138.41, 130.43, 130.27, 119.60, 115.20, 114.89, 114.35, 66.61, 54.81, 52.21, 49.79, 49.53; ESI-MS m/z: [M-H]⁻ 538.25.

Preparation of Gd Complex, **Tyr-GBCA 1**: Gd complex of ligand **7** was prepared from respective solution of the ligand (50mg, 0.093 mmol) in 1 mL of water and solution of GdCl₃·6H₂O (51.7 mg, 0.139 mmol) in 1 mL water. The reaction mixture was stirred at 40 °C for 48 h. The pH was periodically checked and adjusted to 5.0–6.0 using a solution of NaOH (0.1 M), and the generated precipitate was fully dissolved by adding 1 mL of methanol. After 2 days, the pH of solution brought to over pH 8. Precipitation of Gd(OH)₃ was filtered and the pH of the solution brought to pH 7 by adding 0.1 M HCl. After adjusting pH, the solvent was evaporated in vacuo. The crude mixture was further purified by reverse phase silica gel column chromatography with 10% acetonitrile in water. Collected fractions were monitored by absorbance spectrum and HPLC at 280 nm, and the product fractions were collected. **Tyr-GBCA 1** was obtained as white solid (45 mg, 70%); ESI-MS m/z: [M+Na]⁺ 717.15.

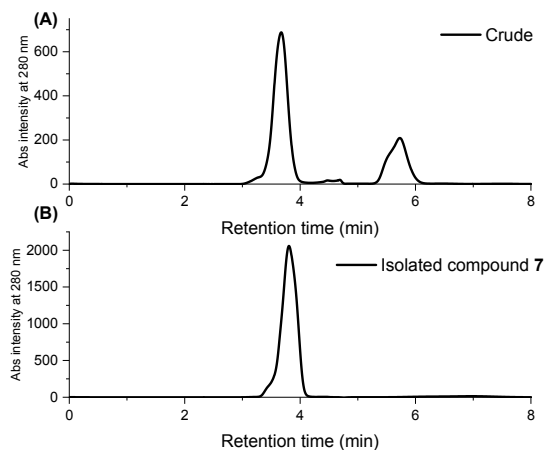


Figure S1. Chromatograms of the compound 7 (A) before and (B) after purification by reverse phase silica gel column chromatography. Detection wavelength: 280 nm, flow rate of mobile phase: 0.5 mL/min. Isocratic elution: 30% acetonitrile/water

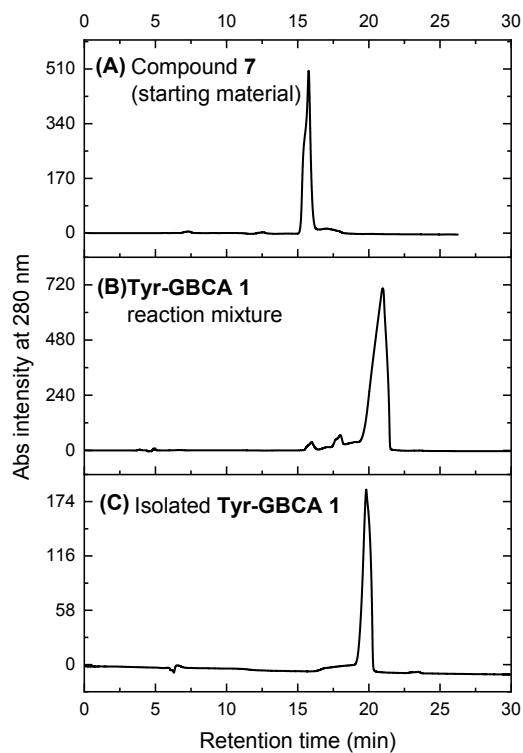


Figure S2. Chromatograms of the Gd-complexation. (A) Starting material (compound 7); (B) reaction crude; (C) after purification by reverse phase silica gel column chromatography. Detection wavelength: 280 nm, flow rate of mobile phase: 0.5 mL/min. Gradient elution: Acetonitrile/water (5% to 40%) over 30 min.

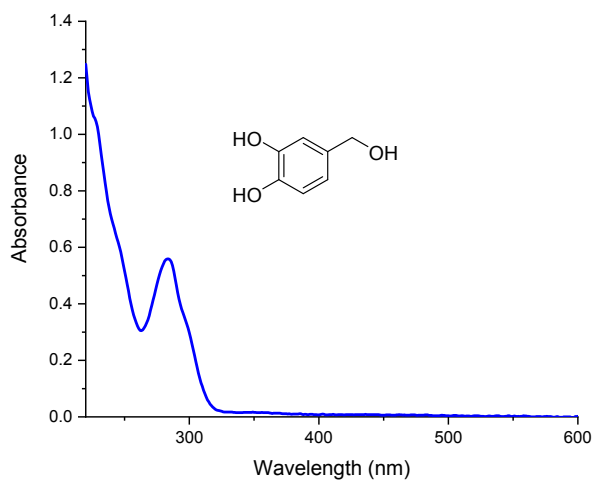


Figure S3. Absorbance of 3,4-dihydroxybenzyl alcohol (0.2 mM) in ACSF.

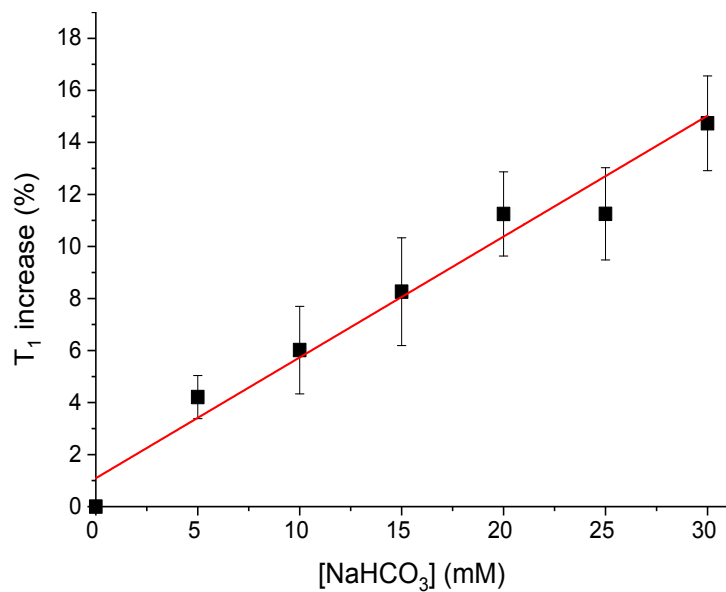


Figure S4. T_1 change (%) as a function of carbonate concentration. **Gd-GBCA 1** (0.5 mM in MOPS, pH 7.4, 1.0% DMSO) was treated with various concentrations of sodium bicarbonate. Error bars on data are the standard deviation of three independent measurements.

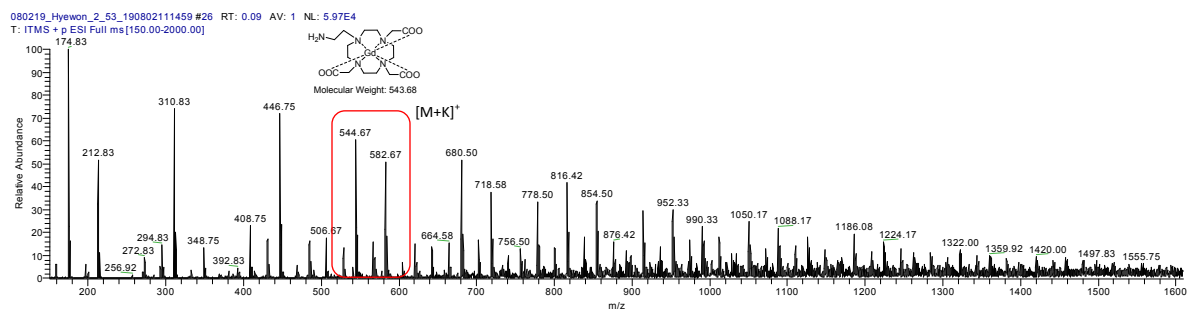
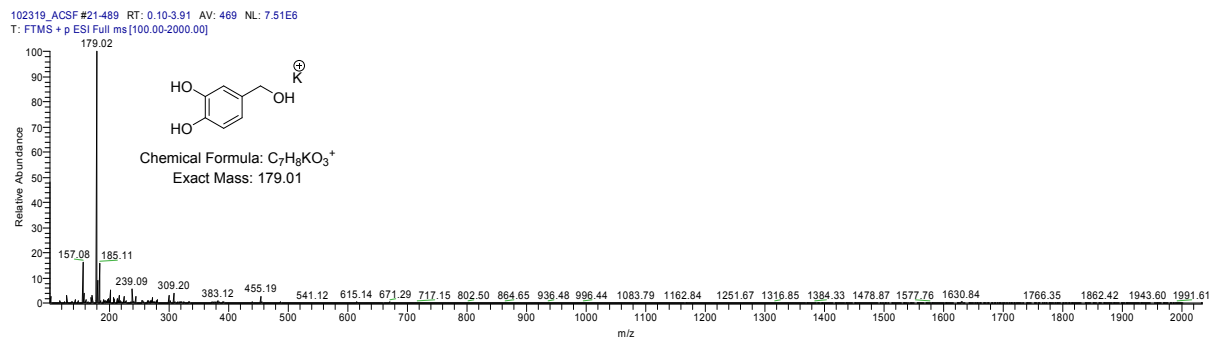


Figure S5. (A) Tyr-GBCA 1(0.2 mM) with 0.1 mg/mL of tyrosinase in pH 7.4 ACSF. (B) Tyr-GBCA 1 (0.2 mM) with 0.1 mg/mL of tyrosinase in pH 6.5 potassium phosphate buffer. All spectra measured after incubation of 2 h.

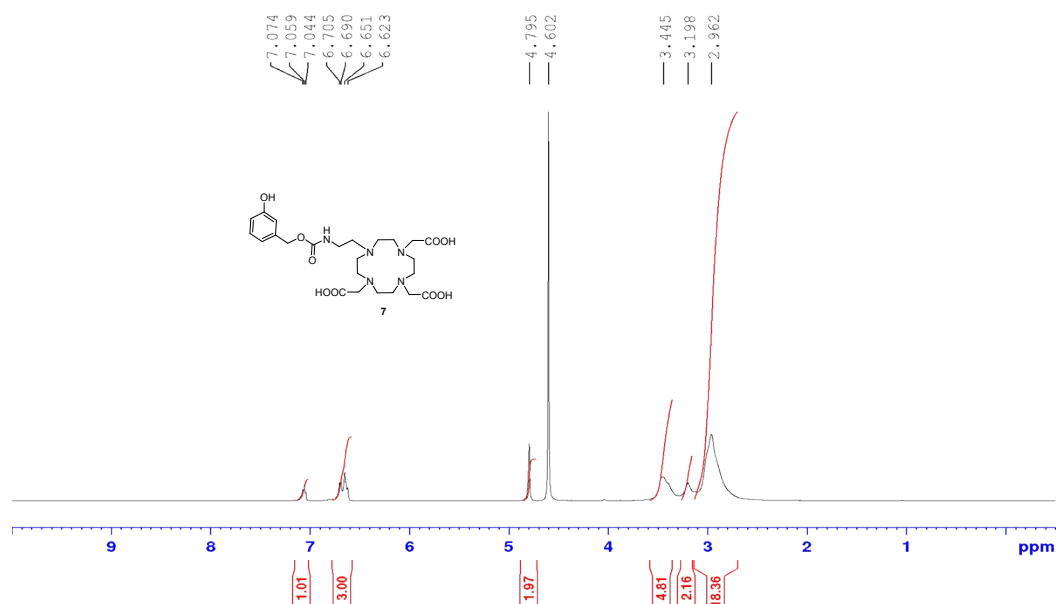


Figure S6. 1H NMR of ligand 7 in D_2O

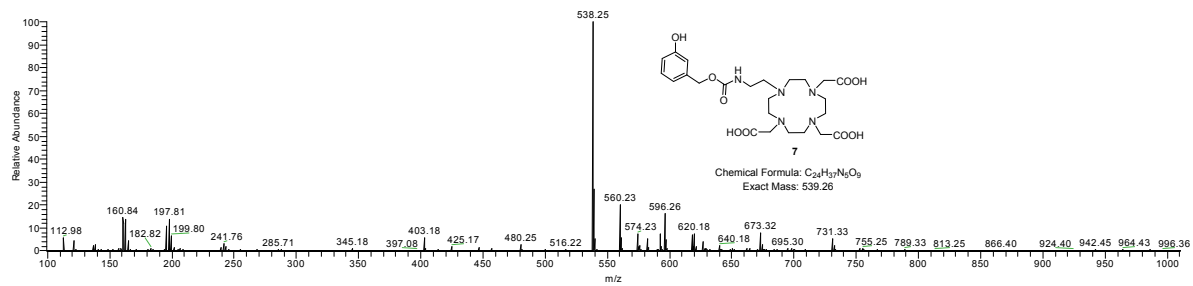


Figure S7. ESI-MS of ligand **7**

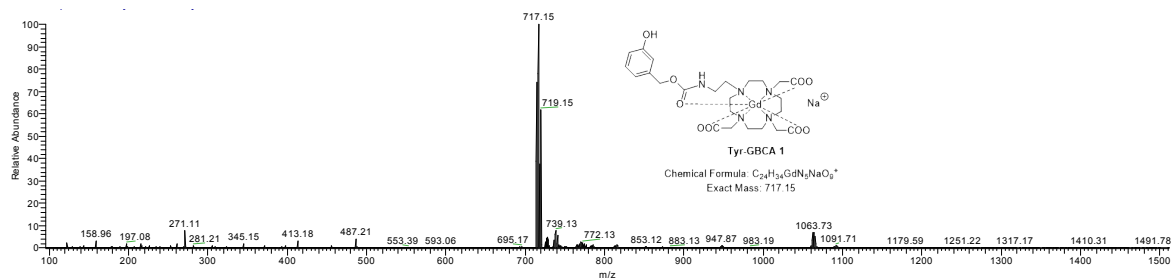


Figure S8. ESI-MS of Tyr-GBCA **1**

1. H. W. Duckworth and J. E. Coleman, *Journal of Biological Chemistry*, 1970, **245**, 1613-1625.
2. *Cold Spring Harbor Protocols*, 2011, **2011**, pdb.rec065730.