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ORIGINAL ARTICLE

Synthesis, binding affinity, and relaxivity of target-specific MRI contrast agents

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Abstract Although magnetic resonance imaging (MRI) is one of the most important imaging modalities of the central nervous system (CNS), one of the main drawbacks of MRI is its limited specificity. This can potentially be partially alleviated by target-specific contrast agents. In the present paper we describe a simple high yield synthesis of two such gadolinium-based spiperone targeted MRI contrast agents, **1a** and **1b**. The R_1 relaxivities of **1a** and **1b** were evaluated and found to be 5.94 and 8.31 mM^{-1} s^{-1}, respectively at 9.4T, while their R_2 relaxivities at the same magnetic field were found to be 18.05 and 22.60 mM^{-1} s^{-1}, respectively. In addition and very importantly compound **1a,** which is a gadolinium-based, spiperone-targeted MRI contrast agent, was found to preserve some of the spiperone affinity toward the dopamine D2 receptor. Compounds **1a** and **1b** thus represent potential agents for in vitro dopamine receptor imaging using MRI in experimental models.

Keywords Dopamine receptor · Gadolinium complexes · MRI contrast agents · MR molecular imaging · Targeted contrast agents

Introduction

Magnetic resonance imaging (MRI) is one of the most important imaging modalities of the central nervous system (CNS) [1]. This is partially due to the non-invasiveness of the technique, its relatively high temporal and spatial resolution, and the fact that several physical parameters of water can be used to construct MR images. An additional important characteristic of MRI is the nearly unlimited tissue penetration of the MRI technique. However, since MRI images water protons, one of the major drawbacks of MRI is its limited specificity. This can, in principle, be partially alleviated by the use of contrast agents with specific tissue distributions and indeed many such contrast-agents were prepared in the last two decades [2]. These MRI contrast-agents were mostly intravascular agents [2]. A more rewarding and challenging approach to increase MRI specificity is to design target-specific MRI contrast agents [3] with a high affinity to specific molecular species (enzymes, receptors etc.,), thus expanding the scope of MRI into the field of molecular imaging [4]. Such an approach may, in principle, open the way for indirect MR imaging of specific enzymes and receptors. To date, molecular imaging using non-MRI methods is much more developed [4, 5] mainly due to sensitivity issues. However, molecular imaging based on fluorescence and bioluminescence techniques suffers from low tissues penetration, and are inadequate for imaging of internal organs and tissues [4, 5]. Another technique for molecular imaging is positron emission tomography (PET) [4, 5]. This method has high

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sensitivity and a good tissues penetration; however it uses radiolabeled compounds, which are harmful both for the patient and the environment. In addition PET uses labeled compounds having a relatively short half-life. Therefore there is an increasing need to develop target-specific contrast agents, which will allow performing molecular imaging based on MRI technology. Indeed in recent years a few elegant demonstrations of the feasibility of this approach, using MRI methodology, were reported [6]. These include, *inter alia*, an elegant example for indirect MR imaging of gene expressions [6], and imaging of the engineered version of the transferring receptor on the surface of 9L glioma cells [6]. Very recently, a new, transgene reporter for in vivo MRI, was reported [6].

In the present paper we describe a simple, high-yield, modular synthesis of target-specific MRI contrast agents, their relaxivity as well as their binding affinity to the dopamine D2 receptor. We selected the D2 dopamine receptor since the dopamine system is believed to be relevant to many neurological diseases, and due to the relative high concentration of this receptor and its uneven distribution in the brain [7].

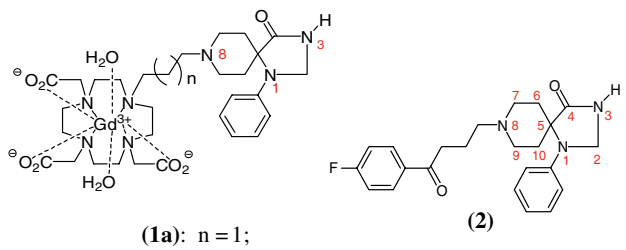
Results and discussion

Synthesis and characterization

We decided to undertake the synthesis of complexes **1a** and **1b** (Scheme 1), based on the structure of (8-[3-(*p*-Fluorobenzoyl)propyl]-1-phenyl-1,3,8-triazaspiro[4,5]-decan-4-one, known also as spiperone (**2** in Scheme 1), a strong ligand of the dopamine D2 receptor [8].

Compounds **1a** and **1b** were synthesized from 1-phenyl-1,3,8-triazaspiro-[4,5]-decan-4-one (**3**) in five steps with an overall yield of about 50% according to the synthesis outlined in Scheme 2.

First, compound **3** was reacted with 3-bromopropanol (**4a**) or 8-bromooctanol (**4b**) in DMF under reflux in the presence of a minute amount of K_2CO_3, affording **5a** and **5b** in 93 and 87% yield, respectively. In this case, we could not use an excess of the alkylating agent since it resulted in dialkylation at position 8 and, in some cases, residual alkylation at position 3 was observed when a large excess of **4a** or **4b** were used.



Scheme 1 The structure of **1a**, **1b** and spiperone (**2**)

Many reagents were then tried in order to transform alcohols **5a** and **5b** to the respective bromides and the best reagent was found to be freshly distilled PBr_3 that was used as a reagent and a solvent. The next step was the N-alkylation of the commercially available compound **7** with compound **6a** or **6b**.

The triesters **8a** and **8b** were then hydrolyzed by TFA to give the ligands **9a** and **9b** in nearly quantitative yield. These compounds afforded less-informative ^{1}H NMR spectra because of the very many nearly equivalent protons and dynamic processes, which broaden the line in the ^{1}H NMR spectra (See Figures S20 and S24 in the supporting information). Therefore these complexes were also characterized by high-resolution mass spectrometry (See Figures S19 and S23 in the supporting information). Finally, these compounds were quantitatively converted to their respective gadolinium complexes **1a** and **1b** by a known literature procedure (Scheme 2) [9].

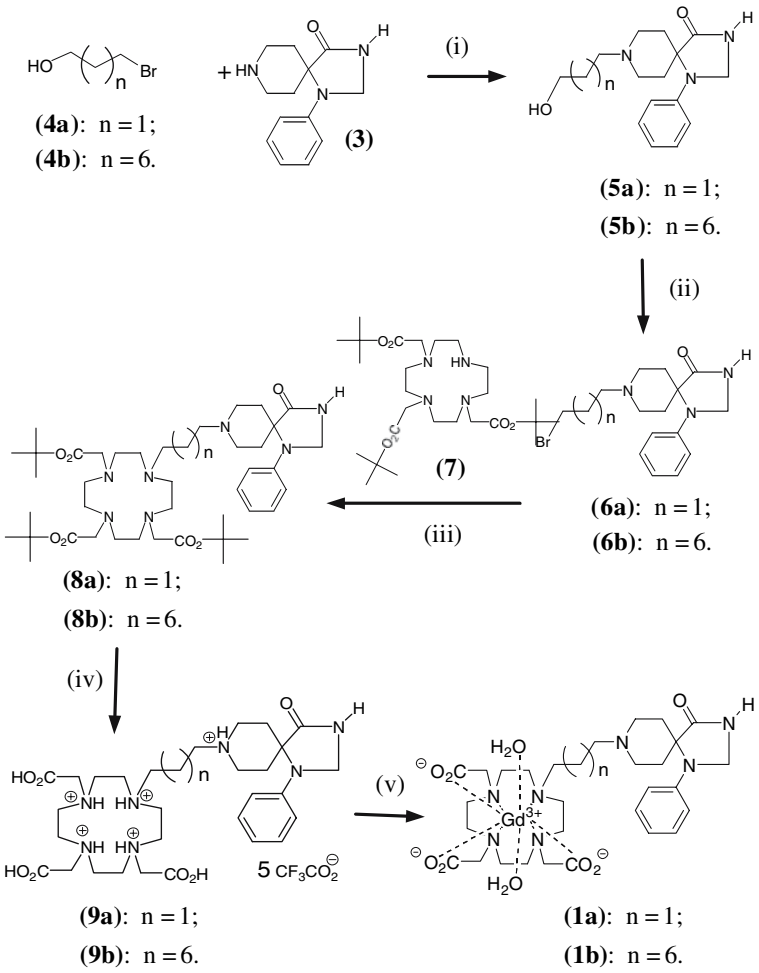
Clear and conclusive evidence for the formation of the paramagnetic complexes **1a** and **1b** was obtained from high-resolution mass spectrometry (HRMS), as shown in Fig. 1. This figure shows, for example, the very good agreement between the calculated and experimental isotope distribution of the molecular peaks of **1a** and **1b**.

Binding and relaxivity of **1a** and **1b**

To have a chance to perform their task, complexes **1a** and **1b** should preserve both their relaxivity and at least some of the spiperone affinity toward the dopamine D2 receptor. Due to the large chemical modification performed we evaluated the binding affinity of **1a** toward the dopamine D2 receptor. To assess the affinity of **1a** to the D2 receptor we performed an assay of [^{3}H]-spiperone binding [5×10^{-10} M] to isolated mouse striatal membranes as shown in Fig. 2 [10]. Complete inhibition was seen at a concentration of 10^{-4} M while 50% inhibition was obtained at a concentration of 10^{-5} M.

These results imply that **1a** bound specifically to the striatal dopamine receptor, although with somewhat lower affinity as compared to spiperone. Despite the relatively large chemical modification performed to convert the system into and MRI active compound, compound **1a** preserves specific binding toward the dopamine D2 receptor. This observation is in line with the fact that recent experimental and computational studies showed that different ligands, having different structures, bind to this class of receptors [11].

Scheme 2 Synthesis of compounds **9a, 9b** and their respective gadolinium complexes **1a, 1b**



(i) K_2CO_3; reflux in DMF for 8 hours; (ii) PBr_3; (iii) dry MeOH / dry $CHCl_3$; (iv) TFA; (v) $GdCl_3$ in H_2O; pH 6-6.5

Figure 3 shows the 300 MHz (7T) T_1-weighted MR images of several NMR tubes containing a 1:1 H_2O:D_2O solution, and such solutions containing different concentrations of **1a** and **1b**, collected at different repetition times (TRs). These images clearly demonstrate that indeed the higher is the concentration of **1a** and **1b**, the higher is the signal intensity of the NMR tube in the T_1-weighted MR images acquired with the short TR.

We also characterized the R_1 and R_2 relaxivity of **1a** and **1b** at 400 MHz (9.4T) by measuring the effect of different concentrations of **1a** and **1b** on the T_1 and T_2 of water. As shown in Fig. 4 we found, as expected, a clear linear dependency of the relaxivity on the concentrations of **1a** and **1b**. From these experiments we extracted the R_1 and R_2 relaxivity of **1a** and **1b**. The R_1 values were found to be 5.94 and 8.31 mM^{-1} s^{-1}, respectively, while the R_2 values were found to be 18.05 and 22.60 mM^{-1} s^{-1} for **1a** and **1b**, respectively. These relaxation studies, performed at 400 MHz (9.4T) show, as expected, that the effect of **1a** and **1b** on water relaxation (T_1 and T_2) is somewhat higher than that of Gadolinium DOTA (1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetic acid).

In addition, it should be noted that at last for **1b**, the intercepts of the regression lines of the R_1 and R_2 relaxivities do not pass through the origin thus suggesting some aggregation of the this system under the used experimental conditions.

Conclusion

In conclusion, we described a simple, yet efficient, modular synthesis of target-specific MRI contrast agents with the potential to be used in indirect MR imaging of the dopamine D2 receptors in animal brains. In vitro MRI animal studies as well as the development of additional target specific MR contrast agents for the dopamine D2 receptors are underway.

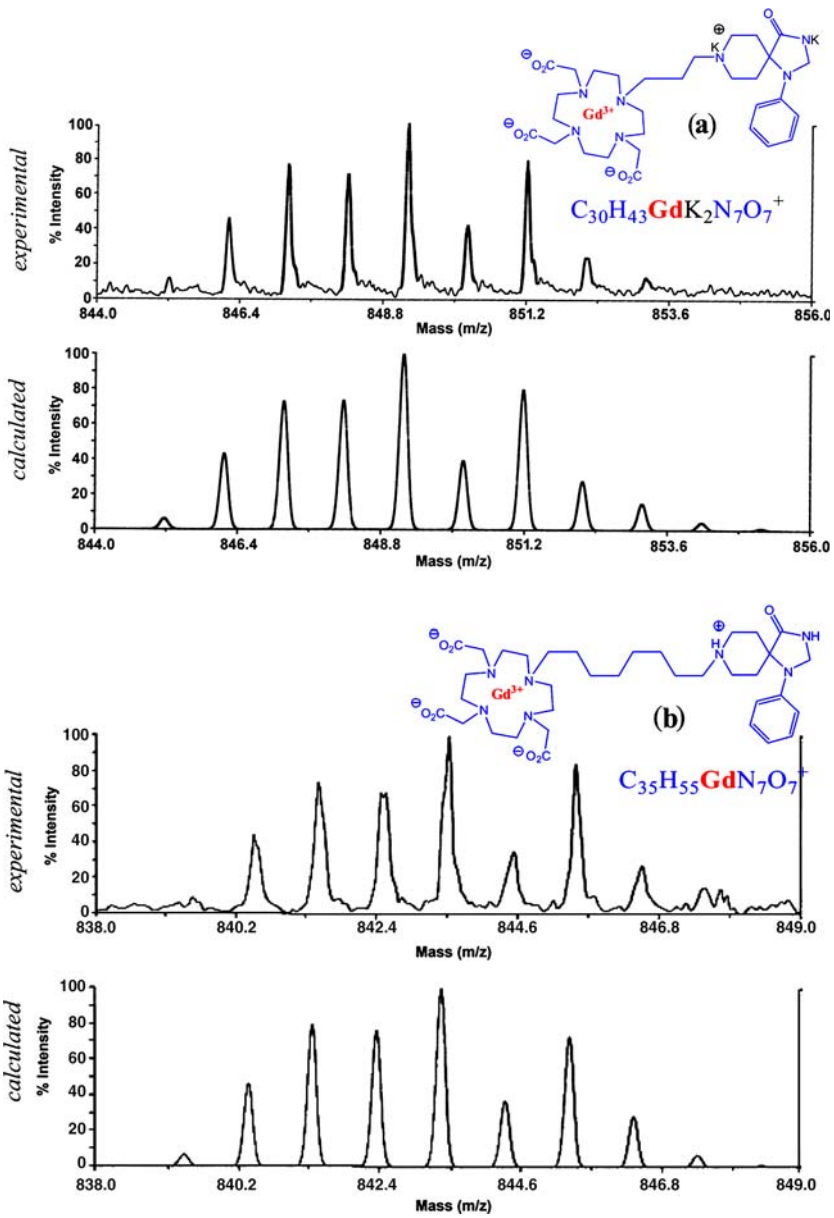


Fig. 1 Calculated and experimental isotope distributions, as obtained from MALDI-TOF mass spectrometry, of the molecular peaks of compounds **1a** and **1b**

Experimental section

General

All reagents (including compound **3**), solvents and deuterated solvents were purchased from Aldrich. Compound **7** was purchased from Macrocyclics. [^{3}H]-Spiperone (91 Ci/mmol) was purchased from Amersham Bio Science UK. Spiperone and sulpiride were purchased from Sigma Chemicals Co. St. Louis, MO, USA. ^{1}H, ^{19}F, and ^{13}C NMR spectra and T_1 and T_2 relaxation experiments were all acquired on a 400 MHz (9.4T) Avance NMR Spectrometer. The T_1-weighted MRI images were collected on a 300 MHz (7T/30 cm) Biospec System equipped with a BG 20 gradient system.

Preparation of **5a** and **5b**

To a solution of **3** (1.00 gr, 4.32 mmol) in dry DMF (30 mL) and K_2CO_3 (0.60 gr, 4.32 mmol), **4a** (780 μL, 4.32 mmol) or **4b** (740 μL, 4.32 mmol) were added under argon. After stirring for 8 h at reflux the reaction mixture was poured onto ice. Thereafter, the aqueous phase was extracted with dichloromethane (3 × 80 mL). The combined organic phase was dried with magnesium sulfate, filtered, and evaporated. The obtained solid was triturated from methanol. A white powdered solid of **5a** was obtained; yield 1.17 gr (93%); mp 198–199 °C; ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ_{ppm} 7.25 (t, J = 7.3 Hz, 2H), 6.70 (d, J = 7.9 Hz, 2H), 6.84 (t, J = 7.3 Hz, 1H), 4.69 (s, 2H), 3.64 (t, J = 6.2 Hz, 2H), 2.81–2.92 (m, 4H), 2.49–

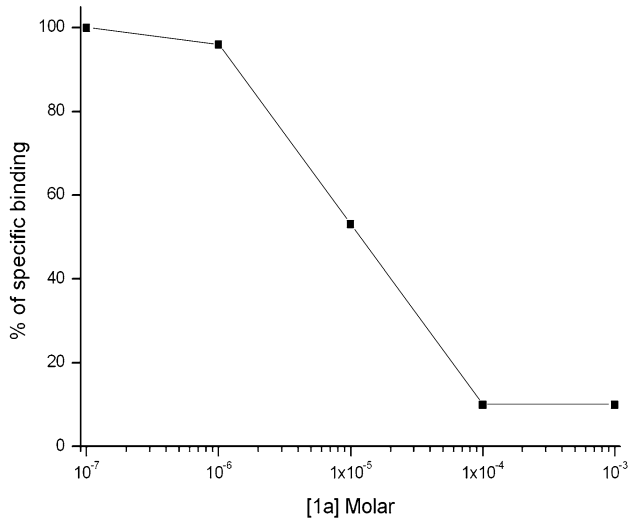


Fig. 2 Inhibiting effect of **1a** on [^{3}H]-spiperone specific binding (5×10^{-10} M) to rat striatal membranes

2.71 (m, 4H), 1.69–1.81 (m, 4H); ^{13}C NMR (100.6 MHz, CD_3OD, 25 °C): δ_{ppm} 179.3, 144.7, 130.1, 120.5, 117.4, 61.9, 60.7, 60.6, 57.0, 50.8, 30.3, 29.9; FAB$^+$-MS *m/z* 290.2 [M+H]$^+$. Compound **5b** (1.35 gr, 87% yield) was obtained as a white solid; mp 155 °C; ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ_{ppm} 7.25 (t, J = 7.3 Hz, 2H), 7.02 (d, J = 7.9 Hz, 2H), 6.84 (t, J = 7.3 Hz, 1H), 4.68 (s, 2H), 3.54 (t, J = 6.4 Hz, 2H), 2.84–2.89 (m, 4H), 2.56–2.71 (m, 2H), 2.38–2.46 (m, 2H), 1.75 (br s, 1H), 1.68 (br s, 1H), 1.56 (m, 4H), 1.36 (m, 8H); ^{13}C NMR (100.6 MHz, CD_3OD, 25 °C): δ_{ppm} 179.3, 144.8, 130.1, 120.5, 117.5, 63.0, 60.8, 60.6, 59.8, 50.7, 33.7, 30.6, 30.5, 29.9, 28.8, 27.7, 26.9; FAB$^+$-MS *m/z* 360.1 [M+H]$^+$.

Preparation of **6a** and **6b**

The solution of **5a** (372 mg; 1.28 mmol) or **5b** (468 mg, 1.30 mmol) in 7 mL of phosphorus tribromide was stirred at 100 °C for 52 h under argon, and then poured onto ice. The pH of the aqueous phase was adjusted to 14 with powdered KOH. After extraction with chloroform (4 × 50 mL), drying with magnesium sulfate, filtering, and evaporation of the chloroform, **6a** was purified on a Sephadex LH20 column (eluent 1:1 methanol/chloroform solution) and afforded 337 mg (75%) of **6a** as a glass-like solid. mp 240 °C; ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ_{ppm} 7.3 (m, 2H), 6.9 (m, 3H), 4.74 (s, 2H), 4.46 (t, J = 8.3 Hz, 2H), 4.33 (t, J = 8.3 Hz, 2H), 4.08 (dt, J = 12.8 Hz, J = 3.5 Hz, 2H), 3.79 (m, 2H), 2.5–2.7 (m, 4H), 1.99 (m, 2H); ^{13}C NMR (100.6 MHz, CD_3OD, 25°C): δ_{ppm} 177.1, 144.1, 130.7, 122.3, 119.0, 65.8, 60.9, 60.6, 58.0, 50.3, 26.4, 14.4; DEI-MS *m/z* 352.1 and 354.1 [M$^+$ with ^{79}Br and ^{81}Br].

Compound **6b** was obtained following chromatography with a preparative silica-gel plate (eluent 5:95 methanol/chloroform solution) as a yellowish solid; yield 422 mg (77%); mp > 245 °C$_{(decomp)}$; ^{1}H NMR (400 MHz, $CDCl_3$ with 5–10% of CD_3OD, 25 °C): δ_{ppm} 7.20 (t, J = 7.5 Hz, 2H), 6.9 (d, J = 8.1 Hz, 2H), 6.78 (t, J = 7.2 Hz, 1H), 4.62 (s, 2H), 3.33 (t, J = 6.8 Hz, 2H), 2.82 (b s, 4H), 2.5–2.7 (m, 2H), 2.3–2.45 (m, 2H), 1.62–1.84 (m, 4H), 1.05–1.55 (m, 12H), ^{13}C NMR (100.6 MHz, $CDCl_3$ with 5–10% of CD_3OD, 25 °C): δ_{ppm} 178.1, 142.9, 129.0, 118.7, 115.2, 59.3, 58.3, 49.4, 33.8, 32.5, 29.1, 28.8, 28.5, 27.9, 27.3, 26.6; FAB$^+$-MS *m/z* 422.1 and 424.1 [M$^+$ with ^{79}Br and ^{81}Br].

Preparation of **8a**

The clear solution of **7** (293 mg; 0.57 mmol) in 1 mL of dry chloroform was added to the solution of **6a** (200 mg; 0.57 mmol) in 5 mL of a dry chloroform/methanol mixture (4:1 v/v). The obtained mixture was stirred at room temperature for 20 h under argon. After chromatographic purification (Sephadex LH20; eluent 1:1 methanol/chloroform solution) a clear glass-like solid was obtained; yield

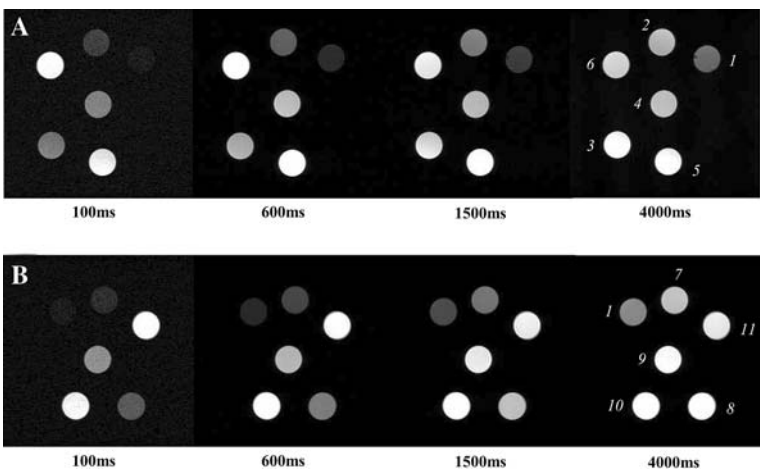


Fig. 3 (300MHz, 7T) T_1-weighted MR images of NMR tubes containing 1:1 H_2O:D_2O solutions without and with different concentrations of **1a** (**A**) and **1b** (**B**). The following concentrations were used: (1) neat, (2, 7) 0.05 mM, (3, 8) 0.13 mM, (4, 9) 0.25 mM, (5, 10) 0.38 mM, (6, 11) 0.5 mM. The different TRs used to collect the T_1-weighted MR images are depicted in the bottom of the MR images

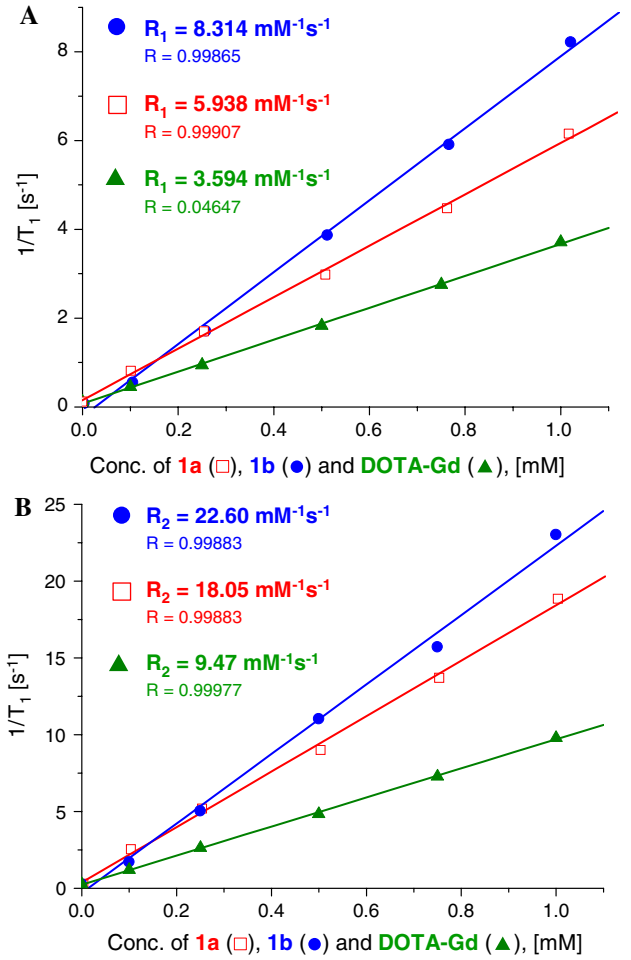


Fig. 4 400 MHz (9.4T) (**A**) longitudinal relaxivity values (R_1) and (**B**) transverse relaxivity values (R_2) of **1a**, **1b** and Gadolinium-DOTA

336 mg (75%); m.p 182 °$C_{(decomp)}$; ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ_{ppm} 7.26 (t, J = 7.3 Hz, 2H), 7.01 (d, J = 7.9 Hz, 2H), 6.86 (t, J = 7.3 Hz, 1H), 4.69 (s, 2H), 3.51 (bs, 2H), 3.39 (s, 4H), 3.37 (s, 2H), 3.35 (s, 2H), 2.5–3.0 (bm, 22H), 1.7–2.1 (bs, 4H), 1.47 (s, 27H); ^{13}C NMR (100.6 MHz, CD_3OD, 25 °C): δ_{ppm} 179.2, (172.6, 172.5, 172.0), 144.7, 130.2, 120.7, 117.9, (82.5, 82.4), 79.5, (60.8, 60.6), (57.9, 57.7), 54.7, 54.2, 53.6, 51.8, 51.3, 50.8, 47.3, 30.1, 28.5; FAB$^+$-MS *m/z* 786.4 $[M+H]^+$, 808.4 $[M+Na]^+$.

Preparation of **8b**

The clear solution of **7** (360 mg; 0.70 mmol) in 1 mL of dry chloroform was added to the clear yellow solution of **6b** (300 mg; 0.71 mmol) in the mixture of dry chloroform (6 mL) with dry methanol (2 mL). The obtained mixture was stirred at room temperature for 20 h under argon. The product was purified chromatographically as described above for **8a**; yield 490 mg (82%); m.p 220 °$C_{(decomp)}$; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ_{ppm} 7.25 (t, J = 7.3 Hz, 2H), 7.02 (d, J = 7.9 Hz, 2H), 6.84 (t, J = 7.3 Hz, 1H), 4.68 (s, 2H), 3.54 (t, J = 6.4 Hz, 2H), 2.84–2.89 (m, 4H), 2.56–2.71 (m, 2H), 2.38–2.46 (m, 2H), 1.75 (br s, 1H), 1.68 (br s, 1H), 1.56 (br s, 4H), 1.36 (br s, 8H); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 °C): δ_{ppm} 176.2, 170.1, 169.9, 169.5, 169.2, 142.6, 128.7, 117.9, 114.5, 81.2, 81.1, 81.0, 77.2, 59.0, 58.4, 57.6, 57.4, 56.7, 56.4, 55.8, 55.1, 54.9, 54.2, 53.8, 52.6, 52.4, 52.3, 50.9, 50.6, 49.7, 48.9, 48.6, 47.1, 46.8, 28.6, 28.4, 27.63, 27.58, 27.4, 27.3, 26.6, 26.0, 25.5, 22.5; FAB$^+$-MS *m/z* 856.5 $[M+H]^+$, 878.5 $[M+Na]^+$.

Preparation of **9a**

Compound **8a** was dissolved in trifluoroacetic acid. After stirring at ambient temperature for four hours the solution was evaporated in vacuum. Methanol was added and evaporated, chloroform was added and evaporated (until the completely disappearance of TFA). Compound **9a** was obtained nearly quantitatively as a light brown viscous substance. HRMS (MALDI-TOF) *m/z* 618.6 $[M+H]^+$, 640.6 $[M+Na]^+$, 662.6 $[M-H+2Na]^+$**.**

Preparation of **9b**

Compound **9b** was obtained like **9a** from **8b**. Compound **9b** was obtained nearly quantitatively as a light brown viscous substance. HRMS (MALDI-TOF) *m/z* 688.4 $[M+H]^+$, 610.4 $[M+Na]^+$, 632.4 $[M-H+2Na]^+$**.**

Preparation of **1a**

The pH of the suspension of **9a** (158 mg; 125 μmol) in 8 mL of distilled water was adjusted to 6.5 with 0.36% HCl. Thereafter Gd_2O_3 (22 mg; 62.5 μmol) was added. The obtained suspension was stirred for 2 weeks at room temperature. A grayish powdered solid was obtained. The reaction proceeded nearly quantitatively; m.p 278 °$C_{(decomp)}$; HRMS (MALDI-TOF) *m/z* 849.2 $[M-H+2K]^+$. Our synthetic procedure was adapted from Ref. 9.

Preparation of **1b**

The compound was prepared using the same procedure as that used for **1a**. A grayish powdered solid was obtained. This reaction also proceeded nearly quantitatively; m.p > 300 °$C_{(decomp)}$; HRMS (MALDI-TOF) *m/z* 843.3 $[M+H]^+$.

Dopamine receptor binding was evaluated according to Ref. 10. Striatal tissues was isolated from C57 bl. mouse and homogenized in 100 volumes of ice-cold Tris-HCl 50 mM pH 7.4 buffer, using Brinkman Polytron. The homogenate was centrifuged three times (and resuspended twice in equal volumes of buffer) for 20 min at 3000 g.

Final reconstitution of the pellet was done to yield a tissue concentration of 10 mg wt weight per ml buffer. To analyze the dopamine receptor binding, 0.1 ml [^{3}H]-spiperone (5×10^{-10} M) was added to 0.1 ml striatal membranes and 0.8 ml Tris-HCl buffer was incubated at 25 °C. One hour later, the mixture was diluted in 3 ml ice-cold buffer and filtered under vacuum through glass fiber filters (Whatman GF/C). The filters were washed three times with 3 ml of ice-cold buffer. The bound radioactivity was counted in a liquid scintillation cocktail (Optiflour) using a scintillation counter (Tri carb 300c Packard). The specific binding was defined as the difference between the binding in the presence and absence of 1 μM sulpiride.

Supporting Information Available. The NMR spectra and MS of compounds **5a**, **5b**, **6a**, **6b**, **8a**, **8b**, **9a**, **9b**, and HRMS of **1a** and **1b**. T_1-weighted MR images of aqueous solutions of **1a** and **1b** at different concentrations along with the change in the SNR as a function of TR and the T_1 and T_2 data of D_2O at 400 MHz with various concentrations of **1a** and **1b**.

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