Photochromically-controlled, reversibly-activated MRI and optical contrast agent

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The contrast agent which tethers a spiropyran group to a Gd-DO3A moiety has higher relaxivity and fluorescence intensity in the dark; the relaxivity and fluorescence intensity decrease after irradiation with visible light.

Magnetic resonance imaging (MRI) is a non-invasive, multi-dimensional imaging modality used to explore the internal structural features of living systems.[1] To increase contrast between pathological and normal tissue, enhancement agents are often introduced in MRI.[2] Contrast agents currently used in a clinical setting are generally non-specific and as such must be administered in relatively large amounts. Another disadvantage of non-specificity is that many tissues in the body then contain contrast agent and so exhibit enhanced signals in MRI scans, which makes diagnosis of contrast due to disease or structural abnormality difficult.[3]

Current efforts in the literature are focused on the development of target-specific and activatable contrast agents.[4] Recently, a new class of contrast agents was developed in which the access of water to the first coordination sphere of a chelated gadolinium ion is blocked with a galactopyranose residue that can be cleaved by β-galactosidase, the product of a common marker gene. Following cleavage, the block is removed and the agent becomes "active". Unlike existing contrast agents that are usually constitutively active, providing a bright signal wherever they are present, these new agents are MRI "dark" until cleaved by a target enzyme.[4a,b] The utility of MRI could be expanded by a toolbox of activatable probes that respond to a variety of biochemical events. In this work we develop a photoactivable contrast agent. Such an agent could have applications as an indicator for gene expression in conjunction with light emitting gene markers such as lucerase-luciferin.[5]

Spirobenzopyrans are one of the most widely studied classes of photochromic compounds.[6] Their ability to be reversibly "activated" brought them to our attention as a possible way of introducing controlled, reversible activation when tethered to an MRI contrast agent such as Gd-DO3A. Spiropyran (**5**-SP) and its merocyanine (**5**-MC) isomer can be reversibly interchanged following light irradiation.[7] The structural change of the spirobenzopyran moiety in the suggested contrast agent **5** influences the accessibility of water to Gd^{3+}, thus causing the two isomers to have different contrast enhancement properties. Herein, we report the synthesis and characterization of compound **5**, a reversibly activated T_1-weighted MRI contrast agent that also shows interesting activatable fluorescence.

Contrast agent **5** consisting of (i) a Gd-DO3A chelator moiety providing seven coordination sites for a paramagnetic Gd(III) ion and (ii) a tethered spirobenzopyran moiety was synthesized as shown in Scheme 1. Compounds **1**[8] and **2**[9] were refluxed in dry THF to afford compound **3** in excellent yield. The tri-ester **3** was decomposed in acidic conditions to give tri-acid **4**.[10] Complexation was carried out in methanol using gadolinium(III) trifluoromethanesulfonate to yield complex **5**.[11]

The photoisomerization of complex **5** is shown in Fig. 1. Irradiation with UV at 365 nm or storage in the dark affords a yellow colored merocyanine isomer (at higher concentrations this is orange) and irradiation with visible light gives a pale yellow colored solution, representing a mixture of merocyanine and spirobenzopyran isomers. Longer irradiation time with visible light (more than 1 minute) or laser irradiation (HeNe, 2 W) cannot further decolor the solution. After the compound **5** is irradiated with visible light for 1 minute, UV irradiation (8 W) at 365 nm can only partially restore the absorption at 468 nm (MC form) and longer irradiation time at 365 nm does not induce further conformational change. This is likely due to the power limit of the UV lamp. However, the dark can gradually restore the MC form and the absorption at 468 nm was completely restored after the sample was put in the dark for 20 minutes. Compared with complex **5**, the photoisomerization of ligand **4** is more efficient. Irradiation with UV at 365 nm or in the dark affords the red colored merocyanine isomer and irradiation with visible light immediately gives the colorless spiropyran isomer; this behavior has been observed in other free or crowned spirobenzopyrans.[7] The observed favor towards the **5**-MC isomer may come from the strong electrostatic interaction between the high charge density, cyclen-complexed gadolinium cation and the phenoxide oxygen,[7,12] which restricts the conversion of complex **5** from the MC form to the SP form, as shown in Scheme 2.

Complex **5**-MC gives strong fluorescence at 582 nm when excited at 500 nm, as shown in Fig. 2. After irradiation with visible light, the fluorescence was partially quenched because a certain amount of **5**-SP isomer was formed (as shown in Fig. 1). For compound **4** the conversion to the SP form was complete as evidenced by the degree of color change and disappearance of absorption at 493 nm, and complete fluorescence quenching is observed. Photochromically switchable fluorescence has been observed for the merocyanine dye encapsulated in polymer nanoparticles,[13] but no appreciable fluorescence has been observed in water for free dye and only weak emission has been seen in its crowned derivative.[14] A weak, switchable fluorescence emission

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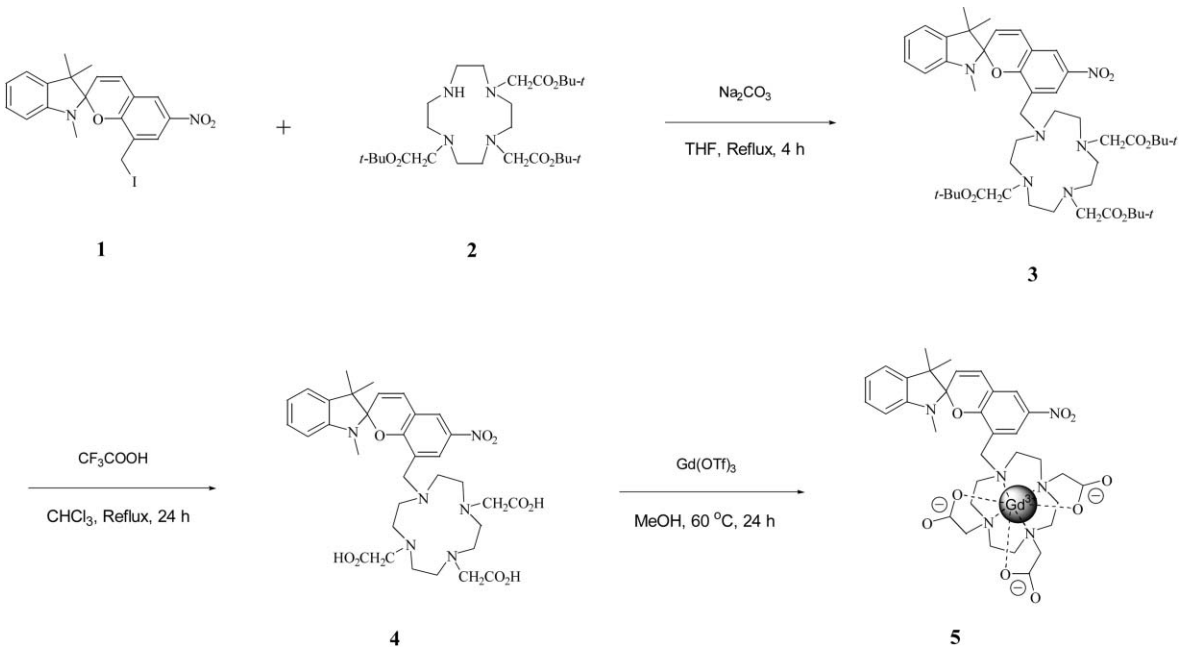


Scheme 1 Synthesis of compound **5**.

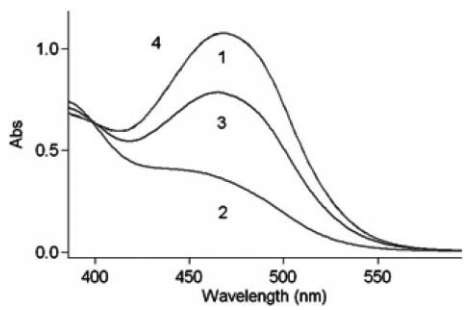


Fig. 1 Absorption spectrum of compound **5** (7.665×10^{-5} M) in water. 1: in the dark; 2: irradiated with visible light for 1 minute; 3: irradiated with UV at 365 nm for 10 minutes after being irradiated with visible light for 1 minute; 4: in the dark for 20 minutes after being irradiated with visible light for 1 minute.

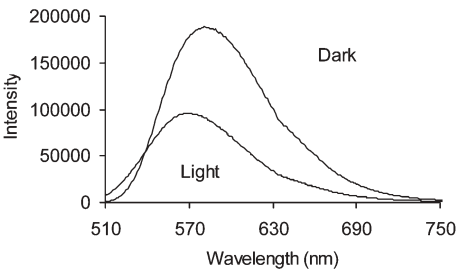
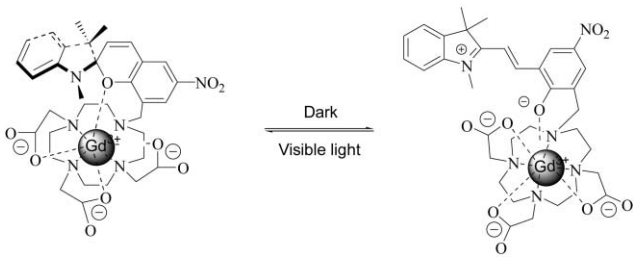


Fig. 2 Emission spectrum of compound **5** (7.665×10^{-5} M) in water with excitation at 500 nm.



Scheme 2 Proposed isomerization of Gd-SPDO3A (left) and Gd-MCDO3A (right).

was observed for the crowned derivative when a pyrene moiety was added to the molecule. The addition of the pyrene group resulted in a molecule that, in the presence of certain metal ions, emitted light due to intramolecular FRET from the pyrene group to MC in the molecule but not to the SP form. The interesting effect of conformation change on emission observed here suggests that compound **5** acts as an activatable fluorescence agent. We are investigating other environmental factors that induce this conformation change to explore applications of this molecule as a biochemical reporter.

The effect of limited isomerization of compound **5** on the relaxivity of water protons (r_1) was evaluated. The presence of the MC form of compound **5** was achieved by storing a series of concentrations in the dark prior to measuring r_1, and the SP form of compound **5** was maximized by irradiating the solutions with visible light for 1 minute prior to measuring r_1. Experiments were performed in water on a 1.5 T Bruker Minispec relaxometer. Plotting $1/T_1$ values against the respective varying concentrations of **5**-MC gave an r_1 (**5**-MC) value of 3.72 mM^{-1} s^{-1}, which is similar to the r_1 value of Gd-DOTA.[15] By the same method an r_1 (**5**-SP) value of 2.93 mM^{-1} s^{-1} was obtained. Thus, visible light irradiation of **5**-MC results in a relaxivity decrease of *ca.* 21%.

From the results of spectroscopy and relaxivity, we observed that the relaxivity decrease is related to the extent of isomerization to the SP form. The decrease in the relaxivity of the contrast agent **5** following visible light irradiation may be explained by the structural distinction between **5**-MC and **5**-SP isomeric forms. In the **5**-SP form, the indoline part is orthogonal to the benzopyran

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part (Scheme 2).[16] The complexed Gd^{3+} may be attracted to the indoline part of the molecule where the metal cation has electrostatic interaction with lone pairs of electrons in oxygen and nitrogen atoms, and perhaps the π electrons on the aromatic ring.[17] Thus the indoline part "covers" Gd^{3+} and the "indoline cap" prevents the water molecules from accessing the metal cation, and inhibits MRI contrast enhancement. Altering the hydration state of gadolinium is a known mechanism for modifying relaxivity.[2,4a,18] In the **5**-MC form, the breaking of the C_{sp^3}–O bond and rehybridization of the spiro carbon atom from sp^3 to sp^2 gives coplanar rings. The removal of the "indoline cap" allows the water molecules to access the metal cation more readily and increases relaxivity.

In conclusion, this preliminary work describes a novel, activatable, T_1-weighted MRI contrast agent that also displays controllable fluorescence properties. We demonstrate that photochromism of an SP/MC motif, when tethered to a Gd-DO3A moiety, induces changes to relaxivity. When in the dark, the contrast agent is in its MC form and has higher relaxivity. After irradiation with visible light, the contrast agent experiences a color change due to partial isomerization to the SP form and relaxivity decreases. This photochromically-controlled, reversibly activated MRI and optical contrast agent may have unique potential to respond to light generated by luciferase-catalyzed reactions thus allowing interrogation of the luciferase marker gene in deep tissues where it is not accessible by optical methods. Studies are ongoing to determine the intensity of light required to produce measurable contrast by MRI and to increase the ease of conversion between the MC and SP isomers. Because the relaxivity decrease is related to the extent of isomerization to the SP form, a more efficient conversion between the MC and SP isomers would increase the effect on relaxivity. The potential ability to turn this type of contrast agent "ON" and "OFF" would be a useful addition to the recent reports of biochemical reporter contrast agents.

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10 Compound **4**: UV–vis: 515 (ε 1.373 × 10^4), 342 (ε 1.127 × 10^4), 265 (ε 1.242 × 10^4), 204 nm (ε 3.869 × 10^4). ESI^+ MS (50% MeOH and 0.1% formic acid): *m/z* 681 (M + H, 100%), 335 (48%). IR: 1632 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6, 300 K) δ_H 1.12 (3H, s, $C(CH_3)_2$), 1.20 (3H, s, $C(CH_3)_2$), 2.64–2.85 (13H, m, NCH_2 and NCH_3), 3.10 (2H, br s, NCH_2), 3.17 (2H, s, NCH_2), 3.30–3.39 (5H, m, NCH_2), 3.56–3.58 (3H, m, NCH_2), 3.95 (1H, s, NCH_2), 4.16 (1H, s, NCH_2), 6.00 (1H, d, *J* 9.7, CH_2=CH_2), 6.59 (1H, d, *J* 5.8, CH_2=CH_2), 6.81 (1H, s, ArH), 7.13 (2H, s, ArH), 7.25 (1H, d, *J* 9.7, ArH), 8.17 (2H, s, ArH). ^{13}C NMR (126 MHz, DMSO-d_6, 300 K) δ_C 20.37, 26.37, 27.05, 29.19, 34.06, 49.10, 49.33, 50.14, 50.44, 51.63, 51.90, 52.18, 52.34, 55.91, 107.53, 107.67, 119.72, 120.28, 121.47, 122.29, 122.81, 124.69, 128.46, 129.61, 136.50, 140.73, 147.83, 158.40, 170.62, 171.78. Found C, 54.44; H, 5.69; N, 11.06; calc. for $C_{46}H_{68}O_9N_6 \cdot CF_3COOH$: C, 54.40; H, 5.70; N, 10.57%.

11 The procedure to prepare compound **5**: A solution of compound **2** (1.1 equiv., 1.540 g) and sodium carbonate (3.0 equiv., 0.748 g) in 25 mL of dry THF was stirred in a flask equipped with a calcium chloride drying tube for 30 minutes. Compound **1** (1.0 equiv., 1.087 g) in 15 mL of dry THF was added to the flask. The mixture was refluxed for 4 hours. The solid was filtered out and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in dichloromethane and the resulting solution was washed with 1 M hydrochloric acid, 5% aqueous sodium bicarbonate and water. The organic layer was separated, dried and evaporated *in vacuo* to give 1.86 g (93%) of compound **3** as a purple solid. A mixture of compound **3** (1.0 g) in chloroform (10 mL) and trifluoroacetic acid (15 mL) was refluxed for 24 hours. The solvent was evaporated *in vacuo*. The residue was dissolved in methanol, and then the solvent was evaporated to dryness. The process was repeated three times. The crude product was purified by column chromatography on silica gel with MeOH–H_2O–CH_2Cl_2 (4 : 1 : 1) as eluent to give 0.63 g (78%) of compound **4** as a red solid. A mixture of compound **4** (0.124 g) and $Gd(OTf)_3$ (0.11 g) in 6 mL of methanol was stirred for 24 hours at 60 °C. The solvent was evaporated *in vacuo*. Methanol was added to the residue and the resulting solution was evaporated to dryness *in vacuo* to give 0.143 g of compound **5** as an orange solid.

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