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Synthesis and Comparative Evaluation of Photoswitchable Magnetic Resonance Imaging Contrast Agents

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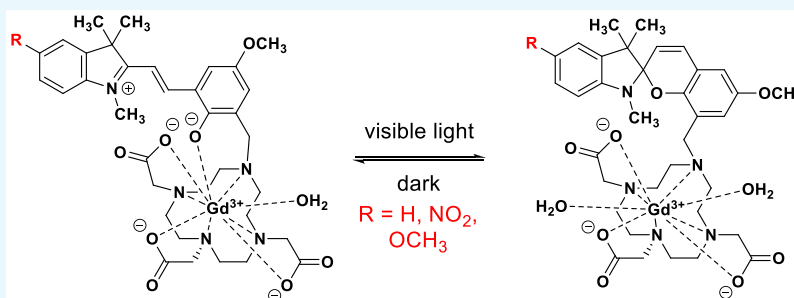
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ABSTRACT: A series of spiropyran (SP)-based magnetic resonance imaging (MRI) contrast agents have been synthesized and evaluated for changes in relaxivity resulting from irradiation with visible light. Both electron-donating and electron-withdrawing substituents were appended to the SP ring in order to study the electronic effects on the photochromic and relaxivity properties of these photoswitchable MRI contrast agents. Photoswitches lacking an electron-withdrawing substituent isomerize readily between the merocyanine and SP forms, while the addition of a nitro group prevents this process. Complexes capable of isomerizing were demonstrated to effect a change in the relaxivity of the appended gadolinium complex.

INTRODUCTION

Magnetic resonance imaging (MRI) represents one of the most common diagnostic tools in modern medicine, and it is widely used to explore the structural features in living systems because of its strengths for noninvasive and three-dimensional imaging with high spatial resolution.¹ To further the advantages of MRI, there is interest in developing activatable contrast agents that can increase the MRI contrast between target tissues and their surroundings as a result of the agent's response to specific biological processes.^{2,3} An early example was galactopyranosyl-substituted 1-hydroxyethyl-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid, which is an analogue of DOTA.⁴ The number of coordination sites for water changes when β -galactosidase enzymatically cleaves the galactopyranose moiety. In recent decades, much effort has been dedicated on the development of target-specific and activatable contrast agents. Kikuchi reported pH-responsive polymers achieved by the alteration of the molecular-tumbling rate.⁵ Herges reported a light-activatable MRI contrast agent based on an intramolecular light-driven coordination-induced spin state switch.⁶ Our lab previously developed a redox-sensitive spironaphthoxazine-based MRI contrast agent based on a photochromic switch tethered to a gadolinium chelator that responds to NADH with a hydration number alteration.⁷ Previous studies have also examined contrast agents that respond to other stimuli such as enzymatic processes,^{8–10} redox,^{11–13} and pH.^{3,14,15}

Photochromic compounds are a large class of compounds that can respond to light reversibly, and spiropyran (SP) is one of the most interesting subtypes.¹⁶ The photochromic behavior of SPs has been well-studied (Scheme 1a). The compound appears colorless or pale yellow in its closed-ring (SP) isomeric form, while an open-ring [merocyanine (MC)] isomer is generated with an optical absorption peak at 550–600 nm after UV irradiation or incubation in the dark.¹⁶ This new absorbance peak is due to the transformation of the photoswitch from its orthogonal configuration (SP form) to the planar one (MC form).¹⁷ In 2007 and 2009, we reported nitro- and dinitro-substituted SP-based contrast agents, where a difference in the hydration number and a significant relaxivity change were observed before and after light irradiation.^{18,19} The responsiveness of the SP-based contrast agent to light encouraged further investigation of the relation between the structure and photochromic properties. Although a comprehensive study in 2016 discussed the electronic effect on the responsiveness to visible light for free SPs, the chemical and photochromic properties could be significantly altered by

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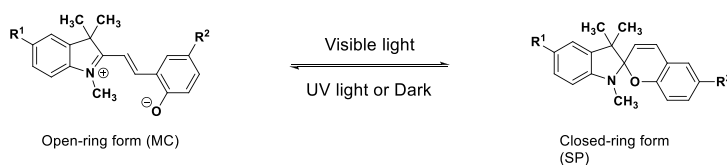
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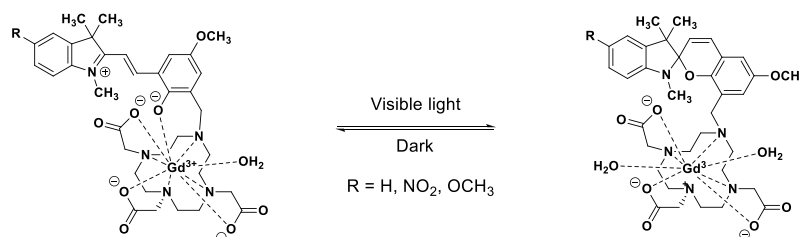


Scheme 1. (a) Photochromic Behavior of SPs; (b) Photochromic Behavior of Photoswitchable MRI Contrast Agents

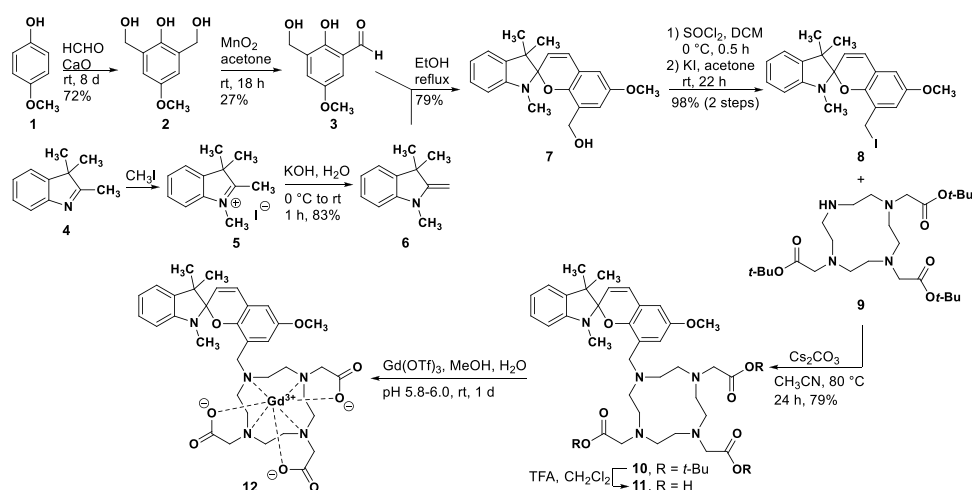
a. Photochromic behavior of spiropyrans



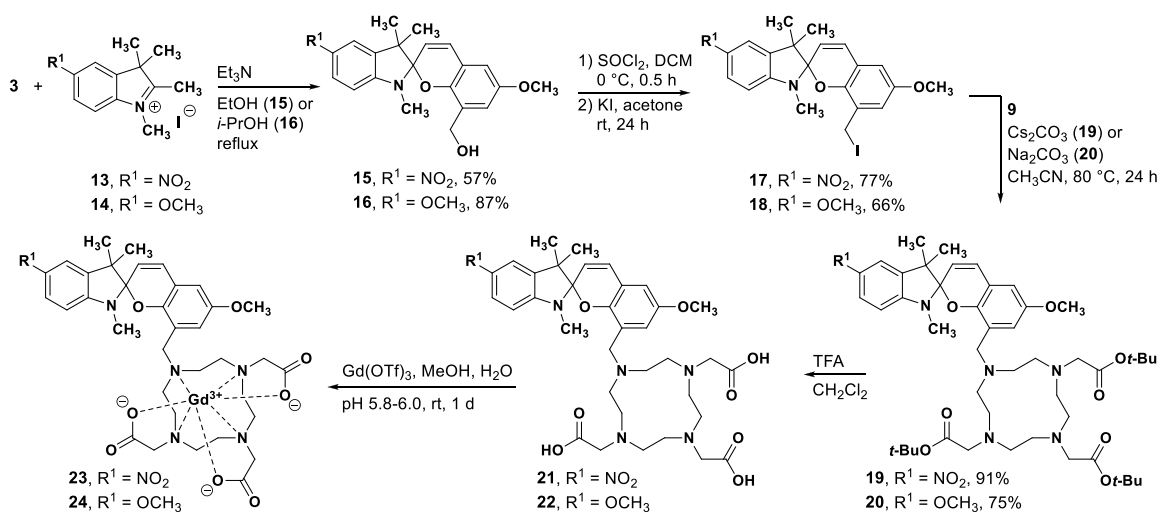
b. This work



Scheme 2. Synthesis of Complex 12



Scheme 3. Synthesis of Complexes 23 and 24



conjugation with a Gd-coordinated DO3A ligand.¹⁷ As such, it is of great value to understand the electronic effects of substituents on photoswitchable MRI contrast agents. Herein, we report our recent efforts on the synthesis and detailed

mechanistic investigation of light-sensitive SP-based MRI contrast agents in which the indoline substituents are varied in their ability to donate or withdraw electron density (Scheme 1b).

RESULTS AND DISCUSSION

Synthesis of SPs Appended to DO3A. Photoswitchable MRI contrast agents were synthesized as illustrated in Scheme 2. Commercially available 4-methoxyphenol **1** was used as the starting material and afforded dihydroxymethylation product **2** in the presence of 37% formaldehyde aqueous solution and CaO, which was further oxidized into aldehyde **3** by MnO₂. Indole **4** was treated with CH₃I and a base to generate intermediate **6**. Spirocyclic intermediate **7** was prepared in 79% yield after the reaction of **3** and **6** in refluxing EtOH. The benzylic hydroxyl group of this spirocycle **7** was converted to benzylic iodide **8** by successively chlorinating and displacing with iodide under Finkelstein conditions. The amination proceeded smoothly in the presence of Cs₂CO₃ and heating in acetonitrile with the *t*-butyl ester of DO3A (**9**). After deprotection and coordination to gadolinium, complex **12** was obtained and characterized by mass spectroscopy. The gadolinium content was determined by using a microwave plasma-atomic emission spectrometer (MP-AES).¹¹ With a similar pathway, we also succeeded in making complexes **23** and **24** from NO₂- and OCH₃-substituted indolium salts, respectively (Scheme 3).

Photochromic Analysis. We investigated the photochromic properties of complexes **12**, **23**, and **24** (Figure 1). Both complexes **12** and **24** turned from yellow to purple after the coordination step with Gd, and both exhibited significant absorbance peaks at 510 and 545 nm, which indicated isomerization of the SP groups from the closed-ring SP to open-ring MC form and that the generated MC form was stabilized by Gd³⁺.²⁰ It is hypothesized that the stabilization is

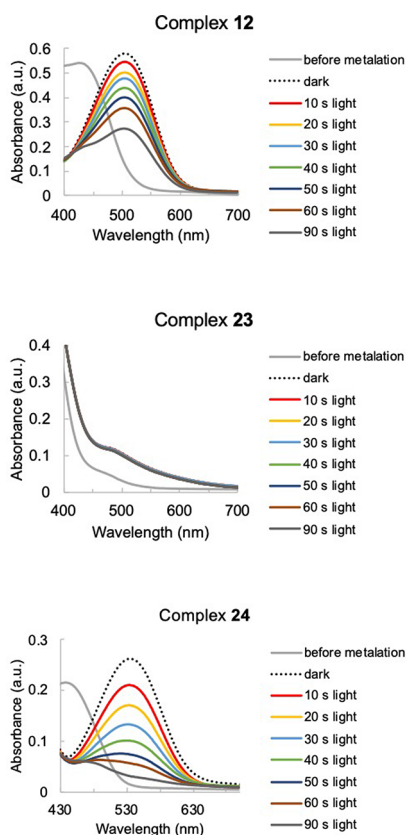


Figure 1. Absorbance spectra of complexes **12**, **23**, and **24** in nanopure water (pH = 7.4) at a concentration of 50 μM [Gd³⁺].

attributed to the newly formed interaction between phenolate oxygen and Gd³⁺. With visible light irradiation of the compound in aqueous solution for 1.5 min, 53 and 88% absorbance decrease at 510 and 545 nm was observed for complexes **12** and **24**, respectively. The absorbance changes suggested that the SP form for the SPs was regenerated from the MC form. Complex **23** remained yellow even after coordination and exhibited no significant peak above 500 nm in the absorbance spectrum. Furthermore, no absorbance change was observed after visible light irradiation. These results indicated that complex **23** might not be responsive to light; furthermore, there was a minimal absorbance difference above 400 nm before and after coordination for **23**. The electron-withdrawing nitro group on the indoline side of the SP appears to inhibit ring-opening. On the other hand, no significant absorbance above 500 nm was observed for free photoswitchable molecules without the Gd-DO3A complex.¹⁷ This suggested that the MC form could be stabilized through the coordination of Gd³⁺ and the phenolate anion.

Fluorescence measurements were also conducted for complexes **12** and **24** under different excitation wavelengths (Figure 2). The excitation wavelengths were chosen based on

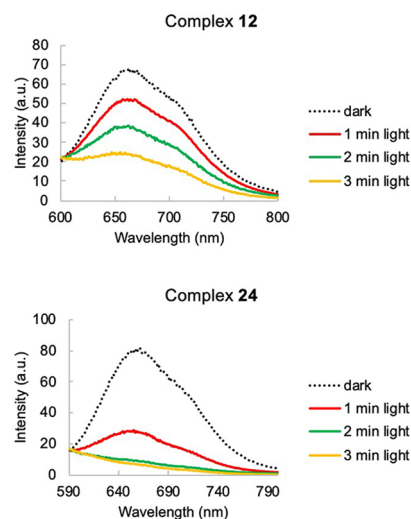


Figure 2. Fluorescence spectra using MC peak wavelength excitation of complexes **12** (510 nm) and **24** (545 nm) in nanopure water (pH = 7.4) at a concentration of 80 μM [Gd³⁺].

the MC absorbance peaks. Fluorescence peak decreases at 664 nm (complex **12**) and 663 nm (complex **24**) were observed after visible light irradiation, which further supports the conclusion drawn from the absorbance spectral experiments that the open-ring MC form can be isomerized to the closed-ring SP form after visible light irradiation.

Fluorescence spectra were also acquired for excitation at the SP absorbance wavelengths of complexes **12** (310 nm) and **24** (390 nm, Figure 3). Interestingly, a new peak appeared after visible light irradiation, which can be assigned to fluorescence from the SP form. Normally, there is no fluorescence from the SP/closed-ring form of the compound/complex (see Supporting Information), because the lone pair electrons on the nitrogen atoms can quench the fluorescence by the photo-induced electron transfer (PET) effect.^{21,22} However, the lone pair electrons on nitrogen or oxygen atoms are hypothesized to be stabilized by Gd³⁺ in this case,¹⁴ resulting in the suppression

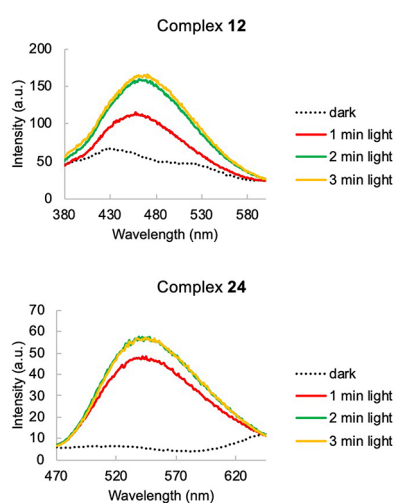


Figure 3. Fluorescence spectra obtained using SP peak wavelength excitation of complexes **12** (340 nm) and **24** (390 nm) in nanopure water (pH = 7.4) at a concentration of 80 μM [Gd^{3+}].

of the PET effect and the enhancement of SP fluorescence intensity.

Reversibility determination was performed for complexes **12** and **24** (Figure 4). New absorbance peaks appeared at 440 and

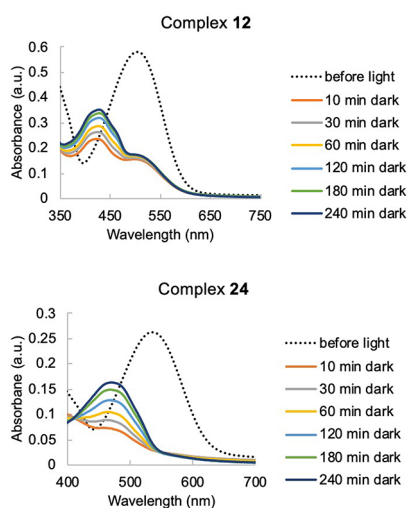


Figure 4. Absorbance spectra of complexes **12** and **24** in nanopure water (pH = 7.4) incubated in the dark after light irradiation for 90 s at a concentration of 50 μM [Gd^{3+}].

480 nm for complexes **12** and **24**, respectively, after subsequent incubation in the dark at room temperature, which did not overlap with the original absorbance peak prior

to light irradiation. We hypothesized that a block to reversibility, such as a twisted MC structure or intermediate, may have occurred.

Relaxivity and Statistical Analysis. The effect of irradiation on the longitudinal (r_1) relaxivity of complexes **12**, **23**, and **24** in an aqueous solution was evaluated as shown in Table 1. The r_1 values of the three compounds were 5.29 ± 0.11 , 2.09 ± 0.09 , and $2.79 \pm 0.05 \text{ mM}^{-1} \text{ s}^{-1}$, respectively, under the dark conditions in water, pH = 7.4. Complex **12** exhibited larger relaxivity than complexes **23** and **24**. This may be because the MC isomer being more stabilized for complex **12** under the dark conditions. Relaxivities for all complexes were determined statistically through a single gamma-generalized linear-mixed model with the identity link function (GGLMM-ID).

Based on the results of absorbance and fluorescence spectral experiments, the effect of light on the relaxivity properties of complexes **12** and **24** was investigated. The relaxivity change of complex **12** was larger than that of **24** after visible light irradiation. We propose that the methoxy group leads to a higher electron density on the indoline ring of complex **24**, generating a stronger electrostatic interaction between Gd^{3+} and indoline rings. The resulting closer distance between GdDO3A and the indoline “cap” makes it more difficult for water to access Gd^{3+} , inhibiting the relaxivity enhancement.¹⁹

MR Imaging. The effect of visible light on the MR properties of complexes **12** and **24** was evaluated by MRI (Figure 5). T_1 -weighted imaging was performed before and

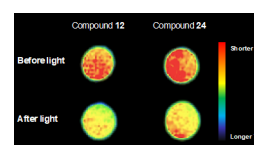


Figure 5. The pseudocolor MRI of complexes **12** and **24** before/after visible light irradiation.

after 1.5-min-long visible light irradiation. After the irradiation, both complexes **12** and **24** exhibited a lower signal intensity, which indicates that the longitudinal relaxation time increases after visible light irradiation for both cases.

CONCLUSIONS

In this work, we have synthesized three SP-based MRI contrast agents. Carefully optimized conditions were required for the challenging nucleophilic substitution of the secondary amine to the benzylic iodides to provide SP-based DO3A ligands **11**, **21**, and **22** leading to complexes **12**, **23**, and **24**. Compounds **12** and **24** exhibited photoswitching behavior upon irradiation with visible light, whereas compound **23**, with a nitro-

Table 1. r_1 Relaxivity before and after Visible Light Irradiation of Complexes **12**, **23**, and **24**^a

contrast agents	r_1 ($\text{mM}^{-1} \text{ s}^{-1}$)		r_1 change (%)	p -value
	in the dark	light		
complex 12	5.29 ± 0.11	4.57 ± 0.10	13.4 ± 1.7	$2.2 \times 10^{-13}***$
complex 23	2.09 ± 0.09			
complex 24	2.79 ± 0.05	2.53 ± 0.05	9.3 ± 1.6	$9.8 \times 10^{-8}***$

^aThe relaxivity was determined using five gradient concentration sample solutions. Each concentration was measured three times with independently prepared solutions. Two-tail unpaired t -test was performed with complexes **12** and **24** before/after visible light irradiation. *** p -value less than 0.001

substituted indoline ring, did not. This effect probably originates from the reduced basicity of the indoline nitrogen. The differential behavior of **12** and **24** compared to the free photoswitches lacking the pendant Gd complex suggests that the MC form is stabilized by coordination of Gd³⁺ and the phenolate oxygen anion. Conjugation to the chelated gadolinium also prevented PET in **12** and **24** and allowed the SP form to exhibit fluorescence, suggesting close interaction with the nitrogen lone pair. This result is consistent with relaxivity measurements on **12** and **24**, which demonstrate little change between the open and closed forms of the photoswitches. Although photoswitching between the SP and MC forms produces a dramatic structural change, both forms have strong Lewis basic interactions with the gadolinium complex, resulting in relatively small changes in relaxivity. This work represents the first example of studies on the electronic effect of substituents on the light-responsive MRI contrast agents. Further work on the evaluation of other photoswitch-based MRI contrast agents is underway.

EXPERIMENTAL SECTION

General Experimental Methods. All reagents were purchased from commercial sources and used without further purification unless stated otherwise. Solvents were dried over an activated alumina solvent system or purchased anhydrous where required. Reactions requiring anhydrous conditions were performed under argon; glassware was flame-dried under vacuum immediately prior to use and allowed to cool under reduced pressure; liquid reagents, solutions, or solvents were added via a syringe through rubber septa; solid reagents were added under a flow of argon. Reactions were monitored by TLC on silica gel 60 F254, and detected by examination under UV light (254 and 365 nm). Flash column chromatography was performed using silica gel [230–400 mesh (40–63 μm)], unless otherwise stated. Accurate mass measurements were recorded in the positive ESI mode in CH₃OH or CH₃CN. Extracts were concentrated in vacuo using both a rotary evaporator at a pressure of 15 mmHg (diaphragm pump), and a high vacuum line at a pressure of 0.1 mmHg (oil pump) at room temperature. ¹H and ¹³C spectra were measured in the solvent stated at 400, 599, or 800 MHz, and 101, 151, and 201 MHz, respectively. ¹H and ¹³C NMR chemical shifts are quoted in parts per million (ppm) and referenced to the residual solvent peak (CDCl₃: ¹H = 7.26 ppm and ¹³C = 77.16 ppm), and coupling constants (*J*) are given in hertz (Hz). Multiplicities are abbreviated as br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet) or combinations thereof.

2-Hydroxy-3-(hydroxymethyl)-5-methoxybenzaldehyde (3).²³ To a 100 mL round bottom flask were added 4-methoxyphenol **1** (4.48 g, 36 mmol), HCHO (37 wt % in H₂O, 6.4 mL, 90 mmol), CaO (1.02 g, 18 mmol), and H₂O (30 mL). The mixture was stirred in the dark for 8 days. Glacial AcOH (4 mL) was then added and the reaction was heated until all the solid was dissolved. After cooling to ambient temperature, the reaction was placed in a freezer at –30 °C overnight. The precipitated pale yellow solid was then filtered and washed with cold water. After drying under high vacuum, the product **2** was obtained (4.76 g, 72%). The solid **2** (921 mg, 5 mmol) was then dissolved in acetone (50 mL), followed by the addition of MnO₂ (2.16 g, 25 mmol). The reaction was stirred at room temperature for 18 h. After filtration and further purification by flash column chromatog-

raphy, the product **3** was obtained as a yellow solid (243.7 mg, 27%). ¹H NMR (599 MHz, CDCl₃): δ 10.99 (s, 1H), 9.88 (s, 1H), 7.23 (s, 1H), 6.96 (s, 1H), 4.75 (s, 2H), 3.83 (s, 3H), 2.36 (s, 1H). ¹³C NMR (151 MHz, CDCl₃): δ 196.5, 153.9, 152.8, 130.9, 123.9, 120.0, 114.5, 60.8, 56.1.

(6-Methoxy-1',3',3'-trimethylspiro[chromene-2,2'-indolin]-8-yl)methanol (7). To a 50 mL round bottom flask were added 2,3,3-trimethyl-3H-indole **4** (398.1 mg, 2.5 mmol), CH₃I (0.23 mL, 3.75 mmol), and CH₃CN (15 mL). The mixture was then stirred at 83 °C for 16 h. After cooling to room temperature, the solvent was removed. The residue was dissolved in CHCl₃ and hexane, which was sonicated for 30 min. After filtration, the iodide **5** was obtained (669.3 mg, 89%). The mixture of **5** (301.2 mg, 1 mmol) and H₂O (10 mL) was placed in an ice bath. KOH (101.0 mg, 1.8 mmol) was then added. The reaction was warmed to room temperature and stirred for 30 min. Upon completion, the reaction was extracted by ether (3 \times 10 mL) and washed by brine. The combined organic phase was then dried over anhydrous Na₂SO₄. After concentrating, compound **6** was obtained (122.8 mg, 71%). The mixture of **6** (71.8 mg, 0.41 mmol), **3** (75.5 mg, 0.41 mmol), and EtOH (4 mL) was reacted at 80 °C for 16 h. Upon the completion of the reaction, the product **7** was obtained (109.1 mg, 79%) after flash column chromatography (hexane/EtOAc = 5:1). ¹H NMR (599 MHz, CDCl₃): δ 7.15 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 7.2 Hz, 1H), 6.88–6.80 (m, 2H), 6.69 (s, 1H), 6.58 (s, 1H), 6.50 (d, *J* = 7.7 Hz, 1H), 5.76 (d, *J* = 10.2 Hz, 1H), 4.50 (dd, *J* = 13.3, 5.8 Hz, 1H), 4.34 (dd, *J* = 13.1, 7.7 Hz, 1H), 3.76 (s, 3H), 2.68 (s, 3H), 1.98 (d, *J* = 7.3 Hz, 1H), 1.31 (s, 3H), 1.19 (s, 3H). ¹³C NMR (101 MHz, CDCl₃): δ 153.2, 147.9, 146.1, 136.8, 129.6, 127.8, 127.7, 121.6, 119.9, 119.6, 119.3, 114.6, 111.0, 107.1, 104.4, 61.8, 56.0, 51.4, 29.1, 25.9, 20.4. AMM (ESI-TOF) *m/z*: calcd for C₂₁H₂₄NO₃⁺ [*M* + *H*]⁺, 338.1751; found, 338.1741.

Tri-tert-butyl 2,2',2''-(10-((6-Methoxy-1',3',3'-trimethylspiro[chromene-2,2'-indolin]-8-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (10). To a flame-dried round bottom flask were added **7** (102.9 mg, 0.3 mmol) and DCM (6 mL). SOCl₂ (4 drops) was then added at 0 °C. After reacting for 30 min, the reaction was quenched by the saturated NaHCO₃ solution (5 mL) and extracted with DCM (3 \times 10 mL). The combined organic phase was then dried over anhydrous Na₂SO₄ and concentrated. The residue was dissolved in acetone (10 mL), followed by the addition of KI (199.2 mg, 1.2 mmol). Upon the completion of reaction after 22 h, the mixture was concentrated and dissolved in DCM. After filtration of the insoluble solid, the filtrate was concentrated and dried without further purification, affording compound **8** as a brown solid (131.5 mg, 98% for 2 steps). Compound **8** (131.5 mg, 0.29 mmol) was dissolved in CH₃CN (3 mL). Tri-tert-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate **9** (100.9 mg, 0.2 mmol) and Cs₂CO₃ (130.3 mg, 0.4 mmol) were then added. The mixture was reacted at 80 °C for 24 h. Upon the completion, the product **10** was obtained as a brown oil (131.8 mg, 79%) by flash column chromatography (DCM/MeOH = 30:1). ¹H NMR (599 MHz, CDCl₃): δ 7.14 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.84–6.79 (m, 3H), 6.56 (d, *J* = 3.0 Hz, 1H), 6.46 (d, *J* = 7.8 Hz, 1H), 5.72 (d, *J* = 10.3 Hz, 1H), 3.75 (s, 3H), 3.52 (d, *J* = 13.0 Hz, 1H), 3.32 (d, *J* = 12.8 Hz, 1H), 3.15–2.44 (m, 19H), 2.34–2.05 (m, 8H), 1.48–1.40 (m, 27H), 1.23 (s, 3H), 1.14 (s, 3H). ¹³C NMR (201 MHz, CDCl₃): δ 172.8,

152.7, 147.9, 147.0, 136.5, 129.8, 127.8, 122.7, 121.4, 119.6, 119.2, 116.8, 112.2, 106.5, 82.3, 77.3, 55.8, 55.5, 50.9, 29.0, 28.2, 28.1, 28.0, 27.95, 27.85, 27.84, 25.9, 20.3. AMM (ESI-TOF) m/z : calcd for $C_{47}H_{72}N_5O_8^+$ $[M + H]^+$, 834.5375; found, 834.5385.

Synthesis of Compound 12. Compound **10** (50 mg) was dissolved in DCM (0.2 mL), and TFA (2 mL) was then added to the solution. The solution was stirred at room temperature for 24 h. The solvent was evaporated in vacuo. The residue was taken up in methanol (3×5 mL) and each time the resulting solution was evaporated to dryness to give product **11** as a red solid. The product was dissolved in MeOH (0.2 mL), followed by the addition of nanopure water (2 mL) and $Gd(OTf)_3$ (2.0 equiv based on the yield of previous step). The pH of the solution was adjusted to 5.8–6.0 with 0.1 M NH_4OH and the solution was stirred at room temperature for 24 h. After the reaction, 5 g of Chelex 100 was added and the solution was stirred for another 30 min at room temperature. The solvent was collected using a 50 mL polypropylene conical tube after filtering out the solid residues and dried in a lyophilizer for 3 d to yield product **12** as a yellow solid. The final products were characterized with mass spectroscopy. AMM (ESI-TOF) m/z : calcd for complex **12** $[M + H]^+$, 821.2504; found $[M + H]^+$, 821.2513 and other gadolinium isotope patterns.

(6-Methoxy-1',3',3'-trimethyl-5'-nitrospiro[chromene-2,2'-indolin]-8-yl)methanol (15). To a round bottom flask were added 1,2,3,3-tetramethyl-5-nitro-3H-indol-1-ium **13** (103.9 mg, 0.3 mmol), 2-hydroxy-3-(hydroxymethyl)-5-methoxybenzaldehyde **3** (60.1 mg, 0.33 mmol), Et_3N (84 μ L, 0.6 mmol), and *i*-PrOH (1.5 mL). The mixture was stirred at 80 °C for 24 h. After cooling to room temperature, the product **15** was obtained (65.0 mg, 57%) by flash column chromatography (toluene/ $EtOAc = 4:1$). 1H NMR (599 MHz, $CDCl_3$): δ 8.17 (d, $J = 8.7$ Hz, 1H), 7.95 (s, 1H), 6.90 (d, $J = 10.4$ Hz, 1H), 6.79 (s, 1H), 6.60 (s, 1H), 6.47 (d, $J = 8.8$ Hz, 1H), 5.72 (d, $J = 10.7$ Hz, 1H), 4.52 (d, $J = 13.3$ Hz, 1H), 4.41 (d, $J = 13.3$ Hz, 1H), 3.78 (s, 3H), 2.81 (s, 3H), 1.77 (s, 1H), 1.36 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 153.7, 153.0, 144.9, 140.7, 137.5, 130.4, 127.9, 126.5, 118.8, 118.5, 118.4, 114.7, 111.2, 105.5, 104.2, 60.9, 56.0, 51.0, 29.0, 25.8, 20.2. AMM (ESI-TOF) m/z : calcd for $C_{21}H_{23}N_2O_5^+$ $[M + H]^+$, 383.1601; found, 383.1596.

Tri-tert-butyl 2,2',2''-(10-((6-Methoxy-1',3',3'-trimethyl-5'-nitrospiro[chromene-2,2'-indolin]-8-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (19). To a flame-dried round bottom flask were added **15** (58.7 mg, 0.15 mmol) and DCM (3 mL). $SOCl_2$ (2 drops) was then added at 0 °C. After reacting for 30 min, the reaction was quenched by the saturated $NaHCO_3$ solution (5 mL) and extracted with DCM (3×10 mL). The combined organic phase was then dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in acetone (6 mL), followed by the addition of KI (99.6 mg, 0.6 mmol). Upon the completion of the reaction after 24 h, the mixture was concentrated and dissolved in DCM. After filtration of the insoluble solid, the filtrate was concentrated and dried without further purification, affording compound **17** as a brown solid (56.6 mg, 77% for 2 steps). Compound **17** (56.6 mg, 0.11 mmol) was dissolved in CH_3CN (2 mL). Tri-tert-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate **9** (45.5 mg, 0.09 mmol) and Cs_2CO_3 (58.6 mg, 0.18 mmol) were then added. The mixture was reacted at 80 °C overnight. Upon the completion, the product **19** was obtained as a brown oil

(71.7 mg, 91%) by flash column chromatography (DCM/MeOH = 30:1). 1H NMR (599 MHz, $CDCl_3$): δ 8.20 (d, $J = 8.6$ Hz, 1H), 7.92 (s, 1H), 6.91 (d, $J = 9.9$ Hz, 2H), 6.61 (s, 1H), 6.55 (d, $J = 8.7$ Hz, 1H), 5.71 (d, $J = 10.2$ Hz, 1H), 3.77 (s, 3H), 3.55 (d, $J = 12.7$ Hz, 1H), 3.37 (d, $J = 13.2$ Hz, 1H), 3.29–2.66 (m, 13H), 2.65–1.88 (m, 12H), 1.58–1.37 (m, 27H), 1.30 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.9, 173.6, 172.8, 153.3, 153.1, 146.2, 140.4, 137.3, 130.7, 126.6, 123.1, 119.2, 118.3, 112.7, 105.5, 104.5, 83.0, 82.8, 82.4, 56.1, 55.9, 55.7, 50.9, 29.3, 28.03, 28.01, 27.9, 26.0, 20.3. AMM (ESI-TOF) m/z : calcd for $C_{47}H_{71}N_6O_{10}^+$ $[M + H]^+$, 879.5226; found, 879.5242.

Synthesis of Compound 23. Compound **19** (50 mg) was dissolved in DCM (0.2 mL), and TFA (2 mL) was then added to the solution. The solution was stirred at room temperature for 24 h. The solvent was evaporated in vacuo. The residue was taken up in methanol (3×5 mL) and each time the resulting solution was evaporated to dryness to give product **21** as a red solid. The product was dissolved in MeOH (0.2 mL), followed by the addition of nanopure water (2 mL) and $Gd(OTf)_3$ (2.0 equiv based on the yield of the previous step). The pH of the solution was adjusted to 5.8–6.0 with 0.1 M NH_4OH and the solution was stirred at room temperature for 24 h. After the reaction, 5 g of Chelex 100 was added and the solution was stirred for another 30 min at room temperature. The solvent was collected using a 50 mL polypropylene conical tube after filtering out the solid residues and dried in a lyophilizer for 3 d to yield product **23** as a red solid. The final products were characterized with mass spectroscopy. AMM (ESI-TOF) m/z : calcd for complex **23** $[M + H]^+$, 866.2354; found $[M + H]^+$, 866.2336 and other gadolinium isotope patterns.

(5',6-Dimethoxy-1',3',3'-trimethylspiro[chromene-2,2'-indolin]-8-yl)methanol (16). To a round bottom flask were added 5-methoxy-1,2,3,3-tetramethyl-3H-indol-1-ium **14** (82.8 mg, 0.25 mmol), 2-hydroxy-3-(hydroxymethyl)-5-methoxybenzaldehyde **3** (45.5 mg, 0.25 mmol), Et_3N (70 μ L, 0.5 mmol), and EtOH (1.0 mL). The mixture was stirred at 80 °C for 18 h. After cooling to room temperature, the product **16** was obtained (79.9 mg, 87%) by flash column chromatography (hexane/ $EtOAc = 4:1$). 1H NMR (599 MHz, $CDCl_3$): δ 6.83 (d, $J = 10.2$ Hz, 1H), 6.73–6.64 (m, 3H), 6.57 (s, 1H), 6.40 (d, $J = 8.3$ Hz, 1H), 5.75 (d, $J = 10.2$ Hz, 1H), 4.54–4.47 (m, 1H), 4.36–4.30 (m, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 2.62 (s, 3H), 2.08 (t, $J = 6.4$ Hz, 1H), 1.29 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 154.0, 153.1, 146.1, 142.1, 138.4, 129.5, 127.6, 119.8, 119.3, 114.5, 111.3, 110.9, 109.7, 107.2, 104.7, 61.8, 55.96, 55.92, 51.5, 29.5, 25.8, 20.3. AMM (ESI-TOF) m/z : calcd for $C_{22}H_{26}NO_4^+$ $[M + H]^+$, 368.1856; found, 368.1850.

Tri-tert-butyl 2,2',2''-(10-((5',6-Dimethoxy-1',3',3'-trimethylspiro[chromene-2,2'-indolin]-8-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (20). To a flame-dried round bottom flask were added **16** (79.9 mg, 0.22 mmol) and DCM (6 mL). $SOCl_2$ (3 drops) was then added at 0 °C. After reaction for 30 min, the reaction was quenched by the saturated $NaHCO_3$ solution (5 mL) and extracted with DCM (3×10 mL). The combined organic phase was then dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in acetone (8 mL), followed by the addition of KI (146.1 mg, 0.88 mmol). Upon the completion of the reaction after 24 h, the mixture was concentrated and dissolved in DCM. After filtration of the insoluble solid, the filtrate was concentrated and dried without

further purification, affording compound **18** as a brown solid (68.9 mg, 66% for 2 steps). Compound **18** (34.0 mg, 0.07 mmol) was dissolved in CH_3CN (1 mL). Tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate **9** (24.4 mg, 0.05 mmol) and Na_2CO_3 (10.0 mg, 0.1 mmol) were then added. The mixture was reacted at 80 °C for 17 h. Upon the completion, the product **20** was obtained as a brown oil (30.6 mg, 75%) by flash column chromatography (DCM/MeOH = 30:1). ^1H NMR (599 MHz, CDCl_3): δ 6.90 (s, 1H), 6.81 (d, J = 10.5 Hz, 1H), 6.73–6.64 (m, 2H), 6.56 (s, 1H), 6.37 (d, J = 7.5 Hz, 1H), 5.73 (d, J = 10.1 Hz, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 3.48 (d, J = 12.5 Hz, 1H), 3.32 (d, J = 12.5 Hz, 1H), 3.15–1.98 (m, 25H), 1.51–1.40 (m, 27H), 1.22 (s, 3H), 1.15 (s, 3H). ^{13}C NMR (201 MHz, CDCl_3): δ 173.8, 173.7, 172.9, 153.9, 152.8, 147.2, 142.2, 138.3, 129.7, 123.1, 119.8, 116.2, 112.5, 111.0, 109.9, 106.7, 82.9, 82.6, 82.3, 77.3, 56.2, 56.0, 55.9, 55.6, 51.1, 29.4, 28.1, 28.03, 28.02, 27.95, 20.4. AMM (ESI-TOF) m/z : calcd for $\text{C}_{48}\text{H}_{74}\text{N}_5\text{O}_9^+$ [$\text{M} + \text{H}$] $^+$, 864.5481; found, 864.5491.

Synthesis of Compound 24. Compound **20** (50 mg) was dissolved in DCM (0.2 mL), and TFA (2 mL) was then added to the solution. The solution was stirred at room temperature for 24 h. The solvent was evaporated in vacuo. The residue was taken up in methanol (3×5 mL) and each time the resulting solution was evaporated to dryness to give product **22** as a red solid. The product was dissolved in MeOH (0.2 mL), followed by the addition of nanopure water (2 mL) and $\text{Gd}(\text{OTf})_3$ (2.0 equiv based on the yield of the previous step). The pH of the solution was adjusted to 5.8–6.0 with 0.1 M NH_4OH and the solution was stirred at room temperature for 24 h. After the reaction, 5 g of Chelex 100 was added and the solution was stirred for another 30 min at room temperature. The solvent was collected using a 50 mL polypropylene conical tube after filtering out the solid residues and dried in a lyophilizer for 3 d to yield product **24** as a red solid. The final products were characterized with mass spectroscopy. AMM (ESI-TOF) m/z : calcd for complex **24** [$\text{M} + \text{H}$] $^+$, 851.2609; found [$\text{M} + \text{H}$] $^+$, 851.2598 and other gadolinium isotope patterns.

Spectroscopic Analysis. Solutions were prepared by dissolving the SP-based GdDO3A complex in nanopure water. The Gd content was determined by using a MP-AES (4210 MP-AES, Agilent Technologies, Malaysia). The solution was carefully adjusted to pH = 7.4 with 0.1 M HCl or 0.1 M $\text{NH}_3 \cdot \text{H}_2\text{O}$. The final concentrations of all complexes used in absorbance and fluorescence measurements were 50 and 80 μM [Gd^{3+}].

The stock solutions were incubated in the dark overnight before addition to quartz cuvettes. Absorbance (Cary 100-Bio UV-vis spectrophotometer, Varian, USA) and fluorescence (Cary Eclipse fluorescence spectrophotometer, Varian, Australia) spectra were recorded in the dark and the cuvette containing sample solution was irradiated with visible light at ambient temperature before taking another absorbance and fluorescence measurements. The light source was a Schott Fostec ACE white lamp. The light source was fixed and the quartz cuvette containing sample solution was irradiated under a flexible arm (see Supporting Information). The reversibility measurements of SP-based GdDO3A were performed with additional 15, 30, 60, 120, 180, and 240 min incubation in the dark, after light irradiation, before final absorbance measurements.

Relaxivity Measurements. A series of aqueous sample solutions (0.2 mL each) with gradient gadolinium (complex

12: 0, 20, 39, 59, and 78 μM ; complex **23**: 0, 21, 42, 62, and 83 μM ; and complex **24**: 0, 27, 55, 109, and 136 μM) concentration were prepared and incubated in the dark for 18 h. T_1 relaxation time was measured on a 1.5 T Minispec relaxometer (Bruker) at 37 °C. The T_1 relaxation measurements were performed before and after irradiation with visible light for 1.5 min. Relaxivities for all complexes were determined statistically through a single GGLMM-ID.

Statistical Analysis. The gamma distribution was used to account for the heteroscedasticity with constant coefficient of variation in the data. Mixed models allow accounting for correlation of r_1 in light on/off repeated measurement situation as well as correlation within the same trial. Furthermore, they allow us to account for unobserved nuisance factors such as light positioning from the tube to tube or correlated concentration errors within the same trial. A random intercept was fitted for each Minispec tube ID and a random slope for each trial. Fixed effects included concentration, SP, and light status (on/off). Bonferroni-corrected Wald Z tests were performed on the fitted model to obtain p -values for the change in relaxivity for complexes **12** and **24** when exposed to light.

MR Imaging. T_1 -weighted MR images of complexes **12** and **24** were taken on a Biospec 7 T (300 MHz) system (Bruker, Billerica, MA) in an aqueous solution at room temperature. Aqueous solutions of complex **12** and **24** were prepared and stored in the dark for 18 h before imaging. Images were taken before and after irradiation with visible light for 1.5 min. Pulse sequence: MSME, TR = 50 ms, and TE = 15 ms; the concentration of the complex is 80 μM [Gd^{3+}].

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c01534>.

^1H and ^{13}C NMR spectra of **3**, **7**, **10**, **15**, **16**, **19**, and **20**; fluorescence spectra of photoswitches without Gd^{3+} ; and set-up of the white lamp and sample solutions (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Plewes, D. B.; Kucharczyk, W. *Physics of MRI: A Primer*. *J. Magn. Reson. Imag.* **2012**, *35*, 1038–1054.
- (2) Tu, C.; Louie, A. Y. Strategies for the Development of Gadolinium-Based 'q'-Activatable MRI Contrast Agents. *NMR Biomed.* **2013**, *26*, 781–787.
- (3) Tu, C.; Osborne, E. A.; Louie, A. Y. Activatable T₁ and T₂ Magnetic Resonance Imaging Contrast Agents. *Ann. Biomed. Eng.* **2011**, *39*, 1335–1348.
- (4) Moats, R. A.; Fraser, S. E.; Meade, T. J. A "Smart" Magnetic Resonance Imaging Agent That Reports on Specific Enzymatic Activity. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 726–728.
- (5) Okada, S.; Mizukami, S.; Kikuchi, K. Switchable MRI Contrast Agents Based on Morphological Changes of PH-Responsive Polymers. *Bioorg. Med. Chem.* **2012**, *20*, 769–774.
- (6) Dommaschk, M.; Peters, M.; Gutzeit, F.; Schütt, C.; Näther, C.; Sönnichsen, F. D.; Tiwari, S.; Riedel, C.; Boretius, S.; Herges, R. Photoswitchable Magnetic Resonance Imaging Contrast by Improved Light-Driven Coordination-Induced Spin State Switch. *J. Am. Chem. Soc.* **2015**, *137*, 7552–7555.
- (7) Tu, C.; Nagao, R.; Louie, A. Y. Multimodal Magnetic-Resonance/Optical-Imaging Contrast Agent Sensitive to NADH. *Angew. Chem., Int. Ed.* **2009**, *48*, 6547–6551.
- (8) Carril, M. Activatable Probes for Diagnosis and Biomarker Detection by MRI. *J. Mater. Chem. B* **2017**, *5*, 4332–4347.
- (9) Haedicke, I. E.; Li, T.; Zhu, Y. L. K.; Martinez, F.; Hamilton, A. M.; Murrell, D. H.; Nofiele, J. T.; Cheng, H.-L. M.; Scholl, T. J.; Foster, P. J.; et al. An Enzyme-Activatable and Cell-Permeable MnIII-Porphyrin as a Highly Efficient T₁ MRI Contrast Agent for Cell Labeling. *Chem. Sci.* **2016**, *7*, 4308–4317.
- (10) Daryaei, I.; Mohammadebrahim Ghaffari, M.; Jones, K. M.; Pagel, M. D. Detection of Alkaline Phosphatase Enzyme Activity with a CatalyCEST MRI Biosensor. *ACS Sens.* **2016**, *1*, 857–861.
- (11) Tsitovich, P. B.; Sperryak, J. A.; Morrow, J. R. A Redox-Activated MRI Contrast Agent That Switches Between Paramagnetic and Diamagnetic States. *Angew. Chem., Int. Ed.* **2013**, *52*, 13997–14000.
- (12) Wang, H.; Jordan, V. C.; Ramsay, I. A.; Sojoodi, M.; Fuchs, B. C.; Tanabe, K. K.; Caravan, P.; Gale, E. M. Molecular Magnetic Resonance Imaging Using a Redox-Active Iron Complex. *J. Am. Chem. Soc.* **2019**, *141*, 5916–5925.
- (13) Yu, M.; Ward, M. B.; Franke, A.; Ambrose, S. L.; Whaley, Z. L.; Bradford, T. M.; Gorden, J. D.; Beyers, R. J.; Cattley, R. C.; Ivanović-Burmazović, I.; et al. Adding a Second Quinol to a Redox-Responsive MRI Contrast Agent Improves Its Relaxivity Response to H₂O₂. *Inorg. Chem.* **2017**, *56*, 2812–2826.
- (14) Thorarinsdottir, A. E.; Du, K.; Collins, J. H. P.; Harris, T. D. Ratiometric PH Imaging with a CoII MRI Probe via CEST Effects of Opposing PH Dependences. *J. Am. Chem. Soc.* **2017**, *139*, 15836–15847.
- (15) Giovenzana, G. B.; Negri, R.; Rolla, G. A.; Tei, L. Gd-Aminoethyl-DO₃A Complexes: A Novel Class of PH-Sensitive MRI Contrast Agents. *Eur. J. Inorg. Chem.* **2012**, 2035–2039.
- (16) Berkovic, G.; Krongauz, V.; Weiss, V. Spiropyran and Spirooxazines for Memories and Switches. *Chem. Rev.* **2000**, *100*, 1741–1754.
- (17) Balmond, E. I.; Tautges, B. K.; Faulkner, A. L.; Or, V. W.; Hodur, B. M.; Shaw, J. T.; Louie, A. Y. Comparative Evaluation of

Substituent Effect on the Photochromic Properties of Spiropyrans and Spirooxazines. *J. Org. Chem.* **2016**, *81*, 8744–8758.

(18) Tu, C.; Osborne, E. A.; Louie, A. Y. Synthesis and Characterization of a Redox- and Light-Sensitive MRI Contrast Agent. *Tetrahedron* **2009**, *65*, 1241–1246.

(19) Tu, C.; Louie, A. Y. Photochromically-Controlled, Reversibly-Activated MRI and Optical Contrast Agent. *Chem. Commun.* **2007**, 1331–1333.

(20) Fedorova, O. A.; Gromov, S. P.; Strokach, Y. P.; Pershina, Y. V.; Sergeev, S. A.; Barachevskii, V. A.; Pepe, G.; Samat, A.; Guglielmetti, R.; Alfimov, M. A. Crown-Containing Spirooxazines and Spiropyrans. *Russ. Chem. Bull.* **1999**, *48*, 1950–1959.

(21) Guo, X.; Zhang, D.; Zhou, Y.; Zhu, D. Reversible Modulation of the Fluorescence of Pyrenemethylamine Hydrochloride by Light in the Presence of Spiropyran: Signal Communication between Two Molecular Switches through Proton Transfer. *Chem. Phys. Lett.* **2003**, *375*, 484–489.

(22) Ahmed, S. A.; Tanaka, M.; Ando, H.; Tawa, K.; Kimura, K. Fluorescence Emission Control and Switching of Oxymethylcrowned Spirobenzopyrans by Metal Ion. *Tetrahedron* **2004**, *60*, 6029–6036.

(23) Xie, R.-G.; Zhang, Z.-J.; Yan, J.-M.; Yuan, D.-Q. Selective Mono- and Bis-Oxidation of 2,6-Bis(Hydroxy-Methyl) Phenols with Active Manganese Dioxide. *Synth. Commun.* **1994**, *24*, 53–58.