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Synthesis and Optical Properties of Aqueous Soluble Indolylfulgimides

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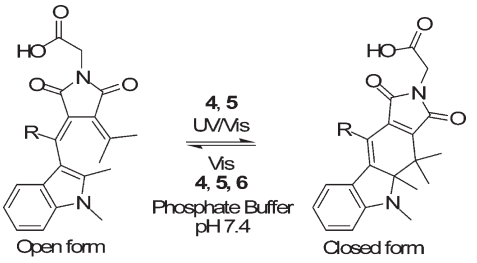

4. R = CF_3, **5**. R = COOH, **6**. R = H

Three novel aqueous soluble fulgimides, trifluoromethyl carboxylic acid indolylfulgimide **4**, dicarboxylic acid indolylfulgimide **5**, and carboxylic acid indolylfulgimide **6**, were synthesized. Both **4** and **5** can switch back and forth between open and closed forms upon illumination with specific wavelengths of light, whereas **6** can only switch from the closed form to the open form. In sodium phosphate buffer (pH 7.4) at 37 °C, an unusual hydrolysis of the trifluoromethyl group of the closed form of **4** resulted in **5**, which has an additional carboxylic acid group. The closed form of **5** was further decarboxylated to generate **6**, which was not photochromic. In buffer, the open form of **4** degraded 20% after 10 days, while the closed form of **4** was converted to **5** rapidly. In buffer, both forms of **5** degraded less than 20% after 21 days at 37 °C, and **5** underwent 670 photochemical cycles before degrading by 20%. It is the most robust fulgimide yet reported in aqueous solution.

Introduction

Photochromic compounds have potential applications in high capacity optical information storage devices, optical molecular switches, and biological sensors.[1−3] All of these applications depend on the binary nature of photochromic compounds. The interconversion between two key forms upon exposure to specific wavelengths of light is known as photochromism (Scheme 1).

Fulgides and fulgimides, promising photochromic compounds, have been considered as potential optical memory materials because of the readily distinguishable absorption spectrum for each key form, efficient photoreactions, and thermal and photochemical stabilities.[1,4] Studies have been conducted to optimize the photochromic properties of fulgides for specific applications.[5−7] Optimization has resulted in more thermally and photochemically stable compounds such as fluorinated indolylfulgide **1**, which was originally synthesized by Yokoyama and Takahashi.[8−10] The most photochemically stable fulgide, a fluorinated indolylfulgide synthesized by Lees et al., undergoes 10,000 photochemical cycles (back and forth conversion between the two key forms) before degrading by 13% in toluene.[11]

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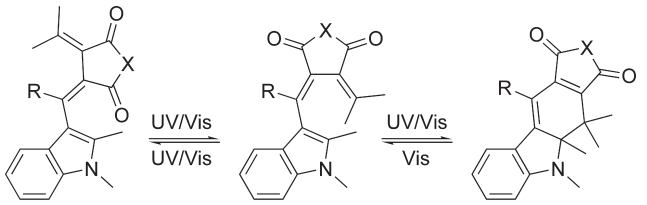
SCHEME 1. Photoreactions of Fulgides and Fulgimides

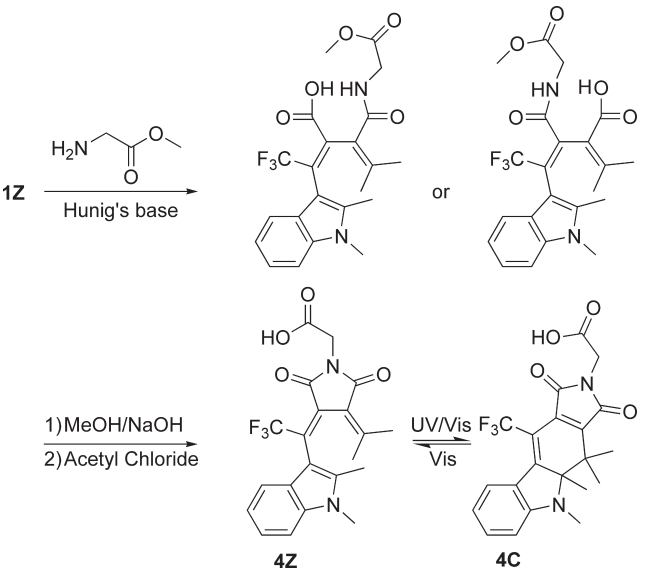


Fulgide **1**. R = CF_3, X = O
Fulgimides **2**. R = CF_3, X = N−C_6H_6
3. R = CF_3, X = N−CH_2−COOEt
4. R = CF_3, X = N−CH_2−COOH
5. R = COOH, X = N−CH_2−COOH

Optimization in almost all cases has been performed in aprotic solvents. However, the properties of fulgides or fulgimides in aqueous solutions have not been thoroughly examined.

Stability in protic environments is an important property of photochromic compounds for their application in optical memory devices and biological optical switches.[1,2] Materials used for memory devices are required to maintain stability and function in humid environments. In many biological applications, optical switches need to function in aqueous solvent systems.[2,12] Previous studies demonstrated that fulgides were unstable in protic solvents as a result of the highly reactive succinic anhydride ring in their structure.[13,14] Fulgimides, the most important fulgide derivatives, were synthesized to improve stability by replacing the succinic anhydride ring with a succinimide ring.[14,15] The closed form of *N*-phenyl fulgimide **2** displayed 3 orders of magnitude greater stability in 70/30 ethanol/water relative to that of the parent fulgide **1** at 25 °C (Scheme 1).[14] One of our recent studies indicated that the open form of ethyl ester fulgimide **3** lost 22% of its absorbance at the absorbance maxima, while **2** lost 52% after 21 days in 70/30 ethanol/water at 50 °C.[15] Furthermore, ethyl ester fulgimide **3** underwent 360 photochemical cycles in 70/30 ethanol/water before degrading by 20%, while **2** underwent 170 cycles.[15] The photochemical stability of fulgides in ethanol/water was not reported because of their rapid decomposition. Although several studies have determined the photochemical properties of fulgimides in protic solvents,[2,12−15] only a few of these studies have reported the properties of fulgimides in aqueous solution.[2,12,16] In one particular study fulgimide derivatives were covalently attached to the lysine residues on concanavalin A, where the open form of the fulgimide was shown to be stable in aqueous solution for 48 h at 25 °C.[12] This report also indicated that the fulgimide can cycle back and forth between the open and the closed forms at least twice. A recent study in live cells demonstrated that fulgimides can switch back and forth seven times in cellular membranes but not very well in water.[2] Therefore, a more systematic study of the photochemical and thermal properties of fulgimides in aqueous solution would accelerate their applications as biological optical switches and sensors.

SCHEME 2. Synthesis of Indolylfulgimide 4



Herein, we have synthesized and characterized two new photochromic indolylfulgimides **4** and **5** (Scheme 1). Fulgimide **4** was prepared from fluorinated indolylfulgide **1**. An unusual hydrolysis of **4** in sodium phosphate buffer resulted in fulgimide **5**, while further decarboxylation of **5** yielded a nonphotochromic fulgimide **6**. Fulgimides **4** and **5** were water-soluble at physiological pH because of the hydrophilicity of the carboxylate anion. The absorption spectra and thermal and photochemical stabilities for **4** and **5** have also been analyzed.

Results and Discussion

Synthesis. Trifluoromethyl indolylfulgide **1**[17] was used as the starting material for the synthesis of carboxylic acid indolylfulgimide **4** (Scheme 2). The anhydride ring of **1** was opened via addition of glycine methyl ester. The resulting methyl ester succinamic acid, one of the two possible regioisomers,[18] was saponified to generate the corresponding carboxylic acid succinamic acid. Subsequent dehydration of the succinamic acid intermediate with acetyl chloride yielded carboxylic acid indolylfulgimide **4Z**. Fulgimide **4C** was obtained by irradiating **4Z** with 405 nm light (Scheme 2).

During thermal stability measurements of **4C** in 50 mM sodium phosphate buffer (pH 7.4) at 37 °C, an unexpected reaction was observed. The reaction was followed by ^1H and ^{19}F NMR spectroscopy. The NMR data indicated an unusually high reactivity for **4C**. Previously reported *C*-forms of fluorinated indolylfulgimides have proven to be very stable under various conditions.[14,15] In the case of **4C**, before incubation at 37 °C, only one resonance at −58 ppm in the

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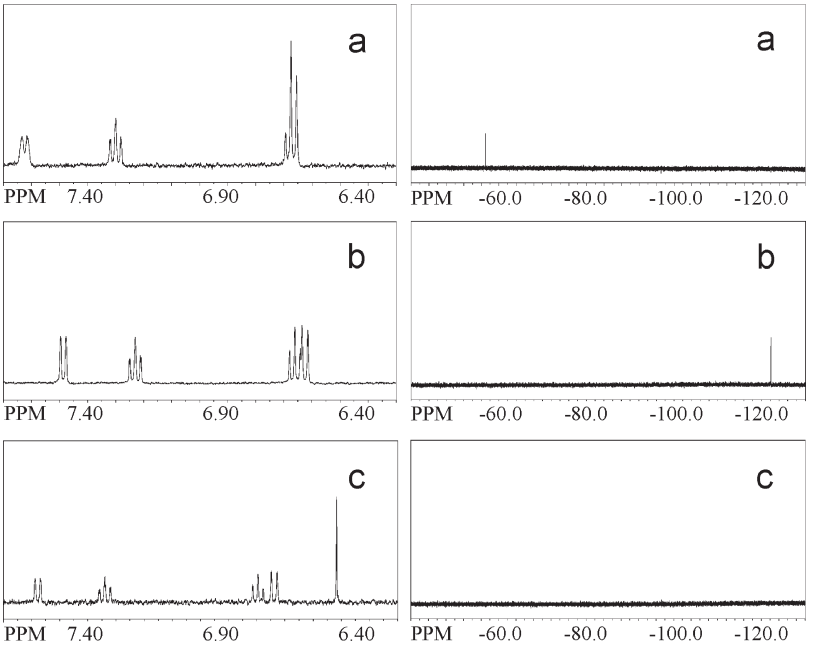
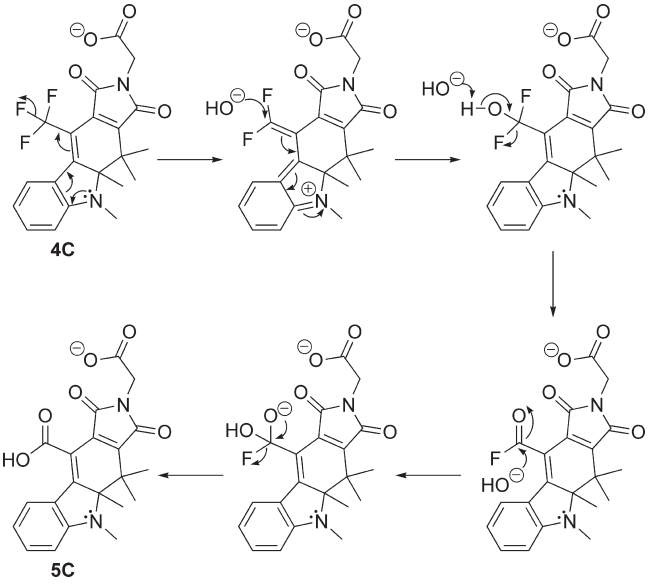


FIGURE 1. ^{1}H and ^{19}F NMR spectra of **4C** in 50 mM D_2O sodium phosphate buffer, pD 7.4, at 37 °C after (a) 0 h, (b) 6 h, and (c) following extraction and purification.

^{19}F NMR spectrum corresponding to the fluorines of the trifluoromethyl group was observed (Figure 1a). After 6 h at 37 °C, the resonance at −58 ppm disappeared, and a new fluorine signal appeared at −122 ppm, which is consistent with the chemical shift of the fluoride anion. The ^{1}H NMR spectrum showed a downfield shift of all hydrogen and methyl resonances (Figure 1b). In order to provide further support for the structure of the resulting product, ^{13}C NMR spectroscopy was performed. The quartet for the carbon of the trifluoromethyl group of **4C** at 122 ppm disappeared, and a new singlet at 170 ppm appeared, suggesting a carboxylic acid group. Furthermore, the resulting product still maintained photochromic properties and was stable in buffer at 37 °C for several days. Therefore, the most plausible mechanism was the hydrolysis of the trifluoromethyl group to form a carboxylic acid group.[19−21] The reaction yielded the photochromic dicarboxylic acid indolylfulgimide **5C** (Scheme 3).

Interestingly, when we initially attempted to isolate **5C** by EtOAc extraction from an acidified aqueous solution, the organic layer did not contain **5C** (Figure 1c). Instead, an extra hydrogen resonance appeared at 6.41 ppm in the ^{1}H NMR spectrum, and the ^{13}C NMR spectrum showed only 20 carbon resonances in comparison with the 21 carbon resonances for **4C** and **5C**, indicating another compound had been formed during the acidic extraction. The missing carbon resonance occurred in the carboxylic acid region. The lack of any ^{19}F NMR resonance suggested that the fluoride anion was removed in the aqueous layer during extraction. Therefore, we propose that the carboxylic acid

SCHEME 3. Mechanism for the Hydrolysis of 4C



group generated from the hydrolysis of the trifluoromethyl group can be decarboxylated to form compound **6C** (Scheme 4).[22−23]

Photochromic studies demonstrated that **6C** can be converted to **6E** (*E*-form due to IUPAC priority rules), but the reverse reaction was not observed (Scheme 4). Previously reported indolylfulgides substituted at the 3-position on the indole and having hydrogen at the bridging position were initially obtained in their *E*-form and also could not be

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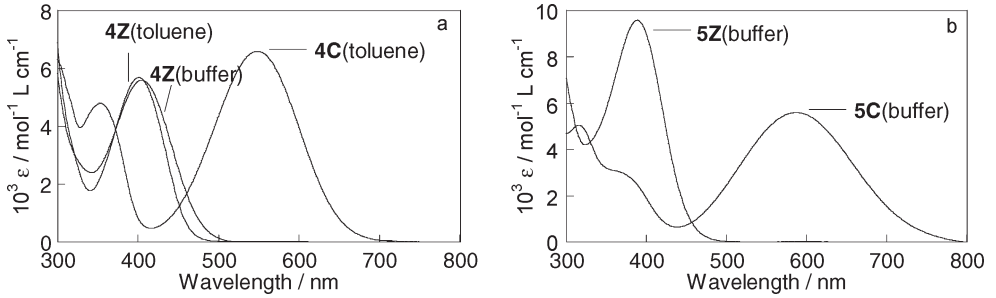
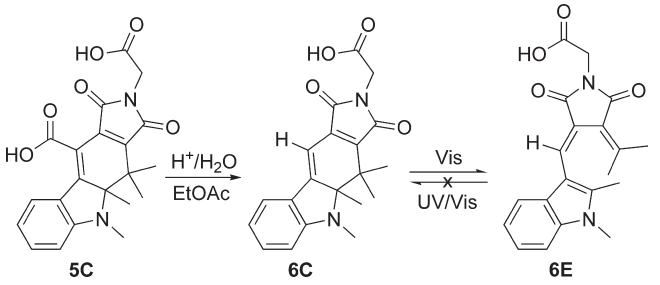


FIGURE 2. UV−vis absorption spectra of (a) **4Z** and **4C** in toluene and **4Z** in 50 mM sodium phosphate buffer (pH 7.4); (b) **5Z** and **5C** in 50 mM sodium phosphate buffer (pH 7.4).

SCHEME 4. Decarboxylation of 5C and Photochemical Reaction of 6



converted to the *C*-form.[24−26] For the first time, we obtained the *C*-form of such a fulgimide with a hydrogen at the bridging position. We did not investigate the optical properties of **6** further as it was not photochromic.

Syntheses of the dicarboxylic acid indolylfulgimide **5** and the carboxylic acid indolylfulgimide **6** were then carried out as described above. The reaction of **4C** to **5C** occurred quantitatively and rapidly in buffer (pH 7.4) at 37 °C. Fulgimide **5C** was relatively stable in acidic aqueous solution, but CO_2 was lost during prolonged extraction with EtOAc.

UV−vis Absorption Spectra. The UV−vis absorption spectra of **4Z** and **4C** were measured in toluene. The spectra of **4Z**, **5Z**, and **5C** were obtained in 50 mM sodium phosphate buffer (pH 7.4) (Figure 2). No UV−vis measurements for **4C** in buffer and **5** in toluene were performed because of the instability of **4C** in buffer (see above) and the poor solubility of **5** in toluene. The wavelengths of maximum absorbance and the extinction coefficients are shown in Table 1. Fulgimide **4Z** showed a small bathochromic shift (4 nm) as the solvent was switched from toluene to buffer. In comparison with **4Z**, **5Z** in buffer demonstrated a 16 nm hypsochromic shift at its absorbance maxima.

Thermal Stability. Thermal stability is one of the most important characteristics of fulgides and fulgimides for their applications in optical memory devices or optical switches.[10,11,13] Previously, the thermal stability of fulgides and fulgimides was examined in toluene at 80 °C.[14,15] Therefore, the thermal stability of **4Z** and **4C** was determined under these conditions. We also examined the stability of **4** and **5** at 37 °C in sodium phosphate buffer (pH 7.4) as this mimics

TABLE 1. Extinction Coefficients at λ_{max} for 4 in Toluene and 50 mM Sodium Phosphate Buffer (pH 7.4) and for 5 in 50 mM Sodium Phosphate Buffer (pH 7.4)

		λ_{max} (nm) (ε_{max} (mol^{-1} L cm^{-1}))		
compd	medium	*Z*-form	*C*-form	PSS_{405nm}[a] (*C*:*Z*:*E*)
4	toluene	401 (5.7×10^3)	549 (6.6×10^3)	90:7:3
4	buffer	405 (5.6×10^3)	unstable[b]	unstable[b]
5	buffer	389 (9.6×10^3)	588 (5.6×10^3)	87:6:6

[a]Photostationary state (PSS): *C*:*Z*:*E* ratio reached by prolonged exposure to 405 nm light; evaluated by ^{1}H NMR spectroscopy. [b]Hydrolysis of **4C** to **5C** was completed in 3 h.

TABLE 2. Thermal Decomposition Rate Constants for 4 in Toluene and 4 and 5 in 50 mM Sodium Phosphate Buffer (pH 7.4)

		rate constants (h^{-1})			
		UV−vis[a]		^{1}H NMR	
compd	medium	*Z*-form	*C*-form	*Z*-form	*C*-form
4	toluene	0.023	0.3×10^{-4}	0.010	0.5×10^{-4}
4	buffer	12×10^{-4}	[b]	7×10^{-4}	0.7
5	buffer	0.9×10^{-4}	2.8×10^{-4}	2.4×10^{-4}	0.6×10^{-4}

[a]Decomposition was followed at λ_{max}. [b]UV−vis spectra of **4C** and **5C** are very similar.

physiological conditions to some extent. The thermal decomposition of **4** and **5** was followed by both ^{1}H NMR and UV−vis spectroscopy. The results are presented in Table 2.

In pure toluene at 80 °C, the decomposition of **4Z** was fit to a single exponential decay (Figure 3a). The decomposition rate constants were 0.023 and 0.010 h^{-1} by UV−vis and ^{1}H NMR spectroscopy, respectively (Table 2). These values are similar to those observed for the parent fulgide **1Z** (0.023 h^{-1}) and ethyl ester fulgimide **3Z** (0.009 h^{-1}).[3,15] The UV−vis spectra also showed a similar pattern, an initial drop in absorbance followed by a red shift and subsequent increase in absorbance.[3,8,15] Our previous studies demonstrated that the thermal decomposition pathway for the *Z*-form of fluorinated indolylfulgides in toluene involves either a reversible *Z*-to-*E* isomerization or the conversion of the *Z*-form to an intermediate via a 1,5-hydrogen shift from the isopropylidene group.[8,11] The intermediate then subsequently rearranges to form a mixture of two isomers. On the basis of similar spectral kinetics, we postulate that fulgimide **4Z** undergoes the same degradation pathway as fulgide **1Z**. Therefore, the offset observed in the UV−vis data in Figure 3a is due to the absorbance of the decomposition products at the λ_{max} of **4Z**.[8,11] In the case of fulgimide **4C** in toluene at 80 °C, a double exponential fit was applied since a relatively rapid

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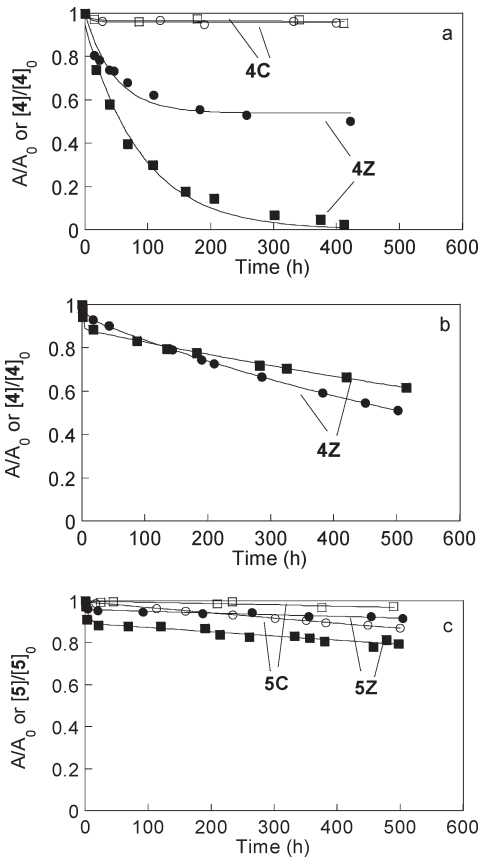

FIGURE 3. Thermal decomposition of *Z*- (closed symbols) and *C*-forms (open symbols) of **4** and **5** as a function of time as measured by UV−vis (circles) and ^{1}H NMR spectroscopy (squares): (a) **4** in toluene at 80 °C, (b) **4** in 50 mM sodium phosphate buffer (pH 7.4) at 37 °C, and (c) **5** in 50 mM sodium phosphate buffer (pH 7.4) at 37 °C.

decomposition of 3% was observed followed by a slow decomposition (Figure 3a). Fulgimide **4C** showed much higher stability than **4Z** in toluene, consistent with previously reported fulgides and fulgimides.[9,14,15]

In 50 mM sodium phosphate buffer (pH 7.4) at 37 °C, single and double exponential fits were applied to the *C*- and *Z*-forms, respectively. **4Z** and **5Z** showed a relatively rapid decline in concentration, which corresponded to *Z*-to-*E* isomerization, followed by a slower decline that corresponds to decomposition (Figure 3b,c). According to ^{1}H NMR data, **5Z** decomposed three times slower than **4Z** in buffer. **4C** is unstable and completely converted to **5C** in buffer after 3−6 h at 37 °C. **5C** showed great thermal durability and very little decomposition was observed after prolonged time in buffer at 37 °C. To account for the difference between the UV−vis and NMR data for **5C** in buffer, we measured the decomposition of **5C** by UV−vis spectroscopy in both D_2O and H_2O buffers and determined a solvent isotope effect of 3−4.

Photochemical Stability. The repeatability of the photochemical opening and closing of fulgimides **4** and **5** was measured in toluene and 50 mM sodium phosphate buffer (pH 7.4), respectively. Photochemical stability is required for many applications.[10] In toluene, the most stable fulgide reported to date can be switched back and forth over 10,000 times before degrading by 13%,[11] although for most

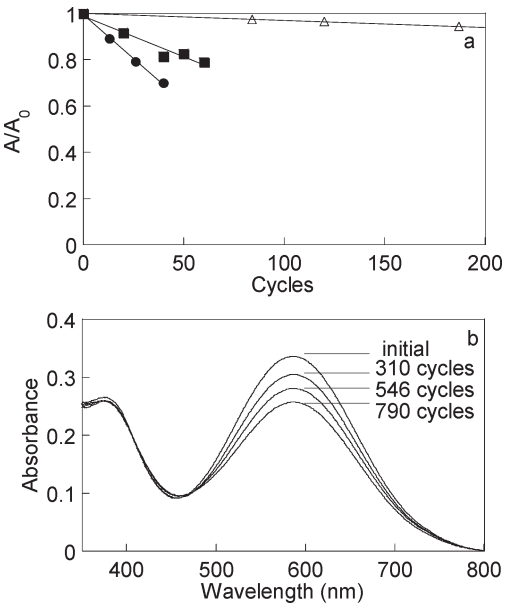

FIGURE 4. (a) Photochemical decomposition of **4** (closed symbols) in pure toluene (circles) and in toluene in the presence of tributylamine (squares) and **5** (open triangles) in 50 mM sodium phosphate buffer (pH 7.4). (b) PSS spectra of **5** in 50 mM sodium phosphate buffer (pH 7.4) after the indicated number of cycles.

fulgides the number is less.[3,9] In the case of fluorinated indolylfulgmides, a previous study indicated that they can be cycled back and forth between 700 and 3,000 times in toluene before degrading by 20%.[14,15]

In protic solvent systems, such as methanol, ethanol/water, or water, fulgides are too unstable and/or insoluble to measure their photochemical stability.[27] On the other hand, fulgimides previously examined in protic solvents only cycled back and forth a limited number of times.[2,13,14] A recent study in our group reported that the ethyl ester fulgimide **3** can be cycled back and forth 360 times before degrading by 20% in 70/30 ethanol/water.[15] Several reports about applications of fulgimides in aqueous biological systems have demonstrated that fulgimides can be cycled back and forth several times.[2,12]

The photochemical stability of fulgimide **4** was initially measured in pure toluene where it degraded by 20% after being cycle back and forth 21 times (Figure 4a), much less stable than its ethyl ester **3**.[15] We speculate that the rapid photochemical decomposition was affected by the carboxylic acid group and that the addition of base would increase the stability. In the presence of tributylamine (27 mM) in toluene, **4** cycled back and forth 55 times before degrading by 20% (Figure 4a). The cycling times were approximately 35 s (*Z*- to *C*-form) and 20 s (*C*- to *Z*-form) in both cases, suggesting that the addition of tributylamine slowed down the photochemical decomposition but not the photochemical reaction. Addition of acetic acid (27 mM) did not affect photochemical stability. The photochemical stability of **4** was not measured in buffer because of the instability of **4C**. Fulgimide **5** cycled back and forth 670 times before degrading by 20% in buffer (Figure 4) with cycling times of 80 s (*Z*- to *C*-form) and 600 s (*C*- to *Z*-form). The increased

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photochemical stability of **5** makes it promising for applications in aqueous solution.

Conclusion

In summary, we have synthesized three novel aqueous soluble indolylfulgimides, **4**, **5**, and **6**. Hydrolysis of the trifluoromethyl group of **4C** was observed in a fluorinated indolylfulgimide for the first time. Hydrolysis of **4C** resulted in **5C**, which was further decarboxylated to **6C** upon extraction. **6C** lacked any photochromic properties. The absorbance maxima of **4Z** varied only slightly between toluene and buffer. A notable blue shift in the absorbance maxima of **5Z** compared to **4Z** was observed in buffer due to the additional carboxylic acid group on the bridging carbon. Fulgimide **5** displayed great thermal and photochemical stabilities in sodium phosphate buffer (pH 7.4). **5Z** and **5C** degraded less than 20% after 500 h at 37 °C, and **5** underwent 670 photochemical cycles before degrading by 20%. Fulgimide **5** is the most robust fulgimide yet reported in aqueous solution.

Experimental Section

General Procedures and Materials. All commercially available materials were used without further purification. ^{1}H and ^{13}C NMR spectra were internally referenced to TMS (0.00 ppm) or solvent (7.26 and 77.00 ppm for $CDCl_3$; 3.31 and 49.00 ppm for CD_3OD; 4.79 ppm for D_2O).

(*Z*)-2-(3-(1-(1,2-Dimethyl-1*H*-indol-3-yl)-2,2,2-trifluoroethylidene)-2,5-dioxo-4-(propan-2-ylidene)pyrrolidin-1-yl)acetic Acid (4). *N*,*N*-Diisopropylethylamine (2.7 g, 20.8 mmol) was added slowly with stirring to a mixture of the HCl salt of glycine methyl ester (1.28 g, 10 mmol) and trifluoromethyl indolylfulgide **1Z** (1.84 g, 5.2 mmol) in 100 mL of acetonitrile at 0 °C. After stirring overnight, the solvent was removed in vacuo. The residue was added to 0.5 M HCl (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by silica gel chromatography (60:40:2 hexane/EtOAc/AcOH) provided 1.94 g of the crude amide acid ester (orange solid). NaOH (1.1 g, 26.5 mmol) was added to the crude amide acid ester in 250 mL of methanol, and the reaction mixture was stirred for 2 h at room temperature. The solvent was then removed in vacuo. The residue was added to 100 mL of Na_2CO_3 (0.19 M) and extracted with ethyl acetate (2×75 mL). The aqueous solution was acidified with 8.0 mL of concd HCl and extracted with ethyl acetate (3×75 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The solvent was concentrated in vacuo to provide 1.75 g of the crude amide acid. AcCl (6.1 g, 78 mmol) was added to the crude amide acid in 100 mL of dichloromethane at reflux, and the reaction mixture was refluxed under Ar for 48 h. The solution was cooled down to room temperature and stirred for 7 d under Ar. The solvent was then removed in vacuo. The residue was added to 100 mL of H_2O and extracted with ethyl acetate (3×75 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (70:30:2 hexane/EtOAc/AcOH) and recrystallized from methanol to provide 0.73 g (35% from **1**) of the carboxylic acid indolylfulgimide **4**. *Z*-form: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.39 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.19 (td, J = 7.4, 1.1 Hz, 1H), 7.09 (td, J = 7.5, 0.9 Hz, 1H), 4.36 (s, 2H), 3.73 (s, 3H), 2.26 (s, 3H), 2.11 (s, 3H), 0.96 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 169.4, 167.9, 165.7, 156.1, 139.1, 138.5, 133.8, 130.5 (q, J = 36 Hz), 126.8, 124.0 (q, J = 272 Hz), 123.8, 122.9, 121.7, 120.2, 110.5, 108.3, 39.7, 30.2, 26.8, 22.3, 12.0. Anal. Calcd for $C_{21}H_{19}F_3N_2O_4$: C, 60.00; H, 4.56; N, 6.66. Found: C, 60.28; H, 4.89; N, 6.39. *C*-form: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.67 (d, J = 8.3 Hz, 1H), 7.38 (td, J = 7.8, 1.1 Hz, 1H), 6.77 (t, J = 7.9 Hz, 2H), 4.24 (d, J = 17.7 Hz, 1H), 4.20 (d, J = 17.7 Hz, 1H), 2.96 (s, 3H), 1.81 (s, 3H), 1.37 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 171.2, 170.0, 167.1, 161.7, 161.6, 141.4, 137.1, 136.3, 129.1 (q, J = 7 Hz), 124.2 (q, J = 272 Hz), 120.5, 119.9, 110.9, 106.8 (q, J = 37 Hz), 77.3, 40.2, 39.6, 33.0, 19.9, 19.6, 14.8. HRMS (ESI$^+$) calcd for $C_{21}H_{19}F_3N_2O_4$ $(M + Na)^+$ 443.1195, obsd 443.1195.

2-(Carboxymethyl)-4,4,4a,5-tetramethyl-1,3-dioxo-1,2,3,4,4a,5-hexahydropyrrolo[3,4-*b*]carbazole-10-carboxylic Acid (5). Carboxylic acid indolylfulgimide **4Z** (0.19 g, 0.45 mmol) in 250 mL of toluene was irradiated with 405 nm light to obtain the photostationary state. Purification of the resulting **4C** was performed via silica gel chromatography (70:30:2 hexane/EtOAc/AcOH) followed by recrystallization from CH_2Cl_2/hexane to provide 0.14 g (74%) of **4C**. Fulgimide **4C** (0.10 g, 0.24 mmol) in 50 mL of 50 mM sodium phosphate buffer (pH 7.4) was incubated at 37 °C for 12 h. The solution was then acidified with dilute HCl (1 M) to pH 5 and extracted with ethyl acetate (3×25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification was performed via recrystallization from CH_2Cl_2/toluene to provide 40 mg (42%) of the dicarboxylic acid indolylfulgimide **5**. *C*-form: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.72 (d, J = 8.3 Hz, 1H), 7.30 (td, J = 8.1, 1.2 Hz, 1H), 6.67 - 6.71 (m, 2H), 4.22 (d, J = 17.3 Hz, 1H), 4.17 (d, J = 17.8 Hz, 1H), 2.98 (s, 3H), 1.81 (s, 3H), 1.40 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.3, 170.5, 169.9, 168.2, 159.7, 156.6, 137.8, 137.6, 135.4, 126.8, 122.1, 119.5, 110.9, 110.2, 74.3, 41.4, 39.3, 32.1, 20.4, 19.2, 15.8. Anal. Calcd for $C_{21}H_{20}N_2O_6$: C, 63.63; H, 5.09; N, 7.07. Found: C, 63.63; H, 5.34; N, 6.89. *Z*-form: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.46 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.20 (t, J = 7.5, 1H), 7.07 (t, J = 7.7, 1H), 4.34 (s, 2H), 3.74 (s, 3H), 2.29 (s, 3H), 2.26 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.6, 170.7, 169.0, 168.1, 154.1, 140.2, 138.5, 136.8, 126.8, 123.6, 123.4, 123.2, 121.6, 120.3, 110.6, 110.1, 39.7, 30.2, 26.6, 22.6, 12.0. HRMS (ESI$^+$) calcd for $C_{20}H_{20}N_2O_6$ $(M + Na)^+$ 419.1246, obsd 419.1233.

2-(4,4,4a,5-Tetramethyl-1,3-dioxo-4a,5-dihydropyrrolo[3,4-*b*]carbazol-2(1*H*,3*H*,4*H*)-yl)acetic Acid (6). Fulgimide **4C** (0.142 g, 0.32 mmol) in 250 mL of 50 mM sodium phosphate buffer (pH 7.4) was incubated at 37 °C for 12 h. The solution was then acidified with concd HCl to pH 1 and extracted with ethyl acetate (3 × 75 mL). The combined organic layers were left overnight, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification was performed via recrystallization from CH_2Cl_2/hexane to provide 76 mg (64%) of the carboxylic acid indolylfulgimide **6**. *C*-form: ^{1}H NMR (D_2O, 400 MHz) δ 7.58 (d, J = 7.6 Hz, 1H), 7.34 (td, J = 7.7, 1.2 Hz, 1H), 6.79 (td, J = 7.5, 0.8 Hz, 1H), 6.74 (d, J = 8.3, 1H), 6.52 (s, 1H), 4.00 (s, 2H), 3.93 (s, 3H), 1.74 (s, 3H), 1.33 (s, 3H), 1.14 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0, 170.0, 168.5, 157.8, 157.4, 138.9, 135.2, 133.4, 123.5, 123.4, 118.8, 109.1, 100.3, 72.8, 41.3, 38.6, 31.9, 20.2, 19.3, 16.1. HRMS (ESI$^+$) calcd for $C_{20}H_{20}N_2O_4$ $(M + Na)^+$ 375.1321, obsd 375.1323. *E*-form: ^{1}H NMR (D_2O, 400 MHz) δ 7.87 (s, 1H), 7.51 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.30 (td, J = 7.4, 1.0 Hz, 1H), 7.17 (td, J = 7.6, 1.0 Hz, 1H), 4.17 (s, 2H), 3.75 (s, 3H), 2.45 (s, 3H), 2.31 (s, 3H), 1.19 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2, 169.6, 168.2, 152.1, 141.2, 136.9, 127.5, 126.4, 123.4, 122.1, 121.3, 121.0, 120.1, 110.4, 109.4, 38.9, 30.2, 26.7, 22.2, 11.6. HRMS (ESI$^+$) calcd for $C_{20}H_{20}N_2O_4$ $(M + Na)^+$ 375.1321, obsd 375.1305.

Spectral Determination. Concentrated, air-saturated stock solutions of **4Z** in toluene and 50 mM sodium phosphate buffer (pH 7.4) were prepared in duplicate or triplicate. From each stock solution, five samples ranging in concentration from

0.25 to 0.05 mM were then prepared by dilution with toluene or buffer. A UV−vis spectrum was then acquired for each sample. Extinction coefficients and λ_{max} were determined. According to ^{1}H NMR data, Z/E isomerization in D_2O with sodium phosphate buffer at room temperature in 1 h was insignificant (1−2%). No isomerization was observed in toluene.

A concentrated, air-saturated stock solution of **4C** (see synthesis) in toluene was diluted to four or five different concentrations, and their UV−vis spectra were obtained. Each **4C** solution was then quantitatively converted to **4Z** solution by illumination with 515 nm light, and the concentration of fulgimide present was ascertained using the predetermined extinction coefficient of **4Z**. Since the original concentration of **4C** will be equivalent to the final concentration of **4Z**, the original concentration of **4C** was determined. The extinction coefficient and λ_{max} for **4C** were then determined from the initial spectra.

The extinction coefficient and λ_{max} for **5C** in 50 mM sodium phosphate buffer (pH 7.4) were determined in the same manner as for **4Z**. To obtain these values for **5Z**, four or five diluted **5C** solutions in buffer were then quantitatively converted to **5Z** solutions by irradiation with 515 nm light. UV−vis spectra of freshly prepared **5Z** solutions were measured, and the extinction coefficient was obtained using the previously determined extinction coefficient of **5C**. Typical error was 3%.

Photostationary State (PSS) Measurements. A solution of **4Z** in toluene-d_8 was illuminated with 405 nm light, and the *Z*:*E*:*C* ratio was monitored via ^{1}H NMR spectroscopy until PSS_{405nm} was achieved. To measure the PSS_{405nm} of **5** in D_2O with 50 mM sodium phosphate buffer (pD 7.4), a solution of **5C**, which was initially obtained from **4C**, was converted to **5Z** using 515 nm light. PSS was then achieved by irradiation of **5Z** with 405 nm light. *Z*:*E*:*C* ratio was monitored by ^{1}H NMR spectroscopy.

Thermal/Hydrolytic Stability. The thermal/hydrolytic stability of the *Z*- and *C*-forms of fulgimides **4** and **5** was measured using UV−vis and ^{1}H NMR spectroscopy. Solutions of **4Z** were prepared in toluene or 50 mM sodium phosphate buffer (pH 7.4) and transferred into several ampules. NMR samples of **4Z** were prepared in toluene-d_8 or D_2O with 50 mM sodium phosphate buffer (pD 7.4). UV−vis and ^{1}H NMR spectra of these initial samples were then acquired. Ampules and NMR tubes were sealed and incubated in water baths that were maintained at 80 °C (toluene) or at 37 °C (buffer). At predetermined times, ampules and NMR tubes were removed, and their contents were analyzed by UV−vis and ^{1}H NMR spectroscopy, respectively. UV−vis and ^{1}H NMR spectra were then compared to the initial spectra. The thermal stability of **4C** in toluene was measured using a PSS_{405nm} solution and evaluated as described for **4Z**. In the case of **4C** in buffer, the UV−vis spectra of **4C** and its decomposition product were almost identical, and thus UV−vis spectroscopy was not used to follow the decomposition of **4C**; only the ^{1}H NMR experiment was performed. Several pure **4C** solutions were prepared in D_2O with buffer (pD 7.4) and transferred into several ampules, which were then placed in a water bath maintained at 37 °C. At prescribed times, solutions were transferred into NMR tubes, and their spectra were taken immediately. To determine the stability of **5C** and **5Z** in buffer, pure **5C** solutions were used while **5Z** solutions were prepared by irradiation of a **5C** solution with 515 nm light. These **5C** and **5Z** solutions were then analyzed in the same manner as **4Z**. In addition, decomposition of **5C** in D_2O with buffer (pD 7.4) was also followed by UV−vis spectroscopy. Typical error was 5×10^{-5} h^{-1} with the exception of **4Z** in toluene which was 0.003 h^{-1}.

For ^{1}H NMR spectroscopy, the residual toluene resonance (toluene) or added DMSO resonance (buffer) were utilized as internal standards, and signals corresponding to the individual species were integrated relative to the internal standards. To confirm the solvent isotope effect for **5C** in buffer, two experiments in D_2O and H_2O buffer solutions were performed simultaneously and followed by UV−vis spectroscopy.

Photochemical Stability. Air-saturated solutions of **4Z** in toluene and in toluene in the presence of an excess of tributylamine (27 mM) or acetic acid (27 mM) were prepared with initial absorbencies of 0.6−0.8 at the absorption maxima. Samples were irradiated to PSS_{405nm} with 405 nm light, and the absorbencies at λ_{max} were measured. Then, in three cases (toluene, toluene/tributylamine, toluene/acetic acid), fresh **4Z** solutions were irradiated to 90% of PSS_{405nm}. The time taken to achieve 90% of the absorbance at PSS_{405nm} was then recorded (coloration reaction *Z* to *C*). The 90% PSS mixture was then irradiated with 515 nm light using a separate filter. The time taken for the absorbance at λ_{max} of the *C*-form to reach $<1\%$ was recorded (decolorization reaction *C* to *Z*). Once the duration of irradiation was established for both the 90% PSS_{405nm} coloration and $<1\%$ *C*-form decolorization reactions, the system was automated through the use of a filter switch. All solutions were capped and stirred. After a designated number of irradiation cycles (coloration followed by decolorization), the samples were fully converted to PSS_{405nm}, and their UV−vis spectra scanned. The photochemical stability was then determined by comparison with the initial PSS_{405nm} (PSS at zero irradiation cycles) absorption spectra.

To measure the photochemical stability of **5** in 50 mM sodium phosphate buffer (pH 7.4) the freshly obtained **5C** solutions were quantitatively converted to **5Z** solutions by irradiating with 515 nm light. The same procedure as described for **4** was then applied. A control experiment to investigate the thermal decomposition of **5Z** at room temperature after 120 h was also performed. After 120 h, besides *Z* to *E* isomerization, the thermal decomposition was determined by ^{1}H NMR to be 1%. *Z* to *E* isomerization will not affect the photochemical decomposition results as these two forms are interconverted photochemically under our conditions. Typical error was 20%.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **4**, **5**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.