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# Preparation and Characterization of Thermochemiluminescent Acridine-Containing 1,2-Dioxetanes as Promising Ultrasensitive Labels in Bioanalysis

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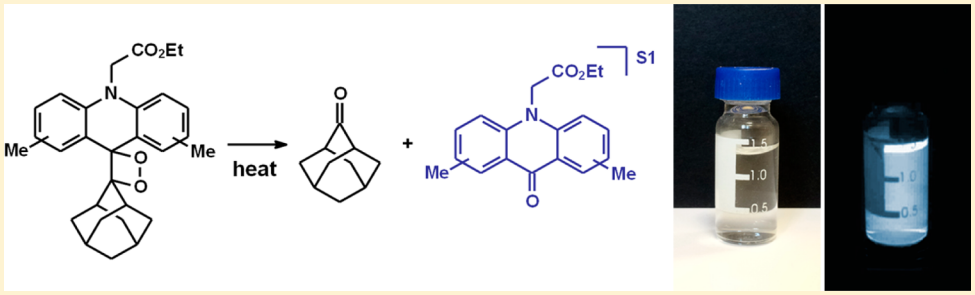
Supporting Information




**ABSTRACT:** Thermochemiluminescence is the luminescence process in which a thermodynamically unstable molecule decomposes with light emission when heated above a threshold temperature. We recently reported the thermochemiluminescence properties of an acridine-containing 1,2-dioxetane, which emits at relatively low temperatures (i.e., below 100 °C). Herein, we explored the effect of the introduction of methyl substituents in the acridine system. The methyl group did not determine an excessive destabilization of 1,2-dioxetane ring nor significantly affect the general physical properties of the molecule. Monosubstituted methyl derivatives and a series of derivatives bearing several combinations of two, three, and four methyl groups were prepared. The rate of formation of 1,2-dioxetane derivatives **1b**−**k** strongly depended on the methyl substitution pattern. All members of this library of mono-, di-, tri-, and tetramethyl-substituted derivatives were characterized in terms of photophysical and thermochemiluminescence properties. The introduction of methyl groups into the acridine ring caused a marked decrease in the activation energy of the thermochemiluminescent reaction. Tri- and tetramethyl-substituted acridones had the highest fluorescence quantum yields, in the range 0.48−0.52, and the corresponding 1,2-dioxetanes **1h** and **1j** showed in thermochemiluminescence imaging experiments limit of detection values more than ten times lower with respect to the unsubstituted derivative.

## INTRODUCTION

Thermochemiluminescence (TCL) is the luminescence process in which a thermodynamically unstable molecule decomposes with light emission when heated above a threshold temperature. This process is closely related to other luminescence phenomena such as photoluminescence, biochemiluminescence (BL-CL), and electrochemiluminescence (ECL), in which the excited-state molecule responsible for light emission is generated through different processes (i.e., light absorption and chemical or electrochemical reactions) rather than heating of a thermally unstable precursor. Even though TCL was proposed in the late 1980s as a luminescence detection technique for bioanalytical applications (e.g., immunoassays),[1] it did not achieve the success of other luminescence detection techniques. The main drawbacks that limited the use of TCL were the high temperatures required to trigger the emission (typically in the 200−250 °C range) and the relatively low emission intensities, which determined a poor sensitivity of TCL-based bioanalytical applications. Nevertheless, TCL still could offer interesting and largely unexplored analytical opportunities. For instance, as in BL-CL and ECL, there is no nonspecific signal due to the matrix components; this simply triggered by heat, TCL would allow for highly sensitive reagent-free detection.

Since the late 1960s, when 3,3,4-trimethyl-1,2-dioxetane was synthesized,[2] a number of 1,2-dioxetanes have been proposed as CL and TCL substrates.[3] 1,2-Dioxetanes undergo thermally induced decomposition into two carbonyl compounds, one of which can be formed in an electronically excited state. The excited product can thus emit light, either directly through its radiative decay to the ground state (direct CL) or indirectly via

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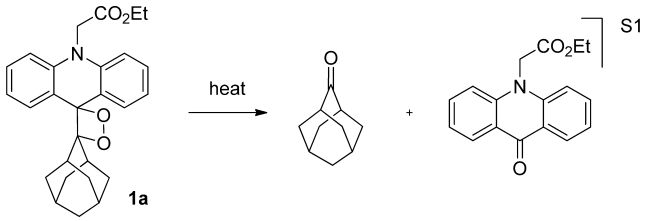
a nonradiative energy transfer to a fluorescent acceptor molecule (indirect CL). The latter case is exploited to amplify the CL signal when the excited product is a poor fluorophore.

Adamantylidene adamantane 1,2-dioxetane was the first 1,2-dioxetane proposed as a TCL label for bioanalytical applications,[1] and even now 1,2-dioxetanes represent the main TCL species. We recently reported an efficient synthesis of the thermochemiluminescent acridine-containing 1,2-dioxetane **1a**, which decomposes at relatively low temperature (<100 °C) into 2-adamantanone and ethyl 9-oxo-10(9*H*)-acridine acetate. The latter compound, obtained in the singlet excited state, is responsible for the TCL emission (Scheme 1).[4] Two

**Scheme 1. Thermally Induced Fragmentation of 1a**




structural properties of **1a** deserve a comment: (i) the crucial stabilizing role played, as in the majority of 1,2-dioxetane substrates, by the spiro-bonded adamantylidene group, which sterically protects the dioxetane ring from spontaneous decomposition at room temperature,[5] and (ii) the presence of an ester group, which provides a binding site useful for bioanalytical applications of **1a**, e.g., for labeling analytes with this TCL molecule.

It should be pointed out that the reaction mechanism of acridine-containing 1,2-dioxetanes is different from that of "classical" TCL 1,2-dioxetanes such as the adamantylidene adamantane 1,2-dioxetane. Indeed, alkyl- and aryl-substituted 1,2-dioxetanes decompose upon heating at relatively high temperatures with low singlet excited state formation efficiency.[3a,6] Different reaction pathways have been proposed, including a synchronous, concerted mechanism[7] and the formation of a biradical intermediate.[8] Further studies suggested that neither extreme is in accordance with the reaction mechanism and an intermediate asynchronous concerted mechanism has been described.[9] *N*-Substituted acridine-containing 1,2-dioxetanes are generally less stable than alkyl- and aryl-substituted 1,2-ones and show higher singlet excited-state formation efficiency.[10] Their proposed thermal decomposition mechanism[5,10,11] postulated an intramolecular electron transfer from the lone electron pair of the acridinic nitrogen to the 1,2-dioxetane ring, followed by cleavage of the O−O bond and formation of a pair of radical ions. Then, a back-electron transfer from the radical anion to the radical cation produced the singlet excited-state *N*-alkylacridone carbonyl fragment. Recently, the involvement of an intramolecular electron transfer from the acridinium moiety to the peroxide ring has been shown experimentally to occur.[12]

To improve the performance of **1a**, we introduced suitable structural modifications in order to (i) decrease the TCL emission triggering temperature and (ii) increase the TCL emission intensity by producing more efficient fluorophores as excited state-products, thus optimizing detectability with no need of an energy acceptor. A classic approach in optimization of emission properties of aromatic molecules relies on the introduction of substituents onto the aromatic scaffold. Therefore, we explored the effect of methyl substituents in the acridine system. The methyl group was selected because of its relatively small electro-donating effect, which should not determine an excessive destabilization of the 1,2-dioxetane ring. Moreover, contrary to strong electron-withdrawing or -donating groups, it does not significantly affect the general physical properties of the molecule, such as the solubility profile. In more detail, the four different monosubstituted methyl derivatives and a series of derivatives bearing several combinations of two, three, and four methyl groups were prepared. All members of this library of mono-, di-, tri-, and tetramethyl-substituted derivatives were characterized in terms of photophysical and TCL properties.

## RESULTS AND DISCUSSION

We report here the synthesis of a family of methyl-substituted 1,2-dioxetane derivatives with formulas **1b−k** (Figure 1) and the photophysical and TCL properties of these temperature-sensitive molecules with the aim to correlate TCL properties, i.e., triggering temperatures and TCL emission intensity, to structural features.

The general synthetic pathway followed to obtain the methyl-substituted acridine-containing 1,2-dioxetanes **1b−k** is reported in Scheme 2. An Ullmann-type coupling[13] of properly substituted anilines and 2-bromo- or 2-iodobenzoic acids provided 2-aminoarylbenzoic acids **2b−k** successively treated with $POCl_3$ to give the corresponding substituted acridones **3b−k**.[14] After *N*-alkylation with ethyl bromoacetate,[15] the resulting ethyl 9-oxo-10(9*H*)-acridineacetates **4b−k** were reductively coupled with 2-adamantanone under McMurry conditions[16] to give **5b−k**, which were photooxygenated using

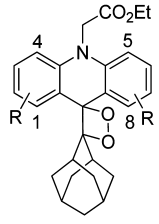
|   **1a** R = H **1b-k** R = Me | Monosubstituted derivatives | Disubstituted derivatives | Trisubstituted derivatives | Tetrasubstituted derivatives |
|---|---|---|---|---|
| | **1b**: 1-Me | **1f**: 2,7-diMe | **1h**: 2,3,7-triMe | **1j**: 2,3,6,7-tetraMe |
| | **1c**: 2-Me | **1g**: 1,5-diMe | **1i**: 1,2,7-triMe | **1k**: 1,2,6,7-tetraMe |
| | **1d**: 3-Me | | | |
| | **1e**: 4-Me | | | |

**Figure 1.** Structures of the methyl-substituted 1,2-dioxetane derivatives. Each member of this family is identified on the basis of the methyl substitution pattern on the acridine ring system.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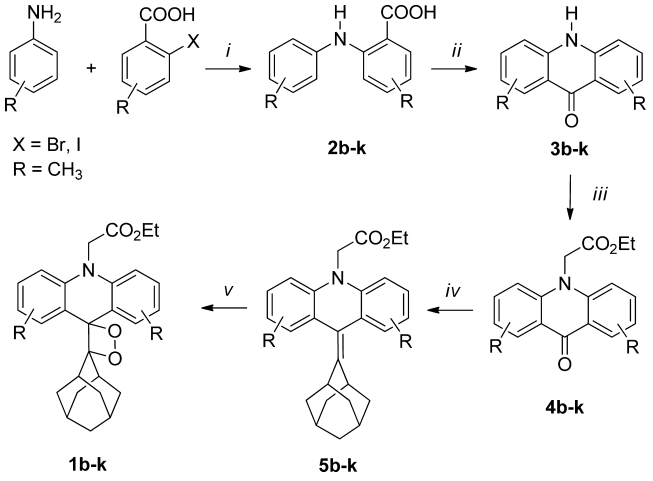
**Scheme 2**[a]




[a]Conditions: (i) $Cu/K_2CO_3$, 1-pentanol, reflux; (ii) $POCl_3$, $CH_3CN$/water, reflux; (iii) NaH, $BrCH_2CO_2Et$, $Bu_4NI$, DMF, rt; (iv) 2-adamantanone, $TiCl_3/LiAlH_4$, $Et_3N$, THF, reflux; (v) polymer-bound Bengal Rose or Methylene Blue, $h\nu$, $O_2$, $CH_2Cl_2$, 0 °C.

Bengal Rose or Methylene Blue as sensitizers to yield the 1,2-dioxetane derivatives **1b–k**.[3j,17]

The critical step of the whole synthetic pathway is the photooxygenation of the tetrasubstituted double bond of compounds **5**. The reaction is very sensitive to the $\pi$-electron density of the double bond; thus, electron-rich olefins undergo easier [2 + 2] cycloaddition reactions with singlet oxygen.[18]

The rate of formation of 1,2-dioxetane derivatives **1b–k** strongly depended on the methyl substitution pattern. While the olefinic precursor of the unsubstituted 1,2-dioxetane **1a** required about 12 h to undergo quantitative photooxygenation, under the same experimental conditions the monomethyl olefins **5c–e** required 7–10 h, the disubstituted olefin **5f** 2 h, and the tri- and tetrasubstituted olefins **5h** and **5j** only 1 h. On the other hand, as demonstrated by HPLC–MS analysis, only trace amounts of 1,2-dioxetane derivatives were observed after photooxygenation of olefins **5b**, **5g**, **5i**, and **5k**, even after 20 h of irradiation using polymer-bound Bengal Rose as sensitizer. Furthermore, no products were observed in the same conditions using Methylene Blue as sensitizer. Since these four substrates share a methyl substituent on position 1 of the acridine system, thus in proximity to the carbon–carbon double bond, it can be concluded that in these compounds the [2 + 2] cycloaddition reaction is inhibited for steric reasons. For the other substrates, the reaction rate increases with the number of methyl groups onto the acridine moiety, indicating that the higher electron density on the double bond promotes a faster [2 + 2] cycloaddition of the singlet oxygen.

We already demonstrated that the TCL emission of **1a** was due to the formation of singlet excited-state acridone.[4] According to this finding, to investigate the TCL properties of the 1,2-dioxetanes **1b–k** we first studied the photophysical properties of substituted acridone derivatives **4b–k**, i.e., the expected emitting species of the TCL reactions. Absorption and fluorescence data (maximum absorption wavelengths, molar absorption coefficients, maximum emission wavelengths and fluorescence quantum yields) obtained for the compounds **4b–k** are collected in Table 1. For comparison, the unsubstituted acridone derivative **4a** has absorption maxima at $\lambda$ = 250 and 390 nm with molar absorption coefficients of 46300 and 11100

**Table 1. Photophysical Properties of Acridone Derivatives 4b–k**

| compd | $\lambda_{max}$[a] (nm) | $\varepsilon$[a] (L mol$^{-1}$ cm$^{-1}$) | $\lambda_{em}$[a] (nm) | $\phi_F$[a,b] |
|---|---|---|---|---|
| **4b** | 255 | 51900 | 406 | 0.02 |
| | 390 | 10400 | | |
| **4c** | 252 | 54300 | 411 | 0.30 |
| | 397 | 11400 | | |
| **4d** | 255 | 55500 | 401 | 0.21 |
| | 387 | 12000 | | |
| **4e** | 254 | 23100 | 422 | 0.09 |
| | 390 | 3000 | | |
| **4f** | 256 | 77000 | 418 | 0.42 |
| | 403 | 12300 | | |
| **4g** | 255 | 55100 | 424 | 0.04 |
| | 388 | 8500 | | |
| **4h** | 253 | 50600 | 416 | 0.52 |
| | 399 | 10500 | | |
| **4i** | 254 | 48400 | 422 | 0.10 |
| | 395 | 8900 | | |
| **4j** | 254 | 63700 | 411 | 0.48 |
| | 404 | 12400 | | |
| **4k** | 261 | 59500 | 418 | 0.08 |
| | 399 | 7800 | | |

[a]Determined in acetonitrile solution. [b]Determined using quinine sulfate as standard ($\phi_F$ = 0.53 in $H_2SO_4$ 0.05 mol L$^{-1}$).

L mol$^{-1}$ cm$^{-1}$, respectively, $\lambda_{em}$ = 403 nm and $\phi_F$ = 0.11. The experimental results suggest that the introduction of methyl groups onto the aromatic system of acridone affects the fluorescence quantum yield through different mechanisms. Compounds containing methyl groups onto positions 2, 3, 6, and 7 (**4c**, **4d**, **4f**, **4h**, **4j**) show a significant increase of the fluorescence quantum yields, which could be due to a decrease of the intersystem crossing rate constant and thus to a lower efficiency of nonradiative deactivation processes, as already reported for aromatic compounds such as anthracene.[19] Tri- and tetramethyl-substituted acridones have the highest fluorescence quantum yield values, in the range 0.48–0.52. On the other hand, the acridone derivatives with methyl groups in positions 4 (compound **4e**) and 1 (compounds **4b**, **4g**, **4i**, **4k**) show quite low fluorescence quantum yields. This behavior can be attributed to the steric hindrance of the methyl substituent, which causes distortion from planarity of the aromatic system.[20]

Upon heating of compounds **1c**, **1d**, **1e**, **1f**, **1h**, and **1j**, a weak TCL emission was observed starting from relatively low temperatures (40–60 °C). With increasing temperature, the TCL reaction became faster and the emission intensity increased. To confirm the nature of the emitting species, we compared the TCL spectra of the 1,2-dioxetane derivatives with the fluorescence spectra of the corresponding acridones **4c**, **4d**, **4e**, **4f**, **4h**, and **4j**. In all cases, the TCL spectra closely matched the fluorescence spectra of the corresponding acridones, thus demonstrating that the TCL emission was primarily due to the singlet excited state of acridone (Figure 2). Singlet excited-state 2-adamantanone could also be produced in the TCL reaction. However, the possible emission of excited 2-adamantanone could not be detected due to the overlap with the fluorescence emission of acridone derivatives and the weak intensity (for 2-adamantanone $\lambda_{max}$ = 425 nm[21] and $\phi_F$ = 0.015[22]).

In the temperature interval between 70 and 120 °C, the TCL emission showed a first-order decay. The activation parameters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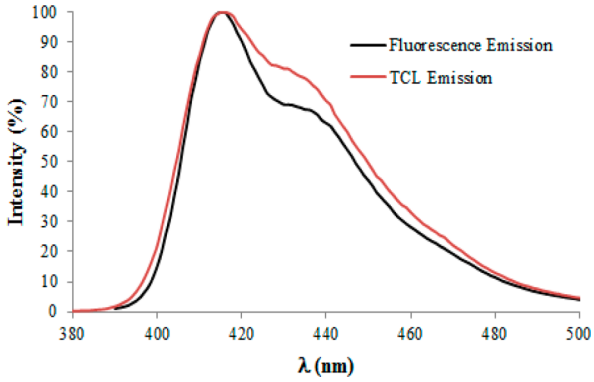


**Figure 2.** Comparison between the TCL emission spectrum of compound **1j** recorded in acetonitrile solution upon heating to 60 °C and the fluorescence emission spectrum of compound **4j** measured in acetonitrile solution ($\lambda_{exc}$ = 380 nm).

of the reaction were determined by a standard isothermal kinetic method: the measurement of the emission decay kinetics at different temperatures allowed us to obtain the kinetic constants $k$ of the TCL reaction, and then the temperature dependence of the kinetic constants was analyzed according to a standard Arrhenius equation to evaluate the activation energies ($E_a$) and the pre-exponential coefficients ($A$) of the TCL reactions (Table 2).

**Table 2. Activation Parameters for the Thermal Decomposition of 1,2-Dioxetane Derivatives 1c–f, 1h, and 1j**

| compd | $E_a$[a] (kcal mol$^{-1}$) | ln $A$[a] (s$^{-1}$) |
|---|---|---|
| **1c** | 25.7 ± 1.6 | 30.9 ± 1.9 |
| **1d** | 24.5 ± 0.3 | 28.1 ± 0.1 |
| **1e** | 23.0 ± 0.4 | 26.2 ± 0.6 |
| **1f** | 19.3 ± 0.5 | 21.4 ± 0.2 |
| **1h** | 18.4 ± 0.2 | 20.9 ± 0.8 |
| **1j** | 16.7 ± 1.6 | 18.6 ± 2.3 |

[a]Mean ± SD of three independent measurements.

For comparison, the parent unsubstituted 1,2-dioxetane **1a** has $E_a$ = 29.7 kcal mol$^{-1}$ and ln $A$ = 38.5 s$^{-1}$, and the activation energies reported for other 1,2-dioxetane derivatives ranged between 12.2 and 32.5 kcal mol$^{-1}$.[3d,j,12a,23] These results clearly show that the introduction of methyl groups into the acridine ring causes a marked decrease in the activation energy of the TCL reaction, which determined a lower triggering temperature of the TCL emission. Nevertheless the methyl-substituted compounds remained stable enough for their handling and storage. Indeed, as shown in Table 2, the decrease in the activation energy due to the presence of methyl groups is paralleled by a reduction in the pre-exponential coefficient. As a result, despite the quite different activation energies, at a given temperature the kinetic rate constants of the TCL reactions of the investigated compounds differ by no more than 1 order of magnitude.

To preliminarily evaluate the suitability of the synthesized 1,2-dioxetane derivatives as labels for biospecific probes in bioassays, we measured their limit of detection (LOD) by TCL imaging. Measurements were performed by imaging arrays of spots containing different amounts of compound using a low-light luminograph equipped by an ultrasensitive, double Peltier-cooled CCD sensor. Samples were deposited as acetonitrile solutions on conventional microscope glass slides glued to a flat heating element or on high-resistivity (70–100 Ω) indium–tin oxide (ITO)-coated microscope glass slides. Then, the TCL signal was imaged upon heating the support. According to the calibration curves so obtained (Figure 3), the LOD values for

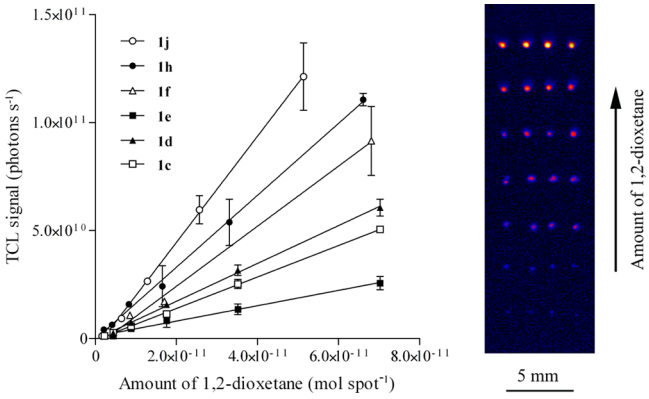


**Figure 3.** Left: TCL imaging calibration curves obtained for the 1,2-dioxetane derivatives **1c–f**, **1h**, and **1j**. Right: TCL image of an ITO-coated glass with spots containing different amounts of the 1,2-dioxetane **1j** (four replicates for each amount of compound). The TCL image is shown in pseudocolors, each color corresponding to a different TCL emission intensity.

the 1,2-dioxetane derivatives **1c–f**, **1h**, and **1j**, defined as the amount of compound giving a signal higher than the background signal plus three times its standard deviation, were calculated (Table 3). For comparison, for the unsubstituted 1,2-dioxetane **1a** the LOD value obtained under similar experimental conditions using an ITO-coated glass slide was 3.5 × 10$^{-13}$ mol spot$^{-1}$.

**Table 3. LOD Values for 1,2-Dioxetane Derivatives 1c–f, 1h, and 1j**

| compd | support | LOD[a] (mol spot$^{-1}$) |
|---|---|---|
| **1c** | glass | (6.0 ± 0.5) × 10$^{-14}$ |
| | ITO-coated glass | (5.4 ± 0.4) × 10$^{-14}$ |
| **1d** | glass | (5.6 ± 0.2) × 10$^{-14}$ |
| | ITO-coated glass | (7.6 ± 0.8) × 10$^{-14}$ |
| **1e** | glass | (1.1 ± 0.3) × 10$^{-13}$ |
| | ITO-coated glass | (4.7 ± 0.7) × 10$^{-13}$ |
| **1f** | glass | (5.1 ± 1.0) × 10$^{-14}$ |
| | ITO-coated glass | (4.1 ± 0.4) × 10$^{-14}$ |
| **1h** | glass | (3.2 ± 0.6) × 10$^{-14}$ |
| | ITO-coated glass | (2.7 ± 0.4) × 10$^{-14}$ |
| **1j** | glass | (1.9 ± 0.3) × 10$^{-14}$ |
| | ITO-coated glass | (2.1 ± 0.3) × 10$^{-14}$ |

[a]Mean ± SD of four measurements.

The highest detectability was obtained for compound **1j**, whose LOD value is 17 times lower than that of the unsubstituted 1,2-dioxetane **1a**. The values reported in Table 3 show a good correlation between the LODs of 1,2-dioxetane derivatives and the fluorescence quantum yields of the corresponding acridone derivatives (i.e., the greater is the fluorescence quantum yield the smaller is the LOD). This suggests that, as expected, the fluorescence quantum yield of the emitting carbonyl fragment is an important factor in


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determining the overall TCL emission intensity of 1,2-dioxetane derivatives.

## CONCLUSIONS

With the aim to design new TCL labels potentially useful in bioanalysis, a family of dispirodioxetanes containing an acridine moiety **1b−k** has been synthesized. The acridine ring system was decorated with one or more methyl groups in different positions to investigate the effect of weak electron donating groups on the photophysical and TCL properties. The behavior of the [2 + 2] cycloaddition reaction and the TCL properties of the 1,2-dioxetanes markedly depend on the substitution pattern of the acridine ring. Indeed, a methyl group in position 1 disfavors both 1,2-dioxetane formation and the cycloreversion responsible for the TCL emission, as expected for a concerted mechanism. On the other hand, an increasing number of methyl groups located in positions 2, 3, 6, and 7 facilitates the [2 + 2] cycloaddition and causes a decrease of the activation energy of the TCL reaction. Moreover, the LOD value of the tetrasubstituted derivative **1j** was 17 times lower than that of the unsubstituted 1,2-dioxetane **1a**. With these compounds in hands, we are investigating their application in ultrasensitive bionalytical applications by binding the TCL molecules to biospecific probes through the properly installed acetic acid appendage. Results will be published in due time.

## EXPERIMENTAL SECTION

**Materials.** All of the chemicals were used as received.

**Characterization of Compounds.** $^1$H and $^{13}$C NMR spectra were recorded on a 200 or 400 NMR instrument with a 5 mm probe. All chemical shifts have been quoted relative to deuterated solvent signals, chemical shifts ($\delta$) are reported in ppm, and coupling constants ($J$) are reported in hertz. HPLC−MS analysis was performed using an HPLC system coupled with a single-quadrupole mass spectrometer. A ZOBRAX-Eclipse XDB-C8 column was employed for the chromatographic separation; mobile phase: $H_2O/CH_3CN$, gradient from 30% to 80% of $CH_3CN$ in 8 min, 80% of $CH_3CN$ until 25 min, 0.4 mL min$^{-1}$. Mass spectrometric detection was performed in full-scan mode from $m/z$ 50 to $m/z$ 2600, scan time 0.1 s in positive ion mode, ESI spray voltage 4500 V, nitrogen gas 35 psi, drying gas flow 11.5 mL min$^{-1}$, fragmentor voltage 20 V. Flash-chromatography was carried out using Merck silica gel 60 (230−400 mesh particle size). Thin-layer chromatography was performed on Merck 60 F254.

**Spectrophotometric Measurements.** Absorption spectra were recorded using a UV−vis spectrophotometer. Fluorescence emission spectra and TCL emission spectra were recorded using a spectrofluorimeter. Fluorescent quantum yields of compounds **4a−k** were measured using quinine sulfate as standard ($\phi_F$ = 0.53 in $H_2SO_4$ 0.05 mol L$^{-1}$).[24]

**TCL Measurements.** TCL imaging experiments were performed with a low-light luminograph. Microscope glass slides glued to a flat heating element or high-resistivity (70−100 Ω) ITO-coated microscope glass slides were used as solid supports. In the latter case, heating was provided by a suitable electrical current flowing through the conductive ITO coating. The temperature of the support was controlled by varying the applied current and monitored by a copper/constantan thermocouple. Samples were deposited on the supports as acetonitrile solutions either with a micropipet or using a manual microarrayer. The manual microarrayer deposited arrays of spots of about 10 nL with diameters in the range of 500−800 $\mu$m depending on the nature of the surface. The spots were allowed to dry before the TCL measurement. TCL images were acquired upon heating the support to the desired temperature (90−100 °C) using integration times varying from 5 s (for evaluation of the TCL decay kinetics) to 5 min (for assessment of the detectability by TCL imaging). Images were then analyzed to measure the TCL signals using the image analysis software provided with the instrument. The kinetic and thermodynamic parameters of the TCL process were obtained by measuring the TCL emission decay kinetics in the temperature range between 70 and 120 °C. For each temperature, the kinetic constant $k$ of the TCL process (i.e., the inverse of the decay time in seconds) was calculated by fitting the decay profile of the TCL emission with the first-order decay equation

$$I_{TCL} = (I_{TCL})_0 e^{-kt} \quad (1)$$

in which $I_{TCL}$ is the TCL signal at time $t$ and $(I_{TCL})_0$ is the TCL signal at zero time. Then, the activation energy $E_a$ and the pre-exponential factor $A$ of the TCL process were calculated from the kinetic constants $k$ measured at different temperatures using the logarithmic form of the Arrhenius equation

$$\ln k = \ln A - (E_a/RT) \quad (2)$$

in which $R$ is the universal gas constant and $T$ is the temperature.

**Synthesis of 2-Iodo-4,5-dimethylbenzoic Acid.**[25] A solution of $NaClO_2$ (80% purity, by iodometric titration, 500 mg, 4.4 mmol) in 4.5 mL of water was added dropwise in 2 h to a stirred mixture of 2-iodo-4,5-dimethylbenzaldehyde (820 mg, 3.1 mmol) in 3.5 mL of acetonitrile, $NaH_2PO_4$ (110 mg) in 1.2 mL of water, and 30% $H_2O_2$ (370 $\mu$L, 3.6 mmol), keeping the temperature at 10 °C with water cooling. After 1 h, a small amount of $Na_2SO_3$ (~30 mg) was added to destroy the unreacted HOCl and $H_2O_2$. Acidification with 10% aqueous HCl afforded 2-iodo-4,5-dimethylbenzoic acid (850 mg, 98% yield) as a crystalline solid: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.27 (s, 6H), 7.84 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 19.3, 19.4, 91.4, 130.0, 133.3, 136.9, 142.8, 143.7, 170.7; MS (ESI) $[M + H]^+$ $m/z$ 277.0. Anal. Calcd for $C_9H_9IO_2$ (275.96): C, 39.16; H, 3.29. Found: C, 39.26; H, 3.31.

**General Procedure for the Synthesis of Compounds 2b−k.** A solution of substituted aniline (6.5 mmol), substituted 2-bromo- or 2-iodobenzoic acid (4.7 mmol), anhydrous $K_2CO_3$ (0.9 g, 6.5 mmol), and copper (57 mg, 0.9 mmol) in anhydrous 1-pentanol (5.0 mL) was heated under reflux for 3 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in hot $H_2O$ (100 mL) and then filtered through Celite. The Celite was washed with $H_2O$ (300 mL), and the filtrate was acidified with concentrated HCl to pH 6. The precipitate was isolated by filtration and washed with $H_2O$. The solid was recrystallized from $CHCl_3$ to give compounds **2b−k**.

**2-(*m*-Tolylamino)benzoic acid (2b):**[26] yield 825 mg (77%).

**5-Methyl-2-(phenylamino)benzoic acid (2c):**[27] yield 761 mg (71%).

**4-Methyl-2-(phenylamino)benzoic acid (2d):** yield 825 mg (77%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.28 (s, 3H), 6.59 (d, $J$ = 8.0, 1H), 7.05 (s, 1H), 7.15 (t, $J$ = 7.6, 1H), 7.28 (d, $J$ = 8.4, 2H), 7.39 (t, $J$ = 7.6, 2H), 7.94 (d, $J$ = 8.4, 1H), 9.30 (br, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 22.1, 107.9, 114.0, 118.6, 123.2, 123.9, 129.4, 132.5, 140.4, 146.3, 148.9, 173.3; MS (ESI) $[M + H]^+$ $m/z$ 228.1. Anal. Calcd for $C_{14}H_{13}NO_2$ (227.09): C, 73.99; H, 5.77; N, 6.16. Found: C, 73.94; H, 5.76; N, 6.16.

**2-(*o*-Tolylamino)benzoic acid (2e):**[26a] yield 793 mg (74%).

**5-Methyl-2-(*p*-tolylamino)benzoic acid (2f):**[28] yield 967 mg (85%).

**6-Methyl-2-(*o*-tolylamino)benzoic acid (2g):** yield 762 (67%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.27 (s, 3H), 2.61 (s, 3H), 6.64 (d, $J$ = 7.6, 1H), 6.76 (d, $J$ = 8.4, 1H), 7.06−7.22 (m, 3H), 7.28 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 18.0, 23.8, 112.8, 121.2, 123.8, 124.4, 126.7, 131.1, 132.5, 133.0, 139.5, 142.1, 148.8, 175.0; MS (ESI) $[M + H]^+$ $m/z$ 242.1. Anal. Calcd for $C_{15}H_{15}NO_2$ (241.11): C, 74.67; H, 6.27; N, 5.81. Found: C, 74.35; H, 6.29; N, 5.87.

**2-((3,4-Dimethylphenyl)amino)-5-methylbenzoic acid (2h):** yield 951 mg (79%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.27 (s, 9H), 7.02 (m, 2H), 7.12 (m, 2H), 7.18 (d, $J$ = 8.8, 1H), 7.84 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 19.1, 19.8, 20.2, 110.0, 114.3, 120.4, 124.5, 125.8, 130.3, 132.0, 132.1, 136.2, 137.6, 138.4, 147.3, 173.6; MS (ESI)

$[M + H]^+$ $m/z$ 256.1. Anal. Calcd for $C_{16}H_{17}NO_2$ (255.13): C, 75.27; H, 6.71; N, 5.49. Found: C, 74.90; H, 6.72; N, 5.44.

**2-((3,4-Dimethylphenyl)amino)-4,5-dimethylbenzoic acid (2j):** yield 1.05 g (83%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.19 (s, 6H), 2.28 (s, 6H), 7.02 (m, 3H), 7.13 (d, $J$ = 8.4, 1H), 7.78 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 18.6, 19.1, 19.8, 20.5, 115.0, 120.5, 124.6, 125.3, 130.3, 132.0, 132.5, 137.6, 138.4, 145.2, 147.6, 173.7; MS (ESI) $[M + H]^+$ $m/z$ 270.0. Anal. Calcd for $C_{17}H_{19}NO_2$ (269.14): C, 75.81; H, 7.11; N, 5.20. Found: C, 75.60; H, 7.07; N, 5.18.

**General Procedure for the Synthesis of Compounds 3b–k.** Compounds **2b–k** (4.0 mmol) were dissolved in $CH_3CN$ (9 mL) and heated to reflux. Phosphorus(V) oxychloride (1.35 g, 8.8 mmol) was added over 1 h. The solution was refluxed for further 2 h and then cooled to 10–15 °C. $H_2O$ (5 mL) was added, and the mixture was heated to reflux for 2.5 h. The suspension was cooled to 10 °C and filtered. The solid was washed with $H_2O$ and $CH_3CN$ and then dried under vacuum to obtain **3b–k**.[29]

**1-Methylacridin-9(10*H*)-one (3b):**[30] yield 301 mg (36%).
**2-Methylacridin-9(10*H*)-one (3c):**[31] yield 829 mg (99%).
**3-Methylacridin-9(10*H*)-one (3d):**[31] yield 578 mg (69%).
**4-Methylacridin-9(10*H*)-one (3e):**[31] yield 720 mg (86%).

**2,7-Dimethylacridin-9(10*H*)-one (3f):** yield 887 mg (99%); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.41 (s, 3H), 7.43 (d, $J$ = 8.4, 2H), 7.54 (d, $J$ = 8.4, 2H), 8.00 (s, 2H), 11.58 (s, 1H); $^{13}$C NMR (100 MHz, $d_6$-DMSO) $\delta$ 21.3, 118.0, 122.2, 125.8, 130.6, 135.4, 139.7, 170.6; MS (ESI) $[M + H]^+$ $m/z$ 224.1. Anal. Calcd for $C_{15}H_{13}NO$ (223.10): C, 80.69; H, 5.87; N, 6.27. Found: C, 80.25; H, 5.93; N, 6.24.

**1,5-Dimethylacridin-9(10*H*)-one (3g):** yield 161 mg (18%); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.56 (s, 3H), 2.86 (s, 3H), 6.96 (d, $J$ = 6.8, 1H), 7.11 (t, $J$ = 7.6, 1H), 7.52 (m, 2H), 7.75 (d, $J$ = 8.4, 1H), 8.06 (d, $J$ = 7.6, 1H), 10.32 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 17.6, 23.7, 116.3, 118.9, 120.4, 122.0, 123.8, 124.1, 124.7, 132.2, 133.7, 138.8, 140.1, 142.7, 179.1; MS (ESI) $[M + H]^+$ $m/z$ 224.1. Anal. Calcd for $C_{15}H_{13}NO$ (223.10): C, 80.69; H, 5.87; N, 6.27. Found: C, 81.07; H, 5.88; N, 6.22.

**2,3,7-Trimethylacridin-9(10*H*)-one (3h) and 1,2,7-trimethylacridin-9(10*H*)-one (3i):** yield 494 mg (52%) (mixture of **3h** and **3i**).

**2,3,6,7-Tetramethylacridin-9(10*H*)-one (3j) and 1,2,6,7-tetramethylacridin-9(10*H*)-one (3k):** yield 653 mg (65%) (mixture of **3j** and **3k**).

**General Procedure for the Synthesis of Compounds 4b–k.** A solution of **3b–k** (0.9 mmol) in DMF (4 mL) was added to a suspension of NaH (29 mg, 1.2 mmol) in dry DMF (2 mL). The mixture was stirred for 30 min at room temperature, and then, after the mixture was cooled to 0 °C, ethyl 2-bromoacetate (234 mg, 1.4 mmol) and tetrabutylammonium iodide (4 mg, 0.01 mmol) were added. The solution was stirred for further 24 h at room temperature and then poured into cold water. The precipitate was collected by filtration, dried under vacuum, and purified by flash chromatography on silica gel using 9:1 (v/v) dichloromethane/ethyl acetate as the eluent to obtain **4b–k**.

**1-Methyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4b):** yield 173 mg (65%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.31 (t, $J$ = 7.0, 3H), 3.00 (s, 3H), 4.33 (q, $J$ = 7.0, 2H), 5.00 (s, 2H), 7.07 (d, $J$ = 7.2, 1H), 7.11 (d, $J$ = 8.4, 1H), 7.25 (m, 2H), 7.53 (t, $J$ = 8.0, 1H), 7.66 (t, $J$ = 7.2, 1H), 8.48 (dd, $J_1$ = 7.6, $J_2$ = 8.0, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 24.5, 49.4, 62.1, 112.3, 113.8, 121.4, 121.6, 124.1, 125.2, 127.8, 132.8, 133.5, 141.8, 143.0, 144.0, 168.5, 180.1; MS (ESI) $[M + H]^+$ $m/z$ 296.1. Anal. Calcd for $C_{18}H_{17}NO_3$ (295.12): C, 73.20; H, 5.80; N, 4.74. Found: C, 72.90; H, 5.79; N, 4.75.

**2-Methyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4c):**[32] yield 184 mg (69%); $^{13}$C NMR (100 MHz, CDCl3) $\delta$ 14.2, 20.6, 48.4, 62.1, 114.0, 114.1, 121.5, 122.5, 127.3, 128.0, 131.5, 133.9, 135.4, 140.4, 142.2, 168.3, 178.1; MS (ESI) $[M + H]^+$ $m/z$ 296.1. Anal. Calcd for $C_{18}H_{17}NO_3$ (295.12): C, 73.20; H, 5.80; N, 4.74. Found: C, 73.54; H, 5.81; N, 4.71.

**3-Methyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4d):** yield 99 mg (37%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.28 (t, $J$ = 7.0, 3H), 2.49 (s, 3H), 4.30 (q, $J$ = 7.0, 2H), 5.02 (s, 2H), 7.05 (s, 1H), 7.11 (d, $J$ = 8.4, 1H), 7.27 (m, 2H), 7.67 (m, 1H), 8.42 (d, $J$ = 8.0, 1H), 8.53 (dd, $J_1$ = 8.0, $J_2$ = 8.0, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 22.5, 48.4, 62.1, 114.0, 114.1, 120.7, 121.7, 122.7, 123.5, 127.9, 129.7, 133.8, 142.3, 142.5, 145.1, 168.4, 177.9; MS (ESI) $[M + H]^+$ $m/z$ 296.1. Anal. Calcd for $C_{18}H_{17}NO_3$ (295.12): C, 73.20; H, 5.80; N, 4.74. Found: C, 73.39; H, 5.81; N, 4.74.

**4-Methyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4e):** yield 51 mg (19%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.19 (t, $J$ = 7.0, 3H), 2.62 (s, 3H), 4.22 (q, $J$ = 7.0, 2H), 4.89 (s, 2H), 7.29 (m, 3H), 7.53 (d, $J$ = 6.8, 1H), 7.67 (m, 1H), 8.36 (d, $J$ = 7.6, 1H), 8.43 (dd, $J_1$ = 7.6, $J_2$ = 8.0, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.0, 21.8, 55.8, 61.8, 116.5, 122.3, 122.8, 123.9, 125.5, 125.9, 126.2, 127.3, 133.9, 137.7, 145.4, 146.4, 169.9, 179.2; MS (ESI) $[M + H]^+$ $m/z$ 296.1. Anal. Calcd for $C_{18}H_{17}NO_3$ (295.12): C, 73.20; H, 5.80; N, 4.74. Found: C, 73.13; H, 5.78; N, 4.73.

**2,7-Dimethyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4f):** yield 165 mg (59%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.28 (t, $J$ = 7.0, 3H), 2.48 (s, 6H), 4.30 (q, $J$ = 7.0, 2H), 5.04 (s, 2H), 7.22 (d, $J$ = 8.4, 2H), 7.53 (dd, $J_1$ = 8.4, $J_2$ = 8.4, 2H), 8.37 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.1, 20.5, 48.3, 62.1, 114.0, 122.4, 127.3, 131.2, 135.3, 140.3, 168.4, 178.0; MS (ESI) $[M + H]^+$ $m/z$ 310.3. Anal. Calcd for $C_{19}H_{19}NO_3$ (309.14): C, 73.77; H, 6.19; N, 4.53. Found: C, 73.64; H, 6.18; N, 4.52.

**1,5-Dimethyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4g):** yield 223 mg (80%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.10 (t, $J$ = 7.0, 3H), 2.55 (s, 3H), 2.88 (s, 3H), 4.12 (q, $J$ = 7.0, 2H), 4.81 (s, 2H), 7.01 (d, $J$ = 7.2, 1H), 7.19 (m, 2H), 7.44 (m, 2H), 8.20 (d, $J$ = 8.0, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.9, 21.3, 23.4, 56.3, 61.6, 114.9, 122.8, 125.2, 125.5, 126.2, 128.2, 132.6, 136.8, 141.8, 144.7, 148.1, 169.8, 181.4; MS (ESI) $[M + H]^+$ $m/z$ 310.1. Anal. Calcd for $C_{19}H_{19}NO_3$ (309.14): C, 73.77; H, 6.19; N, 4.53. Found: C, 73.86; H, 6.14; N, 4.75.

**2,3,7-Trimethyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4h):** yield 90 mg (31%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.29 (t, $J$ = 7.0, 3H), 2.37 (s, 3H), 2.42 (s, 3H), 2.46 (s, 3H), 4.30 (q, $J$ = 7.0, 2H), 5.03 (s, 2H), 7.05 (s, 1H), 7.19 (d, $J$ = 8.8, 1H), 7.50 (dd, $J_1$ = 8.8, $J_2$ = 8.8, 1H), 8.28 (s, 1H), 8.34 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.0, 20.5, 21.1, 48.2, 62.0, 114.0, 114.5, 120.7, 122.3, 127.2, 127.7, 130.7, 131.1, 135.0, 140.2, 140.6, 144.2, 168.5, 177.8; MS (ESI) $[M + H]^+$ $m/z$ 324.0. Anal. Calcd for $C_{20}H_{21}NO_3$ (323.15): C, 74.28; H, 6.55; N, 4.33. Found: C, 74.19; H, 6.54; N, 4.32.

**1,2,7-Trimethyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4i):** yield 140 mg (48%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.30 (t, $J$ = 7.0, 3H), 2.39 (s, 3H), 2.46 (s, 3H), 2.92 (s, 3H), 4.31 (q, $J$ = 7.0, 2H), 4.96 (s, 2H), 7.01 (d, $J$ = 8.8, 1H), 7.13 (d, $J$ = 8.4, 1H), 7.45 (m, 2H), 8.27 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 18.6, 20.3, 20.6, 49.2, 62.0, 111.1, 113.6, 121.5, 124.1, 127.3, 130.5, 130.9, 134.6, 135.1, 139.7, 140.5, 142.3, 168.7, 180.5; MS (ESI) $[M + H]^+$ $m/z$ 324.0. Anal. Calcd for $C_{20}H_{21}NO_3$ (323.15): C, 74.28; H, 6.55; N, 4.33. Found: C, 74.62; H, 6.53; N, 4.34.

**2,3,6,7-Tetramethyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4j):** yield 100 mg (33%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.31 (t, $J$ = 7.0, 3H), 2.37 (s, 6H), 2.42 (s, 6H), 4.32 (q, $J$ = 7.0, 2H), 5.03 (s, 2H), 7.04 (s, 2H), 8.29 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.0, 21.1, 48.2, 62.0, 114.5, 120.8, 127.7, 130.6, 140.6, 143.9, 168.6, 177.5; MS (ESI) $[M + H]^+$ $m/z$ 338.1. Anal. Calcd for $C_{21}H_{23}NO_3$ (337.17): C, 74.75; H, 6.87; N, 4.15. Found: C, 74.60; H, 6.88; N, 4.17.

**1,2,6,7-Tetramethyl-9-oxo-10(9*H*)-acridineacetic acid, ethyl ester (4k):** yield 85 mg (28%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.30 (t, $J$ = 7.0, 3H), 2.38 (m, 9H), 2.92 (s, 3H), 4.32 (q, $J$ = 7.0, 2H), 4.96 (s, 2H), 6.98 (m, 2H), 7.42 (d, $J$ = 8.4, 1H), 8.20 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 18.6, 19.0, 20.3, 21.0, 49.1, 61.9, 111.2, 114.1, 121.5, 122.5, 127.8, 130.4, 130.5, 134.9, 140.1, 140.5, 142.3, 143.4, 168.8, 180.3; MS (ESI) $[M + H]^+$ $m/z$ 338.1. Anal. Calcd for $C_{21}H_{23}NO_3$ (337.17): C, 74.75; H, 6.87; N, 4.15. Found: C, 74.74; H, 6.85; N, 4.14.

**General Procedure for the Synthesis of Compounds 5b−k.** Under a nitrogen atmosphere, $LiAlH_4$ (41 mg, 1.08 mmol) was added to a suspension of $TiCl_3$·3THF (438 mg, 1.08 mmol) in anhydrous THF (1.5 mL) at 0 °C, and the suspension was stirred for 10 min. After addition of triethylamine (109 mg, 1.08 mmol) at room temperature, the reaction mixture was refluxed for 1 h. A solution of compounds **4b−k** (0.36 mmol) and 2-adamantanone (54 mg, 0.36 mmol) in dry THF (3.0 mL) was added dropwise over a period of 30 min. Then, the reaction mixture was refluxed for 17 h, cooled to room temperature, and filtered over silica gel. Silica was washed with dichloromethane, and the filtrate was evaporated under vacuum. The crude product was purified by flash chromatography on silica gel using 1:1 (v/v) dichloromethane/hexane as the eluent to obtain **5b−k**.

**1-Methyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5b):** yield 72 mg (48%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.23 (t, $J$ = 7.2, 3H), 1.42 (m, 2H), 1.60 (m, 2H), 1.78 (s, 3H), 1.93−2.16 (m, 5H), 2.34 (s, 3H), 2.59 (s, 1H), 3.27 (s, 1H), 4.23 (q, $J$ = 7.2, 2H), 4.62 (AB, 2H), 6.60 (d, $J$ = 8.0, 1H), 6.78 (d, $J$ = 8.0, 1H), 6.87 (d, $J$ = 7.6, 1H), 6.98 (t, $J$ = 7.6, 1H), 7.04 (t, $J$ = 8.0, 1H), 7.13 (t, $J$ = 7.2, 1H), 7.20 (d, $J$ = 7.2, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.2, 20.3, 27.8, 27.9, 32.6, 33.4, 36.9, 37.3, 39.1, 39.7, 40.0, 48.8, 61.2, 109.2, 112.1, 119.8, 120.5, 123.1, 125.5, 125.6, 125.9, 127.1, 127.5, 134.7, 143.1, 143.7, 145.8, 169.8; MS (ESI) $[M + H]^+$ $m/z$ 414.2. Anal. Calcd for $C_{28}H_{31}NO_2$ (413.24): C, 81.32; H, 7.56; N, 3.39. Found: C, 81.11; H, 7.54; N, 3.40.

**2-Methyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5c):** yield 54 mg (36%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.29 (t, $J$ = 7.2, 3H), 1.45−2.25 (m, 12H), 2.33 (s, 3H), 3.45 (ds, 2H), 4.28 (q, $J$ = 7.2, 2H), 4.64 (s, 2H), 6.69 (d, $J$ = 8.0, 1H), 6.77 (d, $J$ = 8.0, 1H), 6.98 (d, $J$ = 7.2, 2H), 7.04 (s, 1H), 7.16 (dt, $J_1$ = 7.2, $J_2$ = 8.0, 1H), 7.25 (d, $J$ = 7.2, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.2, 20.8, 22.7, 27.5, 28.1, 28.7, 31.9, 32.2, 36.3, 37.2, 39.3, 39.7, 47.0, 48.7, 61.2, 112.1, 120.1, 120.2, 126.0, 126.1, 126.7, 127.4, 127.9, 129.6, 141.0, 143.4, 144.3, 169.9; MS (ESI) $[M + H]^+$ $m/z$ 414.2. Anal. Calcd for $C_{28}H_{31}NO_2$ (413.24): C, 81.32; H, 7.56; N, 3.39. Found: C, 81.17; H, 7.55; N, 3.38.

**3-Methyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5d):** yield 106 mg (71%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.29 (t, $J$ = 7.2, 2H), 1.40−2.30 (m, 12H), 2.35 (s, 3H), 3.45 (s, 2H), 4.29 (q, $J$ = 7.2, 2H), 4.65 (s, 2H), 6.59 (s, 1H), 6.78 (d, $J$ = 8.0, 1H), 6.82 (d, $J$ = 7.6, 1H), 6.99 (t, $J$ = 7.6, 1H), 7.14 (m, 2H), 7.23 (d, $J$ = 7.6, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.2, 21.7, 28.0, 32.2, 37.1, 39.5, 48.8, 61.2, 112.3, 113.1, 119.9, 120.3, 121.3, 123.5, 126.1, 127.2, 127.4, 135.8, 143.2, 144.0, 169.9; MS (ESI) $[M + H]^+$ $m/z$ 414.2. Anal. Calcd for $C_{28}H_{31}NO_2$ (413.24): C, 81.32; H, 7.56; N, 3.39. Found: C, 81.53; H, 7.59; N, 3.36.

**4-Methyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5e):** yield 84 mg (56%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.17 (t, $J$ = 7.2, 3H), 1.25−2.25 (m, 12H), 2.42 (s, 3H), 3.44 (s, 1H), 3.50 (s, 1H), 4.12 (q, $J$ = 7.2, 2H), 4.58 (AB, 2H), 6.98−7.28 (m, 7H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.1, 19.9, 27.6, 28.4, 32.5, 32.7, 32.9, 36.2, 37.1, 38.5, 38.7, 40.0, 54.6, 60.7, 118.8, 121.5, 121.9, 122.6, 125.4, 126.0, 127.1, 128.8, 129.3, 133.5, 134.6, 143.7, 144.2, 146.4, 170.2; MS (ESI) $[M + H]^+$ $m/z$ 414.2. Anal. Calcd for $C_{28}H_{31}NO_2$ (413.24): C, 81.32; H, 7.56; N, 3.39. Found: C, 81.26; H, 7.61; N, 3.37.

**2,7-Dimethyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5f):** yield 71 mg (46%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.26 (t, $J$ = 7.2, 3H), 1.43−2.20 (m, 12H), 2.31 (s, 6H), 3.44 (s, 2H), 4.26 (q, $J$ = 7.2, 2H), 4.60 (s, 2H), 6.65 (d, $J$ = 8.0, 2H), 6.95 (d, $J$ = 8.0, 2H), 7.01 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.1, 20.5, 20.8, 21.8, 29.7, 31.9, 32.2, 33.7, 36.3, 37.2, 39.2, 46.9, 48.6, 61.1, 111.9, 114.0, 126.6, 127.3, 127.8, 129.3, 131.3, 134.8, 135.3, 140.3, 141.1, 144.0, 170.0; MS (ESI) $[M + H]^+$ $m/z$ 428.3. Anal. Calcd for $C_{29}H_{33}NO_2$ (427.25): C, 81.46; H, 7.78; N, 3.28. Found: C, 81.92; H, 7.79; N, 3.30.

**1,5-Dimethyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5g):** yield 89 mg (58%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.22 (t, $J$ = 7.2, 3H), 1.83 (s, 4H), 1.96 (s, 3H), 1.99−2.18 (m, 5H), 2.32 (s, 3H), 2.40 (s, 3H), 2.62 (s, 1H), 3.37 (s, 1H), 4.15 (m, 2H), 4.57 (AB, 2H), 6.90 (d, $J$ = 7.2, 1H), 6.98 (m, 2H), 7.06 (m, 2H), 7.11 (d, $J$ = 7.6, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.9, 20.1, 27.8, 28.0, 33.0, 33.7, 37.0, 37.6, 39.0, 39.1, 39.3, 54.3, 60.7, 115.2, 120.9, 122.5, 124.2, 125.1, 125.5, 128.3, 129.2, 132.3, 134.4, 135.4, 143.9, 144.9, 146.7, 170.2; MS (ESI) $[M + H]^+$ $m/z$ 428.1. Anal. Calcd for $C_{29}H_{33}NO_2$ (427.25): C, 81.46; H, 7.78; N, 3.28. Found: C, 81.45; H, 7.74; N, 3.31.

**2,3,7-Trimethyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5h):** yield 97 mg (61%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.28 (t, $J$ = 7.2, 3H), 1.50−2.20 (m, 12H), 2.22 (s, 3H), 2.24 (s, 3H), 2.31 (s, 3H), 3.45 (s, 2H), 4.27 (q, $J$ = 7.2, 2H), 4.60 (s, 2H), 6.54 (s, 1H), 6.66 (d, $J$ = 8.4, 1H), 6.94 (d, $J$ = 8.4, 1H), 6.98 (s, 1H), 7.01 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.1, 20.1, 20.8, 26.9, 32.1, 32.2, 37.2, 48.7, 61.1, 111.9, 113.5, 119.9, 123.7, 126.1, 126.5, 127.8, 128.0, 128.3, 129.1, 134.0, 141.2, 141.4, 143.3, 170.1; MS (ESI) $[M + H]^+$ $m/z$ 442.3. Anal. Calcd for $C_{30}H_{35}NO_2$ (441.27): C, 81.59; H, 7.99; N, 3.17. Found: C, 81.76; H, 7.95; N, 3.17.

**1,2,7-Trimethyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5i):** yield 81 mg (51%); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.26 (t, $J$ = 7.2, 3H), 1.44−2.11 (m, 12H), 2.23 (s, 3H), 2.26 (s, 3H), 2.33 (s, 3H), 2.56 (s, 1H), 3.30 (s, 1H), 4.24 (q, $J$ = 7.2, 2H), 4.60 (AB, 2H), 6.53 (d, $J$ = 8.4, 1H), 6.68 (d, $J$ = 8.0, 1H), 6.94 (m, 2H), 7.02 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 18.1, 19.7, 20.7, 27.8, 28.0, 29.7, 32.6, 33.4, 36.9, 37.2, 39.1, 39.8, 40.0, 48.6, 61.1, 108.7, 111.8, 120.4, 126.4, 126.7, 127.5, 129.3, 129.4, 133.1, 141.1, 141.9, 145.1, 170.0; MS (ESI) $[M + H]^+$ $m/z$ 442.3. Anal. Calcd for $C_{30}H_{35}NO_2$ (441.27): C, 81.59; H, 7.99; N, 3.17. Found: C, 81.30; H, 7.99; N, 3.16.

**2,3,6,7-Tetramethyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5j):** yield 34 mg (21%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.28 (t, $J$ = 7.2, 3H), 1.44−2.10 (m, 12H), 2.22 (s, 6H), 2.24 (s, 6H), 3.45 (s, 2H), 4.28 (q, $J$ = 7.2, 2H), 4.60 (s, 2H), 6.54 (s, 2H), 6.97 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.1, 20.1, 32.2, 37.2, 48.7, 61.0, 113.6, 119.7, 123.9, 127.8, 128.3, 133.9, 141.4, 142.7, 170.2; MS (ESI) $[M + H]^+$ $m/z$ 456.2. Anal. Calcd for $C_{31}H_{37}NO_2$ (455.28): C, 81.72; H, 8.19; N, 3.07. Found: C, 81.76; H, 8.17; N, 3.07.

**1,2,6,7-Tetramethyl-9-(tricyclo[3.3.1.13,7]dec-2-ylidene)-10(9*H*)-acridineacetic acid, ethyl ester (5k):** yield 62 mg (38%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.26 (t, $J$ = 7.2, 3H), 1.30−2.10 (m, 12H), 2.22 (s, 3H), 2.23 (s, 3H), 2.24 (s, 3H), 2.25 (s, 3H), 2.55 (s, 1H), 3.31 (s, 1H), 4.25 (m, 2H), 4.59 (AB, 2H), 6.52 (d, $J$ = 8.4, 1H), 6.57 (s, 1H), 6.92 (d, $J$ = 8.4, 1H), 6.98 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 18.1, 19.1, 19.7, 20.1, 26.9, 27.8, 28.0, 32.6, 33.3, 36.9, 37.2, 39.1, 39.7, 40.0, 48.7, 61.0, 108.7, 113.4, 120.2, 125.3, 125.8, 126.6, 128.0, 128.1, 129.2, 133.0, 133.7, 141.3, 142.0, 144.5, 170.1; MS (ESI) $[M + H]^+$ $m/z$ 456.2. Anal. Calcd for $C_{31}H_{37}NO_2$ (455.28): C, 81.72; H, 8.19; N, 3.07. Found: C, 81.58; H, 8.20; N, 3.07.

**General Procedure for the Synthesis of Compounds 1b−k.** Compounds **5b−k** (0.07 mmol) were dissolved in $CH_2Cl_2$ (6.0 mL), 0.05 g of polymer-bound Bengal Rose or Methylene Blue were added, and the suspension was cooled to 0 °C. The solution was bubbled with oxygen and irradiated under stirring using a 500 W halogen lamp equipped with an UV cutoff-filter (0.5% transmission at 400 nm). The irradiation was continued until the starting material disappeared, and the conversion was monitored by means of HPLC−MS. The reaction mixture was treated with activated charcoal, filtered, and dried under vacuum. The products **1c−f**, **1h**, and **1j** were isolated pure or almost pure (≥95%, on the basis of the NMR spectra); conversely, the products **1b**, **1i**, and **1k** were observed only in trace amounts by HPLC−MS, and compound **1g** was never observed.

**Dispiro[2-methylacridine-9(10*H*),3′-[1,2]dioxetane-4′,2″-tricyclo[3.3.1.13,7]decane]-10-acetic acid, ethyl ester (1c):** yield 17 mg (56%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.65 (m, 2H), 1.26 (t, $J$ = 7.2, 3H), 1.47−2.11 (m, 6H), 2.29 (s, 2H), 2.43 (s, 3H), 2.55 (s, 4H), 4.27 (q, $J$ = 7.2, 2H), 4.63 (s, 2H), 6.73 (d, $J$ = 8.0, 1H), 6.80 (d, $J$ = 8.4, 1H), 7.18 (m, 2H), 7.35 (m, 1H), 8.02 (s, 1H), 8.21 (d, $J$ = 6.8, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.2, 20.8, 25.5, 25.7, 29.7, 31.8, 33.0, 36.2, 39.3, 47.0, 48.4, 61.5, 86.9, 97.8, 111.6, 111.7, 120.6, 121.5,

128.4, 128.5, 129.0, 129.8, 130.3, 137.2, 139.5, 169.4; MS (ESI) $[M + H]^+$ $m/z$ 446.3. Anal. Calcd for $C_{28}H_{31}NO_4$ (445.23): C, 75.48; H, 7.01; N, 3.14. Found: C, 75.46; H, 7.00; N, 3.13.

**Dispiro[3-methylacridine-9(10*H*),3′-[1,2]dioxetane-4′,2″-tricyclo[3.3.1.13,7]decane]-10-acetic acid, ethyl ester (1d):** yield 21 mg (66%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.65 (m, 2H), 1.16 (m, 2H), 1.28 (t, $J$ = 7.2, 3H), 1.38−2.11 (m, 6H), 2.29 (ds, 2H), 2.40 (s, 3H), 2.56 (s, 2H), 4.29 (q, $J$ = 7.2, 2H), 4.64 (s, 2H), 6.62 (s, 1H), 6.82 (d, $J$ = 8.0, 1H), 7.02 (d, $J$ = 7.6, 1H), 7.19 (t, $J$ = 7.2, 1H), 7.36 (m, 1H), 8.09 (d, $J$ = 8.0, 1H), 8.22 (dd, $J_1$ = 8.0, $J_2$ = 8.0, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 21.8, 25.5, 25.7, 27.4, 31.7, 31.8, 32.9, 33.0, 36.2, 36.3, 39.2, 47.0, 48.5, 61.5, 86.8, 97.8, 111.8, 112.4, 118.9, 120.8, 121.8, 121.9, 128.3, 128.4, 129.0, 139.1, 139.2, 169.3; MS (ESI) $[M + H]^+$ $m/z$ 446.1. Anal. Calcd for $C_{28}H_{31}NO_4$ (445.23): C, 75.48; H, 7.01; N, 3.14. Found: C, 75.33; H, 7.02; N, 3.15.

**Dispiro[4-methylacridine-9(10*H*),3′-[1,2]dioxetane-4′,2″-tricyclo[3.3.1.13,7]decane]-10-acetic acid, ethyl ester (1e):** yield 15 mg (46%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.59 (m, 1H), 0.79 (m, 1H), 1.16−2.25 (m, 11H), 2.42 (s, 3H), 2.55 (s, 4H), 4.31−4.54 (m, 4H), 7.02 (d, $J$ = 7.6, 1H), 7.10−7.36 (m, 5H), 8.13 (m, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 14.4, 20.7, 25.5, 25.7, 27.6, 29.8, 31.8, 32.0, 32.8, 32.9, 33.0, 33.2, 36.4, 39.4, 47.0, 56.5, 61.7, 87.1, 97.1, 116.3, 119.0, 122.0, 122.4, 126.3, 126.5, 128.0, 129.5, 129.8, 133.3, 141.0, 143.4, 172.1; MS (ESI) $[M + H]^+$ $m/z$ 446.1. Anal. Calcd for $C_{28}H_{31}NO_4$ (445.23): C, 75.48; H, 7.01; N, 3.14. Found: C, 74.83; H, 6.95; N, 3.11.

**Dispiro[2,7-dimethyl-acridine-9(10*H*),3′-[1,2]dioxetane-4′,2″-tricyclo[3.3.1.13,7]decane]-10-acetic acid, ethyl ester (1f):** yield 7 mg (23%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.66 (m, 2H), 1.17 (m, 2H), 1.20−2.30 (m, 9H), 2.30 (s, 2H), 2.43 (s, 6H), 2.48 (s, 1H), 2.56 (s, 1H), 4.26 (q, $J$ = 7.2, 2H), 4.60 (s, 2H), 6.70 (d, $J$ = 8.0, 2H), 7.16 (d, $J$ = 8.0, 2H), 8.00 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 20.8, 22.7, 25.5, 25.7, 27.4, 29.7, 31.8, 32.9, 36.2, 39.2, 46.9, 48.3, 61.5, 87.0, 97.7, 111.5, 118.8, 121.3, 128.5, 129.7, 137.3, 169.5; MS (ESI) $[M + H]^+$ $m/z$ 460.4. Anal. Calcd for $C_{29}H_{33}NO_4$ (459.24): C, 75.79; H, 7.24; N, 3.05. Found: C, 76.19; H, 7.25; N, 3.03.

**Dispiro[2,3,7-trimethylacridine-9(10*H*),3′-[1,2]dioxetane-4′,2″-tricyclo[3.3.1.13,7]decane-10-acetic acid, ethyl ester (1h):** yield 24 mg (72%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.67 (m, 2H), 1.16 (m, 2H), 1.27 (t, $J$ = 7.2, 3H), 1.38−2.11 (m, 9H), 2.30 (s, 3H), 2.33 (s, 3H), 2.42 (s, 3H), 2.56 (s, 1H), 4.27 (q, $J$ = 7.2, 2H), 4.60 (s, 2H), 6.57 (s, 1H), 6.69 (d, $J$ = 8.4, 1H), 7.14 (d, $J$ = 8.4, 1H), 7.92 (s, 1H), 8.00 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.1, 20.3, 20.8, 25.5, 25.8, 27.4, 31.8, 31.9, 32.9, 33.0, 36.2, 39.2, 47.0, 48.3, 61.4, 86.9, 97.7, 111.5, 112.9, 118.9, 121.5, 128.4, 128.7, 129.0, 129.6, 129.7, 137.4, 137.5, 137.6, 169.6; MS (ESI) $[M + H]^+$ $m/z$ 474.2. Anal. Calcd for $C_{30}H_{35}NO_4$ (473.26): C, 76.08; H, 7.45; N, 2.96. Found: C, 76.50; H, 7.46; N, 2.97.

**Dispiro[2,3,6,7-tetramethylacridine-9(10*H*),3′-[1,2]-dioxetane-4′,2″-tricyclo[3.3.1.13,7]decane]-10-acetic acid, ethyl ester (1j):** yield 29 mg (84%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.67 (m, 2H), 1.15 (m, 2H), 1.28 (t, $J$ = 7.2, 3H), 1.35−2.11 (m, 9H), 2.30 (s, 6H), 2.33 (s, 6H), 2.56 (s, 1H), 4.28 (q, $J$ = 7.2, 2H), 4.59 (s, 2H), 6.56 (s, 2H), 7.92 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 19.1, 20.3, 25.5, 25.7, 27.4, 31.8, 33.0, 36.2, 36.3, 39.2, 47.0, 48.3, 61.3, 86.8, 97.8, 112.9, 119.1, 128.6, 129.0, 137.3, 137.6, 169.7; MS (ESI) $[M + H]^+$ $m/z$ 488.2. Anal. Calcd for $C_{31}H_{37}NO_4$ (487.27): C, 76.36; H, 7.65; N, 2.87. Found: C, 76.84; H, 7.65; N, 2.88.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1$H and $^{13}$C NMR spectra of new compounds, absorption and fluorescence spectra of acridone derivatives, and TCL spectra of 1,2-dioxetane derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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