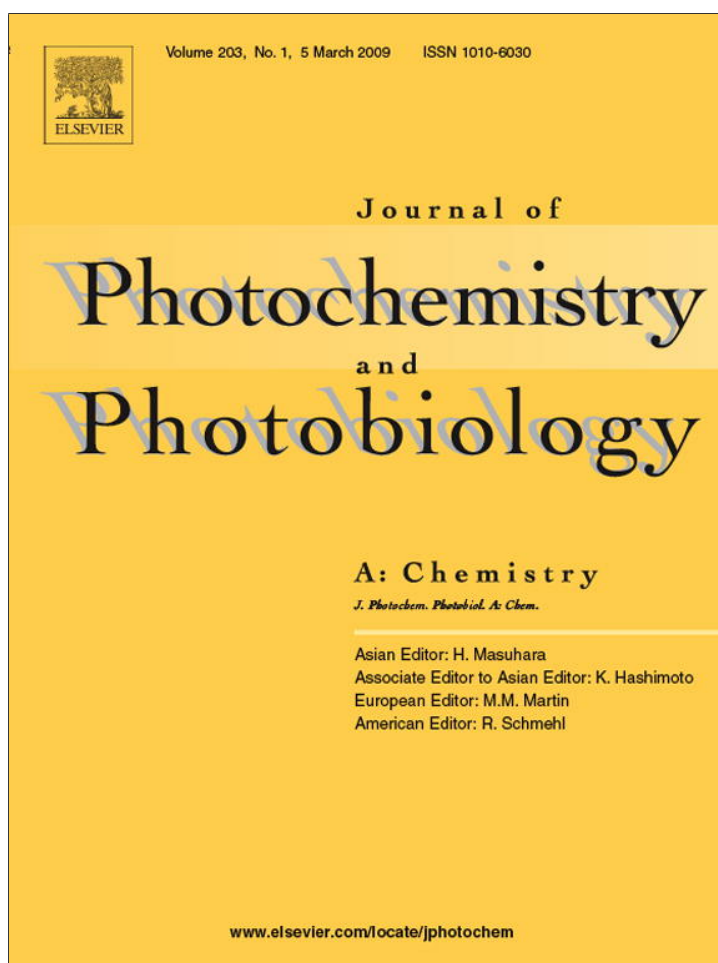


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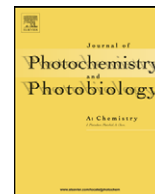
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## Synthesis and properties of novel chemiluminescent biological probes: 2- and 3-(2-Succinimidylloxycarbonyl)ethyl)phenyl acridinium esters

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## ABSTRACT

Five new chemiluminescent aryl acridiniumcarboxylate esters **6–10**, with a linker group in an *ortho*- or *meta*-position of the phenoxy ring, have been synthesized. The *ortho*- derivatives **6**, **7** and **9** show significant improvement in the quantum yield of chemiluminescence and slower chemiluminescence kinetics compared to the unsubstituted *para*- derivative, **1**, while the *ortho*- derivative **8** shows quicker chemiluminescence kinetics and a lower quantum yield of chemiluminescence. The *meta*- derivative **10** shows similar chemiluminescent properties to that of its *para*-substituted analogue.

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## 1. Introduction

Chemiluminescent acridinium esters are widely used as labels in biochemical binding assays such as immunoassay [1] and nucleic acid hybridisation assays [2]. Accordingly, it is desirable to understand the structural features that influence the parameters of the chemiluminescent reaction, such as kinetics and intensity, which will ultimately govern the utility of this class of molecules as labels. In particular, it is desirable to be able to quantify the chemiluminescence emission both rapidly and with a high signal-background ratio to enable convenient and high sensitivity assays to be developed. In a preceding paper [3], we discussed the synthesis and application of an initial series of acridinium esters (Scheme 1, compounds **2–5**) that differed from an established acridinium ester (compound **1**) in having substituents in the *ortho*-position of the phenyl group. It would be of interest to know whether placement of the linker group at the *ortho*- or *meta*- rather than the *para*-position of the phenyl ring would influence the chemiluminescent reaction. As a continuation of our previous work, we report here the synthesis and chemiluminescent properties of a second series of acridinium esters (Scheme 1, compounds **6–10**). This study should aid understanding of the factors influencing acridinium ester chemiluminescence and ultimately permit the design of labels having improved properties, relative to those labels described to date, for any given application.

## 2. Experimental

## 2.1. General methods

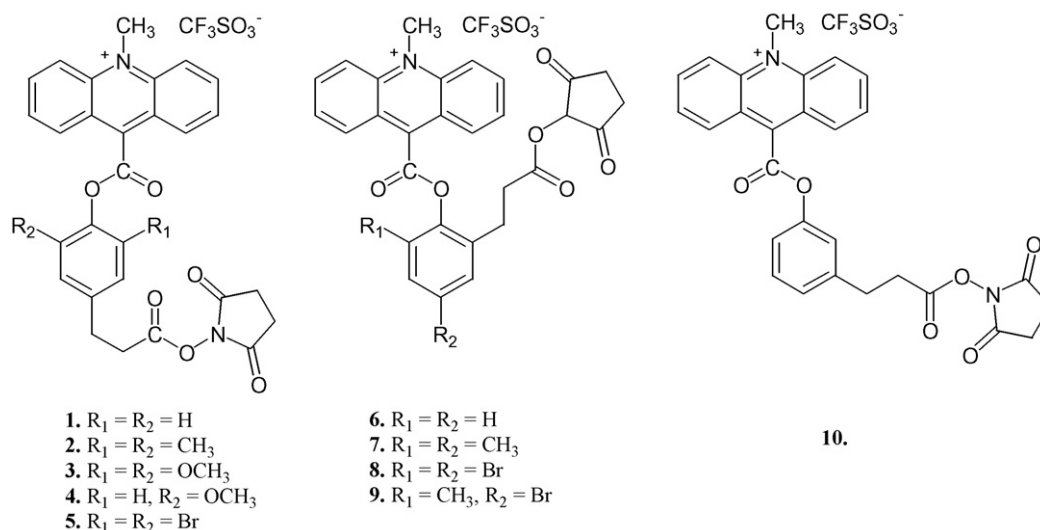
Melting points (mp) were recorded on a Griffin Melting Point Apparatus and are reported uncorrected. IR spectra were obtained using a Perkin Elmer FT-IR spectrometer 1725x. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were measured on a Bruker AC 400 spectrometer with tetramethylsilane as internal standard. Chemical shifts ( $\delta$ ) are expressed in ppm, and coupling constants (*J*) are in Hz. Mass spectra were recorded on a VG 12/253J mass spectrometer for low-resolution electron impact (EI) and chemical ionisation (CI) measurements and a VG AutoSpec mass spectrometer for fast atom bombardment (FAB) mass spectra. The data are presented as *m/z* ratios for the molecular ion and several of the most abundant other ions with their percent relative intensities given in brackets. Microanalyses were obtained from a Carlo Erba 1106 instrument by the microanalysis laboratory at Cardiff University. Column chromatography was carried out with silica gel 60 (230–400 mesh, Merck). Chromatotron separation was carried out on chromatotron Model 7924T from TC Research using 4 mm, 2 mm or 1 mm thick silica gel 60 GF<sub>258</sub> (Merck) layers. In order to help in the assignments, expected chemical shifts were calculated using additivity values and model compounds. [4] Assignments of signals having similar chemical shifts and coupling patterns have not been rigorously confirmed.

## 2.2. Syntheses

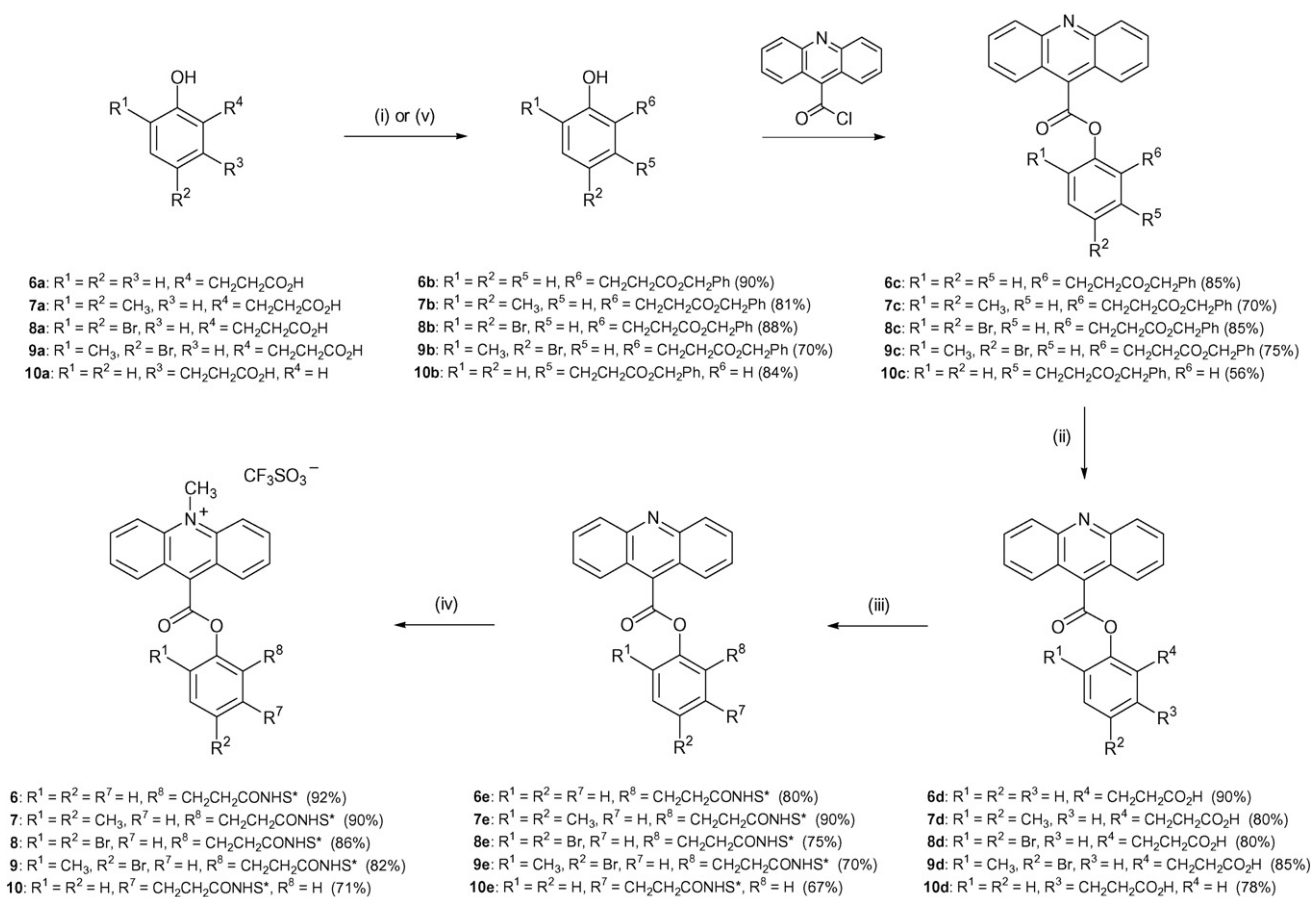
The synthetic routes are given in Schemes 2 and 3.

\* Corresponding author. Tel.: +44 2920-870600; fax: +44 2920 870600.

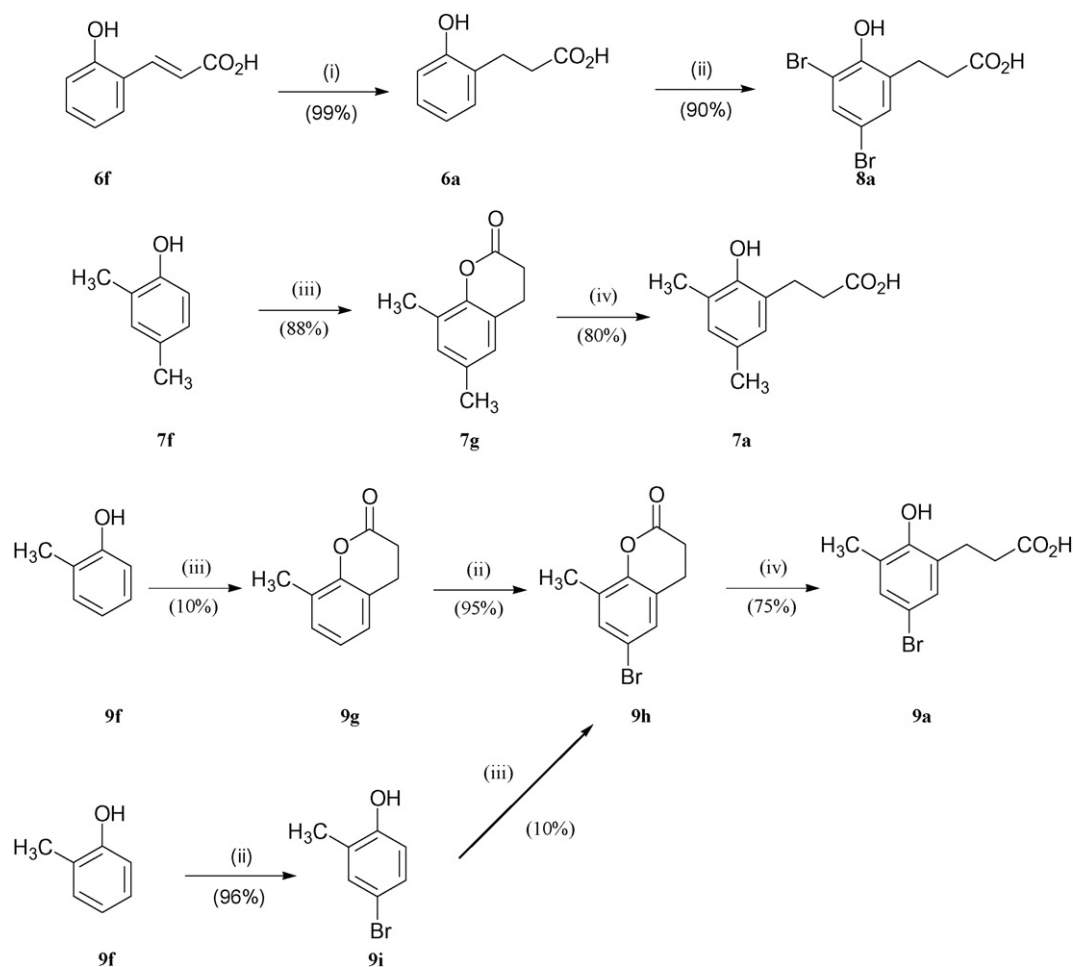
E-mail address: [smithk13@cardiff.ac.uk](mailto:smithk13@cardiff.ac.uk) (K. Smith).



Scheme 1. Acridinium esters 1–10.



Scheme 2. General strategy for the synthesis of acridinium ester labels.



Reagents: (i)  $\text{H}_2$ , Pd/C; (ii)  $\text{Br}_2$ , glacial acetic acid (or  $\text{C}_1_2\text{H}_2\text{CH}_2\text{CH}_2\text{Cl}_2$  or  $\text{CH}_2\text{Cl}_2$ ); (iii)  $\text{CH}_2=\text{CH-CN}$ ,  $\text{AlCl}_3$  (or  $\text{AlBr}_3$ ),  $\text{HCl}$  (gas),  $\text{C}_6\text{H}_4\text{Cl}_2$ ; (iv)  $\text{NaOH}$  (2.5 M),  $\text{MeOH}:\text{H}_2\text{O}$  (1:1), 10%  $\text{HCl}$ ;

**Scheme 3.** Syntheses of substituted 3-(2-hydroxyphenyl)propanoic acids.

### 2.2.1. 3-(2-Hydroxyphenyl)propanoic acid (**6a**)

2-Hydroxycinnamic acid **6f** (1.002 g, 6.10 mmol) was dissolved in absolute ethanol (20 mL). After addition of the catalyst, Pd/C (10%, 50 mg), hydrogenation was conducted for 2 h under atmospheric pressure of hydrogen, after which time TLC analysis (ethyl acetate/hexane, 20:80) showed the reaction to be complete. The catalyst was removed by filtration and the ethanol was evaporated under vacuum, affording **6a** (1.001 g, 99%), mp 89–90 °C, (lit. [5] mp 83–85 °C). IR  $\nu_{\text{max}}$  (KBr) 3400, 3350, 1700;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  10.00 (b, 1H), ca. 7.0 (m, 4H), 2.78 (t, 7 Hz, 2H), 2.50 (t, 7 Hz, 2H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  174.3, 155.2, 129.7, 127.1, 126.9, 118.9, 114.9, 33.7, 25.6; EI-MS  $m/z$  166 [ $\text{M}^+$ , 18%], 148, [( $\text{M}-\text{H}_2\text{O}$ ) $^+$ , 82], 120 [100], 107 [25], 91 [70].

### 2.2.2. Benzyl 3-(2-hydroxyphenyl)propanoate (**6b**)

A solution of anhydrous benzyl alcohol (6.20 mL, 6.514 g, 60.2 mmol), **6a** (1.011 g, 6.01 mmol) and trifluoroacetic anhydride (4.80 mL, 7.085 g, 33.7 mmol) was heated at 60 °C for 3 h, then cooled and poured into  $\text{NaHCO}_3$  (50 mL of 8% aq.). The mixture was extracted with chloroform (50 mL  $\times$  3) and the solvent was evaporated from the combined extracts to leave a syrup (1.801 g). This residue was subjected to chromatography using a chromatatron [ethyl acetate:hexane (1:8; 1:4; 1:2 sequentially)]. After evaporation of the solvent from the appropriate combined fractions, **6b** was obtained as a pure colourless oil (1.401 g, 90%). IR  $\nu_{\text{max}}$  (film) 3400, 1740;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.79–7.37 (m, 9H), 5.08 (s, 2H), 2.92 (t, 7 Hz,

2H), 2.74 (t, 7 Hz, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  175.1, 154.2, 135.4, 130.5, 128.5, 128.3, 128.0, 127.1, 120.7, 116.9, 116.7, 66.9, 34.9, 25.0; EI-MS  $m/z$  256 [ $\text{M}^+$ , 100%], 239 [( $\text{M}-\text{OH}$ ) $^+$ , 40], 179 [( $\text{M}-\text{C}_6\text{H}_5$ ) $^+$ , 41], 148 [40], 120 [32], 91 [99]; Anal. Calcd. for  $\text{C}_{16}\text{H}_{16}\text{O}_3$ : C, 74.98%; H, 6.29%; Found: C, 74.88%; H, 6.43%.

### 2.2.3. 2-(2-Benzyloxycarbonyl)ethyl)phenyl acridine-9-carboxylate (**6c**)

A mixture of **6b** (594 mg, 2.319 mmol) and 4-dimethylaminopyridine (35 mg, 0.29 mmol) in anhydrous pyridine (10 mL, previously stored over 3A molecular sieves) was heated at 90 °C for 30 min. The solution was cooled and pipetted into a flask containing dried acridine-9-carbonyl chloride [**1**] (560 mg, 2.319 mmol). The reaction mixture was stirred at 100 °C for 24 h, then at 20 °C for 24 h. After removal of solvent, the crude product was dissolved in  $\text{CHCl}_3$  (150 mL). The solution was washed with water (70 mL  $\times$  3), dried with anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated. The product was recrystallized from  $\text{CHCl}_3$ /hexane, and **6c** was obtained as yellow-white crystals (909 mg, 85%), mp 135–136 °C. (Note: without the catalyst, 4-dimethylaminopyridine, the yield was only 15%). IR  $\nu_{\text{max}}$  (KBr) 1730, 1700;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.31 (d, 9 Hz, 2H), 8.25 (d, 8 Hz, 2H), 7.83 (m, 2H), 7.58 (m, 2H), 7.19–7.56 (m, 9H), 5.03 (s, 2H), 3.10 (t, 7 Hz, 2H), 2.70 (t, 7 Hz, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 172.1, 166.1, 148.8, 148.6, 135.7, 132.3, 130.6, 130.4, 130.3, 130.1, 128.5, 128.2, 128.1, 127.9, 127.6, 127.0, 124.7, 122.4, 122.2, 66.3, 34.3, 25.9; FAB-MS  $m/z$  462 [( $\text{M}+1$ ) $^+$ , 94%], 371

[(M–CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)<sup>+</sup>, 6], 224 [14], 206 [23], 179 [100]; Anal. Calcd. for C<sub>30</sub>H<sub>23</sub>NO<sub>4</sub>: C, 78.08%; H, 5.02%; N, 3.04%; Found: C, 78.42%; H, 5.18%; N, 3.10%.

#### 2.2.4. 2-(2-Carboxyethyl)phenyl acridine-9-carboxylate (**6d**)

A mixture of **6c** (1.012 g, 2.17 mmol), glacial acetic acid (16 mL) and 48% hydrobromic acid (4 mL) was heated at 100 °C for 3 h and then cooled. The reaction mixture was added to water (300 mL) and extracted with 20% MeOH/CHCl<sub>3</sub> (100 mL × 3). The organic extracts were combined and evaporated. The residue was suspended in chloroform and neutralized with a slight excess of triethylamine. The mixture was washed with water (70 mL × 3), and the organic layer dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was purified by recrystallization from CHCl<sub>3</sub> to give **6d** (725 mg, 90%), mp 231–232 °C. IR ν<sub>max</sub> (KBr) 3450, 1750, 1720; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 12.41 (b, 1H), 8.37 (d, 9 Hz, 2H), 8.35 (d, 8 Hz, 2H), 8.03 (m, 2H), 7.85 (m, 2H), 7.76 (d, 8 Hz, 1H), ca. 7.5 (m, 3H), 3.08 (t, 7 Hz, 2H), 2.73 (t, 7 Hz, 2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 173.4, 165.6, 148.3, 148.0, 135.1, 132.6, 130.8, 130.3, 129.7, 128.1, 127.8, 127.0, 124.5, 122.47, 121.44, 33.8, 24.9; FAB-MS *m/z* 372 [(M+H)<sup>+</sup>, 100%], 224 [4], 206 [40], 179 [22]; Anal. Calcd. for C<sub>23</sub>H<sub>17</sub>NO<sub>4</sub>: C, 74.38%; H, 4.61%; N, 3.77%; Found: C, 74.41%; H, 4.44%; N, 3.51%.

#### 2.2.5. 2-(2-Succinimidylloxycarbonyl)ethyl)phenyl acridine-9-carboxylate (**6e**)

A solution of **6d** (1.001 g, 2.70 mmol) in DMF (50 mL) was cooled in ice for 10 min. A solution of *N,N'*-dicyclohexylcarbodiimide (613 mg, 2.97 mmol) in DMF (10 mL) was added and the mixture was stirred in an ice bath for 30 min. A solution of *N*-hydroxysuccinimide (342 mg, 2.97 mmol) in DMF (10 mL) was added. The solution was stirred at 20 °C overnight then evaporated to dryness. The residue was extracted with dichloromethane (DCM, 50 mL) and filtered. The filtrate was evaporated to give a crude product (1.502 g) that was recrystallized from CHCl<sub>3</sub> to yield pure **6e** (1.011 g, 80%), mp 180 °C. IR ν<sub>max</sub> (KBr) 3000, 1780, 1720, 1650; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.30 (d, 9 Hz, 2H), 8.22 (d, 8 Hz, 2H), 7.85 (m, 2H), 7.68 (m, 2H), 7.53 (d, 8 Hz, 1H), ca. 7.4 (m, 2H), 7.33 (d, 8 Hz, 1H), 3.18 (t, 7 Hz, 2H), 2.93 (t, 7 Hz, 2H), 2.74 (s, 4H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 168.9, 167.6, 166.0, 148.8, 148.6, 135.4, 131.0, 130.5, 130.48, 130.1, 128.4, 127.8, 127.2, 124.5, 122.4, 31.1, 27.8, 25.5; FAB-MS *m/z* 469 [(M+1)<sup>+</sup>, 100%], 225 [10], 206 [21], 179 [10]; Anal. Calcd. For C<sub>27</sub>H<sub>20</sub>N<sub>2</sub>O<sub>6</sub>: C, 69.28%; H, 4.31%; N, 5.98%; Found: C, 69.30%; H, 4.09%; N, 5.99%.

#### 2.2.6. 2-(2-Succinimidylloxycarbonyl)ethyl)phenyl 10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**6**)

To a solution of **6e** (400 mg, 85.45 mmol) in DCM (10 mL) was added CF<sub>3</sub>SO<sub>3</sub>Me (0.680 mL, 0.995 g, 41.86 mmol) under argon. After 1 h stirring, the product precipitated as a bright yellow solid. The mixture was stirred for 24 h. The precipitate was filtered, washed with benzene and CHCl<sub>3</sub> and dried to give **6** (500 mg, 92%) as a yellow solid, mp 200–201 °C. IR ν<sub>max</sub> (KBr) 1840, 1800, 1760; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 8.98 (d, 9 Hz, 2H), ca. 8.6 (m, 4H), 8.18 (m, 2H), 7.94 (d, 8 Hz, 1H), 7.63 (d, 8 Hz, 1H), 7.54 (m, 1H), 7.48 (m, 1H), 4.99 (s, 3H), 3.09 (apparent s, 4H), 2.81 (s, 4H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 170.1, 168.2, 166.2, 147.8, 146.6, 141.9, 139.3, 131.0, 130.5, 129.9, 128.3, 127.6, 127.1, 122.5, 122.4, 120.0, 39.8, 29.9, 25.4, 23.9 (CF<sub>3</sub> signal not observed); FAB-MS *m/z* 483 [(M–SO<sub>3</sub>CF<sub>3</sub>)<sup>+</sup>, 100%], 221 [16], 193 [37]; Anal. Calcd. for C<sub>29</sub>H<sub>23</sub>N<sub>2</sub>F<sub>3</sub>O<sub>9</sub>S: C, 55.06%; H, 3.67%; N, 4.43%; Found: C, 54.98%; H, 3.86%; N, 4.23%.

#### 2.2.7. 3-(3,5-Dimethyl-2-hydroxyphenyl) propanoic acid lactone (**7g**)

2,4-Dimethylphenol (**7f**, 2.441 g, 0.0200 mol) was dissolved in acrylonitrile (1.30 mL, 1.048 g, 0.0200 mol). Anhydrous AlCl<sub>3</sub>

(5.341 g, 0.0400 mol) and 1,2-dichlorobenzene (10 mL) were added and dry HCl gas was bubbled through at 110 °C for 1 h. The mixture was added to water (100 mL) and the whole was extracted with CHCl<sub>3</sub> (70 mL × 3). The combined extract was washed with sat. NaCl aq., dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to give a yellow oil (4.002 g), which was subjected to chromatography by chromatotron (EtOAc:hexane = 1:8; 1:4; 1:2 sequentially) to give **7g** (3.061 g, 88%) as a yellow gum. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.89 (s, 1H), 6.88 (s, 1H), 2.90 (t, 8 Hz, 2H), 2.72 (t, 8 Hz, 2H), 2.26 (s, 3H), 2.25 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 169.1, 148.2, 133.4, 131.5, 130.3, 127.2, 125.9, 29.3, 23.9, 20.6, 15.5; EI-MS *m/z* 176 [M<sup>+</sup>, 100%], 148 [(M–CO)<sup>+</sup>, 90], 134 [70], 106 [50], 91 [90], 77 [40]; Anal. Calcd. for C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>: C, 74.98%; H, 6.86%; Found: C, 74.74%; H, 6.99%.

#### 2.2.8. 3-(3,5-Dimethyl-2-hydroxyphenyl)propanoic acid (**7a**)

To **7g** (1.001 g, 5.68 mmol) in MeOH:H<sub>2</sub>O (1:1, 25 mL) was added dropwise NaOH (227 mg, 5.68 mmol) in H<sub>2</sub>O (2.5 mL). The mixture was stirred at 50 °C for 10 h and 20 °C overnight, then evaporated. The residue was washed with acetone and air dried to give a white salt (1.301 g), which was dissolved in water and acidified with 10% HCl aq. to pH 6. The solution was extracted with CHCl<sub>3</sub> (70 mL × 3). The combined extracts were washed with sat. NaCl aq., dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to give a yellow oil (1.401 g), which crystallized from CHCl<sub>3</sub>/hexane to give a white solid (**7a**, 880 mg, 80%), mp 92–93 °C. IR ν<sub>max</sub> (KBr) 3543, 3450, 1706; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.80 (s, 1H), 6.74 (s, 1H), 2.85 (t, 8 Hz, 2H), 2.72 (t, 8 Hz, 2H), 2.22 (s, 3H), 2.19 (s, 3H), (exchangeable proton signals too broad to identify); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 180.6, 149.7, 129.9, 129.7, 128.4, 126.2, 124.8, 34.8, 24.6, 20.4, 16.1; EI-MS *m/z* 194 [M<sup>+</sup>, 100%], 177 [(M–H<sub>2</sub>O+1)<sup>+</sup>, 45], 148 [12], 57 [30]; Anal. Calcd. for C<sub>11</sub>H<sub>14</sub>O<sub>3</sub>: C, 68.02%; H, 7.27%; Found: C, 68.12%; H, 7.13%.

#### 2.2.9. Benzyl 3-(3,5-dimethyl-2-hydroxyphenyl)propanoate (**7b**)

The procedure was analogous to that used to prepare **6b** except for the different starting material. Pure **7b** was obtained as a colourless oil in 81% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ca. 7.3 (m, 5H), 6.81 (s, 1H), 6.73 (s, 1H), 6.72 (s, 1H), 5.09 (s, 2H), 2.80 (m, 2H), 2.70 (m, 2H), 2.22 (s, 3H), 2.20 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.5, 150.2, 135.4, 130.3, 129.4, 128.6, 128.5, 128.4, 128.3, 126.4, 125.6, 67.6, 35.4, 24.6, 20.4, 16.6; EI-MS *m/z* 284 [M<sup>+</sup>, 80%], 267 [40], 194 [60], 176 [30], 148 [50], 91 [100]; Anal. Calcd. for C<sub>18</sub>H<sub>20</sub>O<sub>3</sub> + 1/4H<sub>2</sub>O: C, 74.85%; H, 7.15%; Found: C, 74.96%; H, 7.27%.

#### 2.2.10. 4,6-Dimethyl-2-(2-benzylloxycarbonyl)ethyl)phenyl acridine-9-carboxylate (**7c**)

The preparation was analogous to that used to prepare **6c**. **7c** was obtained as a yellow gum in 70% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ca. 8.4 (m, 4H), 7.84 (apparent t, 8 Hz, 2H), 7.60 (d, 8 Hz, 2H), ca. 7.3 (m, 5H), 7.04 (s, 1H), 7.02 (s, 1H), 5.05 (s, 2H), 3.10 (t, 7.5 Hz, 2H), 2.72 (t, 7.5 Hz, 2H), 2.39 (s, 3H), 2.33 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 172.3, 165.6, 148.3, 145.7, 136.6, 135.7, 132.0, 130.7, 130.6, 130.1, 129.6, 129.1, 128.6, 128.5, 128.2, 128.1, 127.7, 127.2, 125.1, 66.4, 34.5, 26.3, 20.8, 18.0; FAB-MS *m/z* 490 [(M+1)<sup>+</sup>, 100%], 461 [7], 400 [20], 224 [8]; Anal. Calcd. for C<sub>32</sub>H<sub>27</sub>NO<sub>4</sub> + 1/2H<sub>2</sub>O: C, 77.09%; H, 5.66%; N, 2.81%; Found: C, 77.15%; H, 5.66%; N, 2.80%.

#### 2.2.11. 4,6-Dimethyl-2-(2-carboxyethyl)phenyl acridine-9-carboxylate (**7d**)

The procedure was similar to that used to prepare **6d**. **7d** was obtained in 80% yield, mp 189–190 °C. IR ν<sub>max</sub> (KBr) 3450, 1750, 1713; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) (acidic proton not observed) δ ca. 8.3 (m, 4H), 8.00 (m, 2H), 7.80 (m, 2H), 7.02 (s, 1H), 7.00 (s, 1H), 2.90 (t, 7.5 Hz, 2H), 2.50 (t, 7.5 Hz, 2H), 2.54 (s, 3H), 2.38 (s, 3H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 173.3, 165.0, 148.0, 145.2, 135.9, 134.8, 132.3, 130.7, 130.0, 129.8, 129.4, 128.2, 128.0, 124.6, 121.7, 33.8, 25.6, 20.3, 17.2; FAB-MS *m/z* 400 [(M+1)<sup>+</sup>, 100%], 356 [(M+1–CO<sub>2</sub>)<sup>+</sup>, 60], 224 [29];



Anal. Calcd. for  $C_{25}H_{21}NO_4$ : C, 75.17%; H, 5.23%; N, 3.51%; Found: C, 75.20%; H, 5.25%; N, 3.74%.

#### 2.2.12. 4,6-Dimethyl-2-(2-succinimidylloxycarbonyl)phenylacridine-9-carboxylate (**7e**)

The procedure was similar to that used for **6e** and gave **7e** in 90% yield, mp 149–150 °C. IR  $\nu_{\max}$  (KBr) 1785, 1737;  $^1H$  NMR ( $CDCl_3$ )  $\delta$  8.39 (d, 9 Hz, 2H), 8.33 (d, 9 Hz, 2H), 7.86 (m, 2H), 7.68 (m, 2H), 7.08 (apparent s, 2H), 3.18 (t, 7.5 Hz, 2H), 2.96 (t, 7.5 Hz, 2H), 2.80 (s, 4H), 2.39 (s, 3H), 2.38 (s, 3H);  $^{13}C$  NMR  $\delta$  168.7, 167.6, 165.7, 148.7, 145.7, 136.8, 135.2, 131.0, 130.9, 130.3, 130.2, 130.1, 128.4, 127.7, 124.9, 127.8, 31.4, 26.9, 25.6, 20.8, 17.8; FAB-MS  $m/z$  497 [(M+1)<sup>+</sup>, 100%], 400 [7], 225 [40], 206 [75], 179 [40]; Anal. Calcd. for  $C_{29}H_{24}N_2O_6$ : C, 70.15%; H, 4.87%; N, 5.64%; Found: C, 69.89%; H, 4.96%; N, 5.53%.

#### 2.2.13. 4,6-Dimethyl-2-(2-succinimidylloxycarbonyl)phenyl 10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**7**)

The preparation was similar to that used for **6** and gave **7** as a yellow solid in 90% yield, mp 179–180 °C. IR  $\nu_{\max}$  (KBr) 1820, 1800, 1750;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.97 (d, 9.0 Hz, 2H), 8.52 (m, 4H), 8.17 (m, 2H), 7.23 (s, 1H), 7.16 (s, 1H), 4.99 (s, 3H), 3.08 (app s, 4H), 2.80 (s, 4H), 2.46 (s, 3H), 2.39 (s, 3H);  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  169.9, 168.1, 162.7, 146.6, 144.7, 141.8, 139.1, 136.8, 130.8, 130.7, 129.7, 129.6, 128.2, 127.0, 126.9, 122.5, 120.0, 39.9, 29.9, 25.3, 24.7, 20.3, 17.4; FAB-MS  $m/z$  511 [(M-SO<sub>3</sub>CF<sub>3</sub>)<sup>+</sup>, 100%], 428 [20], 414 [5], 221 [58], 207 [7], 193 [61]; Anal. Calcd. for  $C_{31}H_{27}N_2F_3O_9S$ : C, 56.36%; H, 4.12%; N, 4.24%; Found: C, 56.37%; H, 4.38%; N, 4.04%.

#### 2.2.14. 3-(3,5-Dibromo-2-hydroxyphenyl)propanoic acid (**8a**)

To 3-(2-hydroxyphenyl)propanoic acid (882 mg, 5.31 mmol) in glacial AcOH (50 mL) was added Br<sub>2</sub> (1.701 g, 0.54 mL, 10.62 mmol) and the mixture stirred in the dark for 72 h. Evaporation *in vacuo* left a residue (2.011 g). This was dissolved in CHCl<sub>3</sub> (50 mL), washed with sat. NaCl (40 mL × 3), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to a white powder (1.901 g). Recrystallization from CHCl<sub>3</sub> gave white needles of **8a** (1.551 g, 90%), mp 105 °C. IR  $\nu_{\max}$  (KBr) 3500, 3000, 1740;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  12.0–12.4 (b, 1H), 9.38 (s, 1H), 7.53 (d, 2 Hz, 1H), 7.29 (d, 2 Hz, 1H), 2.85 (t, 7.5 Hz, 2H), 2.52 (t, 7.5 Hz, 2H);  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  173.8, 151.2, 132.4, 132.2, 131.7, 112.2, 111.0, 32.2, 25.8; EI-MS  $m/z$  326 [M<sup>+</sup> (<sup>81</sup>Br<sub>2</sub>), 35%], 324 [M<sup>+</sup> (<sup>81</sup>Br<sup>79</sup>Br), 70%], 322 [M<sup>+</sup> (<sup>79</sup>Br<sub>2</sub>), 35%], 306 [80], 278 [100]; Anal. Calcd. for  $C_9H_8Br_2O_3$ : C, 33.37%; H, 2.49%; Found: C, 33.19%; H, 2.59%.

#### 2.2.15. Benzyl 3-(3,5-dibromo-2-hydroxyphenyl)propanoate (**8b**)

The preparation was similar to that used to prepare **6b**. **8b** was obtained as a colourless oil in a yield of 88%. IR  $\nu_{\max}$  (KBr) 3500, 1750;  $^1H$  NMR ( $CDCl_3$ )  $\delta$  7.45 (d, 2 Hz, 1H), 7.34 (m, 5H), 7.18 (d, 2 Hz, 1H), 6.04 (s, 1H), 5.11 (s, 2H), 2.94 (t, 7.5 Hz, 2H), 2.70 (t, 7.5 Hz, 2H);  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  173.4, 149.9, 135.5, 132.5, 132.4, 130.0, 128.6, 128.5, 128.3, 112.3, 111.5, 66.7, 35.9, 25.9; EI-MS  $m/z$  306 [5%], 278 [2], 91 [100]; CI-MS 434 [(M+NH<sub>4</sub>)<sup>+</sup> (<sup>81</sup>Br<sub>2</sub>), 20%], 432 [(M+NH<sub>4</sub>)<sup>+</sup> (<sup>81</sup>Br<sup>79</sup>Br), 40%], 430 [(M+NH<sub>4</sub>)<sup>+</sup> (<sup>79</sup>Br<sub>2</sub>), 20%], 415 [(M+1)<sup>+</sup>, 15%], 324 [8], 306 [5], 278 [2], 108 [100], 91 [52]; Anal. Calcd. for  $C_{16}H_{14}Br_2O_3$ : C, 46.40%; H, 3.41%; Found: C, 46.23%; H, 3.22%.

#### 2.2.16. 4,6-Dibromo-2-(2-benzyloxycarbonyl)phenyl acridine-9-carboxylate (**8c**)

The procedure was analogous to that used for **6c** and gave **8c** as a yellow gum (85% yield).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  8.70 (d, 9 Hz, 2H), 8.30 (d, 8 Hz, 2H), 7.80 (m, 2H), 7.74 (d, 3 Hz, 1H), 7.60 (m, 2H), 7.46 (d, 3 Hz, 1H), 7.25 (m, 5H), 5.04 (s, 2H), 3.10 (t, 8 Hz, 2H), 2.70 (t, 8 Hz, 2H);  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  171.4, 164.4, 148.6, 146.2, 136.7, 135.4, 134.4, 133.7, 132.4, 130.3, 130.2, 128.8, 128.5, 128.2, 128.1, 127.7, 125.3, 120.5,

117.8, 66.6, 33.8, 26.5; FAB-MS  $m/z$  622 [(M+1)<sup>+</sup> (<sup>81</sup>Br<sub>2</sub>), 17%], 620 [(M+1)<sup>+</sup> (<sup>79</sup>Br<sup>81</sup>Br), 35%], 618 [(M+1)<sup>+</sup> (<sup>79</sup>Br<sub>2</sub>), 17%], 206 [55], 179 [32]; Anal. Calcd. for  $C_{30}H_{21}NBr_2O_4$ : C, 58.18%; H, 3.42%; N, 2.26%; Found: C, 58.05%; H, 3.32%; N, 2.15%.

#### 2.2.17. 4,6-Dibromo-2-(2-carboxyethyl)phenyl acridine-9-carboxylate (**8d**)

The preparation was similar to that used for **6d** and gave **8d** in 80% yield, mp 228–230 °C. IR  $\nu_{\max}$  (KBr) 3500, 1750, 1700;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.56 (d, 9 Hz, 2H), 8.34 (d, 8 Hz, 2H), 8.05 (d, 3 Hz, 1H), 8.00 (m, 2H), 7.86 (m, 2H), 7.82 (d, 3 Hz, 1H), 3.20 (t, 7.5 Hz, 2H), 2.70 (t, 7.5 Hz, 2H), (the exchangeable proton was not observed);  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  173.1, 164.1, 148.1, 145.6, 137.6, 133.6, 133.2, 132.5, 130.9, 129.9, 128.4, 124.8, 121.8, 120.1, 117.2, 33.1, 25.7; FAB-MS  $m/z$  530 [(M+1)<sup>+</sup> (<sup>79</sup>Br<sup>81</sup>Br), 35%], 512 [(M+1-H<sub>2</sub>O)<sup>+</sup>, 17%], 224 [4], 206 [8], 179 [15]; Anal. Calcd. for  $C_{23}H_{15}NBr_2O_4$ : C, 52.20%; H, 2.86%; N, 2.65%; Found: C, 52.10%; H, 2.74%; N, 2.80%.

#### 2.2.18. 4,6-Dibromo-2-(2-succinimidylloxycarbonyl)phenyl acridine-9-carboxylate (**8e**)

The procedure was similar to that for **6e** and gave **8e** in 75% yield, mp 189–190 °C.  $^1H$  NMR ( $CDCl_3$ )  $\delta$  8.53 (d, 9 Hz, 2H), 8.35 (d, 8 Hz, 2H), 7.84 (m, 2H), 7.82 (d, 3 Hz, 1H), 7.70 (m, 2H), 7.54 (d, 3 Hz, 1H), 3.20 (t, 7.5 Hz, 2H), 2.95 (t, 7.5 Hz, 2H), 2.81 (s, 4H);  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  168.6, 167.5, 164.5, 148.8, 146.4, 135.7, 135.1, 133.7, 132.5, 130.4, 128.0, 125.3, 123.0, 120.8, 118.2, 31.0, 26.3, 25.6; FAB-MS  $m/z$  629[(M+1)<sup>+</sup> (<sup>81</sup>Br<sub>2</sub>), 10%], 627[(M+1)<sup>+</sup> (<sup>79</sup>Br<sup>81</sup>Br), 20%], 625[(M+1)<sup>+</sup> (<sup>79</sup>Br<sub>2</sub>), 10%], 114 [100]; Anal. Calcd. for  $C_{27}H_{18}N_2Br_2O_6$ : C, 51.78%; H, 2.90%; N, 4.47%; Found: C, 51.71%; H, 2.97%; N, 4.42%.

#### 2.2.19. 4,6-Dibromo-2-(2-succinimidylloxycarbonyl)phenyl 10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**8**)

The preparation was similar to that for **6** and gave **8** as a yellow solid in 80% yield, mp 224–225 °C. IR  $\nu_{\max}$  (KBr) 1800, 1760, 1750;  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  8.98 (d, 9 Hz, 2H), 8.76 (d, 8 Hz, 2H), 8.56 (m, 2H), 8.20 (m, 2H), 8.09 (d, 3 Hz, 1H), 7.93 (d, 3 Hz, 1H), 5.00 (s, 3H), 3.16 (apparent s, 4H), 2.79 (s, 4H);  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  169.9, 167.9, 161.7, 145.2, 144.9, 141.8, 139.1, 136.1, 134.1, 132.6, 129.8, 128.6, 127.1, 124.6, 122.7, 120.9, 117.1, 39.8, 29.3, 25.3, 24.7; FAB-MS  $m/z$  643 [(M-SO<sub>3</sub>CF<sub>3</sub>)<sup>+</sup> (<sup>81</sup>Br<sub>2</sub>), 50%], 641 [(M-SO<sub>3</sub>CF<sub>3</sub>)<sup>+</sup> (<sup>79</sup>Br<sup>81</sup>Br), 100%], 639 [(M-SO<sub>3</sub>CF<sub>3</sub>)<sup>+</sup> (<sup>79</sup>Br<sub>2</sub>), 50%], 627 [14], 221 [10], 193 [76], 179 [14]; Anal. Calcd. for  $C_{29}H_{21}N_2Br_2F_3O_9S$ : C, 44.07%; H, 2.68%; N, 3.54%; Found: C, 44.19%; H, 2.62%; N, 3.61%.

#### 2.2.20. Synthesis of 4-bromo-2-methylphenol (**9i**)

To *o*-cresol (1.302 g, 12 mmol) in 1,2-dichloroethane (20 mL) was added dropwise bromine (1.611 g, 10 mmol) in DCM (20 mL) at 0 °C, over 30 min. The mixture was stirred 2 h at 0 °C, then 20 °C overnight. Evaporation left a residue (2.012 g) that was dissolved in CHCl<sub>3</sub> (70 mL), washed with saturated NaCl aq. (40 mL × 3), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give a white powder (1.901 g). Recrystallization from PhH/hexane gave **9i** (white needles, 1.811 g, 96%), mp 63 °C, [lit. [6] mp 63 °C]. IR  $\nu_{\max}$  (KBr) 3500;  $^1H$  NMR ( $CDCl_3$ )  $\delta$  7.23 (d, 2 Hz, 1H), 7.15 (dd, 8, 2 Hz, 1H), 6.22 (d, 8 Hz, 1H), 4.89 (s, 1H), 2.20 (s, 3H);  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  152.8, 133.5, 129.8, 126.2, 116.5, 112.6, 15.6; EI-MS 188 [M<sup>+</sup> (<sup>81</sup>Br), 98%], 186 [M<sup>+</sup> (<sup>79</sup>Br), 98%], 107 [(M-Br)<sup>+</sup>, 100%], 89 [20], 77 [70], 51 [38].

#### 2.2.21. Synthesis of 3-(2-hydroxy-3-methylphenyl)propanoic acid lactone (**9g**)

*o*-Cresol (1.012 g, 9.25 mmol) and acrylonitrile (0.61 mL, 9.25 mmol) in 1,2-dichlorobenzene (DCB, 5 mL) was added dropwise to a suspension of anhydrous AlCl<sub>3</sub> (1.231 g, 18.5 mmol) in DCB (20 mL). Dry HCl gas was passed into the mixture (at 120 °C) for 1 h. The mixture was heated for 15 h at 110 °C, added

to water (100 mL) and the whole was extracted with  $\text{CHCl}_3$  (70 mL  $\times$  3). The combined extracts were washed with sat. NaCl aq. (70 mL  $\times$  3), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give a yellow solid (2.012 g). This was chromatographed using a chromatatron (hexane:EtOAc = 100:0, 4:1, 1:1 sequentially) to give two pure white solids. One [Rf = 0.4 in EtOAc:hexane (1:4)] was recrystallized with  $\text{CHCl}_3$ /hexane and gave white crystals of **9g** (150 mg, 10%), mp 73 °C. IR  $\nu_{\text{max}}$  (KBr) 2959, 1763;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.11 (dd, 1, 9 Hz, 1H), 7.02 (m, 2H), 2.97 (t, 7 Hz, 2H), 2.76 (t, 7 Hz, 2H), 2.30 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  168.8, 150.3, 129.7, 126.2, 125.4, 123.9, 122.4, 29.3, 23.9, 15.6; EI-MS 162 [ $\text{M}^+$ , 90%], 134 [95], 120 [40], 105 [(M20), 91 [100]]. The other [Rf = 0.6 in EtOAc:hexane (1:4)] was recrystallized from  $\text{CHCl}_3$ /hexane to give white crystals of 3-(3-methyl-4-hydroxyphenyl)propionitrile (840 mg, 60%), mp 94 °C. IR  $\nu_{\text{max}}$  (KBr) 3450, 2258;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.94 (d, 2H, 1H), 6.90 (dd, 2, 8 Hz, 1H), 6.69 (d, 8 Hz, 1H), 5.61 (s, 1H), 2.82 (t, 7 Hz, 2H), 2.55 (t, 7 Hz, 2H), 2.20 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 153.3, 130.9, 129.8, 126.7, 124.5, 119.5, 115.2, 30.6, 19.7, 15.8; EI-MS 161 [ $\text{M}^+$ , 20%], 121 [100], 91 [18], 77 [20].

### 2.2.22. Synthesis of

#### 3-(5-bromo-2-hydroxy-3-methylphenyl)propanoic acid lactone (**9h**)

Method 1: A solution of **9i** (1.012 g, 5.35 mmol) and acrylonitrile (0.35 mL, 0.282 g, 5.35 mmol) in DCB (5 mL) was added dropwise to anhyd.  $\text{AlBr}_3$  (2.701 g, 10.7 mmol) suspended in DCB (20 mL). The mixture was heated at 110 °C as dry HCl gas was passed for 5 h, then heated for a further 43 h at 110 °C, cooled and added to water (100 mL). The mixture was extracted with  $\text{CHCl}_3$  (70 mL  $\times$  3) and the combined extracts were washed with sat. NaCl aq., dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give a yellow solid (200 mg). Recrystallization from  $\text{CHCl}_3$ /hexane give white crystalline **9h** (120 mg, 10%), mp 93 °C. IR  $\nu_{\text{max}}$  (KBr), 1772;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.24 (d, 2.5 Hz, 1H), 7.16 (d, 2.5 Hz, 1H), 2.96 (t, 7 Hz, 2H), 2.76 (t, 7 Hz, 2H), 2.27 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 167.9, 149.4, 132.4, 128.5, 128.2, 124.4, 116.3, 28.6, 23.7, 15.4; EI-MS 242 [ $\text{M}^+$ , ( $^{81}\text{Br}$ ), 100%], 240 [ $\text{M}^+$ , ( $^{79}\text{Br}$ ), 100], 214 [84], 212 [84]; 200 [20], 198 [20], 172 [22], 170 [22], 162 [15], 133 [35], 91 [95].

Method 2: To a solution of **9g** (100 mg, 0.617 mmol) in DCM (2 mL) was added dropwise a solution of  $\text{Br}_2$  (0.98 mg, 0.617 mmol) in 1,2-dichloroethane (2 mL) at 0 °C, over 30 min. The mixture was stirred for 2 h at 0 °C, then at 20 °C overnight. Evaporation under reduced pressure left a residue (300 mg), which was dissolved in  $\text{CHCl}_3$  (70 mL). The solution was washed with sat. NaCl aq. (40 mL  $\times$  3), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give a white powder (200 mg). Crystallization from benzene/hexane gave white needles of **9h** (141 mg, 95%), mp 93 °C.

### 2.2.23. Synthesis of

#### 3-(5-bromo-2-hydroxy-3-methylphenyl)propanoic acid (**9a**)

The procedure was like that for **7a** and gave **9a** as a yellow solid in 75% yield, mp 100 °C. IR  $\nu_{\text{max}}$  (KBr) 3500, 1750;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  12.09 (b, 1H), 8.54 (s, 1H), 7.09 (d, 2.5 Hz, 1H), 7.06 (d, 2.5 Hz, 1H), 2.76 (t, 8 Hz, 2H), 2.46 (t, 8 Hz, 2H), 2.15 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  174.0, 152.3, 130.8, 130.3, 129.6, 127.4, 110.4, 33.6, 25.4, 16.5; EI-MS 260 [ $\text{M}^+$ , ( $^{81}\text{Br}$ ), 11%], 258 [ $\text{M}^+$ , ( $^{79}\text{Br}$ ), 11%], 242 [45], 240 [45], 214 [40], 212 [40], 200 [10], 198 [10], 188 [68], 186 [70], 162 [80], 134 [70], 120 [20], 107 [80], 91 [100], 77 [72].

### 2.2.24. Synthesis of benzyl

#### 3-(5-bromo-2-hydroxy-3-methylphenyl)propanoate (**9b**)

The procedure was like that for **6b**. **9b** was a colourless oil, 70% yield. IR  $\nu_{\text{max}}$  (KBr) 3350, 1713;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.33 (m, 5H), 7.12 (d, 2 Hz, 1H), 7.04 (d, 2 Hz, 1H), 5.12 (s, 2H), 2.84 (m, 2H), 2.75 (m, 2H), 2.22 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  175.7, 151.8, 135.1, 131.8, 130.5, 129.3, 128.9, 128.5, 128.33, 128.32, 112.1, 67.3, 35.2, 24.4, 16.3;

CI-MS 368 [(M+ $\text{NH}_4$ ) $^+$ , ( $^{81}\text{Br}$ ), 30%], 366 [(M+ $\text{NH}_4$ ) $^+$ , ( $^{79}\text{Br}$ ), 30], 351 [(M+1) $^+$ , 15], 349 [(M+1) $^+$ , 15], 288 [5], 261 [29], 180 [100].

### 2.2.25. Synthesis of

#### 4-bromo-6-methyl-2-(2-benzoyloxycarbonyl)ethyl)phenyl acridine-9-carboxylate (**9c**)

The procedure was like that for **6c**. **9c** was a yellow crystalline solid, 75% yield, mp 105–106 °C. IR  $\nu_{\text{max}}$  (KBr), 1744;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 8.37 (d, 9 Hz, 2H), 8.32 (d, 8 Hz, 2H), 7.82 (m, 2H), 7.60 (m, 2H), 7.4–7.2 (m, 7H), 5.05 (s, 2H), 3.09 (t, 7.5 Hz, 2H), 2.71 (t, 7.5 Hz, 2H), 2.40 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 171.8, 165.3, 148.7, 147.1, 135.6, 134.8, 134.7, 132.9, 132.7, 130.7, 130.4, 130.3, 128.9, 128.5, 128.3, 128.2, 127.8, 122.8, 120.0, 66.5, 34.1, 26.1, 18.0; CI-MS 556 [(M+H) $^+$  ( $^{81}\text{Br}$ ) 100%], 554 [(M+H) $^+$  ( $^{79}\text{Br}$ ), 100], 476 [10], 270 [2], 206 [50], 108 [2], 91 [5].

### 2.2.26. Synthesis of 4-bromo-6-methyl-2-(2-carboxyethyl)phenyl acridine-9-carboxylate (**9d**)

The preparation was like that for **6d**. **9d** was a yellow crystalline solid, 85% yield, mp 179–180 °C. IR  $\nu_{\text{max}}$  (KBr) 3500, 1736, 1715; The NMR spectra were more complex than expected, possibly due to mixtures of forms in solution, but the major observed resonances are given;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  12.28 (s, 1H), 8.3–8.4 (m, 4H), 7.96 (m, 2H), 7.80 (m, 2H), 7.54 (s, 1H), 7.29 (m, 1H), 3.02 (m, 2H), 2.62 (m, 2H), 2.40 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ) 173.1, 164.7, 148.1, 146.7, 135.4, 134.1, 132.8, 132.0, 130.7, 129.9, 128.1, 126.8, 124.5, 121.8, 119.2, 33.4, 25.4, 17.0; CI-MS 466 [(M+H) $^+$  ( $^{81}\text{Br}$ ), 30%], 464 [(M+H) $^+$  ( $^{79}\text{Br}$ ), 30], 386 [100], 224 [5], 206 [10], 180 [60].

### 2.2.27. Synthesis of

#### 4-bromo-6-methyl-2-(2-succinimidylloxycarbonyl)ethyl)phenyl acridine-9-carboxylate (**9e**)

Compound **9e**, 70% yield, a yellow gum, was prepared like **6e**. IR  $\nu_{\text{max}}$  (KBr) 1787, 1737, 1669;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.3–8.4 (m, 4H), 7.84 (m, 2H), 7.68 (m, 2H), 7.45 (br s, 2H), 3.17 (t, 7.5 Hz, 2H), 2.93 (t, 7.5 Hz, 2H), 2.75 (s, 4H), 2.44 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 168.8, 167.4, 165.3, 148.7, 147.1, 134.5, 133.5, 133.2, 133.1, 130.8, 130.43, 130.36, 128.0, 124.7, 122.7, 120.2, 31.0, 25.7, 25.5, 17.8; FAB-MS 585 [(M+Na) $^+$ , ( $^{81}\text{Br}$ ), 10%], 583 [(M+Na) $^+$ , ( $^{79}\text{Br}$ ), 10], 563 [(M+H) $^+$ , 54], 561 [(M+H) $^+$ , 54], 225 [96], 206 [100], 179 [64].

### 2.2.28. Synthesis of

#### 4-bromo-6-methyl-2-(2-succinimidylloxycarbonyl)ethyl)phenyl 10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**9**)

A procedure as for **6** gave yellow solid **9**, 82%, mp 190–192 °C. IR  $\nu_{\text{max}}$  (KBr) 1800, 1760, 1750; NMR ( $\text{DMSO}-d_6$ )  $^1\text{H}$   $\delta$  8.95 (d, 9 Hz, 2H), 8.55 (d, 9 Hz, 2H), 8.35 (m, 2H), 8.15 (m, 2H), 7.57 (s, 1H), 7.56 (s, 1H), 4.97 (s, 3H), 2.97 (m, 2H), 2.63 (m, 2H), 2.59 (s, 4H), 2.50 (s, 3H);  $^{13}\text{C}$   $\delta$  173.7, 164.0, 162.6, 141.9, 145.3, 146.7, 139.2, 136.7, 135.5, 133.3, 132.2, 131.4, 130.5, 129.8, 127.0, 122.3, 120.1, 40.1, 33.3, 29.5, 25.2, 17.5; FAB-MS 577 [(M-SO<sub>3</sub>CF<sub>3</sub>) $^+$ , ( $^{81}\text{Br}$ ), 5%], 575 [(M-SO<sub>3</sub>CF<sub>3</sub>) $^+$ , ( $^{79}\text{Br}$ ), 5], 562 [10], 480 [100], 464 [34], 221 [58], 207 [7], 193 [35], 179 [61].

### 2.2.29. Synthesis of benzyl 3-(3-hydroxyphenyl)propanoate (**10b**)

A mixture of **10a** (0.780 g, 4.70 mmol) in MeOH (5 mL) and KOH (0.268 g, 4.70 mmol) in MeOH (25 mL) was stirred at 20 °C for 1 h. The solvent was evaporated and the residue dried under vacuum. This K $^+$  salt (0.800 g, 3.90 mmol) and dibenzo-18-crown-6 (0.143 g, 0.44 mmol) were stirred at 80–90 °C for 30 min in DMF-acetonitrile (16 mL, 1:2, v/v), benzyl chloride (0.63 mL, 4.4 mmol) was added and stirring maintained for 5 h. The solid formed was filtered off and the filtrate evaporated to give a thick brown liquid. The crude product was purified by column chromatography (silica,  $\text{CHCl}_3$ ) and fractions with a component having Rf = 0.63 (silica

TLC, toluene–ethyl acetate, 4:1) were collected. Evaporation of solvent gave **10b** (0.844 g, 84%) as a thick yellow liquid. IR  $\nu_{\max}$  (thin film), 3385, 1732;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ) 7.27–7.40 (m, 5H), 7.14 (apparent t, 8 Hz, 1H), 6.68–6.76 (m, 3H), 5.86 (b, 1H), 5.14 (s, 2H), 2.93 (t, 7.5 Hz, 2H), 2.71 (t, 7.5 Hz, 2H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ )  $\delta$  173.2, 155.9, 142.1, 135.8, 129.4, 128.7, 128.3, 120.5, 115.3, 113.4, 66.6, 35.8, 30.8; FAB-MS  $m/z$  279 [(M+Na) $^+$ , 26%], 256 [M $^+$ , 99], 165 [65], 107 [100]; Anal. Calcd. for  $\text{C}_{16}\text{H}_{16}\text{O}_3$ : C, 74.98%; H, 6.29%; Found: C, 74.66%; H, 6.18%.

#### 2.2.30. Synthesis of 3-(2-benzyloxycarbonylethyl)phenyl acridine-9-carboxylate (**10c**)

A mixture of **10b** (0.613 g, 2.40 mmol) and acridine-9-carboxylic chloride (0.610 g, 2.52 mmol) in anhyd. pyridine (previously stored over 3A molecular sieves, 10 mL) was stirred at 20 °C overnight then poured into hydrochloric acid (50 mL, 1 M). **3c** (0.680 g, 56%), was collected as a pale yellow solid by filtration, mp 106–108 °C. This was used in the subsequent synthesis, but a sample was purified by column chromatography (silica,  $\text{CHCl}_3$ –ethyl acetate 4:1). Fractions having  $R_f = 0.64$  (silica, toluene–ethyl acetate, 4:1) were combined. Evaporation gave pale yellow solid **3c**. IR  $\nu_{\max}$  (KBr), 1755, 1722;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  8.31 (d, 9 Hz, 2H), 8.21 (d, 8.5 Hz, 2H), 7.83 (dd, 8.5, 7 Hz, 2H), 7.66 (dd, 9, 7 Hz, 2H), 7.43 (apparent t, 8 Hz, 1H), 7.24–7.35 (m, 7H), 7.15 (d, 8 Hz, 1H), 5.14 (s, 2H), 3.08 (t, 8 Hz, 2H), 2.77 (t, 8 Hz, 2H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ )  $\delta$  172.6, 165.9, 150.5, 148.6, 142.6, 135.8, 135.8, 130.5, 130.0, 129.9, 128.6, 128.5, 128.3, 127.5, 126.7, 124.9, 122.4, 121.4, 119.4, 66.5, 35.7, 30.8; FAB-MS  $m/z$  484 [(M+Na) $^+$ , 11%], 462 [(M+H) $^+$ , 100], 206 [49]; Anal. Calcd. for  $\text{C}_{30}\text{H}_{23}\text{NO}_4$ : C, 78.08%; H, 5.02%, N, 3.03%; Found: C, 77.90%; H, 5.11%, N, 2.99%.

#### 2.2.31. Synthesis of 3-(2-carboxyethyl)phenyl acridine-9-carboxylate (**10d**)

The procedure was like that for **6d**, giving **10d** in 78% yield, mp 194–195 °C. IR  $\nu_{\max}$  (KBr) 3056, 1749, 1729;  $^1\text{H NMR}$  ( $\text{DMSO}-d_6$ ) 8.31 (d, 9 Hz, 2H), 8.30 (d, 9 Hz, 2H), 8.01 (m, 2H), 7.84 (m, 2H), 7.44–7.52 (m, 3H), 7.30 (d, 7 Hz, 1H), 2.98 (t, 8 Hz, 2H), 2.66 (t, 8 Hz, 1H) (the exchangeable proton was not observed);  $^{13}\text{C NMR}$  ( $\text{DMSO}-d_6$ )  $\delta$  173.6, 165.5, 150.1, 147.9, 143.4, 135.6, 131.0, 129.7, 129.5, 128.1, 126.7, 124.9, 121.7, 121.5, 119.5, 35.1, 30.2; FAB-MS  $m/z$  394 [(M+Na) $^+$ , 12%], 372 [(M+H) $^+$ , 100], 206 [46], 179 [54]; Anal. Calcd. for  $\text{C}_{23}\text{H}_{17}\text{NO}_4$ : C, 74.38%; H, 4.61%; N, 3.77%; Found: C, 74.58%; H, 4.39%; N, 3.98%.

#### 2.2.32. Synthesis of 3-(2-succinimidylloxycarbonylethyl)phenyl acridine-9-carboxylate (**10e**)

**10e** was prepared like **4e**. Yield 67%; mp 202–203 °C. IR  $\nu_{\max}$  (KBr), 1818, 1784, 1753, 1734;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  8.31 (d, 9 Hz, 2H), 8.25 (d, 9 Hz, 2H), 7.85 (dd, 9, 7 Hz, 2H), 7.68 (dd, 9, 7 Hz, 2H), 7.49 (m, 1H), 7.33–7.35 (m, 2H), 7.26 (s, 1H), 3.18 (t, 8 Hz, 2H), 3.00 (t, 8 Hz, 2H), 2.83 (s, 4H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ )  $\delta$  169.0, 167.7, 165.9, 150.7, 148.7, 141.3, 135.8, 130.4, 130.2, 130.1, 127.6, 126.6, 125.0, 122.4, 121.5, 119.9, 32.5, 30.3, 25.6; FAB-MS  $m/z$  491 [(M+Na) $^+$ , 11%], 469 [(M+H) $^+$ , 100], 206 [17], 179 [40]; Anal. Calcd. for  $\text{C}_{27}\text{H}_{20}\text{N}_2\text{O}_6$ : C, 69.23%; H, 4.30%; N, 5.98%; Found: C, 69.04%; H, 4.08%; N, 5.87%.

#### 2.2.33. Synthesis of 3-(2-succinimidylloxycarbonylethyl)phenyl 10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**10**)

The procedure was like that for **6**, giving **10** as a yellow solid, 71% yield, mp 158–161 °C. IR  $\nu_{\max}$  (KBr), 1813, 1785, 1757, 1738;  $^1\text{H NMR}$  ( $\text{DMSO}-d_6$ )  $\delta$  8.96 (d, 9 Hz, 2H), 8.64 (d, 9 Hz, 2H), 8.57 (m, 2H), 8.24 (m, 2H), 7.71 (s, 1H), 7.54–7.64 (m, 2H), 7.44 (d, 7.5 Hz, 1H), 4.97 (s, 3H), 3.10–3.19 (m, 4H), 2.84 (s, 4H);  $^{13}\text{C NMR}$  ( $\text{DMSO}-d_6$ )  $\delta$  170.2, 168.4, 163.6, 149.6, 146.9, 142.1, 142.0, 139.3, 130.1, 129.8, 127.7, 127.7, 127.6, 122.3, 121.7, 120.0, 39.9, 31.5, 29.6, 25.5 ( $\text{CF}_3$  carbon not observed); FAB-MS  $m/z$  483 [(M– $\text{SO}_3\text{CF}_3$ ) $^+$ , 99%], 221 [35],

193 [100]; Anal. Calcd. for  $\text{C}_{29}\text{H}_{23}\text{N}_2\text{F}_3\text{O}_9\text{S}$ : C, 55.06%; H, 3.66%; N, 4.43%; Found: C, 55.03%; H, 3.71%, N, 4.34%.

### 2.3. Chemiluminescent kinetics and efficiency

A standard procedure was employed for estimation of chemiluminescent kinetics and efficiency. The samples (compounds **1** and **6–10**, around 1 mg each) were separately dissolved in anhydrous acetonitrile (1.0 mL). The solutions were diluted to  $1 \times 10^{-10}$  mol/L using  $1.00 \times 10^{-3}$  M hydrochloric acid. To a test tube containing the diluted sample solution (10  $\mu\text{L}$ ), hydrogen peroxide solution (0.3 mL of 0.5%, w/v in 0.1 mol/L  $\text{HNO}_3$ ) was delivered automatically, followed by NaOH solution (0.3 mL of 0.25 mol/L) containing a surfactant. The output of photons was counted for 3 s (compounds **1**, **8**, **10**), 10 s (compound **6**), or 200 s (compounds **7**, **9**). Chemiluminescent intensity versus time curves were plotted, from which chemiluminescent half peak times and life times were measured. The total counts were used to calculate the relative chemiluminescent efficiencies for all compounds.

## 3. Results and discussion

### 3.1. Syntheses of compounds **6**, **7**, **8**, **9** and **10**

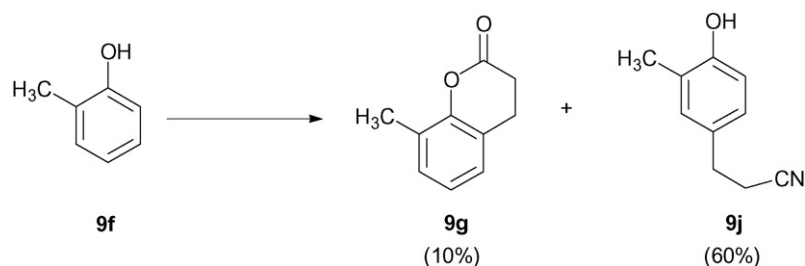
**Scheme 2** outlines the syntheses of the acridinium ester labels from the appropriate 3-(2-hydroxyphenyl)propanoic acid derivatives **6a–9a** or 3-(3-hydroxyphenyl)propanoic acid, **10a**. Firstly, the carboxylic acid groups were protected by benzylation. The benzyl esters **6b–10b** were then coupled to acridine-9-carbonyl chloride, which was prepared by reacting acridine-9-carboxylic acid with thionyl chloride. [7] Deprotection of **6c–10c** with a mixture of ethanoic acid and HBr yielded acids **6d–10d**, which were then esterified with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC) to give **6e–10e**. Finally, methylation by treatment with methyl trifluoromethanesulfonate under an atmosphere of nitrogen afforded the target molecules **6–10**. All of the reactions gave good yields (given in parentheses after the structure number for the compounds synthesized as recorded in **Scheme 2**).

Compound **10a** was commercially available. The syntheses of the substituted 3-(2-hydroxyphenyl)propanoic acids **6a–9a** are summarised in **Scheme 3**. Figures in parentheses under the reaction arrows are the yields obtained.

3-(2-Hydroxyphenyl)propanoic acid (**6a**) was prepared by hydrogenation of 3-(2-hydroxyprop-2-enoic acid (**6f**) over a palladium on carbon catalyst. The synthesis of 3-(2-hydroxy-3,5-dimethylphenyl)propanoic acid (**7a**) was begun with conversion of 2,4-dimethylphenol (**7f**) to 3-(2-hydroxy-3,5-dimethylphenyl)propanoic acid lactone (**7g**) by reaction with acrylonitrile under catalysis by  $\text{AlCl}_3$  and HCl. The resulting lactone was subsequently hydrolysed in base and the salt formed was converted to **7a** by acidification. 3-(3,5-Dibromo-2-hydroxyphenyl)propanoic acid (**8a**) was obtained by direct bromination of 3-(2-hydroxyphenyl)propanoic acid (**6a**).

The synthesis of 3-(5-bromo-2-hydroxy-3-methyl-phenyl)propanoic acid (**9a**) was carried out by initial selective monobromination of *o*-cresol (**9f**) to 4-bromo-2-methylphenol (**9i**), subsequent formation of the lactone (**9b**) by reaction with acrylonitrile under catalysis by  $\text{AlCl}_3$  and HCl, and finally by hydrolysis of the lactone to **9a**. However, the step of making the lactone (**9b**) suffered from a low yield (10%). No improvement in yield was achieved even after variation of the solvent, catalyst, temperature and reaction time. The reason for the low yield appears to be that the electrophilic substitution reaction is deactivated by the electron withdrawing nature of the Br group. An alternative approach was tried. In this approach, the Friedel–Crafts substitution and subsequent cyclisation to form the lactone was carried out prior to the bromination step, thereby





**Scheme 4.** Selectivity problem in the synthesis of **9g**.

avoiding the deactivating effect of the bromo group. However, this approach had a problem of selectivity in the step involving preparation of the lactone. The desired product was obtained in only 10% yield, while undesired 3-(3-methyl-4-hydroxyphenyl)propionitrile (**9j**, Scheme 4) was produced by the reaction in a yield of 60%.

Various attempts to improve the yield of **9g** by variation of the reaction conditions failed to give significant improvement. Therefore, it was decided to continue the route with the small amount of material available. The final product **9** was produced in sufficient quantity to allow evaluation of its chemiluminescent properties.

### 3.2. Chemiluminescent properties

#### 3.2.1. Chemiluminescence kinetics

The emission of light on oxidation of each of the compounds **1** and **6** through **10** was studied over time under a particular set of conditions (see Section 2) and the period of time for which luminescence could be detected (chemiluminescent lifetime) was determined. The results are recorded in Table 1, which illustrates that the chemiluminescent lifetimes are in the order  $7 > 9 > 6 > 10 \approx 1 > 8$ .

The rate-determining step of the chemiluminescent reaction of acridinium esters involves cleavage of the phenoxy moiety. [8] Electron withdrawing substituents like Br would lead to the phenoxy moiety becoming a better leaving group, whereas electron donating groups like CH<sub>3</sub> would cause the phenoxy moiety to become a relatively poor leaving group. Therefore, on electronic grounds the order of reactivity would be expected to be like the observed order. In addition, the results shown in Table 1 suggest that the effects of steric hindrance could also play a role in determining the kinetics of the chemiluminescence reaction because the more sterically hindered **6** showed significantly slower reaction kinetics than its less sterically hindered isomers **1** and **10**.

**Table 1**  
Chemiluminescent lifetime for the new compounds.

Compound	Lifetime (sec)
<b>1</b>	3
<b>6</b>	10
<b>7</b>	180
<b>8</b>	2
<b>9</b>	122
<b>10</b>	3

**Table 2**  
Relative chemiluminescent efficiencies of the new compounds.

Compound	Relative chemiluminescent efficiency
<b>1</b>	100
<b>6</b>	166
<b>7</b>	252
<b>8</b>	45
<b>9</b>	428
<b>10</b>	85

#### 3.2.2. Chemiluminescent efficiency

Chemiluminescent efficiency of acridinium esters is generally affected by structural modifications, but the results are not always predictable. The relative chemiluminescent efficiencies of **6**, **7**, **8**, **9** and **10** have therefore been examined in comparison with that of **1**, and the results are summarised in Table 2. As the results show, compounds **6**, **7**, and **9** show a much higher chemiluminescent efficiency than compound **1**, especially compound **9**, which has over four-fold higher chemiluminescence efficiency than compound **1**. Compounds **8** and **10** are less efficient, with compound **8** having the lowest efficiency, half of that of compound **1**.

### 4. Conclusions

Several *ortho*- and *meta*-linked phenyl acridinium ester labelling compounds, differing in the substituents on the phenyl group, have been successfully prepared in reasonable yields. All exhibit chemiluminescence when reacted with hydrogen peroxide and the *ortho*-linked compounds except for the dibromo derivative show significantly improved quantum yields of luminescence. The 4-bromo-6-methyl derivative exhibits a quantum yield of luminescence that is over four-fold higher than the unsubstituted *para*-linked analogue, which is widely used in biological assays. Both steric and particularly electronic effects are important in determining the rate at which the chemiluminescent reaction occurs, and this has thereby provided a range of labels which are differentiated in the time over which light is emitted. This provides opportunities for choosing a label with properties appropriate for a particular application. For example, the dibromo compound flashes quickly while the 4,6-dimethyl and 4-bromo-6-methyl compounds glow slowly, as a result of the relative ease of expulsion of the corresponding phenoxide anions.

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