Synthesis and properties of chemiluminescent acridinium esters with different N-Alkyl groups

Supplementary Material

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1. Figures S1-S13: HPLC traces, $^1$H-NMR spectra and HRMS (high resolution mass spectra) of synthetic intermediates and acridinium esters. Analytical HPLC was performed with a Phenomenex, Kinetex C$_{18}$, 5 micron, 150 x 4.6 mm column and using a 20 minute gradient of 10% → 100% acetonitrile/water (each with 0.05% trifluoroacetic acid, TFA) at a flow rate of 1 mL/min and UV detection at 260 nm.

2. Figure S14a-f: Chemiluminescence emission spectra of acridinium esters 1a-6a.

3. Figure S15: Chemiluminescence stability of BSA conjugates of 1b-6b.

4. Tables S1 and S2: Fractional non-specific binding (fNSB) experimental data.
**Compound 8**

![Chemical Structure of Compound 8]

**Figure S1a.** HPLC trace of compound 8.
Figure S1b. $^1$H-NMR of compound 8 in trifluoroacetic acid-d.
Figure S1c. HRMS of compound 8.
Compound 9

Figure S2a. HPLC trace of compound 9.
Figure S2b. $^1$H-NMR of compound 9 in trifluoroacetic acid-d.
Figure S2c. HRMS of compound 9.
Compound 2a

Figure S3a. HPLC trace of compound 2a.
Compound 2a

Figure S3b. $^1$H-NMR of compound 2a in trifluoroacetic acid-d.
Compound 2a

Figure S3c. HRMS of compound 2a.
Compound 2b

Figure S4a. HPLC trace of compound 2b.
Figure S4b. $^1$H-NMR of compound 2b in trifluoroacetic acid-d.
Compound 2b

Figure S4c. HRMS of compound 2b.
Compound 10

Figure S5a. HPLC trace of compound 10.
Figure S5b. $^1$H-NMR of compound 10 in trifluoroacetic acid-d._d.
Figure S5c. HRMS of compound 10.
Compound 3a

Figure S6a. HPLC trace of compound 3a.
Figure S6b. $^1$H-NMR of compound 3a in trifluoroacetic acid-d.
Compound 3a

Figure S6c. HRMS of compound 3a.
Compound 3b

Figure S7a. HPLC trace of compound 3b.
Compound 3b

Figure S7b. $^1$H-NMR of compound 3b in trifluoroacetic acid-d.
Compound 3b

Figure S7c. HRMS of compound 3b.
Compound 11

![Chemical Structure of Compound 11]

**Figure S8a.** HPLC trace of compound 11.
Compound 11

Figure S8b. $^1$H-NMR of compound 11 in trifluoroacetic acid-d.
Compound 11

Figure S8c. HRMS of compound 11.
Compound 4a

Figure S9a. HPLC trace of compound 4a.
Figure S9b. $^1$H-NMR of compound 4a in trifluoroacetic acid-d.
Compound 4a

Figure S9c. HRMS of compound 4a.
Figure S10a. HPLC trace of compound 4b.
Figure S10b. $^1$H-NMR of compound 4b in trifluoroacetic acid-d.
Figure S10c. HRMS of compound 4b.
Compound 13

Figure S11a. HPLC trace of compound 13.
Figure S11b. $^1$H-NMR of compound 13 in trifluoroacetic acid-d.
Compound 13

Figure S11c. HRMS of compound 13.
Compound 5a

![Chemical structure of compound 5a](image)

**Figure S12a.** HPLC trace of compound 5a.
Figure S12b. $^1$H-NMR of compound 5a in trifluoroacetic acid-d.
Compound 5a

Figure S12c. HRMS of compound 5a.
Compound 5b

Figure S13a. HPLC trace of compound 5b.
Figure S13b. $^1$H-NMR of compound 5b in trifluoroacetic acid-d.
Compound 5b

Figure S13c. HRMS of compound 5b.
Chemiluminescence emission spectra of acridinium ester labels 1a-6a

Figure S14a. Chemiluminescence emission spectrum of compound 1a.
Figure S14b. Chemiluminescence emission spectrum of compound 2a.
Figure S14c. Chemiluminescence emission spectrum of compound 3a.
Figure S14d. Chemiluminescence emission spectrum of compound 4a.
Figure S14e. Chemiluminescence emission spectrum of compound 5a.
Figure S14f. Chemiluminescence emission of compound 6a.
Figure S15. Chemiluminescence stability of BSA conjugates of labels 1b-6b at 37°C in pH 7.4 phosphate buffer. Compared to the reference compound 1b with an N-sulfopropyl group, better chemiluminescence stability was observed with bulkier N-alkyl groups.
Table S1. fNSB measurements of acridinium ester labels 1a-6a to 4 particles.

<table>
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<tr>
<th>Label</th>
<th>M-270 (amino surface)</th>
<th>M-270 (carboxylate surface)</th>
<th>M-280 (hydrophobic surface)</th>
<th>PMP (amino surface)</th>
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<tr>
<td>1a</td>
<td>7.9×10^{-4}</td>
<td>1.3×10^{-6}</td>
<td>6.6×10^{-5}</td>
<td>1.9×10^{-4}</td>
</tr>
<tr>
<td>2a</td>
<td>3.0×10^{-4}</td>
<td>9.1×10^{-6}</td>
<td>9.4×10^{-5}</td>
<td>4.4×10^{-5}</td>
</tr>
<tr>
<td>3a</td>
<td>7.4×10^{-5}</td>
<td>8.6×10^{-7}</td>
<td>3.3×10^{-5}</td>
<td>4.9×10^{-5}</td>
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<tr>
<td>4a</td>
<td>2.6×10^{-4}</td>
<td>3.4×10^{-6}</td>
<td>8.5×10^{-5}</td>
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<tr>
<td>5a</td>
<td>2.0×10^{-3}</td>
<td>4.8×10^{-6}</td>
<td>4.0×10^{-4}</td>
<td>7.3×10^{-5}</td>
</tr>
<tr>
<td>6a</td>
<td>1.2×10^{-4}</td>
<td>1.4×10^{-6}</td>
<td>5.8×10^{-5}</td>
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Table S2. fNSB measurements of BSA conjugates of 1b-6b to 4 particles.

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<tr>
<th>BSA conjugates</th>
<th>M-270 (amino surface)</th>
<th>M-270 (carboxylate surface)</th>
<th>M-280 (hydrophobic surface)</th>
<th>PMP (amino surface)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b conjugate</td>
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<td>1.9×10^{-6}</td>
<td>2.4×10^{-5}</td>
<td>5.1×10^{-5}</td>
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<tr>
<td>2b conjugate</td>
<td>4.8×10^{-5}</td>
<td>6.3×10^{-6}</td>
<td>2.7×10^{-4}</td>
<td>2.8×10^{-5}</td>
</tr>
<tr>
<td>3b conjugate</td>
<td>1.2×10^{-5}</td>
<td>1.9×10^{-6}</td>
<td>9.3×10^{-5}</td>
<td>1.8×10^{-5}</td>
</tr>
<tr>
<td>4b conjugate</td>
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<td>4.9×10^{-6}</td>
<td>4.9×10^{-4}</td>
<td>1.7×10^{-5}</td>
</tr>
<tr>
<td>5b conjugate</td>
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<td>2.8×10^{-6}</td>
<td>4.2×10^{-4}</td>
<td>2.5×10^{-5}</td>
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<tr>
<td>6b conjugate</td>
<td>2.3×10^{-5}</td>
<td>1.7×10^{-6}</td>
<td>1.1×10^{-4}</td>
<td>2.6×10^{-5}</td>
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