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

groups

Supplementary Material

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- Figures S1-S13: HPLC traces, ¹H-NMR spectra and HRMS (high resolution mass spectra) of synthetic intermediates and acridinium esters. Analytical HPLC was performed with a Phenomenex, Kinetex C₁₈, 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.







COOMe 8



Figure S1b. ¹H-NMR of compound 8 in trifluoroacetic acid-d.





Figure S1c. HRMS of compound 8.











Figure S2b. ¹H-NMR of compound 9 in trifluoroacetic acid-d.





Figure S2c. HRMS of compound 9.

Compound 2a







Compound 2a



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

Compound 2a



SW1 16916129.00000000		23-Apr-2014 16:25:30
20140422EXT9 76 (1.646) AM (Cen,4, 80.00, Ar,9000.0,6	309,28,0.70,LS 10); Sm (SG, 2x4.00); Cm (76:83) 515,2534	16:25:30 1: TOF MS ES+ 1.32e3
8		
141.1315 181.6218 229.1449 261.1300 305.1584	516.2604 667.4409	

Figure S3c. HRMS of compound 2a.

Compound 2b







Compound 2b





Figure S4b. ¹H-NMR of compound 2b in trifluoroacetic acid-d.

Compound 2b





Figure S4c. HRMS of compound 2b.











Figure S5b. ¹H-NMR of compound **10** in trifluoroacetic acid-d.





Figure S5c. HRMS of compound 10.

Compound 3a







Compound 3a



Figure S6b. ¹H-NMR of compound 3a in trifluoroacetic acid-d.

Compound 3a



W1 17716129.00000000		25-Apr-2014 11:42:50
00- 6	wi (Ceri,4, 60.00, Ar,9000.0,609.28,0.70,LS 10); Sm (SG, 2x4.00) 37.2416	1: TOF MS ES+
00		3.0360
-		
	r	
8		
-	638.2590	
	659 2462	
606 0047 620 5207	639.2679 677.2533	
0		
600 610 620 630	640 650 660 670 680 690 700 710 720 730 740 750 760 770 780	790 800

Figure S6c. HRMS of compound 3a.

Compound 3b







Compound 3b



Figure S7b. ¹H-NMR of compound **3b** in trifluoroacetic acid-d.

Compound 3b



SW1	18016129.00000000							2	3-Apr-2014
20140	0422EXT15 43 (0.942) AM (Cen,4,	80.00, Ar,9000.	.0,609.28,0).70,LS 10); Sm (SG, 2x4. 73	.00); Cm (26:54) 4.2891		1: 1	OF MS ES+ 4.34e3
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%									
						735.2924			
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	141 1226								
	236 1066 310.1304			612.2775		736 2908			
	273.1751 310.6	322 426.1833		613.287	7	COLUCIO C			
100) 200 300	400	500	600	700	800	900	1000	m/z 1100

Figure S7c. HRMS of compound 3b.











Figure S8b. ¹H-NMR of compound **11** in trifluoroacetic acid-d.





Figure S8c. HRMS of compound 11.

Compound 4a







Compound 4a





Figure S9b. ¹H-NMR of compound 4a in trifluoroacetic acid-d.

Compound 4a



SW1 173161	129.0000000					23-Apr-2014
20140422EXT	Г11 69 (1.509) АМ (Cen,4, 80.00,	Ar,9000.0,609.28,	0.70,LS 10); Sm	(SG, 2x4.00); C 77	m (69:73) 9.4128	16:34:24 1: TOF MS ES4 1.33e4
%						
-					801.4017	
0	264.1267 412 264.6336 401.1967 200 300 40	2.1849 412.6906 10 500	677.35	⁰³ 710.4100 700	802.4122 819.4173 800 900	m/z 1000 1100

Figure S9c. HRMS of compound 4a.

Compound 4b







Compound 4b



Figure S10b. ¹H-NMR of compound 4b in trifluoroacetic acid-d.

Compound 4b



SW1 182	16129.00000	000								23-Apr-201
201404228	EXT16 39 (0.854	4) AM (Cen,4	4, 80.00, Ar,90	00.0,609.28,0	0.70,LS 10); Sm	(SG, 2x4.00))	070 (100	1:	TOF MS ES+
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-										
8										
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								877 4510		
								011.4510		
								878.4594		
0					625.3707					
100	200	300	400	500	600	700	800	900	1000	1100 m/z

Figure S10c. HRMS of compound 4b.









Figure S11b. ¹H-NMR of compound 13 in trifluoroacetic acid-d.





Figure S11c. HRMS of compound 13.

Compound 5a







Compound 5a



Figure S12b. ¹H-NMR of compound 5a in trifluoroacetic acid-d.

Compound 5a





Figure S12c. HRMS of compound 5a.

Compound 5b







Compound 5b

O H

ot^{Me}

ò 6 0 O Ċ 6.08 6.07 6.06 55.25 55.25 55.25 55.23 55.255 $<^{1.87}_{1.86}$ NAME F2 - Acc Date_ Instrum FROBHD FULPROG DD SOLVENT NS SOLVENT NS SWH FIDRES AQ RG AQ DB TE D1 TE D1 TD0 SF01 PLW1 AE SW1-179 isition Pa 1.00000 ANNEL f1 === MHz 11.00 use 19.79999924 W F2 - Pro SI SF WDW SSB LB GB PC ssing parameters 65536 600.1231821 MHz EM 0.30 Hz 1.00 1 ĥ 2 7 0 ppm 9 8 6 5 4 3 i 3.799 1.960 5.921 1.008 JIII 1.845 5.996 20.401 1.997 1.977 4.959 1.170 1.898 0.941 888 . 974

Figure S13b. ¹H-NMR of compound **5b** in trifluoroacetic acid-d.

Compound 5b



SW1	17916	129.0	00000	00																23-Apr-2014 17:03:17
20140	0422EX	T17 38	(0.836	6) AM (Cen,4,	80.00,	Ar,900	0.0,609	9.28,0. 926.3	.70,LS 919	10); Sm	(SG, :	2x4.00)					1:	TOF MS ES+ 4.40e3
%										927.4	1004									
0	910	912	914	916	918	920	922	924	926	928	28.4174 930	932	934	936	938	940	942	944	946	949.4050 948 m/z

Figure S13c. HRMS of compound 5b.



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 part	ticles.
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Label	M-270 (amino surface)	M-270 (carboxylate surface)	M-280 (hydrophobic surface)	PMP (amino surface)
1a	7.9×10 ⁻⁴	1.3×10⁻ ⁶	6.6×10⁻⁵	1.9×10 ⁻⁴
2a	3.0×10 ⁻⁴	9.1×10 ⁻⁶	9.4×10 ⁻⁵	4.4×10 ⁻⁵
3a	7.4×10 ⁻⁵	8.6×10 ⁻⁷	3.3×10⁻⁵	4.9×10⁻⁵
4a	2.6×10 ⁻⁴	3.4×10⁻ ⁶	8.5×10⁻⁵	2.2×10⁻⁵
5a	2.0×10 ⁻³	4.8×10 ⁻⁶	4.0×10 ⁻⁴	7.3×10⁻⁵
6a	1.2×10 ⁻⁴	1.4×10 ⁻⁶	5.8×10⁻⁵	7.4×10 ⁻⁵

		M-270	M-280	
BSA	M-270 (amino	(aarbayy data	(b) dranhahia	PMP (amino
coniugates	surface)	(Carboxylate	(hyarophobic	surface)
Conjugates	50110007	surface)	surface)	5010007
1b conjugate	5.0×10 ⁻⁵	1.9×10⁻ ⁶	2.4×10⁻⁵	5.1×10⁻⁵
2b conjugate	4.8×10 ⁻⁵	6.3×10⁻ ⁶	2.7×10 ⁻⁴	2.8×10⁻⁵
3b conjugate	1.2×10⁻⁵	1.9×10 ⁻⁶	9.3×10⁻⁵	1.8×10⁻⁵
4b conjugate	3.5×10⁻⁵	4.9×10 ⁻⁶	4.9×10 ⁻⁴	1.7×10⁻⁵
5b conjugate	1.6×10 ⁻⁴	2.8×10 ⁻⁶	4.2×10 ⁻⁴	2.5×10⁻⁵
6b conjugate	2.3×10⁻⁵	1.7×10⁻ ⁶	1.1×10 ⁻⁴	2.6×10 ⁻⁵

 Table S2. fNSB measurements of BSA conjugates of 1b-6b to 4 particles.