

**A comparison of chemiluminescent acridinium dimethylphenyl ester labels with different  
conjugation sites**

Supplementary Material

Anand Natrajan\* and David Wen

*Siemens Healthcare Diagnostics*

*Advanced Technology and Pre-Development*

*333 Coney Street*

*East Walpole, MA 02032*

\* Author to whom correspondence should be directed.

E-mail: [anand.natrajan@siemens.com](mailto:anand.natrajan@siemens.com)

Phone: 1-508-660-4582

Fax: 1-508-660-4591

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1. HPLC traces and NMR spectra of compounds **2a-2c** and **3a-3c** (Figures S1-S6). HPLC analysis was performed using a Phenomenex, Kinetex C<sub>18</sub>, 50 x 4.6 mm, 2.6 micron column and a 10 minute gradient of 10 → 90% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL/minute and UV detection at 260 nm. NMR spectra were recorded in CF<sub>3</sub>CO<sub>2</sub>D using a 600 MHz Bruker NMR spectrometer.
2. Chemiluminescence emission profiles of labels **2a-2c**, amine derivative of **4**, and protein conjugates of **3a-3c** and **4** in the absence of CTAC (Figures S7-S10).
3. Emission spectra of anti-TSH Mab and anti-HBsAg Mab conjugates of **4** and **3a-3c** (Figures S11-S12).

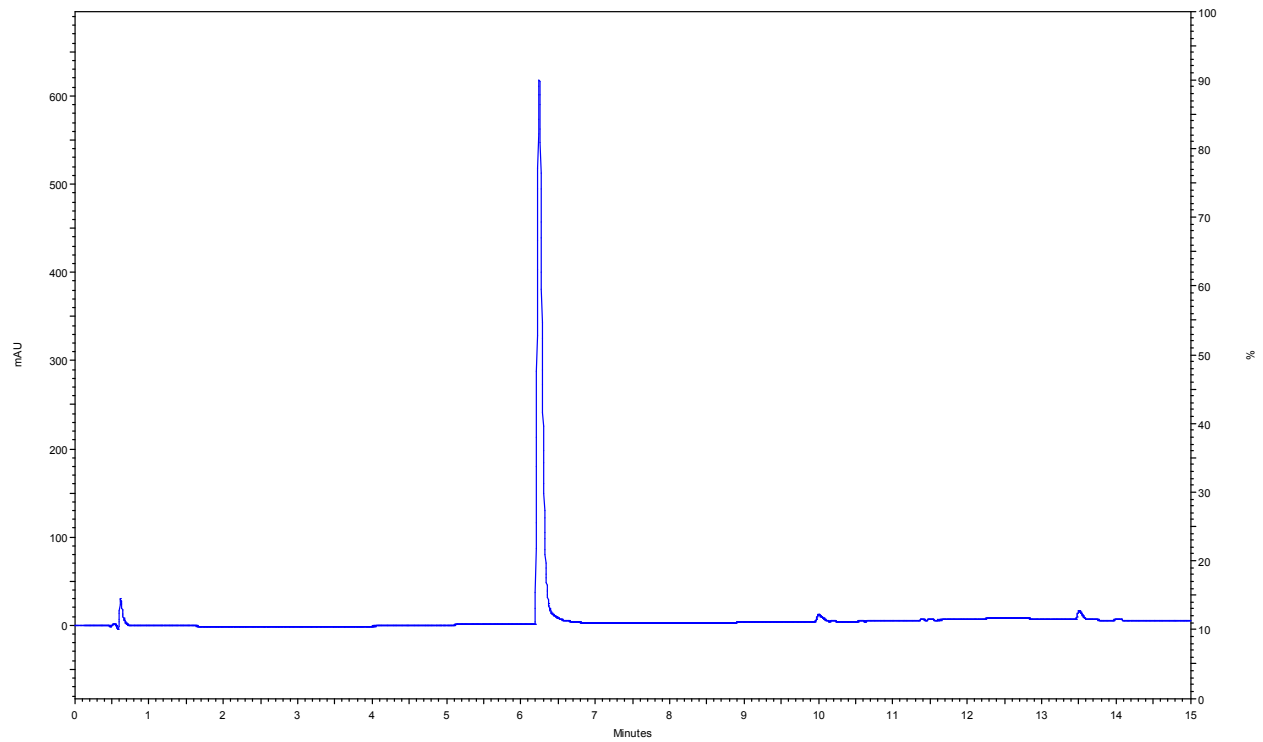
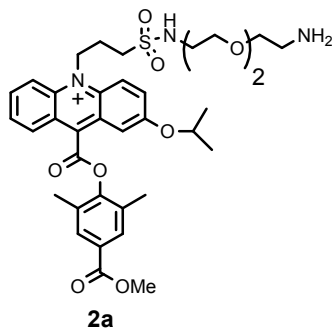


Figure S1A. HPLC trace of **2a**.



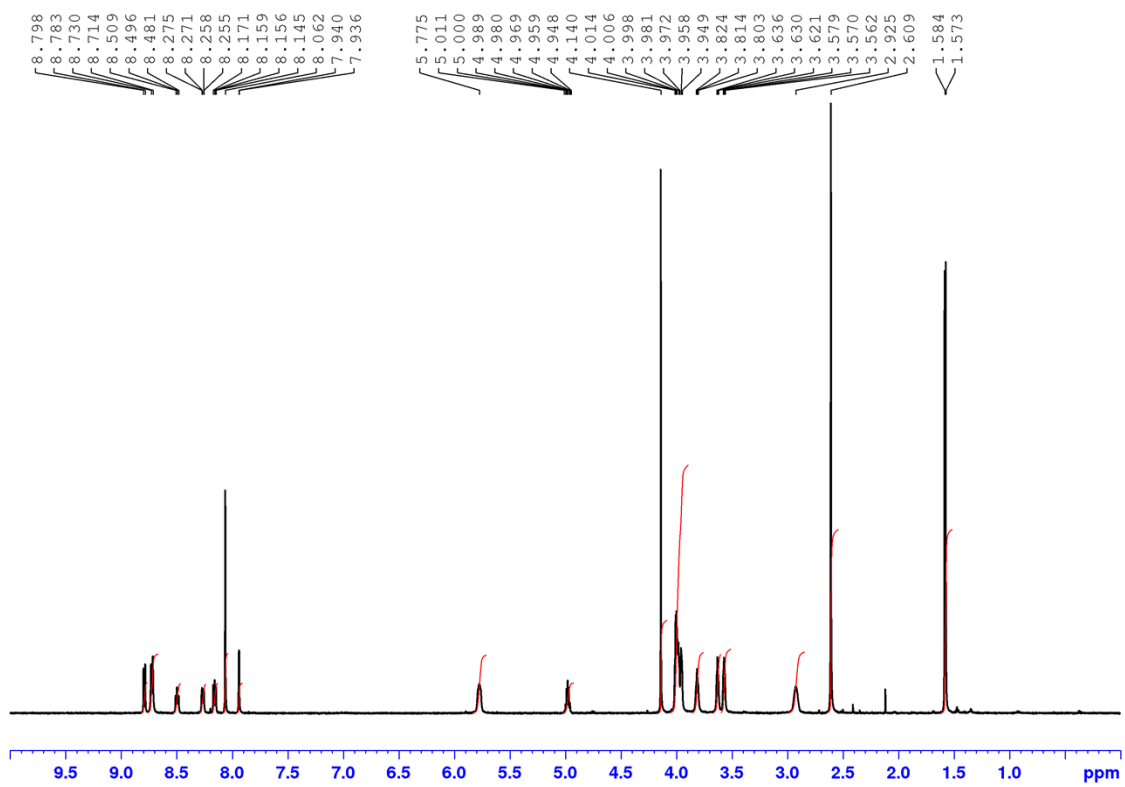
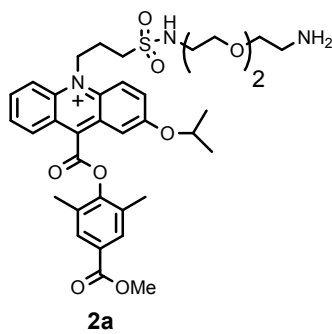


Figure S1B.  $^1\text{H-NMR}$  spectrum of **2a**.



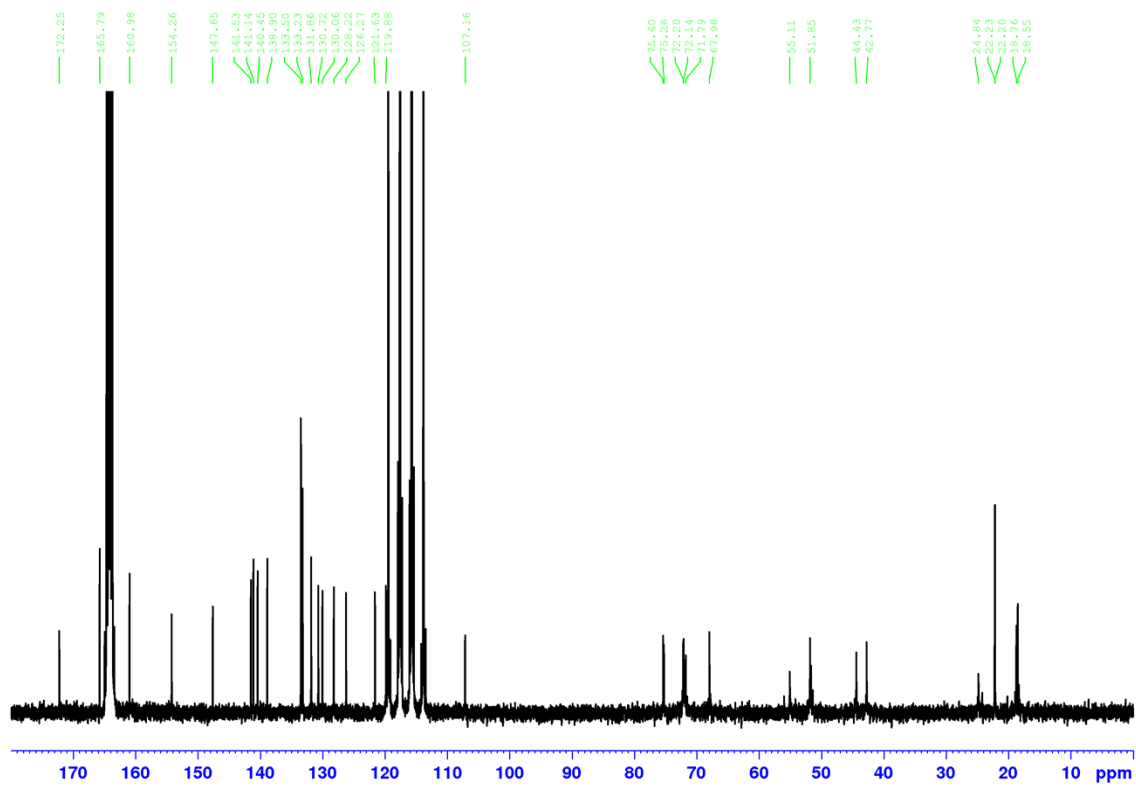
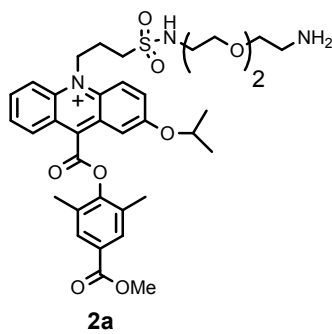


Figure S1C.  $^{13}\text{C}$ -NMR spectrum of **2a**.



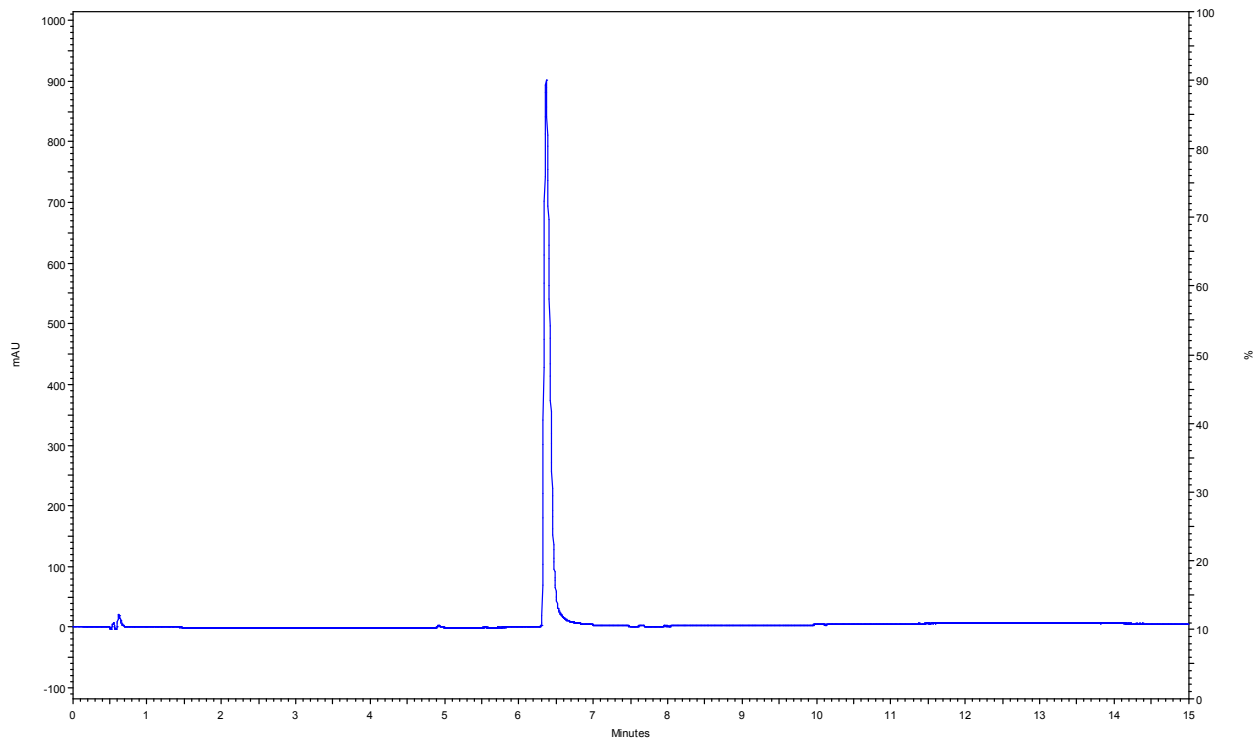
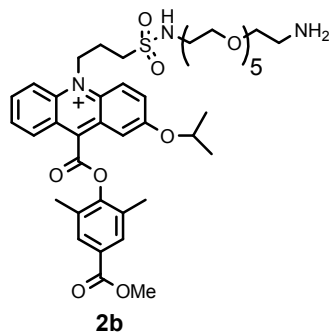


Figure S2A. HPLC trace of **2b**.



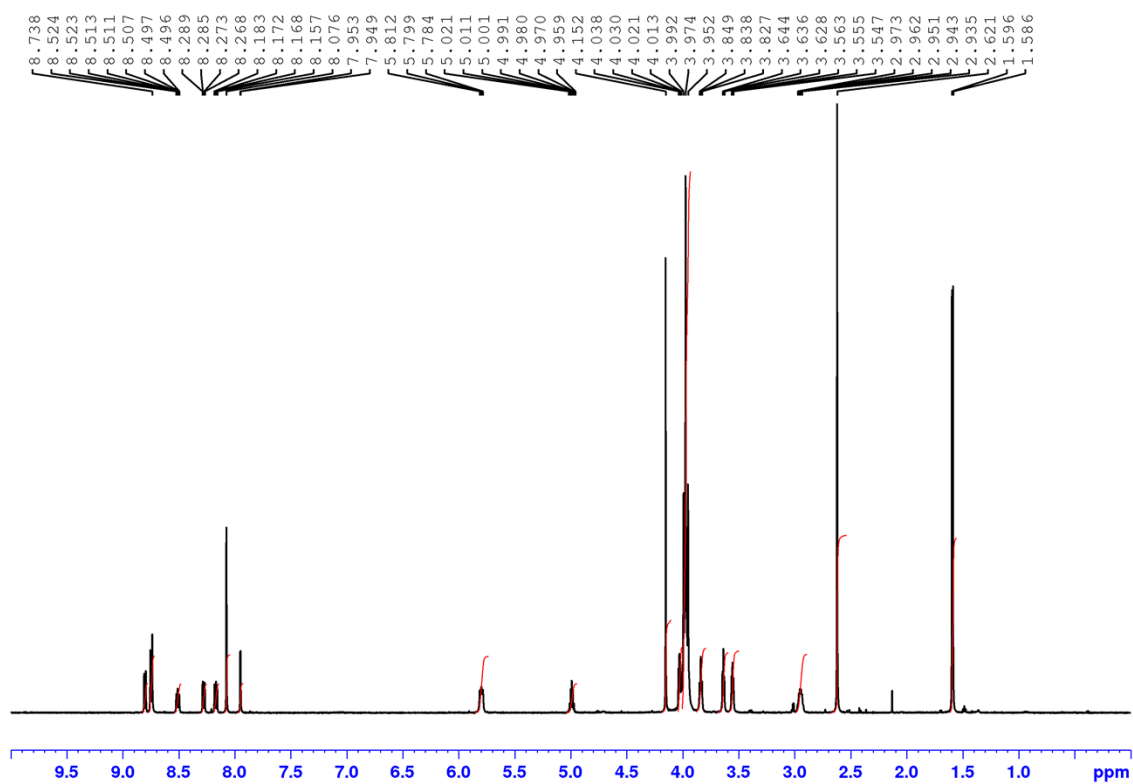
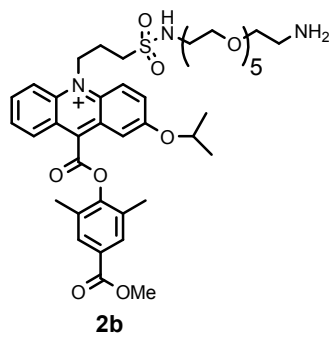


Figure S2B.  $^1\text{H-NMR}$  spectrum of **2b**.



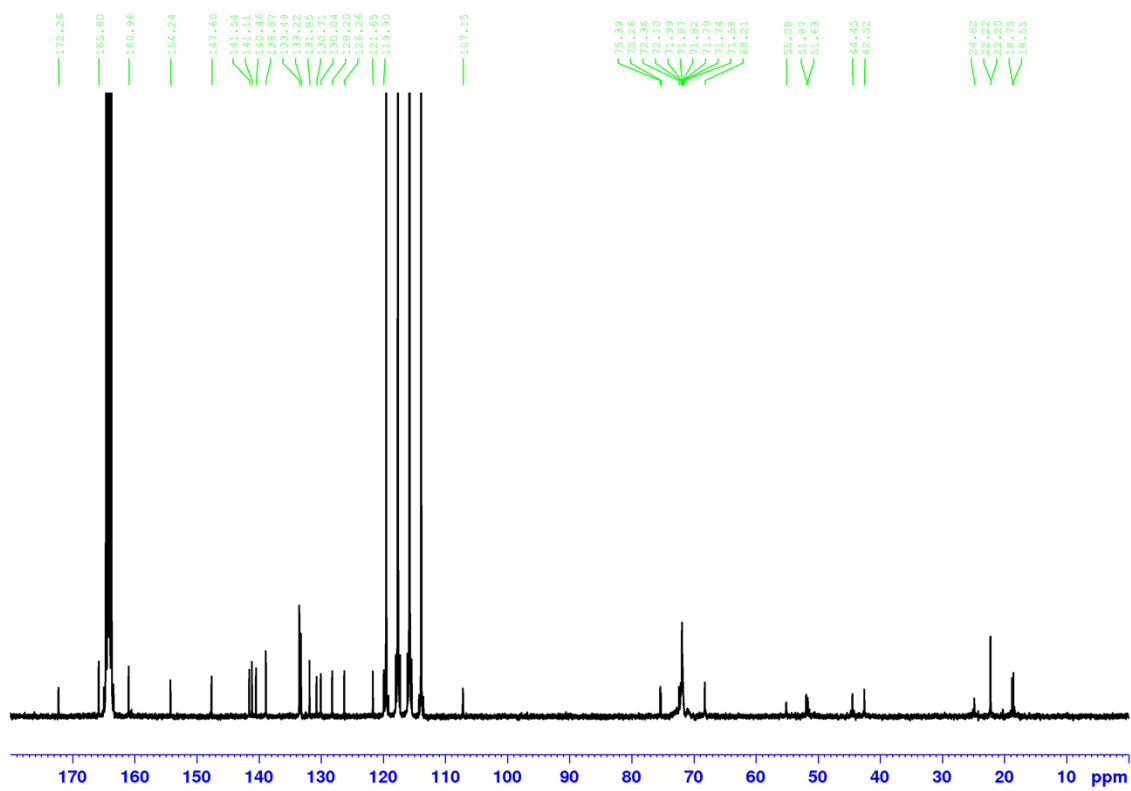
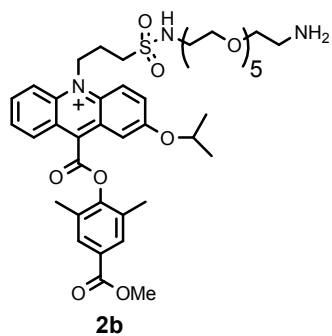


Figure S2C.  $^{13}\text{C}$ -NMR spectrum of **2b**.





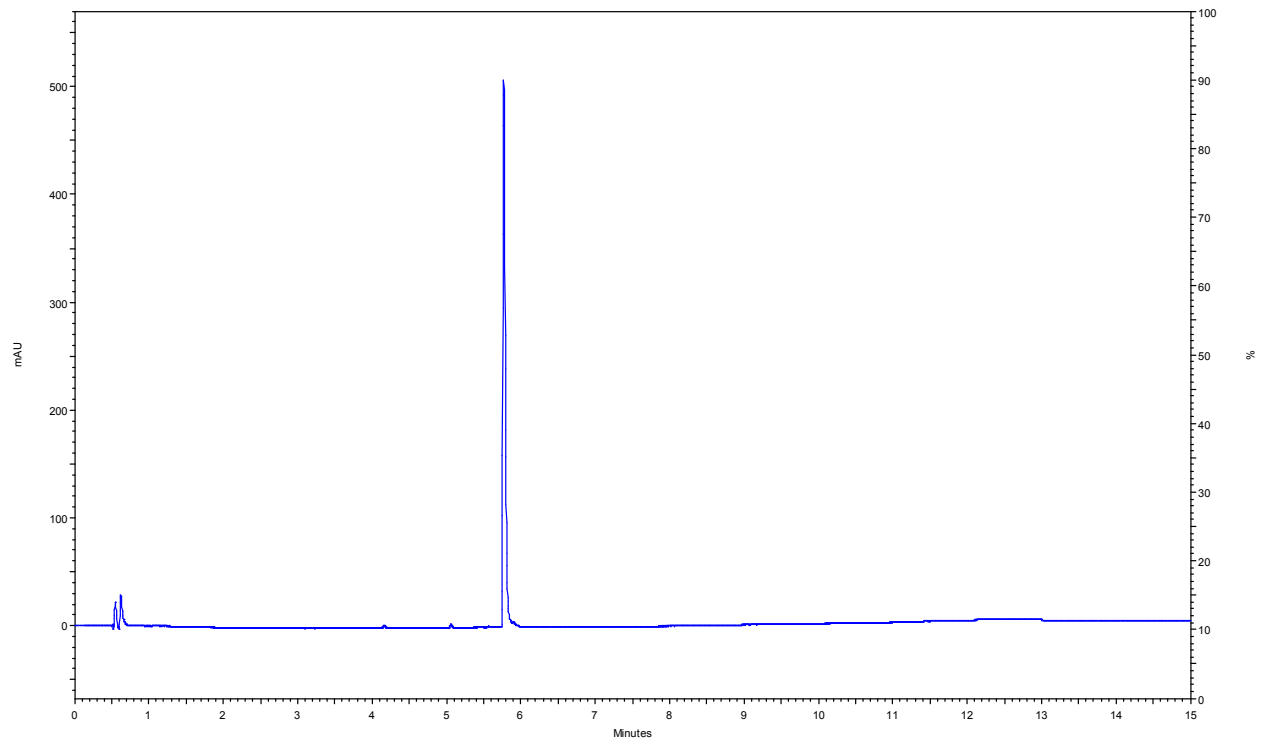
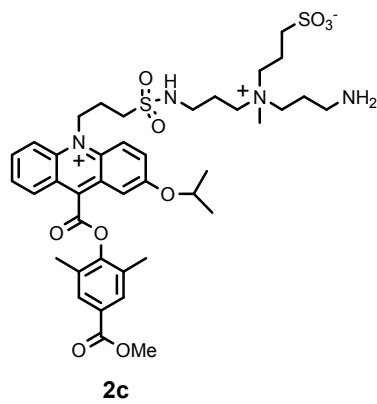
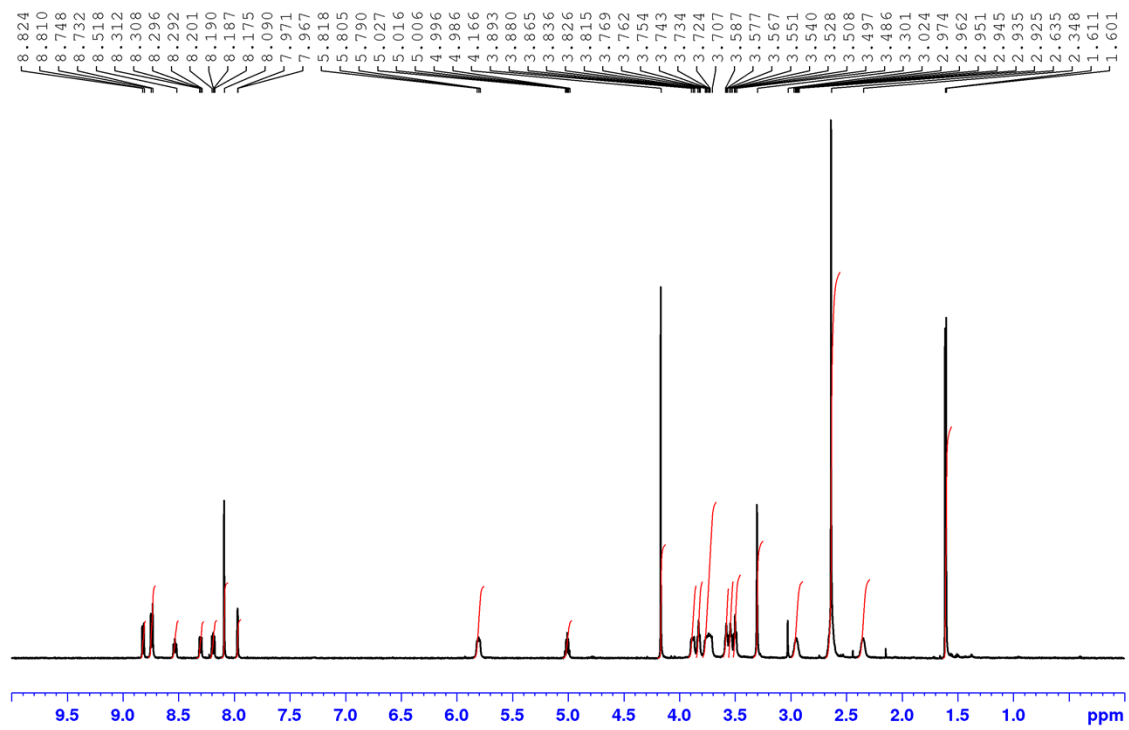
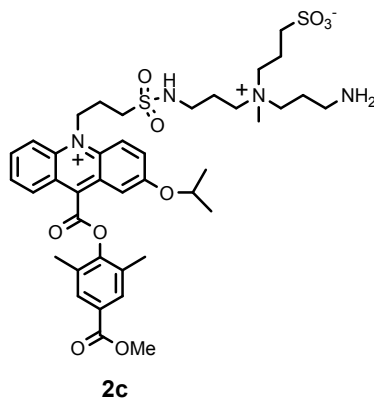
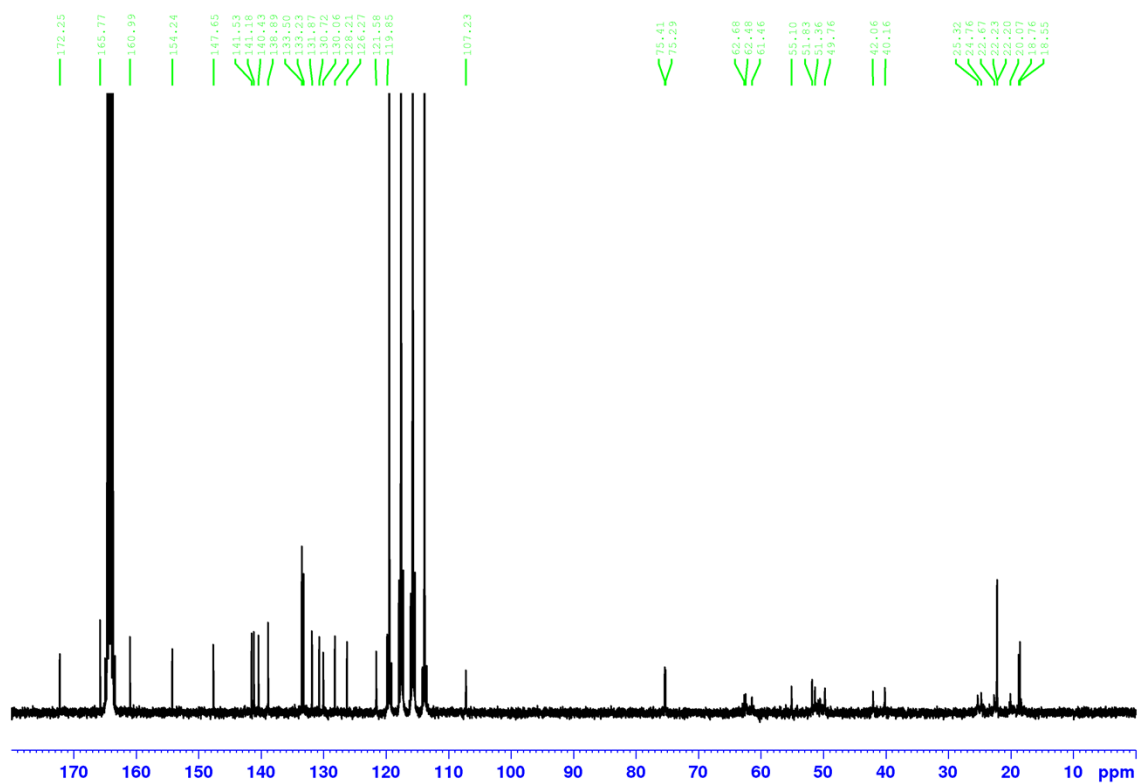
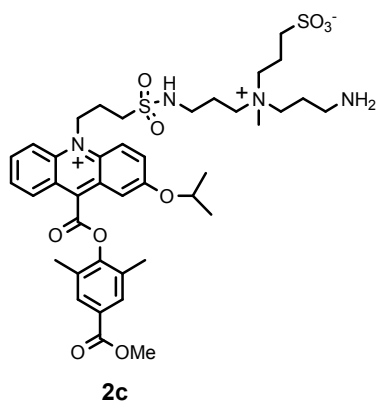
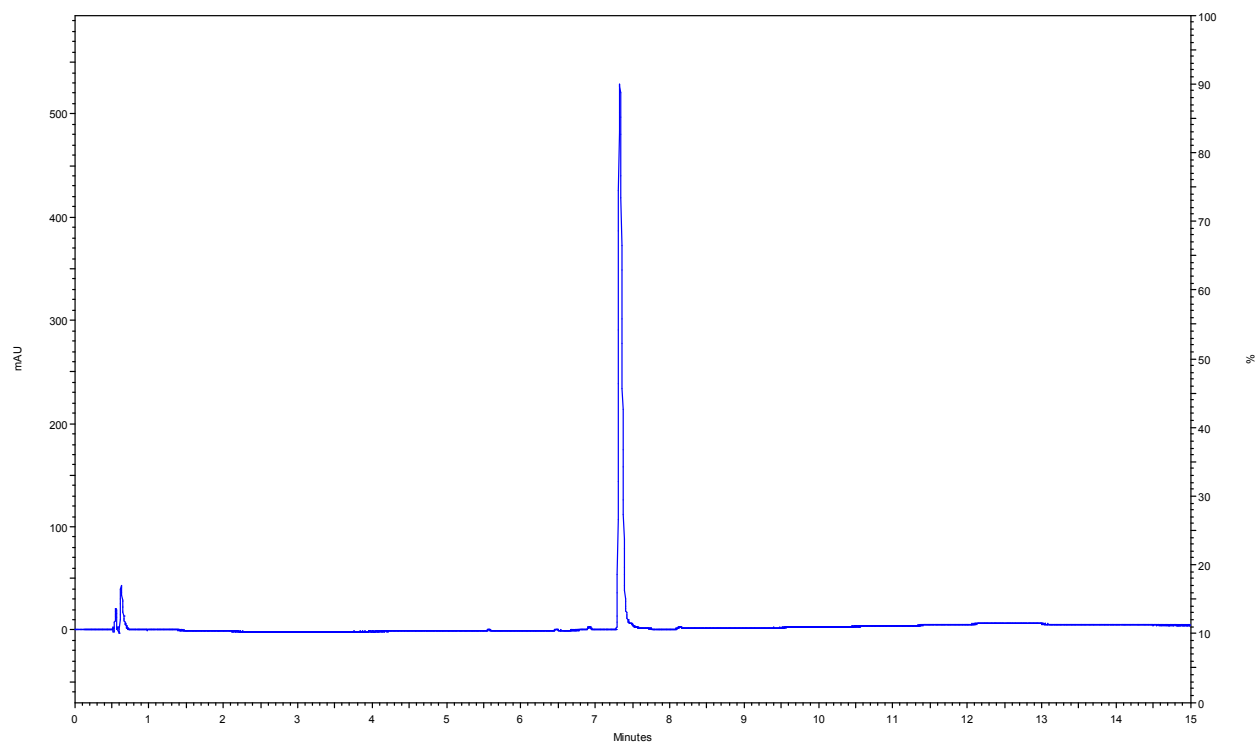
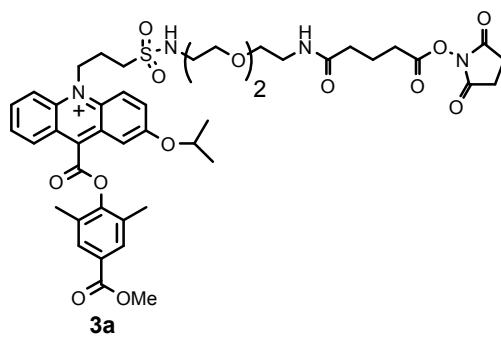


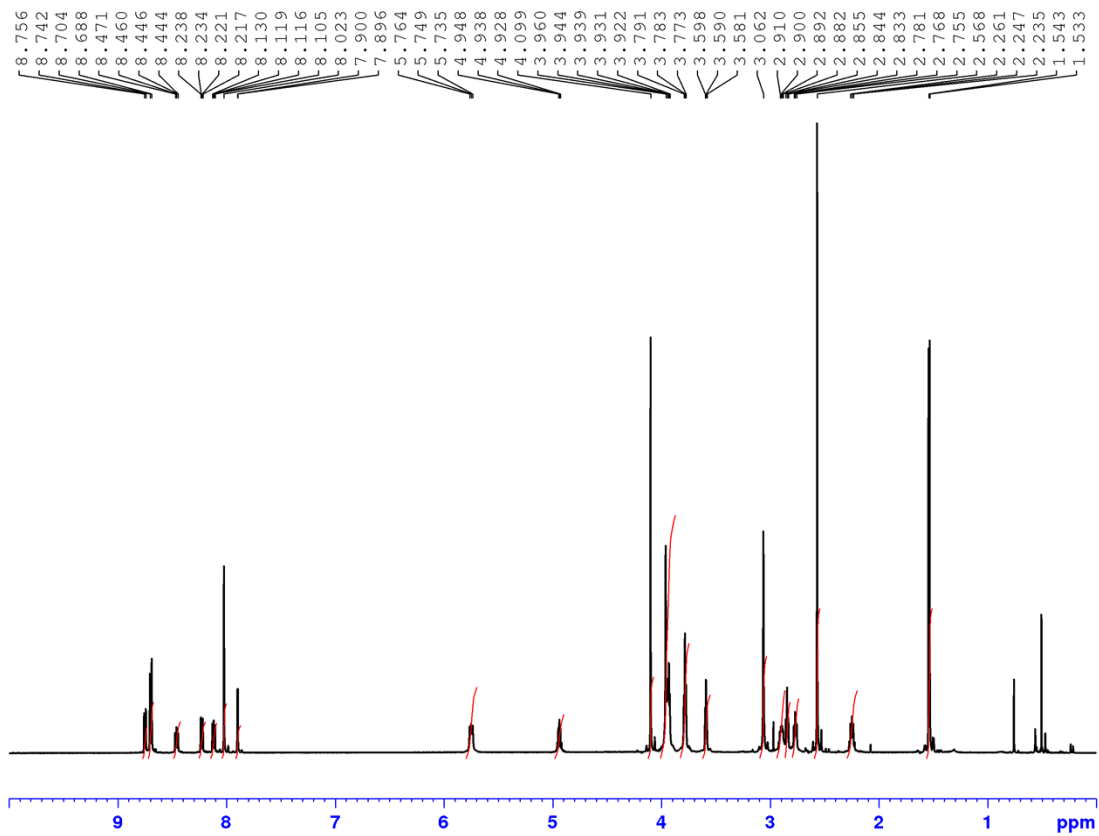
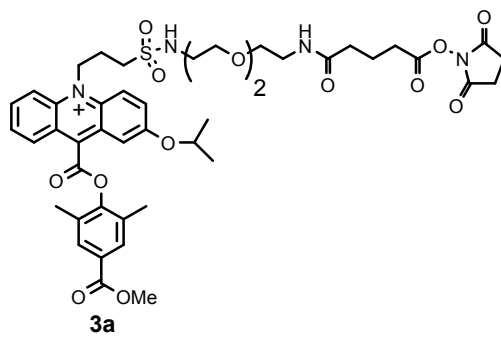
Figure S3A. HPLC trace of **2c**.

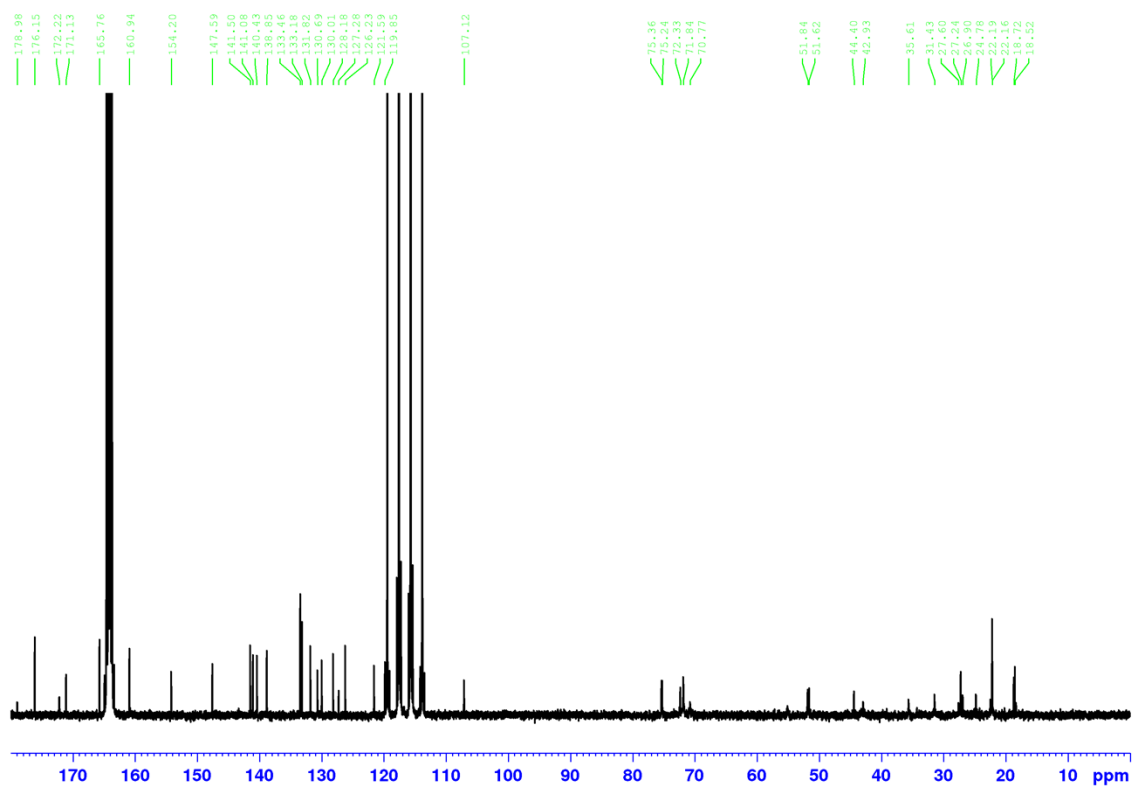
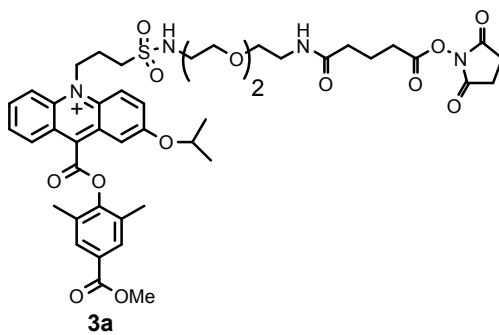


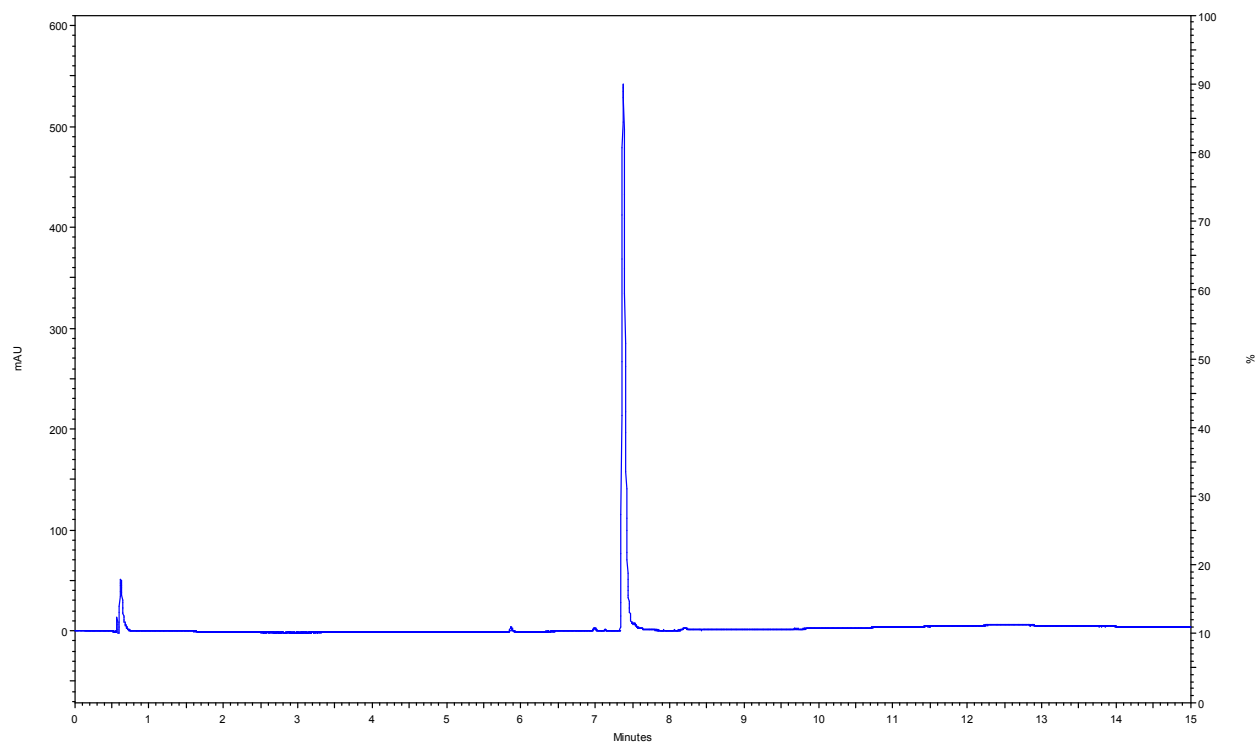
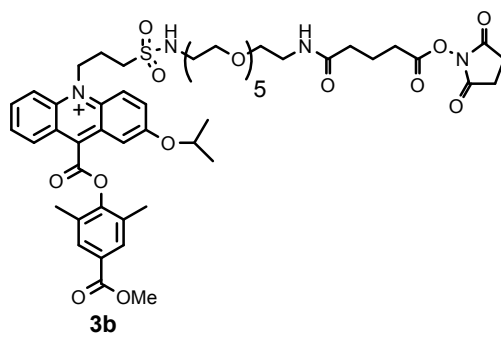
Figure S3B.  $^1\text{H-NMR}$  spectrum of **2c**.

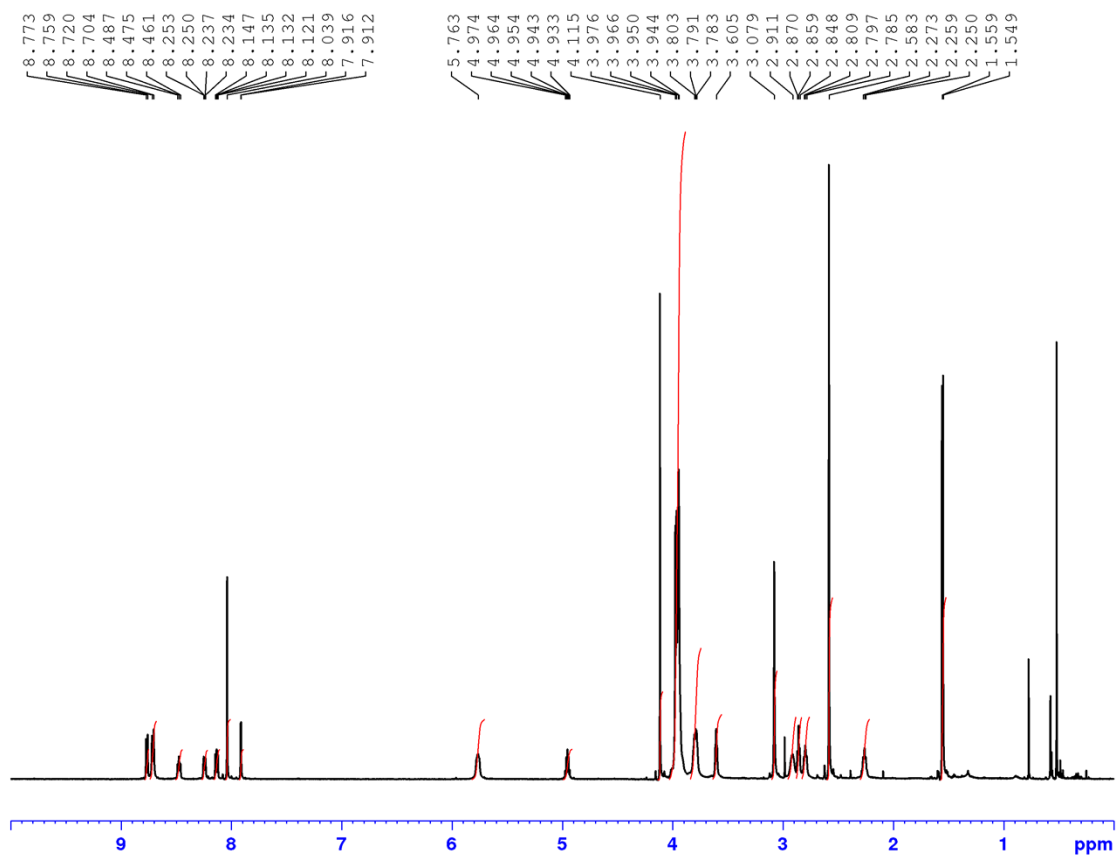
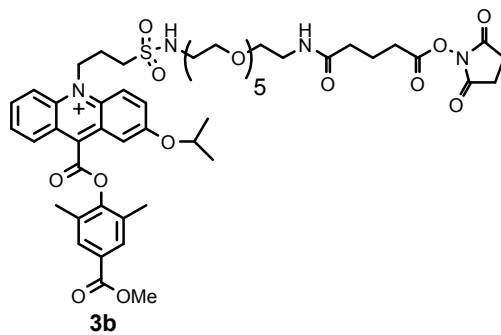
Figure S3C.  $^{13}\text{C}$ -NMR of **2c**.

Figure S4A. HPLC trace of **3a**.

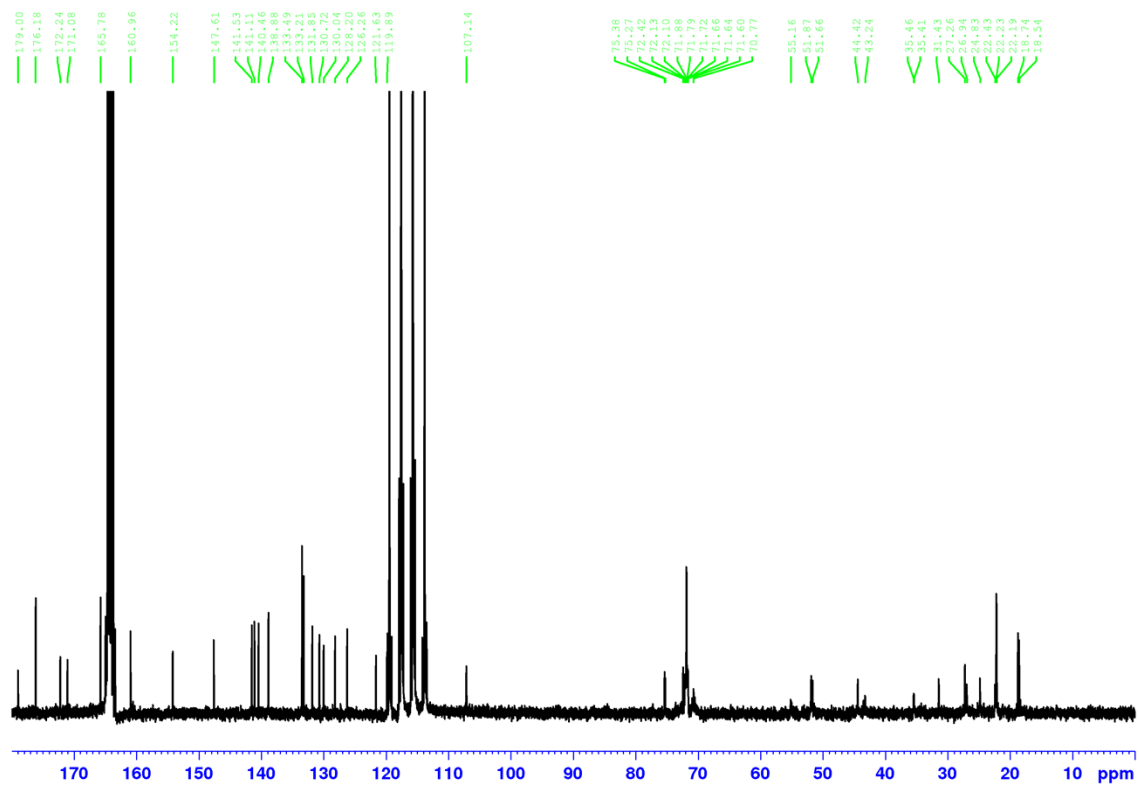
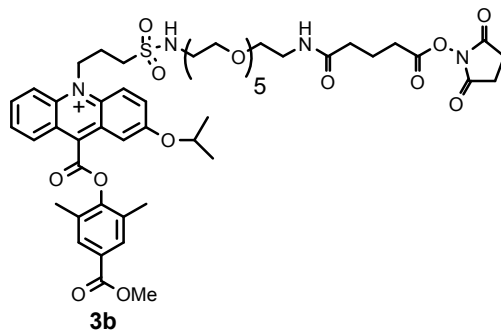
Figure S4B.  $^1\text{H-NMR}$  spectrum of **3a**.

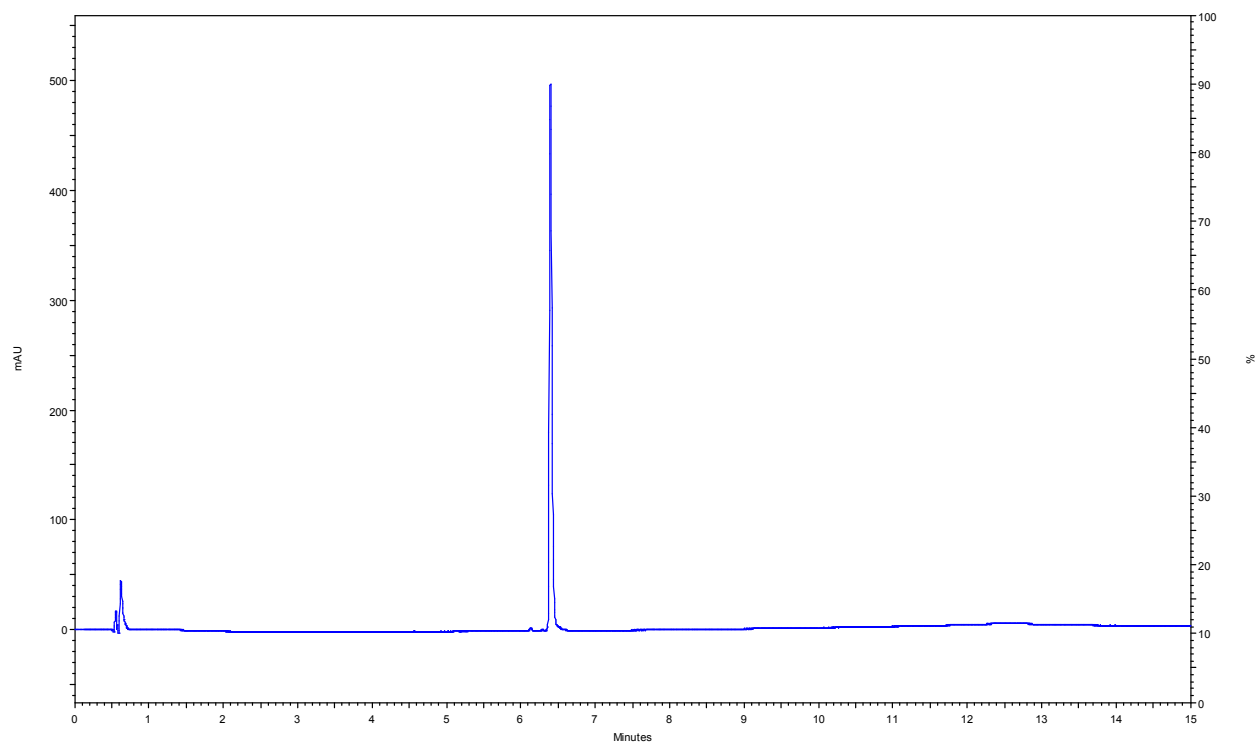
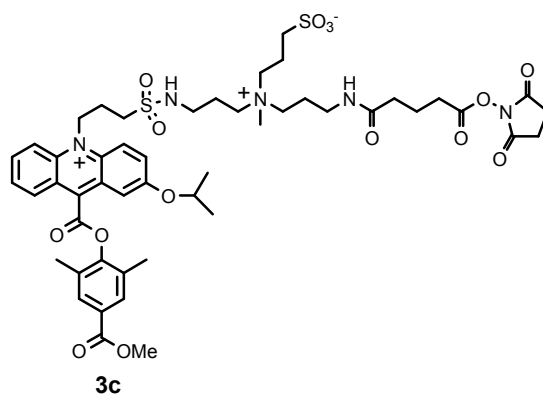
Figure S4C.  $^{13}\text{C}$ -NMR spectrum of **3a**.

Figure S5A. HPLC trace of **3b**.

Figure S5B. <sup>1</sup>H-NMR spectrum of **3b**.



Figure S5C.  $^{13}\text{C}$ -NMR spectrum of **3b**.

Figure S6A. HPLC trace of **3c**.

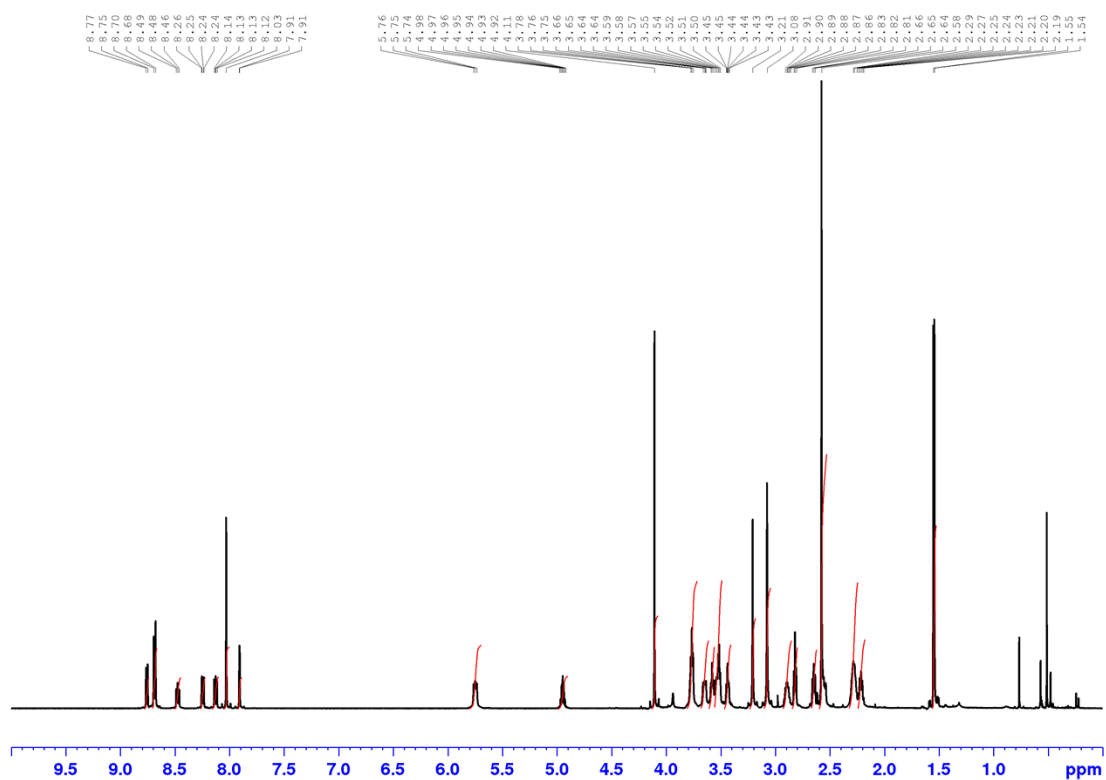
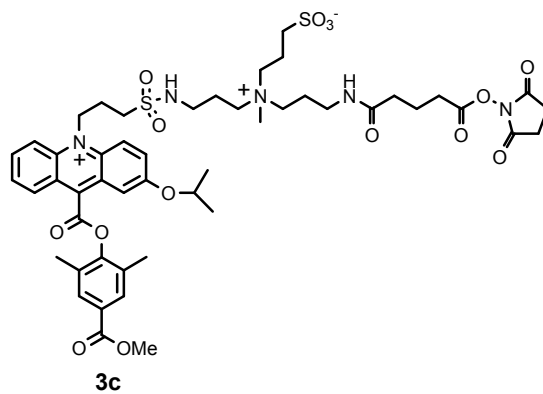
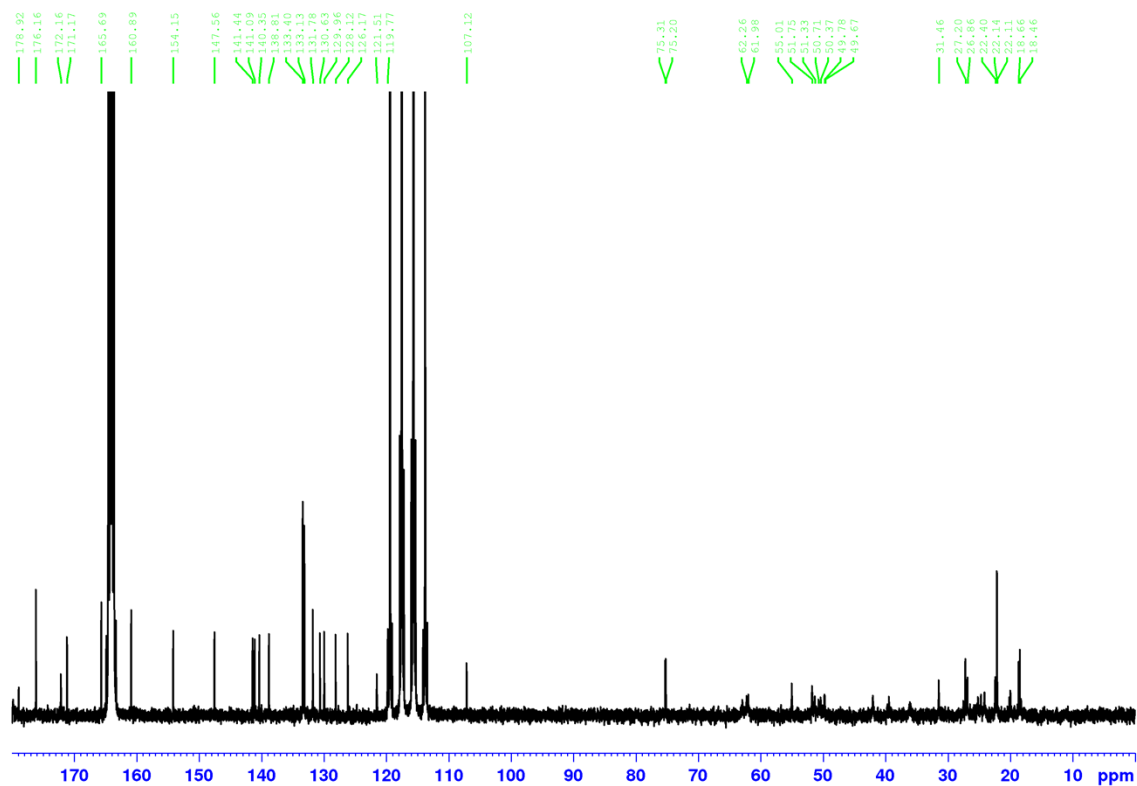
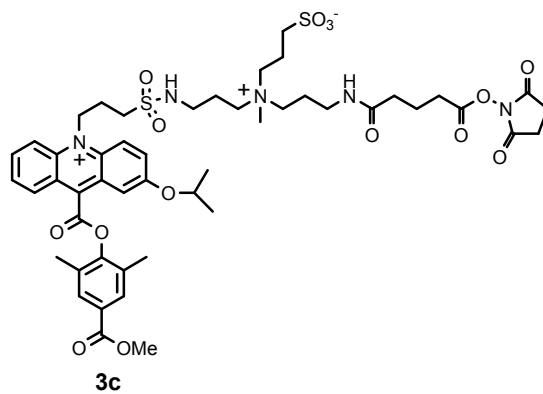


Figure S6B.  $^1\text{H-NMR}$  spectrum of **3c**.



Figure S6C.  $^{13}\text{C}$ -NMR spectrum of **3c**.

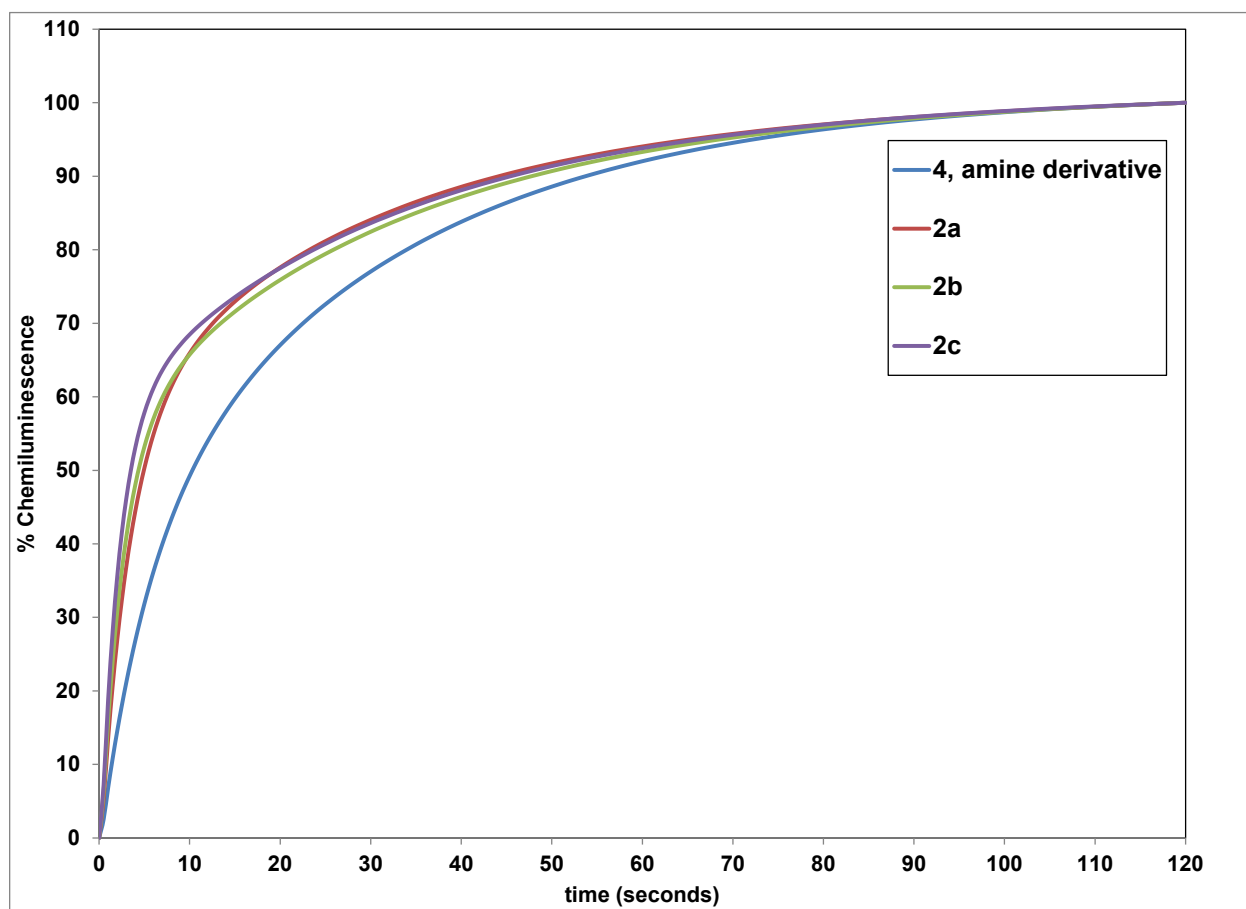


Figure S7. Chemiluminescence emission profiles of amine precursor of **4** and **2a-2c** in the absence of CTAC. Chemiluminescence was initiated by the sequential addition of 0.3 mL of 0.1 M nitric acid containing 0.5% hydrogen peroxide followed by 0.3 mL of 0.25 M sodium hydroxide.

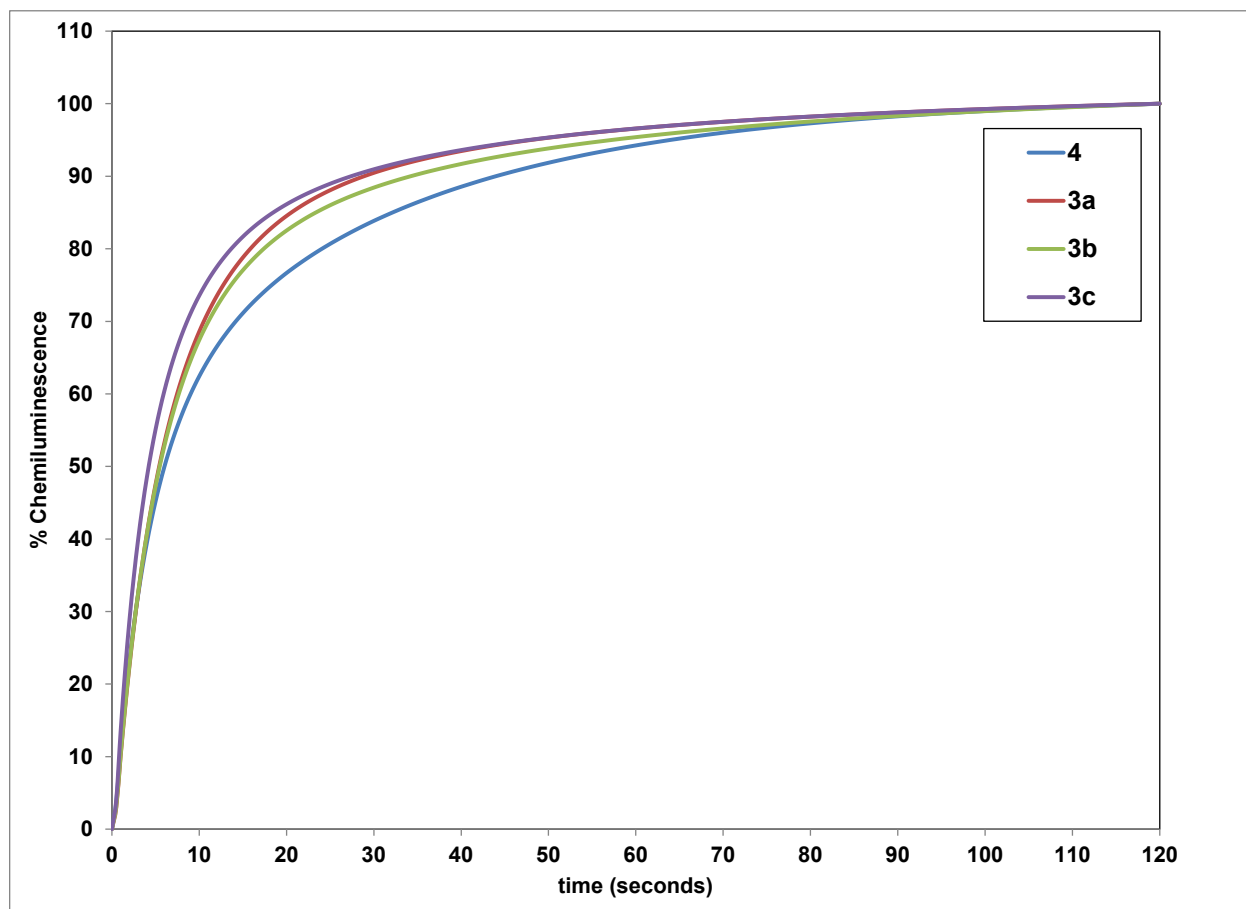


Figure S8. Chemiluminescence emission profiles of BSA conjugates of compound **4** and **3a-3c** in the absence of CTAC.

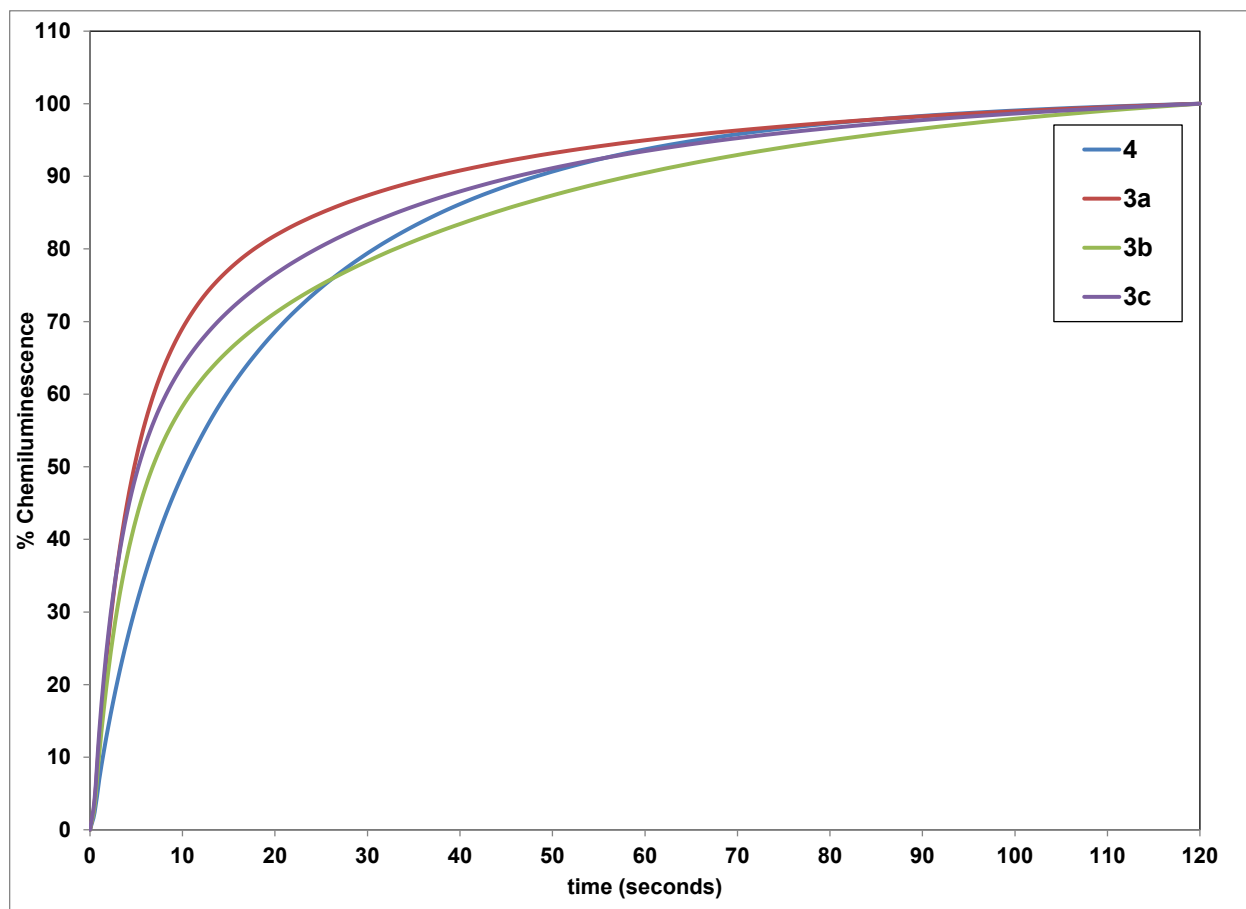


Figure S9. Chemiluminescence emission profiles of anti-TSH Mab conjugates of compound **4** and **3a-3c** in the absence of CTAC.

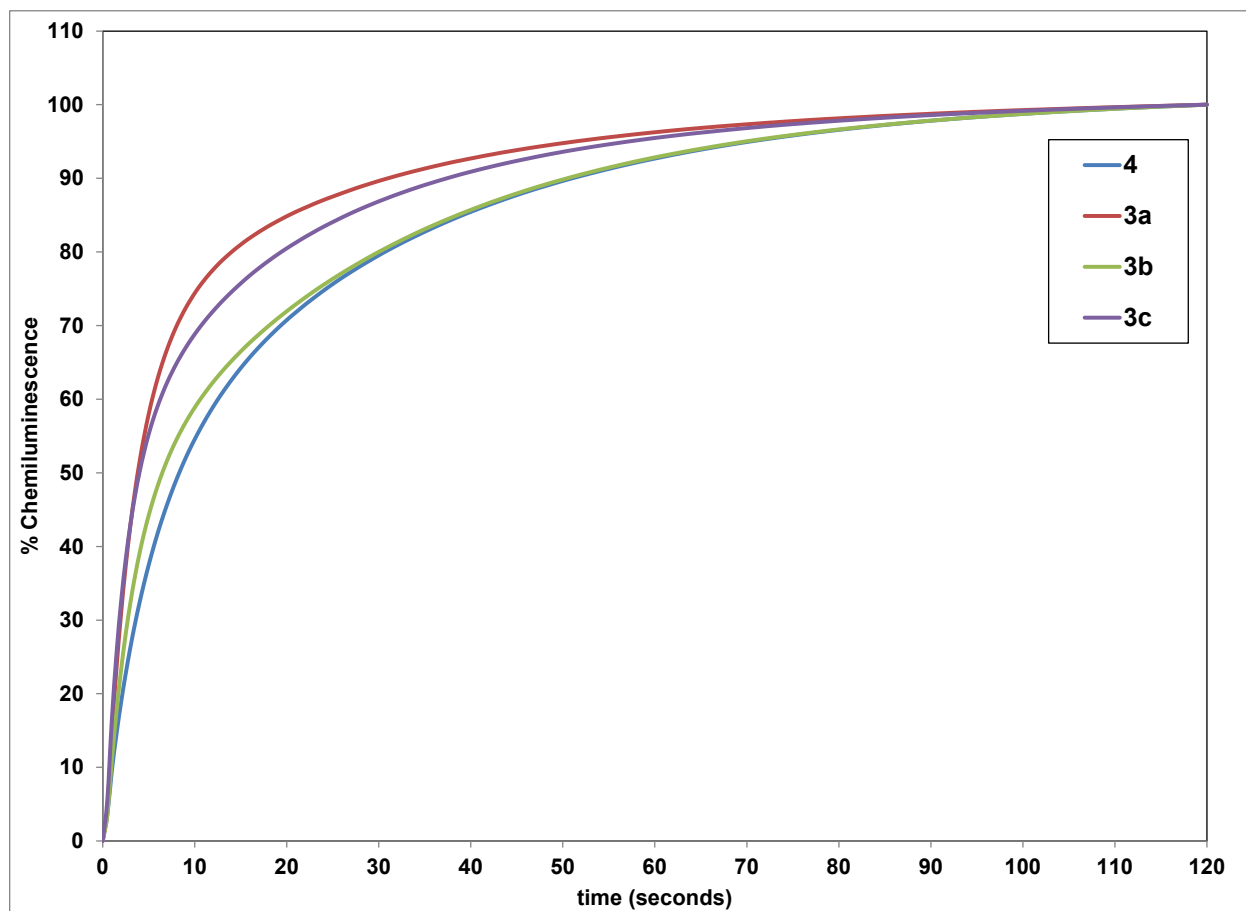


Figure S10. Chemiluminescence emission profiles of anti-HBsAg Mab conjugates of compound **4** and **3a-3c** in the absence of CTAC.



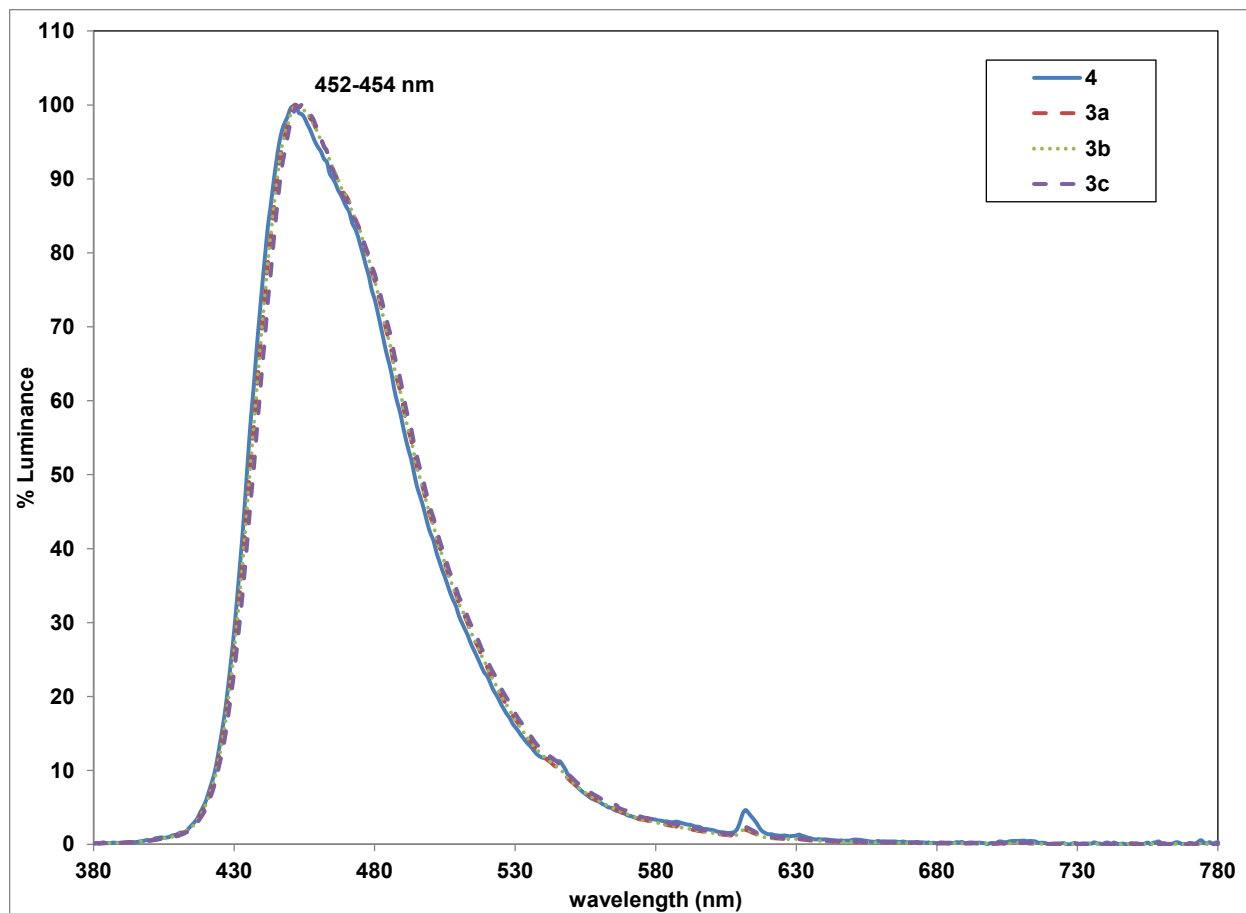


Figure S11. Emission spectra of anti-TSH antibody conjugates of **4**, **3a-3c**. Similar to the BSA conjugates, all acridinium ester conjugates showed very similar emission spectra whether conjugation was performed at the phenol (**4**) or at the acridinium nitrogen (**3a-3c**).

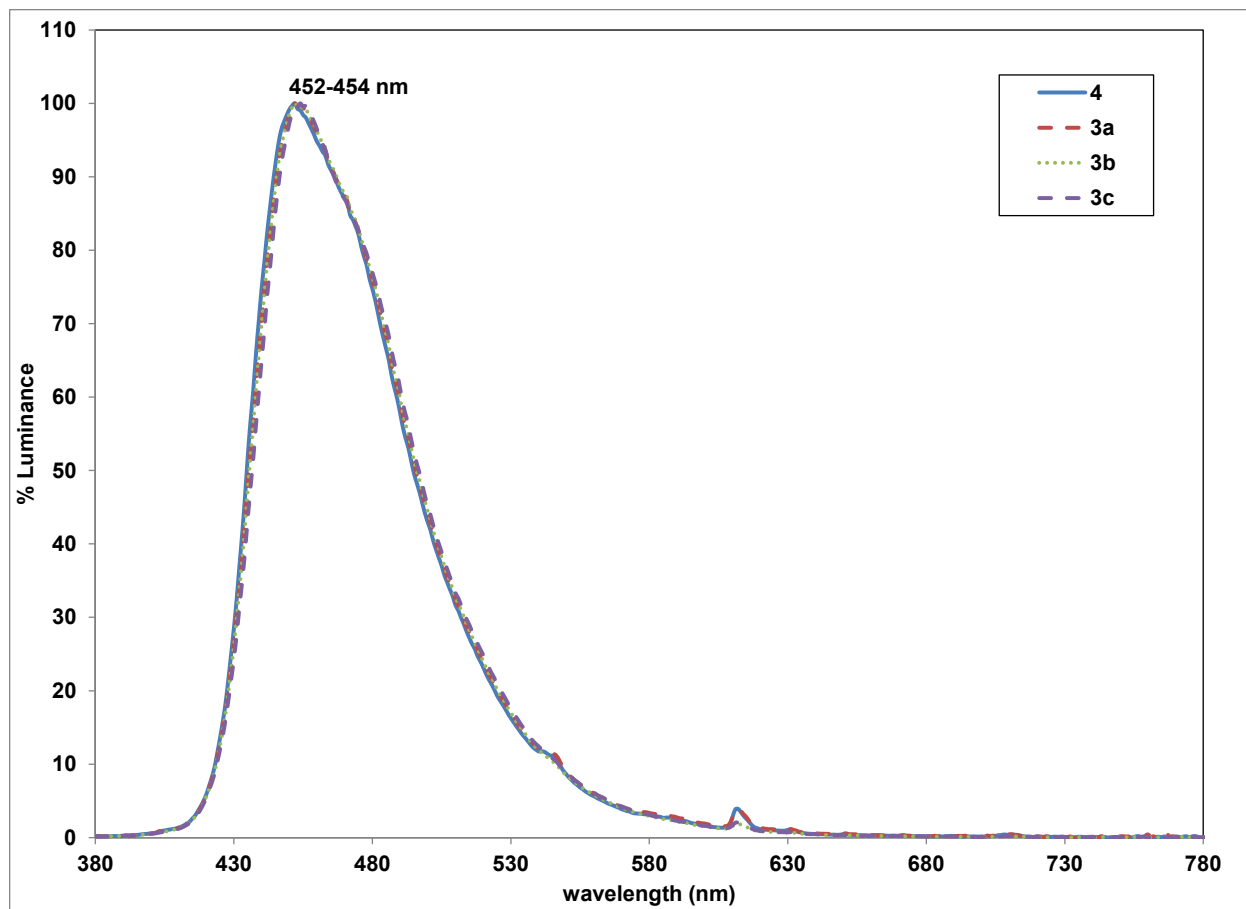


Figure S12. Emission spectra of anti-HBsAg antibody conjugates of **4**, **3a-3c**. Similar to the BSA conjugates, all acridinium ester conjugates showed very similar emission spectra whether conjugation was performed at the phenol (**4**) or at the acridinium nitrogen (**3a-3c**).