Supplementary Material

Chemiluminescence from Alkoxy-Substituted Acridinium Dimethylphenyl Ester Labels

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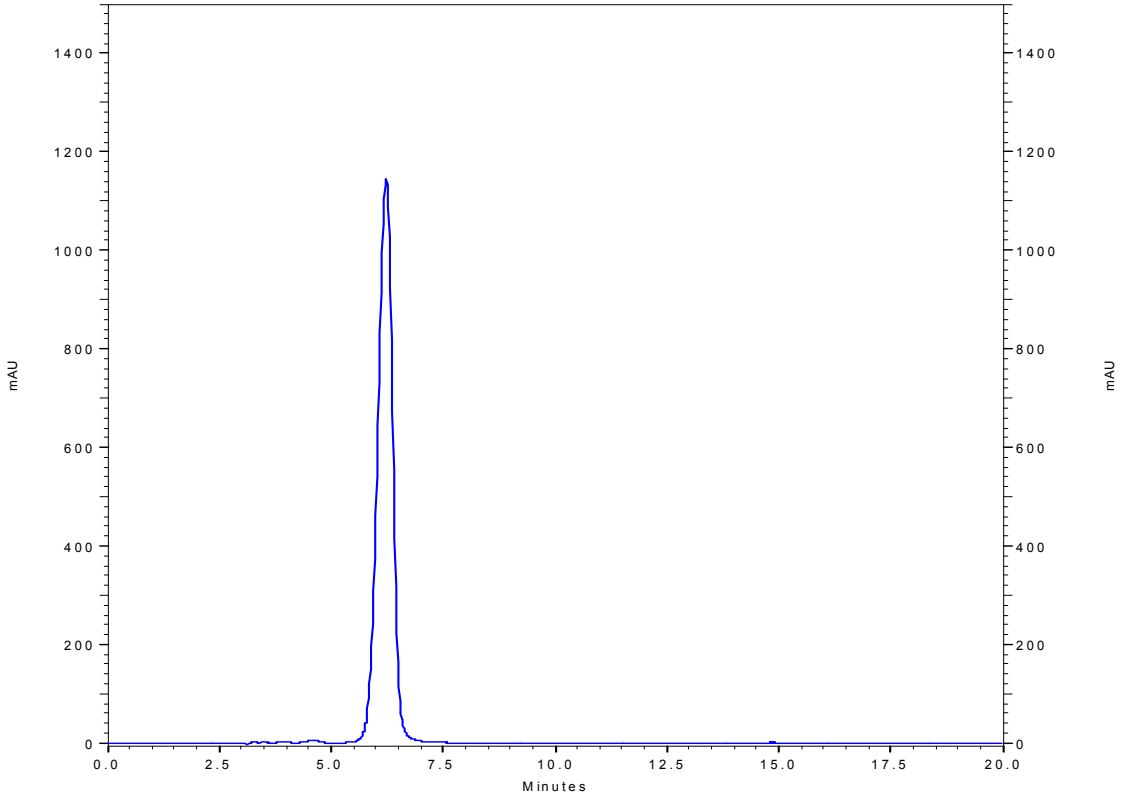
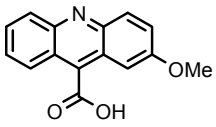


Figure S1. HPLC chromatogram of compound **2**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 40 minute gradient of 10 → 40 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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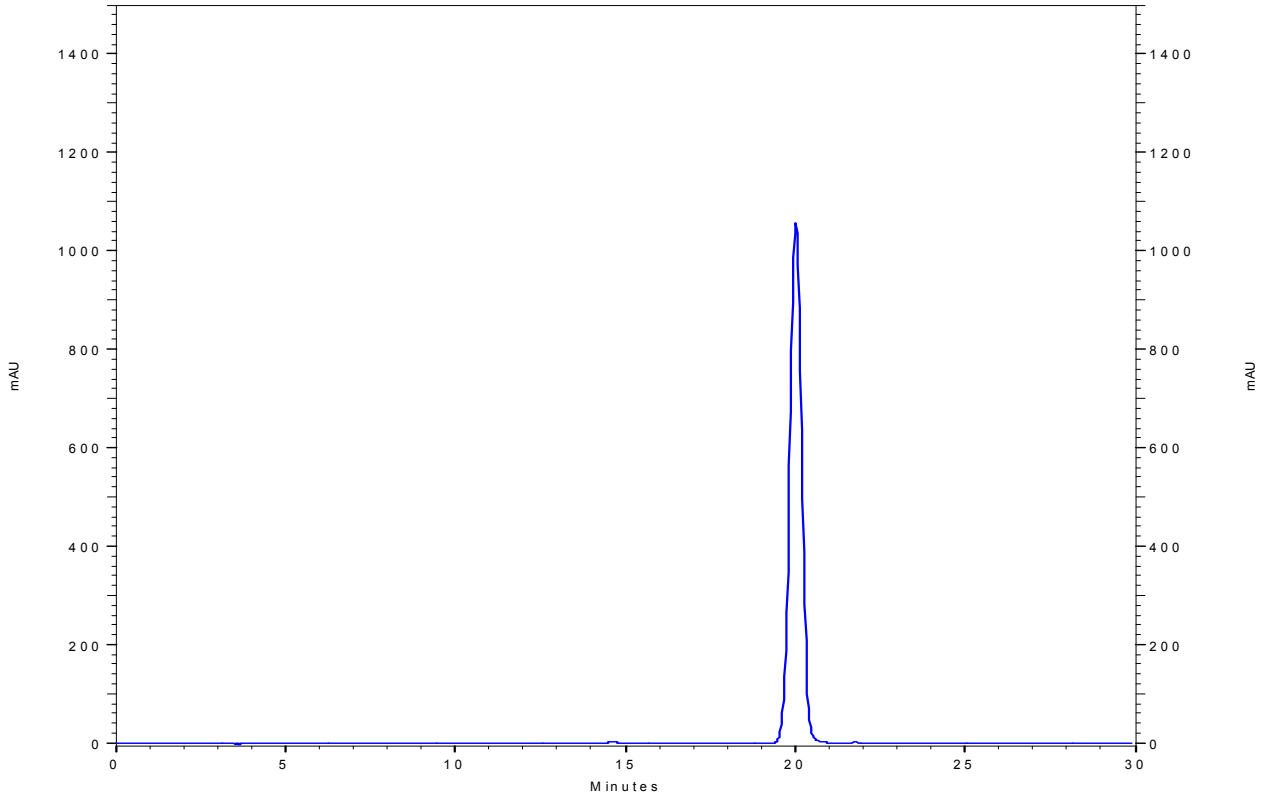
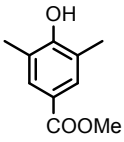


Figure S2. HPLC chromatogram of compound **3**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 40 minute gradient of 10 → 60 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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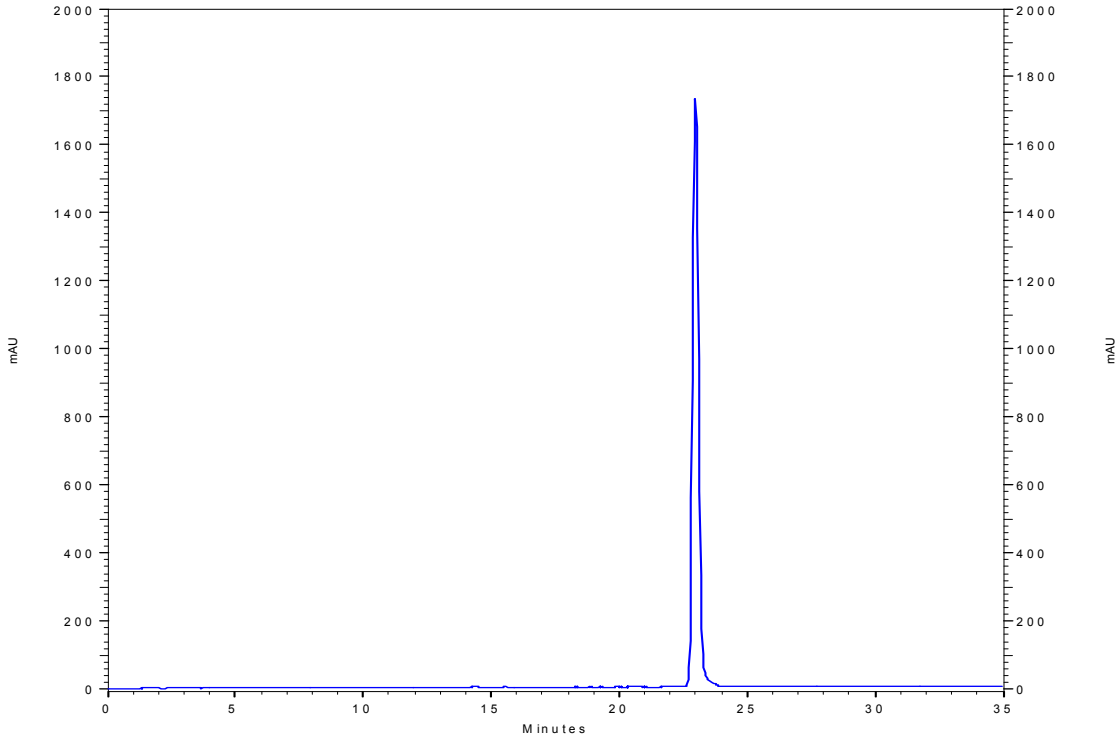
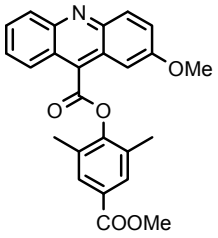


Figure S3. HPLC chromatogram of compound **4** after purification by flash chromatography. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 100 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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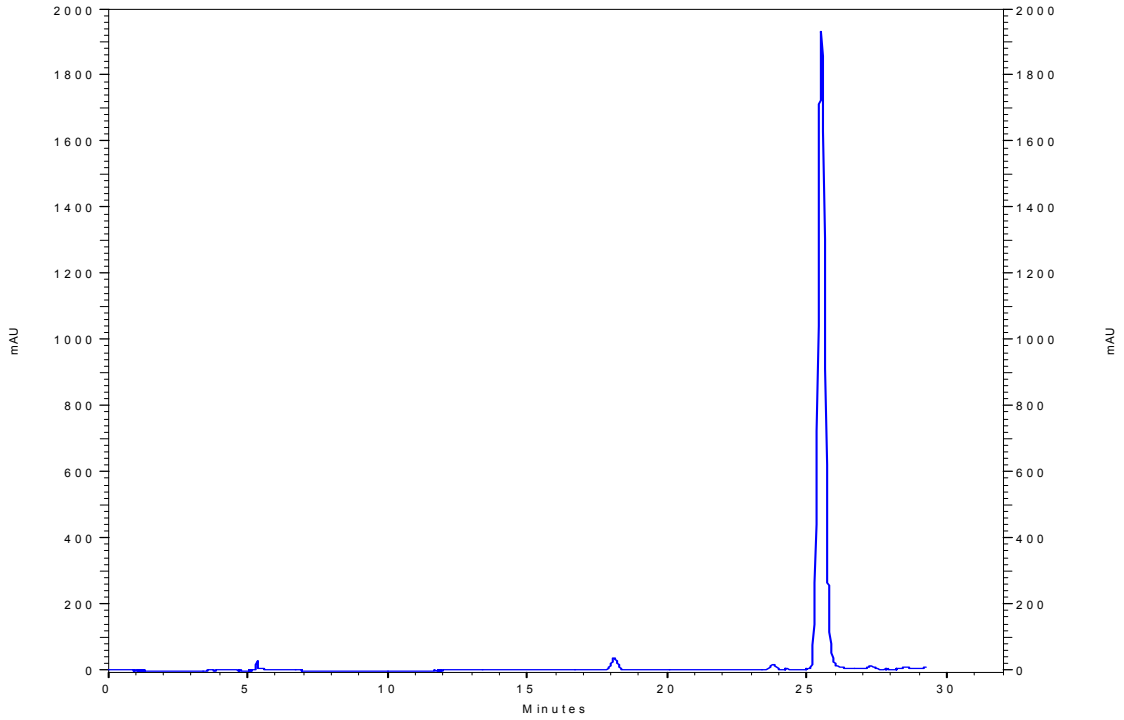
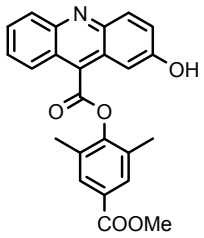


Figure S4. HPLC chromatogram of compound **5** after purification by flash chromatography. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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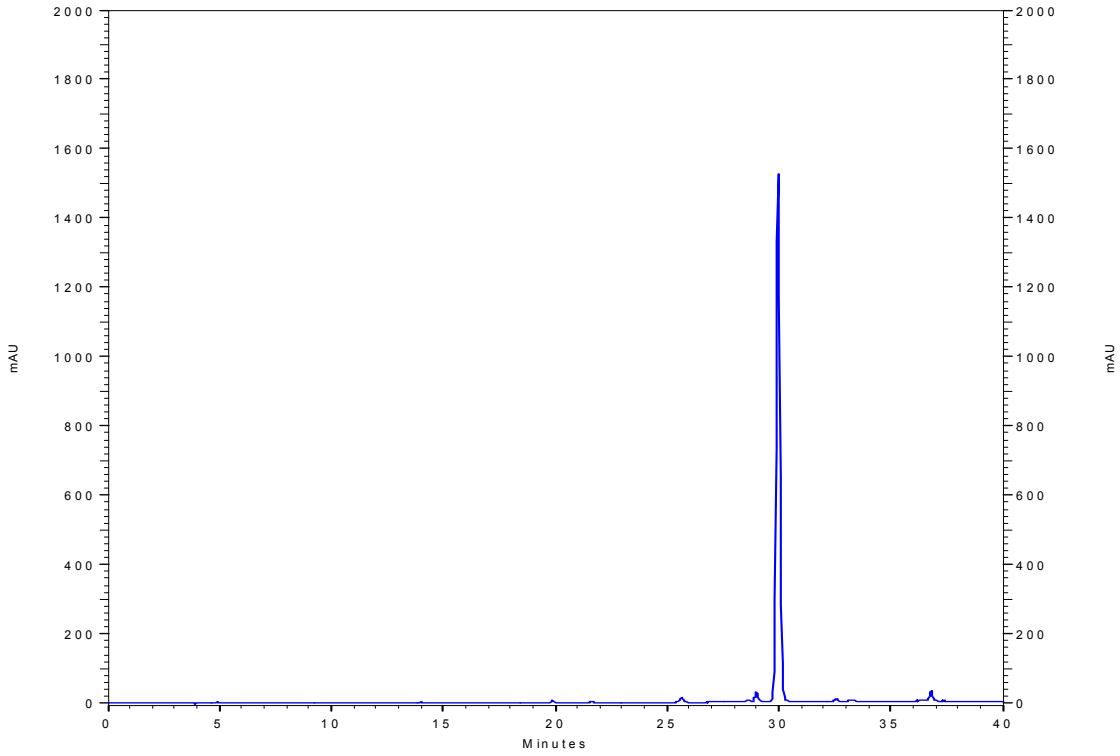
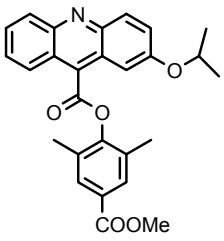


Figure S5. HPLC chromatogram of compound **6a** after purification by flash chromatography. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 100 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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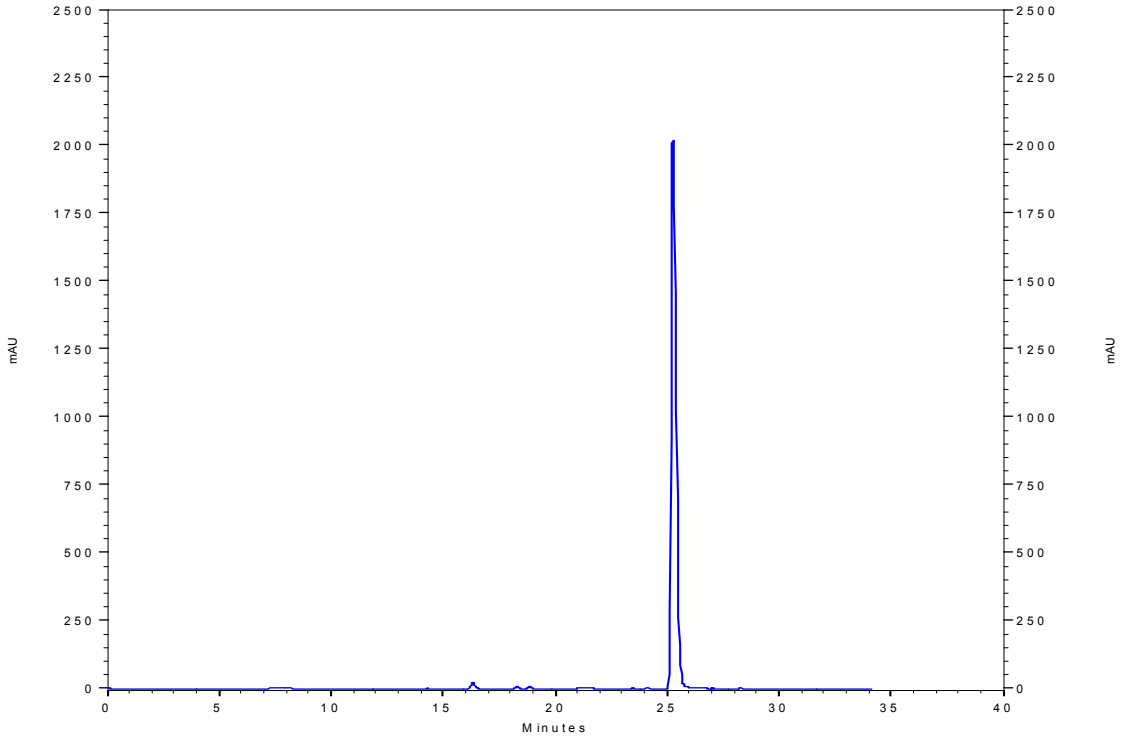
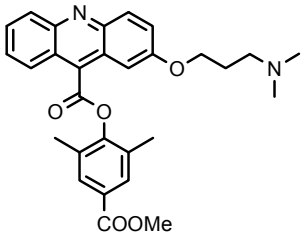


Figure S6. HPLC chromatogram of compound **6b**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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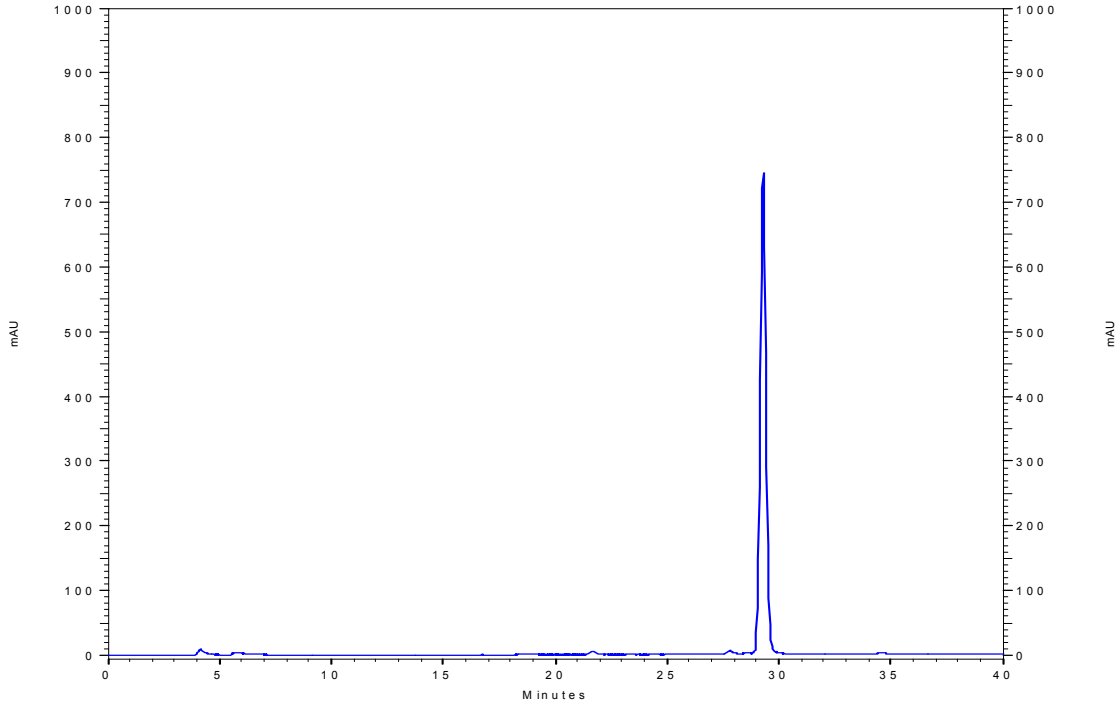
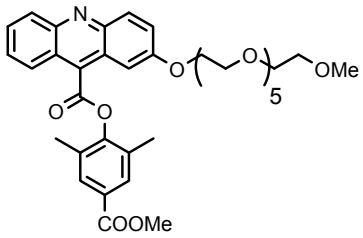


Figure S7. HPLC chromatogram of compound **6c**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



6c

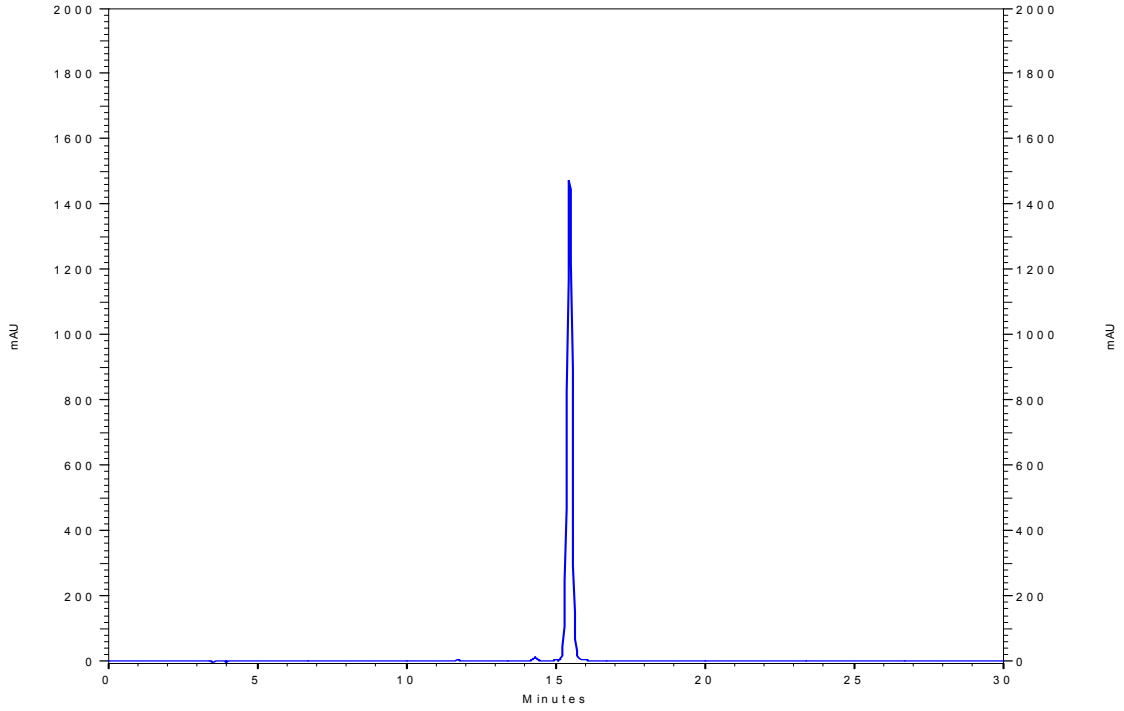
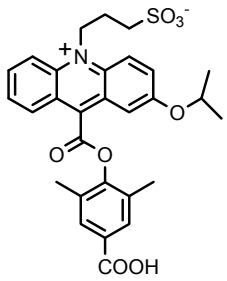


Figure S8. HPLC chromatogram of compound **7a**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 100 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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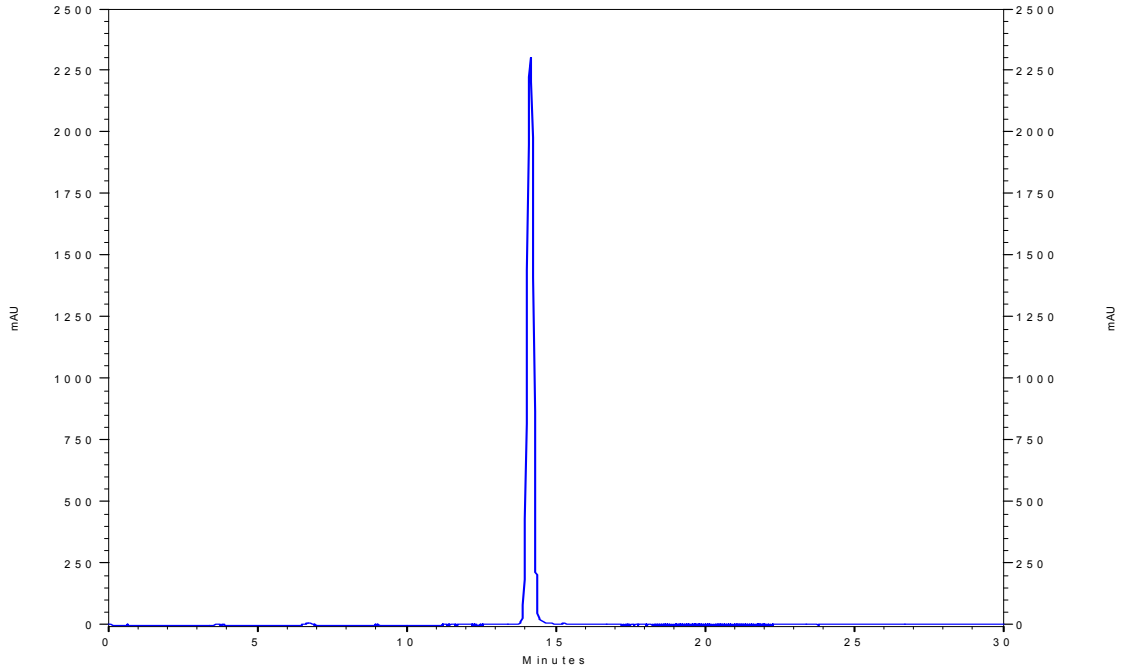
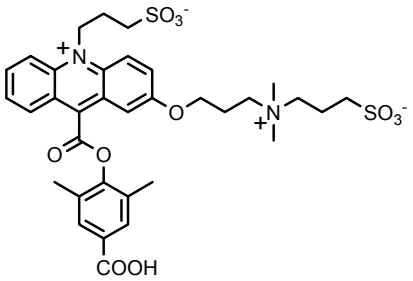


Figure S9. HPLC chromatogram of compound **7b**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70% MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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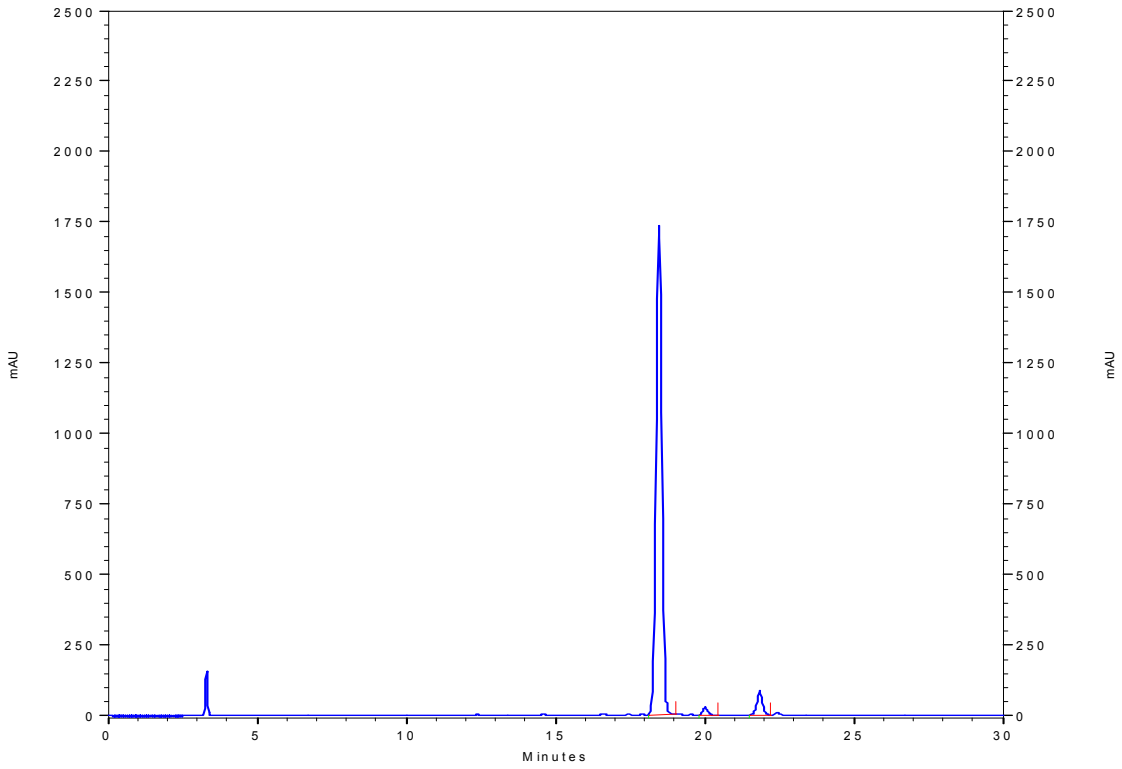
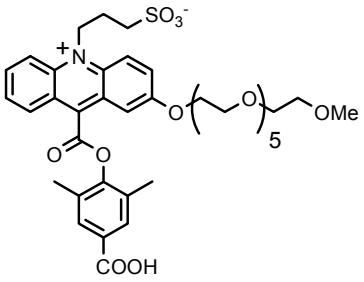


Figure S10. HPLC chromatogram of compound **7c**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



7c

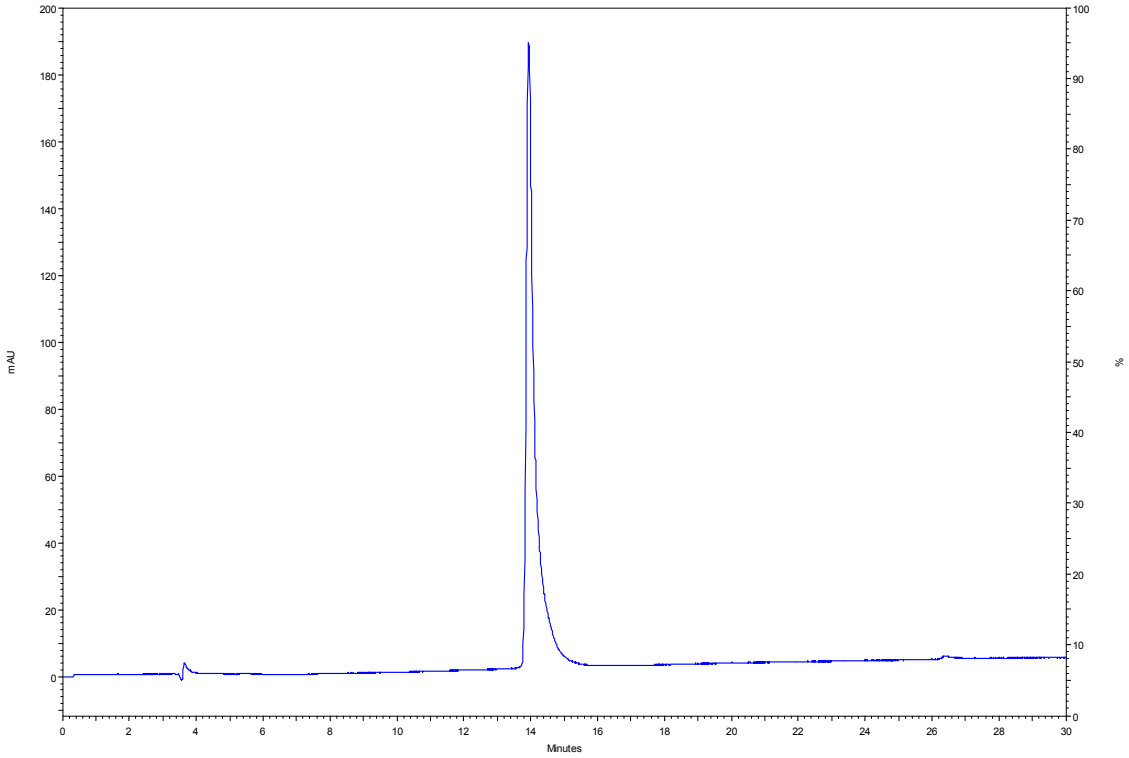
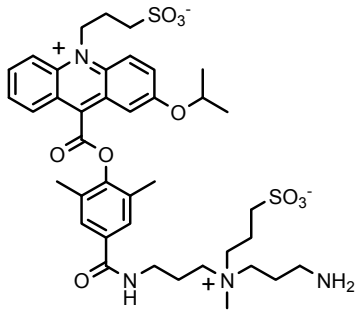


Figure S11. HPLC chromatogram of compound **9a**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



9a

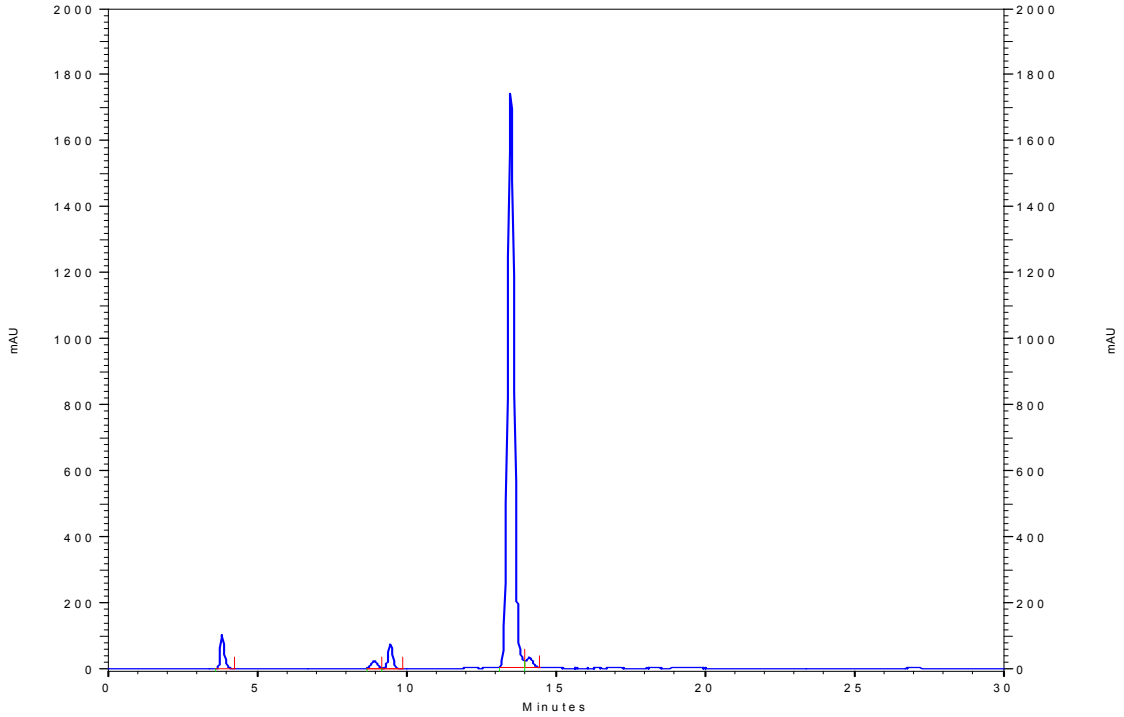
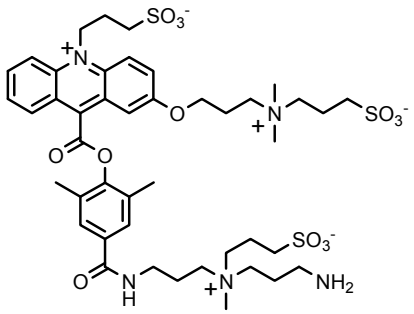


Figure S12. HPLC chromatogram of compound **9b**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



9b

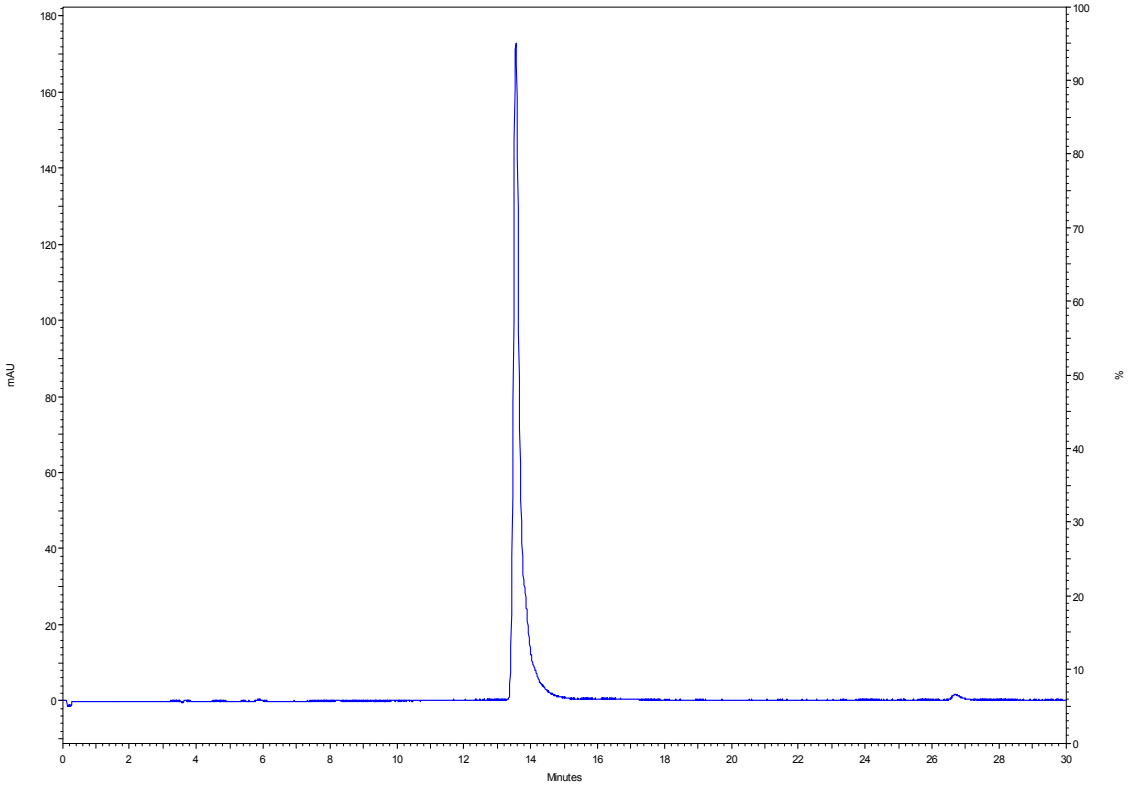
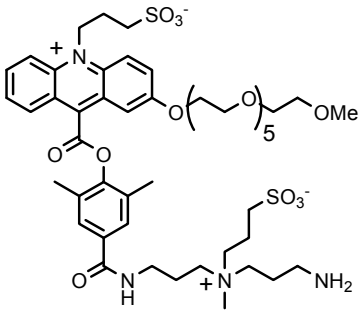


Figure S13. HPLC chromatogram of compound **9c**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



9c

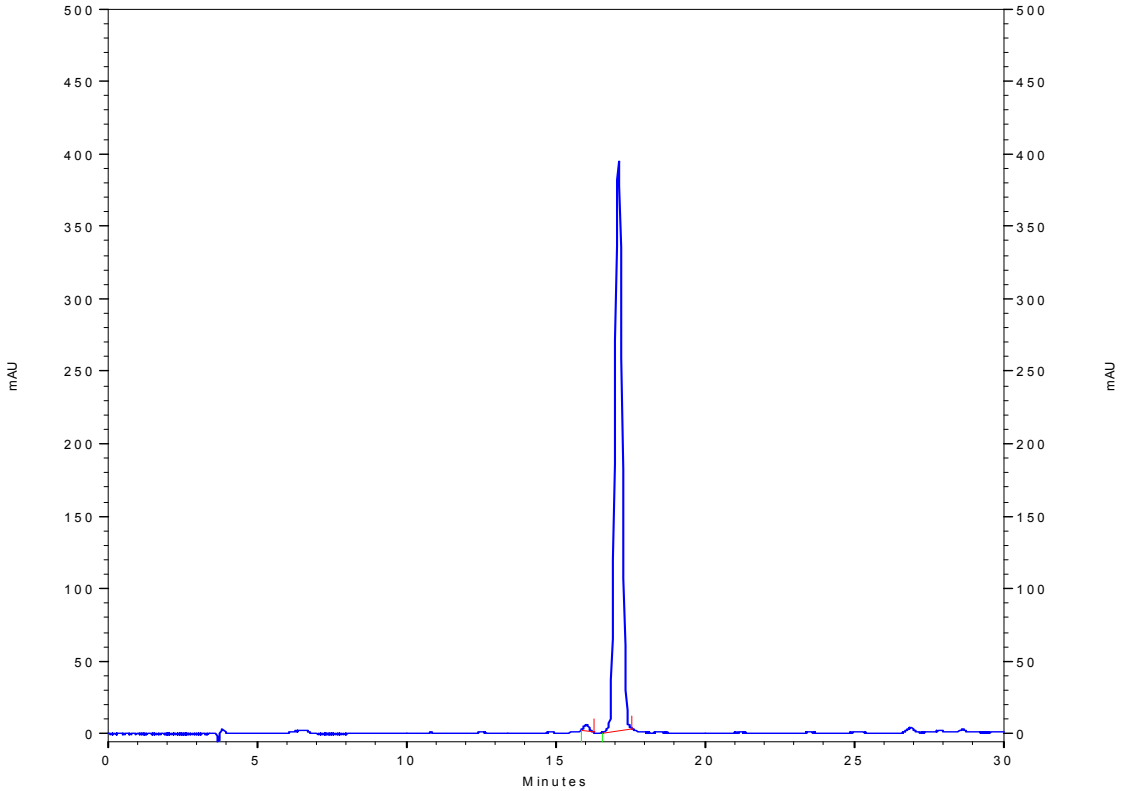
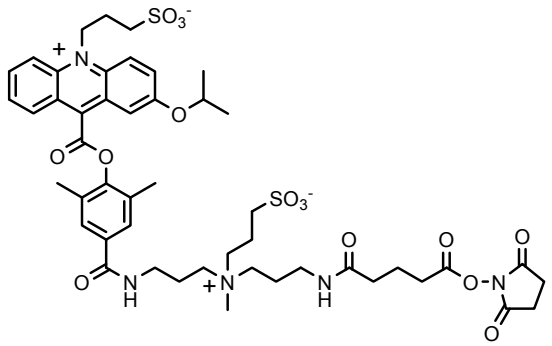


Figure S14. HPLC chromatogram of compound **10a**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % B MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



10a

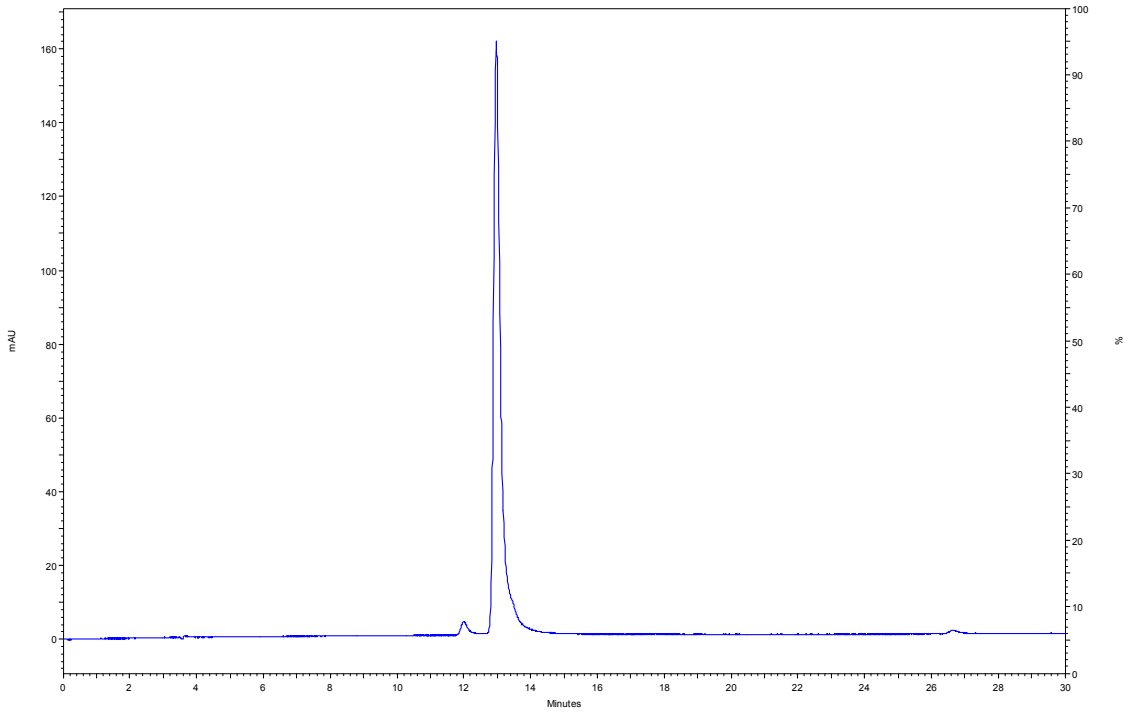
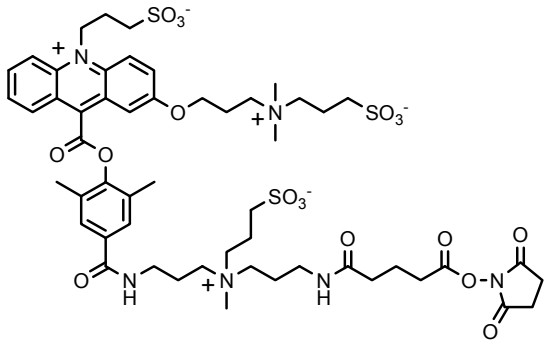


Figure S15. HPLC chromatogram of compound **10b**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 100 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



10b

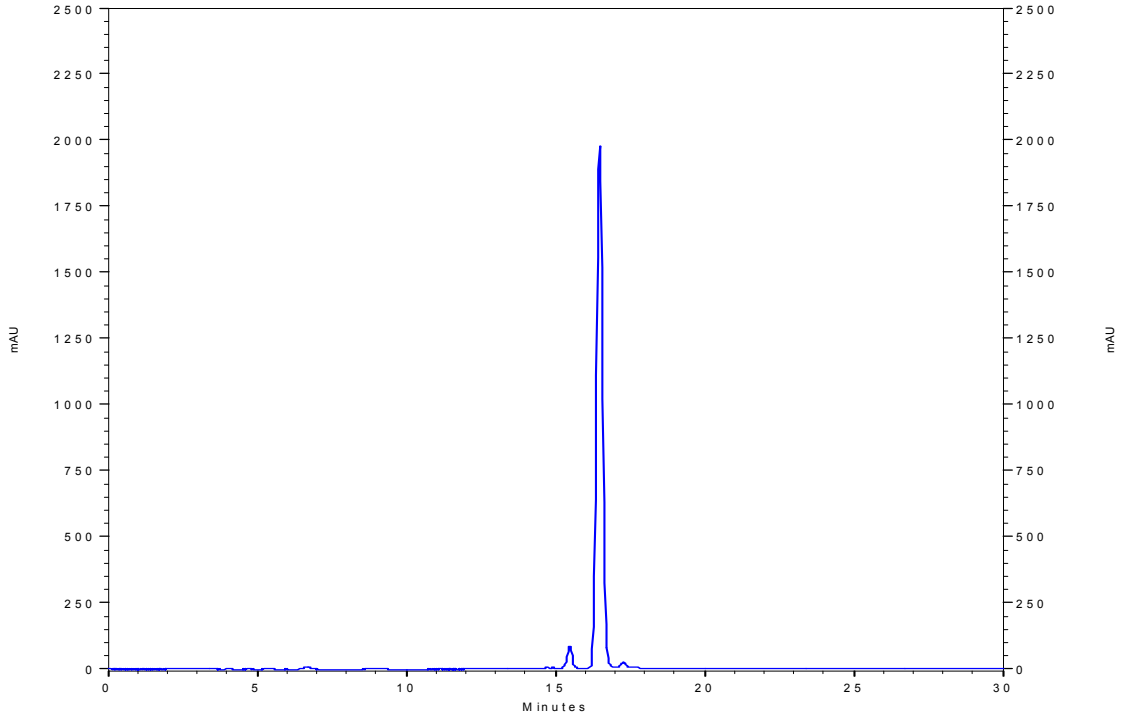
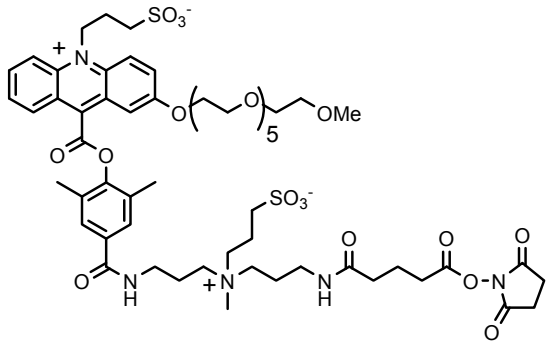


Figure S16. HPLC chromatogram of compound **10c**. HPLC conditions: Phenomenex, C_{18}, 10 micron, 3.9 x 300 mm column, 30 minute gradient of 10 → 70 % MeCN/water (each with 0.05% TFA), 1 mL/min, UV detection at 260 nm.



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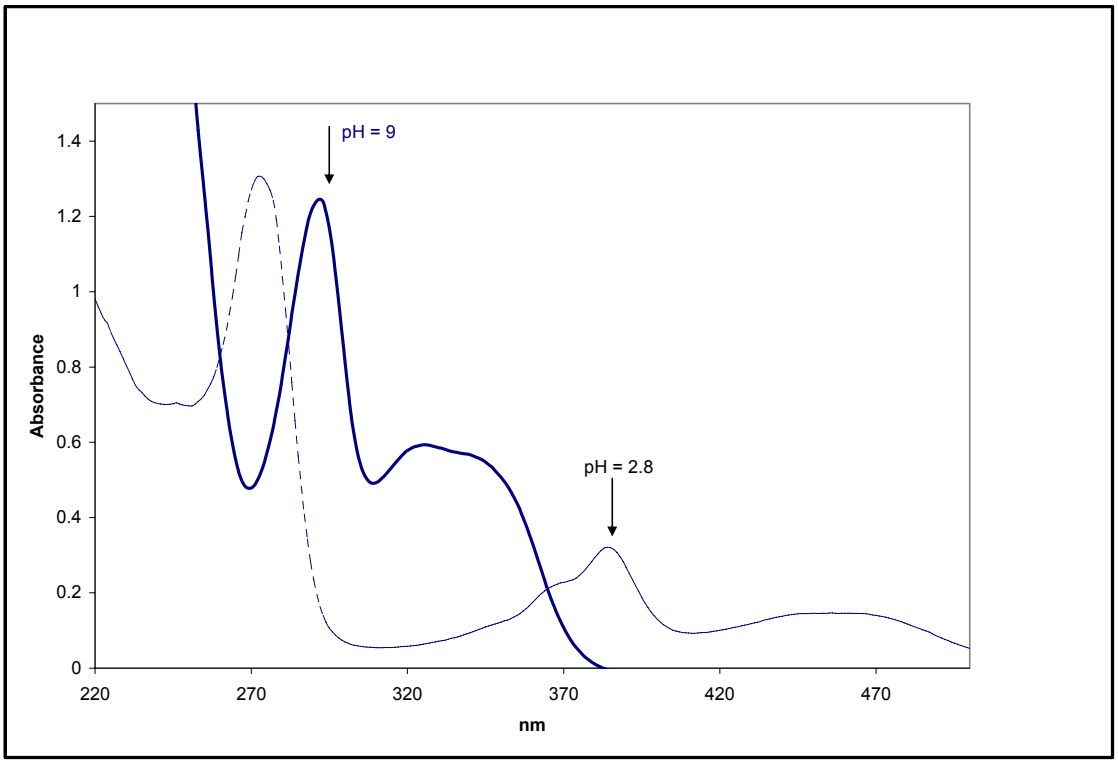
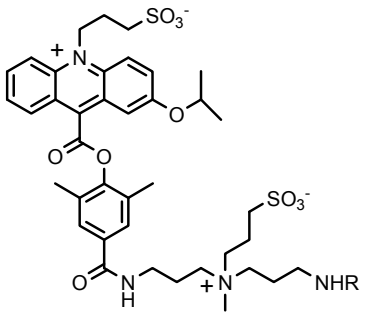


Figure S17. UV-Visible spectra of compounds **9a** and **10a** in 0.1 M phosphate pH 9.0 and pH 2.8 respectively. The absorption band at 383 nm is due to the acridinium chromophore.



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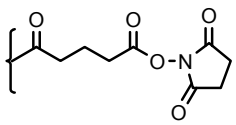
R =  **10a**

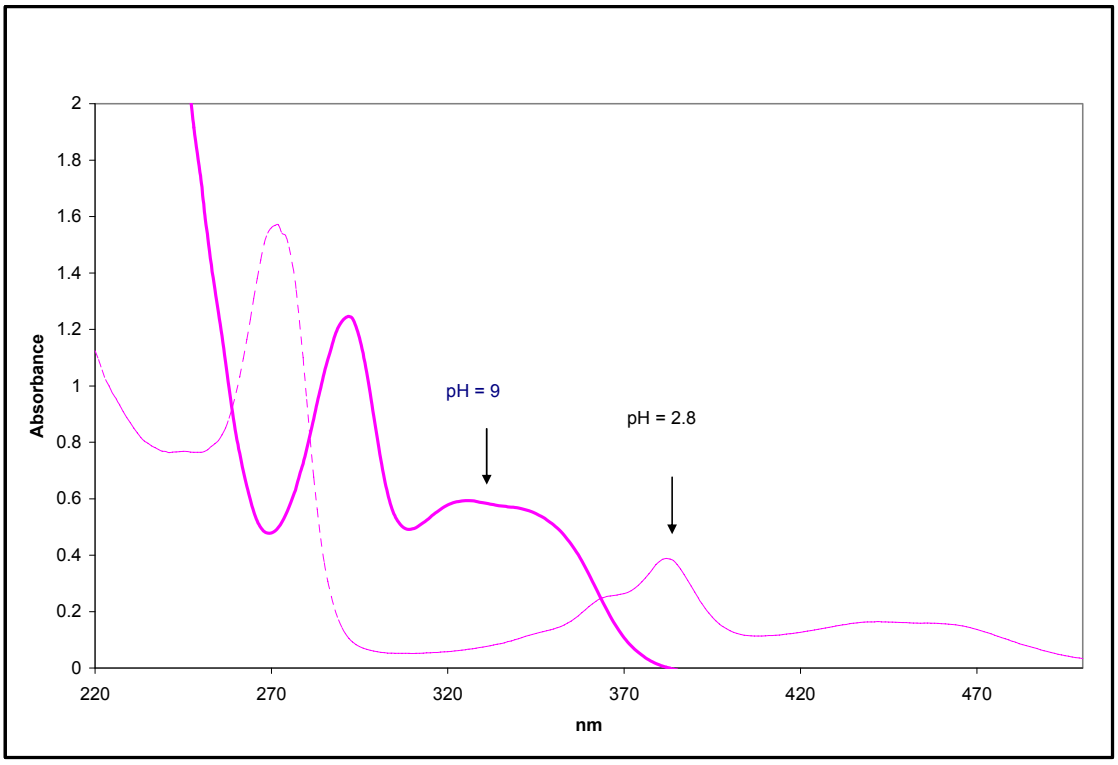
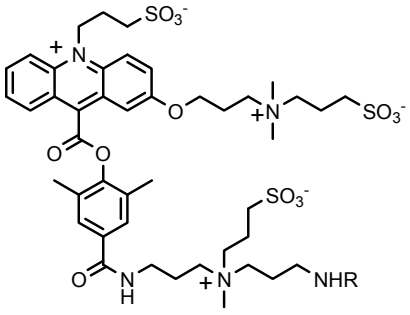


Figure S18. UV-Visible spectra of compounds **9b** and **10b** in 0.1 M phosphate pH 9.0 and pH 2.8 respectively. The absorption band at 383 nm is due to the acridinium chromophore.



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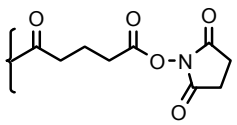
R =  **10b**

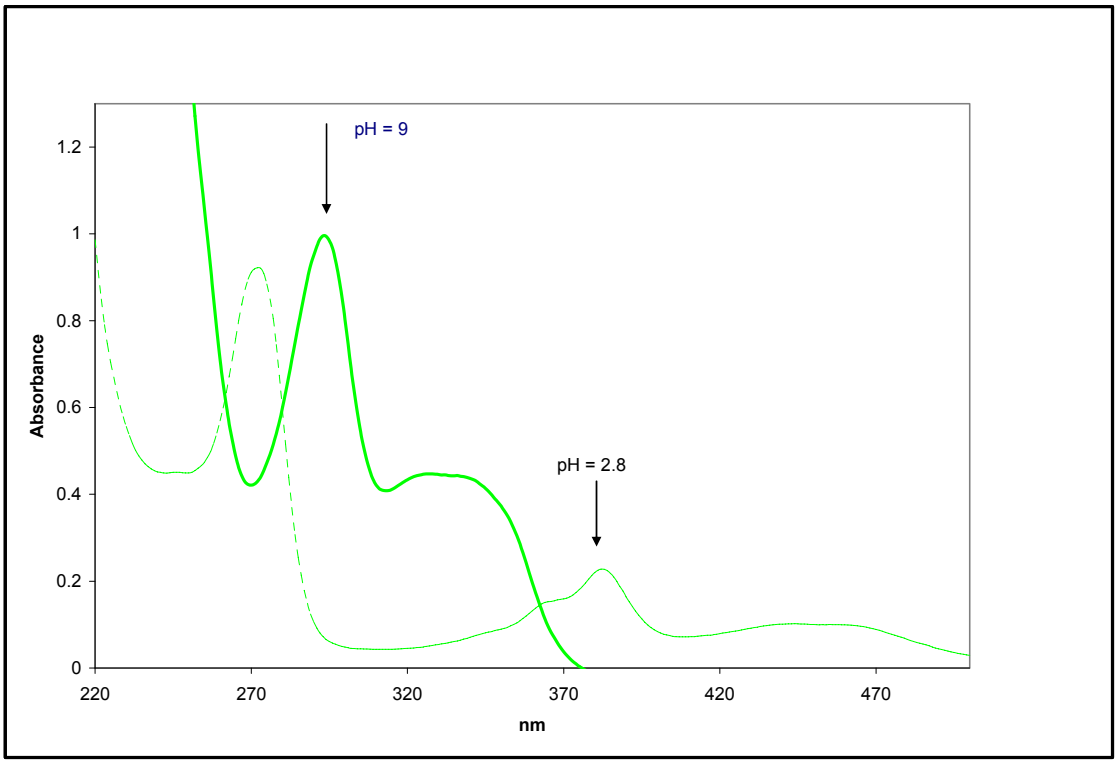
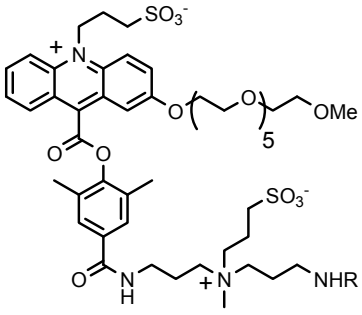


Figure S19. UV-Visible spectra of compounds **9c** and **10c** in 0.1 M phosphate pH 9.0 and pH 2.8 respectively. The absorption band at 383 nm is due to the acridinium chromophore.



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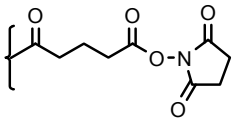
R =  **10c**

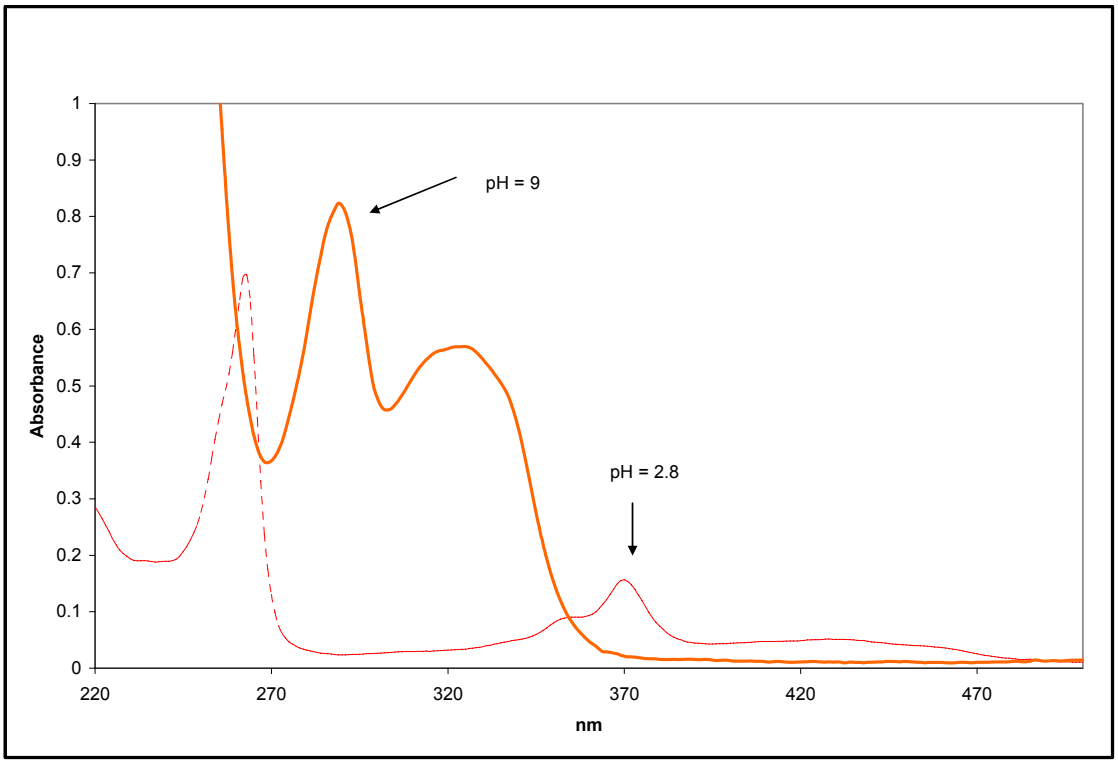
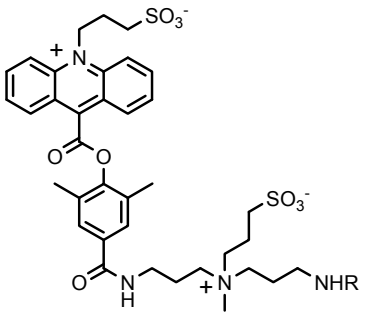


Figure S20. UV-Visible spectra of compounds **9d** and **10d** in 0.1 M phosphate pH 9.0 and pH 2.8 respectively. The absorption band at 371 nm is due to the acridinium chromophore.



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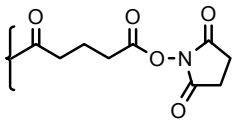
R =  **10d**

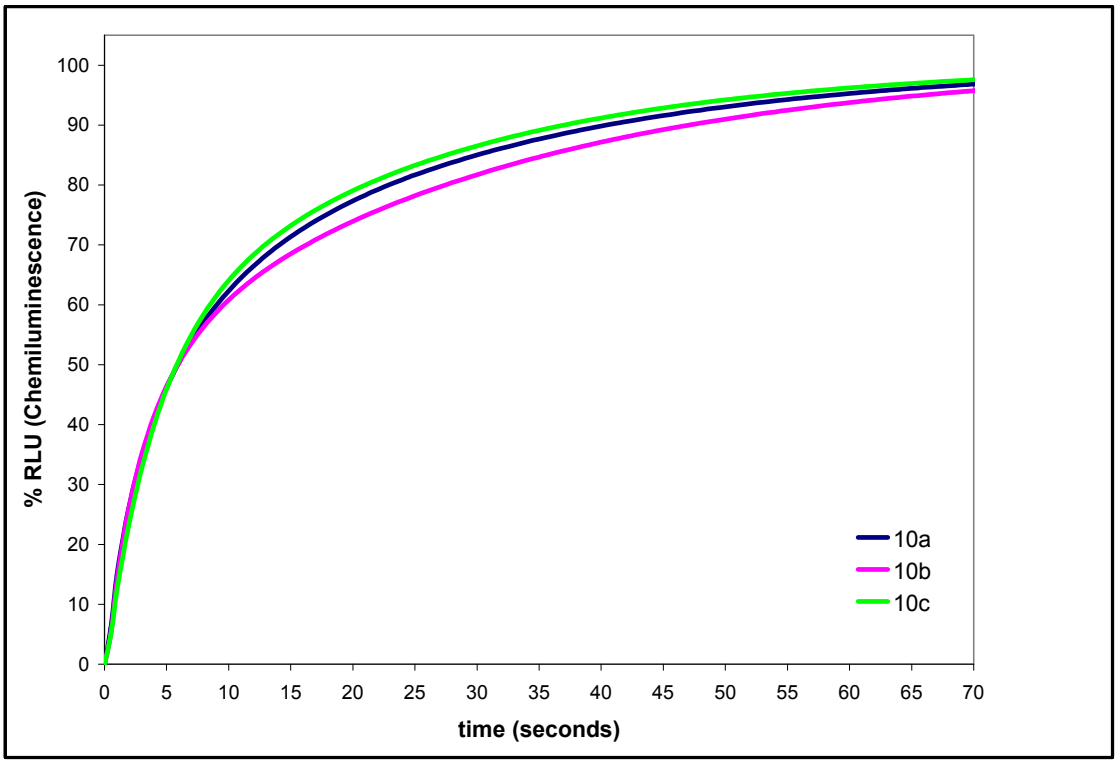


Figure S21. Chemiluminescence profiles of anti-TSH antibody conjugates of alkoxy-substituted acridinium esters in the absence of surfactant.

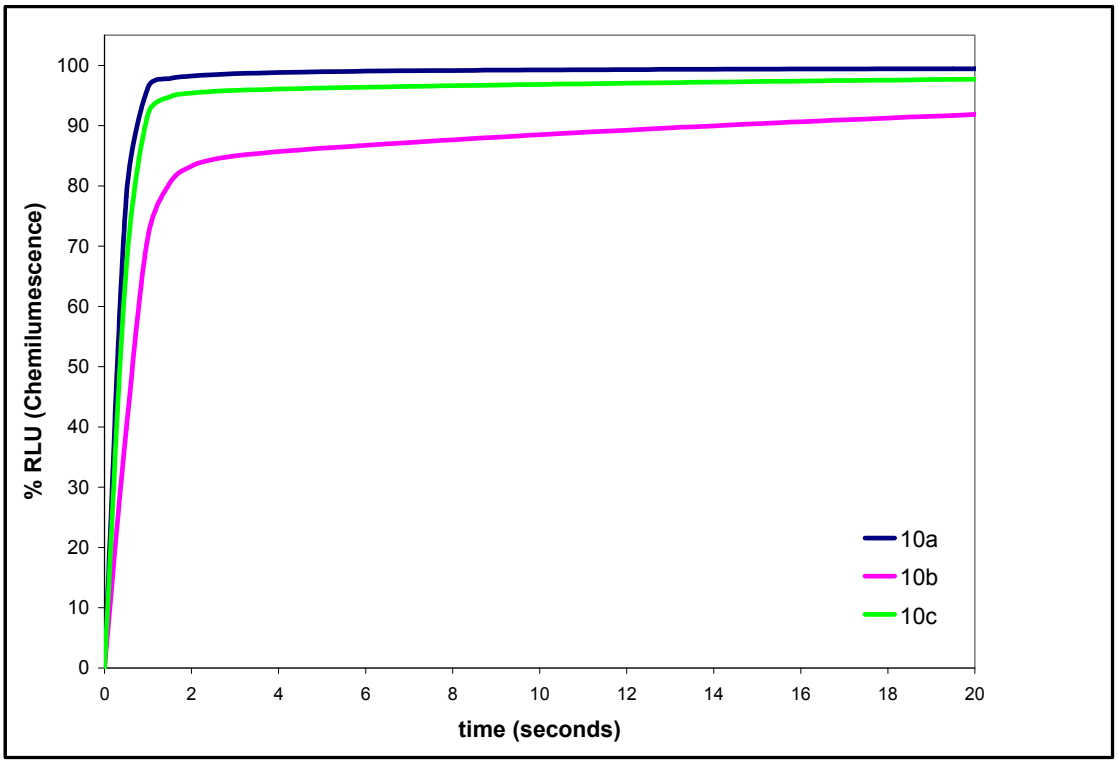


Figure S22. Chemiluminescence profiles of anti-TSH antibody conjugates of alkoxy-substituted acridinium esters in the presence of the cationic surfactant CTAC.

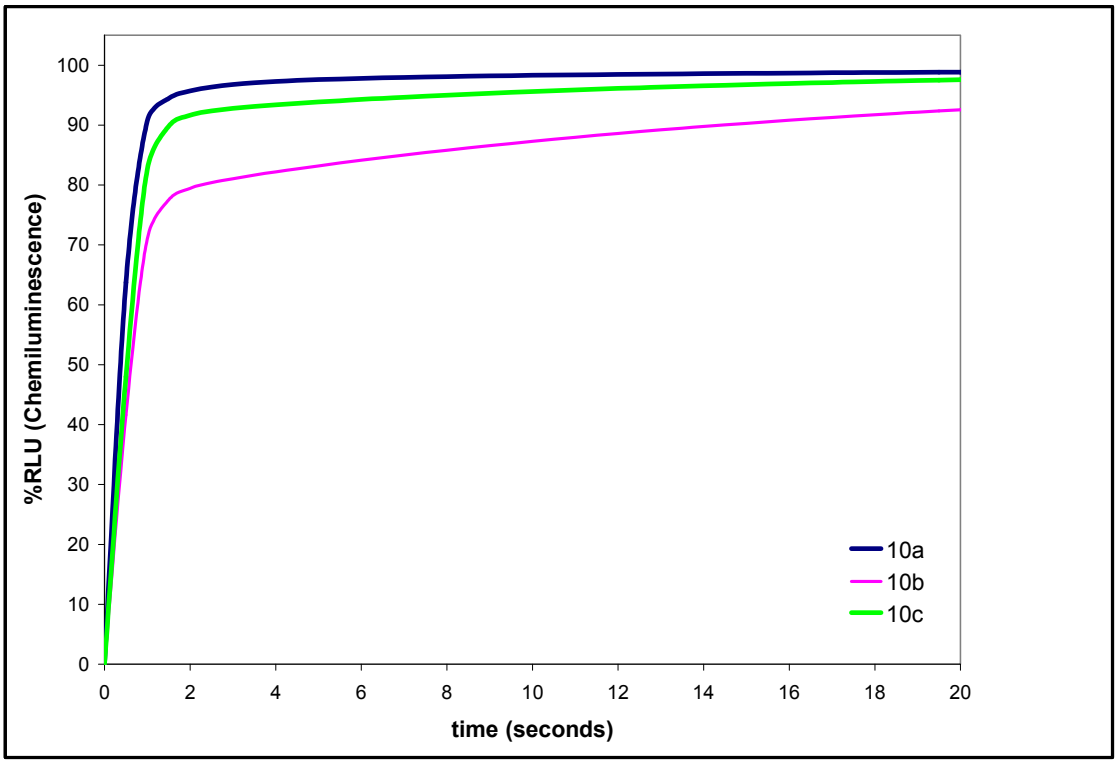


Figure S23. Chemiluminescence profiles of anti-TSH antibody conjugates of alkoxy-substituted acridinium esters in the presence of the cationic surfactant CTPAC.

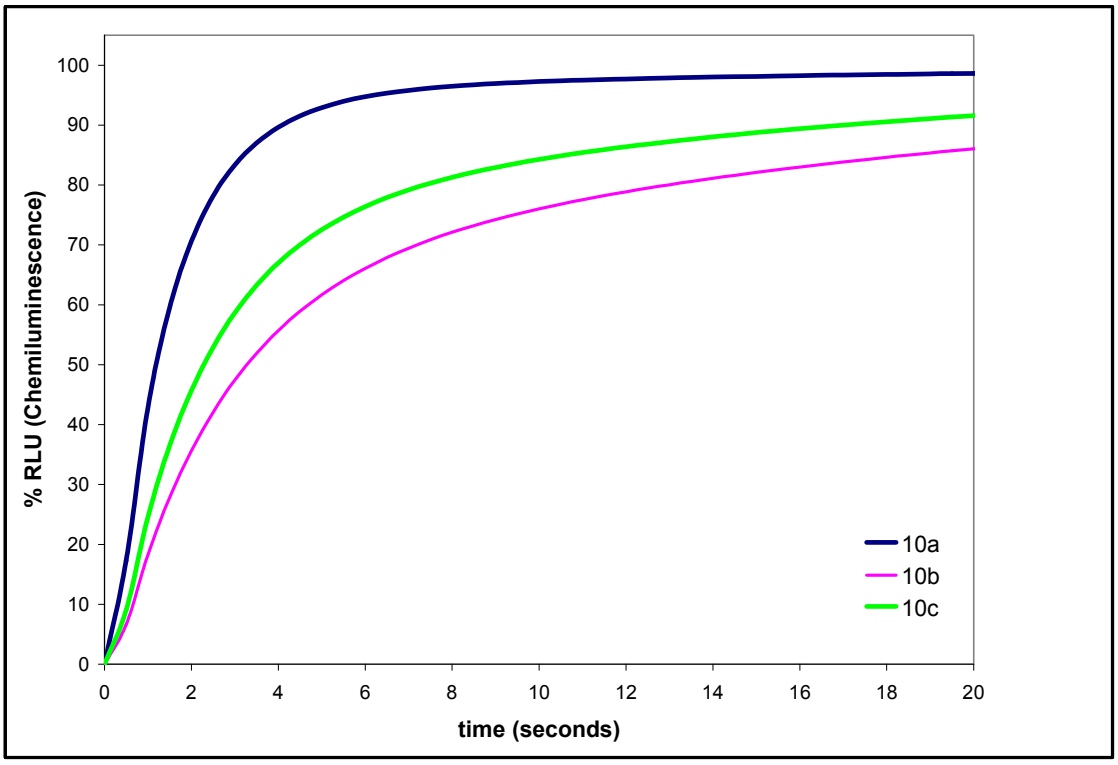


Figure S24. Chemiluminescence profiles of anti-TSH antibody conjugates of alkoxy-substituted acridinium esters in the presence of the zwitterionic surfactant DDAPS.

Micelle-water partition coefficients of acridinium esters

Partition coefficients of the acridinium ester labels **9a**-**9d** and **10a**-**10d** to CTAC micelles were measured by HPLC using the method described by Armstrong and Nome[17]. Briefly, this method entails measuring the retention volumes of organic compounds as a function of surfactant concentration. If a compound partitions into micelles, then the retention volume of the compound decreases with increasing surfactant (micelle) concentration in the mobile phase. By measuring the retention volumes at different surfactant concentrations, the micelle-water partition coefficient (K_{MW}) of a compound is calculated from the following equation derived by Armstrong and Nome[17]:

$$\frac{Vs}{Ve - Vm} = \frac{v(K_{MW} - 1)}{K_{SW}} Cm + \frac{1}{K_{SW}}$$

where Vs is the volume of the stationary phase of the HPLC column,

Ve is the retention volume,

Vm is the void volume of the HPLC column,

Cm is the concentration of surfactant in micelles in g/mL, (Cm = total surfactant concentration – critical micelle concentration),

v is the partial specific volume of the surfactant (for CTAC, *v* = 0.977 mL/g[22]) and,

K_{SW} is the partition coefficient of the compound to the stationary phase.

According to this equation, a plot of $\frac{Vs}{Ve - Vm}$ versus Cm should give a straight line and the ratio of the slope to intercept = $v(K_{MW} - 1)$. Micellar HPLC has its drawbacks that include poor peak efficiency (broad peaks) the causes of which have been addressed in a review by Berthod[23]. Moreover, for substrates that partition very strongly into micelles, an accurate estimate of the partition coefficient is not possible because partition coefficient plots of these substrates have intercepts close to zero or even negative[22,24].

A Phenomenex, polymer-x, 5 micron, 4.6 x 100 mm column with a 20 μL injection loop was used for these measurements. The void volume (Vm) of the column (0.88 mL) was determined by injecting air in the column and noting the first deviation from baseline. The volume of the stationary phase (Vs) was calculated to be 1.61 mL. For partition coefficients of compounds **10a**-**10d** (Figure 1, NHS ester labels), the compounds were dissolved in 2:1 de-ionized water/MeCN (with 0.05% TFA) at pH 2.5 to give approximately 1 mg/mL solutions. For partition coefficients of compounds **9a**-**9d** (Figures 1 and 3, amine intermediates), the compounds were dissolved in 1:1 0.1 M phosphate/MeOH at pH 9.0 to give approximately 1 mg/mL solutions.

The retention volumes of the acridinium forms of the compounds **10a**-**10d** and, the pseudobase forms of compounds **9a**-**9d** were determined as a function of surfactant (CTAC) concentration at pH 2.8 and pH 9 respectively. Specifically, 0.1 M CTAC solutions in either 0.1 M phosphate pH 2.8 or pH 9 were prepared (solvent B). The concentration of CTAC was varied by varying the percentage of solvent B where solvent A was 0.1 M phosphate pH 2.8 or pH 9. The retention volumes at different CTAC concentrations were used to generate the plots illustrated in Figures S25-S31.

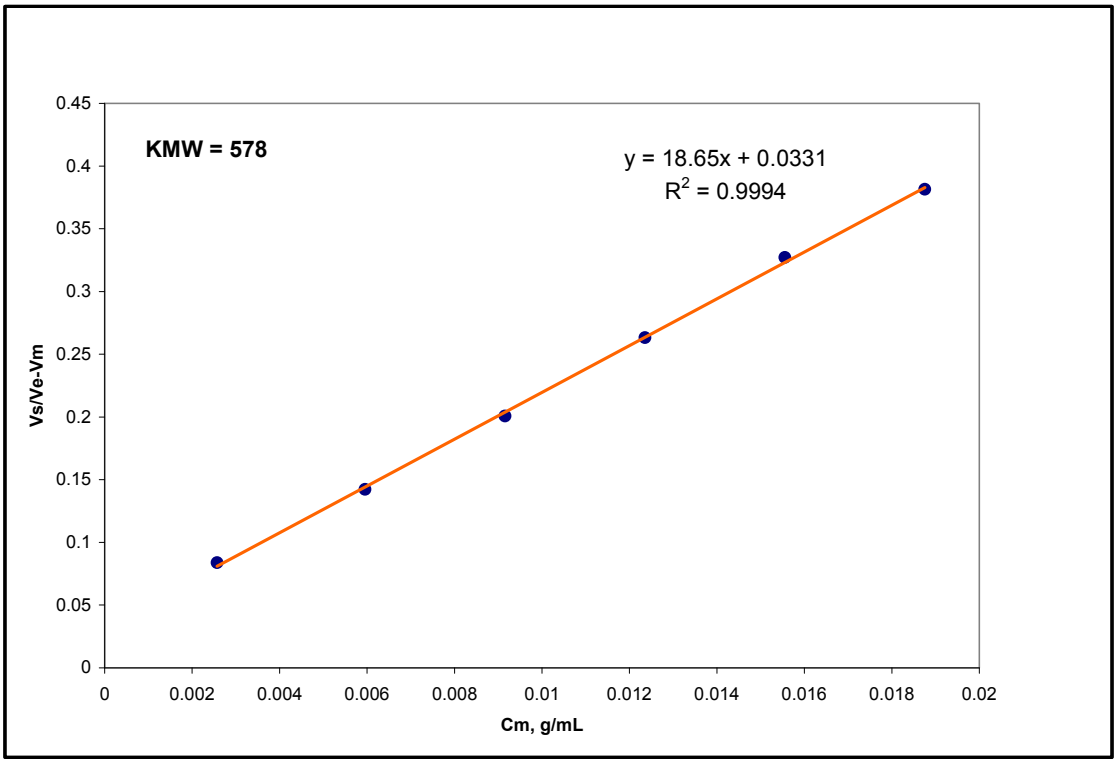
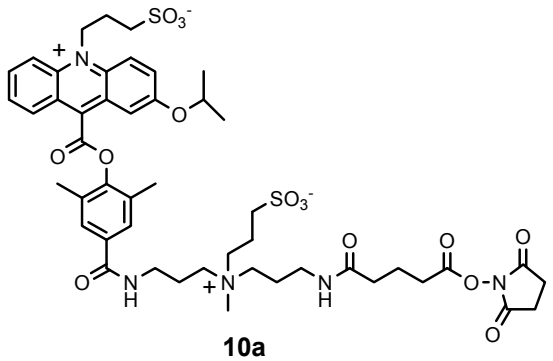


Figure S25. Partition coefficient plot of compound **10a** to CTAC micelles at pH 2.8.



10a

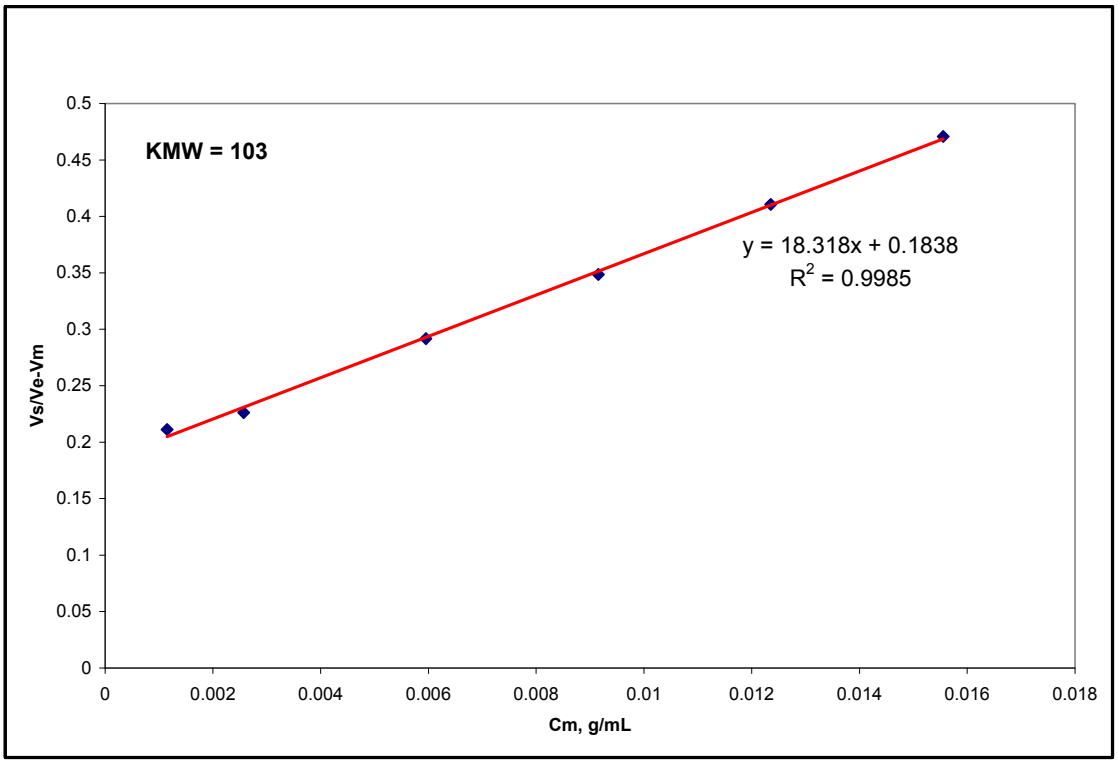
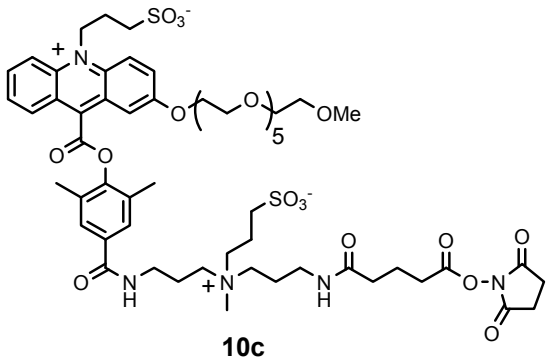


Figure S26. Partition coefficient plot of compound **10c** to CTAC micelles at pH 2.8.



10c

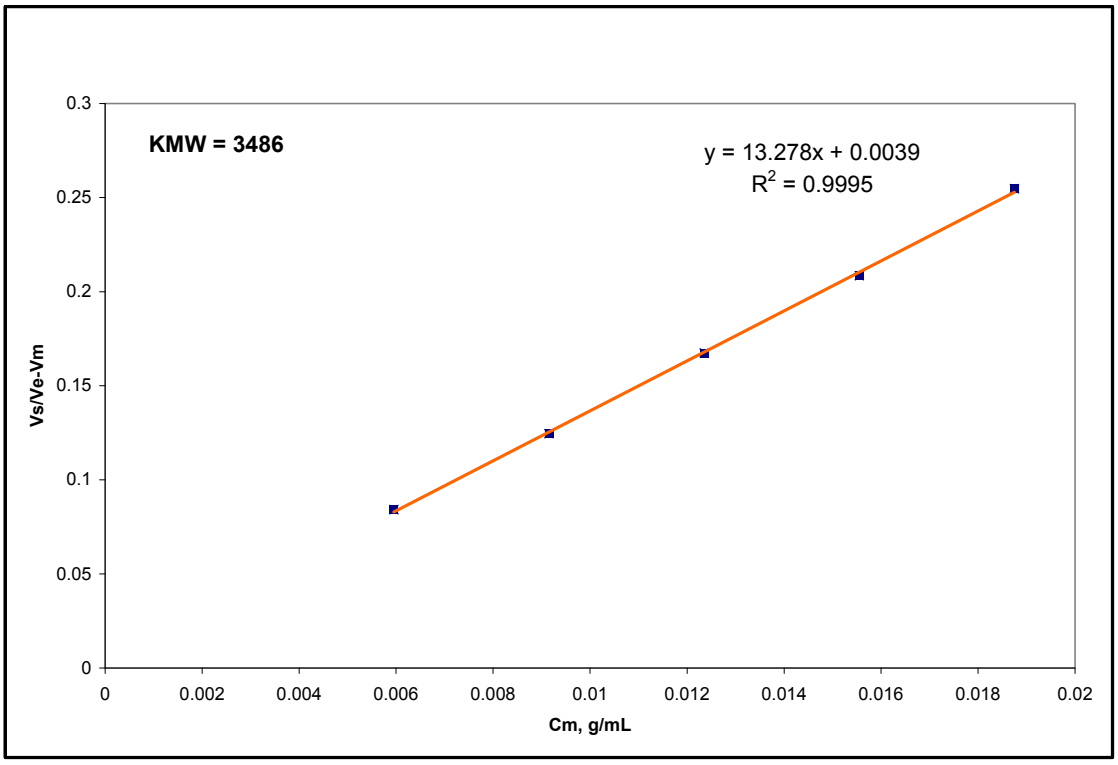
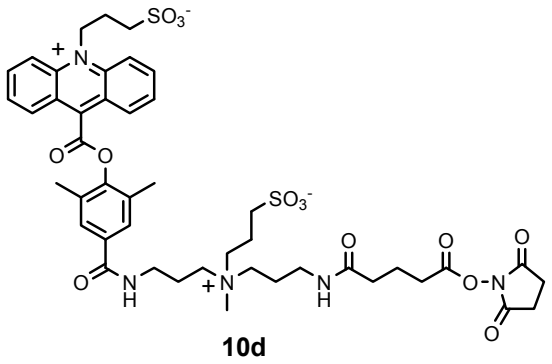


Figure S27. Partition coefficient plot of compound **10d** to CTAC micelles at pH 2.8.



10d

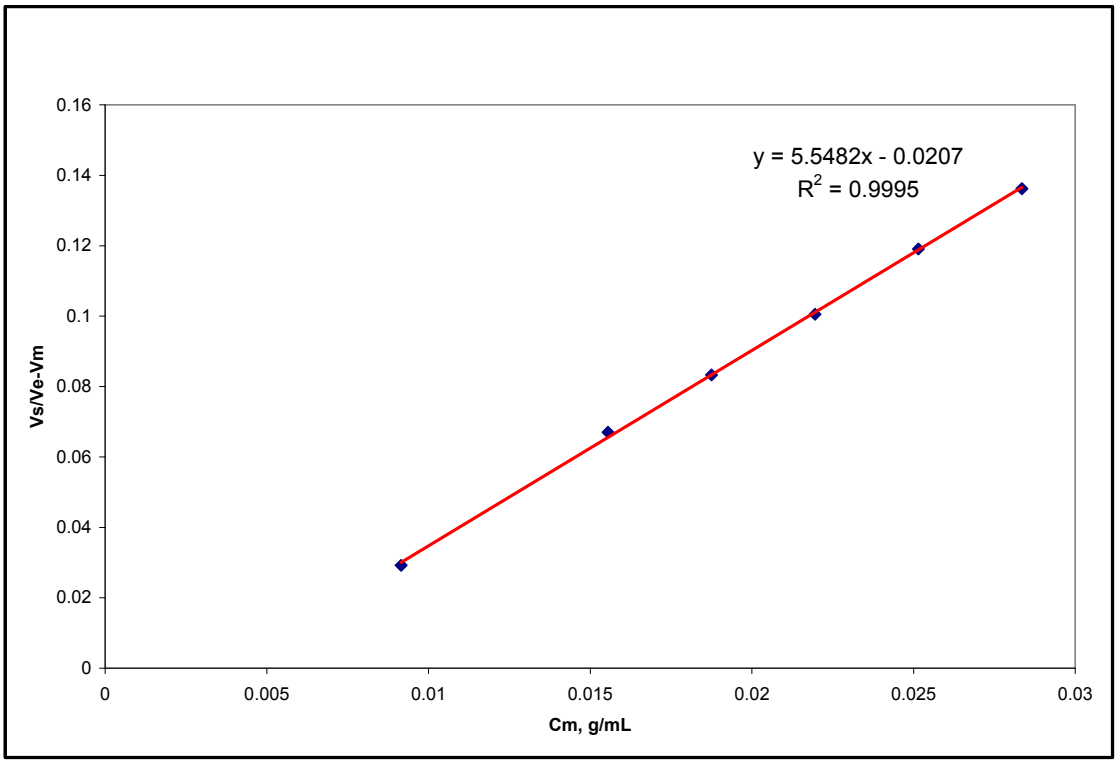
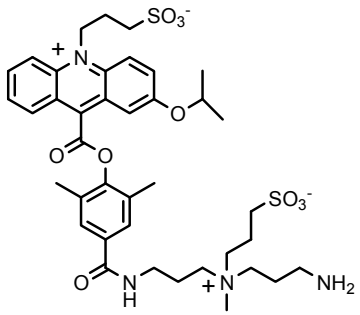


Figure S28. Partition coefficient plot of compound **9a** to CTAC micelles at pH 9.0.



9a

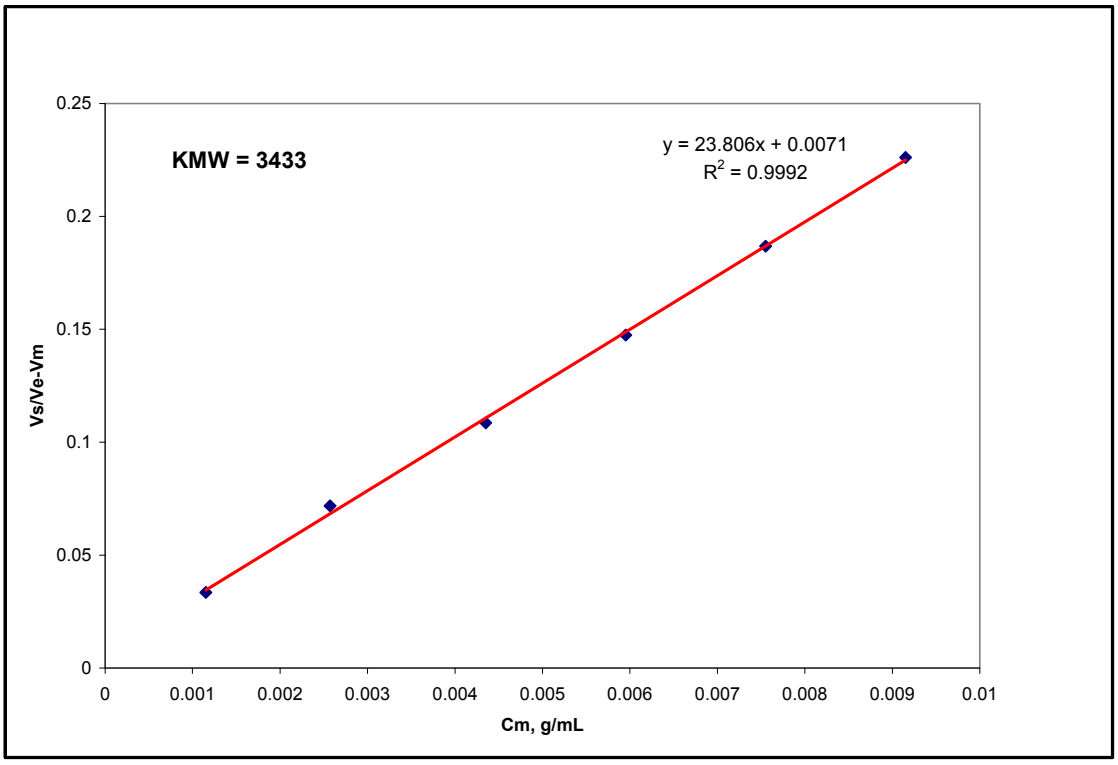
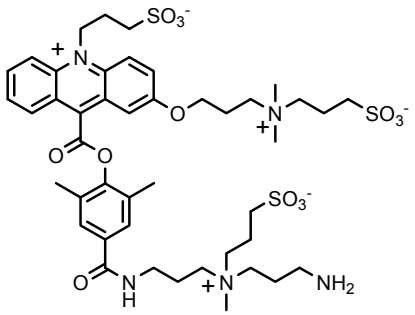


Figure S29. Partition coefficient plot of compound **9b** to CTAC micelles at pH 9.0.



9b

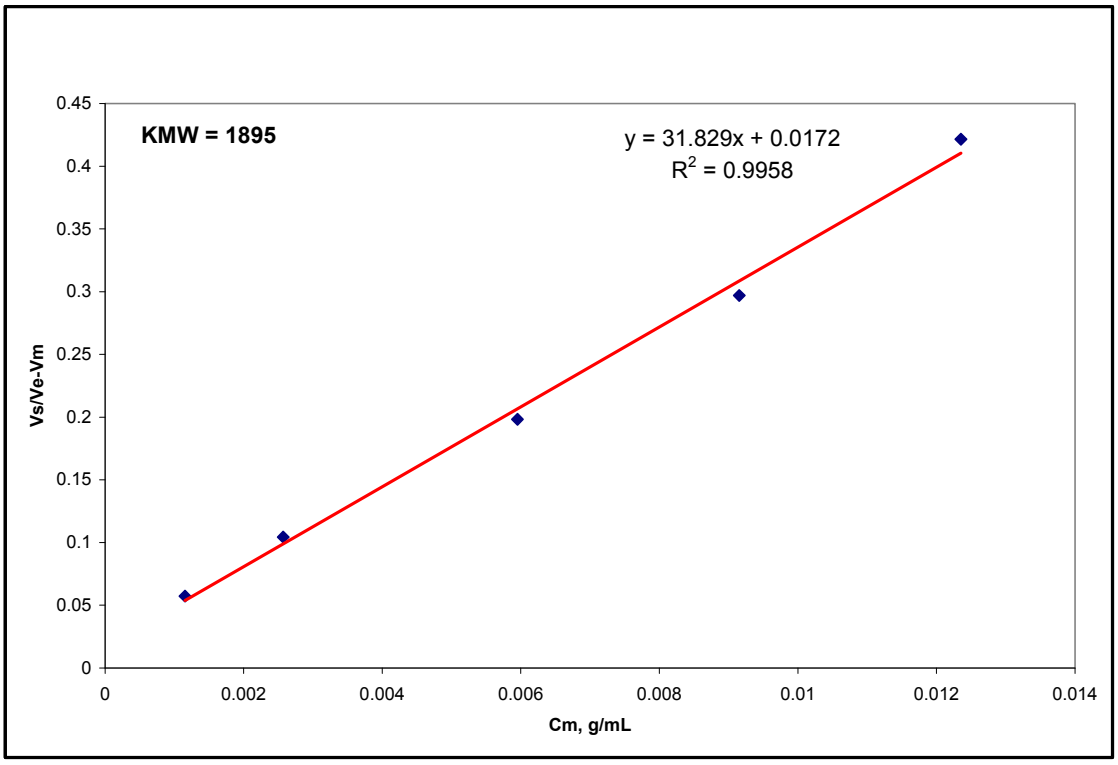
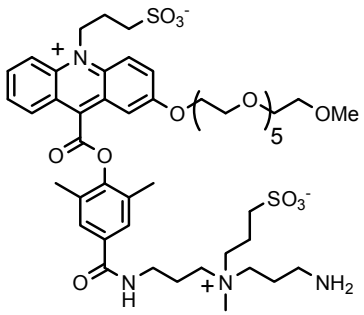


Figure S30. Partition coefficient plot of compound **9c** to CTAC micelles at pH 9.0.



9c

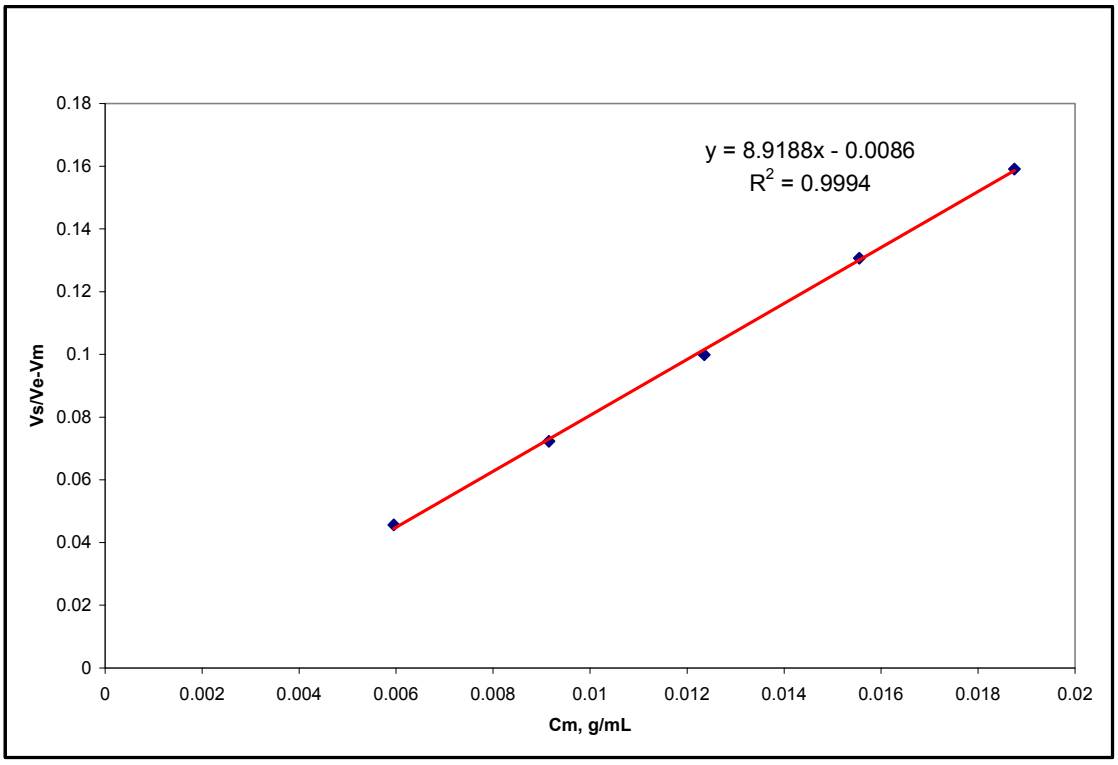
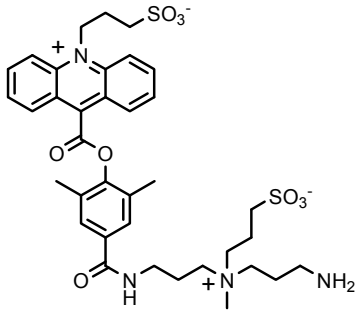


Figure S31. Partition coefficient plot of compound **9d** to CTAC micelles at pH 9.0.



9d