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

Supplementary Material

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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$_{18}$, 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$_3$CO$_2$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. $^1$H-NMR spectrum of 2a.
Figure S1C. $^{13}$C-NMR spectrum of 2a.
Figure S2A. HPLC trace of 2b.
Figure S2B. $^1$H-NMR spectrum of 2b.
Figure S2C. $^{13}$C-NMR spectrum of 2b.
Figure S3A. HPLC trace of 2c.
Figure S3B. $^1$H-NMR spectrum of 2c.
Figure S3C. $^{13}$C-NMR of 2c.
Figure S4A. HPLC trace of 3a.
Figure S4B. $^1$H-NMR spectrum of 3a.
Figure S4C. $^{13}$C-NMR spectrum of 3a.
Figure S5A. HPLC trace of 3b.
Figure S5B. $^1$H-NMR spectrum of 3b.
Figure S5C. $^{13}$C-NMR spectrum of 3b.
Figure S6A. HPLC trace of 3c.
Figure S6B. $^1$H-NMR spectrum of 3c.
Figure S6C. $^{13}$C-NMR spectrum of 3c.

![Figure S6C. $^{13}$C-NMR spectrum of 3c.](image_url)
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).