Electronic Supplementary Material (ESI) for RSC Advances.

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

Supplementary Material

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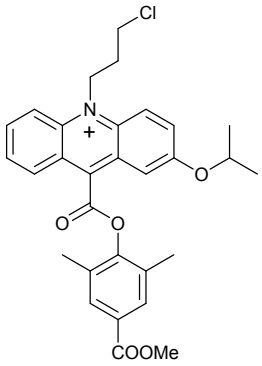

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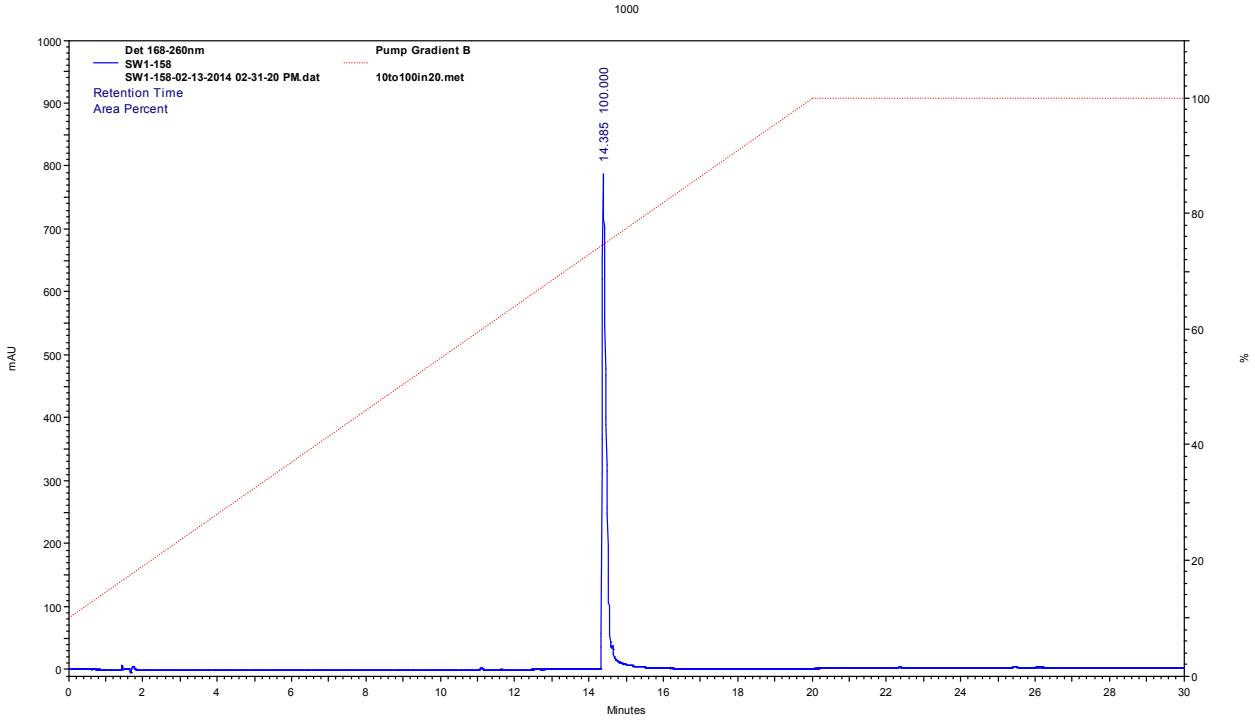
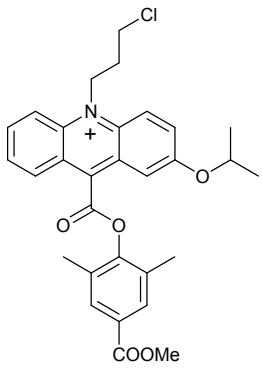


Figure S1a. HPLC trace of compound **8**.

Compound 8



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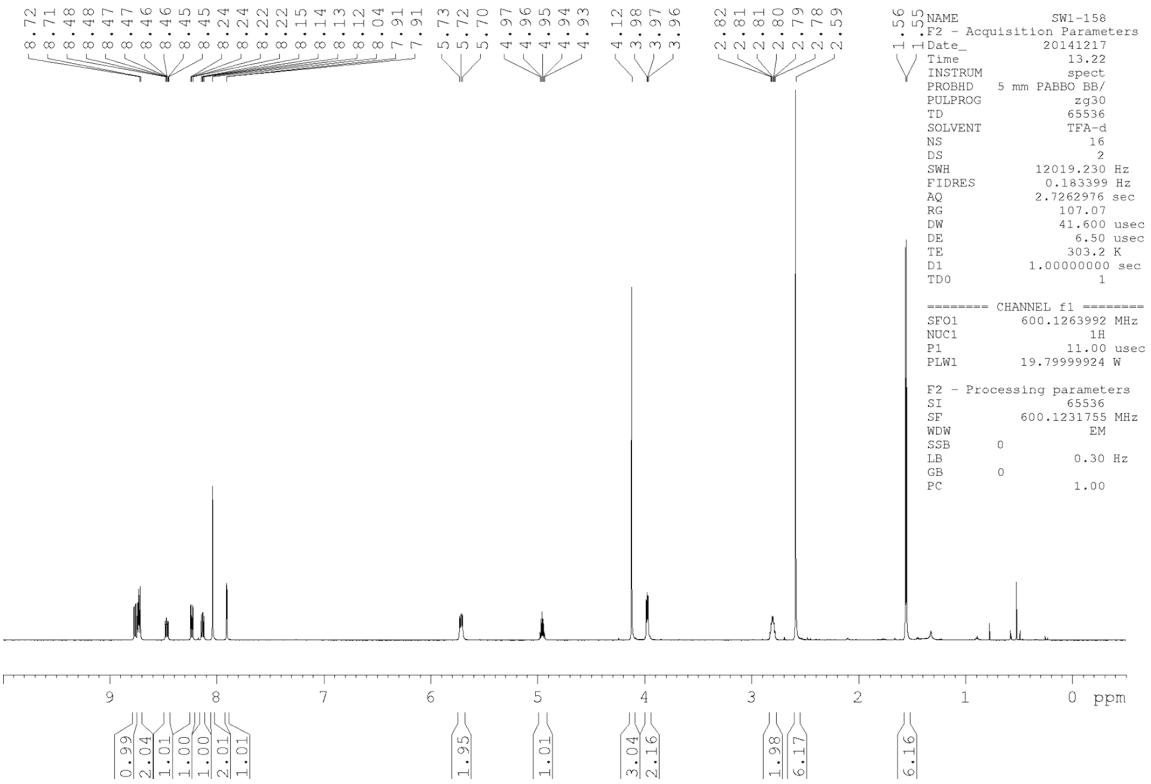
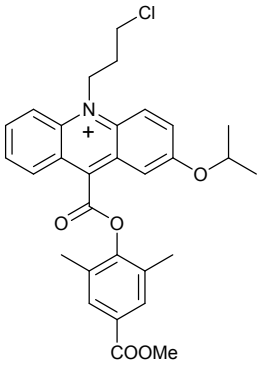


Figure S1b. ^{1}H-NMR of compound **8** in trifluoroacetic acid-d.

Compound 8



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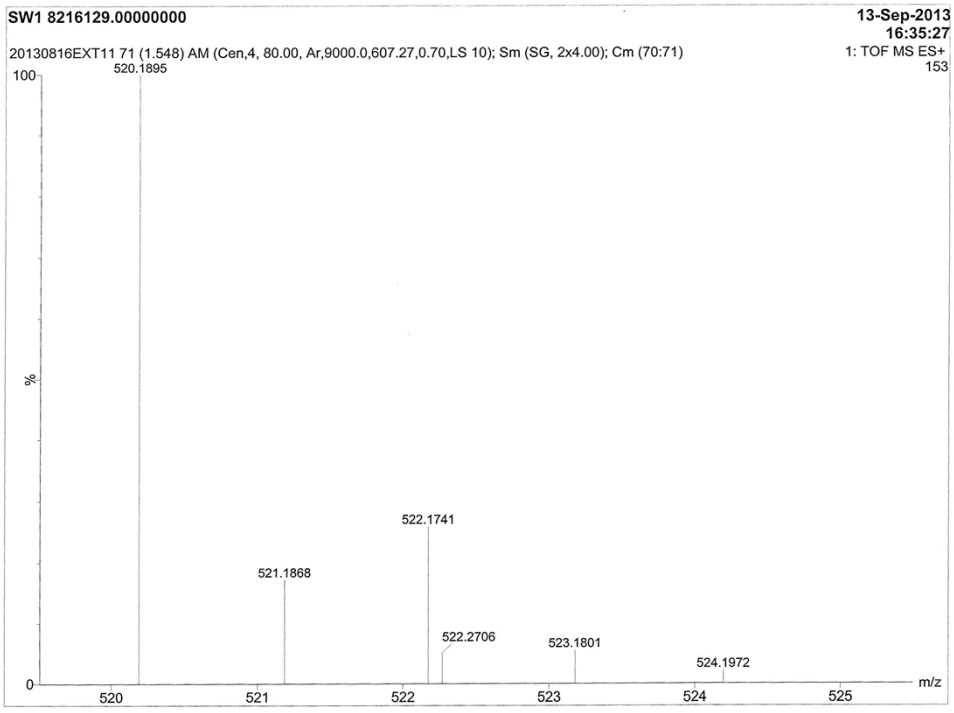
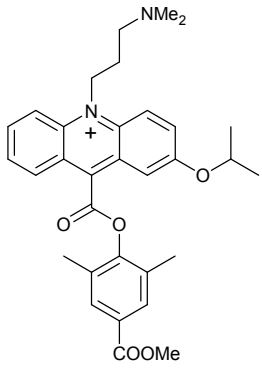


Figure S1c. HRMS of compound **8**.

Compound 9



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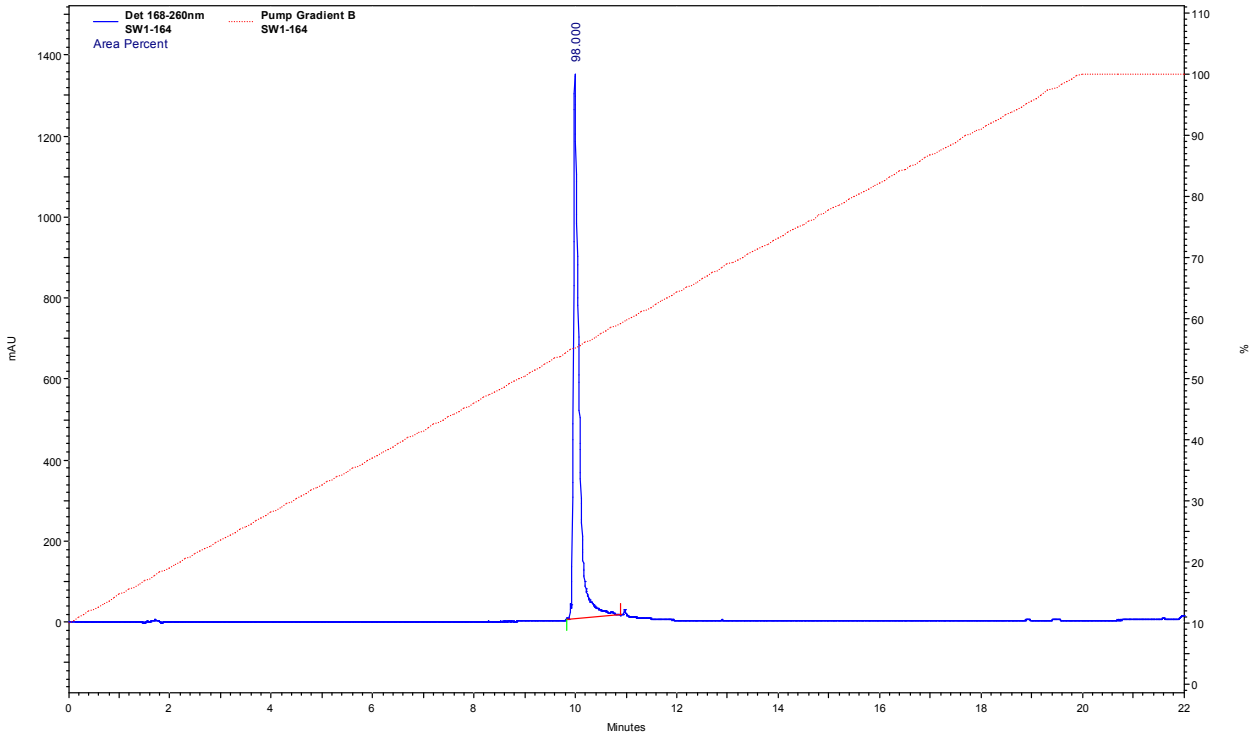
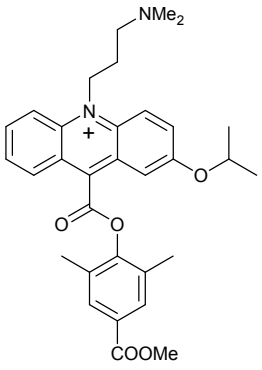


Figure S2a. HPLC trace of compound **9**.

Compound 9



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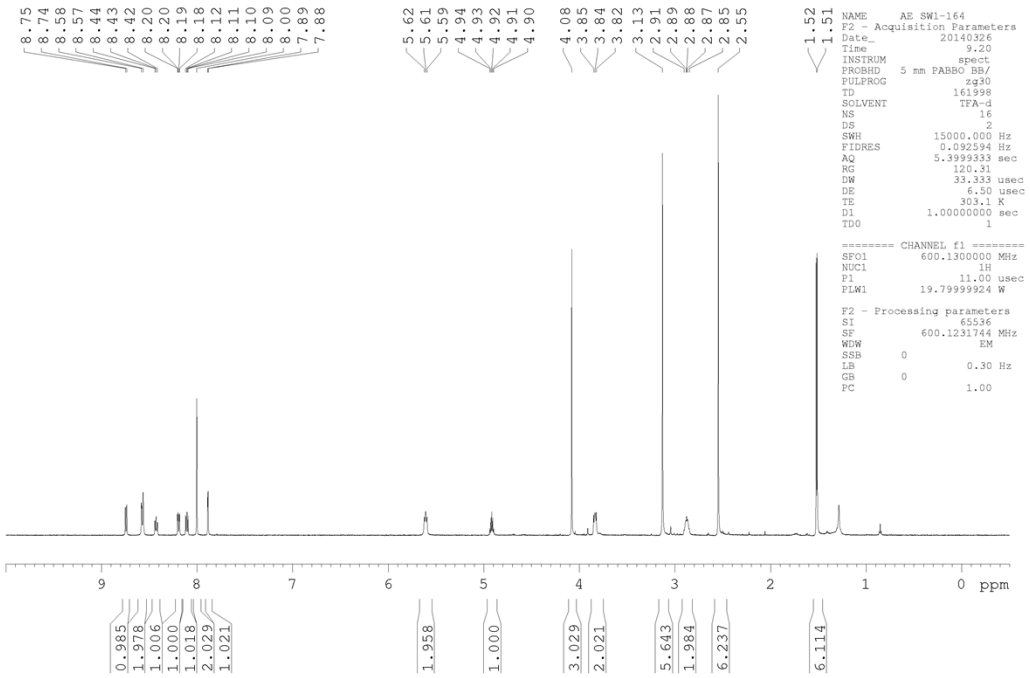
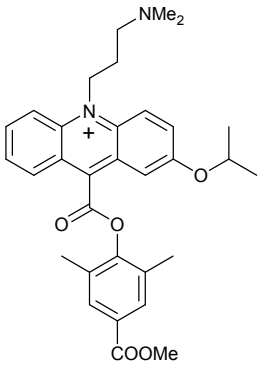


Figure S2b. ^{1}H-NMR of compound **9** in trifluoroacetic acid-d.

Compound 9



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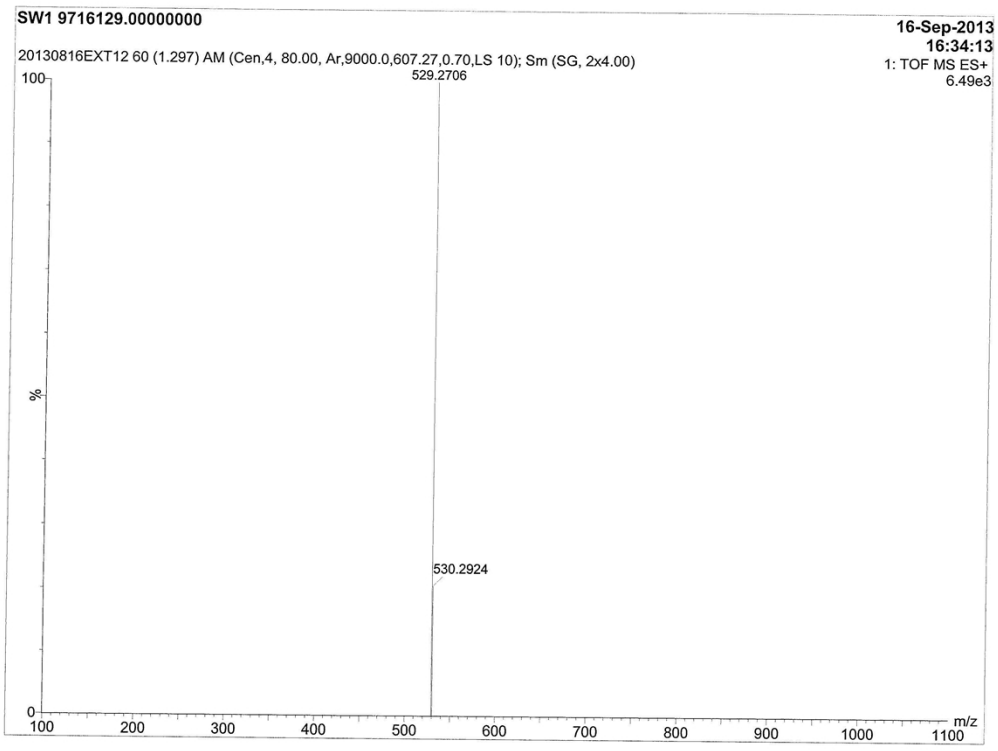
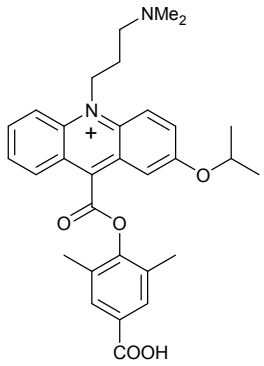


Figure S2c. HRMS of compound **9**.

Compound 2a



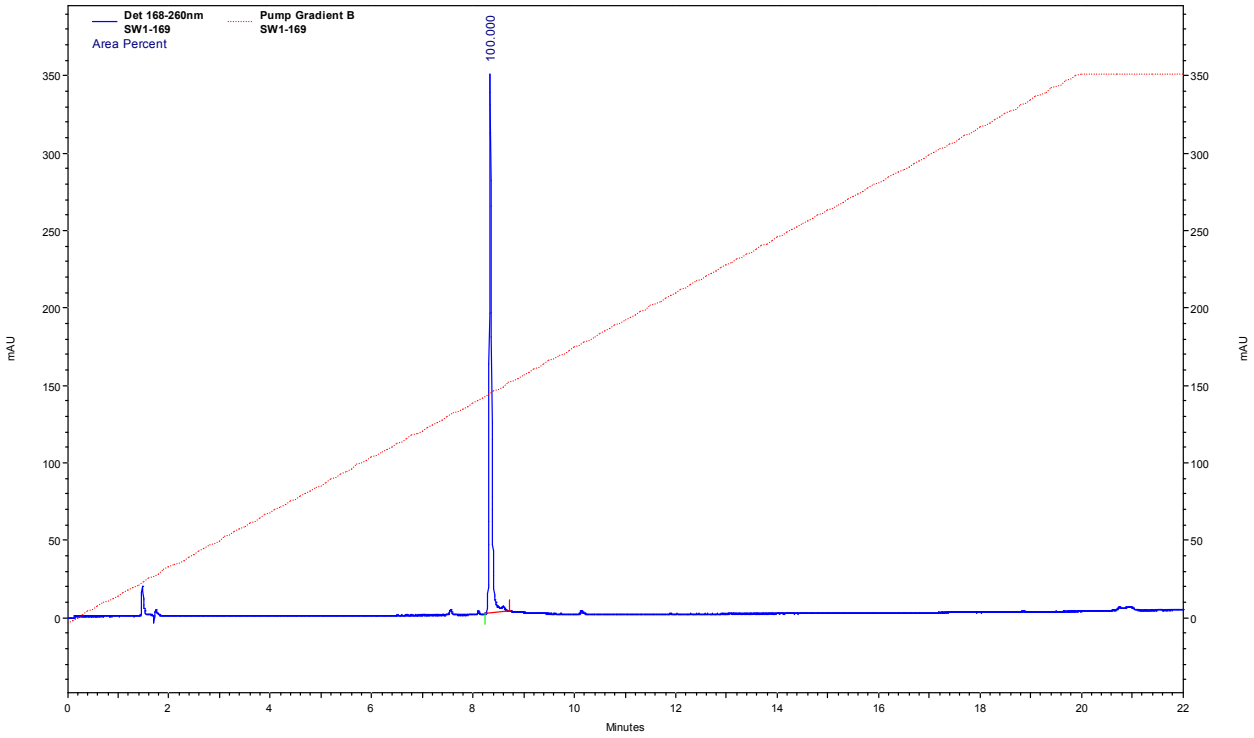


Figure S3a. HPLC trace of compound **2a**.

Compound 2a

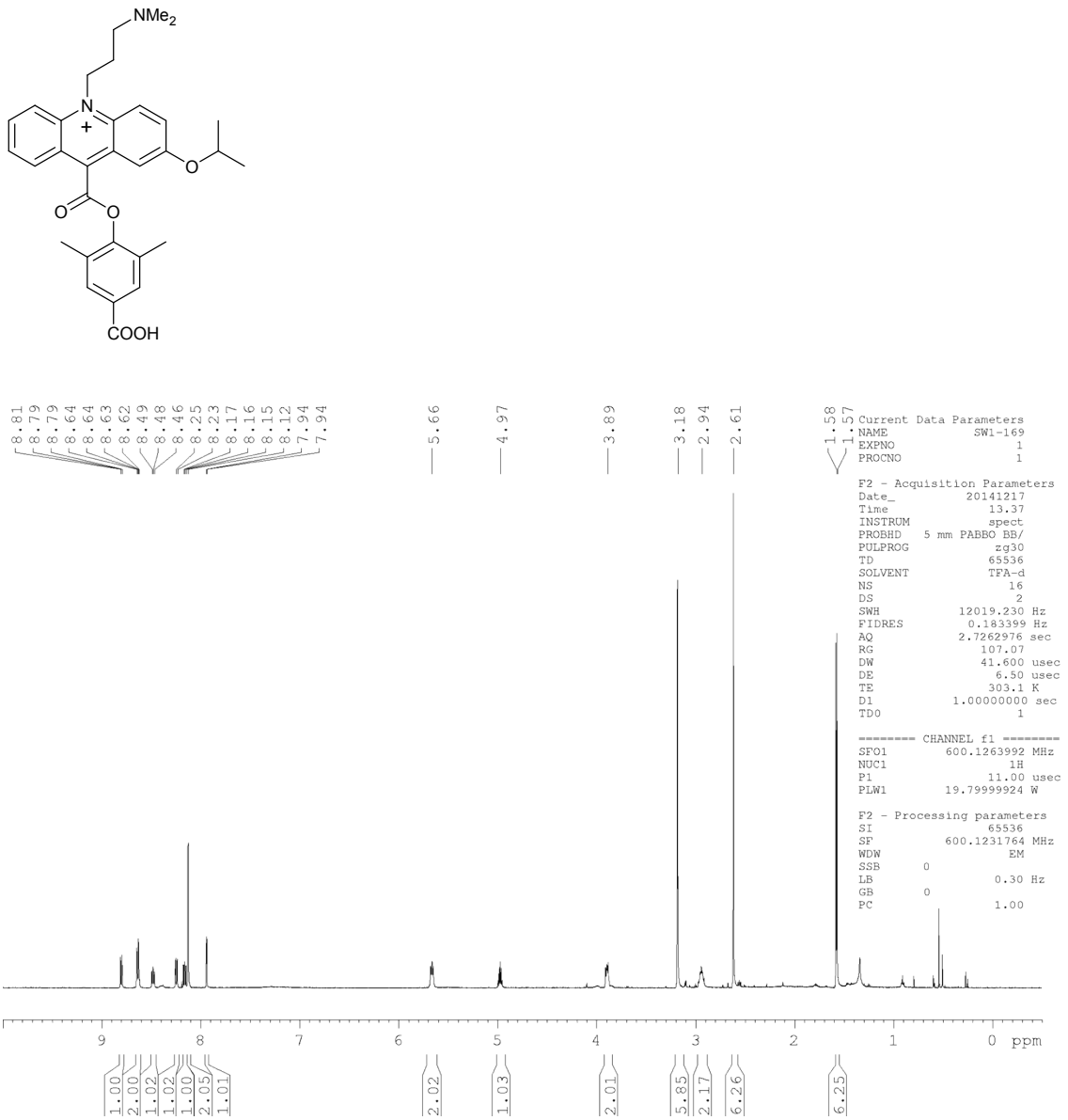
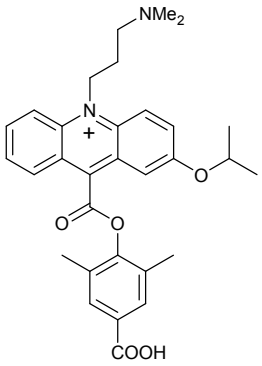


Figure S3b. ^{1}H-NMR of compound **2a** in trifluoroacetic acid-d.

Compound 2a



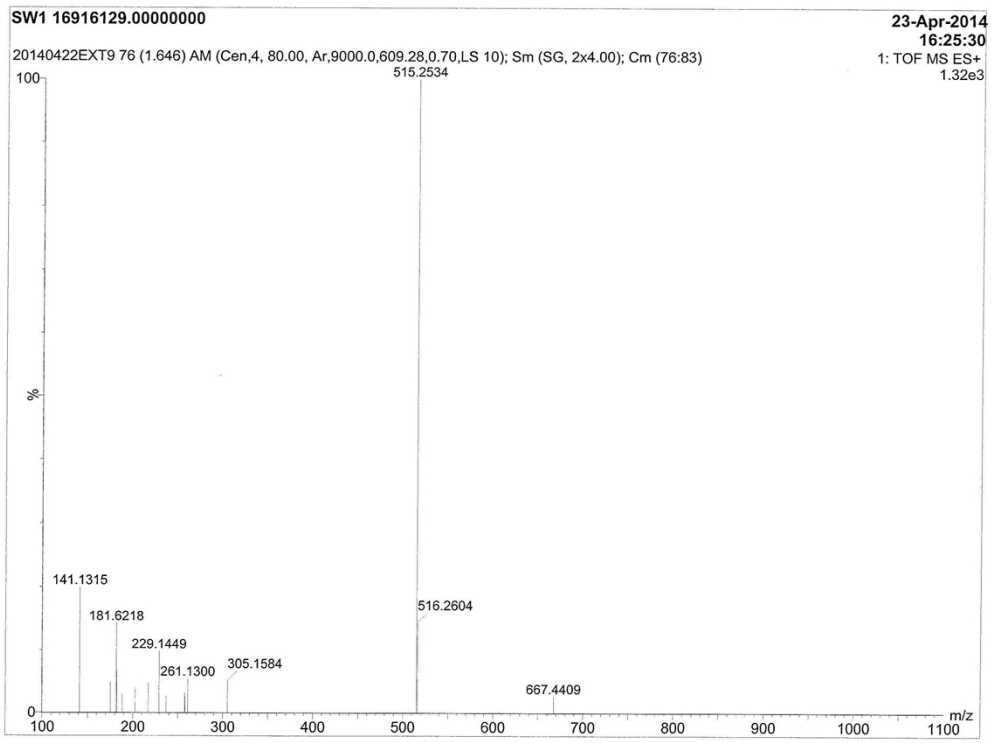
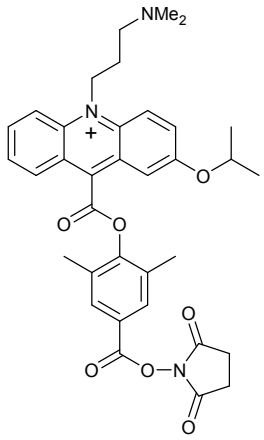


Figure S3c. HRMS of compound **2a**.

Compound 2b



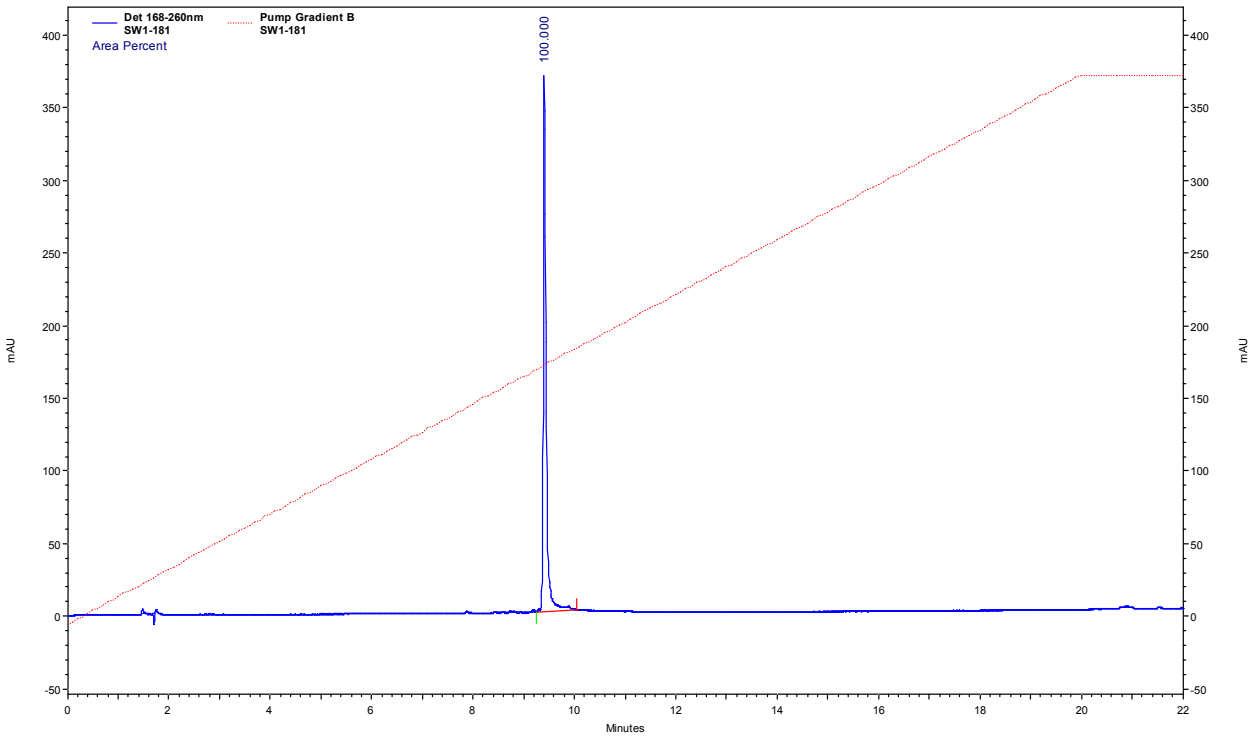
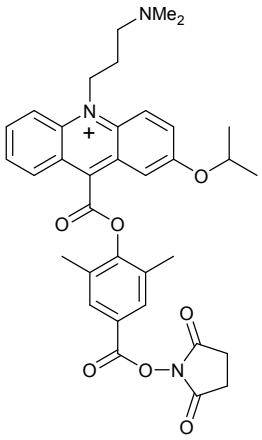


Figure S4a. HPLC trace of compound **2b**.

Compound 2b



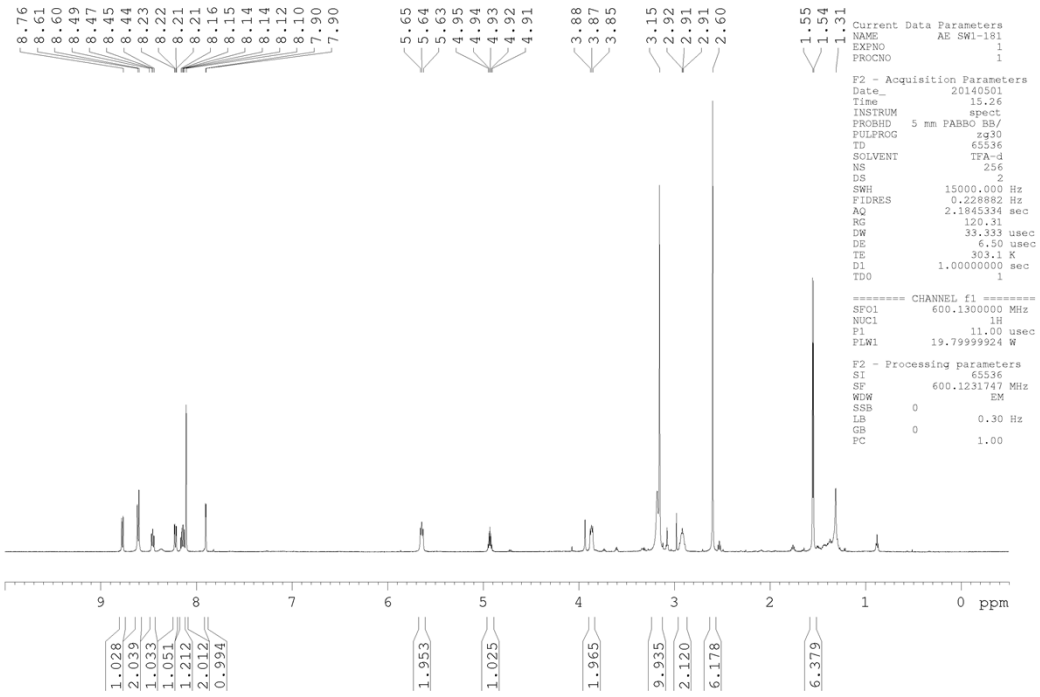
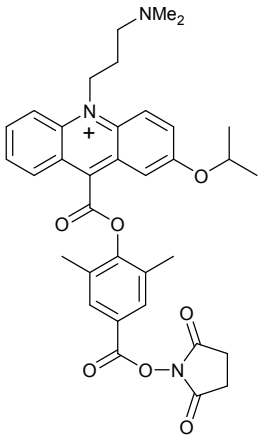


Figure S4b. ^{1}H-NMR of compound **2b** in trifluoroacetic acid-d.

Compound 2b



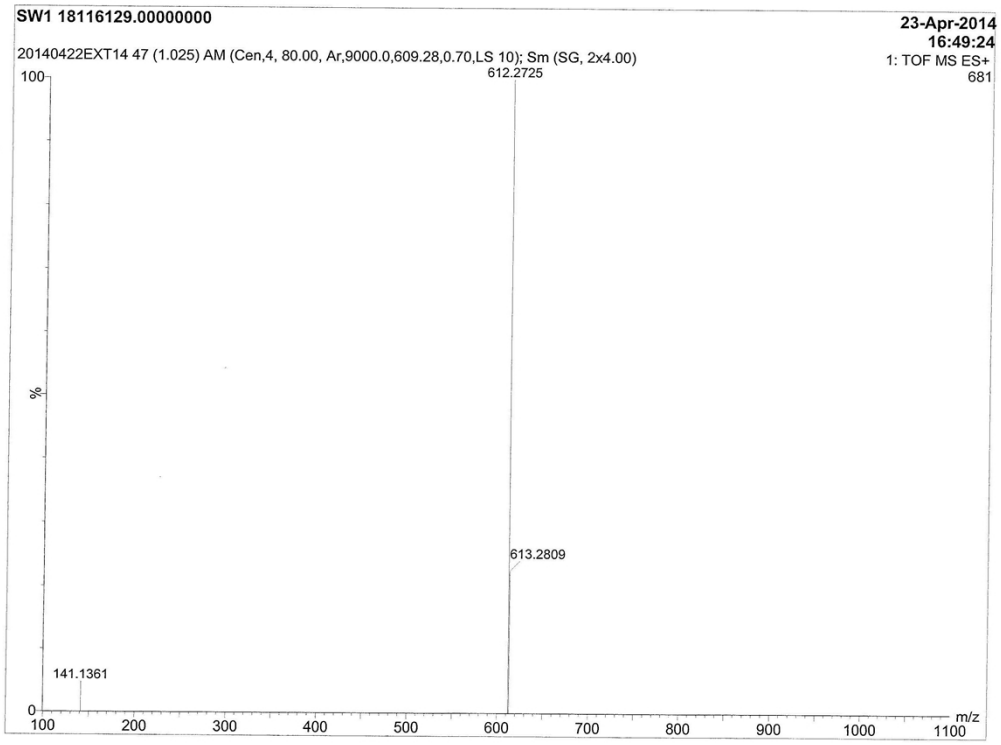
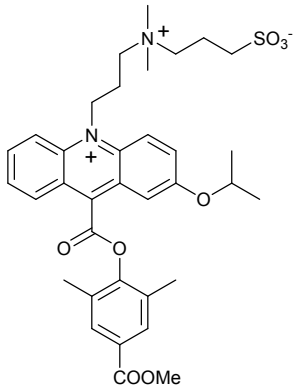


Figure S4c. HRMS of compound **2b**.

Compound 10



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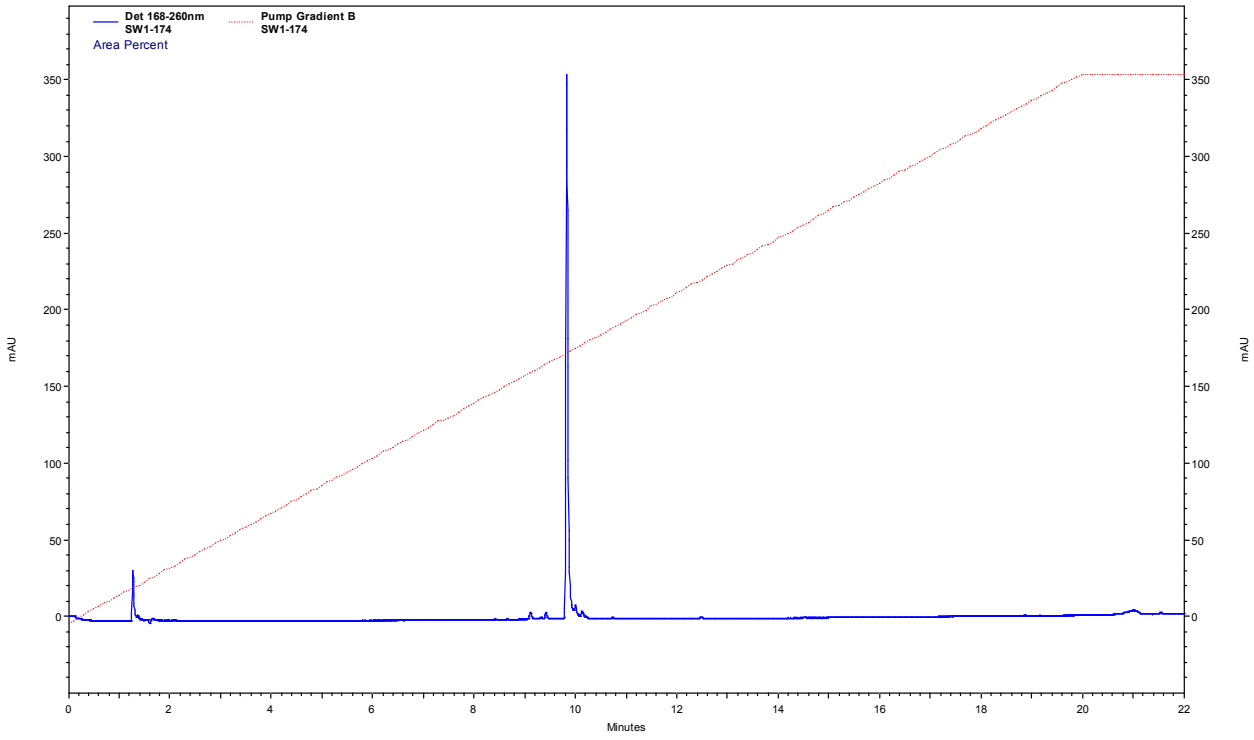
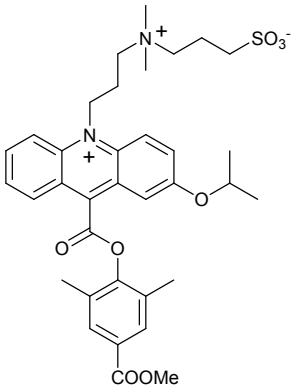


Figure S5a. HPLC trace of compound **10**.

Compound 10



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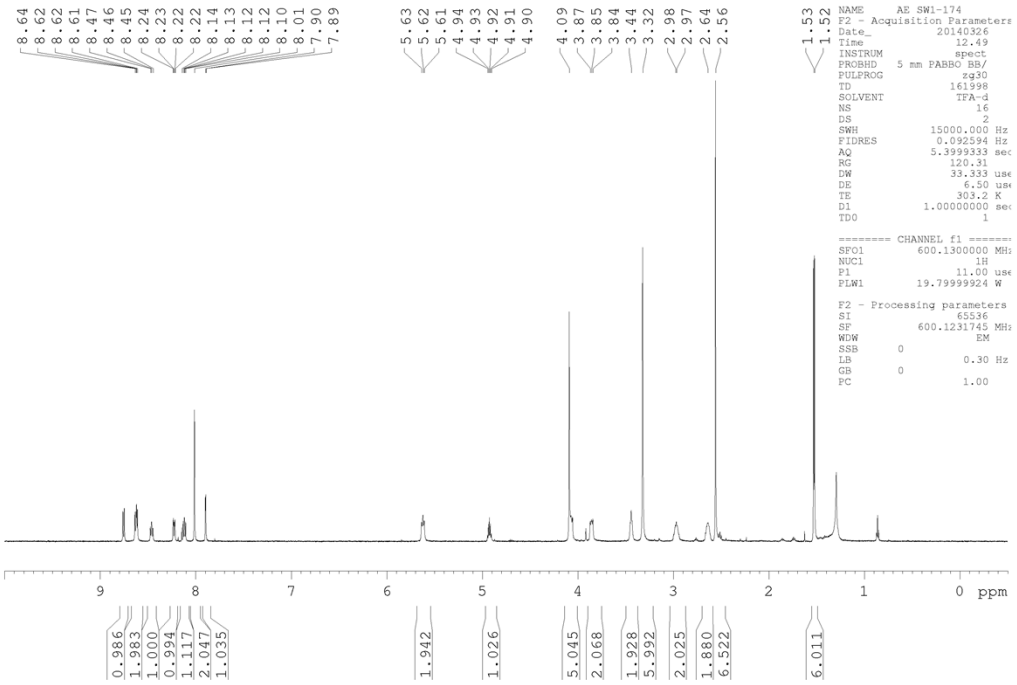
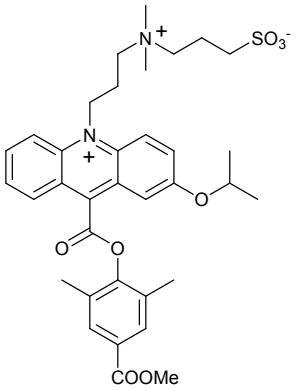


Figure S5b. ^{1}H-NMR of compound **10** in trifluoroacetic acid-d.

Compound 10



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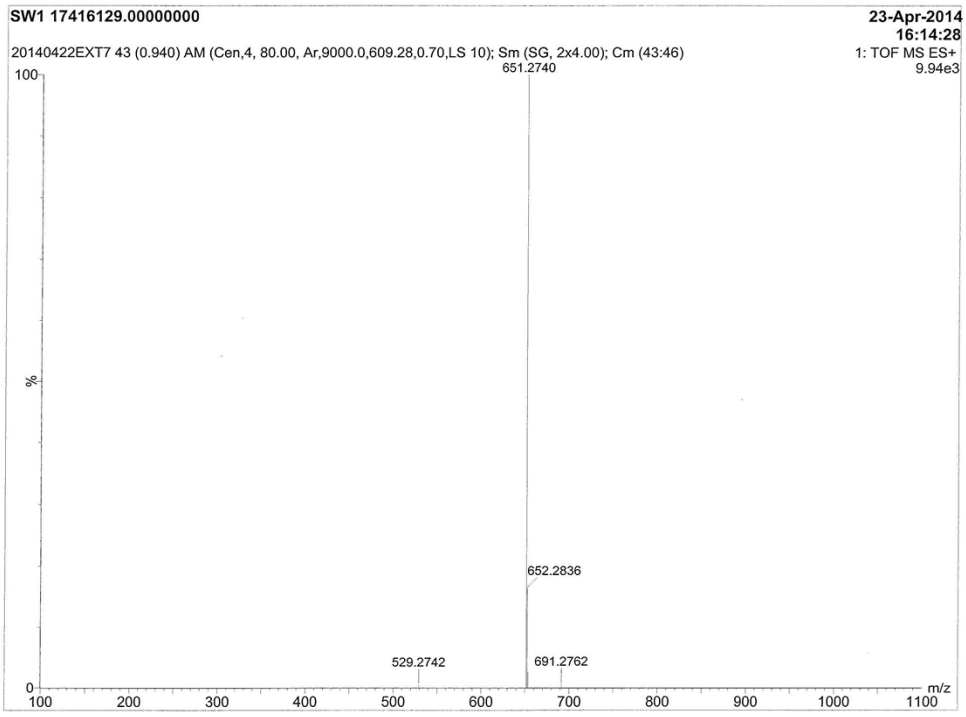
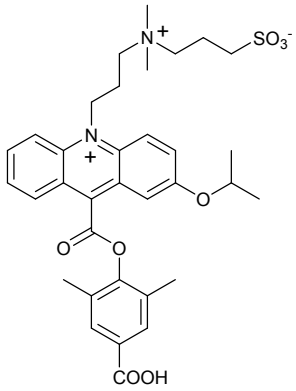


Figure S5c. HRMS of compound **10**.

Compound 3a



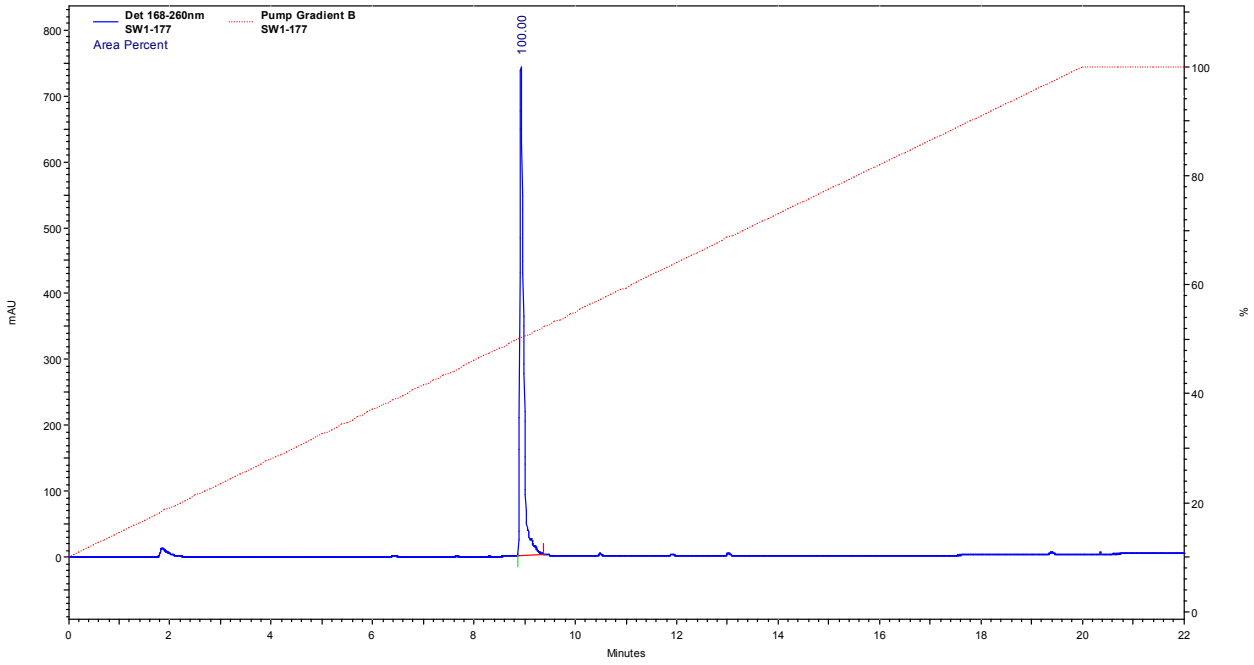


Figure S6a. HPLC trace of compound **3a**.

Compound 3a

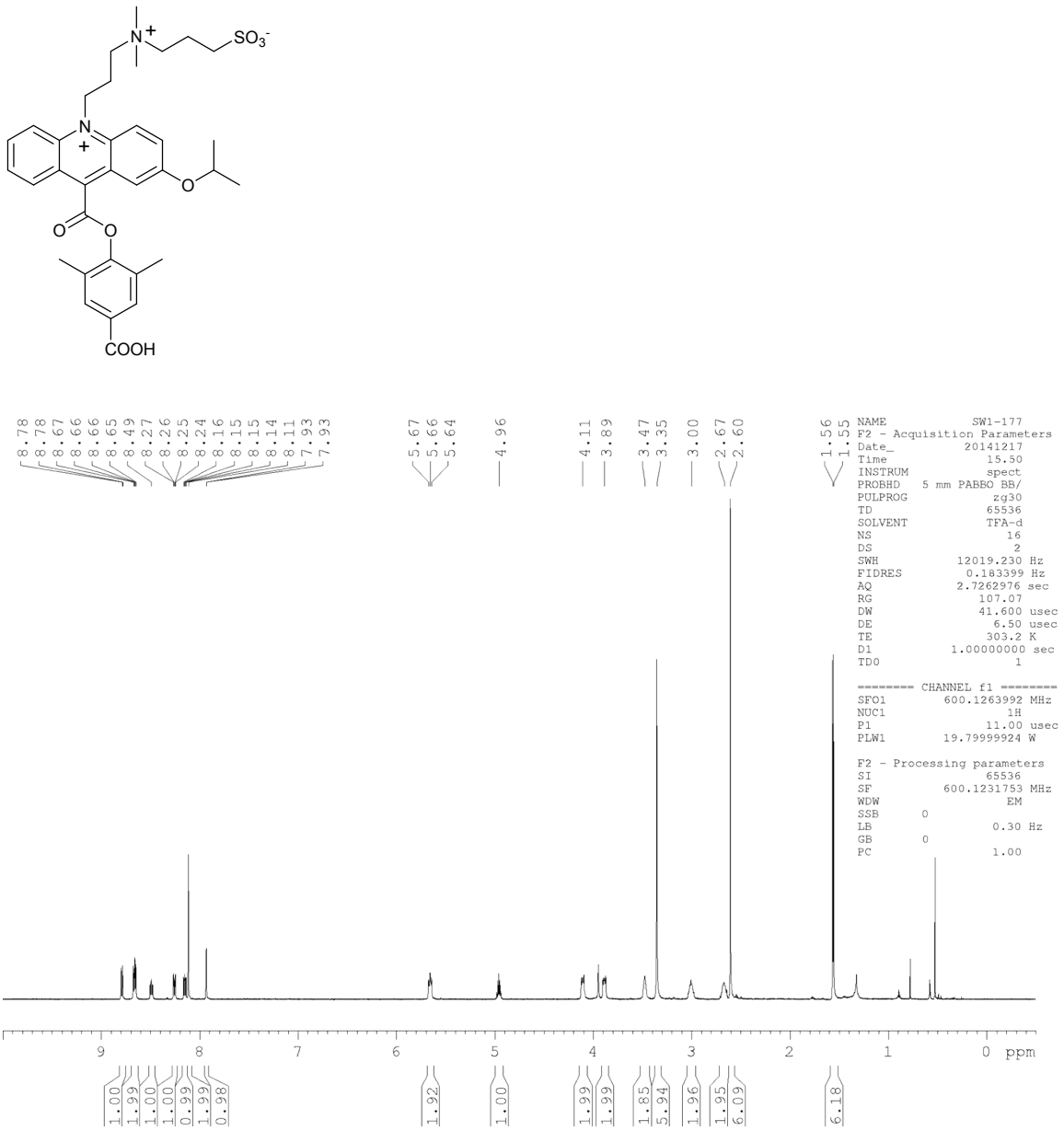
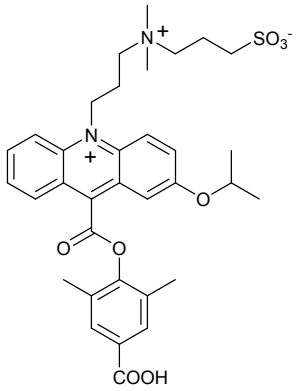


Figure S6b. ^{1}H-NMR of compound **3a** in trifluoroacetic acid-d.

Compound 3a



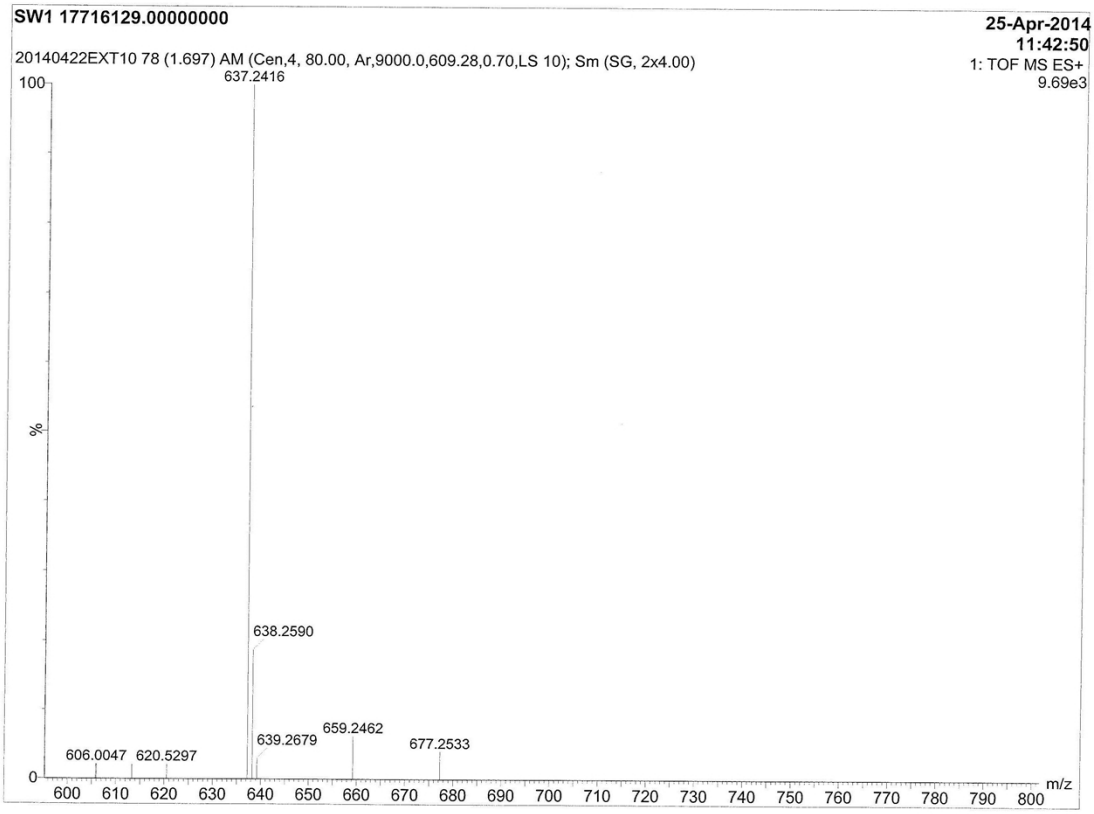
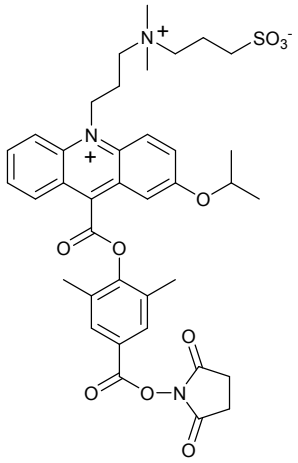


Figure S6c. HRMS of compound **3a**.

Compound 3b



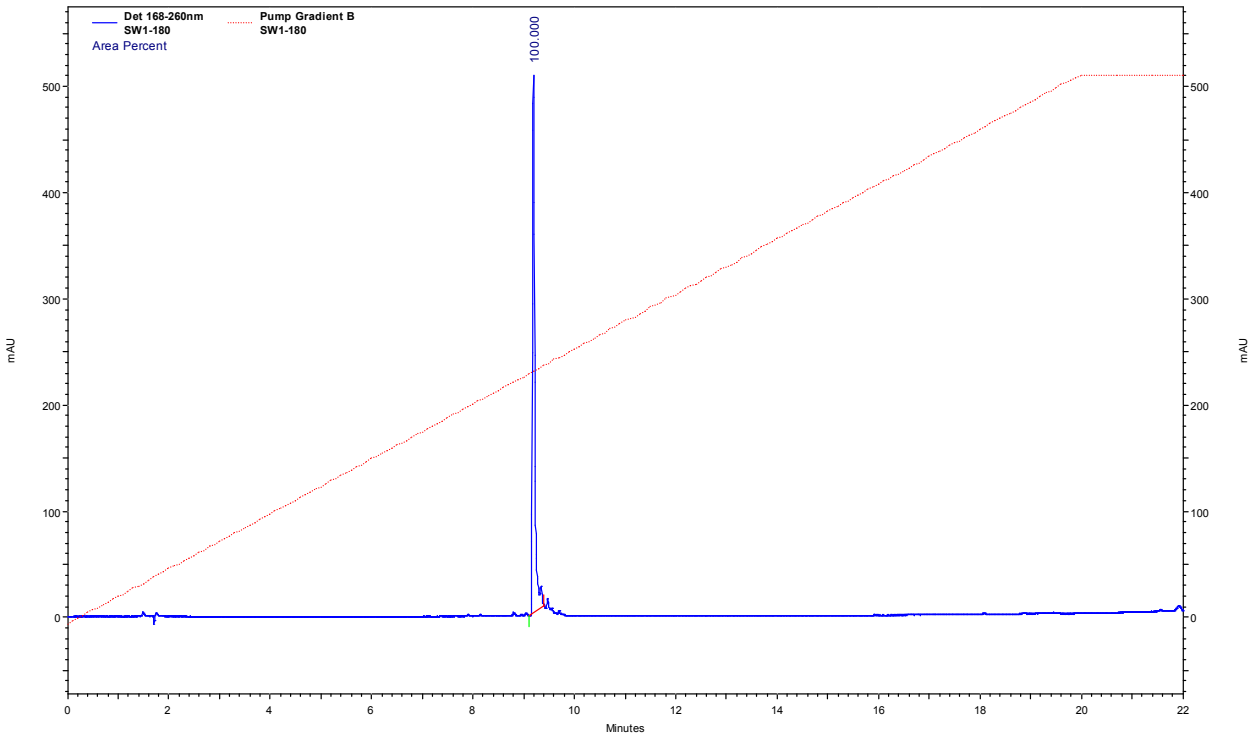
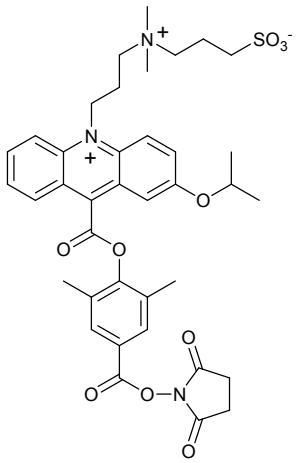


Figure S7a. HPLC trace of compound **3b**.

Compound 3b



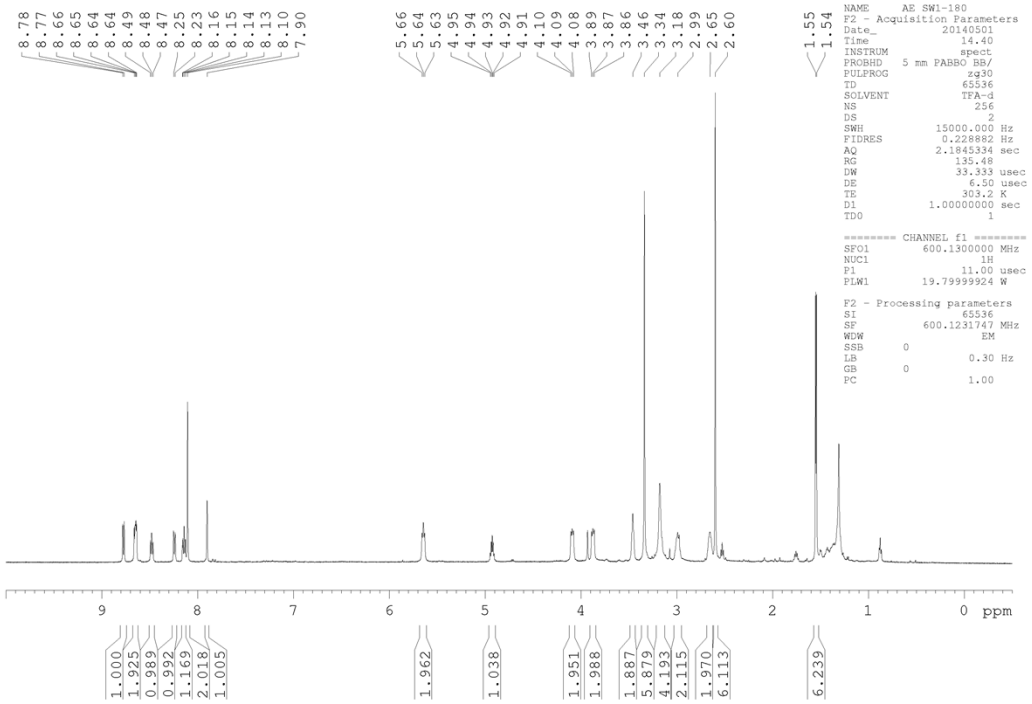
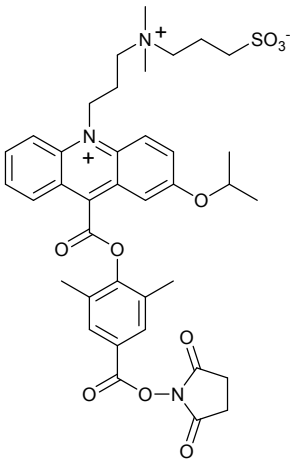


Figure S7b. ^{1}H-NMR of compound **3b** in trifluoroacetic acid-d.

Compound 3b



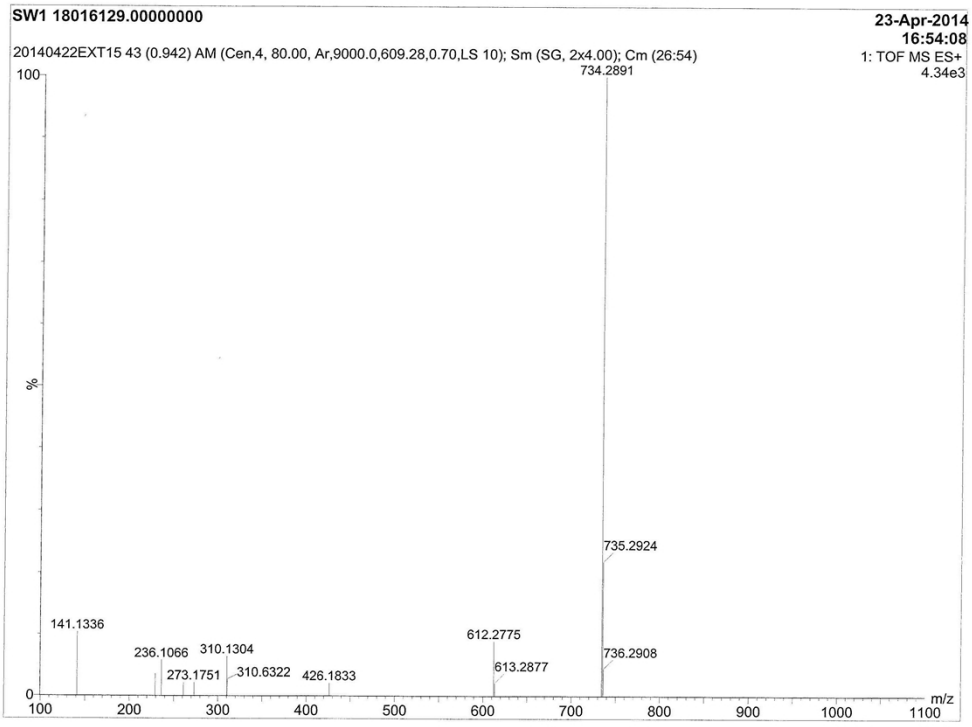
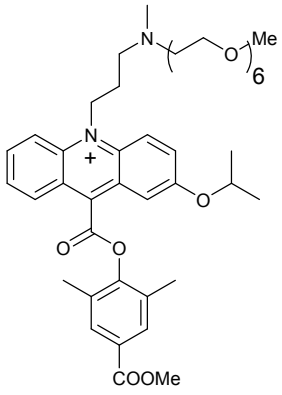


Figure S7c. HRMS of compound **3b**.

Compound 11



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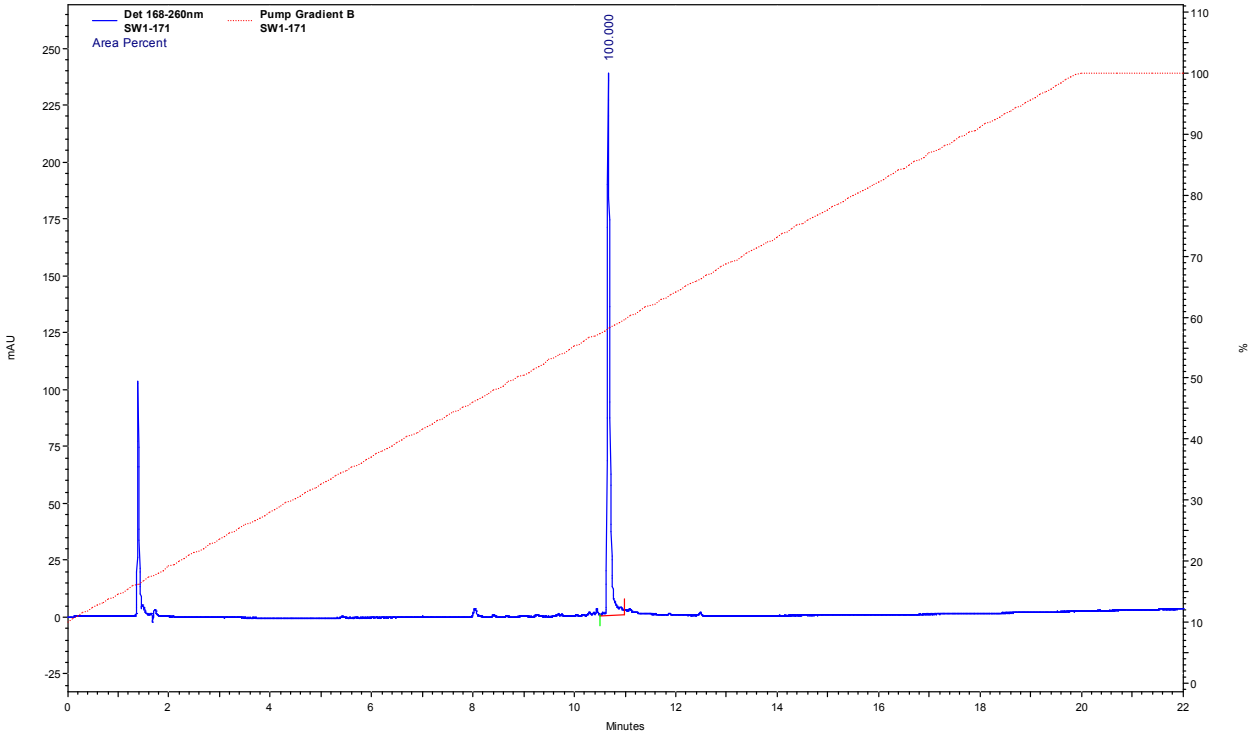
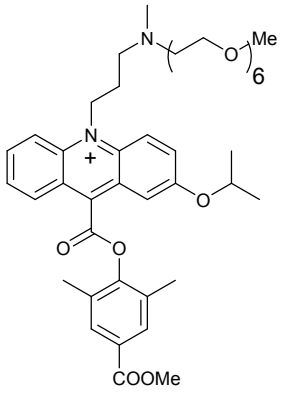


Figure S8a. HPLC trace of compound **11**.

Compound 11



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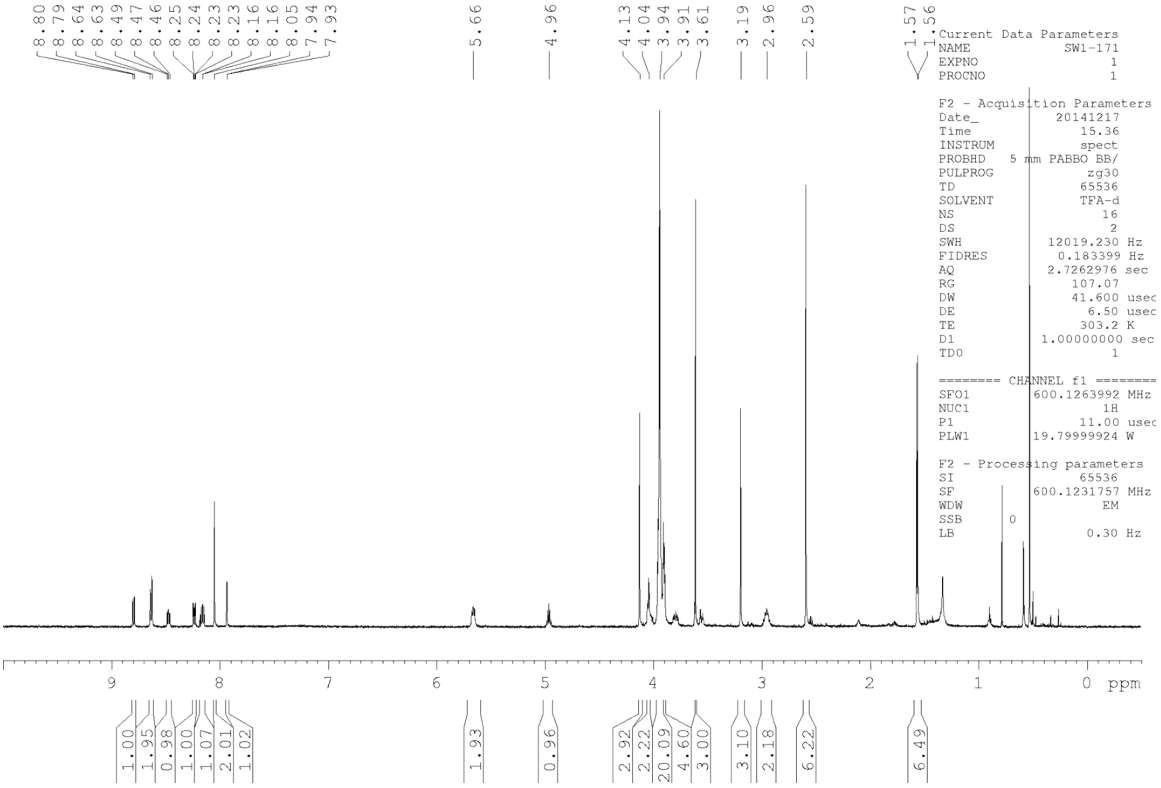
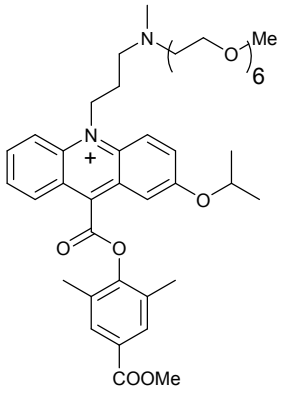


Figure S8b. ^{1}H-NMR of compound **11** in trifluoroacetic acid-d.

Compound 11



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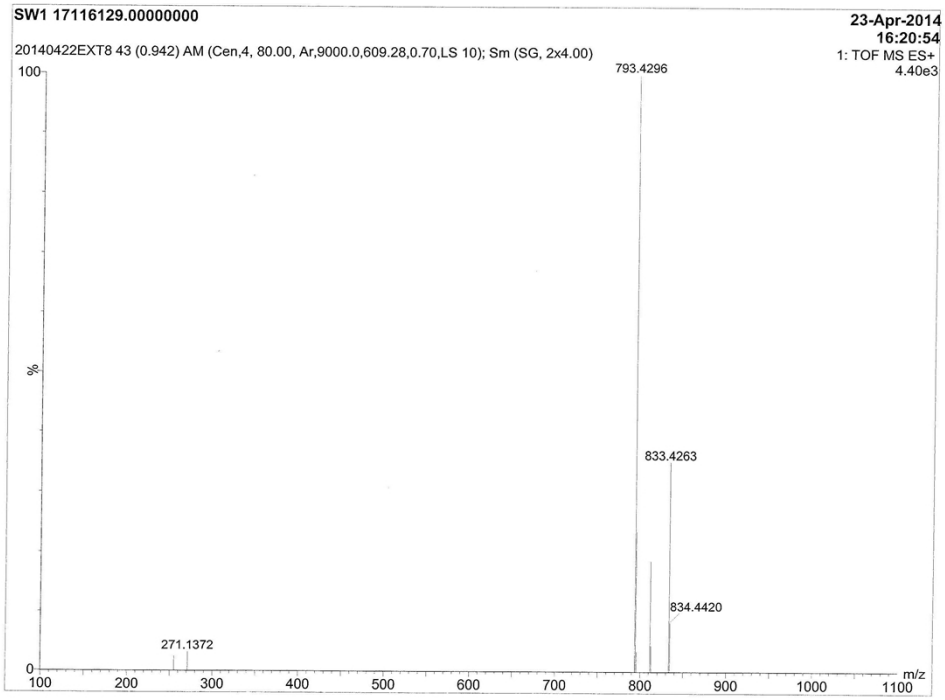
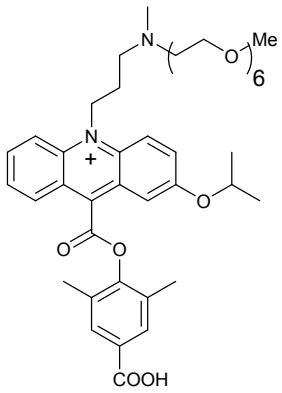


Figure S8c. HRMS of compound **11**.

Compound 4a



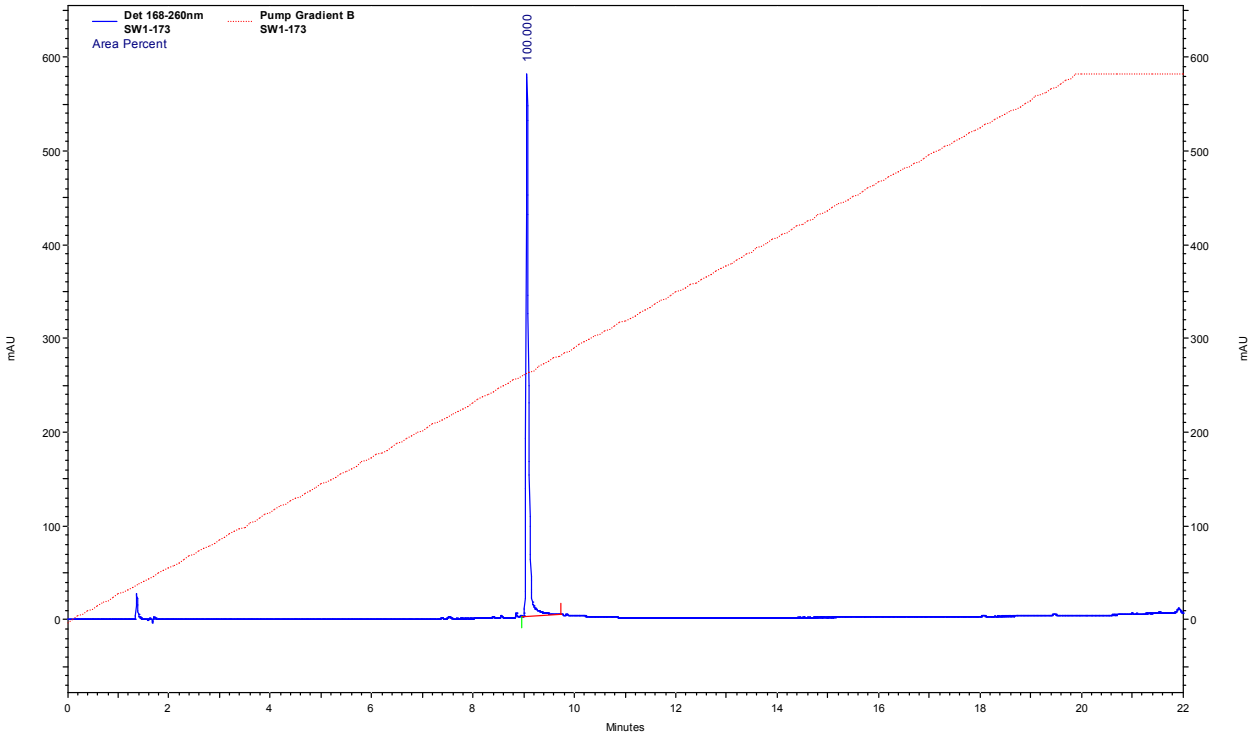
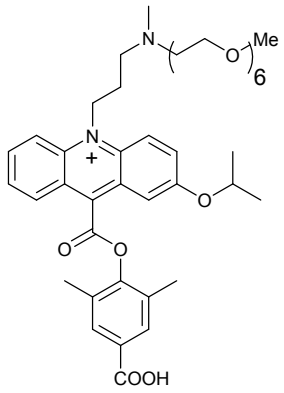


Figure S9a. HPLC trace of compound **4a**.

Compound 4a



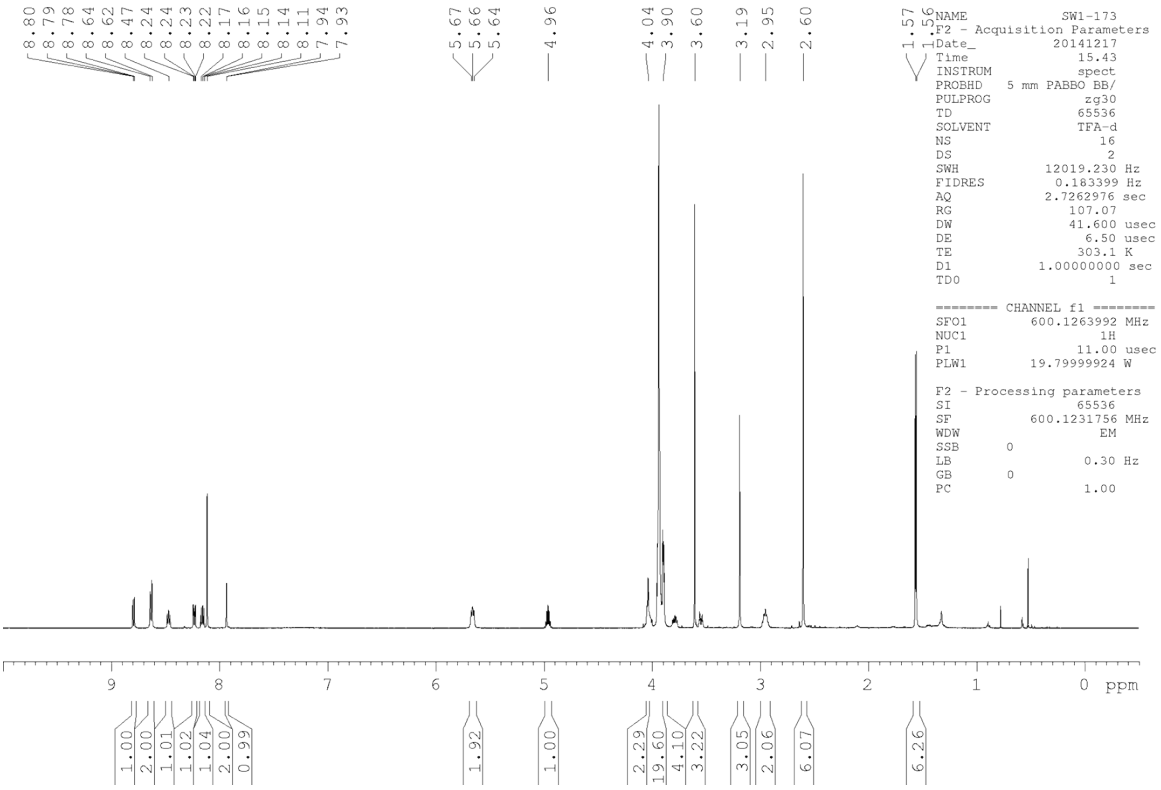
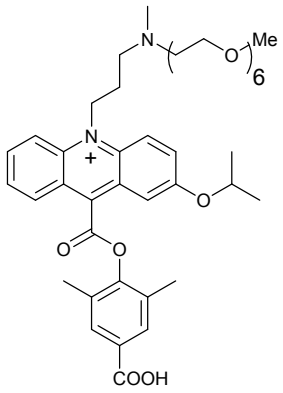


Figure S9b. ^{1}H-NMR of compound **4a** in trifluoroacetic acid-d.

Compound 4a



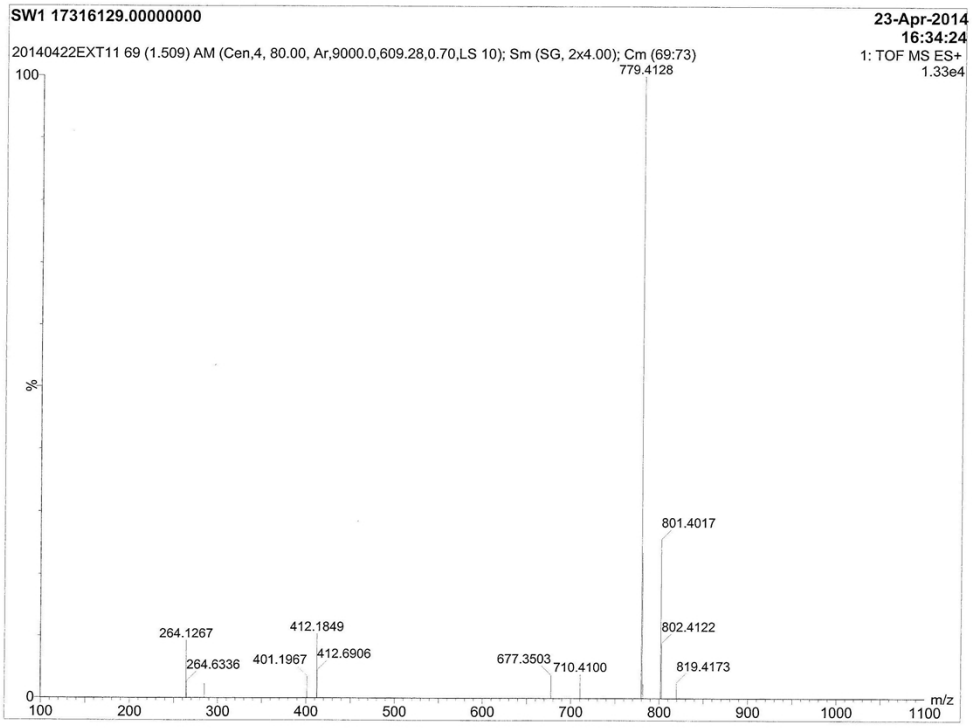
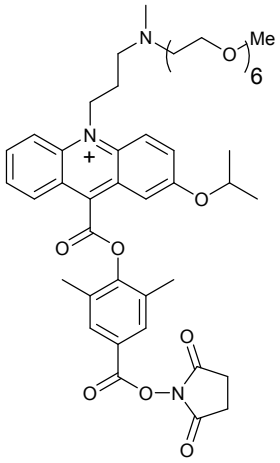


Figure S9c. HRMS of compound **4a**.

Compound 4b



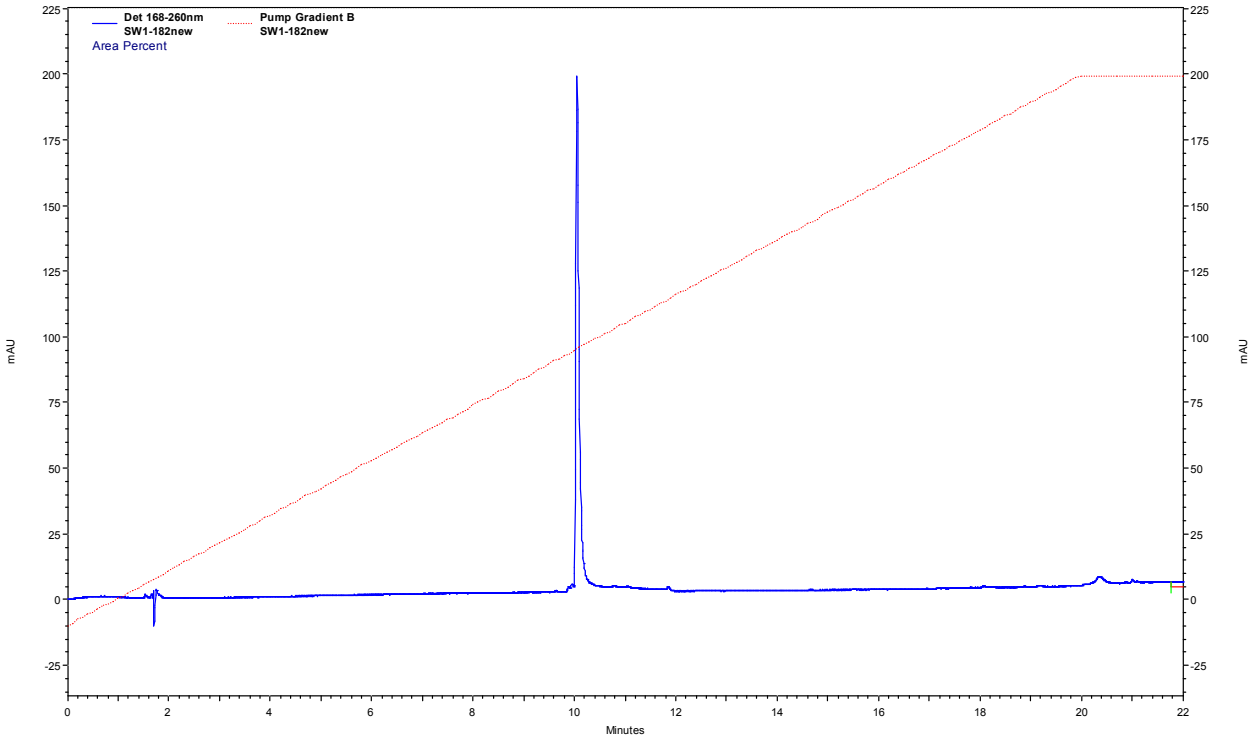
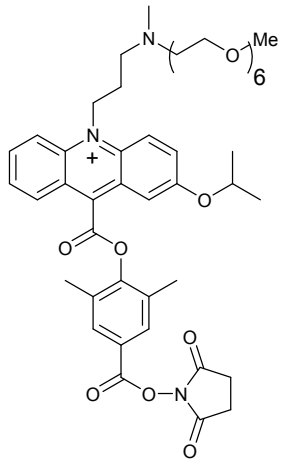


Figure S10a. HPLC trace of compound **4b**.

Compound 4b



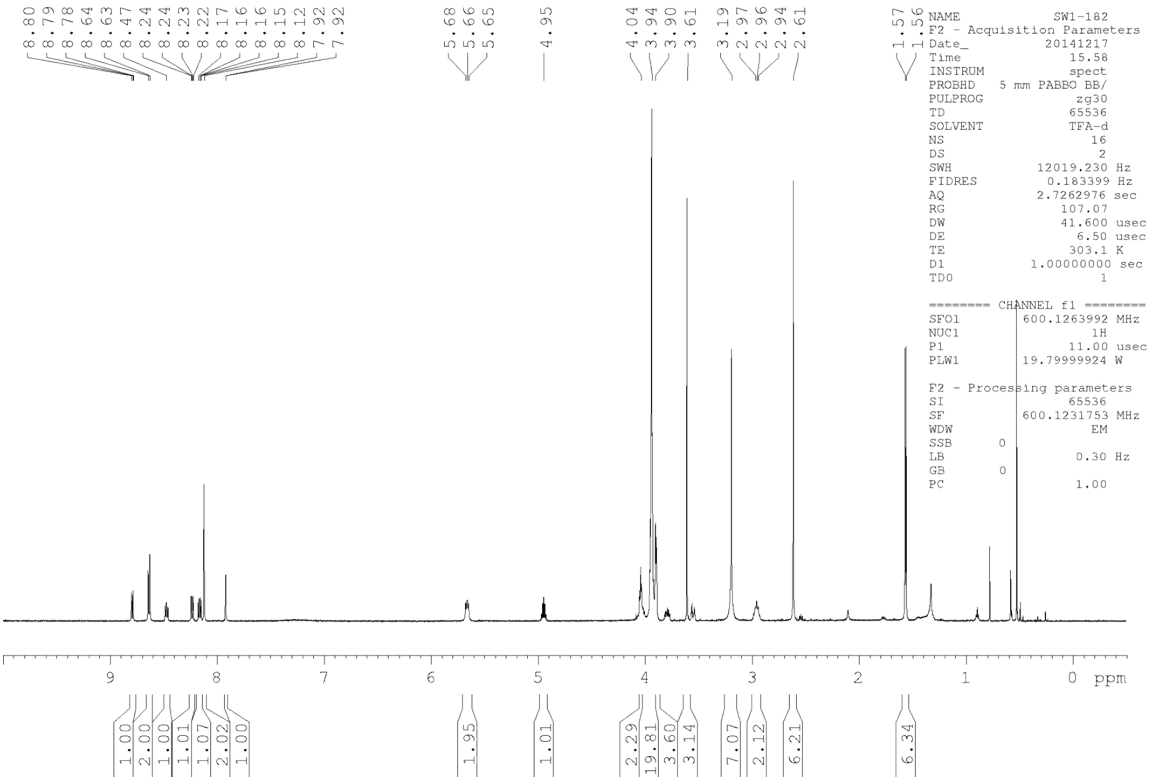
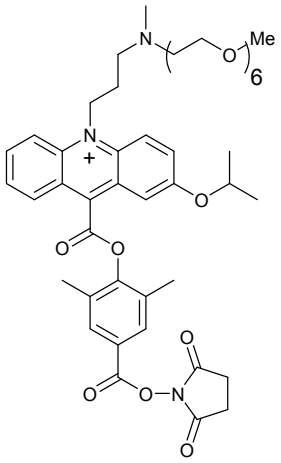


Figure S10b. ^{1}H-NMR of compound **4b** in trifluoroacetic acid-d.

Compound 4b



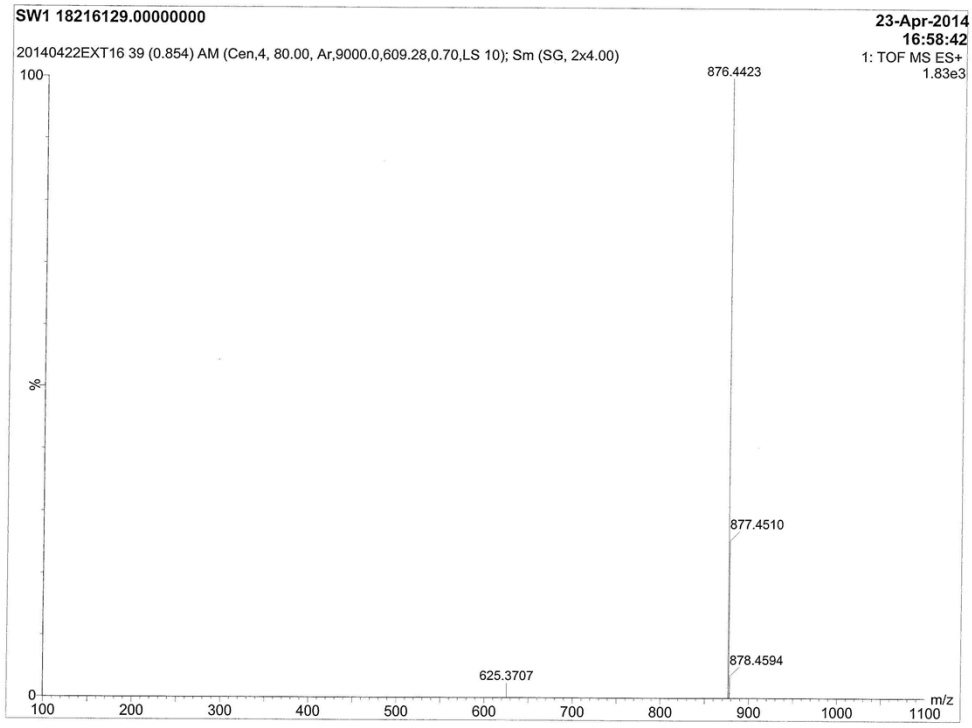
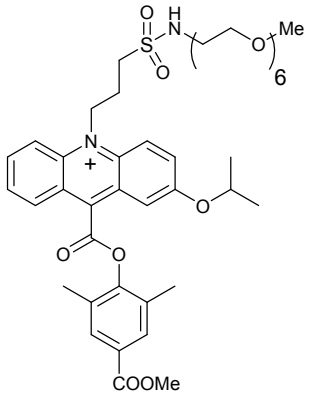


Figure S10c. HRMS of compound **4b**.

Compound 13



13

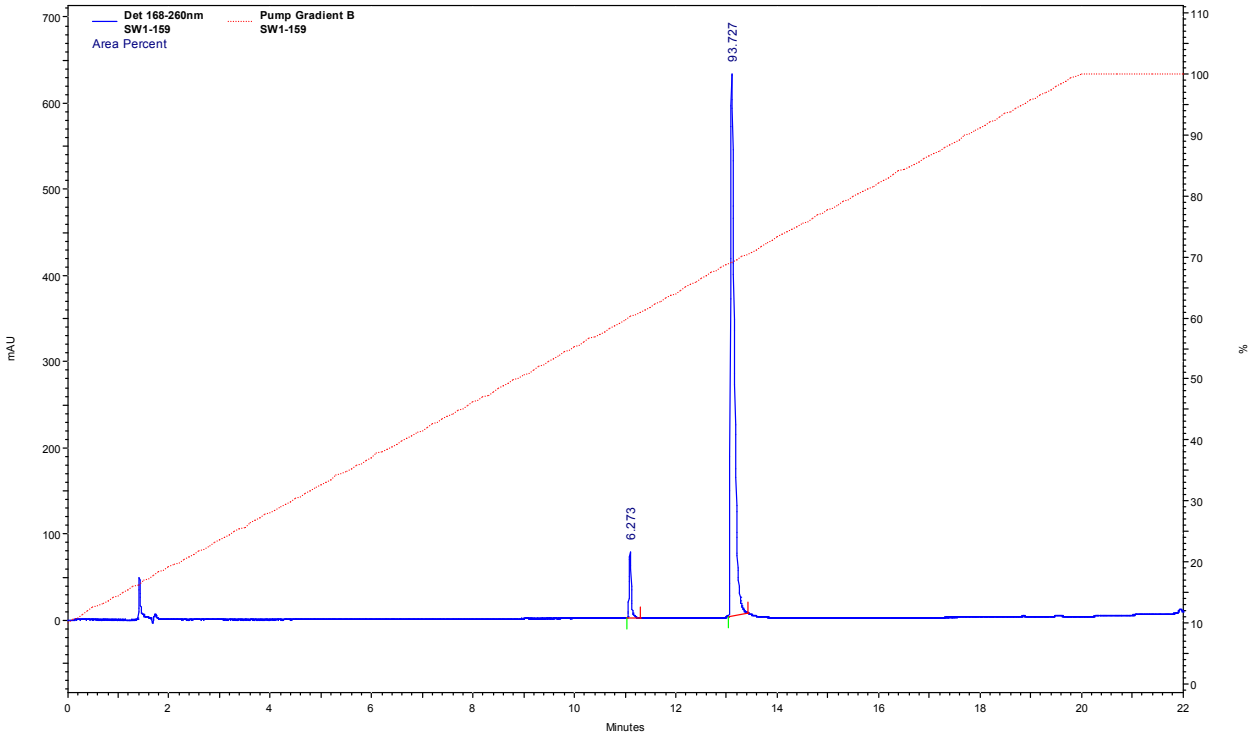
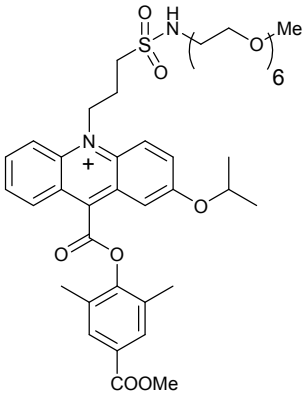


Figure S11a. HPLC trace of compound **13**.

Compound 13



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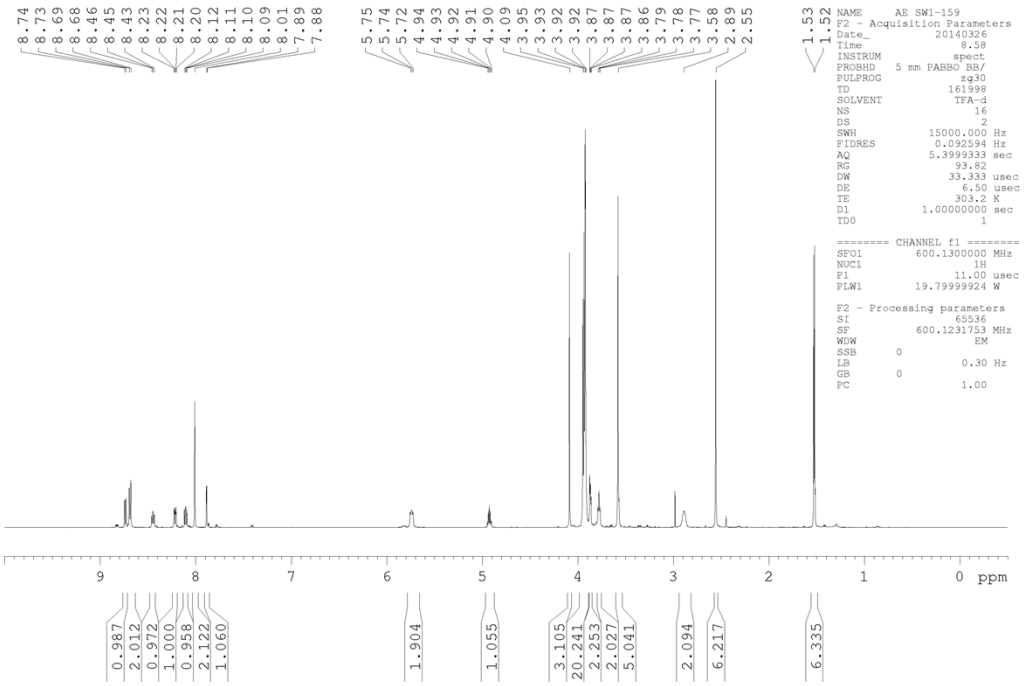
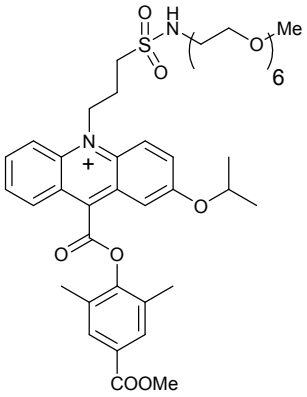


Figure S11b. ^{1}H-NMR of compound **13** in trifluoroacetic acid-d.

Compound 13



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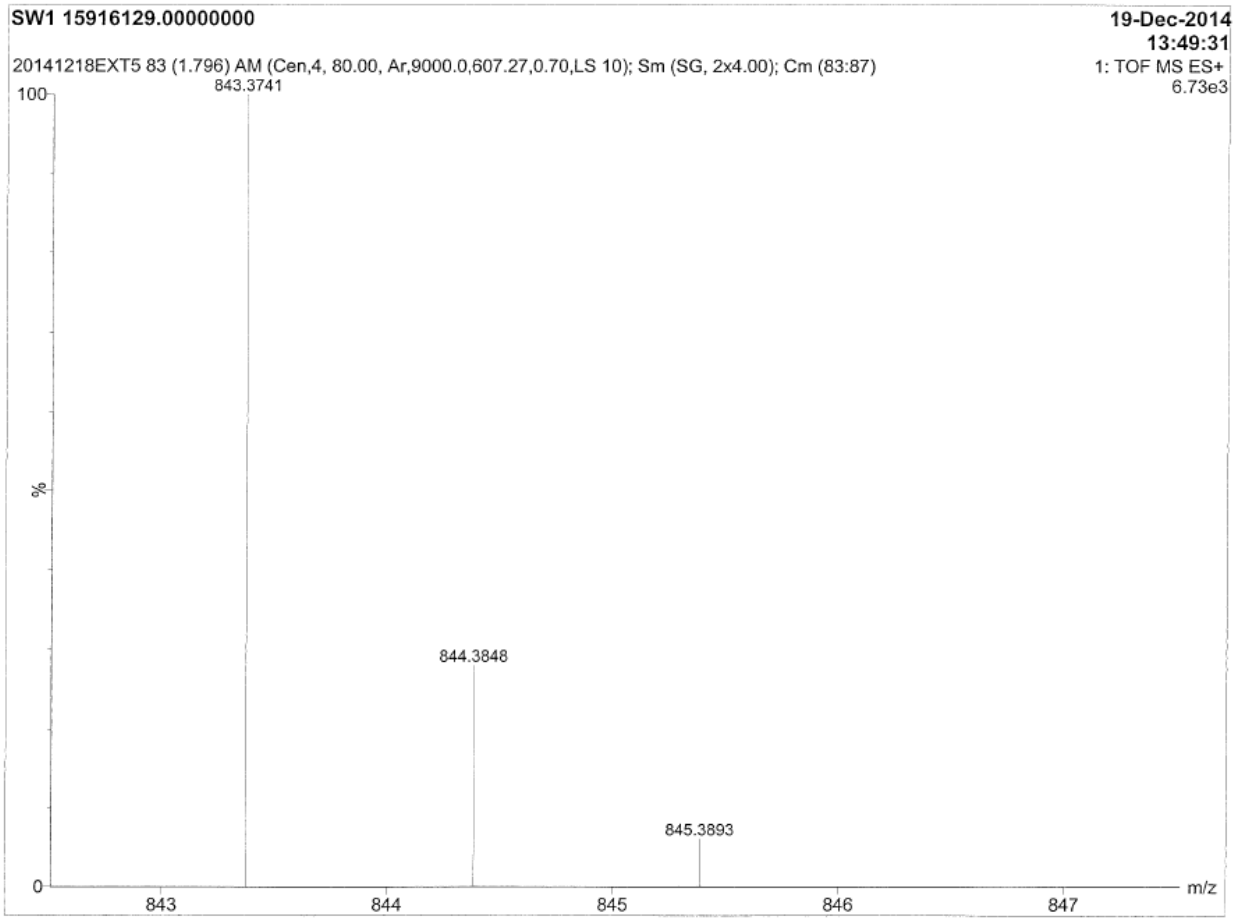
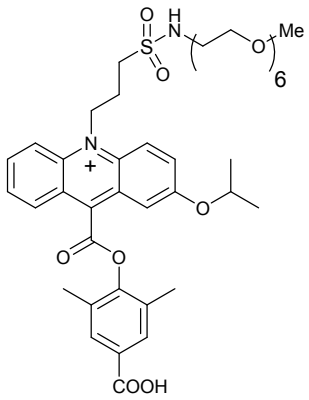


Figure S11c. HRMS of compound **13**.

Compound 5a



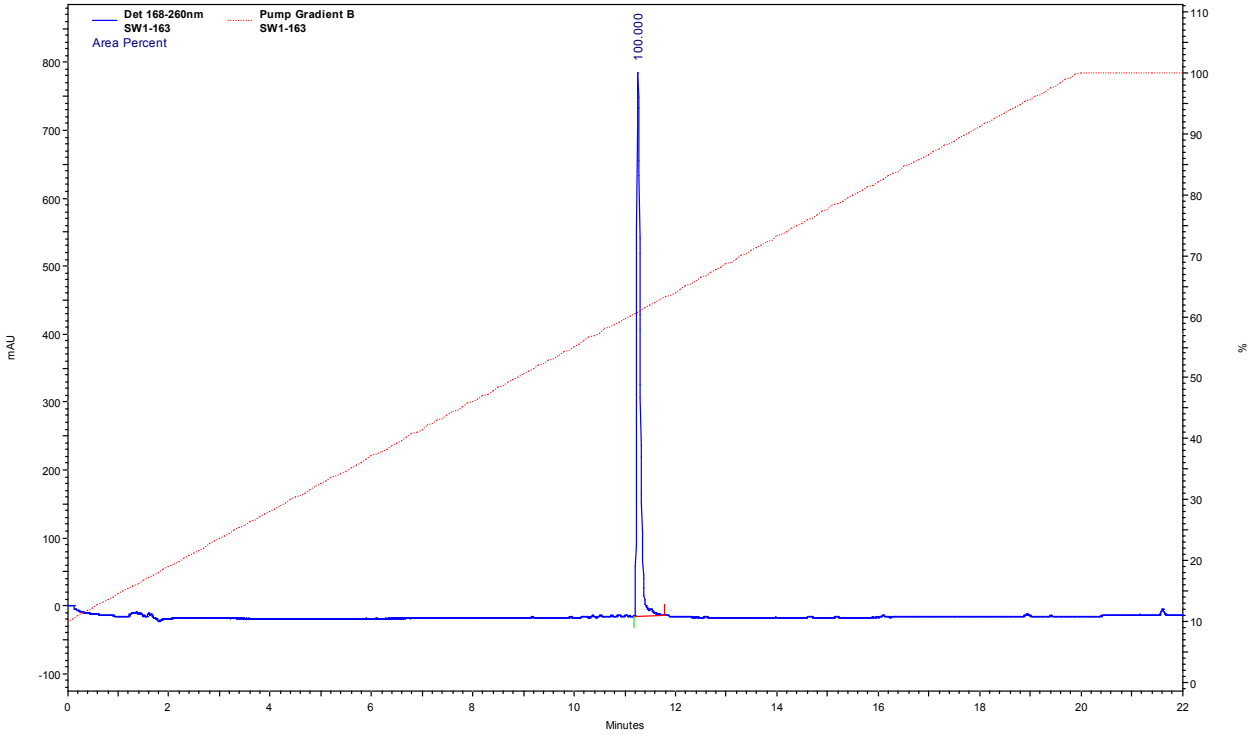
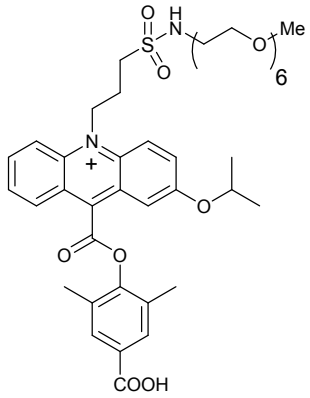


Figure S12a. HPLC trace of compound **5a**.

Compound 5a



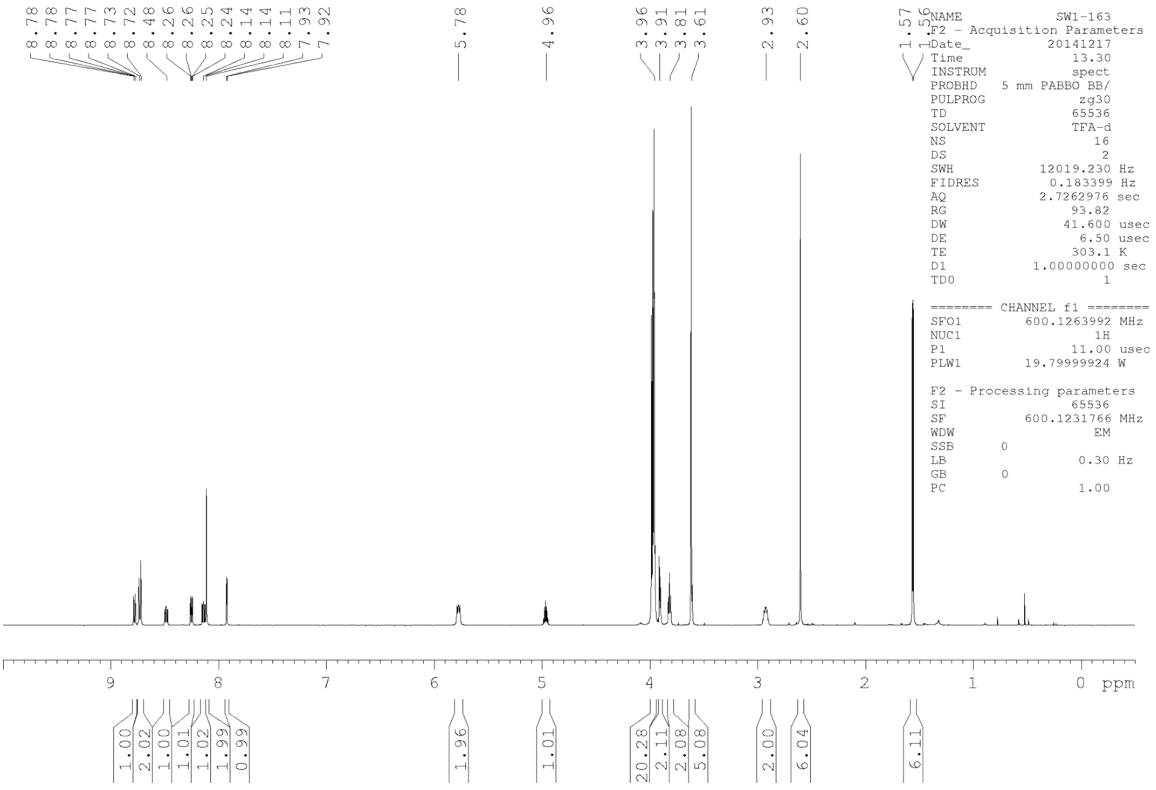
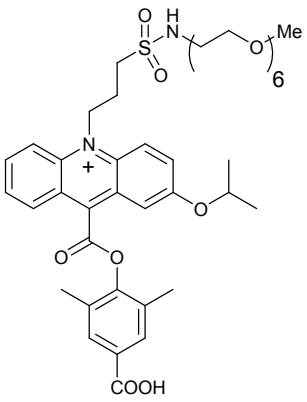


Figure S12b. ^{1}H-NMR of compound **5a** in trifluoroacetic acid-d.

Compound 5a



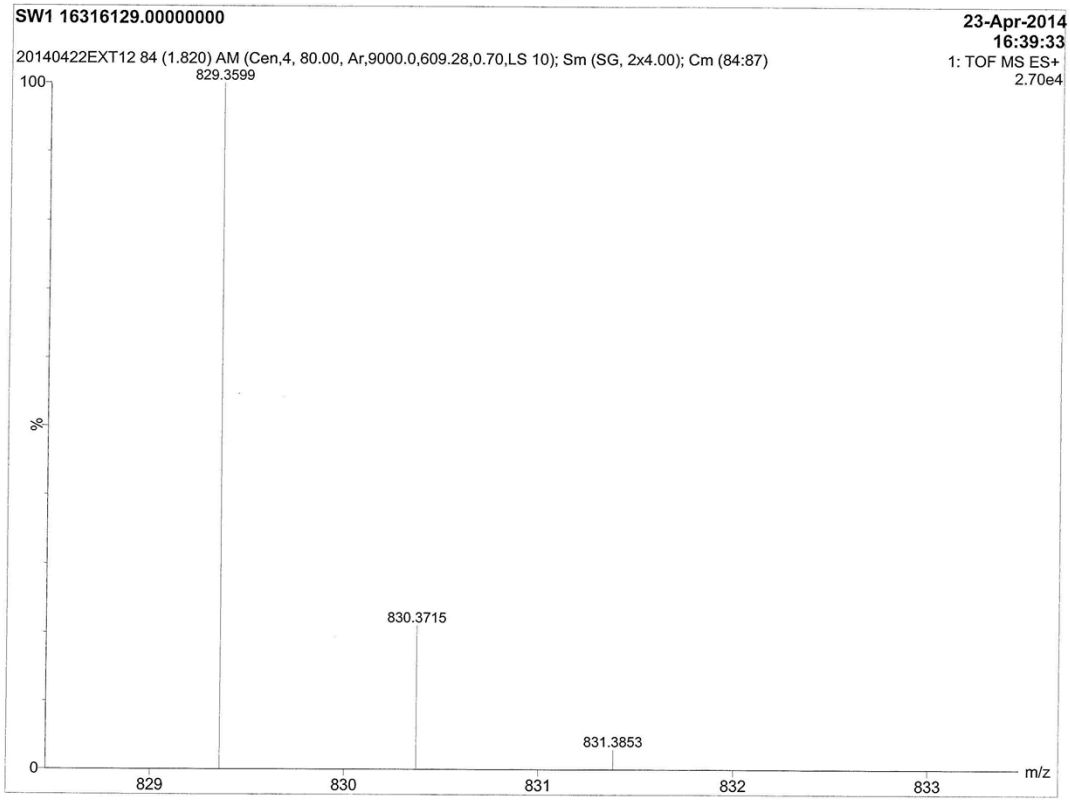
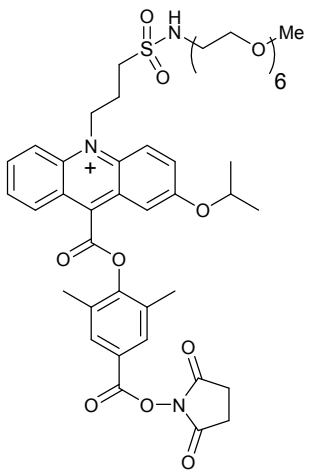


Figure S12c. HRMS of compound **5a**.

Compound 5b



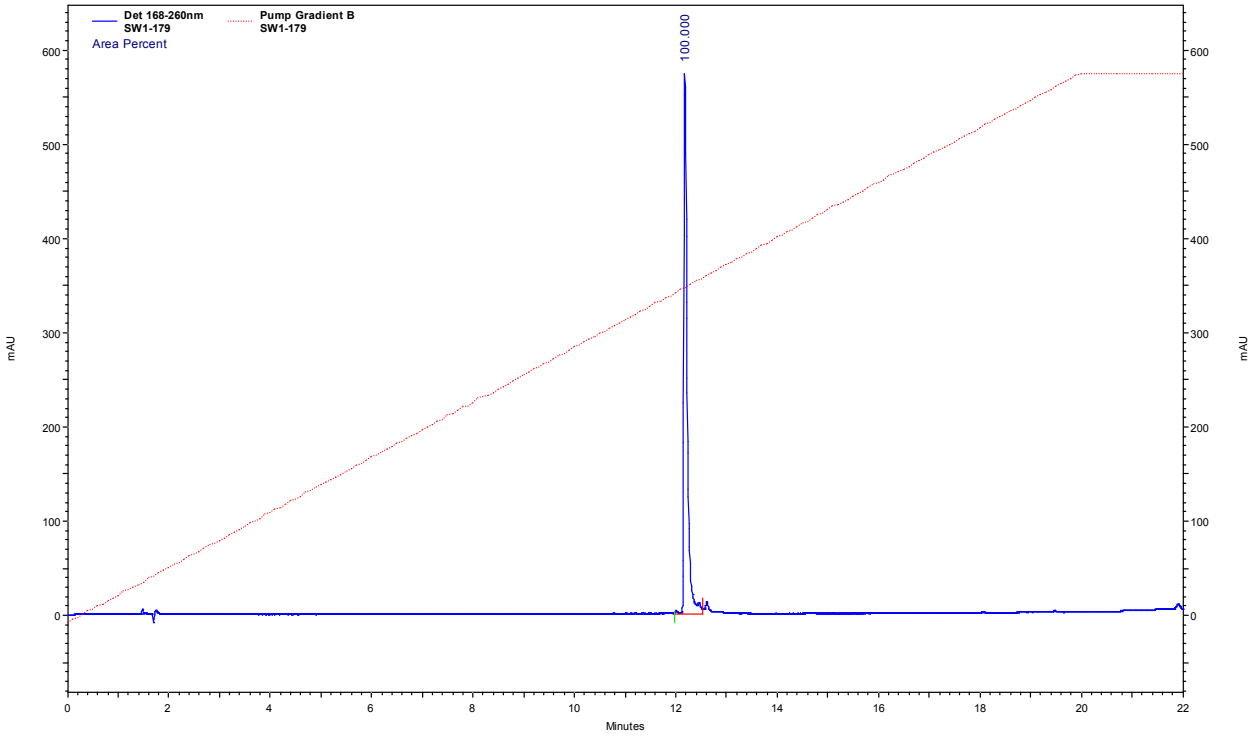
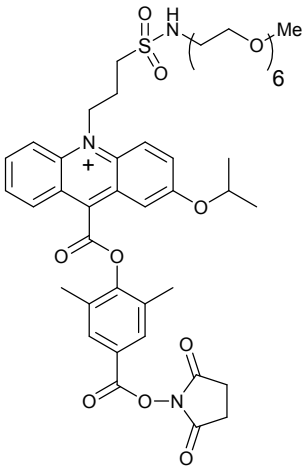


Figure S13a. HPLC trace of compound **5b**.

Compound 5b



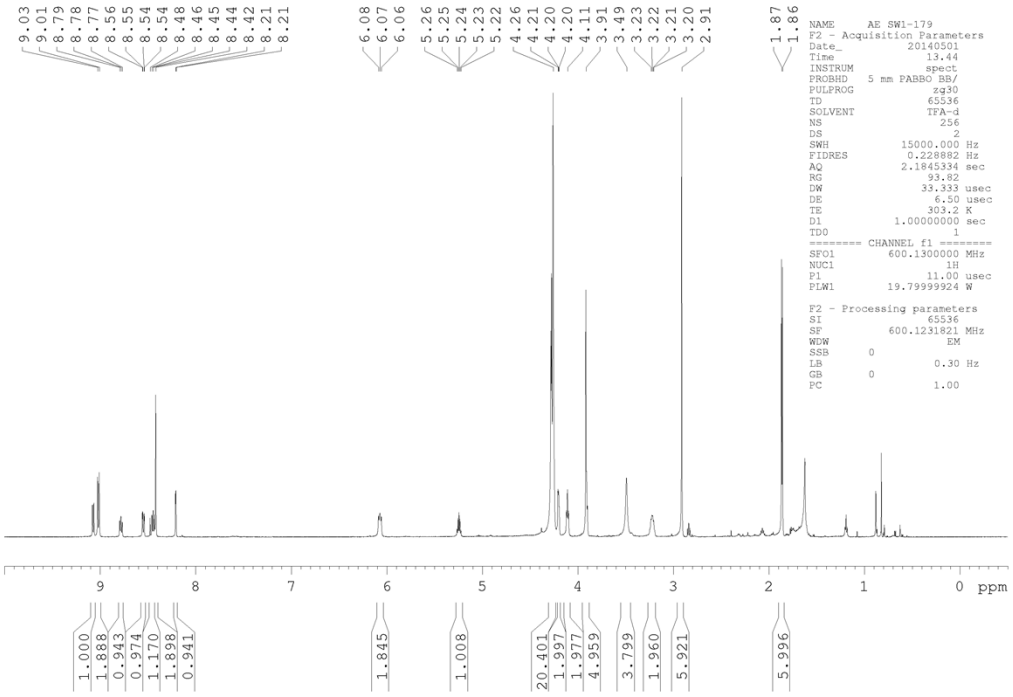
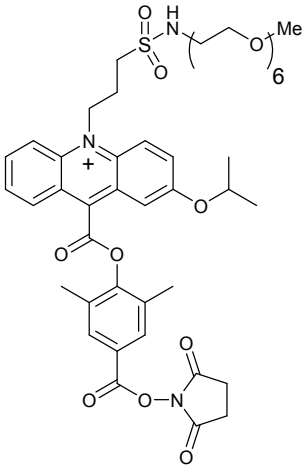


Figure S13b. ^{1}H-NMR of compound **5b** in trifluoroacetic acid-d.

Compound 5b



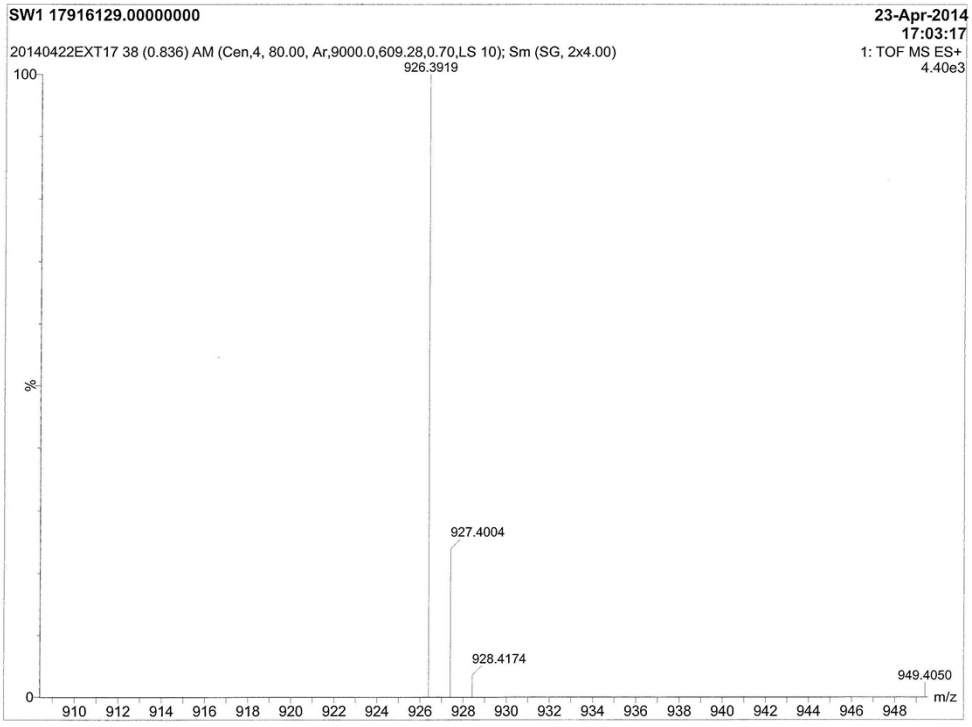


Figure S13c. HRMS of compound **5b**.

Chemiluminescence emission spectra of acridinium ester labels **1a**-**6a**

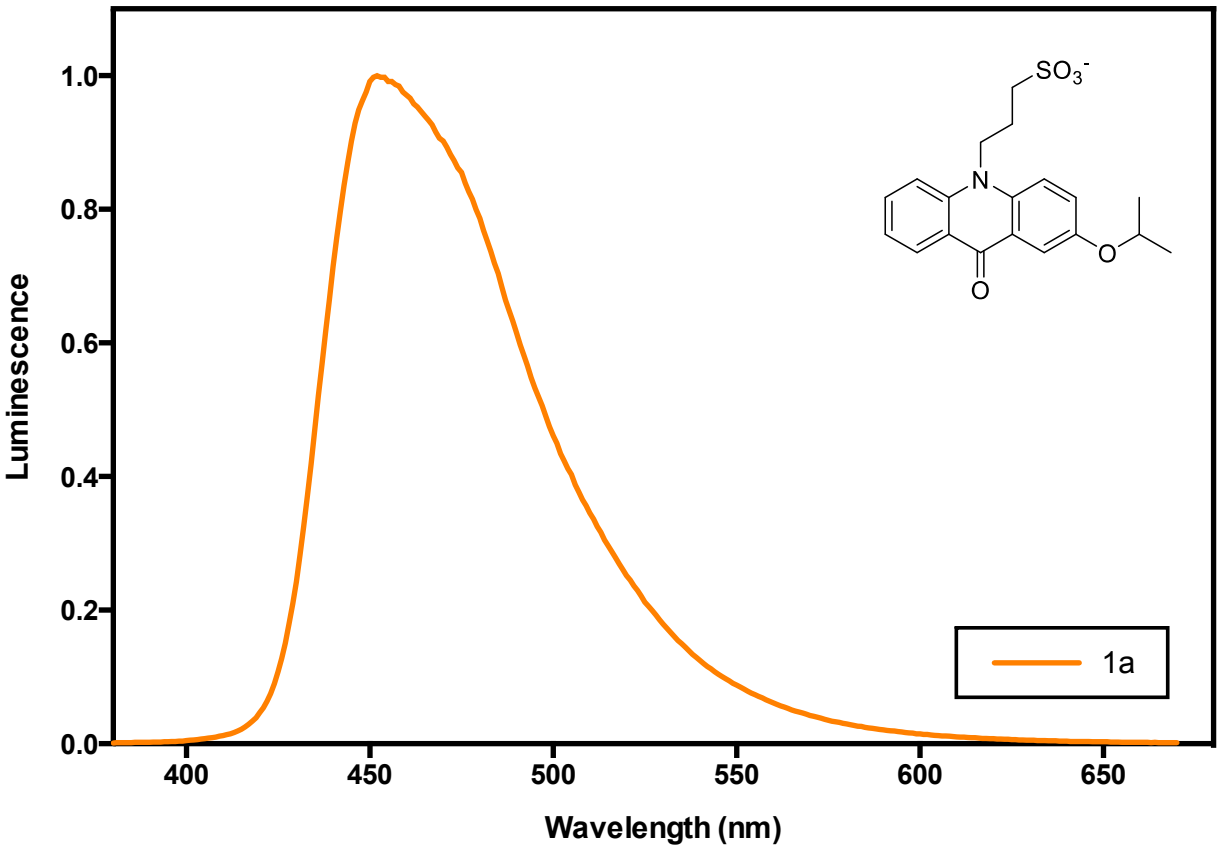


Figure S14a. Chemiluminescence emission spectrum of compound **1a.**

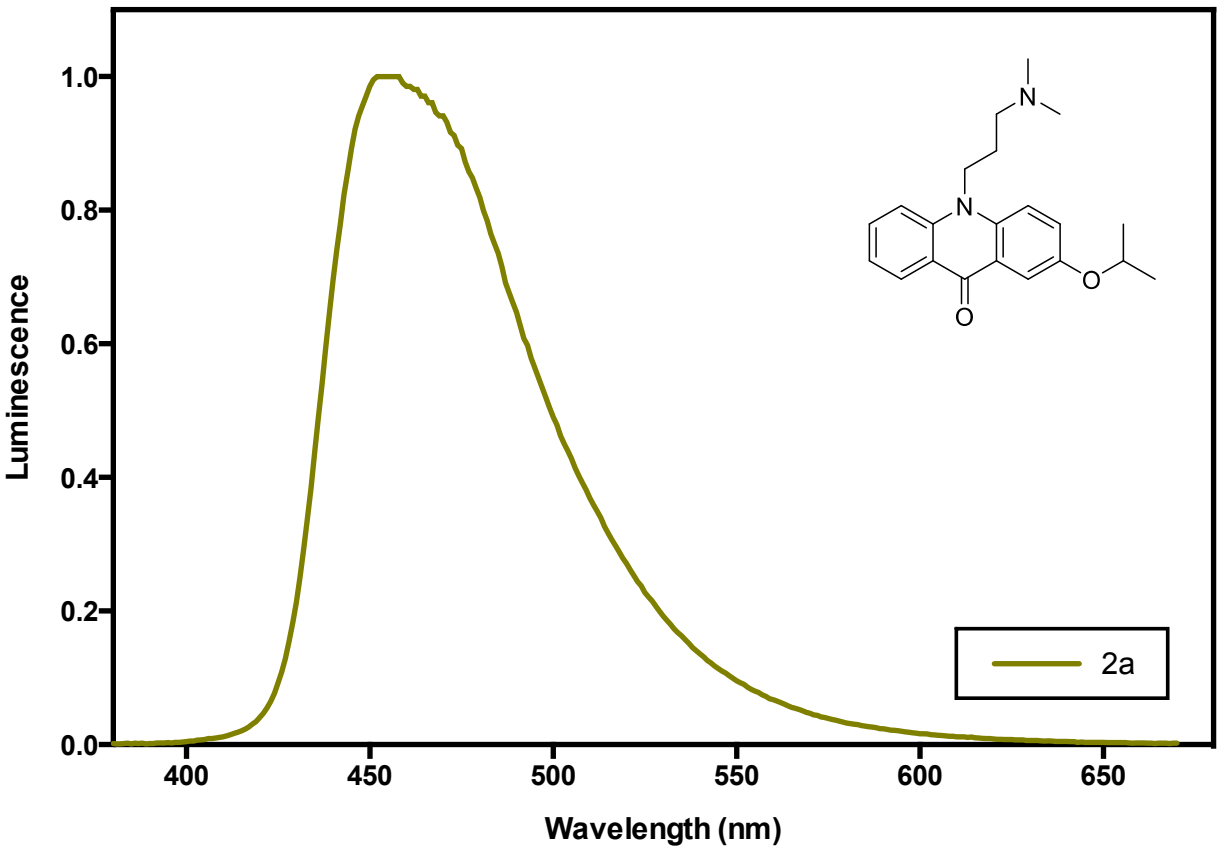


Figure S14b. Chemiluminescence emission spectrum of compound **2a.**

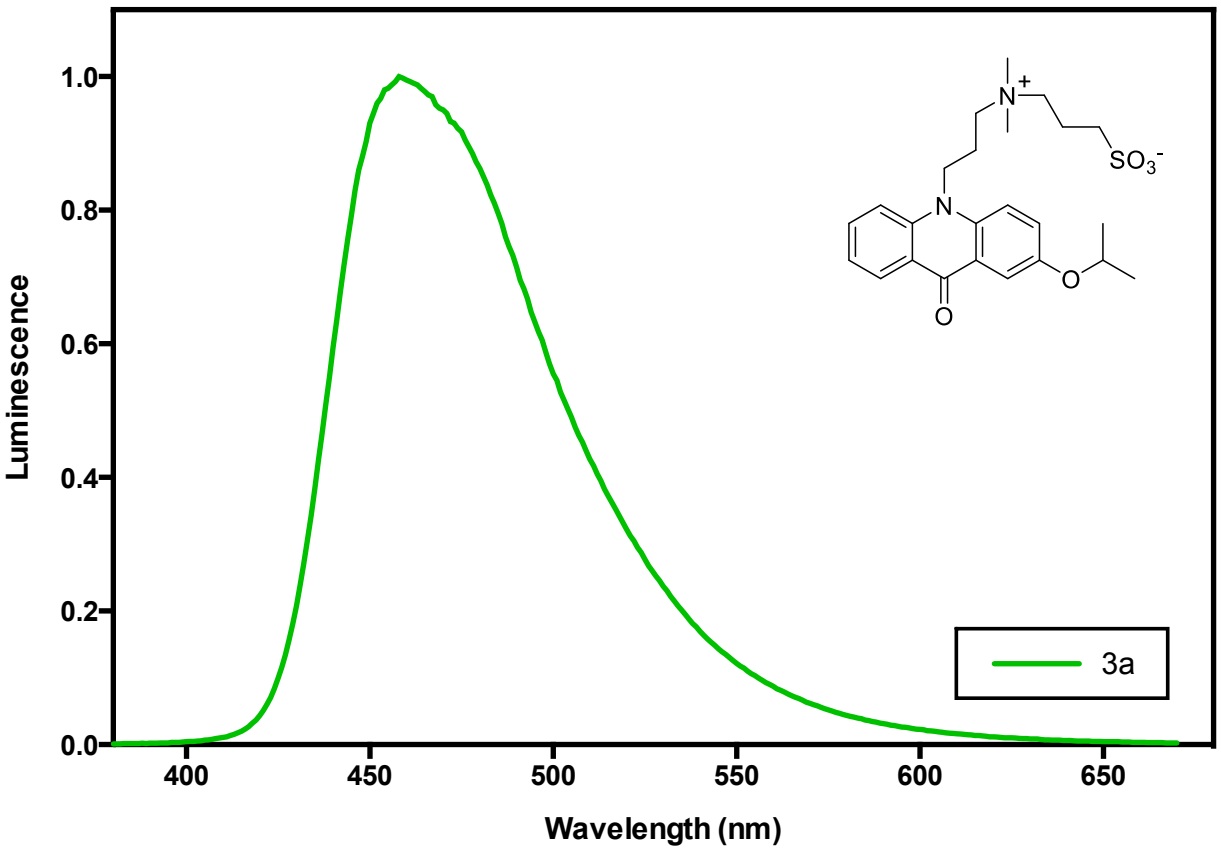


Figure S14c. Chemiluminescence emission spectrum of compound **3a.**

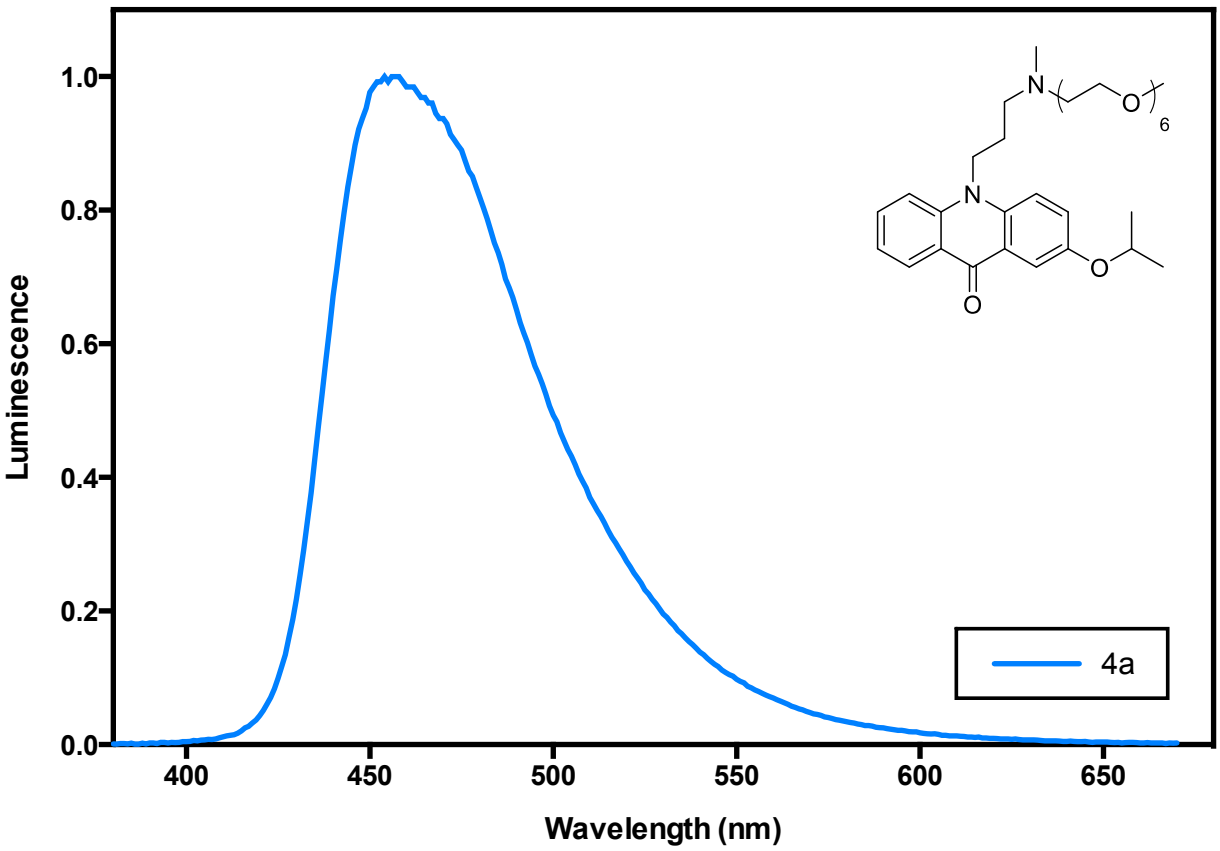


Figure S14d. Chemiluminescence emission spectrum of compound **4a.**

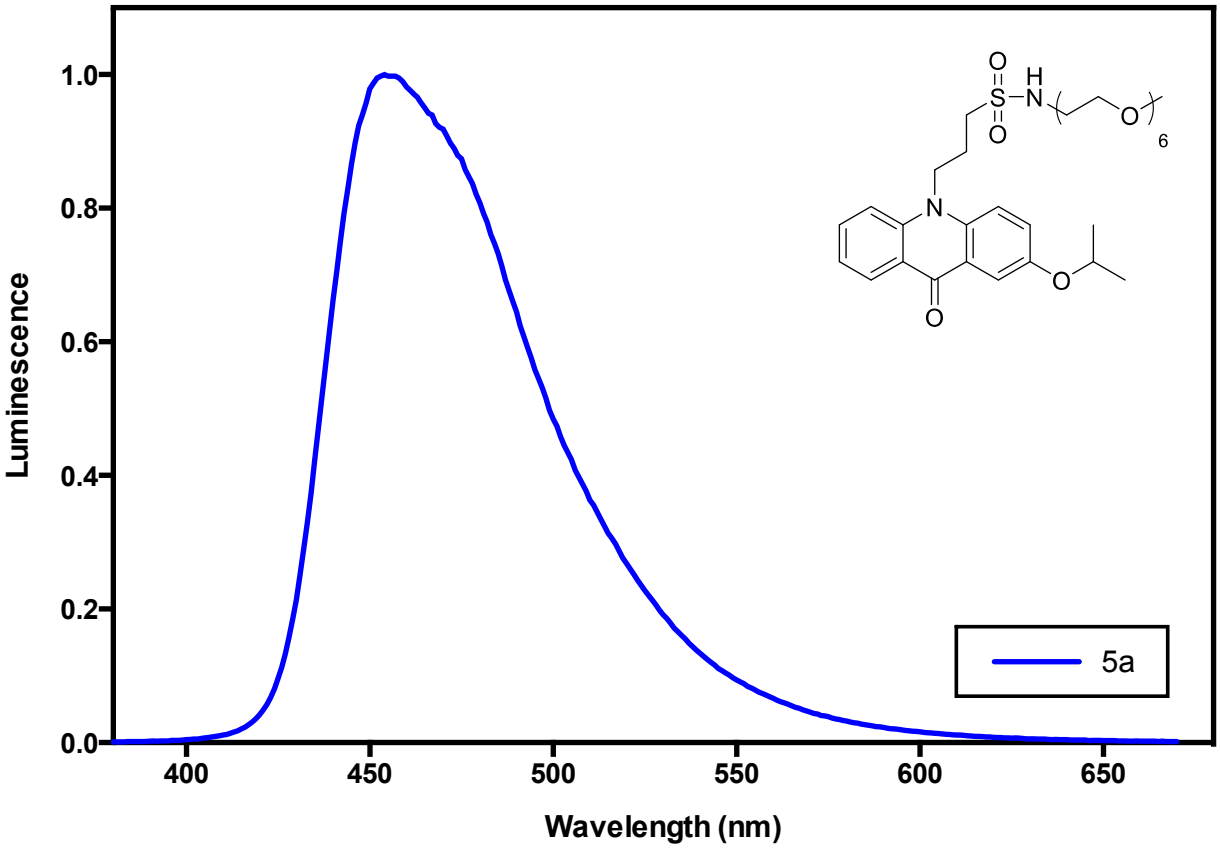


Figure S14e. Chemiluminescence emission spectrum of compound **5a.**

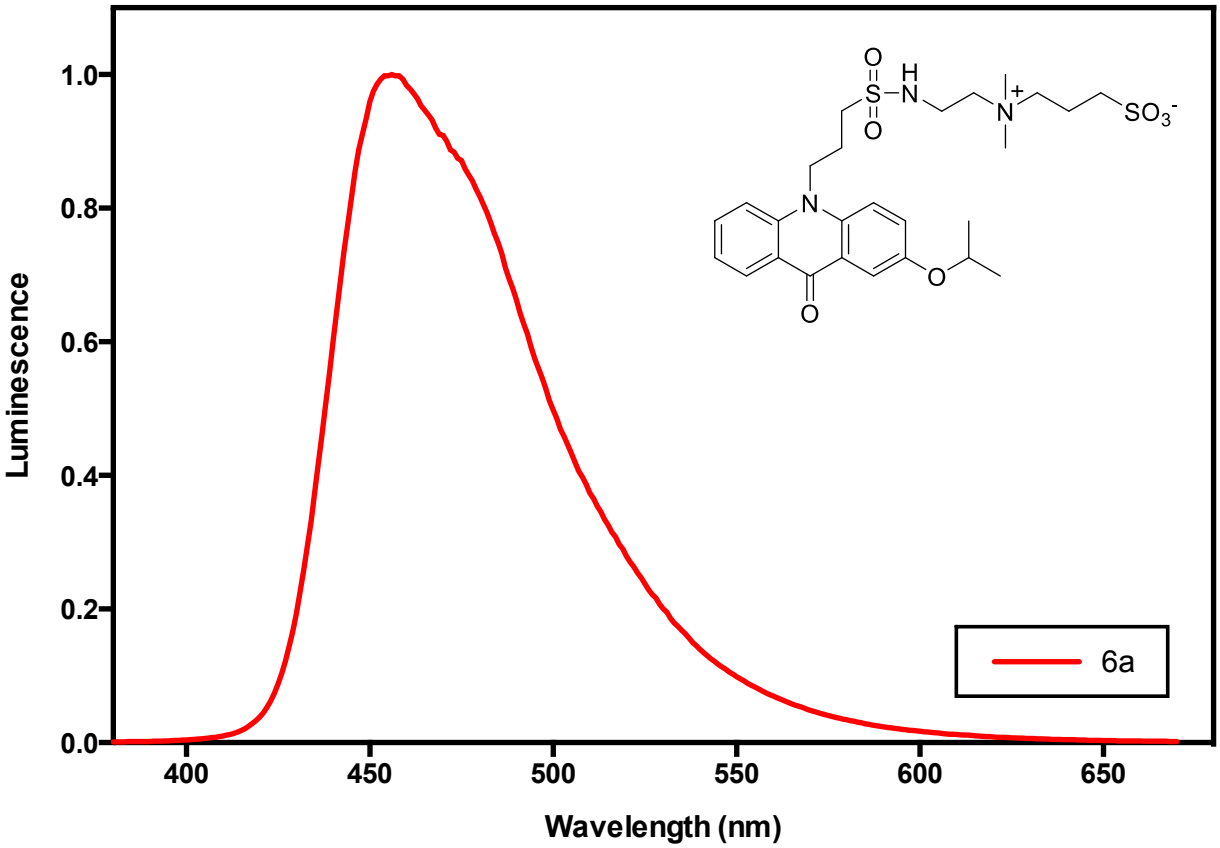


Figure S14f. Chemiluminescence emission of compound **6a.**

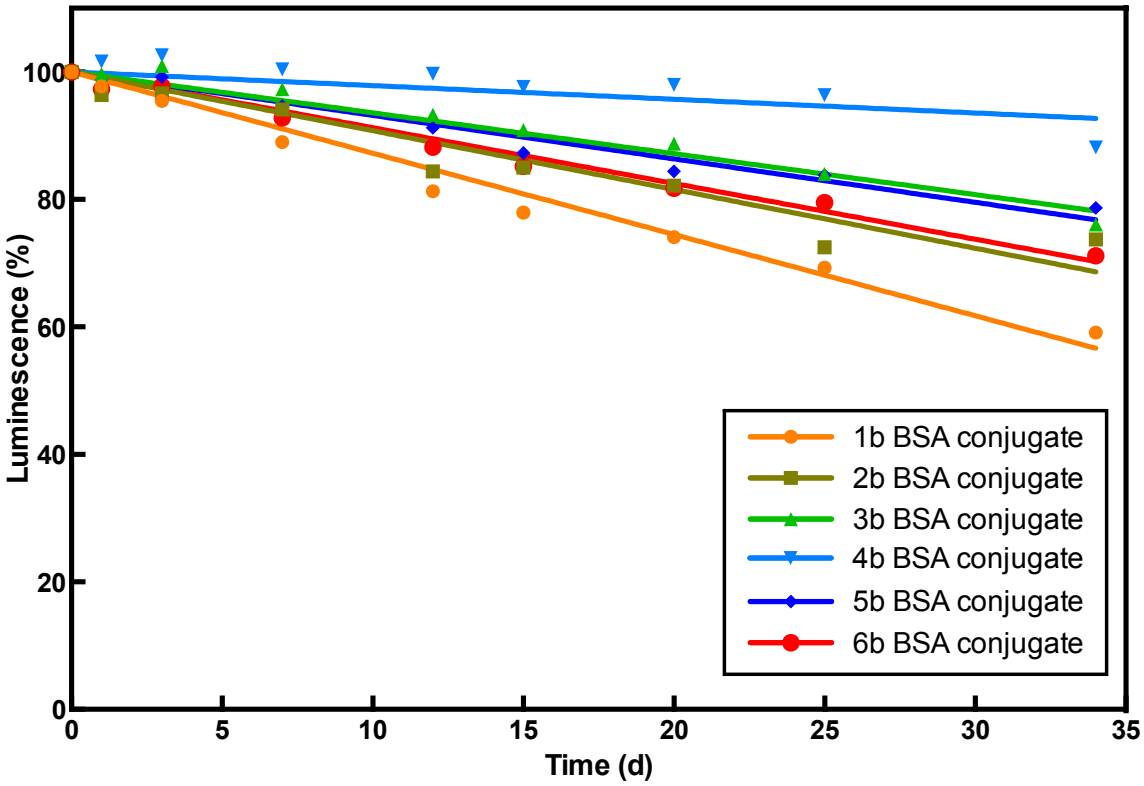


Figure S15. Chemiluminescence stability of BSA conjugates of labels **1b**-**6b** at 37°C in pH 7.4 phosphate buffer. Compared to the reference compound **1b** with an N-sulfopropyl group, better chemiluminescence stability was observed with bulkier N-alkyl groups.

Table S1. fNSB measurements of acridinium ester labels **1a**-**6a** to 4 particles.

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}

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

BSA conjugates	M-270 (amino surface)	M-270 (carboxylate surface)	M-280 (hydrophobic surface)	PMP (amino surface)
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}