Electronic Supplementary Information

Synthesis and utility of fluorogenic acetoxymethyl ethers

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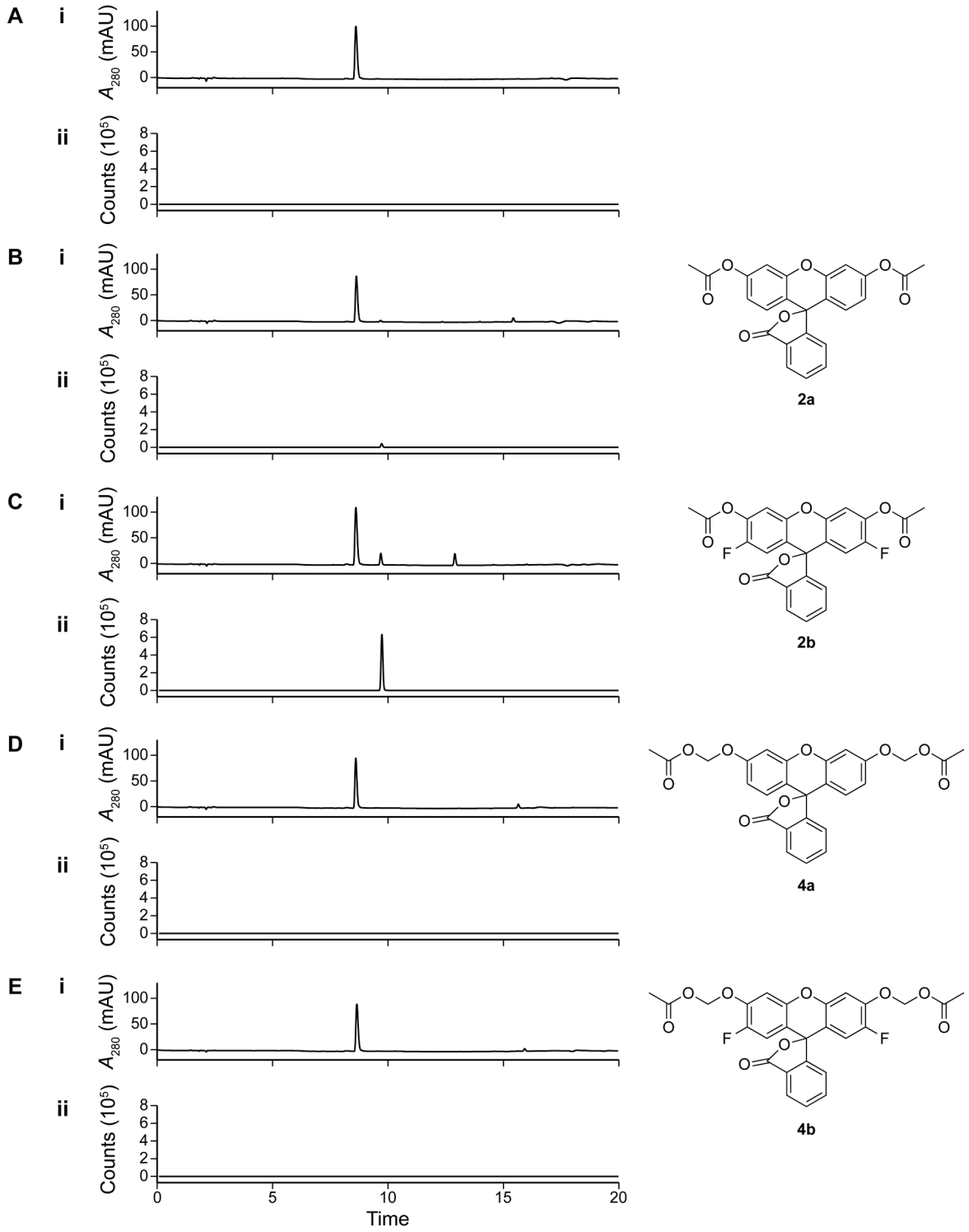


Fig. S1 Chemical reactivity of acetate esters **2a** and **2b** (100 μM), and AM ethers **4a** and **4b** (100 μM) with Ac-Arg–Phe–Met–Trp–Met–Lys-NH_2 (1 mg/mL) in 10 mM HEPES buffer, pH 7.3, for 2 h. (i) HPLC chromatogram of absorbance at 280 nm. (ii) LC–MS chromatogram with single ion monitoring–mass spectrometry (SIM–MS) for acylated peptide (m/z = 981.47). (A) Peptide only. (B) Acetate ester **2a**. (C) Acetate ester **2b**. (D) AM ether **4a**. (E) AM ether **4b**.

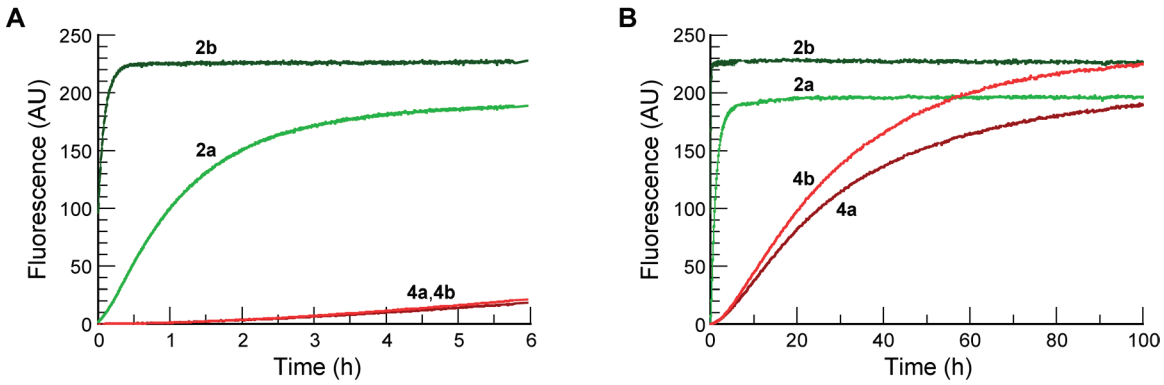


Fig. S2 Time course for the spontaneous generation of fluorescence (λ_{ex} 490 nm, λ_{em} 520 nm) from acetate esters **2a** and **2b** (25 nM), and AM ethers **4a** and **4b** (25 nM) in DMEM containing 10% v/v FBS. (A) 0–6 h; this panel replicates Figure 1B. (B) 0–100 h.

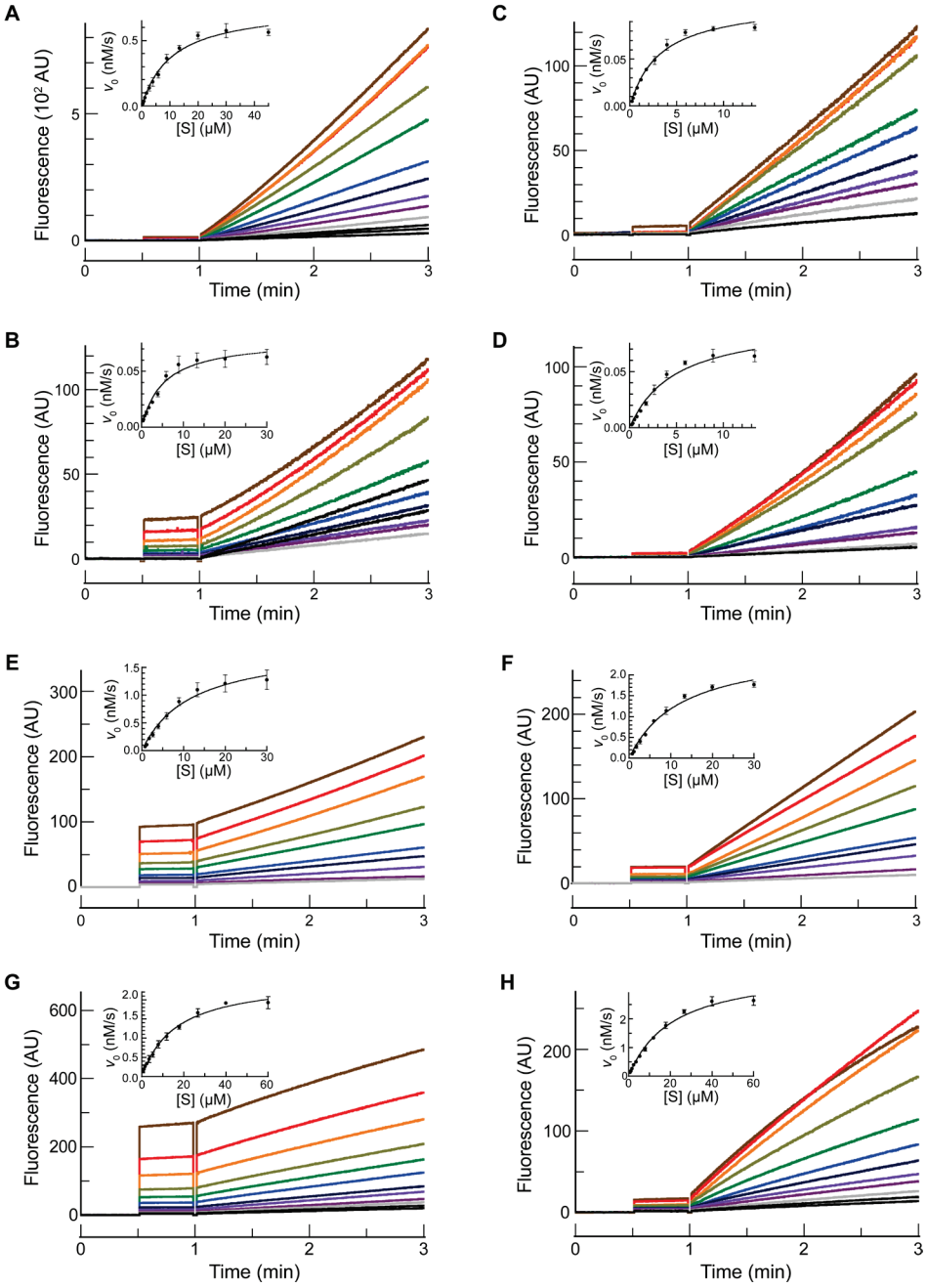


Fig. S3 Kinetic traces and Michaelis–Menten plots (inset) for the hydrolysis of profluorophores by pig liver esterase (8.3 ng/mL) in 10 mM HEPES buffer at pH 7.3. Substrate was added at 30 s; enzyme was added at 1 min. (A) Acetate ester **2a** (45 μM→347 nM); $k_{cat}/K_M = 1.4 \times 10^6$ $M^{-1}s^{-1}$ and $K_M = 11$ μM. (B) Acetate ester **2b** (30 μM→347 nM); $k_{cat}/K_M = 2.9 \times 10^5$ $M^{-1}s^{-1}$ and $K_M = 5.3$ μM. (C) AM ether **4a** (13.3 μM→231 nM); $k_{cat}/K_M = 6.8 \times 10^5$ $M^{-1}s^{-1}$ and $K_M = 3.2$ μM. (D) AM ether **4b** (13.3 μM→231 nM); $k_{cat}/K_M = 3.8 \times 10^5$ $M^{-1}s^{-1}$ and $K_M = 4.9$ μM. (E) Acetate ester **7** (30 μM→780 nM); $k_{cat}/K_M = 3.2 \times 10^6$ $M^{-1}s^{-1}$ and $K_M = 12$ μM. (F) AM ether **6** (30 μM→780 nM); $k_{cat}/K_M = 4.2 \times 10^6$ $M^{-1}s^{-1}$ and $K_M = 13$ μM. (G) Acetate ester **10** (60 μM→694 nM); $k_{cat}/K_M = 3.1 \times 10^6$ $M^{-1}s^{-1}$ and $K_M = 16$ μM. (H) AM ether **9** (60 μM→694 nM); $k_{cat}/K_M = 3.6 \times 10^6$ $M^{-1}s^{-1}$ and $K_M = 21$ μM.

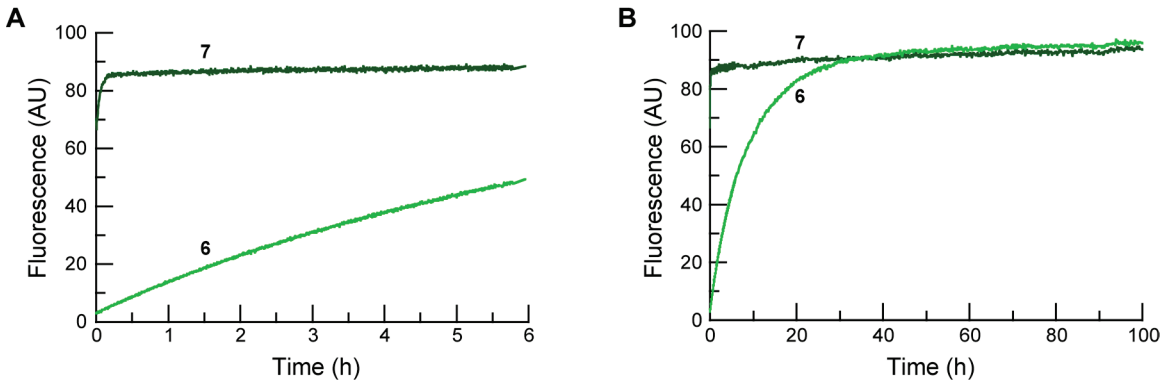


Fig. S4 Time course for the spontaneous generation of fluorescence (λ_{ex} 490 nm, λ_{em} 520 nm) from AM ether **6** and acetate ester **7** (25 nM) in DMEM containing 10% v/v FBS. (A) 0–6 h; this panel replicates Figure 3B. (B) 0–100 h.

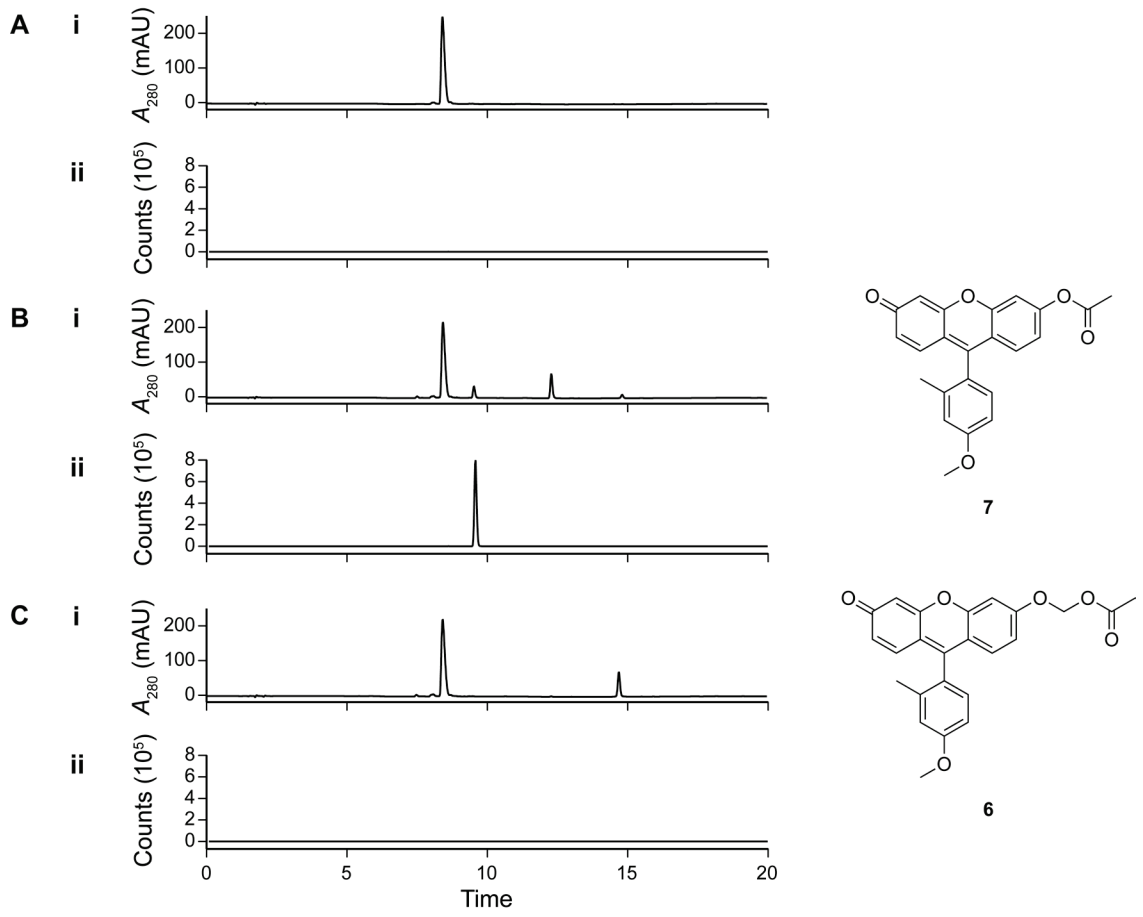


Fig. S5 Chemical reactivity of acetate ester **7** and AM ether **6** (100 μM) with Ac-Arg–Phe–Met–Trp–Met–Lys-NH_2 (1 mg/mL) in 10 mM HEPES buffer, pH 7.3, for 2 h. (i) HPLC chromatogram of absorbance at 280 nm. (ii) LC–MS chromatogram with single ion monitoring–mass spectrometry (SIM–MS) for acylated peptide (m/z = 981.47). (A) Peptide only. (B) Acetate ester **7**. (C) AM ether **6**.

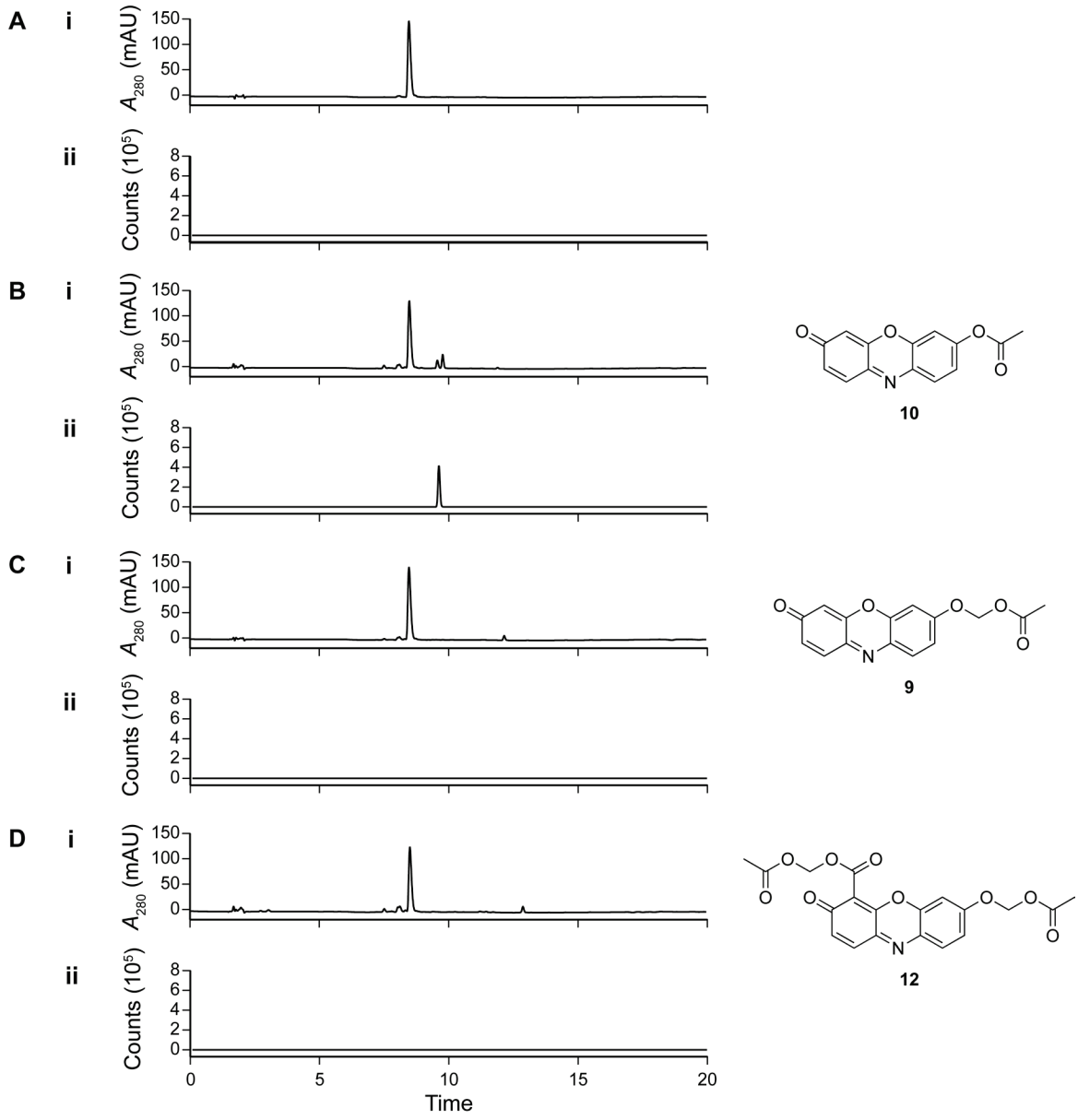
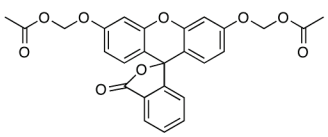


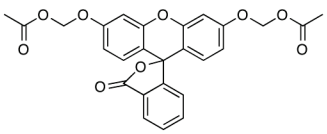
Fig. S6 Chemical reactivity of acetate ester **10** and AM ethers **9** and **12** (100 μM) with Ac-Arg–Phe–Met–Trp–Met–Lys-NH_2 (1 mg/mL) in 10 mM HEPES buffer, pH 7.3, for 2 h. (i) HPLC chromatogram of absorbance at 280 nm. (ii) LC–MS chromatogram with single ion monitoring–mass spectrometry (SIM–MS) for acylated peptide (m/z = 981.47). (A) Peptide only. (B) Acetate ester **10**. (C) AM ether **9**. (D) AM ether **12**.

4a



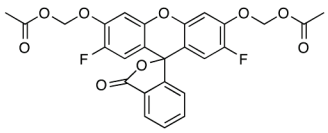
Proton NMR, 400 MHz
$CDCl_3$

4a



Carbon-13 NMR, 100 MHz
$CDCl_3$

4b



Proton NMR, 400 MHz
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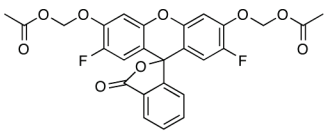
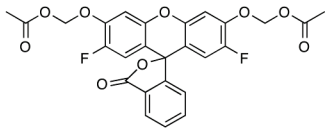
4b

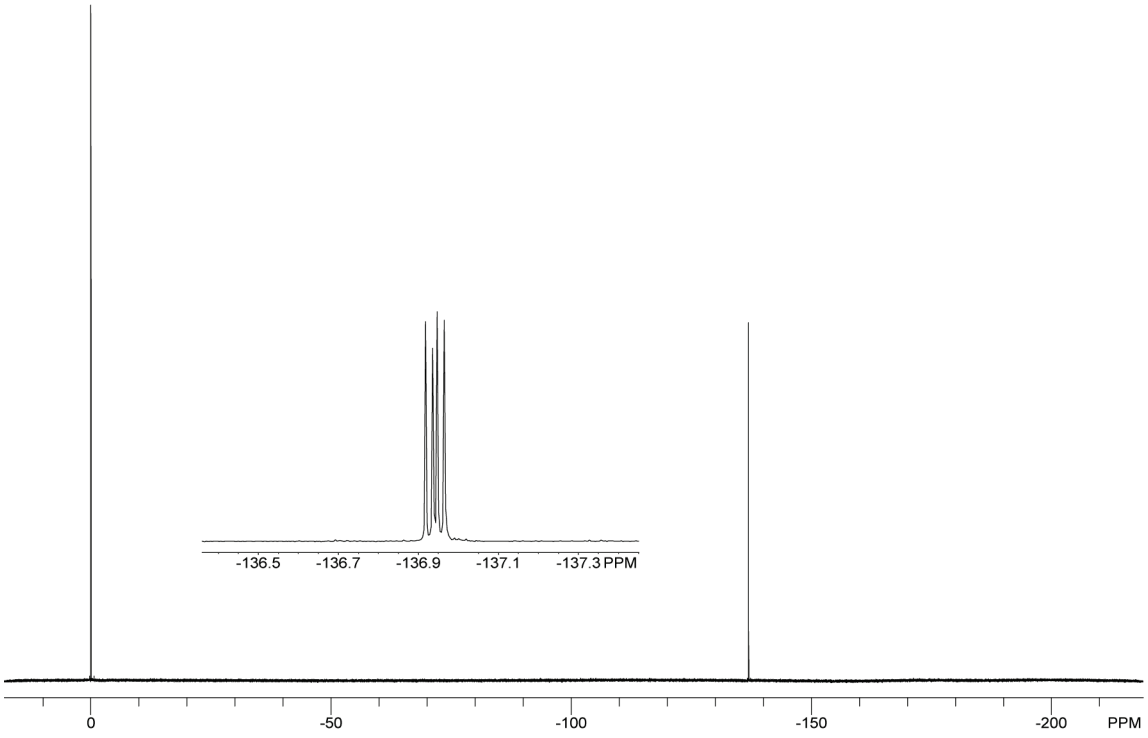


Carbon-13 NMR, 100 MHz
$CDCl_3$

4b



Fluorine NMR, 400 MHz
$CDCl_3$



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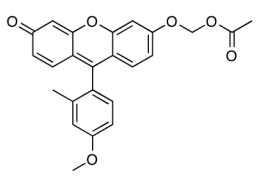

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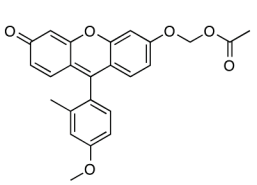

Carbon-13 NMR, 100 MHz
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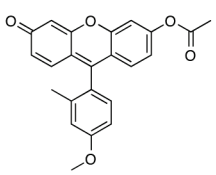

Proton NMR, 400 MHz
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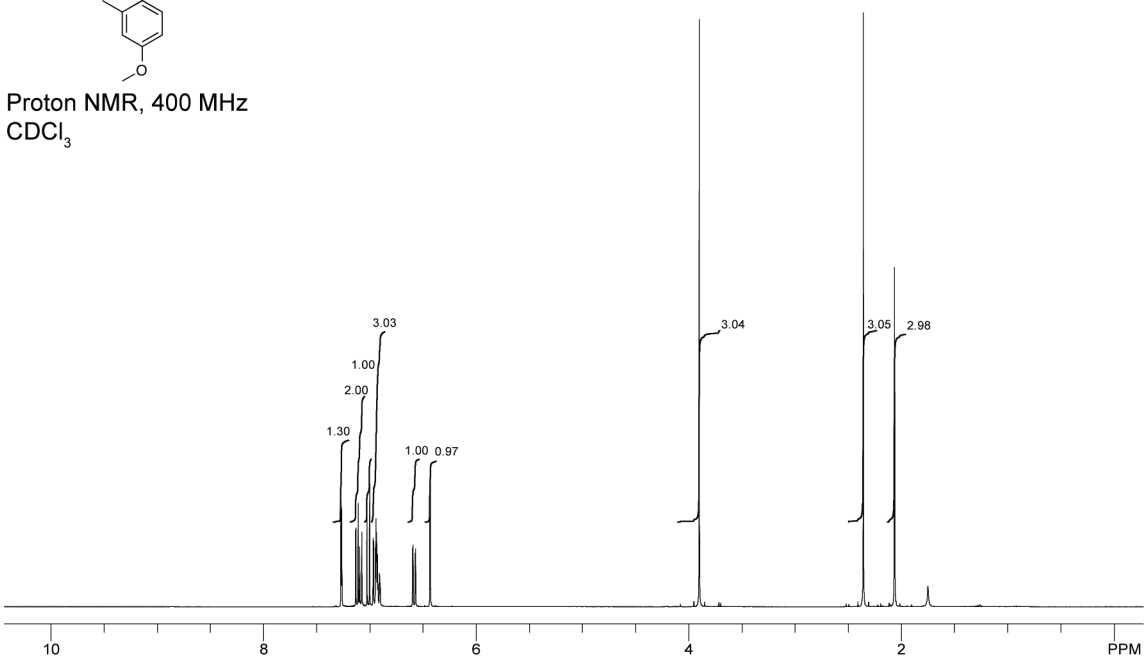

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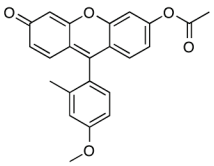

Carbon-13 NMR, 100 MHz
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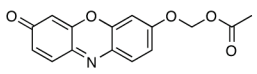

Proton NMR, 400 MHz
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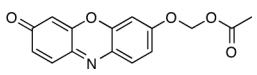

Carbon-13 NMR, 100 MHz
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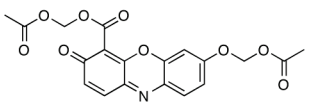

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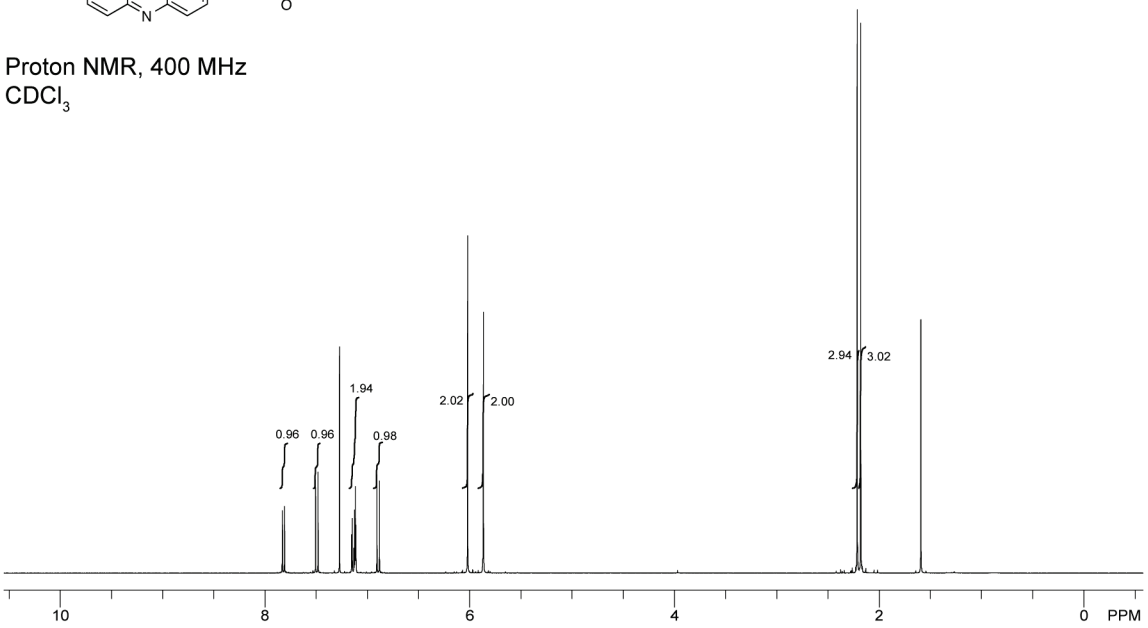

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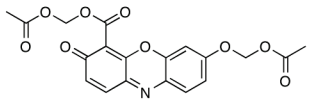

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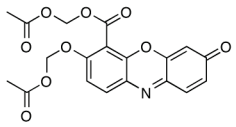

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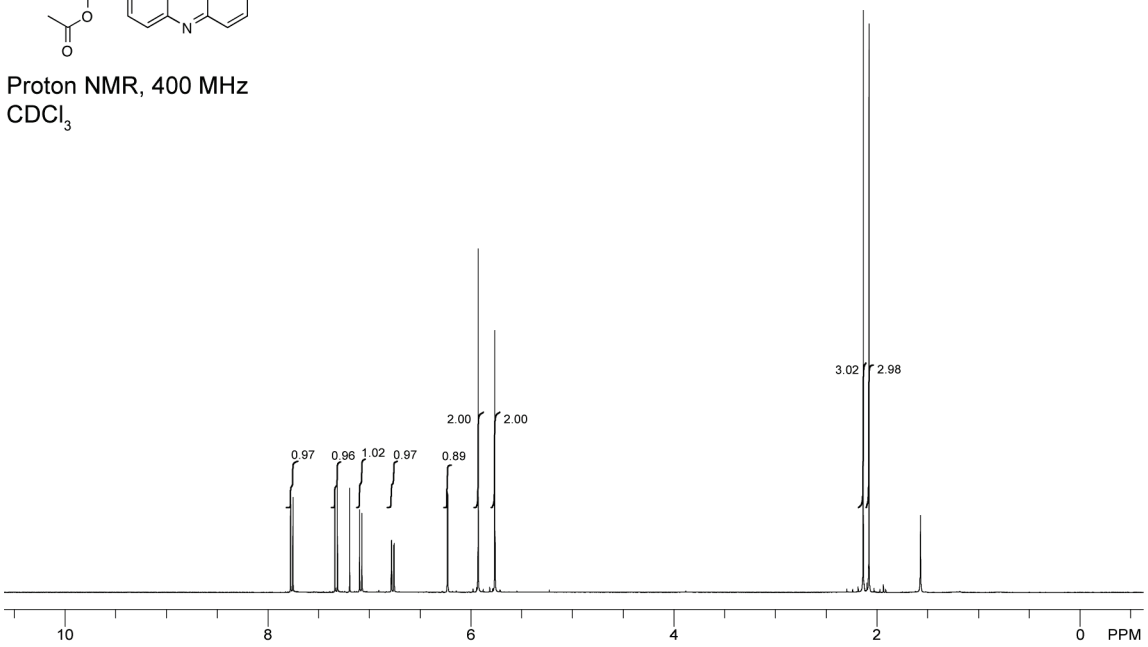

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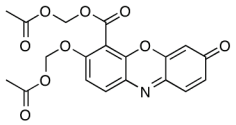

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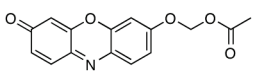

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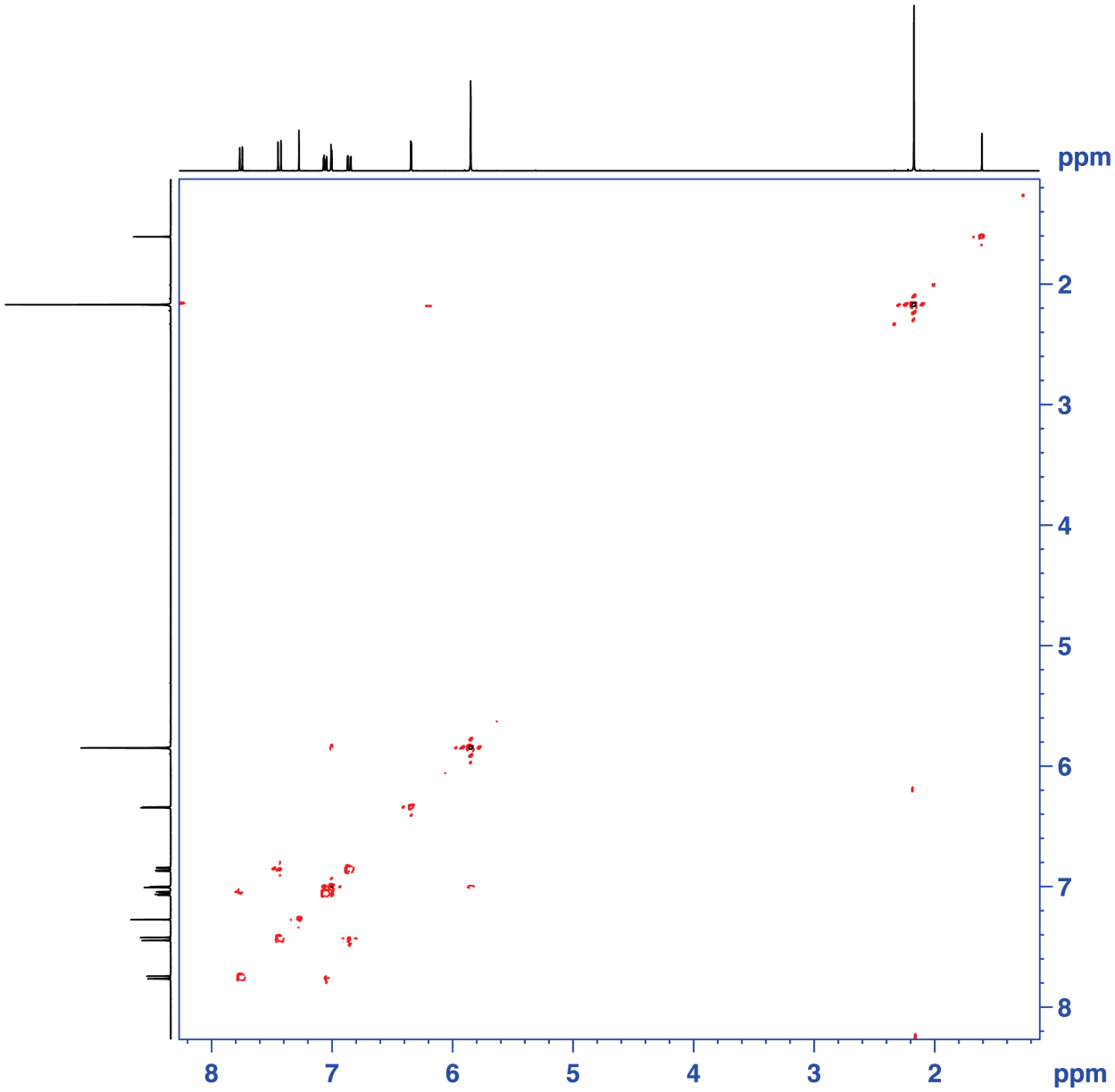

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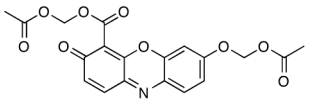

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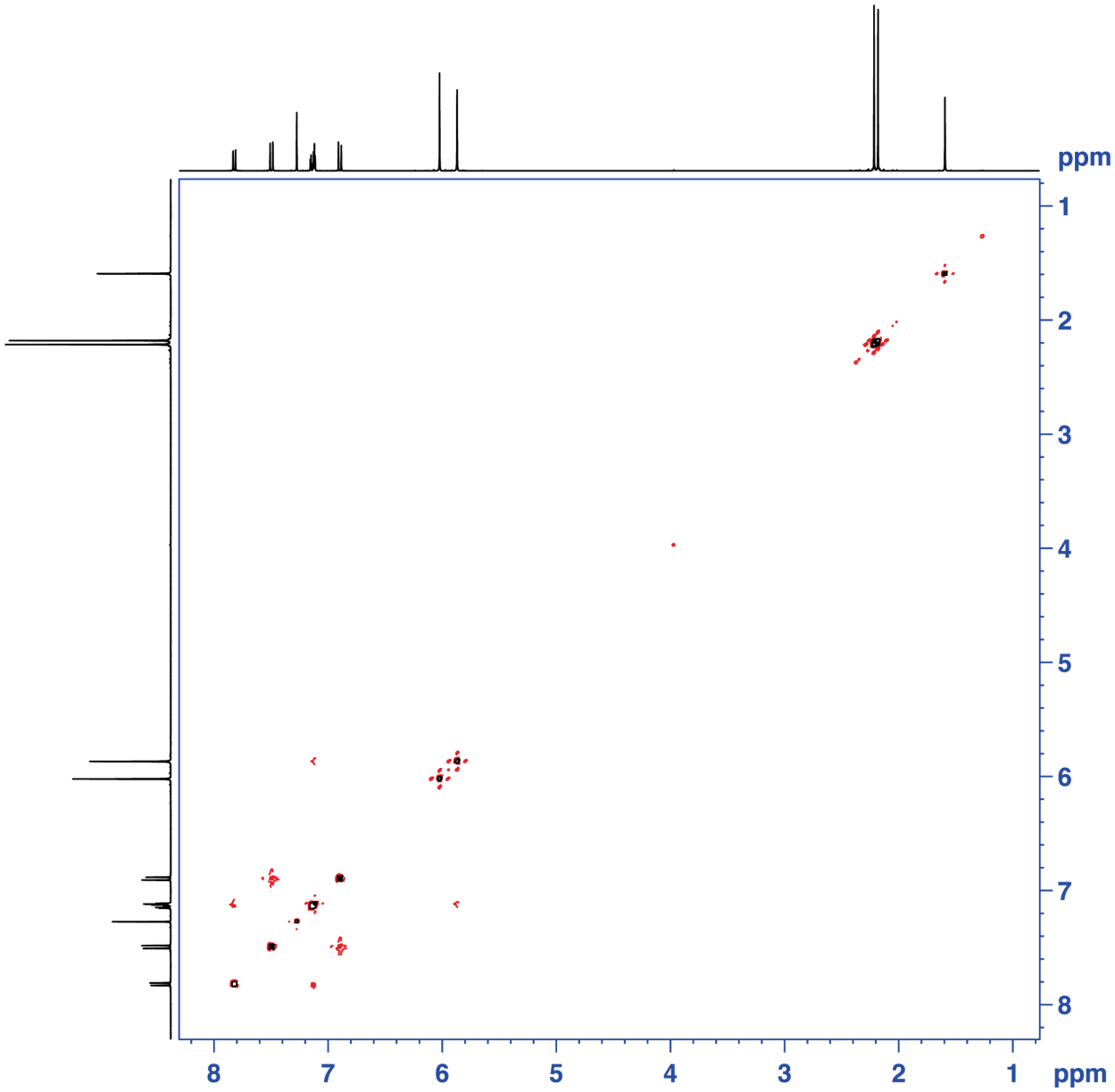

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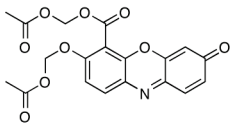

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