Supporting Material for

# Design and application of esterase-labile sulfonate protecting groups

Laert Rusha and Stephen C. Miller*

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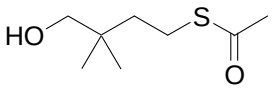
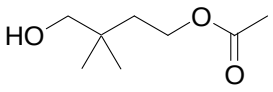
## General

All reactions were performed under an argon atmosphere in oven-dried glassware using anhydrous solvents unless otherwise noted. All chemicals were purchased from Aldrich or Acros unless otherwise noted. HPLC was performed on an Agilent 1100 with a Grace Vydac RP-C18 analytical column (250 x 4.6 mm, 5 micron) or Vydac RP-C18 column (250 x 22 mm, 10 micron). HPLC buffer A is 0.1% TFA in water; buffer B is acetonitrile with 0.1% TFA. The following compounds were synthesized as previously described: ethyl 4-chloro-2,2-dimethylbutanoic acid (**1**),[1] 4-chloro-2,2-dimethylbutanol (**2**),[1] 2,2-dimethyl-1,4-butanol (**4**),[2] Neo-Dan[3], and TFMB-Dan.[3]

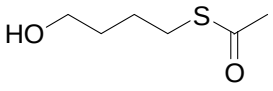
## Experimental Procedures



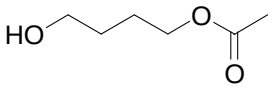

*4-acetylthio-2,2-dimethylbutanol (**3**).* To a solution of **2** (5 mmol) in DMF (6 ml) was added potassium thioacetate (857 mg, 7.5 mmol). The solution was heated to 50°C and stirred for 16h, then cooled to rt and poured into water (50 ml). The product was extracted with ethyl acetate (50 ml), washed with water (3 x 30 ml), and dried with sodium sulfate. The solvent was removed by rotary evaporation and the crude material purified by flash chromatography (0-12.5% ethyl acetate in hexanes stepwise gradient) to afford 531 mg of **3** as a light yellow oil (60% overall yield from **1**). $^{1}$H-NMR ($CDCl_3$): $\delta$ 3.38 (d, 2H, *J* = 6.4 Hz), 2.8 (m, 2H), 2.33 (s, 3H), 1.98 (t, 1H, *J* = 6.4 Hz), 1.52 (m, 2H), 0.91 (s, 6H). $^{13}$C-NMR ($CDCl_3$): $\delta$ 197.1, 71.0, 38.6, 35.9, 30.9, 24.8, 24.2. HR-EIMS *m/z* calculated for $C_8H_{16}O_2SNa$: 199.0769, found: 199.0742.



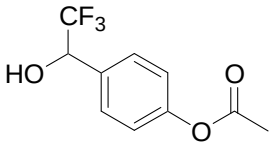

*4-acetoxy-2,2-dimethyl-1-butanol (**5**).* A solution of 2,2-dimethyl-1,4-butanediol (**4**; 154 mg, 1.3 mmol) and pyridine (106 μl, 1.3 mmol) in dichloromethane (20 ml) was stirred at -78°C. Acetyl chloride (93 μl, 1.3 mmol) in dichloromethane (2 ml) was added dropwise, and the solution was allowed to slowly warm to rt. The volatiles were removed by rotary evaporation, and the crude material purified by flash chromatography (0-33% ethyl acetate/hexanes) to afford 57 mg of the product as a clear oil (27%). $^{1}$H-NMR ($CDCl_3$): $\delta$ 4.14 (t, 2H, *J* = 7.2 Hz), 3.34 (s, 2H), 2.05 (s, 3H), 1.63 (t, 2H, *J* = 7.2 Hz), 0.93 (s, 6H). $^{13}$C-NMR ($CDCl_3$): $\delta$ 171.4, 71.8, 61.9, 36.9, 34.8, 24.4, 21.3.




*4-acetylthiobutanol (**6**).* To 4-chlorobutanol (10 mmol, 1.18 ml) in DMF (10 ml) was added potassium thioacetate (1.71 g, 15 mmol). The solution was heated to 50°C and stirred for 16h, then cooled to rt and poured into water (100 mL). The product was extracted with ethyl acetate (2 x 100 mL), washed with water (3 x 100 mL), and dried over sodium sulfate. The solvent was removed by rotary evaporation and the crude material purified by flash chromatography (0-20% ethyl acetate in hexanes stepwise gradient) to afford 1.06 g of **6** as an amber oil (72%). $^{1}$H-NMR ($CDCl_3$): $\delta$ 3.67 (br q, 2H, *J* = 6.4 Hz), 2.90 (t, 2H, *J* = 7.2 Hz), 2.33 (s, 3H), 1.7-1.6 (m, 4H), 1.44 (br s, 1H). $^{13}$C-NMR ($CDCl_3$): $\delta$ 196.6, 61.9, 31.7, 30.8, 29.0, 26.1.

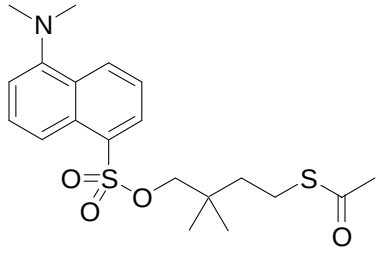


*4-acetoxybutanol (**7**).* To a mixture of acetic anhydride (2 mmol, 204 mg) and 1,4-butanediol (2 mmol, 180 mg) in acetonitrile (2 mL) was added 1 mol% indium triflate (11.2 mg). The reaction was stirred at room temperature overnight, then poured into water (50 mL) and extracted with ethyl acetate (2 x 50 mL). TLC indicated a mixture of bis-acetoxy and mono-acetoxybutanol. The solvent was dried over sodium sulfate and removed in vacuo. Purification by flash chromatography (0-50% ethyl acetate/hexanes) afforded 101.4 mg of product as a clear oil (38%). $^{1}$H-NMR ($CDCl_3$): $\delta$ 4.10 (t, 2H, $J$ = 6.4 Hz), 3.69 (dt, 2H, $J$ = 6.4, 5.6 Hz), 2.05 (s, 3H), 1.77-1.60 (m, 4H), 1.31 (t, 1H, $J$ = 5.6 Hz). $^{13}$C-NMR ($CDCl_3$): $\delta$ 171.3, 64.5, 62.4, 29.3, 25.3, 21.1. This spectral data is consistent with previously reported values.[4]

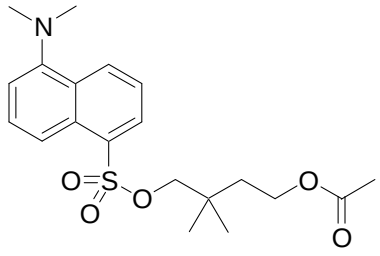


*4-(2,2,2-trifluoro-1-hydroxy-ethyl)-phenyl acetate (**8**).* To a solution of 4-acetoxybenzaldehyde (296 mg, 1.8 mmol) in THF (3 mL) was added 1 mL of trifluoromethyl-trimethyl silane (2M in THF). The rapidly stirred solution was cooled on ice, followed by the addition of a single drop (~20 uL) of 1M TBAF. The reaction was slowly warmed to room temperature. After 3h, 1M HCl (4 mL) was added, and the solution was vigorously stirred at room temperature for 2 hours. The reaction was diluted into water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with 0.1M HCl (100 mL), water (100 mL), and brine (50 mL), then dried over sodium sulfate. After rotary evaporation, the crude material was purified by silica gel chromatography (0-20% ethyl acetate/hexanes) to afford 190 mg of the pure white crystalline product. However, TLC indicated that a significant amount of product eluted as a mixture with starting material. The impure material was further purified by HPLC (10-90% B over 50 minutes) to yield an extra 153 mg, for a total of 343 mg of product (81% yield). $^{1}$H-NMR ($CDCl_3$): $\delta$ 7.48 (d, 2H, $J$ = 8 Hz), 7.13 (m, 2H), 4.98 (m, 1H), 2.86 (d, 1H, $J$ = 4.4 Hz), 2.31 (s, 3H). $^{19}$F-NMR ($CDCl_3$): $\delta$ -78.83 (d, $J_{HF}$ = 6 Hz). $^{13}$C-NMR ($CDCl_3$): $\delta$ 169.8, 151.6, 131.8, 128.9, 124.3 (q, $J^{1}_{CF}$ = 280 Hz), 122.03, 72.4 ($J^{2}_{CF}$ = 32.3 Hz), 21.4. HR-EIMS *m/z* calculated for $C_{10}H_9F_3O_3Na$: 257.0402, found: 257.0378.

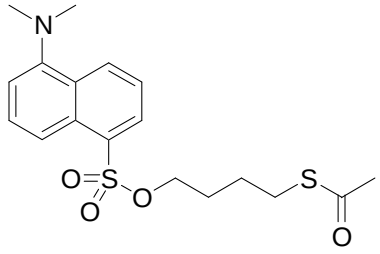
*General procedure for synthesis of dansyl sulfonate esters:* Dansyl chloride (0.2 mmol, 54 mg) and an alcohol (0.2 mmol) were dissolved in 1 ml dichloromethane. DABCO (0.25 mmol, 28 mg) in 1 ml dichloromethane was added, resulting in rapid warming and precipitate formation. After completion, the entire reaction solution was directly loaded on a silica gel column and purified by flash chromatography (0-25% ethyl acetate in hexanes).



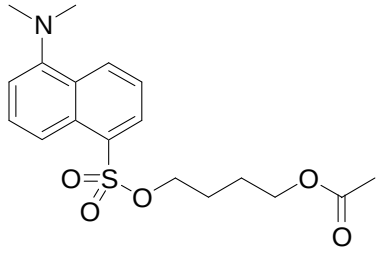
*4-acetylthio-2,2-dimethylbutyl dansylate (**9**).* Yellow oil [0.5 mmol scale] (109 mg, 53%). $^{1}$H-NMR ($CDCl_3$): $\delta$ 8.60 (dt, 1H, *J* = 0.8, 8 Hz), 8.25 (m, 2H), 7.57 (m, 2H), 7.20 (dd, 1H, *J* = 8, 0.8 Hz), 3.64 (s, 2H), 2.89 (s, 6H), 2.57 (m, 2H), 2.27 (s, 3H), 1.43 (m, 2H), 0.88 (s, 6H). $^{13}$C-NMR ($CDCl_3$): $\delta$ 195.5, 152.0, 131.7, 131.5, 130.6, 130.10, 130.09,128.9, 123.2, 119.7, 115.8, 77.9, 45.6, 38.5, 34.8, 30.7, 24.2, 23.9. HR-EIMS *m/z* calculated for $C_{20}H_{28}NO_4S_2$: 410.1460, found: 410.1452.



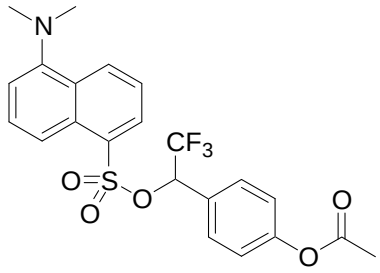
*4-acetoxy-2,2-dimethylbutyl dansylate (**10**).* Yellow oil (50.6 mg, 64%). $^{1}$H-NMR ($CDCl_3$): $\delta$ 8.60 (dt, 1H, *J* = 1.2, 8.4 Hz), 8.25 (m, 2H), 7.57 (m, 2H), 7.19 (dd, 1H, *J* = 8, 0.8 Hz), 3.94 (t, 2H, *J* = 7.2 Hz), 3.66 (s, 2H), 2.89 (s, 6H), 1.96 (s, 3H), 1.57 (t, 2H, *J* = 7.2 Hz), 0.87 (s, 6H). $^{13}$C-NMR ($CDCl_3$): $\delta$ 170.9, 152.0, 131.7, 131.6, 130.6, 130.1, 128.8, 123.2, 119.7, 115.8, 78.2, 61.0, 45.6, 36.9, 33.7, 24.3, 21.1. HR-EIMS *m/z* calculated for $C_{20}H_{28}NO_5S$: 394.1688, found: 394.1684.



*4-acetylthiobutyl dansylate (**11**).* Yellow oil [0.5 mmol scale] (109 mg, 57%). $^{1}$H-NMR ($CDCl_3$): $\delta$ 8.60 (dt, 1H, *J* = 0.8, 8 Hz), 8.25 (m, 2H), 7.57 (m, 2H), 7.21 (dd, 1H, *J* = 8, 0.8 Hz), 3.99 (t, 2H, *J* = 6 Hz), 2.89 (s, 6H), 2.71 (t, 2H, *J* = 7 Hz), 2.28 (s, 3H), 1.7-1.6 (m, 2H), 1.56-1.49 (m, 2H). $^{13}$C-NMR ($CDCl_3$): $\delta$ 195.5, 152.0, 131.73, 131.68, 130.6, 130.13, 130.10,128.9, 123.2, 119.6, 115.8, 70.2, 45.6, 30.7, 28.3, 28.0, 25.8. HR-EIMS *m/z* calculated for $C_{18}H_{24}NO_4S_2$: 382.1147, found: 382.1140.



*4-acetoxybutyl dansylate (**12**).* Yellow oil (46 mg, 42%). $^{1}$H-NMR ($CDCl_3$): δ 8.60 (dt, 1H, *J* = 1.2, 8.4 Hz), 8.26 (m, 2H), 7.57 (m, 2H), 7.21 (dd, 1H, *J* = 7.6, 0.8 Hz), 4.02 (t, 2H, *J* = 6.4 Hz), 3.93 (t, 2H, *J* = 6.4 Hz), 2.90 (s, 6H), 1.98 (s, 3H),1.73-1.56 (m, 4H). $^{13}$C-NMR ($CDCl_3$): δ 171.0, 152.1, 131.75, 131.69, 130.6, 130.1, 128.8, 123.2, 119.6, 115.8, 70.3, 63.6, 45.6, 25.8, 25.0, 21.0. HR-EIMS *m/z* calculated for $C_{18}H_{24}NO_5S$: 366.1375, found: 366.1374.



*4-[1-(5-dimethylamino-naphthalene-1-sulfonyloxy)-2,2,2-trifluoro-ethyl]-phenyl acetate (**13**).* Yellow solid (57.8 mg, 62%). $^{1}$H-NMR ($CDCl_3$): δ 8.49 (d, 1H, *J* = 8.4 Hz), 8.20 (d, 1H, *J* = 8.8 Hz), 8.08 (dd, 1H, *J* = 1.2, 7.6 Hz), 7.57 (t, 1H, *J* = 8 Hz), 7.39 (dt, 1H, *J* = 7.6, 8.4 Hz), 7.17 (d, 1H, *J* = 8 Hz), 7.13 (d, 2H, *J* = 8.4 Hz), 6.78 (m, 2H), 5.63 (q, 1H, *J* = 6.4 Hz), 2.85 (s, 6H), 2.24 (s, 3H). $^{19}$F-NMR ($CDCl_3$): δ -76.29 (d, *J* = 6.8 Hz). $^{13}$C-NMR ($CDCl_3$): δ 168.8, 152.0, 151.9, 132.3, 131.6, 130.4, 129.9, 129.8, 129.5, 129.1, 126.6, 122.9, 122.3 (q, $J^1_{CF}$ = 279 Hz), 121.5, 119.5, 115.8, 78.1 (q, $J^2_{CF}$ = 34 Hz), 45.6, 21.3. HR-EIMS *m/z* calculated for $C_{22}H_{21}F_3NO_5S$: 468.1093, found: 468.1074.

Cell Culture and Dye Incubation

HeLa cells were maintained using DMEM supplemented with 10% FBS, 100 units/mL penicillin and 100 μg/mL streptomycin. CHO-K1 cells were maintained with F-12 Kaighn's media supplemented with 10% FBS, 100 units/mL penicillin and 100 μg/mL streptomycin. All cells were incubated in a 5% $CO_2$ atmosphere. Before incubation with fluorescent dye, the growth media was removed and cells were washed twice with Hanks Buffered Saline Solution (HBSS). Cells were then incubated with 2.5-10 μM fluorescent dye in OPTI-MEM I media for 30 minutes at 37 °C unless otherwise described. Dye labeling media was removed by aspiration and the cells were quickly washed twice with HBSS. Cells were examined on a Zeiss Axiovert 200 fluorescence microscope with a 40X objective using a Hamamatsu ORCA-ER digital camera and excitation from an X-Cite 120 light source through Zeiss filter cube #2 (G 365 excitation filter, FT395 beam splitter, LP 420 long pass emission filter). Where indicated, after observation the cells were switched back to normal growth media, and were observed again 1 hour after the initial incubation in the presence or absence of inhibitors of anionic drug transporters (50 μM MK571 or 100 nM Ko-143).

Stability studies of dansyl sulfonate esters:

Eight solutions were prepared: 1) 5 mg/ml ovalbumin in PBS, 2) 5 mg/ml ovalbumin and 2.7 units/ml of PLE in PBS, 3) 5 mg/ml BSA in PBS, 4) 5 mg/ml BSA and 2.7 units/ml of PLE in PBS, 5) PBS, 6) 2.7 units/ml of PLE in PBS, 7) 5 mM glutathione in PBS, and 8) DMEM supplemented with 10% FBS, 100 units/mL penicillin and 100 μg/mL streptomycin. To 0.2 ml of each solution was added 0.2 μl of a 10 mM DMSO stock solution of each dansyl sulfonate ester (final concentration of 10 μM). After incubation at room temperature or 37°C for times ranging from 1 minute to 16 hours, a 1 μl aliquot was removed from each solution and analyzed by TLC (50% ethyl acetate in hexanes). Determination of the stability of dansyl sulfonate esters to 1M sodium iodide in refluxing acetone was performed as previously described.[3] Stability to storage in DMSO was ascertained by both TLC analysis of the stock solution and by direct fluorescence excitation of the stock solution itself.

Kinetics of dansyl ester cleavage by pig liver esterase

To a fluorescence cuvette was added 3 mL of 25% Methanol/PBS followed by a dansyl sulfonate ester (AcOTFMB, AcSNeo, AcONeo, TFMB, or Neo) from a 10 mM DMSO stock to a final concentration of 20 μM. The fluorescence excitation and emission wavelengths for each dye are shown in Figure S4. The cuvette was placed inside a Fluoromax-3 spectrofluorimeter and 0.25 units of pig liver esterase was added in a small volume of PBS (15 μL) to a final PLE concentration of 0.083 units/ml or 5 μg/mL. Formation of the cleaved dansyl sulfonate was monitored over a period of 900 seconds (excitation at 322 nm, emission at 498 nm). Cleavage of AcONeo-Dan was significantly slower than the AcSNeo and AcOTFMB esters; monitoring of cleavage over 16,000 seconds revealed a half-life of over 160 minutes (Figure S5). The data was plotted using GraphPad Prism 5.0, and fit to one-phase decay to determine half-lives.

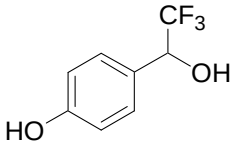
HPLC Analysis

10 μM dye standards were prepared from 20 mM stock solutions in DMSO (final DMSO concentration 0.05%). AcOTFMB-Dan (**13**) was treated with 1 unit/mL (0.037 mg/mL) of pig liver esterase for 10 minutes at room temperature in 25% methanol/75% PBS buffer. The reaction was analyzed by HPLC (0% B for five minutes, 0-100% B over 20 minutes, 100% B for 7 minutes, then return to 0% B over two minutes) at a flow rate of 1 mL per minute and wavelength of 260 nm. The dansyl sulfonate and **13** eluted at ~5.4 and ~26.5 minutes respectively (Figure S6).

Isolation of AcOTFMB cleavage products after treatment with pig liver esterase

To enable a preparative-scale reaction, a compromise had to be made between the solubility of AcOTFMB-Dan and a suitable medium for PLE. AcOTFMB-Dan (30 mg, 0.064 mmol) was added to 107 mL of 25% methanol/PBS. Pig liver esterase was then added to a final concentration of 17 U/mL and the reaction mixture was stirred at 37 ºC. Subsequent experiments (vide supra) indicate that much less PLE is required. After 30 minutes, the fluorescence of the solution was checked with a hand-held long-wave UV lamp, and found to have changed from yellow-green to blue. However, the stir bar was coated with yellow-green fluorescent material, so the reaction mixture was stirred for an additional 16 hours at 37 ºC. Despite this extended incubation, some of the AcOTFMB-Dan remained. The mixture was made slightly acidic (pH 6) with 1M HCl and extracted with EtOAc (3 x 100 mL). The organic layers were combined, dried with $Na_2SO_4$ and

concentrated by rotary evaporation. TLC analysis (50% acetone/hexanes) of the crude mixture indicated the presence of some uncleaved AcOTFMB-Dan ester ($R_f$ = 0.61) and one new UV-absorbent compound ($R_f$ = 0.50). The crude mixture was purified by silica gel flash chromatography (0-25% acetone/hexanes) to afford uncleaved AcOTFMB-Dan (12 mg, 0.026 mmol) and 4-(2,2,2-Trifluoro-1-hydroxy-ethyl)phenol (6.4 mg, 0.033 mmol).

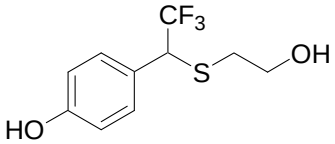



4-(2,2,2-Trifluoro-1-hydroxy-ethyl)phenol

White solid. $^1$H-NMR ($CD_3OD$): $\delta$ 7.28 (d, 2H, $J$ = 8.7 Hz), 6.78 (m, 2H), 4.89 (q, 1H, $J_{HF}$ = 7.2 Hz, overlap with water peak). $^{13}$C-NMR ($CD_3OD$): $\delta$ 158.0, 128.9, 126.4, 125.2 (q, $J^1_{CF}$ = 280 Hz), 114.9, 71.7 (q, $J^2_{CF}$ = 31 Hz). $^{19}$F-NMR ($CD_3OD$): $\delta$ -80.4 (d, $J_{HF}$ = 7.2 Hz).

Isolation of AcOTFMB cleavage products after treatment with pig liver esterase and 2-mercaptoethanol

To enable a preparative-scale reaction, a compromise had to be made between the solubility of AcOTFMB-Dan and a suitable medium for PLE. AcOTFMB-Dan (68 mg, 0.15 mmol) was added to 240 mL of 25% methanol/PBS, followed by the addition of 2-mercaptoethanol (44 mg, 0.58 mmol). Pig liver esterase was then added to a final concentration of 17 U/mL and the reaction mixture was stirred at 37 ºC. Subsequent experiments (vide supra) indicated that much less PLE is required. After 30 minutes, the fluorescence of the solution was checked with a hand-held long-wave UV lamp, and found to have changed from yellow-green to blue. However, the stir bar was coated with yellow-green fluorescent material, so the reaction mixture was stirred for an additional 16 hours at 37 ºC. Despite this extended incubation, some of the AcOTFMB-Dan remained. The mixture was made slightly acidic (pH 6) with 1M HCl and extracted with EtOAc (3 x 100 mL). The organic layers were combined, dried with $Na_2SO_4$ and concentrated by rotary evaporation. TLC analysis (50% acetone/hexanes) of the crude mixture indicated the presence of some uncleaved AcOTFMB-Dan ester ($R_f$ = 0.61) and two new UV absorbent compounds ($R_f$ 0.50, 0.43). The crude mixture was purified by silica gel flash chromatography (0-25% acetone/hexanes). Three compounds were isolated: uncleaved AcOTFMB-Dan (16 mg, 0.034 mmol), 4-(2,2,2-Trifluoro-1-hydroxy-ethyl)phenol (12 mg, 0.063 mmol), and 4-[2,2,2-Trifluoro-1-(2-hydroxy-ethylsulfanyl)-ethyl]-phenol (11 mg, 0.044 mmol).




4-[2,2,2-Trifluoro-1-(2-hydroxy-ethylsulfanyl)-ethyl]-phenol

White solid. $^1$H-NMR ($CD_3OD$): $\delta$ 7.24 (d, 2H, $J$ = 8.6 Hz), 6.75 (m, 2H), 4.62 (q, 1H, $J_{HF}$ = 8.8 Hz), 3.65 (t, 2H, $J$ = 6.5 Hz), 2.71 (m, 2H). $^{13}$C-NMR ($CD_3OD$): $\delta$ 157.9, 130.2, 126.4 (q, $J^1_{CF}$ = 277 Hz), 124.7, 115.2, 61.2, 51.0 (q, $J^2_{CF}$ = 29 Hz), 34.3. $^{19}$F-NMR ($CD_3OD$): $\delta$ -70.4 (d, $J_{HF}$ = 8.8 Hz). HR-EIMS *m/z* calculated for $C_{10}H_{11}F_3O_2SNa$: 275.0330, found 275.0299.

Treatment of AcOTFMB-Dan with 2-mercaptoethanol and ovalbumin

AcOTFMB-Dan (20 mg, 0.043 mmol) was added to 71 mL of 25% methanol in PBS, followed by 2-mercaptoethanol (13.4 mg, 0.17 mmol; 2.4 mM final concentration) and ovalbumin (71.4 mg; 1 mg/mL final concentration). The reaction was stirred at 37 ºC for 16 hours. The mixture was made slightly acidic (pH 6) with 1M HCl and extracted 3 times with 100 mL portions of EtOAc. The organic layers were combined, dried with $Na_2SO_4$ and concentrated by rotary evaporation. Only uncleaved AcOTFMB-Dan was isolated (17.7 mg, 0.038 mmol).

Detection of AcOTFMB cleavage products after treatment with pig liver esterase and 2-mercaptoethanol (small scale)

AcOTFMB-Dan (1 mg, 0.002 mmol) was added to 5 mL of 25% methanol/PBS, followed by the addition of 2-mercaptoethanol (0.6 mg, 0.008 mmol) and pig liver esterase (1U; 0.2 U/ml). The reaction mixture was stirred at 37 ºC for 2 hours. The mixture was made slightly acidic (pH 6) with 1M HCl and extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried with $Na_2SO_4$ and concentrated by rotary evaporation. TLC analysis (50% acetone/hexanes) of the crude mixture indicated the presence of some uncleaved AcOTFMB-Dan ester ($R_f$ = 0.61), 4-(2,2,2-trifluoro-1-hydroxy-ethyl)phenol ($R_f$ = 0.50), and 4-[2,2,2-trifluoro-1-(2-hydroxy-ethylsulfanyl)-ethyl]-phenol ($R_f$ = 0.43), confirmed by co-spotting with the above isolated products.

Stability of AcOTFMB-Dan to 2-mercaptoethanol (small scale)

AcOTFMB-Dan (1 mg, 0.002 mmol) was added to 10 mL of 25% methanol/PBS, followed by the addition of 2-mercaptoethanol (3.9 mg, 0.05 mmol; 50 mM final concentration). The reaction mixture was stirred at 37 ºC for 16 hours. The mixture was made slightly acidic (pH 6) with 1M HCl and extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried with $Na_2SO_4$ and concentrated by rotary evaporation. TLC analysis (50% acetone/hexanes) of the crude mixture indicated only the presence of uncleaved AcOTFMB-Dan ester ($R_f$ = 0.61).

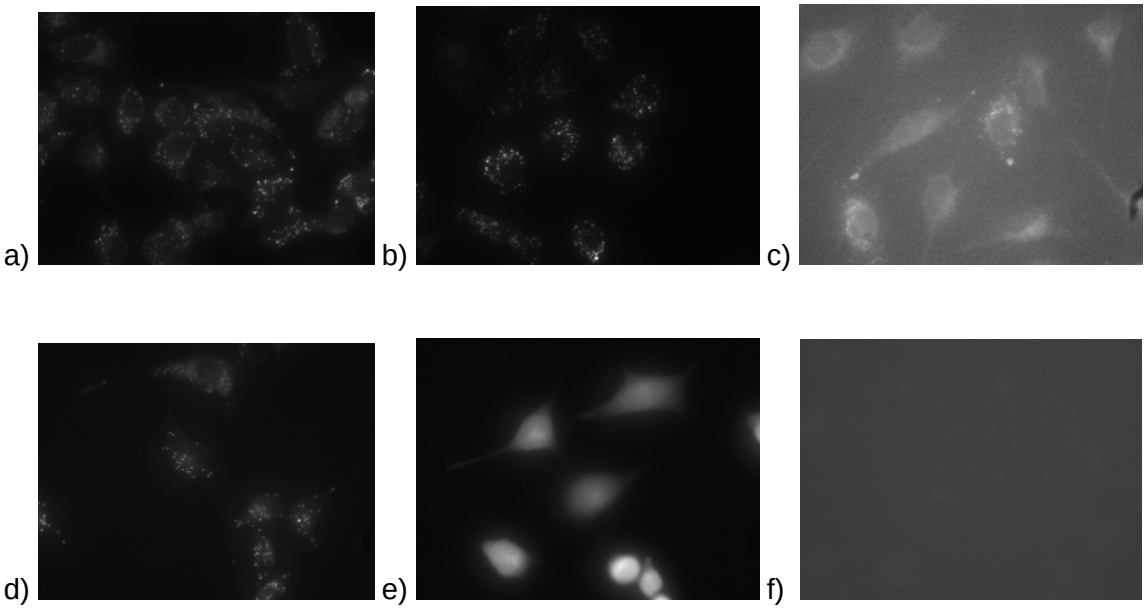



Figure S1. Cellular labeling of HeLa cells with sulfonate esters a) Neo-Dan; b) AcSNeo-Dan; c) AcONeo-Dan; d) TFMB-Dan; e) AcOTFMB-Dan; f) sodium dansylate.

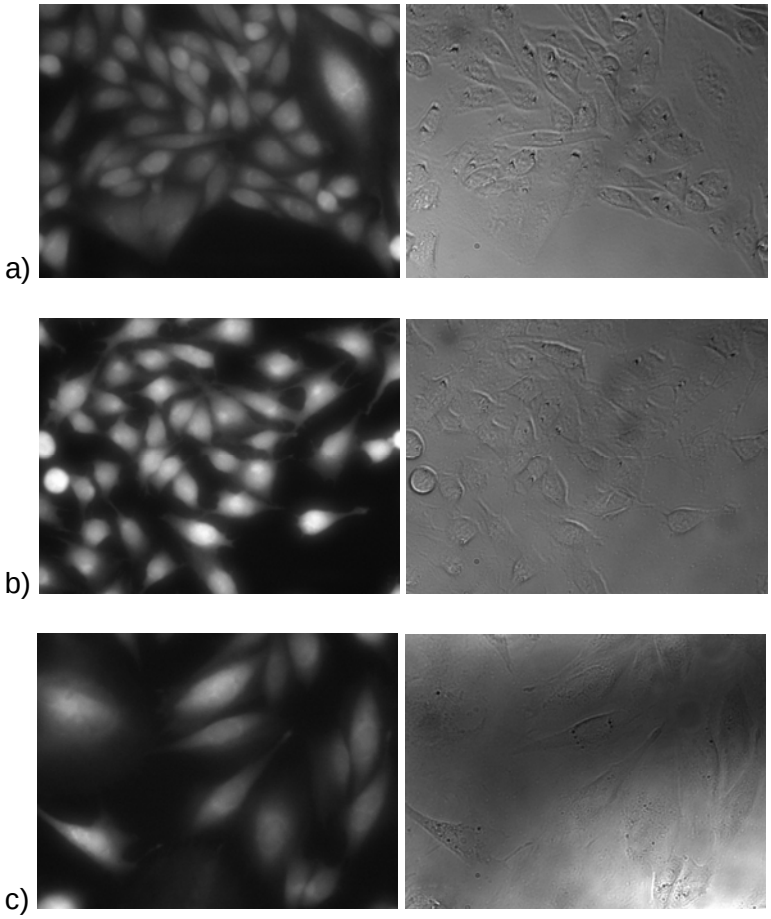



Figure S2. AcOTFMB-Dan is rapidly loaded into all cells, in the presence or absence of serum. Fluorescence and brightfield images of CHO cells after labeling at 37 °C under the following conditions: a) 15 minutes with 5 μM AcOTFMB-Dan in OptiMem; b) 30 minutes with 5 μM AcOTFMB-Dan in OptiMem; c) 30 minutes with 10 μM AcOTFMB-Dan in F12K media containing 10% FBS.

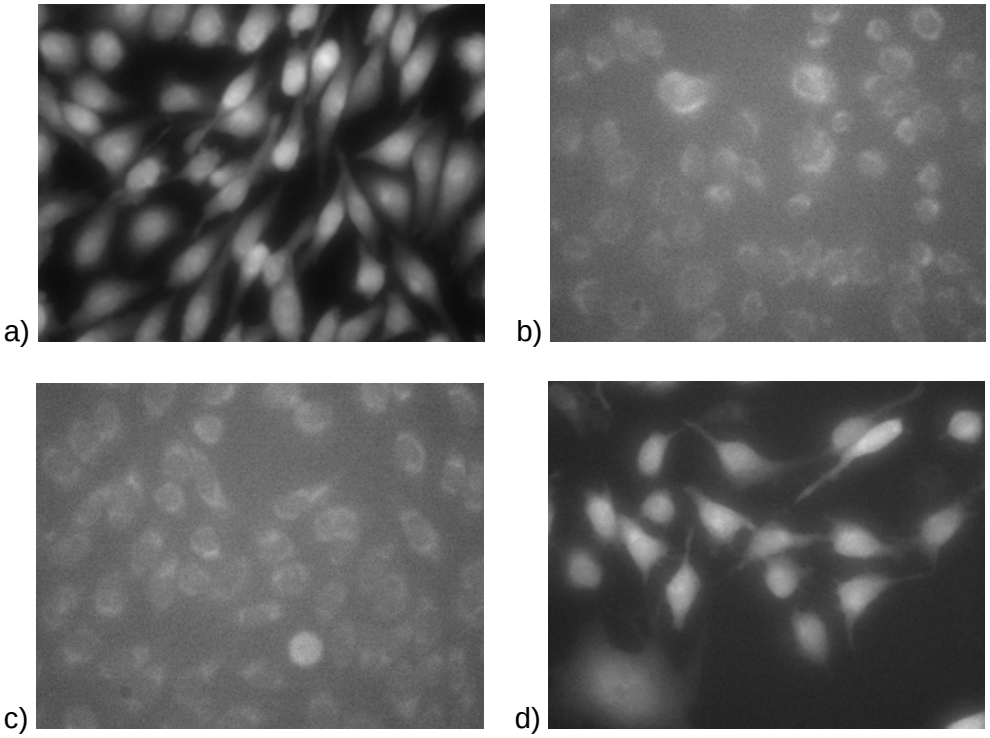
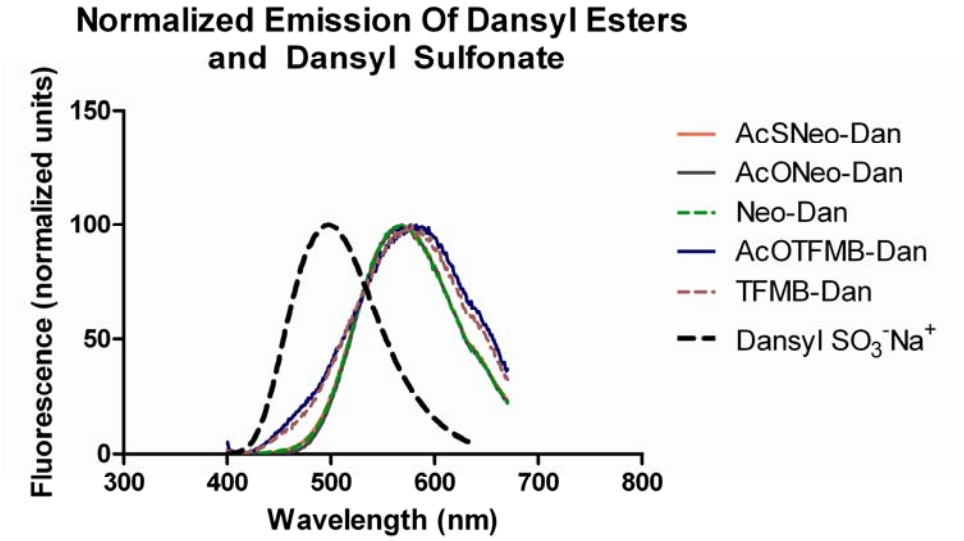



Figure S3. Dye efflux from CHO cells in the absence and presence of inhibitors of ABC transporters. a) cells treated with 5 μM AcOTFMB-Dan (**13**) for 30 minutes and imaged immediately after labeling; b) labeled cells after 1h incubation in growth media at 37°C; c) labeled cells after 1h incubation in growth media containing 100 nM Ko-143; d) labeled cells after 1h incubation in growth media containing 50 μM MK-571.

| Compound | Excitation (nm) | Emission (nm) |
|---|---|---|
| Neo-Dan | 340 | 566 |
| AcONeo-Dan (**9**) | 342 | 566 |
| AcSNeo-Dan (**10**) | 342 | 569 |
| TFMB-Dan | 348 | 578 |
| AcOTFMB-Dan (**13**) | 346 | 577 |
| Sodium dansyl sulfonate | 322 | 498 |



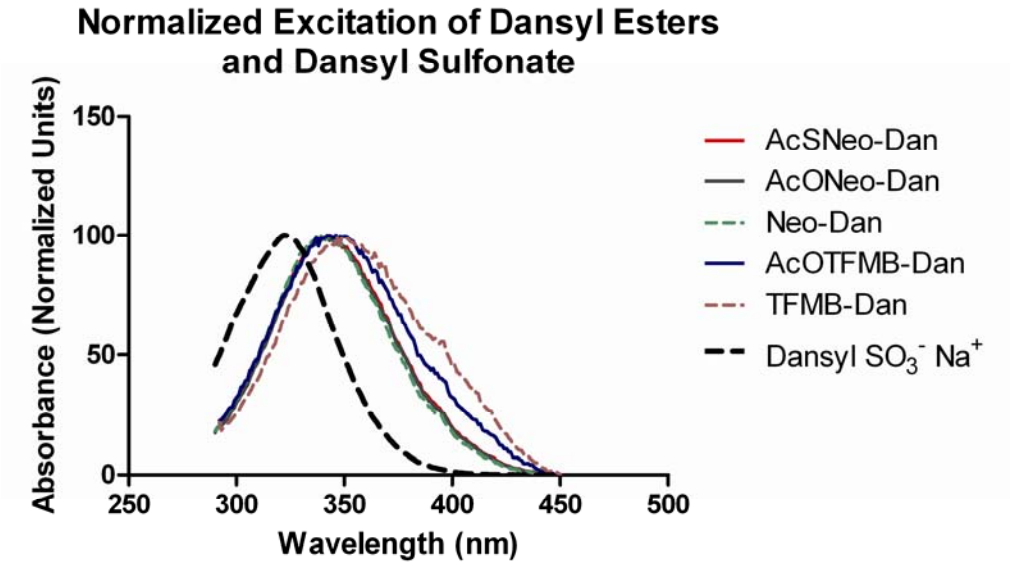




Figure S4. Normalized excitation and emission spectra for dansyl sulfonate and dansyl esters in 25% methanol/PBS.

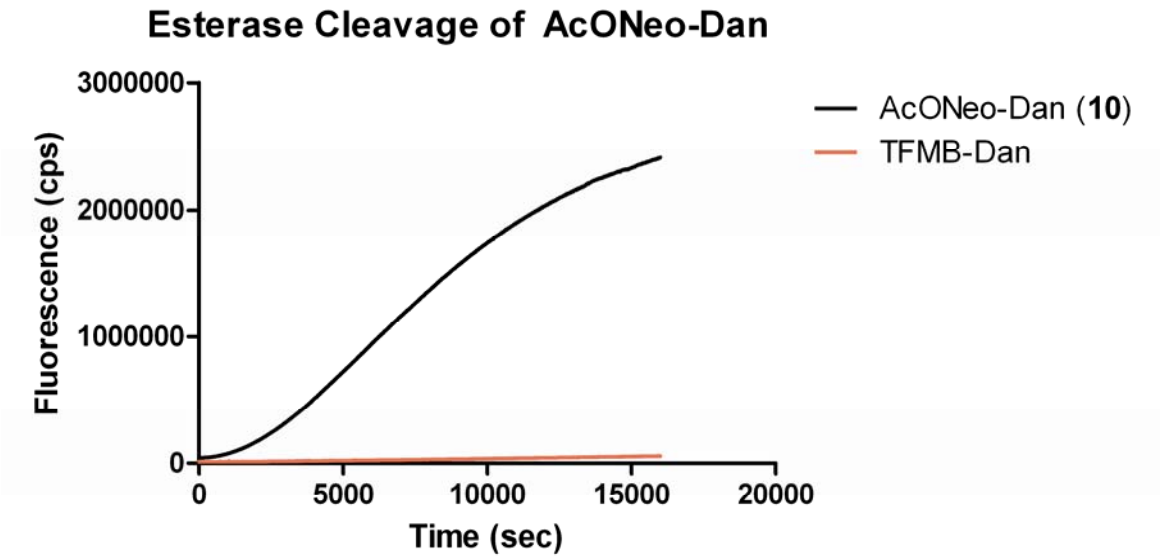



Figure S5. Pig liver esterase cleavage of AcONeo-Dan (**10**) to dansyl sulfonate is significantly slower than AcSNeo and AcOTFMB (see Figure 2 in the main text).

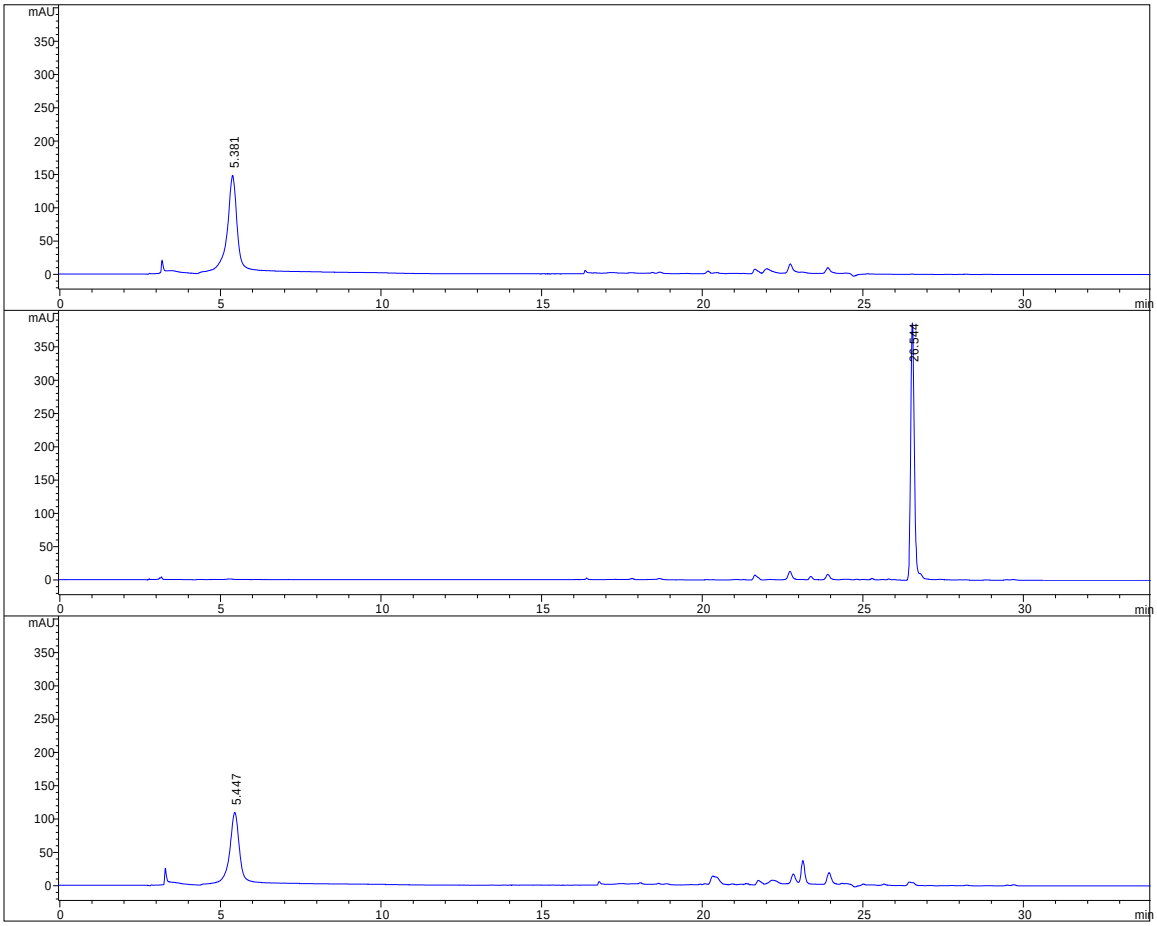



Figure S6. Treatment of 10 μM AcOTFMB-Dan **13** with one unit of pig liver esterase for 10 minutes at room temperature results in complete cleavage to the free dansyl sulfonate (bottom). AcOTFMB-Dan **13** (middle) and dansyl sulfonate (top) are shown for reference.

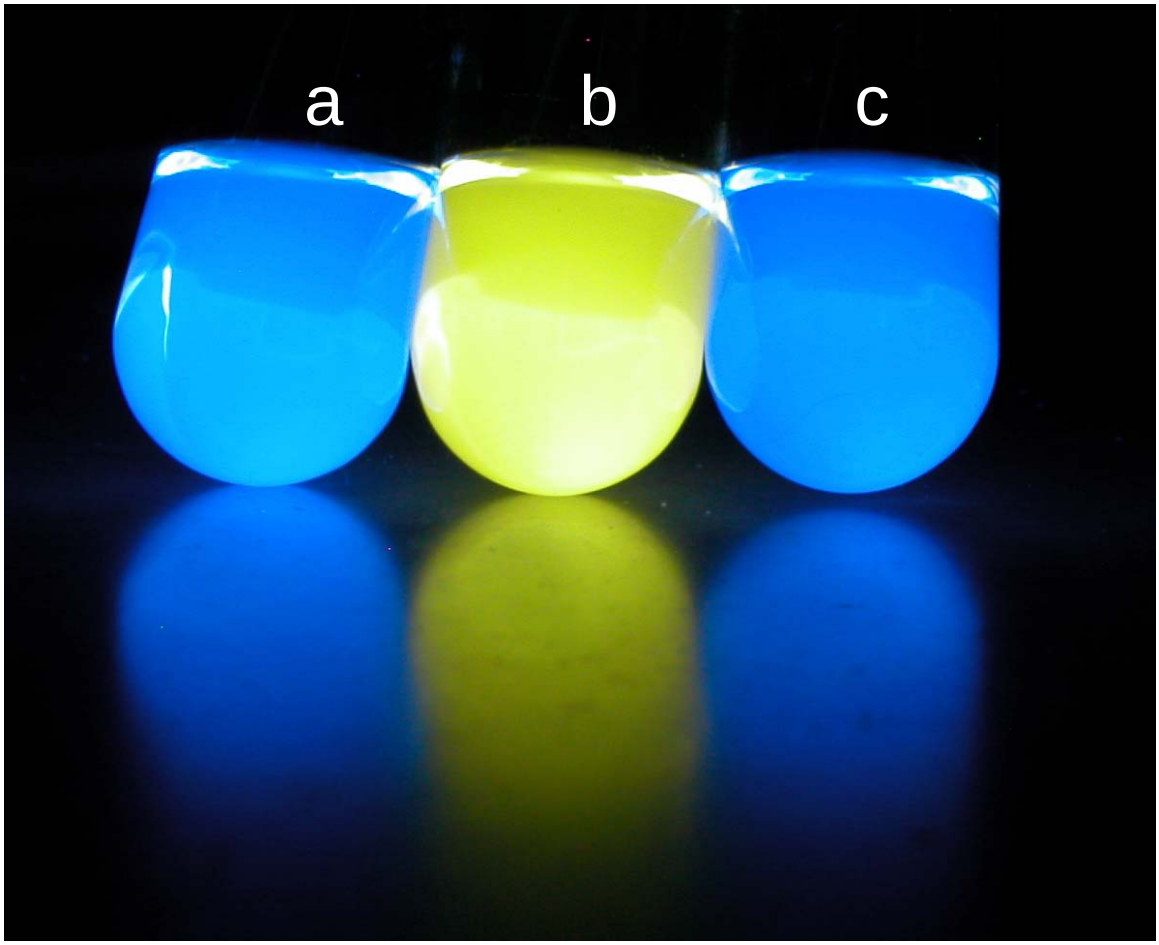
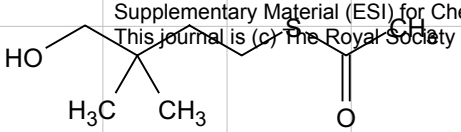
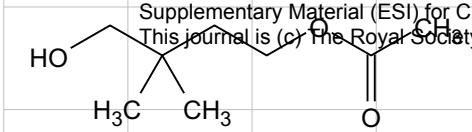
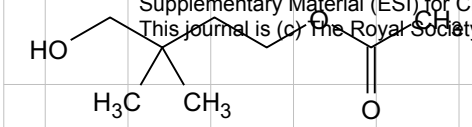



Figure S7. Visual discrimination between dansyl sulfonate and sulfonate esters: a) sodium dansyl sulfonate in PBS buffer; b) AcSNeo-Dan (**9**) in PBS buffer; c) AcSNeo-Dan treated with pig liver esterase in PBS for 40 minutes at room temperature. Dyes were excited with a hand-held long-wave UV light (365 nm).

[1] Dasseux, J.-L.; Oniciu, C.D. WO 2003/087040, Oct 23, **2003**.
[2] Seeberger, S.; Griffin, R.J.; Hardcastle, I.R.; Golding, B.T. *Org. Biomol. Chem.* **2007**, *5*, 132-138.
[3] Miller, S.C. *J. Org. Chem.* **2010**, *75*, 4632-4635.
[4] Tsukamoto, H.; Suzuki, T.; Sato, M.; Kondo, Y. *Tetrahedron Lett.* **2007**, *48*, 8438-8441.



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CH3
H3C
CH3
O


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21.33
1000
900
800
700
600
500
400
300
200
100
0
-100
Intensity
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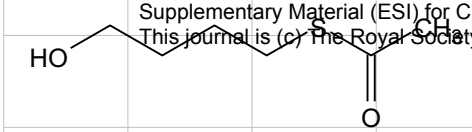
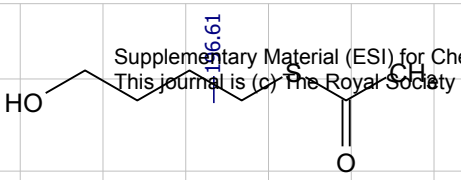
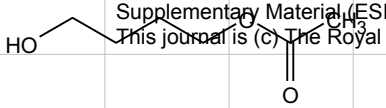
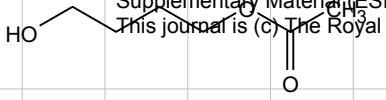
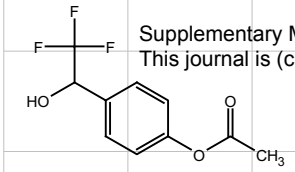
f1 (ppm)

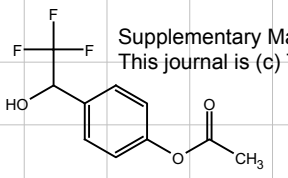


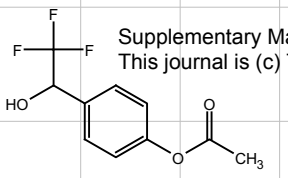










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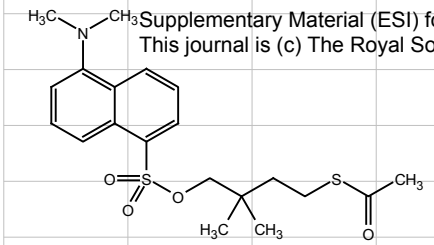

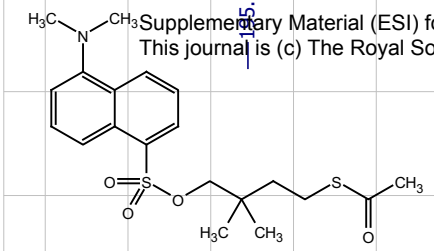

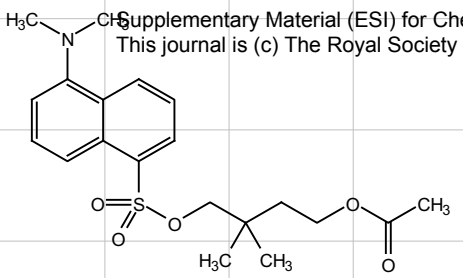

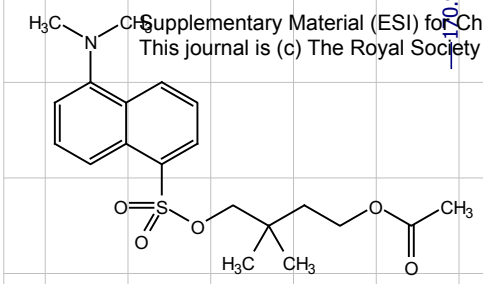

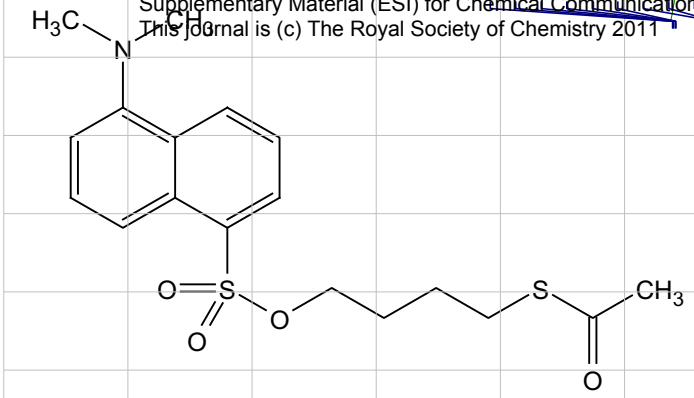

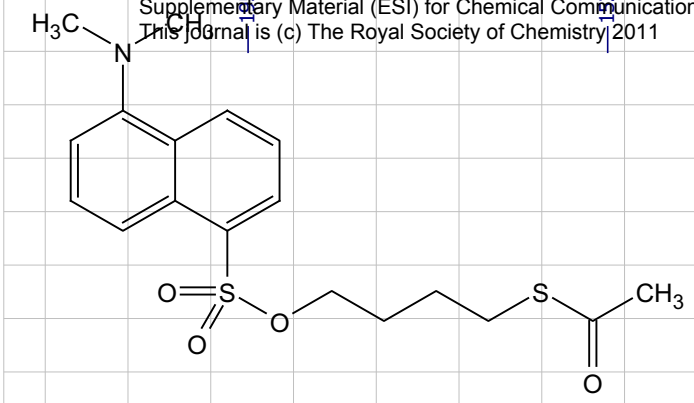

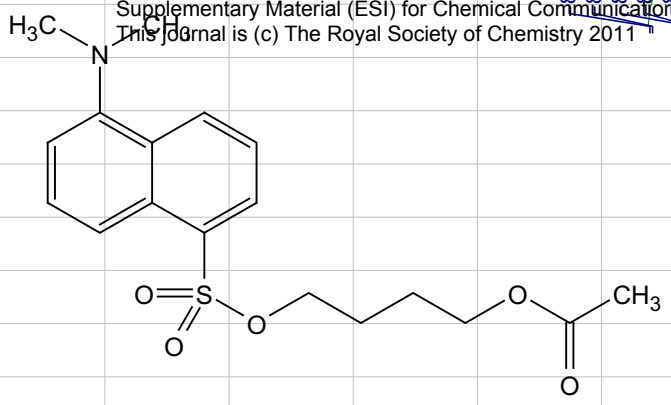

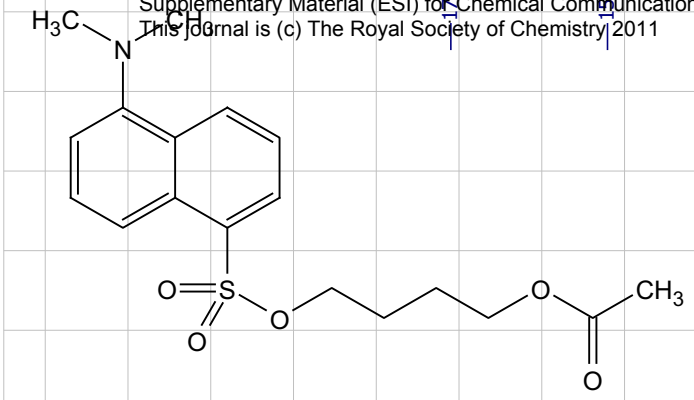

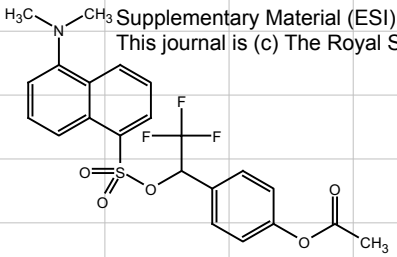

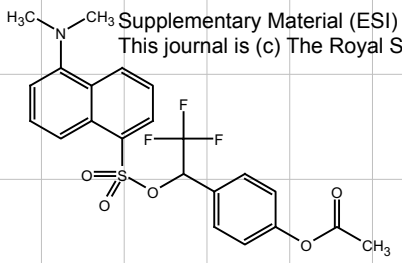


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120.90
119.47
115.79
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Intensity


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120.90
118.10
f1 (ppm)


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77.46
77.25
76.94
f1 (ppm)


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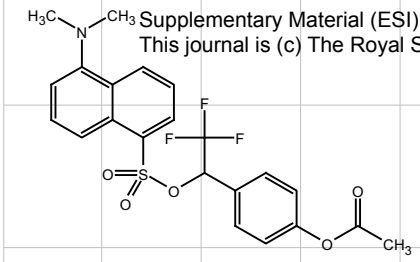
f1 (ppm)

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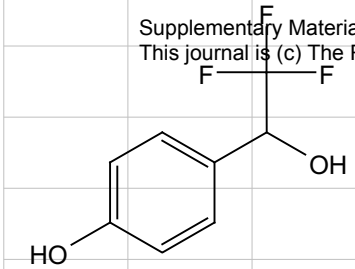

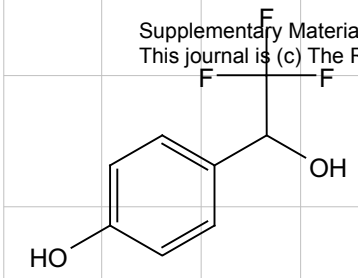


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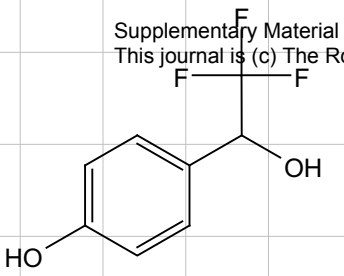

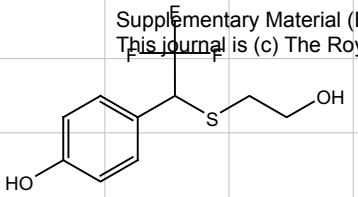

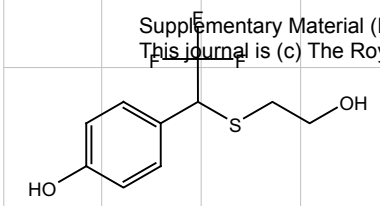


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Intensity

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f1 (ppm)