Latent Fluorophore Based on the Trimethyl Lock
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General Experimental
Pyridine used was dried by storage for 24 h over activated Linde 4A molecular sieves under Ar(g). Anhydrous DMF was obtained from a CYCLE-TAINER solvent delivery system (J. T. Baker; Phillipsburg, NJ). EDCI was from Novabiochem. Rhodamine 110 was from Aldrich Chemical (Milwaukee, WI). Silica gel 60 (230–400 mesh) for flash chromatography was from Silicycle (Québec City, Québec, Canada).

1H NMR and 13C NMR spectra were obtained with a Bruker AC+ 300 spectrometer at the University of Wisconsin–Madison Chemistry Instrument Center. All kinetic evaluations were performed in phosphate-buffered saline (PBS, pH 7.3), which contained (in 1 L) KCl (0.2 g), KH2PO4 (0.2 g), NaCl (8.0 g), and Na2HPO4·7H2O (2.16 g). Pig liver esterase (PLE; MW 163 kDa (ref 1)) was obtained from Sigma Chemical (St. Louis, MO; product number E2884) as a suspension in 3.2 M ammonium sulfate buffer, and was diluted to appropriate concentrations in PBS before use. Stock solutions of pro-fluorophore 3 were prepared in DMSO and added to PBS for the kinetic experiments such that DMSO concentrations never exceeded 1% (v/v). Fluorometric measurements were made with using fluorescence grade quartz or glass cuvettes from Starna Cells (Atascadero, CA) and a QuantaMaster1 photon-counting spectrofluorometer from Photon Technology International (South Brunswick, NJ) equipped with sample stirring.

Synthesis of Pro-Fluorophore 3
Pro-Fluorophore 3 was synthesized by the route shown in Scheme 1. Specifically, acetylated 1 (ref 2; 5 g, 18.8 mmol) was dissolved in a 100 mL of a 1:1 mixture of dry pyridine/DMF at room temperature under argon and treated with EDCI (3.6 g, 18.8 mmol). After stirring the resulting solution at room temperature for 1 h, rhodamine 110 (1.72 g, 4.7 mmol) was added. The reaction mixture was stirred at room temperature for 2 days under Ar(g), during which time the deep red solution slowly turned to a light peach color. The reaction mixture was treated with 100 mL of EtOAc and incubated at −20 °C for 12 h. The clear liquid was removed by decantation and washed with 100 mL of 0.1 M HCl, followed by 100 mL of water. The organic layer was dried over MgSO4(s) and concentrated to dryness by rotary evaporation under reduce pressure. The red residue was purified by flash chromatography (Rf, 0.44; 4:1 EtOAc/hexanes) to yield pro-fluorophore 3 as a white solid (1.12 g, 29% yield). 1H NMR (CDCl3) δ 1.69 (s, 12H), 2.24 (s, 6H), 2.38 (s, 6H), 2.41 (s, 6H), 2.55 (s, 4H), 6.53–6.63 (m, 6H), 6.80 (s, 2H), 7.06 (d, J = 6.9 Hz, 1H), 7.34 (s, 2H), 7.42 (s, 2H), 7.59 (m, 2H), 7.97 (d, J = 6.9 Hz, 2H); 13C NMR (CDCl3) δ 172.2, 169.8, 153.1, 151.6, 150.1, 140.1, 139.1, 137.3, 134.9, 133.2, 132.8, 129.6, 128.1, 124.9, 123.9, 123.4, 115.1, 113.7, 107.2, 51.1, 40.4, 32.2, 32.1, 25.5, 21.9, 20.2. MS (MALDI) m/z 845.3535 (MNa+ = 845.3414).
Supporting Information

Excitation–Emission Spectra of Pro-Fluorophore 3 in the Absence and Presence of Pig Liver Esterase

A solution of pro-fluorophore 3 (5 \(\mu\)M) in 2 mL of PBS containing PLE (0.25 mg/mL) was incubated at room temperature for 4 h. The fluorescence excitation–emission spectra of the solution were then recorded. The procedure was repeated for a solution of pro-fluorophore 3 treated in an identical manner, except for the presence of PLE.

Kinetic Parameters for Activation of Pro-Fluorophore 3 by Pig Liver Esterase

All kinetic measurements were performed at room temperature with an excitation wavelength of \(\lambda_{\text{ex}} = 492\) nM and an emission wavelength of \(\lambda_{\text{em}} = 520\) nM in 2.0 mL of PBS containing PLE (2.5 \(\mu\)g/mL). A calibration curve was created by measuring the fluorescence of known concentrations of rhodamine-110 in the aforementioned reaction mixture. The rate of cleavage of pro-fluorophore 3 by PLE was measured adding known concentrations of 3 (50 nM–5 mM) to the reaction mixture and recording the fluorescence as a function of time, as shown in Figure S1. The reaction rate was calculated by using the calibration curve for rhodamine-110. Enzymatic parameters were calculated by fitting the linear portion of the data (which corresponds to the unmasking of the second amino group) to the Michaelis–Menten equation.

Figure S1. Raw data showing the change in fluorescence (\(\lambda_{\text{ex}} = 492\) nM, \(\lambda_{\text{em}} = 520\) nM) upon addition of PLE (0.5 mg/mL) to solutions containing various concentrations of pro-fluorophore 3.

References