Visual and fluorogenic detection of nerve agent simulant via Lossen rearrangement of rhodamine-hydroxamate

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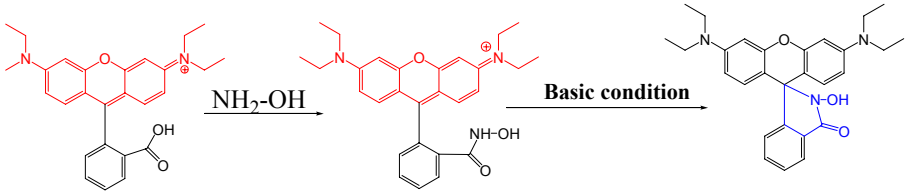
Supplementary Materials

Experimental section

Materials and Methods

Unless otherwise noted, all reagents were obtained from Alfa Aesar and used without further purification. Column chromatography was performed on silica gel (300-400 mesh). NMR spectra (^{1}H at 400 MHz and ^{13}C at 100 MHz) were recorded on a Bruker instrument using tetramethyl silane as the internal reference. The fluorescence emissions spectra and Uv-vis absorbance spectra were performed on a spectrofluorimeter (Spectamax M5, Molecular Device, USA) using the excitation wavelength (λex) of 560 nm. The high resolution mass spectra were performed in Stanford University, California.

Synthesis of rhodamine-hydroxamate



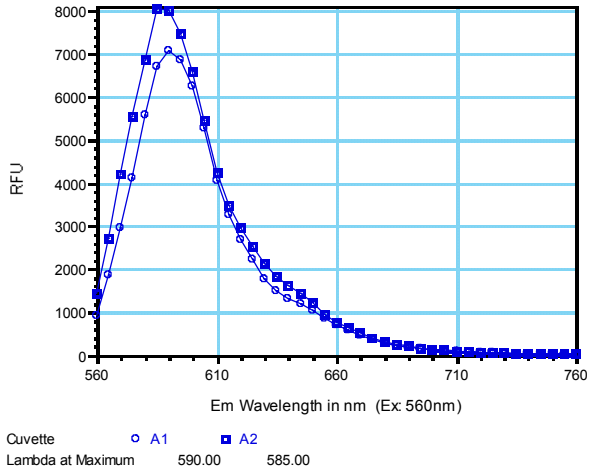
Scheme 1: Synthesis of nonfluorescent rhodamine-hydroxamate

The solution of rhodamine B (10 g) in ethanol (50ml) was added to an aqueous solution (80 mL) of hydroxylamine (10 g) and sodium hydroxide (15 g). The mixture was stirred at room temperature for 2 hours, and then poured into water (300 mL). The

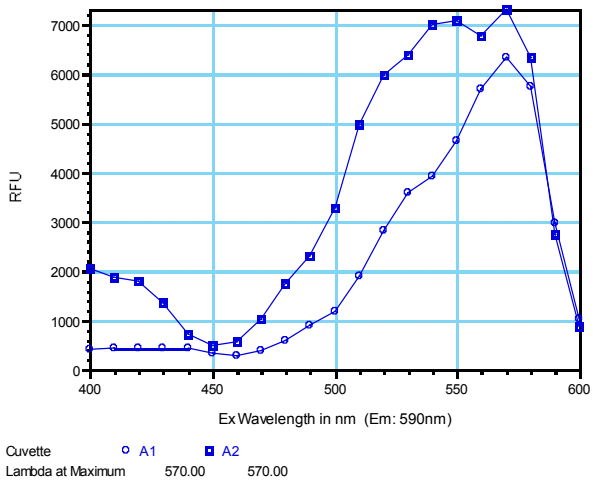
mixture was extracted with methylene dichloride (300 mL). The organic layer was collected, washed with water (250 mL) and then dried over sodium sulfate. The solution was filtered and evaporated to get a brownish solid. The residue was purified by silica gel chromatography using ethyl acetate: TEA (50: 1) as the eluent to yield the desired product in 80% yield. ^{1}H-NMR ($CDCl_3$): δ 1.03 (t, 9H, J = 6.8 Hz), 1.11 (t, 6H, J = 6.4 Hz), 2.82 (q, 6H, J = 7.2Hz), 3.28 (q, 8H, J = 7.6 Hz), 6.13 (dd, 1H, J = 2.4, 9.2 Hz), 6.29 (d, 1H, J = 2.4 Hz), 6.33 (d, 1H, J = 8.8 Hz), 6.33 (d, 1H, J = 8.8 Hz), 6.62 (d, 1H, J = 8.8 Hz), 7.20 (d, 1H, J = 8.8 Hz), 7.34 (m, 2H, J = 2.4 Hz), 7.47 (m, 1H), 7.89 (m, 1H); ^{13}C-NMR ($CDCl_3$): 172.50, 165.73, 161.21, 153.20, 151.45, 140.35, 138.24, 132.34, 130.69, 130.10, 129.27, 129.09, 103.18, 102.06, 44.83, 44.98； HRMS ($C_{28}H_{31}N_3O_3$): calculated (MH^+): 458.2444, found: 458.2433

Comparison of the fluorescent spectral properties of the colored species in the assay solution with rhodamine B

Diethyl chlorophosphate (2 μL) was added into 20 mL of DMF solutions containing rhodamine-hydroxamate (1 mg/mL) and 3% (v/v) triethylamine. The solution was incubated at room temperature for 20 minutes and then an aliquot was taken for the fluorescence excitation spectrum (Em 590 nm) and fluorescence emission spectrum as compared to rhodamine B in DMF solution.



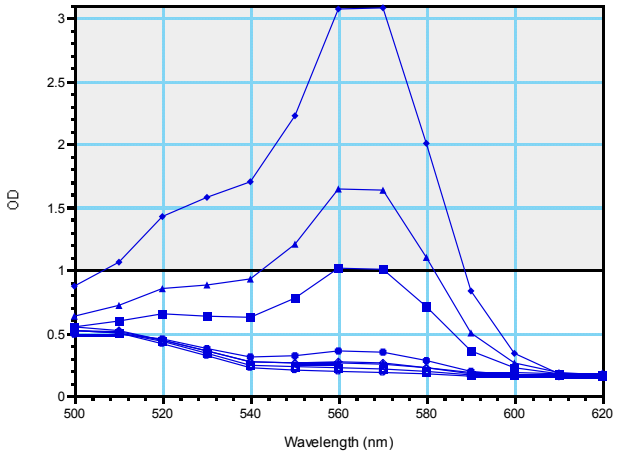
SFigure 1: Fluorescence emission spectra of the rhodamine-hydroxamate assay solution as compared to rhodamine B in DMF



SFigure 2: Fluorescence excitation spectrum of rhodamine-hydroxamate assay solution (A1) as compared to rhodamine B in DMF (A2)

Assay sensitivity determination:

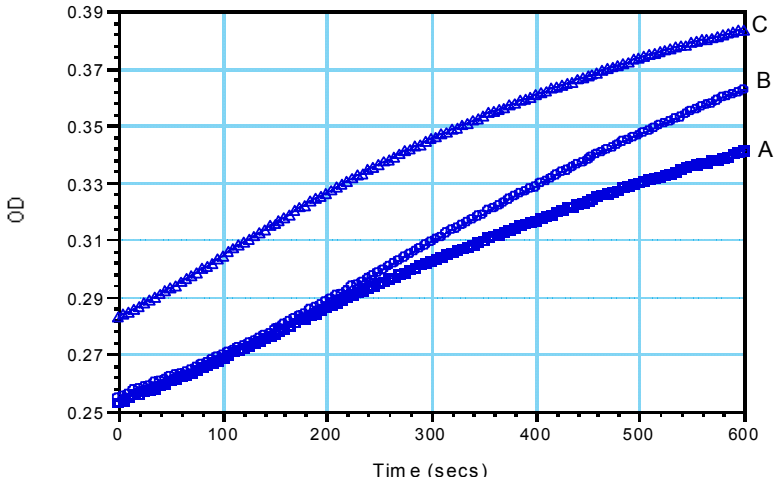
Diethyl chlorophosphate (2 μL) was added into a serial of DMF solutions containing rhodamine-hydroxamate (1 mg/mL) and 3% (v/v) triethylamine to a final volume of 4 mL, 8 mL, 12 mL, 20 mL, 30 mL, 40 mL and 80 mL (which is calculated to be 500 ppm, 250 ppm, 170 ppm, 100 ppm, 67 ppm, 50 ppm, and 25 ppm). Using rhodamine-hydroxamate solution as the control, the reaction solutions were either directly detected for fluorescence emission (Ex 560 nm) or UV-vis absorbance on Spectamax-M5 (Molecular Devices) to monitor the rate of color development, or incubated at room temperature for about 20 minutes and then subjected for fluorescence emission (for assay sensitivity determination).



SFigure 3: Uv-vis absorbance spectra of Rhodamine-hydroxamate based colorimetric detection of diethyl chlorophosphate at various concentrations (from top to bottom: 500 ppm, 250ppm,170 ppm, 100 ppm, 50 ppm, 25 ppm, and 0ppm)

Effects of water on the Assay

Diethyl chlorophosphate (2 µL) was added into three DMF solutions containing rhodamine-hydroxamate (1mg/mL) and 3% (v/v) triethylamine containing that were pre-included with no water, 400 µL or 1mL of water. The rates of color development of the three reaction solutions were directly detected for UV-vis absorbance at 560 nm on Spectamax-M5 (Molecular Devices).



SFigure 4: Time dependant color development of rhodamine-hydroxamate (1 mg/mL) based detection of diethyl chlorophosphate (100 ppm) in anhydrous DMF (A) as compared to the assays in presence of 2% (v/v, line B) and 5% (v/v, line C) water. The OD was recorded at 560 nm.