Chromo-fluorogenic Detection of Aldehydes with a Rhodamine Based Sensor Featuring an Intramolecular Deoxylactam

Zhu Li,[1,2,3] Zhongwei Xue,[1,3] Zhisheng Wu,[1] Jiahuai Han[2] and Shoufa Han[1,*]

[1]Department of Chemical Biology, College of Chemistry and Chemical Engineering, and the Key Laboratory for Chemical Biology of Fujian Province; and [2]State Key Laboratory of Cellular Stress Biology and School of Life Sciences, Xiamen University, Xiamen, Fujian 361005, China; Tel: 86-0592-2181728;

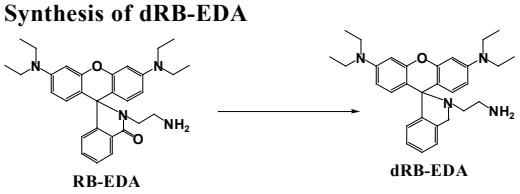
E-mail: shoufa@xmu.edu.cn

Experimental Procedures

Bafilomycin A was purchased from Sigma. All other chemical reagents were obtained from Alfa Aesar and used without further purification. *N*-(rhodamine B)-lactam-ethylenediamine was prepared according to a published procedure.[1] *N*-(rhodamine B)-deoxylactam-amine was prepared according to a literature.[2] The synthesis of *N*-(rhodamine B)-deoxylactam-2-aminoethanol will be reported elsewhere. Column chromatography was performed on silica gel (300-400 mesh). NMR spectra (^{1}H-400 MHz and ^{13}C-100 MHz) were recorded on a Bruker instrument using tetramethyl silane as the internal reference. The fluorescence spectra and Uv-vis absorption spectra were performed on a spectrofluorimeter (Spectamax M5, Molecular Device) using the excitation wavelength (λex) of 560 nm. Melting points were determined on a Yanaco MP-500 melting point apparatus，IR spectra were recorded on a Nicolet Avatar 330 FT-IR spectrophotometer. The mass analysis was performed on Bruker En Apex ultra 7.0T FT-MS.

L929 cells, obtained from American Type Culture Collection, were grown at 37 °C under 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco; Invitrogen) supplemented with 10% fetal bovine serium. Confocal microscopic images were obtained on LeicaSP2 using the following filters: λex@560 nm and λem@580-650 nm. Fluorescence images were merged using Photoshop CS 3.0. Graph by GraphPad Prism5 software.

Synthesis of dRB-EDA



Scheme S1. synthesis of dRB-EDA

N-(rhodamine B)-lactam-ethylenediamine (2 g) was dissolved in anhydrous THF (30 ml). To the solution was added lithium aluminium hydride (1 g). The mixture was stirred at room temperature under argon overnight. 1-Butanol (200 ml) was added gradually to the solution to quench residual lithium aluminium hydride, and then the mixture was washed with water (200 ml). The organic layer was collected, dried over sodium sulfate, and then concentrated to remove the solvent. The residue was purified by silica gel coloum chromatography using dichloromethane/hexanes/triethylamine (10:10:1 v/v/v) as the eluent to give 0.8 g of pale yellow solid as the desired product (40% yield, melting points: 60-61°C). ^{1}H-NMR (400 MHz, $CDCl_3$), δ:7.35 (d, 1H, J = 7.00 Hz), 7.30~7.25 (m, 1H), 7.20 (t, 1H, J = 7.30 Hz), 6.92 (d, 1H, J = 7.52 Hz), 6.68 (d, 2H, J = 8.72 Hz), 6.37~6.31 (m, 4H), 4.19 (s, 2H), 3.35 (q, 8H, J = 6.98 Hz), 2.58 (t, 2H, J = 5.30 Hz), 2.34 (t, 2H, J = 5.64 Hz), 1.93 (s, 2H), 1.18 (t, 12H, J = 7.02 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$):152.88, 149.46, 147.91, 139.08, 130.37, 127.55, 126.77, 124.59, 121.78, 111.65, 107.52, 97.51, 67.98, 56.08, 51.55, 44.30, 40.12, 12.71 ppm; MS ($C_{30}H_{38}N_4O$): calculated (MH^+): 471.3118, found: 471.3122.

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

Formaldehyde was added into 20 mL of DMF solutions containing dRB-EDA (1 mg ml^{-1}) to a final concentration of 17.5 mM. The solution was incubated at room temperature for 2 hours and then an aliquot was taken for analysis by mass spectrometry and fluorescence emission as compared to rhodamine B in DMF solution.

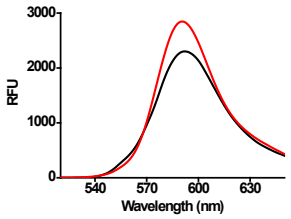


Fig. S1 Fluorescence emission spectra of dRB-EDA assay solution (in dark) as compared to that of rhodamine B in DMF (in red)

Reaction rates of dRB-EDA in selected solvents

dRB-EDA, was respectively added into various solvents containing formaldehyde (17.5 mM) to a final concentration of 1 mg ml^{-1}. The rates of color development of the correponding solutions were directly monitored as function of time by UV-vis absorbance at 560 nm.

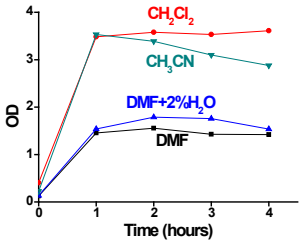


Fig. S2 Effects of solvents on the chromogenic reaction between dRB-EDA and formaldehyde

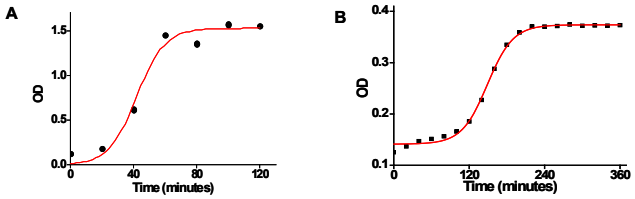


Fig. S3 Time dependant color formation of dRB-EDA (1 mg ml^{-1}) in DMF containing formaldehyde (17.5 mM, **A**; or 3.5 mM, **B**).

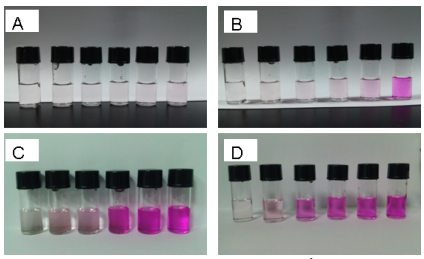
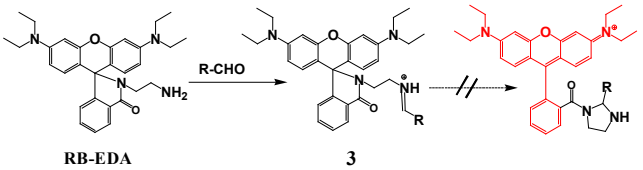


Fig. S4 Time dependant color formation of dRB-EDA (1 mg ml^{-1}) in DMF containing formaldehyde (0, 3.5, 7, 10.5, 14, 17.5). Incubation time: 0 (A), 0.5 (B), 1 (C), or 1.5 hour (D).



Scheme S2. Possible mechanism for nonchromogenic response of RB-EDA with aldehydes.

Differential chromogenic resoponses of dRB-EDA and its structural analogs with formaldehyde in DMF

dRB-EDA, dRB-EA, dRM-amine and RB-EDA were respectively added into DMF containing formaldehyde (17.5 mM) to a final concentration of 1 mg ml^{-1}. Using dRB-EDA solution as the control, the rates of color development of the correponding solutions were directly monitored as function of time by UV-vis absorbance at 560 nm.

Assay sensitivity of dRB-EDA for formadehyde in DMF:

Formaldehyde was added into a serial of DMF solutions containing dRB-EDA (1 mg ml^{-1}) to a final concentration of 0, 3.5, 7, 10.5, 14, 17.5, 35 mM. Using dRB-EDA solution as the control, the reaction solutions incubated at room temperature for about 2 hours and then directly detected by UV-vis absorbance.

Assay sensitivity for aldehydes in DMF

Aliquots of the stock slution of hexanaldehyde, 4-hydroxylbenzaldehyde, D-(+)-glucose, or acetone were respectively added into a serial of DMF solutions containing dRB-EDA (1 mg ml^{-1}) to various concentrations. Using dRB-EDA solution as the control, the reaction solutions incubated at room temperature for about 2 hours and then directly detected by UV-vis absorbance at 560 nm.

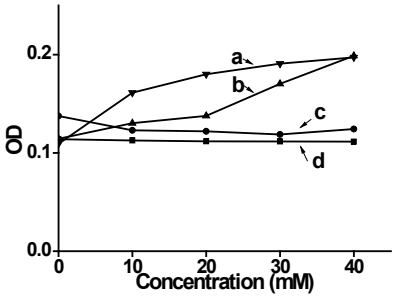


Fig. S5 Chromogenic detection of hexanaldehyde (a), 4-hydroxylbenzaldehyde (b), glucose (c) and acetone (d) with dRB-EDA in DMF. The absorbance of dRB-EDA in the presence of analytes at 560 nm were respectively recorded. The concentration of the analytes uased was 0, 10, 20, 30, 40 mM as indicated in the figure.

Labeling of cell-surface sialoproteins with dRB-EDA in live cells

L929 cells, incubated with DMEM containing bafilomycinA1(50nM) for 6 hours, were treated with or without sodium periodate(1mM, pH 6.8) for 5-20 minutes on ice. Cells were then washed with PBS for 2 times and incubated in dRB-EDA (0.1mg/ml) for 1 minute. Cells were then washed with cold PBS for 3 times and analyzed with Leica SP2 laser confocol scaning microscope.

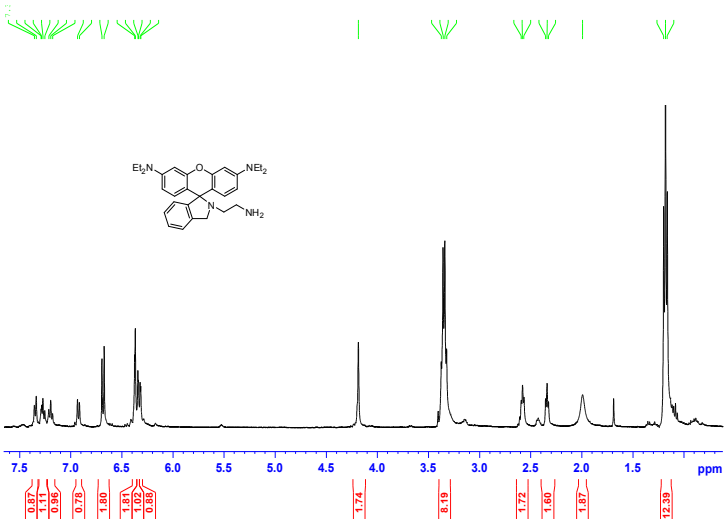


Fig. S6 ^{1}H-NMR spectrum of dRB-EDA

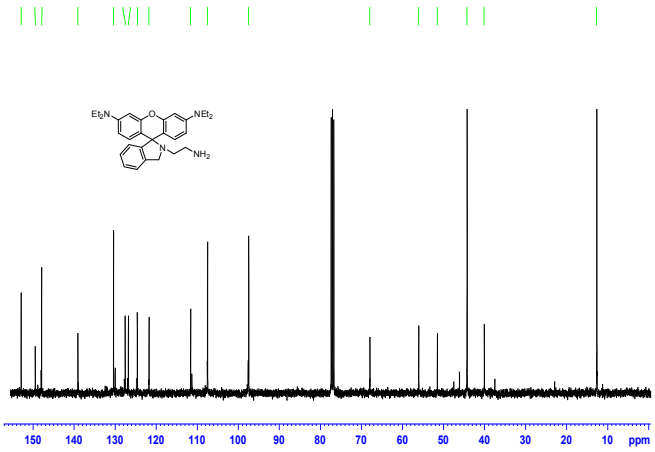


Fig. S7 ^{13}C-NMR spectrum of dRB-EDA

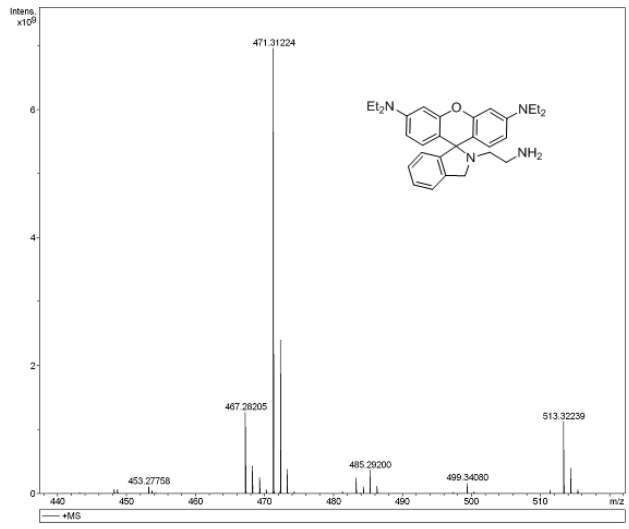


Fig. S8 Mass spectrometry spectrum of dRB-EDA

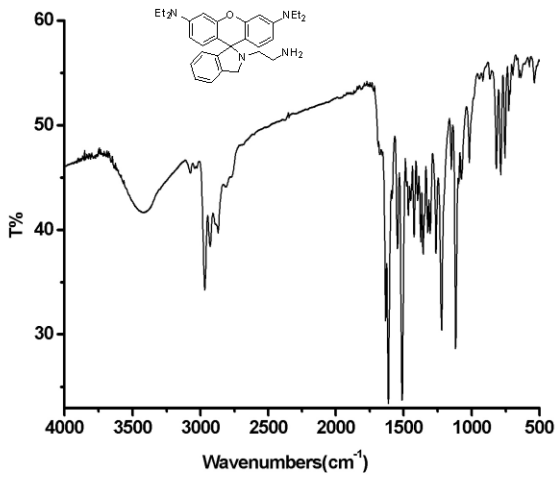


Fig. S9 IR spectrum of dRB-EDA

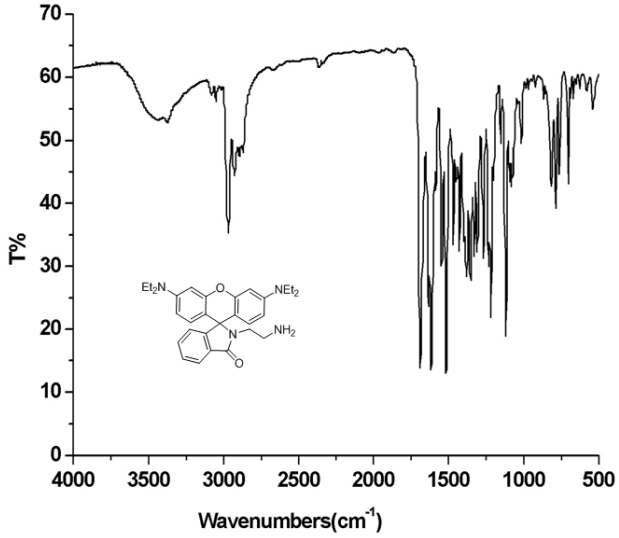


Fig. S10 IR spectrum of RB-EDA

References

1 J. S. Wu, I. C. Hwang, K. S. Kim, J. S. Kim, *Org. Lett.,* 2007, **9**, 907.

2 Q. A. Best, R. Xu, M. E. McCarroll, L. Wang, D. J. Dyer, *Org. Lett.,* 2010, **12**, 3219.