

Supplementary data

A rhodamine B-based lysosomal pH probe

Shi-Li Shen^a, Xin-Peng Chen^b, Xiao-Fan Zhang^a, Jun-Ying Miao^{*,b} and Bao-Xiang

Zhao^{*,a}

*^aInstitute of Organic Chemistry, School of Chemistry and Chemical Engineering,
Shandong University, Jinan 250100, P.R. China*

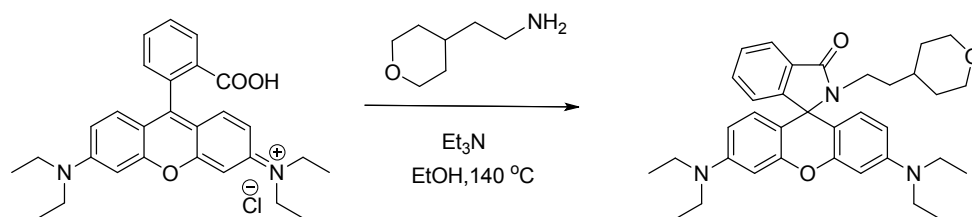
*^bInstitute of Developmental Biology, School of Life Science, Shandong University,
Jinan 250100, P.R. China*

Table of Contents

1. Synthesis of compound R1	2
2. Table S1 The properties of RML and other pH probes.....	3

* Corresponding authors. Tel.: +86 531 88366425; fax: +86 531 88564464;
e-mail addresses: bxzhao@sdu.edu.cn and miaojy@sdu.edu.cn

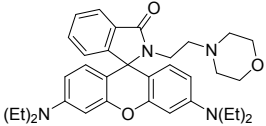
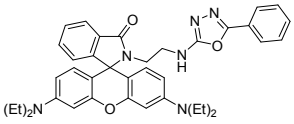
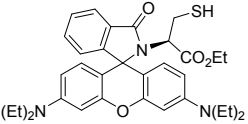
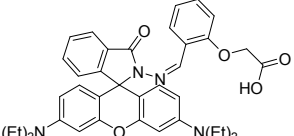
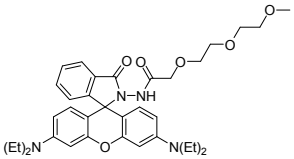
1. Synthesis of compound **R1**.



Scheme S1. Synthesis of compound **R1**

Rhodamine B (0.153 g, 0.32 mmol), 4-(2-aminoethyl)tetrahydropyran (0.074 g, 0.57 mmol) and Et_3N (0.101 g, 1 mmol) were dissolved in ethanol (5 mL), and the reaction mixture was stirred at $140\text{ }^\circ\text{C}$ for 60 h. After the solution was cooled to room temperature, the solution was concentrated under reduced pressure and the residue was purified by column chromatography (Petroleum ether/Ethyl acetate = 1/1, v/v) to afford a white solid **R1** in 41% yield. Mp: $58\text{-}61\text{ }^\circ\text{C}$. IR (KBr), v/cm^{-1} : 2968, 2926, 1689, 1613, 1514, 1266, 1223, 1117, 1015, 788. ^1H NMR (DMSO, 300 MHz), δ : 0.75-1.00 (m, 4H, $\text{CH}(\text{CH}_2\text{CH}_2)_2\text{O}$), 1.08 (t, 12H, $J = 6.9\text{ Hz}$, NCH_2CH_3), 1.23-1.28 (m, 3H, $\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_2)_2\text{O}$), 2.95-3.10 (m, 4H, $(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.32 (q, 8H, $J = 6.9\text{ Hz}$, NCH_2CH_3), 3.62-3.66 (m, 2H, $\text{CONCH}_2\text{CH}_2$), 6.26-6.38 (m, 6H, Xanthene-H), 7.04-7.09 (m, 1H, ArH), 7.48-7.55 (m, 2H, ArH), 7.76-7.79 (m, 1H, ArH); ^{13}C NMR (DMSO, 75 MHz), δ : 166.64, 152.90, 152.77, 148.33, 132.51, 131.09, 128.50, 128.28, 123.66, 122.12, 108.14, 105.26, 97.12, 66.79, 63.98, 43.66, 36.57, 34.53, 32.27, 31.34, 12.31; HRMS calcd for $\text{C}_{35}\text{H}_{44}\text{N}_3\text{O}_3$ $[\text{M}+\text{H}]^+$: 554.3383, found: 554.3383.

2. Table S1. The properties of **RML** and other pH probes.

Probes	In vitro			Cell experiment		Reference	
	Probe concentration	Solvent	Emission intensity increase	Response time	Probe concentration		Incubation time
	1 μ M	DMSO: buffer = 1:99 (v:v)	80-fold	1.5 min	2.5 μ M	1h	This work
	10 μ M	EtOH: buffer = 1:9 (v:v)	46-fold	About 1 min	3 μ M	12h	32
	10 μ M	EtOH: buffer = 1:4 (v:v)	150-fold	5 min	5 μ M	3h	33
	10 μ M	EtOH: buffer = 1:9 (v:v)	20-fold	About 2 min	0.5 μ M	12h	37
	10 μ M	DMSO: buffer = 0.33:99.67 (v:v)	50-fold	several seconds	10 μ M	0.5 h	14