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Fused oxazolidine-based dual optical probe for galactosidase with a dramatic chromogenic and fluorescence turn-on effect

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ABSTRACT

A dual optical probe based on a fused oxazolidinoindocyanine moiety was prepared for detection of β-galactosidase in the biological condition. The probe displayed both a dramatic colorless-to-red optical change and a fluorescence turn-on response in the visible region, exhibiting a high sensitivity for β-galactosidase with a very low limit of detection (LOD = 0.071 U/L) in neutral PBS buffer.

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1. Introduction

β-Galactosidase (β-Gal) is a kind of glycoside hydrolase enzyme. Substrates for β-Gal include carbohydrates containing β-galactose such as ganglioside GM1, lactosylceramides, and lactose. β-Gal is a very important enzyme as the energy provider in the organisms. It produces energy through the breakdown of lactose to galactose and glucose. Moreover, the enzyme has been reported to have a close relationship with cellular senescence and is widely used as a biomarker to monitor senescence and aging cells in biology [1–3]. A well-known biomarker for β-Gal is 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Scheme 1) [4]. X-Gal is cleaved by β-Gal, producing a blue indigo dye but the resulting dye does not display either fluorescence nor good solubility in water with a poor detection limit for β-Gal (0.26 mM). In order to obtain in vivo imaging as well as good limit of detection of β-Gal activity, it is necessary to develop a water soluble and fluorescent dye for the hydrolysis product as well as the substrate of β-Gal.

Recently, several groups have tried to synthesize fluorescent probes for β-Gal and successfully applied them for in vivo detection of the enzyme [5–11]. However, the sensitivity of reported probes for β-Gal was not such a better value than 0.17 unit/L [5]. Moreover, the water solubility of probes and their hydrolysis products still remains challenging. Therefore, we tried to prepare a dual optical probe with a good water solubility even for the hydrolysis product and to enhance the sensitivity for β-Gal. Herein we report an oxazolidinoindocyanine-based dual optical probe, which exhibits both a chromogenic response and a fluorescence turn-on effect in the presence of β-Gal with high sensitivity for β-Gal as well as good water solubility for the hydrolysis product.

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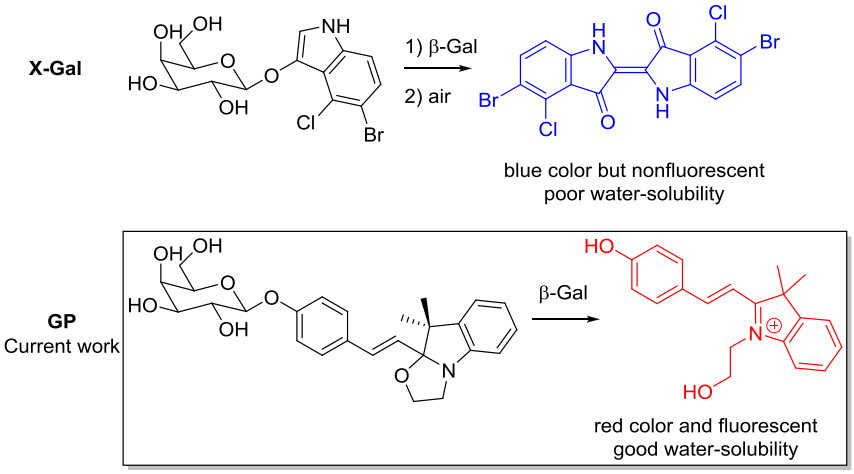
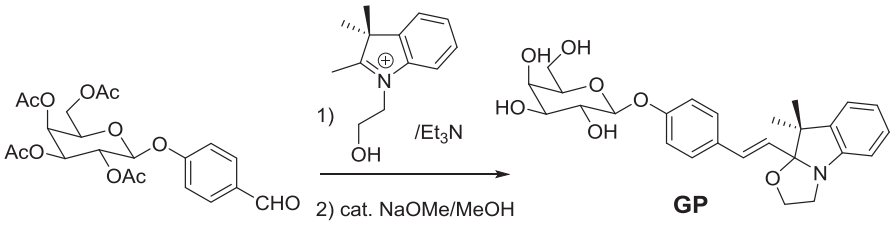


Scheme 1. Comparison of X-Gal and **GP.**

2. Materials and methods



2.1. Preparation of *AcGP*

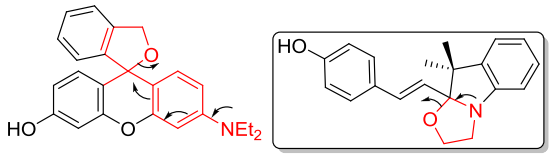
To a solution of acylated galactose tethered *p*-hydroxybenzaldehyde (450 mg, 1 mmol) and pyrrolidine (0.01 mL, 0.2 mmol) in 4 mL CH_3CN, was added hydroxyethylindolium bromide (283 mg, 1 mmol) [12]. The resulting solution was refluxed for 12 h and then evaporated under reduced pressure. Further purification was performed by column chromatography to afford **AcGP** as a yellowish solid in 8% yield (54 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, J = 8.8 Hz, 2 H), 7.19 (t, J = 7.6 Hz, 1 H), 7.10 (d, J = 7.6 Hz, 1 H), 7.00 (d, J = 8.8 Hz, 2 H), 6.97 (t, J = 7.6 Hz, 1 H), 6.87–6.81 (m, 2 H), 6.21 (d, J = 16.0 Hz, 1 H), 5.53–5.48 (m, 2 H), 5.14 (dd, J = 10.4 Hz, J = 3.6 Hz, 1 H), 5.06 (d, J = 8.0 Hz, 1 H), 4.28–4.16 (m, 2 H), 4.08 (t, J = 6.4 Hz, 1 H), 3.82–3.39 (m, 4 H), 2.21 (s, 3 H), 2.09 (s, 6 H), 2.04 (s, 3 H), 1.46 (s, 3 H), 1.18 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 179.1, 169.3, 156.6, 150.5, 139.7, 131.8, 131.2, 127.9, 127.6, 125.6, 122.4, 121.7, 117.1, 112.0, 109.8, 99.7, 71.0, 70.8, 68.6, 66.8, 63.5, 61.3, 50.1, 47.96, 47.93, 28.4, 20.7, 20.6, 20.5, 20.3. HRMS (MALDI$^+$, DHB): m/z obsd. 638.2598 ([M+H]$^+$, calcd. 638.2597 for $C_{34}H_{39}N_1O_{11}$).

2.2. Preparation of *GP*

To a solution of **AcGP** (54 mg) in MeOH was added catalytic amount of NaOMe (2 mg) and the reaction mixture was further stirred at rt for 1 h. After all volatiles were removed under reduced pressure, the mixture was purified by column chromatography to afford **GP** as a yellowish solid in 90% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.42 (d, J = 8.8 Hz, 2 H), 7.15 (t, J = 7.6 Hz, 1 H), 7.12–7.08 (m, 3 H), 6.92 (t, J = 7.6 Hz, 1 H), 6.85–6.81 (m, 2 H), 6.20 (d, J = 16.0 Hz, 1 H), 4.90 (d, J = 8.0 Hz, 1 H), 3.93–3.39 (m, 10 H), 1.42 (s, 3 H), 1.15 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): δ 157.6, 150.4, 139.7, 131.7, 130.7, 127.4, 127.2, 123.8, 121.9, 121.5, 116.5, 111.9, 109.8, 101.4, 75.6, 73.4, 70.8, 68.8, 63.1, 61.0, 53.4, 40.5, 27.4, 19.3. HRMS (MALDI$^+$, DHB): m/z obsd. 469.2093 ([M]$^+$, calcd. 469.2096 for $C_{26}H_{31}N_1O_7$).

2.3. UV–vis and fluorescence measurements

A stock solution (10 mM) of **GP** in DMSO was prepared and used by diluting with a PBS buffer (1X, pH 7.4). For UV–vis measurement, a sample solution was prepared by mixing 10 μL of a stock solution of **GP** (10 mM in DMSO) with an appropriate amount of enzyme and finally diluted with 10 mL of PBS buffer (1X, pH 7.4) to afford the desired concentration of **GP** (10 μM). In a similar manner, fluorescence measurement was carried out with a slit width of 5 nm × 5 nm.



Scheme 2. Design principle of **GP** relative to rhodol.