

Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI) for

Synthesis of Precipitating Chromogenic/Fluorogenic β -Glucosidase/ β -Galactosidase Substrates by a New Method and Their Application in the Visual Detection of Foodborne Pathogenic Bacteria

Xianhu Wei,^{a,b,c} Qingping Wu,^{*b} Jumei Zhang,^b Youxiong Zhang,^{a,b,c} Weipeng Guo,^b Moutong Chen,^b Qihui Gu,^{b,d} Zhihe Cai^e
and Mianfei Lu^e

^a. Guangzhou Institute of Chemistry, Chinese Academy of Sciences, Guangzhou 510650, China.

^b. Guangdong Institute of Microbiology, State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, Guangzhou 510070, China. E-mail: wuqp203@163.com

^c. University of Chinese Academy of Sciences, Beijing 100039, China.

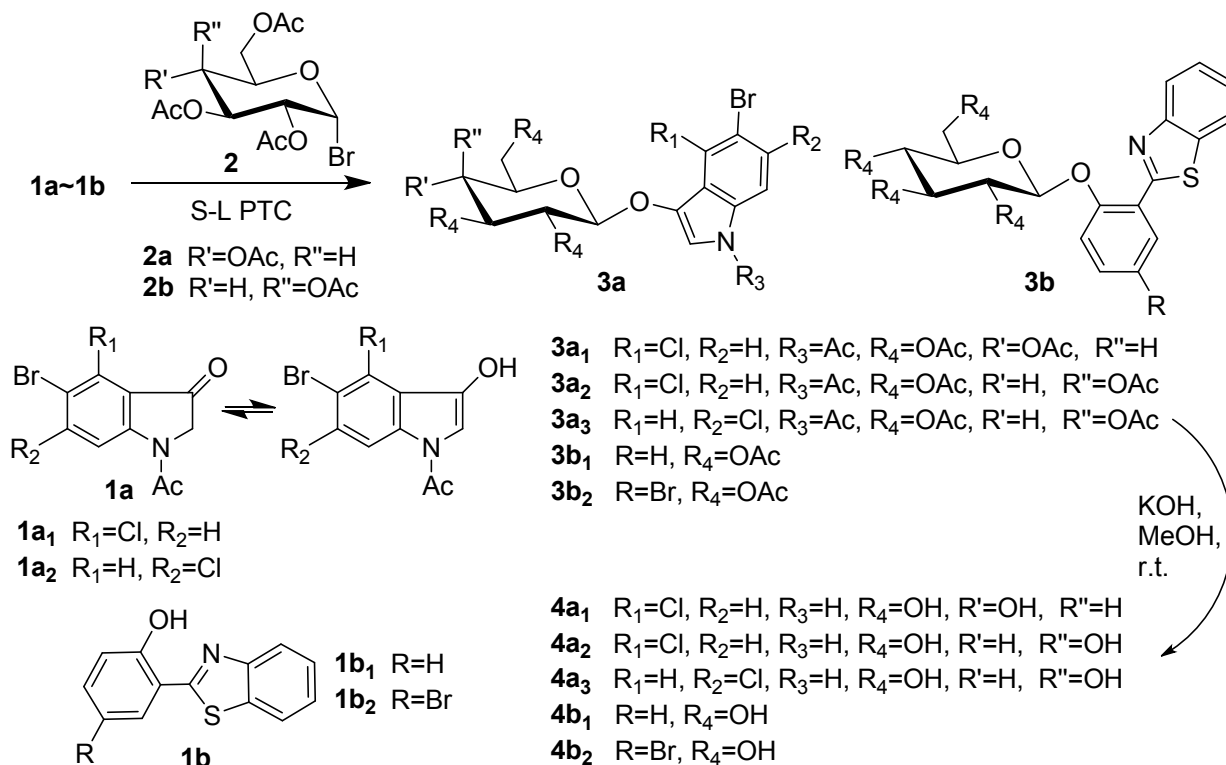
^d. School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, China.

^e. Guangdong Huankai Microbial Sci. & Tech. Co., Ltd., Guangzhou 510663, China.

Experimental section

Materials and apparatus

All chemicals were purchase from commercial sources. Deionized water was used to prepare all aqueous solutions. Petroleum ether (PE) refers to the fraction boiling in the 60–90 °C range. Purified β -glucosidase from almonds was supplied by Fluka. Columbia agar, nutrient broth, *Klebsiella pneumonia* CMCC 46117, *Salmonella typhimurium* ATCC 14028, *Streptococcus faecalis* ATCC 29212 and *Bacillus cereus* CMCC 63301 were obtained from Guangdong Huankai Microbial Sci. & Tech. Co., Ltd. Stock solution of 10 mM each synthetic substrate was obtained by dissolving the material in DMSO. 365 nm UV-light is from UV analyzer with a 365 nm UV-light lamp. Thin-layer chromatography (TLC) was performed on silica gel GF-254 plates. Column chromatography was performed using silica gel (300–400 mesh; Haiyang, Qingdao) using a PE/EtOAc system as eluent. Specific rotations were measured with Anton Paar MCP 500 automatic digital polarimeter. Melting points were measured with a SGW X-4A microscope melting point apparatus (Jingke, Shanghai). NMR spectra in CDCl₃ or DMSO-d₆ were measured with a 300 or 500 MHz Bruker spectrometer using TMS as reference for ¹H and ¹³C NMR. High-resolution mass spectra (HRMS) were measured with Bruker maXis or maXis impact. All bacteria cell images were taken by Carl Zeiss LSM 700 confocal laser microscopic system.



Scheme 1 Synthesis of some indoxyl glycosides and novel precipitating fluorogenic glucosides

Synthesis (Scheme 1)

General procedure for the glycosylation step

To a mixture of the acceptor (1.00–8.00 mmol), TBAHS or TBAB (0.20 equiv.), K_3PO_4 (5.00 equiv.) under an argon-containing atmosphere, water (5% w/w, based on the base weight), and CH_2Cl_2 (10–80 mL) were added successively (without addition of water when using TBAHS [0.2 equiv.]/KOH [5.00 equiv.]). After about 10 min of stirring at room temperature (RT, 25–28°C), the respective donor (2.50 equiv.) was added, and the reaction mixture was then stirred at RT or under reflux until TLC showed that the acceptor disappeared or that there were no additional changes for a certain time. The organic phase was separated and dried by anhydrous sodium sulphate, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (petrol ether/EtOAc, from 5/1 to 9/4, [v/v]), and the desired product was then crystallized from ethanol. The yields were calculated based on the acceptors.

General procedure for the deprotection step

A solution of the starting material (0.485–3.587 mmol, 0.300–2.000 g) in anhydrous MeOH or MeOH/THF (1:1; 30 mL/g) was treated with KOH (1.0 M in anhydrous MeOH; 0.15 equiv.). The solution was stirred for 1 h at RT. If the product precipitated in large amounts, it was collected by filtration; otherwise, the reaction mixture was concentrated. The product was then washed with dichloromethane (CH_2Cl_2) or a small amount of ice-cold water and dried.

Visual detection of purified β -glucosidase activity

To a mixture of acetic acid-sodium acetate buffer solution (0.2 M, pH 5.0, 1974 μL) and a stock solution of the synthetic substrate (10 mM, 16 μL), we added a stock solution of purified β -glucosidase (1 $\mu\text{g}/\mu\text{L}$, 20 μL). The mixture was then incubated at 37°C under aerobic conditions. Control incubations were carried out using inactive β -glucosidase obtained by boiling treatment of E^+ for 5 min. The responses of the substrates were examined under visible light and UV light (365 nm).

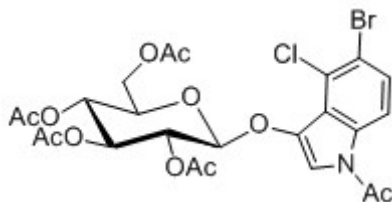
General procedure for the visual detection of foodborne pathogenic bacteria

The substrates (100 μM each) were prepared in Columbia agar or nutrient broth with or without isopropyl- β -D-thiogalactoside (IPTG; 0.06 g/L). The media were autoclaved at 115°C for 15 min. Agar plates were then prepared and dried to remove excess moisture. Plates and test tubes containing nutrient broth were inoculated with the bacteria under,

sterile conditions, and incubated at 37°C under aerobic conditions for 24–70 h.

Cell imaging of the bacteria

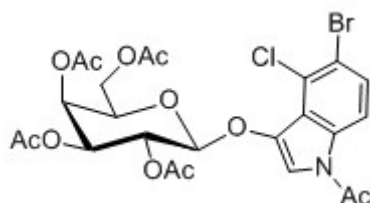
Colonies from respective bacterial strains on Columbia agar were sampled using small loops or pipette tips and suspended equally in 0.85% sterile physiological saline under sterile conditions. A small amount of the suspension was dripped on a large, clean glass cover slip and covered by a small, clean glass cover slip. After drying in air, the sample was examined by confocal laser microscopy.



(*N*-Acetyl-5-bromo-4-chloroindol-3-yl) 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranoside (**3a₁**)

Prepared According to General Procedure for the glycosylation step: Compound **1a₁** (289 mg, 1.00 mmol), TBAHS (68 mg, 0.20 mmol) or TBAB (65 mg, 0.20 mmol), K₃PO₄ (1062 mg, 5.00 mmol), H₂O [53.1 μ L, 5% (w/w)], CH₂Cl₂ (10 mL), compound **2a** (1028 mg, 2.50 mmol), reaction for 10 h at RT, or for 4 h under reflux. [or Compound **1a₁** (289 mg, 1.00 mmol), TBAHS (68 mg, 0.20 mmol), KOH (281 mg, 5.00 mmol), without addition of H₂O, CH₂Cl₂ (10 mL), compound **2a** (1028 mg, 2.50 mmol), reaction for 3 h at RT.] Colourless solid; yield: 285 mg (46%); mp 162–163 °C; $[\alpha]_D^{25} = -72.5^\circ$ ($c =$

0.20, MeCN); $R_f = 0.16$ (PE/AcOEt = 2/1); {lit.,¹⁶ mp 161–162 °C; $[\alpha]_D^{25} = -29.3^\circ$ ($c = 0.15$, CHCl₃); $R_f = 0.20$ (PE/AcOEt = 1/1)}; ¹H-NMR (300 MHz, CDCl₃): $\delta = 8.21$ (d, $J = 8.9$ Hz, 1H, CH_{arom}), 7.53 (d, $J = 8.9$ Hz, 1H, CH_{arom}), 7.28 (s, 1H, =CH-N), 5.37 (m, 1H, 2-H), 5.30 (t, $J = 9.0$ Hz, 1H, 3-H), 5.19 (t, $J = 9.3$ Hz, 1H, 4-H), 5.00 (d, $J_{1,2} = 7.3$ Hz, 1H, 1-H), 4.40 (dd, $J_{6a,6b} = 12.3$, $J_{5,6a} = 2.1$ Hz, 1H, 6a-H), 4.18 (dd, $J_{6a,6b} = 12.4$, $J_{5,6b} = 5.0$ Hz, 1H, 6b-H), 3.85 (ddd, $J_{4,5} = 9.8$, $J_{5,6b} = 4.8$, $J_{5,6a} = 2.2$ Hz, 1H, 5-H), 2.61 (s, 3H, NCOCH₃), 2.10, 2.10, 2.06, 2.05 (4xs, 4x3H, 4xOCOCH₃) ppm; ¹³C-NMR (75 MHz, CDCl₃): $\delta = 170.52$ (NCO), 170.21, 169.43, 169.26, 168.17 (4xOCO), 139.59, 133.17, 130.36, 124.86, 122.36, 118.30, 116.05, 112.65 (8xC_{arom}), 100.44 (C-1), 72.48, 72.48, 70.63, 68.29, 61.90 (C-2, C-3, C-4, C-5, C-6), 23.86 (NCOCH₃), 20.81, 20.77, 20.62, 20.61 (4xOCOCH₃) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₂₄H₂₅BrClNNaO₁₁: 640.0192; found 640.0196.

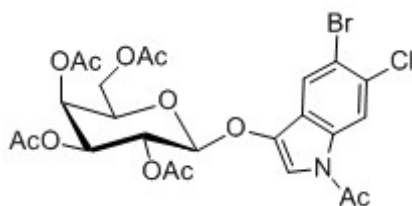


(*N*-Acetyl-5-bromo-4-chloroindol-3-yl) 2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranoside (**3a₂**)

Prepared According to General Procedure for the glycosylation step: Compound **1a₁** (289 mg, 1.00 mmol), TBAHS (68 mg, 0.20 mmol), K₃PO₄ (1062 mg, 5.00 mmol), H₂O [53.1 μ L, 5% (w/w)], CH₂Cl₂ (10 mL), compound **2b** (1028 mg, 2.50 mmol), reaction for 4 h at RT. White crystal; yield: 353 mg (57%); mp 184–185 °C; $[\alpha]_D^{25} = -44.8^\circ$ ($c = 0.30$, MeCN); $R_f = 0.19$

(PE/AcOEt = 2/1); {lit.,¹⁶ mp 184–186 °C; $[\alpha]_D^{25} = -40^\circ$ ($c = 0.5$, CHCl₃); $R_f = 0.24$ (PE/AcOEt = 1/1)}; ¹H-NMR (500 MHz, DMSO-*d*₆): $\delta = 8.21$ (d, $J = 8.9$ Hz, 1H, CH_{arom}), 7.66 (d, $J = 8.9$ Hz, 1H, CH_{arom}), 7.54 (s, 1H, C=CH-N), 5.37 (m, 2H, 1-H, 4-H), 5.35 (dd, $J_{2,3} = 10.0$, $J_{1,2} = 8.0$ Hz, 1H, 2-H), 5.28 (dd, $J_{2,3} = 10.0$, $J_{2,3} = 3.5$ Hz, 1H, 3-H), 4.49 (dd, $J_{5,6b} = 6.5$, $J_{5,6a} = 5.5$ Hz, 1H, 5-H), 4.21 (dd, $J_{6a,6b} = 11.5$, $J_{5,6a} = 5.0$ Hz, 1H, 6a-H), 4.13 (dd, $J_{6a,6b} = 11.3$, $J_{5,6b} = 7.3$ Hz, 1H, 6b-H), 2.63 (s, 3H, NCOCH₃), 2.17, 2.06, 2.04, 1.97 (4xs, 4x3H, 4xOCOCH₃) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): $\delta = 170.49$ (NCO), 170.47, 170.00,

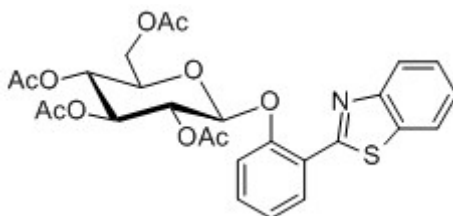
169.52, 169.52 (4×OCO), 139.24, 133.14, 130.49, 124.08, 122.02, 117.57, 116.63, 112.47 (8×C_{arom}), 100.00 (C-1), 71.33, 70.53, 68.40, 67.81, 62.24 (C-2, C-3, C-4, C-5, C-6), 24.28 (NCOCH₃), 21.01, 21.01, 20.88, 20.81 (4×OCOCH₃) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₂₄H₂₅BrClNNaO₁₁: 640.0192; found 640.0191.



(N-Acetyl-5-bromo-6-chloroindol-3-yl) 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside (3a₃)

Prepared According to General Procedure for the glycosylation step: Compound **1a₂** (289 mg, 1.00 mmol), TBAHS (68 mg, 0.20 mmol), K₃PO₄ (1062 mg, 5.00 mmol), H₂O [53.1 μL, 5% (w/w)], CH₂Cl₂ (10 mL), compound **2b** (1028 mg, 2.50 mmol), reaction for 3 h at RT. Colourless solid; yield: 400 mg (65%); mp 189–191 °C; $[\alpha]_D^{25} = -37.1^\circ$ (c = 0.30, MeCN); *R_f* = 0.17

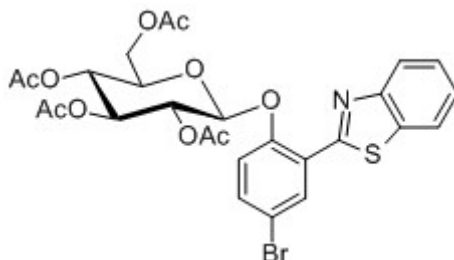
(PE/AcOEt = 2/1); {lit.,¹¹ mp 178–179 °C; $[\alpha]_D^{23} = -20^\circ$ (c = 1.0, acetone); lit.,¹⁶ $[\alpha]_D^{29} = -11.4^\circ$ (c = 0.28, CHCl₃); *R_f* = 0.57 (Et₂O)}; ¹H-NMR (500 MHz, DMSO-d₆): δ = 8.47 (s, 1H, H_{arom}), 7.71 (s, 1H, H_{arom}), 7.62 (s, 1H, H_{arom}), 5.42 (d, *J*_{1,2} = 7.5 Hz, 1H, 1-H), 5.38 (d, *J*_{3,4} = 3.0 Hz, 1H, 4-H), 5.30 (dd, *J*_{2,3} = 10.3, *J*_{3,4} = 3.5 Hz, 1H, 3-H), 5.26 (dd, *J*_{2,3} = 10.5, *J*_{1,2} = 7.5 Hz, 1H, 2-H), 4.43 (t, *J* = 6.2 Hz, 1H, 5-H), 4.16 (dd, *J*_{6a,6b} = 11.4, *J*_{5,6a} = 5.2 Hz, 1H, 6a-H), 4.11 (dd, *J*_{6a,6b} = 11.4, *J*_{5,6b} = 7.0 Hz, 1H, 6b-H), 2.61 (s, 3H, NCOCH₃), 2.17, 2.09, 2.00 (s), 1.97 (4×s, 4×3H, 4×OCOCH₃) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): δ = 170.44 (NCO), 170.41, 170.00, 169.81, 169.77 (4×OCO), 139.10, 132.33, 130.38, 124.24, 122.18, 117.91, 116.38, 113.24 (8×C_{arom}), 100.51 (C-1), 71.23, 70.41, 68.79, 67.67, 62.01 (C-2, C-3, C-4, C-5, C-6), 24.08 (NCOCH₃), 20.97, 20.97, 20.87, 20.81 (4×OCOCH₃) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₂₄H₂₅BrClNNaO₁₁: 640.0192; found 640.0205.



[2-(benzothiazol-2'-yl)-phenolyl] 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside (3b₁)

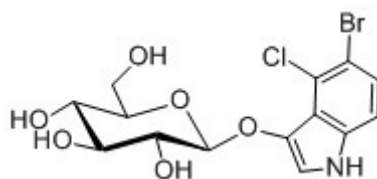
Prepared According to General Procedure for the glycosylation step: Compound **1b₁** (1135 mg, 5.00 mmol), TBAHS (400 mg, 1.00 mmol), K₃PO₄ (5307 mg, 25.00 mmol), H₂O [265 μL, 5% (w/w)], CH₂Cl₂ (50 mL), compound **2a** (5140 mg, 12.50 mmol), reaction for 7 h at reflux. White powder; yield: 2064 mg (74%); mp 173–175 °C; $[\alpha]_D^{25} = -85.8^\circ$ (c = 0.40, MeCN);

R_f = 0.32 (PE/AcOEt = 2/1); ¹H-NMR (500 MHz, DMSO-d₆): δ = 8.42 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{arom}), 8.08 (d, *J* = 8.0 Hz, 1H, H_{arom}), 8.03 (d, *J* = 7.5 Hz, 1H, H_{arom}), 7.63–7.60 (m, 1H, H_{arom}), 7.57–7.54 (m, 1H, H_{arom}), 7.49–7.45 (m, 1H, H_{arom}), 7.38 (d, *J* = 8.0 Hz, 1H, H_{arom}), 7.31–7.28 (m, 1H, H_{arom}), 5.98 (d, *J*_{1,2} = 7.5 Hz, 1H, 1-H), 5.45 (t, *J*_{2,3} = 9.5 Hz, 1H, 2-H), 5.36 (dd, *J*_{3,4} = 9.5, *J*_{4,5} = 8.0 Hz, 1H, 4-H), 5.10 (t, *J*_{2,3} = 9.7 Hz, 1H, 3-H), 4.37 (ddd, *J*_{4,5} = 8.0, *J*_{5,6a} = 5.5, *J*_{5,6b} = 2.5 Hz, 1H, 5-H), 4.22 (dd, *J*_{6a,6b} = 12.4, *J*_{5,6a} = 5.6 Hz, 1H, 6a-H), 4.12 (dd, *J*_{6a,6b} = 12.3, *J*_{5,6b} = 2.2 Hz, 1H, 6b-H), 2.04, 1.99, 1.98, 1.86 (4×s, 4×3H, 4×OCOCH₃) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): δ = 170.40, 170.15, 169.80, 169.54 (4×OCO), 162.07, 154.09, 152.02, 135.85, 132.82, 129.71, 126.89, 125.82, 123.65, 123.19, 122.48, 121.91, 115.72 (13×C_{arom}), 97.09 (C-1), 72.97, 71.52, 71.30, 68.21, 62.00 (C-2, C-3, C-4, C-5, C-6), 20.92, 20.88, 20.86, 20.77 (4×OCOCH₃) ppm; HRMS (ESI): m/z [M+H]⁺ calcd. for C₂₇H₂₈NO₁₀S: 558.1428; found 558.1434.



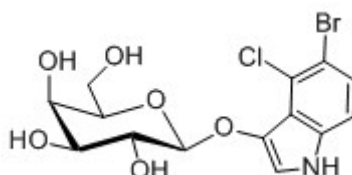
[2-(benzothiazol-2'-yl)-4-bromophenolyl] 2,3,4,6-Tetra-O-acetyl- β -D-glucopyranoside (3b₂**)**

Prepared According to General Procedure for the glycosylation step: Compound **1b₂** (2450 mg, 8.00 mmol), TBAHS (544 mg, 1.60 mmol), K₃PO₄ (8491 mg, 40.00 mmol), H₂O [425 μ L, 5% (w/w)], CH₂Cl₂ (80 mL), compound **2a** (8224 mg, 20.00 mmol), reaction for 7 h at reflux. White crystal; yield: 4582 mg (90%); mp 224.5–226.5 °C; $[\alpha]_D^{25} = -83.0^\circ$ (c = 0.40, MeCN); $R_f = 0.40$ (PE/AcOEt = 2/1); ¹H-NMR (500 MHz, DMSO-d₆): δ = 8.52 (d, J = 2.5 Hz, 1H, H_{arom}), 8.11 (d, J = 8.0 Hz, 1H, H_{arom}), 8.05 (d, J = 8.0 Hz, 1H, H_{arom}), 7.81 (dd, J = 9.0, 2.5 Hz, 1H, H_{arom}), 7.60–7.56 (m, 1H, H_{arom}), 7.51–7.48 (m, 1H, H_{arom}), 7.35 (d, J = 9.0 Hz, 1H, H_{arom}), 5.99 (d, $J_{1,2}$ = 8.0 Hz, 1H, 1-H), 5.43 (t, $J_{4,5}$ = 9.5 Hz, 1H, 4-H), 5.36 (dd, $J_{2,3}$ = 9.5, $J_{1,2}$ = 8.0 Hz, 1H, 2-H), 5.10 (t, $J_{2,3}$ = 9.5 Hz, 1H, 3-H), 4.35 (ddd, $J_{4,5}$ = 10.0, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.5 Hz, 1H, 5-H), 4.21 (dd, $J_{6a,6b}$ = 12.5, $J_{5,6a}$ = 5.5 Hz, 1H, 6a-H), 4.11 (dd, $J_{6a,6b}$ = 12.4, $J_{5,6b}$ = 2.3 Hz, 1H, 6b-H), 2.03, 1.99, 1.98, 1.88 (4xs, 4x3H, 4xOCOCH₃) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): δ = 170.42, 170.14, 169.79, 169.54 (4xOCO), 160.44, 153.20, 151.80, 135.97, 135.05, 131.51, 127.13, 126.20, 124.41, 123.43, 122.02, 118.17, 115.43 (13xC_{arom}), 97.18 (C-1), 72.87, 71.64, 71.20, 68.11, 61.94 (C-2, C-3, C-4, C-5, C-6), 20.93, 20.88, 20.85, 20.76 (4xOCOCH₃) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₂₇H₂₆BrNNaO₁₀S: 658.0353; found 658.0358.



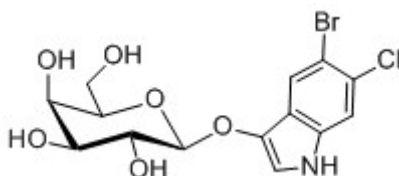
(5-bromo-4-chloroindol-3-yl) β -D-glucopyranoside (4a₁**, X-Glu)**

Prepared according to general procedure for deprotection step: Compound **3a₁** (2000 mg, 3.23 mmol), MeOH (60 mL), KOH-MeOH (1 M, 485 μ L, 0.15 molar equivalent). White powder; yield: 1175 mg (89%); mp 235–237 °C (decomp.); $[\alpha]_D^{25} = -81.2^\circ$ [c = 0.25, DMF (50% in H₂O)]; $R_f = 0.38$ (CHCl₃/MeOH = 5/1); {lit.,¹¹ mp 240–243 °C (decomp.); $[\alpha]_D^{23} = -89.0^\circ$ [c = 1.0, DMF (50% in H₂O)]; lit.,¹⁶ mp 230 °C (decomp.); $[\alpha]_D^{29} = -85.0^\circ$ [c = 0.5, DMF (50% in H₂O)], $[\alpha]_D^{25} = -10.0^\circ$ (c = 0.5, DMSO)}; ¹H-NMR (300 MHz, DMSO-d₆): δ = 11.09 (d, J = 2.4 Hz, 1H, NH), 7.31 (d, J = 8.7 Hz, 1H, H_{arom}), 7.25–7.19 (m, 2H, 2xH_{arom}), 5.13 (d, J = 5.2 Hz, 1H, OH), 5.07 (d, J = 4.6 Hz, 1H, OH), 5.02 (d, J = 5.2 Hz, 1H, OH), 4.65 (d, $J_{1,2}$ = 7.4 Hz, 1H, 1-H), 4.62 (t, J = 5.9 Hz, 1H, 6-OH), 3.73 (ddd, J = 11.7, 5.4, 1.8 Hz, 1H, 6a-H), 3.53–3.41 (m, 1H, 6b-H), 3.33–3.20 (m, 3H, 2-H, 3-H, 5-H), 3.20–3.10 (m, 1H, 4-H) ppm; ¹³C-NMR (75 MHz, DMSO-d₆): δ = 136.62, 133.02, 125.37, 123.07, 117.53, 112.86, 112.19, 111.47 (8xC_{arom}), 103.52 (C-1), 77.14, 76.80, 73.41, 69.96, 60.88 (C-2, C-3, C-4, C-5, C-6) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₁₄H₁₅BrClNNaO₆: 429.9663; found 429.9665.



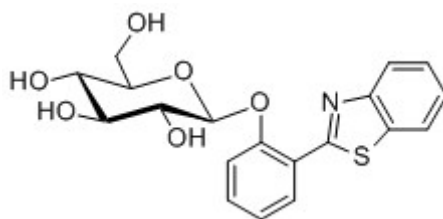
(5-bromo-4-chloroindol-3-yl) β -D-galactopyranoside (4a₂**, X-Gal)**

Prepared according to general procedure for deprotection step: Compound **3a₂** (300 mg, 0.485 mmol), MeOH (9 mL), KOH-MeOH (1 M, 72.7 μ L, 0.15 molar equivalent). White powder; yield: 171 mg (86%); mp 178–179 °C (decomp.); $[\alpha]_D^{25} = -61.3^\circ$ [$c = 0.21$, DMF (50% in H₂O)]; $R_f = 0.31$ (CHCl₃/MeOH = 5/1); {lit.,¹¹ mp 237–239 °C (decomp.); $[\alpha]_D^{24} = -69^\circ$ [$c = 1.0$, DMF (50% in H₂O)]; lit.,¹⁶ mp 177 °C (decomp.); $[\alpha]_D^{29} = -60^\circ$ [$c = 0.25$, DMF (50% in H₂O)]}; ¹H-NMR (500 MHz, DMSO-d₆): $\delta = 11.06$ (d, $J = 2.0$ Hz, 1H, NH), 7.30 (d, $J = 8.6$ Hz, 1H, H_{arom}), 7.21 (d, $J = 8.6$ Hz, 1H, H_{arom}), 7.19 (d, $J = 2.5$ Hz, 1H, H_{arom}), 4.93 (s, 1H, OH), 4.81 (s, 1H, OH), 4.66 (s, 1H, OH), 4.62 (d, $J_{1,2} = 8.0$ Hz, 1H, 1-H), 4.51 (s, 1H, OH), 3.69 (d, $J_{4,5} = 3.0$ Hz, 1H, 4-H), 3.62 (t, $J_{1,2} = 8.5$ Hz, 1H, 2-H), 3.56 (m, 2H, 6a-H, 6b-H), 3.51 (t, $J = 6.0$ Hz, 1H, 3-H), 3.38 (dd, $J_{5,6a} = 9.0$, $J_{4,5} = 3.0$ Hz, 1H, 5-H) ppm; ¹³C-NMR (126 MHz, DMSO): $\delta = 137.17$, 133.54, 125.84, 123.60, 118.08, 113.17, 112.68, 111.94 (8 \times C_{arom}), 104.61 (C-1), 76.14, 73.97, 70.91, 68.73, 61.02 (C-2, C-3, C-4, C-5, C-6) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₁₄H₁₅BrClNNaO₆: 429.9663; found 429.9668.



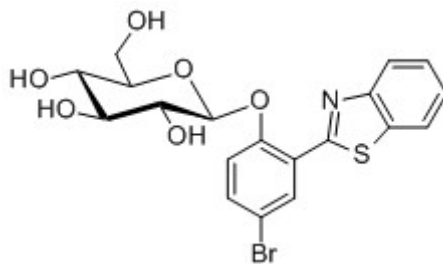
(5-bromo-6-chloroindol-3-yl) β-D-galactopyranoside (4a₃, Magenta-Gal)

Prepared according to general procedure for deprotection step: Compound **3a₃** (300 mg, 0.485 mmol), MeOH (9 mL), KOH-MeOH (1 M, 72.7 μ L, 0.15 molar equivalent). White powder; yield: 196 mg (99%); mp 137–138 °C; $[\alpha]_D^{25} = -49.8^\circ$ [$c = 0.17$, DMF (50% in H₂O)]; $R_f = 0.30$ (CHCl₃/MeOH = 5/1); {lit.,¹¹ mp 180–181 °C; $[\alpha]_D^{24} = -41^\circ$ ($c = 1.3$, EtOH); lit.,¹⁶ $[\alpha]_D^{29} = -34^\circ$ ($c = 0.125$, MeOH)}; ¹H-NMR (500 MHz, DMSO-d₆): $\delta = 7.97$ (s, 1H, H_{arom}), 7.56 (s, 1H, H_{arom}), 7.18 (s, 1H, H_{arom}), 4.49 (d, $J_{1,2} = 7.7$ Hz, 1H, 1-H), 3.68 (d, $J_{4,5} = 3.0$ Hz, 1H, 4-H), 3.61–3.53 (m, 3H, 2-H, 6a-H, 6b-H), 3.46 (t, $J = 6.1$ Hz, 1H, 5-H), 3.37 (dd, $J_{2,3} = 9.5$, $J_{34} = 3.5$ Hz, 1H, 3-H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): $\delta = 136.77$, 132.92, 125.68, 122.44, 120.78, 114.74, 113.54, 110.79 (8 \times C_{arom}), 105.57 (C-1), 76.10, 73.58, 70.93, 68.60, 60.92 (C-2, C-3, C-4, C-5, C-6) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₁₄H₁₅BrClNNaO₆: 429.9663; found 429.9668.



[2-(benzothiazol-2'-yl)-phenolyl] β-D-glucopyranoside (4b₁)

Prepared according to general procedure for deprotection step: Compound **3b₁** (2000 mg, 3.587 mmol), THF/MeOH=1/1 (60 mL), KOH-MeOH (1 M, 538 μ L, 0.15 molar equivalent). White powder; yield: 1521 mg (99%); mp 232–234 °C; $[\alpha]_D^{25} = -43.6^\circ$ [$c = 0.30$, DMF (50% in H₂O)]; $R_f = 0.75$ (CHCl₃/MeOH = 5/1); ¹H-NMR (500 MHz, DMSO-d₆): $\delta = 8.44$ (d, $J = 7.5$ Hz, 1H), 8.10 (d, $J = 7.5$ Hz, 1H), 8.06 (d, $J = 8.0$ Hz, 1H), 7.53 (t, $J = 7.5$ Hz, 2H), 7.44 (t, $J = 7.5$ Hz, 1H), 7.37 (d, $J = 8.5$ Hz, 1H), 7.21 (t, $J = 7.5$ Hz, 1H), 5.38 (s, 1H, OH), 5.27 (d, $J_{1,2} = 7.7$ Hz, 1H, 1-H), 5.25 (s, 1H, OH), 5.13 (s, 1H, OH), 4.62 (s, 1H, OH), 3.72 (d, $J = 11.0$ Hz, 1H), 3.62 (t, $J = 7.8$ Hz, 1H), 3.51–3.45 (m, 2H), 3.37 (m, 1H), 3.26 (t, $J = 7.5$ Hz, 1H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): $\delta = 162.98$, 155.23, 152.01, 136.30, 132.63, 129.20, 126.62, 125.37, 122.90, 122.45, 122.20, 122.12, 115.33 (13 \times C_{arom}), 100.62 (C-1), 77.68, 77.41, 73.69, 70.07, 61.06 (C-2, C-3, C-4, C-5, C-6) ppm; HRMS (ESI): m/z [M+H]⁺ calcd. for C₁₉H₂₀NO₆S: 390.1006; found 390.1013.



[2-(benzothiazol-2'-yl)-4-bromophenolyl] β-D-glucopyranoside (4b₂**)**

Prepared according to general procedure for deprotection step: Compound **3b₂** (2000 mg, 3.142 mmol), THF/MeOH = 1/1

(60 mL), KOH-MeOH (1 M, 471 μL, 0.15 molar equivalent). White powder; yield: 1603 mg (99%); mp 231–233 °C; $[\alpha]_D^{25} =$

-33.8° [$c = 0.30$, DMF (50% in H₂O)]; $R_f = 0.76$ (CHCl₃/MeOH = 5/1); ¹H-NMR (500 MHz, DMSO-*d*₆): $\delta = 8.55$ (d, $J = 2.5$ Hz, 1H, H_{arom}), 8.11 (dd, $J = 12.3, 8.0$ Hz, 2H, 2×H_{arom}), 7.70 (dd, $J = 9.0, 3.0$ Hz, 1H, H_{arom}), 7.58–7.54 (m, 1H, H_{arom}), 7.49–7.45 (m, 1H, H_{arom}), 7.36 (d, $J = 9.5$ Hz, 1H, H_{arom}), 5.42 (d, $J = 5.5$ Hz, 1H, OH), 5.29 (d, $J_{1,2} = 7.5$ Hz, 1H, 1-H), 5.26 (s, 1H, OH), 5.13 (d, $J = 3.5$ Hz, 1H, OH), 4.62 (t, $J = 5.3$ Hz, 1H, OH), 3.71 (dd, $J = 10.5, 5.5$ Hz, 1H), 3.62 (dd, $J_{6a,6b} = 13.7, J_{5,6a} = 8.2$ Hz, 1H, 6a-H), 3.53–3.45 (m, 2H), 3.37 (d, $J = 9.5$ Hz, 1H), 3.26 (dt, $J_{6a,6b} = 13.2, J_{5,6b} = 6.7$ Hz, 1H, 6b-H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): $\delta = 161.31, 154.34, 151.81, 136.44, 134.79, 130.95, 126.86, 125.76, 124.04, 123.17, 122.32, 117.88, 114.10$ (13×C_{arom}), 100.71 (C-1), 77.72, 77.31, 73.63, 69.98, 61.01 (C-2, C-3, C-4, C-5, C-6) ppm; HRMS (ESI): m/z [M+Na]⁺ calcd. for C₁₉H₁₈BrNNaO₆S: 489.9930; found 489.9933.

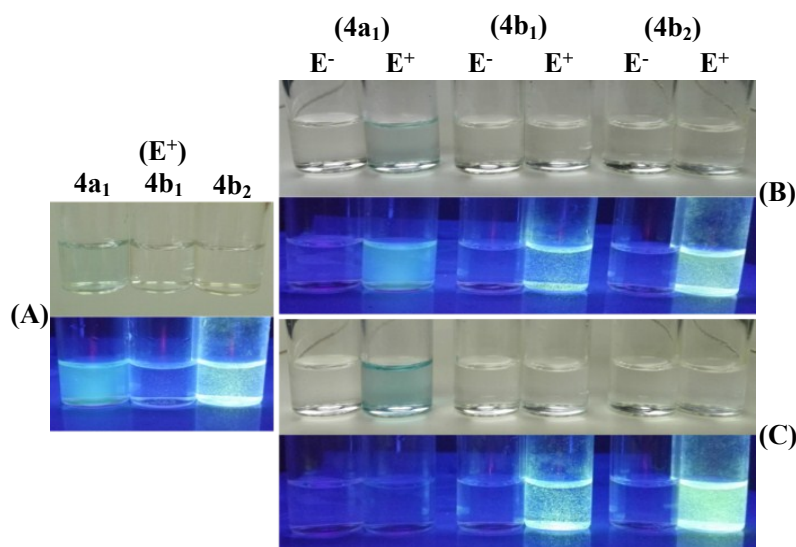


Fig. S1. Responses of **4a₁** (left), **4b₁** (middle), and **4b₂** (right) to active β-Glu (E⁺) after 45 min (A). Responses of substrate groups **4a₁** (left), **4b₁** (middle), and **4b₂** (right) to active β-Glu (E⁺, right) or inactive β-Glu (E⁻, left) after 90 min (B), followed by continuous placement at RT for 10.5 h (C). Each substrate was used at 80 μM; incubation was carried out at 37°C in air under visible light (top) or UV light (365 nm) (bottom).

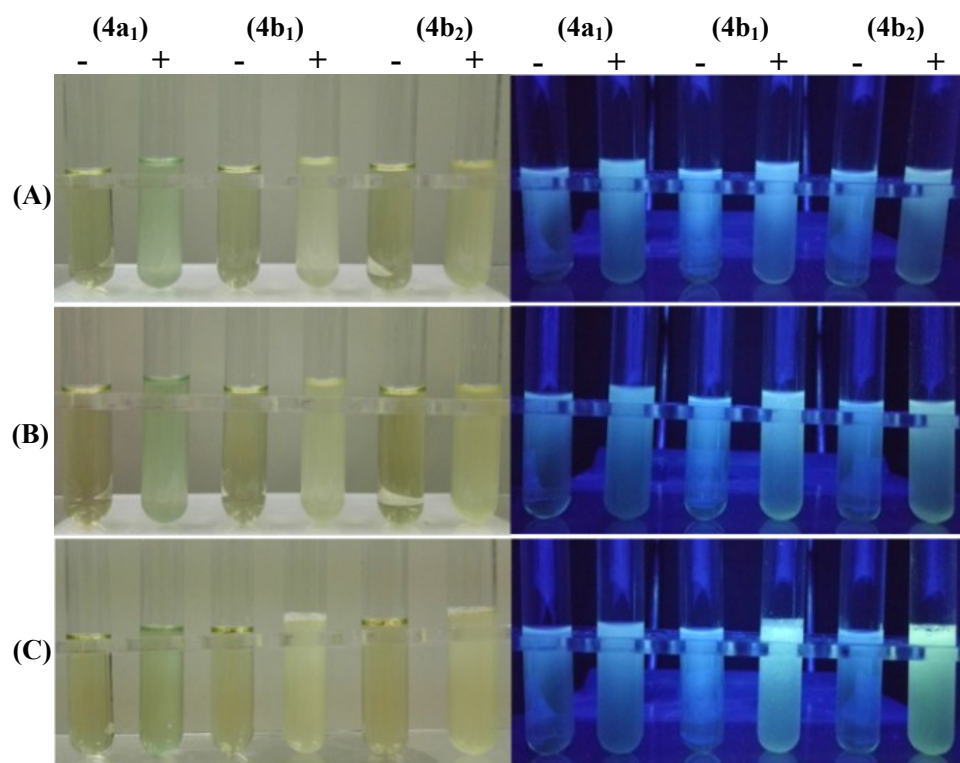


Fig. S2. Photographs of substrate groups **4a₁** (left), **4b₁** (middle), and **4b₂** (right) (100 μ M) in the absence (-) and presence (+) of *K. pneumoniae* CMCC 46117 showing β -glucosidase activity after cultivation in nutrient broth for 24 h (A), 48 h (B), or 70 h (C) at 37°C in air under visible light (left) and UV light (365 nm) (right).

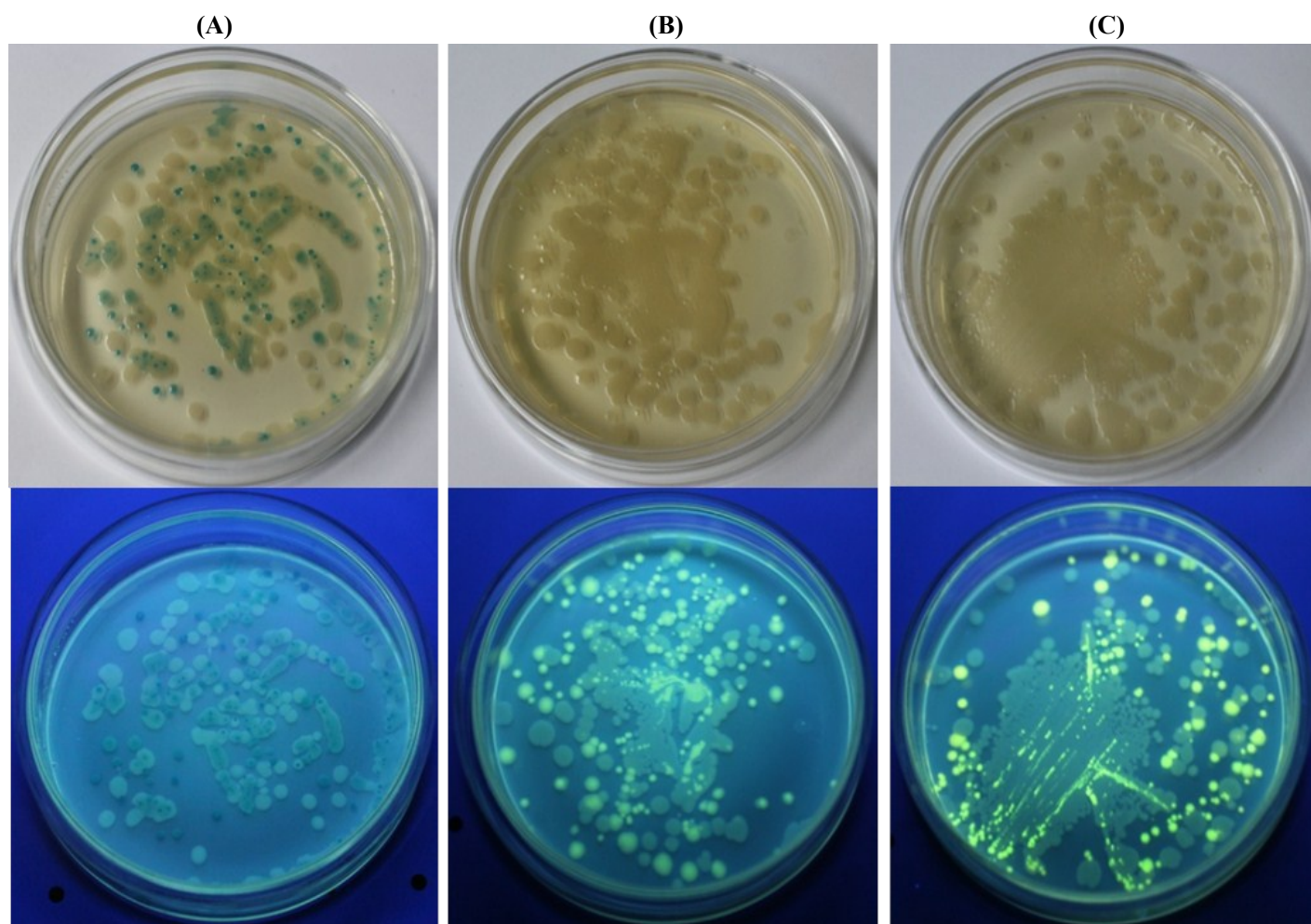


Fig. S3. Responses of substrates **4a₁** (A), **4b₁** (B), and **4b₂** (C) to *K. pneumonia* CMCC 46117 (with β -glucosidase activity) and *Salmonella typhimurium* ATCC 14028 (without β -glucosidase activity). Columbia agar plates were used with 100 μ M of each substrate, and cultivation was carried out for 24 h at 37°C in air under visible light (top) and UV light (365 nm) (bottom).

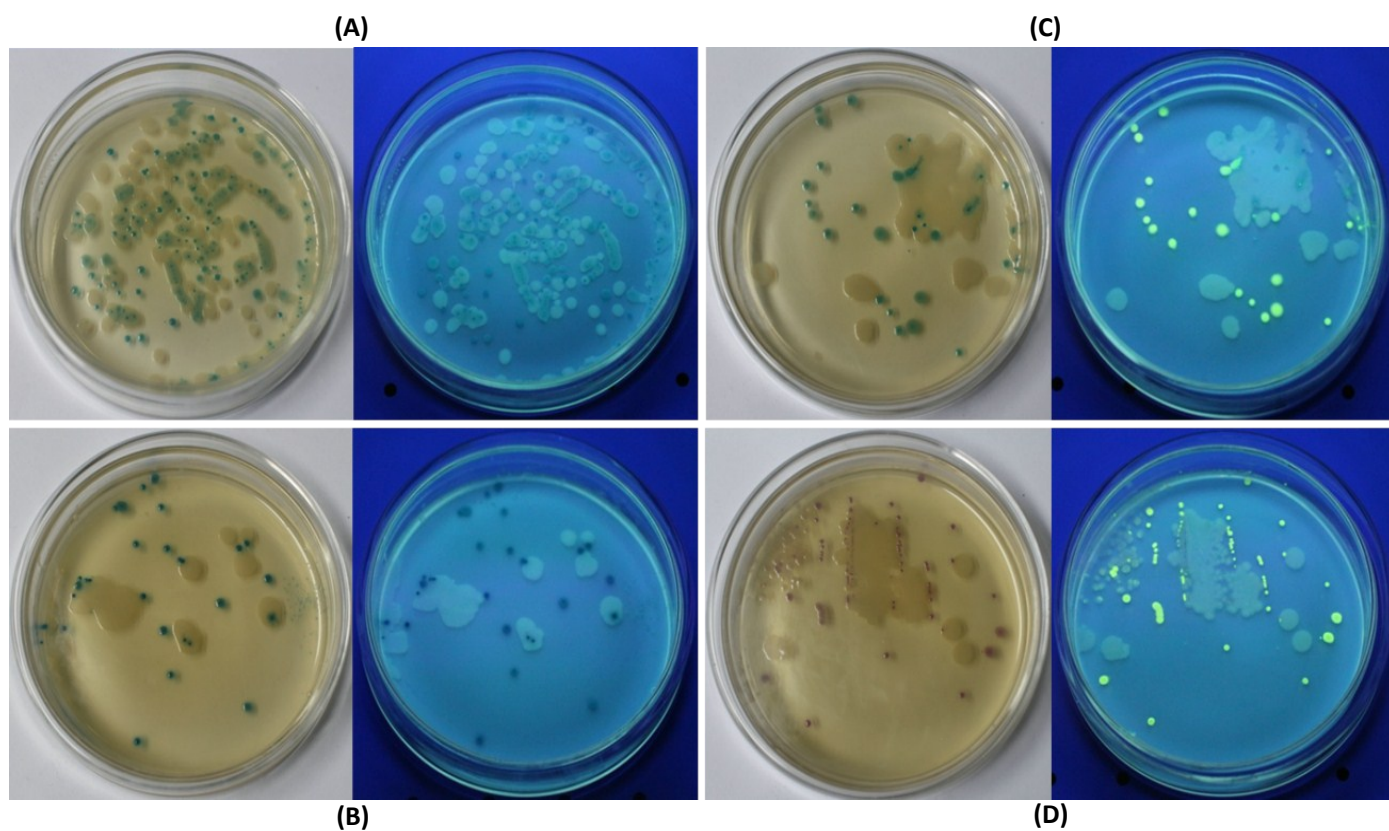


Fig. S4. Responses of substrates **4a₁** (100 μ M) (A), **4a₁** + **4a₃** (100 μ M + 100 μ M, 0.06 g/L IPTG) (B), **4a₂** + **4b₂** (100 μ M + 100 μ M, 0.06 g/L IPTG) (C), and **4a₃** + **4b₂** (100 μ M + 100 μ M, 0.06 g/L IPTG) (D) to *K. pneumonia* CMCC 46117 (with β -glucosidase and β -galactosidase activities) and *Salmonella typhimurium* ATCC 14028 (without β -glucosidase and β -galactosidase activities). Columbia agar plates were used, and cultivation was carried out for 24 h at 37°C in air under visible light (left) and UV light (365 nm) (right).

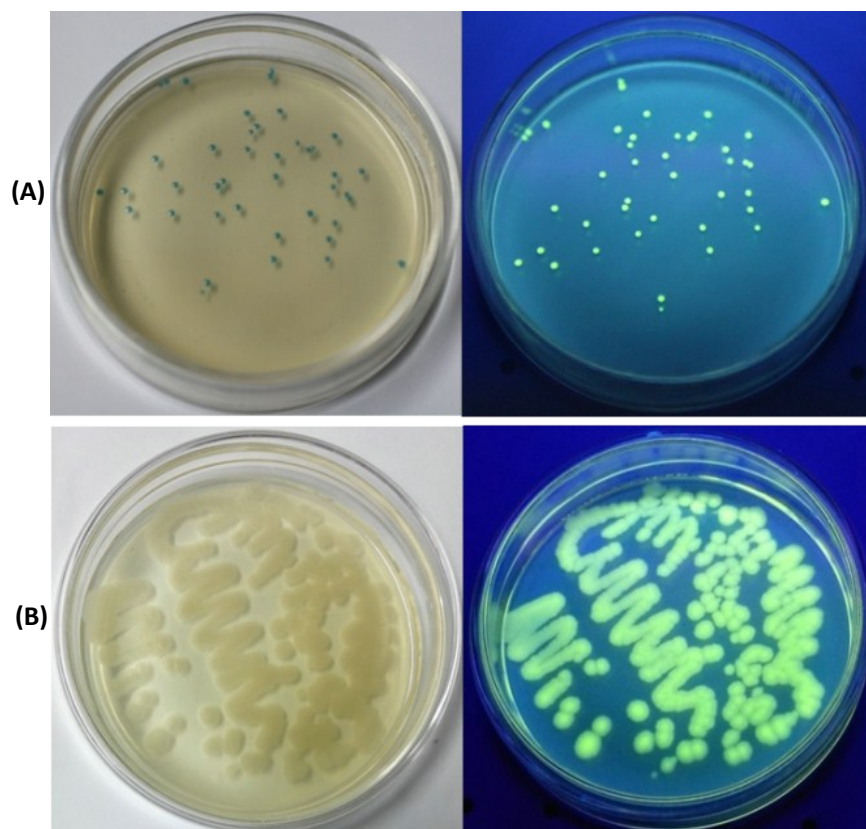


Fig. S5. Responses of substrates **4a₁** + **4b₂** (100 μ M + 100 μ M) to *Streptococcus faecalis* ATCC 29212 (A) and *B. cereus* CMCC 63301 (B). Columbia agar plates were used, and cultivation was carried out for 24 h at 37°C in air under visible light (left) and UV light (365 nm) (right).

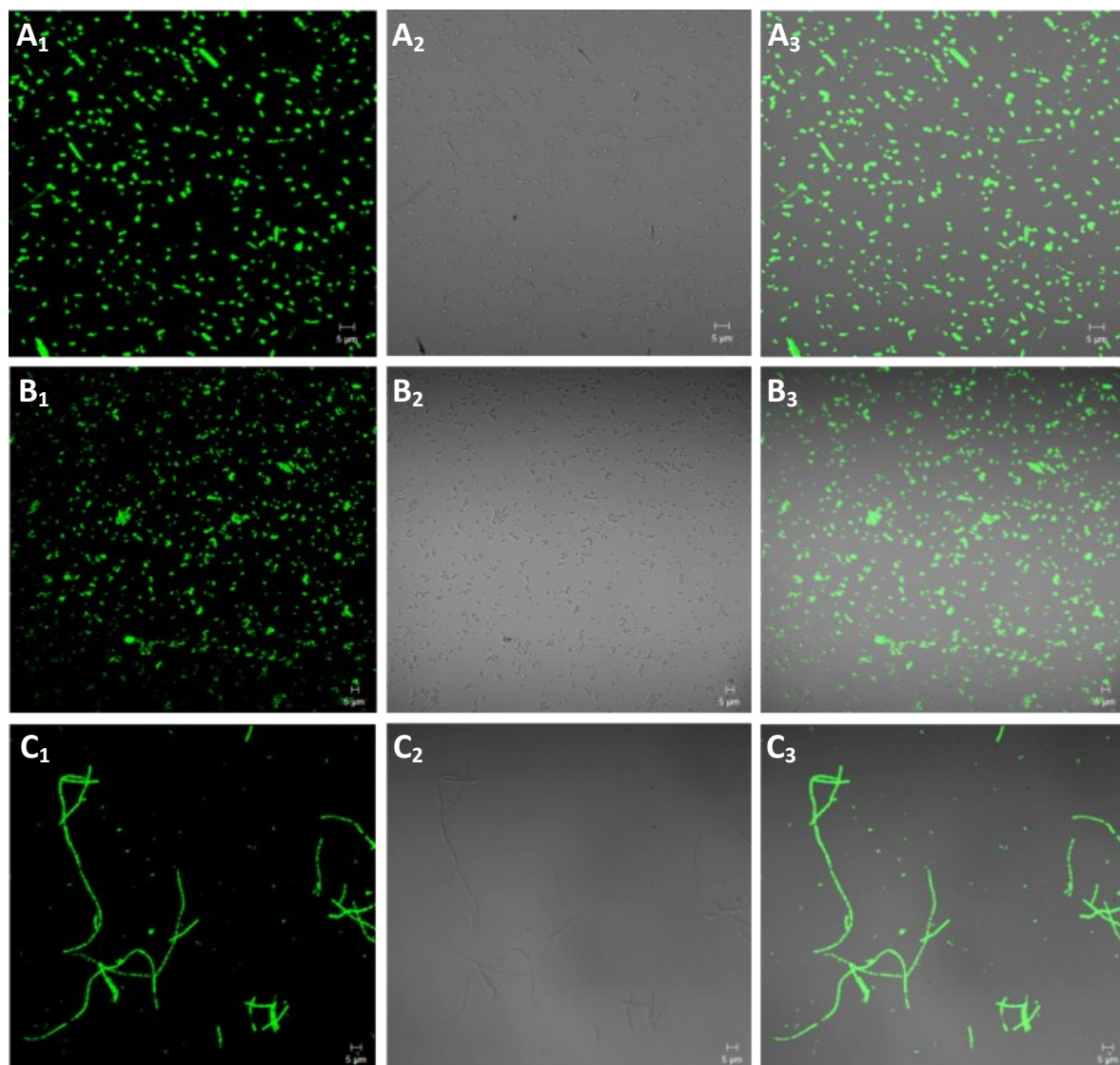


Fig. S6. Confocal fluorescence images of colonies of *K. pneumonia* CMCC 46117 (A₁-A₃) from Fig. S4D, *Streptococcus faecalis* ATCC 29212 (B₁-B₃) from Fig. S5A, and *B. cereus* CMCC 63301 (C₁-C₃) from Fig. S5B. (A₁), (B₁), (C₁) Green emission (DAPI); (A₂), (B₂), (C₂) bright-field images; (A₃), (B₃), (C₃) merged images. $\lambda_{\text{ex}} = 405 \text{ nm}$. 40 \times magnification; NA: 1.30, oil immersion objective.

