

# Electronic Supplementary Information

## Glycopeptide Antibiotic Analogs for Selective Inactivation and Two-Photon Imaging of Vancomycin-Resistant Strains

*Shinya Ariyasu,<sup>a</sup> Pei Chui Too,<sup>a</sup> Jing Mu,<sup>a</sup> Chi Ching Goh,<sup>b</sup> Yichen Ding,<sup>c</sup> Ya Lin Tnay,<sup>a</sup> Edwin Kok Lee  
Yeow,<sup>a</sup> Liang Yang,<sup>c</sup> Lai Guan Ng,<sup>b</sup> Shunsuke Chiba,<sup>a\*</sup> and Bengang Xing<sup>a,d\*</sup>*

<sup>a</sup>Division of Chemistry & Biological Chemistry, School of Physical & Mathematical Sciences, Nanyang Technological University, Singapore 637371, Singapore

<sup>b</sup>Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A\*STAR), Biopolis, Singapore, 138648 (Singapore)

<sup>c</sup>School of Biological Sciences, Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, Singapore 637551, Singapore

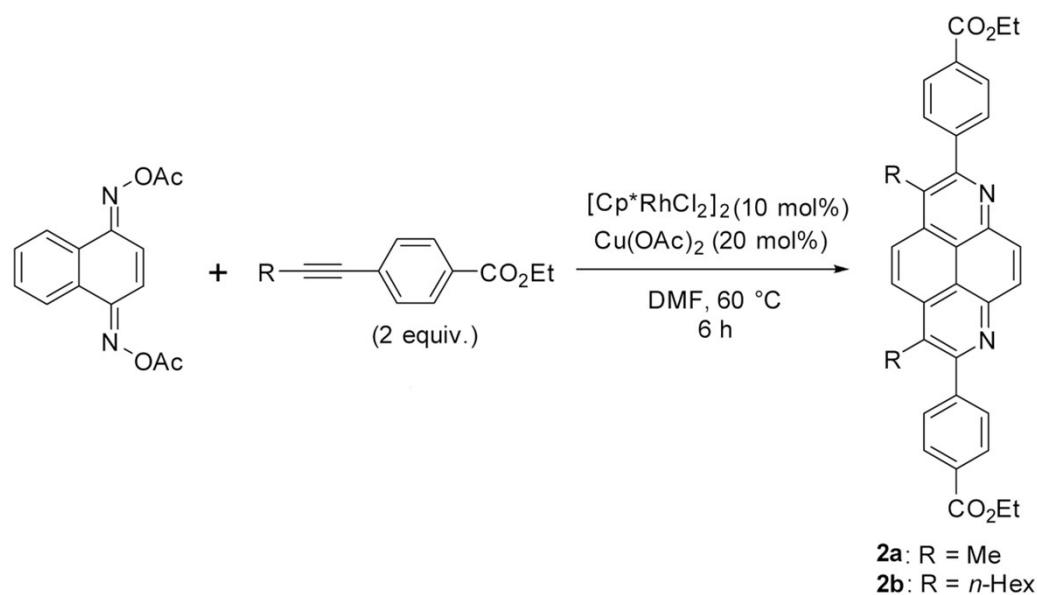
<sup>d</sup>Institute of Materials Research and Engineering (IMRE), A\*STAR (Agency for Science, Technology and Research), Singapore 117602, Singapore.

\*Corresponding authors: E-mail, [shunsuke@ntu.edu.sg](mailto:shunsuke@ntu.edu.sg), [bengang@ntu.edu.sg](mailto:bengang@ntu.edu.sg)

## Experimental Section

**General Information:** All the chemicals were purchased from Sigma Aldrich. Commercially available reagents were used without further purification. Four bacterial strains (*Bacillus subtilis* (*B. subtilis*) (ATCC 33677), Van-resistant *Enterococcus faecium* (*E. faecium*) (VanA genotype, ATCC 51559), Van-resistant *Enterococcus faecalis* (*E. faecalis*) (VanB genotype, ATCC 51299), *Escherichia coli* (*E. coli*) DH5 $\alpha$  (ATCC 53868)) were purchased from American Type Culture Collection (ATCC), USA. Two Van-sensitive strains (*E. faecium* and *E. faecalis*) and two emissive bacteria strains (green fluorescent protein (GFP)-expressed *Staphylococcus aureus* (*S. aureus*) and red fluorescent protein (RFP)-expressed *E. coli*) were constructed following a previously reported method.<sup>1</sup> <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded using a Bruker Avance 300 spectrometer or Bruker Avance 400 spectrometer. Mass spectra (MS) were measured with Thermo LCQ Deca XP Max or Thermo Finnigan MAT 95 XP mass spectrometer for electrospray ionization mass spectra (ESI). Melting points were recorded on a Buchi B-54 melting-point apparatus. Flash column chromatography was performed by using Merck silica gel 60 with distilled solvents. Reverse-phase HPLC analysis was performed on a Shimadzu HPLC system using an Alltima C-18 (250  $\times$  10 mm) column at a flow rate of 3.0 mL/min for preparation and a C-18 (250 $\times$ 4.6 mm) at a flow rate of 1.0 mL/min for analysis. Fluorescence emission spectra were performed on a Varian Cary eclipse Fluorescence Spectrophotometer. UV absorption spectra were recorded in a 10 mm path quartz cell on a Beckman coulter DU800 spectrometer. Scanning electron microscopy (SEM) images were performed on JEOL JSM-7600F operated at 5 kV. OD values in MIC tests and MTT assays were measured by Tecan's Infinite M200 microplate reader. Fluorescence microscopic imaging and confocal laser scanning microscopic imaging were conducted with a Nikon Eclipse TE2000 Confocal Microscope. Two-photon images were acquired on A TriM Scope II single-beam two-photon microscope (LaVision BioTec) with a laser (Coherent Chameleon Ultra II One Box Ti:sapphire) and water-dipping objectives (100 $\times$ , Olympus).<sup>2</sup>

## Synthesis of 1,8-Diazapyrene 2a and 2b:



### Diethyl 4,4'-(3,6-dimethylbenzo[*lmn*][2,9]phenanthroline-2,7-diyl)dibenzoate (**2a**):

$[Cp^*RhCl_2]_2$  (124 mg, 0.20 mmol) and  $Cu(OAc)_2$  (72.7 mg, 0.40 mmol) were added to the solution of naphthalene-1,4-dione *O,O*-diacetyl dioxime<sup>3</sup> (551 mg, 2.00 mmol) and ethyl 4-(prop-1-yn-1-yl)benzoate<sup>4</sup> (753 mg, 4.00 mmol) in DMF (10 mL) and the reaction mixture was stirred at 60 °C under a nitrogen atmosphere for 6 h. The reaction mixture was then cooled to room temperature and quenched with pH 9 aqueous ammonium buffer. The organic materials were extracted thrice with  $CH_2Cl_2$ . The combined extracts were washed thrice with water and once with brine, and dried over  $MgSO_4$ . The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 60:40), that is followed by recrystallization from  $CH_2Cl_2$  to afford **2a** as a white solid (545 mg, 1.03 mmol, yield 52%).

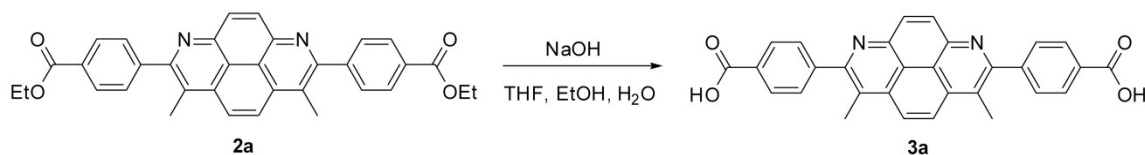
White solid, m.p. 255-256 °C; <sup>1</sup>H NMR (400 MHz,  $CDCl_3$ ):  $\delta$  = 8.53 (2H, s), 8.50 (2H, s), 8.26 (4H, d,  $J$  = 8.4 Hz), 7.80 (4H, d,  $J$  = 8.4 Hz), 4.45 (4H, q,  $J$  = 7.2 Hz), 2.92 (6H, s), 1.46 (6H, t,  $J$  = 7.2 Hz); <sup>13</sup>C NMR (100 MHz,  $CDCl_3$ ):  $\delta$  = 166.5, 156.5, 146.2, 145.8, 134.4, 132.4, 130.1, 129.9, 129.6, 127.1, 124.4, 117.4, 61.1, 16.1, 14.4; HRMS (ESI):  $m/z$  calcd for  $C_{34}H_{29}N_2O_4$  : 529.2127  $[M+H]^+$ ; found: 529.2126.

### Diethyl 4,4'-(3,6-dihexylbenzo[lmn][2,9]phenanthroline-2,7-diyl)dibenzoate (**2b**):

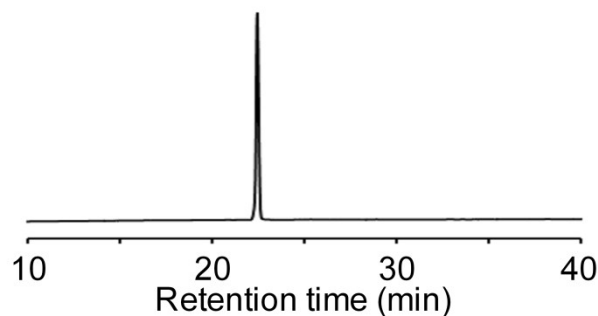
Synthesized from ethyl 4-(oct-1-yn-1-yl)benzoate<sup>5</sup> according to similar procedure as **2a** and purified by silica-gel flash column chromatography (*n*-hexane/ethyl acetate=80:20), followed by recrystallization from CH<sub>2</sub>Cl<sub>2</sub> to afford **2b** as a white solid (553 mg, 0.827 mmol, yield 41%).

White solid, m.p. 143-144°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.56 (2H, s), 8.51 (2H, s), 8.25 (4H, d, *J* = 8.0 Hz), 7.73 (4H, d, *J* = 8.4 Hz), 4.46 (4H, q, *J* = 7.2 Hz), 3.28 (4H, t, *J* = 8.0 Hz), 1.68-1.76 (4H, m), 1.46 (6H, t, *J* = 7.2 Hz), 1.33-1.40 (4H, m), 1.23-1.28 (8H, m), 0.85 (6H, t, *J* = 6.8 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 166.5, 156.8, 146.13, 146.09, 133.9, 132.4, 130.0, 129.8, 129.6, 129.5, 127.2, 118.1, 61.1, 32.3, 31.4, 29.5, 29.1, 22.5, 14.4, 14.0; HRMS (ESI): *m/z* calcd for C<sub>44</sub>H<sub>49</sub>N<sub>2</sub>O<sub>4</sub> : 669.3692 [M+H]<sup>+</sup>; found: 669.3693.

### Hydrolysis of ethyl ester **2a** (**3a**):

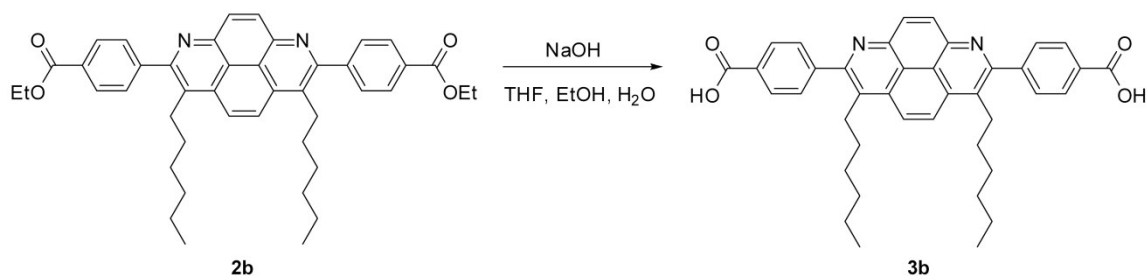


A solution of DP ethyl ester **2a** (5.3 mg, 10 μmol) in THF (1.6 mL)/EtOH (1.6 mL) was treated with a 2 N aqueous solution of NaOH (1 mL) at room temperature overnight.<sup>6</sup> The reaction mixture was concentrated down to water layer under reduced pressure and was neutralized by addition of a 2 N aqueous solution of HCl. The precipitation of **3a** (4.5 mg, 9.5 μmol, 95%) was obtained by centrifugation and dried under reduced pressure. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 8.82 (2H, s), 8.50 (2H, s), 8.17 (4H, d, *J* = 8.4 Hz), 7.90 (4H, d, *J* = 8.4 Hz), 2.96 (6H, s). HRMS (ESI): *m/z* calcd for C<sub>30</sub>H<sub>21</sub>N<sub>2</sub>O<sub>4</sub> : 473.1501 [M+H]<sup>+</sup>; found: 473.1506.

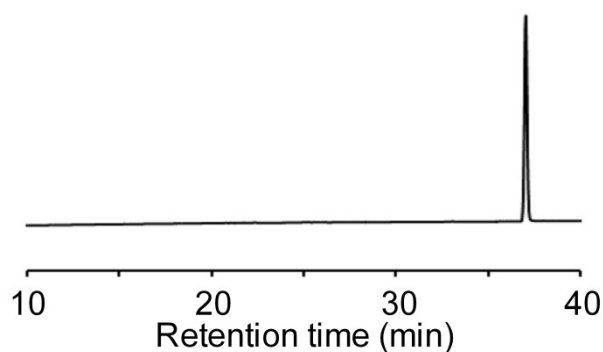


**Fig. S1.** HPLC chromatogram of **3a** monitored at absorbance of 380 nm.

**Hydrolysis of ethyl ester **2b** (**3b**):**

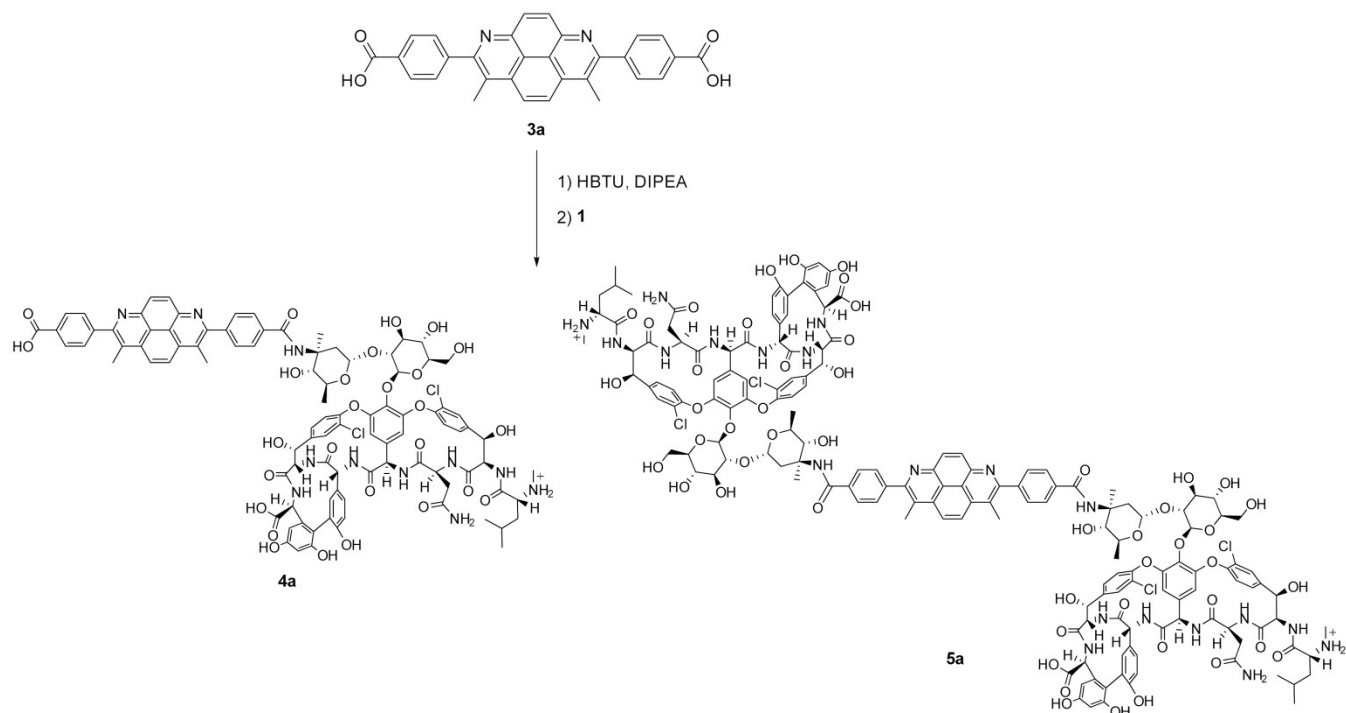


A solution of DP ethyl ester **2b** (6.7 mg, 10  $\mu$ mol) in THF (1.6 mL)/EtOH (1.6 mL) was treated with a 2 N aqueous solution of NaOH (1 mL) at room temperature overnight.<sup>6</sup> The reaction mixture was concentrated down to water layer under reduced pressure and was neutralized by addition of a 2 N aqueous solution of HCl. The precipitation of **3b** (6.0 mg, 9.8  $\mu$ mol, 98%) was obtained by centrifugation and dried under reduced pressure. **3b**: <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.77 (2H, s), 8.48 (2H, s), 8.17 (4H, d, *J* = 8.2 Hz), 7.78 (4H, d, *J* = 8.2 Hz), 2.55-2.50 (4H, m), 1.63-1.95 (4H, m), 1.30-1.34 (4H, m), 1.17-1.19 (8H, m), 0.81 (6H, t, *J* = 6.7 Hz). HRMS (ESI): *m/z* calcd for C<sub>40</sub>H<sub>41</sub>N<sub>2</sub>O<sub>4</sub> : 613.3066 [M+H]<sup>+</sup>; found: 613.3076.



**Fig. S2.** HPLC chromatogram of **3b** monitored at absorbance of 380 nm.

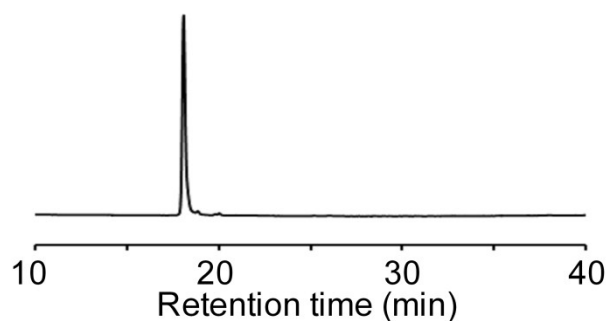
### Conjugation of vancomycin with **3a** (**4a** and **5a**):



Vancomycin hydrochloride (23.8 mg, 16  $\mu\text{mol}$ , 2.0 equiv.) and **3a** (3.8 mg, 8.0  $\mu\text{mol}$ , 1.0 equiv.) were dissolved in 1 mL of DMSO. The mixture was cooled to 0  $^{\circ}\text{C}$ , and *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) (6.1 mg, 16  $\mu\text{mol}$ , 2.0 equiv.) in 1 mL DMSO was added, followed by *N,N*-diisopropylethylamine (DIEA) (10.3 mg, 14.0  $\mu\text{L}$ , 80  $\mu\text{mol}$ , 10 equiv.). The mixture was allowed to rise to room temperature and stirred overnight.<sup>7</sup> The reaction was quenched by adding dropwise 40 mL of acetone. Pale yellowish precipitation was collected by centrifugation. The

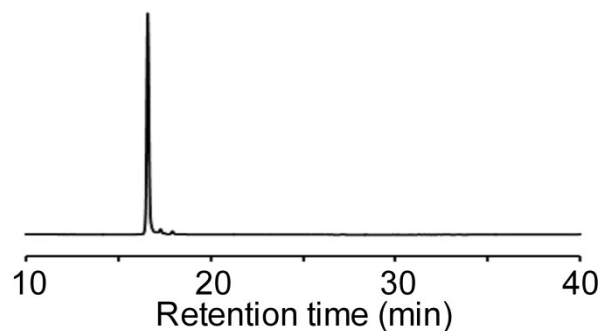
crude product containing Van-DP monomer and dimer was purified by reversed-phase HPLC (RP-HPLC).

Compound **4a**: yield: 21%;  $^1\text{H NMR}$  (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  9.42 (br, s), 9.15 (br, s), 9.02 (br, s), 8.80 (s), 8.72 (br, s), 8.55 (br, s), 8.49 (s), 8.32 (d,  $J = 8.4$  Hz), 7.93-7.98 (m), 7.88 (s), 7.82 (d,  $J = 8.4$  Hz), 7.08-7.53 (m), 6.71-6.78 (m), 6.40 (s), 6.28 (s), 5.93 (br, s), 5.79 (br, m), 5.68 (s), 5.13-5.39 (m), 4.97 (br, s), 4.81 (br, m), 4.45 (br, m), 4.23 (br, s), 3.90 (br, s), 3.75 (br, m), 2.65 (br, m), 2.65 (br, m), 2.08 (s), 2.01 (br, m), 1.60-1.72 (br, m), 1.24 (s), 0.91 (d,  $J = 6.5$  Hz), 0.88 (d,  $J = 6.5$  Hz). HRMS (ESI):  $m/z$  calcd for  $\text{C}_{96}\text{H}_{95}\text{Cl}_2\text{N}_{11}\text{O}_{27}$  : 952.2899  $[\text{M}+2\text{H}]^{2+}$ ; found: 952.2957.



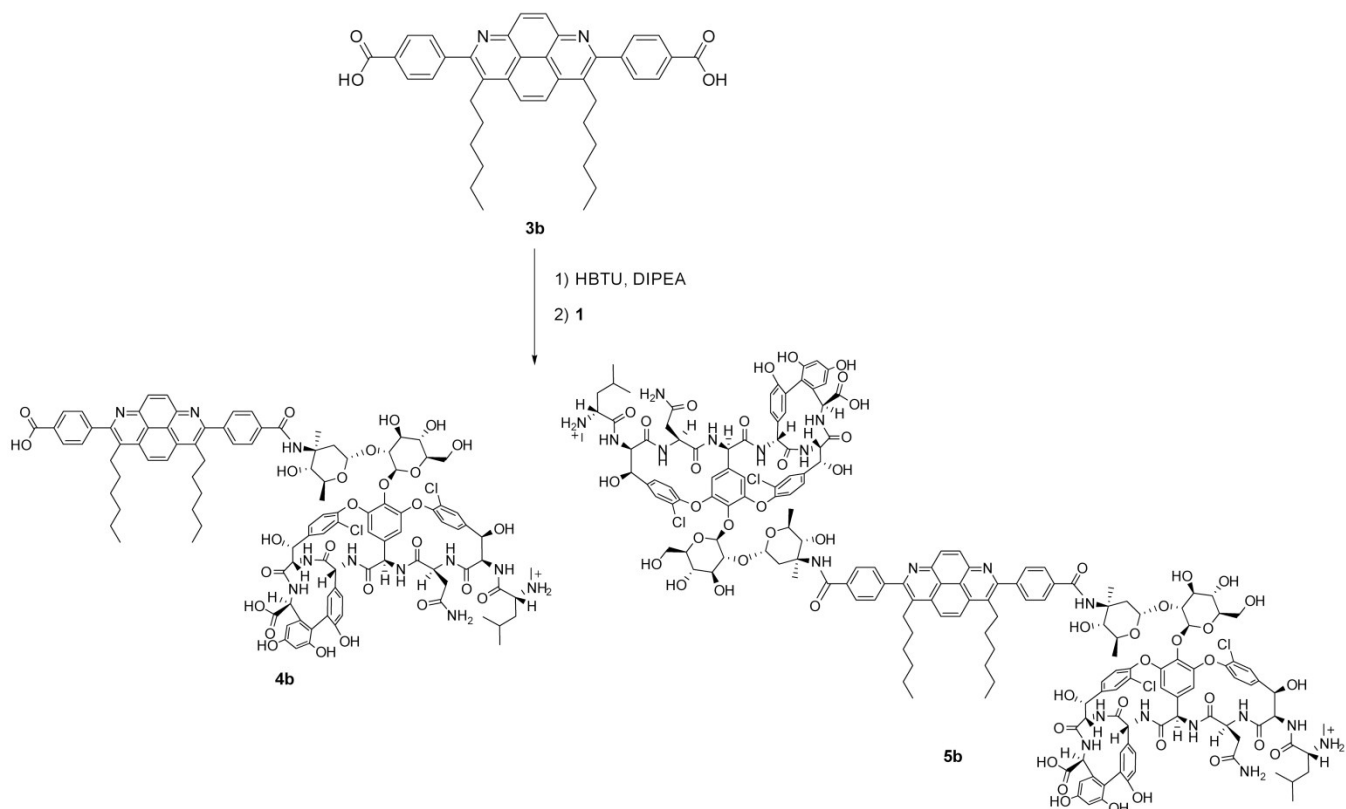
**Fig. S3.** HPLC chromatogram of **4a** monitored at absorbance of 380 nm.

Compound **5a**: yield: 16%;  $^1\text{H NMR}$  (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  9.42 (br, s), 9.15 (br, s), 9.03 (br, s), 8.77 (s), 8.72 (br, s), 8.55 (br, s), 8.47 (s), 8.14 (d,  $J = 8.4$  Hz), 7.94 (d,  $J = 8.4$  Hz), 7.88 (s), 7.72-7.75 (br, m), 7.08-7.53 (m), 6.71-6.78 (m), 6.39 (s), 6.27 (s), 5.93 (br, s), 5.79 (br, m), 5.67 (s), 5.13-5.39 (m), 4.97 (br, s), 4.81 (br, m), 4.46 (br, m), 4.23 (br, s), 3.90 (br, s), 3.75 (br, m), 2.65 (br, m), 2.66 (br, m), 2.08 (s), 2.01 (br, m), 1.60-1.72 (br, m), 1.24 (s), 0.91 (d,  $J = 6.5$  Hz), 0.88 (d,  $J = 6.5$  Hz). HRMS (ESI):  $m/z$  calcd for  $\text{C}_{162}\text{H}_{169}\text{Cl}_4\text{N}_{20}\text{O}_{50}$  : 1112.3346  $[\text{M}+3\text{H}]^{3+}$ ; found: 1112.3394.



**Fig. S4.** HPLC chromatogram of **5a** monitored at absorbance of 380 nm.

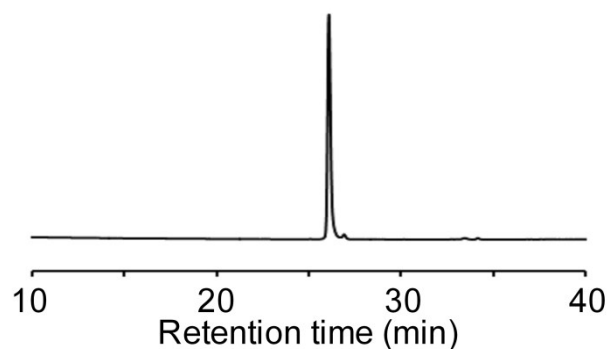
### Conjugation of vancomycin with **3b** (**4b** and **5b**).



Vancomycin hydrochloride (23.8 mg, 16  $\mu\text{mol}$ , 2.0 equiv.) and **3b** (4.9 mg, 8.0  $\mu\text{mol}$ , 1.0 equiv.) were dissolved in 1 mL of DMSO. The mixture was cooled to 0  $^{\circ}\text{C}$ , and HBTU (6.1 mg, 16  $\mu\text{mol}$ , 2.0 equiv.) in 1 mL DMSO was added, followed by DIEA (10.3 mg, 14.0  $\mu\text{L}$ , 80  $\mu\text{mol}$ , 10 equiv.). The mixture was allowed to rise to room temperature and stirred overnight.<sup>7</sup> The reaction was quenched by adding dropwise 40 mL of acetone. Pale yellowish precipitation was collected by centrifugation. The crude product containing Van-DP monomer and dimer was purified by reversed-phase HPLC (RP-HPLC).

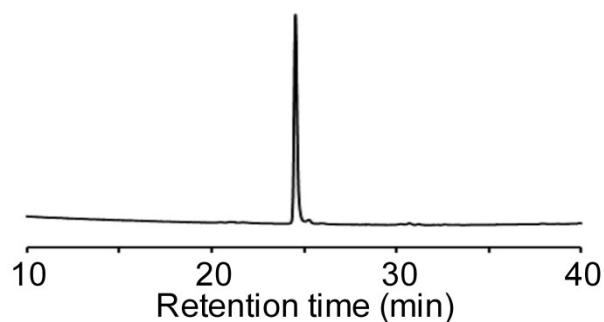


Compound **4b**: yield: 33%;  $^1\text{H NMR}$  (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  9.42 (br, s), 9.15 (br, s), 9.02 (br, s), 8.80 (s), 8.72 (br, s), 8.55 (br, s), 8.49 (s), 8.33 (d,  $J = 8.3$  Hz), 7.93-7.98 (m), 7.88 (s), 7.73 (d,  $J = 8.3$  Hz), 7.08-7.53 (m), 6.71-6.78 (m), 6.40 (s), 6.28 (s), 5.93 (br, s), 5.79 (br, m), 5.68 (s), 5.13-5.39 (m), 4.97 (br, s), 4.81 (br, m), 4.45 (br, m), 4.23 (br, s), 3.90 (br, s), 3.75 (br, m), 2.65 (br, m), 2.65 (br, m), 2.08 (s), 2.01 (br, m), 1.60-1.72 (br, m), 1.30-1.34 (m), 1.24 (s), 1.11-1.17 (m), 0.91 (d,  $J = 6.5$  Hz), 0.88 (d,  $J = 6.5$  Hz), 0.80 (t,  $J = 6.2$  Hz). HRMS (ESI):  $m/z$  calcd for  $\text{C}_{106}\text{H}_{115}\text{Cl}_2\text{N}_{11}\text{O}_{27}$  : 1022.3694  $[\text{M}+2\text{H}]^{2+}$ ; found: 1022.3651.



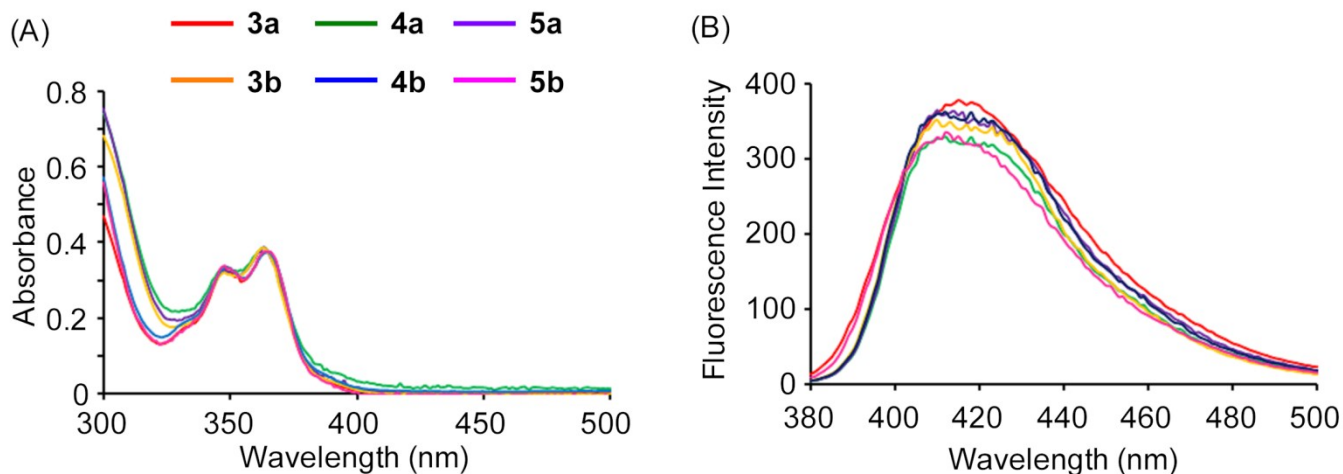
**Fig. S5.** HPLC chromatogram of **4b** monitored at absorbance of 380 nm.

Compound **5b**: yield: 15%;  $^1\text{H NMR}$  (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  9.42 (br, s), 9.15 (br, s), 9.03 (br, s), 8.77 (s), 8.72 (br, s), 8.55 (br, s), 8.47 (s), 8.10 (d,  $J = 8.3$  Hz), 7.96 (d,  $J = 8.3$  Hz), 7.88 (s), 7.72-7.75 (br, m), 7.08-7.53 (m), 6.71-6.78 (m), 6.39 (s), 6.27 (s), 5.93 (br, s), 5.79 (br, m), 5.67 (s), 5.13-5.39 (m), 4.97 (br, s), 4.81 (br, m), 4.46 (br, m), 4.23 (br, s), 3.90 (br, s), 3.75 (br, m), 2.65 (br, m), 2.66 (br, m), 2.08 (s), 2.01 (br, m), 1.60-1.72 (br, m), 1.30-1.34 (m), 1.24 (s), 1.11-1.17 (m), 0.91 (d,  $J = 6.5$  Hz), 0.88 (d,  $J = 6.5$  Hz), 0.80 (t,  $J = 6.2$  Hz). HRMS (ESI):  $m/z$  calcd for  $\text{C}_{172}\text{H}_{189}\text{Cl}_4\text{N}_{20}\text{O}_{50}$  : 1159.0534  $[\text{M}+3\text{H}]^{3+}$ ; found: 1159.0580.



**Fig. S6** HPLC chromatogram of **5b** monitored at absorbance of 380 nm.

**General Spectroscopic Measurements.** The concentration of DMSO stock solution of DP derivatives **3a**, **3b**, **4a**, **4b**, **5a** or **5b** was determined by the absorbance at 365 nm ( $\epsilon_{365}$ : 75000 M<sup>-1</sup>cm<sup>-1</sup>).<sup>3</sup> Each stock solution was diluted to 5.0  $\mu$ M in DMSO. The UV-Visible spectra were recorded using a Beckman coulter DU800 spectrometer. Wavelength interval: 1.0 nm. Scan Speed: 1200 nm/min. Fluorescence spectroscopic studies were also performed using a Varian Cary eclipse Fluorescence Spectrophotometer at the excitation wavelength of 365 nm.



**Fig. S7** UV-Visible absorption and fluorescent emission spectra of **3a**, **3b**, **4a**, **4b**, **5a** and **5b**. (A) UV-Visible absorption spectrum of **3a**, **3b**, **4a**, **4b**, **5a** and **5b** (5.0  $\mu$ M in DMSO). (B) Fluorescent emission spectrum of **3a**, **3b**, **4a**, **4b**, **5a** and **5b** (5.0  $\mu$ M in DMSO) excited at 365 nm. The UV-Visible and fluorescence spectra of **3a**, **3b**, **4a**, **4b**, **5a** and **5b** are shown with red, orange, green, blue, purple and pink lines, respectively.

**Bacteria and Cell Culture.** Six wild-type bacteria strains: *B. subtilis*, Van-sensitive and resistant *E. faecium*, Van sensitive and resistant *E. faecalis* and *E. coli* DH5 $\alpha$  and two emissive bacteria strains: GFP-expressed *S. aureu* and RFP-expressed *E. coli* were used in this study. Luria-Bertani (LB) medium was used for culture of *B. subtilis*, Van-sensitive *E. faecium*, Van-sensitive *E. faecalis*, and *E. coli* DH5 $\alpha$ . LB medium containing 4  $\mu$ g/mL vancomycin was used for culture of two vancomycin resistant strains. Tryptic Soy Broth (TSB) medium containing 20  $\mu$ g/mL chloramphenicol or LB containing 10  $\mu$ g/mL tetracycline were used for culture of GFP-expressed *S. aureus* or RFP-expressed *E. coli*, respectively. Single colony from the stock agar plate was added to 2 mL of liquid medium, then was grown at 37 °C on a shaker incubator (200 rpm) overnight followed by a subculture until an OD<sub>600</sub> of approximately 0.5-0.7 was reached.

The fibroblast NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) under humidified atmosphere of 5% CO<sub>2</sub> at 37 °C.

**Minimum Inhibitory Concentration (MIC) Test.<sup>8</sup>** 1.0 mL aliquots of bacterial strains cultured in LB solution were collected and centrifuged. The cell pellets were washed twice and resuspended in sterile phosphate-buffered saline (PBS pH 7.2) at OD<sub>600</sub> of 0.5, then further diluted to a final concentration of 2 $\times$ 10<sup>5</sup> cfu/mL. Aliquots of this suspension (25  $\mu$ L) were placed into a 96-well plate. Van and synthesized DP derivatives were diluted in 25  $\mu$ L PBS and then added into the bacteria suspensions to give the desired concentration. After treating with compounds at 37 °C for 2 h, the cultures were then added 2  $\times$  LB solutions and further incubated at 37 °C for 16 h. The wells containing the same number of cells but no compounds and the wells containing the same culture solution but without bacterial cells were set as control groups. The plate was then read using a 96-well plate reader at 600 nm. Each concentration had triplicate values, and the whole experiment was done at three times and the MIC value was determined by taking the average of triplicate OD<sub>600</sub> values for each concentration

and plotting it against concentration. The data was then subjected to sigmoidal fitting. From the curve the MIC value was determined, as the point in the curve where the OD<sub>600</sub> is similar to that of control having no bacteria.

**Determination of binding Constants of Van derivatives to bacterial surface precursors through the Fluorescence Titration method.**<sup>9</sup> We prepared the different peptide sequences (e.g. fluorescein-labelled L-Lys-D-Ala-D-Ala (AcK(F)DADA), Diacetyl L-Lys-D-Ala-D-Ala (Ac<sub>2</sub>KDADA) and Diacetyl L-Lys-D-Ala-D-Lac (Ac<sub>2</sub>KDADLAc)) to mimic the Van sensitive and resistant bacterial surface peptide precursors accordingly. These peptide sequences were used separately to titrate with different concentration of our Van derivatives including Van (**1**), **4a**, and **5a**. The fluorescent changes were used to calculate the binding constants. The Dissociation constants between Van (**1**), **4a**, and **5a** analogs and bacterial surface precursors were determined using fluorescence titration method described in the literature.<sup>9</sup> Titration were carried out at 25 °C in 10 mM HEPES, 6 mM NaCl buffer pH 7.0 and monitored at 515 nm with the peptide (AcK(F)DADA) labeled with FITC fluorescent dye. The obtained experimental data were fitted with following calculation curve to determine dissociation constants ( $K_d$ ) against AcK(F)DADA.

$$F = F_0 + (\Delta Q/2) \{ ([AcK(F)DADA]_0 + [Van^*]_t + K_d) - \sqrt{([AcK(F)DADA]_0 + [Van^*]_t + K_d)^2 - 4[AcK(F)DADA]_0[Van^*]_t} \}$$

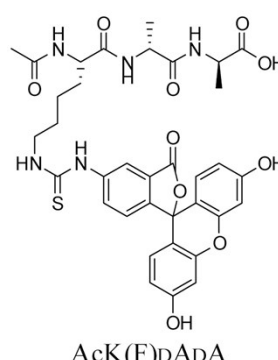
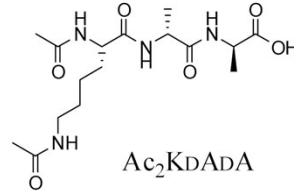
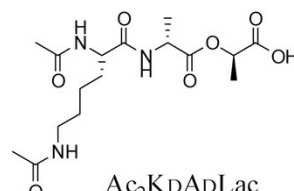
In this equation,  $F$  is the fluorescence observed after each addition of Van derivatives,  $F_0$  is the fluorescence of AcK(F)DADA alone,  $\Delta Q$  is a constant proportional to the difference between the quantum yields of AcK(F)DADA and AcK(F)DADA:Van derivatives,  $[AcK(F)DADA]_0$  is the concentration of AcK(F)DADA, and  $[Van^*]_t$  is the total concentration of Van derivatives added, where Van\* is Van, monovalent Van-DP (**4a**), or divalent Van-DP (**5a**).  $\Delta Q$  and  $K_d$  are adjustable parameters in the analysis.

Dissociation constants of complexes with Ac<sub>2</sub>KDADA and Ac<sub>2</sub>KDADLac were determined by competitive titration methods. Titration were performed as described above, except that the sample cell contained a mixture of AcK(F)DADA and either Ac<sub>2</sub>KDADA (300 μM) or Ac<sub>2</sub>KDADLac (4000 μM).

$$F = F_0 + \Delta Q \left\{ \frac{[\text{AcK(F)DADA}]_0(1/2)\{-K_i[\text{Van}^*]_t + [\text{Ac}_2\text{KDADX}]_0 + ((K_i[\text{Van}^*]_t + [\text{Ac}_2\text{KDADX}]_0)^2 + 4K_i[\text{Van}^*]_t)^{1/2}\}}{K_d + (1/2)\{-K_i[\text{Van}^*]_t + [\text{Ac}_2\text{KDADX}]_0 + ((K_i[\text{Van}^*]_t + [\text{Ac}_2\text{KDADX}]_0)^2 + 4K_i[\text{Van}^*]_t)^{1/2}\}} \right\}$$

Typically, [Ac<sub>2</sub>KDADX] is the initial concentration of either Ac<sub>2</sub>KDADA or Ac<sub>2</sub>KDADLac. K<sub>d</sub> is the dissociation constant for Van\*: AcK(F)DADA complex, K<sub>i</sub> is dissociation constant for Van\*: Ac<sub>2</sub>KDADX complex. ΔQ and K<sub>i</sub> are adjustable parameters in the analysis.

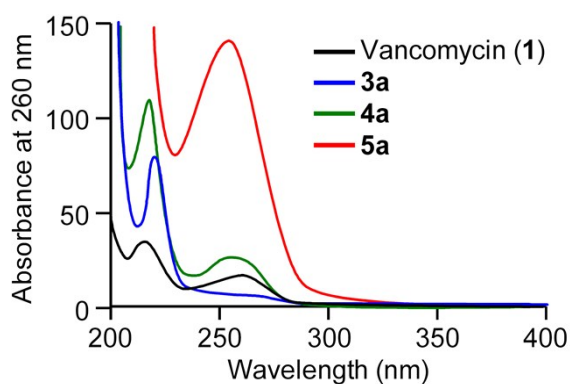
**Table S1.** Dissociation constants for complex of Van derivatives with Ac<sub>2</sub>KDADA and Ac<sub>2</sub>KDADLac

	K <sub>d</sub> (μM)	
	Ac <sub>2</sub> KDADA	Ac <sub>2</sub> KDADLac
		
		
		
<b>1</b> (Van)	1.3 ± 0.1	1900 ± 50
<b>4a</b>	1.2 ± 0.1	1300 ± 80
<b>5a</b>	0.83 ± 0.08	170 ± 20

Dissociation constants determined by competitive titration with AcK(F)DADA in 10 mM HEPES, 6 mM NaCl, pH 7.0, buffer. For **5a**, K<sub>d</sub> values refer to the noncooperative association of individual Van subunits to the ligands.

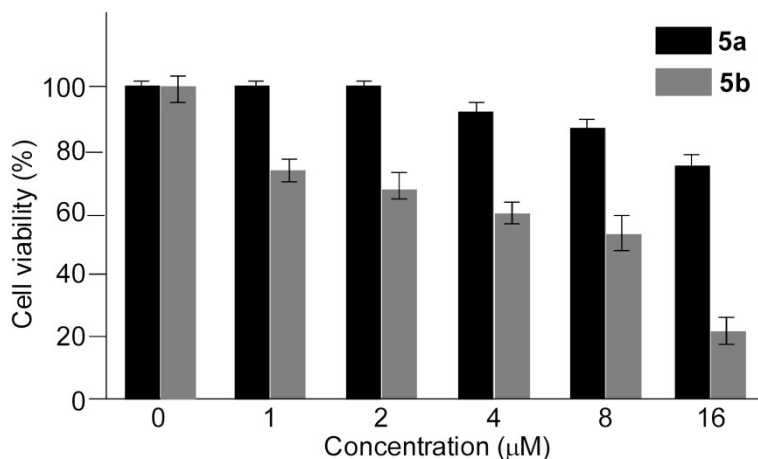
**Investigation of Inhibition of Cell Wall Biosynthesis.**<sup>10</sup> In order to investigate the inhibition of bacterial cell wall biosynthesis, analysis of the cytoplasmic peptidoglycan nucleotide precursor (UDP-*N*-acetyl-muramyl-pentadepsipeptide) was examined based on the process of Van-resistant *E. faecalis* grown in 10 mL LB. Cells were cultured to an OD<sub>600</sub> of 0.6 and followed by incubation with 130 μg/mL

of chloramphenicol for 15 min. Then, Van analogs (5  $\mu$ M) were added and incubated for another 60 min. The bacterial samples were centrifuged and washed with sterile water to remove the antimicrobial agents. After removal of the agents, bacterial cells were subjected to boiling water treatment for about 30 min. The cell extract was then centrifuged and the supernatant was further lyophilized. Finally, the as-lyophilized product was dissolved in 0.2 mL of water and pH was adjusted to 2.0 with 20 % phosphoric acid. The final UDP-linked cell wall precursors in the solution were analyzed by RP-HPLC monitoring the UV absorbance peak at 260 nm.



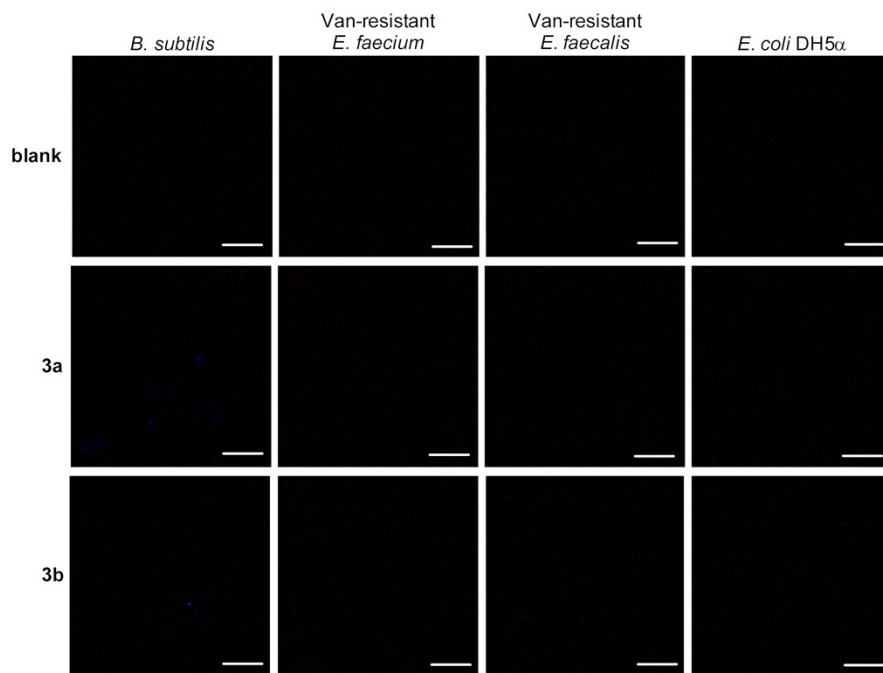
**Fig. S8.** Biosynthesis inhibition of the cell-wall precursor after treatment of VRE with DP and Van analogs including **1**, **3a**, **4a** and **5a** at 5  $\mu$ M by monitoring absorbance at 260 nm wavelength.

**MTT assay.** All the solutions for the MTT assay were freshly prepared in sterile PBS. The fibroblast NIH3T3 cells were seeded on a 96-wells containing 7500 cells per well in 80  $\mu$ L DMEM media and incubated for overnight before adding **5a** or **5b**. Upon incubation with different concentration of **5a** or **5b** at 37  $^{\circ}$ C for 24 h, the media were removed, and cells were washed with PBS and then incubated with cell culture medium containing 20% MTT (3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After 3.5 hours incubation at 37  $^{\circ}$ C, the medium was removed and the cells were lysed with 100  $\mu$ L DMSO. The absorbance was measured at 570 nm using a Tecan's Infinite M200 microplate reader. Cell viabilities at various Van-DP concentrations were given as the percentage of control sample without Van-DP. Each experiment was repeated three times.



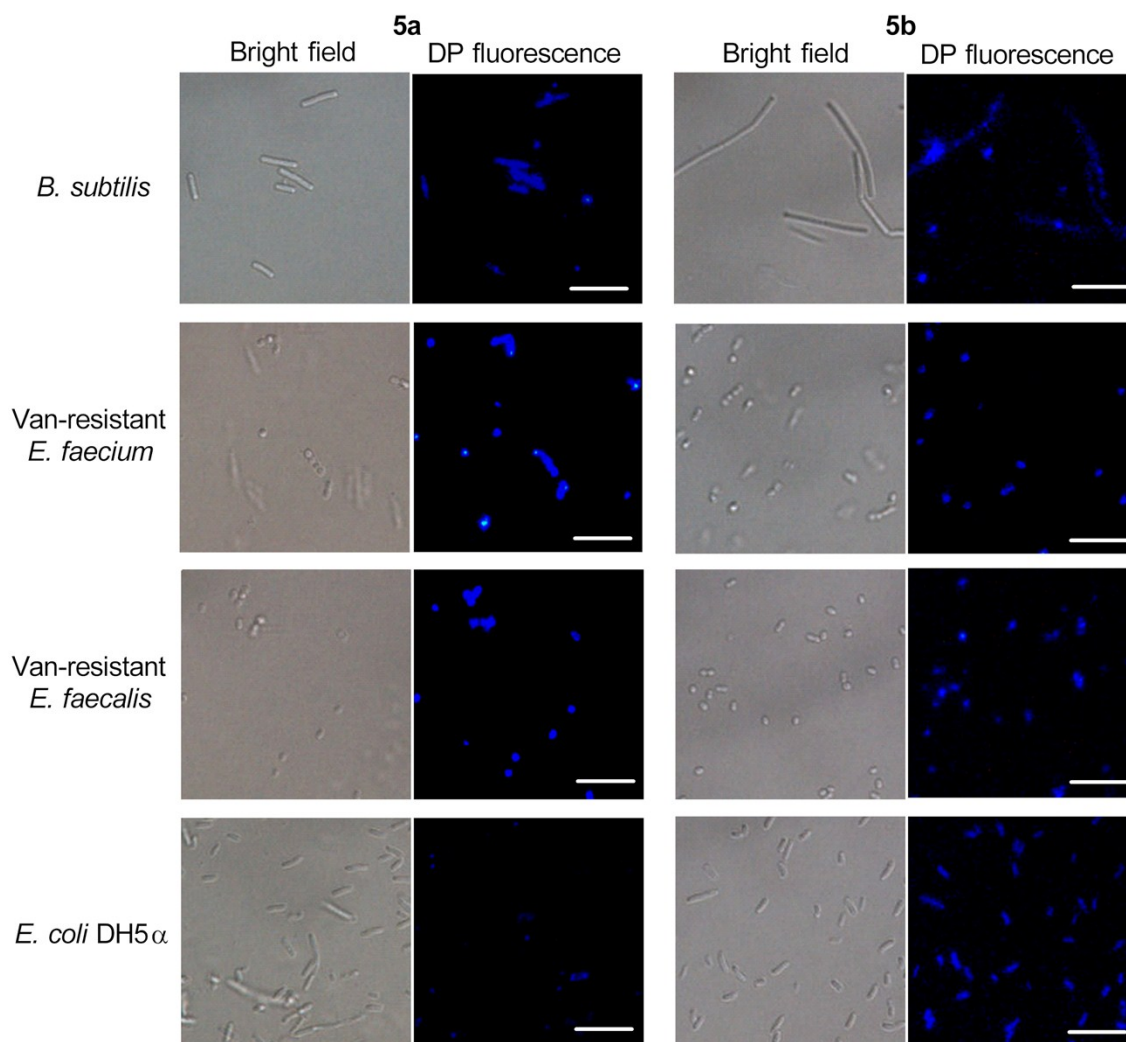
**Fig. S9.** The cytotoxicity assays of **5a** and **5b** in NIH3T3 cells.

**Bacterial Cell Imaging.** Bacterial strains cultured overnight in LB solution were harvested and washed twice with PBS (pH 7.2). The washed cells were resuspended in PBS with an OD<sub>600</sub> of 0.5-0.7. Then 100 μL aliquots were treated with 2 μM of DP derivatives **3a** or **3b** (Fig. S10.) and Van-DP divalent **5a** or **5b** (Fig. S11.). After incubation at 37 °C for 1 h, the cells were washed with PBS by centrifugation to remove the unbound reagents and then a drop of the suspension was immobilized on poly(L-lysine)-treated coverslips followed by covering with another coverslip. Fluorescence images were acquired with fluorescence microscope (Nikon Eclipse TE2000-E, CFI VC 100× oil immersed optics), using a high pressure Hg lamp for excitation and DAPI filter ( $\lambda_{\text{ex}} = 360 \pm 20$  nm and  $\lambda_{\text{em}} = 460 \pm 25$  nm).



**Fig. S10.** Fluorescence images of bacteria staining with the controlled DP derivatives alone (**3a** and **3b**). The bacterial cells were treated with 2  $\mu$ M compounds at 37 °C for 1 h.  $\lambda_{\text{ex}} = 360 \pm 20$  nm and  $\lambda_{\text{em}} = 460 \pm 25$  nm. Scale bar = 10  $\mu$ m.

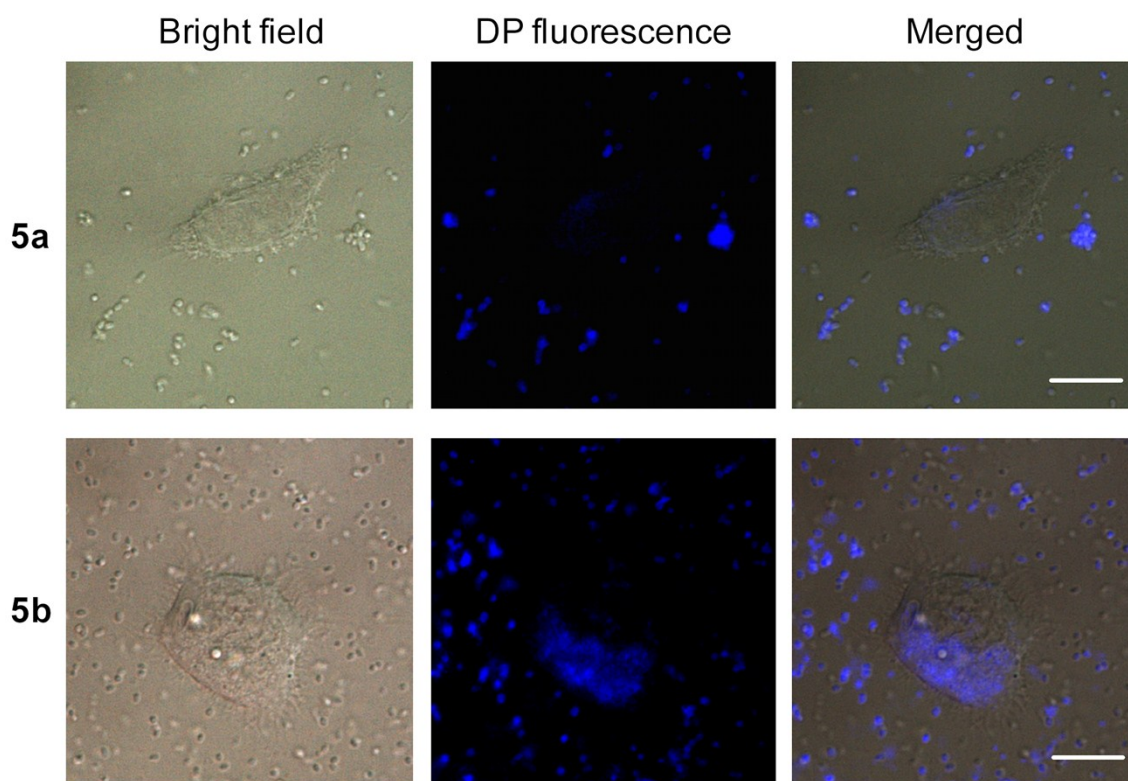




**Fig. S11.** Fluorescence images of bacteria staining with Van-DP analogs of **5a** or **5b** ( $2 \mu\text{M}$ ) at  $37^\circ\text{C}$  for 1 h.  $\lambda_{\text{ex}} = 360 \pm 20 \text{ nm}$  and  $\lambda_{\text{em}} = 460 \pm 25 \text{ nm}$ . Scale bar =  $10 \mu\text{m}$ .

**Fluorescence Microscopic Imaging of Mixture of Bacterial and Mammalian Cells.<sup>11</sup>** Upon reaching 80 % confluence, the NIH3T3 cells ( $1 \text{ mL}$ ,  $1 \times 10^5 \text{ cells/mL}$ ) were transferred into a 6-well plate containing sterile coverslips at the bottom. After overnight culture at  $37^\circ\text{C}$ , the medium was replaced with  $0.5 \text{ mL}$  of Van-resistant *E. faecalis* suspended in PBS with a density of  $1 \times 10^6 \text{ CFU/mL}$ . Simultaneously, Van-DP analogs **5a** or **5b** was added into the wells containing NIH3T3 cells and VRE with a final Van-DP concentration of  $2 \mu\text{M}$ . After incubation at  $37^\circ\text{C}$  for 1 h, the bacteria in the suspension were isolated, followed by washing with PBS at least for three times to remove unbound Van-DP molecules, and the adhesive NIH3T3 cells were also washed with PBS. Finally,  $10 \mu\text{L}$  of the

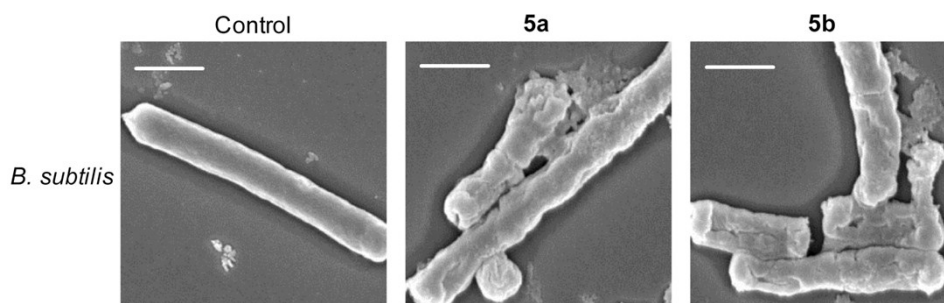
bacterial suspension was dropped onto the coverslip covered with NIH3T3 cells and the distribution of Van-DP was observed with a Nikon Eclipse TE2000-E fluorescence microscopy equipped with an oil immersion lens.



**Fig. S12.** Fluorescence images of the mixture of VanB and NIH3T3 cells staining with **5a** or **5b**. The bacterial and mammalian cells were treated with 2  $\mu\text{M}$  compounds at 37  $^{\circ}\text{C}$  for 1 h.  $\lambda_{\text{ex}} = 360 \pm 20$  nm and  $\lambda_{\text{em}} = 460 \pm 25$  nm. Scale bar = 10  $\mu\text{m}$ .

**Scanning Electron Microscopic Imaging of bacteria treated with 5a or 5b.**<sup>12</sup> *B. subtilis*, Van-resistant *E. faecalis* and *E. coli* DH5 $\alpha$  cells were cultured for 12 h in LB media at 37  $^{\circ}\text{C}$ . The cells were centrifuged and resuspended in LB media. The suspension was added 10  $\mu\text{M}$  of **5a** or **5b** and incubated at 37  $^{\circ}\text{C}$  for 2 h (at 200 rpm shaking speed). After incubation, the cells were harvested by centrifugation at 5000 rpm. The cells were dehydrated sequentially with 30, 50, 70, 80, 90 and 100% ethanol for 15 min. Later, 5  $\mu\text{L}$  of dehydrated cells were dropped on cover glasses and dried at room temperature.

Before SEM observation, the cover glasses were sputter Pt coated (40 mA, 100 sec). Images were recorded by using JEOL JSM-7600F operated at 5 kV.



**Fig. S13.** Scanning electron microscopy images of *B. subtilis* after treatment with 10  $\mu$ M of **5a** or **5b** at 37  $^{\circ}$ C for 2 h. Scale bar = 1  $\mu$ m.

**Selective Treatment of Gram (+) Bacteria.** Emissive bacterial strains (GFP-expressed *S. aureus* and RFP-expressed *E. coli*) cultured overnight in each suitable media were harvested and washed twice with PBS (pH 7.2). The cell pellets were washed twice and resuspended in PBS at OD<sub>600</sub> of 0.5, then further diluted by LB media to a final concentration of  $2 \times 10^5$  cfu/mL. Then 100  $\mu$ L LB media containing 1:1 mixture of GFP-expressed *S. aureus* and RFP-expressed *E. coli* were treated with 10  $\mu$ M of Van-DP dimers **5a** or **5b**. After incubation at 37  $^{\circ}$ C for 12 h, the cells were washed with PBS by centrifugation to remove the unbound compounds and then a drop of the suspension was immobilized on poly(L-lysine)-treated coverslips. Fluorescence images were acquired with confocal laser scanning microscope (Nikon Eclipse TE2000-E, CFI VC 100 $\times$  oil immersed optics), using a 488 nm laser and 515  $\pm$  30 nm filter for GFP-expressed *S. aureus* and 543 nm laser and 590  $\pm$  60 nm filter for RFP-expressed *E. coli*.

**Two Photon Bacterial Cell Imaging.**<sup>2</sup> Bacterial strains cultured overnight in LB solution were harvested and washed twice with PBS (pH 7.2). The washed cells were resuspended in PBS with an OD<sub>600</sub> of 0.5-0.7. Then 100  $\mu$ L aliquots were treated with 2  $\mu$ M of divalent Van-DP **5a**. After incubation at 37  $^{\circ}$ C for 1 h, the cells were washed with PBS by centrifugation to remove the unbound reagents and then a drop of the suspension was immobilized on poly(L-lysine)-treated coverslips. Fluorescence

images were acquired with A TriM Scope II single-beam two-photon microscope (LaVision BioTec) with a laser (Coherent Chameleon Ultra II One Box Ti:sapphire) and water-dipping objectives (100×, Olympus).

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