Supporting Information

Combinatorial approach toward synthesis of small molecule libraries as bacterial transglycosylase inhibitors

Hao-Wei Shih, Kuo-Ting Chen, Shao-Kang Chen, Chia-Ying Huang, Ting-Jen R Cheng, Che Ma, Chi-Huey Wong,* Wei-Chieh Cheng.*

The Genomics Research Center, Academia Sinica, No. 128, Academia Road Sec. 2, Nankang District,Taipei, 11529, Taiwan.

Fax: (+886)2–2789–8771

E–mail: wcheng@gate.sinica.edu.tw

Abbreviations. (1) MurNAc: *N*-acetylmuramic acid; (2) GlcNAc: *N*-acetylglucosamine; (3) *C. difficle*: *Clostridium difficle*; (4) *E. Coli.*: *Escherichia coli*; (5) HTS-FA: high–throughput screening-fluorescence anisotropy; (6) MRSA: methicllin-resistant *Staphylococcus asrues*; (7) PBP: pencillin-binding protein; (8) TGase: transglycosylase; (9) VRE: vancomycin-resistant *Enterococcus*; (10) UDP: uridine diphosphate; (11) C55 alcohol: undecaprenyl alcohol

Experimental Section

General Information. All the solvents and reagents were obtained commercially and used without further purification. NMR spectra (^{1}H at 600 MHz; ^{13}C at 150 MHz) were recorded on a spectrometer in d-solvent at ambient temperature. Mass spectra were obtained by BRUKER Daltonics Bio-TOF III. CC refers to column chromatography.

4. To a solution of freshly prepared phosphoryl chloride **7** (4.9 mmol) and alcohol (1 g, 4.9 mmol) in DCM (20 mL) at 0 ^{o}C was added Et_3N (1 mL, 7.4 mmol). The reaction was slowly warmed to RT and stirred 3 h. The mixture was diluted with DCM and extracted with and water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (50% EtOAc in hexanes) to give a yellow oil in 61% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.68 (t, 2H, J = 9 Hz), 2.58 (dd, 2H, J = 10.8 and 33.0 Hz), 2.71–2.82 (m, 1H), 3.23 (br, 2H), 3.93–4.08 (m, 2H), 5.03–5.25 (m, 10H), 5.63–5.79 (m, 1H), 7.23–7.53 (m, 10H); HRMS calcd for $[C_{21}H_{26}N_1O_5P+H]^+$ 404.4165, found 404.4158.

8. A solution of nitron **3** (0.89 g, 2.3 mmol) and allyl **4** (0.86 g, 2.2 mmol) in tetrachloroethylene (TCE) (5 mL) was heated to 80 ^{o}C and stirred for 2 days. After the reaction was complete, the solvent was evaporated. The residue was purified by CC (50% EtOAc in hexanes) to give yellow oil in 42% yield. ^{1}H NMR (600 MHz, $CDCl_3$)

δ 1.75 (t, 2H, J = 9 Hz), 1.94–2.30 (m, 4H), 2.58 (dd, 2H, J = 10.8 and 33.0 Hz), 3.56 (dd, 1H, J = 6 and 10.2 Hz), 3.61 (dd, 1H, J = 6 and 10.2 Hz), 3.84 (t, 1H, J = 4.8 Hz), 4.0 (t, 1H, J = 6 Hz), 4.33 (br, 1H), 4.44–4.60 (m, 6H), 4.92–5.03 (m, 4H), 7.33–7.34 (m, 25H); HRMS calcd for $[C_{47}H_{53}N_2O_9P+H]^+$ 821.9134, found 821.9128.

9. A solution of **8** (0.2 g, 0.5 mmol), $Pd(OH)_2$ and 1 drop AcOH in MeOH (10 mL) was stirred 72 h under hydrogen atmosphere. After the reaction was complete, the solution was filtrated and evaporated to dryness to give the title compound as colorless oil in 72% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.77 (s, 3H), 1.80–2.01 (m, 5H), 3.00 (t, 2H, J = 7.2 Hz), 3.20–3.48 (m, 2H), 3.73 (dd, 1H, J = 6 and 12.6 Hz), 3.78 (dd, 1H, J = 3.6 and 12.6 Hz), 3.79–3.91 (m, 3H), 3.93 (t, 1H, J = 7.8 Hz), 3.96–3.99 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 77.6, 74.1, 64.1, 61.9, 58.3, 58.1, 37.3, 34.6, 27.8, 27.7, 23.2; HRMS calcd for $[C_{11}H_{25}N_2O_7P+H]^+$ 329.3071, found 329.3066.

14. To a solution of freshly prepared phosphoryl chloride **7** (6.6 mmol) and alcohol (1.6 g, 6.6 mmol) in DCM (50 mL) at 0 ^{o}C was added Et_3N (2 mL, 14 mmol). The reaction was slowly warmed to RT and stirred 3 h. The mixture was diluted with DCM and extracted with and water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (50% EtOAc in hexanes) to give unseparable diastereomers as yellow oil in 55% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.43 (s, 3H), 2.51–2.67 (m, 2H), 4.28–4.44 (m, 2H), 4.93–5.25 (m, 4H), 5.52–5.79 (m, 2H), 6.68–6.81 (m, 1H), 7.36–7.44 (m, 10H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 136.3, 134.9, 129.2, 128.6 (× 2), 128.5 (× 2), 128.2 (× 2), 127.2 (× 4), 120.1, 69.1, 67.6, 67.4, 67.1, 37.7, 20.1; HRMS calcd for $[C_{21}H_{24}N_3O_5P+H]^+$ 430.1532, found 430.1538.

15. The procedure used for the synthesis of **15** was similar to **8**. Compound **15** was obtained as unseparable diastereomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.36–1.38 (m,

3H), 2.12–2.28 (br, 2H), 3.56–3.68 (br, 2H), 3.82–3.91 (m, 2H), 4.02–4.08 (br, 1H), 4.09–4.13 (m, 1H), 4.20–4.24 (m,1 H), 4.42–4.68 (m, 6H), 4.82–5.19 (m, 4H); HRMS calcd for $[C_{47}H_{53}N_2O_9P+H]^+$ 821.3567, found 821.3561.

16. The procedure used for the synthesis of **16** was similar to **9**. Compound **16** was obtained as unseparable diastereomers.^{1}H NMR (600 MHz, $CDCl_3$) δ 1.53 (s, 3H), 1.91–2.14 (m, 5H), 3.54–3.61 (m, 2H), 3.82 (dd, 1H, J = 6 and 13.2 Hz), 3.91 (dd, 1H, J = 3.6 and 12.6 Hz), 3.97–4.06 (m, 4H), 4.25 (dd, 1H, J = 5.4 and 11.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2, 77.2, 73.6, 66.3, 66.2, 63.8, 63.6, 62.1, 62.0, 60.3, 58.1, 57.9, 48.8, 36.9, 33.7, 33.6, 17.9; HRMS calcd for $[C_{12}H_{25}N_2O_9+H]^+$ 373.1376, found 373.1379.

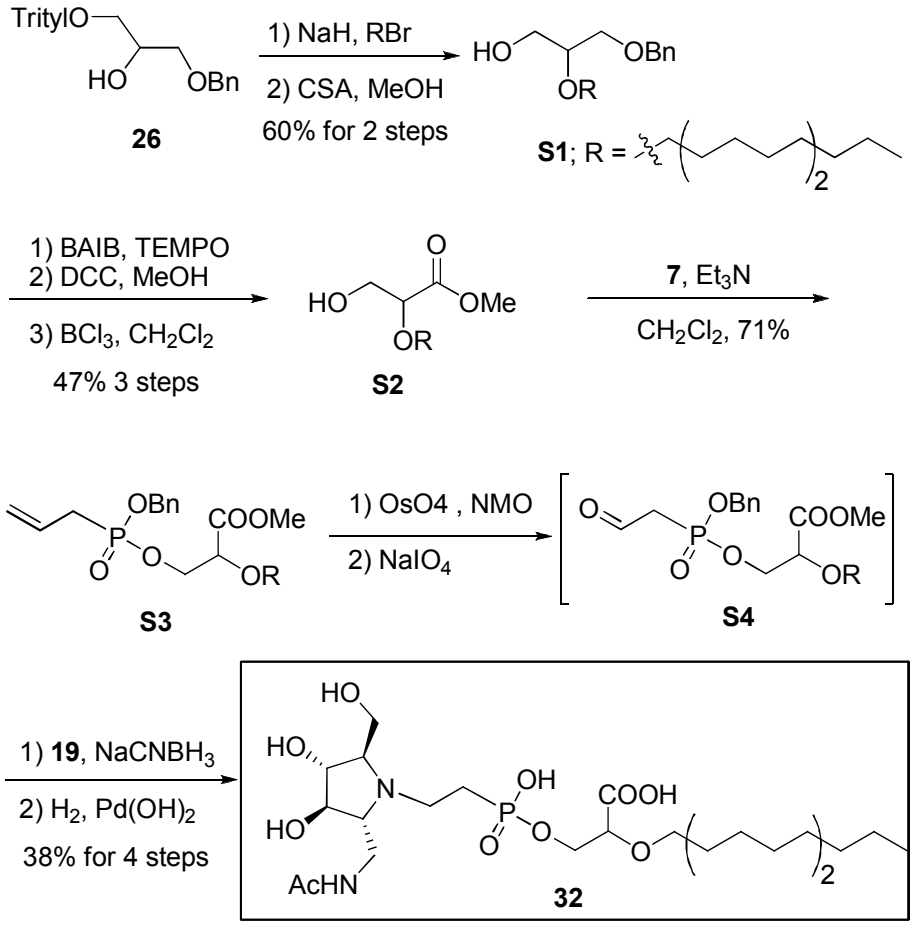
33. The procedure used for the synthesis of **33** was similar to **22.** Yield : 77%. ^{1}H NMR (600 MHz, MeOH-d4) δ 0.85–0.90 (m, 12H), 1.07-1.66 (m, 16H), 2.02-2.12 (m, 2H), 3.42-3.70 (m, 5H), 3.87 (t, 1H, J = 3.6 Hz), 3.91-4.08 (m, 4H), 4.14 (br, 3H); ^{13}C NMR (150 MHz, MeOH-d4) δ 177.6, 82.0, 77.4, 72.7, 72.5, 70.0, 67.1, 64.8, 58.6, 58.4, 47.1, 46.9, 40.6, 38.9, 38.7, 38.6, 37.9, 37.8, 34.1, 31.3, 31.2, 29.2, 26.0, 25.7, 23.2, 23.1, 20.3, 20.2; HRMS calcd for $[C_{26}H_{52}NO_{10}P+H]^+$ 570.3407, found 570.3413.

34. The procedure used for the synthesis of **34** was similar to **22.** Yield : 83%. ^{1}H NMR (600 MHz, MeOH-d4) δ 0.85–1.07 (m, 12H), 1.08-1.56 (m, 16H), 2.01 (s, 3H), 2.02-2.12 (m, 6H), 3.42-3.70 (m, 5H), 4.06 (t, 1H, J = 4.2 Hz), 4.11-4.20 (m, 2H); ^{13}C NMR (150 MHz, MeOH-d4) δ 174.4, 172.6, 80.1,70.5, 66.1, 55.2, 52.6, 40.7, 38.8, 38.6, 38.5, 38.0, 34.1, 31.2, 31.1, 29.3, 26.0, 25.6, 25.4, 24.4, 24.3, 23.3, 23.2, 20.3; HRMS calcd for $[C_{27}H_{53}N_2O_8P+H]^+$ 565.3618, found 565.3622.

35. The procedure used for the synthesis of **35** was similar to **22.** Yield : 91%. ^{1}H NMR (600 MHz, MeOH-d4) δ 0.96–1.01 (m, 12H), 1.20–1.76 (m, 16H), 2.02 (m, 2H), 2.78 (m, 1H), 3.23 (m, 2H), 3.59 (m, 2H), 4.01 (br, 1H), 4.20–4.28 (m, 2H); ^{13}C NMR (150 MHz, MeOH-d4) δ 176.0, 81.1, 70.2, 66.4, 64.8, 38.8, 38.6, 38.5, 37.9, 37.8,

36.9, 34.1, 31.3, 31.2, 29.2, 26.8, 26.1, 26.0, 25.6, 25.2, 23.2, 23.1, 20.3, 20.2; HRMS calcd for $[C_{20}H_{42}NO_6P+H]^+$ 424.2828, found 424.2821.

36. The procedure used for the synthesis of **36** was similar to **22.** Yield : 88%. ^{1}H NMR (600 MHz, MeOH-d4) δ 0.97–1.00 (m, 12H), 1.22–1.78 (m, 16H), 2.01 (m, 2H), 3.22 (m, 2H), 3.57 (m, 2H), 3.78 (m, 1H), 4.11 (br, 1H), 4.21–4.29 (m, 2H); ^{13}C NMR (150 MHz, MeOH-d4) δ 176.0, 81.1, 70.2, 66.4, 64.8, 48.0, 38.8, 38.6, 38.5, 37.9, 37.8, 34.1, 31.3, 31.2, 29.2, 26.8, 26.1, 26.0, 25.6, 25.2, 23.2, 23.1, 20.3, 20.2; HRMS calcd for $[C_{20}H_{41}O_7P+H]^+$ 425.2668, found 425.2259.



S1. To a solution of alcohol **26** [S1] (5.1 g, 12 mmol) and 1-bromododecane (4.5 mL, 18 mmol) in dried DMF (150 mL) at 0^{o}C was added NaH (0.58 g, 14 mmol) in one

portion. The reaction was slowly warmed to RT and stirred for 5 h. The mixture was poured into water, diluted with ether and extracted with water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (10% EtOAc in hexane) to give unseparable enantiomers as yellow oil in 89% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7.2 Hz), 1.24–1.32 (br, 16H), 1.56 (t, 4H, J = 9.6 Hz), 3.20 (d, 2H, J = 4.2 Hz), 3.51–3.62 (m, 5H), 4.52 (d, 2H, J = 12.6 Hz), 7.21–7.45 (m, 20H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 144.1 (× 3), 138.3, 128.7 (× 6), 128.4 (× 2),128.3 (× 2), 127.7 (× 6), 127.5, 126.9 (× 3), 86.5, 78.3, 73.2, 70.7, 70.5, 63.4, 31.9, 30.1, 29.7 (× 4), 29.6, 29.5, 29.4, 22.7, 14.1; HRMS calcd for $[C_{41}H_{53}O_3+H]^+$ 593.3994, found 593.3999.

A solution of alkane (4.5 g, 7.6 mmol) and camphorsulfonic acid (CSA) (0.53 g, 2.2 mmol) in MeOH (100 mL) was reflux for 1 h. The reaction was quenched with TEA and evaporated to dryness. The mixture was extracted with water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (30% EtOAc in hexane) to give unseparable enantiomers as yellow oil in 92% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7.2 Hz), 1.24–1.32 (br, 16H), 1.56 (t, 4H, J = 9.6 Hz), 3.47–3.63 (m, 7H), 4.52 (d, 2H, J = 3 Hz), 7.26–7.34 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.0, 128.4 (× 2), 127.7, 127.6 (× 2), 78.4 73.5, 70.4, 69.9, 62.9, 31.9, 30.1, 29.7 (× 4), 29.6, 29.5, 29.4, 22.7, 14.1; HRMS calcd for $[C_{22}H_{38}O_3+H]^+$ 351.2899, found 351.2907.

S2. To a solution of alcohol **S1** (2.2 g, 6.3 mmol) in acetone (30 mL) and water (5 mL) at 0 ^{o}C was added (bis-acetoxyiodobenzene) (BAIB) (5.4 g, 16 mmol) and TEMPO (0.2 g, 1.3 mmol). The reaction was slowly warmed to RT and stirred for 3 h. The mixture was poured into water, diluted with EtOAc and extracted with water. The aqueous layer was extracted with EtOAc twice and the combined EtOAc was further extracted with $Na_2S_2O_3$ and water. The organic layer was dried with $MgSO_4$ and

concentrated. The residue was used directly without further purification. To a solution of acid in DCM (20 mL) was added DCC (2.7 g, 13 mmol), MeOH (5 mL) and catalytic amount of DMAP. The mixture was stirred at RT overnight. The reaction was concentrated, washed with hexane and filtrated. The filtrate was concentrated and purified by CC (10% EtOAc in hexane) to give unseparable enantiomers as yellow oil in 62% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7.2 Hz), 1.24–1.32 (br, 16H), 1.56 (t, 4H, J = 9.6 Hz), 3.39 (dd, 1H, J = 1.9 and 6.9 Hz), 3.61 (dd, 1H, J = 1.9 and 6.9 Hz), 3.70–3.73 (m, 5H), 4.05 (dd, 1H, J = 4.3 and 5.2 Hz), 4.57 (dd, 2H, J = 12.0 and 20.4 Hz), 7.25–7.32 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 137.9, 128.3 (× 2), 127.7, 127.6 (× 2), 79.1, 73.4, 71.3, 70.4, 52.0, 31.9, 30.1, 29.7 (× 4), 29.6, 29.5, 29.4, 22.7, 14.1; HRMS calcd for $[C_{23}H_{38}O_4+H]^+$ 379.2848, found 379.2851.

A solution of benzyl protected glycerate (1.1 g, 2.9 mmol) in DCM (50 mL) was cooled to – 50 ^{o}C and BCl_3 (1M in hexane, 4.5 mL, 4.5 mmol) was added dropwisely. After the reaction was complete (~ 1h), the solution was quenched with MeOH (10 mL) and TEA (10 mL) and evaporated to dryness. The residual was purified by CC (20% EtOAc in hexane) to give unseparable enantiomers in 92% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7.2 Hz), 1.24–1.32 (br, 18H), 1.56 (dt, 2H), 3.38 (dt, 1H), 3.68 (dt, 1H), 3.73 (s, 3H), 3.75 (dd, 1H, J = 4.2 and 12 Hz), 3.83 (dd, 1H, J = 4.2 and 12 Hz), 3.94 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 79.5, 71.3, 63.3, 52.0, 31.9, 30.1, 29.7 (× 4), 29.6, 29.5, 29.4, 22.7, 14.1; HRMS calcd for $[C_{16}H_{32}O_4+H]^+$ 289.2379, found 289.2388.

S3. The procedure used for the synthesis of **S3** was similar to **4**. Yield : 73%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7.2 Hz), 1.22–1.24 (br, 18H), 1.55 (dt, 2H), 2.62–2.68 (dd, 2H, J = 7.4 and 22.7 Hz), 3.40 (dt, 1H), 3.61 (dt, 1H), 3.70–3.73 (m, 3H), 4.03 (m, 1H), 4.16–4.33 (m, 3H), 5.01–5.08 (m, 2H), 5.14–5.18 (m, 2H), 5.74 (m,

1H), 7.29–7.37 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 136.2, 128.7 (× 2), 128.6, 128.1, 127.9 (× 2), 120.4, 78.1, 71.4, 65.8, 63.3, 60.3, 52.0, 31.9, 30.1, 29.7 (× 4), 29.6, 29.5, 29.4, 22.7, 14.1; HRMS calcd for $[C_{26}H_{43}O_6P+H]^+$ 483.2876, found 483.2868.

Enzymatic synthesis of Lipid II and its analogs

6-(*N*-(7-Nitrobenzyl-2-oxa-1,3-diazol-4-yl) amino) hexanoyl (NBD)-lipid II.[S2] M. flavus vesicles (100 μL, 320 mg/mL) were added to UDP–GlcNAc (100 μL, 100 nmol), UDP MurNAc–pentapeptide (100 μL, 100 nmol), undecaprenyl phosphate (10mg) and buffer (100 μL, 100 mM Tris HCl, pH 8, 5 mM $MgCl_2$, and 1% (w/v) Triton X–100) in dd-H_2O (600 μL). The suspension was sonicated at 37 ^oC for 15 min, followed by extraction of the mixture by butanol (200 μL) and pyridine–acetate (200 μL, 6 M, pH 4.2). The butanol (top) phase was collected after brief centrifugation. The residual was extracted with butanol (200 μL) again and the butanol layer was collected and evaporated to dryness. The crude mixture was purified by CC ($CHCl_3$: MeOH : H_2O : 2 N NH_4OH = 88 : 58 : 12.5 : 1) to give crude lipid II (0.7 mg) which was used for fluorescence-label without further HPLC purification.

A solution of NBD reagent (10.1 mg, 26 μmol) in DMF (2 mL) was added in one portion to a solution of lipid II (2.4 mg, 1.2 μmol) in aq. $NaHCO_3$ (0.25 M, 250 μL). The mixture, which turns turbid immediately, was stirred at room temperature overnight. ESI-MS and TLC showed complete conversion to product. The mixture was concentrated in vacuo, redissolved in 1:1 water/acetonitrile and purified by semi-preparative chromatography on a ZORBAX Bx-C8 column (9.4 mm x 25 cm, P.N. 880967.201) using a gradient of 85:15 to 100:0 methanol/50 mM ammonium acetate over 30 minutes, monitored by fluorescence detector ($\lambda_{ex}/\lambda_{em}$ = 466/535 nm). The appropriate fractions were combined and lyophilized to provide NBD-lipid II (1.7 mg, 63% yield). HRMS calcd for $[C_{106}H_{168}N_{12}O_{30}P_2–2H)^{2-}$ 1075.0675, found

1075.0652.

Lipid I 37. The procedure was similar to NBD-lipid II but UDP-GlcNAc is no need. HRMS calcd for $[C_{86}H_{143}N_7O_{21}P_2–2H)^{2–}$ 834.9833, found 834.9828.

4-Fluorinated (4-F) lipid II 38. The procedure was similar to NBD-lipid II but using 4-F UDP-GlcNAc[s3] instead of UDP-GlcNAc. HRMS calcd for $[C_{94}H_{155}FN_8O_{25}P_2–2H)^{2-}$ 938.0225, found 938.0236.

HTS–FA Measurements (binding assay). The FA assay was used to screen primary library prepared by microtiter-plate based amide-bond formation. The compounds were transferred from 96-well plates to 1536-well plates, using a multi-dispenser (Labcyte) to prepare the compound plates for screening. The *H*. pylori PBP1a (10 μg/ml) in 100 nM F–Moe, 10 mM Tris, 100 mM NaCl, pH 8.0, at a final volume of 9 μl was added to 1536-well black plates (Greiner). One microliter of 10 mM stock solution of compound was added to wells. The last four columns of every plate were controls containing 10 μM moenomycin and 10% DMSO, respectively. After 30 min incubation, changes in F were determined with ViewLux (Perkin Elmer). Hits that showed > 75% reduction compared with the control anisotropy values were selected for further confirmamtion.

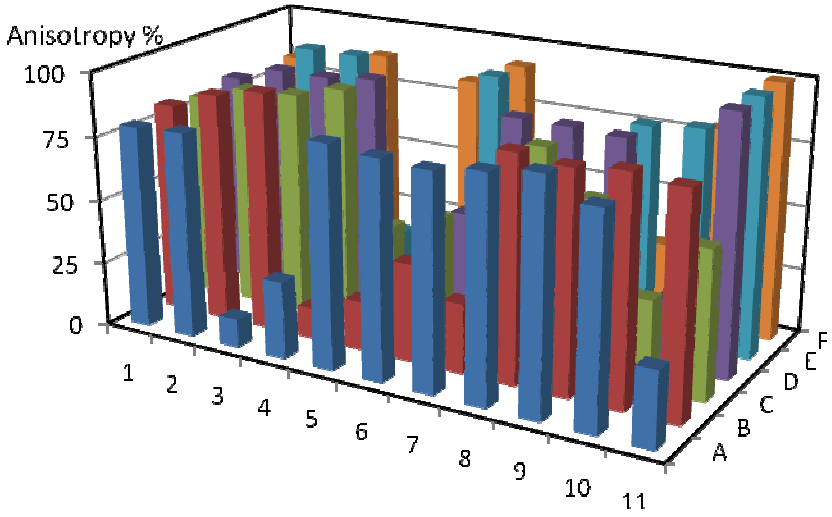


Figure S1. Library B : 17 hits were found at 1 mM.

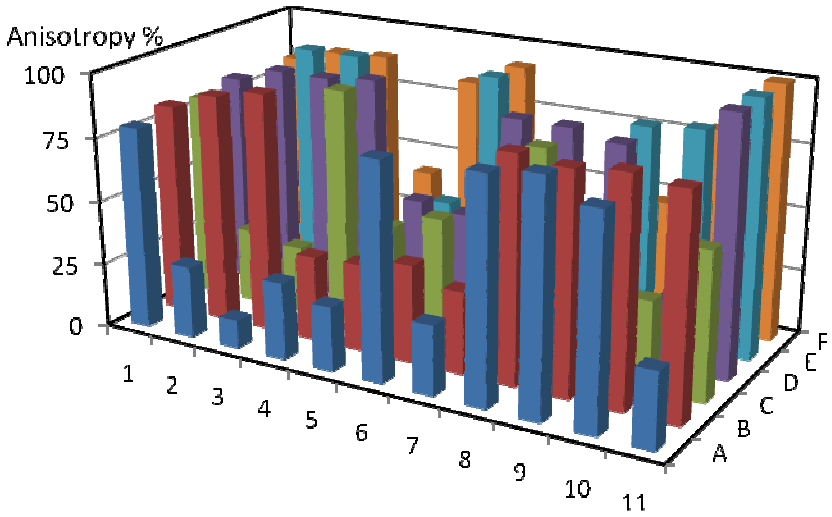


Figure S2. Library C : 15 hits were found at 1 mM.

Inhibition Assay of Synthetic compounds against *E. Coli.* PBP1b[s4] Assays consisted of 1 μL of buffer (0.085% decyl-PEG, 50 mM Tris HCl, pH 8.0), 10% DMSO, 0.5 μL NBD-Lipid II (200 μM), 1 μL inhibitors (concentration as indicated in Table 3), and 2 μL PBP (0.02 mg/mL). After incubation at 30 ^{o}C for 1 h, reactions were stopped by adding 1 μL Moenomycin A (100 μM), followed by further incubation with 1 μL muramidase (0.02 mg/mL) for 3 h. The reaction mixture was analyzed by HPLC analysis performed on an anion exchange column (SAX1, Supelco

Co.). A linear gradient of 20 mM to 1 M ammonium acetate (in methanol) was used as eluant. The retention time of NBD-lipid II is about 13.8 min, whereas the product GlcNAc-MurNAc-pentapeptide-NBD is 13 min.

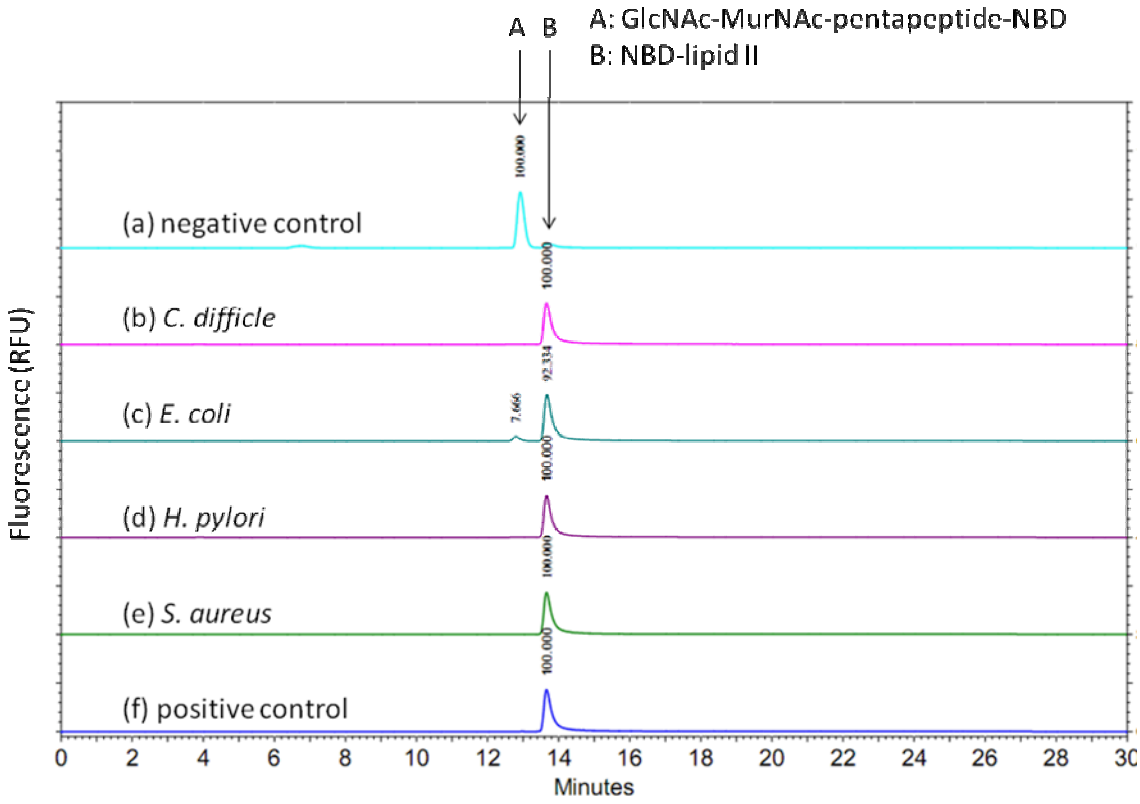


Figure S3. Inhibition activity of **31** at 100 μM toward bacterial TGase. Incubation of NBD-lipid II with (a) TGase (*E. coli*) (negative control) (b) TGase (*C. difficle*) and **31** (c) TGase (*E. coli*) and **31** (d) TGase (*H. pylori*) and **31** (e) TGase (*S. aureus*) and **31** (f) TGase (*E. coli*) and Moe A (positive control), was shaken at 25°C in 50 mM Tris buffer containing 10 mM MgCl2, 10 % DMSO, 15% MeOH, and muramidase (0.02 mg/mL). The reaction was terminated by the addition of moenomycin A (1 μL of 100 μM) after 1 h. The reaction mixture was analyzed by reverse-phase HPLC.

References:

s1. C. Bolchi, M. Pallavicini, L. Fumagalli, B. Moroni, C. Rusconi and E. Valoti, *Tetrahedron: Asymmetry*, 2005, **16**, 3380-3384.

s2. H. E. Hasper, N. E. Kramer, J. L. Smith, J. D. Hillman, C. Zachariah, O. P. Kuipers, B. de Kruijff and E. Breukink, *Science*, 2006, **313**, 1636-1637.

s3. M. T. Sung, Y. T. Lai, C. Y. Huang, L. Y. Chou, H. W. Shih, W. C. Cheng, C. H. Wong and C. Ma, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 8824-8829.

s4. T. Ueda, F. Feng, R. Sadamoto, K. Niikura, K. Monde and S. I. Nishimura, *Org. Lett.*, 2004, **6**, 1753-1756.