An Efficient Chemo-enzymatic Strategy for the Synthesis of Wild-Type and Vancomycin-Resistant Bacterial Cell-Wall Precursors: UDP-N-Acetylmuramyl-Peptides

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Supporting Information

Experimental Materials and Methods:

General Information: Plasmid for constructing MurA, MurB and MurC enzymes was from New England Biolabs. UDP-(^{14}C)GlcNAc were purchased from NEN DuPont. All amino acids were purchased from Novabiochem. NHS-activated fluorescein and rhodamine were purchased from Pierce. Unless otherwise stated, all chemicals were obtained from Aldrich or Sigma and used without further purification. Glucose dehydrogenase was a generous gift from Amano enzyme USA Co. LTD.

Reactions were monitored by thin-layer chromatography (TLC) utilizing ninhydrin or cerium molybdate stain as the developing reagent. All non-aqueous reactions were carried out in oven-dried glassware under an inert Ar atmosphere. All non-aqueous solvents were distilled before use.

NMR spectra were recorded on Bruker AMX-400, AMX-500 or AMX-600 MHz spectrometers and were referenced to residual solvent peaks ($CDCl_3$: ^{1}H δ 7.24; CD_3OD: ^{1}H δ 3.30; D_2O: ^{1}H δ 4.76)

Purification of *E. coli* MurA, MurB and MurC: Vector pMALc2E (New England Biolabs) was used for expression and purification of MurA, MurB and MurC. MurA, MurB and MurC were expressed as fusion proteins that had maltose-binding protein and

His6 tag for easy purification. After expression, enzymes were purified by amylose and nickel chromatography.

Synthesis of UDP-MurNAc pentapeptide 3 from compound 1: A solution of muramyl pentapetide **1** (100 mg, 0.081 mmol) and trioctylamine (35 uL, 0.081 mmol) in 5 mL of methanol was stirred in the presence of 10% Pd/C under H_2 (1 atm) for 12 h. The mixture was filtered and concentrated in vacuo. The residue was dissolved in dry pyridine and the solvent was evaporated three times. The dried solid was combined with 4-morpholine-N,N'-dicyclohexylcarboxamidium uridine 5'-monophosphomorpholidate (81.7 mg, 0.119 mmol) and dried again in the same fashion. ^{1}H-tetrazole (16.6 mg, 0.23 mmol) and dry 1.1 mL of pyridine were added and the mixture was stirred at room temperature under Ar. After 2 days, the reaction was quenched with water and the solvent was evaporated. 1.3 mL of water and 0.7 mL of 2N NaOH aq. were added to the residue and stirred for 30 min. The aqueous solution was washed with 1.5 mL of ether and loaded on Bio-Gel P-4 column, eluted with 100 mM NH_4HCO_3. After lyophilization of the corresponding fraction, white powder 90 mg was obtained in quantitative yield from compound **1**. Spectral analysis in agreement with ref. 3.

Compound 6: 1. **Boc-D-Ala-D-Lac-O-Bzl**: Diethyl azodicarboxylate (124.8 uL, 0.79 mmol) was added to a solution of Boc-D-Ala (100 mg, 0.53 mmol), L-lactate benzyl ester (94.5 uL, 0.53 mmol), and triphenyl phosphine (207.9 mg, 0.79 mmol) in 10 mL of ether during 30 min. The reaction was stirred at room temperature for 2 h. The mixture was filtered and concentrated in vacuo. Purification by flash chromatography on silica gel eluting with 15% EtOAc/Hexanes afforded Boc-D-Ala-D-Lac-O-Bzl 236 mg as a white solid (85%): R_f=0.6 (15% EtOAc/Hexanes); ^{1}H NMR ($CDCl_3$, 500MHz) δ 7.31 (m, 5H),

5.21 (m, 3H), 4.9 (bs 1H), 4.37 (m, 1H), 1.58 (d, *J*=2.60, 3H), 1.53 (s, 9H), 1.39 (d, *J*=6.95, 3H); ES-MS (pos) calcd for $C_{16}H_{25}NO_6$ $[M+H]^+$=352, found 352.

Compound **6** was synthesized by standard HOBt/EDC method with BOC-protected amino acids: R_f=0.4 (70% EtOAc/Hexanes); ^{1}H NMR (MEOD, 500MHz) δ 5.06 (q, *J*=7.0, 1H), 4.48 (m, 1H), 4.41 (dd, *J*=4.75, 4,75, 1H), 4.34 (m, 1H), 4.27-4.18 (m, 4H), 4.12 (m, 2H), 4.04 (q, *J*=6.95, 1H), 3.3 (m, 2H), 3.08 (m, 2H), 2.38 (m, 2H), 2.26 (m, 1H), 2.00 (m, 1H), 1.81 (m, 1H), 1.70 (m, 1H), 1.57 (d, *J*=6.95, 3H), 1.48-1.36 (m, 9H), 1.05-0.95 (m, 6H), 0.05-0.02 (3s, 27H); ES-MS (pos) calcd for $C_{33}H_{71}N_5O_{11}Si_3$ $[M+H]^+$=834, found 834.

Compound 7: DBU (19 uL, 0.125 mmol) was added in one portion to a stirred solution of the muramyl phosphate (101 mg, 0.125 mmol) in 3 mL of CH_2Cl_2 under Ar. After stirring for 30 min, the mixture was quenched with 3 mL of 1N HCl, the organic layer washed with water (2 x 5 mL) and brine (1 x 5 mL) and then dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in 5 mL of DMF under Ar and depsipentapeptide salt (104 mg, 0.125 mmol), HOBt (19 mg, 0.125 mmol), DIEA (21 uL 0.125 mmol) and EDC (24 mg, 0.125 mmol) were added at 0°C. The mixture was allowed to warm up to room temperature in 2 h. The mixture was stirred at room temperature for 12 h. The reaction was washed with brine (2 x 7 mL) and concentrated in vacuo. Purification by flash chromatography on silica gel eluting with 3% to 10% MeOH/CH_2Cl_2 yielded compound **7** 118 mg (65%) as a white powder: R_f=0.5 (5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500MHz) δ 7.40-7.29 (m, 15H), 5.64 (m, 1H), 5.48 (s, 1H), 5.06-4.91 (m, 4H), 4.49 (m, 1H), 4.34 (m, 1H), 4.29 (m, 2H), 4.18 (m, 4H), 4.09 (m, 4H), 3.84 (ddd, *J*=4.75, 4.75, 4.75, 1H), 3.65 (m, 2H), 3.05 (m, 2H), 2.29 (m, 1H),

2.18 (m, 2H), 1.93 (s, 3H), 1.44 (m, 14H), 1.31 (m, 5H), 0.96 (m, 8H), 0.89 (m, 3H), 0.04-0.02 (3s, 27); ES-MS (pos) cald for $C_{68}H_{105}N_6O_{21}PSi_3$ $[M+H]^+$=1458, found 1458.

Compound 8: The procedure is the same as synthesis of UDP-MurNAc pentapeptide **3** from **1**. After coupling compound **7** with uridine 5'-monophosphomorpholidate, the reaction was purified by preparative TLC with 3% NH_4HCO_3/^{i}PrOH. The corresponding part was cut from the plate and stirred with MeOH. The mixture was filtered and concentrated in vacuo to give a white powder which was used in the next reaction without further purification. R_f=0.8 (3% NH_4HCO_3/^{i}PrOH). To the solution of the white solid obtained (21 mg, 0.014 mmol) in 2 mL DMF was added TBAF (5 uL, 0.42 mmol) and the mixture stirred at room temperature for 4 h. The reaction was then concentrated in vacuo and dissolved in 1 mL of water. The mixture was filtered and purified by reverse phase HPLC on a Vydac 218TP^{TM} C18 10 mm x 250 mm column employing a gradient elution of 100:0 A:B to 10:90 A:B over 30 min at a flow rate of 4 mL min^{-1} where A=0.05M aq. NH_4HCO_3 and B=MeCN. The retention time of the desired product was 11 min. Lyophilization of the column fractions afforded pure compound **8** 13 mg (80%) as a white powder; ^{1}H NMR (D_2O, 600MHz) δ 7.85 (d, *J*=8.34, 1H), 5.90 (d, *J*=4.80, 1H), 5.88 (d, *J*=8.34 1H), 5.40 (dd, *J*=3.0, 3.0, 1H), 4.38 q, *J*=7.02, 1H), 4.29-4.25 (m, 2H), 4.20-4.04 (m, 8H), 3.87 (m, 1H), 3.80-3.74 (m, 2H), 3.71 (t, *J*=9.60, 1H), 3.58 (t, *J*=9.66, 1H), 2.92 (t, *J*=7.80, 2H), 2.24 (m, 2H), 2.07 (m, 1H), 1.90 (s. 3H), 1.83-1.60 (m, 5H), 1.37 (m, 2H), 1.36 (d, *J*=7.02, 6H), 1.33 d, *J*=6.54, 6H). ES-MS (neg) calc for $C_{56}H_{98}N_8O_{29}P_2Si_3$ $[M-H]^-$=1494, found 1494.

Synthesis of compound 10 by MurA and MurB with cofactor NADPH regeneration: A reaction mixture in 15 mL of Tris-HCl (pH8.0, 100 mM) containing $MgCl_2$ (7 mM),

DTT (0.7 mM), PEP (63 mg, 0.306 mmol), NADP (57 mg, 0.027 mmol), Glucose (270 mg, 30.6 mmol), UDP-GluNAc (100 mg, 0.153 mmol), MurA (0.7 mL 27 mg/mL), MurB (1.0 mL, 24 mg/mL) and Glucose Dehydrogenase (0.5 mg) was stirred under Ar at room temperature overnight. After removing enzymes by filtration, the product was purified by anion-exchange Q sepharose column and eluted with a linear gradient from 50 mM to 1.5 mM NH_4OAc (pH5.0). The active fractions were pooled and lyophilized. The lyophilized powder was dissolved in 2 mL of water and loaded onto a Bio-Gel P-2 size exclusion column, eluting with water. Fractions containing product were lyophilized to gave compound **10** 83.7 mg (80%) as a whiter powder. Spectral analysis in agreement with ref. 7.

Coupling pentapeptide with compound 10: Compound **10** (9.2 mg, 0.014 mmol) was dissolved in 5 mL of DMF and pentapeptide salt (8.3 mg, 0.014 mmol), DIEA (48 uL, 0.028 mmol) and HBTU (5.1 mg, 0.014 mmol) was added at 0°C and was allowed to warm up to room temperature in 2 h. The reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and 1 mL of water followed by 2N NaOH (70 uL, 0.14 mmol) was added and the mixture was stirred for 1 h. Then the mixture was filtered and loaded on Bio-Gel P-4 size exclusion column and eluted with 50 mM NH_4HCO_3. The active fractions were pooled and lyophilized to give compound **3** 10.9 mg (70%) as a white powder. Spectral analysis in agreement with ref. 7.

Compound 11: To a solution of compound **3** (5 mg, 0.0044 mmol) in anhydrous MeOH (0.5 mL) was added DIEA (2.2 uL, 0.013 mmol). Next, a solution of NBD-F (3.2 mg, 0.017 mmol) in anhydrous THF (0.2 mL) was added at 0°C; the reaction was carried out

in the dark. The mixture was stirred overnight at room temperature and concentrated in vacuo. The brown residue was dissolved in H_2O (0.5 mL) and loaded on Bio-Gel P-4 column, eluted with 100 mM NH_4HCO_3. After lyophilization of the corresponding fractions, yellow powder 3.5 mg was obtained in 60% yield: ^{1}H NMR (D_2O, 600MHz) δ 7.91 (m. 2H), 6.43 (d, J=9.2Hz, 1H), 5.92 (d, J=4.8Hz, 1H), 5.91 (d, J=8.3, 1H), 5.45 (d, J=4.3, 1H), 4.33-4.10 (m, 11H), 3.94 (d, J=9.6Hz, 1H), 3.90-3.76 (m, 4H) 3.62-3.59 (m, 3H), 2.25 (m, 2H), 2.22 (m, 1H), 1.99 (s, 3H), 1.88-1.80 (m, 4H), 1.67-1.49 (m, 3H), 1.41 (d, J=7.4 3H), 1.38 (d, J=6.6, 3H), 1.32 (m, 6H). ES-MS (pos) calcd for $C_{46}H_{66}N_{12}O_{29}P_2$ $[M+H]^+$=1313, found 1313.

Compounds 12 and 13: Compound **3** (4 mg, 0.0035 mmol) was dissolved in 1 mL PBS buffer (pH 8.3). NHS-fluorescein (16.6 mg, 0.035 mmol) or NHS-Rhodamine (18.5 mg, 0.035 mmol) in 1.6 mL DMF was added to the buffered solution at 0°C. After stirring for 2 h at 0°C, the reaction was quenched by the addition of 5 uL ethanolamine (150 mM, pH 9.5) and stirred at 0°C for another 1 h. The mixture was purified by Bio-Gel P-4 column, eluted with 100 mM NH_4HCO_3. After lyophilization of the corresponding fractions, yellow powder 2.7 mg was obtained in 50% yield (compound **11**): ES-MS (neg) calcd for $C_{65}H_{85}N_{11}O_{30}P_2$ $[M-H]^-$=1560, found 1560. For compound **13**, pink powder 0.5 mg was obtained in 10% yield: ES-MS (neg) calcd for $C_{61}H_{75}N_9O_{32}P_2$ $[M-H]^-$=1506, found 1506.