Supporting Information

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Synthetic Peptidoglycan Motifs for Germination of Bacterial Spores


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Experimental Data for Compounds

General Information. All organic reagents were purchased from either Sigma-Aldrich Chemical Company or Acros Organics, unless otherwise stated. All reactions were performed under an atmosphere of nitrogen unless noted otherwise. Reactions were monitored by thin-layer chromatography (TLC) carried out on Whatman reagents 0.25 mm silica gel 60-F plates that were visualized using UV light and/or aqueous cerium sulfate staining, followed by heating. Flash chromatography was carried out with silica gel 60, 230-400 mesh (0.040-0.063 mm particle size) purchased from EM Science. NMR spectra, including \(^1\)H, \(^{13}\)C, DEPT, H-H COSY, H-H TOCSY and H-C HETCOR experiments, were recorded on a Varian INOVA-500, or Varian DirectDrive 600 spectrometer. NMR signal assignments for the compounds were performed on the basis of H-H COSY, H-C HETCOR, and DEPT experiments. High-resolution mass spectra were obtained at the Department of Chemistry and Biochemistry, University of Notre Dame by FAB ionization, using a JEOL AX505HA mass spectrometer or ESI ionization, using Bruker microTOF/Q2 mass spectrometer.

Analytical high-performance liquid chromatography (HPLC) was performed on Waters 2414 instrument with SunFire C18 reversed-phased column (Waters) or delta-pak C18 reversed-phased column (Waters) using a linear gradient of 2-15% acetonitrile in water supplemented with 0.1% TFA over 40 min at 1 mL/min. Detection of the samples was by UV at 205 nm. Preparative HPLC purifications were performed using delta-pak C18 reversed-phased column, 100 Å pore size, 19 × 300 mm.

Crystals were examined under Infineum V8512 oil and placed on a MiTeGen mount, then transferred to the 100 K N\(_2\) stream of either a Bruker SMART Apex CCD diffractometer. Unit cell parameters were determined from reflections with I > 10σ(I) harvested from three orthogonal sets of 30 0.5° ω scans. Data collection strategy was calculated using COSMO, included in the Apex2 suite of programs\(^7\) to maximize coverage of reciprocal space in a minimum amount of time. Average four-fold redundancy of measurements was sought. Data were corrected for Lorentz and polarization effects, as well as for absorption. Structure solution and refinement utilized the programs of the SHELXTL software package.\(^8\)
Syntheses of compounds 1b, 2b, and 3b

7-Benzyl 1-methyl (2S,6R)-6-azido-2-tert-butoxycarbonylamino-heptanedioate (5). Diol 4\(^{[9]}\) (4.2 g, 12 mmol) was dissolved in a mixed solvent system (50 mL of 2:2:3, CH\(_3\)CN:CCl\(_4\):water) and treated sequentially with H\(_2\)IO\(_6\) (11 g, 48 mmol) and RuCl\(_3\) (37 mg, 0.18 mmol). The resulting suspension was stirred for 2 h at RT, followed by sequential addition of CH\(_2\)Cl\(_2\) (50 mL) and water (50 mL). The layers were let to separate. The aqueous layer was washed with CH\(_2\)Cl\(_2\), and the combined organic layers were dried over MgSO\(_4\). Evaporation of the solvents gave the crude acid, which was dissolved in DMF (50 mL), followed by the addition of benzyl bromide (2.8 mL, 22 mmol) and KHCO\(_3\) (2.3 g, 23 mmol). The reaction mixture was stirred at RT for 16 h, diluted with diethyl ether and washed with water. The organic layer was dried (MgSO\(_4\)), filtered, and concentrated to dryness. The crude product was purified by silica gel column chromatography to obtain the benzyl ester 5 (2.5 g, 49% yield). \(^1\)H NMR (500 MHz, CDCl\(_3\), 21 °C, TMS) \(\delta= 1.37 - 1.92 \) (m, 6H), 1.44 (s, 9H), 1.56 - 1.67 (m, 1H), 1.70 - 1.91 (m, 2H), 3.72 (s, 3H), 3.86 (dd, \(J = 8.7, 5.1\) Hz, 1H), 4.29 (q, \(J = 5.6\) Hz, 1H), 5.04 (d, \(J = 8.4\) Hz, 1H), 5.20 (d, \(J = 12.4\) Hz, 1H), 5.22 (d, \(J = 12.2\) Hz, 1H), 7.33 - 7.40 (m, 5H); \(^13\)C NMR (126 MHz, CDCl\(_3\), 21 °C) \(\delta= 21.8, 28.4, 31.0, 32.3, 52.5, 53.2, 61.9, 67.7, 80.1, 128.6, 128.8, 135.1, 155.5, 171.3, 172.8\); HRMS-FAB (m/z): [M+H]\(^+\), calcd. for C\(_{20}\)H\(_{29}\)N\(_4\)O\(_6\), 421.2087; found, 421.2090.

**Compound 8.** Compound 4 (9.6 g, 28 mmol) was dissolved in MeOH (60 mL) and treated with Ba(OH)\(_2\)-8H\(_2\)O (8.7 g, 28 mmol). The resultant suspension was stirred for 4 h at RT. Solvents were removed and the residue was taken up into EtOAc and water. The pH of the solution was adjusted to ~2 with 10% KHSO\(_4\) in an ice-water bath. Layers were separated and the aqueous layer was washed with EtOAc. The combined organic layer was dried (MgSO\(_4\)), filtered, and concentrated to dryness. The crude acid was dissolved in CH\(_2\)Cl\(_2\) (100 mL), followed by the addition of 2-methoxypropene (5.0 mL, 52 mmol) and a catalytic amount of pyridinium \(p\)-toluenesulfonate (PPTS). The mixture was stirred at RT for 2 h before it was quenched with three drops of Et\(_3\)N. The resulting solution was washed with brine, dried (MgSO\(_4\)), and concentrated. The crude acetonide was dissolved in DMF (50 mL) and was treated with \(N\)-hydroxysuccinimide (4.8 g, 42 mmol), followed by the addition of EDCI (8.1 g, 42 mmol) in an ice-water bath. The mixture was stirred at RT for 20 h.
Meanwhile, the Boc-protected d-Ala-d-Ala-OBn (9.7 g, 28 mmol) in CH₂Cl₂ (50 mL) was treated with trifluoroacetic acid (10 mL) in an ice-water bath. Temperature was gradually increased to RT over 1 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in toluene (30 mL). This was followed by evaporation to dryness. The residue was dissolved in iPr₂NEt (9.6 mL, 55 mmol) and DMF (10 mL), and the solution was then added to the NHS-ester of the tripeptide, as prepared above. The resulting mixture was stirred at RT for 20 h. The mixture was diluted with CH₂Cl₂ and water was added. Layers were separated. The organic layer was dried over MgSO₄, filtered, concentrated and the sample was subjected to column chromatography on silica gel chromatography to obtain compound 8 as a white solid (8.7 g, 52% yield).¹H NMR (500 MHz, CDCl₃, 21 °C, TMS) δ= 1.32 - 1.33 (s, 3H), 1.35 (d, J = 6.8 Hz, 3H), 1.39 (d, J = 7.2 Hz, 3H), 1.40 (s, 9H), 1.43 - 1.44 (s, 3H), 1.50 - 1.63 (m, 2H), 3.47 (m, 1H), 3.83 (dd, J = 8.4, 6.4 Hz, 1H), 3.99 (dd, J = 8.2, 6.6 Hz, 1H), 4.04 (dd, J = 11.8, 6.2 Hz, 1H), 4.20 (m, 1H), 4.54 - 4.62 (quintet, J = 7.2 Hz, 1H), 4.66 (quintet, J = 7.0 Hz, 1H), 5.12, 5.17 (2 × d, J = 12.3 Hz, 2H), 5.55 (d, J = 8.8 Hz, 1H), 7.26 - 7.35 (m, 5H), 7.45 (d, J = 7.2 Hz, 1H); ¹³C NMR (126 MHz, CDCl₃, 21 °C) δ= 18.0, 18.7, 22.4, 25.2, 26.3, 28.4, 30.6, 32.7, 48.2, 48.7, 54.2, 63.6, 65.8, 67.1, 77.8, 80.0, 109.7, 128.2, 128.5, 128.6, 135.4, 155.7, 172.0, 172.5; HRMS-FAB (m/z): [M+H⁺], calcd. for C₂₉H₄₅N₆O₈, 605.3299; found, 605.3291.

Compound 9. Compound 8 (4.0 g, 6.6 mmol) was stirred in 70% AcOH (50 mL) at RT for 16 h. The solution was concentrated to dryness and the crude compound was subjected to the same condition used for transformation of diol 4 to benzyl ester 5, yielding the desired compound 9 (2.0 g, 47%).¹H NMR (500 MHz, CDCl₃, 21 °C, TMS) δ= 1.36 (d, J = 7.0 Hz, 3H), 1.41 (d, J = 7.4 Hz, 3H), 1.42 - 1.92 (m, 6H), 1.43 (s, 9H), 3.88 (dd, J = 8.8, 5.0 Hz, 1H), 4.13 (q, J = 7.1 Hz, 1H), 4.60 (m, 1H), 5.16 (q, J = 12.4 Hz, 2H), 5.22 (s, 2H), 5.37 (d, J = 6.8 Hz, 1H), 7.11 (d, J = 6.6 Hz, 1H), 7.23 (d, J = 6.8 Hz, 1H), 7.32 - 7.41 (m, 10H); ¹³C NMR (126 MHz, CDCl₃, 21 °C) δ= 18.1, 18.5, 22.0, 28.4, 31.0, 32.2, 48.3, 48.8, 54.3, 61.9, 67.2, 67.6, 80.2, 128.3, 128.5, 128.7, 128.8, 135.1, 135.5, 155.8, 170.4, 171.3, 171.9, 172.6; HRMS-FAB (m/z): [M+H⁺], calcd. for C₃₂H₄₅N₆O₈, 639.3142; found, 639.3151.

Compound 10. Compound 9 (1.9 g, 3.0 mmol) in CH₂Cl₂ (20 mL) was treated with TFA (5 mL) in an ice-water bath. The mixture was stirred for 30 min and the ice-water bath was
removed and stirring was continued for another 30 min. The reaction mixture was concentrated to dryness, toluene (20 mL) was added to the residue and the mixture was evaporate in vacuo. The resultant oil was dissolved in anhydrous DMF (20 mL) and treated with iPr₂NEt (1.6 mL, 9.0 mmol), followed by the addition of Boc-L-Ala-γ-D-Glu(ONHS)-OBn (3.4 g, 4.6 mmol) in DMF (10 mL). The reaction mixture was stirred for 16 h at RT. The mixture was diluted with CH₂Cl₂ and water. The separated organic layer was dried over MgSO₄, filtered, concentrated and the sample was subjected to column chromatography on silica gel to give the title compound (1.6 g, 56%).

1H NMR (500 MHz, CDCl₃, 21 °C, TMS) δ= 1.28 (d, J = 6.8 Hz, 3H), 1.31 - 2.48 (m, 10H), 1.37 (d, J = 7.2 Hz, 3H), 1.40 (d, J = 7.2 Hz, 3H), 1.43 (s, 9H), 3.87 (m, 1H), 4.11 (m, 1H), 4.28 (m, 1H), 4.44 - 4.60 (m, 3H), 5.06 - 5.24 (m, 6H), 7.06 (d, J = 6.8 Hz, 1H), 7.30 - 7.38 (m, 17H); 13C NMR (151 MHz, CDCl₃, 21 °C) δ= 17.5, 17.6, 22.2, 27.0, 28.3, 30.8, 31.4, 36.5, 48.2, 49.1, 50.6, 51.2, 53.5, 54.2, 61.7, 66.8, 66.9, 67.4, 80.2, 128.0, 128.2, 128.3, 128.5, 128.6, 135.0, 135.4, 135.5, 162.6, 170.2, 171.3, 172.3, 172.5; HRMS-FAB (m/z): [M+H]+, calcd. for C₄₇H₆₁N₈O₁₂, 929.4409; found, 929.4437.

1-Methyl 7-(p-nitrobenzyl) (2S,6R)-6-azido-2-p-nitrophenylsulfonylaminoheptane-dioate (12). Transformation of diol 4 to its p-nitrobenzyl ester was accomplished in the same manner as described for the preparation of 5, with the exception that p-nitrobenzyl bromide was used in place of benzyl bromide. The resulting compound (1-methyl 7-p-nitrobenzyl (2S,6R)-6-azido-2-tert-butoxycarbonylamino-heptanedioate, 0.40 g, 0.86 mmol) in CH₂Cl₂ (2 mL) was treated with TFA (0.5 mL) in ice-water bath. The mixture was stirred for 30 min and ice-water bath was removed and stirring was continued for another 30 min. The reaction mixture was concentrated to dryness, 2 mL of toluene was added and the solution was evaporated to dryness again. The resultant oil was dissolved in CH₂Cl₂ (5 mL) and was treated with iPr₂NEt (0.45 mL, 2.6 mmol), followed by the addition of p-nitrophenyl sulfonyl chloride (0.29 g, 1.3 mmol). The solution was stirred at RT for 16 h. The mixture was diluted with CH₂Cl₂ and water was added. The separated organic layer was dried over MgSO₄, filtered, concentrated and the sample was subjected to column chromatography on silica gel to obtain the desired compound 12 as a white solid (0.30 g, 63%).

1H NMR (500 MHz, 10% CD₃OD in CDCl₃, 21 °C, TMS) δ= 1.35 - 1.88 (m, 6H), 3.37 (s, 2H), 3.41 (s, 3H), 3.88 (td, J = 8.9, 5.1 Hz, 2H), 5.26 (s, 2H), 7.51 (d, J = 9.0 Hz, 2H), 7.96 (d, J = 9.2 Hz, 2H), 8.19 (d, J = 8.8 Hz, 2H), 8.28 (d, J = 9.2 Hz, 2H); 13C NMR (126 MHz, 10% CD₃OD in CDCl₃, 21 °C, TMS) δ= 17.5, 17.6, 22.2, 27.0, 28.3, 30.8, 31.4, 36.5, 48.2, 49.1, 50.6, 51.2, 53.5, 54.2, 61.7, 66.8, 66.9, 67.4, 80.2, 128.0, 128.2, 128.3, 128.5, 128.6, 135.0, 135.4, 135.5, 162.6, 170.2, 171.3, 172.3, 172.5; HRMS-FAB (m/z): [M+H]+, calcd. for C₄₇H₆₁N₈O₁₂, 929.4409; found, 929.4437.
CD3OD in CDCl3, 21 °C) δ = 21.6, 30.5, 32.4, 52.5, 55.5, 61.7, 66.1, 124.0, 124.2, 128.4, 128.8, 142.2, 146.2, 150.0, 171.7; HRMS-FAB (m/z): [M+H]⁺, calcd. for C23H23N6O10S, 551.1196; found, 551.1200.

**Compound 18.** EDCI (61 mg, 0.32 mmol) was added to a mixture of N-hydroxy-succinimde (38 mg, 0.33 mmol) and 15 (400 mg, 0.26 mmol) in CH₂Cl₂ (4 mL) in an ice-water bath. The mixture was stirred at RT 20 h. Meanwhile, the Boc-protected pentapeptide 10 (290 mg, 0.31 mmol) in CH₂Cl₂ (5 mL) was treated with trifluoroacetic acid (2 mL) in an ice-water bath. Temperature was gradually increased to RT over 1 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in toluene. This was followed by evaporation to dryness. The residue (compound 11) was dissolved in iPr₂NEt (140 µL, 0.79 mmol) and DMF (5 mL), and the solution was then added to the NHS-ester of the tetrasaccharide, prepared above. The resulting mixture was stirred at RT for 20 h. The mixture was diluted with CH₂Cl₂ and water was added. Layers were separated. The organic layer was dried over MgSO₄, filtered, concentrated and the sample was subjected to column chromatography on silica gel (CH₂Cl₂/MeCN/MeOH, 10:3:0.5) to give the title compound (530 mg, 64%).

1H NMR (500 MHz, DMSO-d₆, 21 °C) δ = 1.09 - 1.32 (8 × d, 24H), 1.33 - 1.91 (m, 14 H), 1.71 - 1.85 (4 × s, 12H), 1.98 (dq, J = 13.2, 6.6 Hz, 2H), 2.12 - 2.27 (m, 4H), 3.06 - 3.20 (m, 3H), 3.32 (s, 3H), 3.35 - 3.93 (m, 20H), 4.11 - 4.63 (m, 27H), 4.71 (d, J = 12.0 Hz, 1H), 4.95 (d, J = 11.6 Hz, 1H), 4.98 - 5.25 (m, 12H), 5.59 (s, 1H, CHPh), 7.09 - 7.48 (m, 62H), 7.65 - 8.48 (m, 7H); 13C NMR (126 MHz, DMSO-d₆, 21 °C) δ = 16.6, 16.7, 18.1, 18.5, 18.9, 19.1, 22.8, 22.9, 23.0 (12 × q), 21.5, 27.0, 27.1, 27.2, 30.4, 31.2, 31.3 (10 × t), 47.4 (d), 47.5 (d), 47.6 (d), 47.7 (d), 51.7 (d), 51.8 (d), 52.3 (d), 52.4 (d), 55.6 (q, OCH₃), 61.1 (d), 65.4 (d), 65.8, 66.0, 66.8, 71.2, 71.7, 73.4, 73.6 (11 × t, CH₂Ph), 67.9, 68.0, 68.2, 68.5 (4 × t, C-6), 73.8 (d), 74.1 (d), 74.2 (d), 74.4 (d), 75.1 (d), 76.9, 77.6 (2 × d, Lac-α-C), 78.8 (d), 79.3 (d), 79.4 (d), 81.2 (d), 99.2, 99.7, 100.1, 101.6 (4 × d, C-1), 100.1(d, CHPh), 126.0, 126.7, 126.9, 127.1, 127.3, 127.4, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7, 131.6, 131.7, 135.5, 135.8, 136.0, 137.6, 138.5, 138.6, 138.7, 138.8, 139.4, 169.1, 169.2, 169.3, 169.5, 170.2, 171.1, 171.2, 171.3, 171.5, 171.6, 171.7, 172.0, 172.1, 172.2, 172.3, 172.8; ESI-MS (m/z): [M+K]⁺, calcd. for C₁₆₅H₁₉₈N₂₀O₄₃K, 3186.36; found, 3186.32.
Methyl β-D-N-acetylglucosamine-(1→4)-β-D-N-acetylmuramyl-L-Ala-γ-D-Glu-meso-DAP-d-Ala-d-Ala-(1→4)-β-D-N-acetylglucosamine-(1→4)-β-D-N-acetylmuramyl-L-Ala-γ-D-Glu-meso-DAP-d-Ala-d-Ala (3b). Compound 18 (0.50 g, 0.16 mmol) in AcOH (5 mL) was stirred at 60 °C for 2 h. A portion of Pd/C (10%, 0.1 g) was added to the reaction mixture and was stirred under an atmosphere of hydrogen at 50 °C for 3 h. The stirring was continued for an additional 2 h at RT. The reaction mixture was filtered through a layer of Celite. The combined filtrate was concentrated to dryness under reduced pressure. The crude product was subjected to HPLC purification to afford compound 3b (0.18 g, 56%). Preparative HPLC purifications were performed on delta-pak C18 reversed-phase d column, 100 Å pore size, 19 × 300 mm using a linear gradient of 5-15% CH3CN in water supplemented with 0.1% TFA over 0.5 h.

1H NMR (600 MHz, D2O, 21 °C) δ= 1.35 - 1.45 (8 × d, 24H), 1.44 - 2.02 (m, 14H), 1.92, 1.95, 2.04 (4 × s, 12H), 2.10 - 2.23 (m, 2H), 2.31 - 2.42 (m, 4H), 3.37 - 3.44 (m, 2H), 3.46 (s, 3H; OCH3) 3.50 - 3.97 (m, 16H), 4.23 (m, 3H), 4.26 - 4.46 (m, 11H), 4.47 - 4.55 (m, 3H); 13C NMR (151 MHz, D2O, 21 °C) δ= 16.2, 16.3, 16.4, 16.8, 16.9, 17.8, 17.9, 22.0, 22.2 (12 × q), 20.8, 27.1, 27.2, 29.8, 30.3, 31.3 (10 × t), 49.0 (d), 49.4 (d), 49.5 (d), 52.8 (d), 53.9 (d), 54.1, 54.6, 55.2, 55.8 (4 × d, C-2), 57.0 (q, OCH3), 59.7 (d), 59.8 (d), 60.2 (d), 61.0 (4 × t, C-6), 70.1 (d), 72.1 (d), 73.4 (d), 74.6 (d), 74.9 (d), 75.0 (d), 75.3 (d), 76.0 (d), 77.9, 78.1 (2 × d, Lac-α-C), 79.2 (d), 79.4 (d), 100.3, 101.3, 101.9 (4 × d, C-1), 173.9, 174.1, 174.3, 174.5, 174.6, 174.7, 175.1, 175.3, 175.4, 176.4, 176.6; HRMS-ESI (m/z): [M+Na]+, calcd. for C81H132N16O43Na, 2039.8532; found, 2039.8558.

Compound 16. This material was prepared in the same manner as described for 18, with the exception that the protected muramyl derivative 13 was used in place of 15. 1H NMR (500 MHz, 10% CD3OD in CDCl3, 21 °C) δ= 1.28 (d, J = 7.2 Hz, 3H), 1.31 (d, J = 6.6 Hz, 3H), 1.31 (d, J = 7.2 Hz, 3H), 1.33 (d, J = 7.4 Hz, 3H), 1.36 - 1.91 (m, 7H), 1.89 (s, 3H), 2.13 - 2.32 (m, 3H), 3.39 - 3.44 (m, 1H; H-5), 3.43 (s, 3H), 3.56 (t, J = 9.2 Hz, 1H; H-4), 3.59 (t, J = 8.6 Hz, 1H; H-2), 3.75 (t, J = 10.3 Hz, 1H; H-6a), 3.81 (dd, J = 8.8, 4.8 Hz, 1H; DAP), 3.87 (t, J = 9.4 Hz, 1H; H-3), 4.06 (q, J = 6.7 Hz, 1H; Lac-α-H), 4.18 (dd, J = 8.4, 5.6 Hz, 1H; DAP), 4.24 (q, J = 7.0 Hz, 1H; Ala-α-H), 4.30 (dd, J = 10.5, 4.9 Hz, 1H; H-6b), 4.36 (q, J = 7.2 Hz, 1H; Ala-α-H), 4.39 (dd, 1H; Glu-α-H), 4.43 (q, J = 7.2 Hz, 1H; Ala-α-H), 4.59 (d, J = 8.2 Hz, 1H; H-1), 5.00 - 5.16 (m, 6H; CH2Ph), 5.50 (s, 1H; CHPh), 7.21 - 7.44 (m, 20H); 13C NMR (126 MHz, 10% CD3OD...
in CDCl₃, 21 °C) δ= 17.3, 17.4, 17.5, 19.4, 23.2 (5 × q), 22.2, 27.2, 30.8, 31.0, 31.3 (5 × t), 48.3, 49.0, 49.4 (3 × d, 3Ala-α-C), 51.4 (d, Glu-α-C), 53.7 (d, DAP-C-6), 56.4 (C-2), 57.0 (q, OCH₃), 61.9 (d, DAP-C-2), 65.9 (d, C-5), 67.0, 67.3, 67.6 (3 × t, 3CH₂Ph), 68.7 (t, C-6), 78.1 (d, Lac-α-C), 79.3 (d, C-3), 81.5 (d, C-4), 101.4 (d, CHPh), 101.9 (d, C-1), 126.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 129.2, 135.0, 135.2, 135.4, 137.0, 170.5, 171.5, 172.1, 172.4, 172.6, 172.7, 173.2, 174.1.

Methyl β-D-N-acetylmuramyl-L-Ala-γ-D-Glu-meso-DAP-d-Ala-d-Ala (1b). Deprotection of compound 16 to 1b was carried out in the same manner as described for 18 to 3b. ¹H NMR (500 MHz, D₂O, 21 °C) δ= 1.37 (d, J = 7.0 Hz, 3H), 1.38 (d, J = 7.4 Hz, 3H), 1.43 (d, J = 7.2 Hz, 3H), 1.43 (d, J = 7.4 Hz, 3H), 1.47 - 1.56 (m, 2H), 1.72 - 2.05 (m, 5H), 1.96 (s, 3H), 2.16 - 2.26 (m, 1H), 2.34 - 2.42 (m, 2H), 3.44 - 3.54 (m, 3H; H-3, H-4, H-5), 3.49 (s, 3H; OCH₃), 3.77 (dd, J = 12.4, 5.6 Hz, 1H; H-6a), 3.82 (dd, J = 9.8, 8.8 Hz, 1H; H-2), 3.94 (dd, J = 12.4, 2.2 Hz, 1H; H-6b), 4.04 (t, J = 6.3 Hz, 1H; DAP-H-6), 4.21 (q, J = 6.8 Hz, 1H; Lac-α-H), 4.25 (dd, J = 8.2, 6.8 Hz, 1H; DAP-H-2), 4.27 - 4.39 (m, 4H; 3Ala-α-H, Glu-α-H), 4.40 (d, J = 8.6 Hz, 1H; H-1);

¹³C NMR (126 MHz, D₂O, 21 °C) δ= 16.2, 16.6, 16.9, 18.8, 22.3 (5 × q), 20.9, 26.7, 29.5, 30.4, 31.4 (5 × t), 48.7, 49.6, 49.8 (3 × d, Ala-α-C), 52.0 (d, Glu-α-C), 52.9, 54.0 (2 × d, DAP-C-2, DAP-C-6), 55.1 (d, C-2), 57.3 (q, OCH₃), 60.8 (t, C-6), 68.9 (d, C-4), 75.8 (d, C-5), 78.3 (d, Lac-α-C), 82.8(d, C-3), 102.1 (d, C-1), 163.0, 163.3, 172.3, 174.0, 174.3, 174.7, 175.0, 175.3, 175.7, 176.3; HRMS-FAB (m/z): [M+H]⁺, calcd. for C₃₃H₅₆N₇O₁₇, 822.3733; found, 822.3728.

Compound 17. This material was prepared in the same manner as described for 18, with the exception that the protected muramyl derivative 14 was used in place of 15. ¹H NMR (500 MHz, 10% CD₃OD in CDCl₃, 21 °C) δ= 1.11 - 1.26 (4 × d, 12H), 1.23 - 1.88 (m, 7H), 1.73 (s, 3H), 1.82 (s, 3H), 2.02 - 2.16 (m, 3H), 3.17 (br. s., 1H; H-5), 3.26 (s, 3H), 3.38 (t, J = 6.1 Hz, 1H; H-3’), 3.40 - 3.47 (m, 2H; H-5’, H-3), 3.52 (m, J = 9.2 Hz, 1H; H-4), 3.56 - 3.64 (m, 3H; H-6a, H-6a’, H-6b’), 3.66 - 3.77 (m, 3H; H-2, H-4’, DAP), 3.79 (m, 1H; H-2’), 4.04 (m, 1H), 4.08 - 4.21 (m, 3H; Lac-α-H, H-6b, H-1’), 4.21 - 4.32 (m, 5H; Ala-α-H, Glu-α-H, H-1), 4.36, 4.46 (AB, J = 11.2 Hz, 2H; CH₂Ph), 4.48, 4.69 (AB, J = 11.7 Hz, 2H; CH₂Ph), 4.84 - 5.08 (m, 6H; 3 × CH₂Ph), 5.41 (s, 1H; CHPh), 7.00 - 7.40 (m, 30H; Ar); ¹³C NMR (126 MHz, 10% CD₃OD in CDCl₃, 21 °C) δ= 16.8, 17.1, 17.2, 18.0, 22.5, 22.7 (6 × q), 22.0, 26.8, 30.7, 31.1 (5 × t), 48.2, 48.7, 49.1 (3
× d, Ala-α-C), 51.3 (2 × d, C-2´), Glu-α-C), 53.5 (d, DAP), 55.1 (d, C-2), 56.2 (q, OCH₃), 61.7 (d, DAP), 65.8 (C-5), 66.8, 67.0, 67.4 (3 × t, 3 CH₂Ph), 68.5 (t, C-6), 69.0 (t, C-6´), 73.4, 74.1 (2 × t, 2 CH₂Ph), 74.0 (d, C-4´), 74.6 (d, C-5´), 76.1 (d, Lac-α-C), 77.4 (d, C-3´), 77.7 (d, C-3), 82.1 (d, C-4), 100.2 (d, C-1), 101.1 (d, CHPh), 101.7 (d, C-1´), 125.8, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.9, 134.8, 135.1, 135.3, 137.0, 137.7, 138.1, 170.4, 171.4, 171.7, 171.9, 172.3, 172.5, 172.7, 172.9, 173.2, 173.8.

Methyl β-d--N-Acetylglucosamine-(1→4)-β-d-N-acetylmuramyl-L-Ala-γ-d-Glu-meso-DAP-d-Ala-d-Ala (2b). Deprotection of compound 17 to 2b was carried out in the same manner as described for 18 to 3b. ¹H NMR (600 MHz, D₂O, 21 °C) δ = 1.36 - 1.51 (m, 2H), 1.39 (d, 3H), 1.40 (d, J = 7.3 Hz, 3H), 1.43 (d, J = 7.3 Hz, 3H), 1.45 (d, J = 7.0 Hz, 3H), 1.74 - 2.11 (m, 7H), 1.94 (s, 3H), 2.07 (s, 3H), 2.14 - 2.24 (m, 1H), 2.39 (t, J = 7.6 Hz, 2H), 3.41 - 3.44 (m, 2H; H-4 and H-5), 3.45 - 3.52 (m, 1H; H-5´), 3.48 (s, 3H), 3.54 (d, J = 10.3, 8.5 Hz, 1H; H-3), 3.62 (dd, J = 10.0, 9.1 Hz, 1H; H-3´). 3.70 - 3.82 (m, 4H; H-6a, H-6b, H-6´a, H-6´b), 3.79 (q, J = 6.2, 1H; DAP), 3.85 (t, J = 9.1 Hz, 1H; H-4´), 3.90 - 3.96 (m, 2H; H-6b, H-6´b), 4.25 (dd, J = 8.1, 6.3 Hz, 1H; DAP), 4.29 - 4.40 (m, 4H), 4.39 (d, J = 8.2 Hz, 1H; H-1´), 4.43 (q, J = 6.8 Hz, 1H; Lac-α-H), 4.53 (d, J = 8.2 Hz, 1H; H-1); ¹³C NMR (151 MHz, D₂O, 21 °C) δ = 16.2, 16.4, 16.8, 17.9, 22.0, 22.2 (6 × q), 20.8, 27.1, 29.8, 30.3, 31.2 (5 × t), 48.9, 49.4, 49.5 (3 × d, Ala-α-C), 52.6 (d, 3 × d, Ala-α-C), 53.9 (d, DAP), 54.0 (d, DAP), 54.2 (d, C-2´), 55.8 (d, C-2), 57.0 (q, OCH₃), 59.8 (t, C-6´), 60.9 (t, C-6), 70.1 (d, C-4), 73.4 (d, C-3), 75.0 (d, C-5´), 75.1 (d, C-4´), 76.0 (C-5), 78.0 (d, Lac-α-C), 79.4 (d, C-3´), 100.4 (d, C-1), 101.9 (d, C-1´), 173.9, 174.0, 174.2, 174.3, 174.6, 174.7, 175.1, 175.4, 176.6; HRMS-ESI (m/z): [M+Na]+ calcd. for C₄₁H₆₈N₈O₂₂Na, 1047.4346; found, 1047.4391.
References

DMF-DS-pentapeptide

Sample: Carboxyl

Date: Jan 12, 2008
Temp: 25.0

Spectrometer: Varian INOVA 500 MHz
Resolution: Auto
Field: 14.06 T
Dipolar decoupling: OFF

Acquisition:
-tau: 2.1 ms
-Filter: 30 Hz
-Points: 10944
-Transients: 2000
-Processing: 512

Decoupler: H1 VCD 250

Ch3 carbons:

CH2 carbons:

All remaining carbons:

ppm