Supporting Information

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Experimental section

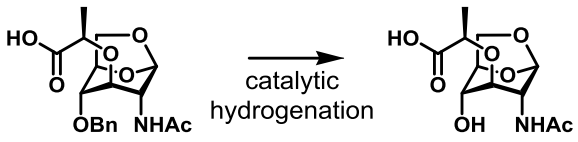
Strains, media and growth conditions. *Pseudomonas aeruginosa* PAO1 and *P. aeruginosa* Z61 (ATCC 35151) were used in this study. *P. aeruginosa* was cultured in Luria Bertani broth (5 g tryptone, 10 g sodium chloride, and 5 g yeast extract per liter) supplemented with 1.5% agar when needed at 37 °C.[1] The antibiotic cefoxitin was added when required.

Induction and β-lactamase assay. Cefoxitin at half minimal-inhibitory concentration (512 µg/mL) was used for induction of β-lactamase in *P. aeruginosa* PAO1.[2] The antibiotic was added when cell culture was at OD_{600} 0.2 and grown until OD_{600} of 1.2. The culture was harvested, washed once and resuspended in 50 mM sodium phosphate buffer (pH 8). The suspension was sonicated. The sonicated suspension was centrifuged and the supernatant was used for analysis of β-lactamase activity. A 7.5-µL portion of the supernatant of both induced and non-induced samples were added to 1 mL of the assay buffer with nitrocefin in a final concentration of 100 µM. The absorbance at 500 nm was observed for 5 min.[3] The ΔA_{500}/min was recorded for both induced and non-induced solution. The protein concentrations of the supernatants were estimated by BCA assay.[4] The enzyme activity was expressed as the nanomole of nitrocefin hydrolyzed per min per mg of protein.[5]

Compounds **2e** and **4e** were used for induction of β-lactamase in *P. aeruginosa* Z61 (ATCC 35151) at 100 µg/mL. Cefoxitin at quarter minimal-inhibitory concentration (0.0156 µg/mL) was used as control. Compounds **2a** and **4a** were used (as negative control). The culture was grown to OD_{600} of 0.1 (~10^8 CFU/mL) in LB medium and, subsequently, diluted 100-fold. LB medium (2.5 mL) containing the compound or the antibiotic were inoculated with 2.5 mL of the inoculum. Various cultures were allowed to grow within the range of OD_{600} of ~1.0 – 1.8. The rest of the protocol is the same as for the wild type. The result is summarized in Table 2.

Bacterial cell counting. *P. aeruginosa* PAO1 was grown in LB broth until early stationary phase (OD_{600} of 1.2) was reached. Serial dilutions of the bacterial culture were plated on LB agar plates and incubated at 37 °C for 16-18 h. Colonies were counted and expressed as colony-forming units (CFU) per mL. Only plates with 30-300 colonies were considered. The average number was $4.9 \pm 0.5 \times 10^8$ cell/mL of culture.

Syntheses of authentic muropeptides. The chemical structure of authentic compounds used in this study is given in Chart S1. Synthetic methods for **2e**, **4c**, **4d**, **4e**, **7**, and **8** were reported previously by our laboratory and were followed for the samples used in the present study.[6, 7] Compound **4a** was synthesized for this study by catalytic hydrogenation of the intermediate **9**. ^{1}H NMR (500 MHz, CD_3CN) δ 1.37 (d, *J* = 6.8 Hz, 3H), 1.93 (s, 3H), 3.37 (s, 1H), 3.55 - 3.72 (m, 3H), 3.90 (d, *J* = 8.4 Hz, 1H), 4.11 (d, *J* = 7.4 Hz, 1H), 4.24 (q, *J* = 6.8 Hz, 2H), 4.50 (d, *J* = 5.2 Hz, 1H), 5.31 (s, 1H), 6.79 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (126 MHz, CD_3CN) d 18.7, 23.0, 50.5, 66.1, 69.7, 74.8, 77.2, 79.8, 101.5, 118.3, 171.3, 175.0; HRMS (ESI-QTOF) *m/z* $[M+H]^+$ Calcd 276.1078 for $C_{11}H_{17}NO_7$; found 276.1101.



9 **4a**

Compound **2a** was obtained from incubation of compound **2e** with an amidase AmpDh3 of *P. aeruginosa*.[7, 8] The reaction product **2a** was confirmed by high-resolution mass spectrometry, which is consistent with the chemical formula of $C_{19}H_{30}N_2O_{12}$: HRMS (ESI-QTOF) *m/z* $[M+H]^+$ Calcd 479.1872; found 479.1844. Its chemical nature was further confirmed by LC/MS/MS experiment (Figure S1A). Compound **2d** was obtained from incubation of compound **2e** with penicillin-binding protein 4 of *P. aeruginosa*.[9] The reaction product **2d** was confirmed by high-resolution mass spectrometry, which is consistent with the chemical formula of $C_{37}H_{59}N_7O_2$: HRMS (ESI-QTOF) *m/z* $[M+H]^+$ Calcd 922.3888; found 922.3840. Its chemical nature was further confirmed by LC/MS/MS experiment (Figure S1B).

Sample preparation: release of muramyl peptides from whole-cell. *P. aeruginosa* PAO1 with and without induction (cefoxitin 512 µg/mL) was grown until late log to early stationary phase (OD_{600} 1.2). The culture was harvested by centrifugation at 4,500 *g* for 20 min and the supernatant discarded. The cell pellet was resuspended gently in cold sucrose solution (20% sucrose, 1.2 mM EDTA, 30 mM Tris, pH 8) and left at 4 °C for 10 min. This was centrifuged and the pellet resuspended in ice-cold water. The suspension was incubated at 4 °C for 10 min, and then centrifuged at 11,000 *g* for 20 min and the supernatant was discarded. The pellet was then boiled for 3 min and sonicated to ensure complete release of muropeptides. The sample was filtered using the 30-kDa molecular weight cut off mini-column. The flow-through was boiled for 6 min to remove any residual enzymatic activity, concentrated by speed vacuum at 50 °C and used for LC/MS analyses.

Detection and quantification of muropeptides by LC/MS. LC/MS conditions for muropeptide detection corresponded to those in the method developed by our laboratory.[10] Peak areas from extracted ion chromatograms of corresponding muropeptides were integrated and normalized to internal standard (compound **8**). Quantification of muropeptide was done from the standard curve of synthetic compound **2e**. Standard curves for **2e**, **4c**, **4d**, **4e**, and **7** were determined and they were very similar within 7% variation of each other (Figure S2). The collection of our synthetic standards covers distinctive chemical structures of >95% of detected muropeptide. So, we chose **2e** as a synthetic standard for quantification, as it has the closest structure to **2d**, the most abundant detected species.

Calculation of number of muropeptides per cell. The concentration of **2d** was calculated from the standard curve of authentic standard **2e**, which is 4.5×10^{-11} mol/mL of culture. This was converted to mol/cell (*x*) using the value obtained from bacterial cell counting, described above. The value *x* then was converted to number of molecules (*y*) using Avogadro's number, 6.022×10^{23}/mol. The rest of muropeptides were calculated the same way as **2d** and numbers are given in Table 1.

$$x = \frac{4.5 \text{ X } 10^{-11}\ mol/mL}{4.9 \text{ X } 10^{8}\ cell/mL} = 9.2 \text{ X } 10^{-20}\ mol/cell$$

$$1\ mol : 6.022 \text{ X}10^{23} = 9.2 \text{ X } 10^{-20}\ mol : y$$

$$y = 5.5 \text{ X } 10^{4} \text{ molecules/cell}$$

References

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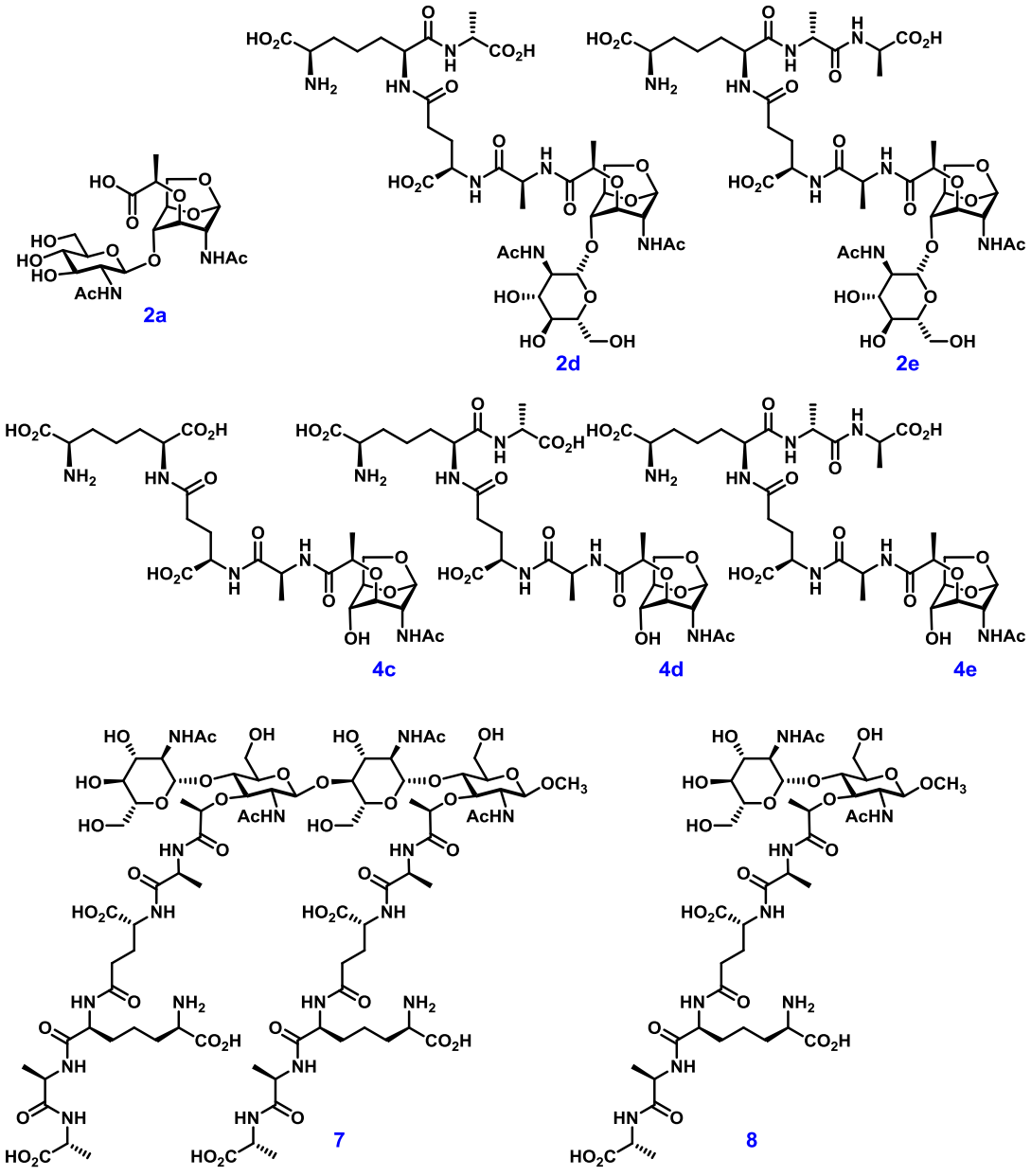

Chart S1. The chemical structure of authentic muropeptides used in this study.

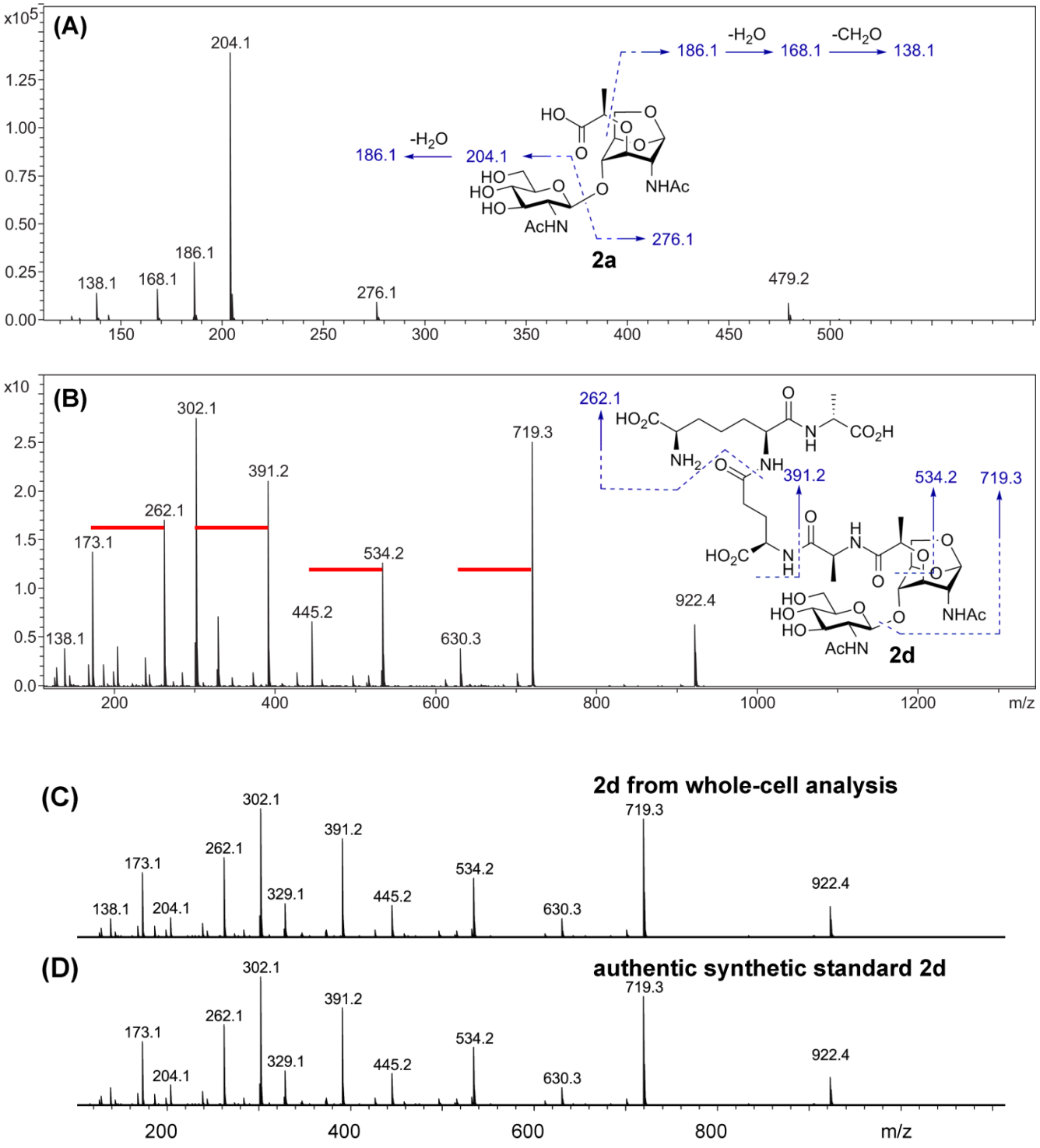

Figure S1. Collision-induced dissociation spectra of **2a** (A), **2d** (B), and side-by side comparison to authentic synthetic standard **2d** (C and D). Losses of Ala are indicated in red bar in (B).

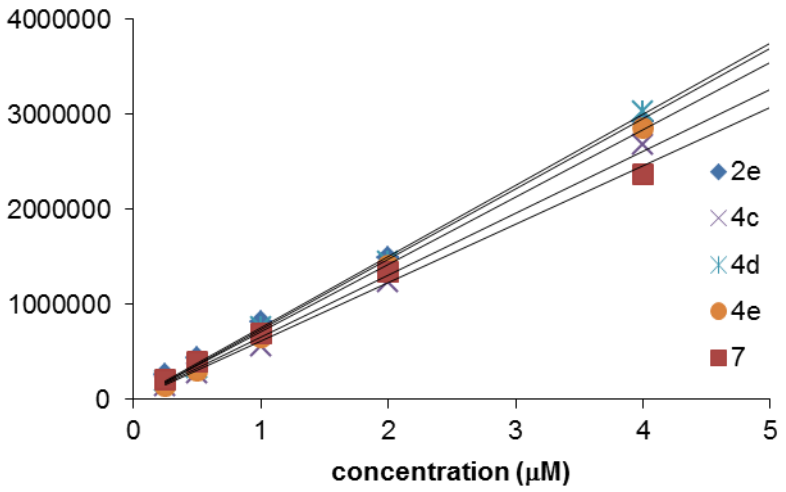

Figure S2. Standard curves of synthetic standards, **2e**, **4c**, **4d**, **4e**, and **7**.

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PA      MDSRLLSGIGAGPDS-GSYTDLNRLNQLKVGKDRDGEANIRKVAQEFESLFLNEMLKSMR
SE      -------MIGDGKLLASAAWDAQSLNELKAKAGQDPAANIRPVARQVEGMFVQMMLKSMR
                ** *    .:  * : **:**.   :*  **** **::.*.:*:: ******

PA      SANEALGDGNFMNSQTTKQYQDMYDQQLSVSLSKNAGGIGLADVLVRQLSKMKQGSRGNG
SE      EA---LPKDGLFSSDQTRLYTSMYDQQIAQQMTA-GKGLGLADMMVKQMTSGQTMPADD-
        .*   * .  ::.*: *: * .*****:: .::  . *:****::*:*::. :     :

PA      ENPFARVAENGAGRWPSNPSAQAGKALPMPEAGRDDSKLLNQRRLALPGKLAERMLAGIV
SE      -------------------APQ----VPLKFS------------LETVNSYQNQALTQLV
                          : *    :*:  :            *    .  :: *: :*

PA      PSASPAASQMQSLGQDSYLPAQSYPAASRRGFSTDGVDSQGSRRIAQPPLARGKSMFASA
SE      RK----------------------------------------AIPKTPDSSDAPLSGDS
         .                                         * : * :    : ..:

PA      DEFIATMLPMAQKAAERIGVDARYLVAQAALETGWGKSIIRQQDGGSSHNLFGIKTGSRW
SE      KDFLARLSLPARLASEQSGVPHHLILAQAALESGWGQRQILRENGEPSYNVFGVKATASW
        .:*:* :   *: *:*: **  : ::******:***:  * :::*  *:*:**:*: : *

PA      DGASARALTTEYEGGKAVKEIAAFRSYSSFEQSFHDYVSFLQGNDRYQNALDSAANPERF
SE      KGPVTEITTTEYENGEAKKVKAKFRVYSSYLEALSDYVALLTRNPRYAAVTT-AATAEQG
        .*  :.  ***** *:* *  * ** ***: ::: ***::*  * **  .   **. *:

PA      MQELQRAGYATDPQYARKVAQIARQMQTYQAVAAAG-TPPLG---
SE      AVALQNAGYATDPNYARKLTSMIQQLKAMSEKVSKTYSANLDNLF
           **.*******:****::.: :*::: .  .:   :  *
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Figure S3. Alignment of PA1085 (PA) and FlgJ (SE) sequence