Axinellamines as Broad Spectrum Antibacterial Agents: Scalable synthesis and Biology

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**General Procedures.** All reactions were carried out with dry solvents unless otherwise stated. Dry dichloromethane (DCM), tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and triethylamine (NEt₃) were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (*¹H-NMR) homogeneous material, unless otherwise stated. Reactions were monitored by HPLC-MS on a reverse phase column, using acetonitrile/water/0.1% formic acid as the mobile phase. NMR spectra were recorded on Bruker DRX-600, DRX-500 and AMX-400 instruments and are calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time of flight reflectron experiments. IR experiments were recorded on a Perkin Elmer Spectrum BX FTIR spectrometer. Preparative HPLC was performed using a Waters Atlantis dC₁₈ OBD 10 µm column with dimension 30 x 250 mm, unless otherwise noted.
Experimental Procedures and Characterization for Compounds

**Triboc allylic guanidine (20).** Bisboc allylic guanidine 52 (102 mg, 0.3 mmol, 1.0 equiv.), DMAP (4 mg, 0.03 mmol), di-tert-butyl dicarbonate (85 mg, 0.4 mmol, 1.3 equiv.) were dissolved in DCM (7 mL) followed by the addition of triethylamine (43 µL). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was transferred to a separatory funnel and water (5 mL) was added. The two layers were partitioned and the organic layer was washed with 10% aq. HCl (5 mL 2X). The organic layer was dried with sodium sulfate, concentrated and purified by silica chromatography to give a white solid (40 mg, 30% yield), m.p.: 112-114 °C; Rf = 0.3 (silica gel, 10% ethyl acetate in hexanes); IR (neat) ν = 2877, 2931, 2359, 1758, 1605, 1366, 1280, 1235, 1135, 1116, 729 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 5.65 (brs, 1 H), 4.60 (apps, 2 H), 2.50 − 2.30 (m, 4 H), 2.10 − 1.90 (m, 2 H), 1.62 (s, 18 H), 1.60 (s, 9 H); ¹³C-NMR (151 MHz, CDCl₃) δ 154.0, 152.42, 149.2, 140.6, 126.5, 84.30, 83.6, 47.9, 33.9, 32.7, 28.5, 28.2, 28.0, 23.8; HRMS (ESI-TOF) calc’d C₂₂H₃₈N₃O₆ [M + H⁺] 440.2761; found 440.2758.

**Alcohol (24).** Propargyl alcohol was dissolved in DCM (20 mL) and cobalt octacarbonyl was added under argon and allowed to stir for 2 h at room
temperature. This alkyne-cobalt complex was kept under argon at 0 °C and used as a stock solution in DCM. To a round bottom flask containing DCM/alkyne-cobalt complex stock solution (~3 mmol propargyl alcohol) was added bis-allylic-methoxyether 23 (1.1 mL, 9 mmol, 3 equiv.), ethylene glycol (1.5 mL) and DCM (total volume 10 mL). Lastly, NMO was added (2.1 g, 12 mmol, 4 equiv.) in 3 portions and the reaction mixture was allowed to stir at room temperature for 12h. The crude reaction mixture was directly loaded onto a hexanes packed silica gel plug and filtered using 30% ethyl acetate as the eluent. The organic layers were combined and concentrated and the resulting dark oil was purified by silica gel chromatography (ethyl acetate/hexanes 1:20) to give alcohol 24 (112 mg, 56% yield) as a colorless oil (yield of 20% obtained without the use of ethylene glycol). Rf = 0.2 (silica gel, 75% ethyl acetate in hexanes); IR (neat) ν = 3394, 2869, 1692, 1638, 1381, 1108, 1003, 953 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.44 (brs, 1 H), 4.31 (brs, 2 H), 3.60 (ddd, J = 26.6, 9.3, 4.7 Hz, 2 H), 3.52 – 3.40 (m, 2 H), 3.30 (s, 3 H), 3.25 (s, 3 H), 3.10 – 3.00 (m, 2 H), 2.40 – 2.30 (m, 1 H); ¹³C-NMR (151 MHz, CDCl₃) δ 208.1, 159.2, 145.5, 74.0, 71.0, 59.1, 57.3, 50.5, 43.9; HRMS (ESI-TOF) calc’d C₁₀H₁₇O₄ [M + H⁺] 201.1127; found 201.1121.

![Enone (25).](image_url)

**Enone (25).** Alcohol 24 (180 mg, 0.9 mmol, 1.0 equiv.) and triphenylphosphine (367 mg, 1.4mmol, 1.5 equiv.) and N, N’-bis-Boc-guanidine (363 mg, 1.4mmol, 1.5 equiv.) were dissolved in THF (5 mL). Diisopropyl
azodicarboxylate (0.27 mL, 1.4 mmol, 1.5 equiv) was then added and the reaction mixture was stirred for 2h at room temperature. The solvent was evaporated and the crude reaction mixture was directly purified by silica gel chromatography (ethyl acetate/hexanes 1:3) to give enone 25 (390 mg, 88% yield) as a colorless oil. Rf = 0.4 (silica gel, 25% ethyl acetate in hexanes); IR (neat) ν = 3389, 3242, 2979, 2932, 1709, 1637, 1611, 1541, 1286, 1239, 1123, 787 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.19 (brs, 1H), 4.73 (brs, 2H), 3.70 – 3.57 (m, 2H), 3.53 – 3.37 (m, 2H), 3.31 (s, 3H), 3.26 (s, 3H), 3.08 – 3.01 (m, 1H), 2.37 – 2.29 (m, 1H), 1.46 (s, 9H), 1.46 (s, 9H); ¹³C-NMR (151 MHz, CDCl₃) δ 163.8, 160.6, 157.7, 154.6, 143.6, 84.4, 79.0, 74.1, 71.1, 59.1, 59.0, 50.1, 43.7, 40.0, 28.4, 28.2, 28.0, 27.7; HRMS (ESI-TOF) calcd C₂₁H₃₆N₃O₇ [M + H⁺] 442.2553; found 442.2558.

** Allylic alcohol (26).** Enone 25 (441 mg, 1.0 mmol, 1 equiv.) was dissolved in methanol (10 mL) then CeCl₃·7H₂O was added (372 mg, 1.0 mmol, 1 equiv.). The reaction mixture was cooled to 0 °C and sodium borohydride was added in two portions (152 mg, 4.0 mmol, 4 equiv.) then the reaction was allowed to warm up to room temperature by removing the ice bath. Stirring continued for 12h and the solvent was removed under reduced pressure. The resulting crude oil was re-dissolved and partitioned between ethyl acetate and aqueous NH₄Cl (saturated). The organic layers was collected, dried using sodium sulfate and concentrated. The resulting oil was purified using silica gel
chromatography (Acetone/Hexanes 1:4) to give allylic alcohols $26\alpha$ and $26\beta$ as a mixture (~2:1) of diastereomers (268 mg, 60% yield) as colorless oils. $R_f = 0.3$ (silica gel, 30% ethyl acetate in hexanes); IR (neat) $\nu = 3380, 2976, 2926, 1712, 1607, 1507, 1366, 1236, 1141, 1116, 544$ cm$^{-1}$; $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 9.17 (brs, 2H), 9.36 (brs, 3H), 5.67 (s, 2H), 5.64 (s, 1H), 4.79 (brs, 1H), 4.75 (brs, 2H), 4.60 (brs, 1H), 4.59 (brs, 1H), 4.36–4.16 (m, 4H), 3.73 (dd, $J = 9.3, 6.7$ Hz, 2H), 3.49–3.40 (m, 8H), 3.36 (s, 6H), 3.33 (s, 3H), 3.32 (s, 5H), 3.30 (s, 6H), 3.18 (t, $J = 8.0$ Hz, 1H), 2.87–2.75 (m, 2H), 2.63 (q, $J = 6.2$ Hz, 1H), 2.26–2.15 (m, 3H), 2.12–2.02 (m, 2H), 1.48 (s, 12H), 1.48 (s, 18H), 1.46 (s, 15H), 1.45 (s, 19H); $^{13}$C-NMR (151 MHz, CDCl$_3$) $\delta$ 163.3, 161.3, 161.2, 155.2, 155.1, 143.1, 143.0, 132.1, 132.0, 79.6, 79.6, 76.8, 76.6, 75.0, 74.5, 72.4, 59.3, 59.3, 59.2, 59.1, 49.9, 48.4, 46.9, 44.9, 43.8, 43.5, 28.5, 28.4, 28.3, 28.2; HRMS (ESI-TOF) calc’d C$_{21}$H$_{38}$N$_3$O$_7$ [M + H$^+$] 444.2710; found 444.2709.

**Allylic acetate (27).** Allylic alcohols $45\alpha$ and $45\beta$ (~2:1 mixture) of diastereomers (16 mg, 0.04 mmol, 1 equiv.) and DMAP (10 mg, 20mol%) was dissolved in DCM. Acetic anhydride (4uL, 0.044 mmol, 1.1 equiv.) was then added at room temperature. After stirring for 1h, acetyl chloride (7 uL, 0.08 mmol, 2.0 equiv) was added and the reaction mixture was stirred at room temperature for an additional 2 h. The solvent was removed under reduced pressure. The resulting crude oil was directly purified using silica gel chromatography to give allylic acetates $27\alpha$ and $27\beta$ as a mixture of diastereomers.
(8.5 mg, 45% yield) as colorless oils. \( R_f = 0.7 \) (silica gel, 25% ethyl acetate in hexanes); IR (neat) \( \nu = 3388, 3244, 2976, 2933, 1709, 1611, 1284, 1240, 1122, 788, 733 \, \text{cm}^{-1}; \) \(^1\text{H}-\text{NMR} \) (400 MHz, CDCl\(_3\)) \( \delta \) 9.42 (s, 1H), 9.25 (s, 2H), 5.86 – 5.80 (m, 1H), 5.72 (s, 1H), 5.66 (s, 0.6H), 5.52 (d, \( J = 3.6 \, \text{Hz} \), 0.6H), 4.75 – 4.56 (m, 3H), 3.48 – 3.41 (m, 3H), 3.37 (dd, \( J = 9.3 \), 7.0 Hz, 2H), 3.32 (s, 4H), 3.30 (s, 3H), 3.28 (s, 4H), 3.22 (dd, \( J = 8.9 \), 7.1 Hz, 1H), 2.86 – 2.77 (m, 1H), 2.75 – 2.68 (m, 1H), 2.46 – 2.30 (m, 2H), 2.04 (s, 3H), 2.02 (s, 2H), 1.50 – 1.44 (m, 32H); \(^{13}\text{C}-\text{NMR} \) (151 MHz, CDCl\(_3\)) \( \delta \) 171.1, 170.9, 85.6, 73.8, 71.8, 59.2, 59.1, 49.2, 48.3, 44.5, 34.8, 30.0, 28.6, 28.2, 28.1, 28.1, 21.3; HRMS (ESI-TOF) calc'd C\(_{23}\)H\(_{40}\)N\(_3\)O\(_8\) [M + H\(^+\)] 486.2815; found 486.2814.

**Allylic chloride (29).** Mixture (~2:1) of allylic alcohols 26\( \alpha \) and 26\( \beta \) (97 mg, 0.22 mmol, 1 equiv.) and triphenylphosphine (66 mg, 0.25 mmol, 1.1 equiv) were dissolved in DCM (2 mL) and NCS (33 mg, 0.25 mmol, 1.1 equiv) was then added to the reaction mixture and stirred for 2 h at room temperature. Upon complete consumption of the starting material, the crude reaction mixture was concentrated and directly loaded to silica gel. The desired allylic chloride 29 was found to be unstable to silica and air sensitive, thus it was used crude for the next step and was not characterized.
Homoallylic alcohol (30). Crude allylic chloride 29 was concentrated and re-dissolved in THF (2.2 mL) followed by the addition of propionaldehyde (79 mg, 1.1 mmol), zinc dust (215 mg, 3.3 mmol), indium (50 mg, 0.44 mmol) and finally 0.7 mL of 6% NH₄Cl (aqueous). The reaction mixture was stirred 3 h at room temperature until complete consumption of the starting material was observed. The crude mixture was filtered over a short silica plug to remove the excess metals and the resulting mixture was purified by silica gel chromatography (ethyl acetate/hexanes 1:6) to give homoallylic alcohol 30 (19 mg, 12% yield from allylic alcohol 26) as a colorless oil. Rᵣ = 0.15 (silica gel, 15% ethyl acetate in hexanes); IR (neat) ν = 3379, 2976, 2929, 2873, 1712, 1608, 1239, 1142, 1116, 730 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 5.30 (brs, 1H), 4.57 – 4.48 (m, 1H), 4.47 – 4.34 (m, 1H), 3.84 (ddd, J = 8.5, 4.8, 2.1 Hz, 1H), 3.44 – 3.38 (m, 1H), 3.32 (s, 3H), 3.30 (s, 3H), 3.27 (d, J = 7.0 Hz, 2H), 2.58 (brs, 1H), 2.39 (brs, 1H), 2.30 (tt, J = 7.0, 3.5 Hz, 1H), 1.50 (s, 9H), 1.47 (s, 9H), 1.00 (t, J = 7.4 Hz, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ 126.1, 71.1, 59.0, 58.8, 54.9, 48.7, 43.9, 40.2, 28.3, 28.0, 28.0, 27.8, 11.3; HRMS (ESI-TOF) calc’d C₂₄H₄₄N₃O₇ [M + H⁺] 486.3179; found 486.3179.
Diol-N-oxide (33)

Diol-N-oxide (33). 3-(Diethylamino)propane-1,2-diol (3g, 20 mmol, 1 equiv.) was added to 7.5 mL 37% H₂O₂ (aqueous) at 0 °C and allowed to warm to room temperature. After stirring for an additional 30 min at room temperature, the crude reaction mixture was directly loaded onto a hexanes packed silica gel column and the desired diol-N-oxide was purified using two sequential solvents for elution: the first fraction was washed with ethyl acetate (discarded this fraction) and the desired diol-N-oxide 33 was collected using methanol as the eluent. The methanol fraction was checked for any remaining peroxide using hydrogen peroxide test strip then concentrated to give diol-N-oxide 33 as a colorless oil, which could be recrystallized into colorless plates from acetonitrile upon standing at 0 °C (2.6 g, 89% yield). Rf = 0.40 (silica gel, 100% methanol); IR (neat) ν = 3185, 2945, 1458, 1385, 1049, 909, 724 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 4.17 (brs, 1H), 4.25 − 4.09 (m, 2H), 3.37 − 3.07 (m, 6H), 1.20 (dt, J = 14.4, 7.2 Hz, 6H); ¹³C-NMR (151 MHz, CDCl₃) δ 67.4, 65.6, 64.5, 61.3, 59.2, 9.1, 8.2; HRMS (ESI-TOF) calc’d C₇H₁₈F₃NO₃ [M + H⁺] 164.1287; found 164.1284.

Triol (41). Cobalt octacarbonyl (3 mmol, 1.0 equiv) was dissolved in DCM and propyne gas was bubbled into the solution until complete formation of
propyne-cobalt complex was observed. Using a septum, the reaction flask was purged several times with argon before adding (E)-2,2,9,9-tetramethyl-3,8-dioxa-2,9-disiladec-5-ene (2.4 mL, 9 mmol, 3 equiv.) and ethylene glycol (1.5 mL) and finally NMO (2.4 g, 12 mmol, 4.0 equiv.). The reaction mixture was allowed to stir at room temperature for 12 h. Upon complete consumption of the propyne-cobalt complex, the crude reaction mixture was filtered over a silica plug (ethyl acetate/hexanes 1:1). The fractions were concentrated and the resulting oil was purified by silica gel chromatography (ethyl acetate/hexanes 1:20). The resulting product was of sufficient purity to be used directly in the next operation. The corresponding enone was concentrated directly from the ethyl acetate/hexanes fractions and re-dissolved in methanol (7 mL). Cerium trichloride heptahydrate (633 mg, 1.7 mmol) was added and the reaction mixture was cooled to 0 °C before adding sodium borohydride in three portions (85 mg; 257 mg total). The crude reaction mixture was concentrated to a minimum solvent amount and purified using silica gel chromatography (methanol/ethyl acetate 1:10) to give triol 41 as a diastereomeric mixture (~2:1) (150 mg, 32% yield). Triol 41 was isolated as a waxy oil which could be crystallized from dichloromethane (slow evaporation). Rf = 0.50 (silica gel, 10% methanol in ethyl acetate); IR (neat) ν = 3306, 2917, 2877, 2469, 1440, 1028, 519 cm⁻¹; ¹H-NMR (400 MHz, CD₃OD) δ 5.49 (t, J = 1.8 Hz, 1.5H), 5.39 (q, J = 1.6 Hz, 1H), 4.90 (s, 1H), 4.54 – 4.46 (m, 1.5H), 4.24 (dd, J = 5.2, 1.6 Hz, 1H), 3.89 – 3.80 (m, 2H), 3.74 – 3.68 (m, 1.5H), 3.68 – 3.61 (m, 2H), 3.61 – 3.52 (m, 4.5H), 3.48 (tt, J = 10.4, 5.6 Hz, 3H), 3.31 (brs, 1H), 2.74 – 3.57 (m, 1.5H), 2.51 – 2.41 (m, 1H), 2.05 (p, J = 6.9 Hz, 2H), 1.87 (dq, J = 7.5, 5.5 Hz, 1.5H), 1.78 (s, 3H), 1.74 (s, 2H), 1.60
(dq, J = 6.1, 2.9 Hz, 3H), 1.01 (q, J = 6.3, 5.9 Hz, 2H); $^{13}$C-NMR (151 MHz, CD$_3$OD) δ 144.0, 143.9, 130.6, 128.3, 81.8, 80.6, 66.9, 66.7, 64.6, 62.9, 62.8, 56.2, 52.0, 50.7, 50.3, 30.3, 14.3, 14.0; HRMS (ESI-TOF) calc'd C$_8$H$_{13}$O$_3$ [M + H$^+$] 157.0865; found 157.0848.

**Trichloride (42).** Triol 41 (160 mg, 1 mmol, 1.0 equiv.) was dissolved in DCM (5 mL) and cooled to 0 °C then triphenylphosphine (1.2 g, 4.5 mmol, 4.5 equiv) was added. The reaction mixture was stirred at 0 °C for 10 minutes, degassed with argon and NCS (603 mg, 4.5 mmol, 4.5 equiv) was added. The reaction mixture was allowed to warm to room temperature and stirred for an additional 12 h. Solvent was evaporated and the crude reaction mixture was directly purified by silica gel chromatography (ethyl acetate/hexanes 1:10) to give trichloride 42 (194 mg, 92% yield) as a yellow oil. $R_f$ = 0.30 (silica gel, 100% hexanes); IR (neat) ν = 2950, 2917, 1739, 1439, 1290, 1230, 844, 731 cm$^{-1}$; $^1$H-NMR (400 MHz, CDCl$_3$) δ 5.56 (s, 0.2H), 5.52 (s, 1H), 4.88 (d, J = 6.1 Hz, 0.4H), 4.69 (d, J = 2.7 Hz, 2H), 3.93 – 3.88 (m, 0.4H), 3.81 – 3.74 (m, 6H), 3.71 – 3.64 (m, 6H), 3.62 – 3.54 (m, 12H), 2.93 (brs, 3H), 2.74 (p, J = 4.8 Hz, 5H), 1.87 (s, 1H), 1.83 (s, 6H), 1.57 (s, 1H); $^{13}$C-NMR (151 MHz, CDCl$_3$) δ 141.9, 131.2, 129.1, 69.0, 55.1, 50.1, 49.9, 49.7, 47.5, 46.5, 46.0, 14.5.
Dichloride (43). Trichloride 42 (150 mg, 0.7 mmol, 1 equiv.), and propionaldehyde (300 mg, 4.25 mmol, 6.0 equiv.), were dissolved in THF/~20% aqueous NH₄Cl (2 mL/1 mL), followed by the addition of zinc dust (732 mg, 11.2 mmol, 16 equiv.) and indium powder (100 mesh, 156 mg, 1.36 mmol, 2 equiv.) to the reaction mixture and then stirred vigorously for 3 h at 23 °C without the use of an inert atmosphere (reaction vessel simply capped). Purification was performed by filtering crude reaction over short silica plug using 100% ethyl acetate as the eluent. The organic layers were collected and concentrated under reduced pressure and the resulting crude oil was purified by flash chromatography on silica (ethyl acetate/Hexanes 8:1) to yield dichloride 43 (103 mg, 62%) as a yellow oil. Rᵣ = 0.65 (silica gel, 15% ethyl acetate in hexanes); IR (neat) ν = 3500, 2961, 2875, 1439, 1290, 959, 725 cm⁻¹;¹H-NMR (400 MHz, CDCl₃) δ 5.62 (s, 1H), 5.42 (s, 2H), 3.86 – 3.80 (m, 5H), 3.76 (ddd, J = 8.4, 5.1, 2.0 Hz, 5H), 3.68 – 3.61 (m, 4H), 3.61 – 3.51 (m, 17H), 3.47 – 3.40 (m, 2H), 2.91 (brs, 2H), 2.82 (brs, 6H), 2.49 (s, 2H), 2.42 (td, J = 6.0, 2.9 Hz, 5H), 1.85 (s, 3H), 1.73 (s, 2H), 1.63 – 1.37 (m, 18H), 1.04 – 0.99 (m, 17H);¹³C-NMR (151 MHz, CDCl₃) δ 141.6, 140.5, 131.3, 127.6, 72.6, 71.2, 57.9, 54.4, 52.1, 50.9, 50.2, 49.8, 48.7, 47.9, 44.6, 43.3, 30.4, 27.9, 18.5, 15.0, 11.6, 11.1; HRMS (ESI-TOF) calc’d C₁₁H₁₈Cl₂O [M + H⁺] 237.0813; found 237.0816.
Bis-allylic alcohol (46). Butadiene was condensed (92 g, 1.7 mol, 1.4 equiv.) in a pre-weighed round bottom flask at -78 °C. Chloroform (400 mL) was cooled to 0 °C in a separate Erlenmeyer flask and slowly added to the condensed butadiene at -78 °C. Bromine (62.4 mL, 1.21 mol, 1.0 equiv) was then slowly added at -78 °C and the reaction was allowed to warm to room temperature by removing the acetone/dry ice bath. The reaction mixture was allowed to stir for an additional 30 minutes at room temperature before the solvent was concentrated to a minimal amount and formation of crystals were observed. The crystals were collected using a fritted filter and washed with cold ether (this process was repeated 4-6 times) to give the corresponding (E)-1,4-dibromobut-2-ene in quantitative yield. To following step was performed in several batches (~40-50 g each) (E)-1,4-dibromobut-2-ene (45 g, 0.26 mol, 1 equiv.) was dissolved in THF (900 mL) followed by the addition of potassium acetate (62 g, 0.8 mol, 3.0 equiv.) and tetrabutylammoniumbromide (3.4 g, 5 mol%). The reaction was capped with a yellow cap (open to air) and allowed to stir 14 h at room temperature. The formation of bis-acetate product was carefully monitored (compared to mono-acetate) by TLC analysis and additional potassium acetate (0.5 equiv.) and tetrabutylammoniumbromide (10 mol%) were added if necessary. Upon no detection of mono-acetate product, the crude reaction mixture was concentrated and purified using silica plug (eluted with ethyl acetate/hexanes 1:4) to give the corresponding bis-allylic acetate. (42 g, 92% yield). Spectroscopic
and chromatographic data for this compound exactly matched the published data. (Stehouwer, J. S.; Daniel, L. M.; Chen, P.; Voll, R. J.; Williams, L.; Plott, S. J.; Votaw, J. R.; Owens, M. J.; Howell, L. Goodman, M. M. J. Med. Chem. 2010, 53, 5549.) Bis-allylic-acetate (21.7 g, 0.126 mol, 1.0 equiv) was dissolved in ethanol (400 mL) and HCl in dioxanes was added to the round bottom flask (4.7 mL, 4M). The round bottom flask was equipped with a reflux condenser and heated to 80 °C for 12 h. Upon complete consumption of the starting material, the reaction mixture was allowed to cool to room temperature and triethylamine (4 mL) was added to neutralize the acidic media. The crude reaction mixture was concentrated to a minimum amount and the resulting oil was purified by silica gel chromatography (gradient using ethyl acetate/hexanes 1:1 to 100 ethyl acetate) to give bis-allylic alcohol 46 (8 g, 72% yield). Spectroscopic and chromatographic data for this compound exactly matched the published data. (Stehouwer, J. S.; Daniel, L. M.; Chen, P.; Voll, R. J.; Williams, L.; Plott, S. J.; Votaw, J. R.; Owens, M. J.; Howell, L. Goodman, M. M. J. Med. Chem. 2010, 53, 5549.)

![Chemical structure](attachment:image)

**Cyclopentenone (47).** Bis-allylic alcohol 46 (8.4 g, 96 mmol, 3.0 equiv.) was premixed with BSA (46 g, 7 equiv.) in DCM (200 mL) then heated to 40 °C and stirred for 3 h. To a separate flask, N-(tert-butoxycarbonyl)propargyl amine (5 g, 32.22 mmol, 1.0 equiv.) was dissolved in DCM (300 mL) and premixed with
dicobalt octacarbonyl (11.02 g, 33.22 mmol, 1.0 equiv.) at 23 °C and the reaction mixture was stirred for 2 h under argon to ensure dicobalt-alkyne complex formation. After complete formation of dicobalt-alkyne complex formation (judged by TLC), the crude reaction solution was added under argon to the flask containing bis-allylic alcohol/BSA mixture via cannula followed by addition of 4Å mol sieves (10 g), ethylene glycol (50 mL), and finally NMO (22.65 g, 193.32 mmol, 6.0 equiv.). The resulting reaction mixture was stirred under argon at 23 °C for 12 h. Partial removal of the solvent (300 mL) under reduced pressure allowed direct purification onto a hexane packed silica gel column (5% to 15% ethyl acetate in hexanes gradient as eluent) to yield recovered 2-Butene-1,4-diol bis(trimethylsilyl) ether (10.2 g) and desired cyclopentenone 47 (6.17 g, 45%) as a brownish oil. Spectroscopic and chromatographic data for this compound exactly matched the published data. (Su, S.; Rodriguez, R. A.; Baran, P. S. *J. Am. Chem. Soc.* 2011, 133, 13922.)

![Chemical Structure](image)

**Trichloride (36).** Cyclopentenone 47 (12.4 g, 30 mmol, 1.0 equiv.) was dissolved in MeOH (300 mL), added CeCl₃•7H₂O (11 g, 30 mmol, 1.0 equiv.) in a single portion then stirred at 23 °C for 5 min. The reaction mixture was cooled to 0 °C and stirred for an additional 5 min. NaBH₄ (4.5 g, 120 mmol, 4.0 equiv.) was then added at 0 °C in three equal portions and the mixture was allowed to warm to 23 °C
by removing the ice bath and stirring for an additional 12 h without the use of an inert atmosphere. The resulting reaction mixture was diluted with ethyl acetate (120 mL) and filtered over a large silica plug (600 g) (eluted with 30% methanol in ethyl acetate until no remaining product was detected in the eluent by TLC analysis) to yield crude triol as a clear colorless oil, which was of sufficient purity to be used directly in the next step. The resulting crude triol was dissolved in DCM (400 mL) followed by the addition of triphenylphosphine (27.2 g, 104 mmol, 3.5 equiv. based on 47 at 23 °C. The reaction mixture was next cooled to 0 °C and stirred for an additional 10 min before adding N-chlorosuccinimide (14 g, 104 mmol, 3.5 equiv. based on 47). After 10 min at 0 °C, the ice bath was removed and the mixture was allowed to stir for 12 h at 23 °C under argon. Silica gel (230 g) was directly added into the reaction flask followed by the addition of hexanes (2400 mL). The resulting slurry was loaded onto a large column containing hexane packed silica gel (400 g) and eluted with 25% ethyl acetate in hexanes, providing the desired trichloride 36 as a crude yellow oil, which was of sufficient purity to carry on directly to the next step. Spectroscopic and chromatographic data for this compound exactly matched the published data. (Su, S.; Rodriguez, R. A.; Baran, P. S. J. Am. Chem. Soc. 2011, 133, 13922.)
**Dichloride (35).** Crude trichloride 36 and 2,2,2-trifluoro-\(N\)-(2-oxoethyl)-acetamide (15 g, 97.5 mmol, 4.37 equiv. based on 47), were dissolved in THF, followed by the addition of zinc dust (24 g, 367.5 mmol, 17 equiv. based on 47) and indium powder (100 mesh, 4.80 g, 42 mmol, 1.9 equiv. based on 47) to the reaction mixture. Lastly, a 6% aqueous solution of ammonium chloride (100 mL) was added and the reaction mixture was stirred vigorously for 3 h at 23 °C without the use of an inert atmosphere (reaction vessel simply capped). Purification was performed by adding silica gel (180 g) and ethyl acetate (460 mL) directly to the reaction vessel followed by a filtration over hexanes packed silica gel (240 g) using 100% ethyl acetate as the eluent. The solvent was removed under reduced pressure to give a crude oil, which was purified by flash chromatography on silica (35% ethyl acetate in hexanes) to yield dichloride 35 (5.6 g, 59% over three steps) as a colorless oil. Spectroscopic and chromatographic data for this compound exactly matched the published data. (Su, S.; Rodriguez, R. A.; Baran, P. S. *J. Am. Chem. Soc.* 2011, 133, 13922.)

**Bis azide (34).** Dichloride 35 (9 g, 19.8 mmol) and sodium azide (13 g, 200 mmol, 10.0 equiv.) were dissolved in DMF (450 mL). A blast shield was
used as a safety precaution and the reaction mixture was heated to 88 °C using large oil bath equipped with external thermometer (internal temperature was measured to be around 84-86 °C at starting time of reaction set up as well as end of reaction) and stirred for 18 h. The reaction mixture was then concentrated, dissolved by EtOAc, and filtered over hexanes packed silica (50 g) using 100% ethyl acetate as the eluent to give bis-azide 34 as a yellow oil, which was of sufficient purity to be used directly in the next step. Spectroscopic and chromatographic data for this compound exactly matched the published data. (Su, S.; Rodriguez, R. A.; Baran, P. S. *J. Am. Chem. Soc.* **2011**, 133, 13922.)

**Allylic guanidine (15).** Bis-azide 34 (~6.2 g crude, 13.7 mmol, 1.0 equiv.) was dissolved in a 1:1 mixture of DCM /TFA (50 mL) and the reaction mixture was stirred at 23 °C for 4 h. The solvent was evaporated under reduced pressure and the reaction mixture was brought to complete dryness before dissolving the crude oil again in DCM (320 mL). To this solution, TEA (10 mL, 72.3 mmol, 5.0 equiv. based on dichloride 35) was added followed by *N,N’*-bis-BOC-*N”*-triflylguanadine (8.8 g, 21.6 mmol, 1.5 equiv. based on 35) and stirred at 23 °C for 12 h. The reaction mixture was transferred directly into a separatory funnel and washed with water (180 mL, 2X). The organic layer was collected, dried with anhydrous sodium sulfate and the solvent removed under reduced pressure. The
resulting brown oil was purified by flash chromatography on silica with 100% dichloromethane to remove excess \(N,N'\)-bis-BOC-\(N''\)-triflylguanadine and with 25% ethyl acetate in hexanes to afford allylic guanidine 15 (4.9 g, 56% over 3 steps) as a pale yellow oil. Spectroscopic and chromatographic data for this compound exactly matched the published data. (Su, S.; Rodriguez, R. A.; Baran, P. S. *J. Am. Chem. Soc.* 2011, 133, 13922.)

![Allylic guanidine](image)

**Allylic guanidine (52).** Cyclopent-1-en-1-ylmethanol (290 mg, 3 mmol, 1 equiv.) was dissolved in THF (15 mL) and phthalimide (588 mg, 4 mmol, 1.3 equiv.) followed by triphenylphosphine (1.1 g, 4 mmol, 1.3 equiv.) was added and stirred at room temperature for 5 minutes. Diethyl azodicarboxylate (0.78 mL, 4 mmol, 1.3 equiv.) was added at room temperature and the reaction mixture was allowed to stir for 2 h. Solvent was evaporated and the crude reaction mixture was filtered by silica gel plug (ethyl acetate/hexanes 1:7). The resulting oil was redissolved in ethanol (10 mL) and hydrazine (0.6 mL, 20 mmol) was added to the reaction mixture. The reaction was heated to reflux until complete consumption of the starting material was observed. The crude reaction mixture was filtered over cotton and then basified with 3N NaOH. The reaction mixture was transferred to a separatory funnel and extracted with ether (4 X 7 mL). The organic layers were combined, dried and concentrated with caution (desired amine is volatile) under reduced pressure. To the resulting ether/amine solution, DCM (10 mL), 1,3-Di-Boc-
2-(trifluoromethylsulfonyl)guanidine (1.5 g, 4 mmol, 1.3 equiv) and triethylamine (0.56 mL, 4 mmol, 1.3 equiv.) was added. The reaction mixture was allowed to stir for 1h at room temperature before evaporating the solvent and directly purifying by silica gel chromatography (ethyl acetate/hexanes 1:6) to give allyl guanidine 52 (810 mg, 85% 3 steps) as a light yellow solid, m.p.: 110-113 °C; Rf = 0.65 (silica gel, 10% ethyl acetate in hexanes); IR (neat) ν = 3383, 2975, 2931, 1709, 1605, 1235, 1139, 1111, 537 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 8.45 (s, 1H), 5.56 (p, J = 2.0 Hz, 1H), 4.10 – 4.06 (m, 2H), 2.42 – 2.24 (m, 4H), 1.90 (tt, J = 8.2, 6.7 Hz, 2H), 1.49 (d, J = 2.1 Hz, 18H); ¹³C-NMR (151 MHz, CDCl₃) δ 156.3, 153.6, 140.2, 126.0, 83.5, 42.1, 39.4, 34.0, 32.7, 30.7, 28.6, 28.4, 25.5, 23.6. HRMS (ESI-TOF) calc'd C₁₇H₃₀N₃O₄ [M + H⁺] 340.2236; found 340.2244.

![Chlorospirocycle (56) - Bis-boc allylic guanidine 52](image)

**Chlorospirocycle (56).** Bis-boc allylic guanidine 52 (340 mg, 1 mmol, 1.0 equiv.) was dissolved in DCM (20 mL) and cooled to 0 °C. tBuOCl (0.25 mL, 2.2 mmol) was added and the reaction mixture was allowed to stir for 20 minutes at 0 °C. The solvent and excess tBuOCl were removed under reduced pressure and the reaction mixture was re-dissolved in DCM (20 mL). TfNH₂ (164 mg, 1.1 mmol) was added as the reducing agent and the mixture was allowed to stir at room temperature for 15 minutes. TFA (1.5 mL) was added at room temperature and chlorospirocycle formation was monitored by LCMS. Upon complete Boc deprotection, the solvent was evaporated and the crude mixture was purified by
silica gel chromatography (methanol/DCM 1:4) to give chlorospirocycle 76 (136 mg, 94% yield) as an off-white solid, m.p.: 119-123 °C; IR (neat) ν = 3197, 1667, 1439, 1186, 1129, 721, 643 cm⁻¹; Rf = 0.9 (silica gel, 20% methanol in dichloromethane);

1H-NMR (600 MHz, CD₃OD) δ 4.24 (t, J = 7.1 Hz, 1H), 4.00 (d, J = 10.2 Hz, 1H), 3.53 (d, J = 10.2 Hz, 1H), 3.31 (p, J = 1.6 Hz, 4H), 2.33 – 2.26 (m, 1H), 2.13 – 2.05 (m, 1H), 2.02 – 1.95 (m, 1H), 1.93 – 1.80 (m, 4H); 13C-NMR (151 MHz, CD₃OD) δ 72.7, 66.4, 51.3, 19.5. HRMS (ESI-TOF) calc’d C₇H₁₂ClN₃ [M + H⁺] 174.0793; found 174.0792.

[58] Chlorobis(t-butoxycarbonyl)guanidine (58, CBBG). N, N’-bis-Boc-guanidine (518 mg, 2 mmol, 1.0 equiv.) was dissolved as best as possible in dichloromethane (100 mL). This cloudy solution was added tBuOCl (245 uL, 2.2 mmol, 1.1 equiv.) at room temperature. The cloudy reaction turned clear after about 10 minutes and the clear mixture was allowed to stir for 1 hr. Solvent was removed under reduced pressure and the crude reaction was dried under reduced pressure to give pure CBBG (58) as a white solid, m.p. 142-145 °C in quantitative yield. Rf = 0.8 (silica gel, 50% ethyl acetate in hexanes); IR (neat) ν = 3230, 3067, 2978, 2933, 1726, 1626, 1487, 1231, 1137, 745, 574 cm⁻¹; 1H-NMR (400 MHz, CDCl₃) δ 9.75 (brs, 1H), 7.88 (brs, 1H), 1.52 (s, 9H), 1.48 (s, 9H); 13C-NMR (151 MHz, CDCl₃) δ 151.8, 150.4, 149.3, 85.3, 82.6, 28.3, 28.3; HRMS (ESI-TOF) calc’d C₁₁H₂₀ClN₃O₄ [M + H⁺] 294.1221; found 294.1222.
3-chlorimidazo[1,2-\textit{a}]pyrazine (72). To a solution of imidazo[1,2-
\textit{a}]pyrazine (0.1 mmol, 1.0 equiv.) in chloroform (1.0 mL) at r.t. was added
chlorinating reagent (NCS, CBBG, CBPG or CBMG, 0.12 mmol, 1.2 equiv.) with
stirring. The reaction was monitored by thin layer chromatography until
completion. Upon consumption of starting material, the reaction was concentrated
and directly purified by column chromatography on silica gel to provide 72 in 5%,
30%, 18%, 90% yields respectively as a white solid. Spectroscopic and
chromatographic data for this compound exactly matched the published data.
(Rodriguez, R. A.; Pan, C.-M.; Yabe, Y.; Kawamata, Y., Eastgate, M. D.; Baran, P. S. \textit{J.
Am. Chem. Soc.} \textbf{2014}, \textit{136}, 6908)

Chlorospirocycle (73). Allylic guanidine 15 (6 g, 9.93 mmol,
1.0 equiv.) and trifluoromethylsulfonylamine (370 mg, 2.48 mmol, 0.25 equiv.) were
dissolved in DCM (580 mL). The reaction flask was wrapped in aluminum foil then
cooled to 0 °C and stirred for 10 min before adding \textit{tert}-butyl hypochlorite (2.25 mL,
19.8 mmol, 2.0 equiv.). The reaction mixture was stirred at 0 °C for 30 min,
whereupon an analytical sample was taken and determined by LCMS to be complete.
Solvent was evaporated under reduced pressure without removal of the aluminum
foil cover and without the use of the water bath. The crude product was brought to complete dryness over high vacuum before the aluminum cover was removed, and the resulting crude foam was re-dissolved in a 10:1 mixture of DCM/TFA (100 mL). To this solution, Dess–Martin periodinane (5 g, 12 mmol, 1.2 equiv.) was added and allowed to stir at 23 °C for 12 h. The solvent was evaporated under reduced pressure, at which point, acetonitrile (150 mL) and water (38 mL) were added and the crude reaction mixture was filtered over a fritted funnel and washed with 4:1 acetonitrile/water (375 mL). The solvent was removed under reduced pressure and final purification was performed by preparative HPLC (5% to 50% MeCN in H₂O, 0.1% TFA, 25 minute ramp) to afford the desired chlorospirocycle 73 as a colorless oil (4.6 g, 83% 2 steps, Rₜ = 23 min). Spectroscopic and chromatographic data for this compound exactly matched the published data. (Su, S.; Rodriguez, R. A.; Baran, P. S. *J. Am. Chem. Soc.* **2011**, *133*, 13922.)

![Tricycle (76) and tetracycle (75).](image)

2-Aminimidazole 74 (2.2 g, 3.72 mmol, 1.0 equiv) was dissolved in 95:5 DCM/TFA (100 mL) at 0 °C and was added DMDO (52 mL, c = 0.08 M, 1.25 equiv.) and the reaction was stirred for 1 hr. After removing the solvent under reduced pressure, 1:1 DCM/TFA (50 mL) was added and the reaction stirred for 12 hrs, after which the solvent was removed under reduced pressure. The resulting oil was
purified by preparative HPLC (5% to 50% MeCN in H₂O, 0.1% TFA, 25 minute ramp) to afford the desired tetracycle (75) (1.6 g, 75% yield). Spectroscopic and chromatographic data for this compound exactly matched the published data (O’Malley, D. P.; Yamaguchi, J.; Young, I. S.; Seiple, I. B.; Baran, P. S. Angew. Chem. Int. Ed. 2008, 47, 3581.) and tricycle (76) (~5 mg, <1% yield) which was isolated as a colorless oil and could be recrystallized from 1:1 H₂O/TFA. IR (neat) ν = 3172, 2110, 1704, 1680, 1559, 1432, 1270, 1200, 1138, 846, 800, 724 cm⁻¹; ¹H-NMR (500 MHz, CD₃OD) δ 5.43 (d, J = 6.8 Hz, 1H), 4.75 (d, J = 4.4 Hz, 1H), 4.23 (d, J = 11.4 Hz, 1H), 4.14 (d, J = 10.7 Hz, 1H), 3.80 – 3.72 (m, 1H), 3.72 – 3.54 (m, 5H), 2.73 (dd, J = 7.0, 4.4 Hz, 1H), 2.02 - 1.91 (m, 2H), 0.98 - 0.87 (m, 1H); HRMS (ESI-TOF) calc’d C₁₂H₁₁ClN₁₂O [M + H⁺] 381.1410; found 381.1414.
Materials and Methods (Biological studies)

Materials. Strains tested were *E. coli* K-12 MG1655, *Y. pestis* KIM6+, *P. aeruginosa* PAO1, *S. aureus* NCTC 8325, *S. epidermidis* RP62A, MRSA strain USA300, MRSA strain N315, *S. pneumoniae* D39, *C. efficiens* DSM 44549, *E. faecalis* ATCC 33186, and *C. albicans* BWP17. Bacteria were routinely grown at 37 °C on Mueller-Hinton II agar (MHIIA) and cation-adjusted Mueller-Hinton II broth (MHIIB) or on trypticase soy agar (TSA) or broth (TSB). *C. albicans* was grown at 34.4 °C on yeast nitrogen base (YNB) agar supplemented with 80 μg/mL uridine (YNB-Ura) and YAD (YNB with ammonium sulfate and dextrose), pH 7.0, supplemented with arginine, histidine, and uridine (YAD-Arg, His, Ura). Stock solutions were prepared at the following concentrations: compounds 4 and 5, 20 mg/ml (DMSO); nitrocefin (H$_2$O), 20 mM; and melittin (H$_2$O), 10 mg/mL. Nitrocefin was obtained from Calbiochem (San Diego, CA) and melittin was obtained from Sigma-Aldrich (St. Louis, MO). Human serum was obtained from Sigma-Aldrich (St. Louis, MO).

Susceptibility Determinations. Drug susceptibilities were determined for the bacterial strains listed above by measuring minimum inhibitory concentrations (MICs) using the Clinical Laboratory Standards Institute (CLSI) broth microdilution method (CLSI; Document M07-A8: CLSI (2009). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard--Eighth Edition. CLSI Document M07-A8, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, Clinical Laboratory Standards Institute). Briefly, 2-fold serial dilutions of antibiotics were prepared in 96-well plates containing 100 μl of
cation-adjusted MHIIB. Inocula were prepared by suspending colonies grown for 20 hours on MHIIA to a final density of about $10^7$ colony-forming units (CFU) per ml in MHIIB. Wells containing the antibiotic dilutions were inoculated to a final density of approximately $10^5$ CFU per ml and MICs were defined as the lowest drug concentration at which no visible growth occurred following 22 h of incubation at 37 °C. To test the effect of serum binding on the activities of 4 and 5, MHIIB was supplemented with 3 to 50% of human serum. To test the inoculum effect on the activities of 4 and 5 against E. coli K-12 MG1655 and S. aureus NCTC 8325, inocula corresponding to approximately $10^8$ to $10^{10}$ CFU per ml were prepared by suspending colonies in MHIIB. These were diluted and inoculated appropriately in the drug-containing wells to test $10^5$, $10^6$, $10^7$, and $10^8$ CFU per mL. Bacterial suspensions were appropriately diluted and plated on MHIIA in order to count viable CFU and confirm the tested inoculum concentrations. Susceptibility determinations were performed in duplicate if the MIC readings were identical and, if the readings varied, the MIC was taken as the median value of at least triplicate determinations. The drug susceptibility testing of C. albicans was performed similarly to the bacterial protocol outlined above with a few modifications. Testing was performed in YAD-Arg, His, Ura. Inocula were prepared by suspending colonies grown for 48 hours at 34.4 °C on YNB-Ura to a final density of about $10^6$ colony-forming units (CFU) per ml in YAD. Wells containing the antibiotic dilutions were inoculated to a final density of approximately $10^5$ CFU per ml and MICs were defined as the lowest drug concentration at which no visible growth occurred following 24-48 h of incubation at 34.4 °C.
**Frequency of mutation studies.** Overnight cultures of *E. coli* K-12 MG1655 and *S. aureus* NCTC 8325 in trypticase soy broth (TSB) were diluted 1:100 into fresh TSB and grown to an OD$_{590}$ of approximately 0.4–0.5 for *E. coli* and 1–3 for *S. aureus*. Approximately 10$^7$ *E. coli* cells and 10$^9$ *S. aureus* cells were plated on TSA containing 24 μg/mL of 4. Resistant colonies were counted after a 24 hr incubation at 37 °C.

**E. coli outer membrane (OM) destabilization study.** We studied OM destabilization using the method described by Epand et al. (Epand, R.F.; Pollard, J.E.; Wright, J.O.; Savage, P.B.; Epand, R.M. *Antimicrob. Agents Chemother.* **2010.** 54, 3708) with a few modifications. Upon OM perturbation, nitrocefin is able to access the periplasm and undergo cleavage by periplasmic β-lactamase. To obtain an *E. coli* strain that expresses sufficient levels of periplasmic β-lactamase, *E. coli* K-12 MG1655 was electroporated with the plasmid pBR322, from which the β-lactamase is constitutively expressed. A 1-mL volume of TSB was inoculated with 3–5 colonies of pBR322-containing *E. coli* K-12 MG1655 grown on plates containing 50 μg/mL ampicillin. The resulting culture was grown at 37 °C with shaking until it reached approximately OD 0.5 (~10$^8$ CFU/mL). After 2 washings in the incubation buffer (1× phosphate-buffered saline, pH 7.4, with 300 μg/mL of TSB), the culture was diluted to 10$^6$ CFU/mL in this buffer. A 96-well plate was prepared with wells containing a constant nitrocefin concentration of 30 μM and variable concentrations of drug (4 or the control, melittin). The 10$^6$ CFU/mL bacterial dilution was added to test wells, whereas the equivalent volume of incubation buffer was added to corresponding no bacteria-control (NBC) wells. The plate was incubated at 37 °C and absorbance was
measured at 492 nm using a Perkin-Elmer Envision multilabel reader every 2–4 min for 1 hr. The corrected absorbance was determined as $\text{ABS}_{\text{test}} - \text{ABS}_{\text{NBC}}$, where “test” refers to drug-containing wells. Negative values obtained from this calculation were set to zero.

**Microscopic studies.** Cell morphology was assessed using light microscopy. Three to five colonies of *E. coli* K-12 MG1655 were inoculated into fresh TSB and grown until OD$_{590}$ reached approximately 0.3. Cultures were divided into two groups. The first group was treated with 4 $\mu$g/ml of 4; the second group was treated with the equivalent volume of DMSO. Small aliquots were withdrawn just prior to treatment and at timed intervals post-treatment, heat-fixed onto glass slides, and stained with safranin. Slides were viewed and digital images were collected using a Nikon E600W microscope equipped with a 100× oil objective and IPLab version 3.9 software. Digital images were loaded into MatLab (The Mathworks, Inc. MATLAB and Statistics Toolbox Release 2012b) and processed using custom software. Briefly, images were converted to a gray-level matrix and median-filtered to correct for noise and intensity in-homogeneities. The images were then thresholded using Otsu’s method (Otsu, N. *IEEE Trans. Syst., Man, Cybern., Syst.* **1979**, 9, 62.) to determine the adequate intensity threshold that would separate background intensities from the bacterial intensities, effectively binarizing the images. Individual cells were then segmented and labeled using spatial connectivity. Any cells that overlapped with a 15-pixel image border were excluded from the study in order to avoid segmentation of cells which were partially obscured by the field of
view. This analysis segmented 126 vehicle-treated cells and 129 compound 4treated cells. The parameters perimeter, major axis, minor axis, and aspect ratio (i.e. the major axis divided by the minor axis) were calculated for the segmented cells using labeled binarized masks with ellipsoid fitting. Measurements were made in pixels (1 pixel = 0.064 μm). All image segmentations were reviewed for accuracy.
Figure SI-1. Plots showing outer membrane permeabilization of *E. coli* K-12 MG1655 containing pBR322 as a function of [4], μg/mL, using the cleavage of nitrocefin by periplasmic β-lactamase as the reporter. Melittin was used as the positive control.
X-ray crystallographic notes

Crystal structure(s) were deposited at the Cambridge Crystallographic Data Centre. The data have been assigned the following deposition numbers.

CCDC 1014254-1014258

Summary of Data CCDC 1014254
Compound Name: Compound 22 (Bromospirocycle)
Formula: C17 H27 Br1 N2 O5
Unit Cell Parameters: a 10.0808(12) b 10.9400(13) c 17.225(2) P21/n

Table 1. Crystal data and structure refinement for Baran149.

| Identification code     | RRI-288A                      |
|-------------------------|--------------------------------|
| Empirical formula       | C17 H27 Br N2 O5               |
| Formula weight          | 419.32                         |
| Temperature             | 100(2) K                       |
| Wavelength              | 0.71073 Å                      |
| Crystal system          | Monoclinic                     |
| Space group             | P2(1)/n                        |
| Unit cell dimensions    | a = 10.0808(12) Å a= 90°.      |
|                         | b = 10.9400(13) Å b= 94.659(2)°|
|                         | c = 17.225(2) Å g = 90°.       |
| Volume                  | 1893.3(4) Å³                   |
| Z                       | 4                              |
| Density (calculated)    | 1.471 Mg/m³                    |
| Absorption coefficient  | 2.200 mm⁻¹                     |
| F(000)                  | 872                            |
Crystal size: 0.45 x 0.18 x 0.11 mm³
Crystal color, habit: Colorless Rod
Theta range for data collection: 2.21 to 28.28°.
Index ranges: -12<=h<=13, -14<=k<=13, -22<=l<=21
Reflections collected: 32055
Independent reflections: 4400 [R(int) = 0.0327]
Completeness to theta = 25.00°: 100.0 %
Absorption correction: Multi-scan
Max. and min. transmission: 0.7939 and 0.4376
Refinement method: Full-matrix least-squares on F²
Data / restraints / parameters: 4400 / 0 / 241
Goodness-of-fit on F²: 1.051
Final R indices [I>2sigma(I)]: R1 = 0.0266, wR2 = 0.0576
R indices (all data): R1 = 0.0338, wR2 = 0.0602
Largest diff. peak and hole: 0.540 and -0.319 e.Å⁻³

Summary of Data CCDC 1014258
Compound Name: Compound 33 (diol-N-oxide)
Formula: C7 H17 N1 O3
Unit Cell Parameters: a 5.6142(5) b 12.7026(8) c 12.0019(8) P21/n

Table 1. Crystal data and structure refinement for Baran313.
Identification code: Baran313
Empirical formula: C7 H17 N O3
Formula weight 163.21
Temperature 100 K
Wavelength 0.71073 Å
Crystal system Monoclinic
Space group P 1 21/n 1
Unit cell dimensions a = 5.6142(5) Å a = 90°.
b = 12.7026(8) Å b = 95.341(3)°.
c = 12.0019(8) Å g = 90°.
Volume 852.20(11) Å³
Z 4
Density (calculated) 1.272 Mg/m³
Absorption coefficient 0.098 mm⁻¹
F(000) 360
Crystal size 0.32 x 0.15 x 0.08 mm³
Crystal color, habit Colorless Plate
Theta range for data collection 2.340 to 26.402°.
Index ranges -7<=h<=5, -15<=k<=15, -14<=l<=14
Reflections collected 5994
Independent reflections 1740 [R(int) = 0.0245]
Completeness to theta = 25.000° 99.9 %
Absorption correction Semi-empirical from equivalents
Max. and min. transmission 0.7454 and 0.6499
Refinement method Full-matrix least-squares on F²
Data / restraints / parameters 1740 / 1 / 115
Goodness-of-fit on F² 1.044
Final R indices [I>2sigma(I)] R1 = 0.0324, wR2 = 0.0846
R indices (all data) R1 = 0.0383, wR2 = 0.0872
Extinction coefficient n/a
Largest diff. peak and hole 0.315 and -0.188 e.Å⁻³

Summary of Data CCDC 1014255
Compound Name: Compound 41 (Triol)
Formula: C8 H12 O3
Unit Cell Parameters: a 12.0535(13) b 7.1631(7) c 9.0112(9) P21/c

S-34
Colorless crystal of Baran306 was mounted on a Cryoloop with Paratone-N oil and data was collected at 100K with a Bruker APEX II CCD using Mo K alpha radiation. Data was corrected for absorption with SADABS and structure was solved by direct methods. All non-hydrogen atoms were refined anisotropically by full matrix least squares on F2. Hydrogen atoms H2N and H3N were found from a Fourier difference map and were refined isotropically with N-H distance 0.87(0.02) angstroms and 1.20 Ueq of parent N atom. All other hydrogen atoms were placed in calculated positions with appropriate riding parameters.
Highest peak 0.25 at 0.1001 0.1954 0.0265 [0.64 Å from C1]  
Deepest hole -0.24 at 0.6290 0.2824 0.0900 [0.83 Å from C3]  

Table 1. Crystal data and structure refinement for baran306.  
| Identification code       | baran306 (Yu Kawamata) |
|---------------------------|------------------------|
| Empirical formula         | C11 H20 Cl N3 O4       |
| Molecular formula         | C11 H20 Cl N3 O4       |
| Formula weight            | 293.75                 |
| Temperature               | 100(2) K               |
| Wavelength                | 0.71073 Å              |
| Crystal system            | Monoclinic             |
| Space group               | P2(1)/n                |
| Unit cell dimensions      | a = 9.2786(11) Å       |
|                           | a = 90°.               |
|                           | b = 8.7907(12) Å       |
|                           | b = 101.237(4)°.       |
|                           | c = 18.680(3) Å        |
|                           | g = 90°.               |
| Volume                    | 1494.5(3) Å³           |
| Z                         | 4                      |
| Density (calculated)      | 1.306 Mg/m³            |
| Absorption coefficient    | 0.269 mm⁻¹             |
| F(000)                    | 624                    |
| Crystal size              | 0.28 x 0.22 x 0.15 mm³ |
| Crystal color, habit      | block / colorless      |
| Theta range for data collection | 2.22 to 26.39°.    |
| Index ranges              | -11<=h<=11, -10<=k<=10, -23<=l<=23 |
| Reflections collected     | 16056                  |
| Independent reflections   | 3046 [R(int) = 0.0658] |
| Completeness to theta = 25.00° | 100.0 %                |
| Absorption correction     | multi-scan / sadabas   |
| Max. and min. transmission | 0.9607 and 0.9284      |
| Refinement method         | Full-matrix least-squares on F² |
| Data / restraints / parameters | 3046 / 2 / 184         |
| Goodness-of-fit on F²     | 1.069                  |
| Final R indices [I>2sigma(I)] | R₁ = 0.0299, wR₂ = 0.0808 |
| R indices (all data)      | R₁ = 0.0333, wR₂ = 0.0830 |
| Largest diff. peak and hole | 0.245 and -0.239 eÅ⁻³   |
Summary of Data CCDC 1014256

Compound Name: Compound 76 (Tricycle)

Formula: C12 H19 Cl1 N12 O1 2+,2(C2 F3 O2 1-)

Unit Cell Parameters: a 12.5877(3) b 13.9945(4) c 13.4289(4) P21/c

Table 1. Crystal data and structure refinement for Baran211.

| Identification code | AX-B-Azide                           |
|---------------------|--------------------------------------|
| Empirical formula   | C16 H19 Cl F6 N12 O5                 |
| Formula weight      | 608.88                               |
| Temperature         | 100(2) K                             |
| Wavelength          | 0.71073 Å                            |
| Crystal system      | Monoclinic                           |
| Space group         | P2(1)/c                              |
| Unit cell dimensions| a = 12.5877(3) Å b= 13.9945(4) Å c = 13.4289(4) Å |
|                     | a= 90°. b= 92.471(2)°. g = 90°.       |
| Volume              | 2363.42(11) Å³                      |
| Z                   | 4                                    |
| Density (calculated)| 1.711 Mg/m³                          |
| Absorption coefficient| 0.268 mm⁻¹                        |
| F(000)              | 1240                                 |
| Crystal size        | 0.15 x 0.11 x 0.08 mm³               |
Crystal color, habit | Colorless Block
Theta range for data collection | 3.24 to 25.46°.
Index ranges | -15<=h<=15, -13<=k<=16, -16<=l<=16
Reflections collected | 16039
Independent reflections | 4359 [R(int) = 0.0549]
Completeness to theta = 25.00° | 99.6 %
Absorption correction | Semi-empirical from equivalents
Max. and min. transmission | 0.9789 and 0.9610
Refinement method | Full-matrix least-squares on F^2
Data / restraints / parameters | 4359 / 9 / 393
Goodness-of-fit on F^2 | 1.012
Final R indices [I>2sigma(I)] | R1 = 0.0417, wR2 = 0.0870
R indices (all data) | R1 = 0.0767, wR2 = 0.0992
Extinction coefficient | multi-scan
Largest diff. peak and hole | 0.477 and -0.329 e.Å^-3
Copies of NMR spectra for compounds

1H NMR spectrum (400 MHz, CDCl₃) of triboc allylic guanidine (20).
$^{13}$C NMR spectrum (151 MHz, CDCl$_3$) of triboc allylic guanidine (20).
1H NMR spectrum (400 MHz, CDCl$_3$) of alcohol (24).
$^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of alcohol (24).
1H NMR spectrum (400 MHz, CDCl₃) of enone (25).
$^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of enone (25).
1H NMR spectrum (400 MHz, CDCl₃) of allylic alcohol (26).
13C NMR spectrum (101 MHz, CDCl₃) of allylic alcohol (26).
1H NMR spectrum (400 MHz, CDCl₃) of allylic acetate (27).
13C NMR spectrum (151 MHz, CDCl$_3$) of allylic acetate (27).
1H NMR spectrum (600 MHz, CDCl₃) of homoallylic alcohol (30).
13C NMR spectrum (151 MHz, CDCl₃) of homoallylic alcohol (30).
1H NMR spectrum (400 MHz, CDCl₃) of diol-N-oxide (33).
13C NMR spectrum (101 MHz, CDCl₃) of diol-N-oxide (33).
1H NMR spectrum (400 MHz, CDCl₃) of triol (41).
$^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of triol (41).
1H NMR spectrum (400 MHz, CDCl₃) of trichloride (42).
13C NMR spectrum (101 MHz, CDCl₃) of trichloride (42).
1H NMR spectrum (400 MHz, CDCl₃) of dichloride (43).
13C NMR spectrum (101 MHz, CDCl₃) of dichloride (43).
1H NMR spectrum (400 MHz, CDCl₃) of allylic guanidine (52).
13C NMR spectrum (151 MHz, CDCl₃) of allylic guanidine (52).
$^{1}H$ NMR spectrum (400 MHz, CDCl$_3$) of chlorospirocycle (56).
$^{13}$C NMR spectrum (151 MHz, CDCl$_3$) of chlorospirocycle (56).
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of CBBG (58).
$^{13}$C NMR spectrum (151 MHz, CDCl$_3$) of CBG (58).
1H NMR spectrum (500 MHz, CDCl₃) of tricycle (76).
Chlorination of \textit{N}-methylbenzenesulfonamide.} Due to the poor solubility of benzenesulfonamide, \textit{N}-methylbenzenesulfonamide was used for the chlorination comparisons between $\text{tBuOCl}$ and corresponding \textit{N}-chloroguanidine reagent (CBBG/CBMG) crude $^1$H-NMR data shown below. The \textit{N}-chloro product was characterized by x-ray analysis (Rodriguez, R. A.; Pan, C.-M.; Yabe, Y.; Kawamata, Y.; Eastgate, M. D.; Baran, P. S. \textit{J. Am. Chem. Soc.} 2014. 136, 6908.)