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Synthesis of sulfonyl piperazine LpxH inhibitors

General Chemistry Procedures. All reactions were conducted in oven-dried glassware under nitrogen. Unless otherwise stated all reagents were purchased from commercial suppliers and used without further purification. All solvents were American Chemical Society (ACS) grade or better and used without further purification. Analytical thin layer chromatography (TLC) was performed with glass backed silica gel (60 Å) plates with fluorescent indication (Whatman). Visualization was accomplished by UV irradiation at 254 nm and/or by staining with *p*-anisaldehyde solution. Flash column chromatography was performed by using silica gel (particle size 230−400 mesh, 60 Å). All ^{1}H spectra were recorded with a Varian 400 (400 MHz) and a Bruker 500 (500 MHz) spectrometer. All NMR δ values are given in parts per million (ppm) and are referenced to the residual isotopomer solvent signals ($(CD_3)_2CO$: δ = 2.05 ppm, CD_3OD: δ = 3.31 ppm, $CDCl_3$: δ = 7.26 ppm) for ^{1}H NMR spectra. Coupling constants (*J*) are given in Hertz (Hz) and multiplicities are indicated using the conventional abbreviation (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of non-equivalent resonances, br = broad). Electrospray ionization (ESI) mass spectrometry (MS) was recorded with an Agilent 1100 series (LC/MSD trap) spectrometer to obtain the molecular masses of compounds.

5-(5-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indoline-1-carboxamido)pentanoic acid (7). [Phosgenation Reaction] To a solution of **6** (120 mg, 0.26 mmol) in saturated aqueous $NaHCO_3/CH_2Cl_2$ (1/1, 10 mL) was added triphosgene (160 mg, 0.54 mmol). After stirring at 25 °C for 1 h, the reaction was quenched by an addition of H_2O and the organic layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford trichloromethyl 5-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indoline-1-carboxylate (220 mg) as a yellowish sticky solid: HRMS (ESI) *m/*z 605.9794 [$(M+H)^+$ calcd for $C_{21}H_{18}Cl_4F_3N_3O_4S$ 605.9797]; [Urea Formation] To a solution of the indoline-1-carboxylate intermediate (110 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (7 mL) was added DIPEA (0.09 mL, 0.54 mmol) and ethyl 5-aminopentanoate (39 mg, 0.27 mmol). After stirring at 25 °C for 1 h, the reaction was quenched by an addition of H_2O and the organic layer was extracted with CH_2Cl_2. The combined organic layers were dried

over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford ethyl 5-(5-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indoline-1-carboxamido)pentanoate (43 mg, 53% for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.7 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.50 (s, 1H), 7.05 (s, 1H), 6.93 (s, 1H), 6.90 (s, 1H), 5.01–4.93 (m, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 4.00 (t, *J* = 8.6 Hz, 2H), 3.37–3.23 (m, 8H), 3.15–3.12 (m, 4H), 2.40–2.31 (m, 2H), 1.70–1.68 (m, 2H), 1.64–1.60 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) *m/z* 617.1806 [$(M+H)^+$ calcd for $C_{27}H_{32}ClF_3N_4O_5S$ 617.1806]; [Hydrolysis] To a solution of the ester intermediate (43 mg, 0.06 mmol) in THF/MeOH (2/1, 2 mL) was added 1 N NaOH (0.35 mL) at 25 °C. After stirring for 30 min, the reaction was quenched by an addition of 1 N HCl, and the resulting mixture was diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, hexanes/EtOAc (5/1) to 100% MeCN) to afford **7** (26 mg, 73%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.03–7.95 (m, 1H), 7.57–7.49 (m, 2H), 7.12 (s, 1H), 7.05 (s, 1H), 7.01 (s, 1H), 3.99 (t, *J* = 8.8 Hz, 2H), 3.27–3.15 (m, 8H), 3.08–3.05 (m, 4H), 2.32–2.30 (m, 2H), 1.68–1.53 (m, 4H); HRMS (ESI) *m/z* 589.1488 [$(M+H)^+$ calcd for $C_{25}H_{28}ClF_3N_4O_5S$ 589.1493].

5-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)-*N*-(5-(hydroxyamino)-5-oxopentyl)indoline-1-carboxamide (8). [Coupling Reaction] To a solution of **7** (18 mg, 0.03 mmol) in anhydrous THF (1.6 mL) were added ethyl chloroformate (6 μL, 0.06 mmol) and Et_3N (8 μL, 0.06 mmol). After stirring at 25 °C for 1.5 h, NH_2OTBS (8.8 mg, 0.06 mmol) in anhydrous MeOH (0.3 mL) was added to the reaction mixture. After stirring at 25 °C for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20/1) to afford *N*-(5-(((*tert*-butyldimethylsilyl)oxy)amino)-5-oxopentyl)-5-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indoline-1-carboxamide (19 mg, 88%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.7 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.49 (s, 1H), 7.04 (s, 1H), 6.92 (s, 1H), 6.89 (s, 1H), 4.02 (t, *J* = 8.8 Hz, 2H), 3.36–3.21 (m, 8H), 3.15–3.10 (m, 4H), 1.76–1.70 (m, 2H), 1.67–1.58 (m, 2H), 1.32–1.22 (m, 2H), 0.94 (s, 9H), 0.17 (s, 6H); HRMS (ESI) *m/z* 718.2460 [$(M+H)^+$ calcd for $C_{31}H_{43}ClF_3N_5O_5SSi$ 718.2467]; [TBS Deprotection] To a cooled (0 °C) solution of TBS protected hydroxamic acid (19 mg, 0.02 mmol)

in anhydrous CH_2Cl_2 (3 mL) was added dropwise TFA (0.3 mL) at 0 °C. After stirring at 25 °C for 25 min, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, (10/1) to 100% MeCN) to afford **8** (9.5 mg, 78%) as a light beige solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, *J* = 9.0 Hz, 1H), 7.58–7.52 (m, 2H), 7.14 (s, 1H), 7.06 (s, 1H), 7.02 (s, 1H), 4.03–3.95 (m, 2H), 3.27–3.11 (m, 8H), 3.10–3.07 (m, 4H), 2.15–2.12 (m, 2H), 1.71–1.61 (m, 2H), 1.60–1.51 (m, 2H); HRMS (ESI) *m/z* 604.1594 [$(M+H)^+$ calcd for $C_{25}H_{29}ClF_3N_5O_5S$ 604.1602].

7-(5-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indolin-1-yl)-7-oxoheptanoic acid (9). [Acylation] To a solution of **6** (280 mg, 0.63 mmol) in anhydrous CH_2Cl_2 (17.5 mL) were added Et_3N (0.17 mL, 1.26 mmol) and ethyl 7-chloro-7-oxoheptanoate (194 mg, 0.94 mmol). After stirring at 25 °C for 20 min, the reaction was quenched by saturated aqueous NH_4Cl, and the organic layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to afford ethyl 7-(5-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indolin-1-yl)-7-oxoheptanoate (260 mg, 67%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 9.0 Hz, 1H), 7.62 (d, *J* = 8.6 Hz, 1H), 7.57 (s, 1H), 7.06 (s, 1H), 6.94 (s, 1H), 6.91 (s, 1H), 4.23–4.08 (m, 4H), 3.34–3.28 (m, 4H), 3.27–3.24 (m, 2H), 3.23–3.09 (m, 4H), 2.51–2.41 (m, 2H), 2.38–2.29 (m, 2H), 1.80–1.70 (m, 2H), 1.68–1.65 (m, 2H), 1.49–1.38 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H); [Hydrolysis] To a solution of the ester intermediate (260 mg, 0.42 mmol) in THF/MeOH (2/1, 10 mL) was added 1 N LiOH (1.4 mL) at 25 °C. After stirring for 1.5 h, the reaction was quenched by an addition of 1 N HCl, and the resulting mixture was diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, (30/1) to 100% MeCN) to afford **9** (245 mg, 99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 7.7 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.56 (s, 1H), 7.06 (s, 1H), 6.94 (s, 1H), 6.92 (s, 1H), 4.15 (t, *J* = 8.5 Hz, 2H), 3.34–3.30 (m, 4H), 3.28–3.25 (m, 2H), 3.16–3.12 (m, 4H), 2.51–2.43 (m, 2H), 2.42–2.33 (m, 2H), 1.87–1.79 (m, 2H), 1.75–1.65 (m, 2H), 1.52–1.42 (m, 2H).

7-(5-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)indolin-1-yl)-*N*-hydroxy-7-oxoheptanamide (10). To a solution of **9** (65 mg, 0.11 mmol) in anhydrous THF (5.9 mL) were added ethyl chloroformate (10.5 μL, 0.22 mmol) and Et_3N (30 μL, 0.22 mmol). After stirring at 25 °C for 1 h, NH_2OTBS (32 mg, 0.22 mmol) in MeOH (1.18 mL) was added and the reaction mixture was left to stir for an additional 1 h. The reaction mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (0.7 mL), and treated with TFA (0.7 mL) at 0 °C. After stirring for 25 min at 25 °C, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 10/1) to afford **10** (14 mg, 21% for 2 steps) as a yellow solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.35–8.30 (m, 1H), 7.63–7.57 (m, 2H), 7.18 (s, 1H), 7.15 (s, 1H), 7.05 (s, 1H), 4.24 (t, *J* = 8.7 Hz, 2H), 3.47–3.43 (m, 4H), 3.29 (t, *J* = 8.7 Hz, 2H), 3.12–3.08 (m, 4H), 2.54–2.48 (m, 2H), 2.14–2.08 (m, 2H), 1.69–1.60 (m, 4H), 1.41–1.37 (m, 2H); HRMS (ESI) *m/z* 603.1650 [$(M+H)^+$ calcd for $C_{26}H_{30}ClF_3N_4O_5S$ 603.1650].

6-(3-(4-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)hexanoic acid (12). [Coupling Reaction] Ethyl 6-isocyanatohexanoate (0.098 mL, 0.76 mmol) was added to a solution of **11** (100 mg, 0.23 mmol) in anhydrous CH_2Cl_2/MeCN (1/1, 9 mL) at 25 °C under an argon atmosphere. After refluxing the reaction mixture for 18 h, the reaction mixture was quenched by an addition of H_2O, and the resulting mixture was diluted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford ethyl 6-(3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)hexanoate (50 mg, 35%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 8.9 Hz, 2H), 7.06 (s, 1H), 6.94 (s, 1H), 6.91 (s, 1H), 6.83 (s, 1H), 5.05–5.03 (m, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.35–3.25 (m, 6H), 3.17–3.09 (m, 4H), 2.37–2.29 (m, 2H), 1.65 (t, *J* = 7.7 Hz, 2H), 1.57–1.53 (m, 2H), 1.43–1.35 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) *m/z* 605.1806 [$(M+H)^+$ calcd for $C_{26}H_{32}ClF_3N_4O_5S$ 605.1806]; [Hydrolysis] To a solution of the ester (50 mg, 0.08 mmol) in THF/MeOH (2/1, 2.25 mL) was added 1 N NaOH (0.3 mL) at 25 °C. After stirring for 3 h, the reaction was quenched by an addition of 1 N HCl, and the resulting mixture was diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The

residue was purified by column chromatography (silica gel, hexanes/EtOAc, (1/1) to 100% MeCN) to afford **12** (43 mg, 93%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.65 (d, *J* = 9.0 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 2H), 7.14 (s, 1H), 7.06 (s, 1H), 7.02 (s, 1H), 3.36–3.25 (m, 4H), 3.23–3.15 (m, 2H), 3.12–3.02 (m, 4H), 2.30–2.21 (m, 2H), 1.66–1.58 (m, 2H), 1.50–1.43 (m, 2H), 1.40–1.30 (m, 2H); HRMS (ESI) *m/z* 577.1497 [$(M+H)^+$ calcd for $C_{24}H_{28}ClF_3N_4O_5S$ 577.1493].

6-(3-(4-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)-*N*-hydroxyhexanamide (13). [Coupling Reaction] To a solution of **12** (40 mg, 0.06 mmol) in anhydrous THF (3.6 mL) were added ethyl chloroformate (13 μL, 0.13 mmol) and Et_3N (19 μL, 0.13 mmol). After stirring at 25 °C for 1 h, NH_2OTBS (20 mg, 0.13 mmol) in anhydrous MeOH (0.7 mL) was added to the reaction mixture. After stirring at 25 °C for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 30/1) to afford *N*-((*tert*-butyldimethylsilyl)oxy)-6-(3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)hexanamide (40 mg, 94%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.67 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.9 Hz, 2H), 7.15 (s, 1H), 7.07 (s, 1H), 7.03 (s, 1H), 4.15–4.07 (m, 1H), 3.37–3.28 (m, 4H), 3.23–3.21 (m, 2H), 3.13–3.04 (m, 4H), 2.15–2.12 (m, 2H), 1.69–1.60 (m, 2H), 1.58–1.51 (m, 2H), 1.43–1.34 (m, 2H), 0.92 (s, 9H), 0.14 (s, 6H); HRMS (ESI) *m/z* 706.2460 [$(M+H)^+$ calcd for $C_{30}H_{43}ClF_3N_5O_5SSi$ 706.2467]; [TBS Deprotection] To a cooled (0 °C) solution of TBS protected hydroxamic acid (40 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (1 mL) was added dropwise TFA (1 mL) at 0 °C. After stirring at 25 °C for 25 min, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20/1) to afford **13** (24.2 mg, 81%) as a yellow solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.67 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.15 (s, 1H), 7.08 (s, 1H), 7.04 (s, 1H), 3.31–3.28 (m, 4H), 3.22–3.20 (m, 2H), 3.13–3.06 (m, 4H), 2.12–2.08 (m, 2H), 1.74–1.60 (m, 2H), 1.58–1.51 (m, 2H), 1.38–1.29 (m, 2H); HRMS (ESI) *m/z* 592.1603 [$(M+H)^+$ calcd for $C_{24}H_{29}ClF_3N_5O_5S$ 592.1602].

1-(4-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)-3-(4-(1-hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)butyl)urea (15). To a solution of **11** (22.6 mg, 0.054 mmol) and CDI (43.7 mg, 0.27 mmol) in anhydrous THF (0.27 mL) was added DIPEA (34.8 mg,

0.27 mmol). After stirring at 25 °C for 20 h, the reaction mixture was transferred to **14** (23 mg, 0.13 mmol). After stirring at 25 °C for 20 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 30/1) to afford **15** (15 mg, 43%) as a white solid: ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 12.62 (br s, 1H), 8.44 (d, *J* = 6.0 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 7.9 Hz, 2H), 7.53 (d, *J* = 7.1 Hz, 1H), 7.21 (s, 1H), 7.20 (s, 1H), 7.08 (s, 1H), 6.98 (br s, 1H), 6.04 (br s, 1H), 3.48–3.41 (m, 4H), 3.32–3.26 (m, 2H), 3.15–3.10 (m, 4H), 2.91–2.85 (m, 2H), 1.78–1.68 (m, 2H), 1.67–1.58 (m, 2H); HRMS (ESI) *m/z* 644.1374 [$(M+H)^+$ calcd for $C_{27}H_{29}ClF_3N_5O_4S_2$ 644.1369].

(*E*)-6-(3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)hex-2-enoic acid (17). [Coupling Reaction] To a solution of **11** (15 mg, 0.035 mmol) and CDI (28 mg, 0.178 mmol) in anhydrous THF (0.175 mL) was added DIPEA (31 μL, 0.178 mmol). After stirring at 25 °C for 20 h, the reaction mixture was transferred to **16** (33 mg, 0.178 mmol). After stirring at 25 °C for 20 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford *tert*-butyl (*E*)-6-(3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)hex-2-enoate (5 mg, 22%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.9 Hz, 2H), 7.36 (s, 1H), 7.09 (s, 1H), 6.99 (s, 1H), 6.96 (s, 1H), 6.82 (dt, *J* = 15.5, 6.9 Hz, 1H), 5.77 (d, *J* = 15.6 Hz, 1H), 5.19 (br s, 1H), 3.34–3.31 (m, 4H), 3.27 (t, *J* = 7.0 Hz, 2H), 3.18–3.12 (m, 4H), 2.23 (q, *J* = 7.1 Hz, 2H), 1.68–1.64 (m, 2H), 1.48 (s, 9H); HRMS (ESI) *m/z* 653.1791 [$(M+Na)^+$ calcd for $C_{28}H_{34}ClF_3N_4O_5S$ 653.1783]; [Deprotection of *tert*-Butyl Group] To a solution of the *t*-butyl ester intermediate (6.5 mg, 0.01 mmol) in CH_2Cl_2 (0.63 mL) was added TFA (0.063 mL) at 0 °C. After stirring at 25 °C for 30 min, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 30/1) to afford **17** (6 mg, 100%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.9 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.17 (s, 1H), 7.07 (s, 1H), 7.02 (dt, *J* = 15.5, 6.9 Hz, 1H), 6.96 (s, 1H), 6.94 (s, 1H), 5.84 (d, *J* = 15.6 Hz, 1H), 5.31 (br s, 1H), 3.34–3.25 (m, 6H), 3.16–2.99 (m, 4H), 2.30 (q, *J* = 7.3 Hz, 2H), 1.76–1.70 (m, 2H); HRMS (ESI) *m/z* 575.1329 [$(M+H)^+$ calcd for $C_{24}H_{26}ClF_3N_4O_5S$ 575.1337].

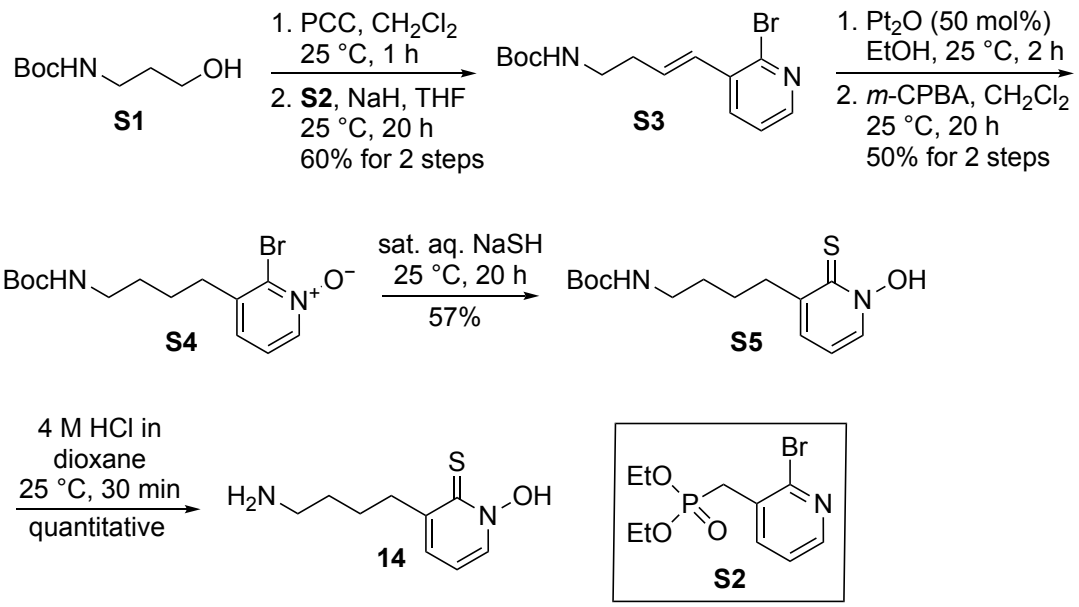
(*E*)-6-(3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)-*N*-hydroxyhex-2-enamide (18). To a solution of **17** (3.3 mg, 0.0057 mmol) in anhydrous THF (0.25 mL) were added ethyl chloroformate (2.6 μL, 0.028 mmol) and Et_3N (3.9 μL, 0.028 mmol). After stirring at 25 °C for 1 h, NH_2OTBS (4 mg, 0.028 mmol) in THF (0.25 mL) was added to the reaction mixture. After stirring at 25 °C for 1 h, the reaction mixture was concentrated *in vacuo* to afford (*E*)-*N*-((*tert*-butyldimethylsilyl)oxy)-6-(3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)hex-2-enamide (3 mg) as a white solid. TBS protected hydroxamic acid was used in the following step without further purification; To a solution of TBS protected hydroxamic acid (6.5 mg, 0.01 mmol) in CH_2Cl_2 (0.625 mL) was added TFA (0.0625 mmol) at 0 °C. After stirring at 25 °C for 30 min, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20/1) to afford **18** (2.5 mg, 74% for 2 steps): ^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 8.5 Hz, 2H), 7.17 (s, 1H), 7.09 (s, 2H), 7.05 (s, 1H), 6.82 (br s, 1H), 5.84 (d, *J* = 15.3 Hz, 1H), 3.38–3.32 (m, 4H), 3.24 (t, *J* = 6.8 Hz, 2H), 3.13–3.11 (m, 4H), 2.27 (q, *J* = 7.2 Hz, 2H), 1.74–1.66 (m, 2H); HRMS (ESI) *m/z* 590.1442 [$(M+H)^+$ calcd for $C_{24}H_{27}ClF_3N_5O_5S$ 590.1446].

1-(2-Azidoethyl)-3-(4-((4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)urea (19). [Urea Formation] To a solution of **11** (1.17 g, 2.79 mmol) in CH_2Cl_2/MeCN (1/1, 10 mL) was added 2-chloroethyl isocyanate (0.88 g, 8.37 mmol). The reaction mixture was stirred at 40 °C for 20 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford the urea intermediate (1.31 g, 89%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.19 (s, 1H), 7.08 (s, 1H), 6.97 (s, 1H), 6.94 (s, 1H), 5.51 (br s, 1H), 3.68 (m, 1H), 3.63 (m, 2H), 3.56 (m, 1H), 3.31 (m, 4H), 3.16 (m, 4H); [Azide Formation] The urea intermediate (663 mg, 1.26 mmol) in anhydrous DMF (7.0 mL) was treated with TBAI (51.0 mg, 0.14 mmol) and NaN_3 (897 mg, 13.80 mmol). The reaction mixture was stirred at 45 °C for 48 h. The reaction was quenched by an addition of brine, and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed twice with brine, dried over anhydrous Na_2SO_4, and condensed *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **19** (397 mg, 59%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.8

Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H), 7.44 (s, 1H), 7.07 (s, 1H), 6.95 (s, 1H), 6.92 (s, 1H), 5.58 (br s, 1H), 3.49 (m, 2H), 3.46 (m, 2H), 3.30 (m, 4H), 3.13 (m, 4H).

1-(2-(3-(4-((4-(3-Chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)phenyl)ureido)ethyl)-N-hydroxy-1H-1,2,3-triazole-4-carboxamide (20). To a solution of **19** (125 mg, 0.24 mmol) in EtOH/*t*-BuOH/H_2O (2/1/1, 6.0 mL) was added anhydrous $CuSO_4$ (64.9 mg, 0.26 mmol), *N*-hydroxypropiolamide (40.1 mg, 0.47 mmol), and sodium L-ascorbate (51.5 mg, 0.26 mmol). The reaction was stirred at 25 °C for 20 h. The reaction mixture was concentrated *in vacuo*. The reaction mixture was reconstituted in brine and extracted with CH_2Cl_2. The organic layers were dried with anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 10/1) to afford **20** (40.3 mg, 28%): ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 10.61 (br s, 1H), 8.66 (br s, 1H), 8.44 (br s, 1H), 8.29 (br s, 1H), 7.73 (br s, 2H), 7.68 (br s, 2H), 7.21 (s, 1H), 7.18 (s, 1H), 7.06 (s, 1H), 6.30 (br s, 1H), 4.66 (m, 2H), 3.78 (m, 2H), 3.46 (m, 4H), 3.12 (m, 4H); HRMS (ESI) *m/z* 617.1304 [$(M+H)^+$ calcd for $C_{23}H_{24}ClF_3N_8O_5S$ 617.1304].

Preparation of 14



***tert*-Butyl (*E*)-(4-(2-bromopyridin-3-yl)but-3-en-1-yl)carbamate (S3).** To a solution of **S1**[1] (1.3 g, 7.42 mmol) in CH_2Cl_2 (35 mL) was added PCC (2.04 g, 9.50 mmol). The reaction mixture was stirred at 25 °C for 1 h, and Et_2O was added. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to give *tert*-butyl (3-oxopropyl)carbamate (1.2 g). The aldehyde was used in the following step without further purification; To a cooled (0 °C) solution of **S2**[2] (1.2 g, 3.9 mmol) and NaH (149 mg, 3.9 mmol, 60% in oil) in anhydrous THF (17 mL) was added dropwise a solution of aldehyde (676 mg, 3.9 mmol) in THF (17 mL). The reaction mixture was stirred at 25 °C for 20 h and diluted with CH_2Cl_2. The mixture was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to afford **S3** (730 mg, 60% for 2 steps) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (dd, J = 4.6, 1.9 Hz, 1H), 7.74 (dd, J = 7.7, 1.9 Hz, 1H), 7.22 (dd, J = 7.7, 4.7 Hz, 1H), 6.69 (d, J = 15.0 Hz, 1H), 6.15 (dt, J = 14.3, 7.2 Hz, 1H), 4.64 (s, 1H), 3.34–3.28 (m, 2H), 2.46 (q, J = 6.8 Hz, 2H), 1.42 (s, 9H).

2-Bromo-3-(4-((*tert*-butoxycarbonyl)amino)butyl)pyridine 1-oxide (S4). [Hydrogenation] To a solution of **S3** (229 mg, 0.70 mmol) in EtOH (20 mL), Pt_2O (15.9 mg, 0.07 mmol) was added. The reaction mixture was stirred at 25 °C for 2 h and the mixture was filtered through a pad of

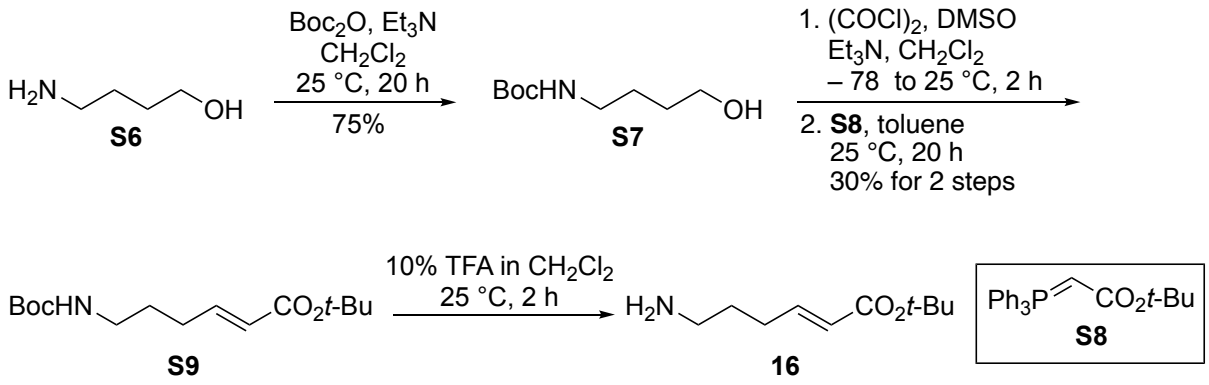
Celite and concentrated *in vacuo* to afford *tert*-butyl (4-(2-bromopyridin-3-yl)butyl)carbamate (185 mg) as a colorless oil. *tert*-Butyl (4-(2-bromopyridin-3-yl)butyl)carbamate was used in the following step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23–8.20 (m, 1H), 7.50 (dd, *J* = 7.4, 2.0 Hz, 1H), 7.19–7.16 (m, 1H), 4.56 (br s, 1H), 3.17–3.16 (m, 2H), 2.73 (t, *J* = 7.6 Hz, 2H), 1.68–1.63 (m, 2H), 1.59–1.52 (m, 2H), 1.44 (s, 9H); [Oxidation] To a solution of *tert*-butyl (4-(2-bromopyridin-3-yl)butyl)carbamate (15 mg, 0.046 mmol) in anhydrous CH_2Cl_2 (1 mL) was added *m*-CPBA (15.8 mg, 0.092 mmol). After stirring at 25 °C for 20 h, the reaction was quenched by saturated $NaHCO_3$ and diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 30/1) to afford **S4** (10 mg, 50% for 2 steps) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (dd, *J* = 6.2, 1.7 Hz, 1H), 7.15 (dd, *J* = 6.4, 1.5 Hz, 1H), 7.10 (dd, *J* = 7.7, 1.6 Hz, 1H), 4.52 (br s, 1H), 3.20–3.16 (m, 2H), 2.79 (t, *J* = 7.7 Hz, 2H), 1.68–1.63 (m, 2H), 1.60–1.52 (m, 2H), 1.44 (s, 9H); HRMS (ESI) *m/z* 345.0810 [$(M+H)^+$ calcd for $C_{14}H_{21}BrN_2O_3$ 345.0808].

***tert*-Butyl (4-(1-hydroxy-2-thioxo-1,2-dihydropyridin-3-yl)butyl)carbamate (S5).** Saturated aqueous solution of NaSH (2.5 mL) was added to **S4** (100 mg, 0.29 mmol) at 25 °C under an argon atmosphere. After being stirred at 25 °C for 20 h, the reaction mixture was quenched by an addition of 1 N HCl at 0 °C, and the resulting mixture was diluted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 30/1) to afford **S5** (50 mg, 57%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.51 (br s, 1H), 8.07–8.00 (m, 1H), 7.25–7.24 (m, 1H), 6.77–6.68 (m, 1H), 4.62 (br s, 1H), 3.22–3.16 (m, 2H), 2.89 (t, *J* = 7.7 Hz, 4H), 1.76–1.68 (m, 2H), 1.44 (s, 9H); HRMS (ESI) *m/z* 299.1425 [$(M+H)^+$ calcd for $C_{14}H_{22}N_2O_3S$ 299.1424].

3-(4-Aminobutyl)-1-hydroxypyridine-2(1*H*)-thione (14). To a cooled (0 °C) solution of **S5** (50 mg, 0.16 mmol) was added dropwise 4 M HCl in dioxane (3 mL). After stirring for 30 min at 25 °C, the reaction mixture was concentrated *in vacuo* to afford **14** (55 mg, quantitative) as a brown solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.31 (d, *J* = 7.0 Hz, 1H), 7.45 (d, *J* = 6.8 Hz, 1H), 6.90 (t, *J*

= 6.2 Hz, 1H), 2.98 (t, *J* = 7.4 Hz, 2H), 2.93 (t, *J* = 8.0 Hz, 2H), 1.83–1.67 (m, 4H); HRMS (ESI) *m/z* 199.0900 [$(M+H)^+$ calcd for $C_9H_{14}N_2OS$ 199.0904].

Preparation of 16



***tert*-Butyl (4-hydroxybutyl)carbamate (S7).** To a solution of **S6** (500 mg, 5.6 mmol) in CH_2Cl_2 (5.5 mL), Boc_2O (1.57 g, 7.2 mmol) and Et_3N (2.34 mL, 16.8 mmol) were added dropwise at 0 °C. The solution was allowed to warm to 25 °C and stirred for 20 h. The reaction mixture was quenched by an addition of 10% aqueous NH_4Cl and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to give **S7** (800 mg, 75%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.89 (br s, 1H), 3.55 (t, J = 5.9 Hz, 2H), 3.13 (s, 1H), 3.04 (t, J = 6.0 Hz, 2H), 1.52–1.46 (m, 4H), 1.35 (s, 9H).

***tert*-Butyl (*E*)-6-((*tert*-butoxycarbonyl)amino)hex-2-enoate (S9).** To a solution of oxalyl chloride (0.69 mL, 8.14 mmol) in CH_2Cl_2 (16 mL) was added dimethyl sulfoxide (1.04 mL) at −78 °C. The mixture was stirred for 30 min followed by addition of **S7** (770 mg, 4.07 mmol) in CH_2Cl_2 (16 mL). The reaction mixture was stirred at −78 °C for 30 min and then Et_3N (4.7 mL, 33.7 mmol) was added. After stirring for an additional 1 h, and the mixture was warmed to 25 °C. The reaction mixture was quenched by addition of H_2O and extracted with CH_2Cl_2 to afford aldehyde (780 mg) as a yellowish oil. Aldehyde was used in the following step without further purification; To a solution of aldehyde (780 mg, 4.16 mmol) in anhydrous toluene (8 mL) was added **S8** (3.13 g, 8.33 mmol). After stirring at 25 °C for 20 h, toluene was removed by evaporation. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford **S9** (370 mg, 30% for 2 steps) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (dt, J = 15.6, 6.9 Hz, 1H), 5.74 (d, J = 15.7 Hz, 1H), 4.51 (br s, 1H), 3.14 (t, J = 5.9 Hz, 2H), 2.24–2.15 (m, 2H), 1.66–1.60 (m,

2H), 1.46 (s, 9H), 1.43 (s, 9H); HRMS (ESI) *m/z* 308.1840 [$(M+H)^+$ calcd for $C_{15}H_{27}NO_4$ 308.1832].

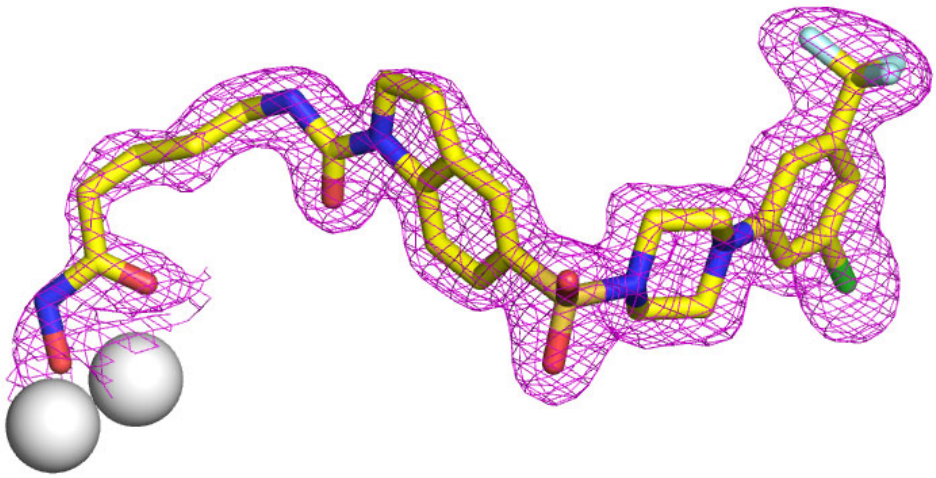
***tert*-Butyl (*E*)-6-aminohex-2-enoate (16).** To a solution of **S9** (803 mg, 2.81 mmol) in CH_2Cl_2 (4.0 mL) was added TFA (861 μL, 11.26 mmol) at 0 °C. After stirring at 25 °C for 2 h, the solvents were removed by evaporation to afford a mixture of **16** and **S9** (2.5:1) as a colorless oil. The mixture was used in the following step without further purification: ^{1}H NMR (500 MHz, CD_3OD) δ 6.76 (dt, *J* = 15.9, 6.9 Hz, 1H), 5.75 (d, *J* = 15.9 Hz, 1H), 3.00–2.92 (m, 2H), 2.31–2.20 (m, 2H), 1.99–1.60 (m, 2H), 1.40 (s, 9H).

Supplementary Table S1: Data collection and statistics of the *K. pneumoniae* LpxH-inhibitor complexes

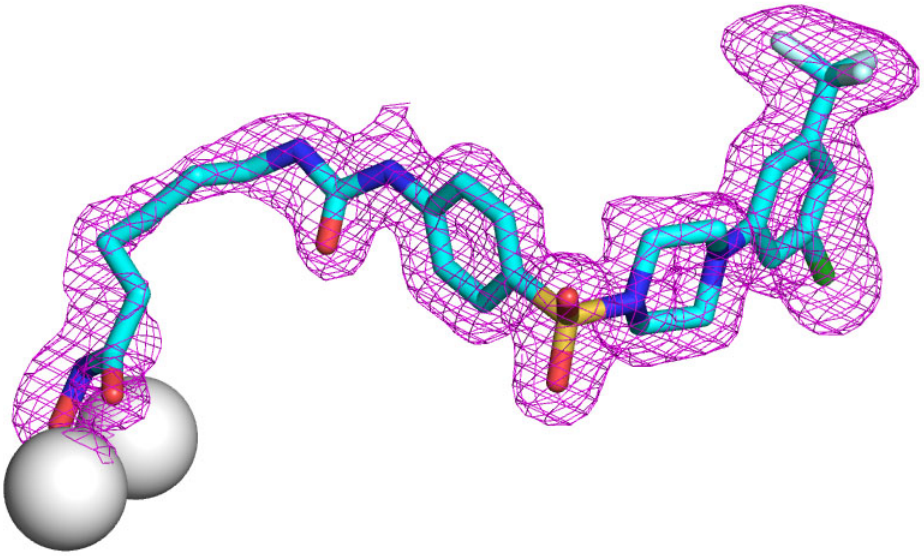
	***K. pneumoniae* LpxH/ JH-LPH-45 (8) Complex**	***K. pneumoniae* LpxH/ JH-LPH-50 (13) Complex**
PDB	**7SS6**	**7SS7**
Data collection		
Wavelength (Å)	0.9791	0.9791
Space group	P 32 2 1	P 32 2 1
Cell dimensions		
a, *b*, *c* (Å)	105.82, 105.82, 53.62	106.53, 106.53, 53.56
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	45.82-1.74 (1.80-1.74)	46.13-1.73 (1.79-1.73)
R_{meas}	0.058 (0.844)	0.075 (0.936)
Mean $I/\sigma I$	24.31 (3.18)	21.98 (3.53)
Completeness (%)	99.95 (99.83)	99.94 (99.86)
Redundancy	11.2 (11.4)	11.6 (11.0)
Total reflections	398707 (40334)	426281 (39932)
Unique reflections	35718 (3524)	36774 (3624)
Refinement		
R_{work} / R_{free}	0.1662/0.1932	0.1676/0.1911
No. atoms	2234	2314
Protein	2024	2001
Ligand/ion	58	65
Water	152	248
Average *B*-factors	35.95	28.24
Protein	35.22	26.52
Ligand/ion	39.59	37.73
Water	44.29	39.65
R.m.s. deviations		
Bond lengths (Å)	0.007	0.007
Bond angles (°)	0.85	0.81
Ramachandran		
Favored (%)	97.49	97.39
Allowed (%)	2.51	2.61
Outliers (%)	0.00	0.00

*Values in parentheses are for highest-resolution shell.

Supplementary Figure S1. Omit map of JH-LPH-45 (**8**) in the *K. pneumoniae* LpxH-bound complex. JH-LPH-45 and the di-manganese cluster are shown in the stick and sphere models, respectively. The purple mesh represents the 2mFo-DFc map of JH-LPH-45 at 1σ.



Supplementary Figure S2. Omit map of JH-LPH-50 (**13**) in the *K. pneumoniae* LpxH-bound complex. JH-LPH-50 and the di-manganese cluster are shown in the stick and sphere models, respectively. The purple mesh represents the 2mFo-DFc map of JH-LPH-50 at 0.8σ.



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