Supporting Information

Discovery of a cyclic choline analog that inhibits anaerobic choline metabolism by human gut bacteria

Maud Bollenbach,[†] Manuel Ortega,[‡] Marina Orman,[†] Catherine L. Drennan,[‡, §, ǁ, ⊥]

Emily P. Balskus[†*]

[†]Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, United States

[‡]Department of Chemistry, [§]Department of Biology,

[ǁ]Howard Hughes Medical Institute, and

[⊥]Center for Environmetal Health, Sciences, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

*Correspondence to: balskus@chemistry.harvard.edu.

Table of Contents

1. General materials and methods

Unless stated otherwise, all chemicals and solvents were purchased from Sigma-Aldrich and were used without further purification. Analytical TLC was performed using silica gel plates (Merck 60F254) and plates were visualized either by exposure to ultraviolet light or staining with potassium permanganate. Compounds were purified on SiliaFlash® P60 (particle size 0.040-0.063nm) silica gel. Yields refer to isolated compounds, estimated to be >95% pure as determined by ^{1}H NMR. ^{1}H and ^{13}C NMR spectra were recorded in the Magnetic Resonance Laboratory in the Harvard University Department of Chemistry and Chemical Biology on a Joel J-400 (400 MHz, 100 MHz) NMR spectrometer operating at 400 MHz and 100 MHz, respectively. All chemical shift values δ and coupling constants *J* are quoted in ppm and in Hz, respectively, multiplicity (s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet, br= broad). High-resolution liquid chromatography-mass spectrometry (LC-MS) analyses of were performed on an Agilent 6530 QTOF Mass Spectrometer fitted with a dual-spray electrospray ionization (ESI) source. The capillary voltage was set to 3.5 kV, the fragmentor voltage to 175 V, the skimmer voltage to 65 V, and the Oct 1 RF to 750 V. The drying gas temperature was maintained at 300 °C with a flow rate of 8 L/min and a nebulizer pressure of 35 psi. A standard calibrant mix was introduced continuously during all experiments via the dual-spray ESI source. The injection volume of all samples was 5 µL. Samples were injected onto a Kinetex (Phenomenex) HILIC column (2.6 µm, 30 mm × 2.1 mm, 100 Å). The LC conditions were: 0% B for 1 min, a gradient increasing to 100% B over 2 min, 100% B for 1.5 min, a gradient decreasing to 0% B over 1.5 min, and, finally, 0% B for 2 min (solvent A = 95% acetonitrile / 5% of 100 mM ammonium formate in water, with 0.02% formic acid; solvent B = 50% acetonitrile / 45% water / 5% of 100 mM ammonium formate in water, with 0.02% formic acid). The flow rate was maintained at 0.6 mL/min for each run. Data analysis was performed with Mass Hunter Workstation Data Acquisition software (Agilent Technologies).

Trimethylamine-d_9 hydrochloride was purchased from CDN Isotopes, choline chloride-(trimethyl-d_9) was purchased from Cambridge Isotope Laboratories, β-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH) was purchased from EMD Millipore and NADH-dependent yeast alcohol dehydrogenase (YADH) was purchased from Sigma-Aldrich. Lennox Luria-Bertani (LB) medium and Lennox LB agar were purchased from EMD Millipore, BBL Brain Heart Infusion (BHI) broth and BBL BHI agar were purchased from BD Biosciences, Reinforced Clostridial Medium (RCM) was purchased from BD Biosciences, and Dulbecco's Phosphate Buffered Saline (PBS) was purchased from ThermoFisher Scientific. Acetonitrile used for liquid chromatography-mass spectrometry (LC-MS) was a B&J Brand high-purity solvent (Honeywell Burdick & Jackson). NMR solvents were purchased from Cambridge Isotope Laboratories.

LC-MS/MS to measure trimethylamine-d_9 (d_9-TMA) and choline chloride-(trimethyl-d_9) (d_9-choline) in whole cell assays was performed on an Agilent 6410 Triple Quadrupole LC-MS instrument (Agilent Technologies) using electrospray ionization. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode with positive ionization monitoring. The precursor-product ion pairs used in MRM mode were: m/z 69.1 → m/z 49.1 (d_9-TMA) and m/z 113.2 → m/z 69.1 (d_9-choline). The capillary voltage was set to 4.0 kV and the fragmentor voltage to 110 V. The drying gas temperature was maintained at 200 °C, with a flow rate of 10 L/min and a nebulizer pressure of 25 psi. The collision energy for the precursor-product ion pairs was set to 21 V. MS^1 resolution was set to wide and MS^2 resolution to unit. The time filter width used was 0.07 min, and the ΔEMV (Electron Multiplier Voltage) was 400 V. The injection volume of all samples was 5 µL. Samples were injected onto a Kinetex (Phenomenex) HILIC

column (2.6 µm, 30 mm × 2.1 mm, 100 Å). The LC conditions were: 0% B for 1 min, a gradient increasing to 100% B over 2 min, 100% B for 1.5 min, a gradient decreasing to 0% B over 1.5 min, and, finally, 0% B for 2 min (solvent A = 95% acetonitrile / 5% of 100 mM ammonium formate in water, with 0.02% formic acid; solvent B = 50% acetonitrile / 45% water / 5% of 100 mM ammonium formate in water, with 0.02% formic acid). The flow rate was maintained at 0.6 mL/min for each run. Data analysis was performed with Mass Hunter Workstation Data Acquisition software (Agilent Technologies).

Optical densities of bacterial cultures (for plasmid overexpression) were determined with a DU 730 Life Sciences UV/Vis spectrophotometer (Beckman Coulter) by measuring absorbance at 600 nm. *E. coli* BL21 (DE3) cells overexpressing CutC were lysed with a cell disruptor (Avestin EmulsiFlex-C3). Sonication for disrupting *E. coli* BL21 (DE3) cells overexpressing CutD was performed with a Branson Sonifier S-450D equipped with a 1⁄2 inch horn in a glove box (Coy Laboratory Products) under an atmosphere of 98% N_2 and 2% H_2 at 4 °C. Nickel-nitrilotriacetic acid agarose (Ni-NTA) resin was purchased from Qiagen. SDS-PAGE gels were purchased from BioRad.

All *in vitro* experiments with CutC-52aa and CutD were conducted anaerobically in an MBraun glovebox (MBraun) under an N_2 atmosphere with less than 5 ppm of O_2. Activity assays were set up in 96-well plates (Corning) and performed at 20 °C. The absorbance at 340 nm was monitored using a PowerWave HT Microplate Spectrophotometer (BioTek).

Other than cultures for protein overexpression, all bacteria were grown anaerobically, either in a vinyl glovebox (Coy Laboratory Products) under an atmosphere of 95% N_2 and 5% H_2 or an MBraun glovebox (described above). For measuring optical densities of bacteria grown on 96-well plates, plates were brought into an anaerobic glovebox and the absorbance of each well was monitored at 600 nm using a PowerWave HT Microplate Spectrophotometer (BioTek). Absorbance values were corrected for a path length of 1 cm. A Genesys™ 20 Visible spectrophotometer was used for measuring optical densities of bacteria grown in Hungate tubes (16×125 mm, Chemglass Life Sciences).

Samples were made anaerobic as follows. Solids were brought into anaerobic chambers (MBraun and Coy Laboratory) in perforated 1.7 mL microcentrifuge tubes. CutC protein solutions were made anaerobic by flushing the headspace of the solution with argon for 30 min. Buffers and other solutions were made anaerobic by bubbling argon through the liquid for 30 min. Media solutions of 200 to 500 mL in volume were first autoclaved and then sparged with argon for 30 min.

2. Chemical synthesis procedures and characterization data

General procedures

General procedure A: Ring-closing metathesis. In an oven-dried round-bottom flask under argon, the reactant (1.0 equiv) was dissolved in anhydrous DCM (0.06 M). Grubbs 2nd generation catalyst (2 mol%) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum, and the crude mixture was purified by chromatography on silica gel (hexanes:EtOAc).

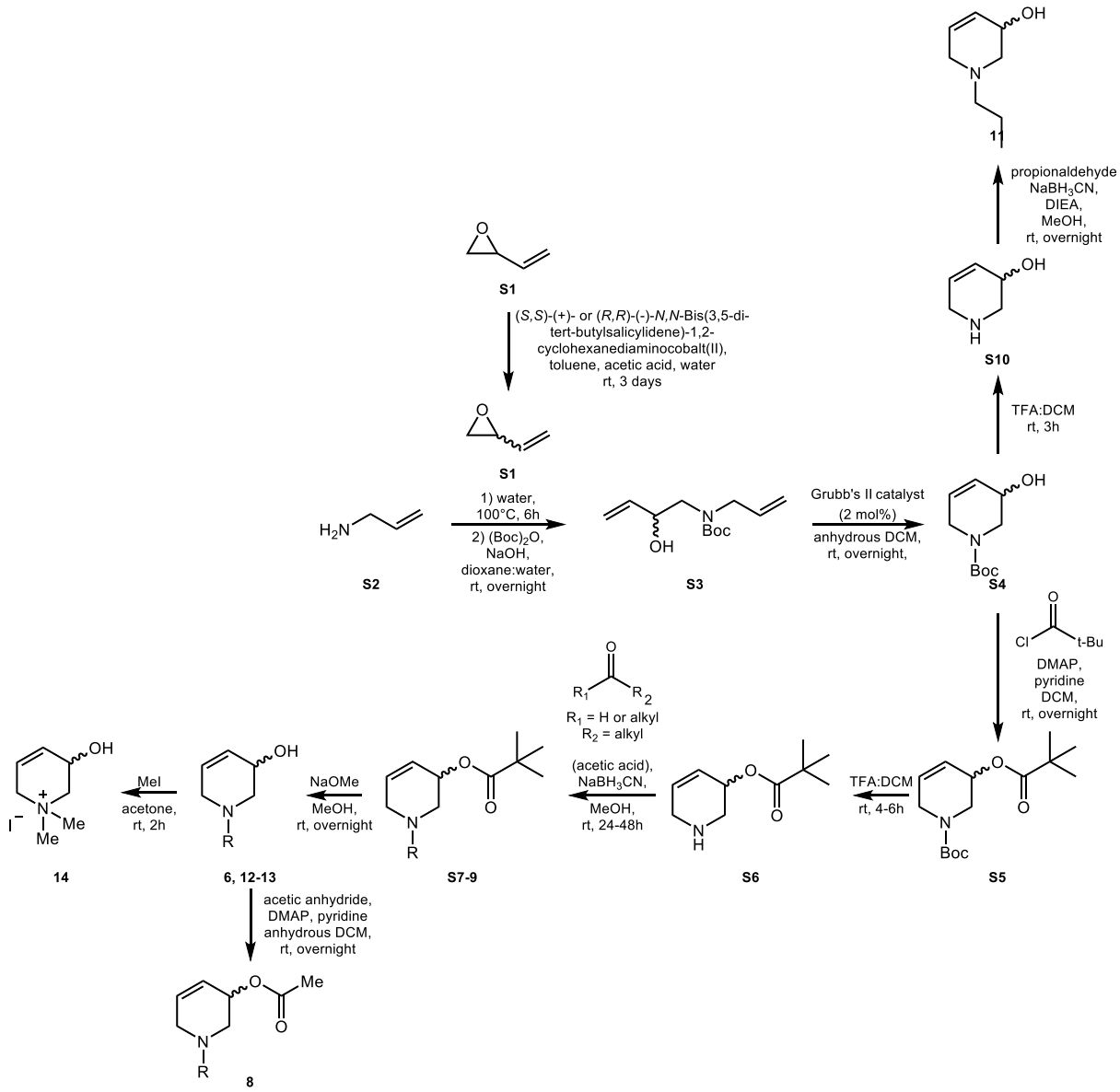
General procedure B: *tert*-Butyloxycarbonyl (Boc) deprotection. The protected amine (1.0 equiv) was dissolved in DCM (0.35 M) and cooled to 0 °C. TFA (14.6 equiv) was added dropwise, and the resulting mixture was stirred at room temperature for 4 hours. The reaction mixture was concentrated under vacuum, and the residue was resuspended in EtOAc. The

organic phase was washed twice with a saturated solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under vacuum. The residue was used without further purification.

General procedure C: Reductive amination using acetic acid. In an oven-dried round-bottom flask under argon, the free amine (1.0 equiv) was dissolved in anhydrous methanol (0.18 M) and cooled to 0 °C. The aldehyde (3.0 equiv) and acetic acid (1.0 equiv) were added and the resulting mixture stirred at 0 °C for 30 minutes. $NaBH_3CN$ (2.5 equiv) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum, and the residue was resuspended in water. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (hexanes:EtOAc).

General procedure D: Reductive amination. In an oven-dried round-bottom flask under argon, the free amine (1.0 equiv) was dissolved in anhydrous methanol (0.18 M) and cooled to 0 °C. The aldehyde (3.0 equiv) was added, and the resulting mixture was stirred at 0 °C for 30 minutes. $NaBH_3CN$ (2.5 equiv.) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum, and the residue was resuspended in water. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (hexanes:EtOAc).

General procedure E: Pivaloyl deprotection. In an oven-dried round-bottom flask under argon, the reactant (1.0 equiv) was dissolved in anhydrous methanol (0.76 M). A 25% solution of sodium methoxide in methanol (5.0 equiv) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum. The residue was purified by chromatography on silica gel (EtOAc:methanol:NH_4OH 28% in water).



Scheme S1. Synthesis of compounds 6, 11-14.

(S)-2-vinyloxirane ((*S*)-**S1**)[1]: (*S,S*)-(+)-*N,N′*-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (899 mg, 1.49 mmol, 1.5 mol%) was dissolved in toluene (7 mL, 13 M) and treated with acetic acid (2.8 mL, 49.60 mmol, 0.5 equiv). The resulting mixture was stirred in air for 30 minutes. The solution was concentrated under vacuum. Butadiene monoxide (8.0 mL, 99.00 mmol, 1.0 equiv) was added and the solution was cooled to 0 °C. Water (1.2 mL, 69.50 mmol, 0.7 equiv) was added and the resulting mixture was allowed to warm up slowly to room temperature and stirred for 3 days. The reaction mixture was distilled under atmospheric pressure, placing the collecting round-bottom flask in an ice-bath, to obtain (*S*)-**S1** as a colorless oil (3.22 g, 45.90 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.65 (dd, 1H, J = 5.3 Hz, J = 2.6 Hz), 2.96 (t, 1H, J = 5.3 Hz), 3.32-3.35 (m, 1H), 5.28-5.31 (m, 1H), 5.48-5.55 (m, 2H). The NMR data were in accordance with literature values.

(R)-2-vinyloxirane ((*R*)-**S1**)[1]: (*R,R*)-(-)-*N,N′*-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (450 mg, 0.74 mmol, 1.5 mol%) was dissolved in toluene (4 mL, 13 M) and treated with acetic acid (1.4 mL, 24.82 mmol, 0.5 equiv). The resulting mixture was

stirred in air for 30 minutes. The solution was concentrated under vacuum. Butadiene monoxide (4.0 mL, 49.60 mmol, 1.0 equiv) was added and the solution was cooled down at 0°C. Water (626 μ, 34.80 mmol, 0.7 equiv) was added and the resulting mixture was allowed to warm up slowly to room temperature and stirred for 3 days. The reaction mixture was distilled under atmospheric pressure, placing the collecting round-bottom flask in an ice-bath, to obtain (*R*)-**S1** as a colorless oil (1.75 g, 24.97 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.65 (dd, 1H, J = 5.3 Hz, J = 2.6 Hz), 2.96 (dd, 1H, J = 4.3 Hz, J = 5.1 Hz), 3.32-3.35 (m, 1H), 5.28-5.31 (m, 1H), 5.48-5.55 (m, 2H). The NMR data were in accordance with literature values.

tert-butyl allyl(2-hydroxybut-3-en-1-yl)carbamate (**S3**): To a solution of allylamine **S2** (5.6 mL, 74.50 mmol, 3.0 equiv) in water (358 μL, 19.86 mmol, 0.8 equiv) was added butadiene monoxide **S1** (2.0 mL, 24.82 mmol, 1.0 equiv). The resulting mixture was heated at 80 °C for 6 hours. After cooling to room temperature, the reaction mixture was concentrated under vacuum. The residue was dissolved in a mixture of dioxane:water (1:1, 66 mL, 0.37 M). A 50% solution of NaOH (2.6 mL, 49.60 mmol, 2.0 equiv.) and di-*tert*-butyl decarbonate (10.8 g, 49.60 mmol, 2.0 equiv.) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc and washed twice with a 20% citric acid solution followed by brine. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to afford **S3** as a colorless oil (5.64 g, 24.82 mmol, 100%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 3.29 (br s, 2H), 3.87 (br s, 2H), 4.32 (q, 1H, J = 5.6 Hz), 5.10-5.19 (m, 3H), 5.33 (d, 1H, J = 17.3 Hz), 5.75-5.90 (m, 2H).

tert-butyl (S)-allyl(2-hydroxybut-3-en-1-yl)carbamate ((*S*)-**S3**): To a solution of allylamine **S2** (5.0 mL, 67.00 mmol, 3.0 equiv) and water (322 μL, 17.87 mmol, 0.8 equiv) was added butadiene monoxide (*S*)-**S1** (1.8 mL, 22.34 mmol, 1.0 equiv). The mixture was heated at 80°C for 6 hours. Once cooled down, the reaction mixture was concentrated under vacuum. The residue was dissolved in a mixture dioxane:water (1:1, 60 mL, 0.37 M). A 50% solution of NaOH (2.3 mL, 44.70 mmol, 2.0 equiv) and di-*tert*-butyl decarbonate (9.8 g, 44.70 mmol, 2.0 equiv) were added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc and washed twice with a 20% citric acid solution and then brine. The organic layer was dried over MgSO4 and concentrated under vacuum to afford (*S*)-**S3** as a colorless oil (5.64 g, 24.82 mmol, 100%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H), 3.28 (br s, 2H), 3.87 (br s, 2H), 4.32 (q, 1H, J = 5.6 Hz), 5.10-5.19 (m, 3H), 5.33 (d, 1H, J = 17.2 Hz), 5.75-5.90 (m, 2H).

tert-butyl (R)-allyl(2-hydroxybut-3-en-1-yl)carbamate ((*R*)-**S3**): To a solution of allylamine **S2** (1.9 mL, 26.50 mmol, 3.0 equiv) and water (127 μL, 7.05 mmol, 0.8 equiv) was added butadiene monoxide (*R*)-**S1** (618 mg, 8.82 mmol, 1.0 equiv). The mixture was heated at 80°C for 6 hours. Once cooled down, the reaction mixture was concentrated under vacuum. The residue was dissolved in a mixture dioxane:water (1:1, 24 mL, 0.37 M). A 50% solution of NaOH (931 μL, 17.63 mmol, 2.0 equiv) and di-*tert*-butyl decarbonate (1.4 g, 17.63 mmol, 2.0 equiv) were added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc and washed twice with a 20% citric acid solution and then brine. The organic layer was dried over MgSO4 and concentrated under vacuum to afford (*R*)**-S3** as a colorless oil (5.64 g, 24.82 mmol, 100%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H), 3.28 (br s, 2H), 3.87 (br s, 2H), 4.32 (q, 1H, J = 5.5 Hz), 5.10-5.19 (m, 3H), 5.34 (d, 1H, J = 17.0 Hz), 5.75-5.90 (m, 2H).

tert-butyl 3-hydroxy-3,6-dihydropyridine-1(2H)-carboxylate (**S4**): Following general procedure A, starting from **S3** (7.0 g, 31.00 mmol, 1.0 equiv) and purifying by chromatography on silica gel using 1:1 hexanes:EtOAc as eluent, **S4** was obtained as a brown oil (5.3 g, 26.90 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 3.50 (dd, 1H, J = 13.3 Hz, J = 5.3 Hz), 3.60 (dd, 1H, J = 13.2 Hz, J = 4.0 Hz), 3.79 (dq, 1H, J = 18.8 Hz, J = 2.2 Hz), 3.97 (d, 1H, J = 18.8 Hz), 4.19 (br s, 1H), 5.82 (dt, 1H, J = 9.9 Hz, J = 2.7 Hz), 5.91 (dq, 1H, J = 10.2 Hz, J = 3.2 Hz).

tert-butyl (S)-3-hydroxy-3,6-dihydropyridine-1(2H)-carboxylate ((*S*)-**S4**): Following general procedure A, starting from (*S*)-**S3** (8.7 g, 38.40 mmol, 1.0 equiv) and purifying by chromatography on silica gel using 1:1 hexanes:EtOAc as eluent, (*S*)-**S4** was obtained as a brown oil (5.2 g, 26.00 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 3.53 (d, 1H, J = 13.3 Hz), 3.58 (d, 1H, J = 13.2 Hz), 3.79 (dq, 1H, J = 19.0 Hz, J = 2.2 Hz), 3.98 (d, 1H, J = 18.1 Hz), 4.19 (br s, 1H), 5.82 (d, 1H, J = 9.8 Hz), 5.91 (dq, 1H, J = 10.2 Hz, J = 3.1 Hz).

tert-butyl (R)-3-hydroxy-3,6-dihydropyridine-1(2H)-carboxylate ((*R*)-**S4**): Following general procedure A, starting from (*R*)-**S3** (8.1 g, 35.70 mmol, 1.0 equiv) and purifying by chromatography on silica gel using 1:1 hexanes:EtOAc as eluent, (*R*)-**S4** was obtained as a brown oil (4.82 g, 24.09 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 3.50 (br s, 1H), 3.58 (br s, 1H), 3.79 (dq, 1H, J = 18.9 Hz, J = 2.2 Hz), 3.97 (d, 1H, J = 18.3 Hz), 4.19 (br s, 1H), 5.82 (d, 1H, J = 9.9 Hz), 5.91 (dq, 1H, J = 9.9 Hz, J = 1.8 Hz).

tert-butyl 3-(pivaloyloxy)-3,6-dihydropyridine-1(2H)-carboxylate (**S5**): **S4** (5.3 g, 26.60 mmol, 1.0 equiv) and DMAP (325 mg, 2.66 mmol, 0.1 equiv) were dissolved in anhydrous DCM (89 mL, 0.3 M). Pyridine (10.7 mL, 133 mmol, 5.0 equiv) and pivaloyl chloride (4.6 mL, 37.20 mmol, 1.4 equiv) were added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water. The aqueous phase was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (4:1 hexanes:EtOAc), to obtain **S5** as a colorless oil (7.0 g, 24.70 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.19 (s, 9H), 1.46 (s, 9H), 3.46 (dd, 1H, J = 13.7 Hz, J = 3.5 Hz), 3.78 (dd, 2H, J = 13.7 Hz, J = 4.3 Hz), 4.02-4.26 (m, 2H), 5.17 (s, 1H), 5.83-5.97 (m, 3H).

tert-butyl (S)-3-(pivaloyloxy)-3,6-dihydropyridine-1(2H)-carboxylate ((*S*)-**S5**): (*S*)-**S4** (1.4 g, 7.08 mmol, 1.0 equiv) and DMAP (86 mg, 0.71 mmol, 0.1 equiv) were dissolved in anhydrous DCM (23 mL, 0.3 M). Pyridine (2.8 mL, 35.40 mmol, 5.0 equiv) and pivaloyl chloride (1.2 mL, 9.91 mmol, 1.4 equiv) were added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water. The aqueous phase was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by chromatography on silica gel (4:1 hexanes:EtOAc), to obtain (*S*)-**S5** as a colorless oil (1.9 g, 6.74 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.19 (s, 9H), 1.46 (s, 9H), 3.46 (dd, 1H, J = 13.7 Hz, J = 3.5 Hz), 3.78 (dd, 2H, J = 13.7 Hz, J = 4.3 Hz), 4.02-4.26 (m, 2H), 5.17 (s, 1H), 5.83-5.97 (m, 3H).

tert-butyl (R)-3-(pivaloyloxy)-3,6-dihydropyridine-1(2H)-carboxylate ((*R*)-**S5**): (*R*)-**S4** (758 mg, 3.80 mmol, 1.0 equiv) and DMAP (93 mg, 0.76 mmol, 0.2 equiv) were dissolved in anhydrous DCM (13 mL, 0.3 M). Pyridine (1.5 mL, 19.02 mmol, 5.0 equiv) and pivaloyl chloride (703 μL, 5.71 mmol, 1.4 equiv) were added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water. The aqueous phase was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by chromatography on silica gel (4:1

hexanes:EtOAc), to obtain *tert*-butyl (*R*)-**S5** as a colorless oil (1.1 g, 3.74 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.19 (s, 9H), 1.46 (s, 9H), 3.46 (dd, 1H, J = 13.7 Hz, J = 3.5 Hz), 3.78 (dd, 2H, J = 13.7 Hz, J = 4.3 Hz), 4.02-4.26 (m, 2H), 5.17 (s, 1H), 5.83-5.97 (m, 3H).

1,2,3,6-tetrahydropyridin-3-yl pivalate (**S6**): Following general procedure B and starting from **S5** (2.6 g, 9.07 mmol, 1.0 equiv), **S6** was obtained as a brown oil (1.3 g, 9.07 mmol, 76%).

(S)-1,2,3,6-tetrahydropyridin-3-yl pivalate ((*S*)-**S6**): Following general procedure B and starting from (*S*)-**S5** (4.5 g, 15.88 mmol, 1.0 equiv), (*S*)-**S6** was obtained as a brown oil (2.2 g, 11.84 mmol, 75%).

(R)-1,2,3,6-tetrahydropyridin-3-yl pivalate ((*R*)-**S6**): Following general procedure B and starting from (*R*)-**S5** (1.1 g, 3.71 mmol, 1.0 equiv), (*R*)-**S6** was obtained as a brown oil (0.7 g, 3.55 mmol, 96%).

1-methyl-1,2,3,6-tetrahydropyridin-3-yl (**S7**): Following general procedure C, starting from **S6** (540 mg, 2.95 mmol, 1.0 equiv) and formaldehyde (37% in water, 658 μL, 8.84 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 1:2 hexanes:EtOAc as eluent, **S7** was obtained as an orange oil (381 mg, 1.93 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (s, 9H), 2.36 (s, 3H), 2.46 (dd, 1H, J = 11.7 Hz, J = 5.4 Hz), 2.79 (dd, 1H, J = 11.7 Hz, J = 4.9 Hz), 2.88 (d, 1H, J = 16.7 Hz), 2.98 (dd, 1H, J = 16.7 Hz, J = 1.1 Hz), 5.31 (s, 1H), 5.73 (dd, 1H, J = 10.1 Hz, J = 1.5 Hz), 5.95 (d, 1H, J = 10.1 Hz).

(S)-1-methyl-1,2,3,6-tetrahydropyridin-3-yl pivalate ((*S*)-**S7**): Following general procedure C, starting from (*S*)-**S6** (272 mg, 1.48 mmol, 1.0 equiv) and formaldehyde (37% in water, 332 μL, 4.45 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 1:2 hexanes:EtOAc as eluent, (*S*)-**S7** was obtained as a colorless oil (193 mg, 0.96 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (s, 9H), 2.36 (s, 3H), 2.46 (dd, 1H, J = 11.7 Hz, J = 5.4 Hz), 2.79 (dd, 1H, J = 11.7 Hz, J = 4.9 Hz), 2.88 (d, 1H, J = 16.7 Hz), 2.98 (dd, 1H, J = 16.7 Hz, J = 1.1 Hz), 5.31 (s, 1H), 5.73 (dd, 1H, J = 10.1 Hz, J = 1.5 Hz), 5.95 (d, 1H, J = 10.1 Hz).

(R)-1-methyl-1,2,3,6-tetrahydropyridin-3-yl pivalate ((*R*)-**S7**): Following general procedure C, starting from (*R*)-**S6** (487 mg, 2.66 mmol, 1.0 equiv) and formaldehyde (37% in water, 594 μL, 7.97 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 1:2 hexanes:EtOAc as eluent, (*R*)-**S7** was obtained as a colorless oil (292 mg, 1.48 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (s, 9H), 2.36 (s, 3H), 2.46 (dd, 1H, J = 11.7 Hz, J = 5.4 Hz), 2.79 (dd, 1H, J = 11.7 Hz, J = 4.9 Hz), 2.88 (d, 1H, J = 16.7 Hz), 2.98 (dd, 1H, J = 16.7 Hz, J = 1.1 Hz), 5.31 (s, 1H), 5.73 (dd, 1H, J = 10.1 Hz, J = 1.5 Hz), 5.95 (d, 1H, J = 10.1 Hz).

1-cyclobutyl-1,2,3,6-tetrahydropyridin-3-yl pivalate (**S8**): Following general procedure C, starting from **S6** (323 mg, 1.76 mmol, 1.0 equiv) and cyclobutanone (395 μL, 5.29 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 4:1 hexanes:EtOAc as eluent, **S8** was obtained as a colorless oil (186 mg, 0.78 mmol, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (s, 9H), 1.65-1.74 (m, 2H), 1.90-2.04 (m, 4H), 2.30-2.37 (m, 3H), 2.61-2.68 (m, 2H), 5.31 (br s, 1H), 5.72 (dq, 1H, J = 10.1 Hz, J = 2.2 Hz), 5.95 (dt, 1H, J = 9.9 Hz, J = 3.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 13.2, 14.7, 27.2, 27.5, 36.8, 48.7, 50.5, 59.6, 67.2, 125.0, 130.1, 178.7.

1-benzyl-1,2,3,6-tetrahydropyridin-3-yl pivalate (**S9**): Following general procedure D, starting from **S6** (180 mg, 0.98 mmol, 1.0 equiv) and benzaldehyde (300 μL, 2.95 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 8:1 hexanes:EtOAc as eluent, **S9** was obtained as a colorless oil (198 mg, 0.72 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.18 (s, 9H),

2.61 (dd, 1H, *J* = 11.9 Hz, *J* = 4.7 Hz), 2.70 (dd, 1H, *J* = 11.9 Hz, *J* = 4.5 Hz), 2.94 (d, 1H, *J* = 16.8 Hz), 3.11 (d, 1H, *J* = 16.5 Hz), 3.51 (d, 1H, *J* = 13.4 Hz), 3.73 (d, 1H, *J* = 13.4 Hz), 5.26 (br s, 1H), 5.76 (dq, 1H, *J* = 9.9 Hz, *J* = 2.9 Hz), 5.97 (dt, 1H, *J* = 9.9 Hz, *J* = 3.1 Hz), 7.24 (t, 1H, *J* = 7.0 Hz), 7.31 (t, 2H, *J* = 7.1 Hz), 7.36 (d, 2H, *J* = 7.3 Hz).

1,2,3,6-tetrahydropyridin-3-ol 2,2,2-trifluoroacetate (**S10**): **S4** (540 mg, 2.70 mmol, 1.0 equiv) was dissolved in DCM (7 mL, 0.38 M) and cooled to 0 °C. TFA (3.4 mL, 43.50 mmol, 16.0 equiv) was added dropwise, and the resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated under vacuum to afford **S10** as a brown oil (576 mg, 2.70 mmol, 100%), which was used in the next step without further purification.

1-methyl-1,2,3,6-tetrahydropyridin-3-ol (**6**):[2] Following general procedure E, starting from **S7** (536 mg, 2.72 mmol, 1.0 equiv) and purifying by chromatography on silica gel using 9:1:0.1 EtOAc:methanol:NH_4OH 28% in water as eluent, **6** was obtained as a yellow oil (207 mg, 1.83 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.35 (s, 3H), 2.46 (dd, 1H, *J* = 11.7 Hz, *J* = 3.4 Hz), 2.65-2.70 (m, 2H), 3.07 (d, 1H, *J* = 16.8 Hz), 4.07 (s, 1H), 5.82 (d, 1H, *J* = 11.8 Hz), 5.88 (d, 1H, *J* = 11.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 46.1, 54.8, 60.4, 64.9, 128.0, 129.0. The NMR data were in accordance with literature values.

(S)-1-methyl-1,2,3,6-tetrahydropyridin-3-ol ((*S*)-**6**): Following general procedure E, starting from (*S*)-**S7** (315 mg, 1.60 mmol, 1.0 equiv) and purifying by chromatography on silica gel using 9:1:0.1 EtOAc:methanol:NH_4OH 28% in water as eluent, (*S*)-**6** was obtained as a yellow oil (92 mg, 0.81 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.35 (s, 3H), 2.46 (dd, 1H, *J* = 11.7 Hz, *J* = 3.4 Hz), 2.65-2.70 (m, 2H), 3.07 (d, 1H, *J* = 16.8 Hz), 4.07 (s, 1H), 5.82 (d, 1H, *J* = 11.8 Hz), 5.88 (d, 1H, *J* = 11.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 46.1, 54.8, 60.4, 64.9, 128.0, 129.0; HRMS ($M+H^+$) 114.0910 (calcd for $C_6H_{11}NOH^+$ 114.0913).

(R)-1-methyl-1,2,3,6-tetrahydropyridin-3-yl pivalate ((*R*)-**6**): Following general procedure E and starting from (*R*)-**S7** (271 mg, 1.37 mmol, 1.0 equiv) and purified by chromatography on silica gel using 9:1:0.1 EtOAc:methanol:NH_4OH 28% in water as eluent, (*R*)-**6** was obtained as a yellow oil (121 mg, 1.07 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.35 (s, 3H), 2.46 (dd, 1H, *J* = 11.7 Hz, *J* = 3.4 Hz), 2.65-2.70 (m, 2H), 3.07 (d, 1H, *J* = 16.8 Hz), 4.07 (s, 1H), 5.82 (d, 1H, *J* = 11.8 Hz), 5.88 (d, 1H, *J* = 11.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 46.1, 54.8, 60.4, 64.9, 128.0, 129.0 ; HRMS ($M+H^+$) 114.0913 (calcd for $C_6H_{11}NOH^+$ 114.0913).

1-methyl-1,2,3,6-tetrahydropyridin-3-yl acetate (**8**)[3]: **6** (46 mg, 0.41 mmol, 1.0 equiv) and DMAP (10 mg, 0.08 mmol, 0.2 equiv) was dissolved in pyridine (164 μL, 2.03 mmol, 5.0 equiv) and anhydrous DCM (1.3 mL, 0.3 M) under an argon atmosphere. Acetic anhydride (57.5 μL, 0.61 mmol, 1.5 equiv) was added dropwise, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water, and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (1:1 hexanes:EtOAc) to obtain **8** as a yellow oil (17 mg, 0.11 mmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.68 (s, 3H), 2.97 (s, 3H), 3.20 (dd, 1H, *J* = 12.2 Hz, *J* = 4.0 Hz), 3.36 (d, 1H, *J* = 15.9 Hz), 3.78 (d, 1H, *J* = 16.8 Hz), 5.86 (br s, 1H), 6.41 (dq, 1H, *J* = 10.1 Hz, *J* = 1.8 z), 6.60 (dt, 1H, *J* = 9.9 Hz, *J* = 3.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 21.6, 45.8, 54.1, 56.7, 67.5, 123.5, 131.3, 171.2. The NMR data were in accordance with literature values.

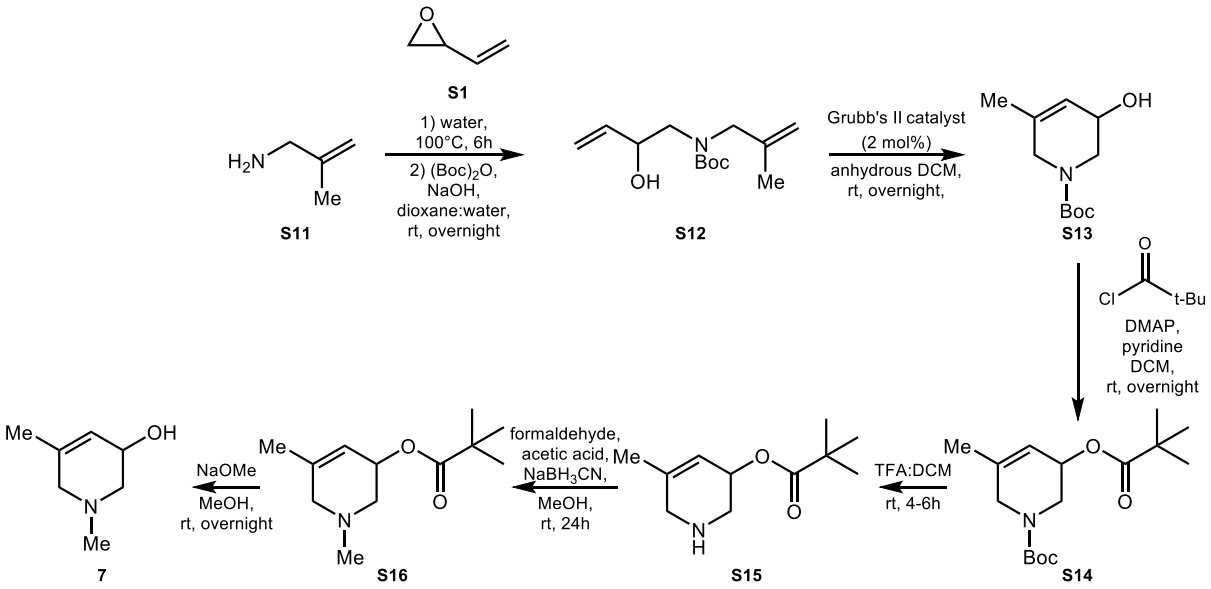
1-propyl-1,2,3,6-tetrahydropyridin-3-ol (**11**): In an oven-dried round-bottom flask under argon, **S10** (576 mg, 2.70 mmol, 1.0 equiv) was dissolved in anhydrous methanol (15 mL, 0.18 M)

and cooled to 0 °C. Propionaldehyde (581 μL, 8.11 mmol, 3.0 equiv) and DIEA (1.9 mL, 10.81 mmol, 4.0 equiv) were added, and the resulting mixture was stirred at 0 °C for 30 minutes. $NaBH_3CN$ (425 mg, 6.76 mmol, 2.5 equiv) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum. The residue was resuspended in water, and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by chromatography on silica gel (1:0 to 8:2 EtOAc:methanol), to afford **11** as an orange oil (95 mg, 0.67 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.94 (t, 3H, J = 7.4 Hz), 1.55 (q, 2H, J = 7.4 Hz), 2.40-2.52 (m, 3H), 2.75-2.84 (m, 3H), 3.19 (dd, 1H, J = 17.0 Hz, J = 4.1 Hz), 4.08 (br s, 1H), 5.84 (dq, 1H, J = 9.8 Hz, J = 2.2 Hz), 5.91 (dt, 1H, J = 9.9 Hz, J = 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 12.1, 20.2, 53.1, 58.1, 60.2, 64.7, 128.3, 128.9; HRMS ($M+H^+$) 142.1223 (calcd for $C_8H_{15}NOH^+$ 142.1226).

1-cyclobutyl-1,2,3,6-tetrahydropyridin-3-ol (**12**): Following general procedure E, starting from **S8** (185 mg, 0.78 mmol, 1.0 equiv) and purifing by chromatography on silica gel using 9:1 EtOAc:methanol as eluent, **12** was obtained as a yellow oil (29 mg, 0.19 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.63-1.76 (m, 2H), 1.95 (sept., 2H, J = 10.3 Hz), 2.01-2.08 (m, 2H), 2.34 (dd, 1H, J = 11.5 Hz, J = 3.4 Hz), 2.61 (d, 1H, J = 16.7 Hz), 2.73 (dd, 1H, J = 11.5 Hz, J = 3.3 Hz), 2.89 (quint., 1H, J = 7.8 Hz), 3.12 (dd, 1H, J = 16.9 Hz, J = 3.9 Hz), 4.08 (br s, 1H), 5.83 (dq, 1H, J = 9.9 Hz, J = 1.8 Hz), 5.90 (dt, 1H, J = 9.9 Hz, J = 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.7, 27.1, 27.3, 27.8, 49.4, 54.3, 60.0, 64.3, 128.3, 128.4; HRMS ($M+H^+$) 154.1227 (calcd for $C_9H_{15}NOH^+$ 154.1226).

1-benzyl-1,2,3,6-tetrahydropyridin-3-ol (**13**)[4]: Following general procedure E, starting from **S9** (197 mg, 0.72 mmol, 1.0 equiv) and purifying by chromatography on silica gel using EtOAc as eluent, **13** was obtained as a yellow oil (84 mg, 0.44 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.51 (dd, 1H, J = 11.4 Hz, J = 3.1 Hz), 2.74-2.82 (m, 2H), 3.13 (dd, 1H, J = 17.0 Hz, J = 4.0 Hz), 3.61 (s, 2H), 4.05 (br s, 1H), 5.82 (dq, 1H, J = 9.8 Hz, J = 4.0 Hz), 5.90 (dt, 1H, J = 9.8 Hz, J = 2.2 Hz), 7.25-7.28 (m, 1H), 7.31-7.33 (m, 4H). The NMR data were in accordance with literature values.

3-hydroxy-1,1-dimethyl-1,2,3,6-tetrahydropyridin-1-ium iodide (**14**): **6** (53 mg, 0.47 mmol, 1.0 equiv) was dissolved in acetone (1.8 mL, 0.25 M). The resulting mixture was bubbled with argon for 10 minutes and MeI (35.0 μL, 0.56 mmol, 1.2 equiv) was added dropwise. The reaction mixture was stirred at room temperature for 3 hours. The formed precipitate was filtrated under vacuum, to afford **14** as a white solid (68 mg, 0.27 mmol, 57%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 2.59 (s, 3H), 2.62 (s, 3H), 2.64-2.67 (m, 2H), 2.85 (dd, 1H, J = 12.9 Hz, J = 3.9 Hz), 3.06 (dd, 1H, J = 13.0 Hz, J = 5.1 Hz), 3.84 (br s, 1H), 5.23 (d, 1H, J = 10.5 Hz), 5.47 (dt, 1H, J = 10.4 Hz, J = 1.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ ppm 53.2, 54.3, 61.6, 61.8, 66.0, 121.3, 129.2 ; HRMS ($M+H^+$) 128.1072 (calcd for $C_7H_{14}NO^+$ 128.1070).



Scheme S2. Synthesis of compound 7

tert-butyl (2-hydroxybut-3-en-1-yl)(2-methylallyl)carbamate (**S12**): To a solution of 2-methylprop-2-en-1-amine **S11** (1.0 mL, 11.24 mmol, 3.0 equiv) in water (54 μL, 2.98 mmol, 0.8 equiv) was added 2-vinyloxirane **S1** (300 μL, 3.72 mmol, 1.0 equiv). The resulting mixture was heated at 80 °C for 6 hours. After cooling to room temperature, the reaction mixture was concentrated under vacuum. The residue was dissolved in a mixture of dioxane:water (1:1, 10 mL, 0.37 M). A 50% solution of NaOH (393 μL, 7.45 mmol, 2.0 equiv.) and di-*tert*-butyl decarbonate (1.6 g, 7.45 mmol, 2.0 equiv.) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc and washed twice with a 20% citric acid solution followed by brine. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to afford **S12** as a colorless oil (899 mg, 3.72 mmol, 100%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H), 1.68 (s, 3H), 3.21-3.36 (m, 1 H), 3.70 (s, 2H), 2.78-3.83 (m, 2H), 4.33 (q, 1H, J = 5.4 Hz), 4.76 (s, 1H), 5.14-5.23 (m, 1H), 5.33 (d, 1H, J = 16.9 Hz), 5.78-5.87 (m, 1H).

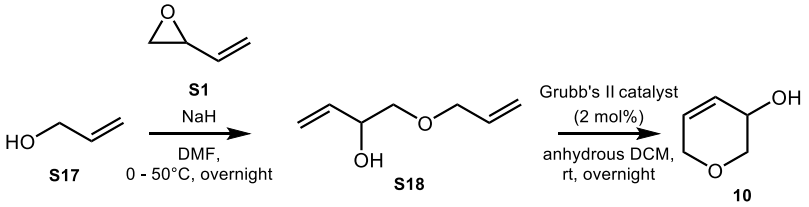
tert-butyl 3-hydroxy-5-methyl-3,6-dihydropyridine-1(2H)-carboxylate (**S13**): Following general procedure A and starting from **S12** (899 mg, 3.73 mmol, 1.0 equiv), **S13** was obtained as a brown oil (585 mg, 2.74 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 9H), 1.72 (s, 3H), 3.42 (d, 1H, J = 12.1 Hz), 3.55-3.65 (m, 2H), 3.89 (br s, 1H), 5.64 (br s, 1H).

tert-butyl 5-methyl-3-(pivaloyloxy)-3,6-dihydropyridine-1(2H)-carboxylate (**S14**): **S13** (560 mg, 263 mmol, 1.0 equiv) and DMAP (64 mg, 0.53 mmol, 0.1 equiv) were dissolved in anhydrous DCM (8.7 mL, 0.3 M) under an argon atmosphere. Pyridine (1.0 mL, 13.13 mmol, 5.0 equiv) and pivaloyl chloride (485 μL, 3.94 mmol, 1.5 equiv) were added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water, and the aqueous phase was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (4:1 hexanes:EtOAc) to obtain **S14** as a colorless oil (636 mg, 2.14 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.18 (s, 9H), 1.46 (s, 9H), 1.74 (s, 3H), 3.30 (dd, 1H, J = 14.0 Hz, J = 3.5 Hz), 3.57 (d, 1H, J = 18.0 Hz), 3.84 (dd, 1H, J = 13.8 Hz, J = 3.7 Hz), 4.07-4.13 (m, 1H), 5.11 (br s, 1H), 5.57 (br s, 1H).

5-methyl-1,2,3,6-tetrahydropyridin-3-yl pivalate (**S15**): Following general procedure B and starting from **S14** (635 mg, 2.14 mmol, 1.0 equiv), **S15** was obtained as a brown oil (320 mg, 1.62 mmol, 76%).

1,5-dimethyl-1,2,3,6-tetrahydropyridin-3-yl pivalate (**S16**): Following general procedure C, starting from **S15** (320 mg, 1.62 mmol, 1.0 equiv) and formaldehyde (37% in water, 362 μL, 4.87 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 1:2 hexanes:EtOAc as eluent, **S16** was obtained as an orange oil (116 mg, 0.55 mmol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (s, 9H), 1.72 (s, 3H), 2.35 (s, 3H), 2.46 (dd, 1H, J = 4.8 Hz, J = 11.8 Hz), 2.66-2.75 (m, 2H), 2.89 (d, 1H, J = 16.1 Hz), 5.26 (br s, 1H), 5.45 (br s, 1H).

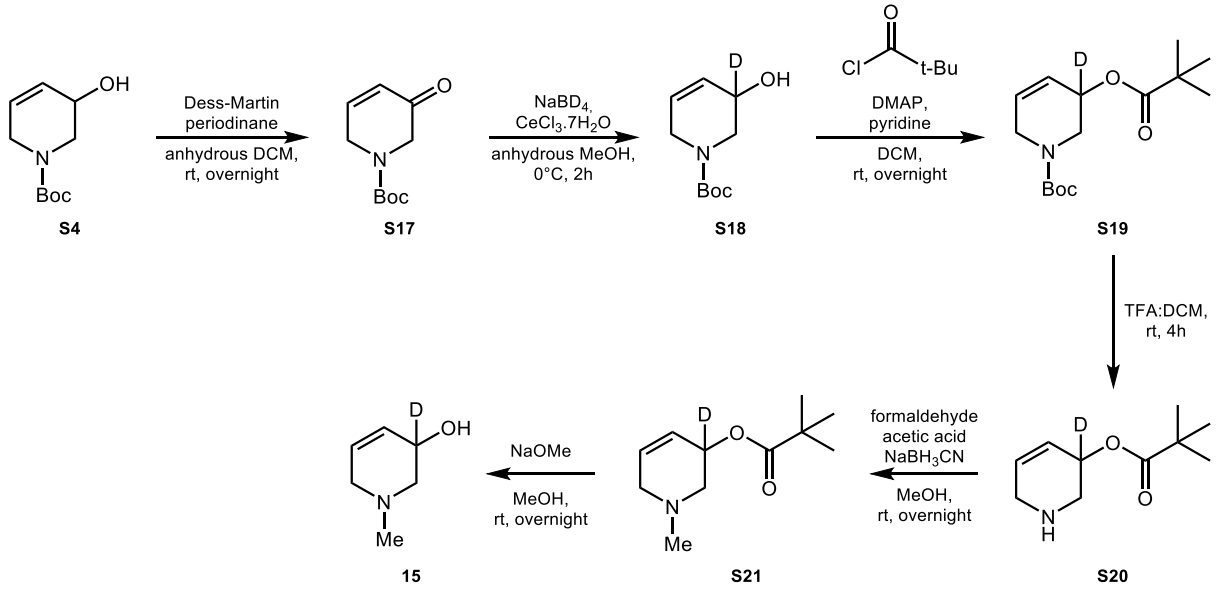
1,5-dimethyl-1,2,3,6-tetrahydropyridin-3-ol (**7**): Following general procedure E, starting from **S16** (116 mg, 0.55 mmol, 1.0 equiv) and purifying by chromatography on silica gel using 9:1:0.1 EtOAc:methanol:NH_4OH 28% in water as eluent, **7** was obtained as a colorless oil (38 mg, 0.30 mmol, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.35 (s, 3H), 2.38 (dd, 1H, J = 11.4 Hz, J = 3.3 Hz), 2.57 (d, 1H, J = 16.1 Hz), 2.67 (dd, 1H, J = 11.4 Hz, J = 3.2 Hz), 2.95 (d, 1H, J = 16.1 Hz), 4.04 (d, 1H, J = 2.9 Hz), 5.60 (d, 1H, J = 2.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 21.0, 45.9, 59.2, 60.1, 65.2, 122.7, 137.2; HRMS ($M+H^+$) 128.1074 (calcd for $C_7H_{13}NOH^+$ 128.1070).



Scheme S3. Synthesis of compound 10

1-(allyloxy)but-3-en-2-ol (**S18**): 2-Vinyloxirane **S1** (500 μL, 6.21 mmol, 1.0 equiv) and prop-2-en-1-ol **S17** (844 μL, 12.41 mmol, 2.0 equiv) were dissolved in anhydrous DMF (12 mL, 0.5 M) and cooled to 0 °C. NaH (60%, 496 mg, 12.41 mmol, 2.0 equiv) was added portion wise, and the resulting mixture was heated at 50 °C overnight. The reaction mixture was quenched with aqueous hydrochloric acid at 0 °C and was stirred at 0 °C for 45 mins. The aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with a 10% solution of LiCl and brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (4:1 hexanes:EtOAc) to obtain **S18** as a colorless oil (382 mg, 2.98 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.24 (br s, 1H), 3.34 (dd, 1H, J = 9.5 Hz, J = 8.1 Hz), 3.52 (dd, 1H, J = 9.5 Hz, J = 3.4 Hz), 4.04 (dt, 1H, J = 5.6 Hz, J = 1.5 Hz), 4.31-4.35 (m, 1H), 5.20 (dt, 2H, J = 10.5 Hz, J = 1.7 Hz), 5.28 (dq, 1H, J = 17.1 Hz, J = 1.7 Hz), 5.37 (dt, 1H, J = 17.4 Hz, J = 1.7 Hz), 5.80-5.95 (m, 2H).

3,6-dihydro-2H-pyran-3-ol (**10**)[5]: Following general procedure A and starting from **S18** (375 mg, 2.93 mmol, 1.0 equiv), **10** was obtained as a yellow oil (112 mg, 1.12 mmol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.23 (br s, 1H), 3.73 (dd, 1H, J = 11.7 Hz, J = 2.4 Hz), 3.83 (dd, 1H, J = 12.9 Hz, J = 2.6 Hz), 3.96 (br s, 1H), 4.04 (dt, 1H, J = 16.8 Hz, J = 1.8 Hz), 4.15 (dd, 1H, J = 16.9 Hz, J = 1.6 Hz), 5.90 (dt, 1H, J = 10.7 Hz, J = 2.6 Hz), 5.97 (dt, 1H, J = 10.2 Hz, J = 2.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 62.9, 65.6, 71.1, 127.0, 130.2. The NMR data were in accordance with literature values.



Scheme S4. Synthesis of compound 15.

tert-butyl 3-oxo-3,6-dihydropyridine-1(2H)-carboxylate (**S17**): **S4** (1.0 g, 5.02 mmol, 1.0 equiv). was dissolved in anhydrous DCM (20 mL, 0.25 M) and Dess-Martin periodinane (2.4 g, 5.77 mmol, 1.2 equiv) was added. The resulting mixture was stirred at room temperature overnight. The reaction mixture was washed with a 20% solution of $Na_2S_2O_3$, saturated solutions of $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography on silica gel (2:1 to 1:1 hexanes:EtOAc) to afford **S17** as an orange oil (555 mg, 2.81 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 4.11 (s, 2H), 4.23 (t, 2H, J = 2.4 Hz), 6.17 (dt, 1H, J = 10.3 Hz, J = 2.2 Hz), 7.02 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 28.6, 42.9, 81.4, 127.9, 147.7, 154.5, 193.7.

tert-butyl 3-hydroxy-3,6-dihydropyridine-1(2H)-carboxylate-3-*d* (**S18**): In an oven dried round-bottom flask under argon, **S17** (400 mg, 2.03 mmol, 1.0 equiv) and $CeCl_3.7H_2O$ (756 mg, 2.03 mmol, 1.0 equiv) were dissolved in anhydrous methanol (20 mL, 0.1 M) and cooled to 0 °C. $NaBD_4$ (212 mg, 5.07 mmol, 2.5 equiv) was added portion wise and the resulting mixture was stirred at 0 °C for 2 hours. The reaction mixture was quenched with water and concentrated under vacuum. The residue was diluted in water and extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum to afford **S18** as a colorless oil (345 mg, 1.72 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 3.48-3.57 (m, 2H), 3.79 (d, 1H, J = 18.8 Hz), 3.97 (d, 1H, J = 17.6 Hz), 5.82 (d, 1H, J = 10.1 Hz), 5.90 (d, 1H, J = 10.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 28.7, 43.6 (d, J = 47.2 Hz), 47.9 (d, J = 103.1 Hz), 63.7 (t, J = 22.2 Hz), 80.4, 128.4, 128.5, 155.6.

tert-butyl 3-(pivaloyloxy)-3,6-dihydropyridine-1(2H)-carboxylate-3-*d* (**S19**): **S18** (343 mg, 1.71 mmol, 1.0 equiv) and DMAP (21 mg, 0.17 mmol, 0.1 equiv) were dissolved in anhydrous DCM (5.7 mL, 0.3 M). Pyridine (693 μL, 8.56 mmol, 5.0 equiv) and pivaloyl chloride (295 μL, 2.40 mmol, 1.4 equiv.) were added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water. The aqueous phase was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and

concentrated under vacuum. The crude was purified by chromatography on silica gel (4:1 hexanes:EtOAc) to obtain **S19** as a colorless oil (413 mg, 1.45 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.18 (s, 9H), 1.46 (s, 9H), 3.44 (d, 1H, J = 13.7 Hz), 3.73-3.80 (m, 2H), 4.09-4.15 (m, 1H), 5.84 (d, 1H, J = 9.6 Hz), 5.97 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 26.8, 27.4, 28.7, 39.1, 42.8 (t, J = 142.1 Hz), 60.7, 80.3, 124.7, 130.6, 155.0, 178.4.

1,2,3,6-tetrahydropyridin-3-yl-3-*d* pivalate (**S20**): Following general procedure B and starting from **S19** (413 mg, 1.45 mmol, 1.0 equiv), **S20** was obtained as a brown oil (268 mg, 1.45 mmol, 100%).

1-methyl-1,2,3,6-tetrahydropyridin-3-yl-3-*d* pivalate (**S21**): Following general procedure C, starting from **S20** (268 mg, 1.45 mmol, 1.0 equiv) and formaldehyde (37% in water, 325 μL, 4.36 mmol, 3.0 equiv) and purifying by chromatography on silica gel using 1:3 hexanes:EtOAc as eluent, **S21** was obtained as a colorless oil (124 mg, 0.62 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (s, 9H), 2.35 (s, 3H), 2.46 (d, 1H, J = 11.8 Hz), 2.76 (d, 1H, J = 11.7 Hz), 2.87 (dt, 1H, J = 16.8 Hz, J = 2.6 Hz), 2.98 (dt, 1H, J = 16.8 Hz, J = 2.6 Hz), 5.72 (dt, 1H, J = 10.1 Hz, J = 1.8 Hz), 5.95 (dt, 1H, J = 9.9 Hz, J = 3.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 27.5, 39.0, 45.9, 54.3, 56.6, 67.2 (t, J = 23.1 Hz), 124.4, 130.5, 178.7.

1-methyl-1,2,3,6-tetrahydropyridin-3-*d*-3-ol (**15**): Following general procedure E, starting from **S21** (111 mg, 0.56 mmol, 1.0 equiv), and purifying by chromatography on silica gel using 8:2:0.1 EtOAc:methanol:NH_4OH 28% in water as eluent, **15** was obtained as a colorless oil (36 mg, 0.31 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.37 (s, 3H), 2.51 (d, 1H, J = 11.8 Hz), 2.71 (dt, 1H, J = 16.7 Hz, J = 2.1 Hz), 2.78 (d, 1H, J = 11.8 Hz), 3.15 (dd, 1H, J = 16.7 Hz, J = 4.0 Hz), 4.03 (br s, 1H), 5.82 (dq, 1H, J = 10.1 Hz, J = 2.2 Hz), 5.90 (d, 1H, J = 9.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 45.7, 54.4, 60.2, 64.2 (t, J = 22.2 Hz), 127.9, 128.2; HRMS ($M+H^+$) 115.0973 (calcd for $C_6H_{10}DNOH^+$ 115.0976).

3. LC-MS/MS assay for determining choline TMA-lyase (CutC) activity in *E. coli* MS 200-1

A frozen stock of *E. coli* MS 200-1 was streaked out onto a BHI agar plate inside an anaerobic chamber (Coy Laboratory) and incubated at 37 °C overnight. A single colony was inoculated into 5 mL of liquid BHI medium supplemented with 1 mM choline chloride (to induce expression of the *cut* genes) and grown anaerobically at 37 °C overnight. The starter culture was inoculated (2%) into fresh BHI medium containing 1 mM of d_9-choline. The bacterial culture was distributed among the wells of a 96-well plate (Corning). Inhibitors were dissolved in 50 mM potassium phosphate buffer, pH 7.4, and aliquoted into the wells so that the final volume of each well was 200 μL. All experiments were performed in triplicate. Plates were covered with aluminum seals (VWR 60941-126) and incubated at 37 °C for 3.5 h inside an anaerobic chamber (Coy Laboratory). An aliquot of each culture was diluted (up to 3750-fold) in a mixture composed of 95% acetonitrile and 5% of 100 mM ammonium formate buffer (final formic acid concentration of 0.02%) and analyzed by LC-MS/MS for d_9-TMA and d_9-choline. One technical replicate was performed.

Concentrations of d_9-TMA and d_9-choline in each sample were determined by comparing the peak integrations to standard curves of d_9-TMA and d_9-choline (0.1, 0.3, 0.5, 0.7 and 1.0 mM). The standard curves were generated for every plate. The calculated [d_9-

TMA] values were plotted against the logarithm of inhibitor concentration in each sample and fit to a nonlinear regression model in Graphpad Prism to obtain IC_{50} values.[6]

4. Overexpression and purification of CutC and CutD

Plasmids encoding the wild-type *D. desulfuricans* G20 *cutC* gene with an 18- ora 52-residue, N-terminal truncation of the resulting protein sequence were constructed previously.[6–8] The N-terminal truncations remove a putative microcompartment localizing sequence. These constructs incorporate an N-terminal hexahistidine tag and thrombin cleavage site. They were previously found to have similar activity to wild-type protein, but are more soluble and less prone to aggregation.[7] CutC was purified in 50 mM potassium phosphate, pH 8.0, 50 mM KCl, and 10% glycerol according to the previously described protocol,[7,8] but was not concentrated after dialysis since the process of concentration contributed to aggregation. The 18-residue truncated construct was used for crystallization and the 52-residue construct was used for all enzyme assays. The 52-residue construct was rendered anoxic by sparging with argon prior to its use in EPR and kinetics assays. A NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific) was used to measure the absorbance at 280 nm of CutC variants (ε = 128 870 M^{-1} cm^{-1}). The extinction coefficients were obtained using the ExPASy protparam tool (http://www.expasy.org/tools/protparam.html).

CutD was also purified as previously described[7,8] except for two modifications to the protocol. Upon induction with 500 µM IPTG (Teknova), cells expressing CutD were sparged with nitrogen for 30 mins, sealed and incubated at 15 °C for 16 to 18 h without shaking. As previously described, cells were subsequently harvested by centrifugation, brought inside a Coy anaerobic chamber (containing an atmosphere of 98% N_2/2% H_2 and kept at 4 °C), and re-suspended in 45-50 mL of anoxic lysis buffer. However, instead of removing the re-suspension from the anaerobic chamber and lysing cells with a cell disruptor, the cells were disrupted using a sonicator inside the oxygen-free Coy chamber. Purification with Ni-NTA resin, reconstitution of iron-sulfur clusters, and dialysis were performed as previously described.[7,8] CutD was used for *in vitro* EPR and kinetics assays. The concentration of CutD was determined according to the method of Bradford,[9] using bovine serum albumin (New England Biolabs) as a standard and dye reagent concentrate from BioRad. All proteins were judged to be of high purity by denaturing polyacrylamide gel electrophoresis.

5. Glycyl radical quantification by electron paramagnetic resonance (EPR) spectroscopy

CutC-52aa was prepared for EPR spectroscopy as follows. Assays were set up in an anaerobic chamber (MBraun) kept at 20 °C in 1.5 mL polypropylene Eppendorf tubes. All assay concentrations are final concentrations. CutD (40 µM) and sodium dithionite (150 µM) were incubated together in buffer (50 mM potassium phosphate, pH 8.0, 50 mM KCl) for 20 min. CutC was rendered anoxic by sparging the headspace of the solution with argon for 30 min. Anoxic CutC (20 μM total monomer concentration) and SAM (200 µM) were added to the activating enzyme mixture and incubated for 1 h. The entire volume of 220 μL for each sample was used for analysis. EPR assays were performed in triplicate.

Perpendicular mode X-band EPR spectra were recorded on a Bruker ElexSysE500 EPR instrument fitted with a quartz dewar (Wilmad Lab-Glass) for measurements at 77 K. All samples were loaded into EPR tubes with 4 mm outer diameter and 8" length (Wilmad Lab-Glass, 734-LPV-7), sealed, and frozen in liquid N_2. Data acquisition was performed with Xepr software (Bruker). The magnetic field was calibrated with a standard sample of α,γ-bisdiphenylene-β-phenylallyl (BDPA), g = 2.0026 (Bruker). The experimental spectra for the glycyl radical were modeled with EasySpin (Version 5.0.22)[10] or Matlab (MathWorks) to obtain g values, hyperfine coupling constants, and line widths. Spin concentration measurements were performed by numerically calculating the double integral of the simulated spectra and comparing the area with that of a $K_2(SO_3)_2NO$ standard. This standard was freshly prepared before each set of EPR measurements by dissolving solid $K_2(SO_3)_2NO$ under anaerobic conditions in anoxic 0.5 M $KHCO_3$ and diluting to a final concentration of 0.3–0.5 mM. To account for any decomposition during dissolution, the concentration was measured at 248 nm (ε = 1690 $M^{-1}cm^{-1}$) using a NanoDrop 2000 UV-Vis Spectrophotometer. For glycyl radical-containing samples and the $K_2(SO_3)_2NO$ standard, EPR spectra represent the average of 3 scans and were recorded under the following conditions: temperature, 77 K; center field, 3370 Gauss; sweep width, 200 Gauss; microwave power, 20 μW; microwave frequency, 9.45 MHz; modulation amplitude, 0.4 mT; modulation frequency, 100 kHz; time constant, 20.48 ms; conversion time, 20.48 ms; scan time, 20.97 s; receiver gain, 60 dB (for enzymatic assays) or 30 dB (for standards). Normalization for the difference in receiver gain was performed by the spectrometer. EPR experiments showed that CutD activated 18 ± 1% of CutC monomers, consistent with the results of previous studies.[8]

6. *In vitro* assay for measuring CutC inhibition

The activity of CutC was coupled to the reduction of acetaldehyde by NADH-dependent yeast alcohol dehydrogenase (YADH). Assays were conducted in a buffer of 50 mM potassium phosphate, pH 8.0, 50 mM KCl and were set up in an anaerobic chamber (MBraun) kept at 20 °C in 1.5 mL polypropylene Eppendorf tubes. CutD (40 μM) and sodium dithionite (150 μM) were incubated for 20 min prior to the addition of 200 μM of *S*-(5'-adenosyl)-L-methionine (SAM, purchased from Sigma-Aldrich as a *p*-toluenesulfonate salt) and 20 μM of CutC monomer in a total volume of 50 μL. Glycyl radical formation was carried out for 1 h, after which the activated CutC mixture was immediately diluted 20-fold into phosphate buffer. An aliquot of the diluted mixture was added to a master mix containing NADH (200 μM) and YADH (0.4 μM) such that the final concentration of CutC monomer was 5 nM (after addition of inhibitor/buffer and choline). The master mix was distributed to the wells of a 96-well plate (180 μL per well), to which was added either inhibitor or buffer (10 μL per well), followed by choline (10 μL per well). The final concentration of choline was 200 μM (~K_M value). All experiments were performed in triplicate.

Following the addition of choline, the absorbance of NADH at 340 nm was immediately recorded, with measurements taken every 20 s for 10 min. Initial rates were calculated from absorbance measurements that decreased linearly. Pathlengths were corrected to 1 cm and absorbance values were converted to concentrations assuming ε_{340} = 6,220 $M^{-1}cm^{-1}$ for NADH. The rate from assays containing no substrate was subtracted from assays containing substrate to account for background activity. Assays were performed in triplicate. All of the data was fit to the Michaelis–Menten equation simultaneously using nonlinear regression in Graphpad Prism. Initial rates at each inhibitor concentration were normalized to control samples without

inhibitor. Normalized activity values were plotted against the logarithm of inhibitor concentration and fit to a nonlinear regression model in Graphpad Prism to obtain IC_{50} values.[6]

7. Determining choline TMA-lyase activity in *cut* gene cluster-harboring bacteria

Frozen stocks of *Escherichia coli* MS 69-1, *Proteus mirabilis* ATCC 29906 and *Klebsiella* sp. MS 92-3 were streaked out onto BHI agar plates inside an anaerobic chamber (Coy Laboratory) and incubated at 37 °C overnight. A single colony of each strain was inoculated into 5 mL of liquid BHI medium supplemented with 1 mM choline chloride (to induce expression of the *cut* genes) and was grown anaerobically at 37 °C overnight. The same conditions were used for culturing *C. sporogenes* ATCC 15579 except that an RCM agar plate and RCM liquid broth were used. For each strain, BHI (or RCM) medium containing 1 mM of choline chloride-(trimethyl-d_9) was inoculated with 2% of the saturated bacterial starter culture. Cultures were distributed among the wells of a 96-well plate (Corning) and inhibitors, dissolved in 50 mM potassium phosphate buffer, pH 7.4, were added to the samples in the wells such that the final volume of each well was 200 µL. All experiments were performed in triplicate. Plates were covered with aluminum seals (VWR) and incubated at 37 °C inside a vinyl anaerobic chamber (Coy Laboratory) for 3.5 hours. An aliquot of each culture was diluted (up to 3750-fold) in a mixture composed of 95% acetonitrile and 5% of 100 mM ammonium formate buffer (with a final formic acid concentration of 0.02%) and analyzed by LC-MS/MS for d_9-TMA and d_9-choline.[6] One technical replicate was performed.

8. Determining choline TMA-lyase activity in a fecal suspension *ex vivo*

A frozen fecal sample from a healthy individual was diluted 1:10 (wt/v) in sterile anaerobic PBS. This sample was collected by Federico E. Rey (University of Wisconsin at Madison). The resulting suspension was vortexed for 3 mins and centrifuged at 500 rpm at 4 °C for 5 mins. The supernatant was resuspended 1:100 (v/v) in BHI medium containing 1 mM d_9-choline. A 190 µL aliquot of the resulting supernatant was transferred to a 96-well plate containing 10 µL of different concentrations of inhibitor. All experiments were performed in triplicate. The mixtures were incubated for 18 h at 37 °C. An aliquot of each culture was diluted 3750-fold in a mixture composed of 95% acetonitrile and 5% of 100 mM ammonium formate buffer (with a final formic acid concentration of 0.02%) and analyzed by LC-MS/MS for d_9-TMA and d_9-choline. one technical replicate was performed.

9. Protein purification, crystallization, and structure determination of CutC bound to (*S*)-1- methyl-1,2,3,4-tetrahydropyridin-3-ol ((*S*)-**6**)

Protein expression, purification, and crystallization conditions for CutC carrying an N-terminal 52-amino acid truncation and a hexahistidine tag were identified and described previously.[8] To obtain the co-crystal structure of CutC with (*S*)-**6,** Δ52aa-CutC (100 µM, 8.2 mg mL^{-1}) in buffer (50 mM potassium phosphate, pH 8.0, 50 mM KCl, and 10% (v/v) glycerol) was pre-incubated with (*S*)-**6** (final concentration of 10 mM) for 1 h at 4 °C. Protein and

precipitant (1.3 M sodium malonate pH 7.6) were mixed in a 1:1 ratio in a final volume of 2 µL and incubated aerobically for 3-6 days in a sitting drop set up at room temperature. Crystals were cryoprotected in a stabilization solution containing 3.2 M sodium malonate pH 7.6 supplemented with 50 mM (*S*)-**6** and cryo-cooled in liquid N_2.

A full data set was collected at the Northeastern Collaborative Access Team (NE-CAT) beamline at the Advanced Photon Source (APS) at a wavelength of 0.9795 Å. Diffraction data were indexed, integrated, and scaled in HKL2000.[11] Crystals diffracted to 2.28-Å resolution in space group P 42 21 2 with cell edges a = b =228.5 Å, c = 78.8 Å. The structure was solved by molecular replacement in the Phenix implementation of Phaser,[12] using as a search model a wild type monomer from model PDB 5FAU with all ligands removed.[8] The asymmetric unit contains two protomers. Refinement was performed in phenix.refine[13] using NCS restraints. Following each round of refinement, manual building was performed in Coot.[14] The final model contains residues 53-846 (chain A and B) out of 846 residues. In addition, portion of the thrombin recognition sequence can be seen at the N-terminus for both chains. Water molecules were placed manually into positive difference density in Coot and refined. Additionally, (*S*)-**6** was modeled into positive difference density toward the final rounds of refinement, and refined using a parameter file that was made in Phenix eLBOW[13] by imputing information about the stereochemistry that was determined experimentally (See section 2). (*S*)-**6** geometry was optimized using the eLBOW AM1 QM method, which calculates the most stable conformation based on semi-empirical calculations.[15] All additional parameters within eLBOW were kept as default. The restraint file generated by eLBOW was then utilized during final rounds of refinement after (*S*)-**6** was manually built into the CutC active site. The structure of CutC bound to (*S*)-**6** and the position of (*S*)-**6** was verified with composite omit maps generated by Phenix.[13] Data were deposited on the worldwide Protein Data Bank under accession code 6VUE.

Table S1. Data Collection and refinement statistics

	CutC w/ (*S*)-1-methyl-1,2,3,4-tetrahydropyridin-3-ol
Wavelength (Å)	0.979
Space group	P 4_2 2_1 2
Resolution (Å)[a]	50.00 – 2.28 (2.36 – 2.28)
Completeness (%)	99.4 (94.4)
<I/σI>	16.9 (3.3)
R_{sym}	0.177 (0.608)
R_{pim}[b]	0.036 (0.194)
Unique reflections	94805
Redundancy	21.8 (7.8)
$CC_{1/2}$	0.997 (0.797)
Refinement	
Reflections used in refinement	93178 (8571)
R_{work}/ R_{free}	0.1637 / 0.1973
Protein chains in asymmetric unit	2
No of atoms	
Protein	12578
Inhibitor	16
Solvent	476
RMSD (bonds)	0.010
RMSD (angles)	1.30
Ramachandran favored (%)	97.75
Ramachandran allowed (%)	2.00
Ramachandran outliers[c] (%)	0.25
Rotamer outliers (%)	0.75
B-factors (Å²)	
Average	24.93
Protein	24.98
Inhibitor	22.07
Solvent	23.81

[a] Values in parenthesis are for the highest resolution shell.
[b] R_{pim} is reported due to the high redundancy of this dataset.
[c]Outliers are Val819B, Ser691B, Ser691A, Pro47B

10. References

(1) Schaus, S. E.; Brandes, B. D.; Larrow, J. F.; Tokunaga, M.; Hansen, K. B.; Gould, A. E.; Furrow, M. E.; Jacobsen, E. N. Highly Selective Hydrolytic Kinetic Resolution of Terminal Epoxides Catalyzed by Chiral (Salen)Co ^{III} Complexes. Practical Synthesis of Enantioenriched Terminal Epoxides and 1,2-Diols. *J. Am. Chem. Soc.* **2002**, *124* (7), 1307–1315. https://doi.org/10.1021/ja016737l.

(2) Lubbers, D. J. Synthetic and Computational Efforts toward the Understanding and Development of Novobiocin-Derived Inhibitors of Hsp90, B.S., Saint Mary's College, Notre Dame, 2005.
(3) Sançon, J.; Sweeney, J. B. Probing the Effect of Allylic Substitution on Cyclic Ammonium Ylid Rearrangements. *Synlett* **2010**, *2010* (04), 664–666. https://doi.org/10.1055/s-0029-1219348.
(4) Grishina, G. V.; Borisenko, A. A.; Veselov, I. S.; Petrenko, A. M. Synthesis and Stereochemistry of 3-Hydroxy-1,2,3,6-Tetrahydropyridines. *Russ. J. Org. Chem.* **2005**, *41* (2), 272–278. https://doi.org/10.1007/s11178-005-0156-4.
(5) Ge, Y.; Cui, X.-Y.; Tan, S. M.; Jiang, H.; Ren, J.; Lee, N.; Lee, R.; Tan, C.-H. Guanidine-Copper Complex Catalyzed Allylic Borylation for the Enantioconvergent Synthesis of Tertiary Cyclic Allylboronates. *Angew. Chem. Int. Ed.* **2019**, *58* (8), 2382–2386. https://doi.org/10.1002/anie.201813490.
(6) Orman, M.; Bodea, S.; Funk, M. A.; Campo, A. M.; Bollenbach, M.; Drennan, C. L.; Balskus, E. P. Structure-Guided Identification of a Small Molecule That Inhibits Anaerobic Choline Metabolism by Human Gut Bacteria. *J. Am. Chem. Soc.* **2019**, *141* (1), 33–37. https://doi.org/10.1021/jacs.8b04883.
(7) Craciun, S.; Marks, J. A.; Balskus, E. P. Characterization of Choline Trimethylamine-Lyase Expands the Chemistry of Glycyl Radical Enzymes. *ACS Chem. Biol.* **2014**, *9* (7), 1408–1413. https://doi.org/10.1021/cb500113p.
(8) Bodea, S.; Funk, M. A.; Balskus, E. P.; Drennan, C. L. Molecular Basis of C–N Bond Cleavage by the Glycyl Radical Enzyme Choline Trimethylamine-Lyase. *Cell Chemical Biology* **2016**, *23* (10), 1206–1216. https://doi.org/10.1016/j.chembiol.2016.07.020.
(9) Stoscheck, C. M. [6] Quantitation of Protein. In *Methods in Enzymology*; Deutscher, M. P., Ed.; Guide to Protein Purification; Academic Press, 1990; Vol. 182, pp 50–68. https://doi.org/10.1016/0076-6879(90)82008-P.
(10) Stoll, S.; Schweiger, A. EasySpin, a Comprehensive Software Package for Spectral Simulation and Analysis in EPR. *J. Magn. Reson.* **2006**, *178* (1), 42–55. https://doi.org/10.1016/j.jmr.2005.08.013.
(11) Otwinowski, Z.; Minor, W. [20] Processing of X-Ray Diffraction Data Collected in Oscillation Mode. In *Methods in Enzymology*; Macromolecular Crystallography Part A; Academic Press, 1997; Vol. 276, pp 307–326. https://doi.org/10.1016/S0076-6879(97)76066-X.
(12) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser Crystallographic Software. *J. Appl. Crystallogr.* **2007**, *40* (Pt 4), 658–674. https://doi.org/10.1107/S0021889807021206.
(13) Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; et al. PHENIX: A Comprehensive Python-Based System for Macromolecular Structure Solution. *Acta Crystallogr. D. Biol. Crystallogr.* **2010**, *66* (Pt 2), 213–221. https://doi.org/10.1107/S0907444909052925.
(14) Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and Development of Coot. *Acta Crystallogr. D. Biol. Crystallogr.* **2010**, *66* (Pt 4), 486–501. https://doi.org/10.1107/S0907444910007493.
(15) Moriarty, N. W.; Grosse-Kunstleve, R. W.; Adams, P. D. Electronic Ligand Builder and Optimization Workbench (ELBOW): A Tool for Ligand Coordinate and Restraint Generation. *Acta Cryst. D.* **2009**, *65* (10), 1074–1080. https://doi.org/10.1107/S0907444909029436.