Supporting Information for:

Structure-Activity Relationship of Penem Antibiotic Sidechains for Use Against Mycobacteria Reveal Highly Active Compounds

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General Methods and Instrumentation

All reagents and starting materials were purchased and used without further purification unless otherwise indicated. Anhydrous solvents were dried using an LC Technology Solutions (Salisbury, MA) SPBT-1 solvent purification system. Silica gel chromatography was performed using Sorbtech Silica Gel (60 Å, 40-75mm particle size) or RediSep Rf disposable flash columns (60 Å, 40-63 μm irregular particle size) on a Teledyne ISCO (Lincoln, NE) CombiFlash EZ Prep. Preparative HPLC was carried out on the same instrument outfitted with a Phenomenex (Torrance, CA) Luna 10μ C18(2) 100 Å column (250 × 21.20 mm ID). All ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker (Billerica, MA) UltraShield 400 MHz or 300 MHz Avance spectrometer. The Johns Hopkins Chemistry Department Mass Spectrometry Facility determined exact masses by high resolution ultra-performance liquid chromatography–electrospray ionization mass spectrometry (UPLC-ESIMS) using a Waters (Milford, MA) Acquity/Xevo-G2.

General procedure for allyl carbamate protection of nitrogen:

A round bottom flask was charged with the amino alcohol (1 equiv.) and a 1:1 mixture of water: tetrahydrofuran (THF) (1 M). The mixture was cooled to 0 ˚C and allyl chloroformate (1.2 equiv.) was added dropwise while stirring. Upon completion of addition, the pH of the mixture was adjusted to 10 with 4N aqueous NaOH. The reaction was allowed to proceed for 1 h. Aqueous brine solution was then added to the mixture and the organics were extracted three times with ethyl acetate (EtOAc). Organics were dried with anhydrous sodium sulfate and removed *in vacuo*. Product was then purified from the organic mixture using silica gel flash chromatography with an EtOAc/Hexanes mobile phase.

General procedure for mesylation of an alcohol:

Round bottom flask was charged with allyl carbamate-protected amino alcohol (1 equiv.). The oil was dissolved in dichloromethane (DCM) (0.2 M) and cooled to 0 ˚C. Methanesulfonyl chloride (1.2 equiv.) was then added to the flask dropwise while stirring, followed by the dropwise addition of triethylamine (2 equiv.). The reaction was allowed to proceed for 1 h or until completion was indicated by thin layer chromatography. The reaction was quenched with water and the organics were extracted with three times with DCM. The organics were dried with anhydrous sodium sulfate and the solvent was removed *in vacuo*. The resulting oil was then purified using silica gel flash chromatography with an EtOAc/Hexanes mobile phase.

General procedure for installation of a thioester:

The methanesulfonate (1 equiv.) added to a round bottom flask and dissolved in a 1:1 mixture of EtOAc to dimethylformamide (0.5 M). Potassium thioacetate (1.3 equiv.) was added to the solution in one portion. The flask was fitted with a reflux condenser and mixture was then stirred at 60 ˚C for 16 h. The mixture was poured into EtOAc and the salts were extracted with an aqueous brine solution. The aqueous layer was then back extracted three times with EtOAc. The organics were combined, dried with anhydrous sodium sulfate, and solvent was removed *in*

vacuo. The resulting oil was then purified using silica gel flash chromatography with an EtOAc/Hexanes mobile phase.

General procedure for methyl ester formation:

The carboxylic acid (1 equiv.) was added to a round bottom flask and dissolved in methanol (0.6 M). A drop of conc. H_2SO_4 was added to the mixture. The flask was then fit with a reflux condenser and heated to 60 ˚C for 16 h. The reaction was allowed to cool to room temperature before the pH was adjust to 5 using 5% aqueous NaOH solution. The mixture was poured into EtOAc and the organic layer was washed with an aqueous brine solution. Organic layer was then dried with anhydrous sodium sulfate and concentrated *in vacuo* to afford the product as an oil.

General procedure for methyl ester reduction:

The methyl ester (1 equiv.) was dissolved in methanol (0.4 M) and cooled to 0 ˚C. To the mixture $NaBH_4$ (2 equiv.) was added portion-wise. The reaction was stirred for 4 h before being quenched with 5% H_2SO_4 solution. EtOAc was added to the mixture and the organic layer was separated, dried with anhydrous sodium sulfate, and concentrated *in vacuo*. The oil was then purified used silica gel flash chromatography with an EtOAc/Hexanes mobile phase.

General procedure for sidechain thiol deprotection and β-addition-elimination into penem core:

The thioester protected side chain (1.5 equiv.) was dissolved in MeOH (0.4 M) and cooled to 0 ˚C. While stirring, a sodium methoxide 5.4 M solution (9 equiv.) was added dropwise. The reaction was allowed to proceed for 2 h before being quenched with a dropwise addition of 6 N HCl until a pH of 4 was reached. The mixture was diluted with degassed EtOAc and washed with brine solution. The resulting organic layer was concentrated *in vacuo*. The resulting oil was dissolved in CH_3CN (0.3 M) and cooled to 0 ˚C before adding diisopropylethylamine (3 equiv.) dropwise to the flask. The mixture was added dropwise to a stirring mixture of the penem core **5** (1 equiv.) in CH_3CN (0.2 M) at 0 ˚C and allowed to react for 3 h. The mixture was then diluted into EtOAc and washed with brine. The aqueous layer was back extracted three times with EtOAc and the resulting organics were dried with anhydrous sodium sulfate. The solvent was removed *in vacuo* and the resulting oil was then purified using silica gel flash chromatography with an EtOAc/Hexanes mobile phase.

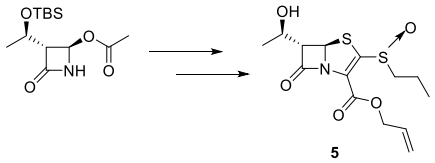
General procedure for a one-pot allyl carbamate and allyl ester deprotection:

The penem protected with an allyl ester and an allyl carbamate (1 equiv.), barbituric acid (1.2 equiv.), and sodium benzenesulfinate (1.2 equiv.) were dissolved in THF (0.05 M). The mixture was degassed by bubbling of nitrogen gas through the mixture for 15 min. Palladium-tetrakis(triphenylphosphine) (0.1 equiv.) was then added to the mixture under nitrogen atmosphere and the reaction was allowed to proceed for 3 h. The solvent was then removed from the reaction *in vacuo,* and the resulting oil was partitioned into EtOAc and water. The organic layer was back extracted twice with water and the aqueous layers were combined. The

mixture was then purified by HPLC using a C18 stationary phase and water/ACN + 0.1% TFA mobile phase. The product fraction was then lyophilized to yield the powder form of the final penem antibiotic.

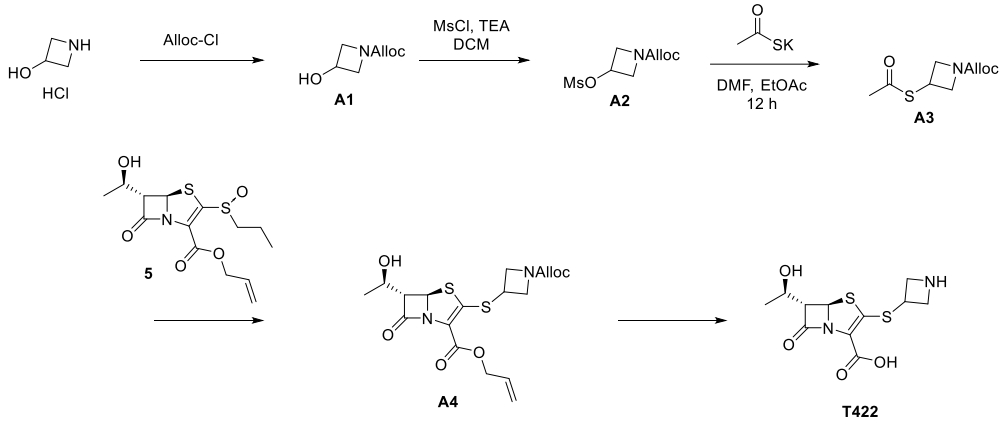
General procedure for reductive amination:

The deprotected penem bearing a secondary amine (1 equiv.) was dissolved in a 3:1 water containing 0.1% formic acid to isopropyl alcohol (0.3 M). Either acetyl aldehyde (3 equiv.) or 4-oxotetrahydropyran (3 equiv.) was added to the mixture at 0 ˚C and let equilibrate for 5 min. $NaBH(OAc)_3$ (3 equiv.) was then added in one portion and the reaction was allowed to proceed for 16 h. Reaction progress was checked by UPLC-HRMS and upon completion, the product was purified using HPLC using a C18 stationary phase and water/ACN + 0.1% TFA mobile phase. The product fraction was then lyophilized to yield the powder form of the final penem antibiotic.



Allyl (5*S*,6*R*)-6-((R)-1-hydroxyethyl)-7-oxo-3-(propylsulfinyl)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (5).

Multistep synthesis of compound **5** was performed as previously described.[1]



Allyl 3-hydroxyazetidine-1-carboxylate (A1):

General procedure for allyl carbamate formation of an amino alcohol was used to convert 3-hydroxyazetidine hydrochloride to **A1** (2.5 g, 96%). **A1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.91 (ddt, *J* = 5.6, 10.4, 17.2 Hz, 1H), 5.29 (dq, *J* = 1.6, 17.2 Hz, 1H), 5.21 (dq, *J* = 1.3, 10.4 Hz, 1H), 4.63 (dddd, *J* = 4.4, 6.6, 10.9, 10.9 Hz, 1H), 4.55 (ddd, *J* = 1.4, 1.5, 5.6 Hz, 2H), 4.23 (ddd, *J* = 1.0, 6.7, 9.7 Hz, 2H), 3.88 (ddd, *J* = 1.2, 4.4, 9.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.6, 132.6, 117.7, 65.8, 61.4, 59.0. HRMS (UPLC/MS), $C_7H_{11}NO_3$ [M+H] calculated: 158.0812; found: 158.0822.

Allyl 3-methylsulfonyloxyazetidine-1-carboxylate (A2):

General procedure for mesylation of an alcohol was performed to convert **A1** to **A2** (3.3 g, 86%). **A2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.91 (ddt, *J* = 5.7, 10.4, 17.2 Hz, 1H), 5.27 (m, 4H), 4.57 (dt, *J* = 1.5, 5.6 Hz, 2H), 4.36 (ddd, *J* = 1.1, 6.6, 10.3 Hz, 2H), 4.18 (ddd, *J* = 1.2, 4.2, 10.6 Hz, 2H), 3.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.0, 132.5, 117.8, 67.5, 65.9, 56.6, 38.2. HRMS (UPLC/MS), $C_8H_{13}NO_5S$ [M+H] calculated: 236.0587; found: 236.0592.

Allyl 3-acetylthioazetidine-1-carboxylate (A3):

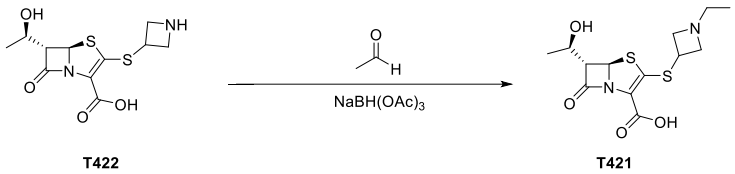
General procedure for installation of a thioester was used to convert **A2** to **A3** (1.8 g, 65%). **A3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.90 (ddt, *J* = 5.2, 11.0, 17.1, 1H), 5.23 (ddd, *J* = 0.5, 1.52, 17.2 Hz, 1H), 5.21 (dd, *J* = 0.8, 10.4 Hz, 1H), 4.55 (d, *J* = 5.6 Hz, 2H), 4.44 (t, *J* = 8.7 Hz, 2H), 4.21 (tt, *J* = 5.5, 8.1 Hz, 1H), 3.89 (dd, *J* = 5.6, 9.4, 2H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 194.2, 155.6, 132.7, 117.4, 65.4, 55.9, 30.8, 30.2. HRMS (UPLC/MS), $C_9H_{13}NO_3S$ [M+H] calculated: 216.0689; found: 216.0683.

Allyl (5*R*,6*S*)-3-((1-((allyloxy)carbonyl)azetidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A4):

General procedure for sidechain thiol deprotection and β-addition-elimination into the penem core was used to incorporate sidechain **A3** into the penem core, **A4** (815 mg, 61%). **A4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.93 (m, 2H), 5.68 (d, *J* = 2.0 Hz, 1H), 5.42 (dq, *J* = 1.6, 22.9 Hz, 1H), 5.24 (m, 1H), 5.26 (m, 2H), 4.73 (dddABq, *J* = 0.6, 2.2, 7.4, 17.9 Hz, 2H), 4.56 (dt, *J* = 2.1, 7.5 Hz, 2H), 4.40 (ddd, *J* = 9.9, 12.0, 22.4 Hz, 2H), 4.24 (quin, *J* = 8.7 Hz, 1H), 4.05 (m, 4H), 3.73 (dd, *J* = 2.0, 9.1 Hz, 1H), 1.30 (d, *J* = 8.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.2, 159.6, 155.8, 152.0, 132.5, 131.6, 118.6, 118.0, 117.8, 71.5, 66.0, 65.8, 65.5, 65.1, 35.3, 21.8. HRMS (UPLC/MS), $C_{18}H_{22}N_2O_6S_2$ [M+H] calculated: 427.0992; found: 427.1000.

(5*R*,6*S*)-3-(azetidin-3-ylthio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T422):

The general procedure for a one-pot allyl carbamate and allyl ester deprotection was used to convert **A4** to **T422** (97 mg, 51%). **T422:** ^{1}H NMR (400 MHz, D_2O) δ = 5.75 (s, 1H), 4.56 (m, 4H), 4.22 (m, 1H), 4.15 (m, 3H), 3.98 (d, *J* = 5.5 Hz, 1H), 1.26 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 175.8, 162.7, 117.7, 114.8, 69.9, 64.9, 64.4, 52.2, 52.0, 37.2, 19.9. HRMS (UPLC/MS), $C_{11}H_{14}N_2O_4S_2$ [M+H] calculated: 303.0468; found: 303.0478.

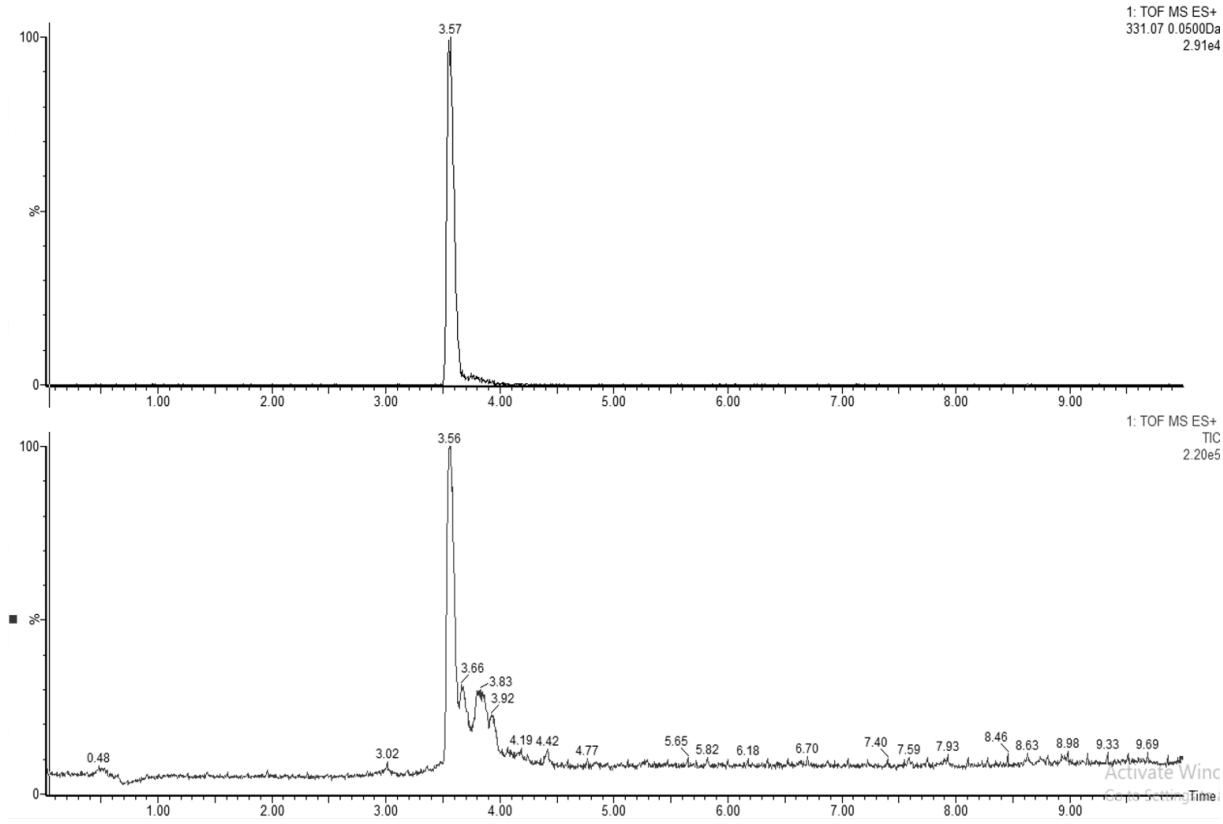


T422 T421

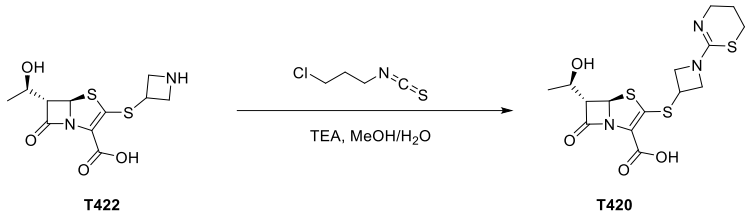
(5*R*,6*S*)-3-((1-ethylazetidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T421):

General procedure for reductive amination was used with acetaldehyde to convert **T422** to **T421** (4 mg, 18%). **T421**: ^{1}H NMR (400 MHz, D_2O) δ = 5.78 (s, 1H), 4.50 (m, 3H), 4.29 (m, 1H), 4.23 (t, *J* = 6.0 Hz, 1H), 4.12 (m, 2H), 4.01 (dd, *J* = 1.2, 5.6 Hz, 1H), 3.30 (m, 2H), 1.27 (d, *J* = 6.4 Hz, 3H), 1.16 (dt, *J* = 2.2, 7.2 Hz, 3H). HRMS (UPLC/MS), $C_{13}H_{18}N_2O_4S_2$ [M+H] calculated: 331.0781; found: 331.0778.

HRMS UPLC-MS:



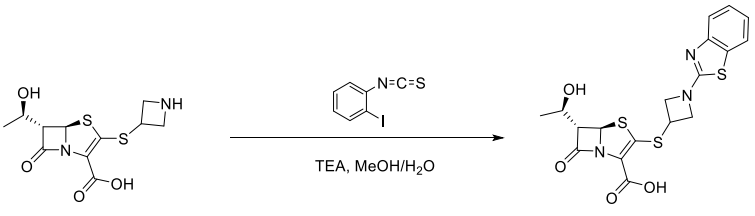
The UPLC-MS traces above indicate the extracted ion chromatogram 331.07 +/- 0.05 = m/z (top) and the total ion chromatogram (bottom) of **T421.**



T422 T420

(5*R*,6*S*)-3-((1-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)azetidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T420):

The deprotected penem **T422** (100 mg, 0.33 mmol) was dissolved in a methanol (220 µL) and water (166 µL) mixture and cooled to 0 ˚C. To the solution 3-chloropropyl isothiocyanate (34 µL, 0.33 mmol) was added followed by triethylamine (92 µL, 0.66 mmol). The reaction was allowed to proceed for 16 h. The mixture was then purified by HPLC using a C18 stationary phase and water/ACN + 0.1% TFA mobile phase. The product fraction was then lyophilized to yield the powder form of the final penem antibiotic **T420** (41 mg, 32%). **T420**: ^{1}H NMR (400 MHz, D_2O) δ = 5.72 (d, *J* = 1.3 Hz, 1H), 4.59 (m, 2H), 4.37 (m, 1H), 4.12 (m, 3H), 3.94 (dd, *J* = 1.3, 5.6 Hz, 1H), 3.38 (t, *J* = 5.6 Hz, 2H), 3.15 (t, *J* = 5.8 Hz, 2H), 2.05 (m, 2H), 1.21 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 175.9, 163.7, 162.4, 154.8, 117.8, 117.2, 114.9, 70.1, 65.1, 64.5, 40.8, 35.2, 26.1, 20.2, 20.0. HRMS (UPLC/MS), $C_{15}H_{19}N_3O_4S_3$ [M+H] calculated: 402.0610; found: 402.0607.

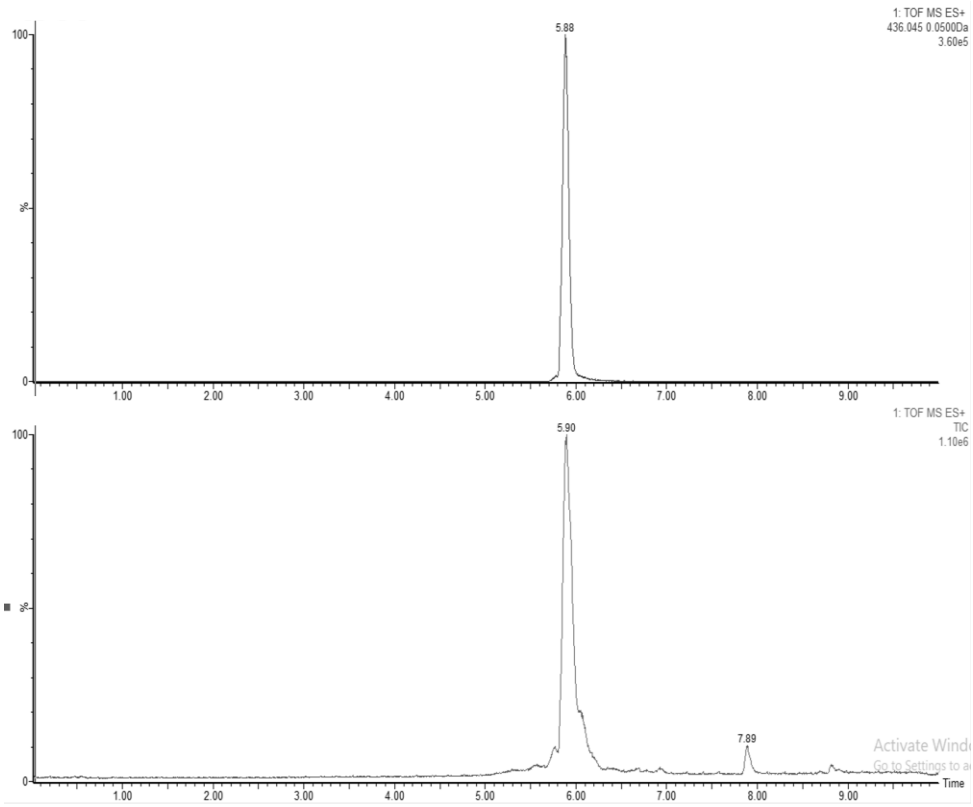


T422 T423

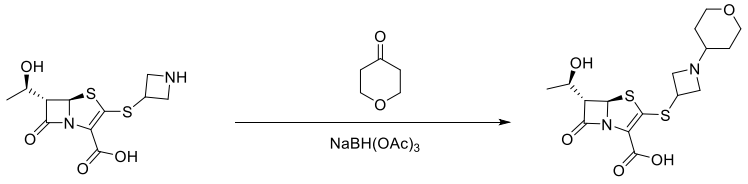
(5*R*,6*S*)-3-((1-(benzo[*d*]thiazol-2-yl)azetidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T423):

The deprotected penem T422 (15 mg, 50 µmol) was dissolved in a methanol (12 µL) and water (12 µL) mixture and cooled to 0 ˚C. To the solution 2-iodophenyl isothiocyanate (14 mg, 55 µmol) was added followed by the addition of triethylamine (14 µL, 100 µmol). The reaction was allowed to proceed for 16 h. The mixture was then purified by HPLC using a C18 stationary phase and water/ACN + 0.1% TFA mobile phase. The product fraction was then lyophilized to yield the powder form of the final penem antibiotic **T423** (4 mg, 19%). **T423:** ^{1}H NMR (400 MHz, DMSO-d6) δ = 7.80 (d, J = 7.5 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.10 (t, J = 6.9 Hz, 1H), 6.56 (s, 1H), 5.69 (m, 1H), 5.19 (d, J = 4.6 Hz, 1H), 4.55 (m, 2H), 4.03 (m, 2H), 3.97 (m, 1H), 3.50 (m, 1H), 1.15 (d, J = 6.2 Hz, 3H). HRMS (UPLC/MS), $C_{18}H_{17}N_3O_4S_3$ [M+H] calculated: 436.0454; found: 436.0453.

HRMS UPLC-MS:



The UPLC-MS traces above indicate the extracted ion chromatogram 436.04 +/- 0.05 = m/z (top) and the total ion chromatogram (bottom) of **T423.**



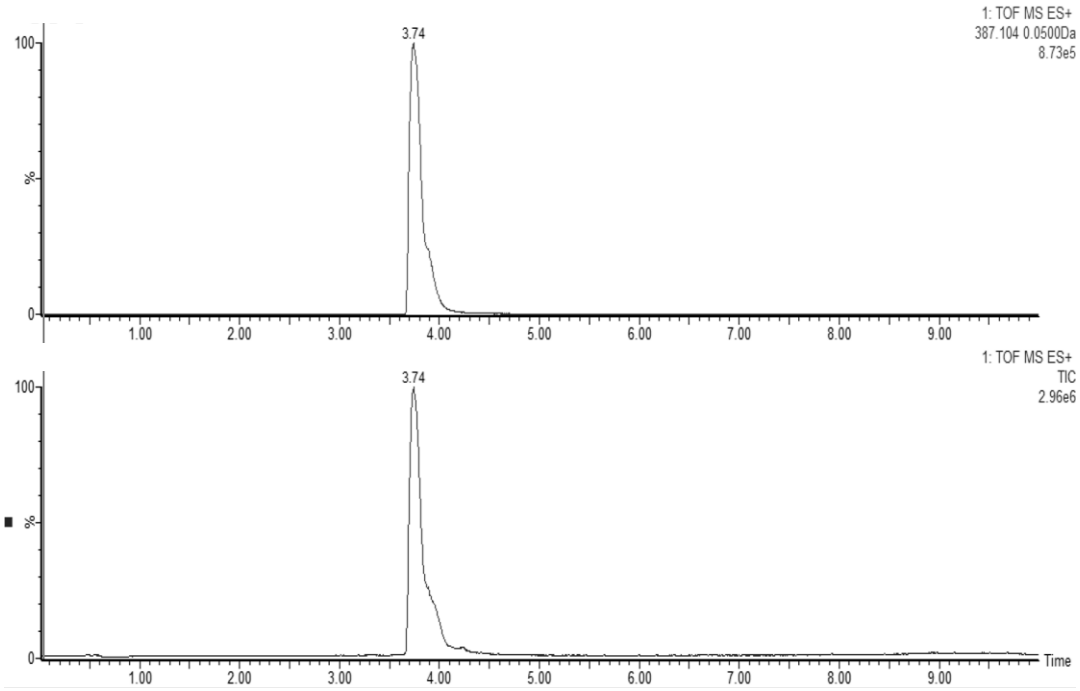
T422 **T430**

(5*R*,6*S*)-6-((R)-1-hydroxyethyl)-7-oxo-3-((1-(tetrahydro-2H-pyran-4-yl)azetidin-3-yl)thio)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T430):

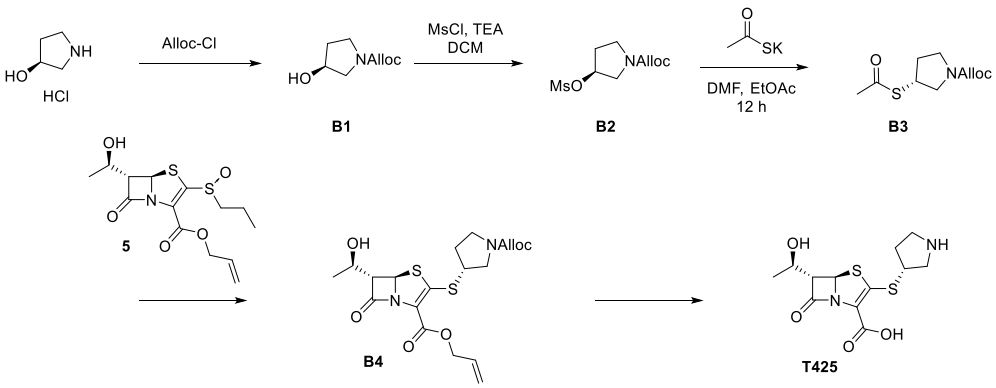
General procedure for reductive amination was used with 4-oxotetrahydropyran as the ketone to convert **T422** to **T430** (13 mg, 59%). **T430**:

^{1}H NMR (400 MHz, D_2O) δ = 5.78 (d, *J* = 1.4 Hz, 1H), 4.70 (m, 1H), 4.62 (m, 1H), 4.52 (quin., *J* = 8.0 Hz, 1H), 4.38 (quin. *J* = 3.8 Hz, 1H), 4.22 (m, 2H), 4.05 (d, *J* = 8.6 Hz, 2H), 4.01 (d, *J* = 5.6 Hz, 1H), 3.55 (m, 1H), 3.43 (t, *J* = 11.2 Hz, 2H), 1.98 (m, 2H), 1.47 (dq, *J* = 4.5, 11.9 Hz, 2H), 1.26 (d, *J* = 6.5 Hz, 3H). HRMS (UPLC/MS), $C_{16}H_{22}N_2O_5S_2$ [M+H] calculated: 387.1043; found: 387.1047.

HRMS UPLC-MS:



The UPLC-MS traces above indicate the extracted ion chromatogram 387.10 +/- 0.05 = m/z (top) and the total ion chromatogram (bottom) of **T430.**



Allyl (*S*)-3-hydroxypyrrolidine-1-carboxylate (B1): General procedure for allyl carbamate formation of an amino alcohol was used to convert (*S*)-3-hydroxypyrrolidine hydrochloride to **B1** (1.9 g, 80%). **B1** (mixture of conformational isomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.94 (ddt, *J* = 5.5, 10.4, 17.2 Hz, 1H), 5.30 (dq, *J* = 1.6, 17.2 Hz, 1H), 5.30 (s, 1H), 5.20 (dq, *J* = 1.4, 10.4 Hz, 1H), 4.59 (dt, *J* = 1.5, 5.5 Hz, 2H), 4.48 (m, 1H), 3.54 (m, 3H), 3.44 (s, 0.7H), 3.41 (s, 0.3H), 2.00 (m, 2H), 1.86 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.0, 154.9, 132.9, 117.1, 70.6, 69.7, 67.0, 65.9, 65.6, 54.4, 53.9, 44.0, 43.7, 41.2, 33.8, 33.3. HRMS (UPLC/MS), $C_8H_{14}NO_3$ [M+H] calculated: 172.0968; found: 172.0975.

Allyl (S)-3-((methylsulfonyl)oxy)pyrrolidine-1-carboxylate (B2): General procedure for mesylation of an alcohol was performed to convert **B1** to **B2** (2.8 g, 52%). **B2** (mixture of conformational isomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.80 (br s, 1H), 5.12 (m, 3H), 4.45 (br s, 2H), 3.49 (m, 4H), 2.92 (s, 3H), 2.13 (br s, 2H), 4.56 (d, *J* = 6.6 Hz, 2H), 3.96 (m, 1H), 3.58 (m, 1H), 3.46 (m, 1H), 3.31 (m, 1H), 2.30 (s, 3H), 1.88 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.8, 153.7, 132.4, 116.6, 79.7, 79.1, 65.1, 51.7, 51.4, 43.2, 42.8, 37.8, 31.8, 30.9. HRMS (UPLC/MS), $C_9H_{15}NO_5S$ [M+H] calculated: 250.0744; found: 250.0757.

Allyl (*R*)-3-(acetylthio)pyrrolidine-1-carboxylate (B3):

General procedure for installation of a thioester was used to convert **B2** to **B3** (1.6 g, 63%). **B3**: (mixture of conformational isomers) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.91 (m, 1H), 5.27 (d, *J* = 22.9 Hz, 1H), 5.17 (d, *J* = 13.9, 1H), 4.56 (d, *J* = 6.6 Hz, 2H), 3.96 (m, 1H), 3.81 (m, 1H), 3.46 (m, 1H), 3.31 (m, 1H), 2.30 (s, 3H), 1.88 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 195.1, 195.0, 154.4, 133.0, 117.3, 117.2, 65.7, 51.6, 51.4, 45.0, 44.6, 41.1, 40.5, 32.0, 30.9, 30.6. HRMS (UPLC/MS), $C_{10}H_{15}NO_3S$ [M+H] calculated: 230.0845; found: 230.0849.

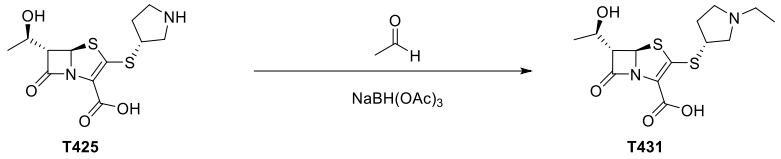
Allyl (5*R*,6*S*)-3-(((R)-1-((allyloxy)carbonyl)pyrrolidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (B4):

General procedure for sidechain thiol deprotection and β-addition-elimination into penem core **5** was used to incorporate sidechain **B3** into the penem core, **B4** (200 mg, 52%). **B4** (mixture of rotational isomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.89 (m, 2H), 5.67 (d, *J* = 1.4 Hz, 1H), 5.37 (dq, *J* = 1.3, 17.2 Hz, 1H), 4.69 (dABq, *J* = 5.4, 13.4 Hz, 2H), 4.54 (d, *J* = 5.5 Hz, 2H), 4.17 (quin, *J* = 6.4 Hz, 1H), 3.87 (m, 1H), 3.78 (m, 1H), 3.69 (m, 1H), 3.51 (m, 3H), 2.32 (sept, *J* = 6.8 Hz, 1H), 1.98 (m, 1H), 1.30 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.2, 159.7, 154.5, 154.4, 152.8, 152.8, 132.9, 132.8, 118.5, 118.0, 117.7, 117.6, 71.5, 71.4, 66.1, 65.8, 65.6, 64.7, 53.0, 52.6, 446.6, 45.9, 44.9, 44.5, 32.7, 32.0, 21.9. HRMS (UPLC/MS), $C_{19}H_{24}N_2O_6S_2$ [M+H] calculated: 441.1149; found: 441.1152.

(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-7-oxo-3-(((R)-pyrrolidin-3-yl)thio)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T425)

The general procedure for a one-pot allyl carbamate and allyl ester deprotection was used to convert **B4** to **T425** (21.4 mg, 30%). **T425:** ^{1}H NMR (400 MHz, D_2O) δ = 5.75 (d, *J* = 1.3 Hz, 1H), 4.21 (quin, *J* = 6.3 Hz, 1H), 4.11 (tt, *J* = 5.2, 7.2 Hz, 1H), 3.98 (dd, *J* = 1.4, 5.7 Hz, 1H), 3.73 (dd, *J* = 7.4, 12.9 Hz, 1H), 3.47 (m, 1H), 3.37 (m, 2H), 2.57 (sex, *J* = 7.4 Hz, 1H), 2.14 (sex, *J* = 6.2 Hz, 1H), 1.25 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 175.9, 162.5, 154.3, 118.1, 70.1, 64.6, 64.5,

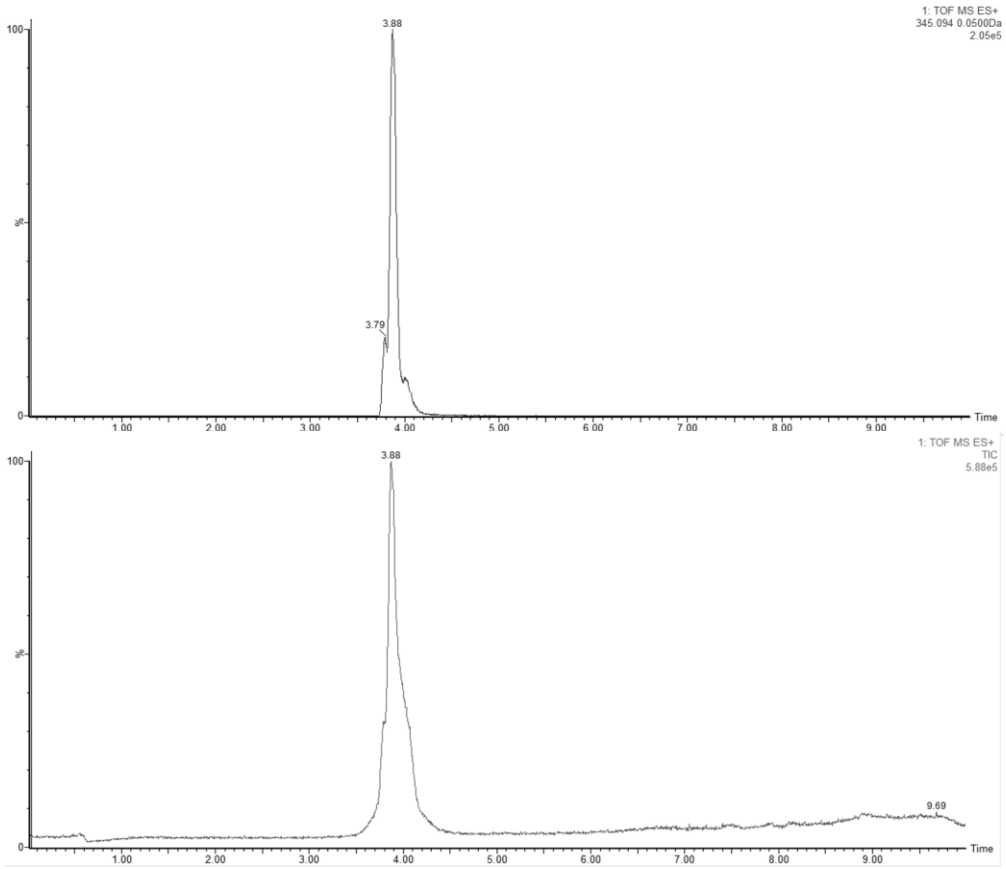
51.1, 44.8, 44.7, 31.3, 20.0. HRMS (UPLC/MS), $C_{12}H_{16}N_2O_4S_2$ [M+H] calculated: 317.0624; found: 317.0627.



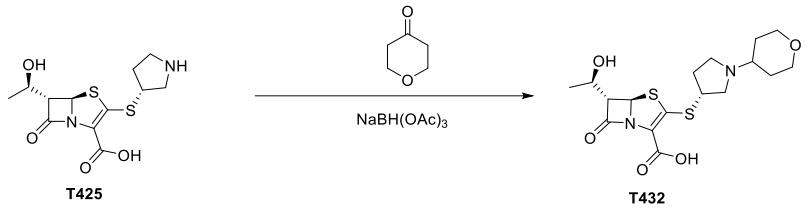
(5*R*,6*S*)-3-(((*R)*-1-ethylpyrrolidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T431):

General procedure for reductive amination was used with acetaldehyde to convert **T425** to **T431** (8.5 mg, 44%). **T431**: ^{1}H NMR (400 MHz, D_2O) δ = 5.69 (s, 1H), 4.15 (t, *J* = 5.6 Hz, 1H), 3.93 (d, *J* = 5.6 Hz, 1H), 3.64 (m, 3H), 3.20 (m, 5H), 2.51 (m, 1H), 1.96 (m, 1H), 1.20 (m, 6H). HRMS (UPLC/MS), $C_{19}H_{20}N_4O_4S_2$ [M+H] calculated: 345.0937; found: 345.0939.

HRMS UPLC-MS:

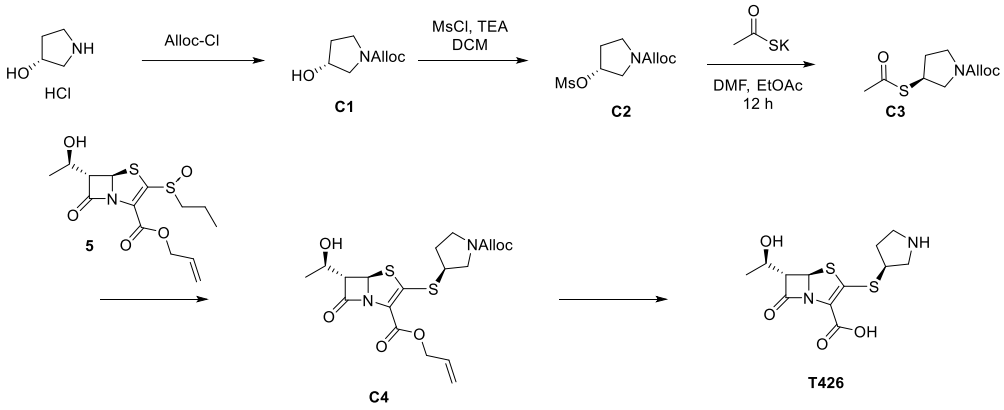


The UPLC-MS traces above indicate the extracted ion chromatogram 345.09 +/- 0.05 = m/z (top) and the total ion chromatogram (bottom) of **T431.**



(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-7-oxo-3-(((*R*)-1-(tetrahydro-2H-pyran-4-yl)pyrrolidin-3-yl)thio)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T432):

General procedure for reductive amination was used with 4-oxotetrahydropyran as the ketone to convert **T425** to **T432** (31 mg, 41%). **T432**: ^{1}H NMR (400 MHz, D_2O) δ = 5.78 (d, *J* = 1.04 Hz, 1H), 4.24 (t, *J* = 5.96 Hz, 1H), 4.06 (br s, *J* = 9.92, 4H), 3.72 (m, 3H), 3.46 (t, *J* = 12.0 Hz, 4H), 3.27 (m, 1H), 2.73 (m, 1H), 2.08 (m, 4H), 1.73 (m, 2H), 1.23 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ = 173.4, 160.9, 71.3, 65.2, 64.5, 63.9, 60.6, 60.5, 48.6, 43.0, 30.8, 28.9, 21.5. HRMS (UPLC/MS), $C_{17}H_{24}N_2O_5S_2$ [M+H] calculated: 401.1199; found: 401.1197.



Allyl (*R*)-3-hydroxypyrrolidine-1-carboxylate (C1):

General procedure for allyl carbamate formation of an amino alcohol was used to convert (*R*)-3-Hydroxypyrrolidine hydrochloride to **C1** (3.5 g, 85%). **C1** (mixture of conformational isomers)**:** ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.87 (tdd, *J* = 4.7, 11.4, 17 Hz, 1H), 5.24 (d, *J* = 17.2 Hz, 1H), 5.14 (d, *J* = 10.4 Hz, 1H), 4.51 (d, *J* = 5.4 Hz, 2H), 4.37 (br s, 1H), 3.46 (m, 2H), 3.41 (t, *J* = 3.9 Hz, 1H), 3.35 (m, 1H), 1.89 (br s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.1, 155.0, 133.0, 133.0, 117.2, 70.7, 69.8, 65.8, 54.5, 54.0, 44.1, 43.8, 33.9, 33.4. HRMS (UPLC/MS), $C_8H_{14}NO_3$ [M+H] calculated: 172.0968; found: 172.0967.

Allyl (*R*)-3-((methylsulfonyl)oxy)pyrrolidine-1-carboxylate (C2):

General procedure for mesylation of an alcohol was performed to convert **C1** to **C2** (4.5 g, 87%). **C2** (mixture of conformational isomers)**:** ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.87 (tdd, *J* = 5.3, 10.6,

16.6 Hz, 1H), 5.24, (m, 2H), 5.16 (d, *J* = 10.3 Hz, 1H), 4.53 (d, *J* = 4.7, 2H), 3.63 (m, 3H), 3.47 (m, 1H), 2.99 (s, 3H), 2.23 (br s, 1H), 2.11 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.6, 132.7, 117.5, 79.6, 66.0, 52.1, 43.6, 38.6, 31.5. HRMS (UPLC/MS), $C_9H_{15}NO_5S$ [M+H] calculated: 250.0744; found: 250.0752.

Allyl (*S*)-3-(acetylthio)pyrrolidine-1-carboxylate (C3):

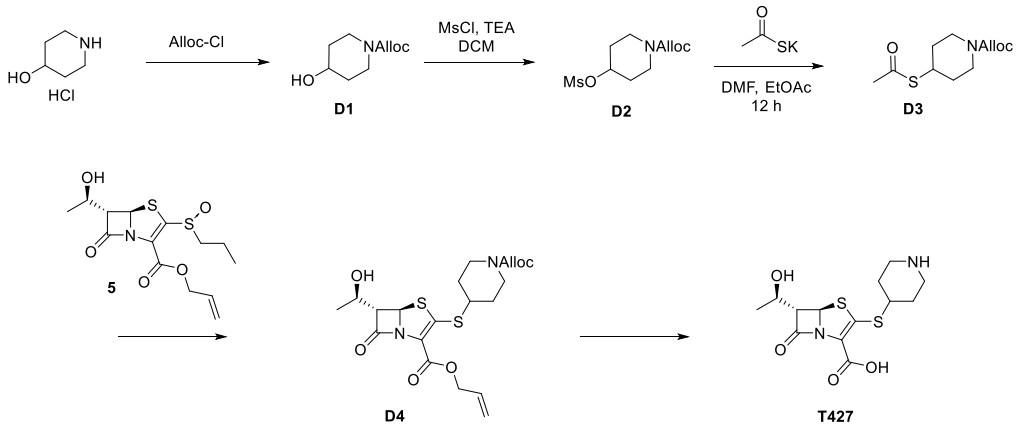
General procedure for installation of a thioester was used to convert **C2** to **C3** (1.65, 40%). **C3** (mixture of conformational isomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.89 (tdd, *J* = 4.6, 10.6, 17 Hz, 1H), 5.25 (d, *J* = 17.2 Hz, 1H), 5.16 (d, *J* = 10.4 Hz, 1H), 4.54 (d, *J* = 4 Hz, 2H), 3.95 (quin, *J* = 6.5 Hz, 2H), 3.78 (dt, *J* = 6.5, 11.5 Hz, 1H), 3.46 (m, 2H), 3.29 (m, 1H), 2.29 (s, 3H), 2.26 (m, 1H), 1.86 (oct, *J* = 6.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 195.2, 195.1, 154.5, 133.1, 133.0, 117.4, 117.3, 65.8, 51.7, 51.5, 45.1, 44.7, 41.2, 40.6, 32.1, 31.0, 30.7. HRMS (UPLC/MS), $C_{10}H_{15}NO_3S$ [M+H] [M+H] calculated: 230.0845; found: 230.0856.

Allyl (5*R*,6*S*)-3-(((*S*)-1-((allyloxy)carbonyl)pyrrolidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (C4):

General procedure for sidechain thiol deprotection and β-addition-elimination into penem core was used to incorporate sidechain **C3** into the penem core, **C4** (670 mg, 34%). **C4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.89 (m, 2H), 5.67 (d, *J* = 1.4 Hz, 1H), 5.37 (dq, *J* = 1.4, 17.2 Hz, 1H), 5.26 (dq, *J* = 1.5, 17.3 Hz, 1H), 5.19 (t, *J* = 10.4 Hz, 2H), 4.74 (dtABq, *J* = 1.4, 5.4, 13.5 Hz, 1H), 4.62 (dABq, *J* = 5.8, 13.3 Hz, 1H), 4.54 (d, *J* = 5.5 Hz, 2H), 4.17 (quin, *J* = 5.9 Hz, 1H), 3.87 (dd, *J* = 6.6, 11.6 Hz, 1H), 3.78 (m, 1H), 3.69 (m, 1H), 3.51 (m, 3H), 2.32 (quin, *J* = 6.6 Hz, 1H), 1.98 (m, 1H), 1.30 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.3, 159.6, 154.5, 154.3, 152.9, 132.8, 131.7, 130.8, 118.4, 118.0, 117.7, 117.6, 71.4, 66.0, 65.7, 65.5, 65.0, 64.8, 52.9, 52.3, 46.6, 45.9, 44.9, 44.4, 32.6, 32.2, 21.8. HRMS (UPLC/MS), $C_{19}H_{24}N_2O_6S_2$ [M+H] calculated: 441.1149; found: 441.1144.

(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-7-oxo-3-(((*S*)-pyrrolidin-3-yl)thio)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T426):

The general procedure for a one-pot allyl carbamate and allyl ester deprotection was used to convert **C4** to **T426** (97 mg, 51%). **T426**: ^{1}H NMR (400 MHz, D_2O) δ = 5.67 (s, 1H), 4.13 (quin, *J* = 6.0 Hz, 1H), 4.03 (m, 1H), 3.89 (d, *J* = 5.7 Hz, 1H), 3.73 (dd, *J* = 7.0, 12.8 Hz, 1H), 3.35 (m, 4H), 2.43 (sex, *J* = 7.64 Hz, 1H), 1.98 (sex, *J* = 7.2 Hz, 1H), 1.17 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 175.8, 162.3, 154.5, 118.1, 70.0, 64.8, 64.5, 51.4, 44.9, 44.7, 30.9, 20.0. HRMS (UPLC/MS), $C_{12}H_{16}N_2O_4S_2$ [M+H] calculated: 317.0624; found: 317.0622.



Allyl 4-hydroxypiperidine-1-carboxylate (D1):

General procedure for allyl carbamate formation of an amino alcohol was used to convert 4-hydroxypiperidine hydrochloride to **D1** (1.7 g, 93%). **D1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.94 (m, 1H), 5.29 (m, 1H), 5.21 (m, 1H), 4.59 (m, 2H), 3.89 (m, 3H), 3.13 (ddd, *J* = 3.4, 9.5, 13.2 Hz, 2H), 1.87 (m, 2H), 1.65 (br s, 1H), 1.49 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ **=** 155.0, 133.1, 117.3, 65.8, 54.4, 43.9, 33.7. HRMS (UPLC/MS), $C_9H_{15}NO_3$ [M+H] calculated: 186.1125; found: 186.1146.

Allyl 4-((methylsulfonyl)oxy)piperidine-1-carboxylate (D2):

General procedure for mesylation of an alcohol was performed to convert **D1** to **D2** (1.5 g, 63%). **D2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.66 (ddt, *J* = 5.4, 10.3, 17.2 Hz, 1H), 5.01 (dd, *J* = 1.6, 17.3 Hz, 1H), 4.93 (dd, *J* = 1.4, 10.4 Hz, 1H), 4.60 (sept, *J* = 3.6 Hz, 1H), 4.29 (d, *J* = 5.4 Hz, 2H), 3.45 (m, 2H), 3.11 (m, 2H), 2.79 (s, 3H), 1.71 (m, 2H), 1.53 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ **=** 154.5, 133.0, 117.0, 77.3, 65.7, 40.4, 38.3, 31.3. HRMS (UPLC/MS), $C_{10}H_{17}NO_5S$ [M+H] calculated: 264.0900; found: 264.0897.

Allyl 4-(acetylthio)piperidine-1-carboxylate (D3):

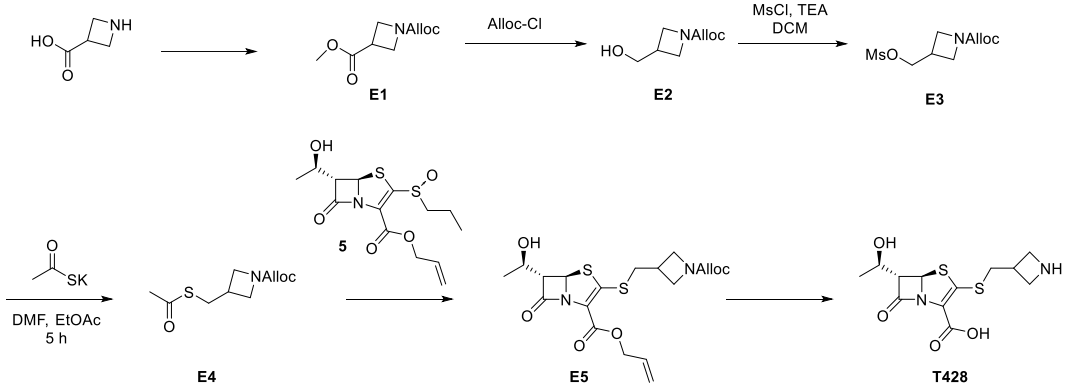
General procedure for installation of a thioester was used to convert **D2** to **D3** (470 mg, 34%). **D3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.92 (ddt, *J* = 5.5, 10.4, 17.2 Hz, 1H), 5.29 (dq, *J* = 1.6, 17.2 Hz, 1H), 5.20 (dq, *J* = 1.3, 10.4 Hz, 1H), 4.57 (dt, *J* = 1.4, 5.5 Hz, 2H), 3.91 (d, *J* = 12.7 Hz, 2H), 3.62 (tt, *J* = 4.0, 10.2 Hz, 1H), 3.13 (t, *J* = 10.8 Hz, 2H), 2.31 (s, 3H), 1.92 (m, 2H), 1.56 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 195.0, 155.0, 133.1, 117.4, 66.1, 43.5, 39.9, 31.8, 30.8. HRMS (UPLC/MS), $C_{11}H_{17}NO_3S$ [M+H] calculated: 244.1002; found: 244.0998.

Allyl (5*R*,6*S*)-3-((1-((allyloxy)carbonyl)piperidin-4-yl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (D4):

General procedure for sidechain thiol deprotection and β-addition-elimination into penem core was used to incorporate sidechain **D3** into the penem core, **D4** (185 mg, 47%). **D4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.9 (m, 2H), 5.63 (s, 1H), 5.38 (d, *J* = 17.2 Hz, 1H), 5.26 (d, *J* = 17.2 Hz, 1H), 5.19 (t, *J* = 10.2 Hz, 2H), 4.68 (dABq, *J* = 5.4, 13.4 Hz, 2H), 4.54 (d, *J* = 5.4 Hz, 2H), 4.18 (t, *J* = 6.2 Hz, 1H), 3.99 (d, *J* = 12.8 Hz, 2H), 3.69 (d, *J* = 7.1 Hz, 1H), 3.31 (m, 1H), 3.03 (br s, 3H), 2.07 (m, 2H), 1.62 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.1, 159.7, 155.0, 152.7, 132.9, 131.8, 118.4, 118.0, 117.6, 71.3, 66.3, 65.7, 65.5, 64.3, 46.3, 43.1, 43.1, 32.7, 32.7, 21.9. HRMS (UPLC/MS), $C_{20}H_{26}N_2O_6S_2$ [M+H] calculated: 455.1305; found: 455.1300.

(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-7-oxo-3-(piperidin-4-ylthio)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T427)

The general procedure for a one-pot allyl carbamate and allyl ester deprotection was used to convert **D4** to **T427** (37.7 mg, 52%). **T427**: ^{1}H NMR (400 MHz, D_2O) δ = 5.76 (d, *J* = 1.4, 1H), 4.25 (quin, *J* = 6.0 Hz, 1H), 4.00 (dd, *J* = 1.4, 5.8 Hz, 1H), 3.60 (tt, *J* = 3.9, 10.2 Hz, 1H), 3.46 (m, 3H), 3.15 (m, 3H), 2.38 (m, 2H), 2.23 (m, 1H), 1.91 (m, 1H), 1.30 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 174.5, 161.0, 153.6, 113.6, 68.7, 63.3, 63.0, 41.6, 41.5, 27.8, 27.5, 18.8. HRMS (UPLC/MS), $C_{13}H_{18}N_2O_4S_2$ [M+H] calculated: 331.0781; found: 331.0792.



1-Allyl 3-methyl azetidine-1,3-dicarboxylate (E1):

The general procedure for allyl carbamate protection of nitrogen was first performed azetidine-3-carboxylic acid followed by the general procedure for methyl ester formation to give **E1** (1.8 g, 93%). **E1** (Mixture of conformational isomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.74 (m, 1H), 5.12 (dt, *J* = 1.6, 17.2 Hz, 1H), 5.03 (d, *J* = 10.4, 1H), 4.37 (m, 2H), 4.01 (m, 4H), 3.58 (m, 3H), 3.26 (quin, *J* = 7.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.2, 155.6, 132.4, 117.0, 65.1, 51.8, 51.3, 31.9. HRMS (UPLC/MS), $C_9H_{13}NO_4$ [M+H] calculated: 200.0917; found: 200.0918.

Allyl 3-(hydroxymethyl)azetidine-1-carboxylate (E2):

The general procedure for methyl ester reduction was used to convert **E1** to **E2** (1.1 g, 70%). **E2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.82 (ddt, *J* = 5.5, 11.5, 17.2 Hz, 1H), 5.20 (d, *J* = 17.2 Hz, 1H), 5.12 (d, *J* = 10.4 Hz, 1H), 4.45 (d, *J* = 5.4 Hz, 2H), 3.96 (t, *J* = 8.4 Hz, 2H), 3.66 (m, 5H), 2.67 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.5, 132.7, 117.4, 65.5, 63.8, 51.6, 51.1, 30.8. HRMS (UPLC/MS), $C_8H_{13}NO_3$ [M+H] calculated: 172.0968; found: 172.0966.

Allyl 3-(((methylsulfonyl)oxy)methyl)azetidine-1-carboxylate (E3):

The general procedure for mesylation of an alcohol was used to convert **E2** to **E3** (1.6 g, 98%). **E3:** ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.72 (ddt, *J* = 4.5, 10.5, 17.2 Hz, 1H), 5.11 (dq, *J* = 1.6, 17.2 Hz, 1H), 5.02 (dq, *J* = 1.3, 10.4 Hz, 1H), 4.34 (dt, *J* = 1.4, 5.5 Hz, 2H), 4.17 (d, *J* = 6.5 Hz, 2H), 3.92 (m, 2H), 3.61 (m, 2H) 2.87 (s, 3H), 2.81 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.7, 132.5, 116.9, 70.0, 65.0, 50.7, 36.8, 27.9. HRMS (UPLC/MS), $C_9H_{15}NO_5S$ [M+H] calculated: 250.0744; found: 250.0765.

Allyl 3-((acetylthio)methyl)azetidine-1-carboxylate (E4):

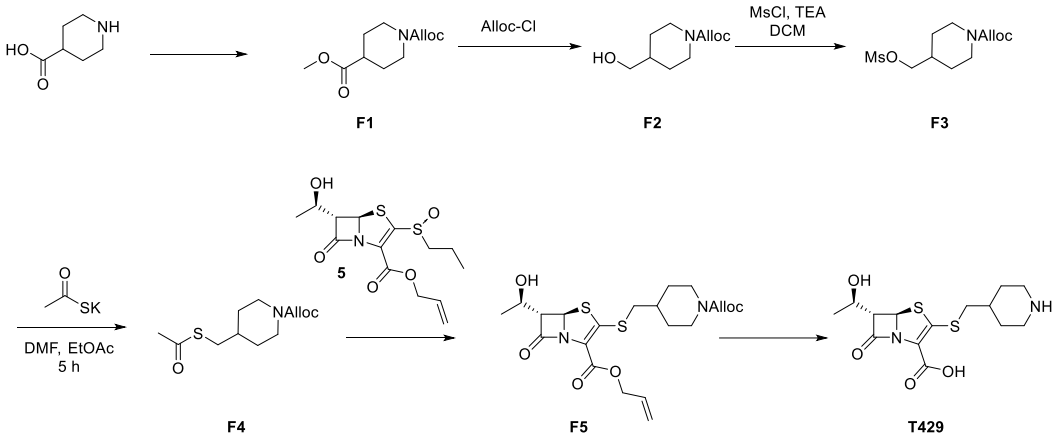
The general procedure for installation of a thioester was used to convert **E3** to **E4** (923 mg, 66%). **E4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.75 (ddt, *J* = 5.5, 10.5, 17.2 Hz, 1H), 5.13 (dd, *J* = 1.6, 17.2 Hz, 1H), 5.03 (dd, *J* = 1.4, 10.4 Hz, 1H), 4.37 (dt, *J* = 1.4, 5.5 Hz, 2H), 3.91 (t, *J* = 8.5 Hz, 2H), 3.49 (dd, *J* = 5.5, 8.7 Hz, 2H), 2.96 (d, *J* = 7.5 Hz, 2H), 2.65 (m, 1H), 2.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 194.6, 155.8, 132.7, 117.1, 65.2, 53.5, 32.2, 30.3, 28.7. HRMS (UPLC/MS), $C_{10}H_{15}NO_3S$ [M+H] calculated: 230.0845; found: 230.0846.

Allyl (5*R*,6*S*)-3-(((1-((allyloxy)carbonyl)azetidin-3-yl)methyl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (E5):

The general procedure for sidechain thiol deprotection and β-addition-elimination into penem core **5** was used to incorporate sidechain **E4** into the penem core to generate **E5** (245 mg, 48%). **E5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.91 (m, 2H), 5.65 (br s, 1H), 5.39 (d, *J* = 17.2 Hz, 1H), 5.27 (d, *J* = 17.2 Hz, 1H), 5.21 (m, 2H), 4.70 (ddABq, *J* = 1.1, 5.4, 13.5 Hz, 2H), 4.53 (dd, *J* = 1.0, 5.5 Hz, 2H), 4.20 (quin, J = 6.2 Hz, 1H), 4.12 (t, *J* = 8.6 Hz, 2H), 3.72 (m, 3H), 3.18 (dABq, *J* = 8.0, 12.8 Hz, 2H), 2.87 (m, 1H), 1.33 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.1, 159.7, 156.3, 153.6, 132.8, 131.8, 118.5, 117.8, 71.4, 65.8, 65.8, 65.6, 64.6, 53.9, 39.6, 29.3, 22.0. HRMS (UPLC/MS), $C_{19}H_{24}N_2O_6S_2$ [M+H] calculated: 441.1149; found: 441.1157.

(5*R*,6*S*)-3-((azetidin-3-ylmethyl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T428):

The general procedure for a one-pot allyl carbamate and allyl ester deprotection was used to convert **E5** to **T428** (74.1 mg, 89%). **T428**: ^{1}H NMR (400 MHz, D_2O) δ = 5.75 (s, 1H), 4.26 (t, *J* = 6.1 Hz, 1H), 3.99 (d, *J* = 11.5 Hz, 2H), 2.99 (m, 2H), 2.11 (d, *J* = 13.8 Hz, 2H), 2.03 (m, 1H), 1.48 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 175.9, 162.5, 117.7, 114.8, 69.8, 64.6, 43.7, 40.7, 34.0, 27.4, 20.1. HRMS (UPLC/MS), $C_{12}H_{16}N_2O_4S_2$ [M+H] calculated: 317.0624; found: 317.0626.



1-Allyl 4-methyl piperidine-1,4-dicarboxylate (F1):

The general procedure for allyl carbamate protection of nitrogen was first performed on piperidine-4-carboxylic acid followed by the general procedure for methyl ester formation to give **F1** (1.7 g, 96%). **F1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.93 (ddt, *J* = 5.8, 10.4, 17.2 Hz, 1H), 5.29 (tt, *J* = 1.6, 17.2 Hz, 1H), 5.20 (tt, *J* = 1.4, 10.4 Hz, 1H), 4.58 (td, *J* = 1.4, 5.5 Hz, 2H), 4.09 (m, 2H), 3.69 (s, 3H), 2.92 (br s, 2H), 2.48 (tt, *J* = 3.9, 11.0 Hz, 1H), 1.91 (br s, 1H), 1.89 (br s, 1H), 1.65 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 174.8, 155.0, 133.0, 117.3, 66.0, 51.8, 43.1, 40.8, 27.8. HRMS (UPLC/MS), $C_{11}H_{17}NO_4$ [M+H] calculated: 228.1230; found: 228.1229.

Allyl 4-(hydroxymethyl)piperidine-1-carboxylate (F2):

The general procedure for methyl ester reduction was used to convert **F1** to **F2** (1 g, 70%). **F2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.77 (ddt, *J* = 5.5, 10.5, 17.2 Hz, 1H), 5.13 (dq, *J* = 1.5, 17.2 Hz, 1H), 5.05 (dq, *J* = 1.3, 10.5 Hz, 1H), 4.41 (d, *J* = 5.4 Hz, 2H), 4.01 (br d, *J* = 12.2 Hz, 2H), 3.54 (br s, 1H), 3.28 (d, *J* = 6.3 Hz, 2H), 2.62 (br s, 2H), 1.59 (br d, *J* = 13.2 Hz, 2H), 1.50 (m, 1H), 0.99 (dq, *J* = 4.4, 8.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.0, 132.8, 117.0, 66.7, 65.7, 43.6, 38.4, 28.4. HRMS (UPLC/MS), $C_{10}H_{17}NO_3$ [M+H] calculated: 200.1281; found: 200.1283.

Allyl 4-(((methylsulfonyl)oxy)methyl)piperidine-1-carboxylate (F3):

The general procedure for mesylation of an alcohol was used to convert **F2** to **F3** (783 mg, 86%). **F3:** ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.80 (ddt, *J* = 5.5, 10.2, 17.2 Hz, 1H), 5.16 (dq, *J* = 1.6, 17.2 Hz, 1H), 5.07 (dq, *J* = 1.2, 10.5 Hz, 1H), 4.46 (d, *J* = 5.5 Hz, 2H), 4.07 (br d, *J* = 9.3 Hz, 2H), 3.94 (d, *J* = 6.4 Hz, 2H), 2.89 (s, 3H), 2.67 (br s, 2H), 1.83 (m, 1H), 1.64 (br d, *J* = 12.9 Hz, 2H), 1.11 (dq, *J* = 4.5, 12.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.7, 132.9, 117.0, 73.2, 65.6, 43.1, 36.9, 35.5, 27.8**.** HRMS (UPLC/MS), $C_{11}H_{19}NO_5S$ [M+H] calculated: 278.1057; found: 278.1067.

Allyl 4-((acetylthio)methyl)piperidine-1-carboxylate (F4):

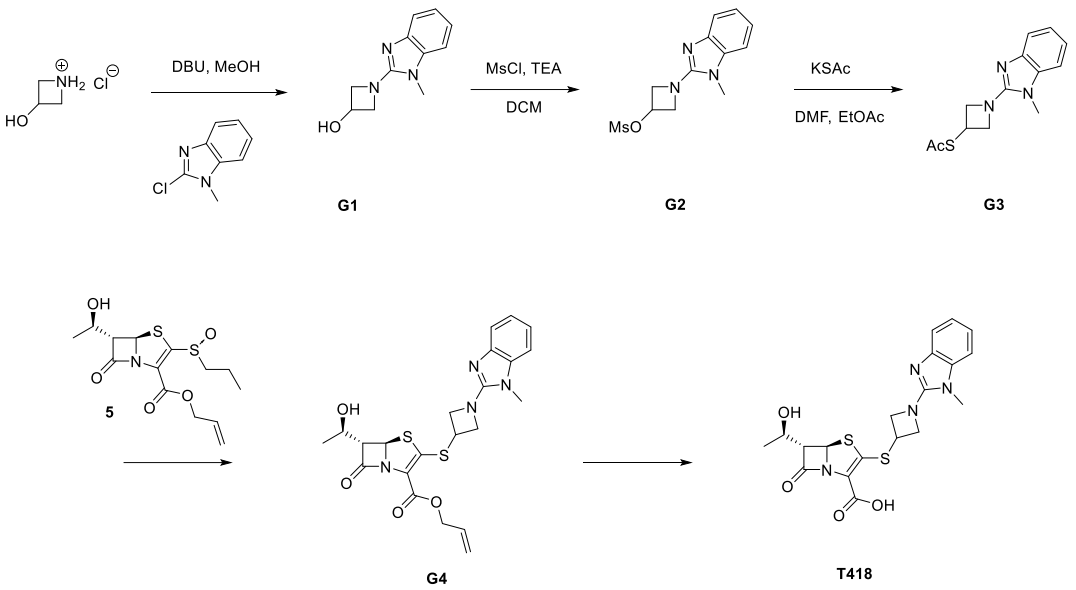
The general procedure for installation of a thioester was used to convert **F3** to **F4** (448 mg, 64%). **F4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.82 (ddt, *J* = 5.5, 10.4, 17.2 Hz, 1H), 5.17 (dd, *J* = 1.6, 17.2 Hz, 1H), 5.08 (dd, *J* = 1.4, 10.4 Hz, 1H), 4.46 (d, *J* = 5.5 Hz, 2H), 4.05 (br s, 2H), 2.72 (d, *J* = 6.7 Hz, 2H), 2.63 (br s, 2H), 2.22 (s, 3H), 1.65 (br d, *J* = 12.9 Hz, 2H), 1.53 (m, 1H), 1.06 (dq, *J* = 4.4, 12.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 195.1, 154.8, 133.1, 117.0, 65.7, 43.6, 36.2, 34.7, 31.0, 30.4. HRMS (UPLC/MS), $C_{12}H_{19}NO_3S$ [M+H] calculated: 258.1158; found: 258.1163.

Allyl (5*R*,6*S*)-3-(((1-((allyloxy)carbonyl)piperidin-4-yl)methyl)thio)-6-((*R*)-1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (F5):

The general procedure for sidechain thiol deprotection and β-addition-elimination into penem core was used to incorporate sidechain **F4** into the penem core to generate **F5** (210 mg, 39%). **F5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.93 (m, 2H), 5.63 (d, *J* = 1.4 Hz, 1H), 5.40 (dd, *J* = 1.4, 17.2 Hz, 1H), 5.28 (dd, *J* = 1.5, 17.2 Hz, 1H), 5.23 (dd, *J* = 1.5, 10.5 Hz, 1H), 5.19 (dd, *J* = 1.4, 10.6 Hz, 1H), 4.71 (dABq, *J* = 5.4, 13.5 Hz, 2H), 4.56 (d, *J* = 5.5 Hz, 2H), 4.19 (m, 1H), 3.70 (dd, *J* = 1.0, 6.7 Hz, 1H), 2.83 (m, 4H), 2.39 (br s, 1H), 1.86 (m, 2H), 1.76 (m, 2H), 1.34 (d, *J* = 6.3 Hz, 3H), 1.17 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.1, 159.9, 155.5, 155.2, 133.2, 132.0, 118.4, 117.5, 71.2, 66.2, 65.7, 64.4, 43.9, 42.1, 37.1, 31.3, 22.1. HRMS (UPLC/MS), $C_{21}H_{28}N_2O_6S_2$ [M+H] calculated: 469.1462; found: 469.1476.

(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-7-oxo-3-((piperidin-4-ylmethyl)thio)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T429):

The general procedure for a one-pot allyl carbamate and allyl ester deprotection was used to convert **F5** to **T429** (53.4 mg, 74%). **T429**: ^{1}H NMR (400 MHz, D_2O) δ = 5.75 (s, 1H), 4.26 (t, *J* = 6.2 Hz, 1H), 3.99 (d, *J* = 5.6 Hz, 1H), 3.46 (m, 3H), 3.00 (m, 4H), 2.06 (m, 4H), 1.48 (m, 4H), 1.48 (m, 3H), 1.31 (d, *J* = 6.2 Hz, 3H).^{13}C NMR (101 MHz, D_2O) δ = 175.9, 162.5, 117.7, 114.8, 69.8, 64.5, 64.2, 43.7, 40.7, 34.0, 27.4, 27.3, 20.1. HRMS (UPLC/MS), $C_{14}H_20N_2O_4S_2$ [M+H] calculated: 345.0937; found: 345.0940.



1-(1-Methyl-1*H*-benzo[*d*]imidazol-2-yl)azetidin-3-ol (G1):

A flask was charged with 3-hydroxyazetidine hydrochloride (492 mg, 4.5 mmol), 1-*N*-methyl-2-chlorobenzimidazole (reagent prepare as previously described[2]) (679 mg, 4.1 mmol), and 3 Å molecular sieves. The mixture was then suspended in methanol (12 mL) and stirred under nitrogen for 10 min. To the mixture, 1,8-diazabicyclo[5.4.0]undec-7-ene (1.3 mL, 8.5 mmol) was added dropwise and the mixture was heated to 65 ˚C for 16 h. The reaction mixture was then filtered, and the retentate was washed with methanol. The filtrate was then concentrated, suspended in EtOAc, and extracted with water. The aqueous layer was then washed twice with EtOAc, the organic layers were combined, dried with anhydrous sodium sulfate, and concentrated *in vacuo*. The mixture was then purified using silica gel flash chromatography with isopropanol/dichloromethane mobile phase to yield **G1** (629 mg, 76%). **G1**: ^{1}H NMR (400 MHz, CDCl3) δ = 7.46 (d, J = 7.5 Hz, 1H), 7.08 (m, 3H), 4.80 (tt, J = 6.4, 5.0 Hz, 1H), 4.44 (dd, J = 8.0, 7.0 Hz, 2H), 4.15 (dd, J = 5.0, 8.7 Hz, 2H), 3.40 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 157.21, 141.21, 135.44, 121.55, 120.27, 116.53, 107.60, 62.72, 62.57, 29.56. HRMS (UPLC/MS), $C_{11}H_{13}N_3O$ [M+H] calculated: 204.1137; found: 204.1137.

1-(1-Methyl-1*H*-benzo[*d*]imidazol-2-yl)azetidin-3-yl methanesulfonate (G2):

General procedure for mesylation of an alcohol was performed to convert **G1** to **G2** (720 mg, 43%). **G2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 (d, *J* = 6.8 Hz, 1H), 7.14 (m, 3H), 5.38 (tt, *J* = 4.6, 6.5 Hz, 1H), 4.55 (dd, J = 6.8, 9.8 Hz, 2H), 4.39 (dd, *J* = 4.6, 9.9 Hz, 2H), 3.51 (s, 3H), 3.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.37, 141.35, 135.57, 121.71, 120.70, 117.27, 107.82, 68.31, 59.51, 38.34, 29.53. HRMS (UPLC/MS), $C_{12}H_{15}N_3O_3S$ [M+H] calculated: 282.0907; found: 282.0947.

_S_-(1-(1-Methyl-1_H_-benzo[_d_]imidazol-2-yl)azetidin-3-yl) ethanethioate (G3):

The general procedure for installation of a thioester was used to convert **G2** to **G3** (546 mg, 82%). **G3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49 (d, *J* = 7.4 Hz, 1H), 7.09 (m, 3H), 4.62 (t, *J* = 8.1 Hz, 2H), 4.39 (m, 1H), 4.12 (dd, *J* = 5.9, 8.2 Hz, 2H), 3.45 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 194.65, 156.36, 141.11, 135.29, 121.51, 120.38, 116.88, 107.57, 59.13, 32.20, 30.26, 29.43. HRMS (UPLC/MS), $C_{13}H_{15}N_3OS$ [M+H] calculated: 262.1009; found: 262.1050.

Allyl (5_R_,6_S_)-6-((_R_)-1-hydroxyethyl)-3-((1-(1-methyl-1_H_-benzo[_d_]imidazol-2-yl)azetidin-3-yl)thio)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (G4):

General procedure for sidechain thiol deprotection and β-addition-elimination into penem core **5** was used to add side chain **G3** into penem core to give **G4** (51.8 mg, 13%). **G4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.54 (d, *J* = 7.4 Hz, 1H), 7.18 (m, 3H), 5.96 (tdd, *J* = 5.5, 10.6, 17.1 Hz, 1H), 5.78 (s, 1H), 5.43 (dd, *J* = 1.2, 17.2 Hz, 1H), 5.26 (dd, *J* = 0.9, 10.4 Hz, 1H), 4.77 (m, 5H), 4.28 (m, 4H), 3.77 (d, *J* = 7.24, 1H), 3.58 (s, 3H), 1.38 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.3, 159.6, 152.0, 131.7, 122.6, 122.5, 121.6, 121.5, 118.6, 117.8, 116.2, 116.1, 108.2, 71.7, 65.8, 65.6, 60.3, 59.6, 37.0, 29.9, 22.0, 14.2. HRMS (UPLC/MS), $C_{22}H_{24}N_4O_4S_2$ [M+H] calculated: 473.1312; found: 473.1320.

(5_R_,6_S_)-6-((_R_)-1-hydroxyethyl)-3-((1-(1-methyl-1_H_-benzo[_d_]imidazol-2-yl)azetidin-3-yl)thio)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (T418):

The allyl ester-protected penem **G4** (46.6 mg, 99 µmol) and sodium benzenesulfinate (21.1 mg, 129 µmol) was dissolved in THF (2 ml). The mixture was degassed via bubbling of nitrogen gas through the mixture for 15 min. Palladium-tetrakis(triphenylphosphine) (10 mg, 9 µmol) was then added to the mixture under nitrogen atmosphere and the reaction was allowed to proceed for 3 h. The solvent was then removed *in vacuo,* and the resulting oil was partitioned into EtOAc and water. The organic layer was back extracted twice with water and the aqueous layers were combined. The mixture was then purified by HPLC using a C18 stationary phase and water/ACN + 0.1% TFA mobile phase. The product fraction was then lyophilized to yield the powder form of the final penem antibiotic **T418** (19 mg, 44%). ^{1}H NMR (400 MHz, D_2O) δ = 7.38 (m, 1H), 7.30 (m, 1H), 7.26 (m, 2H), 5.64 (d, *J* = 1.3 Hz, 1H), 4.92 (m, 2H), 4.44 (m, 2H), 4.00 (dq, *J* = 6.4, 6.4 Hz, 1H), 3.67 (dd, *J* = 1.3, 6.8 Hz, 1H), 3.63 (s, 3H), 3.19 (quin, *J* = 1.6 Hz, 1H), 1.17 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 174.6, 159.8, 151.2, 133.4, 130.5, 125.8, 125.3, 112.8, 11.4, 72.9, 66.7, 66.4, 61.9, 61.5, 38.0, 30.6, 21.9. HRMS (UPLC/MS), $C_{19}H_{20}N_4O_4S_2$ [M+H] calculated: 433.0999; found: 433.1015.

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