Toward Orally Absorbed Prodrugs of the Antibiotic Aztreonam. Design of Novel Prodrugs of Sulfate Containing Drugs Part 2

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Supporting Information

GENERAL

All reagents were purchased from commercial suppliers and used without further purification. All solvents were reagent, or HPLC grade. Analytical TLC was performed on silica gel 60 F254 plates and visualized by UV if possible, or by staining with $KMnO_4$ dip, or phosphomolybdic acid in EtOH dip. Flash chromatography was carried out using an automated system with pre-packed silica columns. Yields refer to isolated yields of pure compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer at ambient temperature. Chemical shifts are reported in parts per million (ppm) relative to deuterated solvent, or a TMS internal standard. Multiplicities are reported as follows: s = singlet; d = doublet, t = triplet; m = multiplet; br = broad; f = fine. High-resolution mass spectrometry was obtained on a Waters Xevo G2 QTOF with Acquity LC system. All final compounds are of ≥95% purity as assessed by ^{1}H and ^{13}C NMR, together with HPLC (20 minute method). Mass spectrometry and high-resolution mass-spectrometry (key compounds) were also used to assess final compounds.

LC-MS 6 minute method:

Autosampler: Finnigan Surveyor Autosampler Plus
MS Pump: Finnigan Surveyor MS Pump Plus
UV Detector: Finnigan Surveyor PDA Plus Detector
Mass Spectrometer: Finnigan LTQ
Ionization Method: APCI
Column: Phenomenex, Gemini 5µm C18 110Å, 50 x 3 mm
Solvent system:
Solvent A: water +0.1% formic acid
Solvent B: 90% acetonitrile / 10% water +0.1% formic acid

Gradient time table:

Time (min)	Flow (mL/min)	%A	%B
0.00	0.50	90.00	10.00
0.10	0.50	90.00	10.00
3.50	0.50	0.00	100.00
4.50	0.50	0.00	100.00
5.00	0.50	90.00	10.00
6.00	0.50	90.00	10.00

LC-MS 10 minute method:

Autosampler: Finnigan Surveyor Autosampler Plus
MS Pump: Finnigan Surveyor MS Pump Plus
UV Detector: Finnigan Surveyor PDA Plus Detector
Mass Spectrometer: Finnigan LTQ
Ionization Method: APCI
Column: Phenomenex, Gemini 5µm C18 110Å, 50 x 3 mm
Solvent system:
Solvent A: water +0.1% formic acid
Solvent B: 90% acetonitrile / 10% water +0.1% formic acid

Gradient time table:

Time (min)	Flow (mL/min)	%A	%B
0.00	0.50	90.00	10.00
0.10	0.50	90.00	10.00
7.50	0.50	0.00	100.00
8.50	0.50	0.00	100.00
9.00	0.50	90.00	10.00
10.00	0.50	90.00	10.00

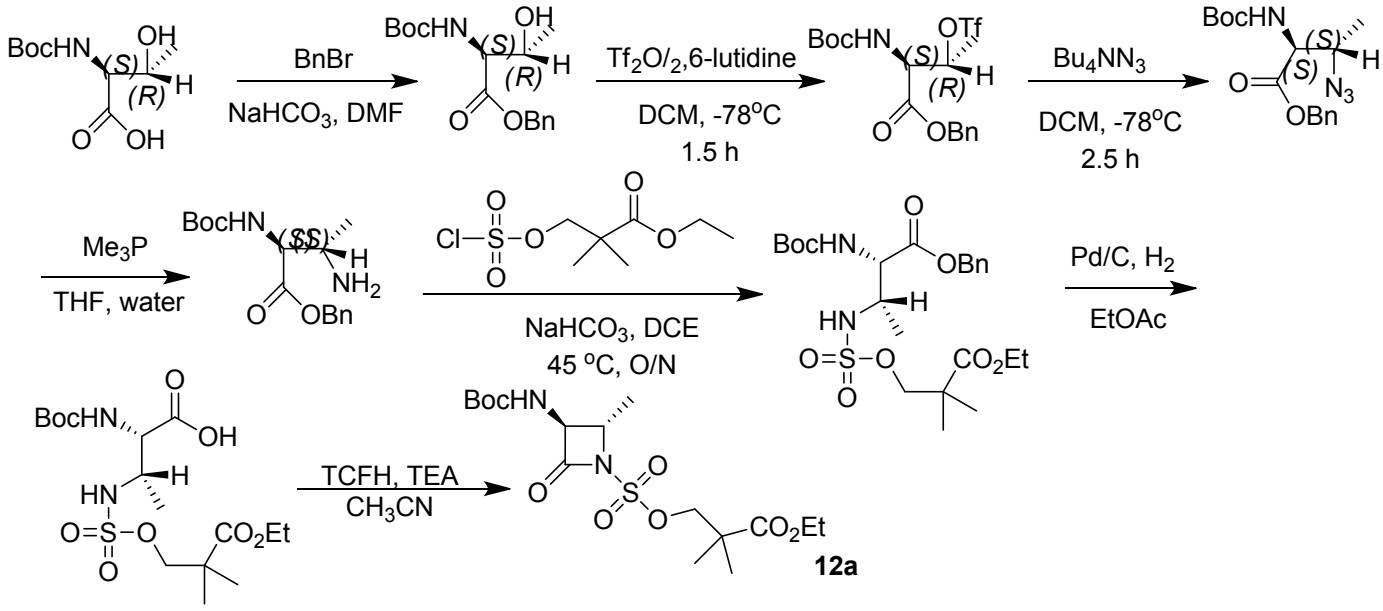
HPLC 20 minute method: Used to assess purity of final compounds

Pump: Varian ProStar Model 210
UV Detector: Varian ProStar (Photo Diode Array)
Column: Phenomenex, Gemini-NX 5µm C18 110Å, 150 x 4.60 mm
Solvent system:
Solvent A: water +0.1% trifluoroacetic acid
Solvent B: acetonitrile

Gradient time table:

Time (min)	**Flow (mL/min)**	**%A**	**%B**
Prerun	1.20	90.00	10.00
1.00	1.20	90.00	10.00
16.00	1.20	10.00	90.00
22.00	1.20	10.00	90.00
22.50	1.20	90.00	10.00

SYNTHESIS OF BETA-LACTAM INTERMEDIATE 12A



Preparation of 2-tert-butoxycarbonylamino-3-hydroxy-butyric acid benzyl ester 5

To a solution of Boc-L-Threonine (15.0 g, 68.4 mmol) in DMF (465 mL) at 0°C were added $NaHCO_3$ (16.0 g, 190.2 mmol) and benzyl bromide (40.6 mL, 342.1 mmol). After stirring overnight at rt, water was added and the mixture was extracted with EtOAc. The organic layers were washed with water and brine, and concentrated. The dried residue was purified with flash chromatography over silica gel (0-60%, EtOAc / Hexanes) to obtain the title compound **5** (18. 8 g, 89% yield) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43-7.29 (m, 5H), 5.35 (d, J = 9.2 Hz, 1H), 5.32-5.11 (m, 2H), 4.29 (d, J = 9.4 Hz, 2H), 1.44 (s, 9H), 1.31-1.19 (m, 3H).

LC-MS: 310 $[M+H]^+$

Preparation of 3-azido-2-tert-butoxycarbonylamino-butyric acid benzyl ester 7

To a solution of 2-*tert*-butoxycarbonylamino-3-hydroxy-butyric acid benzyl ester **5** (9.28 g, 30.0 mmol, 1.0 eq) in anhydrous dichloromethane (150 mL) at -78 ºC were added trifluoromethanesulfonic anhydride (6.06 mL, 36.0 mmol, 1.2 eq) dropwise and 2,6-lutidine (4.54 mL, 39.0 mL, 1.3 eq) respectively. The reaction was stirred at the same temperature for 1.5 h. Bu_4NN_3 (21.3 g, 75.0 mmol, 2.5 eq) in anhydrous dichloromethane (30 mL) was added. The mixture was stirred at -78 ºC for 1h and the reaction was warmed to room temperature for 1.5 h. The reaction mixture was diluted

with dichloromethane and washed with saturated sodium bicarbonate. The organic phase was separated, dried over sodium sulfate, and concentrated to dryness. The residue was purified by flash chromatography (silica, Hexanes / EtOAc = 4:1) to give the title compound **7** (8.82 g, 88%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.36 (d, *J* = 2.6 Hz, 4H), 5.29 (s, 1H), 5.29-5.11 (m, 2H), 4.46 (d, *J* = 8.5 Hz, 1H), 3.84 (d, *J* = 8.1 Hz, 1H), 1.45 (d, *J* = 0.8 Hz, 9H), 1.34-1.16 (m, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.5, 155.0, 134.9, 128.7, 128.6, 128.5, 80.5, 67.6, 58.8, 57.5, 28.3, 28.3, 15.4.

Preparation of 3-amino-2-tert-butoxycarbonylamino-butyric acid benzyl ester 8

To a solution of 3-azido-2-*tert*-butoxycarbonylamino-butyric acid benzyl ester **7** (11.3 g, 33.8 mmol, 1.0 eq) in tetrahydrofuran (40 mL) was added trimethylphosphine (1.0 M in THF, 67.6 mmol, 67.6 mmol, 2.0 eq) at 0 ºC. The reaction was warmed to room temperature for 2h. Water (4 mL) was added to the reaction and the mixture was stirred at room temperature for 2h. The reaction mixture was concentrated to dryness. The residue was purified by flash chromatography (silica, EtOAc) to give the title compound **8** (8.05 g, 77%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (t, *J* = 1.5 Hz, 5H), 7.26 (s, 1H), 5.30 (m, 1H), 5.28–5.10 (m, 2H), 4.32 (s, 1H), 3.30 (s, 1H), 1.47 (s, 9H), 1.02 (d, *J* = 6.7 Hz, 3H).

LC-MS: 309 $[M+H]^+$

Preparation of 2-tert-butoxycarbonylamino-3-(2-ethoxycarbonyl-2-methyl-propoxysulfonyl amino)-butyric acid benzyl ester 10a

To a solution of 3-amino-2-*tert*-butoxycarbonylamino-butyric acid benzyl ester **8** (4.09 g, 13.3 mmol, 1.0 eq) in 1,2-dichloroethane (50 mL) was added a saturated aqueous solution of sodium bicarbonate (50 mL). To the mixture was added ethyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate **9a** (6.49 g, 26.2 mmol, 2.0 eq) and the mixture was stirred rapidly at 45 ºC for 20 h. The reaction mixture was cooled to room temperature and the layers were separated. The organic layer was dried with anhydrous sodium sulfate and concentrated to dryness. The residue was purified by flash column chromatography (silica, Hexanes / EtOAc = 3:1) to give the title compound **10a** (5.07 g, 74%) as a solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37-7.27 (m, 5H), 6.03-5.96 (m, 1H), 5.52-5.46 (m, 1H), 5.18 (s, 2H), 4.57-4.52 (m, 1H), 4.18-4.07 (m, 4H), 4.01-3.95 (m, 1H), 1.42 (s, 9H), 1.33-1.22 (m, 9H), 1.16 (d, J = 6.7 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8, 169.6, 134.7, 128.8, 128.7, 128.5, 81.0, 75.3, 67.9, 61.0, 57.7, 53.4, 52.8, 42.6, 28.2, 22.1, 16.6, 14.1.

Preparation of 2-tert-butoxycarbonylamino-3-(2-ethoxycarbonyl-2-methyl-propoxysulfonyl-amino)-butyric acid 11a

To a solution of 2-*tert*-butoxycarbonylamino-3-(2-ethoxycarbonyl-2-methyl-propoxysulfonyl amino)-butyric acid benzyl ester **10a** (4.20 g, 8.14 mmol, 1.0 eq) in ethyl acetate (50 mL) was added Pd/C (420 mg). The suspension was degassed 3 times and refilled with hydrogen. The mixture was stirred at room temperature for 2h. The reaction mixture was filtered though a pad of Celite. The filtrate was concentrated to dryness, affording the title compound **11a** (3.47 g, 100%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.23-1.18 (m, 1H), 5.68-5.63 (m, 1H), 4.45-4.40 (m, 1H), 4.19-4.05 (m, 4H), 3.97-3.89 (m, 1H), 1.50 (s, 9H), 1.39-1.22 (m, 12H).

^{13}C NMR (75 MHz, acetone-*d6*) δ 174.3, 170.8, 156.1, 79.0, 74.8, 60.5, 59.8, 57.8, 44.2, 27.6, 21.5, 21.4, 21.4, 16.1, 13.6, 13.6.

LC-MS: 427 $[M+H]^+$

Preparation of 3-(3-tert-butoxycarbonylamino-2-methyl-4-oxo-azetidine-1-sulfonyloxy)-2,2-dimethyl-propionic acid ethyl ester 12a

To a solution of 2-*tert*-butoxycarbonylamino-3-(2-ethoxycarbonyl-2-methyl-propoxysulfonyl-amino)-butyric acid **11a** (3.47 g, 8.14 mmol, 1.0 eq) in acetonitrile (240 mL) was added *N*,*N*,*N*',*N*'-tetramethylchloroformamidinium hexafluorophosphate [TCFH] (3.43 g, 12.2 mmol, 1.5 eq) and triethylamine (2.95 mL, 21.2 mmol, 2.6 eq) a 0 ºC. The reaction was stirred at 0 ºC for 10 min and concentrated to dryness. The residue was diluted to ethyl acetate and washed with water and brine. The organic phase was separated, dried, and concentrated to dryness. The residue was purified by flash column chromatography (silica, Hexanes / EtOAc = 3:2) to give the title compound (2.97 g, 89%).

^{1}H NMR (300 MHz, acetone-d_6) δ 7.05-6.97 (m, 1H), 4.56-4.51 (m, 1H), 4.49-4.40 (m, 3H), 4.17 (dd, J = 7.5, 6.9 Hz, 2H), 1.55 (d, J = 6.6 Hz, 3H), 1.42 (s, 9H), 1.33-1.22 (m, 9H).

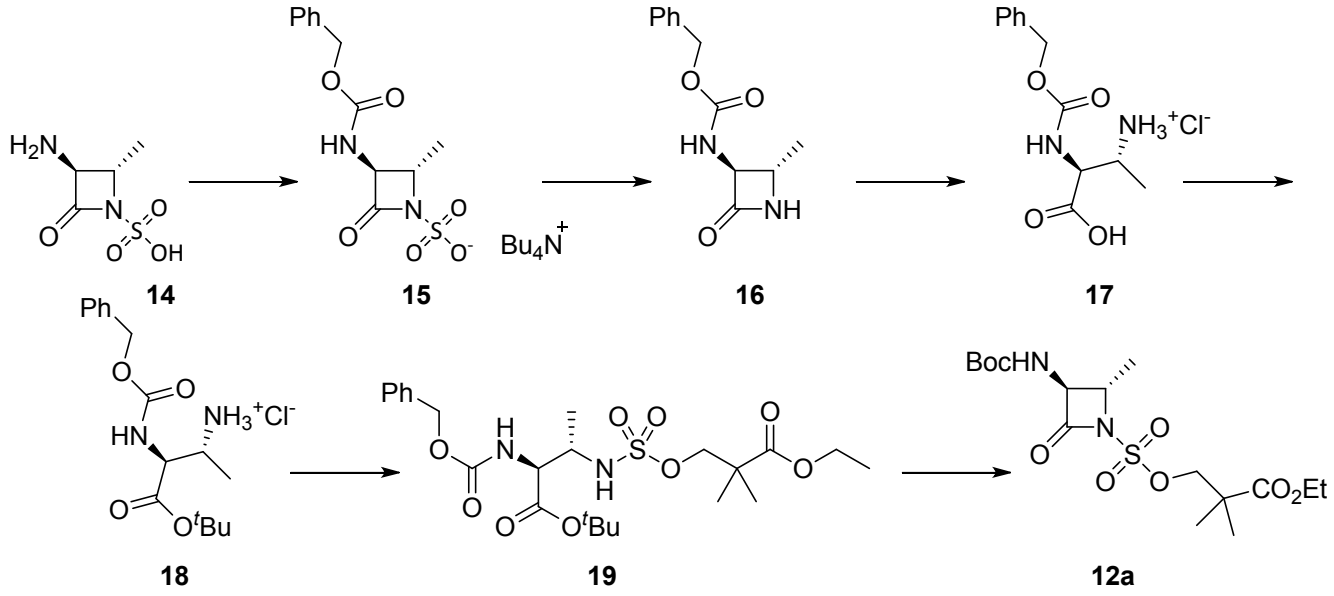
^{13}C NMR (75 MHz, acetone-d_6) δ 174.1, 164.1, 156.1, 79.9, 76.9, 64.6, 60.6, 59.1, 42.4, 27.5, 21.3, 21.1, 16.7, 13.5.

LC-MS: 409 $[M+H]^+$

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (d, J = 8.7 Hz, 1H), 4.54 (d, J = 10.2 Hz, 1H), 4.45 (dd, J = 8.9 Hz, 3.5 Hz, 1H), 4.24-4.11 (m, 4H), 1.58 (d, J = 5.7 Hz, 3H), 1.42 (s, 9H), 1.29-1.24 (m, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 165.2, 154.6, 81.0, 77.2, 64.5, 61.6, 60.7, 42.8, 28.3, 22.1, 21.8, 18.0, 14.1.

ALTERNATIVE SYNTHESIS OF BETA-LACTAM 12A FROM FIGURE 4



Preparation of tetrabutylammonium (2S, 3S)-3-(((benzyloxy)carbonyl)amino)-2-methyl-4-oxoazetidine-1-sulfonate 15

To a solution of (2*S*, 3*S*)-3-amino-2-methyl-4-oxo-1-azetidinesulfonic acid (55.0 g, 305.2 mmol) in a mixture of EtOH (600 mL) and H_2O (300 mL) was added Et_3N (159.5 mL, 915.7 mmol) followed by benzyloxycarbonyl *N*-succinimide (83.7 g, 335.8 mmol). The reaction mixture was stirred at rt for 16 hours. Ethanol was removed under vacuum and the residue was diluted with H_2O (200 mL). The aqueous layer was extracted with EtOAc (2 x 100 mL). The EtOAc was discarded. Tetrabutyl ammonium hydroxide (207.9 g, 320.5 mmol) as 40% w/v in H_2O was added and the resulting aqueous layer was extracted with $CHCl_3$ (5 x 150 mL). The organic extract was dried ($MgSO_4$) and concentered to give the product (160 g, 94%) as a solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38-7.31 (m, 5H), 4.31 (d, *J* = 7.2 Hz, 1H), 6.01 (s, 2 H) 5.50 (s, 1H), 5.12 (s, 2H), 4.31 (d, *J* = 7.2 Hz, 1H), 3.28-3.22 (m, 8H), 1.62-1.59 (m, 8H), 1.46-1.39 (m, 11H), 1.01-0.96 (m, 12H)

^{13}C-NMR (75 MHz, $CDCl_3$) δ 162.9, 155.7, 136.1, 135.7, 128.5, 128.3, 128.2, 128.0, 67.0, 62.7, 59.2, 58.5, 23.9, 19.6, 18.2, 13.7.

Preparation of benzyl ((2S, 3S)-2-methyl-4-oxoazetidin-3-yl)carbamate 16

(2*S*, 3*S*)-3-(((benzyloxy)carbonyl)amino)-2-methyl-4-oxoazetidine-1-sulfonate tetrabutylammonium salt (131 g, 235.7 mmol) was dissolved in dry THF (2.8 L) and H_2O (12.8 mL, 710.5 mmol), then the resulting solution was cooled in an ice bath. Trifluoroacetic acid (280 mL) was added dropwise, and the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was concentrated under vacuum, and the residue was diluted with DCM (1.5 L), then chilled with an ice bath. 10 % Aqueous NaOH solution was added slowly until pH = 8-9. The organic layer was washed with H_2O, dried ($MgSO_4$) and concentered under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / hexanes (1:9 to 1:0) to give the product (30.1 g, 54%) as a solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38-7.31 (m, 5H), 6.01 (s, 1 H) 5.50 (s, 1H), 5.12 (s, 2H), 4.31 (d, *J* = 7.2 Hz, 1H), 3.74-3.71 (m, 1H), 1.42 (d, *J* = 6 Hz, 3H)

^{13}C-NMR (75 MHz, $CDCl_3$) δ 167.4, 155.9, 136.1, 67.2, 64.7, 54.0, 19.2.

LC-MS: *m/z* = 280.1 $[M+HCO_2H]^+$

Preparation of (2S, 3R)-3-amino-2-(((benzyloxy)carbonyl)amino)butanoic acid HCl salt 17

Benzyl ((2*S*, 3*S*)-2-methyl-4-oxoazetidin-3-yl)carbamate azetidin-3-yl)carbamate (6.0 g, 25.6 mmol) was dissolved in neat formic acid (50mL). H_2O (50 mL) was added and the resulting mixture was stirred at rt for 18 hours. The mixture was concentrated under vacuum to give (2*S*, 3*R*)-3-amino-2-(((benzyloxy)carbonyl)amino)butanoic formic acid salt as a solid. The formic acid salt was dissolved in MeCN (10 mL). 4N HCl in dioxane (15 mL) was added and the resulting solution was concentrated to dryness under vacuum. Another portion of 4N HCl in dioxane (15 mL) was added and the mixture was stirred for 1 hour at rt – a solid formed. Et_2O (50 mL) was added and the solid was collected by filtration to give the product (6.8 g, 92%).

^{1}H-NMR (300 MHz, CD_3OD) δ 7.59 (d, *J* = 8.7 Hz, 1H), 7.41-7.30 (m, 5H), 5.15 (q, *J* = 21 Hz, 2H), 4.68-4.64 (m, 1H), 3.88-3.84 (m, 1H), 1.25 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, CD_3OD) δ 171.4, 159.1, 137.8, 129.5, 129.1, 129.0, 68.3, 56.9, 49.9, 14.1.

LC-MS: *m/z* = 253.3 $[M+H]^+$

Preparation of tert-butyl (2S, 3R)-3-amino-2-(((benzyloxy)carbonyl)-amino)butanoate 18

To a solution of (2*S*, 3*S*)-3-amino-2-(((benzyloxy)carbonyl)amino)butanoic acid hydrochloride (4.5 g, 15.5 mmol) in 1,4-dioxane (36 mL) at -10 °C was added dropwise concentrated H_2SO_4 (4.9 ml, 92.8 mmol). The reaction mixture was placed in a dry ice-acetone bath and iso-butylene (36 g, 622.5 mmol) was added. The reaction vessel was capped, and the reaction mixture was stirred at rt for 48 hours. Iso-butylene was removed by passing nitrogen through the solution, and the mixture was poured into a mixture of H_2O (150 mL) and saturated aqueous Na_2CO_3 (50 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL), and the combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and concentered under vacuum. The residue was purified by column chromatography on silica gel using DCM / MeOH (1:0 to 9:1) to the product (2.83 g, 59%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37-7.31 (m, 5H), 5.52 (d, *J* = 6.9 Hz, 1H), 5.11 (s, 2H), 4.28-4.24 (m, 1H), 3.33-3.30 (m, 1H), 1.46 (s, 9H), 1.05 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 156.7, 136.3, 128.6, 128.3, 128.2, 82.5, 67.1, 60.5, 49.5, 28.1, 18.9.

LC-MS: *m/z* = 309.6 $[M+H]^+$

Alternative preparation of tert-butyl (2S, 3R)-3-amino-2-(((benzyloxy)carbonyl)-amino)butanoate 18

A reactor equipped with a mechanical stirrer was prepared and flushed with Argon. Under Argon, (2*S*,3*S*)-3-amino-2-(((benzyloxy)carbonyl)amino)butanoic acid hydrochloride (28.77 g, 0.10 mol) and *tert*-butyl acetate (288 mL, 2.15 mol, 21.5 eq) were added to the reactor, stirred mechanically, and cooled in an ice bath. H_2SO_4 (12.1 g, 0.12 mol, 1.20 eq) was dripped in over 10 minutes. The resulting slurry was immersed in a 20 °C bath for 10 minutes, then AcOH (72 mL) was dripped in over 20 minutes. After

17 hours, the homogeneous reaction solution was cooled with an ice bath while dripping in a solution of NaOH (96.8 g) in H_2O (450 mL). The resulting solution was extracted with EtOAc (600 mL). The extract was washed with brine, dried (Na_2SO_4), and concentrated to give the product (14.95 g). Data as above.

Preparation of tert-butyl (2S,3S)-2-(((benzyloxy)carbonyl)amino)-3-(((3-ethoxy-2,2-dimethyl-3-oxopropoxy)sulfonyl)amino)butanoate 19

(2*S*,3*S*)-*tert*-butyl 3-amino-2-(((benzyloxy)carbonyl)amino)butanoate 1 (8.90 g, 28.9 mmol) was dissolved in 1,2-dichloroethane (150 mL) and combined with ethyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (10.40 g, 37.4 mmol). This solution was combined in a round-bottomed flask with 200 mL of saturated aq. sodium bicarbonate. A stir bar was added and the flask sealed with a rubber septum into which a needle connected to a balloon containing Argon was introduced. The mixture was heated in a 40 °C and stirred rapidly for 46 hours. Stirring was stopped, the phases were partitioned, and the aq. phase was extracted with DCM (50 mL). The organic phases were combined, dried with sodium sulfate, and concentrated to an oil (17.0 g). The oil was purified by column chromatography on silica gel (330-g column) using Et_2O / Hexanes (1:9 to 2:3) as eluent [monitoring for absorbances at 210-nM] to give pure product (6.40 g), together with impure fractions (3.15 g). A significant amount of non-transformed starting material remained on top of the column. This was recovered by washing the column with MeOH / DCM (1:9) as eluent to give starting material (2.27 g). The impure fractions (3.15 g) from the column were re-purified to provide additional pure product (2.17 g). Considering recovery of starting material, the percent yield of this reaction is 77%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.33 (m, 5H), 6.02 (d, J = 7.5 Hz, 1H), 5.73 (d, J = 5.7 Hz, 1H), 5.13 (s, 2H), 4.50 (dd, J = 7.1, 5.7 Hz, 1H), 4.20-4.11 (m, 4H), 3.99-3.95 (m, 1H), 1.47 (s, 9H), 1.27-1.23 (m, 9H), 1.17 (d, J = 6.6 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 168.5, 157.3, 135.8, 128.7, 128.5, 128.4, 84.0, 75.4, 67.8, 61.2, 58.5, 53.0, 42.8, 28.0, 22.2, 16.5, 14.2.

LC-MS: 517.0 $[M+H]^+$

Preparation of 3-(3-tert-butoxycarbonylamino-2-methyl-4-oxo-azetidine-1-sulfonyloxy)-2,2-dimethyl-propionic acid ethyl ester 12a

Into a reactor equipped with a bubbler and an inlet of Argon was added *tert*-butyl (2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-3-(((3-ethoxy-2,2-dimethyl-3-oxopropoxy) sulfonyl)amino)butanoate (15.29 g, 30.0 mmol). An Argon atmosphere was established and anhydrous MeCN (460 mL) was added, stirring to dissolve. The resulting solution was cooled in an ice bath while 4 M HCl in 1,4-dioxane (153 mL, 612 mmol, 20.7 eq) was added dropwise over 20 minutes. The reaction mixture was heated at 40 °C and stirred for 2 hr [note: a constant stream of Argon passing into and out of the reactor for the duration of the reaction]. The solution was concentrated on a rotary evaporator then re-concentrated with MeCN (150 mL) and re-evaporated. This procedure was repeated x 4. The crude, de-*tert*-butylated product (15.51 g) was used without further purification.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.32 (m, 5H), 6.07 (d, J = 7.5 Hz, 1H), 5.91 (d, J = 6.6 Hz, 1H), 5.14 (s, 2H), 4.63-4.61 (m, 1H), 4.24-4.05 (m, 4H), 3.93 (m, 1H), 1.30-1.24 (m, 12H).

LCMS: 460.97 $[M+H]^+$

The crude de-*tert*-butylated product above (10.38 g used in this step) was combined with 10% palladium-on-carbon [50% w/w with H_2O] (2.33 g) and MeOH (120 mL) in a round-bottomed flask equipped with a stir bar. The mixture was placed under an atmosphere of hydrogen and the solution stirred for 1 hr. The mixture was filtered and the filter cake was washed with MeOH (80 mL). The filtrate was concentrated under vacuum and dried further in a vacuum oven set to 60 °C to give a crude amino-acid product (7.21 g) that was used without further purification.

^{1}H NMR (300 MHz, d_6-DMSO) δ 7.97 (s, 3H), 4.12-3.97 (m, 4H), 3.87-3.84 (m, 1H), 3.45 (d, J = 1.8 Hz, 1H), 1.18-1.14 (m, 9H), 1.07 (d, J = 6.9 Hz, 3H)

^{13}C NMR (75 MHz, d_6-DMSO) δ 174.3, 169.0, 74.5, 60.6, 58.4, 50.3, 42.2, 21.8, 21.7, 14.7, 14.0.

LC-MS (ESI): 327.2 $[M+H]^+$

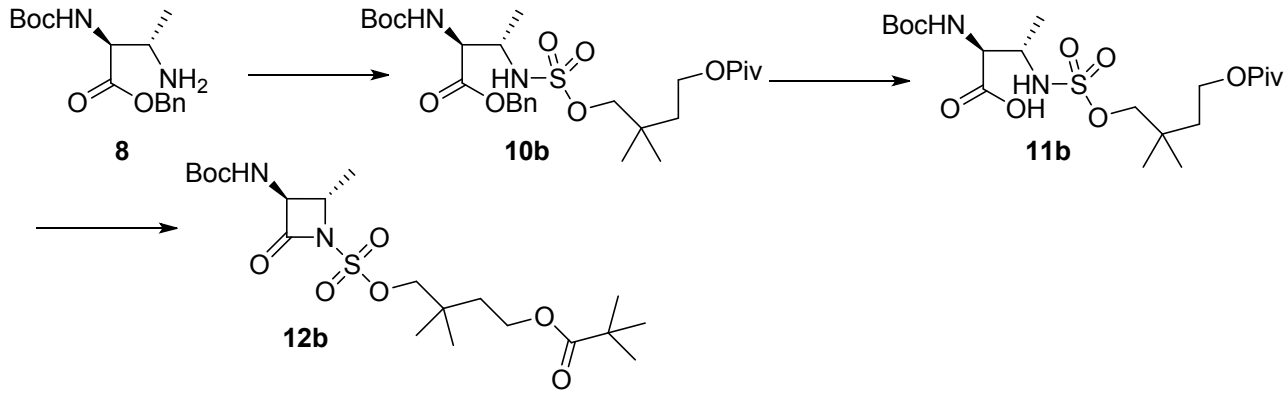
This crude amino-acid product above (7.38 g) was combined with di-*tert*-butyl dicarbonate and tetrahydrofuran (120 mL). To this stirred solution was added Et_3N (7.9

mL) and a balloon of Argon was placed above the reaction. The mixture was stirred at 40 °C for 4 hr. The mixture was concentrated under vacuum, then mixed with H_2O (150 mL) and extracted with hexanes (150 mL). The aqueous phase was combined with EtOAc (150 mL) and stirred vigorously while 7.90 g of citric acid was added in one portion. The aqueous and organic layers were partitioned, and the aqueous phase was extracted with EtOAc (150 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. The product was dried further in a vacuum oven (set at 55 °C) to give the product (9.0 g) that was used without further purification.

LC-MS: 426.7 $[M+H]^+$

The crude Boc-amino acid from above (5.18 g of the sample) was dissolved in anhydrous MeCN (300 mL) and placed under an atmosphere of Argon. The solution was stirred and cooled in an ice-water bath for 5 min, then *N*,*N*,*N*',*N*'-tetramethylchloroformamidinium hexafluorophosphate [TCFH] (4.77 g, 17.0 mmol) was added in one portion. The mixture was stirred in the ice bath for 30 min, then at room temperature for an additional 15 min. The mixture was concentrated under vacuum. The residue was mixed with H_2O (40 mL) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under vacuum. This material was purified by column chromatography on silica gel (120 g column) using EtOAc / Hexanes (0:1 to 1:0) as eluent to give the product **12a** (3.78 g, 79% yield over 4 steps) after drying in a vacuum oven (set at 55 °C). Data as above.

SYNTHESIS OF BETA-LACTAM INTERMEDIATE 12B



Preparation of Compound (2S,3S)-benzyl 2-((tert-butoxycarbonyl)amino)-3-(((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)amino)butanoate 10b

A solution of (2*S*,3*S*)-benzyl 3-amino-2-((*tert*-butoxycarbonyl)amino)butanoate **8** (2.7 g, 8.76 mmol) in DCE (25 mL) was treated with saturated aq. $NaHCO_3$ (29 mL) and TBAB (284 mg, 0.88 mmol). The mixture was warmed to 40 °C then a solution of 4-((chlorosulfonyl)oxy)-3,3-dimethylbutyl pivalate **9b** (3.95 g, 13.13 mmol) in DCE (4 mL) was added dropwise. The reaction was stirred rapidly overnight, then cooled to rt. The organic phase was isolated and dried ($NaSO_4$), filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / DCM (0:1 to 7:93) as eluent to give the title compound **10b** (2.07 g, 41%) as an oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.35 (br, 5H), 5.91 (s, 1H), 5.43 (s, 1H), 5.21 (s, 2H), 4.53 (q, *J* = 6.0 Hz, 1H) 4.12 (q, *J* = 7.1 Hz, 2H), 3.99 (s, 1H), 3.92-3.77 (m, 2H), 1.65 (t, *J* = 7.0 Hz, 2H), 1.44 (s, 9H), 1.18 (s, 9H), 1.12 (d, *J* = 6.0 Hz, 3H), 0.99 (d, *J* = 1.0 Hz, 6H).

Preparation of (2S,3S) 2-((tert-butoxycarbonyl)amino)-3-(((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)amino)butanoic acid 11b

To a solution (2*S*,3*S*)-benzyl 2-((*tert*-butoxycarbonyl)amino)-3-(((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)amino)butanoate **10b** (2.07 g, 3.61 mmol) in EtOAc (18 mL) was added Pd/C (769 mg, 10% dry) under a N_2 balloon. The N_2 balloon was replaced with a H_2 balloon, and the reaction mixture was evacuated under vacuum, then

placed under H_2 – this cycle was repeated x3. The reaction was then stirred for 2 h under H_2 and monitor by TLC (5 : 95 EtOAc / DCM). The Pd/C was removed by filtration through a pad of Celite, and the filter cake was washed with EtOAc (x3). The solvent was removed under vacuum to give the crude product **11b** (1.66 g). LCMS: *m/z* = 483 $[M+H]^+$

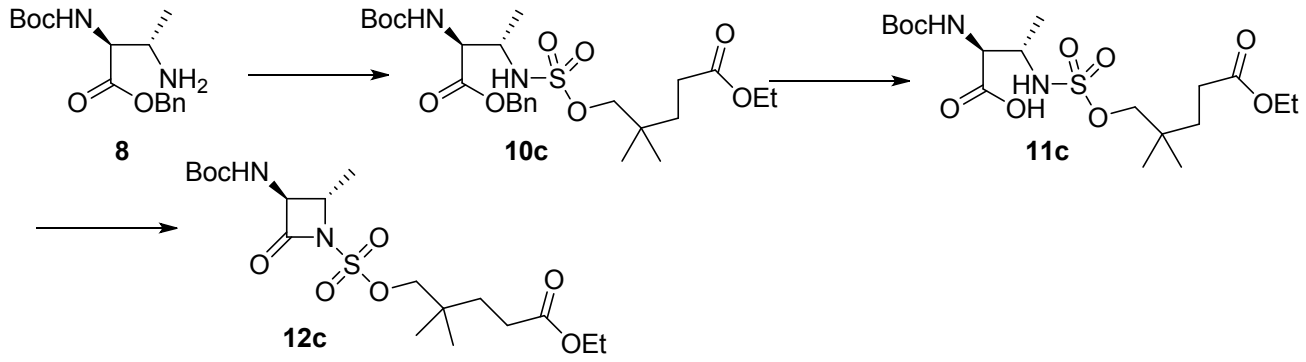
Preparation of 4-((((2S, 3S)-3-((tert-butoxycarbonyl)amino)-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-3,3-dimethylbutyl pivalate 12b

A solution of (2*S*,3*S*) 2-((*tert*-butoxycarbonyl)amino)-3-(((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)amino)butanoic acid **11b** (1.66 g) in MeCN (100 mL) was treated with Et_3N (1.05 mL, 7.57 mmol) at 0 ^oC, followed by addition of TCFH (1.45 g, 5.16 mmol) in MeCN (15 mL). The reaction was then stirred for 2h at rt then concentrated under vacuum (Note: water bath temperature <28 ^oC). The residue was re-dissolved in DCM and then purified by column chromatography on silica gel using EtOAc / DCM (0:1 to 1:9) as eluent to give the title compound **12b** (1.28 g, 80% for 2 steps).

^1H NMR (300 MHz, $CDCl_3$) δ 5.46 (d, *J* = 6.0 Hz, 1H), 4.35-4.08 (m, 6H), 1.69 (dt, *J* = 7.0, 3.9 Hz, 2H), 1.62 (d, *J* = 6.0 Hz, 3H), 1.44 (s, 9H), 1.19 (s, 9H), 1.04 (d, *J* = 4.1 Hz, 6H).

LCMS: *m/z* = 465 $[M+H]^+$, 482 $[M+H+17]^+$.

SYNTHESIS OF BETA-LACTAM INTERMEDIATE 12C



Preparation of (2S,3S)-2-tert-butoxycarbonylamino-3-[2-(2-methoxy-ethoxycarbonyl)-2-methyl-propoxysulfonylamino]-butyric acid benzyl ester 10c

To a solution of (2*S*,3*S*)-benzyl 3-amino-2-((*tert*-butoxycarbonyl)amino)butanoate **8** (2.90 g, 9.39 mmol) in DCE (15 mL) was added a saturated aqueous solution of $NaHCO_3$ (15 mL) and tetrabutylammonium bromide (757 mg, 2.35 mmol). To the solution was added 5-chlorosulfonyloxy-4,4-dimethyl-pentanoic acid ethyl ester **9c** (5.12 g, 18.8 mmol) in DCE (15 mL) at 0 ºC. The mixture was stirred at 0 ºC for 2h and warmed up to rt and stirred for 20 h. The reaction mixture was diluted with DCM (50 mL) and washed with brine and H_2O. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / Hexanes (3:2) as eluent to give the title compound **10c** (654 mg, 13%) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34-7.29 (m, 5H), 5.94-5.91 (m, 1H), 5.44-5.42 (m, 1H), 5.21 (s, 2H), 4.55-4.52 (m, 1H), 4.13 (app. q, *J* = 6.9, 5.4 Hz, 2H), 3.99-3.97 (m, 1H), 3.85-3.76 (m, 2H), 2.34-2.27 (m, 2H), 1.72-1.67 (m, 2H), 1.48 (s, 9H), 1.26 (t, *J* = 6.0 Hz, 3H), 1.12 (d, *J* = 7.2 Hz, 3H), 1.00 (s, 6H).

Preparation of Compound 11c

To a solution of (2*S*,3*S*)-2-*tert*-butoxycarbonylamino-3-[2-(2-methoxy-ethoxycarbonyl)-2-methyl-propoxysulfonylamino]-butyric acid benzyl ester **10c** (5.18 g, 9.47 mmol) in EtOAc was added Pd/C (500 mg). The suspension was degassed 3 times and refilled with hydrogen. The mixture was stirred under an atmosphere of H_2 at rt for 2h. The mixture

was filtered through a pad of Celite, and the filtrate was concentrated to under vacuum, affording the title compound **11c** (4.23 g, 98%).

LCMS: *m/z* = 457 $[M+H]^+$.

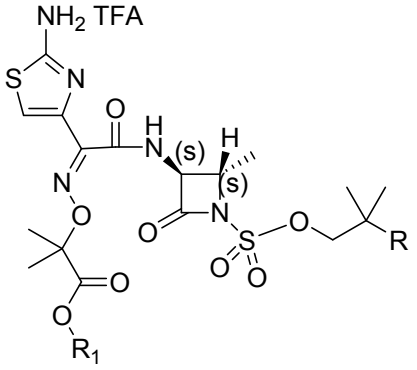
Preparation of ethyl 5-((((2S,3S)-3-((tert-butoxycarbonyl)amino)-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-4,4-dimethylpentanoate 12c

To a solution of compound **11c** (1.16 g, 2.55 mmol) in MeCN (100 mL) at 0 °C was added TCFH (1.08 g, 3.82 mmol) and Et_3N (924 μL, 6.63 mmol) at 0 ºC. The reaction was stirred at 0 ºC for 10 min and then concentrated under vacuum. The residue was diluted to EtOAc (200 mL) and washed with H_2O and brine. The organic phase was separated, dried, and concentrated under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / Hexanes (3:2) as eluent to give the title compound **12c** (905 mg, 82%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.40-4.26 (m, 2H), 4.18-4.09 (m, 4H), 2.34-2.28 (m, 2H), 1.72-1.67 (m, 2H), 1.60 (d, *J* = 6.6 Hz, 3H), 1.44 (s, 9H), 1.25 (t, *J* = 6.9 Hz, 3H), 1.00 (s, 3H), 0.98 (s, 3H).

LCMS: *m/z* = 437 $[M+H]^+$.

SYNTHESIS OF FINAL PRODRUGS 27A TO 27C, 28A TO 28C AND 29B TO 29C



27a R_1= Et, R=
27b R_1= *t*-C$(CH_3)_3$ R= —$CO_2CH_2CH_3$
27c R_1= H, R=

28a R_1= Et, R=
28b R_1= *t*-C$(CH_3)_3$ R= —$CH_2CH_2OCOC(CH_3)CH_3$
28c R_1= H, R=

29a R_1= Et, R=
29b R_1= *t*-C$(CH_3)_3$ R= —$CH_2CH_2\ CO_2CH_2CH_3$
29c R_1= H, R=

Part 1: General method of Boc deprotection

12a —$CO_2CH_2CH_3$
12b —$CH_2CH_2OCOC(CH_3)_3$
12c —$CH_2CH_2\ CO_2CH_2CH_3$

13a —$CO_2CH_2CH_3$
13b —$CH_2CH_2OCOC(CH_3)_3$
13c —$CH_2CH_2\ CO_2CH_2CH_3$

Preparation of 3-(3-amino-2-methyl-4-oxo-azetidine-1-sulfonyloxy)-2,2-dimethyl-propionic acid ethyl ester methanesulfonic acid salt 13a

To a solution of 3-(3-*tert*-butoxycarbonylamino-2-methyl-4-oxo-azetidine-1-sulfonyloxy)-2,2-dimethyl-propionic acid ethyl ester **12a** (1.30 g, 3.44 mmol) in DCM (15 mL) was treated with MsOH (330 mg, 3.44 mmol) at rt for 20 h. The reaction mixture was concentrated to dryness and the residue was used in the next step without further purification.

LCMS: *m/z* = 309 $[M+H]^+$.

Preparation of 4-((((2S,3S)-3-amino-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-3,3-dimethylbutyl pivalate 13b

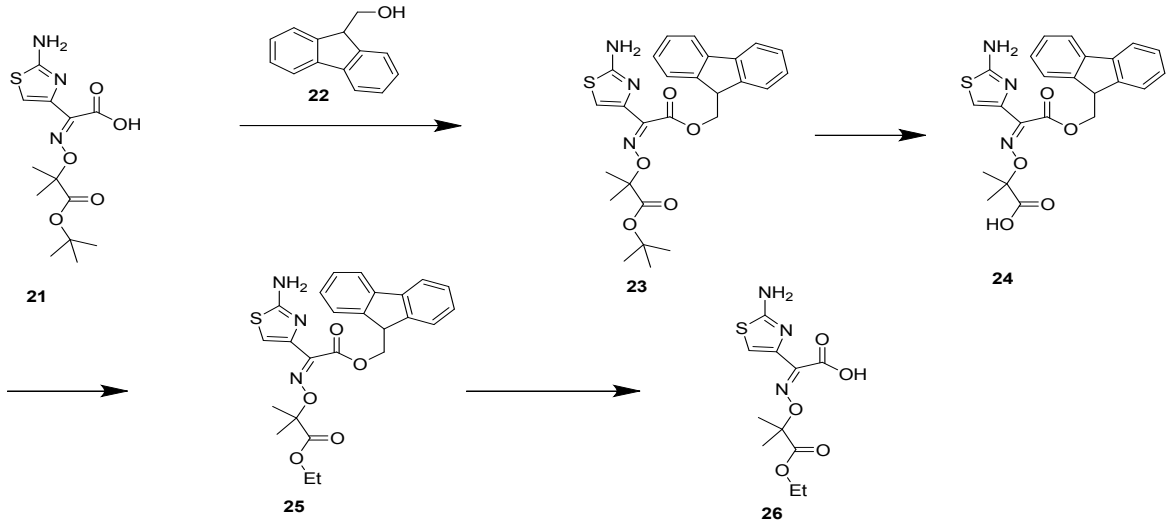
Undertaken on 1.24 g. (no data).

Preparation of ethyl 5-((((2S,3S)-3-amino-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-4,4-dimethylpentanoate 13c

Undertaken on 905 mg.

LCMS: *m/z* = 337 $[M+H]^+$.

Part 2: Synthesis of compound 26



Preparation of 2-[(2-amino-thiazol-4-yl)-(9H-fluoren-9-ylmethoxycarbonyl)-methyleneaminooxy]-2-methyl-propionic acid tert-butyl ester 23

To a suspension of 2-[(2-amino-thiazol-4-yl)-carboxy-methyleneaminooxy]-2-methyl-propionic acid *tert*-butyl ester (19.1 g, 57.9 mmol) in DCM (200 mL) was added (9*H*-fluoren-9-yl)-methanol (9.91 g, 57.9 mmol), EDCI (16.6 g, 86.9 mmol) and pyridine (14.0 mL, 174 mmol). The clear solution was stirred at rt as solid started to form. After 2-3 h, the mixture was filtered and the solid was rinsed with DCM (30 mL), affording the title compound **23** (27.3 g, 93%) as a solid.

LCMS: *m/z* = 508.0 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.35 (t, *J* = 7.5 Hz, 2H), 6.93 (br. s, 2H), 6.41 (s, 1H), 4.62 (d, *J* = 6.9 Hz, 2H), 4.33 (t, *J* = 7.2 Hz, 1H), 1.58 (s, 6H), 1.56 (s, 9H)

^{1}H-NMR (300 MHz, d_6-DMSO), δ 7.88 (d, *J* = 7.2 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.33-7.30 (m, 4H), 6.55 (s, 1H), 4.64 (d, *J* = 6.3 Hz, 2H), 4.32 (t, *J* = 6.5 Hz, 1H), 1.35 (s, 15H)

^{13}C-NMR (75MHz, d_6-DMSO), δ 172.1, 169.3, 162.9, 147.0, 143.8, 141.5, 141.2, 128.3, 127.6, 125.7, 120.7, 109.5, 82.9, 81.0, 67.0, 46.6, 28.0, 24.0.

Preparation of 2-{(9H-fluoren-9-ylmethoxycarbonyl)-[2-(2,2,2-trifluoro-acetylamino)-thiazol-4-yl]-methyleneaminooxy}-2-methyl-propionic acid TFA salt 24

A solution of 2-[(2-amino-thiazol-4-yl)-(9*H*-fluoren-9-ylmethoxycarbonyl)-methyleneaminooxy]-2-methyl-propionic acid *tert*-butyl ester **23** (27.3 g, 53.8 mmol) in TFA (50 mL) was stirred at rt for 2h. The reaction mixture was concentrated to dryness. The residue was triturated with Et_2O (200 mL) and filtered to afford the title compound **24** (29.3 g, 100%) as a solid.

LCMS: *m/z* = 452.2 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.5 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 2H), 6.08 (s, 1H), 4.71 (d, *J* = 6.6 Hz, 2H), 4.28 (t, *J* = 6.6 Hz, 1H), 1.56 (s, 6H)

^{1}H-NMR (300 MHz, d_6-DMSO) δ 7.88 (d, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 6.54 (s, 1H), 4.66 (d, *J* = 6.6 Hz, 2H), 4.33 (t, *J* = 6.5 Hz, 1H), 1.35 (s, 6H)

^{13}C-NMR (75MHz, d_6-DMSO) δ 174.7, 169.5, 162.6, 146.2, 143.8, 141.2, 140.2, 128.3, 127.7, 125.7, 120.6, 110.3, 82.6, 67.1, 46.6, 24.1.

Preparation of 2-[(2-amino-thiazol-4-yl)-(9H-fluoren-9-ylmethoxycarbonyl)-methyleneaminooxy]-2-methyl-propionic acid ethyl ester 25

To a suspension of 2-{(9*H*-fluoren-9-ylmethoxycarbonyl)-[2-(2,2,2-trifluoro-acetylamino)-thiazol-4-yl]-methyleneaminooxy}-2-methyl-propionic acid TFA salt **24** (9.02 g, 16.0 mmol) in DCM (200 mL) and EtOH (5 mL) at 0 ºC was added EDCI (4.60 g, 24.0 mmol) and pyridine (6.47 mL, 80.0 mmol). The clear solution was allowed to warm to rt and stirred for 18h. The mixture was washed with saturated $NaHCO_3$ and H_2O. The organic phase was separated, dried, and concentrated to dryness. The residue

was purified by flash column chromatography (silica, Hexanes / EtOAc = 2:1) to afford the title compound **25** (3.50 g, 46%).

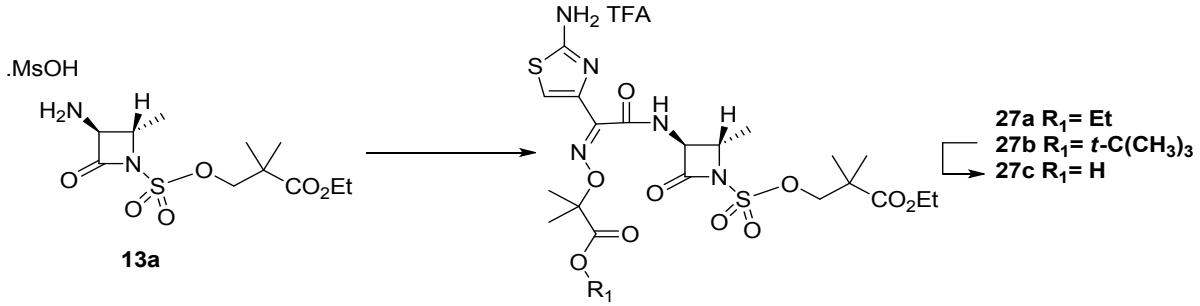
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.5 Hz, 2H), 6.58 (s, 1H), 6.22 (br. s, 2H), 4.62 (d, *J* = 6.9 Hz, 2H), 4.31 (t, *J* = 7.2 Hz, 1H), 4.22 (dd, *J* = 7.5, 6.9 Hz, 2H), 1.56 (s, 6H), 1.24 (t, *J* = 7.5 Hz, 3H).

Preparation of 2-[(2-amino-thiazol-4-yl)-carboxy-methyleneaminooxy]-2-methyl-propionic acid ethyl ester 26

To a solution of 2-[(2-amino-thiazol-4-yl)-(9*H*-fluoren-9-ylmethoxycarbonyl)-methyleneaminooxy]-2-methyl-propionic acid ethyl ester **25** (1.84 g, 3.84 mmol) in DCM (20 mL) was treated with piperidine (654 mg, 7.68 mmol). The reaction mixture was stirred at rt for 3h, then concentrated to dryness. The residue was purified by preparative HPLC to give the title compound **26** (1.17 g, 73%) as a solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1H), 4.16 (dd, *J* = 7.5, 6.9 Hz, 2H), 1.57 (s, 6H), 1.24 (t, *J* = 7.5 Hz, 3H).

Part 3: General method for the synthesis of finals compounds, illustrated for compounds 27a to 27c



Preparation of ethyl 3-((((2S,3S)-3-((Z)-2-(2-aminothiazol-4-yl)-2-(((1-ethoxy-2-methyl-1-oxopropan-2-yl)oxy)imino)acetamido)-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-2,2-dimethylpropanoate TFA salt 27a

To a solution of 3-(3-amino-2-methyl-4-oxo-azetidine-1-sulfonyloxy)-2,2-dimethyl-propionic acid ethyl ester methanesulfonic acid salt **13a** (1.19 g, 2.95 mmol) and 2-[(2-amino-thiazol-4-yl)-carboxy-methyleneaminooxy]-2-methyl-propionic acid ethyl ester **26** (1.22 g, 2.95 mmol) in DCM (100 mL) was added EDCI (847 mg, 4.43 mmol) at 0 ºC. The reaction was stirred for 10 min and concentrated to dryness. The residue was purified by preparative HPLC to give the title compound **27a** (1.20 g, 58%) as a solid.

LCMS: *m/z* = 592 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.38-9.25 (br. s, 2H), 9.12 (d, *J* = 6.9 Hz, 1H), 7.33 (s, 1H), 4.53-4.40 (m, 4H), 4.25-4.11 (m, 4H), 1.73 (s, 3H), 1.65 (d, *J* = 6.9 Hz, 3H), 1.28-1.23 (m, 6H)

^{1}H-NMR (300 MHz, CD_3OD) δ 7.02 (s, 1H), 4.58 (d, *J* = 3.5 Hz, 1H), 4.51-4.42 (m, 2H), 4.38 (d, *J* = 9.1 Hz, 1H), 4.23-4.04 (m, 4H), 1.62-1.51 (m, 9H), 1.29-1.14 (m, 12H)

^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.9, 174.4, 170.7, 162.7, 159.6, 141.7, 132.5, 117.9, 114.1, 110.2, 85.3, 63.5, 62.3, 61.5, 59.2, 42.9, 23.7, 22.0, 17.9, 14.2, 14.1

HRMS (ESI): [M+H]+ calc'd for $C_{22}H_{33}N_5O_{10}S_2$ m/z 592.1747, found 592.1743.

Preparation of 2-(((Z)-(1-(2-aminothiazol-4-yl)-2-(((2S,3S)-1-((3-ethoxy-2,2-dimethyl-3-oxopropoxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic acid TFA salt 27b

To a solution of 3-(3-amino-2-methyl-4-oxo-azetidine-1-sulfonyloxy)-2,2-dimethyl-propionic acid ethyl ester methane sulfonic acid salt **13a** (1.06 g, 3.44 mmol) and 2-[(2-amino-thiazol-4-yl)-carboxy-methyleneaminooxy]-2-methyl-propionic acid *tert*-butyl ester **21** (1.13 g, 3.44 mmol) in DCM (140 mL) was added EDCI (988 mg, 5.16 mmol) at 0 ºC for 10 min. The reaction was concentrated to dryness and the residue was purified by preparative HPLC to give the title compound **27b** (783 mg, 37%) as a solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.15 (d, *J* = 6.9 Hz, 1H), 7.35 (s, 1H), 4.57-4.41 (m, 4H), 4.16 (dd, *J* = 7.2, 6.9Hz, 2H), 1.72-1.68 (m, 9H), 1.46 (s, 9H), 1.28-1.24 (m, 9H)

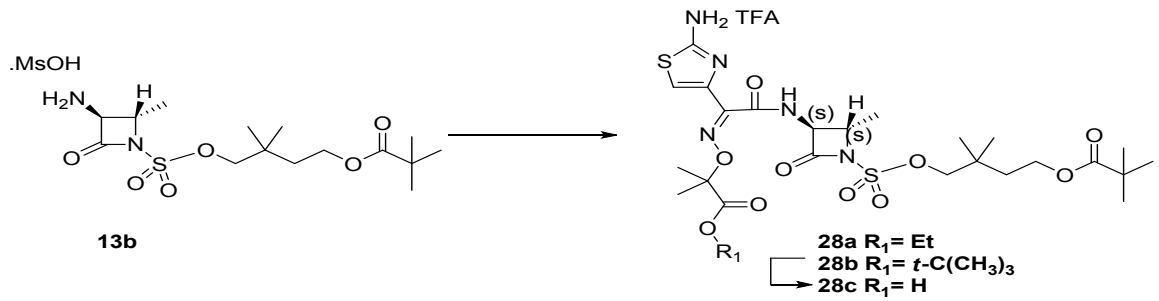
^{1}H-NMR (300 MHz, CD_3OD) δ 7.03 (s, 1H), 4.57 (d, *J* = 3.5 Hz, 1H), 4.49-4.42 (m, 2H), 4.37 (d, *J* = 9.1 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 1.54 (d, *J* = 8.3 Hz, 9H), 1.41 (s, 9H), 1.27-1.13 (m, 9H)

^{13}C-NMR (75 MHz, $CDCl_3$) δ 175.0, 173.7, 170.6, 162.6, 159.6, 141.5, 132.5, 117.8, 113.9, 110.2, 85.6, 83.4, 63.6, 61.5, 59.1, 43.1, 27.9, 23.7, 23.4, 22.0, 18.0, 14.2
HRMS (ESI): [M+H]+ calc'd for $C_{24}H_{37}N_5O_{10}S_2$ m/z 620.2060, found 620.2085.

Preparation of 2-(((Z)-(1-(2-aminothiazol-4-yl)-2-(((2S,3S)-1-((3-ethoxy-2,2-dimethyl-3-oxopropoxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic acid TFA salt 27c

A sample of the above reaction mixture to form compound **27b** was slowly added to a solution of TFA/DCM (15/100, 8.7 mL/58 mL) at rt. The reaction was stirred for 2-3 h and monitored by LCMS. When completed, the reaction was concentrated *in vacuo* and the residue dissolved in MeCN and purified by preparative HPLC using 10-100% MeCN / H_2O with 0.1% TFA (30 min, flow rate 20 mL/min) to give the title compound **27c** (0.77 g, 71% for 3 steps) as a solid.
LC-MS: *m/z* = 564 $[M+H]^+$
^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.83 (s, 1H), 7.04 (s, 1H), 4.51-4.46 (m, 1H), 4.40 (dd, *J* = 27.7, 9.1 Hz, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 1.61 (d, *J* = 9.9 Hz, 9H), 1.31-1.19 (m, 9H)
^{1}H-NMR (300 MHz, CD_3OD) δ 7.03 (s, 1H), 4.59 (d, *J* = 3.5 Hz, 1H), 4.51-4.40 (m, 2H), 4.38 (d, *J* = 9.1 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 1.64-1.47 (m, 9H), 1.26-1.12 (m, 9H)
^{13}C-NMR (75 MHz, $CDCl_3$) δ 176.7, 175.2, 170.5, 163.4, 159.7, 141.4, 131.4, 110.8, 85.2, 77.7, 77.4, 63.3, 61.7, 59.6, 42.9, 41.0, 23.6, 23.3, 21.9, 17.7, 14.1
HRMS (ESI): $[M+H]^+$ calc'd for $C_{20}H_{29}N_5O_{10}S_2$ m/z 564.1434, found 564.1470.

Part 4: Synthesis of finals compounds 28a to 28c using the general methods outlined above



Preparation of 4-((((2S,3S)-3-((Z)-2-(2-aminothiazol-4-yl)-2-(((1-(tert-butoxy)-2-methyl-1-oxopropan-2-yl)oxy)imino)acetamido)-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-3,3-dimethylbutyl pivalate TFA salt 28a

(0.57 g, 59% for 2 steps) as a solid.

LC-MS: *m/z* = 648 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.30 (s, 2H), 8.94 (d, *J* = 7.1 Hz, 1H), 7.20 (s, 1H), 5.55 (s, 2H), 4.51 (m, 1H), 4.37 (q, *J* = 3.0 Hz, 1H), 4.30-4.07 (m, 6H), 1.74-1.60 (m, 9H), 1.30 (t, *J* = 6.0 Hz, 3H), 1.18 (s, 9H), 1.05 (s, 6H)

^{19}F-NMR (282 MHz, $CDCl_3$) δ -76.0

^{13}C-NMR (75 MHz, $CDCl_3$) δ 178.8, 174.6, 170.7, 162.1, 159.7, 142.1, 133.0, 122.0, 118.2, 110.0, 85.2, 80.5, 63.8, 63.5, 62.2, 60.9, 58.5, 38.6, 36.9, 33.8, 27.3, 27.0, 24.1, 24.0, 23.5, 17.9, 14.1.

HRMS (ESI): [M+H]+ calc'd for $C_{26}H_{41}N_5O_{10}S_2$ m/z 648.2373, found 648.2399.

Preparation of 2-(((Z)-(1-(2-aminothiazol-4-yl)-2-(((2S,3S)-1-((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic acid TFA salt 28b

(1.32 g, 73% for 2 steps) as a solid.

LCMS: *m/z* = 678 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.20 (s, 1H), 9.14 (d, *J* = 7.1 Hz, 1H), 7.28 (s, 1H), 4.53 (ddd, *J* = 7.5, 6.2, 3.5 Hz, 1H), 4.35 (dd, *J* = 7.1, 3.5 Hz, 1H), 4.30-4.07 (m, 4H), 1.79-1.60 (m, 15H), 1.47 (s, 9H), 1.25-1.14 (m, 9H), 1.06 (s, 6H).

Preparation of 2-(((Z)-(1-(2-aminothiazol-4-yl)-2-(((2S,3S)-1-((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic 28c

(1.14 g, 70% over 3 steps) as a solid.

LCMS: *m/z* = 620 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.08 (br s, 2H), 7.09 (s, 10H), 4.49 (br, 2H), 4.33-4.03 (m, 4H), 1.92-1.55 (m, 9H), 1.18 (s, 9H), 1.05 (d, *J* = 1.7 Hz, 6H)

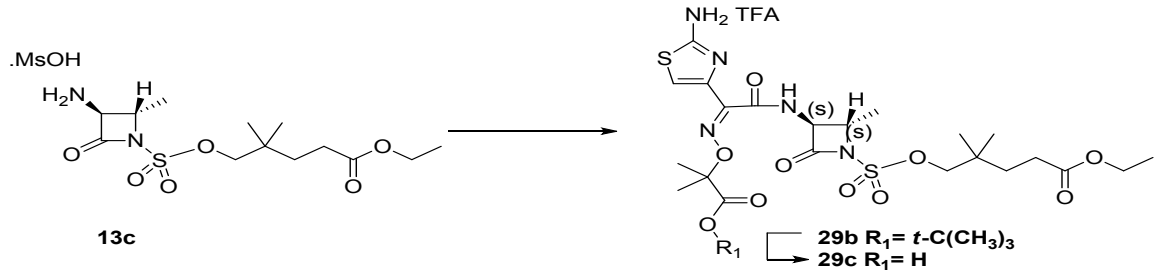
^{1}H-NMR (300 MHz, CD_3OD) δ 7.03 (s, 1H), 4.54 (d, *J* = 3.5 Hz, 1H), 4.48 (dd, *J* = 6.2, 3.5 Hz, 1H), 4.24 (d, *J* = 9.1 Hz, 1H), 4.15 (d, *J* = 9.2 Hz, 1H), 4.10 (t, *J* = 6.3 Hz, 2H), 1.66 (t, *J* = 6.9 Hz, 2H), 1.51-1.59 (m, 9H), 1.13 (d, *J* = 1.9 Hz, 9H), 1.00 (s, 6H)

^{19}F-NMR (282 MHz, $CDCl_3$) δ -75.7

^{13}C-NMR (75 MHz, $CDCl_3$) δ 179.6, 170.5, 162.9, 159.3, 141.4, 131.5, 110.9, 80.5, 63.31, 61.3, 59.3, 38.7, 36.8, 33.8, 27.1, 24.1, 24.0, 23.6, 23.0, 17.8

HRMS (ESI): [M+H]+ calc'd for $C_{24}H_{37}N_5O_{10}S_2$ m/z 620.2060, found 620.2085.

Part 5: Synthesis of finals compounds 29b to 29c using the general methods outlined above



Note: compound 29a was not prepared

Preparation of ethyl 5-((((2S,3S)-3-((Z)-2-(2-aminothiazol-4-yl)-2-(((1-(tert-butoxy)-2-methyl-1-oxopropan-2-yl)oxy)imino)acetamido)-2-methyl-4-oxoazetidin-1-yl)sulfonyl)oxy)-4,4-dimethylpentanoate TFA salt 29b

(1.21 g, 84% for 2 steps) as a solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.95 (d, *J* = 6.9 Hz, 1H), 7.33 (s, 1H), 4.55-4.51 (m, 1H), 4.37-4.33 (m, 1H), 4.26-4.17 (m, 2H), 4.14-4.10 (m, 2H), 2.34-2.29 (m, 2H), 1.71-1.65 (m, 11H), 1.48 (s, 9H), 1.26 (t, *J* = 6.9 Hz, 3H), 0.99 (s, 6H).

Preparation of 2-(((Z)-(1-(2-aminothiazol-4-yl)-2-(((2S,3S)-1-(((5-ethoxy-2,2-dimethyl-5-oxopentyl)oxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic acid TFA salt 29c

(810 mg, 65% over 3 steps) as a solid.

LC-MS: *m/z* = 592 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.05 (d, *J* = 6.9 Hz, 1H), 7.15 (s, 1H), 4.62-4.59 (m, 1H), 4.45-4.42 (m, 1H), 4.17-4.10 (m, 4H), 2.37-2.31 (m, 2H), 1.72-1.65 (m, 11H), 1.26 (t, *J* = 7.2 Hz, 3H), 0.99 (s, 3H), 0.98 (s, 3H)

^{1}H-NMR (300 MHz, CD_3OD) δ 7.03 (s, 1H), 4.54 (d, *J* = 3.5 Hz, 1H), 4.42-4.52 (m, 1H), 4.19 (d, *J* = 9.2 Hz, 1H), 4.11 (d, *J* = 9.1 Hz, 1H), 4.06 (q, *J* = 7.1 Hz, 2H), 2.24-2.33 (m, 2H), 1.59-1.67 (m, 2H), 1.50-1.59 (m, 9H), 1.19 (t, *J* = 7.1 Hz, 3H), 0.94 (s, 6H)

^{13}C-NMR (75 MHz, $CDCl_3$) δ 176.6, 174.6, 170.5, 163.1, 159.6, 141.4, 131.4, 117.7, 113.8, 85.1, 80.5, 61.0, 34.0, 33.1, 29.2, 23.4, 17.9, 17.6, 14.2, 13.9

HRMS (ESI): [M+H]+ calc'd for $C_{22}H_{33}N_5O_{10}S_2$ m/z 592.1747, found 592.1743.

TABLE S1. STABILITY OF PRODRUG 28C IN 2.5% ACETONITRILE IN 0.05 M CITRATE BUFFER AT PH 4.7 AT 37 °C (NO CES1).

Prodrug	Timepoint[a]						
	0 min	1 min	2 min	5 min	10 min	20 min	30 min
28c	100	94	94	95	87	66	54

[a] Percentage of remaining prodrug when 0.5 mg of prodrug / mL of 2.5% acetonitrile in 0.05 M citrate buffer at pH 4.7 incubated at 37 °C (percentage peak area by HPLC analysis).

TABLE S2. RELEASE OF AZTREONAM FROM PRODRUG 28C USING CES1

Prodrug	Timepoint after treatment with CES1[a]						
	0 min	1 min	2 min	5 min	10 min	20 min	30 min
28c	BQL	80	>95	>95	>95	>95	>95

[a] Percentage release of aztreonam when 0.5 mg of prodrug / mL of 2.5% acetonitrile in 0.05 M citrate buffer at pH 4.7 incubated at 37 °C is treated with 150 Units / mL of CES1 enzyme (percentage peak area by HPLC analysis).

Conclusion from Tables S1 and S2: Prodrug **28c** is stable in 2.5% acetonitrile in 0.05 M citrate buffer for up to 30 min and releases aztreonam within 1 min when the buffer contains CES1 (full release of aztreonam within 2 min).

Table S3. Stability of prodrug 29c in 2.5% acetonitrile in 0.05 M citrate buffer at pH 4.7 at 37 °C (no CES1).

Prodrug	Timepoint[a]						
	0 min	1 min	2 min	5 min	10 min	20 min	30 min
29c	100	100	100	83	70	49	36

[a] Percentage of remaining prodrug when 0.5 mg of prodrug / mL of 2.5% acetonitrile in 0.05 M citrate buffer at pH 4.7 incubated at 37 °C (percentage peak area by HPLC analysis).

TABLE S4. RELEASE OF AZTREONAM FROM PRODRUG 29C USING CES1

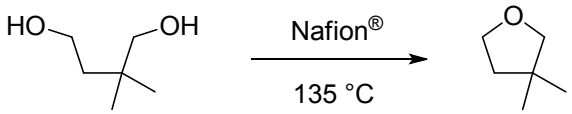
Prodrug	Timepoint after treatment with CES1[a]						
	0 min	1 min	2 min	5 min	10 min	20 min	30 min
29c	BQL	24	45	76	86	90	90

[a] Percentage release of aztreonam when 0.5 mg of prodrug / mL of 2.5% acetonitrile in 0.05 M citrate buffer at pH 4.7 incubated at 37 °C is treated with 150 U / mL of CES1 enzyme (percentage peak area by HPLC analysis).

Conclusion from Tables S3 and S4: Prodrug **29c** is stable in 2.5% acetonitrile in 0.05 M citrate buffer for up to 30 min and releases aztreonam within 1 min when the buffer contains CES1 (full release of aztreonam within 10 min).

Conclusion from Tables S3 and S4: Prodrug **29c** is stable in 2.5% acetonitrile in 0.05 M citrate buffer

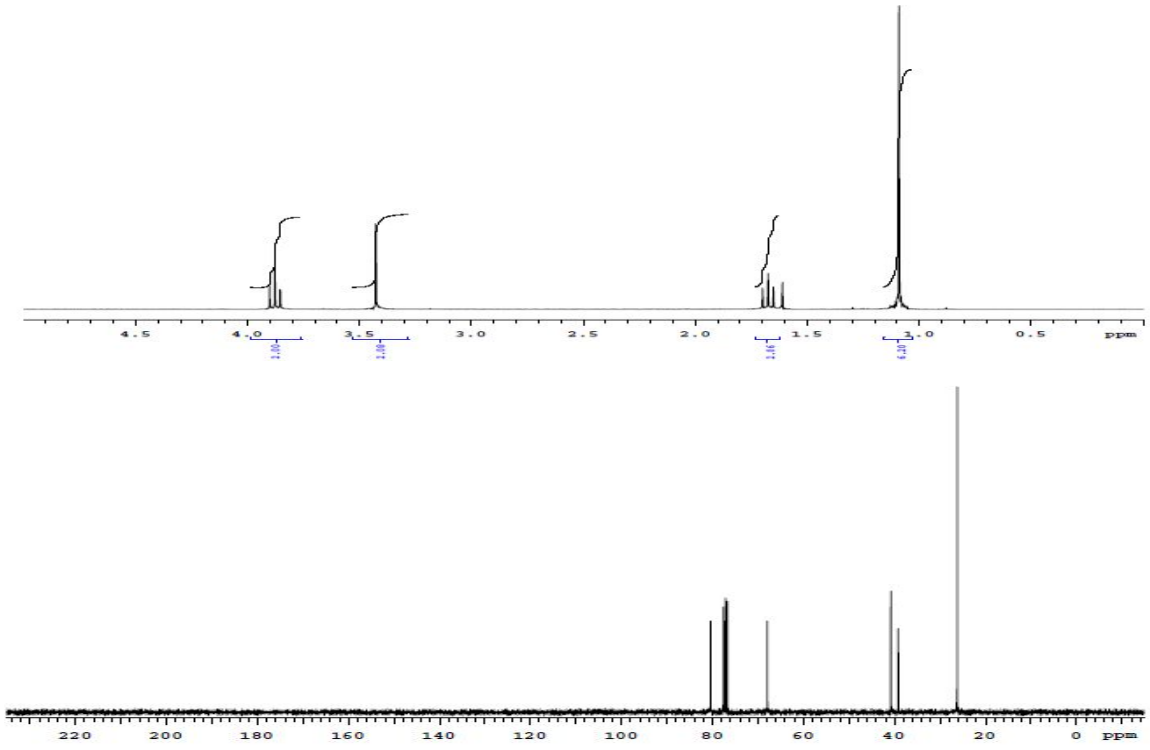
SYNTHESIS OF 3,3-DIMETHYLTETRAHYDROFURAN[1]



A flask equipped with a distillation head was charged with 2,2-dimethylbutane-1,4-diol (1.0 g, 8.5 mmol) and Nafion® perfluorinated resin powder (50 mg). The stirred mixture was heated to 135 °C. As the reaction progressed, the product was collected as a distillate. The distillate was purified by a second distillation to give 3,3-dimethyltetrahydrofuran as a clear and colorless liquid (286 mg, 33% yield).

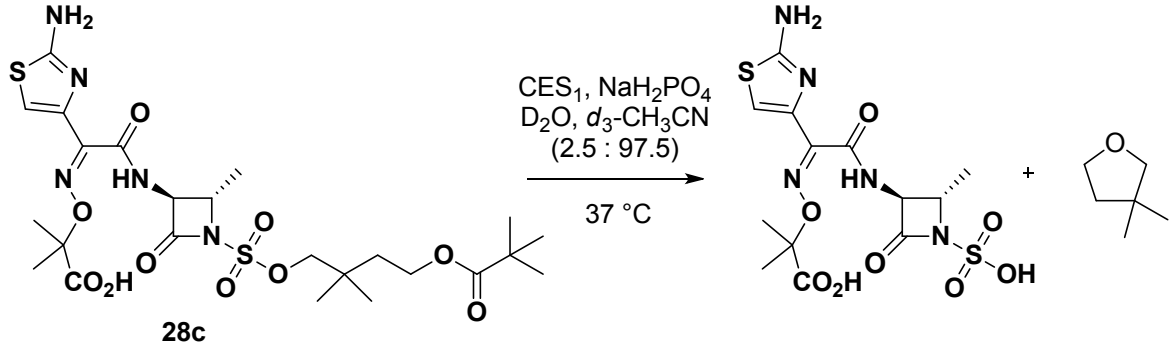
^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.88 (t, J = 7.0 Hz, 2H), 3.43 (s, 2H), 1.68 (t, J = 7.3 Hz, 2H), 1.09 (s, 6H).

^{13}C-NMR (300 MHz, $CDCl_3$) δ 80.4, 67.9, 40.8, 39.2, 26.2.



3,3-Dimethyltetrahydrofuran was used as a reference standard for ^{1}H-NMR enzymatic release studies (detailed on pages S7-S8).

^{1}H-NMR STUDY 1: RELEASE OF 3,3-DIMETHYLTETRAHYDROFURAN AND AZTREONAM FROM PRODRUG 28C



A stirring mixture of CES1 (20 mg, 2200 Units) in a 15 mM solution of sodium phosphate monobasic (enzyme grade) in acetonitrile-d_3 / D_2O (1 mL; ratio of 2.5 : 97.5) was heated at 37 °C for 5 min. 2-(((*Z*)-(1-(2-aminothiazol-4-yl)-2-(((2*S*,3*S*)-1-((2,2-dimethyl-4-(pivaloyloxy)butoxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic acid TFA salt **28c** (10 mg, 14 µmol) was added, and the suspension was stirred for 70 min at 37 °C. Over the course of the reaction a fine precipitate formed. The mixture was filtered through a 25 mm, 0.45 µM glass fiber syringe filter (Pall Corporation Acrodisc). The filtrate was analyzed by ^{1}H-NMR spectroscopy to reveal that 3,3-dimethyltetrahydrofuran was released as one of the products. The presence of 3,3-dimethyltetrahydrofuran was confirmed by ^{1}H-NMR analysis of the same sample spiked with 2 µL of authentic material.

Figure S1. ^{1}H-NMR spectrum of reaction mixture after treatment of **28c** with CES1.

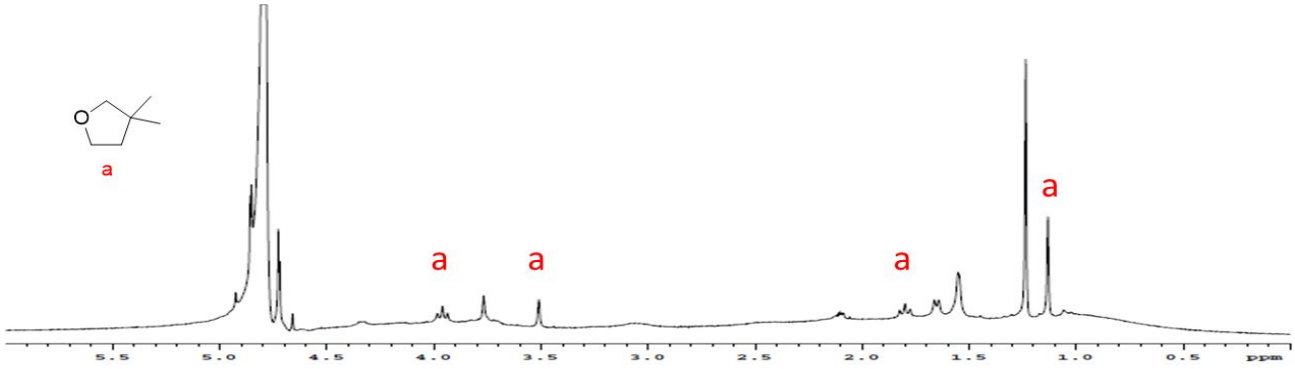


Figure S2. ^{1}H-NMR spectrum of authentic 3,3-dimethyltetrahydrofuran (from synthesis on page S6).

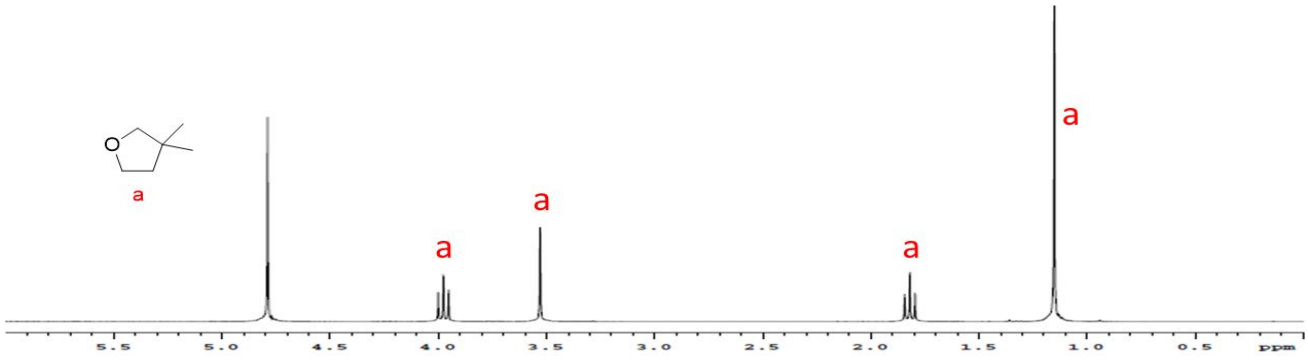
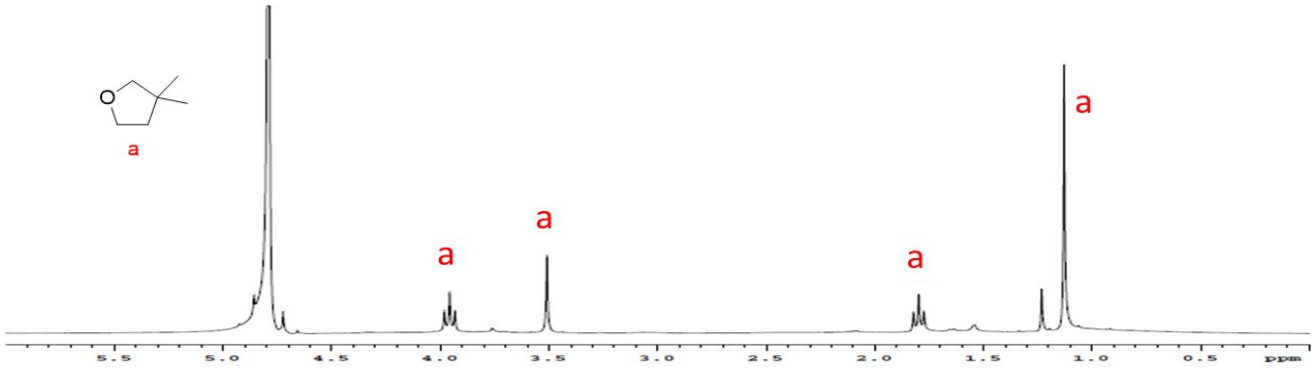
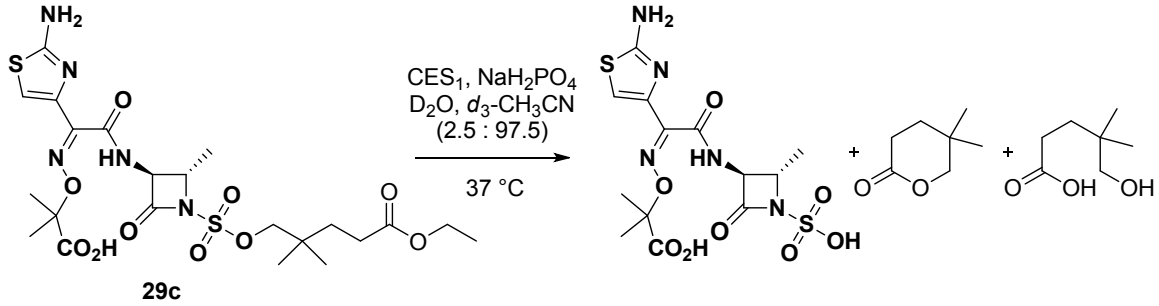


Figure S3. ^{1}H-NMR spectrum of reaction mixture spiked with 2 μL of 3,3-dimethyltetrahydrofuran.



^{1}H-NMR STUDY 2: RELEASE OF 5,5-DIMETHYLTETRAHYDRO-2*H*-PYRAN-2-ONE / 5-HYDROXY-4,4-DIMETHYLPENTANOIC ACID AND AZTREONAM FROM COMPOUND 29C



A stirring mixture of CES1 (20 mg, 2200 Units) in a 15 mM solution of sodium phosphate monobasic (enzyme grade) in 2.5% acetonitrile-d_3 / D_2O (1 mL) was heated at 37 °C for 5 min. 2-(((*Z*)-(1-(2-aminothiazol-4-yl)-2-(((2*S*,3*S*)-1-(((5-ethoxy-2,2-dimethyl-5-oxopentyl)oxy)sulfonyl)-2-methyl-4-oxoazetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic acid TFA salt **29c** (10 mg, 14 µmol) was added, and the suspension was stirred for 19 h at 37 °C. Over the course of the reaction a fine precipitate formed. The mixture was filtered through a 25 mm, 0.45 µM glass fiber syringe filter (Pall Corporation Acrodisc). The filtrate was analyzed by ^{1}H-NMR spectroscopy to reveal that 5,5-dimethyltetrahydro-2*H*-pyran-2-one and 5-hydroxy-4,4-dimethylpentanoic acid were released from compound **29c**. The presence of 5,5-dimethyltetrahydro-2*H*-pyran-2-one and 5-hydroxy-4,4-dimethylpentanoic acid were confirmed by ^{1}H-NMR analysis of the same sample spiked with 2 mg of authentic material (mixture of the two compounds).

Figure S4. ^{1}H-NMR spectrum of reaction mixture after treatment of **29c** with CES1.

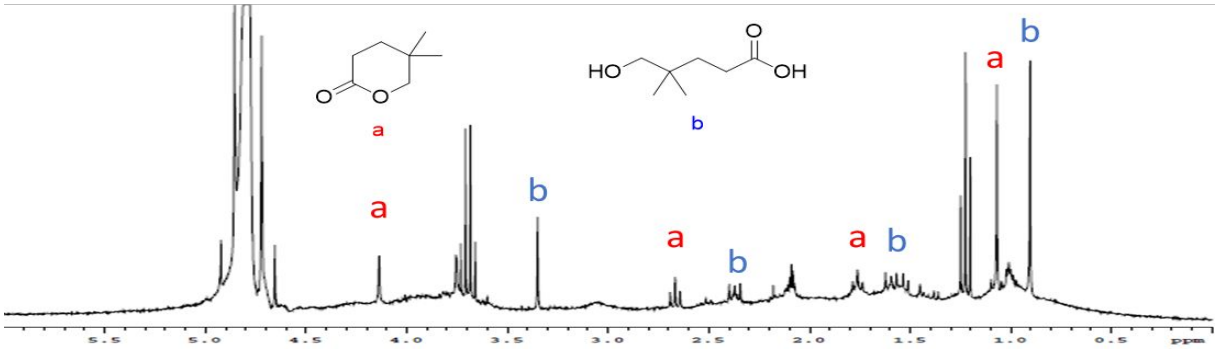


Figure S5. ^{1}H-NMR spectrum of authentic 5,5-dimethyltetrahydro-2*H*-pyran-2-one and 5-hydroxy-4,4-dimethylpentanoic acid mixture.

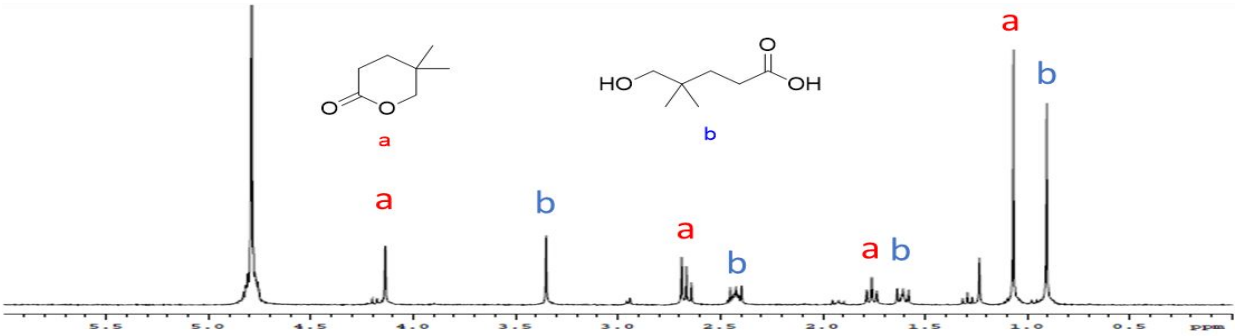
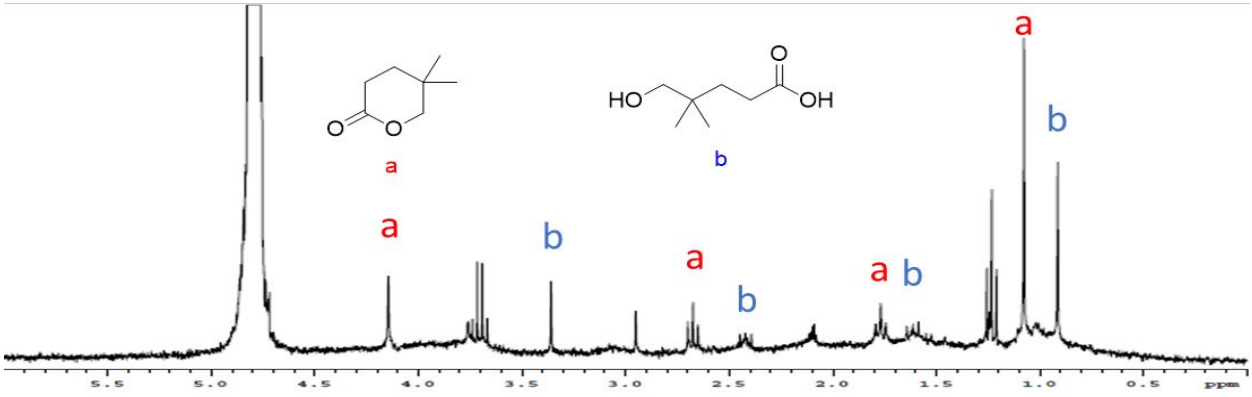


Figure S6. ^{1}H-NMR spectrum of reaction mixture spiked with 2 mg of a 5,5-dimethyltetrahydro-2*H*-pyran-2-one and 5-hydroxy-4,4-dimethylpentanoic acid mixture.



REFERENCES

1. Olah, G. A.; Fung, A. P.; Ripudaman, H. *Synthesis* **1981**, 474-476.