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Alternate "Drug" Delivery Utilizing β-Lactam Cores: Syntheses and Biological Evaluation of β-Lactams Bearing Isocyanate Precursors

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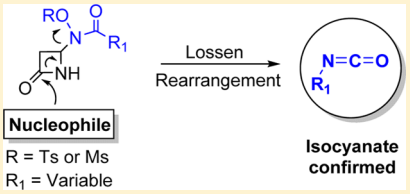
Supporting Information

ABSTRACT: The synthesis of a small set of β-lactams containing isocyanate precursors is described. The release of the isocyanate precursor in model hydrolysis experiments was substantiated by trapping experiments, thus confirming that β-lactams can be designed that are capable of releasing alternatively reactive species. Preliminary biological assessments are also briefly discussed.



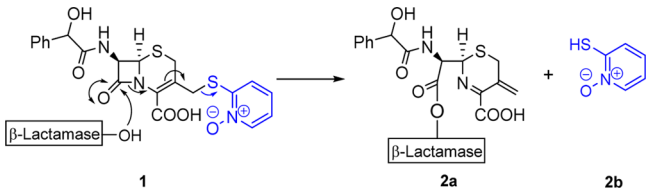
Although serendipitously discovered over 80 years ago, the β-lactam family remains one of the most widely prescribed classes of antibiotics.[1] β-Lactams inhibit the formation of peptidoglycan cross-linkages in the bacterial cell wall by acting as mimics of the terminal D-ala-D-ala dipeptide of peptidoglycan. Unfortunately, bacterial resistance continues to be on the rise and threatens modern healthcare. Bacteria have developed a myriad of mechanisms to stymie successful antibiotic therapy, highlighted by the presence of β-lactamases. These rather promiscuous enzymes catalyze the hydrolysis of β-lactam antibiotics and have received considerable attention.[2] Many drug resistant strains of bacteria produce β−lactamases and more than 900 unique forms of these enzymes have been identified.[3]

There are a number of ways to remain one step ahead of bacterial resistance mechanisms. One approach involves chemical modification of existing cores to make them less susceptible to β-lactamase induced hydrolysis and other bacterial resistance mechanisms.[4] More aggressive methods involve coadministration of an antibiotic and β-lactamase inhibitor, an example being the combination of clavulanic acid (β-lactamase inhibitor) with amoxicillin (antibiotic), known as Augmentin.[5] With this combination, the enzyme inhibitor protects the antibiotic from competitive detrimental hydrolysis and inactivation. A third method of circumventing resistance capitalizes on both the efficiency of bacterial enzymatic machinery and the novel ring opening chemistry of β-lactams. Often, this involves the use of prodrugs.[6] The β-lactam literature is replete with examples in which the core has ultimately been used for this purpose. O'Callaghan and Sykes reported a cephalosporin (**1**) that upon hydrolysis, generated 2-mercaptopyridine-*N*-oxide, **2b**, a species that exhibited separate antibacterial activity (Scheme 1).[7]

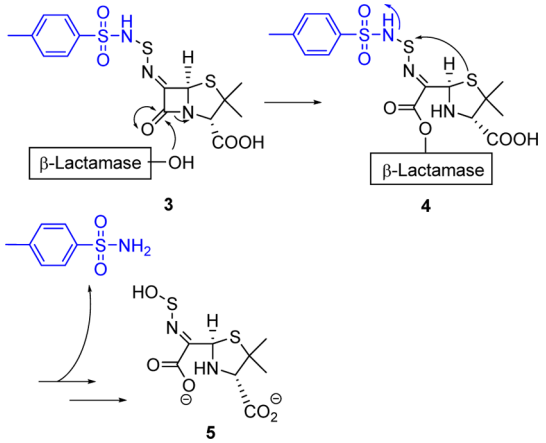
This concept of enzymatically triggered release was subsequently extended to penicillins by Smyth with the report of penicillin scaffolds, such as **3**, that expelled a sulfonamide upon β-lactamase catalyzed ring opening (Scheme 2).[8]

With the above precedence in mind, the aim of this work centered on the design and syntheses of β-lactam scaffolds that exhibit a unique type of reactivity, specifically, release of a broadly reactive isocyanate upon hydrolysis. As illustrated in Scheme 3, compounds such as **6** or **7**, synthesized separately from starting material **A** or **B**, respectively, may exert antibiotic activity or β-lactamase deactivation through an enzymatically induced Lossen rearrangement[9] to generate an isocyanate (**8**). Although not a drug in itself, it is hypothesized that upon release the isocyanate would react with nucleophiles in the

Scheme 1. Utilization of the Cephalosporin Core for Drug Delivery



Scheme 2. Penam Core as Triggered Release System



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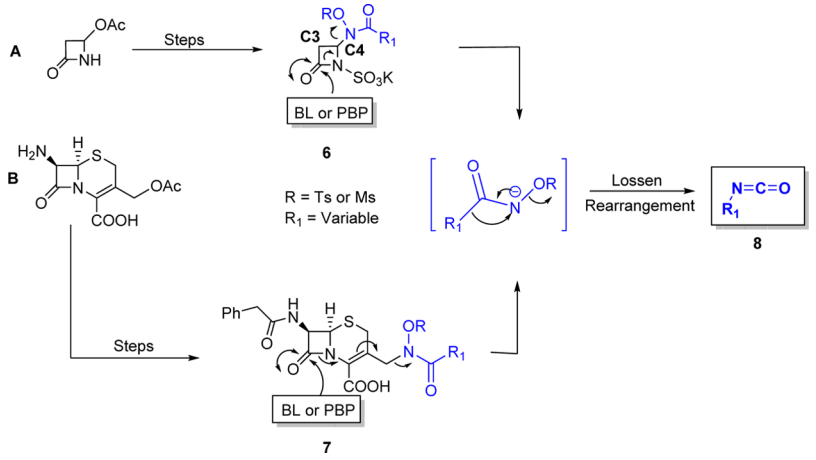
Scheme 3. Hypothesis for Enzymatic Triggered "Drug" Release[a]



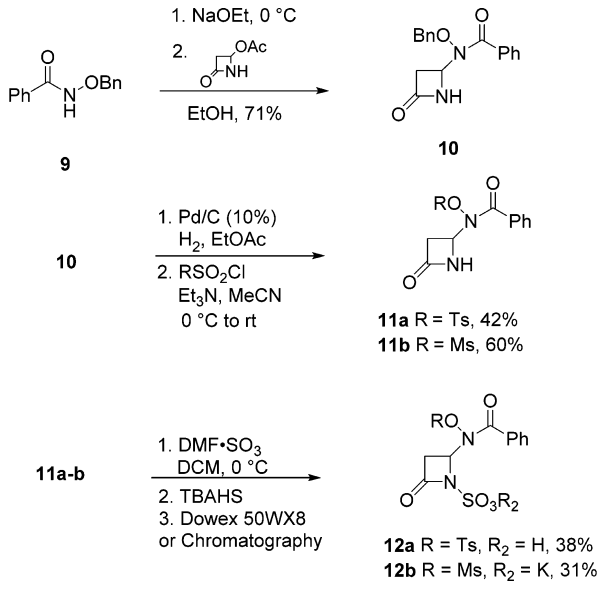
[a]BL = β-lactamase, PBP = penicillin binding protein.

vicinity and lead to either penicillin binding protein (PBP) inhibition, β-lactamase inhibition, or other nonspecific target modifications. Utilizing β-lactam cores to fulfill this purpose is ideal, since it has been shown that cleavage of the ring can result in the release of leaving groups.[10] However, to the best of our knowledge, few reports exist that have applied the concept of triggered release systems to monocyclic β-lactams (monobactams).[11] This concept of using an isocyanate produced from the Lossen rearrangement for purposes of inhibition in biological systems was previously reported by Groutas.[12] In that study, succinimides were shown to be mechanism-based inhibitors of a serine proteinase, specifically human leukocyte elastase (HLE), and operated via an enzyme induced Lossen rearrangement in order to exert activity. Herein we report the syntheses and studies of β-lactams with pendant hydroxamic acids that are appropriately substituted to be isocyanate precursors.

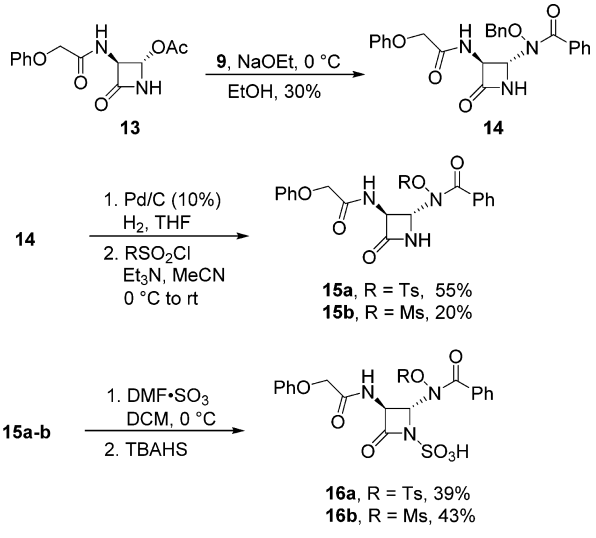
Syntheses of racemic monobactams devoid of C-3 functionality (see structure **6**, Scheme 3 for numbering) are shown in Scheme 4. Starting with appropriately functionalized hydroxamate **9**, reaction with 4-acetoxy-2-azetidinone under conditions first reported by Clauss[13] gave hydroxamate containing monobactam **10** in good yield. The structure for **10** was confirmed by X-ray crystallography to ensure desired *N*-alkylation (see Supporting Information). Hydrogenolysis, followed by tosylation and mesylation gave intermediates **11a,b.** Lastly, nitrogen sulfonation was carried out using DMF·SO_3 complex[14] followed by conversion to the tetrabutylammonium (TBA) salts with tetrabutylammonium hydrogen sulfate (TBAHS). These TBA salts were then either purified with normal phase silica gel to give **12a**, or stirred in a prepared potassium Dowex WX8 resin to give potassium salt **12b**.

The next stage was to generate compounds with classic β-lactam side chains, anticipating that such side chains would likely be necessary to potentiate antibacterial activity. Beginning with azetidinone **13** (Scheme 5), obtained from the degradation of penicillin V,[15] reaction with **9** gave intermediate **14** with the expected *anti* stereochemistry, verified by NMR homo decoupling experiments.[15] Hydrogenolysis, followed by separate tosylation and mesylation gave compounds **15a,b** and sulfonation gave final compounds **16a,b** in modest yields.

Scheme 4. Racemic Syntheses of Monobactams 12a–b



Scheme 5. Syntheses of Monobactams 16a,b



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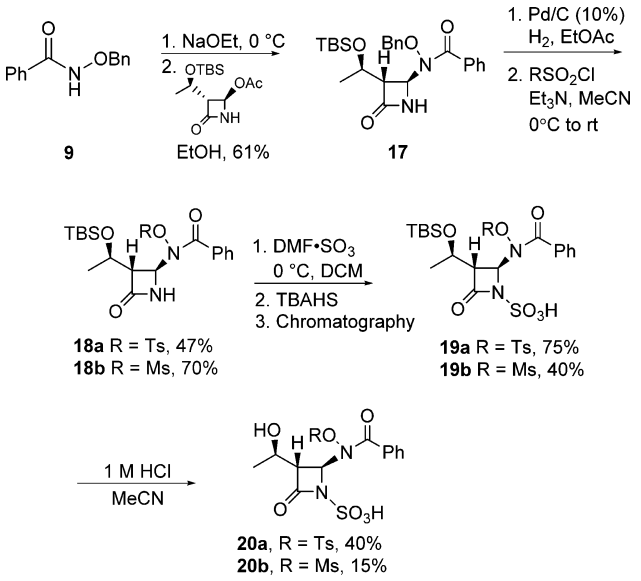
To further expand structure−activity-relationship studies we were also interested in incorporating the classic hydroxyethyl side chain, common in many carbapenem antibiotics, into our systems. Although incorporation of hydroxyethyl side chains onto the periphery of monobactams does not impart antibiotic activity, related studies on β-lactamase susceptibilty have not been reported. As shown in Scheme 6, the commercially available stereopure azetidinone was reacted with hydroxamate **9** to give intermediate **17**. Hydrogenolysis, followed by tosylation and mesylation gave **18a,b** and sulfonation produced penultimate compounds **19a,b**. Removal of the silyl protecting group was accomplished with 1 M HCl to give monobactams **20a,b**.

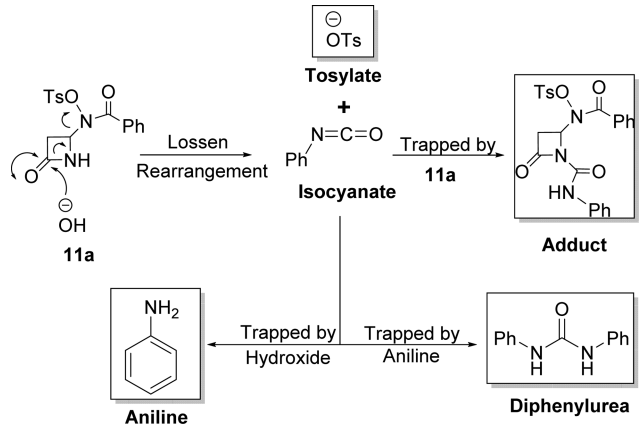
Scheme 6. Syntheses of Monobactams 20a,b



The syntheses of hydroxamate substituted cephalosporins started with commercially available GCLE. As shown in Scheme 7, the silyl protected hydroxamate was directly installed by means of Pd(0) chemistry to give **21**[16] in good yield. Desilyation with HF·pyridine gave hydroxamic acid **22** and tosylation, followed by deprotection with TFA, gave final cephalosporin **23**.

Selected monobactams were then subjected to alkaline hydrolysis conditions followed by LC/MS analysis of products in order to confirm whether hydrolysis of the β-lactam ring ultimately produced products that were consistent with the Lossen rearrangement hypothesis (Scheme 8). Attack by hydroxide was anticipated to expel the activated hydroxamate that would spontaneously engage in the Lossen rearrangement to produce the transient isocyanate. This species would then be trapped by hydroxide to give an unstable carbamic acid and subsequent decarboxylation would produce aniline. Aniline, in turn, could then react with additional isocyanate to ultimately give diphenylurea. Alternatively, **11a** itself might be expected to trap the isocyanate to give an adduct. Our studies indicated that **11a,b** readily hydolyzed under these conditions to produce products consistent with our proposed Lossen hypothesis (see Supporting Information). Additionally, we also observed a competing reaction in which the leaving group (i.e., tosyl or mesyl) was hydrolytically cleaved to give the monobactam with free hydroxamic acid. Studies on sulfonated compounds, specifically **16b**, **19b**, and **20b**, showed that these compounds were stable at pH 10 after 2−4 h of stirring. This is likely the result of the incorporation of the ionizable group onto the β-lactam nitrogen, thus making the β-lactam less prone to nonenzymatic hydrolytic opening. After 24 h of stirring, these compounds showed only slight degradation. Overall, these studies served as a proof of concept for the novel path of reactivity using the monobactam core.

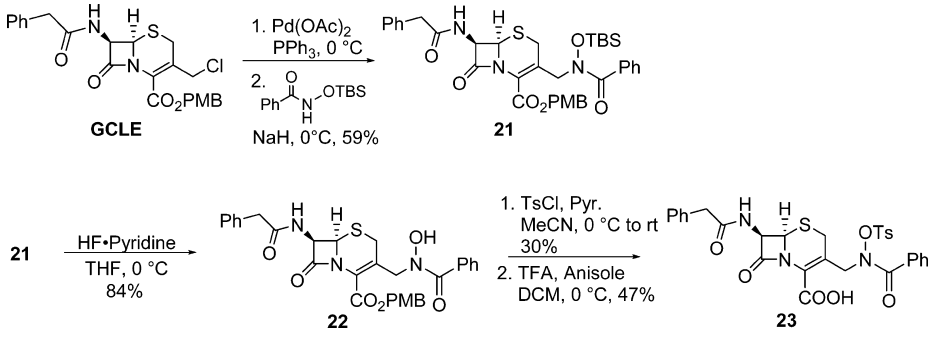
Scheme 8. Hydrolysis of 11a and Expected Products[a]



[a]All confirmed with LC/MS monitoring.

With these encouraging results, compounds were screened against a panel of Gram-positive and Gram-negative bacteria using agar diffusion assays, with final compounds also tested for β-lactamase inhibition via synergy studies with known β-lactam antibiotics using standard broth microdilution assays. While the monobactams were generally inactive, the cephalosporins selectively targeted Gram-positive bacteria, with notable zones of inhibition and minimum inhibitory concentrations (MICs)

Scheme 7. Synthesis of Cephalosporin 23



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observed (Table 1). Highlighted among the compounds were the activities of compounds **21**, **22**, and **23**, with MIC values of 0.4, 0.4, and 3.0 μM against *B. subtilis*, respectively. Cephalosporins **21**, **22**, and **23** also targeted *S. aureus*, with MIC values of 1.6, 0.8, and 12.6 μM, respectively.

Table 1. Zone of Growth Inhibition (mm) with MIC (μM) in Bold[a]

cmpd.	*B. subtilis* ATCC 6633	*S. aureus* SG511	*M. luteus* ATCC 10240
21	18	16	0
	0.4	**1.6**	
22	30	29	27
	0.4	**0.8**	**6.0**
23	25	26	22
	3.0	**12.6**	
Ciprofloxacin	21/25P	21	0

[a]Compounds were dissolved in MeOH/DMSO at a concentration of 2 mM. Ciprofloxacin used in water at 1.66 μg/mL. For diffusion assays, all zones represent 100% inhibition. All wells are not subtracted from reported zones

In conclusion, the syntheses of functionally dense monobactams and cephalosporins bearing isocyanate precursors have been reported. Hydrolysis experiments on model compounds have substantiated our Lossen rearrangement hypothesis and thus a novel reactivity pathway.

EXPERIMENTAL PROCEDURES

General Comments. Commercial grade reagents and solvents were used without further purification except as indicated below. Tetrahydrofuran (THF) was distilled from sodium and both DCM and MeCN were distilled from calcium hydride. All reactions were carried out in oven or flame-dried glassware under an atmosphere of dry argon only when specified in the experimental details. For reactions in which a hydroxamic acid was made, all glassware used was washed with 6 M HCl and dried prior to use to minimize contamination due to iron binding. All reactions were magnetically stirred and monitored by analytical thin-layer chromatography using aluminum-backed 0.2 mm silica gel 60 F-254 plates. Visualization was accomplished by UV light (254 nm), $KMnO_4$, and vanillin spray. Column chromatography was performed with silica gel 60 (230−400 mesh). Both ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature with the residual solvent peaks as internal standards on a 300, 400, 500, or 600 MHz instrument. All IR spectra were recorded on a FT IR by making thin films on NaCl plates. High-resolution mass spectra (HRMS, ESI-TOF) data were obtained with electrospray ionization and a time-of-flight analyzer. Melting points were performed with a capillary melting point apparatus and are uncorrected. All NMR spectra were processed with ACD/academic edition version 12 software and reported using the software's JOC report format.

*N-(Benzyloxy)-N-(2-oxoazetidin-4-yl)benzamide (**10**).* To a flame-dried round bottomed flask under argon was added *N*-(benzyloxy)-benzamide (**9**, 1.19 g, 5.23 mmol). The flask was cooled in an ice bath for 5 min and a solution of 0.8 M NaOEt in EtOH (10 mL, 8 mmol) was slowly added. The reaction was stirred for 5 min, 4-acetoxy-2-azetidinone (1.00 g, 7.74 mmol) in anhydrous EtOH (5 mL) was added dropwise, and the reaction was stirred overnight warming to room temperature. The reaction was diluted with EtOAc (75 mL) and then washed with H_2O, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give crude product that was triturated with hexanes and then purified with column chromatography (90/10 ether/hexanes) to give **10** (1.10 g, 3.71 mmol) as a white solid in 71% yield. mp = 124−126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.28−7.73 (m, 8 H), 7.11 (dd, J = 7.22, 1.84 Hz, 2 H), 6.48−6.52 (br. s., 1 H), 5.99−6.05 (fine m, 1 H), 4.87 (d, J = 9.39 Hz, 1 H), 4.70 (d, J = 9.39 Hz, 1 H), 3.49 (dd, J = 14.9, 1.91 Hz, 1 H), 3.14−3.23 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.7, 166.6, 133.9, 133.6, 131.7, 130.0, 129.4, 128.8, 128.7, 128.6, 80.5, 60.5, 42.4; IR (FT-IR) ν 1772, 1650 cm^{-1}; HRMS (ESI-TOF) calcd. for $C_{17}H_{16}N_2O_3$ ($M+H^+$) 297.1234, found 297.1223.

*N-(2-Oxoazetidin-4-yl)-N-(tosyloxy)benzamide (**11a**).* To a flame-dried round bottomed flask was added **10** (202 mg, 0.681 mmol) and EtOAc (20 mL). Argon gas was bubbled through the solution and then Pd/C (10%) was added (45 mg). The contents were once again purged with argon by immersing the needle directly into the solution. Once purged, the argon line was removed and a vacuum line was inserted through the septum and held above the solution. A balloon was filled with H_2 gas and inserted through the rubber septum and into the solution momentarily (∼1 min) then held above the solution. The reaction was left to stir under an atmosphere of H_2. Upon completion (TLC: 100% EtOAc), the reaction was purged with argon prior to filtration. The reaction mixture was filtered and the filter papers were washed with MeOH. The filtrate was concentrated under reduced pressure to give crude material that was then purified with iron-free normal phase silica gel (90/10 EtOAc/hexanes) to give *N*-hydroxy-*N*-(2-oxoazetidin-4-yl)benzamide (110 mg, 0.533 mmol) as a white solid in 78% yield. mp = 113−115 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ = 9.91 (s, 1 H), 8.31 (s, 1 H), 7.61 (d, J = 7.3 Hz, 2 H), 7.56−7.37 (m, 3 H), 5.87 (br. s., 1 H), 3.08 (dq, J = 2.1, 7.9 Hz, 1 H), 3.04 (dd, J = 1.5, 12.3 Hz, 1 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ = 170.1, 166.7, 134.8, 131.0, 128.7, 128.4, 58.6, 41.4; HRMS (ESI-TOF) calcd. for $C_{10}H_{10}NaN_2O_3$ ($M+Na^+$) 229.0584, found 229.0579. To a flame-dried round bottomed flask under argon was added *N*-hydroxy-*N*-(2-oxoazetidin-4-yl)benzamide (102 mg, 0.494 mmol) and anhydrous MeCN (10 mL). TsCl (102 mg, 0.535 mmol) was added and the solution was cooled in an ice bath for 10 min. Triethylamine (0.535 mmol, 0.0750 mL) was then added dropwise and the reaction was stirred for 3 h. The solvent was removed under reduced pressure to give a yellow-white slurry that was dissolved in $CHCl_3$ (25 mL) and then washed with H_2O, saturated $NaHCO_3$, and brine. The organic layer was collected, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give crude material that was purified with column chromatography (50/50 hexanes/EtOAc) to give **11a** (73.0 mg, 0.202 mmol) as a white solid in 42% yield. mp = 101−103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.65−7.17 (m, 9 H), 6.25 (br. s., 1 H), 5.79 (dd, J = 1.9, 4.8 Hz, 1 H), 3.44 (d, J = 15.2 Hz, 1 H), 3.19 (dd, J = 2.5, 15.2 Hz, 1 H), 2.42 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.2, 165.0, 146.7, 132.6, 132.0, 130.5, 130.1, 129.3, 128.9, 128.7, 64.0, 42.9, 21.9; HRMS (ESI-TOF) calcd. for $C_{17}H_{16}N_2O_5S$ ($M+H^+$) 361.0853, found 361.0876.

*N-((Methylsulfonyl)oxy)-N-(2-oxoazetidin-4-yl)benzamide (**11b**).* To a flame-dried round-bottom flask under argon was added *N*-hydroxy-*N*-(2-oxoazetidin-4-yl)benzamide (1.35 g, 6.50 mmol) and anhydrous MeCN (40 mL). The solution was cooled in an ice bath for 10 min and MsCl (0.55 mL, 7.1 mmol) and Et_3N (0.92 mL, 6.5 mmol) were added dropwise. The reaction was stirred for 2 h and then the solvent was removed under reduced pressure to give a yellow-white slurry that was dissolved in $CHCl_3$ (50 mL), washed with H_2O, saturated $NaHCO_3$, and brine. The organic layer was collected, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow solid that was purified with column chromatography (60/40 EtOAc/hexanes) to give **11b** (1.11 g, 3.90 mmol) as a white solid in 60% yield. mp = 112−113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.68−7.48 (m, 5 H), 6.41 (br. s., 1 H), 5.79 (dd, J = 2.0, 4.7 Hz, 1 H), 3.42 (d, J = 14.8 Hz, 1 H), 3.25−3.16 (m, 1 H), 3.14 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.2, 164.7, 132.9, 132.1, 129.3, 128.6, 63.8, 42.9, 39.3; HRMS (ESI-TOF) calcd. for $C_{11}H_{12}N_2O_5S$ ($M+H^+$) 285.0540, found 285.0528.

*2-Oxo-4-(N-(tosyloxy)benzamido)azetidine-1-sulfonic Acid (**12a**).* To a flame-dried round-bottom flask under argon was added **11a** (100 mg, 0.2 mmol) and anhydrous DCM (10 mL). The solution was cooled in an ice bath for 5 min and DMF·SO_3 (167 mg, 1.10 mmol) was added in portions. The reaction was stirred for 1.5 h on ice and then diluted with DCM and 1 M K_2HPO_4. Tetrabutylammonium

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hydrogen sulfate (TBAHS, 94.0 mg, 0.277 mmol) was added, the separatory funnel shaken vigorously and vented, and the layers separated. The aqueous layer was extracted with EtOAc and the pooled organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give crude salt that was purified with normal phase silica gel (85/15 EtOAc/MeCN) to give **12a** (46.0 mg, 0.104 mmol) as a hydroscopic white solid in 38% yield. mp = 104−105 °C; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.76−7.65 (br. s., 2 H), 7.55−7.44 (m, 3 H), 7.39−7.27 (m, 4 H), 6.00−5.83 (br. s., 1 H), 3.55 (dd, J = 1.5, 14.1 Hz, 1 H), 3.33 (d, J = 5.6 Hz, 1 H), 2.40 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 171.8, 163.4 (DMF), 146.7, 132.4, 131.6, 130.2, 129.6, 129.1, 128.9, 127.8, 67.8 (broad), 41.1 (broad), 35.5 (DMF), 30.2 (DMF), 20.2; HRMS (ESI-TOF) calcd. for $C_{17}H_{16}N_2O_8S_2$ ($M-H^+$) 439.0275, found 439.0251.

*Potassium 4-(N-((methylsulfonyl)oxy)benzamido)-2-oxoazetidine-1-sulfonate (**12b**).* To a flame-dried round-bottom flask under argon was added **11b** (200 mg, 0.7 mmol) and anhydrous DCM (10 mL). The solution was cooled in an ice bath for 5 min and DMF·SO_3 (324 mg, 2.10 mmol) was added in portions. The reaction was stirred for 2 h on ice and then diluted with DCM and 1 M K_2HPO_4. TBAHS (240 mg, 0.703 mmol) was added, the separatory funnel was shaken vigorously and vented, and the layers were separated. The aqueous layer was extracted with EtOAc and the pooled organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude TBA salt. The salt was stirred in a prepared Dowex resin (15 g, 50 WX8, 50−100 mesh, K^+ form) for 3 h, the resin was filtered off, and the filtrate was concentrated under reduced pressure to give **12b** (88.0 mg, 0.218 mmol) as fine oil droplets in 31% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.79 (br. s., 2 H), 7.68−7.57 (m, 2 H), 7.57−7.45 (m, 2 H), 5.72 (d, J = 4.8 Hz, 7 H), 3.49−3.39 (m, 3 H), 3.25 (dd, J = 5.6, 10.0 Hz, 2 H), 3.09 (dd, J = 2.2, 13.2 Hz, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ = 171.4, 162.6, 132.7, 129.6, 129.0, 128.4, 67.4, 40.4 (overlap with solvent), 38.2; IR (FT-IR) ν 1771, 1688 cm^{-1}; HRMS (ESI-TOF) calcd. for $C_{11}H_{11}N_2O_8S_2$ ($M-H^+$) 362.9962, found 362.9985.

*N-(Benzyloxy)-N-((3S,4S)-2-oxo-3-(2-phenoxyacetamido)-azetidin-4-yl)benzamide (**14**).* To a flame-dried round-bottom flask under argon was added **9** (700 mg, 3. mmol). The flask was cooled in an ice bath for 5 min and a solution of 0.8 M NaOEt in anhydrous EtOH (3.90 mL, 3.08 mmol) was added dropwise. The reaction was stirred for 5 min and then a solution of 4-acetoxy-2-azetidinone (900 mg, 3 mmol) in anhydrous EtOH was added slowly. The reaction was left to stir overnight under argon, warming to room temperature. The solvent was removed under reduced pressure to give crude material that was dissolved in EtOAc (40 mL), washed with H_2O, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow oil that was purified with column chromatography (70/30 EtOAc/hexanes) to give **14** (414 mg, 0.930 mmol) as a white solid in 30% yield. mp = 144−145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.79−6.79 (m, 15 H), 6.15 (s, 1 H), 5.39 (d, J = 6.5, 1.6 Hz, 1 H), 4.79 (overlapping dd, J = 9.6, 13.2 Hz, 2 H), 4.44 (s, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.9, 169.7, 165.7, 157.1, 133.8, 133.3, 131.8, 130.1, 130.0, 129.4, 128.8, 128.7, 128.5, 122.4, 114.9, 80.4, 67.2, 66.5, 60.0; IR (FT-IR) ν 1762, 1652 cm^{-1}; HRMS (ESI-TOF) calcd. for $C_{25}H_{23}N_3NaO_5$ ($M+Na^+$) 468.1530, found 468.1552.

*N-((3S,4S)-2-Oxo-3-(2-phenoxyacetamido)azetidin-4-yl)-N-(tosyloxy)benzamide (**15a**).* To a round-bottom flask was added **14** (400 mg, 0.9 mmol) and THF (18 mL). The resulting solution was subjected to hydrogenolysis using the same procedure as for the synthesis of **11a** to give crude *N*-hydroxy-*N*-((3*S*,4*S*)-2-oxo-3-(2-phenoxyacetamido)azetidin-4-yl)benzamide (343 mg, 0.965 mmol) as a yellow sticky solid. To a flame-dried round bottomed flask under argon was added *N*-hydroxy-*N*-((3*S*,4*S*)-2-oxo-3-(2-phenoxyacetamido)azetidin-4-yl)benzamide (220 mg, 0.61 mmol) and 20 mL of anhydrous THF. The resulting suspension was cooled in an ice bath for 5 min, TsCl (165 mg, 0.867 mmol) and Et_3N (0.103 mL, 0.743 mmol) were added, and the reaction was stirred for 2.5 h, warming to room temperature. The solvent was removed under reduced pressure to give crude material that was dissolved in DCM, washed with H_2O, saturated $NaHCO_3$, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give crude material that was purified with column chromatography (60/40 EtOAc/hexanes) to give **15a** (192 mg, 0.377 mmol) as a white solid in 55% yield. mp = 87−89 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.65−6.83 (m, 15 H), 6.43 (s, 1 H), 5.91 (br. s., 1 H), 5.22 (d, J = 7.0 Hz, 1 H), 4.45 (s, 2 H), 2.40 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.8, 169.3, 156.9, 146.6, 132.3, 131.6, 129.94, 129.92, 129.73, 129.2, 128.8, 128.4, 122.1, 114.1, 70.1, 66.1, 60.8, 31.5, 21.7; HRMS (ESI-TOF) calcd. for $C_{25}H_{24}N_3O_7S$ ($M+H^+$) 510.1329, found 510.1348.

*N-((Methylsulfonyl)oxy)-N-((3S,4S)-2-oxo-3-(2-phenoxyacetamido)azetidin-4-yl)benzamide (**15b**).* To a flame-dried round-bottom flask under argon was added crude *N*-hydroxy-*N*-((3*S*,4*S*)-2-oxo-3-(2-phenoxyacetamido) azetidin-4-yl)benzamide (155 mg, 0.436 mmol) and anhydrous MeCN (10 mL). The solution was cooled in an ice bath and then MsCl (0.047 mL, 0.61 mmol) and Et_3N (0.080 mL, 0.57 mmol) were added dropwise and the reaction was stirred for 2 h, warming to room temperature. The solvent was removed under reduced pressure and the crude material was dissolved in DCM, washed with H_2O, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give the crude product that was purified twice with column chromatography (70/30 EtOAc/hexanes first column, 55/45 hexanes/EtOAc second column) to give **15b** (38 mg, 0.087 mmol) as a very sticky white foam in 20% yield. ^{1}H NMR (600 MHz, CD_3OD) δ = 7.67−7.57 (m, 3 H), 7.53−7.48 (m, 2 H), 7.28 (s, 2 H), 7.02−6.95 (m, 2 H), 6.93 (d, J = 8.2 Hz, 1 H), 5.81 (s, 1 H), 5.15−5.09 (s, 1 H), 4.48 (s, 2 H), 3.32−3.28 (s, 3 H, overlap with CD_3OD); ^{13}C NMR (150 MHz, CD_3OD) δ = 170.7, 165.4, 157.6, 132.3, 132.1, 129.2, 128.7, 128.0, 121.4, 114.4, 114.3, 69.2, 66.5, 63.3, 37.5; HRMS (ESI-TOF) calcd. for $C_{19}H_{19}N_3NaO_7S$ ($M+Na^+$) 456.0836, found 456.0824.

*(3S,4R)-2-Oxo-3-(2-phenoxyacetamido)-4-(N-(tosyloxy)-benzamido)azetidine-1-sulfonic Acid (**16a**).* To a flame-dried round-bottom flask under argon was added **15a** (70.0 mg, 0.137 mmol) and anhydrous DCM (10 mL). The solution was cooled in an ice bath for 10 min, DMF·SO_3 (63.0 mg, 0.412 mmol) was added, and the reaction was stirred for 1.5 h. The reaction was then diluted with DCM and 1 M K_2HPO_4. TBAHS (47.0 mg, 0.137 mmol) was added, the separatory funnel was shaken vigorously and vented, and the layers separated. The aqueous layer was extracted with EtOAc and the pooled organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude salt that was purified with normal phase silica gel (90/10 EtOAc/MeCN) to give **16a** (31 mg, 0.050 mmol) as a white solid in 39% yield. mp = 112−114 °C; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.63−7.48 (br. s. 2 H), 7.43−7.18 (m, 10 H), 7.07−6.96 (m, 3 H), 5.95−5.65 (very br. s., 1 H), 4.61 (s, 2 H), 2.38 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 170.8, 170.5 (broad), 162.8, 157.7, 146.9, 132.0, 131.3, 129.7, 129.28, 129.23, 128.7, 127.4, 121.5, 114.5, 76.5 (broad), 66.6, 60.7, 48.4, 20.3; HRMS (ESI-TOF) calcd. for $C_{25}H_{22}N_3O_{10}S_2$ ($M-H^+$) 588.0752, found 588.0737.

*(3S,4R)-4-(N-((Methylsulfonyl)oxy)benzamido)-2-oxo-3-(2-phenoxyacetamido) azetidine-1-sulfonic Acid (**16b**).* To a flame-dried round-bottom flask under argon was added **15b** (51.0 mg, 0.117 mmol) and anhydrous DCM (10 mL). The solution was cooled in an ice bath for 10 min, DMF·SO_3 (62.0 mg, 0.409 mmol) was added, and the reaction was stirred for 1.5 h. The reaction was then diluted with DCM and 1 M K_2HPO_4. TBAHS (40.0 mg, 0.117 mmol) was added, the separatory funnel was shaken vigorously and vented, and the layers separated. The aqueous layer was extracted with EtOAc and the pooled organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude salt that was purified with normal phase silica gel (100% EtOAc to 90/10 EtOAc/MeCN) to give **16b** (26 mg, 0.050 mmol) as a white solid in 43% yield. mp = 108−110 °C; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.74 (d, J = 7.3 Hz, 2 H), 7.56 (t, J = 7.6 Hz, 1 H), 7.45 (t, J = 7.9 Hz, 2 H), 7.33−7.27 (m, 2 H), 7.03−6.91 (m, 3 H), 6.04 (br. s., 1 H), 5.29 (d, J = 2.1 Hz, 1 H), 4.54 (s, 2 H), 3.36−3.31 (br. s., 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 171.4, 171.0, 162.8, 157.6, 132.2, 132.0, 129.2, 128.8, 128.4, 121.5, 114.4, 72.8, 66.5, 59.3, 37.0; HRMS (ESI-TOF) calcd. for $C_{19}H_{18}N_3O_{10}S_2$ ($M-H^+$) 512.0439, found 512.0415.

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*N-(Benzyloxy)-N-((3R,4S)-4-((R)-1-((tert-butyldimethylsilyl)oxy)-ethyl)-2-oxoazetidin-4-yl)benzamide (**17**).* To a flame-dried round-bottom flask under argon was added *N*-(benzyloxy)benzamide (**9**, 4.25 g, 18.7 mmol). The flask was cooled in an ice bath for 10 min and a solution of 0.8 M NaOEt in anhydrous EtOH (23.5 mL, 18.8 mmol) was added slowly. The reaction was stirred for 5 min and (3*R*,4*R*)-3-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-2-oxoazetidin-4-yl acetate (5.67 g, 19.8 mmol), dissolved in anhydrous EtOH (40 mL), was added dropwise over 5 min. The reaction was left to stir overnight, warming to room temperature. The solvent was removed under reduced pressure and the crude material was dissolved in EtOAc (75 mL), washed with H_2O, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow oil that was purified with column chromatography (85/15 DCM/EtOAc) to give **17** (5.17 g, 11.4 mmol) as a colorless oil in 61% yield. The product slowly solidified into a waxy white solid at 0 °C. mp = 79−80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.69−6.96 (m, 10 H), 6.00−5.91 (m, 1 H), 5.90−5.83 (m, 1 H), 4.81 (d, *J* = 9.3 Hz, 1 H), 4.67 (d, *J* = 9.6 Hz, 1 H), 4.24 (m, 1 H), 3.61−3.53 (dd, 2.2, 1.2 Hz, 1 H), 1.08 (d, *J* = 6.2 Hz, 3 H), 0.72 (s, 9 H), −0.04 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.5, 167.6, 134.1, 133.9, 131.6, 130.1, 129.4, 128.94, 128.92, 128.8, 128.6, 128.4, 78.63, 64.3, 62.4, 25.8, 22.6, 18.0, −4.13, −4.86; IR (FT-IR) ν 1751, 1669 cm^{-1}; HRMS (ESI-TOF) calcd. for $C_{25}H_{35}N_2O_4Si$ ($M+H^+$) 455.2361, found 455.2351.

*N-((3R,4S)-4-((S)-1-((tert-Butyldimethylsilyl)oxy)ethyl)-2-oxoazetidin-4-yl)-N-(tosyloxy)benzamide (**18a**).* To a flame-dried round-bottom flask was added **17** (3.58 g, 7.87 mmol) and EtOAc (25 mL). The resulting solution was subjected to hydrogenolysis under the same conditions used in the synthesis of **11a** to give crude *N*-((3*R*,4*S*)-4-((*R*)-1-((*tert*-butyldimethylsilyl) oxy)ethyl)-2-oxoazetidin-4-yl)-*N*-hydroxybenzamide (2.77 g, 7.59 mmol) as an off-white sticky paste. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.66−7.30 (m, 5 H), 7.00−6.89 (br. s., 1 H), 5.89 (br. s., 1 H), 4.26−4.08 (m, 1 H), 3.60 (br. s., 1 H), 1.12 (d, *J* = 6.2 Hz, 3 H), 0.73 (s, 9 H), −0.03 (d, *J* = 5.5 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.2, 168.9, 133.0, 131.5, 128.7, 128.2, 63.8, 63.2, 62.1, 25.8, 22.7, 17.9, −4.23, −4.95; IR (FT-IR) ν 1763, 1667 cm^{-1}; HRMS (ESI-TOF) calcd. for $C_{18}H_{29}N_2O_4Si$ ($M+H^+$) 365.1891, found 365.1879. To a flame-dried round-bottom flask under argon was added *N*-((3*R*,4*S*)-4-((*R*)-1-((*tert*-butyldimethylsilyl) oxy)ethyl)-2-oxoazetidin-4-yl)-*N*-hydroxybenzamide (1.30 g, 3.57 mmol) and anhydrous MeCN (40 mL). The solution was cooled in an ice bath for 5 min, TsCl (880 mg, 4.6 mmol) and Et_3N (0.65 mL, 4.6 mmol, dropwise) were added, and the reaction was stirred, warming to room temperature. After 4 h, the solvent was removed under reduced pressure and the crude material was dissolved in DCM (100 mL). The reaction was washed with H_2O, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give crude material that was purified with column chromatography (70/30 hexanes/EtOAc) to give **18a** (870 mg, 1.6 mmol) as a white solid in 47% yield. mp = 75−77 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.79−7.16 (m, 9 H), 6.12 (br. s., 1 H), 5.79 (d, *J* = 1.7 Hz, 1 H), 4.24−4.13 (m, 1 H), 3.59 (br. s., 1 H), 2.40 (s, 3 H), 1.07 (br. s., 3 H), 0.68 (s, 9 H), −0.05 (d, *J* = 2.2 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.2, 166.0, 146.6, 132.8, 132.2, 130.8, 130.1, 129.4, 128.9, 128.8, 66.1, 63.5, 62.8, 25.7, 22.8, 22.0, 17.9, −4.30, −4.96; HRMS (ESI-TOF) calcd. for $C_{25}H_{35}N_2O_6SSi$ ($M+H^+$) 519.1974, found 519.1980.

*N-((3R,4S)-4-((S)-1-((tert-Butyldimethylsilyl)oxy)ethyl)-2-oxoazetidin-4-yl)-N-((methylsulfonyl)oxy)benzamide (**18b**).* To a flame-dried round-bottom flask under argon was added *N*-((3*R*,4*S*)-4-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)ethyl)-2-oxoazetidin-4-yl)-*N*-hydroxybenzamide (300 mg, 0.8 mmol) and anhydrous MeCN. The solution was cooled in an ice bath for 10 min, MsCl (0.0825 mL, 1.06 mmol) and Et_3N (0.135 mL, 0.983 mmol, dropwise) were added, and the reaction was left to stir for 2.5 h, warming to room temperature. The solvent was removed under reduced pressure and the crude reaction was dissolved in DCM (50 mL). The reaction was washed with H_2O, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give crude material that was purified with column chromatography (60/40 hexanes/EtOAc) to give **18b** (255 mg, 0.576 mmol) as a white solid in 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.73−7.41 (m, 5 H), 6.54 (br. s., 1 H), 5.89−5.78 (fine m, 1 H), 4.25−4.10 (m, 1 H), 3.55 (br. s., 1 H), 3.19 (s, 3 H), 1.06 (d, *J* = 4.3 Hz, 3 H), 0.65 (s, 9 H), −0.07 (d, *J* = 2.9 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.2, 166.1, 132.9, 132.2, 129.3, 128.4, 65.8, 63.4, 63.0, 39.3, 25.7, 22.9, 17.8, −4.34, −4.99; HRMS (ESI-TOF) calcd. for $C_{19}H_{31}N_2O_6SSi$ ($M+H^+$) 443.1667, found 443.1694.

*(3S,4S)-3-((R)-1-((tert-Butyldimethylsilyl)oxy)ethyl)-2-oxo-4-(N-(tosyloxy) benzamido)azetidine-1-sulfonic Acid (**19a**).* To a flame-dried round-bottom flask under argon was added **18a** (125 mg, 0.241 mmol) and anhydrous DCM (10 mL). The solution was cooled in an ice bath for 10 min, DMF·SO_3 (107 mg, 0.722 mmol) was added, and the reaction was stirred for 2.5 h. The reaction was then diluted with DCM and 1 M K_2HPO_4. TBAHS (88.0 mg, 0.260 mmol) was added, the separatory funnel shaken vigorously and vented, and the layers were separated. The aqueous layer was extracted with EtOAc and the pooled organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude salt that was purified with normal phase silica gel (85/15 EtOAc/hexanes) to give **18a** (108 mg, 0.181 mmol) as a white solid in 75% yield. mp = 116−118 °C; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.88−7.68 (br. s., 2 H), 7.58−7.46 (m, 3 H), 7.33 (m, 4 H), 6.12−5.91 (br. s., 1 H), 4.32 (q, *J* = 6.2 Hz, 1 H), 3.87 (br. s., 1 H), 2.39 (s, 3 H), 1.36 (d, *J* = 6.5 Hz, 3 H), 0.75 (br. s., 9 H), 0.05 (s, 3 H), 0.01 (br. s., 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 172.0, 164.8, 146.7, 132.7, 131.5, 130.3, 129.6, 129.2, 128.7, 127.9, 69.1, 63.8, 61.0, 24.8, 21.7, 20.2, 17.2, −5.88, −6.06; HRMS (ESI-TOF) calcd. for $C_{25}H_{33}N_2O_9S_2Si$ ($M-H^+$) 597.1402, found 597.1429.

*(3S,4S)-4-((R)-1-((tert-Butyldimethylsilyl)oxy)ethyl)-4-(N-((methylsulfonyl)oxy) benzamido)-2-oxoazetidine-1-sulfonic Acid (**19b**).* To a flame-dried round-bottom flask under argon was added **18b** (190 mg, 0.42 mmol) and anhydrous DCM (10 mL). The solution was cooled in an ice bath for 5 min, DMF·SO_3 (262 mg, 1.71 mmol) was added, and the reaction was stirred for 1.5 h, warming to rt. The reaction was then diluted with DCM and 1 M K_2HPO_4. TBAHS (145 mg, 0.190 mmol) was added, the separatory funnel was shaken vigorously and vented, and the layers were separated. The aqueous layer was extracted with EtOAc and the pooled organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude salt that was purified with normal phase silica gel (100% EtOAc to 90/10 EtOAc/MeCN) to give **19b** (89 mg, 0.15 mmol) as a very hydroscopic white solid in 40% yield. mp = 121−123 °C; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.87−7.77 (m, 2 H), 7.60 (t, *J* = 7.3 Hz, 1 H), 7.51 (t, *J* = 7.9 Hz, 2 H), 6.11−6.02 (br. s., 1 H), 4.27 (dd, *J* = 4.4, 6.2 Hz, 1 H), 3.66 (t, *J* = 2.2 Hz, 1 H), 3.48−3.34 (br. s., 3 H), 1.19 (d, *J* = 5.3 Hz, 3 H), 0.68 (s, 9 H), −0.03 (d, *J* = 17.6 Hz, 6 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 171.9, 164.5, 132.6, 131.9, 128.7, 128.4, 68.8, 63.5, 60.6, 37.3, 24.8, 21.8, 17.1, −6.00, −6.11; HRMS (ESI-TOF) calcd. for $C_{19}H_{29}N_2O_9S_2Si$ ($M-H^+$) 521.1089, found 521.1098.

*(3S,4S)-3-((R)-1-Hydroxyethyl)-2-oxo-4-(N-(tosyloxy)benzamido)-azetidine-1-sulfonic Acid (**20a**).* To a round-bottom flask was added **19a** (40.0 mg, 0.0660 mmol) and MeCN (5 mL). To the resulting solution was added 1 M HCl (0.093 mL, 0.093 mmol) and the reaction was stirred for 3 h. After 3 h, the reaction was not complete and additional 1 M HCl (1.22 mL, 1.22 mmol) was added. After stirring for an additional 3 h, the reaction was neutralized to pH 7 with dropwise addition of saturated $NaHCO_3$ and then concentrated under reduced pressure to give crude material that was purified with normal phase silica gel (75/25 EtOAc/MeCN) to give **20a** (13.0 mg, 0.0270 mmol) as a white solid in 40% yield. mp = 117−118 °C; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.85−7.67 (m, 2 H), 7.56 (d, *J* = 7.3 Hz, 2 H), 7.51 (t, *J* = 7.6 Hz, 1 H), 7.37 (t, *J* = 7.3 Hz, 2 H), 7.32 (d, *J* = 7.3 Hz, 2 H), 6.01−5.82 (br. s., 1 H), 4.15−4.08 (m, 1 H), 3.82−3.73 (br. s., 1 H), 2.40 (s, 3 H), 1.33 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 172.1, 164.5, 146.6, 132.5, 131.6, 130.3, 129.6, 129.2, 129.0, 127.8, 70.3, 63.2, 60.9, 20.5, 20.2; HRMS (ESI-TOF) calcd. for $C_{19}H_{19}N_2O_9S_2$ ($M-H^+$) 483.0556, found 483.0537.

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*(3S,4S)-3-((R)-1-Hydroxyethyl)-4-(N-((methylsulfonyl)oxy)-benzamido)-2-oxoazetidine-1-sulfonic Acid (**20b**).* To a round-bottom flask was added **19b** (45 mg, 0.086 mmol) and MeCN (5 mL). To the resulting solution was added 1 M HCl (0.103 mL, 0.103 mmol) and the reaction was stirred for 3 h. Additional 1 M HCl (1.6 mL, 1.6 mmol) was added and the reaction was stirred for 2 h. The reaction was then neutralized to pH 7 with dropwise addition of saturated $NaHCO_3$ and concentrated under reduced pressure to give crude material that was purified with normal phase silica gel (65/35 EtOAc/MeCN) to give **20b** (4.7 mg, 0.011 mmol) as a white solid in 15% yield. ^{1}H NMR (600 MHz, CD_3OD) δ = 7.86 (d, J = 7.0 Hz, 2 H), 7.61 (s, 1 H), 7.51 (t, J = 7.9 Hz, 2 H), 5.94−5.84 (m, 1 H), 4.08−3.97 (m, 1 H), 3.63 (dd, J = 2.3, 3.2 Hz, 1 H), 3.38 (br. s., 3 H), 1.22 (d, J = 6.5 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 172.1, 164.4, 133.3, 132.2, 129.0, 128.4, 70.4, 63.0, 60.3, 37.0, 20.7; HRMS (ESI-TOF) calcd. for $C_{13}H_{15}N_2O_9S_2$ ($M-H^+$) 407.0224, found 407.0206.

*(6R,7R)-4-Methoxybenzyl 3-((N-hydroxybenzamido) methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (**22**).* To a flame-dried round-bottom flask under argon was added **21** (170 mg, 0.24 mmol) and anhydrous THF (6 mL). HF·Pyridine (70% HF, 2 drops) was added and the reaction was stirred for 2.5 h. The reaction was diluted with EtOAc (30 mL), washed with 0.1 M HCl, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **22** (120 mg, 0.20 mmol) as a sticky paste in 84%. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.57−6.80 (m, 14 H), 6.47 (br. s., 1 H), 5.72 (dd, J = 4.8, 9.0 Hz, 1 H), 5.07 (s, 2 H), 4.91 (d, J = 16.0 Hz, 1 H), 4.86 (d, J = 4.8 Hz, 1 H), 4.59 (d, J = 16.0 Hz, 1 H), 3.60−3.51 (overlapping signals, 3 H), 3.36 (d, J = 19.1 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.7, 169.2 (broad), 164.9, 162.0 (broad), 160.1, 133.9, 131.5, 130.9, 130.8, 129.6, 129.3, 128.77, 128.74, 128.4, 127.9, 126.7, 125.6, 114.2, 68.4, 59.4, 57.6, 55.5, 51.7 (very broad), 43.4, 26.8; HRMS (ESI-TOF) calcd. for $C_{31}H_{29}N_3NaO_7S$ ($M+Na^+$) 610.1618, found 610.1613.

*(6R,7R)-8-Oxo-7-(2-phenylacetamido)-3-((N-(tosyloxy)-benzamido)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**23**).* To a flame-dried round-bottom flask under argon was added **22** (100 mg, 0.2 mmol) and anhydrous MeCN (10 mL). The solution was cooled in an ice bath for 10 min, TsCl (42.0 mg, 0.221 mmol) and anhydrous pyridine (0.0130 mL, 0.187 mmol) were added, and the reaction was stirred for 2 h, warming to room temperature. The reaction was then concentrated under reduced pressure at room temperature to give crude material that was purified with column chromatography (80/20 ether/hexanes to 100% ether) to give intermediate 4-methoxybenzyl (6*R*,7*R*)-8-oxo-7-(2-phenylacetamido)-3-((*N*-(tosyloxy)benzamido)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (38.0 mg, 0.0510 mmol) as a white solid in 30% yield. mp = 78−80 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48−7.23 (m, 14 H), 7.10 (d, J = 8.0 Hz, 2 H), 6.88 (d, J = 9.0 Hz, 2 H), 6.12 (d, J = 9.2 Hz, 1 H), 5.80 (dd, J = 4.2, 5.0 Hz, 1 H), 5.35 (d, J = 16.2 Hz, 1 H), 5.16 (s, 2 H), 4.95 (d, J = 4.8 Hz, 1 H), 4.82 (d, J = 16.2 Hz, 1 H), 3.80 (s, 3 H), 3.68−3.57 (overlapping signals, 3 H), 3.28 (d, J = 18.1 Hz, 1 H), 2.37 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 171.0, 170.9, 164.4, 161.2, 159.8, 146.4, 133.6, 131.8, 131.6, 130.7, 129.7, 129.6, 129.4, 129.19, 129.11, 128.4, 128.1, 127.7, 126.7, 126.6, 113.9, 68.1, 59.1, 57.4, 55.2, 52.8, 43.3, 26.9, 21.7 (apparent overlap of 1 aromatic carbon); HRMS (ESI-TOF) calcd. for $C_{38}H_{35}N_3NaO_9S_2$ ($M+Na^+$) 764.1707, found 764.1692. To a flame-dried round-bottom flask under argon was added 4-methoxybenzyl (6*R*,7*R*)-8-oxo-7-(2-phenylacetamido)-3-((*N*-(tosyloxy)benzamido)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (30.0 mg, 0.040 mmol) and anhydrous DCM (3 mL). The solution was cooled in an ice bath for 10 min. Anisole (0.01 mL, 0.1 mmol) and TFA (1 mL) were added slowly, and the reaction was stirred on ice for 1 h. The reaction was then concentrated under reduced pressure at room temperature to give crude material that was purified with column chromatography (100% DCM to 90/10 DCM/IPA w/0.3% AcOH) to give **23** (10.0 mg, 0.016 mmol) as an off-white very fine powder in 47% yield. ^{1}H NMR (600 MHz, CD_3OD) δ = 7.57 (d, J = 8.2 Hz, 2 H), 7.50−7.46 (m, 1 H), 7.38−7.21 (m, 11 H), 5.72 (dd, J = 3.8, 4.7 Hz, 1 H), 5.26 (d, J = 15.8 Hz, 1 H), 5.08 (d, J = 4.7 Hz, 1 H), 4.79 (d, J = 15.8 Hz, 1 H), 3.67 (d, J = 17.6 Hz, 1 H), 3.58 (overlapping d, J = 14.2 Hz, 2 H), 3.37 (d, J = 17.3 Hz, 1 H), 2.40 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ = 173.1, 171.6, 164.6, 162.9, 146.8, 134.9, 131.8, 131.6, 129.9, 129.7, 128.8, 128.7, 128.2, 128.1, 128.0, 127.2, 126.5, 124.0, 59.2, 57.6, 52.8, 41.7, 26.5, 20.2; HRMS (ESI-TOF) calcd. for $C_{30}H_{26}N_3O_8S_2$ ($M-H^+$) 620.1167, found 620.1180.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02272.

Agar diffusion assay procedure, MIC procedure, and copies of ^{1}H and ^{13}C NMR spectra for new compounds (PDF)

X-ray crystallographic data for compound **10** (CIF)

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Notes

The authors declare no competing financial interest.

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