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A Synthetic Dual Drug Sideromycin Induces Gram-Negative Bacteria to Commit Suicide with a Gram-Positive Antibiotic

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ABSTRACT. Many antibiotics lack activity against Gram-negative bacteria because they cannot permeate the outer membrane or suffer from efflux and, in the case of β-lactams, are degraded by β-lactamases. Herein, we describe the synthesis and studies of a dual drug conjugate (**1**) of a siderophore linked to a cephalosporin with an attached oxazolidinone. The cephalosporin component of **1** is rapidly hydrolyzed by purified ADC-1 β-lactamase to release the oxazolidinone. Conjugate **1** is active against clinical isolates of *Acinetobacter baumannii* as well as strains producing large amounts of ADC-1 β-lactamase. Overall, the results are consistent with siderophore-mediated active uptake, inherent activity of the delivered dual drug and, in the presence of β-lactamases, intracellular release of the oxazolidinone upon cleavage of the cephalosporin to allow the freed oxazolidinone to inactivate its target. The ultimate result demonstrates that Gram-positive oxazolidinone antibiotics can be made to be effective against Gram-negative bacteria by β-lactamase triggered release.

INTRODUCTION

The need for new antibiotics and strategies for treating multi-drug resistant bacterial infections is urgent![1] While bacterial resistance has developed to essentially all of our current antibiotics, few new antibiotics have been developed over the last several decades. A primary cause of drug resistance is the overuse of antibiotics that can result in alteration of microbial permeability, alteration of drug target binding sites, induction of enzymes that destroy antibiotics (ie., β-lactamases) and even induction of efflux mechanisms.[2,3,4] Herein, we report that a dual drug sideromycin (siderophore-cephalosporin-oxazolidinone conjugate **1**, Figure 1) circumvents these resistance mechanisms by siderophore-mediated active uptake promoting inherent activity of the delivered antibiotic combination. Furthermore, β-lactamase induced destructive hydrolysis of the cephalosporin component initiates intracellular release of the oxazolidinone warhead (**3**) thus allowing this normally Gram positive only class of antibiotics to be effective against targeted Gram-negative bacteria, including cephalosporinase-producing *Acinetobacter baumannii*. The World Health Organization (WHO) recently labeled infections due to β-lactamase producing strains of *A. baumannii* and *Pseudomonas aeruginosa* as being of critical concern world-wide.[5]

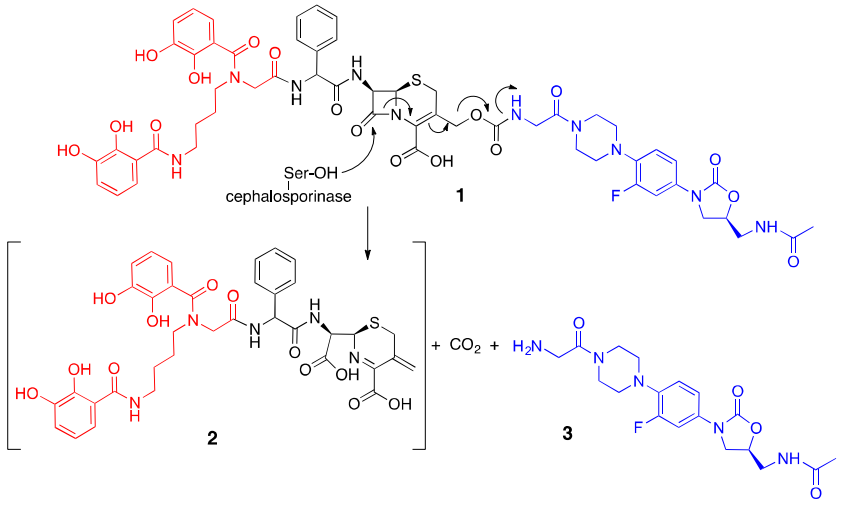


Figure 1. Siderophore-cephalosporin-oxazolidinone conjugate **1** and proposed cephalosporinase triggered release of oxazolidinone **3**.

In order to develop and sustain an infection, bacteria must sequester iron that is needed for essential physiological processes.[6] In fact, bacteria require micromolar concentrations of iron yet the concentration of free iron (Fe^{+3}) in an aerobic, neutral pH environment is ~10^{-18} molar.[6] Thus, bacteria synthesize and excrete iron chelating agents called siderophores that bind ferric iron with high affinity.[7] The resulting complex is then recognized by specific outer membrane proteins that initiate internalization by an active transport of the ferric siderophore. Subsequent reductive or hydrolytic release of the iron then makes it available to the bacteria. Since mammals do not utilize siderophores, exploitation of this essential bacterial reliance on iron uptake for virulence has potential for development of selective new antibiotic therapies.[8]

Studies of natural and synthetic siderophore-antibiotic conjugates (sideromycins) are of interest for development of potential Trojan Horse antibiotics to especially target multidrug resistant bacterial infections.[9] Most reported synthetic sideromycins consist of a siderophore or siderophore mimic directly attached to a β-lactam antibiotic. Many such constructs, including representatives (**4-11**) shown in Figure 2,[10,11,12,13,14,15] show enhanced and strain-selective antibacterial activity because of siderophore-receptor mediated active transport and reduced susceptibility to antibiotic efflux mechanisms.[10,16] While some of these conjugates also are less prone to deactivation by β-lactamases, the activity of other more susceptible conjugates is augmented by co-administration of β-lactamase inhibitors.

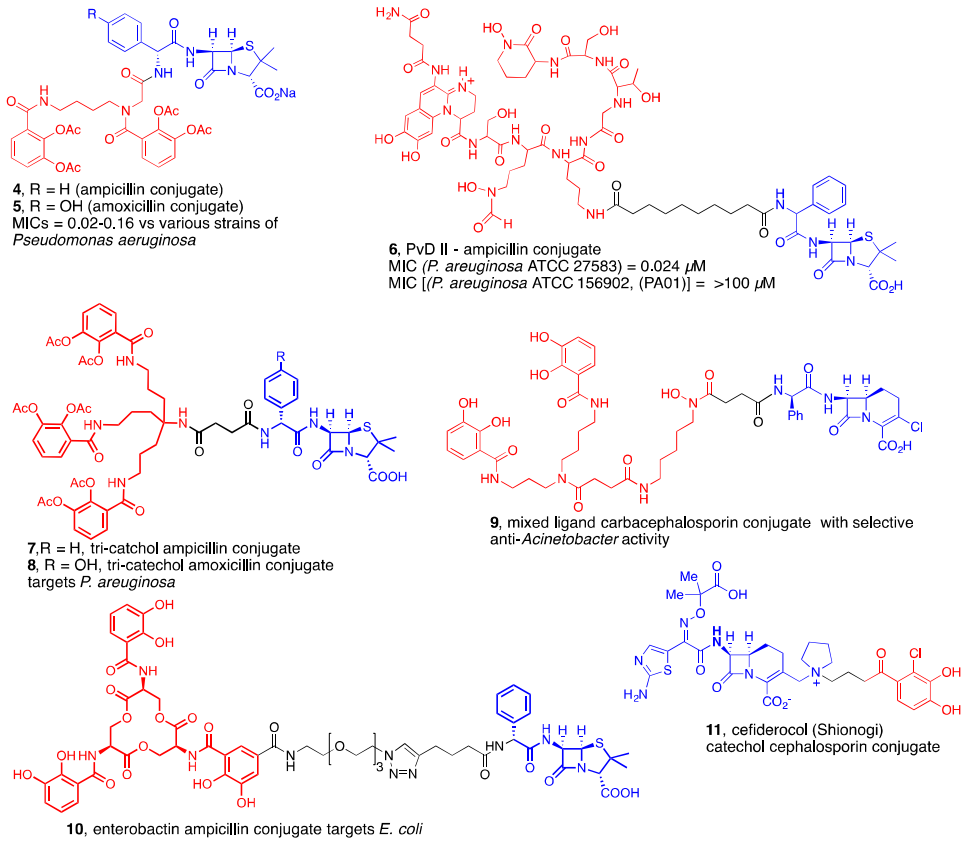


Figure 2. Selected representative examples of synthetic sideromycins containing β-lactam warheads.

To further circumvent problems associated with β-lactamases, siderophore conjugates with non-β-lactam antibiotics have been designed and synthesized. Some siderophore conjugates with non-releasable linkers to other antibiotics, especially those with cytoplasmic targets, might not be recognized by the targets while the warhead remains attached to the siderophore and thus do not have enhanced activity.[17] However, representative compounds shown in Figure 3 have remarkably potent activity against bacteria targeted by the bacteria-selective siderophore component. In fact, this strategy allows delivery of antibiotics, regardless of size or charge, into microbial cells and induces efficacy of these drugs against pathogens that are normally not susceptible to the antibiotic alone. For example, while artemisinin, a small uncharged

antimalarial drug, has no anti-tuberculosis activity itself, its mycobactin conjugate **12**, is a potent anti-TB agent that is completely selective for *Mycobacterium tuberculosis* (*Mtb*).[18] It is not active against any other strain of mycobacteria nor any other Gram-negative or Gram-positive bacteria because the siderophore component is specific for *Mtb*. In another case, the mixed ligand, bis-catechol, monohydroxamate, conjugate (**13**) of daptomycin, a large, polyanionic antibiotic, confers potent and selective *in vitro* and *in vivo* activity of daptomycin against multi-drug resistant strains of *A. baumannii*, even though the parent daptomycin itself is only active against Gram-positive bacteria.[19]

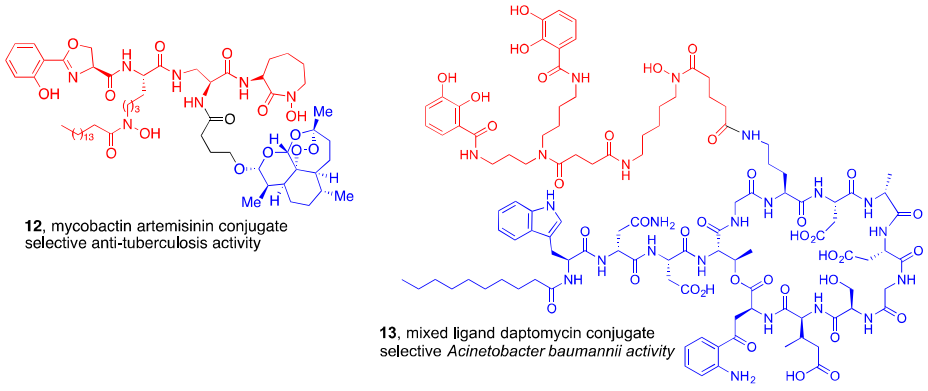


Figure 3. Non β-lactam synthetic and highly targeted sideromycins.

In all cases shown in Figures 2 and 3, the synthetic sideromycins were made by direct coupling of the siderophore component to the antibiotic warhead and no intracellular drug release process was necessary since the intact sideromycin is recognized by the antibiotic target. However, as shown in Figure 4, most natural sideromycins require an intracellular process that allows release of the antibiotic component once the conjugate is sequestered and actively transported into the bacterial cell.[20] Albomycins (**14**)[21] and salmycins (**15**)[22] are the most well studied natural sideromycins.[23] The tRNA synthetase inhibitor portion of albomycin and the aminoglycoside component of salmycin have no antibiotic activity alone, presumably since they cannot reach

their cytoplasmic targets. However, the natural siderophore conjugates are potently active. In the case of the albomycins, bacterial peptidases cleave the seryl tRNA synthetase inhibitor from the conjugate after active transport allowing the inhibitor to exert its toxic antibiotic effect both *in vitro* and *in vivo*. Studies of albomycin have demonstrated clinical efficacy.[21,24,25] Intracellular reductive removal of the iron from salmycins to desferri salmycins (**16**) may trigger intramolecular cyclization with concomitant release of the aminoglycoside antibiotic. Thus, further expansion of the sideromycin-based Trojan Horse approach must consider the nature of the linker between the iron binding component and the warhead to be delivered.

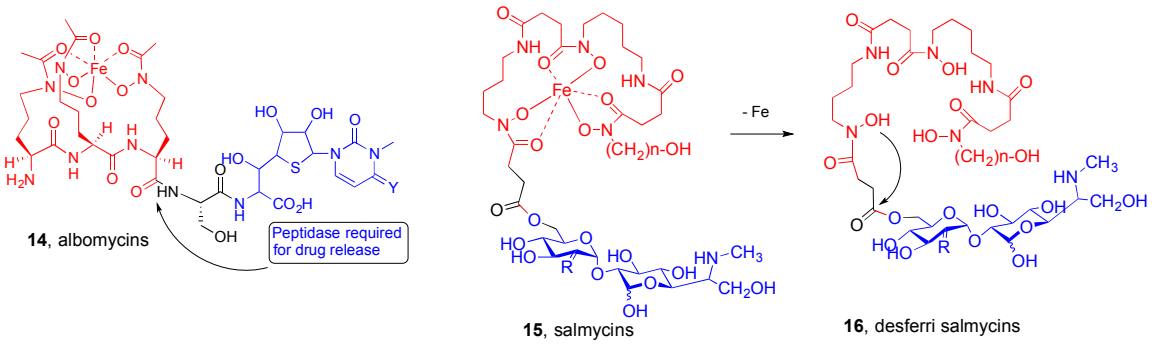


Figure 4. The natural sideromycins, albomycins and salmycins, require drug release.

Early attempts to incorporate releasable linkers included introduction of randomly hydrolysable linkers, but these are prone to premature release before bacterial assimilation.[26] More recent efforts include examples of esterase-triggered[27] and reductive-cyclization[28] -induced release following bacterial internalization of the synthetic sideromycin. As indicated earlier, β-lactams conjugated to siderophores can still be susceptible to hydrolysis by destructive β-lactamase enzymes. Many studies have demonstrated that non-siderophore derivatives of appropriate covalent combinations of cephalosporins or penicillins, themselves, with other drugs create dual action agents with activity due to the β-lactam component but, if that is susceptible to

a β-lactamase, the destructive ring opening results in release of the additional attached drug.[29,30,31,32,33] β-Lactamase-triggered hydrolysis of cephalosporin-antitumor conjugates has also been widely studied,[34] indicating that cephalosporinases can recognize and cleave cephalosporins even when conjugated to large molecules. Thus, we reasoned that a siderophore conjugate of a cephalosporin with an additional antibiotic attached to the 3'-methyl position would create a new class of multiple action sideromycin (**1**) that could target both, bacteria that are susceptible to its β-lactam component and the β-lactamase-producing bacteria which would hydrolyze the β-lactam thus releasing the additional antibiotic warhead (**3**) capable of killing bacteria. Thus, when susceptible to β-lactamases, the cephalosporin component should serve as a releasable linker. Essentially, the normally deleterious β-lactamase enzyme would be utilized to induce the targeted bacteria to commit suicide.

In order to test this, we decided to incorporate an oxazolidinone as the antibiotic that would ultimately be released upon hydrolytic opening of the cephalosporin linker. Oxazolidinones, like eperezolid,[35,36] have a ribosomal target and either cannot permeate Gram-negative outer membranes or, if they do get into Gram-negative bacteria, they are rapidly effluxed.[36] Thus, while oxazolidinone antibiotics are effective against clinically important Gram-positive bacteria, they are not effective against most Gram-negative bacteria. We anticipated that siderophore-mediated transport of oxazolidinones would allow for the efficient passage of these drugs through the bacterial outer membrane. We also hypothesized that incorporation of a cephalosporin linker that is prone to hydrolysis by bacterial β-lactamases would allow efficient release of oxazolidinone so it can reach its intracellular target. Enhanced concentration of the delivered oxazolidinone might also help to overwhelm efflux pumps to allow the drug to exert its lethal effect.

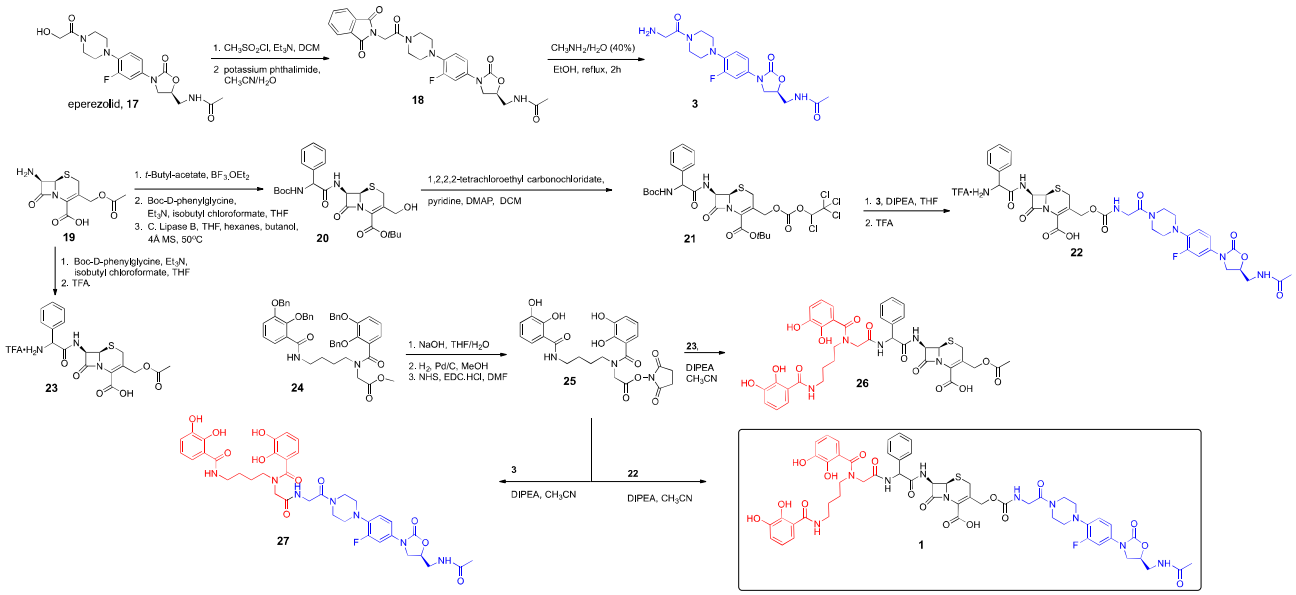
RESULTS AND DISCUSSION

The specific siderophore-cephalosporin-oxazolidinone **1** was designed to test our hypothesis related to siderophore-mediated dual drug delivery and β-lactamase triggered release of an oxazolidinone to Gram-negative bacteria. The synthesis of **1** required access to all three components, the siderophore, an appropriately functionalized cephalosporin and an oxazolidinone with suitable pendant functionality for coupling to the cephalosporin moiety. As shown in detail below (Scheme 1), the primary alcohol of commercially available eperezolid (**17**) was converted to the mesylate which was then displaced with potassium phthalimide to provide protected amine (**18**). Standard deprotection with $MeNH_2$ provided oxazolidinone **3** with a free amine for subsequent reaction with an appropriately activated cephalosporin (**21**).[35] Cephalosporin **21** was prepared in four steps from readily available 3-aminocephalosporanic acid (**19**). Thus, **19** was first converted to the *t*-butyl ester by BF_3-catalyzed reaction with *t*-butyl acetate. D-Phenylglycine, a common side chain in cephalosporins, including cefaclor,[37] was protected with a Boc group and then coupled to the free amine of the *t*-butyl cephalosporinate. Finally, the 3'-acetoxy group was removed enzymatically using Candida lipase B[38] to give protected cephalosporin **20** with a free 3'-hydroxyl group. Reaction of the hydroxyl group with 1,2,2,2-tetrachloroethyl carbonochloridate gave the isolable activated cephalosporin **21**. Treatment of cephalosporin carbonate **21** with aminooxazolidinone **3** followed by reaction with TFA to remove the Boc group and *t*-butyl ester gave cephalosporin oxazolidinone **22**.

Protected bis-catechol **24**[10] was first saponified to give the free acid which was hydrogenolytically debenzylated and then treated with EDC/NHS to give active ester **25**. Direct reaction of the active ester with cephalosporin oxazolidinone **22** gave the final conjugate **1**. Separate coupling of the activated siderophore with D-phenylglycyl cephalosporin (**23**) and

aminooxazolidinone (**3**) gave the corresponding siderophore-cephalosporin (**26**) and siderophore-oxazolidinone (**27**) conjugates for use as controls in subsequent bioassays.

Scheme 1. Syntheses of siderophore conjugates.



With the final siderophore-cephalosporin-oxazolidinone conjugate (**1**) along with the separate cephalosporin and oxazolidinone conjugates (**26** and **27**) as well as the corresponding cephalosporin (**23**), oxazolidinone (**3**) and eperezolid (**17**) available, we performed enzymatic and anti-bacterial assays to test our hypothesis. Our plan and design critically depended on the susceptibility of conjugate **1** to the hydrolytic activity of β-lactamases from Gram-negative bacteria. Thus, we evaluated whether the oxazolidinone could be efficiently released from the final siderophore-cephalosporin-oxazolidinone conjugate (**1**) as a result of cephalosporin hydrolysis by a β-lactamase. For this purpose, we chose one of the ADC-type β-lactamases (ADC-1), as ADC enzymes are intrinsic to *A. baumannii*, an important clinical pathogen, and are capable of hydrolyzing cephalosporin antibiotics. As anticipated, incubation of the purified

ADC-1 with conjugate **1** resulted in its rapid and complete hydrolysis and release of the oxazolidinone, as determined by LC/MS (**Figure 5**).

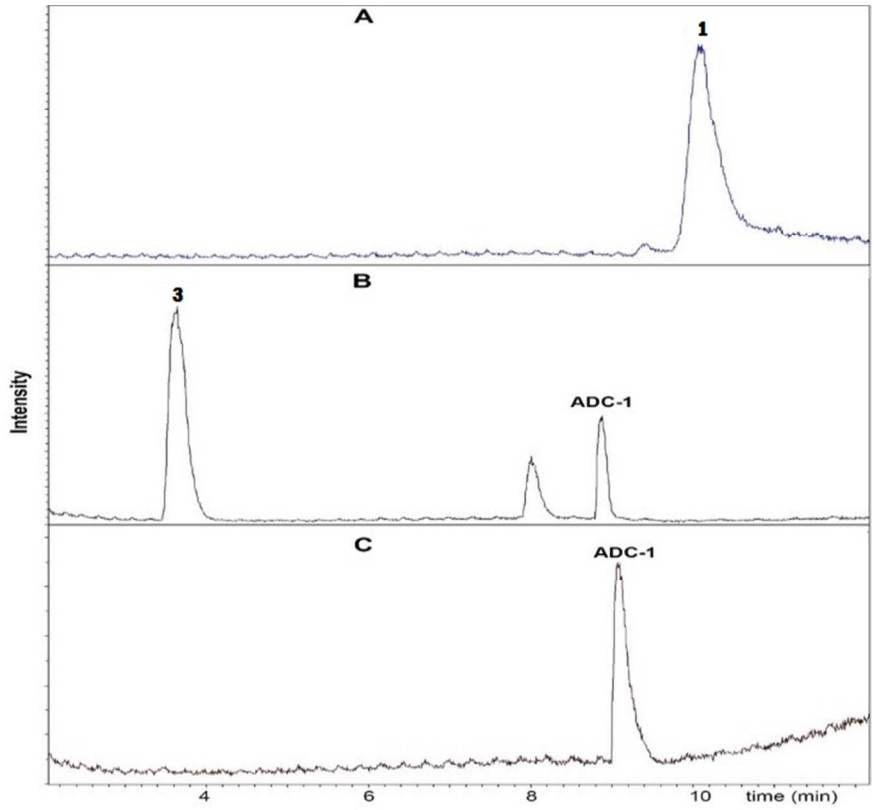


Figure 5. Cephalosporinase (ADC-1)-induced hydrolytic release of oxazolidinone **3** from conjugate **1**. LC/MS Base Peak Chromatograms showing pure conjugate (A), product formed following reaction with ADC-1 (B) and ADC-1 only (C).

Next, we tested whether the siderophore-cephalosporin-oxazolidinone conjugate (**1**) exhibits antimicrobial activity against *A. baumannii* producing the ADC-1 β-lactamase. For this purpose, we used *A. baumannii* ATCC 17978 which produces a small amount of the chromosomally-encoded enzyme and the same strain that produces a large amount of ADC-1 from the pNT255 vector (Table 1). As expected, the non-conjugated oxazolidinones (**3** and **17**) themselves as well as the cephalosporin-oxazolidinone (**22**) and simple cephalosporin (**23**) were not active against

this strain of *A. baumannii*, due to inadequate delivery or efflux. The siderophore-oxazolidinone (**27**, with a non-releasable linkage between the transport agent and antibiotic component) also was not active, suggesting that without release from the siderophore, the oxazolidione either could not reach its target or was not recognized by its target. The siderophore-cephalosporin (**26**) was remarkably active against *A. baumannii* producing a low amount of ADC-1. Dual conjugate, **1**, retained similarly potent activity which indicates that activity of both compounds (**26** and **1**) is due to the cephalosporin. The dramatic improvement in MICs (more than 125-fold) of the cephalosporin or cephalosporin-oxazolidinone when conjugated to the siderophore indicates that the antibiotics are very efficiently delivered inside the bacterial cell.

When the production of the enzyme was significantly increased by the introduction of the plasmid-encoded β-lactamase (pNT255:adc1), this *A. baumannii* was rendered highly resistant to the siderophore-cephalosporin (**26**), an indication that large amounts of ADC-1 protected bacteria by hydrolyzing the incoming cephalosporin antibiotic, as anticipated from our studies that showed that the isolated ADC enzyme rapidly hydrolyzed the cephalosporin component. Addition of the oxazolidinone warhead to the siderophore-cephalosporin to result in final conjugate **1** significantly decreased the MIC (at least 8-fold) when compared to the siderophore-cephalosporin. These results are consistent with the scenario in which cleavage of the cephalosporin by the ADC-1 β-lactamase results in release of the oxazolidinone that can then reach its target, perhaps minimize the influence of efflux pumps and allow the oxazolidinone to exert a lethal effect on targeted Gram-negative bacteria.

Table 1. Activity (in μM) of tested compounds against *A. baumannii* ATCC 17978 without and with plasmid-encoded β-lactamase.

	3	17	27	22	23	26	1
	oxazolidinone	eperezolid	siderophore + oxazolidinone	cephalosporin + oxazolidinone	cephalosporin	siderophore + cephalosporin	siderophore + cephalosporin + oxazolidinone
A. baumannii ATCC 17978	>500	>50	>50	>50	>50	0.4	0.4
A. baumannii ATCC 17978 pNT255	>500	>50	>50	>50	>50	0.4	0.4
A. baumannii ATCC 17978 pNT225: ADC-1	>500	>50	>50	>50	>50	>50	6

Subsequently, we evaluated activity of our compounds against four clinical isolates of *A. baumannii* (ATCC 17961, ATCC BAA1793, ATCC BAA1797 and ATTC BAA1800). All four *A. baumannii* isolates were resistant to compounds **3**, **22**, **23**, and **27**. Strain ATCC 17961 was susceptible to both **26** and **1**, indicating lack of or low β-lactamase content and/or activity. Conjugate **26**, with the cephalosporin alone, had detectable activity against two clinical isolates (ATCC BAA1793 and 1800). Notably, the final siderophore-cephalosporin-oxazolidinone conjugate **1** was very active against all of the clinical isolates, especially the siderophore-cephalopsorin (**26**) resistant clinical strain ATCC BAA 1797 (Table 2).

Table 2. Activity (in μM) of compounds against *A. baumannii* and other bacteria tested.

strain	3	22	23	27	26	1
	oxazolidinone	cephalosporin + oxazolidinone	cephalosporin	siderophore + oxazolidinone	siderophore + cephalosporin	siderophore + cephalosporin + oxazolidinone
A. baumannii ATCC 17961	>50	>50	>50	>50	0.8	0.8

A. baumannii ATCC BAA 1793	>50	>50	>50	>50	12.5	0.8-1.6
A. baumannii ATCC BAA 1797	>50	>50	>50	>50	>50	6.25
A. baumannii ATCC BAA 1800	>50	>50	>50	>50	25	0.8
E. coli DC0	>50	>50	50	>50	<0.025	<0.025
P. aeruginosa KW799/wt	>50	>50	>50	>50	0.4	0.2-0.4

Further studies with two other Gram-negative bacteria (*Escherichia coli* DC0 and *Pseudomonas aeruginosa* KW799) revealed that non-conjugated oxazolidinone **3**, cephalosporin-oxazolidinone **22**, cephalosporin **23**, or the siderophore-oxazolidinone **27** were not active against the *E. coli* and *Pseudomonas aeruginosa* tested. However, the siderophore-cephalosporin (**26**, MIC: < 0.025 μM vs *E. coli* DC0 and 0.4 μM vs *P. aeruginosa* KW799/wt) and siderophore-cephalosporin-oxazolidinone (**1**, MIC: < 0.025 μM vs *E. coli* DC0 and 0.2-0.4 μM vs *P. aeruginosa* KW799/wt) were remarkably active.

Conclusion

Overall, these results are consistent with siderophore-mediated active transport that facilitates uptake of the cephalosporin (conjugate **26**) to improve its activity relative to the cephalosporin (**23**) itself. The lack of activity of the siderophore-oxazolidinone conjugate (**27**) reflects previous studies discussed earlier that indicate cytoplasmic targets do not tolerate intact conjugates to other drugs without releasable linkers. Notably, the activity and cephalosporinase susceptibility of the final siderophore-cephalosporin-oxazolidinone conjugate (**1**) is consistent with siderophore-mediated active uptake, inherent activity of the delivered dual drug and, in the presence of β-lactamase, intracellular release of the oxazolidinone upon cleavage of the

cephalosporin to allow the freed oxazolidinone to inactivate its target. The final result demonstrates that oxazolidinone antibiotics are active against Gram-negative bacteria when they are efficiently delivered to their target. Importantly, we chose to demonstrate this approach by targeting β-lactamase producing *A. baumannii*, which is a pathogen of major concern world-wide.[5,39]

These studies demonstrate that β-lactams, and cephalosporins in particular, can serve as β-lactamase-triggered releasable linkers to allow intracellular delivery of Gram-positive antibiotics to Gram-negative bacteria and thereby provide new methods to inhibit proliferation of multi-drug resistant bacteria.

Experimental Section

General Methods. All solvents and reagents were obtained from commercial sources and used without further purification unless otherwise stated. Silica gel (230−400 mesh) was purchased from Silicycle, Quebec City, Canada. All compounds are >98% pure by HPLC analysis, and MIC values reported are the average of three individual measurements. All compounds were characterized by ^{1}H and ^{13}C NMR using Bruker 400 MHz, 500 MHz NMR, and/or Varian 600 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra, and coupling constants (J) are reported in hertz (Hz). The mass spectra values are reported as m/z, and ultrahigh pressure liquid chromatography mass spectrometry (UPLCMS) analyses of the reaction of **1** with **ADC-1** were carried out with a Bruker MicrOTOF-QII, quadrupole time-of-flight mass spectrometer coupled via electrospray ionization with a Dionex Ultimate 3000 RSLC system. A 20 minute binary gradient separation on a Zorbax® RX-C8 narrow bore 2.1x150 mm 5 micron column kept at 37 C was run under the following conditions: solvent A = water with 0.1% formic acid, solvent B = acetonitrile with

0.1% formic acid, A:B = 90:10 for 2 minutes followed by a 16 minute linear ramp to A:B 0:100 before returning to initial conditions for 2 minutes. Additional liquid chromatography mass spectrum (LC/MS) analyses for all compounds, except **1**, **26**, and **27**, were carried out on a Waters ZQ instrument consisting of chromatography module Alliance HT, photodiode array detector 2996, and mass spectrometer Micromass ZQ, using a 3 × 50 mm Pro C18 YMC reverse phase column. Mobile phases: 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 min at 0.7 mL/min. The MS electrospray source operated at capillary voltage 3.5 kV and a desolvation temperature of 300 °C. For compounds **1** and **26**, LCMS analysis was performed on a Waters 2695 Separations Module integrated 3100 Mass Detector system employing positive ion ESI mode, equipped with a Waters 996 Photodiode Array Detector, using a Waters XBridge BEH C18 2.5 μm column (3.0 × 30 mm), DAD detected at 254 nm, at 50 °C. The operation software is MassLynx Software License V4.1 (Waters, Milford, MA, USA). Mobile phases: 0.1% TFA in HPLC grade water (A) and 0.06% TFA in HPLC grade acetonitrile (B). A gradient was formed from 3% to 97% of B in 4 min at 0.5 mL/min. For compound **27**, LCMS analysis was performed on a Waters 2695 Separations Module integrated 3100 Mass Detector system employing positive ion ESI mode, equipped with a Waters 996 Photodiode Array Detector, using a Waters XBridge BEH C18 2.5 μm column (3.0 × 30 mm), DAD detected at 254 nm, at 50 °C. The operation software is MassLynx Software License V4.1 (Waters, Milford, MA, USA). Mobile phases: 0.1% TFA in HPLC grade water (A) and 0.06% TFA in HPLC grade acetonitrile (B). A gradient was formed from 3% to 97% of B in 4 min at 0.5 mL/min. Reverse phase chromatographic purification was performed on a Waters 1525 Binary pump equipped with a Waters 2998 Photodiode Array Detector, Waters 2707 Autosampler, and Water Fraction Collector III utilizing

Empower 3 Chromatography Manager software (Waters, Milford, MA, USA). A Waters SymmetryPrep C18 7 μm OBD column (300 × 7.8 mm) was used for separation. DAD was detected at 254 nm at 20 °C, eluting with a linear gradient of 0.5−20% acetonitrile in 0.1% aqueous TFA (flow rate 5 mL/min).

(*6R*,*7R*)-3-((((2-(4-(4-((*S*)-5-(Acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazin-1-yl)-2-oxoethyl)carbamoyl)oxy)methyl)-7-(2-(2-(N-(4-(2,3-dihydroxybenzamido)butyl)-2,3-dihydroxybenzamido)acetamido)-2-phenylacetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1).

To a suspension of **22** (0.1 mmol, 88 mg) in anhydrous acetonitrile (5 mL), was added diisoprpylethylamine (0.2 mmol, 35 μL) at room temperature. To this mixture was added a DMF solution of **25** (0.1 mmol) and the mixture was stirred for 4h. The reaction was then concentrated under reduced pressure. The residue was purified using a C_{18} reverse phase silica gel column, eluting with a gradient of water and acetonitrile to give **1** (0.01 mmol, 11 mg) as a white solid in 10% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.23-1.29 (m, 3H), 1.46-1.56 (m, 3H), 1.83 (s, 3H), 2.92-2.96 (m, 4H), 3.08-3.16 (m, 2H), 3.40 (t, 2H, J = 5 Hz), 3.5-3.59 (m, 4H), 3.68-3.71 (m, 1H), 3.87-3.93 (m, 2H), 4.08 (t, 1H, J = 10 Hz), 4.14-4.19 (m, 1H), 4.57-4.71 (m, 2H), 4.84 (s, 1H), 4.94-5.12 (m, 2H), 5.46 (s, 1H), 5.59 (s, 1H), 5.70 (brs, 1H), 6.46-6.77 (m, 5H), 6.87-6.90 (m, 1H), 7.07 (t, 1H, J = 10 Hz), 7.17-7.29 (m, 5H), 7.46-7.52 (m, 2H), 8.01 (s, 1H), 8.24 (t, 1H, J = 4 Hz), 8.64 (brs, 2H), 8.80-8.83 (m, 1H), 9.07-9.11 (m, 1H), 9.40-9.48 (m, 2H), 12.79-12.90 (m, 1H), 13.64 (brs, 1H). ^{13}C NMR (500 MHz, DMSO-d_6): δ 22.30, 24.68, 25.26, 41.34, 41.92, 43.93, 47.26, 50.12, 50.42, 55.43, 57.39, 58.66, 62.62, 71.40, 106.50, 110.04, 113.65, 114.06, 117.02, 117.40, 117.64, 118.00, 119.73, 124.71, 125.70, 126.72, 126.99, 127.35, 128.05, 133.60, 135.13, 138.02, 149.83, 150.90, 151.06, 151.16, 153.74, 153.92, 155.19, 155.36, 155.95,

156.14, 157.79, 158.00, 162.60, 164.14, 166.95, 168.86, 169.06, 169.85, 170.47. HRMS (m/z): $[M+Na]^+$ calcd for $C_{55}H_{59}FN_{10}NaO_{17}S$, 1205.3665; found, 1205.3656. LC/MS retention time 6.32 min.

(*S*)-*N*-((3-(3-Fluoro-4-(4-glycylpiperazin-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3).

A mixture of **18** (0.59 mmol, 308 mg) and 0.52 mL of 40% methylamine in water (5.9 mmol, 10 eq) and 30 mL of EtOH was heated at reflux for 3h. When the starting material **18** was consumed, the mixture was concentrated under reduced pressure and the residue was purified using a silica gel column, eluting with DCM/MeOH (5/1, v/v) to give **3** as a yellow foamy solid in 43% yield (99 mg, 0.25 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.81 (s, 3H), 2.94-2.98 (m, 4H), 3.38 (t, 2H, J = 8.0 Hz), 3.65-3.70 (m, 4H), 3.91-3.92 (m, 2H), 4.06 (t, 2H, J = 8.0 Hz), 4.67-4.71 (m, 1H), 7.06 (t, 1H, J = 8.0 Hz), 7.16-7.19 (m, 1H), 7.47 (d, 1H, J = 12 Hz), 8.05 (brs, 2H), 8.24 (t, 1H, J = 8.0 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.13, 42.12, 42.27, 44.88, 48.03, 50.83, 51.07, 72.27, 107.25-107.51, 114.86, 120.59, 134.52-134.62, 135.74-135.83, 154.16, 154.76, 156.58, 165.33, 170.70. LC/MS (m/z): $[m+H]^+$ calcd for $C_{18}H_{25}FN_5O_4$, 394.19; found, 394.25; retention time: 1.23 min.

(*S*)-*N*-((3-(4-(4-(2-(1,3-Dioxoisoindolin-2-yl)acetyl)piperazin-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (18)

To a solution of Eperezolid (**17**) (394 mg, 1 mmol) and Et_3N (418 µL, 3 mmol) in 20 mL of anhydrous DCM at 0^{o}C was added methanesulfonyl chloride (155 µL, 2 mmol) dropwise. The mixture was then stirred at 0^{o}C for 1h and then at room temperature for 1h. When the starting material was disappeared, the mixture was washed with 100 mL water, and the aqueous layer

was extracted with DCM (30 mL). The combined organic layers were dried with Na_2SO_4, filtered, then concentrated under reduced pressure to give the corresponding mesylate as a yellow foamy solid (472 mg, 1 mmol) which was used directly without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.83 (s, 3H), 2.96-3.00 (m, 4H), 3.07-3.14 (m, 1H), 3.27 (s, 3H), 3.40 (t, 2H, J = 8.0 Hz), 3.51-3.62 (m, 4H), 3.69-3.72 (m, 1H), 4.08 (t, 1H, J = 8.0 Hz), 4.69-4.73 (m, 1H), 5.07 (s, 1H), 7.08 (t, 1H, J = 8.0 Hz), 7.18-7.20 (m, 1H), 7.48-7.53 (m, 1H), 8.24 (t, 1H, J = 4.0 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.13, 38.48, 42.12, 43.78, 44.77, 46.52, 48.02, 50.80-51.01, 67.43, 72.25, 107.21-107.47, 114.83, 120.59, 134.44, 135.83, 154.14, 154.75, 164.51, 170.68. LC/MS (m/z): $[m+H]^+$ calcd for $C_{19}H_{26}FN_4O_7S$, 473.15; found, 473.29; retention time: 4.30 min.

To a solution of the mesylate in CH_3CN (50 mL) and H_2O (0.25 mL) was added potassium phthalimide (556 mg, 3 mmol) and then the reaction was refluxed for 48h. After the reaction was complete as monitored by TLC, it was filtered to remove the solid, and the solvent was concentrated under reduced pressure. The residue was purified by silica gel column, eluting with DCM / MeOH (100/1, v/v) to give **18** as a white solid in 59% yield (308 mg, 0.59 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.83 (s, 3H), 2.96 (brs, 2H), 3.06 (brs, 2H), 3.40 (t, 2H, J = 8.0 Hz), 3.60 (brs, 2H), 3.69-3.72 (m, 3H), 4.09 (t, 1H, J = 8.0 Hz), 4.59 (s, 2H), 4.68-4.74 (m, 1H), 7.11 (t, 1H, J = 8.0 Hz), 7.18-7.20 (m, 1H), 7.49 (dd, 1H, J_1 = 4 Hz, J_2 =16 Hz), 7.87-7.94 (m, 4H), 8.24 (t, 1H, J = 8.0 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.14, 42.13, 42.41, 44.92, 48.02, 50.84, 51.30, 72.26, 107.20-107.46, 114.81, 120.61-120.65, 123.96, 132.38, 134.47-134.57, 135.38, 135.87-135.96, 154.18, 154.75, 156.60, 164.87, 168.31, 170.68. LC/MS (m/z): $[m+H]^+$ calcd for $C_{26}H_{27}FN_5O_6$, 524.19; found, 524.35; retention time: 5.53 min.

tert-Butyl (6*R*,7*R*)-7-(2-((_tert_-butoxycarbonyl)amino)-2-phenylacetamido)-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (20)

To a flame dried pressure tube under argon was added *t*-butyl acetate (18.5 mL, 138 mmol). Argon was bubbled through the *t*-butyl acetate for 5 min and compound **19** (1.00 g, 3.67 mmol) was added. To this suspension was added $BF_3{\cdot}Et_2O$ (2.8 mL, 22.0 mmol), the tube was capped, and the reaction was stirred until homogeneous (~1.25 h). The reaction was poured into 50 mL of stirring ice water and the organic layer was removed. The aqueous layer was washed with EtOAc/hexanes (1:1, v/v) and this organic layer was also discarded. The aqueous layer was transferred to a flask, placed in an ice bath, and EtOAc (75 mL) was added. To this stirring suspension was slowly added Na_2CO_3 until the pH was 8.5. The organic layer was removed and the aqueous layer was extracted with EtOAc (3 times). The pooled organic layers were dried with Na_2SO_4, filtered, and concentrated under reduced pressure to give a yellow oil that was stirred in EtOAc/hexanes (1:10, v/v) for 10 min. Most of the solvent was decanted and the product was dried under reduced pressure to give the *t*-butyl ester as a light-yellow solid in 68% yield (822 mg, 2.5 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.49 (s, 9H), 2.02 (s, 3H), 2.28 (s, 2H), 3.40 (d, 1H, J = 20 Hz), 3.56 (d, 1H, J = 20 Hz), 4.59 (d, 1H, J = 12 Hz), 4.79 (s, 1H), 4.87 (d, 1H, J = 12 Hz), 4.98 (d, 1H, J = 4 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 21.25, 26.00, 28.11, 59.90, 63.41, 64.46, 83.15, 122.66, 127.74, 161.42, 170.68, 170.89.

A solution of Boc-D-phenylglycine (126 mg, 0.5 mmol) and Et_3N (70 µL, 0.5 mmol) in THF (10 mL) was cooled to -10 ^{o}C. While stirring, 65 µL (0.5 mmol) isobutyl chloroformate was added and the temperature was maintained at -10 ^{o}C for 10 min. A cold solution of the *t*-butyl ester in 3 mL of THF was added with stirring to the mixed anhydride solution. The mixture was stirred at 5 ^{o}C for 1h and then at room temperature for 1h. When the reaction was finished, as monitored by

TLC, the THF was evaporated and the residue was purified using a silica gel column eluting with hexanes / EtOAc (4:1, v/v) to give ***tert*-Butyl (*6R*,*7R*)-3-(acetoxymethyl)-7-(2-((*tert*-butoxycarbonyl)amino)-2-phenylacetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate** as a white solid in 77% yield (216 mg, 0.38 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.39 (s, 9H), 1.47 (s, 9 H), 2.01 (s, 3H), 3.42 (d, 1H, J = 20 Hz), 3.55 (d, 1H, J = 20 Hz), 4.61 (d, 1H, J = 12 Hz), 4.89 (d, 1H, J = 12 Hz), 5.04 (d, 1H, J = 4 Hz), 5.35 (d, 1H, J = 8 Hz), 5.73 (brs, 1H), 7.29-7.44 (m, 5H), 9.17-9.18 (d, 1H, J = 4 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 21.21, 26.32, 28.10, 28.86, 58.07, 58.31, 59.43, 63.22, 79.16, 83.46, 122.89, 127.52, 127.91, 128.33, 128.40, 128.87, 129.02, 138.75, 155.58, 161.01, 164.91, 170.81, 171.73. LC/MS (m/z): $[M-H]^+$ calcd for $C_{27}H_{34}N_3O_8S$, 560.20; found, 560.36; retention time: 8.88 min.

To an Erlenmeryer flask was added ***tert*-butyl (*6R*,*7R*)-3-(acetoxymethyl)-7-(2-((*tert*-butoxycarbonyl)amino)-2-phenylacetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate** (0.2 mmol, 112 mg), 1 mL of THF, and 10 mL of hexanes. The flask was swirled to give a suspension. To this suspension was added CAB Lipase (44 mg), 4Å molecular sieves (90 mg) and s-butanol (0.37 mL). The flask was stopped with a septum (with a small air vent) and the reaction was shaken in an incubated shaker at 50 ^{o}C for 3-4 days. The reaction was diluted with DCM and filtered using vacuum filtration. The filtrate was concentrated under reduced pressure to give **20** as a white solid in 85% yield (88 mg, 0.17 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.39 (s, 9H), 1.47 (s, 9 H), 3.39-3.50 (m, 2H), 4.11-4.21 (m, 2H), 4.98 (d, 1H, J = 4 Hz), 5.04 (brs, 1H), 5.34 (d, 1H, J = 8 Hz), 5.66-5.69 (m, 1H), 7.27-7.44 (m, 5H), 9.14 (d, 1H, J = 8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 26.05, 28.18, 28.86, 58.02, 58.30, 59.20, 60.53, 79.14, 82.85, 124.42, 127.89, 128.30, 128.86, 138.80, 155.63, 161.49, 164.79, 171.74. LC/MS (m/z): $[M-H]^+$ calcd for $C_{25}H_{32}N_3O_7S$, 518.19; found, 518.20; retention time: 7.72 min.

tert-Butyl (6R,7R)-7-(2-((_tert_-butoxycarbonyl)amino)-2-phenylacetamido)-8-oxo-3-((((1,2,2,2-tetrachloroethoxy)carbonyl)oxy)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (21).

To a solution of compound **20** (0.1 mmol, 52 mg) in anhydrous DCM at room temperature was added 1,2,2,2-tetrachloroethyl carbonochloridate (0.11 mmol, 17 μL), pyridine (0.13 mmol, 11 μL) and 2 mg of DMAP. The mixture was stirred for 2h. When the starting material was consumed based on TLC analysis, the reaction was concentrated under reduced pressure. The residue was dissolved in 1 mL of EtOAc, and the crude compound, **21**, was precipitated as a light pink solid in 77% yield by adding 5 mL of hexanes. Compound **21** was used directly for the next step without further purification.

(*6R*,*7R*)-3-((((2-(4-(4-((*S*)-5-(Acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazin-1-yl)-2-oxoethyl)carbamoyl)oxy)methyl)-7-(2-amino-2-phenylacetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (22).

To a solution of **3** (0.1 mmol, 39 mg) in anhydrous THF (3 mL) was added DIPEA (0.2 mmol, 34 μL) at room temperature. The mixture was stirred for 2 min, then a solution of crude **21** (0.1 mmol, 73 mg) in THF (2 mL) was added. The reaction was stirred at room temperature for 6h. After evaporation, the residue was purified using a silica gel column eluting with DCM/MeOH (40:1, v/v) to give the corresponding protected cephalosporin oxazolidinone as a white solid in 50% yield (0.05 mmol, 47 mg). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.39 (s, 9H), 1.47 (s, 9H), 1.81 (s, 3H), 2.93 (d, 4H, J = 12 Hz), 3.40-3.70 (m, 5H), 3.89 (brs, 2H), 4.08 (t, 1H, J = 8.0 Hz), 4.56-4.61 (m, 1H), 4.70 (s, 1H), 4.84 (d, 1H, J = 8.0 Hz), 5.03 (s, 1H), 5.33 (s, 1H), 5.71 (brs, 1H), 7.07 (t, 1H, J = 8.0 Hz), 7.17 (d, 1H, J = 4.0 Hz), 7.27-7.31 (m, 2H), 7.43-7.51 (m, 5H),

8.17 (s, 1H), 8.24 (s, 1H), 9.16 (s, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.13, 26.78, 28.14, 28.86, 42.12, 42.70, 44.72, 48.01, 50.93, 51.28, 58.30, 59.40, 63.30, 72.25, 79.18, 83.43, 107.20-107.46, 114.81, 120.50, 124.02, 126.98, 127.91, 128.22, 128.87, 129.88, 134.40-134.51, 135.90-135.99, 137.05, 138.75, 154.14, 154.75, 156.56-156.92, 161.05, 164.90, 167.77, 169.27, 170.68, 171.74. LC/MS (m/z): $[M+H]^+$ calcd for $C_{44}H_{56}FN_8O_{12}S$, 939.37; found, 939.61; retention time: 7.72 min.

To 5 mL of anhydrous trifluoroacetic acid at room temperature was added 40 mg of the white solid from the last step (0.04 mmol) with stirring. After 30 min, pouring the reaction solution into anhydrous ether afforded the trifluoroacetic salt of **22** (30 mg, 0.03 mmol) as a white solid in 75% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.83 (s, 3H), 2.92-2.96 (m, 4H), 3.40 (brs, 2H), 3.48-3.59 (m, 5H), 3.70 (t, 2H, J = 8.0 Hz), 3.87 (d, 2H, J = 4.0 Hz), 4.08 (t, 1H, J = 8.0 Hz), 4.62 (d, 1H, J = 16.0 Hz), 4.71 (t, 1H, J =8.0 Hz), 4.93 (d, 1H, J = 12.0 Hz), 5.04 (d, 2H, J = 4.0 Hz), 5.78 (t, 1H, J = 8.0 Hz), 7.07 (t, 1H, J = 12.0 Hz), 7.17 (d, 1H, J = 4.0 Hz), 7.28 (t, 1H, J = 4.0 Hz), 7.43-7.52 (m, 5H), 8.12 (brs, 1H), 8.27 (brs, 1H), 8.78 (brs, 2H), 9.56 (d, 1H, J = 8.0 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.12, 25.98, 42.11, 42.69, 44.68, 50.93, 51.24, 56.03, 57.88, 59.32, 63.39, 72.23, 107.21-107.47, 114.82, 120.53, 125.37, 126.37, 128.45, 129.43, 130.00, 134.14, 134.40-134.50, 135.90-135.99, 154.14, 154.76, 156.57-156.99, 158.63-158.94, 163.38, 164.41, 165.33, 167.78, 168.93, 170.72. LC/MS (m/z): $[M+H]^+$ calcd for $C_{35}H_{40}FN_8O10S$, 782.25; found, 783.41; retention time: 4.17 min.

(*6R*,*7R*)-3-(Acetoxymethyl)-7-(2-(2-(*N*-(4-(2,3-dihydroxybenzamido)butyl)-2,3-dihydroxybenzamido)acetamido)-2-phenylacetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (26).

Compound 23 was synthesized according to the literature reported method.[40] To a suspension of **23** (0.1 mmol, 50 mg) in anhydrous acetonitrile (5 mL), was added diisopropylethylamine (0.2 mmol, 35 μL) at room temperature. To this mixture was added a DMF solution of **25** (0.1 mmol) and the mixture was stirred for 4h. The reaction was then concentrated under reduced pressure. The residue was purified using a C_{18} reverse phase silica gel column, eluting with a gradient of water and acetonitrile to give **26** as a white solid in 12% yield (0.012 mmol, 10 mg). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.23-1.32 (m, 2H), 1.45-1.56 (m, 2H), 2.01 (s, 3H), 3.09-3.17 (m, 2H), 3.30-3.54 (m, 4H), 3.92-3.94 (m, 1H), 4.17-4.20 (m, 1H), 4.64 (d, 1H, J = 10 Hz), 4.95-5.02 (m, 2H), 5.59-5.71 (m, 2H), 6.46-6.77 (m, 5H), 6.87-6.90 (m, 1H), 7.22-7.31 (m, 5H), 7.46 (brs, 1H), 8.62-8.80 (m, 2H), 9.12 (s, 1H), 9.36-9.49 (m, 2H), 12.79 (d, 1H, J = 50.0 Hz), 13.73 (s, 1H). ^{13}C NMR (500 MHz, DMSO-d_6): δ 20.37, 25.42, 25.83, 28.44, 28.83, 47.47, 48.74, 55.69, 57.37, 58.67, 62.52, 114.94, 115.68, 115.89, 117.03, 117.45, 117.69, 117.83, 118.62, 119.17, 123.34, 126.20, 126.72, 126.91, 127.54, 128.10, 137.79, 141.25, 145.21, 146.05, 149.53, 162.59, 164.11, 168.13, 169.20, 169.56, 170.00, 170.30. HRMS (m/z): $[M+Na]^+$ calcd for $C_{38}H_{39}N_5NaO_{13}S$, 828.2167; found, 828.2157. LC/MS retention time 6.18 min.

(*S*)-*N*-(2-((2-(4-(4-(5-(Acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazin-1-yl)-2-oxoethyl)amino)-2-oxoethyl)-*N*-(4-(2,3-dihydroxybenzamido)butyl)-2,3-dihydroxybenzamide (27).

To a suspension of **3** (0.1 mmol, 39 mg) in anhydrous acetonitrile (5 mL), was added diisoprpylethylamine (0.2 mmol, 35 μL) at room temperature. To this mixture was added a DMF solution of **25** (0.1 mmol) and the mixture was stirred for 4h. The reaction was then concentrated under reduced pressure. The residue was purified using a C_{18} reverse phase silica gel column, eluting with a gradient of water and acetonitrile to give **27** (0.01 mmol, 8 mg) as a white solid in

10% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.23-1.32 (m, 2H), 1.48-1.58 (m, 2H), 1.83 (s, 3H), 2.93-2.98 (m, 4H), 3.11-3.20 (m, 2H), 3.38-3.52 (m, 4H), 3.58 (s, 4H), 3.69 (dd, 1H, J_1 = 10.0 Hz, J_2 = 5.0 Hz), 3.86-3.96 (m, 2H), 4.06-4.14 (m, 2H), 4.67-4.73 (m, 1H), 6.58-6.67 (m, 3H), 6.77 (dd, 1H, J_1 = 20.0 Hz, J_2 = 10.0 Hz), 6.89 (d, 1H, J = 5.0 Hz), 7.06 (brs, 1H), 7.16 (d, 1H, J = 10.0 Hz), 7.21-7.30 (m, 1H), 7.49 (dd, 1H, J_1 = 15.0 Hz, J_2 = 5.0 Hz), 8.06 (s, 1H), 8.24 (t, 1H, J = 5.0 Hz), 8.67 (t, 1H, J = 15.0 Hz), 8.80-8.97 (m, 1H), 9.13 (s, 1H), 9.53 (s, 1H), 12.77 (d, 1H, J = 55.0 Hz). ^{13}C NMR (500 MHz, DMSO-d_6): δ 23.12, 24.82, 25.97, 26.56, 26.89, 29.70, 39.10, 39.46, 41.24, 42.08, 44.78, 45.33, 47.95, 48.16, 49.44, 50.89, 51.19, 51.55, 72.22, 107.15-107.36, 114.74, 115.58, 116.28, 116.61, 117.75, 118.25, 118.51, 118.67, 119.40, 119.96, 120.15, 120.48, 125.22, 125.66, 134.37-134.45, 135.88-135.95, 141.92, 145.95, 146.88, 150.38, 154.34, 154.73, 156.28, 158.44, 158.81, 167.33, 169.30, 169.93, 170.37, 170.69. HRMS (m/z): $[M+Na]^+$ calcd for $C_{38}H_{44}FN_7NaO_{11}$, 816.2982; found, 816.2975. LC/MS retention time 2.08 min.

Enzymatic hydrolysis experiments

The ADC-1 β-lactamase was expressed and purified as previously described.[41] Conjugate **1** (10 mM in DMSO) was diluted 5-fold in DMSO and then another 10-fold in 50 mM sodium phosphate, pH 7.4 to give a 200 μM solution with 10% DMSO. Subsequently, a 60 μL reaction was prepared consisting of 20 μM of **1** (6 μL of a 200 μM solution) and 20 μM ADC-1 β-lactamse (8.1 μL of 148 μM enzyme) in 50 mM sodium phosphate, pH 7.4 (the final concentration of DMSO was 1%). The reaction was incubated at room temperature for 40 min prior to analysis by LC/MS. Two control reactions, one containing only **1** (20 μM) and the other only ADC-1 (1 μM enzyme), were also prepared using the same conditions.

Antimicrobial susceptibility assays

The gene encoding the ADC-1 enzyme from *A. baumannii* with its leader (GenBank accession number NG_048633.1) was custom synthesized (GenScript) and cloned into the NdeI-HindIII restriction sites of the pNT255 shuttle vector, which is a derivative of the pNT221 shuttle vector in which the ISAba3 promoter sequence has been replaced with that of ISAba1.[42] To remove two HindIII sites within the ADC-1 nucleotide sequence, silent mutations were made using the codon usage of *A. baumannii*. This plasmid was then introduced by electroporation into *A. baumannii* ATCC 17978 for MIC measurements. MIC analyses were performed using iron depleted media as described previously.[19]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information (^{1}H NMR, ^{13}C NMR, LC/MS, MS, EIMS of ADC, Charge Deconvoluted Electrospray Mass Spectrum of ADC and Molecular Formula Strings) is available free of charge on the ACS Publications website at DOI: …….

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Author Contributions

The manuscript was written through contributions of all authors. RL performed the syntheses and PAM and NS performed the bioassays. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interests.

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ABBREVIATIONS

ADC, Acinetobacter-derived cephalosporinase; *Mtb*, *Mycobacterium tuberculosis*; Boc, *tert*-butyloxycarbonyl; TFA, trifluoroacetic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, *N*-hydroxysuccinimide; MIC, minimum inhibitory concentration; Et_3N, triethylamine; DCM, dichloromethane; THF, tetrahydrofuran; DIPEA, *N,N*-diisopropylethylamine; CAB Lipase, *Candida* lipase B; DMF, *N,N*-dimethylformamide; TLC, thin layer chromatography; DMSO, dimethyl sulfoxide.

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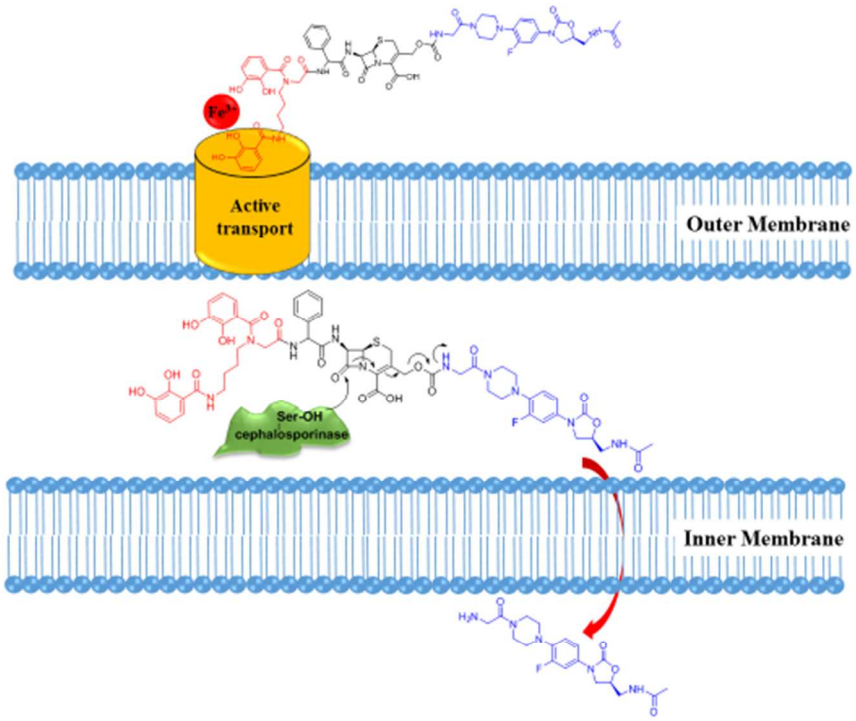
Table of Contents Graphic


Fe3+
Active transport
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Ser-OH cephalosporinase
Inner Membrane