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Discovery of Novel Pyridone-Conjugated Monosulfactams as Potent and Broad-Spectrum Antibiotics for Multidrug-Resistant Gram-Negative Infections

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ABSTRACT: Conjugating a siderophore to an antibiotic is a promising strategy to overcome the permeability-mediated resistance of Gram-negative pathogens. Based on the structure of BAL30072, novel pyridone-conjugated monosulfactams incorporating diverse substituents into the methylene linker between the 1,3-dihydroxypyridin-4(1*H*)-one and the aminothiazole oxime were designed and synthesized. Structure–activity relationship studies revealed that a variety of substituents were tolerated, with isopropyl (compound **12c**) and methylthiomethyl (compound **16a**) showing the best efficacy against multidrug-resistant (MDR) Gram-negative pathogens. In addition, compound **12c** exhibits a good free fraction rate in an in vitro human plasma protein binding test, along with a low clearance and favorable plasma exposure in vivo. In a murine systemic infection model with MDR *Klebsiella pneumoniae*, compound **12c** shows an ED_{50} of 10.20 mg/kg. Taken together, the results indicate that compound **12c** is a promising drug candidate for the treatment of serious infections caused by MDR Gram-negative pathogens.

■ INTRODUCTION

Infections caused by multidrug-resistant (MDR) bacteria, particularly by Gram-negative pathogens, represent a serious and growing threat to human health. The "ESKAPE" pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, are emerging as threatening pathogens.[1, 2] In particular, the carbapenem-resistant *Enterobacteriaceae* (CRE, mostly contributed by *K. pneumoniae*) represent the most worrisome antibiotic resistance crisis, which typically exhibit extremely drug-resistant (XDR) phenotypes, and CRE infections are associated with high mortality rates.[2] Despite the dramatic increase prevalence of highly resistant Gram-negative pathogens, there are few treatment options for related infections, besides polymyxins, an old antibiotic class with serious nephrotoxicity issues.[2, 3] Furthermore, the number of new Gram-negative antibiotic candidates under development is limited and these candidates are not capable of providing coverage against all of the four serious pathogens described above.[4, 5] Therefore, there is an urgent need for new and broad-spectrum Gram-negative antibiotics.

The reduction of outer membrane permeability represents a major mechanism of resistance of Gram-negative pathogens,[6-8] and one strategy to circumvent the penetration issue is to exploit the iron uptake pathway of Gram-negative pathogens by conjugating a siderophore to an antibiotic.[9] Siderophores are small iron-chelating molecules secreted by bacteria to acquire iron from the environment.[10, 11] In response to icon deficiency, siderophores are excreted and sequester iron (III), the resulting complexes are recognized by specific outer membrane receptors and translocated into the microbial cell. A plenty of studies have been reported in the past in understanding the iron binding and recognition processes mentioned above.[12-18] This

siderophore-mediated iron uptake mechanism is very efficient, microbially selective and essential for bacteria, making it an important target to circumvent the permeability-mediated resistance of Gram-negative pathogens through a "Trojan Horse" approach.[9, 19] β-lactam antibiotics target the penicillin-binding proteins (PBPs), which are located in the periplasm, thus only the outer membrane has to be crossed by β-lactam antibiotics for these compounds to be effective antibacterial agents. Therefore, siderophore-conjugated β-lactam antibiotics comply with the "Trojan Horse" strategy perfectly.[9] Natural siderophores most often contain three bidentate ligands for stoichiometric binding of iron (III)[11]. In order to reduce this inherent complexity, a large number of simplified conjugates, which incorporate a single bidentate ligand such as a catechol or a hydroxypyridone, have been studied since 1980s.[20-22] One precedent example is compound **1** (S-649266, Figure 1),[23] a catechol-conjugated cephalosporin, which has progressed into phase II/III clinical trials.[24, 25]

Among all β-lactam antibiotics, the monocyclic β-lactam subclass is supposedly a preferred drug moiety to conjugate to a siderophore because of their potent antimicrobial activity against Gram-negative bacteria, as well as their resistance to β-lactamases, including the metallo-β-lactamases.[26] Structures of representative pyridone-conjugated monocyclic β-lactams are shown in Figure 1. Compounds such as **2** (U-78608),[27] **3** (MC-1)[28] and **4**[29] are potent monocarbams against *P. aeruginosa* and resistant to β-lactamases.[30] However, the therapeutic potential of these compounds might be limited by their poor hydrolytic stability and high plasma protein binding (PPB).[28] Monobactam **5** (MB-1) demonstrates excellent in vitro activity against *P. aeruginosa* along with an improved PPB profile; however, it was reported to be ineffective in vivo.[31, 32] None of the these compounds have progressed into clinical trials. Compound **6** (BAL30072, Figure 2),[33] a monosulfactam discovered by Basilea Pharmaceutica, has ever been

investigated in phase I clinical studies.[34] It exhibits potent activity against MDR *P. aeruginosa* and *A. baumannii*, including carbapenem-resistant strains.[35] In a rat soft tissue infection model, compound **6** was active against most of tested *A. baumannii* strains.[36] However, it was reported to be ineffective against some *Enterobacteriaceae*, especially for *K. pneumoniae*,[35, 37] which would limit its application in the treatment of associated infections.

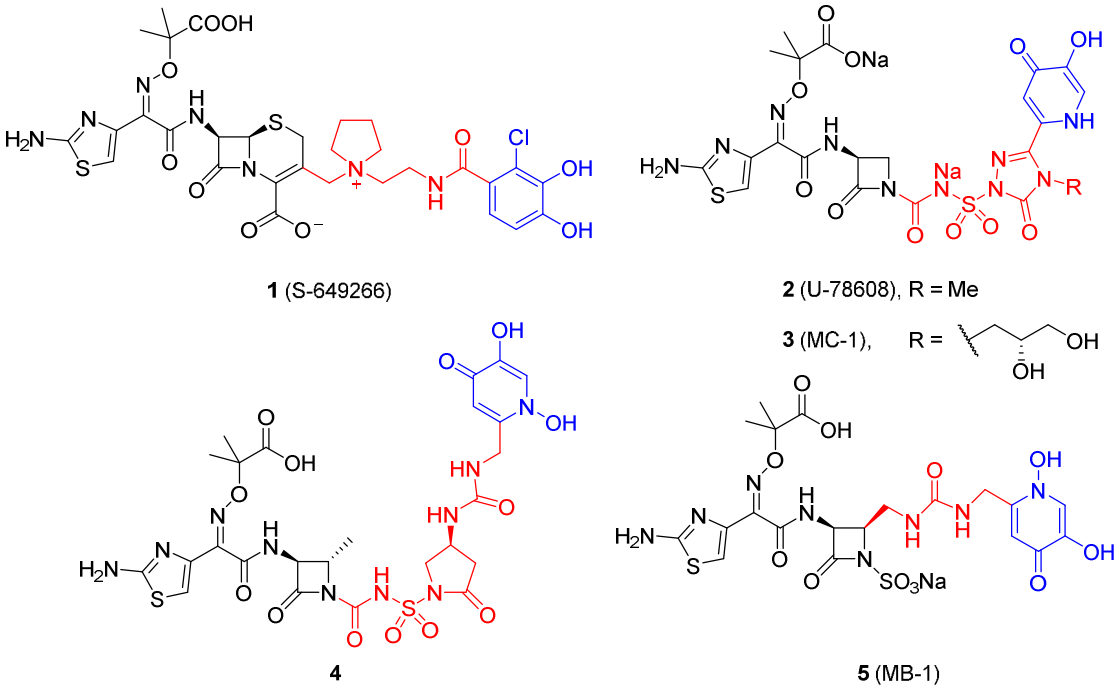


Figure 1. Compound **1** and pyridone-conjugated monocyclic β-lactams **2**–**5**.

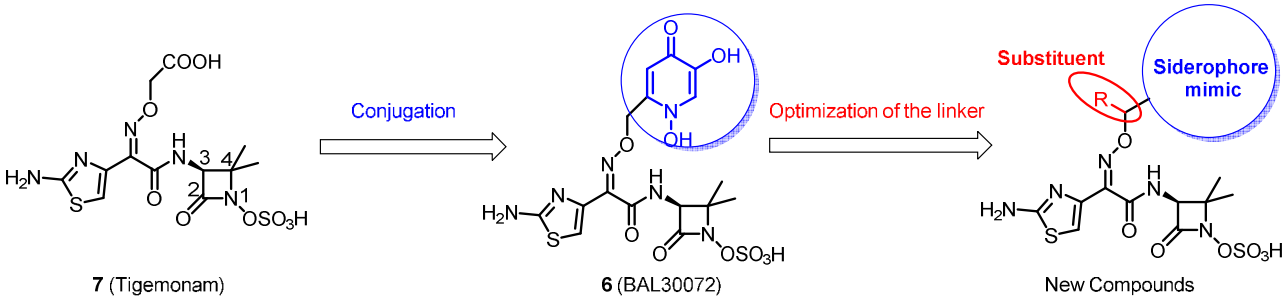


Figure 2. Design of new compounds based on compound **6**.

Discovered from products of saprophytic bacteria in 1979, monobactam is a new class of β-lactams characterized by 2-oxoazetidine-1-sulfonic acid moiety.[38, 39] Initially, extensive structural modifications around the C-3 amide side chain and C-4 positions of the azetidinone ring led to the discovery of aztreonam.[40] Subsequently, other acidic and electronegative groups such as phosphonate and O-sulfate groups were investigated for their activating potential at the N-1 position.[41-43] As shown in Figure 2, compound **7** (tigemonam) is a monosulfactam activated by an O-sulfate group, and it exhibits high level of oral absorption in laboratory animals.[44] Compound **6** was derived from **7** through conjugating 1,3-dihydroxypyridin-4(1*H*)-one to the aminothiazole oxime side chain.[33] To date, there has been limited structure–activity relationships (SAR) generated on **6**. [33, 45] The clinical potential of **6**, along with its drawbacks, which have been discussed above, encourages further optimization of its structure, which we anticipate would provide opportunities to discover better drug candidates. In this article, we report our recent efforts on this topic, which include the following two parts: (1) siderophore mimics other than 1,3-dihydroxypyridin-4(1*H*)-one were explored to optimize that part of the molecule; and (2) the linker connecting the pyridone moiety and the β-lactam core, which has been reported to influence significantly the antibacterial activity and the physicochemical properties of pyridone-conjugates,[28-31, 46] was extensively studied to determine the SAR. As a result, a number of novel compounds with excellent and broad-spectrum antibacterial activity have been discovered, including a preclinical candidate **12c**, which qualifies for further evaluation.

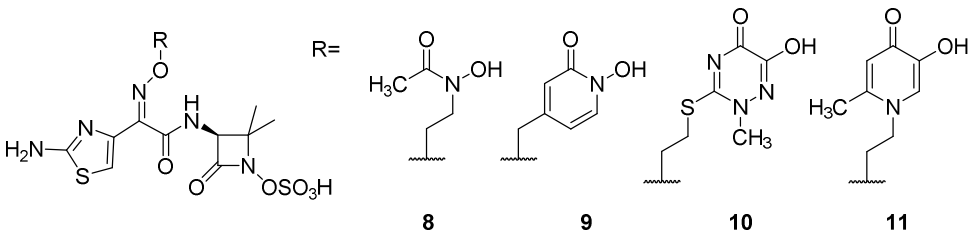
■ RESULTS AND DISCUSSION

Structure–Activity Relationship of monosulfactams. To better correlate the in vitro activity of the new compounds with their future clinical application, the tested bacterial strains were all

clinical isolates. These strains include extended spectrum β-lactamases (ESBLs) producing *E. coli*, *Klebsiella pneumoniae* carbapenemase (KPC-2) producing MDR *K. pneumoniae*, oxacillinase (OXA-23) producing MDR *A. baumannii*, and Imipenemase (IMP-4) producing MDR *P. aeruginosa*, with five strains in each family, respectively. All new compounds were phenotypically screened against these strains and their activities are presented as minimum inhibitory concentrations (MICs). The results are shown in Table 1–3 as ranges of MIC values and the details can be found in Supporting Information.

Bacteria express a wide variety of siderophores with different structural types, thus hydroxamic acid as a common structural type of siderophore[9] was introduced initially to the aminothiazole oxime of **6** to replace the 1,3-dihydroxypyridin-4(1*H*)-one, affording the hydroxamate analogue **8**. As shown in Table 1, compound **8** showed moderate activity against *E. coli* but a significant loss of activity against *A. baumannii* and *P. aeruginosa* when compared with that of **6**. Further modifications exemplified by the replacement of the 1,3-dihydroxypyridin-4(1*H*)-one with other plausible iron-chelating groups provided analogues **9**, **10** and **11**; however, all three compounds also displayed marked loss of activity against *A. baumannii* and *P. aeruginosa* (Table 1). Together with previous results,[31, 33, 46] we considered 1,3-dihydroxypyridin-4(1*H*)-one as the optimal siderophore mimic for monocyclic β-lactams.

Table 1. Antibacterial Activity of Monosulfactams **8**–**11**

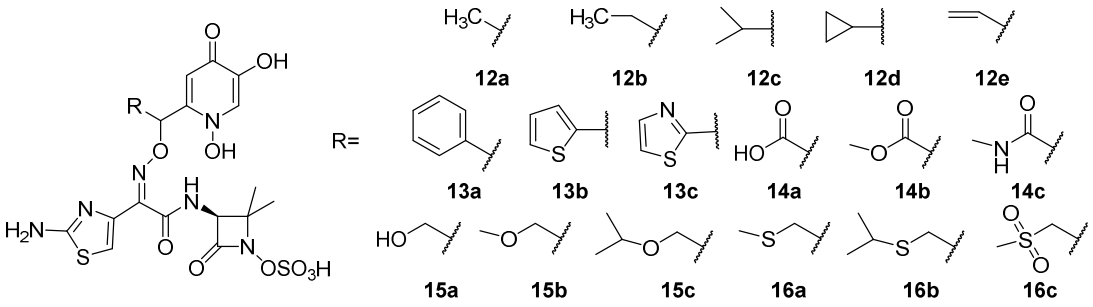


Compd	MIC (μg/mL)[a]			
	Eco	Kpn	Aba	Pae
8	4−32	32−>64	16−64	≥64
9	0.25−4	4−>64	4−64	64
10	1−8	16−64	16−64	16−64
11	1−>64	8−64	8−64	64
6	0.25−1	0.25−>64	0.5−>64	1−4
aztronam	0.5−32	>64	≥64	4−64
meropenem	0.125−8	32−>64	64	32−64
ceftizoxime sodium	0.5−2	32−>64	32−>64	>64

[a]Eco: *E. coli*, 5 strains; Kpn: MDR *K. pneumoniae*, 5 strains; Aba: MDR *A. baumannii*, 5 strains; Pae: MDR *P. aeruginosa*, 5 strains.

We next turned our attention to the modification of the methylene linker between the aminothiazole oxime and the 1,3-dihydroxypyridin-4(1*H*)-one. The *gem*-dimethyl group is a common structural feature for β-lactam drugs such as aztronam and ceftazidime, and it has been reported to play an important role in drug-PBP3 interactions.[47] However, all of our attempts to introduce a *gem*-dimethyl group to the linker failed to afford any compound. Thus, compounds containing a monosubstitution on the linker were designed and synthesized. As shown in Table 2, all the alkyl substituent analogues **12a**–**e** displayed improved potency against *Enterobacteriaceae* strains and equivalent activity against *A. baumannii* and *P. aeruginosa* strains compared with that of **6**. Especially, the isopropyl analogue **12c** showed the most excellent antibacterial activity, with a >100-fold boost in activity against *K. pneumoniae* compared with that of **6**. Aromatic substituents such as the phenyl analogue **13a** and heterocyclic aryl analogues (**13b** and **13c**) also showed excellent antibacterial activity, albeit moderate activity against *K. pneumoniae* was observed. Since free drug exposure is a key factor for the efficacy of β-lactams,[48] the PPB profile was chosen as an important parameter to enable compound selection for advanced studies. As shown in Table 2, all tested alkyl analogues (**12a**–**d**) showed decreased free fractions relative to **6** in the human PPB test, likely because of the increase in lipophilicity of these compounds.

Table 2. Antibacterial Activity and Physiochemical Properties of Monosulfactams **12**–**16**



Compd	MIC (μg/mL)[a]				Human PPB (fu)[b]	Stability[c] (degradation)
	Eco	Kpn	Aba	Pae		
12a	0.06−0.25	0.03−64	0.5−64	0.25−8	0.41 ± 0.03	24%
12b	0.25−2	0.03−>64	0.25−2	0.125−4	0.35 ± 0.02	27%
12c	<0.03−0.25	<0.03−0.5	0.125−1	0.25−1	0.35 ± 0.06	29%
12d	0.06−4	0.06−64	0.5−2	0.25−4	0.39 ± 0.03	24%
12e	<0.03−0.125	<0.03−>64	0.25−2	0.5−2	NT[d]	NT
13a	<0.03−0.06	<0.03−8	0.5−2	0.5−2	NT	NT
13b	<0.03−0.25	0.06−16	0.5−2	0.5−2	NT	NT
13c	<0.03−0.125	<0.03−8	0.5−2	1−2	NT	NT
14a	0.125−4	0.25−1	0.5	1−4	ND[e]	ND
14b	0.25−1	0.25−2	0.5−1	1−4	0.61 ± 0.03	66%
14c	0.06−4	0.25−2	0.25−1	4	0.69 ± 0.05	36%
15a	0.06−4	0.25−>64	0.5−2	0.25−16	0.59 ± 0.10	50%
15b	<0.03−0.25	0.06−2	0.125−1	1−4	0.67 ± 0.03	57%
15c	<0.03−0.25	0.25−>64	1−8	0.5−8	NT	NT
16a	<0.03−0.25	<0.03−1	0.125−1	0.125−8	0.32 ± 0.01	28%
16b	<0.03−0.125	0.06−1	0.25−2	0.25−4	0.21 ± 0.01	34%
16c	<0.03−2	0.06−2	0.25−2	1−32	0.64 ± 0.05	34%
6	0.25−1	0.25−>64	0.5−>64	1−4	0.47 ± 0.02	12%

[a]Eco: *E. coli*, 5 strains; Kpn: MDR *K. pneumoniae*, 5 strains; Aba: MDR *A. baumannii*, 5 strains; Pae: MDR *P. aeruginosa*, 5 strains; [b]n = 4, data represent as mean ± standard deviation; Fu,

fraction unbound; [c]stability: the degradation percentage after 4 h incubation in human plasma at 37 °C; [d]NT: not tested; [e]ND: not determined.

To further explore the SAR of this series of compounds and increase the free drug fraction in plasma, several polar functionalities were introduced. As shown in Table 2, all the carboxylic acid derivatives **14a–c** displayed well-balanced antibacterial activity against all the tested microorganisms. Hydroxymethyl analogue **15a** exhibited good antibacterial activity across all strains except *K. pneumoniae*, and methylation of the hydroxymethyl group of **15a** to methoxymethyl (**15b**) resulted in a boost in potency against *K. pneumoniae*. However, further increasing the size of the substituent by replacing the methoxymethyl with isopropoxymethyl resulted in substantially reduced activity against *K. pneumoniae* (**15c** *vs* **15b**). It is of interest that introduction of a sulfur atom led to improved antibacterial activity, especially against *K. pneumonia,* as shown with **16b** in comparison to **15c**. Oxidization of the methylthiomethyl to sulfonyl afforded analogue **16c**, which exhibited desirable activity against *E. coli* and *K. pneumoniae*, albeit moderate activity against *P. aeruginosa* was observed.

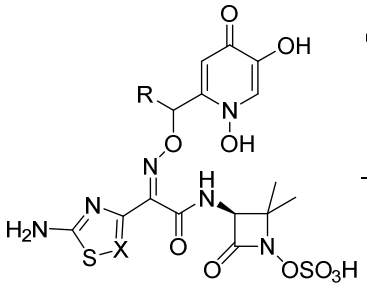
In terms of the PPB profile and plasma stability, compound **14b**, **14c**, **15a** and **15b** exhibited favorable free fractions (0.61, 0.69, 0.59 and 0.67, respectively, Table 2), however, all of them suffer from plasma instability except for **14c**. Compounds containing a sulfur atom showed higher PPB but better plasma stability compared with their O-counterparts. For example, the methylthiomethyl analogue **16a** showed marked improvement in plasma stability compared with that of **15b** (degradation, 28% for **16a** and 57% for **15b**, Table 2). As expected, as the lipophilicity of the substituent increases, the free fraction decreases (0.32 for **16a** and 0.67 for **15b**). The sulfonyl compound **16c** showed a greatly enhanced free fraction (0.64) compared with that of **16a**.

Since small changes of the aminothiazole of monocyclic β-lactams or cephalosporins have been reported to affect significantly their antibacterial activity and their physicochemical properties,[49, 50] compounds **12a** and **12c** were further explored by modifying the thiazole moiety. As shown in Table 3, the chlorothiazole analogues showed comparable potency to the thiazole analogues (**17** *vs* **12a**, **19** *vs* **12c**), and it is particularly interesting that compound **17** demonstrated a marked increase in potency against *K. pneumoniae* when compared with that of **12a**. The thiadiazole analogues exhibited diminished antibacterial activity compared with the thiazole analogues (**18** *vs* **12a**, **20** *vs* **12c**). On the other hand, neither chlorothiazole analogues nor thiadiazole analogues showed improvement in human PPB profiles. However, their stability in plasma was improved, especially for the thiadiazole analogues, which showed comparable stability to that of **6** (degradation, 13% for **18**, 11% for **20**, and 12% for **6**, Table 3).

Finally, as a result of the well-balanced antibacterial activity and favorable plasma stability of compounds **12c**, **16a**, **19** and **20**, the influence of the stereochemistry of the methylene linker on antibacterial activity and physicochemical properties was studied. As shown in Table 3, the diastereomers (**12c**, **16a**, **19** and **20**) exhibit the best overall potency and spectrum, and the preferred configuration depends on the tested panels. For example, as for *E. coli*, *A. baumannii* and *P. aeruginosa*, the *S*-isopropyl analogues (**21**, **23** and **25**) and *R*-methylthiomethyl analogue **27** showed better MICs. This tendency is especially obvious for *A. baumannii* that the *S*-isopropyl analogues demonstrated significantly superior activity when compared with the *R*-isopropyl analogues (**21** *vs* **22**, **23** *vs* **24**, **25** *vs* **26**). However, in terms of *K. pneumoniae*, the *R*-isopropyl analogues (**22** and **26**) and *S*-methylthiomethyl analogue **28** displayed greater activity than the other isomers, and a marked decrease in activity against *K. pneumoniae* was observed particularly for the *R*-methylthiomethyl analogue **27**. The reasons for this phenomenon are not clear, and it

could be attributed to different Gram-negative bacteria possessing their own unique special and sophisticated resistance mechanisms. On the other hand, similar human PPB profiles and subtle differences of plasma stability between diastereomers and their single isomers (**12c** *vs* **21**, **19** *vs* **23**, **20** *vs* **25**, **16a** *vs* **27**, Table 3) indicate that the stereochemistry of the linker has little impact on human PPB and plasma stability.

Table 3. Antibacterial Activity and Physiochemical Properties of Monosulfactams **17**–**28**



Compd	R	X
12a	H_3C	CH
17	H_3C	CCl
18	H_3C	N
12c		CH
19		CCl
20		N
21		CH
22		CH
23		CCl
24		CCl
25		N
26		N
16a	S	CH
27	S	CH
28	S	CH

Compd	MIC (μg/mL)[a]				Human PPB (fu)[b]	Stability[c] (degradation)
	Eco	Kpn	Aba	Pae		
12a	0.06−0.25	<0.03−64	0.5−64	0.25−8	0.41 ± 0.03	24%
17	<0.03−0.25	0.06−1	0.25−2	0.5−4	0.20 ± 0.01	20%
18	<0.03−1	0.25−64	2−64	0.5−64	0.37 ± 0.02	13%
12c	<0.03−0.25	<0.03−0.5	0.125−1	0.25−1	0.35 ± 0.06	29%
19	0.06−1	0.06−0.5	0.06−2	0.25−8	0.23 ± 0.02	22%
20	0.06−2	0.06−1	0.5−4	0.5−32	0.28 ± 0.01	11%
21	0.06−0.25	0.125−1	1−2	0.125−1	0.27 ± 0.01	23%
22	0.06−2	0.06−0.5	0.25−64	1−4	NT[d]	NT
23	0.06−1	0.25−0.5	0.5−1	0.06−1	0.23 ± 0.02	33%
24	0.125−1	0.5−2	0.25−>64	0.5−4	NT	NT
25	0.5−2	1−4	1−4	1−2	0.27 ± 0.01	16%
26	0.5−8	0.5−2	0.5−64	1−4	NT	NT
16a	<0.03−0.25	<0.03−1	0.125−1	0.125−1	0.32 ± 0.01	28%
27	<0.03−0.25	0.06−>64	0.5−4	0.5−2	0.29 ± 0.01	39%
28	<0.03−0.5	0.125−0.5	0.5−2	1−8	NT	NT
6	0.25−1	0.25−>64	0.5−>64	1−4	0.47 ± 0.02	12%

[a]Eco: *E. coli*, 5 strains; Kpn: MDR *K. pneumoniae*, 5 strains; Aba: MDR *A. baumannii*, 5 strains; Pae: MDR *P. aeruginosa*, 5 strains; [b]n = 4, data represent as mean ± standard deviation; Fu, fraction unbound; [c]stability: the degradation percentage after 4 h incubation in human plasma at 37°C;[d]NT: not tested.

Pharmacokinetics. Having identified a number of monosulfactams with excellent in vitro antibacterial activity and physicochemical properties, pharmacokinetic evaluations were conducted in rats following intravenous (iv) administration at a dose of 1 mg/kg, and the results are summarized in Table 4. Compound **12c** exhibited favorable plasma exposure (AUC_{0-t} = 1730 ng·h/mL) and relatively low clearance (CL = 11.0 mL/min/kg), which were comparable to that of **6**. Compound **16a** displayed poor pharmacokinetic properties with low plasma exposure (AUC_{0-t} = 661 ng·h/mL) and relatively high clearance (CL = 26.1 mL/min/kg), whereas the sulfonyl analogue **16c** showed 2-fold higher plasma exposure and a marked decrease of clearance when compared with that of **16a**. Interestingly, the chlorothiazole analogue **19** exhibited the longest elimination half-life ($t_{1/2}$ = 1.5 h) and largest volume of distribution (Vss = 1.41 L/kg) among all these monosulfactams, while its plasma exposure was very low (AUC_{0-t} = 634 ng·h/mL). Although the optically pure form **21** demonstrated equivalent antibacterial activity when compared with that of **12c**, it showed a much higher clearance and lower plasma exposure. Therefore, the racemic form **12c** was chosen for further in vivo studies.

Table 4. Pharmacokinetic Properties of Selected Monosulfactams[a]

Compd	AUC_{0-t} (ng·h/mL)	$t_{1/2}$ (h)	CL (mL/min/kg)	Vss (L/kg)
6	1714	0.27	9.8	0.14
12c	1730	0.45	11.0	0.30
16a	661	0.25	26.1	0.37
16c	1890	0.33	9.3	0.21
19	634	1.50	26.9	1.41
21	675	0.26	28.5	0.36

[a]Sprague Dawley rat (male), iv, vehicle was water, 1 mg/kg, n = 3. Data are the mean values. Abbreviations: AUC_{0-t}: area under the concentration-time curve up to last sampling time; $t_{1/2}$: elimination half-life; CL: clearance; Vss: volume of distribution at steady state.

In Vivo Efficacy of Compounds 12c, 16c and 19. Compound **12c**, **16c** and **19** were evaluated for their in vivo efficacy using a murine systemic infection model. Compound **6** and meropenem were chosen as positive controls. As shown in Table 5, compounds **12c** and **16c** displayed comparable in vivo efficacy (ED_{50} = 3.37, and 5.32 mg/kg, respectively) with that of **6** (ED_{50} = 4.19 mg/kg) against clinical *E. coli* 210737. Compound **19** demonstrated an efficacy (ED_{50} = 6.94 mg/kg) inferior to that of **6** in the *E. coli* infection model, and this might be attributed to its low plasma exposure and reduced in vitro antibacterial activity. Based on its excellent in vivo efficacy in the *E. coli* infection model, compound **12c** was selected for further evaluation in the MDR *K. pneumoniae* 212232 infection model. As shown in Table 5, compound **12c** also displayed a potent

in vivo efficacy (ED_{50} = 10.20 mg/kg), whereas **6** exhibited extremely low activity in this model. This excellent in vivo efficacy of **12c** makes it an ideal preclinical candidate for further studies.

Table 5. In Vivo Efficacy of Compound **12c**, **16c** and **19** in the Mouse Systemic Infection Models[a]

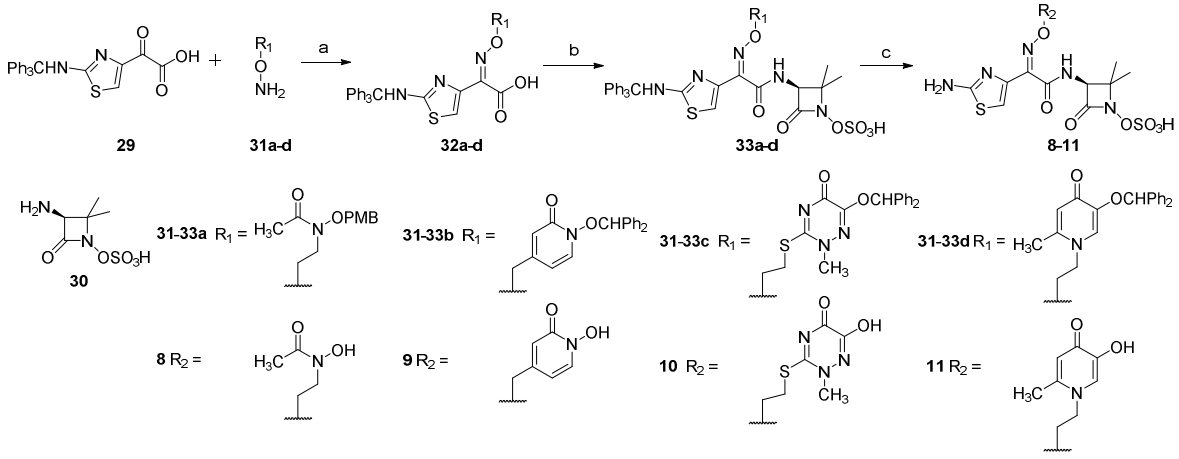
compd	*E. coli* 210737[b]		*K. pneumoniae* 212232[c]	
	MIC (μg/mL)	ED_{50} (mg/kg)[d] (95% CI)	MIC (μg/mL)	ED_{50} (mg/kg)[d] (95% CI)
12c	<0.03	3.37 (1.12–6.85)	<0.03	10.20 (5.28–18.23)
16c	0.06	5.32 (2.99–9.51)	0.06	NT[e]
19	1	6.94 (3.85–13.7)	0.06	NT
6	0.25	4.19 (1.99–11.25)	0.25	>100[f]
meropenem	0.125	2.04 (1.05–3.75)	>64	>100[f]

[a]Mice were inoculated intraperitoneally, and the drug was given subcutaneously at 0.5 and 4.0 h post-infection. [b]*E. coli* 210737, clinically isolated. [c]*K. pneumoniae* 212232, MDR clinically isolated. [d]The efficacy criterion, ED_{50}, was calculated as the dose at which mice survival rate was 50%. Numbers in parentheses are 95% confidence ranges. [e]NT: not tested. [f] Dosing at 100 mg/kg resulted in 40% survival for **6** and 0% survival for meropenem.

■ CHEMISTRY

The synthesis of monosulfactams **8**–**11** is shown in Scheme 1. Condensation of alkoxyamines **31a**–**d** with 2-oxo-2-(2-(tritylamino)thiazol-4-yl) acetic acid **29**[51] provided oxime acids **32a**–**d**, which were coupled with amine **30** to give intermediates **33a**–**d**. Subsequent deprotection of the protecting groups provided the target compounds **8**–**11**.[52] Alkoxyamine **31a** was prepared according to a published procedure,[53] and details for the synthesis of alkoxyamines **31b**–**d** can be found in Supporting Information.

Scheme 1. Synthesis of Compounds **8**–**11**[a]

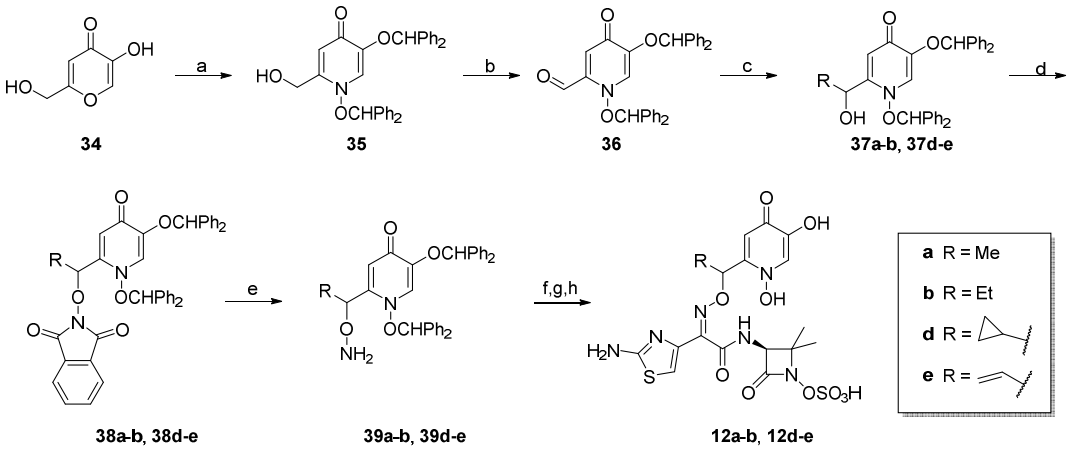


[a]Reagents and conditions: (a) MeOH/CH_2Cl_2, rt; (b) **30**, HATU, $NaHCO_3$, DMSO; (c) TFA, Et_3SiH, CH_2Cl_2.

The syntheses of monosulfactams **12a**, **12b**, **12d** and **12e** are depicted in Scheme 2. Selective protection of the phenolic hydroxyl group of kojic acid (**34**) with diphenyldiazomethane, followed by heating with hydroxylamine and then reacting with chlorodiphenylmethane afforded intermediate **35**,[54] which was oxidized to give aldehyde **36** under Parikh–Doering conditions.

Reactions of various Grignard reagents (RMgBr) with intermediate **36** provided alcohols **37a**–**b** and **37d**–**e** in good yields. Mitsunobu reactions between alcohols (**37a**–**b** and **37d**–**e**) and *N*-hydroxyphthalimide produced phthalimides **38a**–**b** and **38d**–**e**, and subsequent removal of the phthaloyl groups with hydrazine hydrate obtained the key alkoxyamines **39a**–**b** and **39d**–**e**. Then the alkoxyamines were converted to target monosulfactams **12a**, **12b**, **12d** and **12e** using a similar method as described above.

Scheme 2. Synthesis of Compounds **12a**, **12b**, **12d** and **12e**[a]

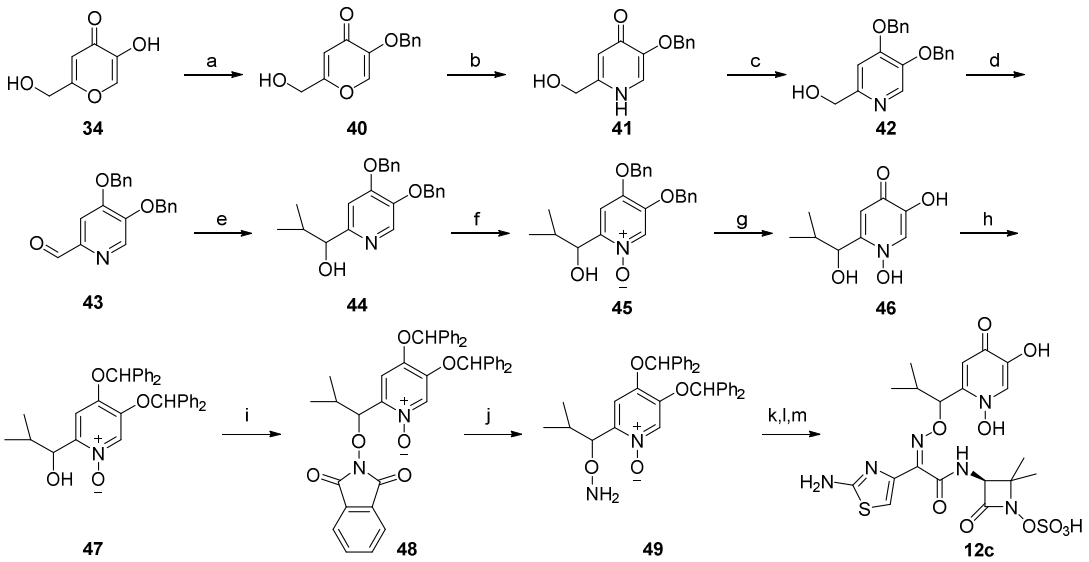


[a]Reagents and conditions: (a) (i) diphenyldiazomethane, EtOH, 40 °C; (ii) hydroxylamine hydrochloride, sodium acetate trihydrate, EtOH/H_2O, 60 °C; (iii) chlorodiphenylmethane, NaI, K_2CO_3, DMSO, rt; (b) pyridine–SO_3, DMSO, NEt_3, CH_2Cl_2; (c) RMgBr, THF; (d) *N*-hydroxyphthalimide, PPh_3, DEAD, THF; (e) N_2H_4, MeOH, rt; (f) **29**, MeOH/CH_2Cl_2, rt; (g) **30**, HATU, $NaHCO_3$, DMSO; (h) for **12a**, **12b**, and **12e**: TFA, Et_3SiH, CH_2Cl_2, −15 °C; for **12d**: HCOOH, 0 °C.

When the R group is changed to an isopropyl (Scheme 2), we failed to get the corresponding phthalimide analogue through Mitsunobu conditions, and we speculated that an increase in steric

hindrance could be the reason. Thus, compound **12c** was synthesized in a modified way as described in Scheme 3. Intermediate **42** was prepared according to published procedures with kojic acid (**34**) as starting material,[55] which was oxidized to give **43** in excellent yields. Nucleophilic addition of **43** with isopropylmagnesium bromide afforded alcohol **44**, which was converted to **45** with *m*-CPBA. Subsequent deprotection with borane trichloride and reprotection with diphenyldiazomethane, led to the formation of intermediate **47,** which lacked the characteristic peak of a carbonyl group at about δ 170 ppm in the ^{13}C NMR spectra (see Supporting Information). After the Mitsunobu reaction with *N*-hydroxyphthalimide and the removal of the phthaloyl group, the key alkoxyamine **49** was obtained. Alkoxyamine **49** was converted to the target compound **12c** using the same method as described above.

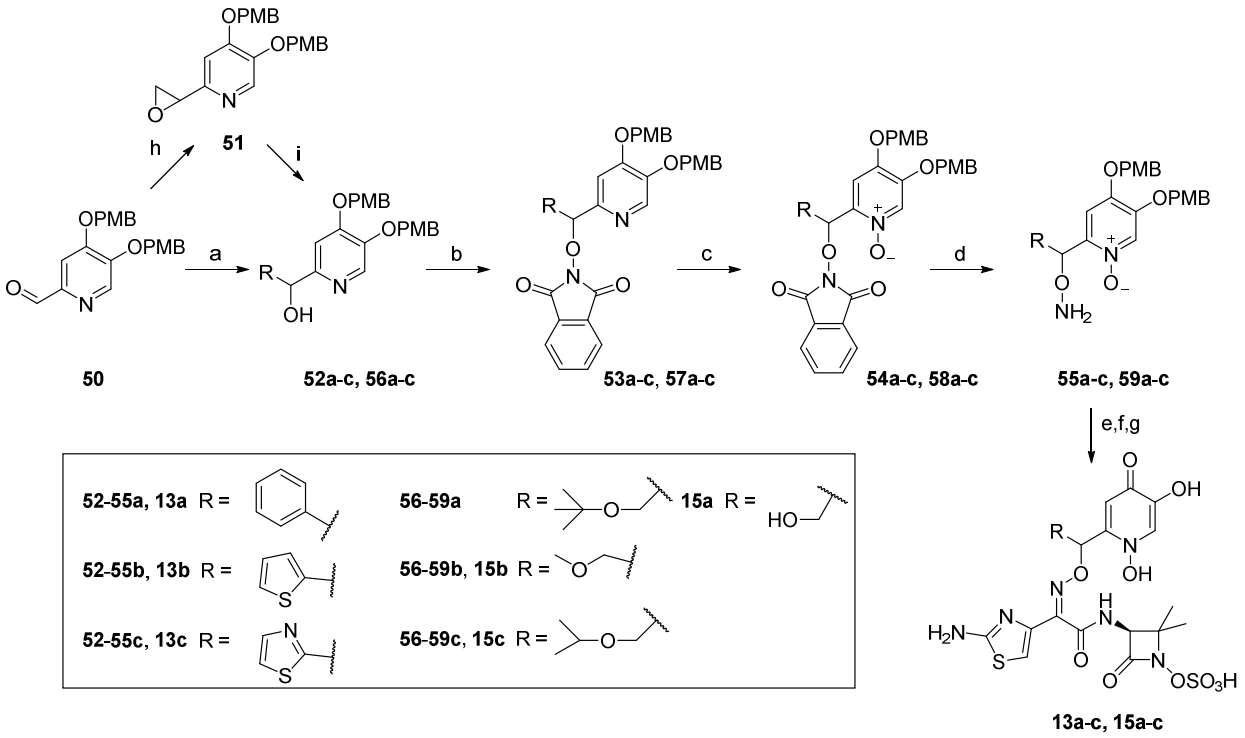
Scheme 3. Synthesis of Compound **12c**[a]



[a]Reagents and conditions: (a) BnCl, NaOH, MeOH, 60 °C; (b) NH_4OH, MeOH, 55 °C; (c) BnCl, K_2CO_3, DMSO, 50 °C; (d) pyridine–SO_3, DMSO, NEt_3, CH_2Cl_2; (e) *i*-PrMgBr, THF; (f) *m*-CPBA, CH_2Cl_2, rt; (g) BCl_3, CH_2Cl_2, −10 °C; (h) diphenyldiazomethane, MeOH/CH_2Cl_2, rt; (i) *N*-hydroxyphthalimide, PPh_3, DIAD, THF; (j) N_2H_4, MeOH, rt; (k) **29**, MeOH/CH_2Cl_2, rt; (l) **30**, HATU, $NaHCO_3$, DMSO; (m) TFA, Et_3SiH, CH_2Cl_2, −15 °C.

The synthesis of monosulfactams **13a**–**c** and **15a**–**c** was achieved using an alternative route. As illustrated in Scheme 4, intermediate **50** was prepared using similar procedures as described above for **43**. Nucleophilic addition of **50** with phenylmagnesium chloride or aryl lithium afforded alcohols **52a**–**c**, which were further converted to **53a**–**c** through Mitsunobu conditions. Oxidization of **53a**–**c** with *m*-CPBA followed by removal of the phthaloyl groups gave the key alkoxyamines **55a**–**c**. Final target compounds **13a**–**c** were then obtained using the same method as described above. Alternatively, intermediate **50** was subjected to Corey–Chaykovsky epoxidation to generate **51**, and subsequent nucleophilic ring opening of **51** with alkoxides provided alcohols **56a**–**c**, which were then converted to the target monosulfactams **15a**–**c** by the same manner as described for **13a**–**c**.

Scheme 4. Synthesis of Compounds **13a–c** and **15a–c**[a]

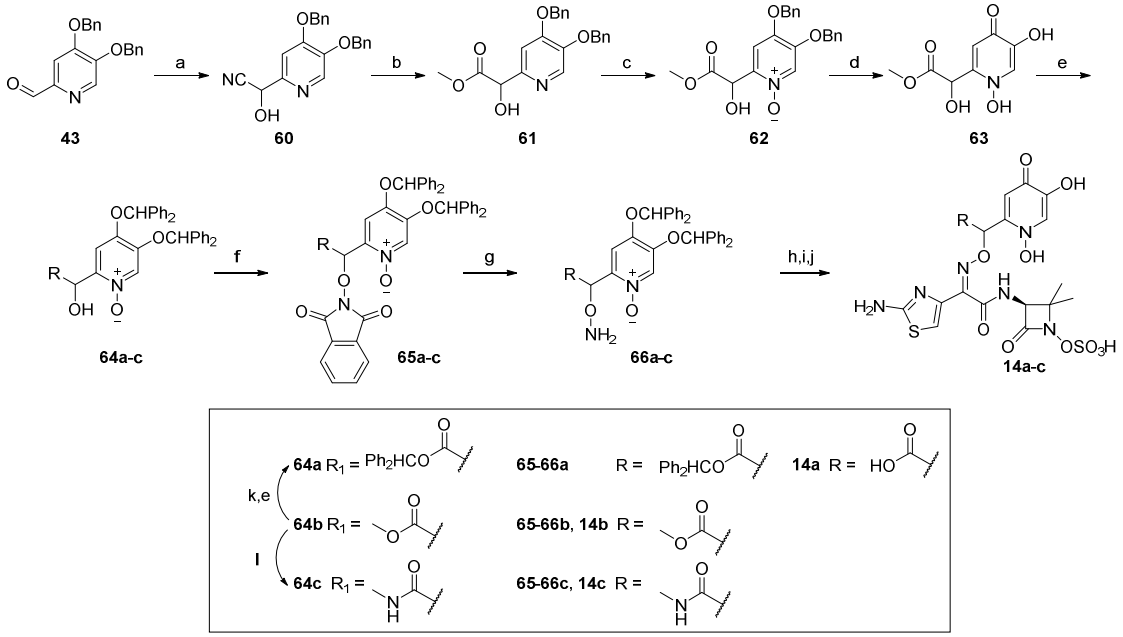


[a]Reagents and conditions: (a) for **52a**: phenylmagnesium chloride, THF, –20 °C; for **52b** and **52c**: aryl lithium, THF, –78 °C; (b) *N*-hydroxyphthalimide, PPh_3, DEAD or DIAD, THF; (c) *m*-CPBA, CH_2Cl_2, rt; (d) N_2H_4, MeOH, rt; (e) **29**, MeOH/CH_2Cl_2, rt; (f) **30**, HATU, $NaHCO_3$, DMSO; (g) TFA, Et_3SiH, CH_2Cl_2, 0 °C; (h) $Me_3S^+(O)I^-$, NaH, DMSO, 0 °C; (i) alkoxides.

The synthesis of monosulfactams **14a–c** is depicted in Scheme 5. Compound **43** was treated with sodium cyanide to give the α-hydroxynitrile **60**, which was converted to ester **61** by alcoholysis with methanol under hydrochloric conditions. Intermediate **61** was oxidized with *m*-CPBA, followed by deprotection with borane trichloride and reprotection with diphenyldiazomethane to give intermediate **64b**. Hydrolysis of **64b** and subsequent protection with diphenyldiazomethane furnished intermediate **64a**, whereas aminolysis of **64b** with

methylamine provided **64c**. Intermediates **64a–c** were then converted to the target monosulfactams **14a–c** by the same manner as described for **12c**.

Scheme 5. Synthesis of Compounds **14a–c**[a]

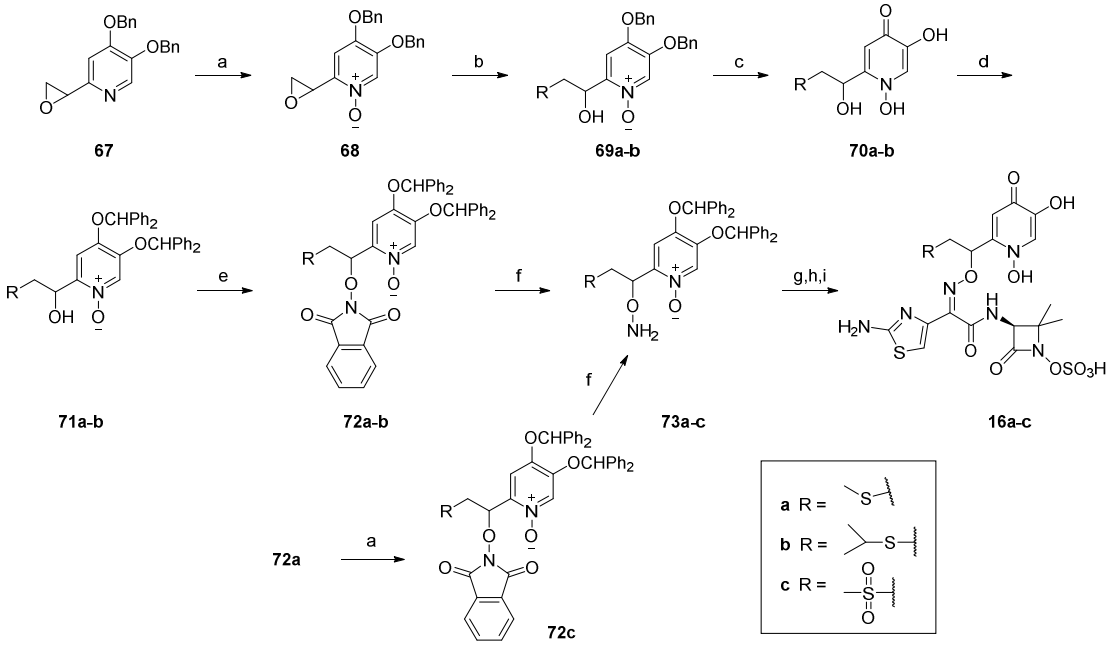


[a]Reagents and conditions: (a) NaCN, $NaHSO_3$,THF/H_2O, 0 °C; (b) 4 *M* HCl, MeOH and then $NaHCO_3$ aqueous; (c) *m*-CPBA, CH_2Cl_2, rt; (d) BCl_3, CH_2Cl_2, −10 °C; (e) diphenyldiazomethane, MeOH/CH_2Cl_2, rt; (f) *N*-hydroxyphthalimide, PPh_3, DIAD, THF; (g) N_2H_4, MeOH, rt; (h) **29**, MeOH/CH_2Cl_2, rt; (i) **30**, HATU, $NaHCO_3$, DMSO; (j) TFA, Et_3SiH, CH_2Cl_2, −15 °C; (k) LiOH, THF/H_2O, rt; (l) methylamine, MeOH, rt.

The synthesis of monosulfactam**s 16a–c** is outlined in Scheme 6. The starting material **67** was synthesized using the same method as compound **51**. Oxidization of **67** with *m*-CPBA provided **68**, which was converted to **69a** and **69b** subsequently by nucleophilic ring opening reactions. Deprotection of **69a** and **69b** with borane trichloride and treatment with diphenyldiazomethane afforded **71a** and **71b**, respectively. Compounds **71a** and **71b** were then converted to **72a** and **72b**

through Mitsunobu conditions as described above. Oxidization of **72a** with *m*-CPBA afforded **72c**. The final monosulfactams **16a**–c were then prepared in a manner similar to that described for **12c**.

Scheme 6. Synthesis of Compounds **16a**–**c**[a]

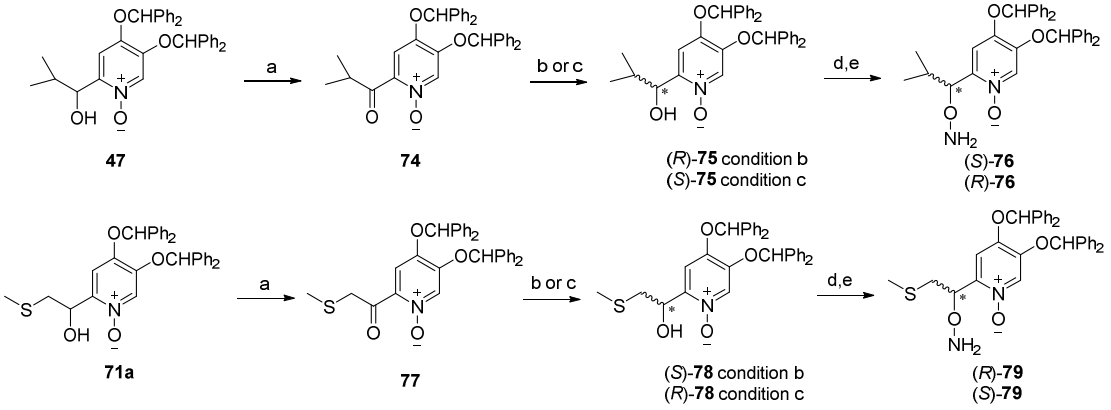


[a]Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, rt; (b) for **69a**: sodium thiomethoxide, MeOH, rt; for **69b**: 2-propanethiol, NaH, dioxane, rt; (c) BCl_3, CH_2Cl_2, −10 °C; (d) diphenyldiazomethane, MeOH/CH_2Cl_2, rt; (e) *N*-hydroxyphthalimide, PBu_3, DIAD, THF, −10 °C; (f) N_2H_4, MeOH, rt; (g) **29**, MeOH/CH_2Cl_2, rt; (h) **30**, HATU, $NaHCO_3$, DMSO; (i) TFA, Et_3SiH, CH_2Cl_2, −15 °C.

The preparation of the chiral building blocks for compounds **21**–**28** is illustrated in Scheme 7. A Parikh–Doering treatment of compound **47** generated the chiral precursor **74**, which was further converted to chiral isobutanols (*R*)–**75** and (*S*)–**75** through the Noyori asymmetric reduction of **74** using a pair of chiral catalysts. Greater than 98% *ee* values were achieved for both enantiomers after recrystallization in ethyl acetate. The absolute configuration of (*S*)–**75** was confirmed by

X-ray crystallography (see Supporting Information). Mitsunobu reactions of these intermediates were conducted at −10 °C to provide phthalimides with good *ee* values, which were subsequently converted to compounds (*R*)–**76** and (*S*)–**76** after hydrazinolysis with hydrazine hydrate. The same synthetic procedures were used for the preparations of (*R*)–**79** and (*S*)–**79**.

Scheme 7. Asymmetry synthesis of chiral fragments (*R*)–**76**, (*S*)–**76**, (*R*)–**79** and (*S*)–**79**[a]



[a]Reagents and conditions: (a) pyridine–SO_3, DMSO, Et_3N, CH_2Cl_2; (b) HCOOH, Et_3N, dichloro(*p*-cymene)ruthenium(II) dimer, (1*S*, 2*S*)-(+)-*N*-(4-toluenesulphonyl)-1, 2-ethane diamine, MTBE/DMF, rt; (c) HCOOH, Et_3N, dichloro(*p*-cymene)ruthenium(II) dimer, (1*R*, 2*R*)-(+)-*N*-(4-toluenesulphonyl)-1, 2-ethane diamine, MTBE/DMF, rt; (d) *N*-hydroxyphthalimide, PBu_3, DIAD, THF, −10 °C; (e) N_2H_4, MeOH, rt.

■ CONCLUSION

In summary, based on the structure of compound **6**, our exploration of its pyridone moiety and optimization of the linker between the pyridone and the β-lactam core have led to the discovery of a series of novel pyridone-conjugated monosulfactams. Various substituents were tolerated on the methylene linker and a number of new compounds showed potent antibacterial activity against a variety of clinical MDR Gram-negative pathogens, including *K. pneumoniae*. The isopropyl-substituted compound **12c** and the methylthiomethyl-substituted compound **16a** exhibited excellent and broad-spectrum antibacterial activity, whereas the *N*-methyl formamide analogue **14c** and sulfonyl analogue **16c** demonstrated more favorable PPB profiles. Among all of these compounds, **12c** showed favorable plasma exposure and relatively low clearance in rats. Furthermore, **12c** displayed a potent in vivo efficacy (ED_{50} = 10.20 mg/kg) in a murine systemic infection model with MDR *Klebsiella pneumoniae*, whereas the precedent compound **6** showed very weak efficacy in the same model. The excellent in vitro and in vivo activity of **12c**, combined with its desirable pharmacokinetic properties, make it a promising drug candidate for the treatment of serious infections caused by MDR Gram-negative pathogens including the CREs. Further comprehensive evaluations of compound **12c** are underway.

■ EXPERIMENTAL SECTION

Chemistry. All solvents and chemicals were used as purchased without further purification. Inert atmosphere operations were conducted under argon in flame-dried glassware. Room temperature refers to 20–25 °C. All reaction mixtures were monitored using thin-layer chromatography (TLC) on silica gel F-254 TLC plates. Column chromatography was carried out using silica gel (200–300 mesh). Melting points (uncorrected) were determined on an X-4 melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 NMR or a Bruker 500 NMR spectrometer using solvent residue as the internal standards. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz). EI-MS spectra were obtained on a Finnigan MAT95 spectrometer and ESI-MS spectra were obtained on a Krats MS 80 mass spectrometer. Purity of all final compounds was determined by analytical HPLC (PLATISIL ODS 250 × 4.6 mm, particle size 5μm) with methanol/buffer (0.1% CF_3COOH and 0.1% NH_4OH in water, pH 3.5) as the mobile phase. The *ee* values were determined using chiral HPLC (CHIRALPAK AD-H column 250 × 4.6 mm, particle size 5μm or CHIRALCEL OD-H column 250 × 4.6 mm, particle size 5μm) with ethanol/*n*-hexane as the mobile phase. A purity of >95% was achieved for all tested compounds. Detailed synthetic procedures and spectral characterization data for all intermediates is provided in the Supporting Information.

(*S*,*Z*)-3-(2-(2-Aminothiazol-4-yl)-2-((2-(N-hydroxyacetamido)ethoxy)imino)acetamido)-2, 2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (8). A suspension of **33a** (210 mg, 0.25 mmol) in anhydrous dichloromethane (3 mL) was treated with triethylsilane (0.13 mL, 0.75 mmol) and cooled to −5 °C. Then trifluoroacetic acid (1.9 mL, 25.0 mmol) was added dropwise. The reacting mixture was stirred overnight and warmed to 0 °C and stirred for 2 h. After completion of the

reaction, a mixture solvent of ethyl acetate and hexane (4:1, 15 mL) was added dropwise, the resulting precipitate was further stirred for 20 min at room temperature and collected by filtration and then the cake was washed with ethyl acetate, dried in vacuum at room temperature to afford **8** as a white solid (45 mg, 38%), mp: 155 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 9.36 (s, 1H), 6.87 (s, 1H), 4.62 (d, J = 7.9 Hz, 1H), 4.23 (t, J = 6.0 Hz, 2H), 3.78 (t, J = 6.1 Hz, 2H), 1.98 (s, 3H), 1.44 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.83, 169.47, 162.43, 161.91, 160.45, 148.71, 110.40, 70.75, 66.44, 61.08, 47.35, 23.12, 20.80, 20.06. HRMS (ESI) *m/z* calcd for $C_{14}H_{19}N_6O_9S_2$ [M − H] $^-$ 479.0660, found 479.0668.

(*S*,*Z*)-3-(2-(2-Aminothiazol-4-yl)-2-(((1-hydroxy-2-oxo-1,2-dihydropyridin-4-yl)methoxy) imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (9). A suspension of **33b** (110 mg, 0.12 mmol) in anhydrous dichloromethane (2 mL) was treated with triethylsilane (0.05 mL, 0.24 mmol) and cooled to −15 °C. Then trifluoroacetic acid (0.9 mL, 12.1 mmol) was added dropwise. The reacting mixture was stirred at −15 °C for 5 h. After completion of the reaction, the reacting mixture was slowly warmed to 0 °C. Then, a mixture solvent ethyl acetate and hexane (4:1, 20 mL) was added dropwise, the resulting precipitate was further stirred for 20 min and collected by filtration and then the cake was washed with ethyl acetate, dried in vacuum at room temperature to afford **9** as a white solid (60 mg, 74%), mp: 178 °C decomp.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 6.84 (s, 1H), 6.49 (s, 1H), 6.15 (dd, J = 7.2, 2.3 Hz, 1H), 5.03 (s, 2H), 4.65 (d, J = 7.8 Hz, 1H), 1.44 (s, 3H), 1.25 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.77, 162.14, 161.91, 158.70, 157.98, 149.02, 148.63, 135.86, 116.25, 111.03, 103.20, 74.18, 68.28, 61.24, 23.87, 20.90. HRMS (ESI) *m/z* calcd for $C_{16}H_{17}N_6O_9S_2$ [M − H] $^-$ 501.0498, found 501.0492.

(*S*,*Z*)-3-(2-(2-Aminothiazol-4-yl)-2-((2-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (10). Compound **10** (48 mg, 83%) was prepared from **33c** (100 mg, 0.10 mmol) in the same manner as described for **9**. mp: 172 °C decomp. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.66 (d, J = 7.6 Hz, 1H), 6.93 (s, 1H), 4.63 (d, J = 7.6 Hz, 1H), 4.40 – 4.35 (m, 2H), 3.62 (s, 3H), 3.52 – 3.42 (m, 2H), 1.45 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ172.50, 170.15, 161.71, 161.51, 160.72, 156.80, 154.03, 146.96, 111.10, 73.12, 68.32, 61.33, 42.89, 30.53, 23.78, 21.05. HRMS (ESI) *m/z* calcd for $C_{16}H_{19}N_8O_9S_3$ $[M - H]^-$ 563.0443, found 563.0433.

(*S*,*Z*)-3-(2-(2-Aminothiazol-4-yl)-2-((2-(5-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl) ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (11). Compound **11** (87 mg, 77%) was prepared from **33d** (200 mg, 0.21 mmol) in the same manner as described for **9**. mp: 171 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.46 (d, J = 7.9 Hz, 1H), 8.13 (s, 1H), 7.08 (s, 1H), 6.76 (s, 1H), 4.60 – 4.54 (m, 3H), 4.51 – 4.42 (m, 2H), 2.55 (s, 3H), 1.41 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.15, 162.65, 162.04, 160.99, 150.48, 148.80, 144.25, 141.31, 131.66, 114.37, 110.39, 73.19, 68.16, 61.06, 54.32, 23.91, 20.67, 19.50. HRMS (ESI) *m/z* calcd for $C_{18}H_{22}N_6O_9S_2Na$ $[M + Na]^+$ 553.0787, found 553.0790.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl) ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (12a). Compound **12a** (a mixture of diastereomer (approximately 1:1), 117 mg, 81%) was prepared from (3*S*)-3-((*Z*)-2-((1-(1,5-Bis(benzhydryloxy)-4-oxo-1,4-dihydropyridin-2-yl)ethoxy)imino)-2-(2-(tritylamino)thiazol-4-yl)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (300 mg, 0.27 mmol) in the same manner as described for **9**. mp: 154 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (d, J = 7.7 Hz, 1/2H), 9.67 (d, J = 7.7 Hz, 1/2H), 8.24 (s, 1H), 7.16 (s, 1/2H),

7.01 (s, 1/2H), 6.86 (s, 1/2H), 6.84 (s, 1/2H), 5.67 – 5.53 (m, 1H), 4.67 (t, *J* = 7.5 Hz, 1H), 1.53 – 1.43 (m, 6H), 1.32 (s, 3/2H), 1.29 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ169.34 , 162.63 and 162.49, 161.95 and 161.83, 159.11 and 158.82, 157.88 and 157.85, 147.48, 145.10 and 145.06, 140.57 and 140.50, 128.18 and 128.04, 111.78 and 111.58, 109.98 and 109.32, 75.29 and 75.11, 68.48 and 68.42, 61.50 and 61.34, 23.88 and 23.82, 21.15 and 21.05, 19.26 and 19.24. HRMS (ESI) *m/z* calcd for $C_{17}H_{21}N_6O_{10}S_2$ $[M + H]^+$ 533.0755, found 533.0753.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl) propoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (12b). Compound **12b** (a mixture of diastereomer (approximately 1:1), 75 mg, 61 %) was prepared from (3*S*)-3-((*Z*)-2-((1-(1,5-Bis(benzhydryloxy)-4-oxo-1,4-dihydropyridin-2-yl)propoxy)imino)-2-(2-(tritylamino)thiazol-4-yl)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (250 mg, 0.22 mmol) in the same manner as described for **9**. mp: 164 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (d, *J* = 7.7 Hz, 1/2H), 9.65 (d, *J* = 7.7 Hz, 1/2H), 8.21 (d, *J* = 1.8 Hz, 1H), 7.35 – 7.19 (m, 2H), 7.07 (s, 1/2H), 6.99 (s, 1/2H), 6.83 (s, 1/2H), 6.81 (s, 1/2H), 5.49 (dd, *J* = 7.8, 4.0 Hz, 1/2H), 5.43 (dd, *J* = 8.3, 4.0 Hz, 1/2H), 4.68 (d, *J* = 7.6 Hz, 1H), 1.98 – 1.85 (m, 1H), 1.80 – 1.66 (m, 1H), 1.49 (s, 3/2H), 1.47 (s, 3/2H), 1.33 (s, 3/2H), 1.31 (s, 3/2H), 1.01 – 0.90 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 168.94, 162.58 and 162.47, 161.78 and 161.70, 157.16 and 157.09, 151.04 and 150.99, 146.26 and 146.06, 144.82 and 144.79, 129.16 and 128.01, 127.88 and 127.79, 111.47 and 111.15, 110.11 and 109.71, 79.75 and 79.48, 68.08 and 68.05, 61.15 and 61.06, 26.37 and 26.18, 23.65 and 23.60, 20.74 and 20.67, 9.80 and 9.69. HRMS (ESI) *m/z* calcd for $C_{18}H_{21}N_6O_{10}S_2$ $[M - H]^-$ 545.0761, found 545.0768.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (12c). Compound **12c** (a mixture of diastereomer (approximately 1:1), 100 mg, 69%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (294 mg, 0.26 mmol) in the same manner as described for **9**. mp: 151 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.69 (d, J = 8.0 Hz, 1/2H), 9.65 (d, J = 7.5 Hz, 1/2H), 8.24 (s, 1/2H), 8.23 (s, 1/2H), 7.02 (s, 1/2H), 7.00 (s, 1/2H), 6.83 (s, 1/2H), 6.81 (s, 1/2H), 5.44 (d, J = 4.8 Hz, 1/2H), 5.37 (d, J = 5.4 Hz, 1/2H), 4.71 (d, J = 7.9 Hz, 1/2H), 4.68 (d, J = 7.6 Hz, 1/2H), 2.23 – 2.09 (m, 1H), 1.48 (s, 3/2H), 1.47 (s, 3/2H), 1.35 (s, 3/2H), 1.34 (s, 3/2H), 1.00 – 0.96 (m, 3H), 0.92 – 0.85 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.35, 162.58, 162.02 and 161.98, 157.35 and 157.31, 150.93 and 150.90, 145.72 and 145.54, 145.13, 140.55 and 140.39, 127.97, 111.87 and 111.60, 110.97 and 110.88, 83.04 and 82.90, 68.17 and 68.05, 61.33 and 61.29, 31.90 and 31.67, 23.88 and 23.86, 21.13 and 21.06, 19.15 and 19.06, 17.32 and 17.08. HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_6O_{10}S_2$ $[M - H]^-$ 559.0917, found 559.0923.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((cyclopropyl(1,5-dihydroxy-4-oxo-1,4-dihydro-pyridin-2-yl)methoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (12d). In formic acid (3 mL) at 0 °C, (3*S*)-3-((*Z*)-2-(((1,5-Bis(benzhydryloxy)-4-oxo-1,4-dihydropyridin-2-yl)(cyclopropyl)methoxy)imino)-2-(2-(tritylamino)thiazol-4-yl)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (237 mg, 0.21 mmol) was added and the solution was stirred for 9 h at 0 °C. Then a solution of ethyl acetate and hexane (2:1, 30 mL) was added dropwise and the resulting precipitate was filtrated off. The white precipitate is washed with

additional ethyl acetate (10 mL). After drying under vacuum at room temperature for 4 h, **12d** was obtained as a white solid (a mixture of diastereomer (approximately 1:1), 76 mg, 65%), mp: 182 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (t, J = 8.5 Hz, 1H), 8.12 (s, 1H), 7.72 (d, J = 3.4 Hz, 1H), 7.44 – 7.30 (m, 2H), 6.76 – 6.63 (m, 1H), 5.34 (d, J = 7.5 Hz, 1/2H), 5.29 (d, J = 7.1 Hz, 1/2H), 4.65 (d, J = 7.8 Hz, 1/2H), 4.63 (d, J = 7.6 Hz, 1/2H), 1.47 (s, 3/2H), 1.45 (s, 3/2H), 1.36 (s, 3/2H), 1.27 (s, 3/2H), 1.20 – 1.12 (m, 1H), 0.60 – 0.25 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 168.92 and 168.88, 163.11 and 163.04, 162.02, 150.28, 144.26 and 144.16, 143.37 and 143.33, 142.52 and 142.47, 127.10 and 126.93, 110.31 and 110.28, 110.22 and 109.81, 80.40 and 80.17, 68.12 and 68.10, 61.16 and 61.13, 23.88 and 23.82, 20.72 and 20.64, 13.89 and 13.83, 3.30 and 3.06, 1.64 and 1.57. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}N_6O_{10}S_2$ $[M - H]^-$ 557.0766, found 557.0769.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl) allyl)oxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (12e). Compound **12e** (a mixture of diastereomer (approximately 1:1), 90 mg, 83%) was prepared from (3*S*)-3-((*Z*)-2-(((1-(1,5-Bis(benzhydryloxy)-4-oxo-1,4-dihydropyridin-2-yl)allyl)oxy) imino) -2-(2-(tritylamino)thiazol-4-yl)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (220 mg, 0.20 mmol) in the same manner as described for **9**. mp: 160 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (d, J = 7.3 Hz, 1/2H), 9.66 (d, J = 8.6 Hz, 1/2H), 8.23 (s, 1H), 7.37 – 7.25 (m, 1H), 7.09 (s, 1/2H), 6.85 (s, 1/2H), 6.15 – 5.97 (m, 2H), 5.48 – 5.32 (m, 2H), 4.67 (t, J = 7.6 Hz, 1H), 1.45 (s, 3/2H), 1.27 (s, 3/2H), 1.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.07, 162.32 and 162.25, 161.76 and 161.68, 158.84 and 158.55, 157.05, 150.99, 145.11 and 143.93, 132.21, 129.17, 127.98 and 127.75, 120.83 and 120.44, 111.69 and 111.51, 110.55 and 110.18, 78.65 and 78.43, 68.16 and

68.11, 61.14 and 60.98, 23.65 and 23.60, 21.00 and 20.92. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}N_6O_{10}S_2$ $[M - H]^-$ 543.0604, found 543.0618.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)(phenyl)methoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (13a). Compound **13a** (a mixture of diastereomer (approximately 1:1), 81 mg, 65%) was prepared from 2-(((((*Z*)-2-(((*S*)-2,2-Dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(trityl- amino) thiazol-4-yl)ethylidene)amino)oxy)(phenyl)methyl)-4,5-bis((4-methoxybenzyl)oxy) pyridine 1-Oxide (230 mg, 0.21 mmol) in the same manner as described for **8**. mp: 162 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (d, J = 8.1 Hz, 1/2H), 9.63 (d, J = 8.0 Hz, 1/2H), 8.15 (s, 1/2H), 8.14 (s, 1/2H), 7.43 – 7.31 (m, 5H), 7.20 (s, 1/2H), 7.16 (s, 1/2H), 6.87 (s, 1/2H), 6.84 (s, 1/2H), 6.60 (s, 1/2H), 6.57 (s, 1/2H), 4.62 (dd, J = 7.8, 1.6 Hz, 1H), 1.37 (s, 3/2H), 1.34 (s, 3/2H), 1.05 (s, 3/2H), 0.80 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.33, 162.67 and 162.57, 162.10 and 161.98, 156.81 and 156.71, 151.45 and 151.28, 145.30, 144.75 and 144.60, 140.77 and 140.68, 136.06 and 135.69, 129.61 and 129.46, 129.15, 129.11, 128.62, 128.31, 128.12 and 128.01, 112.02 and 111.86, 111.14 and 110.42, 80.53 and 80.15, 68.25 and 68.08, 61.25 and 61.05, 24.03 and 23.85, 20.69 and 20.18. HRMS (ESI) *m/z* calcd for $C_{22}H_{21}N_6O_{10}S_2$ $[M - H]^-$ 593.0766, found 593.0781.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)(thiophen-2-yl)methoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (13b). Compound **13b** (a mixture of diastereomer (approximately 1:1), 62 mg, 57%) was prepared from 2-(((((*Z*)-2-(((*S*)-2,2-Dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)(thiophen-2-yl)methyl)-4,5-bis((4-methoxy benzyl)oxy)pyridine 1-Oxide (200 mg, 0.18 mmol) in the same manner as described for **8**. mp:

148 °C decomp. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.70 (d, J = 7.7 Hz, 1/2H), 9.66 (d, J = 7.9 Hz, 1/2H), 8.15 (s, 1/2H), 8.14 (s, 1/2H), 7.66 – 7.62 (m, 2H), 7.23 (s, 1/2H), 7.16 (s, 1/2H), 7.12 (d, J = 3.5 Hz, 1/2H), 7.09 (d, J = 3.6 Hz, 1/2H), 7.05 – 7.02 (m,1H), 6.88 (s, 1/2H), 6.86 (s, 1/2H), 6.81 (d, J = 1.9 Hz, 1H), 4.65 – 4.61 (m, 1H), 1.39 (s, 3/2H), 1.37 (s, 3/2H), 1.10 (s, 3/2H), 0.91 (s, 3/2H). ^{13}C NMR (151 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.35, 162.58 and 162.33, 162.11 and 161.95, 156.39 and 156.18, 151.56 and 151.31, 145.42, 144.42 and 144.21, 140.55 and 140.51, 137.91 and 137.60, 129.07 and 128.77, 128.67 and 128.59, 128.22 and 128.02, 127.53 and 127.48, 112.22 and 112.01, 110.71 and 109.86, 75.85 and 75.73, 68.14, 61.27 and 61.07, 24.02 and 23.85, 20.69 and 20.09. HRMS (ESI) *m/z* calcd for $C_{20}H_{19}N_6O_{10}S_3$ $[M - H]^-$ 599.0330, found 599.0347.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)(thiazol-2-yl)methoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (13c). Compound **13c** (a mixture of diastereomer (approximately 1:1), 56 mg, 72%) was prepared from 2-(((((*Z*)-2-(((*S*)-2,2-Dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)(thiazol-2-yl)methyl)-4,5-bis((4-methoxybenzyl)oxy)pyridine 1-Oxide (140 mg, 0.13 mmol) in the same manner as described for **8**. mp: 137 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.76 – 9.69 (m, 1H), 8.18 (s, 1/2H), 8.15 (s, 1/2H), 7.92 (d, J = 3.3 Hz, 1/2H), 7.84 (d, J = 3.2 Hz, 1/2H), 7.28 – 7.23 (m, 1/2H), 7.20 – 7.17 (m, 1/2H), 7.09 (s, 1/2H), 6.99 (s, 1/2H), 6.95 (s, 1/2H), 6.93 (s, 1/2H), 4.63 (d, J = 8.0 Hz, 1/2H), 4.61 (d, J = 8.0 Hz, 1/2H), 1.46 (s, 3/2H), 1.39 (s, 3/2H), 1.26 (s, 3/2H), 0.91 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.37 and 168.98, 162.32 and 162.21, 161.94 and 161.84, 151.99 and 151.90, 146.87 and 146.15, 145.59, 143.24 and 143.17, 140.50 and 140.45, 129.80 and 128.22, 124.33 and 123.53,

112.56 and 112.41, 111.91, 111.63 and 110.87, 77.32 and 77.18, 68.27 and 68.13, 61.30 and 61.19, 60.95, 24.00 and 23.98, 20.51 and 20.46. HRMS (ESI) *m/z* calcd for $C_{19}H_{18}N_7O_{10}S_3$ [M − H] $^-$ 600.0277, found 600.0262.

2-((((*Z*)-1-(2-Aminothiazol-4-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)acetic Acid (14a). Compound **14a** (a mixture of diastereomer (approximately 1:1), 100 mg, 80%) was prepared from 4,5-Bis(benzhydryloxy)-2-(2-(benzhydryloxy)-1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino) oxy)-2-oxoethyl)pyridine 1-Oxide (290 mg, 0.22 mmol) in the same manner as described for **9**. mp: 130 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (d, J = 7.7 Hz, 1/2H), 9.63 (d, J = 7.8 Hz, 1/2H), 8.08 (s, 1H), 7.09 (s, 1/2H), 7.00 (s, 1/2H), 6.88 (s, 1/2H), 6.87 (s, 1/2H), 5.99 (d, J = 1.1 Hz, 1H), 4.67 (dd, J = 7.8, 5.6 Hz, 1H), 1.46 (s, 3/2H), 1.44 (s, 3/2H), 1.29 (s, 3/2H), 1.20 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ169.34, 167.58 and 167.35, 162.28 and 162.15, 162.00, 154.19, 151.59 and 151.14, 145.39 and 145.32, 140.40 and 140.37, 139.79 and 139.46, 128.08 and 128.02, 111.98 and 111.95, 111.25 and 110.42, 78.85 and 78.52, 68.33 and 68.31, 61.30 and 61.25, 24.02 and 23.97, 20.80 and 20.67. HRMS (ESI) *m/z* calcd for $C_{17}H_{19}N_6O_{12}S_2$ [M + H] $^+$ 563.0502, found 563.0494.

Methyl2-((((*Z*)-1-(2-aminothiazol-4-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)acetate (14b). Compound **14b** (a mixture of diastereomer (approximately 1:1), 92 mg, 84%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy) azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-methoxy-2-o

xoethyl)pyridine 1-Oxide (220 mg, 0.19 mmol) in the same manner as described for **9**. mp: 134 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (d, J = 7.8 Hz, 1/2H), 9.63 (d, J = 7.8 Hz, 1/2H), 8.09 (s, 1/2H), 8.08 (s, 1/2H), 7.15 (s, 1/2H), 7.02 (s, 1/2H), 6.91 (s, 1/2H), 6.89 (s, 1/2H), 6.07 (s, 1/2H), 6.05 (s, 1/2H), 4.63 (dd, J = 7.8, 4.0 Hz,1H), 3.70 (s, 3/2H), 3.68 (s, 3/2H), 1.44 (s, 3/2H), 1.42 (s, 3/2H), 1.22 (s, 3/2H), 1.11 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.38, 166.91 and 166.50, 162.14 and 162.00, 161.97 and 161.84, 153.61 and 153.49, 151.86 and 151.29, 145.61 and 145.45, 140.23 and 140.19, 139.15 and 138.69, 128.17 and 128.13, 112.20 and 112.15, 111.06, 78.97 and 78.48, 68.29, 61.31 and 61.26, 53.34, 23.91, 20.61 and 20.48. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}N_6O_{12}S_2$ $[M-H]^-$ 575.0508, found 575.0523.

(3*S*)-3-((*Z*)-2-(2-aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-(methylamino)-2-oxoethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (14c). Compound **14c** (a mixture of diastereomer (approximately 1:1), 43 mg, 78%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy) azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-(methylamino)-2-oxoethyl)pyridine 1-Oxide (110 mg, 0.10 mmol) in the same manner as described for **9**. mp: 128 °C decomp.^{1}H NMR (400 MHz, CD_3OD) δ 9.65 (t, J = 8.0 Hz, 1H), 8.37 – 8.30 (m, 1/2H), 8.17 (s, 3/2H), 7.13 (s, 1/2H), 6.98 (s, 1/2H), 6.92 (s, 1/2H), 6.90 (s, 1/2H), 6.04 (s, 1/2H), 6.02 (s, 1/2H), 4.68 (d, J = 7.8 Hz, 1/2H), 4.64 (d, J = 8.0 Hz, 1/2H), 2.70 (d, J = 4.9 Hz, 3H), 1.45 (s, 3/2H), 1.43 (s, 3/2H), 1.19 (s, 3/2H), 1.17 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.29, 166.02 and 165.88, 162.37 and 162.16, 161.95 and 161.92, 155.62, 151.84 and 151.56, 145.58 and 145.49, 141.08 and 140.97, 140.67, 128.35 and 128.15, 112.42 and 112.10, 111.93 and 111.60, 79.73 and 79.19, 68.45 and

68.33, 61.42 and 61.23, 26.38 and 26.29, 23.88 and 23.84, 20.70 and 20.58. HRMS (ESI) *m/z* calcd for $C_{18}H_{22}N_7O_{11}S_2$ $[M + H]^+$ 576.0819, found 576.0817.

(3*S*)-3-((*Z*)-2-(2-aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-hydroxyethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (15a). Compound **15a** (a mixture of diastereomer (approximately 1:1), 133 mg, 71%) was prepared from 2-(2-(*tert*-Butoxy)-1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)ethyl)-4,5-bis((4-methoxybenzyl)oxy) pyridine 1-Oxide (340 mg, 0.31 mmol) in the same manner as described for **8**. mp: 154 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (t, J = 7.6 Hz, 1H), 8.24 (s, 1H), 7.13 (s, 1/2H), 7.02 (s, 1/2H), 6.85 (s, 1/2H), 6.84 (s, 1/2H), 5.62 – 5.55 (m, 1H), 4.68 (dd, J = 7.7, 3.4 Hz, 1H), 3.89 – 3.71 (m, 2H), 1.48 (s, 3/2H), 1.47 (s, 3/2H), 1.33 (s, 3/2H), 1.29 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ169.02, 162.32 and 162.26, 161.80 and 161.75, 156.78, 150.74 and 150.65, 144.95 and 144.90, 143.69 and 143.54, 140.65 and 140.60, 127.95 and 127.81, 111.62 and 111.45, 111.25 and 110.98, 80.46 and 80.08, 68.31 and 68.20, 61.30 and 61.17, 61.05, 23.72 and 23.63, 20.71 and 20.67. HRMS (ESI) m/z calcd for $C_{17}H_{21}N_6O_{11}S_2$ $[M + H]^+$ 549.0710, found 549.0698.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methoxyethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl hydrogen sulfate (15b). Compound **15b** (a mixture of diastereomer (approximately 1:1), 153 mg, 79%) was prepared from 2-(1-((((*Z*)-2-(((*S*)-2,2-Dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(trityl-amino)thiazol-4-yl)ethylidene)amino)oxy)-2-methoxyethyl)-4,5-bis((4-methoxybenzyl)oxy) pyridine 1-Oxide (350 mg, 0.33 mmol) in the same manner as described for **8**. mp: 152 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (d, J = 7.9 Hz, 1/2H), 9.69 (d, J = 8.1 Hz, 1/2H),

8.22 (s, 1H), 7.12 (s, 1/2H), 7.00 (s, 1/2H), 6.85 (s, 1/2H), 6.84 (s, 1/2H), 5.72 – 5.65 (m, 1H), 4.67 (dd, J = 7.7, 2.8 Hz, 1H), 3.76 – 3.65 (m, 2H), 3.28 (s, 3/2H), 3.23 (s, 3/2H), 1.48 (s, 3/2H), 1.47 (s, 3/2H), 1.34 (s, 3/2H), 1.31 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.29, 162.63 and 162.50, 161.99 and 161.94, 156.54 and 156.45, 151.30 and 151.22, 145.23 and 145.20, 143.30 and 143.24, 140.76 and 140.71, 128.17 and 128.08, 111.91 and 111.72, 111.64 and 111.24, 79.16 and 78.96, 71.47, 68.35 and 68.34, 61.39 and 61.30, 59.44 and 59.07, 23.99 and 23.89, 20.88 and 20.67. HRMS (ESI) *m/z* calcd for $C_{18}H_{21}N_6O_{11}S_2$ $[M - H]^-$ 561.0715, found 561.0731.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-isopropoxyethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (15c). Compound **15c** (a mixture of diastereomer (approximately 1:1), 153 mg, 79%) was prepared from 2-(1-((((*Z*)-2-(((*S*)-2,2-Dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-isopropoxyethyl)-4,5-bis((4-methoxybenz-yl)oxy)pyridine 1-Oxide (350 mg, 0.33 mmol) in the same manner as described for **8**. mp: 173 °C decomp. ^{1}H NMR (500 MHz, CD_3OD) δ 8.12 (s, 1/2H), 8.11 (s, 1/2H), 7.18 (s, 1/2H), 7.17 (s, 1/2H), 7.14 (s, 1/2H), 7.09 (s, 1/2H), 5.96 (dd, J = 5.4, 3.0 Hz, 1/2H), 5.89 (t, J = 4.6 Hz, 1/2H), 4.87 (s, 1/2H), 4.80 (s, 1/2H), 4.01 – 3.83 (m, 2H), 3.63 (dp, J = 18.5, 6.1 Hz, 1H), 1.63 (s, 3/2H), 1.60 (s, 3/2H), 1.49 (s, 3/2H), 1.39 (s, 3/2H), 1.13 (dd, J = 6.1, 2.9 Hz, 3H), 1.10 (d, J = 6.1 Hz, 3/2H), 1.08 (d, J = 6.1 Hz, 3/2H). ^{13}C NMR (126 MHz, CD_3OD, most carbons show two peaks because of diastereomers) δ171.77, 163.02 and 162.87, 161.68 and 161.60, 156.31 and 156.21, 147.29 and 147.13, 145.96 and 145.92, 143.59 and 143.28, 134.01 and 133.69, 128.66 and 128.44, 113.01 and 112.91, 111.96 and 111.63, 80.75 and 80.11, 73.59 and 73.43, 70.20 and 70.08, 67.38 and 67.14, 62.07 and 61.98, 23.01 and 22.88, 21.68 and 21.66, 21.58 and 21.46,

20.70 and 20.60. HRMS (ESI) *m/z* calcd for $C_{20}H_{27}N_6O_{11}S_2$ $[M + H]^+$ 591.1174, found 591.1177.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-(methylthio)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (16a). Compound **16a** (a mixture of diastereomer (approximately 1:1), 140 mg, 90%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy) azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-(methylthio) ethyl)pyridine 1-Oxide (285 mg, 0.25 mmol) in the same manner as described for **9**. mp: 153 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (dd, J = 10.0, 7.7 Hz, 1H), 8.24 (s, 1H), 7.13 (s, 1/2H), 7.12 (s, 1/2H), 6.88 (s, 1/2H), 6.87 (s, 1/2H), 5.79 – 5.73 (m, 1H), 4.68 (d, J = 7.8 Hz, 1/2H), 4.66 (d, J = 7.9 Hz, 1/2H), 3.08 (td, J = 15.2, 4.1 Hz, 1H), 2.95 (dt, J = 14.9, 7.0 Hz, 1H), 2.13 (s, 3/2H), 2.06 (s, 3/2H), 1.47 (d, J = 3.4 Hz, 3H), 1.33 (s, 3/2H), 1.30 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.48, 162.28, 161.94 and 161.88, 157.03, 150.88 and 150.82, 145.33 and 145.28, 144.48 and 144.44, 139.67 and 139.54, 128.09, 112.30 and 112.08, 111.24 and 111.03, 79.89 and 79.12, 68.26, 61.27 and 61.23, 36.06 and 35.91, 23.93 and 23.88, 21.01, 16.91 and 16.53. HRMS (ESI) *m/z* calcd for $C_{18}H_{23}N_6O_{10}S_3$ $[M + H]^+$ 579.0632, found 579.0631.

(3*S*)-3-((*Z*)-2-(2-aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-(isopropylthio)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (16b). Compound **16b** (a mixture of diastereomer (approximately 1:1), 160 mg, 91%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy) azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-(isopropyl thio)ethyl)pyridine 1-Oxide (343 mg, 0.29 mmol) in the same manner as described for **9**. mp:

146 °C decomp.[1]H NMR (600 MHz, DMSO-d_6) δ 9.71 (d, J = 7.8 Hz, 1/2H), 9.69 (d, J = 7.9 Hz, 1/2H), 8.24 (d, J = 2.4 Hz, 1H), 7.15 (s, 1/2H), 7.09 (s, 1/2H), 6.88 (s, 1/2H), 6.87 (s, 1/2H), 5.72 (dd, J = 6.9, 4.1 Hz, 1/2H), 5.69 (dd, J = 7.2, 4.0 Hz, 1/2H), 4.68 (d, J = 7.8 Hz, 1/2H), 4.64 (d, J = 7.7 Hz, 1/2H), 3.11 (dt, J = 14.5, 3.9 Hz,1H), 3.04 – 2.85 (m,2H), 1.48 (s, 3/2H), 1.47 (s, 3/2H), 1.33 (s, 3/2H), 1.31 (s, 3/2H), 1.20 – 1.16 (m, 3/2H), 1.13 (d, J = 6.7 Hz, 3/2H). [13]C NMR (151 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 169.50, 162.28 and 162.19, 161.95 and 161.85, 156.99 and 156.88, 150.72, 145.29 and 145.25, 144.45 and 144.38, 128.10 and 128.04, 112.15 and 112.01, 111.28 and 111.02, 79.96 and 79.32, 68.32 and 68.27, 61.32 and 61.27, 35.74 and 35.66, 32.47 and 32.28, 23.96 and 23.89, 23.61, 23.59 and 23.56, 21.23 and 21.02. HRMS (ESI) *m/z* calcd for $C_{20}H_{25}N_6O_{10}S_3$ $[M - H]^-$ 605.0800, found 605.0807.

(3*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-(methylsulfonyl)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (16c). Compound **16c** (a mixture of diastereomer (approximately 1:1), 209 mg, 93%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((Z)-2-(((S)-2,2-dimethyl-4-oxo-1-(sulfooxy) azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-(methyl-sulfonyl)ethyl)pyridine 1-Oxide (440 mg, 0.37 mmol) in the same manner as described for **9**. mp: 150 °C decomp.[1]H NMR (500 MHz, DMSO-d_6) δ 9.81 (d, J = 7.6 Hz, 1/2H), 9.71 (d, J = 7.7 Hz, 1/2H), 8.26 (s, 1H), 7.19 (s, 1/2H), 7.07 (s, 1/2H), 6.95 (s, 1/2H), 6.92 (s, 1/2H), 6.05 – 5.95 (m, 1H), 4.76 – 4.62 (m, 1H), 3.95 – 3.79 (m, 1H), 3.71 – 3.53 (m, 1H), 3.10 (s, 3/2H), 3.07 (s, 3/2H), 1.48 (s, 3/2H), 1.33 (s, 3/2H), 1.17 (t, J = 7.1 Hz, 3H). [13]C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ170.80 and 169.59, 162.18 and 162.09, 161.90 and 161.77, 159.17 and 158.87, 156.49 and 156.47, 151.30 and 151.05, 145.40, 142.53

and 142.36, 128.21, 112.97 and 112.89, 111.28 and 110.88, 75.34 and 75.13, 68.21, 61.32 and 61.11, 55.77 and 55.63, 43.64 and 43.25, 23.93, 21.03. HRMS (ESI) *m/z* calcd for $C_{18}H_{21}N_6O_{12}S_3$ $[M-H]^-$ 609.0380, found 609.0374.

(3*S*)-3-((*Z*)-2-(2-Amino-5-chlorothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (17). Compound **17** (a mixture of diastereomer (approximately 1:1), 140 mg, 77%) was prepared from (3*S*)-3-((*Z*)-2-((1-(1,5-Bis(benzhydryloxy)-4-oxo-1,4-dihydropyridin-2-yl)ethoxy) imino)-2-(2-((*tert*-butoxycarbonyl)amino)-5-chlorothiazol-4-yl)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (320 mg, 0.32 mmol) in the same manner as described for **8**. mp: 153 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (d, *J* = 7.8 Hz, 1/2H), 9.59 (d, *J* = 7.9 Hz, 1/2H), 8.22 (s, 1H), 7.44 (s, 2H), 7.18 (s, 1/2H), 7.01 (s, 1/2H), 5.68 – 5.58 (m, 1H), 4.61 (d, *J* = 7.8 Hz, 1/2H), 4.58 (d, *J* = 7.9 Hz, 1/2H), 1.50 (t, *J* = 7.0 Hz, 3H), 1.46 (s, 3/2H), 1.44 (s, 3/2H), 1.32 (s, 3/2H), 1.27 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.07 and 165.00, 162.12 and 162.05, 161.89, 157.51, 150.19, 147.19 and 146.98, 145.02, 137.15, 128.10 and 127.96, 111.47 and 111.39, 110.35 and 109.56, 75.29 and 75.10, 68.56 and 68.50, 61.52 and 61.25, 23.85 and 23.82, 21.24 and 21.11, 19.02. HRMS (ESI) *m/z* calcd for $C_{17}H_{18}ClN_6O_{10}S_2$ $[M-H]^-$ 565.0214, found 565.0219.

(3*S*)-3-((*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (18). Compound **18** (a mixture of diastereomer (approximately 1:1), 89 mg, 80%) was prepared from (3*S*)-3-((*Z*)-2-((1-(1,5-Bis(benzhydryloxy)-4-oxo-1,4-dihydropyridin-2-yl)ethoxy)imino)-2-(5-((*tert*-butoxycarbonyl)amino)-1,2,4-thiadiazol-3-yl)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (200 mg, 0.21 mmol) in the same manner as described for **8**. mp: 160 °C

decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (d, J = 7.6 Hz, 1/2H), 9.64 (d, J = 7.7 Hz, 1/2H), 8.14 (d, J = 5.9 Hz, 2H), 8.00 (s, 1H), 6.96 (s, 1/2H), 6.80 (s, 1/2H), 5.72 – 5.59 (m, 1H), 4.64 (d, J = 7.6 Hz, 1/2H), 4.62 (d, J = 7.7 Hz, 1/2H), 1.47 (t, J = 3.4 Hz, 3H), 1.45 (s, 3/2H), 1.41 (s, 3/2H), 1.32 (s, 3/2H), 1.29 (s, 3/2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 183.23 and 183.20, 161.68 and 161.58, 161.52 and 161.47, 161.36, 158.71 and 158.45, 149.84 and 149.79, 145.67 and 145.62, 144.36 and 144.27, 127.38 and 127.20, 109.19 and 108.55, 75.54 and 75.37, 68.05, 60.94 and 60.80, 23.36 and 23.34, 20.62 and 20.54, 18.54 and 18.50. HRMS (ESI) *m/z* calcd for $C_{16}H_{18}N_7O_{10}S_2$ $[M - H]^-$ 532.0557, found 532.0552.

(3*S*)-3-((*Z*)-2-(2-Amino-5-chlorothiazol-4-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydro-pyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (19). Compound **19** (a mixture of diastereomer (approximately 1:1), 148 mg, 69%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-1-(2-((*tert*-butoxycarbonyl)amino)-5-chlorothiazol-4-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (370 mg, 0.36 mmol) in the same manner as described for **8**. mp: 166 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.65 (d, J = 7.7 Hz, 1/2H), 9.56 (d, J = 7.8 Hz, 1/2H), 8.25 (d, J = 4.5 Hz, 1H), 7.07 (s, 1/2H), 7.05 (s, 1/2H), 5.43 (d, J = 5.7 Hz, 1/2H), 5.34 (d, J = 6.4 Hz, 1/2H), 4.62 (dd, J = 7.9, 1.7 Hz, 1H), 2.22 – 2.11 (m, 1H), 1.46 (s, 3/2H), 1.44 (s, 3/2H), 1.34 (s, 3/2H), 1.32 (s, 3/2H), 0.97 – 0.89 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ170.58, 164.76 and 164.72, 161.97 and 161.80, 161.77 and 161.71, 157.76 and 157.73, 150.25 and 150.16, 145.50 and 145.31, 144.98, 136.78, 127.71 and 127.68, 111.41 and 111.20, 82.89, 68.00 and 67.89, 61.14 and 60.98, 31.65 and 31.45, 23.71 and 23.62, 21.00 and 20.92, 18.67 and 18.62, 17.60 and 17.28. HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_6O_{10}S_2Cl$ $[M - H]^-$ 593.0533, found 593.0542.

(3*S*)-3-((*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-((1-(1,5-dihydroxy-4-oxo-1,4-dihydro pyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (20). Compound **20** (a mixture of diastereomer (approximately 1:1), 73 mg, 72%) was prepared from 4,5-Bis(benzhydryloxy)-2-(1-((((*Z*)-1-(5-((*tert*-butoxycarbonyl)amino)-1,2,4-thiadiazol-3-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethy lidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (180 mg, 0.18 mmol) in the same manner as described for **8**. mp: 175 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (d, J = 7.7 Hz, 1/2H), 9.61 (d, J = 7.7 Hz, 1/2H), 8.21 (s, 1/2H), 8.20 (s, 1/2H), 8.12 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 3.9 Hz, 1H), 5.51 (d, J = 5.1 Hz, 1/2H), 5.42 (d, J = 5.8 Hz, 1/2H), 4.67 (d, J = 7.9 Hz, 1/2H), 4.64 (d, J = 7.7 Hz, 1/2H), 2.23 – 2.10 (m, 1H), 1.47 (s, 3/2H), 1.46 (s, 3/2H), 1.34 (s, 3/2H), 1.33 (s, 3/2H), 1.01 – 0.95 (m, 3H), 0.93 – 0.86 (m,3H). ^{13}C NMR (126 MHz, DMSO-d_6, most carbons show two peaks because of diastereomers) δ 183.22 and 183.19, 172.32, 161.52, 161.48 and 161.45, 156.43 and 156.33, 150.38 and 150.23, 144.71 and 144.66, 144.59 and 144.52, 127.52, 110.41 and 110.24, 83.11 and 82.89, 67.80 and 67.63, 60.81 and 60.79, 31.45 and 31.17, 23.37 and 23.31, 20.72 and 20.61, 18.54 and 18.47, 16.90 and 16.62. HRMS (ESI) *m/z* calcd for $C_{18}H_{23}N_7O_{10}S_2Na$ $[M + Na]^+$ 584.0846, found 584.0833.

(*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((*S*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (21). Compound **21** (135 mg, 72%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*S*)-1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thia zol-4-yl)ethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (380 mg, 0.34 mmol) in the same manner as described for **9**. mp: 154 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.71 (d, J = 7.8 Hz, 1H), 8.24 (s, 1H), 7.03 (s, 1H), 6.85 (s, 1H), 5.45 (d, J = 5.0 Hz, 1H), 4.71 (d, J = 7.8

Hz, 1H), 2.22 – 2.13 (m, 1H), 1.48 (s, 3H), 1.35 (s, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.97, 161.91, 161.47, 157.02, 150.13, 145.03, 144.66, 139.39, 127.51, 111.24, 110.37, 82.58, 67.55, 60.80, 31.21, 23.37, 20.64, 18.54, 16.58. HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_6O_{10}S_2$ $[M - H]^-$ 559.0917, found 559.0928.

(*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((*R*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (22). Compound **22** (110 mg, 60%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*R*)-1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino)thiazol-4-yl)ethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (370 mg, 0.33 mmol) in the same manner as described for **9**. mp: 162 °C decomp. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.68 (d, *J* = 7.8 Hz, 1H), 8.25 (s, 1H), 7.01 (s, 1H), 6.84 (s, 1H), 5.37 (d, *J* = 5.5 Hz, 1H), 4.68 (d, *J* = 7.7 Hz, 1H), 2.22 – 2.10 (m, *J* = 6.7 Hz, 1H), 1.47 (s, 3H), 1.33 (s, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.51, 162.36, 161.92, 157.57, 150.64, 145.69, 145.17, 139.51, 128.01, 112.05, 110.96, 82.95, 68.17, 61.33, 31.92, 23.83 , 21.07, 19.14, 17.30. HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_6O_{10}S_2$ $[M - H]^-$ 559.0917, found 559.0926.

(*S*)-3-((*Z*)-2-(2-Amino-5-chlorothiazol-4-yl)-2-(((*S*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (23). Compound **23** (145 mg, 81%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*S*)-1-((((*Z*)-1-(2-((*tert*-butoxycarbonyl)amino)-5-chlorothiazol-4-yl)-2(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (310 mg, 0.30 mmol) in the same manner as described for **9**. mp: 161 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (d, *J* = 7.8 Hz, 1H), 8.25 (s, 1H), 7.05 (s, 1H), 5.44 (d, *J* = 5.8 Hz, 1H), 4.62 (d, *J* = 7.7 Hz, 1H), 2.24 – 2.11 (m, 1H), 1.46 (s, 3H), 1.35 (s, 3H), 0.95 (d, *J* =

7.0 Hz, 3H), 0.92 (d, *J* = 6.9Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.35, 164.47, 161.51, 161.47, 157.37, 149.89, 145.02, 144.70, 136.52, 127.48, 110.53, 82.62, 67.61, 60.87, 31.17, 23.34, 20.75, 18.34, 17.00. HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_6O_{10}S_2Cl$ $[M - H]^-$ 593.0533, found 593.0529.

(*S*)-3-((*Z*)-2-(2-Amino-5-chlorothiazol-4-yl)-2-(((*R*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (24). Compound **24** (130 mg, 68%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*R*)-1-((((*Z*)-1-(2-((*tert*-butoxycarbonyl)amino)-5-chlorothiazol-4-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (330 mg, 0.32 mmol) in the same manner as described for **9**. mp: 157 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (d, *J* = 7.9 Hz, 1H), 8.26 (s, 1H), 7.07 (s, 1H), 5.35 (d, *J* = 6.4 Hz, 1H), 4.62 (d, *J* = 7.9 Hz, 1H), 2.18 (dp, *J* = 13.6, 6.9, 6.4 Hz, 1H), 1.45 (s, 3H), 1.33 (s, 3H), 0.94 (t, *J* = 6.3 Hz, 6H).^{13}C NMR (126 MHz, DMSO-d_6) δ170.29, 164.43, 161.68, 161.44, 157.40, 149.99, 145.21, 144.69, 136.54, 127.44, 110.88, 82.55, 67.72, 60.71, 31.37, 23.42, 20.65, 18.39, 17.33. HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_6O_{10}S_2Cl$ $[M - H]^-$ 593.0533, found 593.0532.

(*S*)-3-((*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(((*S*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (25). Compound **25** (140 mg, 83%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*S*)-1-((((*Z*)-1-(5-((*tert*-butoxycarbonyl)amino)-1,2,4-thiadiazol-3-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (298 mg, 0.30 mmol) in the same manner as described for **9**. mp: 174 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.67 (d, *J* = 7.7 Hz, 1H), 8.26 (s, 1H), 8.12 (s, 2H), 7.02 (s, 1H), 5.51 (d, *J* = 5.2 Hz, 1H), 4.67 (d, *J* = 7.6 Hz, 1H), 2.24 – 2.11 (m, 1H), 1.48 (s, 3H), 1.35 (s, 3H),

0.99 (d, *J* = 6.7 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 183.24, 170.29, 161.47, 161.40, 157.45, 150.39, 144.86, 144.79, 127.64, 110.28, 83.04, 67.63, 60.79, 31.35, 23.30, 20.71, 18.41, 16.63. HRMS (ESI) *m/z* calcd for $C_{18}H_{22}N_7O_{10}S_2$ [M − H] $^-$ 560.0875, found 560.0867.

(*S*)-3-((*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(((*R*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-methylpropoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (26). Compound **26** (100 mg, 68%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*R*)-1-((((*Z*)-1-(5-((*tert*-butoxycarbonyl)amino)-1,2,4-thiadiazol-3-yl)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxoethylidene)amino)oxy)-2-methylpropyl)pyridine 1-Oxide (260 mg, 0.26 mmol) in the same manner as described for **9**. mp: 168 °C decomp. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.62 (d, *J* = 7.7 Hz, 1H), 8.26 (s, 1H), 8.10 (s, 2H), 7.02 (s, 1H), 5.42 (d, *J* = 5.8 Hz, 1H), 4.64 (d, *J* = 7.6 Hz, 1H), 2.16 (h, *J* = 6.8 Hz, 1H), 1.46 (s, 3H), 1.33 (s, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 183.20, 170.61, 161.48, 161.44, 157.27, 150.50, 144.99, 144.77, 127.59, 110.45, 82.85, 67.81, 60.82, 31.58, 23.36, 20.62, 18.50, 16.92. HRMS (ESI) *m/z* calcd for $C_{18}H_{22}N_7O_{10}S_2$ [M − H] $^-$ 560.0875, found 560.0868.

(*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((*R*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-(methylthio)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (27). Compound **27** (118 mg, 87%) was prepared from 4,5-Bis(benzhydryloxy)-2-((*R*)-1-((((*Z*)-2-(((*S*)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino) thiazol-4-yl)ethylidene)amino)oxy)-2-(methylthio)ethyl)pyridine 1-Oxide (270 mg, 0.23 mmol) in the same manner as described for **9**. mp: 166 °C decomp. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (d, *J* = 7.9 Hz, 1H), 8.24 (s, 1H), 7.12 (s, 1H), 6.89 (s, 1H), 5.77 (dd, *J* = 6.4,

3.9 Hz, 1H), 4.69 (d, *J* = 7.8 Hz, 1H), 3.12 – 3.03 (m, 1H), 2.96 (dd, *J* = 14.6, 6.5 Hz, 1H), 2.06 (s, 3H), 1.47 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.03, 161.74, 161.44, 156.44, 150.18, 144.80, 143.88, 138.97, 127.59, 111.56, 110.53, 78.62, 67.76, 60.77, 35.53, 23.43, 20.49, 16.00. HRMS (ESI) *m/z* calcd for $C_{18}H_{23}N_6O_{10}S_3$ $[M + H]^+$ 579.0632, found 579.0628.

(*S*)-3-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(((*S*)-1-(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)-2-(methylthio)ethoxy)imino)acetamido)-2,2-dimethyl-4-oxoazetidin-1-yl Hydrogen Sulfate (28). Compound **28** (109 mg, 71%) was prepared from 4,5-Bis(benzhydryloxy)-2-((S)-1-((((Z)-2-(((S)-2,2-dimethyl-4-oxo-1-(sulfooxy)azetidin-3-yl)amino)-2-oxo-1-(2-(tritylamino) thiazol-4-yl)ethylidene)amino)oxy)-2-(methylthio)ethyl)pyridine 1-Oxide (304 mg, 0.26 mmol) in the same manner as described for **9**. mp: 155 °C decomp. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.73 (d, *J* = 7.8 Hz, 1H), 8.24 (s, 1H), 7.13 (s, 1H), 6.87 (d, *J* = 1.3 Hz, 1H), 5.75 (dd, *J* = 6.7, 4.1 Hz, 1H), 4.66 (d, *J* = 7.8 Hz, 1H), 3.10 (dd, *J* = 14.9, 4.1 Hz, 1H), 2.94 (dd, *J* = 14.9, 6.8 Hz, 1H), 2.13 (s, 3H), 1.47 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.53, 162.25, 161.88, 157.04, 150.81, 145.27, 144.45, 139.43, 128.08, 112.32, 111.23, 79.90, 68.26, 61.21, 35.89, 23.87, 21.01, 16.90. HRMS (ESI) *m/z* calcd for $C_{18}H_{23}N_6O_{10}S_3$ $[M + H]^+$ 579.0632, found 579.0630.

Minimum Inhibitory Concentration Testing. The minimum inhibitory concentrations (MICs) of the target compounds against Gram-negative bacteria were determined using aztreonam, meropenem, ceftizoxime sodium (provided by Sichuan Primed Bio-Tech Group Co., Ltd) and **6** (synthesized according to published procedures[52]) as reference compounds. The Gram-negative bacteria strains, including five *E. coli* strains, five *K. pneumoniae* strains, five *A. baumannii* strains, and five *P. aeruginosa* strains, were obtained from hospitals in Sichuan and

Beijing. The MIC values were determined using the broth microdilution protocol according to the methods of the Clinical and Laboratory Standards Institute (CLSI),[56] in the Mueller-Hinton broth (Oxoid) supplemented with 16 mg/L 2, 2′-bipyridyl (BPL).[35] All of the test compounds were dissolved in DMSO except for meropenem and ceftizoxime sodium which were dissolved in water, and then serially diluted in growth medium. The strains were incubated at 35–37 °C, and the MIC values were determined at 18 h.

Plasma protein binding determination. Fraction unbound (Fu) rate was determined in human plasma using a rapid equilibrium dialysis device (Thermo Scientific). Plasma loaded with compound at 10 μM (n = 4 replicates per concentration) or blank buffer, was added to opposing compartments of the device and incubated for 4 h at 37 °C in a CO_2 incubator (5% CO_2). Compound concentrations in plasma and buffer were determined by LC–MS/MS. Fraction unbound was calculated as the concentration in the buffer side divided by the total concentration in the plasma side. At the same time, the remaining T0 plasma sample were placed into the CO_2 incubator (5% CO_2) for 4 h at 37 °C to acquire the T4h plasma samples, and the degradation percentage was calculated as 100*(1− Average concentration of T4h samples/Average concentration of T0 samples).

Rat Pharmacokinetic Studies. Pharmacokinetic parameters of compounds **12c**, **16a**, **16c**, **19**, **21**, and compound **6** (positive control) were measured in male Sprague–Dawley rats weighing between 200 and 220 g, with three animals in each group. The tested compounds were dissolved in water and administered intravenously at a dose of 1.0 mg/kg. Serial specimens were collected via the retrobulbar vein 2, 5, 15, 30, 45, 60, 90, and 180 min after administration and quantified by LC–MS/MS. Pharmacokinetic parameters were calculated from the mean plasma concentration by noncompartmental analysis. The protocol for this study was reviewed and approved by the

Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals.

Murine Systemic Infection Model. Clinical *E. coli* 210737. Kunming mice (half male and half female) weighing between 18 and 22 g were used in this study, with 5 mice in each group. A lethal systemic infection was given to the mice by intraperitoneal injection of 0.5 mL of inoculum of *E. coli* 210737, 5×10^4 CFU/mL. Compounds were administered subcutaneously at 0.5 and 4.0 h post-infection at doses of 1.25, 2.5, 5, 10, and 20 mg/kg for **12c**, **16c** and **19**, doses of 1.25, 2.5, 5, 10 mg/kg for compound **6** and doses of 0.625, 1.25, 2.5, 5, 10 mg/kg for meropenem. The ED_{50} was calculated 48 h after treatment by the method of Bliss. The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Sichuan Primed Bio-Tech Group Co., Ltd.

MDR *K. pneumoniae* 212232. Kunming mice (half male and half female) weighing between 18 and 22 g were used in this study, with 5 mice in each group. A lethal systemic infection was given to the mice by intraperitoneal injection of 0.5 mL of inoculum of *K. pneumoniae* 212232, 3×10^6 CFU/mL. Compounds were administered subcutaneously at 0.5 and 4.0 h post-infection at doses of 3.125, 6.25, 12.5, 25, 50, and 100 mg/kg for **12c**, doses of 12.5, 25, 50, and 100 mg/kg for compound **6** and meropenem. The ED_{50} was calculated 48 h after treatment by the method of Bliss. The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Sichuan Primed Bio-Tech Group Co., Ltd.

■ ASSOCIATED CONTENT

Supporting Information

Individual MIC data for tested compounds, experimental details for the preparation of all intermediates, spectral data of compound **6**, X-ray crystallography of (*S*)–**75**, and HPLC analysis for tested compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MDR, multidrug-resistant; CRE, carbapenem–resistant *Enterobacteriaceae*; XDR, extremely drug-resistant; PBP, penicillin binding protein; PPB, plasma protein binding; ESBLs,

extended-spectrum β-lactamases; SAR, structure-activity relationship; MIC, minimum inhibitory concentration; iv, intravenous; Fu, fraction unbound; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acid; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; *m*-CPBA, 3-chloroperbenzic acid; DMF, N,N-dimethylformamide; MTBE, *tert*-butyl methyl ether.

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