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Discovery of ANT3310, a Novel Broad-Spectrum Serine β-Lactamase Inhibitor of the Diazabicyclooctane Class, Which Strongly Potentiates Meropenem Activity against Carbapenem-Resistant Enterobacterales and *Acinetobacter baumannii*

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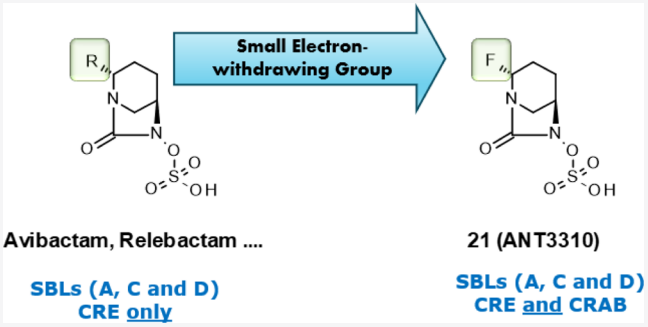
ABSTRACT: The diazabicyclooctanes (DBOs) are a class of serine β-lactamase (SBL) inhibitors that use a strained urea moiety as the warhead to react with the active serine residue in the active site of SBLs. The first in-class drug, avibactam, as well as several other recently approved DBOs (e.g., relebactam) or those in clinical development (e.g., nacubactam and zidebactam) potentiate activity of β-lactam antibiotics, to various extents, against carbapenem-resistant Enterobacterales (CRE) carrying class A, C, and D SBLs; however, none of these are able to rescue the activity of β-lactam antibiotics against carbapenem-resistant *Acinetobacter baumannii* (CRAB), a WHO "critical priority pathogen" producing class D OXA-type SBLs. Herein, we describe the chemical optimization and resulting structure−activity relationship, leading to the discovery of a novel DBO, **ANT3310**, which uniquely has a fluorine atom replacing the carboxamide and stands apart from the current DBOs in restoring carbapenem activity against OXA-CRAB as well as SBL-carrying CRE pathogens.



INTRODUCTION

Multidrug-resistant Gram-negative bacteria have become an increasingly serious healthcare threat, exacerbated by major structural and economic issues underlying the development of new antibacterial drugs.[1] The effectiveness of the most important class of antibacterial agents, the β-lactam antibiotics, has been compromised by a main resistance mechanism, the production of β-lactamase enzymes.[2] Co-dosing of β-lactams with a β-lactamase inhibitor (BLI) has been used as a successful strategy to combat this type of resistance as far back as 1981, when the combination of clavulanic acid and amoxycillin was launched.[3] Other established BLIs, such as tazobactam and sulbactam (Figure 1), have become less clinically useful because of the increasing resistance attributable to bacterial production of extended spectrum β-lactamases.[4] In 2015, the novel diazabicyclooctane (DBO) BLI avibactam entered the clinic[5] in fixed combination with ceftazidime and several "fast followers", including relebactam (approved June 2020), plus nacubactam, zidebactam, and durlobactam, which are in clinical development (Figure 1). To date, all BLIs in clinical use are specific to serine β-lactamases (SBLs), which include class A, C, and D enzymes. Resistance is also increasing because of the occurrence of the class B metallo β-lactamase (MBL) enzymes, such as NDM-1,[6] and currently, there are no MBL inhibitors in clinical use.[7] Significant advances have been achieved with the development of boronate SBLIs, such as vaborbactam (VAB),[8] mainly a *Klebsiella pneumoniae* carbapenemase (KPC) inhibitor, and taniborbactam,[9] which possesses broader spectrum β-lactamase inhibitory properties, including VIM-2 from the MBL family. Recently, a series of publications have described a new boronate QPX7728,[10] representing an

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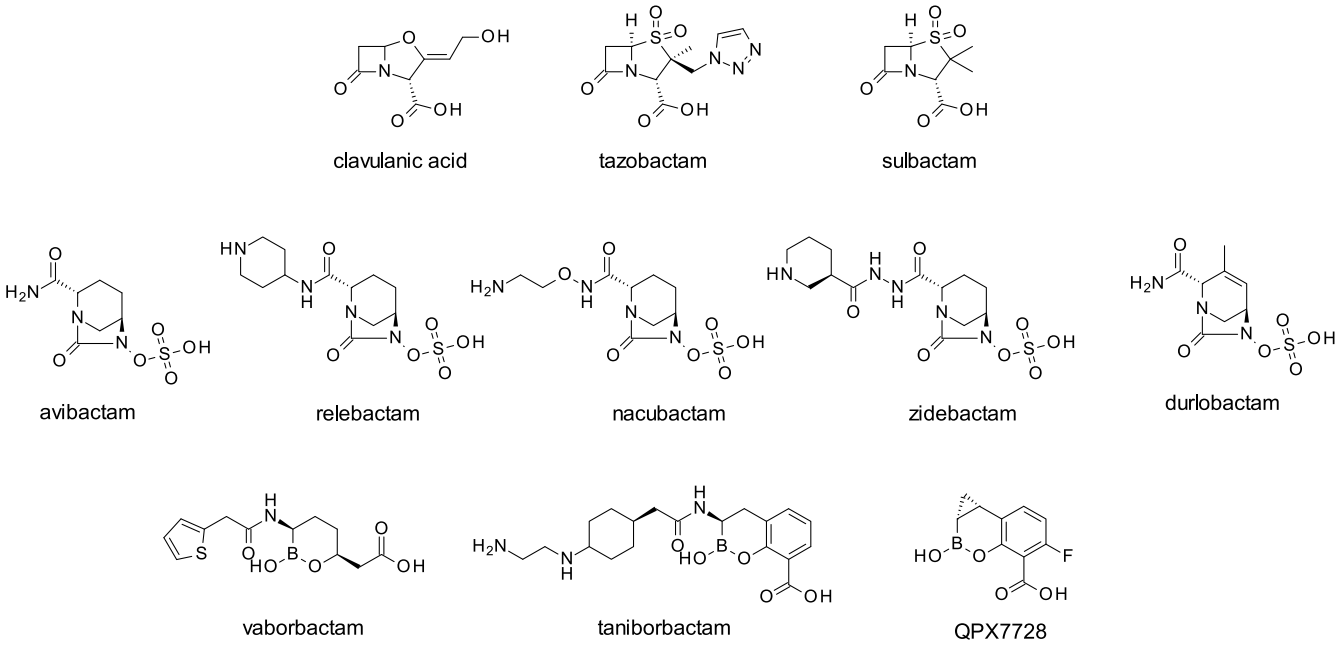
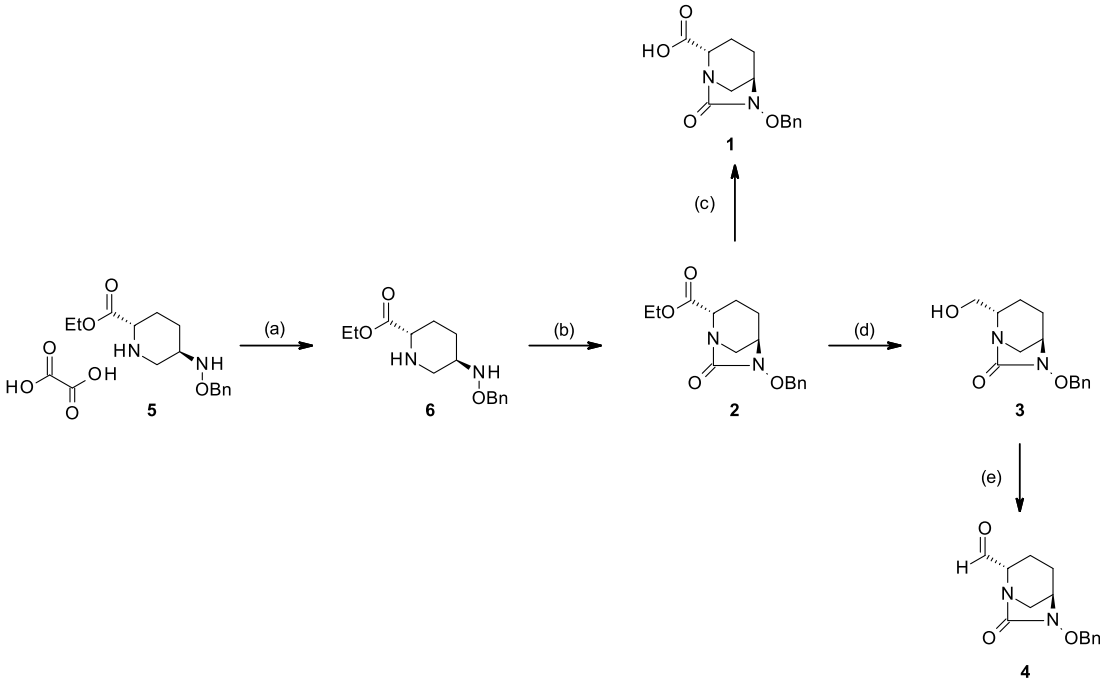


Figure 1. Various BLIs.

Scheme 1. Synthesis of the Key Acid, Alcohol, and Aldehyde Intermediates[a]



[a](a) $KHCO_3$ and THF; (b) triphosgene, DIPEA, and DCM, 0 °C to RT, 16 h; (c) $LiOH{\cdot}H_2O$ and THF/H_2O, RT; (d) $LiBH_4$ and THF/EtOH; and (e) trichloroisocyanuric acid, TEMPO, and DCM.

advance over taniborbactam as it has improved inhibition of the NDM-type MBLs.[10−12] The whole area has been recently reviewed.[13]

In the DBO series, the reactive β-lactam ring of earlier BLIs has been replaced by a similarly reactive strained bicyclic urea. Avibactam has proved to be the inspiration for other additions to the DBO series, such as relebactam, nacubactam, zidebactam, and durlobactam (Figure 1),[14−19] all of which contain a basic substituent appended to the carboxamide. However, none of these, with the exception of durlobactam, penetrate/accumulate into *Acinetobacter* strains, and because durlobactam is not active against *Enterobacterales*, a combination to treat both carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections remains elusive. Consequently, CRAB remains a "critical priority pathogen" as designated by the WHO.[20] Carbapenem resistance in CRAB is mediated by class D OXA-type enzymes, so a successful inhibitor should be able to penetrate into *Acinetobacter* and have activity against OXA-type enzymes. At the outset of this research, we set ourselves

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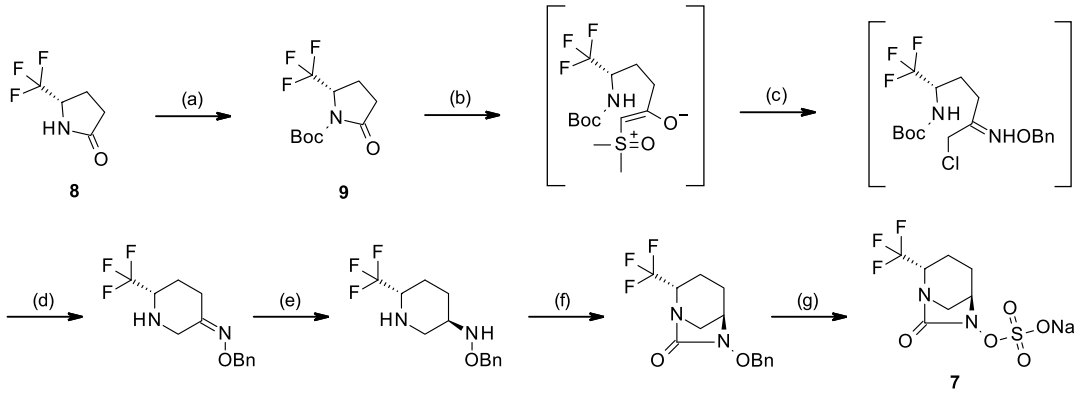
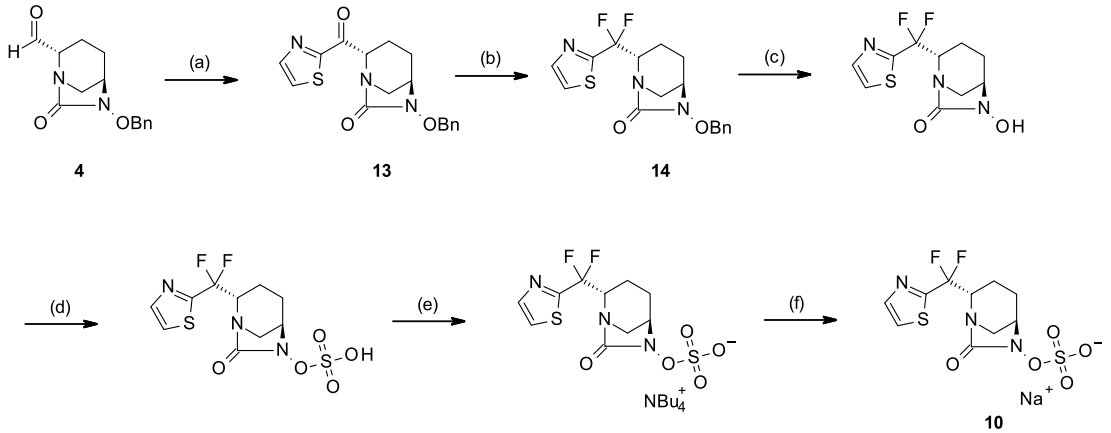
Scheme 2. Synthesis of the CF_3 Analogue[a]



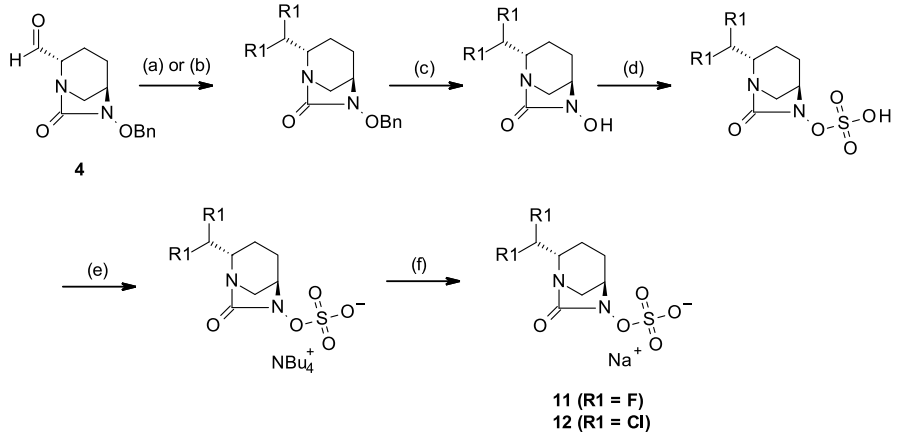
[a](a) $(Boc)_2O$, TEA, and DCM, 0 °C to RT, 5 h; (b) Me_3SOI, KOtBu, and DMSO/THF; (c) $BnONH_2$-HCl and EtOAc; (d) MsOH and EtOAc then $KHCO_3$ aq; (e) $NaBH_3CN$, H_2SO_4, and EtOAc; (f) triphosgene, DIPEA, and DCM, 0 °C to RT, 16 h; (g) H_2, Pd/C then Me_3NSO_3, and TEA then $NaHCO_3$ aq.

Scheme 3. Synthesis of CF_2-Thiazolyl Analogue 10[a]



[a](a) TMS-thiazole, Ac_2O, and DMSO; (b) DAST and DCM; (c) $TiCl_4$ and DCM; (d) SO_3-pyridine, TEA, and DCM; (e) $n$$Bu_4NOAc$ and DCM; and (f) Dowex-Na and H_2O.

Scheme 4. Synthesis of the CHF_2 and $CHCl_2$ Analogues 11 and 12[a]



[a](a) DAST and DCM; (b) PCl_5 and DCM, 0 °C to RT, 8 h; (c) H_2 and Pd/C; (c) SO_3-pyridine, TEA, and DCM; (d) $n$$Bu_4NOAc$ and DCM; and (e) Dowex-Na, H_2O.

the objective of achieving a broad-spectrum DBO that restored carbapenem activity against CRAB and which ideally possessed little or no intrinsic antibacterial activity in order to minimize the potential for resistance.

RESULTS AND DISCUSSION

Chemistry. Many of the analogues were accessed from either key acid **1**, obtained after the basic hydrolysis of ethyl ester **2**, the primary alcohol intermediate **3**, itself prepared by

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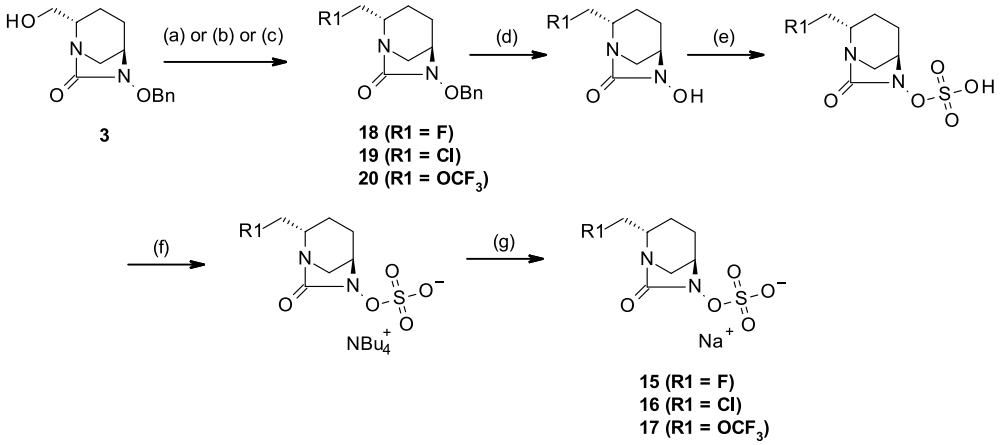
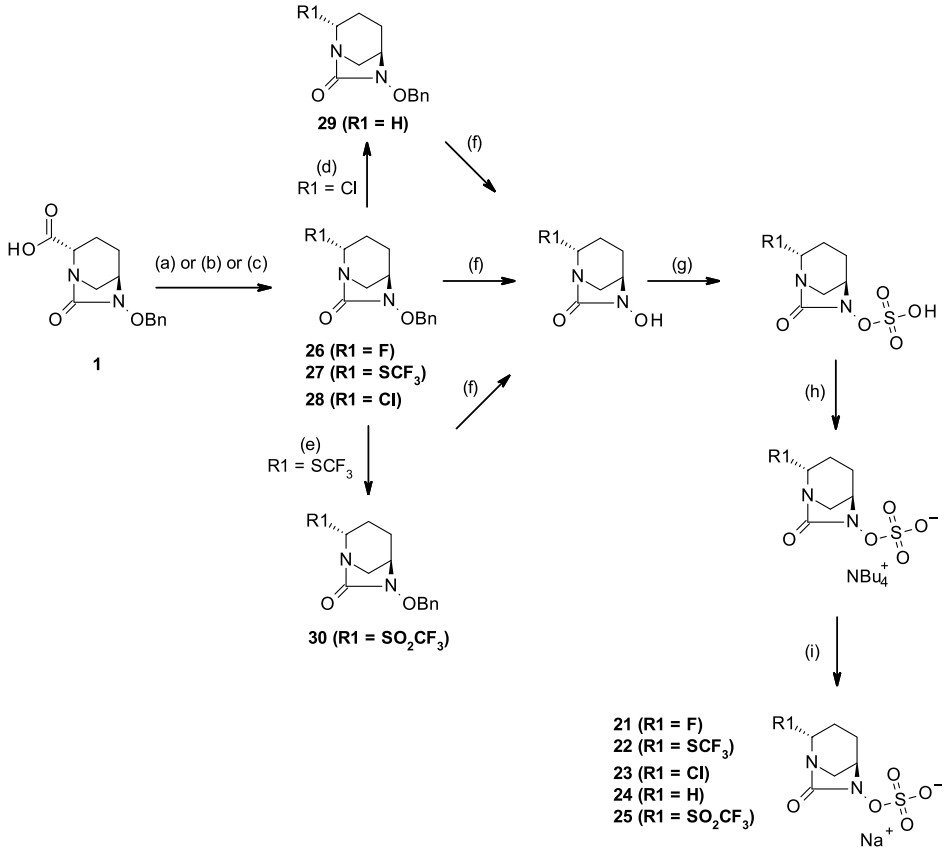
Scheme 5. Synthesis of the CH_2F, CH_2Cl, and CH_2OCF_3 Analogues 15, 16, and 17[a]



[a](a) Mesyl chloride, TEA, DMAP, DCM then TBAF, and MeCN, 70 °C; (b) mesyl chloride, TEA, DMAP, DCM then TBACl, and MeCN, 70 °C (c) $TMSCF_3$, AgOTf, 2-fluoropyridine, KF, Selectfluor, and EtOAc, RT; (d) H_2 and Pd/C; (e) SO_3-pyridine, TEA, and DCM; (f) $n$$Bu_4NOAc$ and DCM; and (g) Dowex-Na and H_2O.

Scheme 6. Synthesis of the F, Cl, SCF_3, H, and SO_2CF_3 Analogues 21 to 25[a]



[a](a) $AgNO_3$, selectfluor, and acetone/H_2O 4:1, 50 °C, 3 h; (b) $Pb(OAc)_4$, NCS, AcOH, and DMF, 60 °C, 4 h; (c) $AgSCF_3$, selectfluor, 2,6-lutidine, and acetone; (d) Bu_3SnH, AIBN, and Toluene, reflux; (e) mCPBA and DCM, RT; (f) H_2 and Pd/C; (g) SO_3-pyridine, TEA, and DCM; (h) $n$$Bu_4NOAc$ and DCM; (i) Dowex-Na and H_2O.

the $LiBH_4$ reduction of ester **2**, or its corresponding aldehyde **4** obtained by the oxidation of alcohol **3** using trichloroisocyanuric acid and (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO). Ethyl ester **2** was prepared from commercially available diamine oxalate salt **5** by conversion to free base **6** and cyclization with triphosgene (Scheme 1).

Certain analogues required bespoke syntheses. For example, CF_3 analogue **7** was synthesized from commercially available (5*S*)-5-(trifluoromethyl)pyrrolidine-2-one **8**, which was Boc protected and then converted to **7**, following the sequence reported for avibactam in the literature,[21] with the minor modification that the sulfur trioxide trimethylamine complex was used to introduce the sulfonic acid functionality and the

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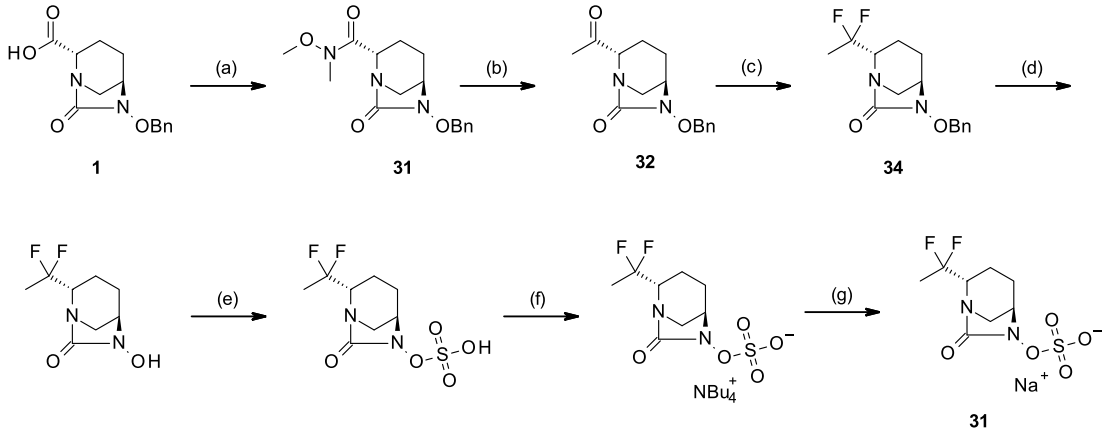
Scheme 7. Synthesis of CF_2CH_3 Analogue 31[a]



[a](a) MeNHOMe; (b) MeMgBr and THF; (c) DAST and DCM; (d) H_2 and Pd/C; (e) SO_3-pyridine, TEA, and DCM; (f) nBu$_4$NOAc and DCM; and (g) Dowex-Na and H_2O.

sodium salt was obtained by treatment with sodium bicarbonate (Scheme 2).

Analogues **10** to **12** were prepared from aldehyde **4**. The reaction of **4** with trimethylsilyl thiazole in the presence of acetic anhydride afforded the thiazolyl alcohol which was oxidized *in situ* to give ketone **13**, based on the chemistry of Dondoni.[22] This was converted to the bis-fluoro methylene derivative **14** by treatment with diethylaminosulfur trifluoride (DAST). Difluoromethylthaizolyl analogue **10** was then obtained following the usual synthetic route,[21] (Scheme 3) involving debenzylation, sulfonation/tetrabulylammonium salt formation, and then ion exchange to give the desired sodium salt. Difluoromethyl **11** and dichloromethyl **12** analogues were obtained after treatment of the aldehyde with DAST or PCl_5, respectively, followed again by the usual sequence (Scheme 4).

Compounds **15** to **17** were prepared from alcohol **3**. The activation of the alcohol as a mesylate, then treatment with TBAF or TBACl in acetonitrile at 70 °C afforded the fluoromethyl and chloromethyl derivatives **18** and **19**, respectively, which were converted to **15** and **16** by the usual synthetic pathway. Trifluoromethoxy compound **17** was obtained following the conditions reported in the literature, leading to trifluoromethoxy intermediate **20**[23] (Scheme 5).

Compounds **21** to **25** were prepared from acid **1**. Fluoro and trifluoromethylthio derivatives **26** and **27** were prepared by decarboxylation and radical treatment with Selecfluor in the presence of $AgNO_3$ or $AgSCF_3$. Chloro derivative **28** was produced by the reaction with $Pb(OAc)_4$ in the presence of *N*-chlorosuccinimide. This intermediate was reduced to H analogue **29** with Bu_3SnH, and SCF_3 intermediate **27** oxidized using mCPBA to give the corresponding sulfone **30**. All these different intermediates were submitted to the usual chemistry sequence described previously, affording analogues **21** to **25** (Scheme 6).

CF_2CH_3 analogue **31** was prepared also from carboxylic acid intermediate **1**. This was activated through Weinreb amide **32** and treated with Grignard reagent MeMgBr to afford methyl ketone **33**. The reaction with DAST afforded $-CF_2CH_3$ intermediate **34**, which was converted to **31** following the usual synthetic route (Scheme 7).

Evaluation of Available Structural Data, Initial Chemistry Strategy, and Screening Cascade. The inspection of the X-ray structures of covalent adducts of DBOs with various OXA enzymes (e.g., PDB entries 4WMC, 4WM9, 42SJ, and 5FAQ) shows several interesting features (Figure 2). First, the orientation of the primary carboxamide

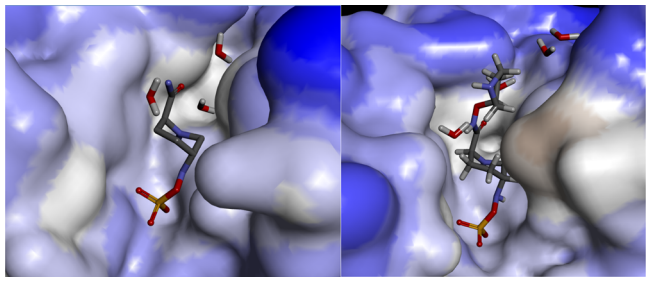


Figure 2. (A) Covalently bound avibactam in OXA-48 showing water molecules in the pocket (PDB entry 4WMC) and (B) covalently bound FPI1465 (close analogue of nacubactam) in OXA-48 (PDB entry 5FAQ) showing the inhibitor side chain exiting the pocket.

substituent in the avibactam adduct is not crucial as it does not seem to make any obvious H-bond with class D β-lactamase enzymes, and alternative conformations can be seen in the secondary carboxamides derived from other DBOs (Figure 2). Second, in OXA-48 there are two water molecules in the active site pocket around the carboxamide. The original plan was to make a range of substituents other than amide, which retained the electron-withdrawing properties of the carboxamide and which with increasing size might also fill the small pocket around the carboxamide by the extrusion of the water molecules—an established strategy in lead optimization.[24−26]

Our primary screening cascade comprised a panel of SBL enzymes (*Enterobacter cloacae* AmpC, CTX-M-15, TEM-1, OXA-48, and KPC-2) for enzyme inhibition studies plus the potentiation of the carbapenem antibiotic meropenem (MEM) against a series of complementary clinical strains of bacteria (see Table 1). The enzyme inhibition is reported as IC_{50} values—however, this data must be interpreted with caution because of the irreversible nature of the reaction forming a covalent product, that is, it is not an equilibrium situation. The results from this type of assay are dependent on the assay conditions (in particular, the pre-incubation time of the enzyme-inhibitor mixture used in the experiment) but nevertheless provided useful data to rank the order of the

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Table 1. Enzyme Inhibition Data and Potentiation of MEM Tested on Various Carbapenemase-Producing Clinical Isolates (Indicated in Bold) in the Presence of the 4 μg/mL Inhibitor[a]

			IC_{50} values (μM)						MEM MIC (μg/mL)				
compound	*R*	volume (Å^3)	AmpC	CTX-M-15	TEM-1	OXA-48	KPC-2	OXA-23	*E. cloacae* (TEM-1, KPC-2)	*K. pneumoniae* (TEM-OSBL, CTX-M-14, OXA-48)	*K. pneumoniae* (TEM-1, SHV-11, KPC-3)	*K. pneumoniae* (SHV-11, OXA-181)	*A. baumannii* (OXA-23)
none									8	16	128	16	32
avibactam	$CONH_2$	35.2	0.008	0.001	0.005	0.252	0.008	>3.0	0.03	0.50	0.25	1	16
24	H	5.1	>3.0	1.200	0.511	1.337	>3.0	>3.0	2	4	64	8	32
15	CH2F	26.9	0.304	0.119	0.492	0.154	0.051	>3.0	0.125	1	2	1	32
16	CH_2Cl	36.0	0.026	0.084	0.135	0.443	0.028	>3.0	0.06	8	2	16	32
11	CHF_2	31.9	0.035	0.017	0.208	0.064	0.007	1.604	0.06	2	1	1	16
12	$CHCl_2$	51.2	0.052	0.178	0.403	0.178	0.004	1.684	0.06	16	2	16	32
7	CF_3	36.9	0.004	0.010	0.810	0.019	0.006	0.810	0.06	2	2	2	16
22	SCF_3	55.7	0.002	0.010	0.026	0.099	0.002	1.320	0.03	8	4	8	32
25	SO_2CF_3	72.8	<0.001	0.002	0.001	0.015	0.015	N.D.	0.06	8	8	16	32
WCK4234	CN	23.5	0.003	0.004	0.067	0.002	0.008	0.059	0.06	0.06	0.5	0.25	4
31	CF_2CH_3	50.0	0.313	0.200	0.267	0.169	0.043	>3.0	0.06	8	2	16	32
10	CF_2-thiaz.	90.9	0.001	0.019	0.216	1.107	0.003	>3.0	0.125	16	8	16	32
17	CH_2OCF_3	64.3	0.023	0.011	0.117	0.015	0.013	>3.0	0.25	8	32	16	32
23	Cl	19.1	0.004	0.008	0.001	0.269	0.031	N.D	0.06	4	64	4	32
21	F	10.0	0.010	0.002	0.001	0.175	0.019	0.050	0.03	0.25	1	1	2

[a](N.D = not done).

potency of compounds. The objective was to achieve a broad-spectrum SBLI but with particular emphasis on the class D carbapenemase OXA-48 in the panel because of the prevalence of OXA-type variants as resistant elements in *A. baumannii*.[27,28]

The whole cell potentiation assay involved measuring the MEM MIC against a panel of resistant clinical strains in the presence of 4 μg/mL of the inhibitor, with the rationale that functionally active inhibitors would significantly lower the MEM MIC against these resistant strains (Table 1). A note of caution regarding this assay is that clinical strains often harbor more than one resistance mechanism, for example, multiple enzymes or porin/efflux changes, making interpretation difficult.

Structure–Activity Relationship of Non-amidic Substituents. The H analogue **24** was used as a baseline. As anticipated, this compound was a significantly worse enzyme inhibitor than avibactam, across this panel (Table 1), but remarkably it did show very modest MEM potentiation against some of the clinical strains. The poor enzyme inhibition may be due to a lower interaction with the target because the amide substituent is absent, or to a reduced rate of the reaction with the catalytic serine in the active site because of the absence of the electron-withdrawing substituent. We decided to explore novel "non-amidic" substituents at this position because the −CONH− amido functionality itself does not seem to engage in significant direct interactions with OXA enzymes, whereas, in contrast, the various larger side chains on the amide derivatives (as in nacubactam and zidebactam) do make significant enzyme interactions. An early first compound, trifluoromethyl (CF_3) analogue **7**, showed good enzyme inhibition and potentiation of MEM against the panel of clinical strains. In particular, **7** is apparently a better inhibitor of OXA-48 than avibactam, with an IC_{50} of 19 nM. This CF_3 analogue, however, was not as active in the potentiation assays as avibactam or the cyano analogue WCK4234.[29,30] Nevertheless, encouraged by the activity of **7**, we made the mono- and difluoromethyl analogues **15** and **11**, which exhibited a broadly similar profile, particularly in the potentiation assays. However, as the monochloro- and dichloromethyl analogues **16** and **12** did not perform well in the potentiation assays, even though the enzyme data were encouraging, the corresponding trichloromethyl analogue was not synthesized.

We investigated the introduction of the electron-withdrawing trifluoromethylthio (SCF_3) group. Both the simple thioether **22** and the corresponding sulphone **25** exhibited very good enzyme inhibition. However, apart from the *E. cloacae* strain, the performance of these analogues in the potentiation assays was mediocre. Similarly, poor MEM potentiation was seen with the modestly potent inhibitor **17** bearing the trifluoromethoxymethyl (CH_2OCF_3) substituent. In an attempt to completely fill the small pocket around the avibactam carboxamide by displacing the water molecules, we made (1,3-thiazol-2-yl)-difluoromethyl analogue **10**, which modeled extremely well into OXA-48, once the two water molecules were removed (Figure 3). However, despite being a good inhibitor of other enzymes in the panel, this compound was an extremely poor inhibitor of OXA-48. Once again, the potentiation of this analogue was generally poor. Similarly, the

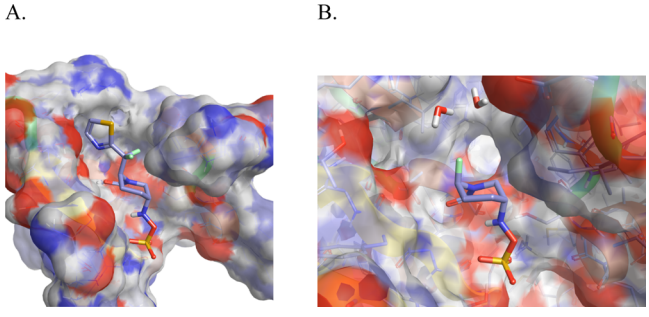

Figure 3. (A) (1,3-Thiazol-2-yl)-difluoromethyl analogue **10** modeled into OXA-48 (PDB entry 4S2K), waters removed prior to minimization and (B) fluoro analogue **21** modeled into OXA-48, waters left in place before minimization.

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replacement of a fluorine atom in trifluoromethyl with a methyl group gave methyldifluoromethyl (CF_2CH_3) analogue **31**, which did show good inhibition of OXA-48; however, once more, there was disappointing potentiation of MEM.

Re-evaluation of the Chemistry Strategy—Small is Beautiful and Stereoelectronic Control. Because of the disappointing potentiation results, the chemistry strategy was re-evaluated. The accumulation of antibiotics in the periplasm of Gram-negative bacteria is affected by two opposing processes—entry though water-filled porins[31] and efflux via transporter systems such as the AcrAB-TolC system present in *Escherichia coli*.[32] In a recent seminal study,[33] experimentally determined guidelines for bacterial accumulation were proposed, which invoke low globularity, low numbers of rotatable bonds, and the presence of a primary amine as being key factors, facilitating accumulation in the periplasm. In our series, it is apparent that the larger the size of the substituent, the worse is the potentiation of MEM, for similar levels of enzyme inhibition. This is unlikely to be because of reduced access by slower diffusion through the porins because other analogues with quite large substituents are able to penetrate effectively. More likely, this phenomenon is related to the enhanced recognition of the drug molecules by the efflux pumps. Even the weak enzyme inhibitor **24**, with a simple hydrogen atom substituent, achieves marginally improved potentiation against the *K. pneumoniae* strain expressing TEM, CTX, and OXA variants than does the sulfone analogue **25**, which is a very potent enzyme inhibitor. This structure–activity relationship (SAR) encouraged us to look at even smaller electron-withdrawing substituents than trifluoromethyl; consequently, we considered placing simple halogens at this position, even though, a priori, this did not seem to be a chemically attractive option because the RCON-C-halogen motif is notoriously reactive because of the facile elimination of the halide, generating an iminium species. Although there are examples in the literature of such functional group juxtapositioning, such as in chloroazetidinone[34] and fluorinated glycosyl donor[35] (Figure 4), in all cases these molecules are functioning as reactive reagents and not as pharmacological agents.

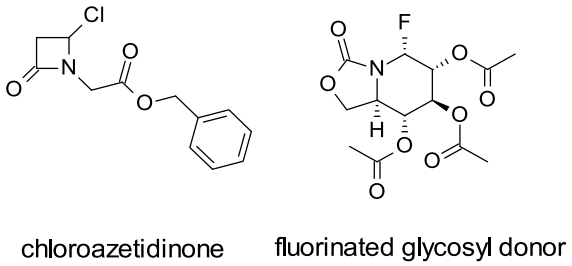

Figure 4. Examples of RCON-C-halogen.

However, the importance of the stereoelectronic control is sometimes underestimated in the organic synthesis.[36−38] If we consider the 3D molecular structure of the proposed halo analogues (Figure 5), it can be seen that the lone pair (shown in red) and the C-Hal bond are at approximately 60° to each other, not a suitable orientation for the orbital overlap required to eliminate halide and form the iminium species. In classical terms, the resulting iminium species contravenes Bredt's law, which states you cannot have a double bond at the bridgehead position of a small bicyclic system.[39]

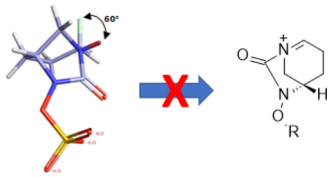

Figure 5. Stereoelectronic control prevents the elimination of the halide.

Encouraged by these considerations, we synthesized and evaluated both chloro analogue **23** and fluoro analogue **21**. These have substituent volumes of 19.1 and 10.0 Å^3, respectively, both smaller than the primary carboxamide of avibactam (35.2 Å^3) and obviously much smaller than the (1,3-thiazol-2-yl)-difluoromethyl substituent (90.9 Å^3). Not only were both analogues chemically stable but they were modestly potent enzyme inhibitors. However, there were huge differences in the potentiation between the two; whereas **23** did not perform well, **21** showed an excellent profile, even potentiating the effect of MEM against the difficult-to-kill *A. baumannii* strain (Table 1). Subsequent studies on OXA-23 showed that **21** was a very good inhibitor of OXA-23, as well as OXA-48 (Table 1). In contrast, avibactam had no activity against OXA-23 even though it was a reasonable inhibitor of OXA-48, offering an explanation for the lack of effectiveness of the MEM−avibactam combination against OXA-23 producing *A. baumannii* (Table 1). The overall profile of **21** in our primary screens resembled that of WCK4234[30] but on further analysis of the potentiation of MEM against a larger panel of clinical isolates **21** proved to be equipotent against KPC- and OXA-CRE while superior against OXA-CRAB (Table 2). In fact, the combination of MEM/**21** compares favorably to that of ceftazidime/avibactam, a drug marketed for the treatment of KPC-and OXA-CRE, and has the additional coverage of OXA-CRAB (Table 2).

Table 2. Potentiation of MEM MIC against KPC- and OXA-Producing CRE and OXA-Producing CRAB at the 4 μg/mL Concentration of SBLI[a]

	MIC (μg/mL)			
antibiotic (combination)	MIC_{50}	MIC_{90}	minimum	maximum
KPC-Positive CRE (n = 50)				
MEM	>32	>32	4	>32
MEM/**21**	0.06	1	≤0.03	16
MEM/WCK4234	0.06	1	≤0.03	16
CAZ−Avi	1	2	0.25	16
OXA-Positive CRE (n = 48)				
MEM	16	>32	2	>32
MEM/**21**	0.12	1	≤0.03	2
MEM/WCK4234	0.12	0.25	≤0.03	0.5
CAZ−Avi	0.12	2	0.25	4
OXA-Positive CRAB (n = 50)				
MEM	>32	>32	8	>32
MEM/**21**	4	8	0.25	>32
MEM/WCK4234	16	32	0.25	>32
CAZ−Avi	>32	>32	4	>32

[a]Ceftazidime−avibactam (CAZ−Avi) was used as a comparator.

As anticipated, in contrast to the intrinsic antibacterial activities of avibactam and zidebactam, the antibacterial activity of **21** was negligible (Table 3).

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Table 3. Intrinsic Antibacterial Activity (μg/mL) of Avibactam, Zidebactam, and 21 on Various Carbapenemase-Producing Clinical Isolates (Indicated in Bold)

	MIC (μg/mL)				
compound	*E. cloacae* (TEM-1, **KPC-2**)	*K. pneumoniae* (TEM-OSBL, CTX-M-14, **OXA-48**)	*K. pneumoniae* (TEM-1, SHV-11, **KPC-3**)	*K. pneumoniae* (SHV-11, **OXA-181**)	*A. baumannii* (**OXA-23**)
avibactam	16	16	16	32	>128
zidebactam	0.5	0.25	2	2	>128
21	128	64	128	128	>128

ADMET Properties of 21. The ADMET properties of **21** were investigated (Table 4). Compound **21** was stable in mouse, dog, and human plasma (79−90% remaining after 1 h), although less so in the rat plasma (55% remaining after 1 h) and was mostly present as a free drug (>93% unbound). Hepatocyte clearance and cytochrome P450 inhibition (time dependent) were low for all species, indicating high metabolic stability and low potential for drug−drug interactions. Compound **21** showed a low *in vitro* toxicity profile, with no observed cytotoxicity (HepG2 cell line), cardiotoxicity (inhibition of the hERG potassium ion channel), or genotoxicity (Ames test). Furthermore, **21** was well tolerated (up to 1000 mg/kg) following a single iv dose in rats.

Table 4. ADMET Properties of Compound 21

ADME	plasma stability, % remaining after 1 h (mouse/rat/dog/human)	90, 55, 85, 79
	hepatocyte Clint (μL/min/million cells) (mouse/rat/dog/human)	<3.5/<3.4/<3.0/<3.4
	PPB, % unbound (mouse/rat/dog/human)	>93% for all species
in vitro toxicity	CYP inhibition (TDI) IC_{50} (μM) 1A2, 2C9, 2C19, 2D6, 3A4	>50
	HepG2, CC_{50} (μM)	>100
	hERG, IC_{50} (μM) patch clamp	>100
	ames genotoxicity test	negative
single dose rat MTD	doses tested: 30, 100, 300, and 1000 mg/kg	tolerated up to 1000 mg/kg

Pharmacokinetics. The IV PK of **21** in mice compared favorably to that of avibactam (Figure 6 and Table 5), showing a longer half-life, greater exposure, slower clearance, and higher volume of distribution. Again, this is in accord with the physicochemical properties of the series as a whole. The PK is compatible to that of MEM, which is the crucial requirement for an IV drug to be co-dosed with MEM and supported the investigation of *in vivo* efficacy of **21** in combination with MEM.

Table 5. Mouse IV PK of 21 and Avibactam

parameter	avibactam	**21**
dose (mg/kg)	1.0	1.0
$T_{1/2}$ (h)	0.37	0.64
AUC (ng·h/mL)	279	412
Cl (mL/min/kg)	59	40
Vd (L/kg)	1.9	2.2

Efficacy of 21 in a Murine Thigh Infection Model. The ability of **21** to restore MEM efficacy was investigated in a 9 h neutropenic murine thigh infection model, with two *K. pneumoniae* clinical strains expressing either KPC-2 or OXA-48 SBLs. This model was specifically developed to demonstrate the efficacy of carbapenems, which have a very short half-life in rodents and need to be dosed frequently in order to maintain plasma levels above the MIC for the correct percent time of the dosing interval (40% T > MIC). A MEM dose of 300 mg/kg administered every 2 h (q2 h) was used in these studies. This dose results in a % T > MIC in mice similar to that of a 2 g dose administered every 8 h by a 3 h infusion in humans,[40] and this is the clinical MEM dose/dosing regimen expected to be used with this combination. The addition of 25, 50, and 100 mg/kg **21** to 300 mg/kg MEM-reduced bacterial burdens (colony forming units, CFU) in a dose-dependent manner to levels below that of the initial starting inoculum at the highest dose (Figure 7a,b). Compared to treatment with MEM alone, which was not efficacious, a statistically significant reduction in bacterial counts could be observed in all the groups treated with the combination, even those that received the lower dose. In fact, the combination demonstrated similar efficacy to

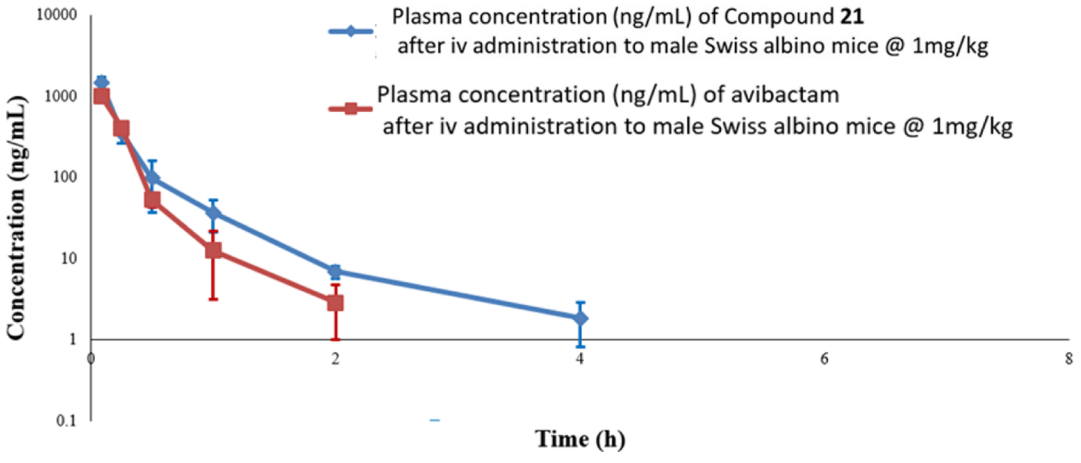

Figure 6. Mouse IV PK of **21** and avibactam.

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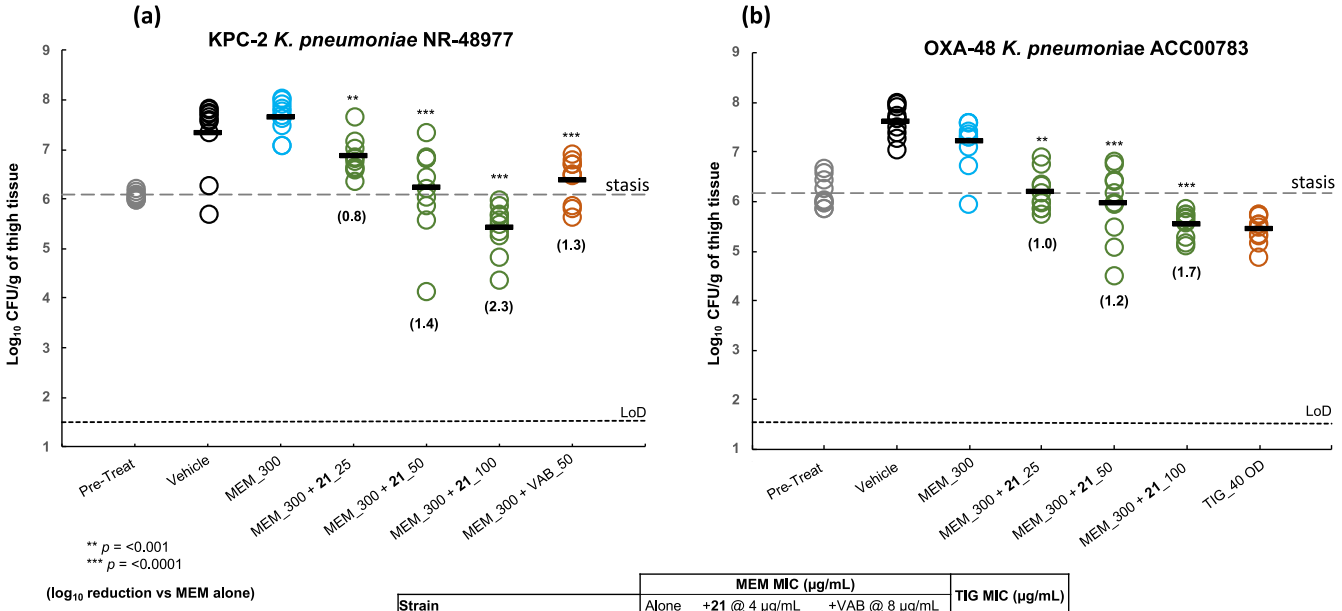

Strain	MEM MIC (μg/mL)			TIG MIC (μg/mL)
	Alone	+21 @ 4 μg/mL	+VAB @ 8 μg/mL	
K. pneumoniae NR-48977 (KPC-2)	64	1	2	NA
K. pneumoniae AC00783 (OXA-48)	32	0.25	NA	≤0.5

Figure 7. Efficacy of **21** in a murine thigh infection model. Neutropenic mice (five/group) were inoculated by IM administration into both thighs with suspensions of either (a) KPC-2 *K. pneumoniae* NR-48977 or (b) OXA-48 *K. pneumoniae* AC00783. MEM was dosed IV at 300 mg/kg alone or in combination with **21** at 25, 50, or 100 mg/kg at 1, 3, 5, and 7 h postinfection. MEM/VAB (dosed IV at 300/50 mg/kg at 1, 3, 5, and 7 h postinfection) was used as a positive control in (a) tigecycline (dosed IV at 40 mg/kg at 1 h postinfection) was used as a positive control in (b). Animals were euthanized at 9 h postinfection and thigh CFUs determined. Three-dimensional structure of the OXA-48 class D carbapenemase covalently bound to **21**.

MEM/VAB (Figure 7a) and tigecycline (Figure 7b), which were used as positive controls in these studies. These data provided the *in vivo* proof-of-concept needed to continue the progression of the compound through preclinical development.

A crystal structure of the OXA-48:**21** covalent complex was obtained at a maximum resolution of 1.85 Å (see Supporting Information for methods and data collection and refinement statistics). The asymmetric unit included a single OXA-48 dimer, in which **21** was found to be covalently bound to the catalytic serine in both subunits (Figure 8A). In addition, the Lys73 residue was found to be decarboxylated in both subunits, and a linear CO_2 molecule could be fitted in the extra electron density found in the active site of subunit A (Figure 8B), as previously observed in the OXA-48:avibactam complex.[41] The carboxylated Lys73 bears functional relevance, as it is important in both the acylation and deacylation steps of the catalytic mechanism of class D β-lactamases.[42−44] Furthermore, the substituent of the piperidine ring, consisting of a single F atom and despite being shorter than that of avibactam (where a carboxamide is found), could be clearly observed in the electron density map, supporting that the compound would not undergo significant chemical modifications after the opening of the five-membered carbamide-containing ring.

Stabilizing interactions were found between compound **21** and OXA-48 residues, essentially (1) between the oxygen atoms of the sulfate moiety with the side chains of Ser118 (SxV-conserved motif), Lys208 and Thr209 (KTG-conserved motif), and Arg250, the latter involved in a strong electrostatic interaction, and (2) the carbonyl oxygen atom of the covalent adduct interacting with the N backbone atom of residues Ser70 and Tyr211, forming the so-called oxyanion hole (Figure 8B). These interactions were very similar to those already observed in the avibactam complex (Figure 8C).[41] The F substituent was found in a hydrophobic pocket made of residues Val120, Leu158, and Trp105 but did not create any significant interaction with such residues (closest distance, 3.9 Å), only interacting with a water molecule (observed in subunit B only) (Figure 8B,C).

When compared to the native enzyme,[44] the binding of the inhibitor was associated with several modifications in the active site that were similar to that observed in the avibactam complex, that is, the decarboxylation of the Lys73, the rotation of the Ser118 side chain, the displacement of the Leu158 side chain, and minor shifts of the region between helices α3 and α5.

Overall, these data are in agreement with the predicted binding mode from earlier modeling, reflecting the conserved binding mode of strained DBO compounds, mostly differing (expect durlobactam[45]) by the nature of the piperidine side chain. As anticipated, the region of the active site around the fluorine atom of **21** may still contain two water molecules (observed in subunit B).

CONCLUSIONS

A rapid and focused medicinal chemistry campaign successfully delivered a new drug in the DBO space, which is clearly differentiated from previous analogues by its expanded microbiological spectrum to include the restoration of MEM antibacterial activity against OXA-producing *Acinetobacter* strains (CRAB). A reductionist approach led to the identification of a single fluorine atom as being the optimum substituent to achieve the desired properties, and while, superficially, the fluoro analogue might be expected to have stability issues, a consideration of the stereoelectronic aspects of the system mitigated against this possibility. The X-ray

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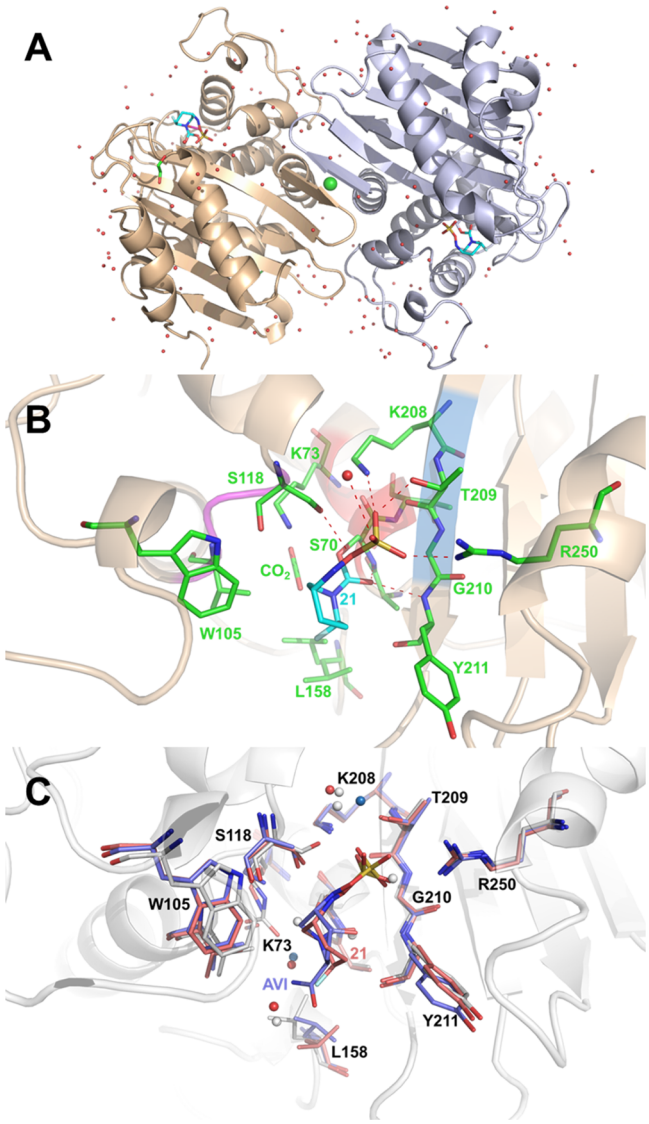

Figure 8. Crystal structure of OXA-48 inhibited by **21**. (A) Content of the asymmetric unit, including an OXA-48 dimer, a chloride anion (green sphere) at the interface between the subunits (shown in different colors) in which the catalytic Ser70 was covalently bound to compound **21** (cyan sticks), as well as water molecules (red spheres) and ethylene glycol (green sticks). (B) View of the active site of the enzyme (subunit A) showing the network of interactions between the OXA-48 residues (green sticks) and the inhibitor **21** (see text for details), the decarboxylated Lys73, and the presence of a linear CO_2 molecule (the conserved motifs Ser^{70}-x-x-Lys, Ser^{118}-x-Val and Lys^{208}-Thr/Ser−Gly are colored in red, magenta, and blue, respectively). (C) View of the active site of the OXA-48:**21** complex (subunit A, salmon; water molecules, red spheres) and its superimposition with the native OXA-48 (white, subunit B of PDB entry 3HBR) and the OXA-48:avibactam complex (blue, subunit C of PDB entry 4WMC), showing the impact of inhibitor binding on the position of active residues.

crystal structure of OXA-48 bound to **21**, revealing a similar binding and network of interaction with that of other DBO compounds, also supports the concept that the inhibitor does not undergo major chemical modifications upon ring opening. Compound **21**, designated as **ANT3310**, is effective against CREs and *A. baumannii* strains and is efficacious in mouse infection models. **ANT3310** is currently being progressed through preclinical development as a broad-spectrum DBO, with coverage of KPC- and OXA-CRE but with the added benefit of activity against OXA-CRAB. From a medicinal chemistry perspective, it is noteworthy that all the synthesized compounds from this project are described in this paper. From this very short campaign, we discovered **ANT3310**, a novel DBO, with clear differentiating advantages over other drugs in this chemical class and addressing the increasingly important global threat of antibiotic resistance.

EXPERIMENTAL SECTION

General Procedures. Reactions were performed under argon or nitrogen using dried glassware and solvents. Commercially available reagents and solvents were used as supplied or purified using standard protocols. Reactions were conducted at room temperature unless otherwise stated and monitored by standard thin-layer chromatography or liquid chromatography−mass spectrometry (LC−MS) techniques. Chromatography was performed with standard silica columns packed with silica gel (Merck silica gel 40−63 μm) and eluting with solvent combinations as described. The Dowex Na resin was obtained from Sigma-Aldrich. ^{1}H spectra were recorded using 500 MHz (Bruker), 400 MHz (Bruker), 400 MHz (Varian), and 300 MHz (Varian) instruments in the deuterated solvents as indicated. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane or alternatively using the residual solvent peak as an internal standard. Multiplicity is given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Coupling constants (J) are reported in Hertz (Hz). Preparative high-performance liquid chromatography (HPLC) conditions are individually reported as appropriate. All final compounds tested were >95% pure as determined by NMR and LC−MS. Modeling of potential targets into OXA-48 were performed with Flare from the Cresset suite of software and Discovery Studio 2019 from Biovia. Substituent volume calculations were undertaken with Marvin from ChemAxon.

*Sodium (2R,5R)-2-Fluoro-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**21**), (**ANT3310**). Ethyl (2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (**2**).* A suspension of commercially available ethyl (2*S*,5*R*)-5-[(benzyloxy)amino]-piperidine-2-carboxylate oxalate salt (15.0 g, 40.7 mmol) in tetrahydrofuran (THF) (150 mL) at 0 °C was treated with a solution of potassium hydrogen carbonate (16.3 g, 163 mmol) in water (150 mL). After 1 h, the mixture was extracted with ethyl acetate and the combined extracts washed with water then brine, dried (Na_2SO_4), and evaporated affording ethyl (2*S*,5*R*)-5-[(benzyloxy)amino]piperidine-2-carboxylate as brown oil (10.0 g, 88%).

A solution of ethyl (2*S*,5*R*)-5-[(benzyloxy)amino]piperidine-2-carboxylate (5.0 g, 18 mmol) in DCM (100 mL) at 0 °C was treated with *N*,*N*-diisopropylethylamine (DIPEA) (12.5 mL, 72 mmol) followed by the addition of a solution of triphosgene (2.6 g, 9 mmol) in DCM (10 mL). The mixture was stirred at ambient temperatures for 16 h, then saturated aqueous potassium bicarbonate solution (100 mL) was added. After 1 h, the phases were separated and the DCM phase washed with water then brine, dried (Na_2SO_4), and evaporated affording brown oil (5.0 g, 92%). LC−MS Anal. Calcd for $C_{16}H_{20}N_2O_4$, 304.1. Found [M + H] = 305.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.35 (m, 5H), 5.06 (d, J = 11.5 Hz, 1H), 4.90 ((d, J = 11.5 Hz, 1H), 4.25−4.22 (m, 2H), 4.09 (d, J = 6.5 Hz, 1H), 3.31 (d, J = 1.5 Hz, 1H), 3.05−3.00 (m, 1H), 2.93 (d, J = 12 Hz, 1H), 2.11−2.04 (m, 3H), 1.71−1.60 (m, 1H), 1.29 (t, J = 7.0 Hz, 3H).

*(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic Acid (**1**).* To a solution of ethyl (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (7.0 g, 23.0 mmol) in acetone/water (1:1, 120 mL) was added LiOH·H_2O (0.97 g, 23.0 mmol) at 0 °C. The resulting reaction mixture was stirred for 2 h. The mixture was diluted with water (50 mL) and washed with EtOAc (2 × 100 mL). The aqueous layer was acidified with 1N HCl to approximately pH3 and then extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with water, brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to obtain (2*S*,5*R*)-

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6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (5 g, 79%) as a white solid, which was used in the next step without purification.

LC−MS Anal. Calcd for $C_{14}H_{16}N_2O_4$, 276.1. Found [M + H] = 277.2. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 13.01 (br s, 1H), 7.45−7.32 (m, 5H), 4.92 (AB q, J = 11 Hz, 2H), 3.86 (d, J = 7 Hz, 1H), 3.62 (s, 1H), 2.95−2.89 (m, 2H), 1.99−1.95 (m, 1H), 1.88−1.79 (m, 2H), 1.70−1.66 (m, 1H).

*(2R,5R)-6-(Benzyloxy)-2-fluoro-1,6-diazabicyclo[3.2.1]octan-7-one (**26**) (A) and (2S,5R)-6-(Benzyloxy)-2-fluoro-1,6-diazabicyclo-[3.2.1]octan-7-one (B).* To a stirred solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1, 6-diazabicyclo [3.2.1] octane-2-carboxylic acid (2.5 g, 9.04 mmol) in acetone: water (4:1, 100 mL) was added Selectfluor (6.4 g, 18.0 mmol) and $AgNO_3$ (153 mg, 0.90 mmol) at room temperature. The reaction mixture was heated at 50 °C for 3 h then evaporated. The resulting residue was extracted with EtOAc (100 mL), filtered through a Celite pad, and washing the pad with further EtOAc (10 mL). The filtrate was washed with the $NaHCO_3$ solution (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to obtain oil. This was chromatographed on silica gel eluting with 10% EtOAc in hexane as the eluent affording the desired isomer (2*R*,5*R*)-6-(benzyloxy)-2-fluoro-1,6-diazabicyclo[3.2.1]octan-7-one (A) (550 mg, 24%) as pale yellow viscous oil. Further elution with 50−60% EtOAc in hexane afforded the undesired isomer (2*S*,5*R*)-6-(benzyloxy)-2-fluoro-1,6-diazabicyclo[3.2.1]octan-7-one (B) (300 mg, 13%) as a pale yellow solid.

(2R,5R)-6-(Benzyloxy)-2-fluoro-1,6-diazabicyclo[3.2.1]octan-7-one (A). LC−MS Anal. Calcd for $C_{13}H_{15}FN_2O_2$, 250.1. Found [M + H] = 251.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.34 (m, 5H), 5.45 (dd, J = 4.5, 46 Hz, 1H), 5.04 (d, J = 11.5 Hz, 1H), 4.89 (d, J = 11.5 Hz, 1H), 3.36 (dd, J = 2.5, 5.5 Hz, 1H), 3.25 (d, J = 12 Hz, 1H), 3.02−2.98 (m, 1H), 2.13−1.98 (m, 2H), 1.92−1.86 (m, 1H), 1.73−1.66 (m, 1H). ^{19}F NMR (470 MHz, $CDCl_3$): δ ppm −155.49−155.69 (m).

(2S,5R)-6-(Benzyloxy)-2-fluoro-1,6-diazabicyclo[3.2.1]octan-7-one (B). LC−MS Anal. Calcd for $C_{13}H_{15}FN_2O_2$, 250.1. Found [M + H] = 251.1. ^{1}H NMR (500 MHz, CF_3COOD): δ ppm 7.49−7.48 (m, 5H), 5.95 (ddd, J = 5, 10.5, 46 Hz, 1H, CHF), 5.19 (d, J = 11.5 Hz, 1H), 5.12 (d, J = 11.5 Hz, 1H), 4.10−4.04 (m, 1H), 3.71 (d, J = 3 Hz, 1H), 3.60 (d, J = 11.5 Hz, 1H), 2.69−2.64 (m, 1H), 2.45−2.42 (m, 1H), 2.22−2.15 (m, 1H), 2.03−1.96 (m, 1H). ^{19}F NMR (376 MHz, $CDCl_3$): δ ppm −140.10−140.25 (m).

Please see the Supporting Information for the NMR experiments to distinguish and characterize these two isomers.

(2R,5R)-2-Fluoro-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one Procedure (a). To a solution of (2*R*,5*R*)-6-(benzyloxy)-2-fluoro-1,6-diazabicyclo[3.2.1]octan-7-one (300 mg, 1.19 mmol) in methanol (30 mL) was added 10% palladium on charcoal (300 mg). The reaction mixture was hydrogenated at room temperature for 1 h using hydrogen balloon pressure. The reaction mixture was filtered through a Celite pad washing with MeOH (10 mL). The filtrate was evaporated to give (2*R*,5*R*)-2-fluoro-6-hydroxy-1,6-diazabicyclo-[3.2.1]octan-7-one (180 mg, 94%) as an off-white solid, which was used in the next step without purification. LC−MS Anal. Calcd for $C_6H_9FN_2O_2$, 160.1. Found [M + H] = 161.0.

Tetrabutylammonium (2R,5R)-2-Fluoro-7-oxo-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate Procedure (b). To a solution of (2*R*,5*R*)-2-fluoro-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (180 mg, 1.12 mmol) in DCM (20 mL) was added Et_3N (1.5 mL, 11.2 mmol), followed by SO_3.Pyr (1.07 g, 6.74 mmol) at 0 °C and stirred for 4 h. Then, a solution of tetra(*n*-butyl)ammonium acetate (TBAA) (2.7 g, 8.99 mmol) in water (20 mL) was added and stirred at room temperature for 2 h. The reaction mixture was diluted with DCM (50 mL) and the organic layer was separated. The organic layer was washed with water (5 × 25 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting residue was chromatographed on silica eluting with 0−100% EtOAc in hexane followed by 5% MeOH in DCM affording colorless oil (220 mg, 40% over 2 steps). LC−MS Anal. Calcd for $C_6H_8FN_2O_5S$, 239.0. Found [M] = 239.0.

*Sodium (2R,5R)-2-Fluoro-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**21**) Procedure (c).* A stirred solution tetrabutylammonium (2*R*,5*R*)-2-fluoro-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (220 mg, 0.45 mmol) in water (10 mL) was treated with a Dowex Na resin (1 g). After 1 h, the mixture was filtered through a pad of the Dowex Na resin, washing with H_2O (5 mL). The combined filtrate was again treated with the Dowex Na resin (1 g) for 1 h, filtered through a bed of the Dowex Na resin, and washing with H_2O (5 mL). This process was repeated for another 3 times. The combined filtrates were lyophilized to obtain the title compound as a white solid (90 mg, 75%). HRMS Anal. Calcd for $C_6H_8O_5N_2FS$ [M], 239.0132. Found 239.0143. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 5.35 (ddd, 1H, J = 46 Hz, 5 Hz, 2.5 Hz, CHF), 4.08−4.06 (m, 1H), 3.26−3.24 (m, 1H), 3.06−3.04 (m, 1H), 1.99−1.68 (m, 4H). Anal. Calcd for $C_6H_8O_5N_2FSNa$, C, 27.48; H, 3.08; N, 10.68; Na, 8.77. Found: C, 28.32; H, 3.22; N, 10.52; Na, 8.49.

*Sodium {7-Oxo-1,6-diazabicyclo[3.2.1]octan-6-yl}-oxidanesulfonate (**24**). (5R)-6-(Benzyloxy)-1,6-diazabicyclo[3.2.1]-octan-7-one.* To a solution of (2*R*,5*R*) charcol-6-(benzyloxy)-2-chloro-1,6-diazabicyclo[3.2.1]octan-7-one **28** (500 mg, 1.87 mmol) in toluene (15 mL) was added AIBN (30.8 mg, 0.18 mmol) and Bu_3SnH (1.09 g, 3.75 mmol). The reaction mixture was heated at 120 °C for 2 h. The reaction mixture was evaporated. The resulting oil was chromatographed on silica eluting with 50% EtOAc in hexane as the eluent, affording (5*R*)-6-(benzyloxy)-1,6-diazabicyclo[3.2.1]octan-7-one (350 mg, 81%) as an off-white solid. LC−MS Anal. Calcd for $C_{13}H_{16}N_2O_2$, 232.1. Found [M + H] = 233.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43 (d, J = 6.5 Hz, 2H), 7.39−7.34 (m, 3H), 5.06 (d, J = 11.5 Hz, 1H), 4.89 (d, J = 11.5 Hz, 1H), 3.42−3.38 (m, 1H), 3.29 (s, 1H), 3.18−3.16 (m, 1H), 2.97−2.91 (m, 1H), 2.77−2.73 (m, 1H), 2.12−2.00 (m, 2H), 1.62−1.56 (m, 2H).

(5R)-6-Hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one. According to Procedure (a): (5*R*)-6-(benzyloxy)-1,6-diazabicyclo[3.2.1]octan-7-one (350 mg, 1.50 mmol) was converted the desired compound (190 mg, 90%) as an off-white solid, which was used immediately next step without purification. LC−MS Anal. Calcd for $C_6H_{10}N_2O_2$, 142.1. Found [M + H] = 143.2.

Tetrabutylammonium (5R)-7-Oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b) (5*R*)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (190 mg, 1.33 mmol) was converted to the desired compound as colorless oil (100 mg, 16% over 2 steps). LC−MS Anal. Calcd for $C_6H_9N_2O_5S$, 221.0. Found [M] = 220.9. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 4.36 (s, 1H), 3.49−3.47 (m, 1H), 3.35−3.26 (m, 9H), 3.00−2.96 (1H, m), 2.84 (d, J = 11.5 Hz, 1H), 2.32−2.28 (m, 1H), 1.97−1.88 (m, 1H), 1.70−1.62 (m, 9H), 1.48−1.41 (m, 9H), 1.01−0.99 (m, 12H).

Sodium (5R)-7-Oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (c): (5*R*)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl sulfate (100 mg, 0.21 mmol) was converted to a white solid, which was then purified by preparative HPLC [column: KROMOSIL-C18 (150 × 25), 10 μ; mobile phase: H_2O: acetonitrile; gradient: (T % B): −0/1, 3/1, 3.1/98, 8/98, 8.1/1, 10/1; flow : 25 mL/min], and the pure fractions were lyophilized to afford the title compound (15.0 mg, 28%) as an off-white solid. HRMS Anal. Calcd for $C_6H_9N_2O_5S$ [M], 221.0227. Found 221.0235. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 3.97−3.96 (m, 1H), 3.10−3.05 (m, 2H), 2.98−2.92 (m, 2H), 1.93−1.92 (m, 1H), 1.72−1.65 (m, 2H), 1.50−1.48 (m, 1H).

*Sodium (2S,5R)-7-Oxo-2-(trifluoromethyl)-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate (**7**).* This was prepared using essentially the same methodology as reported for avibactam (Ball, M. *et al. Organic Process Research and Development,* **2016,** *20,* 1799.) except starting from commercially-available (5*S*)-5-(trifluoromethyl)-2-pyrrolidinone.

tert-Butyl (5S)-2-Oxo-5-(trifluoromethyl)pyrrolidine-1-carboxylate. A solution of (5*S*)-5-(trifluoromethyl)-2-pyrrolidinone (5 g, 32.7 mmol) in DCM (60 mL) at 0 °C was treated with Et_3N (5.5 mL, 39.2 mmol) and 4-dimethylaminopyridine (DMAP) (0.4 g, 0.1 mmol). Then, a solution of Boc_2O (8.6 g, 39.2 mmol) in DCM (20 mL) was added dropwise over 10 min. After 0.5 h, the reaction

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mixture was partitioned between DCM and 10% aqueous citric acid. The organic extract was washed with water, dried (Na_2SO_4), and evaporated affording oil (9.4 g), which was chromatographed on silica eluting with 0−25% EtOAc in toluene affording *tert*-butyl (*S*)-2-oxo-5-(trifluoromethyl)pyrrolidine-1-carboxylate as oil (7.9 g, 96%). LC−MS Anal. Calcd for $C_{10}H_{14}F_3NO_3$, 253.1. Found [M + Na] = 276.

(3R,6S)-N-(Benzyloxy)-6-(trifluoromethyl)piperidin-3-amine. For a detailed explanation of this reaction sequence see Ball, M. *et al. Organic Process Research and Development,* **2016,** *20,* 1799. These steps, optimized for the manufacturing route of avibactam, were carried out in a one-pot manner without full isolation/characterization.

Dimethyl sulfoxide (DMSO) (10 mL) was added to a mixture of trimethylsulfoxonium iodide (1.6 g, 7.3 mmol) and potassium *t*-butoxide (0.72 g, 6.4 mmol) in THF (7 mL). The reaction mixture was stirred at room temperature for 1 h, then cooled to −12 °C (internal temperature). A solution of *tert*-butyl (5*S*)-2-oxo-5-(trifluoromethyl)pyrrolidine-1-carboxylate (1.5 g, 5.8 mmol) in THF (4 mL) was added dropwise over 5 min and the mixture stirred at −12 °C for 1 h. The reaction mixture was treated with 20% aqueous ammonium chloride (13 mL), and the stirred mixture was allowed to warm to room temperature and then extracted twice with EtOAc. The combined extracts were washed with 10% aqueous NaCl solution and then concentrated to approximately a 20 mL solution which was used directly in the next stage.

The above EtOAc solution (20 mL) was treated with O-benzylhydroxylamine hydrochloride, and the mixture heated at 60 °C for 2.75 h then allowed to cool to room temperature before washing with a 10% aqueous NaCl solution. This solution was reduced in volume to approximately 10 mL and used directly in the next step.

The above EtOAc solution (10 mL) was treated with methanesulfonic acid (1.1 mL, 1.7 g, 17.4 mmol), and the mixture heated at 45 °C for 1 h then allowed to cool to room temperature. This was added to a solution of potassium bicarbonate (2.9 g, 29 mmol) in water (10 mL) and stirred at 45 °C for 3 h. After cooling, the phases were separated and the EtOAc phase was washed with 10% aqueous NaCl solution. The EtOAc solution was used as such in the next stage.

The above EtOAc solution was cooled to −15 °C and concentrated H_2SO_4 (0.6 g, 0.3 mL, 6 mmol) was added. Then, sodium triacetoxyborohydride (0.5 g, 2.4 mmol) was added portionwise, allowing the temperature to rise from −15 to −5 °C for over 1 h. Water was added followed by concentrated aqueous ammonia (1 mL). The mixture was extracted with EtOAc, and the organic extract washed with brine, dried, and evaporated, affording oil, which was chromatographed on silica eluting with 15−40% EtOAc in DCM affording (3*R*,6*S*)-*N*-(benzyloxy)-6-(trifluoromethyl)piperidin-3-amine as oil (184 mg, 12% over 4 stages). LC−MS Anal. Calcd for $C_{13}H_{17}F_3N_2O$, 274.1. Found [M + H] = 275.

(2S,5R)-6-(Benzyloxy)-2-(trifluoromethyl)-1,6-diazabicyclo-[3.2.1]octan-7-one. A mixture of (3*R*,6*S*)-*N*-(benzyloxy)-6-(trifluoromethyl)piperidin-3-amine (0.18 g, 0.67 mmol) and potassium carbonate (0.53 g, 3.82 mmol) in DCM (20 mL) was treated with triphosgene (0.2 g, 0.67 mmol) at −10 °C. After 0.5 h, DMAP (3 mg, 0.03 mmol) was added. The reaction mixture was stirred overnight and then washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica eluting with an EtOAc−DCM gradient affording a colorless solid (0.16 g, 81%). LC−MS Anal. Calcd for $C_{14}H_{15}F_3N_2O_2$, 300.1. Found [M + H] = 301.4.

Sodium (2S,5R)-7-Oxo-2-(trifluoromethyl)-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate. A solution of (2*S*,5*R*)-6-(benzyloxy)-2-(trifluoromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (163 mg, 0.54 mmol) in isopropyl alcohol (IPA) (5 mL) was treated with a sulfur trioxide trimethylamine complex (85 mg, 0.61 mmol), Et_3N (11 mg, 0.11 mmol), 10% palladium on charcoal (10 mg), and water (0.6 mL). The mixture was hydrogenated under balloon pressure for 4.5 h and then more sulfur trioxide trimethylamine complex (82 mg, 0.3 mmol) was added. The mixture was stirred under nitrogen for 2 h, then filtered, and concentrated to approximately 1.5 mL. This was diluted with water and treated with a saturated aqueous sodium bicarbonate solution (3 mL). The mixture was loaded onto a reverse phase C18 cartridge (10 g size) and eluted with 0−40% ACN in water. Evaporation of product-containing fractions gave a white solid that was redissolved in water and rechromatographed using the same chromatography conditions. The evaporation of product-containing fractions gave a white solid that was redissolved in water and rechromatographed using similar chromatography conditions except that THF was used in place of ACN. Evaporation gave the title compound as a white solid (86 mg, 51%). HRMS Anal. Calcd for $C_7H_8F_3N_2O_5S$ [M], 289.0101. Found 289.0115. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.09−4.06 (m, 1H), 3.89−3.78 (m, 1H), 3.24 (d, *J* = 12.0 Hz, 1H), 3.13−3.08 (m, 1H), 1.92−1.74 (m, 4H). ^{19}F NMR (376.4 MHz, DMSO-d_6): δ ppm −74.7.

*Sodium (2S,5R)-2-(Fluoromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl Sulfate (**15**). (2S,5R)-6-(Benzyloxy)-2-(hydroxymethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (**3**).* A solution of ethyl (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (5.0 g, 16.4 mmol) in THF (75 mL) and EtOH (75 mL) was treated at 0 °C with a solution of lithium borohydride in THF (4 M; 24 mL, 96 mmol). After 1 h at room temperature, the mixture was re-cooled to 0 °C, and a further portion of a solution of lithium borohydride in THF (4 M; 12 mL, 48 mmol) was added. After a further 16 h, the mixture was treated with saturated aqueous monopotassium phosphate (200 mL), and the mixture extracted several times with DCM. The combined DCM extracts were washed with water then brine, dried (Na_2SO_4), and evaporated affording brown oil (5 g). This was chromatographed on silica eluting with 0−100% EtOAc in hexane affording colorless oil (2.2 g, 47%). LC−MS Anal. Calcd for $C_{14}H_{18}N_2O_3$, 262.1. Found [M + H] = 263.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.43−7.66 (m, 5H), 5.05 (d, *J* = 11.6 Hz, 1H), 4.90 (d, *J* = 11.6 Hz, 1H), 3.71−3.68 (m, 1H), 3.60−3.57 (m, 2H), 3.33 (s, 1H), 3.01−2.89 (m, 2H), 2.04−1.93 (m, 3H), 1.39−1.35 (m, 1H).

((2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)-methyl Methanesulfonate. To a solution of (2*S*,5*R*)-6-(benzyloxy)-2-(hydroxymethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (3 g, 11.4 mmol) in DCM (40 mL) was added Et_3N (4.8 mL, 34.3 mmol) at 0 °C followed by DMAP (140 mg, 1.14 mmol). Then, methanesulfonyl chloride (1.3 mL, 17.1 mmol) was added at 0 °C and the reaction mixture was stirred for 2 h. The reaction mixture was diluted with DCM (40 mL), washed with 1 N HCl (40 mL), water (40 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to obtain ((2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)methyl methane sulfonate as oil (3.2 g) which was taken through to the next step without further purification. LC−MS Anal. Calcd for $C_{15}H_{20}N_2O_5S$, 340.1. Found [M + H] = 341.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.35 (m, 5H), 5.04 (d, *J* = 11.5 Hz, 1H), 4.89 (d, *J* = 11.5 Hz, 1H), 4.40 (dd, *J* = 7.5, 11 Hz, 1H), 4.24 (dd, *J* = 5.5, 11 Hz, 1H), 3.72−3.64 (m, 1H), 3.34 (s, 1H), 3.09 (s, 3H), 3.00−2.99 (m, 2H), 2.03−1.98 (m, 2H), 1.59−1.56 (m, 2H).

(2S,5R)-6-(Benzyloxy)-2-(fluoromethyl)-1,6-diazabicyclo[3.2.1]-octan-7-one. To a solution of ((2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)methyl methane sulfonate (3.2 g, 9.4 mmol) in ACN (20 mL) was added dropwise tetrabutyl ammonium fluoride (1.0 M in THF, 8 mL, 15.9 mmol). The reaction mixture was heated at 70 °C for 10 h and then concentrated under reduced pressure, and the residue was dissolved in DCM (50 mL) and washed with water (2 × 40 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by preparative HPLC [column: ATLANTIS-T3 (250 × 20), 5 μ; mobile phase: (A) 10 mM ammonium bicarbonate in H_2O (B) acetonitrile; flow rate: 20 mL/min, gradient (*T* % B): 0/40, 8/55, 10/55, 10.1/98, 12/98, 12.1/40, 15/40] the pure fractions were combined and lyophilized to obtain (2*S*,5*R*)-6-(benzyloxy)-2-(fluoromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (700 mg, 28%) as an off-white solid. LC−MS Anal. Calcd for $C_{14}H_{17}FN_2O_2$, 264.1. Found [M + H] = 265.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.44−7.35 (m, 5H), 5.05 (d, *J* = 11.5 Hz, 1H), 4.90 (d, *J* = 11.5 Hz, 1H),

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4.72−4.55 (m, 2H), 3.62−3.50 (m, 1H), 3.33−3.16 (m, 1H), 3.17 (d, J = 11.5 Hz, 1H), 2.98 (d, J = 11.5 Hz, 1H), 2.08−2.03 (m, 2H), 1.73−1.60 (m, 2H).

(2S,5R)-2-(Fluoromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]-octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-(fluoromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (500 mg, 1.89 mmol) was converted into the desired compound as an off-white solid (329 mg, 100% yield), which was used immediately in the next step without purification. LC−MS Anal. Calcd for $C_7H_{11}FN_2O_2$, 174.1. Found [M + H] = 175.1.

Tetrabutylammonium (2S,5R)-2-(Fluoromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*S*,5*R*)-2-(fluoromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (340 mg, 1.95 mmol) was converted into the desired compound as colorless oil (201 mg, 21% over 2 steps). LC−MS Anal. Calcd for $C_7H_{10}FN_2O_5S$, 253.0. Found [M] = 253.0. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 4.70−4.57 (m, 2H), 4.36−4.35 (m, 1H), 3.60−3.42 (m, 1H), 3.33−3.20 (m, 9H), 3.19−3.17 (m, 1H), 2.25−2.21 (m, 1H), 1.99−1.97 (m, 1H), 1.75−1.63 (m, 10H), 1.45−1.34 (m, 8H), 1.00 (t, J = 7.5 Hz, 12H).

Sodium (2S,5R)-2-(Fluoromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl Sulfate. According to procedure (c): (2*S*,5*R*)-2-(fluoromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (200 mg, 0.40 mmol) was converted to the title compound as a white solid (64 mg, 57%). HRMS Anal. Calcd for $C_7H_{10}FN_2O_5S$ [M], 253.0289. Found 253.0301. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 4.70−4.46 (m, 2H), 3.99−3.97 (m, 1H), 3.44−3.35 (m, 1H), 3.18 (d, J = 11.5 Hz, 1H), 2.94−2.91 (m, 1H), 1.84−1.80 (m, 1H), 1.79−1.69 (m, 2H), 1.51−1.48 (m, 1H).

*Sodium (2S,5R)-7-Oxo-2-(difluoromethyl)-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate (**11**). (2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbaldehyde (**4**).* A solution of (2*S*,5*R*)-6-(benzyloxy)-2-(hydroxymethyl)-1,6-diazabicyclo[3.2.1]-octan-7-one (1.5 g, 5.7 mmol) in DCM (50 mL) was treated at 0 °C with trichloroisocyanuric acid (1.9 g, 8.6 mmol) and TEMPO (90 mg, 0.6 mmol). The mixture was stirred at 0 °C for 2 h, then filtered through Celite, and washed with DCM. The combined DCM filtrates were washed with saturated aqueous sodium bicarbonate solution, and brine, dried (Na_2SO_4), and evaporated to afford the known aldehyde (**4**) (WO2015/136474) as oil (1.5 g, 100%), which was used directly in the next step. LC−MS Anal. Calcd for $C_{14}H_{16}N_2O_3$, 260.1. Found [M + H] = 261.4. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 9.73 (s, 1H), 7.44−7.36 (m, 5H), 5.07 (d, J = 11.5 Hz, 1H), 4.91 (d, J = 11.5 Hz, 1H), 3.89 (d, J = 8.0 Hz, 1H), 3.27 (s, 1H), 3.14−3.12 (m, 1H), 2.56 (d, J = 12.0 Hz, 1H), 2.19−2.15 (m, 1H), 2.04−1.92 (m, 3H).

(2S,5R)-6-(Benzyloxy)-2-(difluoromethyl)-1,6-diazabicyclo[3.2.1]-octan-7-one. A solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbaldehyde (1.5 g, 5.7 mmol) in DCM (30 mL) at 0 °C was treated with DAST (1.5 mL, 12 mmol). The mixture was stirred for 4 h, then the solvent was removed by purging with nitrogen. The residue was dissolved in EtOAc and added to ice-cold water. The organic phase was separated, washed with saturated aqueous sodium bicarbonate solution and brine, dried (Na_2SO_4), and evaporated affording oil. This was chromatographed on silica eluting with 20% EtOAc in hexane affording yellow oil (0.7 g, 44% over 2 steps). LC−MS Anal. Calcd for $C_{14}H_{16}F_2N_2O_2$, 282.1. Found [M + H] = 283.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.35 (m, 5H), 5.93 (td, J = 2, 55.5 Hz, 1H), 5.04 (d, J = 11.5 Hz, 1H), 4.89 (d, J = 11.5 Hz, 1H), 3.64−3.52 (m, 1H), 3.34 (t, J = 3.0 Hz, 1H), 3.12 (d, J = 12.0 Hz, 1H), 2.98 (d, J = 11.5 Hz, 1H), 2.07−2.03 (m, 1H), 1.94−1.89 (m, 2H), 1.68−1.65 (m, 1H).

(2S,5R)-2-(Difluoromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]-octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-(difluoromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (0.60 g, 2.1 mmol) was converted to the desired compound as a white solid (0.4 g, 100%), which was used directly in the next step. LC−MS Anal. Calcd for $C_7H_{10}F_2N_2O_2$, 192.1. Found [M + H] = 193.1. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.73 (s, 1H), 6.24 (td, J = 4.4, 55.5 Hz, 1H), 3.65−3.63 (m, 1H), 3.33−3.28 (m, 1H), 3.17−3.14 (m, 1H), 2.99 (d, J = 12.0 Hz, 1H), 1.94−1.69 (m, 4H).

Tetrabutylammonium (2S,5R)-2-(Difluoromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*S*,5*R*)-2-(difluoromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (0.40 g, 2.1 mmol) was converted to the desired compound as colorless oil (0.45 g, 42% over 2 steps). LC−MS Anal. Calcd for $C_7H_9F_2N_2O_5S^-$, 271.0. Found [M] = 271.4. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 6.24 (td, J = 4.4, 55.5 Hz, 1H), 4.03−4.02 (m, 1H), 3.36−3.30 (m, 1H), 3.19−3.14 (m, 9H), 3.00 (d, J = 10.8 Hz, 1H), 1.88−1.83 (m, 1H), 1.77−1.71 (m, 3H), 1.60−1.52 (m, 8H), 1.35−1.28 (m, 8 H), 0.98−0.87 (m, 12H).

Sodium (2S,5R)-7-Oxo-2-(difluoromethyl)-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate. According to procedure (c): tetrabutylammonnium (2*S*,5*R*)-2-(difluoromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl sulfate (0.45 g, 0.88 mmol) was converted to the title compound as a white solid (202 mg, 78%). HRMS Anal. Calcd for $C_7H_9F_2N_2O_5S$ [M], 271.0195. Found 271.0208. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 6.24 (t, J = 4.4 Hz, 1H), 4.06 (m, 1H), 3.40 (m, 1H), 3.20 (m, 1H), 3.05 (m, 1H), 1.95−1.75 (m, 4H). ^{19}F NMR (470.59 MHz, DMSO-d_6): δ ppm −122.87 (ddd, J = 13, 55, 283 Hz, 1F), −127.36 (ddd, J = 15, 55, 283 Hz, 1F).

*Sodium (2S,5R)-2-(Chloromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl Sulfate (**16**). (2S,5R)-6-(Benzyloxy)-2-(chloromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one.* To a solution of ((2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)methyl methane sulfonate (2.6 g, 7.63 mmol) in ACN (30 mL) was added tetrabutyl ammonium chloride (4.2 g, 15.3 mmol). The resulting reaction mixture was heated at 70 °C for 16 h. The reaction mixture was concentrated under reduced pressure, then the resulting residue was dissolved in DCM (50 mL), washed with water (2 × 40 mL) and brine (20 mL), and dried (Na_2SO_4). The filtrate was evaporated to obtain oil, which was purified by chromatography on silica eluting with 0−50% EtOAc in hexane as the eluent to obtain the desired compound as an off-white solid (1.3 g, 59%). LC−MS Anal. Calcd for $C_{14}H_{17}ClN_2O_2$, 280.1. Found [M + H] = 281.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.42−7.34 (m, 5H), 5.02 (d, J = 11.5 Hz, 1H), 4.90 (d, J = 11.5 Hz, 1H), 3.70−3.57 (m, 3H), 3.32 (d, J = 2.5 Hz, 1H), 2.97−2.90 (m, 2H), 2.05−2.01 (m, 2H), 1.69−1.57 (m, 2H).

(2S,5R)-2-(Chloromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]-octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-(chloromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (500 mg, 1.78 mmol) was converted to the desired compound as a white solid (330 mg), which was used directly in the next step. LC−MS Anal. Calcd for $C_7H_{11}ClN_2O_2$, 190.1. Found [M + H] = 191.1.

Tetrabutylammonium (2S,5R)-2-(Chloromethyl)-7-oxo-1,6 diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*S*,5*R*)-2-(chloromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (390 mg, 2.05 mmol) was converted to the desired compound as colorless oil (110 mg, 10% over 2 steps). LC−MS Anal. Calcd for $C_7H_{10}ClN_2O_5S^-$, 269.0. Found [M] = 269.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 4.37−4.35 (m, 1H), 3.74−3.71 (m, 1H), 3.64−3.58 (m, 2H), 3.33−3.24 (m, 9H), 2.95 (d, J = 12 Hz, 1H), 2.20−2.15 (m, 1H), 2.00−1.91 (m, 1H), 1.71−1.63 (m, 10H), 1.48−1.41 (m, 8H), 1.00 (t, J = 7.5 Hz, 12H).

Sodium (2S,5R)-2-(Chloromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl Sulfate. According to procedure (c): tetrabutylammonium (2*S*,5*R*)-2-(chloromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (110 mg, 0.21 mmol) was converted to the title compound as an off-white solid (45 mg, 71%). HRMS Anal. Calcd for $C_7H_{10}ClN_2O_5S$ [M], 268.9993. Found 269.0006. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 3.97−3.96 (m, 1H), 3.90−3.86 (m, 1H), 3.82−3.79 (m, 1H), 3.35−3.30 (m, 1H), 3.15 (d, J = 12 Hz, 1H), 2.85 (dt, J = 3.5, 12 Hz, 1H), 1.82−1.71 (m, 3H), 1.53−1.50 (m, 1H).

*Sodium (2S,5R)-2-(Dichloromethyl)-7-oxo-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate (**12**). (2S,5R)-6-(Benzyloxy)-2-(dichloromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one.* A solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbaldehyde (2.2 g, 8.4 mmol) in DCM (100 mL) was treated with phosphorus pentachloride (3.5 g, 16.9 mmol). After 16 h, the mixture was diluted

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with DCM and washed with ice-cold water and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica eluting with 0−20% EtOAc in hexane affording a white solid (130 mg, 5%).

LC−MS Anal. Calcd for $C_{14}H_{16}C_{l2}N_2O_2$, 314.1. Found [M + H] = 315.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.42−7.33 (m, 5H), 5.84 (d, J = 4.8 Hz, 1H), 5.01 (d, J = 11.6 Hz, 1H), 4.86 (d, J = 11.6 Hz, 1H), 3.81−3.76 (m, 1H), 3.39−3.37 (m, 1H), 3.25 (d, J = 12.0 Hz, 1H), 3.04−3.00 (m, 1H), 2.11−1.97 (m, 3H), 1.75−1.65 (m, 1H).

(2S,5R)-2-(Dichloromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]-octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-(dichloromethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (130 mg, 0.4 mmol) was converted to the desired compound an off-white solid (85 mg, 92%), which was used immediately in the next stage. LC−MS Anal. Calcd for $C_7H_{10}Cl_2N_2O_2$, 224.0. Found [M + H] = 225.0.

Tetrabutylammonnium (2S,5R)-2-(Dichloromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*S*,5*R*)-2-(dichloromethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (85 mg, 0.37 mmol) was converted to the desired compound as colorless oil (68 mg, 33%). LC−MS Anal. Calcd for $C_7H_9Cl_2N_2O_5S^-$, 303.0. Found [M] = 303.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.87 (d, J = 5.0 Hz, 1H), 4.38 (br s, 1H), 3.71−3.70 (m, 1H), 3.31−3.26 (m, 10H), 2.09−2.02 (m, 3H), 1.80−1.70 (m, 1H), 1.69−1.63 (m, 8H), 1.48−1.40 (m, 8H), 1.01−0.98 (m, 12H).

Sodium (2S,5R)-2-(Dichloromethyl)-7-oxo-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate. According to procedure (c): tetrabutylammonnium (2*S*,5*R*)-2-(dichloromethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl sulfate (68 mg, 0.12 mmol) was converted to the title compound as a white solid (36 mg, 88%). HRMS Anal. Calcd for $C_7H_9Cl_2N_2O_5S$ [M], 302.9604. Found 302.9619. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 6.25 (d, J = 0.8 Hz, 1H, C<u>H</u>Cl_2), 4.02 (m, 1H), 3.45 (m, 1H), 3.22 (d, 1H), 2.95 (m, 1H), 1.95−1.75 (m, 4H).

*Sodium (2R,5R)-7-Oxo-2-[(trifluoromethyl)sulfanyl]-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**22**). (2R,5R)-6-(Benzyloxy)-2-(trifluoromethylthio)-1,6-diazabicyclo[3.2.1]octan-7-one.* A solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (1 g, 3.62 mmol) in acetone (10 mL) was treated with $AgSCF_3$ (1.51 g, 7.24 mmol), Selectofluor (5.13 g, 14.5 mmol), and 2,6-lutidine (1.04 mL, 7.25 mmol). The reaction mixture was purged with nitrogen and heated at 90 °C for 0.5 h in a sealed tube. The reaction mixture was allowed to cool and then quenched with saturated aqueous K_2CO_3. The mixture was extracted with diethyl ether (2 × 50 mL), then the combined organic phases were washed with brine (20 mL), dried (Na_2SO_4), filtered, and evaporated. The crude material was purified by column chromatography, eluting with 20% EtOAc in hexane to afford a pale yellow solid (150 mg, 12%). LC−MS Anal. Calcd for $C_{14}H_{15}F_3N_2O_2S$, 332.1. Found [M + H] = 333.0. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.43−7.32 (m, 5H), 5.18 (d, J = 8.0 Hz, 1H), 5.04 (d, J = 11.6 Hz, 1H), 4.90 (d, J = 11.6 Hz, 1H), 3.50 (d, J = 12.0 Hz, 1H), 3.34 (s, 1H), 3.00 (d, J = 12.0 Hz, 1H), 2.48−2.40 (m, 1H), 2.08−2.02 (m, 1H), 1.69−1.61 (m, 2H).

(2R,5R)-6-Hydroxy-2-(trifluoromethylthio)-1,6-diazabicyclo-[3.2.1]octan-7-one. According to procedure (a): (2*R*,5*R*)-6-(benzyloxy)-2-(trifluoromethylthio)-1,6-diazabicyclo[3.2.1]octan-7-one (200 mg, 0.602 mmol) was converted to the desired compound as an off white solid (100 mg), which was used in the next step without purification. LC−MS Anal. Calcd for $C_7H_9F_3N_2O_2S$, 242.0. Found [M + H] = 243.0.

Tetrabutylammonium (2R,5R)-7-Oxo-2-(trifluoromethylthio)-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*R*,5*R*)-6-hydroxy-2-(trifluoromethylthio)-1,6-diazabicyclo-[3.2.1]octan-7-one (100 mg, 0.413 mmol) was converted to the desired compound as colorless oil (80 mg, 34% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.13 (d, J = 7.0 Hz, 1H), 4.40 (s, 1H), 3.54 (d, J = 12.0 Hz, 1H), 3.29−3.26 (m, 9H), 2.40−2.38 (m, 1H), 2.26−2.23 (m, 1H), 1.75−1.66 (m, 9H), 1.48−1.39 (m, 9 H), 1.09 (t, J = 7.5 Hz, 12H).

Sodium (2R,5R)-7-Oxo-2-((trifluoromethyl)thio)-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (c): Tetrabutyl ammonium (2*R*,5*R*)-7-oxo-2-(trifluoromethylthio)-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (80 mg, 0.24 mmol) was converted to the title compound as a white solid (36 mg, 73%). HRMS Anal. Calcd for $C_7H_8F_3N_2O_5S_2$ [M], 320.9821. Found 320.9836. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 5.10 (dd, J = 3.5, 8 Hz, 1H), 4.07−4.06 (m, 1H), 3.50 (d, J = 12 Hz, 1H), 3.05 (d, J = 12.5 Hz, 1H), 2.26−2.18 (m, 1H), 1.90−1.86 (m, 1H), 1.80−1.69 (m, 2H). ^{19}F NMR (470 MHz, DMSO-d_6): δ ppm −39.5.

*Sodium (2R,5R)-7-Oxo-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**25**). (2R,5R)-6-(Benzyloxy)-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (**30**).* To a solution of (2*R*,5*R*)-6-(benzyloxy)-2-(trifluoromethylthio)-1,6-diazabicyclo[3.2.1]octan-7-one (550 mg, 1.65 mmol) in CCl_4 (5 mL), ACN (5 mL) and water (10 mL) was added sodium periodate (1.4 g, 6.61 mmol) followed by ruthenium trichloride (34 mg, 0.16 mmol) at 0 °C. The reaction mixture was stirred for 3 h, then was filtered through Celite, and washed with DCM (10 mL). The filtrate was washed with water (10 mL), aqueous sodium thiosulfate (10 mL), and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated. The resulting crude material was triturated with *n*-pentane followed by diethyl ether to give an off-white solid (350 mg, 58%). LC−MS Anal. Calcd for $C_{14}H_{15}F_3N_2O_4S$, 364.1. Found [M + H] = 365.2. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.40−7.37 (m, 5H), 5.00 (d, J = 11.6 Hz, 1H), 4.89−4.86 (m, 2H), 3.52 (d, J = 12.4 Hz, 1H), 3.44 (t, J = 4 Hz, 1H), 3.11 (d, J = 12.4 Hz, 1H), 2.37−2.28 (m, 1H), 2.23−2.17 (m, 2H), 1.91−1.85 (m, 1H).

(2R,5R)-6-Hydroxy-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo-[3.2.1]octan-7-one. According to procedure (a): (2*R*,5*R*)-6-(benzyloxy)-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (350 mg, 0.96 mmol) was converted to the desired compound as an off-white solid (250 mg, 95%), which was used in the next step without purification. LC−MS Anal. Calcd for $C_7H_9F_3N_2O_4S$, 274.0. Found [M + H] = 275.2.

Tetrabutylammonium (2R,5R)-7-Oxo-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*R*,5*R*)-6-hydroxy-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (250 mg, 0.91 mmol) was converted to the desired compound as oil (200 mg). A portion of this material (50 mg) was further purified by preparative HPLC [column: KROMOSIL-C18 (150 × 25), 10 μ; mobile phase: (A) 10 mM NH_4OAc in H_2O, (B) acetonitrile; flow rate: 25 mL/min; gradient (T % B): 0/5, 8/40, 8.1/98, 10/98, 10.1/5, 12/5] to afford a white solid (27 mg). LC−MS Anal. Calcd for $C_7H_8F_3N_2O_7S_2^-$, 353.0. Found [M] = 353.0. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 5.35 (t, J = 9 Hz, 1H), 4.13−4.12 (m, 1H), 3.45 (d, J = 12.0 Hz, 1H), 3.20−3.14 (m, 9H), 2.28−2.23 (m, 1H), 2.13−2.11 (m, 1H), 1.97−1.84 (m, 2H), 1.59−1.53 (m, 8H), 1.34−1.27 (m, 8H), 0.95−0.92 (m, 12H).

Sodium (2R,5R)-7-Oxo-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-6-yl. According to procedure (c): tetrabutylammonium (2*R*,5*R*)-7-oxo-2-(trifluoromethylsulfonyl)-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (25 mg, 0.041 mmol) was converted to the title compound as a white solid (13 mg, 86%). HRMS Anal. Calcd for $C_7H_8F_3N_2O_7S_2$ [M], 352.9720. Found 352.9735. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 5.37−5.33 (m, 1H), 4.13−4.12 (m, 1H), 3.45 (d, J = 12.0 Hz, 1H), 3.19 (d, J = 1.5, 10.5 Hz, 1H), 2.29−2.24 (m, 1H), 2.14−2.10 (m, 1H), 1.99−1.85 (m, 2H).

*Sodium (2S,5R)-2-(1,1-Difluoroethyl)-7-oxo-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate (**31**). (2S,5R)-6-(Benzyloxy)-N-methoxy-N-methyl-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide.* To a stirred solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo-[3.2.1]octane-2-carboxylic acid (1.5 g, 5.43 mmol) in THF (50 mL) was added DIPEA (3.7 mL, 21.7 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (794 mg, 8.15 mmol) at 0 °C followed by T_3P (50% in EtOAc, 6.9 mL, 10.8 mmol). The resulting reaction mixture was stirred for 4 h, then diluted with EtOAc (100 mL), washed with water (50 mL), sat. $NaHCO_3$ solution (50 mL), and brine (50 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to give oil. This was purified by silica chromatography eluting with 40% EtOAc in hexane affording brown oil (900 mg, 53%). LC−MS Anal. Calcd for $C_{16}H_{21}N_3O_4$, 319.2. Found [M + H] = 320.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.44−7.34 (m, 5H), 5.04 (d, J = 11.6 Hz, 1H), 4.90 (d, J = 11.6 Hz, 1H), 4.53 (br s, 1H), 3.80 (s, 3H), 3.34 (s,

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1H), 3.26−3.10 (m, 4H), 2.91 (d, J = 11.2 Hz, 1H), 2.04−1.88 (m, 4H).

(2S,5R)-2-Acetyl-6-(benzyloxy)-1,6-diazabicyclo[3.2.1]octan-7-one. To a stirred solution of (2*S*,5*R*)-6-(benzyloxy)-*N*-methoxy-*N*-methyl-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (900 mg, 2.81 mmol) in THF (20 mL) was added a solution of methylmagnesium bromide in diethylether (3 M; 1.8 mL, 5.63 mmol) at −78 °C under a nitrogen atmosphere. The reaction mixture was stirred at −78 °C for 1 h, then quenched with EtOAc (10 mL) at −78 °C, followed by water (10 mL). The reaction mixture was allowed to warm to room temperature, then diluted with EtOAc (100 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated. The crude material was purified by silica chromatography eluting with 25% EtOAc in hexane to afford a white solid (500 mg, 64%). LC−MS Anal. Calcd for $C_{15}H_{18}N_2O_3$, 274.1. Found [M + H] = 275.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.35 (m, 5H), 5.06 (d, J = 11.5 Hz, 1H), 4.90 (d, J = 11.5 Hz, 1H), 3.92 (d, J = 7.5 Hz, 1H), 3.27 (d, J = 1.5 Hz, 1H), 3.03−3.00 (m, 1H), 2.48 (d, J = 11.5 Hz, 1H), 2.30 (s, 3H), 2.20−2.15 (m, 1H), 2.00−1.90 (m, 1H), 1.87−1.82 (m, 1H), 1.57−1.55 (m, 1H).

(2S,5R)-6-(Benzyloxy)-2-(1,1-difluoroethyl)-1,6-diazabicyclo-[3.2.1]octan-7-one. To a stirred solution of (2*S*,5*R*)-2-acetyl-6-(benzyloxy)-1,6-diazabicyclo[3.2.1]octan-7-one (500 mg, 1.82 mmol) in DCM (10 mL) was added DAST (2.4 mL, 18.2 mmol) at 0 °C under a N_2 atmosphere and stirred for 48 h. The volatile components were removed by purging with nitrogen. The resulting residue was partitioned between EtOAc (50 mL) and ice-cold water (50 mL). The organic phase was separated, washed with saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL), dried (Na_2SO_4), and evaporated. The crude material was purified by chromatography eluting with 10% EtOAc in hexane to afford yellow oil (400 mg, 74%). LC−MS Anal. Calcd for $C_{15}H_{18}F_2N_2O_2$, 296.1. Found [M + H] = 297.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.43−7.34 (m, 5H), 5.03 (d, J = 11.6 Hz, 1H), 4.88 (d, J = 11.6 Hz, 1H), 3.51−3.44 (m, 1H), 3.35−3.33 (m, 1H), 3.13 (d, J = 11.6 Hz, 1H), 2.94 (d, J = 12.0 Hz, 1H), 2.07−1.88 (m, 3H), 1.88−1.57 (m, 4H).

(2S,5R)-2-(1,1-Difluoroethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]-octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-(1,1-difluoroethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (280 mg, 0.94 mmol) was converted to the desired compound as a white solid (175 mg), which was used directly in the next step. LC−MS Anal. Calcd for $C_8H_{12}F_2N_2O_2$, 206.1. Found [M + H] = 207.1. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.68 (s, 1H), 3.64−3.62 (m, 1H), 3.36−3.32 (m, 1H), 3.11 (d, J = 11.5 Hz, 1H), 3.00 (d, J = 11.5 Hz, 1H), 1.92−1.77 (m, 3H), 1.72−1.61 (m, 4H).

Tetrabutylammonium (2S,5R)-2-(1,1-Difluoroethyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2S,5R)-2-(1,1-difluoroethyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]-octan-7-one (170 mg, 0.82 mmol) was converted to the desired compound as colorless oil (200 mg, 46%). LC−MS Anal. Calcd for $C_8H_{11}F_2N_2O_5S^-$, 285.0. Found [M] = 285.2. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 4.35 (br s, 1H), 3.51−3.39 (m, 1H), 3.31−3.23 (m, 10H), 2.19−2.12 (m, 1H), 2.00−1.86 (m, 2H), 1.78−1.63 (m, 12H), 1.48−1.41 (m, 8H), 1.02−0.99 (m, 12H).

Sodium (2S,5R)-2-(1,1-Difluoroethyl)-7-oxo-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate. According to procedure (c): tetrabutylammonium (2*S*,5*R*)-2-(1,1-difluoroethyl)-7-oxo-1,6-diazabicyclo[3.2.1]-octan-6-yl sulfate (50 mg, 0.09 mmol) was converted to the title compound as a white solid (17 mg, 63%). HRMS Anal. Calcd for $C_8H_{11}F_2N_2O_5S$ [M], 285.0351. Found 285.0365. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 4.04−4.02 (m, 1H), 3.38−3.35 (m, 1H), 3.16 (d, J = 12 Hz, 1H), 3.02−3.00 (d, J = 11.5 Hz, 1H), 1.87−1.81 (m, 2H), 1.78−1.71 (m, 2H), 1.69 (t, J = 19.5 Hz, 3H).

*Sodium (2S,5R)-2-[Difluoro(1,3-thiazol-2-yl)methyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**10**). (2S,5R)-6-(Benzyloxy)-2-(thiazole-2-carbonyl)-1,6-diazabicyclo[3.2.1]octan-7-one (**13**).* To a stirred solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbaldehyde **4** (4.0 g, 12.5 mmol) in DCM (40 mL) was added 2-(trimethylsilyl)thiazole (2.5 mL, 18.8 mmol) at 0 °C, and the reaction mixture was stirred for 4 h. The reaction mixture was evaporated, and then DMSO (70 mL) and Ac_2O (16 mL) were added. The resulting mixture was stirred for 48 h, then the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution, and extracted with diethyl ether (2 × 100 mL). The organic layer was washed with water (50 mL) and brine (50 mL), dried (Na_2SO_4) filtered, and evaporated. The residue was chromatographed on silica, eluting with 25% EtOAc in hexane to afford a yellow solid (1.3 g, 24%). LC−MS Anal. Calcd for $C_{17}H_{17}N_3O_3S$, 343.1. Found [M + H] = 344.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.07 (d, J = 3.2 Hz, 1H), 7.69 (d, J = 3.2 Hz, 1H), 7.45−7.34 (m, 5H), 5.19 (d, J = 6.4 Hz, 1H), 5.07 (d, J = 11.6 Hz, 1H), 4.92 (d, J = 11.6 Hz, 1H), 3.33 (d, J = 2.8 Hz, 1H), 3.11−3.00 (m, 2H), 2.29−2.18 (m, 1H), 2.14−2.06 (m, 2H), 1.81−1.68 (m, 1H).

*(2S,5R)-6-(Benzyloxy)-2-(difluoro(thiazol-2-yl)methyl)-1,6-diazabicyclo[3.2.1]octan-7-one (**14**).* To a stirred solution of (2*S*,5*R*)-6-(benzyloxy)-2-(thiazole-2-carbonyl)-1,6-diazabicyclo-[3.2.1]octan-7-one (1.3 g, 3.79 mmol) in DCM (25 mL) was added DAST (4.9 mL, 37.9 mmol) at 0 °C under a N_2 atmosphere. The resulting reaction mixture was stirred for 16 h. The reaction mixture was diluted with DCM (50 mL), washed with saturated aqueous $NaHCO_3$ (25 mL), brine (25 mL), dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica eluting with 25% EtOAc in hexane to afford yellow oil (0.8 g, 57%). LC−MS Anal. Calcd for $C_{17}H_{17}F_2N_3O_2S$, 365.1. Found [M + H] = 366.0. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.92 (d, J = 1.6 Hz, 1H), 7.49 (d, J = 2.8 Hz, 1H), 7.41−7.33 (m, 5H), 4.99 (d, J = 11.6 Hz, 1H), 4.86 (d, J = 11.6 Hz, 1H), 4.20−4.04 (m, 1H), 3.38 (s, 1H), 3.25 (d, J = 12.0 Hz, 1H), 2.97 (d, J = 12.4 Hz, 1H), 2.14−2.02 (m, 3H), 1.77−1.65 (m, 1H).

(2S,5R)-2-(Difluoro(thiazol-2-yl)methyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-(difluoro(thiazol-2-yl)methyl)-1,6-diazabicyclo[3.2.1]octan-7-one (800 mg, 2.19 mmol) was converted to the desired compound as an off-white solid (301 mg, 49%). This crude material was used in the next step without purification. LC−MS Anal. Calcd for $C_{10}H_{11}F_2N_3O_2S$, 275.1. Found [M + H] = 276.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.93 (d, J = 3.5 Hz, 1H), 7.51 (d, J = 3.5 Hz, 1H), 4.25−4.15 (m, 1H), 3.81 (s, 1H), 3.36 (d, J = 12.0 Hz, 1H), 3.13 (d, J = 11.5 Hz, 1H), 2.23−2.15 (m, 2H), 2.08−2.03 (m, 1H), 1.89−1.80 (m, 1H).

Tetrabutylammonium (2S,5R)-2-(Difluoro(thiazol-2-yl)methyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*S*,5*R*)-2-(difluoro(thiazol-2-yl)methyl)-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (301 mg, 1.45 mmol) was converted to the desired compound as colorless oil (250 mg, 38% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.90 (d, J = 3.0 Hz, 1H), 7.49 (d, J = 3.0 Hz, 1H), 4.38 (s, 1H), 4.18−4.12 (m, 1H), 3.33−3.23 (m, 10H), 2.21−2.09 (m, 2H), 1.99−1.91 (m, 1H), 1.86−1.80 (m, 1H), 1.68−1.62 (m, 8H), 1.47−1.39 (m, 8H), 1.01−0.98 (m, 12H).

Sodium (2S,5R)-2-(Difluoro(thiazol-2-yl)methyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (c): tetrabutylammonium (2*S*,5*R*)-2-(difluoro(thiazol-2-yl)methyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (100 mg, 0.16 mmol) was converted to the title compound as a white solid (21 mg, 33%). HRMS Anal. Calcd for $C_{10}H_{10}F_2N_3O_5S_2$ [M], 354.0024. Found 354.0039. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.04 (s, 2H), 4.04−3.97 (m, 2H), 3.21 (d, J = 12 Hz, 1H), 3.00 (dd, J = 10.5 Hz, J = 1.0 Hz, 1H), 2.03−1.96 (m, 1H), 1.88−1.76 (m, 3H). ^{19}F NMR (470 MHz, DMSO-d_6): δ ppm −96. 97 (dd, J = 260 Hz, J = 12 Hz), −99. 25 (dd, J = 260 Hz, J = 16 Hz).

*Sodium (2S,5R)-7-Oxo-2-[(trifluoromethoxy)methyl]-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**17**). (2S,5R)-6-(Benzyloxy)-2-((trifluoromethoxy)methyl)-1,6-diazabicyclo[3.2.1]octan-7-one.* A solution of (2*S*,5*R*)-6-(benzyloxy)-2-(hydroxymethyl)-1,6-diazabicyclo[3.2.1]octan-7-one (1 g, 3.81 mmol) in EtOAc (50 mL) was treated with KF (0.88 g, 15.3 mmol), AgOTf (1.78 g, 11.4 mmol), Selectofluor (2.02 g, 5.72 mmol), 2-fluoro pyridine (1.21 mL, 11.4 mmol), and TMS-CF_3 (1.45 mL, 11.4 mmol). The reaction mixture was purged with nitrogen for 10 min and then heated at 80

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°C for 16 h in a sealed tube. The reaction mixture was allowed to cool to room temperature, filtered, and washed with EtOAc. The combined filtrates were washed with water (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude material was purified by chromatography on silica eluting with 40% EtOAc in hexane to afford a yellow solid (200 mg, 16%). LC−MS Anal. Calcd for $C_{15}H_{17}F_3N_2O_3$, 330.1. Found [M + H] = 331.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.34 (m, 5H), 5.03 (d, J = 11.5 Hz, 1H), 4.89 (d, J = 11.5 Hz, 1H), 4.13−4.05 (m, 2H), 3.67−3.65 (m, 1H), 3.33 (s, 1H), 2.99 (s, 2H), 2.06−2.00 (m, 2H), 1.66−1.61 (m, 2H).

(2S,5R)-6-Hydroxy-2-((trifluoromethoxy)methyl)-1,6-diazabicyclo[3.2.1]octan-7-one. According to procedure (a): (2*S*,5*R*)-6-(benzyloxy)-2-((trifluoromethoxy)methyl)-1,6-diazabicyclo[3.2.1]octan-7-one (200 mg, 0.60 mmol) was converted to the desired compound as an off-white solid (100 mg). LC−MS Anal. Calcd for $C_8H_{11}F_3N_2O_3$, 240.1. Found [M + H] = 241.0.

Tetrabutylammonium (2S,5R)-7-Oxo-2-((trifluoromethoxy)-methyl)-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate. According to procedure (b): (2*S*,5*R*)-6-hydroxy-2-((trifluoromethoxy)methyl)-1,6-diazabicyclo[3.2.1]octan-7-one (120 mg, 0.5 mmol) was converted to the desired compound as colorless oil (100 mg, 34% over 2 steps). LC−MS Anal. Calcd for $C_8H_{10}F_3N_2O_6S^-$, 319.0. Found [M] = 319.0.

Sodium (2R,5R)-7-Oxo-2-(trifluoromethoxy)-1,6-diazabicyclo-[3.2.1]octan-6-yl Sulfate. According to procedure (c): tetrabutylammonium (2*S*,5*R*)-7-oxo-2-((trifluoromethoxy)methyl)-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (100 mg, 0.18 mmol) was converted to the desired compound as a white solid. This was further purified by preparative HPLC [column: X-SELECET-C18 (150 × 19), 5 μ; mobile phase: (A) 10 mM ammonium bicarbonate in H_2O, (B) acetonitrile; flow rate: 18 mL/min; gradient (T % B): 0/5, 6/60, 6.1/98, 8/98, 8./5, 11/5]. The product-containing fractions were lyophilized to afford the title compound as a white solid (31 mg, 51%). HRMS Anal. Calcd for $C_8H_{10}F_3N_2O_6S$ [M], 319.0206. Found 319.0220. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 4.32 (dd, J = 10.5 Hz, J = 9.5 Hz, 1H), 4.16 (dd, J = 10.5 Hz, J = 5.5 Hz, 1H), 3.98 (d, J = 3 Hz, 1H), 3.45−3.42 (m, 1H), 3.20 (d, J = 12.0 Hz, 1H), 2.91 (d, J = 12.0 Hz, 1H), 1.85−1.69 (m, 3H), 1.50−1.45 (m, 1H). ^{19}F NMR (470 MHz, DMSO-d_6): δ ppm −58.68.

*Sodium (2R,5R)-2-Chloro-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl Sulfate (**23**). (2R,5R)-6-(Benzyloxy)-2-chloro-1,6-diazabicyclo-[3.2.1]octan-7-one.* To a solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (1 g, 3.62 mmol) in DMF (20 mL) and AcOH (4 mL) was added *N*-chlorosuccinimide (4.83 g, 36.2 mmol). The reaction mixture was purged with nitrogen gas for 5 min and then $Pb(OAc)_4$ (2.4 g, 5.43 mmol) was added. The reaction mixture was purged with nitrogen gas for further 5 min and then heated at 60 °C for 4 h. The cooled mixture was treated with saturated aqueous K_2CO_3 and extracted with diethyl ether (2 × 50 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica eluting with 10% EtOAc in hexane affording yellow oil (270 mg, 28%). LC−MS Anal. Calcd for $C_{13}H_{15}ClN_2O_2$. 266.1. Found [M + H] = 267.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.43−7.36 (m, 5H), 5.49 (d, J = 5.5 Hz, 1H), 5.05 (d, J = 11.5 Hz, 1H), 4.90 (d, J = 11.5 Hz, 1H), 3.55 (d, J = 12.0 Hz, 1H), 3.36 (dd, J = 5.5 Hz, J = 3.5 Hz, 1H), 2.98 (dd, J = 11.5 Hz, J = 3.0 Hz, 1H), 2.44−2.37 (m, 1H), 1.98−1.85 (m, 2H), 1.83−1.78 (m, 1H).

NMR experiments showed coupling constants for the H atom on the same carbon as the Cl of 5.5 Hz (coupling to the axial proton on the adjacent carbon), thereby establishing that this H atom has an equatorial disposition and the molecule has the stereochemistry as shown. See the Supporting Information for related experiments in the F analogue series.

(2R,5R)-2-Chloro-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one. According to procedure (a): (2*R*,5*R*)-6-(benzyloxy)-2-chloro-1,6-diazabicyclo[3.2.1]octan-7-one (220 mg, 0.824 mmol) was converted to the desired compound as a white solid (150 mg), which was used without purification. LC−MS Anal. Calcd for $C_6H_9ClN_2O_2$, 176.0. Found [M + H] = 177.0.

Tetrabutylammonium (2R,5R)-2-Chloro-7-oxo-1, 6-Diazabicyclo [3.2.1] Octan-6-yl Sulfate. According to procedure (b): (2*R*,5*R*)-2-chloro-6-hydroxy-1,6-diazabicyclo[3.2.1]octan-7-one (150 mg, 0.849 mmol) was converted to the desired compound as colorless oil (130 mg, 30% over 2 steps). LC−MS Anal. Calcd for $C_6H_8ClN_2O_5S^-$, 255.0. Found [M] = 254.9. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 5.47 (d, J = 5.6 Hz, 1H), 4.42 (s, 1H), 3.64 (d, J = 12.0 Hz, 1H), 3.30−3.22 (m, 9H), 2.38−2.34 (m, 1H), 2.21−2.10 (m, 1H), 1.96−1.80 (m, 2H), 1.70−1.62 (m, 8H), 1.49−1.39 (m, 8H), 1.02−0.98 (m, 12H).

Sodium (2R,5R)-2-Chloro-7-oxo-1, 6-Diazabicyclo [3.2.1] Octan-6-yl Sulfate. According to procedure (c): tetrabutylammonium (2*R*,5*R*)-2-chloro-7-oxo-1, 6-diazabicyclo [3.2.1] octan-6-yl sulfate (130 mg, 0.26 mmol) was converted to the desired compound as a white solid (20 mg), which was further purified by preparative HPLC [X-SELECT-C18 (150 × 19), 5 μ, mobile phase: H_2O: MeCN]. The collected fractions were freeze-dried to afford the title compound (5.0 mg, 7%) as an off-white solid. LC−MS Anal. Calcd for $C_6H_8ClN_2O_5S^-$, 255.0. Found [M] = 255.0. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 5.51 (dd, J = 6.0 Hz, J = 2.0 Hz, 1H), 4.10 (d, J = 3.0 Hz, 1H), 3.51−3.49 (obs, 1H), 3.07−3.05 (d, J = 12.0 Hz, 1H), 2.26−2.20 (m, 1H), 1.94−1.80 (m, 3H)..

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01535.

Tested compounds including Smiles strings (CSV)

Molecular modeling for Figure 3A (PDB)

Molecular modeling for Figure 3B (PDB)

Includes synthesis and characterization of key compounds, experimental procedures for enzyme inhibition assays, *in vitro* antimicrobial susceptibility testing, efficacy studies, protein crystallization, and collection and processing of diffraction data (with resulting statistics) (PDF)

Accession Codes

The coordinates and structure factors of the OXA-48:**ANT3310** (compound **21**) complex were deposited to the Protein Data Bank under accession code 6ZXI.

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D.T.D. and S.L. designed the compounds and performed the molecular modeling; C.P., R.E., S.V., R.P., R.V., B.K., and S.P. synthesized the compounds; J.C., J.B., C.L., and A.L. performed the enzymology and MIC experiments; M.Z., N.S., M.L., and M.E. devised the screening cascades and biological strategies; M.Z., C.D.P., and I.M. devised and performed the large-scale MIC profiling experiments; M.Z., K.H., and P.W. devised and performed the efficacy experiments; F.M., M.B., C.P., S.M., G.T., and J.-D.D. performed the protein crystallography and X-ray determination. All the authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ACN, acetonitrile; AcOH, acetic acid; Ac_2O, acetic anhydride; $AgNO_3$, silver nitrate; AgOTf, silver triflate; AIBN, azobisisobutyronitrile; Boc, *tert*-butoxycarbonyl; Boc_2O, di-*tert*-butyl decarbonate; Bu_3SnH, tributyltin hydride; brine, saturated aqueous sodium chloride solution; DAST, diethylaminosulfur trifluoride; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; dppf, 1,1′-bis(diphenylphosphino)-ferrocene; EtOAc, ethyl acetate; EtOH, ethanol; Et_3N, triethylamine; IPA, isopropyl alcohol; MEM, meropenem; MeOH, methanol; Na_2SO_4, sodium sulfate; $Pb(OAc)_4$, lead tetraacetate; SO_3.pyr, pyridine sulfur trioxide complex; Selectfluor, 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo-[2.2.2]octane bis(tetrafluoroborate); TBAA, tetra(*n*-butyl)-ammonium acetate; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl; $TMSCF_3$, trimethyl(trifluoromethyl)silane; THF, tetrahydrofuran; T3P, |propylphosphinic anhydride

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