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Antimicrobial Effects of Novel Siderophores Linked to β-Lactam Antibiotics

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Abstract—As a strategy to increase the penetration of antibiotic drugs through the outer membrane of Gram-negative pathogens, facilitated transport through siderophore receptors has been frequently exploited. Hydroxamic acids, catechols, or very close isosteres of catechols, which are mimics of naturally occurring siderophores, have been used successfully as covalently linked escorting moieties, but a much wider diversity of iron binding motifs exists. This observation, coupled to the relative lack of specificity of siderophore receptors, prompted us to initiate a program to identify novel, noncatechol siderophoric structures. We screened over 300 compounds for their ability to (1) support growth in low iron medium of a *Pseudomonas aeruginosa* siderophore biosynthesis deletion mutant, or (2) compete with a bactericidal siderophore-antibiotic conjugate for siderophore receptor access. From these assays we identified a set of small molecules that fulfilled one or both of these criteria. We then synthesized these compounds with functional groups suitable for attachment to both monobactam and cephalosporin core structures. Siderophore-β-lactam conjugates then were tested against a panel of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* strains. Although several of the resultant chimeric compounds had antimicrobial activity approaching that of ceftazidime, and most compounds demonstrated very potent activity against their cellular targets, only a single compound was obtained that had enhanced, siderophore-mediated antibacterial activity. Results with *tonB* mutants frequently showed increased rather than decreased susceptibilities, suggesting that multiple factors influenced the intracellular concentration of the drugs.

Introduction

Bacteria must acquire iron by competing with environmental chelation. One mechanism for bacterial iron acquisition utilizes siderophores, small-molecule chelating agents that have evolved in parallel with siderophore receptors, a class of iron regulated outer membrane proteins. The siderophore/siderophore receptor systems, thus, capture and escort iron through the outer membrane and deliver it into the periplasm. Iron affinity constants for the *Pseudomonas aeruginosa* siderophores range from as weak as 10^{-6} M for pyochelin to as strong as 10^{-32} M for pyoverdin.[1] In *Escherichia coli* and several *Pseudomonas* species, uptake of ferrisiderophores requires *tonB*, an essential protein that couples cytoplasmic membrane promotive force to active transport across the outer membrane.[1] Following subsequent delivery of the ferrisiderophore through the inner membrane, the iron is reductively released in the cytoplasm, and the siderophore is recycled.[2] While many siderophores are associated with a cognate receptor, there exists a high degree of promiscuity among siderophore receptors, allowing cross-recognition of a variety of siderophores beyond the genus.[3] The siderophores and receptors for several Gram-negative species, particularly *E. coli*[2,4] and *P. aeruginosa*[1] have been reviewed.

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Gram-negative bacteria in general, and *P. aeruginosa* in particular, present a formidable physical barrier to antibiotics by means of an outer membrane traversed by restrictive porins.[5] Alternative access through the outer membrane via siderophore receptors has been exploited as a strategy for "illicit transport" of antibiotics covalently linked to microbial siderophores.[6–11] Mimicry of naturally occurring siderophores led to use of hydroxamic acids,[10–13] catechols,[6,8,9,14–21] or very close isosteres of catechols,[6,16] as the escorting moiety. Because their target is periplasmic, β-lactams have been the most commonly selected antibiotic core structures for this strategy. Many cephalosporins and monobactams showed increased activity against Gram-negative pathogens, notably *E. coli* and *P. aeruginosa*, when covalently linked to a siderophoric moiety.[15,18] An attempt to extend this strategy to macrolides failed to decrease the resultant MIC values against *P. aeruginosa* or other Gram-negative organisms.[22] Recently, Miller has described the application of this strategy to nucleoside antifungal and antiviral agents as well.[23]

A variety of structural motifs bind iron; this observation, coupled with the relative lack of specificity of siderophore receptors, prompted us to initiate a program to identify novel, noncatechol siderophoric structures. These structures would function as escorting agents for β-lactams through the outer membrane of Gram-negative bacteria. Herein, we report the results of this study.

Results

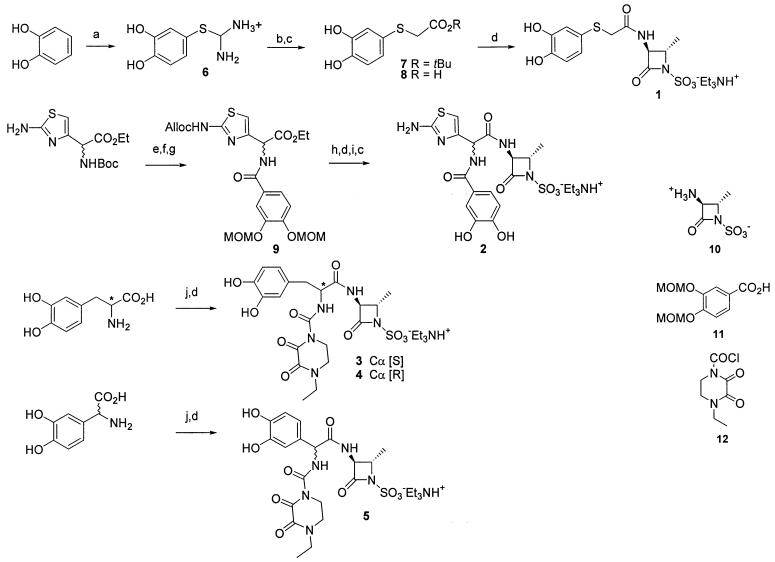
Assays for siderophores have been described.[24–26] We established two independent determinants of siderophore activity and developed assays for each of these. In the first, referred to as the growth promotion assay, we measured the ability of compounds to compensate for the lack of endogenous siderophores in a siderophore-deficient mutant. We constructed PGO 2812, a genetically defined *P. aeruginosa* mutant deficient in the production of pyochelin and pyoverdin. Growth of PGO 2812 in the medium in which the iron is bound by dipyridyl reflects the ability of the exogenously added compounds to compete the iron away and transport it though the bacterial membrane.

In parallel to this assay, we developed an assay that would measure the competition at siderophore receptors between a siderophore-antibiotic and a panel of small molecule siderophore candidates. Attenuation of the bactericidal activity of the siderophore-antibiotic could be interpreted as a measure of the ability of the siderophore to interfere with the antibiotic conjugate's binding and uptake via the siderophore receptor.[27,28] This assay thus obviates the need to identify the actual receptor(s) involved in illicit transport, while at the same time facilitates the discovery of compounds that access these receptors.

For the competition assay, we first synthesized (Scheme 1) a panel of catechol monobactams in order to obtain an antibiotic ligand with improved Gram-negative activity that could be abrogated in a *tonB* mutant of *P. aeruginosa.*

Whereas **1**, **2**, **3** and **4** showed no activity against *P. aeruginosa*, and very limited activity against other Gram-negative species, **5**, a catechol monobactam analogue of piperacillin, was active against both *E. coli* and *P. aeruginosa* (Table 1). The MIC values of **5**, particularly in *P. aeruginosa*, were increased significantly in the absence of *tonB*.

With **5** in hand, we proceeded to screen approximately 300 compounds obtained from commercial sources. Our selection criteria for these compounds was driven by either structural homology to a catechol, e.g. **31**, or



Scheme 1. (a) Thiourea, $K_3Fe(CN)_6$, NaOAc (b) (i) NaOH, H_2O (ii) $BrCH_2CO_2{}^tBu$ (c) TFA, CH_2Cl_2 (d) (i) NHS, DCC, THF (ii) **10**, NEt_3, H_2O (e) allyl chloroformate, DIEA, CH_2Cl_2 (f) TFA (g) **11**, HBTU, DMF, NMM (h) (i) NaOH (ii) H^+ (i) $(Ph_3P)_4Pd$, 2-ethyl octanoic acid, Ph_3P (j) (i) $[(Me_3N)_2Si]_2$, Me_3SiCl (ii) **12**, CH_2Cl_2.

Table 1. Antibacterial activity of catechol monobactams in wild-type and *tonB* mutants of *E. coli* and *P. aeruginosa*. Details of the assay are given in Experimental

Compound	*E. coli* ATCC 25922	*E. coli* Δ *tonB*	*P. aeruginosa* PAO-1	*P. aeruginosa* Δ *tonB*
1	>50	>50	>50	ND
2	6	12	>50	>50
3	>50	>50	>50	ND
4	>50	>50	>50	>50
5	0.4	1.56	6.25	>50

metal-binding capability described in another biological context (e.g. **19**[29,30], **29**[31]). The screening results for key compounds, including catechols and noncatechols, are shown in Table 2. Of the compounds screened, 69 out of 352, 20%, were able to support growth of PGO 2812 in dipyridyl-containing media. In competition with **5**, 48 out of 310, 15%, were able to protect PA01 from the bactericidal effects of the drug when the drug was present at its MIC value. Of these two classes, 25 out of 310, 8%, were active in both assays.

Although diverse in structure, and even more diverse in biochemical application, nearly all of the noncatechol compounds in Table 2 have in common a salicylimine motif that may serve as a general metal binding function. Presumably recognition at the siderophore receptor(s) is determined by other distinguishing features of these compounds. To better understand the predictive value of the assays, we selected structural motifs from compounds that were either active in one assay alone or active in both assays. These structural motifs were then covalently incorporated into β-lactam antibiotics, and the minimal inhibitory concentration (MIC) values against a panel of selected organisms was determined.

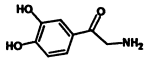
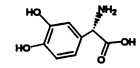
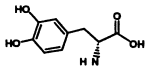
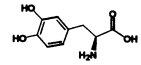
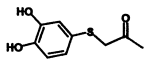
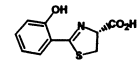
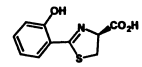
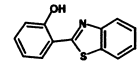
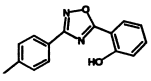
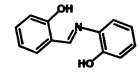
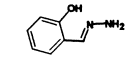
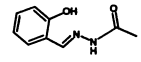
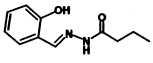
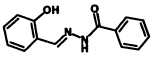
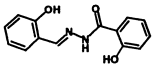
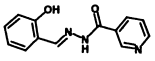
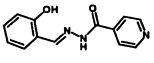
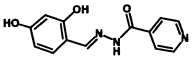
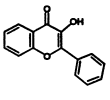
Our expectation was that a β-lactam linked to a functional siderophore would have cidal properties against wild type *E. coli* and *P. aeruginosa*, but be far less effective in *tonB* mutants, since facilitated transport would be unavailable to these mutant strains. Use of the hypersusceptible ATCC 35151 strain of *P. aeruginosa* would allow us to measure the intrinsic effect of that compound at the molecular target, the penicillin-binding proteins (PBPs) since in ATC 35151 there is a substantially reduced permeability barrier. Activity against *S. aureus*, a prototypical Gram-positive species, would also be a measure of the activity of the compound against the PBPs in the absence of an outer membrane barrier.

Chemistry

Synthesis of $ArCH_2Br$ moieties

While a few of the siderophores in Table 2 had functional groups that could be recruited for direct attachment to an antibiotic core, the remainder were synthesized as suitably functionalized derivatives, particularly halomethyl compounds (Scheme 2) that

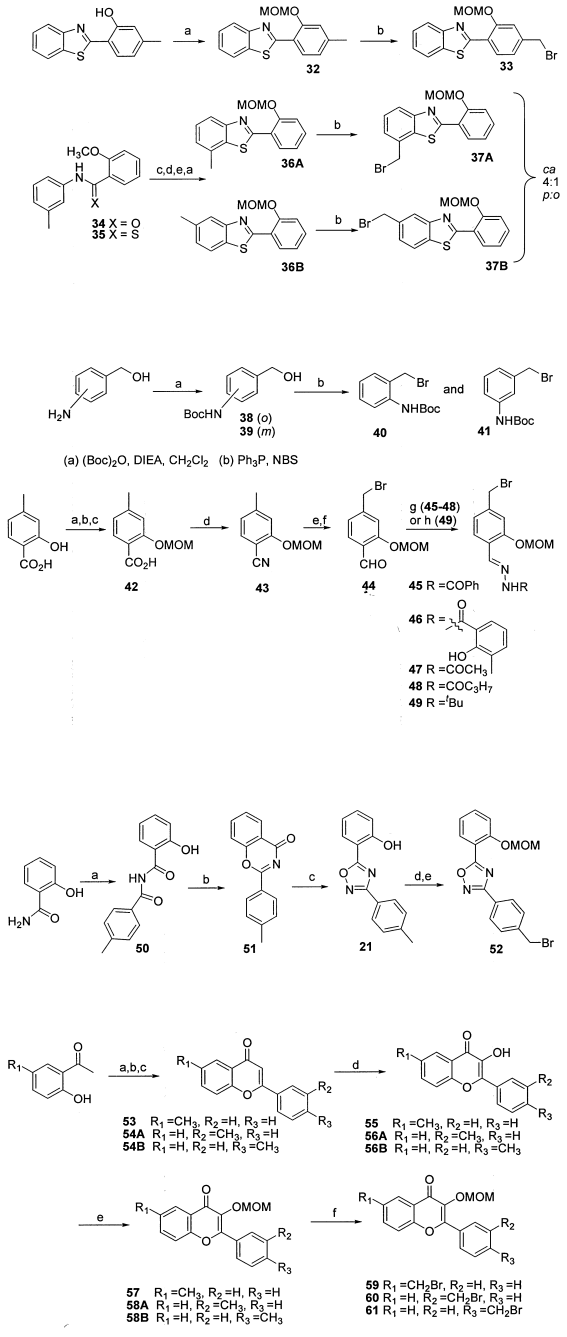
Table 2. Siderophore effects, as determined by the two assays, for catechols and selected noncatechols[a]

Compound		Growth in low iron	Competition with 5
13		+	+ +
14		+	+ +
15		–	–
16		–	+ +
17		–	+
18		+	+ +
19		+	+ +
20		+	–
21		+	+
22		+	–
23		–	–
24		+	–
25		+	–
26		+	+ +
27		+	+ +
28		+	+ +
29		+	+
30		+	+ +
31		+	–

[a]Values are considered + for growth in low iron if the compound is able to stimulate sufficient *Pseudomonas aeruginosa* PGO 2812 to give an optical density (OD_{600}) of ≥ 0.5 A.U. at ≤ 300 μg/mL. Values are considered + for competition if the bactericidal activity at compound concentrations of ≤ 300 μg/ml, as measured by an OD_{600} of ≥ 0.50–070. Values are + + if the $OD_{600} \geq 0.71$. Details of the assays are given in Experimental.

could be readily appended onto β-lactams at various positions.

For benzthiazole **20**, two alternative attachment sites were investigated. The synthesis of 2-(4′-bromomethyl-2′-(methoxymethyloxy))phenylbenzthiazole **33** from 2-(4′-methyl-2′-methoxy) phenylbenzthiazole required simply protection of the phenolic hydroxyl and radical bromination. Synthesis of the complementary 2-(2′-(methoxymethyloxy)) phenyl 5-bromomethyl benzthiazole **37** followed the procedure of Stevens et al.[32] The two regiomeric ring closure products **36A** and **B**, produced in approximately 4:1 *para:ortho* ratio, were carried forward as a mixture throughout the preparation of the bromomethyl derivatives. Although regiomeric enrichments could be achieved at each stage of synthesis, separation was most conveniently carried out at the oxime ether intermediate **81** shown in Scheme 5.



Scheme 2. A.(a) MOMCI, DBU, THF 0 °C to rt (b) NBS, AIBN, Δ, CCl_4 (c) Lawesson's reagent (d) NaOH, $K_3Fe(CN)_6$ (e) BBr_3, CH_2Cl_2, −78 °C. B.(a) MeOH, DCC, Et_2O (b) MOMCl, DIEA, CH_3CN (c) (i) 1N NaOH, EtOH (ii) H^+ (d) (i) MsCl, pyridine (ii) NH_3, MsCl(e) NBS, AIBN, Δ CCl_4 (f) DIBAL, toluene (g) $RCONHNH_2$, AcOH, EtOH (h) $^tBuNHNH_2$. C.(a) (i) pyridine, 0 °C (ii) p-$CH_3C_4H_4COCl$ (b) 200 °C (c) $HCl.NH_2OH$, NaOAc, EtOH (d) NaH, MOMCl, CH_2Cl_2 (e) NBS, AIBN, Δ CCl_4. D.(a) pyridine, $R_2R_3C_6H_3COCl$ (b) KOH, pyridine (c) AcOH, H_2SO_4 (d) (i) $PhI(OAc)_2$, KOH, MeOH (ii) HCl, acetone (e) MOMCl, NaH, CH_2Cl_2 (f) NBS, AIBN, Δ CCl_4.

N-protected 2- and 3-bromomethyl anilines **40** and **41** were synthesized as shown from commercially available anilines.

The key *O*-protected bromomethylsalicylaldehyde **44** was prepared in 6 steps from 4-methylsalicylic acid, and converted to the desired acylhydrazones **45**–**49** either before or after alkylation of the thiopyridyl intermediate **89** in Scheme 7.

Oxadiazole **52** was prepared by acylation and cyclodehydration of salicylic amide,[33] followed by protection of the phenolic hydroxyl and benzylic bromination.

Flavones **59**, **60** and **61** were synthesized from the corresponding *ortho* hydroxy acetophenones and substituted benzoyl chlorides.[34,35]

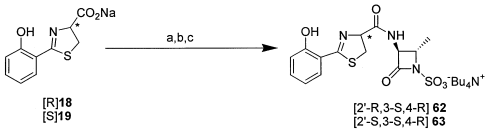
Synthesis of antibiotic siderophore conjugates Schemes 3, 4, 5, 6, and 7

(*S*)-Dihydroaeruginoic acid **19** is the biogenic precursor to the *Pseudomonas* siderophore pyochelin,[36] and was one of the most potent siderophores identified in our screens. We first explored direct acylation of a monobactam by both epimeric dihydroaeruginoic acids in a linkage conceptually analogous to the siderophore-monobactam relationship of **5**.

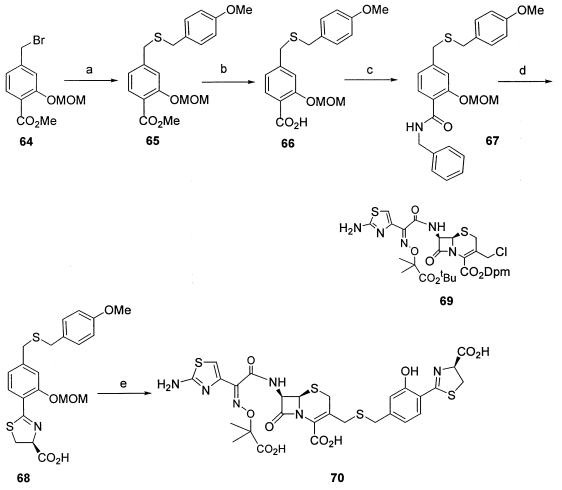
Dihydroaeruginoic acids **18** and **19** were synthesized according to Ankenbauer et al.,[37] and used to acylate directly (3S)-*trans*-3-amino 4-methyl 2-oxoazetidine-1-sulfonic acid **10**[38] (Scheme 3).

The same structural motif was also used in the construction of **70** (Scheme 4) where a 3-thioether linkage was used at the attachment site.

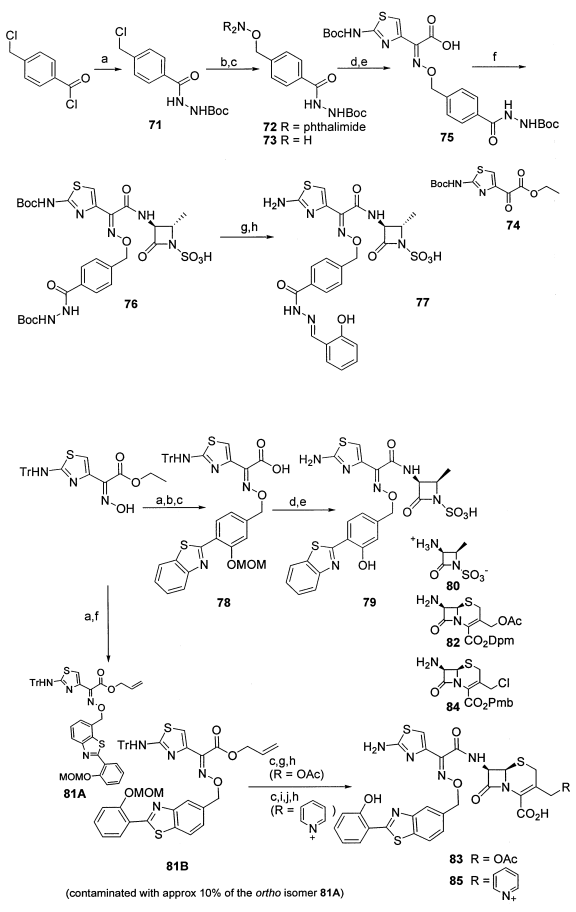
A frequently used mode of linkage for siderophores to both monobactams and to cephalosporins is the oxime ether α to the amide bond between 2-aminothiazole acetic acid and the β-lactam.[39–44]



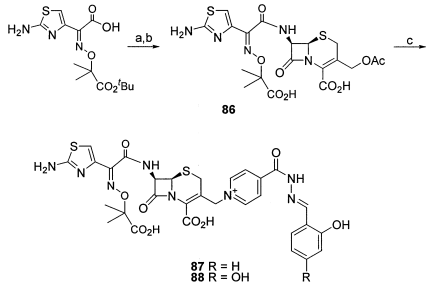
Scheme 3. (a) NHS, DCC (b) **10**, DBU (c) $(Bu)_4N^+SO_3H^-$.



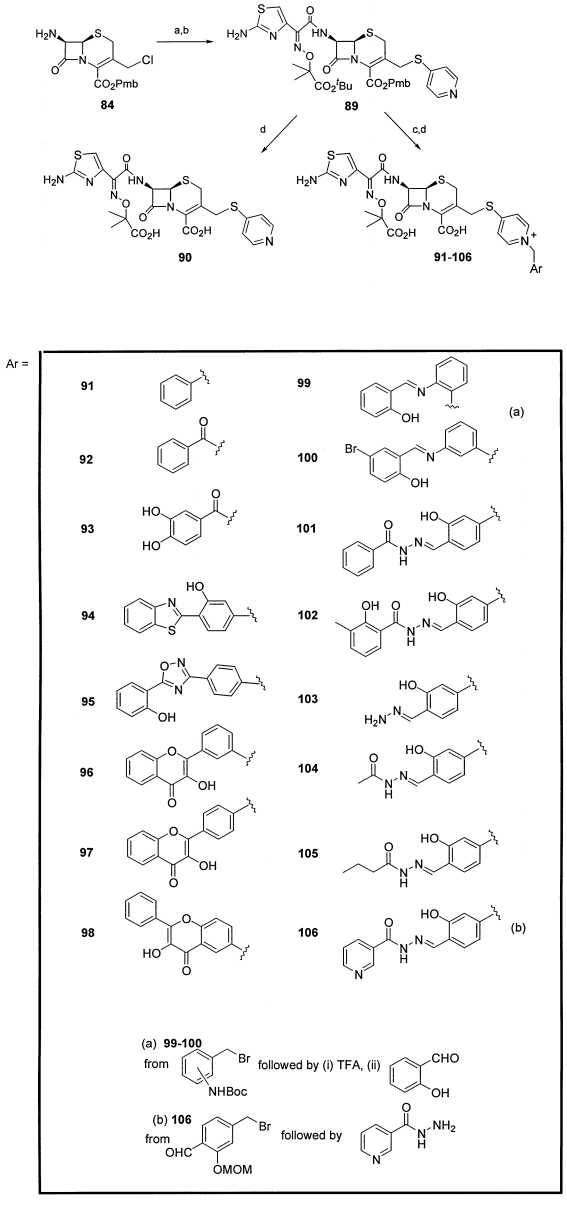
Scheme 4. (a) NaH, *p*-$MeOC_4H_4CH_2SH$, DMF, NaI (b) (i) NaOH, H_2O/THF (ii) H^+ (c) $BnNH_2$, EDCl, Et_3N, CH_2Cl_2 (d) (i) Tf_2O, pyridine, −50–0 °C (ii) L-Cysteine methyl ester, −30 °C (iii) NaOH, H_2O/THF (iv) H^+ (e) (i) TFA, anisole (ii) **69**, K_2CO_3, DMF, NaI (iii) TFA, anisole.



Scheme 5. A(a)′butyl carbazate, DIEA (b) *N*-hydroxypthalimide, K_2CO_3, NaI (c) H_2NNH_2, EtOH (d) **74**, EtOH, AcOH (e) 50% NaOH, Δ (f) **10**, DCC, HOAt, DMAP, DMF (g) TFA (h) salicylaldehyde, EtOH, AcOH. B(a) allyl alchohol, NaOEt, 60 °C (b) **33**, K_2CO_3, KI, DMF, rt (c) (i) $(Ph_3P)_4Pd$, $(Bu)_3SnH$, THF (ii) AcOH, CH_3CN (d) DCC, HOAt, **80** , Et_3N, DMAP, DMF, rt (e) 3N HCl, THF-H_2O, rt (f) **37**, K_2CO_3, Kl, KMF, rt (g) **82**, $POCl_3$, pyr, 0 °C (h) TFA, anisole, CH_2Cl_2 (i) **84** $POCl_3$, pyr, Et_3N, CH_2Cl_2 (j) pyr, Nal, THF.



Scheme 6. (a) DCC, HOAt, **82**, THF (b) 5:1 TFA-anisole, 0 °C to rt (c) (i) $CF_3CON(Me)_3$, CH_2Cl_2 (ii) $(Me)_3SiI$ (iii) THF (iv) **29** (for **87**) or **30** (for **88**) CH_3CN.



Scheme 7. (a)(i) 1 N NaOH (ii) 4-mercaptopyridine (b) ref. 13 (c) Ar(MOM)CH_2X (d) 5:1 TFA-anisole.

Bromomethyl derivatives of acylhydrazone **26** and benzthiazole **20** were incorporated as oximinoethers into, respectively, the *trans* (**77**) and *cis* (**79**) monobactams; **20** was additionally incorporated into the cephalosporins **83** and **85** (Scheme 5).

The key intermediate, aminothiazole derivative **78**, was prepared by transesterification of the commercially available ethyl ester to the allyl ester, followed by alkylation of the oxime with bromide **33**, and finally Pd[0]-promoted deallylation. Acid **78** was coupled to **80** to provide monobactam **79**. The cephalosporin synthesis began with the corresponding transesterication and alkylation reactions. Facile separation of the alkylation products **81A** and **81B** on silica gel provided the individual regiomeric benzthiazole derivatives in a 1:10 ratio. Only the major isomer **81B** was carried forward to cephalosporins **83** and **85**.

Ceftazidime, as well as a variety of siderophore–cephalosporin conjugates, exploit the beneficial properties of a 3-heterocyclic onium group for enhancing Gram-negative activity.[35,45–49] Isonicotinyl hydrazones **87** and **88** were synthesized by direct quaternization of the 3-acetoxy cephalosporin derivative **86**, by the method of Brown et al.[47] (Scheme 6).

For the majority of the bromomethyl derivatives, attachment to the cephalosporin nucleus at the 3-methylene could be achieved by a convergent route from intermediate **89** (Scheme 7). We also synthesized **90**, **91** and **92** as control analogues lacking the siderophore, and **93** as a positive control.[12,13]

Antimicrobial activity

Siderophores **24** and **25** that were active as determined by growth promotion alone were able to give rise to antibiotics (**104** and **105** respectively) having antipseudomonal activity. Several additional analogues in this class had modest activity against *E. coli* strains, although this activity was *tonB*-insensitive. In general, we observed the *E. coli* strains to be more susceptible than *P. aeruginosa* strains to all our novel compounds.

Mixed results were obtained from siderophores determined to be positive in both assays. Chimeric compounds in this class included inactive (**62**, **63**, **102**) as well as active (**77**, **87**, **88**, **101**, **106**) analogues against *P. aeruginosa* and *E. coli*. Of these, isonicotinyl hydrazone **87** alone gave the expected profile for *tonB*-sensitive transport. Two additional compounds in this class, **70** and **94**, were active only against *E. coli*.

Hydrazone **23**, the associated siderophore of **103** was inactive in both assays, yet the antibiotic conjugate showed Gram-negative activity comparable to compounds (e.g. **87**, **101**) with strong siderophores.

The MIC values of the most active antipseudomonal compounds, **104** and **106**, approached the MIC value for ceftazidime, but were independent of any influence of the conjugate siderophores. When tested against the hypersusceptible *P. aeruginosa* ATCC 35151 strain that is considered to be membrane-permeable, all three compounds were still over an order of magnitude more active than in the wild type PAO-1. This indicated that for these compounds permeability remained an obstacle to high activity.

Unexpected results were obtained when we tested our conjugates against organisms intended to serve as controls. When tested against the *tonB* mutants of both *E. coli* and *P. aeruginosa*, several compounds showed increased rather than decreased susceptibilities. However, similar results have been reported for the β-lactams carbenicillin and cefepime, and can be rationalized by considering mechanisms for efflux as well as influx of drugs through the membrane.[45–49] Upregulation of the MexAB-OprM efflux pump system is a significant mechanism of *P. aeruginosa* resistance to β-lactam drugs. While *tonB* is not essential for the expression of MexAB-OprM resistance, this energy-coupling protein nonetheless facilitates MexAB-OprM function. Mutants deficient in *tonB* have been shown to be orders of magnitude more sensitive to drugs from several structural classes. This sensitivity has been attributed to loss of TonB enhancement of pump activity.[50] Thus the effect of increased drug concentration (from compromised efflux pumps) might have offset the effect of decreased penetration (from loss of siderophore receptor activity) to give rise to the MIC values measured for many of the conjugates in Table 3. A second unexpected result was the robust antistaphylococcal activity of compounds **79**, **83** and **85**, since Gram-positive activity in β-lactams is often inferior to Gram-negative activity.[51] Again, there is some precedent for the *S. aureus* susceptibility: the benzthiazole nucleus has been reported to impart improved activity to cephalosporins,[51] carbapenems,[25] and carbacephems against both methicillin sensitive (MSSA) and resistant (MRSA) *S. aureus*.[53] We did not observe any significant activity in MRSA strains, however, suggesting that the mechanism of methicillin resistance was still maintained against these compounds.

We next directed our attention to the nature of the membrane transport of the siderophores when covalently bound to the antibiotic core. We focused on three representative compounds that, despite attachment to promising siderophores, were inactive (**63**), weakly active (**70**), or *tonB* insensitive (**70** and **104**). The results are shown in Figure 1.

Siderophore **19** was re-examined as its conjugate **63** in both siderophore assays. Compound **63** neither supported growth in low iron nor competed with **5**, although the conjugate was present at concentrations (50–100 μg/mL) above those required for the siderophoric effects of **19** (30 μg/mL). At 50 μg/mL, **63** had no detectable bactericidal effects. Attachment of **24** (active in the growth promotion assay at 30 μg/mL) to a cephalosporin (**104**) abrogated the siderophoric properties of the acylhydrazone. This observation confirmed and explained our observation that the Gram-negative activity of **104** was insensitive to *tonB*. More complex results were obtained with **70** in which we had attached

Table 3. Antibacterial activity of siderophore-β-lactam conjugates in wild-type and *tonB* mutants of *E. coli* and *P. aeruginosa*, hypersusceptible *P. aeruginosa* ATCC 35151, and *S. Aureus*. Details of the assay are given in Experimental

Category	Compound	Strains, MIC in μg/mL					
		E. coli ATCC 25922	*E. coli* Δ *tonB*	*P.aeruginosa* PAO-1	*P. aeruginosa* Δ *tonB*	*P. aeruginosa* ATCC 35151	*S. aureus* ATCC 29213
Positive in growth assay	**79**	12.5	6.25	>50	>50	>50	6.25
	83	12.5	3.13	>50	25	0.4	0.78
	85	>50	>50	>50	>50	3.13	1.56
	94	6.25	6.25	50	25	0.2	0.4
	96	12.5	12.5	>50	3.13	0.4	3.13
	97	6.25	6.25	50	25	<0.1	6.25
	98	25	12.5	>50	25	1.56	6.25
	99	12.5	12.5	>50	>50	<0.1	50
	100	1.56	0.78	50	>50	<0.1	12.5
	104	1.56	0.78	3.13	3.13	<0.1	6.25
	105	3.13	3.13	12.5	6.25	<0.1	12.5
Positive in both assays	**62**[a]	>50	>50	>50	ND[b]	ND	>50
	63[a]	>50	>50	>50	ND	ND	>50
	70	12.5	6.25	50	50	<0.1	>50
	77	12.5	6.25	12.5	25	0.2	6.25
	87	3.13	25	12.5	>50	<0.1	50
	88	12.5	12.5	12.5	25	0.2	25
	94	12.5	12.5	>50	25	0.78	1.56
	101	3.13	3.13	12.5	12.5	<0.1	12.5
	102	>50	>50	>50	>50	25	>50
	106	3.13	3.13	6.25	0.78	<0.1	12.5
Negative in both assays	**103**	3.13	1.56	25	6.25	<0.1	6.25
Controls	**90**	1.56	0.78	25	12.5	<0.1	3.13
	91	0.78	0.4	25	6.25	<0.1	3.13
	92	1.56	0.78	25	6.25	<0.1	6.25
	93	0.2	1.56	0.78	12.5	<0.1	12.5
	Ceftazidime	0.13–0.25	0.13	2–4	0.25	0.03	8

[a]Microbroth dilution MIC.
[b]ND = not done.

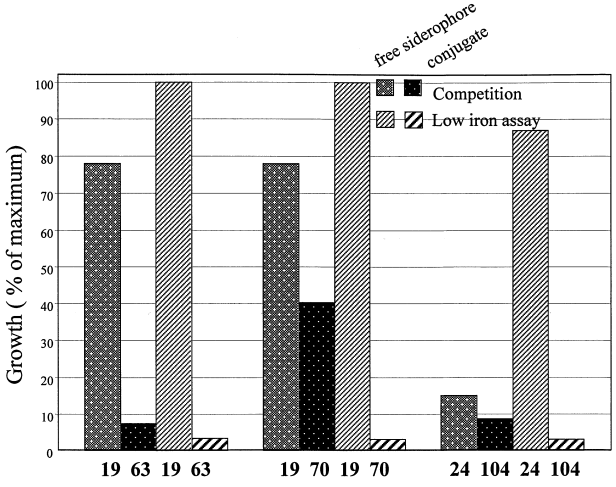


Figure 1. Comparison, in both indices of activity, of the siderophoric effects of **19** and **24**, first as discrete compounds and then after covalent attachment to antibiotic core structures. The growth in low iron data assay data represent a single experiment; competition assays were performed in triplicate. Details of the assay are given in Experimental.

thiazoline siderophore **19** to a cephalosporin at C-3, a position others have used for catechol siderophores: Although **70** no longer supported the growth of PGO 2812, it did afford modest protection against **5**. The siderophore was apparently recognized at the cognate receptor(s), but either not transported across the outer membrane, not ligated with sufficient iron to support growth, or both.

Conclusions

By the use of our assays, novel noncatechols have been identified that can function as siderophores in Gram-negative bacteria. Most of these compounds have an embedded salicylimine motif within their structure. In general, more active conjugate compounds were obtained using siderophores that had shown activity in both assays than were obtained from siderophores active in growth promotion alone. Significant bactericidal activity was not, however, generally attributable to facilitated transport. The nature of the linkage of the siderophore to the antibiotic core did not appear to influence the activity of the conjugate.

Thus, our screens identified a wide variety of novel siderophores, but did not predict which of these compounds would serve as escorting agents for β-lactam antibiotics. Indeed, many of the most effective compounds lost their siderophoric capabilities when covalently linked to the β-lactam. In most cases the siderophore-β-lactam conjugates retained good activity against the hypersusceptible ATCC 35151 strain. Activity against *S. aureus* ranged from modest to very good. Taken together, the ATC 35151 and *S. aureus* results

suggest that the direct effects of the antibiotic moieties at their molecular targets, the penicillin-binding proteins (PBPs), were unimpaired by the siderophores. The fact that antibiotic potencies were largely retained, or even improved, in the *tonB* mutants of both *E. coli* and *P. aeruginosa* raised the possibility that the MexAB/OprM efflux system may have contributed to the activities of the siderophore-antibiotic conjugates. These intriguing possibilities are being investigated.

Experimental

Chemistry

General. Unless otherwise stated, all reactions were run under a nitrogen atmosphere. THF was distilled from benzophenone ketyl; all other anhydrous solvents were obtained from Aldrich in sure-seal® bottles and stored under a nitrogen atmosphere. Commercially-obtained reagents were of the highest available purity. NMR spectra were taken on a GE QE-300 MHz or Bruker 400 MHz spectrometer; with proton chemical shifts reported in ppm referenced to tetramethylsilane. Mass spectra were taken on a Finnigan SSQ-3000 (low resolution electrospray), Micromass Quattro II Tandem Quadrupole (low resolution electrospray), Micromass 70 SEQ (EI, CI, low and high resolution FAB), or Perceptive Biosystems Mariner Electrospray TOF (high resolution electrospray) instrument. Organic solutions were dried over anhydrous $MgSO_4$ unless otherwise stated. Volatiles were removed in vacuo on a rotary evaporator at ca. 1–10 Torr. HPLC purifications on the Beckman Gold Nouveau system were performed on a 250×21 mm 10 μm 330 Å Phenomenex Jupiter octadecylsilane (ODS) column; HPLC purifications on the Gilson system were performed on a 250×21 mm 10 μm 300 Å Phenomenex Jupiter ODS column. Analytical HPLC data were obtained on the Beckman system using a Phenomenex Columbus 250×4.6 mm 5 μm 100 Å ODS column. HPLC eluant A was 0.05% TFA in water; HPLC eluant B was 0.05% TFA in CH_3CN. Preparative silica gel chromatography was performed under medium-pressure flash conditions[54] with Merck 40–63 μm silica or on a Biotage system using pre-packed 32–63 μm 60 Å silica cartridges.

***trans*-3-[2-(3,4-Dihydroxyphenylthioacetyl)amino]-4-methyl-2-oxoazetidine-1-sulfonic acid triethylammonium salt (1).** Catechol (2.6 g, 110 mmol) was dissolved in 40 mL of water. To it thiourea (1.52 g, 20 mmol) was added at room temperature. A solution of potassium ferricyanide (13.0 g, 40 mmol) and sodium acetate (20.0 g, 240 mmol) in 60 mL of water was added to the solution, and additional sodium acetate (20 g) was added to the mixture. After stirring overnight, the reaction mixture was dissolved in 1N HCl, and the insoluble material was filtered off. To the aqueous filtrate were added sodium acetate (20.0 g) and water (40 mL). The mixture was stirred to precipitate a salt that was filtered and dried in vacuo to give 2.67 g. The reaction was repeated twice more, for a total of 8.77 g (11%) of **6**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.77 (3H, s), 6.78–6.82 (2H, m), 6.83–6.84 (1H, bs). To a solution of **6** (8.77 g, 40 mmol), stirred in 144 mL of 1 N NaOH for 15 min, was added *t*-butyl bromoacetate (5.81 mL, 40 mmol), and the reaction mixture stirred for 3 h, then extracted four times with ethyl acetate. The combined extracts were washed with NaCl, dried, and concentrated in vacuo to a residue that was purified by silica gel chromatography (10:1, hexane:ethyl acetate) to give 5.67 g (62%) of **7**. *t*-Butyl ester **7** (123 mg, 0.53 mmol) was treated with 1 mL of TFA for 1.5 h. The reaction mixture was concentrated in vacuo, and the residue dissolved in 2 mL of THF. To this solution, DCC (119 mg, 0.58 mmol) and NHS (67 mg, 0.58 mmol) were added. After stirring for 2 h, the precipitate was removed by filtration, and the filtrate was added to a solution of monobactam **10** (81 mg, 0.45 mmol) and triethylamine (82 μl, 59 mmol) in 1 mL water. The reaction mixture was stirred at room temperature for 3 h, then concentrated in vacuo, and the residue purified by chromatography on a Sephadex LH-20 column (MeOH) to give 124 mg (59%) of **1**. ^{1}H NMR (300 MHz, CD_3OD) δ 1.28 (9H, t, J = 7.0 Hz), 1.48 (3H, d, J = 6.0 Hz), 3.18 (6H, q, J = 7.0 Hz), 3.44 (2H, s), 3.82–3.84 (1H, m), 4.38 (1H, d, J = 3.0 Hz), 6.71–6.72 (1H, m), 6.82–6.84 (1H, m), 6.91–6.92 (1H, m). HRMS, m/z for $C_{12}H_{14}N_2O_7S_2$ calculated, 362.2117, found, 361.0181(MH^-).

***trans*-3-[2-(3,4-Dihydroxyphenylcarbonyl)amino-2-(aminothiazol-4-yl)acetyl]-4-methyl-2-oxoazetidine-1-sulfonic acid (2).** Ethyl 2-amino-α-(*N*-(*t*butoxycarbonyl)amino)-4-thiazole acetate (1.23 g, 4.0 mmol) and diisopropylethylamine (1.96 mL, 11.2 mmol) were stirred in 12 mL CH_2Cl_2, and allyl chloroformate (1.62 mL, 15 mmol) in 2 mL CH_2Cl_2 was added slowly. The mixture was stirred at room temperature for 3 h. The crude product was isolated and purified by silica gel chromatography (8:1 hexane:ethyl acetate) to give 1.66 g (75%) of **107**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (3H, t, J = 7.3 Hz), 1.44 (9H, s), 4.16 (2H, q, J = 7.3 Hz), 4.70 (2H, d, J = 5.0 Hz), 4.88 (2H, s), 5.26 (1H, m), 5.32–5.34 (1H, m), 5.88–6.12 (1H, m), 7.00 (1H, s).

The 2-allyloxycarbonyl amine **107** (143 mg, 0.37 mmol) was stirred with 0.5 mL TFA for 20 min. Excess acid was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with sodium bicarbonate, dried, and concentrated in vacuo to give the crude α-amine (99 mg, 94%) as a residue that was dissolved in 1 mL DMF. To this solution 3,4-di-(methoxymethyloxy)-benzoic acid **11** (102 mg, 0.42 mmol), HBTU (158 mg, 0.42 mmol) and *N*-methylmorpholine (46 μl, 0.42 mmol) were added. The reaction mixture was stirred overnight, then concentrated in vacuo. The crude product was purified by silica gel (4:1 hexane:ethyl acetate) to give 145 mg (77%) of **9**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.28 (3H, m), 3.44 (3H, s), 3.48 (3H, s), 4.06–4.20 (2H, m), 4.70 (2H, d, J = 5.0 Hz), 5.20 (2H, s), 5.28 (2H, s), 5.20–5.44 (2H, m), 5.86–5.98 (2H, m), 6.99 (1H, s), 7.10 (1H, d, J = 10.0 Hz), 7.32–7.36 (1H, m), 7.51–7.53 (1H, m), 7.62 (1H, d, J = 2.0 Hz), 9.00 (1H, bs).

Compound **9** (173 mg, 0.34 mmol) was refluxed in 2 mL 0.5N NaOH for 1 h. After cooling, the reaction mixture

was acidified to pH ~2 with 0.1 N HCl and extracted into ethyl acetate. The organic layer was washed with water, dried, and concentrated in vacuo to give 141 mg (87%) of the free acid that was coupled to *trans* β-lactam **10** as described for **1**. To the crude amide (125 mg, 0.17 mmol) in 0.5 mL CH_2Cl_2 was added triphenylphosphine (2 mg), tetrakis(triphenylphosphine) palladium (2 mg), and then 2-ethyl octanoic acid (28 mg, 0.19 mmol), and the reaction mixture stirred for 2 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue purified on Sephadex LH-20 column (CH_3OH) to give 50 mg (45%) of **108**. ^{1}H NMR (300 MHz, $CDCl_3$) 1.30 (9H, t, J = 7.3 Hz), 1.50 (3H, d, J = 8.5 Hz), 3.10 (6H, q, J = 7.3 Hz), 3.46 (6H, two s), 4.00–4.06 (1H, m), 4.36–4.42 (1H, m), 5.20 (4H, two s) 5.60–5.70 (1H, m), 6.40 (2H, bs), 6.48–5.02 (1H, m), 7.08–7.12 (1H, m), 7.54–7.60 (1H, m), 7.66–7.68 (1H, m), 7.94–8.04 (1H, m), 8.54–8.60 (1H, m).

Compound **108** (50 mg, 0.08 mmol) was stirred with 1:1 TFA:CH_2Cl_2 at room temperature for 30 min. and concentrated in vacuo. The residue was purified on a Sephadex LH-20 column (CH_3OH) to give 8 mg (22%) of **2**. ^{1}H NMR (300 MHz, CD_3OD) δ 1.52 (3H, d, J = 8.5 Hz), 4.16–4.22 (1H, m), 4.38–4.44 (1H, m), 4.80 (1H, s), 5.78 (1H, s), 6.78–6.80 (1H, m), 7.30–7.40 (2H, m). MS, m/z 470.4 (M^-).

***trans*-3-[α-(4-Ethyl-2,3-dioxopiperazine-1-carbonyl)-3-(3,4-dihydroxyphenyl)-L-alanine]amino-4-mehtyl-2-oxoazetidine-1-sulfonic acid (3).** L-DOPA (0.99 g, 5.0 mmol) was refluxed in 1,1,1,3,3,3-hexamethyldisilazane (8 mL) and trimethylsilyl chloride (2 mL) for 2 h. After cooling, excess reagent was removed by concentration in vacuo. The residue was dissolved in dry CH_2Cl_2 (10 mL) and cooled to 0 °C. A solution of 4-ethyl-2, 3-dioxopiperazine-1-carbonyl chloride **12** (1.12 g, 5.5 mmol) in 8 mL CH_2Cl_2 was added dropwise. The mixture was stirred for 1.5 h at room temperature, and concentrated in vacuo. The residue was dissolved in 2:1 acetone:water (30 mL) at pH 1~2 with vigorous stirring for 18 min. After the removal of acetone, the aqueous solution was extracted with 1:1 ethyl acetate:*n*-butanol. The aqueous phase was saturated with NaCl and extracted again with 1:1 ethyl acetate:*n*-butanol. The combined extracts were dried and concentrated in vacuo to give 0.85 g (49%) of **109**. ^{1}H NMR (400 MHz, CD_3OD) 1.19 (3H, t, J = 7.2 Hz), 2.96 (1H, dd, J = 6.8, 7.2 Hz), 3.08 (1H, dd, J = 4.9, 8.1 Hz), 3.50 (2H, q, J = 7.2 Hz), 3.59–3.62 (2H, m), 3.98–4.02 (2H, m), 4.56–4.60 (1H, m), 6.56 (1H, dd, J = 2.1, 5.9 Hz), 6.67–6.69 (1H, m), 9.29 (1H, d, J = 7.1 Hz).

Coupling of **109** to *trans* β-lactam **10** as described for **1** gave **3** in 84% yield. ^{1}H NMR (400 MHz, DMSO) δ 1.06 (3H, t, J = 7.1 Hz), 1.17 (9H, t, J = 7.3 Hz), 1.35 (3H, d, J = 6.1 Hz), 2.82–2.83 (1H, m), 2.86–2.87 (1H, m), 3.09 (6H, q, J = 7.3 Hz), 3.37 (2H, q, J = 7.1 Hz), 3.51–3.58 (3H, m), 3.85–3.88 (2H, m), 4.26–4.28 (1H, m), 4.40–4.42 (1H, m), 6.43–6.45 (1H, m), 6.57–6.61 (2H, m) 8.71 (1H, s), 8.74 (1H, s), 8.93 (1H, d, J = 7.8 Hz), 9.08 (1H, d, J = 7.4 Hz). HRMS, m/z $C_{20}H_{25}N_5O_{10}S$ calculated, 527.2255, found, 526.1246 (M^-).

***trans*-3-[α-(4-Ethyl-2,3-dioxopiperazine-1-carbonyl)-3-(3,4-dihydroxyphenyl)-D-alanine]amino-4-methyl-2-oxoazetidine-1-sulfonic acid triethylammonium salt (4).** D-DOPA (500 mg, 2.4 mmol) was converted to 129 mg (50%) of **4** according to the procedure described for **3**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.16 (3H, t. J = 7.5 Hz), 1.24 (9H, t, J = 7.3 Hz), 1.46 (3H, d, J = 8.5 Hz), 2.94–2.96 (2H, m), 3.18 (6H, q, J = 7.3 Hz), 3.48 (2H, q, 7.5 Hz), 3.58–3.62 (2H, m), 3.85–3.86 (1H, m), 3.38–3.40 (2H, m), 4.32–4.39 (1H, m), 4.49–4.51 (1H, m), 6.53–6.54 (1H, m), 6.65–6.66 (2H, m). HRMS, m/z for $C_{26}H_{25}N_5O_{10}S$ calculated, 527.2255, found, 526.1246 (M^-).

***trans*-3-[α-(4-Ethyl-2,3-dioxopiperazine-1-carbonyl)-3,4-D,L-dihydroxyphenylglycine]amino-4-methyl-2-oxoazetidine-1-sulfonic acid triethylammonium salt (5).** α-Amino-(3,4-dihydroxyphenyl)acetic acid HBr[55] (73 mg, 0.28 mmol) was converted to 61 mg, (36%) of **5** following the same procedures described for **3**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.31 (3H, t, J = 7.2 Hz), 1.41 (9H, t, J = 7.3 Hz), 1.60–1.64 (3H, m), 3.32 (6H, q, J = 7.3 Hz), 3.64 (2H, q, J = 7.2 Hz), 3.75–3.76 (2H, m), 3.93–4.06 (1H, m), 4.14–4.15 (2H, m), 4.56–4.57 (1H, m), 5.35–5.36 (1H, m), 6.86–6.88 (2H, m), 6.97–6.99 (1H, m). HRMS, m/z for $C_{19}H_{23}N_5O_9S$ calculated, 513.2099, found, 512.1088 (M^-).

2-(2-Methoxymethyloxy-4-methyphenyl) benzthiazole (32). To an icebath-cooled solution of 2-(2-hydroxy-4-methylphenyl) benzthiazole (50.0 g, 20.7 mmol) in 50 mL THF was added 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU, 3.1 mL, 20.7 mmol) followed by a solution of chloromethyl methyl ether (1.75 g, 21.7 mmol) in 10 mL THF. The reaction mixture was allowed to equilibrate to ambient temperature and was stirred overnight. The THF was removed in vacuo and the residue dissolved in CH_2Cl_2, washed successively with saturated NH_4Cl, $NaHCO_3$, NaCl, dried, and concentrated in vacuo to give 5.78 g (99%) of **32** as a waxy solid that was homogenous by tlc (R_F = 0.25 in 100% hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (3H, s), 3.57 (3H, s), 5.51 (2H, s), 6.99 (1H, d, J = 8.1 Hz), 7.07 (1H, s), 7.46–7.48 (2H, m), 7.91 (2H, dd, J = 8.1, 9.7 Hz), 8.41 (1H, d, J = 8.1 Hz). MS m/z 286.1 (MH^+)

2-(2-Methoxymethyloxy-4-bromomethylphenyl) benzthiazole (33). To a solution of **32** (5.78 g, 20 mmol) in 200 mL CCl_4 was added *N*-bromosuccinimide (3.98 g, 22.3 mmol) followed by 2,2-azobisisobutyronitrile (AIBN, 333 mg). The solution was refluxed while being illuminated by a 150 W halogen light for 16 h, and additional AIBN was added at 6 and 12 h. The reaction mixture was washed with water, the aqueous phase re-extracted with CH_2Cl_2, and the combined organic phases washed with NaCl, dried, and concentrated in vacuo to an oily residue. The product was purified on flash silica (99:2 hexane:ethyl acetate) to give 1.1 g of recovered starting material, followed by elution with 96:5 hexane:ethyl acetate to give 3.47 g. (58%) of **33**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (3H, s), 4.52 (2H, s), 5.45 (2H, s), 7.26 (1H, d, J = 16.2 Hz), 7.38–7.40 (1H, m), 7.50 (2H, d, J = 16.0 Hz), 8.06 (2H, dd, J = 8.1,

8.8 Hz), 8.51 (1H, d, $J=8.1$ Hz). MS, m/z 363.9/365.9 (MH^+).

2-(2-Methoxymethyloxyphenyl)-2-methyl-benzthiazole (36A) and 2-(2-Methoxylmethyloxyphenyl)-4-methyl-benzthiazole (36B). To an icebath-cooled solution of *m*-toluidine (6.37 mL, 58.8 mmol) and triethylamine (8.20 mL, 58.8 mmol) in 20 mL dry CH_2Cl_2, anisoyl chloride (10.0 mL, 58.8 mmol) was added dropwise. The reaction mixture was allowed to equilibrate to ambient temperature overnight, and washed successively with 1 N HCl, saturated $NaHCO_3$, and NaCl, dried, and concentrated in vacuo to give 13.7 g (99%) of **34** as an off-white solid. MS, m/z 242.0 (MH^+).

Amide **34** was combined with Lawesson's reagent (13.2 g, 32.6 mmol) in 100 mL of toluene, and refluxed for 11 h. After cooling, a mixture of 98:3 hexane:ethyl acetate was added to the solution, and the product precipitated upon standing at room temperature for several hours. Recrystallization from EtOH–hexane gave 7.4 g (53%) of the thioamide **35**. MS, m/z 258.0 (MH^+).

To a suspension of **35** (7.4 g, 28.9 mmol) in 20 mL EtOH was added a solution of 7.5 M NaOH (30.8 mL) followed by an additional 62.5 mL of water. This suspension was added in 1 mL aliquots to a 90 °C solution of $K_3Fe(CN)_6$ (38 g, 115.6 mmol) in 200 mL of water. The reaction mixture was stirred an additional 90 min following complete addition, then cooled to room temperature, and the product filtered off. Recrystallization from MeOH gave 5.6 g (90%) of **110** as light brown crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 and 2.62 (3H, two s, 1:2 ratio), 4.02 and 4.06 (3H, two s), 6.99–7.25 (3H, m), 7.39–7.51 (2H, m), 7.77 (1H, d, $J=16.0$ Hz), 7.98 (1H, t, $J=15.5$ Hz), 8.49–8.52 (1H, m). MS, m/z 256.0 (MH^+).

To a −78 °C solution of **110** (3.45 g, 13.5 mmol) in 15 mL CH_2Cl_2 a solution of 1 M BBr_3 in CH_2Cl_2 (70 mL) was added dropwise over 1 h. The reaction mixture was allowed to equilibrate to ambient temperature overnight, then re-cooled to −78 °C and treated dropwise with MeOH until cessation of effervescence. The quenched reaction mixture was poured into 8% aqueous NaOH (50 mL), and the organic and aqueous phases separated. The aqueous phase was acidified with 5 N HCl and extracted with 4:1 CH_2Cl_2:MeOH. The combined organic phases were concentrated in vacuo to a bright yellow solid that was recrystallized from ethyl acetate–hexane to give 2.54 g (83%) of **111** that was ca. 4:1 by integration of the ^{1}H NMR methyl signals at δ 2.5 and 2.6. ^{1}H NMR (400 MHz, $CDCl_3$) 2.51 and 2.62 (3H, two s, 1:4), 6.89–7.90 (7H, m).

HPLC (60 to 90% B in A over 40 min): T_R 22 min and 23 min. MS, m/z 242.1 (MH^+).

The phenolic group of **111** (2.54 g, 11.2 mmol) was protected as described for **32**. Chromatography on silica gel (98:2 hexane:ethyl acetate) gave 1.84 g (58%) of **36**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 and 2.61 (3H, two s, 1:2), 3.54 and 3.56 (3H, two s, 1:2 ratio), 5.41 (2H, s), 7.14 (1H, s), 7.22 (2H, d, $J=3.5$ Hz), 7.40 (1H, dd, $J=1.6$, 1.9 Hz), 7.95 (1H, d, $J=8.2$ Hz), 8.53 (1H, d, $J=2.8$ Hz). MS, m/z 286.0 (MH^+), 308.2 (MNa^+).

2-(2-Methoxymethyloxyphenyl)-2-bromomethylbenzthiazole (37A) and 2-(2-))-4-methoxy-methyloxyphenyl bromomethylbenzthiazole (37B). Radical bromination of **36** (1.84 g, 6.4 mmol) was performed as described for **33**. Chromatography on silica gel using successively 99:2, then 96:5, hexane:ethyl acetate gave 2.2 g (94%) of **37**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.54 and 3.56 (3H, two s, 1:3 ratio), 4.62 and 4.71 (2H, two s 1:3 ratio), 5.37–5.40 (2H, two s), 7.15 (1H, s), 7.25 (2H, dd, $J=1.6$, 8.3 Hz), 7.37–7.42 (2H, m), 7.93–7.96 (1H,m), 8.53 (1H, d, $J=2.8$ Hz).

1-(2-[*N*-*tert*-Butoxycarbonyl]aminophenyl)methyl bromide (40). To a rapidly stirring solution of 2-aminobenzyl alcohol (5.00 g, 40.6 mmol) in CH_2Cl_2 (100 mL) was added di-*t*butyldicarbonate (9.75 g, 44.6 mmol) and DIEA (7.8 mL, 44.6 mmol). After 18 h the solution was washed with aqueous HCl (1 M, 3×35 mL) and water (25 mL). The organic phase was dried and concentrated to give 10.9 g of a brown oil. Chromatography (80:20 hexane:ethyl acetate) afforded 9.1 g (quantitative) of **38** as pale yellow oil. To a solution of this material (3.18 g, 14.2 mmol) in THF (50 mL) at −20 °C was added triphenylphosphine (4.49 g, 17.1 mmol), followed by *N*-bromosuccinimide (3.04 g, 17.1 mmol). After 3 h the solvent was removed in vacuo, and the residue chromatographed (90:10 hexane:ethyl acetate) to provide 3.14 g (77%) of **40** as a tan oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (9H, s), 4.53 (2H, s), 6.70 (1H, bs), 7.08 (1H, dt, $J=0.9$, 7.5 Hz), 7.30 (1H, dd, $J=1.4$, 7.6 Hz), 7.36 (1H, dt, $J=1.5$, 7.8 Hz), 7.85 (1H, d, $J=8.1$ Hz).

1-(3-[*N*-*tert*-Butoxycarbonyl]aminophenyl)methyl bromide (41). Intermediate **39** was prepared as described for **38** from 3-aminobenzyl alcohol (5.00 g, 40.6 mmol) to give 3.6 g (40%) of **39**. Bromination of **39** (230 mg, 1.03 mmol) as described for **40** provided 257 mg (87%) of **41** as an orange oil that solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (9H, s), 4.47 (2H, s), 6.63 (1H, bs), 7.07 (1H, d, $J=7.1$ Hz), 7.23–7.28 (2H, m), 7.53 (1H, s).

4-Bromomethyl-2-(methoxymethyloxy)benzaldehyde (44). 4-Methyl salicylic acid (1.86 g, 12.2 mmol) was dissolved in 20 mL diethyl ether. To it EtOH (5 mL, 122 mmol) was added, the flask was cooled in an icebath. DCC (3.02 g, 14.6 mmol) was added in portions. After the addition the mixture was stirred at room temperature overnight. Solid was removed by filtration, the filtrate was washed with water and dried to give a crude methyl ester. The ester was dissolved in a solution of 20 mL dry CH_3CN and diisopropylethylamine (4.7 mL, 27.0 mmol). Chloromethyl methyl ether (2 mL, 26.3 mmol) in CH_3CN solution was added dropwise. The mixture was refluxed overnight. Solvents were removed in vacuo, and the residue was purified by silica gel column (80:10 hexane:ethyl acetate) to give 2.26 g (88%) of **106**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (3H, s), 3.52 (3H, s), 3.87 (3H, s), 5.24 (2H, s), 6.85 (1H, d, J = 7.9 Hz), 7.00 (1H, s), 7.70 (1H, d, J = 7.9 Hz).

Methyl ester **106** (13.4 g, 63.7 mmol) was refluxed with 1N NaOH (95 mL) and EtOH (95 mL) for 2 h. The mixture was cooled to room temperature. EtOH was removed in vacuo, and the aqueous solution was washed with CH_2Cl_2, then acidified to pH 1 with 0.1N HCl layered with ethyl acetate. The organic layer was separated and dried to give 11.24 g (90%) of **42** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (3H, s), 3.56 (3H, s), 5.41 (3H, s), 6.99 (1H, d, J = 8.0 Hz), 7.07 (1H, s), 8.06 (1H, d, J = 8.0 Hz).

Acid **42** (5.03 g, 25.7 mmol) was dissolved in 65 mL pyridine. The flask was cooled in an ice-bath. To it methanesulfonyl chloride (2.2 mL, 28.3 mmol) was added dropwise. The mixture was allowed to stir for 1 h. Ammonia gas was bubbled for 2 min. An additional 13 mL of methanesulfonyl chloride was added slowly, and the reaction mixture stirred overnight. After concentration in vacuo, the residue was partitioned between 0.01N HCl and ethyl acetate. The organic phase was washed successively with saturated $CuSO_4$ and water, dried, and concentrated in vacuo. The residue was purified on silica gel (90:10 hexane:ethyl acetate) to give 3.4 g (75%) of **43**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (3H, s), 3.52 (3H, s), 5.27 (2H, s), 6.86 (1H, d, J = 8.0 Hz), 7.03 (1H, s), 7.44 (1H, d, J = 8.0 Hz).

Nitrile **43** (3.4 g, 19.2 mmol) was brominated as described for **33**. Purification on silica gel (96:5 then 90:10 hexane:ethyl acetate) gave 2.57 g (52%) of bromide **112**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.54 (3H, s), 4.43 (2H, s), 5.31 (2H, s), 7.10 (1H, q, J = 1.5, 7.9 Hz), 7.26 (1H, d, J = 1.5 Hz), 7.54 (1H, d, J = 7.9 Hz).

Bromide **112** (175 mg, 0.68 mmol) was dissolved in 1 mL dry toluene under nitrogen. To it DIBAL (1.5 M in toluene, 680 μl) was introduced at 0 °C. After the addition, the mixture was stirred at room temperature for 2 h, then 1 N HCl was added to acidify the mixture to pH 2, and stirring was continued for 15 min. The reaction mixture was extracted with ethyl acetate, washed with water, dried, and concentrated in vacuo to give 126 mg (71%) of aldehyde **44**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (3H, s), 4.45 (2H, s), 5.32 (2H, s), 7.10 (1H, d, J = 8.0 Hz), 7.25 (1H, d, J = 1.3 Hz), 7.81 (1H, d, J = 8.0 z), 10.45 (1H, s).

Benzoyl (4-bromomethyl) salicylhydrazone (50). Aldehyde **44** (126 mg, 0.48 mmol) was stirred at ambient temperature with benzoyl hydrazide (65 mg, 0.48 mmol) in EtOH for 2 h, and 112 mg (43%) of **45** was collected by filtration. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (3H, s), 4.45 (2H, s), 5.32 (2H, s), 7.10 (1H, d, J = 7.9 Hz), 7.25 (1H, s), 7.81 (1H, d, J = 7.9 Hz), 10.45 (1H, s).

***N*-(4-Methyl)-benzoylsalicylamide (50).** To a solution of salicylamide (5.00 g, 36.4 mmol) in CH_2Cl_2 (100 mL) was added pyridine (3.12 g, 40.0 mmol). The reaction was cooled to 0 °C and *p*-toluoyl chloride (5.63 g, 36.4 mmol) added. The reaction was stirred at 0 °C for 30 min then allowed to stir at room temperature for 1 h. The resulting mixture was washed with 1N HCl, H_2O, NaCl, dried, and concentrated in vacuo. The resulting oil was purified on silica gel (50:50 hexane:ethyl acetate) to give 8.1 g (87%) of **50** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (3H, s), 5.77 (1H, bs), 6.38 (1H, bs), 7.23 (1H, d, J = 8.1 Hz), 7.32–7.38 (3H, m), 7.54 (1H, dt, J = 1.7, 7.8 Hz), 7.95 (1H, dd, J = 1.7, 7.8 Hz), 8.09 (2H, d, J = 8.2 Hz).

2-(4-Methylphenyl)-1,3-benzoxazin-4-one (51). Intermediate **50** (3.00 g 11.7 mmol) was heated in a heating mantle over its melting point (~200 °C) for 1 h. The reaction was cooled, and the resulting mass slurried in CH_2Cl_2 (100 mL), then extracted with water, NaCl, dried, and concentrated in vacuo. The resulting residue was purified on silica gel (80:20 hexanes:ethyl acetate) to give 1.21 g (43%) of **51** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (3H, s), 7.35 (1H, d, J = 8.0 Hz), 7.47–7.49 (2H, m), 7.74–7.78 (1H, m), 7.99 (1H, d, J = 8.1 Hz), 8.21–8.23 (1H, m), 8.29–8.31 (2H, m). MS, m/z 238.0 (MH^+).

3-(4-Methylphenyl)-5-(2-hydroxyphenyl)-1,2,4-oxadiazole (21). Intermediate **51** (1.20 g, 5.06 mmol) was suspended in absolute EtOH (25 mL). Sodium acetate (0.50 g, 6.07 mmol) and hydroxylamine hydrochloride (0.42 g, 6.07 mmol) were added to the suspension at ambient temperature. The reaction was stirred for 16 h, diluted with CH_2Cl_2 (75 mL), and washed with water, NaCl, dried, and concentrated in vacuo. The resulting solid was purified via chromatography (90:10 hexane:ethyl acetate) to give 0.77 g (64%) of **52** as a white, flocculant solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (3H, s), 7.04 (1H, dt, J = 0.7, 7.9 Hz), 7.15 (1H, d, J = 8.4 Hz), 7.34 (2H, d, J = 8.0 Hz), 7.52 (1H, dt, J = 1.6, 7.9 Hz), 7.99–8.02 (3H, m), 10.57 (1H, s). HRMS, m/z for $C_{15}H_{13}N_2O_2$ calculated, 253.0977, found, 253.0977 (MH^+).

3-(4-Bromomethylphenyl)-5-(2-methoxymethyloxyphenyl)-1,2,4-oxadiazole (52). To a solution of **21** (0.76 g, 3.16 mmol) in CH_2Cl_2 (25 mL) at 0 °C was added NaH (0.15 g, 3.80 mmol). The reaction was stirred at 0 °C for 30 min, then chloromethyl methyl ether (0.28 g, 3.50 mmol) was added. The reaction was warmed to ambient temperature and stirred for 2 h. The reaction was quenched with water and washed with NaCl, dried, and concentrated in vacuo to give 0.78 g (83%) of **113** as a white solid which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (3H, s), 3.56 (3H, s), 5.36 (2H, s), 7.16 (1H, t, J = 7.6 Hz), 7.29–7.32 (3H, m), 7.51–7.54 (1H, m), 8.07 (2H, d, J = 8.1 Hz), 8.16 (1H, dd, J = 1.6, 7.8 Hz). MS, m/z 297.1 (MH^+). Intermediate **113** (0.5 g, 1.69 mmol) was brominated according to the general procedure described for **33**. Chromatography (90:10 hexane:ethyl acetate) gave 200 mg (32%) of **52** as an off white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (3H, s), 4.55 (2H, s), 5.36 (2H, s), 7.17 (1H, dt, J = 0.9, 8.0 Hz), 7.31 (1H, d, J = 8.5 Hz), 7.52–7.56 (3H, m), 8.14–8.17 (3H, m).

6-Methyl-3-methoxymethyloxyflavone (57). To a solution of **55**[31] (500 mg, 2.0 mmol) in CH_2Cl_2 (20 mL) at

0 °C was added NaH (90 mg of a 60% suspension, 2.2 mmol) followed by chloromethylmethyl ether (180 L, 2.4 mmol). The solution was allowed to slowly warm to 23 °C over 2.5 h. The reaction mixture was diluted with NH_4Cl and ethyl acetate. The phases were separated, and the organic phase dried and concentrated in vacuo to give a colorless oil that was purified by chromatography (80:20 hexane:ethyl acetate) to give 500 mg (85%) of **57** as a white solid. $R_f = 0.17$ in 90:10 hexane:ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (3H, s), 3.02 (3H, s), 5.12 (2H, s), 7.40–7.44 (5H, m), 7.96–7.98 (3H, m).

6-Bromomethyl-3-methoxymethyoxylflavone (59). A solution of **57** (500 mg, 1.69 mmol) in CCl_4 (17 mL) was brominated with NBS (330 mg, 1.86 mmol) and AIBN (28 mg, 0.17 mmol) as described for **33** to give, purification on silica gel (90:10 hexane:ethyl acetate), 400 mg (63%) of **59** as a white solid. $R_f = 0.20$ in 90:10 hexane:ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (3H, s), 4.48 (2H, s), 5.11 (2H, s), 7.42–7.45 (5H, m), 7.64 (1H, dd, $J = 2.4$, 8.6 Hz), 7.94–7.97 (2H, m), 8.16 (1H, d, $J = 2.3$ Hz). HRMS, m/z for $C_{18}H_{16}O_4Br$ calculated, 375.0232, found, 375.0235 (MH^+).

3-Methoxymethyloxy-3′-methylflavone (58A). Intermediate **58A** was prepared from **56A**[33] (500 mg, 2.0 mmol) as described for **57** to give, after chromatography (80:20 hexane:ethyl acetate) 540 mg (92%) of **58A** as a white solid: $R_f = 0.12$ in 90:10 hexane:ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (3H, s), 3.15 (3H, s), 5.22 (2H, s), 7.34 (1H, d, $J = 7.4$ Hz), 7.43 (2H, t, $J = 7.7$ Hz), 7.56 (1H, d, $J = 8.4$ Hz), 7.64–7.74 (1H, m), 7.87–7.89 (2H, m), 8.28 (1H, d, $J = 8.0$ Hz).

3-Methoxymethyloxy-3′-bromomethylflavone (60). Intermediate **60** was prepared from **58A** (540 mg, 1.82 mmol) as described for **59** to give, after chromatography (90:10 hexane:ethyl acetate), 420 mg (62%) of **60** as a white solid. $R_f = 0.18$ in (90:10 hexane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.14 (3H, s), 4.60 (2H, s), 5.25 (2H, s), 7.43–7.45 (1H, m), 7.53–7.57 (3H, m), 7.68–7.71 (1H, m), 8.00–8.03 (1H, m), 8.12 (1H, s), 8.28 (1H, dd, $J = 1.6$, 8.0 Hz). HRMS, m/z for $C_{18}H_{16}O_4Br$ calculated, 375.0232, found, 375.0227(MH^+).

3-Methoxymethyloxy-4′-methylflavone (58B). Intermediate **58B** was prepared from **56B**[33] (500 mg, 2.0 mmol) as described for **58A** to give, after chromatography (80:20 hexane:ethyl acetate) 460 mg (78%) of **58B** as a white solid. $R_f = 0.24$ (90:10 hexane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.55 (3H, s), 7.09 (1H, s), 7.45 (2H, d, $J = 8.1$ Hz), 7.50–7.54 (1H, m), 7.68–7.83 (2H, m), 8.25–8.28 (2H, m), 8.35 (1H, dd, $J = 1.6$, 8.0 Hz).

3-Methoxymethyl-4′-bromomethylflavone (61). Intermediate **61** was prepared from **57C** (460 mg, 1.55 mmol), NBS (304 mg, 1.71 mmol) and AIBN (26 mg, 0.15 mmol) as described for **59** to give, after chromatography (90:10 hexane-ethyl acetate) 300 mg (51%) of **61** as a white solid. $R_f = 0.20$ (90:10 hexane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.07 (3H, s), 4.48 (2H, s), 5.16 (2H, s), 7.33–7.37 (1H, m), 7.45–7.49 (3H, m), 7.60–7.65 (2H, m), 7.98–8.00 (2H, m), 8.19 (1H, dd, $J = 1.5$, 8.0 Hz). HRMS, m/z for $C_{18}H_{16}O_4Br$ calculated, 375.0232, found, 375.0236 (MH^+).

***N*-(4*R*)-[[2-[2-(2-Hydroxy)phenyl]thiazolin-4-yl]carbonyl]-(3*S*,4*S*)-*trans*-3-amino-4-methyl-2-azetidinone-1-sulfonic acid, tetrabutylammonium salt (59).** To a rapidly stirring suspension of [R]-sodium dihydroaeruginoate **18**[36] (75 mg, 0.3 mmol) in THF (3 mL) in a flask under N_2, was added DCC (63 mg, 0.3 mmol) and NHS (53 mg, 0.46 mmol). The reaction mixture was stirred for 2 h and filtered. In a separate flask under N_2, DBU (46 μL, 0.3 mmol) was added to a suspension of **10** in CH_2Cl_2 (2 mL) for 5 min until a clear solution was obtained. The above filtrate was added to this solution, and the reaction mixture allowed to stir at ambient temperature for 18 h. The reaction was diluted with CH_2Cl_2 to 20 mL and was extracted into water (4×10 mL). The solution was neutralized to pH 7 with dilute aqueous NaOH, and tetrabutylammonium hydrogen sulfate (170 mg, 0.5 mmol) was added. The product was extracted with CH_2Cl_2 (3×15 mL) and concentrated in vacuo to provide 89 mg (46%) of **62** as the tetrabutylammonium salt. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (12H, t, $J = 7.3$ Hz), 1.41 (8H, sext., $J = 7.3$ Hz), 1.58–1.66 (11H, m), 3.21–3.26 (8H, m), 3.60–3.74 (2H, m), 3.87–3.99 (1H, m), 4.55–4.63 (1H, m), 5.34 (1H, t, $J = 8.9$ Hz), 6.87–6.92 (1H, m), 6.98 (1H, d, $J = 8.4$ Hz), 7.35–7.47 (3H, m), 11.84 (1H, bs). HRMS, m/z for $C_{14}H_{14}N_3O_6S_2$ calculated, 384.0324, found, 384.0339 (MH^+).

***N*-(4*S*)-[[2-[2-(2-Hydroxy)phenyl]thiazolin-4-yl]carbonyl]-(3*S*,4*S*)-*trans*-3-amino-4-methyl-2-azetidinone-1-sulfonic acid, tetrabutylammonium salt (63).** The epimeric amide was prepared in an analogous manner using (*S*)-sodium dihydroaeruginoate[36] **19** (47 mg, 0.19 mmol) and [*S*]-*trans*-3-amino-4-methyl-2-oxoazetidine-1-sulfonic acid[37] (32 mg, 0.18 mmol) to give 86 mg (80%) of **63** after chromatography (90:10 CH_2Cl_2:MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (12H, t, $J = 7.3$ Hz), 1.41 (8H, sext., $J = 7.3$ Hz), 1.60–1.64 (11H, m), 3.19–3.24 (8H, m), 3.63–3.70 (1H, m), 3.89–4.00 (1H, m), 4.55–4.63 (1H, m), 5.34 (1H, t, $J = 8.9$ Hz), 6.87–6.92 (1H, m), 6.99 (1H, d, $J = 8.3$ Hz), 7.31–7.40 (2H, m), 7.43–7.46 (1H, m). HRMS, m/z for $C_{14}H_{14}N_3O_6S_2$ calculated, 384.0324, found, 384.0335 (MH^+).

Methyl (4-((4-methoxyphenyl)methylthio)methyl)-3-(methoxymethyloxy) benzoate (65). Ester **64** was brominated as described for **33**, and the product (1.00 g, 3.5 mmol) was dissolved in DMF (5 mL), and to it NaH (0.15 g, 3.8 mmol) was added under nitrogen. The reaction was stirred for 20 min, then 4-methoxy-α-toluenethiol (0.59 g, 3.8 mmol) added followed by NaI (catalytic). The reaction was stirred for 2 h, H_2O added (20 mL), and the aqueous layer extracted with ethyl acetate (3×20 mL). The organic phases were combined, washed with H_2O, brine, dried, and concentrated in vacuo. Purification on silica gel (80:20 hexane:ethyl acetate) gave 0.43 g (34%) of **65** as an off white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (3H, s), 3.55 (4H, s), 3.79 (3H, s), 3.88 (3H, s), 5.25 (2H, s), 6.84 (2H, d, $J = 8.6$ Hz),

6.99 (1H, dd, $J=1.4$, 8.0 Hz), 7.1 (1H, d, $J=1.3$ Hz), 7.18 (2H, d, $J=8.6$ Hz), 7.74 (1H, d, $J=8.0$ Hz). MS, m/z 385 (MNa^+)

4-((4-Methoxyphenyl)methylthio)methyl)-3-(methoxymethyloxy)benzoic acid (66). 65 (0.42 g, 1.2 mmol) was dissolved in a solution of 1:1 THF-1N NaOH (10 mL) and EtOH (2 mL). The reaction was stirred at ambient temperature for 2 h, then extracted with Et_2O (10 mL). The aqueous layer was acidified with 1 N HCl to pH 3. The aqueous layer was extracted with CH_2Cl_2 (2×15 mL), the organic layers combined, dried, and concentrated in vacuo to give 0.37 g (90%) of **66** as a pale yellow solid which required no further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 3.58 (7H, s), 3.80 (3H, s), 5.41 (2H, s), 6.84 (2H, d, $J=8.7$ Hz), 7.07 (1H, dd, $J=1.4$, 8.1 Hz), 7.17–7.19 (2H, m), 8.11 (1H, d, $J=8.1$ Hz).

***N*-Benzyl(4-((4-methoxyphenyl)methylthio)methyl)-3-(methoxymethyloxy) benzamide (67).** Acid **66** (0.36 g, 1.0 mmol) was dissolved in CH_2Cl_2 (5 mL) and $BnNH_2$ (0.12 g, 1.1 mmol) added followed by EDCl (0.21 g, 1.1 mmol) and Et_3N (0.11 g, 1.1 mmol). The reaction was stirred at ambient temperature for 16 h, then washed with H_2O, brine, dried, and concentrated in vacuo. The resulting residue was purified on silica gel (70:30 hexane:ethyl acetate) to give 0.28 g (62%) of **67** as a pale yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 3.41 (3H, s), 3.56 (4H, d, $J=5.4$ Hz), 3.80 (3H, s), 4.69 (2H, d, $J=5.6$ Hz), 5.25 (2H, s), 6.83–6.85 (2H, m), 7.02–7.06 (3H, m), 7.17–7.19 (2H, m), 7.26–7.28 (1H, m), 7.33–7.38 (3H, m), 8.07 (1H, bs), 8.17 (1H, d, $J=8.0$ Hz). MS, m/z 438.3 (MH^+).

[*R*] 2-(2-(Methoxymethyloxy)-(4-(4-methoxyphenyl)methylthiomethyl)phenyl)) thiazoline-4-carboxylic acid (68). Amide **67** (0.27 g, 0.62 mmol), was dissolved in anhydrous CH_2Cl_2 (25 mL) and cooled to −50 °C under an atmosphere of N_2. Pyridine (49 mg, 0.68 mmol) was added, followed by slow addition of Tf_2O (0.18 g, 0.68 mmol). After complete addition of Tf_2O the reaction was allowed to warm to 0 °C and stirred at that temperature for 4 h. The reaction was cooled to −30 °C and pyridine (49 mg, 0.68 mmol) added followed by L-cysteine methyl ester hydrochloride (0.12 g, 0.68 mmol). The reaction was slowly allowed to warm to ambient temperature and stirred for 16 h. The reaction was washed with H_2O, brine, dried, and concentrated in vacuo. The resulting residue was purified on silica gel (70:30 hexane:ethyl acetate) to give 80 mg (30%) of **114** as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 3.53–3.63 (9H, m), 3.79 (3H, s), 3.83 (3H, s), 5.16 (1H, t, $J=9.3$ Hz), 5.25 (2H, s), 6.83 (2H, d, $J=8.6$ Hz), 6.99 (1H, dd, $J=1.4$, 8.1 Hz), 7.08–7.11 (1H, d, $J=1.3$ Hz), 7.18 (2H, d, $J=8.7$ Hz), 7.94 (1H, d, $J=8.0$ Hz). Ester **114** (80 mg, 0.18 mmol) was dissolved in a mixture of 1:1 THF:1 M NaOH (5 mL). The reaction was stirred for 1 h, washed with Et_2O (5 mL), and the aqueous layer acidified with 1N HCl to pH 3. This was extracted with CH_2Cl_2 (2×10 mL), the organic layers combined, dried, and concentrated in vacuo to give 60 mg (75%) of **68** as a yellow solid which was used without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 3.52–3.78 (12H, m), 5.26–5.30 (3H, m), 6.83 (2H, d, $J=8.5$ Hz), 6.99 (1H, d, $J=8.1$ Hz), 7.10 (1H, s), 7.17 (2H, d, $J=8.5$ Hz), 7.87 (1H, d, $J=8.0$ Hz), 10.12 (1H, bs). HRMS, m/z for $C_{22}H_{26}NO_5S_2$ calculated, 448.1252, found, 448.1241 (MH^+).

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[1-(carboxymethylethyloxy]imino]acetamido]-3-[4(3-hydroxy-4-[4-carboxy-2-thiazolinyl]phenylmethyl]thio]methyl]-8-oxo-1-aza-5-oct-2-ene-2-carboxylic acid (70). Acid **68** (0.05 g, 0.11 mmol) was dissolved in TFA (1 mL) and anisole (0.1 mL) added. This was stirred for 2 h, then the TFA and anisole removed in vacuo, and the crude thiol dissolved in DMF (1 mL). Diphenylmethyl-protected cephalosporin **69**[56] (0.81 g, 0.11 mmol), K_2CO_3 (0.017 g, 0.12 mmol) were added, followed by NaI (catalytic). The reaction was stirred under N_2 at ambient temperature for 16 h. The protected intermediate **115** was precipitated by addition of HO (5 mL), collected via filtration, and washed further with HO and hexanes. Intermediate **115** was deprotected by treatment with 5:1 TFA:anisole (0.6 mL). The product was precipitated by addition of Et_2O, collected via filtration, further washed with Et_2O, and dried in vacuo to give the 5 mg (8%) of **70** as a brown solid. ^1H NMR (400 MHz, DMSO-d_6) δ 1.28–1.31 (6H, m), 3.40–3.65 (9H, m), 5.13–5.19 (1H, m), 5.52–5.64 (1H, m), 6.72–6.95 (4H, m), 7.32–7.52 (3H, m), 9.29–9.33 (2H, m). HRMS, m/z for $C_{28}H_{29}N_6O_{10}S_4$ calculated, 737.0828, found, 737.0837 (MH^+).

4-[[2-(*tert*-Butoxycarbonyl)hydrazino]carbonyl]benzyl-chloride (71). 4-Chloromethyl benzoyl chloride (10.0 g, 53 mmol) and *t*-butyl carbazate (8.38 g, 63.5 mmol) were stirred in 200 mL CH_2Cl_2 and cooled in an icebath. Diisopropylethylamine (9.2 mL, 53 mmol) was added slowly, and the reaction mixture stirred at room temperature for 2 h, diluted with another 200 mL of CH_2Cl_2, washed with water, dried, filtered, and concentrated in vacuo. The residue was recrystallized from ethyl acetate:hexane to give 12 g (80%) of **71**. ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (9H, s), 4.50 (2H,s), 6.74 (1H, bs), 7.33 (2H, d, $J=8.0$ Hz), 7.71 (2H, d, $J=8.0$ Hz), 8.43 (1H, bs).

4-[[2-(*tert*-Butoxycarbonyl)hydrazino]carbonyl]benzyloxy phthallimide (72). Intermediate **71** (3.58 g, 12.6 mmol) and *N*-hydroxyphthallimide (2.47 g, 15.1 mmol), were dissolved in 15 mL DMF, and K_2CO_3 (189 mg, 1.26 mmol) and NaI (catalytic) were added. The reaction was stirred at 80 °C for 30 min, filtered, and the filtrate poured into 0.1 N HCl (500 mL). The precipitate was collected by filtration, washed with water, dried, and recrystallized from ethyl acetate to give 3.36 g (62%) of **72**. ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (9H, s), 5.16 (2H, s), 6.83 (1H, s). 7.52 (2H, d, $J=8.0$ Hz), 7.66 (2H, dd, $J=3.0$, 6.0 Hz), 7.72–7.76 (5H, m), 8.34 (1H, bs).

4-[[2-(*tert*-Butoxycarbonyl)hydrazino]carbonyl]benzylhydroxylamine (73). An ethanolic solution of compound **72** was treated with hydrazine and refluxed for 2 h. After cooling the precipitate was filtered off, and the filtrate was dried and concentrated in vacuo to give a crude

benzyloxyamine **73** which was used directly in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 4.86 (2H, s), 7.46 (2H, d, J = 8.0 Hz), 7.84 (2H, d, J = 8.0 Hz).

2-(Butoxycarbonylamino)-2-[[(*Z*)-4-[[2-butoxycarbonyl)-hydrazino]carbonyl]benzylhydroxyl]imino] - 4 - thiazole acetic acid (75). Benzyloxyamine **73** (2.14 g, 7.67 mmol) and ethyl 2-*N*-Boc-amino-4-thiazole glyoxalate **74** (2.09 g, 6.79 mmol) were refluxed in 40 mL EtOH with a catalytic amount of acetic acid for 4 h. After concentration in vacuo the residue was purified by silica gel column (using a step gradient of 70:30, then 60:40, then 40:60 hexane:ethyl acetate) to give 2.1 g (53%) of oxime **115**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, t, J = 7.1 Hz), 1.45 (9H, s), 1.51 (9H, s), 4.42 (2H, q, J = 7.1 Hz), 5.15 (2H, s), 7.46 (3H, m), 7.84 (2H, d, J = 7.6 Hz), 9.74 (1H, bs.), 10.33 (1H, bs).

Oxime **115** was refluxed with 3.5 mL 2N NaOH and 3.5 mL EtOH for 2 h. After cooling, the reaction mixture was acidified with 0.1 N HCl to pH∼3, and extracted with ethyl acetate. The extract was concentrated in vacuo to give 1.12 g (97%) of acid **75**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (9H, s,), 1.53 (9H, s), 5.06 (2H, s), 7.09 (2H, d, J = 7.5 Hz), 7.17 (1H, s), 7.47 (2H, d, J = 7.5 Hz), 7.59 (1H, s), 9.79 (1H, s).

***trans*-3-[4-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-[(*Z*)-4-butoxycarbonyl)hydrazino]carbonyl]]benzlhydroxyl]-imino]acetamido]-4-methyl-2-oxoazetidine-1-sulfonic acid triethylammonium salt (76).** Acid **75** (200 mg, 0.374 mmol) was stirred with HOAt (50 mg, 0.374 mmol), DCC (77 mg, 0.374 mmol), and DMAP (12 mg, catalytic amount) in 2 mL DMF for 1 h. Simultaneously **10** (67 mg, 0.374 mmol) was stirred in 0.5 mL DMF with triethylamine (52 μL. 0.374 mmol) for 1 h, then added to the activated acid solution. The mixture was stirred at room temperature overnight. DMF was removed in vacuo. The residue was purified by Sephadex LH-20 column (MeOH) to give 160 mg (53%) of amide **76**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.28 (9H, t, J = 7.3 Hz), 1.34 (9H, s), 1.51–1.58 (12H, m), 3.19 (6H, q, J = 7.3 Hz), 4.07 (1H, m), 4.61 (1H, d, J = 2.7 Hz), 5.30 (2H, s), 7.37 (1H, s), 7.52 (2H, d, J = 7.9 Hz), 7.86 (2H, d, J = 7.9 Hz).

***trans*-3-[4-(2-Aminothiazol-4-yl)-2-[(*Z*)-4-hydroxybenzyl-idenehydrazide)benzlhydroxyl] imino]acetamido]-4-methyl-2-oxoazetidine-1-sulfonic acid triethylammonium salt (77).** Amide **76** (25 mg, 0.03 mmol) was treated with 2 mL 1:1 TFA:CH_2Cl_2 for 15 min at room temperature. The reaction mixture was neutralized with triethylamine, concentrated in vacuo, and the residue purified by Sephadex LH-20 column (MeOH). The hydrazide was dissolved in 1 mL EtOH. To it salicylaldehyde (5 μL, 0.047 mmol) and one drop of acetic acid were added, and the mixture was refluxed for 30 min, then stirred at room temperature an additional 30 min. After concentration in vacuo the residue was purified on Sephadex LH-20 (MeOH) to give 8 mg (36% for two steps) of **77**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (9H, t, J = 7.5 Hz), 1.40 (3H, d, J = 6.1 Hz), 3.04 (6H, m), 3.70 (1H, m), 4.47 (1H, m), 5.23 (2H, s), 6.93 (3H, m), 7.22 (1H, m), 7.30 (1H, m), 7.52 (2H, d, J = 8.3 Hz), 7.93 (2H, d, J = 8.3 Hz), 8.64 (1H, s). MS, m/z 602 (MH^+).

2-(Tritylamino)-α-((3-methoxymethyloxy-4-(2-benzthiazolyl)benzyloxy)imino)-4-thiazole acetic acid (78). The free base (obtained by treatment of ethyl-2-(tritylamino)-α-(hydroxylimino)-4-thiazolyleacetate monohydrochloride (6.8 g, 15.5 mmol) with $NaHCO_3$ and extraction into CH_2Cl_2) was suspended in 86 mL allyl alcohol and warmed to 60 °C. Sodium ethoxide (420 mg, 6.2 mmol) was dissolved in 2 mL allyl alcohol and added to the reaction. Stirring was maintained at 60 °C for 4 h, then allowed to continue at room temperature overnight. After adjusting the pH to 7 with 2 N HCl, the reaction mixture was concentrated in vacuo, and the crude allyl ester precipitated with methyl-*t*-butyl ether. The product was dissolved in $CHCl_3$, washed with NaCl, dried, and concentrated in vacuo to give 4.8 g (70%) of allyl ester **116** sufficiently pure to carry on in the subsequent reaction. ^{1}H NMR (400 MHz, $CDCl_3$) 4.83 (2H, dd, J = 0.9, 5.0 Hz), 5.39 (2H, dd, J = 10.4, 17.1 Hz), 5.93–6.02 (1H, m), 6.48 (1H, s), 6.96 (1H, bs), 7.26–7.52 (15H, m), 8. 56 (1H, bs). MS, m/z 470.4 (MH^+).

A mixture of bromomethyl intermediate **33** (1.65 g, 4.5 mmol) and allyl ester **116** (2.15 g, 5 mmol) was stirred at room temperature overnight in 10 mL dry DMF. The reaction mixture was diluted with $CHCl_3$, washed with NaCl, dried, and concentrated in vacuo to an oil that was purified on silica gel (80:20 hexane:ethyl acetate) to give 2.1 g (61%) of **117**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (3H, s), 4.82 (2H, d, J = 5.7 Hz), 5.33 (2H, dd, J = 3.7, 17.5 Hz), 5.35 (2H, s), 5.43 (2H, s), 5.92–5.99 (1H, m), 6.53(1H, s), 6.99–7.50(19H, m), 7.93 (1H, d, J = 7.8 Hz), 8.09 (1H, d, J = 8.0 Hz), 8.49 (1H, d, J = 8.1 Hz). MS, m/z 753.2 (MH^+).

A solution of **117** (1.05 g, 1.4 mmol) in dry THF was added under Argon via canula to a solution of tetrakis(triphenylphospine)palladium (generated in situ from palladium acetate (31.4 mg, 0.14 mmol) and triphenylphosphine (183 mg, 0.7 mmol) in 4 mL dry THF). After 15 min of stirring, tributyltin hydride (420 μL, 1.54 mmol) was added, and the reaction stirred at room temperature for 5 h. After concentration in vacuo, the residue was applied to a silica column, and the tributylstannate ester eluted with 93:8 $CHCl_3$:MeOH. The eluting solvent was removed, and the residue was dissolved in CH_3CN and washed with hexane. The CH_3CN phase was treated with a 5:1 CH_3CN-AcOH solution (pH 3) at room temperature for 2 h to hydrolyze the tributylstannate ester. After washing the reaction mixture with hexane, the CH_3CN was removed in vacuo, the product dissolved in $CHCl_3$, washed with water, dried, and the solvent removed in vacuo to give 660 mg (66%) of **78** that was >90% free acid, <10% tributylstannate ester by integration of the ^{1}H NMR signals. ^{1}HNMR (400 MHz, $CDCl_3$) δ 0.84–0.92 (1H, m), 1.31–1.39 (2H m), 1.60–2.17 (1H, m), 3.45 (3H, s), 5.25 (2H, s), 5.42 (2H, s), 6.57 (1H, s), 7.10–7.69 (19H, m), 7.89 (1H, d, J = 7.9 Hz), 8.07 (1H, d, J = 7.9 Hz), 8.43 (1H, d, J = 7.9 Hz). MS, m/z 713.3 (MH^+).

***cis*-3-[2-(2-Aminothiazol-4-yl)-2-((*Z*)-(3-hydroxy-4-(2-thiazolyl)benzyl)oxyimino)acetamido]-4-methyl-2-azetidinone-1-sulfonic acid (67).** A solution of acid **78** (660 mg, 0.93 mmol), DCC (209 mg, 1.10 mmol), HOAt (125 mg, 0.93 mmol), and DMAP (35 mg) in 2.5 mL dry DMF was stirred at room temperature for 30 min. Simultaneously, a solution of the *cis* β-lactam **80**[34] (166 mg, 0.93 mmol) and NEt_3 (128 μL, 0.93 mmol) in 1 mL dry DMF was stirred at room temperature. The amine solution was added to the activated ester solution, and the reaction mixture was stirred overnight. After removing the solvent in vacuo, the residue was purified on silica gel (95.5:5:0.5 $CHCl_3$:-MeOH:NEt_3) to give 430 mg (54%) of **118**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (3H, t, J = 6.2 Hz), 3.49 (3H, s), 3.94–4.00 (1H, m), 5.06 (1H, d, J = 4.4 Hz), 5.19 (2H, s), 5.52 (2H, s), 6.73 (1H, s), 7.00–7.52 (19H, m), 8.11 (1H, dd, J = 8.1, 16.6 Hz), 8.05 (1H, d, J = 7.9, 16.6 Hz), 8.46 (1H, d, J = 8.2 Hz), 8.83–8.84 (1H, bs). MS, m/z 875.3 (MH^+), 873.4 (MH^-).

A solution of **118** (165 mg, 0.19 mmol) in 1 mL THF was cooled to 0 °C and treated with 1 mL 6 N aqueous HCl dropwise. The icebath was removed, and the reaction mixture stirred at room temperature overnight. After lyophilization, the residue was purified on reverse phase HPLC (linear gradient of 20 to 35% B in A over 40 min) to give 7 mg (6%) of **79**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29–1.32 (3H, s), 4.00–4.11 (1H, m), 5.09–5.13 (1H, m), 5.17 (2H, s), 6.80 (1H, s), 7.03–7.68 (4H, m), 8.07 (1H, d, J = 8.1 Hz), 8.14 (1H, d, J = 7.9 Hz), 9.29–9.30 (1H, s). MS m/z 589.1(MH^+), 587.0 (MH^-). HRMS, m/z for $C_{23}H_{21}N_6O_7S_3$ calculated, 589.0634, found, 589.0650 (MH^+).

Allyl-2-(tritylamino)-α-methoxymethyloxy)phenyl)benzthiazolyl)methyloxy)imino)-4-thiazole acetate (81) and allyl-2-(tritylamino)methoxymethyloxy)phenyl)benzthiazolyl)methyloxy)imino)-4-thiazole acetate (81B). Alkylation of **116** (1.47 g, 3.4 mmol) with the regiomeric bromides **37** (1.13 g, 3.1 mmol) in 10 mL dry DMF was performed as for **117**. Chromatography on silica gel (90:10 hexane:ethyl acetate) gave 1.13 (49%) of **81B**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (3H, s) 4.72–4.73 (2H, d, J = 5.1 Hz), 5.05–5.38 (2H, m), 5.42–5.46 (2H, m), 5.57 (2H, s), 5.76–5.81 (1H, m), 6.54 (1H, s), 6.54–7.46 (20H, m), 8.03 (1H, d, J = 7.9 Hz), 8.53 (1H, d, J = 8.0 Hz). MS, m/z 753.1 (MH^+).

Further elution with 85:15 hexane:ethyl acetate gave 121 mg (5%) of **81A**. ^{1}H NMR. (400 MHz, $CDCl_3$) δ 3.52 (3H, s), 4.73 (2H, d, J = 5.1 Hz), 5.05–5.38 (2H, m), 5.42 (2H, s), 5.44 (2H, s), 5.76–5.81 (1H, m), 6.54 (1H, s), 6.54–7.46 (19H, m), 7.98 (1H, s), 8.04 (1H, s), 8.53 (1H, s).

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[2-(2-hydroxy)-phenyl-5-benthiazolyl)methyloxy]imino]acetamido]-3-acetoxymethyl)-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid (83). Allyl ester **81B** (1.03 g, 1.37 mmol) was treated with $[Ph_3P]_4Pd$ and n-Bu_3SnH as described for **117** to give 595 mg (61%) of free acid **119**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (3H, s), 5.46 (2H, s), 5.57 (2H, s) 6.55 (1H, s), 6.92–7.83 (20H, m), 8.05 (1H, d, J = 8.0 Hz), 8.53 (1H, d, J = 7.9 Hz).

Diphenylmethyl ester **82** was prepared by reaction of 7-amino cephalosporonic acid (5.0 g, 18.4 mmol) with p-toluenesulfonic acid (3.85 g, 20.2 mmol) and diphenyldiazomethane (prepared fresh from diphenylhydrazine (5.9 g, 30 mmol) according to Holton and Shechter[57]) in 150 mL 2:1 CH_3CN:H_2O for 48 h. Excess diphenyldiazomethane was quenched by dropwise addition of AcOH until the deep purple reaction mixture had decolorized. The reaction mixture was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was washed with NaCl, dried, and concentrated in vacuo to give a residue. Purification on silica gel (60:40 hexane:ethyl acetate, then 100% ethyl acetate) gave 4.0 g (50%) of **82**. ^{1}H NMR (400 MHz,$CDCl_3$) δ 2.00 (3H, s), 3.46 (2H, dd, J = 18.4, 18.5 Hz), 4.76 (1H, d, J = 17.6 Hz), 4.78 (2H, d, J = 4.6 Hz), 5.00 (1H, d, J = 16.6 Hz), 6.98 (1H, s), 7.26–7.45 (10H, m). MS m/z 439.1 (MH^+).

To a 0 °C solution of **82** (76 mg, 0.17 mmol) in 2 mL CH_2Cl_2 was added pyridine (67 mL, 0.85 mmol), then **119** (136 mg, 0.19 mmol) in 1 mL CH_2Cl_2, followed by $POCl_3$ (20 mL, 0.22 mmol). After 100 min of stirring at 0 °, the reaction mixture was diluted with CH_2Cl_2, washed successively with 0.02 N HCl, then NaCl, dried, and concentrated in vacuo. The crude product was purified on silica gel (75:25, then 60:40, then 90:10 hexane:ethyl acetate) to give 69 mg (36%) of pure **120**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (3H, s), 3.03 (2H, dd, J = 13.2, 18.6 Hz), 3.49 (3H, s), 4.78 (1H, d, J = 5.0 Hz), 4.80 (2H, d, J = 13.6 Hz), 5.38 (2H, d, J = 6.2 Hz), 5.65 (2H, dd, J = 11.7, 14.7 Hz), 5.83 (1H, dd, J = 4.4, 4.9 Hz), 6.84 (1H, s), 6.94 (1H, d, J = 12.2 Hz) 7.03–7.52 (30H, m), 8.04 (1H, d, J = 7.9 Hz), 8.50 (1H, d, J = 1.7 Hz). MS, m/z 1133.4 (MH^+).

Intermediate **120** was treated with 5:1 TFA-anisole for 2.5 h, the product precipitated with diisopropyl ether, and the crude precipitate lyophilized from 50:50 CH_3CN:H_2O (0.05% TFA) to give 24 mg (70%) of **83**. ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 3.33–3.61 (2H, m), 4.97 (1H, dd, J = 3.8, 4.5 Hz), 5.14 (1H, dd, J = 4.8, 5.8 Hz), 5.39–5.54 (2H, m), 5.74 (1H, dd, J = 3.1, 4.7 Hz), 5.83 (1H, dd, J = 4.8, 4.9 Hz), 6.75 (1H, s), 6.99–7.64 (4H, m), 8.04 (1H, d, J = 7.9 Hz), 8.18 (1H, d, J = 2.3 Hz), 9.69-9.71 (1H, m), HRMS, m/z for $C_{29}H_{24}N_6O_8S_3$ calculated, 681.0899, found, 681.0882 (MH^+).

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[2-(2-hydroxy)-phenyl-5-benthiazolyl)methyloxy]imino]acetamido]-3-pyridiniummethyl)-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (85). An ice-cooled solution of p-methoxybenzyl-[[[7-amino-3-chloromethyl-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]-oct-2-ene-2-carboxylate hydrochloride (**84**) (100 mg, 0.25 mmol), NEt_3 (35 mL, 0.25 mmol), **119** (180 mg, 0.25 mmol), and pyridine (88 L, 1.25 mmol) in 10 mL dry CH_2Cl_2 was stirred for 1 h, then further cooled to −10 °. $POCl_3$ (28 μL, 0.31 mmol) was added via syringe,

and the reaction mixture stirred for 80 min at −10 °, then concentrated in vacuo and dissolved in ethyl acetate. The organic phase was washed with NaCl, dried, and concentrated in vacuo to give a residue that was purified on silica gel (75:25, then 65:35 hexane:ethyl acetate) to give 36.6 mg (14%) of **121** as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89–3.33 (2H, m), 3.50 (3H, s), 3.80 (3H, s), 4.20–4.49 (2H, m), 4.75 (1H, d, J = 5.0 Hz), 5.11 (2H, s), 5.39 (2H, d, J = 8.0 Hz), 5.63 (2H, dd, J = 11.6, 14.8 Hz), 5.78 (1H, dd, J = 4.3, 4.9 Hz), 6.80 (1H, s), 6.86–7.49 (24H, m), 8.05 (1H, d, J = 8.1 Hz), 8.52 (1H, d, J = 9.6 Hz). MS m/z 1063.3. (MH^+).

To a solution of intermediate **121** (36 mg, 0.04 mmol) in 350 mL THF was added NaI (5.6 mg, 0.04 mmol) and dry pyridine (50 μL, 0.6 mmol). The reaction mixture was stirred for 5 h at room temperature, concentrated in vacuo, and the product precipitated with diisopropyl ether and lyophilized from 50:50 CH_3CN:H_2O (0.05% TFA) to give a tan solid that was homogeneous by HPLC (3 to 70% B in A over 30 min) T_R = 33.5 min. MS m/z 1106.4 (MH^+).

Treatment with 5:1 TFA-anisole gave, upon precipitation from diisopropyl ether, 5 mg (18%) of **85**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.18–3.48 (2H, m), 3.63 (2H, s), 5.10 (1H, d, J = 5.0 Hz), 5.32 (2H, s), 5.86 (1H, dd, J = 3.0, 5.0 Hz), 6.68 (1H, s), 6.72–8.24 (9H, m), 8.62–8.66 (1H, m), 8.99 (1H, d, J = 5.6 Hz), 9.68 (1H, d, J = 8.0 Hz). HPLC (3 to 70% B in A over 30 min) T_R = 19.1 min. MS, m/z 700.2 (MH^+).

(*Z*)-2-Aminoa[1-(carboxy)-1-methylethyloxyimino]-4-acetamido]-3-(4-(2-hydrazido-1-pyridylmethyl)-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (87). To a 0 ° solution of (*Z*)-2-amino a[1-(*t*-butoxycarbonyl)-1-methylethyloxyimino]-4-acetic acid (905 mg, 2.73 mmol) and HOAt (375 mg, 2.75 mmol) in 20 mL THF was added DCC (570 mg, 2.75 mmol), and the reaction stirred for 1h at room temperature. To the activated ester solution was added **82** in 10 mL THF, and the reaction stirred overnight. The solvent was removed in vacuo, and the residue dissolved in $CHCl_3$, washed with 0.1 N citric acid, saturated $NaHCO_3$, NaCl, dried, and concentrated in vacuo. Purification on silica gel (60:40 hexane:ethyl acetate) gave 1.36 g (66%) of **122**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s), 1.59 (3H, s), 1.60 (3H, s), 2.02 (3H, s), 3.37–3.59 (2H, m), 4.82 (1H, d, J = 13.8 Hz), 5.07 (2H, dd, J = 4.2, 5.0 Hz), 6.04 (1H, dd, J = 4.0, 4.9 Hz), 6.50 (2H, bs), 6.89 (1H, s), 6.95 (1H, s), 7.32–7.65 (10H, m).

Protected cephalosporin **122** (1.0 g, 1.34 mmol) was treated with 5:1 TFA-anisole for 2.5 h to give 743 mg (quant) of **86**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (6H, s), 2.03 (3H, s) 3.48–3.76 (2H m), 5.00 (2H, d, J = 12.9 Hz), 5.19 (1H, d, J = 4.9 Hz), 5.87 (1H, dd, J = 3.5, 4.9 Hz), 6.77 (1H, s) 9.45 (1H, d, J = 7.8 Hz). MS, m/z 528 (MH^+).

Acetate **86** (264 mg, 0.5 mmol) was suspended in CH_2Cl_2 and treated with trifluoromethyl(*N*-methyl *N*-trimethylsilyl)acetamide (MSTFA, 620 L, 3.5 mmol), warmed briefly to 40 °, then cooled to room temperature. Trimethylsilyliodide (TMSI, 193 mL, 2.25 mmol) was added and the reaction stirred at room temperature for 30 min, then concentrated in vacuo, and the residue dissolved in 1 mL CH_3CN. THF (250 mL) was added to consume excess TMSI, followed by addition of a suspension of 2-hydroxyphenylisonicotinylhydrazide (**29**) in 3 mL CH_3CN. A clear solution was obtained within 30 min, and the reaction stirred for 3 h at room temperature, then poured into a solution of 96:5 acetone-MeOH. The precipitate was collected and purified on reverse phase HPLC (linear gradient 10 to 35% B in A over 40 min) to give 7 mg (2%) of **87**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (3H, s), 1.43 (3H, s), 3.45–3.83 (2H, m), 5.24 (1H, d, J = 5.5 Hz), 5.65 (2H, dd, J = 14.7, 17.4 Hz), 5.97 (1H, bd, J = 5.7 Hz), 6.73 (1H, s), 6.95–6.99 (1H, m), 7.33–7.62 (2H, m), 7.69 (1H, d, J = 7.6 Hz), 8.39–8.84 (2H, m), 9.17 (1H, d, J = 5.8 Hz), 9.48 (1H, d, J = 7.7 Hz), 10.86 (1H, bs). HPLC (3 to 70% B in A over 30 min) T_R = 15.7 min. MS, m/z 709.2 (MH^+). HRMS, m/z for $C_{30}H_{29}N_8O_9S_2$ calculated, 709.1499, found, 709.1479 (MH^+).

(*Z*)-2-Amino-α-[1-(carboxy-1-methylethyloxyimino]-4-acetamido]-3-(4-(2,4-dihydroxyphenyl)hydrazido-1-pyridylmethyl)-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid (88). Compound **88** was synthesized from **30** (90 mg, 0.35 mmol) and **86** (185 mg, 0.35 mmol) as described for **87** to give, after HPLC purification on a linear gradient of 3–30% B in a over 30 min, 2 mg (1%) of **88**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (3H, s), 1.35 (3H, s), 3.28–3.74 (2H, m), 5.14 (1H, d, J = 5.0 Hz), 5.53–5.58 (2H, m), 5.88 (1H, dd, J = 3.1, 5.0 Hz), 6.64 (1H, s), 7.39–7.47 (2H, m), 7.46 (1H, d, J = 16.6 Hz), 8.51 (2H, dd, J = 6.7, 16.7 Hz), 9.15–9.41 (2H, m), 9.86 (1H, s), 10.83 (2H, bs), 10.89 (2H, bs). HPLC (3 to 70% B in A over 30 min) T_R = 15.8 min. MS, m/z 725.1 (MH^+).

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[1-(carboxymethylethyloxy]imino]acetamido]-3-[[[1-[*N*-((3-hydroxy-4-(2-benzthiazolyl)benzyl)carbamoylmethyl]pyridinium-4-yl]-thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (94). A solution of **89**[13](482 mg, 0.64 mmol) and **33** (717 mg, 1.99 mmol) in 4 mL dry DMF was stirred at room temperature for 24 h. Trituration with 50:50 diethyl ether:hexane, followed by 50:50 diisopropyl ether:hexane, followed by diisopropyl ether, gave a precipitate that was collected and purified on a column of silica gel ($CHCl_3$, followed by 10:90 MeOH:$CHCl_3$,) to give 470 mg (71%) of **123**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (9H, s), 1.53 (3H, s), 1.56 (3H,s), 3.52 (3H, s), 3.73 (3H, s), 3.48–3.82 (4H, m), 4.11–4.43 (2H, m), 5.08–5.16 (1H, m), 5.16 (2H, s), 5.51 (2H, s), 5.84–5.95 (3H, m), 6.78–7.89 (13H, m), 8.04 (1H, d, J = 8.1 Hz), 8.49 (1H, d, J = 7.7 Hz), 8.96 (1H, d, J = 7.9 Hz), 9.05–9.09 (2H, bs). MS m/z 1038.3 (MH^+).

A solution of **123** (280 mg, 0.25 mmol) in 2 mL CH_2Cl_2 was cooled on ice, and a mixture of 5:1 TFA-anisole (2.4 mL) was added. After 30 min the icebath was removed, and the reaction mixture allowed to stir for an additional 2.5 h. Trituration with diisopropyl ether

gave, upon cooling, 240 mg of crude product. Purification on HPLC (linear gradient of 30–40% B in A over 40 min, B = 0.05%TFA in CH_3CN, A = 0.05% TFA in H_2O) gave 8 mg (4%) of **94**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (3H, s), 1.38 (3H, s), 3.45–3.72 (2H, m), 4.32 (2H, s), 5.16 (1H, d, J = 4.9 Hz), 5.65 (2H, s), 5.79 (1H, dd, J = 4.8, 4.9 Hz), 6.67 (1H, s), 7.04 (2H, dd, J = 1.4, 6.8 Hz), 7.38–7.51 (2H, m), 8.00 (2H, d, J = 7.0 Hz), 8.09 (1H, d, J = 7.8 Hz), 8.19 (1H, d, J = 8.1 Hz), 8.84 (2H, d, J = 7.0 Hz), 9.40 (1H, d, J = 8.1 Hz). HPLC (3–70% B in A over 30 min) T_R = 20.4 min. HRMS, m/z for $C_{36}H_{32}N_7O_3S_4$ calculated, 818.1195, found, 818.1221 (MH^+).

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[1-(carboxymethylethyloxy]imino]acetamido]-3-[[pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (90). Thiopyridine **90** was prepared by TFA deprotection of **89** (210 mg, 0.28 mmol) as described for **94**. HPLC purification (5–25% B in A over 40 min) gave 10.1 mg. (6%) **90**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.53 (3H, s), 1.55 (3H, s), 3.61–3.87(2H, m), 4.43 (2H, dd, J = 11.0, 12.8 Hz), 5.31 (1H, d, J = 4.9 Hz), 5.96 (1H, d, J = 4.9 Hz), 6.83 (1H, s), 7.83 (2H, d, J = 6.7), 8.67 (2H, d, J = 6.5 Hz), 9.54 (1H, d, J = 8.3 Hz). HPLC (3–70% B in A over 30 min) T_R = 12.2 min. HRMS, m/z $C_{22}H_{23}N_6O_7S_3$ calculated, 579.0790 (MH^+), found, 579.0789.

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[1-(carboxymethylethyloxy]imino]acetamido]-[3-[4-methylphenyl)-5-(2-hydroxyphenyl)-1,2,4-oxadiazolyl]pyridinium-4-yl]]-thiomethyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (95). Bromomethyl intermediate **52** was converted to **124** (68 mg, 0.0065 mmol), which was deprotected according to the procedure described for **94** to give 15 mg (28%) of **95**. ^{1}H NMR (400 MHz, DMSO d_6) δ 1.33–1.36 (6H, m), 3.62–3.71 (2H, m), 4.07–4.11 (1H, m), 4.28–4.33 (1H, m), 5.13–5.16 (1H, m), 5.69–5.78 (3H, m), 6.79 (1H, s), 6.99 (1H, t, J = 7.5 Hz), 7.06 (1H, d, J = 8.3 Hz), 7.47 (1H, dt, J = 1.6, 8.6 Hz), 7.59–7.62 (2H, m), 7.93–7.98 (3H, m), 8.07–8.10 (2H, m), 8.77–8.82 (2H, m).

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-benzyl]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]-oct-2-ene-2-carboxylate (91). A solution of **125** (50 mg, 0.06 mmol) in 2 mL of CH_2Cl_2 was treated with anisole (200 L), cooled to 0 °C, and neat TFA (2.0 mL) added. The reaction was allowed to stir at 0 °C for 2 h followed by slow warming to rt over 30 min. Addition of ethyl ether (20 mL) to the reaction mixture gave 35 mg (81%) of **91** as brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50 (3H, s), 1.51 (3H, s), 3.57–3.84 (2H, m), 4.44 (2H, d, J = 1.7 Hz), 5.29 (1H, t, J = 5.4 Hz), 5.74 (2H, s), 5.93 (1H, dd, J = 4.9, 8.3 Hz), 6.80 (1H, s), 7.48–7.58 (7H, m), 8.05–8.10 (4H, m), 8.95 (2H, d, J = 7.0 Hz), 9.51 (1H, d, J = 8.4 Hz). HRMS, m/z for $C_{29}H_{29}N_6O_7S_3$ calculated, 669.1260, found, 669.1255 (M^+).

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino] acetamido]-3-[[[1-[*N*-acetophenone]-pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo-[4.2.0]oct-2-ene-2-carboxylate (92). A solution of **126** in 2 mL of CH_2Cl_2 (58 mg, 0.06 mmol) was reacted with 10:1 TFA:anisole as described for **91** to give 37 mg (71%) of **92** as a light yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45 (6H, t, J = 5.5 Hz), 3.56–3.84 (1H, m), 4.19–4.41 (3H, m), 4.43 (2H, s), 5.08 (1H, s), 5.26 (1H, m), 5.60–5.63 (1H, m), 6.29 (2H, d, J = 8.1 Hz), 6.77 (1H, d, J = 11.6 Hz), 7.67 (2H, t, J = 7.6 Hz), 7.80 (1H, t, J = 7.6 Hz), 8.04–8.13 (5H, m), 8.67 (2H, dd, J = 7.1, 9.4 Hz), 9.50 (1H, d, J = 7.7 Hz). HRMS, m/z for $C_{30}H_{29}N_6O_8S_3$ calculated, 697.1209, found, 697.1216 (M^+).

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-(3-hydroxy-3′-methylflavone)]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (96). Bromomethyl intermediate **59** (55 mg, 0.15 mmol) was reacted with **89** (100 mg, 0.14 mmol) in 2 mL DMF as described for **123** to give 73 mg (50%) of **127** that was homogeneous by TLC. R_f = 0.18 (90:10 CH_2Cl_2:MeOH). A solution of **127** (43 mg, 0.04 mmol) in 2 mL of CH_2Cl_2 was treated with 10:1 TFA:anisole for 3.5 h. An additional 10:1 TFA:anisole was added, and the reaction continued at 0 °C for 2 h, then precipitated as for **91** to give 25 mg (66%) of **96** as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47–1.55 (6H, m), 3.61–3.84 (4H, m), 4.43 (2H, s), 5.27–5.28 (1H, m), 5.87–5.90 (3H, m), 6.77 (1H, s), 7.38 (1H, s), 7.54–7.58 (1H, m), 7.64–7.71 (2H, m), 7.80–7.82 (1H, m), 7.89–7.91 (1H, m), 8.10 (2H, d, J = 7.1 Hz), 8.19–8.21 (1H, m), 8.33 (2H, d, J = 7.8 Hz), 8.39 (1H, s), 9.00 (2H, d, J = 7.0 Hz), 9.50 (1H, d, J = 8.3 Hz), 9.86 (1H, s). HRMS, m/z for $C_{38}H_{33}N_6O_{10}S_3$ calculated, 829.1420, found, 829.1433 (M^+).

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-(3-hydroxy-4′-methylflavone)]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (97). The protected intermediate was prepared from **60** (55 mg, 0.15 mmol) and **89** (100 mg, 0.14 mmol) as described for **127**, to give 80 mg (54%) of **128** that was homogeneous by tlc. R_f = 0.18 (90:10 CH_2Cl_2:MeOH). A solution of **128** (50 mg, 0.04 mmol) in 2 mL CH_2Cl_2 was treated with 10:1 TFA:anisole as described for **96** to give 30 mg (72%) **97** as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (6H, m), 3.52–3.56 (1H, m), 3.72–3.79 (1H, m), 4.16–4.20 (1H, m), 4.28–4.42 (2H, m), 5.77–5.78 (2H, m), 5.83–5.88 (1H, m), 6.71–6.77 (1H, m), 7.47–7.53 (2H, m), 7.67–7.70 (2H, m), 7.72–7.77 (2H, m), 7.79–7.85 (2H, m), 8.04 (2H, dd, J = 7.0, 12.4 Hz), 8.13 (2H, dd, J = 1.4, 8.0 Hz), 8.22–8.28 (2H, m), 8.89–8.94 (2H, m), 9.43–9.46 (1H, m), 9.79 (1H, s); HRMS, m/z calcd. for $C_{38}H_{33}N_6O_{10}S_3$ 829.1420, found, 829.1408 (M^+).

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-(3-hydroxy-6-methylflavone)]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate(98). The protected intermediate was prepared from **61** (55 mg, 0.15 mmol) and **89** (100 mg, 0.14 mmol) as described for **127**, to give 85 mg (57%) of **129** that was homogeneous

by TLC. $R_f = 0.18$ (90:10 CH_2Cl_2:MeOH). A solution of **129** (50 mg, 0.04 mmol) in 2 mL of CH_2Cl_2 was treated with 10:1 TFA:anisole for 3.5 h, and the product precipitated as for 96 to give 20 mg (48%) of **98** as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41–1.44 (6H, m), 3.51–3.55 (2H, m), 3.70–3.78 (2H), 4.16–4.19 (2H, m), 5.03 (2H, s), 5.21–5.24 (2H, m), 5.59–5.62 (1H, dd, $J = 3.9$, 8.0 Hz), 5.83–5.88 (2H, m), 6.87 (1H, s), 7.50–7.64 (2H, m), 7.85–7.88 (1H, m), 7.93–7.98 (1H, m), 8.01–8.08 (2H, m), 8.20–8.23 (2H, m), 8.38–8.41 (1H, m), 8.94–8.99 (2H, m), 9.43–9.46 (1H, m), 9.79 (1H, s). HRMS, m/z for $C_{38}H_{33}N_6O^{10}S_3$ calculated, 829.1420, found, 829.1429 (M^+).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-2-[(2-hydroxy)-benzylidene] aminobenzyl] pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (99). Bromomethyl intermediate **40** (76 mg, 0.27 mmol) was reacted with **89** (200 mg, 0.27 mmol) as described for **123** to give 217 mg (79%) of a brown glassy residue, of which 101 mg, (0.097 mmol.) was treated with 5:1 TFA:anisole at −10 ° for 4 h. Precipitation with diethyl ether gave 86 mg (79%) of **130** as a beige solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.48 (3H, s), 1.49 (3H, s), 3.52–3.58 (2H, m), 4.32–4.36 (2H, m), 5.08 (1H, d, $J = 4.8$ Hz), 5.45 (2H, s), 5.76 (1H, d, $J = 4.8$ Hz), 6.64 (1H, t, $J = 7.3$ Hz), 6.70 (1H, d, $J = 7.8$ Hz), 6.96 (1H, s), 7.10 (2H, d, $J = 7.7$ Hz), 7.76 (2H, d, $J = 7.0$ Hz), 8.35 (2H, d, $J = 6.8$ Hz). HRMS, m/z for $C_{29}H_{30}N_7O_7S_3$ calculated, 684.1368, found, 684.1352 (M^+). A suspension of **130** (25 mg, 0.036 mmol) in EtOH (1.0 mL) was treated with salicylaldehyde (39 mL, 0.36 mmol). After 48 h the reaction was diluted with CH_2Cl_2 and filtered. The resulting filtrate was concentrated in vacuo, suspended in CH_2Cl_2, and filtered to afford 15 mg (53%) of **99** as an amber solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (3H, d, $J = 6.0$ Hz), 1.49 (3H, d, $J = 6.5$ Hz), 3.63–3.79 (2H, m), 4.30–4.39 (2H, m), 5.17–5.30 (1H, m), 5.46 (1H, s), 5.82–5.90 (2H, m), 6.99 (2H, d, $J = 6.2$ Hz), 7.35 (2H, d, $J = 7.0$ Hz), 7.40–7.54 (3H, m), 7.76 (1H, d, $J = 6.5$ Hz), 7.84 (1H, d, $J = 5.8$ Hz), 7.94–8.01 (1H, m), 8.69–8.75 (1H, m), 8.80–8.87 (2H, m), 11.62 (1H, s), 11.90 (1H, s), 12.74 (1H, bs). HRMS, m/z for $C_{36}H_{34}N_7O_8S_3$ calculated, 788.1631, found, 788.1632 (M^+).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-3-[(2-hydroxy-5-bromo)benzylidene]aminobenzyl]pyridinium-4-yl]thio]-methyl]-8-oxo-1-aza-5-thiabicyclo [4.2.0]oct-2-ene-2-carboxylate (100). Bromomethyl intermediate **41** (225 mg, 0.79 mmol) was reacted with **89** (230 mg, 0.31 mmol) as described for **123** to give 217 mg (68%) of quaternary intermediate **131**. The crude product (217 mg, 0.21 mmol) was treated with 10:1 TFA:anisole to give, upon precipitation, 190 mg of a peach solid. HPLC purification of 50 mg (30–70% B in A over 30 min.) provided 24 mg (64%) of **132**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.60 (3H, s), 1.61 (3H, s), 3.65–3.69 (2H, m), 4.46–4.50 (2H, m), 5.24 (1H, d, $J = 4.9$ Hz), 5.54 (2H, s), 5.88 (1H, d, $J = 4.9$ Hz), 6.97–7.03 (2H, m), 7.09 (1H, s), 7.27–7.35 (1H, m), 7.33 (1H, t, $J = 8.0$ Hz), 7.93 (2H, d, $J = 7.2$ Hz), 8.63 (2H, d, $J = 7.1$ Hz). HRMS, m/z $C_{29}H_{30}N_7O_7S_3$ calculated, 684.1368, found, 684.1348 (M^+).

5-Bromosalicylaldehyde (200 mg, 1.0 mmol) was added to a solution of **132** (24 mg, 0.037 mmol) in DMF (0.5 mL) and the resulting mixture was allowed to stir at room temperature for 72 h, diluted with CH_2Cl_2, and filtered. The filtrate was concentrated in vacuo, suspended in warm EtOAc, and allowed to stand for 1 h to give 16 mg (50%) of **100** as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (3H, d, $J = 5.0$ Hz), 1.48 (3H, d, $J = 5.5$ Hz), 3.72–3.79 (2H, m), 4.37 (2H, s), 5.19–5.25 (2H, m), 5.71 (2H, s), 6.97 (2H, d, $J = 7.4$ Hz), 7.26–7.29 (1H, m), 7.45 (2H, d, $J = 7.6$ Hz), 7.53–7.60 (2H, m), 7.86–7.89 (1H, m), 8.01–8.06 (2H, m), 8.90–8.92 (3H, m), 12.68 (1H, bs). HRMS, m/z for $C_{36}H_{33}N_7O_8S_3Br$ calculated, 866.0736, found, 866.0750 (M^+).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[1-carbonyl-1-(methylethyloxy)imino]acetamido]-3-[[[1-[*N*-(3-hydroxy-4-(benzoylhydrazonomethyl)benzyl)]pyridinium-4-yl]thio]-methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (101). Cephalosporin **89** (7.5 mg, 0.01 mmol) was reacted with bromomethyl intermediate **45** (11 mg, 0.01 mmol) to give, after 5:1 TFA:anisole deprotection, 5 mg (59%) of **101**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.50 (3H, s), 1.51 (3H, s), 3.47–3.51 (1H, m), 3.64–3.68 (1H, m), 4.30 (1H, d, $J = 13.7$ Hz), 4.49 (1H, d, $J = 12.8$ Hz), 5.10 (1H, d, $J = 4.7$ Hz), 5.47–5.54 (2H, m), 5.78 (1H, d, $J = 4.7$ Hz), 6.86–6.88 (2H, m), 6.98 (1H, s), 7.41–7.43 (3H, m), 7.50–7.52 (1H, m), 7.83–7.85 (4H, m), 8.42 (1H, s), 8.56 (2H, d, $J = 6.6$ Hz). HRMS, m/z for $C_{37}H_{36}N_8O_9S_3$ calculated, 832.4577 found, 831.1689 (M^-).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[1-carbonyl-1-(methylethyloxy)[*N*-(3-hydroxy-4-[(2-hydroxy-3-methyl-benzoylhydrazonomethyl)benzyl)]pyridinium-4-yl]thio]-methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (102). Cephalosporin **89** (91 mg, 0.12 mmol) was reacted with bromomethyl intermediate **46** (49 mg, 0.12 mmol) in dry DMF to give, after 5:1 TFA:anisole deprotection, 27 mg (26%) of **102**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.25 (6H, s), 2.05 (3H, s), 3.38–3.42 (1H, m), 3.57–3.59 (1H, m), 4.09–4.12 (1H, m), 4.21–4.22 (1H, m), 5.01–5.03 (1H, m), 5.46–5.47 (2H, m), 5.71 (1H, d, $J = 4.8$ Hz), 6.61 (2H, d, $J = 8.6$ Hz), 6.80–6.82 (1H, m), 7.12 (2H, d, $J = 8.6$ Hz), 7.50–7.52 (1H, m), 7.64–7.66 (2H, m), 8.00 (1H, d, $J = 8.1$ Hz), 8.45–8.47 (2H, m), 8.63–8.65 (1H, m). MS, m/z 861.5 (M^-).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[1-carbonyl-1-(methylethyloxy)imino]acetamido]-3-[[[1[*N*-(3-hydroxy-4-hydrazonomethyl)benzyl)]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (103). Cephaolosporin **89** (87 mg, 0.115 mmol) was reacted with **49** (43 mg, 0.115 mmol) in dry DMF to give, after 5:1 TFA:anisole deprotection, 5 mg (6%) of **103**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (3H, s), 1.43 (3H, s), 3.55 (5H, bs), 3.74 (1H, d, $J = 5.3$ Hz), 4.37–4.38 (2H, m), 5.21–5.22 (1H, m), 5.67–5.68 (2H, m), 5.85–5.87 (1H, m), 6.71 (1H, s), 7.01–7.04 (2H, m), 7.28–7.29 (2H, m), 7.74 (1H, d, $J = 5.1$ Hz), 8.02–8.03 (2H, m),

8.87–8.88 (2H, m), 8.99 (1H, s), 9.44 (1H, s), 11.26 (1H, s). HRMS, *m*/*z* for $C_{30}H_{32}N_8O_8S_3$ calculated, 728.4316, found, 727.1447 (M^+).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[1-carbonyl-1-(methylethyloxy)imino]acetamido]-3-[[[1[*N*-(3-hydroxy-4-acetylhydrazonomethyl)benzyl]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (104). Cephalosporin **89** (271 mg, 0.36 mmol) was reacted with **47** (115 mg, 0.36 mmol) in dry DMF to give, after 5:1 TFA:anisole deprotection, 30 mg (26%) of **104**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.52 (3H, s), 1.53 (3H, s), 1.99 (3H, s), 3.51 (1H, d, J = 8.0 Hz), 3.69–3.71 (1H, m), 4.30–4.34 (1H, m), 4.50–4.52 (1H, m), 5.12 (1H, d, J = 4.9 Hz), 5.48–5.51 (2H, m), 5.80–5.81 (1H, m), 6.86 (2H, d, J = 5.6 Hz), 7.03 (1H, s), 7.39–7.41 (1H, m), 7.80–7.81 (2H, m), 8.17 (1H, s), 8.56–8.57 (2H, m). MS, *m*/*z* 770.0 (M^+).

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[1-carbonyl-1-(methylethyloxy)imino]acetamido]-3-[[[1[*N*-(3-hydroxy-4-[(2-hydroxy-4-butyrylhydrazonomethyl)benzyl)]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (105). Cephalosporin **89** (157 mg, 0.2 mmol) was reacted with bromomethyl intermediate **48** (151 mg, 0.2 mmol) in dry DMF to give, after 5:1 TFA:anisole deprotection, 20 mg (12%) of **105**. ^{1}H NMR (400 MHz, CDOD) δ 1.00–1.02 (3H, m), 1.61–1.75 (8H, m), 2.30–2.32 (2H, m), 3.72–3.75 (1H, m), 3.79–3.82 (1H, m), 4.39–4.42 (1H, m), 4.58–4.61 (1H, m), 5.22 (1H, d, J = 4.7 Hz), 5.60–5.63 (2H, m), 5.88–5.89 (1H, m), 6.78–6.80 (1H, m), 6.96–6.97 (1H, m), 7.18 (1H, d, J = 12 Hz), 7.48–7.50 (1H, m), 7.95 (2H, d, J = 6.9 Hz), 8.17 (1H, d, J = 2.3 Hz), 8.31 (2H, d, J = 3.6 Hz). MS, *m*/*z* 797.2 (M^+).

(6*R*,7*R*)-7-[2-Aminothiazol-4-yl)-2-[(*Z*)-[1-(*t*-butoxycarbonyl)-methylethyloxy]imino]acetamido]-3-[[[1-[*N*-(3-methoxymethyloxy-4-formyl)benzyl)methyl]pyridinium-4-yl]-thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (106). Cephalosporin **89** (518 mg, 0.67 mmol) was reacted with bromomethyl aldehyde **44** (380 mg, 1.46 mol) in 3 mL dry DMF to give intermediate **133** that was used directly in the following reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (9H, s), 1.44–1.46 (6H, two s), 3.45–3.55 (2H, m), 3.45 (3H, s), 3.71 (3H, s), 4.32 (2H, m), 5.20 (2H, s), 5.27–5.37(1H, m), 5.40 (2H, s), 5.75–6.00 (3H, m), 6.84–7.31 (4H, m), 7.32–7.33 (4H, m), 7.96–7.99 (2H, m), 8.92–9.00 (2H, m), 10.22 (1H, s). MS, *m*/*z* 933.2 (M^+).

To a solution of aldehyde **133** (56 mg, 0.06 mmol) in 3 mL MeOH was added nicotinoyl hydrazide (84 mg, 0.6 mmol) and the reaction mixture stirred at room temperature for 6 h. Additional hydrazide (0.8 mmol) was added, and the reaction stirred overnight. After concentration in vacuo, intermediate **134** was purified away from unreacted nicotinyl hydrazide and aldehyde on silica gel (9:1 $CHCl_3$:MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (9H, s), 1.41 (3H, s), 1.42 (3H, s), 3.45 (3H, s), 3.52–3.81 (2H, m), 3.71 (3H, s), 4.32 (2H, s), 5.21 (2H, s), 5.24 (1H, d, J = 4.9 Hz), 5.36 (2H, s), 5.72 (2H, s), 5.87–5.90 (1H, dd, J = 3.3, 4.9 Hz), 6.71 (1H, s), 6.83–6.86 (2H, m), 7.18–7.59 (5H, m), 7.96–8.00 (2H, m), 8.28–8.33 (2H, m), 8.83–8.96 (3H, m), 9.44 (1H, d, J = 8.3 Hz), 12.14 (1H, s). MS, *m*/*z* 1052.3 (M^+).

The entire crude hydrazide product **134** was stirred with 5:1 TFA:anisole in CH_2Cl_2 for 3 h, and the product precipitated with diisopropyl ether to give 4 mg (8% from **133**) of **106**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (3H, s), 1.38 (3H, s), 3.38–3.72 (4H, m), 4.31 (2H, s), 5.16–5.17 (1H, d, J = 2.9 Hz), 5.63 (2H, s), 5.80 (1H, bs), 6.66 (1H, s), 6.88–7.00 (2H, m), 7.62–7.64 (1H, m), 7.99–8.00 (2H, m), 8.79–8.84 (2H, m), 9.39–9.40 (1H, d, J = 7.4 Hz), 10.19 (1H, s). HPLC (15 to 25% B in A over 40 min) T_R 17.6 in. HRMS, *m*/*z* for $C_{36}H_{34}N_9O_9S_3$ calculated, 832.1642, found, 832.1646 (M^+).

Biology

Construction of siderophore-deficient *P. aeruginosa* strains

Gene replacements disrupting pyochelin and pyoverdin synthesis were performed essentially as described.[58,59] Briefly, pMJ19, a derivative of vector pEX18T (GenBank #AF004910) with an insert consisting of the 5′ portion of the *pch*D open reading frame (ORF) from PAO1, a gentamicin-resistance (GmR) marker, and the 3′ portion of the *pch*A ORF, was mobilized into strain PAO1 by conjugation. Transconjugants were selected on L agar containing 200 μg/mL Gm and patched onto L agar with 500 μg/mL carbenicillin (Cb) to identify and eliminate merodiploid organisms. DNA from clones with the appropriate Gm^R, Cb^S phenotype was purified and the alteration of the *pch* locus (truncation of *pch*D and *pch*A and the deletion of *pch*CB ORFs) was confirmed by polymerase chain reaction (PCR) and Southern blotting using previously described hybridization conditions.[60] This pyochelin-deficient strain was designated PAO1Δ*pch*.

Interruption of a gene required for pyoverdin synthesis was accomplished by inserting the *pvd*D ORF from PAO1 into pEX100T[55] and cloning a tetracycline-resistance (Tc^R) marker into the unique Pst I site of that insert, creating a vector designated pWS92-29. Introduction of this construct into PAO1Δ*pch* was again performed by conjugation and the selection of organisms required initial plating on media containing 100 μg/mL Tc, 200 μg/mL Gm, 5% sucrose, and 5 mM $FeCl_3$. The identity of the resultant strain, PAO1(Δ*pch*, pvdD::Tc), assigned PGO 2812, was confirmed by PCR and Southern blotting as described above.

Growth in low iron assay

The influence of the various siderophore-like compounds on bacterial growth in low iron conditions was studied using the *P. aeruginosa* strain PGO 2812 described above. Cells from a saturated overnight culture were diluted to approximately 0.01 OD_{600} in LB (Luria Broth Base, Miller; Gibco #0414-05-5) containing 2 mM 2,2-dipyridyl (Sigma #D-7505). The LB-dipyridyl

media was prepared at least 24 h in advance. Each assay contained 4 dilutions of compound at final concentrations of 11, 33, 100, 300 μg/mL. Each sample contained compound (resuspended in DMSO), DMSO to 3% total volume, bacteria and LB in a final volume of 1 mL. After shaking overnight at 37 °C, the OD_{600} was measured and compared to controls. Compounds were scored as positive if cultures had OD600 values $\geq$0.5.

Competition assay

Overnight cultures of *P. aeruginosa* strain PA01 were diluted to an OD_{492} of 0.0003 in LB and compound **5** was added to a concentration of 6.25 μg/mL (approximately the MIC value). Candidate siderophore compounds (in DMSO) were added to 1 mL aliquots to a final concentration of 50 μg/mL. Cultures were grown with shaking for 20 h at 37 ° in 96-well culture blocks (AGTC, Gaithersburg, MD) and turbidities were measured with an HT7000 Bio Assay Reader (Perkin–Elmer, Foster City, CA). Compounds resulting in cultures with OD492 <0.5 were considered negative; 0.5 to 0.7 were considered positive and above 0.7 were considered strongly positive.

Determination of MIC values

Screening panel. For the screening panel, bacterial isolates were cultivated from −70 ° frozen stocks by 2 consecutive overnight passages (P1, P2) at 37 ° on 5% blood agar (Remel, Lenexa, KS). The *E. coli* BR158 *tonB* mutant was received from Klaus Hantke, Tubingen, Germany. The *P. aeruginosa* PAO1 was received from Steven Lory, Seattle, WA. The *P. aeruginosa* PAO1 Cox Δ*tonB* was created at PathoGenesis for this study by William Schwan. All other strains were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and are designated *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 35151.

Compounds. All compounds for screening were provided at a concentration of 2.5 mg/mL in DMSO (Sigma). Any further dilutions were carried out in DMSO.

Susceptibility testing broth microdilution. Broth microdilution susceptibility testing was performed in accordance with National Committee for Clinical Laboratory Standards (NCCLS) standards by broth microdilution.[61] Bacterial inoculum (~5×105 CFU/mL final concentration) was added to the cation-adjusted Mueller Hinton (MH) broth (Remel), and microdilution trays were incubated at 37 ° overnight. The MIC was defined as the lowest drug concentration that inhibited visible bacterial growth. Tobramycin was included in all assays for quality control.

Agar dilution. Agar dilution susceptibility testing was performed in accordance with NCCLS standards by agar dilution.[62] Bacterial inoculum (~1×104 CFU per spot) was applied onto the MH agar (Remel), and the plates were incubated at 37 °C overnight. A Multipoint Inoculator (MAST Group LTD., Merseyside, UK) was used to spot the plates. The MIC was defined as the lowest drug concentration that inhibited bacterial growth. Tobramycin was included in all assays for quality control.

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