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A novel series of parenteral cephalosporins exhibiting potent activities against *Pseudomonas aeruginosa* and other Gram-negative pathogens: Synthesis and structure–activity relationships

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Abstract—A series of 7β-[2-(2-aminothiazol-4-yl)-2-(*Z*)-(carboxymethoxyimino)acetamido]cephalosporins bearing a 1-(substituted)-1*H*-pyrrolo[3,2-*b*]pyridinium group at C-3′ position was synthesized and their in vitro antibacterial activities against *Pseudomonas aeruginosa* and other Gram-negative pathogens were evaluated. Among the cephalosporins prepared, 7β-[2-(2-amino-5-chlorothiazol-4yl)-2(*Z*)-((*S*)-1-carboxyethoxyimino)acetamido]cephalosporins (**42d**) showed potent antibacterial activities against *P. aeruginosa* and other Gram-negative pathogens including the strains which produce class C β-lactamase and extended spectrum β-lactamase (ESBL). These results imply that both the Cl atom on the C-7 aminothiazole moiety and the α-substituent at the iminoether moiety are essential for the stability against β-lactamase and the potent activity against Gram-negative bacteria including *P. aeruginosa*.

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1. Introduction

Cephalosporins bearing aminothiazolyl or aminothiadiazolyl acetamide at the C-7 position and the quaternary ammonium group at the C-3′ position such as cefepime (CFPM),[1] cefozopran (CZOP),[2] and ceftazidime (CAZ)[3] have broad spectrum and potent activities against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa* and are widely used for treatment of infections caused by these pathogens.[4] However, only a limited number of antibacterial agents are available for nosocomial and opportunistic infections caused by Gram-negative bacteria compared to those used for the treatment of Gram-positive infection. Moreover, in case of Gram-negative bacteria, the extensive clinical application of cephalosporin and penicillin antibiotics has resulted in an increasing emergence of resistance caused by β-lactamase production. Recently, Takeda et al. reported a novel cephalosporin derivative FR264205 which has potent antibacterial activity against *P. aeruginosa* and other Gram-negative bacteria including β-lactamase-producing strains.[5] This prompted us to pursue research for a novel series of cephalosporins that can overcome the resistance by β-lactamase and show potent antibacterial activity against Gram-negative bacteria including *P. aeruginosa*.

In the course of searching for novel cephalosporins having more potent antibacterial activity against Gram-negative bacteria, we found that 7β-[2-(2-amino-5-chlorothiazol-4-yl)-2(*Z*)-(carboxymethoxyimino)acetamido]-3-[1-(3-(methylamino)propyl)-1*H*-pyrrolo[3,2-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylates showed potent antibacterial activities against *P. aeruginosa* and other Gram-negative pathogens including the strains which produce class C β-lactamase and ESBL. We report herein the preparation of cephalosporins bearing various C-7 groups and their antibacterial activities, and the

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Keywords: Cephalosporin; β-Lactamase; Resistant; Gram-negative bacterium; *Pseudomonas aeruginosa*; Extended spectrum β-lactamase.

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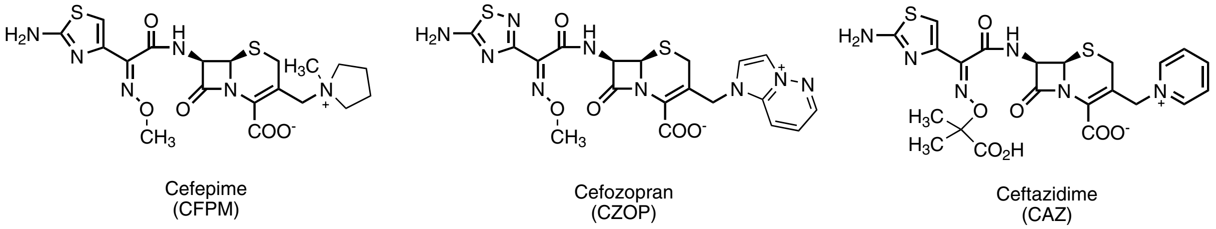


Figure 1. Structures of CFPM, CZOP, and CAZ.

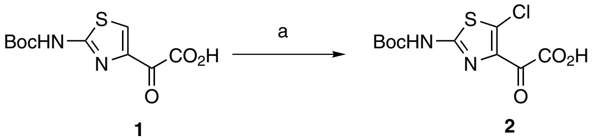
effects of various substituents on the C-7 aminothiazole and iminoether moieties for stability against β-lactamase (Fig. 1).

2. Chemistry

We synthesized novel cephalosprins as shown by the outline in Scheme 1. Aminothiazolylacetic acid derivatives as C-7 side chains were coupled with the 7-aminocephalosporin, and then the C-3′ side chain of imidazopyridine or pyrropyridine derivatives was introduced followed by deprotection.

Aminochlorothiazole derivative **2** which was used for the synthesis of C-7 side chains was prepared by chlorination of **1** using *N*-chlorosuccinimide in 1,4-dioxane as shown in Scheme 2.

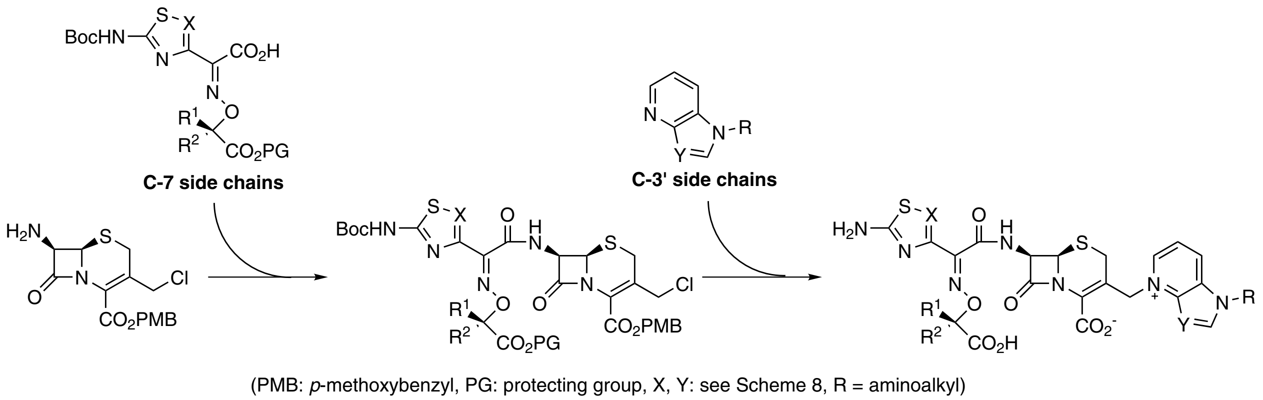
Scheme 3 outlines the synthesis of the C-7 side chains **8** and **9** bearing the chloro substituent and no substituent on the aminothiazole moiety, respectively. 2-Halo or hydroxyl acetate **3** was allowed to react with *N*-hydroxyphthalimide under the conditions of alkylation or the Mitsunobu reaction to afford the corresponding *N*-phthaloyl-alkoxyamines **4**. Treatment of **4** with *N*-methylhydrazine in dichloromethane gave alkoxyamines **7**, which were immediately allowed to react with α-keto carboxylic acid **1** or **2** to afford **8a**, **8d**, **9a–g**, and **9i–j**. Cyclopropyl derivative **9h** was prepared from commercially available hydroxycyclopropane carboxylic acid **5**, which was treated with diphenyldiazomethane in tetrahydrofuran to give **6**. Ester **6** was subjected to O-amination reaction using *O*-(mesitylsulfonyl)hydroxylamine[6] to afford **7h** in good yield. The resulting alkoxyamine **7h** was then condensed with α-keto acid **2** to give **9h**.



Scheme 2. Reagents: (a) NCS, 1,4-dioxane.

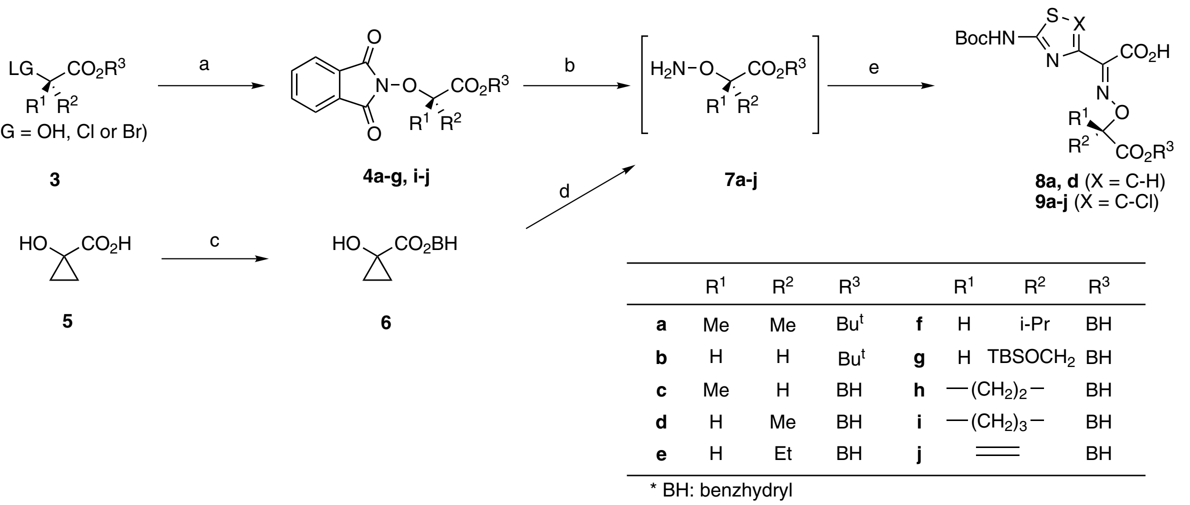
Synthesis of C-7 side chains bearing bromo and methyl on the aminothiazole is shown in Scheme 4. Keto acid **1** was brominated with *N*-bromosuccinimide to afford bromoaminothiazolyl derivative **10**, which was converted to the iminoacetic acid **11** by a method similar to that used for **8** or **9**. Ketoacid **10** was treated with methylboronic acid under the conditions of Suzuki–Miyaura coupling to give methylthiazole derivative **12** in moderate yield. Similarly, the ketoacid **12** was converted to iminoacid **13**.

Scheme 5 shows the synthesis of the iminocarboxylic acid having fluoroaminothiazole derivative **20** by a method similar to that reported by Yamamoto et al.[7] Malonic acid half-allylester **14** and acid chloride **15** were treated with isopropylmagnesium chloride in dichloromethane to afford β-ketoester **16**, which was then treated with sodium nitrite in aqueous acetic acid to afford hydroxyiminoester **17** as *E* and *Z* isomers. Compound



(PMB: *p*-methoxybenzyl, PG: protecting group, X, Y: see Scheme 8, R = aminoalkyl)

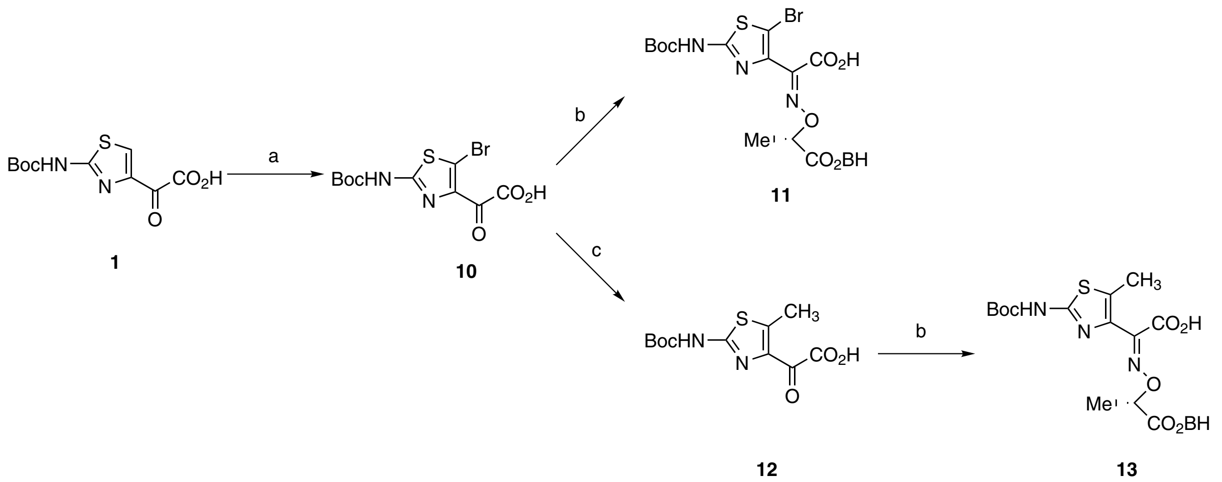
Scheme 1. Outline of the synthesis for novel cephalosporins.



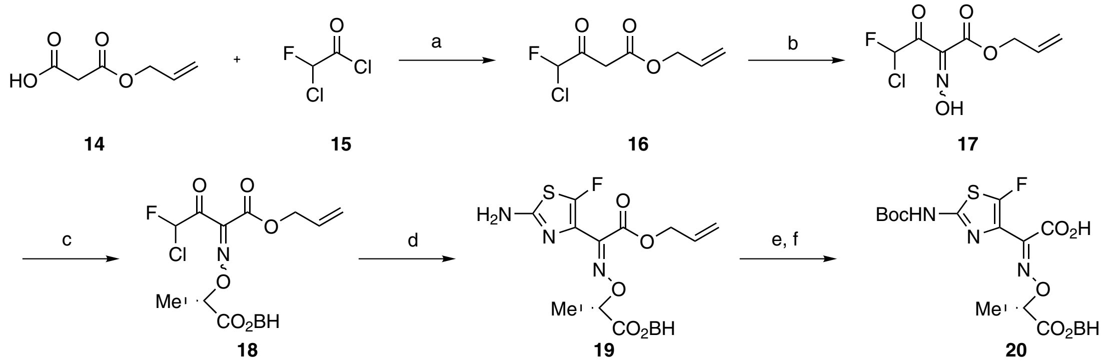
	R^1	R^2	R^3		R^1	R^2	R^3
a	Me	Me	Bu^t	**f**	H	i-Pr	BH
b	H	H	Bu^t	**g**	H	$TBSOCH_2$	BH
c	Me	H	BH	**h**	—$(CH_2)_2$—		BH
d	H	Me	BH	**i**	—$(CH_2)_3$—		BH
e	H	Et	BH	**j**	═		BH

* BH: benzhydryl

Scheme 3. Reagents: (a) RX/base or Mitsunobu reaction with *N*-hydroxyphthalimide; (b) *N*-methylhydradine, CH_2Cl_2; (c) diphenyldiazomethane, THF; (d) NaH, *O*-(mesitylsulfonyl)hydroxylamine, THF; (e) **1** or **2**, CH_2Cl_2–MeOH.



Scheme 4. Reagents: (a) NBS, 1,4-dioxane; (b) **7d**, CH_2Cl_2–MeOH; (c) $MeB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane.



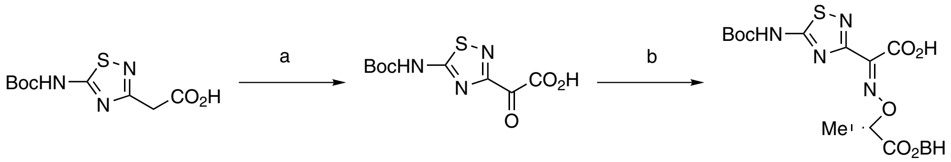
Scheme 5. Reagents: (a) *i*-PrMgCl, CH_2C1_2; (b) $NaNO_2$, AcOH, H_2O; (c) (*R*)-benzhydryl 2-hydroxypropanoate; DIAD, PPh_3, THF; (d) thiourea, DMA; (e) triphosgene, *t*-BuOH, CH_2C1_2; (f) $Pd(PPh_3)_4$, morpholine, THF.

17 was allowed to react with benzhydryl (*R*)-lactate under the conditions of the Mitsunobu reaction to afford alkoxyimino ester **18**. Ester **18** was cyclized with thiourea in dimethylacetamide to afford fluoroaminothiazolylester derivative **19** after separation of *E*/*Z* isomers by silica gel column chromatography. Protection of the amino group in compound **19** by *tert*-butyl carbamate followed by removal of the allyl group using tetrakis(triphenylphosphine)palladium in the presence of morpholine gave **20** in moderate yield.

Scheme 6 shows the synthesis of the aminothiadiazolylacetic acid derivative **23**. The N-protected aminothiadiazolylacetic acid **21** was oxidized with selenium dioxide in 1,4-dioxane to give keto acid **22**,[8] which was converted to the desired iminoacetic acid **23** by the same method used for **8** or **9**.

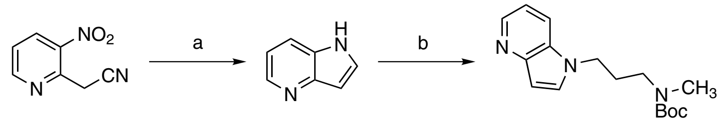
Synthesis of *tert*-butyl 3-(1*H*-pyrrolo[3,2-*b*]pyridin-1-yl)propyl(methyl)carbamate **26**, a C-3′ moiety, was accomplished as shown in Scheme 7. 2-(3-Nitropyridin-2-yl)acetonitrile **24**, prepared by the reported method,[9] was converted to the 1*H*-pyrrolo[3,2-*b*]pyridine **25**[10] under the conditions of hydrogenation using palladium carbon as a catalyst in methanol. Pyrropyridine **25** was then reacted with mesylate, which was derived from corresponding aminoalcohol, using sodium hydride in dimethylformamide to afford pyrrolopyridine derivative **26**.

Having C-7 and C-3′ moieties in hand, we then completed the synthesis of target compounds **40–46** starting from commercially available 7β-aminocephalosporin derivative **27**[11] as shown in Scheme 8. Iminoacids of



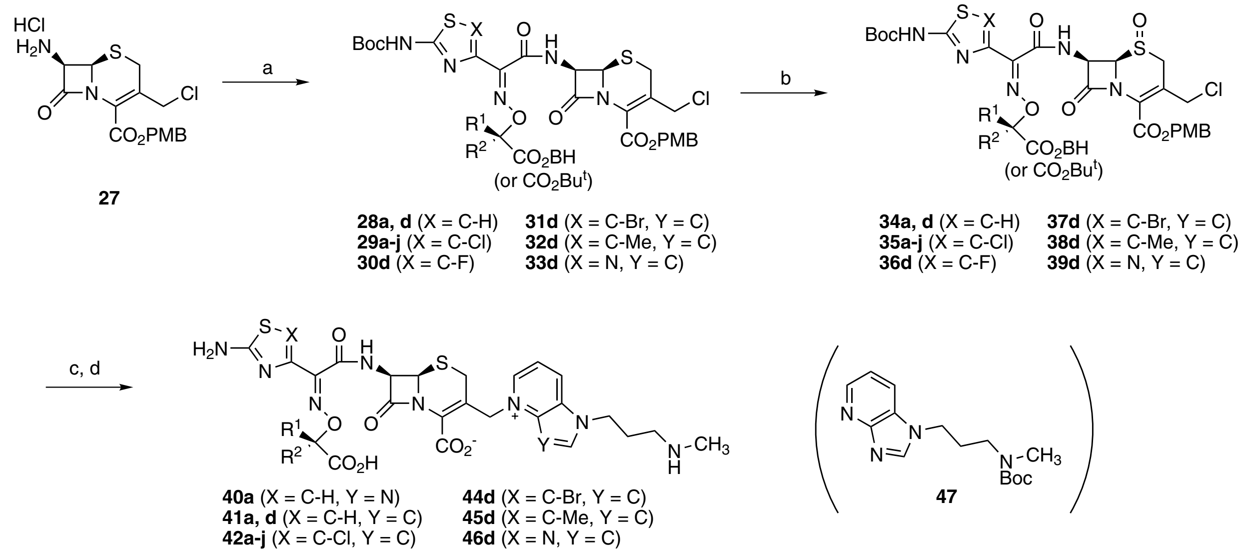
21 **22** **23**

Scheme 6. Reagents: (a) SeO_2, 1,4-dioxane; (b) **7d**, CH_2Cl_2–MeOH.



24 **25** **26**

Scheme 7. Reagents: (a) H_2, Pd/C, MeOH; (b) NaH, $MsO(CH_2)_3NMeBoc$, DMF.



27

28a, d (X = C-H) **31d** (X = C-Br, Y = C)
29a-j (X = C-Cl) **32d** (X = C-Me, Y = C)
30d (X = C-F) **33d** (X = N, Y = C)

34a, d (X = C-H) **37d** (X = C-Br, Y = C)
35a-j (X = C-Cl) **38d** (X = C-Me, Y = C)
36d (X = C-F) **39d** (X = N, Y = C)

40a (X = C-H, Y = N) **44d** (X = C-Br, Y = C)
41a, d (X = C-H, Y = C) **45d** (X = C-Me, Y = C)
42a-j (X = C-Cl, Y = C) **46d** (X = N, Y = C)
43d (X = C-F, Y = C)

47

	R^1	R^2		R^1	R^2
a	Me	Me	**f**	H	i-Pr
b	H	H	**g**	H	$TBSOCH_2$
c	Me	H	**h**	$—(CH_2)_2—$	
d	H	Me	**i**	$—(CH_2)_3—$	
e	H	Et	**j**	═	

Scheme 8. Reagents: (a) coupled with **8**, **9**, **11**, **13**, **20**, and **23**: Method A: EDC hydrochloride, pyridine, DMF, Method B: $Cl_2P(O)OPh$, *N*-methylmorpholine. CH_2Cl_2; (b) *m*-CPBA, CH_2Cl_2; (c) (i) **26** or **47**, NaBr, DMF; (ii) AcCl, KI, DMF; (d) $AlCl_3$, anisole, CH_2Cl_2, CH_3NO_2.

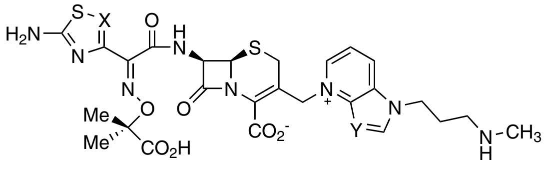
the C-7 moiety (**8a**, **8d**, **9a–j**, **11**, **13**, **20**, and **23**) were coupled with **27** using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC hydrochloride) in the presence of pyridine or dichlorophenylphosphate in the presence of *N*-methylmorpholine to give **28a**,[12] **28d**, **29a–j**, **30d**, **31d**, **32d**, and **33d**, respectively. The chloromethyl derivatives **28–33** were easily converted to *S*-oxides **34–39** using *m*-chloroperbenzoic acid. The resulting sulfoxides (mixture of α- and β-sulfoxide) were reacted with C-3′ moiety pyrrolopyridine **26** or imidazopyridine **47**[13a] followed by reduction using acetyl chloride and potassium iodide to give pyridinium salts, which were then treated with aluminum trichloride in the presence of anisole in dichloromethane/nitromethane to remove all protecting groups to obtain **40–46**.

3. Results and discussion

The in vitro antibacterial activity (MICs) of the new cephalosporins, along with CAZ and CFPM as reference compounds, against selected various Gram-positive and Gram-negative bacteria is shown in Tables 1–3. MICs were determined by the standard serial agar dilution method using Mueller–Hilton agar. As can be deduced from the data, most of the compounds synthesized exhibited potent antibacterial activity against Gram-positive bacteria including penicillin-resistant *Streptococcus pneumoniae* (PRSP) and Gram-negative bacteria including *P. aeruginosa*. In particular, the activity of several compounds against CAZ resistant *P. aeruginosa* and *Enterobacter cloacae* was markedly superior to the reference compounds (Fig. 2).

Aminothiazolylacetamidocephalosporins having a 1-carboxyethoxyimino group on the C-7 side chain, including CAZ, are known to have potent antibacterial activity against Gram-negative bacteria including *P. aeruginosa*.[3,14] Another compound having broad spectrum and potent antibacterial activities against Gram-positive including methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria including *P. aeruginosa*, S-3578 (Fig. 2),[13] as been reported by our research group. We therefore hypothesized that it might be possible to obtain a novel cephalosporin having more potent antibacterial activity against Gram-negative pathogens if the advantages of CAZ and S-3578 could be combined. Table 1 shows the in vitro antibacterial activity for the MIC of various CAZ or S-3578 derivatives **40a**, **41a**, and **42a**. We first prepared **40a** which has the C-3′ side chain of S-3678 and the C-7 side chain of CAZ. Unexpectedly, the antibacterial activity of **40a** against several Gram-negative strains tested was slightly inferior to S-3578. In the course of searching for a novel C-3′ side chain, we found that cephalosporins having pyrrolopyridinium as a C-3′ side chain also exhibited potent antibacterial activity. Therefore, we next prepared **41a** and attained great improvement of the antibacterial activity, which was equal or slightly superior to CAZ. Thus, the pyrrolopyridinium C-3′ side chain seemed to be suitable for cephalosporins bearing the carboxyethoxyimino moiety as a C-7 side chain. However, the antibacterial activity of **41a** against CAZ-resistant or β-lactamase-producing strains was not sufficient. Other work showed that cephalosporins with chloroaminothiazolylacetamido on the C-7 side chain exhibited antibacterial activity against cephalosporin-resistant strains.[15] This led us to try introducing the chloroaminothiazolyl moiety to our compound. The antibacterial activity of **42a** having this chloroaminothiazolyl moiety against CAZ-susceptible strains decreased somewhat but, compared with **41a**, it was markedly better against CAZ-resistant strains including *P. aeruginosa* and *E. cloacae*. This result prompted us to prepare other analogs based on **42a**.

Table 1. In vitro antibacterial activities (MIC, μg/mL) of compounds **40a**, **41a** and **42a**



	X = C–H, Y = N, **40a**	X = C–H, Y = C, **41a**	X = C–Cl, Y = C, **42a**	S-3578	CAZ
E. coli NIHJJC-2	0.5	0.25	1	0.25	0.25
E. coli SR21003[a]	2	2	8	64	1
E. cloacae ATCC 13047	4	1	2	0.5	8
E. cloacae SR4321[b]	128	16	4	32	64
P. aeruginosa SR24	1	1	2	2	0.5
P. aeruginosa SR24-12[c]	>128	32	8	64	64
P. aeruginosa SR5393	4	2	4	4	2
P. aeruginosa SR6554[d]	128	16	16	>128	32

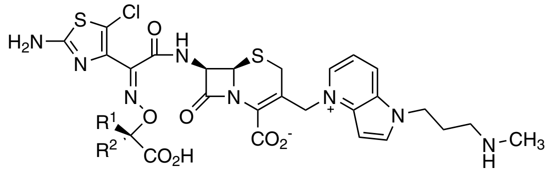
[a] Toho 2 (ESBL) producing *Escherichia coli*.

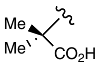
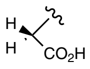
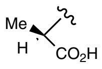
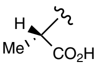
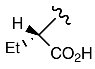
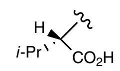
[b] AmpC producing *E. cloacae*.

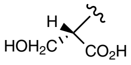
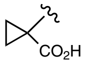
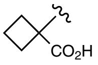
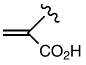
[c] AmpC hyperproducing *P. aeruginosa*.

[d] Imipenem resistant *P. aeruginosa*.

Table 2. In vitro antibacterial activities (MIC, μg/mL) of compounds **42a–j**



	R^1,R^2					
	 **42a**	 **42b**	 **42c**	 **42d**	 **42e**	 **42f**
S. aureus SMITH	2	2	2	1	1	1
S. pneumoniae SR16675[a]	4	2	4	2	4	4
E. coli NIHJ JC-2	1	0.25	0.5	0.25	1	2
E. coli SR21003[b]	8	2	4	4	4	8
E. cloacae ATCC 13047	2	0.25	1	0.5	1	4
E. cloacae SR4321	4	8	4	2	4	8
P. aeruginosa SR24	2	0.5	1	1	1	2
P. aeruginosa SR24-12[c]	8	8	4	4	4	8
P. aeruginosa SR5393	4	2	4	2	4	8
P. aeruginosa SR6554[d]	16	8	16	8	16	16
H. influenzae ATCC49766	0.032	0.032	0.063	0.032	0.032	0.032
H. influenzae SR11435[e]	0.063	0.125	0.25	0.063	0.063	0.063
M. catarrhalis ATCC43617	0.008	0.016	0.032	0.016	0.016	0.016

	 **42g**	 **42h**	 **42i**	 **42j**	CAZ	CFPM
S. aureus SMITH	2	1	1	0.5	8	2
S. pneumoniae SR16675[a]	8	4	4	2	8	2
E. coli NIHJ JC-2	0.25	0.5	1	0.25	0.25	0.063
E. coli SR21003[b]	2	4	4	1	1	4
E. cloacae ATCC 13047	0.25	0.5	2	0.25	8	0.125
E. cloacae SR4321	2	2	4	1	64	4
P. aeruginosa SR24	1	1	1	2	0.5	0.5
P. aeruginosa SR24-12[c]	4	4	4	8	64	32
P. aeruginosa SR5393	4	4	4	8	2	4
P. aeruginosa SR6554[d]	16	16	16	32	32	64
H. influenzae ATCC49766	0.063	0.016	0.032	0.032	0.063	0.032
H. influenzae SR11435[e]	0.125	0.032	0.063	0.063	0.125	0.5
M. catarrhalis ATCC43617[f]	0.032	0.008	0.016	0.016	0.063	0.5

[a] Penicillin resistant *S. pneumoniae*.

[b] Toho 2 (ESBL) producing *E. coli*.

[c] AmpC hyperproducing *P. aeruginosa*.

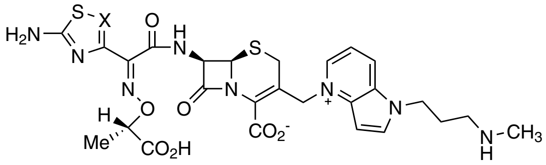
[d] Imipenem resistant *P. aeruginosa*.

[e] β-Lactamase negative ampicillin resistant *Haemophilus influenzae* (BLNAR).

[f] *M. catarrhalis*, *Moraxella* subgenus *Branhamella catarrhalis*.

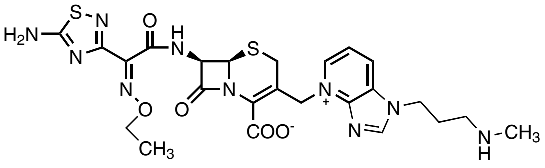
Table 2 shows the effect of various carboxymethyloxime substituents (R^1, R^2) on the MIC of 7β-[2-(2-amino-5-chlorothiazol-4-yl)-2(*Z*)-(alkoxyimino)acetamido]-3-[1-(3-(methylamino)propyl)-1*H*-pyrrolo[3,2-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylates (**42a–j**). The antibacterial activity of **42b** (R^1, R^2 = H) which has no substituents at the α-position of the carboxyl group against several strains tested, in particular CAZ-susceptible *P. aeruginosa* and *E. cloacae*, was superior to **42a** (R^1, R^2 = Me). The antibacterial activity of mono-methyl substituted **42c** (R^1 = Me, R^2 = H) and **42d** (R^1 = H, R^2 = Me) against CAZ-resistant *P. aeruginosa* SR24-12 and *E. cloacae* SR4321 producing AmpC β-lactamase was slightly improved compared to that of **42a**. The stereochemistry of the chiral carboxymethyl moiety was found to be important for the activity, and *S* isomer **42d** appeared to be superior to *R* isomer **42c**. Compound **42d** exhibited well-balanced antibacterial activity against Gram-negative including AmpC β-lactamase and ESBL producing strains and Gram-positive strains. This result prompted us to prepare several analogs of compound **42d**. The length of the carbon chain or bulkiness of the substituents also had some influence on the MIC. The ethyl derivative **42e** (R^1 = H, R^2 = Et) of the *S*

Table 3. In vitro antibacterial activities (MIC, μg/mL) of compounds **41d**–**46d**



	X					
	C–Cl, **42d**	C–H, **41d**	C–F, **43d**	C–Br, **44d**	C–Me, **45d**	N, **46d**
S. aureus SMITH	1	2	2	1	4	2
S. pneumoniae SR16675	2	8	16	4	8	4
E. coli NIHJJC-2	0.25	0.063	0.031	0.5	2	0.031
E. coli SR21003	4	1	16	4	32	8
E. cloacae ATCC 13047	0.5	0.25	1	1	4	0.25
E. cloacae SR4321	2	16	>128	4	16	8
P. aeruginosa SR24	1	0.5	1	1	1	0.5
P. aeruginosa SR24-12	4	32	>128	4	8	16
P. aeruginosa SR5393	2	2	8	4	4	2
P. aeruginosa SR6554	8	16	128	8	64	4
H. influenzae ATCC49766	0.032	0.032	0.063	0.063	0.125	0.031
H. influenzae SR11435	0.063	0.063	0.25	0.125	2	0.25
M. catarrhalis ATCC43617	0.016	0.125	0.5	0.016	0.5	0.5

Abbreviations: see footnote in Table 2.



S-3578

Figure 2. Structure of S-3578.

configuration showed relatively weak activity to **42d**, and the activity of the **42f** ($R^1 = H$, $R^2 = i$-Pr) bearing the isopropyl group decreased further. Although the hydroxyl substituent, such as in **42g** ($R^1 = H$, $R^2 = CH_2OH$), hardly affected the antibacterial activity against Gram-negative bacteria, the activity against Gram-positive bacteria including *S. aureus* and *S. pneumoniae* was lower than that of **42d**.

The cyclic compounds **42h** and **42i** with a cyclopropyl and a cyclobutyl moiety, respectively, showed activities superior to that of dimethyl derivative **42a** which was fully substituted, but had somewhat lower antibacterial activity against several strains, including *P. aeruginosa*, than that of **42d**. The acrylic acid derivative **42j** appeared much less active against *P. aeruginosa*, but exhibited the most potent activity against Gram-positive bacteria of all the compounds in Table 2. These results indicated that the substituents on the carboxymethoxyimino moiety and its absolute configuration were important for the antibacterial activity, especially against CAZ-resistant *P. aeruginosa*.

We next examined the effect of the substituent on aminothiazole. Table 3 presents the antibacterial activity of **42d**, which showed the best activity against Gram-negative bacteria including *P. aeruginosa* and *E. cloacae*, and its derivatives **41d**–**46d**. Compound **41d** which had no substituent on aminothiazole showed a marked decrease of the activity against CAZ-resistant *P. aeruginosa*, as expected. Replacement of the chloro substituent by fluorine on the aminothiazole moiety (**43d**) resulted in a marked decrease of antibacterial activity, especially against CAZ-resistant *P. aeruginosa* and *E. cloacae*, which was inferior to that of CAZ. Compound **44d** with a bromo substituent showed well-balanced activity against Gram-negative and Gram-positive strains, but was relatively weaker than **42d** with a chloro substituent. Introduction of an alkyl substituent to the aminothiazole moiety appeared to be unfavorable for increasing antibacterial activity. Compound **45d** bearing a methyl substituent showed decreased activity against most strains tested. In contrast, conversion of the aminothiazole to aminothiadiazole (**46d**) was found to be beneficial for the activity against CAZ-susceptible *P. aeruginosa* SR24 and *E. cloacae* ATCC13047, although the activity of **46d** exhibited much less activity against strains producing AmpC β-lactamase, *P. aeruginosa* SR24-12 and *E. cloacae* SR4321 than that of **42d**. These results indicated that the combination between the substituted aminothiazole or aminothiadiazole and the iminoacetic acid moiety was important for the antibacterial activity.

In conclusion, we found compound **42d** having a chlorine on aminothiazole and an α-methyl substituent on the oxyiminoacetic acid moiety to be the optimized compound with potent and well-balanced activity against antibacterial activities against *P. aeruginosa* and other Gram-negative pathogens including the strains which produce class C β-lactamase and ESBL.

Table 4. In vivo efficacy of **42d** in the mouse systemic infection model

	P. aeruginosa SR-24		*P. aeruginosa* SR24-12	
	MIC (μg/mL)	ED_{50} (mg/kg)	MIC (μg/mL)	ED_{50} (mg/kg)
42d	1	1.40	4	5.32
CAZ	1	2.83	64	127

The in vivo efficacy of **42d**, as well as that of CAZ, was evaluated and the results are shown in Table 4. The therapeutic effect was evaluated with the mouse systemic infection model using two strains, CAZ-susceptible *P. aeruginosa* SR-24 and CAZ-resistant *P. aeruginosa* SR24-12. The efficacy of each compound was expressed as 50% effective dose values (ED_{50}) which were calculated by the prohibit method from the number of mice surviving seven days after infection. The in vivo antibacterial activity of **42d** against SR24 was twice that of CAZ. Furthermore, **42d** showed activity twenty times more potent than CAZ against SR24-12 as predicted from its MIC value. This finding indicates the importance of the combination of a chloro substituent on aminothiazole and a methyl substituent on the oxyiminoacetic acid moiety on the in vivo efficacy as well as the in vitro antibacterial activity.

4. Conclusion

Chemical modification and optimization of the aminothiazole and alkoxyimino moieties at the C-7 side chain of a cephalosporin demonstrated that novel cephalosporins having both a 1-carboxyalkoxyimino moiety and a chloroaminothiazole moiety at the C-7 exhibit potent antibacterial activity. Among the prepared cephalosporins, 7β-[2-(2-amino-5-chlorothiazol-4-yl)-2(*Z*)-((*S*)-1-carboxyethoxyimino)acetamido]-3-[1-(3-(methylamino) propyl)-1*H*-pyrrolo[3,2-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylate **42d** showed the most potent antibacterial and well-balanced activity against Gram-negative bacteria including *P. aeruginosa* and *E. cloacae* and Gram-positive bacteria including *S. aureus* and *S. pneumoniae*. Furthermore, **42d** was found to have dramatically improved activity against CAZ-resistant *P. aeruginosa* SR24-12 and SR6554.

5. Experimental

Infrared (IR) spectra were recorded on a JASCO FT/IR-700 spectrometer. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini-300 spectrometer. Chemical shifts are reported in ppm using tetramethylsilane (TMS in $CDCl_3$ and DMSO-d_6) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS in D_2O) as internal standards. Analytical thin layer chromatography (TLC) was carried out on Merck precoated TLC plates silica gel 60 F_{254} and visualized with UV light or 10% H_2SO_4 containing 5% ammonium molybdate and 0.2% ceric sulfate. Column chromatography was carried out on Merck Kieselgel 60 (230–400 mesh) and Mitsubishi Chemical HP-20.

5.1. Synthesis of C-7 side chains

5.1.1. 2-(2-(*tert*-Butoxycarbonylamino)-5-chlorothiazol-4-yl)-2-oxoacetic acid (2). To a solution of 2-(2-(*tert*-butoxycarbonylamino)thiazol-4-yl)-2-oxoacetic acid (**1**) (140 g, 0.51 mol) in 1,4-dioxane (1 L), *N*-chlorosuccinimide (72.8 g, 0.55 mol) was added portionwise. The mixture was stirred at 40 °C for 8 h. The reaction mixture was cooled to room temperature and then filtered to remove insoluble material. The filtrate was concentrated and to the residue were added diethyl ether (200 mL) and *n*-hexane (100 mL). The insoluble material was filtered off and washed with diethyl ether. The filtrate was concentrated and *n*-hexane was added to the residual oil. The precipitate was collected by filtration and dried in vacuo to give compound **2** (128 g, 87%) as an amorphous solid. ^1H NMR (DMSO-d_6) δ: 1.48 (9H, s), 12.2 (1H, br s).

5.1.2. *tert*-Butyl 2-(1,3-dioxoisoindolin-2-yloxy)-2-methylpropanoate (4a). To a suspension of NaH (11.3 g, 290 mmol, 60%w/w) in DMF (400 mL) were added *N*-hydroxyphthalimide (44 g, 270 mmol) and *tert*-butyl 2-bromo-2-methylpropanoate (66 g, 283 mmol) at 0 °C. The reaction mixture was heated to 100 °C and stirred for 5 h. The solvent was removed by evaporation and AcOEt (300 mL) and water were added to the residue. The organic layer was washed with 10% aqueous $NaHCO_3$, water, and saturated brine. The aqueous layer was successively extracted with AcOEt and the combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The resulting precipitated material was collected by filtration and dried in vacuo to give **4a** (42 g, 51%) as a solid. ^1H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.59 (6H, s), 7.74–7.78 (2H, m), 7.83–7.86 (2H, m). IR (KBr) cm^{-1}: 1788, 1740, 1717, 1466, 1388, 1370, 1328, 1298, 1253, 1186, 1153, 1121.

5.1.3. *tert*-Butyl 2-(1,3-dioxoisoindolin-2-yloxy)acetate (4b). To a suspension of *N*-hydroxyphthalimide (32.6 g, 0.20 mol) in THF (400 mL) were added NEt_3 (30.7 mL, 0.22 mol) and *tert*-butyl bromoacetate (42.9 g, 0.22 mol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for a further 5 h. The resulting mixture was evaporated to remove THF and then $CHCl_3$ (300 mL) and water (100 mL) were added to the residue. The whole mixture was poured into saturated aqueous $NaHCO_3$ (400 mL). The aqueous layer was extracted with $CHCl_3$ and combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to give a crude crystalline solid, which was triturated in *n*-hexane/Et_2O and collected by filtration. The collected solid was dried in vacuo to give **4b** (55 g, 90%). ^1H NMR ($CDCl_3$) δ: 1.49 (9H, s), 4.71 (2H, s), 7.70–7.90 (4H, m). IR (KBr) cm^{-1}: 2980, 2939, 1788, 1745, 1730, 1465, 1441, 1374, 1247, 1186, 1160, 1137, 1043.

5.1.4. (*R*)-Benzhydryl 2-(1,3-dioxoisoindolin-2-yloxy)propanoate (4c). To a solution of (*S*)-2-bromopropanoic acid (20.0 g, 130 mmol) in THF (100 mL) was added dropwise a solution diphenyldiazomethane (25.2 g, 130 mmol) in THF (300 mL) over 1 h and the reaction mixture was stirred an additional 1 h at room temperature. The reaction mixture was concentrated, and then AcOEt (200 mL) and saturated aqueous $NaHCO_3$were added. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give (*S*)-benzhydryl 2-bromopropanoate (49.7 g) which was used for the next step without further purification.

To a suspension of *N*-hydroxyphthalimide (25.8 g, 158 mmol) in H_2O were added K_2CO_3(18.5 g, 134 mmol), *n*-Bu_4NBr (3.93 g, 12.2 mmol), and a solution of 2-bromopropanoate (49.0 g, 122 mmol) in AcOEt (490 mL). The reaction mixture was vigorously stirred for 15 h. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The precipitated material was collected with Et_2O/*n*-hexane and dried in vacuo to give **4c** (46.6 g, 95%). ^1H NMR ($CDCl_3$) δ: 1.67 (3H, d, $J = 6.9$ Hz), 5.05 (1H, q, $J = 6.9$ Hz), 6.93 (1H, s), 7.21–7.35 (10H, m), 7.70–7.79 (4H, m). IR (KBr) cm^{-1}: 1791, 1731, 1494, 1452, 1444, 1385, 1368, 1283, 1185, 1117.

5.1.5. (*S*)-Benzhydryl 2-(1,3-dioxoisoindolin-2-yloxy)propanoate (4d). To a stirred solution of (*S*)-benzhydryl 2-hydroxypropanoate (40 g, 156 mmol) in DMF (160 mL), sulfuryl chloride (23.2 g, 172 mmol) was added dropwise over 30 min. Next, the reaction mixture was warmed to room temperature and stirred at room temperature for 1.5 h before being partitioned between AcOEt (350 mL) and ice water (350 mL) containing $NaHCO_3$ (55 g). The organic layer was separated and washed with saturated $NaHCO_3$ and brine. The separated organic layer was dried over $MgSO_4$, filtered, and concentrated to give (*R*)-benzhydryl 2-chloropropanoate (47.5 g) which was used for the next step without further purification.

To a stirred solution of (*R*)-benzhydryl 2-chloropropanoate (47.5 g) in DMF (240 mL) were added *N*-hydroxyphthalimide (26.7 g, 164 mmol) and K_2CO_3 (22.6 g, 164 mmol). The reaction mixture was stirred at room temperature for 18 h and then poured into ice water (500 mL). The mixture was extracted with 20% THF in AcOEt (500 mL) three times and combined organic layer was washed with brine (×5), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residual solid was recrystallized from *n*-hexane/AcOEt to give **4 d** (35.0 g, 56%) as a crystalline solid. ^1H NMR ($CDCl_3$) δ: 1.66 (3H, d, $J = 7.2$ Hz), 5.05 (1H, q, $J = 7.2$ Hz), 6.93 (1H, s), 7.20–7.35 (10H, m), 7.70–7.79 (4H, m). IR (KBr) cm^{-1}: 1793, 1733, 1497, 1455, 1443, 1386, 1285, 1187, 1118.

5.1.6. (*S*)-Benzhydryl 2-(1,3-dioxoisoindolin-2-yloxy)butanoate (4e). To a solution of (*R*)-2-aminobutanoic acid (25.0 g, 242 mmol) in 2.5NH_2SO_4 (600 mL) was added KBr (100.8 g, 847 mmol) and the mixture was cooled to −10 °C. To this mixture was added $NaNO_2$ (25.0 g, 362 mmol) portionwise over 1 h. The reaction mixture was stirred at −5 °C for 1.5 h before AcOEt was added. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give (*R*)-2-bromobutanoic acid (30.0 g) which was used without further purification.

To a solution of (*R*)-2-bromobutanoic acid (30.0 g) in THF (100 mL), the solution of diphenyldiazomethane (30 g, 154 mmol) in THF (200 mL) was added dropwise. The reaction mixture was left standing at room temperature overnight and then concentrated. The residue was purified by column chromatography on silica gel to give (*R*)-benzhydryl 2-bromobutanoate (41.6 g, 52%). ^1H NMR ($CDCl_3$) δ: 0.99 (3H, t, $J = 7.5$ Hz), 1.96–2.21 (2H, m), 4.27 (1H, dd, $J = 7.8$, 6.6 Hz), 6.89 (1H, s), 7.25–7.38 (10H, m).

To a solution of *N*-hydroxyphthalimide (32.6g, 0.20 mol) in DMF (200 mL) were added NEt_3 (17.3 mL, 124 mmol) and the solution of 2-bromobutanoate (41.6 g, 124 mmol) in DMF (80 mL). The reaction mixture was stirred for 1 h and then poured into water. The mixture was extracted with AcOEt and the organic layer was washed with brine, dried over MgSO4, filtered, and concentration in vacuo to give **4e** (44.1 g, 86%) as a solid. ^1H NMR ($CDCl_3$) δ: 1.07 (3H, t, $J = 7.2$ Hz), 2.00–2.11 (2H, m), 4.87 (1H, t, $J = 6.0$ Hz), 6.95 (1H, s), 7.22–7.33 (10H, m), 7.72–7.80 (4H, m). IR (KBr) cm^{-1}: 2980, 2939, 2878, 1791, 1736, 1495, 1463, 1455, 1371, 1341, 1301, 1273.

5.1.7. (*S*)-Benzhydryl 2-(1,3-dioxoisoindolin-2-yloxy)-3-methylbutanoate (4f). Compound **4f** was prepared from (*R*)-2-amino-3-methylbutanoic acid by a procedure similar to that used for **4e**: an amorphous solid. 32% yield (overall). ^1H NMR ($CDCl_3$) δ: 0.98 (3H, d, $J = 6.6$ Hz), 1.16 (3H, d, $J = 6.9$ Hz), 2.37 (1H, m), 4.63 (1H, d, $J = 6.9$ Hz), 6.96 (1H, s), 7.18–7.36 (10H, m), 7.69–7.77 (4H, m). IR (KBr) cm^{-1}: 2974, 1792, 1732, 1497, 1465, 1456, 1372, 1349, 1274, 1187, 1118.

5.1.8. (*S*)-Benzhydryl 3-(*tert*-butyldimethylsilyloxy)-2-(1,3-dioxoisoindolin-2-yloxy)propanoate (4g). (*R*)-Benzhydryl 2-bromo-3-hydroxypropanoate was prepared from D-serine by a procedure similar to that used for (*R*)-benzhydryl 2-bromobutanoate in the synthesis **4e**: an oil. 62% yield. ^1H NMR ($CDCl_3$) δ: 3.95 (1H, dd, $J = 5.7$, 12.0 Hz), 4.05 (1H, dd, $J = 7.2$, 12.0 Hz), 4.45 (1H, dd, $J = 5.7$, 7.2 Hz), 6.90 (1H, s), 7.28–7.38 (10H, m).

To a solution of (*R*)-benzhydryl 2-bromo-3-hydroxypropanoate (20.9 g, 62.0 mmol) in THF (200 mL) were added imidazole (4.22 g, 62.0 mmol), *tert*-butyldimethylsilylchloride (9.35 g, 62.0 mmol), and DMF (20 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The resulting mixture was concentrated and water was added. The whole mixture was extracted with AcOEt and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give (*R*)-benzhydryl 2-bromo-3-(*tert*-butyldimethylsilyloxy) propanoate as an oil (24.3 g, 87%). ^{1}H NMR ($CDCl_3$) δ: 0.01 (3H, s), 0.03 (3H, s), 0.81 (9H, s), 3.91 (1H, dd, $J = 5.1$, 10.2 Hz), 4.05 (1H, dd, $J = 9.0$, 10.2 Hz), 4.31 (1H, dd, $J = 5.1$, 9.0 Hz), 6.92 (1H, s), 7.28–7.38 (10H, m).

Compound **4g** was prepared from (*R*)-benzhydryl 2-bromo-3-(*tert*-butyldimethylsilyloxy)propanoate described above by a procedure similar to that used for **4e**: oil. 72% yield. ^{1}H NMR ($CDCl_3$) δ: 0.03 (6H, s), 0.80 (9H, s), 4.19 (2H, q, $J = 4.8$ Hz), 6.97 (1H, s), 7.23–7.35 (10H, m), 7.70–7.79 (4H, m). IR (KBr) cm^{-1}: 2955, 2930, 2885, 2858, 1794, 1738, 1496, 1469, 1455, 1372, 1258.

5.1.9. Benzhydryl 1-(1,3-dioxoisoindolin-2-yloxy)cyclobutanecarboxylate (4i). To a solution of ethyl 1-bromocyclobutanecarboxylate (10.2 g, 49.3 mmol) in MeOH (100 mL) was added 8 N aqueous NaOH (31 mL) at 0 °C. The reaction mixture was stirred for 45 min before concentrated HCl (22 mL) was added. The resulting mixture was evaporated to remove MeOH and then the residual mixture was extracted with AcOEt (×2). The combined organic layer was washed with saturated brine, dried over $MgSO_4$, filtered, and concentrated. To the residual oil in THF (30 mL), a solution of diphenyldiazomethane (14.4 g, 73.9 mmol) in THF (30 mL) was added dropwise over 2 h. The reaction mixture was left standing at room temperature overnight. The resulting solution was concentrated and the residue was purified by silica gel column chromatography to give benzhydryl 1-bromocyclobutanecarboxylate (9.69 g, 57%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.78–1.92 (1H, m), 2.14–2.28 (1H, m), 2.60–2.71 (2H, m), 2.88–2.98 (2H, m), 6.89 (1H, s), 7.25–7.38 (10H, m).

To a solution of benzhydryl 1-bromocyclobutanecarboxylate (8.65 g, 25 mmol) in DMSO (70 mL) were added *N*-hydroxyphthalimide (3.26 g, 20 mmol) and potassium carbonate (8.29 g, 60 mmol). The reaction mixture was stirred at 80 °C for 2 h and poured into ice water. The whole mixture was extracted with AcOEt and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residual solid was triturated with Et_2O/*n*-hexane and collected by filtration to give **4i** (5.10 g, 48%) as a solid. ^{1}H NMR ($CDCl_3$) δ: 1.65–1.80 (1H, m), 1.94–2.07 (1H, m), 2.54–2.65 (4H, m), 6.93 (1H, s), 7.24–7.40 (10H, m), 7.72–7.76 (4H, m). IR (KBr) cm^{-1}: 2951, 1794, 1730, 1495, 1454, 1367, 1344, 1306, 1254, 1208, 1182, 1149, 1119.

5.1.10. Benzhydryl 2-(1,3-dioxoisoindolin-2-yloxy)acrylate (4j). Compound **4j** was prepared according to the reported procedure.[16]

5.1.11. (*Z*)-2-(1-*tert*-Butoxycarbonyl-1-methylethoxyimino)-2-(2-(*tert*-butoxycarbonylamino)thiazol-4-yl)acetic acid (8a). Compound **8a** was prepared from **7a** using a procedure similar to that used for **9d** with **1**: solid. ^{1}H NMR (DMSO-d_6) δ: 1.43 (9H, s), 1.47 (14H, s), 7.38 (1H, s), 11.8 (1H, br s). IR (KBr) cm^{-1}: 3205, 2983, 1726, 1560, 1473, 1456, 1394, 1369, 1298, 1245, 1157.

5.1.12. (*S*,*Z*)-2-(1-(Benzhydryloxycarbonyl)ethoxyimino)-2-(2-(*tert*-butoxycarbonylamino)thiazol-4-yl)acetic acid (8d). Compound **8d** was prepared using a procedure similar to that used for **9d** with **1**: solid. ^{1}H NMR ($CDCl_3$) δ: 1.54 (9H, s), 1.60 (3H, d, $J = 7.2$ Hz), 5.16 (1H, q, $J = 7.2$ Hz), 6.91 (1H, s), 7.25–7.36 (10H, m). IR (KBr) cm^{-1}: 3194, 1723, 1560, 1497, 1455, 1372, 1246, 1153.

5.1.13. (*S*,*Z*)-2-(1-(Benzhydryloxycarbonyl)ethoxyimino)-2-(2-(*tert*-butoxycarbonylamino)-5-chlorothiazol-4-yl)acetic acid (9d). To a solution of **4d** (32.1 g, 80.0 mmol) in CH_2Cl_2 (96 mL) was added methylhydrazine (3.68 g, 80.0 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h and filtered to remove insoluble material. The filtrate was concentrated to approximately 50 mL. To the resulting solution, MeOH (200 mL) and **2** (24.5 g, 80.0 mmol) were added with stirring at 0 °C, and the mixture was allowed to warm to room temperature. After 3 h, the reaction mixture was evaporated to remove MeOH and the residual oil was diluted in AcOEt. The whole mixture was washed with 0.1 N HCl and saturated brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residual solid was triturated with *n*-hexane and collected by filtration to give **9d** (44.9 g, 100%) as a solid. ^{1}H NMR (DMSO-d_6) δ: 1.46 (3H, d, $J = 6.9$ Hz), 1.47 (9H, s), 5.00 (1H, q, $J = 6.9$ Hz), 6.85 (1H, s), 7.26–7.42 (10H, m), 12.06 (1H, s). IR (KBr) cm^{-1}: 3422, 3193, 3062, 3032, 2983, 1740, 1719, 1602, 1554, 1453, 1370, 1250, 1155.

Compounds **9a–c**, **9e–g**, and **9i–j** were prepared by the same procedure as that described for **9d** with**4a–c**, **4e–g**, and **4i–j**, respectively.

5.1.13.1. Compound 9a. An amorphous solid. 97% yield. ^{1}H NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.52 (9H, s), 1.58 (6H, s), 5.20–6.20 (2H, br s). IR (KBr) cm^{-1}: 3426, 3220, 3081, 2981, 2937, 1720, 1556, 1455, 1394, 1369, 1249, 1155.

5.1.13.2. Compound 9b. Amorphous solid. 95% yield. ^{1}H NMR (DMSO-d_6) δ: 1.42 (9H, s), 1.46 (9H, s), 4.36 (2H, s), 11.9 (1H, br s). IR (KBr) cm^{-1}: 3429, 3136, 2982, 2936, 1739, 1715, 1626, 1557, 1458, 1392, 1381, 1370, 1249, 1157.

5.1.13.3. Compound 9c. Amorphous solid. 100% yield. ^{1}H NMR (DMSO-d_6) δ: 1.48–1.49 (12H, m), 5.01 (1H, q, $J = 6.9$ Hz), 6.86 (1H, s), 7.25–7.45 (10H, m), 12.08 (1H, s). IR (KBr) cm^{-1}: 3197, 2983, 1762, 1717, 1548, 1495, 1451, 1368, 1295, 1242, 1199, 11171, 1145, 1102.

5.1.13.4. Compound 9e. Foam. 100% yield. ^{1}H NMR (DMSO-d_6) δ: 0.89 (3H, t, $J = 7.5$ Hz), 1.46 (9H, s), 1.78 (2H, quintet-like), 4.52 (1H, t, $J = 6.9$ Hz), 6.84 (1H, s), 7.23–7.46 (10H, m), 12.0 (1H, br s). IR (KBr) cm^{-1}: 3431, 3180, 3064, 3033, 2978, 2934, 1736, 1715, 1621, 1557, 1496, 1455, 1391, 1370, 1295, 1250, 1211, 1158, 1118, 1064, 1034.

5.1.13.5. Compound 9f. Amorphous solid. 100% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (3H, d, $J = 6.6$ Hz), 0.93 (3H, d, $J = 6.6$ Hz), 1.46 (9H, s), 2.07 (1H, sextet-like), 4.35 (1H, d, $J = 7.2$ Hz), 6.87 (1H, s), 7.1–7.5 (11H, m), 12.0 (1H, br s). IR (KBr) cm^{-1}: 3422, 3207, 3064, 3032, 2976, 2933, 2876, 1717, 1629, 1555, 1495, 1455, 1393, 1370, 1295, 1248, 1156, 1055, 1032.

5.1.13.6. Compound 9g. Amorphous solid. 99% yield. ^{1}H NMR (DMSO-d_6) δ: −0.03 (3H, s), −0.01 (3H, s), 0.77 (9H, s), 1.46 (9H, s), 3.87–3.99 (2H, m), 4.63 (1H, t-like), 6.83 (1H, s), 7.22–7.48 (11H, m), 11.1 (1H, br s). IR (KBr) cm^{-1}: 3450, 3159, 3078, 2955, 2794, 1772, 1697, 1428, 1417, 1373, 1294, 1240, 1191, 1002.

5.1.13.7. Compound 9i. Amorphous solid. 100% yield. ^{1}H NMR (DMSO-d_6) δ: 1.47 (9H, s), 1.75–2.00 (2H, m), 2.20–2.38 (2H, m), 2.44–2.54 (2H, m), 6.82 (1H, s), 7.1–7.5 (10H, m), 12.0 (1H, br s). IR (KBr) cm^{-1}: 3209, 3064, 3031, 2980, 2955, 1719, 1619, 1554, 1495, 1454, 1394, 1370, 1295, 1249, 1204, 1155, 1067, 1037.

5.1.13.8. Compound 9j. Foam. 89% yield. ^{1}H NMR (CDCl$_3$) δ: 1.48 (9H, s), 5.65 (1H, d, $J = 2.4$), 5.75 (1H, d, $J = 2.4$), 6.93 (1H, s), 7.27–7.34 (10H, m). IR (CHCl$_3$) cm^{-1}: 3602, 3404, 1723, 1603, 1550, 1285, 1253, 1227, 1155.

5.1.13.9. Compound 9h. To a solution of 1-hydroxycyclopropanecarboxylic acid (4.55 g, 44.6 mmol) in THF (100 mL), a solution of diphenyldiazomethane (10.4 g, 53.5 mmol) in THF (50 mL) was added. The reaction mixture was stirred at room temperature for 6 h, and then the solvent was removed by evaporation. The precipitated solid was collected by filtration and dried in vacuo to give **6** (11.36 g, 95%). ^{1}H NMR (CDCl$_3$) δ: 1.21 (2H, m), 1.40 (2H, m), 3.00 (1H, br s), 6.91 (1H, s), 7.25–7.38 (10H, m).

To a stirring solution of **6** (4.02 g, 15.0 mmol) in THF (30 mL) in an ice bath, sodium hydride (720 mg, 18 mmol, 60%w/w) was carefully added, and then the mixture was stirred for 20 min. To a resulting suspension was added a solution of *O*-(mesitylsulfonyl)hydroxylamine (4.20 g, 19.5 mmol) in THF (30 mL), which was used after drying over MgSO$_4$. After this was stirred for 1 h in an ice bath, AcOEt and water were added. The organic layer was washed with brine, dried over MgSO$_4$, filtered, and concentrated by evaporation. The residual oil was subjected to column chromatography on silica gel to give **7** (2.72 g, 64%) as a solid. ^{1}H NMR (CDCl$_3$) δ: 1.30 (2H, m), 1.46 (2H, m), 6.93 (1H, s), 7.26–7.34 (10H, m).

To a stirred solution of **2** (920 mg, 3.0 mmol) in MeOH (10 mL) in an ice bath was added **7** (850 mg, 3.0 mmol), and then the reaction mixture was allowed to warmed to room temperature and stirred for 1.5 h. The resulting mixture was concentrated and diisopropyl ether/*n*-hexane (1:1) was added. The precipitated material was collected by filtration and dried in vacuo to give **9h** (1.59 g, 93%) as an amorphous solid. ^{1}H NMR (CDCl$_3$) δ: 1.52 (9H, s), 1.67 (4H, s), 6.91 (1H, s), 7.24–7.33 (10H, m). IR (KBr) cm^{-1}: 3190, 2983, 1716, 1555, 1497, 1454, 1367, 1272, 1249, 1151.

5.1.14. 2-(2-(*tert*-Butoxycarbonylamino)-5-bromothiazol-4-yl)-2-oxoacetic acid (10). This compound was prepared by a procedure similar to that used for **2** with *N*-bromosuccinimide: amorphous solid. 64% yield. ^{1}H NMR (DMSO-d_6) δ: 1.46 (9H, s), 12.2 (1H, br s). IR (KBr) cm^{-1}: 3193, 2983, 1719, 1691, 1552, 1481, 1454, 1371, 1300, 1275, 1253.

5.1.15. (*S*,*Z*)-2-(1-(Benzhydryloxycarbonyl)ethoxyimino)-2-(5-bromo-2-(*tert*-butoxycarbonylamino)thiazol-4-yl)acetic acid (11). This compound was prepared by a procedure similar to that used for **9d** with **10**: solid. 99% yield. ^{1}H NMR (CDCl$_3$) δ: 1.52 (9H, s), 1.61 (3H, d, $J = 6.9$ Hz), 5.08 (1H, q, $J = 6.9$ Hz), 6.93 (1H, s), 7.25–7.33 (10H, m). IR (KBr) cm^{-1}: 3185, 1751, 1719, 1556, 1495, 1453, 1370, 1278, 1243, 1197.

5.1.16. 2-(2-(*tert*-Butoxycarbonylamino)-5-methylthiazol-4-yl)-2-oxoacetic acid (12). To a solution of **10** (1.76 g, 5.0 mmol) in 1,4-dioxane (50 mL) were added methylboronic acid (448 mg, 7.5 mmol) and K$_3$PO$_4$ (4.25 g, 20 mmol). The mixture was evacuated and backfilled with nitrogen (three cycles). Pd(PPh$_3$)$_4$ (578 mg, 0.50 mmol) was added before the reaction mixture was stirred under reflux for 4.5 h. The resulting mixture was concentrated and water was added. The pH was adjusted to approximately 1.0–1.5 and then the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine, dried over MgSO$_4$, filtered, and concentrated in vacuo to give 10 (1.45 g) which was used for the next step without further purification.

Compound **13** was prepared using a procedure similar to that used for **9d** with **12**: solid. ^{1}H NMR (CDCl$_3$) δ: 1.52 (9H, s), 1.60 (3H, d, $J = 6.9$ Hz), 5.01 (1H, q, $J = 6.9$ Hz), 6.93 (1H, s), 7.22–7.33 (10H, m). IR (KBr) cm^{-1}: 3195, 2984, 1756, 1719, 1563, 1498, 1453, 1371, 1290, 1245, 1152.

5.1.17. (*S*,*Z*)-2-(1-(Benzhydryloxycarbonyl)ethooxyimino)-2-(2-(*tert*-butoxycarbonylamino)-5-fluorothiazol-4-yl)acetic acid (20). To a stirred solution of **14** (17.3 g, 120 mmol) in CH$_2$Cl$_2$ (87 mL) was added isopropylmagnesium bromide (120 mL, 2.0 M in THF) dropwise over 1 h at 0 °C. The reaction mixture was stirred at same temperature for 30 min, and then a solution of **15** (10.0 g, 76.4 mmol) in CH$_2$Cl$_2$ (17 mL) was added dropwise over 20 min at 0 °C. After 30 min, 1 N aqueous HCl (300 mL) was added and then the mixture was extracted with AcOEt (×2). The organic layer was washed with water and saturated brine, dried over MgSO$_4$, filtered, and concentrated in vacuo to give **16** (24.1 g) which was used for the next step without further purification.

To a stirred solution of **16** (24.1 g) in acetic acid (67 mL), a solution of sodium nitrite (9.1 g, 132 mmol) in water

(200 mL) was added dropwise over 30 min, and the reaction mixture was stirred for a further 1 h. The reaction was quenched with water (200 mL) and the aqueous layer was extracted with AcOEt. The organic layer was washed successively with saturated aqueous $NaHCO_3$, water, and saturated brine, then dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel to give **17** (14.4 g) as a mixture of the respective *E* and *Z* isomers.

To a solution of **17** (6.04 g, 20 mmol) in THF (60 mL) were added (*R*)-benzhydryl 2-hydroxypropanoate (5.64 g, 22 mmol) and triphenylphosphine (5.77 g, 22 mmol). To this mixture was added diisopropyl azodicarboxylate (11.8 mL, 40% in toluene solution, 22 mmol) dropwise and then the reaction mixture was stirred at 0 °C for 2 h. The solvent was removed by evaporation before Et_2O was added. The precipitate produced was removed by filtration, and then the filtrate was concentrated. The residue was purified by silica gel column chromatography to obtain **18** (3.21 g) as a mixture of the respective *E* and *Z* isomers, which were separated in the next step.

To a solution of **18** (3.21 g, 6.95 mmol) in DMA was added thiourea (3.17 g, 41.7 mmol) and the mixture was stirred at 30 °C for 18 h. The resulting mixture was poured into AcOEt and aqueous $NaHCO_3$ and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residual oil was purified by column chromatography to **19** (1.55 g, 48%) as a foam. ^1H NMR ($CDCl_3$) δ: 1.53 (3H, d, $J = 6.9$ Hz), 4.82 (2H, m), 4.93 (1H, q, $J = 6.9$ Hz), 5.26 (1H, m), 5.41 (1H, m), 5.94 (1H, m), 6.88 (1H, s), 7.19–7.36 (10H, m).

To a solution of **19** (515 mg, 1.10 mmol) in CH_2Cl_2 (5 mL) were added triethylamine (0.38 mL, 2.75 mmol) and triphosgene (131 mg, 0.44 mmol) at 0 °C. The reaction mixture was stirred for 30 min at the same temperature. The whole mixture was allowed to warm to room temperature after *tert*-butanol (0.53 mL, 5.51 mmol) was added. After 1.5 h, AcOEt and water were added and the organic layer was washed with 0.5 N aqueous HCl, water, and saturated brine. The extract was dried over $MgSO_4$, filtered, and concentrated in vacuo. To the residual oil in THF (7 mL) were added morpholine (0.45 mL, 5.17 mmol) and $Pd(PPh_3)_4$ (120 mg, 0.10 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. AcOEt and 0.5 N aqueous HCl were added and the organic layer was washed with water and saturated brine. The extract was dried over $MgSO_4$, filtered, and concentrated in vacuo to give **20** as an oil which was used for the next step without further purification. ^1H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.59 (3H, d, $J = 7.2$ Hz), 5.09 (1H, q, $J = 7.2$ Hz), 6.91 (1H, s), 7.25–7.36 (10H, m).

5.1.18. (*S*,*Z*)-2-(1-(Benzhydryloxycarbonyl)ethoxyimino)-2-(5-(*tert*-butoxycarbonylamino)-1,2,4-thiadiazol-3-yl)acetic acid (23). To a solution of **21** (6.50 g, 25.1 mmol) in 1,4-dioxane (100 mL) was added selenium dioxide (5.5 g, 50 mmol), and the mixture was stirred at 100 °C. After 2 h, the insoluble material was removed by filtration through Celite and the filtrate was concentrated in vacuo to give **22**. To an ice-cooled solution of **22** in MeOH (50 mL) was added a solution of **7 d**, which was prepared according to the procedure for **8d**, in CH_2Cl_2 (50 mL). The whole mixture was stirred at room temperature for 12 h and concentrated by evaporation. AcOEt (150 mL) was added and then the mixture was washed with 1 N hydrochloric acid and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **23** (14.7 g) which was used for the next step without further purification. A small amount of the sample was purified by column chromatography on silica gel for compound identification. ^1H NMR ($CDCl_3$) δ: 1.55–1.58 (11H, m), 5.13 (1H, q, $J = 7.2$ Hz), 6.88 (1H, s), 7.21–7.35 (10H, m), 9.10 (1H, br s). IR (KBr) cm^{-1}: 3183, 1717, 1548, 1455, 1372, 1244, 1150, 1115.

5.2. Synthesis of C-3′ side chain 1*H*-pyrrolo[3,2-*b*]pyridine (25)

To a solution of **24** (16.3 g, 100 mmol) in MeOH (320 mL) was added 10% Pd–C (1.63 g) and the mixture was stirred under hydrogen atmosphere at room temperature for 24 h. The resulting mixture was filtered and concentrated to give **25** (11.8 g, 89%).

5.2.1. *tert*-Butyl 3-(1H-pyrrolo[3,2-*b*]pyridin-1-yl)propyl(methyl)carbamate (26). To a solution of 3-(*tert*-butoxycarbonyl(methyl)amino)propyl methanesulfonate (42.8 g, 160 mmol), which was derived from methyl acrylate for four steps, in DMF (100 mL) was added **25** (11.8 g, 100 mmol). To this mixture was added NaH (6.0 g, 60%w/w, 150 mmol) portionwise below 55 °C. The reaction mixture was stirred for a further 30 min before quenching with ice water. The mixture was extracted with AcOEt (×2) and the combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel to give **26** (14.8 g, 54%) as an oil. ^1H NMR ($CDCl_3$) δ: 1.43 (9H, br s), 2.06 (2H, seq, $J = 6.9$ Hz), 2.82 (3H, br s), 3.26 (2H, br s), 4.14 (2H, t, $J = 6.9$ Hz), 6.70 (1H, d, $J = 3.0$ Hz), 7.11 (1H, dd, $J = 4.5$, 8.1 Hz), 7.37 (1H, br s), 7.62 (1H, dd, $J = 1.5$, 8.1 Hz), 8.46 (1H, dd, $J = 1.5$, 4.5 Hz). IR (KBr) cm^{-1}: 2973, 2932, 2836, 1683, 1605, 1556, 1482, 1454, 1420, 1393, 1365, 1291.

5.3. Synthesis of 7β-[2-(2-aminothiazol-4-yl)-2(*Z*)-(alkoxyimino)acetamide]-3-pyridiniummethyl-3-cephem-4-carboxylate

5.3.1. Compound 29b (Method A). To a solution of **9b** (1.36 g, 2.93 mmol) and (6*R*,7*R*)-4-methoxybenzyl 7β-amino-3-(chloromethyl)cephalosporincarboxylate hydrochloride **27** (1.18 g, 2.93 mmol) in DMF (13 mL) were added EDC hydrochloride (674 mg, 3.52 mmol) and pyridine (0.26 mL) at room temperature. The reaction mixture was stirred for 4 h and then quenched with 0.1 N HCl. The whole mixture was extracted with AcOEt (×2), and the extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residual oil was purified by silica gel column chromatography to give

29b (1.20 g, 52%) as a foam. ^{1}H NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.53 (9H, s), 3.47 and 3.63 (2H, ABq, J = 18.0 Hz), 3.82 (3H, s), 4.45 (2H, s), 4.68 and 4.75 (2H, ABq, J = 16.8Hz), 5.05 (1H, d, J = 4.8 Hz), 5.20 and 5.27 (2H, ABq, J = 12.0 Hz), 5.98 (1H, dd, J = 4.8 and 9.3 Hz), 6.91 (2H, d, J = 8.7 Hz), 7.35 (2H, d, J = 8.7 Hz), 8.11 (1H, br s), 8.49 (1H, d, J = 9.3 Hz). IR (KBr) cm^{-1}: 3382, 3277, 2979, 2935, 2837, 1791, 1722, 1613, 1551, 1515, 1455, 1369, 1302, 1246, 1157, 1085, 1062, 1036, 1021.

5.3.2. Compound 29d (Method B). To a solution of **9d** (43.7 g, 78.1 mmol) and ACLE-HCl in CH_2Cl_2 (520 mL) were added phenyl dichlorophosphate (19.8 g, 93.7 mmol) and *N*-methylmorpholine (23.7 g, 234.2 mmol) at −30 °C. The reaction mixture was stirred at −20 °C for 4 h and quenched with a 10% aqueous solution of citric acid (300 mL). The whole mixture was concentrated to half volume and extracted with AcOEt (×2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated to give **29d** (70.7 g) which was used for the next step without further purification. The sample was purified by silica gel column chromatography: foam. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 1.64 (3H, d, J = 7.2 Hz), 3.39 and 3.58 (2H, ABq, J = 18.3 Hz), 3.81 (3H, s), 4.42 and 4.59 (2H, ABq, J = 12 Hz), 4.97 (1H, d, J = 5.1 Hz), 5.08 (1H, q, J = 7.2 Hz), 5.20 and 5.27 (2H, ABq, J = 11.7 Hz), 6.01 (1H, dd, J =5.1, 9.3 Hz), 6.88–6.91 (3H, m), 7.06–7.35 (12H, m), 7.85 (1H, d, J = 9.3 Hz), 8.15 (1H, br s). IR (KBr) cm^{-1}: 3281, 2980, 2935, 2836, 1790, 1719, 1612, 1552, 1515, 1454, 1369, 1247.

The following compounds were prepared by a procedure similar to that used for **29b** (Method A) or **29d** (Method B).

5.3.3. Compound 28d. Method B: Foam. 74% yield. ^{1}H NMR ($CDCl_3$) δ: 1.54 (9H, s), 1.62 (3H, d, J = 7.2 Hz), 3.39 and 3.59 (2H, ABq, J = 18.0 Hz), 3.81 (3H, s), 4.42 and 4.61 (2H, ABq, J = 11.7 Hz), 5.00 (1H, d, J = 6.3 Hz), 5.13 (1H, q, J = 7.2 Hz), 5.19 and 5.27 (2H, ABq, J = 11.7 Hz), 5.95 (1H, dd, J = 6.3, 8.7 Hz), 6.90 (1H, d, J = 8.7 Hz), 6.91 (1H, s), 7.23–7.30 (11H, m), 7.35 (2H, d, J = 8.7 Hz), 8.10 (1H, d, J = 8.7 Hz), 8.16 (1H, br s). IR (KBr) cm^{-1}: 3279, 2984, 2935, 1790, 1722, 1690, 1615, 1549, 1518, 1456, 1370, 1244, 1155, 1032.

5.3.4. Compound 29a. Method A: Foam. 76% yield. ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.52 (9H, s), 1.60 (6H, s), 3.48 and 3.65 (2H, ABq, J = 18.0 Hz), 3.82 (3H, s), 4.45 and 4.55 (2H, ABq, J = 11.7 Hz), 5.04 (1H, d, J = 5.1 Hz), 5.20 and 5.27 (2H, ABq, J = 12.0 Hz), 6.03 (1H, dd, J = 5.1 and 9.3 Hz), 6.91 (2H, d, J = 8.7 Hz), 7.35 (2H, d, J = 8.7 Hz), 8.03 (1H, d, J = 9.3 Hz), 8.13 (1H, br s). IR (KBr) cm^{-1}: 3396, 3284, 2979, 2937, 2836, 1791, 1722, 1614, 1550, 1515, 1455, 1384, 1369, 1301, 1247, 1155, 1035.

5.3.5. Compound 29c. Method B: Foam. 97% yield. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 1.65 (3H, d, J = 7.2 Hz), 3.23 and 3.47 (2H, ABq, J = 18.3 Hz), 3.82 (3H, s), 4.39 and 4.55 (2H, ABq, J = 12 Hz), 4.99 (1H, d, J = 5.1 Hz), 5.10 (1H, q, J = 7.2 Hz), 5.21 and 5.27 (2H, ABq, J = 12 Hz), 5.99 (1H, dd, J = 5.1, 9.9 Hz), 6.91 (3H, m), 7.16–7.37 (12H, m), 7.76 (1H,d, J = 9.9 Hz), 8.20 (1H, br s). IR (KBr) cm^{-1}: 3373, 3286, 2979, 2937, 1791, 1720, 1612, 1550, 1515, 1248, 1155, 1035.

5.3.6. Compound 29e. Method A: Foam. 53% yield. ^{1}H NMR ($CDCl_3$) δ: 1.02 (3H, t, J = 7.2Hz), 1.53 (9H, s), 1.96–2.08 (2H, m), 3.40 and 3.59 (2H, ABq, J = 18.0 Hz), 3.81 (3H, s), 4.43 and 4.58 (2H, ABq, J = 11.7 Hz), 4.93 (1H, t, J = 6.3 Hz), 4.99 (1H, d, J = 5.1 Hz), 5.20 and 5.28 (2H, ABq, J = 11.7 Hz), 6.01 (1H, dd, J = 5.1 and 9.0 Hz), 6.90 (2H, d, J = 9.0 Hz), 6.95 (1H, s), 7.25–7.31 (10H, m), 7.35 (2H, d, J = 9.0 Hz), 7.91 (1H, d, J = 9.0 Hz), 7.93 (1H, br s). IR (KBr) cm^{-1}: 3283, 3063, 3031, 2976, 2936, 2836, 1791, 1721, 1613, 1550, 1515, 1455, 1384, 1369, 1301, 1246, 1155, 1058, 1033, 1004.

5.3.7. Compound 29f. Method A: Foam. 32% yield. ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, d, J = 7.2 Hz), 1.04 (3H, d, J = 6.9 Hz), 1.53 (9H, s), 2.35 (1H, m), 3.43 and 3.59 (2H, ABq, J = 18.3 Hz), 3.81 (3H, s), 4.45 and 4.57 (2H, ABq, J = 11.7 Hz), 4.84 (1H, d, J = 4.5 Hz), 4.99 (1H, d, J = 4.8 Hz), 5.21 and 5.28 (2H, ABq, J = 12.0 Hz), 5.99 (1H, dd, J = 4.8 and 9.0 Hz), 6.91 (2H, d, J = 8.7 Hz), 6.98 (1H, s), 7.25–7.32 (10H, m), 7.35 (2H, d, J = 8.7Hz), 7.92 (1H, s), 7.99 (1H, d, J = 9.0 Hz). IR (KBr) cm^{-1}: 3392, 3283, 3062, 3032, 2969, 2934, 2835, 1791, 1721, 1613, 1585, 1551, 1514, 1455, 1387, 1368, 1302, 1246, 1155, 1096, 1061, 1030.

5.3.8. Compound 29g. Method B: Foam. 85% yield. ^{1}H NMR ($CDCl_3$) δ: 0.32 (3H, s), 0.36 (3H, s), 0.85 (9H, s), 1.53 (9H, s), 3.40 and 3.59 (2H, ABq, J = 18.0 Hz), 3.81 (3H, s), 4.15 (2H, m), 4.44 and 4.59 (2H, ABq, J = 11.7 Hz), 4.98 (1H, d, J = 5.10 Hz), 5.10 (1H, m), 5.20 and 5.25 (2H, ABq, J = 12.0 Hz), 5.95 (1H, dd, J = 5.1, 9.0 Hz), 6.89 (2H, d, J = 8.7 Hz), 6.94 (1H, s), 7.21–7.36 (12H, m), 7.60 (1H, d, J = 9.0 Hz), 7.97 (1H, br s). IR (KBr) cm^{-1}: 3277, 2933, 1789, 1717, 1614, 1550, 1516, 1455, 1388, 1367, 1301, 1245, 1153, 1097, 1063, 1031.

5.3.9. Compound 29h. Method B: Foam. 52% yield. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 1.64 (4H, br s), 3.25 and 3.51 (2H, ABq, J = 18.3 Hz), 3.82 (3H, s), 4.39 and 4.58 (2H, ABq, J = 12.0 Hz), 4.96 (1H, d, J = 5.1 Hz), 5.21 and 5.28 (2H, ABq, J = 11.7 Hz), 5.95 (1H, dd, J = 5.1, 9.6 Hz), 6.89 (1H, s), 6.91 (2H, d, J = 8.7 Hz), 7.21–7.32 (10H, m), 7.35 (2H, d, J = 8.7 Hz), 7.75 (1H, d, J = 9.6 Hz), 8.02 (1H, br s). IR (KBr) cm^{-1}: 3274, 2980, 1789, 1716, 1614, 1548, 1516, 1454, 1367, 1243, 1152, 1030.

5.3.10. Cmpound 29i. Method A: Foam. 34% yield. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 2.05–2.18 (2H, m), 2.47–2.78 (4H, m), 3.26 and 3.51 (2H, ABq, J = 18.3 Hz), 3.82 (3H, s), 4.40 and 4.56 (2H, ABq, J = 12.0 Hz), 4.96 (1H, d, J = 4.8 Hz), 5.24 (1H, d,

J = 5.1 Hz), 5.21 and 5.27 (2H, ABq, J = 12.0 Hz), 5.97 (1H, dd, J = 5.1 and 9.6 Hz), 6.90 (2H, d, J = 8.7 Hz), 6.92 (1H, s), 7.25–7.31 (10H, m), 7.35 (2H, d, J = 8.7Hz), 7.44 (1H, d, J = 9.6 Hz), 8.00 (1H, s). IR (KBr) cm^{-1}: 3378, 3285, 3063, 3031, 2978, 2836, 1790, 1722, 1613, 1585, 1549, 1515, 1454, 1385, 1368, 1300, 1247, 1203, 1156, 1112, 1098, 1063, 1034.

5.3.11. Compound 29j. Method A: Foam. 56% yield. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 3.23 and 3.43 (2H, ABq, J = 18 Hz), 3.80 (3H, s), 4.36 and 4.55 (2H, ABq, J = 12 Hz), 4.75 (1H, d, J = 5.1 Hz), 5.16 and 5.25 (2H, ABq, J = 11.4 Hz), 5.61 (1H, d, J = 1.8), 5.81 (1H, d, J = 1.8), 5.88 (1H, dd, J = 5.1, 9.0 Hz), 6.87–6.92 (3H, m), 7.16–7.39 (12H, m), 8.56 (1H, br s). IR (CHCl3) cm^{-1}: 3403, 1793, 1725, 1613, 1550, 1517, 1248, 1215, 1155.

5.3.12. Compound 30d. Method B: Foam. 41% yield. ^{1}H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.62 (3H, d, J = 7.2 Hz), 3.40 and 3.59 (2H, ABq, J = 18 Hz), 3.81 (3H, s), 4.42 and 4.60 (2H, ABq, J = 12 Hz), 4.98 (1H, d, J = 4.8 Hz), 5.08 (1H, q, J = 7.2 Hz), 5.19 and 5.27 (2H, ABq, J = 12 Hz), 5.99 (1H, dd, J = 4.8, 9.3 Hz), 6.90 (3H, m), 7.24–7.34 (12H, m), 7.78 (1H, br s), 7.93 (1H, d, J = 9.3 Hz). IR ($CHCl_3$) cm^{-1}: 3277, 1788, 1717, 1613, 1543, 1516, 1455, 1368, 1243, 1152.

5.3.13. Compound 31d. Method B: Foam. 88% yield. ^{1}H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.65 (3H, d, J = 6.9 Hz), 3.39 and 3.58 (2H, ABq, J = 18.3 Hz), 3.81 (3H, s), 4.43 and 4.58 (2H, ABq, J = 11.7 Hz), 4.97 (1H, d, J = 5.1 Hz), 5.07 (1H, q, J = 6.9 Hz), 5.19 and 5.26 (2H, ABq, J = 12.0 Hz), 6.01 (1H, dd, J = 5.1, 9.3 Hz), 6.89 (1H, d, J = 8.7 Hz), 6.92 (1H, s), 7.25–7.32 (10H, m), 7.34 (2H, d, J = 8.7 Hz), 7.79 (1H, d, J = 9.3 Hz), 8.08 (1H, br s). IR (KBr) cm^{-1}: 3285, 2981, 1787, 1717, 1614, 1547, 1516, 1454, 1368, 1242, 1151.

5.3.14. Compound 32d. Method B: Foam. 62% yield. ^{1}H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.63 (3H, d, J = 7.2 Hz), 2.30 (3H, s), 3.39 and 3.59 (2H, ABq, J = 18.3 Hz), 3.81 (3H, s), 4.43 and 4.58 (2H, ABq, J = 11.7 Hz), 4.97–5.02 (2H, m), 5.19 and 5.26 (2H, ABq, J = 11.7 Hz), 6.02 (1H, dd, J = 5.1, 9.3 Hz), 6.89–6.92 (3H, m), 7.25–7.36 (12H, m), 7.64 (1H, d, J = 9.3 Hz), 8.20 (1H, br s). IR (KBr) cm^{-1}: 3290, 2981, 1787, 1720, 1613, 1551, 1516, 1453, 1367, 1243, 1152.

5.3.15. Compound 33d. Method B: Foam. 61% yield. ^{1}H NMR ($CDCl_3$) δ: 1.58 (9H, s), 1.65 (3H, d, J = 7.2 Hz), 3.20 and 3.59 (2H, ABq, J = 18.3 Hz), 3.83 (3H, s), 4.09 and 4.75 (2H, ABq, J = 12.0 Hz), 4.98 (1H, d, J = 4.5 Hz), 5.12–5.28 (3H, m), 6.19 (1H, dd, J = 4.5, 9.0 Hz), 6.79 (1H, s), 6.89–6.95 (2H, m), 7.19–7.37 (13H, m), 8.16 (1H, br s). IR (KBr) cm^{-1}: 3222, 2983, 1762, 1716, 1613, 1542, 1516, 1454, 1369, 1243, 1150, 1030.

5.3.16. Compound 35d. To a solution of **29d** (68.4 g, 75.1 mmol) in CH_2Cl_2 (700 mL), *m*-chloroperbenzoic acid (17.8 g, 65%/w/w, 67 mmol) in CH_2Cl_2 (63 mL) was added portionwise at below −40 °C. After 30 min, the reaction mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (100 mL) and concentrated by evaporation to half volume. The resulting mixture was extracted with AcOEt (×2) and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give **35d** (17.6 g) as a mixture of α, β-oxide, which was used for the next step without separation.

5.3.17. Compound 42d. To a solution of **26** (305 mg, 1.11 mmol) in DMF (1.8 mL) were added **35d** (1.02 g, 1.10 mmol) and NaBr (271 mg, 2.63 mmol). The reaction mixture was stirred for 3 h, and then KI (1.28 g, 7.71 mmol) and DMF (2 mL) were added. The mixture was cooled to −40 °C and acetyl chloride (432 mg, 5.50 mmol) was added. The mixture was allowed to warm to 0 °C and stirred for a further 1 h. The resulting mixture was poured into a cooled aqueous solution of NaCl (100 mL) containing $Na_2S_2O_55H_2O$ (1.0 g). The mixture was stirred at 0 °C for 20 min and the insoluble material was collected by filtration and dried in vacuo to give a pyrrolopyridinium salt (1.27 g) which was used for the next step without further purification.

To a solution of the pyrrolopyridinium salt (1.27 g), which was prepared in the previous step, in CH_2Cl_2 (15 mL) and CH_3NO_2 (15 mL) were added anisole (1.7 mL) and $AlCl_3$ (7 mL, 2.0 M in CH_3NO_2, 14 mmol). The reaction mixture was allowed to warm to 0 °C and stirred for 1 h before being poured into an ice-cooled mixture of 1 N HCl (20 mL), CH_3CN (20 mL), and Et_2O (50 mL) with stirring. The aqueous layer was concentrated by evaporation, chromatographed on HP-20ss column with aqueous CH_3CN. The fractions containing the desired compounds were collected and lyophilized to give **42d** (450 mg, 52%) as an amorphous powder. ^{1}H NMR (D_2O) δ: 1.43 (3H, d, J = 7.2 Hz), 2.31 (2H, q like), 2.68 (3H, s), 3.05 (2H, t, J = 8 Hz), 3.18 and 3.37 (2H, ABq, J = 18 Hz), 4.53 (2H, t like), 4.65 (1H, q, J = 7.2 Hz), 5.17 (1H, d, J = 4.8 Hz), 5.54 and 5.70 (2H, ABq, J = 15 Hz), 5.86 (1H, d, J = 4.5 Hz), 7.03 (1H, d, J = 3.6 Hz), 7.69 (1H, dd, J = 6.0, 8.4 Hz), 8.13 (1H, d, J = 3.6 Hz), 8.60 (1H, d, J = 8.4 Hz), 8.64 (1H, d, J = 6.0 Hz). IR (KBr) cm^{-1}: 3398, 1775, 1603, 1541, 1392, 1363, 1320, 1286, 1033, 762. Anal. Calcd for $C_{27}H_{29}N_8O_7S_2Cl\cdot6.2H_2O$: C, 41.11; H, 5.29; N, 14.20; S, 8.13; Cl, 4.49. Found: C, 40.88; H, 4.88; N, 14.23; S, 8.05; Cl, 4.57.

The following compounds were prepared by a procedure similar to that used for **42d**.

5.3.18. Compound 40a. Compound **40a** was obtained using imidazopyridine **47** instead of pyrropyridine **26** as an amorphous powder. 41% yield. ^{1}H NMR (DMSO-d_6–D_2O) δ: 1.36 (3H, s), 1.40 (3H, s), 2.22 (2H, br s), 2.47 (3H, s), 2.91 (2H, br s), 3.10 and 3.52 (2H, ABq, J = 16.8 Hz), 4.54 (2H, br s), 5.07 (1H, d, J = 4.8 Hz), 5.56 and 5.68 (2H, ABq, J = 12.9 Hz), 5.78 (1H, d, J = 4.8 Hz), 6.69 (1H, s), 7.69 (1H, t like), 8.80 (1H, d, J = 7.8), 9.02 (1H, s), 9.18 (1H, d, J = 6.3 Hz). IR(KBr) cm^{-1}: 3409, 2462, 1774, 1606, 1535, 1488, 1465, 1390, 1359, 1234, 1193, 1159. Anal. Calcd for $C_{27}H_{30}N_9O_7S_2\cdot 5.0H_2O$: C, 43.37; H, 5.53; N, 16.86; S, 8.58. Found: C, 43.21; H, 5.49; N, 16.73; S, 8.82.

5.3.19. Compound 41a. Amorphous powder. 41% yield. ^1H NMR(DMSO-d_6–D_2O) δ: 1.36 (3H, s), 1.40 (3H, s), 2.22 (2H, br s), 2.47 (3H, s), 2.91 (2H, br s), 3.10 and 3.52 (2H, ABq, *J* = 16.8 Hz), 4.54 (2H, br s), 5.07 (1H, d, *J* = 4.8 Hz), 5.56 and 5.68 (2H, ABq, *J* = 12.9 Hz), 5.78 (1H, d, *J* = 4.8 Hz), 6.69 (1H, s), 7.69 (1H, t like), 8.80 (1H, d, *J* = 7.8), 9.02 (1H, s), 9.18 (1H, d, *J* = 6.3 Hz). IR(KBr) cm^{-1}:3409, 2462, 1774, 1606, 1535, 1488, 1465, 1390, 1359, 1234, 1193, 1159. Anal. Calcd for $C_{27}H_{30}N_9O_7S_2\cdot 5.0H_2O$: C, 43.37; H, 5.53; N, 16.86; S, 8.58. Found: C, 43.21; H, 5.49; N, 16.73; S, 8.82.

5.3.20. Compound 41d. Amorphous powder. 62% yield. ^1H NMR (D_2O) δ: 1.44 (2H, d, *J* = 6.9 Hz), 2.31 (2H, m), 2.69 (3H, s), 2.68 (3H, s), 3.06 (2H, m), 3.16 and 3.39 (2H, ABq, *J* = 18.0 Hz), 4.53 (2H, m), 4.67 (1H, q, *J* = 6.9 Hz), 5.18 (1H, d, *J* = 4.8 Hz), 5.55 and 5.69 (2H, ABq, *J* = 15.3 Hz), 5.81 (1H, d, *J* = 4.8 Hz), 6.92 (1H, s), 7.03 (1H, d, *J* = 3.3 Hz), 7.68 (1H, dd, *J* = 6.3, 8.1 Hz), 8.12 (1H, d, *J* = 3.3 Hz), 8.59 (1H, d, *J* = 8.1 Hz), 8.63 (1H, d, *J* = 6.3 Hz). IR (KBr) cm^{-1}:3101, 2980, 1774, 1671, 1611, 1532, 1497, 1458, 1383, 1285, 1120. Anal. Calcd for $C_{27}H_{30}N_8O_7S_2\cdot 6.0\ H_2O$: C, 43.19; H, 5.64; N, 14.92; S, 8.54. Found: C, 43.11; H, 5.38; N, 14.97; S, 8.51.

5.3.21. Compound 42a. Amorphous powder. 35% yield. ^1H NMR (D_2O) δ: 1.46 (6H, s), 2.31 (2H, m), 2.68 (3H, s), 3.05 (2H, m), 3.18 and 3.39 (2H, ABq, *J* = 17.7 Hz), 4.54 (2H, t, *J* = 7.2 Hz), 5.18 (1H, d, *J* = 4.8 Hz), 5.56 and 5.69 (2H, ABq, *J* = 15.0 Hz), 5.83 (1H, d, *J* = 4.8 Hz), 7.04 (1H, d, *J* = 3.3 Hz), 7.70 (1H, dd, *J* = 6.0 and 8.4 Hz), 8.13 (1H, d, *J* = 3.3 Hz), 8.60 (1H, d, *J* = 8.4 Hz), 8.65 (1H, d, *J* = 6.0 Hz). IR (KBr) cm^{-1}: 3398, 1775, 1603, 1541, 1392, 1363, 1320, 1286, 1033, 762. Anal. Calcd for $C_{28}H_{31}ClN_8O_7S_2\cdot 0.1\ HCl\cdot 6.4\ H_2O$: C, 41.51; H, 5.46; N, 13.83; Cl, 4.81; S, 7.92. Found: C, 41.50; H, 4.93; N, 13.71; Cl, 4.68; S, 7.85.

5.3.22. Compound 42b. Amorphous powder. 53% yield. ^1H NMR (D_2O) δ: 2.30 (2H, m), 2.68 (3H, s), 3.05 (2H, m), 3.15 and 3.38 (2H, ABq, *J* = 17.7 Hz), 4.52 (2H, t, *J* = 6.9 Hz), 4.54 (2H, s), 5.16 (1H, d, *J* = 4.8 Hz), 5.56 and 5.67 (2H, ABq, *J* = 15.0 Hz), 5.83 (1H, d, *J* = 4.8 Hz), 7.04 (1H, d, *J* = 3.6 Hz), 7.68 (1H, dd, *J* = 6.0 and 8.1 Hz), 8.12 (1H, d, *J* = 3.6 Hz), 8.59 (1H, d, *J* = 8.1 Hz), 8.65 (1H, d, *J* = 6.0 Hz). IR (KBr) cm^{-1}: 3394, 2817, 1773, 1604, 1539, 1498, 1466, 1391, 1361, 1317, 1163, 1121. Anal. Calcd for $C_{26}H_{27}N_8O_7S_2Cl\cdot 3.7H_2O$: C, 42.79; H, 4.75; N, 15.35; S, 8.79; Cl, 4.86. Found: C, 42.78; H, 4.66; N, 15.42; S, 9.02; Cl, 4.81.

5.3.23. Compound 42c. Amorphous powder. 56% yield. ^1H NMR (D_2O) δ: 1.40 (3H, d, *J* = 6.9 Hz), 2.31 (2H, q like), 2.68 (3H, s), 3.05 (2H, t like), 3.14 and 3.39 (2H, ABq, *J* = 17.7 Hz), 4.52 (2H, t like), 4.61 (1H, q, *J* = 6.9 Hz), 5.19 (1H, d, *J* = 4.8 Hz), 5.57 and 5.67 (2H, ABq, *J* = 15 Hz), 5.80 (1H, d, *J* = 4.5 Hz), 7.06 (1H, d, *J* = 3.6), 7.69 (1H, dd, *J* = 6.0, 8.1 Hz), 8.12 (1H, d, *J* = 3.6 Hz), 8.59 (1H, d, *J* = 8.1 Hz), 8.64 (1H, d, *J* = 6.0 Hz). IR (KBr) cm^{-1}: 3411, 1774, 1606, 1539, 1498, 1392, 1363, 1034, 759. Anal. Calcd for $C_{27}H_{29}N_8O_7S_2Cl\cdot 6.2H_2O$: C, 41.11; H, 5.29; N, 14.20; S, 8.13; Cl, 4.49. Found: C, 40.99; H, 5.07; N, 14.15; S, 8.21; Cl, 4.76.

5.3.24. Compound 42e. Amorphous powder. 55% yield. ^1H NMR (D_2O) δ: 0.93 (3H, t, *J* = 7.5 Hz), 1.83 (2H, quintet-like), 2.30 (2H, quintet-like), 2.69 (3H, s), 3.05 (2H, t, *J* = 8.1 Hz), 3.16 and 3.37 (2H, ABq, *J* = 17.7 Hz), 4.52 (1H, t, *J* = 6.0 Hz), 4.52 (2H, t, *J* = 6.3 Hz), 5.17 (1H, d, *J* = 4.8 Hz), 5.55 and 5.68 (2H, ABq, *J* = 15.0 Hz), 5.85 (1H, d, *J* = 4.8 Hz), 7.03 (1H, d, *J* = 3.6 Hz), 7.69 (1H, dd, *J* = 6.0 and 8.4 Hz), 8.12 (1H, d, *J* = 3.6 Hz), 8.58 (1H, d, *J* = 8.4 Hz), 8.64 (1H, d, *J* = 6.0 Hz). Anal. Calcd for $C_{28}H_{31}ClN_8O_7S_2\cdot 5.6\ H_2O$: C, 42.46; H, 5.37; N, 14.15; Cl, 4.48; S, 8.10. Found: C, 42.38; H, 5.02; N, 14.25; Cl, 4.41; S, 8.02.

5.3.25. Compound 42f. Amorphous powder. 60% yield. ^1H NMR (D_2O) δ: 0.94 (3H, d, *J* = 7.2 Hz), 0.98 (3H, d, *J* = 6.9 Hz), 2.13 (1H, sextet-like), 2.31 (2H, quintet-like), 2.68 (3H, s), 2.91 (2H, t, *J* = 7.8 Hz), 3.15 and 3.37 (2H, ABq, *J* = 17.7 Hz), 4.35 (1H, d, *J* = 5.4 Hz), 4.52 (2H, t, *J* = 6.9 Hz), 5.17 (1H, d, *J* = 4.8 Hz), 5.55 and 5.67 (2H, ABq, *J* = 15.3 Hz), 5.87 (1H, d, *J* = 4.8 Hz), 7.04 (1H, d, *J* = 3.3 Hz), 7.69 (1H, dd, *J* = 6.0 and 8.1 Hz), 8.12 (1H, d, *J* = 3.3 Hz), 8.59 (1H, d, *J* = 8.1 Hz), 8.64 (1H, d, *J* = 6.0 Hz). IR (KBr) cm^{-1}:3389, 2965, 1777, 1601, 1539, 1498, 1466, 1391, 1364, 1223, 1120, 1062, 1019. Anal. Calcd for $C_{29}H_{33}ClN_8O_7S_2\cdot 6.5\ H_2O$: C, 42.36; H, 5.64; N, 13.63; Cl, 4.31; S, 7.80. Found: C, 42.01; H, 4.82; N, 13.51; Cl, 4.26; S, 7.89.

5.3.26. Compound 42g. Amorphous powder. 31% yield. ^1H NMR (D_2O) δ: 2.31 (2H, quintet-like), 2.68 (3H, s), 3.05 (2H, t, *J* = 8.1 Hz), 3.17 and 3.38 (2H, ABq, *J* = 17.7 Hz), 3.94 (2H, m), 4.53 (2H, t, *J* = 7.2 Hz), 4.70 (1H, m), 5.18 (1H, d, *J* = 4.8 Hz), 5.55 and 5.68 (2H, ABq, *J* = 15.0 Hz), 5.88 (1H, d, *J* = 4.8 Hz), 7.04 (1H, d, *J* = 3.3 Hz), 7.69 (1H, dd, *J* = 6.3 and 8.4 Hz), 8.12 (1H, d, *J* = 3.3 Hz), 8.60 (1H, d, *J* = 8.4 Hz), 8.64 (1H, d, *J* = 6.3 Hz). IR (KBr) cm^{-1}:3398, 1774, 1603, 1538, 1498, 1466, 1392, 1362, 1320, 1064. Anal. Calcd for $C_{27}H_{29}ClN_8O_8S_2\cdot 9.0\ H_2O$: C, 37.92; H, 5.54; N, 13.10; Cl, 4.15; S, 7.50. Found: C, 37.77; H, 4.42; N, 13.09; Cl, 4.24; S, 7.49.

5.3.27. Compound 42h. Amorphous powder. 56% yield. ^1H NMR (D_2O) δ: 1.26–1.32 (4H, m), 2.31 (2H, q like), 2.68 (3H, s), 3.06 (2H, t, *J* = 8.1 Hz), 3.15 and 3.39 (2H, ABq, *J* = 17.7 Hz), 4.54 (2H, t like), 5.17 (1H, d, *J* = 4.5 Hz), 5.57 and 5.68 (2H, ABq, *J* = 15 Hz), 5.80 (1H, d, *J* = 4.5 Hz), 7.05 (1H, d, *J* = 3.3 Hz), 7.70 (1H, t, *J* = ca7 Hz), 8.13 (1H, d, *J* = 2.4 Hz), 8.60 (1H, d, *J* = 8.4 Hz), 8.65 (1H, d, *J* = 6 Hz). IR (KBr) cm^{-1}:

3398, 2820, 1773, 1608, 1540, 1395, 1225, 1033, 968, 761. Anal. Calcd for $C_{28}H_{29}N_8O_7S_2Cl\cdot 6.0H_2O$: C, 42.18; H, 5.18; N, 14.05; S, 8.04; Cl, 4.45. Found: C, 42.22; H, 4.85; N, 14.18; S, 8.19; Cl, 4.66.

5.3.28. Compound 42i. Amorphous powder. 45% yield. ^{1}H NMR (D_2O) δ: 1.90 (2H, m), 2.31 (4H, m), 2.44 (2H, m), 2.68 (3H, s), 3.05 (2H, t, $J = 8.1$ Hz), 3.17 and 3.39 (2H, ABq, $J = 18.0$ Hz), 4.54 (2H, t, $J = 6.9$ Hz), 5.20 (1H, d, $J = 4.8$ Hz), 5.56 and 5.69 (2H, ABq, $J = 15.0$ Hz), 5.83 (1H, d, $J = 4.8$ Hz), 7.04 (1H, d, $J = 3.3$ Hz), 7.69 (1H, dd, $J = 6.3$ and 8.4 Hz), 8.12 (1H, d, $J = 3.3$ Hz), 8.60 (1H, d, $J = 8.4$ Hz), 8.64 (1H, d, $J = 6.3$ Hz). IR (KBr) cm^{-1}:3398, 2948, 1774, 1610, 1538, 1498, 1458, 1392, 1287, 1236, 1158, 1120, 1064, 1032. Anal. Calcd for $C_{29}H_{31}ClN_8O_7S_2\cdot 6.5\ H_2O$: C, 42.46; H, 5.41; N, 13.66; Cl, 4.32; S, 7.82. Found: C, 42.34; H, 4.87; N, 13.71; Cl, 4.39; S, 7.79.

5.3.29. Compound 42j. Amorphous powder. 45% yield. ^{1}H NMR (D_2O) δ: 2.31 (2H, q like, $J = 7.5$ Hz), 2.68 (3H, s), 3.04 (2H, t like), 3.17 and 3.31 (2H, ABq, $J = 17.7$ Hz), 4.53 (2H, t like), 5.10 (1H, d, $J = 2.1$ Hz), 5.12 (1H, d, $J = 4.5$ Hz), 5.27 (1H, d, $J = 2.1$ Hz), 5.51 and 5.76 (2H, ABq, $J = 15$ Hz), 5.88 (1H, d, $J = 4.5$ Hz), 6.99 (1H, d, $J = 3.6$), 7.67 (1H, dd, $J = 6.4$, 8.1 Hz), 8.12 (1H, d, $J = 3.6$ Hz), 8.59 (1H, d, $J = 8.1$ Hz), 8.63 (1H, d, $J = 6.4$Hz). IR (KBr) cm^{-1}: 3398, 1774, 1606, 1539, 1498, 1468, 1392, 1203. Anal. Calcd for $C_{27}H_{27}N_8O_7S_2Cl\cdot 5.5H_2O$: C, 41.89; H, 4.95; N, 14.47; S, 8.28; Cl, 4.58; H, 5.29; N, 14.20; S, 8.13; Cl, 4.49. Found: C, 41.92; H, 4.72; N, 14.49; S, 8.38; Cl, 4.66.

5.3.30. Compound 43d. Amorphous powder. 45% yield. ^{1}H NMR (D_2O) δ: 1.42 (3H, d, $J = 6.9$ Hz), 2.31 (2H, m), 2.68 (3H, s), 3.05 (2H, m), 3.17 and 3.37 (2H, ABq, $J = 17.7$ Hz), 4.54 (2H, t like), 4.62 (1H, q, $J = 6.9$ Hz), 5.16 (1H, d, $J = 4.8$ Hz), 5.54 and 5.70 (2H, ABq, $J = 15.0$ Hz), 5.82 (1H, d, $J = .5$ Hz), 7.03 (1H, d, $J = 3.3$ Hz), 7.69 (1H, dd, $J = 6.3$, 8.4 Hz), 8.12 (1H, d, $J = 3.3$ Hz), 8.60 (1H, d, $J = 8.4$ Hz), 8.64 (1H, d, $J = 6.3$ Hz). IR (KBr) cm^{-1}: 3306, 1771, 1583, 1532, 1498, 1458, 1389, 1361, 1215. Anal. Calcd for $C_{27}H_{28}N_8O_7S_2F\cdot 3.2H_2O$: C, 45.21; H, 4.83; N, 15.62; S, 8.94; F, 2.65. Found: C, 45.2; H, 4.82; N, 12.68; S, 8.76; F, 2.51.

5.3.31. Compound 44d. Amorphous powder. 62% yield. ^{1}H NMR (D_2O) δ: 1.44 (3H, d, $J = 7.2$ Hz), 2.30 (2H, m), 2.69 (3H, s), 3.06 (2H, m), 3.17 and 3.37 (2H, ABq, $J = 17.4$ Hz), 4.50 (2H, t like), 4.67 (1H, q, $J = 7.2$ Hz), 5.17 (1H, d, $J = 4.5$ Hz), 5.54 and 5.69 (2H, ABq, $J = 15.0$ Hz), 5.86 (1H, d, $J = 4.5$ Hz), 7.02 (1H, d, $J = 3.3$ Hz), 7.68 (1H, m), 8.11 (1H, d, $J = 3.3$ Hz), 8.56 (1H, d, $J = 8.4$ Hz), 8.64 (1H, d, $J = 6.0$ Hz). IR (KBr) cm^{-1}: 3397, 1773, 1604, 1532, 1498, 1459, 1392, 1363, 1286. Anal. Calcd for $C_{27}H_{29}N_8O_7S_2Br\cdot 6.0H_2O$: C, 39.09; H, 4.98; N, 13.51; S, 7.73; Br, 9.63. Found: C, 39.21; H, 4.72; N, 13.74; S, 7.59; Br, 9.72.

5.3.32. Compound 45d. Amorphous powder. 72% yield. ^{1}H NMR (D_2O) δ: 1.42 (3H, d, $J = 6.9$ Hz), 2.26–2.36 (5H, m), 2.68 (3H, s), 3.05 (2H, m), 3.17 and 3.37 (2H, ABq, $J = 17.7$ Hz), 4.53 (2H, t like), 4.62 (1H, q, $J = 6.9$ Hz), 5.17 (1H, d, $J = 4.8$ Hz), 5.54 and 5.69 (2H, ABq, $J = 15.0$ Hz), 5.85 (1H, d, $J = 4.8$ Hz), 7.03 (1H, d, $J = 3.3$ Hz), 7.69 (1H, dd, $J = 6.0$, 8.4 Hz), 8.12 (1H, d, $J = 3.3$ Hz), 8.59 (1H, d, $J = 8.4$ Hz), 8.63 (1H, d, $J = 6.0$ Hz). IR (KBr) cm^{-1}: 3307, 1769, 1585, 1498, 1456, 1364, 1217. Anal. Calcd for $C_{28}H_{31}N_8O_7S_2\cdot 3.8H_2O$: C, 46.44; H, 5.37; N, 15.47; S, 8.86. Found: C, 46.48; H, 5.38; N, 15.50; S, 8.93.

5.3.33. Compound 46d. Amorphous powder. 31% yield. ^{1}H NMR (D_2O) δ: 1.45 (3H, d, $J = 7.2$ Hz), 2.31 (2H, m), 2.69 (3H, s), 3.06 (2H, m), 3.18 and 3.37 (2H, ABq, $J = 17.7$ Hz), 4.53 (2H, t like), 4.71 (1H, q, $J = 7.2$ Hz), 5.18 (1H, d, $J = 4.8$ Hz), 5.53 and 5.70 (2H, ABq, $J = 15.0$ Hz), 5.85 (1H, d, $J = 4.8$ Hz), 7.02 (1H, d, $J = 3.3$ Hz), 7.68 (1H, dd, $J = 6.3$, 8.7 Hz), 8.12 (1H, d, $J = 3.3$ Hz), 8.56 (1H, d, $J = 8.7$ Hz), 8.63 (1H, d, $J = 6.3$ Hz). IR (KBr) cm^{-1}: 1768, 1589, 1525, 1456, 1363, 1285. Anal. Calcd for $C_{26}H_{28}N_9O_7S_2\cdot 4.1H_2O$: C, 43.58; H, 5.09; N, 17.59; S, 8.95. Found: C, 43.46; H, 5.04; N, 17.76; S, 8.98.

5.4. Method for in vitro antibacterial activity evaluation

MICs were determined using a serial twofold dilution method in Sensivity Disk Agar-N (Nissui Pharmaceutical, Tokyo, Japan). Overnight cultures of antibacterial strains in Mueller–Hilton broth (Becton–Dickinson) were diluted to about 10^6 CFU/mL. Bacterial suspensions of 1 μL were spotted onto agar plates containing various concentrations of an antibiotic and incubated for 20 h at 37 °C before the MICs were scored.

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