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TAK-599, a Novel *N*-Phosphono Type Prodrug of Anti-MRSA Cephalosporin T-91825: Synthesis, Physicochemical and Pharmacological Properties

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Abstract—Crystalline **1** (TAK-599) is a novel *N*-phosphono prodrug of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) cephalosporin **2a** (T-91825) that has high affinity for penicillin-binding protein (PBP) 2′ (IC_{50}; 0.90 μg/mL) and shows potent in vitro anti-MRSA activity (MIC against MRSA N133; 1.56 μg/mL), comparable to that of vancomycin (1.56 μg/mL). Although **2a** had insufficient water solubility (2.3 mg/mL) for parenteral administration, **1** showed excellent water solubility (>100 mg/mL, pH 7) as well as good chemical stability in the solid state and solution. In pharmacokinetic studies, when **1** was administered intravenously to rats and monkeys, it was rapidly converted into **2a** in the blood. These results show that **1** (TAK-599) is a highly promising parenteral cephalosporin targeted for MRSA infection.

Introduction

Life threatening infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious problem for the management of inpatients all over the world.[1] Several studies have reported that MRSA accounts for about 35% in the United States[2] and 60% in Japan[3] of all clinically isolated *S. aureus* strains. These situations are closely related to the fact that MRSA has acquired resistance relevant to almost all clinically used antibiotics. Although many types of the multi-drug resistant mechanism of MRSA have been reported,[4] production of a genetically controlled alternate penicillin-binding protein (PBP) 2′, for which the currently used β-lactam antibiotics have very low affinity, is thought to be one of the main reasons for the survival of MRSA strains in hospitals. Very recently, a last-resort anti-MRSA antibiotic, vancomycin (VCM), resistant *S. aureus* (VRSA) was reported in the United States.[5] Development of a new effective drug against these resistant *S. aureus* strains is an urgent subject.

Our research program was started with the aim of discovering a clinically effective agent for MRSA infection. Considering the safety and bactericidal properties of cephalosporin derivatives compared with those of the other classes of antibiotics such as VCM, we have examined chemical modifications of cefozopran (CZOP), a so-called fourth generation cephalosporin. It is expected that cephalosporins with high affinity for PBP2′ will be active against most MRSA, including intermediate and highly resistant strains, because the resistance mechanism to β-lactam antibiotics involves the production of PBP2′ in common (vide supra). In our previous papers,[6–9] we reported CZOP derivatives, which possess anti-MRSA activities comparable to that of VCM, both in vitro and in vivo, based on their high affinities for PBP2′. However, in view of their unsatisfactory physicochemical and pharmacokinetic properties, none of the compounds was suitable for further development. One of the reported compounds, the *N*-phosphono type prodrug **II** of C-3 4-pyridinio thiovinyl derivative **I**, did possess sufficient water-solubility for intravenous administration,[9] but not sufficient chemical stability for drug storage in any formulation (Fig. 1).

Having continued chemical modification of the series of CZOP, we focused on the optimization of the spacer

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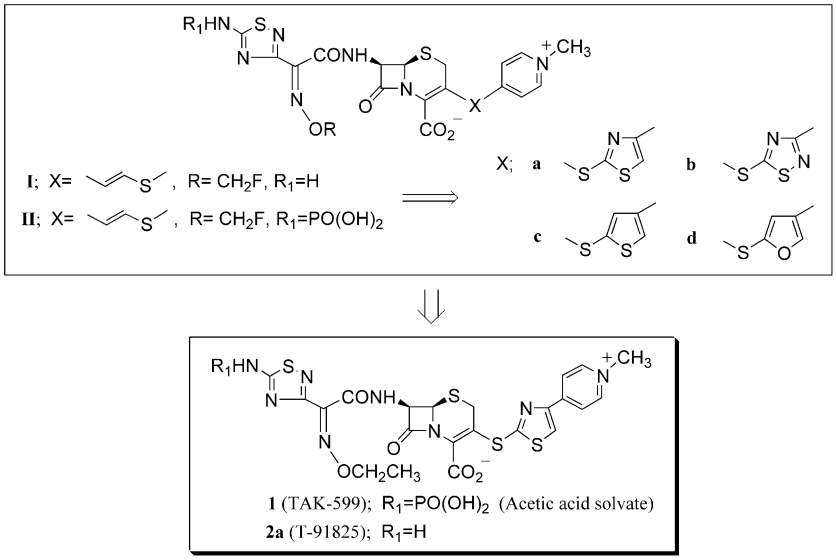


Figure 1. The course of our studies on the development of anti-MRSA cephalosporins.

group. The preliminary structure–activity relationship (SAR) study of the spacer group X was limited to aliphatic or heteroatom-containing aliphatic linker moieties and led us to examine thio five-membered heteroaromatic spacer groups such as **a**–**d** (Fig. 1), which were expected to adopt a similar conformation to that of the optimal derivative **I**. Although the selected thiazole derivative **2a** (T-91825) had a comparable anti-MRSA activity to that of VCM in vitro and in vivo, the intrinsic water-insolubility problem, ascribed to the zwitterionic structure, still remained. In order to improve its water solubility, we applied the reported *N*-phosphono prodrug strategy. Ultimately, our efforts were rewarded with the discovery of a crystalline form of the *N*-phosphono prodrug, 3-[4-(1-methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-7β-[2-(5-phosphonoamino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-cephem-4-carboxylate acetic acid solvate (**1**: TAK-599), which has not only practical water solubility, but also good chemical stability in the solid state and solution.

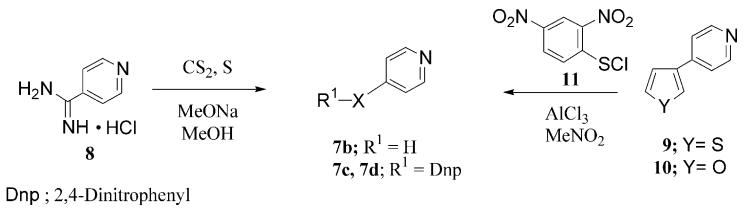
In this paper, we describe the synthesis and SAR of **2a** (T-91825) as well as the synthesis and characterization of **1** (TAK-599).

Chemistry

We selected 1,3-thiazole (**a**), 1,2,4-thiadiazole (**b**), thiophene (**c**) and furan (**d**) as representative five-membered heteroaromatic moieties in the spacer X. In order to examine the synergistic effect between the spacer group and the oxyimino substituent of the acyl moiety on antibacterial activity, we decided to prepare the representative eight derivatives **2a**–**6a** and **3b**–**3d** (Scheme 2). The synthesis of the requisite heteroaromatic thiols, except **7a**, is shown in Scheme 1. The thiazole derivative **7a** was prepared from 4-acetylpyridine by the reported method.[10] The thiadiazole derivative, 3-(4-pyridyl)-1,2,4-thiadiazol-5-thiol (**7b**) was obtained by reaction of 4-amidinopyridine (**8**)[11] with carbon disulfide and sulfur in the presence of sodium methoxide in MeOH. For the 3-(4-pyridyl)thiophene and 3-(4-pyridyl)furan derivatives, the corresponding 2,4-dinitrophenyl (Dnp) derivatives **7c** and **7d** were prepared by Friedel–Crafts reaction of 3-(4-pyridyl)thiophene (**9**) or 3-(4-pyridyl)furan (**10**) with 2,4-dinitrophenylsulfenyl chloride (**11**) in the presence of aluminum chloride.

Preparation of C-3 modified cephalosporin derivatives **2a**–**6a** and **3b**–**3d** is summarized in Scheme 2. The condensation reaction of thiol derivatives **7a**–**d** with the cephalosporin nucleus was carried out via the sodium salts **12a**–**d**, generated in situ by treatment of **7a**–**d** with sodium hydride (method A) or sodium methoxide (method B). The known triflate **13**[8,12] reacted with the sodium salts **12a**–**d** to give the coupling products **15a**–**d** in moderate yields. In addition, considering the industrial scale production, we selected the reported mesylate derivative **14**[13] as an alternative starting material. Condensation of **14** with the sodium salt **7a** gave the desired product **16a** in a comparable purity and yield to that obtained using the triflate **13** as a starting material.[14] After methylation of the resulting compounds **15a**–**d** and **16a**, removal of the phenylacetyl group was carried out using standard methods. Without purification of the crude products **19a**–**d** and **20a**, *p*-methoxybenzyl and benzhydryl groups were cleaved by TFA/anisole (method A) or by conc HCl/acetonitrile (method B). In method A, the 7β-amino derivatives **21a**–**d** were obtained as lyophilized forms after chromatographic purification. When method B was applied, the dihydrochloride salt of **21a** was isolated as a stable crystalline form from the reaction medium. The acylation of **21a**–**d** with the previously reported 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetyl chlorides[15,16] gave the desired C-3 modified cephalosporin derivatives (**2a**–**6a**, **3b**–**3d**).

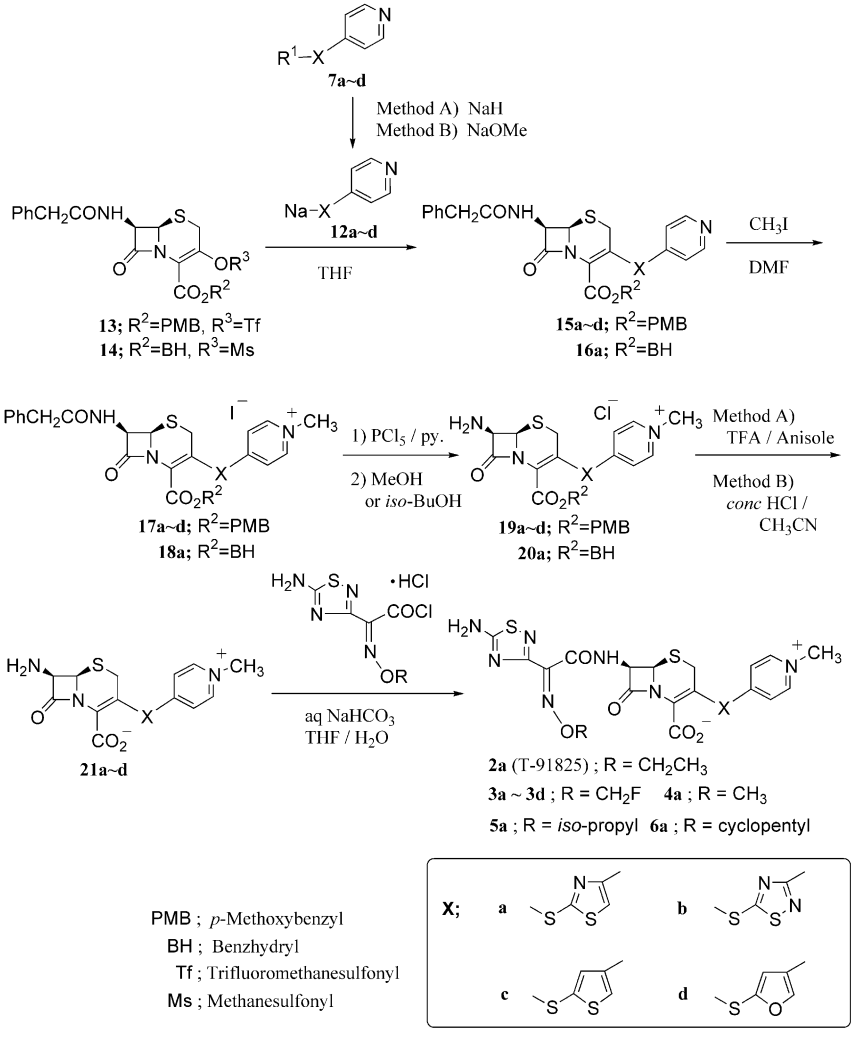
Since **2a** (T-91825) showed the most promising antibacterial activity among the synthesized derivatives, we investigated the preparation of the corresponding



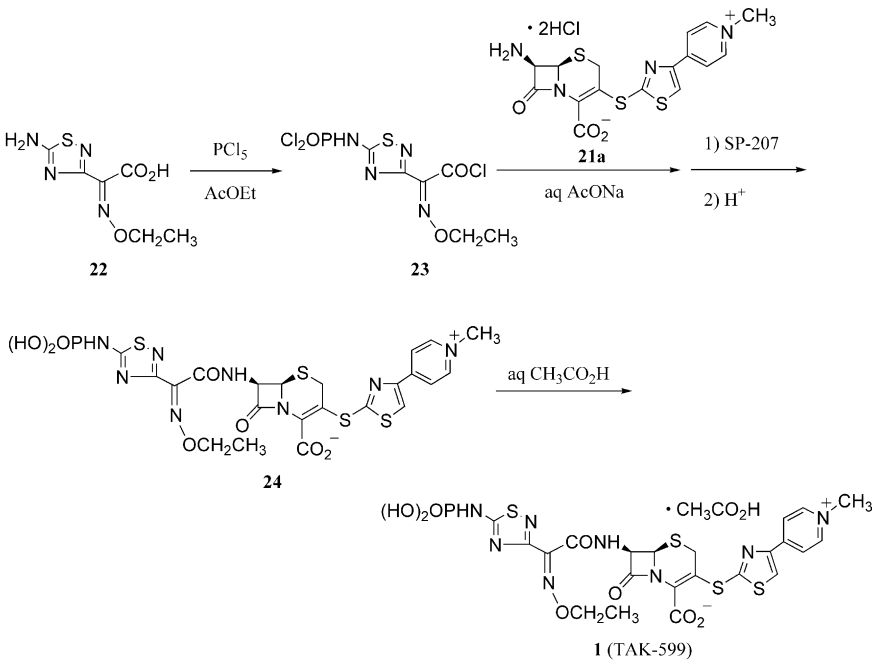
Scheme 1. Synthesis of C-3 heteroaromatic thiols.

N-phosphono prodrug **1** (TAK-599) shown in Scheme 3. When 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetic acid (**22**) was treated with over two equivalents of phosphorus pentachloride in ethyl acetate, dichlorophosphorylation of the amino group in the thiadiazole moiety occurred concomitant with acid chloride formation. The resulting acid chloride **23** was reacted with the dihydrochloride of 7β-amino cephalosporin **21a** described above in the presence of sodium acetate followed by chromatographic purification to give *N*-phosphono derivative **24**. Although a P–N bond is generally thought to be chemically unstable, compound **24** could be crystallized from an aqueous acetic acid solution as a stable acetic acid solvate **1** (TAK-599).

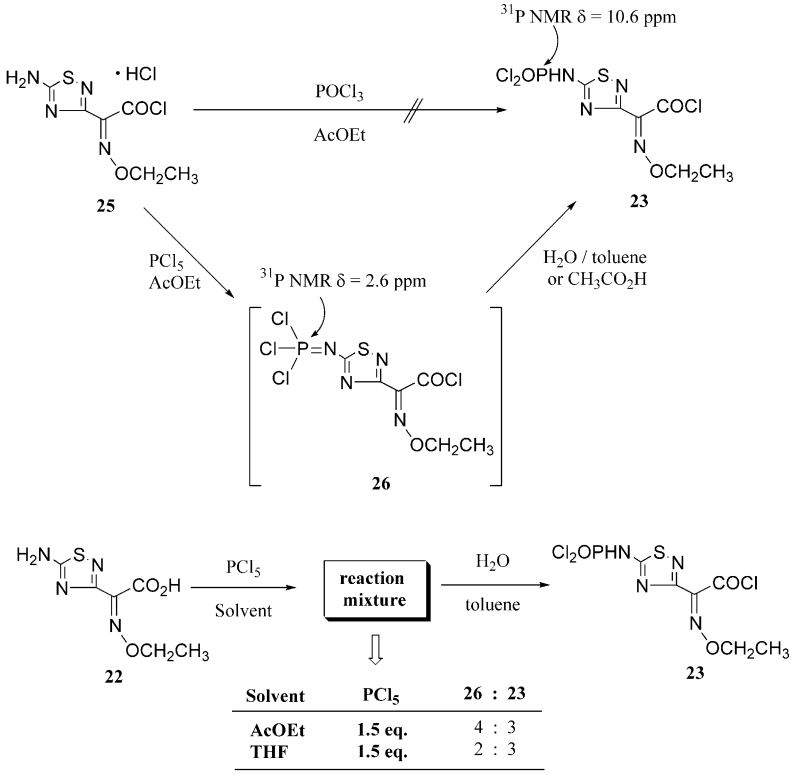
In order to elucidate the reaction mechanism of dichlorophosphorylation, we followed the reaction by ^{31}P NMR measurement (Scheme 4). Although there are some examples[16,17] of dichlorophosphorylation of an amino group with phosphorus pentachloride, its mechanistic considerations have not been discussed. A plausible mechanism for the formation of the desired product **23** was considered to be reaction of the amino group with phosphorus oxychloride, generated in situ from reaction between the carboxylic acid and phosphorus pentachloride. However, this mechanism was ruled out because no dichlorophosphorylation of the reported acid chloride intermediate **25**[15] was observed with phosphorus oxychloride under the identical reaction conditions.



Scheme 2. Synthesis of C-3 modified cephalosporins.



Scheme 3. Synthesis of *N*-phosphono prodrug (**1**, TAK-599).



Scheme 4. Mechanistic consideration of *N*-dichlorophosphorylation.

On the other hand, while there are only a few reports about the mechanism of dichlorophosphorylation of an amino group with phosphorus pentachloride, the phosphorimidic trichloride group has been proposed to be a precursor for a dichlorophosphoryl group by simple hydration.[18] In fact, when acid chloride **25** was reacted with phosphorus pentachloride in ethyl acetate, ^{31}P NMR measurement detected a characteristic chemical shift (δ 2.6 ppm) comparable to that of the reported phosphorimidic trichloride group (δ 1.9 ppm).[19] Furthermore, treatment of the reaction mixture with water generated an identical peak (δ 10.6 ppm) to that of the dichlorophosphoryl group of **23**. When a carboxylic acid, such as acetic acid, was added to the solution instead of water, the latter peak (δ 10.6 ppm) appeared simultaneously with the disappearance of the former peak (δ 2.6 ppm).[20]

Next, based on the observed chemical shifts, we examined the ratio of the phosphorimidic trichloride group (δ 2.6 ppm) and dichlorophosphoryl group (δ 10.6 ppm) during the reaction of the starting carboxylic acid **22** with phosphorus pentachloride. When 1.5 equivalents of phosphorus pentachloride was added to **22** in ethyl acetate, the reaction did not go to completion,[21] and a 4:3 ratio of products **26**:**23** was observed. When ethyl acetate was replaced with THF, which was reported to be another suitable solvent for dichlorophosphorylation,[9,16] the observed ratio decreased to 2:3. These results suggested that the mechanism proceeded via initial formation of the phosphorimidic trichloride group which was converted to the dichlorophosphoryl group by aqueous work up and/or the coexisting carboxyl group.

Results and Discussion

Table 1 shows the SAR of the prepared five-membered heteroaromatic thio derivatives **3a**–**3d**, which have a 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-fluoromethoxyiminoacetyl group at the C-7 position. Among these compounds, **3a**, which contains the 2-thiazolylthio group as the spacer group X, showed the most potent anti-MRSA activity. In addition to the in vitro activity, **3a** also exhibited excellent protective effect against MRSA infection in mice comparable to that of VCM.

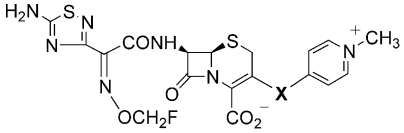
Having selected 2-thiazolylthio group as the optimal spacer, we investigated the SAR of the alkoxyimino groups R in the C-7 acyl moiety. As shown in Table 2, the in vitro anti-MRSA activities of all prepared derivatives **2a**–**6a** except the methoxyimino derivative **4a** were 16 times more potent than that of CZOP, and comparable to that of VCM. On the other hand, in vivo protective effects of these derivatives against MRSA infections did not correlate with their in vitro activities. Cyclopentyloxyimino derivative **6a** did not exhibit an in vivo protective effect at a dose of 12.5 mg/kg. Among the tested compounds, ethoxyimino derivative **2a** (T-91825) showed the most potent protective effect with an ED_{50} value of 1.74 mg/kg which was superior or comparable to that of VCM. In addition, **2a** showed much higher affinity than CZOP for PBP2′, which suggested that this series of chemical modifications restored high affinity for the target enzyme, PBP2′.

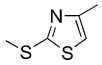
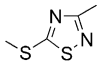
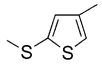
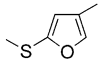
Because, unlike VCM and other clinically used anti-MRSA agents, **2a** (T-91825) also exhibits excellent activities against Gram-negative bacteria, **2a** (T-91825) has the potential to be an ideal therapy for immunocompromised and hospitalized patients suffering from opportunistic infection and/or superinfection due to microbial substitution.

Nevertheless, a critical drawback for **2a** was its insufficient water solubility (2.3 mg/mL) to allow preparation of a parenteral injectable solution. This problem was addressed by the reported *N*-phosphono prodrug strategy, which resulted in the preparation of crystalline *N*-phosphono derivative **1** (TAK-599). As we expected, **1** showed excellent water solubility (>100 mg/mL) in pH 7 solution. Furthermore, we examined the chemical stability of **1** (TAK-599). In solution at pH 7, good stability was observed for 8 h (purity >98%), and in the solid state, **1** remained in high quality for at least 16 weeks at 40 °C (purity >96%).

Figure 2 displays the pharmacokinetics of **1** (TAK-599) and **2a** (T-91825) in rats and monkeys after intravenous

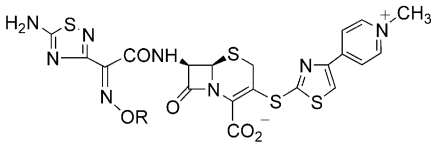
Table 1. Antibacterial activities (MIC, μg/mL) and protective effects (ED_{50}, mg/kg) of the prepared cephem derivatives (**3a**–**3d**) and VCM



Compd	X	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*P.a.*	ED_{50}[a]
3a		0.2	0.78	1.56	1.56	0.05	0.2	12.5	2.21
3b		0.39	1.56	3.13	3.13	0.2	0.39	>100	4.37
3c		0.1	1.56	6.25	6.25	0.05	3.13	0.78	NT
3d		0.2	6.25	50	50	0.025	3.13	1.56	NT
VCM		0.78	0.78	1.56	0.78	>100	>100	>100	2.21–5.05

S.a., *Staphylococcus aureus* 308A-1; MRSA1, *S. aureus* N295; MRSA2, *S. aureus* N241; MRSA3, *S. aureus* OFU4; *E.c.*, *Escherichia coli* NIHJ JC-2; *E.cl.*, *Enterobacter cloacae* GN5788; P.a., *Pseudomonas aeruginosa* IFO 3445.
[a]Protective effects against experimental systemic infection caused by *S. aureus* N133 in mice. NT, not tested.

Table 2. Antibacterial activities (MIC, μg/mL), protective effects (ED_{50}, mg/kg) and affinities for PBP2′ (IC_{50}, μg/mL) of the prepared cephem derivatives (**2a**–**6a**), CZOP and VCM



Compd	R	*S.a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*P.a.*	ED_{50}[a]	IC_{50}[b]
2a (T-91825)	CH_2CH_3	0.39	0.78	1.56	1.56	0.1	0.39	3.13	1.74	0.90
3a	CH_2F	0.2	0.78	1.56	1.56	0.05	0.2	12.5	2.21	NT
4a	CH_3	0.39	3.13	3.13	6.25	0.1	0.39	25	3.90	NT
5a		0.39	0.78	1.56	1.56	0.39	0.78	3.13	3.41	NT
6a		0.39	0.78	1.56	3.13	0.78	1.56	6.25	>12.5	NT
VCM		0.78	0.78	1.56	0.78	>100	>100	>100	2.21–5.05	NT
CZOP		0.78	12.5	50	100	0.05	0.1	0.78	>12.5	120

Details of each strain are shown in Table 1.
[a]Protective effects against experimental systemic infection caused by *S. aureus* N133 in mice.
[b]Affinity for PBP2′ of *S. aureus* N200P.

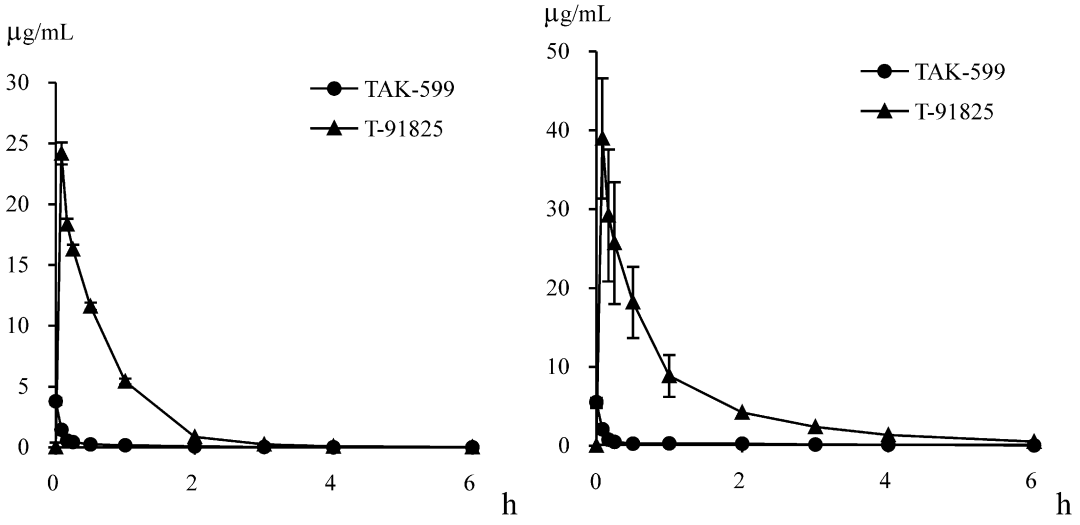


Figure 2. Plasma concentrations of **1** (TAK-599)* (●) and **2a** (T-91825) (▲) after administration of a 10 mg/kg intravenous dose of **1** (TAK-599) in rats (left) and monkeys (right). * **1** (TAK-599) was calculated as an acetic acid free compound.

administration of **1** at a dose of 10 mg/kg. Compound **1** disappeared rapidly and the main compound detected was **2a**, suggesting that **1** was converted smoothly into **2a** in blood. This result shows that **1** (TAK-599) functions as an excellent prodrug of **2a** (T-91825) in animals.

Table 3 shows the protective effects of **1** (TAK-599) against MRSA infections compared with those of VCM. Reflecting both the pharmacokinetics of **1** and the potent anti-MRSA activity of **2a**, **1** (TAK-599) exhibited excellent in vivo anti-MRSA activity, which was superior to that of VCM.

Table 3. Protective effects of compound **1** (TAK-599) and VCM against experimental systemic infection caused by *S. aureus* N133 in mice

Compd	ED_{50}[a] (mg/kg)
1 (TAK-599)	1.60–2.37
VCM	3.37–4.82

[a]Compounds were administered subcutaneously immediately after the bacterial challenge.

In conclusion, these results show that **1** (TAK-599) is a highly promising parenteral cephalosporin targeted for MRSA.

Experimental

General methods

MPs were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 215 or Horiba FT-200 spectrophotometer. ^{1}H NMR spectra were recorded on a Var-

ian Gemini 200 (200MHz) spectrometer using TMS as the internal standard. ^{31}P NMR spectra were recorded on a Bruker DRX500 (500 MHz) spectrometer using 85% phosphoric acid as an external standard. Column chromatography was carried out on Merck Kieselgel 60 (Art No. 7734), and Mitsubishi Chemical MCI gel CHP-20P and SP-207.

Antibacterial activity in vitro

The MICs against selected strains of Gram-positive and Gram-negative bacteria were determined by the standard serial 2-fold agar dilution method with Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) as the test medium. The agar plates were inoculated with about 10^4 CFU of microorganisms per spot and were incubated overnight at 37 °C.

Antibacterial activity in vivo

S. aureus strain N133 was cultured overnight at 37 °C in brain heart infusion broth (Difco Laboratories), suspended in 5% mucin (Difco Laboratories) and inoculated intraperitoneally into ICR male mice (Japan SLC Inc., Japan). Compounds were dissolved in a 10% aq DMSO solution (**2a**–**6a** and **3b**) or saline containing two equivalents of $NaHCO_3$ to the compound **1** and administered subcutaneously immediately after the bacterial challenge. The 50% effective dose (ED_{50}) was calculated from the survival rate recorded on day 5 after infection.

Affinity for penicillin binding protein 2′

Membrane was prepared from *S. aureus* N200P cells grown to the late exponential phase in trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD, USA) and incubated with [^{14}C]benzylpenicillin. Binding affinity of antibiotic for PBP2′ was assessed by a competition assay, in which the membrane was incubated with dilutions of the antibiotic at 30 °C for 10 min and then labeled with [^{14}C]benzylpenicillin for 10 min. PBPs were separated by SDS-polyacrylamide gel electrophoresis and detected by fluorography. Binding affinity was expressed in terms of the concentration required to prevent [^{14}C]benzylpenicillin binding by 50% (IC_{50}).

Pharmacokinetic profiles

^{14}C-Labelled **1** ([^{14}C]**1**) prepared by Amersham Pharmacia Biotech UK Ltd. (Buckinghamshire, UK) was used. The animals used in this study were male Crj/IGS rats (Charles river Japan Inc., Japan), and male cynomolgus monkeys (Keari Co. Ltd., Japan). [^{14}C]**1** diluted appropriately with unlabeled compound was dissolved in saline containing 2.5-fold mole of sodium bicarbonate for intravenous administrations at a dose of 10 mg/kg. After dosing, blood samples were taken from the tail vein of rats and the femoral vein of monkeys. Immediately after the blood sampling, the plasma obtained by centrifugation was added with half a volume of 10 mmol/L sodium phosphate buffer (pH 7.0), and the mixture was extracted by ethanol with 2 volumes of plasma. The ethanolic supernatants obtained by centrifugation were kept frozen at −20 °C until analysis.

The ^{14}C in plasma, organic solvent extracts, and effluent from HPLC were determined by a liquid scintillation counter (LSC-5100; Aloka, MA, USA). [^{14}C] **1** and ^{14}C-labelled **2a** ([^{14}C]**2a**) in the plasma were quantified by HPLC. The column used was an Inertsil ODS-3 (5-μm particle size, 150×4.6 mm I.D.; GL Sciences, Japan). The mobile phase (A) [MP(A)] was 10 mmol/L $CH_3CO_2NH_4$–acetonitrile (95:5, v/v) and the mobile phase (B) [MP(B)] was 10 mmol/L $CH_3CO_2NH_4$–acetonitrile (50:50, v/v). The column temperature and the flow-rate were 40 °C and 1.0 mL/min, respectively. The time program for the gradient elution was as follows: the concentration of MP(B) was held at 8% for 10 min and linearly increased from 8 to 80% over a period of 15 min and then cycled back to the initial condition (8%). Under these conditions, unchanged [^{14}C]**1** and [^{14}C]**2a** were eluted at 12 and 21 min, respectively.

3-(4-Pyridyl)-1,2,4-thiazole-5-thiol (7b). To a mixture of 4-amidinopyridine hydrochloride[11] (**8**, 3.14 g 19.9 mmol), carbon disulfide (3.8 g, 49.9 mmol), sulfur (0.8 g, 25.0 mmol) in MeOH (24 mL) was added 28% sodium methoxide in MeOH (12 mL), and the reaction mixture was refluxed for 6 h. After concentration of the reaction mixture under reduced pressure, the residue was diluted with water (5 mL), and adjusted to pH 4 with 2 N HCl. The resulting precipitate was collected by filtration to give **7b** (1.90 g, 50%): ^{1}H NMR (DMSO-d_6) δ 8.08 (2H, d, J=6 Hz), 8.82 (2H, d, J=6 Hz).

2-Dinitrophenylthio-4-(4-pyridyl)thiophene (7c). Under ice-cooling, aluminum chloride (1.33 g, 10.0 mmol) was added portionwise to a mixture of 3-(4-pyridyl)thiophene[22] (**9**, 0.8 g, 5.0 mmol) and 2,4-dinitrophenylsulfenyl chloride (**11**, 1.29 g, 4.8 mmol) in nitromethane (15 mL). The reaction mixture was stirred at room temperature for 2 h and poured into ice-water (100 mL). The mixture was diluted with a mixture of THF (20 mL) and EtOAc (80 mL), and neutralized with aqueous $NaHCO_3$. The insoluble precipitate was filtered off, and the separated organic layer of the filtrate was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give **7c** as a brown solid (0.85 g, 48%): ^{1}H NMR ($CDCl_3$) δ 7.25 (1H, d, J=9 Hz), 7.51 (2H, d, J=6 Hz), 7.77 (1H, d, J=2 Hz), 8.02 (1H, d, J=2 Hz), 8.26 (1H, dd, J=9 Hz, 3 Hz), 8.70 (2H, d, J=6 Hz), 9.13 (1H, d, J=3 Hz).

Compound **7d** was obtained in 90% yield from 3-(4-pyridyl)furan (**10**)[22] by a similar procedure to that used for preparation of **7c**. ^{1}H NMR (DMSO-d_6) δ 7.15 (1H, d, J=9 Hz), 7.79 (2H, d, J=6 Hz), 7.98 (1H, d, J=1 Hz), 8.44 (1H, dd, J=9 Hz, 3 Hz), 8.66 (2H, d, J=6 Hz), 8.94 (1H, d, J=3 Hz), 8.96 (1H, d, J=1 Hz).

***p*-Methoxybenzyl 7β-phenylacetamido-3-[4-(4-pyridyl)-1,3-thiazol-2-yl]thio-3-cephem-4-carboxylate (15a) Method A.** Under ice-cooling, 4-(4-pyridyl)thiazole-2-thiol[10] (**7a**,

4.27 g, 22 mmol) was added to a mixture of sodium hydride (60% in oil, 0.96 g, 24 mmol) in THF (77 mL), and the mixture was stirred at room temperature for 2 h. Maintaining ice-cooling, **13** (11.7 g, 20 mmol) was added to the reaction mixture (containing **12a**) and then stirred at room temperature for 2 h. After the reaction mixture was concentrated under reduced pressure, the residue was diluted with a mixture of water and EtOAc (40 mL/20 mL). The resulting precipitate was collected by filtration, washed with EtOAc (10 mL) and Et_2O (20 mL) successively, and dried under vacuum to give **15a** (11.1 g, 79%) as a THF solvate: ^{1}H NMR (DMSO-d_6) δ 3.46–3.69 (3H, m), 3.70 (3H,s), 3.94 (1H, d, J = 18 Hz), 5.23 (2H,s), 5.28 (1H, d, J = 5 Hz), 5.82 (1H, dd, J = 8 Hz, 5 Hz), 6.84 (2H, d, J = 8 Hz), 7.22–7.30 (7H, m), 7.89 (2H, d, J = 6 Hz), 8.52 (1H, s), 8.66 (2H, d, J = 6 Hz), 9.27 (1H, d, J = 8 Hz).

Compound **15b** was obtained in 32% yield from **7b** by a similar procedure to that used for preparation of **15a**. ^{1}H NMR ($CDCl_3$) δ 3.51 (1H, d, J = 18 Hz), 3.67 (2H, s), 3.69 (3H, s), 3.91 (1H, d, J = 18 Hz), 5.09 (1H, d, J = 5 Hz), 5.20 (1H, d, J = 5 Hz), 5.94 (1H, dd, J = 8 Hz, 5 Hz), 6.19 (1H, d, J = 8 Hz), 6.76 (2H, d, J = 8 Hz), 7.19–7.40 (7H, m), 8.05 (2H, d, J = 6 Hz), 8.77 (2H, d, J = 6 Hz).

***p*-Methoxybenzyl 7β-phenylacetamido-3-[4-(4-pyridyl)-furan-2-yl]thio-3-cephem-4-carboxylate (15d) Method B.** Under ice-cooling, 1 M sodium methoxide in MeOH (2.5 mL, 2.5 mmol) was added to a solution of **7d** (858 mg, 2.5 mmol), and the mixture was stirred at room temperature for 1 h. After the mixture was concentrated under reduced pressure, the residual solid was collected by filtration. The obtained powder containing **12d** was added portionwise to a suspension of **13** (1.46 g, 2.5 mmol) in THF (16 mL) at −30 °C, and the reaction mixture was stirred at −30 °C for 30 min. The mixture was poured into water (30 mL), and extracted with EtOAc (50 mL). The separated organic layer was washed with brine (30 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Treatment of the solid residue with diethyl ether (10 mL) gave **15d** (599 mg, 40%): ^{1}H NMR ($CDCl_3$) δ 3.29–3.80 (4H, m), 3.81 (3H, s), 4.91 (1H, d, J = 5 Hz), 5.25 (2H, s), 5.77 (1H, dd, J = 9 Hz, 5 Hz), 6.09 (1H, d, J = 9 Hz), 6.90 (2H, d, J = 9 Hz), 7.09 (1H, s), 7.21–7.39 (9H, m), 8.02 (1H, s), 8.63 (2H, d, J = 6 Hz).

Compound **15c** was obtained in 91% yield from **7c** by a similar procedure to that used for preparation of **15d**. NMR ($CDCl_3$) δ 3.34 (2H, s), 3.62 (2H, s), 3.80 (3H, s), 4.91 (1H, d, J = 5 Hz), 5.26 (2H, s), 5.76 (1H, d, J = 5 Hz), 5.94 (1H, dd, J = 9 Hz, 5 Hz), 6.11 (1H, d, J = 9 Hz), 6.88 (2H, d, J = 8 Hz), 7.39 (9H, m), 7.57 (1H, d, J = 1 Hz), 7.80 (1H, d, J = 1 Hz), 8.64 (2H, d, J = 6 Hz).

7β-Amino-3-[4-(1-methyl-4-pyridinio)-1,3-thiazol-2-yl]-thio-3-cephem-4-carboxylate (21a). Iodomethane (42 mL) was added to a solution of **15a** (28.4 g, 45 mmol) in DMF (85 mL), and the reaction mixture was stirred at room temperature for 16 h. Diethyl ether (1 L) was added to the mixture, and the mixture was stirred at room temperature for 30 min. The resulting upper layer was removed by decantation. After treatment of the residual oil with diethyl ether (250 mL), the resulting solid mass of **17a** was collected by filtration, washed with diethyl ether (50 mL), and dried under vacuum. In a separate vessel, under ice-cooling, pyridine (18.2 mL, 225 mmol) was added to a suspension of phosphorus pentachloride (46.9 g, 225 mmol) in CH_2Cl_2 (300 mL), and the mixture was stirred at 5 °C for 30 min. To the suspended mixture was added portionwise the above **17a** at 5 °C, and the reaction mixture was stirred at 5 °C for 1 h. After cooling at −20 °C, MeOH (60 mL) was added, and the mixture was stirred at the same temperature for 30 min. The mixture was diluted with diethyl ether (900 mL), and the resulting upper layer was removed by decantation. To a suspension of the residual oil in CH_2Cl_2 (150 mL) was added TFA (150 mL) and anisole (15 mL) successively, and the reaction mixture was stirred at room temperature for 1 h. After the mixture was diluted with diethyl ether (750 mL), the resulting upper layer was removed by decantation. The residue was suspended in water (1.5 L), and the mixture was adjusted to pH 7 with aqueous $NaHCO_3$. The precipitate was filtered off, and the filtrate was purified by MCI gel SP-207 (750 mL) column chromatography. The fractions eluted by aqueous 15% EtOH were concentrated under reduced pressure and the concentrate was lyophilized to give **21a** (8.28 g, 45%): ^{1}H NMR (D_2O) δ 3.48, 3.87 (2H, ABq, J = 18 Hz), 4.30 (3H, s), 4.79 (1H, d, J = 5 Hz), 5.16 (1H, d, J = 5 Hz), 8.27 (2H, d, J = 7 Hz), 8.44 (1H, s), 8.69 (2H, d, J = 7 Hz).

Compounds **21b–d** were obtained from **15b–d** by a similar procedure to that used for the preparation of **21a**.

Compound **21b** (48% yield): ^{1}H NMR (D_2O) δ 3.49, 3.93 (2H, ABq, J = 18 Hz), 4.35 (3H, s), 4.78 (1H, d, J = 5 Hz), 5.18 (1H, d, J = 5 Hz), 8.52 (2H, d, J = 6 Hz), 8.83 (2H, d, J = 6 Hz).

Compound **21c** (29% yield): ^{1}H NMR (D_2O) δ 3.08, 3.41 (2H, ABq, J = 17 Hz), 4.25 (3H, s), 4.63 (1H, d, J = 5 Hz), 4.89 (1H, d, J = 5 Hz), 7.59 (1H, s), 7.99 (2H, d, J = 6 Hz), 8.27 (1H, s), 8.57 (2H, d, J = 6 Hz).

Compound **21d** (38% yield): ^{1}H NMR (D_2O) δ 3.30, 3.50 (2H, ABq, J = 18 Hz), 4.27 (3H, s), 4.76 (1H, d, J = 5 Hz), 4.99 (1H, d, J = 5 Hz), 7.34 (1H, s), 8.05 (2H, d, J = 6 Hz), 8.49 (1H, s), 8.60 (2H, d, J = 6 Hz).

Benzhydryl 7β-phenylacetamido-3-[4-(4-pyridyl)-1,3-thiazol-2-yl]thio-3-cephem-4-carboxylate (15a). To a suspension of **7a** (402 g, 2.0 mol) in THF (600 mL) was added 28% sodium methoxide in MeOH (400 g, 2.0 mol), and the mixture was stirred at room temperature for 1 h. Under cooling at −5 °C, the resulting mixture containing **12a** was added to a suspension of **14** (1.0 kg, 1.73 mol), and stirred at −5 °C for 2 h. After neutralization with acetic acid (20.8 g, 347 mmol), MeOH (10 L) and water (6 L) were successively added to the mixture at 0 °C. After stirring at 0 °C for 1 h, the resulting powder was collected by filtration, washed

with MeOH (6 L), and dried under vacuum to give **16a** (943 g, 81%): ^{1}H NMR ($CDCl_3$) δ 3.41–3.73 (4H, m), 5.02 (1H, d, J = 5 Hz), 5.84 (1H, dd, J = 8 Hz, 5 Hz), 6.23 (1H, d, J = 8 Hz), 6.97 (1H, s), 7.27–7.72 (17H, m), 7.73 (1H, s), 8.67 (2H, d, J = 6 Hz). Anal. calcd for $C_{36}H_{28}N_4O_4S_3 \cdot 0.5H_2O$: C, 63.05; H, 4.11; N, 8.17; S, 14.02. Found: C, 63.16; H, 4.15; N, 8.27; S, 13.98.

Benzhydryl 7β-phenylacetamido-3-[4-(1-methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-3-cephem-4-carboxylate iodide (18a). Iodomethane (324 g, 2.17 mol) was added dropwise to a solution of **16a** (300 g, 0.43 mol) in DMF (0.6 L), and the reaction mixture was stirred at room temperature for 8 h. The mixture was added dropwise to EtOAc (6 L) with stirring, and the resulting precipitate was collected by filtration, washed with EtOAc (500 mL) and diethyl ether (1 L) successively, and dried under vacuum to give **18a** (351 g, 97%): ^{1}H NMR (DMSO-d_6) δ 3.55 (2H, d, J = 4 Hz), 3.70, 4.01 (2H, ABq, J = 18 Hz), 4.35 (3H, s), 5.33 (1H, d, J = 5 Hz), 5.89 (1H, dd, J = 8 Hz, 5 Hz), 6.99 (1H, s), 7.18–7.42 (15H, m), 8.54 (2H, d, J = 7 Hz), 9.02 (1H, s), 9.03 (2H, d, J = 7 Hz), 9.32 (1H, d, J = 8 Hz).

Benzhydryl 7β-amino-3-[4-(1-methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-3-cephem-4-carboxylate chloride (20a). Under ice-cooling, pyridine (115 g, 1.44 mol) was added dropwise to a suspension of phosphorus pentachloride (312 g, 1.44 mol) in CH_2Cl_2 (2.8 L), and the mixture was stirred at 5 °C for 30 min. To the mixture **18a** (400 g, 0.48 mol) was added portionwise, and the reaction mixture was stirred at 5 °C for 1 h. Under cooling at −10 °C, the mixture was added dropwise to *iso*-butanol (5.6 L), and stirred at room temperature for 3 h. The resulting precipitate was collected by filtration, washed with EtOAc (500 mL) and diethyl ether (1L) successively, and dried under vacuum to give **20a** (270 g, 93%): ^{1}H NMR (DMSO-d_6) δ 3.94 (2H, s), 4.35 (3H, s), 5.32 (1H, d, J = 5 Hz), 5.45 (1H, d, J = 5 Hz), 6.99 (1H, s), 7.25–7.40 (10H, m), 8.59 (2H, d, J = 7 Hz), 9.07 (2H, d, J = 7 Hz), 9.19 (1H, s).

Dihydrochloride salt of 7β-amino-3-[4-(1-methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-3-cephem-4-carboxylate (21a). To a suspension of **20a** (430 g, 0.66 mol) in acetonitrile (3.5 L) was added dropwise concd HCl (3.5 L), and the mixture was stirred at room temperature for 30 min. EtOAc (7 L) was added, and the mixture was stirred at room temperature for 5 h. The resulting crystals were collected by filtration, washed twice with acetonitrile (1 L), and dried under vacuum to give **21a** as the dihydrochloride form (236 g, 76%): MP 202 °C; ^{1}H NMR (DMSO-d_6) δ 3.78, 3.98 (2H, ABq, J = 17 Hz), 4.35 (3H, s), 5.26 (1H, d, J = 5 Hz), 5.42 (1H, d, J = 5 Hz), 8.61 (2H, d, J = 7 Hz), 9.05 (2H, d, J = 7 Hz), 9.17 (1H, s). Anal. calcd for $C_{16}H_{14}N_4O_3S_3 \cdot 2HCl$: C, 40.08; H, 3.36; N, 11.69. Found: C, 39.83; H, 3.43; N, 11.78.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-[4-(1-methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-3-cephem-4-carboxylate (2a, T-91825). Under ice-cooling, **21a** (2.0 g, 4.92 mmol) was suspended in a mixture of THF (80 mL) and water (80 mL). To the suspension were successively added 0.6 M $NaHCO_3$ (26 mL) and 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetyl chloride hydrochloride[15] (2.0 g, 7.38 mmol), and the reaction mixture was stirred at 5 °C for 30 min. After concentration under reduced pressure, the concentrate was purified by MCI gel SP-207 (300 mL) column chromatography. The fractions eluted by aqueous 20% EtOH were concentrated under reduced pressure and the concentrate was lyophilized to give **2a** (1.83 g, 62%): ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7 Hz), 3.34, 3.89 (2H, ABq, J = 17 Hz), 4.18 (2H, q, J = 7 Hz), 4.30 (3H, s), 5.18 (1H, d, J = 5 Hz), 5.73 (1H, dd, J = 8 Hz, 5 Hz), 8.13 (2H, br s), 8.49 (2H, d, J = 7 Hz), 8.88 (1H, s), 8.95 (2H, d, J = 7 Hz), 9.62 (1H, d, J = 8 Hz); IR (KBr) cm^{-1} 1775, 1640, 1615. Anal. calcd for $C_{22}H_{20}N_8O_5S_4 \cdot 4.5H_2O$: C, 38.53; H, 4.26; N, 16.34. Found: C, 38.39; H, 4.06; N, 16.04.

Compounds **3a–d**, **4a–6a** were obtained from **21a–d** by a similar procedure to that used for preparation of **2a**.

3a (23% yield): ^{1}H NMR (DMSO-d_6) δ 3.34, 3.89 (2H, ABq, J = 17 Hz), 4.30 (3H, s), 5.19 (1H, d, J = 5 Hz), 5.74 (1H, dd, J = 8 Hz, 5 Hz), 5.79 (2H, d, J = 55 Hz), 8.22 (2H, br s), 8.49 (2H, d, J = 7 Hz), 8.88 (1H, s), 8.95 (2H, d, J = 7 Hz), 9.84 (1H, d, J = 8 Hz); IR (KBr) cm^{-1} 1775, 1640, 1610. Anal. calcd for $C_{21}H_{17}FN_8O_5S_4 \cdot 5.0H_2O$: C, 36.10; H, 3.89; N, 16.04. Found: C, 36.14; H, 3.83; N, 15.69.

3b (30% yield): ^{1}H NMR (D_2O) δ 3.55, 4.00 (2H, ABq, J = 18 Hz), 4.35 (3H, s), 5.38 (1H, d, J = 5 Hz), 5.79 (2H, d, J = 55 Hz), 5.88 (1H, d, J = 5 Hz), 8.57 (2H, d, J = 7 Hz), 8.82 (2H, d, J = 7 Hz); IR (KBr) cm^{-1} 1770, 1670, 1630, 1610. Anal. calcd for $C_{20}H_{16}FN_9O_5S_4 \cdot 4.0H_2O$: C, 35.24; H, 3.55; N, 18.49. Found: C, 35.15; H, 3.37; N, 18.21.

3c (52% yield): ^{1}H NMR (DMSO-d_6) δ 3.19, 3.54 (2H, ABq, J = 17 Hz), 4.29 (3H, s), 5.02 (1H, d, J = 5 Hz), 5.62 (1H, dd, J = 8 Hz, 5 Hz), 5.76 (2H, d, J = 56 Hz), 8.04 (1H, d, J = 1 Hz), 8.25 (2H, br s), 8.43 (2H, d, J = 7 Hz), 8.78 (1H, d, J = 1 Hz), 8.95 (2H, d, J = 7 Hz), 9.74 (1H, d, J = 8 Hz); IR (KBr) cm^{-1} 1760, 1670, 1635, 1605. Anal. calcd for $C_{22}H_{18}FN_7O_5S_4 \cdot 3.0H_2O$: C, 39.93; H, 3.66; N, 14.82. Found: C, 39.82; H, 3.52; N, 14.56.

3d (14% yield): ^{1}H NMR (DMSO-d_6) δ 3.12, 3.27 (2H, ABq, J = 17 Hz), 4.26 (3H, s), 5.01 (1H, d, J = 5 Hz), 5.61 (1H, dd, J = 9 Hz, 5 Hz), 5.75 (2H, d, J = 55 Hz), 7.60 (1H, s), 8.20 (2H, br s), 8.33 (2H, d, J = 6 Hz), 8.93 (2H, d, J = 6 Hz), 8.96 (1H, s), 9.67 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1765, 1670, 1640, 1610. Anal. calcd for $C_{22}H_{18}FN_7O_6S_3 \cdot 4.5H_2O$: C, 39.25; H, 4.01; N, 14.57. Found: C, 39.67; H, 3.79; N, 14.30.

4a (38% yield): ^{1}H NMR (DMSO-d_6) δ 3.53, 3.91 (2H, ABq, J = 18 Hz), 3.92 (3H, s), 4.31 (3H, s), 5.17 (1H, d, J = 5 Hz), 5.74 (1H, dd, J = 8 Hz, 5 Hz), 8.15 (2H, br s), 8.47 (2H, d, J = 7 Hz), 8.89 (1H, s), 8.97 (2H, d, J = 7 Hz), 9.66 (1H, d, J = 8 Hz); IR (KBr) cm^{-1} 1770, 1640, 1610. Anal. calcd for $C_{21}H_{18}N_8O_5S_4 \cdot 4.0H_2O$: C, 38.06; H, 3.95; N, 16.91. Found: C, 38.26; H, 4.01; N, 16.87.

5a (41% yield): ^{1}H NMR (DMSO-d_6) δ 1.25 (6H, d, J=7 Hz), 3.32, 3.90 (2H, ABq, J=17 Hz), 4.32 (3H, s), 4.39 (1H, m), 5.19 (1H, d, J=5 Hz), 5.74 (1H, dd, J=8 Hz, 5 Hz), 8.17 (2H, br s), 8.46 (2H, d, J=7 Hz), 8.89 (1H, s), 8.97 (2H, d, J=7 Hz), 9.61 (1H, d, J=8 Hz); IR (KBr) cm^{-1} 1765, 1660, 1620. Anal. calcd for $C_{23}H_{22}N_8O_5S_4 \cdot 3.5H_2O$: C, 40.52; H, 4.29; N, 16.44. Found: C, 40.87; H, 4.46; N, 16.13.

6a (38% yield): ^{1}H NMR (DMSO-d_6) δ 1.42–1.98 (8H, m), 3.33, 3.90 (2H, ABq, J=17 Hz), 4.30 (3H, s), 4.74 (1H, m), 5.18 (1H, d, J=5 Hz), 5.70 (1H, dd, J=8 Hz, 5 Hz), 8.14 (2H, br s), 8.49 (2H, d, J=7 Hz), 8.89 (1H, s), 8.95 (2H, d, J=7 Hz), 9.57 (1H, d, J=8 Hz); IR (KBr) cm^{-1} 1770, 1640, 1615. Anal. calcd for $C_{25}H_{24}N_8O_5S_4 \cdot 4.5H_2O$: C, 41.37; H, 4.58; N, 15.44. Found: C, 41.27; H, 4.47; N, 15.39.

2-(5-Dichlorophosphorylamino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetyl chloride (23). Under ice-cooling, 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetic acid (**22**, 64.8 g, 0.30 mol) was added to a suspension of phosphorus pentachloride (156 g, 0.75 mol) in EtOAc (450 mL), and the mixture was stirred at 5 °C for 30 min. The reaction mixture was diluted with toluene (1.8 L), and washed with brine (1.2 L) cooled below −5 °C. The separated organic layer was dried over $MgSO_4$, and filtered. The filtrate was evaporated under reduced pressure and diisopropyl ether (200 mL) was added to the solid residue, and the mixture was stirred at 5 °C for 30 min. The resulting crystals were collected by filtration, washed with diisopropyl ether (40 mL), and dried under vacuum to give **23** (63.5 g, 60%): MP 118–120 °C; ^{31}P NMR ($CDCl_3$) δ 10.6; ^{1}H NMR ($CDCl_3$) δ 1.42 (3H, t, J=7 Hz), 4.45 (2H, q, J=7 Hz), 8.81 (1H, br s); IR (KBr) cm^{-1} 3063, 1784, 1593, 1223, 1057. Anal. calcd for $C_6H_6N_4O_3SCl_3P$: C, 20.50; H, 1.72; N, 15.94; P, 8.81. Found: C, 20.52; H, 1.77; N, 15.99; P, 8.90.

3-[4-(1-Methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-7β-[2-(5-phosphonoamino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-cephem-4-carboxylate (24). Under ice-cooling, to a solution of **21a** (dihydrochloride salt, 60 g, 0.125 mol) in water (3.6 L) were added 2 M sodium acetate (700 mL) and **23** (52.8 g, 0.15 mol) successively, and the mixture was stirred at room temperature for 1 h. The reaction mixture was washed with EtOAc (3 L), and the separated aqueous layer was concentrated to about 600 mL under reduced pressure. EtOH (1.2 L) was added to the concentrate, and the mixture was allowed to stand at 5 °C for 15 min. The resulting precipitate was collected by filtration, washed with a mixture of water (150 mL) and EtOH (300 mL), and EtOH (600 mL) successively, and air-dried. The resulting powder was dissolved in water (1.3 L) containing 1% acetic acid, and purified by MCI gel SP-207 (2.5 L) column chromatography. The fractions eluted by aqueous 10% EtOH were concentrated to about 700 mL under reduced pressure, and the pH of the concentrate was adjusted to 0.5 with 6N HCl. The resulting precipitate was collected by filtration, washed with water (300 mL), and dried under vacuum to give **24** (68.4 g, 77%): ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J=7 Hz), 3.58, 3.94 (2H, ABq, J=18 Hz), 4.17 (2H, q, J=7 Hz), 4.33 (3H, s), 5.32 (1H, d, J=5 Hz), 5.90 (1H, dd, J=5 Hz, 8 Hz), 8.51 (2H, d, J=6 Hz), 8.99 (3H, m), 9.30 (1H, m), 9.70 (1H, d, J=8 Hz).; IR (KBr) cm^{-1} 1778, 1682, 1643. Anal. calcd for $C_{22}H_{21}N_8O_8S_4P \cdot 2.0H_2O$: C, 36.66; H, 3.50; N, 15.55; P, 4.30. Found: C, 36.94; H, 3.46; N, 15.57; P, 3.95.

3-[4-(1-Methyl-4-pyridinio)-1,3-thiazol-2-yl]thio-7β-[2-(5-phosphonoamino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-cephem-4-carboxylate acetic acid solvate (1, TAK-599). To a suspension of **24** (10 g, 14.6 mmol) in water (30 mL) was added 2 M sodium acetate (14.6 mL) to give a clear solution, to which were added acetic acid (60 mL) and 1 M sulfuric acid (14.6 mL) successively, and the mixture was stirred at room temperature for 3 h. The resulting crystals were collected by filtration, washed with 50% aqueous acetic acid (30 mL) and water (50 mL) successively, and dried under vacuum to give **1** (5.55 g, 57%): MP, 221–223 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, J=7 Hz), 1.91 (3H, s), 3.58, 3.95 (2H, ABq, J=17 Hz), 4.17 (2H, q, J=7 Hz), 4.34 (3H, s), 5.32 (1H, d, J=5 Hz), 5.92 (1H, dd, J=5 Hz, 8 Hz), 8.51 (2H, d, J=6 Hz), 8.99 (3H, m), 9.30 (1H, m), 9.70 (1H, d, J=8 Hz); IR (KBr) cm^{-1} 1755, 1668, 1645. Anal. calcd for $C_{24}H_{25}N_8O_{10}S_4P \cdot 1.0H_2O$: C, 37.79; H, 3.57; N, 14.69; P, 4.06. Found: C, 37.97; H, 3.30; N, 14.37; P, 3.88.

Experiments for mechanistic consideration of *N*-dichlorophosphorylation

Reaction of the acid chloride 25 with phosphorus pentachloride. Under ice-cooling, to a suspension of 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetyl chloride hydrochloride[15] (**25**, 542 mg, 2.0 mmol) in EtOAc (3 mL) was added phosphorus pentachloride (416.5 mg, 2.0 mmol), and the mixture was stirred at 5 °C for 1 h. [Acetic acid (0.115 mL, 2.0 mmol) was added to the mixture with ice-cooling, and the reaction mixture was stirred at 5 °C for 1 h.] The reaction mixture was evaporated under reduced pressure. The residue was dissolved in $CDCl_3$ (4 mL), and ^{31}P NMR spectra were taken: δ 2.6 (when not added acetic acid), δ 10.6 (when added acetic acid).

Reaction of the carboxylic acid 22 with phosphorus pentachloride. Under ice-cooling, to a suspension of 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetic acid (**22**, 432 mg, 2.0 mmol) in EtOAc or THF (3 mL) was added phosphorus pentachloride (625 mg, 3.0 mmol), and the mixture was stirred at 5 °C for 1 h. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in $CDCl_3$ (4 mL), and ratios of **26** (δ 2.6 ppm) and **23** (δ 10.6 ppm) in the mixture were determined by ^{31}P NMR spectra.

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