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New broad-spectrum parenteral cephalosporins exhibiting potent activity against both methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Part 3: 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-ethoxyiminoacetamido] cephalosporins bearing 4-[3-(aminoalkyl)-ureido]-1-pyridinium at C-3′

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Abstract—Among the prepared C-3′ substituted-pyridinium cephalosporins, a series of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-ethoxyiminoacetamido] cephalosporins bearing 4-[3-(aminoalkyl)-ureido]-1-pyridinium at C-3′ showed highly potent antibacterial activity against MRSA and *Pseudomonas aeruginosa.*

1. Introduction

In our previous papers, we reported that C-3′ 1-(aminoalkyl)-imdazo[4,5-*b*]pyridinium cephalosporins, such as S-3578 and **1a** (Fig. 1), exhibit potent antibacterial activity against both MRSA and *Pseudomonas aeruginosa*, and display good water solubility. In the course of studies of S-3578, we found that introduction of a basic functionality such as an aminoalkyl group to the 1-position of C-3′ imidazo[4,5-*b*]pyridinium moiety enhances anti-MRSA activity.[1]

Subsequently, we found that **1a** analogs, in which the aminoalkyl-imidazo[4,5-*b*]pyridinium moiety of **1a** is replaced by other condensed-heterocyclic pyridinium derivatives bearing an aminoalkyl group, showed also potent antibacterial activity against MRSA and *P. aeruginosa*.[2]

These findings led interested in not only C-3′ condensed-heterocyclic pyridinium cephalosporins such as S-3578 but also structurally more simple C-3′ pyridinium cephalosporins bearing a basic functionality.

C-3′ unsubstituted-pyridinium cephalosporin **1b** (Fig. 1) has been reported to show potent antibacterial activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa* (anti-MRSA activity of **1b** was not reported).[3]

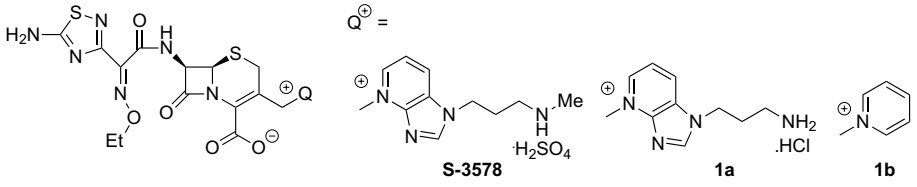

Figure 1. Structures of S-3578, **1a**, and **1b**.

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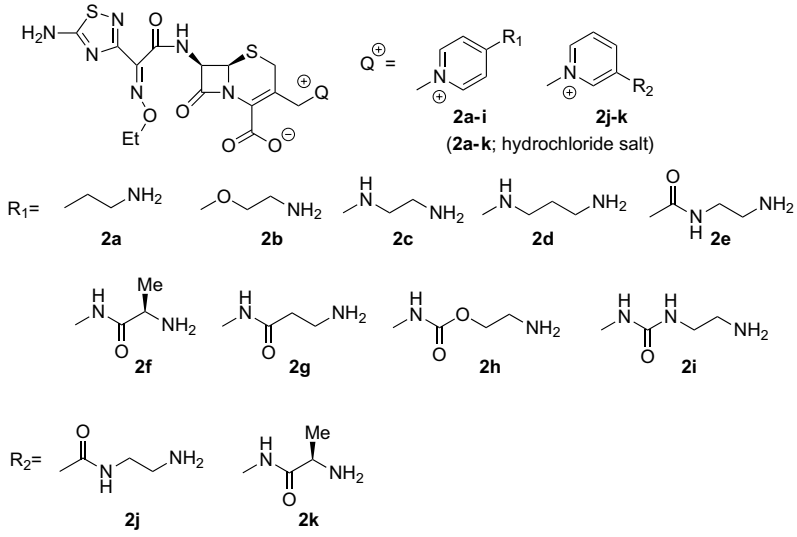
Table 1. Antibacterial activities (MIC, μg/mL) of **2a**–**k**, S-3578, CZOP, CFSL and VCM



Compound	*S. a.**	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
2a	1.56	12.5	12.5	0.2	0.78	3.13
2b	0.78	25	25	0.05	1.56	6.25
2c	0.2	12.5	12.5	0.05	0.78	3.13
2d	0.2	6.25	6.25	0.05	3.13	6.25
2e	1.56	12.5	12.5	0.2	1.56	3.13
2f	0.78	12.5	12.5	0.05	1.56	3.13
2g	1.56	6.25	6.25	0.1	0.78	3.13
2h	1.56	6.25	6.25	0.1	0.78	3.13
2i	0.78	3.13	6.25	0.1	0.78	3.13
2j	3.13	12.5	12.5	0.78	1.56	6.25
2k	1.56	12.5	12.5	0.39	1.56	6.25
S-3578	0.78	3.13	3.13	0.39	1.56	6.25
1b	1.56	12.5	12.5	0.05	0.39	1.56
CZOP	0.78	50	50	0.05	0.39	1.56
CFSL	0.78	25	25	0.05	3.13	6.25
VCM	1.56	0.78	1.56	>100	>100	>100

*Abbreviations: *S. a.*, *Staphylococcus aureus* SMITH; MRSA 1, *S. aureus* SR3626; MRSA 2, *S. aureus* SR3637; *E. c.*, *Escherichia coli* NIHJ JC-2; *P. a.* 1, *Pseudomonas aeruginosa* SR24; *P. a.* 2, *P. aeruginosa* SR5393; CZOP, cefozopran; CFSL, cefoselis; VCM, vancomycin.

We prepared a series of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-ethoxyiminoacetamido]-3-pyridinomethyl-cephalosporins bearing a basic functionality on the C-3′ pyridinium ring (**2a**–**s** in Tables 1 and 2) in order to discover novel cephalosporins exhibiting potent antibacterial activity against MRSA and *P. aeruginosa*.

Here we report the synthesis and antibacterial activity of novel C-3′ pyridinium cephalosporins.

2. Chemistry

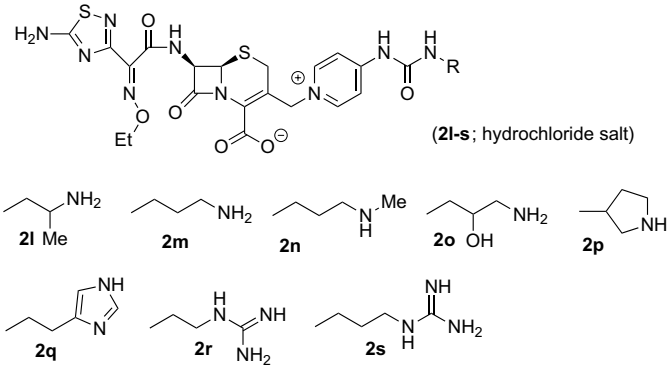
Novel C-3′ substituted-pyridinium cephems (**2a**–**s**) were synthesized by a method similar to one reported.[1] The method for the preparation of C-3′ pyridinium cephem **2i** is shown in Scheme 1. The C-3 iodomethyl cephalosporin intermediate **3**[1] was displaced by pyridine derivative **2i′** to afford iodide salt **3′**, which was treated with an $AlCl_3$-anisole system. Purification by reversed phase (HP-20) column chromatography yielded cephalosporin derivatives **2i** as the hydrochloride salt. Other cephems **2a**–**h** and **2j**–**s** were synthesized from iodomethy **3** and the corresponding pyridine derivative **2a′**–**h′** and **2j′**–**s′** by a method similar to that for preparing of **2i** (the structures of **2a′**–**c′**, **2h′**, and **2r′** are shown in Schemes 2–5, and those of **2d′**–**g′**, **2j′**–**q′**, and **2s′** are shown in Fig. 2).

The syntheses of selected pyridine derivatives are shown in Schemes 2–5.

Pyridine derivative **2a′** was prepared as shown in Scheme 2. Alkene **4** was reduced by palladium-catalyzed hydrogenation in ethanol, affording ester **5**, which was hydrolyzed by sodium hydroxide to give acid **6**. This acid **6** was converted to **2a′** using a 2-methyl-2-propanol and diphenylphosphoryl azide (DPPA)–triethylamine (Et_3N) system.[4]

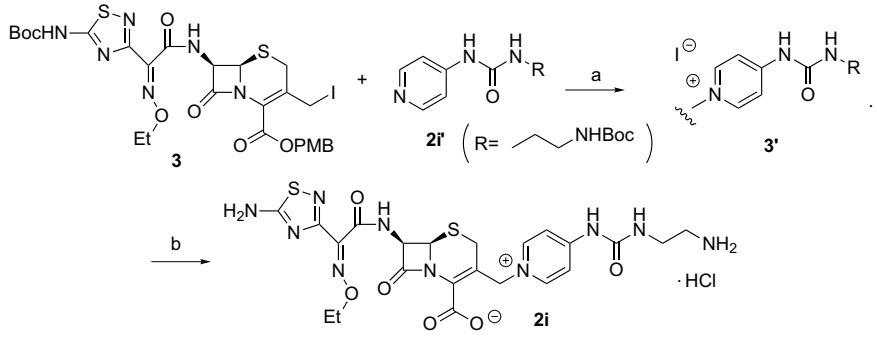
Pyridine derivative **2b′** and **2c′** were synthesized as shown in Scheme 3. Chloropyridine **7** was treated with 2-aminoethanol in the presence of sodium hydride, and the resulting amine was allowed to react with di-*tert*-butyl dicarbonate (Boc_2O) to give **2b′**. On the other

Table 2. Antibacterial activities of **21**–**s**

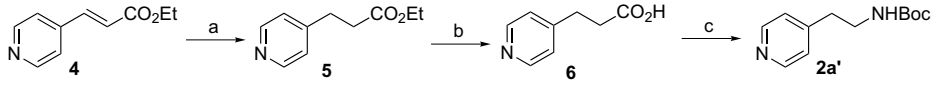


Compound	*S. a.**	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
2l	0.78	3.13	3.13	0.1	1.56	3.13
2m	0.78	3.13	3.13	0.05	1.56	3.13
2n	1.56	6.25	6.25	0.1	1.56	6.25
2o	1.56	6.25	6.25	0.1	1.56	3.13
2p	0.78	3.13	3.13	0.05	0.78	3.13
2q	0.78	6.25	6.25	0.1	1.56	6.25
2r	0.78	1.56	3.13	0.1	1.56	3.13
2s	0.78	3.13	3.13	0.05	1.56	3.13

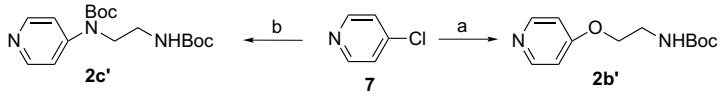
*Abbreviations: see footnote in Table 1.



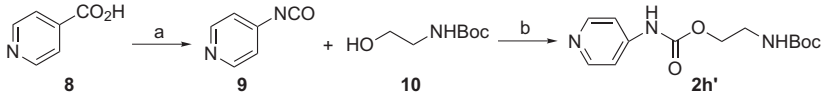
Scheme 1. Reagents and conditions: (a) **2i'**, MeCN, rt, 90 min; (b) (i) $AlCl_3$-anisole, CH_2Cl_2, −40–0 °C, (ii) purification by HP-20 chromatography.



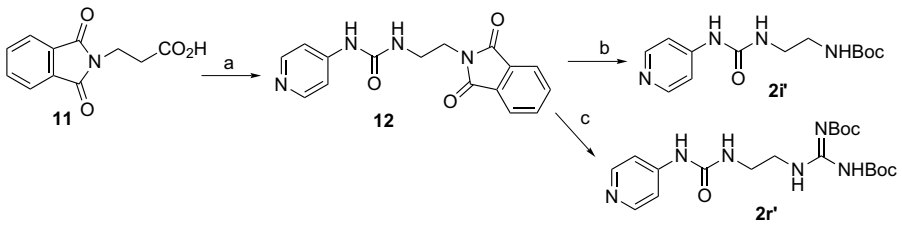
Scheme 2. Reagents and conditions: (a) H_2, Pd(C), EtOH; (b) 2 N NaOH, EtOH; (c) 2-methyl-2-propanol, DPPA, Et_3N, reflux.



Scheme 3. Reagents and conditions: (a) (i) 2-aminoethanol, NaH, (ii) Boc_2O, EtOAc, H_2O; (b) (i) ethylenediamine, K_2CO_3, 130 °C, 3 days, (ii) Boc_2O, H_2O, THF.



Scheme 4. Reagents and conditions: (a) DPPA, Et_3N, MeCN, 75 °C, 2 h; (b) Et_3N, DMF, rt.



Scheme 5. Reagents and conditions: (a) (i) DPPA, Et_3N, DMF, 60 °C, (ii) 4-aminopyridine, rt; (b) (i) hydrazine hydrate, EtOH, 80 °C, (ii) Boc_2O, H_2O, THF; (c) (i) hydrazine hydrate, EtOH, 80 °C, (ii) 1*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine, DMF, DMSO.

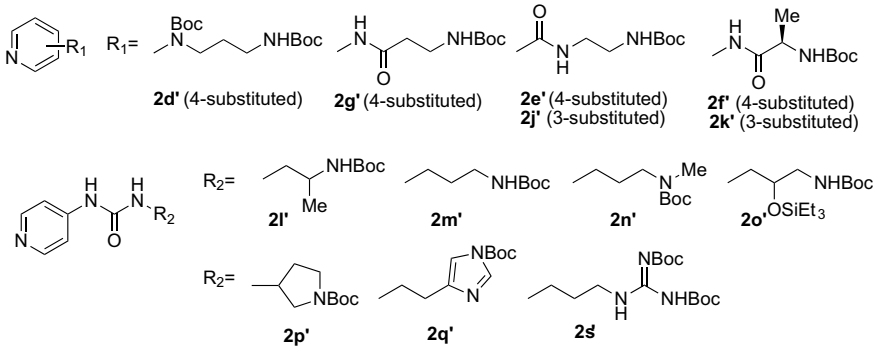


Figure 2. Structures of pyridine derivatives **2d**′–**g**′, **j**′–**q**′, **s**′.

hand, **7** was treated with ethylenediame in the presence of potassium carbonate in DMF at 130 °C for 3 days, and the resulting diamine was treated with Boc_2O to yield **2c**′.

Pyridine derivative **2h**′ was prepared as shown in Scheme 4. Isocyanate **9**[5] was synthesized from carboxylic acid **8** using the DPPA–Et_3N system. The isocyanate **9** was allowed to react with alcohol **10** in the presence of Et_3N to afford **2h**′.

Pyridine derivative **2i**′ and **2r**′ were prepared as shown in Scheme 5. 4-Aminopyridine was allowed to react with the isocyanate generated in situ from carboxylic acid **11** using the DPPA–Et_3N system to yield the pyridine **12**. After deprotection by treatment of **12** with hydrazine hydrate, the resulting amine was treated with Boc_2O or 1*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine to give ureido-pyridine **2i**′ or **2r**′, respectively.

3. Antibacterial activities

Table 1 shows the antibacterial activities (MICs) of C-3′ substituted-pyridinium cephalosporins (**2a**–**k**), and reference compounds **1b**, cefozopran (CZOP),[6] cefoselis (CFSL),[7] S-3578, and vancomycin (VCM). MICs were determined by the standard serial twofold agar dilution method using Mueller–Hinton agar.

All C-3′ substituted-pyridinium cephalosporins **2a**–**k** contain an aminoalkyl group as a basic functionality at the 3- or 4-position of the C-3′ pyridinium ring with a variety of linkers between the aminoalkyl group and the pyridinium ring.

Most of these compounds (**2a**–**k**) showed good antibacterial activity against Gram-positive bacteria including MRSA and Gram-negative bacteria including *P. aeruginosa*, although these anti-MRSA activities were inferior to S-3578.

Among the 4-substituted compounds (**2a**–**i**), amino-linked pyridinium **2d**, amide-linked pyridinium **2g**, carbamate-linked pyridinium **2h**, and ureido-linked pyridinium **2i** showed more potent activity against MRSA than the reference compound **1b**. Comparison of amino-linked pyridinium **2c** and **2d** indicates that elongation of the spacer carbon chain between the C-3′ pyridinium moiety and the terminal amino group enhances anti-MRSA activity, but significantly decreases antibacterial activity against *P. aeruginosa* SR24.

On the other hand, 3-substituted pyridinium **2j** and **2k** were less active against *Staphylococcus aureus* SMITH and Gram-negative bacteria including *P. aeruginosa* than the corresponding 4-subsituted pyridinium **2e** and **2f**, respectively. Both anti-MRSA activities of **2j** and **2k** were as weak as those of **2e** and **2f**.

Among compounds **2a**–**k**, ureido-linked pyridinium **2i** showed the most potent activity against MRSA and *P. aeruginosa*. Therefore, we continued modification of the aminoalkyl moiety of **2i** to try to improve anti-MRSA activity.

Table 2 shows the antibacterial activity of **2i** analogs.

Anti-MRSA activity could be enhanced by elongating the carbon chain of the aminoalkyl moiety (**2i** vs **2l**, **2m**, **2p**), or by replacing the amino group with another basic group, guanidine (**2i** vs **2r**, **2s**). Anti-MRSA activity was reduced by introducing another polar group such as a hydroxy group on the spacer carbon chain (**2m** vs **2o**), introducing a methyl group on the terminal amine (**2m** vs **2n**) or replacing the amino group with imidazole as a basic function (**2i** vs **2q**). The antibacterial activities against *P. aeruginosa*, except for that of **2p**, were reduced compared to that of **2i**, but still strong comparable to that of S-3578.

Among the prepared novel cephalosporins, **2i**, **2l**, **2m**, **2p**, **2r**, and **2s** showed highly potent antibacterial activity against Gram-positive bacteria including MRSA and Gram-negative bacteria including *P. aeruginosa*. In particular, guanidine derivative **2r** showed more potent anti-MRSA activity than S-3578. However, subsequent evaluation of **2i**, **2l**, **2m**, **2p**, **2r**, and **2s** revealed that their water solubility was inferior to that of S-3578 and their mouse acute toxicity (i.v.) was stronger than that of S-3578.

4. Experimental

Mps were determined on a Yanagimoto micro melting point apparatus and uncorrected. IR spectra were taken on JASCO IR-700. ^{1}H NMR spectra were recorded with a Varian Gemini-300 (300 MHz) or Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts are reported in ppm from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS in D_2O) or TMS (in $CDCl_3$ and DMSO-d_6) as internal standard. The following abbreviations are used: s singlet, d doublet, dd double doublet, t triplet, q quartet, m multiplet, ABq AB quartet, br s broad singlet. Column chromatography was carried out on Merck Kieselgel and Mitsubushi Chemical HP-20.

4.1. Measurement of in vitro antibacterial activity

MIC was determined by a serial twofold dilution method with Sensitivity Disk Agar-N (Nissui Pharmaceutical, Tokyo, Japan). The overnight cultures of bacterial strains in Mueller–Hinton 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.

4.2. 3-Pyridin-4-yl-propionic acid ethyl ester (5)

To a solution of (*E*)-3-pyridin-4-yl-acrylic acid ethyl ester (**4**) (177 mg, 1 mmol) in EtOH was added 10% Pd–C (26 mg). The mixture was stirred under hydrogen atmosphere for 30 min under ambient pressure and at room temperature. The catalyst was filtered off and the filtrate was concentrated in vacuo to give **5** (177 mg, 99% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, $J = 8$ Hz), 2.64 (2H, t, $J = 7.8$ Hz), 2.95 (2H, t, $J = 7.8$ Hz), 4.23 (2H, q, $J = 8$ Hz), 7.14 (2H, d, $J = 6.4$ Hz), 8.51 (2H, d, $J = 6.4$ Hz).

4.3. 3-Pyridin-4-yl-propionic acid (6)

To a solution of **5** (134 mg, 0.75 mmol) in EtOH (0.6 mL) was added 2 N NaOH (0.41 mL) and the reaction mixture was stirred at room temperature for 30 min. After addition of 1 N HCl (0.83 mL), the mixture was concentrated and the precipitate was filtered to give **6** as a crystalline solid (71 mg, 63% yield); mp 227–230 °C; ^{1}H NMR (DMSO-d_6) δ 2.58 (2H, t, $J = 6.6$ Hz), 2.83 (2H, t, $J = 6.6$ Hz), 7.25 (2H, d, $J = 5.8$ Hz), 8.45 (2H, d, $J = 5.8$ Hz); Anal Calcd for $C_8H_9NO_2 \cdot 0.1H_2O$: C, 62.82; H, 6.06; N, 9.16. Found: C, 62.9; H, 6.04; N 9.32.

4.4. (2-Pyridin-4-yl-ethyl)-carbamic acid *tert*-butyl ester (2a′)

To a mixture of **6** (453 mg, 3 mmol) and 2-methyl-2-propanol (9 mL) was added diphenylphoshorylazide (0.72 mL, 3.3 mmol) and Et_3N (0.46 mL, 3.3 mmol) and this was refluxed for 4 h. After addition of H_2O and EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on a silica gel column to give **2a′** (370 mg, 55% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 2.81 (2H, t, $J = 6.9$ Hz), 3.41 (2H, m), 4.60 (1H, m), 7.13 (2H, d, $J = 5.7$ Hz), 8.52 (2H, d, $J = 5.7$ Hz).

4.5. [2-(Pyridin-4-yloxy)-ethyl]-carbamic acid *tert*-butyl ester (2b′)

To 2-aminoethanol (2 mL) was added 60% NaH (480 mg) under cooling on ice-water bath. The mixture was stirred at room temperature for 40 min. To this was added 4-chloropyridine (**7**) (600 mg, 4 mmol), and the reaction mixture was stirred for 16 h and stirred at 60 °C for 1 h. After evaporation, the residue was dissolved in a mixture of H_2O (4 mL) and EtOAc (4 mL). To this was added Boc_2O (2.3 mL), and the reaction mixture was stirred at room temperature for 2 h. After addition of EtOAc and H_2O, the organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography to give the title compound **2b′** (712 mg, 75% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 3.55 (2H, m), 4.07 (2H, t, $J = 5.1$ Hz), 5.01 (1H, br s), 6.81 (2H, d, $J = 6$ Hz), 8.44 (2H, d, $J = 6$ Hz).

4.6. (2-*tert*-Butoxycarbonylamino-ethyl)-pyridin-4-yl-carbamic acid *tert*-butyl ester (2c′)

To a mixture of 4-chloropyridine (**7**) hydrochloride (30 g, 0.2 mol) and ethylenediamine (150 mL) was added K_2CO_3 (55 g, 0.4 mol). The reaction mixture was stirred at 130 °C for 3 days and concentrated in vacuo. To the

residue was added, in succession, concd HCl (13 mL), H_2O (100 mL), THF (60 mL), and Boc_2O (230 mL). The mixture was stirred at room temperature for 1 h. After addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2c′** (39.7 g, 59% yield) as a crystalline solid; mp 103–104 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (9H, s), 1.49 (9H, s), 3.45 (2H, m), 3.82 (2H, t, $J = 6.6$ Hz), 4.78 (1H, m), 7.26 (2H, d, $J = 6.3$ Hz), 8.52 (2H, d, $J = 6.3$ Hz).

4.7. (3-*tert*-Butoxycarbonylamino-propyl)-pyridin-4-yl-carbamic acid *tert*-butyl ester (2d′)

Compound **2d′** was obtained as a colorless oil in 41% yield from 4-chloropyridine hydrochloride (3.6 g) using 1,3-diaminopropane (20 mL) and K_2CO_3 (6.6 g) by a similar procedure to that for the synthesis of **2c′**; ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 1.48 (9H, s), 1.74 (2H, m), 3.14 (2H, m), 3.78 (2H, t, $J = 6.9$ Hz), 4.85 (1H, m), 7.20 (2H, m), 8.53 (2H, m).

4.8. {2-[(Pyridine-4-carbonyl)-amino]-ethyl}-carbamic acid *tert*-butyl ester (2e′)

To a mixture of isonicotinic acid (1.23 g, 10 mmol) and CH_2Cl_2 (25 mL) was added water-soluble carbodiimide hydrochloride (WSCD·HCl) (2.3 g, 12 mmol) under cooling in an ice-water bath. The mixture was stirred at room temperature for 1 h. To this was added a solution of (2-amino-ethyl)-carbamic acid *tert*-butyl ester (1.92 g, 12 mmol) in CH_2Cl_2 (5 mL) under cooling on an ice-water bath. The mixture was stirred for 3 days. After addition of EtOAc and H_2O, the organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2e′** (1.1 g, 41%) as a crystalline solid; mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 3.43 (2H, m), 3.55 (2H, m), 5.08 (1H, m), 7.68 (2H, m), 7.75 (1H, m), 8.73 (2H, m).

4.9. [(*R*)-1-(Pyridin-4-ylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (2f′)

Compound **2f′** (1.61 g) was obtained as a crystalline solid in 60% yield from 4-aminopyridine (0.94 g, 10 mmol) using *N*-Boc-D-alanine (2.08 g, 11 mmol) and WSCD·HCl (2.11 g, 11 mmol) by a similar procedure to that for the synthesis of **2e′**; mp 164–165 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, d, $J = 6.9$ Hz), 1.38 (9H, s), 4.11 (1H, m), 7.17 (1H, d, $J = 6.6$ Hz), 7.57 (2H, m), 8.42 (2H, m), 10.3 (1H, s).

4.10. [2-(Pyridin-4-ylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (2g′)

Compound **2g′** (4.66 g) was obtained as a crystalline solid in 60% yield from 4-aminopyridine (1.9 g, 20 mmol) using *N*-Boc-β-alanine (4.16 g, 22 mmol), WSCD·HCl (4.21 g, 22 mmol), and THF as the solvent by a similar procedure to that for the synthesis of **2e′**; mp 121–122 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 2.15 (1H, m), 2.60 (2H, m), 3.44 (2H, m), 5.35 (1H, m), 7.55 (2H, m), 8.43 (2H, m).

4.11. 4-Isocyanato-pyridine (9)

To a mixture of isonicotinic acid (24.6 g, 0.2 mol), Et_3N (29.2 mL, 0.21 mL), and MeCN (170 mL) was added dropwise DPPA (45.2 mL, 0.21 mol) under cooling on an ice-water bath, and the mixture was stirred at the same temperature for 30 min and stirred at 75 °C for 2 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was collected by filtration and rinsed with EtOAc. The amorphous solid was dried under reduced pressure to give **9** (13.4 g, 56% yield), which was used for synthesis without further purification. Using this compound **9** and aniline, 1-phenyl-3-pyridin-4-yl-urea was prepared by the following procedure to confirm the structure of **9**. To a mixture of **9** (120 mg, 1 mmol) and THF (1.5 mL) was added aniline (0.18 mL, 2 mmol), and the mixture was stirred at room temperature for 4 h. After evaporation of the solvents, the residue was chromatographed on silica gel to give 1-phenyl-3-pyridin-4-yl-urea (136 mg, 63% yield) as a crystalline solid; mp 160–161 °C (lit. mp 150 °C,[5b] 166–167 °C;[5c]) ^{1}H NMR ($CDCl_3$) δ 7.07 (1H, t, $J = 7.2$ Hz), 7.26–7.38 (6H, m), 8.05 (1H, br s), 8.35 (2H, d, $J = 5.7$ Hz), 8.69 (1H, br s); Anal Calcd for $C_{12}H_{11}N_3O$: C, 67.59; H, 5.20; N, 19.71. Found: C, 67.31; H, 5.23; N, 19.50; HR-FABMS calcd for $C_{12}H_{12}N_3O$ [$(M+H)^+$]: 214.0980, found: 214.0975.

4.12. Pyridin-4-yl-carbamic acid 2-*tert*-butoxycarbonyl-amino-ethyl ester (2h′)

To a solution of (2-hydroxyethyl)-carbamic acid *tert*-butyl ester (**10**) (1.61 g, 10 mmol) in DMF (10 mL) was added Et_3N (1.4 mL, 10 mmol) and 4-isocyanato-pyridine (**9**) (1.2 g, 10 mmol). The reaction mixture was stirred at room temperature for 16 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography to give the title compound **2h′** (2.0 g, 71% yield) as a pale yellow oil; ^{1}H NMR (DMSO-d_6) δ 1.1.37 (9H, s), 3.22 (2H, m), 4.11 (2H, m), 6.96 (1H, m), 7.43 (2H, d, $J = 5.7$ Hz), 8.36 (2H, d, $J = 5.7$ Hz), 10.1 (1H, s).

4.13. 1-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethyl]-3-pyridin-4-yl-urea (12)

To a mixture of 3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propionic acid (**11**) (245 mg, 1.12 mmol) in MeCN (5 mL) was added Et_3N (0.1 mL, 1.23 mmol) and diphenylphosphorylazide (0.265 mL, 1.23 mmol), and the reaction mixture was stirred at 60 °C for 80 min. To the reaction mixture was added 4-aminopyridine (100 mg, 1.06 mmol) under cooling in an ice-water bath, and the mixture was stirred at room temperature for 16 h. After

addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **12** (225 mg, 68% yield) as a crystalline solid; mp 181–183 °C; ^{1}H NMR (DMSO-d_6) δ 3.37 (2H, m), 3.70 (2H, m), 6.52 (1H, m), 7.27 (2H, d, $J = 6.4$ Hz), 7.82–8.25 (4H, m), 8.24 (2H, d, $J = 6.4$ Hz), 8.96 (1H, s).

4.14. [2-(3-Pyridin-4-yl-ureido)-ethyl]-carbamic acid *tert*-butyl ester (2i′)

To a mixture of **12** (2.32 g, 7.48 mmol) and EtOH (80 mL) was added hydrazine hydrate (3.64 mL, 75 mmol), and the reaction mixture was stirred at 80 °C for 150 min and cooled in an ice-water bath. After filtration of the precipitate, the filtrate was concentrated in vacuo to give 1-(2-amino-ethyl)-3-pyridin-4-yl-urea, which was dissolved in H_2O (4 mL) and THF (2 mL) then Boc_2O (1.84 mL, 8 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 120 min. After addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2i′** (850 mg, 39% from **12**) as a crystalline solid; mp 146–148 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 3.28 (2H, m), 3.36 (2H, m), 5.25 (1H, m), 6.13 (1H, m), 7.35 (2H, d, $J = 6.4$ Hz), 8.30 (2H, d, $J = 6.4$ Hz), 8.34 (1H, m).

4.15. {2-[(Pyridine-3-carbonyl)-amino]-ethyl}-carbamic acid *tert*-butyl ester (2j′)

Compound **2j′** (1.70 g) was obtained as a crystalline solid in 64% yield from nicotinic acid (1.23 g, 10 mmol) using (2-amino-ethyl)-carbamic acid *tert*-butyl ester (2.40 g, 12 mmol) and WSCD·HCl (2.3 g, 12 mmol) in a similar procedure to that for the synthesis of **2e′**; mp 163–165 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 3.45 (2H, m), 3.55 (2H, m), 5.15 (1H, m), 7.38 (1H, m), 7.65 (1H, m), 8.17 (1H, m), 8.72 (1H, m), 9.06 (1H, m).

4.16. [(*R*)-1-(Pyridin-3-ylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (2k′)

Compound **2k′** (2.25 g) was obtained as a crystalline solid in 84% yield from 3-aminopyridine (0.94 g, 10 mmol) using *N*-Boc-D-alanine (2.08 g, 11 mmol) and WSCD·HCl (2.11 g, 11 mmol) by a similar procedure to that for the synthesis of **2e′**; mp 195–197 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, d, $J = 6.9$ Hz), 1.32 (9H, s), 4.12 (1H, m), 7.12 (1H, d, $J = 6.9$ Hz), 7.34 (1H, m), 8.05 (1H, m), 8.26 (1H, m), 8.75 (1H, d, 1H, $J = 2.7$ Hz), 10.1 (1H, s).

4.17. [1-Methyl-2-(3-pyridin-4-yl-ureido)-ethyl]-carbamic acid *tert*-butyl ester (2l′)

Compound **2l′** (0.96 g) was obtained in 61% yield from 4-aminopyridine (1.51 g, 16 mmol) using 3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-butyric acid (4.66 g, 20 mmol) by a similar procedure to that for the synthesis of **2i′** as a crystalline solid; mp 173–174 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (3H, d, $J = 6.3$ Hz), 1.42 (9H, s), 3.1–3.5 (2H, m), 3.72 (1H, m), 4.85 (1H, m), 6.0 (1H, m), 7.41 (2H, m), 8.20 (1H, m), 8.37 (2H, d, $J = 6$ Hz).

4.18. [3-(3-Pyridin-4-yl-ureido)-propyl]-carbamic acid *tert*-butyl ester (2m′)

Compound **2m′** (3.76 g) was obtained in 82% yield from 4-aminopyridine (1.47 g, 15.6 mmol) using 4-*tert*-butoxycarbonylamino-butyric acid (3.95 g, 19.5 mmol) by a similar procedure to that of the synthesis of **12** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 1.63 (2H, m), 3.20 (2H, m), 3.28 (2H, m), 5.88 (1H, m), 6.03 (1H, m), 7.36 (2H, m), 8.34 (2H, d, $J = 5.7$ Hz).

4.19. Methyl-[3-(3-pyridin-4-yl-ureido)-propyl]-carbamic acid *tert*-butyl ester (2n′)

To a solution of (3-amino-propyl)-methyl-carbamic acid *tert*-butyl ester (0.94 g, 5 mmol) in DMF (5 mL) was added 4-isocyanato-pyridine (**9**) (0.60 g, 5 mmol). The reaction mixture was stirred at room temperature for 3 h. After addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2n′** (0.90 g, 59%) as a crystalline solid; mp 93–94 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.69 (2H, m), 2.83 (3H, s), 3.22 (2H, m), 3.31 (2H, m), 6.25 (1H, m), 7.36 (2H, d, $J = 4.6$ Hz), 7.72 (1H, br s), 8.36 (2H, m); Anal Calcd for $C_{15}H_{24}N_4O_3$: C, 58.42; H, 7.84; N, 18.17. Found: C, 58.47; H, 7.78; N, 17.94.

4.20. [3-(3-Pyridin-4-yl-ureido)-2-triethylsilanyloxy-propyl]-carbamic acid *tert*-butyl ester (2o′)

[2-(*tert*-Butyl-dimethyl-silanyloxy)-3-(3-pyridin-4-yl-ureido)-propyl]- carbamic acid *tert*-butyl ester (3.83 g, 90% yield) was obtained as a pale yellow oil from 4-aminopyridine (0.94 g, 10 mmol) using 4-*tert*-butoxycarbonylamino-3-(*tert*-butyl-dimethyl-silanyloxy)-butyric acid (3.34 g, 10 mmol) by a similar procedure to that for the synthesis of **2i′**; ^{1}H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.89 (9H, s), 1.44 (9H, s), 2.8–3.1 (2H, m), 3.2–3.7 (2H, m), 3.82 (1H, m), 5.04 (1H, m), 5.91 (1H, m), 7.34 (2H, d, $J = 6.2$ Hz), 7.80 (1H, m), 8.33 (2H, d, $J = 6.2$ Hz). This compound (13.7 g, 32.4 mmol) was dissolved in MeCN (65 mL). To the solution was added 12 N HCl (5.41 mL) under cooling in an ice-water bath. The mixture was stirred at the same temperature for 20 min. After addition of Et_2O and H_2O, the aqueous layer was alkalized with $NaHCO_3$ (6.81 g). After addition of EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give [2-hydroxy-3-(3-pyridin-4-yl-ureido)-propyl]-carbamic acid *tert*-butyl ester (3.10 g, 31%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$+DMSO-d_6) δ 1.44 (9H, s), 3.18 (2H, m),

3.30 (2H, m), 3.73 (1H, m), 5.65 (1H, m), 6.31 (1H, m), 7.35 (2H, d, $J = 6.3$ Hz), 8.32 (2H, d, $J = 6.3$ Hz), 8.76 (1H, br s). To a solution of this compound (1.73 g, 5.57 mmol) in THF (17 mL) was added Et_3N (0.85 mL, 6.1 mmol) and chlorotriethylsilane (1.03 mL, 6.1 mmol) and the mixture was stirred at room temperature for 16 h. After addition of Et_3N (0.19 mL) and chlorotriethylsilane (0.15 mL), the mixture was stirred for 3 h. After addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2o′** (2.43 g, 99%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.63 (6H, q, $J = 7.2$ Hz), 0.96 (9H, t, $J = 7.2$ Hz), 1.45 (9H, s), 2.7–3.0 (2H, m), 3.4–3.7 (2H, m), 3.83 (1H, m), 5.06 (1H, m), 5.89 (1H, m), 7.34 (2H, d, $J = 6$ Hz), 7. 60 (1H, br s), 8.37 (2H, d, $J = 6$ Hz).

4.21. 3-(3-Pyridin-4-yl-ureido)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (2p′)

Compound **2p′** (0.85 g) was obtained in 57% yield from 4-isocyanato-pyridine (**9**) (0.64 g, 5.4 mmol) using 3-amino-pyrrolidine-1-carboxylic acid *tert*-butyl ester (0.91 g, 4.9 mmol) in a similar procedure to that for the synthesis of **2n′** as a crystalline solid; mp 182–184 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 2.08 (2H, m), 3.24–3.56 (4H, m), 4.43 (1H, m), 6.22 (1H, m), 7.35 (2H, d, $J = 4.8$ Hz), 8.28 (1H, m), 8.36 (2H, m).

4.22. 4-[2-(3-Pyridin-4-yl-ureido)-ethyl]-imidazole-1-carboxylic acid *tert*-butyl ester (2q′)

Compound **2q′** (0.84 g) was obtained in 36% yield from 4-aminopyridine (0.65 g, 6.9 mmol) using 4-(2-carboxyethyl)-imidazole-1-carboxylic acid *tert*-butyl ester (1.68 g, 7 mmol) in a similar procedure to that of the synthesis of **12** as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.57 (9H, s), 2.66 (2H, m), 3.46 (2H, m), 6.25 (1H, m), 7.0 (1H, m), 7.10 (1H, s), 7.27 (2H, m), 7.98 (1H, d, $J = 1.2$ Hz), 8.20 (2H, d, $J = 6.3$ Hz).

4.23. 1-[2-*N*,*N*′-Bis(*tert*-butoxycarbonyl)guanidino-ethyl]-3-pyridin-4-yl-urea (2r′)

1-(2-Amino-ethyl)-3-pyridin-4-yl-urea, which was obtained by the treatment of **12** (0.20 g, 0.64 mmol) with hydrazine hydrate, was dissolved in DMF (2 mL) and DMSO (2 mL), then 1*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine (0.414 g, 1.4 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 16 h. After addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2r′** (0.212 g, 78% from **12**) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 1.52 (9H, s), 3.47 (2H, m), 3.54 (2H, m), 7.03 (1H, m), 7.31 (2H, d, $J = 6.2$ Hz), 8.39 (2H, d, $J = 6.2$ Hz), 8.71 (1H, m).

4.24. 1-[3-*N*,*N*′-Bis(*tert*-butoxycarbonyl)guanidino-propyl]-3-pyridin-4-yl-urea (2s′)

[3-(3-Pyridin-4-yl-ureido)-propyl]-carbamic acid *tert*-butyl ester (**2m′**) (1.47 g, 5 mmol) was dissolved in TFA (15 mL), and the solution was stirred at room temperature for 30 min. After evaporation, the residue was dissolved in THF (10 mL). After successive addition of Et_3N (3.5 mL) and 1*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine (1.48 g, 4.99 mmol), the solution was stirred for 5 h. After addition of EtOAc and H_2O, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the title compound **2s′** (1.83 g, 84%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.51 (9H, s), 1.54 (9H, s), 1.72 (2H, m), 3.27 (2H, q, $J = 5.4$ Hz), 3.49 (2H, q, $J = 6.6$ Hz), 6.88 (1H, br s), 7.35 (2H, d, $J = 6.6$ Hz), 7.51 (1H, m), 8.38 (2H, d, $J = 6.6$ Hz), 8.54 (1H, m).

4.25. 7-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-{4-[3-(2-aminoethyl)-ureido]-pyridinium-1-yl}methyl-3-cephem-4-carboxylate hydrochloride (2i)

To a solution of iodomethyl **3** (1.48 g, 1.95 mmol) in MeCN (2 mL) was added pyridine derivative **2i′** (420 mg, 1.5 mmol) under cooling in ice-water bath and the reaction mixture was stirred at room temperature for 90 min. After addition of MeCN (2 mL), the mixture was poured into *i*-Pr_2O (200 mL). The precipitate was filtered and dried in vacuo to quantitatively give pyridinium salt **3′**. To a solution of **3′** in CH_2Cl_2 (20 mL) was added a solution of $AlCl_3$ (1.2 g, 9 mmol) in anisole (10 mL) at −30 °C and the reaction mixture was stirred under cooling in an ice-water bath for 60 min. After addition of H_2O (30 mL) and MeOH (10 mL), aqueous layer was washed with Et_2O. It was then concentrated and chromatographed on HP-20 resin. The target product was eluted with 4% MeCN/0.001 N HCl. The fraction containing the target product was concentrated and lyophilized to give **2i** (185 mg, 18% yield from **2i′**) as an amorphous powder; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.23(3H, t, $J = 6.9$ Hz), 2.96(2H, t, $J = 6.0$ Hz), 3.22 and 3.59 (2H, ABq, $J = 17.7$ Hz), 3.41 (2H, t, $J = 6.0$ Hz), 4.16 (2H, q, $J = 6.9$ Hz), 4.94 and 5.27 (2H, ABq, $J = 15.3$ Hz), 5.10 (1H, d, $J = 4.8$ Hz), 5.76 (1H, d, $J = 4.8$ Hz), 7.83 (2H, d, $J = 7.8$ Hz), 8.80 (2H, d, $J = 7.8$ Hz); IR (KBr) cm^{-1} 1772, 1714, 1608, 1518, 1465, 1390; Anal Calcd for $C_{22}H_{26}N_{10}O_6S_2 \cdot 1HCl \cdot 3.4H_2O$: C, 38.39; H, 4.95; Cl, 5.15; N, 20.35; S, 9.32. Found: C, 38.32; H, 4.89; Cl, 5.24; N, 20.45; S, 9.29.

The other cephalosporins **2a**–**h**, **j**–**s**, and reference compound **1b** were prepared by a procedure similar to that described for the preparation of **2i**.

4.26. Compound 1b

1b was obtained as an amorphous powder in 31% yield from pyridine; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.20 (3H,

t, $J = 7.2$ Hz), 3.05 and 3.49 (2H, ABq, $J = 18.0$ Hz), 4.11 (2H, q, $J = 7.2$ Hz), 5.04 (1H, d, $J = 4.2$ Hz), 5.13 and 5.61 (2H, ABq, $J = 13.8$ Hz), 5.69 (1H, d, $J = 4.2$ Hz), 8.05–8.2 (2H, m), 8.45–8.6 (1H, m), 9.25–9.4 (2H, m); IR (KBr) cm^{-1} 1775, 1666, 1617, 1526, 1485, 1455, 1390; Anal Calcd for $C_{19}H_{19}N_7O_5S_2{\cdot}2.6H_2O$: C, 42.55; H, 4.55; N, 18.28; S, 11.96. Found: C, 42.3; H, 4.58; N, 18.55; S, 11.94.

4.27. Compound 2a

2a was obtained as an amorphous powder in 19% yield from **2a′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.21 (3H, t, $J = 7.5$ Hz), 3.2–3.25 (4H, m), 3.05 and 3.55 (2H, ABq, $J = 18$ Hz), 4.12 (2H, q, $J = 7.5$ Hz), 5.1 (1H, d, $J = 4.8$ Hz), 5.12 and 5.62 (2H, ABq, $J = 13.5$ Hz), 5.73 (1H, d, $J = 4.8$ Hz), 8.10 (2H, d, $J = 6.6$ Hz), 9.31 (2H, d, $J = 6.6$ Hz); IR (KBr) cm^{-1} 1773, 1616, 1521, 1466, 1395; Anal Calcd for $C_{21}H_{24}N_8O_5S_2{\cdot}1.1HCl{\cdot}4H_2O$: C, 39.13; H, 5.16; Cl, 6.05; N, 17.38; S, 9.95. Found: C, 39.18; H, 5.17; Cl, 6.13; N, 17.26; S, 9.95.

4.28. Compound 2b

2b was obtained as an amorphous powder in 12% yield from **2b′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.21 (3H, t, $J = 6.9$ Hz), 3.05 and 3.52 (2H, ABq, $J = 18$ Hz), 3.32 (2H, m), 4.16 (2H, q, $J = 6.9$ Hz), 4.57 (2H, m), 5.0 and 5.45 (2H, ABq, $J = 14.1$ Hz), 5.08 (1H, d, $J = 5.1$ Hz), 5.73 (1H, d, $J = 5.1$ Hz), 7.66 (2H, d, $J = 7.5$ Hz), 9.15 (2H, d, $J = 7.5$ Hz); IR (KBr) cm^{-1} 1773, 1642, 1519, 1484, 1456, 1398; Anal Calcd for $C_{21}H_{24}N_8O_6S_2{\cdot}1.1HCl{\cdot}5.2H_2O$: C, 36.96; H, 5.24; Cl, 5.71; N, 16.42; S, 9.4. Found: C, 36.69; H, 5.18; Cl, 5.63; N, 16.40; S, 9.36.

4.29. Compound 2c

2c was obtained as an amorphous powder in 25% yield from **2c′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.21 (3H, t, $J = 6.9$ Hz), 2.99 and 3.50 (2H, ABq, $J = 18$ Hz), 3.04 (2H, m), 3.61 (2H, m), 4.15 (2H, q, $J = 6.9$ Hz), 4.70 and 5.22 (2H, ABq, $J = 13.2$ Hz), 5.07 (1H, d, $J = 5.1$ Hz), 5.73 (1H, d, $J = 5.1$ Hz), 6.91 (1H, m), 7.11 (1H, m), 8.43 (1H, m), 8.65 (1H, m); IR (KBr) cm^{-1} 1767, 1649, 1556, 1520, 1455, 1389; Anal Calcd for $C_{21}H_{25}N_9O_5S_2{\cdot}1HCl{\cdot}5H_2O$: C, 37.41; H, 5.38; Cl, 5.26; N, 18.7; S, 9.51. Found: C, 37.61; H, 5.14; Cl, 5.39; N, 18.98; S, 9.37.

4.30. Compound 2d

2d was obtained as an amorphous powder in 22% yield from **2d′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.22 (3H, t, $J = 7.2$ Hz), 1.86 (2H, m), 2.89 (2H, t, $J = 7.5$ Hz), 2.96 and 3.47 (2H, ABq, $J = 18$ Hz), 3.36 (2H, t, $J = 7.5$ Hz), 4.15 (2H, q, $J = 7.2$ Hz), 4.67 and 5.16 (2H, ABq, $J = 14.1$ Hz), 5.04 (1H, d, $J = 5.1$ Hz), 5.69 (1H, d, $J = 5.1$ Hz), 6.88 (1H, m), 6.96 (1H, m), 8.42 (1H, m), 8.61 (1H,m); IR (KBr) cm^{-1} 1773, 1649, 1556, 1523, 1460, 1385; Anal Calcd for $C_{22}H_{27}N_9O_5S_2{\cdot}1.1HCl{\cdot}4H_2O$: C, 39.22; H, 5.4; Cl, 5.79; N, 18.71; S, 9.52. Found: C, 39.16; H, 5.34; Cl, 5.90; N, 18.85; S, 9.43.

4.31. Compound 2e

2e was obtained as an amorphous powder in 48% yield from **2e′**; ^1H NMR (D_2O) δ 1.34 (3H, t, $J = 7.2$ Hz), 3.3 and 3.75 (2H, ABq, $J = 18$ Hz), 3.34 (2H, t, $J = 6.3$ Hz), 3.82 (2H, t, $J = 6.3$ Hz), 4.39 (2H, q, $J = 7.2$ Hz), 5.34 (1H, d, $J = 5.1$ Hz), 5.48 and 5.73 (2H, ABq, $J = 15$ Hz), 5.93 (1H, d, $J = 5.1$ Hz), 8.43 (2H, d, $J = 6.6$ Hz), 9.19 (2H, d, $J = 6.6$ Hz); IR (KBr) cm^{-1} 1775, 1668, 1616, 1527, 1456, 1393; Anal Calcd for $C_{22}H_{25}N_9O_6S_2{\cdot}1.3HCl{\cdot}4.5H_2O$: C, 37.53; H, 5.05; Cl, 6.55; N, 17.90; S, 9.11. Found: C, 37.48; H, 4.98; Cl, 6.63; N, 18.17; S, 8.95.

4.32. Compound 2f

2f was obtained as an amorphous powder in 16% yield from **2f′**; ^1H NMR (D_2O) δ 1.34 (3H, t, $J = 6.9$ Hz), 1.70 (3H, d, $J = 7.5$ Hz), 3.30 and 3.70 (1H, ABq, $J = 17.7$ Hz), 4.36 (2H, q, $J = 6.9$ Hz), 4.4 (1H, m), 5.26 and 5.50 (2H, ABq, $J = 14.4$ Hz), 5.33 (1H, d, $J = 4.8$ Hz), 5.93 (1H, d, $J = 4.8$ Hz), 8.18 (2H, d, $J = 7.2$ Hz), 8.79 (2H, d, $J = 7.2$ Hz); IR (KBr) cm^{-1} 1777, 1727, 1669, 1608, 1517, 1465, 1397; Anal Calcd for $C_{22}H_{25}N_9O_6S_2{\cdot}1.6HCl{\cdot}4.2H_2O$: C, 37.24; H, 4.95; Cl, 7.99; N, 17.76; S, 9.04. Found: C, 37.03; H, 5.02; Cl, 7.91; N, 17.85; S, 8.92.

4.33. Compound 2g

2g was obtained as an amorphous powder in 53% yield from **2g′**; ^1H NMR (D_2O) δ 1.34 (3H, t, $J = 6.9$ Hz), 3.06 (2H, t, $J = 6.6$ Hz), 3.26 and 3.70 (2H, ABq, $J = 18$ Hz), 3.42 (2H, t, $J = 6.6$ Hz), 4.37 (2H, q, $J = 6.9$ Hz), 5.24 and 5.43 (2H, ABq, $J = 14.7$ Hz), 5.31 (1H, d, $J = 4.5$ Hz), 5.91 (1H, d, $J = 4.5$ Hz), 8.12 (2H, d, $J = 7.5$ Hz), 8.74 (2H, d, $J = 7.5$ Hz); IR (KBr) cm^{-1} 1774, 1718, 1604, 1517, 1461, 1396; Anal Calcd for $C_{22}H_{25}N_9O_6S_2{\cdot}1.2HCl{\cdot}4H_2O$: C, 38.22; H, 4.99; Cl, 6.15; N, 18.23; S, 9.27. Found: C, 38.55; H, 4.91; Cl, 6.27; N, 18.47; S, 9.35.

4.34. Compound 2h

2h was obtained as an amorphous powder in 14% yield from **2h′**; ^1H NMR (DMS0-d_6) δ 1.22 (3H, t, $J = 7.2$ Hz), 3.14 (2H, m), 3.37 and 3.52 (2H, ABq, $J = 17.7$ Hz), 4.18 (2H, q, $J = 7.2$ Hz), 4.43 (2H, m), 5.19 (1H, d, $J = 5.1$ Hz), 5.30 and 5.41 (2H, ABq, $J = 14$ Hz), 5.9 (1H, m), 8.03 (2H, d, $J = 6.9$ Hz), 8.16 (1H, m), 8.36 (2H, br s), 8.76 (2H, d, $J = 6.9$ Hz), 9.56 (1H, d, $J = 8.1$ Hz); IR (KBr) cm^{-1} 1783, 1752, 1676, 1637, 1593, 1532, 1464, 1383; HR-FABMS calcd for $C_{22}H_{26}N_9O_7S_2$ [$(M+H)^+$]: 592.1397, found: 592.1395.

4.35. Compound 2j

2j was obtained as an amorphous powder in 38% yield from **2j′**; ^{1}H NMR (D_2O) δ 1.34 (3H, t, $J = 6.6$ Hz), 3.29 and 3.78 (2H, ABq, $J = 18$ Hz), 3.34 (2H, t, $J = 5.7$ Hz), 3.81 (2H, t, $J = 5.7$ Hz), 4.38 (2H, q, $J = 6.6$ Hz), 5.35 (1H, d, $J = 4.8$ Hz), 5.48 and 5.74 (2H, ABq, $J = 14.7$ Hz), 5.92 (1H, d, $J = 4.8$ Hz), 8.28 (1H, m), 8.96 (1H, d, $J = 8.4$ Hz), 9.2 (1H, d, $J = 6.3$ Hz), 9.47 (1H, s); IR (KBr) cm^{-1} 1774, 1670, 1623, 1527, 1457, 1395; Anal Calcd for $C_{22}H_{25}N_9O_6S_2 \cdot 1.2HCl \cdot 4H_2O$: C, 38.22; H, 4.99; Cl, 6.15; N, 18.23; S, 9.27. Found: C, 37.93; H, 5.00; Cl, 6.40; N, 18.23; S, 9.14.

4.36. Compound 2k

2k was obtained as an amorphous powder in 37% yield from **2k′**; ^{1}H NMR (D_2O) δ 1.34 (3H, t, $J = 6.9$ Hz), 1.70 (3H, d, $J = 7.2$ Hz), 3.29 and 3.72 (2H, ABq, $J = 18$ Hz), 4.37 (2H, q, $J = 6.9$ Hz), 4.38 (1H, m), 5.34 (1H, d, $J = 5.1$ Hz), 5.40 and 5.66 (2H, ABq, $J = 14.4$ Hz), 5.92 (1H, d, $J = 5.1$ Hz), 8.11 (1H, m), 8.53 (1H, m), 8.78 (1H, d, $J = 6.3$ Hz), 9.58 (1H,s); IR (KBr) cm^{-1} 1775, 1710, 1669, 1615, 1564, 1505, 1459, 1395; Anal Calcd for $C_{22}H_{25}N_9O_6S_2 \cdot 1.3HCl \cdot 4.5H_2O$: C, 37.53; H, 5.05; Cl, 6.55; N, 17.90; S, 9.11. Found: C, 37.52; H, 5.03; Cl, 6.54; N, 18.06; S, 9.10.

4.37. Compound 2l

2l was obtained as an amorphous powder in 26% yield from **2l′**; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.19 (3H, d, $J = 1.8$ Hz), 1.22 (3H, t, $J = 6.9$ Hz), 3.21 and 3.59 (2H, ABq, $J = 17.4$ Hz), 3.31–3.35 (3H, m), 4.16 (2H, q, $J = 6.9$ Hz), 4.97 and 5.28 (2H, ABq, $J = 12.9$ Hz), 5.11 (1H, d, $J = 5.4$ Hz), 5.77 (1H, d, $J = 5.4$ Hz), 7.84 (2H, m), 8.78 (2H, m); IR (KBr) cm^{-1} 1773, 1716, 1607, 1519, 1465, 1396; Anal Calcd for $C_{23}H_{28}N_{10}O_6S_2 \cdot 1.15HCl \cdot 4.7H_2O$: C, 37.78; H, 5.31; Cl, 5.58; N, 19.15; S, 8.77. Found: C, 37.73; H, 5.21; Cl, 5.48; N, 19.37; S, 8.80.

4.38. Compound 2m

2m was obtained as an amorphous powder in 22% yield from **2m′**; ^{1}H NMR (DMS-d_6+D_2O) δ 1.23 (3H, t, $J = 7$ Hz), 1.78 (2H, m), 2.85 (2H, t, $J = 6.6$ Hz), 3.22 (2H, t, $J = 6.6$ Hz), 3.16 and 3.55 (2H, ABq, $J = 17.6$ Hz), 4.16 (2H, q, $J = 7$ Hz), 5.04 and 5.29 (2H, ABq, $J = 16.2$ Hz), 5.13 (1H, d, $J = 4.6$ Hz), 5.80 (1H, d, $J = 4.6$ Hz), 7.88 (2H, m), 8.68 (2H, m); IR (KBr) cm^{-1} 1777, 1710, 1671, 1644, 1606, 1520, 1466, 1398; Anal Calcd for $C_{23}H_{28}N_{10}O_6S_2 \cdot 1.35HCl \cdot 4.7H_2O$: C, 37.4; H, 5.29; Cl, 6.48; N, 18.96; S, 8.68. Found: C, 37.12; H, 5.01; Cl, 6.60; N, 18.96; S, 8.59.

4.39. Compound 2n

2n was obtained as an amorphous powder in 33% yield from **2n′**; ^{1}H NMR (DMS-d_6+D_2O) δ 1.23 (3H, t, $J = 7.2$ Hz), 1.80 (2H, m), 2.57 (3H, s), 2.94 (2H, m), 3.20 and 3.55 (2H, ABq, $J = 18$ Hz), 3.23 (2H, m), 4.17 (2H, q, $J = 7.2$ Hz), 4.98 and 5.24 (2H, ABq, $J = 13.2$ Hz), 5.11 (1H, d, $J = 4.8$ Hz), 5.78 (1H, d, $J = 4.8$ Hz), 7.82 (2H, d, $J = 7.2$ Hz), 8.73 (2H, d, $J = 7.2$ Hz); IR (KBr) cm^{-1} 1777, 1713, 1671, 1606, 1518, 1466, 1395; Anal Calcd for $C_{24}H_{30}N_{10}O_6S_2 \cdot 1.35HCl \cdot 4H_2O$: C, 38.96; H, 5.36; Cl, 6.47; N, 18.93; S, 8.67. Found: C, 38.89; H, 5.36; Cl, 6.41; N, 19.1; S, 8.81.

4.40. Compound 2o

2o was obtained as an amorphous powder in 44% yield from **2o′**; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.23 (3H, t, $J = 7.2$ Hz), 2.69–2.97 (2H, m), 3.15 and 3.56 (2H, ABq, $J = 17.7$ Hz), 3.24 (2H, m), 3.6 (1H, m), 4.17 (2H, q, $J = 7.2$ Hz), 4.97 and 5.29 (2H, ABq, $J = 14.4$ Hz), 5.10 (1H, d, $J = 4.8$ Hz), 5.76 (1H, d, $J = 4.8$ Hz), 7.83 (2H, m), 8.74 (2H, m); IR (KBr) cm^{-1} 1773, 1713, 1606, 1517, 1465, 1396; Anal Calcd for $C_{23}H_{28}N_{10}O_7S_2 \cdot 1.2HCl \cdot 4.2H_2O$: C, 37.33; H, 5.12; Cl, 5.75; N, 18.93; S, 8.66. Found: C, 37.21; H, 5.07; Cl, 5.61; N, 19.03; S, 8.78.

4.41. Compound 2p

2p was obtained as an amorphous powder in 31% yield from **2p′**; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.23 (3H, t, $J = 7.0$ Hz), 1.8–2.3 (2H, m), 3.09–3.65 (6H, m), 4.16 (2H, q, $J = 7.0$ Hz), 4.34 (1H, m), 5.06 and 5.27 (2H, ABq, $J = 14.4$ Hz), 5.15 (1H, d, $J = 5.0$ Hz), 5.82 (1H, d, $J = 5.0$ Hz), 7.86 (2H, d, $J = 7.0$ Hz), 8.72 (2H, d, $J = 7.0$ Hz); IR (KBr) cm^{-1} 1778, 1715, 1671, 1608, 1520, 1465, 1396; Anal Calcd for $C_{24}H_{28}N_{10}O_6S_2 \cdot 1.5HCl \cdot 4H_2O$: C, 38.78; H, 5.08; Cl, 7.15; N, 18.84; S, 8.63. Found: C, 38.68; H, 5.01; Cl, 7.28; N, 18.98; S, 8.40.

4.42. Compound 2q

2q was obtained as an amorphous powder in 41% yield from **2q′**; ^{1}H NMR (D_2O) δ 1.32 (3H, t, $J = 7.2$ Hz), 3.02 (2H, t, $J = 6.3$ Hz), 3.25 and 3.66 (2H, ABq, $J = 18$ Hz), 3.58 (2H, t, $J = 6.3$ Hz), 4.35 (2H, q, $J = 7.2$ Hz), 5.14 and 5.35 (2H, ABq, $J = 14.7$ Hz), 5.30 (1H, d, $J = 5.1$ Hz), 5.89 (1H, d, $J = 5.1$ Hz), 7.33 (1H, d, $J = 1.2$ Hz), 7.82 (2H, d, $J = 7.2$ Hz), 8.54 (2H, d, $J = 7.2$ Hz), 8.63 (1H, d, $J = 1.5$ Hz); IR (KBr) cm^{-1} 1775, 1715, 1671, 1606, 1519, 1466, 1398; Anal Calcd for $C_{25}H_{27}N_{11}O_6S_2 \cdot 1.3HCl \cdot 4H_2O$: C, 39.45; H, 4.81; Cl, 6.06; N, 20.24; S, 8.43. Found: C, 39.20; H, 4.84; Cl, 5.82; N, 20.44; S, 8.43.

4.43. Compound 2r

2r was obtained as an amorphous powder in 30% yield from **2r′**; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.22 (3H, t, $J = 7.4$ Hz), 3.20–3.40 (4H, m), 3.20 and 3.55 (2H, ABq,

$J = 18$ Hz), 4.16 (2H, q, $J = 7.4$ Hz), 4.92 and 5.30 (2H, ABq, $J = 12.9$ Hz), 5.12 (1H, d, $J = 4.8$ Hz), 5.77 (1H, d, $J = 4.8$ Hz), 7.93 (2H, m), 8.58 (2H, m); IR (KBr) cm^{-1} 1780, 1716, 1645, 1520, 1464, 1386; Anal Calcd for $C_{23}H_{28}N_{12}O_7S_2 \cdot 2HCl \cdot 4H_2O$: C, 34.81; H, 4.83; Cl, 8.93; N, 21.18; S, 8.08. Found: C, 34.59; H, 4.63; Cl, 9.10; N, 21.07; S, 8.17.

4.44. Compound 2s

2s was obtained as an amorphous powder in 19% yield from **2s′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.23 (3H, t, $J = 7.2$ Hz), 1.70 (2H, m), 3.0–3.3 (4H, m), 3.20 and 3.45 (2H, ABq, $J = 18$ Hz), 4.15 (2H, q, $J = 7.2$ Hz), 4.97 and 5.27 (2H, ABq, $J = 13.5$ Hz), 5.14 (1H, d, $J = 5.1$ Hz), 5.83 (1H, d, $J = 5.1$ Hz), 7.82 (2H, m), 8.69 (2H, m); IR (KBr) cm^{-1} 1774, 1713, 1646, 1521, 1466, 1387; Anal Calcd for $C_{24}H_{30}N_{12}O_6S_2 \cdot 1.3HCl \cdot 3.5H_2O$: C, 38.07; H, 5.1; Cl, 6.09; N, 22.2; S, 8.47. Found: C, 37.90; H, 5.07; Cl, 6.18; N, 22.35; S, 8.36.

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