STUDIES ON MONOCYCLIC β-LACTAM ANTIBIOTICS

III†. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF *N*-(AROMATIC HETEROCYCLIC SUBSTITUTED)AZETIDIN-2-ONES

CHOSAKU YOSHIDA*, KIYOSHI TANAKA, JOJI NAKANO, YOZO TODO, TETSUO YAMAFUJI, RIKIZO HATTORI, YOSHIKAZU FUKUOKA and ISAMU SAIKAWA

Research Laboratory, Toyama Chemical Co., Ltd.
2-4-1 Shimookui, Toyama 930, Japan

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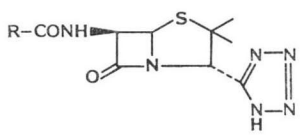
The relationship between structure and antibacterial activity among monocyclic β-lactams having a pyridyl, pyrimidinyl, thiazolyl, imidazolyl, or a tetrazolyl group at N-1 position was investigated. *N*-(Tetrazol-5-yl)azetidin-2-ones were found to posses excellent activity.

The introduction of electron withdrawing groups at N-1 position of monocyclic β-lactam antibiotics has been extensively reported[1~8]. In our previous paper[9], we reported on the antibacterial activity and the β-lactamase inhibitory activity of 2-azetidinone-1-oxysulfonic acids. They had an insufficient stability against penicillinase. On the other hand, tetrazolyl penam compounds[10] (Fig. 1) obtained by replacing the C-3 carboxyl group of 6-aminopenicillanic acid with a tetrazolyl group, have been found to have excellent stability against β-lactamases. Thus, we attempted to synthesize monocyclic β-lactams with aromatic heterocyclic groups including a tetrazolyl group at N-1, and investigated the structure-activity relationships regarding their antibacterial and β-lactamase inhibitory activities.

Fig. 1.



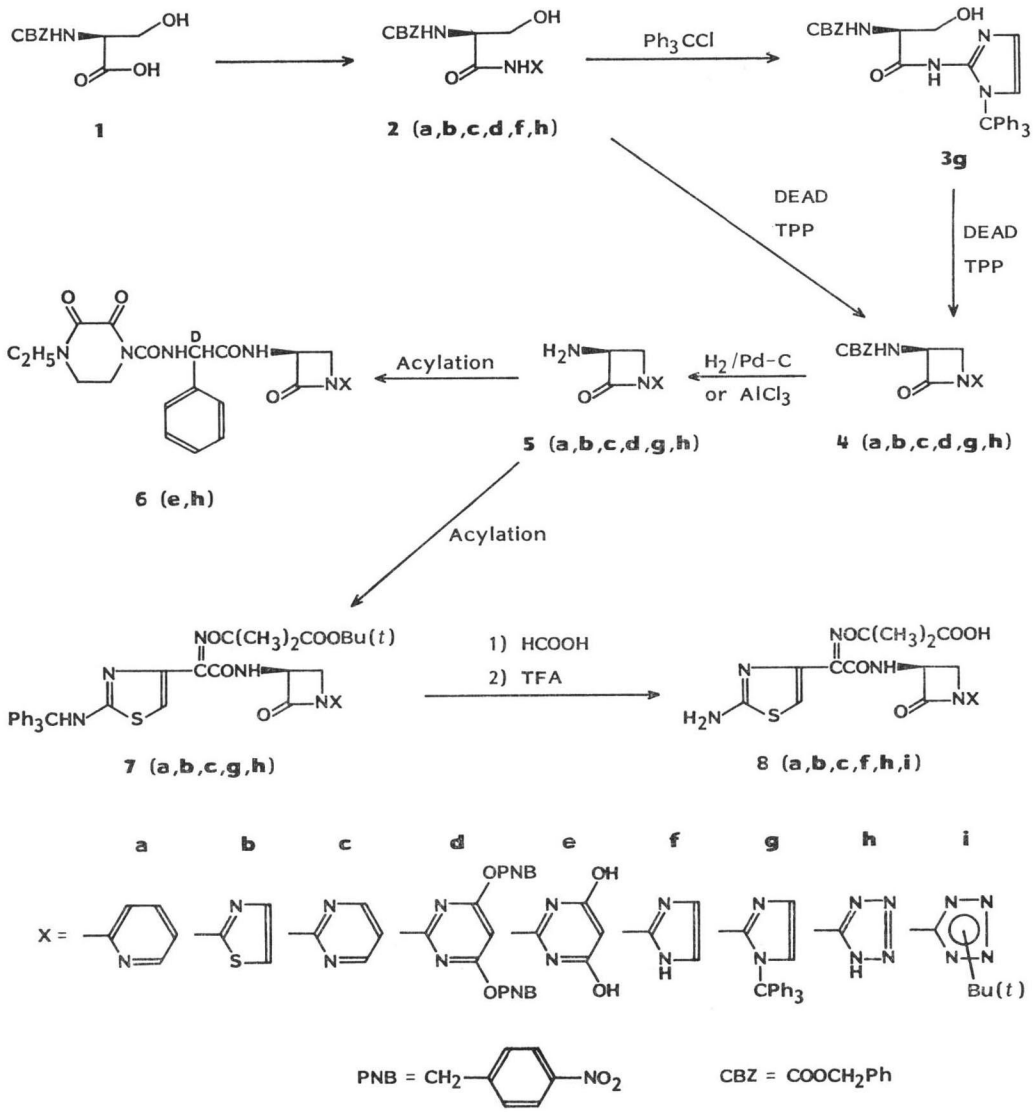
We were also interested in the effect of functional groups on the tetrazole ring and synthesized compounds **23** and **24** having a carboxymethyl group.

Chemistry

The synthesis of 3-amino-2-azetidinones (**5**), key intermediates for preparation of various compounds are shown in Scheme 1. The synthesis was carried out by using *N*-CBZ-L-serine (**1**) as a starting material. Compounds **2a**, **2c**, **2d** and **2f** were not able to be obtained by the mixed anhydride method using compound **1** and ethyl chloroformate. Instead compound **1** was silylated with trimethylchlorosilane (TMS-Cl) - Et_3N and reacted with trichloroethyl chloroformate (TCF) to obtain the chloride, which was reacted with *N*-trimethylsilyl heteroaromatic amines (TMS-HN-X (**a**, **c**, **d**, **f**)) to afford **2** in 34~46% yield. MILLER *et al.*[11] modification of the MITSUNOBU reaction[12] was very applica-

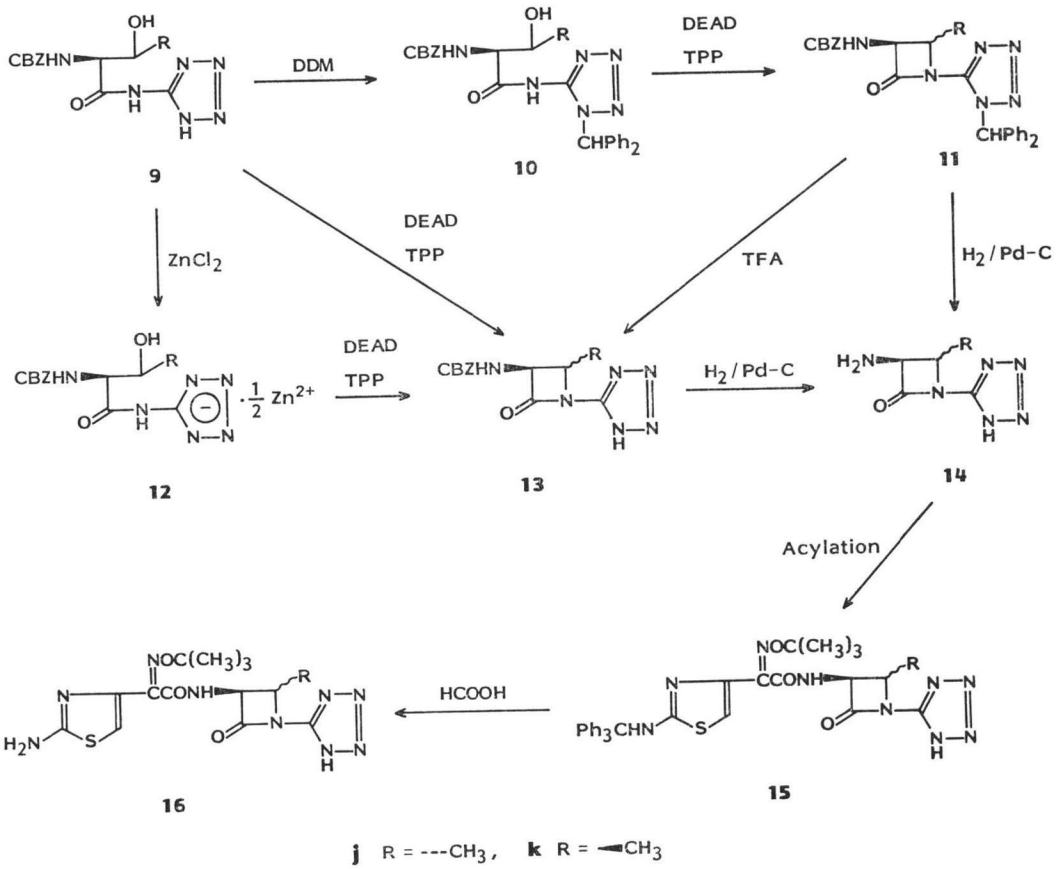
† Paper II. YOSHIDA, C.; T. HORI, K. MOMONOI, K. NAGUMO, J. NAKANO, T. KITANI, Y. FUKUOKA & I. SAIKAWA: Studies on monocyclic β-lactam antibiotics. II. Synthesis and antibacterial activity of 3-acylamino-2-azetidinone-1-oxysulfonic acids. J. Antibiotics 38: 1536~1549, 1985

Scheme 1.



ble to the synthesis of the *N*-(aromatic heterocyclic substituted)azetidin-2-ones. However, this method could not be applied to aromatic compounds **2f** and **2h** having an active ring proton (NH group). Especially, β-lactam was not obtained at all from **2f** using diethyl azodicarboxylate (DEAD) and triphenyl phosphine (TPP). Tritylation of **2f** gave **3g** which could be cyclized to β-lactam (**4g**). 3-Amino-β-lactams (**5**) were easily obtained from the β-lactams (**4**) by removing the CBZ group using hydrogenolysis or aluminum trichloride[13]. Subsequently, 3-acylamino-β-lactams (**6e**, **6h**) were synthesized by established acylation methods. Despite of the successful condensation of **5d** and the α-oxyiminoacetic acid, compound **7e** could not be isolated because of its instability. When compound **7h** was reacted with TFA - CH_2Cl_2 (3: 1) to remove the *tert*-butyl group, not only the expected compound **8h** but also **8i** having a *tert*-butyl group in the tetrazole ring were formed. Isobutene formed in the cleavage of the *tert*-butyl ester is considered to react with the tetrazole ring in presence of the

Scheme 2.

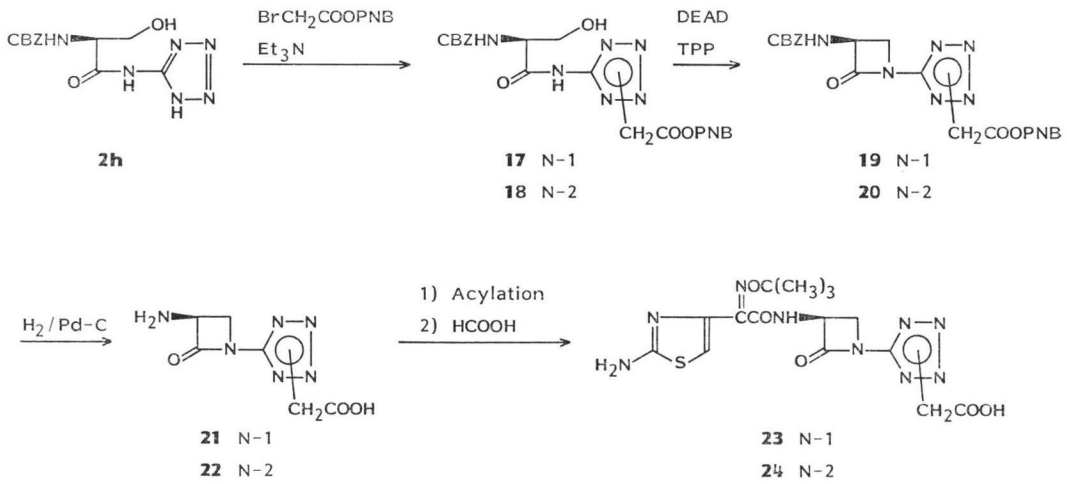


catalyst of TFA to form **8i**. However, the position of the *tert*-butyl group was not identified.

Scheme 2 describe the synthetic route to 3-amino-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinones (**14**). Cyclization of compounds **9j** and **9k** obtained from *N*-CBZ-L-threonine and *N*-CBZ-L-allothreonine, respectively, in a similar manner as in Scheme 1, gave only 20% of **13**. Protection of the nitrogen atom of the tetrazole ring increased the yield of the cyclization. Diphenyl diazomethane (DDM) was found to react selectively and quantitatively at the N-1 position of the tetrazole ring to afford **10**, whereas the trityl group as reported by CHRISTENSEN *et al.*[5] was selectively introduced at the N-2 position. Other alkyl halides were found not to be selective regents in terms of substitution position. The protected compounds (**10**) were cyclized to afford β-lactams (**11**) in a yield of 70%. Alternatively, Zn salts (**12**) formed by reacting **9j** and **9k** with zinc chloride were directly cyclized to **13** in high yields of (63~70%). Removal of CBZ and benzhydryl groups by hydrogenolysis and TFA, respectively, gave **14**, which was acylated in a similar manner as in Scheme 1. The azetidinone **26**, without substituent at C-4 was synthesized in a similar way.

1-(Carboxymethyltetrazol-5-yl)azetidin-2-ones (**23**, **24**) were prepared from compound **2h** (Scheme 3). The alkylation of **2h** with *p*-nitrobenzyl bromoacetate and Et_3N in dimethylformamide gave the N-1 isomer (**17**) and the N-2 isomer (**18**) in a ratio of 1 to 2. Structural confirmation of the N-1 and N-2 substituted tetrazole isomers were made according to the report of ISIDA *et al.*[14] in that the chemi-

Scheme 3.



cal shift of methylene group at the N-2 position was reported to appear at lower field than that at the N-1 position. The ^1H NMR spectrum of the N-1 substituted tetrazole (**17**) in $CDCl_3$ - CF_3COOD (1 : 1) solution showed the resonance of the methylene protons adjacent to the tetrazole ring as a singlet at δ 5.43, while the N-2 isomer (**18**) appeared as a singlet at δ 5.58. The substituted compounds (**17, 18**) were respectively cyclized to afford β-lactams (**19, 20**) in 68 and 72% yield. Deprotection of **19** and **20** by hydrogenolysis of the CBZ and PNB-protecting group was followed by coupling with the α-oxyiminoacetic acid *via* the acid chloride formed with VILSMEYER-reagent. The triphenylmethyl group was removed under the mild conditions by 50% aqueous formic acid to give **23** and **24** in 41 and 47% yield, respectively.

The structure of the 3-amino-β-lactams and the 3-acylamino-β-lactams were confirmed by IR and NMR. Physical properties were listed in Tables 3~6.

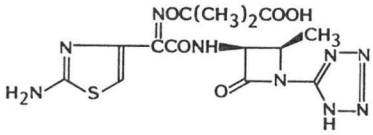
Biological Properties and Discussion

Antibacterial Activity

The minimum inhibition concentration (MIC) values of the 3-acylamino-1-(1*H*-tetrazol-5-yl)-2-azetidinones against two staphylococci and several strains of Gram-negative bacteria were determined by the agar dilution method (Tables 1 and 2). Aztreonam[15] was used as a reference compound.

Table 1 shows the structure-activity relationship of *N*-(aromatic heterocyclic substituted)azetidin-2-ones bearing the same acyl group as that of aztreonam. Compounds **8a~c** and **8f** having aromatic heterocyclic groups other than tetrazole hardly showed any activity. Compounds **8h, 25**, having an acidic tetrazolyl group, and aztreonam showed excellent activity against Gram-negative bacteria, but did not show activity against *Staphylococcus aureus* and *S. epidermidis*. Compounds **8h** and **25** were inferior to aztreonam, against *Pseudomonas aeruginosa*. Compound **25** with a CH_3 group in the *R*-configuration at C-4 showed higher activity than the unsubstituted analog (**8h**). The non-acidic compound **8i** showed activity to medium extent.

Table 2 shows the structure-activity relationships of compounds with a CH_3 group at C-4 or a carboxymethyltetrazole at N-1. The α-(*tert*-butyloxyimino)acetyl group which has proved to show



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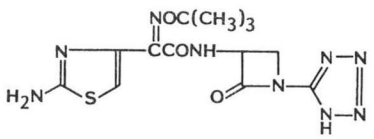

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Table 1. Antibacterial (MIC μg/ml) and cephalosporinase inhibitory activities of *N*-(aromatic heterocyclic substituted)azetidin-2-ones.

Organisms[a]	8a	8b	8c	8f	8h	25	8i	Aztreonam (Control)
S.e. IID 866	200	>200	>200	200	>200	>200	>200	>200
S.a. F-137*	200	>200	>200	>200	>200	>200	>200	>200
E.c. NIHJ JC-2	200	>200	>200	>200	0.2	0.2	12.5	0.2
E.c. TK-3*	200	>200	>200	>200	0.39	≦0.1	12.5	≦0.1
E.c. GN 5482**	200	200	200	>200	0.78	0.2	200	6.25
K.p. Y-50	>200	>200	>200	>200	≦0.1	≦0.1	6.25	≦0.1
K.p. Y-4*	200	>200	>200	>200	0.39	0.39	25	≦0.1
E.cl. IID 977	>200	>200	>200	>200	≦0.1	≦0.1	200	3.13
S.m. IID 620	>200	200	>200	>200	6.25	0.78	3.13	≦0.1
S.m. W-8**	200	200	12.5	>200	6.25	0.78	>200	6.25
P.m. T-111	>200	>200	>200	>200	≦0.1	≦0.1	3.13	≦0.1
P.v. GN 76**	200	50	200	>200	≦0.1	≦0.1	3.13	≦0.1
P.a. IFO 3445	>200	>200	>200	>200	100	12.5	>200	3.13
P.a. GN 918**	200	>200	>200	>200	25	0.78	>200	12.5
I_{50}[b]	N.T.[c]	160	400	N.T.	0.62	0.15	460	0.02

[a] Organisms included in the Table are: *S.e.*, *Staphylococcus epidermidis*; *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *P.m.*, *Proteus mirabilis*; *P.v.*, *Proteus vulgaris*; *P.a.*, *Pseudomonas aeruginosa*.

[b] I_{50} values were determined using 100 μM cephaloridine as substrate for *E. cloacae* H-27.

[c] Not tested.

* Penicillinase producer. ** Cephalosporinase producer.

relatively broad spectrum of activity and the α-ureidoacetyl group of piperacillin was used in the acylamino group at C-3. Compounds with a CH_3 group at C-4 differed very little in activity against sensitive strains. Compounds **23** and **24** having carboxymethyltetrazole had no activity.

Compounds of the ureido, **6e** having a 4,6-dihydroxypyrimidinyl group at N-1 did not exhibit activity, whereas the tetrazole compound **6h** show inferior activity compared to the compound having the α-oxyiminoacetyl side chain. The change of acyl group seems to contribute more to the antibacterial activity in compounds with tetrazolyl groups than in the previously reported compounds with 1-oxysulfonic acid[9].

β-Lactamase Inhibitory Activity

Compounds **8h**, **16j**, **16k** and **23**~**26** showed relatively strong inhibitory activity against cephalosporinase, being about 100 times as active as sulbactam[17] (I_{50}, 14 μg/ml not described in paper), but still inferior to aztreonam. All compounds had I_{50} values >500 μg/ml against the penicillinase produced by *Escherichia coli*, TK-3, even though having appreciable activity against the organism. Similarly, compounds **23** and **24** exhibiting little antibacterial activity showed inhibitory activity against the cephalosporinase.

As described above, *N*-(tetrazol-5-yl)azetidin-2-ones derivatives possesed excellent antibacterial

Table 2. Antibacterial (MIC μg/ml) and cephalosporinase inhibitory activities of *N*-(tetrazol-5-yl)azetidin-2-ones.

Organisms[a]	**26**	**16j**	**16k**	**23**	**24**	**6e**	**6h**	Aztreonam (Control)
S.e. IID 866	100	50	100	>200	>200	>200	6.25	>200
S.a. F-137*	>200	>200	>200	>200	>200	>200	100	>200
E.c. NIHJ JC-2	1.56	1.56	1.56	>200	>200	>200	12.5	0.2
E.c. TK-3*	6.25	1.56	1.56	>200	>200	50	100	≦0.1
E.c. GN 5482**	3.13	0.78	0.39	>200	>200	200	100	6.25
K.p. Y-50	0.78	0.78	0.78	100	100	>200	6.25	≦0.1
K.p. Y-4*	6.25	6.25	6.25	>200	>200	>200	200	≦0.1
E.cl. IID 977	6.25	1.56	3.13	>200	>200	>200	50	3.13
S.m. IID 620	6.25	12.5	6.25	>200	>200	200	25	≦0.1
S.m. W-8**	25	3.13	0.78	>200	>200	>200	>200	6.25
P.m. T-111	1.56	3.13	3.13	>200	200	50	12.5	≦0.1
P.m. GN 76**	0.78	0.78	0.78	100	12.5	50	50	≦0.1
P.a. IFO 3445	>200	200	>200	>200	100	>200	>200	3.13
P.a. GN 918**	12.5	25	3.13	>200	50	>200	>200	12.5
I_{50}[b]	0.12	0.10	0.19	0.42	0.48	N.T.[c]	400	0.02

[a~c] See the footnotes in the Table 1.

activity against Gram-negative bacteria. In a forthcoming report, *N*-(tetrazol-5-yl)azetidin-2-ones which have substituents at C-3 and C-4 and shows activity against Gram-positive bacteria as well will be described[18].

Experimental

IR spectra were recorded on a Hitachi model 260-30 spectrophotometer. NMR spectra were recorded on a Hitachi R-24 (60 MHz) spectrometer using TMS as an internal standard. Melting points are uncorrected. Organic solvents were dried over anhydrous $MgSO_4$, and all concentration and evaporation of solvents were carried out under reduced pressure. Column chromatography was carried out on Wako silica gel (C-200).

In Vitro Antibacterial Activity

Minimum inhibitory concentration (MIC) was determined by the agar dilution method using heart infusion agar (Eiken). MIC (μg/ml) was determined after incubation for 20 hours at 37°C and an inoculum size of about 10^4 cfu.

In Vitro β-Lactamase Inhibitory Activity

I_{50} values were determined by the method of MAK *et al.*[10]. Experimental details for the β-lactamase inhibitory activity studies have been described previously[9].

Materials

Compounds **2h**, **4h**~**8h** and **9**~**26** were prepared as previously reported in the reference[4,5,8,20]. The following examples comprise a representative selection of preparative procedures and further examples may be found in the patent literature[20].

Preparation of (2*S*)-2-Benzyloxycarbonylamino-3-hydroxy-*N*-heteroaromatic Substituted Propionamides (**2**)

(Method A) Preparation of **2b**: To a solution of *N*-CBZ-L-serine (**1**) (10 mmol) in CH_2Cl_2 (25 ml) was added *N*-methylmorpholine (10 mmol) under ice-cooling. ClCOOEt (10.5 mmol) in CH_2Cl_2 (2 ml) was added dropwise to the resulting solution at −30~−20°C over 10~15 minutes, and stirred at −20~−15°C for 1 hour. 2-Aminothiazole (10 mmol) in CH_2Cl_2 (7 ml) was added dropwise to the resulting mixture at −30~−20°C over 15~20 minutes and, stirred at −15~5°C for 1 hour.

Table 3. Yield, mp, ^{1}H NMR and IR data of L-seryl heteroaromatic amides [**2**(**a**~**d**, **f**, **h**) and **3g**].

Compound No.	Yield (%)	MP (°C)	^{1}H NMR (Solvent) δ (J=Hz)	IR $\nu_{C=O}^{KBr}$ (cm^{-1})
2a	37.9	110~112	($CDCl_3$); 3.70~4.26 (2H, m), 4.50~4.90 (2H, m), 5.18 (2H, s), 6.88~7.10 (2H, m), 7.36 (5H, s), 7.68 (1H, m), 8.20~8.36 (2H, m), 10.03 (1H, br s)	1725, 1690
2b	71.9	168~170	(DMSO-d_6); 3.80 (2H, m), 4.36~4.75 (2H, m), 5.15 (2H, s), 7.29~7.64 (7H, m), 12.5 (1H, br s), 10.0 (1H, d, 8)	1720 (sh), 1680
2c	45.5	Amorphous	(DMSO-d_6); 3.45~3.84 (3H, m) 4.59 (1H, m), 5.17 (2H, s), 7.23~7.48 (6H, m), 8.25 (1H, d, 8), 8.85 (2H, d, 5), 10.55 (1H, br s)	1720, 1700
2d	34.3	126~128	($CDCl_3$); 3.56~4.07 (9H, m), 4.93~5.30 (7H, m), 5.95 (1H, s), 6.17 (1H, d, 8), 6.88 (4H, d, 9), 7.22~7.45 (9H, m), 8.90 (1H, br s)	1710, 1690
2f	43.8	182~184 (dec)	(DMSO-d_6+D_2O); 3.83 (2H, m), 4.47 (1H, m), 5.15 (2H, s), 6.90 (2H, s), 7.43 (5H, s)	1690, 1670
2h	83	203~205 (dec)	(DMSO-d_6); 3.69~4.00 (2H, m), 4.44 (1H, m), 5.11 (2H, s), 7.43 (6H, m), 12.30 (1H, s)	1720, 1685
3g	28.9	Amorphous	($CDCl_3$); 3.24~3.63 (2H, m), 3.90 (1H, m), 5.03 (2H, s), 5.46 (1H, d, 6), 6.53 (1H, d, 2.5), 6.75 (1H, d, 2.5), 7.00~7.55 (20H, m), 7.85 (2H, br s)	1715, 1690

The reaction mixture was evaporated to give a residue, which was dissolved in EtOAc (30 ml) and H_2O (30 ml). The organic layer was washed successively with saturated $NaHCO_3$ solution and brine, dried, and evaporated to give a residue, which was crystalized from 2-PrOH (20 ml) to afford **2b** in 71.9%.

(Method B) Preparation of **2a**, **c**, **d** and **2f**: To a solution of *N*-CBZ-L-serine (**1**) (10 mmol) in CH_2Cl_2 (40 ml) was added Et_3N (20.5 mmol), and added dropwise TMS-Cl (21 mmol) under ice-cooling, and stirred at the same temp for 1 hour. TCF (5.5 mmol) in CH_2Cl_2 (2 ml) was added dropwise to the resulting solution at −30~−25°C over 10 minutes, and stirred at −15~−10°C for 2 hours. Separately, heteroaromatic amines (H_2N-X (**a**, **c**, **d**, **f**)) (11 mmol) and Et_3N (11.6 mmol) in CH_2Cl_2 (10 ml) were treated dropwise with TMS-Cl (12.1 mmol) under ice-cooling, and stirred at room temp for 1 hour, and then added dropwise to the solution prepared from **1** at −20~−15°C over 10 minutes, and stirred at 0~10°C for 2 hours. H_2O (30 ml) was added dropwise and the mixture was extracted with CH_2Cl_2. The organic layer was treated as above to afford **2a**, **c**, **d**, **f** in 34.3~45.5%. The physical properties of **2** are summarized in Table 3.

(2*S*)-2-Benzyloxycarbonylamino-3-hydroxy-*N*-(1-triphenylmethylimidazol-2-yl)propionamide (**3g**)

To a solution of **2f** (1.65 mmol) in DMF (5 ml) were added triphenylmethylchloride (1.8 mmol) and Et_3N (1.7 mmol) under ice-cooling, and stirred at 15~20°C for 12 hours. H_2O (30 ml) and EtOAc (30 ml) were added into the reaction mixture. The organic layer was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatograpy (toluene - EtOAc, 2: 1) to afford **3g** as an amorphous powder in a yield of 28.9%.

3-Benzyloxycarbonylamino-2-azetidinones (**4**)

To a solution of propionamide **2a**~**d** and **3g** (1 mmol) and TPP (1.3 mmol) in THF (15 ml) was added dropwise DEAD (1.3 mmol) at 5~10°C over 15 minutes and the mixture was stirred for 3 hours at room temp. The reaction mixture was evaporated and the residue dissolved in EtOAc (10 ml), washed succesively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatography (benzene - EtOAc, 50: 1) to afford **4** in 21.5~51.3% yield. The physical properties of **4** are summarized in Table 4.

Table 4. Yield, mp, ^{1}H NMR, IR and analytical data of β-lactams (**4**).

Compound No.	Yield (%)	MP (°C)	^{1}H NMR (Solvent) δ (J=Hz)	IR $\nu_{C=O}^{KBr}$ (cm^{-1})	*Anal* [a]
4a	44.4	189~189.5	(DMSO-d_6); 3.77 (1H, m), 4.10 (1H, m), 4.93 (1H, m), 5.17 (2H, s), 7.16~7.98 (8H, m), 8.26 (1H, d, 8), 8.48 (1H, m)	1770, 1685	$C_{16}H_{15}N_3O_3$
4b	51.3	179~180	(DMSO-d_6); 3.92 (1H, m), 4.24 (1H, m), 4.98~5.27 (3H, m), 7.32~7.62 (7H, m), 8.30 (1H, d, 9)	1770, 1695	$C_{14}H_{13}N_3O_3S$
4c	28.6	148~149	(DMSO-d_6); 3.81 (1H, m), 4.61 (1H, m), 4.97 (1H, m), 5.16 (2H, s), 7.24~7.50 (6H, m), 8.24 (1H, d, 8), 8.83 (2H, d, 5)	1775, 1705	$C_{15}H_{14}N_4O_3$
4d	21.5	171~172	(DMSO-d_6); 3.55~3.80 (7H, m), 4.04 (1H, m), 4.93 (1H, m), 5.10 (2H, s), 5.30 (4H, s), 5.92 (1H, s), 6.97 (4H, d, 9), 7.40~7.68 (9H, m), 8.17 (1H, d, 9)	1770, 1715	$C_{31}H_{30}N_4O_7$
4g	50	204~208 (dec)	(DMSO-d_6); 2.17 (1H, m), 2.86 (1H, m), 4.09 (1H, m), 5.10 (2H, s), 6.82 (1H, d, 2), 7.09~7.72 (21H, m), 8.04 (1H, d, 8)	1780, 1725	$C_{33}H_{28}N_4O_3$
4h	24	153~156	(DMSO-d_6+D_2O); 3.75~4.30 (2H, m), 4.95~5.31 (3H, m), 7.49 (5H, s)	1800, 1780, 1700	$C_{12}H_{12}N_6O_3$

[a] All the compounds given the formula were analyzed for C, H and N; analytical results obtained for these elements were within $\pm$0.4% of calculated values.

General Preparation of 3-Amino-β-lactams **5a, c, d** and **5g**

β-Lactams (**4**) (2 mmol) were hydrogenated in MeOH (30 ml) for 30 minutes over 10% Pd-C catalyst (500 mg) at room temp and 5 atm pressure. The catalyst was filtered and washed with MeOH. The combined filtrates were evaporated to give a residue, which was triturated with isopropyl ether to afford **5** in 30~90% yield. Crude 3-amino-β-lactams (**5**) were used in subsequent steps without further purification. Compound **5d** was acylated immediately because of its instability.

(3*S*)-3-Amino-1-(thiazol-2-yl)-2-azetidinone (**5b**)

To a solution of **4b** (250 mg, 0.82 mmol) in CH_2Cl_2 (2.5 ml) and anisole (2.5 ml) was added dropwise $AlCl_3$ (550 mg, 4.12 mmol) in CH_3NO_2 at -20~-10°C, and stirred at -10°C for 30 minutes. The reaction mixture was dropped into a mixture of EtOAc (100 ml) and H_2O (10 ml) keeping pH at 7.0 with saturated aqueous $NaHCO_3$. The organic layer was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatography ($CHCl_3$-MeOH, 30: 1) to afford **5b** as a white powder in 28.8% yield. The physical properties of **5** are summarized in Table 5.

General Procedure for the Acylation of 3-Amino-β-lactams (**5**)

(Method A) Synthesis of (3*S*)-3-[D(−)-2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)phenylacetamido]-1-(4,6-dihydroxypyrimidin-2-yl)-2-azetidinone (**6e**): To a solution of **5d** (200 mg, 0.43 mmol) in DMF (4 ml) was added D(−)-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)phenylacetic acid (150 mg, 0.47 mmol) and DCC (100 mg, 0.48 mmol) under ice-cooling, and stirred for 3 hours at room temp. The precipitate was filtered off, and the filtrate was evaporated to afford a residue, which was purified by column chromatography ($CHCl_3$ - Me_2CO, 10: 1) to afford (3*S*)-3-[D-(−)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)phenylacetamido]-1-[4,6-di-(*p*-methoxybenzyloxy)pyrimidin-2-yl]-2-

Table 5. Yield, mp, ^{1}H NMR and IR data of 3-amino-β-lactams (**5**).

Compound No.	Yield (%)	MP (°C)	^{1}H NMR (Solvent) δ (J=Hz)	IR $\nu_{C=O}$ (cm^{-1})
5a	85.7	Amorphous	(DMSO-d_6); 3.24 (2H, br s), 3.50 (1H, m), 4.05 (1H, m), 4.37 (1H, m), 7.23 (1H, m), 7.53~8.10 (2H, m), 8.48 (1H, m)	1750 (KBr)
5b	28.8	127~129	(DMSO-d_6); 2.70 (2H, br s), 3.53 (1H, m), 4.08 (1H, m), 4.40 (1H, m), 7.32 (1H, d, 3), 7.40 (1H, d, 3)	1745 (KBr)
5c	87.9	90~95	(DMSO-d_6); 3.40~3.58 (4H, m), 4.00 (1H, m), 7.20 (1H, d, 5), 8.78 (2H, d, 5)	1780 (THF)
5g	68.5	180~181 (dec)	(DMSO-d_6); 2.30 (1H, m), 2.75~3.65 (4H, m), 6.80~7.69 (17H, m)	1770 (KBr)
5h	73	179~180 (dec)	(D_2O+DCl); 4.23~4.68 (2H, m), 5.24 (1H, m)	1785, 1765 (KBr)

azetidinone (**6d**) (70 mg, 21%) as a white powder. The powder was dissolved in a mixture of anisole (0.5 ml) and TFA (1.5 ml) under ice-cooling, and stirred at room temp for 30 minutes. The solvent was evaporated and the obtained residue was triturated with isopropyl ether to afford **6e** as a white powder in 15% yield. Crude **6e** was subjected to antibacterial activity test without further purification. MP 205~210°C (dec); IR $\nu_{C=O}^{KBr}$ 1775, 1720, 1710, 1685, 1660 cm^{-1}; NMR (DMSO-d_6) δ 1.12 (3H, t, J=7.0 Hz), 3.25~4.31 (8H, m), 5.25 (1H, m), 5.55 (1H, d, J=8.0 Hz), 7.35~7.60 (8H, m), 9.56 (1H, d, J=8.0 Hz), 10.05 (1H, d, J=8.0 Hz).

(Method B) Synthesis of (3*S*)-3-{2-(2-Aminothiazol-4-yl)-(*Z*)-2-[(1-carboxy-1-methylethoxy)imino]-acetamido}-1-substituted Azetidin-2-ones (**8a**, **b**, **c**, **d** and **8f**): i) To a solution of **5a**, **b**, **c**, **g** (2 mmol) in DMF (5 ml) were added successively 1-hydroxybenzotriazole hydrate (2 mmol), molecular sieves 4A (1 g), DCC (2.2 mmol) and (*Z*)-2-[(1-*tert*-butoxycarbonyl-1-methylethoxy)imino]-2-(2-triphenylmethylaminothiazol-4-yl)acetic acid (2 mmol) under ice-cooling, and stirred for 1 hour at room temp. The precipitate was filtered off, and the filtrate was evaporated. The residue was dissolved in EtOAc (20 ml), which was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatography to afford **7a**, **b**, **c** and **7g** in 50~75% yield, which were used in the following step.

ii) The compounds **7a**, **b**, **c** and **7g** were dissolved in THF (2 ml) and 50% aqueous HCOOH (4 ml) and kept at 40~50°C for 1 hour. The solvent was evaporated to afford a residue, which was dissolved in EtOAc (20 ml) and H_2O (10 ml), and adjusted to pH 7.5 with saturated $NaHCO_3$ solution. The separated organic layer was washed with brine, dried, and evaporated to afford a residue, which was dissolved in CH_2Cl_2 (0.5 ml), and treated with TFA (1.5 ml) under ice-cooling. The mixture was stirred at room temp for 1.5 hours, then the solvent was evaporated and the residue triturated with isopropyl ether to afford the TFA salt of **8a**, **b**, **c**, **f** as a white powder in 35~55% yield. Crude TFA salt of **8a**, **b**, **c**, **f** were subjected to antibacterial activity test, without further purification. The physical properties of **8a**, **b**, **c**, **f** are summarised in Table 6.

(3*S*)-3-{2-(2-Aminothiazol-4-yl)-(*Z*)-2-[(1-carboxy-1-methylethoxy)imino]acetamido}-1-(1*H*-tetrazol-5-yl)-2-azetidinone (**8h**) and (3*S*)-3-{2-(2-Aminothiazol-4-yl)-(*Z*)-2-[(1-carboxyl-1-methylethoxy)imino]acetamido}-1-(1 or 2-*tert*-butyl-1*H*-tetrazol-5-yl)-2-azetidinone (**8i**)

To a solution of (3*S*)-3-{2-(2-aminothiazol-4-yl)-(*Z*)-2-[1-*tert*-butoxycarbonyl-1-methylethoxy)-imino]acetamido}-1-(1*H*-tetrazol-5-yl)-2-azetidinone (1.05 g, 2.32 mmol) in CH_2Cl_2 (2.1 ml) was added TFA (6.3 ml) under ice-cooling, and stirred at room temp for 1.5 hours. The solvent was evaporated to afford a residue, which was dissolved in EtOAc (50 ml) and H_2O (50 ml), and adjusted to pH 4.0 with saturated $NaHCO_3$ solution. The layers were separated. The organic layer was washed with brine, dried, and evaporated to give a residue, which was purified by column chromatography (toluene-EtOAc, 1:1) to afford **8i** as a white powder in 15% yield. The aqueous layer was adjusted to pH

Table 6. MP, ^{1}H NMR and IR data of 3-acylamino-1-(aromatic heterocyclic substituted)azetidin-2-ones [**8(a, b, c, f, h, i)**, **16(j, k)**, **25** and **26**].

Compound No.	MP (°C, dec)	^{1}H NMR (Solvent) δ (J=Hz)	IR $\nu_{C=O}^{KBr}$ (cm^{-1})
8a*	187~190	(DMSO-d_6); 1.53 (6H, s), 3.87 (1H, m), 4.22 (1H, m), 5.26 (1H, m), 7.03 (1H, s), 7.30 (1H, m), 7.73~8.23 (7H, m), 8.50 (1H, m), 9.42 (1H, d, 8)	1755, 1705, 1660
8b*	170~174	(DMSO-d_6); 1.55 (6H, s), 3.76~4.38 (2H, m), 5.28 (1H, m), 7.03 (1H, s), 7.16~7.73, (5H, m), 8.88 (2H, d, 5), 9.40 (1H, d, 7)	1775, 1740, 1660
8c*	138~142	(DMSO-d_6); 1.51 (6H, s), 3.97 (1H, m), 4.37 (1H, m), 5.29 (1H, m), 7.01 (1H, s), 7.40~7.65 (2H, m), 8.20 (4H, br s), 9.60 (1H, d, 8)	1775, 1720, 1660, 1630
8f*	150~155	(DMSO-d_6+D_2O); 1.50 (6H, s), 3.45 (1H, m), 4.20 (1H, m), 4.97 (1H, m), 7.00 (1H, s), 7.1~7.5 (2H, m)	1790, 1715, 1665, 1630
8h	115~120	(DMSO-d_6+D_2O); 1.50 (6H, s), 3.80~4.30 (2H, m), 5.30 (1H, m), 6.87 (1H, s)	1770, 1660
25*	120~140	(DMSO-d_6+D_2O); 1.58 (6H, s), 1.69 (3H, d, 6.5), 4.7 (1H)[a], 5.62 (1H, d, 5), 7.10 (1H, s)	1760, 1660
8i	183~187	(DMSO-d_6); 1.48 (6H, s), 1.68 (9H, s), 3.89~4.33 (2H, m), 5.28 (1H, m) 6.90 (1H, s), 7.40 (3H, br s), 9.30 (1H, d, 8)	1775, 1720, 1660
16j	145~150	(DMSO-d_6); 1.30 (9H, s), 1.63 (3H, d, 7), 4.45 (1H, m), 4.96 (1H, m), 6.87 (1H, s), 7.25 (3H, br s), 9.25 (1H, d, 7)	1760, 1650, 1620
16k	130~140	(DMSO-d_6+D_2O); 1.28 (9H, s), 1.46 (3H, d, 6.5), 4.65 (1H, m), 5.55 (1H, d, 5), 6.87 (1H, s)	1760, 1650, 1620
26	95~97	(DMSO-d_6); 1.47 (9H, s), 3.81~4.41 (2H, m), 5.41 (1H, m), 7.03 (1H, s), 9.47 (1H, d, 7) 10.1 (3H, br s)	1775, 1660

[a] It was difficult to read the δ value because the signal overlapped with those of water.
* TFA salt.

2.5 with 2 N HCl, extracted with EtOAc, washed with brine, dried, and evaporated to give a residue, which was triturated with Et_2O to afford **8h** as a white powder in 60% yield. The physical properties are summarized in Table 6.

(2*S*,3*S*)-2-Benzyloxycarbonylamino-3-hydroxy-*N*-(1-diphenylmethyl-1*H*-tetrazol-5-yl)butyramide (**10k**)

To a solution of **9** (10 g, 31.2 mmol) in MeOH (50 ml) and acetone (50 ml) was added diphenyl diazomethane (11 g, 57 mmol) at room temp, and stirred for 24 hours at the same temp. The reaction mixture was evaporated and the residue was dissolved in EtOAc (200 ml) and H_2O (100 ml), which was adjusted to pH 7.0 with saturated $NaHCO_3$ solution. The organic layer was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatography (benzene - EtOAc, 50: 1) to afford **10k** as an amorphous powder in 80% yield. IR $\nu_{C=O}^{KBr}$ 1720, 1700 cm^{-1}; NMR (DMSO-d_6+D_2O) δ 1.31 (3H, d, J=6.0 Hz), 3.39 (1H, m), 4.30 (1H, m), 5.14 (2H, s), 7.04 (1H, s), 7.37 (15H, s).

(3*S*,4*R*)-3-Benzyloxycarbonylamino-1-(1-diphenylmethyl-1*H*-tetrazol-5-yl)-4-methyl-2-azetidinone (**11k**)

To a solution of **10k** (17.2 g, 35.4 mmol) and TPP (12 g, 46 mmol) in THF (400 ml) was added dropwise DEAD (7.2 ml, 46 mmol) at 5~10°C over 15 minutes, and stirred for 3 hours at room temp. The reaction mixture was evaporated and the residue dissolved in EtOAc (100 ml) and H_2O (50 ml). The organic layer was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was triturated with benzene (80 ml). The solid was filtered off, and the filtrate was evaporated. The residue was purified by column chromatography (benzene - EtOAc, 50: 1) to afford **11k** as colorless

crystals in 70% yield. MP 158~160°C; IR $\nu_{C=O}^{KBr}$ 1780, 1720 cm^{-1}; NMR ($CDCl_3$) δ 1.31 (3H, d, J=6.0 Hz), 4.54 (1H, m), 5.16~5.25 (3H, m), 5.71 (1H, d, J=9.0 Hz), 7.38 (5H, s), 7.42 (10H, s), 7.77 (1H, s).

(3*S*,4*R*)-Benzyloxycarbonylamino-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (**13k**)

Method A: To a solution of **11k** (3 g, 6.4 mmol) in anisole (15 ml) was added TFA (45 ml) at room temp, and stirred for 2 hours. The solvent was evaporated to afford a residue, which was dissolved in Et_2O (20 ml) and ice-water (2 ml) and adjusted to pH 7.5 with saturated $NaHCO_3$ solution. The aqueous layer was adjusted to pH 2.0 with 2 N HCl, and extracted with EtOAc. The organic layer was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was triturated with a mixture of EtOAc (5 ml) and Et_2O (10 ml) to afford **13k** as colorless crystals in 80% yield. MP 147~149°C; IR $\nu_{C=O}^{THF}$ 1770, 1720 cm^{-1}; NMR ($CDCl_3$+D_2O) δ 1.52 (3H, d, J=6.0 Hz), 4.67 (1H, t, J=6.0 Hz), 5.19 (2H, s), 5.37 (1H, d, J=6.0 Hz), 7.42 (5H, s).

Method B: Cyclization of **9k** was carried out in a similar manner as for **11k**. Yield 20%.

Method C: To a solution of **12k** (2.8 g, 7.1 mmol) in THF (70 ml) and acetone (70 ml) was added anhydrous $MgSO_4$ (5 g), and stirred for 30 minutes. The solid was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in THF (120 ml), TPP (2.69 g, 10.3 mmol) added, followed by DEAD (1.62 ml, 10.3 mmol) in THF (10 ml) dropwise at 20~25°C over 30 minutes. The reaction mixture was stirred for 12 hours at room temp, and evaporated to give a residue, which was triturated with a mixture of EtOAc (20 ml) and Et_2O (20 ml). The separating crystals were collected by filtration and suspended in EtOAc (30 ml) and H_2O (30 ml), which was adjusted to pH 1.5 with 6 N HCl. The organic layer was separated and added to H_2O (30 ml), and adjusted to pH 7.0 with saturated $NaHCO_3$ solution. The aqueous layer was separated, added to EtOAc (30 ml), and adjusted to pH 2.0 with 2 N HCl. The organic layer was washed successively with H_2O (10 ml) and brine (10 ml), dried, and evaporated to give a residue, which was triturated with Et_2O (20 ml) to afford **13k** in 63% yield.

(3*S*,4*R*)-3-Amino-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (**14k**)

Method A: The compound **13k** (2 g, 6.6 mmol) was hydrogenated in MeOH (50 ml) for 1.5 hours over 5% Pd-C (0.5 g) at atmospheric pressure and room temp. The catalyst was filtered and washed with aqueous $NaHCO_3$ (0.83 g, 9.9 mmol) solution (20 ml). The combined filtrates were treated with 6 N HCl (2 ml) and evaporated to give a residue, which was dissolved in hot EtOH (30 ml). The solid was filtered off and the filtrate cooled to room temp to afford the HCl salt of **14k** (1 g) as colorless crystals in 74.1% yield. To a suspention of the HCl salt (1 g, 4.9 mmol) in EtOH (40 ml) pyridine (0.6 ml, 7.4 mmol) was added, and stirred for 5 hours at room temp. The precipitate was collected by filtration and washed with EtOH to afford **14k** as a white powder in 83% yield. MP 192°C (dec); IR $\nu_{C=O}^{KBr}$ 1810, 1775 cm^{-1}; NMR (D_2O+DCl) δ 1.72 (3H, d, J=7.0 Hz), 4.64~5.09 (2H, m).

Method B: The compound **11k** (1 g, 2.1 mmol) was hydrogenated in MeOH (20 ml) for 5 hours over 10% Pd-C (0.5 g) at room temp and 5 atm pressure. The title compound was obtained in a similar manner as above in 30% yield.

Zinc Salt of (2*S*,3*S*)-2-Benzyloxycarbonylamino-3-hydroxy-*N*-(1*H*-tetrazol-5-yl)butyramide·$\frac{1}{2}H_2O$ (**12k**)

To a suspension of **9k** (3 g, 9.4 mmol) in H_2O (60 ml) was added $NaHCO_3$ (0.83 g, 9.9 mmol) to obtain a clear solution. $ZnCl_2$ (0.77 g, 5.65 mmol) was added, and stirred for 30 minutes at room temp. The crystals formed were collected by filtration and washed with H_2O (10 ml) to afford **12k** as white crystals in 97.3% yield. MP >240°C; IR $\nu_{C=O}^{KBr}$ 1700, 1670 cm^{-1}.

(3*S*,4*R*)-4-Methyl-1-(1*H*-tetrazol-5-yl)-3-[2-(2-triphenylmethylaminothiazol-4-yl)-(*Z*)-2-*tert*-butoxyiminoacetamido]-2-azetidinone (**15k**)

To a solution of **14k** (0.34 g, 2 mmol) in DMF (3 ml) was added Et_3N (0.28 ml, 2 mmol) under ice-cooling. Molecular sieves 4A (0.5 g), 1*H*-hydroxybenzotriazole hydrate (0.27 g, 2 mmol), (*Z*)-2-*tert*-butoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetic acid (1 g, 2 mmol) and DCC (0.46 g,

2.2 mmol) were added successively to the solution at the same temp, and stirred for 2 hours at room temp. The precipitate was filtered off and the filtrate evaporated. The residue was dissolved in H_2O (15 ml) and EtOAc (15 ml) and adjusted to pH 2.0 with 2 N HCl. The organic layer was washed with brine, dried, and evaporated to give a residue, which was purified by column chromatography ($CHCl_3$ - MeOH, 50: 1) to afford **15k** as a white powder in 49% yield. MP 155~175°C (dec); IR $\nu_{C=O}^{KBr}$ 1765, 1660 cm^{-1}; NMR (DMSO-d_6) δ 1.22 (9H, s), 1.38 (3H, d, J=6.0 Hz), 4.48 (1H, m), 5.28~5.55 (2H, m), 6.68 (1H, s), 7.31 (15H, s), 9.26 (1H, d, J=9.0 Hz).

(3*S*,4*R*)-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-*tert*-butoxyiminoacetamido]-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (**16k**)

The compound **15k** (0.38 g, 0.6 mmol) was dissolved in a mixture of THF (2 ml) and 50% aqueous HCOOH (4 ml) and kept at 40~50°C for 1 hour. The solvent was evaporated to afford a residue, which was dissolved in EtOAc (10 ml) and H_2O (10 ml), and adjusted to pH 7.5 with saturated $NaHCO_3$ solution. The aqueous layer was adjusted to pH 2.5 with 2 N HCl, saturated with NaCl, and extrated with EtOAc (10 ml) twice. The combined extracts were washed with brine (10 ml), and evaporated to give a residue, which was triturated with Et_2O to afford **16k** as a white powder in 55% yield. MP 130~140°C (dec); IR $\nu_{C=O}^{KBr}$ 1760, 1650, 1620 cm^{-1}; NMR (DMSO-d_6+D_2O) δ 1.29 (9H, s), 1.45 (3H, d, J=6.0 Hz), 4.65 (1H, m), 5.55 (1H, d, J=5.0 Hz), 6.87 (1H, s).

(2*S*)-2-Benzyloxycarbonylamino-3-hydroxy-*N*-[1-(4-nitrobenzyloxycarboxymethyl)-1*H*-tetrazol-5-yl]propionamide (**17**) and N-2 Isomer (**18**)

To a solution of **2h** (3 g, 0.98 mmol) in DMF (15 ml) and Et_3N (1.35 ml, 1 mmol) was added *p*-nitrobenzyl bromoacetate (2.74 g, 1 mmol) at room temp, and stirred for 4 hours. EtOAc (300 ml) and H_2O (200 ml) was added and the organic layer was separated, washed successively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatography ($CHCl_3$ - acetone, 10: 1) to afford **17** (0.95 g, 19.4%) and **18** (2.05 g, 41.9%).

17: MP 117~119°C (EtOAc - Et_2O); Rf 0.09 [Merck Silica gel 60 F_{254}, ($CHCl_3$ - acetone, 2: 1)]; IR $\nu_{C=O}^{KBr}$ 1740, 1700 cm^{-1}; NMR ($CDCl_3$ - CF_3COOD, 1: 1) δ 4.0~4.3 (2H, m), 4.55~4.85 (1H, m), 5.20 (2H, s), 5.36 (2H, s), 5.43 (2H, s), 7.30 (5H, s), 7.52 (2H, d, J=9.0 Hz), 8.21 (2H, d, J=9.0 Hz).

Anal Calcd for $C_{21}H_{21}N_7O_8$: C 50.50, H 4.24, N 19.63.
Found: C 50.61, H 4.22, N 19.35.

18: MP 164~165°C (EtOAc - Et_2O); Rf 0.29 [Merck Silica gel 60 F_{254}, ($CHCl_3$ - acetone, 2: 1)]; IR $\nu_{C=O}^{KBr}$ 1750, 1680 cm^{-1}; NMR ($CDCl_3$ - CF_3COOD, 1: 1) δ 4.1~4.33 (2H, m), 4.6~4.87 (1H, m), 5.21 (2H, s), 5.39 (2H, s), 5.58 (2H, s), 7.28 (5H, s), 7.55 (2H, d, J=9.0 Hz), 8.23 (2H, d, J=9.0 Hz).

Anal Calcd for $C_{21}H_{21}N_7O_8$: C 50.50, H 4.24, N 19.63.
Found: C 50.18, H 4.31, N 19.88.

(3*S*)-3-Benzyloxycarbonylamino-1-[1-(4-nitrobenzyloxycarboxymethyl)-1*H*-tetrazol-5-yl]-2-azetidinone (**19**) and N-2 Isomer (**20**)

Cyclization of **19** and **20** were carried out in a similar manner as for **11k**.

19: Yield 68%; mp 138~140°C (EtOAc - Et_2O); IR $\nu_{C=O}^{CH_2Cl_2}$ 1785, 1765, 1730 cm^{-1}; NMR (DMSO-d_6) δ 3.76~4.20 (2H, m), 4.90~5.20 (3H, m), 5.40 (2H, s), 5.94 (2H, s), 7.37 (5H, s), 7.69 (2H, d, J=10.0 Hz), 8.27 (2H, d, J=10.0 Hz), 8.65 (1H, d, J=8.0 Hz).

Anal Calcd for $C_{21}H_{19}N_7O_7$: C 52.39, H 3.98, N 20.37.
Found: C 52.31, H 3.77, N 20.51.

20: Yield 72%; mp 122~126°C (EtOAc - Et_2O); IR $\nu_{C=O}^{CH_2Cl_2}$ 1790, 1760, 1705 cm^{-1}; NMR (DMSO-d_6) δ 3.95~4.20 (2H, m), 5.10~5.26 (3H, m), 5.40 (2H, s), 5.92 (2H, s), 7.35 (5H, s), 7.72 (2H, d, J=10.0 Hz), 8.25 (2H, d, J=10.0 Hz), 8.60 (1H, d, J=8.0 Hz).

Anal Calcd for $C_{21}H_{19}N_7O_7$: C 52.39, H 3.98, N 20.37.
Found: C 52.58, H 3.91, N 20.61.

(3*S*)-3-Amino-1-(1-carboxymethyl-1*H*-tetrazol-5-yl)-2-azetidinone (**21**) and N-2 Isomer (**22**)

Compound **19** (50 mg, 0.1 mmol) was hydrogenated in MeOH (5 ml) for 30 minutes over 10%

Pd-C (10 mg) at room temp and 5 atm pressure. The catalyst was filtered and washed with MeOH. The combined filtrates were evaporated to give a residue, which was triturated with EtOH to afford **21** as a white powder in 77% yield. MP 172~174°C (dec); IR $\nu_{C=O}^{KBr}$ 1780, 1610 cm^{-1}; NMR (D_2O) δ 4.20 (2H, m), 5.10 (1H, m), 5.40 (2H, s).

Compound **22** was synthesized in a similar manner as above. Yield 95%; mp 195°C (dec); IR $\nu_{C=O}^{KBr}$ 1785, 1635 cm^{-1}; NMR (D_2O) δ 4.30 (2H, m), 5.07 (1H, m), 5.36 (2H, s).

(3*S*)-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(*tert*-butoxyimino)acetamido]-1-(1-carboxymethyl-1*H*-tetrazol-5-yl)-2-azetidinone (**23**) and N-2 Isomer (**24**)

To a solution of (*Z*)-2-*tert*-butoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetic acid (0.97 g, 2 mmol) in DMF (5 ml) was added dropwise a solution of $POCl_3$ (0.19 ml, 2 mmol) in CH_2Cl_2 (1 ml) at −15~−10°C over 5 minutes, and stirred for 30 minutes at that temp. Separately, a mixture of **21** (0.42 g, 2 mmol) and Et_3N (0.28 ml, 2 mmol) in DMF (5 ml) was stirred for 30 minutes at the same temp to give a solution, which was cooled to −20°C and dropped into the above reaction mixture. After stirring for 1 hour at room temp, EtOAc (20 ml) and H_2O (20 ml) was added. The organic layer was washed successively with H_2O and brine, dried, and evaporated to give a residue, which was purified by column chromatography ($CHCl_3$ - MeOH, 50: 1) to afford a white powder (0.75 g). These materials were dissolved in THF (4 ml) and 50% aqueous HCOOH (8 ml) and kept at 40~50°C for 1 hour. The solvent was evaporated to afford a residue, which was triturated with Et_2O to afford **23** as an amorphous powder in 41% yield. IR $\nu_{C=O}^{KBr}$ 1775, 1650, 1620 cm^{-1}; NMR (DMSO-d_6) δ 1.35 (9H, s), 4.10 (2H, m), 5.05~6.05 (5H, m), 6.65 (1H, s), 7.40 (1H, br s), 9.20 (1H, br s).

Compound **24** was synthesized in a similar manner as above. Yield 47%; mp 197~201°C (dec); IR $\nu_{C=O}^{KBr}$ 1775, 1650, 1620 cm^{-1}; NMR (DMSO-d_6) δ 1.32 (9H, s), 4.02 (2H, m), 4.82~5.92 (5H, m), 6.83 (1H, s), 7.32 (1H, br s), 9.12 (1H, br s).

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