CEPHALOSPORINS. III
7-(*O*-AMINOMETHYLPHENYLACETAMIDO)CEPHALOSPORANIC ACIDS WITH BICYCLIC HETEROAROMATICS IN THE C-3 SIDE CHAIN

TAKAYUKI NAITO, JUN OKUMURA, HAJIME KAMACHI,
HIDEAKI HOSHI and HIROSHI KAWAGUCHI

Bristol-Banyu Research Institute, Ltd.
Meguro, Tokyo, Japan

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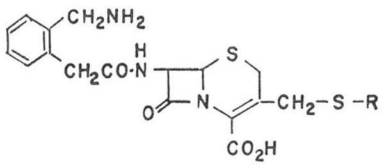
Bicyclic heteroaromatic thiols with a bridge-head nitrogen atom were used for nucleophilic substitution of 7-ACA at the C-3 acetoxy function followed by N-acylation of the 7-amino group with *o*-aminomethylphenylacetic acid to afford a series of new cephalosporins (**24**) with potent antibacterial activity against gram-positive and gram-negative organisms. The most active member of this series was 7-(*o*-aminomethylphenylacetamido)-3-(tetrazolo-[4,5-b]pyridazin-6-ylthiomethyl)-3-cephem-4-carboxylic acid (BB-S 226) (**24e**) with antibacterial activity superior to cephalothin and cefazolin.

The preceding paper[1] described 7-(*o*-aminomethylphenylacetamido)cephalosporanic acids with a mono-cyclic azine in the C-3 side chain which showed the antibacterial activity varying with the heterocycle involved. In general pyridazines gave cephalosporin derivatives with promising activity. This paper describes a series of cephalosporins in which the 7-amino group was acylated with the same amino acid, *o*-aminomethylphenylacetic acid, and the C-3 acetoxy group was replaced by a bicyclic heteroaromatic ring.

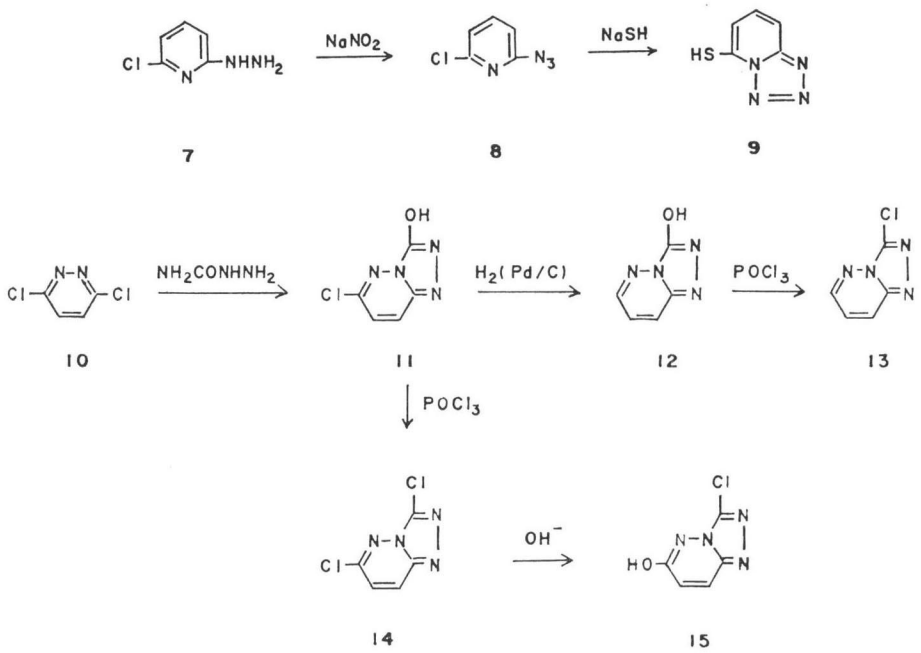


R = Bicyclic heteroaromatic

Chemistry

Bicyclic heteroaromatics used in this study are all fused ring systems consisting of an azine (pyridine, pyridazine or pyrimidine) and an azole (triazole or tetrazole) with a bridge-head nitrogen atom. The preparation of these ring systems was, in general, carried out by synthesis of an appropriate azine derivative followed by formation of the azole moiety. Cyclization of an appropriate hydrazino azine with formic acid[2], nitrous acid[2] and carbon disulfide[3,4] was applied to obtain a variety of bicyclic compounds listed in Table 1.

Treatment of 6-chloro-2-hydrazinopyridine (**7**)[5] with sodium nitrite in 30% acetic acid gave 6-chloro-2-azidopyridine (**8**) whose infrared spectrum showed the azide stretching band at 2130 cm^{-1}. Upon reaction of **8** with sodium hydrogen sulfide in ethanol a ring closure occurred by valence isomerization to form tetrazolo[4,5-a]pyridin-2-ylthiol(**9**) in 10% yield with disappearance of the azide stretching band in the infrared spectrum.



7 8 9

10 11 12 13

14 15

Treatment of 3,6-dichloropyridazine (**10**) with semicarbazide hydrochloride, followed by refluxing in ethanol containing a small amount of mineral acid gave 6-chloro-3-hydroxy-s-triazolo[4,3-b]pyridazine (**11**)[6]. Compound **11** was dehalogenated with Pd/C to **12** which was chlorinated with phosphorous oxychloride to give **13**[6]. Compound **11** was also chlorinated with phosphorous oxychloride to the dichloro derivative **14** which was refluxed in 5% aqueous sodium hydroxide to give **15**.

Table 1. Cyclization of heterocyclic hydrazines

Compound		M.p. °C	Yield,%	Hydrazines	Reagent
	1[2]	209~210	40		HCOOH
	2	73~78	57		HCOOH
	3	>250	50		HCOOH
	4[2]	104~105	64		$NaNO_2$
	5[3]	218~219	72		CS_2
	6[4]	260~265	47		CS_2

Thiolation of the bicyclic chlorides **1**, **2**, **4**, **11**, **13** and **15**, was carried out by reaction with alkali hydrogen sulfide by refluxing in alcohol or heating in a sealed tube to give **16**[2,7], **17**, **18**[8], **19**, **20** and **21**, respectively. Compound **4** was more easily converted to the corresponding thiol **18** by reaction with sodium hydrogen sulfide in water at room temperature. The results are given in Table 2.

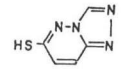
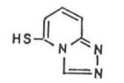
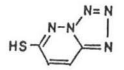
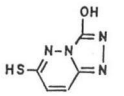
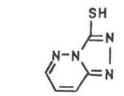
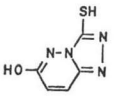
The bicyclic thiols reacted with 7-aminocephalosporanic acid (**22**) in 0.1 M phosphate buffer (pH 6.4) at 60~70°C by nucleophilic displacement of the acetoxy group to provide **23** in 39~83% yield (Table 3). These were characterized by their infrared spectra showing the β-lactam C=O absorption at 1800~1805 cm^{-1} and UV maxima at 265~270 nm due to the dihydrothiazine ring of the 7-ACA nucleus. The compounds **23** were acylated with N-BOC protected *o*-aminomethylphenylacetic acid[1] by the active ester method using 2,4-dinitrophenol followed by deblocking with trifluoroacetic acid to pro-

vide the new cephalosporins **24**, which showed a β-lactam carbonyl stretching band in a range of 1760~1780cm^{-1}. Physicochemical data of **24** are summarized in Table 4.

Antimicrobial Activity

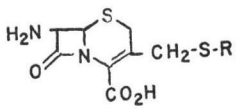
The minimum inhibitory concentrations (MIC) of this series of cephalosporins **24** against a variety of gram-positive and gram-negative bacteria were determined by the two-fold serial tube dilution method using nutrient broth and the results are given in Table 6 compared with cefazolin (CEZ) and cephalothin (CET).

Table 2. Bicyclic heteroaromatic thiols from the corresponding chlorides

Thiol		M.p. °C	Yield, %	Reagent	Solvent	Reaction temp.
	**16**[2,7]	190~193 (dec.)	89	KSH	MeOH	reflux
	**17**	247~248	56	KSH	EtOH	120°*
	**18**[8]	142~144	97	NaSH	H_2O	room temp.
	**19**	>300	95	NaSH	EtOH	120°*
	**20**	260~270	62	KSH	EtOH	120°*
	**21**	280~285	50	NaSH	EtOH	120°*

* heated in a sealed tube.

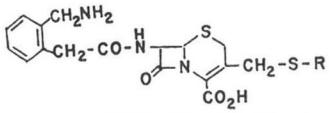
Table 3. 3-Substituted 7-aminocephalosporanic acids (**23**)



Compound	Yield, %	Mp, °C (dec.)	λ_{max} nm (ε)[a]	Formula	Analyses[b]
23a	56	>300	270 (12300) 310 (8700)	$C_{13}H_{13}N_5O_5S_2 \cdot H_2O$	C, H, N, S
23b	75	210~215	268 (12500) 297 (10000)	$C_{13}H_{12}N_6O_5S_2 \cdot \frac{1}{2}H_2O$	C, H, N, S
23c	33	215~225	244 (19200) 270 (13600) 310 (6000)	$C_{13}H_{12}N_6O_5S_2 \cdot \frac{1}{2}H_2O$	C, H, N
23d	79	>300	257 (17700) 310 (6000)	$C_{13}H_{12}N_6O_4S_2 \cdot H_2O$	C, H, N, S
23e	83	245~250	237 (19500) 275 (12000) 310 s (5700)	$C_{12}H_{11}N_7O_5S_2 \cdot \frac{1}{2}H_2O$	C, H, N, S
23f	68	200~210	298 (12100)	$C_{13}H_{12}N_6O_5S_2 \cdot \frac{1}{2}H_2O$	C, H, N, S
23g	39	215~220		$C_{13}H_{12}N_6O_5S_2 \cdot \frac{1}{2}H_2O$	C, H, N, S
23h	64	215~220		$C_{15}H_{16}N_6O_5S_2 \cdot \frac{1}{2}H_2O$	C, H, N, S
23i	49	>300	275 (13100)	$C_{13}H_{12}N_6O_5S_2 \cdot H_2O$	C, H, N, S[c]
23j	47	>300		$C_{13}H_{12}N_6O_5S_2 \cdot H_2O$	C, H, N, S

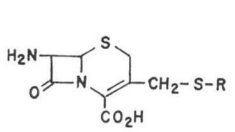
a: determined in 1% $NaHCO_3$ solution. b: Symbols of the elements indicate that analyses are coincident with the calculated value within $\pm 1\%$ deviation unless otherwise states. c: S, calcd, 16.78; found 17.90.

Table 4. 3-Substituted 7-(*o*-aminomethylphenylacetamido)cephalosporanic acids (**24**)



Compound	Yield, %	Mp, °C (dec)	λ_{max} nm (ε)[a]	Formula	Analyses[b]
24a	39	190~198	265 (9300) 305 (6400)	$C_{23}H_{22}N_6O_4S_2$	C, H, N
24b	60	180~187	265 (10000) 300 (9200)	$C_{22}H_{21}N_7O_4S_2 \cdot H_2O$	C, H, N, S
24c	66	205~210	256 (14000) 270 (13000) 310 s (6500)	$C_{22}H_{21}N_7O_4S_2 \cdot 2H_2O$	C, H, N
24d	85	>300	258 (19300) 310 s (6600)	$C_{22}H_{21}N_7O_5S_2 \cdot 2\frac{1}{2}H_2O$	C, H, N, S
24e	74	190~193	245 (18300) 270 (12800) 310 (6000)	$C_{21}H_{20}N_8O_4S_2 \cdot \frac{3}{2}H_2O$	C, H, N, S
24f	75	190	270 (18000) 295 s (12800)	$C_{22}H_{21}N_7O_4S_2 \cdot 3H_2O$	C, H, N, S
24g	36	175~183	273 (11000)	$C_{23}H_{22}N_6O_4S_2 \cdot 2\frac{1}{2}H_2O$	C, H, N, S
24h	71	190~200	270 (12000) 295 s (8300)	$C_{24}H_{25}N_7O_4S_2 \cdot H_2O$	C, H, N
24i	85	210~220	272 (12500)	$C_{22}H_{21}N_7O_4S_2 \cdot \frac{3}{2}H_2O$	C, H, N[c], S
24j	90	234~242	270 (13500)	$C_{22}H_{21}N_7O_5S_2 \cdot \frac{3}{2}H_2O$	C, H, N, S

a: determined in 1% $NaHCO_3$ solution. b: see, footnote b of Table 3. c: N, calcd. 18.20; found 16.68.



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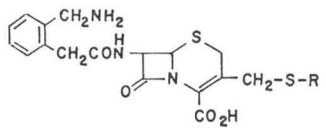

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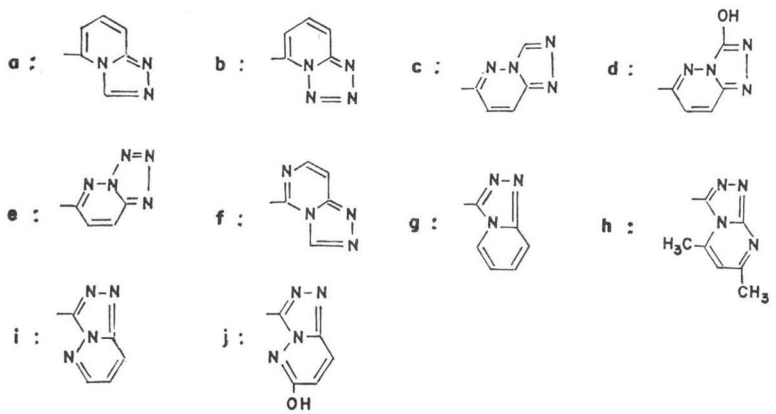
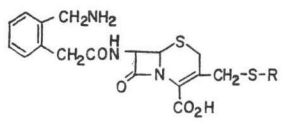
R



When this series of cephalosporins is classified by the heterocyclic ring which is directly attached to the sulfur atom in the 3-side chain (Table 5), a structure-activity relationship is observed similar to that with the monocyclic six-membered heterocyclic derivatives described in the previous paper[1]. As shown in Table 6, the pyridazine derivatives (**24c**, **24d** and **24e**) provided that most active members of this series. They were 2~8 times as active as CEZ and CET against staphylococci and 8~32 times against streptococci. Against gram-negative organisms **24d** and **24e** were 2~8 fold more active than CEZ and **24c** was nearly as active as CEZ. CET was generally less active than CEZ against the gram-negative organisms tested. The pyridine derivatives **24a** and **24b** were the second most active group. Compound **24b** was as active as CEZ against both gram-positive and gram-negative test organisms, while **24a** was slightly less active. The pyrimidine derivative **24f** was the least active one of the present family, although the gram-positive activity was at least equal to CEZ. The triazole derivatives (**24g**,

Table 5. Classification of cephalosporins **24**



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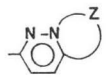
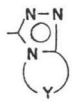
Class	R	Compd.
Pyridine derivatives		**24a** **24b**
Pyridazine derivatives		**24c** **24d** **24e**
Pyrimidine derivatives		**24f**
Triazole derivatives		**24g** **24h** **24i** **24j**

 = Pyridine, pyridazine or pyrimidine

 = Triazole or tetrazole

Table 6. *In vitro* activity of 3-substituted 7-(*o*-aminomethylphenylacetamido)cephalosporanic acids (**24**) by tube dilution method in nutrient broth

Comp.	MIC (mcg/ml)								
	Staphylococcus aureus Smith	*Staphylococcus aureus* BX-1633-2	*Streptococcus pyogenes* A 9604	*Streptococcus pneumoniae* A 9585	*Escherichia coli* NIHJ	*Escherichia coli* Juhl	*Klebsiella pneumoniae* A 9977	*Proteus mirabilis* A 9900	*Salmonella enteritidis* A 9531
24a	0.2	0.8	0.1	0.02	3.1	3.1	3.1	25	1.6
24b	0.1	0.4	0.01	0.01	1.6	3.1	3.1	6.3	0.8
24c	0.05	0.2	0.01	0.02	1.6	3.1	3.1	6.3	3.1
24d	0.1	0.2	0.0025	0.005	0.4	1.6	0.8	3.1	0.8
24e (BB-S226)	0.1	0.2	0.0025	0.005	0.4	0.8	0.8	1.6	0.8
24f	0.2	0.4	0.0025	0.01	0.8	12.5	12.5	25	3.1
24g	0.1	0.4	0.0025	0.0025	0.4	3.1	1.6	3.1	0.8
24h	0.4	0.8	0.005	0.02	0.8	3.1	3.1	12.5	1.6
24i	0.4	0.8	0.01	0.01	0.8	3.1	3.1	6.3	0.8
24j	0.8	1.6	0.31	0.31	3.1	12.5	3.1	3.1	1.6
CEZ	0.2	0.4	0.08	0.08	3.1	3.1	1.6	6.3	1.6
CET	0.4	0.8	0.08	0.16	12.5	25	6.3	6.3	1.6

Table 7. Cumulative percentage of 32 test organisms inhibited at indicated antibiotic concentrations

Antibiotic	Minimal inhibitory concentration (mcg/ml)														
	0.0125	0.025	0.05	0.1	0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	50	100	>100
BB-S 226	3.1	6.3	9.4	12	12	38	53	66	78	78	81	81	84	94	100
CET	0	0	0	6.3	13	25	34	38	53	69	69	69	75	78	100
CEZ	0	0	0	3.1	13	13	44	63	69	75	78	78	78	91	100

24h, **24i** and **24j**) had about the same range of antibacterial activity as the pyridine derivative **24a** and CEZ, and superior to CET against gram-negative bacteria. With an exception of **24j**, they were as active as CET against *Staphylococcus aureus* and more active against streptococcal strains. The observations described above indicate that the heterocycle linked directly to the sulfur atom in the 3-side chain plays a more important role for the antibacterial activity of this series than does the remaining ring of the bicyclic system.

The most active compound **24e** designated BB-S 226 was also evaluated by STEERS' agar dilution method on MUELLER-HINTON agar plates against 4 strains of gram-positive (3 *S. aureus* and 1 *Streptococcus faecalis*) and 28 strains of gram-negative bacteria (7 *E. coli*, 4 *K. pneumoniae*, 7 *Proteus* sp., 2 *P. aeruginosa*, 3 *Shigella* sp., 1 *Serratia marcescens*, 1 *Enterobacter cloacae*, 2 *Salmonella* sp. and 1 *Bacillus anthracis*). The results were compared with CEZ and CET by the cumulative percentage of MIC values (Table 7). This shows that BB-S 226 is distinctly more active than both of these clinically-used cephalosporins. More detailed evaluation on BB-S 226 will be reported in a separate paper.

Experimental

5-Chloro-*s*-triazolo[4,3-a]pyridine (**2**)

A mixture of 2-chloro-6-hydrazinopyridine[5] (4.7 g, 0.02 mol) and formic acid (30 ml) was refluxed for 6 hours and then evaporated to dryness. Water (10 ml) was added to the residue and the mixture was made alkaline with conc. NH_4OH and extracted with ethyl acetate (3 × 50 ml). The extracts were evaporated to dryness and the residue was crystallized from ethyl acetate (10 ml) - *n*-hexane (10 ml) to give 3 g (57%) of **2**. M.p. 73~78°C.

IR: ν_{max}^{nujol} 1670, 1630, 1495, 770 cm^{-1}.

NMR: $\delta_{ppm}^{DMSO\text{-}d_6}$ 6.9~7.4 (3H, m, pyridine-H), 9.3 (1H, s, triazole-H).

Anal. Calcd. for $C_6H_4ClN_3$: C, 46.93; H, 2.63; N, 27.36; Cl, 23.09.
Found: C, 46.79; H, 2.31; N, 27.56; Cl, 22.90.

5-Mercapto-*s*-triazolo[4,3-c]pyrimidine (**3**)

A mixture of 4.26 g (0.04 mole) of 4-hydrazino-2-mercaptopyrimidine[9] and 40 ml of formic acid was refluxed for 3 hours and evaporated to dryness. The residue was washed with water and crystallized from 50% aqueous DMSO to give 3 g (49.5%) of colorless needles **3** melting at >250°C.

IR: ν_{max}^{KBr} 1620, 1550, 1375, 1330, 1270 cm^{-1}.

UV: $\lambda_{max}^{0.1NNaOH}$ 301 nm (ε, 19000)

NMR: $\delta_{ppm}^{DMSO\text{-}d_6}$ 7.06 (1H, d, J=7.5 Hz, pyrimidine-H), 7.57 (1H, d, J=7.5 Hz, pyrimidine-H), 8.44 (1H, s, triazole-H), 13.5 (1H, br, SH).

Anal. Calcd. for $C_5H_4N_4S$: C, 39.46; H, 2.65; N, 37.82; S, 21.07.
Found: C, 39.21; H, 2.46; N, 37.36; S, 21.26.

2-Azido-6-chloropyridine (**8**)

To a mixture of 2-chloro-6-hydrazinopyridine (**7**)[5] (14.3 g, 0.1 mole) in 30% aqueous acetic acid (60 ml) was added dropwise a solution of sodium nitrite (8.97 g, 0.13 mole) in water (30 ml) with

stirring at 0~10°C. The reaction mixture was stirred for 45 minutes at room temperature to precipitate **8** which was collected by filtration, washed with water (20 ml), dried in the air and crystallized from ethyl acetate - *n*-hexane (50 ml, 1 : 1). Yield 8.0 g (52%). M.p. 79~80°C.

IR: ν_{max}^{nujol} 2130, 1580, 770 cm^{-1}.

Anal. Calcd. for $C_5H_3ClN_4$: C, 38.85; H, 1.96; N, 36.25; Cl, 22.94.
Found: C, 38.91; H, 1.65; N, 36.10; Cl, 22.21.

5-Mercapto-tetrazolo[4,5-a]pyridine (**9**)

A mixture of 2-azido-6-chloropyridine (**8**) (2.0 g, 0.013 mol) and KSH (1.5 g, 0.02 mole) in ethanol (30 ml) was refluxed for 2 hours and evaporated to dryness. The residue was dissolved in 20 ml of water and washed with ethyl acetate (3×10 ml). The aqueous layer was acidified to pH 2 with dil. HCl to precipitate **9** which was collected by filtration, dissolved in 2 N aqueous KOH (10 ml) and filtered. The filtrate was acidified to pH 2 with dil. HCl to give yellow needles **9**. Yield 0.12 g (10%). M.p. 126~128°C (dec.).

IR: ν_{max}^{nujol} 2400, 1616, 785 cm^{-1}.

Anal. Calcd. for $C_5H_4N_4S$: C, 39.46; H, 2.65; N, 36.82; S, 21.07.
Found: C, 39.28, 39.54; H, 2.30, 2.25; N, 36.54; S, 20.81.

3-Chloro-6-hydroxy-*s*-triazolo[4,3-b]pyridazine (**15**)

A solution of 3,6-dichloro-*s*-triazolo[4,3-b]pyridazine[6] (**14**) (5.0 g, 0.026 mole) in 50 ml of 5% aqueous sodium hydroxide was refluxed for 4 hours and acidified to pH 2 with dil. hydrochloric acid to precipitate the crude product. Crystallization from methanol gave colorless plates **15** melting at 255~261.°C. Yield 4.0 g (80%).

Anal. Calcd. for $C_5H_3ClN_4O$: C, 35.21; H, 1.77; N, 32.85; Cl, 20.79.
Found: C, 35.39; H, 1.14; N, 33.28; Cl, 20.76.

5-Mercapto-*s*-triazolo[4,3-a]pyridine (**17**)

A mixture of 5-chloro-*s*-triazolo[4,3-a]pyridine (**2**) (2.0 g, 0.013 mole) and KSH (2.0 g, 0.028 mole) in ethanol was heated in a sealed tube for 8 hours at 150°C. The reaction mixture was evaporated to dryness and the residue was dissolved in water (10 ml). The solution was filtered and the filtrate acidified to pH 1 with dil. HCl to precipitate **17** which was collected by filtration. The crude product was dissolved in aqueous 3 N KOH solution and, after filtration, acidified again with dil. HCl to give pure **17** which was collected by filtration, washed with water (20 ml) and dried *in vacuo* over P_2O_5. Yield 1.1 g (56%). M.p. 247~248°C.

Anal. Calcd. for $C_6H_5N_3S$: C, 47.66; H, 3.33; N, 27.79; S, 21.21.
Found: C, 47.64; H, 3.07; N, 27.74; S, 21.66.

6-Mercapto-3-hydroxy-*s*-triazolo[4,3-b]pyridazine (**19**)

A mixture of **11** (1.7 g, 0.01 mole), and KSH (1.44 g, 0.02 mole) in ethanol (30 ml) was heated in a sealed tube for 8 hours at 120°C to give 1.6 g (95%) of **19**. M.p. >300°C.

Anal. Calcd. for $C_5H_4N_4OS$: C, 35.71; H, 2.40; N, 33.31; S, 19.07.
Found: C, 35.17; H, 2.28; N, 33.38; S, 19.70.

3-Mercapto-*s*-triazolo[4,3-b]pyridazine (**20**)

A mixture of **13** (1.2 g, 0.08 mole) and KSH (1.2 g, 0.16 mole) in 20 ml of ethanol was heated for 8 hours at 130°C in a sealed tube to give 0.75 g (62%) of **20**. M.p. 260~270°C (dec.).

Anal. Calcd. for $C_5H_4N_4S \cdot \frac{1}{2}H_2O$: C, 37.26; H, 3.31; N, 34.76.
Found: C, 37.35; H, 3.32; N, 34.81.

3-Mercapto-6-hydroxy-*s*-triazolo[4,3-b]pyridazine (**21**)

A mixture of **15** (2.0 g, 0.012 mole) and NaSH (2.0 g, 0.036 mole) in ethanol (20 ml) was heated in a sealed tube for 20 hours at 160°C to give 1.03 g (50%) of **21**. M.p. 280~285°C.

3-Substituted 7-(*o*-aminomethylphenylacetamido)cephalosporanic acids (**23** and **24**)

The preparation of the 7-ACA derivatives **23** and the final products **24** was carried out in essentially the same manner for all the compounds listed in the Tables. The followings are representative procedures for **23** and **24**.

7-Amino-3-(tetrazolo[4,5-b]pyridazin-6-ylthiomethyl)-3-cephem-4-carboxylic acid (**23e**)

A stirred solution of 16.8 g (0.11 mole) of **18** and 18.48 g (0.22 mole) of $NaHCO_3$ in 1 liter of 0.1M phosphate buffer (pH 6.4) was heated at 50°C and to the solution was added portionwise 30 g (0.11 mole) of 7-ACA. The mixture was heated at 80°C for 2.5 hours. After the reaction mixture was cooled to room temperature, the precipitate **23e** was collected by filtration, washed thoroughly with 200 ml of water and air-dried. An additional amount of **23e** was obtained from the filtrate and the washings by acidifying to pH 5 with dil. HCl. Total yield 32.9 g (83%). M.p. 245~250°C (dec.).

Anal. Calcd. for $C_{12}H_{11}N_7O_3S_2 \cdot \frac{1}{2}H_2O$: C, 38.50; H, 3.23; N, 26.19; S, 17.13.
Found: C, 38.46; H, 2.75; N, 25.99; S, 17.35.

7-(*o*-Aminomethylphenylacetamido)-3-(tetrazolo[4,5-b]pyridazin-6-ylthiomethyl)-3-cephem-4-carboxylic acid (BB-S 226; **24e**)

To a solution of 20.26 g (0.047 mole) of 2,4-dinitrophenyl *o*-*t*-butoxycarbonylaminomethylphenylacetate in 150 ml of THF was added in one portion a solution of 14.4 g (0.039 mole) of 7-amino-3-(tetrazolo[4,5-b]pyridazin-6-ylthiomethyl)-3-cephem-4-carboxylic acid (**23e**) and 19.19 g (0.19 mole) of triethylamine in 150 ml of 50% aqueous tetrahydrofuran at 0~5°C. The reaction mixture was stirred for 18 hours and concentrated under reduced pressure below 30°C to remove the THF. The aqueous concentrate was washed with two 200-ml portions of ether, acidified to pH 2 with dil. hydrochloric acid and extracted with five 200-ml portions of ethyl acetate. The combined extracts were washed with two 200-ml portions of water, dried with anhydrous sodium sulfate, treated with active carbon and filtered. Evaporation of the solvent gave a pale yellow oil which was triturated with ether to give 13.89 g of the N-*t*-BOC-blocked cephalosporin. M.p. 166~173°C (dec.).

Trifluoroacetic acid (20 ml) was mixed with 13.8 g (0.022 mole) of the blocked cephalosporin and the mixture was stirred for 45 minutes at 0~10°C. To the mixture was added 300 ml of ether to precipitate the trifluoroacetate of the desired product, which was dissolved in 20 ml of water. The solution was adjusted to pH 5 with dil. ammonium hydroxide to give the zwitter-ion form of **24e** as a gummy oil. The oily product was separated by decantation, triturated with water, washed with 200 ml of acetonitrile and dried *in vacuo* to give 5.1 g of BB-S 226 (**24e**). The amorphous powder of BB-S 226 (3.5 g) was dissolved in 400 ml of 50% aqueous THF at 60~70°C under vigorous stirring. The solution was treated with a small amount of active carbon. The filtrate was cooled, scratched with a glass rod and allowed to stand overnight in a refrigerator to afford 2.27 g of fine needles **24e** melting at 190~193°C (dec.).

Anal. Calcd. for $C_{21}H_{20}N_8O_4S_2 \cdot \frac{3}{2}H_2O$: C, 46.74; H, 4.30; N, 20.77.
Found: C, 47.18, 47.37; H, 4.08, 3.88; N, 20.93; 20.23.

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