SEMISYNTHETIC β-LACTAM ANTIBIOTICS

I. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW UREIDOPENICILLIN DERIVATIVES HAVING CATECHOL MOIETIES

NOBUHIRO OHI*, BUNYA AOKI, TEIZO SHINOZAKI, KANZI MORO,
(The late) TAKAO NOTO, TOSHIYUKI NEHASHI,
HIROSHI OKAZAKI and ISAO MATSUNAGA

New Drug Research Laboratories, Chugai Pharmaceutical Co., Ltd.,
3-41-8 Takada, Toshima-ku, Tokyo 171, Japan

(Received for publication July 22, 1985)

The synthesis and antibacterial activity of new ureidopenicillin derivatives having catechol moieties in the 6-acyl side chain are described. These compounds showed remarkably strong activities against *Pseudomonas aeruginosa*. Especially, 6-[(*R*)-2-[3-(3,4-dihydroxybenzoyl)-3-methyl-1-ureido]-2-phenylacetamido]penicillanic acid (**7a**) had the most potent activity *in vitro* against Gram-negative bacteria, its activity being 30~60-fold greater than that of piperacillin against most strains of *P. aeruginosa*.

In the investigations of semisynthetic penicillin antibiotics, it has been reported that carbenicillin (CBPC)[1], sulbenicillin[2], ticarcillin (TIPC)[3], azlocillin (AZPC)[4], mezlocillin (MZPC)[5], piperacillin (PIPC)[6,7], furazlocillin (FRPC)[8] and apalcillin (APPC)[9] are especially effective against Gram-negative bacteria including *Pseudomonas aeruginosa*.

It is also well known that many microorganisms produce iron-sequestering substances (siderophores) which enhance the growth of bacteria. Some siderophores consisting of 2,3-dihydroxybenzoic acid conjugated with the amino group of specific amino acids have been isolated from several bacteria: *N*-(2,3-dihydroxybenzoyl)glycine from *Bacillus subtilis*[10] and *N,N'*-bis(2,3-dihydroxybenzoyl)-L-lysine from *Azotobacter vinelandii*[11]. A cyclic trimer of *N*-(2,3-dihydroxybenzoyl)-L-serine, named enterochelin[12] or enterobactin[13], was also isolated from *Escherichia coli* or *Salmonella typhimurium*. Furthermore, it was reported a few years ago that *P. aeruginosa* produces an iron-chelating phenolic compound, pyochelin[14~17]. We have been interested in these siderophores and have designed some catechol-containing ureidopenicillins in which the urea side chain is a constituent of AZPC, MZPC, PIPC and FRPC. Speculating on the fact that the siderophore-iron complex is readily incorporated into bacterial cells[18], we postulated that penicillins having an iron-sequestering moiety also be taken into a bacterial cell with the same facility.

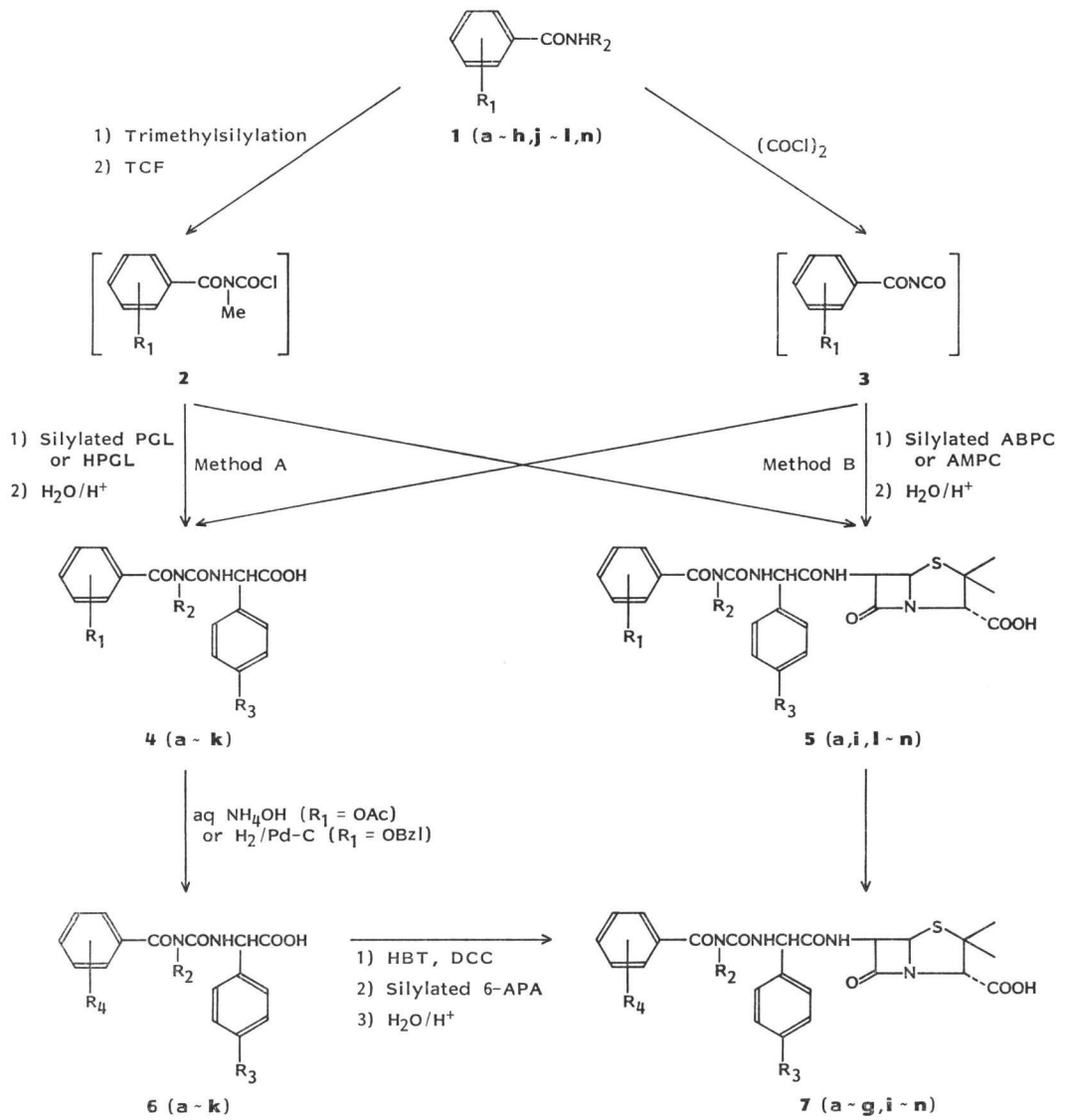
We observed that penicillins having catechol moieties showed strong activity against *P. aeruginosa*. We report here the synthesis and antibacterial activity of these new ureidopenicillins.

Chemistry

As outlined in Scheme 1, we prepared some ureidopenicillins having benzoyl moieties which are substituted with 1~3 hydroxyl groups.

These penicillins (**7**) were synthesized by acylation of silylated 6-aminopenicillanic acid (6-APA) with 2-ureidophenylacetic acids (**6**) activated with 1-hydroxybenzotriazole (Method A). The acids

Scheme 1.



	R_1	R_2	R_3
a	3,4-$(OAc)_2$	Me	H
b	3,4,5-$(OAc)_3$	Me	H
c	3,5-$(OAc)_2$	Me	H
d	3-Cl, 4-OAc	Me	H
e	3-OMe, 4-OAc	Me	H
f	3-OBzl	Me	H
g	4-OBzl	Me	H
h	2-OBzl	Me	H
i	3,4-$(OAc)_2$	Me	OH
j	2,3-$(OAc)_2$	H	H
k	2-OBzl	H	H
l	3,4-$(OAc)_2$	H	H
m	3,4-$(OAc)_2$	H	OH
n	3,4,5-$(OAc)_3$	H	H

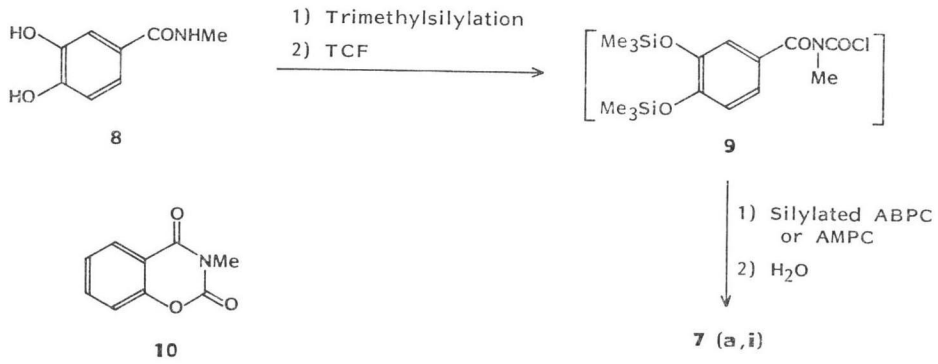
(for compounds **4**, **5** and **1**)

	R_4	R_2	R_3
a	3,4-$(OH)_2$	Me	H
b	3,4,5-$(OH)_3$	Me	H
c	3,5-$(OH)_2$	Me	H
d	3-Cl, 4-OH	Me	H
e	3-OMe, 4-OH	Me	H
f	3-OH	Me	H
g	4-OH	Me	H
h	2-OH	Me	H
i	3,4-$(OH)_2$	Me	OH
j	2,3-$(OH)_2$	H	H
k	2-OH	H	H
l	3,4-$(OH)_2$	H	H
m	3,4-$(OH)_2$	H	OH
n	3,4,5-$(OH)_3$	H	H

(for compounds **6**, **7**)

TCF: Trichloromethyl chloroformate, PGL: D-phenylglycine, HPGL: *p*-hydroxy-D-phenylglycine, HBT: 1-hydroxybenzotriazole, DCC: *N*,*N*-dicyclohexylcarbodiimide.

Scheme 2.



(**6a**~**i**; R_2=Me) were prepared by acylation of silylated D-phenylglycine (PGL) or *p*-hydroxy-D-phenylglycine (HPGL) with the *N*-methyl-*N*-substituted benzoylcarbamoyl chlorides (**2**) followed by removal of the acetyl or benzyl group by hydrolysis with 28% aqueous ammonia or hydrogenation, respectively. The carbamoyl chlorides (**2**) were prepared from the *N*-methylbenzamides (**1**) by silylation and chlorocarbonylation with trichloromethyl chloroformate (TCF). Alternatively, the acids (**6j**~**n**; R_2=H) were obtained by acylation of PGL or HPGL with the substituted benzoylisocyanates (**3**)[10] followed by similar treatments. The isocyanates (**3**) were derived from the benzamides (**1**) and oxalyl chloride. Another route (Method B) to those penicillins (**7**) was achieved by the reaction of silylated ampicillin (ABPC) or amoxicillin (AMPC) with the carbamoyl chlorides (**2**) or the benzoylisocyanates (**3**), followed by the removal of the acetyl groups with methanolic ammonia or tertiary amines bearing 1~3 hydroxyalkyl groups such as *N*,*N*-diethylethanolamine, *N*-ethyldiethanolamine or triethanolamine in dimethylformamide (DMF). The use of these tertiary amines is preferable to that of methanolic ammonia because the β-lactam cleavage by-products can be washed out with dilute acid. In the case of preparing **7a** and **7i**, Scheme 2, *N*-methyl-3,4-dihydroxybenzamide (**8**) was reacted with three molar equivalents of trimethylsilyl chloride for protecting the hydroxyl groups and simultaneously activating the amino group. The trimethylsilyl groups were easily removed *in situ* without β-lactam cleavage by treatment with water or cold aqueous sodium bicarbonate. (*R*)-2-[3-(*o*-Benzyloxybenzoyl)-3-methyl-1-ureido]phenylacetic acid (**4h**) obtained from *N*-methyl-*o*-benzyloxybenzamide (**1h**), TCF and PGL was hydrogenated to give (*R*)-2-[3-(*o*-hydroxybenzoyl)-3-methyl-1-ureido]phenylacetic acid (**6h**) as colorless glass. However, **6h** was gradually decomposed to *N*,*O*-carbonylsalicylamide (**10**) and D-phenylglycine during attemped crystallization. This decomposition was probably caused by attack of the *ortho* hydroxyl group on the ureido carbonyl.

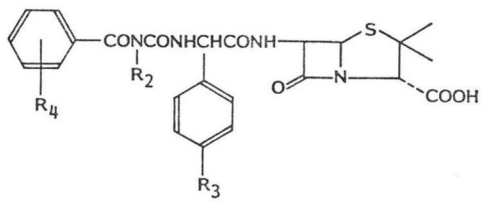
Curiously, (*R*)-2-[3-(2,3-dihydroxybenzoyl)-1-ureido]phenylacetic acid (**6j**) and (*R*)-2-[3-(*o*-hydroxybenzoyl)-1-ureido]phenylacetic acid (**6k**) also having an *ortho* hydroxyl group on the benzene ring could be obtained without decomposition.

Antibacterial Activity

The minimum inhibitory concentration (MIC) of the penicillins (**7**) against several Gram-positive and Gram-negative bacteria are shown in Table 1. For comparison, the MIC values of CBPC and PIPC are listed at the bottom of the table.

The following structure-activity relationships can be drawn from Table 1.

Table 1. Antibacterial activity of penicillins (7).



Compound No.	R_4	R_2	R_3	MIC (μg/ml)									
				S. aureus		*E. coli* NIHJ	*K. pneumoniae* 15c	*S. typhi* Tanaka	*P. mirabilis* 9	*P. vulgaris* Vu-4	*P. aeruginosa*		*S. marcescens* FU-104
				209P	JU-5[a]						J-272	J-169	
7a	3,4-$(OH)_2$	Me	H	0.2	3.12	<0.2	0.4	<0.2	0.78	6.25	0.2	0.4	3.12
7b	3,4,5-$(OH)_3$	Me	H	1.56	25	0.78	6.25	0.78	3.12	100	12.5	6.25	50
7c	3,5-$(OH)_2$	Me	H	<0.2	3.12	<0.2	6.25	3.12	6.25	12.5	50	100	25
7d	3-Cl, 4-OH	Me	H	<0.2	6.25	0.4	12.5	6.25	6.25	6.25	50	100	12.5
7e	3-OMe, 4-OH	Me	H	<0.2	6.25	0.4	25	6.25	12.5	12.5	50	50	25
7f	3-OH	Me	H	<0.2	1.56	<0.2	25	3.12	3.12	6.25	50	50	12.5
7g	4-OH	Me	H	<0.2	1.56	<0.2	12.5	3.12	3.12	6.25	50	50	12.5
7i	3,4-$(OH)_2$	Me	OH	0.4	6.25	<0.2	1.56	<0.2	0.78	25	0.2	0.4	25
7j	2,3-$(OH)_2$	H	H	0.4	6.25	<0.2	1.56	0.2	0.78	25	<0.2	0.2	12.5
7k	2-OH	H	H	<0.2	1.56	0.78	25	6.25	6.25	12.5	25	25	50
7l	3,4-$(OH)_2$	H	H	<0.2	3.12	0.4	6.25	0.2	1.56	>200	0.2	0.4	200
7m	3,4-$(OH)_2$	H	OH	0.2	3.12	<0.2	1.56	<0.2	0.78	>200	<0.2	0.2	100
7n	3,4,5-$(OH)_3$	H	H	0.4	100	1.56	>200	1.56	50	>200	0.78	1.56	>200
CBPC				<0.2	6.25	3.12	200	0.78	0.78	12.5	200	>200	>200
PIPC				<0.2	3.12	0.2	3.12	0.4	0.78	3.12	12.5	12.5	6.25

[a] Penicillinase producer.

Table 2. Antibacterial and therapeutic activities of **7a**, PIPC and CBPC.

Strains	Challenge dose (cfu/mouse)	ED_{50} (mg/mouse)			MIC (μg/ml)		
		7a	PIPC	CBPC	**7a**	PIPC	CBPC
E. coli 41	1×10^4 (100 LD_{50})	0.08	0.08	0.08	0.4	0.78	1.56
K. pneumoniae 3K25	1×10^3 (1,000 LD_{50})	0.72	1.11	27	1.56	3.12	200
P. aeruginosa NC-5	1×10^4 (1,000 LD_{50})	0.57	3.34	4.17	0.1	6.25	100
J-276	4×10^3 (80 LD_{50})	0.37	0.57	3.0	0.1	3.12	—
G-94	1×10^4 (120 LD_{50})	0.30	0.57	4.17	0.1	1.56	25
J-272	1×10^3 (9 LD_{50})	0.24	2.68	11.33	0.2	12.5	200

1) The number and the position of hydroxyl groups on the benzene ring showed considerable influence upon the activities against some of the Gram-negative bacteria. Against *P. aeruginosa*, the compounds (**7a, i, j, l, m**) with two adjacent hydroxyl groups were more active than **7b, n** having three adjacent hydroxyl groups, and the compounds (**7f, g, k**) having only one hydroxyl group were less active than **7b, n**. When two adjacent hydroxyl groups were separated or one of two adjacent groups was replaced by a methoxy group or a chlorine atom, the activities were definitely decreased. The penicillins (**7a, i, j, l, m**) proved strongly active *in vitro* against *P. aeruginosa* with a 30~60-fold increase in activity compared with PIPC. The catechol moiety was considered to be indispensable for a strong activity against *P. aeruginosa*.

2) Compound **7l**, having two adjacent hydroxyl groups located at the *meta* and *para* positions of the benzene ring, was generally less potent against Gram-negative bacteria including *P. aeruginosa* than the corresponding **7j**, having two hydroxyl groups at the *ortho* and *meta* positions.

3) In case of the penicillins with two or three adjacent hydroxyl groups, the compounds (**7a, b, i**) bearing a methyl group as the substituent (R_2) showed higher or equal activity compared to the *N*-unsubstituted analogs (**7l, m, n**) against the Gram-negative bacteria tested, except **7b** which was less active than **7n** against *P. aeruginosa*.

Following these results, **7a**, which had the most potent *in vitro* activity among the penicillins prepared, was tested *in vivo* against experimental infections in mice due to *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa*, Table 2 shows the ED_{50} (mg/mouse) and MIC of **7a**, PIPC and CBPC.

The compound, **7a**, was more effective than PIPC and CBPC against 4 strains of *P. aeruginosa* and *K. pneumoniae* 3K25. Against *E. coli* 41, it was similar in activity to PIPC and CBPC.

These results demonstrate that the new ureidopenicillin derivatives with catechol moieties possess powerful *in vitro* and *in vivo* activities against opportunistic, siderophore producing bacteria, especially *P. aeruginosa*.

Experimental

All melting points are uncorrected. IR spectra were recorded on a Hitachi EPI-G3 spectrometer. The NMR spectra were measured on a Hitachi R-20A spectrometer using TMS as internal standard. All chemical shifts are reported in δ ppm.

Table 3.

Compound No.	Yield (%)	MP (°C)	Formula	Analysis (%)*		
				C	H	N
1b	76.2	124	$C_{14}H_{15}N_7$	54.37	4.89	4.53
				(54.48	4.82	4.57)
1c	77.7	107~108	$C_{12}H_{13}NO_5$	57.37	5.22	5.58
				(57.30	5.10	5.51)
1d	78.5	147~148	$C_{10}H_{10}NO_3Cl$	52.76	4.43	6.15
				(52.49	4.41	6.27)
1e	78.0	121	$C_{11}H_{13}NO_4$	59.18	5.87	6.28
				(59.24	5.90	6.30)

* Calcd. Found in parentheses.

Determination of *In Vitro* Antibacterial Activity

All the *in vitro* antibacterial activities are reported as MIC in μg/ml. MIC's were determined by the agar dilution method using heart infusion agar (Difco) or Antibiotic Medium No. 3 agar after incubation at 37°C for 20 hours, with an inoculum size of about 10^6 cfu/ml. The latter medium were used to culture *Proteus vulgaris* Vu-4 and *Serratia marcescens* FU-104.

Therapeutic Activity in Experimental Infections in Mice

Male *dd*Y/slc mice, 5~6 week-old, were used. Bacteria, cultured on heart infusion agar plates overnight, were suspended in 5% gastric mucin and injected intraperitoneally into mice. The compounds were serially diluted 3-fold with saline and 0.2 ml of each dilution was administered subcutaneously to mice at 1 and 3 hours after the infection. Each experimental group consisted of 5 mice.

The dose in mg/mouse required to protect 50% of the mice from death (ED_{50}) for 7 days was calculated by the BEHRENS-KARBER method[20].

N-Methyl-3,4-diacetoxybenzamide (**1a**)

To a stirred suspension of 3,4-diacetoxybenzoic acid (70.0 g) and ethyl chloroformate (38.3 g) in CH_2Cl_2 (500 ml) was added triethylamine (35.7 g) at −15~−10°C. After stirring at the same temperature for 30 minutes, a solution of methylamine (13.7 g) in CH_2Cl_2 (150 ml) was added dropwise to the reaction mixture below −10°C. The mixture was stirred at −15~−10°C for 50 minutes and the organic layer was acidified by adding AcOH. After H_2O (100 ml) was added to the mixture, the organic layer was separated, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was triturated with ether and the resulting crystals were collected by filtration to give **1a** (55.0 g) as colorless granules: mp 141°C.

Anal Calcd for $C_{12}H_{13}NO_5$: C 57.37, H 5.22, N 5.58.
Found: C 57.55, H 5.26, N 5.63.

Other *N*-methyl substituted benzamides (**1b~e**) were also obtained from the corresponding benzoic acids. Yields, melting points and analytical data of these compounds are given in Table 3.

(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methyl-1-ureido]-2-phenylacetic Acid (**4a**)

To a solution of **1a** (25.0 g) in CH_2Cl_2 (200 ml) were added trimethylchlorosilane (16.2 g) and triethylamine (15.1 g) at 5~15°C. The mixture was stirred under reflux for 1 hour. After cooling, a solution of trichloromethyl chloroformate (14.8 g) in CH_2Cl_2 (20 ml) was added to the reaction mixture below −10°C. Stirring was continued for 2 hours at room temperature and for 1 hour at 25~30°C. The reaction mixture containing *N*-(3,4-diacetoxybenzoyl)-*N*-methylcarbamoyl chloride (**2a**) was used for the following reaction.

To a stirred suspension of D-phenylglycine (22.5 g) in CH_2Cl_2 (240 ml) was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (85 ml) at room temperature. After stirring for 15 minutes, trimethylchlorosilane (0.5 ml) was added to the mixture. Stirring was continued for 2 hours at room temperature. To the resulting solution was added the mixture containing (**2a**) described above at 0~10°C. After stirring at room temperature for 1 hour, the reaction mixture was evaporated *in vacuo*. To the

Table 4.

Compound No.	MP (°C)	Formula	Analysis (%)*		
			C	H	N
4e	183 (dec)	$C_{20}H_{20}N_2O_7$	59.99	5.04	7.00
			(60.08	5.11	7.04)
4f	160~161	$C_{24}H_{22}N_2O_5$	68.89	5.30	6.70
			(68.67	5.41	6.65)
4g	190~191	$C_{24}H_{22}N_2O_5$	68.89	5.30	6.70
			(68.60	5.32	6.71)
4h	163	$C_{24}H_{22}N_2O_5$	68.89	5.30	6.70
			(68.60	5.31	6.72)

* Calcd. Found in parentheses.

residue were added H_2O (100 ml) and EtOAc (200 ml). The organic layer was separated and extracted with saturated $NaHCO_3$ solution. The aqueous layer was adjusted to pH 2~3 with 2 N HCl and re-extracted with EtOAc (300 ml). The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was triturated with C_6H_6 to give **4a** (45.3 g) as colorless crystals: mp 130~131°C (dec).

Anal Calcd for $C_{21}H_{20}N_2O_8 \cdot C_6H_6$: C 64.02, H 5.17, N 5.53.
Found: C 64.08, H 5.21, N 5.52.

Compounds (**4b~h**) were also obtained from **1b~h** by treatment similar to that described for **4a**.

4b: Pale brown glass; NMR (DMSO-d_6) 2.21 (9H, s, $OCOCH_3$), 3.14 (3H, s, NCH_3), 5.49 (1H, d, J=6 Hz, -C*H*NH-), 7.1~7.5 (7H, m, phenyl protons), 9.88 (1H, d, J=6 Hz, -CONH-).

4c: Colorless glass; NMR (DMSO-d_6) 2.24 (6H, s, $OCOCH_3$), 3.09 (3H, s, NCH_3), 5.37 (1H, d, J=7 Hz, -C*H*NH-), 7.1~7.6 (8H, m, phenyl protons), 9.64 (1H, d, J=7 Hz, -CONH-).

4d: Colorless glass; NMR (DMSO-d_6) 2.33 (3H, s, $OCOCH_3$), 3.12 (3H, s, NCH_3), 5.36 (1H, d, J=7 Hz, -C*H*NH-), 7.2~7.9 (8H, m, phenyl protons), 9.65 (1H, d, J=7 Hz, -CONH-).

Melting points and analysis data of **4e~h** are given in Table 4.

(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methyl-1-ureido]-2-(*p*-hydroxyphenyl)acetic Acid (**4i**)

Compound (**4i**) was similarly prepared from **1a** and D-*p*-hydroxyphenylglycine by the method as described for **4a**, colorless crystals: mp 123~124°C (dec).

Anal Calcd for $C_{21}H_{20}N_2O_9 \cdot C_6H_6$: C 62.06, H 5.01, N 5.36.
Found: C 61.95, H 5.09, N 5.63.

(*R*)-2-[3-(2,3-Diacetoxybenzoly)-1-ureido]-2-phenylacetic Acid (**4j**)

To a solution of 2,3-diacetoxybenzamide (**1j**, 4.0 g) in 1,2-dichloroethane (40 ml) was added oxalyl chloride (5.3 g). The mixture was stirred under reflux for 10 hours. Excess oxalyl chloride and the solvent were evaporated *in vacuo*, and CH_2Cl_2 (40 ml) was added to the residue. To the solution was added silylated D-phenylglycine (5.3 g) in CH_2Cl_2 (100 ml) at 5~10°C. After stirring at the same temperature for 1.5 hours, 1 N HCl (100 ml) was added to the reaction mixture, and stirring was continued for 15 minutes. The precipitates formed were collected by filtration, and dissolved in saturated $NaHCO_3$ solution with cooling. After insoluble material was removed by filtration, the aqueous layer was adjusted to pH 2.5 with 2 N HCl. The precipitates were collected by filtration, washed successively with H_2O and Et_2O, and dried to give **4j** (4.5 g) as colorless powder: mp 200~201°C (dec).

Anal Calcd for $C_{20}H_{18}N_2O_8$: C 57.97, H 4.38, N 6.76.
Found: C 57.64, H 4.39, N 6.65.

Compound (**4k**) was also obtained from **1k** by the treatment similar to that described for **4j**.

4k: mp 215~218°C (dec).

Anal Calcd for $C_{23}H_{20}N_2O_5$: C 68.31, H 4.98, N 6.93.
Found: C 67.99, H 4.94, N 6.92.

6-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methyl-1-ureido]-2-phenylacetamido]penicillanic Acid (**5a**)

To a stirred suspension of ABPC (9.0 g) in CH_2Cl_2 (150 ml) was added BSA (12.8 ml) at room

Table 5.

Compound No.	Appearance	IR (KBr, cm^{-1})
6b	Colorless glass	1720, 1680, 1605, 1520, 1330
6c	Colorless glass	1730, 1690, 1600, 1520, 1365
6d	Colorless glass	1730, 1680, 1600, 1515, 1335
6e	Colorless glass	1730, 1680, 1595, 1510, 1330
6i	Colorless glass	1720, 1680, 1600, 1510, 1340

temperature. After stirring for 5 minutes, trimethylchlorosilane (0.5 ml) was added to the mixture under ice-cooling. Stirring was continued for 1 hour at room temperature. To the resulting solution was added the reaction mixture consisting of **2a** prepared from **1a** (5.0 g) at 0~10°C and stirring was continued at the same temperature for 1 hour. The mixture was poured into cold 1 N HCl (100 ml) and extracted with EtOAc. The extract was washed with brine. The organic layer was separated and extracted with saturated $NaHCO_3$ solution (200 ml). The aqueous layer was adjusted to pH 2.5 with 2 N HCl and re-extracted with EtOAc, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was triturated with *n*-hexane (100 ml) to give **5a** (7.5 g) as colorless powder: IR (KBr) 1780, 1720~1620, 1510 cm^{-1}; NMR ($CDCl_3$) 1.48 (6H, br s, C2-CH_3), 2.27 (6H, s, $OCOCH_3$), 3.18 (3H, s, NCH_3), 4.34 (1H, s, C3-H), 5.3~5.8 (3H, m, C5, C6-H and -C*H*NH-), 7.1~7.6 (9H, m, phenyl protons and -CONH-), 9.97 (1H, d, J=7 Hz, -CONH-).

Compound (**5i**) was also prepared by the method described for **5a.**

IR (KBr) 1770, 1720~1620, 1510 cm^{-1}; NMR (DMSO-d_6) 1.45 (3H, s, C2-CH_3), 1.57 (3H, s, C2-CH_3), 2.28 (6H, s, $OCOCH_3$), 3.11 (3H, s, NCH_3), 4.23 (1H, s, C3-H), 5.3~5.8 (3H, m, C5, C6-H and -C*H*NH-), 6.6~7.8 (7H, m, phenyl protons), 9.11 (1H, d, J=7 Hz, -CONH-), 9.56 (1H, d, J=7 Hz, -CONH-).

To a solution of silylated ABPC prepared from ABPC (12.8 g) and BSA (20.6 ml) in CH_2Cl_2 (100 ml) was added the reaction mixture consisting of **3l** prepared from **1l** (7.5 g) at 5~10°C. Stirring was continued at the same temperature for 2 hours. The same work-up as that described for **5a** was carried out to give **5l** (11.6 g) as colorless powder: IR (KBr) 1770, 1700~1620, 1525 cm^{-1}; NMR (DMSO-d_6) 1.42 (3H, s, C2-CH_3), 1.54 (3H, s, C2-CH_3), 2.28 (6H, s, $OCOCH_3$), 4.23 (1H, s, C3-H), 5.3~6.1 (3H, m, C5, C6-H and -C*H*NH-), 7.0~8.0 (8H, m, phenyl protons), 8.96 (1H, d, J=7 Hz, -CONH-), 9.07 (1H, d, J=7 Hz, -CONH-).

Compounds (**5m, n**) were similarly prepared by the method as described for **5l.**

5m: IR (KBr) 1770, 1710~1630, 1510 cm^{-1}; NMR (DMSO-d_6) 1.47 (3H, s, C2-CH_3), 1.56 (3H, s, C2-CH_3), 2.28 (6H, s, $OCOCH_3$), 4.24 (1H, s, C3-H), 5.3~6.0 (3H, m, C5, C6-H and -C*H*NH-), 6.6~8.2 (7H, m, phenyl protons), 9.0 (2H, m, -CONH-).

5n: IR (KBr) 1775, 1700~1600, 1520 cm^{-1}; NMR (DMSO-d_6) 1.46 (3H, s, C2-CH_3), 1.55 (3H, s, C2-CH_3) 2.30 (9H, s, $OCOCH_3$), 4.26 (1H, s, C3-H), 5.3~6.0 (3H, s, C5, C6-H and -C*H*NH-), 7.2~7.7 (5H, m, phenyl protons), 7.83 (2H, s, phenyl protons), 9.1 (2H, m, -CONH-).

(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-methyl-1-ureido)-2-phenylacetic Acid (**6a**)

To a solution of **4a** (2.0 g) in MeOH (20 ml) was added 28% NH_4OH (3 ml) under ice-cooling. After stirring at 0~5°C for 30 minutes, the solution was poured into 1 N HCl (50 ml) and extracted with EtOAc. The extracts were combined and washed with brine, dried over $MgSO_4$ and evaporated to dryness to give **6a** (1.3 g) as a colorless glass: IR (KBr) 1720, 1675, 1595, 1515 cm^{-1}.

Compounds (**6b, e, i, j**) were obtained from **4b, e, i, j** by the treatment similar to that described for **6a.** IR spectral data of **6b, e, i** are given in Table 5.

6j: mp 211~212°C (dec).

Anal Calcd for $C_{16}H_{14}N_2O_6 \cdot H_2O$: C 55.17, H 4.63, N 8.04.
Found: C 55.44, H 4.38, N 8.08.

(*R*)-2-[3-(*m*-Hydroxybenzoyl)-3-methyl-1-ureido]-2-phenylacetic Acid (**6f**)

To a solution of **4f** (10.0 g) in MeOH (200 ml) was added 10% palladium on carbon (1.0 g). The

Table 6. NMR and IR spectral data of **7**.

Compound No.	NMR δ value (DMSO-d_6)					IR$_{max}^{KBr}$ (cm^{-1})	
	2-CH_3 ($\times$2) (6H, each s)	3-H (1H, s)	6-NH (1H, d)	α-NH (1H, d)	R_2, 5-H, 6-H, α-H, R_4 and phenyl protons	β-Lactam	CONH
7a	1.41 1.54	4.20	9.21 (J=7 Hz)	9.68 (J=8 Hz)	3.10 (3H, s, NCH_3), 5.3~5.8 (3H, m, 5, 6 and α-H), 6.6~7.5 (8H, m, C_6H_3 and C_6H_5)	1775	1675
7b	1.42 1.56	4.20	9.19 (J=7 Hz)	9.67 (J=7 Hz)	3.10 (3H, s, NCH_3), 5.2~5.8 (3H, m, 5, 6 and α-H), 6.51 (2H, s, C_6H_2), 7.32 (5H, s, C_6H_5)	1770	1680
7c	1.41 1.54	4.19	9.16 (J=7 Hz)	9.70 (J=7 Hz)	3.02 (3H, s, NCH_3), 5.3~5.8 (3H, m, 5, 6 and α-H), 6.29 (3H, s, C_6H_3), 7.29 (5H, s, C_6H_5)	1775	1680
7d	1.41 1.56	4.22	9.22 (J=7 Hz)	9.58 (J=7 Hz)	3.11 (3H, s, NCH_3), 5.3~5.8 (3H, m, 5, 6 and α-H), 6.9~7.7 (8H, m, C_6H_3 and C_6H_5)	1775	1680
7e	1.42 1.55	4.24	9.28 (J=7 Hz)	9.76 (J=7 Hz)	3.14 (3H, s, NCH_3), 3.79 (3H, s, OCH_3), 5.3~5.9 (3H, m, 5, 6 and α-H), 6.7~7.6 (8H, m, C_6H_3 and C_6H_5)	1780	1680
7f*	1.49 1.56	4.30	8.10 (J=8 Hz)	10.04 (J=8 Hz)	3.11 (3H, s, NCH_3), 5.4~5.8 (3H, m, 5, 6 and α-H), 6.8~7.7 (9H, m, C_6H_4 and C_6H_5)	1780	1685
7g*	1.49 1.57	4.30	8.14 (J=8 Hz)	9.97 (J=7 Hz)	3.19 (3H, s, NCH_3), 5.4~5.9 (3H, m, 5, 6 and α-H), 6.8~7.7 (9H, m, C_6H_4 and C_6H_5)	1775	1675
7i	1.42 1.55	4.20	9.05 (J=7 Hz)	9.55 (J=7 Hz)	3.10 (3H, s, NCH_3), 5.3~5.7 (3H, m, 5, 6 and α-H), 6.5~7.4 (7H, m, C_6H_3 and C_6H_4)	1775	1675
7j	1.41 1.56	4.23	9.32 (J=7 Hz)	9.58 (J=7 Hz)	5.3~6.0 (3H, m, 5, 6 and α-H), 6.6~7.7 (8H, m, C_6H_3 and C_6H_5)	1770	1675
7k	1.42 1.57	4.26	9.37 (J=7 Hz)	9.62 (J=8 Hz)	5.3~6.0 (3H, m, 5, 6 and α-H), 6.8~8.1 (9H, m, C_6H_4 and C_6H_5)	1775	1670
7l	1.41 1.53	4.22	8.42 (J=8 Hz)	9.00 (J=7 Hz)	5.3~6.0 (3H, m, 5, 6 and α-H), 6.6~7.7 (8H, m, C_6H_3 and C_6H_5)	1770	1675
7m	1.43 1.57	4.22	9.16 (J=7 Hz)	9.57 (J=8 Hz)	5.3~5.9 (3H, m, 5, 6 and α-H), 6.5~7.6 (7H, m, C_6H_3 and C_6H_4)	1770	1675
7n	1.42 1.53	4.23	8.33 (J=7 Hz)	8.99 (J=8 Hz)	5.3~6.0 (3H, m, 5, 6 and α-H), 6.91 (2H, s, C_6H_2), 7.37 (5H, s, C_6H_5)	1770	1675

* Acetone-d_6.

The presence of catechol moiety in these compounds followed from the dark green color in the $FeCl_3$ test.

mixture was stirred at room temperature in hydrogen gas for 1 hour under atmospheric pressure. After the reaction, insoluble materials were removed by filtration, and the filtrate was evaporated under reduced pressure to give **6f** as colorless powder: NMR (DMSO-d_6) 3.09 (3H, s, NCH_3), 5.37 (1H, d, J=7 Hz, –C*H*NH–), 6.8~7.7 (9H, m, phenyl protons), 9.82 (1H, d, J=7 Hz, –CONH–).

Compounds (**6g, k**) were similarly prepared from **4g** and **4k** by the method as described for **6f**.

6g: mp 162~163°C (dec).

Anal Calcd for $C_{17}H_{16}N_2O_5$: C 62.19, H 4.91, N 8.53.
Found: C 61.94, H 4.91, N 8.43.

6k: mp 210~211°C (dec).

Anal Calcd for $C_{16}H_{14}N_2O_5$: C 61.14, H 4.49, N 8.91.
Found: C 60.96, H 4.38, N 8.90.

(*R*)-2-[3-(*o*-Hydroxybenzoyl)-3-methyl-1-ureido]-2-phenylacetic Acid (**6h**)

To a solution of **4h** (1.4 g) in THF (60 ml) was added 10% palladium on carbon (0.14 g). The mixture was stirred at room temperature in hydrogen gas for 7 hours under atmospheric pressure. After the reaction, insoluble materials were removed by filtration, and the filtrate was evaporated *in vacuo* to give oily substance of crude **6h**: NMR (DMSO-d_6) 3.01 (3H, s, NCH_3), 5.38 (1H, d, J= 7 Hz, –C*H*NH–), 6.8~8.1 (9H, m, phenyl protons), 9.98 (1H, d, J=7 Hz, –CONH–).

Crude **6h** was dissolved in EtOH (30 ml) and stirring was continued for 5 minutes under reflux. After removing the solvent *in vacuo*, THF (100 ml) was added to the residue, and the precipitate (0.35 g) of D-phenylglycine was collected by filtration. The mother liquor was concentrated *in vacuo* and treated with EtOH (10 ml) to give *N*-methyl-*N*,*O*-carbonylsalicyl amide (**10**) (0.37 g): mp 148°C (Ref 146°C).

6-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-methyl-1-ureido]phenylacetamido]penicillanic Acid (**7a**)

To a solution of **6a** (1.00 g) and 1-hydroxybenzotriazole (0.39 g) in THF (15 ml) was added *N*,*N*-dicyclohexylcarbodiimide (DCC) (0.72 g) under cooling, and the mixture was stirred at 0~5°C for 4 hours. The dicyclohexyl urea was removed by filtration. Then, the filtrate was used for the following reaction.

To a stirred suspension of 6-APA (1.26 g) in CH_2Cl_2 (50 ml) was added BSA (2.9 ml) at room temperature. After stirring for 5 minutes, trimethylchlorosilane (0.5 ml) was added to the mixture under ice-cooling. Stirring was continued for 1 hour at room temperature. To the resulting solution was added the filtrate containing activated ester described above at 5~10°C, and the mixture was stirred at the same temperature for 4 hours. The same work-up as that described for **5a** was carried out to give **7a** (0.80 g) as pale yellowish powder.

The penicillins (**7b~g, i~k**) were also obtained from **6b~g, i~k**, respectively, by the treatment similar to that described for **7a**. IR and NMR spectral data of these compounds are given in Table 6.

Preparation of **7a** from **5a**

Method 1: To a MeOH solution (5 ml) of **5a** (1.0 g) was added dropwise 3 ml of methanolic ammonia (0.075 g/ml), the mixture was stirred at −15~−10°C for 20 minutes. When the reaction was over, the solution was poured into 5% HCl (20 ml), layered with EtOAc (20 ml), and shaken under cooling. The EtOAc extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was triturated with *n*-hexane to afford **7a**.

Method 2: To a solution of **5a** (10.0 g) in DMF (10 ml) was added 2-diethylaminoethanol under ice-cooling, and stirring was continued at 25~30°C for 90 minutes. The same work-up as that described for Method 1 was carried out to give **7a** (6.7 g).

The penicillins (**7l~n**) were also obtained from **6l~n** by the treatment similar to that described for Method 1 or Method 2.

N-Methyl-3,4-dihydroxybenzamide (**8**)

To a solution of **1a** (50.0 g) in MeOH (150 ml) was added triethylamine (10 ml) under ice-cooling. After stirring at room temperature for 40 minutes, the resulting solution was concentrated *in vacuo* and the residue was triturated with EtOAc to give **8** (30.0 g) as colorless granules: mp 191°C.

Anal Calcd for $C_8H_6NO_3$: C 57.48, H 5.43, N 8.38.
Found: C 57.46, H 5.54, N 8.51.

Preparation of **7a** from **8**

To a stirred suspension of **8** (50.2 g) and trimethylchlorosilane (109 g) in EtOAc (800 ml) was dropwise added triethylamine (101 g) at 10~20°C. The mixture was stirred at 35~40°C for 1 hour. After cooling, trichloromethyl chloroformate (35.6 g) was added to the reaction mixture at 0~10°C. Stirring was continued for 3 hours at 20~30°C and then for 1 hour at the same temperature under reduced pressure to remove excessive phosgene. To the residue was added silylated ABPC (131 g) in EtOAc (900 ml) at 0~5°C. After stirring at 5~10°C for 1.5 hours, 1 N HCl (500 ml) was added to the reaction mixture and stirring was continued for 10 minutes. The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated to dryness to give a crude product, which was dissolved in aqueous $NaHCO_3$ (200 ml) and chromatographed over Diaion HP-20 with elution by H_2O and 30% aqueous acetone in order. The fractions containing the product were combined and adjusted to pH 2.5 with 2 N HCl after the addition of EtOAc (1.5 liters). The EtOAc layer was separated, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was triturated with *n*-hexane to give **7a** (97.6 g).

7i was similarly prepared from **8** and silylated AMPC by the method as described above.

Acknowledgments

The authors would like to dedicate this paper to Dr. T. NOTO, who died in 1984. We wish to thank Dr. S. TOMIOKA and Dr. T. MORI for their encouragment throughout this work.

References

1) BODEY, G. P. & L. M. TERRELL: *In vitro* activity of carbenicillin against Gram-negative bacilli. J. Bacteriol. 95: 1587~1590, 1968
2) TSUCHIYA, K.; T. OISHI, C. IWAGISHI & T. IWAHI: *In vitro* antibacterial activity of disodium α-sulfobenzylpenicillin. J. Antibiotics 14: 607~619, 1971
3) SUTHERLAND, R.; J. BURNETT & G. N. ROLINSON: α-Carboxy-3-thienylmethylpenicillin (BRL2288), a new semisynthetic penicillin: *In vitro* evaluation. Antimicrob. Agents Chemother. -1970: 390~395, 1971
4) STEWART, D. & G. P. BODEY: Azlocillin: *In vitro* studies of a new semisynthetic penicillin. Antimicrob. Agents Chemother. 11: 865~870, 1977
5) BODEY, G. P. & T. PAN: Mezlocillin: *In vitro* studies of a new broad-spectrum penicillin. Antimicrob. Agents Chemother. 11: 74~79, 1977
6) UEO, K.; Y. FUKUOKA, T. HAYASHI, T. YASUDA, H. TANI, M. TAI, Y. WATANABE, I. SAIKAWA & S. MITSUHASHI: *In vitro* and *in vivo* antibacterial activity of T-1220, a new semisynthetic penicillin. Antimicrob. Agents Chemother. 12: 455~460, 1977
7) FU, K. P. & H. C. NEU: Piperacillin, a new penicillin active against many bacteria resistant to other penicillins. Antimicrob. Agents Chemother. 13: 358~367, 1978
8) VERBIST, L.: Comparison of the activities of the new ureidopenicillins piperacillin, mezlocillin, azlocillin, and bay k 4999 against Gram-negative organisms. Antimicrob. Agents Chemother. 16: 115~119, 1979
9) NOGUCHI, H.; Y. EDA, H. TOBIKI, T. NAKAGOME & T. KOMATSU: PC-904, a novel broad-spectrum semisynthetic penicillin with marked antipseudomonal activity: Microbiological evaluation. Antimicrob. Agents Chemother. 9: 262~273, 1976
10) ITO, T. & J. B. NEILANDS: Products of "Low-iron Fermentation" with *Bacillus subtilis*: Isolation, characterization and synthesis of 2,3-dihydroxybenzoylglycine. J. Am. Chem. Soc. 80: 4645~4647, 1958
11) CORBIN, J. L. & W. A. BULEN: The isolation and identification of 2,3-dihydroxybenzoic acid and 2-*N*,6-*N*-di(2,3-dihydroxybenzoyl)-L-lysine formed by iron-deficient *Azotobacter vinelandii*. Biochemistry 8: 757~762, 1969
12) O'BRIEN, I. G. & F. GIBSON: The structure of enterochelin and related 2,3-dihydroxy-*N*-benzoylserine conjugates from *Escherichia coli*. Biochim. Biophys. Acta 215: 393~402, 1970
13) POLLACK, J. R. & J. B. NEILANS: Enterobactin, an iron transport compound from *Salmonella typhimurium*. Biochem. Biophys. Res. Commun. 38: 989~993, 1970
14) COX, C. D. & R. GRAHM: Isolation of an iron-binding compound from *Pseudomonas aeruginosa*. J.

Bacteriol. 137: 357~364, 1979
15) LIU, P. V. & F. SHOKRANI: Biological activities of pyochelins: Iron-chelating agents of *Pseudomonas aeruginosa*. Infect. Immun. 22: 878~890, 1978
16) COX, C. D.: Iron uptake with ferripyochelin and citrate by *Pseudomonas aeruginosa*. J. Bacteriol. 142: 581~587, 1980
17) COX, C. D.; K. L. RINEHART, M. L. MOORE & J. C. COOK: Pyochelin: Novel structure of an iron-chelating growth promotor for *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U.S.A. Biol. Sci. 78: 4256~4260, 1981
18) HOLLIFIELD, W. C., Jr. & J. B. NEILANDS: Ferric enterobactin transport system in *Escherichia coli* K-12. Extraction, assay and specificity of the outer membrane receptor. Biochemistry 17: 1922~1928, 1978
19) SPEZIALE, A. J. & L. R. SMITH: The reaction of oxalyl chloride with amides. II. Oxazolidinedione and acyl isocyanates. J. Org. Chem. 28: 1805~1811, 1963
20) KARBER, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihen-Versuche. Arch. Exp. Pharm. 162: 480~483, 1931