SEMISYNTHETIC β-LACTAM ANTIBIOTICS

II. EFFECT ON ANTIBACTERIAL ACTIVITY OF UREIDO *N*-SUBSTITUENTS IN THE 6-[(*R*)-2-[3-(3,4-DIHYDROXYBENZOYL)-1-UREIDO]-2-PHENYLACETAMIDO]PENICILLANIC ACIDS

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The synthesis and the relationship between *in vitro* and *in vivo* activities of 6-[(*R*)-2-[3-(3,4-dihydroxybenzoyl)-3-R_1-1-ureido]-2-phenylacetamido]penicillanic acids having $C_{2\sim8}$ alkyl or substituted alkyl groups as the substituents (R_1) are described. In this series, 6-[(*R*)-2-[3-(3,4-dihydroxybenzoyl)-3-(3-hydroxypropyl)-1-ureido]-2-phenylacetamido]penicillanic acid (**1b**, AO-1100) showed the most potent protective effect on mice in experimental *Pseudomonas aeruginosa* infections, although it did not have the strongest *in vitro* activity among the penicillins we synthesized.

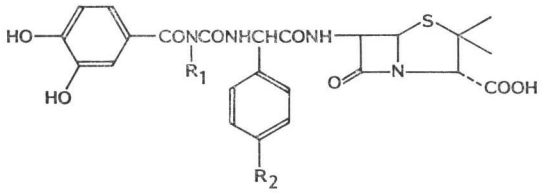
In our previous publication,[1] we reported that 6-[(*R*)-2-[3-(3,4-dihydroxybenzoyl)-3-methyl-1-ureido]-2-phenylacetamido]penicillanic acid (**1a**), bearing a catechol moiety, showed strong *in vitro* activities against Gram-negative bacteria including *Pseudomonas aeruginosa*, but it had weaker *in vivo* activity than expected from the *in vitro* activities. Therefore, we continued the study on the relationship between *in vitro* and *in vivo* activity of 6-[(*R*)-2-[3-(3,4-dihydroxybenzoyl)-3-R_1-1-ureido]-2-phenylacetamido]penicillanic acids bearing $C_{2\sim8}$ alkyl or substituted alkyl groups as the substituents (R_1) on the urea bond in order to improve the *in vivo* activity.

This paper describes the synthesis of new penicillins (**1**) and the results of our structure-activity studies.

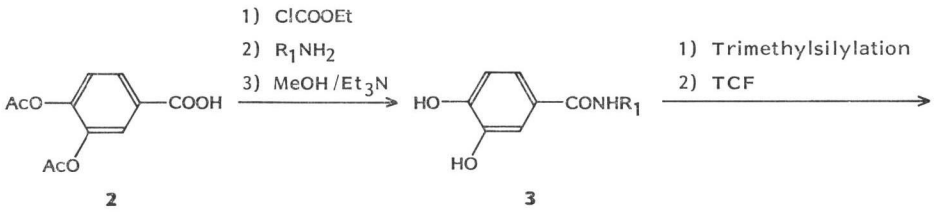
Chemistry

As outlined in Scheme 1, the penicillins (**1**) were prepared by the reaction of silylated ampicillin (ABPC) or amoxicillin (AMPC) with *N*-R_1-*N*-(3,4-bistrimethylsilyloxybenzoyl)carbamoyl chlorides (**4**) obtained by chlorocarbonylation of silylated *N*-R_1-3,4-dihydroxybenzamides (**3**) with trichloromethyl chloroformate (TCF). The benzamides (**3**) were derived by amidation of 3,4-diacetoxybenzoic acid (**2**) with various primary amines by the mixed anhydride method followed by deacetylation. In case of preparing the penicillins (**1b**, **1h**, **1i**, **1j**) having a hydroxyl group in the substituent (R_1), the hydroxyl group was protected with trimethylsilyl by the addition of one additional equivalent of trimethylsilyl chloride. 6-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-(3-hydroxypropyl)-1-ureido]-2-phenylacetamido]penicillanic acid (**1b**) was obtained in 89.2% yield by using dichlorodimethylsilane as silylating agent. The yield was about 30% higher than that achieved with trimethylsilyl chloride. This reaction proceeds probably *via* the cyclic intermediate (**5**). The selective cleavage of the two acetoxy groups on the

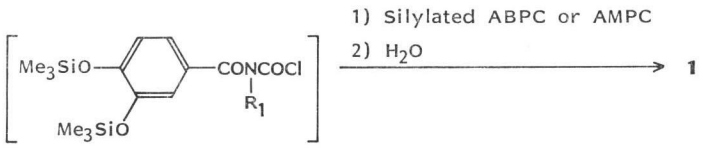
Scheme 1.



1a R_1 = Me, R_2 = H
1b R_1 = $(CH_2)_3OH$, R_2 = H



2 **3**



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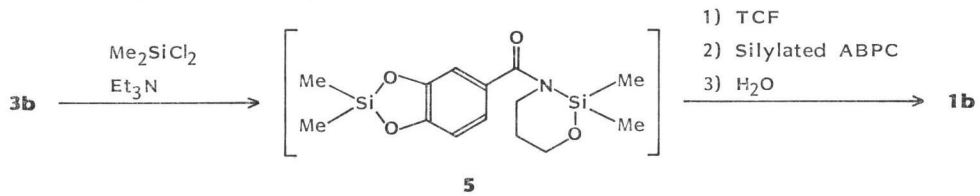

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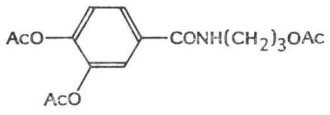
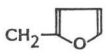
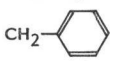
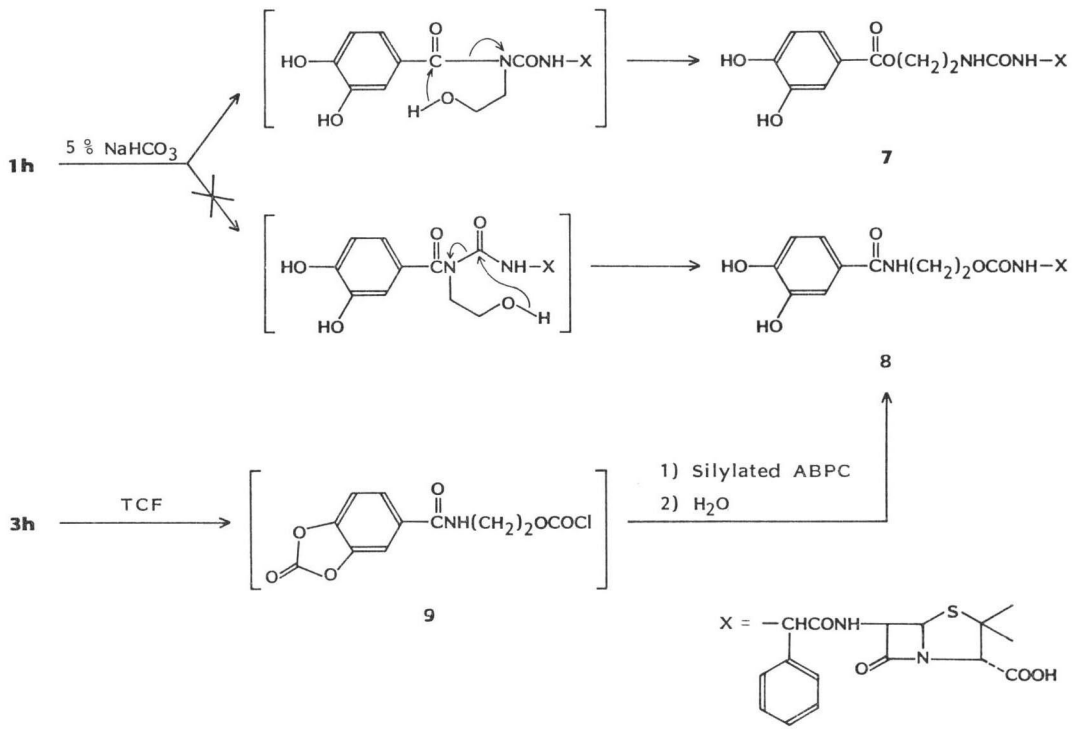


6

	R_1		R_1
a	CH_3	**k**	$(CH_2)_2OCH_3$
b	$(CH_2)_3OH$	**l**	$CH_2COOC_2H_5$
c	C_2H_5	**m**	CH_2CN
d	*n*-C_3H_7	**n**	$(CH_2)_2CN$
e	*n*-C_4H_9	**o**	
f	*n*-C_6H_{13}		
g	*n*-C_8H_{17}	**p**	
h	$(CH_2)_2OH$		
i	$(CH_2)_5OH$	**q**	$(CH_2)_3OAc$
j	$(CH_2)_2O(CH_2)_2OH$		

TCF: Trichloromethyl chloroformate.

Scheme 2.



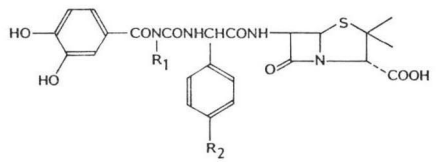
benzene ring of *N*-(3-acetoxypropyl)-3,4-diacetoxybenzamide (**6**) was carried out by treatment with 28% NH_4OH at $-20\sim-15°C$ to afford *N*-(3-acetoxypropyl)-3,4-dihydroxybenzamide (**3q**). 6-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-(2-hydroxyethyl)-1-ureido]-2-phenylacetamido]penicillanic acid (**1h**) was very unstable under basic conditions and trans-acylated easily to 6-[(*R*)-2-[3-[2-(3,4-dihydroxybenzoyloxy)ethyl]-1-ureido]-2-phenylacetamido]penicillanic acid (**7**) as shown in Scheme 2. The structure of compound (**7**) was deduced from a similar, previously reported conversion,[2] and is supported by IR and NMR spectral data. In order to exclude the possibility of the formation of isomeric compound (**8**), we synthesized **8** by reaction of silylated ABPC with 2-(3,4-*o*,*o'*-carbonyldioxybenzamido)ethyloxycarbonyl chloride (**9**) and demonstrated the spectral properties of **8** to be different from those of **7**.

Antimicrobial Activity

Table 1 lists the minimum inhibitory concentrations (MIC) of the penicillins (**1**) against several bacteria, and the results of their protective effect on mice against *P. aeruginosa* J-272 infections.

When the substituents (R_1) are alkyl groups ($C_{1\sim8}$), the length of carbon chains had little effect on *in vitro* activity against *Staphylococcus aureus* 209P, *Escherichia coli* NIHJ and *Klebsiella pneumoniae* 3K25. However, the longer the carbon chains, the weaker the *in vitro* activities were against *P. aeruginosa*. When R_1 were alkyl groups substituted with a hydroxyl, ethoxycarbonyl or cyano group, the penicillins (**1b**, **1i**, **1j**, **1l**, **1m**, **1n**) were found more active against *P. aeruginosa* J-169 than the penicillins (**1k**, **1o**, **1p**) with a methoxy, phenyl or furfuryl group at R_1. The penicillin (**1h**) bearing a hydroxyethyl group as the substituent (R_1) generally showed weak activity against all bacteria tested. This weak activity may be attributed to the conversion of **1h** to **7**. On the other hand, the penicillin (**1d**) with an *n*-propyl group showed more potent *in vivo* activity against *P. aeruginosa* J-272 infections

Table 1. Antibacterial and therapeutic activities of penicillins (**1**).



Compound No.	R_1	R_2	Configuration of C	MIC (μg/ml)								ED_{50}[b] (mg/mouse)
				S. aureus		*E. coli* NIHJ	*K. pneumoniae* 3K25	*P. mirabilis* 9	*S. marcescens* FU-104	*P. aeruginosa*		
				209P	JU-5[a]					J-272	J-169	
1a	CH_3	H	*R*	0.2	3.12	≤0.2	0.1	0.78	3.12	0.2	0.4	2.30
1b	$(CH_2)_3OH$	H	*R*	0.78	3.12	0.4	0.4	1.56	12.5	0.4	0.78	0.61
1c	C_2H_5	H	*R*	0.4	3.12	0.78	0.4	1.56	12.5	0.2	0.78	2.91
1d	n-C_3H_7	H	*R*	0.78	3.12	0.4	0.2	0.2	6.25	0.78	3.12	1.14
1e	n-C_4H_9	H	*R*	0.78	3.12	0.2	0.2	0.4	3.12	0.78	6.25	>3.0
1f	n-C_6H_{13}	H	*R*	0.78	3.12	0.4	0.2	0.1	3.12	1.56	12.5	>3.0
1g	n-C_8H_{17}	H	*R*	0.78	3.12	0.4	0.4	0.4	3.12	3.12	100	>3.0
1h	$(CH_2)_2OH$	H	*R*	3.12	3.12	12.5	6.25	1.56	50	1.56	12.5	n.t.
1i	$(CH_2)_5OH$	H	*R*	1.56	3.12	0.4	0.4	0.78	3.12	0.4	0.4	>3.0
1j	$(CH_2)_5O$–$(CH_2)_2OH$	H	*R*	0.4	3.12	0.4	0.78	1.56	3.12	0.2	0.78	1.48
1k	$(CH_2)_2OCH_3$	H	*R*	0.78	6.25	0.4	0.4	0.2	6.25	0.78	3.12	1.52
1l	$CH_2COOC_2H_5$	H	*R*	0.4	1.56	0.78	0.4	0.4	50	0.2	0.4	2.20
1m	CH_2CN	H	*R*	0.78	3.12	3.12	3.12	12.5	>200	0.4	0.4	2.00
1n	$(CH_2)_2CN$	H	*R*	0.78	3.12	0.4	0.2	0.78	3.12	0.1	0.78	>3.0
1o		H	*R*	0.78	3.12	0.4	0.2	0.78	3.12	0.1	0.78	>3.0
1p		H	*R*	0.4	3.12	0.4	0.2	1.56	3.12	0.4	3.12	2.70
1q	$(CH_2)_3OAc$	H	*R*	1.56	6.25	0.78	0.78	0.78	12.5	0.4	3.12	1.71
1r	$(CH_2)_3OH$	OH	*R*	0.78	6.25	0.78	0.4	1.56	50	0.2	0.78	1.18
1s	$(CH_2)_3OH$	H	*S*	1.56	6.25	3.12	100	25	n.t.	100	100	n.t.
PIPC				0.2	3.12	0.2	1.56	0.78	6.25	12.5	12.5	10.0
APPC				0.4	3.12	0.2	1.56	0.78	25	6.25	6.25	n.t.

[a] Penicillinase producer. [b] *P. aeruginosa* J-272 (challenge dose: 1×10^4 cfu/ml). n.t.: Not tested.

Table 2. Protective effect of **1b** against systemic infection in mice.

Strains	Challenge dose (cells/mouse)	ED_{50} (mg/mouse)			MIC (μg/ml)		
		1b	PIPC	APPC	**1b**	PIPC	APPC
E. coli 41	2×10^5 (1,000 LD_{50})	0.062	0.096	0.70	1.56	0.78	3.12
K. pneumoniae 3K25	1.1×10^4 (5,000 LD_{50})	0.12	0.30	0.061	0.4	1.56	1.56
P. aeruginosa							
NC-5	1×10^4 (500 LD_{50})	0.46	3.35	3.35	0.05	6.25	3.12
J-166	1.2×10^5 (100 LD_{50})	0.24	3.35	3.35	0.4	6.25	6.25
GNB-70	3×10^2 (100 LD_{50})	0.72	0.90	1.39	0.4	3.12	3.12

PIPC: Piperacillin, APPC: apalcillin.
IP challenge with 5% mucin, subcutaneous administration at 1 and 3 hours after challenge.

than the penicillins (**1a**, **1c**) with a methyl or ethyl group, although **1d** was less active *in vitro* than **1a** and **1c**. Furthermore, the penicillin (**1b**) which has a terminal hydroxyl group in the *n*-propyl group of **1d** showed the most potent *in vivo* activity among the all penicillins we synthesized. The penicillin (**1q**) with an acetoxy group, derived from **1b**, was three times less potent than **1b** against *P. aeruginosa* J-272 infection, and the penicillin (**1s**), the L-diastereoisomer of **1b**, was not active *in vitro*.

Based on these results, the penicillin (**1b**), which showed the most potent protective effect on mice against experimental *P. aeruginosa* J-272 infection, was evaluated *in vivo* in mice against other Gram-negative bacteria in comparison with piperacillin (PIPC) and apalcillin (APPC). The results are shown in Table 2.

The penicillin (**1b**) was remarkably more active than PIPC and APPC against *P. aeruginosa* NC-5 and J-166; its activity was 2.5 times that of PIPC against *K. pneumoniae* 3K25. Against *P. aeruginosa* GNB-70 and *E. coli* 41, it was as active as PIPC.

Recently, the significance of iron in infection has aroused interest,[3] and it has been reported that the iron-binding proteins transferrin and lactferrin, in combination with antibodies, often show powerful bacteriostatic effects *in vitro* and are essential for protection against many infections.[4~9]

The new ureido penicillin (**1b**, AO-1100) contains a catechol moiety which is capable of binding to iron. We expect that this penicillin (**1b**, AO-1100) applied *in vivo* may behave like those iron-binding proteins to show bacteriostatic activity. Further studies are in progress to evaluate this penicillin.

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.

Determination of *In Vitro* Antibacterial Activity

All the *in vitro* antibacterial activities are given as MIC in μg/ml required to prevent growth of the bacterial culture. 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 was used to culture *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

Table 3.

Compound No.	Yield (%)	MP (°C)	Formula	Analysis (%)*		
				C	H	N
3c	67.2	147	$C_9H_{11}NO_3$	59.66	6.12	7.73
				(59.42	6.12	7.88)
3d	68.5	157	$C_{10}H_{13}NO_3$	61.52	6.72	7.18
				(61.33	6.69	7.27)
3e	66.3	174~175	$C_{11}H_{15}NO_3$	63.14	7.23	6.69
				(63.39	7.23	6.83)
3f	69.1	140	$C_{13}H_{19}NO_3$	65.80	8.07	5.90
				(66.01	8.04	5.95)
3g	70.2	122~123	$C_{15}H_{23}NO_3$	67.90	8.74	5.28
				(68.16	8.84	5.59)
3h	35.1	154~155	$C_9H_{11}NO_4$	54.82	5.62	7.10
				(54.74	5.61	7.00)
3i	45.2	113~114	$C_{12}H_{17}NO_4$	60.24	7.16	5.85
				(60.27	7.19	6.07)
3k	42.0	142~143	$C_{10}H_{13}NO_4$	56.86	6.20	6.63
				(56.84	6.17	6.64)
3l	58.3	161~163	$C_{11}H_{13}NO_5$	55.23	5.48	5.86
				(55.13	5.43	6.10)
3m	45.0	193~194	$C_9H_8N_2O_3$	56.25	4.20	14.58
				(55.95	4.16	14.49)
3n	60.4	155~157	$C_{10}H_{10}N_2O_3$	58.25	4.89	13.58
				(57.95	4.99	13.40)
3o	68.2	193~194	$C_{12}H_{11}NO_4$	61.80	4.75	6.01
				(61.35	4.66	6.10)
3p	70.1	194~195	$C_{14}H_{13}NO_3$	69.12	5.39	5.76
				(68.82	5.35	5.83)

* Calcd. Found in parentheses.

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.[10]

N-(3-Hydroxypropyl)-3,4-dihydroxybenzamide (**3b**)

To a stirred suspension of 3,4-diacetoxybenzoic acid (30.0 g) and ethyl chloroformate (15.0 g) in EtOAc (300 ml) was added triethylamine (14.0 g) at −15~−10°C. After stirring at the same temperature for 30 minutes, 3-aminopropanol (12.2 g) in EtOAc (60 ml) was dropwise added to the reaction mixture below −10°C. The mixture was stirred at −15~−10°C for 1 hour and the organic layer was acidified by adding AcOH. After H_2O (200 ml) was added to the mixture, the organic layer was separated, washed successively with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was dissolved in MeOH (200 ml) and triethylamine (1.0 ml) was added to the solution at room temperature. After stirring at the same temperature for 40 minutes, the reaction solution was concentrated *in vacuo* and the residue was treated with EtOAc. The resulting crystals were collected by filtration to give **3b** (11.7 g) as colorless granules: mp 176~178°C.

Anal Calcd for $C_{10}H_{13}NO_4$: C 56.87, H 6.20, N 6.63.
Found: C 56.64, H 6.12, N 6.43.

Other *N*-substituted alkyl-3,4-dihydroxybenzamides (**3c~p**) were similarly obtained from 3,4-diacetoxybenzoic acid and the corresponding amines by the mixed anhydride method following deacetylation. Yield, melting points and analytical data of these compounds except **3j** are given in Table 3. **3j** was obtained as colorless glass: IR (KBr) 3700~2200, 1620, 1590, 1540, 1500, 1110, 1060

cm^{-1}; NMR (DMSO-d_6) 3.48 (8H, br s), 6.6~7.5 (3H, m).

N-(3-Acetoxypropyl)-3,4-diacetoxybenzamide (**6**)

Compound **3b** (20.0 g) was suspended in acetic anhydride (150 ml) and a catalytic amount of pyridine was added to the stirred suspension. After stirring at 40~50°C for 1 hour, the reaction mixture was poured into ice-water and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$ to give **6** (25.5 g) as colorless crystals: mp 63~64°C.

Anal Calcd for $C_{16}H_{19}NO_7$: C 56.97, H 5.68, N 4.15.
Found: C 56.98, H 5.66, N 4.18.

N-(3-Acetoxypropyl)-3,4-dihydroxybenzamide (**3q**)

To a solution of **6** (3.0 g) in MeOH (50 ml) was added 28% NH_4OH (2.2 ml) at −20~−15°C. After stirring at the same temperature, AcOH was added to the reaction mixture until the organic layer was acidified. The mixture was poured into ice-water and **3q** (1.7 g) was obtained. The crude product was recrystallized from MeOH - EtOAc: mp 160°C.

Anal Calcd for $C_{12}H_{15}NO_5$: C 56.91, H 5.97, N 5.53.
Found: C 56.90, H 6.02, N 5.49.

6-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-(3-hydroxypropyl)-1-ureido]-2-phenylacetamido]penicillanic Acid (**1b**)

The Preparation of **1b** by Using Trimethylsilyl Chloride as Silylating Agent (Method A): To a stirred suspension of **3b** (21.1 g) and trimethylsilyl chloride (52.2 g) in EtOAc (600 ml) was added triethylamine (48.6 g). After stirring at 40~45°C for 1 hour, trichloromethyl chloroformate (10.9 g) in EtOAc (30 ml) was added to the reaction mixture at 0~2°C. Stirring was continued while allowing the temperature to rise to room temperature over 2 hours and furthermore for 1 hour at 25~30°C. Silylated ABPC which was prepared from ABPC (42.0 g), trimethylsilyl chloride (28.7 g) and triethylamine (26.8 g) in EtOAc (600 ml) was added to the mixture at 0~5°C. After stirring at the same temperature for 1.5 hours, the reaction mixture was poured into ice-water. The organic layer was separated after the addition of THF (300 ml) and extracted with cold saturated $NaHCO_3$ solution (500 ml). The aqueous layer was adjusted to pH 2.5 with 2 N HCl and re-extracted with EtOAc. The extract was washed with brine, separated, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was treated by *n*-hexane to give **1b** (35.0 g) as pale yellow powder.

The Preparation of **1b** by Using Dichlorodimethylsilane as Silylating Agent (Method B): A suspension of **3b** (21.1 g), dichlorodimethylsilane (32.3 g) and triethylamine (50.5 g) in THF (600 ml) was stirred under reflux for 1 hour and trichloromethyl chloroformate (10.9 g) in THF (30 ml) was added to the mixture at 5~10°C. After stirring at room temperature for 4 hours, silylated ABPC (40.2 g) in THF was added to the reaction mixture at 0~5°C and stirring was continued for 1 hour. The same work-up as that described for Method A was carried out to give **1b** (52.3 g) as pale yellow powder.

Purification of **1b**: Crude **1b** (35.0 g) was dissolved in 5% $NaHCO_3$ solution (120 ml) and chromatographed over Diaion HP-20 with elution by H_2O and then 25% aqueous acetone. The fractions containing the product were combined and adjusted to pH 2.5 with 2 N HCl after the addition of EtOAc. The organic layer was separated, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was triturated with *n*-hexane to give **1b** (21.0 g) as colorless powder.

Compounds (**1c~g, i~s**) were prepared according to Method A. In the case of preparing **1r** and **1s**, AMPC and 6-[(*S*)-2-amino-2-phenylacetamido]penicillanic acid, respectively, were utilized in place of ABPC.

NMR and IR spectral data of these compounds are given in Table 4.

6-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-(3-hydroxyethyl)-1-ureido]-2-phenylacetamido]penicillanic Acid (**1h**)

Compound **1h** was prepared from **3h** (4.0 g) by Method A. However, as **1h** was very unstable under basic conditions, the purification was carried out by chromatography on Sephadex LH-20 eluted

Table 4. IR and NMR spectral data of **1**.

Compound No.	IR ν_{max}^{KBr} cm^{-1} (C=O)	NMR δ value (DMSO-d_6)
1b	1775, 1700~1640	1.4~2.0 (2H, m), 1.41 (3H, s), 1.55 (3H, s), 3.2~4.0 (4H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.2 (2H, m)
1c	1775, 1700~1630	0.8~1.3 (3H, m), 1.41 (3H, s), 1.56 (3H, s), 3.4~4.0 (2H, m), 4.21 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.13 (1H, d, J=7 Hz), 9.30 (1H, d, J=7 Hz)
1d	1770, 1700~1640	0.6~1.9 (5H, m), 1.43 (3H, s), 1.56 (3H, s), 3.4~3.9 (2H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.6 (8H, m), 9.11 (1H, d, J=7 Hz), 9.21 (1H, d, J=7 Hz)
1e	1770, 1710~1630	0.7~1.7 (7H, m), 1.42 (3H, s), 1.56 (3H, s), 3.5~3.9 (2H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.11 (1H, d, J=7 Hz), 9.21 (1H, d, J=7 Hz)
1f	1770, 1710~1640	0.7~1.8 (11H, m), 1.43 (3H, s), 1.57 (3H, s), 3.5~3.9 (2H, m), 4.21 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.16 (1H, d, J=7 Hz), 9.27 (1H, d, J=7 Hz)
1g	1770, 1700~1640	0.7~1.8 (15H, m), 1.41 (3H, s), 1.55 (3H, s), 3.4~3.9 (2H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.14 (1H, d, J=7 Hz), 9.27 (1H, d, J=7 Hz)
1h	1770, 1700~1640	1.41 (3H, s), 1.56 (3H, s), 3.2~4.0 (4H, m), 4.21 (1H, s), 5.3~5.8 (3H, m), 6.7~7.7 (8H, m), 9.23 (1H, d, J=7 Hz), 9.31 (1H, d, J=7 Hz)
1i	1770, 1700~1630	1.3 (6H, br s), 1.41 (3H, s), 1.55 (3H, s), 3.1~3.9 (4H, m), 4.21 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.14 (1H, d, J=7 Hz), 9.24 (1H, d, J=7 Hz)
1j	1775, 1700~1640	1.41 (3H, s), 1.55 (3H, s), 3.3~4.1 (8H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.6 (8H, m), 9.08 (1H, d, J=7 Hz), 9.20 (1H, d, J=7 Hz)
1k	1775, 1700~1650	1.41 (3H, s), 1.56 (3H, s), 3.18 (3H, s), 3.40 (2H, m), 3.85 (2H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.6 (8H, m), 9.11 (1H, d, J=7 Hz), 9.18 (1H, d, J=7 Hz)
1l	1770, 1730, 1700~1650	0.8~1.4 (3H, m), 1.41 (3H, s), 1.55 (3H, s), 3.8~4.7 (4H, m), 4.20 (1H, s), 5.3~5.9 (3H, m), 6.6~7.7 (8H, m), 8.7~9.8 (2H, m)
1m	1770, 1700~1660	1.41 (3H, s), 1.56 (3H, s), 4.20 (1H, s), 4.6 (2H, br s), 5.3~5.8 (3H, m), 6.8~7.6 (8H, m), 9.21 (2H, d, J=7 Hz)
1n	1770, 1700~1650	1.41 (3H, s), 1.55 (3H, s), 2.78 (2H, m), 3.98 (2H, m), 4.20 (1H, s), 5.3~5.8 (3H, m), 6.7~7.5 (8H, m), 9.09 (1H, d, J=7 Hz), 9.14 (1H, d, J=7 Hz)
1o	1770, 1700~1650	1.41 (3H, s), 1.55 (3H, s), 4.20 (1H, s), 4.8~5.1 (2H, br s), 5.3~5.8 (3H, m), 6.1~7.6 (11H, m), 9.18 (2H, d, J=7 Hz)
1p	1770, 1700~1640	1.42 (3H, s), 1.55 (3H, s), 4.21 (1H, s), 4.8~5.1 (2H, br s), 5.3~5.8 (3H, m), 6.7~7.6 (13H, m), 9.16 (1H, d, J=7 Hz), 9.24 (1H, d, J=7 Hz)
1q	1775, 1730, 1700~1640	1.41 (3H, s), 1.5~2.1 (2H, m), 1.55 (3H, s), 1.88 (3H, s), 3.6~4.1 (4H, m), 4.21 (1H, s), 5.3~5.8 (3H, m), 6.8~7.6 (8H, m), 9.17 (1H, d, J=7 Hz), 9.30 (1H, d, J=7 Hz)
1r	1770, 1700~1640	1.3~1.9 (2H, m), 1.42 (3H, s), 1.56 (3H, s), 3.1~4.0 (4H, m), 4.20 (1H, s), 5.3~5.7 (3H, m), 6.5~7.3 (7H, m), 9.0 (2H, m)
1s	1775, 1700~1640	1.3~1.9 (2H, m), 1.49 (3H, s), 1.61 (3H, s), 3.2~4.0 (4H, m), 4.21 (1H, s), 5.3~5.7 (3H, m), 6.7~7.5 (8H, m), 9.09 (1H, d, J=7 Hz), 9.19 (1H, d, J=7 Hz)

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

with acetone to give **1h** (1.2 g) and **7** (1.5 g) as colorless powder.

6-[(*R*)-2-[3-(2-(3,4-Dihydroxybenzoyloxy)ethyl)-1-ureido]-2-phenylacetamido]penicillanic Acid (**7**)

Compound **1h** (2.0 g) was dissolved in saturated $NaHCO_3$ solution (50 ml). After stirring at room temperature for 10 minutes, the aqueous solution was acidified by adding AcOH at 0~5°C and extracted with a mixture of EtOAc (50 ml) and THF (50 ml). The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed over Diaion HP-20 with elution by H_2O and then 25% aqueous acetone to give **7** (0.7 g) as colorless powder.

IR (KBr) 1775, 1695, 1650, 1550, 1530 cm^{-1}; NMR (DMSO-d_6) 1.45 (3H, s), 1.59 (3H, s), 3.2~3.7 (2H, m), 4.0~4.5 (2H, m), 4.27 (1H, s), 5.3~5.8 (3H, m), 6.52 (1H, br s), 6.8~7.7 (9H, m), 9.20 (1H, d, J=7 Hz).

6-[(*R*)-2-[[2-(3,4-Dihydroxybenzamido)ethyloxycarbonyl]amino]-2-phenylacetamido]penicillanic Acid (**8**)

To a stirred suspension of **1h** (30.0 g) and triethylamine (5.1 g) in EtOAc (100 ml), trichloromethyl chloroformate (2.7 ml) was added dropwise below −5°C. Stirring was continued for 3 hours and then for 1 hour at 25~30°C under reduced pressure to remove excess phosgene. To the residue was added silylated ABPC (6.9 g) in EtOAc (100 ml) at 0~5°C and the mixture was stirred for 1 hour at the same temperature. The same work-up as that described for **1b** was carried out to give **8** (1.5 g) as colorless powder: IR (KBr) 1770, 1715, 1670, 1505 cm^{-1}; NMR (DMSO-d_6) 1.42 (3H, s), 1.56 (3H, s), 3.3~3.8 (2H, m), 3.9~4.4 (2H, m), 4.22 (1H, s), 5.3~5.8 (3H, m), 7.1~8.0 (8H, m), 8.3 (1H, br s), 9.05 (1H, d, J=7 Hz).

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