DIRECT INTRODUCTION OF A FORMAMIDO GROUP INTO THE 7α(6α)-POSITION OF CEPHALOSPORINS (PENICILLINS)†

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A novel direct introduction of a formamido group into the 7α(6α)-position of cephalosporins (penicillins) was achieved by treatment of 7β(6β)-[(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)methylimino]cephem (penam) esters with *N*,*N*-bis(trimethylsilyl)formamide, followed by deblocking with Girard reagent T to give the corresponding 7α(6α)-formamido-7β(6β)-amino derivatives. Three 7α-formamidocephalosporins were prepared by the conventional *N*-acylation of 7α-formamidocephem. All of them were resistant to β-lactamases, showing similar MIC values against both of a pair of a β-lactamase-producing strain and the corresponding non or low-producing strain of the same species of bacteria, when tested on *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Enterobacter cloacae* and *Citrobacter freundii*.

A new semisynthetic penicillin (BRL 36650)[1] and cephalosporin[2] having a formamido group at the 6α and 7α-positions, respectively, were reported to be active against Gram-negative bacteria including *Pseudomonas aeruginosa* and resistant to β-lactamases. On the other hand, naturally occurring 7α-formamidocephalosporins have been isolated independently from culture filtrates of *Flavobacterium* sp. SC12,154[3], *Flavobacterium chitinovorum* sp.[4], *Lysobacter lactamgenus* YK-90[5~8] and *Xanthomonas lactamgena* YK-280 and YK-278[5~8]. The formamido derivatives of monocyclic β-lactams were also isolated from fermentation broth of *Flexibacter alginoliquefaciens* sp. YK-49[9,10]. These naturally occurring compounds were reported to be resistant to β-lactamases, although their intrinsic activity was weak.

Herein we wish to report a simple and direct introduction of a formamido group at the 7α-position of cephalosporin and the 6α-position of penicillin.

Chemistry

There have been developed several methods[11~15] for the introduction of a formamido function into the 7α(6α)-position of cephalosporins and penicillins. However, most of them are indirect; they require functionalization of the 7α(6α)-position by conversion to methylsulfide[11], sulfoxide[12], amino[11] or succinimido-oxy groups[13], before the incorporation of a formamido moiety. There have been no methods available for direct introduction of a formamido group at the 7α(6α)-position, except those reported by PEARSON *et al.*[14,15]. They achieved direct conversion of 7β(6β)-*N*,*N*-disubstituted derivatives into the corresponding 7α(6α)-formamido derivatives by treatment with *N*,*N*-bis(trimethylsilyl)formamide (BSF) and triethylamine. Although this method was applied to the preparation of penicillin derivatives in good yield, some problems were encountered in the case of cephalosporins. 7α-Formamido cephems were obtained only in moderate yield with concomitant formation of the inseparable Δ^2 isomer. As reported by PEARSON and his colleagues[15], putative imine intermediates play a key role in introduction of a formamido group at the 7α-position. It was anticipated that if this imine analog could be formed without the presence of

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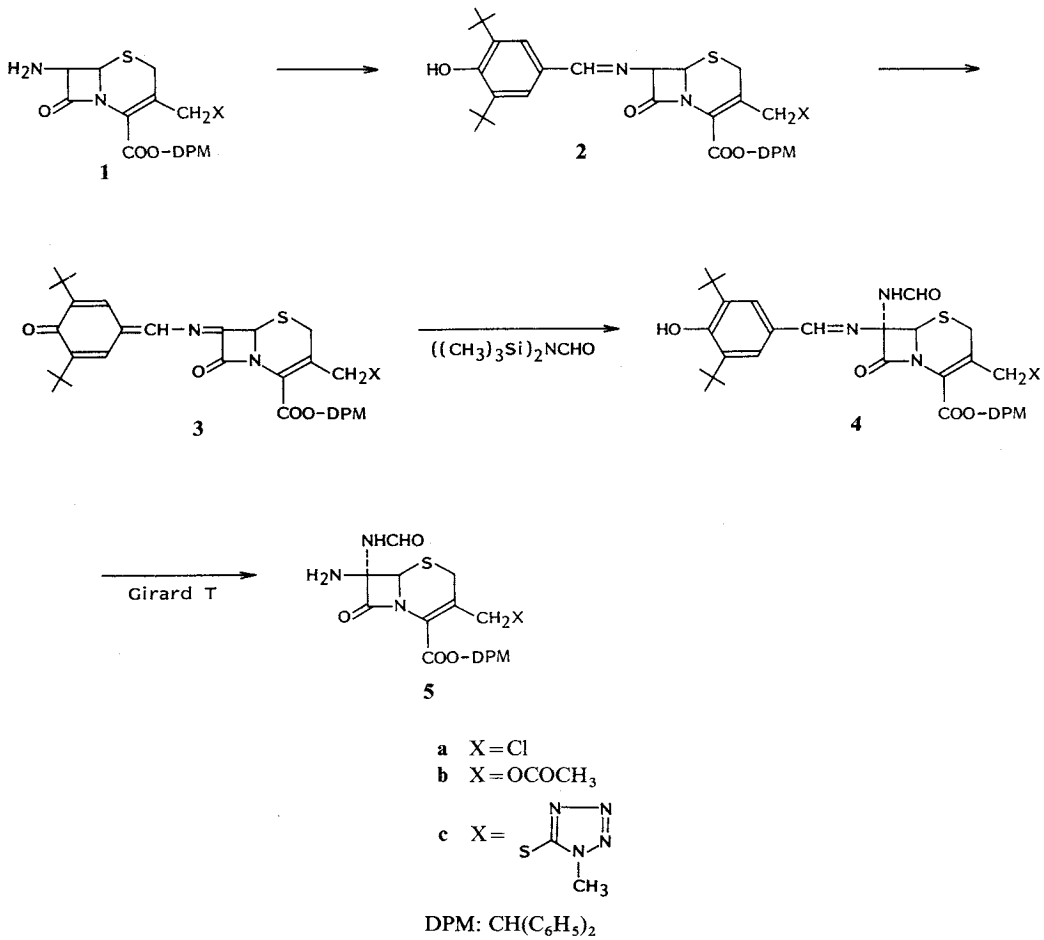
base, subsequent reaction with BSF might yield the desired 7α-formamidocephem avoiding the generation of the thermodynamically stable Δ^2 isomer. Therefore, we tried to apply the quinoid intermediates **3** having a 7-iminocephem substructure, which were reported by YANAGISAWA *et al.*[16,17], to the introduction of a formamido group at the 7α-position by attack from the convex side and successfully obtained the 7α-formamido cephems (Scheme 1).

The Schiff base **2a**, which was prepared from diphenylmethyl 3-chloromethylcephalosporanate **1a**[18], was oxidized with PbO_2 in benzene to give the quinoidal cephem intermediate **3a**. After removal of PbO_2 and the solvent, **3a** was treated with BSF in CH_2Cl_2 and chromatographed on a column of silica gel to give the 7α-formamido derivative **4a** in 58% yield without formation of the Δ^2 isomer. Reaction of the quinoid **3a** with BSF in benzene was much more sluggish than in CH_2Cl_2. Removal of the benzylidene group by treatment with Girard reagent T gave 7β-amino-3-chloromethyl-7α-formamidocephalosporanate **5a** as colorless crystals in 49% overall yield from **1a**. Similarly crystalline 7α-formamido cephem **5b** and 7α-formamido-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl cephem **5c** were also prepared from the corresponding quinoid **3**[16,17] in 61 and 52% overall yields, respectively. The *Z* and *E*-rotamers (4:1) around the formamido C-N bond were observed in the ^1H NMR spectra of **5a**, **5b** and **5c**[11].

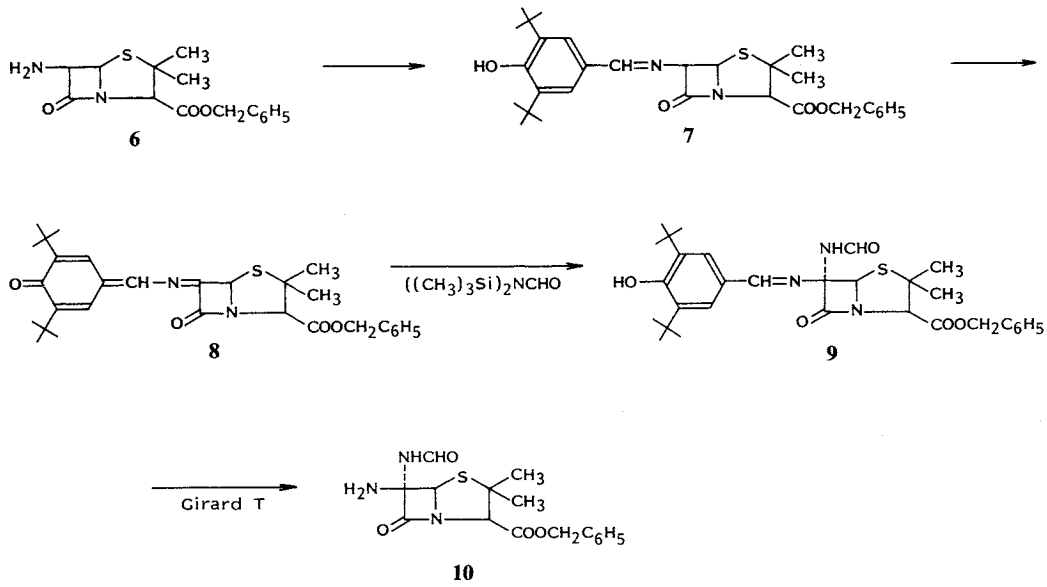
Scheme 1. Preparation of 7β-amino-7α-formamidocephalosporanates (**5**).



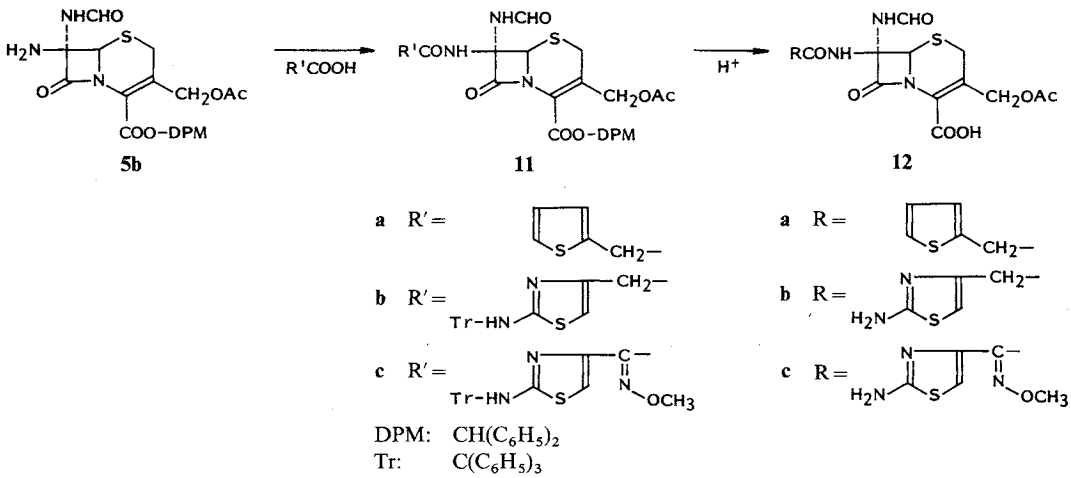
This method has some remarkable features compared with the reported one. First, quinoid **2** was prepared from the corresponding diphenylmethyl 7β-aminocephalosporanate in two steps without purification and then 7α-formamido cephems **5** were simply obtained in a good yield; secondly, the undesired Δ^2 isomer, which is frequently inseparable, was not detected in the products.

This methodology was also applied to penicillin derivatives (Scheme 2). The quinoidal penam intermediate **8**, prepared by oxidation of the Schiff base **7** with PbO_2, was treated with BSF to give the 6α-formamido derivative **9** in 46% yield. Compound **9** was deblocked with Girard reagent T and chromatographed on a silica gel column to afford a 93% yield of **10**, which was identical with that prepared by the reported method[11] in IR and ^{1}H NMR spectra, and HPLC behavior. The ^{1}H NMR spectrum of

Scheme 2. Preparation of 6β-amino-6α-formamidopenicillanate (**10**).



Scheme 3. Preparation of 7α-formamidocephalosporins (**12**)



10 also showed two rotamers (*Z* and *E*) about the formamido N-C bond in the ratio of 4:1 as described in the literature.

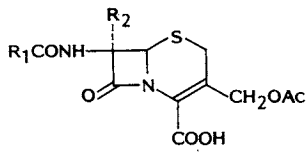
Compound **5b** was acylated with thienylacetic acid and deblocked with TFA in CH_2Cl_2 to give 7α-formamidocephalothin (**12a**)[19] (Scheme 3). In a similar way, 7β-[2-(2-aminothiazol-4-yl)acetamido]-7α-formamidocephalosporanic acid (**12b**) and 7α-formamidocefotaxime (**12c**)[20] were synthesized by 7-*N*-acylation of **5b** followed by deblocking.

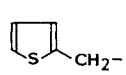
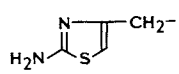
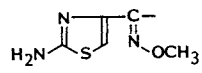
Microbiological Activity

Table 1 shows MICs of three 7α-formamidocephalosporins, **12a**, **12b** and **12c**, compared with those of the corresponding 7α-unsubstituted derivatives, cephalothin (CET), **13**[21] and cefotaxime (CTX), against selected pairs of β-lactamase-non or low-producing organisms and the corresponding β-lactamase-producing strains. All pairs except for the *Staphylococcus aureus* group consist of a clinical isolate producing β-lactamase constitutively and its mutant lacking or having reduced β-lactamase activity[22], which were kindly provided by Prof. SAWAI of Chiba University.

Comparing the activity of the 7α-formamidocephalosporins of Table 1 with that of the corresponding 7α-unsubstituted derivatives, introduction of a formamido group to the 7α-position of the cephem nucleus results in decrease of the activity against the strains susceptible to 7α-unsubstituted derivatives. However,

Table 1. *In vitro* activity of 7α-formamidocephalosporins (**12a**, **12b** and **12c**) and the corresponding 7α-unsubstituted derivatives.



Organism	R_1						
	R_2	NHCHO **12a**	H CET	NHCHO **12b**	H **13**	NHCHO **12c**	H CTX
		MIC (μg/ml)					
Staphylococcus aureus Smith		3.1	0.4	12.5	0.8	25	1.6
S. aureus BX-1633[a]		3.1	0.4	12.5	1.6	25	3.1
Klebsiella pneumoniae GN69/2-1		50	3.1	25	0.8	50	0.05
K. pneumoniae GN69[a]		50	6.3	25	0.8	50	0.05
Escherichia coli 255/L-7		25	50	25	0.8	50	0.1
E. coli 255[b]		25	>100	25	>50	50	6.3
Proteus mirabilis N-29/2		12.5	6.3	25	0.4	25	0.05
P. mirabilis N-29[b]		12.5	>100	25	12.5	25	0.4
P. vulgaris GN76/C-1/2		6.3	6.3	25	0.8	12.5	0.05
P. vulgaris GN76/C-1[b]		6.3	>100	25	>50	25	6.3
Morganella morganii 1510/9		6.3	25	25	3.1	25	0.05
M. morganii 1510[b]		12.5	>100	25	>50	25	6.3
Enterobacter cloacae 363/1		12.5	3.1	25	0.8	25	0.05
E. cloacae 363[b]		50	>100	50	>50	>50	25
Citrobacter freundii GN346/16		25	25	25	6.3	50	0.1
C. freundii GN346[b]		50	>100	50	>50	50	25

[a] Penicillinase producing strain.
[b] Cephalosporinase producing strain.

the MIC values of the 7α-formamidocephalosporins against β-lactamase-high-producting strains of the present study were same as or higher by 2-fold or at most 4-fold than those against the corresponding β-lactamase-non or low-producing strains. On the contrary, the 7α-unsubstituted derivatives showed big differences between their MIC values against cephalosporinase-producing strains (*Escherichia coli* 255, *Proteus mirabilis* N-29, *Proteus vulgaris* GN76/C-1, *Morganella morganii* 1510, *Enterobacter cloacae* 363 and *Citrobacter freundii* GN346) and the corresponding non- or low-producing strains, although the activity against penicillinase-producing strains (*Staphylococcus aureus* BX-1633 and *Klebsiella pneumoniae* GN69) was similar to that against the corresponding non- or low-producing strains of the same species.

These results show that **12a**, **12b** and **12c** are stable to all of the β-lactamases of test organisms used in this study, whereas the corresponding 7α-unsubstituted derivatives (CET, **13** and CTX) are stable to penicillinases produced by *S. aureus* BX-1633 and *K. penumoniae* GN69, but susceptible to the β-lactamases produced by the other six Gram-negative organisms tested.

Experimental

MP's were determined with a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra were recorded on a Jasco IRA-1 and UV spectra on a Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on a Jeol CL-60HL (60 MHz), Varian FT-80A (80 MHz) or Jeol GX-400 (400 MHz) spectrometer and mass spectra on a Jeol AX505H mass spectrometer. MICs were determined on solid medium by the standard 2-fold agar dilution method[23] in Mueller-Hinton Agar (Difco) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml.

Diphenylmethyl 7β-(3,5-Di-*tert*-butyl-4-hydroxybenzylideneamino)-3-chloromethyl-3-cephem-4-carboxylate (**2a**)

A mixture of diphenylmethyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate **1a** (2.07 g, 5 mmol) and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (1.17 g, 5 mmol) in dry benzene (40 ml) was distilled during a period of 1 hour (bath temperature, 95°C) to remove water azeotropically. The resulting oil was dried under reduced pressure to give 3.24 g of the product. IR ν_{max} (KBr) cm^{-1} 3600, 2950, 1780, 1720, 1630, 1240; ^1H NMR (80 MHz, $CDCl_3$) δ 1.44 (18H, s, *tert*-Bu), 3.36, 3.65 (2H, ABq, J=18 Hz, 2-H), 4.22, 4.44 (2H, ABq, J=13 Hz, 3-CH_2), 5.13 (1H, d, J=5 Hz, 6-H), 5.37 (1H, dd, J=2 and 5 Hz, 7-H), 5.50 (1H, s, OH), 6.95 (1H, s, Ph_2CH), 7.32 (10H, br s, Ph), 7.62 (2H, s, aromatic), 8.46 (1H, d, J=2 Hz, CH=N).

Diphenylmethyl 7β-[3,5-Di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)methylimino]-3-chloromethyl-3-cephem-4-carboxylate (**3a**)

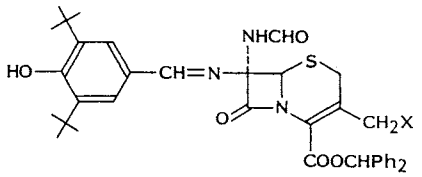
A mixture of **2a** (2.0 g, 3.2 mmol) and freshly prepared PbO_2 (2.0 g) in dry benzene (20 ml) was stirred for 1 hour and then filtered. The filtrate was evaporated under reduced pressure to give 2.0 g of product. IR ν_{max} (KBr) cm^{-1} 2950, 1770, 1725, 1610, 1360; ^1H NMR (80 MHz, $CDCl_3$) δ 1.34 (18 H, s, *tert*-Bu), 3.47, 3.76 (2H, ABq, J=18 Hz, 2-H), 4.26, 4.57 (2H, ABq, J=14 Hz, 3-CH_2), 5.40 (1H, s, 6-H), 6.97 (2H, d, J=3 Hz, cyclohexadienyl), 7.04 (1H, s, Ph_2CH), 7.35 (10H, br s, Ph), 7.87 (1H, d, J=3 Hz, =CH–N=).

Diphenylmethyl 7α-Formamido-7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-3-chloromethyl-3-cephem-4-carboxylate (**4a**)

To a solution of **3a** (700 mg, 1.1 mmol) in CH_2Cl_2 (7 ml) was added BSF (642 mg, 3.4 mmol); the mixture was stirred for 1 hour at room temperature. The mixture was diluted with EtOAc, washed with water and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel. The column was eluted with EtOAc-*n*-hexane (1:4) to give 430 mg (58%) of the product as an amorphous powder.

Compounds **4b** and **4c** were prepared in a similar way. Yields, mp's and spectral data of the products are summarized in Table 2.

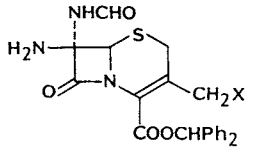
Table 2. Spectral and physical data of compound **4**.



Compound	X	Yield (%)	MP (°C, dec)	UV $\lambda_{max}^{ClCH_2CH_2Cl}$ nm (ε)	IR ν_{max} KBr (cm^{-1})	FAB-MS m/z $(M+H)^+$ m/z $(M+Na)^+$	^1H NMR (80 MHz, $CDCl_3$)[a] CH=N 1H, s	CHO 1H, d (J=1 Hz)	Aromatic 2H, s 10H, m	$-CHPh_2$ 1H, s	6-H 1H, s	3-CH_2 2H, ABq (J=13 Hz)	2-H 2H, ABq (J=18 Hz)	*tert*-Bu 18H, s	X
4a	Cl	58	110~115	227 (19,000), 293 (16,200)	1775 (β-lactam), 1685 (NHCHO)	674 696	8.34	8.32	7.60 7.1~7.6	6.96	5.46	4.28 (2H, s)	3.40, 3.60	1.43	
4b	OAc	74	105~109	230 (17,900), 292 (17,200)	1775, 1685	698 —	8.33	8.32	7.60 7.1~7.6	6.95	5.44	4.72, 4.93	3.23, 3.53	1.40	1.95 (3H, s)
4c		74	120~127	228 (23,200), 294 (19,300)	1775, 1690	754 776	8.37	8.32	7.60 7.1~7.6	6.95	5.43	4.19, 4.36	3.53, 3.65	1.40	3.80 (3H, s)

[a] Only the major *Z*-rotamer is quoted.

Table 3. Spectral and physical data of compound **5**.



Compound	X	Yield (%)	MP (°C, dec)	UV $\lambda_{max}^{ClCH_2CH_2Cl}$ nm (ε)	IR ν_{max} KBr (cm^{-1})	FAB-MS m/z $(M+H)^+$ m/z $(M+Na)^+$	^1H NMR (400 MHz, DMSO-d_6)[a] NH 1H, d (J=1 Hz)	CHO 1H, d (J=1 Hz)	Ph_2 10H, m	$-CHPh_2$ 1H, s	6-H 1H, s	3-CH_2 2H, ABq (J=13 Hz)	2-H 2H, ABq (J=18 Hz)	X
5a[b]	Cl	84	169~172	227 (13,700), 269 (10,000)	1790 (β-lactam), 1675 (NHCHO)	458 480	8.91	8.11	7.25~7.7	6.95	5.20	4.38 (s)	3.48 3.68	
5b[b]	OAc	83	120~122	227 (10,900), 265 (7,200)	1790, 1685	482 504	8.91	8.10	7.2~7.6	6.91	5.16	4.58, 4.79	3.43 3.68	1.99 (3H, s)
5c		70	89~93	228 (21,300), 275 (11,400)	1775, 1685	538 560	8.93	8.10	7.2~7.6	6.88	5.13	4.15, 4.35	3.55 3.75	3.87 (3H, s)

[a] Only the major Z-rotamer is quoted.
[b] Colorless crystals.

Diphenylmethyl 7α-Formamido-7β-amino-3-chloromethyl-3-cephem-4-carboxylate (**5a**)

A mixture of **4a** (300 mg, 0.44 mmol), Girard reagent T (112 mg, 0.67 mmol) and acetic acid (0.1 ml) in EtOAc (6 ml) and MeOH (6 ml) was stirred for 1 hour at room temperature. The mixture was diluted with EtOAc, washed with water and dried. After concentration under reduced pressure, precipitated crystalline product (169 mg, 84%) was collected by filtration.

Compounds **5b** and **5c** were prepared in a similar way. Yields, mp's and spectral data of the products are summarized in Table 3.

Benzyl 6β-(3,5-Di-*tert*-butyl-4-hydroxybenzylideneamino)penicillanate (**7**)

A mixture of benzyl 6-aminopenicillanate **6**[24] (1.53 g, 5 mmol) and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (1.17 g, 5 mmol) in benzene (40 ml) was heated under reflux for 1 hour using a water separator. After cooling, the mixture was concentrated under reduced pressure. The concentrate was diluted with benzene-cyclohexane, 1:5 (30 ml) and insoluble materials were filtered off. The filtrate was concentrated and chromatographed on a column of silica gel. Elution with *n*-hexane-EtOAc (5:1) afforded 1.59 g (61%) of product as an amorphous powder: IR ν_{max} (KBr) cm^{-1} 1770, 1745; UV $\lambda_{max}^{ClCH_2CH_2Cl}$ nm (ε) 230 (16,700), 282 (16,500); ^1H NMR (60 MHz, $CDCl_3$) δ 1.50 (21H, s, *tert*-Bu and 2-CH_3), 1.63 (3H, s, 2-CH_3), 4.41 (1H, s, 3-H), 5.20 (2H, s, CH_2-Ph), 5.43 (1H, dd, J=2 and 4 Hz, 6-H), 5.50 (1H, s, OH), 5.63 (1H, d, J=4 Hz, 5-H), 7.37 (5H, s, Ph), 7.60 (2H, s, aromatic), 8.51 (1H, d, J=2 Hz, CH=N); FAB-MS m/z 523 $(M+H)^+$.

Benzyl 6α-Formamido-6β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)penicillanate (**9**)

A mixture of **7** (310 mg, 0.59 mmol) and freshly prepared PbO_2 (310 mg, 1.07 mmol) in benzene (3.1 ml) was stirred for 1 hour at room temperature. The mixture was filtered; the filtrate was concentrated under reduced pressure and dissolved in dry CH_2Cl_2 (2 ml). BSF (230 mg, 1.2 mmol) was added to the mixture. Then, the mixture was allowed to stand for 1 hour at room temperature. The reaction mixture was diluted with EtOAc, washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel. Elution with *n*-hexane-EtOAc (3:1) and removal of the solvent under reduced pressure gave 156 mg (46%) of the desired product as an amorphous powder: IR ν_{max} (KBr) cm^{-1} 1775, 1745, 1685; UV $\lambda_{max}^{ClCH_2CH_2Cl}$ nm (ε) 228 (14,300), 293 (14,300); ^1H NMR (80 MHz, $CDCl_3$) δ 1.43 (21H, s, *tert*-Bu and 2-CH_3), 1.60 (3H, s, 2-CH_3), 4.50 (1H, s, 3-H), 5.17 (2H, s, CH_2-Ph), 5.51 (0.3H, s, 5-H), 5.58 (1H, br s, OH), 5.86 (0.7H, s, 5-H), 6.38 (0.7H, br s, N*H*CHO), 6.41 (0.3H, d, J=11 Hz, N*H*CHO), 7.33 (5H, s, Ph), 7.76 (2H, s, aromatic), 8.30 (0.7H, br s, CHO), 8.34 (1H, s, CH=N), 8.43 (0.3H, d, J=11 Hz, CHO); FAB-MS m/z 566 $(M+H)^+$, 588 $(M+Na)^+$.

Benzyl 6β-Amino-6α-formamidopenicillanate (**10**)

To a solution of **9** (300 mg, 0.53 mmol) in EtOAc (6 ml) was added a mixture of Girard reagent T (102 mg, 0.61 mmol) and AcOH (0.03 ml) in MeOH (6 ml); the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with a small amount of EtOAc and washed successively with aq $NaHCO_3$ and water. After concentration under reduced pressure, the resulting oil was purified by silica gel column chromatography and elution with *n*-hexane-EtOAc (1:1) to give 153 mg (83%) of the product. The identity was confirmed by direct comparison with an authentic sample prepared by the reported method[11].

7α-Formamido-7β-[(2-thienyl)acetamido]cephalosporanic Acid (**12a**)

A solution of 356 mg (0.74 mmol) of **5b**, 116 mg (0.83 mmol) of thienylacetic acid and 170 mg (0.83 mmol) of dicyclohexylcarbodiimide in THF (15 ml) was stirred for 4 hours at room temperature and then filtered. The filtrate was diluted with EtOAc (30 ml), washed with water and dried. Removal of the solvent gave an oil, which was subjected to column chromatography (silica gel). Elution with EtOAc-toluene (1:2) and evaporation of the fractions containing the desired product gave 376 mg (74%) of diphenylmethyl 7α-formamido-7β-(2-thienylacetamido)cephalosporanate (**11a**) as an amorphous powder. IR ν_{max} (KBr) cm^{-1} 1790, 1740, 1680; ^1H NMR (80 MHz, $CDCl_3$) δ 2.00 (3H, s, OAc), 3.06, 3.27 (2H, ABq, J=18 Hz, 2-H), 3.82 (2H, s, thiophene-CH_2), 4.89, 5.08 (2H, ABq, J=13 Hz, 3-CH_2), 5.15 (1H, s, 6-H), 6.90 (2H, m, thiophene), 6.95 (1H, s, C*H*Ph_2), 7.1 ~ 7.6 (11H, m, Ph and thiophene), 8.05 (1H, s, CHO).

A mixture of 309 mg (0.51 mmol) of **11a** and TFA (1 ml) in CH_2Cl_2 (1 ml) was allowed to stand for 1.5 hours at room temperature and then concentrated *in vacuo*. The residue was triturated with isopropyl ether. The crude product was collected by fitration and purified by column chromatography using high-porous polymer resin Diaion HP-20 which was eluted with water, 30% aq MeOH and 50% aq MeOH, successively. The fractions containing the desired product were concentrated under reduced pressure. The aqueous residue was freeze-dried to give 67 mg (30%) of **12a** as an amorphous powder. MP 120°C (dec); IR ν_{max} (KBr) cm^{-1} 1775, 1720, 1670; UV $\lambda_{max}^{(pH\ 7\ phosphate\ buffer)}$ nm (ε) 236 (10,900), 265 (sh, 7,000); ^1H NMR (80 MHz, $D_2O+NaHCO_3$) δ 2.17 (3H, s, OAc), 3.35, 3.70 (2H, ABq, $J=18$ Hz, 2-H), 4.00 (2H, s, thiophene-CH_2), 4.67, 4.96 (2H, ABq, $J=13$ Hz, 3-CH_2), 5.37 (1H, s, 6-H), 7.0~7.3 (2H, m, thiophene), 7.3~7.5 (1H, m, thiophene), 8.20 (1H, s, CHO); FAB-MS m/z 440 $(M+H)^+$, 462 $(M+Na)^+$.

GUEST *et al.*[19] prepared **12a** sodium salt by a different route. Their NMR spectral data are nearly identical with ours except for the 3-CH_2 and 6-H, which are probably assigned incorrectly.

7β-[(2-Aminothiazol-4-yl)acetamido]-7α-formamidocephalosporanic Acid (**12b**)

Phosphorous pentachloride (104 mg, 0.5 mmol) was added to a solution of 2-tritylaminothiazol-4-ylacetic acid (194 mg, 0.48 mmol) in CH_2Cl_2 (4 ml) at 0°C. After stirring for 30 minutes, the mixture was added to a cooled solution of **5b** (195 mg, 0.40 mmol) and *N,O*-bis(trimethylsilyl)acetamide (244 mg, 1.2 mmol) in CH_2Cl_2 (4 ml). After stirring for 1 hour at room temperature, the reaction mixture was diluted with EtOAc, washed with water, dried and evaporated under reduced pressure. The residue was purified by silica gel column chromatography. Elution with EtOAc - toluene (1 : 2) and concentration of the desired fraction under diminished pressure gave 218 mg (62%) of diphenylmethyl 7α-formamido-7β-[(2-tritylaminothiazol-4-yl)acetamido]cephalosporanate (**11b**). IR ν_{max} (KBr) cm^{-1} 1790, 1730, 1690; ^1H NMR (80 MHz, $CDCl_3$) δ 1.95 (3H, s, OAc), 3.25 (2H, s, 2-H), 3.55 (2H, s, CH_2-thiazole), 4.84, 5.10 (2H, ABq, $J=13$ Hz, 3-CH_2), 5.22 (1H, s, 6-H), 6.05 (1H, s, thiazole), 6.88 (1H, s, CHPh$_2$), 7.0~7.5 (25H, m, Ph), 8.13 (1H, s, CHO).

A mixture of **11b** (207 mg, 0.24 mmol) and TFA (1 ml) in CH_2Cl_2 (1 ml) was allowed to stand for 1 hour at room temperature. After concentration *in vacuo*, the residue was triturated with isopropyl ether (10 ml) to precipitate the crude trifluoroacetate of **12b**, which was collected by filtration and purified by Diaion HP-20 column chromatography. The column was eluted with 50% aq MeOH and the desired fraction was concentrated under reduced pressure. The aqueous residue was freeze-dried to give 56 mg (51%) of **12b** as an amorphous powder. MP 160°C (dec); IR ν_{max} (KBr) cm^{-1} 1785, 1660, 1630; UV $\lambda_{max}^{(pH\ 7\ phosphate\ buffer)}$ nm (ε) 257 (12,900); ^1H NMR (80 MHz, $D_2O+NaHCO_3$) δ 2.17 (3H, s, OAc), 3.53 (2H, ABq, $J=18$ Hz, 2-H), 3.75 (2H, s, thiazole-CH_2), 5.39 (1H, s, 6-H), 6.64 (1H, s, thiazole), 8.23 (1H, s, CHO); FAB-MS m/z 456 $(M+H)^+$, 478 $(M+Na)^+$.

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-7α-formamidocephalosporanic Acid (**12c**)

Compound **12c** was obtained in the same manner as **12b** in a 59% yield from **5b**. MP 155°C (dec); IR ν_{max} (KBr) cm^{-1} 1775, 1670; UV $\lambda_{max}^{(pH\ 7\ phosphate\ buffer)}$ nm (ε) 238 (16,000), 256 (sh, 15,600); ^1H NMR (80 MHz, DMSO-d_6) δ 2.02 (3H, s, OAc), 3.82 (3H, s, OCH_3), 4.70, 4.95 (2H, ABq, $J=15$ Hz, 3-CH_2), 5.22 (1H, s, 6-H), 6.86 (1H, s, thiazole), 8.05 (1H, s, CHO), 9.41 (1H, s, NH), 9.80 (1H, s, NH).

The above NMR data showed good agreement with those reported[20] for the compound prepared by a somewhat different procedure.

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