NEW BROAD-SPECTRUM CEPHALOSPORINS WITH ANTI-PSEUDOMONAL ACTIVITY

I. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[D-2-[(4-HYDROXY-1,5-NAPHTHYRIDINE-3-CARBONYLAMINO)- AND (4-HYDROXYPYRIDINE-3-CARBONYLAMINO)]-2-(4-HYDOXYPHENYL)ACETAMIDO]CEPHALOSPORINS

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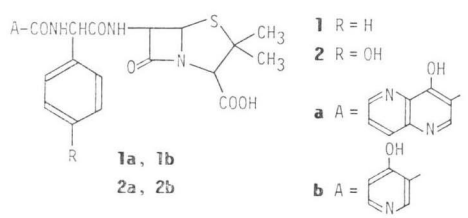
The synthesis and the antibacterial activity of 7β-[D-2-[(4-hydroxy-1,5-naphthyridine-3-carbonylamino)- and (4-hydroxypyridine-3-carbonylamino)]-2-(4-hydroxyphenyl)acetamido]-cephalosporins with various substituents at the 3-position in the cephem nucleus are described. These compounds exhibited strong antibacterial activities against a variety of Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Enterobacter aerogenes*, which are insensitive to cefazolin and cefmetazole. The compounds (**3e**, **4e**) having a 1-methyl-1*H*-tetrazolylthiomethyl group at the 3-position appeared to show the best activity in each series. The 4-hydroxypyridine-3-carbonylamino derivative **4e** gave higher peak serum concentrations and urinary recovery rates than those of the 4-hydroxy-1,5-naphthyridine derivative **3e** when administered subcutaneously to mice and intramuscularly to rats.

In recent years, the incidence of infections caused by multiply antibiotic-resistant bacteria and opportunistic pathogens, *e.g.*, Pseudomonas and Serratia species has become a serious therapeutic problem. One approach to solve this problem has been to synthesize new β-lactam antibiotics which have an expanded antibacterial spectrum and greater β-lactamase stability together with good pharmacokinetic characteristics.

Our previous work with this aim resulted in the preparation of 6-[D-(−)-α-[(acylamino)-phenyl- and (acylamino)-*p*-hydroxyphenyl]acetamido]penicillanic acids,[1,2] (**1**, **2**) which had an expanded antibacterial spectrum and showed remarkable activity against *Pseudomonas aeruginosa*.



These findings encouraged us to synthesize cephalosporins with the same acylamino-*p*-hydroxyphenyl acetamido side chains to see whether these compounds also had expanded antibacterial spectra. Studies on the structure-activity relationships of the penicillins indicated that the *N*-acylamino moiety, *i.e.*, A–CONH–, in **1** and **2** greatly influenced the antibacterial activities, spectra, and the pharmacokinetic characteristics of the compounds. Among the various acylamino residues tested, the 4-hydroxy-1,5-naphthyridine-3-carbonylamino group gave compounds (**1a**, **2a**) with remarkable *in vitro* activities, particularly against Enterobacteriaceae and *P. aeruginosa*, whereas the 4-hydroxypyridine-3-carbonylamino

group appeared to give compounds (**1b**, **2b**) which were not so active but which gave higher serum levels and urinary recovery rates than those of the naphthyridine derivatives when administered subcutaneously to mice or intramuscularly to rats.[8]

In synthesizing the cephalosporins, the 4-hydroxy-1,5-nathyridine-3-carbonylamino group and the 4-hydroxypyridine-3-carbonylamino group in the side chain at C-7 were combined with 3-cephem derivatives with an acetoxymethyl, a carbamoyloxymethyl, and hetero-aromatic thiomethyl groups as 3-substituents to give compounds **3a**~**e** and **4a**~**e** (Table 1).

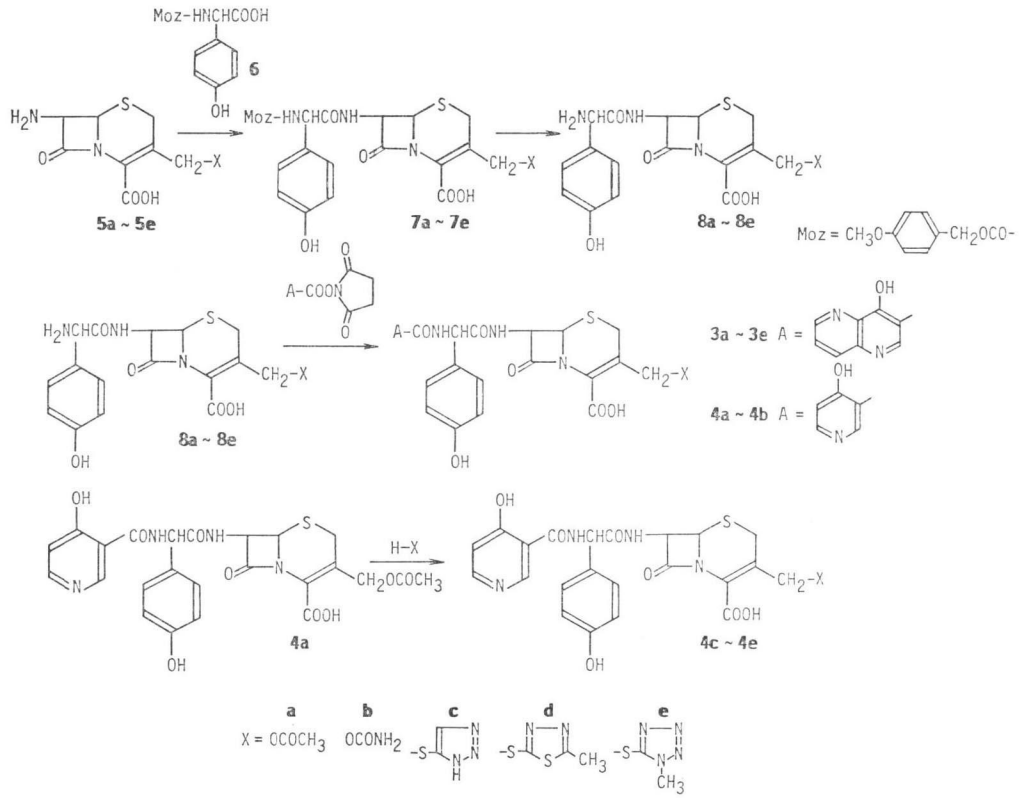
The compounds have elicited considerable interest because of their potent antibacterial activity against species of *P. aeruginosa, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus faecalis.*

Chemistry

The cephalosporins listed in Table 1 were prepared by the two general methods outlined in Scheme 1.

The *p*-hydroxyphenylglycylcephalosporins **8a**~**e** were prepared by acylation of disilylated 7-aminocephalosporanic acid **5a** or its derivatives **5b**~**e** with the mixed anhydride derived from D-2-(4-methoxybenzyloxycarbonylamino)-2-(4-hydroxyphenyl)acetic acid and isobutyl chloroformate, and subsequent removal of the 4-methoxybenzyloxycarbonyl protecting group using trifluoroacetic acid-anisole. Another synthesis of **8a**, **c**~**e** had been reported by DUNN *et al.*[4] and that of **8e** had been also reported by BREUER *et al.*[10]

Scheme 1.



The cephalosporins **3a, b, c, d, e**, and **4a, b** were prepared by acylation of the corresponding *p*-hydroxyphenylglycylcephalosporins **8a**~**e** with active esters derived from *N*-hydroxysuccinimide and the appropriate carboxylic acids. The cephalosporins **4c, d, e**, were prepared by displacement of the 3-acetoxy group of cephalosporin **4a** with an appropriate hetero-aromatic thiol by the widely used general procedure.[5] It was confirmed by HPLC that no racemization at the asymmetric carbon of the 7-acyl side chain occurred during the displacement.

Biological Results and Discussion

Antibacterial Activity

The minimum inhibitory concentration (MIC) values of the cephalosporins were determined by the serial two-fold agar dilution method against three species of Gram-positive and seven species of Gram-negative bacteria.

The *in vitro* activities of **3a**~**e** and **4a**~**e** compared to those of cefazolin and cefmetazole are listed in Table 1.

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

1. Effect of 3-Substituents on *In Vitro* Activity

The carbamoyloxymethyl analogs (**3b, 4b**) were about as active as or slightly less active than the corresponding acetoxymethyl analogs (**3a, 4a**) against the bacteria tested except *P. aeruginosa*. It is noteworthy that the carbamoyloxymethyl analogs showed the highest antipseudomonal activity in both series.

It is often observed that displacement of the 3-acetoxy group with hetero-aromatic thiols, such as 1-methyl-1*H*-tetrazole-5-thiol, enhances the antibacterial activity against Gram-negative bacteria.[4,6] By the displacement of the 3-acetoxy group of **3a** and **4a** with 1-methyl-1*H*-tetrazole-5-thiol, the activity against *E. coli, K. pneumoniae, P. vulgaris*, and *S. marcescens* was greatly improved, but no significant changes in activity were observed against *S. aureus, S. epidermidis, S. faecalis, P. mirabilis, E. aerogenes* and *P. aeruginosa*. A similar pattern of activity was seen on displacement of the 3-acetoxy group with 2-methyl-1,3,4-thiadiazole-5-thiol. In the case of the displacement with 1,2,3-triazole-5-thiol, the effect on activity was more complicated: the activity of **3c** was superior to that of **3a** against *K. pneumoniae, P. vulgaris*, and *S. marcescens*, but the activity of **4c** was of the same order as that of **4a** against all the bacteria tested.

2. Comparison of the *In Vitro* Activities of the 4-Hydroxy-1,5-naphthyridine Derivatives (**3a**~**e**) with Those of the 4-Hydroxypyridine Derivatives (**4a**~**e**)

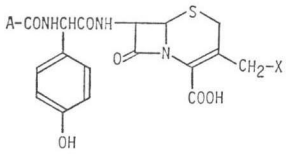
The naphthyridine derivatives (**3a**~**e**) were two to four times less active than the pyridine derivatives (**4a**~**e**) against Gram-positive bacteria. On the other hand, the naphthyridine derivatives (**3a**~**e**) were more active against some of the Gram-negative bacteria tested than the corresponding pyridine derivatives (**4a**~**e**): 16 to 64 times more active against *E. coli*, 8 to 32 times more active against *S. marcescens*, and 4 to 16 times more active against *P. aeruginosa*.

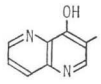
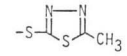
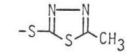
3. Comparison of the *In Vitro* Activities of Compounds **3e** and **4e** with Those of Cefazolin and Cefmetazole.

Compound **3e** was more potent than cefazolin and cefmetazole against *S. epidermidis, S. faecalis* and all Gram-negative bacteria tested, while it was less active against *S. aureus*.

Compound **4e** had higher activity than cefazolin and cefmetazole against *S. epidermidis, S. faecalis,*

Table 1. Comparative *in vitro* activity of the cephalosporins.



Compound No.	A	X	MIC (μg/ml)[a]									
			S. a.	*S. e.*	*S. f.*	*E. c.*	*K. p.*	*P. m.*	*P. v.*	*E. a.*	*P. a.*	*S. m.*
3a		$-OCOCH_3$	0.78	1.56	12.5	0.39	0.20	3.13	0.10	1.56	0.20	12.5
3b		$-OCONH_2$	0.78	0.78	12.5	0.78	0.10	6.25	0.39	12.5	0.05	6.25
3c			1.56	3.13	25	0.39	≦0.025	3.13	≦0.025	1.56	0.78	3.13
3d			0.39	1.56	6.25	0.025	≦0.025	6.25	≦0.025	1.56	0.39	3.13
3e			0.39	1.56	6.25	0.025	≦0.025	1.56	≦0.025	0.39	0.20	0.39
4a		$-OCOCH_3$	0.39	1.56	3.13	12.5	0.20	12.5	0.20	3.13	1.56	100
4b		$-OCONH_2$	0.20	0.78	3.13	25	0.39	6.25	3.13	6.25	0.78	>100
4c			0.39	3.13	6.25	6.25	0.10	12.5	0.10	6.25	3.13	>100
4d			0.10	0.39	1.56	1.56	0.05	12.5	≦0.025	3.13	1.56	50
4e			0.20	1.56	3.13	1.56	≦0.025	6.25	≦0.025	1.56	1.56	12.5
Cefazolin			≦0.025	3.13	12.5	0.78	0.39	6.25	6.25	>100	>100	>100
Cefmetazole			0.20	25	>100	0.20	0.10	1.56	0.78	>100	>100	1.56

[a] The MIC's were determined by the serial two-fold agar dilution method.[9]
Test organisms and abbreviations: *S. a.*, *Staphylococcus aureus* 209P; *S. e.*, *Staphylococcus epidermidis* IAM 1296; *S. f.*, *Streptococcus faecalis* NCTC 8213; *E. c.*, *Escherichia coli* NIHJ JC-2; *K. p.*, *Klebsiella pneumoniae* ATCC 10031; *P. m.*, *Proteus mirabilis* GN 2425; *P. v.*, *Proteus vulgaris* OX-19; *E. a.*, *Enterobacter aerogenes* ATCC 13048; *P. a.*, *Pseudomonas aeruginosa* IFO 3451; *S. m.*, *Serratia marcescens* X100.

K. pneumoniae, *P. vulgaris*, *E. aerogenes*, and *P. aeruginosa*, while it was less active than cefazolin against *S. aureus* and less active than cefmetazole against *E. coli*, *P. mirabilis*, and *S. marcescens*.

It is noteworthy that compounds **3e** and **4e** displayed potent activity against strains insensitive to cefazolin or cefmetazole: *P. aeruginosa*, *E. aerogenes*, *S. marcescens*, and *S. faecalis*.

Serum Levels and Urinary Recovery Rates in Experimental Animals

Table 2 shows the antibiotic peak serum levels and urinary recovery rates of compounds **3e** and **4e** when administered subcutaneously to mice and intramuscularly to rats.

Peak serum concentration of compound **4e**, having the 4-hydroxypyridine-3-carbonyl group, was approximately five times higher than that of compound **3e**, having the 4-hydroxy-1,5-naphthyridine-3-

carbonyl group in mice and rats. In addition to this finding, urinary recovery rates of compound **4e** were approximately 3.5 times higher than those of compound **3e** in mice and twice higher in rats. Thus compound **4e** appears to have more favorable pharmacokinetic characteristics than compound **3e**.

Table 2. Mean peak serum levels and urinary recovery rates[a] of **3e** and **4e** after administration to mice[b] (s. c.) and rats[c] (i. m.) at a dose of 50 mg/kg.

Compound No.	Peak serum level (Mean±S.D.)(μg/ml)		Urinary recovery rate (Mean±S.D.)(%)	
	mice[d] (n=1)	rats (n=3)	mice[e] (n=3)	rats (n=3)
3e	15.4	10.3± 0.2	16.4±2.7	21.1±1.7
4e	71.3	57.0±13.2	56.6±5.9	41.8±1.4

a) The antibiotic concentration was determined by disk plate method using *Bacillus subtilis* ATCC 6633 as test organism.
b) Male ICR strain mice.
c) Male SD-SLC strain rats.
d) Serum of four mice was pooled and assayed.
e) A group of eight mice was housed in each cage. A urine sample of each cage was pooled and assayed.

The foregoing findings of the superior activities and the inferior pharmacokinetic characteristics of the 4-hydroxy-1,5-naphthyridine-3-carbonyl derivatives compared to those of the 4-hydroxypyridine-3-carbonyl derivatives are consistent with the results of previous studies of the corresponding penicillins. This suggests that the structure-activity relationships of penicillins might be useful for deducing the characteristics of cephalosporins with the same acyl moieties. However in the cephalosporins, the 3-substituent of the cephem nucleus also was of importance for the activity. Of the different substituents tested 1-methyl-1*H*-tetrazol-5-ylthiomethyl substituent was the most effective one in increasing the activity. As a result, compound **4e**, for example, became an attractive compound having good antibacterial activity and good pharmacokinetic characteristics.

The studies showed that the *N*-acyl moiety, A–CO–, and the 3-substituent greatly influenced the antibacterial activity and spectrum, and the pharmacokinetic characteristics of the cephalosporins and suggested that it could be worthy to examine further cephalosporins having other acyl moieties or other 3-substituents to find even better compounds.

Such further studies will be presented in subsequent papers.

Experimental

Infrared spectra were recorded on a Hitachi model EPI-G3 spectrophotometer. NMR spectra were recorded on a JEOL FX-90Q (90 MHz) spectrometer or a Varian T-60 (60 MHz) spectrometer

Table 3. Empirical formulas and IR data of the cephalosporins.

Compound No.	Formula[a]	IR(KBr) β-lactam (cm^{-1})	Compound No.	Formula[a]	IR(KBr) β-lactam (cm^{-1})
3a	$C_{27}H_{23}N_5O_9S\cdot 2H_2O$[b]	1770	**4a**	$C_{24}H_{22}N_4O_9S\cdot H_2O$	1780
3b	$C_{26}H_{22}N_6O_9S\cdot H_2O$	1777	**4b**	$C_{23}H_{21}N_5O_9S\cdot 2H_2O$	1765
3c	$C_{27}H_{22}N_8O_7S_2\cdot 3.5H_2O$[c]	1770	**4c**	$C_{24}H_{21}N_7O_7S_2\cdot 2H_2O$[e]	1770
3d	$C_{28}H_{23}N_7O_7S_3\cdot 2H_2O$	1770	**4d**	$C_{25}H_{22}N_6O_7S_3\cdot 2.5H_2O$	1776
3e	$C_{27}H_{23}N_9O_7S_2\cdot 3H_2O$[d]	1777	**4e**	$C_{24}H_{22}N_8O_7S_2\cdot 1.5H_2O$	1770

a) Compounds were analyzed for C, H, N. Unless otherwise indicated, analyses are within ±0.4% of the theoretical values.
b) H: calcd. 4.32; found 3.81.
c) H: calcd. 4.19; found 3.52.
d) H: calcd. 4.15; found 3.60.
e) N: calcd. 15.83; found 15.24.

using TMS as an internal standard; all chemical shifts are reported in δ values. Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Empirical formulas and IR data, and NMR data of the cephalosporins synthesized are shown in Tables 3 and 4, respectively.

D-2-(4-Methoxybenzyloxycarbonylamino)-2-(4-hydroxyphenyl)acetic Acid (**6**)

To a solution of D-(−)-*p*-hydroxyphenylglycine (4.175 g, 25 mmole) and triethylamine (3.79 g, 37.5 mmole) in 13 ml of water was added 2-(4-methoxybenzyloxycarbonylthio)-4,6-dimethylpyrimidine[7] (8.36 g, 27.5 mmole) in 17 ml of dioxane. The mixture was stirred at room temperature for 2 hours. To the resulting mixture 33 ml of water was added and unreacted carbonate was extracted with 45 ml of EtOAc. The aqueous layer was cooled to 0~5°C, adjusted to pH 2 by the addition of 6 N HCl, and then extracted once with 45 ml of EtOAc and twice with 20 ml of EtOAc. The combined extracts were washed successively twice with 25 ml of 5% aqueous HCl and three times with 40 ml of saturated aqueous NaCl and dried over $MgSO_4$. Concentration of the extract to one-third of its original volume and cooling to below 10°C gave the product **6**, which was filtered off, washed with 12 ml of EtOAc, and dried *in vacuo*: yield 7.47 g (90.2%). mp 136~137°C (dec.); $[\alpha]_D^{27}$ −105.2° (*c* 1, DMF); IR (Nujol) 1740, 1665, 1615, 1520 cm^{-1}; NMR (DMSO-d_6) δ 3.73 (s, 3H, OCH_3), 5.00 (s, 2H, CH_2), 5.08 (d, 1H, J=8 Hz, CH), 6.68~7.40 (m, 8H, phenyl protons), 7.77 (d, 1H, J=8 Hz, NH).

Anal. Calcd. for $C_{17}H_{17}NO_6$: C 61.63, H 5.17, N 4.23.
Found: C 61.84, H 5.19, N 4.24.

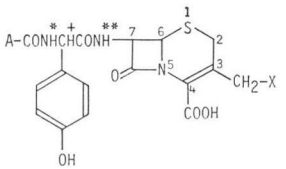
7-[D-2-(4-Methoxybenzyloxycarbonylamino)-2-(4-hydroxyphenyl)acetamido]cephalosporanic Acid (**7a**)

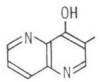
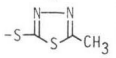
A solution of D-2-(4-methoxybenzyloxycarbonylamino)-2-(4-hydroxyphenyl)acetic acid (66.3 g, 0.2 mole) and *N*-methylmorpholine (20.23 g, 0.2 mole) in MeCN (420 ml) was stirred and chilled to −20~−15°C. Isobutyl chloroformate (27.32 g, 0.2 mole) was added and the temperature was maintained at −20~−15°C for 10 minutes. A cold solution of 7-ACA disilyl derivatives, prepared by adding *N*,*O*-bis(trimethylsilyl)acetamide (85.3 g, 0.42 mole) to a suspension of 7-ACA (**5a**) (54.4 g, 0.2 mole) in MeCN (350 ml), was added with stirring to the mixed anhydride solution. The mixture was stirred at −15°C for 2 hours, then allowed to warm slowly to 5°C and stirred at 5°C for 1 hour. MeOH (25 ml) was added to the resulting mixture. Unreacted 7-ACA (7.0 g) precipitated and was removed by filtration. The filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in EtOAc (480 ml). The solution was washed three times with water (218 ml) and dried with $MgSO_4$. Methylene chloride (335 ml) was added and the solution was stored at 0~5°C overnight. The precipitate was collected, washed with CH_2Cl_2 and dried *in vacuo*: yield 71.0 g (60.7%). mp 142~143°C (dec.); IR (KBr) 1775, 1735~1670, 1650, 1610, 1510 cm^{-1}; NMR (DMSO-d_6) δ 2.01 (s, 3H, $COCH_3$), 3.47 (broad s, 2H, C_2-H_2), 3.76 (s, 3H, OCH_3), 4.98 (s, 2H, $-CH_2$–Ph), 4.55~5.13 (m, 3H, C_3-CH_2 & C_6-H), 5.30 (d, 1H, J= 8Hz, –C*H*NH–), 5.71 (dd, 1H, C_7-H), 6.70, 7.27 (each d, 4H, phenyl protons), 6.91, 7.31 (each d, 4H, phenyl protons), 7.63 (d, 1H, CONH), 9.03 (d, 1H, CONH).

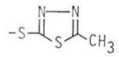
Anal. Calcd. for $C_{27}H_{27}O_{10}N_3S$: C 55.37, H 4.66, N 7.18.
Found: C 55.16, H 4.88, N 6.74.

7b: Compound **7b** was prepared from **5b** and **6** in a similar way except for the following work-up. After removal of unreacted **5b**, the filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in 10% aqueous $KHCO_3$ (twice the molar quantity of **5b**). The solution was washed twice with EtOAc. The aqueous solution was layered with fresh EtOAc and adjusted to pH 2 by the addition of 2 N HCl with stirring at 0~5°C. The EtOAc layer was separated and the aqueous layer was extracted twice with EtOAc. The combined extracts were washed with aqueous NaCl and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was treated with CH_2Cl_2. The precipitate was collected by filtration, washed with CH_2Cl_2, and dried *in vacuo* to give **7b**. mp 149~152°C (dec.); IR (KBr) 1775, 1700, 1610, 1510 cm^{-1}; NMR (DMSO-d_6) δ 3.37 (broad s, 2H, C_2-H_2), 3.74 (s, 3H, OCH_3), 4.95 (s, 2H, $-CH_2$–Ph), 4.46~5.08 (m, 3H, C_3-CH_2 & C_6-H), 5.26 (d, 1H, –C*H*NH–), 5.63 (dd, 1H, C_7-H), 6.66 (d, 2H, phenyl protons), 6.89 (d, 2H, phenyl protons), 7.14~7.31 (m, 4H, phenyl protons), 7.71 (d, 1H, J= 8Hz, CONH), 9.03 (d, 1H, J=8Hz, CONH). In a similar manner, **7c**, **7d** and **7e** were prepared.

Table 4. ^{1}H NMR data of **3a**~**e** and **4a**~**e**.



Compound No.	A	X	NMR δ value (DMSO-d_6)[a]								
			CONH* 1H, d, J=8Hz	CONH** 1H, d, J=9Hz	C_7-H, -CH$^+$- 2H, m	C_6-H 1H, d, J=5Hz	C_3-CH_2 2H, ABq, J=13Hz	C_2-H_2 2H, ABq, J=18Hz	Phenyl protons 2H×2, each d, J=9Hz	A ring protons	X
3a		-$OCOCH_3$	10.86	9.31	5.66~5.86	5.02	4.97 4.65	3.57 3.36	7.29 6.71	8.77 (m, 2H), 8.17 (d, 1H, J=9.4Hz), 7.74 (dd, 1H, J=9.4Hz, 4.7 Hz)	1.97 (s, 3H)
3b		-$OCONH_2$	10.83	9.31	5.66~5.83	5.05	4.89 4.57	[b]	7.29 6.71	8.77 (m, 2H), 8.17 (d, 1H), 7.74 (dd, 1H)	6.60 (s, 2H)
3c			10.74	9.31	5.60~5.83	5.00	4.04 3.80	3.71 3.43	7.29 6.71	8.76 (m, 2H), 8.20 (dd, 1H), 7.77 (dd, 1H)	7.89 (s, 1H)
3d			10.80	9.31	5.60~5.83	5.00	4.48 4.17	3.69 3.46	7.29 6.69	8.74 (m, 2H), 8.17 (d, 1H), 7.71 (dd, 1H)	2.69 (s, 3H)
3e			10.77	9.29	5.63~5.83	4.97	4.34 4.14	3.71 3.48	7.26 6.69	8.74 (m, 2H), 8.11 (dd, 1H), 7.71 (dd, 1H)	3.90 (s, 3H)

4a		$-OCOCH_3$	11.00	9.23	5.60~5.77	4.97	4.97 4.63	b)	7.20 6.69	8.34 (m, 1H), 7.71 (m, 1H), 6.37 (d, 1H, J=7Hz)	2.00 (s, 3H)
4b		$-OCONH_2$	11.00	9.20	5.57~5.74	5.00	4.86 4.54	b)	7.20 6.67	8.34 (m, 1H), 7.71 (m, 1H), 6.37 (d, 1H)	6.54 (s, 2H, NH_2)
4c			11.03	9.23	5.57~5.71	4.97	4.04 3.74	3.69 3.43	7.20 6.69	8.36 (br, 1H), 7.74 (m, 1H), 6.37 (d, 1H)	7.89 (s, 1H)
4d			11.03	9.23	5.60~5.77	4.97	4.48 4.14	3.69 3.45	7.20 6.66	8.34 (m, 1H), 7.74 (m, 1H), 6.37 (d, 1H)	2.66 (s, 3H)
4e			11.08	9.31	5.66~5.83	5.00	4.40 4.17	3.60 (br)	7.23 6.71	8.43 (br, 1H), 7.83 (m, 1H), 6.40 (d, 1H)	3.91 (s, 3H)

a) In NMR descriptions, s=singlet, d=doublet, dd=double doublet, m=multiplet, br=broad, ABq=AB quartet.

b) It was difficult to read the δ value because the signals overlapped with those of water.

7c: mp 104~106°C (dec.); IR (KBr) 1775, 1720~1655, 1612, 1512 cm^{-1}; NMR (DMSO-d_6) δ 3.31 (broad s, 2H, C_2-H_2), 3.72 (s, 3H, OCH_3), 3.94 (ABq, 2H, C_3-CH_2), 4.94~5.00 (m, 3H, $-CH_2Ph$ & C_6-H), 5.26 (d, 1H, J=8Hz, C*H*NH), 5.63 (dd, 1H, C_7-H), 6.66, 6.89 (each d, 2H×2, J=9Hz, phenyl protons), 7.16~7.34 (m, 4H, phenyl protons), 7.69 (d, 1H, J=8Hz, CONH), 7.89 (broad s, 1H, triazole C_4-H), 9.06 (d, 1H, J=8Hz, CONH).

7d: mp 149~153°C (dec.); IR (KBr) 1780, 1725~1655, 1613, 1512 cm^{-1}; NMR (DMSO-d_6) δ 2.67 (s, 3H, thiadiazole-CH_3), 3.49, 3.65 (ABq, 2H, C_2-H_2), 3.73 (s, 3H, OCH_3), 4.17, 4.47 (ABq, 2H, J=16Hz, C_3-CH_2), 4.95~5.01 (m, 3H, $-CH_2Ph$ & C_6-H), 5.23 (d, 1H, J=8Hz, $-CH$NH–), 5.66 (dd, 1H, C_7-H), 6.66, 6.89 (each d, 2H×2, J=9Hz, phenyl protons), 7.14~7.31 (m, 4H, phenyl protons), 7.69 (d, 1H, J=8Hz, CONH), 9.06 (d, 1H, J=8Hz, CONH).

7e: mp 127~134°C (dec.); IR (KBr) 1775, 1710~1670, 1610, 1510 cm^{-1}; NMR (DMSO-d_6) δ 3.60 (broad s, 2H, C_2-H_2), 3.75 (s, 3H, OCH_3), 3.93 (s, 3H, N–CH_3), 4.27 (broad s, 2H, C_3-CH_2), 4.93~5.03 (m, 3H, C_6–H & $-CH_2$–Ph), 5.28 (d, 1H, J=8Hz, $-CH$NH–), 5.68 (dd, 1H, C_7-H), 6.68, 7.23 (each d, 4H, J=9Hz, phenyl protons), 6.90, 7.28 (each d, 4H, J=9Hz, phenyl protons), 7.62 (d, 1H, CONH), 9.07 (d, 1H, CONH).

Removal of Protecting Groups

Synthesis of **8a~e**: A solution of **7a~e** (10 mmole) in trifluoroacetic acid (30 ml) and anisole (6 ml) was stirred at room temperature for 20 minutes. The solution was added to ethyl ether (400 ml) with stirring. The resulting precipitates were filtered off, washed with ethyl ether, and dried *in vacuo* to give the trifluoroacetic acid salt of **8a~e**. The product was used in subsequent steps without further purification.

General Procedure for Acylation of Cephalosporins **8a~e** with Active Esters (Method A)

Synthesis of Cephalosporins **3a, b, c, d, e** and **4a, b**: To a stirred suspension of triethylamine (30 mmole) and *N*-(4-hydroxy-1,5-naphthyridine-3-carbonyloxy)succinimide[8] (10 mmole) or *N*-(4-hydroxypyridine-3-carbonyloxy)succinimde[2] (10 mmole) in 30~50 ml of dimethylsulfoxide was added cephalosporin **8a~e** (trifluoroacetic acid salt: 10 mmole). The reaction mixture was stirred at room temperature for 30 minutes and then filtered to remove traces of insoluble material. The clear filtrate was added to stirred acetone (600~1,000 ml). The precipitate was collected, washed on a filter with acetone and dried *in vacuo*. Each triethylamine salt of the cephalosporins **3a~e** or **4a~b** thus obtained was dissolved in a mixture of H_2O (40~80 ml) and MeOH (40~80 ml) or H_2O (40~80 ml) and MeCN (40~80 ml), any trace amount of an insoluble material, if present, being removed by filtration. The solution was adjusted to pH 2 by the addition of 2 N HCl at 5~15°C. The precipitate was collected, washed on a filter with H_2O - MeOH or H_2O - MeCN and dried *in vacuo*. It was further purified by preparative liquid chromatography on a reverse phase column, LiChroprep RP-8 or μBondapak C-18, with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8) and MeOH, MeCN, or THF (see below). The fractions containing the product were concentrated *in vacuo* to a small volume and adjusted to pH 2 by the addition of 2 N HCl at 0~5°C. The precipitate was collected, washed on a filter with H_2O, and dried *in vacuo* over phosphorus pentoxide.

Mobile phases used were as follows. (Buffer in all cases was 0.01 M potassium dihydrogen phosphate adjusted to pH 6.8 with 1N NaOH). **3a**, 75% Buffer - 25% MeOH (by volume); **3b**, 78% Buffer - 22% MeOH; **3c**, 92% Buffer - 8% THF; **3d**, 70% Buffer - 30% MeOH; **3e**, 75% Buffer - 25% MeOH; **4a**, 83% Buffer - 17% MeOH; **4b**, 90% Buffer - 10% MeOH.

General Procedure for Displacing the 3-Acetoxy Group of the Cephalosporins **4a** with a Heterocyclic Thiol (Method B)

Synthesis of Cephalosporins **4c, d, e**: A solution containing the triethylamine salt of the cephalosporin **4a** (10 mmole), the heterocyclic thiol (15 mmole) and $NaHCO_3$ (15 mmole) in 140 ml of phosphate buffer (pH 6.4) was heated at 55°C for 18~24 hours. When the reaction was complete (monitored by HPLC), the solution was cooled to room temperature and adjusted to pH 2 by the addition of 2 N HCl. The precipitate was collected, washed on a filter with water and dried *in vacuo*. It was further purified by the preparative liquid chromatography as described in Method A. Mobile phases used were as follows. **4c**, 80% Buffer - 20% MeOH; **4d**, 75% Buffer - 25% MeOH; **4e**, 91% Buffer - 9% MeCN.

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