SYNTHESIS AND BIOLOGICAL PROPERTIES OF SOME 3-[(*N*-SUBSTITUTED-AMINO)PYRIDINIUM-4-THIOMETHYL]-7-[2-(2-AMINO-THIAZOL-4-YL)-2-(*Z*)-(METHOXYIMINO)ACETAMIDO]CEPH-3-EM-4-CARBOXYLATES

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The synthesis and antibacterial activity of a series of β-lactamase stable, broad spectrum 7-[2-(2-amino-thiazol-4-yl)-2-(*Z*)-(methoxyimino)acetamido]-cephalosporins, characterised by a C-3-[*N*-(substituted-amino)pyridinium-4-thiomethyl] group, is described. Gram-positive and Gram-negative bacteria including extended spectrum β-lactamase-producing strains were most susceptible to the *N*-amino- and *N*-methylamino derivatives (**3a**) and (**3b**); with the exception of *Pseudomonas aeruginosa*, (**3b**) was more active *in vitro* and *in vivo* than cefpirome or ceftazidime.

The development of the C-7 (2-aminothiazolyloxyimino)cephalosporins with their inherent good stability to β-lactamases has led to established antibacterial agents such as cefotaxime,[1] ceftazidime[2] and ceftriaxone[3] and the newly marketed cefpirome.[4] A primary concern in the search for new β-lactam antibiotics remains their stability to β-lactamases since these enzymes are still a major cause of treatment failure. The recent emergence of extended spectrum β-lactamase-producing bacteria has further highlighted the need for new potent broad spectrum agents with enhanced β-lactamase stability.

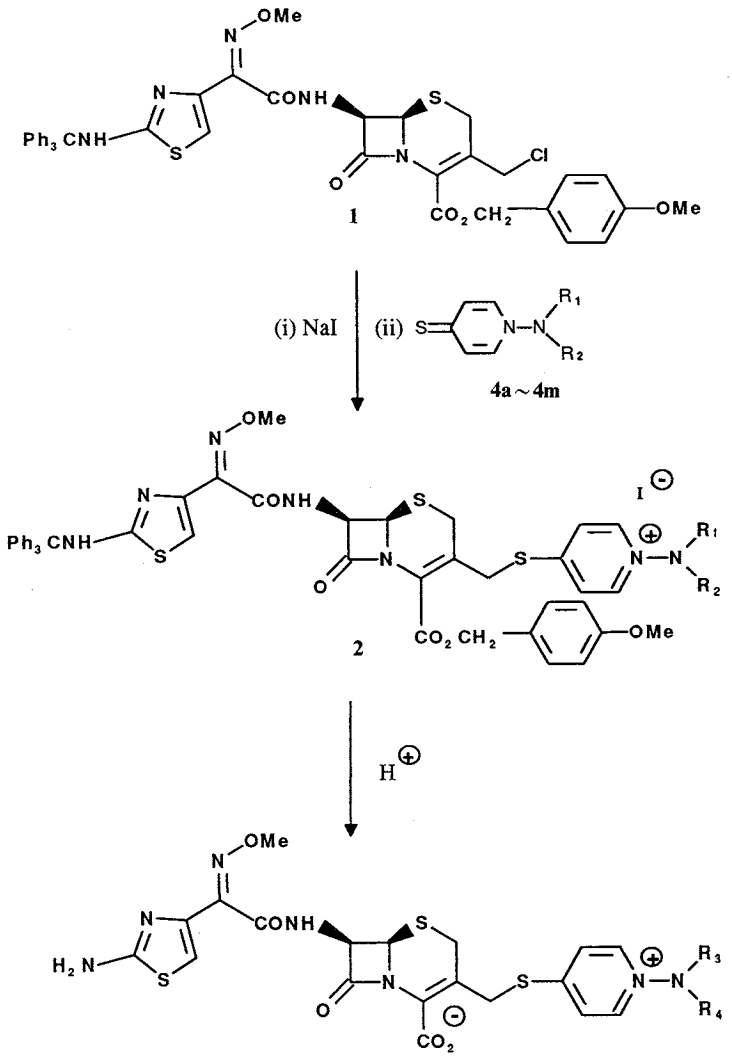
Good activity against Gram-positive and Gram-negative bacteria including β-lactamase producing strains has been reported for 2-aminothiazolyloxyimino cephalosporins with a C-3[*N*-alkylpyridinium-4-thiomethyl]- or [*N*-alkylcyclopenteno[*b*]pyridinium-4-thiomethyl] group.[5~7] Within these series, poor aqueous solubility and relatively high acute toxicity has been found for some derivatives lacking an additional carboxy group. Introduction of such an acidic moiety often improved solubility and toxicity but at the expense of some Gram-positive potency.

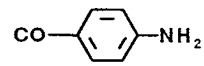
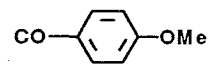
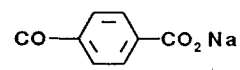
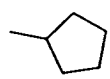
A recent report from these laboratories describes a series of 7α-formamido cephalosporins, characterised by a novel C-3[*N*-(substituted-amino)pyridinium-4-thiomethyl] group.[8] Although these compounds were highly β-lactamase stable agents with potent Gram-negative activity, including *Pseudomonas aeruginosa*, the Gram-positive activity was of borderline clinical utility. It was of interest therefore to investigate the effect of this particular C-3 substitution on the biological properties of conventional cephalosporins, where improved anti-staphylococcal potency might be anticipated. Our initial studies focused on 3-[(*N*-substituted-amino)pyridinium-4-thiomethyl]-7-[2-(2-amino-thiazol-4-yl)-2-(*Z*)-(methoxyimino)acetamido]-ceph-3-em-4-carboxylates (**3**) which form the subject of this paper.

Chemistry

The general synthetic route to the betaine cephalosporins (**3**) is outlined in Scheme 1. Thus, *S*-alkylation of the previously reported 4-thiopyridones (**4a**~**4c**)[8] (Fig. 1) with the 3-chloromethyl cephalosporin (**1**) in

Scheme 1.

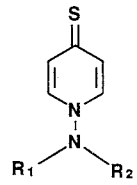


	R_3	R_4		R_3	R_4
a	H	H	**h**	Me	COPh
b	H	Me	**i**	Me	
c	Me	Me	**j**	c Me	
d	H	Hex	**k**	Me	
e	H		**l**	Me	COMe
f	H	CH_2Ph	**m**	Me	$CONH_2$
g	H	CH_2CO_2Na			

the presence of sodium iodide proceeded in high yield to the corresponding ester (**2**), as a mixture of chloride and iodide salts. Subsequent TFA deprotection concomitantly removed all the protecting groups from (**2**), including any *tert*-butoxycarbonyl groups on the *N*-aminopyridinium thiomethyl moiety, to provide the initial target derivatives (**3a**～**3c**).

In the initial route to 4-thiopyridones (**4a**～**4c**), the key synthetic step involved condensation of a

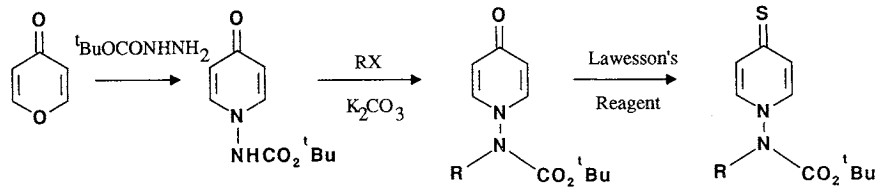
Fig. 1.



4

a $R_1=CO_2{}^tBu$, $R_2=H$
b $R_1=CO_2{}^tBu$, $R_2=Me$
c $R_1=R_2=Me$

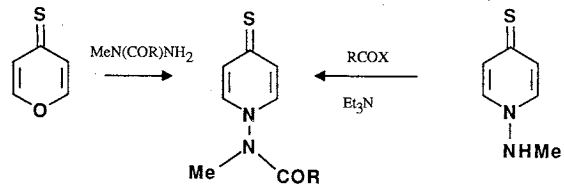
Scheme 2.



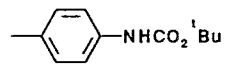
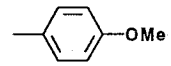
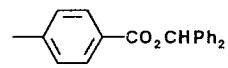
5 **6** **7d**～**7g** **4d**～**4g**

R **d** Hex, **e**  , **f** CH_2Ph, **g** $CH_2CO_2{}^tBu$

Scheme 3.



8 **4h**～**4m** **4n**

	R
h	Ph
i	
j	
k	
l	Me
m	NH_2

hydrazine or hydrazine derived carbazate with 4-thiopyranone (**8**).[8,9] This strategy is, however, limited by the accessibility of appropriate hydrazines and their reactivity with 4-thiopyranone (**8**). An alternative approach was therefore required which would facilitate the preparation of a wide range of alkyl substituted thiones of general structure (**4**). A versatile and efficient 2-stage synthesis was developed from the γ-pyrone derived 4-pyridone derivative (**6**) (Scheme 2). Thus, potassium carbonate mediated *N*-alkylation of pyridone (**6**) with alkyl halides followed by reaction of intermediates (**7**) with LAWESSON's reagent afforded *N*-monoalkylamino-4-thiopyridones such as (**4d**～**4g**).

The *N*-acylamino 4-thiopyridones (**4h**～**4m**) were prepared by two procedures (Scheme 3). Condensation of 4-thiopyranone (**8**) with the appropriate *N*-acylhydrazines or semicarbazide provided thiones (**4h**, **4i**) and (**4m**) respectively. Alternatively, a more convergent and expedient route, exemplified by (**4j**～**4l**), utilised direct acylation of the thione (**4n**).[8] The 4-thiopyridones (**4d**～**4m**) were then elaborated as shown in Scheme 1 to the required cephalosporins (**3d**～**3m**); the final deprotection with TFA unmasked the amino and acidic functionality in cephalosporins (**3g**), (**3i**) and (**3k**).

Results and Discussion

The antibacterial activity of the 3-[*N*-(substituted-amino)pyridinium-4-thiomethyl]cephalosporins (**3**) against a range of Gram-positive and Gram-negative bacteria is shown in Table 1, with cefpirome

Table 1. Antibacterial activity (MIC μg/ml)[a] of cephalosporins (**3**).

	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**
Escherichia coli DCO	0.03	<0.03	0.03	0.5	0.06	0.06	<0.03	0.12
E. coli DCOR TEM[b]	0.06	<0.03	0.03	0.5	0.12	0.12	0.06	0.5
Enterobacter cloacae N1	0.06	0.06	0.06	2	0.25	0.5	0.12	0.5
E. cloacae P99[b]	2	2	4	2	2	2	32	8
Klebsiella pneumoniae T767	<0.03	0.03	0.06	0.5	0.12	0.06	<0.03	0.12
K. pneumoniae 48[b]	0.5	0.5	1	4	2	2	8	4
Proteus mirabilis C997	0.06	0.06	0.25	2	0.5	0.5	0.06	0.5
Serratia marcescens US32	0.12	0.25	0.25	4	0.25	0.25	0.06	0.25
Pseudomonas aeruginosa Dalgleish[b]	4	4	8	>32	32	8	16	16
Staphylococcus aureus Russell[b]	0.5	0.25	0.5	0.5	1	0.5	2	1
Streptococcus pyogenes CN10	0.06	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	0.06

	3i	**3j**	**3k**	**3l**	**3m**	Cefpirome	Ceftazidime
Escherichia coli DCO	0.06	0.25	<0.03	0.03	0.03	0.03	0.12
E. coli DCOR TEM[b]	0.06	0.50	0.12	0.06	0.06	0.06	0.25
Enterobacter cloacae N1	0.25	1	0.12	0.06	0.12	0.06	0.25
E. cloacae P99[b]	8	8	8	4	8	1	128
Klebsiella pneumoniae T767	0.06	0.12	<0.03	0.03	0.03	0.06	0.25
K. pneumoniae 48[b]	4	4	8	2	2	4	32
Proteus mirabilis C997	0.5	0.5	0.25	0.06	0.12	0.06	0.12
Serratia marcescens US32	0.5	2	0.06	0.12	0.25	0.06	0.5
Pseudomonas aeruginosa Dalgleish[b]	8	16	8	8	8	2	1
Staphylococcus aureus Russell[b]	1	2	4	0.5	1	1	8
Streptococcus pyogenes CN10	<0.03	0.03	0.06	<0.03	<0.03	<0.03	0.12

[a] Agar dilution method Oxoid Iso-sensitest agar 10^4 cfu/spot and 10^6 cfu/spot for Gram-negative and Gram-positive bacteria, respectively.

[b] β-Lactamase producing strains.

Table 2. Antibacterial activities of (**3b**) against β-lactamase producing strains (MIC μg/ml).

Test organism	Type of β-lactamase[a]	**3b**	Cefpirome	Ceftazidime
Staphylococcus aureus Russell	2a P	0.25	1.0	8
Escherichia coli E96	2b P	0.03	0.03	0.06
E. coli CF604	2b' P	0.12	0.12	8.0
E. coli CF504	2b' P	2.0	2.0	>32
E. coli 8414-T	2b' P	0.25	4.0	>32
Klebsiella pneumoniae Bed 8	2b P	0.03	0.12	0.25
K. pneumoniae 20	2b' C	1.0	4.0	>32
Enterobacter cloacae V2033	1 C	1.0	2.0	>32
Citrobacter freundii Foxon	1 C	1.0	4.0	>32
Pseudomonas aeruginosa Dalgleish	2c C/P	4.0	2.0	1.0

[a] Karen Bush, AAC 33: 264 (1989). P: Penicillinase. C: Cephalosporinase.

Table 3. *In vivo* efficacy of (**3b**) in experimental mouse infections.

Test organism	Challenge dose (cfu/mouse)	ED_{50} mg/mouse[a] (MIC μg/ml)			
		3b	Cefpirome	Cefotaxime	Ceftazidime
Staphylococcus aureus Smith	4.0×10^8	0.48 (0.25)	2.7 (0.5)	3.3 (0.5)	60 (8.0)
Escherichia coli E96[b]	8.5×10^3	<0.05 (0.03)	0.09 (0.03)	0.09 (0.06)	NT NT
Citrobacter freundii T1739[b]	1.0×10^5	0.5 (1.0)	0.4 (1.0)	7 (>32)	>10 (>32)
Pseudomonas aeruginosa Pu21	8.5×10^6	660 (8.0)	420 (2.0)	NT NT	390 (1.0)

[a] Dosed sc at 1, 3, and 5 hours post challenge.
[b] β-Lactamase producing strain.
NT Not tested.
( ) MIC in parentheses.

and ceftazidime included as reference compounds.

Compounds (**3a**) and (**3b**) with *N*-amino and *N*-methylamino substituents respectively possessed potent broad spectrum activity including moderate activity against *P. aeruginosa*. Both compounds demonstrated good activity against β-lactamase producing organisms such as *Klebsiella pneumoniae* 48 and *Enterobacter cloacae* P99, resistant to ceftazidime. Most Gram-negative bacteria with the exception of *Escherichia coli* were slightly less susceptible to the dialkyl-substituted derivative (**3c**). Similarly introduction of more sterically demanding alkyl, or aryl, groups such as (**3e**) and (**3f**) resulted in reduced overall potency compared to (**3a**) and (**3b**).

Table 4. Pharmacokinetic parameters in mice.

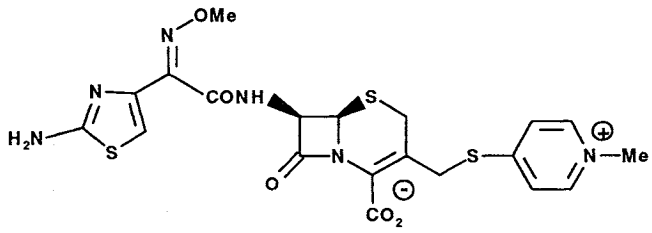
	3b	Cefpirome	Ceftazidime
$T_{1/2}$ (minutes)	9.3	10.7	11.1
AUC (0~2 hours) μg·minute/ml	1,210	717	597

Dose 25 mg/kg sc.

Extension of the alkyl group to *N*-hexyl (**3d**) compromised the activity against all the Gram-negative bacteria and acidic substitution such as (**3g**) gave diminished potency against *Staphylococcus aureus* and a constitutive chromosomal β-lactamase producer, *E. cloacae* P99.

N-Methyl-*N*-acetylamino (**3l**) and *N*-methyl ureido-substitution of the pyridinium nitrogen (**3m**)

Fig. 2.



resulted in similar Gram-negative potency to (**3a**) and (**3b**) but no advantage in anti-staphylococcal activity was observed. In comparison, Gram-negative and Gram-positive organisms were generally less susceptible to the benzoyl derivatives (**3h**～**3k**).

Overall, (**3a**) and (**3b**) were the most potent and broad spectrum compounds. Although the *N*-amino derivative (**3a**) had aqueous solubility of the order of 10%, the *N*-methyl amino counterpart (**3b**) was soluble at 50% w/v resulting in selection of (**3b**) for further evaluation.

The antibacterial activities against β-lactamase producing strains are shown in Table 2. (**3b**) was more active than cefpirome and ceftazidime against the β-lactamase producing organisms including those producing expanded spectrum β-lactamase (Group 2b') and constitutive Group 1 cephalosporinase.

In vivo (**3b**) was more efficacious in a mouse protection test than cefpirome, ceftazidime, or cefotaxime using *S. aureus*, and *E. coli* (Table 3). In the case of *Citrobacter freundii* infection, cefpirome and (**3b**) were of similar efficacy, both being superior to cefotaxime or ceftazidime. Although less effective than the comparators against *P. aeruginosa* infections, (**3b**) gave better results than would be predicted from MIC data. Whilst the half-life in the mouse was similar to cefpirome, the AUC of (**3b**) was higher than either cefpirome or ceftazidime (Table 4).

Of particular concern was the reported high acute toxicity[10] (LD_{50} (iv mice) <1 g/kg) of the 3-[*N*-methylpyridinium-4-thiomethyl]substituted cephalosporin shown in Fig. 2. It was thus reassuring that the acute toxicity (iv mice) of (**3b**) was in excess of 1 g/kg.

Conclusion

Against Gram-positive cocci and most Gram-negative bacteria including extended spectrum β-lactamase producing strains, the *N*-methylamino derivative (**3b**) was the most active compound *in vitro*, and with the exception of *P. aeruginosa*, generally superior to cefpirome. *In vivo*, (**3b**) also demonstrated excellent efficacy in experimental mouse infections.

Experimental

MP's were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded for chloroform solutions on a Perkin-Elmer 197 spectrophotometer and for KBr discs on Perkin-Elmer 457 or Perkin-Elmer 983 grating spectrophotometers. ^{1}H NMR spectra were obtained on Perkin-Elmer R32 (90 MHz) or Bruker WM 250 (250 MHz) instruments using TMS as internal standard, except for D_2O solutions when HOD (250 MHz) was used as internal standard. Mass spectra were recorded on either a VG 7070 or a VG ZAB spectrometer operating in the electron impact mode. Fast atom bombardment spectra were recorded on a VG ZAB spectrometer and the matrix used is stated. Microanalytical data were determined on a Carlo Erba 1106 elemental analyser. Preparative chromatography was carried out on Silica gel 60 (finer than 230 mesh ASTM) (Merck 7729).

The synthesis of the cephalosporin betaine (**3b**) and the *N*-(substituted-amino)-4-thiopyridones (**4g**), (**4i**) and (**4j**) are described as representative procedures.

4-Methoxybenzyl (6*R*,7*R*)-3-[Chloromethyl]-7-[2-(*Z*)-(methoxyimino)-2-(2-tritylamino-thiazol-4-yl)acetamido]ceph-3-em-4-carboxylate (**1**)

To 2-(*Z*)-(methoxyimino)-2-(2-tritylamino-thiazol-4-yl)acetic acid hydrochloride (7.68 g, 0.016 mol) in DMF (60 ml) at −40°C was added *N*,*N*-diisopropylethylamine (5.6 ml, 0.032 mol) and methane sulphonyl chloride (1.2 ml, 0.016 mol) and the reaction warmed to 0°C. After 30 minutes, the reaction was recooled to −40°C and a solution of 4-methoxybenzyl (6*R*,7*R*)-7-amino-3-[chloromethyl]-ceph-3-em-4-carboxylate hydrochloride (6.50 g, 0.016 mol) and *N*,*N*-diisopropylethylamine (5.6 ml, 0.032 mol) in DMF (20 ml) added. After stirring 1.5 hours at room temperature, the reaction mixture was partitioned between ethyl acetate and water. The organic phase was separated, washed with dilute citric acid, water, dilute sodium hydrogen carbonate, brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed eluting with ethyl acetate - hexane mixtures to afford (**1**) (9.58 g, 76%). IR (KBr) cm^{-1} 1783, 1724, 1676 and 1610; ^1H NMR (250 MHz, $CDCl_3$) δ 3.47 and 3.65 (2H, ABq, J=18 Hz), 3.81 (3H, s), 4.07 (3H, s), 4.43 and 4.54 (2H, ABq, J=12 Hz), 5.03 (1H, d, J=5 Hz), 5.22 (2H, AA'), 5.92 (1H, dd, J=5, 9 Hz), 6.69 (1H, s), 6.84 (1H, d, J=9 Hz, exch.), 6.90 (2H, ABq, J=7 Hz), 7.04 (1H, s) and 7.30 (17H, m); FAB-MS (3-NOBA/Na) m/z 816 (M+Na).

(6*R*,7*R*)-7-[2-(2-Amino-thiazol-4-yl)-2-(*Z*)-(methoxyimino)acetamido]-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate (**3b**)

To compound (**1**) (0.16 g, 0.2 mmol) in acetonitrile (10 ml) was added sodium iodide (0.033 g, 0.22 mmol) and compound (**4b**) (0.05 g, 0.2 mmol). After stirring for 2 hours the reaction mixture was evaporated and chromatographed eluting with ethanol - dichloromethane mixtures to give compound (**2b**) (R_1=Me, R_2=CO_2Bu^t) (0.18 g, 78%). IR (KBr) cm^{-1} 1783, 1725, 1677 and 1614; ^1H NMR (250 MHz, $CDCl_3$) δ 1.51 (9H, s), 3.56 and 3.88 (2H, ABq, J=18 Hz), 3.67 (3H, s), 3.79 (3H, s), 4.04 (3H, s), 4.50 and 4.58 (2H, ABq, J=13 Hz), 5.11 (1H, d, J=5 Hz), 5.17 and 5.25 (2H, ABq, J=12 Hz), 5.90 (1H, dd, J=5, 9 Hz), 6.63 (1H, s), 6.87 (2H, d, J=9 Hz), 6.98 (1H, br s, exch.), 7.16~7.36 (18H, m), 8.09 (2H, d, J=7 Hz) and 8.63 (2H, d, J=7 Hz); FAB-MS (thioglycerol) m/z 998 (M).

Compound (**2b**) (0.1 g, 0.088 mmol) was dissolved in TFA (1 ml) and after 10 minutes the mixture was evaporated to dryness and the residue triturated with ether (3 × 30 ml). The product was dissolved in water with sodium bicarbonate to pH 7.0, chromatographed on Diaion HP20SS eluting with 2~5% THF and water and the product-containing fractions were combined and lyophilised to give (**3b**) (0.024 g, 50%). IR (KBr) cm^{-1} 1760, 1665, 1615 and 1530; ^1H NMR (250 MHz, D_2O) δ 3.01 (3H, s), 3.43 and 3.71 (2H, ABq, J=18 Hz), 3.95 (3H, s), 4.11 and 4.40 (2H, ABq, J=14 Hz), 5.14 (1H, d, J=4.5 Hz), 5.74 (1H, d, J=4.5 Hz), 6.96 (1H, s), 7.78 (2H, d, J=7.5 Hz) and 8.50 (2H, d, J=7.5 Hz); FAB-MS (thioglycerol) m/z 536 (M+H).

Cephalosporins (**3a**, **3c**~**3m**) were synthesised from (**1**) by analogous procedures[11] and their spectral data are shown in Table 5.

1-(*tert*-Butyloxycarbonylamino)-4-pyridone (**6**)

A solution of 4-pyranone (**5**) (1 g, 10.4 mmol) and *tert*-butylcarbazate (1.32 g, 10 mmol) in ethanol was refluxed for 48 hours, then concentrated and the residue chromatographed, eluting with ethanol - dichloromethane (1 : 19), to afford (**6**) (1.16 g, 50%); MP 189~190°C (dec.) ($CHCl_3$ - Hexane); IR (KBr) cm^{-1} 1720, 1630 and 1550; ^1H NMR (250 MHz, $CDCl_3$) δ 1.54 (9H, s), 6.47 (2H, d, J=7 Hz) and 7.60 (2H, d, J=7 Hz); MS m/z 210.1006 (M^+, $C_{10}H_{14}N_2O_3$ requires M, 210.1004).

Anal Calcd for $C_{10}H_{14}N_2O_3$: C 57.1, H 6.7, N 13.3.
Found: C 57.4, H 6.9, N 13.4.

1-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(*tert*-butyloxycarbonylmethyl)amino]-4-pyridone (**7g**)

To compound (**6**) (0.2 g, 0.95 mmol) in DMF (10 ml) was added successively potassium carbonate (0.143 g, 1.04 mmol) and *tert*-butyl bromoacetate (0.17 ml, 1.04 mmol). The reaction mixture was stirred

Table 5. Yield and spectral data of Cephalosporins (**3**).

Compound No.	Yield (%) from (1)	IR (KBr) cm^{-1}	MS[a] (*m/z*)	^1H NMR (250 MHz, D_2O, δ ppm)
3a	25	1763 1670 1611	522	3.43, 3.72 (2H, ABq, $J=17.5$ Hz), 3.94 (3H, s), 4.13, 4.41 (2H, ABq, $J=14$ Hz), 5.15 (1H, d, $J=4.5$ Hz), 5.73 (1H, d, $J=4.5$ Hz), 6.96 (1H, s), 7.73 (2H, d, $J=7$ Hz), 8.38 (2H, d, $J=7$ Hz)
3c	43	1760 1670 1610	550[b]	2.97 (6H, s), 3.41, 3.70 (2H, ABq, $J=17.6$ Hz), 3.92 (3H, s), 4.10, 4.39 (2H, ABq, $J=13.7$ Hz), 5.13 (1H, d, $J=4.6$ Hz), 5.72 (1H, d, $J=4.6$ Hz), 6.95 (1H, s), 7.80, 8.65 (4H, ABq, $J=7.2$ Hz)
3d	41	1764 1674 1619	606	0.80 (3H, t, $J=7$ Hz), 1.16~1.57 (8H, m), 3.25 (2H, t, $J=7$ Hz), 3.41, 3.69 (2H, ABq, $J=18$ Hz), 3.93 (3H, s), 4.12, 4.40 (2H, ABq, $J=14$ Hz), 5.12 (1H, d, $J=5$ Hz), 5.71 (1H, d, $J=5$ Hz), 6.94 (1H, s), 7.79 (2H, d, $J=7$ Hz), 8.49 (2H, d, $J=7$ Hz)
3e	18	1761 1726 1675 1613	590	1.35~1.86 (8H, m), 3.42, 3.71 (2H, ABq, $J=17.5$ Hz), 3.94 (3H, s), 4.11, 4.40 (2H, ABq, $J=14$ Hz), 4.70~4.98 (1H, m), 5.15 (1H, d, $J=5$ Hz), 5.73 (1H, d, $J=5$ Hz), 6.97 (1H, s), 7.78 (2H, d, $J=7$ Hz), 8.48 (2H, d, $J=7$ Hz)
3f	26	1772 1671 1617	612	[d]3.40, 3.67 (2H, ABq, $J=17.7$ Hz), 3.96 (3H, s), 4.10, 4.39 (2H, ABq, $J=13.8$ Hz), 4.42 (2H, s), 5.13 (1H, d, $J=4.7$ Hz), 5.74 (1H, d, $J=4.7$ Hz), 7.0 (1H, s), 7.25 (2H, m), 7.35 (3H, m), 7.68 (2H, d, $J=7.1$ Hz, 8.27 (2H, d, $J=7.1$ Hz)
3g	23	1769 1669 1618	580	3.44, 3.72 (2H, ABq, $J=17.5$ Hz), 3.99 (3H, s), 4.02 (2H, s), 4.12, 4.40 (2H, ABq, $J=13.5$ Hz), 5.16 (1H, d, $J=5$ Hz), 5.76 (1H, d, $J=5$ Hz), 7.04 (1H, s), 7.77 (2H, d, $J=7$ Hz), 8.57 (2H, d, $J=7$ Hz)
3h	22	1769 1671 1616	640	[d]3.43, 3.68 (2H, ABq, $J=17.5$ Hz), 3.69 (3H, s), 3.93 (3H, s), 4.24, 4.43 (2H, ABq, $J=14$ Hz), 5.14 (1H, d, $J=4.5$ Hz), 5.74 (1H, d, $J=4.5$ Hz), 6.95 (1H, s), 7.45~7.70 (5H, m), 7.97 (2H, d, $J=7$ Hz), 8.75 (2H, d, $J=7$ Hz)
3i	24	1761 1669 1605	—	[d]3.51, 3.75 (2H, ABq, $J=17$ Hz), 3.81 (3H, s), 3.99 (3H, s), 4.01 (3H, s), 4.35, 4.51 (2H, ABq, $J=13$ Hz), 5.21 (1H, d, $J=5$ Hz), 5.82 (1H, d, $J=5$ Hz), 6.83 (2H, d, $J=8$ Hz), 6.99 (1H, s), 7.54 (2H, d, $J=8$ Hz), 8.09 (2H, d, $J=7$ Hz), 8.81 (2H, d, $J=7$ Hz)
3j	52	1762 1672 1616	670	[d]3.52, 3.76 (2H, ABq, $J=18$ Hz), 3.84 (3H, s), 3.93 (3H, s), 3.99 (3H, s), 4.54, 4.60 (2H, ABq, $J=14$ Hz), 5.22 (1H, d, $J=4$ Hz), 5.84 (1H, d, $J=4$ Hz), 6.9 (1H, s), 7.12 (2H, d, $J=9$ Hz), 7.77 (2H, d, $J=9$ Hz), 8.25 (2H, d, $J=7$ Hz), 8.94 (2H, d, $J=7$ Hz)
3k	17	1759 1670 1617	684[c]	3.42 (1H, d, $J=18$ Hz), 3.69 (3H, s), 3.94 (3H, s), 4.24, 4.40 (2H, ABq, $J=13$ Hz), 5.17 (1H, d, $J=5$ Hz), 5.75 (1H, d, $J=5$ Hz), 6.96 (1H, s), 7.68 (2H, d, $J=8$ Hz), 7.86~8.07 (4H, m), 8.73 (2H, d, $J=7$ Hz)
3l	50	1758 1671 1617	578	2.35 (3H, s), 3.45, 3.73 (2H, ABq, $J=18$ Hz), 3.70 (3H, s), 3.95 (3H, s), 4.21, 4.44 (2H, ABq, $J=14$ Hz), 5.17 (1H, d, $J=4.5$ Hz), 5.75 (1H, d, $J=4.5$ Hz), 6.98 (1H, s), 7.94 (2H, d, $J=7$ Hz), 8.52 (2H, d, $J=7$ Hz)
3m	28	1765 1670 1615	579	3.52 (3H, s), 3.44, 3.72 (2H, ABq, $J=17$ Hz), 3.94 (3H, s), 4.18, 4.43 (2H, ABq, $J=14$ Hz), 5.16 (1H, d, $J=5$ Hz), 5.75 (1H, d, $J=5$ Hz), 6.97 (1H, s), 7.90 (2H, d, $J=7$ Hz)

[a] FAB (thioglycerol) $(M+H)^+$.
[b] Thioglycerol-thiodiethylene glycol matrix.
[c] $M+H^+$ for the corresponding acid.
[d] $+(CD_3)_2CO$.

at room temperature for 1.5 hours, diluted with ethyl acetate and washed with water. The organic phase was separated, dried ($MgSO_4$) and evaporated. Chromatography of the residue eluting with ethanol - dichloromethane (1 : 19) afforded (**7g**) (0.287 g, 93%), MP 163~164°C (EtOAc - hexane); IR (KBr) cm^{-1} 1741, 1725, 1660 and 1589; ^1H NMR (250 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.50 (9H, s), 4.25 (2H, s), 6.40 (2H, d, J=7 Hz) and 7.56 (2H, d, J=7 Hz); MS m/z 324.1681 (M^+, $C_{16}H_{24}N_2O_5$ requires M, 324.1685).

Anal Calcd for $C_{16}H_{24}N_2O_5$: C 59.3, H 7.4, N 8.6.
Found: C 59.3, H 7.6, N 8.8.

In a similar manner were prepared pyridones **7d**~**7f**.[11]

1-[*N*-(*tert*-Butyloxycarbonyl)-*N*-hexylamino]-4-pyridone (**7d**)

93%; off-white amorphous solid; IR (CH_2Cl_2) cm^{-1} 1723, 1644 and 1590; ^1H NMR (250 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.5 Hz), 1.31 (8H, m), 1.44 (9H, s), 3.73 (2H, m), 6.35 (2H, d, J=8 Hz) and 7.21 (2H, d, J=8 Hz); MS m/z 294.1946 (M^+, $C_{16}H_{26}N_2O_3$ requires M, 294.1943).

1-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(cyclopentyl)amino]-4-pyridone (**7e**)

55%; MP 106~108°C (EtOAc - Hexane); IR (CH_2Cl_2) cm^{-1} 1719, 1650 and 1591; ^1H NMR (250 MHz, $CDCl_3$) δ 1.37~1.46 (11H, m), 1.46~1.48 (4H, m), 1.84~1.97 (4H, m, 2H exchangeable), 4.41~4.54 (1H, m), 6.24 (2H, d, J=8 Hz) and 7.14 (2H, d, J=8 Hz); MS m/z 278.1637 (M^+, $C_{15}H_{22}N_2O_3$ requires M, 278.1630).

Anal Calcd for $C_{15}H_{22}N_2O_3 \cdot H_2O$: C 60.8, H 8.1, N 9.5.
Found: C 60.9, H 8.4, N 9.5.

1-[*N*-Benzyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-pyridone (**7f**)

81%; MP 112~113°C (EtOAc - Hexane); IR (KBr) cm^{-1} 1727, 1630 and 1577; ^1H NMR (250 MHz, $CDCl_3$) δ 1.48 (9H, s), 4.79 (2H, s), 6.15 (2H, d, J=8 Hz), 6.92 (2H, d, J=8 Hz), 7.2 (2H, m) and 7.36 (3H, m); MS m/z 300.1477 (M^+, $C_{17}H_{20}N_2O_3$ requires M, 300.1474).

Anal Calcd for $C_{17}H_{20}N_2O_3$: C 68.0, H 6.7, N 9.3.
Found: C 68.1, H 6.8, N 9.4.

1-[*N*-(*tert*-butyloxycarbonyl)-*N*-(*tert*-butyloxycarbonylmethyl)amino]-4-thiopyridone (**4g**)

Pyridone (**7g**) (0.21 g, 0.64 mmol) in toluene (20 ml) was treated with LAWESSON's Reagent (0.17 g, 0.65 mmol). The mixture was heated at 80°C for 1 hour, cooled and chromatographed eluting with ethyl acetate - hexane to give thione (**4g**) as an amorphous solid (0.15 g, 68%); IR ($CHCl_3$) cm^{-1} 1750, 1615, 1150 and 1120; ^1H NMR (250 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.50 (9H, s), 4.26 (2H, s) and 7.38 (4H, s); MS m/z 340.1454 (M^+, $C_{16}H_{24}N_2O_4S$ requires M, 340.1457).

4-Thiopyridones (**4d**~**4f**) were synthesised in a similar manner.[11]

1-[*N*-(*tert*-Butyloxycarbonyl)-*N*-hexylamino]-4-thiopyridone (**4d**)

66%; yellow amorphous solid; IR (CH_2Cl_2) cm^{-1} 1727 and 1618; ^1H NMR (250 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.5 Hz), 1.26~1.63 (8H, m), 1.45 (9H, s), 3.64 (2H, t, J=7.5 Hz), 7.06 (2H, d, J=7 Hz) and 7.42 (2H, d, J=7 Hz); MS m/z 310.1718 (M^+, $C_{16}H_{26}N_2O_2S$ requires M, 310.1715).

1-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(cyclopentyl)amino]-4-thiopyridone (**4e**)

51%; MP 192°C (dec.) (EtOAc - Hexane); IR (CH_2Cl_2) cm^{-1} 1723 and 1618; ^1H NMR (250 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.53~1.71 (6H, m), 1.91~2.05 (2H, m), 4.49~4.62 (1H, m), 6.95~7.0 (2H, m) and 7.34~7.40 (2H, m); MS m/z 294.1404 (M^+, $C_{15}H_{22}N_2O_2S$ requires M, 294.1402).

Anal Calcd for $C_{15}H_{22}N_2O_2S$: C 61.2, H 7.5, N 9.5, S 10.9.
Found: C 61.1, H 7.5, N 9.5, S 10.7.

1-[*N*-Benzyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-thiopyridone (**4f**)

88%; MP 170~172°C (EtOAc - Hexane); IR (KBr) cm^{-1} 1708 and 1612; ^1H NMR (250 MHz, $CDCl_3$) δ 1.49 (9H, s), 4.80 (2H, s), 6.72 (2H, d, J=5.8 Hz), 7.2~7.3 (4H, m) and 7.3~7.4 (3H, m); MS m/z 316.1244 (M^+, $C_{17}H_{20}N_2O_2S$ requires M, 316.1245).

Anal Calcd for $C_{17}H_{20}N_2O_2S$: C 64.5, H 6.4, N 8.9, S 10.1.
Found: C 64.7, H 6.4, N 8.9, S 10.2.

1-{*N*-[4-(*tert*-Butyloxycarbonylamino)benzoyl]-*N*-methylamino}-4-thiopyridone (**4i**)

1-[4-(*tert*-Butyloxycarbonylamino)benzoyl]-1-methyl hydrazine (0.205 g, 0.77 mmol) and compound (**8**) (0.087 g, 0.78 mmol) were heated at reflux in ethanol (20 ml) for 16 hours. The solvent was evaporated and the residue chromatographed eluting with dichloromethane and then ethylacetate - hexane to give (**4i**) as a yellow glass (0.09 g, 32%); IR (KBr) cm^{-1} 1725, 1670 and 1610; ^1H NMR (90 MHz, $CDCl_3+CD_3OD$) δ 1.51 (9H, s), 3.52 (3H, s), 7.35~7.50 (8H, m) and 8.48 (1H, br s, exch.); MS *m/z* 359.1311 (M^+, $C_{18}H_{21}N_3O_3S$ requires M, 359.1304).

In a similar manner were prepared 4-thiopyridones (**4h**, **4m**).

1-[*N*-Benzoyl-*N*-methylamino]-4-thiopyridone (**4h**)

36%; MP 173~180°C (dec.) ($CHCl_3$ - hexane); IR (CH_2Cl_2) cm^{-1} 1684 and 1616; ^1H NMR (250 MHz, $CDCl_3$) δ 3.54 (3H, s), 7.10 (2H, d, J=7 Hz) and 7.2~7.6 (7H, m); MS *m/z* 244.0674 (M^+, $C_{13}H_{12}N_2OS$ requires M, 244.0670).

Anal Calcd for $C_{13}H_{12}N_2OS$: C 63.9, H 5.0, N 11.5, S 13.2.
Found: C 63.9, H 4.9, N 11.4, S 13.2.

1-(1-Methylureido)-4-thiopyridone (**4m**)

13%; orange amorphous solid; IR (CH_2Cl_2) cm^{-1} 1709 and 1618; ^1H NMR (250 MHz, $(CD_3)_2SO$) δ *inter alia* 3.27 (3H, s), 6.74 (2H, s, exch.), 7.13 (2H, d, J=6 Hz) and 7.69 (2H, d, J=6 Hz); MS *m/z* 183.0466 (M^+, $C_7H_9N_3OS$ requires M, 183.0466).

1-[*N*-(4-Methoxybenzoyl)-*N*-methylamino]-4-thiopyridone (**4j**)

To a solution of compound (**4n**) (0.078 g, 0.56 mmol) and triethylamine (0.056 g, 0.56 mmol) in THF (25 ml) at room temperature was added 4-methoxybenzoyl chloride (0.095 g, 0.56 mmol). After 0.5 hour, the reaction mixture was evaporated and chromatographed eluting with methanol - dichloromethane mixtures to afford (**4j**) (0.148 g, 96%) MP 172~173°C (CH_2Cl_2 - hexane); IR (KBr) cm^{-1} 1775, 1673 and 1606; ^1H NMR (250 MHz, $CDCl_3$) δ 3.49 (3H, s), 3.80 (3H, s), 6.87 (2H, d, J=9 Hz), 7.11 (2H, d, J=7 Hz), 7.30 (2H, d, J=7 Hz) and 7.44 (2H, d, J=8 Hz); MS *m/z* 274.0773 (M^+, $C_{14}H_{14}N_2O_2S$ requires M, 274.0776).

In an analogous manner were prepared 4-thiopyridones (**4k**, **4l**).[11]

1-[*N*-(4-Diphenylmethoxycarbonylbenzoyl)-*N*-methylamino]-4-thiopyridone (**4k**)

81%; yellow amorphous solid; IR (KBr) cm^{-1} 1715, 1677, 1615; ^1H NMR (250 MHz, $(CD_3)_2SO$) δ 5.44 (3H, s), 7.05 (1H, s), 7.24~7.59 (16H, m) and 7.96 (2H, d, J=7 Hz); MS *m/z* 454.1358 (M^+, $C_{27}H_{22}N_2O_3S$ requires M, 454.1351).

1-[*N*-Acetyl-*N*-methylamino]-4-thiopyridone (**4l**)

62%; MP 220~222°C (dec.) ($CHCl_3$ - hexane); IR (CH_2Cl_2) cm^{-1} 1700 and 1618; ^1H NMR (250 MHz, $CDCl_3$) δ 2.02 (3H, s), 3.41 (3H, s), 7.08 (2H, d, J=7 Hz) and 7.42 (2H, d, J=7 Hz); MS *m/z* 182.0516 (M^+, $C_8H_{10}N_2OS$ requires M, 182.0514).

Anal Calcd for $C_8H_{10}N_2OS$: C 63.9, H 5.0, N 11.5, S 13.2.
Found: C 63.9, H 4.9, N 11.4, S 13.2.

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References

1) HEYMES, R.; A. LUTZ & E. SHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative.

Pre-clinical study. Infection 5: 259~260, 1977
2) VERBIST, L. & J. VERHAEGEN: GR-20263: A new aminothiazolyl cephalosporin with high activity against *Pseudomonas* and *Enterobacteriaceae*. Antimicrob. Agents Chemother. 17: 807~812, 1980
3) REINER, R.; U. WEISS, U. BROMBACHER, P. LANZ, M. MONTAVON, A. FURLENMEIER, A. ANGEHRN & P. J. PROBST: RO 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiotics 33: 783~786, 1980
4) PROUS, J. R.: Cefpirome. Drugs Future 1984: 252~255, 1984
5) TSURUOKA, T.; T. YOSHIDA, K. KATANO, S. NAKABAYASHI, K. IWAMATSU, H. OGINO, T. OKONOGI, Y. MURAI, I. KOMIYA, M. NISHIO, Y. KAZUNO & S. INOUYE: New aminothiazole cephalosporins with 3-pyridiniumthiomethyl substituents. *In* Recent Advances in Chemotherapy Antimicrobial Section 2. *Ed.*, J. ISHIGAMI, pp. 877~878, University of Tokyo Press, 1985
6) SHIBAHARA, S.; T. OKONOGI, T. YOSHIDA, Y. MURAI, T. KUDO, S. INOUYE & S. KONDO: A new aminothiazolylcephalosporin having 1-carboxyethoxyimino group, ME1228. J. Antibiotics 43: 62~69, 1990
7) KATANO, K.; H. OGINO, K. IWAMATSU, S. NAKABAYASHI, T. YOSHIDA, I. KOMIYA, T. TSURUOKA, S. INOUYE & S. KONDO: Synthesis and biological activity of (cyclopentenopyridinium)thiomethylcephalosporins. J. Antibiotics 43: 1150~1159, 1990
8) GUEST, A. W.; R. G. ADAMS, M. J. BASKER, E. G. BRAIN, C. L. BRANCH, F. P. HARRINGTON, J. E. NEALE, M. J. PEARSON & I. I. ZOMAYA: Synthesis and biological activity of 3-(*N*-substituted pyridinium-4-thiomethyl)-7α-formamido cephalosporins. J. Antibiotics 46: 1279~1288, 1993
9) REID, D. H. & R. G. WEBSTER: Studies of heterocyclic compounds. Pt. XII. A three-step synthesis of 6α-thiathiophthen from γ-pyrone. J. Chem. Soc. Perkin Trans. I. 1972: 1447~1449, 1972
10) SHIBAHARA, S.; T. OKONOGI, Y. MURAI, T. KUDO, T. YOSHIDA, K. NISHIHATA & S. KONDO: Structure-activity relationships of novel cephalosporins leading to ME 1228. Program and Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 643, p. 209, New York, Oct. 4~7, 1987
11) BRANCH, C. L.; R. G. ADAMS & A. W. GUEST (Beecham): Cephalosporin compounds, process for their preparation, pharmaceutical compositions and intermediates. Eur. Pat. Appl. 0 416 814 A2, Sept. 1, 1989