SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3-(*N*-SUBSTITUTED PYRIDINIUM-4-THIOMETHYL)-7α-FORMAMIDO CEPHALOSPORINS

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The synthesis and antibacterial activity of a series of 3-(1-substituted pyridinium-4-thiomethyl)-7α-formamido cephalosporins is described. All the derivatives showed good potency and stability to bacterial β-lactamases. The antibacterial efficacy seen with the *N*-alkyl pyridinium substituents was enhanced by the introduction of a catecholic side chain at C-7 and by preparation of *N*-(substituted amino)pyridinium derivatives.

Previous publications from these laboratories have described a series of semi-synthetic 7α-formamido cephalosporins with (heterocyclylthiomethyl) substituents at C-3[1,2] (**1**). These highly β-lactamase stable antibiotics possess good activity against Gram-negative bacteria, but potency against Gram-positive organisms, in particular *Staphylococci*, was only moderate (MIC's $>8\,\mu g/ml$).

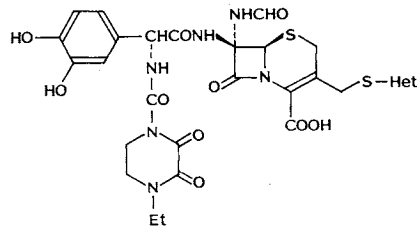
Following recent reports on cephalosporins bearing a C-3 (pyridiniumthiomethyl) group[3,4] we now wish to disclose our own studies on the chemistry and biological properties of the 7α-formamido cephalosporins[1] characterised by this type of C-3 substituent. Although the simple alkyl substituted compound (**2**) possessed moderate antibacterial activity, extending the scope of substitution from *N*-alkyl to *N*-amino proved of interest. Consequently we decided to fully explore the potential of this type of derivative by incorporating a variety of substituents on nitrogen.[5]

Chemistry

The preferred route to the 7α-formamido cephalosporins (**2**)~(**12**) is outlined in Scheme 1 and involves preparation of the halomethyl cephem (**15**) by acylation of nucleus (**14**)[5] with the appropriate side-chain acid chloride (**13**). For the preparation of intermediate (**15c**) bearing a catechol functionality, the side-chain acid chloride was protected as the bistrimethylsilyl derivative (**13b**)[6] during the acylation step. Utilising this temporary protection avoids a second deprotection step in the final stages of the synthesis. During the course of the acylation reaction some halogen exchange occurs to give intermediates (**15a** and **15c**) as a mixture of bromomethyl and chloromethyl compounds. Displacement of halide by an appropriately substituted thiopyridone *e.g.* (**18**) could be carried out on the bromo/chloromethyl cephem (**15**) or more rapidly on the *in situ* generated iodide (**16**) to give the ester salt (**17**). Trifluoroacetic acid mediated deprotection then afforded the betaines (**2**)~(**12**). Experimental details for representative examples are given.

The key step in the synthetic sequence is the preparation of the thiopyridones used in the displacement reaction. The routes to these compounds are shown in Schemes 2 and 3.

The *N*-alkyl-4-thiopyridones (**18**)~(**20**) were available from 4-pyridone (**29**) by the route shown in



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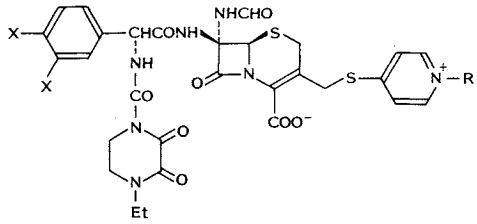
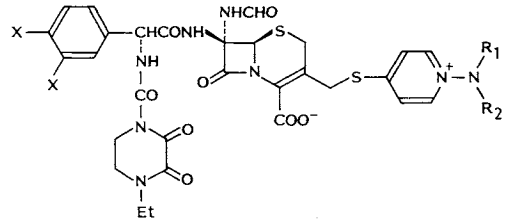
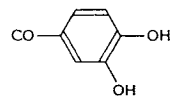
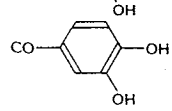
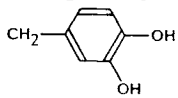




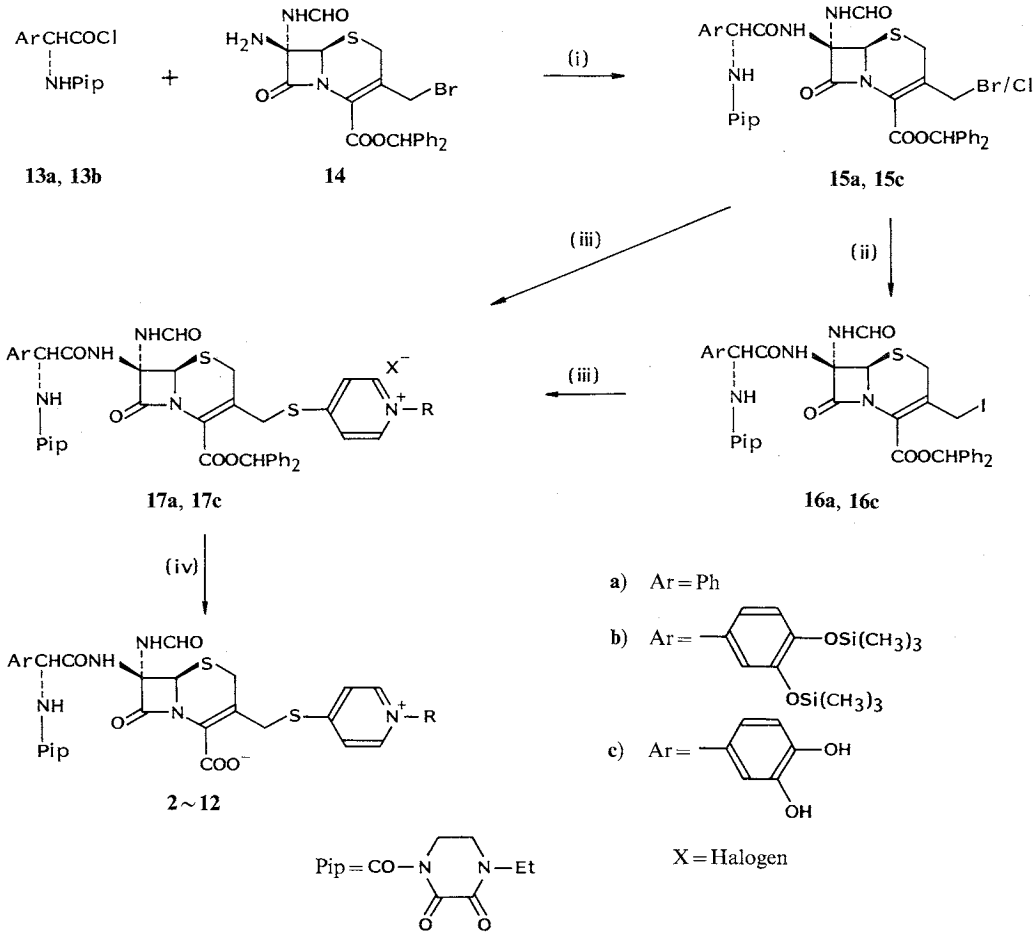
	R	X		R_1	R_2	X
2	CH_3	H	7		H	H
3	CH_3	OH	8		CH_3	H
4	CH_2COOH	H	9	CH_3	CH_3	H
5	CH_2COCH_3	H	10	CH_3	H	H
6		H	11	CH_3	H	OH
			12	H	H	OH

Scheme 2. In fact the first example *N*-methyl-4-thiopyridone (**18**) was prepared by a literature procedure[7] involving fusion of 4-pyridone, methyl iodide, and potassium hydroxide and subsequent heating of the product with phosphorus pentasulphide. In a milder version of this process 4-pyridone (**29**) and alkyl halide were reacted in *N*,*N*-dimethylformamide (DMF) in the presence of potassium carbonate and the subsequent reaction with phosphorus pentasulphide utilised toluene as solvent. In this way thiopyridones (**19**) and (**20**) were obtained.

A more versatile synthesis was provided by treatment of 4-thiopyrone (**31**) with amines[8] or disubstituted hydrazines as shown in Scheme 3, 4-thiopyrone (**31**) being obtained from 4-pyrone (**30**) after treatment with LAWESSON's reagent in toluene at reflux.[9] Thiopyridones (**21**), (**22**), (**23**) and (**24**) were available directly by this route from (**30**) and the appropriate amine, acyl or dialkyl hydrazine. Protection of the hydrazine was required for derivatives (**26**) and (**28**), since 4-thiopyrones are known to give pyrazoles with monoalkyl hydrazines.[10] Use of *N*-(*tert*-butyloxycarbonyl)-*N*-methyl hydrazine and *tert*-butyl carbazate gave the protected 4-thiopyridones (**25**) and (**27**). These were deprotected using trifluoroacetic acid to give thiopyridones (**26**) and (**28**) prior to use in the preparation of betaines (**10**)~(**12**).

Thiopyridone (**23**), protected at the catechol moiety, was selected for the synthesis of betaine (**8**). The 4-methoxybenzyl ether proved ideal since it was not necessary to deprotect the thiopyridone (**23**). Removal

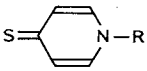
Scheme 1.



Reagents:

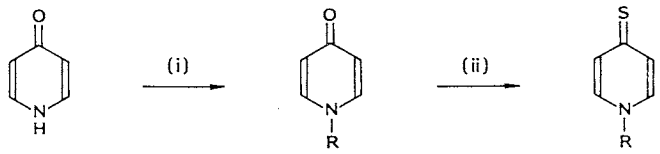
(i) Pyridine, CH_2Cl_2, tetrahydrofuran

(ii) NaI, acetone

(iii)  , CH_2Cl_2

(iv) CF_3COOH

Scheme 2.



29

18 R=CH_3

19 R=$CH_2COOCHPh_2$

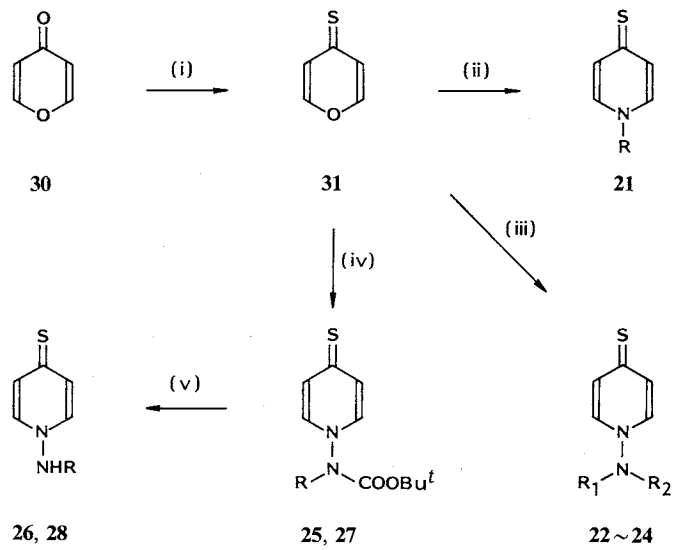
20 R=CH_2COCH_3

Reagents:

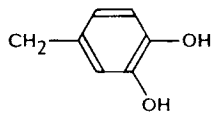
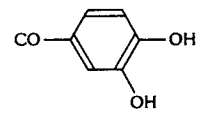
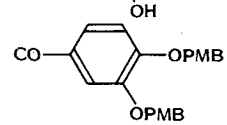
(i) RX, KOH or RX, K_2CO_3, DMF

(ii) P_4S_{10}, Δ or P_4S_{10}, toluene, reflux

Scheme 3.



Reagents:
(i) LAWESSON's reagent, toluene, reflux, 1 hour
(ii) EtOH, RNH_2, reflux
(iii) $R_1(R_2)NNH_2$, DMF, room temperature or $R_1(R_2)NNH_2$, EtOH, reflux
(vi) $RN(COO^tBu)NH_2$, EtOH, reflux
(v) CF_3COOH

	R		R_1	R_2
21		22		H
25, 26	CH_3	23		CH_3
27, 28	H	24	CH_3	CH_3

PMB: 4-methoxybenzyl.

of the 4-methoxybenzyl group occurred concomitantly with ester hydrolysis using trifluoroacetic acid in the final stage of the synthesis of betaine (**8**).

Results and Discussion

The antibacterial activities of a series of C-7α-formamido-substituted piperazinyl cephalosporins with (1-alkylpyridinium)-4-thiomethyl C-3-substituents are shown in Table 1. The methyl substituted compound (**2**) had broad-spectrum activity and was highly stable to a wide range of bacterial β-lactamases, MIC values being in the range 0.25~8.0 μg/ml against many organisms including strains resistant to ceftazidime. The corresponding 3,4-dihydroxyphenyl analogue (**3**) showed much improved activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa* but was about four- to eight-fold less active

Table 1. *In vitro* activity of 7α-formamido-3-(1-alkylpyridinium)thiomethyl cephems (MIC[a], μg/ml).

Organism	2	3	4	5	6	Ceftazidime
Escherichia coli DCO	0.25	0.03	0.25	1.0	0.5	0.12
E. coli DCO RTEM	0.25	0.03	0.5	1.0	0.5	0.25
Klebsiella pneumoniae T767	1.0	0.03	0.5	1.0	1.0	0.25
Enterobacter cloacae P99[b]	1.0	0.25	2.0	1.0	1.0	>32
Serratia marcescens US32	1.0	0.25	1.0	2.0	0.5	0.5
S. marcescens HCN 3956[b]	0.5	0.5	2.0	4.0	1.0	>32
Proteus mirabilis C977	2.0	4.0	2.0	0.5	2.0	0.12
Pseudomonas aeruginosa 10662	8.0	0.12	8.0	8.0	0.5	1.0
P. aeruginosa Badia[c]	4.0	0.06	8.0	4.0	0.5	>32
Streptococcus pyogenes CN10	0.12	0.5	0.5	0.25	2.0	0.12
Staphylococcus aureus Oxford	2.0	16	16	4.0	8.0	8.0

[a] Serial dilution in Diagnostic Sensitest agar containing 5% defibrinated horse blood inoculated with 0.001 ml of an overnight broth culture diluted 1/100 (approx. 10^4 cfu/spot).
[b] Constitutive class I β-lactamase producing strain.
[c] Ceftazidime-resistant strain.

Table 2. *In vitro* activity of 7α-formamido-3-(1-substituted aminopyridinium)thiomethyl cephems (MIC[a], μg/ml).

Organism	7	8	9	10	11	12
Escherichia coli DCO	≤0.03	≤0.03	0.5	0.5	≤0.03	≤0.03
E. coli DCO RTEM	—	≤0.03	0.5	1.0	≤0.03	≤0.03
Klebsiella pneumoniae T767	0.06	≤0.03	1.0	1.0	≤0.03	≤0.03
Enterobacter cloacae P99[b]	0.5	0.12	2.0	4.0	0.5	0.25
Serratia marcescens US32	1.0	0.06	2.0	0.25	0.25	0.06
S. marcescens HCN 3956[b]	1.0	0.5	4.0	1.0	0.5	0.25
Proteus mirabilis C977	8.0	0.25	4.0	4.0	1.0	1.0
Pseudomonas aeruginosa 10662	≤0.03	0.06	4.0	16	0.25	0.5
P. aeruginosa Badia[c]	2.0	0.25	8.0	4.0	0.06	0.06
Streptococcus pyogenes CN10	0.5	0.25	0.12	0.06	0.25	0.12
Staphylococcus aureus Oxford	16	4.0	4.0	8.0	4.0	4.0

[a,b,c] See footnotes to Table 1.

than (**2**) against Gram-positive cocci. Likewise, when the catechol moiety was incorporated into the C-3-substituent (**6**), a marked improvement in potency against *P. aeruginosa* was achieved at the expense of Gram-positive activity. Other alkyl substituents such as carboxymethyl (**4**) or acetonyl (**5**) showed no advantage over (**2**) in antibacterial activity.

Essentially similar results were seen with the corresponding (1-aminopyridinium)thiomethyl series (Table 2). The mono (**10**) and dialkyl (**9**) substituted compounds had a moderate level of antibacterial activity (MIC range 0.06～16 μg/ml) and good β-lactamase stability. Introduction of a 3,4-dihydroxybenzoyl group into the (*N*-amino)pyridinium moiety led to a significant improvement in activity against Gram-negative bacteria, particularly *P. aeruginosa*, but a four-fold loss in Gram-positive activity in compound (**7**) compared to (**9**). However, catecholic compounds (**8**), (**11**) and (**12**) had the most balanced antibacterial spectrum. This level of potency is considerably improved over ceftazidime and results from excellent β-lactamase stability and improved penetration, conferred by the formamido and catechol moieties, respectively.

The β-lactamase stability and potency of antibiotics (**8**), (**11**), and (**12**) against Gram-negative organisms is comparable to that observed with the C-3-(heterocyclylmethylthio)-7-α-formamido cephalosporins (**1**) described previously.[2] The desired improvement in activity against Gram-positive organisms was not

obtained with these betaine structures.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded for dichloromethane solutions on a Perkin-Elmer 197 spectrophotometer and for KBr discs on a Perkin-Elmer 983 grating spectrophotometer. ^{1}H NMR spectra were obtained on a Brucker WM 250 instrument using TMS or HOD as internal standard. Mass spectra were recorded on either VG 7070 or VG ZAB spectrometer operating in the electron impact mode. Fast atom bombardment spectra were recorded on a VG ZAB spectrophotometer and the matrix used is stated. Microanalytical data were determined on a Carlo Erba 1106 elemental analyser.

Preparation of Halomethyl Intermediates

Diphenylmethyl (6*R*,7*R*)-7-{(*R*)-2-[(4-Ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetamido}-7-formamido-3-(halomethyl)ceph-3-em-4-carboxylate (**15a**)

(*R*)-2-[(4-Ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetic acid (1.3 g, 4.1 mmol) in dichloromethane (15 ml) was treated with DMF (1 drop) and oxalyl chloride (1.0 ml, 11.0 mmol). The reaction was stirred for 0.75 hour, then evaporated under reduced pressure. The residue was dissolved in dichloromethane and evaporated to dryness. The resulting acid chloride (**13a**) was dissolved in dichloromethane (10 ml) and added dropwise to a solution of diphenylmethyl (6*R*,7*S*)-7-amino-3-bromomethyl-7-formamidoceph-3-em-4-carboxylate (**14**) (1.0 g, 2.0 mmol) in THF (20 ml) with pyridine (0.24 ml, 3.0 mmol). After addition was complete the mixture was stirred for 1 hour, then evaporated under reduced pressure. The residue was dissolved in ethyl acetate (40 ml) and water (40 ml). The organic phase was separated and washed with dil. HCl (2 × 40 ml), sodium hydrogen carbonate solution (2 × 40 ml), brine (40 ml), dried over magnesium sulphate and evaporated under reduced pressure. Chromatography on Silica gel 60 (< 230 mesh ASTM) eluting with ethanol, dichloromethane mixtures gave the title compound (1.16 g, 77%). The product is obtained as a mixture of 3-chloromethyl and 3-bromomethyl derivatives the relative quantities varying with each preparation. The preparation described contained predominantly diphenylmethyl (6*R*,7*R*)-3-chloromethyl-7-{(*R*)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetamido}-7-formamidoceph-3-em-4-carboxylate; IR ν_{max} (KBr) 1787, 1720, and 1684 cm^{-1}; ^{1}H NMR ($(CD_3)_2CO$) δ 1.16 (3H, t, J=7 Hz), 3.17 and 3.34 (2H, ABq, J=17 Hz), 3.50 (2H, q, J=7 Hz), 3.68 (2H, m), 4.04 (2H, m), 4.54 (2H, s), 5.35 (1H, s), 5.74 (1H, d, J=7 Hz), 6.95 (1H, s), 7.14~7.69 (15H, m), 8.30 (1H, d, J=1 Hz), 8.54 (1H, s), 8.82 (1H, s), and 10.04 (1H, d, J=7 Hz); MS m/z (positive xenon FAB, 3-nitrobenzylalcohol - NaOAc) 781 (M + Na, $C_{37}H_{35}ClN_6O_8S$).

Diphenylmethyl (6*R*,7*R*)-7-{(*R*)-2-(3,4-dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido}-7-formamido-3-(halomethyl)ceph-3-em-4-carboxylate (**15c**)

(*R*)-2-(3,4-Dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetic acid (7.34 g, 20 mmol) in THF (40 ml) was treated with chlorotrimethylsilane (15.2 ml, 120 mmol) and hexamethyldisilazane (12.8 ml, 60 mmol). After stirring for 2 hours the mixture was filtered through celite washing the solids with toluene (40 ml). The filtrate was evaporated under reduced pressure and the residue dried under high vacuum for 1 hour. The residue was taken up in toluene and filtered through celite. The filtrate was diluted with hexane (80 ml) and treated with oxalyl chloride (2.1 ml, 24 mmol) and DMF (2 drops). A precipitate began to form after a few minutes. After 0.5 hour the mixture was filtered and the solids washed with toluene - hexane (1 : 2), then dried to give (*R*)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-[3,4-bis(trimethylsilyloxy)phenyl]acetyl chloride (**13b**) (7.59 g, 73%); IR ν_{max} (CH_2Cl_2) 3440, 1795, 1725 (sh), 1715, and 1690 cm^{-1}, $[\alpha]_D^{20}$ −90.2° (*c* 0.48, THF).

The acid chloride (**13b**)[6] (0.75, 1.5 mmol) in THF (25 ml) was added dropwise to a stirred mixture of diphenylmethyl (6*R*,7*S*)-7-amino-3-(bromomethyl)-7-formamidoceph-3-em-4-carboxylate (**14**) (0.50 g, 1.0 mmol) and pyridine (0.08 ml, 1.0 mmol) in dichloromethane (10 ml), and THF (5 ml). The reaction mixture was stirred for 3 hours, diluted with water (1 ml), then evaporated to dryness. Purification on Silica gel 60 eluting with ethanol, dichloromethane mixtures gave the title compound (0.65 g, 82%). Due to

halogen exchange occurring during the reaction the product is obtained as a mixture of 3-chloromethyl and 3-bromomethyl derivatives. The preparation described contained predominantly diphenylmethyl (6*R*,7*R*)-3-(chloromethyl)-7-{(*R*)-2-(3,4-dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido}-7-formamidoceph-3-em-4-carboxylate; IR ν_{max} (KBr) 1786, 1717, 1675, and 1611 cm^{-1}; ^1H NMR ($CDCl_3+CD_3OD$) δ 1.13 (3H, t, $J=7$ Hz), 2.98 and 3.11 (2H, ABq, $J=17$ Hz), 3.43 (4H, m), 3.91 (2H, m), 4.31 (2H, m), 5.25 (1H, s), 5.41 (1H, br s), 6.71~7.04 (4H, m), 7.12~7.60 (10H, m), 8.13 (1H, s), and 9.82 (1H, br s); MS *m/z* (positive xenon FAB, 3-nitrobenzylalcohol-NaOAc) 813 (M+Na, $C_{37}H_{35}ClN_6O_{10}S$).

Typical Preparations of Betaine Cephalosporins

(6*R*,7*R*)-7-{(*R*)-2-[(4-Ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetamido}-7-formamido-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate (**10**)

Diphenylmethyl (6*R*,7*R*)-7-{(*R*)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetamido}-7-formamido-3-(halomethyl)ceph-3-em-4-carboxylate (**15a**) (0.8 g, 1.0 mmol) in dichloromethane (20 ml) was treated with 1-(methylamino)-4-thiopyridone (**26**) (0.14 g, 1.0 mmol) for 2 hours at room temperature. Chromatography on Silica gel eluting with mixtures of ethanol in dichloromethane gave (**17a**, R=$NHCH_3$) (0.47 g, 50%). Diphenylmethyl (6*R*,7*R*)-7-{(*R*)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetamido}-7-formamido-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate halide (**17a**, R=$NHCH_3$) (0.47 g, 0.5 mmol) in dichloromethane (30 ml) was treated with trifluoroacetic acid (1.25 ml) and stirred for 90 minutes. The mixture was evaporated to dryness and the residue washed with ether (3×40 ml). The product was dissolved in water at pH 7.0 with sodium bicarbonate and the product purified by chromatography on Diaion HP20SS resin eluting with mixtures of water and tetrahydrofuran. Fractions containing product were combined and freeze dried to give (**10**) (0.14 g, 41%); IR ν_{max} (KBr) 1775, 1705, 1676, and 1617 cm^{-1}; ^1H NMR (D_2O and $(CD_3)_2CO$) δ 1.16 (3H, t, $J=7$ Hz), 3.02 (3H, s), 3.00 and 3.38 (2H, ABq, $J=17$ Hz), 3.50 (2H, q, $J=7$ Hz), 3.67 (2H, m), 3.98 (2H, m), 4.06 and 4.14 (2H, ABq, $J=14$ Hz), 5.22 (1H, s), 5.48 (1H, s), 7.36~7.80 (5H, m), 7.79 and 8.51 (4H, ABq, $J=7$ Hz), and 8.10 (1H, s), MS *m/z* (positive xenon FAB, thioglycerol-acetic acid) 696 (M, $C_{30}H_{32}N_8O_8S_2$).

(6*R*,7*R*)-7-{(*R*)-2-(3,4-Dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido}-7-formamido-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate (**11**)

Diphenylmethyl (6*R*,7*R*)-7-{(*R*)-2-(3,4-dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido}-7-formamido-3-(halomethyl)ceph-3-em-4-carboxylate (**15c**) (0.65 g, 0.82 mmol) in acetonitrile (30 ml) was treated successively with 1-(methylamino)-4-thiopyridone (**26**) (0.144 g, 0.99 mmol) and sodium iodide (0.123 g, 0.82 mmol). The reaction mixture was stirred for 1.5 hours, then filtered through celite and evaporated to low volume (5 ml) under reduced pressure. The solution was added to diethyl ether and the precipitate filtered and dried to give (**17c**, R=$NHCH_3$) (0.309 g, 37%); IR ν_{max} (CH_2Cl_2) 1782, 1710, 1676, and 1617 cm^{-1}; ^1H NMR ($CDCl_3+CD_3OD$) δ 1.23 (3H, t, $J=7$ Hz), 2.76 and 3.01 (2H, ABq, $J=16$ Hz), 3.05 (3H, s), 3.40~3.70 (4H, m), 4.04 (4H, m), 5.31 (1H, s), 5.35 (1H, s), 6.75~7.00 (3H, m), 7.20~7.59 (10H, m), 7.70 (2H, d, $J=7$ Hz), 8.21 (1H, s), and 8.50 (2H, d, $J=7$ Hz); MS *m/z* (positive xenon FAB, 3-nitrobenzylalcohol-NaOAc) 895 (M, $C_{43}H_{43}N_8O_{10}S_2$).

The above product (**17c**, R=$NHCH_3$) (0.30 g, 0.29 mmol) was treated with trifluoroacetic acid (2 ml) and stirred for 5 minutes. The mixture was filtered through celite, washing the solids with a little toluene. The filtrate was added to diethyl ether and the precipitate filtered and dried. The crude product was dissolved in acetonitrile and water. The organic solvent was evaporated under reduced pressure and the aqueous mixture purified on Diaion HP20SS resin eluting with tetrahydrofuran, water mixtures to give compound (**11**) (0.088 g, 41%), IR ν_{max} (KBr) 1771, 1678, 1617 and 1512 cm^{-1}; ^1H NMR (D_2O) δ 1.13 (3H, t, $J=7$ Hz), 2.99 (3H, s), 2.94 and 3.33 (2H, ABq, $J=17$ Hz), 3.47 (2H, ABq, $J=7$ Hz), 3.57~3.77 (2H, m), 3.90~4.20 (2H, m), 4.08 and 4.29 (2H, ABq, $J=12$ Hz), 5.21 (1H, s), 5.29 (1H, s), 6.76~7.00 (3H, m), 7.77 (2H, d, $J=7$ Hz), 8.08 (1H, s), and 8.49 (2H, d, $J=7$ Hz); MS *m/z* (positive xenon FAB, thioglycerol) 729 (M+H, $C_{30}H_{32}N_8O_{10}S_2$).

Preparation of Thiopyridones

1-(2-Oxopropyl)-4-thiopyridone (**20**)

4-Pyridone (**30**) (0.25 g, 2.6 mmol) in DMF (5 ml) was treated with chloroacetone (0.31 g, 3.9 mmol) and potassium carbonate (0.44 g, 3.2 mmol). The mixture was heated at 60°C for 4 hours, then evaporated to dryness. The residue was extracted with chloroform and the extracts dried over magnesium sulphate and evaporated under reduced pressure to give 1-(2-oxopropyl)-4-pyridone in quantitative yield.

The pyridone (0.30 g, 2.0 mmol) in pyridine (5 ml) was treated with phosphorus pentasulphide (0.17 g, 0.4 mmol) and heated at 100°C for 1 hour. The mixture was evaporated and the residue partitioned between dilute sodium hydrogen carbonate solution and ethyl acetate. The aqueous phase was again extracted with ethyl acetate. The organic extracts were combined and washed with brine, dried over magnesium sulphate, and evaporated under reduced pressure. Purification on Silica gel 60 eluting with ethanol, ethyl acetate mixtures gave a brown gum (**20**) (0.017 g, 5%); ^{1}H NMR ($CDCl_3$) δ 2.32 (3H, s), 4.76 (2H, s), 6.97 (2H, d, J=7 Hz), and 7.39 (2H, d, J=7 Hz); MS m/z (positive xenon FAB, thioglycerol) 168 (M+H, C_8H_9NOS).

1-(Diphenylmethoxycarbonylmethyl)-4-thiopyridone (**19**)

Compound **19** was similarly prepared as a brown gum (0.07 g, 32%), IR ν_{max} (CH_2Cl_2) 1750 and 1625 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 4.64 (2H, s), 6.96 (1H, s), 7.01 (2H, d, J=7 Hz), 7.10~7.46 (10H, m), 7.42 (2H, d, J=7 Hz). MS m/z 335 (M, $C_{20}H_{17}NO_2S$).

1-(3,4-Dihydroxybenzyl)-4-thiopyridone (**21**)

4-Thiopyranone (**31**) (0.12 g, 1.07 mmol) in acetone (5 ml) was treated with a mixture of 3,4-dihydroxybenzylamine hydrobromide (0.22 g, 1.0 mmol) and triethylamine (0.14 ml, 1.0 mmol) in ethanol (5 ml). The mixture was stirred for 5 minutes then stored at 0°C for 16 hours. The solution was evaporated and the residue purified on Silica gel 60 eluting with ethanol, dichloromethane mixtures. After trituration with dichloromethane the product was obtained as a brown solid (**21**) (0.067 g, 27%); IR ν_{max} (KBr) 1616, 1511 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 5.00 (2H, s), 6.72 (3H, m), 7.17 (2H, d, J=7 Hz), 7.66 (2H, d, J=7 Hz), and 9.09 (2H, br, exch. with D_2O).

1-(3,4-Dihydroxybenzoylamino)-4-thiopyridone (**22**)

4-Thiopyranone (**31**) (0.10 g, 0.89 mmol) in DMF (5 ml) was treated with 3,4-dihydroxybenzhydrazide (0.12 g, 0.71 mmol) and stirred at room temperature for 24 hours. The solvent was evaporated under reduced pressure and the residue was triturated with dichloromethane. Filtration and drying gave the title compound (0.105 g, 51%); IR ν_{max} (KBr) 1662 and 1606 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 6.85 (1H, d, J=8 Hz), 7.17 (2H, d, J=7 Hz), 7.22~7.39 (2H, m), 7.67 (2H, d, J=7 Hz), and 9.00~9.10 (3H, m); MS m/z (positive xenon FAB; thioglycerol) 263 (M+H, $C_{12}H_{10}N_2O_3S$).

1-{*N*-[3,4-bis(4-Methoxybenzyloxy)benzoyl]-*N*-methylamino}-4-thiopyridone (**23**)

3,4-Dihydroxybenzoic acid (3.08 g, 0.02 mol) was dissolved in DMF (50 ml) and treated with 4-methoxybenzyl chloride (10 ml, 0.07 mol) and potassium carbonate (10 g, 0.07 mol). The mixture was warmed to 60°C for 6 hours and then stirred at room temperature overnight. The mixture was diluted with ethyl acetate and water. The organic phase was separated, washed exhaustively with water, then brine, dried and evaporated. The product was purified on Silica gel 60 eluting with mixtures of ethyl acetate and hexane to give 4-methoxybenzyl 3,4-bis(4-methoxybenzyloxy)benzoate. This material (6.48 g, 0.013 mol) was suspended in ethanol and treated with 2.5 M aqueous sodium hydroxide solution (7.6 ml, 0.015 mol). The mixture was warmed to 60°C for 4 hours, then evaporated to low volume. The concentrate was diluted with ethyl acetate and water. The aqueous layer was separated and washed with water, then acidified and extracted with ethyl acetate. The organic extracts were dried and evaporated. As the solution was concentrated the product precipitated from solution. Filtration and drying gave 3,4-bis(4-methoxybenzyloxy)benzoic acid (4.36 g). The acid (0.792 g, 2.0 mmol) in dichloromethane (20 ml) was treated with *N*,*N*-diisopropylethylamine (0.35 ml, 2.0 mmol). The solution was cooled to −40°C and treated with methane sulphonyl chloride (0.15 ml, 2.0 mmol). The mixture was allowed to warm to room temperature

for 10 minutes then cooled to −40°C and added to a solution of methyl hydrazine (0.215 ml, 4 mmol) in dichloromethane (10 ml) at −40°C. The mixture was allowed to warm to room temperature for 1.5 hours and then evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate and washed with water and brine. Drying and evaporation of the solvent gave *N*-[3,4-bis-(4-methoxybenzyloxy)benzoyl]-*N*-methylhydrazine (0.823 g, 94%); ^{1}H NMR ($CDCl_3$) δ 3.08 (3H, s), 3.74 (6H, s), 4.25 (2H, br s), 5.03 (4H, s), and 6.70~7.40 (11H, m).

N-[3,4-bis-(4-Methoxybenzyloxy)benzoyl]-*N*-methylhydrazine (0.80 g, 1.8 mmol) was heated at reflux in ethanol (60 ml) with 4-thiopyranone (**31**) (0.224 g, 2.0 mmol) for 48 hours. After evaporation under reduced pressure, purification on Silica gel 60 gave the title compound (0.404 g; 43%); IR ν_{max} (KBr) 1656, 1612, and 1513 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.40 (3H, s), 3.88 (6H, s), 5.02 (2H, s), 5.07 (2H, s), 6.90~7.30 (7H, m), 6.85 (2H, d, J=9 Hz), and 7.30 (2H, d, J=9 Hz); MS m/z 516 (M, $C_{29}H_{28}N_2O_5S$).

1-(Dimethylamino)-4-thiopyridone (**24**)

4-Thiopyranone (**31**) (0.112 g, 1.0 mmol) in DMF (2.0 ml) was treated with 1,1-dimethylhydrazine (0.76 ml, 10 mmol) and stirred at room temperature for 3 hours. The solvent was evaporated under reduced pressure and the residue chromatographed on Silica gel 60 (<230 mesh ASTM) eluting with ethanol, dichloromethane (9:1) to give the title compound as a dark orange liquid (0.069 g, 45%); ^{1}H NMR ($CDCl_3$) δ 2.88 (6H, s), and 7.55 (4H, s); MS m/z 154 (M, $C_7H_{10}N_2S$).

1-[*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino]-4-thiopyridone (**25**)

N-Methyl hydrazine (1.6 ml, 0.03 mol) in dichloromethane (20 ml) was treated dropwise with a solution of di-*tert*-butyldicarbonate (6.6 g, 0.03 mol) in dichloromethane (25 ml). The mixture was decanted from the sticky residue which had formed and evaporated to dryness under reduced pressure. The residue was diluted with dichloromethane and evaporated to dryness then diluted with dichloromethane and toluene and evaporated to give *tert*-butyl 1-methylhydrazinecarboxylate (4 g, 97%); ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), and 3.03 (3H, s).

tert-Butyl 1-methylhydrazinecarboxylate (0.5 g, 3.4 mmol)[5] and 4-thiopyranone (**31**) (0.34 g, 3 mmol) were dissolved in ethanol (20 ml). The solution was heated at reflux for 24 hours, then evaporated to dryness and the product purified by chromatography on silica gel eluting with mixtures of hexane and ethyl acetate to give the title compound (0.55 g, 76%); mp 156°C.

Anal Calcd for $C_{11}H_{16}N_2O_2S$: C 54.98, H 6.71, N 11.66, S 13.32.
Found: C 55.14, H 6.78, N 11.81, S 13.16.

^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 3.37 (3H, s), 7.19 and 7.32 (4H, ABq, J=7 Hz); λ_{max} (EtOH) nm (E (1 cm, 1%)) 356 (29,050). MS m/z 240.0934 (M^+); $C_{11}H_{16}N_2O_2S$ requires M, 240.0932).

1-(Methylamino)-4-thiopyridone (**26**)

Compound (**25**) (1 g, 4.16 mmol) in dichloromethane (40 ml) was treated with trifluoroacetic acid (5 ml) and stirred for 2.5 hours. On completion of the reaction the mixture was evaporated to dryness and the residue dissolved in ethyl acetate. The product was extracted into water maintained at pH 6.8 with sodium bicarbonate. The water was evaporated to low volume and then the product absorbed onto Silica gel 60 by evaporation. The product was then purified by chromatography on Silica gel 60 eluting with mixtures of ethanol in dichloromethane to give the title compound (0.4 g, 67%); mp 135~136°C, IR ν_{max} (KBr) 1685, 1605, 1523 and 1108 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.95 (3H, d, J=6 Hz), 5.46 (1H, q, J=6 Hz), and 7.34 (4H, s); MS m/z 140 (M, $C_6H_8N_2S$).

Anal Calcd for $C_6H_8N_2S$: C 51.28, H 5.76, N 19.99, S 22.50.
Found: C 51.40, H 5.75, N 19.97, S 22.87.

1-(*tert*-Butyloxycarbonylamino)-4-thiopyridone (**27**)

tert-Butylcarbazate (0.13 g, 1.0 mmol) and 4-thiopyranone (**31**) (0.11 g, 1.0 mmol) were heated at reflux in ethanol for 2 hours. The mixture was allowed to cool, diluted with acetone, then evaporated under reduced pressure. Chromatography on Silica gel 60 eluting with ethanol, dichloromethane (1:19) gave the title compound (0.49 g) as an orange solid; mp 163~166°C, IR ν_{max} (KBr) 1745, 1611, and 1503 cm^{-1};

^{1}H NMR ($CDCl_3$) δ 1.51 (9H, s), and 7.33 (4H, s); MS *m/z* 226 (M, $C_{16}H_{14}N_2O_2S$).

1-Amino-4-thiopyridone (**28**)

Compound (**27**) (1.12 g, 8.9 mmol) in dichloromethane (150 ml) was treated with trifluoroacetic acid (15 ml, 1.95 mol). The mixture was stirred for 1.5 hours, then evaporated to low volume (*ca.* 30 ml) under reduced pressure. The solution was added to diethyl ether (150 ml) and the precipitate filtered off and dried to give the title compound (0.50 g, 80%) as an orange solid; mp 108~110°C, IR ν_{max} (KBr) 1684 and 1624 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 6.80 (2H, s), 7.11 (2H, d, J=7 Hz), and 7.54 (2H, d, J=7 Hz); MS *m/z* 126 (M, $C_5H_6N_2S$).

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References

1) BRANCH, C. L.; M. J. BASKER, S. C. FINCH, A. W. GUEST, F. P. HARRINGTON, A. C. KAURA, S. J. KNOTT, P. H. MILNER & M. J. PEARSON: Studies on semi-synthetic 7α-formamidocephalosporins. III. Synthesis and antibacterial activity of some 7β-[D-2-(aryl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido]-7α-formamidoceph-3-em-4-carboxylate derivatives. J. Antibiotics 40: 646~651, 1987
2) HARRINGTON, F. P.; S. J. KNOTT, P. J. O'HANLON & R. SOUTHGATE: Structure activity relationships in a series of piperazine-2,3-dione containing penicillins and cephalosporins. II. Derivatives substituted at N(4) of the piperazine ring. J. Antibiotics 42: 1241~1247, 1989
3) SHIBAHARA, S.; T. OKONOGI, T. YOSHIDA, Y. MURAI, T. KUDO, S. INOUYE & S. KONDO: A new aminothiazolylcephalosporin having 1-carboxyethoxyimino group, ME 1228. J. Antibiotics 43: 62~69, 1990
4) OGINO, H.; K. IWAMATSU, K. KATANO, S. NAKABAYASHI, T. YOSHIDA, T. TSURUOKA, S. INOUYE & S. KONDO: New aminothiazolylglycylcephalosporins with a 1,5-dihydroxy-4-pyridone-2-carbonyl group. I. Synthesis and biological activity of cephalosporin derivatives leading to MT 0703. J. Antibiotics 43: 174~188, 1990
5) BRANCH, C. L.; A. W. GUEST & S. C. FINCH (Beecham): Eur. Pat. Appl. 416 810 A, Mar. 13, 1991
6) BRANCH, C. L. & A. C. KAURA (Beecham): Eur. Pat. Appl. 219 926, June 25, 1985
7) JONES, R. A. & A. R. KATRITSKY: Tautomeric pyridines. Part 1. Pyrid-2- and -4-thione. J. Chem. Soc. 1958: 3610~3613, 1958
8) EL-KHOLY, I. E.; M. M. MISHRIKEY & R. F. ATMEH: Action of amines and carbonyl reagents on 3,5-diphenyl-4-pyrone and its thio analogue. J. Heterocyclic Chem. 11: 487~490, 1974
9) TSURUOKA, T.; K. IWAMATSU, K. KATANO, H. OGINO, R. OKAMOTO, T. YOSHIDA, M. SEZAKI, FR. KAI, S. INOUYE & S. KONDO (Meiji Seika): Eur. Pat Appl. 209 751, Jan. 28, 1987
10) AINSWORTH, C. & R. G. JONES: Reaction of hydrazines with γ-pyrones. J. Am. Chem. Soc. 76: 3172~3174, 1954