NEW CEPHALOSPORINS AND 7α-METHOXY CEPHALOSPORINS CHEMISTRY AND BIOLOGICAL ACTIVITIES

GUGLIELMO ELITROPI, EZIO PANTÒ, SILVIA TRICERRI, LINO CHIARANI, ROBERTO PEZZALI, MARIO RIVA and GIOVANNA ZANUSO

Pierrel S.p.A., Research Laboratories, Via degli Artigianelli 10, 20159 Milan, Italy

(Received for publication July 13, 1979)

The synthesis and the *in vitro* activity of a number of cephalosporins and 7α-methoxy cephalosporins having 7-acyl substituents derived from 1-methyl-4 (or 5)-nitro-1H-imidazolylthioacetic acids are described. The microbiological profile is influenced by the position of both the nitro group and the side-chain sulfur atom on the 1-methyl imidazole, and by the nature of the 3-substituent.

The discovery of highly active cephalosporins and 7α-methoxy cephalosporins having 7-acyl substituents derived from mercaptoacetic acid[1,2,3], prompted us to record our own work on the use of various 1-methyl-4(or 5)-nitro-1H-imidazolylthioacetic acids as acylating agents in the preparations of semi-synthetic cephalosporins and 7α-methoxy cephalosporins.

This study deals primarily with the synthesis of two new imidazolylthioacetic acids, the preparation of the new antibiotics, and the effects on biological activity of varying both the position of the nitro group on the imidazole ring and that of the attachment of mercaptoacetic acid to the ring. Additionally, the influence of 3-substituent variation on the activity of a cephalosporin having the 7-acyl side-chain derived from 1-methyl-4-nitro-1H-imidazol-5-ylthioacetic acid is examined.

Chemistry

For these studies four acids have been used, and particularly: 1-methyl-4-nitro-1H-imidazol-5-ylthioacetic acid[4], 1-methyl-5-nitro-1H-imidazol-4-ylthioacetic acid[4], 1-methyl-4-nitro-1H-imidazol-2-ylthioacetic acid, and 1-methyl-5-nitro-1H-imidazol-2-ylthioacetic acid (Table 1). The last two compounds have not yet been described in literature, and we synthesized them from 1-methylimidazole according to Scheme 1.

The already known 2-bromo derivative[5] was prepared more readily *via* the carbanion generated at C-2 with *n*-butyllithium according to K. L. KIRK[6]. The nitration gave a mixture of 4- and 5-nitro derivatives[5] which were separated and treated with the potassium salt of ethyl

Scheme 1.

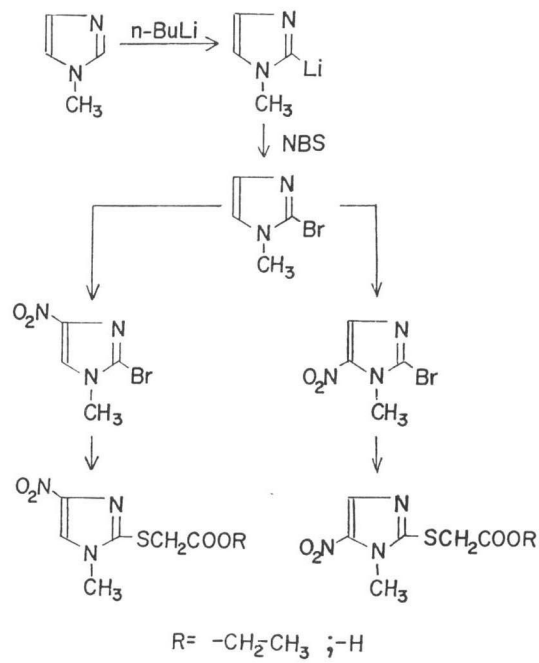
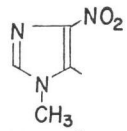
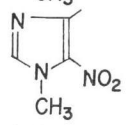
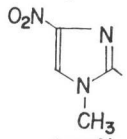
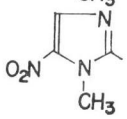


Table 1. 1-Methyl-4(5)-nitro-1H-imidazolylthioacetic acids.

R-S-CH_2-COOH

R	Nmr spectra				Melting points °C	Formula[a]
	Solvent	δ	No. of protons	Assignment		
	DMSO-d_6	3.70 s 3.70 s 7.87 s	3 2 1	CH_3-N CH_2-S H-im	209~210	$C_6H_7N_3O_4S$
	DMSO-d_6	3.87 s 3.93 s 7.93 s	3 2 1	CH_3-N CH_2-S H-im	163~164	$C_6H_7N_3O_4S$
	DMSO-d_6	3.67 s 4.00 s 8.33 s	3 2 1	CH_3-N CH_2-S H-im	139~141	$C_6H_7N_3O_4S$
	DMSO-d_6	3.83 s 4.12 s 8.12 s	3 2 1	CH_3-N CH_2-S H-im	133~139	$C_6H_7N_3O_4S$[b]

[a] All compounds were analysed for C, H, N, S. Except where indicated, analytical results were within $\pm 0.3\%$ of theoretical values.

[b] For analyses see Experimental Section.

thioacetate. Subsequent hydrolysis provided the two relative acids. All cephalosporins and 7α-methoxy cephalosporins synthesized in this study are listed in Tables 2 and 3 and were prepared by the four general methods (A~D) outlined in the Experimental Section.

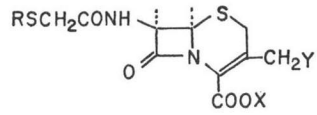
The cephalosporins **1** and **8** having an acetoxymethyl substituent on the 3-position were prepared by coupling the side-chain acid to 7-ACA either *via* the acid chloride (**1**, method A) or using the mixed anhydride derived from ethyl-chloroformate (**8**, method B). The cephalosporins **2~7** and **9** were obtained from **1** and **8** by displacing the 3-acetoxy group either with pyridine or with the selected five-membered heteroaromatic thiols (method C). The 7α-methoxy cephalosporins **10~13** were prepared by first acylating the diphenylmethyl 7β-amino-7α-methoxy-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylate[2] with the appropriate acid using dicyclohexylcarbodiimide (DCC), followed by removal of the protective group with trifluoroacetic acid-anisole at room temperature (method D).

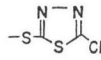
The purity of **1~13**, established by nmr, tlc and analyses, was greater than 90%. The heterocyclic thiols used in this work are known compounds and were synthesized by the methods described in the literature.

Biological Activities

The minimal inhibitory concentrations (MIC) of this series of compounds reported in Tables 4 and 5 were determined by the standard two-fold agar dilution method on Trypticase soy agar (TSA). For all strains of bacteria, MIC were determined as the lowest concentration inhibiting bacterial growth. Bacterial strains used for susceptibility determinations were cultures regularly employed in our primary

Table 2. Cephalosporins.



Compound	R	X	Y	Method	$\nu C{=}O(cm^{-1}\pm5)$ β Lactam	Formula[a]
1		H	$-OCOCH_3$	A	1780	$C_{16}H_{17}N_5O_8S_2$[b]
2	"	"		C	1780	$C_{16}H_{17}N_9O_6S_3$ 0.5 Me_2CO[b]
3	"	(-)		C	1765	$C_{19}H_{19}N_6O_6S_2$
4	"	H		C	1780	$C_{16}H_{15}N_7O_6S_4$
5	"	"		C	1780	$C_{17}H_{17}N_7O_6S_4$
6	"	"		C	1780	$C_{18}H_{19}N_9O_6S_3$
7	"	"		C	1775	$C_{17}H_{17}N_9O_8S_3$
8		H	$-OCOCH_3$	B	1780	$C_{16}H_{17}N_5O_8S_2$
9	"	"		C	1780	$C_{16}H_{17}N_9O_6S_3$

[a] Except for **1** and **2**, the products were not obtained in an analytically pure form and analyses (C, H, N. S) were within $\pm3\%$.

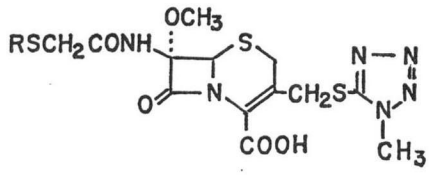
[b] For analyses see Experimental Section.

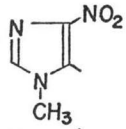
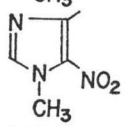
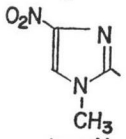
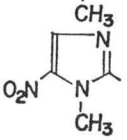
screening of cephalosporins and 7α-methoxy cephalosporins. The strains coded LRP (Laboratories Research Pierrel) have been collected and identified by standard criteria in these laboratories. The results given in Tables 4 and 5 are compared with those obtained with cephalothin (CET), cephapirin (CEP), cephacetrile (CEC), cephaloridine (CER), cefazolin (CEZ), P-75123* and cefoxitin (CFX). The data given in Tables 6 and 7 summarize the antimicrobial activities of two selected compounds (**2** and **10**) against a larger variety of Gram-positive and Gram-negative bacteria. The antimicrobial activity's data confirmed what already observed in the cephalosporin series[1,2,3], *i.e.* the remarkable Gram-negative activity of some compounds with a 7-thioacylamino side-chain bearing electronegative substituents at the sulphur atom.

The effect of placing the nitro group at the 4- or 5- position of the imidazole ring results in a noteworthy difference in individual MIC values against Gram-negative organisms. The 4-nitroimidazole

* Code number of 7-[1-(1H-4-nitroimidazolyl)acetyl]amino-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid, a new Pierrel's cephalosporin[7].

Table 3. 7α-Methoxycephalosporins.



Compound	R	Method	νC=O (cm^{-1} ±5) β Lactam	δNH DMSO-d_6	Formula[a]
10		D	1790	9.30s	$C_{17}H_{19}N_9O_7S_3$[b]
11		D	1780	9.42s	$C_{17}H_{19}N_9O_7S_3$
12		D	1780	9.53s	$C_{17}H_{19}N_9O_7S_3$
13		D	1780	9.50 s	$C_{17}H_{19}N_9O_7S_3$

[a] Except for **10**, the products were obtained as amorphous solids with analyses (C, H, N, S) within ±2%.

[b] For analyses see Experimental Section.

derivatives display activities remarkably higher than those of the corresponding 5-nitro parent compounds, and this effect can be seen by comparing **1** and **2** with **8** and **9**, **10** with **11**, and **12** with **13**.

It has been demonstrated[8] that compounds which are particularly active against *Escherichia coli* are more hydrophilic than those active against *Staphylococcus aureus*. This observation is consistent with the improved *in vitro* activity of **1**, **2**, **10** and **12** in comparison with that of **8**, **9**, **11** and **13**, since the higher polarity and the better water-solubility of 4-nitroimidazole derivatives in relation to 5-nitro isomers is well documented[9] and substantiated by the properties of the 1-methyl-4 (and 5)-nitro-1H-imidazolylthioacetic acids (see[4] and Experimental Section). A more detailed evaluation on the selected **2** and **10** is needed and will be reported in future communications.

Experimental Section

Infrared spectra were determined in KBr on a Perkin-Elmer 577 spectrophotometer. Nmr spectra were obtained in DMSO-d_6 with a Varian 60 MHz instrument using TMS as internal standard. Melting points of the side-chain acids were taken in capillary tubes on a Büchi apparatus and are uncorrected. Melting points of the cephalosporins and 7α-methoxy cephalosporins are not reported, since they are not accurately reproducible owing to extensive decomposition. No effort was made to improve the yields.

2-Bromo-1-methyl-1H-imidazole

1-Methyl-2-lithioimidazole was prepared by the dropwise addition of 4.93 g (60 mmole) of 1-methylimidazole dissolved in 20 ml of dry tetrahydrofuran to a stirred solution of 1.51 M *n*-butyl-lithium in hexane (Aldrich) (40 ml) and tetrahydrofuran (50 ml) at −40°C under nitrogen.

The slightly cloudy solution turned yellow. After 10 minutes a solution of 10.7 g of N-bromosuccinimide in 70 ml of tetrahydrofuran was added dropwise. The reaction mixture was stirred for additional 30 minutes at −40°C and then the temperature was allowed to rise to −10°C. After quenching with 100 ml of water and evaporation of THF *in vacuo*, the product was extracted with 200 ml of ethyl acetate. The organic layer was washed with water and brine just to pH 7 and dried. Removal of the

Table 4. *In vitro* antibacterial activities of cephalosporins.

Compounds	Minimal inhibitory concentration (mcg/ml)[a]									
	S.a.	*S.a.*(R)	*E.c.*	*K.p.*	*Sal. t*	*Sh. d.*	*Sh. s.*	*P.m.*	*P.v.*	*Ps.a.*
1	0.39	1.56	6.25	1.56	0.39	3.12	6.25	6.25	100	>100
2	0.195	3.12	0.78	0.78	0.097	0.195	0.78	3.12	50	>100
3	0.195	3.12	12.5	12.5	3.12	6.25	25	25	50	>100
4	0.097	3.12	3.12	1.56	0.097	0.78	3.12	25	50	>100
5	0.097	1.56	3.12	3.12	0.39	0.78	3.12	12.5	50	>100
6	0.195	1.56	3.12	3.12	0.195	0.39	3.12	25	50	>100
7	0.39	3.12	6.25	0.39	0.012	3.12	6.25	0.39	12.5	>100
8	0.097	0.39	50	100	12.5	6.25	50	>100	100	>100
9	0.097	0.39	12.5	25	1.56	1.56	12.5	>100	50	>100
CET	0.049	0.39	6.25	3.12	0.78	1.56	6.25	3.12	100	>100
CEP	0.049	0.39	6.25	3.12	0.78	1.56	6.25	12.5	100	>100
CEC	0.195	0.78	6.25	6.25	3.12	6.25	12.5	12.5	>100	>100
CER	0.024	0.78	3.12	3.12	1.56	1.56	3.12	3.12	>100	>100
CEZ	0.097	0.39	1.56	0.78	0.78	0.78	3.12	6.25	50	>100
P-75123	0.195	0.78	0.78	0.78	0.39	0.78	0.78	12.5	25	>100

[a] Organisms selected for inclusion in this Table are: *S.a.*, *Staphylococcus aureus* ATCC 6538 P; *S.a.*(R), *Staphylococcus aureus* LRP 14 (penicillin G resistant); *E.c.*, *Escherichia coli* LRP 50; *K.p.*, *Klebsiella pneumoniae* LRP 54; *Sal. t.*, *Salmonella typhi* LRP 8; *Sh. d.*, *Shigella dysenteriae* LRP 41; *Sh. s.*, *Shigella sonnei* LRP 5; *P.m.*, *Proteus mirabilis* LRP 19; *P.v.*, *Proteus vulgaris* ATCC 6380; *Ps. a.*, *Pseudomonas aeruginosa* LRP 9.

Table 5. *In vitro* antibacterial activities of 7α-methoxycephalosporins.

Compounds	Minimal inhibitory concentration (mcg/ml)[a]									
	S.a.	*S.a.*(R)	*E.c.*	*K.p.*	*Sal. t.*	*Sh. d.*	*Sh. s.*	*P.m.*	*P.v.*	*Ps.a.*
10	0.195	1.56	1.56	1.56	0.195	0.195	1.56	6.25	1.56	>100
11	0.78	1.56	50	50	3.12	6.25	100	>100	N.T.[b]	>100
12	0.78	0.78	12.5	25	1.56	1.56	25	25	6.25	>100
13	0.39	0.78	>100	>100	50	25	>100	>100	100	>100
CFX	1.56	3.12	6.25	6.25	0.78	3.12	12.5	6.25	1.56	>100

[a] See footnote a, Table 4. [b] Not tested

solvent by rotary evaporation and chromatography on a silica-gel column with methylene chloride gave 2.03 g of the desired product (21% yield); nmr: δ 3.55 (3H, s, NCH_3), 6.80 (1H, d, imidazole-H), 7.20 (1H, d, imidazole-H).

2-Bromo-1-methyl-4(and 5)-nitro-1H-imidazoles

The two compounds were prepared as previously described[5].

4-Nitro isomer. Ir: 755 cm^{-1} C_5-H^{10}; nmr: δ 3.68 (3H, s, NCH_3), 8.42 (1H, s, imidazole-H).

5-Nitro isomer. Ir: 742 cm^{-1} C_4-H^{10}; nmr: δ 3.88 (3H, s, NCH_3), 7.98 (1H, s, imidazole-H).

Ethyl 1-methyl-4-nitro-1H-imidazol-2-ylthioacetate

A solution of ethyl thioacetate (1.25 g, 10.4 mmole) in 13.5 ml of 0.69 N ethanolic potassium ethoxide was evaporated *in vacuo* to dryness. To the solid residue suspended in acetonitrile (20 ml) and ice-cooled, was added under stirring a solution of 2-bromo-1-methyl-4-nitro-1H-imidazole (1.0 g, 4.85 mmole) in 20 ml of acetonitrile. The mixture turned red and when all the bromo derivative had reacted

Table 6. Comparative *in vitro* activities of cephalosporins **2**, CET, CEZ and P-75123 against 32 bacterial strains.

Organism	No. of strains tested	Drug	Culture medium	Number of strains inhibited at concentration (mcg/ml)											
				0.049	0.097	0.195	0.39	0.78	1.56	3.12	6.25	12.5	25	50	≧100
Staphylococcus aureus	5	**2**	TPB[a]				2	1	1		1				
		CET		1	1	2	1	1							
		CEZ				2	1	1							
		P-75123				1	3		1						
Streptococcus spp.	4	**2**	BHIB +3% h.s.[b]		2	1		1							
		CET		1		3									
		CEZ		1	2	1									
		P-75123		2	2										
Escherichia coli	7	**2**	TPB			1		1	2			1	1		1
		CET							1	1	1	2		1	1
		CEZ							2	2	1	1		1	
		P-75123						3	1	2		1			
Salmonella spp.	6	**2**	TPB		1	1		4							
		CET						1	1	1	1	2			
		CEZ							2	4					
		P-75123					1	4	1						
Shigella spp.	3	**2**	TPB				1	1	1						
		CET							1		1	1			
		CEZ							1	1	1				
		P-75123						1	2						
Klebsiella pneumoniae	3	**2**	TPB				1	1	1						
		CET						1	1		1				
		CEZ							1	1	1				
		P-75123						3							
Proteus mirabilis	4	**2**	MHB[c]							1		2	1		
		CET								1		2	1		
		CEZ									1	3			
		P-75123									1	1		1	1

[a] Tryptose phosphate broth. [b] Brain heart infusion broth+3% horse serum. [c] MUELLER-HINTON broth.

(25 minutes, tlc control) the solution was filtered to remove undissolved substance and the filtrate evaporated *in vacuo*. The residue was taken up in benzene and the suspension was filtered. The yellow solution was washed well with water, dried and evaporated. Chromatography on silica gel, eluting with methylene chloride and ethyl acetate, gave the pure ester in 50% yield (0.595 g); mp 54~55°C; ir: 1730 and 1740 cm^{-1} ($\nu_{C=O}$); nmr: δ 3.70 (3H, s, NCH_3), 4.05 (2H, s, SCH_2), 8.43 (1H, s, imidazole-H).

1-Methyl-4-nitro-1H-imidazol-2-ylthioacetic acid

To 0.500 g (2.04 mmole) of the ethyl ester in methanol (6 ml) were added 20.5 ml of 0.1 N NaOH. After stirring at room temperature (15 minutes) the solution was extracted with 20 ml of chloroform and 20 ml of ethyl acetate (both discarded). The aqueous phase was layered with ethyl acetate (50 ml) and acidified with 0.41 ml of 5 N HCl. The organic layer was washed with water, dried and evaporated *in vacuo*. The crude residue was triturated with ether and crystallized from benzene to give 0.300 g of the pure acid. Only one spot on tlc with acetone - chloroform - acetic acid (8: 2: 1).

Ethyl-1-methyl-5-nitro-1H-imidazol-2-ylthioacetate

The procedure was the same as for the 4-nitro isomer, except that benzene was used as solvent instead of acetonitrile. The pure ester (0.80 g, 70% yield) was an oil; ir: 1745 cm^{-1} ($\nu_{C=O}$); nmr: δ 3.83 (3H, s, NCH_3), 4.13 (2H, s, SCH_2), 8.00 (1H, s, imidazole-H).

Table 7. Comparative activities of the 7α-methoxycephalosporins **10** and CFX against 74 bacterial strains.

Organism	No. of strains tested	Drug	Culture medium	Number of strains inhibited at concentration (mcg/ml)								
				0.39	0.78	1.56	3.12	6.25	12.5	25	50	≧100
Staphylococcus aureus	6	**10**	TPB[a]			1	4		1			
		CFX					4	1	1			
Escherichia coli	8	**10**	TPB				3	3	2			
		CFX					4	2	2			
Salmonella spp.	8	**10**	TPB	1	1		1	3	2			
		CFX				2		3	3			
Citrobacter spp.	15	**10**	TPB				6	3	1	2	1	2
		CFX					1	4	2	1	3	4
Klebsiella pneumoniae	8	**10**	TPB				5	2	1			
		CFX						5	3			
Enterobacter cloacae	8	**10**	MHB[b]						1			7
		CFX									1	7
Serratia spp.	3	**10**	MHB					1	2			
		CFX						1	1	1		
Proteus mirabilis	4	**10**	MHB				2	2				
		CFX				1	2	1				
Proteus spp. indole positive	11	**10**	MHB			1	1	1	2	2	1	3
		CFX				1		4	5	1		
Providencia spp.	3	**10**	MHB								1	2
		CFX						1	1		1	

[a] Tryptose phosphate broth. [b] MUELLER-HINTON broth.

1-Methyl-5-nitro-1H-imidazol-2-ylthioacetic acid

This acid was prepared by the same procedure used for the 4-nitro isomer, with the difference that the product precipitated from the acidified aqueous phase. Crystallization from water afforded pale yellow crystals (60% yield) with a quite broad melting point (see Table 1).

Anal. Calcd. for $C_6H_7N_3O_4S$ (217.204): C, 33.18; H, 3.25; N, 19.35; S, 14.76.
Found: C, 32.69; H, 3.38; N, 18.85; S, 14.27.

Cephalosporins

The synthesis of **1** to **9** listed in Table 2 has been achieved by three general methods. The following are representative procedures for each of the three methods.

7-[(1-Methyl-4-nitro-1H-imidazol-5-yl) thioacetyl] amino cephalosporanic acid (**1**)

Method A. Phosphorus pentachloride (4.17 g, 20 mmole) was added to 2.17 g of 1-methyl-4-nitro-1H-imidazol-5-ylthioacetic acid (10 mmole) in 20 ml of benzene, and the mixture was stirred at room temperature for 2 hours. The precipitated acid chloride was collected, dried *in vacuo* over P_2O_5, slurried in acetone (20 ml) and added to 10 mmole of 7-ACA which has been dissolved in 80 ml of 40% aqueous acetone by adding $NaHCO_3$ (2.68 g, 32 mmole). During addition the temperature was kept at $-4°C$ and the pH was maintained at 7~8 by the addition of saturated $NaHCO_3$ solution. After stirring for 1 hour at 0°C and another hour without external cooling the acetone was evaporated *in vacuo*. The aqueous phase was extracted with ethyl acetate (discarded), cooled in an ice-bath and adjusted to pH 2. The resulting precipitate was collected, washed with water, triturated with ether and dried *in vacuo* to give 4.0 g of **1**. Tlc on silica gel gave only one spot with acetone - chloroform - acetic acid (80: 20: 10).

Anal. Calcd. for $C_{16}H_{17}N_5O_8S_2$ (471.469): C, 40.76; H, 3.63; N, 14.85; S, 13.60.
Found: C, 40.58; H, 3.65; N, 14.80; S, 13.57.

7-[(1-Methyl-5-nitro-1H-imidazol-4-yl)thioacetyl] amino cephalosporanic acid (**8**)

Method B. To a stirred suspension of 1-methyl-5-nitro-1H-imidazol-4-ylthioacetic acid triethylammonium salt (4.14 g, 13 mmole) in dry acetone (43 ml) at $-10°C$ was added 1 drop of N-methyl-

morpholine and then ethylchloroformate (1.4 g, 13 mmole). The red mixture was stirred for 5 minutes and then poured into a vigorously stirred and cold (−10°C) solution of 7-ACA (10 mmole) in 100 ml of 50% aqueous acetone and triethylamine (10 mmole). After stirring for 30 minutes at 0°C and 40 minutes at room temperature, the suspension was filtered and the red solution worked-up according to method A to give 1.07 g of **8**.

7-[(1-Methyl-4-nitro-1H-imidazol-5-yl)thioacetyl]amino-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (**2**)

Method C. A solution of **1** sodium salt (0.987 g, 2 mmole) and 0.290 g of 1-methyl-1H-tetrazole-5-thiol in 25 ml of phosphate buffer (pH 6.4) was stirred for 8 hours at 60°C followed by standing overnight. The pH was adjusted from 3.6 to 5.2 with 2 N NaOH. After washing with ethyl acetate the aqueous layer was filtered through Celite, cooled in an ice-bath and acidified to pH 2 with 4 N HCl. The precipitate was filtered, washed with water, triturated with ether and dried *in vacuo* over P_2O_5. The crude product was dissolved in 100 ml of acetone and treated with a small amount of active carbon. The filtered solution was evaporated *in vacuo* to give 0.415 g of pure **2**.

Anal. Calcd. for $C_{16}H_{17}N_9O_6S_3 \cdot 0.5\ Me_2CO$ (556.605): C, 37.76; H, 3.62; N, 22.65; S, 17.28.
Found: C, 37.71; H, 3.63; N, 22.60; S, 17.21.

7α-Methoxy cephalosporins

The synthesis of **10**～**13** listed in Table 3 has been achieved by the general method outlined for compound **10**.

7β-[(1-Methyl-4-nitro-1H-imidazol-5-yl)thioacetyl] amino-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl) thiomethyl]-3-cephem-4-carboxylic acid (**10**)

Method D. To a cold solution of diphenylmethyl 7β-amino-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate[2] (1.1 g, 2.1 mmole) in 30 ml of 1,2-dichloroethane were added dropwise 0.35 ml of N,N-diethylaniline in 10 ml of 1,2-dichloroethane and 3.15 mmole of 1-methyl-4-nitro-1H-imidazol-5-ylthioacetyl chloride (see preparation under method A) in 10 ml of the same solvent. The mixture was stirred for 20 minutes under cooling and then washed with water, buffer solution pH 7.8, and water successively. The dried (Na_2SO_4) organic layer was concentrated to dryness *in vacuo* to give 1.25 g of a brownish amorphous powder which was chromatographed (silica gel, chloroform - ethyl acetate) to yield the pure ester (0.760 g). To a cold solution of the ester (0.700 g) in 10 ml of 1,2-dichloroethane and 0.7 ml of anisole was added dropwise 1.2 ml of trifluoroacetic acid with stirring. After 30 minutes the solution was evaporated *in vacuo* below 40°C. To the residue were added 20 ml of ethyl acetate and 20 ml of water. The separated organic layer was washed with 10 ml of water and then extracted with 10% aqueous K_2HPO_4 solution (15 ml). The extract was washed with ethyl acetate, then covered with 20 ml of ethyl acetate and adjusted to pH 2 with 2 N HCl. The organic layer was separated and the aqueous phase extracted two times with ethyl acetate (20+20 ml). The combined organic layer was washed with saturated NaCl solution, dried, treated with active carbon, filtered and evaporated *in vacuo* to give 0.210 g of the pure acid **10** as a white powder.

Anal. Calcd. for $C_{17}H_{19}N_9O_7S_3$ (557.588): C, 36.62; H, 3.43; N, 22.61; S, 17.25.
Found: C, 36.47; H, 3.44; N, 22.54; S, 17.20.

References

1) DeMarinis, R. M.; J. R. E. Hoover, G. L. Dunn, P. Actor, J. V. Uri & J. A. Weisbach: A new parenteral cephalosporin. SK&F 59962: 7-Trifluoromethylthioacetamido-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid. Chemistry and structure activity relationships. J. Antibiotics 28: 463～470, 1975
2) Nakao, H.; H. Yanagisawa, B. Shimizu, M. Kaneko, M. Nagano & S. Sugawara: A new semisynthetic 7α-methoxy cephalosporin, CS-1170: 7β-[[(cyanomethyl)thio]acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. J. Antibiotics 29: 554～558, 1976
3) DeMarinis, R. M.; J. V. Uri & J. A. Weisbach: Synthesis and *in vitro* antibacterial activity of 7-trifluoro-

methylthioacetamido cephamycins related to SK&F 59962 (cefazaflur). J. Antibiotics 29: 973~975, 1976
4) FISHER, M. H.; W. H. NICHOLSON & R. S. STUART: Carboxymethylmercapto and carboxymethylsulphonyl derivatives of nitroimidazole. Canad. J. Chem. 39: 785~788, 1961
5) BARLIN, G. B.: The relative electron-releasing power of a singly bound, and the electron-attracting power of a doubly bound nitrogen atom when present in the same five-membered ring. J. Chem. Soc. (B) 1967: 641~647, 1967
6) KIRK, K. L.: Facile synthesis of 2-substituted imidazoles. J. Org. Chem. 43: 4381~4383, 1978
7) ZUMIN-TRICERRI S. & L. CHIARANI: 3,7-Disubstituted cephalosporins and their preparation. British Patent 1,500,010. Feb. 8, 1978
8) BIAGI, G. L.; M. C. GUERRA, A. M. BARBARO & M. F. GAMBA: Influence of lipophilic character on the antibacterial activity of cephalosporins and penicillins. J. Med. Chem. 13: 511~516, 1970
9) ŠUNJIĆ, V.; F. KAJFEŽ, D. KOLBAH & N. N. BLAŽEVIĆ: Physikalisch-chemische Eigenschaften und antitrichomonale Wirkung von 2-,4- und 5-Nitroimidazolen. Pharmazie 27: 131~138, 1972
10) KAJFEŽ, F.; V. ŠUNJIĆ, D. KOLBAH, T. FAJDIGA & M. OKLOBDŽIJA: 1-Substitution in 2-methyl-4(5)-nitroimidazole. 1. Synthesis of compounds with potential antitrichomonal activity. J. Med. Chem. 11: 167~169, 1968