CEPHALOSPORINS. II

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NEW 7-VINYLENETHIOACETAMIDO AND THIOACRYLAMIDO CEPHALOSPORINS

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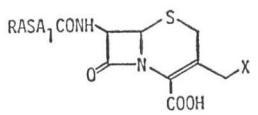
The synthesis and *in vitro* structure-activity relationships of 7-vinylenethioacetamido and thioacrylamido cephalosporins with various substituents at the 3-position are described. 7(*Z*)-β-Vinylenethioacetamido cephalosporins proved the most active against Gram-positive and Gram-negative bacteria. 7-[(*Z*)-β-Cyanovinylenethioacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)-thiomethyl]-3-cephem-4-carboxylic acid (K 13101, **40**) was several times more active *in vitro* than cefazolin.

In a previous paper[1] we described the synthesis and antibacterial activity of new alkylthioacetamido cephalosporins, in which the 7-acyl groups contained a saturated chain substituted by different electron-withdrawing groups. Of these, derivatives with a (cyanomethylthio)acetyl group at the 7-position and methylthiadiazolethiomethyl (K 9227) or methyltetrazolethiomethyl (K 10299) at the 3-position were the most active. The results encouraged us to pursue our initial approach. Among the possible structural changes of the 7- side chain we considered it interesting to introduce one or two double bonds in the alkylthioacetyl group and study the effect of unsaturation on activity, by synthesizing cephalosporins with the following general formula[2].



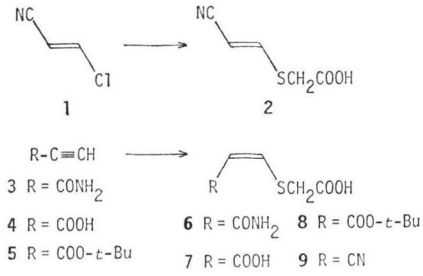
R = CN, $CONH_2$, COOH
$A,A_1 = CH_2$, CH = CH
X = OAc, S-Het (Het = five membered heterocycle)

This paper describes the synthesis of new 7-position side chain acids, the preparation of new cephalosporins, the effects on biological activity of introducing one or two double-bonds, and of altering the electron-withdrawing substituents, such as nitrile, carboxamido or carboxy groups and of varying the heterocycle at the 3-position. The effect on the activity of inverting the stereochemistry of the substituents on the double bond is also presented.

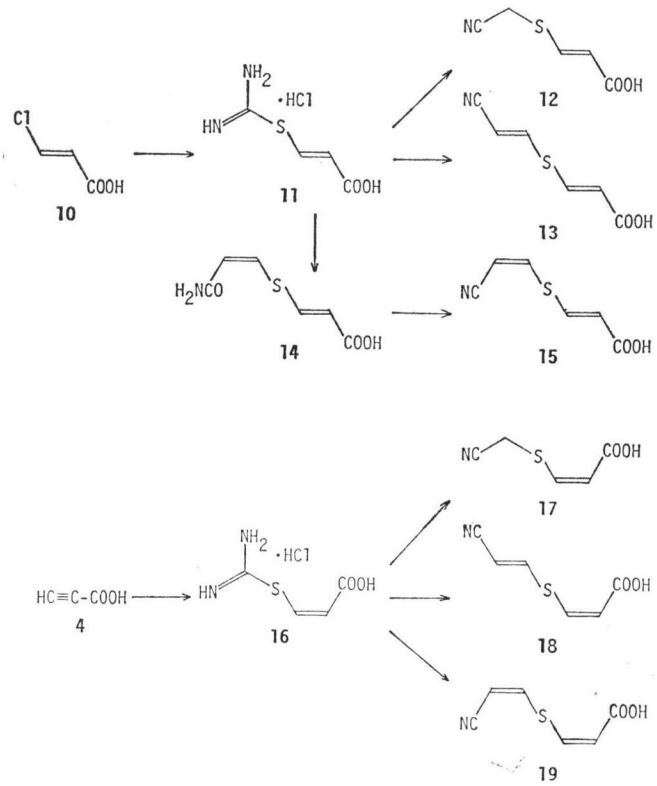
Chemistry

The side chain acids used for our studies have not yet been reported in the literature and we prepared them by different methods depending on the stereochemistry, and the position and the number of double bonds. As shown in Schemes 1 and 2, (*E*)-isomers were obtained by direct reaction of the

Scheme 1. Substituted vinylenethioacetic acids.



Scheme 2. Substituted alkyl- and vinylenethioacrylic acids.



appropriate thiol with the (*E*)-chloroacrylo derivatives. The corresponding (*Z*)-isomers were prepared most satisfactorily by stereoselective addition of the appropriate thiol to an alkyne.

Treatment of (*E*)-β-chloroacrylonitrile (**1**) with thioglycolic acid gave (*E*)-β-cyanovinylenethioacetic acid (**2**). Addition of thioglycolic acid to the appropriate alkyne (**3**~**5**) gave (*Z*)-isomers (**6**~**8**). In order to minimize the formation of (*E*)-isomers, it was necessary to carefully control the temperature (0°~10°C) and to use less than the stoichiometric amount of the thiolate anion (0.95 mole per mole of the alkyne). Compound **9** was obtained upon treatment of **6** with phosphorus pentachloride in a mixture of N,N-dimethylformamide - ethyl ether. Upon reaction of (*E*)-β-chloroacrylic acid (**10**) with thiourea in tetrahydrofuran the isothiuronium salt (**11**) was obtained, which was first converted

Table 1. Physical properties of side-chain acids.

$RASA_1COOH$

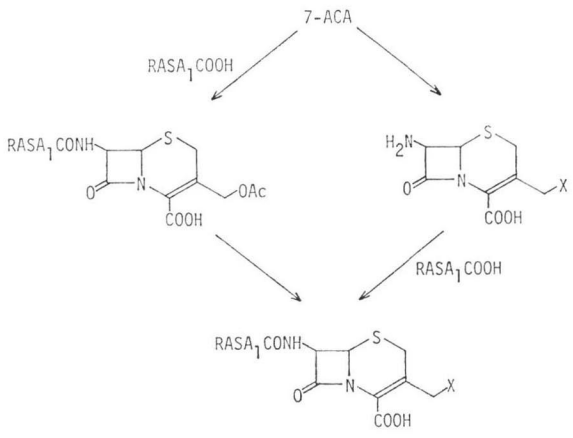
Compound	R	A	A_1	Mp, °C	Yield, %	Formula
2	NC	(*E*) CH=CH	CH_2	84~ 85	88	$C_5H_5NO_2S$
6	H_2NCO	(*Z*) CH=CH	CH_2	180~181	72	$C_5H_7NO_3S$
7	HOOC	(*Z*) CH=CH	CH_2	160~162	85	$C_5H_6O_4S$
8	*t*-BuOOC	(*Z*) CH=CH	CH_2	102~105	82	$C_9H_{14}O_4S$
9	NC	(*Z*) CH=CH	CH_2	90~ 92	77	$C_5H_5NO_2S$
12	NC	CH_2	(*E*) CH=CH	146~147	88	$C_5H_5NO_2S$
13	NC	(*E*) CH=CH	(*E*) CH=CH	171~172	92	$C_{18}H_{28}N_2O_2S$[a]
15	NC	(*Z*) CH=CH	(*E*) CH=CH	175~177	70	$C_6H_5NO_2S$
17	NC	CH_2	(*Z*) CH=CH	161~163	87	$C_5H_5NO_2S$
18	NC	(*E*) CH=CH	(*Z*) CH=CH	203~205	71	$C_6H_5NO_2S$
19	NC	(*Z*) CH=CH	(*Z*) CH=CH	213~215	75	$C_6H_5NO_2S$

[a] Isolated as dicyclohexylamine salt.

into the potassium or sodium salt of (*E*)-β-thioacrylic acid and then into compounds **12**, **13** and **14** by treatment with chloroacetonitrile, **1** and **3** respectively. **15** was obtained from **14** using the method reported for the synthesis of **9** from **6**. By treatment of propiolic acid with thiourea and HCl in water, (*Z*)-β-carboxyvinylisothiuroniumchloride (**16**) was obtained, from which compounds **17** and **18** were prepared using the same method reported above for the corresponding (*E*)-isomers. **19** was obtained by reacting the sodium salt of (*Z*)-β-thioacrylic acid with propiolonitrile. The results are given in Table 1.

The heterocyclic thiols used in this study were known compounds, except for 1-(2-cyanoethyl)-1*H*-tetrazole-5-thiol, which was obtained by treatment of 1-(2-aminocarbonylethyl)-1H-tetrazole-5-thiol with phosphorus pentachloride in a mixture of N,N-dimethylformamide - ethyl ether. Nucleophilic displacement of the C-3 acetoxy group of 7-ACA with the appropriate heterocyclic thiols was achieved in the usual manner[3], also outlined in the Experimental section.

Scheme 3.

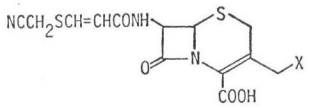


The cephalosporins were prepared by coupling the unsaturated acids with 7-amino cephalosporanic acid (7-ACA) or its 3-heterocyclic thiomethyl analogues (Scheme 3). Acylation was carried out using mixed anhydride derived from ethyl chloroformate (method A) or from pivaloyl chloride (method B). When a second carboxy group was present in the acylating acid (R=COOH), this was protected with a *tert*-butyl group, which was subsequently removed with trifluoroacetic acid - anisole (method C). In some cases we found it advantageous to use alternative methods. Thus **36** was prepared by coupling the diphenylmethyl ester of 7-ACA with the acyl chloride of the acylating acid, in which the second carboxy group was protected with a *tert*-butyl group. The two protective groups were then removed with trifluoroacetic acid -

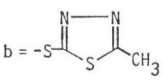
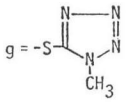
anisole (method D). Some derivatives (**43**~**45**, **54** and **55**) were obtained from **34**~**36** by displacing the 3-acetoxy group with the appropriate thiol (method E).

The cephalosporins synthesized are listed in Tables 2~4. The purity of the cephalosporins, established by nmr, tlc, and analyses, was greater than 90%.

Table 2. 7β-(Cyanomethylthioacrylamido)cephalosporins.



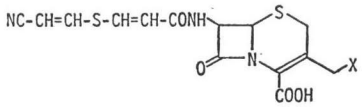
Compound	Configuration	X[a]	Method	ir (β-lactam) KBr, cm^{-1}	Formula[b]
20	*Z*	b	A	1780	$C_{16}H_{15}N_5O_4S_4$[c]
21	*Z*	g	A	1780	$C_{15}H_{15}N_7O_4S_3$
22	*E*	b	A	1775	$C_{16}H_{15}N_5O_4S_4$
23	*E*	g	A	1775	$C_{15}H_{15}N_7O_4S_3$

a) b =  g = 

b) All compounds were analysed for C, H, N, S. Analytical results are coincident with the calculated value within ±1% deviation.

c) For analyses see Experimental section.

Table 3. 7β-(Cyanovinylenethioacrylamido)cephalosporins.



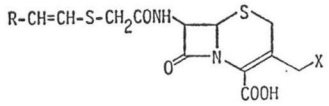
Compound	Configuration	X[a]	Method	ir (β-lactam) KBr, cm^{-1}	Formula[b]
24	*E-E*	b	A	1770	$C_{17}H_{15}N_5O_4S_4$
25	*E-E*	g	A	1770	$C_{16}H_{15}N_7O_4S_3$
26	*E-Z*	b	A	1775	$C_{17}H_{15}N_5O_4S_4$
27	*E-Z*	g	A	1775	$C_{16}H_{15}N_7O_4S_3$
28	*Z-E*	b	A	1775	$C_{17}H_{15}N_5O_4S_4$
29	*Z-E*	g	A	1775	$C_{16}H_{15}N_7O_4S_3$
30	*Z-Z*	b	A	1775	$C_{17}H_{15}N_5O_4S_4$
31	*Z-Z*	g	A	1775	$C_{16}H_{15}N_7O_4S_3$

a),b) See footnotes a, b to Table 2.

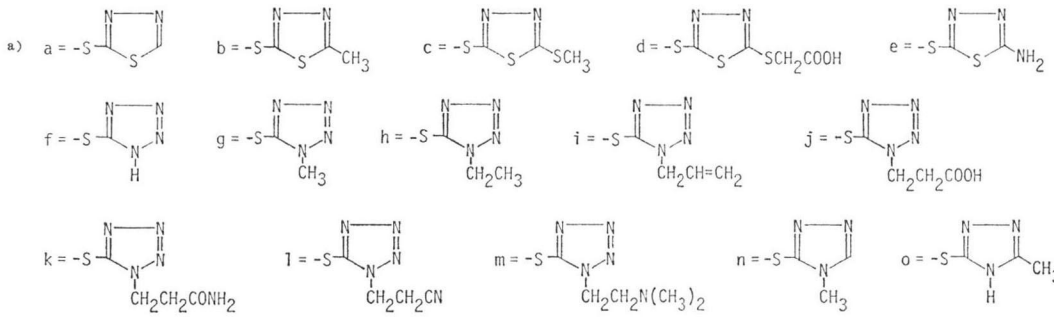
Antimicrobial Activity

The minimum inhibitory concentrations (MICs) of this series of cephalosporins against 3 strains of Gram-positive and 5 strains of Gram-negative bacteria were determined by the standard two-fold serial dilution method using diagnostic sensitivity test agar (Oxoid). The plates were inoculated with about 2×10^5 colony forming units using an automatic inoculator (Deneley Tech. Ltd.). The results are the geometric average of two determinations and are compared with cefazolin (CEZ) (Tables 5~8).

Table 4. 7β-(Substituted vinylenethioacetamido)cephalosporins.



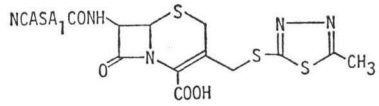
Compound	R	Configuration	X[a]	Method	ir (β-lactam) KBr, cm^{-1}	Formula[b]
32	NC	*E*	b	B	1775	$C_{16}H_{15}N_5O_4S_4$
33	NC	*E*	g	B	1775	$C_{15}H_{15}N_7O_4S_3$
34	NC	*Z*	OAc	B	1775	$C_{15}H_{15}N_3O_6S_2$
35	H_2NCO	*Z*	OAc	B	1770	$C_{15}H_{17}N_3O_7S_2$
36	HOOC	*Z*	OAc	D	1780	$C_{15}H_{16}N_2O_8S_2$
37	NC	*Z*	b	B	1780	$C_{16}H_{15}N_5O_4S_4$
38	H_2NCO	*Z*	b	B	1780	$C_{16}H_{17}N_5O_5S_4$
39	HOOC	*Z*	b	C	1780	$C_{16}H_{16}N_4O_6S_4$
40	NC	*Z*	g	B	1775	$C_{15}H_{15}N_7O_4S_3$
41	H_2NCO	*Z*	g	B	1775	$C_{15}H_{17}N_7O_5S_3$
42	HOOC	*Z*	g	C	1780	$C_{15}H_{16}N_6O_6S_3$
43	NC	*Z*	l	E	1770	$C_{17}H_{16}N_8O_4S_3$
44	H_2NCO	*Z*	l	E	1770	$C_{17}H_{18}N_8O_5S_3$
45	HOOC	*Z*	l	E	1780	$C_{17}H_{17}N_7O_6S_3$
46	NC	*Z*	a	B	1775	$C_{15}H_{13}N_5O_4S_4$
47	NC	*Z*	c	B	1780	$C_{16}H_{15}N_5O_4S_5$
48	NC	*Z*	d	B	1770	$C_{17}H_{15}N_5O_6S_5$
49	NC	*Z*	e	B	1770	$C_{15}H_{14}N_6O_4S_4$
50	NC	*Z*	f	B	1780	$C_{14}H_{13}N_7O_4S_3$
51	NC	*Z*	h	B	1780	$C_{16}H_{17}N_7O_4S_3$
52	NC	*Z*	i	B	1780	$C_{17}H_{17}N_7O_4S_3$
53	NC	*Z*	j	B	1770	$C_{17}H_{17}N_7O_6S_3$
54	NC	*Z*	k	E	1770	$C_{17}H_{18}N_8O_5S_3$
55	NC	*Z*	m	E	1780	$C_{18}H_{22}N_8O_4S_3$
56	NC	*Z*	n	B	1770	$C_{16}H_{16}N_6O_4S_3$
57	NC	*Z*	o	B	1770	$C_{16}H_{16}N_6O_4S_3$

a) 

b) See footnote b to Table 2.

Tables 5 and 6 list compounds containing thiadiazole or tetrazole as the heterocycle at the 3-side chain, while the functional group at the 7-side chain was maintained constant and the sequence -ASA_1- was modified in different ways, as reported above. The data reported in Table 5 show the following relationships between structure and activity:

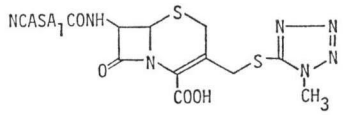
Table 5. *In vitro* activity of 3-[(5-methyl-1,3,4-thiadiazol-2-yl)-thiomethyl]cephalosporins.



Compound	A	A_1	MIC (μg/ml)[a]							
			S.a.	*S.a.* (R)	*S.p.*	*E.c.*	*K.p.*	*S.t.*	*Sh.s.*	*P.m.*
20	CH_2	(*Z*) CH=CH	0.1	1.6	0.1	>100	17.7	70.7	>100	12.5
22	CH_2	(*E*) CH=CH	0.4	1.6	0.28	>100	12.5	100	>100	100
24	(*E*) CH=CH	(*E*) CH=CH	0.28	1.1	0.1	>100	25	>100	>100	>100
26	(*E*) CH=CH	(*Z*) CH=CH	0.1	1.1	0.035	>100	25	>100	>100	50
28	(*Z*) CH=CH	(*E*) CH=CH	0.4	2.2	0.05	>100	17.7	>100	>100	50
30	(*Z*) CH=CH	(*Z*) CH=CH	0.025	0.14	0.004	>100	6.2	70.7	>100	17.7
32	(*E*) CH=CH	CH_2	0.035	0.4	0.004	12.5	0.4	3.1	25	6.2
37	(*Z*) CH=CH	CH_2	0.035	0.14	≤0.006	1.1	0.2	0.4	0.8	1.6
K9227	CH_2	CH_2	0.05	0.2	0.025	3.1	0.4	0.8	1.6	12.5
CEZ			0.05	0.57	0.05	1.6	0.8	1.6	1.6	6.2

[a] Organisms selected for inclusion in this Table are: *S.a.*, *Staphylococcus aureus* Smith (penicillin G sensitive); *S.a.* (R), *Staphylococcus aureus* 39/2 (penicillin G resistant); *S.p.*, *Streptococcus pyogenes* C 203; *E.c.*, *Escherichia coli* G; *K.p.*, *Klebsiella pneumoniae* ATCC 10031; *S.t.*, *Salmonella typhi* Watson; *Sh.s.*, *Shigella sonnei* ATCC 11060; *P.m.*, *Proteus mirabilis* ATCC 9921.

Table 6. *In vitro* activity of 3-[(1-methyl-1H-tetrazol-5-yl)-thiomethyl]cephalosporins.



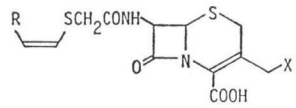
Compound	A	A_1	MIC (μg/ml)[a]							
			S.a.	*S.a.* (R)	*S.p.*	*E.c.*	*K.p.*	*S.t.*	*Sh.s.*	*P.m.*
21	CH_2	(*Z*) CH=CH	0.28	2.2	0.1	17.7	8.8	8.8	100	3.1
23	CH_2	(*E*) CH=CH	0.8	3.1	0.57	50	6.2	17.7	>100	100
25	(*E*) CH=CH	(*E*) CH=CH	0.57	2.2	0.1	>100	8.8	50	>100	50
27	(*E*) CH=CH	(*Z*) CH=CH	0.28	1.1	0.05	>100	17.7	35.4	>100	25
29	(*Z*) CH=CH	(*E*) CH=CH	0.57	3.1	0.14	>100	12.5	35.4	>100	35.4
31	(*Z*) CH=CH	(*Z*) CH=CH	0.035	0.28	0.006	25	2.2	8.8	>100	4.4
33	(*E*) CH=CH	CH_2	0.05	0.4	0.003	1.6	0.1	0.4	6.2	6.2
40	(*Z*) CH=CH	CH_2	0.025	0.14	0.012	0.14	0.1	0.07	0.8	0.4
K10299	CH_2	CH_2	0.07	0.4	0.035	0.8	0.2	0.2	1.6	6.2
CEZ			0.05	0.57	0.05	1.6	0.8	1.6	1.6	6.2

[a] See footnote a to Table 5.

1) The introduction of a double bond between the sulfur and the carboxamido group (A_1 = –CH=CH–) (**20**, **22**) reduced the activity in comparison with K 9227. Conversely, when the double bond was introduced between the sulfur and the functional group (A = –CH=CH–) (**32**, **37**), an improvement of the activity against Gram-positive bacteria was observed. Furthermore (*Z*)-isomer (**37**) was more effective against Gram-negative bacteria than the corresponding (*E*)-isomer (**32**), K 9227 and CEZ.

2) The introduction of two double bonds (**24**, **26**, **28** and **30**) independent of stereochemistry dramatically reduced the activity especially against Gram-negative bacteria. The drop in activity

Table 7. *In vitro* activity of 7-[(*Z*)-β-substituted vinylenethioacetamido]cephalosporins.



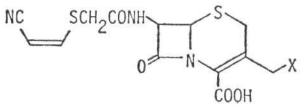
Compound	R	X[a]	MIC (μg/ml)[b]							
			S.a.	*S.a.* (R)	*S.p.*	*E.c.*	*K.p.*	*S.t.*	*Sh.s.*	*P.m.*
34	NC	OAc	0.05	0.2	0.025	1.6	0.57	0.4	12.5	3.1
35	H_2NCO	OAc	0.1	0.4	0.035	12.5	2.2	2.2	50	3.1
36	HOOC	OAc	1.6	4.4	0.57	12.5	0.8	0.4	50	0.8
37	NC	b	0.035	0.14	≤0.006	1.1	0.2	0.4	0.8	1.6
38	H_2NCO	b	0.05	0.14	<0.012	3.1	0.4	0.8	12.5	1.6
39	HOOC	b	0.57	1.6	0.14	6.2	0.57	0.57	35.4	0.8
40	NC	g	0.025	0.14	0.012	0.14	0.1	0.07	0.8	0.4
41	H_2NCO	g	0.1	0.2	<0.012	0.8	0.2	0.2	0.28	0.8
42	HOOC	g	0.8	2.2	0.2	1.1	0.1	0.07	4.4	0.2
43	NC	l	0.05	0.4	0.006	0.28	0.1	0.1	4.4	0.28
44	H_2NCO	l	0.1	0.57	0.012	1.6	0.4	0.2	12.5	0.8
45	HOOC	l	0.8	3.1	0.2	0.57	0.07	0.035	17.7	0.2
CEZ			0.05	0.57	0.05	1.6	0.8	1.6	1.6	6.2

[a] See footnote a to Table 4.
[b] See footnote a to Table 5.

against Gram-positive bacteria was less pronounced and in one case (**30**) there was greater activity. The same consideration holds for the tetrazole derivatives (Table 6). In line with previous observations[1,4] these compounds (Table 6) were always more effective against Gram-negative bacteria than the corresponding thiadiazole ones (Table 5). Derivative **40** was the most active against Gram-positive and Gram-negative bacteria in comparison with K 10299 and CEZ.

Bearing these findings in mind we only developed the (*Z*)-vinylenethioacetamido cephalosporins, on which we modified either the functional group or the substituents at the 3-position. As shown in Table 7 substitution of the –CN group either with $-CONH_2$ or –COOH, led to reduced activity against Gram-positive bacteria and the effect on the activity against Gram-negative bacteria was variable. The general trend was towards a reduction of the activity except for derivatives containing a –COOH group (**36**, **39**, **42** and **45**), which showed significantly better activity against *Proteus mirabilis* ATCC 9921 and in one case (**45**) against *Salmonella typhi* Watson too. Displacement of the 3-acetoxy group with heterocyclic thiols such as 5-methyl-1,3,4-thiadiazolethiol (b), 1-methyltetrazolethiol (g) or 1-(2-cyanoethyl)-tetrazolethiol (l) enhanced the activity against Gram-negative bacteria. The improvement against Gram-positive was less significant. Since compounds containing the –CN group proved to be the most active on the whole, the study on this series was enlarged by making some structural changes at the 3-position. Table 8 summarizes the activity of all the 7-(*Z*)-β-cyanovinylenethioacetamido cephalosporins synthesized. In this series too the tetrazole derivatives were generally more effective than thiadiazole analogs against Gram-negative bacteria. In the tetrazole series substitution of the methyl group (**40**) with more lipophilic groups (**51**, **52**) and with more hydrophilic (**50**) or polar ones (**43**, **53**~**55**) did not improve the activity against Gram-negative bacteria. In one case (**50**) there was actually a marked reduction in activity, except against *Proteus mirabilis* ATCC 9921. The presence of the –COOH group at the side chain of the heterocycle (**48** and **53**) improved the activity against

Table 8. *In vitro* activity of 7-[(*Z*)-β-cyanovinylenethioacetamido] cephalosporins.



Compound	X[a]	MIC (μg/ml)[b]							
		S.a.	*S.a.* (R)	*S.p.*	*E.c.*	*K.p.*	*S.t.*	*Sh.s.*	*P.m.*
46	a	0.025	0.14	<0.006	0.57	0.2	0.2	1.6	0.4
37	b	0.035	0.14	≤0.006	1.1	0.2	0.4	0.8	1.6
47	c	0.012	0.1	<0.006	2.2	0.2	1.1	8.8	12.5
48	d	0.07	0.4	0.025	2.2	1.1	0.4	12.5	0.28
49	e	0.035	0.2	0.012	0.8	0.2	0.4	8.8	3.1
50	f	0.4	0.8	0.1	12.5	1.1	0.8	35.4	0.2
40	g	0.025	0.14	0.012	0.14	0.1	0.07	0.8	0.4
51	h	0.05	0.2	0.1	0.4	0.2	0.28	2.2	0.8
52	i	0.025	0.1	0.003	0.57	0.1	0.28	3.1	1.6
53	j	0.05	0.5	0.017	0.8	0.2	0.1	1.6	0.05
54	k	0.035	0.4	0.012	0.28	0.14	0.07	1.1	0.8
43	l	0.05	0.4	0.006	0.28	0.1	0.1	4.4	0.28
55	m	0.035	0.4	0.012	0.2	0.14	0.1	0.8	0.8
56	n	0.1	0.4	0.025	1.1	0.28	0.2	1.1	1.6
57	o	0.1	0.4	0.012	4.4	0.4	0.8	8.8	3.1
CEZ		0.05	0.57	0.05	1.6	0.8	1.6	1.6	6.2

[a] See footnote a to Table 4.
[b] See footnote a to Table 5.

Table 9. *In vivo* activity of K 13101 (**40**), K 13156 (**53**) and cefazolin in acute systemic infection in mice.[a]

Challenge organism	ED_{50} (mg/kg), Confidence limits for P=0.95		
	K 13101	K 13156	Cefazolin
Staphylococcus aureus Smith	0.15 (0.12~0.18)	0.99 (0.76~1.39)	0.13 (0.10~ 0.18)
Streptococcus pyogenes C 203	0.30 (0.22~0.40)	0.95 (0.65~1.44)	0.47 (0.34~ 0.64)
Escherichia coli G	3.38 (2.4 ~4.58)	1.42 (0.99~1.82)	7.43 (5.88~ 9.25)
Proteus mirabilis ATCC 9921	7.02 (5.88~8.75)	0.66 (0.53~0.84)	10.18 (8.63~12.02)

[a] The mice were challenged intraperitoneally. Antibiotics were given subcutaneously immediately after challenge and three hours later.

Proteus mirabilis ATCC 9921 in line with previous observations (**36**, **39**, **42** and **45**). Triazole derivatives (**56**, **57**) did not improve the activity in comparison with tetrazole ones.

From the compounds selected for *in vivo* evaluation we report the data on two derivatives [K 13101 (**40**) and K 13156 (**53**)] in comparison with CEZ (Table 9). **40** was chosen for its good *in vitro* activity and **53** for its long plasma half-life in mice. The therapeutic activity of **40** was only equal to or slightly higher than that of cefazolin, although *in vitro* it was several times more active than the standard compound. This reduced *in vivo* activity was probably due to the short half-life in mice. *In vivo*, **53** showed less activity against Gram-positive bacteria, but it remained more active than CEZ against Gram-negative ones.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer spectrometer (model 125). The nmr spectra were determined on either a Perkin-Elmer R-24B (60 MHz) or a Bruker HX-90 (90 MHz) spectrometer using tetramethylsilane as internal standard, and chemical shifts are reported in parts per million (δ) relative to Me_4Si. Melting points were established on a Büchi melting point apparatus and are not corrected. Melting points of the cephalosporins are not accurately reproducible because of extensive decomposition.

(*E*)-β-Cyanovinylenethioacetic acid (**2**)

To a stirred solution of 70% thioglicolic acid (2.1 ml, 20 mmole) and triethylamine (5.6 ml, 40 mmole) in water (50 ml), cooled to 5°C, a solution of (*E*)-β-chloroacrylonitrile **1** (1.75 g, 20 mmole) in tetrahydrofuran (7 ml) was added dropwise. The mixture was stirred for 30 minutes at room temperature and washed with ethyl acetate. The organic layer was separated and the aqueous layer was acidified with 20% H_2SO_4. The resulting precipitate was extracted twice with ethyl acetate. The combined organic layers were washed with saturated NaCl solution, dried (Na_2SO_4) and evaporated *in vacuo*, yielding an oil which solidified to give 2.5 g (88%) of **2**; mp 84~85°C, ir (KBr): 2220, 1720, 1575, 930 cm^{-1}; nmr (DMSO-d_6): δ 3.63 (2H, s, CH_2), 5.56 (1H, d, J=16 Hz, NC–CH=), 7.78 (1H, d, J=16 Hz, =CH–S).

Anal. Calcd. for $C_5H_5NO_2S$: C, 41.94; H, 3.52; N, 9.78; S, 22.39.
Found: C, 41.81; H, 3.57; N, 9.71; S, 22.31.

(*Z*)-β-Carboxamidovinylenethioacetic acid (**6**)

To an ice-cooled solution of propiolamide **3** (6.9 g, 0.1 mole) in water (20 ml) a solution of 70% thioglycolic acid (10 ml, 0.095 mole) in 20% aqueous NaOH (18.9 ml) was added. The mixture was stirred for 1 hour at 0°C and another hour at 10°C, maintaining the pH at 9~9.5 by adding NaOH as necessary. The solution was then cooled to 0~5°C and carefully acidified with a stoichiometric amount of 70% sulfuric acid. The solid obtained was immediately filtered, washed with water and dried to give the crude product, which contained about 10% of the (*E*)-isomer. The mixture was then stirred with a suitable amount of water (twice with 50 ml and then with 100 ml) to dissolve all the (*E*)-isomer, leaving still undissolved the major part (about 88%) of the (*Z*)-isomer, which was then collected; the purification process was checked by tlc (acetone - water - CH_3COOH, 180: 20: 10). The pure (*Z*)-isomer **6** was obtained (11.06 g, 72%); mp 180~181°C; ir (KBr): 3450, 3210, 1685, 1625, 1580, 680 cm^{-1}; nmr (DMSO-d_6): δ 3.43 (2H, s, CH_2), 5.94 (1H, d, J=10 Hz, H_2NCO–CH=), 6.97 (1H, d, J=10 Hz, =CH–S–), 7.16 (2H, d, $-CONH_2$), 12.00 (1H, br-s, –COOH).

Anal. Calcd. for $C_5H_7NO_3S$: C, 37.25; H, 4.37; N, 8.69; S, 19.89.
Found: C, 37.22; H, 4.37; N, 8.66; S, 20.00.

(*Z*)-β-Carboxyvinylenethioacetic acid (**7**)

By a similar procedure propiolic acid **4** (7.0 g) was reacted with 70% thioglycolic acid (10 ml) in 2 N KOH (100 ml). At the end of the reaction the solution was cooled to 0~5°C, carefully acidified with 2 N HCl (100 ml) and extracted from the aqueous phase with ethyl acetate, the organic layer was washed, dried (Na_2SO_4), evaporated to small volume and cooled to give the desired compound (13.08 g, 85%); mp 160~162°C; ir (KBr): 3000~2300, 1700, 1570, 1425, 1350, 680 cm^{-1}; nmr (DMSO-d_6): δ 3.62 (2H, s, CH_2), 5.90 (1H, d, J=10 Hz, HOOC–CH=), 7.40 (1H, d, J=10 Hz, =CH–S), 11.80 (2H, br-s, COOH).

Anal. Calcd. for $C_5H_6O_4S$: C, 37.03; H, 3.73; S, 19.77.
Found: C, 36.90; H, 3.78; S, 19.62.

(*Z*)-β-*tert*-Butoxycarbonylvinylenethioacetic acid (**8**)

A solution of 95% thioglycolic acid (0.75 ml, 9.5 mmole) in 2 N NaOH (4.75 ml) and water (5.25 ml) was added dropwise, with stirring, to an ice-cooled solution of *tert*-butyl propiolate **5** (1.26 g, 10 mmole) in acetone (12.6 ml) and water (6.5 ml). After stirring for 30 minutes at 0°C and 1 hour at 10°C the acetone was evaporated *in vacuo*. Water was added and the reaction mixture was adjusted to pH 9~9.5 with a few drops of 2 N aqueous NaOH. The aqueous solution was washed with ethyl acetate, acidified

with 20% H_2SO_4 and extracted with ethyl acetate. The organic layer was washed with saturated NaCl solution, dried (Na_2SO_4) and evaporated. The residual oil was crystallized from carbon tetrachloride to give 1.7 g (82%) of white crystalline **8**; mp 102~105°C; ir (KBr): 1710, 1690, 1575, 1370, 1160~1150 cm^{-1}; nmr ($CDCl_3$): δ 1.48 (9H, s, COO*t*-Bu), 3.42 (2H, s, CH_2), 5.84 (1H, d, J=10 Hz, *t*-BuOOC-CH=), 7.08 (1H, d, J=10 Hz, =CHS), 11.00 (1H, s, COOH).

Anal. Calcd. for $C_9H_{14}O_4S$: C, 49.52; H, 6.46; S, 14.68.
Found: C, 49.31; H, 6.44; S, 14.44.

(*Z*)-β-Cyanovinylenethioacetic acid (**9**)

To an ice-cooled solution of **6** (4.8 g, 30 mmole) in a mixture of dry N,N-dimethylformamide - ethyl ether (3: 2) (120 ml), PCl_5 (6.25 g) was added and the mixture was stirred for 2 hours at 0~10°C. The reaction mixture was poured into ice-water and the ethereal layer was separated. The aqueous layer was extracted with ethyl acetate (4×50 ml). The combined extracts were dried (Na_2SO_4), evaporated *in vacuo* below 40°C. The residual oil was dissolved in methanol (12 ml) and a stoichiometric amount of dicyclohexylamine was added to the resulting solution. The precipitated salt was collected, washed twice with ethyl ether and dried under vacuum to give 7.3 g of the dicyclohexylamine salt; mp 180~183°C. The salt was then dissolved in 50 ml of water and stratified with 70 ml of ethyl acetate at 5°C, the solution was acidified by dropwise addition of 40% H_3PO_4 (10 ml) and extracted three times with ethyl acetate; the combined extracts were washed with saturated NaCl solution, dried (Na_2SO_4) and evaporated to give 3.3 g (77%) of **9**; mp 90~92°C; ir (KBr): 2220, 1720, 1575, 680 cm^{-1}; nmr (DMSO-d_6): δ 3.62 (2H, s, CH_2), 5.4 (1H, d, J=10 Hz, NC-CH=), 7.4 (1H, d, J=10 Hz, =CH-S).

Anal. Calcd. for $C_5H_5NO_2S$: C, 41.94; H, 3.52; N, 9.78; S, 22.39.
Found: C, 41.70; H, 3.63; N, 9.64; S, 22.25.

(*E*)-Cyanomethylthioacrylic acid (**12**)

A solution of thiourea (2.19 g, 28.8 mmole) and (*E*)-β-chloroacrylic acid **10** (3.03 g, 28.4 mmole) in dry tetrahydrofuran (100 ml) was refluxed for 48 hours. After cooling, the precipitate was collected, washed with tetrahydrofuran and then with ethyl ether, to give 4.30 g (83%) of (*E*)-β-carboxyvinyl-isothiuronium chloride (**11**); mp 203~205°C; nmr (DMSO-d_6): δ 6.0 (1H, d, J=16 Hz, =CH-COOH), 7.4 (1H, d, J=16 Hz, S-CH=).

Anal. Calcd. for $C_4H_7ClN_2O_2S$: C, 26.30; H, 3.86; Cl, 19.41; N, 15.34; S, 17.55.
Found: C, 26.41; H, 3.93; Cl, 19.37; N, 15.35; S, 17.64.

A solution of **11** (5.48 g, 30 mmole) in 0.5 N KOH (120 ml) and water (380 ml) was stirred for 90 minutes at room temperature, under N_2 stream, (tlc indicated the absence of starting material) to give the potassium salt of (*E*)-β-thioacrylic acid. To this solution, cooled to 0°C, chloroacetonitrile (2.26 g, 30 mmole) was added and followed by a solution of K_2CO_3 (4.56 g, 33 mmole) in water (60 ml) a few minutes later. After stirring for 90 minutes at room temperature, the reaction mixture was acidified with 20% H_2SO_4, extracted with ethyl acetate (3×300 ml), washed with water, dried (Na_2SO_4) and evaporated *in vacuo* with cooling to give a residue which was triturated with ethyl ether - petroleum ether (2: 1) to yield 3.78 g (88%) of **12**; mp 146~147°C; ir (KBr): 2220, 1720, 1575, 930 cm^{-1}; nmr (DMSO-d_6): δ 4.19 (2H, s, CH_2), 5.87 (1H, d, J=16 Hz, =CH-COOH), 7.7 (1H, d, J=16 Hz, S-CH=), 12.1 (1H, br-s, -COOH).

Anal. Calcd. for $C_5H_5NO_2S$: C, 41.94; H, 3.52; N, 9.78; S, 22.39.
Found: C, 41.76; H, 3.51; N, 9.71; S, 22.22.

(*E*)-β-Cyanovinylene-(*E*)-thioacrylic acid (**13**)

By a similar procedure **11** (5.48 g, 30 mmole) was treated with KOH as reported above and then with (*E*)-β-chloroacrylonitrile **1** (2.63 g, 30 mmole) in tetrahydrofuran at room temperature overnight. After work up in the usual manner, the desired product was isolated as dicyclohexylamine salt (9.28 g, 92%); mp 171~172°C (dec.); ir (KBr): 2220, 1720, 1575, 930 cm^{-1}; nmr ($CDCl_3$): δ 5.44 (1H, d, J=16 Hz, NC-CH=), 6.22 (1H, d, J=16 Hz, =CH-COOH), 7.2 (1H, d, J=16 Hz, S-CH=), 7.42 (1H, d, J=16 Hz, =CH-S).

Anal. Calcd. for $C_{15}H_{28}N_2O_2S$: C, 64.24; H, 8.38; N, 8.32; S, 9.53.
Found: C, 64.27; H, 8.56; N, 8.19; S, 9.52.

(*Z*)-β-Cyanovinylene-(*E*)-thioacrylic acid (**15**)

To a solution of **11** (4.55 g, 25 mmole) in 95% ethanol (150 ml), cooled at −10°C, was added precooled 1 N NaOH (75 ml) within 3 minutes and the mixture was stirred for 5 minutes at −15°C. Subsequently a precooled solution of propiolamide (1.73 g, 25 mmole) in 95% ethanol (25 ml) was added. After stirring for 1 hour at −10°C and another hour at room temperature, the ethanol was removed *in vacuo* without external heating and the aqueous phase was acidified with 23% HCl. After cooling, the precipitate was collected by filtration and dried *in vacuo* at 60°C to give 3.87 g (89.5%) of **14**; mp 232~234°C; ir (KBr): 3400~3170, 1700, 1660, 1580, 975, 670 cm^{-1}; nmr (DMSO-d_6): δ 5.94 (1H, d, J=10 Hz, H_2NCO–CH=), 6.0 (1H, d, J=16 Hz, =CH–COOH), 7.8(1H, d, J=16 Hz, S–CH=), 7.94 (1H, d, J=10 Hz, =CH–S).

Anal. Calcd. for $C_6H_7NO_3S$: C, 41.61; H, 4.07; N, 8.08; S, 18.51.
Found: C, 41.57; H, 4.11; N, 8.10; S, 18.76.

According to the procedure described for the synthesis of **9** from **6**, **15** was obtained from **14** in 70% yield as a white solid; mp 175~177°C; ir (KBr): 2220, 1680 cm^{-1}; nmr (DMSO-d_6): δ 5.80 (1H, d, J=10 Hz, NC–CH=), 6.0 (1H, d, J=16 Hz, =CH–COOH), 7.8 (1H, d, J=16 Hz, S–CH=), 7.92 (1H, d, J=10 Hz, =CHS).

Anal. Calcd. for $C_6H_5NO_2S$: C, 46.44; H, 3.25; N, 9.02; S, 20.66.
Found: C, 46.23; H, 3.35; N, 8.93; S, 20.43.

(*Z*)-Cyanomethylthioacrylic acid (**17**)

To a solution of thiourea (3.80 g, 50 mmole) in 2 N HCl (25 ml), a solution of propiolic acid **4** (3.5 g, 50 mmole) in water (15 ml) was added slowly under cooling to maintain the reaction temperature at about 10~20°C. The reaction mixture was then stirred for 2 hours at room temperature and then evaporated to small volume giving a precipitate, which was collected and washed with ethyl ether. Crystallization from isopropanol - ethyl ether gave 6.94 g (76%) of (*Z*)-β-carboxyvinylisothiuronium chloride (**16**); mp 169~170°C (dec.); nmr (DMSO-d_6): δ 6.1 (1H, d, J=10 Hz, =CH–COOH), 7.5 (1H, d, J=10 Hz, S–CH=).

Anal. Calcd. for $C_4H_7ClN_2O_2S$: C, 26.30; H, 3.86; Cl, 19.41; N, 15.34; S, 17.55.
Found: C, 26.40; H, 3.91; Cl, 19.23; N, 15.26; S, 17.70.

To a stirred suspension of **16** (9.1 g, 50 mmole) in 95% ethanol (300 ml) cooled to −10°C, was quickly added a precooled solution of 1 N NaOH (150 ml) and the reaction mixture was stirred for 3 minutes at −15°C, producing a complete solution. To this solution a precooled solution of chloroacetonitrile (3.77 g, 50 mmole) was added within 3 minutes, maintaining the temperature at −10°C. After stirring for 1 hour at −10°C and another hour at room temperature, the ethanol was removed *in vacuo* without heating. After cooling, the solution was acidified with 23% HCl; the precipitate was collected, washed with cold water and dried under vacuum to give 6.2 g (87%) of **17**; mp 161~163°C; ir (KBr): 2240, 1675, 1575, 680 cm^{-1}; nmr (DMSO-d_6): δ 3.75 (2H, s, CH_2), 5.75 (1H, d, J=10 Hz, =CH–COOH), 7.15 (1H, d, J=10 Hz, S–CH=).

Anal. Calcd. for $C_5H_5NO_2S$: C, 41.94; H, 3.52; N, 9.78; S, 22.39.
Found: C, 41.79; H, 3.49; N, 9.64; S, 22.15.

(*E*)-β-Cyanovinylene-(*Z*)-thioacrylic acid (**18**)

To an ethanolic solution of the sodium salt of (*Z*)-β-thioacrylic acid (prepared from 3.65 g of **16** as reported above for the preparation of **17**) cooled to −10°C, a precooled solution of **1** (1.75 g, 20 mmole) in 95% ethanol was added. After stirring for 1 hour at −10°C and another hour at room temperature, the undissolved material was filtered off and the ethanol was removed *in vacuo* at room temperature. The aqueous phase was acidified with 23% HCl; after cooling, the precipitate was collected washed with cold water and dried at 60°C under vacuum to give 2.2 g (71%) of **18**; mp 203~205°C; ir (KBr): 2210, 1690, 1600, 935 cm^{-1}; nmr (DMSO-d_6): δ 6.09 (1H, d, J=16 Hz, NC–CH=), 6.12 (1H, d, J=10 Hz, =CH–COOH), 7.72 (1H, d, J=10 Hz, S–CH=), 8.20 (1H, d, J=16 Hz, =CH–S).

Anal. Calcd. for $C_6H_5NO_2S$: C, 46.44; H, 3.25; N, 9.02; S, 20.66.
Found: C, 46.31; H, 3.18; N, 8.91; S, 20.52.

(*Z*)-β-Cyanovinylene-(*Z*)-thioacrylic acid (**19**)

To an ethanolic solution of the sodium salt of (*Z*)-β-thioacrylic acid (prepared from 4.55 g of **16**

as reported above for the preparation of **17**), a precooled solution of propiolonitrile (1.27 g, 25 mmole) in ethanol (35 ml) was added. After stirring for 2 hours at −10°C, the ethanol was removed *in vacuo* at room temperature and the aqueous phase was acidified with 23% HCl. The precipitate was collected, washed with water and crystallized from aqueous ethanol to give 2.9 g (75%) of **19**; mp 213~215°C; ir (KBr): 2210, 1690, 1590 cm^{-1}; nmr (DMSO-d_6): δ 5.96 (1H, d, J=10 Hz, NC–CH=), 6.15 (1H, d, J=10 Hz, =CH–COOH), 7.77 (1H, d, J=10 Hz, S–CH=), 8.03 (1H, d, J=10 Hz, =CH–S).

Anal. Calcd. for $C_6H_5NO_2S$: C, 46.44; N, 3.25; N, 9.02; S, 20.66.
Found: C, 46.54; H, 3.22; N, 8.99; S, 20.59.

1-(2-Cyanoethyl)-1H-tetrazole-5-thiol

To a suspension of 1-(2-aminocarbonylethyl)-1H-tetrazole-5-thiol (1.73 g, 10 mmole) in a mixture of dry N,N-dimethylformamide (20 ml) and ethyl ether (50 ml), cooled to 0°C, PCl_5 (2.08 g) was added portionwise and the mixture was stirred for 30 minutes at 0°C. The reaction mixture was then poured into ice; the organic layer was separated and the aqueous phase was extracted with ethyl ether (3× 30 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residue was dissolved in chloroform (50 ml) and precipitated by adding *n*-hexane (200 ml) to give 0.93g (60%) of the desired product; mp 138~140°C (dec.); ir (KBr): 2250, 1515 cm^{-1}; nmr (DMSO-d_6): δ 3.20 (2H, t, $-CH_2-CN$), 4.62 (2H, t, $>N-CH_2-$), 12.50 (1H, br-s, –SH).

Anal. Calcd. for $C_4H_5N_5S$: C, 30.96; H, 3.25; N, 45.13; S, 20.66.
Found: C, 30.90; H, 3.20; N, 45.05; S, 20.60.

7-Amino-3-heterocyclicthiomethyl-3-cephem-4-carboxylic acids

A solution of the appropriate thiol (15 mmole) and $NaHCO_3$ (1.26 g) in water (25 ml) was added to a stirred solution of 7-ACA (10 mmole) and $NaHCO_3$ (0.84 g) in water (25 ml). The mixture was stirred for 6 hours at 55°C, maintaining the pH between 6.8~7.2 by adding 5% $NaHCO_3$ or 3 N HCl if necessary. The solution was treated with a small amount of charcoal and filtered. The filtrate was cooled in an ice bath and acidified to pH 3.5 with diluted HCl. The resulting precipitate was collected, washed with water (15 ml) and acetone (15 ml) and dried *in vacuo*. The crude products were used without further purification.

Method A

7-[(*Z*)-Cyanomethylthioacrylamido]-3-[(5-methyl-1, 3, 4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid (**20**)

A solution of ethyl chloroformate (0.95 ml, 10 mmole) in dry acetone (10 ml) was added dropwise at −10°C to a stirred solution of (*Z*)-cyanomethylthioacrylic acid (1.43 g, 10 mmole) in dry acetone (80 ml) and triethylamine (1.4 ml). After stirring for 30 minutes at 0°C, a cold (0°C) solution of 7-amino-3-[(5-methyl-1,3,4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid (3.44 g, 10 mmole) and triethylamine (1.4 ml) in 50% aqueous acetone (140 ml) was added. After stirring for 1 hour at 0°C and 3 hours at room temperature the acetone was evaporated *in vacuo*. The aqueous phase was washed with ethyl acetate (discarded), adjusted to pH 2 with 20% H_2SO_4 under stirring and extracted with ethyl acetate. The undissolved material was filtered and discarded. The organic layer was separated, washed with aqueous NaCl solution, dried (Na_2SO_4) and evaporated to small volume. Dropwise addition of ethyl ether precipitated the product which was collected and dried *in vacuo* to give 2.8 g of **20**; mp 130~135°C (dec.); tlc on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20): Rf=0.52; ir (KBr): 2220, 1780, 1720, 1660, 1580 cm^{-1}; nmr (DMSO-d_6): δ 2.70 (3H, s, CH_3 on thiadiazole ring), 3.68 (2H, q, 2–CH_2), 3.75 (2H, s, $-CH_2S-$), 4.31 (2H, q, 3–CH_2), 5.10 (1H, d, 6–H), 5.63 (1H, d-d, 7–H), 5.75 (1H, d, J=10 Hz, =CHCO), 7.15 (1H, d, J=10 Hz, S–CH=), 9.2 (1H, d, –CONH).

Anal. Calcd. for $C_{10}H_{15}N_5O_4S_4$: C, 40.92; H, 3.22; N, 14.91; S, 27.31.
Found: C, 40.71; H, 3.28; N, 14.75; S, 27.11.

Method B

7-[(*Z*)-β-Cyanovinylenethioacetamido]-3-[(1-methyl-1 H-tetrazol-5-yl)-thiomethyl]-3-cephem-4-carboxylic acid (**40**)

To a solution of (*Z*)-β-cyanovinylenethioacetic acid (1.43 g, 10 mmole) and triethylamine (1.4 ml)

in dry acetone (80 ml), cooled to 0°C, was added pivaloylchloride (1.22 ml) dissolved in dry acetone (20 ml) with stirring. The mixture was stirred for 30 minutes at 0°C, then a solution of 7-amino-3-[(1-methyl-1H-tetrazol-5-yl)-thiomethyl]-3-cephem-4-carboxylic acid (3.28 g, 10 mmole) and triethylamine (1.4 ml) in 50% aqueous acetone (160 ml) was added. After stirring for 1 hour at 0°C and 2 hours at room temperature the acetone was evaporated *in vacuo*. The residue was taken up with water and washed with ethyl acetate. After separation of the organic layer, the aqueous phase was cooled and adjusted to pH 2 with 20% H_2SO_4 under stirring and extracted with ethyl acetate. The organic layer was worked up according to method A to give 2.71 g of **40**; mp 113~115°C (dec.); tlc on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20): Rf=0.60; ir (KBr): 2210, 1775, 1680, 1540 cm^{-1}; nmr (DMSO-d_6): δ 3.68 (2H, q, 2-CH_2), 3.73 (2H, s, SCH_2CO), 3.94 (3H, s, $-NCH_3$), 4.31 (2H, q, 3-CH_2), 5.10 (1H, d, 6-H), 5.63 (1H, d-d, 7-H), 5.72 (1H, d, J=10 Hz, NC-CH=), 7.63 (1H, d, J=10 Hz, =CHS), 9.2 (1H, d, -CONH).

Anal. Calcd. for $C_{15}H_{13}N_7O_4S_3$: C, 39.72; H, 3.33; N, 21.62; S, 21.21.
Found: C, 39.78; H, 3.43; N, 21.40; S, 20.78.

Method C

7-[(*Z*)-β-Carboxyvinylenethioacetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid (**39**)

To a solution of (*Z*)-β-*tert*-butoxycarbonylvinylenethioacetic acid (2.84 g, 13 mmole) in dry acetone (80 ml), cooled to −5°C were added triethylamine (1.82 ml) and 2 drops of N-methyl morpholine followed by a solution of pivaloyl chloride (1.60 ml) in dry acetone (20 ml). After stirring for 30 minutes at 5°C a solution of 7-amino-3-[(5-methyl-1,3,4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid (3.44 g, 10 mmole) in 50% aqueous acetone (100 ml) and triethylamine (1.4 ml) was added dropwise. After stirring for 1 hour at 0°C and 2 hours at room temperature, the acetone was removed *in vacuo*. The residue was taken up with water, stratified with ethyl acetate and adjusted to pH 2 with 20% H_2SO_4 with stirring. The organic layer was separated and the aqueous layer extracted twice with ethyl acetate. The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was taken up with ethyl ether to give 7-[(*Z*)-β-butoxycarbonylvinylenethioacetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid (70%), which was used for the next step without further purification.

The above ester 2.75 g (5 mmole), was added to a stirred solution of trifluoroacetic acid (30 ml) and anisole (6 ml), cooled to 0°C. After stirring for 45 minutes at 5°C, the mixture was evaporated *in vacuo* below 35°C to remove trifluoroacetic acid. The resulting residue was taken up with ethyl ether and collected. The solid was dissolved in 5% aqueous $NaHCO_3$ solution, covered with ethyl acetate and adjusted to pH 2 with 20% H_2SO_4 under stirring. A small amount of insoluble material was filtered off and discarded. After separation of the organic layer, the aqueous phase was extracted twice with ethyl acetate. The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated *in vacuo* to small volume. After adding ethyl ether a solid precipitated which was collected by filtration and dried *in vacuo* to give 1.47 g of **39**; mp 138~140°C (dec.); tlc on silica gel gave a single spot with chloroform - methanol - formic acid (160: 30: 20): Rf=0.41; ir (KBr): 1780, 1680, 1540 cm^{-1}; nmr (DMSO-d_6): δ 2.70 (3H, s, $-CH_3$ on thiadiazole ring), 3.51 (2H, s, SCH_2CO), 3.68 (2H, q, 2-CH_2), 4.31 (2H, q, 3-CH_2), 5.13 (1H, d, 6-H), 5.71 (1H, d-d, 7-H), 5.85 (1H, d, J= 10 Hz, HOOC-CH=), 7.42 (1H, d, J=10 Hz, =CHS), 9.14 (1H, d, -CONH).

Anal. Calcd. for $C_{16}H_{16}N_4O_6S_4$: C, 39.33; H, 3.30; N, 11.47; S, 26.25.
Found: C, 39.61; H, 3.51; N, 11.15; S, 26.01.

Method D

7-[(*Z*)-β-Carboxyvinylenethioacetamido]cephalosporanic acid (**36**)

Phosphorus pentachloride (1.04 g, 5 mmole) was added to (*Z*)-β-*tert*-butoxycarbonylvinylenethioacetic acid (1.1 g, 5 mmole) in dry ethyl ether (50 ml) and the mixture was stirred for 5 minutes at 0°C and 2 hours at room temperature. The reaction mixture was evaporated *in vacuo* below 30°C, taken up with benzene, evaporated again in order to remove any traces of phosphorus oxychloride and taken up with dry 1,2-dichloromethane (10 ml). This solution was then dropped into a stirred, ice-

cooled solution of the diphenylmethyl ester of 7-aminocephalosporanic acid (2.41 g, 5.5 mmole) and N,N-diethylaniline (0.88 ml) in dry 1,2-dichloroethane (50 ml). After stirring for 20 minutes at 0°C and 1 hour at room temperature, the solution was evaporated *in vacuo* below 40°C. The residue was dissolved in ethyl acetate, washed with 10% aqueous $KHSO_4$ solution, saturated aqueous NaCl solution, aqueous $NaHCO_3$ solution and then with water. The organic layer was separated, dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give 2.5 g of a solid which was chromatographed (silica gel, ethyl ether) to give the pure ester (1.86 g); mp 135~137°C.

A cold solution of the ester (1.75 g) in trifluoroacetic acid (20 ml) and anisole (5 ml) was stirred for 30 minutes and then evaporated *in vacuo* below 40°C. The residue was taken up with ethyl ether (50 ml) and stirred for 10 minutes. The solid was filtered, washed with ethyl ether and dried under vacuum to give 1.1 g (96.6%) of **36**; mp 169~170°C (dec.); tlc on silica gel gave a single spot with chloroform - methanol - formic acid (160: 30: 20): Rf=0.43; ir (KBr): 1780, 1730, 1680, 1535 cm^{-1}; nmr (DMSO-d_6): δ 2.00 (3H, s, $-OCOCH_3$), 3.51 (2H, s, SCH_2CO), 3.68 (2H, q, 2-CH_2), 4.31 (2H, q, 3-CH_2), 5.13 (1H, d, 6-H), 5.71 (1H, d-d, 7-H), 5.85 (1H, d, J=10 Hz, HOOC-CH=), 7.42 (1H, d, J=10 Hz, =CHS), 9.14 (1H, d, -CONH).

Anal. Calcd. for $C_{15}H_{16}N_2O_8S_2$: C, 43.26; H, 3.87; N, 6.72; S, 15.40.
Found: C, 43.06; H, 3.86; N, 6.68; S, 15.51.

Method E

7-[(*Z*)-β-Cyanovinylenethioacetamido]-3-[(1-(2-cyanoethyl)-1H-tetrazol-5-yl)-thiomethyl]-3-cephem-4-carboxylic acid (**43**)

A solution of 1-(2-cyanoethyl)-1H-tetrazole-5-thiol (2.33 g, 15 mmole) and $NaHCO_3$ (1.26 g) in water (80 ml) was added to a stirred solution of 7-[(*Z*)-β-cyanovinylenethioacetamido]cephalosporanic acid **34** (3.97 g, 10 mmole) and $NaHCO_3$ (1.6 g) in water (80 ml). The mixture was stirred for 10 hours at 55°C, maintaining the pH at 6.5. After cooling to room temperature, the reaction mixture was washed with ethyl acetate (discarded), the aqueous phase was separated, stratified with ethyl acetate, cooled in an ice-bath and acidified to pH 2 with 20% H_2SO_4 with stirring. The organic layer was separated and the aqueous phase was extracted with ethyl acetate; the combined organic extracts were washed with water and dried (Na_2SO_4). After evaporating to small volume, ethyl ether was added. The solid precipitated was collected by filtration, dissolved in methanol and dicyclohexylamine was added dropwise to the solution. After adding ethyl ether a solid precipitated which was collected and washed with ethyl ether.

The dicyclohexylamine salt was dissolved in water, stratified with ethyl acetate and acidified to pH 2 with 20% H_2SO_4. The organic layer was separated and worked-up according to Method A to give 2.46 g of **43**; mp 142~144°C (dec.); tlc on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20): Rf=0.49; ir (KBr): 2250, 2220, 1770, 1540 cm^{-1}; nmr (DMSO-d_6): δ 3.18 (2H, t, $-CH_2CN$), 3.50 (2H, s, SCH_2CO), 3.73 (2H, q, 2-CH_2), 4.38 (2H, q, 3-CH_2), 4.64 (2H, t, >N-CH_2-), 5.07 (1H, d, 6-H), 5.67 (1H, d-d, 7-H), 5.70 (1H, d, J=10 Hz, NC-CH=), 7.64 (1H, d, J=10 Hz, =CHS), 9.18 (1H, d, -CONH).

Anal. Calcd. for $C_{17}H_{16}N_8O_4S_3$: C, 41.45; H, 3.27; N, 22.75; S, 19.53.
Found: C, 41.58; H, 3.35; N, 22.51; S, 19.31.

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