CEFODIZIME, AN AMINOTHIAZOLYLCEPHALOSPORIN

V. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN THE CEFODIZIME SERIES†

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The synthesis as well as *in vitro* antibacterial activity and pharmacokinetic behavior of cefodizime (HR 221, **1a**), its analogs and derivatives is described. In this comparison, cefodizime stands out for its balance between its high antibacterial activity, prolonged elimination half-life and high AUC in mice and dogs.

In recent years, a number of β-lactamase stable, highly active broad-spectrum cephalosporins have been developed and introduced into therapy. The first representative of this group was cefotaxime (CTX)[1], which possesses an aminothiazolyl side chain with a *syn*-methoximino group in the 7-position. This specific side chain, which is responsible for the outstanding microbiological properties of CTX[2,3], was subsequently combined with other cephalosporin parent compounds to also yield highly active cephalosporins such as cefmenoxime[4], ceftizoxime[5] or ceftriaxone[6]. The 3′-substituent of these do not substantially alter the antibacterial spectrum compared to that of cefotaxime but affect mainly the pharmacokinetics, β-lactamase stability and metabolism.

Cefodizime (HR 221)[7], a derivative of CTX synthesized in the laboratories of Hoechst AG, contains a mercaptothiazolyl side chain in 3′-position with an additional acid group. As has been demonstrated in our and other laboratories this specific 3′-substituent renders both high antibacterial activity[7,8] and prolonged half-life in laboratory animals[9] to HR 221. As has been found recently[10], HR 221 markedly stimulates the immune-system of laboratory animals.

In this context we were especially interested in how variations in the 7-side-chain and in the substitution of the mercaptothiazolyl moiety would affect the antibacterial and pharmacokinetic properties.

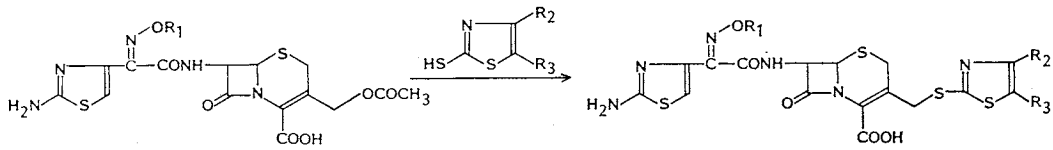
In this paper we wish to describe the synthesis of HR 221 derivatives and closely related aminothiazolylcephalosporins. Their microbiological and pharmacokinetic properties will be discussed.

Chemistry

Compounds **1a**, **2a**～**2k** and **3a**～**3i** were prepared from cefotaxime (CTX) by 3′-acetate displacement with the appropriate 2-mercaptothiazoles in aqueous solution of pH 6.5～7.0 (Scheme 1). In general, the cephalosporanic acids were isolated directly from the reaction mixture by acidification and proved to be sufficiently pure for biological testing.

† Dedicated to Prof. L. HORNER on the occasion of his 75th birthday.

Scheme 1.



$R_1 = CH_3$ (Cefotaxime)
$R_1 = H$

1a, 1d, 2a~2i, 2k, 3a~3i

1a $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOH$
1d $R_1 = H$ $R_2 = CH_3$ $R_3 = CH_2COOH$
2a $R_1 = CH_3$ $R_2 = COOH$ $R_3 = H$
2b $R_1 = CH_3$ $R_2 = CH_2COOH$ $R_3 = H$
2c $R_1 = CH_3$ $R_2 = CH_2CH_2COOH$ $R_3 = H$
2d $R_1 = CH_3$ $R_2 = CH_2CH_2COOCH_3$ $R_3 = H$
2e $R_1 = CH_3$ $R_2 = CH_2COOH$ $R_3 = CH_3$
2f $R_1 = CH_3$ $R_2 = CH_2COOCH_3$ $R_3 = CH_3$
2g $R_1 = CH_3$ $R_2 = CH_2COOC_2H_5$ $R_3 = CH_3$
2h $R_1 = CH_3$ $R_2 = CH_2COOC_2H_5$ $R_3 = COOC_2H_5$
2i $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2CH_2COOH$
2k $R_1 = CH_3$ $R_2 = H$ $R_3 = CH_2COOH$

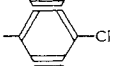
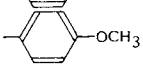
3a $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_3$
3b $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH(CH_3)_2$
3c $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_2CH_2OCH_3$
3d $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_2CH{=}CH_2$
3e $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_2C{\equiv}CH$
3f $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_2$– 
3g $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_2$– 
3h $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2COOCH_2$– 
3i $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = CH_2CONH_2$

Table 1. Configurational assignment of the methoximino group, chemical shift (60 MHz, DMSO-d_6, Hz).

	Thiazol-5-H		$=NOCH_3$	
	Z	*E*	*Z*	*E*
(*Z*)-**6**	434		233	
(*E*)-**6**		450		239
(*Z*)-**7**	411		233	
(*E*)-**7**		454		245
(*Z*)-**8**	—		234	
(*E*)-**8**		—		238

Alternatively, compounds **1** were prepared by a multistep procedure (Scheme 2). (2-Mercapto-4-methylthiazol-5-yl)acetic acid as well as its methyl- and ethyl-ester were reacted with 7-aminocephalosporanic acid (7-ACA) to **4a~4c** which were acylated with 2-aminothiazol acetic acid side chains to the cephalosporins **1**, using the 1-hydroxybenzotriazole (HOBT)-methodology. In the case of the hydroximino derivatives **1d~1f** the acylation step was followed by removal of the trityl groups in formic acid at 0°C.

During cyclization of ethyl-γ-bromo-α-(*Z*)-methoximinoacetoacetate with thiourea in refluxing ethanol, the methoximino group was isomerized to the (*E*)-configuration to yield (*E*)-**6**·HBr. This was saponified in high yield to (*E*)-**7**. As is shown in Table 1, the (*E*)- and (*Z*)-configuration can be assigned unambiguously by ^1H NMR based on the chemical shift of the thiazol-5-H and of the methoximino group.

The synthesis of 2-amino-5-chlorothiazol-4-yl acetic acid (*Z*)-**8** is achieved best by direct chlorination of (*Z*)-**7** in $CHCl_3$ - acetic acid at 0°C, whereby (*Z*)-**8**·HCl crystallizes from the reaction mixture. The precipitate is recrystallized from THF in order to remove acetic acid. After treatment with one equivalent of CH_3ONa the free acid crystallizes as (*Z*)-**8**·MeOH from methanol.

Antibacterial Activity

The *in vitro* antibacterial activity of cefodizime and its analogs and derivatives is shown in Tables 2, 3 and 4.

All analogs of cefodizime (HR 221, **1a**) bearing an acidic substituent on the mercaptothiazole

Scheme 2.

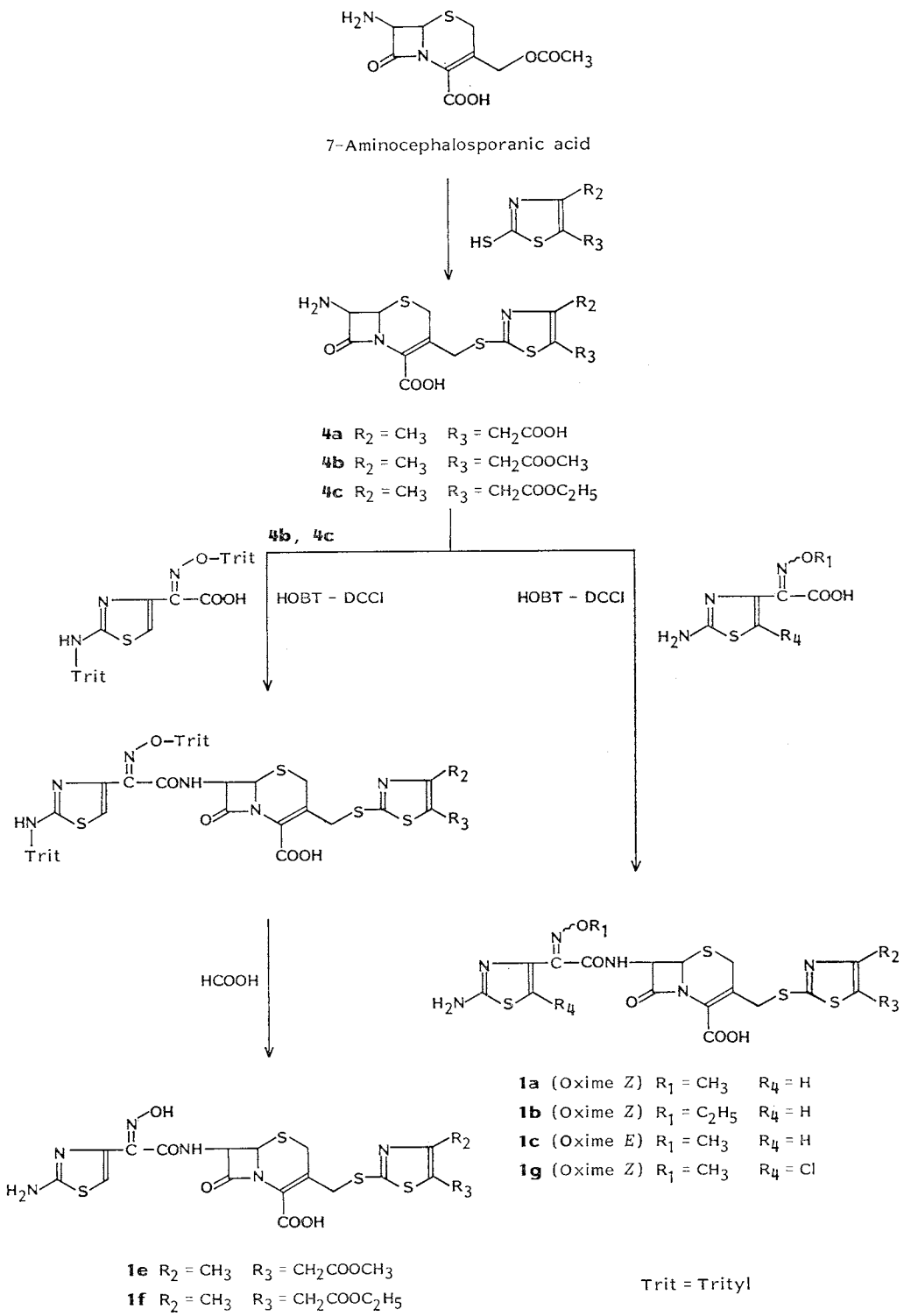
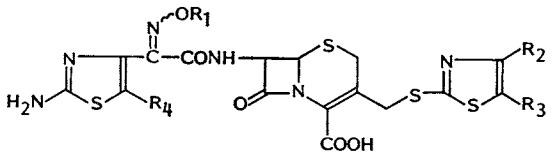

7-Aminocephalosporanic acid
4a R2 = CH3 R3 = CH2COOH
4b R2 = CH3 R3 = CH2COOCH3
4c R2 = CH3 R3 = CH2COOC2H5
4b, 4c
HOBT - DCCI
HOBT - DCCI
HCOOH
1a (Oxime Z) R1 = CH3 R4 = H
1b (Oxime Z) R1 = C2H5 R4 = H
1c (Oxime E) R1 = CH3 R4 = H
1g (Oxime Z) R1 = CH3 R4 = Cl
1e R2 = CH3 R3 = CH2COOCH3
1f R2 = CH3 R3 = CH2COOC2H5
Trit = Trityl

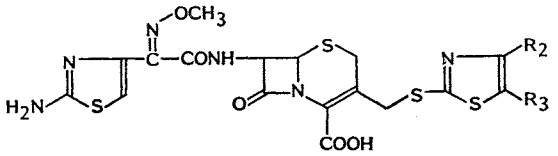
Table 2. Minimal inhibitory concentrations of C-7-acyl side chain isomers of cefodizime.



	1a	1b	1c	1d	1e	1f	1g
R_1:	CH_3	C_2H_5	CH_3	H	H	H	CH_3
R_2:	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3
R_3:	CH_2COOH	CH_2COOH	CH_2COOH	CH_2COOH	CH_2COOCH_3	$CH_2COOC_2H_5$	CH_2COOH
R_4:	H	H	H	H	H	H	Cl
Oxime:	*Z*	*Z*	*E*	*Z*	*Z*	*Z*	*Z*
Staphylococcus aureus SG 511	3.13	12.5	50	1.25	0.391	0.391	5.0
S. aureus 285	6.25	25	100	6.25	1.56	0.781	12.5
Streptococcus pyogenes 308A	0.062	0.078	1.56	0.313	0.019	0.039	0.078
Escherichia coli TEM	0.313	1.95	12.5	2.5	1.95	7.81	1.25
E. coli 1507E	0.313	1.95	6.25	0.625	0.195	1.95	1.25
Salmonella typhimurium	0.156	3.13	6.25	0.195	1.56	6.25	6.25
Klebsiella aerogenes 1082E	125	>100	>100	>100	>100	>100	7.81
K. aerogenes 1522E	0.313	0.781	3.13	0.625	0.781	1.95	2.5
Enterobacter cloacae P99	>500	>500	>500	>500	>500	>500	500
E. cloacae 1321	0.313	0.781	1.56	0.313	0.195	1.95	2.5
Proteus mirabilis ATCC 14273	0.039	0.078	nd	0.039	0.156	0.781	0.391

nd: Not determined.

Table 3. Minimal inhibitory concentrations of 3′-isomers of cefodizime.

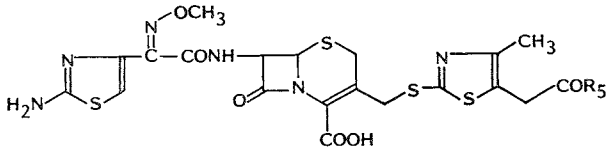


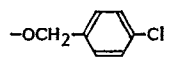
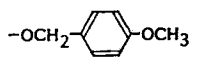
	2a	2b	2c	2d	2e	2f
	R_2: COOH R_3: H	CH_2COOH H	CH_2CH_2COOH H	$CH_2CH_2COOCH_3$ H	CH_2COOH CH_3	CH_2COOCH_3 CH_3
Staphylococcus aureus SG 511	6.25	5.0	6.25	1.56	12.5	1.56
S. aureus 285	12.5	10.0	6.25	3.13	12.5	3.13
Streptococcus pyogenes 308A	0.078	0.062	0.031	0.008	0.039	0.015
Escherichia coli TEM	0.625	0.313	0.391	0.781	0.313	0.781
E. coli 1507E	0.313	0.078	0.195	0.039	0.156	0.039
Salmonella typhimurium	0.391	0.784	0.195	0.781	0.391	0.781
Klebsiella aerogenes 1082E	250	nd	62.5	62.5	250	125
K. aerogenes 1522E	0.625	0.156	0.156	0.781	0.156	0.391
Enterobacter cloacae P99	>500	>500	>500	500	>500	500
E. cloacae 1321	0.156	0.313	0.156	0.195	0.078	0.195
Proteus mirabilis ATCC 14273	0.078	0.031	0.015	0.313	0.008	0.078

	2g	2h	1a	3a	2i	2k
	R_2: $CH_2COOC_2H_5$ R_3: CH_3	$CH_2COOC_2H_5$ $COOC_2H_5$	CH_3 CH_2COOH	CH_3 CH_2COOCH_3	CH_3 CH_2CH_2COOH	H CH_2COOH
Staphylococcus aureus SG 511	1.56	0.781	3.13	1.25	6.25	3.13
S. aureus 285	1.56	1.56	6.25	1.56	12.5	6.25
Streptococcus pyogenes 308A	0.008	0.008	0.062	0.019	0.039	0.002
Escherichia coli TEM	1.56	0.781	0.313	1.56	0.391	0.049
E. coli 1507E	0.313	0.156	0.313	0.391	0.195	0.098
Salmonella typhimurium	0.781	1.56	0.156	1.56	0.313	0.195
Klebsiella aerogenes 1082E	15.63	125	125	250	62.5	6.25
K. aerogenes 1522E	0.781	1.56	0.313	0.781	0.195	0.049
Enterobacter cloacae P99	>500	500	>500	250	>500	>500
E. cloacae 1321	0.781	0.195	0.313	0.195	0.313	0.004
Proteus mirabilis ATCC 14273	0.156	0.313	0.39	0.078	0.019	nd

nd: Not determined.

Table 4. Minimal inhibitory concentrations of esters and amides of cefodizime.



	3a	3b	3c	3d	3e	3f	3g	3h	3i
	R_5: OCH_3	$OCH(CH_3)_2$	$OC_2HCH_2OCH_3$	$OCH_2CH=CH_2$	$OCH_2C\equiv CH$	$OCH_2C_6H_5$			NH_2
S.a. SG 511	1.25	1.56	3.13	0.391	0.781	1.56	6.25	0.781	3.125
S.a. 285	1.56	3.13	6.15	1.56	1.56	3.13	3.13	0.781	6.15
S.p.	0.019	0.008	0.019	0.015	0.031	0.015	0.031	0.004	0.039
E.c. TEM	1.56	2.5	0.391	0.391	0.313	1.95	1.56	1.56	0.781
E.c. 1507E	0.391	0.039	0.078	0.019	0.078	0.313	0.156	0.195	0.078
S.t.	1.56	0.313	0.781	0.781	0.195	3.13	3.13	1.56	0.781
K.a. 1082E	250	62.5	62.5	62.5	62.5	>500	>500	125	31.25
K.a. 1522E	0.781	1.25	0.195	0.391	0.313	0.625	0.781	0.391	1.95
E.c. P99	250	500	>500	125	125	500	250	250	>500
E.c. 1321	0.195	0.156	0.313	0.313	0.156	1.95	0.391	0.195	0.625
P.m.	0.078	0.078	0.019	0.078	0.195	0.195	0.156	0.019	0.195

Abbreviations: *S.a.* SG 511; *Staphylococcus aureus* SG 511, *S.a.* 285; *Staphylococcus aureus* 285, *S.p.*; *Streptococcus pyogenes* 308A, *E.c.* TEM; *Escherichia coli* TEM, *E.c.* 1507E; *Escherichia coli* 1507E, *S.t.*; *Salmonella typhimurium*, *K.a.* 1082E; *Klebsiella aerogenes* 1082E, *K.a.* 1522E; *Klebsiella aerogenes* 1522E, *E.c.* P99; *Enterobacter cloacae* P99, *E.c.* 1321; *Enterobacter cloacae* 1321, *P.m.*; *Proteus mirabilis* ATCC 14273.

Table 5. Pharmacokinetics of cefodizime (**1a**) analogs in mouse (10 mg/kg, sc).

Compound	$T_{1/2}$ (hours)	AUC (mg·hours/liter)
1a	1.28	40.7
2k	0.49	16.4
1g	0.5	17.9
2e	0.52	15.2
2f	0.79	11.0
2b	0.27	8.5
2c	0.31	1.8

moiety exhibit low MIC values in the range of 0.1 to 0.5 μg/ml against most Gram-negative organisms (Tables 2 and 3). They also inhibit bacteria producing the clinically important TEM-type-β-lactamases. Because of their high polarity (2 acidic groups) the MIC values against staphylococci are higher than 1 μg/ml.

Table 1 demonstrates the influence of differently substituted aminothiazolyl side chains on the *in vitro* activities. Among them, HR 221 possesses the lowest MIC especially against Gram-negative strains. A free oximino group as in **1d** lowers the stability against plasmid mediated TEM-type-β-lactamases. The anti-methoximino analog **1c**, as expected, is devoid of both β-lactamase stability and high antibacterial activity. **1g**, the 5-chloroaminothiazolyl derivative, is worth mentioning in that it is highly stable against chromosomally mediated K1-β-lactamase. This specific effect has been observed before in other cephalosporins, bearing this type of chloro substituted side chain†.

Exchange of both 2-mercaptothiazolyl substituents in **2e**, does not influence the antibacterial activity considerably as compared to **1a**. Shortening or lengthening of the acidic side chain in position 4 of the mercaptothiazole, too, does not alter the activity to a great extent. The demethyl analog of **1a**, **2k**, apart from exhibiting broad and high antibacterial activity, is surprisingly active against K1-β-lactamase producing *Klebsiella aerogenes*, comparable to **1g**.

As can be seen in Table 4, transformation of the mercaptothiazolcarboxy group into esters and amides does not have the expected effect against Gram-positive strains: The allyl and propargyl esters (**3d** and **3e**) alone have MIC values distinctly lower than those of **1a**. In the Gram-negative range, the esters are slightly less active than **1a**, whereas the amide **3i** is comparable. In neither compound, antipseudomonal activity could be detected.

Pharmacokinetics

Cefodizime (**1a**) has by far the highest values with respect to both elimination half-life and area under the curve (AUC) (Table 5). The comparison with its analogs, but especially with its positional isomer **2e** and its demethyl analog **2k** shows, that the specific substitution pattern of **1a** is responsible for its outstanding pharmacokinetic properties.

The esters of cefodizime (**1a**), too, have to some extent high elimination half-lives and AUC values in mouse and dogs (Table 6). The chlorobenzyl ester **3g** alone has comparably balanced pharmacokinetic properties as cefodizime (**1a**) has.

On the basis of its antibacterial activity together with its pharmacokinetic behavior, cefodizime (**1a**) was selected for further development.

Experimental

^{1}H NMR spectra were recorded on a Varian T 60 or a Bruker AM 270 spectrometer using tetramethylsilane as internal standard. UV spectra were measured in methanolic solution using a Perkin-Elmer 554 spectrometer. All melting points are uncorrected. The MIC values were determined by

† Unpublished results.

Table 6. Pharmacokinetics of cefodizime (**1a**) esters in mouse and dog.

Compound	Mouse (10 mg/kg, sc)		Dog (10 mg/kg, iv)	
	$T_{1/2}$ (hours)	AUC (mg·hours/liter)	$T_{1/2}$ (hours)	AUC (mg·hours/liter)
1a	1.28	40.7	1.2	59.8
3a	0.71	28.7	nd	nd
3b	1.74	24.9	1.24	18.6
3c	0.88	33.8	nd	nd
3d	0.92	35.1	0.68	45.9
3e	1.08	22.1	0.67	32.8
3f	1.15	57.8	nd	nd
3g	1.29	38.6	1.22	61.9
3h	0.89	49.8	0.77	45.5

nd: Not determined.

an agar dilution method with Mueller-Hinton agar[11]. Pharmacokinetic data were obtained as described[12].

7-Amino-3-[(5-carboxymethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**4a**)

10 g of 7-aminocephalosporanic acid tosylate and 4.7 g of (2-mercapto-4-methylthiazol-5-yl)-acetic acid were suspended in 200 ml of H_2O. 1 N NaOH was added to adjust the pH to 6.5~6.8. The solution was stirred at 60°C for 3 hours. During this period the pH was kept at 6.5~6.8. After cooling to room temperature the solution was shaken 3 times with ethyl acetate and acidified with 2 N HCl to pH 4.0. The precipitate thus formed was filtered off, washed with H_2O, stirred with 200 ml of acetone, filtered and washed with acetone to yield after drying *in vacuo* 6.7 g of the title compound **4a**. ^{1}H NMR (60 MHz, DMSO-d_6) δ 2.2 (3H, s, CH_3), 3.55 (2H, AB, 2-CH_2), 3.75 (2H, s, CH_2), 4.25 (2H, AB, 3-CH_2), 4.7 (1H, d, 6H), 4.9 (1H, d, 7H).

Similarly to **4a** compounds **4b** and **4c** were obtained by treatment of 7-aminocephalosporanic acid tosylate with the methyl and ethyl ester of (2-mercapto-4-methylthiazol-5-yl)acetic acid.

7-Amino-3-[(5-methoxycarbonylmethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**4b**)

^{1}H NMR (60 MHz, DMSO-d_6) δ 2.2 (3H, s, CH_3), 3.5 (2H, AB, 2-CH_2), 3.90 (2H, s, CH_2), 3.6 (3H, s, OCH_3), 4.2 (2H, AB, 3-CH_2), 4.7 (1H, d, 6-H), 4.95 (1H, d, 7-H).

7-Amino-3-[(5-ethoxycarbonylmethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**4c**)

^{1}H NMR (60 MHz, DMSO-d_6) δ 1.2 (3H, t, CH_3), 2.3 (3H, s, CH_3), 3.55 (2H, AB, 2-CH_2), 3.85 (2H, s, CH_2), 4.1 (2H, q, OCH_2), 4.25 (2H, AB, 3-CH_2), 4.75 (1H, d, 6-H), 4.95 (1H, d, 7-H).

7-[α-(*Z*)-Methoximino-α-(2-aminothiazol-4-yl)acetamido-3-[(5-carboxymethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**1a**)

Method A: 7.54 g of 2-(*Z*)-methoximino-2-(2-aminothiazol-4-yl)acetic acid were dissolved in 100 ml of DMF. 5.7 g of HOBT·H_2O and 8.5 g of dicyclohexylcarbodiimide (DCCI) were added and the solution was stirred at room temperature. After 4 hours dicyclohexylurea was filtered off. 15 g of **4**, finely ground, were added and stirring was continued for 18 hours, whereupon the almost clear solution was filtered. 380 ml of H_2O were added, the precipitate was filtered off and discarded. A further 450 ml of H_2O were added to the filtrate, whereupon **2** crystallized. Filtration and washing with H_2O yielded 6 g of **1a**.

^{1}H NMR (60 MHz, DMSO-d_6) δ 2.2 (3H, s, CH_3), 3.6 (2H, AB, 2-CH_2), 3.75 (2H, s, CH_2), 3.85 (3H, s, (*Z*)-$NOCH_3$), 4.25 (2H, AB, 3-CH_2), 5.1 (1H, d, 6-H), 5.75 (1H, dd, 7-H), 6.7 (1H, s, thiazol-H), 7.15 (2H, br s, NH_2), 9.5 (1H, d, NH).

Method B: 6.1 g of (2-mercapto-4-methylthiazol-5-yl)acetic acid were dissolved in H_2O by adjusting the pH to 6.5 with 2 N NaOH. The solution was heated to 70°C and 12 g of cefotaxime dissolved in 75 ml H_2O were added under stirring. Stirring at 70°C was continued for 3 hours, during which time the pH was kept at 6.5 by addition of 2 N NaOH. After cooling to room temperature the solution was acidified to pH 2.8 and filtered. The residue was washed with H_2O and dried *in vacuo* over P_2O_5 to yield 10 g of **1a**.

1-[α-(*Z*)-Ethoximino-α-(2-aminothiazol-4-yl)acetoxy]benzotriazole (**5a**) and 1-[α-*syn*-Ethoximino-α-(2-aminothiazol-4-yl)acetyl]benzotriazol-3-oxide (**5b**)

6.5 g of α-(*Z*)-ethoximino-α-(2-aminothiazol-4-yl)acetic acid were dissolved in 35 ml of DMF. Under stirring, 4.05 g of HOBT and 6.19 g of DCCI were added. After 2 hours at room temperature dicyclohexyl urea was filtered off and washed with 2 ml of DMF. 50 ml of H_2O were added dropwise under stirring at 10°C. The precipitate was collected by filtration, washed with H_2O and dried *in vacuo* to yield 7.9 g (79%) of **5a** and **5b**, mp 118~120°C.

α-(*E*)-Methoximino-α-(2-aminothiazol-4-yl)acetic Acid ((*E*)-**7**)

To a solution of 10 g of (*E*)-**6** in 70 ml MeOH were added 32 ml of 2 N NaOH. The solution was left at room temperature for 1 hour and acidified with conc HCl to pH 3.0. The precipitate was filtered off, washed with MeOH and H_2O and dried to yield 7.7 g (88%) of (*E*)-**7**, mp 192°C (dec).

^{1}H NMR (60 MHz, DMSO-d_6) δ 7.4 (1H, s, thiazol-H), 3.92 (3H, s, (*E*)-$NOCH_3$).

7-[α-(*E*)-Methoximino-α-(2-aminothiazol-4-yl)acetamido]-3-[(5-carboxymethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**1c**)

To a solution of 5 g of α-(*E*)-methoximino-α-(2-aminothiazol-4-yl)acetic acid ((*E*)-**7**) and 3.85 g of HOBT in 60 ml of DMF were added 5.15 g of DCCI dissolved in 10 ml of DMF. The solution was stirred at room temperature for 4 hours whereupon the precipitated dicyclohexylurea was filtered off. 10 g of **4** were added to the filtrate and stirred overnight. The almost clear solution was filtered and evaporated to near dryness. The residue was triturated with 150 ml ethyl acetate, filtered and triturated with 200 ml of ethanol, filtered and dried to yield 10 g of **1c**.

7-[α-(*Z*)-Hydroximino-α-(2-aminothiazol-4-yl)acetamido]-3-[(5-carboxymethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**1d**)

710 mg of 7-[2-(*Z*)-hydroximino-2-(2-aminothiazol-4-yl)acetamido]cephalosporanic acid and 290 mg of 2-(2-mercapto-4-methylthiazol-5-yl)acetic acid were dissolved in 50 ml of H_2O at pH 7 by addition of satd $NaHCO_3$. The solution was stirred for 7 hours at 50~60°C, left overnight at room temperature, and washed with 3 times 20 ml of ethyl acetate. After removal of traces ethyl acetate from the aqueous layer under reduced pressure, 510 mg of **1d** were obtained on acidification to pH 4.2.

7-[α-(*Z*)-Hydroximino-α-(2-aminothiazol-4-yl)acetamido-3-[(5-methoxycarbonylmethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**1e**)

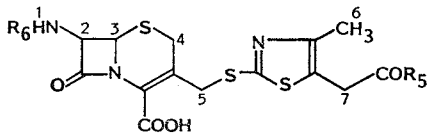
2.35 g of α-(*Z*)-trityloximino-α-(2-tritylaminothiazol-4-yl)acetic acid and 0.47 g of HOBT were dissolved in 14 ml of DMF. 0.721 g of DCCI dissolved in 4.5 ml DMF were added dropwise. After stirring for 3 hours dicyclohexylurea was filtered off. The filtrate was added to a solution of 1.45 g of **4b** in 10 ml of DMF and stirred for 1 hour at room temperature. After filtration, the solution was added to 360 ml of H_2O. The precipitate was filtered off, washed with H_2O, dried and taken up in 6.3 ml of formic acid (98%) and 1.3 ml of H_2O. After stirring at room temperature for 20 minutes, the precipitate was filtered off and the mother liquor was diluted with ethanol to precipitate 557 mg of **1e**.

Similarly to **1e** compound **1f** was obtained.

α-(*Z*)-Methoximino-α-(2-amino-5-chlorothiazol-4-yl)acetic Acid (**8**)

17.5 g of Cl_2 in 200 ml of acetic acid was added dropwise to a solution of 50 g of α-(*Z*)-methoximino-α-(2-aminothiazol-4-yl)acetic acid in 300 ml of $CHCl_3$ and 150 ml of acetic acid at 0~10°C. Stirring at 0°C was continued for 0.5 hour. The precipitate was filtered off, taken up in $CHCl_3$, filtered off, refluxed in 190 ml of THF for 15 minutes, and stirred at room temperature for 2 hours. The

Table 7. ^{1}H NMR spectral data of **1a**~**1g** (DMSO-d_6, 60 MHz, δ values in ppm).



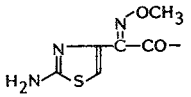
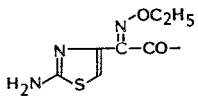
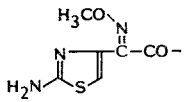
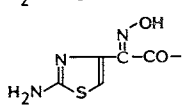
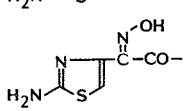
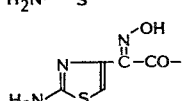
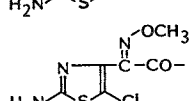
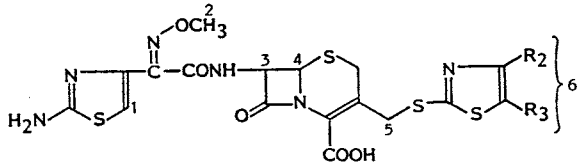
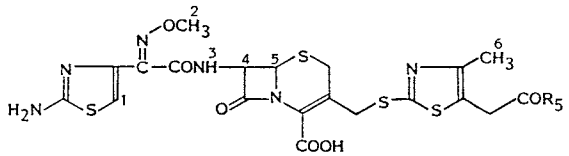
1	R_6		1	2	3	4	5	6	7	R_5
a (Cefodizime)		6.7 (s, thiazol-H), 3.85 (s, $NOCH_3$)	9.5 (d)	5.75 (dd)	5.1 (d)	3.6 (AB)	4.25 (AB)	2.2 (s)	3.75 (s)	OH
b		6.7 (s, thiazol-H), 4.1 (q, CH_2), 1.25 (t, CH_3)	9.5 (d)	5.75 (dd)	5.15 (d)	3.6 (AB)	4.3 (AB)	2.2 (s)	3.75 (s)	OH
c		7.4 (s, thiazol-H), 3.95 (s, $NOCH_3$)	9.4 (d)	5.75 (dd)	5.1 (d)	3.6 (AB)	4.25 (AB)	2.2 (s)	3.73 (s)	OH
d		11.25 (s, NOH), 6.63 (s, thiazol-H)	9.45 (d)	5.7 (dd)	5.1 (d)	3.6 (AB)	4.2 (AB)	2.2 (s)	3.7 (s)	OH
e		11.2 (s, NOH), 6.6 (s, thiazol-H)	9.5 (d)	5.75 (dd)	5.15 (d)	3.6 (AB)	4.25 (AB)	2.2 (s)	3.9 (s)	OCH_3, 3.7 (s)
f		11.3 (s, NOH), 6.6 (s, thiazol-H)	9.5 (d)	5.75 (dd)	5.1 (d)	3.6 (AB)	4.3 (AB)	2.2 (s)	3.9 (s)	OC_2H_5, 4.2 (q, CH_2), 1.3 (t, CH_3)
g		3.85 (230 Hz) (s, $NOCH_3$ *syn*)	9.5 (d)	5.75 (dd)	5.1 (d)	3.7 (AB)	4.3 (AB)	2.2 (s)	3.8 (s)	OH

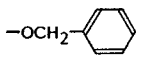
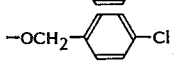
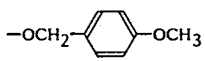
Table 8. ^{1}H NMR spectral data of **2a**~**2k** (DMSO-d_6, 60 MHz, δ values in ppm).



2	1	2	3	4	5	R_2	R_3	6
a	6.75 (s)	3.8 (s)	5.75 (dd)	5.1 (d)	4.3 (AB)	COOH	H	8.4 (s, thiazol-H)
b	6.75 (s)	3.85 (s)	5.75 (dd)	5.1 (d)	4.3 (AB)	CH_2COOH	H	7.4 (s, thiazol-H), 3.7 (s, CH_2)
c	6.7 (s)	3.85 (s)	5.7 (dd)	5.1 (d)	4.3 (AB)	CH_2CH_2COOH	H	7.2 (s, thiazol-H)
d	6.75 (s)	3.8 (s)	5.75 (dd)	5.15 (d)	4.3 (AB)	$CH_2CH_2COOCH_3$	H	7.25 (s, thiazol-H), 3.6 (s, $COOCH_3$)
e	6.75 (s)	3.83 (s)	5.75 (dd)	5.1 (d)	4.15 (AB)	CH_2COOH	CH_3	3.6 (s, CH_2), 2.25 (s, CH_3)
f	6.7 (s)	3.85 (s)	5.75 (dd)	5.1 (d)	4.25 (AB)	CH_2COOCH_3	CH_3	3.75 (s, CH_2), 3.6 (s, OCH_3), 2.33 (s, CH_3)
g	6.7 (s)	3.8 (s)	5.7 (dd)	5.1 (d)	4.2 (AB)	$CH_2COOC_2H_5$	CH_3	4.1 (q, OCH_2), 3.7 (s, CH_2), 2.3 (s, CH_3), 1.2 (t, CH_2CH_3)
h	6.7 (s)	3.8 (s)	5.75 (dd)	5.15 (d)	4.2 (AB)	$CH_2COOC_2H_5$	$COOC_2H_5$	4.1 (2×q, OCH_2), 4.1 (s, CH_2), 1.25 (2×t, CH_2CH_3)
i	6.8 (s)	3.85 (s)	5.7 (dd)	5.1 (d)	4.3 (AB)	CH_3	CH_2CH_2COOH	2.3 (s, CH_3)
k	6.75 (s)	3.76 (s)	5.75 (dd)	5.1 (d)	4.25 (AB)	H	CH_2COOH	7.5 (s, thiazol-H), 3.76 (s, CH_2)

Table 9. ^{1}H NMR spectral data of **3a**~**3i** (DMSO-d_6, 60 MHz, δ values in ppm).



3	1	2	3	4	5	6	7	R_5	
a	6.7 (s)	3.80 (s)	9.53 (d)	5.73 (dd)	5.1 (d)	2.23 (s)	3.9 (s)	OCH_3	3.64 (s, OCH_3)
b	6.65 (s)	3.8 (s)	9.5 (d)	5.65 (dd)	5.0 (d)	2.2 (s)	3.8 (s)	$OCH(CH_3)_2$	4.8 (m, CH), 1.15 (d, CH_3)
c*	6.9 (s)	3.93 (s)	nd	5.7 (d)	5.1 (d)	2.2 (s)	3.65 (s)	$OCH_2CH_2OCH_3$	3.3 (s, OCH_3)
d	6.66 (s)	3.8 (s)	9.5 (d)	5.7 (dd)	5.1 (d)	2.2 (s)	3.9 (s)	$OCH_2CH=CH_2$	4.6 (d, CH_2), 5~5.7 (m, vinyl-H)
e	6.66 (s)	3.8 (s)	9.49 (s)	5.7 (dd)	5.02 (d)	2.2 (s)	3.92 (s)	$OCH_2C\equiv CH$	4.7 (d, CH_2)
f	6.66 (s)	3.8 (s)	9.43 (d)	5.63 (dd)	5.0 (d)	2.13 (s)	3.9 (s)		7.3 (s, C_6H_5), 5.1 (s, CH_2)
g	6.73 (s)	3.8 (s)	9.56 (d)	5.7 (dd)	5.1 (d)	2.2 (s)	3.9 (s)		7.36 (s, C_6H_4), 5.1 (s, CH_2)
h	6.66 (s)	3.8 (s)	9.43 (d)	5.63 (dd)	5.05 (d)	2.13 (s)	3.85 (s)		7.0 (C_6H_4), 3.75 (OCH_3)
i	6.66 (s)	3.76 (s)	9.49 (d)	5.66 (dd)	5.03 (d)	2.2 (s)	3.5 (s)	NH_2	

* Solvent D_2O.
nd: Not detected.

residue was dissolved in 100 ml of MeOH. After addition of 4.9 g of CH_3ONa and stirring for 15 minutes, the solution was evaporated to dryness, and extracted with 3 times 75 ml of boiling THF. The combined extracts were evaporated, and the residue was recrystallized from little MeOH to yield 13.5 g of **8**·MeOH, mp 129~130°C (dec).

^1H NMR (60 MHz, DMSO-d_6) δ 3.1 (3H, s, CH_3OH), 3.9 (234 Hz) (3H, s, (*Z*)-$NOCH_3$).

7-[α-(*Z*)-Methoximino-α-(2-amino-5-chlorothiazol-4-yl)acetamido]-3-[(5-carboxymethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**1g**)

To a solution of 1.07 g of α-(*Z*)-methoxy-α-(2-amino-5-chlorothiazol-4-yl)acetic acid·MeOH (**8**) in 30 ml of THF were added 824 mg of 1-hydroxybenzotriazole. After 2 hours at room temperature the precipitated dicyclohexylurea was filtered off. The filtrate was added to a solution of 2 g of **4** and 2.0 g of triethylamine in 30 ml of CH_2Cl_2, 2.0 ml of DMF and 0.3 ml of H_2O. The mixture was stirred at room temperature overnight. CH_2Cl_2 was evaporated and the residues were taken up in 70 ml of H_2O, acidified to pH 4.5 and extracted 4 times with ethyl acetate. The aqueous phase was cooled to 5°C and acidified to pH 2.0. The precipitate was filtered off and dried to yield 1.24 g of **1g** (51%).

7-[α-(*Z*)-Methoximino-α-(2-aminothiazol-4-yl)acetamido]-3-[(4-carboxythiazol-2-yl)thiomethyl]-ceph-3-em-4-carboxylic Acid (**2a**)

0.8 g of (2-mercaptothiazol-4-yl)carboxylic acid and 2.5 g of cefotaxime were dissolved in 50 ml of H_2O by adjusting the pH to 7.2 with satd $NaHCO_3$. The mixture was stirred at 65°C for 3.5 hours. After cooling to room temperature the solution was acidified to pH 2.5. The precipitate was collected by filtration, washed with H_2O and dried to yield 1.3 g of **2a**.

In a similar way, compounds **2b**~**2k** were obtained by treatment of cefotaxime with the appropriate 2-mercaptothiazoles. ^1H NMR data of compounds **2a**~**2k** are given in Table 8.

7-[α-(*Z*)-Methoximino-(2-aminothiazol-4-yl)acetamido]-3-[(5-methoxycarbonylmethyl-4-methylthiazol-2-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**3a**)

9.5 g of cefotaxime sodium salt in 150 ml of H_2O were adjusted with satd $NaHCO_3$ to pH 7.2. 6.5 g of methyl (2-mercapto-4-methylthiazol-5-yl)acetate dissolved in 150 ml of acetone were added. The solution was stirred at 65°C for 12 hours during which time the pH was kept at 6.0 with satd $NaHCO_3$. After cooling the acetone was removed under reduced pressure. The aqueous solution was extracted twice with ethyl acetate, freed from traces ethyl acetate under reduced pressure and acidified at 5°C with 1 N HCl to pH 2.8. The precipitate was collected by filtration and dried to yield 7.2 g of the title compound.

Similarly to **3a** compounds **3b**~**3i** were obtained by treatment of cefotaxime with the appropriate 2-mercaptothiazoles. Spectral data of compounds **3a**~**3i** are given in Table 9.

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