CEPHALOSPORINS V

SYNTHESIS AND *IN VITRO* ACTIVITY OF SOME 7-[2-METHOXYIMINO-(SUBSTITUTED THIO)ALKANOYL]AMINO CEPHALOSPORANIC ACID DERIVATIVES

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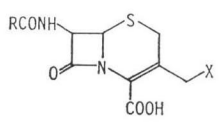
The introduction of the methoxyimino group next to the carbonyl of the 7-position side chain in some alkylthioacetamido and vinylenethioacetamido cephalosporins, and its effect on the *in vitro* antimicrobial activity, are described.

Fresh interest in the field of cephalosporins has been provoked by the discovery that a broader spectrum of antimicrobial activity can be achieved when a methoxyimino group is present next to the amide carbonyl at the 7-position side chain[1].

As part of an effort[2] aimed at providing our 7-substituted alkylthioacetamido cephalosporins[3] and vinylenethioacetamido cephalosporins[4,5] **Ia** with good resistance against β-lactamase producing bacteria, the preparation of some representatives **Ib** containing the methoxyimino group as well as the other elements characteristic of our previous series was undertaken[6].



(I)

a $R=R_1\text{–}A\text{–}S\text{–}CH_2\text{–}$

b $R=R_1\text{–}A\text{–}S\text{–}A_1\text{–}C(=NOCH_3)\text{–}$

R_1=CN, COOH; A=CH_2, CH=CH;
A_1=bond, CH_2, CH=CH, X=OAc, SMTZ

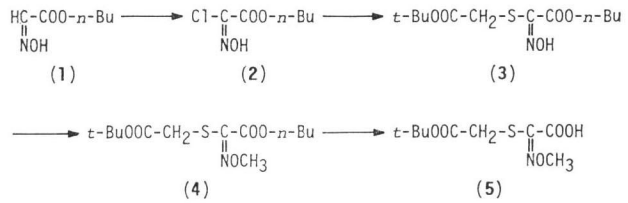
In particular, three series were examined. In the first (A_1=bond), the methoxyimino group replaces the thioacetamido methylene of the parent cephalosporin **Ia**, in the same way as cefuroxime, cefotaxime *etc.* can ideally be derived from the "first generation" arylacetamido cephalosporins. However, owing to the presence of the sulfur atom, in our particular case this structural modification generates a thioester oxime instead of the conventional ketoxime. The latter situation can be restored by leaving the methylene group next to the S atom (A_1=CH_2, second series). In the third series (A_1=vinylene) a situation vinylogous to the first case yet avoiding the thioester oxime functionality was sought.

This paper describes the synthesis of some new cephalosporins **Ib** selected from these three series, and their *in vitro* antimicrobial activity.

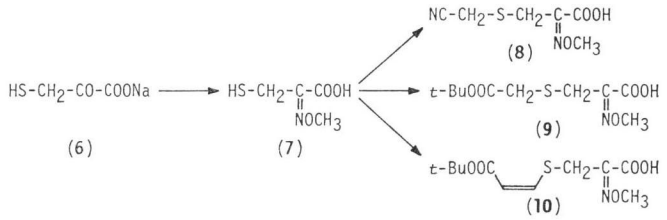
Chemistry

The 7-position side chain acids used for the synthesis of the new cephalosporins have not been reported yet, and we prepared them by different methods, depending on their structure. In the first series (Scheme 1), as preliminary experiments had shown the direct oximation of a thio-oxalic precursor to be

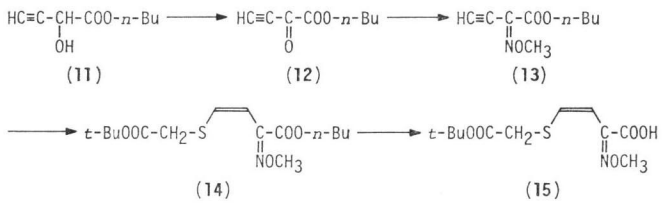
Scheme 1.



Scheme 2.



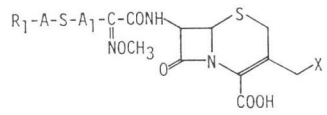
Scheme 3.



impracticable, the hydroxyimidoyl chloride **2** was chosen as the key synthon. Thus, reacting hydroxylamine hydrochloride with *n*-butyl glyoxylate afforded the hydroxyiminoacetate **1**, which was then chlorinated to **2** according to a general method[7]. Displacement of the chlorine atom with *tert*-butyl thioglycolate afforded **3**. Methylation of the hydroxyimino group furnished **4**, which was selectively hydrolysed with K_2CO_3 to give the protected side chain acid **5**. In the second series (Scheme 2), three side chain acids **8**~**10** were selected. 3-Mercapto-2-methoxyimino propionic acid **7**, obtained from its pyruvic precursor **6** by reaction with *O*-methylhydroxylamine, was converted to **8** and **9** upon treatment with chloroacetonitrile or *tert*-butyl chloroacetate, respectively. The (*Z*)-vinylene derivative **10** was obtained by stereoselective addition of **7** to *tert*-butyl propionate. In the third series, compound **15** was selected as a suitable representative, and synthesized from *n*-butyl 2-hydroxy-3-butynoate **11**[8] following the sequence depicted in Scheme 3. Thus, **11** was oxidized with MnO_2 to **12**, which in turn was converted into the methoxyimino derivative **13** by treatment with *O*-methylhydroxylamine hydrochloride. Stereoselective *trans* addition of *tert*-butyl thioglycolate to the triple bond of **13** gave the (*Z*)-alkene **14**, from which **15** was obtained by selective hydrolysis of the *n*-butyl ester.

The cephalosporins **18**~**21** (Table 1) were prepared by first converting the methoxyimino acids **8**~**10** into the corresponding acyl chlorides and then by coupling them with *tert*-butyl-7-aminocephalosporanate or its 3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl derivative (method A). The symmetrical anhydrides, prepared by treatment of the acids **5** and **15** with dicyclohexylcarbodiimide (DCC)[9], gave better results in the synthesis of the cephalosporins **16**, **17**, **22** (Method B). The second carboxyl group present

Table 1.



Compound	R_1	A	A_1	X*	Method**
16	HOOC	CH_2	bond	OAc	B
17	HOOC	CH_2	bond	SMTZ	B
18	NC	CH_2	CH_2	OAc	A
19	HOOC	CH_2	CH_2	OAc	A
20	HOOC	CH_2	CH_2	SMTZ	A
21	HOOC	(*Z*) CH=CH	CH_2	OAc	A
22	HOOC	CH_2	(*Z*) CH=CH	SMTZ	B

* MTZ=1-methyl-1*H*-tetrazol-5-yl.
** A =coupling *via* the acyl chloride.
B =coupling *via* the symmetrical anhydride.

in all the acylating acids except **8** was eventually unmasked in the concomitant hydrolysis of the nuclear and side chain *tert*-butyl esters. Throughout the syntheses care was taken to follow the oximation conditions previously known in the arylglyoxylic acid series to give preferentially the *syn*-CO isomer. Thus, reactions with hydroxylamine or *O*-methylhydroxylamine were carried out in a hydroalcoholic medium, at low temperature and pH value between 4 and 5[1]. Conversion of the acids into the chlorides or symmetrical anhydrides and subsequent coupling were performed under non-isomerizing conditions. In every case a single isomer of the acylating acid and of the final cephalosporin was obtained, as judged by NMR and TLC.

The cephalosporins synthesized are listed in Table 1. Their purity, established by NMR, TLC and analyses, was greater than 90%.

Antimicrobial Activity

The minimum inhibitory concentrations (MIC) of this series of cephalosporins against strains of Gram-positive and Gram-negative bacteria were determined by the standard two-fold serial dilution method starting from 128 μg/ml and using diagnostic sensitivity test agar (Oxoid). The plates were inoculated with about 2×10^5 colony forming units using an automatic inoculator (Denley Tech. Ltd.). The results are the geometric average of two determinations and are compared with cefazolin (CEZ), and with K 11394[3] and FCE 20054[10], two cephalosporins previously synthesized in our laboratories (Table 2).

Activity was disappointingly poor against both Gram-positive and Gram-negative bacteria. In general, the cephalosporins substituted with 1-methyltetrazole thiol performed noticeably better than their 3-acetoxymethyl analogs (**17** *versus* **16** and **20** *versus* **19**). Contrary to our expectations, the new cephalosporins did not compare favourably with their analogs possessing a methylene instead of the methoxyimino group (**17** *versus* K 11394 and **18** *versus* FCE 20054). Of the three series, only the first gave a product with significant antibacterial activity (compound **17**), though it still fell short of our targets of potency and resistance to β-lactamase producing strains.

Table 2. *In vitro* antibacterial activities of the new cephalosporins.

Compound	MIC (μg/ml)*1								
	S.a.	S.p.	E.c.	E.c.t.	K.p.	E.cl.	P.m.	K.ae.	P.v.
16	64	2	128	>128	32	64	16	>128	>128
17	32	0.25	5.7	8	1	4	0.7	>128	>128
18	11.3	2.8	>128	>128	>128	>128	>128	>128	>128
19	>128	22.6	>128	>128	>128	>128	>128	>128	>128
20	32	8	>128	>128	5.6	90	45	>128	>128
21	16	2.83	>128	>128	64	>128	64	>128	>128
22	32	2	128	128	4	64	2	>128	90
CEZ	0.12	0.03	2	8	1	2	4	>128	>128
K 11394*2	2	1.4	1.4	—	0.7	—	—	>128	>128
FCE 20054*3	0.25	0.25	90	128	8	32	8	>128	>128

*1 Organisms selected for inclusion in this table are: S.a., *Staphylococcus aureus* Smith (penicillin G sensitive); S.p., *Streptococcus pyogenes* C 203; E.c., *Escherichia coli* G; E.c.t., *Escherichia coli* TEM; K.p., *Klebsiella pneumoniae* ATCC 10031; E.cl., *Enterobacter cloacae* 1321 E; P.m., *Proteus mirabilis* ATCC 9921; K.ae., *Klebsiella aerogenes* 1082 E; P.v., *Proteus vulgaris* X20.

*2 K 11394 = HOOC-CH_2-S-CH_2-CONH

*3 FCE 20054 = NC-CH_2-S-CH_2-CH_2-CONH

Experimental

Infrared spectra were recorded on a Perkin-Elmer spectrometer (model 125). The NMR spectra were determined on either a Perkin-Elmer R-24 B (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.

n-Butyl Hydroxyiminoacetate (**1**)

Hydroxylamine hydrochloride (3.5 g, 50 mmole) was dissolved in water (25 ml) and the pH of the solution was brought to 5~5.5 by adding solid $NaHCO_3$ (3.8 g). To this solution, cooled to 0~5°C, *n*-butyl glyoxylate (6.5 g, 50 mmole) dissolved in 95% ethanol (25 ml) was added under stirring. The reaction mixture was kept stirring for 3 hours in the cold and afterwards for 1 hour at room temperature. The ethanol was evaporated under vacuum; the aqueous phase was extracted with ethyl ether (3×80 ml). The combined organic extracts were washed with 50 ml of saturated aqueous NaCl solution, dried (Na_2SO_4) and evaporated to dryness *in vacuo*, thus obtaining 7.0 g (96.5%) of *n*-butyl hydroxyiminoacetate (**1**)[7]; IR (KBr) 1740, 1600, 950 cm^{-1}; NMR (acetone-d_6) δ 0.5~1.5 (7H, m, $-CH_2CH_2CH_3$), 4.05 (2H, t, OCH_2), 7.35 (1H, s, H–C=N).

Anal. Calcd. for $C_6H_{11}NO_3$: C 49.64, H 7.64, N 9.65.
Found: C 50.00, H 7.72, N 9.57.

n-Butyl 2-Chloro-2-hydroxyiminoacetate (**2**)

A stream of dry chlorine was bubbled through a solution of **1** (7 g, 48.2 mmole) in anhydrous ethyl ether (100 ml), cooled to −60°C, until about 7 g of chlorine was absorbed. Afterwards the temperature of the solution was allowed to rise to +20°C and the reaction mixture was stirred for 30 minutes at this temperature. The ethyl ether was evaporated *in vacuo* to give 8.55 g (98.7%) of *n*-butyl 2-chloro-2-

hydroxyiminoacetate (**2**) as a syrup; NMR (acetone-d_6) δ 0.5~1.5 (7H, m, $-CH_2CH_2CH_3$), 4.05 (2H, t, OCH_2).

Anal. Calcd. for $C_6H_{10}ClNO_3$: C 40.11, H 5.61, N 7.79, Cl 19.73.
Found: C 40.23, H 5.70, N 7.82, Cl 19.52.

n-Butyl 2-*tert*-Butoxycarbonylmethylthio-2-hydroxyiminoacetate (**3**)

To an ice-cold solution of **2** (6.3 g, 35 mmole) and *tert*-butyl thioglycolate (5.18 g, 35 mmole) in anhydrous THF (50 ml), a solution of triethylamine (4.9 ml) in anhydrous THF (10 ml) was added dropwise under stirring. The reaction mixture was stirred overnight at room temperature and then evaporated to dryness *in vacuo*. The oily residue was taken up with 50 ml of ethyl ether and 50 ml of water. The organic phase was separated, the aqueous phase was extracted with ethyl ether (2×50 ml) and the combined organic extracts were washed with saturated aqueous NaCl solution. Drying over Na_2SO_4 and evaporation to dryness *in vacuo* afforded 8.68 g (85%) of the title product as a syrup; NMR (CCl_4) δ 0.5~1.5 (7H, m, $-CH_2CH_2CH_3$), 1.3 (9H, s, *t*-Bu), 3.6 (2H, s, CH_2S), 4.05 (2H, t, OCH_2), 10.5 (1H, s, =NOH).

Anal. Calcd. for $C_{12}H_{21}NO_5S$: C 49.47, H 7.26, N 4.80, S 11.00.
Found: C 49.23, H 7.16, N 4.71, S 11.20.

n-Butyl 2-*tert*-Butoxycarbonylmethylthio-2-methoxyiminoacetate (**4**)

Anhydrous K_2CO_3 (1.09 g, 7.9 mmole) and a solution of dimethylsulfate (0.75 ml, 7.9 mmole) in anhydrous THF (5 ml) were sequentially added to an ice-cold solution of **3** (2.3 g, 7.9 mmole) in the same solvent (35 ml). The mixture was stirred overnight at room temperature; the undissolved matter was filtered off, the filtrate was evaporated to dryness *in vacuo*, the residue was taken up with 50 ml of water and 100 ml of ethyl ether. The organic layer was separated and the aqueous phase was extracted with ethyl ether (2×100 ml). The combined extracts were washed with saturated aqueous NaCl solution (2× 50 ml), dried (Na_2SO_4) and evaporated to dryness *in vacuo* to obtain an oily residue, which was chromatographed over a silica gel column eluting with CH_2Cl_2 - EtOAc, 7: 1.

The title product (1.5 g, 62%) was thus obtained as a syrup; NMR (CCl_4) δ 0.8~1.8 (7H, m, $-CH_2-CH_2CH_3$), 1.3 (9H, s, *t*-Bu), 3.6 (2H, s, CH_2S), 4.0 (3H, s, OCH_3), 4.05 (2H, t, OCH_2).

Anal. Calcd. for $C_{13}H_{23}NO_5S$: C 51.12, H 7.59, N 4.58, S 10.49.
Found: C 50.82, H 7.41, N 4.38, S 10.83.

2-*tert*-Butoxycarbonylmethylthio-2-methoxyiminoacetic Acid (**5**)

To a solution of **4** (1.3 g, 4.25 mmole) in 50% EtOH (50 ml), K_2CO_3 (4.25 g) was added and the mixture was stirred at 65°C for 1 hour, after which time TLC indicated complete reaction. The ethanol was removed *in vacuo*, the residue was taken up in ice-cold water, acidified with 1 N HCl (8.5 ml) and immediately extracted with ethyl ether (4×20 ml). Evaporation of the solvent gave impure **5** as a syrup in about 85% yield; IR (Nujol) 1730, 1710, 1600, 1380 and 1365 cm^{-1}. This product, stable in alkaline solution, underwent rapid degradation as a free acid, and was immediately used for coupling with the 7-aminocephem (DCC method).

3-Mercapto-2-methoxyiminopropionic Acid (**7**)

A solution of *O*-methylhydroxylamine hydrochloride (1.71 g, 20.5 mmole) in water (20 ml) was dropped within 30 minutes into a cold aqueous solution of sodium β-mercaptopyruvate bihydrate (**6**)[11] (3.56 g, 20 mmole), while adding $NaHCO_3$ (1.7 g) portionwise so as to maintain the pH between 4 and 5. The mixture was then stirred for 3 hours at room temperature, allowed to stand overnight, acidified with 2 N aqueous HCl (10 ml) and extracted with ethyl acetate (3×50 ml). The combined organic extracts were washed with 25% aqueous NaCl solution (2×25 ml), dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The resulting residue was crystallized from cyclohexane (100 ml), to give 2.4 g (82.5%) of **7**; mp 83~84°C; IR (KBr) 1700, 1595, 1050 cm^{-1}; NMR (acetone-d_6) δ 2.3 (1H, t, SH), 3.44 (2H, d, SCH_2), 4.05 (3H, s, OCH_3), 8.85 (br-s, COOH).

Anal. Calcd. for $C_4H_7NO_3S$: C 32.20, H 4.73, N 9.39, S 21.50.
Found: C 32.30, H 4.73, N 9.29, S 21.46.

3-Cyanomethylthio-2-methoxyiminopropionic Acid (**8**)

85% KOH (12 g, 180 mmole) in ethanol (250 ml) and chloroacetonitrile (7.1 g, 93 mmole) in ethanol

(50 ml) were sequentially added to a cold solution of **7** (13.5 g, 90 mmole) in the same solvent (50 ml). The reaction mixture was stirred for 18 hours at room temperature; the solid was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in water (100 ml) and the aqueous solution was washed with ethyl ether (50 ml), made acidic with 20% HCl and then extracted with ethyl ether (3 × 100 ml).

The combined extracts were washed with an aqueous NaCl solution (2 × 50 ml), dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residue was crystallized from benzene (150 ml), to give 12 g (71%) of **8**; mp 63~65°C; IR (KBr) 2250, 1710, 1600, 1050 cm^{-1}; NMR (acetone-d_6) δ 3.60 and 3.74 (each 2H, s, SCH_2), 4.07 (3H, s, OCH_3), 8.0 (1H, br-s, COOH).

Anal. Calcd. for $C_6H_8N_2O_3S$: C 38.29, H 4.28, N 14.89, S 17.03.
Found: C 37.95, H 4.26, N 14.64, S 17.12.

3-*tert*-Butoxycarbonylmethylthio-2-methoxyiminopropionic Acid (**9**)

A solution of 3-mercapto-2-methoxyiminopropionic acid (4.47 g, 30 mmole) in ethanol (50 ml) was sequentially treated under stirring at 5~10°C with 1 N ethanolic potassium hydroxide (60 ml) and a solution of *tert*-butyl chloroacetate (4.65 g, 31 mmole) in ethanol (20 ml). The mixture was stirred for 18 hours at room temperature, then evaporated to dryness *in vacuo*. The residue was dissolved in water (50 ml) and the aqueous solution was extracted with ethyl ether (2 × 50 ml). The ethereal layer was discarded, the aqueous solution was acidified with 3 N aqueous HCl (10 ml) and then extracted with ethyl acetate (3 × 100 ml). The organic extracts were washed with water (2 × 50 ml), dried over Na_2SO_4 and evaporated to dryness *in vacuo*, yielding a semi-solid mass, which recrystallized from cyclohexane (100 ml) to give 6.71 g (85%) of **9**; mp 55~57°C; IR (KBr) 1730, 1600, 1375, 1170, 1050 cm^{-1}; NMR (acetone-d_6) δ 1.45 (9H, s, *t*-Bu), 3.2 and 3.6 (each 2H, s, SCH_2), 3.95 (3H, s, OCH_3), 8.78 (1H, br-s, COOH).

Anal. Calcd. for $C_{10}H_{17}NO_5S$: C 45.61, H 6.51, N 5.32, S 12.17.
Found: C 45.55, H 6.49, N 5.32, S 12.39.

3-*tert*-Butoxycarbonyl-(*Z*)-vinylenethio-2-methoxyiminopropionic Acid (**10**)

A solution of *tert*-butylpropiolate (3.78 g, 30 mmole) in ethanol (15 ml) was added with external cooling to a stirred solution of 3-mercapto-2-methoxyiminopropionic acid (4.47 g, 30 mmole) in a mixture of 95% EtOH (15 ml) and 1 N aqueous KOH (30 ml). After 20 hours at 20°C the ethanol was evaporated *in vacuo* and the aqueous solution acidified with 3 N HCl (10 ml) and extracted with ethyl ether (3 × 100 ml). The ethereal extracts were dried over Na_2SO_4 and evaporated *in vacuo* to give 7.51 g (91%) of the crude product **10** as a mixture (85: 15) of *Z* and *E* isomers; mp 115~120°C. Recrystallization from di-*iso*propyl ether afforded the pure *Z* isomer (4.5 g, 55%); mp 127~129°C, IR (KBr) 1710, 1600, 1375, 1050 cm^{-1}; NMR (acetone-d_6) δ 1.43 (9H, s, *t*-Bu), 3.75 (2H, s, SCH_2), 4.02 (3H, s, OCH_3), 5.65 (1H, d, J=10 Hz, CH=CH–S), 7.27 (1H, d, J=10 Hz, CH=CH–S), 9.3 (1H, br-s, COOH).

Anal. Calcd. for $C_{11}H_{17}NO_5S$: C 47.98, H 6.22, N 5.09, S 11.64.
Found: C 48.20, H 6.26, N 5.04, S 11.79.

n-Butyl 2-Oxo-3-butynoate (**12**)

Manganese dioxide (25 g) was added in several portions within 15 minutes to a stirred solution of *n*-butyl-2-hydroxy-3-butynoate (**11** 4.7 g, 30 mmole) in chloroform (200 ml). After stirring for additional 5 minutes, the suspension was filtered and the filtrate evaporated *in vacuo* without external heating to give the crude product **12** as an oily residue (3.01 g, 65%), which was used without further purification; IR (neat) 3270, 2090, 1740, 1690 cm^{-1}; NMR (CCl_4) δ 1.0~2.0 (7H, m, $-CH_2CH_2CH_3$), 3.8 (1H, s, HC≡C), 4.1 (2H, t, OCH_2).

n-Butyl 2-Methoxyimino-3-butynoate (**13**)

A solution of *O*-methylhydroxylamine hydrochloride (2.0 g, 25 mmole) in water (20 ml) was brought to pH 5.0 by adding the required amount of $NaHCO_3$ and then slowly added to a solution of the oxobutynoate **12** (3.01 g, 19.5 mmole) in 95% ethanol (20 ml).

After stirring overnight at room temperature, 100 ml of water were added and the mixture was extracted three times with ethyl acetate. The organic layers were dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The oily residue was chromatographed (silica gel, petroleum ether - ethyl ether, 2: 1) to

give the title product **13** (2.14 g, 60%); IR (neat) 3260, 2820, 2105, 1730, 1055 cm^{-1}; NMR (CCl_4) δ 0.8~2.00 (7H, m, $-CH_2CH_2CH_3$), 3.6 (1H, s, HC≡C), 4.0 (3H, s, OCH_3), 4.2 (2H, s, OCH_2).

Anal. Calcd. for $C_9H_{13}NO_3$: C 59.00, H 7.15, N 7.65.
Found: C 58.78, H 7.20, N 7.55.

n-Butyl 4-*tert*-Butoxycarbonylmethylthio-2-methoxyimino-3(*Z*)-butenoate (**14**)

tert-Butyl thioglycolate (0.8 g, 5.4 mmole) dissolved in ethanol (2 ml) was added to a stirred solution of *n*-butyl 2-methoxyimino-3-butynoate (1 g, 5.5 mmole) in ethanol (15 ml). After stirring for 3 hours at room temperature, the reaction mixture was diluted with water (100 ml) and extracted with ethyl ether (3×50 ml). The combined extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residue was chromatographed (SiO_2, petroleum ether - ethyl ether, 9: 1) to give 1.55 g (87%) of the title product (**14**) as a colorless oil; IR (neat) 1730, 1720 and 1060 cm^{-1}; NMR (CCl_4) δ 1.0~2.0 (7H, m, OCH_2–$CH_2CH_2CH_3$), 1.40 (9H, s, *t*-Bu), 3.1 (2H, s, CH_2S), 3.9 (3H, s, OCH_3), 4.05 (2H, t, O–CH_2–$CH_2CH_2CH_3$), 6.05 (1H, d, J=10 Hz, CH=CH–S), 6.45 (1H, d, J=10 Hz, CH=CH–S).

4-*tert*-Butoxycarbonylmethylthio-2-methoxyimino-3(*Z*)-butenoic Acid (**15**)

Potassium carbonate (0.2 g, 1.45 mmole) was added to a solution of the ester **14** (0.414 g, 1.25 mmole) in 50% EtOH (8 ml). The mixture was stirred for 90 minutes at 65°C. After cooling to room temperature, the reaction mixture was diluted with 50 ml of water and then extracted three times with ethyl ether. The organic layers were discarded; the aqueous layer was acidified with 1 N HCl to pH 2.0 under cooling. The product was extracted with ethyl ether (3×50 ml), dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give 0.264 g (76.9%) of the title product **15**, which was used without further purification; IR (neat) 1730, 1710, 1050 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (9H, s, *t*-Bu), 3.2 (2H, s, CH_2S), 4.00 (3H, s, OCH_3), 6.15 (1H, d, J=10 Hz, CH=CH–S), 6.65 (1H, d, J=10 Hz, CH=CH–S), 7.5~8.5 (1H, br-s, COOH).

Method A

7β-(3-Cyanomethylthio-2-methoxyimino)propionamido-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**18**)

Triethylamine (1.4 ml) was added to a stirred solution of 3-cyanomethylthio-2-methoxyiminopropionic acid (**8**) (1.88 g, 10 mmole) in anhydrous CH_2Cl_2 (25 ml). The mixture was cooled to 0°C and PCl_5 (2.08 g, 10 mmole) was added in a single portion. The solution was stirred for 1 hour at room temperature, the solvent and the $POCl_3$ formed were removed *in vacuo* at 25°C. The crude 3-cyanomethylthio-2-methoxyiminopropionyl chloride thus obtained was immediately taken up in cold, anhydrous dichloromethane and used for the acylation step.

The solution of the above acyl chloride in CH_2Cl_2 was dropped into a cold (0°C) solution of 7-aminocephalosporanic acid *tert*-butyl ester (3.28 g, 10 mmole) and *N*,*N*-diethylaniline (1.6 ml, 10 mmole) in the same solvent (25 ml). After stirring for 30 minutes at 0°C and 15 minutes at room temperature, the mixture was sequentially washed with 4% HCl, H_2O, 5% aqueous $NaHCO_3$ solution and again with water. The organic phase was dried ($CaCl_2$) and evaporated to dryness to yield 4.5 g of a slimy oil which was purified by silica gel chromatography (EtOAc - C_6H_{12}, 1: 1) to give 3.2 g (64%) of chromatographically homogeneous 7β-(3-cyanomethylthio-2-methoxyimino)propionamido-3-acetoxymethyl-3-cephem-4-carboxylic acid *tert*-butyl ester; NMR ($CDCl_3$) δ 1.54 (9H, s, *t*-Bu), 2.07 (3H, s, $-OCOCH_3$), 3.25 (2H, s, SCH_2), 3.48 (2H, q, 2-CH_2), 3.72 (2H, s, NC-CH_2), 4.05 (3H, s, OCH_3), 4.93 (2H, dd, 3-CH_2), 5.03 (1H, d, 6-H), 5.83 (1H, d-d, 7-H), 7.39 (1H, d, –CONH).

Trifluoroacetic acid (20 ml) was added to the solution of the above ester (1 g, 2 mmole) in CH_2Cl_2 (2 ml) and anisole (5 ml). The mixture was stirred in a stoppered flask for 2 hours at room temperature, then evaporated *in vacuo*; the residue was taken up twice with benzene and the solvent removed to give a foam which was kept under vacuum for 2 hours (0.01 mmHg). This crude material was partitioned between ethyl acetate and an aqueous $NaHCO_3$ solution. The aqueous phase was washed twice with ethyl ether, then stratified with ethyl ether and brought to about pH 2 with 8% HCl. The organic layer was separated and the aqueous phase was extracted again with ethyl ether. The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated to dryness to give 0.75 g (84%) of **18**; mp 65°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160:

20: 20), Rf 0.52; IR (KBr) 2230, 1780, 1730, 1520, 1050 cm^{-1}; NMR (acetone-d_6) δ 2.01 (3H, s, $-OCO-CH_3$), 3.65 (4H, s+ABq, SCH_2 and 2-CH_2), 3.70 (2H, s, $NC-CH_2$), 4.01 (3H, s, OCH_3), 4.91 (2H, ABq, 3-CH_2), 5.14 (1H, d, 6-H), 5.81 (1H, dd, 7-H), 7.95 (1H, d, –CONH).

Anal. Calcd. for $C_{16}H_{18}N_4O_7S_2$: C 43.43, H 4.10, N 12.60, S 14.49.
Found: C 43.32, H 4.15, N 12.41, S 14.32

By a similar procedure **19**, **20** and **21** were prepared, and the data for each compound are as follows:

7β-(3-Carboxymethylthio-2-methoxyimino) propionamido-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**19**)

Mp 90°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 30: 20), Rf 0.57; IR (KBr) 1785, 1730, 1630, 1525, 1050 cm^{-1}; NMR (DMSO-d_6) δ 2.04 (3H, s, $-OCO-CH_3$), 3.32 (2H, s, $S-CH_2CO$), 3.6 (4H, s+ABq, SCH_2 and 2-CH_2), 4.0 (3H, s, OCH_3), 4.81 (2H, ABq, 3-CH_2), 5.18 (1H, d, 6-H), 5.73 (1H, dd, 7-H), 8.79 (1H, d, –CONH).

Anal. Calcd. for $C_{16}H_{19}N_3O_9S_2$: C 41.64, H 4.15, N 9.10, S 13.89.
Found: C 41.33, H 4.22, N 8.91, S 13.22.

7β-(3-Carboxymethylthio-2-methoxyimino) propionamido-3-[(1-methyl-1*H*-tetrazol-5-yl) thiomethyl]-3-cephem-4-carboxylic Acid (**20**)

Mp 70°C (dec.); IR (KBr) 1780, 1730, 1630, 1050 cm^{-1}; NMR (DMSO-d_6) δ 3.32 (2H, s, $S-CH_2CO$), 3.6 (4H, s+ABq, SCH_2 and 2-CH_2), 3.95 (3H, s, $N-CH_3$), 4.05 (3H, s, OCH_3), 4.31 (2H, ABq, 3-CH_2), 5.18 (1H, d, 6-H), 5.73 (1H, dd, 7-H), 8.80 (1H, d, –CONH).

Anal. Calcd. for $C_{16}H_{19}N_7O_7S_3$: C 37.12, H 3.70, N 18.94, S 18.58.
Found: C 36.91, H 3.83, N 18.71, S 18.07.

7β-[3-Carboxy-(*Z*)-vinylenethio-2-methoxyimino] propionamido-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**21**)

Mp 160°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 20: 20), Rf 0.42; IR (KBr) 1780, 1730, 1630, 1520, 1050 cm^{-1}; NMR (DMSO-d_6) δ 2.05 (3H, s, OAc), 3.55 (2H, ABq, 2-CH_2), 3.72 (2H, s, SCH_2), 4.01 (3H, s, OCH_3), 4.82 (2H, ABq, 3-CH_2), 5.11 (1H, d, 6-H), 5.67 (1H, dd, 7-H), 5.75 (1H, d, J=10 Hz, CH=C*H*–S), 7.31 (1H, dd, J=10 Hz, C*H*=CH–S), 8.76 (1H, br-d, CONH).

Anal. Calcd. for $C_{17}H_{19}N_3O_9S_2$: C 43.12, H 4.04, N 8.87, S 13.54.
Found: C 43.61, H 4.12, N 8.62, S 13.27.

Method B

7β-(2-Carboxymethylthio-2-methoxyimino) acetamido-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**16**)

A solution of *N,N'*-dicyclohexylcarbodiimide (0.44 g, 2.1 mmole) in anhydrous CH_2Cl_2 (5 ml) was added dropwise to an ice-cold solution of freshly prepared 2-*tert*-butoxycarbonylmethylthio-2-methoxyiminoacetic acid (**5**) (1 g, 4 mmole) in the same solvent (25 ml). The mixture was stirred for 30 minutes at 0°C and for 30 minutes at room temperature, then cooled to −20°C and treated with a solution of 7-aminocephalosporanic acid *tert*-butyl ester (0.66 g, 2 mmole) in CH_2Cl_2 (25 ml). After stirring for 30 minutes at −20°C and overnight at room temperature, the reaction mixture was filtered, the filtrate was concentrated *in vacuo* and chromatographed on silica gel (cyclohexane - ethyl acetate, 1: 1) to afford the double *tert*-butyl ester of the title product as a yellowish foam (0.86 g, 77%).

A solution of the above ester in trifluoroacetic acid (TFA, 15 ml) was stirred for 2 hours at 0°C, after which time hydrolysis of the two *tert*-butyl carboxylates was virtually complete (TLC).

The mixture was then evaporated to dryness *in vacuo*, taken up with benzene and the solvent removed. This process was repeated several time in order to free the crude product from any trace of TFA, and the residue was partitioned between ethyl acetate and 5% aqueous $NaHCO_3$ solution. The organic layer was discarded; the aqueous phase was made acidic with 2 N HCl and extracted with fresh ethyl acetate. After washing with water and drying over anhydrous Na_2SO_4, the extracts were concentrated *in vacuo*. Trituration from a large amount of ethyl ether gave the title compound **16** as a powder (0.56 g, 82%). TLC on silica gel gave a single spot with chloroform - methanol - formic acid (180: 20: 10), Rf 0.40; IR (KBr) 1775, 1725, 1630, 1510, 1020 cm^{-1}; NMR (acetone-d_6) δ 2.05 (3H, s,

OAc), 3.53 (2H, ABq, 2-CH_2), 3.9 (5H, s, SCH_2 and OCH_3), 4.88 (2H, ABq, 3-CH_2), 5.07 (1H, d, 6-H), 5.76 (1H, dd, 7-H), 8.05 (1H, br-d, CONH).

Anal. Calcd. for $C_{15}H_{17}N_3O_9S_2$: C 40.26, H 3.82, N 9.39, S 14.33.
Found: C 40.61, H 4.17, N 9.01, S 13.97.

By a similar procedure **17** and **22** were prepared, and the data for each compound are as follows:

7β-(2-Carboxymethylthio-2-methoxyimino)acetamido-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (**17**)

TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20), Rf 0.67; IR (KBr) 1770, 1720, 1670 cm^{-1}; NMR (DMSO-d_6) δ 3.65 (2H, ABq, 2-CH_2), 3.95 (3H, s, N–CH_3), 4.05 (5H, s, S–CH_2 and OCH_3), 4.25 (2H, ABq, 3-CH_2), 5.0 (1H, d, 6-H), 5.8 (1H, dd, 7-H), 9.5 (1H, d, CONH).

Anal. Calcd. for $C_{15}H_{17}N_7O_7S_3$: C 35.77, H 3.40, N 19.47, S 19.10.
Found: C 35.91, H 3.65, N 19.11, S 18.81.

7β-(4-Carboxymethylthio-2-methoxyimino)but-3(*Z*)-enamido-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (**22**)

TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 30: 20), Rf 0.46; IR (KBr) 1780, 1730~1710, 1680, 1050 cm^{-1}; NMR (DMSO-d_6) δ 3.26 (2H, s, S–CH_2–CO), 3.6 (2H, ABq, 2-CH_2), 3.8 (3H, s, N–CH_3), 3.95 (3H, s, OCH_3), 4.30 (2H, ABq, 3-CH_2), 4.95 (1H, d, 6-H), 5.75 (1H, dd, 7-H), 6.1 (1H, d, J=10 Hz, CH=C*H*–C), 6.65 (1H, d, J=10 Hz, S–C*H*=CH), 7.25 (1H, d, CONH).

Anal. Calcd. for $C_{17}H_{19}N_7O_7S_3$: C 38.55, H 3.61, N 18.51, S 18.16.
Found: C 38.27, H 3.41, N 18.33, S 17.81.

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