THE SYNTHESIS AND BIOLOGICAL EVALUATION OF 7β-[2-(5-AMINO-[1,2,4]OXADIAZOL-3-YL)-2-*Z*-METHOXIMINOACETAMIDO]-CEPHALOSPORIN DERIVATIVES

WILLIAM J. WHEELER*, JACK B. DEETER, DON R. FINLEY, MICHAEL D. KINNICK, RICHARD KOEHLER, HAROLD E. OBSORNE, JOHN T. OTT, JOHN K. SWARTZENDRUBER and DONN G. WISHKA

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, U.S.A.

(Received for publication August 31, 1985)

A series of 7β-[2-(5-aminooxadiazol-3-yl)-2-*Z*-methoximinoacetamido]-3-cephem-4-carboxylic acids (**7a**~**g**) were synthesized and evaluated microbiologically. Although somewhat less active than cefotaxime **7a**~**g** showed good antimicrobial activity against a wide variety of Gram-positive and Gram-negative bacteria. The β-lactamase stability of **7a** and **7f** was also discussed.

Reports concerning the microbiological evaluation of novel aminoheterocyclic methoxime cephalosporin derivatives[1] have prompted us to describe the synthesis and *in vitro* biological evaluation of a similar series of aminooxadiazole methoxime cephalosporins (**1**).

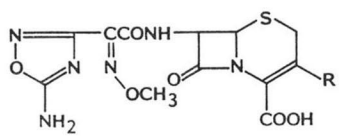
Chemistry

TIEMANN first described the synthesis of alkylated-[1,2,4]oxadiazoles in 1885 by reaction of the appropriately substituted amide oxime with acylhalides or anhydrides[2], presumably *via* the intermediacy of the *O*-acylated amide oxime[3]. More recently, BREUER reported that 5-amino[1,2,4]-oxadiazoles could be prepared by reaction of the corresponding 5-trichloromethyl-substituted-[1,2,4]-oxadiazole with ammonia at −33°C[4].

The requisite aminooxadiazole methoximinoacetic acid (**6**) was prepared from the appropriately substituted amide oxime **3** as outlined in Scheme 1. Ethyl 2-methoximinocyanoacetate (**2a**) was reacted with hydroxylamine to produce **3a** as a single isomer of unknown geometry. Very subtle changes in the procedure and/or the purity of the starting nitrile greatly affected the yield of **3a** presumably in part due to the formation of the corresponding hydroxamic acid. Attempts to circumvent the poor yields of **3a** by first converting **2a** to imidate ester **2b** seemed plausible; however reactions of **2a** with either anhydrous HCl - EtOH[5] or $NaOCH_3$ - EtOH[6] failed to produce **2b**.

Acylation of **3a** with trichloroacetyl chloride and subsequent ring closure yielded 5-trichloro-[1,2,4]oxadiazole **4** as a mixture of isomers. Reaction of **3a** with *p*-toluenesulfonyl chloride (*p*-TsCl) - 2,6-lutidine yielded **3b**. Reaction of **3b** in the manner described above (trichloroacetyl chloride) also yielded **4** as well as TsCl suggesting conversion back to **3a** prior to reaction with trichloroacetyl chloride.

An ether solution of **4** was added dropwise to liquid ammonia at −33°C and the resulting mixture was stirred overnight allowing the ammonia to evaporate. The residue was triturated with Et_2O and filtered, leaving a substantial amount of insoluble material which was shown by NMR to be the *E*-amino carboxamide **5b**. The filtrate was concentrated and recrystallized from EtOH to yield the *Z*-amino ester **5a**. This material was shown by NMR and TLC to be a single isomer, which when

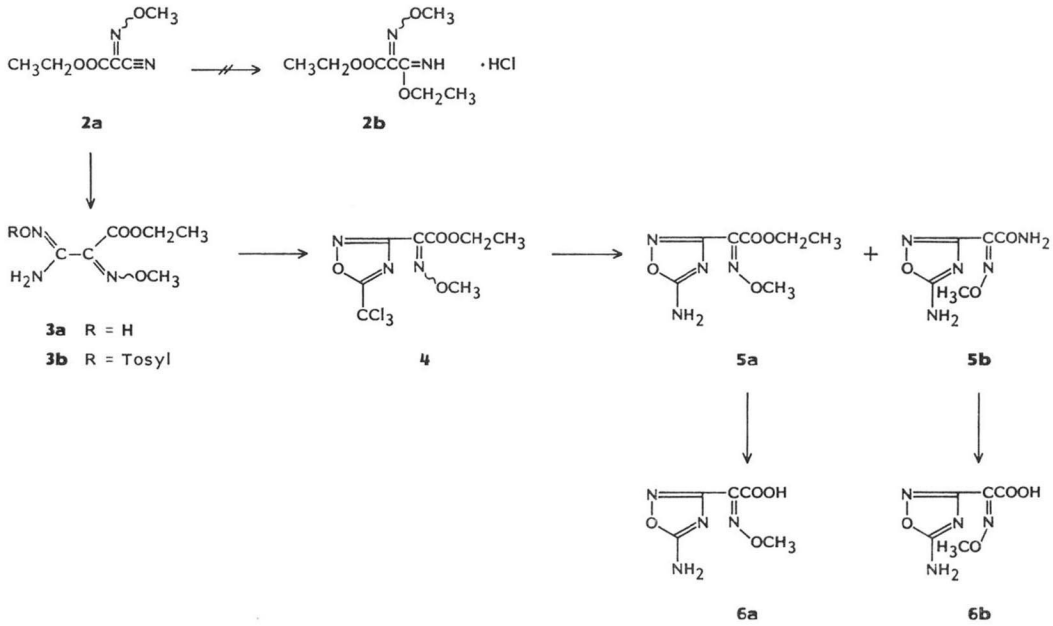


1

Table 1. Crystal data for **5a**.

Formula $C_5H_7N_5O_3$	MW 185.151
a 21.673±0.007Å	Space group $I2_1/C$
b 10.502±0.004Å	D_{calcd} 1.29 g/cm^3
c 8.520±0.002Å	
α 90	
β 100.013±0.02	
Volume 1,909.70±1.11 A^3	

Scheme 1.



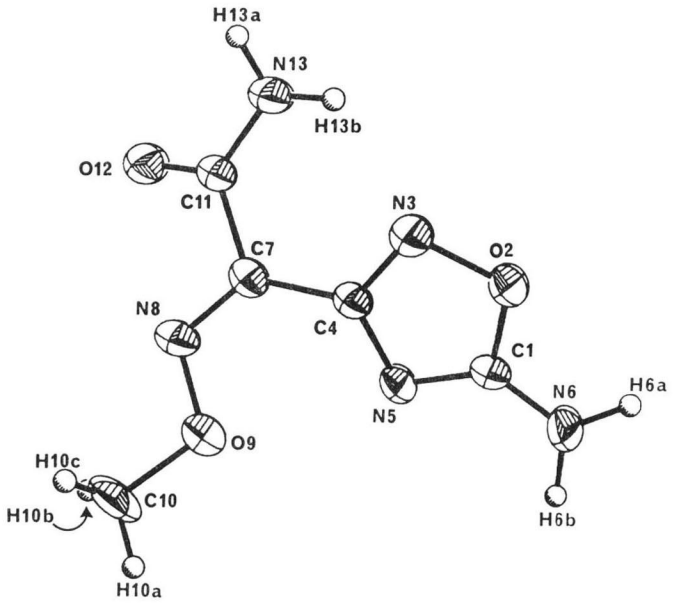
re-dissolved resisted conversion to the corresponding carboxamide, presumably because of the steric interaction between the *Z*-methoximino group and the ester carbonyl. Such differences in relative reactivities between the *Z*- and *E*-isomers of the corresponding aminothiazole methoxime esters have been reported[7]. In this paper the *E*-isomer, when reacted with ethylamine at room temperature for 2 hours afforded *N*-ethyl-2-(2-aminothiazol-4-yl)-2-*E*-methoximinoacetamide; the *Z*-isomer yielded the corresponding *N*-ethylcarboxamide only after reaction with ethylamine at 100°C in a sealed tube for 4 hours.

Single crystal X-ray analysis confirmed the structure of **5b**, firmly establishing the geometry of the methoxime (*E*-isomer). The crystal data is listed in Table 1 and the ORTEP drawing of **5b** is shown in Fig. 1.

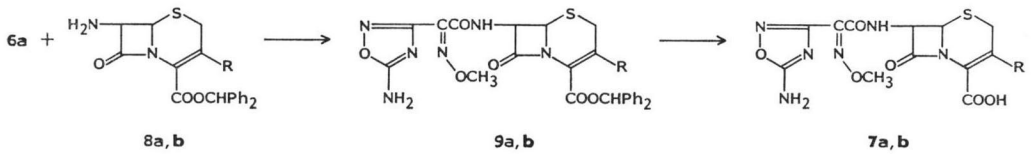
Base hydrolysis of the ester **5a** yielded the desired *Z*-methoxime acid **6a**. The corresponding *E*-methoxime acid **6b** was prepared by hydrolysis of *E*-methoximino carboxamide **5b** with sodium peroxide[8].

The amino group of **6a** was sufficiently unreactive to allow coupling to the various cephem nuclei without protection; in fact, numerous attempts to derivatize the amino group of **6a** with trityl chloride or chloroacetyl chloride were unsuccessful. The synthesis of aminooxadiazole methoxime cephalo-

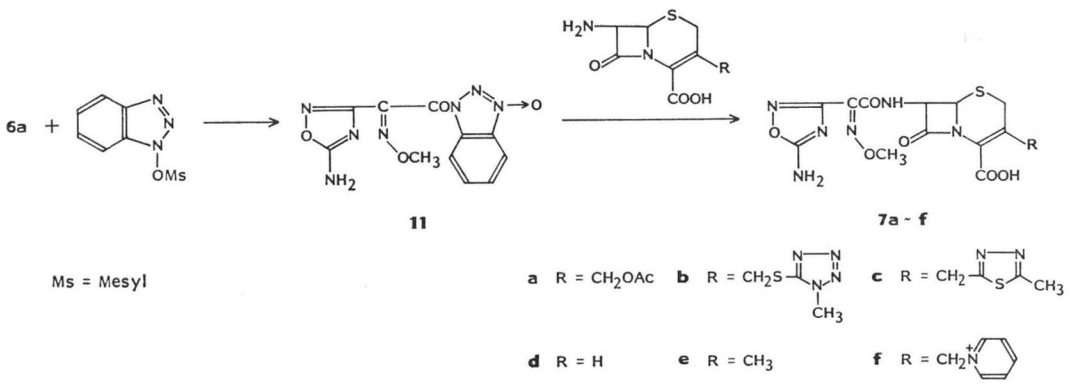
Fig. 1. ORTEP stereo drawing of **5b**.



Scheme 2.



Scheme 3.



sporins **7a**~**g** was accomplished by a variety of procedures as shown in Schemes 2, 3, and 4.

Thus reaction of **6a** with benzhydryl esters **8a,b** (prepared by esterification of the appropriate cephalosporin nucleus with diphenyldiazomethane) in the presence of dicyclohexylcarbodiimide (DCC) yielded the corresponding benzhydryl-protected cephalosporins **9a** and **9b**. Removal of the benzhydryl protecting group by reaction with anhydrous TFA yielded the desired cephalosporins **7a,b** (see Scheme 2).

Table 2. *In vitro* antibacterial activity of aminooxadiazole methoxime cephalosporins (agar dilution, MIC in μg/ml).

		Cefotaxime	7a	7b	7c	7d	7e	7f	7g
Staphylococcus aureus	XI. 1	2	2	2	2	8	32	1	8
〃	V41	4	8	4	4	16	32	2	8
〃	X400	128	16	16	64	128	>128	4	32
〃	S13E	8	8	8	8	64	128	2	16
S. epidermidis	EPI1	8	16	8	3	32	64	2	16
〃	EPI2	32	32	32	2	>128	>128	4	64
Streptococcus pyogenes	C203	0.015	0.015	0.03	0.015	0.06	0.06	0.03	0.03
S. pneumoniae	PARK	0.015	0.015	0.015	0.015	0.125	0.125	0.03	0.03
Enterococcus faecalis	X66	128	>128	>128	>128	>128	>128	>128	64
〃	9960	4	>128	>128	64	128	>128	16	32
Haemophilis influenzae	BRUN	0.03	0.5	0.125	0.5	2	8	2	0.25
〃	251	0.03	0.5	0.125	0.25	0.5	4	2	0.125
Shigella sonnei	N9	0.06	0.5	0.125	0.5	2	8	1	0.5
Escherichia coli	N10	0.125	1	4	1	1	8	1	1
〃	EC14	0.06	0.5	0.125	0.5	0.5	4	0.5	0.25
〃	TEM	0.03	1	0.5	0.5	0.5	8	2	0.5
Klebsiella pneumoniae	X26	<0.008	0.125	0.06	0.125	0.25	4	1	0.125
〃	KAE	2	>128	64	>128	64	128	128	>128
Enterobacter aerogenes	X68	0.06	0.5	0.25	1	0.5	8	2	0.5
〃	EB17	0.125	0.5	0.25	1	1	8	2	1
E. cloacae	EB5	0.25	2	1	8	8	32	8	8
〃	265A	128	>128	32	>128	>128	>128	64	>128
Salmonella heidelberg	X514	0.125	0.5	0.5	2	0.5	8	1	0.25
〃	1335	0.125	2	0.5	4	1	16	2	0.5
Pseudomonas aeruginosa	X528	16	64	64	64	>128	>128	64	64
〃	X239	16	64	64	128	>128	>128	2	64
〃	PS18	32	128	128	>128	>128	>128	4	128
Serratia marcescens	X99	0.5	2	0.25	4	8	64	4	8
〃	SE3	1	16	4	8	16	128	4	32
Morganella morganii	PR15	0.25	—	—	2	128	128	32	16
Providencia stuartii	PR33	0.06	4	0.25	1	0.25	1	1	0.25
P. rettgeri	PR7	<0.008	0.125	0.06	2	0.06	0.5	0.125	<0.008
〃	C24	0.25	8	4	0.5	0.5	4	4	0.125
Citrobacter freundii	CF17	2	—	—	128	64	>128	32	128

Scheme 4.

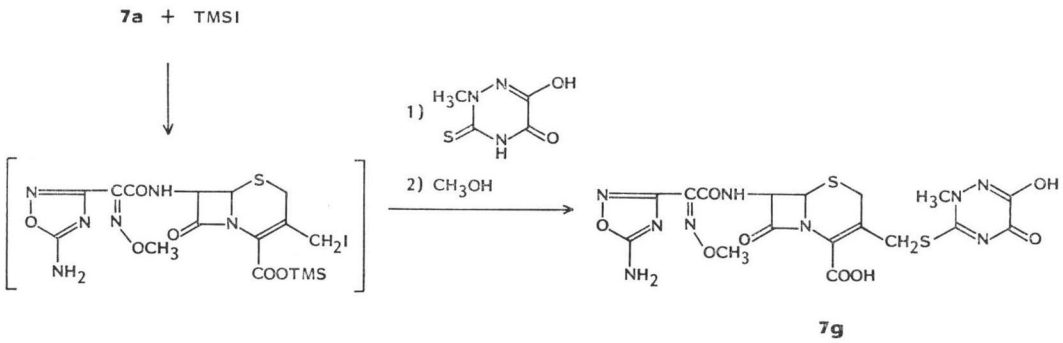


Table 3. β-Lactamase stabilities of **7a**, **7f**, and cefotaxime.

Enzyme source	Relative hydrolysis rates			
	Cephaloridine	**7a**	**7f**	Cefotaxime
Staphylococcus aureus S13E	100	3.65	58.57	10.80
Escherichia coli TEM	100	1.08	2.65	0.18
Klebsiella pneumoniae KAE	100	26.97	15.95	2.50
Enterobacter cloacae 265A	100	0.14	0.32	0.06
Pseudomonas aeruginosa Ps18	100	0.13	0.33	0.09

Acylation of 7-aminocephalosporin nuclei **10a~g** with **11**, hydroxybenzotriazole (HOBT) activated **6a**[9] (Scheme 3) under Schotten-Baumann conditions directly yielded cephem derivatives **7a~g**.

Amino oxadiazole cephem derivative **7g** was prepared from **7a** by the *in situ* generation of the 3-iodomethyl trimethylsilyl derivative **12** (resulting from the reaction of **7a** with trimethylsilyl iodide (TMSI))[10] and subsequent reaction with 6-hydroxy-2-methyl-5-oxo[1,2,4]triazine-6-thione (Scheme 4) followed by methanolysis of the resulting TMS ester.

Antimicrobial Activity

The antimicrobial activity of the 5-amino[1,2,4]oxadiazol-3-yl methoximinocephalosporins **7a~g** were determined against various Gram-positive and Gram-negative strains of aerobic bacteria by an *in vitro* agar dilution technique[11]. Cefotaxime is included as a reference compound (Table 2).

The activity of this series of compounds parallels that of the aminothiazole cephalosporins; however, against *Pseudomonas aeruginosa* with the exception of **7f** this series of compounds is essentially inactive, while cefotaxime has moderate activity against *P. aeruginosa*. Against the more resistant strains of various enterobacteriaceae **7a~f** possess diminished activity relative to that of cefotaxime. Against the more sensitive strains with the notable exception of **7e** (and in some cases **7d**) the activity is excellent (although still usually 1~2 times less active than cefotaxime). The activity of **7a~g** against *Streptococcus pyogenes*, *S. pneumonia*, and *Haemophilus influenzae* is superb; cefotaxime and **7a~g** are inactive against group D streptococci. Against *Staphylococcus aureus* the activity is only moderate although **7f** is rather more active than the other compounds (including cefotaxime). Interestingly **7f** is also the most active compound in the series against *P. aeruginosa*. The 3-methyl analog **7e** has very poor activity against *S. aureus*; the whole series with the exception of **7f** has rather poor

activity against *S. epidermidis*.

Compounds **7a** and **7f** showed good stability against the β-lactamases from several Gram-negative organisms and those from *S. aureus* S13E (Table 3). Similar trends in β-lactamase stability were observed with cefotaxime.

Experimental

IR spectra were measured as a KBr pellet or $CHCl_3$ solution on a Nicolet MX-10 spectrophotometer. ^1H NMR spectra were recorded on a Varian EM-390 (90 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million relative to TMS. UV spectra were determined on a Carry Model 219 spectrophotometer in the solvent indicated. Titration data was determined in 66% DMF - H_2O. Mass spectral data were determined on a CEC 21-110 spectrometer. All melting points are uncorrected. β-Lactamase stabilities were determined by the method of MAHONEY *et al.*[12].

Ethyl 2-Methoximinocyanoacetate (**2a**)

Ethyl 2-hydroximinocyanoacetate[13] (40.2 g, 0.283 mol) was dissolved in acetone (225 ml). Anhydrous K_2CO_3 (58.5 g, 0.423 mol) was added with stirring and the resulting bright red suspension was cooled to 10°C. Dimethyl sulfate (26 ml, 0.283 mol) was added dropwise and the resulting mixture was allowed to stir for 3 hours while warming to room temp. The mixture was poured into ice and extracted twice with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with H_2O, dried (anhydrous $MgSO_4$) and concentrated to a red oil *in vacuo* which was carefully distilled under vacuum to give **2a**, 16 g, bp 69~72°C/0.5 mmHg: NMR ($CDCl_3$) δ 1.35 (3H, s, ester CH_3), 4.25 (3H, s, OCH_3), and 4.35 (2H, q, ester CH_2).

2-Ethoxycarbonyl-2-methoximinoacetoximeamide (**3a**)

An EtOH solution of hydroxylamine was prepared by adding KOH (3.9 g in 75 ml EtOH) to hydroxylamine·HCl (4.86 g in 100 ml EtOH) at 30~40°C. The solution was then cooled to 0°C and filtered to remove the precipitated KCl. An EtOH solution of **2a** (10 g, 64 mmol in 25 ml EtOH) was added dropwise to the filtrate and the resulting mixture was stirred at room temp for 42 hours.

The EtOH was removed *in vacuo*. The residue was dissolved in EtOAc and washed twice with H_2O, dried (anhydrous $MgSO_4$) and concentrated *in vacuo* to an oil which partially crystallized. The semi-solid mass was triturated with cyclohexane several times and then crystallized from $CHCl_3$ to yield 2.16 g (18%) **3a**, mp 156~158°C. *Anal* Calcd for $C_6H_{11}N_3O_4$: C 38.10, H 5.86, N 22.21. Found: C 38.00, H 5.62, N 21.97. NMR (DMSO-d_6) δ 0.82 (3H, t, ester CH_3), 3.5 (3H, s, OCH_3), 3.62 (2H, q, ester CH_2), 5.0 (2H, br s, NH_2), and 10.15 (1H, s, NOH).

2-Ethoxycarbonyl-2-methoximinoacetoximeamide Tosylate (**3b**)

A THF solution (70 ml) of **3a** (1.543 g, 8.2 mmol) was stirred and 2,6-lutidine (0.965 g, 8.2 mmol) was added. Stirring was continued and *p*-TsCl (4.7 g, 24.6 mmol) in 10 ml THF was added dropwise. The mixture was then allowed to stir overnight. The THF was removed *in vacuo* and the residue was redissolved in EtOAc and washed twice with H_2O. The dried EtOAc solution ($MgSO_4$) was concentrated to a slightly yellow oil which subsequently crystallized. Recrystallization from petroleum ether yielded **3b** as a white crystalline solid (2.64 g, 93%); mp 125~127°C. *Anal* Calcd for $C_{13}H_{15}N_3O_6S$: C 45.74, H 4.56, N 12.31. Found: C 45.46, H 4.70, N 12.16. MS 343 (M^+). NMR ($CDCl_3$) δ 1.24 (3H, t, ester CH_3), 2.38 (3H, s, aromatic CH_3), 3.92 (3H, s, OCH_3), 4.27 (2H, q, ester CH_2), 5.32 (2H, br s, amide NH_2), and 7.21 and 7.69 (4H, ABq, aromatic).

Ethyl 2-(5-Trichloromethyl[1,2,4]oxadiazol-3-yl)-2-methoximinoacetate (**4**)

Method A: A mixture of **3a** (7.65 g, 40 mmol) and pyridine (3.6 ml, 45 mmol) were dissolved in dioxane (25 ml) and chilled while trichloroacetyl chloride (5 ml, 45 mmol) was added dropwise. The mixture was allowed to warm to room temp then stirred with refluxing overnight. The solvent was removed *in vacuo* and the resulting residue dissolved in Et_2O - H_2O. The Et_2O layer was washed

with saturated $NaHCO_3$, H_2O, and dried ($MgSO_4$). Evaporation yielded a residue which was shown by TLC (EtOAc - $PhCH_3$, 4: 3) to be a mixture of starting material and a higher Rf material which was dissolved in hexane and filtered. Concentration *in vacuo* yielded **4** as a yellow oil; the NMR ($CDCl_3$) showed **4** to be a mixture of isomers: MS 315 (M^+), 280 (M−Cl), 270 (M−OC_2H_5), 242 (M−$COOC_2H_5$), 212 (base, M−$COOC_2H_5$−CH_2O). The isotope pattern of the M^+ confirmed the presence of 3 chlorines.

Method B: A dioxane solution (25 ml) of **3b** (0.750 g, 2.4 mmol) and trichloroacetyl chloride (0.439 g, 2.4 mmol) were stirred in the cold while pyridine (0.195 ml, 2.4 mmol) in 5 ml of dioxane was added dropwise. The mixture was allowed to warm to room temp and then stirred overnight at reflux. The mixture was filtered and concentrated; the residue was dissolved in hexane and chilled. A crystalline precipitate of *p*-toluenesulfonylchloride (*p*-TsCl) formed. The mother liquors contained additional *p*-TsCl as well as **4** (by TLC on silica gel EtOAc - $PhCH_3$, 4: 3).

Ethyl 2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetate (**5a**) and 2-(5-Amino[1,2,4]-oxadiazol-3-yl-2-*E*-methoximinoacetamide (**5b**)

An ether solution (40 ml) of **4** (7.62 g, 2.42 mmol) was added dropwise to liquid ammonia (250 ml) at −33°C. After the addition was complete stirring was continued overnight allowing the ammonia to evaporate. The mixture was further diluted with Et_2O and filtered to remove 1.1 g of insoluble materials (**5b** contaminated with **5a**). The solid was crystallized from EtOAc and filtered to yield 0.95 g of **5b**, mp 198~200°C. NMR (DMSO-d_6) δ 3.92 (3H, s, OCH_3), 7.15 (2H, br d, amide NH_2), and 7.45 (2H, br s, amino).

The combined filtrates (Et_2O) from above were concentrated and the residue was crystallized from EtOH to yield **5a** as a white crystalline solid, mp 154~155°C. *Anal* Calcd for $C_7H_{10}N_4O_4$: C 39.25, H 4.71, N 26.16. Found: C 39.25, H 4.55, N 25.88. NMR ($CDCl_3$) δ 1.15 (3H, t, ester CH_3), 3.95 (3H, s, OCH_3), 4.25 (2H, q, OCH_2), and 8.05 (2H, br s, NH_2). UV λ_{max}^{EtOH} 227 nm (ε 11,335). IR (Nujol) 3420, 1730, 1670 cm^{-1}.

2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetic Acid (**6a**)

Sodium Salt: An EtOH solution (50 ml) of **5a** (4.28 g, 20 mmol) was treated dropwise with 4 ml of 5 N NaOH (20 mmol) at room temp. A white precipitate slowly formed. After 45 minutes the white solid was collected by filtration. The solid was washed with EtOH and Et_2O and dried *in vacuo* to yield **6a** sodium salt, 3.43 g (82%), mp 230°C; TLC (silica gel BuOH - AcOH - H_2O, 8: 2: 1) showed 1 spot material, Rf 0.38. *Anal* Calcd for $C_5H_5N_4O_4Na \cdot H_2O$: C 26.56, H 3.12, N 24.78. Found: C 26.62, H 2.86, N 24.52. UV λ_{max}^{MeOH} 233 nm (ε 10,391). IR (KBr) 1680, 1665, 1615 cm^{-1}. Titration, *pKa'* 4.02, AMW 204.

Free Acid (**6a**): The sodium salt from above (3.43 g, 16.5 mmol) was dissolved in 5 ml H_2O and layered with EtOAc. The pH of the aqueous layer was adjusted to 2.0 by the dropwise additions of 2 N HCl. The aqueous layer was saturated with NaCl and extracted 3 times with EtOAc. The organic extracts were combined and dried ($MgSO_4$). Evaporations yielded an oily residue which was thoroughly triturated with Et_2O. The white crystalline precipitate was filtered to yield 2.8 g of **6a**, mp 177~179°C. NMR ($CDCl_3$) δ 4.0 (3H, s, OCH_3), 7.05 (2H, s, NH_2), and 8.5 (1H, s, COOH).

2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*E*-methoximinoacetic Acid (**6b**)

An aqueous suspension (10 ml) of **5b** (1.47 g, 7.9 mmol) was warmed on the steam bath and sodium peroxide (0.62 g, 7.9 mmol) was carefully added portionwise. After the addition was complete heating was continued for 3 hours (no additional starting material in evidence by TLC on silica gel Et_2O - AcOH - H_2O, 15: 3: 1). The mixture was chilled to ~10°C and filtered (pH 10). The pH was adjusted to 2 by the dropwise addition of 1 N HCl and the resulting suspension was exhaustively extracted with EtOAc. The combined EtOAc extracts were dried ($MgSO_4$) and concentrated to an oil *in vacuo*. The oil subsequently crystallized upon standing and was recrystallized from EtOAc - hexane to yield 0.133 g (9%) of **6b**, mp 154~155°C. *Anal* Calcd for $C_5H_6N_4O_4$: C 32.37, H 3.25, N 30.10. Found: C 32.02, H 3.36, N 29.88. NMR (DMSO-d_6) δ 3.95 (3H, s, OCH_3) and 7.91 (2H, s, NH_2). MS 186 (M^+), 169 (M−OH), 139 (M−COOH), 111 (base, M−COOH−CH_2O). TLC (BuOH - AcOH - H_2O,

8: 2: 1) Rf 0.5.

2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetic Acid 1-Hydroxybenzotriazole-active Ester (**11**)

A dimethylacetamide solution (10 ml) of **6a** (2.0 g, 10.7 mmol) was stirred while triethylamine (1.63 ml, 11.8 mmol) was added dropwise. A precipitate formed which slowly went back into solution after the portionwise addition of methanesulfonyloxybenzotriazole (2.29 g, 10.7 mmol). After 2.5 hours the mixture was poured into cold water and stirred for an additional 1 hour. A white precipitate slowly formed which was collected by filtration and dried *in vacuo*. The solid was thoroughly triturated with Et_2O and filtered to give 0.84 g (29%) of **11**, mp 193~194°C. *Anal* Calcd for $C_{11}H_9N_7O_4$: C 43.57, H 2.99, N 32.33. Found: C 43.31, H 2.81, N 32.17. NMR (DMSO-d_6) δ 4.02 (3H, s, OCH_3) and 7.65~8.47 (6H, m, NH_2 and aromatic). IR (KBr) carbonyl 1734 cm^{-1}.

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-acetoxymethyl-3-cephem-4-carboxylic Acid Benzhydryl Ester (**9a**)

A solution of **6a** (0.75 g, 4 mmol) in 10 ml of MeCN - THF, 50: 50, was stirred at room temp while a 10 ml MeCN - THF solution of DCC was added dropwise. After stirring for 0.5 hour benzhydryl 7-ACA (**8a**) was added. The reaction was followed by TLC (silica gel EtOAc - $PhCH_3$, 4: 3). Stirring was continued for 56 hours and then the mixture was filtered to remove 0.3 g of dicyclohexylurea. The filtrate was evaporated and the residue triturated 2 times with Et_2O. The Et_2O insoluble residue was dissolved in EtOAc and washed successively with 1 N HCl, saturated $NaHCO_3$ and finally saturated NaCl. The EtOAc was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was triturated with Et_2O to yield 0.640 g of **9a** as an amorphous solid. This material was chromatographed over dry-column silica gel (ICN). The column was first eluted with EtOAc - cyclohexane, 1: 1 (25 ml fractions), and thereafter with EtOAc - cyclohexane, 3: 1. Fractions 33~42 were combined and dissolved in $CHCl_3$ and precipitated with hexane to yield 0.420 g (35%) of **9a**: NMR ($CDCl_3$) δ 1.90 (3H, s, OAc), 3.27 and 3.55 (2H, ABq, J=12 Hz, C-2), 4.68 and 4.95 (2H, ABq, J=5 Hz, C-3 methylene), 4.96 (1H, d, J=3 Hz, C-6), 5.95 (1H, dd, J=3 and 6 Hz, C-7), 6.25 (2H, br s, NH_2), 6.85 (1H, s, $CHPh_2$), 7.20 (10H, s, aromatic), and 8.72 (1H, d, J=6 Hz, amido).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-[(1-methyltetrazol-3-yl)-thiomethyl]-3-cephem-4-carboxylic Acid Benzhydryl Ester (**9b**)

A MeCN - THF, 50: 50, solution (40 ml) of **6a** (0.750 g, 4 mmol) was treated dropwise with DCC (0.906 g, 4.4 mmol) in 10 ml of MeCN - THF. After 0.5 hour the benzhydryl ester **8b** was added and the mixture was stirred for 16 hours. The mixture was filtered and the residue was triturated with Et_2O to leave 2.65 g of an amorphous solid which was chromatographed over dry-column silica gel. The desired product was eluted with EtOAc - cyclohexane, 2: 1. Crystallization from EtOH yielded **9b** as a white crystalline solid, 0.98 g (37%): *Anal* Calcd for $C_{28}H_{26}N_{10}O_6S_2$: C 50.75, H 3.95, N 21.14. Found: C 50.95, H 4.15, N 21.06. NMR ($CDCl_3$) δ 3.67 (2H, ABq, J=9 Hz, C-3 methylene), 5.00 (1H, d, J=4 Hz, C-6), 5.95 (1H, dd, J=4 and 6 Hz, C-7), 6.78 (2H, s, NH_2), 6.85 (1H, s, $CHPh_2$), 7.28 (10H, s, aromatic), and 8.87 (1H, d, J=6 Hz, amido). UV λ_{max}^{MeOH} 260 nm (ε 50,000).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**7a**)

Method A: A formic acid solution (12 ml) of **9a** (0.435 g, 0.72 mmol) and triethylsilane (0.3 ml) was stirred for 3 hours, then concentrated *in vacuo*. The residue was dissolved in MeOH and once again concentrated. The residue was dissolved in EtOAc and extracted with 10% aqueous $NaHCO_3$. The bicarbonate extract was layered with EtOAc and acidified with 1 N HCl to pH 2. The EtOAc layer was washed with saturated NaCl, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was triturated with Et_2O and filtered to yield **7a** as an amorphous solid (0.215 g, 70%). *Anal* Calcd for $C_{15}H_{16}N_6O_8S$: C 40.91, H 3.66, N 19.08. Found: C 40.68, H 3.82, N 19.32. NMR (acetone-d_6) δ 1.7 (3H, s, OAc), 3.15 and 3.42 (2H, ABq, J=12 Hz, C-2), 3.67 (3H, s, OCH_3), 4.57 and 4.80 (2H, ABq, J=10 Hz, C-3 methylene), 4.87 (1H, s, d, J=4 Hz, C-6), 5.65 (1H, dd, J=4 and 5 Hz, C-7), 7.05 (2H,

br s, NH_2), and 8.25 (1H, d, J=5 Hz, amido).

Method B: 7-ACA (1.83 g, 7 mmol) was suspended in 50% aqueous acetone (70 ml) and cooled while triethylamine (0.96 ml, 6.9 mmol) was added dropwise with stirring. After 30 minutes, **11** (2.5 g, 7.4 mmol) was added portionwise. As needed 45% aqueous potassium phosphate was added to keep the pH between 7.5 and 7.8. The reaction was warmed to 35~40°C and the material slowly went into solution. Stirring was continued overnight.

The acetone was removed *in vacuo* and the remaining aqueous solution was layered with EtOAc and acidified to pH 2. The EtOAc extract was combined with several washings, dried ($MgSO_4$) and concentrated *in vacuo*. Silica gel TLC (EtOAc - MeCN - AcOH - H_2O, 21: 7: 7: 9) showed that the desired product was contaminated with HOBT and unreacted side chain. The crude product (2.43 g) was dissolved in EtOH and treated with LiOAc (1 g) in 2 ml MeOH. The resulting lithium salt (1 spot by TLC) was converted back to **7a** (1.17 g, 35.9%) by dissolving in H_2O, acidification to pH 2 and extraction with EtOAc. A white amorphous solid was obtained by dissolving **7a** in THF and precipitating by the addition of cyclohexane.

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-[(1-methyltetrazol-3-yl)-thiomethyl]-3-cephem-4-carboxylic Acid (**7b**)

A formic acid solution (8 ml) of **9b** and triethylsilane (0.2 ml) was stirred at room temp for 3.5 hours and then concentrated *in vacuo*. The residue was dissolved in MeOH, evaporated, and triturated with $CHCl_3$ forming a white amorphous solid of **7b** (0.305 g, 81%): NMR (DMSO-d_6) δ 3.62 (2H, s, C-3 methylene), 5.05 (1H, d, J=4 Hz, C-6), 5.75 (1H, dd, J=4 and 6 Hz, C-7), 7.80 (2H, s, NH_2), and 9.67 (1H, d, J=6 Hz, amido).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-[(2-methyl[1,3,4]thiadiazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (**7c**), Lithium Salt

A 50% aqueous acetone suspension of **10c** (0.664 g, 2 mmol) was treated with **11** (0.7 g, 2.2 mmol) as in Method A above. Upon work-up in a like manner **7c** (0.680 g) was obtained. This material was dissolved in EtOH (4 ml) and treated with 0.084 g of LiOAc·$2H_2O$ in 0.5 ml of MeOH. The lithium salt of **7c** slowly crystallized, mp 210°C (0.260 g, 24%). *Anal* Calcd for $C_{16}H_{15}N_8O_6S_2Li \cdot H_2O$: C 35.82, H 3.19, N 30.89. Found: C 35.59, H 3.01, N 30.69. NMR (DMSO-d_6) δ 2.68 (3H, s, thiadiazole CH_3), 3.2 and 3.70 (2H, ABq, J=6 Hz, C-2), 3.25 (2H, s, H_2O), 3.95 (3H, s, OCH_3), 4.25 and 4.55 (2H, ABq, J=8 Hz, C-3 methylene), 49.5 (1H, d, J=3 Hz, C-6), 5.6 (1H, m, C-7), 7.90 (2H, br s, NH_2), and 9.45 (1H, d, J=6 Hz, amido).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-cephem-4-carboxylic Acid (**7d**)

An aqueous acetone suspension of **10d** (0.5 g, 2.5 mmol) was treated with **11** (0.88 g, 2.6 mmol) as for **7a**, Method A above. A crystalline solid of **10d** was obtained from the aqueous solution (pH 8) by acidification to pH 2 with 1 N HCl, mp 160°C (0.2 g, 23%). NMR (DMSO-d_6) δ 3.52 (2H, br s, C-2), 3.95 (3H, s, OCH_3), 5.05 (1H, d, J=4.5 Hz, C-6), 5.80 (1H, dd, J=4.5 and 9 Hz, C-7), 6.44 (1H, br t, C-3), 7.93 (2H, s, amino), and 9.65 (1H, d, J=9 Hz, 7-amido).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-methyl-3-cephem-4-carboxylic Acid (**7e**)

7-ADCA (**10e**) (0.535 g, 2.5 mmol) was suspended in 50 ml of 50% aqueous acetone and the pH was adjusted to 7.5 with 45% aqueous potassium phosphate after the addition of 0.35 ml (2.5 mmol) of triethylamine. The active ester **11** (0.90 g, 2.66 mmol) was added portionwise as described above in Method A.

After initial purification as its lithium salt, the crystalline **7e** (0.160 g, 17%) was obtained after dissolving in H_2O and adjusting the pH to 2 with 1 N HCl. *Anal* Calcd for $C_{13}H_{14}N_8O_6S$: C 40.84, H 3.69, N 21.98. Found: C 41.07, H 3.80, N 21.79. NMR ($CDCl_3$ and DMSO-d_6) δ 2.07 (3H, s, 3-methyl), 3.25 and 3.55 (2H, ABq, J=12 Hz, C-2), 4.00 (3H, s, OCH_3), 5.05 (1H, d, J=3 Hz, C-6), 5.70 (1H, dd, J=3 and 6 Hz, C-7), 7.7 (2H, s, NH_2), and 9.55 (1H, d, J=6 Hz, amido).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-pyridiniummethyl-3-cephem-4-carboxylic Acid (**7f**)

An aqueous acetone suspension (20 ml) of **10f** (1.47 g, 4 mmol) was cooled and the pH adjusted to 7.5 with 45% aqueous potassium phosphate. The active ester **11** (0.676 g, 2 mmol) was added and the resulting suspension was stirred overnight. The acetone was removed *in vacuo* and the resulting aqueous solution was layered with EtOAc and the pH adjusted to 2 with 1 N HCl. The aqueous layer was charcoal treated and then lyophilized to yield 0.9 g of crude **7f**. Purification by reversed-phase preparative HPLC on C-18 eluting with MeCN - AcOH - H_2O (20: 2: 78) at 2.4 ml/minute yielded 0.1 g of **7f** as an amorphous solid. NMR (DMSO-d_6) δ 3.00 and 3.50 (2H, ABq, J=9 Hz, C-2), 3.95 (3H, s, OCH_3), 5.08 (1H, d, J=4.5 and 9 Hz, C-7), 8~9.7 (5H, m, pyridinium), and 9.70 (1H, d, J=9 Hz, 7-amido). UV λ_{max}^{MeOH} 255 nm (ε 21,549).

7-[2-(5-Amino[1,2,4]oxadiazol-3-yl)-2-*Z*-methoximinoacetyl]amino-3-[(2-methyl-6-hydroxy-5-oxo-5*H*-[1,2,4]triazin-3-yl)thio]methyl-3-cephem-4-carboxylic Acid (**7g**)

A suspension of **7a** (0.440 g, 1 mmol) in CH_2Cl_2 (5 ml) under N_2 was treated with monotrimethylsilyltrifluoroacetamide (MSTFA) (0.43 ml, 2.4 mmol) then warmed to 40°C. After everything was dissolved the solution was cooled to room temp and TMSI (0.38 ml, 2.7 mmol) was added. After stirring at room temp for 1 hour the solvent was removed *in vacuo* and the oily residue was redissolved in 10 ml of MeCN and THF (0.081 ml, 1 mmol) was added.

Thiol **12** was suspended in MeCN and MSTFA (0.43 ml, 2.4 mmol) was added. The resulting solution was added to the above solution of the 3-iodomethylcephalosporin. After stirring for 3 hours under N_2 at room temp the solution was diluted with Et_2O and 3 ml of H_2O was added whereupon a thick tan precipitate formed which was collected by filtration, washed with Et_2O and dried (0.258 g, 47.8%). Purification by reversed-phase preparative HPLC (MeCN - AcOH - H_2O, 8: 2: 90, flow rate 6 ml/minute) yielded 0.153 g of **7g**. *Anal* Calcd for: C 37.85, H 3.18, N 23.37, S 11.89. Found: C 37.83, H 3.21, N 23.15, S 11.84. NMR (DMSO-d_6) δ 3.35 and 3.62 (2H, ABq, J=6 Hz, C-2), 3.60 (3H, s, NCH_3), 3.92 (3H, s, OCH_3), 4.08 and 4.2 (2H, ABq, J=8 Hz, C-3 methylene), 5.10 (1H, d, J=3 Hz, C-6), 5.72 (1H, dd, J=3 and 6 Hz, C-7), 8.05 (2H, br s, NH_2), and 9.70 (1H, d, J= 6 Hz, amido).

References

1) Teraji, T.: Aminothiazole surrogates. Program and Abstracts of the 22nd Intersci. Conf. Antimicrob. Agents Chemother., Session 35, Miami Beach, Oct. 4~6, 1982
2) Tiemann, F.: Über das Verhalten von Amidoximen und Azoximen. Ber. Deutsch. Chem. Ges. 18: 1060~1080, 1885
3) Lenaers, R.; C. Moussebois & F. Eloy: Synthese due derive dimethyle et de quelques derives monosubstitues de l'oxadiazole-1,2,4. Helv. Chim. Acta 45: 441, 1962
4) Breuer, H.: Amino-1,2,4-oxadiazol-3-yl acetylcephalosporins. U.S. 3,960,849, June 1, 1976
5) Pinner, A. & A. R. Klein: Umwandlung der Nitrile in Imide. Ber. Deutsch. Chem. Ges. 10: 1889~1897, 1877
6) Woodburn, H. M.; A. B. Whitehouse & B. G. Pautler: Reaction of cyanogen with organic compounds. XII. Glycols and glycol monoethers. J. Org. Chem. 24: 210~214, 1959
7) Ochiai, M.; A. Morimoto, Y. Matsushita & T. Okada: Synthesis and structure-activity relationships of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporin derivatives. IV. Synthesis of 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid derivatives and related compounds. J. Antibiotics 34: 160~170, 1981
8) Vaughn, H. L. & M. D. Robbins: A rapid procedure for the hydrolysis of amides to acids. J. Org. Chem. 40: 1187~1189, 1975
9) Konig, W. & R. Geiger: Eine neue Methode zur Synthese von Peptiden: Aktivierung der Carboxylgruppe mit Dicyclohexylcarbodiimid unter zusatz von 1-Hydroxybenzotriazolen. Chem. Ber. 103: 788~798, 1970
10) Bonjouklian, R. & M. L. Phillips: Reaction of TMSI with cephalosporin esters. Tetrahedron Lett. 22: 3915~3917, 1981
11) Kirst, H. A.; G. M. Wild, R. H. Baltz, R. L. Hamill, J. L. Ott, F. T. Counter & E. E. Ose: Structure-activity studies among 16-membered macrolide antibiotics related to tylosin. J. Antibiotics 35: 1675~1682, 1982
12) Mahoney, D. F.; G. A. Koppel & J. R. Turner: Substrate inhibition of beta-lactamases, a method for predicting enzymatic stability of cephalosporins. Antimicrob. Agents Chemother. 10: 470~475, 1976
13) Parker, C. O.: Chemistry of dinitroacetonitrile. IV. Preparation and properties of esters of dinitrocyanoacetic acid. Tetrahedron 17: 109~116, 1962