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

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In an effort to improve the antibacterial activity of 7β-[2-(2-aminothiazol-4-yl)acetamido]-cephalosporins by introducing a methoxyimino group into the 7-acyl side chain, geometrically isomeric 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acids and their derivatives were selectively synthesized. Structurally related acid derivatives were also synthesized. A facile and practical synthesis of an important starting material, 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid, for the preparation of SCE-1365 which is now under extensive clinical trial was achieved.

In our previous papers[1] various chemical modifications of 7β-[2-(2-aminothiazol-4-yl)acetamido]-cephalosporins[2] were described expecting to improve their antibacterial activity especially against β-lactamase-producing strains. It was of great interest to us, considering the desirable biological properties of cefuroxime,[3] to investigate an effective method for the introduction of the methoxyimino moiety into the 7-acyl side chain of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins for further improvement of the antibacterial activity.*

In this paper the synthesis and configuration of 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid derivatives and related acids necessary for the acylation of 7-aminocephalosporins will be described.

1. Synthesis of 2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetic Acid Derivatives

Analogously to the synthesis of ethyl 2-hydroxyimino-2-(2-methylthiazol-4-yl)acetate[7], ethyl 4-bromo-2-methoxyimino-3-oxobutyrate (**1**) was reacted with thiourea in ethanol under reflux to give ethyl 2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetate (**2a**) selectively. Careful work up of the reaction mixture, however, gave rise to a small amount of the corresponding (*Z*)-isomer (**2b**). Methyl 4-bromo-2-methoxyimino-3-oxobutyrate (**10**) also gave the similar results. Since **2b** was more desirable for our chemical modification, various efficient syntheses of this ester were subsequently investigated.

* Part of the results was reported as a brief communication[4] and presented at the 18th Interscience Conference on Antimicrobial Agents and Chemotherapy.[5] Another group working independently has reported partially similar results[6].

1–1. Synthesis of **2b** *via* ethyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetate (**4b**)—Method A

In our previous paper[1c] it was shown that the reaction of ethyl 4-chloro-2-hydroxyimino-3-oxobutyrate (**3**) with thiourea gave a mixture of geometric isomers of ethyl 2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate (**4a, 4b**), and separation and characterization of each isomer were done. Conventional methylation of the (*Z*)-isomer (**4b**) with dimethyl sulfate afforded the desired (*Z*)-methoxyimino ester (**2b**) in good yield together with a small amount of a (*Z*)-nitrone compound (**5b**).

Based on these results, the possibility of a selective synthesis of the (*Z*)-isomer (**4b**) from **3** and thiourea was investigated under various conditions which are summarized in Table 1.

Chart 1.

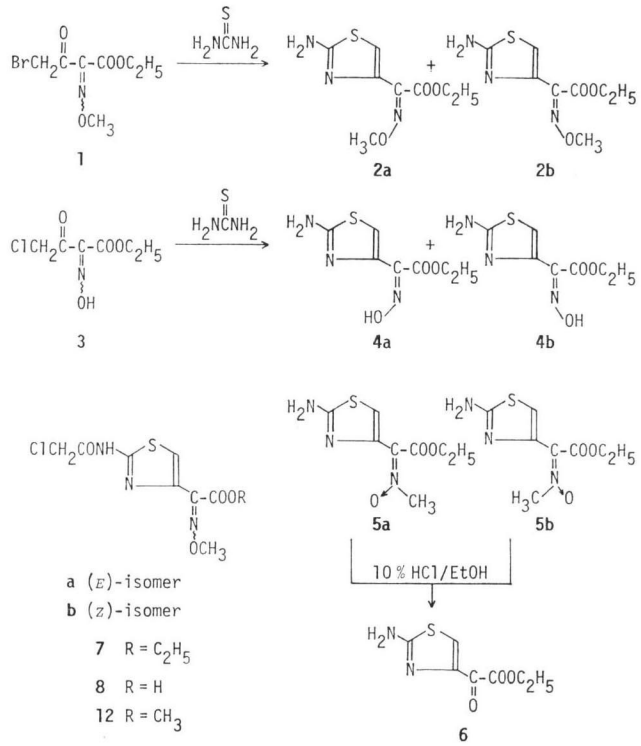


Table 1. Reaction of ethyl 4-chloro-2-hydroxyimino-3-oxobutyrate (**3**) with thiourea.

Run	**3** (g)	Thiourea (g)	$AcONa \cdot 3H_2O$ (g)	Solvent	Temp. (°C)	Time (hours)	Result*	
							4a (*E*)	**4b** (*Z*)
1	3.9	1.3	—	aq. EtOH	5	1	1	1
2	3.9	1.3	—	〃	r.t.	1	1	1
3	3.9	1.3	—	〃	〃	15	7	3
4	3.9	1.3	—	EtOH	40~50	1	7	3
5	5.0	3.9	—	aq. THF	r.t.	3	3	1
6	5.0	3.9	10.5	〃	〃	3	2	9
7	5.0	3.9	10.5	〃	〃	16	2	9
8	2.0	0.7	—	DMA	〃	3	15	85

* Ratio of **4a** to **4b** was determined by integration intensity of thiazole 5-H in NMR spectrum.

It is apparent from Table 1 that selective synthesis of **4b** was well achieved under conditions containing an acid-scavenger (Run 6~8). These results may well be explained in terms of the isomerization of oxyimino compounds under acidic conditions.[8]

1-2. Synthesis of **2b** *via* nitrone compounds (**5a, 5b**)—Method B

Although the formation of the (*Z*)-nitrone compound (**5b**) was sluggish, conventional methylation of (*E*)-hydroxyimino ester (**4a**) with methyl iodide or diazomethane afforded (*E*)-nitrone compound (**5a**) predominantly. Treatment of both isomeric nitrones (**5a, 5b**) with acid readily gave the corresponding carbonyl compound (**6**) as reported for other nitrone compounds.[8]

Reaction of **6** with O-methylhydroxylamine afforded (*Z*)-methoxyimino ester (**2b**) predominantly. Thus selective synthesis of **2b** *via* nitrone compounds (**5a, 5b**) was achieved with emphasis on the conversion of (*E*)-hydroxyimino ester (**4a**) into (*Z*)-methoxyimino ester (**2b**).

Structural assignment of the new nitrones (**5a, 5b**) [(*E*)-hydroxyimino ester (**4a**) giving (*E*)-nitrone (**5a**) and **4b** giving **5b**] was based on the facts that the methylation reactions we have employed were devoid of acidic conditions and that geometric isomerization of nitrone compounds was not observed.[8]

1-3. Synthesis of **2b** *via* ethyl 4-bromo-2-methoxyimino-3-oxobutyrate (**1**)—Method C

The reaction conditions shown in Table 1 for the selective formation of (*Z*)-isomer (**4b**) (Run 6) were applied to the synthesis of **2b**. Thus the reaction of 4-bromo-2-methoxyimino-3-oxobutyrate (**1**) with thiourea in the presence of sodium acetate gave rise to selective formation of the desired (*Z*)-methoxyimino ester (**2b**).

Selective synthesis of **2b** was thus achieved by three different methods (A~C).

1-4. Synthesis of 2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetic acid (**8**)

For the acylation of 7-aminocephalosporins, the aminothiazole esters (**2a, 2b**) were hydrolyzed to free acids (**8**) after protection[1c] (**7**) of the amino moiety by the chloroacetyl group.

Finally, a convenient and practical synthesis of 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**8b**), an important starting material for the preparation of SCE-1365,[5]* was achieved starting from methyl acetoacetate *via* methyl 2-methoxyimino-3-oxobutyrate (**9**). Methyl 4-bromo-2-methoxyimino-3-oxobutyrate (**10**) obtained from **9** by bromination was converted into methyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetate (**11b**) according to Method C followed by chloroacetylation to give methyl 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetate (**12b**). Hydrolysis of **12b** afforded **8b**.

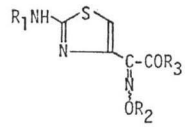
Using this sequence of reactions **8b** was readily obtained without distillation or chromatographic purification of the intermediates (see Experimental).

2. Configuration of 2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetic Acid and Its Derivatives

Geometric isomers of ethyl 2-hydroxyimino-2-(thiazol-4-yl)acetate were separated but without assignment of the structure[7]. NMR spectra of each isomeric pair of 2-(fur-2-yl)-2-hydroxyimino-

* Generic name: cefmenoxime, 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid.

Table 2. Chemical shift of thiazole 5-H of 2-aminothiazole derivatives.



Compound	R_1	R_2	R_3	Solvent	Thiazole 5-H (ppm)
2a (*E*)	H	CH_3	OC_2H_5	$CDCl_3$	7.43
2b (*Z*)					6.74
11a (*E*)	H	CH_3	OCH_3	$CDCl_3$	7.43
11b (*Z*)					6.74
7a (*E*)	$ClCH_2CO$	CH_3	OC_2H_5	$CDCl_3$	7.94
7b (*Z*)					7.15
12a (*E*)	$ClCH_2CO$	CH_3	OCH_3	$CDCl_3$	7.90
12b (*Z*)					7.24
8a (*E*)	$ClCH_2CO$	CH_3	OH	d_6-DMSO	8.00
8b (*Z*)					7.57
4a (*E*)	H	H	OC_2H_5	d_6-DMSO	7.50
4b (*Z*)					6.80
13a (*E*)	H	CH_3	NHC_2H_5	d_6-DMSO	7.44
13b (*Z*)					6.74

and 2-hydroxyimino-2-(thien-2-yl)acetic acids were disclosed in a patent specification.[9] Although in each case chemical shifts of annular protons were not assigned, it may be assumed that annular protons at the 3-position of (*Z*)-isomers in both cases appear at the higher field by *ca.* 0.5 ppm. Similar difference in chemical shift was observed in each pair of 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid derivatives as shown in Table 2.

However, some additional evidence was sought to support the structural assignment of these compounds. Therefore, the difference in reactivity between a pair of isomers towards hydrolysis and aminolysis was examined assuming that steric hindrance might affect the reactivity as reported with a pair of related esters.[10]

Hydrolysis of **7a** in water containing 2 equivalents of potassium hydroxide at room temperature afforded the corresponding acid (**8a**) within 15 minutes, while (*Z*)-isomer (**7b**) was not affected by these reaction conditions. Hydrolysis of **7b** in water containing 5 equivalents of potassium hydroxide at room temperature to give 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**8b**) required 2 hours. Moreover, reaction of **2a** with ethylamine at room temperature for 2 hours gave N-ethyl-2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamide (**13a**). The (*Z*)-isomer (**2b**), by contrast, afforded the corresponding (*Z*)-isomer (**13b**) after reaction with ethylamine at 100°C in a sealed vessel for 4 hours. Thus, the structure of each geometric isomer was assigned* on the basis of these results together with the difference in chemical shift of thiazole 5-proton.

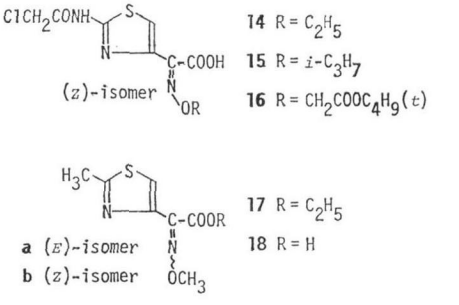
* This assignment was confirmed by X-ray analysis of SCE-1365[5] which will be reported elsewhere.

3. Synthesis of Related Acid Derivatives

In order to expand the profile of structure-activity relationships, several derivatives of related acids were also synthesized.

2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-ethoxyiminoacetic acid (**14**) and the corresponding 2-isopropyloxyiminoacetic acid (**15**) were synthesized using Method C followed by chloroacetylation and hydrolysis. Hydrolysis after protection of the amino moiety of the glyoxylate (**6**) with a chloroacetyl group gave (2-chloroacetamidothiazol-4-yl)glyoxylic acid. Subsequent reaction with O-(*t*-butyloxycarbonyl)methylhydroxylamine[11] (Method B) gave 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-(*t*-butyloxycarbonyl)methoxyiminoacetic acid (**16**).

Chart 2.



Reaction of **1** with thioacetamide gave a mixture of geometric isomers of ethyl 2-(2-methylthiazol-4-yl)-2-methoxyiminoacetate (**17**) which were separated by column chromatography on silica gel. Hydrolysis of each isomer afforded (*E*)- and (*Z*)-isomer of 2-(2-methylthiazol-4-yl)-2-methoxyiminoacetic acid, respectively (**18a**, **18b**).

Acylation of 7-aminocephalosporins with the new acid derivatives prepared in this paper will be reported in a forthcoming paper.

Experimental

NMR spectra were measured on a Varian T-60 (60 MHz) or EM-390 (90 MHz) spectrometer. All melting points are uncorrected.

Ethyl 2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetate (**2a**)

To a solution of ethyl 4-bromo-2-methoxyimino-3-oxobutyrate (**1**)[4] (5.0 g) in EtOH (20 ml) was added thiourea (1.8 g). The reaction mixture was refluxed for 3 hours. After cooling, the separated solids (hydrobromide of **2a**) were collected by suction and washed with EtOH. To a suspension of the solids in AcOEt was added 10% aqueous $NaHCO_3$. The organic layer was separated after vigorous shaking, washed and dried over $MgSO_4$. The residue obtained by evaporation of AcOEt was treated with a small amount of Et_2O to cause crystallization which was collected by suction. Colorless crystals, 2.6 g. Mp 114~115°C. *Anal.* Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.84; N, 18.33. Found: C, 41.71; H, 4.75; N, 18.07. NMR (60 MHz, $CDCl_3$) δ: 1.38 (3H, t, CH_3), 4.07 (3H, s, OCH_3), 4.34 (2H, q, CH_2), 5.80 (2H, bs, NH_2), 7.43 (1H, s, thiazole 5-H).

Similar treatment of the corresponding methyl ester (**10**) gave methyl 2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetate (**11a**) as colorless crystals, mp 133~134°C. *Anal.* Calcd. for $C_7H_9N_3O_3S$: C, 39.06; H, 4.21; N, 19.52. Found: C, 38.73; H, 4.09; N, 19.31. NMR (90 MHz, $CDCl_3$) δ: 3.86 (3H, s, CH_3), 4.05 (3H, s, OCH_3), 5.66 (2H, bs, NH_2), 7.43 (1H, s, thiazole 5-H).

Ethyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetate (**2b**)

(a) The filtrate obtained from the filtration of the hydrobromide of **2a** above was concentrated under reduced pressure. Water was added to the residue and the mixture neutralized with $NaHCO_3$. The mixture was extracted with AcOEt, and washed and dried over $MgSO_4$. The residue obtained by evaporation of AcOEt was chromatographed on silica gel. Elution with *n*-hexane-AcOEt (1:1) gave **2b**, 59 mg. Mp 163~164°C. *Anal.* Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.84; N, 18.33. Found: C, 41.57; H, 4.76; N, 18.07. NMR (60 MHz, $CDCl_3$) δ: 1.40 (3H, t, CH_3), 4.02 (3H, s, OCH_3), 4.43

(2H, q, CH_2), 5.80 (2H, bs, NH_2), 6.74 (1H, s, thiazole 5-H).

Similar treatment of **10** and work-up of the filtrate gave methyl 2-(2-aminothiazol-4-yl)-(*Z*)-methoxyiminoacetate (**11b**).

(b) To a solution of **4b** (10.7 g) in a mixture of THF (50 ml) and MeOH (50 ml) was added a solution of Na_2CO_3 (10.6 g) in water (150 ml). Dimethyl sulfate (12.6 g) was added to this mixture under ice-cooling. After addition the mixture was stirred at room temperature for 3 hours. Most of the solvents were removed under reduced pressure and the residue was cooled with ice. The separated solids were collected by suction, washed with water and dried over P_2O_5 to give **2b** as colorless crystals, 5.7 g.

The filtrate obtained after filtration of **2b** was extracted with a mixture of THF and AcOEt (1: 1). After washing with water and drying over $MgSO_4$, the organic layer was concentrated under reduced pressure. The residue after addition of THF (20 ml) was kept overnight in a refrigerator. The separated solids were collected and recrystallized from AcOEt to give (*Z*)-α-(2-aminothiazol-4-yl)-α-(ethoxycarbonyl)-N-methylnitrone (**5b**), yellow crystals, 1.3 g. Mp 111.6°C. *Anal.* Calcd. for $C_8H_{11}N_3O_5S$: C, 41.91; H, 4.84; N, 18.33. Found: C, 41.89; H, 4.91; N, 18.44. NMR (60 MHz, $CDCl_3$) δ: 1.36 (3H, t, CH_3), 4.14 (3H, s, N-CH_3), 4.42 (4H, q, CH_2), 5.34 (2H, bs, NH_2), 6.62 (1H, s, thiazole 5-H).

(c) A mixture of **6** (200 mg), 50% aqueous EtOH (10 ml), O-methylhydroxylamine hydrochloride (160 mg) and $NaHCO_3$ (168 mg) was heated at 70°C for 5 hours with stirring in a sealed vessel. The mixture was concentrated under reduced pressure and the residue was extracted with AcOEt. Conventional work-up of the AcOEt layer gave a crystalline substance, 165 mg. An NMR spectrum revealed that the product was a mixture of **2a** and **2b** (17: 83).

(d) To a solution of **1** (1.5 g) in a mixture of THF (10 ml) and water (7 ml) were added AcONa·$3H_2O$ (2.4 g) and thiourea (0.9 g). The mixture was stirred at room temperature for 17 hours. After concentration under reduced pressure, the reaction mixture was acidified with dil. HCl to pH 1.5 and washed with AcOEt. After neutralization with $NaHCO_3$, the aqueous layer was then extracted with AcOEt. Conventional work-up to the AcOEt layer afforded **2b**, 0.8 g.

Ethyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetate (**4b**)

To a solution of ethyl 4-chloro-2-hydroxyimino-3-oxobutyrate (**3**) (67.8 g) in 50% aqueous THF (600 ml) were added thiourea (53.2 g) and AcONa·$3H_2O$ (155 g). The mixture was stirred at room temperature for 4 hours. After neutralization with $NaHCO_3$ and addition of NaCl, the reaction mixture was extracted with AcOEt. From the AcOEt layer, after conventional work-up, was obtained **4b** as colorless crystals, 27.5 g. Mp 185.5°C. NMR spectrum was consistent with the reported data[1c].

(*E*)-α-(2-Aminothiazol-4-yl)-α-(ethoxycarbonyl)nitrone (**5a**)

(a) To a solution of **4a** (1.0 g) in a mixture of THF (10 ml) and AcOEt (5 ml) was added a solution of excess diazomethane in Et_2O. After standing for 2 days, the excess diazomethane was quenched with AcOH, and the mixture was concentrated under reduced pressure. To the residue was added AcOEt to cause crystallization of **5a** which was collected as yellow crystals, 0.8 g. Mp 184~185°C. *Anal.* Calcd. for $C_8H_{11}N_3O_5S$: C, 41.91; H, 4.84; N, 18.33. Found: C, 41.86; H, 4.75; N, 18.33. NMR (60 MHz, $CDCl_3$) δ: 1.40 (3H, t, CH_3), 3.82 (3H, s, N-CH_3), 4.99 (2H, q, CH_2), 5.27 (2H, bs, NH_2), 8.49 (1H, s, thiazole 5-H).

(b) To a solution of Na (23 mg) in MeOH (8 ml) were added **4a** (215 mg) and CH_3I (280 mg). After stirring for 45 minutes at room temperature, the mixture was concentrated under reduced pressure. Water was added and the residue was extracted with AcOEt. Conventional work-up of the AcOEt layer gave **5a**, 160 mg.

Ethyl (2-aminothiazol-4-yl)glyoxylate (**6**)

(a) A suspension of **5a** (2.4 g) in 10% (w/v) HCl in EtOH (70 ml) was stirred at room temperature for 16 hours. The reaction mixture was concentrated under reduced pressure and water was added to the residue. The aqueous solution was neutralized with 5% aqueous $NaHCO_3$ and extracted with AcOEt. The residue obtained by evaporation of AcOEt was recrystallized from EtOH to give

6 as colorless crystals, 1.54 g. Mp 143.3°C. *Anal.* Calcd. for $C_7H_8N_2O_3S$: C, 41.98; H, 4.02; N, 13.99. Found: C, 41.83; H, 4.14; N, 13.98. NMR (60 MHz, d_6-DMSO) δ: 1.66 (3H, t, CH_3), 4.73 (2H, q, CH_2), 7.79 (2H, bs, NH_2), 8.30 (1H, s, thiazole 5-H).

(b) Similar treatment of a suspension of **5b** (1.2 g) in 10% (w/v) HCl in EtOH (20 ml) gave **6**, 0.7 g.

Ethyl 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetate (**7b**)

To an ice-cooled solution of **2b** (2.15 g) in DMA (10 ml) was added chloroacetyl chloride (1.27 g). The mixture was stirred under ice-cooling for 30 minutes and at room temperature for 30 minutes. The mixture was poured into water and extracted with AcOEt. Conventional work-up of the AcOEt layer afforded **7b** as colorless crystals, 2.04 g. Mp 111~112°C. *Anal.* Calcd. for $C_{10}H_{12}ClN_3O_4S$: C, 39.29; H, 3.96; N, 13.74. Found: C, 39.15; H, 3.91; N, 13.69. NMR (60 MHz, $CDCl_3$) δ: 1.37 (3H, t, CH_3), 4.00 (3H, s, OCH_3), 4.24 (2H, s, $ClCH_2$), 4.40 (2H, q, CH_2), 7.15 (1H, s, thiazole 5-H).

2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**8b**)

To a suspension of **7b** (458 mg) in EtOH (16.5 ml) was added a solution of KOH (450 mg) in water (1.5 ml). After stirring at room temperature for 2 hours, the mixture was concentrated under reduced pressure. The residue was treated with water (10 ml) and washed with AcOEt. The aqueous layer was then extracted with AcOEt after acidification (pH 1.5) with 15% HCl. Conventional work-up of the AcOEt layer gave **8b** as colorless crystals, 310 mg. Mp 170~171°C. *Anal.* Calcd. for $C_8H_8ClN_3O_4S$: C, 34.60; H, 2.90; N, 15.13. Found: C, 34.97; H, 3.03; N, 14.74. NMR (60 MHz, d_6-DMSO) δ: 3.95 (3H, s, OCH_3), 4.40 (2H, s, $ClCH_2$), 7.57 (1H, s, thiazole 5-H).

Ethyl 2-(2-chloroacetamidothiazol-4-yl)-(*E*)-2-methoxyiminoacetate (**7a**)

To a solution of **2a** (10 g) in DMA (100 ml) was added chloroacetyl chloride (5.9 g) under ice-cooling. After stirring at room temperature for 1 hour, the mixture was poured into water and extracted with AcOEt. Conventional work-up of the AcOEt layer afforded **7a** as colorless crystals, 12.66 g. Mp 81~82°C. *Anal.* Calcd. for $C_{10}H_{12}ClN_3O_4S$: C, 39.29; H, 3.96; N, 13.74. Found: C, 38.74; H, 3.58; N, 13.16. NMR (60 MHz, $CDCl_3$) δ: 1.30 (3H, t, CH_3), 4.10 (3H, s, OCH_3), 4.24 (2H, s, $ClCH_2$), 4.34 (2H, q, CH_2), 7.94 (1H, s, thiazole 5-H).

Similar treatment of **11a** (2.15 g) gave methyl 2-(2-chloroacetamidothiazol-4-yl)-(*E*)-2-methoxyiminoacetate (**12a**), 2.18 g. Mp 118~120°C. *Anal.* Calcd. for $C_9H_{10}ClN_3O_4S$: C, 37.05; H, 3.46; N, 14.40. Found: C, 36.86; H, 3.35; N, 14.48. NMR (90 MHz, $CDCl_3$) δ: 3.87 (3H, s, CH_3), 4.10 (3H, s, OCH_3), 4.23 (2H, s, $ClCH_2$), 7.90 (1H, s, thiazole 5-H), 10.05 (1H, bs, CONH).

2-(2-Chloroacetamidothiazol-4-yl)-(*E*)-2-methoxyiminoacetic acid (**8a**)

To a solution of KOH (5.6 g) in 50% aqueous EtOH (500 ml) was added **7a** (15 g). After stirring 15 minutes at room temperature, the mixture was concentrated under reduced pressure. 1 N HCl was for added to the residue and the separated solids were collected by suction and washed with cold water to give **8a** as colorless crystals, 11.4 g. Mp 182~183°C. *Anal.* Calcd. for $C_8H_8ClN_3O_4S$: C, 34.60; H, 2.90; N, 15.13. Found: C, 34.53; H, 3.00; N, 14.80. NMR (60 MHz, d_6-DMSO) δ: 4.00 (3H, s, OCH_3), 4.38 (2H, s, $ClCH_2$), 8.00 (1H, s, thiazole 5-H).

Practical preparation of **8b** from methyl acetoacetate (**10**)

(a) Methyl 2-methoxyimino-3-oxobutyrate (**9**)

Methyl acetoacetate (**10**) (350 g) and $NaNO_2$ (250 g) were dissolved in water (1.3 liters). To this was added dropwise with stirring a solution of c.H_2SO_4 (146 ml) in water (1.3 liters) while keeping the temperature below 5°C (1.5 hours). After stirring for 2.5 hours at 5~8°C, the mixture was extracted with AcOEt (2 liters×2). The combined extracts were washed twice with satd. aqueous NaCl. The organic layer was then extracted 3 times each with a solution of Na_2CO_3 (212 g) in water (2.1 liters). To the combined aqueous extracts which contain methyl 2-hydroxyimino-3-oxobutyrate was added MeOH (1.3 liters). Dimethyl sulfate (500 ml) was added dropwise to this stirred solution while keeping the temperature below 20°C (1 hour). After addition, the mixture was stirred at room temperature for 1.5 hours and kept in the cold (5°C) overnight. The separated solids were collected by suction and dissolved in $CHCl_3$ (1.7 liters) after washing with water (500 ml×2). The $CHCl_3$ solu-

tion was washed with water (300 ml×2). To the residue obtained by concentration of the dried $CHCl_3$ layer was added *n*-hexane to cause crystallization. Crystals were collected and washed with *n*-hexane to give **9** as colorless crystals, 351 g. Mp 64.5~65°C. *Anal.* Calcd. for $C_6H_9NO_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 44.93; H, 5.61; N, 8.71. NMR (60 MHz, $CDCl_3$) δ: 2.40 (3H, s, $COCH_3$), 3.86 (3H, s, $COOCH_3$), 4.10 (3H, s, OCH_3).

(b) Methyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetate (**11b**)

To a warmed (38°C) solution of **9** (960 g) in $CHCl_3$ (3.6 liters) was added dropwise with stirring a solution of Br_2 (910 g) in $CHCl_3$ (1.1 liters) below 43°C* (3 hours). After stirring for 1 hour at room temperature, the mixture was poured into ice-water (4 liters). The organic layer was separated and washed with satd. aqueous $NaHCO_3$ (2 liters), and then with water (2 liters) and dried over Na_2SO_4. Evaporation of $CHCl_3$ yielded crude methyl 4-bromo-2-methoxyiminoacetate (**10**). NMR (60 MHz, $CDCl_3$) δ: 3.82 (3H, s, $COOCH_3$), 4.09 (3H, s, N-OCH_3), 4.27 (2H, s, $BrCH_2$).

The crude ester (**10**) was dissolved in THF (8.4 liters) and to this was added under ice-cooling a solution of $AcONa \cdot 3H_2O$ (2.45 kg) and thiourea (912 g) in water (6 liters) in one portion. After stirring at room temperature for 10 hours, the mixture was extracted with AcOEt (6 liters×2). The combined AcOEt layer was washed with satd. aqueous $NaHCO_3$ (5 liters) and then with water (10 liters) and dried over Na_2SO_4. To the residue obtained by evaporation of AcOEt was added Et_2O (2 liters) to form a crystalline substance which was collected by suction to afford **11b** as yellowish crystals, 774 g. Mp 169~170°C. *Anal.* Calcd. for $C_7H_9N_3OS$: C, 39.06; H, 4.21; N, 19.52. Found: C, 38.78; H, 4.15; N, 19.33. NMR (60 MHz, $CDCl_3$) δ: 3.84 (3H, s, CH_3), 4.02 (3H, s, OCH_3), 5.74 (2H, bs, NH_2), 6.74 (1H, s, thiazole 5-H).

(c) 2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**8b**)

To an ice-cooled solution of **11b** (721 g) in DMA (3.5 liters) was added dropwise with stirring chloroacetyl chloride (300 ml). After addition, the mixture was stirred at room temperature for 30 minutes and poured into ice-water (7 liters). The separated solids were collected by suction to give methyl 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetate (**12b**). Mp 130.8°C. *Anal.* Calcd. for $C_9H_{11}ClN_3O_4S$: C, 37.05; H, 3.45; N, 14.40 Found: C, 37.30; H, 3.40; N, 14.35. NMR (60 MHz, $CDCl_3$) δ: 3.90 (3H, s, $COOCH_3$), 4.02 (3H, s, N-OCH_3), 4.26 (2H, s, $ClCH_2$), 7.24 (1H, s, thiazole 5-H).

To a suspension of wet **12b** and water (2.5 liters) was added dropwise a solution of KOH (420 g) in water (6 liters) below 15°C. After addition, the mixture was stirred for 1.5 hours to obtain a solution. This solution was acidified (pH 1.5) with 10% HCl (2.5 liters) causing separation of a solid which was extracted with THF - AcOEt (1: 2) (6 liters) and then with THF - AcOEt (1: 4) (6 liters). The combined organic layers were washed with satd. aqueous NaCl (6 liters) and dried over Na_2SO_4.

After evaporation of the organic solvents, the separated solids were collected by suction and washed with Et_2O to give **8b**, 391 g. Concentration of the filtrate afforded additional **8b**, 349 g. Total yield was 740 g.

N-Ethyl-2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamide (**13a**)

To a suspension of **2a** (0.7 g) in EtOH (10 ml) was added 70% aqueous ethylamine (8 ml). After stirring for 2.5 hours at room temperature, the mixture was concentrated under reduced pressure and the residue was extracted with AcOEt. Conventional work-up of the AcOEt extract and purification by chromatography on silica gel gave **13a** as colorless crystals (from AcOEt and benzene), 370 mg. Mp 115.4°C (dec.). *Anal.* Calcd. for $C_8H_{12}N_4O_2S$: C, 42.08; H, 5.29; N, 24.54. Found: C, 42.06; H, 5.25; N, 24.89. NMR (60 MHz, d_6-DMSO) δ: 1.08 (3H, t, $CH_2\underline{CH_3}$), 3.24 (2H, q, $\underline{CH_2}CH_3$), 3.92 (3H, s, OCH_3), 7.06 (2H, bs, NH_2), 7.44 (1H, s, thiazole 5-H), 8.30 (1H, bs, CONH).

N-Ethyl-2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamide (**13b**)

A suspension of **2b** (229 mg) in a mixture of EtOH (6 ml) and 70% aqueous ethylamine (2 ml) was heated at 100°C for 4 hours (reactions at room temperature overnight and at 80°C for 4 hours

* Bromination takes place at room temperature in the presence of a small amount of acid such as *p*-toluenesulfonic acid.

resulted in recovery of **2b**). After concentration under reduced pressure, the residue was extracted with AcOEt. Conventional work-up of the extract gave **13b** as colorless crystals (from MeOH - AcOEt), 65 mg. Mp 199.2°C (dec.). *Anal.* Calcd. for $C_8H_{12}N_4O_2S$: C, 42.08; H, 5.29; N, 24.54. Found: C, 42.07; H, 5.39; N, 23.95. NMR (60 MHz, d_6-DMSO) δ: 1.08 (3H, t, $CH_2C\underline{H}_3$), 3.24 (2H, q, $C\underline{H}_2CH_3$), 3.84 (3H, s, OCH_3), 6.74 (1H, s, thiazole 5-H), 7.20 (2H, bs, NH_2), 8.44 (1H, bs, CONH).

2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-ethoxyiminoacetic acid (**14**)

Application of Method C to the reaction of 4-bromo-2-ethoxyimino-3-oxobutyrate[12] (12.70 g) with thiourea (3.43 g) afforded ethyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-ethoxyiminoacetate as yellowish crystals 6.00 g. Mp 132~133°C. *Anal.* Calcd. for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27. Found: C, 44.35; H, 5.34; N, 17.08. NMR (60 MHz, d_6-DMSO) δ: 1.08 and 1.15 (3H×2, t×2, CH_3×2), 4.02 and 4.18 (4H, q, CH_2×2), 6.75 (1H, s, thiazole 5-H), 7.08 (2H, bs, NH_2).

Reaction of this ester (2.43 g) with chloroacetyl chloride (1.35 g) gave ethyl 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-ethoxyiminoacetate as colorless crystals (from a mixture of *n*-hexane and AcOEt), 2.45 g. Mp 122~123°C. *Anal.* Calcd. for $C_{11}H_{14}ClN_3O_4S$: C, 41.32; H, 4.41; N, 13.14. Found: C, 41.42; H, 4.32; N, 13.39. NMR (60 MHz, $CDCl_3$) δ: 1.29 and 1.35 (3H×2, t×2, CH_3×2), 4.05~4.60 (4H, q, CH_2×2), 4.29 (2H, s, $ClCH_2$), 7.25 (1H, s, thiazole 5-H).

To a solution of the above chloroacetamido ester (1.92 g) in EtOH (15 ml) was added 1 N NaOH (17 ml). After stirring for 30 minutes at room temperature, the mixture was concentrated under reduced pressure. The residue was washed with AcOEt and the aqueous layer was then extracted with AcOEt after acidification (pH 1.5) with 3 N HCl. Conventional work-up of the extract gave **14** as colorless crystals (from a mixture of $CHCl_3$ and *n*-hexane), 1.51 g. Mp 176~177°C (dec.). *Anal.* Calcd. for $C_9H_{10}ClN_3O_4S$: C, 37.06; H, 3.46; N, 14.40. Found: C, 37.08; H, 3.39; N, 14.38. NMR (60 MHz, d_6-DMSO) δ: 1.14 (3H, t, CH_3), 4.07 (2H, q, CH_2), 4.25 (2H, s, $ClCH_2$), 7.40 (1H, s, thiazole 5-H).

2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-isopropyloxyiminoacetic acid (**15**)

Treatment, similar to that employed for the synthesis of **14**, of 4-bromo-2-isopropyloxyimino-3-oxobutyrate[12] (14.0 g) with thiourea (3.81 g) gave ethyl 2-(2-aminothiazol-4-yl)-(*Z*)-2-isopropyloxyiminoacetate as an oil, 10.95 g. NMR (60 MHz, d_6-DMSO) δ: 1.21 (6H, d, CH_3×2), 1.26 (3H, t, $C\underline{H}_3CH_2$), 4.28 (2H, q, $CH_3C\underline{H}_2$), 4.38 (1H, m, CH), 6.77 (1H, s, thiazole 5-H), 7.13 (2H, bs, NH_2).

Reaction of this ester (2.57 g) with chloroacetyl chloride (1.50 g) afforded ethyl 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-isopropyloxyiminoacetate as colorless crystals, 1.99 g. Mp 109~110°C. *Anal.* Calcd. for $C_{12}H_{16}ClN_3O_4S$: C, 43.18; H, 4.83; N, 12.59. Found: C, 43.15; H, 5.00; N, 12.29. NMR (90 MHz, $CDCl_3$) δ: 1.28 (6H, d, CH_3×2), 1.36 (3H, t, $C\underline{H}_3CH_2$), 4.25 (2H, s, $ClCH_2$), 4.42 (1H, m, CH), 7.22 (1H, s, thiazole 5-H), 10.03 (1H, bs, NH).

Hydrolysis of the chloroacetamido ester (4.95 g) gave rise to **15** as colorless crystals, 3.35 g. Mp 161~162°C. *Anal.* Calcd. for $C_{10}H_{12}ClN_3O_4S$: C, 39.29; H, 3.96; N, 13.74. Found: C, 39.04; H, 4.07; N, 13.68. NMR (90 MHz, d_6-DMSO) δ: 1.22 (6H, d, CH_3×2), 4.33 (2H, s, $ClCH_2$), 4.37 (1H, m, CH), 7.47 (1H, s, thiazole 5-H), 12.84 (1H, s, COOH).

(*Z*)-2-(*t*-Butyloxycarbonyl)methoxyimino-2-(2-chloroacetamidothiazol-4-yl)acetic acid (**16**)

To a solution of **6** (0.5 g) in DMA (4 ml) was added chloroacetyl chloride (338 mg). After stirring for 1.5 hours at room temperature, the mixture was poured into water. The separated solids were collected and recrystallized from EtOH to give ethyl (2-chloroacetamidothiazol-4-yl)glyoxylate as colorless crystals, 650 mg. Mp 172.6°C. *Anal.* Calcd. for $C_9H_9ClN_2O_4S$: C, 39.06; H, 3.27; N, 10.12. Found: C, 38.96; H, 3.09; N, 9.95. NMR (60 MHz, d_6-DMSO) δ: 1.28 (3H, t, CH_3), 4.32 (2H, q, CH_2), 4.36 (2H, s, $ClCH_2$), 8.46 (1H, s, thiazole 5-H).

To a stirred suspension of the above chloroacetamido glyoxylate (2.80 g) in EtOH (50 ml) was added 1 N NaOH (20 ml). After stirring for 2 hours at room temperature, the mixture was concentrated under reduced pressure. The residue was washed with AcOEt and the aqueous layer was then extracted with AcOEt after acidification (pH 2.0) with 2 N HCl. Conventional work-up of the extract gave (2-chloroacetamidothiazol-4-yl)glyoxylic acid as yellowish crystals (from a mixture of *n*-hexane and AcOEt), 2.15 g. Mp 205~210°C (dec.). *Anal.* Calcd. for $C_7H_5ClN_2O_4S$: C, 33.81; H, 2.03;

N, 11.27. Found: C, 33.94; H, 2.04; N, 11.23. NMR (60 MHz, d_6-DMSO) δ: 4.29 (2H, s, $ClCH_2$), 8.30 (1H, s, thiazole 5-H).

To a solution of the chloroacetamido acid (249 mg) in a mixture of water (5 ml) and THF (3 ml) was added O-(*t*-butyloxycarbonyl)methylhydroxylamine[11] (160 mg) (Method B). The reaction mixture was adjusted to pH 7.0 with 1 N NaOH. After stirring for 20 hours at room temperature, the mixture was washed with AcOEt. The aqueous layer was then extracted with AcOEt after acidification (pH 2.0) with 2 N HCl. Conventional work-up of the extract afforded **16** as slightly yellowish crystals, 320 mg. Mp 180~182°C (dec.). *Anal.* Calcd. for $C_{13}H_{16}ClN_3O_6S$: C, 41.33; H, 4.27; N, 11.12. Found: C, 41.15; H, 4.21; N, 10.91. NMR (60 MHz, d_6-DMSO) δ: 1.30 (9H, s, C_4H_9), 4.26 (2H, s, $ClCH_2$), 4.52 (2H, s, OCH_2), 7.44 (1H, s, thiazole 5-H).

Ethyl 2-methoxyimino-2-(2-methylthiazol-4-yl)acetate (**17**)

A solution of **1** (25.1 g) and thioacetamide (8 g) in EtOH (200 ml) was refluxed for 2 hours. After concentration under reduced pressure, water was added to the residue. The mixture was neutralized with $NaHCO_3$ and extracted with AcOEt. Conventional work-up of the extract gave an oily product, 16.3 g. The oil was chromatographed on silica gel and eluted with $CHCl_3$ - *n*-hexane (1: 3). From the more mobile fraction was obtained (*Z*)-ester (**17b**) as an oil, 2.1 g. NMR (60 MHz, $CDCl_3$) δ: 1.39 (3H, t, CH_3), 2.72 (3H, s, thiazole 2-CH_3), 4.01 (3H, s, OCH_3), 4.44 (2H, q, CH_2), 7.39 (1H, s, thiazole 5-H).

From the less mobile fraction was obtained (*E*)-ester (**17a**), as an oil, 4.2 g. NMR (60 MHz, $CDCl_3$) δ: 1.39 (3H, s, CH_3), 2.75 (3H, s, thiazole 2-CH_3), 4.05 (3H, s, OCH_3), 4.44 (2H, q, CH_2), 7.92 (1H, s, thiazole 5-H).

(*E*)-2-Methoxyimino-2-(2-methylthiazol-4-yl)acetic acid (**18a**)

To a solution of **17a** (800 mg) in EtOH (10 ml) was added a solution of KOH (0.5 g) in water (1 ml). After stirring for 30 minutes at room temperature, the mixture was poured into water and extracted with AcOEt after acidification (pH 2.0) with 1 N HCl. Conventional work-up of the extract gave **18a** as colorless crystals, 595 mg. Mp 140~141°C. *Anal.* Calcd. for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99. Found: C, 41.95; H, 3.97; N, 13.85. NMR (60 MHz, d_6-DMSO) δ: 2.70 (3H, s, thiazole 2-CH_3), 4.03 (3H, s, OCH_3), 8.26 (1H, s, thiazol 5-H).

(*Z*)-2-Methoxyimino-2-(2-methylthiazol-4-yl)acetic acid (**18b**)

Similar treatment of **17b** (2.1 g) afforded **18b** as colorless crystals, 940 mg. Mp 165~167°C. *Anal.* Calcd. for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99. Found: C, 41.95; H, 3.97; N, 14.02. NMR (60 MHz, d_6-DMSO) δ: 2.69 (3H, s, thiazole 2-CH_3), 3.96 (3H, s, OCH_3), 7.84 (1H, s, thiazole 5-H).

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