4. H. L. Berg and E. M. Purcell, *Biophys. J.*, **20**, 193 (1977).
5. H. Kawauchi, H. Tsubokawa, A. Kanezawa and E. Kitagawa, *Biochem. Biophys. Res. Comm.*, **92**, 1278 (1980).
6. M. J. Sheetz and H. S. Tager, *J. Biol. Chem.*, **263**, 19210 (1988).
7. P. Past, G. Vecchio and G. Carrea, *Biochim. Biophys. Acta*, **953**, 314 (1988).
8. A. M. Albisser and W. D. Lougheed, UK patent application GB 2070620A (1981).
9. W. B. Gratzer, G. H. Beaven, and E. Bailey, *Biochem. Biophys. Res. Comm.*, **28**, 914 (1967).
10. F. Szoka, Jr. and D. Papahadijopoulos, *Proc. Natl. Acad. Sci. (USA)*, **95**, 4194 (1978).
11. V. E. Vaskovsky, E. Y. Kostesky, and I. M. Vasendin, *J. Chromatogr.*, **114**, 129 (1975).
12. M. K. Markwell, S. M. Haas, L. L. Bieber, and N. E. Tolbert, *Anal. Biochem.*, **87**, 206 (1978).
13. A. F. Sikorski and R. B. Daczynska, *Biochim. Biophys. Actd*, **690**, 302 (1982).
14. R. W. Cogwill, *Biochim. Biophys. Acta*, **133**, 6 (1967).
15. W. W. Bromer, A. Staub, L. G. Sinn, and O. K. Behrens, *J. Am. Chem. Soc.*, **79**, 2801 (1957).
16. V. Keil-Dlouha, N. Zlber, J. M. Imhoff, N. T. Tong, and B. Keil, *FEBS Letter*, **16**, 291 (1971).
17. G. Allen, Sequencing of proteins and peptides (Laboratory techniques in biochemistry and molecular biology; vol. 9), Elsevier Elsevier Science Publisher B. V., Elsevier Science Publishing Co., New York, p 172 (1983).
18. M. Schiffer and A. B. Edmundson, *Biophys. J.*, **7**, 121 (1967).
19. W. Braun, G. Wider, K. H. Lee and K. Wuthrich, *J. Mol. Biol.*, **169**, 921 (1983).
20. J. Kim and H. Kim, *Biochemistry*, **25**, 7867 (1986).
21. J. Kim and H. Kim, *Biochim. Biophys. Acta.*, **983**, 1 (1989).
22. J. Kim and H. Kim, *Arch. Biochem. Biophys.*, **274**, 100 (1989).

New Aminothiazolyl Cephalosporins. Synthesis and Biological Evaluation of 7-[Alkoxyiminomethyl(2-aminothiazol-4-yl)acetamido]ceph-3-em-4-carboxylic Acids

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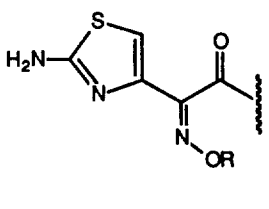
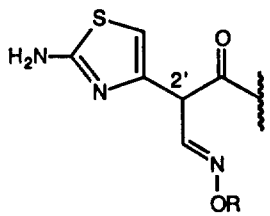
New aminothiazolyl cephalosporins with alkoxyiminomethyl(2-aminothiazol-4-yl)acetyl substituents at 7-position of cephems were synthesized starting from (2-aminothiazol-4-yl)acetate *via* one carbon homologation followed by acylation with 7-aminoceph-3-em-4-carboxylic acid derivatives. These new aminothiazolyl cephalosporins exhibit promising *in vitro* activities against various strains including Gram positive bacteria.

Introduction

Cephalosporin antibiotics with aminothiazole as a part of 7-position substituent have been increasingly popular in recent cephalosporin research.[1] In connection with our studies in developing new oral cephalosporins, we have become interested in structural modification of aminothiazole acetic acid oxime side chain at 7-position of cephalosporins. Typical structure of this 7-substituent is shown in the following formula (I):



(I)



(II)

It has occurred that alteration of the length of the tether chain at 2′ position might lead to change in biological activities. The simplest substituent at 7-position in cephalosporins along this line is shown in the formula (II). We wish to report here the synthesis of the new cephalosporins having modified substituents at 7-position of cephems which can be represented by the general formula (II) and their antibacterial activities.

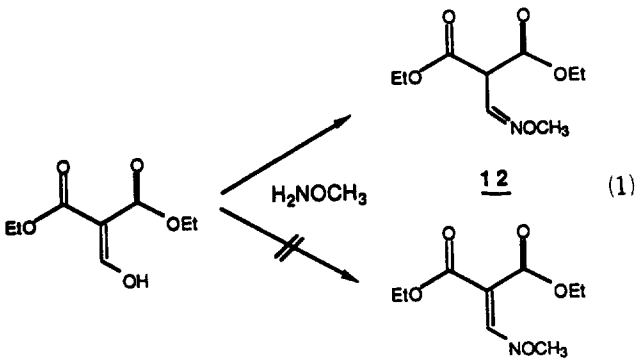
Resutls and Discussion

The synthetic route to the aminothiazolylcephalosporins with one-carbon homologation at 2′-position is shown in Scheme 1. Starting from ethyl (2-aminothiazol-4-yl) acetate (1), *N*-protected compound **2** was prepared.[2] One carbon homologation at 2′-position was achieved by the reaction of **2** with methyl formate in the presence of a base followed by hydrolysis. Successful acylations by DCC promoted coupling were observed between the active esters(5) derived from **3** and the corresponding properly protected 7-aminocephem-4-carboxylic acid **6** to form the coupled products **7**. After acid hydrolysis of **7**, the enols **8** were reacted

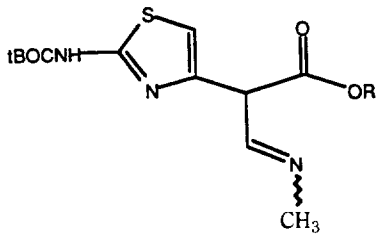
with alkoxyamines (**9**) to provide the imines **10**. Deprotection of **10** furnished the desired cephalosporins **11** and completed the synthetic sequence.

It was not clear whether the products after addition of alkoxyamines were imines (as the structures shown in Scheme 1) or enamines. The imine structure was favored based upon the observed symmetry in structure by the analysis of the ^{1}H NMR spectrum of the product **12**(not **13**) [only one kind of ethyl protons in ^{1}H NMR($CDCl_3$, δ): 1.33(t, 6H), 4.30(q, 4H)] as a model compound prepared from the reaction of diethyl 2-formylmalonate with methoxyamine (Equation 1).[3]

Complication due to the introduction of a stereocenter at 2'-position is possible. In fact the HPLC analysis of **10a** showed four separate isomers. We were able to separate these diastereomers and obtained their ^{1}H-NMR spectra. Two of the four isomers after deprotection were subjected to biological evaluation (*vide infra*).



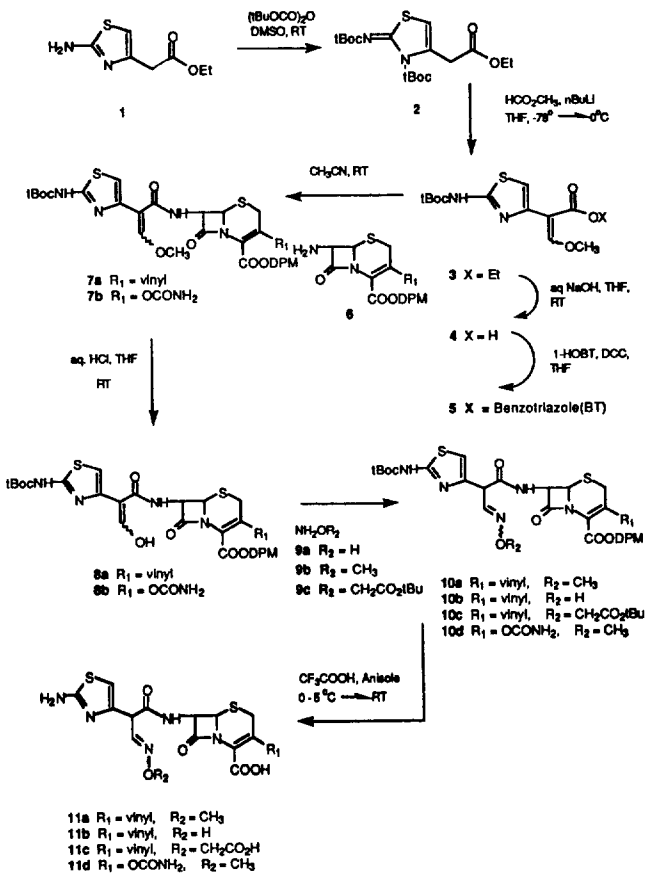
A route which involves the coupling between acid **15** and cephem **6** was also investigated. Although addition of hydroxyamines **9** to enol ether (e.g. **3**) to prepare **14** was proceeded without any incident, synthesis of **15** from **14** proved difficult, and all the attempts including hydrolysis to prepare **15** from the corresponding ester **14** (R = CH_3, benzyl, allyl, and etc) were met with failure presumably due to the unstability of acid **15**.



14 R = CH_3

15 R = H

The representative cephalosporin derivatives prepared herein **11a-d** are shown in Scheme 1. Their MIC values were evaluated and summarized in Table 1. The two of the four isomers in **10a** (designated **10a-1**, **10a-2**, **10a-3**, and **10a-4** in which the last numbers stand for the order of elution in the HPLC separation), that is, **10a-3** and **10a-4** were successfully separated as pure forms and submitted to biological evaluation after deprotection. The separation of the remaining two isomers having shorter retention times (e.g., **10a-1** and **10a-2**) was found to be difficult due to their unstability after separation. The origin of this unstability as well as the assignment



Scheme 1.

of the structures of the four isomers remains to be clarified. The MIC values indicated that **11a-3** and **11a-4** manifested better activities than the mixture of isomers before separation. Therefore, the indication is that purification of the mixture of isomers could improve the biological activities.[4] Changes in the biological activities upon variation of the substitution at 3-position of cephems are also of interest. Carbamoyloxymethyl group at 3-position instead of vinyl brought a considerable improvement of the activities against Gram-positive and negative bacteria. The derivative **11c** was prepared in the hope that it would lead to an increase in the activity against *Pseudomonas* strains. Relatively low activity of **11c** could be attributed to its instability.

In summary, we have described a successful synthetic route to the new cephalosporins having modified substituent at 7-position of cephems and the evaluation of their antibacterial activities. Further elongation of the tether length in aminothiazole acetic acid oxime side chain are under current investigation in order to establish the optimum structure in terms of the biological activities.

Experimental

General. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with one of the following: a Jeol PMX60SI, a Varian FT-80A, or a Bruker AM 200 spectrometer. Infrared (IR) spectra were obtained with a Perkin-Elmer 1310 spectrometer. The *in vitro* biological activity of the cephalosporins prepared was determined by conven-

Table 1. MIC Values of the Compounds Prepared

Strains	11a	11a-3	11a-4	11b	11c	11d
Streptococcus pyogenes A308	0.049	0.049	0.002	1.563	0.049	0.049
Streptococcus pyogenes A77	100	0.025	0.004	0.781	0.025	0.013
Staphylococcus aureus SG511	3.125	3.125	1.563	12.5	0.781	1.563
Staphylococcus aureus 285	3.125	3.125	3.125	12.5	0.781	1.563
Staphylococcus aureus 503	1.563	1.563	1.563	12.5	0.781	0.195
Escherichia coli 055	1.563	1.563	0.781	12.5	3.125	0.195
Escherichia coli DC 0	3.125	3.125	3.125	25	6.25	0.391
Escherichia coli DC 2	0.781	0.781	0.391	25	6.25	0.098
Escherichia coli TEM	3.125	6.25	3.125	25	6.25	0.391
Pseudomonas aeruginosa 1771M	6.25	12.5	6.25	50	25	0.391
Salmonella typhimurium	0.781	1.563	0.781	12.5	1.563	0.098
Klebsiella aerogenes 1522 E	0.781	1.563	0.781	6.25	0.781	0.195
Enterobacter cloacae 1321E	0.781	0.781	0.391	6.25	0.781	0.049

tional agar dilution procedures.

Ethyl(2-*tert*-butoxycarbonylimino-3-*tert*-butoxycarbonylthiazol-4-yl)acetate (2). This compound was prepared according to the method previously reported.[2] A solution of 5 g (0.027 mol) of ethyl (2-aminothiazol-4-yl) acetate and 15 g (0.069 mole) of di-tert-butyldicarbonate in 8 m*l* of dimethyl sulfoxide (DMSO) was stirred for 7 days at room temperature. After the mixture was cooled to 0 °C, white solid was obtaind by the addition of 100 m*l* of ice-cooled water. The solid was filtered and dissolved in 70 m*l* of CH_2Cl_2. The organic layer was washed with water (50 m*l* × 3) and concentrated to provide crude product. Removal of impurities by washing with petroleum ether provided 7.2g (69%) of **2** as solid. ^{1}H NMR($CDCl_3$, δ): 1.23(t, 3H), 1.50(s, 9H), 1.58(s, 9H), 3.70(s, 2H), 4.15(q, 2H), 6.24(s, 1H).

Ethyl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxypropenoate (3). To a solution of 10g (0.026 mol) of **2** in tetrahydrofuran (THF) was added 1.86g (0.029 mol) of n-BuLi(Hexane solution) and 1.91g (0.30 mol) of methyl formate at -50~-60 °C. The mixture was stirred at -40~-30 °C for 1.5 h and warmed to room temperature. Aqueous (10%) citric acid (26 mL, 0.015 mol) was added and stirred for 10 min. After concentration of the mixture, the residue was diluted with 10 m*l* of water and extracted with CH_2Cl_2. Concentration of the organic layer followed by purification by column chromatography provided 6.4g (75%) of **3** as a crystalline solid (mp. 129~130 °C). ^{1}H-NMR($CDCl_3$, δ): 1.15(t, 3H), 1.51(s, 9H), 3.82(s, 3H), 4.18(q, 2H), 7.08(s, 1H), 7.56(s, 1H).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-3-methoxypropenoic acid (4). To a solution of 5.91g (0.018 mol) of the ester **3** in the minimum amount of THF (ca. 100 m*l*) was added 50 m*l* of 2 N NaOH solution. After the solution was stirred for 1 day at room temperature, it was diluted with water and washed with CH_2Cl_2. The pH of the aqueous layer was adjusted to 4.0~5.5 using 1N HCl solution and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography afforded 3.8g (70%) of **4** as a solid [mp. 178 °C (dec)]. ^{1}H NMR ($CDCl_3$, δ): 1.53(s, 9H), 4.03(s, 3H), 7.28(s, 1H), 7.82(s, 1H).

Benzotriazol-1-yl 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxypropenoate (5). A solution of 1.2 g (4.0 mmol) of **4** and 0.54g (4.0 mmol) of 1-hydroxybenzotriazole hydrate in 120 m*l* of THF was cooled to 0 °C. A solution of 0.83 g(4 mmol) of *N,N'*-dicyclohexylcarbodiimide (DCC) in 100 mL of THF was added and the resulting solution was stirred for 3 days at room temperature. After filtration, the filtrate was concentrated to about half volume, and then cooled to precipitate the residual by-products. The deposited impurities were filtered, and the filtrate was concentrated to reduce the volume of the solution to 20~30 m*l*. The crystallization of the desired product was intitiated by stirring at 0 °C. Filtration followed by concentration provided the desired ester. After repeating the crystallization procedure a total of 1.3g (78%) of the desired ester **5** was as a white obtained crystal. IR(KBr, cm^{-1}): 1780, 1730, 1640; ^{1}H-NMR ($CDCl_3$, δ): 1.50(s, 9H), 4.13(s, 3H), 7.33(s, 1H), 7.43~8.13(m, 4H), 8.45(s, 1H).

Benzhydryl 7-[[2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxyl]propenoyl]amino-3-vinylceph-3-em-4-carboxylate (7a). A solution of 0.4g(1.0 mmol) of benzhydryl 7β-amino-3-vinyl-3-cephem-4-carboxylate (**6a**) and 0.42g (1.0 mmol) of benzotriazol-1-yl 2-(2-tert-butoxycarbonylaminothiazol-4-yl)-3-methoxypropenoate (**5**) in 25 m*l* of acetonitrile was stirred for 24 h at room temperature. The insolubles were filtered and the filtrate was concentrated. After the residue was dissolved with 20 m*l* of ethyl acetate, the solution was washed with 10% HCl solution, saturated $NaHCO_3$, and saturated NaCl solution, dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane:ethyl acetate = 2:1) afforded 0.50g (74%) of the acyation product **7a** as a light yellow solid [mp 100-105 °C (dec)]. ^{1}H NMR($CDCl_3$, δ): 5.17(d, 9H, J=5 Hz), 5.33(d, 1H, J=6 Hz), 5.90(dd, 1H), 7.03(m, 2H), 7.40(s, 1H), 7.50(s, 10H), 7.90(s, 1H), 8.97(br s, 1H), 10.13(d, 1H).

Benzhydryl 7-[[2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxy]propenoyl]amino-3-carbamoyloxymethyl-3-cephem-4-carboxylate (7b). A solution of 1.8g (4.36 mmol) of benzhydryl 7β-amino-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**6b**) and 1.91g (4.36 mmol) of benzotriazol-1-yl 2-(2-tert-butoxycarbonylaminothiazol-4-yl)-3-methoxypropenoate (**5**) in 160 m*l* of THF was stirred for 20 h at room temperature. The stirring was continued for additional 3 h at 45~50 °C. The insolubles were filtered

and the filtrate was concentrated. After the residue was dissolved with 20 m*l* of ethyl acetate, the solution was washed with 10% HCl solution, saturated $NaHCO_3$, and saturated NaCl solution, dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (n-hexane: ethyl acetate = 2:1) afforded 1.23g (39%) of the acylation product **7b** as a light yellow solid. No attempt was made for the optimization of the yield. ^{1}H-NMR($CDCl_3$, δ): 1.51(s, 9H), 3.4(m, 2H), 3.8(s, 3H), 4.8(d, 1H), 5.50(dd, 2H), 5.2(br s, 2H), 5.8(dd, 1H, J = 6 Hz), 6.9(s, 1H), 7.2(s, 1H), 7.4(s 10H), 7.8(s, 1H), 9.8(d, 1H).

Benzhydryl 7-[[2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-2-formyl]acetyl]amino-3-vinylceph-3-em-4-caboxylate (8a). A solution of 1.2g (1.78 mmol) of benzhydryl 7-[[2-[2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxy]propenoyl]amino-3-vinylceph-3-em-4-carboxylate (40 m*l*) and 10% aqueous HCl solution (20 m*l*) in 40 m*l* of THF was stirred for 24 h at room temperature. The solution was concentrated and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried ($MgSO_4$), and concentrated. Purification by passing through a short silica gel column followed by the addition of mixed solvent (hexane: ethyl acetate = 20:1) provided the white precipitate. Filtration provided the desired compound **8a** as a white solid (0.59g, 50%). ^{1}H-NMR($CDCl_3$, δ): 1.40(s, 9H), 3.15(q, 2H), 4.73(d, 1H, J = 5 Hz), 4.90-5.50(m, 3H), 6.33(s, 1H), 6.57(s. 1H), 6.90(m, 1H), 7.25(s, 10H), 8.13(d, 1H, J = 7Hz), 10.90(br s, 1H).

Benzhydryl 7-[[2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxyimino]propanoylamino-3-vinylceph-3-em-4-carboxylate (10a). To a solution of methoxyamine hydrochloride (25-30% aqueous solution, 1.8 m*l*, 6.0 mmol) and 0.10g (0.15 mmol) of 8a in 5 m*l* of acetonitrile was added aqueous $NaHCO_3$ solution to adjust the pH to 4~5. After stirring the solution for 3 h at room temperature, white solid was precipitated. Filtration aforded 0.05g (50%) of the desired compound as a white solid.

Separation of Isomers. Column (Partisil), Solvent (n-Hexane:Ethyl ether = 3:1), Flow rate(2 m*l*/min) for analytical scale sepration for ^{1}H-NMR spectra. Preparative scale separation for **10a-3** and **10a-4** were achieved using semipreparative column chromatography: Column (LiChrosorb RP-18), Solvent (n-hexane:ethyl ether = 3:2), Flow rate (4 m*l*/min).

10a-1. ^{1}H-NMR($CDCl_3$, δ): 1.48(s, 9H), 3.41(q, 2H), 3.81(s, 3H), 4.45(d, 1H), 4.93(d, 1H), 5.22(d, 1H), 5.38(d, 1H), 5.75(dd, 1H), 6.67(s, 1H), 6.90(s, 1H), 7.20(s, 10H), 7.55(d, 1H).

10a-2. ^{1}H-NMR($CDCl_3$, δ): 1.56(s, 9H), 3.48(q, 2H), 3.87(s, 3H), 4.50(d, 1H), 5.01(d, 1H), 5.29(d, 11H), 5.46(d, 1H), 5.81(dd, 1H), 6.71(s, 1H), 6.97(s, 1H), 7.26(s. 10H), 7.62(d, 1H).

10a-3. ^{1}H-NMR($CDCl_3$, δ): 1.48(s, 9H), 3.50(q, 2H), 3.82(s, 3H), 4.93(d, 1H), 5.08(d, 1H), 5.22(d, 1H), 5.39(d, 1H), 5.77(dd, 1H), 6.66(s, 1H), 6.91(s, 1H), 7.08(d, 1H), 7.19(s, 10H).

10a-4. ^{1}H-NMR($CDCl_3$, δ): 1.48(s, 9H), 3.57(q, 2H), 3.94(s, 3H), 5.01(d, 1H), 5.17(d, 1H), 5.30(d, 1H), 5.46(d, 1H), 5.84(dd, 1H), 6.74(s, 1H), 6.97(s, 1H), 7.13(d, 1H), 7.26(s, 10H).

7-[[2-(2-Aminothiazol-4-yl)-3-methoxyimino]propanoyl]amino-3vinylceph-3-em-4-carboxylic acid (11a). To a solution of 0.2 m*l* (1.8 mmol) of anisole in 2.0 m*l* (26.0 mmol) of trifluoroacetic acid was added a solution of 0.05g (0.07 mmol) of **9a** in 1.5 m*l* of CH_2Cl_2 dropwise at 0 °C and the resulting solution was stirred for 1 h at room temperature. The solution was concentrated and dried under vacuum. Trituration with ether followed by filtration furnished 0.027g (90%) of the desired compound as light yellow colored solid. **11a-3** and **11a-4** were prepared by the same procedure from **10a-3** (79%) and **10a-4** (54%), respectively. **11a-3:** ^{1}H-NMR(DMSO-d_6, δ): 3.65(q, 2H), 3.88(s, 3H), 5.21(d, 1H), 5.31(d, 1H), 5.44(d, 1H), 5.72(d, 1H), 6.09(q, 1H), 6.46(s, 1H), 7.06(q, 1H), 7.21(br s, 2H), 7.66(d, 1H). **11a-4** ^{1}H-NMR(DMSO-d_6, δ): 3.66(q, 2H), 3.89(s, 3H), 5.21(d, 1H), 5.44(d, 1H), 5.73(d, 1H), 6.10(q, 1H), 6.45(s, 1H), 7.20(br s, 2H), 7.68(d, 1H).

Benzhydryl 7-[[2-[2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-hydroxyimino]propanoyl]-amino-3-vinylceph-3-em-4-carboxylate (10b). Hydroxylamine hydrochloride (0.30g, 4.3 mmol) was dissolved in 5 m*l* of water and 15 m*l* of acetonitrile. While stirring, 0.30g (0.45 mmol) of **8a** was dissolved and 2 N aqueous NaOH solution was added slowly to adjust the pH to 5~6. After stirring for 30 min at room temperature, the solution was extracted with ethyl acetate. The organic layer was washed with water, saturated sodium chloride solution, dried ($MgSO_4$), and concentrated. Addition of hexane to the residue followed by filtration provided 0.25g (82%) of **10b** as a white solid. ^{1}H-NMR ($CDCl_3$, δ): 1.58(s, 9H), 3.46(m, 2H), 5.00(d, 1H), 5.27-5.50(m, 3H), 5.85(dd, 1H), 6.80(s, 1H), 7.03(s, 1H), 7.11(m, 1H), 7.35(s, 10H), 7.80(m, 1H).

7-[[2-Aminothiazol-4-yl)-3-hydroxyimino]propanoyl]amino-3-vinylceph-3-em-4-carboxylic acid (11b). A solution of 0.5 m*l* (4.5 mmol) of anisole in 4.0 m*l* (52.0 mmol) of trifluoroacetic acid was cooled at 0 °C. A solution of 0.20g (0.18 mmol) of **10b** in 3.5 m*l* of CH_2Cl_2 was added dropwise at 0 °C and the resulting solution was stirred for 1 h at room temperature. The solution was concentrated and dried under vacuum. Trituration with ether followed by filtration furnished 0.056g (78%) of the desired compound as a light yellow colored solid. ^{1}H NMR(DMSO-d_6, δ): 3.78(q, 2H), 4.90(d, 1H), 5.12(d, 1H), 5.37(d, 1H), 5.65(d, 1H), 6.98(dd, 1H), 8.40(s, 1H).

Benzhydryl 7-[[2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-2-formyl]acetyl]amino-3-carbamoyloxymethylceph-3-em-4-carboxylate (8b). A solution of 0.2g (0.28 mmol) of benzhydry 7-[[(2-(2-*tert*-tutoxycarbonylaminothiazol-4-yl)-3-methoxy]propenoyl]amino-3-carbamoyloxymethylceph-3-em-4-carboxylate (**7b**) and 3 m*l* of 10% aquous HCl solution in 6 m*l* of THF was stirred for 24 h at room temperature. After removal of THF under reduced pressure, the solution was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl solution, dried($MgSO_4$), and concentrated. Purification by passing the residue through a short silica gel column followed by treatment of ethyl acetate and hexane provided 0.1g (51%) of **8b**. ^{1}H-NMR($CDCl_3$, δ): 1.40(s, 9H), 3.27(q, 2H), 4.50~5.00(m, 3H), 5.60(dd, 1H), 6.40(s, 1H), 7.22(s, 10H), 7.40(d, 1H).

Benzhydryl 7-[[2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-3-methoxyimino]propanoyl]amino-3-carba-

moyloxymethylceph-3-em-4-carboxylate (10d). To a solution of methoxyamine hydrochloride (25-30% aqueous solution, 1.0 m*l*, 3.3 mmol) and 0.08g (0.15 mmol) of **8b** in 4 m*l* of acetonitrile was added 2 N NaOH solution to adjust the pH of the solution to 5~6. After stirring for 30 min at room temperature, the solution was extracted with ethyl acetate. The organic layer was washed with water, saturated sodium chloride solution, dried ($MgSO_4$) and concentrated. Addition of hexane to the residue followed by filtration provided 0.076g (91%) of **10d** as a white solid. ^{1}H-NMR($CDCl_3$, δ): 1.60(s, 9H), 3.40(q, 2H), 3.95(d, 3H), 4.50~5.30(m, 3H), 5.80(m, 1H), 6.68(s, 1H), 6.95(s, 1H), 7.38(s, 10H), 7.75(m, 1H).

7-[[2-(2-Aminothiazol-4-yl)-3-methoxyimino]propanoyl]amino-3-carbamoyloxymethylceph- 3-em-4-carboxylic acid (11d). A solution of 0.1 m*l* (0.9 mmol) of anisole in 1.0 m*l* (13.0 mmol) of trifluoroacetic acid was cooled at 0 °C. A solution of 0.20g (0.18 mmol) of **10d** in 1.0 m*l* of CH_2Cl_2 was added dropwise at 0 °C and stirred for 40 min at room temperature. The solution was concentrated, dried under vacuum. Trituration with ether followed by filtration furnished 0.019g (100%) of **11d** as a light yellow-colored solid. ^{1}H-NMR (DMSO-d_6, δ): 3.30(q, 2H), 3.78(s, 3H).

***N-tert*-Butoxycarbonylmethoxyphthalimide.** To a suspension of 2g of *N*-hydroxyphthalimide (0.012 mol) in 8.4 m*l* of CH_3CN were added 1.4g (0.013 mol) of Et_3N and 2.9g of *t*-butyl bromoacetate. The mixture was heated at reflux for 1.5 h. When the reaction mixture was cooled to room temperature, solid was precipitated. The solid was dissolved with water and extracted with ethyl acetate. Concetration of the organic layer followed by treatment of the resulting residue with hexane produced 3.2g (94%) of the desired imide as a white solid. ^{1}H NMR($CDCl_3$, δ): 1.48(s, 9H), 4.68(s, 2H), 7.79(s, 4H).

***tert*-Butoxycarbonylmethoxyamine (9c).** A solution of 3.07g (0.011 mol) of *N*-*t*-butoxycarbonylmethoxyphtalimide in CH_2Cl_2 was added to a solution of hydrazine monohydrate (770 mg, 15 mmol) in 1.3 m*l* of EtOH. The solution was stirred for 30 min at room temperature after which it was concentrated. The resulting residue was extracted with 5% aqueous HCl solution. The aqueous layer was washed with ethyl ether and the pH was adjusted to 7.5 by addition of ammonia water. After extraction with CH_2Cl_2 and concentration of the organic phase afforded 410 mg (24.4%) of *N*-*t*-butoxycarbonylmethoxyamine (**9c**) as an oil. No effort was made to optimize the yield. ^{1}H NMR($CDCl_3$, δ): 1.50(s, 9H), 4.10(br s, 2H), 5.70(s, 4H.

Benzhydryl 7-[[2-{2-(*N*-*tert*-butoxycarbonyl)aminothiazol-4-yl}-3-{t-butoxycarbonylmethoxyimino} propanoyl]-amino]-3-vinylceph-3-em-4-carboxylate (10c). A solution of 88 mg (0.6 mmol) of *t*-butoxycarbonylmethoxyamine (**9c**) and 200 mg (0.3 mmol) of **8a** in 3 m*l* of acetonitrile was stirred for 18 h at room temperature. After the solution was extracted with ethyl acetate, the organic layer was washed with water, saturated sodium chloride solution, dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane:ethyl acetate = 1:1) afforded 190 mg (80%) of **10c** as a white solid. ^{1}H-NMR($CDCl_3$, δ): 1.53(s, 9H), 1.65(s, 9H), 3.56(q, 2H), 3.71(s, 3H), 5.03(d, 1H), 5.49(d, 1H), 5.93(dd, 1H), 6.79(s, 1H), 7.01(s, 1H), 7.31(d, 1H), 7.45(10H, s).

7-[{2-(2-Aminothiazol-4-yl)-3-(carboxymethoxyimino)propanoyl} amino]-3- vinylceph -3-em-4-carboxylic acid (11c). To a solution of 0.2 m*l* (1.8 mmol) of anisole in 2.0 m*l* (26.0 mmol) of trifluoroacetic acid was added a solution of 85 mg (0.11 mmol) of **10c** in 2.0 m*l* of CH_2Cl_2 dropwise at 0 °C. After stirring for 1 h at room temperature, the solution was concentrated and dried under vacuum. Treatment with methanol-ehtyl acetate furnished 41 mg (79%) of **11c** as a light yellow-colored solid. ^{1}H-NMR (DMSO-d_6, δ): 3.56(q, 2H), 3.65(s, 2H), 4.96(d, 1H), 5.15(d, 1H), 5.41(d, 1H), 5.53(dd, 1H), 6.29(s, 1H), 6.70(q, 1H), 6.71(s, 1H), 8.91(d, 1H).

Acknowledgement. The financial support of the Ministry of Science and Technology, Korea is gratefully appreciated.

References

1. W. Dürckheimer, G. Fisher and R. Laterell, "Recent advances in the chemistry of β-lactam anitibiotics", Fourth International Symposium, P. H. Bently and R. Southgate, Eds., Royal Society of Chemistry, London, pp. 49-76 1988.
2. Kinast, G. German Patent DE 3 145 727, 1983.
3. On the other hand, addition of alkyl amine such as methyl amine to enol 8 afforded enamines which confirmed the fact that the enamine is stabilized and therefore the enamine-imine equilibra is shifted in favor of the enamine. See also (a) B. Capon and Z. P. Wu, *J. Org. Chem.*, **55**, 2317 (1990); (b) R. A. Clark and D. C. Parker, *J. Am. Chem. Soc.*, **93**, 7257 (1971); (c) H. Ahlbrecht and R.-D. Kalas, *Justus Liebigs Ann. Chem.*, 102 (1979).
4. For an example, see S. Shibahara, T. Okonogi, T. Yoshida, Y. Murai, T. Kudo, S. Inouye, and S. Kondo, *J. Antibiot.*, **43**, 62 (1990).