Synthesis and Biological Evaluations of *N*-(2-Substituted-1-carboxyl) vinylazetidinones: A Study on the Essential Structural Element for Biological Activities of β-Lactam Antibiotics

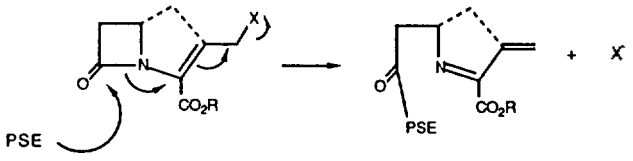
Han-Young Kang*, Ae Nim Pae, Hun Yeong Koh, and Moon Ho Chang*

Chemistry Division, Korea Institute of Science and Technology, Seoul 130-650. *Received September 8, 1990*

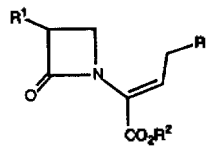
A series of compounds, *N*-(2-substituted-1-carboxy)vinylazetidinones were successfully synthesized in order to test the hypothesis that biological activities of β-lactam antibiotics could be attributed to the smooth flow of electrons after a nucleophilic attack at the carbonyl carbon in the β-lactam ring. After introduction of aminothiazolylacetamido group at 3-position of the azetidinones, their biological activities were evaluated. Their low activities led to the conclusion that the smooth electron flow in β-lactams in not the sufficient source for the biological activities.

Introduction

The significance of understanding structure-activity relationship cannot be overestimated in developing biologically active pharmaceuticals. β-Lactam antibiotics have been targets for extensive research in establishing structure-activity relationships due to their paramount importance in the antibiotics research field. It has been accepted that the activities of the compounds with β-lactam ring could be related to the facility of being attacked by nucleophiles which presumably exist in bacterial proteins (*e.g.*, by a nucleophilic moiety in the penicillin sensitive enzyme). In other words, the smooth electron flow after the nucleophilic attack at the carbonyl carbon in the β-lactam ring could play a crucial role for the biological activities.



As an effort to deduce the essential structural element needed to manifest the biological activities we prepared a series of compounds,[1] that is, *N*-(2-substituted-1-carboxy)vinylazetidinones shown in the following general formula (**I**) in the hope that these azetidinone derivatives would provide an excellent test for the proposed relations between the smooth flow of electrons and the biological activty in the antibiotics containing β-lactam ring.



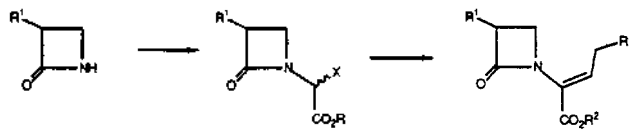
(**I**)

The concept that β-lactam ring is the essential part of the pharmacophore has been challenged by the discovery of lactivicin[2] as well as the recent active research in γ-lactam related compounds[3]. Therefore, as analogs to the azetidinones, cycloserine derivatives, in which the cycloserine moiety is the core structure, are also of interest.

In this article we disclose our effort for development of the synthetic method to the derivatives (**I**) and evaluatioins of their biological activities.

Results and Discussion

Our strategy toward synthesis of **I** is based on the reaction between appropriately substituted azetidinone and glyoxalic acid derivatives followed by Witting olefination. The similar synthetic route was quite well established in the Woodward's penem synthesis.[4]

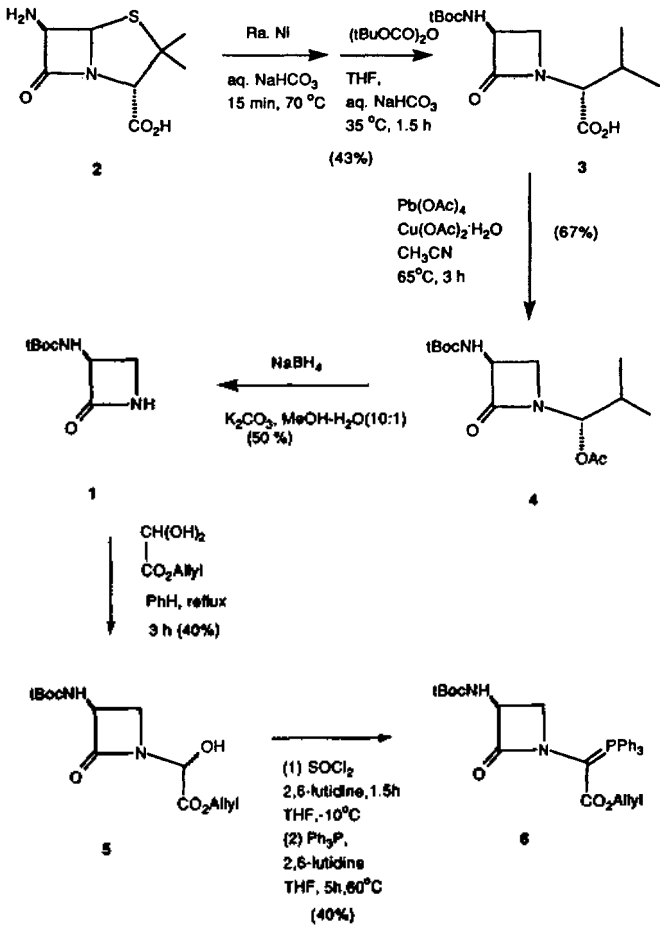


(**II**) (**III**)(X= halogen) (**I**)

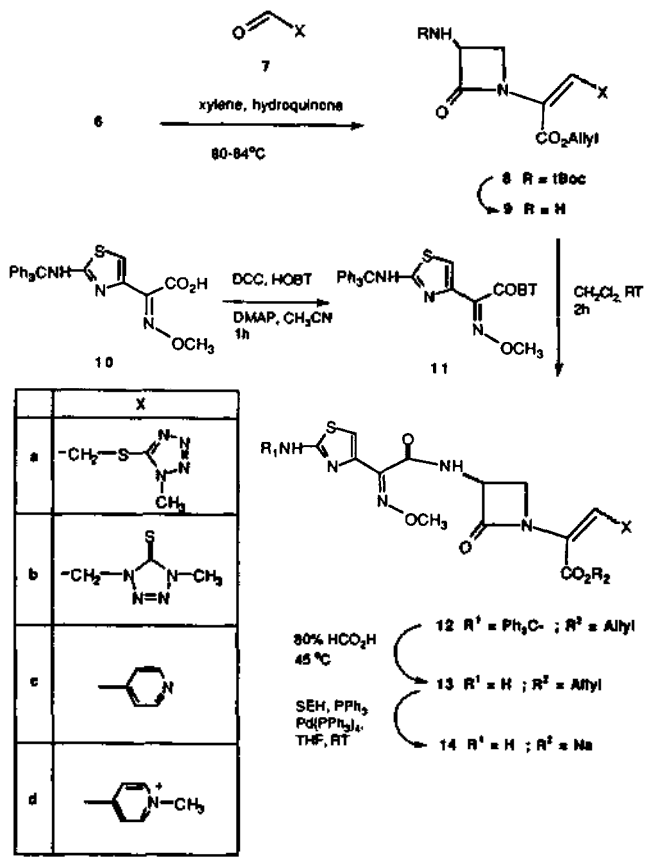
We have initially tested our strategy with the unsubstituted compound at 3-position of azetidinone [*i.e.*, (**II**; R_1=H)]. Thus, a series of the desired compounds (**I**; R_1=H) were prepared by applying the successfully developed synthetic route. Unfortunately the derivatives without any substituent at 3-position exhibited disappointingly low biological activities. The deficiency of the activities eventually prompted us to reinvestigate this synthetic route for the derivatives properly substituted at 3-position. The choice of the substitution can be easily decided, since aminothiazolyl moiety has been increasingly popular in the recent development of β-lactams including cephalosporins as well as monobactam related antibiotics. It is now obvious that we need to have the 3-aminoazetidinone **1** as an starting material.

In this study we have limited the substituent to a (2-aminothiazol-4-yl)methoxyiminoacetyl group in order to confine our investigation to the effect upon variation of *N*-substituent (more specifically X group in **I**). The synthesis of **1** is depeciated in Scheme 1.

The C-S bond in 6-APA (**2**) was cleaved and the 3-amino group was protected with *tert*-butyloxycarbonyl group (tBoc). Protection of 3-amino group with Cbz group proved difficult in the deprotection at the later stage. This led us to use tBoc group as the protecting group. The yield of the protection step was poor when the reaction was performed at room temperature. Heating at 35℃ led to an improvement in yield. The next two steps were carried out without any incident



Scheme 1.



Scheme 2.

to provide the desired tBoc-protected 3-aminoazetidinone **1**.[5] Reaction with glyoxalate followed by chlorination by $SOCl_2$ and formation of phosphorus ylide **6** set stage for the Wittig reaction with the appropriately substituted aldehydes.

The synthetic route to 3-(aminothiazolylacetamido)azetidinone derivatives prepared are shown in Scheme 2. The Yield was subjected to the Witting reaction with aldehyde **7** in xylene in the presence of hydroquinone. The mixtures of two stereoisomers were produced. The ratios for the two isomers were approximately 3:1, 2:1, and 1:1 for **8a**, **8b**, and **8c**, respectively (by ^{1}NMR anslysis). The mixtures were subjected to further reactions without separation. Deprotection of tBoc group in the resulting coupled products **8a-c** to generate amines **9a-c** was achieved by the treatment of acid. Acylation was performed *via* active ester. Thus, the reaction between active ester **11** (prepared from **10**) and amine **9** provided the acylated product **12**. Deprotection of **12** was the only remaining task and this was achieved by a two-step procedure, that is, acid hydrolysis of trityl group and palladium mediated deallylation. The second step of deprotection-deallyation step was accompanied by sodium salt formation (by the treatment with SEH (sodium 2-ethylhexanoate). Four 3-(aminothiazolylacetamido)azetidinone derivertives **14 a-d** were prepared and evaluated their biological activities (MIC).

The MIC values for **14a-d** and summarized in Table 1. Substitution with *N*-methyl-5-thiotetrazole, *N*-methyltetrazoline-5-thione, 4-pyridyl group[6] or N-methyl salt of 4-pyridyl group exhibited low *in vitro* activities against various strains. This result suggested that the simple electron flow after nucleophilic attack on the carbonyl cabon in the β-lactam ring is, at least, not the sufficient source for the biological activities in β-lactam antibiotics.

Table 1. MIC Values of the Azetidinones Prepared

Strains	Azetidinones			
	14a	**14b**	**14c**	**14d**
Streptococcus pyogenes A308	25	3.125	0.781	nd
Streptococcus pyogenes A77	6.25	0.781	0.195	0.391
Staphylococcus aureus SG511	100	100	25	100
Staphylococcus aureus 285	100	100	50	100
Staphylococcus aureus 503	100	100	25	100
Escherichia coli O55	3.125	6.25	6.25	25
Escherichia coli DC0	12.5	25	12.5	100
Escherichia coli DC2	25	12.5	12.5	100
Escherichia coli TEM	6.25	25	12.5	50
Pseudomonas aeruginosa 1771M	100	50	100	100
Salmonella typhimurium	25	50	12.5	100
Klebsiella aerogenes 1522E	3.125	12.5	6.25	25
Enterobacter cloacae 1321E	6.25	25	6.25	50

In summary, we have successfully developed a synthetic route to the *N*-(2-substituted-1-carboxy)vinylazetidinones. Introduction of the aminothiazolylacetamido group at 3-position of azetidinone ring provided the desired compounds **14**

a-d. Low *in vitro* activities of these derivatives led us to conclude that the simple electron flow after the nucleophilic attack (*e.g.*, by a nucleophilic moiety in the PSE) is, at least, not the crucial factor for exhibiting high biological activities. Extension of the results of this study to cephems and penems is currently underway.

Experimental

General. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. 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 azetidinones prepared was determined by conventional agar dilution procedures.

1-[(1*R*)-Carboxy-2-methylpropyl]-(3*S*)-[t-butoxycarbonylamino]azetidin-2-one (3). A mixture of 4.5 g (0.02 mol) of 6-APA and 1.9 g of $NaHCO_3$ in 50 m*l* of water was added into a slurry of Raney Nickel (45 g) at 70℃ with stirring. After stirred for 15 min at 70℃ and the mixture was cooled and filtered. The filtrate was dissolved in 125 m*l* of tetrahydrofuran (THF) and to this solution was added a solution of 5.5 m*l* (0.024 mmol) of di-t-butyl dicarbonate in 20 m*l* of THF dropwise at 0℃, After completion of the reaction and concentration followed by extraction with ethyl ether, the pH of the aqueous layer was adjusted to about 2 with 1 N HCl solution. The solution was extracted with CH_2Cl_2 and the organic phase was dried ($MgSO_4$) and concentrated. The desired acid (3) was obtained by addition of ethyl ether (2.3 g, 40%). ^{1}H-NMR($CDCl_3$, δ); 1.0 (d, 6H, CH_3), 1.5 (s, 9H, $C(CH_3)_3$), 1.8-2.5 (m, 1H, CH-$(CH_3)_2$), 3.3 (m, 1H, C_4-H), 3.9 (t, 1H, C_4-H), 4.2 (d, 1H, N-CH-iPr), 4.8 (m, 1H, C_3-H), 5.7 (br d, 1H, NH).

1-[Acetyloxy-2-methylpropyl]-(3*S*)-[t-Butoxycarbonylamino]azetidin-2-one (4). To a solution of 3 (4.95 g, 17.3 mmol) in acetonitrile (180 m*l*) was added lead tetraacetate (7.64 g, 17.2 mmol) and cupric acetate monohydrate (1.74 g, 8.71 mmol). The mixture was heated at reflux for 3 h. After the reaction was completed the reaction mixture was filtered. The flitrate was concentrated and dried under vacuum. The residue was taken with ethyl acetate and water after which the solution was neutralized (pH=7). The organic phase was concentrated and the resulting residue was triturated with ether to provied a solid. Drying under vacuum furnished acetate 4 (3.14 g, 67%) as a solid: mp. 126-128℃. ^{1}H-NMR ($CDCl_3$, δ); 0.9 (d, 6H, $(CH_3)_2$), 1.5 (s, 9H, $C(CH_3)_3$), 2.1 (s, 3H, $COCH_3$), 2.2 (m, 1H,-CH-iPr), 3.3 (m, 1H, C_4-H), 3.6 (t, 1H, C_4-H), 4.7 (br s, 1H, C_3-H), 5.3 (d, 1H, NH), 5.7 (d, 1H, NCHOAc). IR (KBr, cm^{-1}); 3348, 2970, 1710, 1756.

3-[*N*-(t-Butoxycarbonyl)amino]azetidin-2-one (1). To a solution of 4 (1.61 g, 5.94 mmol) in methanol (41 m*l*) and water (4.1 m*l*) was added K_2CO_3 (762 mg, 5.51 mmol) and sodium borohydride (199 mg, 5.26 mmol). This solution was stirred for 2 h at −5-0℃. The pH of the solution was adjusted to 7 with 1 N HCl and MeOH was evaporated and then pH was adjusted to 6. The solution was extracted with ethyl acetate and the organic layer were separated and concentrated. Trituration with ether afford 1 (553 mg, 50%) as a solid: mp. 210℃ (decomp). ^{1}H-NMR ($CDCl_3$, δ); 1.4 (s, 9H, $-C(CH_3)_3$), 3.2 (m, 1H, C_4-H), 3.5 (t, 1H, C_4-H), 4.7 (br s, 1H, C_3-H), 5.4 (d, 1H, C_3-NH), 6.2(s, 1H, NH). IR (KBr, cm^{-1}) 2979, 1728, 1730.

Allyl [2-(3*S*)-(t-Butoxycarbonylamino)azetidin-2-on-1-yl] hydroxyacetate (5). Allyl glyoxalate monohydrate (564 mg, 4.27 mmol) was added to a solution of 1 (530 mg, 2.8 mmol) in benzene (50 m*l*). The solution was heated at reflux and water was taken off using Dean-Stark apparatus. Approximately 3 h was needed to complete the reaction. Concentration followed by purification by column chromatography provided hydroxyacetate 5 (336 mg, 40%) as an oil. ^{1}H-NMR ($CDCl_3$, δ); 1.4 (s, 9H, $C(CH_3)_3$), 3.5 (m, 1H, C_4-H), 3.7 (m, 1H, C_4-H), 4.7 (d, 2H, $CH_2C=C$), 4.8 (m, 1H, C_3-H), 5.4 (d, 2H, $C=CH_2$), 5.5-6.1 (m, 1H, $CH=C$).

Allyl [2-(3*S*)-(t-Butoxycarbonylamino)azetidin-2-on-1-yl]triphenylphosporanylidene acetate (6). To a solution of 5 (360 mg, 1.2 mmol) in dry THF (14 m*l*) was added 2,6-lutidine (357 μ*l*, 3.06 mmol) and thionly chloride (135 μ*l*, 1.85 mmol) at −20℃. The solution was stirred at −10℃ for 1.5 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in THF (12 m*l*). To this solution 2,6-lutidine (294 μ*l*) and triphenyl phosphine (637 mg, 2.43 mmol) was added. The solution was heated at reflux for 5 h. The solution was extracted with ethyl acetate and 0.3 N HCl, washed with saturated sodium chloride, dried, and concentrated. Purification by column chromatography afforded phosphorane 6 (264 mg, 40%). ^{1}H-NMR($CDCl_3$, δ); 1.3 (s, 9H, $C(CH_3)_3$), 4.2-4.3 (m, 2H, C_4-H), 4.4 (m, 1H, C_3-H), 4.6 (d, 2H, $CH_2C=C$), 5.1-5.3 (m, 2H, $C-C=CH_2$), 5.8-6.0 (m, 1H, $CH=C$), 7.4-7.7 (m, 15 H, CPh_3). IR (KBr, cm^{-1}); 3080, 2961, 1753, 1709.

1-Methyl-5-(ethanal-2-yl) thiotetrazoline (7a). Catalytic amount of osmium tetroxide and *N*-methylmorpholine-*N*-oxide was added to a solutioin of 1-methyl-5-allylthiotetrazoline[7] (630 mg, 4.03 mmol) in acetone and water (4:1, total of 42 m*l*) at room temperature. The solution was stirred for 12 h at room temperature after which sodium thiosulfate was added. Filtration, extraction with ethyl acetate-water (2:1), and dried (Na_2SO_4) yielded a diol. This diol was dissloved in 50% aqueous methanol (42 m*l*). Sodium periodate (1.37 g, 6.4 mmol) was added to this solution and stirred at room temperature for 12 h. Filtration, extraction with ethyl acetate, and concentration of the extract provided aldehyde 7a (382 mg, 60%). This aldehyde was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$, δ); 4.0 (s, 3H, CH_3), 4.2 (d, 2H, $-CH_2-$), 9.8 (t, 1H, CHO).

1-Methyl-4-(ethanal-2-yl)tetrazolin-5-thione (7b). Ozone was bubbled through a solution of 1-methyltetrazolin-5-thione[7] (8.5 g, 54.3 mmol) in CH_2Cl_2 at −78℃ until the reaction was completed. Dimethyl sulfide (3.71 g, 59.8 mmol) was added and the resulting solution was stirred at room temperature for 4 h. Concentration and purification of the residue by column chromatography yielded aldehyde 7b (1.72 g, 20%). No further effort was made to optimize the yield. ^{1}H-NMR ($CDCl_3$, δ); 3.9 (s, 3H, CH_3), 5.1 (d, 2H, CH_2), 9.9 (t, 1H, CHO).

Allyl 2-[2-(3*S*)-(t-Butoxycarbonylamino)azetidin-2-on-1-yl]-3-[(1-methyl-1*H*-tetrazol-5-yl) thio]methyl propenoate (8a). To a solution of phosphorane 6 (150 mg,

0.23 mmol) in xylene (5 m*l*) were added aldehyde **7a** (90 mg, 0.57 mmol) and hydroquinone (catalytic amount). The solution was stirred at 84℃ for 3 h. After concentration purification by flash chromatography (hexane: ethyl acetate = 2:3) afforded **8a** (79 mg, 60%). ^{1}H-NMR ($CDCl_3$, δ); 1.4 (s, 9H, $C(CH_3)_3$), 3.7 (m, 1H, C_4-H), 3.9 (s, 3H, NCH_3), 4.2 (m, 1H, C_4-H), 4.4 (d, 2H, $C=CCH_2S$), 4.7 (d, 2H, CH_2-C=C), 4.9 (br s, 1H, C_3-H), 5.3 (m, 2H, $C=CH_2$), 5.9-6.1 (m, 1H, CH=C), 6.6-6.9 (t, 1H, C=CH-C-S).

Allyl 2-[2-(3*S*)-(t-Butoxycarbonylamino)azetidin-2-on-1-yl]-3-(1-methyl-1,4-dihydro-5*H*-tetrazol-5-thion-4-yl) methyl propenoate (8b). To a solution of phosphorane **6** (300 mg, 0.57 mmol) in xylene (5 m*l*) were added aldehyde **7b** (175 mg, 1.1 mmol) and hydroquinone (catalytic amount). The solution was stirred at 84℃ for 3 h. After concentration purification by flash chromatography (hexane: ethyl acetate = 1:1) afforded **8b** (131 mg, 55%). ^{1}H-NMR ($CDCl_3$, δ); 1.4 (s, 9H, $C(CH_3)_3$), 3.6 (m, 1H, C_4-H), 3.8 (s, 3H, NCH_3), 4.0-4.1 (m, 1H, C_4-H), 4.6 (d, 2H, CH_2-C=C), 4.9 (br s, 1H, C_3-H), 5.1-5.4 (m, 2H, -C=CH_2), 5.8-6.0 (m, 1H, CH=C), 6.6 (t, 1H, $CH=CCH_2S$).

Allyl 2-[2-(3*S*)-(t-Butoxycarbonylamino)azetidin-2-on-1-yl]-3-(pyridin-4-yl) propenoate (8c). To a solution of phosphorane **6** (300 mg, 0.57 mmol) in xylene (5 m*l*) were added pyridine carboxaldehyde **7c** (100 μ*l*, 1.06 mmol) and hydroquinone (catalytic amount). The solution was stirred at 84℃ for 5 h. After concentration purification by flash chromatography (hexane:ethyl acetate = 1:2) afforded **8c** (102 mg, 48%). ^{1}H-NMR ($CDCl_3$, δ); 1.4 (s, 9H, $C(CH_3)_3$), 3.8 (m, 1H, C_4-H), 3.9 (t, 1H, C_4-H), 4.8 (d, 2H, CH_2-C=C), 4.9 (br s, 1H, C_3-H), 5.3 (m, 2H, -CH=CH_2), 5.8-6.1 (m, 1H, CH=C), 6.8 (s, 1H, =CH-Py), 7.2 (d, 1H, Py), 7.5 (d, 1H, Py), 8.5 (d, 1H, Py), 8.6 (d, 1H, Pv).

Allyl 2-[2-(3*S*)-aminoazetidin-2-on-1-yl]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl propenoate (9a). To a cooled solution of trifluoroacetic acetic acid (4.8 m*l*, 62.3 mmol) and anisole (672 μ*l*, 6.2 mmol) were added **8a** (260 mg, 0.61 mmol) in CH_2Cl_2 (1.2 m*l*) dropwise at 0℃. The solution was stirred at room temperature for 2 h. The solution was concentrated and the residue was dissolved in CH_2Cl_2. After the solution was washed with $NaHCO_3$ solution, the organic layer was dried ($MgSO_4$) and concentrated. Trituration with ether furnished **9a** (131 mg, 66%) as a solid which was used without further purification. ^{1}H-NMR ($CDCl_3$, δ); 3.8 (m, 1H, C_4-H), 3.9 (s, 3H, NCH_3), 3.9-4.5 (m, 4H, C_4-H + C_3-H + CH_2), 4.7 (d, 2H, CH_2-CH=C), 5.2 (d, 1H, -C=CH_2), 5.4 (d, 1H, -C=CH_2), 5.8-6.0 (m, 1H, -CH-C=C), 6.7 (m, 1H, -C=CH-CH_2S).

Allyl 2-[2-(3*S*)-aminoazetidin-2-on-1-yl]-3-(1-methyl-1,4-dihydro-5H-tetrazol-5-thion-4-yl) methyl propenoate (9b) and Allyl 2-[2-(3*S*)-aminoazetidin-2-on-1-yl]-3-(pyridin-4-yl) propenoate (9c). The deprotection of tBOC group was achieved as the same procedure described in the prepartion of **9a**. The yield of **9b** and **9c** were 57 and 70%, respectively. **9a**, **9b**, and **9c** were used immediately for the next acylation step without further purification. **9b**: ^{1}H-NMR ($CDCl_3$, δ); 3.7-4.3 (m, 3H, C_3-H + C_4-H), 3.9 (s, 3H, NCH_3), 4.7 (d, 2H, -CH_2-C=C), 5.1 (d, 2H, CH_2-N), 5.4 (d, 2H, C=CH_2), 5.6-6.2 (m, 1H, -CH=C), 6.6 (t, 1H, =CH-CH_2N). **9c**: ^{1}H-NMR ($CDCl_3$, δ); 3.6-4.4 (m, 3H, C_4-H + C_3-H), 4.8 (d, 2H, CH_2-C=C), 5.5 (m, 2H, C=CH_2), 5.7-6.3 (m, 1H, -CH=C), 7.5 (m, 3H, CH-Py), 8.8 (d, 2H, Py).

Allyl 2-[2(3*S*)-[2-tritylaminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl propenoate (12a). To a suspension of 2-[2-tritylaminothiazol-4-yl]-2-methoxyiminoacetic acid (300 mg, 0.623 mmol) in acetonitrile (5 m*l*) were added dicyclohexylcarbodiimide (DCC) (150 mg, 0.72 mmol), 1-hydroxybenzotriazole hydrate (HOBT) (100 mg, 0.74 mmol), and dimethylaminopyridine (DMAP) (catalytic amount). The mixture was stirred for 1 h at room temperature and filtered. Concentration followed by drying under vacuum provided an active ester **11a**. This active ester was dissolved in 3 m*l* of CH_2Cl_2. To this solution was added a solution of amine **9a** (100 mg, 0.31 mmol) in 2 m*l* of CH_2Cl_2. The solution was stirred for 2 h at room temperature and then washed with water. The organic layer was concentrated and the residue was purified by column chromatography (silica gel: hexane: ethyl acetate = 1:3) followed by trituration with isopropyl ether to provide **12a** as a solid (87 mg, 36%). ^{1}H-NMR ($CDCl_3$, δ); 3.4-3.8 (m, 1H, C_4-H), 3.9 (s, 3H, NCH_3), 4.1 (s, 3H, $NOCH_3$), 4.2 (m, 1H, C_4-H), 4.4 (d, 2H, CH_2-S), 4.6-4.8 (d, 2H, CH_2-C=CH_2), 5.1 (br s, 1H, C_3-H), 5.3-5.5 (m, 2H, C=CH_2), 5.8-6.1 (m, 1H, CH=C), 6.7-6.9 (t, 1H, =CH-CH_2S), 6.8 (s, 1H, aminothiazole-H), 7.3 (s, 15H, CPh_3). IR (KBr, cm^{-1}); 3320, 2948, 1760, 1724, 1675.

Allyl 2-[2-(3*S*)-[2-tritylaminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-(1-methyl-1,4-dihydro-5*H*-tetrazol-5-thion-4-yl)methyl propenoate (12b) and Allyl 2-[2-(3*S*)-[2-tritylaminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3 (pyridin-4-yl) propenoate (12c). The coupled products **12b** and **12c** were prepared according to the procedure described in the preparation of **12a** by the acylation of the active ester **11** with **9b** and **9c**, respectively. The yields of 40% for **12b** and 30% for **12c** were obtained. No further attempt to optimize the yields for the coupling was pursued. **12b**: ^{1}H-NMR ($CDCl_3$, δ); 3.3-3.5 (m, 2H, C_4-H), 3.8 (s, 3H, NCH_3), 4.0-4.1 (m, 1H, C_3-H), 4.1 (s, 3H, $NOCH_3$), 4.7 (d, 2H, CH_2 C=C), 5.0-5.3 (m, 2H, C=CH-CH_2N), 5.3 (d, 2H, -C=CH_2), 5.9 (m, 1H, -CH=C), 6.5-6.8 (m, 1H, =CH-CH_2N), 6.7 (s, 1H, aminothiazole-H), 7.3 (s, 15H, -CPh_3). **12c**: ^{1}H-NMR ($CDCl_3$, δ); 3.7 (m, 1H, C_4-H), 3.8-4.1 (m, 1H, C_4-H, 4.1 (s, 3H, $NOCH_3$), 4.8 (d, 2H, CH_2-C=C), 4.9 (m, 1H, C_3-H), 5.3 (m, 2H, C=CH_2), 5.7-6.2 (m, 1H, =CH-Py), 6.7 (s, 1H, aminothiazole-H), 6.8 (m, 1H, C=CH-Py), 7.2 (s, 15H, -CPh_3), 7.3 (d, 2H, Py), 8.4-8.6 (d, 2H, Py).

[2-[2-(3*S*)-[2-tritylaminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-2-[allyloxycarbonyl]ethen-4-yl]pyridinium iodide (12d). A solution of **12c** (70 mg, 0.095 mmol) in acetone (3 m*l*) and methyl iodide (308 μ*l*, 4.95 mmol) was stirred for 5 h at room temperature. The solvent was evaporated. Trituration of the residue with ether provided a salt **12d** (60 mg, 70%). **12d**: ^{1}H-NMR (CD_3OD, δ); 3.6-3.8 (m, 2H, C_4-H), 3.9 (s, 3H, $NOCH_3$), 4.3 (s, 3H, Py-NCH_3), 4.3 (d, 1H, C_3-H), 4.9 (d, 2H, -CH_2-C=C), 5.2-5.5 (m, 2H, C=CH_2), 5.9-6.2 (m, 1H, -CH=C), 6.7 (s, 1H, aminothiazole-H), 7.3 (m, 15H, -CPh_3), 8.1 (d, 2H, Py), 8.7 (d, 2H, Py).

Allyl 2-[2-(3*S*)-[2-aminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-[(1-methyl-1*H*-tetrazol-5-yl)-thio]methyl propenoate (13a). A solution

of **12a** (60 mg, 0.077 mmol) in 80% formic acid (0.6 m*l*) was stirred for 2 h at 45℃. Filtration followed by trituration with ether afforded **13a** (30 mg, 73%).

Allyl 2-[2-(3*S*)-[2-aminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-(1-methyl-1,4-dihydro-5*H*-tetrazol-5-thion-4-yl)methylpropenoate (13b), Allyl 2-[2-(3*S*)-[2-aminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-(pyridin-4-yl) propenoate (13c), and 4-[2-[2-(3*S*)-[2-tritylaminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-2-[allyoxycarbonyl]-2-ethenyl]pyridinium iodide (13d). The amines **13b**, **13c**, and **13d** were prepared as described in the preceding procedure for **13a**. Starting from **12b** (60 mg), **12c** (32 mg), and **12d** (70 mg), the deprotected amines, **13b** (30 mg, 70%), **13c** (15 mg, 70%), and **13d** (30 mg, 67%) were obtained, respectively.

Sodium 2-[2-(3*S*)-[2-aminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methylpropenoate (14a). Allyl ester **13a** (30 mg, 0.56 mmol), sodium ethyl hexanoate (SEH) (15 mg, 0.09 mmol), triphenyl phosphine (3 mg, 0.011 mmol), and palladium tetrakis(triphenylphosphine) (6 mg, 0.052 mmol) were added to 1.6 m*l* of THF at 0℃. The mixture was wared to room temperature and stirred for 20 min. Concentration followed by trituration with ether provided a solid. After dissolving into water, freeze drying provided a sodium salt **14a** (15 mg, 50%). ^{1}H-NMR (D_2O, δ); 3.8 (m, 1H, C_4-H), 4.0 (m, 2H, C_4-H+C_2-H), 4.0 (s, 3H, N-CH_3), 4.0 (s, 3H, N-OCH_3), 4.1 (d, 2H, CH_2S-), 6.6 (t, 1H, C=CH-CH_2S), 7.0 (s, 1H, aminothiazole-H). IR (KBr, cm^{-1}); 3392, 1743, 1627.

Sodium 2-[2-(3*S*)-[2-aminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]-3-(1-methyl-1,4-dihydro-5*H*-tetrazol-5-thion-4-yl)methylpropenoate (14b), Sodium 2-[2-(3*S*)-[2-aminothiazol-4-yl]-2-[methoxyimino]acetamido]azetidin-2-on-1-yl]3-(pyridin-4-yl)propenoate (14c), and 4-[2-[2-(3*S*)-[2-tritylaminothiazol-4-yl]-2-[methyoxyimino]acetamido]azetidin-2-on-1-yl]-2-ethen-yl]pyridinium carboxylate (14d). The desired compounds **14b**, **14c**, and **14d** were prepared as described in the preceding procedure for **14a**. Starting from **13b** (21 mg), **13c** (15 mg), and **13d** (30 mg), the desired compounds, **14b** (10 mg, 50%), **14c** (10 mg, 70%), and **14d** (10 mg, 40%) were obtained, respectively. **14b**: ^{1}H-NMR (D_2O, δ); 3.9 (s, 3H, N-CH_3), 4.0 (s, 3H, $NOCH_3$), 3.9-4.1 (m, 2H, C_4-H), 4.2 (t, 1H, C_3-H), 5.2 (d, 2H, C=CH-CH_2N), 6.5 (t, 1H, C=CH-CH_2N), 7.0(s, 1H, aminothiazole-H). IR (KBr, cm^{-1}); 3398, 1745, 1612. **14c**: ^{1}H-NMR (D_2O, δ); 3.8-4.2 (m, 3H, C_4-H+C_3-H), 3.9 (s, 3H, $NOCH_3$), 6.9 (s, 1H, aminothiazole-H), 7.0 (s, 1H, C=CH-Py), 7.5 (d, 2H, Py), 8.5 (d, 2H, Py). IR (KBr, cm^{-1}); 3425, 1747, 1611. **14d**: ^{1}H-NMR (D_2O, δ); 3.9-4.3 (m, 3H, C_4-H+C_3-H), 3.9 (s, 3H, $NOCH_3$), 4.2 (s, 3H, Py^+-CH_3), 6.9 (s, 1H, aminothiazole-H), 7.0 (s, 1H, C=CH-Py), 7.8 (d, 2H, Py), 8.5 (d, 2H, Py).

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

References

1. The literature survey reveals that there are a few related reports: see: (a) D. K. Herron and A. Whitesitt, *U. S. Patent* 4, 195, 021 (1980); (b) A. K. Bosem, I. F. Fernadez, and K. J. Gala, *U. S. Patent* 4, 440, 682 (1984); (c) C. -C. Wei, J. Borgese, and M. Borgese, *Tetraheron Lett.*, **24**, 1875 (1983).
2. (a) S. Harada, S. Tsbotani, T. Hida, H. Ono, and H. Okazaki, *Tetrahedron Lett.*, **27**, 6229 (1986); (b) Y. Nozaki, N. Katayama, S. Harda, H. Ono, and H. Okazaki, *J. Antibiotics* **42**, 84 (1989).
3. For examples see: (a) L. N. Jungheim, C. J. Barnett, J. E. Gray, L. H. Horcher, T. A. Shepherd, and S. K. Sigmund, *Tetrahedron*, **44**, 3119 (1988); (b) J. E. Baldwin and E. Lee, *ibid.* **42**, 6551 (1986); (c) J. E. Baldwin, C. Lowe, and C. J. Schofield, *Tetrahedron Lett.*, **27**, 3461 (1986); (d) D. B. Boyd, T. K. Elzey, L. D. Hatfield, M. D. Kinnick, and J. M. Morin, *ibid.* **27**, 3453 (1986).
4. Ivan Ernest In *Chemistry and Biology of β-Lactam Antibiotics*, R. B. Morin and M. Gordman, Eds., Academic Press, London, Vol. 2, Chapt. 5, 1982.
5. R. B. Sykes and W. L. Parker, GB 2071650A, 1981.
6. An example of lowering of biological activites as the pyridine is quarternized compared with the neutral form in 2-(pyridyl)penem derivatives is reported. See A. Bedeschi, G. Visentin, E. Perrone, F. Giudici, F. Zarini, G. Franceschi, G. Meinardi, P. Castellani, D. Jabes, R. Rossi, and C. D. Bruna, *J. Antibiotics*, **43**, 306 (1990).
7. H. -Y. Kang and M. H. Chang, Proceedings of the 8th Symposium on Organic Chemistry, Korean Chemical Society, Taejeon, Korea. February 9-11, p. 61, 1989.