http://dx.doi.org/10.5012/bkcs.2013.34.1.121

Design and Synthesis of New 4-Alkylthio Monocyclic β-Lactams

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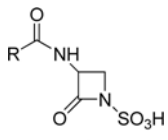
Received August 1, 2012, Accepted October 16, 2012

New types of monocyclic β-lactams constitute an important class of compounds due to their unique structures and natures. Here, the design and synthesis of new 4-alkylthio monocyclic β-lactams **2a** and **3a** are reported. Significantly, compounds **2a** and **3a**, while keeping a monocyclic system, were designed to contain all of the substructures provided by the cleavage of C(2)-C(3) bond in penicillins. Efficient synthetic pathways for compounds **2a** and **3a** were established based on two different strategies. Compound **2a** was synthesized from raw materials, using 4-acetoxyazetidin-2-one as a key intermediate, through a ten-step synthetic sequence in 3% overall yield. Compound **3a** was synthesized from potassium salt of penicillin G (**17**), using the degraded product **20** as a key intermediate, through a six-step synthetic sequence in 11% overall yield. 4-Alkylthioazetidin-2-one derivatives, introduced in this study, could serve as valuable intermediates for the development of new monocyclic β-lactams.

Key Words : Monocyclic β-lactams, Penicillin, Isopropylthioazetidinone, *t*-Butylthioazetidinone, Degradation of penicillin

Introduction

The four-membered ring appears to be the smallest cyclic system that accommodates the amide function as a constituent. Such four-membered cyclic amides, commonly referred to β-lactams, possess physical and chemical properties that diverge sharply from those of acyclic amides, partially due to ring strain. After numerous penicillins and cephalosporins, new types of nonclassical β-lactams have appeared. Monocyclic β-lactam compounds[1] such as monobactams and nocardicins are included in these categories and differ from classical β-lactams in structural features and biological activities. In addition, the most important groups of monocyclic β-lactam antibiotics were discovered in the 1980s. The Imada[2] and Sykes[3] groups independently described a new class of compounds **1**. As characteristic features, these monocyclic β-lactams have a 4-unsubstituted β-lactam ring, a 3-acylamido side chain, and a 1-sulfonate group, which rarely occur in nature.



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Recently, monocyclic β-lactams have been found to display a range of non-antibacterial activities as well as bacterial activities,[4,5] again highlighting the significance of these compounds. The studies on the structures and biological activities of monocyclic β-lactams were reviewed by Galletti and Giacomini.[6] Some of the compounds in this series proved to be biologically active but were chemically unstable and therefore unsuitable for practical use.

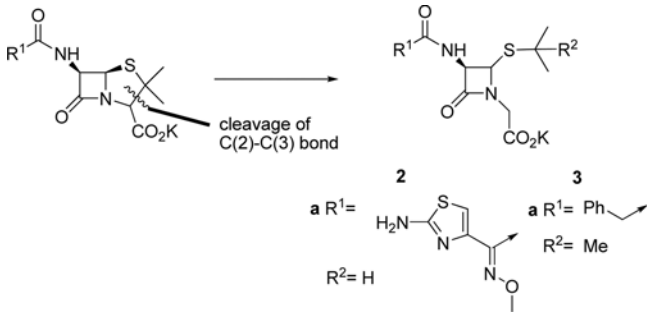
As the biologically active principle of all β-lactam antibiotics is the β-lactam ring, the reactivity and stability of the β-lactam ring must be decisively influenced by substituents. However, the exact influence of each substituent on biological activities remains a puzzle in spite of the development of numerous analogues. Nevertheless, the substituent(s) might have significant effects on amide resonance and molecular geometry and, as a result, govern the amide bond stability and reactivity and the corresponding biological activities.

So, the synthesis of monocyclic β-lactam derivatives that could be more effective than the existing compounds has become an important subject of medicinal interest.[7,8] Accordingly, here I report the design and synthesis of two new monocyclic β-lactams that contain penicillin-similar moieties.

Results and Discussion

Design and Structural Features of 2a and 3a. I wished to design new monocyclic β-lactams and so attempted to breakdown the bicyclic system in the penicillin structure. Based on the importance of the alkylthio group at C(4) and the carboxymethyl group at N(1), I was specifically interested in structures **2** and **3** that would be obtained by the cleavage of C(2)-C(3) bond in the penicillin structure. As a result, **2** and **3** should contain all of the penicillin moieties except the bicyclic ring system. In particular, compound **2a** contained 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido group at C(3) as an acyl side chain and isopropylthio group at C(4) (*trans* to acetamido group), and compound **3a** contained phenylacetamido group at C(3) and *t*-butylthio group at C(4) (*cis* to acetamido group). Notably, compound **2a** and **3a** contained *trans* and *cis* configurations between the acetamido and the alkylthio groups, respectively. Although compound **2a** contained *trans* configuration which is differ-

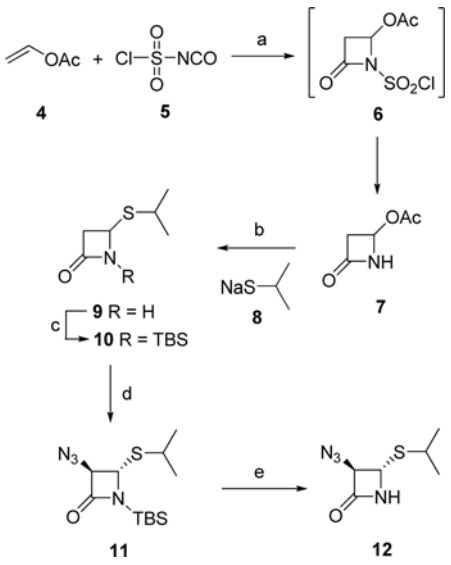
ent from that of penicillins, a number of monocyclic β-lactam derivatives of biological significance were found to have *trans* configurations. Carboxymethyl group (potassium salt) was retained at N(1) in both cases. Taken together, **2a** and **3a** might be structurally similar but less-strained than penicillin, and these new 4-alkylthio monocyclic β-lactams might therefore have comparable characteristics to penicillin. Thus, **2a** and **3a** could be meaningful analogues of monocyclic β-lactams derived from penicillins.



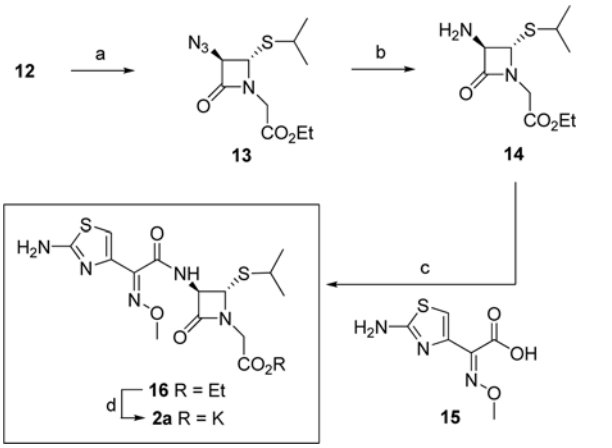
Synthesis of Monocyclic β-Lactam 2a. After considering several synthetic pathways, I established a plausible synthetic scheme through azetidin-2-one as an intermediate, as shown in Scheme 1. In particular, 4-substituted azetidin-2-ones were considered as very important intermediates in the synthesis of various nonclassical β-lactams. The preparations of these compounds were developed by the Clauss group[9] and later further exploited period.[10,11] However, most of these methods suffered from side reactions and low yields (~20%). Thus, I applied minor modifications to these methods. At first, vinyl acetate (**4**) was treated with chlorosulfonyl isocyanate (CSI, **5**) in CH_2Cl_2 to provide azetidinone **6**, which was applied *in situ* for reductive hydrolysis of the N-S bond, leading to the generation of compound **7**[10] in moderate yield (35%, for two steps). Unfortunately, further efforts to improve the yield of this step did not lead to better results. Intermediate **7** could be functionalized by displacing the acetoxy group with various nucleophiles to form new C-S or C-C bond. In our experiments, the acetoxy group was displaced by sulfur-containing nucleophiles such as sodium isopropanethiolate (**8**), which was prepared by the reaction of isopropanethiol and sodium metal in Et_2O at room temperature for 15 h. The reaction of compound **7** with sodium isopropanethiolate at 0 °C for 30 min gave 4-isopropylthio compound **9** in 78% yield. Also, sodium *t*-butanethiolate (or thiol) and thioacetate anions (or thiolacetic acids) were employed to react with compound **7** to give the corresponding thioderivatives.[12] For example, the reaction of **7** with sodium *t*-butanethiolate at room temperature for 1 h afforded 4-*t*-butylthioazetidin-2-one in 65% yield. This type of carbon-sulfur bond forming reaction has been regarded as an important step for further functionalization. Then, for the functionalization at C(3) it was required to protect NH group due to its high reactivity. Accordingly, compound **9** was reacted with *t*-butyldimethylsilyl chloride (TBSCl)[13] in CH_2Cl_2, which smoothly proceeded within 4 h to give **10** in near quantitative yield. Then, the formation of anion at C(3) in **10** with LDA in THF (–78 °C, 2 h) was followed by treatment with *p*-toluenesulfonyl azide[14] and trimethylsilyl chloride (TMSCl), affording the 3-azido compound **11** in 65% yield, along with the minor desilylated compound **12**. At this step I was also interested in the stereochemistry of **11** (*cis* or *trans*-isomer). However, analysis of the NMR signals was not clear since the two proton signals for C(3)H and C(4)H overlapped near δ 4.60-4.40. However, **11** was found to be a *trans*-isomer since the stereochemistry of compound **12** in the next step was determined. Treatment of compound **11** with tetrabutylammonium fluoride (TBAF) in THF at –78 °C for 2 h produced the desilylated compound **12** in 70% yield. Treatment with HF·pyridine in acetonitrile at room temperature also led to the formation of **12**. However, the condition using TBAF gave slightly better results than that using HF·pyridine. As mentioned above, I analyzed the stereochemistry of **12** using ^{1}H NMR and found that it was a *trans*-isomer based on the coupling constant (J = 1.5 Hz) of the signals of C(3)H and C(4)H, which is diagnostic for a *trans*-isomer.[15]

The intermediate **12** was then converted to *N*-alkyl compound **13** in 84% yield by treatment with ethyl bromoacetate and potassium carbonate in DMF at room temperature for 4 h, as shown in Scheme 2. The IR spectrum of the product **13** showed the expected amide, azide, and ester absorptions at 1780, 2120, and 1745 cm^{-1}, respectively.

Then, the azido compound **13** was transformed into primary amine **14**. Among various reducing agents that have been employed in the conversion of aliphatic or aromatic azido groups into corresponding amines, two methods were tested. First, azido derivative **13** was mixed with triphenyl-



Scheme 1. Synthesis of intermediate **12**. Reagents and conditions: (a) CH_2Cl_2, 0 °C, 2 h; then, $NaHCO_3$, Na_2SO_3, 0 °C, 30 min, 35% (for two steps); (b) THF, H_2O, 0 °C, 30 min, 78%; (c) TBSCl, DIEA, room temperature, 4 h, ~100%; (d) LDA, *p*-toluenesulfonyl azide, THF, –78 °C, 2 h; then, TMSCl, 50 °C, 4 h, 65%; (e) TBAF, THF, 2 h, 70%.

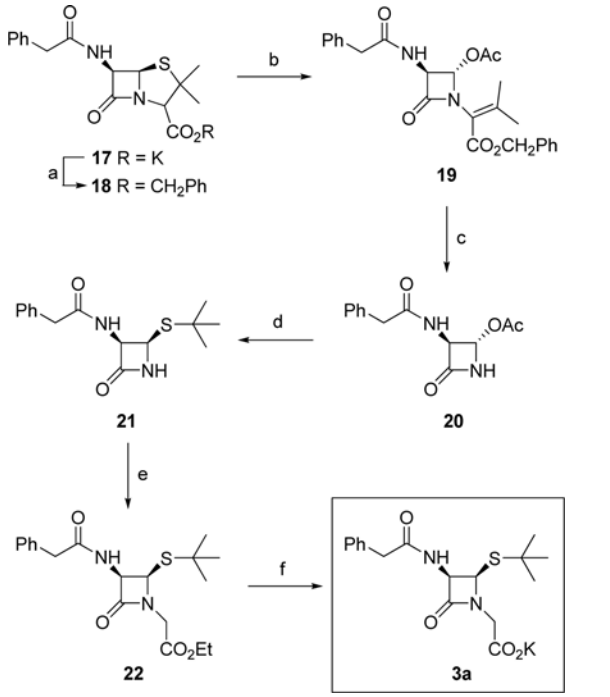


Scheme 2. Synthesis of monocyclic β-lactam **2a**. Reagents and conditions: (a) $BrCH_2CO_2Et$, K_2CO_3, DMF, room temperature, 4 h, 84%; (b) 10% Pd/C, H_2, EtOAc, room temperature, 20 h, 71%; (c) BDP, TEA, DMF, room temperature, 1 h, 63%; (d) KOH, MeOH, H_2O, room temperature, 30 min, 65%.

phosphine in THF[16] and then treated with water to afford the amine product. Second, the azido group was treated with Pd/C in EtOAc under 40 psi (H_2 gas) at room temperature[17] to give the amine product **14** in 71% yield. When compared, the latter method (Pd/C) gave slightly better results.

3-Amino compound **14** was considered a highly important intermediate because it could react with various acids using appropriate coupling agents to give a range of 3-substituted azetidinone compounds. For this purpose, I considered employing organophosphate coupling agents since these agents have attracted a great deal of interest in peptide synthesis. Among these agents, I chose benzotriazol-1-yl diethylphosphate (BDP)[18] as an appropriate coupling agent that was prepared by mixing equimolar amounts of diethylchlorophosphate, 1-hydroxybenzotriazole (HOBT), and triethylamine in THF. On the other hand, I also intended to choose an appropriate acid as an acyl side chain. Among numerous acids, aminothiazole moieties have been extensively explored, and in particular, 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid (**15**) has been widely employed in clinically important β-lactam antibiotics[19] and in investigations of structure-activity relationships.[20] Based on this fact, I chose **15** as an exemplary acid for our study. So, compound **15** was first mixed with 3-amino compound **14** in the presence of triethylamine in DMF at room temperature, and to this mixture was slowly added a solution of BDP in DMF, leading to the formation of the product **16** in 63% yield. Similar results were obtained in the absence of triethylamine, probably due to the role of the amino group attached to the thiazole ring. In general, the size and number of substituents in a small ring molecule could be very important factors that stabilize the ring; thus, more substituted β-lactams would be more stable than less substituted β-lactams. Accordingly, compound **16** would be stabilized more or less by introducing the large acid as a side chain in the monocyclic β-lactam ring. Finally, ethyl ester in **16** was hydrolyzed in methanol by treatment with aqueous potassium hydroxide (KOH). Here, I wished to examine, in advance, the stability of the β-lactam carbonyl group against potassium hydroxide. When treated with aqueous potassium hydroxide, the less substituted monocyclic β-lactam **9** was found to be slightly decomposed after 1 h and severely decomposed after 7 h, while the more substituted monocyclic β-lactam, 3-phenylacetamido-4-isopropylthioazetidin-2-one, was found to be resistant for 5 h. Based on these results, I carefully conducted the hydrolysis reaction using only 1.0 equiv. of potassium hydroxide as a base, for fear of ring opening and further decomposition. Fortunately, the reaction proceeded without severe decomposition of **16** in this condition. However, after the reaction was complete, isolation and purification of the salt were difficult. After extensive efforts, I succeeded in recrystallizing the crude product by fine modulation of the solvent mixture (methanol and Et_2O), affording purified salt **2a** in 65% yield. Consequently, the target compound **2a** was synthesized from **4** and **5** through a 10-step synthetic sequence in 3% overall yield.

Synthesis of Monocyclic β-Lactam 3a. In order to synthesize monocyclic β-lactam **3a**, I wished to apply a different strategy from that for compound **2a**. Oftentimes, penicillin G has been regarded as one of the best starting materials for the synthesis of various β-lactams, because it is currently available at low cost *via* fermentation and could be stereospecifically transformed to important key intermediate(s).[21] In particular, the methods whereby penicillanic acid derivatives could be degraded to monocyclic azetidinones were of my interest. Thus, I intended to transform penicillin G to 4-



Scheme 3. Synthesis of monocyclic β-lactam **3a**. Reagents and conditions: (a) $PhCH_2Br$, DMF, 2 h, 84%; (b) $Hg(OAc)_2$, AcOH, Ac_2O, 80 °C, 2 h, 60%; (c) O_3, methanol-CH_2Cl_2, Me_2S, –78 °C, 2 h, 80%; (d) sodium *t*-butanethiolate, THF, H_2O, room temperature, 4 h, 51%; (e) $BrCH_2CO_2Et$, K_2CO_3, DMF, 60 °C, 10 h, 74%; (f) KOH, MeOH, room temperature, 30 min, 75%.

acetoxyazetidin-2-one derivatives that could be used to synthesize monocyclic β-lactams in many ways.[22] As shown in Scheme 3, potassium salt of penicillin G (**17**) was treated with benzyl bromide in DMF to give benzyl ester of penicillin G (**18**)[23] in 84% yield. Then, according to previous procedures[24,25] I attempted to convert the bicyclic system in **18** to monocyclic compound **19**.[24] Mercuric acetate was heated with acetic anhydride in acetic acid at 80 °C for 30 min and to the resulting solution was added compound **18**, affording monocyclic compound **19** in 60% yield.

The oxidation procedures that were employed for other *N*-alkylidene derivatives of azetidinone were applied to achieve the transformation of compound **19** to compound **20**.[24] When I tested several methods, I found that ozonolysis was the most efficient procedure for oxidation of the side chain. Ozonolysis of compound **19** in CH_2Cl_2-methanol at –78 °C led to the production of compound **20** in 80% yield. Then, 4-acetoxy derivative **20** was transformed to 4-*t*-butylthioazetidin-2-one compound **21**[26] by nucleophilic displacement of the acetoxy group with sodium *t*-butanethiolate that was prepared by dissolving sodium metal in *t*-butanethiol.[26,27] We found that the reaction rate of **20** with *t*-butanethiolate anion at 0 °C was slower than that with isopropanethiolate anion probably due to steric hindrance. This reaction therefore required higher temperature (room temperature) and longer reaction time (4 h) to give compound **21** in 51% yield, compared to the reaction with isopropanethiolate (0 °C, 2 h, 60% yield). Although this reaction could work through nucleophilic displacement and/or elimination-addition processes,[28] we mainly observed the formation of *cis*-isomer **21** due to inversion by nucleophilic displacement, which was identified by the chemical shifts and coupling constants of the signals of C(3)H and C(4)H in ^{1}H NMR.[26,29]

Before the alkylation of the NH group, I was interested in the influence of the alkylthio groups at C(4) on the reactivity of the NH group. I therefore conducted a model study for *N*-alkylation in two cases; 4-isopropylthioazetidin-2-one and 4-*t*-butylthioazetidin-2-one. In the former case, the reaction with ethyl bromoacetate in the presence of potassium carbonate in DMF at room temperature was complete in 5 h to give the corresponding product in 79% yield. However, in the latter case, the reaction did not work efficiently under the same conditions, and higher temperature (60 °C) and longer reaction time (10 h) were required to give the corresponding product in similar yield (77%). Thus, we believed that the reactivity of the NH group was strongly influenced by the substituent at C(4), probably due to steric effect. On the basis of the results of the model study, I performed the reaction under the similar condition, in which compound **21** was treated with ethyl bromoacetate at 60 °C for 10 h to provide compound **22** in 74% yield. Finally, I carefully conducted the hydrolysis reaction using only 1.0 equiv. of potassium hydroxide as a base and further purification by recrystallization in methanol and Et_2O, finally affording the potassium salt **3a** in good yield (75%). Consequently, the target monocyclic ß-lactam **3a** was synthesized from **17** through a six-step synthetic sequence in 11% overall yield.

Conclusions

The design and synthesis of monocyclic β-lactams **2a** and **3a** are reported. These compounds were specifically designed by cleavage of the C(2)-C(3) bond of the penicillin structure and aimed to retain all of the penicillin moieties except the bicyclic ring system. Compound **2a** contained a useful aminothiazolylacetamido group as a side chain at C(3) and an isopropylthio group at C(4). Compound **3a** contained a phenylacetamido group as a side chain at C(3) and a *t*-butylthio group at C(4). Both compounds kept the same carboxymethyl group (its potassium salt) at N(1). These substructures would exactly represent the structures that would be obtained by the cleavage of the C(2)-C(3) bond in the penicillins. Compound **2a** was synthesized from **4** and **5** through a ten-step synthetic sequence in 3% overall yield. Compound **3a** was obtained from the degradation of the potassium salt of penicillin G (**17**) through a six-step synthetic sequence in 11% overall yield.

Experimental

General. ^{1}H NMR spectra were recorded on a Varian FT-80A Spectrometer and chemical shifts are expressed as δ units relative to tetramethylsilane (TMS). Infrared (IR) spectra were measured on a Perkin-Elmer 267 spectrometer and frequencies are given in reciprocal centimeters. Analytical thin layer chromatography (TLC) was performed on glass plates (0.25 mm) coated with silica gel 60F-254 (Merck). Column chromatography was conducted using Merck silica gels (0.040-0.063 mm). Most of the reagent grade chemicals were purchased commercially and distilled before use, if necessary. Some compounds were prepared by known procedures, and spectral and physical data of the products were in accord with reported data.

4-Acetoxyazetidin-2-one (7).[10] To a stirred solution of vinyl acetate (**4**, 5.0 mL, 54 mmol) in dry CH_2Cl_2 (5 mL) was added chlorosulfonyl isocyanate (**5**, 1.0 mL, 11 mmol) dropwise at 0 °C. After stirring for 2 h at 0 °C, the mixture was poured into an aqueous solution (150 mL) of $NaHCO_3$ (8 g) and Na_2SO_3 (8 g) at 0 °C with stirring. Stirring was again continued for 30 min at 0 °C and the mixture was extracted with CH_2Cl_2. Evaporation of the solvent gave a yellow oil that was chromatographed on silica gel to afford the title compound (0.50 g, 35%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.60 (br s, 1H), 6.05-5.80 (m, 1H), 3.60-2.86 (m, 2H), 2.25 (s, 3H); IR (KBr) ν 1750, 1740 cm^{-1}.

4-Isopropylthioazetidin-2-one (9). To a stirred solution of compound **7** (2.4 g, 19 mmol) in THF (30 mL) at 0 °C was added a solution of sodium propanethiolate (**8**, 2.03 g, 21 mmol) in H_2O (10 mL). After being stirred for 30 min at 0 °C, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with brine three times. The organic layer was dried over $MgSO_4$ and evaporated to give the title compound (2.1 g, 78%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.60 (br s, 1H), 5.94-5.70 (m, 1H), 3.61-2.68 (m, 3H), 1.36 (d, J = 6.0 Hz, 6H); IR (KBr) ν 3200, 1750 cm^{-1}.

1-*t*-Butyldimethylsilyl-4-isopropylthioazetidin-2-one (10). To a stirred solution of compound **9** (2.1 g, 15 mmol) in CH_2Cl_2 was added *t*-butyldimethylsilyl chloride (TBSCl, 2.7 g, 18 mmol) in the presence of diisopropylethylamine (DIEA, 3.1 mL, 18 mmol) at 0 °C. Then the ice bath was removed and stirring was continued for 4 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography to give the title compound in quantitative yield. ^{1}H NMR ($CDCl_3$, 80 MHz) δ 4.90-4.62 (m, 1H), 3.80-2.80 (m, 3H), 1.39 (d, J = 6.0 Hz, 6H), 1.09 (s, 9H), 0.35 (s, 6H); IR (KBr) ν 1780 cm^{-1}.

***trans*-3-Azido-1-*t*-butyldimethylsilyl-4-isopropylthioazetidin-2-one (11).** To a stirred solution of lithium diisopropylamide (LDA, 7.9 mmol: diisopropyl amine, *n*-BuLi/THF, –78 °C) in THF (20 mL) was added compound **10** (1.9 g, 7.2 mmol) at –78 °C. After stirring for 2 h at –78 °C, a solution of *p*-toluenesulfonyl azide (1.4 g, 7.2 mmol) in THF was slowly added. After 1 h stirring, TMSCl (1.8 mL, 14 mmol) was added. Then the reaction mixture was maintained at room temperature for 1 h, followed by heating to 50 °C for 4 h. After cooling to room temperature, the reaction mixture was filtered and the filtrate was diluted with CH_2Cl_2 (50 mL) and washed with brine at neutral conditions. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography to afford the title compound (1.4 g, 65%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 4.60-4.40 (m, 2H), 3.25-3.00 (m, 1H), 1.39 (d, J = 6.0 Hz, 6H), 1.09 (s, 9H), 0.35 (s, 6H); IR (KBr) ν 2120, 1778 cm^{-1}.

***trans*-3-Azido-4-isopropylthioazetidin-2-one (12).** To a stirred solution of compound **11** (0.90 g, 3.0 mmol) in THF was added tetrabutyl ammoniumfluoride (TBAF, 4.5 mL of 1 M solution in THF, 4.5 mmol) at –78 °C. After being stirred for 2 h at the same temperature, the reaction mixture was slowly diluted with EtOAc (10 mL) and washed with brine (30 mL) three times. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to give the title compound (0.39 g, 70%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.20 (br s, 1H), 4.64 (d, J = 1.5 Hz, 1H), 4.46 (d, J = 1.5 Hz, 1H), 3.30-3.05 (m. 1H), 1.39 (d, J = 6.0 Hz, 6H); IR (KBr) ν 3300, 2120, 1780 cm^{-1}.

***trans*-3-Azido-1-ethoxycarbonylmethyl-4-isopropylthioazetidin-2-one (13).** To a stirred solution of ethyl bromoacetate (0.26 mL, 2.3 mmol) in DMF (6 mL) was added a solution of compound **12** (0.39 g, 2.1 mmol) in DMF (2 mL) in the presence of potassium carbonate (0.32 g, 2.3 mmol) at room temperature. The reaction mixture was then stirred for 4 h at room temperature. The resulting mixture was diluted with CH_2Cl_2 (35 mL) and subsequently, washed with brine (30 mL). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*, affording an oily residue that was chromatographed on Merck silica gel using EtOAc-hexanes (1:5) as an eluent. The fractions were combined and solvent was removed *in vacuo* to give the title compound (0.48 g, 84%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 4.81 (d, J = 2.0 Hz, 1H), 4.53-3.74 (m, 5H), 3.18-3.00 (m, 1H), 1.34 (d, J = 6.5 Hz, 6H), 1.26 (t, J = 7.0 Hz, 3H); IR (KBr) ν 2120, 1785, 1750 cm^{-1}.

***trans*-3-Amino-1-ethoxycarbonylmethyl-4-isopropylthioazetidin-2-one (14).** Azido lactam **13** (0.48 g, 1.8 mmol) was hydrogenated in EtOAc (10 mL) using a 10% Pd on carbon catalyst (0.32 g, 0.30 mmol) under 40 psi (hydrogen gas). After the mixture was shaken for 20 h at room temperature, hydrogen gas was replaced by nitrogen gas and the catalyst was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was chromatographed on Merck silica gel using EtOAc as an eluent. The fractions with R_f 0.4 were combined and the solvent was removed *in vacuo* to afford the title compound (0.31 g, 71%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 4.60 (d, J = 2.0 Hz, 1H), 4.42-3.73 (m, 5H), 3.15-2.90 (m, 1H), 2.02 (br s, 2H), 1.34 (d, J = 6.5 Hz, 6H), 1.26 (t, J = 7.0 Hz, 3H); IR (KBr) ν 3400, 1780, 1750 cm^{-1}; MS (CI) *m/z* 247 [M+1]$^+$.

***trans*-3-(2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-1-ethoxycarbonylmethyl-4-isopropylthioazetidin-2-one (16).** To a solution of thiazole acetic acid derivative **15** (0.18 g, 0.88 mmol), 3-amino lactam **14** (0.22 g, 0.88 mmol), and TEA (0.12 mL, 0.88 mmol) at room temperature was added benzotriazol-1-yl diethylphosphate (0.26 g, 0.97 mmol) in DMF (4 mL), and the resulting solution was stirred at room temperature until completion of the reaction (2 h). Solvent was removed *in vacuo* and the residue was chromatographed on Merck silica gel using EtOAc as an eluent to give the title compound (0.24 g, 63%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.43 (d, J = 8.0 Hz, 1H), 6.70 (s, 1H), 6.00 (br s, 2H), 5.25-5.00 (m, 2H), 4.40-3.60 (m, 7H), 3.19-2.98 (m, 1H), 1.35 (d, J = 6.5 Hz, 6H), 1.28 (t, J = 7.0 Hz, 3H); IR (KBr) ν 3350, 1775, 1750, 1680 cm^{-1}.

Hydrolysis of 16 to Give Potassium Salt 2a. To a stirred solution of compound **16** (0.15 g, 0.35 mmol) in methanol (3 mL) was added aqueous potassium hydroxide (0.30 mL of 1.0 N solution, 0.30 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was diluted with EtOAc (30 mL) and subsequently poured into distilled water (30 mL). The aqueous layer was washed three times with EtOAc to remove excess starting material. The aqueous layer was dried by freeze dryer to give the corresponding potassium salt. The salt was then purified by recrystallization from a methanol-Et_2O mixture to give the title compound (86 mg, 65%). ^{1}H NMR (D_2O, 80 MHz) δ 6.70 (s, 1H), 5.25-5.00 (m, 2H), 4.40-3.60 (m, 5H), 3.25-2.92 (m, 1H), 1.35 (d, J = 6.5 Hz, 6H); IR (KBr) ν 3380, 1770, 1685, 1620 cm^{-1}.

Benzyl Ester of Penicillin G (18).[23] A suspension of potassium salt of penicillin G (**17**, 3.0 g, 8.1 mmol) in DMF (25 mL) and benzyl bromide (1.1 mL, 9.3 mmol) was stirred for 2 h at room temperature. The mixture was filtered and the filtrate was poured into ice water (100 mL). The resulting oil was extracted with Et_2O (50 mL), and the organic layer was washed with saturated $NaHCO_3$ solution (50 mL) and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* gave the title compound (2.9 g, 84%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.46-7.39 (m, 5H), 7.38-7.29 (m, 5H), 6.36 (br s, 1H), 5.80-5.46 (m, 2H), 5.20 (s, 2H), 4.42 (s, 1H), 3.63 (s,

2H), 1.38 (d, J = 5.5 Hz, 6H); IR (KBr) ν 3400, 1792, 1750, 1685 cm^{-1}.

***trans*-(4*S*)-Acetoxy-1-(benzyloxycarbonyl-2-methylpropenyl)-(3*S*)-phenylacetamidoazetidin-2-one (19).**[24] Mercuric acetate (1.9 mg, 6.0 mmol) was dissolved in acetic acid (8.5 mL) and acetic anhydride (0.85 mL), and the mixture was heated to 80 °C in an oil bath and stirred for 30 min. While the temperature was maintained at 80-85 °C, compound **18** (1.7 g, 4.0 mmol) was added in small portions and stirred for 2 h at the same temperature range. Then, the reaction mixture was cooled, filtered through celite, and evaporated to give an oily residue. The residue was dissolved in CH_2Cl_2 and the resulting mixture was washed with 5% $NaHCO_3$ and 5% NaCl solutions. The organic layer was dried over Na_2SO_4, and evaporated to dryness. Column chromatography of the residue on a silica gel column by EtOAc-haxanes (1:1) as an eluent gave an amorphous solid (1.1 g, 60%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.52-7.40 (m, 5H), 7.16-7.06 (m, 5H), 6.12-6.20 (d, J = 8.0 Hz, 1H), 6.05 (d, J = 1.5 Hz, 1H), 5.15 (s, 2H), 4.91 (dd, J = 8.0, 1.5 Hz, 1H), 3.57 (s, 2H), 2.23 (s, 2H), 2.02 (s, 6H); IR (KBr) ν 3325, 1780, 1720, 1695 cm^{-1}.

***trans*-(4*S*)-Acetoxy-(3*S*)-phenylacetamidoazetidin-2-one (20).**[24] Compound **19** (2.3 g, 5.1 mmol) was dissolved in methanol-CH_2Cl_2 (1:1, 50 mL) and the solution was cooled to –78 °C in dry ice-acetone bath. Ozone was passed through this solution for 2 h with stirring. After degasification of the solution, dimethyl sulfide (20 mL) was added and the resulting mixture was stirred overnight at room temperature. The solvent was removed *in vacuo* to give an oily residue. Column chromatography of the residue on a silica gel column gave the title compound (0.86 g, 80%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.82 (br s, 1H), 7.25-7.45 (m, 5H), 5.85 (q, J = 2.0 Hz, 1H), 4.05 (dd, J = 8.0, 2.0 Hz, 1H), 3.55 (s, 2H), 2.15 (s, 3H); IR (KBr) í 3330, 1795, 1725, 1690 cm^{-1}.

***cis*-(4*R*)-*t*-Butylthio-(3*R*)-phenylacetamidoazetidin-2-one (21).**[26] To a stirred solution of compound **20** (0.28 g, 1.1 mmol) in THF at 0 °C was added a solution of sodium *t*-butanethiolate (0.12 g, 1.1 mmol) in H_2O. At the end of the addition, the ice bath was removed. After being stirred for 4 h at room temperature, the reaction mixture was diluted with EtOAc (15 mL) and washed three times with brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. Column chromatography of the residue on a silica gel column with EtOAc-hexanes (2:1) as an eluent gave the title compound (0.16 g, 51%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.54-7.05 (m, 7H), 4.84 (d, J = 2.5 Hz, 1H), 4.52 (dd, J = 8.0, 2.5 Hz, 1H), 3.60-3.40 (m, 2H), 1.22 (s, 9H); IR (KBr) ν 3300, 1780, 1675 cm^{-1}.

***cis*-(4*R*)-*t*-Butylthio-1-ethoxycarbonylmethyl-(3*R*)-phenylacetamidoazetidin-2-one (22).** A mixture of ethyl bromoacetate (89 μL, 0.80 mmol), compound **21** (0.16 g, 0.53 mmol), and potassium carbonate (0.11 g, 0.80 mmol) in DMF (3 mL) was heated at 60 °C for 10 h. After cooling, the resulting mixture was diluted with EtOAc (15 mL), washed with brine (30 mL), and dried over $MgSO_4$. Evaporation of the solvent gave a yellow oil that was purified by column chromatography using EtOAc-hexanes (1:1) as an eluent to afford the title compound (0.15 g, 74%). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.33-7.24 (m, 5H), 5.04-4.80 (m, 2H), 4.61-3.77 (m, 4H), 3.57 (s. 2H), 1.21-1.04 (m, 12H); IR (KBr) ν 3300, 1785, 1755, 1670 cm^{-1}.

Hydrolysis of 22 to Give Potassium Salt 3a. To a stirred solution of compound **22** (0.15 g, 0.40 mmol) in methanol (3 mL) was added aqueous potassium hydroxide (0.35 mL of 1.0 N solution, 0.35 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was diluted with EtOAc (30 mL) and poured into distilled water (30 mL). The aqueous layer was washed two times with EtOAc to remove excess starting material. The aqueous layer was dried by freeze dryer to afford the corresponding potassium salt. The salt was further purified by recrystallization from methanol-Et_2O mixture to afford the title compound (0.10 g, 75%). ^{1}H NMR (D_2O, 80 MHz) δ 7.46-7.31 (m, 5H), 5.07-4.60 (m, 2H), 4.03-3.35 (m, 4H), 1.33 (s, 9H); IR (KBr) ν 3400, 1765, 1675, 1620 cm^{-1}.

Acknowledgments. This Research was supported by the Duksung Women's University Research Grant 2011.

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