Chemistry of Heterocyclic Compounds, Vol. 34, No. 11, 1998

STUDIES ON MONOBACTAMS
I. SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF 4-SUBSTITUTED 3-[(N-METHYL-1,2,3-TRIAZOL-4-YL)-METHYLENE]-2-AZETIDINONE-1-SULFONATES

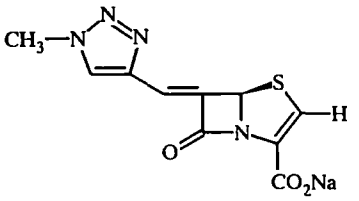
Oludotun A. Phillips, David P. Czajkowski, Kevin Atchison, Ronald G. Micetich, Samarendra N. Maiti, Chieko Kunugita, and Akio Hyodo

Two monobactam derivatives, potassium (3*Z*)-[(N-methyl-1,2,3-triazol-4-yl)methylene]-4-phenylthio-2-azetidinone-1-sulfonate and potassium (3*E*)-[(N-methyl-1,2,3-triazol-4-yl)methylene]-4-(1,2,3-triazol-1-yl)-2-azetidinone-1-sulfonate, were synthesized and tested for β-lactamase inhibitory activity.

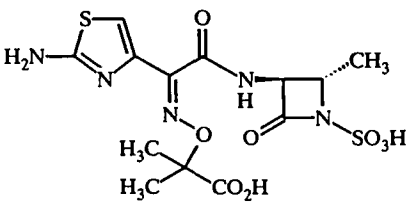
β-Lactamases are enzymes responsible for many failures of antimicrobial therapy because of the hydrolysis of β-lactam antibiotics to inert and ineffective agents. Although the original β-lactamase described was primarily effective in hydrolyzing penicillins, many related enzymes with a wide range of substrate specificities are now recognized. Almost as soon as a new β-lactam antibiotic is introduced into clinical usage, some previously unrecognized β-lactamases with the capability of destroying this antibiotic are identified. To overcome the resistance mediated by β-lactamase the popular approach has been the use of a combination of β-lactam antibiotic with a β-lactamase inhibitor. The success of clavulanic acid [1] stimulated extensive research leading to the discovery of other β-lactamase inhibitors such as sulbactam [2] and YTR-830, now known as tazobactam [3, 4].

A number of 6-(heteroaryl-substituted methylene)penams have been reported in the literature [5-7] as potent inhibitors of cell free β-lactamases, but were not effective in synergistic antibacterial tests probably because of poor penetration through the bacterial cell wall. Due to the widespread use of third-generation cephalosporins, resistance caused by chromosomally-mediated class I cephalosporinase is increasing rapidly and may pose a threat in the future. A major breakthrough on this aspect has been the synthesis of BRL-42,715 [8-10], which is a potent inhibitor of most bacterial β-lactamases including the class I cephalosporinase. This penem derivative I bearing the N-methyltriazolylmethylene group at C-6 position had good synergistic activity with amoxycillin *in vitro* and *in vivo* [11]; however, there is little information about the further development of this compound.

Aztreonam (II) as a representative of the monobactam family has long been recognized as a good inhibitor of cephalosporinase, whereas broad-spectrum β-lactamases and penicillinases generally have weaker affinities for aztreonam than for clavulanic acid [12].



I

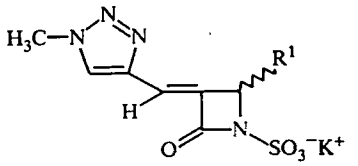


II

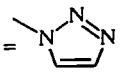
SynPhar Laboratories Inc., #2, 4290-91A Street, Edmonton, Alberta, Canada T6E 5V2. Tokushima Research Institute, Taiho Pharmaceutical Co. Ltd., 224-2 Ebisuno Hiraishi, Kawauchi-cho, Japana 771-01. Published in Khimiya Geterotsiklicheskikh Soedinenii, No. 11, pp. 1536-1547, November, 1998. Original article submitted April 14, 1998.

 0009-3122/98/3411-1308$20.00

This paper outlines the synthesis of two monobactam derivatives III and IV in which we sought to investigate the effect of introducing (N^1-methyl-1,2,3-triazol-4-yl)methylene at the C-3 position of the azetidinone nucleus on the β-lactamase inhibitory activity of the monobactam. The β-lactamase inhibitory data (Table 1) and synergistic data (Table 2) of compound IV are also described.

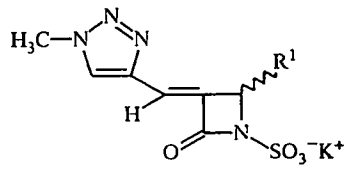


III R^1 = SPh; IV R^1 =



CHEMISTRY

In our initial effort we planned to synthesize the compounds V and VI with the hope that introduction of a good leaving group such as acetate or the phenylsulfonyl group at C-4 will activate the lactam ring and hence will inactivate the β-lactamase rapidly. Though we successfully prepared the 4-acetoxy derivative VII, the introduction of the sulfonate group in the final step by complex Py·SO_3 to afford the target molecule V was not successful. On the other hand, the preparation of the precursor molecule III of the target compound VI was achieved without much difficulty. Unfortunately, the intermediate III was chemically quite unstable; even at refrigeration temperature, it underwent decomposition, and hence its biological evaluation was not possible. Finally we focussed our attention on the synthesis of compound IV. The synthetic scheme (Scheme 1) was first investigated in the racemic series in which the required intermediate VIII was obtained by the method of Leanza et. al [13].



V R^1 = OAc; VI R^1 = O_2SPh

Methyl 6,6-dibromopenicillanate (IX) was readily obtained from 6-aminopenicillanic acid (6-APA) by the diazotation-bromination method [14], followed by esterification of the crude dibromo acid. The methyl ester IX underwent metal—halogen exchange with methyl magnesium bromide in THF at –78°C to give an enolate intermediate which, on quenching with 1-methyl-1,2,3-triazol-4-carboxaldehyde, afforded an inseparable mixture of bromohydrins X. Debromination of X with 4 molar equivalents of zinc in a mixture of THF and aqueous ammonium acetate [15] afforded a mixture of hydroxy adducts XI which were difficult to separate at this stage by column chromatography.

The mixture of alcohols was converted to the corresponding mixture of *t*-butyldimethylsilyl derivatives XII and the thiazolidine ring was then disrupted using an established procedure [16]. Treatment of XII with mercuric acetate in glacial acetic acid at 90°C gave a mixture of acetoxyazetidinones XIII. Oxidation of the mixture with a catalytic amount of potassium permanganate and sodium periodate in 0.1 N phosphate buffer [13] removed the N-isopropylidene acetate group to produce a mixture of 4-acetoxyazetidinones VIII.

The synthetic methodology for the preparation of the compounds III and IV is depicted in Scheme 2 and Scheme 3. Replacement of the acetoxy group of VIII with NaN_3 in acetone–water mixture gave 4-azido derivative XIV. In a similar manner, displacement of the acetoxy group with thiophenol gave compound XV. Heating of compound XIV with acetylene in dimethoxyethane gave the triazole derivative XVI in about 87% yield. Initially, the NH group of compound XVI was protected by 4-nitrobenzyl chloroformate. Removal of the protecting group by hydrogenation at a later stage, however, was not selective; the double bond at C-3 position was also saturated. It was more practical for us to protect the NH group of compounds XV and XVI by the 2,2,2-trichloroethoxycarbonyl group which was removed selectively at the final stage by treating with activated zinc in DMF containing glacial

TABLE 1. β-Lactamase Inhibitory Activity of Compound IV

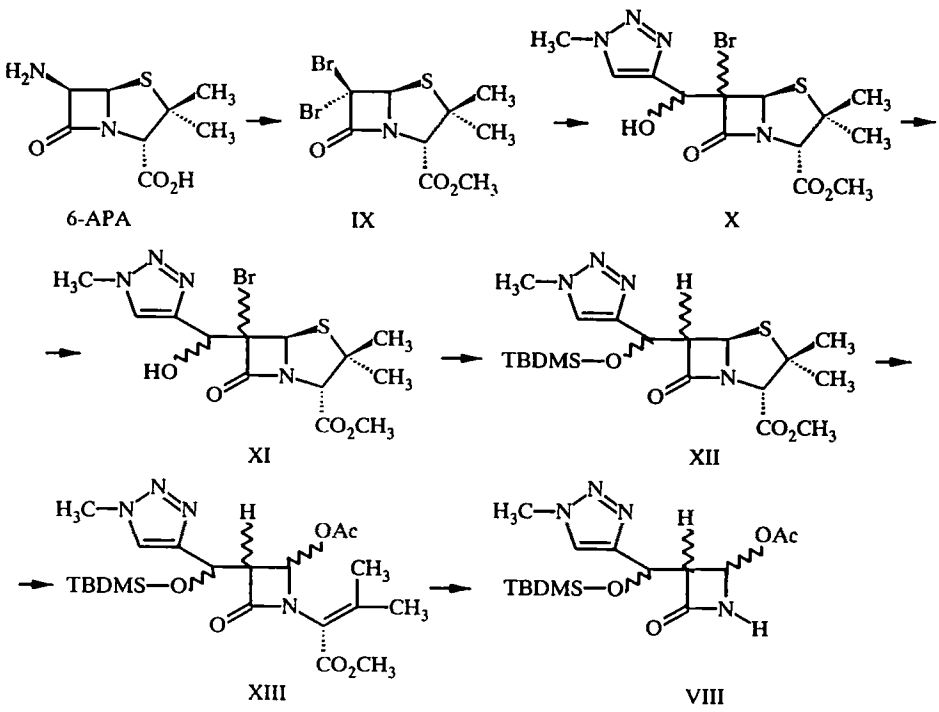
Enzyme type	Enzyme activity (U/ml)	Substrate	Inhibitor dose (μg/ml)	Inhibition percentage (%) TAZ[a]	BRL[b]	IV
TEM-1 (*E. coli*)	1.6328	ABPC[c]	1	93	100	4
CTX-1 (*K. pneumoniae*)	0.7363	CER[d]	1	98	100	0
Ceph-ase (*E. cloacae*)	1.1517	CER[d]	1	7	99	3
Ceph-ase (*P. aeruginosa*)	1.0352	CER[d]	1	14	98	6

[a]TAZ—tazobactam;
[b]BRL—BRL-42,715;
[c]ABPC—ampicillin;
[d]CER—cephaloridine.

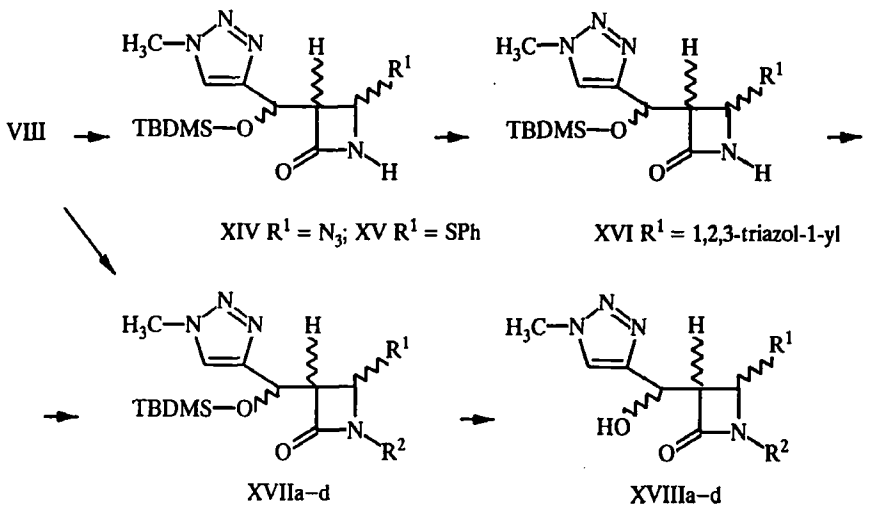
acetic acid. Desilylation was accomplished by the method of J. D. Buynak et al. [17] in which compounds XVIIa-d were treated with an excess of 48% HF in acetonitrile at ambient temperature for 4 days to afford XVIIIa-d.

Elimination of the hydroxy group of the compounds XVIIIa-d by reaction with mesyl chloride in triethylamine provided a mixture of (*E*)- and (*Z*)-triazolylmethylene monobactam derivatives, which were separated by silica gel column chromatography. The structural assignment of the (*E*)-geometry around the double bond was based on a

Scheme 1



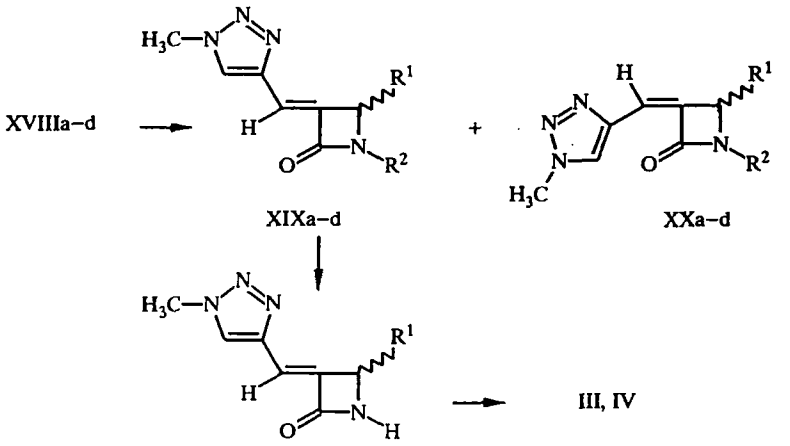
Scheme 2



a R^1 = 1,2,3-triazol-1-yl; $R^2 = CO_2CH_2C_6H_4NO_2$-4
b R^1 = 1,2,3-triazol-1-yl; $R^2 = CO_2CH_2CCl_3$
c R^1 = SPh; $R^2 = CO_2CH_2CCl_3$
d R^1 = OAc; $R^2 = CO_2CH_2CCl_3$

comparison of its ^{1}H-NMR spectrum with that of the (*Z*)-isomer. Thus, the vinyl proton of the (*E*)-isomer of the compound (XIXb) appears at δ 7.70, downfield from that of the corresponding (*Z*)-isomer, which appears at δ 7.27 due to the anisotropic deshielding effect of the β-lactam carbonyl on this proton. On the other hand, the triazole proton of the (*Z*)-isomer, (XXb), is deshielded by the β-lactam carbonyl and appears at δ 8.85, downfield from that of the (*E*)-isomer, (XIXb), which appears at δ 8.28. Finally, the N-1 sulfonation was achieved by the method of Cimarusti et al. [18], Scheme 3, in which the monobactam (XXI) was converted to the N-1-silyl derivative by treat-

Scheme 3



XXI R^1 = 1,2,3-triazol-1-yl
XXII R^1 = SPh
VII R^1 = OAc

TABLE 2. *In vitro* Synergy of Compound IV with Ceftazidime (CAZ) and Piperacillin (PIPC) against β-Lactamase Producing Isolates

Organism	MIC (μg/ml)							
	CAZ	+TAZ[a]	+BRL-42,715	+IV	PIPC	+TAZ	+BRL-42,715	IV
S. a. CT-10	100	100	25	100	>100	100	25	>100
S. a. 54K	12.5	6.25	≤0.2	12.5	25	0.78	≤0.2	50
S. a. 80K	6.25	6.25	<0.2	6.25	25	0.39	≤0.2	12.5
E. c. TEM 1	≤0.2	≤0.2	≤0.2	≤0.2	100	0.78	0.78	100
E. c. TEM 2	0.39	0.39	≤0.2	0.39	>100	50	0.39	>100
E. c. OXA 1	≤0.2	≤0.2	≤0.2	0.39	25	6.25	0.78	25
E. c. SHV 1	0.39	≤0.2	≤0.2	≤0.2	100	1.56	1.56	100
K. p. 336 L	0.78	≤0.2	0.39	0.78	>100	6.25	6.25	>100
K. p. 101 L	≤0.2	≤0.2	≤0.2	≤0.2	>100	1.56	1.56	>100
K. p. CTX 1	100	1.58	1.56	100	>100	12.5	12.5	>100
S. m. 200 L	≤0.2	≤0.2	≤0.2	≤0.2	100	0.78	0.39	100
S. m. CT 98	6.25	6.25	3.13	3.13	>100	>100	6.25	>100
P. v. CT 106	25	0.78	0.39	12.5	>100	1.56	3.13	>100
C. f. 2046 E	≤0.2	≤0.2	≤0.2	≤0.2	>100	0.78	1.56	>100
C. f. CT 76	100	50	1.56	50	>100	50	6.25	>100
C. f. 648 L	≤0.2	≤0.2	≤0.2	≤0.2	>100	3.13	1.56	>100
E. cl. P 99	100	25	0.78	100	>100	50	3.13	>100
E. cl. 212 L	0.39	0.39	≤0.2	0.39	>100	100	1.56	>100
P.a. CT 122	100	50	12.5	50	>100	>100	25	>100
P.a. CT 137	25	25	12.5	25	>100	>100	>100	>100
P.a. CT 144	100	50	3.13	50	>100	>100	25	>100
P. a. PSE 1	3.13	3.13	3.13	3.13	6.25	6.25	6.25	6.25
P. a. PSE 2	1.56	3.13	1.56	3.13	100	50	3.13	50
P. a. PSE 3	3.13	3.13	1.56	3.13	100	6.25	25	100
P. a. PSE 4	1.56	1.56	1.56	1.56	>100	25	100	>100

a TAZ = tazobactam;
S. a. — *Staphylococcus aureus*;
E. c. — *Escherichia coli*;
K. p. — *Klebsiella pneumoniae*;
S. m. — *Serratia marcescens*;
P. v. — *Proteus vulgaris*;
C. f. — *Citrobacter freundii*;
E. cl. — *Enterobacter cloacae*;
P. a. — *Pseudomonas aeruginosa*.

ment with triethylamine and trimethylsilyl chloride. This N-silyl derivative was allowed to react with trimethylsilyl chlorosulfonate to form an intermediate monobactam trimethylsilyl ester, which was then hydrolyzed in buffer solution to afford the potassium salt of the target compound IV.

RESULTS AND DISCUSSION

The β-lactamase inhibitory activity of compound IV was determined against cell free β-lactamase preparation by spectrophotometrically measuring the hydrolysis of the substrate in the presence and absence of the β-lactamase inhibitor. The results are summarized in Table 1.

Compound IV did not demonstrate any time-dependent inactivation of the β-lactamase.

It has been postulated that sulbactam interacts with β-lactamase with high affinity yielding a long-lived acyl-enzyme complex [19-22]. Such a complex can break down by three different pathways, such as hydrolysis and regeneration of the free enzyme, formation of a transiently inhibited enzyme form, and slow irreversible inactivation of the enzyme. The cleavage of the β-lactam ring is a necessary condition for the azetidinone derivative to act as "suicide" substrate of β-lactamases, and this in turn would depend on the relative affinity of the compound for the enzyme and its ability to form a long-lived acyl enzyme complex. Since the compound IV has poor inhibitory activity it is possible to suggest that the affinity of the compound for the β-lactamase is strongly diminished. By the introduction of a substituted double bond at the C-3 position of the azetidinone nucleus one would produce an increase in ring strain. But the increase of the reactivity expected from the additional ring strain created by the introduction of the double bond does not seem sufficient to allow enzymatic ring opening. The lower affinity and other effects produced by the presence of a poor leaving group at C-4 position can explain the lack of β-lactamase inhibitory activity of the compound IV.

The synergistic effects of compound IV with various antibiotics such as piperacillin (PIPC) and ceftazidime (CAZ) are shown in Table 2. The bacteria cultivated in Mueller Hinton Broth (Difco) and diluted to 10^7 cfu/ml were inoculated into the same medium containing the antibiotics and tazobactam (TAZ), BRL-42,715 or compound IV in a specific concentration and incubated at 37 °C for 20 h. The growth of the microorganisms was observed to determine the minimal inhibitory concentration (MIC) for rendering the inoculated medium free from turbidity.

CONCLUSION

Potassium (3*Z*)-[(N-methyl-1,2,3-triazol-4-yl)methylene]-4-(1,2,3-triazol-1-yl)-2-azetidinone-1-sulfonate (IV) was a poor β-lactamase inhibitor. In synergism studies it failed to protect the antibiotics. It showed very weak synergism in combination with ceftazidime against *S. marcescens* CT-98, *P. vulgaris* CT-106, *P. aeruginosa* CT-122, and *P. aeruginosa* CT-144.

EXPERIMENTAL

All column chromatographic purifications were accomplished on silica gel 60 (E. Merck, 230~400 mesh) with the appropriate solvent gradients. ^{1}H-NMR spectra were determined with a Bruker AC-200-F (200 MHz) spectrometer in appropriate deuterated solvents and are expressed in ppm downfield from TMS (internal standard).

Due to synthetic expediency isomeric separations were not performed on the intermediates. The reference compounds, e.g. tazobactam and BRL-42,715, were synthesized in our laboratories. The compound IV was tested as a racemic mixture.

Methyl 6-Bromo-6-[1-hydroxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]penicillanate (X). To a stirred solution of 15.0 g (40.20 mmoles) of methyl 6,6-dibromopenicillanate (IX) in dry THF (250 ml) at –78°C was added dropwise 16.1 ml (48.24 mmoles) of 3.0 M methyl magnesium bromide in Et_2O solution. The solution was stirred at –78°C for an additional 20 min and then treated with a solution of 1-methyl-1,2,3-triazol-4-yl-carboxaldehyde (4.47 g, 40.20 mmoles) in dry THF (80 ml). The mixture was stirred at –78°C for 20 min and quenched with 30 ml of saturated NH_4Cl. The mixture was evaporated under reduced pressure to remove most of THF and the residue was extracted with EtOAc. The combined EtOAc extracts were washed with brine, dried over $MgSO_4$, and concentrated to give a white foam (18.1 g), which was purified over a silica gel column (ethyl acetate—hexane, 10:1) to yield bromohydrin X as a white foam (16.6 g, 98%). ^{1}H NMR of this product indicated the presence of three isomers. Found, %: C 38.60; H 4.20; N 13.70. $C_{13}H_{17}BrN_4O_4S$. Calculated, %: C 38.52; H 4.23; N 13.83.

200 MHz ^{1}H NMR ($CDCl_3$) of the major isomer, δ: 7.83 (1H, s); 5.83 (1H, s); 5.48 (1H, d, J = 4.8 Hz); 4.51 (1H, s); 4.11 (3H, s); 4.02 (1H, d, exchangeable with D_2O, J = 5.1 Hz); 3.77 (3H, s); 1.66 (3H, s); 1.46 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) of the first minor isomer, δ: 7.70 (1H, s); 5.79 (1H, s); 5.62 (1H, d, J = 5.5 Hz); 4.58 (1H, s); 4.12 (3H, s); 3.80 (3H, s); 3.29 (1H, d, exchangeable with D_2O, J = 5.8 Hz); 1.69 (3H, s); 1.47 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) of the second minor isomer, δ: 7.70 (1H, s); 5.79 (1H, s); 5.40 (1H, d, J = 4.6 Hz), 4.53 (1H, s); 4.28 (1H, d, exchangeable with D_2O, J = 6.1 Hz); 4.12 (3H, s); 3.80 (3H, s); 1.69 (3H, s); 1.46 (3H, s).

Methyl 6-[1-Hydroxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]penicillanate (XI). A solution of bromohydrin X (2.0 g, 4.93 mmoles) in 50 ml of THF was treated with 22 ml of 1 M NH_4OAc aq. and powdered zinc (1.29 g). The mixture was stirred at room temperature for 1 h, then filtered through a bed of Celite, and washed thoroughly with EtOAc. The organic layer was separated out and the aqueous layer was extracted with EtOAc. The combined EtOAc extracts were washed with brine, dried over $MgSO_4$, and concentrated to give a white foam (1.5 g). The compound was purified by column chromatography on silica gel (ethyl acetate—hexane, 10:1).

The first eluting component was methyl (6*Z*)-[1-methyl-1,2,3-triazol-4-yl]methylene penicillanate, 20 mg. 200 MHz ^{1}H NMR ($CDCl_3$) δ 8.70 (1H, s); 6.85 (1H, s); 5.83 (1H, s); 4.53 (1H, s); 4.13 (3H, s); 3.80 (3H, s); 1.58 (3H, s); 1.48 (3H, s).

The second eluting component was methyl (6*E*)-[1-methyl-1,2,3-triazol-4-yl]methylene penicillanate, 80 mg. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.65 (1H, s); 7.00 (1H, s); 6.13 (1H, s); 4.56 (1H, s); 4.13 (3H, s); 3.79 (3H, s); 1.59 (3H, s); 1.49 (3H, s).

Further elution of the column with ethyl acetate gave the desired product XI as a mixture of isomers (1.07 g, 75% yield). Found, %: C 47.92; H 5.53; N 17.30. $C_{13}H_{18}N_4O_4S$. Calculated, %: C 47.84; H 5.56; N 17.17.

Methyl 6-[1-*t*-Butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]penicillanate (XII). A solution of compound XI (2.86 g, 8.76 mmoles) in 100 ml of methylene chloride was treated with imidazole (1.79 g, 26.29 mmoles) and *t*-butyldimethylchlorosilane (2.64 g, 17.53 mmoles). The mixture was stirred at room temperature for 28 h, and diluted with water. The organic layer was separated out, washed with water, brine, dried ($MgSO_4$), and concentrated under reduced pressure to provide a semi-solid mass (4.94 g). Purification of the product by silica gel column chromatography eluting with ethyl acetate—hexane, 1:1, afforded the product as a mixture of two isomers, white solid (2.88 g, 75% yield). Found, %: C 51.95; H 7.23; N 12.68. $C_{19}H_{32}N_4O_4SSi$. Calculated, %: C 51.79; H 7.32; N 12.72.

200 MHz ^{1}H NMR ($CDCl_3$) of the major isomer, δ: 7.80 (1H, s); 5.38 (1H, d, J = 2.5 Hz); 5.27 (1H, d, J = 2.0 Hz); 4.48 (1H, s); 4.07 (3H, s); 3.73 (3H, s); 3.60 (1H, dd, J = 2.0 and 3.0 Hz); 1.58 (3H, s); 1.43 (3H, s); 0.86 (9H, s); 0.15 (3H, s); –0.18 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) of the minor isomer, δ: 7.41 (1H, s); 5.34 (1H, d, J = 1.5 Hz); 5.27 (1H, d, J = 2.0 Hz); 4.43 (1H, s); 4.07 (3H, s); 3.78 (1H, dd, J = 2.0 and 3.0 Hz); 3.71 (3H, s); 1.59 (3H, s); 1.41 (3H, s); 0.88 (9H, s); 0.11 (3H, s); –0.04 (3H, s).

Further elution of the column (ethyl acetate—hexane, 2:1) gave a single isomer, methyl (3*S*,5*R*,6*R*)-6-[(*R*)-1-*t*-butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]penicillanate (523 mg, 14% yield); m.p. 109-110 °C.

200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.49 (1H, s); 5.48 (1H, d, J = 4.4 Hz); 5.41 (1H, d, J = 10.3 Hz); 4.36 (1H, s); 4.21 (1H, dd, J = 4.4 and 10.3 Hz); 4.10 (3H, s); 3.76 (3H, s); 1.67 (3H, s); 1.49 (3H, s); 0.81 (9H, s); 0.13 (3H, s); –0.19 (3H, s).

4-Acetoxy-3-[1-*t*-butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-1-(1-methoxycarbonyl-2-methyl-1-propenyl-2-azetidinone (XIII). A solution of compound XII (6.8 g, 15.43 mmoles) in 420 ml of glacial acetic acid was treated with $Hg(OAc)_2$ (14.75 g, 46.29 mmoles), the mixture was heated at 90°C for 90 min, and cooled to room temperature. The white precipitate was removed by filtration. The filtrate was evaporated under reduced pressure. To the semi-solid residue, EtOAc (400 ml) was added, filtered again, and washed with an additional amount of EtOAc (50 ml). The combined EtOAc extracts were washed with water, $NaHCO_3$ solution, brine, dried over $MgSO_4$, and evaporated under reduced pressure to give a foam (6.60 g, 92% yield). Found, *m/z*: 466.6. $C_{21}H_{34}N_4O_6Si$. Calculated, M: 466.61. 200 MHz ^{1}H NMR analysis of the product indicated that it was a mixture of isomers. This product was directly used for the next step without further separation and identification of isomers.

^{1}H NMR ($CDCl_3$) δ: 7.89 (1H, s); 6.28 (1H, d, J = 1.5 Hz); 5.43 (1H, d, J = 3.4 Hz); 4.10 (3H, s); 3.76 (3H, s); 3.71 (1H, dd, J = 1.5 and 3.9 Hz); 2.21 (3H, s); 1.93 (3H, s); 0.88 (9H, s); 0.13 (3H, s); –0.07 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) for other isomer, δ: 7.53 (1H, s); 6.40 (1H, d, J = 1.5 Hz); 5.47 (1H, d, J = 2.9 Hz); 4.10 (3H, s); 3.75 (3H, s); 3.50 (1H, dd, J = 1.5 and 3.5 Hz); 2.06 (3H, s); 1.97 (3H, s); 0.85 (9H, s); 0.10 (3H, s); –0.14 (3H, s).

4-Acetoxy-3-[1-*t*-butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-2-azetidinone (VIII). A solution of compound XIII (1.02 g, 2.19 mmoles) in 45 ml of acetone was added to a solution of $KMnO_4$ (17.3 mg, 0.109 mmoles)

and $NaIO_4$ (2.57 g, 12.023 mmoles) in 22 ml of water and 22 ml of 0.1 N phosphate buffer (pH 7.0). The resulting mixture was stirred overnight at room temperature, filtered, and the filtrate was concentrated under reduced pressure to a volume of 40 ml. The residue was saturated with NaCl and extracted with several portions of EtOAc. The combined EtOAc extracts were washed with brine, dried over $MgSO_4$, filtered, and treated with charcoal. After filtration and concentration under vacuum, a white foam (750 mg) was obtained. Found, *m/z*: 354.5. $C_{15}H_{26}N_4O_4Si$. Calculated, M: 354.48. ^1H NMR analysis of the product indicates that it is a mixture of two isomers.

200 MHz ^1H NMR ($CDCl_3$) δ: 7.45 (1H, s); 6.54 (1H, br. s); 5.95 (1H, br. s); 5.37 (1H, d, J = 2.2 Hz); 4.10 (3H, s); 3.69 (1H, dd, J = 1.3 and 2.7 Hz); 2.04 (3H, s); 0.88 (9H, s); 0.12 (3H, s); –0.04 (3H, s).

200 MHz ^1H NMR ($CDCl_3$) for other isomer, δ 7.94 (1H, s); 6.54 (1H, br, s); 5.80 (1H, br, s); 5.41 (1H, d, J = 2.4 Hz); 4.10 (3H, s); 3.48 (1H, dd, J = 1.4 and 2.8 Hz); 2.12 (3H, s); 0.86 (9H, s); 0.15 (3H, s); –0.19 (3H, s).

4-Azido-3-[1-*t*-butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)-methyl]-2-azetidinone (XIV). A solution of NaN_3 (128 mg, 1.97 mmoles) in water (6 ml) was added dropwise to a solution of compound VIII (350 mg, 0.99 mmoles) in acetone (9 ml) and the mixture was heated at 50°C for 22 h. The mixture was concentrated under reduced pressure to a small volume, saturated with NaCl, and extracted with small portions of ethyl acetate (20 ml×3). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated to give a viscous oil (346 mg). Purification of the product by column chromatography on a silica gel column (hexane—ethyl acetate, 1:1.5) gave the title compound, XIV as a white solid (280 mg, 84% yield). ^1H NMR analysis indicated that it was a mixture of two isomers. Found, %: C 46.20; H 6.66; N 29.20. $C_{13}H_{23}N_7O_2Si$. Calculated, %: C 46.27; H 6.87; N 29.06.

200 MHz ^1H NMR ($CDCl_3$) δ: 7.90 (1H, s); 6.34 (1H, d, J = 7.46 Hz); 5.36 (1H, d, J = 3.0 Hz); 5.27 (1H, d, J = 1.4 Hz); 4.11 (3H, s); 3.65 (1H, t, J = 2.1 Hz); 0.87 (9H, s); 0.14 (3H, s); 0.13 (3H, s).

200 MHz ^1H NMR ($CDCl_3$) for the other isomer δ: 7.44 (1H, s); 6.34 (1H, d, J = 7.46 Hz); 5.36 (1H, d, J = 3.0 Hz); 4.98 (1H, d, J = 1.5 Hz); 4.10 (3H, s); 3.50 (1H, dd, J = 1.8 and 2.8 Hz); 0.86 (9H, s); –0.03 (3H, s); –0.19 (3H, s).

3-[1-*t*-Butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-4-phenylthio-2-azetidinone (XV). To a solution of compound VIII (2.04 g, 5.75 mmoles) in acetone (30 ml) was added dropwise a solution of thiophenol (647 mg, 5.87 mmoles) and NaOH (237 mg, 5.93 mmoles) in water (30 ml). The mixture was stirred at room temperature for 4 h; acetone was removed under reduced pressure. The aqueous layer was extracted with small portions of methylene chloride. The combined methylene chloride layers were washed with water, brine, dried ($MgSO_4$), and concentrated under reduced pressure to give a sticky foam (2.1 g). Purification of the product by column chromatography over a silica gel column (hexane—ethyl acetate, 1:1) gave compound XV as a mixture of two isomers (1.6 g, 69% yield). Found, %: C 56.20; H 6.90; N 13.65. $C_{19}H_{28}N_4O_2SSi$. Calculated, %: C 56.40; H 6.97; N 13.85.

200 MHz ^1H NMR ($CDCl_3$) δ: 7.79 (1H, s); 7.09-7.39 (5H, m); 6.13 (1H, br. s); 5.27 (1H, d, J = 2.7 Hz); 5.19 (1H, d, J = 2.7 Hz); 3.97 (3H, s); 3.23 (1H, br. t); 0.74 (9H, s); –0.14 (3H, s); –0.30 (3H, s).

200 MHz ^1H NMR ($CDCl_3$) of other isomer, δ: 7.28 (1H, s); 7.09-7.39 (5H, m); 6.13 (1H, br. s); 5.03 (1H, d, J = 2.3 Hz); 4.90 (1H, d, J = 2.3 Hz); 3.98 (3H, s); 3.43 (1H, br. t); 0.77 (9H, s); 0.016 (3H, s); –0.007 (3H, s).

3-[1-*t*-Butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)-methyl]-4-triazol-1-yl-2-azetidinone (XVI). The azido derivative XIV (257 mg, 0.762 mmoles) was dissolved in dimethoxyethane (15 ml) and transferred into a steel bomb which was flushed with dry N_2 and cooled to –78°C. Excess acetylene was transferred into the bomb. The mixture was heated at 90°C for 22 h. The steel bomb was cooled to 0°C and the valve was released to remove excess acetylene. The residue was taken in THF and transferred to a round-bottomed flask. Evaporation of the solvent under reduced pressure gave a white foam (240 mg, 87% yield). ^1H NMR analysis of the product indicated it was a mixture of two isomers. Found, %: C 49.58; H 6.94; N 26.89. $C_{15}H_{25}N_7O_2Si$. Calculated, %: C 49.56; H 6.93; N 26.98.

200 MHz ^1H NMR ($CDCl_3$) δ: 7.90 (1H, s); 7.89 (1H, s); 7.57 (1H, s); 6.89 (1H, br. s); 6.58 (1H, br. s); 5.48 (1H, d, J = 2.5 Hz); 4.08 (3H, s) 3.97 (1H, br. t); 0.93 (9H, s); 0.18 (3H, s); –0.0005 (3H, s).

200 MHz ^1H NMR ($CDCl_3$) of the other isomer, δ: 7.85 (1H, s); 7.80 (1H, s); 7.72 (1H, s); 6.89 (1H, br, s); 6.31 (1H, br, s); 5.48 (1H, d, J = 2.5 Hz); 4.13 (3H, s); 3.93 (1H, br, t); 0.91 (9H, s); 0.21 (3H, s); –0.12 (3H, s).

3-[1-*t*-Butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-4-(1,2,3-triazol-1-yl)-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone (XVIIb). To a solution of the azetidinone XVI, (3.70 g, 10.26 mmoles) in dry methylene chloride (100 ml) was added triethylamine (2.08 g, 20.53 mmoles) followed by trichloroethyl chloroformate (4.35 g, 20.53 mmoles) and the mixture was stirred at room temperature overnight. The reaction was quenched by addition of water, the organic layer was separated out, washed with 10% aq. HCl, water, 10% $NaHCO_3$ solution, water,

brine, and finally dried ($MgSO_4$). The solvent was removed under reduced pressure to give a yellow gum which on trituration with a mixture of ether—hexane gave a white solid (4.03 g, 73% yield); m.p. 120-122°C (decomp.). ^{1}H-NMR analysis indicated that it was a mixture of two isomers. Found, %: C 39.98; H 4.80; N 18.30. $C_{18}H_{26}Cl_3N_7O_4Si$. Calculated, %: C 40.12; H 4.86; N 18.20.

200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.92 (1H, s); 7.85 (1H, s); 7.78 (1H, s); 6.50 (1H, d, J = 2.6 Hz); 5.49 (1H, d, J = 3.5 Hz); 4.77 (2H, ABq, J = 12.0 and 23.0 Hz); 4.67 (1H, br. t); 4.12 (3H, s); 0.85 (9H, s); 0.17 (3H, s); –0.16 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) of the other isomer, δ: 8.00 (1H, s); 7.71 (1H, s); 7.44 (1H, s); 7.00 (1H, d, J = 1.4 Hz); 5.47 (1H, d, J = 3.7 Hz); 4.76 (2H, ABq, J = 12.0 and 15.0 Hz); 4.47 (1H, br. t); 4.05 (3H, s); 0.86 (9H, s); 0.13 (3H, s); –0.07 (3H, s).

3-[1-*t*-Butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-4-phenylthio-1-(2,2,2-trichloroethyloxycarbonyl)-2-azetidinone (XVIIc) was prepared in the same manner in 80% yield starting from compound XV. Found, %: C 45.27; H 4.98; N 9.58. $C_{22}H_{29}Cl_3N_4O_4SSi$. Calculated, %: C 45.56; H 5.04; N 9.66.

4-Acetoxy-3-[1-*t*-butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)-methyl]-2-azetidinone (XVIId) was prepared in the same manner in 74% yield starting from compound VIII. Found, %: C 40.63; H 5.08; N 10.59. $C_{18}H_{27}Cl_3N_4O_6Si$. Calculated, %: C 40.80; H 5.14; N 10.57.

3-[1-*t*-Butyldimethylsiloxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-1-(4-nitrobenzyloxycarbonyl)-4-(1,2,3-triazol-1-yl)-2-azetidinone (XVIIa) was prepared from compound XVI by using 4-nitrobenzyl chloroformate instead of 2,2,2-trichloroethyl chloroformate in about 70% yield. Found, %: C 51.02; H 5.54; N 20.60. $C_{23}H_{30}N_2O_6Si$. Calculated, %: C 50.91; H 5.57; N 20.65.

3-[1-Hydroxy-1-(N-methyl-1,2,3-triazol-4-yl)methyl]-4-(1,2,3-triazol-1-yl)-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone (XVIIIb). The silyloxy derivative (XVIIb), (4.03 g, 7.48 mmoles) was dissolved in acetonitrile (200 ml). To this solution, 48% HF (74 ml) was added and the mixture was stirred at room temperature for 4 days. Reaction was quenched by addition of 0.1 N phosphate buffer (60 ml), ethyl acetate (80 ml) was added, and the mixture was carefully neutralized by addition of $NaHCO_3$. The aqueous layer was separated out, saturated with NaCl, and extracted with ethyl acetate. The combined EtOAc extracts were dried ($MgSO_4$), evaporated to dryness to give a white solid, which was triturated with ether. The solid was collected by filtration (2.49 g, 78% yield). ^{1}H-NMR analysis indicated that it was a mixture of two isomers. Found, %: C 33.95; H 2.90; N 23.20. $C_{12}H_{12}Cl_3N_7O_4$. Calculated, %: C 33.94; H 2.25; N 23.09.

200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.61 (1H, d, J = 1.0 Hz); 8.01 (1H, s); 7.85 (1H, d, J = 1.0 Hz); 6.77 (1H, d, J = 2.8 Hz); 6.39 (1H, d, J = 5.0 Hz, exchangeable with D_2O); 5.29-5.40 (1H, m); 4.94 (2H, ABq, J = 12.3 and 19.5 Hz); 4.48-4.56 (1H, m); 4.05 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) for other isomer, δ: 8.57 (1H, d, J = 0.9 Hz); 8.02 (1H, s); 7.81 (1H, d, J = 0.9 Hz); 6.84 (1H, d, J = 2.7 Hz); 6.40 (1H, d, J = 5.2 Hz, exchangeable with D_2O); 5.29-5.40 (1H, m); 4.95 (2H, ABq, J = 12.3 and 18.5 Hz); 4.48-4.56 (1H, m); 4.00 (3H, s).

Compounds XVIIIa, XVIIIc, and XVIIId were prepared in the same manner and have quite satisfactory data of elemental analyses (C, H, N).

***E*- and *Z*-3-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-4-(1,2,3-triazol-1-yl)-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinones (XIXb and XXb).** To an ice-cooled solution of compound XVIIIb (145 mg, 0.34 mmole) in a mixture of acetonitrile (24 ml) and methylene chloride (24 ml) was added triethylamine (276.45 mg, 2.73 mmoles) followed by mesyl chloride (156.47 mg, 1.37 mmol). The mixture was stirred at 0°C for 3 h, the reaction was quenched by addition of water, and methylene chloride was added. The methylene chloride layer was separated out, washed successively with dil. HCl, water, 10% $NaHCO_3$ solution, water, brine, dried ($MgSO_4$), and concentrated to give a solid (100 mg). Found, %: C 35.67; H 2.46; N 23.99. $C_{12}H_{10}Cl_3N_7O_3$. Calculated, %: C 35.44; H 2.48; N 24.11. The (*E*)- and (*Z*)-isomers were separated by preparative TLC; the (3*Z*)-isomer was a white solid, 20 mg (14% yield); m.p. 165-166°C (decomp.) and (3*E*)-isomer was obtained as a white solid (15 mg, 11% yield); m.p. 109-110°C (decomp.).

200 MHz ^{1}H NMR ($CDCl_3$) of (3*E*)-isomer, δ: 8.50 (1H, d, J = 1.0 Hz); 8.28 (1H, s); 7.72 (1H, d, J = 1.0 Hz); 7.70 (1H, d, J = 1.5 Hz); 7.40 (1H, d, J = 1.5 Hz); 4.95 (2H, ABq, J = 12.2 and 21.6 Hz); 4.00 (3H, s).

200 MHz ^{1}H NMR ($CDCl_3$) of (3*Z*)-isomer, δ: 8.85 (1H, s); 8.54 (1H, br. s); 7.84 (1H, br. s); 7.27 (1H, br. s); 7.22 (1H, br. s); 4.96 (2H, ABq, J = 12.2 and 22.0 Hz); 4.17 (3H, s).

Compounds XIXa, c, d and XXa, c, d were prepared and characterized similarly.

***Z*- and *E*-3-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-4-phenylthio-1-(2,2,2-trichloroethoxycarbony)-2-azetidinones (XIXc and XXc).** Found, %: C 43.01; H 2.97; N 12.34. $C_{16}H_{13}Cl_3N_4O_3S$. Calculated, %: C 42.92; H 2.93; N 12.51.

200 MHz ^{1}H NMR ($CDCl_3$) of (3*Z*)-isomer, δ: 8.01 (1H, s); 7.20-7.40 (5H, m); 7.26 (1H, d, *J* = 1.6 Hz); 5.95 (1H, d, *J* = 1.6 Hz); 4.94 (2H, ABq, *J* = 11.8 and 20.7 Hz); 4.22 (3H, s); m.p. 60-61°C (decomp.).

200 MHz ^{1}H NMR ($CDCl_3$) of (3*E*)-isomer, δ: 8.61 (1H, s); 7.20-7.55 (5H, m); 7.11 (1H, d, *J* = 1.7 Hz); 5.85 (1H, d, *J* = 1.7 Hz); 4.94 (2H, ABq, *J* = 12.0 and 20.0 Hz); 4.13 (3H, s); m.p. 136-138°C (decomp.).

***E*- and *Z*-4-Acetoxy-3-[(N-methyl-1,2,3-triazol-4-yl)-methylene]-1-(2,2,2-trichloroethoxy-carbonyl)-2-azetidinones (XIXd and XXd).** Found, %: C 36.31; H 2.83; N 14.27. $C_{12}H_{11}Cl_3N_4O_5$. Calculated, %: C 36.25; H 2.79; N 14.09. 200 MHz ^{1}H NMR ($CDCl_3$) of (3*E*)-isomer, δ: 7.78 (1H, s); 7.48 (1H, d, *J* = 1.1 Hz); 7.32 (1H, d, *J* = 1.2 Hz); 4.88 (2H, ABq, *J* = 12.0 and 54.0 Hz); 4.14 (3H, s); 2.14 (3H, s). 200 MHz ^{1}H NMR ($CDCl_3$) of (3*Z*)-isomer, δ: 8.74 (1H, s); 7.29 (1H, s); 6.93 (1H, s); 4.89 (2H, ABq, *J* = 12.0 and 42.0 Hz); 4.16 (3H, s); 2.18 (3H, s); m.p. 126-128°C (decomp.).

***E*- and *Z*-3-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-1-(4-nitrobenzyloxycarbonyl- 4-(1,2,3-triazol-1-yl)-2-azetidinones (XIXa and XXa).** Found, %: C 49.63; H 3.46; N 27.23. $C_{17}H_{14}N_8O_5$. Calculated, %: C 49.76; H 3.44; N 27.31. 200 MHz ^{1}H NMR (DMSO-d_6) of (3*E*)-isomer, δ: 8.50 (1H, s); 8.27 (1H, s); 8.24 (2H, d); 7.72 (1H, s); 7.67 (1H, br, s); 7.60 (2H, d); 7.40 (1H, br. s); 5.34 (2H, s); 4.00 (3H, s); m.p. 154-155°C (decomp.). 200 MHz ^{1}H NMR (DMSO-d_6) of (3*Z*)-isomer, δ: 8.82 (1H, s); 8.56 (1H, s); 8.23 (2H, d); 7.87 (1H, s); 7.60 (2H, d); 7.27 (1H, s); 7.19 (1H, s); 5.36 (2H, s); 4.17 (3H, s); m.p. 150-152°C (decomp.).

(3*E*)-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-4-(1,2,3-triazol-1-yl)-2-azetidinone (XXI). To a solution of XIXb (281 mg, 0.68 mmole) in a mixture of glacial acetic acid (327 mg) and DMF (9.7 ml), activated zinc (178 mg) was added and the mixture was stirred for 3 h at room temperature. The reaction mixture was filtered through Celite and washed thoroughly with ethyl acetate. The filtrate was concentrated under reduced pressure to give a viscous oil, which was taken in water and partitioned with ethyl acetate. The aqueous layer was separated out and freeze-dried to give a solid (300 mg) which was partially purified over a HP-20 column (water—acetonitrile, 1:1). The solid (170 mg) obtained from the column was redissolved in a small volume of water and ethyl acetate was added. The mixture was stirred overnight under reflux at 70°C. The ethyl acetate layer was separated out, dried ($MgSO_4$), and concentrated to give a white solid (70 mg, 45% yield); m.p. 135-137°C (decomp.). 200 MHz ^{1}H NMR (DMSO-d_6) δ: 9.82 (1H, br. s); 8.26 (1H, s); 8.08 (1H, s); 7.70 (1H, s); 7.16 (1H, s); 6.98 (1H, s); 3.98 (3H, s). Found, %: 46.65; H 3.80; N 42.32. $C_9H_9N_7O$. Calculated, %: C 46.75; H 3.92; N 42.41.

Compounds XXII and VII were prepared in the same manner.

(3*Z*)-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-4-(phenylthio)-2- azetidinone (XXII). 200 MHz ^{1}H NMR (DMSO-d_6) δ: 9.39 (1H, br. s); 8.37 (1H, s); 7.33 (5H, s); 6.86 (1H, d, *J* = 1.2 Hz); 5.81 (1H, d, *J* = 1.2 Hz); 4.13 (3H, s); m. p. 124-126°C (decomp.). Found, %: C 57.50; H 440; N 20.55. $C_{13}H_{12}N_4OS$. Calculated, %: C 57.33; H 4.44; N 20.58.

4-Acetoxy-(3*E*)-[(N-methyl-1,2,3-triazol-4-yl)methylene]-2-azetidinone (VII). 200 MHz ^{1}H NMR (DMSO-d_6) δ: 9.74 (1H, br. s); 8.30 (1H, s); 6.97 (1H, s); 6.47 (1H, s); 4.07 (3H, s); 2.07 (3H, s); m.p. 112-114°C (decomp.). Found, %: C 48.63; H 4.52; N 25.24. $C_9H_{10}N_4O_3$. Calculated, %: C 48.65; H 4,54; N 25.22.

Potassium (3*E*)-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-4-(1,2,3-triazol-1-yl)-2-azetidinone-1-sulfonate (IV). A solution of compound XXI (54 mg, 0.23 mmol) in dry methylene chloride (15 ml) was treated with triethylamine (28.4 mg, 0.28 mmol) followed by trimethylsilyl chloride (30.5 mg, 0.28 mmol) and the mixture was stirred at room temperature overnight under N_2. Trimethylsilyl chlorosulfonate (52.9 mg, 0.28 mmol) was added and the mixture was stirred overnight at room temperature under N_2, then heated under reflux at 40°C for 1 h under N_2. After cooling, the mixture was treated with a solution of KH_2PO_4 (500 mg dissolved in 8 ml of water, 3.67 mmol). The mixture was stirred for 30 min and then pH of the mixture was adjusted to ~7.0 with 5% KOH solution. The aqueous layer was separated out and extracted with methylene chloride followed by ether. The aqueous layer was concentrated under reduced pressure to a small volume which was purified by reverse-phase preparative TLC (acetonitrile—water, 10:1). After freeze-drying the product was obtained as a fluffy white amorphous solid (23 mg, 28% yield). 200 MHz ^{1}H NMR (D_2O) δ: 8.30 (1H, s); 7.96 (1H, s); 7.82 (1H, s); 7.57 (1H, s); 7.32 (1H, s); 4.02 (3H, s). Found, FAB [M+1]: 350. $C_9H_8KN_7O_4S$. Calculated, M: 349.

Potassium (3*E*)-[(N-Methyl-1,2,3-triazol-4-yl)methylene]-4-phenylthio-2-azetidinone-1-sulfonate (III) was prepared in the same manner as described above; 200 MHz ^{1}H NMR (DMSO-d_6) δ: 8.92 (1H, s); 7.60-7.80 (5H, m); 7.23 (1H, d, *J* = 1.4 Hz); 6.22 (1H, d, *J* = 1.4 Hz); 4.58 (3H, s). Found, FAB [M+1]: 391. $C_{13}H_{11}KN_4O_4S_2$. Calculated, M: 390.

ACKNOWLEDGEMENTS

The authors wish to thank Taiho Pharmaceutical Co., Ltd. for financial support and Mrs. Rhonda Sarnoski for typing the manuscript.

REFERENCES

1. T. T. Howarth, A. G. Brown, and T. T. King, J. Chem. Soc. Chem. Commun., 266 (1976).
2. A. R. English, J. A. Retsema, A. E. Girard, J. E. Lynch, and W. E. Barth, Antimicrob. Agents Chemother., **14**, 414 (1978).
3. T. W. Hall, S. N. Maiti, R. G. Micetich, P. Spevak, S. Yamabe, N. Ishida, M. Kajitani, M. Tanaka, and T. Yamazaki, Recent Advances in the Chemistry of β-Lactam Antibiotics, S. M. Roberts and A. G. Brown (eds.), Royal Society of Chemistry, London (1985), p. 285.
4. R. G. Micetich, S. N. Maiti, P. Spevak, T. W. Hall, S. Yamabe, N. Ishida, M. Tanaka, T. Yamazaki, A. Nakai, and K. Ogawa, J. Med. Chem., **30**, 1469 (1987).
5. Y. L. Chen, C. W. Chang, and K. Hedberg, Tetrahedron Lett., **27**, 3449 (1986).
6. Y. L. Chen, C. W. Chang, K. Hedberg, K. Guarino, W. M. Welch, L. Kiessling, J. A. Retsema, S. L. Haskell, M. Anderson, M. Manousos, and J. F. Barrett, J. Antibiot., **40**, 803 (1987).
7. Y. L. Chen, K. Hedberg, J. F. Barrett, and J. A. Retsema, J. Antibiot., **41**, 134 (1988).
8. K. Coleman, D. R. J. Griffin, J. W. J. Page, and P. A. Upshon, Antimicrob. Agents Chemother., **33**, 1580 (1989).
9. G. Woodnutt, V. Berry, and L. Mizen, Antimicrob. Agents Chemother., **36**, 1427 (1992).
10. K. Coleman, D. R. J. Griffin, and P. A. Upshon, Antimicrob. Agents Chemother., **35**, 1748 (1991).
11. I. Bennett, N. J. P. Broom, G. Bruton, S. Calvert, B. P. Clarke, K. Coleman, R. Edmondson, P. Edwards, D. Jones, N. F. Osborne, and G. Walker, J. Antibiot., **44**, 331 (1991).
12. K. Bush, Rev. Infect. Dis., **10**, 681 (1988).
13. W. J. Leanza, F. Dininno, D. A. Muthard, R. R. Wilkening, K. J. Wildonger, R. W. Ratcliffe, and B. G. Christensen, Tetrahedron, **39**, 2505 (1983).
14. R. A. Volkmann, R. D. Carroll, R. B. Drolet, M. L. Elliott, and B. S. Moore, J. Org. Chem., **47**, 3344 (1982).
15. R. G. Micetich, S. N. Maiti, M. Tanaka, T. Yamazaki, and K. Ogawa, J. Org. Chem., **51**, 853 (1986).
16. A. Yoshida, T. Hayashi, N. Takeda, S. Oida, and E. Ohki, Chem. Pharm. Bull., **29**, 2899 (1981).
17. J. D. Buynak, M. N. Rao, H. Pajouhesh, R. Y. Chandrasekaran, and K. Finn, J. Org. Chem., **50**, 4245 (1985).
18. C. M. Cimarusti, D. P. Bonner, H. Breuer, H. W. Chang, A. W. Fritz, D. M. Floyd, T. P. Kissick, W. H. Koster, D. Kronenthal, F. Massa, R. H. Mueller, J. Pluscec, W. A. Slusarchyk, R. B. Sykes, M. Taylor, and E. R. Weaver, Tetrahedron, **39**, 2577 (1983).
19. J. Fisher, R. L. Charnas, S. M. Bradley, and J. R. Knowles, Biochemistry, **20**, 2726 (1981).
20. D. G. Brenner and J. R. Knowles, Biochemistry, **20**, 3680 (1981).
21. C. Kemal and J. R. Knowles, Biochemistry, **20**, 3688 (1981).
22. D. G. Brenner and J. R. Knowles, Biochemistry, **23**, 5833 (1984).