β-LACTAMASE INHIBITORS: SYNTHESIS AND IN VITRO EVALUATION OF 6-[(1-SUBSTITUTED-1,2,3-TRIAZOL-4-YL)METHYLENE]-PENICILLANIC ACID SULFONES

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Abstract - A series of 6-[(1-substituted 1,2,3-triazol-4-yl)methylene]penicillanic acid sulfones were synthesized and tested for β-lactamase inhibitory activity. The (6Z)-isomers were over 100 times more potent than the (6E)-isomers against cell-free β-lactamases and were synergistic with ampicillin against a variety of β-lactamase producing bacteria.

Despite the introduction of new β-lactam antibiotics with extended spectrum of antibacterial activity and improved β-lactamase stability, emergence of resistant pathogenic bacteria continues to be a major problem in clinics.[1,2] β-Lactamase activity is the primary mechanism of high-level resistance to β-lactam antibiotics. The strategy to counter β-lactamase activity by the use of β-lactams resistant to β-lactamases has not been very successful, rather adversely, the extensive and indiscriminate usage of such agents has been shown to confer high levels of resistance in bacteria world-wide. The use of inactivators for β-lactamases has extended the clinical utility of β-lactamase-susceptible penicillins.[3] The success of clavulanic acid[4] stimulated extensive research in this area which resulted in the discovery and introduction of other classes of β-lactamase inhibitors such as sulbactam[5] and tazobactam.[6] Emergence of bacteria producing new extended spectrum β-lactamases including the plasmid and chromosomally mediated AmpC-types pose further threat to the armamentarium of β-lactam antibiotics.[1,7,8] Therefore, the need

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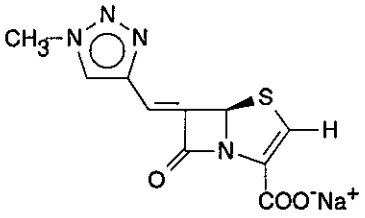
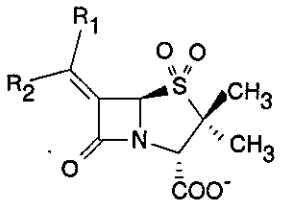
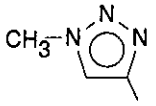
Figure 1

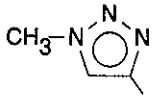


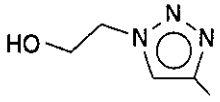
BRL-42,715

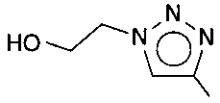
1

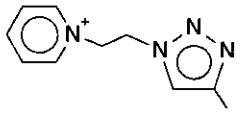


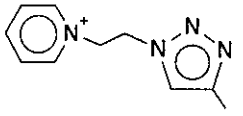
2 a, R_1 =  , R_2 = H

b, R_1 = H , R_2 = 

c, R_1 =  , R_2 = H

d, R_1 = H , R_2 = 

e, R_1 =  , R_2 = H

f, R_1 = H , R_2 = 

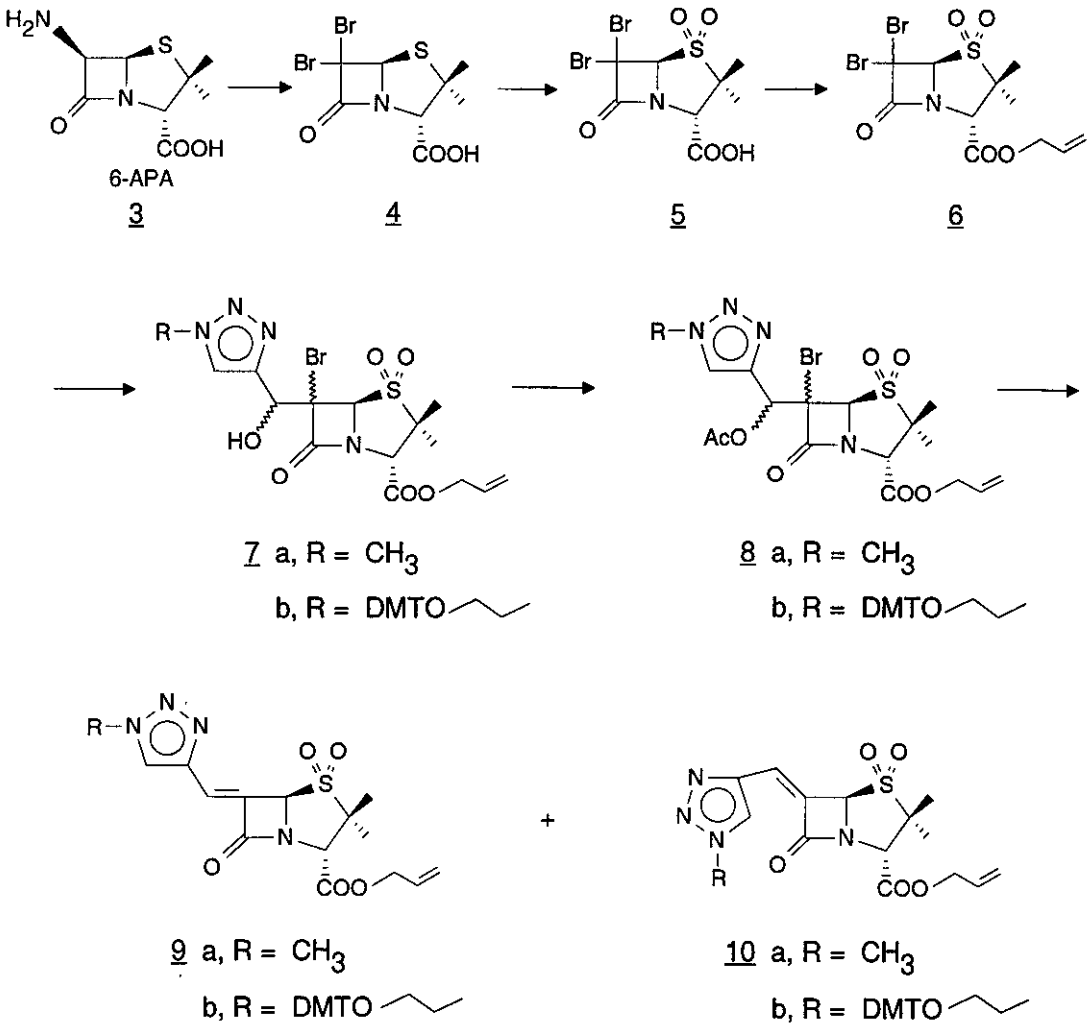
for broad spectrum β-lactamase inhibitors with suitable physico-chemical and pharmacodynamic profile for oral as well as parenteral use remains a subject of high interest among medicinal chemists.

A number of 6-(heteroaryl substituted methylene)penams have been reported in the literature[9,10,11] as potent inhibitors of cell free β-lactamases, but were not effective in synergistic tests probably because of poor penetration through the bacterial cell wall. A recent breakthrough on the β-lactamase research has been the synthesis of BRL-42,715 (1)[12,13,14] which is a potent inhibitor of most bacterial β-lactamases including the class I cephalosporinases. This penem derivative bearing the N_1-methyltriazolylmethylene group at C-6 position had good synergistic activity with amoxycillin[15] in vitro and in vivo, however there is little information about the further development of this compound.

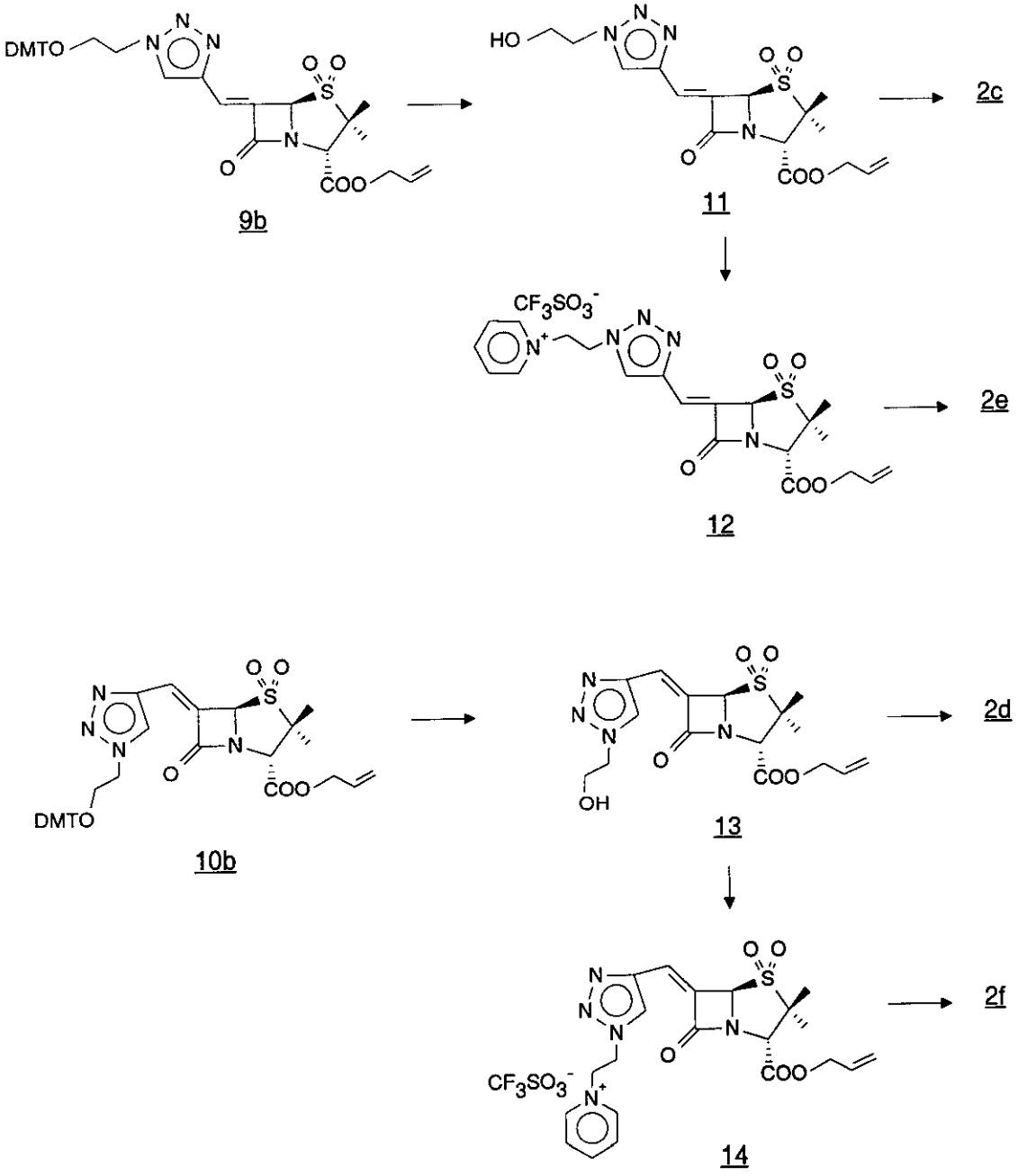
In search for new β-lactamase inhibitors based on the penam sulfone skeleton, we prepared a series of 6-[(1-substituted 1,2,3-triazol-4-yl)methylene]penam sulfones and in this paper we report the synthesis and β-lactamase inhibitory activities of both the Z- and E-isomers of 6-triazolylmethylenepenicillanic acid sulfones (2) containing a substituent at the N_1-position of the triazole moiety. It was anticipated that by introduction of various chemical functions at N_1-position of the triazole ring, the physico-chemical properties of the molecule could be altered to cross the normal permeability barrier of the bacterial cell wall and that specific interactions with the target enzymes could be achieved.

By using the literature procedure,[16] 6-aminopenicillanic acid (6-APA) was converted to 6,6-dibromopenicillanic acid (4) which was oxidized with $KMnO_4$ in a mixture of glacial acetic acid-acetone-water to the corresponding sulfone (5) in 69% yield. Esterification of the crude dibromo acid (5) by allyl bromide in DMF gave the allyl ester (6) in 99.8% yield. The allyl ester (6) underwent metal halogen exchange with methylmagnesium bromide in THF at -78° C to give an enolate intermediate which, on quenching with 1-substituted 1,2,3-triazol-4-carboxaldehyde, afforded an inseparable mixture of the corresponding bromohydrins (7a-b). Acetylation followed by reductive elimination with Zn/HOAc in THF gave the (6Z)- and (6E)-isomers which were separated by column chromatography. The structural assignment of the (E)-geometry around the double bond was based on a comparison of its ^{1}H-nmr spectrum with that of the (Z)-isomer. Thus the vinyl proton of compound (9a) appears at δ 7.32,

Scheme I


6-APA
3
4
5
6
7 a, R = CH3
b, R = DMTO
8 a, R = CH3
b, R = DMTO
9 a, R = CH3
b, R = DMTO
10 a, R = CH3
b, R = DMTO
[9a , 10a] ⟶ [2a , 2b]

Scheme II



downfield from that of the corresponding (6E)-isomer (10a) which appears at δ 7.12 due to the anisotropic deshielding effect of the β-lactam carbonyl on this proton. On the other hand, the triazole proton of the 6E isomer (10a) is deshielded by the β-lactam carbonyl and appears at δ 8.69 downfield from that of the 6Z isomer (9a) which appears at δ 7.77. Removal of the 4,4-dimethoxytrityloxy group of compound (9b) by formic acid gave compound (11) in about 47% yield. Treatment of compound (11) with trifluoromethanesulfonic anhydride in large excess of pyridine gave compound (12) in 73% yield. Finally, the allyl group was removed by treating the esters with palladium tetrakis in presence of triphenylphosphine. Compounds (2e) and (2f) were obtained in zwitterions. Compounds (2a, 2b, 2c and 2d) were obtained as sodium salts by treating the corresponding acids with sodium 2-ethylhexanoate. The sodium salts were purified by passing through a HP-20 column.

Six compounds (2a-f) representing three different substituents at 6-position and two isomeric types for each were synthesized and tested for biological activity. The β-lactamase inhibitory activity against cell-free β-lactamases and synergistic antibacterial activity with ampicillin against β-lactamase producing clinical isolates are given in Tables 1 and 2, respectively.

Table 1. β-Lactamase inhibitory activity of compounds 2 (a-f) against cell free enzymes

	IC_{50} (μM)		
Inhibitor	Penicillinase (*B. cereus*)	R-TEM (*E. coli*)	Cephalosporinase (*E. cloacae*)
2a	>20	0.2	0.7
2b	>20	>20	>20
2c	>20	0.34	0.4
2d	>100	>20	>20
2e	20	0.11	21
2f	>100	>100	1.5
Tazobactam	0.31	0.076	2.4
Sulbactam	23	4.9	20

Table 2. In vitro comparative synergy of 2a, 2c, 2e and tazobactam with ampicillin (ABPC)

Organisms	MIC (μg/ml)				
	ABPC	+ TZB	+ 2a	+ 2c	+ 2e
E. coli SHV-1	>128	2	128	>128	8
E. coli TEM-1	>128	4	>128	>128	>128
E. coli TEM-2	>128	2	>128	>128	>128
E. coli OXA-1	>128	64	128	128	32
E. cloacae [a]	>128	>128	>128	>128	>128
E. aerogenes ATCC 13048 [a]	128	8	64	64	32
F. odoratum ATCC 29979 [a]	32	64	64	64	64
C. freundii (Ceph-R) [a]	128	2	128	128	>128
K. pneumoniae ATCC 13883 [b]	128	4	16	32	4
S. marcescens ATCC 29882 [c]	>128	128	16	16	4
P. aeruginosa ATCC 27853 [c]	>128	>128	>128	>128	>128
S. epidermidis ATCC 27626 [b]	64	8	64	64	64

[a], cephalosporinase producing organism; [b], penicillinase producing organism; [c], penicillinase and cephalosporinase producing organism. Assay conditions: Microbroth dilution (Mueller Hinton Broth), Inoculum 5 x 10^5 CFU/ml; Incubation 18 h at 37° C; Inhibitor concentration, 15 μg/ml (fixed). MICs of 2a, 2c, and 2e were >128 μg/ml.

Interestingly, the (6Z)-isomers (2a, 2c and 2e) were more active than their (6E)-counterparts against the cell-free cephalosporinase and broad spectrum R-TEM β-lactamase isolated from *Enterobacter cloacae* and *Escherichia coli*, respectively. Activity of (Z)-isomers seems to be better than tazobactam and sulbactam against the cephalosporinase, but they are 2-4 times less active than tazobactam against the R-TEM type enzyme. None of the synthesized compounds exhibited good activity against the penicillinase isolated from *Bacillus cereus*. The(Z)-isomers (15 μg/ml), in combination with ampicillin, were synergistic against only a few β-lactamase producing clinical isolates such as *E. coli* SHV-1, *Enterobacter aerogenes, Klebsiella pneumoniae,* and *Serratia marcescens*. The compound (2e), with charged pyridinium moiety, was the most active in the series against cell-free enzymes and against intracellular enzymes. Most

pronounced activity was noted against *Serratia* sp., where 2e was significantly more active than tazobactam.

Significantly superior β-lactamase inhibitory activity of the(Z)-isomers than (E)-isomers suggests that either (Z)-isomers have better affinity for the enzymes or the triazolyl group of the (E)-isomers crowds around the carbonyl functionality of the lactam ring which may prevent or hinder enzymatic attack on the lactam carbonyl. Lack of good synergistic activity against most β-lactamase producing isolates may be attributed to poor diffusion of these compounds through the normal permeability barrier of bacterial cell wall. Compounds with +ve charge, as present in 2e , may facilitate their entry into periplasmic space to be effective inhibitor of intracellular β-lactamases.

It has been postulated that penam sulfones, like sulbactam and tazobactam, interacts with β-lactamases with high affinity yielding a long-lived acyl-enzyme complex.[17] 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. Since the compound (2a) does not have significant inhibitory activity compared to tazobactam, it is possible to suggest that the affinity of the compound for the enzymes is poor. Even the increase of reactivity expected from the additional ring strain created by the introduction of the double bond does not seem sufficient to allow enzymatic ring opening. Our data suggest that substitutions at C-6 position of penam sulfones delivers good inhibitory activity against broad spectrum β-lactamases. Further modification of (Z)-isomers may lead to compounds with desirable physico-chemical properties.

EXPERIMENTAL

All column chromatographic purifications were accomplished on silica gel (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). The POSFAB mass spectra were recorded on a AEI MS9 (modified). Elemental analyses were performed on a Carlo Erba Ea 11108 analyzer. Melting points were determined on an Electrothermal digital apparatus and were uncorrected. Due to synthetic expediency isomeric separations

were not performed on the intermediates. The reference compounds sulbactam and tazobactam were prepared in our laboratory. All new compounds have been characterized from their spectral data. The purity of all title compounds was judged to be >95% by tlc and ^{1}H nmr. The following three enzymes were used for testing the β-lactamase inhibitory - Penicillinase from *Bacillus cereus* (purchased from Sigma); Cephalosporinase from *Enterobacter cloacae* (purchased from Sigma); broad spectrum R-TEM enzyme from *Escherichia coli* (purchased from Boehringer). The β-lactamase inhibitory activity was determined by spectrophotometrically measuring the hydrolysis of the substrates (penicillin G or cephaloridine) in the presence and absence of the β-lactamase inhibitors.[18] The *in vitro* antibacterial activities were determined by the microbroth dilution method as recommended in the National Committee for Clinical Laboratory Standards.[19] Clinical isolates were obtained from various medical centers in Japan. Other cultures were purchased from the American Type Culture Collection, Rockville, MD. All cultures were stored frozen with 15% glycerol at -80° C. Mueller-Hinton II broth (unsupplemented) purchased from Becton Dickinson Microbiology Systems, Cockeysville, MD was used as the medium. Inocula were adjusted to a density of 10^7 CFU/ml and 5 µl volumes were then inoculated into each well containing 0.1 ml solution of ampicillin (128-0.03 µg/ml) alone or mixed with the inhibitor (15 µg/ml). The MIC was defined as the lowest concentration of antibiotic which prevented turbidity after 18 h of incubation at 38° C.

6,6-Dibromopenicillanic acid-1,1-Dioxide (5)

6,6-Dibromopenicillanic acid (20.0 g, 55.7 mmol) was dissolved in a mixture of glacial acetic acid (84 ml) and acetone (240 ml). To this mixture water (84 ml) was added dropwise, $KMnO_4$ (17.6 g, 111.4 mmol) was added in small portions over a period of 20 min. The resulting mixture was stirred at 0°C for 0.5 h and then at room temperature for 4 h. The mixture was cooled again in an ice-bath and 30% H_2O_2 (v/v) (12 ml) was added dropwise until the mixture became colourless, ice-water (60 ml) was added, then extracted with methylene chloride. The combined methylene chloride extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a light yellow solid (15.0 g, 69%) which was directly used for esterification without further purification.

Allyl 6,6-dibromopenicillanate-1,1-Dioxide (6)

6,6-Dibromopenicillanic acid-1,1-dioxide (20.0 g, 51.14 mmol) was dissolved in DMF (150 ml), $NaHCO_3$ (5.17 g, 61.5 mmol) was added and the mixture was stirred under N_2 for 45 min, allyl bromide (8.02 g, 66.34 mmol) in DMF (20 ml) was added dropwise over 15 min and the mixture was stirred overnight at room temperature. Excess allyl bromide and DMF were removed under reduced pressure; water (200 ml) was added to the mixture and stirred for 30 min. The mixture was extracted with ethyl acetate. The combined ethyl acetate extracts were washed successively with saturated $NaHCO_3$ solution, water, brine and dried (Na_2SO_4). After filtration, the filtrate was treated with activated charcoal and stirred for 15 min. Charcoal was removed by filtration through Celite and the filtrate was concentrated under reduced pressure to give an off-white solid, 86-87° C, (22.0 g, 99.8%). Nmr ($CDCl_3$): δ 1.42 (s, 3H), 1.63 (s, 3H), 4.54 (s, 1H, C-3H), 4.71-4.75 (m, 2H, OCH_2), 5.04 (s, 1H, C-5H), 5.33-5.46 (m, 2H, $=CH_2$), 5.84-6.03 (m, 1H, CH=). Anal. Calcd for $C_{11}H_{13}NO_5Br_2S$: C, 30.64; H, 3.04; N, 3.25. Found: C, 30.65; H, 2.81; N, 3.15.

Allyl 6-bromo-6-[1-hydroxy-1-(*N*-methyl-1,2,3-triazol-4-yl)methyl]penicillanate-1,1-dioxide (7a)

To a stirred solution of 1.0 g (2.3 mmol) of allyl 6,6-dibromopenicillanate-1,1-dioxide 6 in dry THF (20 ml) at -78° C was added dropwise 1.0 ml (3.0 mmol) of 3.0 M methylmagnesium bromide in ether solution. The solution was stirred at -78° C for an additional 15 min and then treated with a solution of 1-methyl-1,2,3-triazole-4-carboxaldehyde (306 mg, 2.8 mmol) in dry THF (5 ml). The mixture was stirred at -78° C for 15 min and then quenched with 3 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 (Na_2SO_4) and concentrated to give a foam (970 mg) which was purified over a silica gel column (elution with hexane-ethyl acetate, 2:1) to afford 640 mg (60%) of a mixture of two major isomers in a ratio of 1:2 (^1H nmr), which were inseparable by chromatography. Nmr ($CDCl_3$) of major isomer: δ 1.40 (s, 3H), 1.61 (s, 3H), 4.12 (s, 3H, N-CH_3), 4.49 (s, 1H, C-3H), 4.56-4.85 (m, 2H, OCH_2), 5.16 (s, 1H, C-5H), 5.31-5.47 (m, 3H), 5.82-6.01 (m, 1H), 7.78 (s, 1H, triazole).

Nmr ($CDCl_3$) of minor isomer: δ 1.40 (s, 3H), 1.61 (s, 3H), 4.13 (s, 3H, N-CH_3), 4.51 (s, 1H, C-3H), 4.56-4.85 (m, 2H, OCH_2), 5.15 (s, 1H, C-5H), 5.31-5.47 (m, 3H), 5.82-6.01 (m, 1H), 7.70 (s, 1H, triazole).

Allyl 6-bromo-6-[1-hydroxy-1-{2-(4,4-dimethoxytrityloxy)ethyl-1,2,3-triazol-4-yl}methyl]-penicillanate-1,1-dioxide (7b)

This compound was prepared in a manner similar to that described for the synthesis of (7a); yield 50% as a pale yellow foam; 1-[{2-(4,4-dimethoxytrityloxy)ethyl}-1,2,3-triazol-4-yl]carboxaldehyde was used as the starting aldehyde; nmr ($CDCl_3$) of the major isomer: δ 1.42 (s, 3H), 1.61 (s, 3H), 3.24 (s, 1H, OH), 3.54 (m, 2H, -CH_2-N), 3.78 (s, 6H, OCH_3), 4.35-4.75 (m, 3H), 4.66-4.70 (m, 2H), 5.29-5.42 (m, 3H), 5.82-5.99 (m, 1H), 6.78-7.29 (m, 13H), 7.96 (s, 1H, triazole).

Allyl 6-bromo-6-[1-acetoxy-1-(*N*-methyl-1,2,3-triazol-4-yl)methyl]penicillanate-1,1-dioxide (8a)

Compound (7a) (8.2 g, 17.7 mmol) was dissolved in 80 ml of dry THF and cooled in an ice-bath. To this solution pyridine (15.4 g, 195 mmol) was added followed by acetic anhydride (18.1 g, 177 mmol). The cold bath was then allowed to warm slowly to room temperature and the mixture was stirred overnight, the reaction was quenched by the addition of water (100 ml). The reaction mixture was extracted with methylene chloride (2 x 50 ml). The aqueous layer was saturated with sodium chloride and re-extracted with methylene chloride (2 x 50 ml). The combined methylene chloride layers were washed successively with 0.5 N HCl, water, saturated $NaHCO_3$ solution, water, brine and dried (Na_2SO_4). The solvent was evaporated and the crude residue was subjected to column chromatography (elution with hexane-ethyl acetate, 2:1) to afford 5.07 g (57%) of a mixture of two isomers in a ratio of 3:1 (^1H nmr) which were inseparable by chromatography. Nmr ($CDCl_3$) of the major isomer: δ 1.38 (s, 3H), 1.60 (s, 3H), 2.21 (s, 3H), 4.12 (s, 3H), 4.44 (s, 1H, C-3H), 4.64-4.79 (m, 2H, OCH_2), 5.27 (s, 1H, C-5H), 5.30-5.46 (m, 2H, =CH_2), 5.81-6.03 (m, 1H, CH=), 6.54 (s, 1H, C-8H), 7.69 (s, 1H, triazole). Nmr ($CDCl_3$) of the minor isomer: δ 1.46 (s, 3H), 1.61 (s, 3H), 2.15 (s, 3H), 4.13 (s, 3H, N-CH_3), 4.53 (s, 1H, C-3H), 4.64-4.79 (m, 2H, OCH_2), 5.30-5.46 (m, 2H, =CH_2), 5.60 (s, 1H, C-5H), 5.81-6.03 (m, 1H, CH=), 6.33 (s, 1H, C-8H), 7.71 (s, 1H, triazole).

Allyl 6-bromo-6-[1-acetoxy-1-{2-(4,4-dimethoxytrityloxy)ethyl-1,2,3-triazol-4-yl}methyl]-penicillanate-1,1-dioxide (8b)

This compound was prepared in a manner similar to that described for the synthesis of 8a; yield 97%. This product was used for the next step without further purification.

Allyl (6Z)- and (6E)-[(*N*-methyl-1,2,3-triazol-4-yl)methylene]penicillanate-1,1-dioxide (9a) and (10a)

To a stirred and ice-cooled solution of compound (8a) (1.0 g, 2 mmol) in dry THF (20 ml) was added glacial acetic acid (3 ml), activated zinc (1.01 g) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through a pad of Celite and washed thoroughly with EtOAc. The filtrate was washed successively with water, saturated $NaHCO_3$ solution, water, brine. After drying (Na_2SO_4) the solvent was removed under reduced pressure to afford a white foam (640 mg) which was purified further over a silica gel column (elution with hexane-ethyl acetate, 3:2).

The (6E)-isomer (10a) was eluted first (250 mg, 34%) and was obtained as pale yellow solid. Nmr ($CDCl_3$): δ 1.47 (s, 3H), 1.62 (s, 3H), 4.15 (s, 3H, N-CH_3), 4.46 (s, 1H, C-3H), 4.65-4.84 (m, 2H, OCH_2), 5.21 (br s, 1H, C-5H), 5.30-5.47 (m, 2H, $=CH_2$), 5.86-6.06 (m, 1H, CH=), 7.12 (s, 1H, olefin), 8.69 (s, 1H, triazole). POSFAB, m/z 367 $[M + H]^+$. The (6Z)-isomer (9a) was obtained as a white foam (310 mg, 42%). Nmr ($CDCl_3$): δ 1.49 (s, 3H), 1.62 (s, 3H), 4.12 (s, 3H, N-CH_3), 4.47 (s, 1H, C-3H), 4.68-4.82 (m, 2H, $COOCH_2$), 5.31-5.46 (m, 2H, $=CH_2$), 5.65 (d, 1H, C-5H, J = 1.1 Hz), 5.86-6.05 (m, 1H, CH=), 7.32 (d, 1H, J = 1.1 Hz, olefin), 7.77 (s, 1H, triazole). Anal. Calcd for $C_{15}H_{18}N_4O_5S$: C, 49.17; H, 4.95; N, 15.29. Found: C, 48.92; H, 4.83; N, 14.84.

Allyl (6Z)- and (6E)-[{1-[2-(4,4-dimethoxytrityloxy)ethyl]-1,2,3-triazol-4-yl}methylene]-penicillanate-1,1-dioxide (9b) and (10b)

These compounds were prepared in a manner similar to those described for the synthesis of 9a and 10a. The two isomers were separated on a silver nitrate impregnated silica gel column (elution with benzene-ethyl acetate, 2:1). The (6Z)-isomer was obtained as a white foam in 15% yield and the (6E)-isomer was obtained as a white foam in 25% yield. Nmr ($CDCl_3$) of (6Z)-isomer: δ 1.48 (s, 3H), 1.58 (s,

3H), 3.57 (br t, 2H, J = 5.1 Hz), 3.78 (s, 6H, OCH_3), 4.45 (br t, 2H, J = 5.1 Hz, DMTO-CH_2-), 4.47 (s, 1H, C-3H), 4.71-4.75 (m, 2H, $COOCH_2$), 5.31-5.46 (m, 2H, $=CH_2$), 5.62 (d, 1H, J = 1.1 Hz, C-5H), 5.86-6.06 (m, 1H, C<u>H</u> = CH_2), 6.77-6.87 (m, 6H, aromatic), 7.14-7.35 (m, 7H, aromatic), 7.34 (d, 1H, J = 1.1 Hz, olefin), 7.88 (s, 1H, triazole). Anal. Calcd for $C_{37}H_{38}N_4O_8S$: C, 63.59; H, 5.48; N, 8.02. Found: C, 63.62; H, 5.46; N, 8.05. Nmr ($CDCl_3$) of (6E)-isomer: δ 1.47 (s, 3H), 1.62 (s, 3H), 3.51-3.59 (m, 2H), 3.77 (s, 6H, OCH_3), 4.44-4.55 (m, 2H, DMTO -CH_2), 4.47 (s, 1H, C-3H), 4.70-4.77 (m, 2H, $COOCH_2$), 5.21 (br, s, 1H, C-5H), 5.32-5.47 (m, 2H, $=CH_2$), 5.85-6.06 (m, 1H, C<u>H</u> = CH_2), 6.77-6.85 (m, 6H, aromatic), 7.14-7.35 (m, 8H, aromatic + olefin), 8.86 (s, 1H, triazole). Anal. Calcd for $C_{37}H_{38}N_4O_8S$: C, 63.59; H, 5.48; N, 8.02. Found: C, 63.60; H, 5.50; N, 7.59.

<u>Sodium (6Z)-[(*N*-methyl-1,2,3-triazol-4-yl)methylene)penicillanate-1,1-dioxide (2a)</u>

To a stirred solution of the allyl ester (<u>9a</u>) (250 mg, 0.7 mmol) in a mixture of ethyl acetate (4 ml) and methylene chloride (5 ml), sodium 2-ethylhexanoate (1.4 ml) was added followed by PPh_3 (18 mg, 0.07 mmol) and palladium tetrakis (27 mg 0.028 mmol). The mixture was stirred at room temperature for 1 h. The precipitated solid was collected by filtration. The solid (pale yellow) was dissolved in a small volume of water and filtered again. The filtrate, after freeze-drying, was purified over a HP-20 column (elution with acetone-water). The title compound (<u>2a</u>) was obtained as a pale yellow fluffy mass in 90% yield. Ir (KBr) 1761, 1689, 1616 cm^{-1}; nmr (D_2O): δ 1.54 (s, 3H), 1.60 (s, 3H), 4.13 (s, 3H, N-CH_3), 4.28 (s, 1H, C-3H), 5.94 (br s, 1H, C-5H), 7.47 (br, s, 1H, olefin), 8.19 (s, 1H, triazole). POSFAB, m/z 348 $[M + H]^+$. In a similar manner as described above, compound (<u>2b</u>) was prepared from compound (<u>10a</u>). Ir (KBr) 1751, 1679, 1618, cm^{-1}; nmr (D_2O): δ 1.50 (s, 3H), 1.60 (s, 3H), 4.14 (s, 3H, N-CH_3), 4.27 (s, 1H, C-3H), 5.64 (br, s, 1H, C-5H), 7.20 (br, s, 1H, olefin), 8.67 (s, 1H, triazole). POSFAB, m/z 348 $[M + H]^+$.

<u>Allyl (6Z)-[{1-(2-hydroxyethyl)-1,2,3-triazol-4-yl}methylene]penicillanate-1,1-dioxide (11)</u>

Compound (<u>9b</u>) (26 g, 0.037 mol) was dissolved in methylene chloride (500 ml) and cooled in an ice-bath. To this solution formic acid (49.1 ml, 1.30 mol) was added dropwise. After the addition was over, the ice-bath was removed and the mixture was stirred at room temperature. The progress of the reaction was

followed by tlc. After 2 h, the mixture was diluted with additional volume of methylene chloride (30 ml). The organic layer was washed with 0.25 N NaOH solution, water, brine and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a brown viscous oil (24.4 g) which was purified by column chromatography over a silica gel column (elution with ethyl acetate-hexane, 2:1 and finally with ethyl acetate). The product (11) was obtained as a white foam (7.0 g, 47%). Nmr ($CDCl_3$): δ 1.48 (s, 3H), 1.62 (s, 3H), 2.81 (br t, 1H, J = 5.4 Hz), 4.00-4.16 (m, 2H, CH_2-N), 4.36-4.58 (m, 2H, $C\underline{H}_2OH$), 4.47 (s, 1H, C-3H), 4.64-4.81 (m, 2H, $COOC\underline{H}_2$), 5.30-5.45 (m, 2H, -CH = $C\underline{H}_2$), 5.65 (d, 1H, J = 1.0 Hz, C-5H), 5.85-6.05 (m, 1H, -$C\underline{H}$ = CH_2), 7.30 (d, 1H, J = 1.0 Hz, olefin), 7.95 (s, 1H, triazole). POSFAB m/z 397 $[M + H]^+$.

Compound (13) was prepared in the same manner starting from 10b.

Nmr ($CDCl_3$): δ 1.47 (s, 3H), 1.61 (s, 3H), 3.65 (br, s, 1H, OH), 4.00-4.12 (m, 2H, CH_2-N), 4.45 (s, 1H, C-3H), 4.47-4.52 (m, 2H, $COOC\underline{H}_2$), 4.74 (br t, 2H, J = 4.6 Hz, $C\underline{H}_2OH$), 5.32-5.45 (m, 2H, CH = $C\underline{H}_2$), 5.35 (br s, 1H, C-5H), 5.84-6.05 (m, 1H, $C\underline{H}$ = CH_2), 7.03 (br, s, 1H, olefin), 8.76 (s, 1H, triazole). POSFAB m/z 397 $[M + H]^+$.

Sodium (6Z)- and (6E)-[{1-(2-hydroxyethyl)-1,2,3-triazol-4-yl}methylene]penicillanate-1,1-dioxide (2c) and (2d)

These compounds were prepared in the same manner as described for the preparation of 2a . Nmr (D_2O) of (6Z)-isomer: δ 1.54 (s, 3H), 1.61 (s, 3H), 4.03 (t, 2H, J = 5.0 Hz, -$C\underline{H}_2N$), 4.30 (s, 1H, C-3H), 4.61 (t, 2H, J = 5.0 Hz, -$C\underline{H}_2OH$), 5.99 (d, 1H, J = 1.0 Hz, C-5H), 7.55 (d, 1H, J = 1.0 Hz, olefin), 8.33 (s, 1H, triazole). POSFAB m/z 379 $[M + H]^+$. Nmr (D_2O) of (6E)-isomer: δ 1.51 (s, 3H), 1.61 (s, 3H), 4.04 (t, 2H, J = 5.0 Hz, -$C\underline{H}_2N$), 4.30 (s, 1H, C-3H), 4.62 (t, 2H, J = 5.0 Hz, -CH_2OH), 5.66 (s, 1H, C-5H), 7.25 (s, 1H, olefin), 8.79 (s, 1H, triazole). POSFAB m/z 379 $[M + H]^+$.

Allyl (6Z)-[{1-(2-pyridiniumethyl)-1,2,3-triazol-4-yl}methylene]penicillanate-1,1-dioxide (12)

To a stirred solution of compound (11) (200 mg, 0.505 mmol) in dry methylene chloride (10 ml) and cooled to -40° C, pyridine (1.02 ml) was added dropwise followed by trifluoromethanesulfonic anhydride (0.32 ml, 1.9 mmol). The color of the reaction mixture turned from colorless to yellow. It was allowed to warm to room temperature over a period of 1 h and the color of the mixture became orange. The mixture was diluted with methylene chloride and washed successively with water, 10% HCl, brine, dried (Na_2SO_4). After evaporation of the solvent the title compound was obtained as tan brown crystals (225 mg, 73% yield). This product was directly used for the next step. Nmr ($CDCl_3$): δ 1.42 (s, 3H), 1.56 (s, 3H), 4.43 (s, 1H, C-3H), 4.59-4.72 (m, 2H, $-CH_2N$), 5.15-5.44 (m, 6H), 5.73 (br s, 1H, C-5H), 5.84-6.04 (m, 1H, $CH{=}CH_2$), 7.25 (s, 1H, olefin), 7.92-7.99 (m, 2H, pyridine), 8.27 (s, 1H, triazole), 8.39-8.47 (m, 1H, pyridine), 8.76-8.79 (m, 2H, pyridine).

Compound (14) was prepared in a similar manner as described for the synthesis of compound (12). Nmr ($CDCl_3$): δ 1.43 (s, 3H), 1.57 (s, 3H), 4.60 (s, 1H, C-3H), 4.55-4.79 (m, 2H, $-CH_2N$), 5.16-5.43 (m, 6H), 5.43 (s, 1H, C-5H), 5.83-6.02 (m, 1H, $CH{=}CH_2$), 7.01 (s, 1H, olefin), 7.97-7.99 (m, 2H, pyridine), 8.40-8.47 (m, 1H, pyridine), 8.60 (s, 1H, triazole), 8.88-8.91 (m, 2H, pyridine).

(6Z)-[{1-(2-pyridiniumethyl)-1,2,3-triazol-4-yl}methylene]penicillanate-1,1-dioxide (2e)

To a stirred solution of compound (12) (50 mg, 0.0823 mmol) in a mixture of ethyl acetate (1 ml) and methylene chloride (1 ml) was added PPh_3 (2.16 mg, 0.00823 mmol) followed by $Pd(PPh_3)_4$ (3.80 mg, 0.00329 mmol). After stirring the mixture under N_2 for 5 min, 0.7 ml of 0.99% solution of pyrollidine (5.85 mg, 0.0823 mmol) in methylene chloride (1 ml) was added dropwise. The reaction mixture was stirred for 1 h 50 min, diluted with EtOAc. The resulting precipitate was filtered off, washed thoroughly with EtOAc and air-dried. The solid was dissolved in water (2 ml) and filtered through a small bed of Celite and washed with water (2 x 3 ml). The filtrate was freeze-dried to give a white fluffy mass (30 mg, 87%). Ir (KBr): 1757, 1606, 1490, 1448 cm^{-1}. Nmr(D_2O): δ 1.51 (s, 3H), 1.61 (s, 3H), 4.29 (s, 1H, C-3H), 5.21 (s, 4H, $-CH_2CH_2-$), 5.93 (s, 1H, C-5H), 7.48 (d, 1H, J = 1.0 Hz, olefin), 8.05 (t, 2H, J = 6.81 and 7.49 Hz), 8.27 (s, 1H, triazole, 8.57-8.68 (m, 3H). POSFAB m/z 418 $[M + H]^+$. Compound (2f) was

prepared in the same manner as described above. Nmr (D_2O): δ 1.50 (s, 3H), 1.61 (s, 3H), 4.27 (s, 1H, C-3H), 5.22 (s, 4H, -CH_2CH_2-), 5.65 (s, 1H, C-5H), 7.21 (s, 1H, olefin), 8.02-8.09 (m, 2H, pyridine), 8.57-8.69 (m, 3H, pyridine). POSFAB, m/z 418 $[M + H]^+$.

ACKNOWLEDGMENT

We thank Taiho Pharmaceutical Co., Ltd. for the financial support and Mrs. Rhonda Sarnoski for typing this manuscript.

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Received, 10th April, 1995