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Synthesis of 6-[1-[4-(Benzoxazol-2-yl)thiobuthyl]-1,2,3-triazole-4-yl]methylenepenam as β-Lactamase Inhibitors

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The 6,6-dibromopenam **6** was treated with CH_3MgBr and carbaldehyde **5** to afford the 6-bromo-6-(1-hydroxy-1-methyl)penicillanate **7**, which was reacted with acetic anhydride to give acetoxy compound **8**. The deacetobromination of **8** with zinc and acetic acid gave 6-exomethylenpenams, Z-isomer **9** and E-isomer **10**, which were oxidized to sulfones **11** and **12** by m-CPBA. The p-methoxybenzyl compounds were deprotected by $AlCl_3$ and neutralized to give the sodium salts **13**, **14**, and **15**.

Key words : Triazole, 6-Exomethylenepenam, β-Lactamase Inhibitors

INTRODUCTION

The bacterial β-lactamase enzyme opens β-lactam ring of β-lactam antibiotics to lose their antibacterial activities. New agents that can inhibit the action of the β-lactamase have been developed to overcome this bacterial resistance against β-lactam antibiotics. The success of clavulanic acid (Reading, *et al.*, 1981) stimulated extensive research leading to the development of other β-lactamase inhibitors such as sulbactam (English, *et al.*, 1978) and tazobactam (Micetich, *et al.*, 1987), which are on the market.

A number of 6-(substituted methylene)penams have been reported in the literature (Chen, *et al.*, 1986 and 1987) as potent inhibitors of β-lactamases. Recently, 6-triazolylmethylenepenem **1** (Bennett, *et al.*, 1991a and 1991b) BRL-42715, has been shown to be a very potent inhibitor of most bacterial β-lactamases including the class I β-lactamase, which is resistant to other β-lactamase inhibitors. The N_1-position of the 6-triazolylmethylenepenem was modified further **2** (Broom, *et al.*, 1989) to improve its β-lactamase inhibitory activity and its pharmacological properties.

In our continuous search for potent β-lactamase inhibitors based on the penam sulfone skeleton, we have prepared a series of 6-(substituted methylene)penams and in this paper we wish to report the synthesis of both the Z- and E- isomers of 6-triazolylmethylene penicillanates **3** containing a benzoxazolthiobuthyl side chain at the N_1-position of the triazole moiety. The biological activities of these compounds were reported in separated papers (Park, *et al., 1997).*

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MATERIALS AND METHODS

Melting points were determined with a Büchi Melting Point B 540 and are uncorrected. Analytical thin layer chromatography (TLC) was performed with commercially available silica plates (Merck silica gel 60 F_{254}), and the spots were visualized by UV lamp (Spectroline ENF-240C). The reverse phase thin layer chromatography was performed with Merck RP-18 F_{254s}, and column chromatography was performed by using silica gel (Merck silica gel 60, 230-400 mesh), unless otherwise noted. The reverse phase column chromatography was performed with a Comosil 75 C_{18}-OPN. IR spectra were taken on a Shimadzu IR-435 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX 90A (90 MHz) and Varian Gemini 2000 (300 MHz) using tetramethylsilane as an internal standard.

By using reported methods (Boyer, *et al.*, 1955 and Sauer, 1963), chlorobutanol on treatment with sodium azide gave 4-azidobutanol which underwent a cycloaddition reaction with propargyl aldehyde to give 1-(4-hydroxybutyl)-1,2,3-triazole-4-carbaldehyde **4**. Further chemical modification of the hydroxy group led to the synthesis of 1-[4-(benzoxazol-2-yl)thiobutyl]-1,2,3-triazole-4-carbaldehyde **5**. The 6,6-dibromopenam-3-carboxylate **6** was prepared from 6-aminopenicillanic acid (6-APA) by literature procedures (Kapur *et al.*, 1985).

1-[4-(Benzoxazol-2-yl)thiobutyl]-1,2,3-triazole-4-carbaldehyde (5)

To a stirred mixture of 1-(4-hydroxybutyl)-1,2,3-triazole-

4-carbaldehyde **4** (1.00 g, 5.91 mmol) and triethylamine (0.83 ml, 5.91 mmol) in dichloromethane (20 ml), was added trifluoromethanesulfonic anhydride (0.99 ml, 5.91 mmol) at -15°C and the resulting mixture was stirred for 3 hours under a nitrogen atmosphere. A mixture of 2-mercaptobenzoxazole (0.89 g, 5.91 mmol) and triethylamine (0.83 ml, 5.91 mmol) in dry dichloromethane (20 ml) was added to the reaction mixture dropwise at -15°C for 10 minutes and stirred at room temperature overnight. The reaction mixture was washed with brine and the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was chromatographed on a silica gel column with a mixture of hexane-ethyl acetate, 1:1 (v/v) as eluant to give compound **5** (1.42 g, 79%) as a solid: Rf=0.38 (hexane : ethylacetate=1:1); m.p.: 143~145°C; IR ($CHCl_3$) cm^{-1}: 1698, 1443 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.64~2.23 (4H, m), 3.22 (2H, t), 4.41 (2H, t), 7.11~7.60 (4H, m), 8.19 (1H, s), 10.04 (1H, s).

p-Methoxybenzyl 6-Bromo-6-[1-hydroxy-1-[1-[4-(benzoxazol-2-yl)thiobutyl]-1,2,3-triazol-4-yl]methyl] penicillanate 1,1-Dioxide (7)

To a solution of p-methoxybenzyl 6,6-dibromopenam-3-carboxylate-1,1-dioxide **6** (1.20 g, 2.35 mmol) in dry THF (20 ml), was added CH_3MgBr (1.13 ml, 2.82 mmol, 2.5 M soln in ether) and stirred at -78°C for 15 minutes under a nitrogen atmosphere. To this reaction mixture, a solution of 5 (0.71 g, 2.35 mmol) in dry dichloromethane (20 ml) was added and stirred at -78°C for 6 hours. The reaction was quenched by adding saturated NH_4Cl solution and extracted with ethylacetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on a silica gel column with dichloromethane-ethylacetate, 4:1 (v/v) as eluant to give the stereoisomeric mixture **7** (1.18 g, 68.6%) as a foam: Rf =0.25 (dichloromethane:ethylacetate=4:1); IR ($CHCl_3$) cm^{-1}: 1801, 1755, 1241 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.28 (3H, s), 1.53 (3H, s), 1.76~2.27 (5H, m), 3.33 (2H, t), 3.82 (3H, s), 4.30~4.58 (3H, m), 4.81~4.91 (1H, m), 5.17 (2H, d), 5.41~5.57 (1H, m), 6.82~6.98 (2H, m), 7.21-7.68 (6H, m), 7.74~7.84 (1H, m).

p-Methoxybenzyl 6-Bromo-6-[1-acetoxy-1-[1-[4-(benzoxazol-2-yl)thiobutyl]-1,2,3-triazol-4-yl]methyl] penicillanate 1,1-Dioxide (8)

Acetic anhydride (1.28 ml, 13.61 mmol) was added to a solution of **7** (1.00 g, 1.36 mmol) and pyridine (1.32 ml, 16.34 mmol) in dichloromethane (15 ml) and the mixture was stirred overnight at room temperature. The reaction mixture was extracted with dichloromethane and washed sequentially with 1% HCl, 5% $NaHCO_3$ solution, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel column using hexane-ethylacetate, 1:2 (v/v) as eluant to give stereoisomeric mixture **8** (0.89 g, 84.2%) as a foam: Rf=0.54 (hexane:ethylacetate=1:2); IR ($CHCl_3$): 1806, 1754, 1214 cm^{-1}; ^{1}H-NMR ($CDCl_3$), 1.21 (3H, s), 1.52 (3H, s), 1.80~2.29 (7H, m), 3.34 (2H, t), 3.78 (3H, s), 4.32~4.54 (3H, m), 5.06~5.27 (3H, m), 6.32 and 6.56 (1H, two s), 6.80~7.01 (2H, m), 7.21-7.69 (6H, m), 7.79 and 8.16 (1H, two s).

p-Methoxybenzyl (6Z)-6-[1-[1-[4-(Benzoxazol-2-yl) thiobutyl]-1,2,3-triazol-4-yl]methylene]penicillanate 1,1-Dioxide (9) and p-Methoxybenzyl (6E)-6-[1-[1-[4-(Benzoxazol-2-yl)thiobutyl]-1,2,3-triazol-4-yl]methylene] penicillanate 1,1-Dioxide (10)

Acetic acid (0.74 ml, 12.98 mmol) and Zn powder (2.12 g, 32.44 mmol) was added to a solution of **8** (5.00 g, 6.49 mmol) in acetonitrile (60 ml) at 0°C and reaction mixture was stirred for 3 hours. The solid was filtered off and organic layer was washed with 5% $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified on a silica gel column using ethylacetate-hexane, 1:1 (v/v) as eluant to give the Z- isomer **9** (1.92 g) and E- isomer **10** (0.91 g) as foams.

Z-isomer **9**, 46.4% yield; Rf=0.14 (ethylacetate-hexane=1:1); IR ($CHCl_3$): 1782, 1754, 1248 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.28 (3H, s), 1.43~1.82 (7H, m), 3.85 (3H, s), 4.06 (2H, t), 4.48 (1H, s), 5.06 (2H, t), 5.14~5.29 (3H, m), 6.84~6.99 (2H, m), 7.12 (1H, s), 7.27~7.76 (6H, m), 7.92 (1H, s).

E-isomer **10**, 22.0% yield; Rf=0.31 (ethylacetate-hexane=1:1); IR ($CHCl_3$): 1770, 1752, 1239 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.34 (3H, s), 1.58 (3H, s), 1.99~2.28 (4H, m), 3.38 (2H, t), 3.87 (3H, s), 4.40~4.54 (3H, m), 5.13~5.29 (3H, m), 6.86~7.01 (2H. m), 7.13 (1H, s), 7.27~7.74 (6H, m), 8.84 (1H, s).

p-Methoxybenzyl (6Z)-6-[1-[1-[4-(Benzoxazol-2-yl) sulfonylbutyl]-1,2,3-triazol-4-yl]methylene]penicillanate 1,1-Dioxide (11)

m-Chloroperbenzoic acid (m-CPBA) (0.40 g, 2.32 mmol) was added to a solution of **9** (0.60 g, 0.94 mmol) in dichloromethane (10 ml) at 0°C and the mixture stirred at room temperature overnight. The solid was filtered off and reaction mixture was washed with 5% $NaHCO_3$ solution, water, and dried over anhydrous Na_2SO_4, and concentrated. The residue was purified on a silica gel column using ethylacetate-hexane, 2:1 (v/v) as eluant to give the 11 (0.39 g, 61.9%) as a foam; Rf=0.34 (hexane:ethylacetate=1:2); IR ($CHCl_3$): 1773, 1700, 1260 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.32 (3H, s), 1.45~1.85 (7H, m), 3.85 (3H, s), 4.46~4.50 (3H, m), 5.08 (2H, t), 5.14~5.32 (3H, m), 6.83~7.01 (2H,

m), 7.12 (1H, s), 7.25~7.75 (6H, m), 8.11 (1H, s).

p-Methoxybenzyl (6E)-6-[1-[1-[4-(Benzoxazol-2-yl) sulfonylbutyl]-1,2,3-triazol-4-yl]methylene]penicillanate 1,1-Dioxide (12)

Following the process described for **11**, compound **12** was prepared from **10** in 52.4% yield as a foam; Rf=0.60 (ethylacetate-hexane=2:1); IR ($CHCl_3$): 1775, 1754, 1248 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.30 (3H, s), 1.55 (3H, s), 1.89~2.25 (4H, m), 3.83 (3H, s), 4.26~4.52 (3H, m), 5.03~5.54 (5H, m), 6.80~6.99 (2H, m), 7.06~7.81 (7H, m), 8.67 (1H, s).

Sodium (6Z)-6-[1-[1-[4-(Benzoxazol-2-yl)thiobutyl]-1,2, 3-triazol-4-yl]methylene]penicillanate 1,1-Dioxide (13)

Anhydrous aluminum chloride (0.28 g, 2.12 mmol) was added to a stirred solution of **9** (0.54 g, 0.85 mmol) in an anhydrous mixture of dichloromethane (10 ml) and anisole (5 ml) at -40°C under a nitrogen atmosphere. After 1 hour, reaction was quenched by adding water and pH was adjusted to pH 7.0 with 0.1N NaOH solution. The mixture was filtered and the aqueous layer was separated. The organic layer was extracted with water which was added to the previous aqueous layer. The aqueous solution was freeze-dried and purified by reverse phase chromatography using water-acetonitrile, 2:1 (v/v) as eluant and freeze-dried again to give **13** (0.28 g, 61.3%) as a solid; Rf= 0.51 (water:acetonitrile=1:1); IR (Nujol): 1771 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.37 (3H, s), 1.43 (3H, s), 1.78~1.87 (2H, m), 1.98~2.06 (2H, m), 3.39 (2H, m), 3.77 (1H, s), 4.47~4.56 (2H. m), 5.72 (1H, s), 7.31~7.42 (3H, m), 7.63~7.78 (2H, m), 8.39 (1H, s).

In a similar manner, the following compounds, **14** and **15** were obtained from the corresponding PMB ester compounds **10** and **11**, respectively.

Sodium (6E)-6-[1-[1-[4-(Benzoxazol-2-yl)thiobutyl]-1,2, 3-triazol-4-yl]methylene]penicillanate 1,1-Dioxide (14)

52.4% yield; Rf=0.49 (water:acetonitrile=1:1); IR (Nujol): 1768 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.39 (3H, s), 1.47 (3H, s), 1.67~1.84 (2H, m), 1.96~2.09 (2H, m), 3.71~3.75 (2H, m), 3.85 (1H, s), 4.54 (2H, t, J=6.9 Hz), 5.57 (1H, s), 7.11 (1H, s), 7.25~7.37 (2H, m), 7.63~7.68 (2H, m), 8.75 (1H, s).

Sodium (6Z)-6-[1-[1-[4-(Benzoxazol-2-yl)sulfonylbutyl]-1,2,3-triazol-4-yl]methylene]penicillanate 1,1-Dioxide (15)

63.7% yield; Rf=0.68 (water:acetonitrile=1:1); IR (Nujol): 1774 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.39 (3H, s), 1.45 (3H, s), 1.75~1.93 (4H, m), 3.62~3.74 (2H, m), 3.82 (1H, s), 4.38~4.43 (2H, m), 5.82 (1H, s), 7.30~8.01 (5H, m), 8.42 (1H, s).

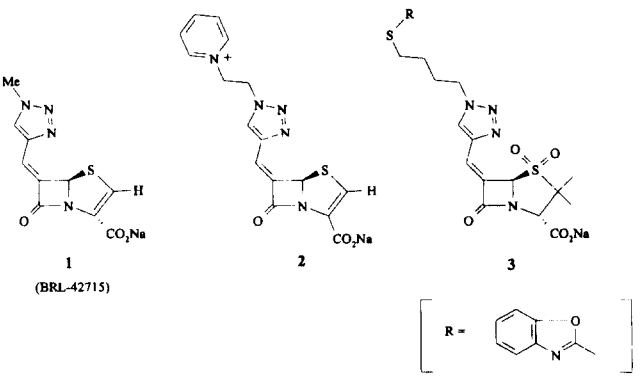


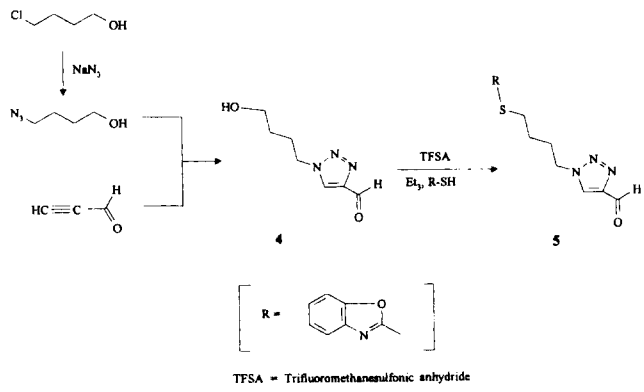
Fig. 1. Structures of β-Lactamase Inhibitors.

RESULTS AND DISCUSSION

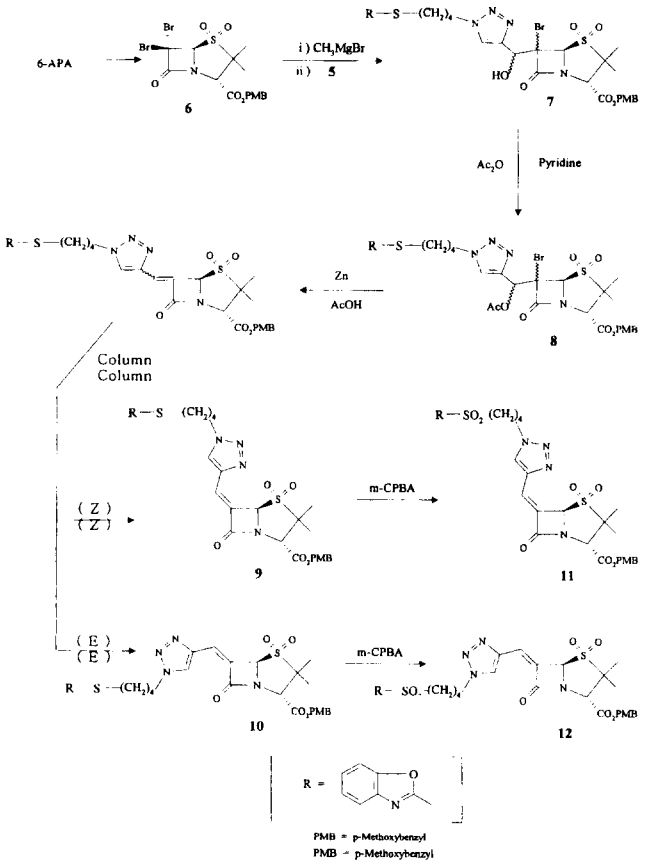
The hydroxybutyl compound **4** was first converted into the trifluorosulfonyl derivatives by treatment with trifluoromethane sulfonic anhydride and then reacted with thiol compound to yield the desired 4-substituted thiobutyl compound **5** in 79% yield (Scheme 1).

The 6,6-dibromopenam compound **6** was first treated with methyl magnesium bromide and then reacted with carbaldehyde compound **5** to afford the stereoisomeric mixture of hydroxy compound **7** in 69% yield. In 90 MHz NMR spectrum of this compound, the hydrogens of C_5, triazole, and CHOH gave multiplet instead of a singlet. This suggested that compound **7** was not a pure isomer, but was a mixture of stereoisomers. This isomeric mixture could not be separated by classical silica gel column chromatography. In 90 MHz NMR spectrum of acetoxy compound **8**, the hydrogens of triazole and CHOAc showed two major single peaks, which suggested that there were two major stereoisomers from the four possible isomers. The integrated intensities suggested that they were a mixture in the ratio of 3:1.

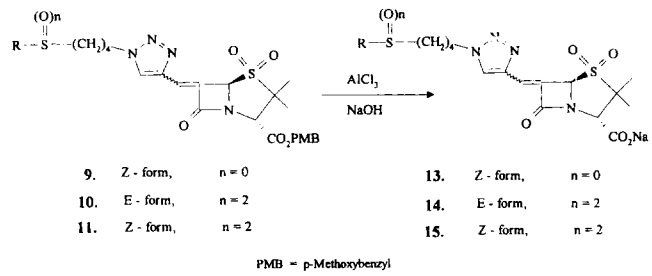
The introduction of a double bond at the 6-position of the penam sulfone was accomplished by the el-



Scheme 1. Synthesis of 1-(substituted thiobutyl)-1,2,3-triazole-4-carbaldehyde.



Scheme 2. Synthesis of 6-Exomethylene Penamsulfones.



Scheme 3. Synthesis of 6-Exomethylene Penamsulfones Sodium Salts.

imination reaction of the acetoxy compounds **8** with acetic acid and Zn to give the two isomers: Z-isomer **9** and E-isomer **10** in 46 and 22% yield, respectively (Scheme 2). In the E-isomer, the hydrogen of the triazole ring is close to the carbonyl group of the β-lactam ring. This carbonyl group might render some anisotropic effect (probably deshielding effect) on the hydrogen of the triazole ring. In the Z-isomer, this proton is too far away to be under the anisotropic effect of the carbonyl group in the β-lactam ring. Therefore, chemical shift values of this proton in E-isomer **10** (δ 8.84) is at a much lower field than that of the Z-isomer **9** (δ 7.92). The similar results were also observed from corresponding sulfonyl compounds **11** and **12**.

The p-methoxybenzyl compounds **9**, **10**, and **11** were first converted into the corresponding free carboxylic compounds by deprotection with aluminum chloride and adjustment of pH to 7.0 with 0.1 N-NaOH solution gave the sodium salt. (Scheme 3). The very slow and careful titration of the NaOH solution was required during the pH adjustment, since the β-lactam ring is sensitive to strong base. The resulting sodium carboxylate solution was freeze-dried to give a solid which was purified on a reverse phase column.

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