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Synthesis of 6-Exomethylenepenams as β-Lactamase Inhibitors

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

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

INTRODUCTION

A successful approach to overcoming the bacterial resistance to β-lactam antibiotics caused by β-lactamase production is to develop agents that can inhibit the action of the β-lactamase. 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)penems have been reported in the literature (Chen, *et al.*, 1986 and 1987) as potent inhibitors of cell free β-lactamases, but were ineffective in synergistic antibacterial tests probably because of poor penetration through the bacterial cell wall. 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 compound **2** (Broom, *et al.*, 1989) to improve its β-lactamase inhibitory activity and penetration of bacterial cell wall.

In our search for potent β-lactamase inhibitors based on the penam sulfone skeleton, we have prepared 6-substituted methylenepenams, which have a polar group at N_1-position like **2** to improve its penetration of bacterial cell wall. In this paper, we wish to report the synthesis of both the *Z*- and *E*- isomers of 6-triazolylmethylene penicillanates **3** containing a thioethyl nicotinate side chain at the N_1-position of the triazole moiety.

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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), bromoethanol on treatment with sodium azide gave 2-azidoethanol which underwent a cycloaddition reaction with propargyl aldehyde to give triazole-4-carbaldehyde **4**. Further chemical modification of the hydroxy group led to the synthesis of substituted thioethyl compound **5**. The 6,6-dibromopenam-3-carboxylate **6** was prepared from 6-aminopenicillanic acid (6-APA) by literature procedures (Kapur *et al.*, 1985).

1-[2-(3-Methoxybenzyl nicotinate-2-yl)thioethyl]-1,2,3-triazole-4-carbaldehyde (5)

To a stirred mixture of 1-(2-hydroxyethyl)-1,2,3-triazole-4-carbaldehyde **4** (2.50 g, 17.72 mmol) and triethylamine (2.50 ml) in dichloromethane (50 ml), was added trifluoromethanesulfonic anhydride (TFSA) (5.00 g, 17.72 mmol) at -20°C and stirred for 4 h under a nitrogen atmosphere. A mixture of 3-methoxybenzyl 2-mercaptonicotinate (4.88 g, 17.72 mmol) and triethyl-

amine (2.50 ml) in dry dichloromethane (50 ml) was added to the reaction mixture dropwise at -20°C 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 using hexanes and ethyl acetate as eluant to give compound **5** (4.50 g, 65%) as a solid: Rf=0.33 (hexanes:ethyl acetate=1:1); m.p.: 147~149°C;

IR ($CHCl_3$) cm^{-1}: 1715, 1690, 1275 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 3.78 (3H, s), 4.39 (2H, s), 4.71~4.97 (4H, m), 6.76~6.94 (2H, m), 7.15 (1H, dd), 7.28~7.43 (2H, m), 8.10 (1H, dd), 8.38 (1H, s), 8.62 (1H, dd), 10.11 (1H, s).

3-Methoxybenzyl 6-bromo-6-[1-hydroxy-1-[1-[2-(3-methoxybenzyl nicotinate-2-yl)thioethyl]-1,2,3-triazol-4-yl] methyl]penicillanate 1,1-Dioxide (7)

To a solution of 3-methoxybenzyl 6,6-dibromopenam-3-carboxylate-1,1-dioxide **6** (1.12 g, 2.19 mmol) in dry THF (20 ml), was added CH_3MgBr (0.92 ml, 2.5 M soln in ether) and stirred at -78°C for 30 min under a nitrogen atmosphere. To this reaction mixture, a solution of **5** (0.92 g, 2.31 mmol) in dry dichloromethane (20 ml) was added and stirred at room temperature overnight. 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 using hexanes and ethyl acetate as eluant to give the stereoisomeric mixture **7** (0.93 g, 51 %) as a foam: Rf=0.51 (hexanes:ethyl acetate=1:2); IR ($CHCl_3$) cm^{-1}: 1805, 1755, 1721 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 1.18 (3H, s), 1.48 (3H, s), 2.15 (1H, s), 3.75 (3H, s), 3.78 (3H, s), 4.37~4.43 (3H, m), 4.64~4.80 (5H, m), 5.04~5.21 (2H, m), 5.39~5.54 (1H, m), 6.76~6.97 (4H, m), 7.07 (1H, dd), 7.21~7.41 (4H, m), 7.90 (1H, s), 8.09 (1H, dd), 8.55 (1H, dd).

3-Methoxybenzyl 6-bromo-6-[1-acetoxy-1-[1-[2-(3-methoxybenzyl nicotinate-2-yl)thioethyl]-1,2,3-triazol-4-yl] methyl]penicillanate 1,1-dioxide (8)

Acetic anhydride (1.13 ml) was added to a solution of **7** (1.00 g, 1.20 mmol) and pyridine (1.22 ml) in dichloromethane (20 ml) and 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 hexanes and ethyl acetate as eluant to give stereoisomeric mixture **8** (0.84 g, 80%) as a

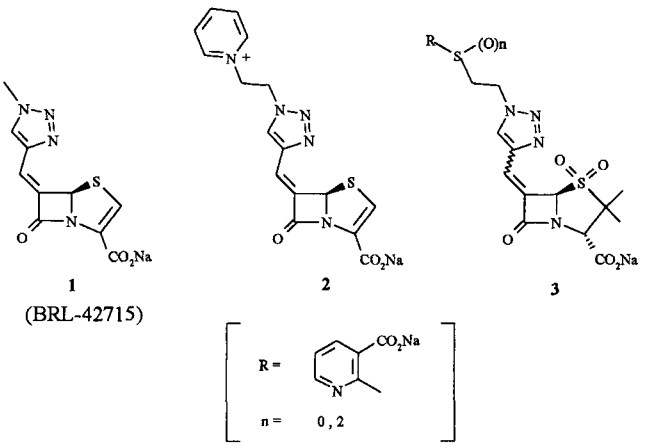


Fig. 1. Structures of β-lactamase inhibitors

foam: Rf=0.61 (dichloromethane:ethyl acetate=4:1); IR ($CHCl_3$): 1807, 1756, 1713 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 1.19 (3H, s), 1.48 (3H, s), 2.18 (3H, s), 3.76 (3H, s), 3.81 (3H, s), 4.32 (1H, s), 4.41 (2H, s), 4.68~4.81 (5H, m), 5.01~5.18 (2H, m), 6.29 and 6.51 (1H, s), 6.77~6.96 (4H, m), 7.15 (1H, dd), 7.27~7.44 (4H, m), 7.80 (1H, s), 8.18 (1H, dd), 8.59 (1H, dd).

3-Methoxybenzyl (6*Z*)-6-[1-[1-[2-(3-methoxybenzyl nicotinate-2-yl)thioethyl]-1,2,3-triazol-4-yl]methylene] penicillanate 1,1-dioxide (9) and 3-methoxybenzyl (6*E*)-6-[1-[1-[2-(3-methoxybenzyl nicotinate-2-yl)thioethyl]-1,2,3-triazol-4-yl] methylene]penicillanate 1,1-dioxide (10)

Acetic acid (0.10 ml) and Zn powder (0.26 g) was added to a solution of 8 (0.70 g, 0.80 mmol) in acetonitrile (10 ml) at 0°C and stirred for 3 h. The solid was filtered off and organic layer was washed with 5% $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified on a silica gel column using ethyl acetate and dichloromethane as eluant to give the *Z*- isomer **9** (0.21 g) and *E*- isomer **10** (0.14 g) as foams.

Z- isomer **9**, 36% yield; Rf=0.37 (ethyl acetate-dichloromethane=1:4); IR ($CHCl_3$): 1782, 1721, 1246 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 1.30 (3H, s), 1.52 (3H, s), 3,77 (3H, s), 3.81 (3H, s), 4.37 (2H, s), 4.43 (1H, s), 4.61~4.80 (4H, m), 5.14~5.26 (2H, m), 5.59 (1H, s), 6.76~6.94 (4H, m), 7.09 (1H, dd), 7.18~7.40 (4H, m), 7.89 (1H, s), 8.11 (1H, dd), 8.59 (1H, dd).

E- isomer **10**, 24% yield; Rf=0.70 (ethyl acetate-dichloromethane=1:4); IR ($CHCl_3$): 1772, 1720, 1247 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 1.31 (3H, s), 1.53 (3H, s), 3.77 (3H, s), 3.82 (3H, s), 4.36~4.44 (3H, m), 4.66~4.85 (4H, m), 5.15~5.28 (3H, m), 6.77~6.98 (4H, m), 7.02~7.16 (2H, m), 7.25~7.41 (4H, m), 8.17 (1H, dd), 8.60 (1H, m), 8.81 (1H, s).

3-Methoxybenzyl (6*Z*)-6-[1-[1-[2-(3-methoxybenzyl nicotinate-2-yl)sulfonylethyl]-1,2,3-triazol-4-yl]methylene] penicillanate 1,1-dioxide (11)

m-Chloroperbenzoic acid (*m*-CPBA) (0.22 g) was added to a solution of **9** (0.40 g, 0.55 mmol) in dichloromethane (10 ml) at 0°C and 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 ethyl acetate and dichloromethane as eluant to give the **11** (0.26 g, 63%) as a foam; Rf= 0.40 (dichloromethane:ethyl acetate=4:1); IR ($CHCl_3$): 1769, 1745, 1251 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 1.24 (3H, s), 1.46 (3H, s), 3.69 (3H, s), 3.74 (3H, s), 4.35 (1H, s), 4.61~4.82 (6H, m), 5.11~5.22 (3H, m), 6.70~ 6.93 (4H, m), 7.01 (1H, s), 7.12~7.36 (4H, m), 7.51 (1H, m), 7.83~7.93 (2H, m), 8.62~8.80 (1H, m).

Disodium (6*Z*)-6-[1-[1-[2-(nicotinate-2-yl)thioethyl]-1, 2,3-triazol-4-yl]methylene]penicillanate 1,1-dioxide (12)

Anhydrous aluminum chloride (0.27 g) was added to a stirred solution of **9** (0.3 g, 0.41 mmol) in an anhydrous mixture of dichloromethane (8 ml) and anisole (4 ml) at -40°C under a nitrogen atmosphere. After 1 h, reaction was quenched by adding water and pH was adjusted to pH 7.0 with 0.1N NaOH solution. The aqueous solution was freeze-dried and purified by reverse phase chromatography using water and acetonitrile as eluant and freeze-dried again to give **12** (0.15 g, 68%) as a solid; Rf=0.55 (water: acetonitrile=1:1); ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 1.40 (3H, s), 1.45 (3H, s), 3.86 (1H, s), 4.61~4.81 (4H, m), 5.85 (1H, s), 6.75~6.81 (1H, m), 7.40 (1H, s), 7.56 (1H, d, *J*=7.0 Hz), 7.83 (1H, d, *J*=4.7Hz), 8.59 (1H, s).

In a similar manner, the following compounds, **13** and **14** were obtained from the corresponding PMB ester compounds **10** and **11**, respectively.

Disodium (6*E*)-6-[1-[1-[2-(nicotinate-2-yl)thioethyl]-1, 2,3-triazol]4-yl]methylene]penicillanate 1,1-dioxide (13)

58% yield; Rf=0.58 (water:acetonitrile=1:1); ^{1}H-NMR (300 MHz, DMSO-d_6), δ: 1.39 (3H, s), 1.47 (3H, s), 3.85 (1H, s), 4.61~4.71 (2H, m), 4.86~4.94 (2H, m), 5.57 (1H, s), 7.14 (1H, s), 7.26 (1H, dd, J_1=4.6 Hz, J_2=7.8 Hz), 8.16 (1H, dd, J_1=1.6 Hz, J_2=7.8 Hz), 8.69 (1H, dd, J_1=1.6 Hz, J_2=4.6 Hz), 8.89 (1H, s).

Disodium (6*Z*)-6-[1-[1-[2-(nicotinate-2-yl)sulfonylethyl]-1, 2,3-triazol-4-yl]methylene]penicillanate 1,1-dioxide (14)

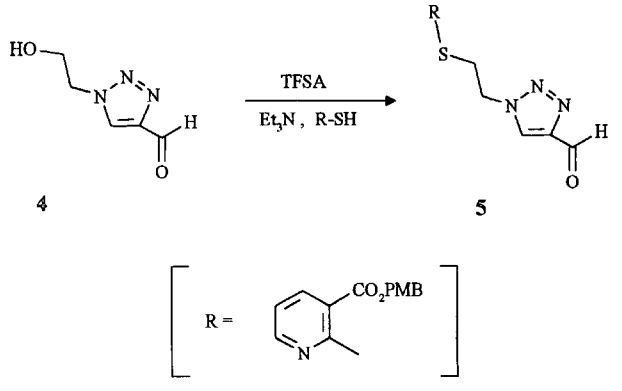
80% yield; Rf=0.45 (water:acetonitrile=1:1); ^{1}H-NMR (300 HMz, DMSO-d_6), δ: 1.37 (3H, s), 1.43 (3H, s), 3.85 (1H, s), 4.60~4.76 (2H, m), 4.84~4.88 (2H, m), 5.91 (1H, s), 7.35 (1H, s), 7.40~7.51 (1H, m), 7.82~ 7.95 (1H, m), 8.63~8.75 (1H, m), 8.82 (1H, s).

RESULTS AND DISCUSSION

The hydroxyethyl compound **4** was first converted into the trifluorosulfonyl derivatives by treatment with trifluoromethanesulfonic anhydride and then reacted with thiol compound to yield the desired 2-substituted thioethyl compound **5** in 65% yield (Scheme 1).

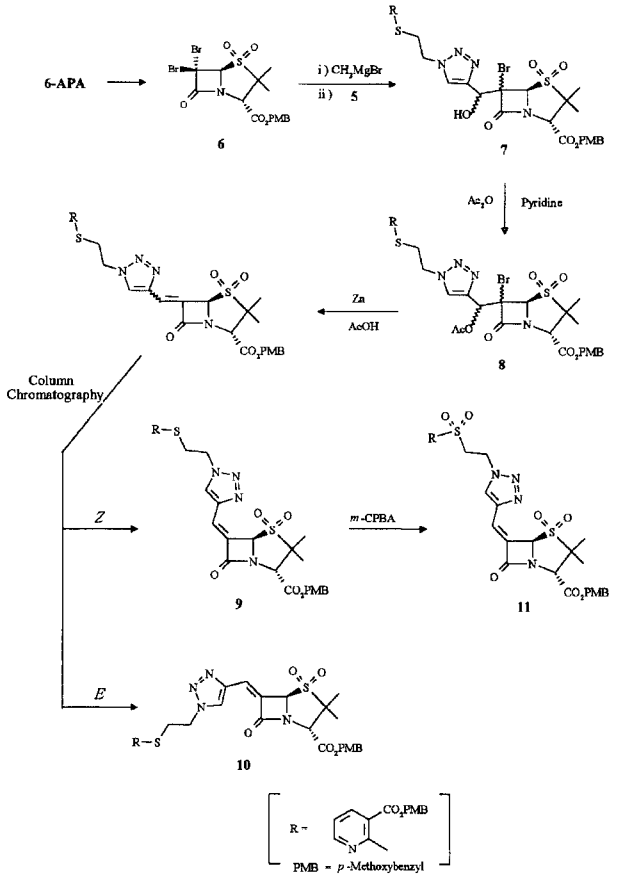
Treatment of 6,6-dibromopenam compound **6** with methyl magnesium bromide, followed by carbaldehyde compound **5** afford the diastereomeric mixture of hydroxy compound **7**, which was acylated by acetic anhydride. In NMR spectrum of hydroxy compound **7**, the hydrogens of C_5, triazole, and CHOH gave multiplet instead of a singlet. This suggested that compound **7** was not a pure isomer, but a mixture of stereoisomers. In 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 7:9.

The introduction of a double bond at the 6-position of the penam sulfone was accomplished by the deacetobromination of acetoxy compound **8** with acetic acid and Zn to give the two isomers: *Z*-isomer **9** and *E*-isomer **10** in 36 and 24% yield, respectively (Scheme **2**). In the *E*-isomer **10**, 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 **9**, this proton is too far away to be under the anisotropic effect of the carbonyl group in the β-lactam ring. Therefore, chemical

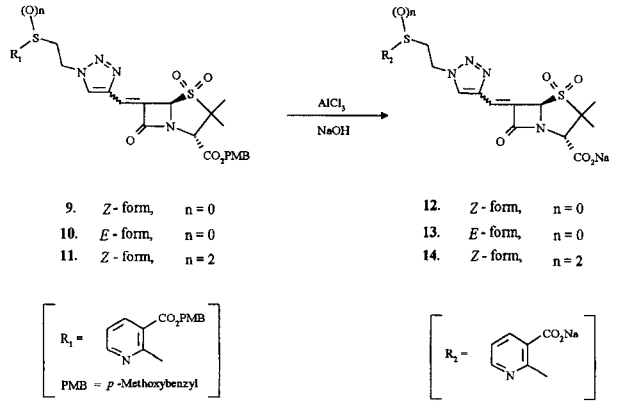


TFSA = Trifluoromethanesulfonic anhydride
PMB = *p*-Methoxybenzyl

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



Scheme 2. Synthesis of 6-exomethylene penams



Scheme 3. Synthesis of 6-exomethylene penam sodium salts

shift value of this proton in *E*-isomer **10** (δ 8.81) is at a much lower field than that of the *Z*-isomer **9** (δ 7.89).

The 3-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 corresponding sodium salt. (Scheme **3**). The careful titration of the 0.1 N-NaOH solution was required during the pH adjustment, since the β-lactam ring is sensitive to strong base. The resulting sodium carboxylate solutions were freeze-dried and purified on a reverse phase column.

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