SYNTHESIS AND BETA-LACTAMASE INHIBITORY ACTIVITY OF 2,2-BIS(MONOSUBSTITUTED) METHYLPENICILLIN SULFONES

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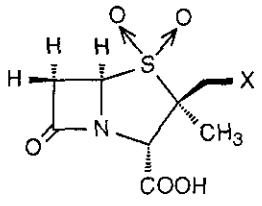
Abstract - A series of 2,2-bis(monosubstituted) methylpenicillin sulfones were prepared from 6,6-dibromopenicillanate by a double sulfoxide rearrangement. β-Lactamase inhibitory activity of 2,2-bis-(chloromethyl)penicillanic acid sulfone was studied and its activity was compared with YTR-830.

Although the development of new broad-spectrum β-lactam antibiotics has resulted in expanded antibacterial activity, β-lactamase mediated resistance continues to pose a clinically significant problem, particularly among G(-) bacilli. Combinations of a β-lactam antibiotic plus a β-lactamase inhibitor are effective in the treatment of a variety of infections associated with β-lactamase producing bacteria. Since the discovery of the β-lactamase inhibitory properties of clavulanic acid, a variety of inhibitors have appeared in the literature.[1] Among them sulbactam[1a] (1, X = H) and its analogues e.g. BL-P 2013[1d] (1, X = Cl) and YTR-830[1g] (1, X = 1,2,3-triazole) have attracted considerable interest as promising β-lactamase inhibitors. To see the effect of stereochemical changes on β-lactamase activity we recently reported[2] the

synthesis of the 2α-isomer (2) of BL-P 2013. Herein we report the synthesis and β-lactamase inhibitory activity of a new class of compounds (3 a-c) which are easily prepared from 6,6-dibromopenicillanate.



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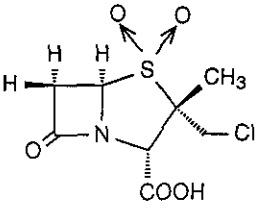

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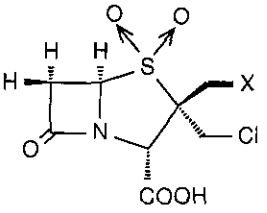
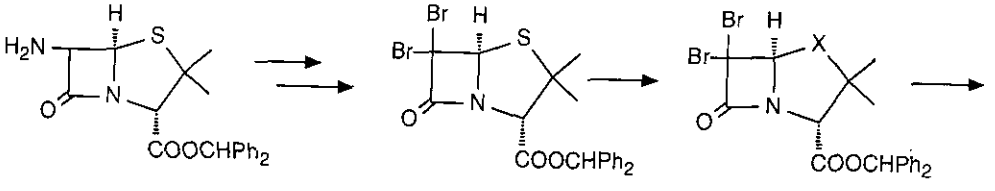


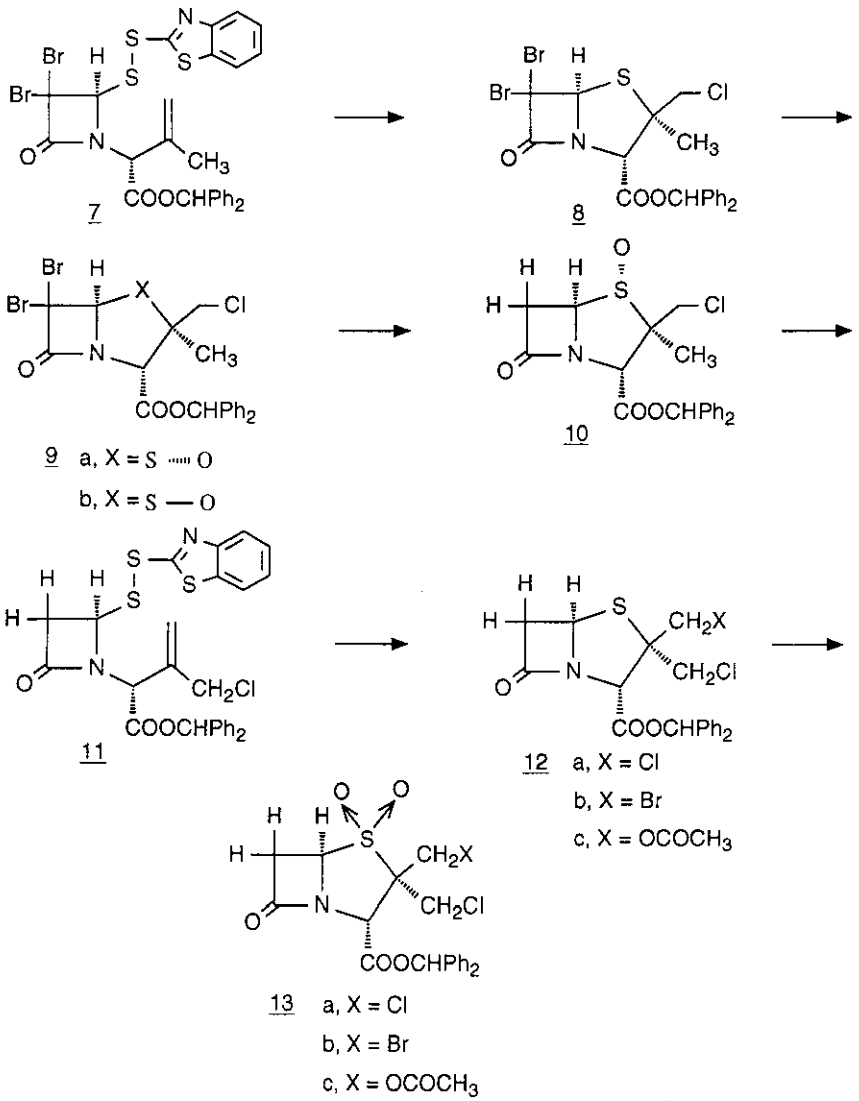
3 a, X = Cl
b, X = Br
c, X = OAc

The approach used to synthesize the compounds (3 a-c) is based on the double sulfoxide rearrangement and the important feature for this kind of rearrangement is the necessity of α-sulfoxide to introduce the second functionality at the 2α-CH_3 group. Spry[3] first utilized this sequential rearrangement to prepare 2-C-bisacetoxymethyl- and 2-C-triacetoxypenicillin derivatives. Later Uyeo et al.[4] used similar approach for functionalization of both the C-2 methyl groups of 6β-phenoxyacetamidopenicillin and converted these compounds to 3'-substituted cephalosporins. As oxidation[5] of 6,6-dibromopenicillanate with m-CPBA is reported to give the 1α-sulfoxide stereoselectively in high yield we thought that a double sulfoxide rearrangement would be possible by using 6,6-dibromopenicillanate 1α-sulfoxide (6a) as the starting material (Scheme).

Scheme



4 5 6 a, X = S ⋯ O
b, X = S — O



Benzhydryl 6,6-dibromopenicillanate (5) was conveniently prepared from 6-APA (4) by diazotization-bromination[6] followed by esterification with diphenyldiazomethane. Oxidation of 5 with m-CPBA in methylene chloride at room temperature for 35 min gave a mixture of the 1α-sulfoxide (6a) and the 1β-sulfoxide (6b) in the ratio of 10:1. The mixture of the sulfoxides (6a and 6b) without prior separation was converted to the disulfide (7) by heating with 2-mercaptobenzothiazole in toluene according to the method developed by Kamiya et al.[7] The disulfide (7) was found to be reluctant to cyclize to give 8 when stirred with an equimolar amount of cupric chloride in methylene chloride at room temperature for 4 h. Use of excess cupric chloride or increased reaction time (8 h) led to only undesired products. However, treatment of the

disulfide (7) with sulfuryl chloride in methylene chloride at -30° C for 20 min gave the desired 2β-chloromethyl-6,6-dibromopenam (8) in good yield. Oxidation of 2β-chloromethyl-6,6-dibromopenam (8) with m-CPBA in methylene chloride again gave a mixture of isomeric sulfoxides (9a and 9b) although the required 1α-sulfoxide (9a) was favoured. Chromatography on silica gel column led to the isolation of the desired 1α-sulfoxide (9a) which upon reductive debromination with four molar equivalents of zinc in aqueous ammonium acetate at room temperature for 1 h gave the sulfoxide (10) in about 68% yield. The stereochemistry of the sulfoxide (10) is very important at this stage, the sulfoxide and the unsubstituted 2-methyl group should be oriented in cis relationship (in this instance α-oriented) to give the desired sulfenic acid. On heating in toluene with 2-mercaptobenzothiazole the sulfoxide (10) was converted to the disulfide (11) in high yield. Cyclization of the disulfide (11) with sulfuryl chloride followed by oxidation with $KMnO_4$ in glacial acetic acid gave the desired disubstituted sulfone (13a). Similarly cyclization with bromine followed by oxidation gave 13b . Reaction of 11 with glacial acetic acid in the presence of silver oxide and iodine[8] gave 12c which on oxidation with $KMnO_4$ in glacial acetic acid gave 13c .

Thus 6,6-dibromopenicillanate 1α-sulfoxide has been found to be a suitable intermediate for synthesizing 2,2-bis(monosubstituted) methylpenicillins via a double sulfoxide rearrangement. The compound (13a) was deprotected by catalytic hydrogenation over Pd/C and converted to the corresponding sodium salt and its β-lactamase inhibitory activity was determined. A comparative β-lactamase inhibitory activity data for compound (3a) and YTR-830 is reported in the Table. The data indicate that the 2,2-bis(chloromethyl)penicillanic acid sulfone (3a) is a very weak β-lactamase inhibitor compared to the YTR-830.

Table: β-Lactamase Inhibitory Activity[a]

Compound	IC_{50}, μM
3a	15.5
YTR-830	0.21

[a]conditions: method UV (λ max at 233 nm); substrate, PCG (200 μM, Sigma); enzyme, penicillinase from Bacillus cereus (5000 units, 30 μl, Tokyo Kasei); preincubation, 30° C, 5 min; incubation, 30° C, 3 min.

EXPERIMENTAL

Benzhydryl 6,6-Dibromopenicillanate 1α-Oxide (6a)

Benzhydryl 6,6-dibromopenicillanate (5) (52.8 g, 0.101 mol) was dissolved in 500 ml of methylene chloride; m-CPBA (20.99 g, 0.101 mol, 83%) was added portionwise and the mixture was stirred at room temperature for 30 min, the precipitated solid was filtered off, the filtrate was washed with 5% $NaHCO_3$ solution, brine, dried over anhydrous Na_2SO_4 and concentrated. The product was purified over silica gel column using hexane-ethyl acetate mixture (7:3) to give a white foam, 49.68 g (91.3%). ^1H Nmr ($CDCl_3$) δ 1.23 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 4.70 (s, 1H, C-3), 5.30 (s, 1H, C-5), 7.00 (s, 1H, $COOCHPh_2$), 7.40 (br s, 10H, ar.).

Preparation of the Disulfide (7)

To a stirred solution of 25.4 g (0.047 mol) of benzhydryl 6,6-dibromopenicillanate 1α-sulfoxide (6a) in 400 ml of dry toluene was added 8.42 g (0.049 mol) of 2-mercaptobenzothiazole. The reaction mixture was heated to reflux for 1 h using a Dean-Stark trap and the toluene was removed under reduced pressure to give a viscous oil. The above viscous oil was dissolved in methylene chloride (200 ml), cooled in ice-bath and diluted with hexane with vigorous stirring to give a white solid which was filtered, washed with hexane and air-dried to give the desired product (7, 24.3 g, 75%) in the pure form which was used in the next step without further purification. ^1H Nmr ($CDCl_3$) δ 1.90 (s, 3H, CH_3), 4.90-5.20 (m, 3H, $=CH_2$ + C-3), 5.82 (s, 1H, C-5), 7.00 (s, 1H, $COOCHPh_2$), 7.22-8.16 (m, 14H, ar.).

Benzhydryl 2β-Chloromethyl-6,6-Dibromopenicillanate (8)

A solution of the disulfide (7) (24.3 g, 0.0352 mol) in methylene chloride (250 ml) was cooled to about -30° C. To this solution sulfuryl chloride (2.94 g, 0.0211 mol) was added dropwise with stirring and the reaction mixture was

stirred at -30° C for 20 min. The precipitated white solid was rapidly filtered through a small bed of Celite and the filtrate was washed successively with 5% $NaHCO_3$ solution, water and brine. After drying (Na_2SO_4) the methylene chloride solution was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, cooled to -40° C; the precipitated solid was removed by quick filtration through a short silica gel column and the filtrate was evaporated to dryness to give a light yellow foam, 18.0 g (90%) which was used in the next step without further purification; ^1H nmr ($CDCl_3$) δ 1.33 (s, 3H, CH_3), 3.63 (br s, 2H, CH_2Cl), 5.20 (s, 1H, C-3), 5.89 (s, 1H, C-5), 7.00 (s, 1H, $COOCHPh_2$), 7.40 (br s, 10H, ar.).

Benzhydryl 2β-Chloromethyl-6,6-dibromopenicillanate 1α-Oxide (9a)

Oxidation of benzhydryl 2β-chloromethyl-6,6-dibromopenicillanate (8) (7.5 g, 0.0134 mol) with m-CPBA (2.9 g, 0.0134 mol) in methylene chloride (100 ml) at room temperature for 35 min gave a mixture of the α- and β-sulfoxides in 95% yield. Purification of the crude product on silica gel column using methylene chloride as the solvent gave the pure 1α-sulfoxide (9a) as a foam (5.48 g, 71%); ^1H nmr ($CDCl_3$) δ 1.20 (s, 3H, CH_3), 3.79 (br s, 2H, CH_2Cl), 5.10 (s, 1H, C-3), 5.26 (s, 1H, C-5), 7.00(s, 1H, $COOCHPh_2$), 7.40 (br s, 10H, ar.).

Benzhydryl 2β-Chloromethyl-6,6-dihydropenicillanate 1α-Oxide (10)

To a stirred solution of 25.48 g (0.0442 mol) of the sulfoxide (9a) in 400 ml of tetrahydrofuran was added 260 ml of 1 M aqueous ammonium acetate. To this solution zinc dust (11.574 g, 0.177 mol) was added portionwise and the mixture was stirred at room temperature for 4 h, then filtered through a bed of Celite. The filtrate was taken in a separatory funnel and the organic layer was separated. The aqueous layer was saturated with sodium chloride and extracted twice with ethyl acetate. The combined organic layers were evaporated under reduced pressure to give a viscous oil which was dissolved in ethyl acetate, washed with water, brine and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a light yellow foam which was purified over a silica gel column using chloroform-ethyl acetate mixture (3:2) to give the desired 1α-sulfoxide (10) as a white foam (12.6 g, 68%) which was used in the next step; ^{1}nmr ($CDCl_3$) δ 1.13 (s, 3H, CH_3), 3.53 (t, J = 3.0 Hz, 2H, C-6), 3.80 (br s, 2H, CH_2Cl), 4.60 (dd, J = 2.0 Hz and 3.0 Hz, 1H, C-5), 4.93 (s, 1H, C-3), 7.00 (s, 1H, $COOCHPh_2$), 7.40 (br s, 10H, ar.).

Preparation of Disulfide (11)

The 2β-chloromethyl-6,6-dihydropenicillanate 1α-oxide (10) (5.5 g, 0.0132 mol) was dissolved in 80 ml of dry toluene and 2-mercaptobenzothiazole (2.364 g, 0.01385 mol) was added. The reaction mixture was heated to reflux using a Dean-Stark trap for 1.5 h, toluene was removed under reduced pressure. The residue was dissolved in methylene chloride and cooled in an ice-bath; to this solution hexane was added slowly with vigorous stirring. After stirring for a while the hexane layer was decanted and the residue was dried in vacuo to give a light brown sticky foam, 6.37 g (85%) which was used in the next step without further purification, since an attempt to purify the above sticky foam over a silica gel column led to isomerization of the double bond; ^{1}H nmr ($CDCl_3$) δ 3.40 (t, J = 4.0 Hz, 2H, $-COCH_2-$), 4.24 (br s, 2H, CH_2Cl), 5.20 (s, 1H, $C\underline{H}COOCHPh_2$), 5.35 (t, merged with a br s, J = 3.0 Hz, 2H, $C\underline{H}SS-$ + olefin), 5.56 (br s, 1H, olefin), 7.00 (s, 1H, $COOC\underline{H}Ph_2$), 7.30-8.10 (m, 14H, ar.).

Preparation of Compound (13a)

The unsymmetrical disulfide (11) (3.0 g, 0.0053 mol), obtained from the previous experiment was dissolved in 30 ml of methylene chloride and cooled to -30° C. To this solution 0.442 g (0.0032 mol) of sulfuryl chloride was added dropwise and the mixture was stirred at -30° C for 20 min. The precipitated solid was rapidly filtered through a bed of Celite. The filtrate was concentrated under reduced pressure and the residue was redissolved in ethyl acetate, cooled and the precipitated solid was filtered off. The filtrate was concentrated and directly used for the next step.

The crude product (12a) (2.66 g, 0.00611 mol) from the previous step was dissolved in a mixture of glacial acetic acid (50 ml) and water (10 ml). To the above reaction mixture was added potassium permanganate (1.931 g, 0.0122 mol) in small portions over a period of 30 min. After stirring for 3 h at room temperature, the reaction mixture was cooled in an ice-bath and hydrogen peroxide (30%) was added dropwise to it to destroy the excess potassium permanganate. The reaction mixture was poured into a mixture of methylene chloride and water and the separated organic layer was washed successively with cold water, 5% $NaHCO_3$ solution, brine and was then dried (Na_2SO_4) and concentrated to afford the title compound as a light yellow foam (1.99 g). The product was purified by silica gel column chromatography with chloroform as an eluant to afford a white foam (0.90 g, 31.47%). Trituration with Et_2O afforded 0.601 g (21%) of 13a ; ^{1}H nmr ($CDCl_3$) δ 3.47-4.37 (m, 6H, β-CH_2Cl + α-CH_2Cl

+ C-6), 4.75 (br s, merged with a triplet, 2H, C-5 + C-3), 6.97 (s, 1H, $COOCHPh_2$), 7.40 (br s, 10H, ar.). Anal. Calcd for ($C_{21}H_{19}Cl_2NO_5S$): C, 53.85; H, 4.09; N, 2.99. Found: C, 53.90; H, 4.13; N, 2.97.

Sodium 2α-(Chloromethyl)-2β-(chloromethyl)-6,6-dihydropenam 3α-Carboxylate 1,1-Dioxide

A mixture of compound (13a, 0.6 g, 0.0013 mol), ethyl acetate (10 ml), 0.5 M $NaHCO_3$ solution (0.25 ml) and water (10 ml) was hydrogenated in the presence of 10% Pd/C (10 mg) under 2-3 atm. The reaction mixture was filtered and the filtrate was concentrated to about 3 ml, which was subjected to column chromatography using MCI gel (CHP-20P, Mitsubishi Chemical Ind. Ltd.) The column was eluated with water-acetone (9:1) and the eluate was lyophilized to afford 0.166 g of the desired sodium salt as an off-white amorphous solid in 40% yield; ^1H nmr (D_2O) δ 4.13 and 4.35 (ABq, J = 12.0 Hz, 2H, 2α-CH_2Cl), 4.25 and 4.43 (ABq, J = 12.0 Hz, 2H, 2β-CH_2Cl), 3.45 (dd, J = 2.0 Hz and 16.0 Hz, 1H, C-6), 3.71 (dd, J = 4.0 Hz and 16.0 Hz, 1H, C-6), 4.52 (s, 1H, C-3), 5.03 (dd, J = 2.0 Hz and 4.0 Hz, 1H, C-5). Anal. Calcd. for ($C_8H_8NaCl_2NO_5S$); C, 29.64; H, 2.49; N, 4.32. Found: C, 29.60; H, 2.51; N, 4.35.

Preparation of Compound (13b)

A solution of compound (11) (3.12 g, 0.0055 mol) in methylene chloride (30 ml) was cooled to -40° C, then bromine (0.528 g, 0.0033 mol) was added dropwise. After the addition was over, the reaction mixture was stirred at -40° C for 20 min and then cooled to -70° C, the precipitated solid was filtered rapidly through a bed of Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and cooled again to -70° C; the precipitated solid was rapidly filtered through a small bed of silica gel. The filtrate was concentrated under reduced pressure to give (12b) as an oil (2.79 g) which was oxidized directly without further purification. The disubstituted intermediate (12b, 2.79 g, 0.0058 mol) was dissolved in a mixture of glacial acetic acid (50 ml) and water (10 ml). To the above reaction mixture was added potassium permanganate (1.838 g, 0.0116 mol) in small portions over a period of 30 min. After stirring at room temperature for 2 h, the reaction mixture was cooled in an ice-bath and hydrogen peroxide (30%) was added dropwise to it to destroy excess potassium permanganate. The reaction mixture was poured into a mixture of methylene chloride and water and the separated organic layer was washed successively with cold water, 5% $NaHCO_3$ solution and brine, and was

then dried (Na_2SO_4) and concentrated (2.11 g). The product was purified by silica gel column chromatography with hexane-ethyl acetate mixture (3:2) as eluant to afford the title compound (13b) as a white foam (0.9 g, 30.20%). Trituration with Et_2O afforded 0.745 g (25%) of 13b ; ^1H nmr ($CDCl_3$) δ 3.50-4.30 (m, 6H, CH_2Br + CH_2Cl + C-6), 4.80 (t, J = 3.0 Hz, 1H, C-5), 4.90 (br s, 1H, C-3), 7.00 (s, 1H, $COOCHPh_2$), 7.40 (br s, 10H, ar.). Anal. Calcd for ($C_{21}H_{19}BrClNO_5S$): C, 49.18; H, 3.74; N, 2.73. Found: C, 49.22; H, 3.77; N, 2.70.

Preparation of Compound (13C)

To a stirred solution of compound (11) (5.71 g, 0.0101 mol) in glacial acetic acid (70 ml) was added silver oxide (2.335 g, 0.0101 mol) and the mixture was stirred at room temperature for 10 min. To the green reaction mixture iodine (2.557 g, 0.0101 mol) was added in small portions while the reaction mixture turned from green to light yellow. After stirring at room temperature for 0.5 h glacial acetic acid was removed under reduced pressure. To the residue methylene chloride was added and the precipitated solid was removed by filtration, the filtrate was washed successively with 10% sodium thiosulfate solution, water, 5% $NaHCO_3$ solution, brine, dried (Na_2SO_4) and concentrated to give (12c) (3.0 g) which was oxidized directly without further purification.

The product (12c) (3.00 g, 0.0065 mol) obtained from the previous step was dissolved in a mixture of glacial acetic acid (45 ml) and water (8 ml). To the above reaction mixture was added potassium permanganate (1.543 g, 0.0098 mol) in small portions over a period of 30 min. After stirring at room temperature for 3 h, the reaction mixture was cooled in an ice-bath and hydrogen peroxide (30%) was added dropwise to it to destroy excess potassium permanganate. The reaction mixture was poured into a mixture of methylene chloride and water and the separated organic layer was washed successively with cold water, 5% $NaHCO_3$ solution and brine, and was then dried (Na_2SO_4) and concentrated (2.46 g). The product was purified by silica gel column chromatography with hexane-ethyl acetate mixture (3:2) as eluant to afford the title compound (13c) as a light yellow foam (1.03 g, 32%). Trituration with Et_2O afforded 0.8 g (24.85%) of 13c ; ^1H nmr ($CDCl_3$) δ 2.00 (s, 3H, $OCOCH_3$), 3.40 (d, J = 3.0 Hz, 2H, C-6), 4.02 (d, J = 4.0 Hz, 2H, CH_2Cl), 4.63-4.77 (m, 4H, CH_2OCOCH_3 + C-3 + C-5), 7.00 (s, 1H, $COOCHPh_2$), 7.35 (br s, 10H, ar.). Anal. Calcd for ($C_{23}H_{22}ClNO_7S$): C, 56.15; H, 4.51; N, 2.85. Found: C, 56.17; H, 4.50; N, 2.92.

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