6α-HYDROXYPENICILLANIC ACID-*S*(*S*)-OXIDE AND ANALOGUES: SYNTHESIS AND ANTIMICROBIAL ACTIVITY

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The synthesis and *in vitro* antibacterial activity of a series of 6-oxygenated penicillanic acid sulfoxides is described. 6α-Hydroxypenicillanic acid-*S*(*S*)-oxide (**1a**) exhibits weak Gram-negative antibacterial activity and appears to be similar to amdinocillin (**5**) in its mode of action. 6α-Hydroxypenicillanic acid-*S*(*R*)-oxide (**4a**) has a broader spectrum of activity, but again is rather weak. The corresponding 6β-hydroxy series is essentially devoid of activity.

As part of a program to evaluate compounds of interest as potential antibiotics and β-lactamase inhibitors, we prepared a number of 6-oxygenated penicillanic acid sulfoxide derivatives. We wish to report herein their synthesis and some interesting observations with respect to their *in vitro* antibacterial activity.

Chemistry

The 6-oxygenated penicillanic acid sulfoxide derivatives used in this study were either prepared from readily available precursors by standard methods as described in the experimental section (**1a**, **2a**, **3a**, **4a**, **6a**, **7a**, **9a**, **10a** and **11a**), or were prepared as described in the literature (**8**[1] and **12**[2]). The routes to the two α-sulfoxides, **3a** and **4a**, involved oxidation of an appropriate precursor to afford a 1 : 1 mixture of α- and β-sulfoxides, followed by chromatographic separation and then removal of protecting groups, since a direct stereospecific oxidation to afford the α-sulfoxide moiety was not possible.

The course of the oxidation of the penicillanic acid derivatives by *meta*-chloroperbenzoic acid followed the general observations outlined in the literature[3,4]. Thus, when α-substituents are present at C-6, the β-sulfoxide is always obtained as essentially the sole product. For compounds with a 6β-substituent that is capable of hydrogen-bonding with the incoming oxidant (or with the sulfoxide of the product), the β-sulfoxide is again the sole product. When a 6β-substituent is present that is not capable of undergoing hydrogen-bonding interactions, steric effects come into play and direct the product ratio to generally favor the α-sulfoxide. In order to obtain any α-sulfoxide in the 6α-substituted penicillins one has to go to a more reactive oxidant that is less selective, such as ozone (which is also less sterically demanding). In both of the latter situations a mixture of α- and β-sulfoxides are generally obtained, which can be separated chromatographically. The assignment of sulfoxide orientation in each series was based on the ^{13}C NMR shifts[3] of the C-2 methyl groups.

Biology

Notwithstanding the original focus of this study, none of the substances tested showed significant activity as β-lactamase inhibitors. However the antimicrobial screening of some of the initially prepared

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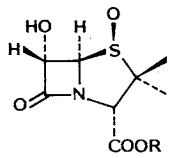
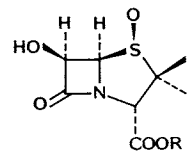
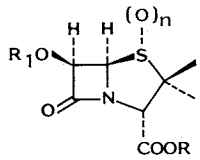


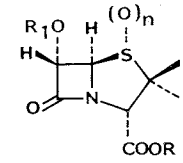
1a R=H
1b R=CH_2Ph
1c R=$CH(Ph)_2$



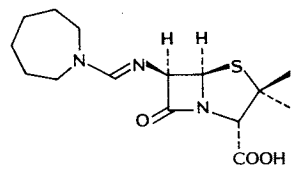
2a R=H
2b R=CH_2Ph



3a n=1, R_1=R=H
3b n=1, R_1=$COOCH_2Ph$, R=CH_2Ph
3c n=0, R_1=$COOCH_2Ph$, R=CH_2Ph
3d n=0, R_1=H, R=CH_2Ph



4a n=1, R_1=R=H
4b n=1, R_1=$COOCH_2Ph$, R=CH_2Ph
4c n=1, R_1=H, R=CH_2Ph
4d n=0, R_1=H, R=CH_2Ph



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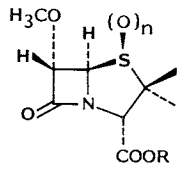
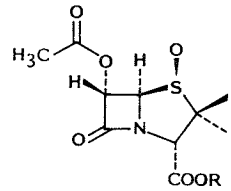
compounds produced some unusual and surprising results. 6α-Hydroxypenicillanic acid β-sulfoxide (**1a**), was found in preliminary screening to exhibit antibacterial activity against *Escherichia coli*, but not against *Staphylococcus aureus* or *Micrococcus luteus*. Compared to the typical activity profile of penams, this was unusual and the results of a fuller antimicrobial spectrum (see Table 1) indicated that **1a** is a moderately active antibacterial, with a spectrum limited to Gram-negative bacteria. As can be seen from the data, **1a** displays a spectrum of activity that is remarkably similar to that of amdinocillin (**5**), although it is less potent. Furthermore it was found that **1a** was β-lactamase resistant, and that it exhibited synergism with ampicillin against *Klebsiella pneumoniae* HE7, similar to that observed with amdinocillin[5~7].

Because of the similarity in antibacterial spectra between **1a** and amdinocillin (**5**), a compound which exhibits a unique mode of action relative to other β-lactam antibacterials, penicillin binding protein (PBP) and cell morphology studies were undertaken. Like **5**, when **1a** was tested in the PBP assay described by SPRATT and PARDEE[8], it was bound specifically by PBP-2 in *Escherichia coli*. Furthermore, it was found that treatment of *E. coli* with sublethal doses of either **1a** or **5** caused transformation of the Gram-negative bacilli into spherical cells. This activity has been associated with inhibition of PBP-2. Thus it seems likely that the new antibacterial **1a** exerts its action in the same manner as amdinocillin[5~7].

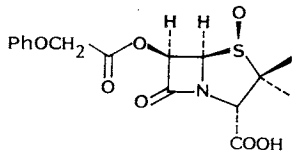
In order to explore more closely the structural elements responsible for this interesting profile of antibacterial activity, three additional hydroxy sulfoxide stereoisomers **2a**, **3a**, and **4a** were prepared and their antibacterial activities determined (see Table 1). Only 6α-hydroxypenicillanic acid α-sulfoxide (**4a**)



6a n=1, R=H
6b n=1, R=CH_2Ph
6c n=0, R=CH_2Ph



7a R=H
7b R=CH_2Ph



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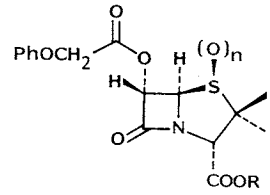
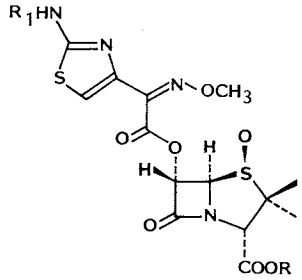
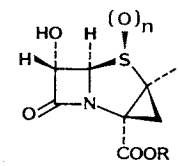


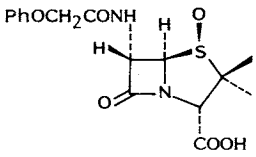
9a n=1, R=H
9b n=1, R=CH_2Ph
9c n=0, R=CH_2Ph



10a R_1=R=H
10b R_1=H, R=$CH(Ph)_2$
10c R_1=$COCH_2Cl$, R=$CH(Ph)_2$



11a n=1, R=H
11b n=1, R=$CH(Ph)_2$
11c n=0, R=$CH(Ph)_2$



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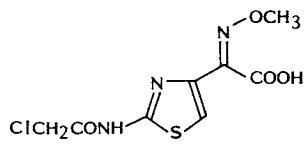

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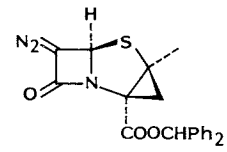

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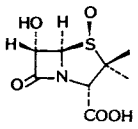
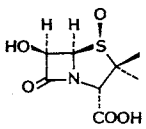
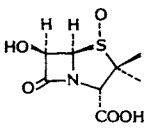
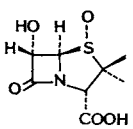
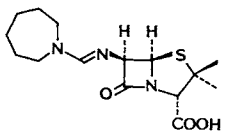
exhibited significant activity. This compound had moderate to weak antibacterial activity, but displayed a broad-spectrum profile.

In an attempt to improve on the activity shown by **1a**, a series of C-6 substituted analogues (**6a**, **7a**, **8**, **9a**, **10a**, **11a** and **12**) was synthesized. With the exception of the 6α-esters, **7a**, **9a** and **10a**, all were either devoid of activity or exhibited extremely weak activity. The esters **7a** and **10a** were weakly active and the ester **9a** was moderately active having MIC values two fold higher than **1a**. All esters had the same antibacterial spectrum as **1a** and may in fact be acting as prodrugs.

Table 1. *In vitro* antibacterial activity[a].

	 **1a**[b]	 **2a**[b]	 **3a**[b]	 **4a**[c]	 **5**[b]	**5**[c]
Escherichia coli ATCC 27856	7.9	250	1,000	—	0.12	—
E. coli ATCC 25922	—	—	—	32	—	0.125
Klebsiella pneumoniae ATCC 27858	15.7	250	1,000	—	0.12	—
K. pneumoniae 4964	—	—	—	16	—	0.25
Proteus vulgaris ATCC 6380	15.7	1,000	>1,000	8	0.12	0.063
Serratia marcescens ATCC 27857	15.7	1,000	>1,000	—	0.49	—
Pseudomonas aeruginosa ATCC 8709	>1,000	1,000	1,000	—	500	—
Acinetobacter calcoaceticus ATCC 10153	250	>1,000	62.5	—	250	—
Streptococcus pyogenes 503-782	—	—	—	4	—	32
S. faecium ATCC 8043	>1,000	>1,000	>1,000	—	>1,000	—
Staphylococcus aureus ATCC 6538P	>1,000	>1,000	250	—	62.5	—
S. aureus Smith	—	—	—	8	—	32
• *Micrococcus luteus* ATCC 9341	>1,000	>1,000	>1,000	—	62.5	—
Bacillus megaterium ATCC 8011	>1,000	>1,000	500	—	31.3	—

[a] MIC values in μg/ml.
[b] Determined by agar-well-diffusion method (see Experimental).
[c] Determined by the broth dilution method (see Experimental).

Although the active compounds in this study displayed only weak to moderate antibacterial activity, the finding of any activity in this series was surprising since the known penicillanic acids without a 6β-nitrogen substituent are in general, devoid of antibacterial activity[9,10]. It would appear that for activity in the series we describe, the α-orientation of the C-6 substituent is required, since all of the compounds in the 6β series are essentially inactive. Furthermore, it appears that the β-sulfoxide orientation tends to favor a Gram-negative spectrum, whereas the sulfoxide with the α-configuration leads to a broader spectrum of antibacterial activity. It is also worth noting that the sulfide and sulfone analogues of **1a**, **2a**, **7a**, **8** and **9a** were devoid of antibacterial activity.

Experimental

MP's were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were measured on a Digilab FTS 15-E spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on a Varian XL-100-FT spectrometer with $Si(CH_3)_4$ as the internal standard. Preparative liquid chromatography was carried out on a Waters Prep-500 instrument with silica as the support and the eluant as indicated.

In Vitro Antimicrobial Assays

Agar-well-diffusion Method

In vitro antimicrobial activity was determined in BBL seed agar using the agar-well-diffusion method. Serial dilutions of the test compound were pipetted into agar wells (80 μl per well); after incubation, the lowest concentration of compound which showed a zone of inhibition was designated the MIC. This concentration approximates the critical concentration obtained by extrapolation[11], and is somewhat higher than that obtained by broth or agar dilution methods. Compounds **1a**, **2a**, **3a**, **5**, **6a**, **7a**, **8**, **9a**, **10a**, **11a** and **12** were tested using this method.

Serial Broth Dilution Method

The diluted agents prepared in Mueller-Hinton broth were dispensed from the Dynatech MIC 2000 in 100 μl volumes into the wells of the Dynatech 96 well trays and used immediately or frozen at $-10°$ to $-70°$ until needed. Using the Dynatech inoculator and inoculum trays supplied by the manufacturer, 1.5 μl of a 10^{-2} dilution of an overnight culture was added to each well of the tray. The tops of the trays were then sealed according to the manufacturer's instructions using the Dynatech tape dispenser, and the trays incubated overnight at 37°C. The trays were examined with the aid of the Dynatech viewer. The lowest concentration at which no growth was observed was considered to be the MIC. It should be noted that in our hands, the broth dilution method of MIC determination generally produces slightly lower values than the agar well diffusion method. Compounds **4a** and **5** were tested by this method.

Synergy Experiments

Synergism with ampicillin against *Klebsiella pneumoniae* HE7 was measured in Mueller-Hinton agar for both **1a** and amdinocillin (**5**), using a 2-disk counter diffusion technique[12] in a manner generally similar to that described by CHATTOPADHYAY and HALL[6].

General Procedure for Synthesis of Sulfoxides *via* Oxidation of the Corresponding Sulfide with *meta*-Chloroperbenzoic Acid

To a cold (0°C) dichloromethane (5 ml/mmol) solution of sulfide (11.8 mmol) was added dropwise over 30 minutes a solution of *meta*-chloroperbenzoic acid (1.05 equiv) in dichloromethane (4 ml/mmol) and the mixture stirred at 0°C for 1 hour. Dichloromethane was added to the reaction to dissolve the precipitate and the mixture was washed successively with 5% aqueous sodium sulfite, 10% aqueous sodium bicarbonate, water, dried (Na_2SO_4), and solvent removed to afford a tacky gum. The residue was dissolved in diethyl ether and evaporated down to dryness. The sulfoxides **1b**, **2b**, **3b**, **6b**, **9b** and **11b** were prepared from their respective sulfides in this manner.

General Procedure for Hydrogenolysis of Benzyl Esters

The benzyl ester (1 g) was dissolved in ethyl acetate (200 ml) and hydrogenated at 3.52 kg/cm^2 on a Parr apparatus over 10% Pd-C (1 g) until uptake of hydrogen ceased. The catalyst was removed by filtration and if the TLC indicated the presence of starting material (usually the case) the process was repeated with fresh catalyst. After separation from catalyst, solvent was removed to afford the free acid. The acids **1a**, **2a**, **3a**, **4a**, **6a**, **7a** and **9a** were prepared by this route (in some instances, for solubility purposes, ethanol - ethyl acetate, 50 : 50, was used as the solvent). Isolated yields ranged from 75 ~ 100%.

[2*S*-(2α,4β,5α,6α)]-6-Hydroxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**1a**)

Benzyl ester precursor **1b**; white crystalline powder; mp 171 ~ 173°C dec; IR (KBr) ν_{max} cm^{-1} 3335, 2520 (br), 1793, 1748, 1707, 1220, 1000; ^{1}H NMR ($CDCl_3$ - DMSO-d_6; 3 : 1) δ 1.28 (3H, s), 1.63 (3H, s), 4.29 (1H, s), 4.96 (1H, d, J = 1.5 Hz), 4.98 (1H, d, J = 1.5 Hz), 8.25 (2H, br s, $-CO_2H$, $-OH$); *Anal* Calcd for $C_8H_{11}NO_5S$: C 41.20, H 4.75, N 6.01. Found: C 41.46, H 4.94, N 5.94.

[2*S*-(2α,4β,5α,6α)]-6-Hydroxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**1b**)

Sulfide precursor **4d**[13]; yield 85%; white crystalline powder; mp 152 ~ 154°C dec (literature[1] 146°C dec); IR (KBr) ν_{max} cm^{-1} 3225, 1788, 1745, 1002, 700; ^{1}H NMR ($CDCl_3$ - DMSO-d_6; 3 : 1) δ 1.10 (3H, s), 1.56 (3H, s), 4.39 (1H, s), 4.97 (1H, dd, J = 10 and 1.5 Hz), 5.01 (1H, d, J = 1.5 Hz), 5.18 (2H, ABq), 6.76 (1H, d, J = 10 Hz, OH), 7.38 (5H, s); ^{13}C NMR ($CDCl_3$ - DMSO-d_6; 3 : 1) δ 170.9 (s), 167.6 (s), 134.8 (s), 128.5 (d), 79.2 (d), 74.6 (d), 73.1 (s), 67.4 (t), 64.0 (d), 19.6 (q), 17.9 (q) (hence β-sulfoxide).

[2*S*-(2α,4β,5α,6α)]-3,3-Dimethyl-6-hydroxy-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Diphenylmethyl Ester-4-oxide (**1c**)

To a solution of a 6α-hydroxypenicillanic acid 4β-sulfoxide, **1a**, (3.56 g, 15.3 mmol) in anhydrous tetrahydrofuran (200 ml) was added diphenyldiazomethane (3.07 g, 15.8 mmol) in tetrahydrofuran (20 ml) and the resulting red solution was stirred at room temperature for 4 hours. The solvent was removed and the resulting tan precipitate was purified by preparative chromatography (dichloromethane - ethyl acetate; 65 : 35) to afford the ester, **1c**, as a white crystalline powder (5.2 g, 85%): mp 162 ~ 164°C; $[\alpha]_D^{25}$ +202.08° (*c* 1.027, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 3520 ~ 3340, 1790, 1750, 1060, 706; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, s), 1.61 (3H, s), 4.58 (1H, s), 4.92 (1H, s, OH), 5.07 (1H, d, J = 1.5 Hz), 5.21 (1H, d, J = 1.5 Hz), 6.97 (1H, s), 7.34, 7.36 (10H, 2s); ^{13}C NMR ($CDCl_3$) δ 170.6 (s), 167.5 (s), 139.0 (s), 138.7 (s), 128.8 (d), 128.7 (d), 128.3 (d), 127.8 (d), 126.7 (d), 79.4 (d), 79.2 (d), 75.1 (d), 73.7 (s), 64.4 (d), 19.9 (q), 17.9 (q); *Anal* Calcd for $C_{21}H_{21}NO_5S$: C 63.14, H 5.30, N 3.51. Found: C 63.15, H 5.52, N 3.26.

[2*S*-(2α,4β,5α,6β)]-6-Hydroxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**2a**)

Benzyl ester precursor **2b**; **2a** was isolated from an incomplete hydrogenolysis *via* a bicarbonate workup. The product, a light tan powder, was unstable and slightly impure; mp 105 ~ 110°C dec; IR (KBr) ν_{max} cm^{-1} 3430, 3380 ~ 3080, 2680 ~ 2400, 1785, 1740, 1650, 1200, 1070; ^{1}H NMR ($CDCl_3$ - DMSO-d_3; 3 : 1) δ 1.28 (3H, s), 1.68 (3H, s), 4.48 (1H, s), 5.11 (1H, d, J = 4 Hz), 5.28 (1H, d, J = 4 Hz), 5.98 (2H, br s, $-CO_2H$, $-OH$).

[2*S*-(2α,4β,5α,6β)]-6-Hydroxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**2b**)

Sulfide precursor **3d**[1,14]; yield 94%, tacky glass; IR ($CHCl_3$) ν_{max} cm^{-1} 3430, 1800, 1750, 1180; ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, s), 1.64 (3H, s), 4.71 (1H, s), 4.77 (1H, d, J = 13 Hz, OH), 4.92 (1H, d, J = 4 Hz), 5.18 (2H, ABq), 5.28 (1H, dd, J = 13 and 4 Hz), 7.46 (5H, s).

[2*S*-(2α,4α,5α,6β)]-6-Hydroxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**3a**)

Benzyl ester precursor **3b**; light yellow powder; mp 178 ~ 180°C dec; IR (KBr) ν_{max} cm^{-1} 3450,

2740~2540, 1781, 1748, 1738, 1300, 1210, 1020; ^{1}H NMR ($CDCl_3$-DMSO-d_6; 3:1) δ 1.36 (3H, s), 1.61 (3H, s), 4.28 (1H, s), 4.66 (1H, d, J=4 Hz), 5.34 (1H, d, J=4 Hz), 8.80 (2H, br s, CO_2H, OH); ^{13}C NMR ($CDCl_3$-DMSO-d_6; 3:1) δ 173.0 (s), 168.9 (s), 79.3 (d), 75.6 (d), 67.3 (s), 63.7 (d), 23.9 (q), 15.4 (q) (hence α-sulfoxide); *Anal* Calcd for $C_8H_{11}NO_5S$: C 41.20, H 4.75, N 6.01. Found: C 41.28, H 4.85, N 5.78.

[2*S*-(2α,4α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]oxy]-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**3b**)

Sulfide precursor **3c**; isolated from a 50:50 mixture of α- and β-sulfoxides by preparative chromatography (CH_2Cl_2-ethyl acetate; 5:3); yield 45% pale yellow gum; $[\alpha]_D^{25}$ +123.5° (*c* 0.954, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 1804, 1757, 1250, 1190, 1068, 698; ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, s), 1.60 (3H, s), 4.60 (1H, s), 4.95 (1H, d, J=4 Hz), 5.22 (2H, ABq), 5.26 (2H, s), 6.06 (1H, d, J=4 Hz), 7.37 (10H, s); ^{13}C NMR ($CDCl_3$) δ 166.66 (s), 166.46 (s), 152.77 (s), 134.19 (s), 133.87 (s), 128.73 (d), 128.65 (d), 128.57 (d), 128.50 (d), 128.39 (d), 79.40 (d), 77.43 (d), 71.15 (s), 69.66 (t), 68.00 (t), 65.24 (d), 24.03 (q), 15.78 (q). Methyl shifts diagnostic for α-sulfoxide; *Anal* Calcd for $C_{28}H_{23}NO_7S$: C 60.38, H 5.07, N 3.06. Found: C 60.58, H 5.23, N 2.87. The corresponding β-sulfoxide [2*S*-(2α,4β,5α,6β)]-3,3-dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]oxy]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid phenylmethyl ester-4-oxide was also isolated from the α- and β-sulfoxide mixture above; yield 40%; pale yellow gum; $[\alpha]_D^{25}$ +213.3° (*c* 1.066, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 1813, 1753, 1250, 1200, 1067; ^{1}H NMR ($CDCl_3$) δ 1.04 (3H, s), 1.61 (3H, s), 4.72 (1H, s), 4.95 (1H, d, J=4 Hz), 5.20 (2H, s), 5.22 (2H, ABq), 5.83 (1H, d, J=4 Hz), 7.35 (10H, s); ^{13}C NMR ($CDCl_3$) δ 168.45 (s), 167.53 (s), 153.24 (s), 134.40 (s), 134.12 (s), 128.68 (d), 128.61 (d), 128.54 (d), 128.38 (d), 128.15 (d), 75.43 (d), 75.38 (d), 74.74 (s), 70.74 (t), 67.83 (t), 66.22 (d), 19.19 (q), 18.40 (q): Methyl shifts diagnostic for β-sulfoxide; *Anal* Calcd for $C_{23}H_{23}NO_7S$: C 60.38, H 5.07, N 3.06. Found: C 60.41, H 5.18, N 3.32.

[2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]oxy]-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic Acid Phenylmethyl Ester (**3c**)

To a cooled (0°C), solution of 6β-hydroxypenicillanic acid benzyl ester (**3d**[1,14], 1.67 g, 5.4 mmol) in anhydrous dichloromethane (70 ml) was simultaneously added solutions of benzyl chloroformate (1.30 g, 7.6 mmol) in dichloromethane (70 ml) and triethylamine (1.0 ml, 7.2 mmol) in dichloromethane (35 ml). The addition rate of each solution was controlled such that both additions were complete after 1 hour. The cooling bath was removed and the reaction stirred at ambient temperature for 3 hours. The solution was then washed successively with aqueous potassium bicarbonate (1 M, 2×75 ml), water (2×150 ml), dried (Na_2SO_4), and solvent removed to afford a pale yellow viscous oil. Purification by preparative chromatography (silica; hexane-ethyl acetate-dichloromethane; 25:5:5, as eluant) afforded the desired protected compound **3c** as a clear gum (1.81 g, 76%); IR ($CHCl_3$) ν_{max} cm^{-1} 1795, 1750, 1255; ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, s), 1.57 (3H, s), 4.53 (1H, s), 5.17 (2H, s), 5.19 (2H, s), 5.58 (1H, d, J=4 Hz), 5.71 (1H, d, J=4 Hz), 7.36 (10H, s); *Anal* Calcd for $C_{23}H_{23}NO_6S$: C 62.57, H 5.25, N 3.17. Found: C 62.25, H 5.46, N 3.13.

[2*S*-(2α,4α,5α,6α)]-3,3-Dimethyl-6-hydroxy-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**4a**)

Benzyl ester precursor **4b**; light tan powder (unstable and slightly impure); IR (KBr) ν_{max} cm^{-1} 3410, 3300, 1783, 1740, 1635, 1300, 1220, 1190, 1030; ^{1}H NMR ($CDCl_3$-DMSO-d_6; 3:1) δ 1.54 (3H, s), 1.58 (3H, s), 4.38 (1H, s), 4.65 (1H, d, J=1 Hz), 5.17 (1H, d, J=1 Hz), 6.80 (2H, br s, COOH, OH); ^{13}C NMR ($CDCl_3$-DMSO-d_6; 3:1) δ 169.7 (s), 168.7 (s), 84.4 (d), 78.7 (d), 70.9 (s), 64.5 (d), 23.8 (q), 16.1 (q) (hence α-sulfoxide).

[2*S*-(2α,4α,5α,6α)]-3,3-Dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]oxy]-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**4b**) and [2*S*-(2α,4β,5α,6α)]-3,3-Dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]oxy]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide

A stirred solution of 6α-hydroxypenicillanic acid benzyl ester (**4d**[13], 1.50 g, 4.9 mmol) in dichloromethane (50 ml) containing methanol (0.5 ml) was cooled to −70°C. Ozone was passed into the solution

for 2 hours and then the excess ozone was removed by bubbling nitrogen through the mixture. Removal of solvent afforded a mixture of **4c** and **1b** as a "tacky" solid (1.60 g). This was dissolved in dichloromethane (60 ml), cooled to 0°, and then benzylchloroformate (1.17 g, 6.88 mmol) in dichloromethane (60 ml) was added. To this mixture was added dropwise, over 30 minutes, a solution of triethylamine (0.90 g, 8.9 mmol) in dichloromethane (30 ml). After the addition was complete the cooling bath was removed and the mixture stirred at room temperature for 2 hours. The organic phase was washed successively with aqueous potassium bicarbonate (1 M, 2 × 75 ml), water (2 × 150 ml), dried (Na_2SO_4) and the solvent removed to afford a mixture of the protected α- and β-sulfoxides. These were separated by preparative chromatography using the recycling technique (silica; dichloromethane-hexane-ethyl acetate; 70:20:10) to afford the α- and β-sulfoxides as indicated below in approximately a 1:1 ratio. α-Sulfoxide (**4b**): Tacky, pale yellow solid; IR ($CHCl_3$) ν_{max} cm^{-1} 1800, 1760, 1270, 1250, 1065, 703; ^1H NMR ($CDCl_3$) δ 1.34 (3H, s), 1.41 (3H, s), 4.51 (1H, s), 4.87 (1H, d, J=1.5 Hz), 5.31 (2H, ABq), 5.33 (2H, s), 5.79 (1H, d, J=1.5 Hz), 7.38 (10H, s); ^{13}C NMR ($CDCl_3$) δ 166.2 (s), 163.9 (s), 153.2 (s), 134.4 (s), 134.1 (s), 129.0 (d), 128.9 (d), 128.7 (d), 128.6 (d), 83.3 (d), 78.5 (d), 71.9 (t), 71.2 (s), 68.1 (t), 65.7 (d), 24.0 (q), 16.3 (q) (hence α-sulfoxide); *Anal* Calcd for $C_{23}H_{23}NO_7S$: C 60.38, H 5.07, N 3.06. Found: C 60.32, H 5.28, N 3.09. β-Sulfoxide: Pale yellow gum containing approximately 0.25 mol of dichloromethane; IR ($CHCl_3$) ν_{max} cm^{-1} 1807, 1757, 1260, 1251, 1063, 703; ^1H NMR ($CDCl_3$) δ 1.07 (3H, s), 1.63 (3H, s), 4.55 (1H, s), 5.02 (1H, d, J=1.5 Hz), 5.15 (2H, s), 5.19 (2H, ABq), 5.75 (1H, d, J=1.5 Hz), 7.38 (10H, s); ^{13}C NMR ($CDCl_3$) δ 167.2 (s), 164.8 (s), 153.5 (s), 134.6 (s), 134.2 (s), 128.8 (d), 128.7 (d), 128.5 (d), 77.1 (d), 75.7 (d), 73.7 (s), 70.9 (t), 68.0 (t), 64.8 (d), 19.7 (q), 18.1 (q) (hence β-sulfoxide); *Anal* Calcd for $C_{23}H_{23}NO_7S \cdot \frac{1}{4}CH_2Cl_2$: C 58.33, H 4.96, N 2.93. Found: C 58.27, H 5.02, N 2.93.

[2*S*-(2α,4β,5α,6α)]-6-Methoxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**6a**)

Benzyl ester precursor **6b**; white crystalline powder; mp 142~143°C dec; $[\alpha]_D^{25}$ +270.2° (*c* 0.929, acetone); IR (KBr) ν_{max} cm^{-1} 2540, 1790, 1738, 1220, 1000; ^1H NMR ($CDCl_3$-DMSO-d_6, 3:1) δ 1.29 (3H, s), 1.64 (3H, s), 3.54 (3H, s), 4.31 (1H, s), 4.85 (1H, d, J=1.5 Hz), 5.25 (1H, d, J=1.5 Hz); *Anal* Calcd for $C_9H_{13}NO_5S$: C 43.72, H 5.30, N 5.66. Found: C 43.46, H 5.14, N 5.48.

[2*S*-(2α,4β,5α,6α)]-6-Methoxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**6b**)

Sulfide precursor **6c**; white powdery solid; yield 87%; an analytical sample was obtained by crystallization from benzene-pentane: mp 109~111°C; $[\alpha]_D^{25}$ +228.8° (*c* 0.972, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 1790, 1752, 1058, 698; ^1H NMR ($CDCl_3$) δ 1.09 (3H, s), 1.61 (3H, s), 3.56 (3H, s), 4.50 (1H, s), 4.96 (2H, s, H5, H6, identical chemical shifts), 5.21 (2H, ABq), 7.38 (5H, s); ^{13}C NMR ($CDCl_3$) δ 169.56 (s), 168.67 (s), 135.58 (s), 129.58 (d), 129.14 (d), 82.68 (d), 77.12 (d), 74.29 (s), 68.31 (t), 64.94 (d), 58.57 (q), 19.90 (q), 18.32 (q). The shift of the two methyl groups is diagnostic of the β-sulfoxide; *Anal* Calcd for $C_{16}H_{19}NO_5S$: C 56.96, H 5.68, N 4.15. Found: C 57.04, H 5.66, N 4.11.

[2*S*-(2α,5α,6α)]-6-Methoxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester (**6c**)

To a dichloromethane (75 ml) solution of benzyl-6-diazopenicillanate[13,15,16] (11.4 g, 0.036 mol) containing dry methanol (10 ml, excess) was added boron trifluoride etherate (0.25 ml) at room temperature. Evolution of nitrogen occurred and the reaction was cooled to maintain the temperature at 20°C. Nitrogen evolution was essentially complete after 30 minutes. The solution was washed with water (2 × 50 ml), saturated aqueous sodium chloride (1 × 50 ml) and dried (Na_2SO_4). Removal of solvent afforded a dark viscous oil which was purified by preparative chromatography (CH_2Cl_2) to afford benzyl-6α-methoxypenicillanate, **6c**, as a colorless gum (5.7 g 50%): $[\alpha]_D^{25}$ +166.4° (*c* 0.984, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 1778, 1750; ^1H NMR ($CDCl_3$) δ 1.35 (3H, s), 1.52 (3H, s), 3.52 (3H, s), 4.51 (1H, s), 4.57 (1H, d, J=1.5 Hz), 5.15 (2H, s), 5.28 (1H, d, J=1.5 Hz), 7.37 (5H, s). *Anal* Calcd for $C_{16}H_{19}NO_4S$: C 59.80, H 5.96, N 4.36. Found: C 59.53, H 6.06, N 4.54.

[2*S*-(2α,4β,5α,6α)]-6-Acetyloxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**7a**)

Benzyl ester precursor **7b**; white solid; mp 47~52°C; $[\alpha]_D^{25}$ +217.3° (*c* 0.912, CH_3OH); IR (KBr) ν_{max} cm^{-1} 2700~2550, 1803, 1757, 1735, 1227, 1057; ^1H NMR ($CDCl_3$) δ 1.31 (3H, s), 1.72 (3H, s), 2.18 (3H, s), 4.57 (1H, s), 4.99 (1H, d, *J*=1.5 Hz), 5.77 (1H, d, *J*=1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 169.46 (s), 166.10 (s), 77.00 (d), 73.79 (s), 73.04 (d), 64.91 (d), 20.25 (q), 19.55 (q), 18.17 (q) (hence β-sulfoxide); *Anal* Calcd for $C_{10}H_{13}NO_6S \cdot \frac{1}{4}H_2O$: C 42.93, H 4.86, N 5.01. Found: C 42.98, H 5.04, N 4.94.

[2*S*-(2α,4β,5α,6α)]-6-Acetyloxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**7b**)

To a dichloromethane (125 ml) solution of benzyl-6α-hydroxypenicillanate-(*S*)*S*-oxide (**1b**)[1] (3.20 g, 10 mmol) maintained at 0°C was simultaneously added separate solutions of acetyl chloride (1.17 g, 15 mmol) in dichloromethane (60 ml) and triethylamine (1.40 g, 14 mmol) in dichloromethane (30 ml). The solutions were added dropwise at rates such that addition of both solutions was completed at the same time. The reaction was then brought to room temperarure and stirred for 1 hour. The mixture was washed successively with 10% aqueous potassium bicarbonate (2×100 ml), water (2×200 ml), saturated aqueous sodium chloride (1×100 ml), dried (Na_2SO_4) and solvent removed to afford the acylated derivative **7b**, as a pale yellow gum (3.64 g, 100%): $[\alpha]_D^{25}$ +197.3° (*c* 1.051, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 3050, 1803, 1754, 1225, 1060, 697; ^1H NMR ($CDCl_3$) δ 1.10 (3H, s), 1.63 (3H, s), 2.16 (3H, s), 4.55 (1H, s), 4.99 (1H, s), 5.24 (2H, ABq), 5.78 (1H, s), 7.38 (s, 5); *Anal* Calcd for $C_{17}H_{19}NO_6S$: C 55.88, H 5.24, N 3.83. Found: C 56.10, H 5.39, N 3.80.

[2*S*-(2α,4β,5α,6α)]-3,3-Dimethyl-7-oxo-6-[(phenoxyacetyl)oxy]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**9a**)

Benzyl ester precursor **9b**; white crystalline solid; mp 160~169°C dec; $[\alpha]_D^{25}$ +176.3° (*c* 0.591, acetone); IR (KBr) ν_{max} cm^{-1} 3050 (br), 1795, 1780, 1190, 1010; ^1H NMR (CD_3COCD_3) δ 1.32 (3H, s), 1.66 (3H, s), 4.44 (1H, s), 4.90 (2H, s), 5.40 (1H, d, *J*=1.5 Hz), 5.85 (1H, d, *J*=1.5 Hz), 6.89~7.08 (3H, m), 7.20~7.38 (2H, m); *Anal* Calcd for $C_{16}H_{17}NO_7S$: C 52.31, H 4.66, N 3.81. Found: C 51.93, H 4.62, N 3.84.

[2*S*-(2α,4β,5α,6α)]-3,3-Dimethyl-7-oxo-6-[(phenoxyacetyl)oxy]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Phenylmethyl Ester-4-oxide (**9b**)

Sulfide precursor **9c**[1]; yield 77%; white crystalline powder; mp 157~158°C (literature[1] 155~156°C); IR ($CHCl_3$) ν_{max} cm^{-1} 1804, 1790, 1753, 1180, 1060; ^1H NMR ($CDCl_3$) δ 1.06 (3H, s), 1.62 (3H, s), 4.56 (1H, s), 4.74 (2H, s), 4.98 (1H, d, *J*=1 Hz), 5.22 (2H, ABq), 5.87 (1H, d, *J*=1 Hz), 6.88~7.10 (3H, m), 7.20~7.30 (2H, m), 7.37 (5H, s). This compound was purified by preparative chromatography (hexane - ethyl acetate; 3:2) to remove a small amount of sulfone.

[2*S*-(2α,4β,5α,6α)-(*Z*)]-6-[[[[2-(Amino)-4-thiazolyl]methoxyimino]acetyl]oxy]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid-4-oxide (**10a**)

A solution of the benzhydryl ester, **10b**, (1.00 g, 1.72 mmol) in trifluoroacetic acid (25 ml) and anisole (5 ml) was stirred at −5°C for 1.5 hours under an argon atmosphere. Anhydrous ether (100 ml) and hexane (150 ml) was added and the mixture triturated. The resulting precipitate was filtered, triturated with fresh ether, and dried under vacuum, to afford slightly impure free acid **10a**, as a yellow powder (0.52 g, 73%); mp 100~103°C dec effervesce; IR (KBr) ν_{max} cm^{-1} 3380~2540, 1790, 1760, 1640, 1055; ^1H NMR (DMSO-d_6) δ 1.25 (3H, s), 1.58 (3H, s), 3.95 (3H, s), 4.38 (1H, s), 5.61 (1H, d, *J*=1.5 Hz), 5.84 (1H, d, *J*=1.5 Hz), 7.01 (3H, br s, CO_2H, NH_2), 7.16 (1H, s); ^{13}C NMR (DMSO-d_6) δ 168.99 (s), 168.62 (s), 165.24 (s), 160.53 (s), 144.16 (s), 138.73 (s), 110.31 (d), 75.80 (d), 73.48 (q), 72.55 (s), 64.63 (d), 63.00 (d), 19.30 (q), 17.42 (q).

[2*S*-(2α,4β,5α,6α)-(*Z*)]-6-[[[[2-(Amino)-4-thiazolyl]methoxyimino]acetyl]oxy]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Diphenylmethyl Ester-4-oxide (**10b**)

To a stirred solution of the chloroacetyl protected aminothiazole compound, **10c**, (1.32 g, 2 mmol) in anhydrous dimethylformamide (14 ml) was added methyldithiocarbamic acid sodium salt hydrate (0.44 g, 3 mmol) and the mixture stirred at ambient temperature for 30 minutes. The solvent was removed under

vacuum, ethyl acetate (250 ml) added and the solution washed successively with water (4 × 120 ml), saturated sodium chloride (120 ml), dried (Na_2SO_4). The solvent was removed to afford a red solid (1.4 g), which was purified by preparative chromatography (dichloromethane - ethyl acetate; 72 : 28) to afford the title compound, **10b**, as a pale yellow solid (0.80 g, 69%); mp 118~121°C; $[\alpha]_D^{25}$ +140.8° (*c* 1.037, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 3500, 3400, 1804, 1754, 1605, 1535, 1155, 1050, 1020, 703; ^1H NMR ($CDCl_3$) δ 0.93 (3H, s), 1.66 (3H, s), 4.01 (3H, s), 4.66 (1H, s), 5.10 (1H, d, J=1.5 Hz), 5.67 (2H, br s, NH_2), 5.98 (1H, d, J=1.5 Hz), 6.80 (1H, s), 6.98 (1H, s), 7.35 (10H, s); ^{13}C NMR ($CDCl_3$) δ 168.77 (s), 166.68 (s), 165.10 (s), 161.43 (s), 144.41 (s), 140.98 (s), 139.0 (s), 138.65 (s), 128.74 (d), 128.32 (d), 127.76 (d), 126.83 (d), 109.95 (d), 79.06 (d), 73.75 (s), 73.75 (q), 65.01 (d), 63.44 (d), 19.75 (q), 17.77 (q); *Anal* Calcd for $C_{27}H_{26}N_4O_7S_2$: C 55.66, H 4.50, N 9.62, S 11.00. Found: C 55.32, H 4.78, N 9.37, S 10.93.

[2*S*-(2α,4β,5α,6α)-(*Z*)]-6-[[[[2-[(Chloroacetyl)amino]-4-thiazolyl]methoxyimino]acetyl]oxy]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Diphenylmethyl Ester-4-oxide (**10c**)

To the iminothiazolylacetic acid, **13** (2.78 g, 10 mmol), in a mixture of anhydrous dichloromethane (20 ml) and anhydrous tetrahydrofuran (20 ml) at 0°C was added dry pyridine (0.79 g, 10 mmol) and the mixture was stirred at 0°C for 5 minutes. A solution of the hydroxysulfoxide **1c** (3.99 g, 10 mmol) in dichloromethane (25 ml) was added and the mixture stirred for a further 5 minutes. Dicyclohexylcarbodiimide (2.06 g, 10 mmol) dissolved in dichloromethane (15 ml) was added dropwise at 0°C over 15 minutes and the solution was then warmed to room temperature and stirred for 48 hours. The precipitate of dicyclohexylurea (2.0 g, 90%) was removed by filtration and solvent removed to afford a pale yellow solid, which was purified by preparative chromatography (dichloromethane - hexane - ethyl acetate; 80 : 11 : 9); yield 65%; pale yellow crystalline solid; mp 123~126°C; $[\alpha]_D^{25}$ +138.5° (*c* 1.02, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1} 3380, 1805, 1753, 1695, 1550, 1165, 1065, 1040, 705; ^1H NMR ($CDCl_3$) δ 0.96 (3H, s), 1.67 (3H, s), 4.06 (3H, s), 4.26 (2H, s), 4.70 (1H, s), 5.14 (1H, d, J=1.5 Hz), 6.01 (1H, d, J=1.5 Hz), 7.00 (1H, s), 7.40 (1H, s), 7.42 (10H, s); *Anal* Calcd for $C_{29}H_{27}ClN_4O_8S_2$: C 52.84, H 4.13, N 8.49. Found: C 52.41, H 4.21, N 8.29.

[2*S*-(2α,4α,5β,6α,7α)]-7-Hydroxy-4-methyl-8-oxo-5-thia-1-azatricyclo[4.2.0.0/2,4/]octane-2-carboxylic Acid-5-oxide (**11a**)

To a cooled (0°C) solution of the benzhydryl ester, **11b** (0.807 g, 2.03 mmol) in anisole (3.8 ml, 35 mmol) was added trifluoroacetic acid (18 ml) and the mixture stirred at 0°C for 1.5 hours. After the solvent was removed under high vacuum, the residue was triturated with ether - petroleum ether, dissolved in methanol, treated with charcoal and then filtered. The resulting clear solution was evaporated and the residue recrystallized from methanol - ether to afford the desired acid, **11a** as a white crystalline powder; mp 150°C dec; $[\alpha]_D^{25}$ +358.26° (*c* 0.41, CH_3OH); IR (KBr) ν_{max} cm^{-1} 3325, 2680~2460, 1778, 1737, 1000; ^1H NMR (CD_3OD) δ 1.80 (3H, s), 2.00 (1H, d, J=7 Hz), 2.35 (1H, d, J=7 Hz), 4.89 (1H, d, J=1.5 Hz), 5.31 (1H, d, J=1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 171.3 (s), 169.2 (s), 90.4 (d), 75.4 (d), 69.6 (s), 52.5 (s), 29.1 (t), 13.6 (q) (hence β-sulfoxide); *Anal* Calcd for $C_8H_9NO_5S$: C 41.56, H 3.92, N 6.06. Found: C 41.08, H 3.90, N 5.94.

[2*S*-(2α,4α,5β,6α,7α)]-7-Hydroxy-4-methyl-8-oxo-5-thia-1-azatricyclo[4.2.0.0/2,4/]octane-2-carboxylic Acid Diphenylmethyl Ester-5-oxide (**11b**)

Sulfide precursor **11c**; white crystalline powder (from $CHCl_3$ - hexane - ether); yield 73%; mp 85~95°C; $[\alpha]_D^{25}$ +229.48° (*c* 0.86, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3240, 1780, 1737, 1210, 1180, 1027, 748, 700; ^1H NMR ($CDCl_3$) δ 1.68 (3H, s), 2.07 (1H, d, J=7 Hz), 2.53 (1H, d, J=7 Hz), 3.63 (1H, d, J=8 Hz, OH), 5.15 (1H, s), 5.16 (1H, d, J=8 Hz), 6.91 (1H, s), 7.34 (10H, m); ^{13}C NMR ($CDCl_3$) δ 168.0 (s), 165.5 (s), 139.1 (s), 128.8 (d), 128.4 (d), 127.1 (d), 88.5 (d), 79.4 (d), 74.5 (d), 68.9 (s), 51.6 (s), 29.3 (t), 13.6 (q) (hence β-sulfoxide by comparison to the α-isomer); *Anal* Calcd for $C_{21}H_{19}NO_5S \cdot 0.37CHCl_3$: C 58.12, H 4.42, N 3.17, S 7.26. Found: C 58.17, H 4.43, N 3.16, S 7.29. Also isolated the corresponding α-isomer; [2*S*-(2α,4α,5α,6α,7α)]-7-hydroxy-4-methyl-8-oxo-5-thia-1-azatricyclo[4.2.0.0/2,4/]octane-2-carboxylic acid diphenylmethyl ester-5-oxide; white crystalline powder (from $CHCl_3$); yield 7%; mp 78~86°C; IR (KBr) ν_{max} cm^{-1} 3440, 3240, 1790, 1730, 1270, 1220, 1180, 1020, 755, 748, 700; ^1H NMR ($CDCl_3$) δ 1.54 (1H, d, J=7 Hz), 1.56 (3H, s), 2.31 (1H, d, J=7 Hz), 3.87 (1H, d, J=8 Hz, OH), 5.09 (1H, d, J=8 Hz), 5.19 (1H, s), 6.94 (1H, s), 7.36 (10H, s); ^{13}C NMR ($CDCl_3$) δ 168.0 (s), 165.6 (s), 139.0

(s), 128.7 (d), 128.4 (d), 127.1 (d), 97.4 (d), 79.5 (d), 78.0 (d), 62.3 (s), 50.6 (s), 32.9 (t), 10.6 (q) (hence α-sulfoxide by comparison to the β-isomer); *Anal* Calcd for $C_{21}H_{19}NO_5S \cdot 0.9CHCl_3$: C 52.09, H 3.97, N 2.77. Found: C 52.03, H 3.96, N 2.85.

[2*S*-(2α,4α,6α,7α)]-7-Hydroxy-4-methyl-8-oxo-5-thia-1-azatricyclo[4.2.0.0/2,4/]octane-2-carboxylic Acid Diphenylmethyl Ester (**11c**)

A solution of trifluoroacetic acid (0.90 ml, 11.7 mmol) in anhydrous dichloromethane (90 ml) was added over 40 minutes to a stirred, cooled (0°C) solution of diazocyclopropyl penicillanic acid ester, **14**[17] (4.6 g, 11.8 mmol) in anhydrous dichloromethane (175 ml). The cooling bath was removed and the reaction mixture stirred at ambient temperature for 16 hours. Saturated aqueous sodium bicarbonate (50 ml) was then added and the mixture stirred for 1 hour more. The organic layer was washed successively with saturated aqueous sodium bicarbonate, saturated aqueous sodium chloride, dried (Na_2SO_4), and the solvent removed. The resulting residue was purified by preparative chromatography (silica; eluant hexane - ethyl acetate; 4 : 1) to afford the desired 6α-hydroxy compound, **11c** (1.85 g, 40%): ^{1}H NMR ($CDCl_3$) δ 1.54 (3H, s), 1.97 (1H, d, J=7 Hz), 2.35 (1H, d, J=7 Hz), 4.68 (1H, d, J=1.5 Hz), 5.85 (1H, d, J=1.5 Hz), 6.95 (1H, s), 7.45 (10H, s).

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