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Synthesis and Elastase Inhibitory Activity of 6α-Chloro-2,2-dimethyl-3α-(pivaloyloxy)methylpenam Sulfone, 6α-Chloro-2,2-dimethyl-3-*exo*-methylenepenam Sulfone, Benzyl and Methyl 6α-Substituted Penicillanate Sulfones

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Abstract—The triflates and pivalates of 3α-hydroxymethyl-6-substituted-2,2-dimethylpenam sulfones **3**, **5**; methyl and benzyl 6-substituted penicillanates **6–9** and 3-*exo*-methylene-6-substituted-2,2-dimethylpenam sulfone **4** were synthesized. These novel compounds were evaluated as elastase inhibitors using porcine pancreatic elastase. The effects that structural modifications of substituents on C-3 and C-6 in the penam nucleus have on elastase activity were examined and several similarities and distinctions were identified when compared to the reported penicillin esters and amides elastase inhibitors.

Introduction

In recent years there has been considerable interest in the development and mechanism of action of human leukocyte elastase (HLE, EC 3.4.21.37) and porcine pancreatic elastase (PPE, EC 3.4.21.36) inhibitors.[1–3] HLE is a serine protease associated with several degenerative diseases.[4–6] This interest has led ultimately to the synthesis of a wide variety of inhibitors based on β-lactam nucleus among other synthetic inhibitors.[7] These β-lactam derivatives include ester and amide derivatives of cephalosporin sulfones,[8] cephem-4-ketone sulfones,[9] *tert*-butyl esters of cephem sulfones,[10] novel bicyclic β-lactams,[11] penem benzyl esters,[12] esters[13] or amides[14] of penam sulfides, sulfoxides and sulfones, and monocyclic β-lactams,[15] as mechanism-based inhibitors of HLE. The cephalosporin sulfone amide L-658,758 has been selected as a clinical candidate for development as a topical aerosol[8f] as well as a monocyclic β-lactam for oral administration.[15e]

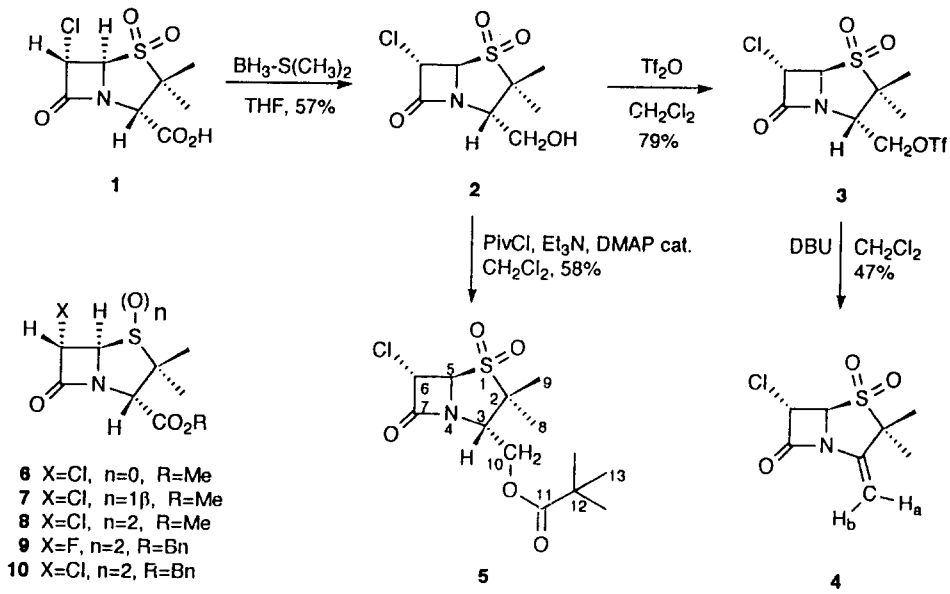
The structure of the complex resulting from the *tert*-butyl 7α-chlorocephalosporinate sulfone and PPE has been solved at 1.84 Å resolution.[16] Several studies have been carried out in which some aspects of the mechanism of action of these β-lactam inhibitors have been elucidated.[8g,15h,17a,b]

The structure–activity relationships (SAR) for penicillin esters and amides substituted at C-6 with either an α- or β-trifluoroacetamido or an α-alkoxy functionalities as HLE inhibitors were reported.[13,14]

Previously, we prepared a series of 6-substituted penicillanic acids which behaved as inhibitors of bacterial serine β-lactamases.[18] We have now designed pivalates and triflates of 3α-hydroxymethyl-6-substituted-2,2-dimethylpenam sulfones, 3-*exo*-methylene-6-substituted-2,2-dimethylpenam sulfones, and benzyl or methyl 6-substituted penicillanates as inhibitors of leukocyte elastase (Scheme 1). These novel penam derivatives were evaluated as elastase inhibitors using PPE. The effects that structural modifications of substituents on C-3 and C-6 in the penam nucleus have on elastase activity were examined in detail and several similarities and distinctions were identified when compared to the reported penicillin esters and amides.[13,14]

Chemistry

Synthetic approaches to the (pivaloyloxy)methyl penam sulfone (**5**) and the 3-*exo*-methylene penam sulfone (**4**) are outlined in Scheme 1. The starting material was the readily available 6α-chloropenicillanic acid sulfone (**1**).[19] In this sequence the penicillanic acid **1** was reduced by diborane–dimethyl sulfide complex yielding the corresponding 3α-hydroxymethylpenam **2**, a versatile common intermediate from which **4** and **5** were prepared. Treatment of the alcohol **2** with trifluoromethanesulfonic anhydride (Tf_2O) afforded the penam triflate **3** in 79% yield. The ^{19}F NMR spectrum showed the characteristic singlet at 74.57 ppm, corresponding to the trifluoromethyl moiety. The elimination reaction was rapidly completed by DBU at low temperature, giving the product **4** in 47% yield. The formation of the exocyclic double bond was confirmed by the presence of signals at 4.73 and 5.27 ppm (olefinic geminal protons, J = 2.7 Hz) in the ^1H NMR spectrum.



Scheme 1.

On the other hand, the alcohol **2** was reacted with pivaloyl chloride and triethylamine at 25 °C in dichloromethane. 4-Dimethylaminopyridine (DMAP, 0.25 eq.) was essential to promote the reaction. The desired (pivaloyloxy)methylpenam **5** was obtained in 58% yield.

Methyl 6α-chloropenicillanate (**6**),[20] benzyl 6α-fluoropenicillanate sulfone (**9**)[18] and benzyl 6α-chloropenicillanate sulfone (**10**)[13] were synthesized as reported in the literature. Methyl 6α-chloropenicillanate (*S*)-sulfoxide (**7**) and methyl 6α-chloropenicillanate sulfone (**8**) were obtained by oxidation of the sulfide **6**.

Biological Activity

The new compounds prepared (**2–9**) have been tested as potential inhibitors of porcine pancreatic elastase (PPE). In Table 1 IC_{50} values calculated from initial velocities are shown. In order to compare the effectiveness of our compounds with that of known inhibitors it has also been included the IC_{50} obtained with compound **10**,[13] under exactly the same experimental conditions. From the data in Table 1 it can be concluded that replacement of the 6α-chloro atom (compound **10**) by a fluorine one (compound **9**) produced a marked decrease in the inhibitory activity. From the comparison of our results with those reported by Thompson *et al.*,[13] obtained under essentially similar experimental conditions with HLE, it can also be concluded that replacement of the 6α-methoxy group in methyl 6α-methoxypenicillanate (a good HLE inhibitor) by a chloro atom (compound **8**) renders a compound that only slightly inhibited PPE activity. Surprisingly (see Ref. 13), for the methyl 6α-chloropenicillanate series, higher inhibitory activity could be observed for the sulfoxide **7** than for the corresponding sulfone **8**. However, compound **7** did not completely inhibit elastase activity, hence it is not likely that it binds at the active site of the enzyme. The comparison of the effect of different C-3 analogues of the 6α-chloro-2,2-dimetilpenam sulfones led us to conclude that the methyl ester **8** is the less active. The free alcohol **2** and the trifluoromethanesulfonic ester **3** are somehow more active. The more potent inhibitors were those with a pivaloyloxymethyl group (compound **5**) and with an *exo* methylene group (compound **4**) in C-3.

Table 1

Compound n°	IC_{50} (μM)
2	650
3	420
4	35
5	15
6	950 (24%)[a]
7	159[b]
8	950 (44%)[a]
9	650 (35%)[a]
10	205

Elastase activity was determined as indicated in the Experimental. Substrate concentration was 145 μM. IC_{50} value is the concentration of compound that inhibited 50% enzyme activity.

[a]The maximum concentration used in the inhibition assays is indicated; between parentheses are shown the inhibition values obtained.

[b]The kinetic data obtained with this compound strongly suggest that it behaved as a partial inhibitor; a maximal inhibition equal to 55% could be derived by extrapolation to infinite inhibitor concentration; half-maximal effect was obtained with 20 μM.

All the inhibitions were instantaneous. A time-dependent inhibition could not be observed, even with the known inhibitor **10**. In addition, similar IC_{50} values were obtained when the enzyme was preincubated 20 min with the inhibitor (data not shown). It has been reported by Thompson *et al.*[14] that some penam

derivatives were capable of inhibiting in a time-dependent manner the enzyme activity of HLE. Unfortunately, the structural differences between those penam derivatives and the compounds discussed in this manuscript do not let us to draw conclusions on the structural reasons responsible for a time-dependent inhibition. Moreover, it has been reported[14] that small structural differences such as replacement of an α-methoxy group by an α-ethoxy group in C-6, resulted in a loss of the time-dependent inhibition. A mechanism-based inhibitor of elastases must fulfil several conditions: (i) it must be able to bind at the active site; (ii) it must be able to acylate the enzyme and (iii) the acyl–enzyme complex must be susceptible to rearrangement reactions leading to an irreversible adduct. Whenever a compound lacks any of the above mentioned conditions, it would be unable to produce a time-dependent, mechanism-based inhibition.

The inhibition produced by the more potent inhibitors (compounds **4** and **5**) was further studied in order to characterize the mechanism of inhibition. The effect of both compounds on enzyme activity was assayed at different substrate and inhibitor concentrations. Both compounds behaved as linear mixed inhibitors (compound **4**: K_i = 56 ± 9 μM, K'_i = 20 ± 5 μM; compound **5**: K_i = 20 ± 3 μM, K'_i = 13 ± 4 μM, see Scheme 2 and Figs 1A and B). These results suggest that these compounds can bind to the free enzyme (**E**) and to the enzyme–substrate (**ES**) complex. According to the mechanism 1 shown in Scheme 2, these penam derivatives bind to a site other than the active site and, since they would not fulfil the conditions mentioned above, they would lack the time-dependent, mechanism-based inhibition.

Mixed inhibition can also be obtained when the inhibitor binds at two different mutually exclusive sites (see Scheme 2, mechanism 2). Mechanisms 1 and 2 cannot be distinguished on a kinetic basis. In any case, compounds **4** and **5** either do not bind at the active site (mechanism 1) or they are displaced from the active site by stronger binding to another different mutually exclusive site (mechanism 2) that becomes available upon substrate binding. We have not been able to detect any time-dependent inhibition with compounds **4** and **5**. Such a result suggests one of the following alternatives: (i) the compounds do not bind at the active site and (ii) they bind to the active site and to a second site and they are not able to acylate the enzyme. The possibility that the compounds bind to the enzyme according to alternative (ii) and that they are able to acylate the enzyme but they do so in such slow manner, that the binding at the second site competes effectively with the mechanism-based inhibition cannot be excluded.

The ability of these penam derivatives of binding to a site different than the active site suggests that they are not good candidates for mechanism-based inhibition. Further studies are being carried out in order to determine the structural features responsible for such behaviour.

Conclusion

Previous structure–activity studies on 6-substituted penicillin esters showed that the 3α-benzyl ester group of penicillin sulfones substituted at C-6 with either an α-trifluoroacetamide, an α-alkoxy, or an α-tosylate functionality are good inhibitors of human leukocyte elastase.[13]

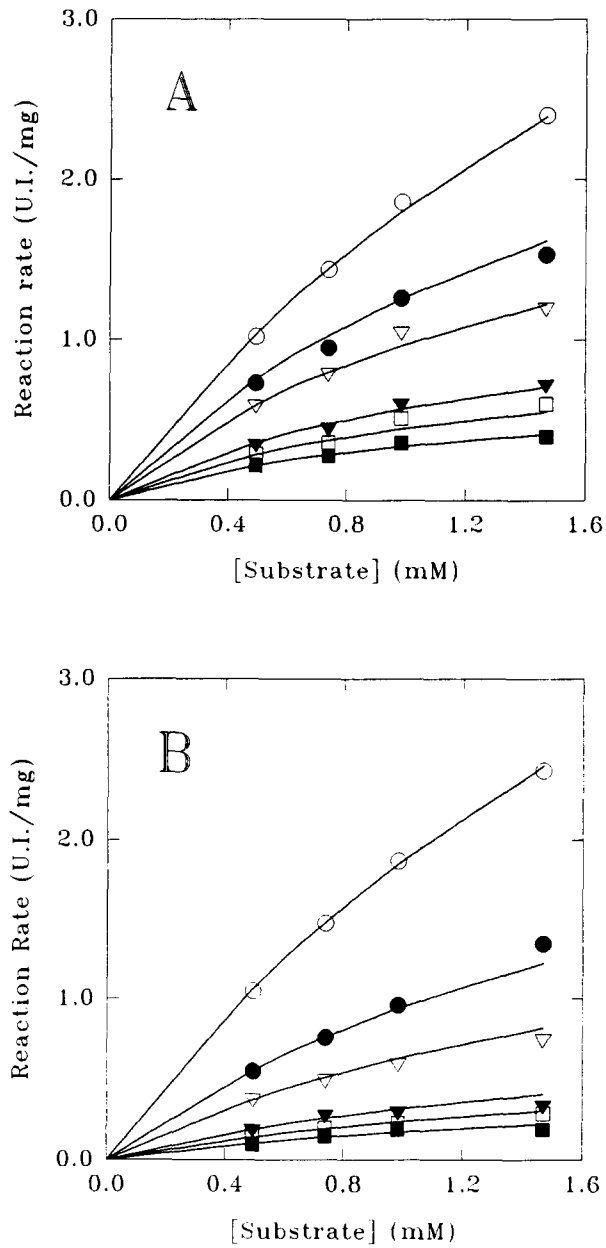
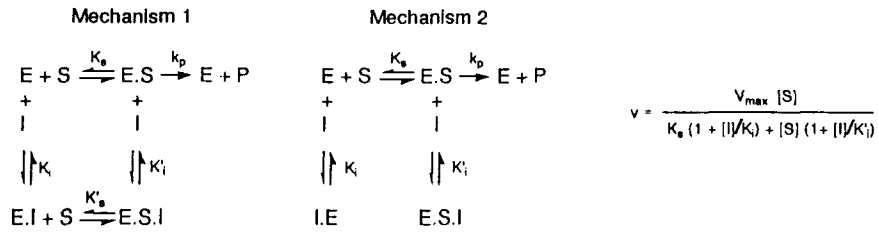


Figure 1. Kinetic study of the inhibition of PPE activity by (A) 6α-chloro-2,2-dimethyl-3-*exo*-methylenepenam sulfone, and (B) 6α-chloro-2,2-dimethyl-3α-(pivaloyloxy)methylpenam sulfone. Enzyme activity was measured as indicated in the Experimental. The symbols indicate the experimental reaction rates obtained at different *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide and at the following inhibitor concentrations: ○ = 0; ● = 16.5; ▽ = 33; ▼ = 82; ❑ = 114.5 and ■ = 163 μM. The solid lines are those obtained by fitting the experimental points to the equation shown in Scheme 2 (linear mixed inhibition) using a non-linear regression procedure.



Scheme 2.

This study provides the first examples of the successful use of the pivalate ester of 3-hydroxymethyl and the 3-*exo*-methylene groups of 6-substituted penam sulfones, in the rational design of biologically active molecules.

Experimental

Infrared spectra (IR) were taken on a Bruker IFS 25 FT-IR spectrometer. Proton and carbon magnetic resonance spectra (^1H and ^{13}C NMR) were taken on a Bruker AC 200 spectrometer. Fluorine magnetic resonance spectrum (^{19}F NMR) was recorded on a Bruker WP 80 SY spectrometer. Melting points were taken on a Ernst Leitz melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was carried out with silica gel 60 F_{254} pre-coated aluminium sheets (Merck); column chromatography was performed on silica gel grade 60 (Merck).

Elastase (EC 3.4.21.36) was purchased from Sigma Chemical Co. (Type III, from porcine pancreas). A stock solution (1 mg mL^{-1}) was prepared in sodium acetate 50 mM (pH 5.5), and frozen at –20 °C until used. *N*-Methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (Sigma Chemical Co.) was dissolved in dimethylsulfoxide (DMSO). Enzyme activity was assayed in potassium phosphate 100 mM, pH 7 (final volume 3 mL). The reaction was started by addition of 30–50 μL of the enzyme stock solution. The release of *p*-nitroaniline was followed spectrophotometrically at 405 nm in a Varian Cary 210 with a thermostatted cell holder (30 °C). The inhibitors were dissolved in DMSO. The maximal concentration of DMSO was 2% in the reaction medium. Control experiments were run in all the cases with equal amounts of DMSO.

All the reagents used were of the highest available analytical grade.

6α-Chloro-3α-hydroxymethyl-2,2-dimethylpenam S,S-*dioxide (2).* To a solution of 6α-chloropenicillanic acid sulfone (**1**) (781 mg, 2.91 mmol) in dry THF (10 mL) was added dropwise borane–methyl sulphide complex (2 M solution in THF, 2.34 mL, 4.68 mmol) at room temperature. The resulting solution was stirred for 45 h, then the solvent was removed and the residue was diluted with ethyl acetate (10 mL), washed with aqueous sodium hydrogen carbonate (2 × 5 mL) and brine (2 × 5 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* and the residue was chromatographed (eluant: ethyl acetate:hexane, 4:6) to give the title compound **2** (420 mg, 57%) as white crystals, mp 112–114 °C; νmax(film) 3566 (OH), 1792 (β-lactam), 1320 and 1122 cm^{-1} (SO2); δ_H (200 MHz; $CDCl_3$; standard Me_4Si) 1.49 (3H, *s*, 8-Me), 1.50 (3H, *s*, 9-Me), 2.33 (1H, *br s*, 10-OH), 3.78 (2H, *m*, 10-CH_2), 3.92 (1H, *m*, 3-H), 4.56 (1H, *d*, *J* = 1.53 Hz, 6-H) and 5.16 ppm (1H, *d*, *J* = 1.53 Hz, 5-H); δ_C (50 MHz; $CDCl_3$; standard $CDCl_3$) 167.46 (C-7), 68.72 (C-6), 63.33 (C-2), 62.68 (C-3), 60.23 (C-10), 54.84 (C-5), 18.71 (C-9) and 18.04 ppm (C-8); LRMS (Cl, methane) 256 (36%), 254 ([M+1]$^+$, 100), 178 (47), 122 (18), 120 (56), 85 (8); HRMS calcd for $C_8H_{13}O_4NSCl$ 254.0254, found 254.0242.

6α-Chloro-2,2-dimethyl-3α-(trifluoromethanesulfonyl-oxy)methylpenam S,S-*dioxide (3).* A solution of the alcohol **2** (105 mg, 0.41 mmol) and pyridine (40 μL, 0.50 mmol) in anhydrous dichloromethane (3 mL) was added dropwise to a solution of triflic anhydride (84 μL, 0.50 mmol) in anhydrous dichloromethane (2 mL) at 0 °C over 10 min. The reaction was allowed to reach room temperature and then stirred for 40 h. To the dark solution obtained saturated aqueous NH_4Cl (2 mL) was added, the layers were separated and the organic layer was washed with brine (2 × 1.5 mL) and dried (Na_2SO_4). The oily product was chromatographed, eluting with ethyl acetate:hexane (3:7), to give **3** (125 mg, 79%) as a colourless oil; ν_{max} (film) 1814 (β-lactam), 1416, 1246, 1210, 1142, 966 (Tf), 1330 and 1128 cm^{-1} (>SO_2); δ_H (200 MHz; $CDCl_3$; standard Me_4Si) 1.51 (3H, *s*, 8-Me), 1.53 (3H, *s*, 9-Me), 4.18 (1H, *dd*, ABX system, J_{vic} = 6.75 Hz, 3-H), 4.49 (1H, *dd*, ABX system, J_{gem} = 10.85 Hz, J_{vic} = 6.75 Hz, 10-H), 4.55 (1H, *d*, *J* = 1.63 Hz, 6-H), 4.59 (1H, *dd*, J_{gem} = 10.85 Hz, J_{vic} = 6.75 Hz, 10-H) and 5.19 ppm (1H, *d*, *J* = 1.63 Hz, 5-H); δ_C (50 MHz; $CDCl_3$; standard $CDCl_3$) 166.69 (C-7), 118.35 (*c*, *J* = 12.71 Hz, CF_3), 71.34 (C-10), 68.81 (C-6), 62.78 (C-2), 59.75 (C-3), 55.56 (C-5), 18.72 (C-9) and 18.60 ppm (C-8); δ_F (80.13 MHz; $CDCl_3$; standard $CFCl_3$) 74.57 ppm (*s*, CF_3); LRMS (Cl, methane) 388 (47%), 386 ([M+1]$^+$, 100), 310 (66), 254 (25), 252 (16), 91 (15); HRMS calcd for $C_9H_{12}O_6NS_2F_3Cl$ 385.9747, found 385.9766.

6α-Chloro-2,2-dimethyl-3-exo-*methylenepenam* S,S-*dioxide (4).* A solution of DBU (12 μL, 0.079 mmol) in anhydrous dichloromethane (1 mL) was slowly added to a solution of the triflate **3** (29 mg; 0.075 mmol) in anhydrous dichloromethane (2 mL) at –84 °C. After stirring for 1 h, the reaction was allowed to reach room

temperature, and then washed with aqueous NH_4Cl (2 mL) and brine (3 mL). After drying (Na_2SO_4), the crude product was chromatographed, eluting with chloroform:ethyl ether (95:5), to give **5** (8 mg, 47%) as a colourless oil; ν_{max} (film) 1810 (β-lactam), 1652 (C=C), 1330 and 1116 cm^{-1} (>SO_2); δ_H (200 MHz; $CDCl_3$; standard Me_4Si) 1.54 (3H, *s*, 8-Me), 1.58 (3H, *s*, 9-Me), 4.72 (1H, *d*, *J* = 2.67 Hz, 10-H_a), 4.74 (1H, *d*, *J* = 1.86 Hz, 6-H), 5.26 (1H, *d*, *J* = 2.67 Hz, 10-H_b), and 5.28 ppm (1H, *d*, *J* = 1.86 Hz, 5-H); δ_C (50 MHz; $CDCl_3$; standard $CDCl_3$) 162.28 (C-7), 142.07 (C-3), 97.29 (C-10), 69.26 (C-6), 64.27 (C-2), 55.66 (C-5), 25.58 (C-9) and 16.55 ppm (C-8); LRMS (EI) 237 (4%), 235 ([M]$^+$, 7), 171 (6), 128 (12), 108 (64), 67 (63), 41 (68), 28 (100); HRMS calcd for $C_8H_{10}O_3NSCl$ 235.0068, found 235.0072.

6α-Chloro-2,2-dimethyl-3α-(pivaloyloxy)methylpenam S,S-dioxide (5). To a solution of the alcohol **2** (50 mg, 0.20 mmol) and catalytic DMAP in dry dichloromethane was added dropwise triethylamine (56 μL, 0.39 mmol) and pivaloyl chloride (49 μL, 0.39 mmol), at room temperature. After stirring 15 h the reaction mixture was diluted with dichloromethane (5 mL) and washed with brine (2 × 3 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a yellow oil, which was chromatographed (eluant: ethyl acetate: hexane, 4:6) affording the pivalate **5** (39 mg, 58%) as white needles (mp 105.5–106.5 °C); ν_{max} (KBr) 1816 (β-lactam), 1720 (ester), 1323 and 1126 cm^{-1} (>SO_2); δ_H (200 MHz: $CDCl_3$; standard Me_4Si) 1.23 (9H, *s*, 13-Me), 1.45 (3H, *s*, 8-Me), 1.50 (3H, *s*, 9-Me), 4.11 (3H, *m*, 3-H and 10-CH_2), 4.52 (1H, *d*, *J* = 1.59 Hz, 6-H) and 5.14 ppm (1H, *d*, *J* = 1.59 Hz, 5-H); δ_C (50 MHz; $CDCl_3$; standard $CDCl_3$) 177.86 (C-11), 166.58 (C-7), 69.07 (C-6), 62.85 (C-2), 61.66 (C-10), 60.46 (C-3), 55.39 (C-5), 38.73 (C-12), 26.98 (3 C-13), 18.87 (C-9) and 18.79 ppm (C-8); LRMS (Cl, methane) 340 (37%), 338 ([M+1]$^+$, 90), 262 (19), 238 (40), 236 (100), 170 (11), 85 (66); HRMS calcd for $C_{13}H_{21}O_5NSCl$ 338.0829, found 338.0818.

Methyl 6α-chloropenicillanate (S)-sulfoxide (7). To a solution of methyl 6α-chloropenicillanate (**6**) (77 mg, 0.31 mmol) in chloroform (5 mL) was added Oxone[21] (190 mg, 0.31 mmol) and wet alumina (310 mg).[22] The resulting slurry was stirred at room temperature for 24 h and then was filtered, washing thoroughly with chloroform. The solvent was evaporated and the residue chromatographed, eluting with ethyl acetate:hexane (4:6), to give the sulfoxide (77 mg, 94%) as a colourless oil, the spectroscopic data being coincident with those reported.[23]

Methyl 6α-chloropenicillanate S,S-dioxide (8). According to the procedure previously described,[18] the methyl 6α-chloropenicillanate (**6**) was oxidized to afford the sulfone (**8**) as a white solid, mp 142–144 °C; ν_{max} (nujol) 1794 (β-lactam), 1757 (ester), 1327 and 1118 cm^{-1} (>SO_2); δ_H (200 MHz; $CDCl_3$; standard Me_4Si) 1.42 (3H, *s*, 8-Me), 1.61 (3H, *s*, 9-Me), 3.85 (3H, *s*, methyl ester), 4.44 (1H, *s*, 3-H), 4.67 (1H, *d*, *J* = 1.60 Hz, 6-H) and 5.17 ppm (1H, *d*, *J* = 1.60 Hz, 5-H); δ_C (50 MHz; $CDCl_3$; standard $CDCl_3$) 166.36 (C-10), 165.85 (C-7), 69.13 (C-5), 63.07 (C-3), 55.35 (C-6), 53.32 (C-2), 53.17 (CO_2Me), 20.03 (C-9) and 18.45 ppm (C-8); LRMS (Cl, methane) 284 (32%), 282 ([M+1]$^+$, 86), 206 (62), 192 (17), 148 (26), 115 (21), 91 (42), 61 (100); HRMS calcd for $C_9H_{13}O_5NSCl$ 282.0203, found 282.0203.

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