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Synthesis and *in vitro* antibacterial activity of 2β and 2α-(substituted methyl) phenoxymethylpenicillins and an oxidation product

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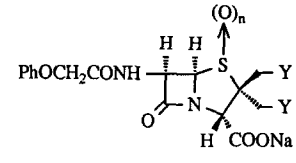
Summary — The effect of stereochemical changes on the antibacterial activity of a series of 2-substituted methyl-6β-phenoxyacetamido penams synthesized in our laboratory was studied. The 2α-heteroarylthiomethyl penams were found to be more active than the 2β-substituted derivatives against a range of Gram-positive and Gram-negative microorganisms. Both the isomers of 2-substituted methyl derivatives were found to be less active than the unsubstituted compound, the phenoxymethyl penicillin. The oxidized product of the 2α-isomer was less active than the unoxidized compound.

Résumé — Synthèse et activité antibactérienne *in vitro* de 2β et 2α-(méthyl substitué) phénoxyméthylpénicillines et d'un produit d'oxydation. *L'effet de changements stéréochimiques sur l'activité antibactérienne d'une série de 2-méthyl substitué-6β-phénoxyacétamidopénems a été étudié. Les 2α-hétéroarylthiométhylpénems se sont révélés plus actifs que les dérivés 2β-substitués vis-à-vis d'une série de microorganismes Gram + et Gram –. Les isomères des dérivés 2-méthyl substitués ont été trouvés moins actifs que le composé non substitué, la phénoxyméthylpénicilline. Le produit oxydé de l'isomère 2-α est moins actif que le composé non oxydé.*

β-lactam antibiotic / 2β-substituted penicillin / 2α-substituted penicillin / oxidation products / antimicrobial activity

Introduction

Many of the most effective and broad-spectrum cephalosporins either introduced on the market or under development have a heteroaryl thiomethyl substitution at the C-3 position [1]. Similar substitutions at the 2β-methyl position in penicillins have also been reported in the patent literature [2] without any details regarding their antimicrobial activity. Our systematic oxidation studies on penicillins and cephalosporins [3–6] have shown that the oxidation patterns of 2-substituted methyl penams and 3-substituted methyl cephems are distinctly different and certain oxidation products are more potent antibacterial agents than the others. Four representative members of the 2β and 2α-substituted methyl phenoxyacetamido penams (general structure 1) were synthesized and the effect of their stereochemistry on the antimicrobial spectrum and activity were determined. One of the 2α-substituted penams was oxidized by *m*-chloroperbenzoic acid and tested for biological activity. The chemistry and preliminary antimicrobial activity were presented in part at the 28th ICAAC meeting [7]. A group from Beecham has recently reported a series of 2α-substituted methyl penicillins having good antibacterial activity [8].



General structure **1**
Y = H or S-Het
n = 0, 1

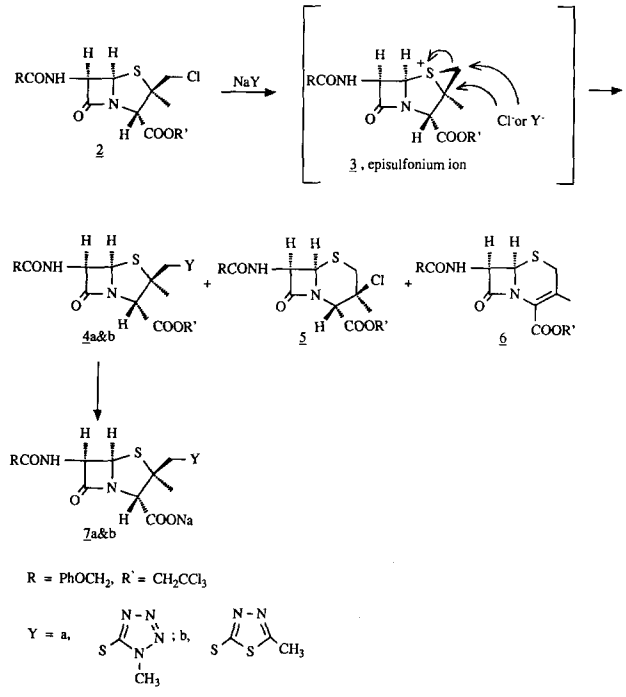
Chemistry

The trichloroethyl 2β-chloromethyl-6β-phenoxyacetamidopenicillin **2** was prepared by the method of Kamiya *et al* [9] using the corresponding *unsym*-azetidinone disulfide and cupric chloride in dichloromethane. The 2α-(substituted methyl) penam systems

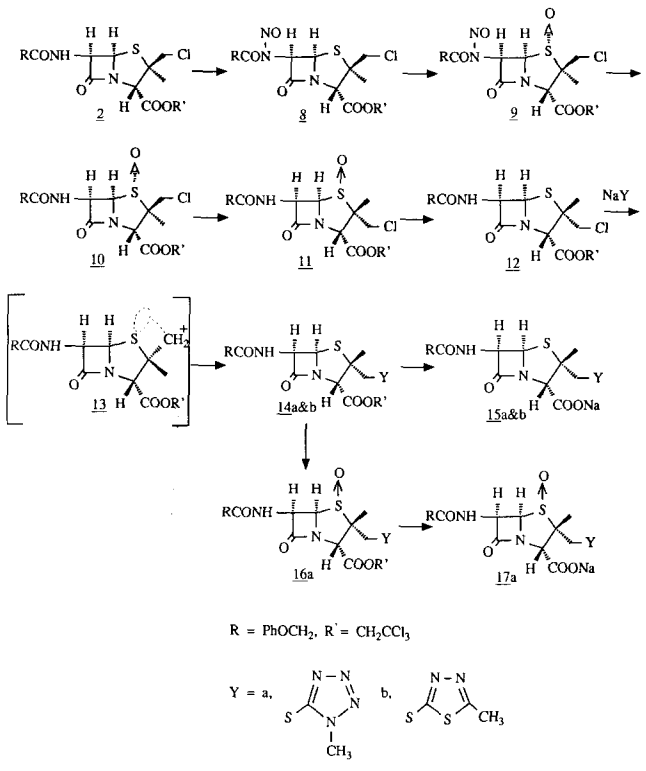
have been synthesized and reported by several workers following different methods [10–13]. We synthesized the 2α-chloromethyl penam ester **12** to be used as a starting material for the 2α-substituted methylpenam **14** by following the steps summarized in scheme 2. In this route the 1R-sulfoxide **10** was prepared by a 3-step process starting from the trichloroethyl 2β-(chloromethyl)-6β-(phenoxyacetamido) penicillin **2** according to the procedure described by Uyeo *et al* [14]. The treatment of compound **2** with dinitrogen tetroxide in dichloromethane at 0°C for 1 h gave the *N*-nitroso derivative **8**, which was oxidized with *m*-chloroperbenzoic acid at 0°C for 1 h to afford the *N*-nitroso (*R*)-sulfoxide **9**. Subsequent removal of the nitroso group by hydrogenation in the presence of 10% Pd-C in ethyl acetate gave the desired 1R-sulfoxide **10** in 90% yield. When the de-nitrosation was attempted by the treatment of compound **9** with zinc and glacial acetic acid in dichloromethane, de-esterification occurred concurrently which was not desired at this stage. The 1R-sulfoxide **10** was converted into the more stable 1S-sulfoxide **11** by a thermal epimerization process in very dilute solution. During this process, a rotation about the C2–C3 bond takes place and the substituent on the 2β-methyl group is transferred to the 2α-methyl position (*eg* **10–11**). The success of this transformation is dependent upon the proper orientation of the sulfoxide and the C2-substituted methyl group. Since a stereospecific opening of the thiazolidine ring takes place by a *cis* (2, 3) sigmatropic reaction, the sulfoxide and the unsubstituted methyl group at the C2 position must be *cis*-oriented (*ie* both should be in α-orientation) to give the desired sulfenic acid. The sulfenic acid forms an intramolecular hydrogen bond with the NH group of 6β-amido side chain forcing the recyclization from the β-face of the molecule resulting in a stereochemically controlled transformation. The 2α-chloromethyl penam 1S-sulfoxide **11** was then deoxygenated by the use of PBr_3 [15] as reducing agent with afforded a clean product **12**.

The 2β and 2α-chloromethyl penam esters **2** and **12** were then substituted with various thiolates to obtain the target compounds **4** and **14**. The substitution of 2β-chlorine and 2α-chlorine of **2** and **12** by nucleophiles like mercapto-1-methyl-1,2,3,4-tetrazole-5-yl and mercapto-2-methyl-1,3,4-thiadiazole-5-yl seems to follow different kinetic profile as each reaction proceeded to yield different end products. The 2-substituted methyl penams **4a**, **4b**, **14a**, and **14b** were obtained in 25, 4.5, 52, and 17% yields respectively, which show that the substitution of both the 2β and 2α-chlorine by mercaptotetrazole was more efficient than by the mercaptothiadiazole, and substitution of 2α-chlorine was more efficient than the 2β-chlorine by both the nucleophiles. It is therefore evident that the 2 isomeric halopenams (**2** and **12**) react differently under the same reaction condition. The difference could be explained by taking a closer look at the mechanisms of substitution reactions of 2β-chloromethyl penam (scheme 1) and 2α-chloromethyl penam (scheme 2) which proceed through different intermediates. In case of the 2β-isomer, the reaction proceeds through the episulfonium ion **3** which gives rise to 3 different products: **4**, **5**, and **6** along with other decomposed products of which only **4** was of interest for our studies. In the case of 2α-isomer, reaction proceeds through a different intermediate **13** which affords the major product **14** along with starting material and some decomposed products. Either of the 2 possible mechanisms: i), participation of the ester group in the stabilization of the carbonium ion; or ii), abnormal type SN1 substitution as suggested by Kukolja *et al* [13] might be the reason for single product formation.

The esters of the 2β and 2α-substituted methyl penams **4** and **14** were then de-esterified by reaction with Zn/AcOH in DMF to obtain the corresponding acids in quantitative yields. The compound **14a** in dichloromethane at 0°C was oxidized by one equivalent of *m*-chloroperbenzoic acid (*m*-CPBA) and the sulfoxide **16a** thus obtained was de-esterified by the same method. The corresponding acids of **4a** and **b**, **14a** and **b** and **16a** were then converted into their sodium salts by treatment with sodium 2-ethylhexanoate (schemes 1 and 2).



Scheme 1. Synthesis of 2β-substituted methyl penams.



Scheme 2. Synthesis of 2α-substituted methyl penams.

Results and discussion

The minimum inhibitory concentrations (MICs) for compounds **7a**, **7b**, **15a**, **15b** and **17a** as compared to penicillin-V (PCV), cefamadole (CMD) and cefoperazone (CPZ) are given in table I. It is clear from table I that 2-substituted methyl penams are less active than the unsubstituted penam and the 3-substituted cephems have much broader spectrum of antibacterial activity as compared to the 2-substituted penams. We have reported earlier that the oxidized products of the 6β-substituted penams have much reduced activity against Gram-positive microorganisms and no activity against Gram-negative microorganisms [6]. A similar result was found with the oxidized product of the 2α-substituted penam **17a** which is reported in table I. However, there is a considerable difference in the antimicrobial activity of 2α and 2β-substituted penicillins. The 2α-substituted penicillins (**15a** and **15b**) have marginally inferior (0–1 dilution factor) antimicrobial activity to the unsubstituted penicillin (PCV) and generally they are slightly (1–2 dilution factor) more active than that 2β-substituted penicillins (**7a** and **7b**) with the exception of *Staphylococcus aureus* 54K and *Streptococcus faecalis* against which 2β-isomer is more active. The difference in activity is more noticeable against *Providencia rettgeri* and *Proteus vulgaris* where 2α-substituted penicillins are significantly more active than their 2β-counterparts (2–5 dilution factor). The activity of 2α and 2β-isomers, when tested at higher concentrations (500, 250, and 100 μg/ml) against Gram-negative bacteria (see table I, MIC > 100), the end points (MICs) for 2α-isomers where within 250–500 μg whereas for 2β-isomers it went beyond 500 μg/ml concentration (data not included in table I).

The substitutions present at the C3-position of cephalosporins help in the improvement of antibacterial property by making the β-lactam ring more susceptible to cleavage and attack by a nucleophile (penicillin-sensitive enzymes, PSEs/PBPs) but such an effect is not possible in the case of C2-substituted penicillins [16]. The substitution at C2-methyl of penam system hinders the attack by PSEs on the β-lactam ring which results in the reduced antibacterial activity. The steric hindrance is expected to be more prominent in the case of 2β-substituted derivatives than that of 2α-counterparts because the 6β-substituent and 2β-substituent would probably fold over the β-lactam ring, whereas the 2α-substituent would tend to stay away in the α-orientation, exerting less protective action on β-lactam ring. It may not be the only reason for 2-substituted penams being less active than unsubstituted penam and of 2α-isomer being more active than 2β-isomer, as there are several other factors (*eg*, permeability through the bacterial cell wall, β-lactamase stability, affinity towards PBPs, vulnerability of the β-lactam ring, etc) which play equally important roles in determining the antibacterial activity of a compound.

Experimental protocols

Melting points were determined on a Electrothermal digital melting point apparatus and are uncorrected. The ^1H NMR spectra (δ ppm) were obtained in $CDCl_3$ (for esters) or D_2O (for salts) with tetramethylsilane as an internal standard on a Bruker AM-300 spectrometer. Infrared spectra were obtained as thin potassium bromide pellet on a Nicolet DXFT-IR spectrophotometer. Analytical results for compounds followed by elemental symbols were ± 0.4 of calculated values and were determined on a Perkin–Elmer-240 elemental analyzer. Chemical purities of synthesized compounds were tested with thin-layer chromatography. Mass spectroscopic analyses (FAB) were performed on Krotos MS-9 coupled to a Data General NOVA-4 computer for the molecular ion peaks. The proposed structures agree with the spectroscopic analyses.

The *in vitro* antibacterial activity of the final compounds was determined by the standard agar plate dilution method recommended by the National Committee for Clinical Laboratory standards [17] using unsupplemented Mueller–Hinton agar (Difco) medium. All the reference antibiotics were

Table I. *In vitro* antibacterial activity of 2-heteroarylthiomethyl phenoxymethyl penicillin as compared to known penam and cephems (MIC, μg/ml).

Test organisms ATCC/clinical	***7a***	***15a***	***17a***	***7b***	***15b***	*PCV*	*CMD*	*CPZ*
S aureus 29213	0.20	0.10	12.5	0.20	0.05	0.05	0.20	1.56
S aureus 209P	0.10	0.05	6.25	–	–	0.025	–	–
S aureus 54K	6.25	1.56	12.5	–	–	1.56	–	–
S aureus 123K	0.20	0.10	6.25	–	–	0.05	–	–
S aureus 66K	1.56	0.78	50	–	–	0.78	–	–
S aureus 86K	> 100	100	100	–	–	25	–	–
S aureus JHH M241	100	50	100	–	–	25	–	–
S epidermidis 12228	0.40	0.20	25	0.40	0.10	0.10	0.20	0.10
Str faecalis 29212	1.56	3.12	50	0.78	3.12	1.56	0.78	1.56
M luteus 9341	≤ 0.05	≤ 0.05	12.5	≤ 0.05	≤ 0.05	≤ 0.05	0.05	0.05
B subtilis 6633	0.05	0.025	6.25	0.05	0.025	0.012	1.56	0.40
B cereus 14579	25	12.5	> 100	25	12.5	6.25	6.25	3.12
E coli 25922	> 100	> 100	> 100	> 100	> 100	100	0.40	0.10
E coli (β-lact + ve)	> 100	> 100	> 100	> 100	> 100	> 100	0.40	0.10
Ent cloacae 23355	> 100	100	> 100	> 100	> 100	50	0.20	0.40
C freundii (clinical)	> 100	> 100	> 100	> 100	> 100	> 100	0.40	0.20
C diversus (clinical)	> 100	> 100	> 100	> 100	> 100	> 100	0.40	0.10
Pr mirabilis (clinical)	> 100	> 100	> 100	> 100	> 100	100	0.78	0.78
Pr vulgaris (clinical)	> 100	6.25	> 100	> 100	12.5	3.12	0.10	0.10
Pv rettgeri 29944	> 100	6.25	> 100	> 100	6.25	3.12	0.10	0.20
Pv rettgeri (clinical)	> 100	50	> 100	> 100	50	50	0.20	0.40
Pv rettgeri NIH 96	> 100	6.25	> 100	> 100	6.25	1.56	0.10	0.20
Ps aeruginosa 27853	> 100	> 100	> 100	> 100	> 100	> 100	> 100	1.56
Se marcescens 8100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	3.12

S: *Staphylococcus*, *Str*: *Streptococcus*, *M*: *Micrococcus*, *B*: *Bacillus*, *E*: *Escherichia*, *Ent*: *Enterobacter*, *C*: *Citrobacter*, *Pr*: *Proteus*, *Pv*: *Providencia*, *Ps*: *Pseudomonas*, *Se*: *Serratia*, PCV: Penicillin-V-K, CMD: Cefamandole -Na, CPZ: Cefroperazone Na, –: not determined.

purchased from Sigma (USA). The Cathra multipoint inoculator (MCT Medical Inc, St Paul, USA) was used to dispense ≈ 10^4 cfu/spot of the test organism onto the agar plate. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of the antibiotic that prevented visible growth after 18 h of incubation at 35°C disregarding the appearance of a single colony or very hazy growth.

Trichloroethyl 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-2α-methyl-6β-(phenoxyacetamido)-penam-3α-carboxylate ***4a***
To a stirred solution of trichloroethyl 2β-(chloromethyl)-2α-methyl-6β-(phenoxyacetamido)-penam-3α-carboxylate **2** (5.0 g, 0.0097 mol) in acetone (20 ml) was added a solution of 5-mercapto-1-methyl-1,2,3,4-tetrazole (1.2 g, 0.01067 mol) made up in 0.1 M sodium phosphate buffer pH 6.8 (8 ml) containing $NaHCO_3$ (0.897 g, 0.01067 mol). The mixture was refluxed at 60°C for 1 h and then further stirred for 24 h at room temperature. Acetone was evaporated *in vacuo* and the residue was extracted with ethyl acetate (100 ml). The ethyl acetate layer was washed successively with water, saturated $NaCHO_3$ solution, saturated brine, and made anhydrous over Na_2SO_4. Solvent was removed *in vacuo* to obtain a dark brown foam (5.2 g) which was purified by silica gel column chromatography using methylene chloride: ethyl acetate (90:10) as eluant. After the concentration to dryness the desired product was obtained as a white foam (1.48 g). Yield: 25.6%, ^{1}H NMR ($CDCl_3$): 1.68 (s, 3H), 3.88 (s, 3H), 3.82 and 3.97 (ABq, 2H, J = 13.2 Hz), 4.64 and 4.68 (ABq, 2H, J = 15.4 Hz), 4.82 and 4.88 (ABq, 2H, J = 12.1 Hz), 5.0 (s, 1H), 5.76 (d, 1H, J = 4.4 Hz), 5.88 (dd, 1H, J_1 = 8.8 and J_2 = 4.4 Hz), 7.00 (m, 3H), 7.34 (t, 2H), 7.98 (d, 1H, J = 8.8 Hz).

Sodium 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl] 2α-methyl-6β-(phenoxyacetamido-penam-3α-carboxylate ***7a***
To a stirred and cooled (0°C) solution of **4a** (0.250 g, 0.0004 mol) in dry dimethylformamide (5 ml) was added zinc dust (0.6 g) followed by glacial acetic acid (2 ml), and the mixture was stirred for 1 h and 15 min. The content was diluted with ethyl acetate (50 ml) filtered through a packed bed of celite and the filter bed further washed with ethyl acetate (15 ml). The filtrate was washed with saturated brine (10 x 50 ml), ethyl acetate layer dried over Na_2SO_4 and then evaporated to a white foam *in vacuo* to obtain the acid of **6a** (0.148 g, yield 76%). The acid thus obtained was dissolved in ethyl acetate (3 ml) and a sufficient amount of sodium 2-ethyl-hexanoate solution in ethanol was added to bring the pH of the solution to 7.0. The resulting solution was diluted with sufficient quantity of ether–hexane (2:1) mixture to precipitate the salt out which was then filtered and dried (0.123 g). mp = 173°C, yield: 83%, ^{1}H NMR (D_2O): 1.60 (s, 3H), 3.70 (s, 2H), 3.90 (s, 3H), 4.56 (s, 1H), 4.70 (bs, 2H), 5.50 (d, 1H, J = 4.15 Hz), 5.68 (d, 1H, J = 4.15 Hz), 7.02 (m, 3H), 7.36 (t, 2H). Anal $C_{18}H_{19}N_6O_5S_2Na$ (C, H, N). MS (FAB): M^+ 487 calcd 486.5.

*Trichloroethyl 2β-[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]-2α-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate **4b***
This was prepared by the same method as described for **4a** using **2** (5 g, 0.0097 mol) in acetone (25 ml) and 5-mercapto 2-methyl-1,3,4-thiadiazole (1.41 g, 0.0107 mol) in 0.1 M sodium phosphate buffer pH 6.8 (10 ml) containing $NaHCO_3$ (0.897 g, 0.0107 mol). A dark brown foam (5.6 g) obtained as crude product was purified by silica gel column chromatography using ethyl acetate (5–30%) in hexane as eluant. The desired product in pure form was obtained as a white foam (0.265 g, yield: 4.46%) by combining and evaporating the fractions 35–38. ^1H NMR ($CDCl_3$): 1.70 (s, 3H), 2.60 (s, 3H), 3.78 and 4.03 (ABQ, 2H, J = 13.5 Hz), 4.63 (s, 2H), 4.83 (s, 2H), 4.97 (s, 1H), 5.66–5.9 (m, 2H), 6.96 (m, 3H), 7.33 (t, 2H), 7.7 (d, 1H, J = 9 Hz).

*Sodium 2β-[(2-methyl-1,3,4-thiadiazol-5-yl)-thiomethyl]2α-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate **7b***
This was prepared by the same method as described for **7a** using **4b** (0.200 g, 0.000326 mol) in dry dimethylformamide (3 ml)–glacial acetic acid (1 ml) mixture and zinc dust (0.400 g). The corresponding acid (0.138 g, yield 87.86%) was converted into sodium salt (0.115 g), mp = 176°C, yield: 83.3%, ^1H NMR (D_2O: 1.59 (s, 3H), 2.58 (s, 3H), 3.62 and 3.68 (ABq, 2H, J = 13.0 Hz), 4.56 (s, 1H), 4.68 (bs, 2H), 5.50 (d, 1H, J = 4.03 Hz), 5.65 (d, 1H, J = 4 Hz), 7.00 (m, 3H), 7.34 (t, 2H). Anal $C_{19}H_{19}N_4O_5S_3Na$ (C, H, N). MS (FAB): M^+ 503 calcd 502.6.

*Trichloroethyl 2β-(chloromethyl)-2α-methyl-6β-(N-nitroso-phenoxyacetamido)penam-3α-carboxylate **8***
To a stirred solution of **2** (24 g, 0.0466 mol) in dry methylene chloride (200 ml) was added 14 g (0.171 mol) of anhydrous sodium acetate, and the mixture was cooled to 0°C in an ice bath. Dinitrogen tetroxide (25 ml) was added in small portions within 2 min and the mixture was stirred at 0°C. After 30 min an additional 5-ml portion of dinitrogen tetroxide was added in 1 portion and stirring was continued at 0°C for an additional 30 min. The mixture was diluted with 150 ml of methylene chloride and the remaining excess of dinitrogen tetroxide was destroyed by the addition of 5 N aqueous $NaHCO_3$ solution. The methylene chloride layer was separated, washed with water followed by saturated brine, dried over Na_2SO_4, and evaporated *in vacuo*. The yellow crystalline solid (22.4 g) obtained upon recrystallization from methylene chloride and hexane gave a pure product (19.5 g). mp = 112°C, yield: 88.4%. ^1H NMR ($CDCl_3$): 1.68 (s, 3H), 3.60 and 4.26 (ABq, 2H, J = 12 Hz), 4.83 (s, 2H), 5.20 (s, 1H), 5.53 (s, 2H), 5.63 (d, 1H, J = 3 Hz), 5.81 (d, 1H, J = 3 Hz), 7.10 (m, 3H), 7.36 (m, 2H). IR (KBr): 3460, 2950, 2004, 1763, 1587, 1489, 1303, 1264, 1201 cm^{-1}. Anal $C_{18}H_{17}N_3O_5SCl_4$ (C, H, N). MS (FAB): M^+ 517 calcd 516.23.

*Trichloroethyl 2β-(chloromethyl)-2α-methyl-6β-(N-nitroso-phenoxyacetamido)penam-3α-carboxylate 1R-oxide **9***
To an ice-cooled (0°C) solution of **8** (19.0 g, 0.0349 mol) in dry methylene chloride (200 ml), was added 7.087 g (0.0349 mol) of *m*-chloroperbenzoic acid in small portions over 5 min and the reaction mixture was stirred for 1 h. The precipitated solid was filtered off, and the filtrate was washed with aqueous $NaHCO_3$ (3 x 150 ml), water and saturated brine, then dried over Na_2SO_4 and evaporated to dryness to obtain the desired compound **9** (19.3 g). mp = 79°C, yield: 98.7%. ^1H NMR ($CDCl_3$): 1.40 (s, 3H), 4.01 and 4.20 (ABq, 2H, J = 12 Hz), 4.70 and 5.00 (ABq, 2H, J = 12 Hz), 4.73 (d, 1H, J = 4.5 Hz), 5.06 (s, 1H), 5.56 (s, 2H), 6.16 (d, 1H, J = 4.5 Hz), 7.03 (m, 3H), 7.36 (m, 2H). IR (KBr): 3360, 3065, 1795, 1774, 1691, 1525, 1493, 1235, 1186 cm^{-1}.

*Trichloroethyl 2β-(chloromethyl)-2α-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate 1R-oxide **10***
To a solution of **9** (14.12 g, 0.025 mol) in dry ethyl acetate (60 ml), 10% Pd-C (11.0 g) was added. Hydrogenation of the above mixture was carried out under a constant H_2 pressure (0.422 kg/cm^2) for 90 min. The reaction mixture was filtered through a packed bed of celite and the filtrate was evaporated *in vacuo* to a foam (12.1 g). mp = 77°C, yield: 90%. ^1H NMR ($CDCl_3$): 1.53 (s, 3H), 4.06 and 4.16 (ABq, 2H, J = 12.4 Hz), 4.60 (s, 2H), 4.73 and 5.00 (ABq, 2H, J = 12 Hz), 4.83 (d, 1H, J = 4.5 Hz), 5.0 (s, 1H), 5.47 (dd, 1H, J_1 = 8.0 Hz, J_2 = 4.5 Hz), 7.0 (m, 3H), 7.43 (m, 3H). IR (KBr): 3360, 3060, 1797, 1750, 1690, 1587, 1523, 1492, 1430, 1367, 1238 cm^{-1}. Anal $C_{18}H_{18}N_2O_6SCl_4$ (C, H, N). MS (FAB): M^+ 533 calcd 532.23.

*Trichloroethyl 2α-(chloromethyl)-2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate 1S-oxide **11***
Compound **10** (4.0 g) dissolved in dry toluene (4 ml) was added to dry toluene (8 l) well stirred and preheated to refluxing temperature. Heating was stopped after 20 min and stirring was continued until the temperature came down to ≈ 45°C. Toluene was then removed under reduced pressure and the residue was collected. The same thermal epimerization procedure was repeated twice with an additional 8 g of compound **10**. The residue from all 3 batches was combined by dissolving in methylene chloride, and an excess of hexane was added to the above solution to precipitate the desired compound. The precipitate was then redissolved in methylene chloride and evaporated *in vacuo* to obtain a light brown foam (10.45 g) which was purified by silica gel column chromatography using methylene chloride–ethyl acetate (90:10) as eluant. After concentration to dryness product **11** was isolated as white foam (5.6 g). mp = 76°C, yield 46.7%. ^1H NMR ($CDCl_3$): 1.92 (s, 3H), 3.78 and 3.86 (ABq, 2H, J = 12 Hz), 4.56 (s, 2H), 4.52 and 5.0 (ABq, 2H, J = 12 Hz), 4.88 (s, 1H), 5.24 (d, 1H, J = 4.2 Hz), 6.22 (dd, 1H, J_1 = 10.10 and J_2 = 4.2 Hz), 7.00 (m, 3H), 7.36 (m, 2H), 8.22 (d, 1H, J = 10.1 Hz). IR (KBr): 3360, 3000, 1804, 1779, 1692, 1586, 1525, 1505, 1200, 1232 cm^{-1}. Anal $C_{18}H_{18}N_2O_6SCl_4$ (C, H, N). MS (FAB): M^+ 533 calcd 532.23.

*Trichloroethyl 2α-(chloromethyl)-2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate **12***
To a stirred and cooled (0°C) solution of **11** (4.25 g, 0.0008 mol) in dry dimethylformamide (175 ml) under nitrogen atmosphere was added phosphorus tribromide (17.32 g, 0.064 mol) dropwise over a period of 10 min. The above mixture was stirred at the same temperature for 1 h and 45 min. The reaction mixture was then slowly poured over chilled $NaHCO_3$ aqueous solution (10% w/v, 500 ml). The neutralized mixture was then extracted with ethyl acetate (4 x 50 ml), ethyl acetate fractions were combined and washed with cold water (2 x 50 ml) followed by saturated brine (10 x 50 ml). The ethyl acetate layer was separated and dried over Na_2SO_4 and then evaporated to yield a white foam of crude product (3.65 g). The crude product was purified on silica gel column using hexane–ethyl acetate (80:20) as eluant. After concentration to dryness product **12** was obtained as a white foam (1.25 g, yield: 30.3%). The last few fractions (30–40) were combined and evaporated to obtain the starting material **11** (0.47 g). ^1H NMR ($CDCl_3$): 1.78 (s, 3H), 3.87 and 3.98 (ABq, 2H, J = 11.2 Hz), 4.6 (bs, 2H), 4.76 (s, 1H), 4.94 (bs, 2H), 5.7 (d, 1H, J = 4.0 Hz), 5.84 (dd, 1H, J_1 = 10.5 and J_2 = 4.0 Hz), 7.05 (m,

3H), 7.4 (m, 3H). Anal $C_{18}H_{18}N_2O_5SCl_4$ (C, H, N). MS (FAB): M^+ 517 calcd 516.23.

Trichloroethyl 2α-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate ***14a***
To a stirred solution of **12** (1.0 g, 0.00194 mol) in acetone (12 ml) was added the solution of 5-mercapto-1-methyl-1,2,3,4-tetrazole (0.281 g, 0.00242 mol) made up in 0.1 M sodium phosphate buffer pH 6.8 (5 ml) containing additional $NaHCO_3$ (0.2033 g, 0.00242 mol), the mixture was refluxed at 60°C for 1 h and then further stirred for 24 h at room temperature. Acetone was then evaporated *in vacuo* and the residue was extracted with ethyl acetate (25 ml). The ethyl acetate layer was washed successively with water, saturated $NaHCO_3$ aqueous solution, saturated brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a foam of crude product (1.1 g) which was purified by silica gel column chromatography using methylene chloride–ethyl acetate (90:10) as eluant. After concentration to dryness compound **14a** (single tlc spot) was obtained as a white foam (0.60 g). mp = 75°C, yield: 52%. ^1H NMR ($CDCl_3$): 1.8 (s, 3H), 3.85 and 4.06 (ABq, 2H, J = 13.1 Hz), 3.96 (s, 3H), 4.54 and 4.62 (ABq, 2H, J = 15.0 Hz), 4.78 (s, 1H), 4.82 and 4.90 (ABq, 2H, J = 11.3 Hz), 5.72 (d, 1H, J = 4.0 Hz), 5.85 (dd, 1H, J_1 = 9.0 and J_2 = 4.0 Hz), 7.0 (m, 3H), 7.35 (m, 2H). IR (KBr): 3375, 3040, 1792, 1765, 1690, 1586, 1521, 1491, 1435, 1204, 1233, 1170 cm^{-1}. Anal $C_{20}H_{21}N_6O_5S_2Cl_3$ (C, H, N). MS (FAB): M^+ 597 calcd 595.9.

Sodium 2α-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate ***15a***
To a stirred and cooled (0°C) solution of **14a** (0.220 g, 0.3698 mmol) in dry dimethylformamide (4 ml) was added zinc dust (0.6 g) followed by glacial acetic acid (1.5 ml), and the mixture was stirred for 2 h. The content was diluted with ethyl acetate (50 ml), filtered through a packed bed of celite and the filter bed further washed with ethyl acetate (15 ml). The filtrate was washed with saturated brine (10 x 50 ml), ethyl acetate layer dried over Na_2SO_4 and then evaporated to a white foam *in vacuo* to obtain the acid of **13a** (0.16 g, yield: 93.3%). The acid thus obtained was dissolved in ethyl acetate (3 ml) and a sufficient amount of sodium 2-ethylhexanoate solution in ethanol was added to bring the pH of the solution to 7.0. The resulting solution was diluted with sufficient quantity of ether–hexane (2:1) mixture to precipitate out the salt which was then filtered and dried (0.135 g). mp = 186°C, yield: 80.4%. ^1H NMR (D_2O): 1.36 (s, 3H), 3.44 and 3.68 (ABq, 2H, J = 14.2 Hz), 3.82 (s, 3H), 4.22 (s, 1H), 4.56 (bs, 2H), 5.29 (d, 1H, J = 4.6 Hz), 5.32 (d, 1H, J = 4.5 Hz), 6.88 (m, 3H), 7.20 (m, 2H). Anal $C_{18}H_{19}N_6O_5S_2Na$ (C, H, N). MS (FAB): M^+ 487 calcd 486.5.

Trichloroethyl 2α-[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]-2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate ***14b***
To a stirred solution of **12** (0.48 g, 0.00093 mol) in acetone (5 ml) was added to the solution of 5-mercapto-2-methyl-1,3,4-thiadiazole (0.154 g, 0.00116 mol) made up in 0.1 M sodium phosphate buffer pH 6.8 (3 ml) containing additional $NaHCO_3$ (0.097 g, 0.00116 mol). The mixture was refluxed at 60°C for 1 h and then further stirred for 24 h at room temperature. A crude product (0.43 g) obtained by the same workup procedure as described for compound **14a**, was further purified by silica gel column chromatography using hexane–ethyl acetate (70:30) as solvent to obtain the desired product **14b** (0.095 g, yield: 16.96%). ^1H NMR ($CDCl_3$): 1.78 (s, 3H), 2.74 (s, 3H), 3.86 and 4.04 (ABq, 2H, J = 13 Hz), 4.57 (ABq, 2H, J = 16 Hz), 4.76 (s, 1H), 4.86 (ABq, 2H, J = 12 Hz), 5.86 (d, 1H, J = 4 Hz), 5.84 (dd, 1H, J_1 = 8.0 and J_2 = 4.0 Hz), 6.94 (m, 3H), 7.4 (m, 2H).

Sodium 2α-[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate ***15b***
This was prepared by the same method as described for **15a** using **14b** (0.095 g, 0.155 mol) in dry dimethyl formamide (1.5 ml) glacial acetic acid (0.5 ml) mixture and zinc dust (0.200 g). The corresponding acid (0.065 g, yield 87%) was converted into sodium salt (0.055 g, mp = 155°C, yield 81%) by the method described earlier. ^1H NMR (D_2O): 1.52 (s, 3H), 2.68 (s, 3H), 3.57 and 3.76 (ABq, 2H, J = 14 Hz), 4.40 (s, 1H), 4.70 (bs, 2H), 5.50 (bs, 2H), 7.00 (m, 3H), 7.34 (m, 2H). IR (KBr): 3394, 2975, 2926, 1778, 1696, 1622, 1245, 1105, 941 908 cm^{-1}. Anal $C_{19}H_{19}N_4O_5S_3Na$ (C, H, N). MS (FAB): M^+ 503. Calcd 502.6.

Trichloroethyl 2α-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-2β-methyl-6β-(phenoxyacetamido)penam-3α-carboxylate 1β-oxide ***16a***
To an ice cooled (0°C) solution of **14a** (0.35 g, 0.00059 mol) in dry methylene chloride (10 ml), 0.12 g (0.00059 mol) of *m*-chloroperbenzoic acid was added and the reaction mixture was stirred for 1 h. The precipitated solid was filtered off, and the filtrate was washed with aqueous $NaHCO_3$, water and saturated brine, and then dried over Na_2SO_4 and evaporation of the solvent *in vacuo* gave the crude product which was purified by silica gel column chromatography using hexane–ethyl acetate (50:50) as eluant. Upon evaporation to dryness the desired product **16a** was obtained as white foam (0.310 g, yield: 86%). ^1H NMR ($CDCl_3$): 1.93 (s, 3H), 3.54 and 3.93 (ABq, 2H, J = 15 Hz), 3.94 (s, 3H), 4.54 (s, 2H), 4.74 and 5.06 (ABq, 2H, J = 12 Hz), 4.92 (s, 1H), 5.83 (d, 1H, J = 5.2 Hz), 6.22 (dd, 1H, J_1 = 10.5 and J_2 = 5.2 Hz), 7.00 (m, 3H), 7.30 (t, 2H), 8.20 (d, 1H, J_1 = 10.5 Hz). IR (KBr): 3360, 3030, 1801, 1765, 1690, 1508, 1486, 1435, 1373, 1278, 1223, 1180, 1051 cm^{-1}. Anal $C_{20}H_{21}N_6O_6S_2Cl_3$ (C, H, N).

Sodium 2α-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-2β-methyl-6β-(phenoxyacetamidopenam-3α-carboxylate 1β-oxide ***17a***
This was prepared by the same method as described for the compound **15a** in 2 steps starting from compound **16a**. mp = 138°C, yield: 69%. ^1H NMR (D_2O): 1.78 (s, 3H), 3.62 and 3.78 (ABq, 2H, J = 14.6 Hz), 4.54 (s, 1H), 4.72 and 4.78 (ABq, 2H, J = 14 Hz), 5.74 (d, 1H, J = 4.2 Hz), 6.07 (d, 1H, J = 4.2 Hz), 7.02 (m, 3H), 7.36 (m, 2H). Anal $C_{18}H_{19}N_6O_6S_2Na$ (C, H, N). MS (FAB): M^+ 503 calcd 502.6.

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