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Synthesis and Human Leukocyte Elastase Inhibitory Evaluation of Phosphate Triesters and Acyl Phosphates of Penam Sulfides and Sulfones

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Abstract—The synthesis of 6,6-dibromo-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam sulfone (**3a**), 6α-chloro-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam sulfone (**3b**), benzyl 6α-(diphenyl-phosphate)oxypenicillanate sulfone (**4**) and 6,6-dibromo-3α-(methylphosphate)carbonyl-2,2-dimethylpenam sulfone (**12**) are reported. When tested as inhibitors of human leukocyte elastase, the compound **4** proved to be the most active.

Introduction

Mechanistic understanding of enzymatic processes is exerting an increasingly important influence on the design of enzyme inhibitors. Transition-state analogues which are among the most effective types of enzyme inhibitors arise from this approach.[1] The family of peptide-bond-cleaving hydrolases, the peptidases (=proteases, EC 3.4), are sub-grouped, according to the reactive group at the active site involved in catalysis. Serine proteinases[2] represent one of the four main classes of proteolytic enzymes, the other three being aspartyl proteinases, cysteine proteinases and metalloproteinases. The serine proteinase family includes many well-studied enzymes, such as elastases. Human leukocyte elastase (HLE, EC 3.4.21.37) is a serine proteinase found in the azurophilic granules of polymorphonuclear leukocytes.[3] Several β-lactam compounds have been found to accomplish HLE inactivation through acylation of the hydroxyl group in the enzyme's active site.[4,5]

Four co-ordinate tetrahedral phosphorous derivatives are known to be inhibitors of proteolytic enzymes by acting as phosphorylating agents of the serine hydroxyl group and thereby produce transition-state analogues of tetrahedral intermediates. Examples include inhibitors of serine proteinases such as human leukocyte elastase,[6] and active-site serine β-lactamases of classes A[7] and C.[8,9]

Some years ago, Pratt et al. demonstrated that the phosphonate monoesters monoanion and phosphonamidates of structure **1** (Fig. 1) (where L is a leaving group) inhibit active-site serine β-lactamases of classes A[7] and C,[8,9] by phosphorylation of the active-site serine hydroxyl group. Song and Kluger[10] reported the synthesis of benzylpenicillin methyl phosphate and found that this compound is a substrate and inactivator of *Escherichia coli* RTEM β-lactamase. These authors proposed that the compound acylate the ϵ-amino group of Lys-234 of the enzyme. More recently, Li and Pratt[11] have demonstrated that acyl phosph(on)ates monoanion of structure **2a**–**c** inhibits the class C β-lactamase of *Enterobacter cloacae* P99 and compound **2c** reacts with the class A TEM-β-lactamase to form an acyl enzyme. Clearly, the compounds **1** and **2a**–**c** have the potential to form both the acyl and phosphor(on)yl enzyme species.

What distinguishes HLE from active site serine β-lactamases (classes A, C, and D according to Ambler's classification) is the mechanism to effect peptide or β-lactam bond cleavage. From the point of view of their mechanism, an active-site serine β-lactamase is not an active-site serine proteinase.

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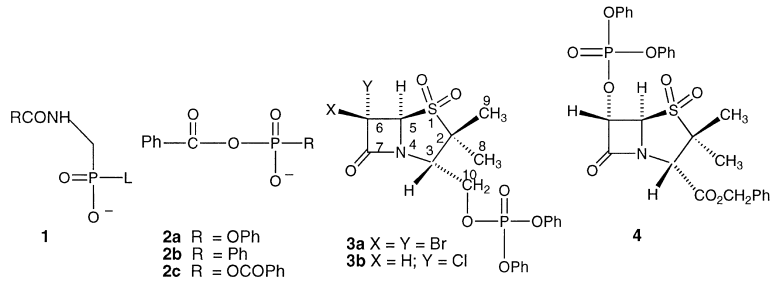


Figure 1.

We envisioned that phosphate triesters of the penam sulfides and sulfones as well as acyl-phosphates of penam sulfides and sulfones could potentially resemble the tetrahedral intermediate or the transition state for the formation and/or breakdown of the intermediate and, therefore, act as inhibitor of HLE as well as serine β-lactamases.

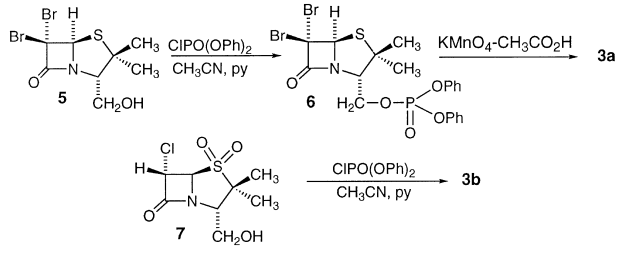
Results and Discussion

We describe here the synthesis of a series of prototypical phosphate triesters of penan sulfides (structures **6** and **9**) sulfones (structures **3a,b** and **4**) and acyl-phosphonate of penam sulfide (structure **11**) and their sulfone (structure **12**). These new phosphate triesters and acyl-phosphate of penam sulfides and sulfones were evaluated as inhibitors of human leukocyte elastase. This enzyme has been the subject of extensive studies, both in terms of its biological role in numerous diseases[12] and of the development of suitable therapeutic inhibitors to supplement the body's elastase inhibitory capacity and thereby shift the proposed proteinase/antiproteinase imbalance in pathogenic conditions.

Chemistry

Synthesis of penam and penicillanate sulfone phosphate triesters

The synthesis of 6,6-dibromo-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam sulfone (**3a**) is shown in Scheme 1. The starting materials was 6,6-dibromo 3α-hydroxymethyl-2,2-dimethylpenam (**5**).[13] Conversion of **5** into the 6,6-dibromo-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam (**6**) in 75% isolated yield was accomplished by phosphorylation with diphenylphosphochloridate[14] in acetonitrile in the presence of catalytic amount of pyridine, at room temperature. Subsequent oxidation of **6** with potassium permanganate in acetic acid afforded the corresponding sulfone **3a**. The 6α-chloro-3α-hydroxymethyl-2,2-dimethyl-penam sulfone (**7**)[15] was phosphorylated by the phosphochloridate methodology. The compound **3b** was obtained in 60% yield after purification by column chromatography.



Scheme 1.

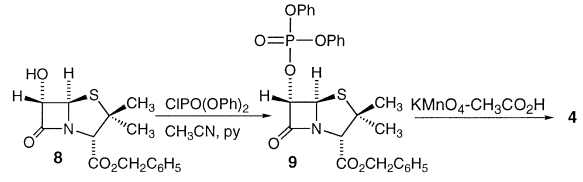
The synthesis of benzyl 6α-(diphenylphosphate) oxypenicillanate sulfone (**4**), was performed by phosphorylation of benzyl 6α-hydroxypenicillanate (**8**)[16] using the phosphochloridate methodology,[14] and subsequent oxidation (Scheme 2). The structure of compounds **3a,b** and **4** were ascertained by ^{31}P, ^{13}C, and ^{1}H NMR spectroscopy.

Synthesis of 6,6-dibromo-3α-(methylphosphate)carbonyl-2,2-dimethylpenam sulfone

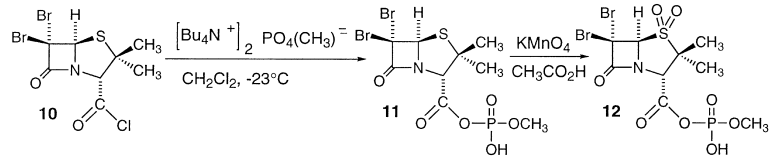
The starting material was the ready available 6,6-dibromo-3α-chlorocarbonyl-2,2-dimethylpenam (**10**), previously reported by us.[17] Conversion of the acid chloride **10** into its corresponding 6,6-dibromo-3α-(methylphosphate)carbonyl-2,2-dimethylpenam (**11**) was achieved with bis(tetrabutylammonium) methylphosphate using the methodology reported by Song and Kluger.[10] Oxidation of the penam sulfide **11** with potassium permanganate in acetic acid gave the corresponding sulfone (**12**) (Scheme 3). The compounds **11** and **12** were unstable at 30 °C under atmospheric conditions.

In vitro HLE inhibition

Activity was measured following the increase in absorbance at 410 nm due to the release of *p*-nitroaniline by hydrolysis of the substrate *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide. Measurements were made at 20 °C in 1 mL of assay medium containing 100 mM KH_2PO_4 (pH 7.0, at 20 °C), 0.05 IU of human leukocyte elastase and concentrations of substrate and inhibitors



Scheme 2.



Scheme 3.

as indicated. Time courses of product formation where tested for linearity and only the initial, linear parts where considered to calculate the rates. Inhibitors were dissolved in dimethyl sulfoxide (DMSO). In all cases, final concentration of DMSO in the incubation media was 2% in volume, to minimize inhibitory effects of this solvent.

We assayed six different compounds. Compound **4** is near 10-fold more potent for the inhibition of human elastase than compounds **3b**, **6**, and **3a**, and near 100-fold than compounds **11** and **12** (Table 1).

All the compounds inhibit through interaction with the substrate according to a simple competitive mechanism, with the following equation for steady-state activity:

$$v = \frac{V_{max}[S]}{[S] + K_M(1 + [I]/K_i)}$$

Where V_{max} is the activity at substrate concentration, [S], tending to infinity, K_M is the substrate concentration for half-maximal activity in the absence of inhibitor and K_i is the equilibrium constant for the dissociation of inhibitor, *I*, from the enzyme.

Since the mechanism of inhibition is due to the competition between the substrate *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide and the different compounds assayed, it seems reasonable that the molecule which is more likely to compete with the substrate is also the most potent. The comparison of compounds **6** and **3a**, **4** and **9** indicate that substitution in position **1** (sulfide and sulfone) is not relevant for the inhibition. Comparison among compounds **4** with **3b** and **3a**, indicates that substitution of the phosphate triester in position **6** with orientation α, for a chlorine or bromine atom modifies the potency of the inhibitor. Differences between the triester phosphate compound **3a** and the acyl-phosphate **12** significantly change the potency for the inhibition of HLE. The stability of all compounds was tested by measuring their spectra under identical conditions as during enzymatic determinations but omitting the addition of substrate. Spectra remained unchanged after 1 h of incubation.

Table 1. Values of $K_i = K_M[I]/[([S] + K_M)\ (v_o/v_i - 1)]$, were obtained by measuring activity either in the absence (v_o) or in the presence (v_i) of inhibitors.[a]

Compound	$K_i \pm$SD (μM)
4	1.3±0.5
9	1.5±0.5
3b	11.0±2
6	12.0±3
3a	14.0±2
12	79.0±25
11	142.0±30

Measurements of v_o versus [S] allowed to calculate a value of $K_M = 1.8 \pm 0{,}15$ mM

[a]Measurements of v_i were performed in media with either 3.125 μM (inhibitors **4**, **9**, **3b**, **6** and **3a**) or 125 μM (inhibitors **12** and **11**) of each inhibitor, for two concentrations of the substrate: 1.25 and 2.5 mM.

Conclusion

The structure–activity relationship studies around the phosphate triesters group at C-3α and C-6α positions as well as acyl-phosphate at C3α position of the penam nucleus as inhibitors of HLE have been studied. It will be interesting to find whether specifically more potent analogues at C-6α with better leaving groups at the phosphate esters and also of phosphate-penam monoesters can be obtained by structural variation.

Experimental

Proton and carbon magnetic resonance spectra (^{1}H and ^{13}C NMR) were taken on a Bruker AC 200 spectrometer operating at 200 and 50.0 MHz, respectively, with samples referenced to internal tetramethylsilane ($CDCl_3$ solutions). Phosphorous magnetic resonance spectra (^{31}P NMR) were recorded on a Bruker 250 MHz Fourier-transform instrument operating at 101.25 MHz with samples referenced to external (capillary) 85% H_3PO_4. Mass spectra were obtained from the Mass Spectrometry Facility at Ohio State University Chemical Instrument Center. Infra-red spectra (IR) were recordedon a Bruker IFS 25 FT-IR spectrometer. Melting point 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} precoated aluminium sheets (Merck); column chromatography was performed on silica gel grade 60 (Merck).

Human Leukocyte Elastase was purchased from Sigma Chemical Co. 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 dimethyl sulfoxide (DMSO). Enzyme activity was assayed in potassium phosphate 100 mM, pH 7 (final volume 1 mL). The reaction was started by addition of 10–30 μL of the enzyme stock solution. The release of *p*-nitroaniline was followed spectrophotometrically at 410 nm in a Beckman spectrophotometer DU 640 (30 °C). The inhibitors were dissolved in DMSO. Control experiments were run in all the cases with equal amounts of DMSO.

6,6-Dibromo-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam (6). Diphenylphosphochloridate (0.31 mL, 1.48 mmol) was added very slowly to a solution of 170 mg, (0.49 mmol) of 6,6-dibromo 3α-hydroxymethyl-2,2-dimethylpenam (**5**) dissolved in a mixture of dry acetonitrile (1.5 mL) and pyridine (0.12 mL) at room temperature in a nitrogen atmosphere. After being stirred for 2 h, the mixture was diluted with chloroform (10 mL) and washed with water (2×5 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The products were purified by column chromatography (eluant: hexane/ethyl acetate 75:25) to give compound **6** as white crystals, mp 67.5–69.0 °C. ^{1}H NMR ($CDCl_3$) δ 1.41 (3H, s, α-CH_3), 1.51 (3H, s, β-CH_3), 4.18–4.20 (3H, m 10-H and 3-H), 5.49 (1H, s, 5-H), 7.19–7.39 (10H, m, Ar–H); ^{13}C NMR ($CDCl_3$) δ 24.14 (C-8), 33.49 (C-9), 58.94 (C-6), 62.91 (C-2), 64.79 (d, $J_{P,C}$ = 5.5 Hz, C-10), 67.18 (d, $J_{P,C}$ = 7.0 Hz, C-3), 78.57 (C-5), 119.84 (d, $J_{P,C}$ = 4.3 Hz, Ph C-2), 125.50 (Ph C-4), 129.78 (Ph C-3), 150.13 (d, $J_{P,C}$ = 7.4 Hz, Ph C-1) and 165.26 (C-7); ^{31}P NMR ($CDCl_3$) δ −12.7. EI mass spectrum: *m*/*z* (M^+) 579 (0.6%), 577 (1.1), 575 (0.6), 498 (20.6), 418 (8.7), 317 (5.2), 284 (66.7), 251 (100) and 170 (28.7). Found: M^+, 576.91453. calcd for $C_{20}H_{20}SO_5PN^{79}Br^{81}Br$: M^+, 576.91447.

6,6-Dibromo-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam sulfone (3a). To a solution of 6,6-dibromo-3α-(diphenylphosphate)oxymethyl-2,2-dimethyl penam **6** (143 mg. 0.20 mmol) in a mixture of acetic acid/water (62.5:37.5) (4.0 mL) was added powdered potassium permanganate (68 mg. 0.43 mmol) and the mixture was stirred for 10 min. The reaction mixture was then quenched with drops of H_2O_2 until disappearance of color, diluted with CH_2Cl_2 (5 mL) and water (5 mL). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (2×5 mL). The combined organic layers were washed with 5% aqueous sodium bicarbonate solution (2×10 mL) and water (1×6 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure to yield **3a** (118 mg, 98%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.38 and 1.41 (each 3H, s, α + β-CH_3), 4.13–4.39 (3H, m, 10-H and 3-H), 4.86 (1H, s, 5-H), 7.17–7.40 (10H, m, Ar–H); ^{13}C NMR ($CDCl_3$) δ 18.18 and 19.18 (C-8 and C-9), 44.02 (C-6), 60.92 (d, $J_{P,C}$ = 9.3 Hz, C-3), 64.17 (C-2), 64.99 (t, $J_{P,C}$ = 5.5 Hz, C-10), 73.32 (C-5), 119.80 (dd, $J_{P,C}$ = 4.5 Hz, Ph C-2), 125.70 (Ph C-4), 129.86 (Ph C-3), 150.02 (d, $J_{P,C}$ = 7.3 Hz, Ph C-1), 164.94 (C-7). ^{31}P NMR ($CDCl_3$) δ −13.17. EI mass spectrum: *m*/*z* (M^+) 612 (0.49), 610 (1.16), 608 (0.48), 464 (7.01), 389 (8.25), 318 (3.24), 251 (85.06), 250 (100), 216 (38.84), 214 (40.68), 170 (46.49). Found M^+, 608.92256. calcd for $C_{20}H_{20}SO_7PN^{79}Br^{81}Br$: M^+, 608.90429.

6α-Chloro-3α-(diphenyl-phosphate)oxymethyl-2,2-dimethyl penam sulfone (3b). According to the procedure previously described, the 6α-chloro-3α-hydroxymethyl-2,2-dimethyl-penam sulfone (**7**) was phosphorylated to afford the compound **3b** in 60% yield, as a white solid. The product was characterized by mp 77.0–79.0 °C. IR (film) γ_{max} 1808 (β-lactam), 1325 and 1186 cm^{-1} (sulfone). ^{1}H NMR ($CDCl_3$) δ 1.39 and 1.41 (each 3H, s, α + β-CH_3), 4.08–4.32 (3H, m, 10-H and 3-H), 4.47 (1H, d, *J* = 1.53 Hz, 6-H), 5.13 (1H, d, *J* = 1.53 Hz. 5-H), 7.18–7.41 (10H, m, Ar–H); ^{13}C NMR ($CDCl_3$) δ 18.55 and 18.82 (C-8 and C-9), 55.44 (C-6), 60.62 (d, $J_{P,C}$ = 4.7 Hz, C-3), 62.97 (C-2), 65.49 (d, $J_{P,C}$ = 5.3 Hz, C-10), 68.84 (C-5), 119.80 (dd, $J_{P,C}$ = 4.1 Hz, Ph C-2), 125.68 (Ph C-4), 129.84 (Ph C-3), 150.00 (d, $J_{P,C}$ = 8.5 Hz, Ph C-1), 166.67 (C-7). LR-MS (EI) *m*/*z* (M^+) 485.04 (0.20), 487.02 (0.14), 105.04 (100). HR-MS (ES). Found (M^+Na) 508.0362. Calcd for $C_{20}H_{21}SO_7PN^{35}Cl$ Na (M^+Na) 508.0363.

Benzyl 6α-(diphenylphosphate)oxypenicillanate (9). According to the procedure previously described, the benzyl 6α-hydroxypenicillanate (**8**)[16] was phosporylated to afford the compound 9 in 73% yield, as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, s, α-CH_3), 1.53 (3H, s, β-CH_3), 4.52 (1H, s, 3-H), 5.17 (2H, d, AB *J* = 1.53 Hz.-CH_2-Ph), 5.29 (1H, d, $J_{5,6H}$ = 1.37 Hz, 5-H) 5.35 (1H, dd, $J_{5,6H}$ = 1.37 Hz, $^2J_{P,H}$ = 10 Hz, 6-H), 7.19–7.38 (15H, m, Ar–H); ^{13}C NMR ($CDCl_3$) δ 25.18 (C-8), 33.70 (C-9), 64.05 (C-2), 67.31 (–*C*H_2–Ph), 69.33 (C-5), 69.40 (C-3), 85.52 (d, $J_{P,C}$ = 7.8 Hz, C-6), 119.90 (d, $J_{P,C}$ = 4.3 Hz,–O–Ph C-2), 125.64 (–O–Ph C-4), 128.44, 128.52, 134.45 (–CH_2–Ph–*C*–), 128.48 (–O–Ph C-3), 150.03 (d, $J_{P,C}$ = 8.5 Hz,–O–Ph C-1), 165.25 (d, $J_{P,C}$ = 8.6 Hz, C-7), 166.60 (–C–*C*O_2–CH_2–); ^{31}P NMR ($CDCl_3$) δ −14.48. Found M^+, 539.11923. Calcd for $C_{27}H_{26}SO_7PN$: M^+, 539.11659.

Benzyl 6α-(diphenylphosphate)oxypenicillanate sulfone (4). According to a similar procedure to that used for the synthesis of **3a**, compound **9** was converted to **4** (77% yield) as colorless oil. IR (film) γ_{max} 1804 (β-lactam), 1754 (ester) 1322, and 1127 cm^{-1} (sulfone). ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, s, α-CH_3), 1.51 (3H, s, β-CH_3), 4.42 (1H, s, 3-H), 4.65 (1H, d, $J_{5,6H}$ = 1.38 Hz, 5-H), 5.22 (2H, m.–CH_2–Ph), 5.75 (1H, $J_{5,6H}$ = 1.32 Hz, $^2J_{P,H}$ = 10.39 Hz, 6-H), 7.18–7.40 (15H, m, Ar–H); ^{13}C NMR ($CDCl_3$) δ 18.37 (C-8), 19.64 (C-9), 62.82 (C-3), 63.25 (C-2), 67.90 (C-5). 68.26 (C-10), 78.11 (d, $J_{P,C}$ = 7.7 Hz, C-6), 119.90 (d, $J_{P,C}$ = 4.0 Hz, Ph C-2), 125.89 (Ph C-4), 128.72, 128.96, 129.94, 134.11 (–CH_2–Ph *C*-), 128.75 (Ph C-3), 149.85 (d, $J_{P,C}$ = 8.0 Hz, Ph C-1), 164.78 (d, *J* = 8.0 Hz, C-7) and 165.75 (–C–*C*O_2–CH_2–).

6,6-Dibromo-3α-(methylphosphate)carbonyl-2,2-dimethylpenam (11). To a stirred suspension of 6,6-dibromopenicillanic acid (2.8 mmol) in a mixture of anhydrous benzene (8 mL) and anhyd DMF (0.15 mL, 1.9 mmol) at 20 °C, oxalyl chloride (1.29 mL, 3.3 mmol) was added dropwise while a slow stream of nitrogen was passed through the mixture. After 50 min the mixture was cooled at −23 °C, then a solution of bis(tetrabutylammonium) methylphosphate[10] (1.98 g, 3.33 mmol) in dry CH_2Cl_2 (16 mL) was added. The mixture was stirred at −23 °C for 3 h. It was then warmed to room temperature, and stirred 2 h. The CH_2Cl_2 phase was washed with H_2O, sat $CuSO_4$, H_2O, 5% $NaHCO_3$, H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The products were purified by column chromatography (eluant: $CHCl_3$/methanol 97:3) to give in 66% yield compound **11**

as white crystals. The product was characterized by mp 99.0–101.0 °C. IR (KBr) γ_{max} 1804 (β-lactam), and 1754 cm^{-1} (ester). ^1H NMR ($CDCl_3$) δ 1.46 (3H, s, α-CH_3), 1.62 (3H, s, β-CH_3), 3.73 (3H, s, –OCH_3), 4.55 (1H, s, 3-H), 5.80 (1H, s, 5-H); ^{13}C NMR ($CDCl_3$) δ 25.77 (C-8), 33.33 (C-9), 52.38 (–O*C*H_3), 56.49 (C-6), 64.45 (C-2), 69.55 (C-3), 80.56 (C-5), 164.34 (C-7), 166.97 (–C–*C*O_2–P–).

6,6-Dibromo-3α-(methylphosphate)carbonyl-2,2-dimethylpenam sulfone (12). According to a similar procedure to that used for the synthesis of **3a** compound **11** was converted to **12** (98% yield) as a white solid. Mp 181.0–183.0 °C. IR (KBr) γ_{max} 1808 (β-lactam), 1749 (acyl phosphate) 1325, and 1127 cm^{-1} (sulfone). ^1H NMR ($CDCl_3$) δ 1.41 (3H, s, α-CH_3), 1.62 (3H, s, β-CH_3), 3.85 (3H, s, –OCH_3), 4.52 (1H, s, 3-H), 5.01 (1H, s, 5-H); ^{13}C NMR ($CDCl_3$) δ 18.46 and 19.58 (C-8 and C-9), 43.33 (C-6), 53.19 (O-*C*H_3), 62.90 (C-3), 64.52 (C-2), 73.30 (C-5), 164.00 (C-7), and 165.84 (C–*C*O_2–P–).

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References and Notes

1. Fersht, A. *In Structure and Mechanism in Protein Science. A Guide to Enzyme Catalysis and Protein Folding*; W.H. Freeman: New York, 1999; pp 54–102.
2. Fersht, A. In *Structure and Mechanism in Protein Science. A Guide to Enzyme Catalysis and Protein Folding*; W.H. Freeman: New York, 1999; pp 26–30, 40–43, 473–482.
3. (a) For recent reviews see: Edwards, P. D.; Bernstein, P. R. *Med. Res. Rev*. **1994**, *14*, 127. (b) Bernstein, P. R.; Edwards, P. D.; Williams, J. C. In *Progress in Medicinal Chemistry;* Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science: Amsterdam, 1994; pp 61–120. (c) Zimmerman, M.; Powers, J. C. In *Elastin and Elastases*; Robert, L., Hornebeck, W., Eds.; CRC: Boca Raton, CA, 1989; Vol. II, pp 109–123. (d) Hlasta, D. J.; Pagani, E. D. *Annu. Rep. Med. Chem*. **1994**, *29*, 195.
4. For recent reviews on β-lactam compounds as inhibitors of elastases, and serine β-lactamases see: (a) Mascaretti, O. A.; Boschetti, C. E.; Danelon, G. O.; Mata, E. G.; Roveri, O. A. *Curr. Med. Chem*. **1995**, *1*, 441. (b) Mascaretti, O. A.; Danelon, G. O.; Setti, E. L.; Laborde, M.; Mata, E. G. *Curr. Pharm. Des*. **1999**, *5*, 939.
5. (a) For recent reports on β-lactam compounds as inhibitors of human leukocite elastase see: Vergely, I.; Boggeto, N.; Okochi, V.; Golpayegani, S.; Reboud-Ravaux, M.; Kobaiter, R.; Joyeau, R.; Wakselman, M. *Eur. J. Med. Chem*. **1995**, *30*, 199. (b) Alpegiani, M.; Bizzolino, P.; Corigli, R.; Rizzo, V.; Perrone, E. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 687. (c) Finke, P. E.; Shah, S. K.; Fletcher, D. S.; Ashe, B. M.; Brause, K. A.; Chandler, G. O.; Dellea, P. S.; Hand, K. M.; Maycock, A. L.; Osinga, D. G.; Underwood, D. J.; Weston, H.; Davies, P.; Doherty, J. B. *J. Med. Chem*. **1995**, *38*, 2449. (d) Green, B. G.; Chabin, R.; Mills, S.; Underwood, D. J.; Shah, S. K.; Kuo, D.; Gale, P.; Maycock, A. L.; Liesch, J.; Burgey, C. S.; Doherty, J. B.; Dorn, C. P.; Finke, P. E.; Hagmann, W. K.; Hale, J. J.; MacCoss, M.; Westler, W. M.; Knight, W. B. *Biochemistry* **1995**, *34*, 14331. (e) Maiti, S. M.; Czajkowski, D. P.; Reddy, N. A. V.; Spevak, P.; Kaleta, J.; Micetich, R. G. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 823. (f) Cvetovich, R. J.; Chartrain, M.; Hartner, F. W.; Roberge, C.; Amato, J. S.; Grabowski, E. J. J. *J. Org. Chem*. **1996**, *61*, 6575. (g) Buynak, J. D.; Rao, A. S.; Ford, G. P.; Carver, Ch.; Adam, G.; Geng, B.; Bachmann, B.; Shobassy, S.; Lackey, S. *J. Med. Chem*. **1997**, *40*, 3423. (h) Maiti, S. M.; Woods, D. E.; Cantin, A. M. *Drugs Future* **1998**, *23*, 635.
6. Durette, P. L.; Chabin, R. M.; Fletcher, D. S.; Green, B. G.; Hanlon, W. A.; Humes, J. L.; Knight, W. B.; Lanza, T. J.; Mumford, R. A.; Pacholok, S.; MacCoss, M. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 271.
7. (a) For the crystal structure of serine β-lactamase of *Staphylococcus aureus* inactivated by a methyl-phosphonate monoester monoanion inhibitor, see: Chen, C. C. H.; Rahil, J.; Pratt, R. F.; Herzberg, O. *J. Mol. Biol*. **1993**, *234*, 165. (b) For a previous report on phosphonate monoester inhibitors of class A serine-β-lactamase see: Rahil, J.; Pratt, R. F. *Biochem. J*. **1991**, *275*, 793.
8. (a) For inhibition of the class C serine-β-lactamase of *Enterobacter cloacae* P99 by phosphonate monoesters see: Pratt, R. F. *Science* **1989**, *246*, 917. (b) Rahil, J.; Pratt, R. F. *Biochemistry* **1992**, *31*, 5869. (c) Lobkovsky, E.; Billings, E. M.; Moews, P. C.; Rahil, J.; Pratt, R. F.; Knox, J. R. *Biochemistry* **1994**, *33*, 6762. (d) Dryjanski, M.; Pratt, R. F. *Biochemistry* **1995**, *34*, 3561. (e) Dryjanski, M.; Pratt, R. F. *Biochemistry* **1995**, *34*, 3569. (f) Li, N.; Rahil, J.; Wright, M.; Pratt, R. F. *Bioorg. Med. Chem*. **1997**, *5*, 1783.
9. (a) For inhibition of the class C serine-β-lactamase of *Enterobacter cloacae* P99 by phosphonoamidates see: Laws, A. P.; Page, M. I.; Slater, M. J. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 2317. (b) Rahil, J.; Pratt, R. F. *Biochemistry* **1993**, *32*, 10763. (c) Bateson, J. H.; Gasson, B. C.; Khushi, T.; Neale, J. E.; Payne, D. J.; Tolson, D. A.; Walker, G. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 1667.
10. Song, Y.; Kluger, R. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 1225.
11. Li, N.; Pratt, R. F. *J. Am. Chem. Soc*. **1998**, *120*, 4264.
12. (a) Weinbaum, G., Giles, R. E., Krell, R. D., Eds.; Pulmonary Emphysema, the Rationale for Intervention; *Ann. N. Y. Acad. Sci*. 1991, *624*, 1. (b) Merrit, T. A.; Cochrane, C. G.; Holconth, K.; Bohl, B.; Hallman, M.; Strayer, D.; Edwards, D.; Gluck, L. *J. Clin. Invest*. **1983**, *72*, 656. (c) Jackson, A. H.; Hill, S. L.; Afford, S. C.; Stockley, R. A. *Eur. J. Respir. Dis*. **1984**, *65*, 114.
13. Mata, E. G.; Setti, E. L.; Mascaretti, O. A.; Boggio, S. B.; Roveri, O. A. *J. Chem. Soc., Perkin Trans. 1*, 1551.
14. Mora, M.; Lacombe, J. M.; Pavia, A. A. *Tetrahedron Lett*. **1993**, *34*, 2461.
15. Boschetti, C. E.; Mascarettii, O. A.; Cricco, J. A.; Roveri, O. A. *Bioorg. Med. Chem*. **1995**, *3*, 95.
16. Sheehan, J. C.; Lo, Y. S.; Loliger, J.; Podewell, C. C. *J. Org. Chem*. **1974**, *39*, 1444.
17. Mata, E. G.; Boschetti, C. E.; Mascaretti, O. A. *Org. Prep. Proced. Int*. **1995**, *27*, 229.