Bioorganic & Medicinal Chemistry Letters, Vol.3, No.11, pp. 2259-2264, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

STUDIES ON CEPHEM SULFONES AS MECHANISM-BASED INACTIVATORS OF HUMAN LEUKOCYTE ELASTASE. III.[1] REACTIONS ENSUING FROM CHEMICAL β-LACTAM CLEAVAGE

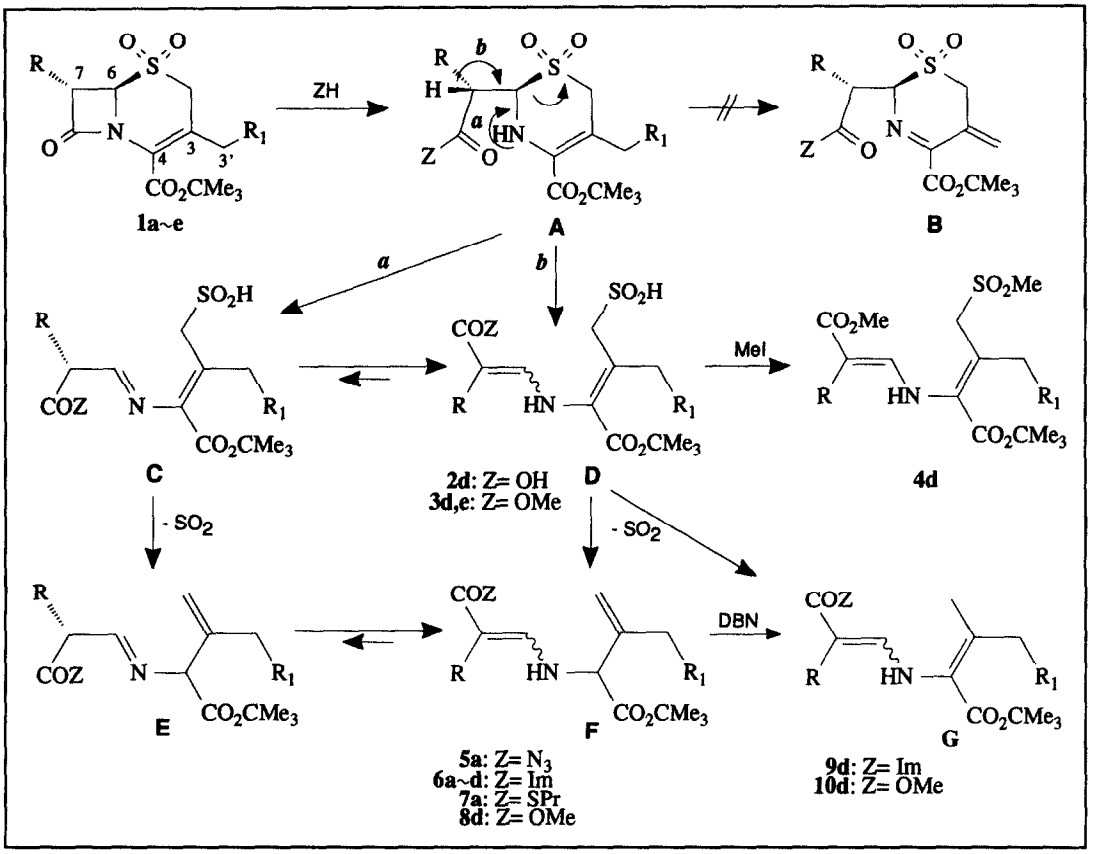
Marco Alpegiani,* Pierluigi Bissolino, Daniela Borghi, Vincenzo Rizzo & Ettore Perrone*
Farmitalia Carlo Erba R&D, 20014 Nerviano (MI), Italy

(*Received* 1 *April* 1993)

Abstract. The molecular events ensuing from β-lactam cleavage of cephem sulfones by chemical nucleophiles have been determined. Alkaline hydrolysis and methanolysis led to opening of the dihydrothiazine ring to produce sulfinate salts. Cleavages promoted by NaN_3, imidazole and *n*-PrSH in MeCN gave products from dihydrothiazine opening and desulfonylation. By contrast with enzyme-promoted reactions, expulsion of potential leaving groups at C3' was not observed.

Modified cephalosporanic sulfones (**1a**, **1b**) were shown to be potent inhibitors of human leukocyte elastase (HLE), a target enzyme for the development of effective drugs in the cure of pulmonary emphysema, cystic fibrosis and rheumatoid arthritis.[2] As for other β-lactam inhibitors of serine proteases (bacterial transpeptidases, β-lactamases) and proteinases (elastase), the effectiveness of cephem sulfones is reputed to rest on the formation of a covalently linked enzyme-inhibitor complex unusually resistant to reactivation by the normal hydrolytic pathway.[3] The structure of the complex resulting from the 7α-chlorocephem sulfone (**1a**) and porcine pancreatic elastase (PPE) has been solved at 1.84 Å resolution,[4] but the chemical mechanisms terminating in inhibition of the human enzyme (HLE) by the more promising class of the 7α-methoxy derivatives are not well understood.[5] In the past, a parallel has been proposed[6] between the reactions observable when β-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam) are cleaved by chemical nucleophiles (hydroxide anion,[7] methanol,[7d,7e,8] imidazole[9]), and the probable sequence of molecular events occurring at the enzyme active site. We wish here to report on reactions occurring after β-lactam cleavage of cephem sulfones (**1a-e**) by sodium azide, *n*-propanethiol, imidazole, sodium hydroxide and sodium methoxide, which set the basis for further studies aimed at determining the possible mechanisms by which these compounds behave either as enzymic substrates or inhibitors.[10]

The sulfone (**1a**),[11,12] activated by the chlorine atom at C7 and the acetoxy group at C3', reacted at room temperature with NaN_3 (2 mol equiv., MeCN-H_2O 3:1, 5 min), imidazole (5 mol equiv., MeCN, 3 h), and *n*-PrSH (large excess, imidazole 1 mol equiv., MeCN, 3 h). Work-up and flash chromatography over silica (gradient elution with *n*-hexane/ethyl acetate mixtures) afforded, respectively, the β-aminoacryloyl azide (**5a**) (25%), the imidazolide (**6a**) (65%), and a mixture of thiolester (**7a**) (20%) and imidazolide (**6a**) (55%).



Scheme 1. Cleavage products of cephem sulfones and possible pathways. Pyr= 4-pyridyl; Im= 1-imidazolyl. **a**: R=Cl, R_1=OAc; **b**: R=OMe, R_1=OAc; **c**: R=H, R_1=OAc; **d**: R=OMe, R_1=H; **e**: R=OMe, R_1=S-Pyr.

Reaction of the 7-unsubstituted sulfone (**1c**)[11] with imidazole (refluxing MeCN, 9 h) was studied to assess the alkene geometry of obtained compounds; the imidazolide (**6c**) was isolated as an inseparable mixture of alkene isomers (ca. 1:1) in 85% yield (based on reacted **1c**; 70% conversion). The ^{1}H NMR spectrum of the *Z*-isomer (J= 7.9 Hz for the vinylic protons) was characterized by a very deshielded enamine proton (δ 9.60 ppm in $CDCl_3$), indicative of intramolecular hydrogen bonding;[8a] in the *E*-isomer (J= 12.8 Hz) the corresponding proton resonated at 6.38 ppm (but moved to 8.22 ppm in DMSO-d_6). Since **5a**, **6a**, **7a** had resonances at $5.80 \leq \delta \leq 6.39$ ppm in $CDCl_3$ (Table 1), they were assigned the *trans*-aminoacrylate configuration analogous to that of (*E*)-**6c**. Equilibration between the two isomers of **6c** was observed in DMSO, where the *E*/*Z* ratio was altered from 1:1 to a final value of ca. 2.5:1 within 3 hours. Similar results were reported for aminoacrylates arising from clavulanic acid[8a] and suggest that enamine-imine tautomerism (**F** ⇌ **E**) is operative. By contrast, isomerization was not observed on the α-chloroacrylate (**6a**).

Reaction of the 7α-methoxycephem sulfones (**1b**)[11] and (**1d**)[12,13] with imidazole (sixfold molar excess; MeCN, reflux temperature) was examined next. The 3'-acetoxy compound (**1b**) reacted within 2.5 h, yielding **6b** (60%)

as a 6:1 mixture of *trans*- and *cis*-aminoacrylates (amine protons at 5.95 and 8.80 ppm in $CDCl_3$, respectively). With the 3-deacetoxycephalosporanate (**1d**) a longer reaction time was required (30 h); under such conditions, the isolated product was a mixture of (*Z*)-**6d**, (*E*)-**6d**, and bis-enamine (**9d**)[14] (7:1.5:2 by NMR integration; 65% overall yield).

Initial attempts to obtain informative products from alkaline hydrolysis (0.01 N NaOH) of cephem sulfones were discouraging. However, one predominant product was observed by HPLC[15] from hydrolysis of **1d**. The ^1H NMR spectrum of the solution (pH 11.5), recorded after 15 min at 600 MHz in H_2O under water presaturation conditions, showed characteristic resonances (all singlets) at 2.00 (vinylic methyl), 3.33 (CH_2SO_2), 3.56 (OMe), and 6.68 ppm (vinylic proton). After lowering pH to neutrality, the rapidly exchanging NH proton became detectable (7.71 ppm) and coupled with the vinylic proton (J= 12.6 Hz). These results are consistent with the bis-enamine sulfinate structure (**D**) (compound **2d** disodium salt). Similarly, methanolysis of **1d** (NaOMe 1.2 mol equiv., MeOH, 15 min) gave **3d** sodium salt, which could be collected as a solid (80%) after removal of the solvent and trituration with isopropyl ether. The same reaction was observed on the 3'-substituted cephem sulfone (**1e**), which underwent methanolysis without expulsion of the 4-pyridylthio group to give an impure sample of **3e** in 50% isolated yield.[16] Alkylation of **3d** with MeI (2.5 mol equiv., MeOH, 36 h) gave the methyl sulfone (**4d**) in over 80% yield, providing unequivocal evidence of the sulfinate moiety in structure (**D**).[17] Sulfone (**4d**), characterized by an enamine proton resonating at relatively high fields in $CDCl_3$ (δ 7.56 ppm), was assigned the *trans*-aminoacrylate structure. ^1H NMR analysis of the parent sulfinate salt (**3d**) could not be run in this solvent (or CD_3CN) because of rapid decomposition. Decomposition was slower ($t_{1/2}$ ca. 1 h) and cleaner in DMSO; two main desulfonylated products, **8d** and **10d** (2:1), were detected (HPLC[18] and NMR), and successively isolated by flash chromatography.

A general representation of the molecular events taking place after chemical β-lactam cleavage of cephem sulfones is given in Scheme 1. In all cases a transient intermediate possessing the intact dihydrothiazine structure (**A**) should be generated first; the alternative interpretation, a concerted mechanism leading to sulfinates (**D**), is unlikely on a stereoelectronic basis.[19] Compounds of this type (**A**) at the sulfide oxidation level are the hydrolysis products of antibacterial cephalosporins and, when R_1 is not a leaving group, are reasonably stable in diluted, non-acidic solutions; otherwise (e.g., R_1= OAc or S-Het) they evolve to the exomethylene structure (**B**).[7a,20] Our results indicate that, under the chemical cleavage conditions, scission of the C6-S bond (cephem numbering) is preferred to expulsion of the C3' group. Conversion of **A** to the enamine-sulfinates (**D**) might either be direct (elimination across the C7-C6 bond, path ***b***) or sequential (elimination across the N-C6 bond, path ***a***, followed by imine-enamine tautomerism **C**→ **D**). The first mechanism has a precedent in penicilloate diesters,[7b,21] the second in the elimination across N-C5 observed on the thiazolidine ring of penicillins[22] and penicillin sulfones.[7c,8b] Hydrolytic cleavages promoted by the enzyme (pH 7.4) follow path ***a***, since decarboxylated products originating from β-iminoacrylic acid (**C**; Z=OH) are generated.[10,23] However, path ***b*** might be operative in the presence of bases, especially in non-hydrolytic cleavages, where abstraction of the C7 proton is facilitated by the electron-withdrawing COZ group. Indeed, monitoring by NMR the reaction of **1a**

with imidazole (CD_3CN) failed to reveal metastable species evolving to the isolated compound (**6a**), but the latter may result from any of the fast sequences **A**→ **D**→ **F**, **A**→ **C**→ **D**→ **F**, or **A**→ **C**→ **E**→ **F**. Thus, understanding the desulfonylation mechanism is essential to a comprehensive interpretation of the cleavage cascade.

Reasonably, sulfinate salts (**C**, **D**) are stabilized by protic solvents; in fact, compounds **2d**, **3d**, **3e** were isolated from reactions run in water or methanol, while in MeCN the desulfonylated derivatives (compounds **5**~**7** and **9**) were directly observed. Thus, the first question is whether desulfonylation of β,γ-unsaturated sulfinate salts occurs spontaneously in aprotic solvents, in particular MeCN, and the second one is whether bis-enamines (**G**) are direct desulfonylation products or arise from base-assisted isomerization (**F**→ **G**). As mentioned above, degradation of **3d** was rapid when this compound was dissolved in MeCN, but formation of **F** and **G** (compounds **8d**, **10d**) was observed in yields much poorer than those obtained from direct cleavage of **1d** (compounds **6d**, **9d**). Expectedly, desulfonylation is aided by the unsaturated ester moiety acting as an electron sink (S_E1 mechanism); species (**F**) and (**G**) would result from protonation of the delocalized carbanion intermediate. Exposure of **8d**, **10d** to triethylamine did not affect the isomeric ratio, but quantitative conversion of the former into the latter was observed with a stronger base (DBN, DMSO overnight). Thus, the isomeric mixture (**F**) and (**G**) appears to be a kinetic protonation result. Since a stabilized carbanion is involved in desulfonylation, it is tempting to speculate that the imine moiety of species (**C**) makes them better substrates than isomers (**D**). That might account for the higher yields of the direct process relative to that performed *via* isolated species (**D**); involvement of **C** (path **A**→ **C**→ **E**→ **F**,**G**) is suggested.

Other possible mechanisms for the formation of enamines (**F**) were briefly considered and discarded. Desulfonylation with shift of the double bond might occur by a concerted mechanism (reverse ene reaction),[24] but that would require a protonated sulfinic species. Indeed, protonation of **3d** with pyridinium chloride led to immediate degradation and **8d** was detected (HPLC) in the reaction mixture, but yields were minimal; above all, the presence of external bases in the investigated cleavages does not support the intermediacy of undissociated sulfinic acids. Desulfonylation concerted with opening of the dihydrothiazine ring (simultaneous cleavage of C6-S and S-C2 bonds) might be considered, but cheletropic reactions usually require high temperatures[25] and, above all, the unlikely intermediacy of the amine anion of **A** would be necessary to fuel the postulated six-electron concerted process.

In conclusion, several peculiarities of the cleavage cascade of cephem sulfones were underscored. First, it has been proved that dihydrothiazine sulfones produced by opening the azetidinone ring of cephem sulfones are bound to C6-S cleavage. Second, loss of SO_2 concomitant with shift of the original C3-C4 unsaturation takes place under well-defined conditions. In particular, enamine-sulfinates (**D**) arising from esters of cephem sulfones appear to be hydrolytically more stable than those arising from carboxylate salts of penicillin sulfones (sulbactam and tazobactam),[7c,7e] owing to a less basic nitrogen atom, but much more prone to desulfonylation, owing to the β,γ-unsaturation. Finally, expulsion of leaving groups at C3' does not take place in chemical cleavages, by contrast with enzyme-promoted reactions.[10] The relevance of these findings to the mechanism of HLE inhibition and turnover is discussed in the accompanying paper.

Table 1. Distinctive spectral data of cleavage products

2d (disodium salt) NMR (600 MHz, H_2O): δ 1.52 (9H,s), 2.00 (3H,s), 3.33 (2H,s), 3.56 (3H,s), 6.68 and 7.71 (each 1H,d,*J*=12.6 Hz). UV (H_2O-CH_3CN, pH 6.2): λ_{max} 278, 310 (*sh*) nm.

3d (sodium salt) NMR (600 MHz, H_2O): δ 1.50 (9H,s), 2.07 (3H,s), 3.35 (2H,s), 3.58 and 3.69 (each 3H,s), 7.02 and 8.40 (each 1H,d,*J*= 11.7 Hz). IR (KBr): ν_{max} 1710, 1640, 1355 cm^{-1}. UV (EtOH): λ_{max} 287, 320 nm.

3e (sodium salt) NMR (600 MHz, H_2O): δ 1.49 (3H,s), 3.42 (2H,s), 3.52 and 3.71 (each 3H,s), 4.10 (2H,s), 6.99 (1H,s), 7.35 and 8.34 (each 2H,d,*J*=5.9 Hz). UV (EtOH) λ_{max} 280, 330 nm.

4d NMR (400 MHz, $CDCl_3$): δ 1.51 (9H,s), 2.09, 2.99, 3.67 and 3.71 (each 3H,s), 3.88 (2H,s), 6.91 (1H,d,*J*=12.6 Hz), 7.56 (1H,d,*J*= 12.6 Hz, exch. D_2O). IR ($CHCl_3$): ν_{max} 3350, 1710, 1650, 1315, 1135 cm^{-1}. UV (EtOH): λ_{max} 275 (sh), 310 nm.

5a NMR (200 MHz, $CDCl_3$): δ 1.48 (9H,s), 2.09 (3H,s), 4.50 (1H,d,*J*=6.4 Hz), 4.55 and 4.65 (each 1H,d,*J*= 13.0 Hz), 5.35 and 5.44 (each 1H,s), 6.05 (1H,dd,*J*=6.4 and 13.8 Hz, exch. D_2O), 7.53 (1H,d,*J*= 13.8 Hz). IR ($CHCl_3$): ν_{max}2150, 1745, 1675, 1630 cm^{-1}.

6a NMR (200 MHz, $CDCl_3$): δ 1.47 (9H,s), 2.06 (3H,s), 4.51 and 4.63 (2H,ABq,*J*=14.0 Hz), 4.56 (1H,d,*J*=6.2 Hz), 5.34 and 5.44 (2H, each br. s), 6.39 (1H, dd,*J*=6.2 and 13.8 Hz, exch. D_2O), 7.05 and 7.46 (each 1H,d,*J*=1.5 Hz), 7.50 (1H,d,*J*=13.8 Hz), 8.12 (1H,s). IR ($CHCl_3$): ν_{max} 3370, 1740, 1635 cm^{-1}.

6b (major isomer: *trans*-acrylate) NMR (400 MHz): δ 1.46 (9H,s), 2.06 and 3.54 (each 3H,s), 4.47 (1H,d,*J*=6.4 Hz), 4.53 and 4.67 (2H, each d,*J*=13.2 Hz), 5.33 and 5.40 (2H, each br. s), 5.95 (1H,dd,*J*=6.4 and 13.5 Hz, exch. D_2O), 7.03 (1H,d,*J*=1.5 Hz), 7.17 (1H,d,*J*=13.5 Hz), 7.62 (1H,d,*J*=1.5 Hz), 8.35 (1H,s). UV (H_2O-CH_3CN): λ_{max}310 nm.
(minor isomer: *cis*-acrylate) NMR (400 MHz): distinct signals, δ 1.46 (9H,s), 2.07 and 3.50 (each 3H,s), 4.45 (1H,d,*J*=6.4 Hz), 4.60 (2H,m), 7.03 (1H,d,*J*=13.5 Hz), 8.80 (1H,dd,*J*=6.4 and 13.5 Hz, exch. D_2O). UV (H_2O-CH_3CN): λ_{max} 319 nm.

6c (*cis*-acrylate) NMR (200 MHz, $CDCl_3$): δ 1.46 (9H,s), 2.07 (3H,s), 4.48 (1H,d,*J*=6.8 Hz), 4.59, 4.63 (2H,ABq,*J*=13.5 Hz), 5.22 (1H,d,*J*=7.9 Hz), 5.34 and 5.40 (each 1H,s), 6.91 (1H,dd,*J*=7.9 and 13.3 Hz), 7.02 and 7.43 (each 1H,d,*J*=1.5 Hz), 8.12 (1H,s), 9.60 (1H,dd,*J*=6.8 and 13.3 Hz, exch. D_2O). IR ($CHCl_3$): ν_{max} 3370, 1740, 1635 cm^{-1}.
(*trans*-acrylate) NMR (200 MHz, DMSO-d_6): δ 1.41 (9H,s), 2.01 (3H,s), 4.56 (2H,m), 4.89 (1H,d,*J*=6.7 Hz), 5.33 and 5.41 (each 1H,s), 5.50 (1H,d,*J*=12.8 Hz), 6.96 and 7.64 (each 1H,d,*J*=1.5 Hz), 7.69 (1H,dd,*J*=12.8 and 13.5 Hz), 8.22 (1H,dd,*J*=6.7 and 13.5 Hz, exch. D_2O), 8.30 (1H,s).

6d (*trans*-acrylate) NMR (400 MHz, $CDCl_3$): δ 1.46 (9H,s), 1.68 and 3.53 (each 3H,s), 4.34 (1H,d,*J*=5.9 Hz), 5.08 (2H,m), 5.95 (1H,dd,*J*=5.9 and 13.8 Hz, exch. D_2O), 7.02 (1H,d,*J*=1.5 Hz), 7.20 (1H,d,*J*=13.8 Hz), 7.61 (1H,d,*J*=1.5 Hz), 8.34 (1H,s).
(minor isomer: *cis*-acrylate) NMR (400 MHz, $CDCl_3$): distinct signals, δ 1.49 (9H,s), 1.70 and 3.49 (each 3H,s), 4.31 (1H,d,*J*=5.9 Hz), 5.08 (2H,m), 7.04 (1H,d,*J*=13.8 Hz), 8.93 (1H,dd,*J*=5.9 and 13.8 Hz, exch. D_2O).

7a NMR (200 MHz, $CDCl_3$): δ 0.97 (3H,t,*J*=7.5 Hz), 1.48 (9H,s), 1.59 (2H,m), 2.08 (3H,s), 2.89 (2H,m), 4.54, 4.64 (2H,ABq,*J*=13.5 Hz), 5.34 and 5.41 (each 1H,s), 5.80 (1H,dd,*J*=6.4 and 13.6 Hz, exch. D_2O), 7.48 (1H,d,*J*=13.6 Hz). IR ($CHCl_3$): ν_{max}1740, 1650 cm^{-1}.

8d NMR (400 MHz, $CDCl_3$): δ 1.47 (9H,s), 1.70 (3H,m), 3.65 and 3.71 (each 3H,s), 4.24 (1H,d,*J*=6.4 Hz), 5.06 (2H,m), 5.31 (1H,dd,*J*=6.4 and 13.2 Hz, exch. D_2O), 6.86 (1H,d, *J*=13.2 Hz). UV (EtOH) λ_{max} 280 nm.

9d (in admixture with *Z*-**6d** and *E*-**6d**) NMR (400 MHz, $CDCl_3$): distinct signals, δ 1.91, 2.11 and 3.59 (each 3H,s), 6.37 (1H,d,*J*=12.8 Hz, exch. D_2O), 7.18 (1H,d,*J*=12.8 Hz).

10d NMR (400 MHz, DMSO-d_6): *inter alia*, δ 1.77 and 1.94 (each 3H,s), 6.70 (1H,d,*J*=12.5 Hz), 7.48 (1H,d,*J*=12.5 Hz, exch. D_2O). IR ($CHCl_3$): ν_{max} 3400, 1715, 1645 cm^{-1}. UV (EtOH): λ_{max} 280 nm.

References and Notes

1. Part II of this series: Alpegiani, M.; Bissolino, P.; Borghi, D.; Corigli, R.; Del Nero, S.; Perrone, E; Razzano, G.; Rizzo, V. *BioMed. Chem. Lett.* **1992**, *2*, 1127.
2. Doherty, J.B.; Ashe, B.M.; Argenbright, L.W.; Barker, P.L.; Bonney, R.J.; Chandler, G.O.; Dahlgren, M.E.; Dorn, C.P.; Finke, P.E.; Firestone, R.A.; Fletcher, D.; Hagmann, W.K.; Mumford, R.A.; O'Grady, L.; Maycock, A.M.; Pisano, J.; Shah, S.; Thompson, K.R.; Zimmerman, M. *Nature* **1986**, *322*, 192.
3. Pratt, R.F. *The Chemistry of β-Lactams*; Page, M.I., Ed.; Blackie Academic & Professional: London, 1992; pp. 229-271.
4. Navia, M.A.; Springer, J.P.; Lin, T.-Y.; Williams, H.; Firestone, R.A.; Pisano, J.M.; Doherty, J.B.; Finke, P.E.; Hogsteen, K. *Nature* **1987**, *327*, 79.
5. Knight, W.B.; Maycock, A.L.; Green, B.G.; Ashe, B.M.; Gale, P.; Weston, H.; Finke, P.E.; Hagmann, W.K.; Shah, S.K.; Doherty, J.B. *Biochemistry* **1992**, *31*, 4980.
6. *a)* Kemal, C.; Knowles, J.R. *Biochemistry* **1981**, *20*, 3688; *b)* Newall, C.E. *β-Lactam Antibiotics: Mode of Action, New Developments and Future Prospects*; Salton, M.; Shockman, G.D., Eds.; Academic Press: New York, 1981; p. 287.
7. *a)* Hamilton-Miller, J.M.; Richards, E.; Abraham, E.P. *Biochem. J.* **1970**, *116*, 385; *b)* Davis, A.M.; Page, M.I. *J. Chem. Soc., Chem. Commun.* **1985**, 1702; *c)* Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. *Chem. Pharm. Bull.* **1985**, *33*, 2035; *d)* Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. *Chem. Pharm. Bull.* **1985**, *33*, 2461; *e)* Marunaka, T.; Matsushima, E.; Minami, Y.; Yoshida, K.-I.; Azuma, R. *Chem. Pharm. Bull.* **1988**, *36*, 4478.
8. *a)* Davies, J.S.; Howarth, T.T. *Tetrahedron Lett.* **1982**, *23*, 3109; *b)* Chen, Y.N.; Chang, C.-W.; Hedberg, K. *Tetrahedron Lett.* **1986**, *27*, 3449.
9. *a)* Bird, A.E.; Bellis, J.M.; Gasson, B.G. *Analyst* (London) **1982**, *107*, 1241; *b)* Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. *Chem. Pharm. Bull.* **1985**, *33*, 4923.
10. Rizzo, V.; Borghi, D.; Sacchi, N.; Alpegiani, M.; Perrone, E. *BioMed. Chem. Lett.*, following paper in this issue.
11. Doherty, J.B.; Ashe, B.M.; Barker, P.L.; Blacklock, T.J.; Butcher, J.W.; Chandler, G.O.; Dahlgren, M.E.; Davies, P.; Dorn, C.P.; Finke, P.E.; Firestone, R.A.; Hagmann, W.K.; Halgren, T.; Knight, W.B.; Maycock, A.L.; Navia, M.A.; O'Grady, L.; Pisano, J.M.; Shah, S.K.; Thompson, K.R.; Weston, H.; Zimmerman, M. *J. Med. Chem.* **1990**, *33*, 2513.
12. Alpegiani, M.; Baici, A.; Bissolino, P.; Carminati, P.; Cassinelli, G.; Del Nero, S.; Franceschi, G.; Orezzi, P.; Perrone, E.; Rizzo, V.; Sacchi, N. *Eur. J. Med. Chem.* **1992**, *27*, 875.
13. Shah, S.K.; Brause, K.A.; Chandler, G.O.; Finke, P.E.; Ashe, B.M.; Weston, H.; Knight, W.B.; Maycock, A.L.; Doherty, J.B. *J. Med. Chem.* **1990**, *33*, 2529.
14. Bis-enamine formation was independently observed in a study on the triethylamine-catalyzed epimerization of phthalimidocephem sulfones. De Angelis, F.; Botta, M.; Antonelli, R.; Dorigo, A.; Nicoletti, R. *Gazz. Chim. Ital.* **1989**, *119*, 375.
15. Chromatographic resolution was achieved under ion pairing conditions on a Whatman partisphere C18 column (5μm, 125×4.6 mm) eluted at 1.5 mL/min flow with a mobile phase containing 0.04 M phosphate buffer (pH 6.2) and 1.8 mM tetrabutylammonium bisulfate as constant components, and an increasing fraction of MeCN (from 14% to 36% in 14 min, and up to 55% in the next 4 min). Compound **2d** eluted at 7.7 min; starting **1d** at 14.2 min.
16. The C3'-heterothiocephem (**1e**) was preferred to analogs (**1a~c**) to avoid complications from the cleavable acetoxy moiety, but reaction of this substrate was made complex by initial Δ^3-Δ^2 isomerization: Alpegiani, M.; Bissolino, P.; Borghi, D.; Sbraletta, P.; Tonani, R.; Perrone, E. *Heterocycles*, submitted to.
17. Methylation of the ambidental sulfinate salts to methyl sulfones (instead of sulfinic esters) was reported previously: Steele, J.; Stoodley, R.J. *J. Chem. Soc. Perkin Trans. 1* **1983**, 2241.
18. Whatman partisphere C18 column (125×4.6 mm) eluted with 0.05 M KH_2PO_4 (brought to pH 2.5 with conc. H_2SO_4), and an increasing fraction of MeCN (from 28% to 40% within 12 min).
19. Crackett, P.H.; Stoodley, R.J. *Tetrahedron Lett.* **1984**, *23*, 1295.
20. Buckwell, S.C.; Page, M.I.; Longridge, J.L.; Waley, S.G. *J. Chem. Soc. Perkin Trans. 2* **1988**, 1823.
21. Davis, A.M.; Layland, N.J.; Page, M.I.; Martin, F.; More O'Ferrall, R. *J. Chem. Soc. Perkin Trans. 2* **1991**, 1225.
22. Davis, A.M.; Jones, M.; Page, M.I. *J. Chem. Soc. Perkin Trans. 2* **1991**, 1219.
23. By contrast, the α,β-unsaturated acid (**2d**) did not undergo decarboxylation at neutral pH.
24. Desulfonylation of 3.4-unsaturated sulfinic acids concerted with shift of the double bond was reported after hydrolysis of the corresponding esters and acidic work-up. Peterson, P.E.; Brockington, R.; Dunham, M. *J. Am. Chem. Soc.* **1975**, *97*, 3517.
25. Mock, W.L. *J. Am. Chem. Soc.* **1969**, *91*, 5682.