Articles

S-Aminosulfeniminopenicillins: Multimode β-Lactamase Inhibitors and Template Structures for Penicillin-Based β-Lactamase Substrates as Prodrugs

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A series of novel penicillins, bearing an *S*-aminosulfenimine (R′(R″)NSN═) side chain at the 6-position, have been prepared by direct reaction of a penicillin ester with sulfur diimides. A set of these structures, with the thiazolidine-ring sulfur in the sulfide oxidation state, exhibited a pattern of reactivity not previously encountered in penicillin chemistry, viz., cleavage of the β-lactam ring resulted in a rapid intramolecular displacement of the *S*-amino moiety as R′(R″)NH. This was found to be the exclusive reaction occurring consequent on cleavage of the β-lactam ring. The mechanism of this process was delineated in a detailed study in basic methanol. That a similar reactivity pattern held for a penicillin salt in aqueous solution was also verified. Thus the salt **5bm** (R′ = CH_3, R″ = *p*-$CH_3C_6H_4SO_2$) behaved as a moderate substrate for β-lactamase type I from *Bacillus cereus* ($k_{cat}/K_m = 6.26 \times 10^5$ M^{-1} min^{-1}). On enzyme-catalyzed hydrolysis of this compound, displacement of *N*-methyl-*p*-toluenesulfonamide (R′(R″)NH) was directly observed (^{1}H NMR) and found to occur faster than displacement of this group from (intact) **5bm** in aqueous buffer, by a factor of at least 600. These findings identified the potential of the *S*-aminosulfeniminopenicillin structure type to be developed as β-lactamase substrates for use as site-specific-release prodrugs. A degree of enzyme inhibition was also observed with this set of thiazolidine-ring-sulfide structures with the most potent inhibitor having the most nucleofugic *S*-amino moiety *p*-$ClC_6H_4SO_2N(CH_3)$, indicating that displacement of this group, at the enzyme active site, played a role in their mode of inhibition. Structures with the thiazolidine-ring sulfur in the sulfone oxidation state were considerably more potent as inhibitors, with the structure **5a$_2$** being the most active. As this compound bore the least nucleofugic *S*-amino moiety $C_2H_5OC(O)NH$, it indicated that the mode of inhibition of the sulfones was distinct from that of the thiazolidine-ring sulfides; it is probable that the sulfones reacted in a manner similar to that shown by sulbactam viz., rapid scission of the thiazolidine-sulfone ring after cleavage of the β-lactam ring. Synergy of action was observed with **5a$_2$** at high concentration (78 μg/mL) against *Escherichia coli* when combined 1:1 with penicillin G; no synergy was observed at low concentration (4 μg/mL) when combined with pipericillin, indicating poor permeation characteristics.

Introduction

The increasing prevalence of bacterial strains resistant to β-lactam antibiotics, due to the production of β-lactamase enzymes, has driven a good deal of research on mechanism-based inhibitors for these enzymes. A feature common to most such inhibitors is the formation of an acylated enzyme at the active site, which is not readily cleaved by hydrolysis. The route leading to the formation of such an acylated species can be quite varied as can the cause for slow hydrolytic cleavage.[1] Passive covalent inhibitors operate on the basis of forming an acylated enzyme which is inherently slow to hydrolyze. Active covalent inhibitors lead to an acylated enzyme which is slow to hydrolyze primarily because, in reactions occurring after initial acylation of the enzyme, some of the catalytic components for hydrolysis are dislocated from their optimum position.[1,2] These factors allow for considerable variation of inhibitor structure particularly in those which are β-lactam based. The mode of action of inhibitors such as clavulanic acid and sulbactam is primarily driven by the enhanced tendency of the non-lactam ring to cleave after opening of the β-lactam moiety. A feature common to a number of inhibitors

(1) Pratt, R. F. In *The Chemistry of β-Lactams*; Page, M. I., Ed.; Blackie Academic and Professional: Glasgow, 1992; Chapter 7.

(2) (a) Bulychev, A.; Massova, I.; Lerner S. A.; Mobashery, S. *J. Am. Chem. Soc.* **1995**, *117*, 4797. (b) Miyashita, K.; Massova, I.; Taibi, P.; Mobashery, S. *J. Am. Chem. Soc.* **1995**, *117*, 11055.

(3) (a) Arisawa, M.; Adam, S. *Biochem. J.* **1983**, *211*, 447. (b) Chen, Y. L.; Chang, C.-W.; Hedberg, K. *Tetrahedron Lett.* **1986**, *27*, 3449. (c) Chen, Y. L.; Chang, C.-W.; Hedberg, K.; Guarino, K.; Welch, W. M.; Kiessling, L.; Retsema, J. A.; Haskell, S. L.; Anderson, M.; Manuosos, M.; Barrett, J. F. *J. Antibiot.* **1987**, *40*, 803. (d) Bennett, I. S.; Brooks, G.; Broom, N. J. P.; Calvert, S. N.; Coleman, K.; Grancois, I. *J. Antibiot.* **1994**, *44*, 969. (e) Buynak, J. D.; Khasnis, D.; Bachmann, B.; Wu, K.; Lamb, G. *J. Am. Chem. Soc.* **1994**, *116*, 10955. (f) Buynak, J. D.; Geng, B.; Bachmann, B.; Hua, L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1513.

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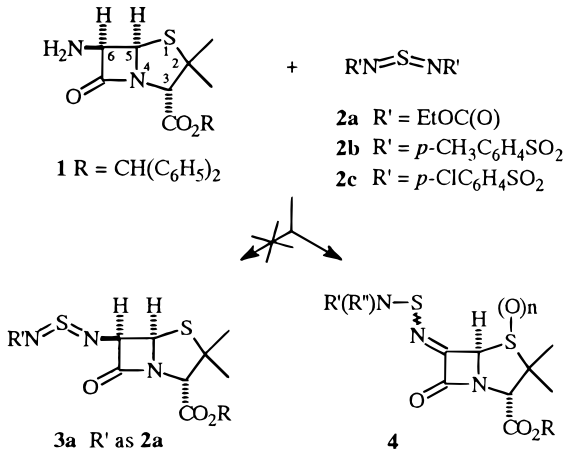
Scheme 1



R				
$CH(C_6H_5)_2$	Na+	R'	R''	n
4a	**5a**	EtOC(O)	H	0
4a$_1$	**5a$_1$**	EtOC(O)	H	1
4a$_2$	**5a$_2$**	EtOC(O)	H	2
4b	**5b**	*p*-$CH_3C_6H_4SO_2$	H	0
4b$_1$	**5b$_1$**	*p*-$CH_3C_6H_4SO_2$	H	1
4bm	**5bm**	*p*-$CH_3C_6H_4SO_2$	CH_3	0
4b$_2$m	**5b$_2$m**	*p*-$CH_3C_6H_4SO_2$	CH_3	2
4cm	**5cm**	*p*-$ClC_6H_4SO_2$	CH_3	0

developed more recently is a side group of latent reactivity which drives further structural rearrangement after cleavage of the β-lactam ring.[3] The inhibitor strategy in the long term must, however, be one of decreasing efficacy as it involves application of a selective pressure for the evolution of new variants of β-lactamase enzymes.

In this paper we report the first penicillin-based structures which behave as β-lactamase-dependent prodrugs. Such structures—together with the dual-acting cephalosporins[4]—offer the possibility of combating β-lactamase-based resistance to antibiotics by exploiting the presence of these enzymes: exploitation of these enzymes—including the metalloenzymes—may present the only strategy which, in the long term, should redress the balance in favor of non-β-lactamase producers in the general population of pathogenic bacteria.

Results and Discussion

The reaction of 6-aminopenicillanic acid ester **1** with the sulfur diimide **2a** at room temperature led to the rapid formation of a structure which we have identified as the *S*-aminosulfeniminopenicillin **4a** (Scheme 1). The previously reported reaction between amines and sulfur diimides is one of transimidation with the equilibrium lying on the side of the less basic amine[5] which, in the reaction above, would have led to **3a**. (An *S*-aminosulfenimine structure has been reported as one of the many products formed in the reaction of amines with elemental sulfur[6] and also as a minor product in the reaction of a cephalosporin with thionyl chloride in base.[7]) Spectroscopic data clearly characterized the experimental reaction product as an *S*-aminosulfeniminopenicillin. The ^{1}H NMR spectrum showed a singlet at 5.79 ppm corresponding to H-5, no signal corresponding to H-6, and one exchangeable hydrogen (N−H) at 6.61 ppm (this chemical shift was concentration dependent). A key feature of the ^{13}C NMR spectrum[8] was the occurrence of four peaks at 166.8, 165.0, 155.5, and 154.0 ppm. These chemical shifts indicated sp^2 carbons, bearing no hydrogens as shown by the off-resonance spectrum,[8] corresponding to the two ester carbonyls, the β-lactam carbonyl, and the carbon of the exocyclic imine,[9] respectively. The appearance of a band at 1665 cm^{-1} in the IR spectrum was characteristic of an imine. The change in the shape of the ^{1}H NMR peak associated with H-5 of **4bm**, from a sharp singlet at 6.20 ppm ($CDCl_3$) at 30 °C to a broad peak at −4 °C and to a baseline peak at −20 °C, indicated an isomerizable system, and one which was rapidly equilibrating at room temperature.[8] The ^{1}H NMR spectrum at −60 °C clearly indicated the predominance of one isomer with a singlet for H-5 at 6.57 ppm; the H-5 peak of the minor isomer appeared to be coincident with that of H-3 at 4.70 ppm. Taking the chemical shift observed at room temperature of 6.20 ppm as a weighted average of the cis and trans isomers, an equilibrium constant of 4 for their interconversion was obtained; in deuterioacetone, at ambient temperature, H-5 appeared as a sharp singlet at 6.31 ppm indicating that the equilibrium constant was solvent dependent. The activation energies for isomerization of sulfenimines—which have a carbon-based group attached to sulfur—are known[10a] and for a selected set vary from 12 to 14 kcal/mol.[10b] A crystal structure of a penicillin compound bearing a sulfenimine revealed that the side chain sulfur had a trans geometry with respect to the lactam carbonyl,[11] indicating that this was the more stable isomeric form, at least in the crystalline solid state.

In Scheme 2 is shown a stepwise mechanism illustrating how the reaction between a sulfur diimide and amine can lead to the formation of an *S*-aminosulfenimine (route **a**) or to a transimidation product (route **b**). A key step in route **a** involves removal of H-6 on the β-lactam ring

(4) The inherent reactivity of cephalosporins, resulting in elimination of the 3′ substitutent after cleavage of the β-lactam ring, has been harnessed in the development of dual-action structures producing effectively, cephalosporin based, β-lactamase substrate prodrugs: (a) O'Callaghan, C. H.; Sykes, R. B.; Staniforth, S. E. *Antimicrob. Agents Chemother*. **1976**, 245. (b) Mobashery, S.; Lerner, S. A.; Johnston, M.; *J. Am. Chem. Soc*. **1986**, *108*, 1685. (c) Albrecht, H. A.; Beskid, G.; Christenson, J. G.; Durkin, J. W.; Fallat, V.; Georgopapadakou, N. H.; Keith, D. D.; Konzelmann, F. M.; Lipshitz, E. R.; McGarry, D. H.; Siebelist, J.-A, Wei, C.-C.; Weigele, M.; Yang, R. *J. Med. Chem*. **1991**, *34*, 669. (d) Albrecht, H. A.; Beskid, G.; Christenson, J. G.; Georgopapadakou, N. H.; Keith, D. D.; Konzelmann, F. M.; Pruess, D. L.; Rossman, P. L.; Wei, C.-C. *J. Med. Chem*. **1991**, *34*, 2857. (e) Vrudhula, V. M.; Svensson, H. P.; Senter, P. D. *J. Med. Chem*. **1995**, *38*, 1380.

(5) Bussas, R.; Kresze, G.; Munsterer, H.; Schwobel, A. In *Sulfur Reports*; Senning, A., Ed.; Harwood Academic Publishers: New York, 1983; Vol. 2 (6), p 215.

(6) Sasaki, Y.; Olsen, F. P. *Can. J. Chem*. **1971**, *41*, 283.

(7) Saito, T.; Hiraoka, T. *Chem. Pharm. Bull*. **1977**, *25*, 784.

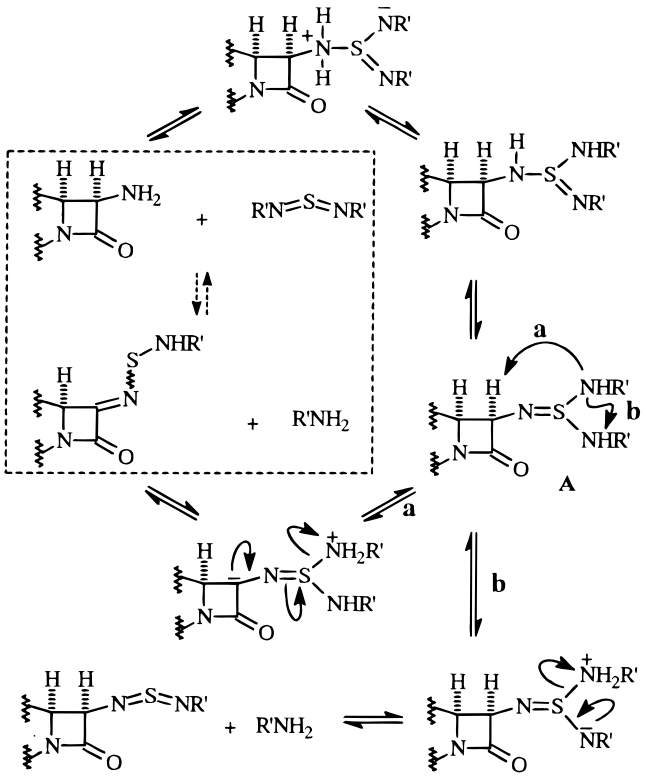
(8) Included in Supporting Information.

(9) Levy, G. C.; Lichter, R. L.; Nelson, G. L.; Eds. *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; John Wiley & Sons: New York, 1980; Chapter 5.

(10) (a) Claus, P. K. In *The Chemistry of Sulphenic Acids and Derivatives*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1990; Chapter 17. (b) The overall electron withdrawing character of the sulfur moiety in our structures may be broadly comparable to that present in sulfinamides $XC_6H_4S(O)N{=}C(C_6H_4CH_3)_2$ for which coalescence temperatures of −10 and −27 °C have been reported for X = H and *p*-NO_2, respectively: Davis, F. A.; Kluger, E. W. *J. Am. Chem. Soc*. **1976**, 302.

(11) Gordon, E. M.; Chang, H. W.; Cimarusti, C. M.; Toeplitz, B.; Gougoutas, J. Z. *J. Am. Chem. Soc*. **1980**, *102*, 1690.

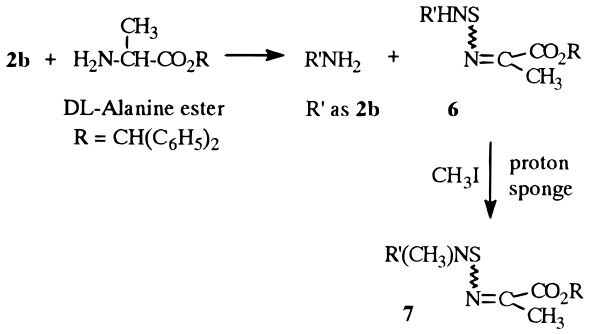
Scheme 2



to form a zwitterionic intermediate, a process for which there is good precedent in the literature.[12] There may be an element of kinetic control in the partitioning of intermediate **A** (Scheme 2) in that H-6 may be more acidic than the relevant N−H. The *S*-aminosulfenimine may also be the thermodynamically favored product in this case; semiempirical calculations (AM1, SAM1, PM3)[13] showed that the *S*-aminosulfenimine structure type was considerably more stable that the sulfur diimide form.

The range of *S*-aminosulfeniminopenicillins which we have been able to produce directly by this method was influenced by two factors. The first was the range of substituted sulfur diimides which could be conveniently prepared and purified, and the second factor was the reactivity of some of the sulfur diimides themselves; diphenyl sulfur diimide did not react with **1**. Our present work is limited to *S*-aminosulfenimines bearing carbethoxy, *p*-toluenesulfonyl (tosyl), and *p*-chlorophenylsulfonyl groups. Further structural variation was achieved by oxidation of the thiazolidine-ring sulfur and also by methylation of the side-chain nitrogen bearing the arylsulfonyl group; this latter was cleanly effected using Proton sponge and methyl iodide. In all cases selective oxidation of the thiazolidine-ring sulfur to the sulfoxide was achieved using 1 equiv of *m*-chloroperbenzoic acid (MCPBA). Further selective oxidation of these sulfoxides to the corresponding sulfones was achieved, except for **4b$_1$**, with $KMnO_4$ in a two-phase system. The chemical shift pattern of H-5 and of the α,*β*-methyl group clearly indicated that the site of the successive oxidations was the thiazolidine-ring sulfur (see Experimental Section for details). This oxidation pattern paralleled that found by Gordon for the sulfeniminopenicillins in that selective oxidation of the thiazolidine-ring sulfur to the sulfoxide was achieved with MCPBA; further oxidation to the corresponding sulfones was not achieved with the sulfeminines using MCPBA and no other oxidation process was reported by Gordon.[11]

Scheme 3



Previously reported reactions of sulfur diimides with amines[5] do not include examples of amines analogous to that present in penicillins. We have found that the reaction of alanine diphenylmethyl ester and sulfur diimide **2b** led uniquely to the formation of the corresponding *S*-aminosulfenimine **6** (Scheme 3) and not the transimidation product, thus showing the generality of this reaction with appropriate amines. The ^{1}H NMR spectrum of **6** had a singlet at 2.10 ppm due to the imine methyl group while in the ^{13}C NMR spectrum[8] peaks occurred at 160.0 and 143.8 ppm due to the conjugated ester carbonyl and the imino carbon, respectively. The variable temperature ^{1}H NMR data[8] obtained for **7** clearly showed an isomerizable structure—considerable line broadening of the *p*-CH_3 peak occurred at −21 °C.

In the following sections the chemical, enzymatic, and bacteriological activity profiles of the *S*-aminosulfeniminopenicillins prepared in this work are presented and discussed.

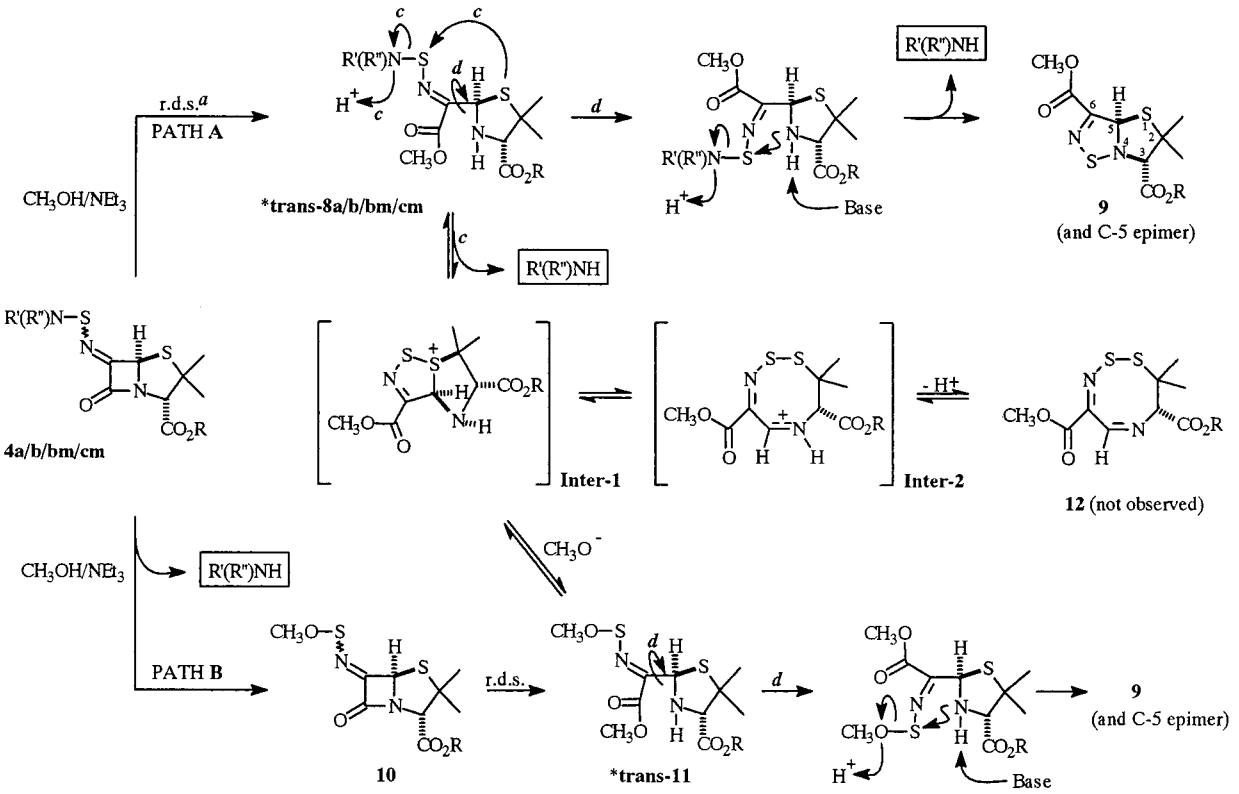
Chemical Reactivity. Cleavage of the *β*-lactam ring of penicillin structures in methanol containing a low concentration of base serves as a simple model for the corresponding reaction by the *β*-lactamase-active-site serine. As such, establishing the pattern of inherent chemical reactivity of a structure in this system can provide information which might be relevant to its interaction with a *β*-lactamase enzyme. We carried out a detailed analysis of the reaction end products, and intermediate structures formed as a function of time, on treatment of the thiazolidine-ring-sulfide esters **4a**, **4b**, **4bm**, and **4cm** in basic methanol (typically 35 mM unless otherwise stated); samples were withdrawn from the reaction solution at varying time intervals and extracted and the ^{1}H NMR spectra were recorded (see Experimental Section for other details). With **4b**, **4bm**, and **4cm** a common set of products was obtained. This set consisted of the arylsulfonamide corresponding to the individual *S*-amino moiety and one other structure in two epimeric forms. The arylsulfonamides were identified by comparison with authentic samples while ^{1}H and ^{13}C NMR data corroborated assignment of **9** (Scheme 4) as the structure of the other product (these data are presented later as the reaction sequences leading to its formation were relevant to the structural assignment).

The overall results were analyzed in terms of the reaction paths shown in Scheme 4: these provided a basis

(12) Grigg, R.; McMeekin, P.; Sridharan, V. *Tetrahedron* **1995**, *48*, 13347.

(13) Ampac 5.0, 1994, Semichem, 7204 Mullen, Shawnee, KS 66216. For details of SAM1 see: Dewar, J. S.; Jie, C.; Yu, J. *Tetrahedron* **1993**, *49*, 5003.

Scheme 4[a]



[a] Rate determing step for **4bm** and **4cm**; trans indicates side-chain S away from original lactam carbonyl.

for proposing **9** as the end product and, as shown later, the mechanistic work provided a set of results consistent with this reaction pattern (**12** was not observed as an end product—no peak attributable to an aldimine hydrogen was apparent in the NMR spectra). The set of reactions shown in Scheme 4 consist of methanolysis of the β-lactam ring and both inter- and intramolecular displacement of the *S*-amino moiety, all of which are well precedented in the literature. Reactions involving displacement of leaving groups from sulfur are well documented in the chemistry of sulfenyl chlorides and related structures.[14] Implicit in the reaction of piperidine-1-sulfenyl chloride with diamines to form (cyclic) thiadiazoles[14a,15] is an intramolecular process which closely parallels that shown leading to **9** from structure type **8**. The formation of five-membered rings via intramolecular nucleophilic displacement enjoys a considerable kinetic advantage over the corresponding intermolecular process leading to acyclic products. This kinetic advantage is quantified in terms of "effective molarity" (EM), and for a variety of cyclizations, involving reaction of secondary amines with a carbonyl group as the electron-deficient center (nucleosite), EM values of the order of 10^3 M are typical;[16a] the same should hold with sulfur as the nucleosite. The efficacy of alkyl sulfides as nucleophiles in intramolecular reactions is well documented in the chemistry of ω-halogenoalkyl sulfides (sulfur mustards) for which the formation of five-membered rings is particularly favored with alkyl as opposed to aryl sulfides.[17] The sequence **8** → **Inter-1** → **11** (Scheme 4) parallels the chemistry of the sulfur mustards closely. The formation of **9** from **11** is an intramolecular counterpart of the established intermolecular reaction of methyl sulfenates ($RSOCH_3$) with primary and secondary amines to form sulfenamides ($RSNR'(R'')$).[14b,18]

The side-chain and thiazolidine-ring sulfur atoms are held at 4.00 Å—i.e., just outside the sum of the van der Waals radii for this atom pair—in a sulfeniminopenicillin of which the crystal structure is known.[11] Cleavage of the lactam ring will release this constraint and could thus trigger reaction between the thiazolidine-ring sulfur and an appropriately functionalized sulfenimine side chain. Conformational analysis (semiempirical methodology using SAM1) of the β-lactam-ring-cleaved structure **8bm** (as the free acid which is relevant to neutral structures such as the corresponding ester, and also as the dicarboxylate dianion which is relevant to reaction in aqueous solution detailed later) provided some substantiation for this hypothesis. The conformational profiles (Figures 1 and 2) showed that, in conformers occupying minima on the potential energy surface, the separation distances of the side-chain sulfur atom to the thiazolidine-ring nitrogen and sulfur atoms, d_{NS} and d_{SS}, respectively, lay well inside the sum of the van der Waals radii of the relevant atom pairs. This is precisely the circumstance which has

(14) (a) Capozzi, C.; Modena, G.; Pasquato, L. In *The Chemistry of Sulphenic Acids and Derivatives*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1990; Chapter 10. (b) Hogg, D. R. In *The Chemistry of Sulphenic Acids and Derivatives*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1990; Chapter 9.

(15) Bryce, M. R. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2591.

(16) (a) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, 183. (b) Kirby, A. J.; Logan, C. J. *J. Chem. Soc., Perkin Trans. 2* **1978**, 642. (c) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128; **1993**, *26*, 206.

(17) (a) Bohme, H.; Sell, K. *Chem. Ber.* **1948**, *81*, 123. (b) Bird, R.; Stirling, J. M. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1221 and references therein.

(18) Armitage, D. A.; Clark. M. J.; Kisey, A. C. *J. Chem. Soc. C* **1971**, 3867.

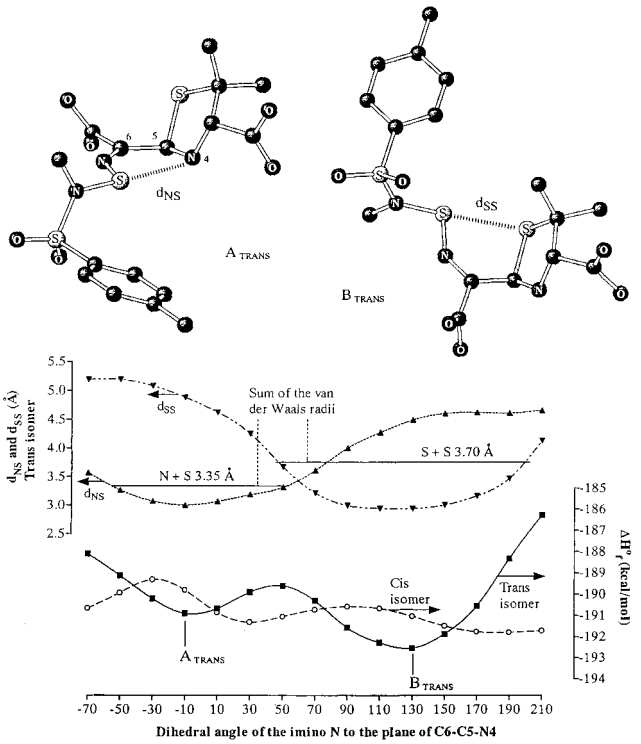


Figure 1. Conformational profile (rotation about C6−C5), and internuclear separations, of **8bm** (free-acid); hydrogen atoms are not shown.

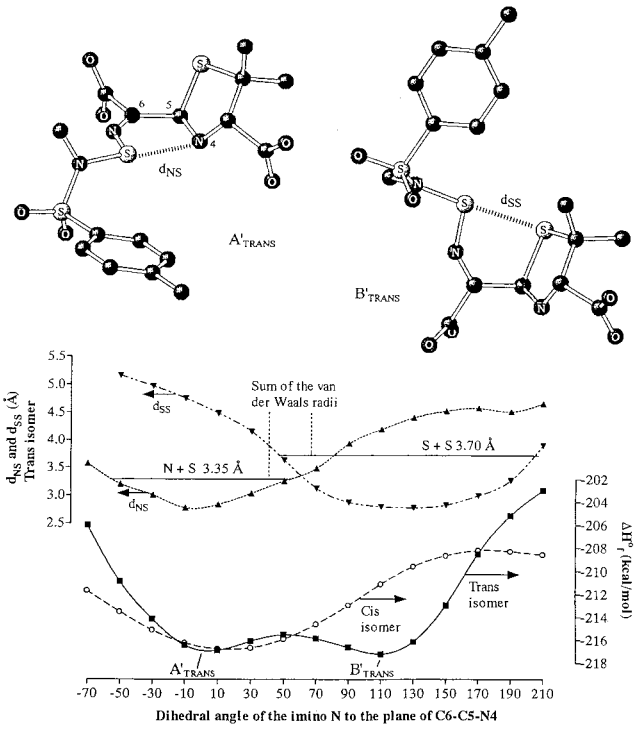


Figure 2. Conformational profile (rotation about C6−C5), and internuclear separations, of **8bm** (dicarboxylate dianion − **8′bm**); hydrogen atoms are not shown.

been identified as providing the impetus unique to intramolecular processes which exhibit exceptionally high EM values.[16b,c]

The experimental detail and its analysis, which provided substantiation of the reaction patterns shown in Scheme 4, follow.

The *β*-lactam-ring-opened structure **8a** was the sole product of the reaction of **4a** in basic methanol; notably, the *S*-amino moiety here was a poor leaving group and in addition bore an ionizable NH group, the relevance of which is elaborated on below. The chemical shift of the α-methyl group at 1.05 ppm and the upfield shift of H-3 to 3.81 ppm were diagnostic of the *β*-lactam-ring-opened structure[19] (Figure 3b). The coupling of H-5 with the thiazolidine-ring nitrogen ($J = 4$ Hz) was readily apparent while the spectrum resultant on addition of D_2O allowed inference of the chemical shifts of this N−H and H-3 as a coupled set of hydrogens ($J = 10$ Hz) to be made (Figure 3b and 3c); the differing coupling constants implied a cis and trans geometry of this N−H with H-5 and H-3, respectively. This compound was found to be quite stable when stored in $CDCl_3$ for up to 10 h. In the case of **4b**, with *p*-toluenesulfonamide as the *S*-amino moiety, the *β*-lactam-ring-opened structure **8b** was isolated but was found to undergo a further reaction in $CDCl_3$ solution. Figure 4b shows the ^{1}H NMR spectrum of a sample obtained from the reaction of **4b** in basic methanol in which the major product was **8b**: this was identifiable primarily from the chemical shift of the α-methyl group at 1.05 ppm and the upfield shift of H-3. Some unreacted starting material was also present together with small amounts of the eventual end products. The ^{1}H NMR spectrum of this sample after 6 h in $CDCl_3$ at room temperature, and in the absence of added bases or nucleophiles, indicated a considerably altered structure (Figure 4c). A fine precipitate which formed during this time was unambiguously identified as *p*-toluenesulfonamide by comparison with an authentic sample; a good deal of this latter material remained in solution, but the chemical shifts of the *p*-methyl group in **4b** and in this latter compound were very close, thus hindering its identification from the ^{1}H NMR data alone. The downfield shift of the α-methyl group (Figure 4c) was an initial indication of the thiazolidine ring being in a ring-fused system,[20] and this together with the formation of *p*-toluenesulfonamide pointed to **9** as a possible structure for the major coproduct. Chromatographic purification (flash technique) of the reaction products readily removed *p*-toluenesulfonamide and yielded the major and minor products as a mixture, roughly in a ratio of 3:1—this corresponded to the ratio in which they were observed in the crude reaction sample. The ^{1}H and ^{13}C NMR spectra of such a purified product sample are shown in Figures 5 and 6, and the assignments are summarized in Tables 1 and 2, respectively, together with data for other relevant structures. On the basis of the chemical shift and integration data in the ^{1}H NMR spectrum, and taking into consideration the reaction in which the products were formed, the presence of the following components was inferred for both the major and minor products: (a) a geminal dimethyl group originating from the α,*β*-methyl groups, (b) a methyl and a diphenylmethyl ester, (c) single, uncoupled, hydrogens originating from H-3 and H-5, (d) *no readily exchangeable (NH) hydrogens* (on addition of D_2O), and (e) the complete

(19) Davis, A. M.; Jones, M.; Page. M. I. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1219.

(20) For examples of a similar pattern of chemical shift changes on opening the *β*-lactam ring of a penicillin followed by closure to yield bicyclic structures with the thiazolidine ring fused to five-membered rings, see: (a) Marchand-Brynaert, J.; Ghosez, L. *Bull. Soc. Chim. Belg.* **1985**, *94*, 1021. (b) Tanaka, R.; Nakatsuka, T.; Ishiguro, M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2299.

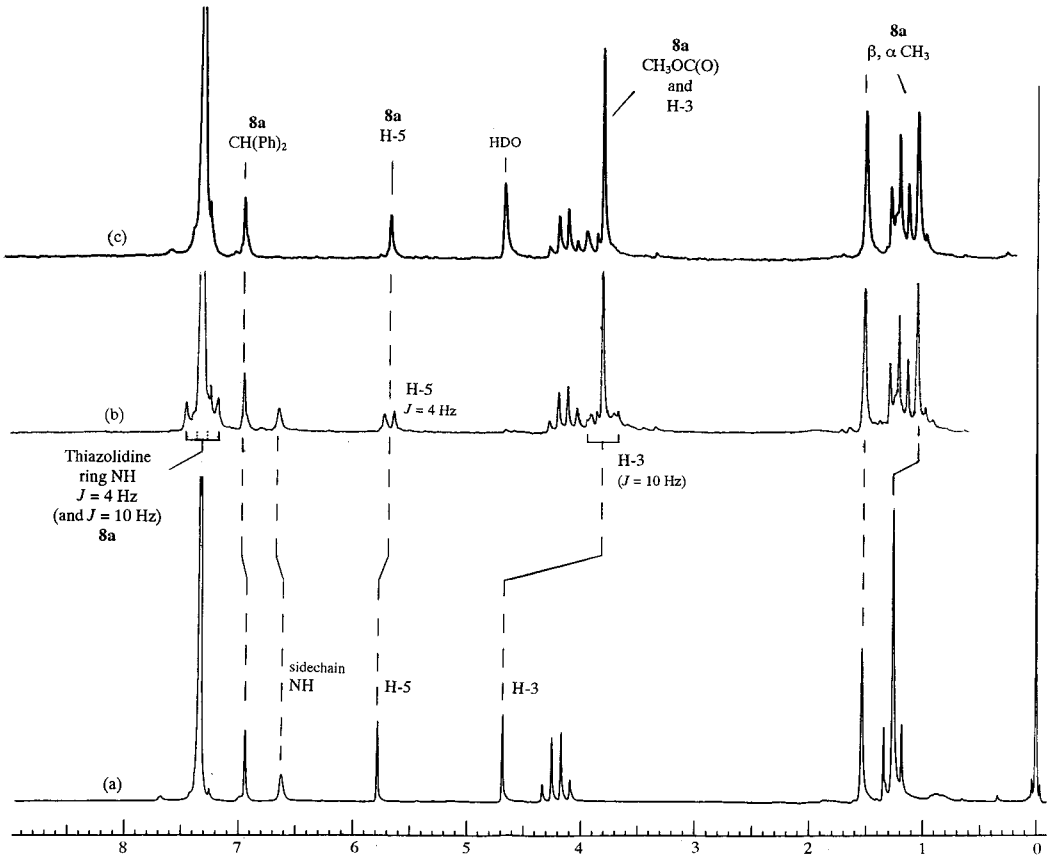
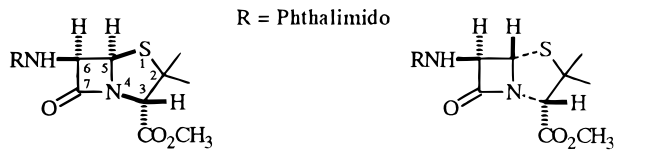


Figure 3. ^{1}H NMR spectra of (a) **4a**, (b) **8a**, and (c) **8a** on addition of D_2O.

absence of the *S*-amino side chain. The ^{13}C NMR spectrum corroborated the presence of the geminal dimethyl group and both the methyl and diphenylmethyl esters, and in the case of the major product the spectrum clearly showed the presence of an imino carbon. In addition peaks attributable to the other required carbons, C-2, C-3, and C-5, were clearly observed.

The finding that no change in the line shape of H-5 occurred from +30 to −60 °C in the ^{1}H NMR spectrum[8] of the major and minor product set indicated that the imino function was not isomerizable which was consistent with its being in a ring system. Furthermore very pronounced line broadening was observed for the geminal dimethyl group below −35 °C. Similar line broadening was seen with **4bm** and was associated with a slowing down of the thiazolidine ring flipping between "open" and "closed" conformations[21] which is well established in ring-fused systems such as penicillin structures.[22] Overall the spectroscopic data were consistent with a structure containing a thiazolidine ring bearing a geminal dimethyl group and fused to a second ring containing an endocyclic imino functionality; sensibly the diphenylmethyl ester must be on the thiazolidine ring and the methyl ester on the ring fused to the thiazolidine ring.

The major and minor products were assigned as stereoisomers of **9** with H-5/H-3 being either cis or trans to each other on the basis of the chemical shift pattern of these hydrogens and the ^{13}C chemical shift pattern of the α,*β*-methyl groups in this product set. First, it has been found that the chemical shifts of H-5/H-3 occurred at 5.03/3.69 ppm and at 5.48/4.37 ppm for stereoisomers of a given penicillin structure, with these hydrogens cis and trans (to each other), respectively.[23a,b] This pattern was matched by the chemical shifts of H-5/H-3 in the major and minor products (Figure 5; Table 1) indicating that these products were, most probably, similarly related



as stereoisomers. Second, it has been shown by Dobson and co-workers[22] that the ^{13}C chemical shifts of the α,*β*-methyl groups are very much closer together in penicillin structures which are predominantly in an open conformation than in penicillins predominantly in a closed conformation; a similar result should hold when the thiazolidine ring is fused to a five-membered ring as in **9**. The observation of just such a pattern for the geminal methyl group in the major and minor product set (Figure

(21) Davern, P.; Sheehy, J.; Smyth, T. *J. Chem. Soc., Perkin Trans. 2* **1994**, 381 and references therein.

(22) Claydon, N. J.; Dobson, C. M.; Lian, L.-Y.; Twyman, M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1933. Twyman, M.; Fattah, J.; Dobson, C. M. *J. Chem. Soc., Chem. Commun.* **1991**, 647.

(23) (a) Busson, R.; Vanderhaeghe, H. *J. Org. Chem.* **1976**, *41*, 2561. (b) A number of other penicillin, and closely related, structures showing this pattern of chemical shifts for H5/H3 are given in *Topics in Antibiotic Chemistry*, Vol. 4; Sammes, P. G., Ed.; Ellis Horwood: Chichester, 1980; Chapter 2. (c) Busson and Vanderhaeghe observed no epimerization at C-3 in the penicillin structures using NEt_3, but were able to so using the stronger base DBN.

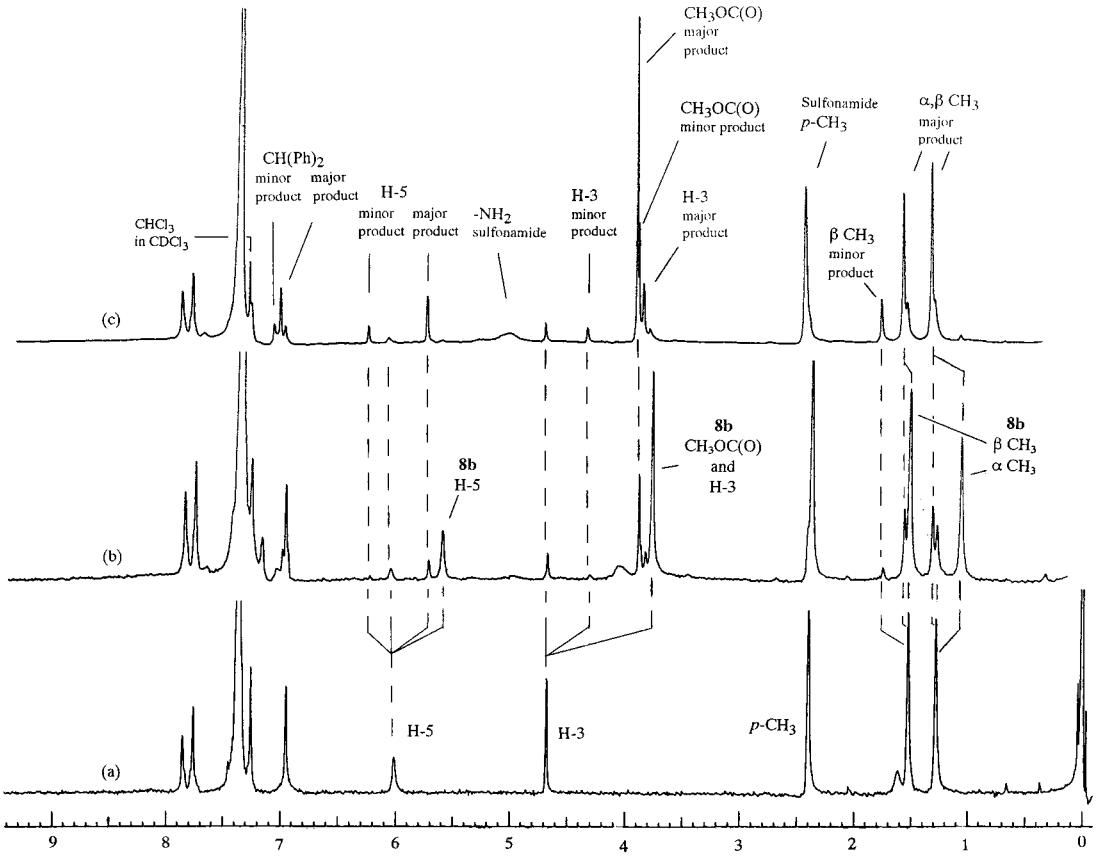


Figure 4. ^{1}H NMR spectra of (a) **4b**, (b) sample isolated from reaction of **4b** in basic methanol, and (c) this isolated sample after 6 h in $CDCl_3$ at 25 °C.

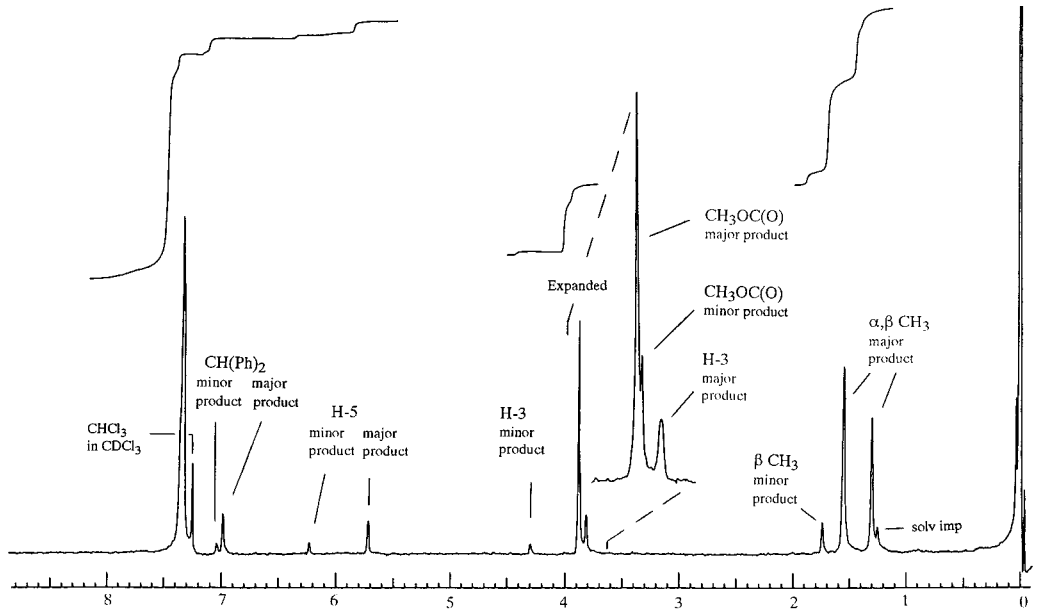


Figure 5. ^{1}H NMR spectra of purified reaction products: the major and minor products were assigned as (3*S*,5*S*)-**9** and (3*S*,5*R*)-**9**, respectively.

6; Table 2) was consistent with interpreting the major and minor products as being bicyclic ring fused systems, with the major product bearing the geminal methyl group in a more open conformation than in the minor product. This was supported by conformational analysis (semiempirical methodology using SAM1) which showed that (3*S*,5*S*)-**9** (cis H-5/H-3) had a considerably more open aspect than (3*S*,5*R*)-**9**, and in addition, was the more stable of the two by a small margin (Figure 7). The finding that a 3:1 ratio of major:minor product was obtained from **4bm**, **4cm**, and **8b** was consistent with this product set being an equilibrated mixture of (stereo)-isomers of marginally differing stability.

The chemical shift pattern of H-5/H-3, the ^{13}C NMR chemical shift pattern of the α/*β*-methyl group, and the results of the molecular modeling collectively supported

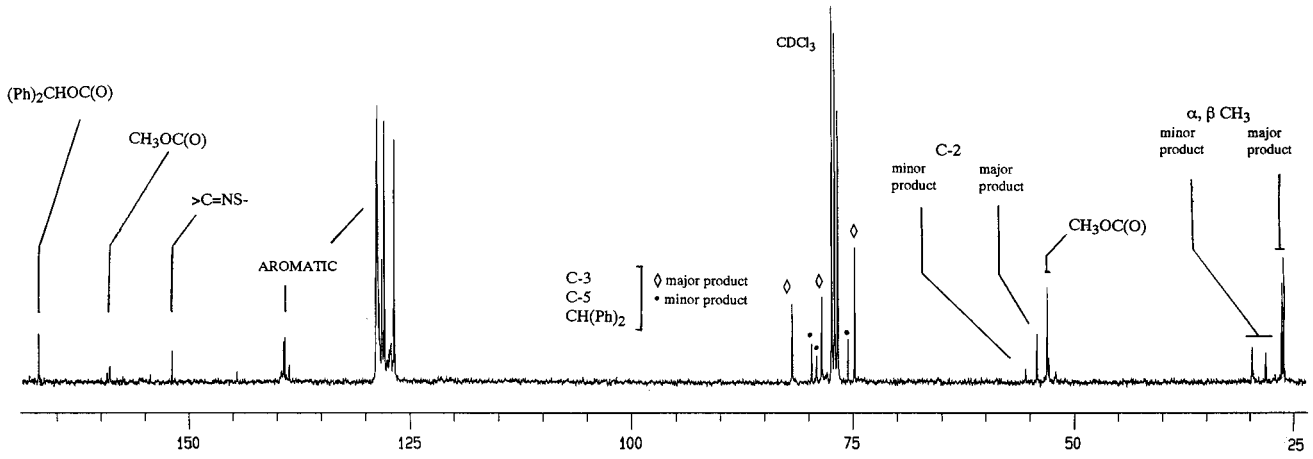


Figure 6. ^{13}C NMR spectra (100 MHz) of purified reaction products: the major and minor products were assigned as (3*S*,5*S*)-**9** and (3*S*,5*R*)-**9**, respectively.

Table 1. ^{1}H NMR Chemical Shifts (ppm)

	(3*S*,5*S*)-**9** major product	(3*S*,5*R*)-**9** minor product	**4bm**[a]/**4cm**[a]	**10**[a]
α,β CH_3	1.30; 1.57	(1.57); 1.70	1.30[b]; 1.57[b]	(1.30; 1.57)[c]
H-3	3.82	4.30	4.70[b]	4.70
CH_3OSN				3.86
$CH_3OC(O)$	3.89	3.88		
H-5	5.72	6.23	6.20/6.12	5.88
$CH(C_6H_5)_2$	7.00	7.05	6.95/6.94	6.95

[a] The configuration here is (3*S*,5*R*). [b] Identical chemical shift for **4bm** and **4 cm**. [c] Close overlap occurred with other peaks.

Table 2. ^{13}C NMR Chemical Shifts (ppm)

	(3*S*,5*S*)-**9** major product	(3*S*,5*R*)-**9** minor product	**4a**[a]
α,β CH_3	26.1, 26.3	28.2, 29.8	25.3, 34.2
CH_3O	53.1	(53.0)	
C-2	54.2	55.5	64.0
C-3	74.8	75.5	71.2
C-5	78.5	79.1	72.0
$OCH(Ph)_2$	81.8	79.6	78.5
C-6 (imine)	151.9	*b*	154.1
aromatic	126.7−128.8; 139.1−139.2	*c*	127.0−129.4; 139.1
lactam			155.5
ester (alkyl)	159.0 (conjugated)	*b*	166.5 (nonconjugated)
ester (diphenylmethyl)	167.2 (nonconjugated)	*b*	165.0 (nonconjugated)

[a] The configuration here is (3*S*,5*R*). [b] Not readily identifiable. [c] Coincident with those of the major product.

assignment of the major product as the stereoisomer bearing H-5/H-3 cis to each other, i.e., either (3*S*,5*S*)-**9** or its enantiomer (3*R*,5*R*)-**9**. As the stereochemistry in the starting penicillin structure was (3*S*,5*R*)—trans H-5/H-3—epimerization must have occurred at some stage in the reaction process, at either C-5 or C-3. The ^{1}H NMR data clearly indicated that epimerization at these positions had not occurred, neither in the ring-opened structures such as **8a** (Figure 3) and **8b** (Figure 4) nor, as seen later, in the intact penicillin structures[23c] such as **4bm** (Figure 9) and **10** (Figure 8). No exchange of H-5 or of H-3 in the product set was observed when **4bm** was reacted in CD_3OD/NEt_3. This finding restricted the epimerization option to the rapid equilibration of **Inter-1**

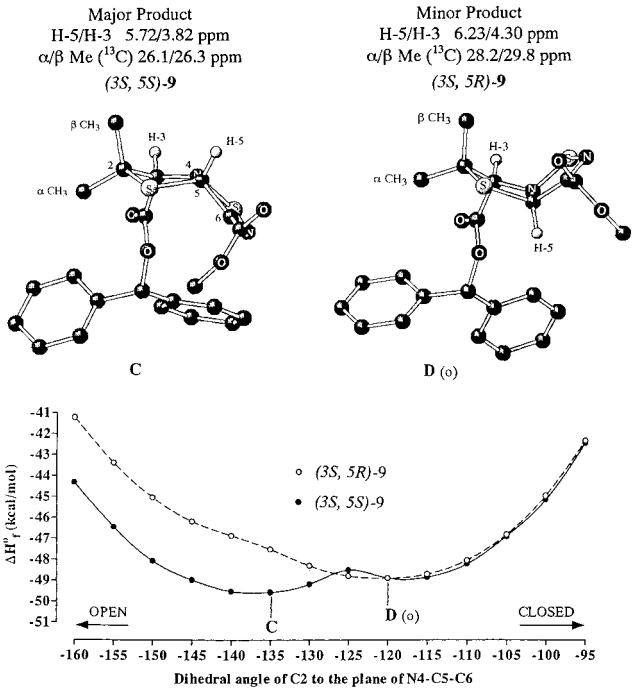


Figure 7. Conformational profile (open/closed aspect) of (3*S*,5*S*)-**9** and (3*S*,5*R*)-**9**; H-3 and H-5 are the only hydrogen atoms shown.

and **Inter-2** (Scheme 4), which would indicate that the major product was (3*S*,5*S*)-**9**. The transannular interaction of a sulfur atom with a carbonium ion center in cyclic structures is well established;[24a] substantiation for the reversibility of the equilibrium between **Inter-1** and **Inter-2** is provided by the observed ready contraction of seven- and eight-membered cyclic sulfides to form fused bicyclic structures, by the nucleophilic addition of the sulfur atom to the carbonium center.[24b]

Having thus substantiated the structure, and basis of formation, of the reaction products, the pattern of reactivity is further delineated in the following sections.

(24) (a) Tabushi, I.; Yoshinao, T.; Yoshida, Z.-i; Sugimoto, T. *J. Am. Chem. Soc.* **1975**, *97*, 2886. Block, E. *Reactions of Organosulfur Compounds*; Academic Press: New York, 1978 Chapter 4 and references therein. (b) deGroot, A.; Boerma, J. A.; Wynberg, H. *Tetrahedron Lett*, **1968**, 2365. Cerè, V.; Peri, F.; Pollicino, S.; Antonio, A. *J. Chem. Soc., Perkin Trans. 2* **1998**, 977 and references therein.

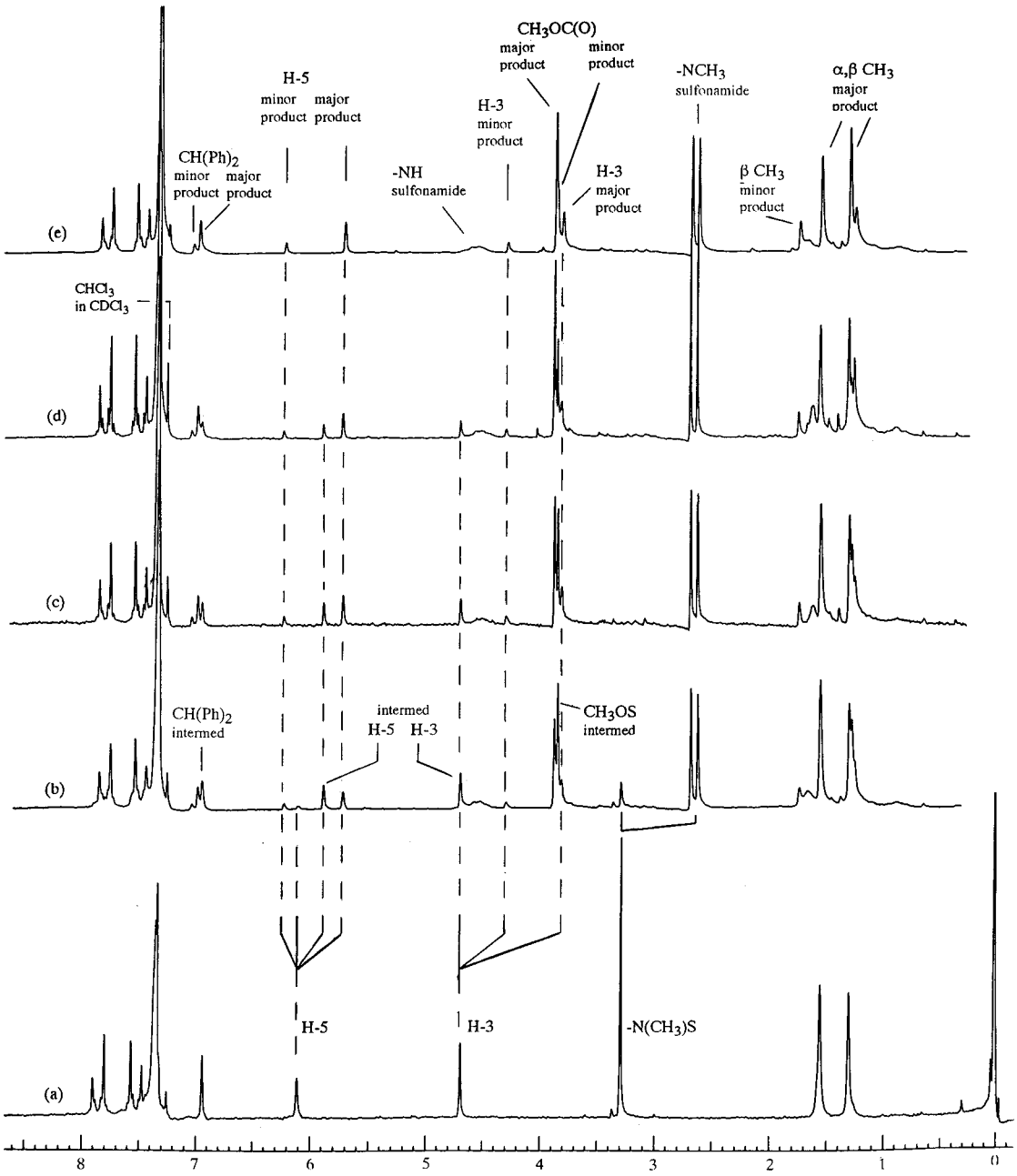


Figure 8. ^{1}H NMR spectra of (a) **4cm** and samples isolated at (b) 10, (c) 30, (d) 60, and (e) 120 min from reaction of **4 cm** in basic methanol.

As a neutral group *p*-toluenesulfonamide is a moderate leaving group; however, ionization of the sulfonamide hydrogen in **4b** was found to occur quite readily in basic methanol,[25] thereby considerably reducing its nucleofugacity. This would also occur for **8b** in basic methanol. Partial ionization of this acidic NH bond in $CDCl_3$, through formation of a strong hydrogen bond with the basic thiazolidine-ring nitrogen in an inter- or intramolecular fashion, would significantly retard the rate of displacement of *p*-toluenesulfonamide from **8b** in this solvent system. These processes were blocked in the case of the *N*-methylated structures, resulting in a modification of the reaction kinetics leading to the same pattern of end products, as discussed below.

A set of ^{1}H NMR data obtained from reaction of the *N*-methylated structures **4cm** and **4bm** in basic methanol, at different time intervals, is shown in Figures 8 and 9, respectively. No β-lactam-ring-opened structures were detected which implied that reactions occurred, after ring opening, which were faster than the ring-opening step itself. Displacement of the *S*-amino moiety, to give the corresponding *N*-methylarylsulfonamide, was readily identified by the change in the chemical shift of the

(25) (a) The structure $\mathbf{4b}_{(\text{side-chain anion})}$ was readily observed (^{1}H NMR)[8] by treatment of **4b** in $CDCl_3$ with Proton Sponge; it was quite stable for an extended period of time (24 h). Cleavage of the β-lactam ring in this structure was particularly slow and was effected using 0.8 M MeO^- for a very short reaction time, see Experimental Section for details. (b) The pK_a of a related compound, TsNHBoc, has been reported as 8.5 in DMSO: Koppel, I.; Koppel, J.; Degerbeck, F.; Grehn, L.; Ragnarsson, U. *J. Org. Chem.* **1991**, *56*, 7172. (c) The ability of sulfur to stabilize an adjacent anion is well documented: Wiberg, K. B.; Castejon, H. *J. Am. Chem. Soc.* **1994**, *116*, 10489 and references therein.

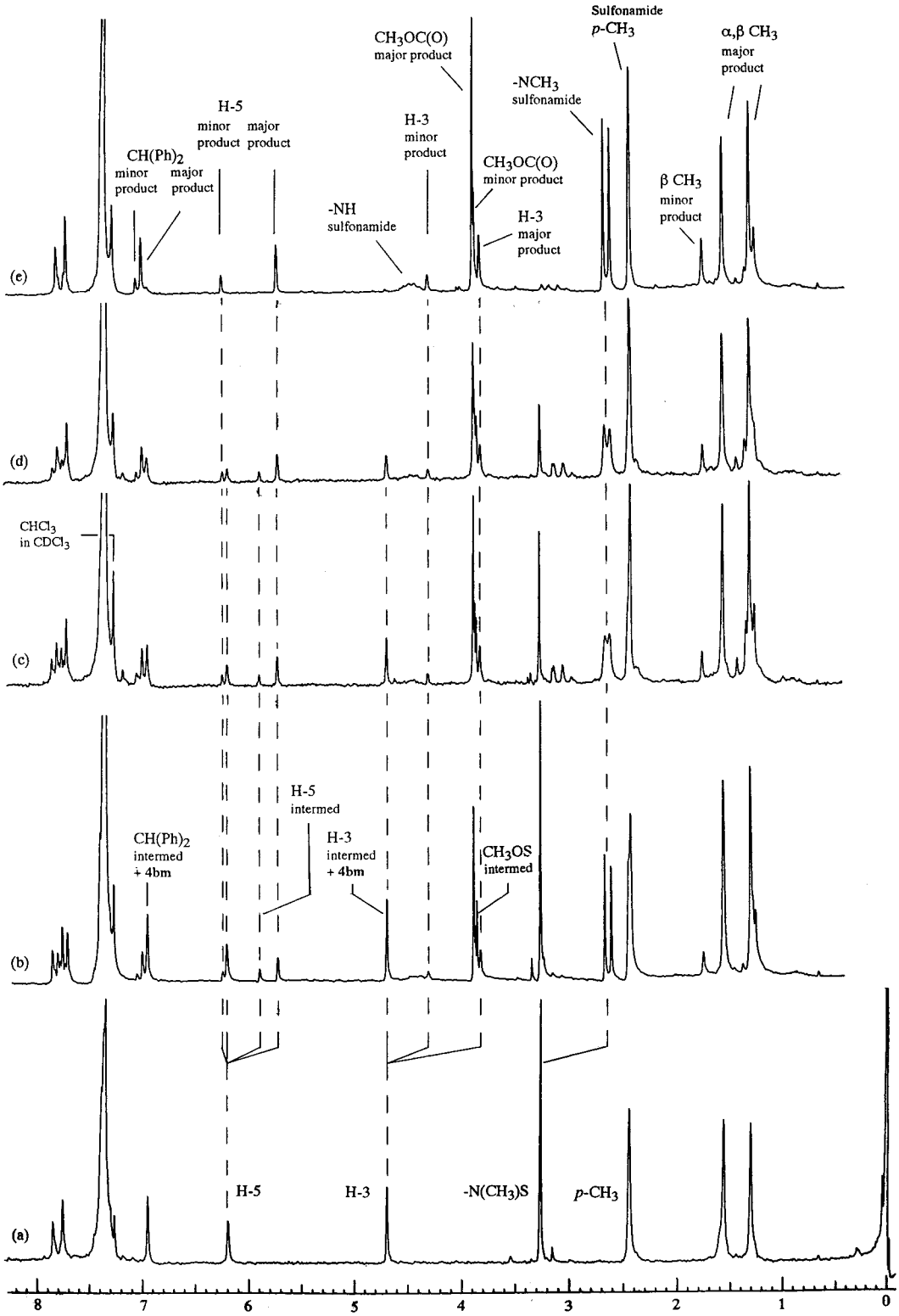


Figure 9. ^{1}H NMR spectra of (a) **4bm** and samples isolated at (b) 10, (c) 30, (d) 60, and (e) 240 min from reaction of **4bm** in basic methanol; the quartet centered at 3.08 ppm in (c) and (d) is due to a trace of triethylamine hydrogen bonded to the sulfonamide.

N-methyl group. In **4cm** this occurred as a singlet at 3.29 ppm which changed during the course of the reaction to a doublet at 2.65 ppm (J = 3.7 Hz; this collapsed to a singlet on addition of D_2O concomitant with the disappearance of the broad peak at 4.55 ppm). Similarly, in the reaction of **4bm** the original singlet at 3.25 ppm (*N*-methyl) changed progressively to a doublet at 2.63 ppm which corresponded accurately with the chemical shift of the *N*-methyl peak in *N*-methyl-*p*-toluenesulfonamide[26] (J = 3.7 Hz; this collapsed to a singlet on addition of D_2O concomitant with the disappearance of the broad peak at 4.45 ppm). The buildup and decay of an intermediate was clearly observed with both **4cm** (Figure 8) and **4bm**

(26) Pouchert, C. J. *The Aldrich Library of NMR Spectra*, 2nd ed.; Aldrich Chemical Co., Inc.: Milwaukee, 1983; Vol. 2.

Table 3. Values of IC_{50} (μM), Partition Number, k_{cat}/K_m, and k_{Inact}/K_m (M^{-1} min^{-1}) at pH 7.2, for the *S*-Aminosulfeniminopenicillin Sodium Salts with β-Lactamase Type I from *B. cereus*

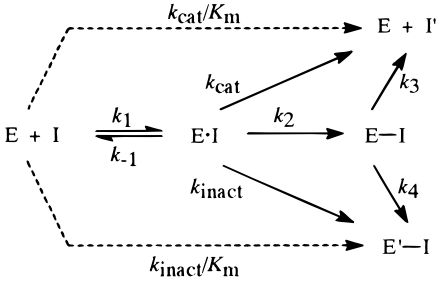
	sulfides				sulfoxides		sulfones		
structure	**5a**	**5b**	**5bm**	**5cm**	**5a$_1$**	**5b$_1$**	**5a$_2$**	**5b$_{2m}$**	sulbactam[a]
IC_{50}	10 000	10 000	1 000	425	1800	1500	15	115	80
partn. no.	>30 000	>30 000	15 000	6800	5600	10 000	100	1000	500
k_{cat}/K_m			6.26×10^5						
k_{inact}/K_m			41.73[b]						

[a] Sulbactam was screened at the same time as the other compounds using the same enzyme stock solution (see Experimental Section for details). [b] Calcutated from the partition number and k_{cat}/K_m.

(Figure 9). A number of peaks were clearly assignable to this intermediate: 6.95 ($CH(Ph)_2$), 5.88 (H-5), 4.70 (H-3), and 3.86 ppm (CH_3OS) while the geminal dimethyl set of peaks were coincident with other such peaks. The coincidence of the chemical shift of H-3 in this structure with that in **4bm** and in **4cm** was strongly indicative of the intermediate being an intact penicillin structure. The chemical shift at 3.86 ppm, assigned to the CH_3OS moiety, was similar to that found for the corresponding hydrogens in closely related structures (ROSN(R′)R″).[27] That the buildup of this intermediate was more pronounced in the case of **4cm** (Figure 8), which contained the better *S*-amino leaving group compared to **4bm** (Figure 9), was consistent with identification of the intermediate as resulting from direct intermolecular displacement of the *S*-amino moiety. That the same end products resulted from the decay of this intermediate, and at a slower rate than their formation directly from **4cm** (see below), was also consistent with the identification of this intermediate as **10**.

It is evident from the data in Figure 8 (spectra of reaction samples at 30, 60, and 120 min) that the rate of disappearance of **10** to give **9** was a good deal slower than the initial reaction of **4cm** itself (Figure 8, spectrum of reaction sample at 10 min) to generate the same end products. This was consistent with the greater electron-withdrawing effect of the *N*-methyl-*p*-chlorophenylsulfonamido group over a methoxy group resulting in the β-lactam ring of **4cm** being more activated toward ring opening[28] compared to **10**. On the basis of the combined integration of the peaks at 5.72 and at 6.23 ppm, it was also evident that a significant amount of **9** had been formed quite rapidly (Figure 8, spectrum of reaction sample at 10 min) and, therefore, could have arisen only via path **A** (Scheme 4). The fact that at this reaction time the amount of **10** formed was somewhat larger than that of **9** (based on the combined integration of the peaks at 5.72 and at 6.23 ppm relative to that of the peak at 5.88 ppm) indicated that direct intermolecular displacement of the *S*-amino moiety in **4cm** had outpaced β-lactam ring opening by a small margin. With **4bm** this situation was reversed. The electron-withdrawing effect of the *N*-methyl-*p*-toluenesulfonamido group was such that the rates of both β-lactam ring opening and direct intermolecular displacement of this group were slower relative to that of **4cm**. This is clear from the data in Figure 9 where after a 30 min reaction time a significantly larger amount of starting material was still present compared with that observed for **4cm** at a 10 min reaction time (Figure 8). On the basis of the combined integration of the peaks at 5.72 and at 6.23 relative to that of the peak at 5.88 ppm in Figure 9 (spectra of reaction samples at 10, 30 and 60 min) it was evident that cleavage of the β-lactam ring, followed by rapid intramolecular displacement of the *S*-amino moiety, had outpaced direct intermolecular displacement of the *S*-amino moiety in **4bm**. The nondetection of a buildup of a β-lactam-ring-opened structure indicated that cleavage of the β-lactam ring was rate determining for formation of **9** via paths **A** and **B** (Scheme 4). *Thus **4bm** and **10** define structure types in which the predominant reaction pattern was rapid intramolecular displacement of the sulfur-attached moiety consequent on cleavage of the β-lactam ring.* The finding that **10** followed this pattern was significant in that it showed the efficiency, and generality, of the intramolecular displacement process.

Scheme 5



Partition No. $= k_3/k_4 = k_{cat}/k_{inact}$

E' = inactivated enzyme; I' = hydrolysed inhibitor

The relevance of this pattern of inherent chemical reactivity to the observed interactions of these structures with a β-lactamase enzyme is elaborated on in the next section.

Enzymatic Activity. The interaction of the salts **5a**−**5cm**[29] with β-lactamase type I from *Bacillus cereus* was studied in aqueous buffers: the results are summarized in Table 3. Evaluation of the their efficacy as mechanism-based inhibitors was quantified in terms of IC_{50} (the concentration of inhibitor required to lower enzyme activity by 50%) and partition number. This latter is the number of inhibitor molecules hydrolyzed by the enzyme per inactivation event[30] and, on a practical basis, corresponds to the (minimum) inhibitor:enzyme molar ratio at which complete inactivation of the enzyme occurs (see also kinetic definition in Scheme 5). Benzylpenicillin was used as the assay substrate, and measurement of the rate of its enzyme catalyzed hydrolysis by samples of β-lactamase, which had been incubated (10 min) with varying concentrations of inhibitor, was carried out in a standard

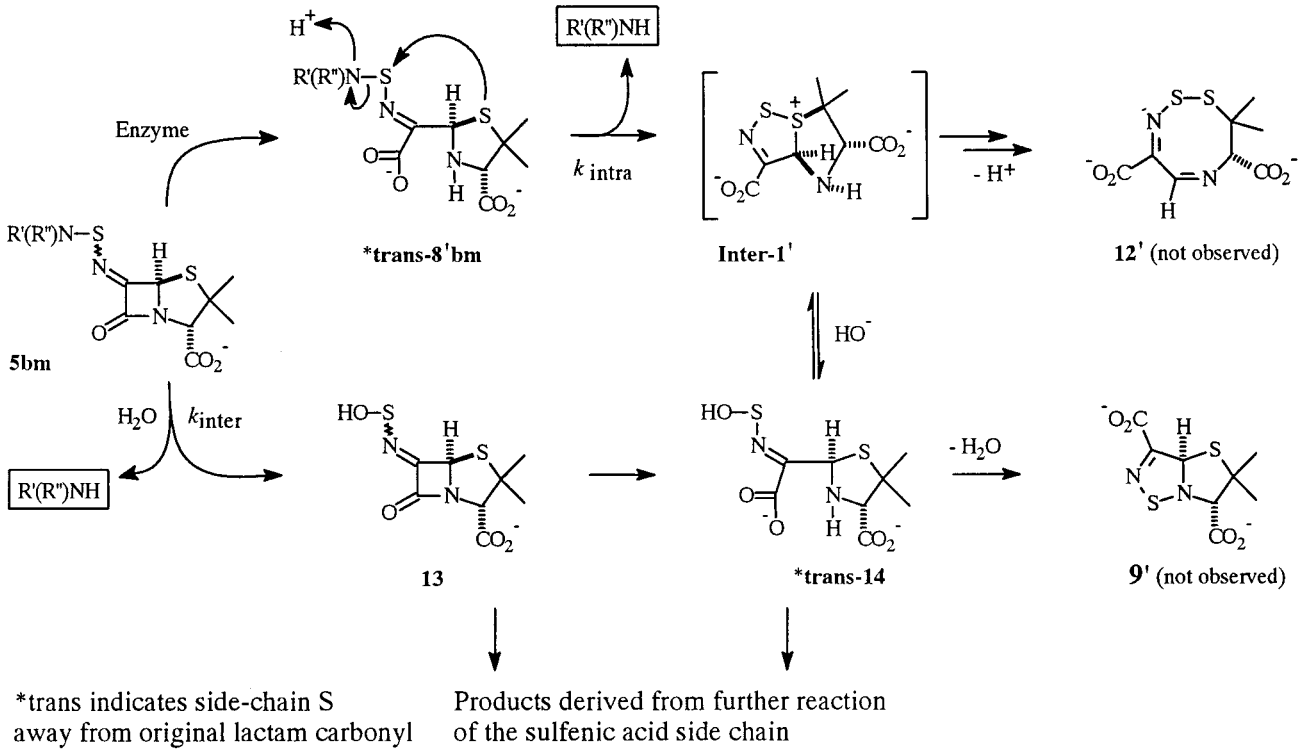
(27) (a) Bassindale, A. R.; Iley, J. In *The Chemistry of Sulphenic Acids and Derivatives*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1990; Chapter 3. (b) Wenschuh, E.; Kuhne, U.; Mikolajczyk, M.; Bujnicki, B. *Z. Chem.* **1981**, *21*, 217.

(28) Rao, S. N.; More O'Ferrall, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 2729.

(29) **5cm** was isolated as the free acid. For enzymatic studies 50 μL of a THF solution of **5cm-free acid** was added to the aqueous buffer containing the β-lactamase enzyme (see Experimental Section).

(30) Walsh, C. *Tetrahedron* **1982**, *38*, 871.

Scheme 6



manner using UV/vis spectroscopy. Using the same assay procedure the minimum concentration of inhibitor required for complete inactivation of a known concentration of enzyme, at the standard 10 min incubation time, was determined and the inhibitor:enzyme molar ratio at this point gave the partition number directly.[31] In addition, in the case of **5bm** the degree to which it behaved as an enzyme substrate was quantified in terms of k_{cat}/K_m,[32a] this term is essentially a second-order rate constant for hydrolysis of the substrate/inhibitor by this enzyme[32b,33] (Scheme 5). The values of k_{cat}/K_m were determined using the expression $k_{cat}/K_m = v/[E_o][S_o]$ by measuring v, the initial rate of hydrolysis (5%) of a known concentration of substrate using a known concentration of enzyme (see Experimental Section for details).

The value of k_{cat}/K_m for **5bm** (Table 3) identified it as a moderate substrate for this enzyme. This allowed us to examine the intramolecular displacement reaction from the carboxylate salt **8′bm** (Scheme 6).[34] We found that the rate of displacement of *N*-methyl-*p*-toluenesulfonamide from **8′bm**, generated by β-lactamase-catalyzed hydrolysis of **5bm** in aqueous buffer, was faster that its direct displacement from **5bm** in this medium, by a factor of at least 600. This was verified by direct observation of the reaction, when run on a small scale in D_2O buffer, using ^1H NMR. To a sample of **5bm** (approximately 5 mg) in D_2O buffer (pD 7.2, 0.1 M phosphate) in an NMR tube was added a portion of enzyme sufficient to effect immediate and complete hydrolysis. A spectrum was recorded as quickly as possible after addition of the enzyme solution, approximately 20 min was required for 200 accumulations. This spectrum (Figure 10b) showed the complete absence of the peak at 3.33 ppm corresponding to the side-chain *N*-methyl (this chemical shift was characteristic of this group while attached to the side-chain sulfur in both **5bm** and **8′bm**) but had a sharp singlet at 2.55 ppm corresponding to the *N*-methyl group of *N*-methyl-*p*-toluenesulfonamide in this solvent; when this experiment was run on a larger scale in H_2O buffer a precipitate formed and was recovered and identified unambiguously as *N*-methyl-*p*-toluenesulfonamide. The experiment in D_2O buffer was rerun using a larger amount of **5bm** (approximately 17 mg) which allowed a sufficiently detailed spectrum to be obtained within 5 min of adding the enzyme (Figure 10c). Although the spectral resolution was not as good as that obtained at the lower concentration, due to the formation of a fine precipitate, both the complete absence of the peak at 3.33 and the appearance of the peak at 2.55 ppm were readily apparent. The half-life for expulsion of *N*-methyl-*p*-toluenesulfonamide from **8′bm** was thus estimated at <0.5 min, on the basis that the ^1H NMR observation made after 5 min, showing complete loss of the side-chain *N*-methyl moiety, corresponded to 10 half-lives; this can only be taken as an upper limit, however, in that the NMR technique did not allow us to make observations at a shorter time interval after addition of the enzyme. Significantly, the half-life for the intermolecular displacement, by water, of *N*-methyl-*p*-toluenesulfonamide from **5bm** in the same D_2O buffer (no enzyme present) was found by direct observa-

(31) Enzyme inactivation was found to be time dependent. An incubation time of 10 min proved to be convenient. The partition number, when evaluated in this way, can vary with the incubation time used: see ref 3e.

(32) (a) Fersht, A., Ed. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and Company: New York, 1985; Chapter 3. (b) Strictly, $K_m = ((k_{-1} + k_2)/k_1)\,((k_3/(k_2 + k_3)) \approx k_{-1}/k_1 = K_s$ when $k_{-1} \gg k_2$ and $k_3 \gg k_2$. An alternative condition under which $K_m \approx K_s$ is when $k_{-1} = k_3$ which has been shown to be the case for a variety of β-lactamases (see ref 33).

(33) Christensen, H.; Martin, M. T.; Waley, S. G. *Biochem. J.* **1990**, *266*, 853.

(34) The thiazolidine-ring in **8′bm** is shown as being unprotonated on nitrogen consistent with the fact that the pK_a of the *N*-conjugate acid of a closely related structure is 5.14: see ref 19.

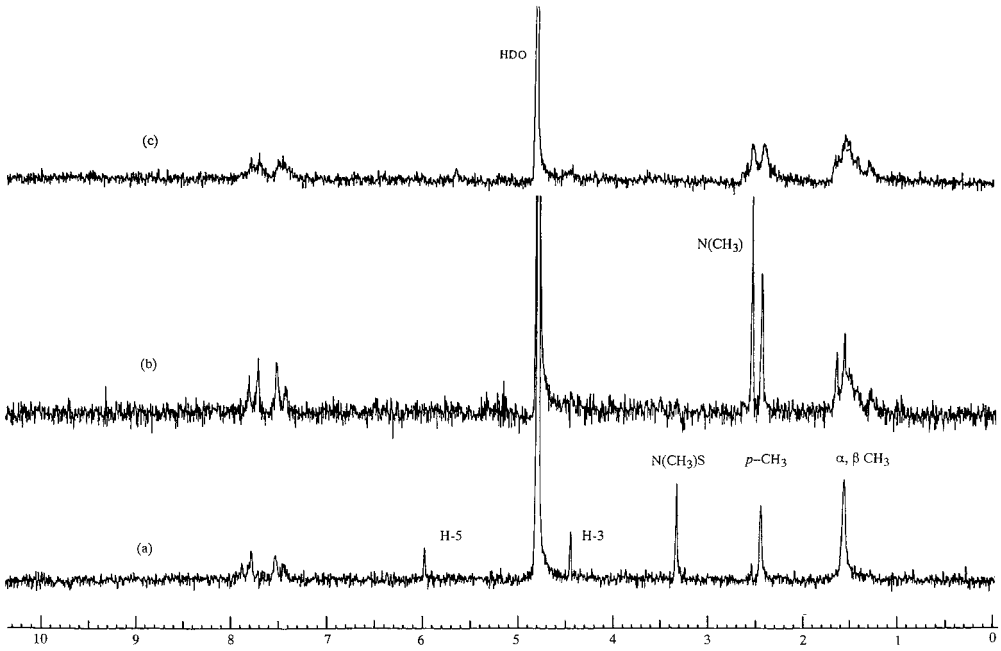


Figure 10. ^{1}H NMR spectra of (a) **5bm** in D_2O buffer (pD 7.2) at 25 °C, (b) reaction mixture of **5bm** (5 mg) 20 min after addition of β-lactamase enzyme, and (c) reaction mixture of **5bm** (17 mg) 5 min after enzyme addition; DSS was used as external reference.

tion (^{1}H NMR) to be approximately 300 min at 25 °C (Figure 11). The ratio of these two half-lives gave a value of 600 for $k_{intra}/(k_{inter}[D_2O])$; as $[D_2O] = 55.3$ M an apparent EM value (k_{intra}/k_{inter}) of 3.32×10^4 M was obtained for the intramolecular displacement of *N*-methyl-*p*-toluenesulfonamide from **8′bm**—for the reasons mentioned above this must be taken only as a lower limit (this value is labeled as an apparent one as the inter- and intramolecular nucleophiles are not the same, water vs the thiazolidine-ring sulfur; however, this EM value gives a practical measure of the kinetic advantage pertaining to the intramolecular process in aqueous solution). The identity of the coproduct formed from **5bm** in aqueous solution was not deducible from the ^{1}H NMR data. Although resonances attributable to the α,*β*-methyl group were obvious (Figure 10b), there were no peaks present which could have been attributed to H-5 or H-3 (albeit the residual water peak could have masked some detail), and neither was the presence of an aldimine hydrogen apparent. The exploitable potential of the *S*-amino sulfeniminopenicillin structure type, detailed in the following section, is dependent only on a rapid intramolecular displacement of the sulfur-attached moiety but not on the coproduct formed from the penicillin part of the molecule.

*The above results clearly identified **5bm** as a prototypic penicillin-based β-lactamase substrate with potential for development as a site-specific-release prodrug.*[35] Such substrates, bearing an appropriate sulfur-attached moiety, would allow exploitation of *β*-lactamase enzymes, present in many pathogenic bacteria, to catalyse the release of a bactericidal agent, or precursor, within the bacterial periplasmic space. In the context of enzymatic behavior such structures should have high k_{cat}/K_m and partition number values; they should behave as good substrates and as poor inhibitors. In the context of chemical reactivity the intramolecular displacement of the sulfur-attached moiety must be characterized by a high EM value. There must be a far greater propensity for intra- over intermolecular displacement in aqueous solution: the actual rate of this displacement would depend on the nature of the leaving group (Scheme 7). Thus **5bm** has a set of kinetic characteristics which match the required pattern shown in Scheme 7: the partition number gave k_3/k_4 directly as 1.5×10^4, while $k_{intra}/k_{inter} = EM_{app} > 3.32 \times 10^4$ M. The value of k_{cat}/K_m (6.26×10^5 M^{-1} min^{-1}) is not optimal, and neither is the *S*-amino moiety of **5bm** a toxic entity or precursor. Nonetheless this compound constitutes a valuable lead structure, and the delineation of its pattern of intrinsic reactivity is significant.[36]

It is apparent that *β*-lactamases are ancient enzymes—certainly they predate human usage of *β*-lactam antibiotics by eons—and there is evidence that they share a common ancestry with the transpeptidase domain of penicillin binding proteins (PBPs).[37] It has been shown[33] that a variety of *β*-lactamases are "fully efficient" enzymes, which means that they have evolved to become "perfect" catalysts[38] for hydrolysis of their natural

(35) For a definition and examples of site-specific-release prodrugs, see: Wermuth, C. G.; Gaignault, J.-C.; Marchandeau, C. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, 1996; Chapter 31.

(36) We are currently preparing a variant of **5bm** designed to directly establish (UV/vis spectroscopy, see ref 45b) if the release of the sulfur-attached moiety inside a *β*-lactamase-producing strain of a bacterium is faster that its release inside the corresponding nonproducing strain. Also, we are investigating how reduction of the imino group might influence k_{cat}/K_m and EM values. In the context of enhancing the value of k_{cat}/K_m it is relevant to note than all penicillin-based structures which are good substrates of *β*-lactamase enzymes have a saturated carbon, with an *R* configuration and bearing a hydrogen atom, at C-6.

(37) (a) Bush, K. In *Antibiotic Resistance: Origins, Evolution, Selection and Spread*; Wiley: Chichester (Ciba Foundation Symposium 207): 1997; p 152 and references therein. (b) Neuhaus, F. C.; Georgopapadakou, N. H. In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*; Sutcliffe, J., Georgopapadakou, N. H., Eds.; Chapman Hall: New York, 1992; Chapter 9 and references therein.

(38) (a) Albery, W. J.; Knowles, J. R. *Biochemistry* **1976**, *25*, 5631. (b) Albery, W. J.; Knowles, J. R. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 285.

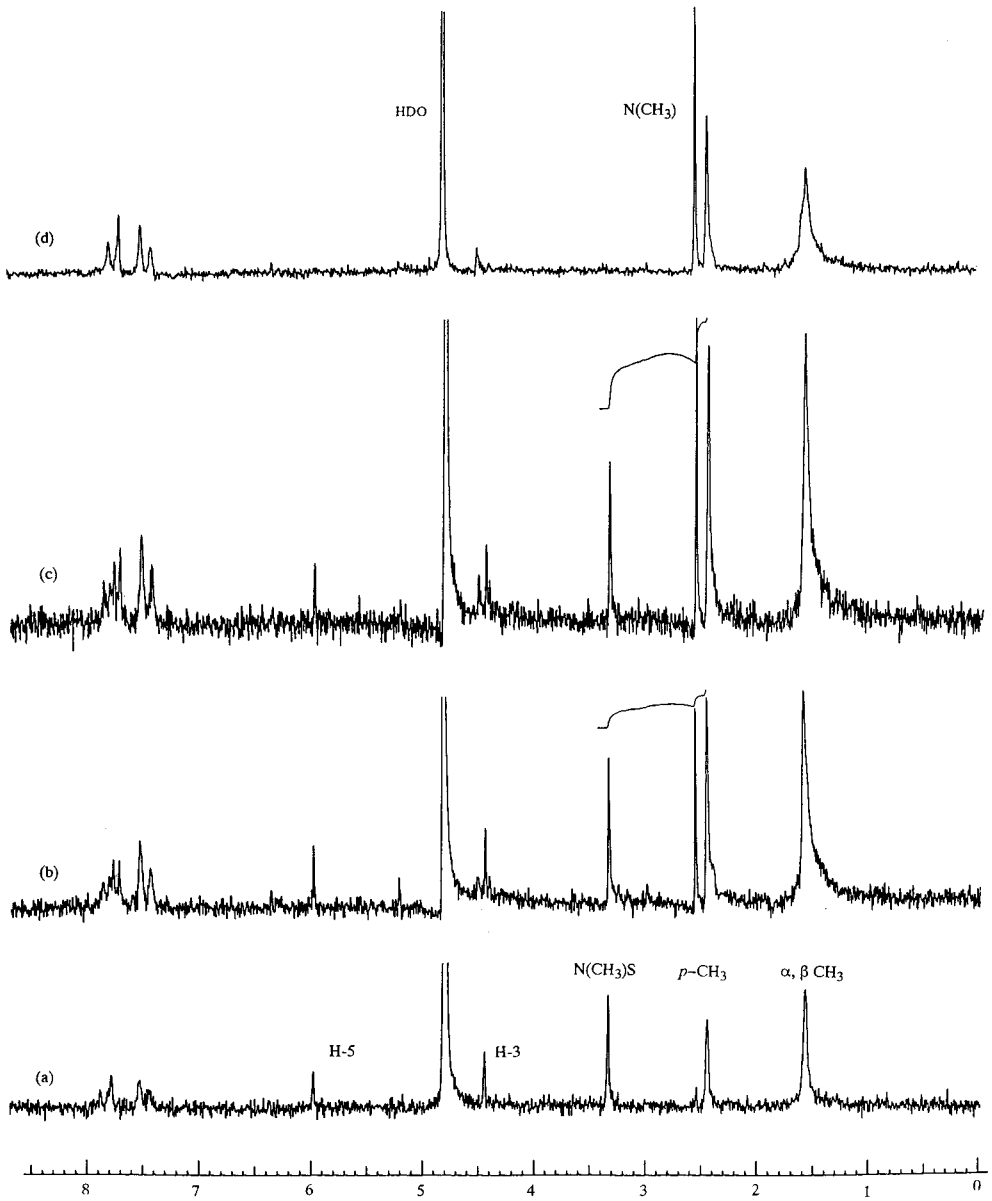


Figure 11. ^{1}H NMR spectra of (a) **5bm** in D_2O buffer (pD 7.2), and this reaction mixture after (b) 4, (c) 6, and (d) 20 h at 25 °C; DSS was used as external reference.

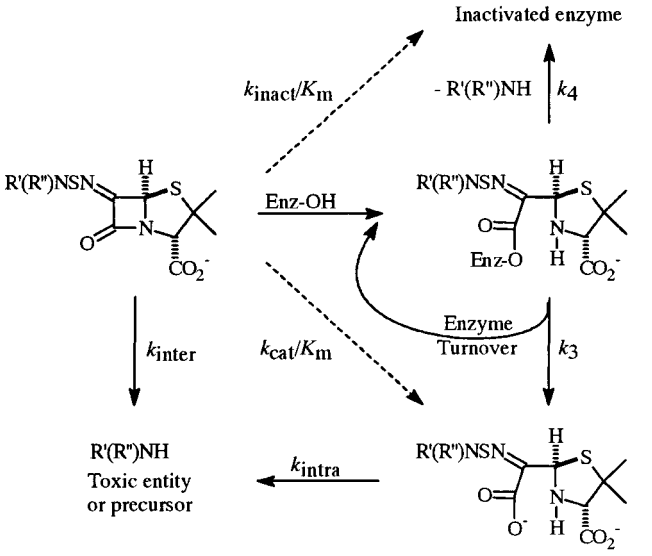
substrates—the naturally occurring inhibitors of transpeptidase enzymes. Therefore, some structures elaborated to be good substrates for β-lactamase enzymes must also behave as transpeptidase inhibitors: such structures, embodying β-lactamase catalyzed release of a bactericidal entity or precursor, would realize a checkmate stratagem with respect to the problem of β-lactamase-based resistance to antibiotics. The fact that a variety of semisynthetic penicillin structures, known to be transpeptidase inhibitors, are hydrolyzed at close to the diffusion-controlled rate by a number of β-lactamases[33] buttresses the strategy of developing penicillin-based β-lactamase substrates as site-specific-release prodrugs. The *S*-aminosulfeniminopenicillin structure constitutes a robust template for the elaboration of such materials. Synthesis of **4bm** and **4cm** is efficient, and variation of the sulfur-attached moiety is readily achievable by activation of the *S*-amino moiety toward direct displacement: cyanomethylation of the side-chain nitrogen in these structures allows for facile displacement of this whole group by a wide variety of nucleophiles.[39]

In the context of β-lactamase inhibition the thiazolidine-ring sulfides were much less active as inhibitors than the corresponding thiazolidine-ring sulfones.[40] Notable, however, was the finding that the inhibitory potency (Table 3) of **5b**, **5bm**, and **5cm** mirrored the nucleofugacity of the *S*-amino moiety as observed in their reactions in basic methanol. This correlation, although qualitative, indicated that displacement of the *S*-amino moiety at the enzyme active site was probably a key factor in the mode of inhibition operating for these structures; this is consistent with the overall reaction pattern shown in Scheme 7. There is a limit on the extent to which inhibitory potency could be enhanced by attachement of better leaving groups, in that displace-

(39) Smyth, T. P.; O'Donnell, M. E.; O'Connor, M. J.; St Ledger, J. O. Unpublished results.

(40) In all cases no recovery of enzyme activity was detected after up to 19 h.

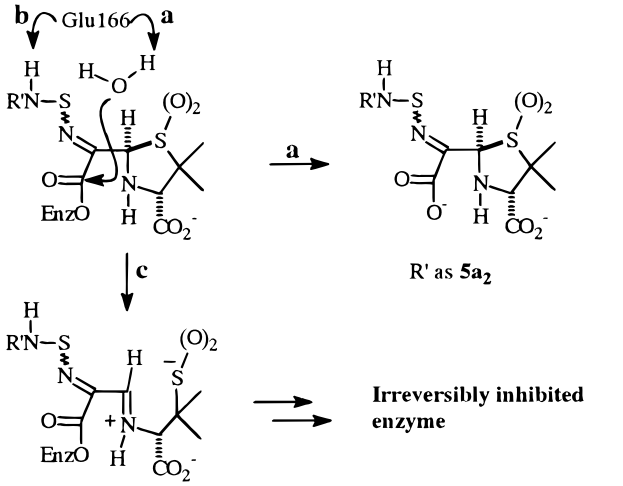
Scheme 7



Penicillin-based β-lactamase substrate as prodrug

Required kinetic pattern:	k_{inter}	<<	k_{intra}	<	k_3	>>	k_4	
Controlling factor:	EM		Leaving group ability of R'(R")NH		Molecular structure with high k_{cat} value			

Scheme 8



ment of such groups by opportunistic nucleophiles would occur prior to binding of the intact structure at the enzyme active site.

The sulfone **5a₂** was clearly the most active of all the structures prepared here, with values of IC_{50} and partition number lower than that of sulbactam by approximately a factor of 5. Significantly **5a₂** contained the poorest *S*-amino leaving group which implied that the mode of action of this sulfone was distinct from that of the thiazolidine-ring sulfides; notably, the thiazolidine-ring sulfur in the sulfone structures cannot behave as a nucleophile. We viewed the mode of action of **5a₂** as being similar to that of sulbactam[41] involving rapid scission of the thiazolidine sulfone ring following cleavage of the β-lactam ring (Scheme 8). It is possible that the greater β-lactamase inhibitory activity of **5a₂** over **5b₂m**, and over sulbactam was primarily due to the presence of a polar, potentially ionizable, hydrogen on the side chain of **5a₂**. The more efficient partitioning of the initial acylated enzyme derived from **5a₂** along an inhibitory route could be due to either slower hydrolytic cleavage (path a, Scheme 8) or more rapid sulfone elimination (path c, Scheme 8). A glutamic acid residue (Glu166) has been implicated as one of the catalytic components in the active site of class A β-lactamases, and it is evident from a combination of X-ray data and molecular modeling[42] that the 6-amino side chain of penicillin structures occupies a position proximate to Glu166. The formation of a strong hydrogen bond between Glu166 and the polar hydrogen on the side chain of **5a₂** (b, Scheme 8) would disrupt its role as a general base in hydrolysis of the acylated enzyme.

Bacteriological Activity. In a preliminary biological screening against three β-lactamase-producing *E. coli* strains (NCIMB 12091, 9517, and 9472),[43] **5a₂** showed synergy of action when combined 1:1 with penicillin G. Thus penicillin G showed no halo of inhibition up to 100 μg/mL whereas at 78 μg/mL **5a₂** plus penicillin G gave inhibition halos of 11−18 mm in diameter: these halo sizes were comparable to those obtained with a 1:1 combination of sulbactam and penicillin G at 78 μg/mL run at the same time. On its own **5a₂** showed no antibiotic activity against these *E. coli* strains (weak antibiotic activity was detected at high concentrations against *S. aureus* (NCIMB 8625 (ATCC 6538P)). In a more stringent screening **5a₂** was held at a fixed concentration of 4 μg/mL and combined, in turn, with varying concentrations of pipericillin (0.06−128 μg/mL) and tested against *E. coli* strains producing nine different β-lactamase types (see Experimental Section).[44] Under these circumstances no synergy of action was observed. The observation of synergy at high but not at low concentration was indicative of slow permeation of the *S*-aminosulfeniminopenicillin structures through the porins of the outer membrane of Gram-negative bacteria; in general the permeation rate of any given solute decreases in proportion to its external concentration when the concentration is low.[45a] The problem of poor permeation of β-lactamase inhibitors has been identified in a number of instances and constitutes a major barrier to the exploitation of many novel inhibitor structures. The combination of a β-lactamase inhibitor and an antibiotic involves competition between the two for diffusion through the porins, and this competition can reduce the therapeutic usefulness of effective β-lactamase inhibitors which have poor permeation characteristics. The monolactam structures synthesized by Miller and co-workers[46] while very effective enzyme inhibitors failed to show any synergy even at high concentration (100 μg/mL). Likewise the penicillin-based structures prepared by Buynak

(41) Knowles, J. R. *Acc. Chem. Res.* **1985**, *18*, 97.

(42) Ishiguru, M.; Imajo, S. *J. Am. Chem. Soc.* **1996**, *39*, 2207 and references therein.

(43) (a) These tests were carried out by CPA Laboratories, Birkenhead, Wirral, U.K. (b) NCIMB (National Collection of Industrial and Marine Bacteria), Aberdeen, Scotland.

(44) These tests were carried out at the Centre for Research in Anti-Infectives and Biotechnology, Creighton University, Omaha, NB.

(45) (a) Nikaido, H.; Rosenberg, E. Y. *J. Bacteriol.* **1983**, *153*, 241. (b) Nikaido, H.; Rosenberg, E. Y.; Foulds, J. *J. Bacteriol.* **1983**, *153*, 232. (c) Nikaido, H.; *Pharmacol. Ther.* **1985**, *27*, 231.

(46) Teng, M.; Miller, M. J.; Nicas, T. I.; Grissom-Arnold, J.; Cooper, R. D. G. *Biorg. Med. Chem. Lett.* **1993**, *1*, 151.

and co-workers[47] showed either none or only weak synergy which was not commensurate with their high level of enzyme inhibition. Permeation rates have been well studied by Nikaido and co-workers for both penicillin and cephalosporin antibiotic structures.[45] Hydrophobicity in particular seems to be critical in determining permeation rates for β-lactam-based structures where molecular weight is not a problem. Cephalosporin zwitterions with proximate opposing charges were found to have higher rates of permeation than either cephalosporin monoanions or zwitterions with remote opposing charges.[45] This identifies a structural feature, the incorporation of which on some of the compounds reported here, may merit evaluation.

Conclusions

An efficient and direct route to the preparation of a novel functionality—an *S*-aminosulfenimine—has been developed involving the reaction of selected amines with sulfur diimides. When incorporated as the side chain of a penicillin nucleus, the *S*-aminosulfenimine moiety has been shown to engender a pattern of reactivity not previously encountered in penicillin chemistry: expulsion of the *S*-amino moiety was found to occur, consequent on cleavage of the β-lactam ring, via a rapid intramolecular nucleophilic displacement reaction. This pattern held for cleavage of the ester derivatives in basic methanol and, significantly, on cleavage of a carboxylate salt structure by a β-lactamase enzyme in aqueous solution. This pattern of inherent chemical and enzymatic reactivity has identified the potential of these materials to be developed as penicillin-based β-lactamase substrates for use as site-specific-release prodrugs. Such materials should allow a strategy of combating β-lactamase-based resistance to antibiotics to be pursued, which is based on exploitation of the presence of these enzymes in pathogenic bacteria.

Experimental Section

General Procedures. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Column chromatography (dry flash technique)[48] was carried out using Kieselgel S (32−63 μm) with light petroleum ether (bp 60−80 °C) and ethyl acetate in various ratios as eluants; the specific solvent ratio in which a pure product was obtained is given below. β-Lactamase enzyme was obtained from Sigma, Type I ex. *Bacillus cereus*, EC 3.5.2.6. Elemental analyses were performed by the Microanalytical Laboratory, U.C.D., Dublin.

Preparation of *N*-Sulfinylcarbamate. Thionyl chloride (18.9 mL, 0.26 mol) in benzene (30 mL) and pyridine (38 mL, 0.42 mol) in benzene (20 mL) were added, with stirring, to ethyl carbamate (22.2 g, 0.25 mol) in benzene (60 mL) over 20 min. After an initial exotherm the reaction mixture was stirred for 1 h after which time the temperature had returned to ambient. Precipitated pyridium hydrochloride was filtered off and washed with benzene (50 mL) and the filtrate collected. The solvent was removed under reduced pressure and the light orange viscous residue distilled to yield pure *N*-sulfinylcarbamate (12.14 g, 0.90 mmol, 36% yield): bp_{20} 48 °C (lit.[49] $bp_{0.5}$ 23 °C); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.25 (t, J = 8 Hz, 3H), 4.4 (q, J = 8 Hz, 2H).

Preparation of *N*-Sulfinylsulfonamide. Thionyl chloride (60 mL, 0.82 mol) was added to *p*-toluenesulfonamide (30 g, 0.175 mol) in a 100 mL round-bottomed flask, and the mixture was heated to reflux for 8 h. Following this unreacted thionyl chloride was distilled off under reduced pressure, and further residual traces were removed by entrainment with benzene (3 × 50 mL) under reduced pressure at 80 °C. Upon cooling *N*-sulfinylsulfonamide solidified as a yellow solid[50] (30.4 g, 0.14 mol, 80%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.47 (s, 3H), 7.40 (d, J = 10 Hz, 2H), 7.94 (d, J = 10 Hz, 2H). This product was sufficiently pure for direct use in further reactions.

Preparation of Bis(ethoxycarbonyl)sulfur Diimide (2a).[51] *N*-Sulfinylcarbamate (6 g, 0.044 mol) and pyridine (0.2 mL, 2.5 mmol) were mixed in a small flask and a $CaCl_2$ drying tube attached. The mixture was allowed to stand at ambient temperature overnight. Crude **2a** was obtained as a light orange liquid (3.65 g, 0.019 mol, 85%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.22 (t, J = 8 Hz, 6H), 4.30 (q, J = 8 Hz, 4H). This material was used without further purification but was freshly prepared from the *N*-sulfinyl precursor as required.

Preparation of Bis(*p*-toluenesulfonyl)sulfur Diimide (2b).[52] *N*-Sulfinylsulfonamide (3.0 g, 13.8 mmol) was added to benzene (4 mL) together with pyridine (100 μL, 1.3 mmol), and the solution was allowed to stand at ambient temperature overnight by which time the reaction mixture had turned bright yellow and **2b** had crystallized out as a yellow solid (2.43 g, 6.55 mmol, 95%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.46 (s, 6H), 7.35 (d, J = 10 Hz, 4H), 7.86 (d, J = 10 Hz, 4H). This material was used without further purification but was freshly prepared from the *N*-sulfinyl precursor as required.

Preparation of Bis(*p*-chlorophenylsulfonyl)sulfur Diimide (2c). Thionyl chloride (20 mL, 280 mmol) was added to *p*-chlorophenylsulfonamide (10 g, 52 mmol) in a 100 mL round-bottomed flask, and the mixture was heated to reflux for 20 h. Following this, unreacted thionyl chloride was distilled off under reduced pressure and further residual traces were removed by entrainment with benzene (3 × 50 mL) under reduced pressure at 80 °C. Upon cooling, *N*-sulfinyl-*p*-chlorophenylsulfonamide solidified as a tan solid (11.9 g, 50.2 mmol, 96.6%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.45 (d, J = 9 Hz, 2H), 7.83 (d, J = 9 Hz, 2H). This product was sufficiently pure for direct use in further reactions. *N*-Sulfinyl-*p*-chlorophenylsulfonamide (1.46 g, 6.2 mmol) was added to benzene (1.6 mL) and pyridine (23 μL, 0.3 mmol) and the mixture was stirred at room temperature for 3 h by which time **2c** had crystallized out as a bright yellow solid (1.19 g, 2.89 mmol, 94%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.55 (d, J = 9 Hz, 4H), 8.00 (d, J = 9 Hz, 4H). This material was used without further purification but was freshly prepared from the *N*-sulfinyl precursor as required.

Preparation of Diphenylmethylpenicillanate (1). This compound was prepared, as the *p*-toluenesulfonate salt, according to the method of Petursson and Waley[53] for the free amine: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.60 (s, 3H), 4.5 (d, J = 4 Hz, 1H), 4.5 (s, 1H), 5.5 (d, J = 4 Hz, 1H), 6.95 (s, 1H), 7.3 (s, 10H).

Preparation of Diphenylmethyl *N*-Carbethoxy-*S*-aminosulfeniminopenicillanate (4a). 1 (as the free amine) (1 g, 2.6 mmol) was dissolved in dichloromethane (25 mL). **2a** (0.56 g, 2.9 mmol) was added dropwise, and the reaction mixture was stirred at ambient temperature for 10 min. The solution was then washed with distilled water (2 × 60 mL), and the organic layer was separated, dried, and concentrated under reduced pressure. Crude **4a** was obtained as a yellow solid. Using dry flash chromatography, the product eluted in the 60/40 fraction. Removal of the solvent under reduced pressure yielded **4a** as a light yellow solid (625 mg, 1.25 mmol,

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(48) Casey, M.; Leonard, J.; Lygo B.; Procter, G. *Advanced Practical Organic Chemistry*, Blackie: Glasgow, 1990; Chapter 9.
(49) Hanson. P.; Stockburn, W. A. *J. Chem. Soc., Perkin Trans. 2* **1985**, 589.
(50) Kresze, G.; Wucherpfennig, W. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 149.
(51) This is a modification of the procedure reported in Katz, T. J.; Shi, S. *J. Org. Chem.* **1994**, *59*, 8297.
(52) This is a modification of the procedure reported in ref 50.
(53) Petrusson, S.; Walley, S. G. *Tetrahedron* **1983**, *39*, 2465.

48%): mp 53−56 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.3 (m, 6H), 1.55 (s, 3H), 4.23 (q, J = 8 Hz, 2H), 4.7 (s, 1H), 5.79 (s, 1H), 6.61 (s, 1H, D_2O exchangeable), 6.95 (s, 1H), 7.3 (s, 10H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 14.5, 25.2, 34.1, 63.9, 65.0, 71.5, 72.0, 78.9, 127.2, 127.5, 128.5−129.0, 139.5, 154.0, 155.5, 165.0, 166.8; IR (KBr) 1785, 1740, 1660 cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_3O_5S_2$: C, 57.71; H, 5.01; N, 8.41. Found: C, 57.99; H, 5.24; N, 8.05.

Preparation of Diphenylmethyl *N*-Carbethoxy-*S*-aminosulfenimino penicillanate-1-sulfoxide (4a$_1$). **4a** (200 mg, 0.40 mmol) was dissolved in chloroform (5 mL) at 0 °C. To this was added a solution of *m*-chloroperbenzoic acid (130 mg, 0.75 mmol) in chloroform (5 mL). The mixture was stirred for 20 min, while allowed the temperature to rise to ambient. The solution was washed successively with 1 M sodium sulfite (50 mL), 5% sodium hydrogen carbonate (50 mL), and distilled water (50 mL). The organic layer was separated and dried and the solvent removed under reduced pressure to leave crude **4a$_1$** as a yellow solid. Using dry flash column chromatography the product eluted in the 25/75 light petroleum ether/ethyl acetate fraction. Removal of the solvent under reduced pressure yielded **4a$_1$** as a white solid (150 mg, 0.29 mmol, 73%): mp 107−110 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.30 (t, J = 7.5 Hz, 3H), 1.68 (s, 3H), 4.24 (q, J = 7.5 Hz, 2H), 4.81 (s, 1H), 5.4 (s, 1H), 7.00 (s, 1H), 7.2 (br s, 1H, D_2O exchangeable), 7.35−7.4 (s, 10H); IR (KBr) 1800, 1750, 1665 cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_3O_6S_2$: C, 55.91; H, 4.88; N, 8.15. Found: C, 56.02; H, 5.09; N, 7.71.

Preparation of Diphenylmethyl *N*-Carbethoxy-*S*-aminosulfeniminopenicillanate-1-sulfone (4a$_2$). A solution of **4a$_1$** (1 g, 1.9 mmol) in dichloromethane (25 mL) was added to an aqueous solution of $KMnO_4$ (300 mg, 1.9 mmol) and tetrabutylammonium hydrogen sulfate (1 mg, 2.9 μmol) as phase transfer catalyst and the mixture vigorously stirred at room temperature for 1 h. The organic layer was separated, and the precipitated manganese dioxide was filtered off. The organic layer was dried and concentrated under reduced pressure to leave **4a$_2$** as a colorless solid. Using dry flash column chromatography **4a$_2$** eluted in the 30/70 light petroleum ether/ethyl acetate fraction. The solvent was removed under reduced pressure to leave **4a$_2$** as a white foamy solid (700 mg, 1.32 mmol, 69%): mp 135 °C (dec); ^{1}H NMR (90 MHz, CD_3OD) δ 1.15 (s, 3H), 1.30 (t, J = 7.5 Hz, 3H), 1.58 (s, 3H), 4.24 (q, J = 7.5 Hz, 2H), 4.62 (s, 1H), 5.1 (s, 1H), 6.98 (s, 1H), 7.29−7.40 (s, 11H); IR (KBr) 1795, 1750 cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_3O_7S_2$: C, 54.24; H, 4.71; N, 7.91. Found: C, 54.37; H, 4.84; N, 7.57.

Preparation of Diphenylmethyl *N*-Tosyl-*S*-aminosulfeniminopenicillanate (4b). **1** (as the free amine) (2 g, 5.2 mmol) was dissolved in dichloromethane (25 mL). **2b** (2.2 g, 6 mmol) was added, and the mixture was stirred for 5 min, followed by the slow addition of light petroleum ether (20 mL). The precipitated sulfonamide was filtered off, and the filtrate was washed with distilled water (3 × 200 mL). On standing **4b** (900 mg) precipitated from the organic layer and was filtered and dried. Further crude **4b** was obtained on concentration of the filtrate. Using dry flash chromatography **4b** eluted in the 55/45 and 50/50 light petroleum ether/ethyl acetate fractions to yield an additional 600 mg (total yield was 1.5 g, 2.58 mmol, 49%): mp 177−179 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.55 (s, 3H), 2.4 (s, 3H), 4.7 (s, 1H), 6.05 (s, 1H), 6.95 (s,1H), 7.05 (s, 1H, D_2O exchangeable), 7.25−7.87 (m, 14 H); IR (film) 3207, 1779, 1758, 1667 cm^{-1}; UV log ϵ = 4.09, λ_{289} (MeOH). Anal. Calcd for $C_{28}H_{27}N_3O_5S_3$: C, 57.83; H, 4.65; N, 7.23. Found: C, 57.59; H, 4.64; N, 7.20.

Preparation of Diphenylmethyl *N*-Methyl-*n*-tosyl-*S*-aminosulfeniminopenicillanate (4bm). **4b** (1 g, 1.72 mmol) was dissolved in chloroform (10 mL). Proton Sponge (400 mg, 1.87 mmol) was added and the mixture stirred until the solution was clear. Methyl iodide (0.25 mL, 4.0 mmol) was added and the mixture stirred briefly. The solution was allowed to stand at ambient temperature for 12 h. The Proton Sponge−HI salt which had crystallized out of solution was filtered off. The filtrate was concentrated, and the product was purified by dry flash chromatography. **4bm** eluted in the 65/35 and 60/40 light petroleum ether/ethyl acetate fractions and on removal of the solvent under reduced pressure the product was obtained as a light yellow solid (700 mg, 1.17 mol, 68%): mp 85−89 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.30 (s, 3H), 1.57 (s, 3H), 2.42 (s, 3H), 3.27 (s, 3H), 4.7 (s, 1H), 6.2 (s, 1H), 6.97 (s, 1H), 7.25−7.85 (m, 14H); IR (KBr) 1775, 1740, 1660 cm^{-1}; UV log ϵ = 4.10, λ_{290} (MeOH). Anal. Calcd for $C_{29}H_{29}N_3O_5S_3$: C, 58.47; H, 4.90; N, 7.05. Found: C, 58.45; H, 4.93; N, 6.81.

Preparation of Diphenylmethyl *N*-Tosyl-*S*-aminosulfeniminopenicillanate-1- sulfoxide (4b$_1$). This was prepared in a manner similar to that given for **4a$_1$** above. Using dry flash column chromatography the product eluted in the 30/70 light petroleum ether/ethyl acetate fraction. Removal of the solvent under reduced pressure gave **4b$_1$** as a foamy white solid (290 mg, 0.48 mmol, 68%): mp 145 °C (dec); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.1 (s, 3H), 1.7 (s, 3H), 2.43 (s, 3H), 4.82 (s, 1H), 6.30 (s, 1H), 7.02 (s, 1H), 7.25−7.85 (m, 15 H); IR (KBr) 1805, 1745, 1660 cm^{-1}.

Preparation of Diphenylmethyl *N*-Methyl-*N*-tosyl-*S*-aminosulfeniminopenicillanate-1-sulfoxide (4b$_1$m). This was prepared in a manner similar to that given for **4a$_1$** above. Using dry flash column chromatography the product eluted in the 60/40 light petroleum ether/ethyl acetate fraction. Removal of the solvent under reduced pressure gave **4b$_1$m** as a foamy white solid (650 mg, 1.06 mmol, 62%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.04 (s, 3H), 1.70 (s, 3H), 2.45 (s, 3H), 3.30 (s, 3H), 4.88 (s, 1H), 6.03 (s, 1H), 7.35 (s, 1H), 7.25−7.85 (m, 14 H).

Preparation of Diphenylmethyl *N*-Methyl-*N*-tosyl-*S*-aminosulfeniminopenicillanate-1-sulfone (4b$_2$m). This was prepared from **4b$_1$m** in a manner similar to that given for **4a$_2$** above. Using dry flash column chromatography the product eluted in the 50/50 light petroleum ether/ethyl acetate fraction. Removal of the solvent under reduced pressure gave **4b$_2$m** as a foamy white solid (110 mg, 0.18 mmol, 63%): mp 105 °C (dec); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.21 (s, 3H), 1.60 (s, 3H), 2.45 (s, 3H), 3.28 (s, 1H), 4.68 (s, 1H), 5.72 (s, 1H), 7.02 (s, 1H), 7.25−7.87 (m, 14 H); IR (KBr) 1800, 1750, 1660 cm^{-1}.

Preparation of Diphenylmethyl *N*-Methyl-*N*-(*p*-chlorophenylsulfonyl)-*S*-aminosulfeniminopenicillanate (4cm). **1** (as the free amine) (1 g, 2.7 mmol) was dissolved in dichloromethane (35 mL), and to this was added **2c** (1.28 g, 3 mmol). The mixture was stirred overnight at room temperature. Material which had precipitated at that point was removed by filtration. Distilled water (2 mL) was added to the supernatant (to decompose unreacted sulfur diimide) followed by the slow addition of light petroleum ether, and the mixture was stirred for 1 h at room temperature by which time a white precipitate had formed. This was removed by filtration and dried under vacuum at ambient temperature to yield a white solid (800 mg) which was found to be a mixture of diphenylmethyl *N*-(*p*-chlorophenylsulfonyl)-*S*-aminosulfeniminopenicillanate and *p*-chlorophenylsulfonamide in a ratio of 1:2 by ^{1}H NMR. This material was methylated, without further purification, in a manner similar to that used to prepare **4bm**. Using dry flash chromatography **4cm** eluted in the 80/20 and 90/10 dichloromethane/light petroleum ether fractions. On removal of the solvent under reduced pressure **4cm** (460 mg, 0.75 mmol, 26% starting from **1**) was obtained as a yellow solid: mp 126−129 C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.30 (s, 3H), 1.57 (s, 3H), 3.20 (s, 3H), 4.70 (s, 1H), 6.12 (s, 1H), 6.94 (s, 1H), 7.20−7.92 (m, 14 H); IR (KBr) 1781, 1745, 1644 cm^{-1}. Anal. Calcd for $C_{28}H_{26}N_3O_5ClS_3$: C, 54.58; H, 4.25; N, 6.82. Found: C, 54.90; H, 3.90; N, 6.46.

Removal of the Protecting Group and Isolation of Salts.[54] Aluminum trichloride (133.5 mg, 1 mmol) in nitromethane (3 mL) was added to dichloromethane (15 mL) containing the required diphenylmethyl *S*-aminosulfeniminopenicillin ester (0.5 mmol) at −80 °C and the mixture stirred for 15 min. The was followed by the addition of sodium bicarbonate (5%, 50 mL) and ethyl acetate or dichloromethane (30 mL), and the mixture was vigorously stirred while allowing the temperature to rise to ambient. The mixture was further diluted by the addition of distilled water (100 mL). The

organic layer was separated and discarded. The aqueous layer was repeatedly filtered (4−6 times) until clear, acidified to pH 2.2 with 1 M HCl, and extracted with ethyl acetate (20 mL). The organic layer was separated and concentrated to yield the free acid of the *S*-aminosulfeniminopenicillin. The sodium salts were obtained by extracting an ethyl acetate solution (20 mL) of an accurately weighed amount the free acid (typically 0.25 mmol) with aqueous sodium bicarbonate (0.20 mmol), i.e., with a deficiency of bicarbonate. The aqueous layer was separated and freeze-dried to yield the sodium salts as yellow solids.

Sodium *N*-carbethoxy-*S*-aminosulfeniminopenicillanate (5a) (84 mg, 0.236 mmol, 32%): ^{1}H NMR (90 MHz, D_2O) δ 1.32 (t, J = 7.5 Hz, 3H), 1.53 (s, 3H), 1.57 (s, 3H), 4.27 (q, J = 7.5 Hz, 2H), 4.4 (s, 1H), 5.95 (s, 1H); IR (KBr) 1770, 1615 cm^{-1}. Anal. Calcd for $C_{11}H_{14}N_3O_5S_2Na\cdot 2H_2O$: C, 33.75; H, 4.60; N, 10.73. Found: C, 33.65; H, 4.36; N, 10.37.

Sodium *N*-carbethoxy-*S*-aminosulfeniminopenicillanate-1-sulfoxide (5a$_1$) (110 mg, 0.296 mmol, 26%): ^{1}H NMR (90 MHz, D_2O) δ 1.30 (t, J = 7.5 Hz, 3H), 1.33 (s, 3H), 1.68 (s, 3H), 4.28 (q, J = 7.5 Hz 2H), 4.50 (s, 1H), 6.00 (s, 1H); IR (KBr) 1780, 1660, 1620 cm^{-1}.

Sodium *N*-carbethoxy-*S*-aminosulfeniminopenicillanate-1-sulfone (5a$_2$) (70 mg, 0.18 mmol, 24%): ^{1}H NMR (90 MHz, D_2O) δ 1.30 (t, J = 7.5 Hz, 3H), 1.43 (s, 3H), 1.55 (s, 3H), 4.2 (q, J = 7.5 Hz, 2H), 4.25 (s, 1H), 5.36 (s, 1H); IR (KBr) 1785, 1620 cm^{-1}. Anal. Calcd for $C_{11}H_{14}N_3O_7S_2Na\cdot 2.5H_2O$: C, 30.55; H, 4.42; N, 9.71. Found: C, 30.19; H, 4.10; N, 9.88.

Sodium *N*-tosyl-*S*-aminosulfeniminopenicillanate (5b) (100 mg, 0.23 mmol, 16%): ^{1}H NMR (90 MHz, D_2O) δ 1.5 (s, 6H), 2.37, (s, 3H), 4.34 (s, 1H), 6.2 (s, 1H), 7.3−7.8 (m, 4H); IR (KBr) 1775, 1615 cm^{-1}.

Sodium *N*-tosyl-*S*-aminosulfeniminopenicillanate-1-sulfoxide (5b$_1$) (205 mg, 0.45 mmol, 33%): ^{1}H NMR (90 MHz, D_2O) δ 1.32 (s, 3H), 1.65 (s, 3H), 2.40 (s, 3H), 4.48 (s, 1H), 6.17 (s, 1H), 7.3−7.8 (m, 4H); IR (KBr) 1785, 1625 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_3O_6S_3Na\cdot 3.5H_2O$: C, 34.84; H, 4.45; N, 8.13. Found: C, 34.41; H, 3.99; N, 7.90.

Sodium *N*-methyl-*N*-tosyl-*S*-aminosulfeniminopenicillanate (5bm) (84 mg, 0.186 mmol, 16%): ^{1}H NMR (90 MHz, D_2O) δ 1.55 (s, 6H), 2.43 (s, 3H), 3.33 (s, 3H), 4.45, (s, 1H), 5.97 (s, 1H), 7.40−7.85 (m, 4H); IR (KBr) 1770, 1620 cm^{-1}; corresponding free acid ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.52 (s, 6H), 2.45 (s, 3H), 3.27 (s, 3H), 4.50, (s, 1H), 6.10 (s, 1H), 7.30−7.85 (m, 4H). Anal. Calcd for $C_{16}H_{19}N_3O_5S_3$: C, 44.74; H, 4.45; N, 9.78. Found: C, 44.96; H, 4.42; N, 9.87.

Sodium *N*-methyl-*N*-tosyl-*S*-aminosulfeniminopenicillanate-1-sulfone (5b$_2$m) (25 mg, 0.052 mmol, 28%): ^{1}H NMR (90 MHz, D_2O) δ 1.45 (s, 6H), 2.37 (s, 3H), 3.30 (s, 3H), 4.44 (s, 1H) 5.85 (s, 1H), 7.3−7.8 (m, 4H); IR (KBr) 1785, 1625 cm^{-1}.

***N*-Methyl-*N*-(*p*-chlorophenylsulfonyl)-*S*-aminosulfeniminopenicillanic acid (5cm-free acid)** (215 mg, 0.455 mmol, 65%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.61 (s, 6H), 3.31 (s, 3H), 3.70 (br s, exchangeable with D_2O), 4.65, (s, 1H), 6.03 (s, 1H), 7.55 (d, J = 6 Hz, 2H), 7.86 (d, J = 6 Hz, 2H).

***N*-Tosyl-*S*-aminosulfeniminoalanine Diphenylmethyl Ester (6).** Alanine diphenylmethyl ester (1.79 g, 7.04 mmol) was added to **2b** (3.12 g, 8.45 mol) in dichloromethane (60 mL) and stirred at room temperature overnight. Distilled water (2 mL) was added (to decompose unreacted sulfur diimide) followed by the addition of light petroleum ether (60 mL) and the solution stirred for 20 m to complete the precipitation of *p*-toluenesulfonamide which was removed by filtration. The filtrate was washed with water (3 × 50 mL), separated, and dried ($MgSO_4$) and the solvent removed under reduced pressure to yield crude **6** as a pale yellow solid (1.56 g, 3.43 mmol, 49%). This was purified by (wet) flash chromatography using 80/20 light petroleum ether/ethyl acetate to yield pure **6** as a pale yellow solid: mp 121−123 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.10 (s, 3H), 2.38 (s, 3H), 6.90 (s, 1H), 7.21 (d, J = 6 Hz, 2H), 7.32 (s, 10H), 7.79 (d, J = 6 Hz, 2H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 21.36 (CH_3C=N and *p*-CH_3), 79.11, 126.94−129.86, 138.96, 143.78, 160.0; UV log ϵ = 3.94 λ_{283} (MeOH); FAB-MS m/z 587 (4.6%) (M + Cs^+). Anal. Calcd for $C_{23}H_{22}N_2O_4S_2$: C. 60.79; H, 4.84; N, 6.16. Found: C, 60.65; H, 4.76; N, 6.03.

***N*-Methyl-*N*-tosyl-*S*-aminosulfeniminoalanine Diphenylmethyl Ester (7).** **6** (338 mg, 0.75 mmol) was added to chloroform (10 mL). To this was added methyl iodide (154 mg, 1.09 mmol) and Proton Sponge (0.179 g, 0.75 mmol), and the solution was stirred at room temperature for 30 min. On standing overnight Proton Sponge−HI salt had precipitated from solution and was filtered off and the solution concentrated under reduced vacuum to leave crude **7** as a brown liquid. Using (wet) flash chromatography the product was eluted with light petroleum ether/ethyl acetate 20/80. A white solid crystallized on standing, and this was filtered and dried to yield pure **7** (216 mg, 0.46 mmol, 62%): mp 125−127 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.10 (s, 3H), 2.38 (s, 3H), 3.29 (s, 3H), 6.92 (s, 1H), 7.22 (d, J = 6 Hz, 2H), 7.37 (s, 10 H), 7.77 (d, J = 6 Hz, 2H). Anal. Calcd for $C_{24}H_{24}N_2O_4S_2$: C. 61.52; H, 5.16; N, 5.98. Found: C, 61.79; H,5.14; N, 6.04.

Preparation of 8a. To a solution of **4a** (50 mg, 0.1 mmol) in methanol (5 mL) was added Et_3N (15 μL). After the solution was briefly stirred, the flask was sealed and allowed to stand at room temperature for 90 min. The reaction mixture was diluted with dichloromethane (20 mL) and washed with distilled water (2 × 50 mL). The organic layer was separated, dried, and concentrated under reduced pressure to yield **8a** as a gel-like solid (42 mg, 0.079 mmol, 79%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.09 (s, 3H), 1.25 (t, J = 7.5 Hz, 3H), 1.55 (s, 3H), 3.83 (s, 3H), 3.88 (s, 1H), 4.20 (q, J = 7.5 Hz, 2H), 5.71 (s, 1H), 6.65 (br s, 1H, D_2O exchangeable), 6.98 (s, 1H), 7.35 (s, 10H) (see Figure 3b).

Preparation of 8b. To a solution of **4b** (50 mg, 0.09 mmol) in methanol (5 mL) was added 0.8 M sodium methoxide in methanol (0.5 mL). (Stronger base was required here compared to **8a** above as ionization of the side chain of **4b** reduced the susceptibility of the β-lactam ring to cleavage by nucleophiles.) The solution was briefly stirred and allowed to stand at room temperature for approximately 1.5 min, or until the reaction mixture turned an orange color. The reaction mixture was diluted with dichloromethane (20 mL) and washed with distilled water (3 × 50 mL). The organic layer was separated, dried, and concentrated under reduced pressure to yield **9b** as a white solid (35 mg): ^{1}H NMR (90 MHz, $CDCl_3$), see Figure 4b.

Formation of 10 from 8b. A solution of **8b** in $CDCl_3$ in an NMR tube was monitored after 6 h. A new set of peaks was observed to appear: δ 1.31 (s, 3H), 1.57 (s, 3H), 3.87 (s, 1H), 3.88 (s, 3H), 5.72 (s, 1H), 7.00 (s, 1H), 7.25−7.38 (m, 11H), see Figure 4c. A fine precipitate which had formed was filtered off and dried: mp 137−139 °C (lit.[55] mp 138−139 °C *p*-toluenesulfonamide); ^{1}H NMR (90 MHz, DMSO/$CDCl_3$) δ 2.41 (s, 3H), 6.95 (br s, 2H, D_2O exchangeable), 7.32 (d, J = 14 Hz), 7.82 (d, J = 14 Hz, 2H). **9** was purified chromatographically as indicated below.

Formation and isolation of 9 from 4bm. To a solution of **4bm** (250 mg, 0.47 mmol) in methanol (15 mL) was added Et_3N (25 μL). The solution was briefly stirred, sealed, and maintained at 25 °C for 4 h. The reaction mixture was diluted with dichloromethane (50 mL) and washed with distilled water (2 × 150 mL). The organic layer was separated, dried, and concentrated under reduced pressure. Using dry flash column chromatography the product eluted in the 50/50 light petroleum ether/ethyl acetate fraction. Removal of the solvent under reduced pressure yielded **9** as a yellow gel-like solid (190 mg, 0.43 mmol, 91%). This sample was further purified by another stage of (wet) flash chromatography using dichloromethane as eluant. Removal of the solvent under reduced pressure yielded a yellow solid which was further dried under vacuum at ambient temperature (40 mg, 0.09 mmol): ^{1}H NMR ($CDCl_3$, 90 MHz), see Figure 5 and Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Figure 6 and Table 2. Anal. Calcd for $C_{22}H_{22}N_2S_2O_4$: C, 59.65; H, 4.97; N, 6.32. Found: C, 58.06; H, 5.11; N, 5.90. For the kinetic profiles shown in Figures 8

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and 9 the above procedure, omitting the chromatographic purification step, was repeated for each reaction time shown using 25−30 mg samples of **4bm** and **4cm** in MeOH (3 mL) and Et_3N (15 μL).

Enzyme Inhibition Studies. Phosphate buffer (0.1 M, pH 7.2) was used for all solutions. EDTA (50 μM) was present to suppress activity of type II β-lactamase (a zinc dependent metalloenzyme)[56] present at low levels in the Sigma type I β-lactamase preparations. The specific activity for the hydrolysis of benzylpenicillin at pH 7.2, 25 °C was 2.71 mmol min^{-1} per mg of protein. The temperature was held constant at 25 ± 0.1 °C for the kinetic runs. A stock solution was made up by dissolving a portion of enzyme (0.25 mg of protein based on log ϵ = 4.41 at 280 nm for *B. cereus* Type I)[57] in 2.5 mL of buffer. From this 250 μL was withdrawn and made up to 5 mL with buffer to provide the working enzyme solution. The incubation mixture was made up by adding the inhibitor to the working enzyme solution to give the desired inhibitor concentration (20−2000 μM). After a standard 10 min incubation time, 50 μL aliquots were withdrawn from this solution and injected into benzylpenicillin solution (0.5 mM) in a cuvette, and the rate of hydrolysis of the benzylpenicillin measured by following the zero order decrease in absorbance at 232 nm with time. Note that **5cm** was isolated as the free acid and for all enzyme experiments 50 μL of a THF solution of **5cm** was added to the aqueous buffer containing the enzyme; this volume of THF had no effect on its own on enzyme activity.

For the measurement of k_{cat}/K_m the enzyme concentration was 0.7 μM, and the concentration of **5bm** was varied from 15 to 60 μM. The decrease in absorbance at 290 nm was monitored as a function of time; the initial rate was determined from the linear absorbance change within the first 10 s (0.3 absorbance units/m with 60 μM **5bm**); Δe_{290} was 11 400 M^{-1} cm^{-1}. Under these conditions the initial rate varied with the substrate concentration; however, as the variation in rate was found to be directly proportional to the substrate concentration over this range, it was not possible to determine accurately a value for K_m.

Bacteriological Screening. Test organisms included nine strains of *E. coli* (C600N) involving producers of seven different β-lactamases[58] (TEM-1, OSBL; TEM-10; SHV-1; SHV-4; MIR-1; AmpC (Ecl-c); AmpC (Ecl-i)).

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Supporting Information Available: ^1H NMR spectra are available for the esters **4b$_1$**, **4b$_2$m**, **4cm-free acid**, and the salts **5a$_1$**, **5b**, and **5b$_2$m** for which satisfactory elemental analysis was not obtained. The following are also included: ^1H NMR spectrum of **4b$_{(side-chain\ anion)}$** and variable temperature spectra of **4bm**, **7**, and the product set (3*S*,5*S*)-**9** and (3*S*,5*R*)-**9**, ^{13}C NMR of **4a** (decoupled and off resonance) and **6** (decoupled) (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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