Extending the β-Lactamase-Dependent Prodrug Armory: *S*-Aminosulfeniminocephalosporins as Dual-Release Prodrugs

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Cephalosporins bearing an *S*-aminosulfenimine (R′(R″)NSN=) side chain at the 7-position are prototypic examples of a novel class of β-lactamase-dependent prodrug. Enzyme-catalyzed hydrolysis of the β-lactam ring in these structures triggers release of both the 3′-acetoxy group and the side chain sulfur-attached *S*-amino moiety as R′(R″)NH. This reactivity pattern should allow site-specific corelease of two distinct drug components from a cephalosporin, thereby providing a singular enhancement to the capacity of a cephalosporin as a prodrug nucleus; a key advantage of a dual-release prodrug is the potential to establish synergy between the coreleased structures. Areas for exploitation of this new structure type are antibody-directed enzyme prodrug therapy (ADEPT), which is a key emerging anticancer therapy, and the further development of site-specific-release prodrugs to combat the problem of β-lactamase-based resistance to antibiotics.

Introduction

The site-specific release of drug substances from nontoxic precursors allows integration of a number of therapeutically desirable objectives. Thus, systemic delivery can be combined with targeted release of a toxic entity in a manner which minimizes damage to the host organism. Implementation of this strategy depends on the presence of an enzyme unique to the invasive organism and on the availability of substrates which, on turnover by this enzyme, undergo a transformation leading to the release of a toxic entity.[1] In this context β-lactamase enzymes, and their substrates, are of particular interest. The presence of these enzymes in many pathogenic bacteria is, now,[2] a naturally occurring instance of a required element of this prodrug strategy, whereas their use in antibody-directed enzyme prodrug therapy (ADEPT) is a man-made arrangement.[3] Until recently the only substrates exploitable as β-lactamase-dependent prodrugs were cephalosporin-based. Expulsion of the 3′-substituent consequent on cleavage of the β-lactam ring is an inherent feature of their chemical reactivity;[4] considerable use of this has been made in the ADEPT approach to cancer chemotherapy,[3] and some potentially useful dual-acting antibiotic agents have also been prepared.[4d] A short time ago we reported on the synthesis and reactivity of *S*-aminosulfeniminopenicillins and identified these as the first penicillins to behave as β-lactamase-dependent prodrugs.[5] Their inherent chemical reactivity involves a rapid intramolecular displacement, characterized by a high effective molarity,[6] of the (side chain) sulfur-attached moiety consequent on cleavage of the β-lactam ring (Scheme 1). The basis of this novel reactivity pattern was identified and is such that this mode could be incorporated into other β-lactam structures,[7] thereby extending the β-lactamase-dependent prodrug armory. In particular, elaboration of cephalosporins bearing an *S*-aminosulfenimine, or related side chain, at the 7-position would provide a singular enhancement of the prodrug strategy, as such compounds would contain two distinct structural fragments, the expulsion of which should be triggered by cleavage of the

(1) (a) Wermuth, C. G.; Gaignault, J.-C.; Marchandeau, C. In *Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, 1996; Chapter 31. (b) Clearance of the intact precursor from the body must be faster than the rate of its background hydrolysis/breakdown; β-lactams are readily cleared from the blood stream via tubular secretion: Ehlert, C.; Strunz, H.; Visser, K.; Wiese, M.; Seydel, J. K. *J. Pharm. Sci.* **1998**, *87*, 101 and references therein.

(2) An alarming proportion of bacterial pathogens exhibit high-level resistance to β-lactam antibiotics due to the presence of β-lactamase enzymes, and to date over 190 varieties of these enzymes have been characterized: (a) Neu, H. C. *Science* **1992**, *257*, 1064. Nicolas-Chanoine, M. H. *Int. J. Antimicrob. Agents* **1996**, *7*, S21−S26. (b) Bush, K.; Jacoby, G. A.; Medeiros, A. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 1211.

(3) For leading references see: (a) Svensson, H. P.; Frank, I. S.; Berry, K. K.; Senter, P. D. *J. Med. Chem.* **1998**, *41*, 1507 and references therein. (b) Bagshawe, K. D.; Begent, R. H. J. *Adv. Drug Delivery Rev.* **1996**, *22*, 365. (c) Bagshawe, K. D.; Sharma, S. K.; Springer, C. J.; Antoniw, P. *Tumour Targeting* **1995**, *1*, 17. (d) Rodrigues, M. L.; Carter, P.; Wirth, C.; Mullins, S.; Lee, A.; Blackburn, B. K. *Chem. Biol.* **1995**, *2*, 223.

(4) (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) Pratt, R. F.; Faraci, W. S. *J. Am. Chem. Soc.* **1986**, *108*, 5328. (d) 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. 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) Expulsion of the C-2′ moiety, following cleavage of the β-lactam ring, has also been shown to occur with penems: Perrone, E.; Jabés, D.; Alpegiani, M.; Andreini, B. P.; Bruna, C. D.; Nero, S. D.; Rossi, R.; Visentin, G.; Zarini, F.; Franceshi, G. *J. Antibiot.* **1992**, *45*, 589.

(5) Smyth, T. P.; O'Donnell, M. E.; O'Connor, M. J.; St Ledger, J. O. *J. Org. Chem.* **1998**, 63, 7600.

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

(7) The basis of, and experimental evidence for, the rapid intramolecular displacement has been elaborated in detail, including participation by the thiazolidine-ring sulfur and nitrogen atoms and the identity of the coproduct formed, in the case of reactions of the penicillin esters in basic methanol. While it did not prove feasible to obtain as detailed a picture in the case of reactions of the penicillin salts in aqueous solution, the key finding of a very rapid intramolecular displacement of the *S*-amino moiety from the β-lactam-ring-cleaved structure was established unambiguously.[5]

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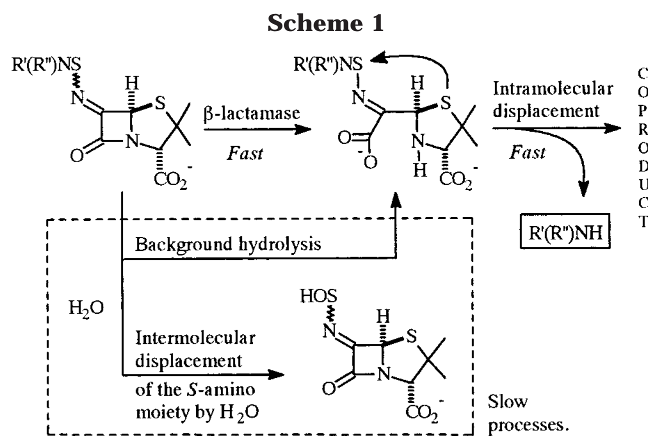
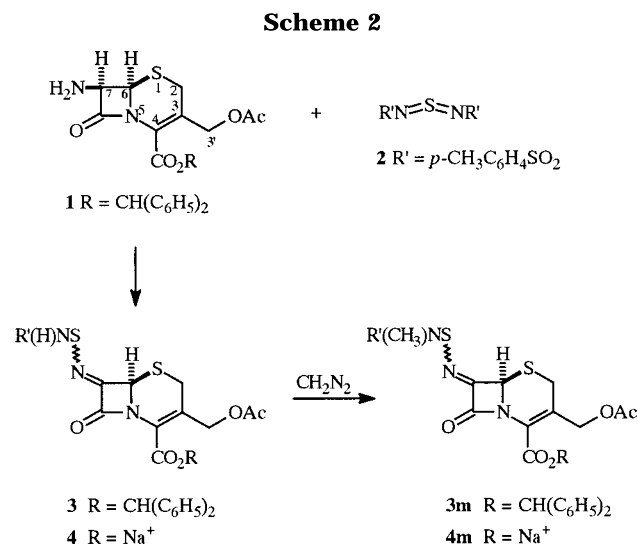
Scheme 1



Scheme 2



β-lactam ring. Herein we report that *S*-aminosulfeniminocephalosporins are prototypic examples of such materials.

Results and Discussion

The *S*-aminosulfeniminocephalosporin **3** (Scheme 2) was prepared in a manner identical with that used for the corresponding penicillin: reaction of **1** with the sulfur diimide **2** gave **3** directly, and treatment of this latter species with diazomethane gave the *N*-methylated material **3m**.[8] Details of the mechanism leading to the formation of the *S*-aminosulfenimine have been presented previously.[5] As with the penicillin structures ^{1}H and ^{13}C NMR spectroscopy provided key structural characterization. Briefly, H-6 appeared as a singlet in the ^{1}H NMR spectrum, while in the ^{13}C NMR spectrum the presence of the imino carbon, in addition to the other required sp^2 carbons, was readily apparent.[9] Furthermore, variable-temperature ^{1}H NMR data[9] indicated that rapid isomerism about the imino group occurred at room temperature for both **3** and **3m**, as had been observed for penicillins bearing the same side chains.[5]

Our prime interest lay in ascertaining if cleavage of the β-lactam ring in these cephalosporins would trigger the rapid expulsion of both the 3′-acetoxy group and the *S*-amino moiety (Scheme 3). This scheme illustrates two distinct reaction pathways which could lead to the sequential expulsion of the side chains: in path c expulsion of the *S*-amino moiety is shown to precede expulsion of the 3′-acetoxy group, and in path d the sequence is reversed. We sought to study this process in a manner similar to that used previously with related penicillin structures.[10a] The salts **4** and **4m** were found to be moderate substrates of β-lactamase type I from *Bacillus cereus*: k_{cat}/K_m values of 1.15×10^5 and 1.85×10^5 M^{-1} s^{-1}, respectively, were obtained.[11] This enzyme was then used to rapidly generate the β-lactam-ring-opened structures **5** and **5m** in a D_2O buffer, and the subsequent expulsion of acetate and of *p*-toluenesulfonamide or *N*-methyl-*p*-toluenesulfonamide was followed using ^{1}H NMR spectroscopy: the chemical shift of the methyl group in the acetate ion is quite distinct from that in acetate esters, such as **4**/**4m** and **5**/**5m**, and likewise the chemical shift of the *N*-methyl group in *N*-methyl-*p*-toluenesulfonamide is very distinct from that in **4m**/**5m**, where this moiety is attached to the sulfur atom.

The time profiles of the background hydrolysis (D_2O buffer, 100 mM phosphate, pH 7.2, 25 °C; no enzyme present) of **4** and **4m** are presented in Figures 1 and 2. The half-lives for hydrolysis were determined to be approximately 5 and 20 h, respectively.[12] The data in Figure 2 show that after 22 h a considerable amount of the intact cephalosporin was still present, clearly indicating that the direct intermolecular displacement of the arenesulfonamide by water from **4m** (Scheme 3, path B) is inherently quite a slow process,[13] and it is logical to consider that the intermolecular displacement of this same group by water in the β-lactam-ring-opened structure **5m** should be equally slow. A sequence of ^{1}H NMR spectra illustrating the enzyme-catalyzed hydrolysis of **4** is shown in Figure 3. These spectral data indicated that addition of the enzyme resulted in release of acetate and of *p*-toluenesulfonamide in an initial rapid burst (Figure 3, **a** → **b**) followed by a stage of lower reaction rate

(8) The acidity of the side chain NH in **3** is such[5,12] that methylation occurred readily with CH_2N_2—this process avoided base-catalyzed Δ^3/Δ^2 isomerization which was found to occur when using CH_3I with Proton Sponge.

(9) Included in the Supporting Information.

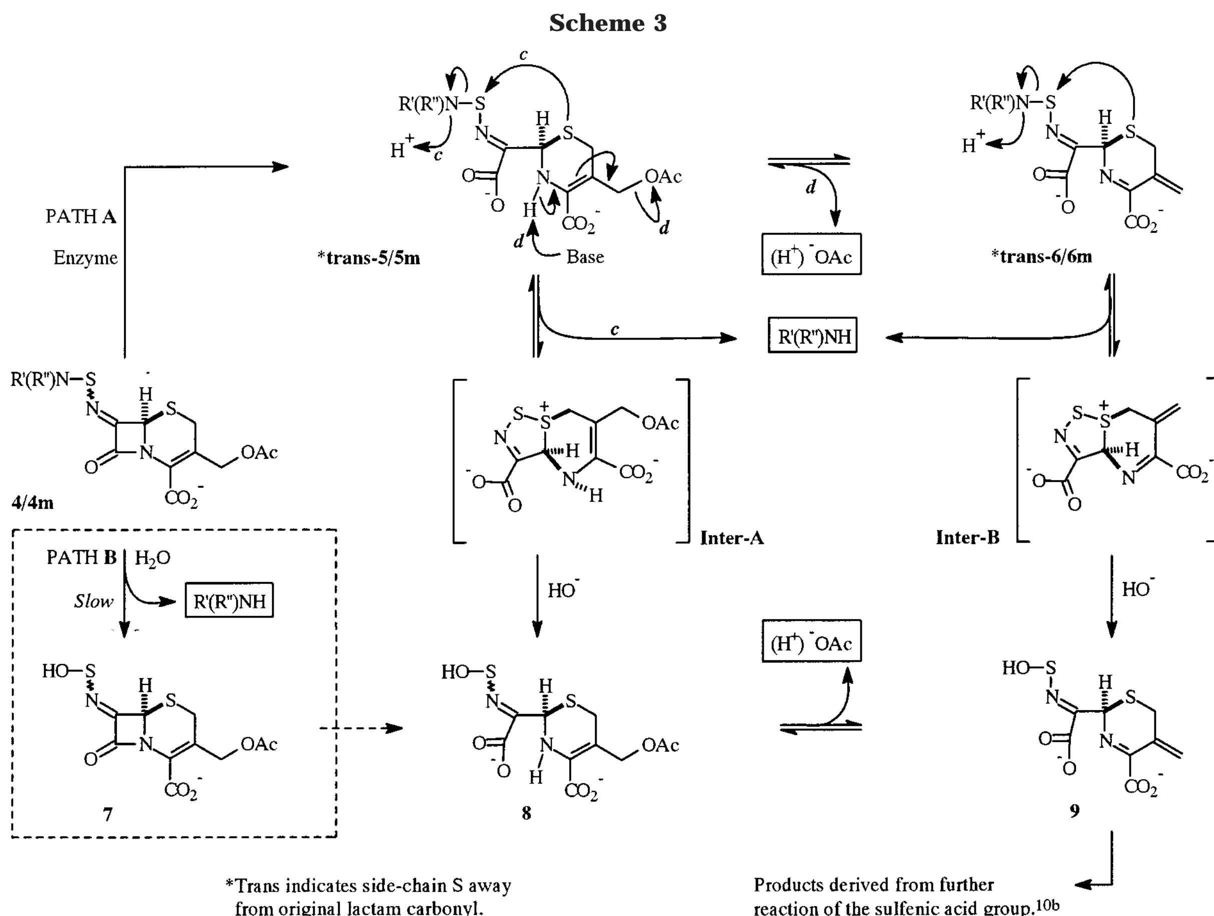
(10) (a) Key information on the details of the intramolecular process was obtained in that work from a study of the penicillin esters in MeOH/NEt_3.[5] An analogous study with the cephalosporin esters used here did not provide a similar level of detail—this was largely due to rapid Δ^3/Δ^2 isomerization which gave rise to product mixtures characterized by complex ^{1}H NMR spectra.[9] This isomerization was not a complicating process when the salts **4** and **4m** were studied in aqueous buffer. The intramolecular displacement in Scheme 3 is shown to occur via the dihydrothiazine-ring sulfur atom by analogy with that shown for the corresponding penicillin structures.[5] A more detailed picture of the reaction mechanism in aqueous solution must await further work. (b) Products derived from reactions of sulfenic acids in aqueous solution are described in: Hogg, D. R.; Vipond, P. W. *Int. J. Sulfur Chem. C* **1971**, *6*, 17. (c) Products derived from further hydrolytic reaction of an *exo*-methylene thiazine type structure (related to **9** in Scheme 3 here) are described in: Taibi-Tranche, P.; Massova, I.; Vakulenko, S. B.; Lerner, S. A.; Mobashery, S. *J. Am. Chem. Soc.* **1996**, *118*, 7441.

(11) The methodology used was as previously described;[5] see the Experimental Section for further details.

(12) The structure $\mathbf{3}_{\text{side-chain anion}}$ was readily observed (^{1}H NMR)[9] by treatment of **3** in $CDCl_3$ with Proton Sponge. In MeOH λ_{max} occurred at 290 cm^{-1} for **3** and this shifted to 359 cm^{-1} on addition of NEt_3; in the aqueous buffer used the salt **4** had a λ_{max} value at 361 cm^{-1} (this shifted to 291 cm^{-1} on acidification), indicating that the side chain was ionized. The involvement of this side chain anion as a general base, acting in an intramolecular fashion, may have been the cause of the more rapid rate of hydrolysis of the β-lactam ring in **4** over that in **4m**.

(13) From our previous work[5] a lower limit of 300 min was obtained as the half-life for this direct displacement reaction by water; that limit was set by the half-life for hydrolysis of the β-lactam ring of the penicillin structure.

Scheme 3



(Figure 3, **b** → **c** → **d**). Addition of a further portion of enzyme resulted in a second rapid reaction in which the remainder of **4** was consumed and the liberation of acetate and of *p*-toluenesulfonamide was, largely, completed (Figure 3, **d** → **e**); the change in the chemical shifts of the aromatic hydrogens was consistent with the formation of *p*-toluenesulfonamide. A similar pattern was observed for **4m**; here the change in the chemical shift of the *N*-methyl peak from 3.33 ppm in **4m** to 2.55 ppm clearly identified the liberation of *N*-methyl-*p*-toluenesulfonamide. A sequence of ^{1}H NMR spectra illustrating the enzyme-catalyzed hydrolysis of **4m** is shown in Figure 4. As above, the spectral data showed an initial rapid release of acetate and of *N*-methyl-*p*-toluenesulfonamide (Figure 4, **a** → **b**) followed by a stage involving a lower rate of reaction (Figure 4, **b** → **c**). Addition of a further portion of enzyme resulted in the rapid hydrolysis of the remainder of **4m** (Figure 4, **c** → **d**); at this point the expulsion of *N*-methyl-*p*-toluenesulfonamide was complete and, although acetate expulsion lagged somewhat, this went to completion within a further 20 min (Figure 4, **e**).

The enzyme-catalyzed reactions observed above for **4** and **4m** were considerably faster than background hydrolysis leading to the same end products. The slower rate of reaction following the initial burst, observed for both **4** and **4m**, was indicative of some process of enzyme inactivation. This appeared to be a feature of the relatively concentrated solutions used for the ^{1}H NMR experiments; a distinct cloudiness and a deepening of the solution color occurred after each addition of enzyme. In dilute solutions—suitable for UV/vis spectroscopic monitoring—the inhibitory potency of **4** and of **4m** toward this enzyme was, in each case, too low to be readily quantifiable.[15]

Overall, the foregoing results allowed delineation of the gross features of the reaction pattern of these *S*-aminosulfeniminocephalosporins. They behave as moderate substrates for the β-lactamase enzyme used, and enzyme-catalyzed hydrolysis of the β-lactam ring is an efficient trigger for the release of both the 3′-acetoxy group and of the sulfur-attached moiety of the *S*-aminosulfenimine side chain: this pattern is summarized in Scheme 4.[16]

The potential to exploit the unique molecular transformation of this structure type to effect site-specific corelease of two distinct drug components, with the intent of establishing synergy, is manifest. Apropos ADEPT possible drug combinations include (i) two distinct toxic entities or precursors,[17] (ii) a potentiating agent and a

(14) The weight refers to the bulk enzyme sample, which contained about 10% protein with the balance made up of buffer salts. The enzyme/substrate ratio here was quite high—the sole objective was to achieve rapid, and selective, cleavage of the β-lactam ring in solutions which were sufficiently concentrated to allow acquisition of ^{1}H NMR data in a short time scale.

(15) The methodology used is described in detail in ref 5. Low levels of inhibition against β-lactamase type IV from *Enterobacter cloacae* (Sigma) were observed.

(16) The data in Figures 2 and 4 indicate that expulsion of acetate from **5m** was slower than expulsion of *N*-methyl-*p*-toluenesulfonamide.

(17) Evidence for synergy between the two cytotoxic agents doxorubicin and paclitaxel has been reported: Holmes, F. A.; Kudelka, A. P.; Kavanagh, J. J.; Huber, M. H.; Ajani, J. A.; Valero, V. In *Taxane Anticancer Agents – Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series 583; American Chemical Society: Washington, DC, 1995; Chapter 3. These two cytotoxic agents have been individually combined with a cephalosporin as (β-lactamase-dependent) site-specific release prodrugs for use in ADEPT; see ref 3d and: Vrudhula, V. M.; Svensson, H. P.; Senter, P. D. *J. Med. Chem.* **1995**, *38*, 1380.

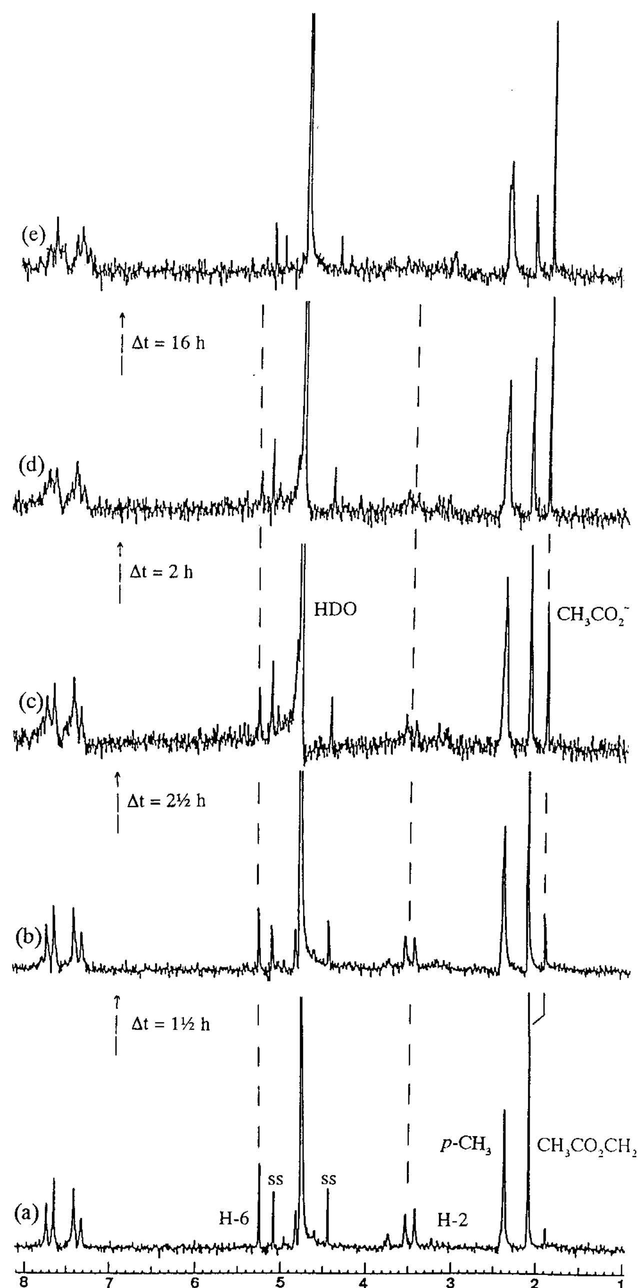


Figure 1. Sequence of ^{1}H NMR spectra showing hydrolysis of **4** in D_2O buffer (pD 7.2, 100 mM phosphate, 25 °C) in the absence of enzyme. The hydrogens on C-3′ are largely masked by the water peak at 4.68 ppm.

toxic entity,[18] and, (iii) a toxic entity and a multidrug-resistance (MDR) inhibitor.[19] The key features to elaborate are (i) optimization of the structures to enhance their fitness as β-lactamase substrates (i.e. increase k_{cat}/K_m) and (ii) variation of the 3′-substituent and of the sulfur-attached moiety as desired. The chemistry associated with varying the 3′-substituent in cephalosporins is well-established, and considerable diversity is tolerated in terms of substrate fitness.[20] The variation tolerated in the sulfur-attached group has yet to be explored. It is relevant to note that we have already demonstrated in the penicillin series that a sulfenate type side chain (CH$_3$-OSN=) behaved in the same manner as an *S*-aminosulfenimine (R(R′)NSN=): viz., rapid intramolecular displacement of methanol followed on cleavage of the β-lactam ring.[21] Thus, the key intramolecular displacement process is not limited to amines alone as the displaceable sulfur-attached group.

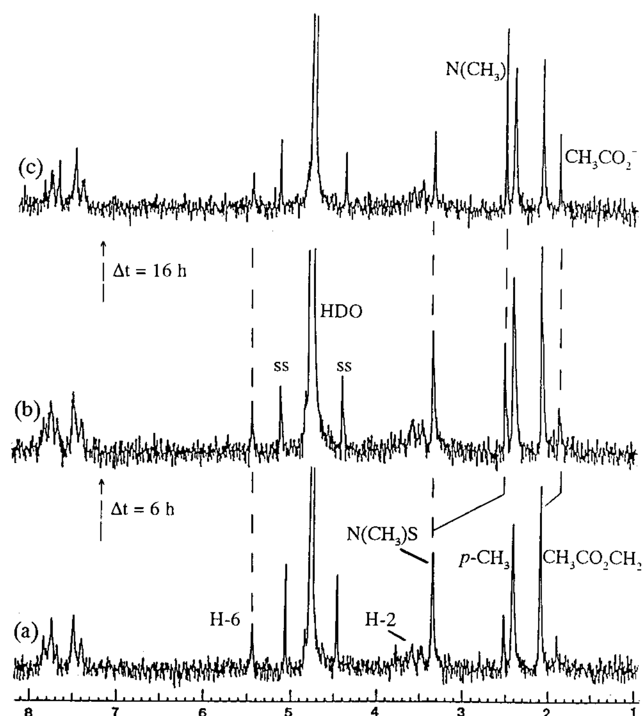


Figure 2. Sequence of ^{1}H NMR spectra showing the hydrolysis of **4m** in D_2O buffer (pD 7.2, 100 mM phosphate, 25 °C) in the absence of enzyme. The hydrogens on C-3′ are largely masked by the water peak at 4.68 ppm. A small amount of *N*-methyl-*p*-toluenesulfonamide was present at the outset (a).

The inherent reactivity of cephalosporins, resulting in the release of the 3′-substituent following cleavage of the β-lactam ring, has been harnessed to produce β-lactamase-dependent prodrugs for use as antibacterial agents. One approach has been to use a known antibiotic, a

(18) L-Histidinol is known to potentiate the cytotoxicity of certain alkylating agents: Warrington, R. C.; Fang, W. D. *Br. J. Cancer* **1989**, *60*, 652; *J. Nat. Can. Inst.* **1989**, *81*, 798. The release of alkylating agents from cephalosporin prodrugs is well-established; see ref 3 and: Alexander, R. P.; Bates, R. W.; Pratt, A. J.; Kraunsoe, J. A. E. *Tetrahedron* **1996**, *52*, 5983.

(19) For details of MDR inhibitors see: Ojima, I.; Bounaud, P.-Y.; Bernacki, R. J. *CHEMTECH* **1998**, *28*, 31 and references therein. A cephalosporin prodrug which would effect corelease of a taxane-based MDR inhibitor and a taxoid is an intriguing prospect—synergy between such inhibitors and paclitaxel has been demonstrated by Ojima et al., while the release of paclitaxel from a cephalosporin prodrug has been described in ref 3d.

(20) See refs 3 and 4d and references therein.

(21) This reactivity pattern was observed for a penicillin ester in MeOH/NEt$_3$.[5]

(22) The bactericidal efficacy of quinolones and β-lactams has been shown to correlate with different parameters. In the case of quinolones, which exert their antibiotic effect inside the cytoplasm, this efficacy is highly correlated with their concentration, whereas the correlation in the case of penicillins in particular, and by extension with cephalosporins, is with the dosage time span during which the β-lactam concentration is moderately higher than the MIC level: Cars, O. *Diagn. Microbiol. Infect. Dis.* **1997**, *27*, 29. The fact that gram positive bacteria express β-lactamases extracellularly, whereas gram negative bacteria express these enzymes within the periplasmic space, may mean that a single broad-spectrum-type β-lactamase-dependent prodrug will not be viable. This should not be a limitation, however, in a scenario where administration of a particular drug will only follow on first characterizing the infectious agent, as is likely to be the case in a regime designed to contain resistance and prolong the viability of future drugs: Chopra, I.; Hodgson, J.; Metcalf, B.; Poste, G. *Antimicrob. Agents Chemother.* **1997**, *41*, 497. Levy, S. B. *Sci. Am.* **1998** (March), 32 and references therein.

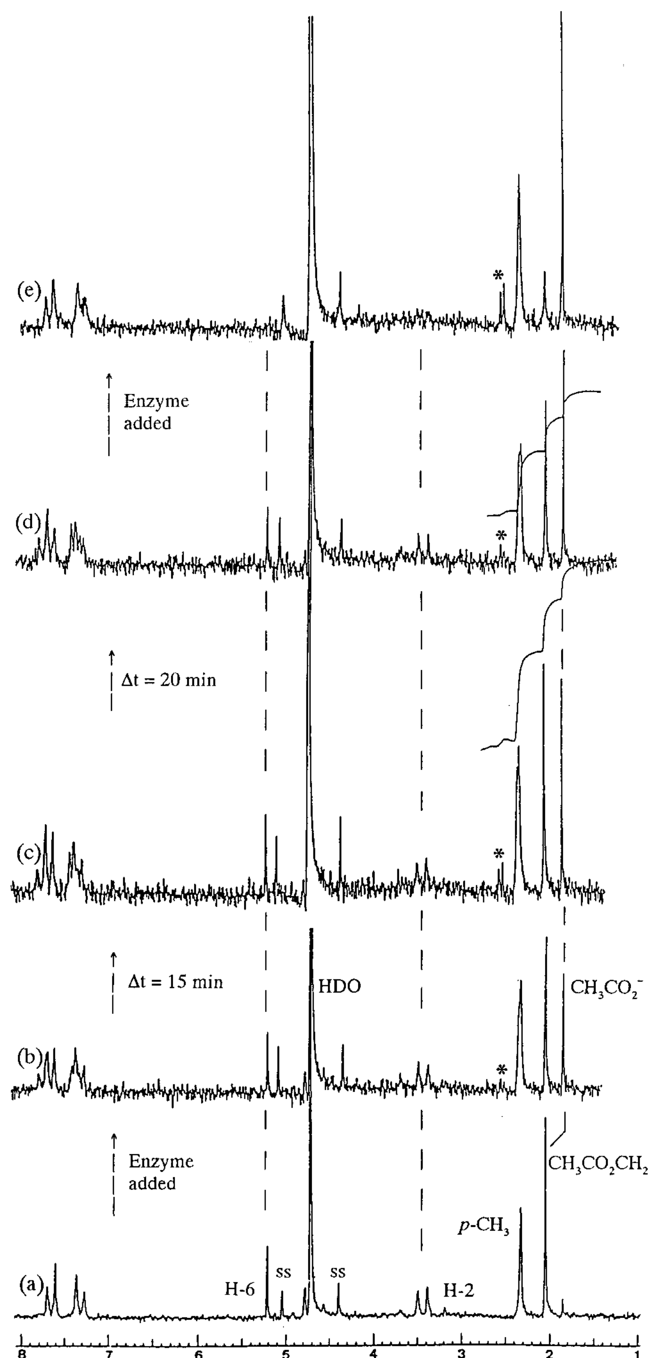


Figure 3. Sequence of ^{1}H NMR spectra showing enzyme-catalyzed hydrolysis of **4** (13 mg) in D_2O buffer (0.5 mL; pD 7.2, 100 mM phosphate, 25 °C). 1.3 and 1.0 mg of enzyme were used in the first and second additions, respectively.[14] Each spectrum required 5 min for data acquistion—this is included in the time intervals shown. The peaks labeled with asterisks originate from the enzyme sample. The hydrogens on C-3′ are largely masked by the water peak at 4.68 ppm.

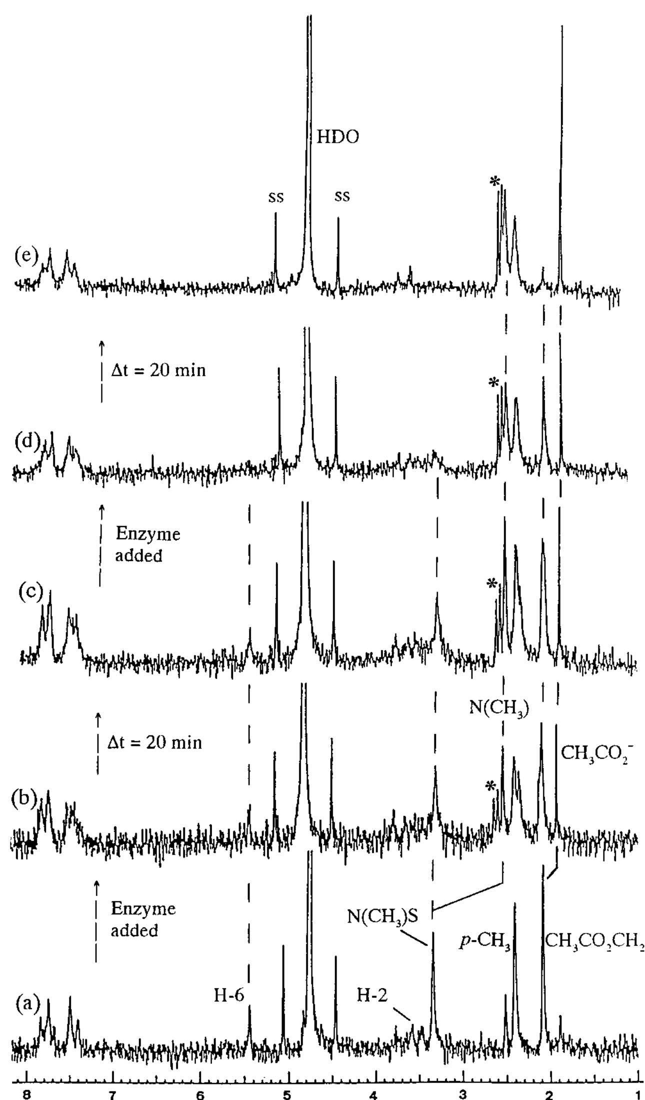
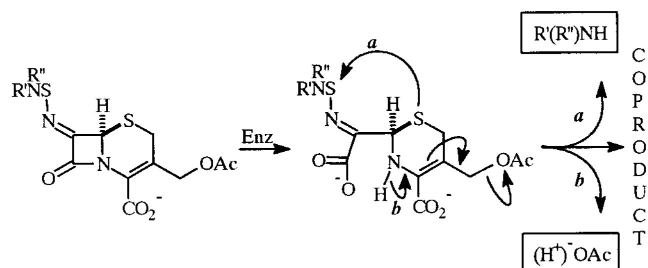


Figure 4. Sequence of ^{1}H NMR spectra showing enzyme-catalyzed hydrolysis of **4m** (11 mg) in D_2O buffer (0.5 mL; pD 7.2, 100 mM phosphate, 25 °C). 2.5 and 1.2 mg of enzyme were used in the first and second additions, respectively.[14] Each spectrum required 5 min for data acquistion—this is included in the time intervals shown. The peaks labeled with asterisks originate from the enzyme sample. The hydrogens on C-3′ are largely masked by the water peak at 4.68 ppm. A small amount of *N*-methyl-*p*-toluenesulfonamide was present at the outset (a).

Scheme 4



fluoroquinolone, as the 3′-component.[4d] To date, however, it does not appear that this approach has led to a generally useful therapeutic agent.[22] Site-specific release of an entity too toxic for systemic delivery could allow for selective action[1b] of such a species as an antibiotic.[23] A key advantage of a dual release prodrug is the potential to establish synergy[24] between the coreleased structures. Both of these aspects may be relevant in focusing on new targets for inhibition in bacteria. One general target that has been identified is the “two-component system” (TCS), which plays a critical role in the survival and growth of a bacterial population in any given environment.[25] A number of TCS inhibitors have been identified, and some have been shown to have inhibitory activity on bacterial growth in vitro.[26a] In the context of disrupting multiple stages of a TCS pathway as a primary or as an adjunct

target,[25-27] the use of a prodrug to corelease two distinct structures could provide a route to establishing useful synergistic effects.

Conclusions

We have demonstrated that *S*-aminosulfeniminocephalosporins are prototypic examples of a novel class of β-lactamase-dependent prodrug. In these the cephalosporin acts as a nucleus from which the corelease of two distinct structural components can be effected through enzyme-catalyzed hydrolysis of the β-lactam ring. The potential to elaborate and exploit this structure type in the ADEPT approach to cancer chemotherapy and in the further development of antibiotic agents is clear.

Experimental Section

Column chromatography (flash technique)[28] was carried out using Kieselgel S (32–63 μm). β-Lactamase type I from *Bacillus cereus* (EC 3.5.2.6) was obtained from Sigma; the specific activity for the hydrolysis of benzylpenicillin at pH 7.2 and 25 °C was 2.70 mmol min^{-1} (mg of protein)$^{-1}$. Elemental analyses were performed by the Microanalytical Laboratory, UCD, Dublin, Ireland.

Diphenylmethyl 7-Aminocephalosporanate (1). To a suspension of 7-ACA (28.9 g, 0.106 mol) in methanol (100 mL) was added a solution of diphenyldiazomethane (0.1 mol) in dichloromethane (40 mL), and the mixture was stirred for 2 days at room temperature until the purple color disappeared. The unreacted solid was removed by filtration, and the organic layer was concentrated under reduced pressure to yield a sticky solid. This was purified by flash column chromatography using 50/50 ethyl acetate/dichloromethane as eluant. Removal of solvent under reduced pressure yielded **1** as a pale yellow powder (23.3 g, 53.2 mmol, 50%): mp 120–121 C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.85 (br s, NH_2), 2.00 (s, 3H), 3.32 and 3.60 (AB q, J = 18 Hz, 2H), 4.73 and 5.01 (AB q, J = 13.5 Hz, 2H), 4.77 (d, J = 5 Hz, 1H), 4.91 (d, J = 5 Hz, 1H), 6.98 (s, 1H), 7.20–7.92 (m, 10 H); IR (film) 1779, 1735 cm^{-1}.

Diphenylmethyl *N*-Tosyl-*S*-aminosulfeniminocephalosporanate (3). **1** (1 g, 2.28 mmol) was dissolved in dichloromethane (35 mL). **2**[5] (1.0 g, 2.7 mmol) was added, and the mixture was stirred for 15 min. Water (2 mL) was added, followed by the slow addition of light petroleum (30 mL); the reaction mixture was then stirred for a further 10 min. Precipitated sulfonamide was filtered off, and the filtrate was washed successively with water (3 × 500 mL) and saturated brine solution (100 mL), separated, dried, and concentrated under reduced pressure to yield crude **3**. This was purified by flash chromatography using 90/10 dichloromethane/ethyl acetate. Removal of solvent under reduced pressure gave **3** as a yellow solid: yield 1.05 g (1.65 mmol, 72%): mp 96–99 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 2.03 (s, 3H), 2.42 (s, 3H), 3.32 and 3.62 (AB q, J = 18 Hz, 2H), 4.67 and 5.03 (AB q, J = 13 Hz, 2H), 5.36 (s, 1H), 7.00 (s, 1H), 7.20–7.85 (m, 14 H); IR (film) 1779, 1735 cm^{-1}; IR (film) 3214, 1780, 1736, 1674 cm^{-1}; UV log ϵ = 4.06, λ_{290} (MeOH). Anal. Calcd for $C_{30}H_{27}N_3O_7S_3$: C, 56.50.; H, 4.27; N, 6.59. Found: C, 56.64; H, 4.29; N, 6.54.

Diphenylmethyl *N*-Methyl-*N*-tosyl-*S*-aminosulfeniminocephalosporanate (3m). A solution of diazomethane was prepared from Diazald.[29] Using a stream of nitrogen the diazomethane was entrained through a cold trap into a solution of **3** (1 g, 1.57 mmol) in dichloromethane (25 mL); reaction progress was monitored using TLC. Removal of solvent under reduced pressure yielded a sticky solid. This was purified by flash chromatography using dichloromethane as eluant. Pure **3m** was obtained as a light yellow solid (650 mg, 1.00 mol, 63%): mp 87–92 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.46 (s, 3H), 3.31 (s, 3H), 3.35 and 3.67 (AB q, J = 18 Hz, 2H), 4.76 and 5.02 (AB q, J = 13 Hz, 2H), 5.50 (s, 1H), 7.00 (s, 1H), 7.22–7.90 (m, 14 H); IR (film) 1781, 1738, 1675 cm^{-1}; UV log ϵ = 4.13, λ_{298} (MeOH). Anal. Calcd for $C_{31}H_{29}N_3O_7S_3$: C, 57.13; H, 4.48; N, 6.44. Found: C, 56.86; H, 4.38; N, 6.18.

Deprotection and Isolation of the Sodium Salts. The cephem esters (typically 0.5 mmol) were deprotected as previously described for the *S*-aminosulfeniminopenicillanates.[5] The sodium salts were obtained by extracting a dichloromethane solution (20 mL) of an accurately weighed amount of the free acid (typically 0.25 mmol) with aqueous sodium bicarbonate (0.20 mmol). The aqueous layer was separated and freeze-dried to yield the sodium salts as yellow solids.

Sodium *N*-tosyl-*S*-aminosulfeniminocephalosporanate (4) was obtained as a bright yellow foam (137 mg, 0.28 mmol, 45%): ^{1}H NMR (90 MHz, D_2O) δ 2.12 (s, 3H), 2.40 (s, 3H), 3.35 and 3.67 (AB q, J = 18 Hz, 2H), 4.70 and 4.92 (AB q J = 12.5 Hz, 2H), 5.30 (s, 1H), 7.3–7.8 (m, 4H); UV, log ϵ = 4.16, λ_{max} 361 nm (H_2O buffer pH 7.2). Anal. Calcd for $C_{17}H_{16}N_3O_7S_3Na\cdot 2H_2O$: C, 38.56; H, 3.81; N, 7.94. Found: C, 38.16; H, 3.47; N, 7.66. Corresponding free acid: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.12 (s, 3H), 2.42 (s, 3H), 3.40 and 3.61 (AB q, J = 18 Hz, 2H), 4.96 and 5.18 (AB q, J = 13.5 Hz, 2H), 5.38 (s, 1H), 7.23–7.90 (m, 4H).

Sodium *N*-methyl-*N*-tosyl-*S*-aminosulfeniminocephalosporinate (4m) was obtained as a bright yellow solid (73 mg, 0.14 mmol, 32%): ^{1}H NMR (90 MHz, D_2O) δ 2.11 (s, 3H), 2.42 (s, 3H), 3.37 (s, 3H), 3.40 and 3.70 (AB q, J = 18 Hz, 2H), 4.71 and 4.92 (AB q, J = 12.5 Hz, 2H) 5.47 (s, 1H), 7.39–7.90 (m, 4H);. UV, log ϵ = 4.11, λ_{max} 298 nm (H_2O buffer pH 7.2). Anal. Calcd for $C_{18}H_{18}N_3O_7S_3Na\cdot 1.75H_2O$: C, 40.11; H, 4.02; N, 7.79. Found: C, 39.77; H, 3.69; N, 7.59. Corresponding free acid: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.12 (s, 3H), 2.43 (s, 3H), 3.32 (s, 3H), 3.41 and 3.72 (AB q, J = 18 Hz, 2H), 4.97 and 5.20 (AB q, J = 13 Hz, 2H), 5.51 (s, 1H), 7.27–7.88 (m, 4H).

Determination of k_{cat}/K_m for 4 and 4m. The measurement of k_{cat}/K_m was carried out as described previously for the corresponding penicillins.[5] For **4** and **4m** the decrease in absorbance at 361 and 298 nm, respectively, was monitored as a function of time; the initial rate was determined from the linear absorbance change within the first 10 s. The enzyme concentration in each case was 28.2 nM, while the concentration of **4** was 112 μM and for **4m** it was 120 μM. $\Delta\epsilon$ values for **4** and **4m** were 10 000 and 6500 M^{-1} cm^{-1}, respectively.

Enzyme Inhibition Studies. Benzylpenicillin was used as the assay substrate, and measurement of the rate of its

(23) Appropriately substituted phenols, and related structures, are very effective inhibitors of oxidative phosphorylation, which is a key metabolic process: Terada, H.; Goto, S.; Yamamoto, K.; Takeuchi, I.; Hamada, Y.; Miyake, K. *Biochim. Biophys. Acta* **1988**, *936*, 504 and references therein. It is relevant to note that some phenolic structures (salicylanilides) which have been used as topical antibiotics have been found to act as inhibitors of autophosphorylation of KinA kinase (which is part of a TCS pathway[26a]). The phenolic group provides a useful linkage for attachment to a prodrug nucleus, and this linkage should also mask the inherent toxicity of the phenolic moiety within the prodrug, as the key acid-dissociable site would be blocked.

(24) Examples of synergy in the field of antibiotics include (i) combination of pristinamycin I_A and II_A in streptogramins (Barrière, J. C.; Paris, J. M. *Drugs of the Future* **1993**, *18*, 833), (ii) combination of trimethoprim with a sulfonamide, and (iii) combination of a β-lactamase inhibitor (e.g. clavulanic acid) with a transpeptidase inhibitor (e.g. amoxycillin).

(25) For leading references see: (a) *Two-Component Signal Transduction*; Hoch, J. A., Silhavy, T. J., Eds.; ASM Press: Washington, DC, 1995. (b) Barrett, J. F.; Isaacson, R. E. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: San Diego, CA, 1995; Chapter 12. (c) Goldschmidt, R. M.; Macielag, M. J.; Hlasta, D. J.; Barrett, J. F. *Curr. Pharm. Des.* **1997**, *3*, 125.

(26) (a) Macielag, M. J.; Demers, J. P.; Fraga-Spano, S. A.; Hlasta, D. J.; Johnson, S. G.; Kanojia, R. M.; Russell, R. K.; Sui, Z.; Weidner-Wells, M. A.; Werblood, H.; Foleno, B. D.; Goldschmidt, R. M.; Loeloff, M. J.; Webb, G. C.; Barrett, J. F. *J. Med. Chem.* **1998**, *41*, 2939. (b) Lam, C.; Turnowsky, F.; Högenauer, G.; Schutze, E. *J. Antimicrob. Chemther.* **1987**, *20*, 37.

(27) Volz, K. In *Two-Component Signal Transduction*; Hoch, J. A., Silhavy, T. J., Eds.; ASM Press: Washington, DC, 1995; Chapter 4, and references therein.

(28) Casey, M.; Leonard, J.; Lygo, B.; Procter, G. *Advanced Practical Organic Chemistry*; Blackie: Glasgow, Scotland, 1990; Chapter 9.

(29) See Chapter 5 of ref 28.

enzyme-catalyzed hydrolysis by samples of β-lactamase, which had been incubated (10 min) with varying concentrations of **4** or **4m**, was carried out in a standard manner using UV/vis spectroscopy. The concentration range of **4** and **4m** used was 40−1000 μM with an enzyme concentration of 0.35 μM. For further details of the procedure, see ref 5.

β-**Lactamase-Catalyzed Hydrolysis of 4 and 4m Using ^{1}H NMR.** To **4** (13 mg) in 0.5 mL of D_2O buffer (phosphate 0.1 M, pD 7.2 at 25 °C in a 5 mm NMR tube) was added type I β-lactamase enzyme (1.3 mg). Spectra were recorded at different time intervals. Acquisition time for each of the spectra was 5 min. A second portion of enzyme (1.0 mg) was added later, as indicated in the text. A similar procedure was used for **4m**, while the background hydrolysis was monitored for **4** and **4m** in this buffer in the absence of enzyme. DSS was used as external reference.

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Supporting Information Available: ^{1}H NMR (variable temperature) spectra of **3** and **3m**, the ^{13}C NMR spectrum of **3**, the ^{1}H NMR spectrum of $\mathbf{3}_{\text{side-chain anion}}$, and ^{1}H NMR spectra of Δ^3/Δ^2 product mixtures obtained from the reaction of **3** and **3m** in $MeOH/NEt_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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