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Toward the Development of a Cephalosporin-Based Dual-Release Prodrug for Use in ADEPT

Jonathan W. Grant and Timothy P. Smyth*

Department of Chemical and Environmental Sciences, University of Limerick, National Technological Park, County Limerick, Ireland

timothy.smyth@ul.ie

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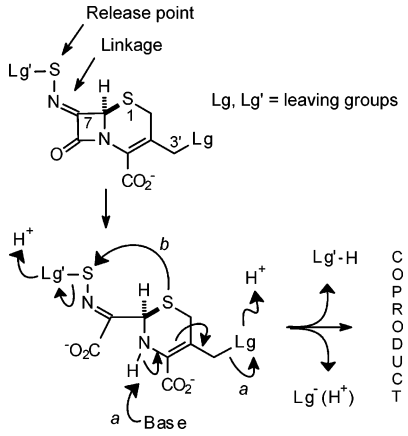
In previous work we have shown that a cephalosporin structure bearing an *S*-aminosulfenimine at the 7-position behaved as a β-lactamase-dependent dual-release prodrug. Scission of the β-lactam ring of such a structure led to the rapid loss of the sulfur-attached side chain moiety via an intramolecular displacement, while the 3′-group was lost via the well-established elimination process at that position. In the present work we report on an evaluation of the scope and limitations of exploiting the *S*-aminosulfenimine functionality to generate a cephalosporin-based prodrug incorporating two biologically active components. Starting from 7-ACA, a viable synthetic cycle was put in place that avoided formation of the Δ^2 isomer throughout and that allowed incorporation of aminoglutethimide at the 3′-position and of a tosyl *S*-aminosulfenimine at the 7-position. The direct incorporation of a biologically active sulfonamide (ethoxzolamide) or a sulfamate (coumate) at this latter position was not achieved as a result of the difficulty of generating the corresponding sulfur diimides. An indirect route for the formation of an *S*-aminosulfenimine was put in place, as was a general method of alkylation (Mitsunobu reaction) of the tosyl *S*-aminosulfenimine following its incorporation.

Introduction

The cephalosporin structure is well-established as a mono-release prodrug nucleus owing to rapid elimination of the 3′-substituent following enzyme-catalyzed scission of the β-lactam ring. Examples are known where antimicrobial (quinolones)[1] and cytotoxic components (melphalan, doxorubicin)[2] have been incorporated at this position. The cephalosporin-cytotoxic conjugates have been shown to be valuable prodrugs in the ADEPT (antibody-directed enzyme prodrug therapy) mode of drug targeting.[2] Previously we reported on a modification that allowed the cephalosporin structure to behave as a dual-release prodrug nucleus.[3] This involved incorporation of an unsaturated linker at the 7-position with a moderately nucleofugic group attached via an electron-deficient center. The generalized structure type and its associated reaction pattern are shown in Scheme 1: elimination of the 3′-substitutent is shown as path *a*, and displacement of the second leaving group is shown as path *b*. The efficacy of this latter process is due to the rapidity of an intramolecular displacement occurring within a five-membered acyclic unit of restricted conformational space (due to the double bond). The specific prototypic structure had *N*-methyl-*p*-toluenesulfonamide as the sulfur-attached moiety and acetate at the 3′-position. Herein, we report on our evaluation of the scope and limitations of using the *S*-aminosulfenimine unit to generate a dual-release cephalosporin prodrug bearing biologically active components relevant to the ADEPT mode of anticancer drug targeting.[4]

SCHEME 1



(1) Albrecht, H. A.; Beskid, G.; Christenson, J. G.; Dietcher, K. H.; Georgopapadakou, H. H.; Keith, D. D.; Konzelmann, F. M.; Pruess, D. L.; Wei, C. C. *J. Med. Chem*. **1994**, *37*, 400−407 and references therein.

(2) (a) Svensson, H. P.; Frank, I. S.; Berry, K. K.; Senter, P. D. *J. Med Chem*. **1998**, *41*, 1507−1512 and references therein. (b) Hudyma, T. W.; Bush, K.; Colson, K. L.; Firestone, R. A.; King, H. D. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 323−328.

(3) (a) Smyth, T. P.; O'Connor, M. J.; O'Donnell, M. E. *J. Org. Chem.* **1999**, *64*, 3132−3138. (b) Smyth, T. P.; O'Donnell, M. E.; O'Connor, M. J.; St Ledger, J. O. *Tetrahedron* **2000**, *56*, 5699−5707. (c) Smyth, T. P.; O'Donnell, M. E.; O'Connor, M. J.; St Ledger, J. O. *J. Org. Chem*. **1998**, *63*, 7600−7618.

(4) For details of an alternative dual-release prodrug nucleus, see: Shamis, M.; Lode, H. L.; Shabat, D. *J. Am. Chem. Soc*. **2004**, *126*, 1726−1731.

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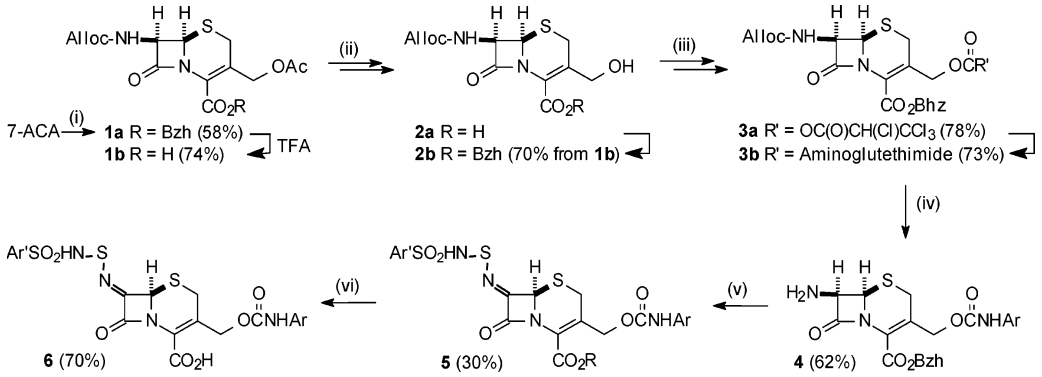
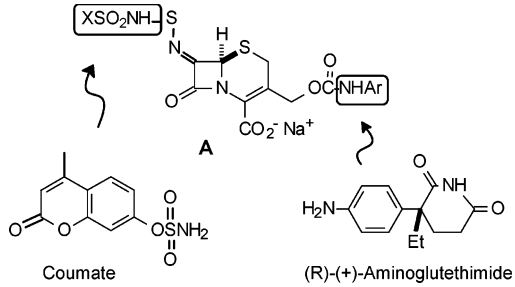
SCHEME 2[a]



[a] (i) (a) $(Ph)_2CN_2$, (b) allyloxychloroformate/Py in CH_2Cl_2, 58% from 7-ACA; (ii) (a) acetylesterase pH 6.5, 2 h at rt, (b) acidification, extraction into ethyl acetate, $(Ph)_2CN_2$, 70% crude from **1b**, Δ^3 isomer; (iii) (a) $ClC(O)OCH(Cl)CCl_3$/Py/DMAP in CH_2Cl_2, 78%, Δ^3 isomer, (b) (*R*)-(+)-aminoglutehimide/Py/DMAP in CH_2Cl_2, 73%, Δ^3 Isomer; (iv) $Pd(P(Ph)_3)_4$/*p*-toluenesulfinic acid in CH_2Cl_2, 62%, Δ^3 isomer (trace impurity present); (v) (*p*-$CH_3C_6H_4SO_2N{=})_2S$ in CH_2Cl_2, 30%; (vi) $AlCl_3$ in CH_3NO_2/CH_2Cl_2 −80 °C, aqueous extraction, 70%.

Results and Discussion

The synthetic challenges addressed here (Scheme 2) were to sequentially functionalize the 3′- and 7-positions of 7-aminocephalosporanic acid (7-ACA) via procedures that were reliable and robust in terms of removal of the 3′-acetate, incorporation of the *S*-aminosulfenimine, and avoiding Δ^3/Δ^2 isomerization throughout. It was envisaged to integrate a biologically active amine via a carbamate linkage at the 3′-position and to incorporate a biologically active sulfonamide or sulfamate as the *S*-aminosulfenimine; in earlier work we had found that direct formation of the *S*-aminosulfenimine occurred readily on reaction of a sulfur diimide, derived from an aryl sulfonamide, with the 7-amino group of a cephalosporin ester. Our synthetic endeavor initially focused on **A**, which bears aminoglutethimide (an aromatase inhibitor)[5] at the 3′-position and coumate (a sulfatase inhibitor)[6] as the *S*-aminosulfenimine. Both inhibitors are used clinically to reduce the production of estrogen in hormone-dependent breast cancer. These estrogen-forming enzymes are present in higher concentrations in tumor tissue than elsewhere in the body[7] and so may be appropriate for the ADEPT mode of targeted drug deliver.



The synthetic plan that was implemented is given in Scheme 2. A key step in the transformation was selective removal of the 3′-acetate. Various protocols have been described in the literature using hydroxide solutions at low temperature;[8] however, we did not find these to be robust or reliable processes. The enzyme acetylesterase from orange peel (commercially available) is known to be selective and efficient[9] (if somewhat expensive) for this transformation and this gave reproducible results in our work. The substrate used was 7-(allyloxycarbonyl)aminocephalosporanic acid **1b**; it was found that 5 mmol of **1b** was efficiently processed using 1000 units of enzyme activity within 2 h. The resulting crude desacetyl structure **2a** was extracted and converted back to the benzhydryl ester **2b** (70% from **1b**). Chromatographic purification of **2b** was avoided as this led to formation of some lactone (via reaction of the 3′-OH and benzhydryl ester) and to some of the Δ^2 isomer. Treatment of crude **2b** with tetrachloroethyl chloroformate in the presence of pyridine and DMAP gave the corresponding carbonate, **3a**, which readily reacted with (*R*)-(+)-aminoglutethimide to give the carbamate **3b**; both **3a** and **3b** were obtained as the Δ^3 isomers exclusively following chromatographic purification.

Removal of the allyl group from **3b** to give **4** was achieved with $Pd(P(Ph)_3)_4$ in the presence of *p*-toluenesulfinic acid; the standard additive for this process, $P(Ph)_3$, was omitted as its inclusion led to extensive formation of the Δ^2 isomer. It was also found that direct chromatographic purification of crude **4** following removal of solvent (entrainment with nitrogen) but without any extraction step was optimal. The material obtained in this manner contained a small amount of triphenylphosphine oxide, but this did not interfere with the next step (use of N_2 for the flash chromatography limited formation of $(Ph)_3P(O)$). The synthetic cycle was initially completed by reaction of **4** with the sulfur diimide derived from

(5) Jones, C. D.; Winter, M. A.; Hirsch, K. S.; Stamm, N.; Taylor, H. M.; Holden, H. E.; Davenport, J. D.; Krumkalns, E. V.; Suhr, R. G. *J. Med. Chem.* **1990**, *33*, 416−429 and references therein.

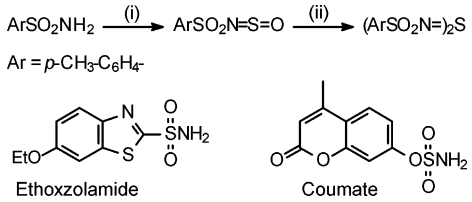
(6) (a) Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Hejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, *41*, 1068−1083. (b) Woo, L. W.; Sutcliffe, O. B.; Bubert, C.; Grasso, A.; Chander, S. K.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2003**, *46*, 3193−3196 and references therein.

(7) Chetrite, G. S.; Phillipe, J. C.; Wright, F.; Pasqualini, J. R. *J. Steroid Biochem. Mol. Biol.* **2000**, *72*, 23−27.

(8) (a) Yamanaka, H.; Chiba, T.; Kawabata, K.; Takasugi, H.; Masugi, T.; Takaya, T. *J. Antibiot.* **1985**, *38*, 1738−1751. (b) Mobashery, S.; Johnson, M. *J. Biol. Chem.* **1986**, *261*, 7879−7887. (c) López, M.; Rodriguez, Z.; Valdés, B., Velez, H.; Agüero, J.; Fini, A.; González, M. *Farmaco* **2001**, *56*, 629−631.

(9) Quante, J. M.; Hoke, R. A.; Mize, P. D.; Woodward, D. L.; Millner, O. E. U.S. Patent 5,514,561, May 1996.

SCHEME 3[a]



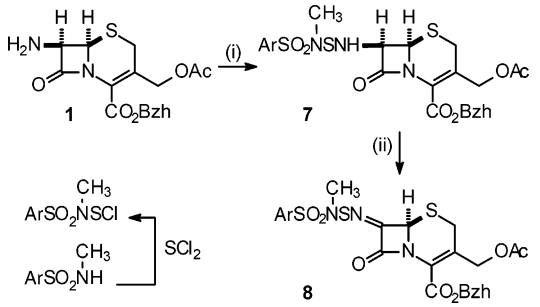
[a] (i) SO_2Cl_2/pyridine in benzene; (ii) pyridine in benzene.

p-toluenesulfonamide to give **5**. A feature of the ^{1}H NMR spectrum of **5** is the quite broad appearance of the H-6 singlet; this is a characteristic feature of *S*-aminosulfeniminocephalosporins[3a] and is associated with dynamic isomerization about the imine bond. Removal of the benzhydryl group using $AlCl_3$ gave the free acid **6** in 70% yield.

The standard route used for sulfur diimide preparation starting from a sulfonamide was via dimerization of the corresponding *N*-sulfinyl derivative; this process worked well with *p*-toluenesulfonamide and *p*-chlorobenzenesulfonamide (Scheme 3).[3c] Despite many attempts we were unsuccessful in forming the sulfur diimide of coumate (prepared from commercially available 7-hydroxy-4-methylcoumarin)[6] via this route. Ethoxzolamide (commercially available), which is a known inhibitor of carbonic anhydrase (CA XI)[10] and has application in cancer chemotherapy, was subjected to the same processes, but it too failed to generate the corresponding sulfur diimide. Some other methods of sulfur diimide synthesis, involving *N*-chlorinated sulfonamides,[11] were examined but these processes gave intractable product mixtures. Clearly, the difficulty of forming a variety of sulfur diimides is a limiting feature for direct incorporation of a biologically active sulfonamide/sulfamate as an *S*-aminosulfenimine at the 7-position of cephalosporins. In a final evaluation of the *S*-aminosulfenimine chemistry two further processes were studied: (a) an indirect method for its formation, and (ii) alkylation of the sulfonamide nitrogen in a Mitsunobu reaction following formation of the *S*-aminosulfenimine. Both of these processes gave some success.

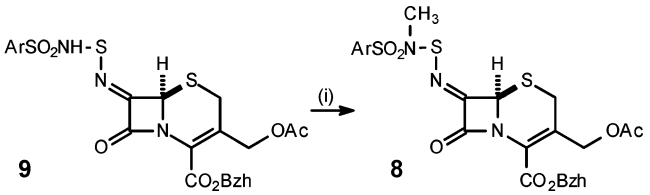
A two-phase, electrochemically driven process was developed by Torii and co-workers for the selective oxidation of a cephalosporin-based sulfenamide to the corresponding sulfenimine.[12] We were successful in applying this process to conversion of the *S*-aminosulfenamide **7**, derived from *p*-toluenesulfonamide, to the corresponding *S*-aminosulfenimine **8** exclusively as the Δ^3 isomer (Scheme 4). The usefulness of this route is limited, however, by the stability of the *N*-sulfenyl chloride required for formation of the *S*-aminosulfenamide and to oxidize this latter structure. An *N*-alkyl group is

SCHEME 4[a]



[a] (i) *p*-$CH_3C_6H_4SO_2N(CH_3)SCl$; (ii) $MgBr_2/H_2O/e^-/CH_2Cl_2$.

SCHEME 5[a]



[a] (i) DIAD, $P(Ph)_3$, CH_3OH in CH_2Cl_2, −23 °C to rt, 45 min.

essential for the selective oxidation process so this approach cannot be applied to coumate or ethoxzolamide. The process may be applicable to *N*-methyl coumate; however, this structure is known to be a less potent sulfatase inhibitor than coumate itself.[7a]

Finally, the Mitsunobu reaction was evaluated as a general alkylation process of the sulfonamide nitrogen (Scheme 5) of the *S*-aminosulfenimine **9** (in previous work we had methylated this position using diazomethane; however, this method lacks generality as an alkylation route). Methanol was chosen as an example of a simple alcohol and also because **8** is a known structure.[3a] With 2 equiv of DIAD/$P(Ph)_3$/MeOH the *N*-methylated structure **8** was obtained in 52% yield after chromatography exclusively as the Δ^3 isomer (prolonged reaction times and/or use of more that 2 equiv of reagents did not improve the yield but did lead to Δ^2 isomer formation). The potential value of this modification of the *S*-aminosulfenimine lies in the fact that sulfonamides, the component that is displaced from the *S*-aminosulfenimine, find wide use as biological templates;[10] the sulfonamide derivatives of epipodophyllotoxin are particularly relevant in this context.[13]

Conclusion

In this work we have evaluated the scope and limitations of exploiting the *S*-aminosulfenimine functionality at the 7-position of a cephalosporin to prepare a β-lactamase-dependent, dual-release prodrug structure bearing two distinct biologically active components. Starting from 7-ACA, a viable synthetic cycle was put in place that avoided formation of the Δ^2 isomer throughout and that allowed incorporation of a biologically active amine

(10) Winum, J.-Y.; Vullo, D.; Casini, A.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem*. **2003**, *46*, 2197−2204 and references therein.

(11) (a) Kresze, G.; Wucherpfennig, W. *Angew. Chem., Int. Ed. Engl.* **1967**, *6* 149. (b) Levtschenko, E. S.; Kirsanov, A. V. *Zh.. Org. Khim*. **1962**, *32*, 161−165. (c) Levtschenko, E. S.; Kirsanov, A. V. *Zh.. Org. Khim*. **1962**, *32*, 2256−2262.

(12) Torii, S.; Tanaka, H.; Hamano, S.; Tada, N.; Nokami, J.; Sasaoka, M. *Chem. Lett*. **1984**, 1823−1826. The mechanism involves formation of Br_2 by oxidation of Br^- in the aqueous phase with the co-formation of $Mg^{2+}(^-OH)_2$ following which the sulfenamide NH is brominated in the organic phase and elimination of HBr is driven by ^-OH at the interface to generate the sulfenimine.

(13) (a) Caddick, S.; Wilden, J. D.; Judd, D. B. *J. Am. Chem. Soc*. **2004**, *126*, 1024−1025 and references therein. (b) Guianvarc'h, D.; Duca, M.; Boukarim, C.; Kraus-Berthier, L.; Léonce, S.; Pierré, A.; Pfeiffer, B.; Renard, P.; Arimondo, P. B.; Monneret, C.; Dauzonne, D. *J. Med. Chem*. **2004**, *47*, 2365−2374.

(aminoglutethimide) at the 3′-position and a tosyl *S*-aminosulfenimine at the 7-position. The direct incorporation of a biologically active sulfonamide or sulfamate at this latter position was not achieved as a result of the difficulty of forming the required sulfur diimide reagent. An indirect route for the formation of an *S*-aminosulfenimine was put in place, as was a general method of alkylating the sulfonamide nitrogen of the tosyl *S*-aminosulfenimine following its incorporation. Given that sulfonamides find varied uses as biological templates this latter process offers the possibility of forming a biologically active sulfonamide[13] (as the displaceable component) following formation of the *S*-aminosulfenimine.[14]

Experimental Section

Benzhydryl 7-Aminocephalosporinate (1). To a suspension of 7-ACA (2 g, 7.35 mmol) in methanol (7 mL) was added a solution of diphenyldiazomethane (14.58 mmol) in dichloromethane (40 mL), and the mixture was stirred for 3 days at room temperature over which time the characteristic purple color of the diphenyldiazomethane disappeared. The solution was filtered and concentrated under reduced pressure to yield a brown oily material that precipitated as a fine solid on addition of diethyl ether. The ether was decanted to remove diphenylmethyl methyl ether (a side product); this suspension/decantation step was repeated twice. Purification by flash chromatography (silica gel, 50/50 ethyl acetate/dichloromethane) gave **1** as a pale yellow solid (2.02 g, 4.77 mmol, 65%): mp 122−123 °C (dec) (lit.[3a] 120−121 °C); ν_{max} (KBr) cm^{-1} 3419, 1770 (lactam), 1727; δ_H (90 MHz, $CDCl_3$) 1.84 (br s, 2H), 2.03 (s, 3H), 3.33 (d, J = 18.16 Hz, 1H), 3.60 (d, J = 18.16 Hz, 1H), 4.66−5.09 (m, 4H), 6.98 (s, 1H), 7.34 (s, 10H). Anal. Calcd for $C_{23}H_{22}N_2O_5S$: C, 63.00; H, 5.06; N, 6.39. Found: C, 62.70; H, 4.98; N, 6.28.

Benzhydryl 7-(Allyloxycarbonyl)aminocephalosporinate (1a). To a solution of **1** (2 g, 4.56 mmol) in dichloromethane (30 mL) were added allyl chloroformate (727 μL, 6.84 mmol), freshly distilled pyridine (369 μL, 4.56 mmol), and DMAP (22.28 mg, 0.18 mmol), and the solution was stirred for 4.5 h. Dichloromethane (20 mL) was added, and the organic layer was washed with brine (2 × 40 mL) and distilled water (2 × 40 mL), separated, dried over anhydrous $MgSO_4$, filtered, and removed under reduced pressure to leave a pale yellow solid (2.27 g). Purification by flash chromatography (silica gel, 60/40 ethyl acetate/hexanes) yielded **1a** as a pale yellow solid (2.12 g, 4.07 mmol, 89%): mp 68−70 °C (dec); ν_{max} (KBr) cm^{-1} 1790 (lactam), 1726, 1684; δ_H (300 MHz, $CDCl_3$) 2.02 (s, 3H), 3.39 (d, J = 18.6 Hz, 1H), 3.56 (d, J = 18.6 Hz, 1H), 4.62 (d, J = 4.68 Hz, 2H), 4.80 (d, J = 13.65 Hz, 1H), 4.98 (d, J = 4.92 Hz, 1H), 5.05 (d, J = 13.65 Hz, 1H), 5.25 (app dd, J = 1.11, 11.40 Hz, 1H), 5.33 (app dd, J = 1.41, 17.19 Hz, 1H), 5.50 (d, J = 11.40 Hz, 1H), 5.70 (dd, J = 5.1, 10 Hz, 1H), 5.92 (ddt, J = 5.7, 7.2, 9.9 Hz, 1H), 6.94 (s, 1H), 7.26−7.45 (m, 10H). Anal. Calcd for $C_{27}H_{26}N_2O_7S$: C, 62.06; H, 5.01; N, 5.36. Found: C, 61.8; H, 5.04; N, 5.24.

7-(Allyloxycarbonyl)aminocephalosporanic Acid (1b). Compound **1a** (2.60 g, 4.99 mmol) was dissolved in dichloromethane (60 mL), and the solution was cooled to 0 °C under nitrogen. Trifluoroacetic acid (23 mL, 0.30 mol) and trifluoroacetic anhydride (7 μL, 0.05 mmol) were added. After 10 min, the solution was washed with aqueous acid (pH 2.5), and the organic layer was separated and extracted with 10% $NaHCO_3$ (2 × 50 mL). This aqueous extract was acidified (2 M HCl) to pH 2 and extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield **1b** as a cream solid (1.32 g, 3.70 mmol, 74%): δ_H NMR (90 MHz, $CDCl_3$) 2.01 (s, 3H), 3.46 (d, J = 18.16 Hz, 1H), 3.71 (d, J = 18.16 Hz, 1H), 4.57 (d, J = 4.32 Hz, 2H), 4.78 (d, J = 12.1 Hz, 1H), 5.09 (d, J = 12.1 Hz, 1H), 5.14 (d, J = 5.19 Hz, 1H), 5.34−6.10 (m, 4H), 7.30 (br d, J = 7.78 Hz, 1H).

Benzhydryl 7-(Allyloxycarbonyl)amino-3-hydroxymethyl Cephalosporinate (2b). Compound **1b** (2 g, 5.61 mmol) was suspended in distilled water (30 mL), and the pH was adjusted to pH 6.5 using 35% ammonia solution. Acetyl esterase (Sigma; 1000 units of activity in 72 mL of ammonium sulfate solution) was added to the cephalosporin solution in one portion with constant stirring. The pH immediately decreased and was maintained between pH 6.7 and 6.4 over the course of the reaction using ammonia solution. After approximately 1.5−2 h the pH of the solution changed at a very slow rate. The pH was then adjusted to 2 with 2 M HCl, and the solution was extracted with ethyl acetate (3 × 75 mL). The organic layer was washed with brine (2 × 60 mL) and distilled water (60 mL), separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to ∼20 mL. A solution of diphenyldiazomethane was added dropwise with stirring until the characteristic color of the diphenyldiazomethane persisted. The reaction mixture was left stirring overnight and then concentrated under reduced pressure to yield crude **2b** as a pale yellow solid (2.08 g): ν_{max} (KBr) cm^{-1} 3298, 1785 (lactam), 1717; δ_H (300 MHz, $CDCl_3$) 2.62 (br s), 3.59 (s, 2H), 3.97 (d, J = 12.66 Hz, 1H), 4.41 (d, J = 13.03 Hz, 1H), 4.62 (d, J = 4.65 Hz, 2H), 4.95 (d, J = 4.86 Hz, 1H), 5.25 (app dd, J = 1.35, 10.35 Hz, 1H), 5.33 (app dd, J = 1.35, 17.4 Hz, 1H), 5.59 (d, J = 9.9 Hz, 1H), 5.71 (dd, J = 4.8, 9.9 Hz, 1H), 5.92 (ddt, J = 5.7, 6.61, 10.5 Hz, 1H), 6.92 (s, 1H), 7.26−7.46 (m, 10H). This material was used without further purification in the following step.

Benzhydryl 7-(Allyloxycarbonyl)amino-3-[[(1′,2′,2′,2′-tetrachloroethoxycarbonyl)oxy] methyl] Cephalosporinate (3a). To a solution of **2b** (1.14 g, 2.38 mmol) in dichloromethane (30 mL) were added tetrachloroethyl chloroformate (384 μL, 2.5 mmol), freshly distilled pyridine (202 μL, 2.5 mmol), and DMAP (11.65 mg, 0.095 mmol). The reaction mixture was stirred for 4.5 h. Dichloromethane (20 mL) was added, and the organic layer was washed with brine (2 × 40 mL) and distilled water (2 × 40 mL), separated, dried over anhydrous $MgSO_4$, filtered, and removed under reduced pressure. Purification by flash chromatography (silica gel, 60/40 hexanes/ethyl acetate) gave **2b** as a pale yellow solid (1.28 g, 1.86 mmol, 78%): mp 69−71 °C (dec); δ_H (300 MHz, $CDCl_3$) 3.43 (d, J = 18.66 Hz, 1H), 3.61 (d, J = 18.58 Hz, 1H), 4.62 (d, J = 5.58 Hz, 2H), 5.0 (m, 2H), 5.29 (app dd, J = 1.2, 10.2 Hz, 1H), 5.33 (app dd, J = 1.5, 17.4 Hz, 1H), 5.34 (d, J = 7.8 Hz, 1H), 5.48 (d, J = 9.6 Hz, 1H), 5.73 (dd, J = 5.1, 9.6 Hz, 1H), 5.93 (ddt, J = 5.7, 6.9, 10.2 Hz, 1H), 6.65 (s, 1H), 6.94 (s, 1H), 7.27−7.44 (m, 10H); δ_C (75.47 MHz, $CDCl_3$) 26.2, 57.6, 61.2, 66.7, 68.3, 80.2, 91.1, 96.9, 118.6, 124.5, 126.5, 127.0, 127.7, 128.2, 128.3, 128.5, 128.6, 131.9, 138.8, 138.9, 151.6, 155.2, 160.5, 165.0. Anal. Calcd for $C_{28}H_{24}Cl_4N_2O_8S$: C, 48.71; H, 3.50; N, 4.06. Found: C, 48.46; H, 3.44; N, 3.82.

Benzhydryl 7-(Allyloxycarbonyl)amino-3-[[3′-[[(4-aminocarbonyl)phenyl]-3′-ethyl-2′,6′-piperidinedione]oxy]-methyl] Cephalosporinate (3b). To a solution of **3a** (0.67 g, 0.97 mmol) in dichloromethane (15 mL) were added aminoglutethimide (231.9 mg, 1.0 mmol), freshly distilled pyridine (80.88 μL, 1.0 mmol), and DMAP (4.75 mg, 0.039 mmol), and the mixture was stirred for 5 h. Dichloromethane (30 mL) was added, and the organic layer was washed with brine and distilled water, separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by gravity chromatography (silica gel, 70/30 dichloromethane/ethyl acetate) gave **3b** as a pale yellow solid (0.5207 g, 0.71 mmol, 73%): mp 116−118 °C (dec); ν_{max} (KBr) cm^{-1} 3315, 1787 (lactam), 1722, 1701, 1647; δ_H (500 MHz, $CDCl_3$) 0.85 (t, J = 7.5 Hz, 3H), 1.90 (m, J = 7.5 Hz, 1H), 2.02 (m, J = 7.5 Hz, 1H), 2.20 (m, 1H), 2.33 (m, 1H), 2.40 (m, 1H), 2.61 (m, 1H),

(14) For details of an alternative side chain linkage, see: Ruddle, C. C.; Smyth, T. P. *Chem. Commun.* **2004**, 2332−2333.

3.44 (d, J = 18.5 Hz, 1H), 3.58 (d, J = 18.5 Hz, 1H), 4.61 (d, J = 4 Hz, 2H), 4.85 (d, J = 13.5 Hz, 1H), 4.97 (d, J = 5 Hz, 1H), 5.14 (d, 1H, J = 13.5 Hz, 1H), 5.24 (app d, 10.5 Hz, 1H), 5.32 (app dd, J = 0.75, 17.25 Hz, 1H), 5.65 (d, J = 9.5 Hz, 1H), 5.68 (dd, J = 5, 9.5 Hz, 1H), 5.91 (ddt, J = 5.5, 6.5, 10.5 Hz, 1H), 6.78 (s, 1H), 6.96 (s, 1H), 7.19−7.44 (m, 14H), 8.05 (s, 1H); δ_C NMR (125 MHz, $CDCl_3$) 9.0, 26.6, 27.0, 29.3, 32.9, 50.6, 57.6, 61.3, 63.6, 66.7, 79.3, 118.5, 119.2, 125.9, 127.0, 127.2, 127.8, 128.2, 128.3, 128.5, 128.6, 132.1, 133.9, 136.9, 139.0, 139.2, 152.9, 155.9, 160.8, 165.1, 172.1, 175.1; MALDIFT-HRMS (DHB) *m/z* 761.2275 (M + Na^+, $C_{39}H_{38}N_4O_9S$ requires 761.2252).

Benzhydryl 7-Amino-3-[[3′-[[(4-aminocarbonyl)phenyl]-3′-ethyl-2′,6′-piperidinedione]oxy] methyl] Cephalosporinate (4). To a stirred solution of *p*-toluenesulfinic acid (167.7 mg, 1.07 mmol) and **3b** (198 mg, 0.27 mmol) in dichloromethane (4 mL) was added $Pd(PPh_3)_4$ (93.06 mg, 0.081 mmol) in six portions. After indication by TLC that the reaction was complete, the solution was concentrated to a volume of 1 mL by entrainment with a current of nitrogen. Purification of the residue by flash chromatography (silica gel, 90/10 ethyl acetate/hexanes, under nitrogen) gave a yellow solid (130.8 mg) containing **4** (116 mg, 0.177, mmol, 66% as determined by integration from the ^{1}H NMR spectrum) contaminated by a small amount of triphenylphosphine oxide: δ_H (300 MHz, $CDCl_3$) 0.86 (t, J = 7.5 Hz, 3H), 1.89 (m, 1H), 2.01 (m, 1H), 2.19 (m, 1H), 2.34 (m, 1H), 2.44 (m, 1H), 2.59 (m, 1H), 3.40 (d, J = 18.6 Hz, 1H), 3.58 (d, J = 18.6 Hz, 1H), 4.78 (d, J = 4.5 Hz, 1H) overlapping with 4.80 (d, J = 13.2 Hz, 1H), 4.92 (d, J = 5.1 Hz, 1H), 5.09 (d, J = 13.2 Hz, 1H), 6.86 (s, 1H), 6.98 (s, 1H), 7.18−7.70 (m, 14H), 8.02 (s, 1H); δ_C (125 MHz, $CDCl_3$) 9.0, 26.3, 26.9, 29.3, 32.9, 50.6, 58.8, 60.4, 63.7, 79.7, 119.1, 125.7, 126.2, 126.9, 127.2, 127.8, 128.2, 128.5, 128.53, 128.6, 132.0, 132.2, 133.7, 137.0, 139.0, 139.2, 152.9, 161.2, 169.0, 172.2, 175.1; ESI-HRMS *m/z* 655.2218 (M + H^+, $C_{35}H_{34}N_4O_7S$ requires 655.2221).

Benzhydryl 7-(*N*-Tosyl-*S*-aminosulfenimino)-3-[[3′-[[(4-aminocarbonyl)phenyl]-3′-ethyl-2′,6′-piperidinedione]-oxy]methyl] Cephalosporinate (5). To a solution of **4** (198 mg, 0.30 mmol) in dichloromethane (10 mL) was added bis-(*p*-toluenesulfonyl) sulfur dimide[3a] (133.5 mg, 0.36 mmol), and the mixture was stirred for 1 h. Water (2 mL) was added followed by the slow addition of light petroleum ether (8 mL), the solution was stirred for 10 min, and the precipitated *p*-toluenesulfonamide was filtered off. Dichloromethane (10 mL) and light petroleum ether (8 mL) were added to the filtrate, which was washed with distilled water (2 × 30 mL) and brine (2 × 30 mL), separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to leave a yellow solid. Purification by gravity chromatography (silica gel, 75/25 dichloromethane/ethyl acetate) gave **5** as a yellow solid (78.2 mg, 0.092 mmol, 30%): mp 125−127 °C; ν_{max} (KBr) cm^{-1} 3306, 1780 (lactam), 1725, 1700, 1637; δ_H (500 MHz, $CDCl_3$) 0.85 (t, J = 7.25 Hz, 3H), 1.89 (m, 1H), 2.02 (m, 1H), 2.19 (m, 1H), 2.35 (m, 1H), 2.41 (s, 3H), 2.42 (m, 1H), 2.57 (m, 1H), 3.39 (d, J = 18 Hz, 1H), 3.62 (d, J = 18 Hz, 1H), 4.82 (d, J = 13.25 Hz, 1H), 5.11 (d, 2H, J = 13.25 Hz, 1H), 5.39 (br s, 1H), 6.86 (s, 1H),7.01 (s, 1H), 7.19−7.52 (m, 16H), 7.75 (d, J = 8 Hz, 2H), 8.08 (s, 1H); δ_C (125 MHz, $CDCl_3$) 9.0, 21.7, 27.0, 28.2, 29.3, 32.9, 50.7, 60.7, 63.5, 80.2, 119.3, 126.8, 127.0, 127.2, 127.7, 127.9, 128.2, 128.3, 128.6, 128.63, 130.0, 136.0, 136.9, 138.8, 139.2, 145.0, 152.1, 152.9, 158.1, 160.7, 172.4, 175.2; ESI-HRMS *m/z* 876.1795 (M + Na^+, $C_{42}H_{39}N_5O_9S_3$ requires 876.1802).

7-(*N*-Tosyl-*S*-aminosulfenimino)-3-[[3′-[[(4-aminocarbonyl)phenyl]-3′-ethyl-2′,6′-piperidinedione]oxy]methyl] Cephalosporanic Acid (6). A solution of aluminum trichloride (25.95 mg, 0.19 mmol) in nitromethane (0.5 mL) was added to dichloromethane (8 mL) containing the benzhydryl ester **24** (83.1 mg, 0.097 mmol) at −84 °C (bath), and the mixture was stirred for 20 min. Next, 5% sodium bicarbonate (25 mL) and ethyl acetate (25 mL) were added, and the mixture was vigorously stirred while allowing the temperature to rise to ambient. The mixture was further diluted by the addition of water (40 mL). The organic layer was separated and discarded. The aqueous layer was filtered through a bed of Celite until clear, acidified to pH 2.2 with 2 M HCl, extracted with dichloromethane (2 × 25 mL), separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield the free acid as a yellow solid (45.5 mg, 0.068 mmol, 70%): mp 123−127 °C (dec); ESI-HRMS (MeOH) *m/z* [M − H]$^-$ calcd for $C_{29}H_{28}N_5O_9S_3$ 686.1055, found 686.1056; ν_{max} (KBr) cm^{-1} 3440, 1776, 1719, 1701; δ_H (300 MHz, $CDCl_3$/5% DMSO-d_6) 0.85 (t, J = 7.05 Hz, 3H), 1.90 (m, 1H), 2.01 (m, 1H), 2.19 (m, 1H), 2.34 (m, 1H), 2.41 (s, 3H), 2.42 (m, 1H), 2.57 (m, 1H), 3.47, (d, J = 16.5 Hz, 1H), 3.69 (d, J = 18.6 Hz, 1H), 4.99 (d, J = 12.9 Hz, 1H), 5.18 (d, J = 13.2 Hz, 1H), 5.51 (br s, 1H), 5.84 (s, 1H), 7.19 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 7.8 Hz, 2H), 8.35 (br s, 1H), 8.85 (br s, 1H); δ_C (75.47 MHz, $CDCl_3$/5% DMSO-d_6) 9.0, 21.1, 27.0, 28.2, 29.3, 32.8, 50.5, 60.6, 63.3, 119.1, 123.6, 126.3, 127.4, 129.5, 129.8, 136.7, 137.6, 144.5, 151.7, 153.5, 156.6, 163.1, 172.8, 175.6.

***N*-Methyl-*p*-toluenesulfonamide Sulfenyl Chloride.** Sulfur dichloride was distilled under nitrogen at 58−60 °C with 0.1% w/v phosphorus pentachloride.[15] Sulfur dichloride (0.12 mL, ∼1.5 mmol) was added to *N*-methyl-*p*-toluenesulfonamide (193 mg, 1 mmol) in chloroform (0.5 mL) under nitrogen, and this mixture was left overnight in a stoppered glass vial at room temperature. The solvent and other volatile components were removed under a stream of nitrogen to leave a yellow solid: δ_H (90 MHz, $CDCl_3$) 2.48 (s, 3H), 3.43 (s, 3H), 8.64 (dd, J = 9.5, 45 Hz, 2H). This material was used directly in the following reaction.

Benzhydryl *N*-Methyl-*N*-tosyl-*S*-aminosulfenamidocephalosporinate (7). To a solution of **8** (0.5 g, 1.14 mmol) in chloroform (15 mL) was added to *N*-methyl-*p*-toluenesulfonamide sulfenyl chloride (0.36 g, 1.43 mmol) in chloroform (15 mL). The reaction mixture was washed immediately with 0.25 M sodium bicarbonate (2 Î 30 mL) and distilled water (2 × 20 mL), separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield a yellow solid. Purification by wet flash chromatography (silica gel, dichloromethane/ethyl acetate 70/30) gave **7** as a yellow solid (0.51 g, 0.8 mmol, 68%): mp 76−78 °C; δ_H (300 MHz, $CDCl_3$) 1.64 (br s, 1H), 2.02 (s, 3H), 2.42 (s, 3H), 3.28 (s, 3H), 3.39 (d, J = 18.6 Hz, 1H), 3.64 (d, J = 18.6 Hz, 1H), 4.79 (d, J = 13.53 Hz, 1H), 5.02 (d, J = 13.8 Hz, 1H), 5.10 (d, J = 4.50 Hz, 1H), 5.29 (dd, J = 4.5, 10.2 Hz, 1H), 6.95 (s, 1H), 7.26−7.45 (m, 12H), 7.75 (d, J = 8.19 Hz, 2H); δ_C NMR (75.47 MHz, $CDCl_3$) 20.7, 21.6, 26.7, 40.9, 58.6, 63.1, 73.1, 79.7, 125.8, 126.3, 127.1, 127.3, 127.7, 128.1, 128.2, 128.5, 128.6, 129.8, 136.2, 139.1, 139.2, 144.0, 160.8, 165.1, 170.5. Anal. Calcd for $C_{31}H_{31}N_3O_7S_3$: C, 56.95; H, 4.78; N, 6.43. Found: C, 56.77; H, 4.73; N, 6.27.

Benzhydryl *N*-Methyl-*N*-tosyl-*S*-aminosulfeniminocephalosporinate (8). Electrochemical Method. The electrolysis was carried out in a biphasic cell system in a beaker-type undivided cell, with two Pt electrodes (2 × 2 cm^2) held in the upper aqueous layer (25 mL) containing $MgBr_2$ (0.5 g, 2.71 mmol) and with sulfenamide **7** (150 mg, 0.23 mmol) in dichloromethane (25 mL) as the (lower) organic layer. Direct current (20−25 mA) was applied, and the mixture was stirred at a rate that was moderate enough to allow the electrodes to remain immersed in the aqueous medium. After 4.5 h the organic layer was separated, washed with brine (2 × 25 mL) and distilled water (25 mL), separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield a dark brown solid. Purification by gravity chromatography (silica gel, 90/10 dichloromethane/ethyl acetate) yielded **8** as a light yellow solid (113.4 mg, 0.17 mmol, 76%): mp 86−89 °C (lit.[3a] 87−92); δ_H NMR[3a] (90 MHz, $CDCl_3$) 2.03 (s, 3H), 2.45 (s, 3H), 3.28 (d, J = 7.95 Hz, 1H) overlapping with (s,

(15) Tardif, S. L.; Harpp, D. N. *J. Org. Chem*. **2000**, *65*, 4791−4795.

3H), 3.50 (d, J = 7.95 Hz, 1H), 4.76 (d, J = 12.54 Hz, 1H), 5.03 (d, J = 12.54 Hz, 1H), 5.49 (s, 1H), 6.99 (s, 1H), 7.22−7.55 (m, 12H), 7.79 (d, J = 7.98 Hz, 2H).

Benzhydryl *N*-Methyl-*N*-tosyl-*S*-aminosulfeniminocephalosporinate (8). Mitsunobu Process. Triphenylphosphine (205.3 mg, 0.78 mmol) was dissolved in a solution of methanol (32 μL, 0.78 mmol) and dichloromethane (1.5 mL) under N_2. The solution was cooled to −23 °C (bath), and diisopropyldiazidodicarboxylate (154.5 μL, 0.78 mmol) in dichloromethane (3 mL) was added dropwise with stirring. DIAD (yellow) decolorized almost instantaneously. **9**[3a] (250 mg, 0.39 mmol) in dichloromethane (2 mL) was added, and the reaction mixture was stirred for 10 min at −23 °C (bath) and then allowed to warm to ambient temperature (total reaction time of 45 min). Dichloromethane (15 mL) was added, and the organic layer was washed with brine (2 × 30 mL) and distilled water (2 × 30 mL), separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield a yellow solid. Purification by flash chromatography (silica gel, dichloromethane) gave **8** as a yellow solid (134.5 mg, 0.21 mmol, 53%). The ^{1}H NMR spectrum was identical to that given above.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for **3a**, **3b**, **4**, **5**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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