Table II. In Vivo Studies. Maximum Doses Administered to Mice

compound	vol injected per 25 g of body wt, mL	max dose, mmol kg^{-1} [c]	limiting factor
16	1.0	0.12 (25 mg kg^{-1})	solubility[a]
18	0.5	0.44 (100 mg kg^{-1})	toxicity[b]
20	0.5	0.24 (50 mg kg^{-1})	toxicity[b]
25	1.0	0.11 (25 mg kg^{-1})	solubility[a]

[a] Injected as suspensions. [b] No acute, severe, or persistent physical or behavorial effects were apparent in C3H/He mice with any of the doses shown in the table. However, following injection of 18, piloerection, an increased respiration rate, and decreased locomotor activity were observed in the mice for approximately 1 h after injection. After higher doses of any of the drugs limited by toxicity severe tremors and convulsions occurred, from which the mice did not recover. [c] Injected ip.

16 and **20** from 0.5 g (2.5 mmol) of **29**. Recrystallization from EtOH gave **30** (0.3 g, 53%) as colorless needles: mp 129–130 °C; NMR ($CDCl_3$) δ 2.8 (s, 3 H, furan-CH_3), 2.75–4.1 (m, 5 H, NCH_2 and 3 × oxirane-H), 6.5 (br, 1 H, NH), 7.45 (s, 1 H, furan 4-H). Anal. ($C_9H_{10}N_2O_5$) C, H, N.

2-Methyl-3-nitro-*N*-(prop-2-enyl)furan-5-carboxamide (31). 2-Methyl-3-nitrofuran-5-carboxylate (prepared by the method of Rinkes[14]) (0.25 g, 1.4 mmol) was stirred with allylamine (3 mL, 69 mmol) and dicyclohexylcarbodiimide (2.1 g, 10 mmol) in THF (7 mL) at 25 °C for 12 h. Excess amine was then evaporated after filtration and the residue was purified by chromatography (silica gel; EtOAc/$CHCl_3$ (1:1, v/v)) to give **31** (0.1 g, 35%) as pale yellow prisms: mp 55–56 °C; NMR ($(CD_3)_2SO$) δ 2.6 (s, 3 H, furan-CH_3), 4.0 (m, 2 H, allylic CH_2), 5.2–5.8 (m, 3 H, $CH{=}CH_2$), 7.6 (s, 1 H, furan 4-H), 8.5 (br t, J = 7 Hz, 1 H, NH). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

Biological Methods. The radiosensitization studies in vitro were carried out as described previously with use of Chinese hamster V79-379A cells.[8] The methods for determining selective toxicity to hypoxic V79-379A cells using the MTT assay are also described elsewhere.[20]

On the basis of results from experiments in vitro, compounds **16**, **18**, **20**, and **25** were selected for evaluation in vivo in C3H/He mice. The compounds were injected as suspensions or solutions in phosphate-buffered saline (pH 7.3). TLC analyses were carried out to show that the compounds were unchanged at the time of injection. Initial studies were carried out to determine the maximum doses of compounds which could be administered (Table II). Subsequently, the maximum single doses of each compound which could be administered (according to toxicity or solubility) were injected at various times (5–90 min) before local irradiation of subcutaneous KHT sarcomas with a 10-Gy dose of X-rays. Tumors were excised 24 h later and clonogenic assays performed in vitro to determine the survival of tumor cells.[8]

Acknowledgment. Financial support from the British Technology Group is gratefully acknowledged. We thank Dr. M. A. Stier and Dr. M. J. Suto of Parke-Davis Pharmaceutical Research, Warner-Lambert Company, Ann Arbor, MI 48105, for the mass spectroscopic data.

(20) Stratford, I. J.; Stephens, M. A. *Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *16*, 973.
(21) O'Neill, P.; Jenkins, T. C.; Stratford, I. J.; Silver, A. R. J.; Ahmed, I.; McNeil, S. S.; Fielden, E. M.; Adams, G. E. *Anti-Cancer Drug Des.* **1987**, *1*, 271.
(22) Wardman, P.; Clarke, E. D. *Biochem. Biophys. Res. Comm.* **1976**, *69*, 942.

Inhibition of Human Leukocyte Elastase. 1. Inhibition by C-7-Substituted Cephalosporin *tert*-Butyl Esters

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Time-dependent inhibitors of the enzyme human leukocyte elastase have been developed based on the cephem nucleus. A series of cephalosporin *tert*-butyl esters has been examined, and the activity of these compounds has been found to be very sensitive to C-7 substituents, with small, α-oriented, electron-withdrawing groups showing greatest activity. Additionally, the oxidation state of the sulfur atom has been found to play a role in potency, with sulfones showing considerably greater activity than the corresponding sulfides or β-sulfoxides. The α-sulfoxides were inactive.

The azurophilic granules of human polymorphonuclear leukocytes (PMN) contain a serine protease referred to as human leukocyte elastase (HLE EC 3.4.21.37) because of its ability to degrade elastin in addition to a number of other connective-tissue substrates.[1] The possible pathological consequences of the release of HLE from the PMN into the extracellular environment have been the subject of considerable research and speculation for the past 25 years.[2,3] Substantial effort has gone into the study of the interaction of HLE with the major naturally occurring protease inhibitor of plasma, α-1 protease inhibitor (α_1-PI).[4] In particular, α_1-PI is considered the primary

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[»] Present address: Vertex Pharmaceuticals, 40 Alston St., Cambridge, MA 02139-4211.

(1) Starkey, P. M.; Barrett, A. J. *Biochem. J.* **1976**, *155*, 265.
(2) Laurell, C. B.; Eriksson, S. *Scand. J. Clin. Invest.* **1963**, *15*, 132.
(3) Janoff, A. *Am. Rev. Respir. Dis.* **1985**, *132*, 417.
(4) Morrison, H. *Clin. Sci.* **1987**, *72*, 151.

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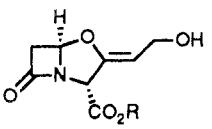
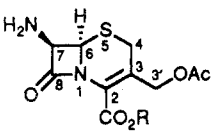
guardian against HLE-mediated connective-tissue destruction and it associates with HLE in a very fast (k_{on} = 8×10^6 M^{-1} s^{-1}) and essentially irreversible manner.[5] Nevertheless, HLE has been implicated in the pathology of a number of disease states, particularly in situations where plasma α_1-PI levels are genetically low[6] or where the efficacy of the α_1-PI has been compromised by oxidation or degradation,[7] or where direct release onto target tissues has made access to HLE difficult for α_1-PI.[8] In such situations, the use of low molecular weight synthetic inhibitors may be of therapeutic benefit. We wish to report here the first in a series of studies of substituted β-lactams which are potent time-dependant inhibitors of HLE.[9]

The β-lactam antibiotics such as penicillin G, cephalothin, and thienamycin acylate and thus inactivate bacterial transpeptidases and carboxypeptidases that are crucial to the viability of the microorganism.[10] Although the bacterial enzymes bear little similarity to members of the chymotrypsin superfamily of serine proteases[11] such as HLE, apart from the utilization of a serine hydroxyl as a key nucleophile at the active site, it is reasonable to hypothesize that acyl enzymes formed by the nucleophilic attack on and ring opening of β-lactam nuclei may have an adequate lifetime to function effectively as HLE inhibitors and as therapeutic agents. Considerable data has been generated in our laboratories and in others demonstrating the effective inhibition of HLE by serine hydroxyl acylation.[12] The seminal observation that benzyl clavulanate (**1b**) *but not clavulanic acid* (**1a**) inhibited HLE[13]



1a: R = H
b: R = CH_2Ph



2a: R = H
b: R = *t*-Bu

led to the hypothesis that, since HLE is an endopeptidase (as opposed to the bacterial enzymes which are carboxypeptidases), it may be critical to disperse or otherwise effectively quench the negative charge that the β-lactam antibiotics normally require in order to create time-dependent HLE inhibitors. Our studies with a variety of β-lactam nuclei have shown this hypothesis to be supported. The combination of the synthetic versatility of the cephem nucleus and its ready commercial availability (as 7-aminocephalosporanic acid, 7-ACA, **2a**) made it particularly attractive to us for exploration, and we report here some results in this series.

Results and Discussion

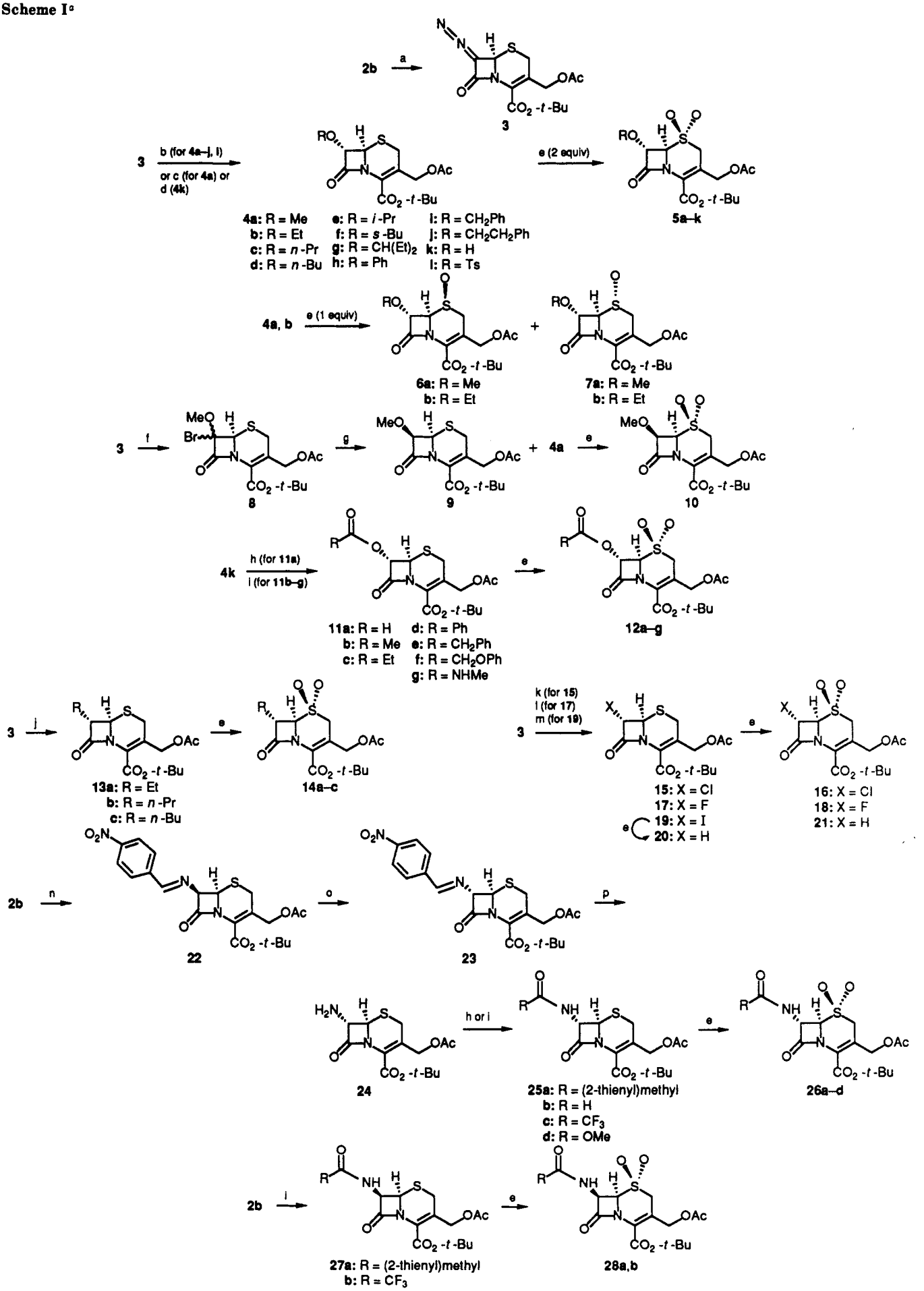
A. Synthesis. The synthesis of the compounds described here followed the general outlines shown in Scheme I. The key intermediate for the preparation of the alkoxy, alkyl, and halo analogues is diazo ketone **3** prepared from 7-aminocephalosporanic acid *tert*-butyl ester with 1 equiv of $NaNO_2$ in aqueous H_2SO_4/CH_2Cl_2.[14] The use of larger excesses of $NaNO_2$ led to lower yields of product. Intermediate **3**, upon treatment with tosic acid in methanol, yielded **4a** in 15–20% yield from **2** after preparative HPLC as the principal product along with variable amounts of the 7α-tosyloxy compound **41**. In our hands, **3** proved to be quite unstable, and this instability is principally responsible for the low yields of **4a**.[15] Other alcohols behaved similarly, although it became impractical to use extreme excesses of alcohols of higher molecular weight, resulting in a further reduction in yields. Alternatively, **4a** could be obtained in somewhat higher yield as the only isolable product by using $Rh_2(OAc)_4$ as catalyst in place of tosic acid. Oxidation of the sulfur was carried out with *m*-chloroperbenzoic acid (*m*-CPBA) to furnish either the mixture of sulfoxides **6** and **7** (1 equiv of *m*-CPBA) or sulfone **5** (2 equiv). The sulfoxides could be easily separated by silica gel chromatography with the more polar isomer being tentatively assigned as α-sulfoxide **7**. In support of this assignment, it was found that only isomer **6** would undergo the Mannich reaction which is reported to work only for β-sulfoxides.[16] For comparison purposes, **10**, the 7β-isomer of **5a**, was synthesized by first reacting **3** with bromine in methanol to give **8** as a mixture of isomers.[17] This mixture was then reduced with Zn–acetic acid to give **4a** and its 7β-isomer **9**, which were easily separated by chromatography. Oxidation of **9** then gave **10**. The 7α-hydroxy analogue **4k** could be acylated in a straightforward (ROCl/pyridine/CH_2Cl_2) fashion to furnish a series of 7α-acyloxy derivatives **11** which were oxidized to sulfones **12** in an uneventful manner.

The synthesis of the 7-alkyl analogues was carried out with **3** and the appropriate trialkylborane in the manner first described by Wynberg et al.[14] Thus, a solution of **3** in CH_2Cl_2 and a solution of triethylborane in THF were added simultaneously to a chilled (−78 °C) THF solution containing 3 equiv of water, followed by addition of H_2O_2. Workup including preparative HPLC typically yielded ca. 15–20% of 7-ethylcephem as a 3/1 mixture of α/β isomers. A sample of pure trans isomer **13a** could be separated from the mixture by multiple-elution preparative TLC. The yields for **13b** and **13c** were similar to that of **13a**, but the product ratio favored the trans product to a greater degree (6/1 α/β for **13b**, 7/1 for **13c**).

The 7α-chloro analogue **16** was prepared by treatment of **3** with anhydrous HCl in CH_2Cl_2 to give **15** followed by oxidation. No 7β-chloro isomer could be detected. The preparation of 7α-fluoro compound **18** required treatment of **3** with HF/pyridine to give intermediate product **17** in 6% yield. The structure of **17** was clear from the diagnostic doublet of doublets for the C-7 proton with coupling constants of 54 Hz for the geminal CHF moiety and 1.2

(5) Stein, R., unpublished results. This number is somewhat lower than that which has previously been reported, see: Travis, J.; Bieth, J. *Annu. Rev. Biochem.* **1982**, *52*, 655.
(6) Heng, M.; Moy, R. L.; Lieberman, J. *Br. J. Dermatol.* **1985**, *112*, 129.
(7) Abrams, W. R.; Eliraz, A.; Kimbel, P.; Weinbaum, G. *Exp. Lung Res.* **1980**, *1*, 211.
(8) Campbell, E. J. *Am. Rev. Respir. Dis.* **1986**, *134*, 435.
(9) Doherty, J. B.; Ashe, B. M.; Argenbright, L. W.; Barker, P. L.; Bonney, R. J.; Chandler, G. O.; Dahlgren, M. E.; Dorn, C. P.; Finke, P. E.; Firestone, R. A.; Fletcher, D.; Hagmann, W. K.; Mumford, R. A.; O'Grady, L.; Maycock, A. M.; Pisano, J.; Shah, S.; Thompson, K. R.; Zimmerman, M. *Nature* **1986**, *322*, 192.
(10) Frere, J.-M.; Joris, B. *CRC Crit. Rev. Microbiol.* **1984**, *11* (*4*), 299.
(11) Barrett, A. J. In *Proteinase Inhibitors. Research Monographs in Cell and Tissue Physiology*; Barrett, A. J., Salvesen, G., Eds.; Elsevier: Amsterdam, Vol. 12, Chapter 1.
(12) Trainor, D. A. *Trends in Pharm. Sci.* **1987**, *8*, 303.
(13) Zimmerman, M., unpublished results.
(14) Wiering, J. S.; Wynberg, H. *J. Org. Chem.* **1976**, *41*, 1574. For a discussion of the effects of other esters and amides on HLE inhibitory activity, see Finke, P. et al. *J. Med. Chem.* following paper in this issue.
(15) Blacklock, T. J.; Butcher, J. W.; Sohar, P.; Lamanec, T. R.; Grabowski, E. J. J. *J. Org. Chem.* **1989**, *54*, 3907.
(16) Jaszberenyi, J.; Petrikovics, I.; Gunda, E. T.; Hosztafi, S. *Acta Chim. Acad. Sci. Hung.* **1982**, *110*, 81.
(17) Cama, L. D.; Leanza, R. J.; Beattie, T. R.; Christensen, B. G. *J. Am. Chem. Soc.* **1972**, *94*, 1408.

Scheme I[a]



[a] (a) $NaNO_2/H_2SO_4$; (b) *p*-tosic acid, ROH; (c) Rh_2OAc_4, MeOH; (d) $HClO_4/H_2O$; (e) *m*-CPBA; (f) Br_2, MeOH; (g) Zn/NH_4OAc, THF; (h) $HCOOCOCH_3$, py; (i) RCOCl, py; (j) R_3B, H_2O_2, H_2O, THF; (k) HCl, EtOH; (l) HF–py; (m) HI; (n) *p*-NO_2PhCHO, $MgSO_4$, CH_2Cl_2; (o) Et_3N; (p) Girard's T, MeOH.

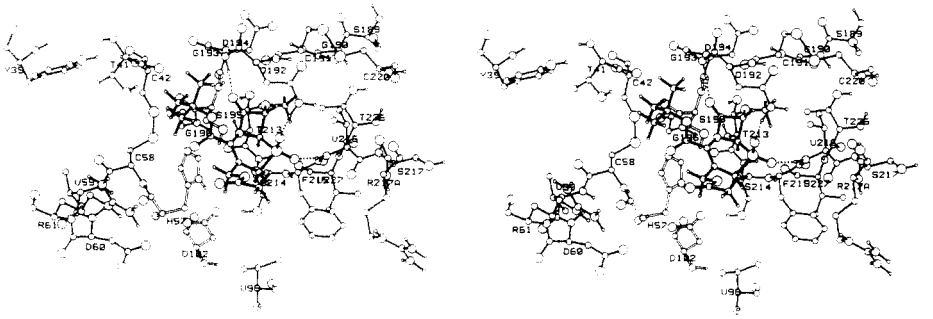


Figure 1. The structure of **5a** as optimized in the PPE active site, showing the methoxy group penetrating into the S1 specificity pocket. The inhibitor is shown in bold, and the Ser 195, His 57, and Asp 102 residues of the catalytic triad are in shown with open bonds. Hydrogen bonds from the carbonyl oxygen to the amide hydrogen of Gly 193 and from the sulfone oxygens to Val 216 and to the Gln 194 side chain are indicated by dotted lines. The PPE structure is that of tosyl elastase (Sawyer, L.; Shotton, D. M.; Watson, H. C. *J. Mol. Biol.* **1978**, *118*, 137) except that His 57 has been repositioned by modifying χ_1 from 160° to 81° and χ_2 from −5° to 121°. The position shown for **5a** resulted from its optimization in the PPE site using an extended version of the MM2 force field.

Hz characteristic of a trans relationship of the C-6 and C-7 protons.[18] While **3** could be converted to 7-iodo analogue **19**[19] (in this case a 1/1 mixture of 7α- and 7β-isomers was observed) by treating **3** with concentrated HI, attempts to convert **19** to the sulfone using *m*-CPBA failed, presumably due to competing oxidation of the iodo moiety. Compound **19** did prove useful as an intermediate for the preparation of the unsubstituted analogue **21** by removal of the iodo group with Zn/NH_4OAc to give **20** followed by oxidation.

7α-Amido analogues could be obtained by treatment of **2b** with *p*-nitrobenzaldehyde to form Schiff base **22**, followed by exposure to Et_3N to yield a 55/45 mixture of **23**/**22** which could be separated chromatographically.[20] Hydrolysis of **23** then gave **24**, which was then acylated with the appropriate activated acid and oxidized to yield **26**. Finally, direct acylation of **2b** followed by oxidation yielded 7β-amides **28**.

B. Inhibitory Activity against HLE. In the cephem series, unlike that in the clavam case, esterification of the acid moiety at C-2 is not in itself sufficient to produce compounds that function as inhibitors of HLE (Table I, entries **1b** and **21**). Esterification of cephem antibiotics such as cephalothin is likewise ineffective (Table I, entry **27a**). However, when a small group such as methoxy is incorporated at the 7α-position of *tert*-butyl cephalosporanate, a marked increase in inhibitory activity against HLE is seen (Table I, **4a**). HLE is an enzyme with a propensity for cleaving peptides at amino acids bearing small hydrophobic residues in the P1-position[21] of its substrates.[22] Moreover, oxidation of the sulfide of **4a** to sulfone **5a** results in a further substantial increase in potency. Molecular modeling of **5a** at the active site of the related enzyme porcine pancreatic elastase (PPE) shows that the 7α-methoxy moiety fits nicely into the relatively small S1 binding pocket of the enzyme (see Figure 1). Experimental results are in accord with this model of binding. First, compound **10**, the 7β-isomer of **5a**, is much less active than **5a**, in agreement with modeling's prediction that the α-orientation is required for a C-7 substituent to interact effectively with the S1 site. Second, examination of the series **5a–d** shows that increasing the chain length of the ether moiety at the 7-position leads to a rapid dropoff in enzymatic activity, presumably because the added steric bulk cannot be accommodated near the active site.[22] 7α-Hydroxy compound **5k** was rapidly turned over and functioned essentially as a substrate for the enzyme. Both phenoxy **5h** and phenethyloxy **5j** (but *not* benzyloxy compound **5i**) showed reasonable inhibitory activity against HLE, suggesting that aromatic rings may be accommodated at the active site where simple aliphatics cannot if the side chain is of appropriate length.

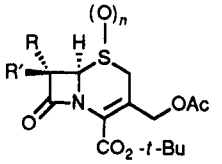
The structure–activity relationship for the 7α-alkanoyloxy series **12a–g** closely mimicked that seen in the alkoxy series. Again, the compound with the smallest substituent sterically, **12a**, was the most potent inhibitor. Benzoate **12d**, isosteric with **5i**, was also a poor inhibitor of HLE whereas **12e** and **12f** showed significant activity. In general, we have found that the measured second-order rate constants of inactivation ($k_{obs}/[I]$) show trends consistent with those of the IC_{50} values. In some cases discrepencies exist, as in the comparison of **5c** with **12c**; **5c** has a greater $k_{obs}/[I]$ (280 M^{-1} s^{-1}) than **12c** (80 M^{-1} s^{-1}), but **12c** has a lower IC_{50} (10 μM) than **5c** (40 μM). These differences can probably be explained by the fact that $k_{obs}/[I]$ values take into account both the binding of the inhibitor to the active site as well as the rate of conversion of that complex to a covalently inhibited form, whereas the IC_{50} may or may not reflect a contribution from covalent binding of the inhibitor to the enzyme.

Replacement of the methoxy group at C-7 with an ethyl group yielded a compound (**14a**) which retained most of the activity of **5a**. As in the alkoxy and alkanoyloxy series, increasing the size of the alkyl substituent led to greatly reduced activity (compare **14a**, **14b**, and **14c**). The importance of the interaction of the C-7 substituent with the enzyme is underscored by the observation that the unsubstituted compound **21** is a very poor inhibitor of HLE. Substitution at C-7 by halogen gave very active compounds (**16**, **18**), probably due in large part to the increased intrinsic reactivity of the β-lactam ring in these compounds.

Virtually all cephalosporins which are effective antibiotics have a requirement for a 7β-amido side chain.[23] Naturally, we were intrigued to see if C-7 amido groups could also lead to effective HLE inhibitors. While it was

(18) Demarco, P. V.; Nagarjan, R. In *Cephalosporins and Penicillins: Chemistry and Biology*; Flynn, E. H., Ed.; Academic Press: New York, 1972; Chapter 8.
(19) DiNinno, F.; Beattie, T. R.; Christensen, B. G. *J. Org. Chem.* **1977**, *42*, 2960.
(20) Firestone, R.; Maciejewicz, N.; Ratcliffe, R.; Christensen, B. G. *J. Org. Chem.* **1974**, *39*, 437.
(21) Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
(22) Zimmerman, M. In *Biological Functions of Proteinases-30. Colloquium der Gesellschaft fur Biologische Chemie*; Holzer, H., Tschesche, H., Eds.; Springer-Verlag: Berlin, 1979; p 186.
(23) Christensen, B. In *β-Lactam Antibiotics*; Salton, M., Schockman, G., Eds.; Academic Press: New York, 1981; p 101.

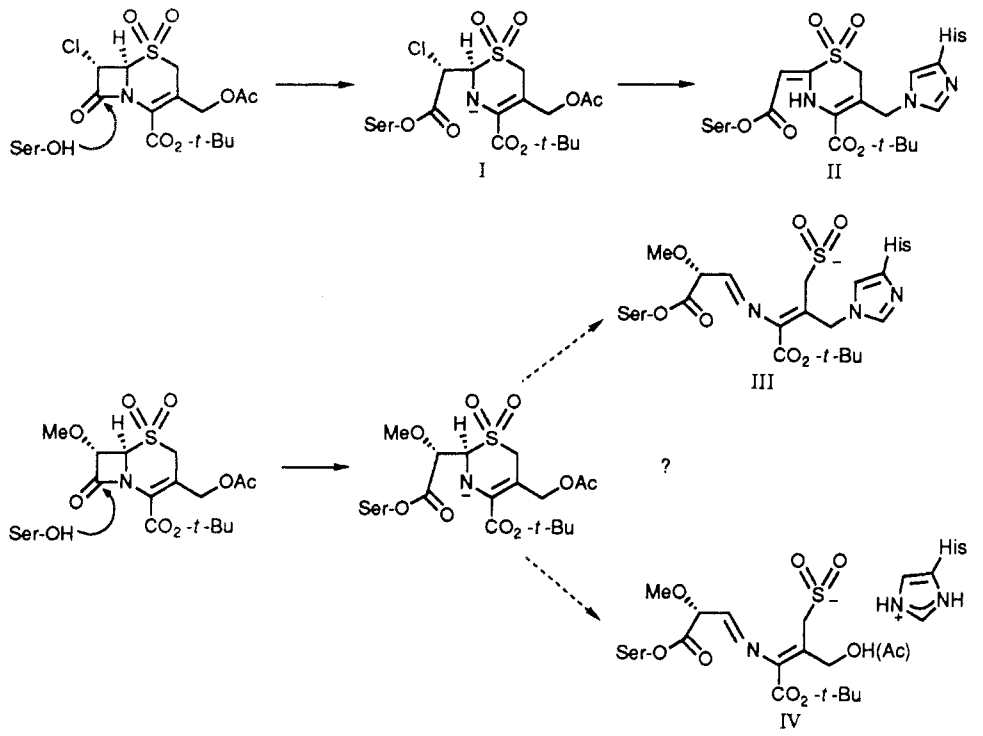
Table I[a]



compd	R	R'	n	IC_{50}, μM	$k_{obs}/[I]$, M^{-1} s^{-1} (SD)	% yield (method)	mp, °C	formula
1a	(clavulanic acid)			<40	ND			
1b	(benzyl clavulanate)			4	ND			
2b	H	NH_2	0	>40	ND			
4a	MeO	H	0	10	ND	19 (A)	oil	$C_{15}H_{21}NO_6S$
4b	EtO	H	0	>40	ND	7 (A)	oil	$C_{16}H_{23}NO_6S$
4c	*n*-PrO	H	0	>40	ND	17 (A)	oil	$C_{17}H_{25}NO_6S$
4d	*n*-BuO	H	0	>40	ND	15 (A)	oil	$C_{18}H_{27}NO_6S$
4e	*i*-PrO	H	0	ND	ND	12 (A)	oil	$C_{17}H_{25}NO_6S$
4f	*s*-BuO	H	0	>40	ND	8.5 (A)	85 dec	$C_{18}H_{27}NO_6S$
4g	$(Et)_2CHO$	H	0	ND	ND	5 (A)	90 dec	$C_{19}H_{29}NO_6S$
4h	PhO	H	0	ND	ND	5 (A)	oil	$C_{20}H_{23}NO_6S$
4i	$PhCH_2O$	H	0	ND	ND	13 (A)	76–79 dec	$C_{21}H_{25}NO_6S$
4j	$PhCH_2CH_2O$	H	0	ND	ND	6 (A)	oil	$C_{22}H_{27}NO_6S$
4k	HO	H	0	ND	ND	13	foam	$C_{14}H_{19}NO_6S$
4l	TsO	H	0	NVT	ND	4 (A)	*	$C_{21}H_{25}NO_8S_2$
5a	MeO	H	2	1	19000 (1,500)	55 (C)	127 dec	$C_{15}H_{21}NO_8S$
5b	EtO	H	2	3	2100 (120)	66 (C)	80–83 dec	$C_{16}H_{23}NO_8S$
5c	*n*-PrO	H	2	40	280 (50)	70 (C)	79–81 dec	$C_{17}H_{25}NO_8S$
5d	*n*-BuO	H	2	>40	ND	79 (C)	84–86 dec	$C_{18}H_{27}NO_8S$
5e	*i*-PrO	H	2	30	ND	75 (C)	oil	$C_{17}H_{25}NO_8S$
5f	*s*-BuO	H	2	28	ND	72 (C)	oil	$C_{18}H_{27}NO_8S$
5g	$(Et)_2CHO$	H	2	>40	ND	77 (C)	85–86 dec	$C_{19}H_{29}NO_8S$
5h	PhO	H	2	1	720 (110)	60 (C)	127–128 dec	$C_{20}H_{23}NO_8S$
5i	$PhCH_2O$	H	2	>10 (a)	ND	90 (C)	183–185 dec	$C_{21}H_{25}NO_8S$
5j	$PhCH_2CH_2O$	H	2	5	ND	86 (C)	116–117 dec	$C_{22}H_{27}NO_8S$
5k	HO	H	2	(substrate)	ND	45 (C)	128–130 dec	$C_{14}H_{19}NO_8S$
6a	MeO	H	1(β)	4	500 (20)	32	87 dec	$C_{15}H_{21}NO_7S$
6b	EtO	H	1(β)	40	ND	30	oil	$C_{15}H_{21}NO_7S$
7a	MeO	H	1(α)	>40	ND	25	oil	$C_{15}H_{21}NO_7S$
7b	EtO	H	1(α)	>40	ND	30	oil	$C_{15}H_{21}NO_7S$
10	H	MeO	2	>40	ND	80 (C)	147 dec	$C_{15}H_{21}NO_8S$
11a	HCOO	H	0	10	ND	43 (B)	140–150 dec	$C_{15}H_{19}NO_7S$
11b	MeCOO	H	0	40	ND	37 (B)	94–95 dec	$C_{16}H_{21}NO_7S$
11c	EtCOO	H	0	36	ND	27 (B)	120–121 dec	$C_{17}H_{23}NO_7S$
11d	PhCOO	H	0	ND	ND	46 (B)	106–107 dec	$C_{21}H_{23}NO_7S$
11e	$PhCH_2COO$	H	0	ND	ND	40 (B)	135–138 dec	$C_{22}H_{25}NO_7S$
11f	$PhOCH_2COO$	H	0	ND	ND	31 (B)	148–149 dec	$C_{22}H_{25}NO_8S$
11g	MeNHCOO	H	0	ND	ND	68	149–150 dec	$C_{16}H_{22}N_2O_7S$
12a	HCOO	H	2	0.3	59000 (6000)	41 (C)	171–172 dec	$C_{15}H_{19}NO_9S$
12b	MeCOO	H	2	6	950 (20)	36 (C)	oil	$C_{16}H_{21}NO_9S$
12c	EtCOO	H	2	10	80 (10)	84 (C)	154–155 dec	$C_{17}H_{23}NO_9S$
12d	PhCOO	H	2	>40	ND	67 (C)	136–138 dec	$C_{21}H_{23}NO_9S$
12e	$PhCH_2COO$	H	2	10	ND	62 (C)	112–113 dec	$C_{22}H_{25}NO_9S$
12f	$PhOCH_2COO$	H	2	6	ND	50 (C)	117–120 dec	$C_{22}H_{25}NO_{10}S$
12g	MeNHCOO	H	2	1	180 (10)	84 (C)	157–158 dec	$C_{16}H_{22}N_2O_9S$
13a	Et	H	0	ND	ND	19 (D)	oil	$C_{16}H_{23}NO_5S$
13b	*n*-Pr	H	0	ND	ND	26 (D)	oil	
13c	*n*-Bu	H	0	ND	ND	21 (D)	oil	
14a	Et	H	2	2	ND	81 (C)	91–94 dec	$C_{16}H_{23}NO_7S$
14b	*n*-Pr	H	2	24	ND	61 (C)	102–105 dec	$C_{17}H_{25}NO_7S$
14c	*n*-Bu	H	2	40	ND	59 (C)	94–97 dec	$C_{18}H_{27}NO_7S$
15	Cl	H	0	2	ND	20	oil	
16	Cl	H	2	0.04	161000 (9500)	71 (C)	140–143 dec	$C_{14}H_{18}ClNO_7S$
17	F	H	0	20	ND	6	oil	
18	F	H	2	0.06	ND	71 (C)	oil	$C_{14}H_{18}FNO_7S$
21	H	H	2	>40	ND	82 (C)	163–164 dec	$C_{14}H_{19}NO_7S$
25a	ThCONH	H	0	ND	ND	48 (E)	oil	
25b	HCONH	H	0	ND	ND	71	132–135 dec	$C_{15}H_{19}N_2O_6S$
25d	MeOCONH	H	0	ND	ND	91 (E)	168–170 dec	$C_{16}H_{21}N_2O_7S$
26a	ThCONH	H	2	>40	ND	71 (C)	foam	$C_{20}H_{23}N_2O_8S_2$
26b	HCONH	H	2	4	1200 (110)	76 (C)	166 dec	$C_{15}H_{19}N_2O_8S$
26c	CF_3CONH	H	2	2	ND	76 (C)	170 dec	$C_{16}H_{18}F_3N_{O8}S$
26d	MeOCONH	H	2	4	1600 (200)	84 (C)	154 dec	$C_{16}H_{21}N_2O_9S$
28a	H	ThCONH	2	>40	ND	64 (C)	foam	$C_{20}H_{23}N_2O_8S_2$
28b	H	CF_3CONH	2	>40	ND	81 (C)	oil	$C_{16}H_{18}F_3N_2O_8S$

[a] Th = 2-thienylmethyl. ND, not determined; NVT, nonvalid test; SD, standard deviation; (a), limit of solubility of this compound; *, low-melting solid. HLE (EC 3.4.21.37) as obtained from Elastin Products, St. Louis, MO. IC_{50} values were determined as previously described.[9] To determine second-order rate constants ($k_{obs}/[I]$) for inactivation, the data were fit by nonlinear regression to the equation $A = v_s t + (v_0 - v_s)(1 - e^{k_{obs}t})/k_{obs} + C$, which describes the amount of cleaved substrate (A) as a function of time (t) in the presence of a concentration of inhibitor [I] which causes first-order enzyme inactivation. Values of the initial velocity (v_0), final velocity (v_s), and first order rate constant k_{obs} are obtained from the curve. Results are expressed in terms of the bimolecular rate constant $k_{obs}/[I]$ in M^{-1} s^{-1}.[32]

Scheme II



found that small α-oriented amides such as **26b–d** had some activity, this class in general was not as potent, possibly due to the rigidity and/or polarity of the amide bond mediating against an optimum interaction of these moieties with the S1 site. In keeping with our observations on the importance of the orientation of the C-7 substituent in the alkoxy series, it was found here also that 7β-substituents (**28a**, **28b**) yielded compounds which showed substantially poorer inhibition than their 7α-counterparts (compare **26a** and **26c**).

The differences in activity between the various oxidation forms of the sulfur cannot be readily explained by inspection of the PPE model. While it would appear that hydrogen-bonding opportunities exist for both α-sulfoxide **6a** (backbone NH of Val-216[24]) and β-sulfoxide **7a** (γ-$CONH_2$ of Gln-192), only **7a** shows noteworthy inhibitory activity. Further, it has recently been shown that while the active site regions of HLE and PPE are highly conserved, Gln-192 in PPE is replaced by a Phe residue in HLE.[25] It is possible that the increased activity of sulfones such as **5a** may be largely attributable to the enhanced chemical reactivity of the β-lactam ring over the corresponding sulfides.[26]

Scheme II depicts the mechanisms thought to be at work when molecules of this type inhibit HLE or PPE. At the present time, it appears that at least two types of inhibited complexes can be formed with PPE. The initial event in all cases is considered to be β-lactam ring opening by the OH of Ser-195 of the enzyme catalytic triad to form complex I. As we have previously reported for PPE,[27] in the case where the C-7 substituent is chloro, expulsion of the 3′-acetate, loss of HCl, and Michael addition of N-1 of the imidazole ring of His-57 lead to formation of inhibited complex II. However, in the case where the C-7 substituent is a poorer leaving group such as methoxy, preliminary crystallographic data[28] suggest that this group is not lost and the thiazoline ring appears to open to generate an inhibited species. At this point in time, it is not possible to discern whether there is a second covalent linkage between the inhibitor and the enzyme (Scheme II, structure III) or alternatively that a critical salt bridge between the liberated sulfinate and the imidazole of His-57 is formed which slows hydrolysis of intermediate IV presumably because the imidazole moiety is not aligned properly to deliver water to the serine ester carbonyl.

Table II[a]

compd	IC_{50}, μM			
	HLE	PPE	ChT	Trp
12a	0.3	0.4	ND	≫40
12b	6	2	≫40	≫40
12e	10	20	4	≫40

[a] ChT, bovine α-chymotrypsin; Trp, bovine trypsin. The assays were carried out as described in ref 9.

Some specificity data on this class of HLE inhibitors have been previously reported.[9] Additional results for some acyloxy-substituted inhibitors are shown in Table II. Each compound shows comparable inhibitory capacity against HLE and PPE, and the trend toward larger substituents at C-7 giving poorer inhibition is seen for both enzymes. None had any activity against bovine trypsin. Interestingly, although **12e** is about 10 times less active than **5j**[9] against bovine chymotrypsin, the importance of the phenyl ring in the side chain to the inhibition of this enzyme is underscored when **12e** is compared to **12b**.

The traditional model of elastase-mediated lung disease has been the hamster emphysema assay.[29] Shortcomings of this model include its lengthy duration (6–8 weeks) and the need for lung histology as a readout, both of which make this assay unsuitable for use in screening large numbers of compounds. We have been developing models

(24) All amino acid residues discussed are numbered relative to chymotrypsinogen (see ref 25).
(25) Sinha, S.; Watorek, W.; Karr, S.; Giles, J.; Bode, W.; Travis, J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2228.
(26) Thompson, K. R., unpublished results.
(27) Navia, M. A.; Springer, J.; Lin, T.-Y.; Williams, H.; Firestone, R.; Pisano, J.; Doherty, J.; Finke, P.; Hoogsteen, K. *Nature* **1987**, *327*, 79.
(28) Navia, M. A., unpublished results.
(29) Karlinsky, J.; Snider, G. *Am. Rev. Respir. Dis.* **1978**, *117*, 1109.

of HLE-mediated tissue damage more appropriate for use in determining an in vivo structure–activity relationship to assist in the selection of a candidate for drug development, and some results of these investigations have been reported.[30]

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Lack of melting point data indicates an indefinite melting point or noncrystallinity. Analytical thin-layer chromatography (TLC) was performed with Analtech 0.25-mm silica gel 60 glass-backed plates with flourescent indicator UV_{254}. Preparative high-pressure liquid chromatography (HPLC) was carried out with a Waters 500A LC System with PrepPAK-500/silica cartridges.

Proton magnetic resonance (^1H NMR) spectra were recorded on Varian T-60 (60-MHz), Varian EM 390 (90-MHz), and Varian XL 200 (200-MHz) spectrometers in deuterochloroform solvent unless otherwise indicated. Chemical shifts are reported in parts per million downfield from tetramethylsilane as internal standard (δ scale). Infrared spectra (IR) were obtained with a Perkin-Elmer 1310 infrared spectrometer. Ultraviolet spectra were recorded on a Perkin-Elmer 553 Fast Scan UV/VIS spectrophotometer. Mass spectra were obtained on an LKB 9000 mass spectrometer. Microanalytical data were provided by the MSDRL Analytical Services Department and were determined on a Control Equipment elemental analyzer 240X.

The majority of results reported were obtained from single experiments, and as a consequence the yields are not optimized.

***tert*-Butyl 3-(Acetyloxymethyl)-7-diazo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3).**[14] Into a 2-L Erlenmeyer flask is placed a solution of 7-ACA *tert*-butyl ester **2b**[31] (22.22 g, 0.067 mol) in CH_2Cl_2 (500 mL). To this solution was added a mixture of $NaNO_2$ (4.68 g, 0.067 mol) in water (500 mL). The resulting two-phase mixture was cooled in an ice bath, then 2 N aqueous H_2SO_4 (51 mL) was added dropwise over 30 min with vigorous stirring. Stirring was continued for 1 h at 0 °C, then the layers were separated, and the aqueous layer was washed with methylene chloride (200 mL). The organic layers were combined, washed with brine (250 mL), dried over $MgSO_4$, and filtered to give a yellow solution of **3**. This solution was typically used immediately in the next step where possible. Evaporation of solvent in vacuo gave crude **3** as a viscous yellow oil which decomposes rapidly on standing: IR 2100 cm^{-1} (diazo stretch). **WARNING!** As with all diazo compounds, the potential for explosion exists in the handling of **3**, and due caution should be observed. For more information on the explosion potential of compounds in this class, see ref 15.

Method A: *tert*-Butyl 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (4a) (Tosic Acid Catalysis). To a solution of **3** [prepared from 19.7 g (60 mmol) of **2b** as described above] in CH_2Cl_2 (650 mL) and MeOH (500 mL, 12.4 mol) at 0 °C was added a solution of tosic acid (12.5 g, 66 mmol) in MeOH (30 mL) over 20 min with stirring (gas evolution). After an additional 40 min, the reaction mixture was concentrated to ca. 100 mL and diluted with EtOAc (500 mL) and then extracted with aqueous saturated $NaHCO_3$ (2 × 200 mL), water (200 mL), and then brine (200 mL). The organic layer was dried over Na_2SO_4 and concentrated to ca. 100 mL. The concentrated solution was filtered through silica gel to remove very polar impurities. The remaining solvent was removed in vacuo and the residue was chromatographed on a flash column with 3/1 hexane/EtOAc to give 3.62 g (19%) of **4a** as a light yellow oil: NMR δ 1.59 (9 H, s), 2.04 (3 H, s), 3.31 (1 H, d, J = 19 Hz), 3.55 (3 H, s), 3.72 (1 H, d, J = 19 Hz), 4.50 (1 H, d, J = 2 Hz), 4.64 (1 H, d, J = 2 Hz), 4.65 (1 H, d, J = 13 Hz), 5.08 (1 H, d, J = 13 Hz); IR (neat film) 1780 cm^{-1} (β-lactam CO stretch). In addition, a more polar product was obtained which was identified as 7α-tosyloxy compound **4l** (1.14 g, 4%), a low melting solid. Anal. C, H, N.

Sulfides **4b–j** were prepared from **3** in the manner described above. The equivalents of alcohol used are indicated below: **4b**, 167 equiv of EtOH; **4c**, 150 equiv of *n*-PrOH; **4d**, 92.5 equiv of *n*-BuOH; **4e**, 150 equiv of *i*-PrOH; **4f**, 73 equiv of 2-BuOH; **4g**, 25 equiv of $(Et)_2CHOH$; **4h**, 10 equiv of PhOH; **4i**, 80 equiv of BzOH; **4j**, 5 equiv of $PhCH_2CH_2OH$.

Data on Sulfides 4. 4b: NMR δ 1.25 (3 H, t, J = 7 Hz), 1.57 (9 H, s), 2.03 (3 H, s), 3.23 (1 H, d, J = 19 Hz), 3.57 (1 H, d, J = 19 Hz), 3.67 (2 H, m), 4.47 (1 H, d, J = 2 Hz), 4.60 (1 H, d, J = 2 Hz), 4.70 (1 H, d, J = 12 Hz), 4.90 (1 H, d, J = 13 Hz).

4d: 15%; oil; NMR δ 1.30 (7 H, m), 1.55 (9 H, s), 2.06 (3 H, s), 3.25 (1 H, d, J = 18 Hz), 3.65 (1 H, d, J = 18 Hz), 3.75 (2 H, m), 4.46 (1 H, d, J = 2 Hz), 4.60 (1 H, d, J = 2 Hz), 4.70 (1 H, d, J = 14 Hz), 4.91 (1 H, d, J = 14 Hz).

4e: NMR δ 12%; oil; NMR δ 1.18 (6 H, d, J = 7 Hz), 1.55 (9 H, s), 2.05 (3 H, s), 3.30 (1 H, d, J = 18 Hz), 3.60 (1 H, d, J = 18 Hz), 3.65 (1 H, m), 4.50 (1 H, d, J = 2 Hz), 4.65 (1 H, d, J = 2 Hz); 4.70 (1 H, d, J = 14 Hz), 4.95 (1 H, d, J = 14 Hz).

4h: NMR δ 1.59 (9 H, s), 2.05 (3 H, s), 3.40 (1 H, d, J = 18 Hz), 3.70 (1 H, d, J = 18 Hz), 4.75 (1 H, d, J = 14 Hz), 4.80 (1 H, d, J = 2 Hz), 5.05 (1 H, d, J = 14 Hz), 5.18 (1 H, d, J = 2 Hz), 7.15 (5 H, m).

4i: NMR δ 1.54 (9 H, s), 2.01 (3 H, s), 3.19 (1 H, d, J = 18 Hz), 3.48 (1 H, d, J = 18 Hz), 4.50 (2 H, s), 4.60 (2 H, m), 4.61 (1 H, d, J = 14 Hz), 4.85 (1 H, d, J = 14 Hz), 7.25 (5 H, s).

4j: NMR δ 1.53 (9 H, s), 2.02 (3 H, s), 2.86 (2 H, t, J = 7 Hz), 3.17 (1 H, d, J = 18 Hz), 3.50 (1 H, d, J = 18 Hz), 3.85 (2 H, m), 4.45 (1 H, d, J = 1 Hz), 4.51 (1 H, d, J = 1 Hz), 4.60 (1 H, d, J = 12 Hz), 4.85 (1 H, d, J = 12 Hz), 7.15 (5 H, br s).

Preparation of 4a Using $Rh_2(OAc)_4$ as Catalyst. The crude solution of **3** (from 22.2 g of **2b** prepared as described above) was cooled in an ice bath and methanol (525 mL, 13 mol) was added. To this chilled mixture was added rhodium(II) acetate dimer (220 mg, 0.5 mmol), and the reaction mixture was stirred for 45 min, during which time the color changed from yellow to green-brown. The reaction mixture was filtered through silica gel, concentrated, and dried in vacuo to give a dark red oil which was then purified by preparative HPLC using 15/85 EtOAc/nexane to give 9.62 g of **4a** (41% from **2b**) as a yellow oil.

***tert*-Butyl 3-(Acetoxymethyl)-7α-hydroxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (4k).** To a solution of crude **3** (prepared from 22.2 g of **2b** as described above) in acetone (400 mL) was added 0.165 N aqueous $HClO_4$ (480 mL) over 1 h at room temperature. The reaction was stirred an additional 3 h and then diluted with an additional 500 mL of water and extracted twice with CH_2Cl_2 (500 mL of each). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ solution and then brine and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was chromatographed on silica gel with 30% EtOAc/hexane to give 2.75 g (13%) of amorphous product: NMR δ 1.53 (9 H, s), 2.07 (3 H, s), 3.20 (1 H, d, J = 18 Hz), 3.60 (1 H, d, J = 18 Hz), 4.75 (4 H, m).

***tert*-Butyl 3-(Acetoxymethyl)-7α-(formyloxy)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (11a).** To a solution of **4k** (1.5 g, 4.6 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added 1.5 mL of acetic formic anhydride reagent (prepared by cooling 2 vol of acetic anhydride to 0 °C, slowly adding 1 vol of 96% formic acid, heating to 50 °C for 15 min, and cooling) followed by pyridine (1.2 mL). The reaction was allowed to warm to room temperature, stirred for 2 h, and then quenched by addition of ice water (50 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL), water (50 mL), and saturated brine (50 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. Crystallization from 30% EtOAc/hexane gave 700 mg (43%) of **11a**: mp 140–150 °C dec; NMR δ 7.99 (1 H, s). Anal. ($0.5H_2O$) C, H, N.

Method B: *tert*-Butyl 3-(Acetoxymethyl)-7α-acetoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (11b). To a solution of **4k** (240 mg, 0.73 mmol) and acetyl chloride (85 mg, 1.1 mmol) in CH_2Cl_2 (10 mL) at 0 °C under N_2 was added pyridine (87 mg, 1.1 mmol). The cooling bath was removed and the reaction was stirred for 3 h. The reaction mixture was then extracted with aqueous saturated $NaHCO_3$ (10 mL), water (10 mL), and saturated brine (10 mL) and dried over Na_2SO_4. The

(30) Bonney, R. J.; Ashe, B.; Maycock, A.; Dellea, P.; Hand, K.; Osinga, D.; Fletcher, D.; Mumford, R.; Davies, P.; Frankenfield, D.; Nolan, T.; Schaeffer, L.; Hagmann, W.; Finke, P.; Shah, S.; Dorn, C.; Doherty, J. *J. Cell. Biochem.* **1989**, *39*, 47.
(31) Stedman, R. J. *J. Med. Chem.* **1966**, *9*, 444.
(32) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.

solvent was removed in vacuo to give a residue which was purified by preparative TLC (4/1 hexane/EtOAc) to give 100 mg (37%) of **11b**; mp 94–95 °C dec. Anal. C, H, N.

Analogues **11c–f** were prepared as described above.

***tert*-Butyl 3-(Acetoxymethyl)-7α-[(*N*-methylcarbamoyl)oxy]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (11g).** A solution of **4k** (124 mg, 0.35 mmol) and *N*-methyl isocyanate (0.5 mL) in CH_2Cl_2 (3 mL) was stirred under N_2 at room temperature for 14 h. The solvent was removed in vacuo and the residue was purified by preparative TLC (1/1 hexane/EtOAc, two elutions) to give **11g** (99 mg, 68%) as a band at R_f 0.45: mp 149–150 °C dec. Anal. C, H, N.

Method C. Preparation of Cephem Sulfones: *tert*-Butyl 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (5a). Into a 50-mL round-bottom flask were placed **4a** (2.07 g, 6.03 mmol) and CH_2Cl_2 (25 mL). The resulting mixture was cooled under nitrogen to 0 °C, then *m*-CPBA (3.1 g, 85% pure, 15 mmol) was added, the cooling bath was removed, and stirring was continued for 2 h. The reaction mixture was diluted with EtOAc (50 mL), filtered, and washed with saturated sodium bicarbonate (100 mL), water (100 mL), and saturated brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated to give 2.20 g of crude product. This product was purified by preparative HPLC using hexane/EtOAc (2/1) to give a white solid (1.23 g, 54.3%) of analytically pure **5a**: mp 127 °C dec. Anal. C, H, N, S.

Sulfones **5b–k**, **10**, **12a–g**, **14a–c**, **16**, **18**, **26a–d**, and **28a,b** were prepared as described above for **5a**.

Method D: *tert*-Butyl 3-(Acetoxymethyl)-7α-ethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (13a).[14] A 2-L, three-necked round-bottom flask fitted with two dropping funnels was charged with THF (200 mL) and cooled to −78 °C under N_2. One dropping funnel was charged with a solution of 6.33 g (15.3 mmol) of **3** in THF (300 mL). The other funnel was charged with triethylborane (32 mL, 1 M in THF), water (1.2 mL), and THF (300 mL). The funnels were adjusted so their contents were added to the flask at 2.5 mL/min and the temperature of the reaction mixture was maintained at −78 °C. After the addition was complete, the cooling bath was removed and the reaction mixture was allowed to warm. When the reaction temperature reached −45 °C, H_2O_2 (6.67 mL, 30%) was added. Stirring was continued until the reaction reached −15 °C, then it was poured into brine (300 mL), and CH_2Cl_2 (300 mL) was added. The resulting organic layer was washed with brine (300 mL), dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo to give 7.46 g of a thick yellow oil which was chromatographed on a flash column with $CHCl_3$/EtOAc (25/1) to yield 1.29 g (19%) **13a** contaminated with about 20% of the 7β-isomer: NMR δ 1.10 (3 H, t, J = 8 Hz), 1.58 (9 H, s), 1.92 (2 H, br q), 2.10 (3 H, s), 3.19 (1 H, m), 3.37 (1 H, d, J = 18 Hz), 3.57 (1 H, d, J = 18 Hz), 4.44 (1 H, d, J = 2 Hz), 4.78 (1 H, d, J = 12.5 Hz), 5.02 (1 H, d, J = 12.5 Hz). Anal. C, H, N.

Sulfides **13b** and **13c** were prepared as described above. For **13b**, 7α/7β = 6/1; for **13c**, 7α/7β = 8/1.

Preparation of Sulfoxides 6a and 7a. A solution of **4a** (1.002 g, 2.92 mmol) in CH_2Cl_2 (10 mL) was cooled to −78 °C. Then a solution of *m*-CPBA (0.505 g, 80–90% pure) in CH_2Cl_2 (10 mL) was added to the cold solution over 5 min. After 1 h, the cold reaction mixture was poured into 7% aqueous $NaHCO_3$ containing excess Na_2SO_3, and the organic layer was separated. The aqueous layer was extracted with additional CH_2Cl_2 (30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The concentrated filtrate was flash chromatographed with 20% acetone/CH_2Cl_2 to obtain 0.837 g (80%) of **6a** and **7a** as an approximately 1/1 mixture. Careful rechromatography yielded pure **6a**: mp 87 °C, NMR δ 1.57 (9 H, s), 2.12 (3 H, s), 3.50 (1 H, d, J = 18 Hz), 3.63 (3 H, s), 4.06 (1 H, d, J = 18 Hz), 4.45 (1 H, d, J = 2 Hz), 4.70 (1 H, d, J = 13 Hz), 4.95 (1 H, br s), 5.07 (1 H, d, J = 13 Hz). Anal. C, H, N. The more polar **7a** was also isolated: NMR δ 1.58 (9 H, s), 2.10 (3 H, s), 3.25 (1 H, d of d, J = 18, 2 Hz), 3.62 (3 H, s), 3.78 (1 H, d, J = 18 Hz), 4.37 (1 H, d, J = 2 Hz), 4.62 (1 H, d, J = 14 Hz), 5.02 (1 H, d, J = 2 Hz), 5.18 (1 H, d, J = 14 Hz).

Sulfoxides **6b** and **7b** were prepared in an analogous manner to that described for **6a** and **7a**. **6b**: NMR δ 1.30 (3 H, t, J = 7 Hz), 1.53 (9 H, s), 2.09 (3 H, s), 3.42 (1 H, d, J = 18 Hz), 3.75 (2 H, q, J = 7 Hz), 3.98 (1 H, d, J = 18 Hz), 4.31 (1 H, d, J = 2 Hz), 4.60 (1 H, d, J = 16 Hz), 4.90 (1 H, d, J = 2 Hz), 4.92 (1 H, d, J = 16 Hz). **7b**: NMR δ 1.25 (3 H, t, J = 7 Hz), 1.55 (9 H, s), 2.02 (3 H, s), 3.20 (1 H, d, J = 20 Hz), 3.72 (1 H, d, J = 20 Hz), 3.75 (3 H, q, J = 7 Hz), 4.25 (1 H, d, J = 2 Hz), 4.55 (1 H, d, J = 15 Hz), 4.98 (1 H, d, J = 2 Hz), 5.10 (1 H, d, J = 15 Hz).

***tert*-Butyl 3-(Acetoxymethyl)-7β-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (9). Step A: Reaction of 3 with *N*-Bromosuccinimide (NBS) in Methanol.**[17] To a solution of **3** (prepared from 3.25 g of **2b** as described above) in CH_2Cl_2 (70 mL) was added MeOH (50 mL). Then solid NBS (1.78 g, 10 mmol) was added in small portions to control N_2 evolution. The reaction was stirred for an additional 30 min at room temperature after addition was complete, then the mixture was concentrated and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was chromatographed on a flash column (4/1 hexane/EtOAc) to give 970 mg of **8** as an oily mixture of isomers which was immediately taken on to the next step.

Step B: Reduction of 8 to 9.[18] To a solution of **8** (480 mg, 1.15 mmol) in THF (4 mL) were added 1 M aqueous NH_4OAc (2 mL) and Zn powder (220 mg, 3.4 mmol). The reaction was stirred at room temperature for 1.5 h. The reaction mixture was then diluted with ether (50 mL) and filtered through Celite. The solid was thoroughly washed with ether. The filtrate was diluted with hexane (50 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was chromatographed on a flash column using hexane/EtOAc (4/1) to give **4a** (162 mg, 46%) and the more polar **9** (28 mg, 8%): NMR δ 1.56 (9 H, s), 2.09 (3 H, s), 3.38 (1 H, d, J = 20 Hz), 3.56 (1 H, d, J = 20 Hz), 3.61 (3 H, s), 4.83 (1 H, d, J = 12 Hz), 4.99 (2 H, AB q, J = 4 Hz), 5.10 (1 H, d, J = 12 Hz).

***tert*-Butyl 3-(Acetoxymethyl)-7α-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (15).** A sample of crude **3** [prepared as described above from 9.84 g (30 mmol) of **2b**] was dissolved in EtOH (60 mL), and concentrated aqueous HCl (2.5 mL) was added. The reaction was stirred at room temperature for 20 s and then poured into 1 M aqueous KH_2PO_4 (200 mL), and the resulting solution was extracted with CH_2Cl_2 (2 × 200 mL). The organic layers were combined, washed with brine (100 mL), and dried over $MgSO_4$. The solvent was removed in vacuo to give crude residue (10.2 g) which was purified by flash chromatography using $CHCl_3$/EtOAc (50/1) to give 2.03 g (20%) of **15** as an oil: NMR δ 1.55 (9 H, s), 2.10 (3 H, s), 3.40 (1 H, d, J = 18 Hz), 3.59 (1 H, d, J = 18 Hz), 4.70 (1 H, d, J = 1.5 Hz), 4.78 (1 H, d, J = 1.5 Hz), 4.79 (1 H, d, J = 13 Hz), 5.03 (1 H, d, J = 13 Hz).

***tert*-Butyl 3-(Acetoxymethyl)-7α-fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (17).** To a solution of **3** [prepared as described above from 3.28 g (10 mmol) of **2b**] in CH_2Cl_2 (25 mL) was added HF in pyridine (0.60 mL of a 70% solution) dropwise over 30 s with rapid stirring. The mixture was stirred for an additional 2.5 min and then washed with aqueous K_2HPO_4 (1 M, 20 mL), water (20 mL), aqueous H_3PO_4 (1 M, 20 mL), and brine (20 mL), and the organic layer was dried over $MgSO_4$. The solvent was removed in vacuo and the residue was flash chromatographed with hexane/EtOAc (1/1) to afford 183 mg (6%) of **17** as an oil: NMR δ 1.54 (9 H, s), 2.08 (3 H, s), 3.34 (1 H, d of d, J = 18, 1.9 Hz), 3.58 (1 H, d of d, J = 18, 0.8 Hz), 4.75 (1 H, d, J = 13 Hz), 4.90 (1 H, d of d, J = 9, 1.6 Hz), 4.97 (1 H, d, J = 13 Hz), 5.32 (1 H, d of d, J = 54, 1.6 Hz).

***tert*-Butyl 3-(Acetoxymethyl)-7-iodo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (19).** To a sample of crude **3** [prepared from 3.28 g (10 mmol) of **2b** by the method described above] dissolved in acetone (150 mL) and cooled to 0 °C was added a solution consisting of aqueous HI (4.5 mL of a 57% solution), NaI (6.0 g, 40 mmol), and water (16 mL) rapidly with stirring. Gas evolution was observed for ca. 10 min. The reaction was stirred for an additional 30 min, then excess solid $NaHCO_3$ was added to consume the acid, and the mixture was filtered. The filter cake was washed with an additional portion of acetone and the combined filtrate was concentrated in vacuo. The residue was taken up in EtOAc (130 mL) and washed with 5% aqueous $Na_2S_2O_4$ (100 mL). The aqueous layer was extracted with EtOAc

(50 mL), and the combined organic phases were washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified by flash chromatography using cyclohexane/EtOAc (3/1) as eluent to give 520 mg (12%) of the 7α-isomer [NMR δ 1.58 (9 H, s), 2.08 (3 H, s), 3.45 (2 H, AB q, J = 19 Hz), 4.70 (1 H, d, J = 13 Hz), 4.80 (2 H, s), 5.05 (1 H, d, J = 13 Hz)] and 180 mg (4%) of 7β-isomer [NMR δ 1.58 (9 H, s), 2.07 (3 H, s), 3.42 (2 H, AB q, J = 20 Hz), 4.75 (1 H, d, J = 14 Hz), 4.80 (1 H, d, J = 5 Hz), 5.15 (1 H, d, J = 14 Hz), 5.62 (1 H, d, J = 5 Hz)].

***tert*-Butyl 3-(Acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (20).** To a stirred solution of **19** (278 mg, 0.63 mmol) in THF (3 mL) under nitrogen were added Zn powder (112 mg, 1.7 mmol) and 1 M aqueous NH_4OAc (1 mL). The resulting suspension was vigorously stirred for 1 h, then filtered through Celite. The filter cake was washed thoroughly with ether, and the combined filtrate was extracted with 1.2 N HCl (10 mL) and then brine (10 mL) and dried over $MgSO_4$. The solvent was removed in vacuo and the residue was purified by preparative TLC using EtOAc/cyclohexane (4/1) as eluent to give 180 mg (91%) of **20** as a solid: mp 98–100 °C; NMR δ 1.56 (9 H, s), 2.09 (3 H, s), 2.96 (1 H, d of d, J = 17, 2 Hz), 3.32 (1 H, d, J = 18 Hz), 3.55 (1 H, d, J = 18 Hz), 3.58 (1 H, d of d, J = 17, 6 Hz), 4.66 (1 H, d of d, J = 6, 2 Hz), 4.74 (1 H, d, J = 13 Hz), 5.02 (1 H, d, J = 13 Hz). Anal. C, H, N.

Synthesis of 7-Amido-Substituted Cephems: *tert*-Butyl 3-(Acetoxymethyl)-7α-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (24). A. Preparation of *p*-Nitrophenyl Schiff Base 22. To a solution of **2** (1.00 g, 3.05 mmol) and *p*-nitrobenzaldehyde (460 mg, 3.05 mmol) in CH_2Cl_2 (40 mL) at room temperature was added $MgSO_4$ (370 mg). The mixture was stirred for 4 h and then filtered, and the solvent was removed in vacuo to give 1.40 g (100%) of **22** as an orange foam which was taken immediately on to the epimerization step: NMR δ 1.55 (9 H, s), 2.08 (3 H, s), 3.26 (1 H, d, J = 19 Hz), 3.56 (1 H, d, J = 19 Hz), 4.71 (1 H, d, J = 14 Hz), 5.01 (1 H, d, J = 14 Hz), 5.12 (1 H, d, J = 5 Hz), 5.42 (1 H, d of d, J = 5, 2 Hz), 7.80 (2 H, d, J = 9 Hz), 8.20 (2 H, d, J = 9 Hz), 8.63 (1 H, d, J = 2 Hz).

B. Epimerization. The material prepared in step A was dissolved in DMF (63 mL) under N_2 and cooled to −20 °C. The Et_3N (1.72 g, 2.38 mL) was added in one portion, and the resulting solution was stirred for 15 min. The reaction mixture was then poured into 0.015 N aqueous HCl (200 mL). The resulting mixture was extracted with EtOAc (2 × 100 mL), and the combined organic layers were washed with 5% KH_2PO_4 (3 × 50 mL) and brine (3 × 100 mL) and dried over $MgSO_4$. The solvent was removed in vacuo to give an orange foam (1.31 g, 94%) of a mixture of **23** and **22** in approximate ratio of 65/35 by NMR. Pure **23** was obtained by preparative HPLC by eluting with hexane/EtOAc (3/1) to give 630 mg (45%) of pure **23** as a light orange foam: NMR δ 1.57 (9 H, s), 2.07 (3 H, s), 3.27 (1 H, d, J = 18 Hz), 3.57 (1 H, d, J = 18 Hz), 4.63 (1 H, d, J = 13 Hz), 4.71 (1 H, d, J = 1.5 Hz), 4.83 (1 H, d, J = 1.5 Hz), 4.85 (1 H, d, J = 13 Hz). In addition, 384 mg (27%) of **22** was recovered, bringing the yield of **23** to 62% based on consumed **22**.

C. Hydrolysis of 23 to 24. A mixture of **23** (630 mg, 1.37 mmol) and Girard's T reagent (550 mg, 3.3 mmol) was dissolved in MeOH (20 mL) at room temperature and allowed to stand for 100 min. The reaction was poured into a mixture of EtOAc (200 mL) and water (200 mL), shaken, and separated. The organic layer was washed with water (2 × 100 mL) and dried over $MgSO_4$. The solvent was removed in vacuo to give 430 mg (96%) of **24** as a light orange gum homogeneous by TLC: NMR δ 1.57 (9 H, s), 2.06 (3 H, s), 2.45 (2 H, br s), 3.22 (1 H, d, J = 18 Hz), 3.53 (1 H, d, J = 18 Hz), 4.10 (1 H, d, J = 2 Hz), 4.40 (1 H, d, J = 2 Hz), 4.55 (1 H, d, J = 14 Hz), 4.80 (1 H, d, J = 14 Hz).

***tert*-Butyl 3-(Acetoxymethyl)-7α-[(2-thienyl)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (25a).** To a solution of 2-thiopheneacetyl chloride (40 mg, 0.25 mmol) and pyridine (50 μL) in CH_2Cl_2 (1 mL) was added a solution of **24** (82 mg, 0.25 mmol) in CH_2Cl_2 (3 mL). The reaction was allowed to stand at room temperature for 12 h, then the solution was diluted with EtOAc (50 mL) and washed sequentially with 10% aqueous HOAc (3 × 15 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified by preparative TLC (60/40 hexane/EtOAc) and the band at R_f 0.4 was removed and eluted to give 50 mg (48%) of product as an orange oil: NMR δ 1.58 (9 H, s), 2.03 (3 H, s), 3.23 (1 H, d, J = 18 Hz), 3.48 (1 H, d, J = 18 Hz), 3.75 (2 H, s), 4.60 (1 H, d, J = 2 Hz), 4.65 (1 H, d, J = 13 Hz), 4.80 (1 H, d, J = 13 Hz), 4.82 (1 H, d, J = 2 Hz), 6.95 (3 H, m).

Substituting **2b** for **24** in the above reaction gives cephalothin *tert*-butyl ester **27a**: oil; 55%.

***tert*-Butyl 3-(Acetoxymethyl)-7α-formamido-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (25b).** To a solution of **24** (1.38 g, 4.2 mmol) in CH_2Cl_2 (15 mL) at 0 °C under N_2 was added pyridine (1.6 g) followed by formic acetic anhydride (1.5 g, 17 mmol) in CH_2Cl_2 (10 mL). The reaction was allowed to stir for 20 min at 0 °C, and it was then diluted with EtOAc (100 mL) and washed with aqueous saturated $NaHCO_3$ (50 mL), water (50 mL), 10% aqueous HOAc (2 × 50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo to give 1.36 g (91%) of product as a yellow foam that was further purified by preparative HPLC to give 1.07 g (71%) of **25b**: mp 132–135 °C dec. Anal. C, H, N.

***tert*-Butyl 7α-(Trifluoroacetamido)-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (25c).** To a solution of **24** (5.03 g, 15.3 mmol) in CH_2Cl_2 (375 mL) at 0 °C were added pyridine (3.6 g, 46 mmol) and trifluoroacetic anhydride (9.65 g, 46 mmol), and the solution was stirred for 20 min. Then the reaction was washed with aqeuous saturated $NaHCO_3$ (200 mL), 10% aqueous HOAc (3 × 150 mL), aqueous saturated $NaHCO_3$ (2 × 100 mL), and then water (500 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo to give **25c** as a yellow foam (6.05 g, 100%) which partially solidified on standing. A sample was purified by preparative TLC (70/30 hexane/EtOAc, R_f 0.5): mp 130–132 °C dec. Anal. C, H, N.

Substituting **2b** for **24** in the above reaction gives **27b**: NMR δ 1.58 (9 H, s), 2.08 (3 H, s), 3.34 (1 H, d, J = 18 Hz), 3.68 (1 H, d, J = 18 Hz), 4.78 (1 H, d, J = 14 Hz), 5.05 (1 H, d, J = 5 Hz), 5.25 (1 H, d, J = 14 Hz), 5.76 (1 H, d of d, J = 8, 5 Hz), 8.40 (1 H, d, J = 8 Hz).

***tert*-Butyl 7α-[(Methoxycarbonyl)amino]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (25d).** Prepared as in **25b** with 2 equiv of methyl chloroformate: 91%; mp 168–170 °C dec. Anal. C, H, N.

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Registry No. **2b**, 6187-87-7; **3**, 58249-92-6; **4a**, 95570-83-5; **4b**, 104163-95-3; **4c**, 127732-88-1; **4d**, 127732-89-2; **4e**, 127732-90-5; **4f**, 127732-91-6; **4g**, 127732-92-7; **4h**, 127732-93-8; **4i**, 127732-94-9; **4j**, 127732-95-0; **4k**, 63599-58-6; **4l**, 127732-87-0; **5a**, 95671-97-9; **5b**, 104163-88-4; **5c**, 104163-89-5; **5d**, 104163-90-8; **5e**, 104163-91-9; **5f**, 127733-00-0; **5g**, 127761-75-5; **5h**, 104263-95-8; **5i**, 104163-92-0; **5j**, 104163-93-1; **5k**, 122189-88-2; **6a**, 95570-71-1; **6b**, 104163-96-4; **7a**, 104196-07-8; **7b**, 104163-97-5; **8** (7α isomer), 127733-10-2; **8** (7β isomer), 127733-11-3; **9**, 127733-12-4; **10**, 104263-94-7; **11a**, 95570-85-7; **11b**, 95570-86-8; **11c**, 127732-96-1; **11d**, 127761-74-4; **11e**, 127732-97-2; **11f**, 127732-98-3; **11g**, 127732-99-4; **12a**, 95671-98-0; **12b**, 95570-87-9; **12c**, 127733-01-1; **12d**, 127733-02-2; **12e**, 127733-03-3; **12f**, 127733-04-4; **12g**, 127733-05-5; **13a**, 95570-69-7; **13a** (7β isomer), 95570-70-0; **13b**, 127733-06-6; **13b** (7β isomer), 127733-09-9; **13c**, 127733-07-7; **13c** (7β isomer), 127733-08-8; **14a**, 104163-87-3; **14b**, 127733-19-1; **14c**, 127733-20-4; **15**, 95570-98-2; **16**, 95672-01-8; **17**, 95570-97-1; **18**, 95672-00-7; **19**, 62263-70-1; **19** (7β isomer), 62263-71-2; **20**, 127733-13-5; **21**, 104163-86-2; **22**, 81533-60-0; **23**, 127733-14-6; **24**, 102253-55-4; **25a**, 127733-15-7; **25b**, 127733-16-8; **25c**, 102253-49-6; **25d**, 102253-57-6; **26a**, 127733-18-0; **26b**, 104196-06-7; **26c**, 102253-56-5; **26d**, 102253-58-7; **27a**, 55151-60-5; **27b**, 127733-17-9; **28a**, 104163-83-9; **28b**, 104196-05-6; $PhOCH_2COCl$, 701-99-5; 2-thiopheneacetyl chloride, 39098-97-0; elastase, 9004-06-2.