Inhibition of Human Leukocyte Elastase. 2. Inhibition by Substituted Cephalosporin Esters and Amides

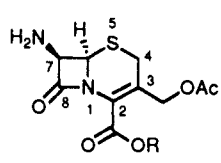
Paul E. Finke,*,† Bonnie M. Ashe,‡ Wilson B. Knight,‡ Alan L. Maycock,‡,§ Manuel A. Navia,‖,⊥ Shrenik K. Shah,† Kevan R. Thompson,† Dennis J. Underwood,∞ Hazel Weston,‡ Morris Zimmerman,‡ and James B. Doherty†

Departments of Medicinal Chemical Research, Enzymology, Biophysics Research, and Molecular Systems Research, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065. *Received July 24, 1989*

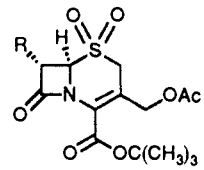
A variety of 7α-methoxycephalosporin ester and amide sulfones were prepared and tested to determine the structure–activity relations for inhibition of human leukocyte elastase (HLE), a serine protease which has been implicated in several degenerative lung and tissue diseases. The most potent IC_{50} values were obtained with neutral, lipophilic derivatives, with the esters being more active than the amides. However, the best time-dependent inhibition in this series was observed with the *p*- and *m*-carboxybenzyl esters **7b** and **7c**. These results are discussed in terms of the proposed mechanism of inhibition as well as a molecular modeling study using the recently solved X-ray crystal structure of HLE.

Human leukocyte elastase (HLE, EC 3.4.21.37) has been the focus of extensive study, both in terms of its possible biological role in numerous diseases and in terms of the development of suitable therapeutic inhibitors.[1] HLE is one of the more potent of several proteases released by polymorphonuclear leukocytes (PMN).[2,3] Under normal circumstances their proteolytic activity in the extracellular environment is effectively limited by an excess of natural inhibitors, predominantly α_1 protease inhibitor (α_1-PI) and α_2 macroglobulin. However, under certain pathological conditions, a protease–antiprotease imbalance can occur and result in uncontrolled proteolysis of structural tissue, primarily of the lung and joints.[3] A pathological role for HLE in particular has been implicated in several of these degenerative diseases including emphysema,[3b,4,5] respiratory distress syndrome (RDS),[6] chronic bronchitis,[7] cystic fibrosis,[8] and rheumatoid arthritis.[9] In the hope of moderating the effect of HLE in these diseases and thus obtaining a beneficial therapeutic response,[10] several research groups have vigorously pursued either an α_1-PI replacement strategy[11] or the synthesis and development of small molecular weight HLE inhibitors.[12]

This group has previously reported[13] our preliminary findings on the use of the cephalosporin nucleus 1 as a key



1a: R = H (7-ACA)
b: R = $C(CH_3)_3$



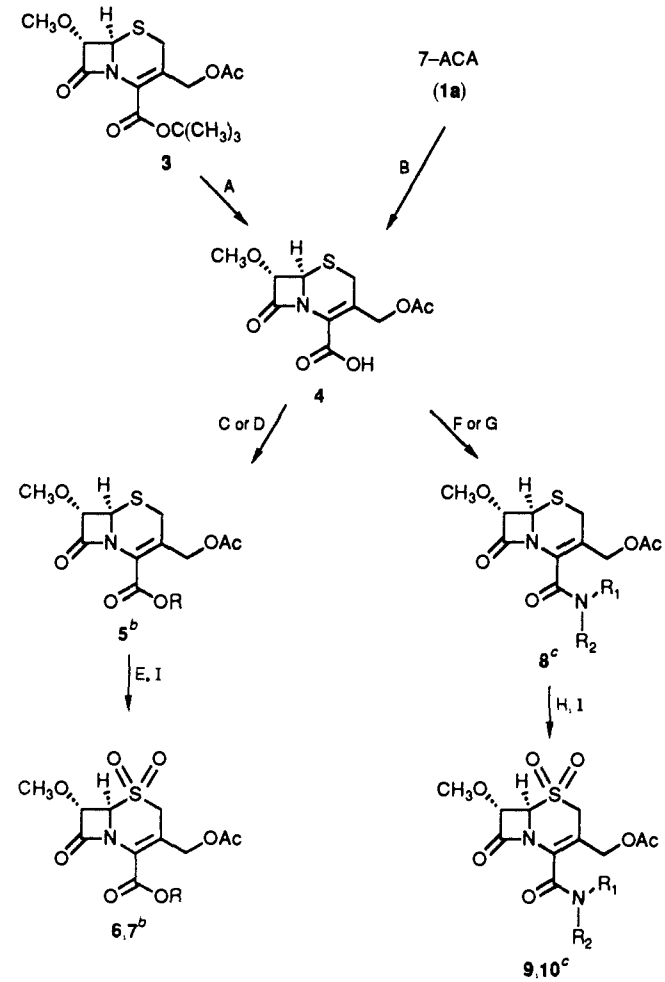
2

to time-dependent inactivation of HLE. In this series of papers[14-16] we offer a detailed description of this lead's development in terms of its initiation and rationale, the synthesis of a wide variety of derivatives at C-2, C-3, C-4, and C-7, and their structure–activity relationships as well as a discussion of their possible mode of binding and mechanism of inhibition.

Chemistry

As reported in the preceding paper,[14] our initial investigation of the C-7 position employed the readily available *t*-Bu ester of 7-aminocephalosporanic acid (7-ACA, **1b**).[17]

Scheme I[a]



[a] General methods: (A) TFA, anisole, 0 °C; (B) CH_3OH, isoamyl nitrite, 7 N $HClO_4$, 50–60 °C; (C) Diazoalkane, CH_2Cl_2/Et_2O; (D) $(CH_3)_2CHNHC(OR)NCH(CH_3)_2$, THF; (E) *m*-CPBA, CH_2Cl_2; (F) $(CH_3)_2CHOCOCl$, acetone/dioxane, −20 °C; R_1R_2NH, room temperature, (G) DCC, R_1R_2NH, CH_2Cl_2; (H) *m*-CPBA, CH_2Cl_2; pyridine; (I) TFA, anisole, 0 °C. [b] R is as in Table I. [c] R_1 and R_2 are as in Table II.

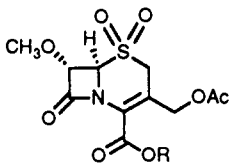
Conversion of the 7-amino to small, α-oriented groups (i.e. 2, R = Cl, CH_3O, CH_3CH_2) and the oxidation of the sulfur

† Department of Medicinal Chemical Research.
‡ Department of Enzymology.
§ Present address: Sterling Research Group, Malvern, PA 19355.
‖ Department of Biophysics Research.
⊥ Present address: Vertex Pharmaceuticals, 40 Alston St., Cambridge, MA 02139-4211.
∞ Department of Molecular Systems Research.

(1) Stein, R. L.; Trainor, D. A.; Wildonger, R. A. *Annu. Rep. Med. Chem.* **1985**, *20*, 237.
(2) Dewald, B.; Rinder-Ludwig, R.; Bretz, U.; Baggiolini, M. *J. Exp. Med.* **1975**, *141*, 709.
(3) (a) Travis, J.; Salvesen, G. S. *Annu. Rev. Biochem.* **1983**, *52*, 655. (b) Mittman, C., Ed. *Pulmonary Emphysema and Proteolysis*; Academic Press: New York, 1972.

0022-2623/90/1833-2522$02.50/0

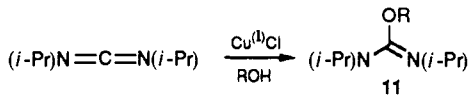
Table I. HLE Activity, Preparation, and Characterization of Cephalosporin Esters 6 and 7



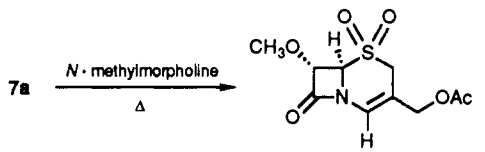
no.	R	IC_{50},[a] μM	$k_{obs}/[I]$,[b] M^{-1} s^{-1} (SD)[c]	methods (%)[d]	formula[e]
6a	t-C_4H_9	1.0	16000 (1500)	*f*	
6b	i-C_3H_7	0.8		D (5), E (98)	$C_{14}H_{19}NO_8S{\cdot}1H_2O$
6c	C_2H_5	0.2		C (91), E (75)	$C_{13}H_{17}NO_8S$
6d	CH_3	0.2	17000 (700)	C (65), E(75)	$C_{12}H_{15}NO_8S$
6e	$CH_2CO_2C_2H_5$	0.1		C (42), E(70)	$C_{15}H_{19}NO_{10}S$
6f	$(CH_2)_2CO_2CH_3$	0.5	7700 (1000)	C (41), E(81)	$C_{15}H_{19}NO_{10}S$
6g	$(CH_2)_3CO_2CH_3$	0.1	30000 (900)	C (13), E (69)	$C_{16}H_{21}NO_{10}S$
6h	$CH_2C_6H_5$	0.07	*g*	D (45), E (50)	$C_{18}H_{19}NO_8S$
6i	$CH_2C_6H_4$-4-OCH_3	0.1	*h*	D (19), E (54)	$C_{19}H_{21}NO_9S$
6j	$CH_2C_6H_4$-4-t-C_4H_9	0.2	*h*	D (5), E (12)	$C_{22}H_{27}NO_8S$
6k	$CH_2C_6H_4$-4-CO_2CH_3	0.1	*h*	D (72), E (71)	$C_{20}H_{21}NO_{10}S$
6l	$CH_2C_6H_4$-4-CO_2-t-C_4H_9	0.4	*g*	D (90), E (94)	$C_{23}H_{27}NO_{10}S$
6m	$CH_2C_6H_4$-3-CO_2CH_3	0.04	*h*	D (98), E (81)	$C_{20}H_{21}NO_{10}S$
7a	H	>50		I (80)	$C_{11}H_{13}NO_8S{\cdot}1H_2O$
7b	$CH_2C_6H_4$-4-CO_2H	0.4	57000 (6000)	I (84)	$C_{19}H_{19}NO_{10}S$
7c	$CH_2C_6H_4$-3-CO_2H	0.4	62000 (3000)	I (96)	$C_{19}H_{19}NO_{10}S$

[a] See ref 13 for methodology. [b] See ref 14 for methodology. [c] Average of two or more determinations at different inhibitor concentration. [d] Isolated yields. See the Experimental Section for representative examples. [e] All compounds were analyzed for C, H, and N. Values were within +/−0.4% of the theoretical values. [f] Reference 14. [g] No time-dependent inhibition was observed. [h] An inverse progress curve was observed.

Scheme II



Scheme III



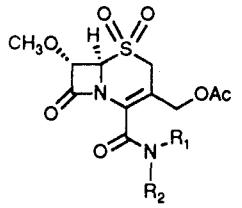
to a sulfone afforded the most potent, time-dependent inhibitors of HLE in this series. For the work reported herein the 7α-methoxy substituent was chosen since it was readily available, showed enhanced activity over the 7α-ethyl derivative, and would avoid any problem inherent in an α-halocarbonyl compound. As shown in Tables I and II, systematic variation of the C-2 carbonyl substituent resulted in further enhancements in HLE binding and inactivation. The effect of further modifications at the C-3 and C-4 positions have been published elsewhere.[15,16]

As outlined in Scheme I, the key intermediate employed for this work was 7α-methoxycephalosporanic acid (4). This intermediate was available from the t-Bu ester **3**[14] [prepared in three steps from 7-ACA (**1a**) in 10–15% yield] on treatment with trifluoroacetic acid (TFA) (method A) or directly from 7-ACA in a lower 7-10% yield by the in situ diazotization of the 7-amino group with isoamyl nitrite in the presence of methanol and 7 N perchloric acid (method B). Esterification or amidation of 4 followed by oxidation of the sulfur to the sulfone afforded the desired esters (Table I) and amides (Table II).

Treatment of 4 with an appropriate diazoalkane[18] (method C) afforded the ester sulfides **5c–g** in low to good yield. For the preparation of **5e** which involved the use of the less reactive ethyl diazoacetate, the esterification was catalyzed with rhodium(II) acetate dimer to give the ester in 42% yield. Alternately, esterification of 4 with *N,N'*-diisopropyl-*O*-alkylisoureas 11 (method D)[19] gave the corresponding ester sulfides **5b,h–m** in low to excellent yield, usually as a mixture of Δ^2- and Δ^3-isomers. The

(4) Janoff, A. *Am. Rev. Respir. Dis.* **1985**, *132*, 417.
(5) Morrison, H. M. *Clin. Sci.* **1987**, *72*, 151.
(6) (a) Sprung, C. L.; Schultz, D. R.; Clerch, A. R. *N. Engl. J. Med.* **1981**, *304*, 1301. (b) Cochrane, C. G.; Spragg, R.; Revak, S. D. *J. Clin. Invest.* **1983**, *71*, 754.
(7) Snider, G. L. *Drug Dev. Res.* **1987**, *10*, 235.
(8) (a) Jackson, A. H.; Hill, S. L.; Afford, S. C.; Stockley, R. A. *Eur. J. Respir. Dis.* **1984**, *65*, 114. (b) Suter, S.; Schaad, U. B.; Roux, L.; Nydegger, U. E.; Waldvogel, F. A. *J. Infect. Dis.* **1984**, *149*, 523.
(9) (a) Velvart, M. *Rheumatol. Int.* **1981**, *1*, 121. (b) Ekerot, L.; Ohlsson, K. *Adv. Exp. Med. Biol.* **1984**, *167*, 335.
(10) Weinbaum, G.; Damiano, V. V. *Trends Pharmacol. Sci.* **1987**, *8*, 6.
(11) Powers, J. C. *Am. Rev. Respir. Dis.* **1983**, *127*, 554.
(12) (a) Trainor, D. A. *Trends Pharmacol. Sci.* **1987**, *8*, 303. (b) Groutas, W. C. *Med. Res. Rev.* **1987**, *7*, 227.
(13) Doherty, J. B.; Ashe, B. M.; Argenbright, L. W.; Barker, P. L.; Bonney, R. J.; Chandler, G. O.; Dahlgren, M. E.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Fletcher, D.; Hagmann, W. K.; Mumford, R.; O'Grady, L.; Maycock, A. L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Zimmerman, M. *Nature* **1986**, *322*, 192.
(14) Doherty, J. B.; Ashe, B. M.; Barker, P. L.; Blacklock, T.; Butcher, J. W.; Chandler, G. O.; Dahlgren, Davies, P.; M. E.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Hagmann, W. K.; Halgren, T.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; O'Grady, L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Weston, H.; Zimmerman, M. *J. Med. Chem.* preceding paper in this issue.
(15) Shah, S. K.; Brause, K. A.; Chandler, G. O.; Finke, P. E.; Ashe, B. M.; Weston, H.; Knight, W. B.; Maycock, A. L.; Doherty, J. B. *J. Med. Chem.* following paper in this issue.
(16) Hagmann, W. K.; O'Grady, L. A.; Ashe, B. M.; Dahlgren, M. E.; Weston, H.; Doherty, J. B. *Eur. J. Med. Chem.* **1989**, *24*, 599.
(17) Stedman, R. J. *J. Med. Chem.* **1966**, *9*, 444.
(18) Braun, L. L.; Looker, J. H. *J. Am. Chem. Soc.* **1958**, *80*, 359.
(19) Mathias, L. J. *Synthesis* **1979**, 561.

Table II. HLE Activity, Preparation, and Characterization of Cephalosporin Amides 9 and 10



no.	R_1	R_2	IC_{50},[a] μM	$k_{obs}/[I]$,[b] $M^{-1}\ s^{-1}$ (SD)[c]	methods (%)[d]	formula[e]
9a	H	$CH_2C_6H_5$	5.0		F (13), H (28)	$C_{18}H_{20}N_2O_7S$[f]
9b	CH_3	$CH_2C_6H_5$	0.1	9600 (800)	F (32), H (85)	$C_{19}H_{22}N_2O_7S{\cdot}0.33H_2O$
9c	H	$(CH_2)_2CO_2$-*t*-C_4H_9	1.0		G (22), H (22)	$C_{18}H_{26}N_2O_9S$
9d	H	$(CH_2)_3CO_2$-*t*-C_4H_9	0.2	3500 (100)	G (11), H (13)	$C_{19}H_{26}N_2O_9S{\cdot}1H_2O$
9e	H	*t*-C_4H_9	0.8	2200 (100)	F (44), H (25)	$C_{15}H_{22}N_2O_7S{\cdot}25H_2O$
9f	CH_3	CH_3	2.0	3900 (100)	F (59), H (84)	$C_{13}H_{18}N_2O_7S$
9g	CH_3	CH_2CO_2-*t*-C_4H_9	0.2		F (10), H (77)	$C_{18}H_{26}N_2O_9S$
9h	CH_3	$CH_2CO_2CH_3$		9500 (500)	C (75)	$C_{15}H_{20}N_2O_9S{\cdot}0.5H_2O$
9i	CH_3	$(CH_2)_2CO_2CH_3$		6200 (700)	C (80)	$C_{16}H_{22}N_2O_9S$
9j	$-(CH_2)_4-$			27000[g]	G (44), H (98)	$C_{15}H_{20}N_2O_7S$
9k	$-(CH_2)_2O(CH_2)_2-$			35000[g]	G (32), H (85)	$C_{15}H_{20}N_2O_8S$
10a	H	CH_2CO_2H	>50		I (70)	$C_{13}H_{16}N_2O_9S$
10b	H	$(CH_2)_2CO_2H$	>50		I (50)	$C_{14}H_{18}N_2O_9S$[h]
10c	CH_3	CH_2CO_2H	5.0	300 (100)	I (95)	$C_{14}H_{18}N_2O_9S{\cdot}0.5H_2O$
10d	CH_3	$(CH_2)_2CO_2H$		500 (100)	I (84)	$C_{15}H_{20}N_2O_9S{\cdot}1.5H_2O$
10e	CH_3	$(CH_2)_3CO_2H$		900[g]	I (68)	$C_{16}H_{22}N_2O_9S{\cdot}2H_2O$
10f	CH_3	$CH_2C_6H_4$-4-CO_2H	0.4	8600 (3000)	I (86)	$C_{20}H_{22}N_2O_9S{\cdot}0.25H_2O$

[a] See ref 13 for methodology. [b] See ref 14 for methodology. [c] Average of two or more determinations at different inhibitor concentrations. [d] All compounds were analyzed for C, H, and N. Values were within +/−0.4% of the theoretical values except were noted. [f] No analysis available. MS *m/e* 408; R_f = 0.35 (50% ethyl acetate/hexanes, 1% acetic acid). [g] Result of a single determination. [h] Anal. N: calcd, 7.18; found, 6.50.

isoureas were prepared from the corresponding alcohol and *N,N'*-diisopropylcarbodiimide with a catalytic amount of cuprous chloride (Scheme II).[20]

Oxidation of sulfides **5b–m** was conveniently carried out with 2.5 equiv of *m*-chloroperbenzoic acid (*m*-CPBA) (method E) and afforded sulfones **6b–m** in 50–90% yields. In the cases where double bond mixtures had been obtained, oxidation afforded the isomerically pure Δ^2-sulfones due to the spontaneous rearrangement of the Δ^3 to the more stable Δ^2-isomer during the reaction.

Amidation of 4 was achieved in low to moderate yield either by formation of the mixed anhydride with isobutyl chloroformate followed by the addition of amine (method F) or through DCC activation of the carboxyl (method G).[21] The sulfides were usually isolated as a mixture of double bond isomers and were directly oxidized to sulfones **9a–g,j–k** with *m*-CPBA (method H). However, unlike with the esters, subsequent pyridine-catalyzed isomerization was required to afford the isomerically pure sulfones. The reason for the unusually low yields for oxidation of secondary amides **8a,c–e** is unclear.

In order to investigate the effects of a free carboxylic acid at various distances from the β-lactam as well as to modify the pharmacokinetic properties, several *t*-Bu ester derivatives were prepared as above and subsequently deblocked by treatment with TFA (method I) to yield sulfone acids **7a–c** and **10f**. In addition, the peptidyl analogues **10a–e** were prepared as their *t*-Bu esters and deprotected as above with TFA. Reesterification of **10c** and **10d** with diazomethane afforded the corresponding methyl esters **9h** and **9i** for comparison.

Finally, base-catalyzed decarboxylation[22] of **7a** afforded the C-2 unsubstituted analogue **12** (Scheme III).

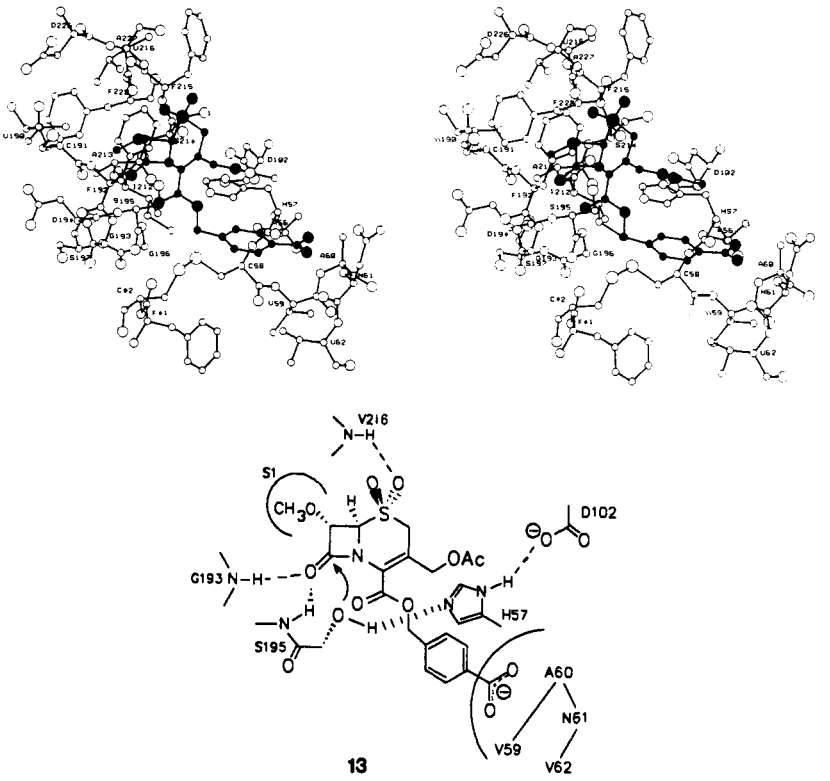
Biological Results

Tables I and II show the in vitro HLE inhibitory activity of the target compounds as nominal 2 min IC_{50} values (μM), which give an approximation of their initial K_I and/or the second-order rate constants $k_{obs}/[I]$ ($M^{-1}\ s^{-1}$), which give a more accurate and meaningful indication of the rate of time-dependent inactivation.[14]

Starting with the lead *t*-Bu ester **6a**, removal of successive methyl groups as in **6b–d** indicated some preference for the unbranched esters as seen in the IC_{50} values although the inactivation rates for **6a** and **6d** were approximately the same. Removal of the ester group entirely, as in C-2 unsubstituted compound **12**, still maintained some activity (IC_{50} = 7 μM) while free carboxylic acid **7a** was completely inactive. Linear extension of the alkyl chain incorporating an additional ester group at varying distances was then investigated as a probe for the optimal chain length and any favorable hydrogen-bonding interactions. While these modifications afforded some increase in activity for **6e** and **6g**, the intermediate compound **6f** was substantially worse. Use of a benzyl ester (**6h**) provided a greater than 10-fold increase over **6a** in potency as measured by its IC_{50} value of 0.07 μM. Para substitution gave comparable or slightly diminished results as seen with the methoxy (**6i**), *t*-Bu (**6j**), and carbomethoxy (**6k**) compounds, as well as the *m*-carbomethoxy (**6m**). However, as evidenced by a straight or even inverse progress curve (due to turnover of the inhibitor and reactivation of the enzyme), the above benzyl derivatives were poor time-dependent inhibitors (see below). In contrast to the above results, the free *p*- and *m*-carboxylate derivatives **7b** and **7c**, while having comparatively poor IC_{50} values of 0.4 μM, were excellent inactivators of HLE as seen in their $k_{obs}/[I]$ values of 57 000 and 61 700 $M^{-1}\ s^{-1}$, respectively.

The HLE inhibitory activities of the prepared amides are listed in Table II. In general, the secondary amides were less active than their corresponding esters while the methyl-substituted tertiary amides gave intermediate ac-

(20) Schmidt, E.; Dabritz, E.; Thulke, K.; Grassmann, E. *Justus Liebigs Ann. Chem.* **1965**, *685*, 161.
(21) Chauvette, R. R.; Flynn, E. H. *J. Med. Chem.* **1966**, *9*, 741.
(22) Sassiver, M. L.; Sheperd, R. G. *Tetrahedron Lett.* **1969**, 3993.



13

Figure 1. Overall stereo view of the HLE active site region incorporating **7b** as shown schematically in structure **13**. Open circles are atoms of the enzyme with the residues numbered and the filled circles are atoms of the inhibitor **7b**.

tivity (compare **6h**, **9a**, and **9b**; **6f**, **9c**, and **9i**; **6g** and **9d**). Interestingly, while ester **6h** and amide **9b** gave comparable IC_{50} values (0.07 and 0.1 μM, respectively), the amide was still a time-dependent inhibitor ($k_{obs}/[I]$ = 9600 M^{-1} s^{-1}). Unlike the alkyl esters, the larger amide groups afforded little improvement in activity as seen with the series **9c–e** as well as **9f–i**. The effect of a cyclic amide was investigated with the preparation of the pyrrolidine- and morpholineamides **9j** and **9k**, both having good inactivation rates. Incorporation of a peptidyl unit at C-2 resulted in the loss of all activity for the glycinamides and β-alaninamides **10a** and **10b**, although some potency was restored with the tertiary amides **10c–e**. While the IC_{50} value for **10f** was the same as that of ester **7b** (0.4 μM), the large increase in the inactivation rate was not observed (8600 M^{-1} s^{-1} vs 57 000 M^{-1} s^{-1}).

Discussion

As described in the previous paper,[14] our investigation into the use of a β-lactam nucleus as an HLE inhibitor began with the observation that benzyl clavulanate was a time-dependent inhibitor of HLE (IC_{50} = 5 μM) while clavulanic acid itself was inactive.[23] As shown in the results above, the presence of a carboxylate at the C-2 position is disfavored with the cephalosporins as well, even at more remote distances from the nucleus. These considerations led to the synthesis of several different cephem C-2 esters and amides in an attempt to delineate the structural requirements for optimal inhibition of HLE.

In addition to the above experimental work, a modeling study[24] of **7b** was carried out by utilizing the X-ray crystal structure of HLE recently determined in these laboratories.[25] Figure 1 shows a stereo view of the active site region of HLE incorporating **7b** in what could be considered as an initial binding complex (**13**, Figure 1). The 7α-methoxy group extends into the S1 specificity pocket as expected from the known preference of HLE for small alkyl groups.[26] The S-5 α-sulfone oxygen is located within hydrogen-bonding distance of the backbone amide NH of valine 216 and might explain some of the beneficial effects of the sulfone vs the sulfide.[14] The C-8 β-lactam carbonyl is situated in the oxyanion pocket formed by the backbone amide NH of serine 195 and glycine 193 and is in position for attack by the hydroxyl of serine 195. The C-2 position is proximate to the active-site catalytic triad formed by serine 195, histidine 57, and aspartate 102, and the C-2 substituents extend into the prime sites of the enzyme. The C-2′ carbonyl oxygen may interact to some extent with the hydrophilic oxyanion region with the remaining ester portion occupying an adjacent hydrophobic area. Examination of the inhibition data and this model led to the following structure–activity relations.

1. As expected from the initial observations with clavulanic acid, there is a prominent decrease in potency for compounds containing a carboxylate at the C-2-position. This effect is evident not only for the parent compound **7a** but also for compounds having a more remote carboxylate as seen in the series **7a**, **10a–f**, **7b**, **7c** and **6h**. This decrease in potency may simply be the result of placing a very hydrophilic residue near or into the hydrophobic prime-site region which, by the presence of a negatively charged species, may also disrupt the effect of the catalytic triad.

(23) Zimmerman, M., unpublished result.
(24) The enzyme-ligand structural modeling was done with a modified version of FRODO[30] and the geometry optimization of the ligands within the context of a rigid enzyme were done using a modified MM2 force field.[31]
(25) (a) Williams, H. R.; Lin, T.-Y.; Navia, M. A.; Springer, J. P.; McKeever, B. M.; Hoogsteen, K.; Dorn, C. P., Jr. *J. Biol. Chem.* **1987**, *262*, 17178. (b) Navia, M. A.; McKeever, B. M.; Springer, J. P.; Lin, T.-Y.; Williams, H. R.; Fluder, E. M.; Dorn, C. P.; Hoogsteen, K. *Proc. Natl. Acad. Sci., U.S.A.* **1989**, *86*, 7–11.
(26) Harper, J. W.; Cook, R. R.; Roberts, C. J.; McLaughlin, B. J.; Powers, J. C. *Biochemistry* **1984**, *23*, 2995.

2. While a C-2 substituent is not required (compound **12**, IC_{50} = 7 μM), the best inhibition occurs with an unbranched ester (see **6a–d**) and linearly extended alkyl chains as seen in the series **6d,c,e–g**. The inactivation rates in these series parallel the IC_{50} values.

3. The orientational demands placed on the intact cephalosporins by the positioning of the β-lactam carbonyl in the oxyanion pocket and the stereospecific requirements of the C-7 substituent at the S1 site dictates that the C-2 substituent be positioned in what is effectively the S1′–S2′ sites of HLE. The large increase in potency for the more lipophilic alkyl and benzyl groups can thus be understood from the model in view of this region's largely hydrophobic nature and the known affinity that HLE has for substrates which are lipophilic in this region.[12b] It remains unclear why the alkyl esters and the free acids **7b** and **7c** are good time-dependent inactivators while benzyl esters **6h–m** are either much slower or very inefficient inactivators.

4. The more hydrophilic amides were generally less potent inhibitors than the esters with some improvement seen for the methyl-substituted tertiary amides (compare **6h**, **9a**, and **9b**; **6f**, **9c**, and **9i**) and cyclic amides **9j** and **9k**. The electron-rich amide oxygen may mimic that of a carboxylate and result in their decreased potency for the above reasons. In addition the amide carbonyl may interact more strongly with the oxyanion region and thus interfere with the initial binding of the C-8 carbonyl in the oxyanion pocket.

5. The inactivation rates are not strictly related to the IC_{50} values but are dependent on specific active-site interactions of the C-2 substituent as seen by the alkyl and benzyl esters and the difference between the carboxylic acids **7b** and **7c** and their methyl esters **6k** and **6m**. While the specific reasons for this are not evident from this model, it is interesting to note that the dramatic effect of carboxylates **7b** and **7c** may be due to a favorable interaction with a hydrophilic region near alanine 60 and asparagine 61. This interaction might stabilize the acyl enzyme intermediate long enough to better allow a second inactivation step to occur (see below). The subtle nature of the inactivation process is again evident in that this effect is not seen with the corresponding amide **10f**.

This model is also consistent with several possible mechanisms for the time-dependent inactivation of HLE as outlined in Scheme II of the previous paper.[14] An initial, reversible binding of the inhibitor to the HLE active site (**13**, Figure 1) would be followed by an irreversible ring opening of the β-lactam by the serine 195 hydroxyl to form an acyl enzyme intermediate. This transient intermediate could then be deacylated as occurs with the normal substrates of HLE (inhibitor turnover) or react further in a subsequent step to give a more fully inhibited species. Evidence that there is indeed a second inactivation event is gleaned from the observation that, on incubation of **6a** with HLE for a short period followed by quenching with hydroxylamine, the inactivation is short-lived and most of the HLE activity rapidly recovers, whereas with longer incubation times the inhibition is more persistent.[27] Crystallographic evidence for a second covalent hit has also been reported[28] in the case of the related 7-chloro derivative **2** (R = Cl) and porcine pancreatic elastase (PPE). Several other possible events may also be operative[14] as have been delineated for the established β-lactamase inhibitors.[29] However, the inactivation is not completely irreversible and eventually active HLE will be regenerated. The reactivation rate is again dependent on the substituents present and when it is very slow as in the case of **7b** ($T_{1/2}$ > 7 days)[27] the inhibition becomes functionally irreversible.

Conclusion

The results presented in this series of papers clearly demonstrate that modification of the cephalosporin nucleus can provide potent, time-dependent inhibitors of HLE. Furthermore, the HLE inhibition is very dependent on the substitution present at C-2, both in terms of their binding characteristics and time-dependent inactivation. These results are currently being applied to the development of therapeutic drugs for possible use in a variety of degenerative lung diseases.

Experimental Section

Proton NMR were recorded on either a Varian EM 390 or XL-200 instrument with tetramethylsilane as internal standard (δ scale). Infrared spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. Mass spectra were obtained on a LKB 9000 mass spectrometer. Elemental analyses were conducted by the Micro-Analytical Laboratory of Merck and Co. and were within 0.4% of the calculated values. Analytical TLC was carried out on Analtech, Inc. silica gel GF 250-μm plates (visualized with UV or cerric ammonium nitrate) and preparative TLC on Analtech, Inc. silica gel GF 1000- and 2000- μm plates. Flash chromatography was performed with EM silica gel 60 (230–400 mesh). Extractions were routinely carried out twice with the given solvent and each washed with water and/or 7% sodium bicarbonate solution followed by brine. The organic layers were then combined, dried over sodium sulfate, and concentrated on a rotary evaporator. IR and NMR spectra were consistent with the assigned structures. The reported yields were generally the result of a single experiment and were not optimized.

Method A. 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (4). Trifluoroacetic acid (TFA; 5 mL) was added to 316 mg (0.92 mmol) of *tert*-butyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (**3**) and 1 mL of anisole with cooling in an ice bath. After stirring for 0.5 h at 0 °C, the TFA was evaporated in vacuo. The residue was taken up in methylene chloride and washed with cold water and brine. The methylene chloride solution was dried and concentrated to give crude acid **4** as an oil in 80–90% yield after vacuum drying. This was routinely used without further purification: NMR ($CDCl_3$) δ 2.09 (s, 3, $COCH_3$), 3.50 (AB q, J = 18 Hz, 2, SCH_2), 3.56 (s, 3, OCH_3), 4.54 (d, J = 2 Hz, 1, 6 H), 4.72 (d, J = 2 Hz, 1, 7 H), 5.01 (AB q, J = 14 Hz, 2, CH_2O). Anal. ($C_{11}H_{13}NO_6S$) C, H, N.

Method B. Alternate Preparation of 4. To a suspension of 22 g (80 mmol) of 7-aminocephalosporanic acid (**1a**) in 300 mL of methanol was added 22 mL (160 mmol) of isoamyl nitrite followed by 14 mL (98 mmol) of 7 N perchloric acid. The reaction was warmed to 50–60 °C for 0.5 h with concurrent evolution of nitrogen. The reaction was cooled, poured into water, and extracted twice with ethyl acetate. The combined organic layers were extracted twice with 7% sodium bicarbonate solution, and the aqueous layers were acidified with 2 N hydrochloric acid in the presence of ethyl acetate. The product was extracted into ethyl acetate, washed with brine, dried, and concentrated to give 6 g of crude **4**. This was purified by flash chromatography (50% ethyl acetate/hexane, then 1% acetic acid) to give 1.6 g (7%) of fairly pure product followed by another 1.0 g of mixed fractions. These were used directly without further purification.

General Method C. Esterification of 4 with Diazoalkanes. Methyl 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (5d, R = CH_3). The crude

(27) Ashe, B. M., unpublished result.
(28) Navia, M. A.; Springer, J. P.; Lin, T.-Y.; Williams, H. R.; Firestone, R. A.; Pisano, J. M.; Doherty, J. B.; Finke, P. E.; Hoogsteen, K. *Nature* **1987**, *327*, 79.
(29) Knowles, J. R. *Acc. Chem. Res.* **1985**, *18*, 97.
(30) (a) Bush, B. L., private communication. (b) Bush, B. L. *Comput. Chem.* **1984**, *8*, 1. (c) Jones, T. A. *Computational Crystallography*; Sayre, D., Ed.; Clarendon Press: Oxford, 1982; p 303.
(31) Halgren, T., private communication.

carboxylic acid **4** (obtained from 0.95 g of *t*-Bu ester **3** by method A) was taken up in 20 mL of methanol and an ether solution of diazomethane was added until the yellow color persisted. The excess diazomethane was quenched with acetic acid and the reaction was concentrated. The residue was flash chromatographed (50% ethyl acetate/hexane, R_f 0.6) to give 0.52 g (65%) of sulfide **5d** (R = CH_3) as an oil: NMR ($CDCl_3$) δ 2.04 (s, 3, $COCH_3$), 3.42 (AB q, J = 18 Hz, 2, SCH_2), 3.53 (s, 3, 7-OCH_3), 3.87 (s, 3, CO_2CH_3), 4.40 (d, J = 2 Hz, 1, 6 H), 4.63 (d, J = 2 Hz, 1, 7 H), 4.80 (AB q, J = 13 Hz, 2, CH_2O). Anal. ($C_{12}H_{15}NO_6S$) C, H, N.

General Method E. Oxidation of Sulfide Esters 5 to Sulfones 6. Methyl 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (6d, R = CH_3). To a solution of 35 mg (0.11 mmol) of **5d** (R = CH_3) in 2 mL of methylene chloride was added 45 mg (0.26 mmol, 2.5 equiv) of 85% *m*-chloroperbenzoic acid (*m*-CPBA). The reaction was stirred at room temperature for 18 h and was then poured into a 7% sodium bicarbonate solution containing excess sodium sulfite to destroy any remaining *m*-CPBA. The product was extracted into methylene chloride, washed with brine, dried, and concentrated. Preparative TLC (50% ethyl acetate/hexane, R_f 0.4) afforded 30 mg (75%) of sulfone **6d** (R = CH_3). Trituration with ether/hexane gave a white solid: mp 79–80 °C; NMR ($CDCl_3$) δ 2.05 (s, 3, $COCH_3$), 3.53 (s, 3, 7-OCH_3), 3.81 (AB q, J = 18 Hz, 2, SCH_2), 3.87 (s, 3, CO_2CH_3), 4.62 (br d, J = 2 Hz, 1, 6 H), 4.80 (AB q, J = 14 Hz, 2, CH_2O), 5.07 (d, J = 2 Hz, 1, 7 H). Anal. ($C_{12}H_{15}NO_8S$) C, H, N.

Preparation of Esters 5c,f,g and 6c,f,g. With the procedure described in method C the ester sulfides **5c,f,g** were prepared and then oxidized as in method E to afford sulfone esters **6c,f,g** as listed in Table I. **6c**: R = CH_3CH_2; partial NMR ($CDCl_3$) δ 1.33 (t, J = 7 Hz, 3, CH_3), 2.33 (q, J = 7 Hz, 2, CH_2). **6f**: R = $CH_2CH_2CO_2CH_3$; partial NMR ($CDCl_3$) δ 2.70 (t, J = 7 Hz, 2, CH_2CO), 3.65 (s, 3, OCH_3), 4.5 (m, 2, OCH_2). **6g**: R = $(CH_2)_3CO_2CH_3$; partial NMR ($CDCl_3$) δ 2.0 (m, 2, $CH_2CH_2CH_2$), 2.38 (t, J = 7 Hz, CH_2CO), 3.65 (s, 3, OCH_3), 4.26 (t, J = 7 Hz, 2, OCH_2).

(Ethoxycarbonyl)methyl 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (6e, R = $CH_2CO_2CH_2CH_3$). A solution of 1 g (3.5 mmol) of crude **4** (obtained by method B), 4 mL of ethyl diazoacetate, and 10 mg of rhodium acetate dimer in 25 mL of methylene chloride was warmed to 35–40 °C for 0.5 h. Evaporation in vacuo and flash chromatography (50% ethyl acetate/hexane, R_f 0.5) afforded 550 mg (42%) of (ethoxycarbonyl)methyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate (**5e**, R = $CH_2CO_2CH_2CH_3$) as an oil: NMR ($CDCl_3$) δ 1.28 (t, J = 7 Hz, 3, CH_2CH_3), 2.05 (s, 3, $COCH_3$), 3.46 (AB q, J = 18 Hz, 2, SCH_2), 3.53 (s, 3, OCH_3), 4.20 (q, J = 7 Hz, 2, CH_2CH_3), 4.47 (d, J = 2 Hz, 1, 6 H) 4.67 (d, J = 2 Hz, 1, 7 H), 4.73 (AB q, J = 14 Hz, 2, SCH_2), 4.89 (AB q, J = 13 Hz, 2, CH_2O). Anal. ($C_{15}H_{19}NO_8S \cdot 0.5\ H_2O$) C, H, N.

Oxidation of 250 mg of the above sulfide as in method E gave 190 mg (70%) of sulfone **6e** (R = $CH_2CO_2CH_2CH_3$): NMR ($CDCl_3$) δ 1.28 (t, J = 7 Hz, 3, CH_2CH_3), 2.05 (s, 3, $COCH_3$), 3.53 (s, 3, OCH_3), 3.89 (AB q, J = 18 Hz, 2, SCH_2), 4.2 (q, J = 7 Hz, 2, CH_2CH_3), 4.74 (d, J = 2 Hz, 1, 6 H), 4.74 (AB q, J = 16 Hz, 2, OCH_2CO), 4.89 (AB q, J = 14 Hz, 2, CH_2O) 5.11 (d, J = 2 Hz, 1, 7 H). Anal. ($C_{15}H_{19}NO_{10}S$) C, H, N.

General Method for the Preparation of *N,N'*-Diisopropyl-*O*-alkylisoureas 11.[19,20] *N,N'*-Diisopropyl-*O*-[*p*-(methoxycarbonyl)benzyl]isourea (11, R = $CH_2C_6H_4$-4-CO_2CH_3). To a mixture of 1.8 g (10.8 mmol) of methyl *p*-(hydroxymethyl)benzoate and 1.35 g (10.8 mmol, 1.0 equiv) of *N,N'*-diisopropylcarbodiimide was added 20 mg (0.2 mmol, 0.02 equiv) of cuprous chloride. The mixture was stirred at room temperature for 3 days. The reaction was then rapidly eluted (30% ethyl acetate/hexane) through a short column of neutral alumina. Evaporation of the solvent afforded crude isourea **11** (R = $CH_2C_6H_4$-4-CO_2CH_3) which was used directly in the esterification of **4**: NMR ($CDCl_3$) δ 1.10 (d, J = 8 Hz, 6, $NHCH(CH_3)_2$), 1.21 (d, J = 8 Hz, 6, $NCH(CH_3)_2$), 3.11 (m, 1, $NHCH$), 3.5 (m, 1, $NHCH$), 3.90 (s, 3, OCH_3), 5.11 (s, 2, OCH_2), 7.32 (d, J = 8 Hz, 2, Ar-2,6 H), 7.90 (d, J = 8 Hz, 2, Ar-3,5 H).

General Method D. Esterification of 4 with Isoureas 11. Benzyl 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (6h, R = $CH_2C_6H_5$). A solution of crude acid **4** (obtained from 145 mg (0.42 mmol) of *t*-Bu ester **3** by method A) and 0.13 mL (0.55 mmol, 1.3 equiv) of *N,N'*-diisopropyl-*O*-benzylisourea[19] (11, R = $CH_2C_6H_5$) in 2 mL of THF was stirred at room temperature for 18 h. The reaction was then poured into 7% sodium bicarbonate solution and the product was extracted into methylene chloride, washed with brine, dried, and concentrated. Preparative TLC (50% ethyl acetate/hexane, R_f 0.5) gave 71 mg (45%) of benzyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate (**5h**, R = $CH_2C_6H_5$) as a 1/1 mixture of Δ^2- and Δ^3-isomers: NMR ($CDCl_3$) δ 1.96, 1.98 (2 s, 3, $COCH_3$), 3.4 (AB q, J = 17 Hz, 1, Δ^2-SCH_2), 3.43, 3.47 (2 s, 3, OCH_3), 4.2–5.3 (m, 6.5, CH_2O, 6 H, 7 H, OCH_2, Δ^3 2 H), 6.34 (br s, 0.5, Δ^3 4 H), 7.1–7.4 (br s, 5, C_6H_5).

Oxidation of 70 mg of the above sulfide as in method E afforded 37 mg (50%) of sulfone **6h** (R = $CH_2C_6H_5$) as an oil: NMR ($CDCl_3$) δ 2.04 (s, 3, $COCH_3$), 3.49 (s, 3, OCH_3), 3.80 (AB q, J = 18 Hz, 2, SCH_2), 4.60 (br s, 1, 6 H), 4.78 (AB q, J = 13 Hz, 2, CH_2O) 5.07 (d, J = 2 Hz, 1, 7 H), 5.17 (AB q, J = 11 Hz, 2, OCH_2), 7.1–7.4 (br s, 5, C_6H_5). Anal. ($C_{18}H_{19}NO_8S$) C, H, N.

Preparation of Esters 5b,i–m and 6b,i–m. With the procedure described in method D the ester sulfides **5b,i–m** were isolated as a mixture of isomers and directly oxidized as in method E to afford ester sulfones **6b,i–m** as listed in Table I. **6b**: R_1 = $CH(CH_3)_2$; partial NMR ($CDCl_3$) δ 1.28 and 1.31 (2 d, J = 6 Hz, 6, CH_3), 5.19 (heptet, J = 6 Hz, 1, CH). **6i**: R_1 = $CH_2C_6H_4$-4-OCH_3; partial NMR ($CDCl_3$) δ 3.82 (s, 3, OCH_3), 5.27 (AB q, J = 12 Hz, 2, CH_2), 6.91 (d, J = 9 Hz, 2, Ar-3, 5 H), 7.36 (d, J = 9 Hz, 2, Ar-2, 6 H). **6j**: R_1 = $CH_2C_6H_4$-4-$C(CH_3)_3$; partial NMR ($CDCl_3$) δ 1.33 (s, 9, $C(CH_3)_3$), 5.22 (AB q, J = 12 Hz, 2, CH_2), 7.3 (m, 4, C_6H_4) **6k**: R_1 = $CH_2C_6H_4$-4-CO_2CH_3; partial NMR ($CDCl_3$) δ 3.89 (s, 3, OCH_3), 5.30 (AB q, J = 13 Hz, 2, CH_2), 7.40 (d, J = 8 Hz, 2, Ar-2, 6 H), 7.90 (d, J = 8 Hz, 2, Ar-3, 5 H). **6l**: R_1 = $CH_2C_6H_4$-4-$CO_2C(CH_3)_3$; partial NMR ($CDCl_3$) δ 1.55 (s, 9, $C(CH_3)_3$), 5.30 (AB q, J = 13 Hz, 2, CH_2), 7.40 (d, J = 8 Hz, 2, Ar-2, 6 H), 7.89 (d, J = 8 Hz, 2, Ar-3, 5 H). **6m**: R_1 = $CH_2C_6H_4$-3-CO_2CH_3; partial NMR ($CDCl_3$) δ 3.87 (s, 3, OCH_3), 5.28 (AB q, J = 12 Hz, 2, CH_2), 7.30 (t, J = 8 Hz, 1, Ar-6 H), 7.50 (br d, J = 8 Hz, 1, Ar-5 H), 7.93 (br d, J = 8 Hz, 1, Ar-4 H), 8.00 (br s, 1, Ar-2 H).

3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid 5,5-Dioxide (7a, R = H). A solution of 100 mg of *tert*-butyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (**6a**, R = $C(CH_3)_3$) and 2 mL of anisole in 10 mL of TFA was stirred at 0 °C for 0.5 h and was then evaporated in vacuo. Two additional aliquots of methylene chloride were evaporated to remove most of the residual TFA before the residue was purified by preparative TLC (1% acetic acid/ethyl acetate) to give 75 mg (88%) of **7a** (*R* = H) as a white solid after trituration in ether: NMR (acetone-d_6) δ 2.03 (s, 3, $COCH_3$), 3.50 (s, 3, OCH_3), 3.90 (AB q, J = 18 Hz, 2, SCH_2), 4.84 (br s, 1, 6 H), 4.87 (AB q, J = 14 Hz, 2, CH_2O), 5.07 (br s, 1, 7 H). Anal. ($C_{11}H_{13}NO_8S \cdot 1H_2O$) C, H, N.

Preparation of Carboxylic Acids 7b,c. With the procedures described in method I, the title compounds were prepared as listed in Table I. **7b**: R_1 = $CH_2C_6H_4$-4-CO_2H; partial NMR ($CDCl_3$) δ 5.31 (br s, 2, CH_2), 7.44 (d, J = 8 Hz, 2, Ar-2, 6 H), 8.01 (d, J = 8 Hz, 2, Ar-3, 5 H). **7c**: R_1 = $CH_2C_6H_4$-3-CO_2H; partial NMR ($CDCl_3$) δ 5.38 (AB q, J = 12 Hz, 2, CH_2), 7.50 (t, J = 8 Hz, 1, Ar-6 H), 7.68 (br d, J = 8 Hz, 1, Ar-5 H), 8.09 (br d, J = 8 Hz, 1, Ar-4 H), 8.14 (br s, 1, Ar-2 H)).

General Method F. Mixed Anhydride Amidation of 4. *N,N*-Dimethyl-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide (8f, R_1 = R_2 = CH_3). A solution of 1.0 g (3.5 mmol) of **4** (prepared by method B), 0.72 mL (5.2 mmol, 1.5 equiv) of isobutyl chloroformate, and 0.42 mL (5.2 mmol, 1.5 equiv) of pyridine in 20 mL of acetone and 10 mL of dioxane was stirred at −20 °C for 0.5 h. To the solution of mixed anhydride was then added 0.78 g (17.5 mmol, 5 equiv) of dimethylamine (as a 40% aqueous solution) and the reaction was stirred for 2 h while warming to room temperature. The reaction was poured into excess dilute hydrochloric acid solution and the product was extracted into methylene chloride, washed with sodium bicarbonate solution and brine, dried, and concentrated. Flash chromatography of the residue (60–70% ethyl

acetate/hexane, R_f 0.4) afforded 650 mg (59%) of **8f** ($R_1 = R_2 = CH_3$) as an oil: NMR ($CDCl_3$) δ 2.05 (s, 3, $COCH_3$), 2.97 (s, 3, NCH_3), 3.03 (s, 3, NCH_3), 3.30 (m, 2, SCH_2), 3.50 (s, 3, OCH_3), 4.41 (br s, 3, 6 H, CH_2O), 4.67 (br s, 1, 7 H).

General Method H. Oxidation of Sulfide Amides 8 to Sulfone Amides 9. *N*,*N*-Dimethyl-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2(and 3)-ene-2-carboxamide 5,5-Dioxide (9f, $R_1 = R_2 = CH_3$). Oxidation of 650 mg of the above sulfide **8f** ($R_1 = R_2 = CH_3$) was carried out as in method E. Before concentration of the crude extracts, 2 drops of pyridine were added to catalyze the complete conversion to the Δ^2-isomer. Preparative TLC (60% ethyl acetate/hexane, R_f 0.3) of the residue afforded 600 mg (84%) of sulfone **9f** ($R_1 = R_2 = CH_3$) as a white solid: NMR ($CDCl_3$) δ 2.10 (s, 3, $COCH_3$), 3.00 (s, 3, NCH_3), 3.10 (s, 3, NCH_3), 3.58 (s, 3, OCH_3), 3.86 (AB q, J = 18 Hz, 2, SCH_2), 4.56 (AB q, J = 13 Hz, 2, CH_2O), 4.85 (br t, 1, 6 H), 5.04 (d, J = 2 Hz, 1, 7 H). Anal. ($C_{13}H_{18}N_2O_7S$) C, H, N.

Preparation of Amides 8a,b,e,g and 9a,b,e,g. With the procedure described in method F amide sulfides **8a,b,e,g** were prepared and directly oxidized as in method H to amide sulfones **9a,b,e,g** as listed in Table II. **9a**: R_1 = H; $R_2 = CH_2C_6H_5$; partial NMR ($CDCl_3$) δ 4.3–4.6 (m, 2, CH_2), 7.2 (br s, 5, C_6H_5), 7.3 (br t, 1, NH). **9b**: $R_1 = CH_3$; $R_2 = CH_2C_6H_5$; partial NMR ($CDCl_3$) δ 2.85 and 2.99 (2 s, NCH_3), 4.3–4.6 (m, 2, CH_2), 7.3 (br s, 5, C_6H_5). **9e**: R_1 = H; $R_2 = C(CH_3)_3$; partial NMR ($CDCl_3$) δ 1.40 (s, 9, $C(CH_3)_3$), 6.8 (br s, 1, NH). **9g**: $R_1 = CH_3$; $R_2 = CH_2CO_2C(CH_3)_3$; partial NMR ($CDCl_3$) δ 1.46 (s, 9, $C(CH_3)_3$), 3.01 and 3.04 (2 s, NCH_3), 3.5–3.8 (m, 2, CH_2).

General Method G. DCC Amidation of 4. *N*-[2-(*tert*-Butoxycarbonyl)ethyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (9c, R_1 = H, $R_2 = CH_2CH_2CO_2C(CH_3)_3$). A solution of 1.0 g (3.4 mmol) of **4** (prepared as in method B) in 25 mL of methylene chloride was stirred with 1.4 g (6.8 mmol, 2 equiv) of dicyclohexylcarbodiimide and 1.4 g (6.8 mmol, 2 eq) of *tert*-butyl β-alanine at room temperature for 4 h. The reaction was concentrated in vacuo and the residue partially purified by elution through a short silica gel column (50% ethyl acetate/hexane, R_f 0.3). The fractions containing the product were combined and further purified by flash chromatography (solvent gradient of 40–60% ethyl acetate/hexane) to give 310 mg (22%) of *N*-[2-(*tert*-butoxycarbonyl)ethyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2(and 3)-ene-2-carboxamide (**8c**, R_1 = H, $R_2 = CH_2CH_2CO_2C(CH_3)_3$) as a mixture of Δ^2- and Δ^3-isomers.

The oxidation of 80 mg of the above sulfide mixture **8c** (R_1 = H, $R_2 = CH_2CH_2CO_2C(CH_3)_3$) was carried out as in method H to give 70 mg (22%) of **9c** (R_1 = H, $R_2 = CH_2CH_2CO_2C(CH_3)_3$) as a semisolid: NMR ($CDCl_3$) δ 1.47 (s, 9, $C(CH_3)_3$), 2.07 (s, 3, $COCH_3$), 2.56 (t, J = 6 Hz, 2, CH_2CO), 3.60 (s, 3, OCH_3), 3.65 (m, 2, OCH_2CH_2), 3.80 (AB q, J = 18 Hz, 2, SCH_2), 4.76 (br s, 1, 6 H), 4.80 (AB q, J = 14 Hz, 2, CH_2O), 5.18 (d, J = 2 Hz, 1, 7 H). Anal. ($C_{18}H_{26}N_2O_9S$) C, H, N.

Preparation of Amides 8d,j,k and 9d,j,k. With the procedure described in method G amide sulfides **8d,j,k** were prepared as a mixture of isomers and oxidized directly as in method H to give amide sulfones **9d,j,k** as listed in Table II **9d**: R_1 = H; $R_2 = (CH_2)_3CO_2C(CH_3)_3$; partial NMR ($CDCl_3$) δ 1.43 (s, 9, $C(CH_3)_3$), 1.4–1.9 (m, 2, $CH_2CH_2CH_2$), 2.30 (t, J = 7 Hz, 2, CH_2CO), 3.2–3.5 (m, 2, NCH_2), 7.3 (br s, 1, NH). **9j**: $R_1, R_2 = -(CH_2)_4-$; partial NMR ($CDCl_3$) δ 1.9–2.1 (m, 4, $CH_2CH_2CH_2CH_2$), 3.3–3.5 and 3.5–3.7 (2 m, 4, NCH_2). **9k**: $R_1, R_2 = -(CH_2)_2O(CH_2)_2-$; partial NMR ($CDCl_3$) δ 3.6–3.9 (m, 8, $-(CH_2)_2O(CH_2)_2-$).

General Method I. *t*-Bu Ester Removal with TFA. *N*-(Carboxymethyl)-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (10b, R_1 = H, $R_2 = CH_2CH_2CO_2H$). A solution of 40 mg of **9c** (R_1 = H, $R_2 = CH_2CH_2CO_2C(CH_3)_3$) and 0.2 mL of anisole in 1 mL of TFA was stirred at 0 °C for 0.5 h and was then evaporated in vacuo. Two additional aliquots of methylene chloride were evaporated to remove most of the residual TFA before the residue was purified by preparative TLC (1% acetic acid in 50% ethyl acetate/hexane, R_f 0.2–0.3) to give 17 mg (50%) of **10b** (R_1 = H, $R_2 = CH_2CH_2CO_2H$) as an oil: NMR ($CDCl_3$) δ 2.12 (s, 3, $COCH_3$), 2.70 (t, J = 7 Hz, 2, CH_2CO_2), 3.56 (s, 3, OCH_3), 3.68 (m, 2, OCH_2CH_2), 3.86 (AB q, J = 17 Hz, SCH_2), 4.76 (AB q, J = 13 Hz, 2, CH_2O), 4.82 (br s, 1, 6 H), 5.18 (d, J = 2 Hz, 1, 7 H), 7.56 (br t, J = 6 Hz, 1, NH). Anal. ($C_{14}H_{18}N_2O_9S$) C, H; N: calcd, 7.18; found, 6.50.

Preparation of Carboxylic Acids 10a,c–f. With the procedure as described in method I, the title compounds were prepared as listed in Table II **10a**: R_1 = H; $R_2 = CH_2CO_2H$; partial NMR (acetone-d_6) δ 3.8–4.1 (m, 2, CH_2), 7.4 (br t, J = 6 Hz, 1, NH). **10c**: $R_1 = CH_3$; $R_2 = CH_2CO_2H$; partial NMR (acetone-d_6) δ 3.06 and 3.10 (2 s, 3, NCH_3), 3.8–4.1 (m, 2, NCH_2). **10d**: $R_1 = CH_3$; $R_2 = (CH_2)_2CO_2H$; partial NMR (acetone-d_6) δ 2.70 (t, J = 8 Hz, 2, CH_2CO), 3.01 and 3.08 (2 s, 3, NCH_3), 3.8 (m, 2, NCH_2). **10e**: $R_1 = CH_3$; $R_2 = (CH_2)_3CO_2H$; partial NMR (acetone-d_6) δ 1.9 (m, 2, $CH_2CH_2CH_2$), 2.4 (m, 2, CH_2CO), 3.01 and 3.04 (2 s, 3, NCH_3), 3.4 (m, 2, NCH_2). **10f**: $R_1 = CH_3$; $R_2 = CH_2C_6H_4$-4-CO_2H; partial NMR (acetone-d_6) δ 2.72 and 2.81 (2 s, 3, NCH_3), 4.40 (m, 2, CH_2), 7.29 (d, J = 8 Hz, 2, Ar-2, 6 H), 7.89 (d, J = 8 Hz, 2, Ar-3, 5 H).

3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (12). A solution of 78 mg (0.25 mmol) of **7a** (R = H) and 25 mg (0.25 mmol, 1 equiv) of *N*-methylmorpholine in 10 mL of THF was heated at 75 °C for 0.5 h. The reaction was then cooled and concentrated in vacuo. Preparative TLC (50% ethyl acetate/hexanes, R_f 0.36) of the residue afforded 60 mg (89%) of **12** as an oil: NMR ($CDCl_3$) δ 2.03 (s, 3, $COCH_3$), 3.53 (s, 3, OCH_3), 4.20 (AB q, J = 17 Hz, 2, SCH_2), 4.47 (br s, 2, CH_2O), 4.65 (br s, 1, 6 H), 5.11 (d, J = 2 Hz, 1, 7 H), 6.69 (br s, 1, 2 H). Anal. ($C_{10}H_{13}NO_6S$) C, H, N.

Acknowledgment. We thank the members of the Micro-Analytical Laboratory for obtaining the chemical analyses.

Registry No. **1a**, 957-68-6; **3**, 95570-83-5; **4**, 95570-59-5; **5b** (isomer 1), 128472-49-1; **5b** (isomer 2), 128472-50-4; **5c**, 128472-52-6; **5d**, 128472-51-5; **5e**, 95570-60-8; **5f**, 128472-53-7; **5g**, 128472-54-8; **5h** (isomer 1), 95570-68-6; **5h** (isomer 2), 95570-67-5; **5i** (isomer 1), 128472-55-9; **5i** (isomer 2), 128472-56-0; **5j** (isomer 1), 128472-57-1; **5j** (isomer 2), 128472-58-2; **5k** (isomer 1), 128472-59-3; **5k** (isomer 2), 128495-27-2; **5l** (isomer 1), 128472-60-6; **5l** (isomer 2), 128472-61-7; **5m** (isomer 1), 128472-62-8; **5m** (isomer 2), 95570-53-9; **6a**, 95671-97-9; **6b**, 104163-98-6; **6c**, 104182-16-3; **6d**, 104263-96-9; **6e**, 95570-61-9; **6f**, 128472-63-9; **6g**, 116660-92-5; **6h**, 95671-92-4; **6i**, 128472-64-0; **6j**, 128472-65-1; **6k**, 116660-89-0; **6l**, 128472-66-2; **6m**, 95570-54-0; **7a**, 104164-02-5; **7b**, 95570-58-4; **7c**, 128472-67-3; **8a** (isomer 1), 128472-68-4; **8a** (isomer 2), 128472-69-5; **8b** (isomer 1), 128472-70-8; **8b** (isomer 2), 95570-65-3; **8c** (isomer 1), 128472-71-9; **8c** (isomer 2), 128495-28-3; **8d** (isomer 1), 128472-72-0; **8d** (isomer 2), 128472-73-1; **8e** (isomer 1), 128472-74-2; **8e** (isomer 2), 128472-75-3; **8f** (isomer 1), 128472-76-4; **8f** (isomer 2), 128472-77-5; **8g** (isomer 1), 128472-78-6; **8g** (isomer 2), 128472-79-7; **8j** (isomer 1), 128472-80-0; **8j** (isomer 2), 128472-81-1; **8k** (isomer 1), 116536-16-4; **8k** (isomer 2), 116536-15-3; **9a**, 104163-99-7; **9b**, 95671-90-2; **9c**, 128472-82-2; **9d**, 116561-52-5; **9e**, 128472-83-3; **9f**, 104263-97-0; **9g**, 116536-12-0; **9h**, 128472-84-4; **9i**, 128472-85-5; **9j**, 128472-86-6; **9k**, 128472-87-7; **10a**, 104164-00-3; **10a** (*t*-Bu ester), 95671-91-3; **10b**, 128472-88-8; **10c**, 128472-89-9; **10d**, 128472-90-2; **10d** (*t*-Bu ester), 128472-93-5; **10e**, 128472-91-3; **10e** (*t*-Bu ester), 128472-94-6; **10f**, 128472-92-4; **10f** (*t*-Bu ester), 128472-95-7; **11** (R = $CH_2C_6H_4$-4-CO_2CH_3), 128472-96-8; **11** (R = $CH_2C_6H_5$), 2978-10-1; **11** (R = *i*-C_3H_7), 63460-32-2; **11** (R = $CH_2C_6H_4$-4-OCH_3), 128472-97-9; **11** (R = $CH_2C_6H_4$-4-*t*-C_4H_9), 128472-98-0; **11** (R = $CH_2C_6H_4$-4-CO_2-*t*-C_4H_9), 128472-99-1; **11** (R = $CH_2C_6H_4$-3-CO_2CH_3), 95570-52-8; **11** (R = $CH_2C_6H_4$-3-CO_2-*t*-C_4H_9), 128473-00-7; **12**, 104164-01-4; HLE, 9004-06-2; $N_2CHCO_2C_2H_5$, 623-73-4; $HOCH_2C_6H_4$-4-CO_2CH_3, 6908-41-4; $H_2N(CH_2)_3CO_2$-*t*-C_4H_9, 50479-22-6; $CH_3NHCH_2CO_2$-*t*-C_4H_9, 5616-81-9; *i*-PrN=C=NPr-*i*, 693-13-0; *tert*-butyl-β-alanine, 15231-41-1.