Inhibition of Human Leukocyte Elastase. 4. Selection of a Substituted Cephalosporin (L-658,758) as a Topical Aerosol†

Paul E. Finke,*,‡ Shrenik K. Shah,‡ Bonnie M. Ashe,§ Richard G. Ball,∞ Thomas J. Blacklock,∥ Robert J. Bonney,⊥,# Karen A. Brause,‡ Gilbert O. Chandler,‡ Meredith Cotton,∞ Philip Davies,§ Pam S. Dellea,⊥ Conrad P. Dorn, Jr.,‡ Daniel S. Fletcher,⊥ Laura A. O'Grady,‡ William K. Hagmann,‡ Karen M. Hand,⊥ Wilson B. Knight,§ Alan L. Maycock,§,° Richard A. Mumford,§ Donald G. Osinga,⊥ Paul Sohar,∥ Kevan R. Thompson,‡ Hazel Weston,§ and James B. Doherty‡

Departments of Medicinal Chemical Research, Enzymology, Immunology and Inflammation, and Biophysical Chemistry and Process Chemistry Research and Product Research and Development, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

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Human leukocyte elastase (HLE) is a serine protease which has been implicated as a causative agent in several pulmonary diseases. The continued modification of our previously reported cephalosporin-based HLE inhibitors has led to the identification of a series of C-2 amides with potent, topical activity in an in vivo hamster lung hemorrhage model. While the most potent in vitro HLE inhibition had previously been obtained with lipophilic ester derivatives, it was found that the less active, but more polar and stable, amide derivatives were much more effective in vivo. The development of the structure–activity relations for optimization of these activities is discussed. These results led to the selection of 3-(acetoxymethyl)-2-[(2(*S*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-dioxide (3, L-658,758) as a selective, potent, time-dependent HLE inhibitor suitable for formulation as a topical aerosol drug for possible clinical use.

The inhibition of human leukocyte elastase (HLE) (EC 3.4.21.37) has been a major therapeutic goal for some time. Since an excess of active HLE has been implicated in several disease states, such as emphysema,[1] chronic bronchitis,[2] acute respiratory distress syndrome (ARDS),[3] and cystic fibrosis,[4] an inhibitor of HLE has the potential of preventing or arresting these conditions.[5] In most cases, the pathogenesis of these diseases has been correlated with the inactivation or an insufficient reserve of HLE's natural inhibitors, notably α_1-protease inhibitor (α_1-PI), in localized environments. The presence of this uninhibited elastase in the intercellular spaces then results in the uncontrolled proteolysis and damage of structural tissue. Given this hypothesis, considerable effort has been directed toward finding treatments which would augment the activity of the natural inhibitors and restore the protease/antiprotease balance.[1a,6] Two methods investigated so far in this regard are the development of recombinant sources of the natural inhibitors for a replacement therapy[7] and the synthesis of mechanism-based peptide or low molecular weight inhibitors.[8] Since many of the proposed HLE-mediated diseases occur in the lung, aerosolization of these agents has been employed. This route of administration circumvents the problems of absorption and metabolism associated with systemic administration and should also lessen any possible side effects.[9]

Modification of the cephalosporin nucleus[10] to afford selective, potent, and time-dependent inhibitors of HLE has been previously reported by these laboratories.[11-15] From these studies 4-carboxybenzyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-

* To whom correspondence should be addressed.

† This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday in recognition of his many scientific accomplishments at Merck and throughout his career.

‡ Department of Medicinal Chemical Research.
§ Department of Enzymology.
∥ Process Chemistry Research.
⊥ Department of Immunology and Inflammation.
∞ Product Research and Development.
° Department of Biophysical Chemistry.
Present address: Bristol-Myers Co., 100 Forest Avenue, Buffalo, NY 14213.
° Present address: Sterling Research Group, 25 Great Valley Parkway, Malvern, PA 19355.

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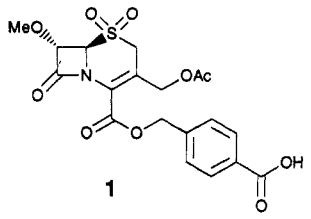

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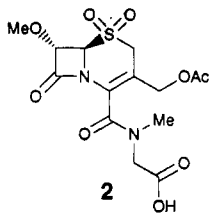

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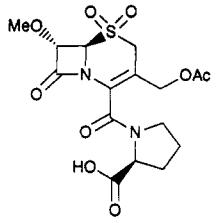
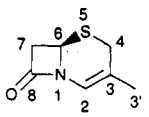
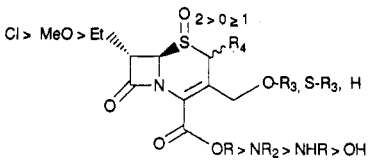


3, L-658,758



A

Cephalosporin Numbering



B

carboxylate 5,5-dioxide (1) was identified[13] as a very potent, time-dependent inhibitor of HLE ($k_{obs}/[I]$ = 57 000 M^{-1} s^{-1}) whereas the sarcosine amide 2 was comparatively much less active ($k_{obs}/[I]$ = 300 M^{-1} s^{-1}) and the C-2 carboxylic acid **19b** was inactive. However, all of these inhibitors were found to be rapidly degraded in blood and no oral absorption could be detected in the rat. These problems were addressed with the subsequent establishment of an intratracheally (IT) administered, HLE-induced lung hemorrhage assay in the hamster which afforded the necessary means to evaluate the efficacy of our cephalosporin inhibitors directly in a lung environment.[16] In contrast to the above in vitro inhibition values, 2 was found to be much more efficacious than 1 in the in vivo assay (Table I) when administered intratracheally prior to HLE instillation. This pivotal finding then led to the synthesis of several amide analogues and ultimately to the selection of the L-proline derivative 3-(acetoxymethyl)-2-[(2(*S*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-dioxide (3, L-658,758) as a more stable, selective, and time-dependent HLE inhibitor having functional in vivo activity in this and several other efficacy models of HLE-mediated tissue damage. In this paper the development of this compound is described in terms of the structure–activity relations (SAR) for enzyme inhibition and lung hemorrhage activity as well as the chemical and physical properties required of an aerosol candidate for possible clinical evaluation.

It has been observed[17] that large, polar compounds are retained in the lung environment better than small, nonpolar entities which can be rapidly absorbed. However, experience has shown that HLE prefers a more hydrophobic, nonpolar substrate[18] or inhibitor.[11,13] As will be seen below, another crucial finding was that the previously optimized esters were in fact very susceptible to hydrolysis of the β-lactam, while the corresponding less potent amides were found to have improved hydrolytic stability. These initial findings explained very well the above results with 1 and 2, and thus the focus of this work became the development of a more polar, stable derivative which retained the previous potent HLE inhibition. The criteria used to evaluate these cephalosporin derivatives were (1) their activity in the hamster lung assay, which was used as the primary screen for efficacy as well as a pharmacokinetic tool, (2) their stability in pH 8 MOPS buffer to determine their relative chemical stability, and (3) their HLE inhibitory potency. As described below, 3 was found to have good overall activity both in vitro ($k_{obs}/[I]$ = 3800 M^{-1} s^{-1}) and in vivo (ED_{50} = 5 μg/animal with a 30-min predose, T_{50} = 3 h with a 200-μg dose), as well as better chemical stability ($t_{1/2}$ = 21 h), suitable physical properties, ease of synthesis, and the required initial safety. In addition, 3 has been found[5] to be potent in several subsequent efficacy studies as well.

Chemistry

As reported in our initial investigations and depicted in Structure B, a small, α-oriented chloro, methoxy, or ethyl group was found to be optimal at the C-7 position for HLE inhibition and the sulfone (oxidation state at S-5) was considerably more potent than the sulfide derivative or either of the sulfoxide isomers.[12] At the C-2 position, lipophilic esters were found to be the most potent inhibitors while tertiary amides were considerably less active and secondary amides were very poor inhibitors. The free carboxylic acids (as is found in most β-lactam antibiotics)

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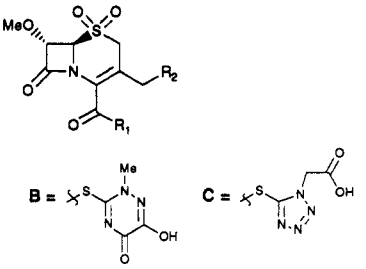
Table I. Chemical Stability and in Vitro and in Vivo HLE Activity of Selected Initial C-2 Ester and Amide Derivatives



compd	R_1	R_2	$t_{1/2}$,[b] h (SD)[d]	$k_{obs}/[I]$,[c] M^{-1} s^{-1} (SD)[e]	lung hemorrhage activity[a] dose, μg	time, min	% inhibn (SD)[f]
1[g]	OCH_2Ph-4-CO_2H	OAc	5 (0.1)	57000 (6000)	100	−5	98 (3)
					100	−30	25 (25)
2[g]	$NMeCH_2CO_2H$	OAc	13 (1)	300 (100)	100	−30	90 (10)
					10	−30	67 (27)
					200	−180	54 (30)
4a[h]	O-*t*-Bu	OAc	nd[i]	16000 (1500)	50	premix[j]	98 (5)
4b[k]	OCH_2Ph-4-CO_2H	H	≫24	K_i = 0.5 μM[l]	100	−30	54 (12)
4c[g]	7α-ethyl derivative of 1		40 (2)	5700 (1400)	100	−30	−32 (24)
4d[m]	4-methyl derivative of 1		<5	25000 (2300)	100	−30	29 (27)
4e[k]	O-*t*-Bu	$OCO(CH_2)_2CO_2H$	nd	13800 (300)	400	−5	98 (2)
4f[k]	O-*t*-Bu	A	nd	63900 (4000)	100	−30	87 (7)
					100	−90	10 (31)
4g[k]	O-*t*-Bu	B	nd	8600 (500)	100	−30	90 (6)
					100	−90	18 (16)
4h[k]	O-*t*-Bu	C	nd	3700 (300)	100	−30	89 (13)
					30	−30	77 (10)
4i[g]	NH-*t*-Bu	OAc	nd	2200 (100)	400	−30	−5 (41)
4j[g]	$N(CH_2CH_2)_2O$	OAc	13 (0.3)	35200[n]	100	−30	−21 (49)
4k[g]	$NMeCH_2Ph$-4-CO_2H	OAc	nd	8600 (3200)	100	−30	65 (20)

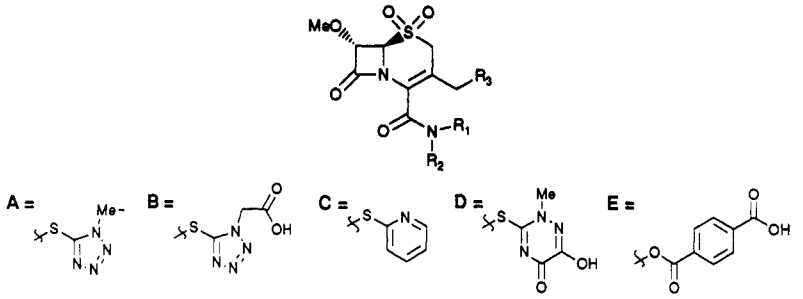
[a] See ref 16 for methodology. The compounds were administered IT at the given dose (per animal) and time prior to HLE. [b] See Experimental Section for methodology. [c] See ref 12 and 22 for methodology. [d] Standard deviation of experimental points from the calculated first-order decay curve. [e] Average of two or more determinations at different inhibitor concentrations. [f] Average of three animals at each dose and time. [g] See ref 13. [h] See ref 12. [i] Not determined. [j] HLE was premixed with compound prior to IT administration. [k] See ref 14. [l] No time-dependent inhibition was observed. [m] See ref 15. [n] Result of a single determination.

were inactive.[13] Also, it was found that substitution at C-4 could enhance activity in some cases[15] and the C-3′ acetoxy group could be replaced by a variety of other oxygen- or sulfur-based leaving groups.[14] The unsubstituted C-3 methyl analogues were much less active, although they could still be time-dependent inhibitors even without a potential leaving group at this position.[14] For this work the sulfone derivatives received the primary emphasis and the 7α-methoxy group at C-7 was chosen over the more potent 7α-chloro so as to avoid possible problems inherent in an α-halocarbonyl compound. A few of the less potent, but more stable, 7α-ethyl analogues were also prepared for comparison. As implied from the results of **1** and **2** mentioned above, the substitution at C-2 was pivotal to the modification of both the in vitro and in vivo activities and was extensively explored. Then, once the SAR at C-2 was delineated, a variety of other C-3′ and C-4 analogues was evaluated.

To help understand the above contradiction with **1** and **2**, a solution-stability assay was developed. Samples were dissolved at 1 mg/mL in 0.5 M MOPS buffer at pH 8.0 and incubated at 25 °C. Aliquots (50 μL) were removed at regular intervals, quenched with 2% aqueous trifluoroacetic acid, and analyzed by reverse-phase HPLC. The disappearance of compound was then monitored for one to two half-lives and the results fit to a first-order decay curve from which the reported $t_{1/2}$ values were derived. Table I lists the structure and pH 8 stability data for some selected, previously described esters as well as several of our initial amides. In general, the esters were readily hydrolyzed (**1**, $t_{1/2}$ = 5 h) while the amides had significantly longer half-lives (**2**, $t_{1/2}$ = 13 h). The more potent, neutral amide **4j** was equally stable ($t_{1/2}$ = 13 h) but was totally inactive in the lung assay. As expected, the C-3 methyl (**4b**) and 7α-ethyl (**4c**) analogues were much more resistant to hydrolysis due to less activation of the β-lactam carbonyl. Also, the C-4 methyl-substituted compounds, such as **4d**, were found to be more readily hydrolyzed compared to the unsubstituted parents, possibly due to steric repulsion of the acetoxy, making it a better leaving group. This also correlates with the improved HLE inhibition observed with some of these C-4 derivatives. For reference, the $t_{1/2}$ of the C-2 carboxylic acid **19b** increased to 85 h and that of cephalothin, a known antibiotic, was >100 h (data not shown).

The amides prepared in this study are listed in Table II along with the hydrolysis results ($t_{1/2}$) for selected compounds. The C-3′ acetoxy amide analogues were routinely prepared from *t*-butyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (**5**)[12] as previously described[13] or as outlined in Scheme I. Since the C-2 carboxylic acid **6** was an oil and difficult to purify, the trifluoroacetic acid (TFA) deprotection of **5** (method A) was usually followed immediately by amide formation to give the sulfides **7** as a mixture of Δ^2 and Δ^3 isomers. This reaction was accomplished through dicyclohexylcarbodiimide (DCC) activation and active ester formation with *N*-hydroxysuccinimide followed by addition of the corresponding amine or amino acid *tert*-butyl ester. It was soon found that it was advantageous to convert the active ester intermediate completely to the Δ^3 form by treatment with excess triethylamine (TEA) prior to addition of the secondary amine (method B). The conversion to the Δ^3 isomer was

Table II. Chemical Stability and in Vitro and in Vivo HLE Activity of C-2 Amide Derivatives



compd	R_1	R_2	R_3	$t_{1/2}$,[b] h (SD)[g]	$k_{obs}/[I]$,[c] M^{-1} s^{-1} (SD)[h]	lung hemorrhage activity: % inhibn[a] screen[d] (SD)[i]	titration[e] of 100/30/10/3 μg at 30 min	duration[f] of 200 μg at 1/2/3/4 h
2	Me	CH_2CO_2H	OAc	13 (1)	300 (100)	90 (10)	90/75/65	97/80/54/20
3		-(*S*)-$CH(CO_2H)(CH_2)_3$-	OAc	21 (1)	3800 (800)	94 (6)	93/86/77	99/97/78/32
8a	Me	$(CH_2)_2CO_2H$	OAc	24 (2)	500 (100)	87 (10)		80/39/-1/33
8b	Me	$(CH_2)_3CO_2H$	OAc	nd[j]	900[k]	98 (2)		92/43/4/-12
8c	Me	(*S*)-$CH(Me)CO_2H$	OAc	nd	500 (50)	97 (1)	96/89/78/50	94/81/47/21
8d	Me	(*R*)-$CH(Me)CO_2H$	OAc	nd	900 (400)	97 (1)	97/90/29	
8e		-(*R*)-$CH(CO_2H)(CH_2)_3$-	OAc	<5	K_i = 3.4 (0.3)[l]	0 (46)		
8f		$-(CH_2)_4-$	OAc	16 (1)	27000[k]	5 (20)		
8g	CH_2Ph	CH_2CO_2H	OAc	nd	K_i = 6.1 (0.05)[l]	70 (15)	42/30/-26	
8h		$-CH_2CH(OH)(CH_2)_2-$	OAc	nd	12000 (600)	72 (23)		
8i		-(*S*)-$CH(CO_2H)CH_2$(R)$CH(OH)CH_2$-	OAc	nd	900 (100)	95 (4)	98/95/78	86/81/4/17
8j		-(*R,S*)-$CH_2CH(CO_2H)CH_2CH_2$-	OAc	nd	1900 (100)	-9 (72)		
8k	Me	$CH_2CONMe(CH_2)_2CO_2H$	OAc	nd	700 (200)	95 (5)	86/77/35	
11a	Me	CH_2CO_2H	A	nd	600 (100)	93 (11)		
11b	Me	CH_2CO_2-*t*-Bu	B	nd	20500 (700)	90 (6)	91/59/20	
11c	Me	CH_2CO_2H	C	nd	K_i = 43[l]	76 (14)	76/46/35	
11d	Me	CH_2CO_2-*t*-Bu	D	nd	19300 (1300)	98 (1)	98/94/65	89/84/38
11e	Me	CH_2CO_2H	D	22 (2)	6200 (1100)	98 (1)	98/94/56	94/90/64/37
11f		$-(CH_2)_4-$	D	20 (0.7)	13400 (3400)	91 (7)	95/60/30	88/87/64/50
11g		-(*S*)-$CH(CO_2H)(CH_2)_3$-	D	46 (3)	K_i = 0.25 (0.07)[l]	99 (1)	100/86/80/61	
11h	Me	$CH_2CH_2CO_2H$	D	nd	1000 (300)	94 (4)	96/74/45	95/87/46/-23
13a	Me	CH_2CO_2H	OBz	nd	600 (300)	79 (6)	87/58/-9	88/58/38/5
13b	Me	CH_2CO_2H	OCONHBn	nd	600 (50)	89 (6)	91/93/27	
13c	Me	Bn	E	nd	10000 (1600)	-27 (99)		
			7α-Ethyl Derivatives					
16a	Me	CH_2CO_2H	OAc	>50	30 (10)	66 (16)		
16b	Me	CH_2CO_2H	E	nd	100 (20)	90 (8)		84/59/57/28
			4-Methyl Derivative					
18	Me	CH_2CO_2H	OAc	8 (0.3)	1400 (100)	25 (30)		

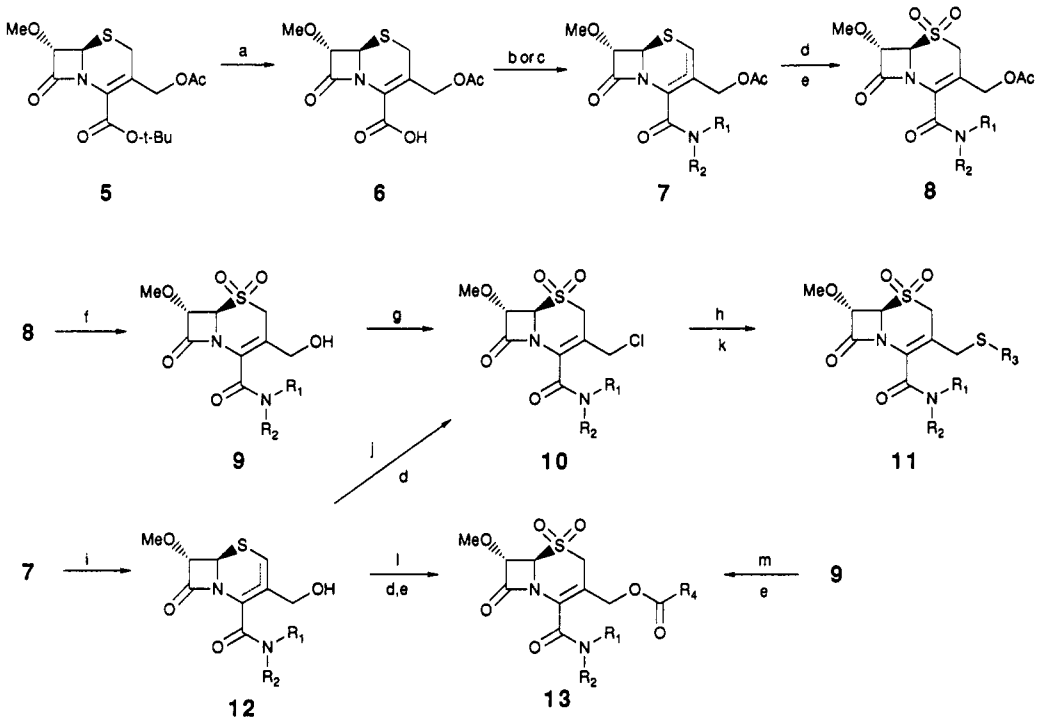
[a] See ref 16 for methodology. The compounds were administered IT at the given dose (per animal) and time prior to HLE. [b] See Experimental Section for methodology. [c] See refs 12 and 22 for methodology. [d] The standard screen was administration of 100 μg of compound/animal 30 min prior to instillation of 50 μg of HLE. Inhibition of the hemorrhage was determined after 3 h. [e] For active compounds from the screen, doses of 100, 30, 10, and 3 μg/animal were given at 30 min prior to HLE and inhibition determined. On repeated assaying, an ED_{50} could be determined. [f] For active compounds, the duration of the compound was determined by dosing at 200 μg/animal 1, 2, 3, and 4 h prior to HLE instillation. The time at which 50% inhibition was still obtained was used as the T_{50} for efficacy. [g] Standard deviation of experimental points from the calculated first-order decay curve. [h] Average of two or more determinations at different inhibitor concentrations. [i] Average of three animals at each dose and time. Similar errors were obtained for the titration and duration experiments. [j] Not determined. [k] Result of a single determination. [l] No time-dependent inhibition was observed.

conveniently monitored by NMR analysis (Δ^3 C-4 H, δ = 6.54) and the product was determined to be almost exclusively the Δ^3 isomer. Later, the use of 1-hydroxybenzotriazole was found to afford a better yield of Δ^3 sulfide without the need for TEA (method C). No evidence was observed for an intermediate Δ^3 ketene as proposed[19] for the preparation of Δ^3 esters from the Δ^2 acid chloride. The products were usually only partially purified by flash chromatography before oxidation to the sulfones 8 with *m*-chloroperbenzoic acid (*m*-CPBA) (method D). Treatment with a trace of pyridine before purification was required to completely convert the sulfones back to the more stable Δ^2 isomers. The amino acid *tert*-butyl ester derivatives were then deesterified with TFA to afford the final amido carboxylic acid products **2**, **3**, **8a–e,g,i,j** (method E). As hoped, these amides retained or had improved stability in the pH 8 hydrolysis assay as observed with the β-alanine derivative **8a** ($t_{1/2}$ = 24 h). The important effect of the carboxylate position on the $t_{1/2}$ values was clearly seen in the pyrrolidine series, with the neutral, unsubstituted compound **8f** having a $t_{1/2}$ of 16 h, and that of the L-proline derivative **3** improved to 21 h while that of the D isomer **8e** was less than 5 h. The instability of the latter compound may actually imply internal catalysis by the carboxylate.

Replacement of the C-3′ acetoxy was accomplished by hydrolysis of the sulfone amides 8 with titanium isopropoxide (method F) before deblocking the carboxylic acid. Treatment of the intermediate alcohols **9** with thionyl

(19) Murphy, C. F.; Koehler, R. E. Chemistry of Cephalosporin Antibiotics. XVIII. Synthesis of 7-Acyl-3-methyl-2-cephem-4-carboxylic Acid Esters. *J. Org. Chem.* 1970, *35*, 2429–2430.

Scheme I[a,b]



[a] The lettered reagents correspond to the methods A–M as described in Chemistry and Experimental Section. [b] Reagents: (a) TFA, anisole, 0 °C; (b) DCC, *N*-hydroxysuccinimide, dioxane; TEA, 0 °C; HNR_1R_2; (c) DCC, 1-hydroxybenzotriazole, DMF; HNR_1R_2; (d) 2.5 equiv of *m*-CPBA, CH_2Cl_2; (e) TFA, anisole, 0 °C; (f) $Ti(O\text{-}i\text{-}Pr)_4$, *i*-PrOH, 50 °C; (g) $SOCl_2$, pyridine, CH_2Cl_2, 0 °C; (h) HSR_3, $NaHCO_3$ or $KHCO_3$, acetone/H_2O; (i) $Ti(O\text{-}i\text{-}Pr)_4$, *i*-PrOH, 50 °C; (j) $SOCl_2$, pyridine, CH_2Cl_2, 0 °C; (k) TFA, anisole, 0 °C; (l) OCNBn, DMAP, CH_2Cl_2, 50 °C; (m) $ClCOR_4$, pyridine, CH_2Cl_2.

chloride afforded the C-3′ chlorides **10** (method G), which were immediately reacted with the sodium or potassium salt of the corresponding thioheterocycle (method H). Alternatively, the intermediate Δ^3 sulfides **7** could be hydrolyzed to the alcohols **12** (method I). Reaction with thionyl chloride followed by *m*-CPBA oxidation (methods J and D) again afforded the sulfone chlorides **10**. The amino acid amides **11a,c,e,g,h** were then obtained by TFA deblocking of the *tert*-butyl esters. Very good hydrolysis results and in vivo activity (see below) were obtained with the thiotriazine compounds **11d–h**, the pH 8 stability being significantly better than their corresponding C-3′ acetoxy compounds.

The alcohols **9** or **12** could also be acylated (method L and M) with subsequent oxidation and/or deblocking with TFA to afford ester and carbamate analogues, e.g. **13a–c**. These compounds were less polar and thus less active in vivo and were therefore not a major emphasis.

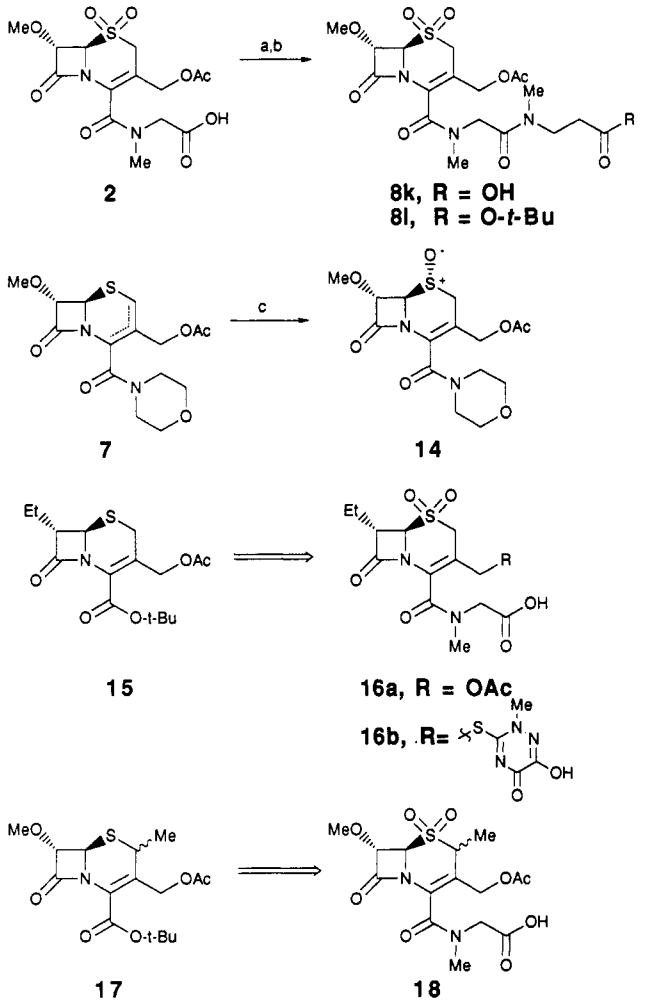
In order to complete the SAR picture, several other derivatives were prepared as shown in Scheme II. Extension of the carboxylic acid further removed from the β-lactam ring was investigated with diamide analogues such as **8k**. The more polar sulfoxide **14** was prepared from the sulfide **7** ($NR_1R_2 = N(CH_2CH_2)_2O$) by partial oxidation with *m*-CPBA. While **14** was found to be very stable ($t_{1/2}$ = 49 h), it was inactive in the lung assay (data not shown). The 7α-ethyl and 4-methyl derivatives **16a**, **16b**, and **18** were prepared via the same routes as above starting with the known sulfides **15**[12] and **17**.[15] The hydrolytic stability of these compounds compared well with the initial results, **16a** having a very long half-life ($t_{1/2}$ > 50 h) and **18** having a shorter one than **2** ($t_{1/2}$ = 8 h).

Once **3** was selected for safety assessment evaluation (see below), a stable, nonhygroscopic crystalline form which could be milled to about 1 μm was necessary for formulation as an aerosol in a metered-dose inhaler. The NMR spectrum of **3** indicated that the isolated free acid existed as a 4:1 mixture of amide rotamers as seen by two resonances for the 7α-methoxy [δ from TMS (acetone-d_6) 3.60 and 3.52]. HPLC also gave two broadened peaks, indicating that the rotamer equilibration was quite slow. For this reason initial attempts at crystallization were unsuccessful and thus several salts were prepared. While all of these salts were too hygroscopic for use in an aerosol formulation, interestingly, the sodium salt afforded crystals which were a single, inactive amide rotamer ($k_{obs}/[I]$ = 88 vs 3800 M^{-1} s^{-1}) while the lithium salt contained a single, active rotamer ($k_{obs}/[I]$ = 3600 M^{-1} s^{-1}). The rate of equilibration for the sodium salt was studied by NMR in D_2O [7-CH_3O δ from DSS (active) 3.60, (inactive) 3.55] at p*D* = 7.1 (pH = 7.5) and 25 °C. The equilibration was found to have a half-life of 1.2 ± 0.5 h and resulted in a 50:50 mixture of the two rotamers. Fortunately, the free carboxylic acid of **3** eventually did crystallize from ethyl acetate as the pure, active rotamer. This material was a nonhygroscopic, crystalline solid, mp 161.5-163 °C, which was microjet milled to a mean particle size of 1.1 μm,[20] suitable for use in an aerosol formulation.

An X-ray crystal structure was also obtained from the above crystals with the results shown in Figure 1. This structure indicates that the amide carbonyl is nearly perpendicular to the plane of the C-2,C-3 double bond (67°) due to the steric bulk of the pyrrolidine ring. This alignment places the partially negative amide oxygen over the β-face of the lactam ring which might shield it from nucleophilic attack, mimicking to some extent the C-2 carboxylate of the more stable antibiotics. Also evident

(20) Andrews, A.; Reynolds, S. Unpublished results.

Scheme II[a]



[a] Reagents: (a) DCC, *N*-hydroxysuccinimide, dioxane; $HNMeCH_2CH_2CO_2$-*t*-Bu; (b) TFA, anisole, 0 °C; (c) 1 equiv of *m*-CPBA, CH_2Cl_2, 0 °C.

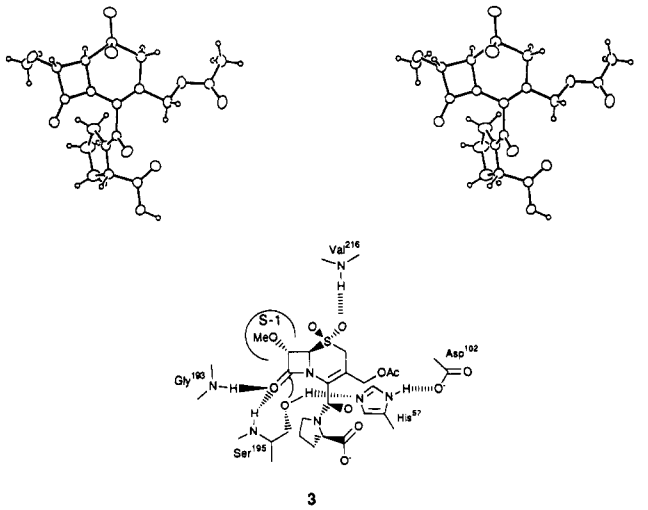
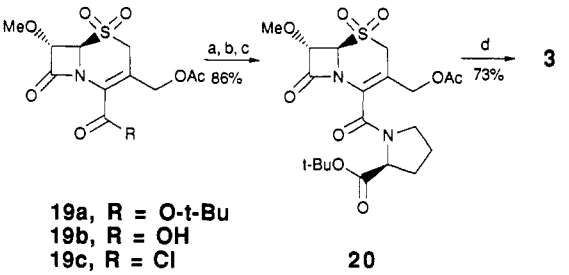


Figure 1. A stereoview of the crystal structure obtained for 3 and a schematic drawing of it in the active site of HLE.

is the planar character of the amide bond (the dihedral angle for the planes of the amide group and the pyrrolidine ring is only 4°) and the positioning of the L-carboxylate away from the lactam carbonyl. The other amide rotamer would place the carboxylate directly under the α-face, thus shielding the lactam from attack. This observation correlates well with both the increased stability of 3 over

Scheme III[a]



[a] Reagents: (a) TFA, 10 °C; (b) $COCl_2$, DMF (cat), CH_2Cl_2, 0 °C; (c) L-proline *tert*-butyl ester, CH_2Cl_2, −15 to 25 °C; (d) TFA, 0 °C, then EtOAc crystallization.

the unsubstituted pyrrolidine **8f** and the observed HLE activities of the two rotamers. The greater hydrolysis rate for the D isomer **8e** might be the placement of the carboxylate in such a manner so as to catalyze the hydrolysis from the α-side. The observed HLE inhibition and hydrolysis rates of the open analogues **2** and **8a–d** would then be the sum of the effects of all four possible carboxylate conformations.

A more direct and improved synthesis of **3** as shown in Scheme III was also investigated for the production of larger quantities. This process work found that the sulfone 2-carbonyl chloride **19c** was the most suitable for the amide preparation, and a much improved sythesis of the sulfone *t*-butyl ester **19a** was developed, as has already been reported.[21] Deprotection with TFA afforded the acid **19b** which was converted to the acid chloride with oxalyl chloride. This was immediately reacted with L-proline *tert*-butyl ester to give the amide **20** in 86% yield and without any need for purification. Again TFA deesterification of the proline *tert*-butyl ester was utilized to afford the desired product **3**. Silica gel filtration followed by crystallization from hot ethyl acetate afforded analytically pure **3** in 73% yield from **19a** and in 28.8% overall yield from commercially available 7-aminocephalosporanic acid (7-ACA).

Biological Results

Tables I and II contain the biochemical and biological results for these compounds in terms of their in vitro HLE inhibition ($k_{obs}/[I]$)[11,22] and their potency in the hamster lung assay.[16] The $k_{obs}/[I]$ is the second-order rate constant for the time-dependent inactivation of HLE by these inhibitors and under these conditions is ≥90% of the K_{inact}/K_i.[22] In some cases no time-dependence was observed and the apparent K_i is reported. The hamster lung hemorrhage assay was used as the primary screening model to determine if the compounds were effective inhibitors of the HLE-induced hemorrhage in the lung when given by intratracheal (IT) administration. The routine screen protocol utilized IT administration of 100 μg/animal of test compound 30 min prior to IT instillation of 50 units of HLE and determination of the lung hemmorhage after

(21) Blacklock, T. J.; Butcher, J. W.; Sohar, P.; Lamanec, T. R.; Grabowski, E. J. J. A Versatile Synthesis of 1,1-Dioxo 7-Substituted Cephems: Preparation of the Human Leukocyte Elastase (HLE) Inhibitor 1,1-Dioxo-trans-7-methoxycephalosporanic Acid *tert*-Butyl Ester. *J. Org. Chem.* **1989**, *54*, 3907–3913.

(22) Green, B. G.; Weston, H.; Ashe, B. M.; Doherty, J.; Finke, P.; Hagmann, W.; Lark, M.; Mao, J.; Maycock, A.; Moore, V.; Mumford, R.; Shah, S.; Walakovits, L.; Knight, W. B. PMN Elastases: A Comparison of the Specificity of Human Isozymes and the Enzyme from Other Species toward Substrates and Inhibitors. *Arch. Biochem. Biophys.* **1991**, *286*, 284–292.

3 h. The animals were sacrificed, the lungs were lavaged with saline, and the amount of hemoglobin in the fluid was measured spectrophotometrically at 414 nm. The percent inhibition was calculated as the ratio of hemorrhage in drug-treated animals to vehicle-treated animals. For those compounds with good activity at the screening dose, a titration at 100, 30, 10, and 3 μg/animal was done to determine a 50% effective dose (ED_{50}) at the 30-min predose time point. This assay was also used as a pharmacokinetic tool by dosing selected compounds at 200 μg/animal at 60, 120, 180, and 240 min prior to HLE instillation. The time at which 50% inhibition (T_{50}) was obtained was then calculated and used as a measure of the effective duration of the compound in the lung environment. In addition, a 20-min postdose treatment of test compound after HLE administration afforded a means to evaluate whether these compounds were still effective after the lung hemorrhage had already been initiated.

The most potent enzyme inhibitors from our initial structure–activity work were generally hydrophobic, neutral, water-insoluble C-2 esters as exemplified by the original lead compound **4a**[12] (see Table I). In order to verify that a cephalosporin-inhibited HLE complex would not reactivate in vivo, **4a** was premixed with HLE before intratracheal administration and in fact a 98% inhibition of the hemorrahge was observed. The topical efficacy of these compounds was first established in vivo with **1**, a very potent, water-soluble derivative containing a 4-carboxybenzyl ester, giving 98% inhibition when adminstered IT at 100 μg/animal 5 min prior to HLE challenge. However, this activity was short-lived and diminished to 25% inhibition when **1** was given 30 min prior to HLE instillation. Interestingly, while its HLE potency in vitro was low ($k_{obs}/[I]$ = 300 M^{-1} s^{-1}), the sarcosine amide derivative **2**, when likewise administered in this assay, gave nearly complete inhibition (90%) even with a 30-min predose. In fact, good activity was seen at 10 μg (67% inhibition) and for up to 180 min at the 200 μg/animal dose (54%). Table I shows the biological results for some other previously reported[12-15] esters and amides that were amenable for testing in this assay. The presence of just the C-2 amide was not sufficient for activity, as can be seen with the simple *tert*-butyl amide **4i** and the morpholine amide **4j** (−5% and −21% inhibition, respectively). The moderate activity of the 4-carboxybenzylamide hybrid **4k** (65%) demonstrates the negative effect of the additional lipophilicity of a phenyl group, when compared to **2**. The importance of the aqueous stability was evident when comparing **1** and **4d** with the more stable, but less potent, C-3 methyl derivative **4b** (54% vs 25% and 29% inhibition at 30 min). The lack of in vivo activity for **4c** was presumed to be its greater lipophilicity and thus faster absorption. Placement of the carboxylate in the C-3′ leaving group afforded some activity at the 30-min time point as seen with the succinate analogue **4e** (98% inhibition at 400 μg) and the very potent carbamate **4f** (87% inhibition at 100 μg). Other previously reported C-3′ modified, water-soluble compounds containing a C-2 *tert*-butyl ester were also effective when administered at 100 μg 30 min prior to HLE instillation, such as **4g** and **4h** (90% and 89% inhibition); however, the activity rapidly diminished at lower doses or longer time intervals. From these initial results it was clear that good in vitro potency was not sufficient for in vivo efficacy, but poor potency on the enzyme could be more than compensated for by improving the stability and increasing the polarity to give a longer lung residence time. Thus, all three of these parameters would have to be optimized for the best effectiveness in the lungs and the amide derivatives were chosen for further investigation.

Table II contains the results for the various tertiary amides prepared in this study. Since previous work[13] had shown very poor in vitro activity for secondary amides, these were not investigated. Extension of the spacer between the amide and carboxyl of **2** as in **8a** (87%), **8b** (98%), and **8k** (95% inhibition) as well as the α-methyl analogues **8c** (97%) and **8d** (97%) showed comparable lung hemorrhage results, slightly improved HLE potency in vitro, and greater hydrolytic stability, e.g. **8a** ($t_{1/2}$ = 24 h). With phenyl substitution on the amide as with **8g**, the apparent time-dependent inhibition was lost and lower in vivo activity was seen, probably due to its greater lipophilicity. The dominant effect on the lung hemorrhage potency of the polarity and position of the carboxylic acid, in contrast to the enzyme inhibition, was very evident in the pyrrolidine series. While the unsubstituted compound **8f** was very potent in vitro, it was inactive in the lung assay. Incorporation of a hydroxyl for increased polarity provided moderate activity as in **8h** (72% inhibition). The best overall activity in this series was observed with the conformationally restrained L-proline analogue **3** (94%), whereas the D-isomer **8e** was essentially inactive. The presence of both a carboxyl and hydroxyl, as in the 4-hydroxyproline derivative **8i**, also gave excellent inhibition (95%). The β-proline analogue **8j** was completely inactive in contrast to **3** or the corresponding open-chain β-alanine analogue **8a** (−9% vs 94% and 87% inhibition, respectively).

As expected, substitution of the acetoxy group with larger, more lipophilic esters (**13a**, 79%) and carbamates (**13b**, 89%) gave little improvement in vitro and these derivatives showed decreased lung activity. Moving the carboxyl to the leaving group, as in **13c**, resulted in the loss of all lung activity (−27%).

Substitution at the C-3′ position with the previously employed thioheterocycles was first investigated in the sarcosine series. The use of the *N*-methylthiotetrazole **11a** (93%) and the acetic acid derivative **11b** (90%) again afforded good initial potency. The thiopyridine **11c** (76%), the only basic compound prepared, showed very poor HLE activity (K_i = 43 μM) and had only moderate in vivo activity (76% inhibition). The best results were obtianed with the very polar triazine ring as seen with **11e** (98%) and even with the intermediate *tert*-butyl ester **11d** (98%). The pyrrolidine derivative **11f** (91%) had very comparable potency even without the carboxyl group in the amide. Once **3** was discovered as above, an additive effect was hoped for with the hybrid analogue **11g**. Indeed, the chemical stability ($t_{1/2}$ = 46 h) was excellent and the duration seemed as good or better than **3** or **11e**; however, the apparent HLE inhibition was found not to be time-dependent (K_i = 0.25 μM).

The 7α-ethyl (**16a** and **16b**) and 4-methyl (**18**) analogues correlated with our previous experience. Thus, **16a** was a poorer inhibitor than **2** in both assays (66% vs 90% inhibition) while **16b** showed enhanced activity over **16a**, but still less than **11e** (90% vs 98%). Even with the expected improvement in the HLE inhibition for the C-4 derivative **18** ($k_{obs}/[I]$ = 1400 vs 300 M^{-1} s^{-1} for **2**), the lung hemorrhage activity was diminished (25% vs 90%).

Discussion

Our earlier work[12-15] had demonstrated that the cephalosporin nucleus could be modified (as depicted in Structure **B**) to afford very potent, time-dependent inhibitors. Since in most cases the reactivation time for these inhibitors was very long (e.g. **1**, $t_{1/2} > 7$ days),[13] the inhibition could be considered functionally irreversible. This type of inhibition was considered a very desirable property of these compounds in as much as the detrimental effects of HLE reactivation would be minimized.[23] With the development of the hamster lung assay (and its variations) a tool became available to evaluate these agents in an in vivo model of HLE-mediated lung damage. However, most of the compounds prepared previously to optimize the in vitro inhibition were neutral, lipophilic esters and thus were not suitable for topical administration or gave only limited duration in the lung. The solution to this dichotomy was then addressed in our subsequent work as described here.

Unlike many of the related antibiotic drugs, a critical factor effecting the use of these modified cephalosporins is their susceptibility to hydrolysis, which is the likely reason for their lack of oral absorption and very short blood half-lives. While the absorption/blood-stability problem could be addressed with the decision to pursue topical administration directly into the lungs, the stability of the more potent esters was still inadequate, as can be seen with the 4-carboxybenzyl ester **1**. While **1** was the more potent inhibitor ($k_{obs}/[I] = 57\,000\ M^{-1}\ s^{-1}$), the less active 3-methyl compound **4b** ($K_i = 1.0\ \mu M$) was actually more effective in the lung assay at the 30 min predose time (54% vs 25% inhibition at 100 μg). Since in many cases an increase in the hydrolysis rate of the β-lactam at pH 8 seemed to correspond to enhanced activity against HLE (serine hydroxyl opening of the β-lactam), optimization of HLE activity in view of increased stability was required. The further requirement for a nonlipophilic, charged compound was evident from the adverse effect on the HLE in vivo potency of the phenyl derivatives **4f** and **4k**, especially since the additional polarity of the simple amides **4i** and **4j**, the addition of the hydroxyl in **8j** (Table II), or the use of the sulfoxide **14** was not sufficient for in vivo activity.

As shown in Table II, the synthesis of a series of *N*-methylamino acids **2** and **8a–d** and in particular the proline derivatives **3**, **8e**, and **8j** delineated the restraints required for the carboxyl group. While moving the charge further out from the β-lactam (**8a,b,k**) resulted in moderate improvement in enzyme activity and chemical stability (e.g. **8a**), activity in the hamster lung hemorrhage assay was diminished at longer times. While the lung hemorrhage activity for **8c** was excellent, the in vitro inhibition was still poor ($K_{obs}/[I] = 500\ M^{-1}\ s^{-1}$). The central finding that only the L-proline analogue **3** gave both enhanced enzyme and functional potency, as well as additional stability, was crucial. This result can be explained by examination of the X-ray crystal structure of the active rotamer and integration of it with the proposed active site model[13,24] for binding of the cephalosporins. As depicted in Figure 1, the α-methoxy group fits into the S-1 binding pocket, the α-oxygen of the sulfone may H-bond to the Val^{216} backbone NH and the β-lactam carbonyl fits into the "oxy-anion" pocket formed by the backbone NH's of Gly^{193} and Ser^{195}. The Ser^{195} hydroxyl is in position to open the β-lactam from the α-side, the attack being aided by the catalytic triad comprising the imidazole of His^{57} and the carboxyl of Asp^{102}. The reduced potency of the amides, as seen in their second-order rate constants, may be due to the amide oxygen's proximity to and competition for the "oxy-anion" region. It is also intriguing to speculate that the inherently lower activity of the amides might also be attributable to destabilization of the tetrahedral intermediate by the partially negative amide carbonyl on the β-face of the lactam. More interesting is the observation that the carboxyl is held away from the lactam carbonyl on the opposite side of the pyrrolidine ring and above the catalytic triad, thus minimizing the unfavorable effects of the carboxylate charge. This rationalization is supported by the lack of enzyme activity for the sodium salt amide rotamer which would have the carboxyl below and thus shield the β-lactam carbonyl from attack by Ser^{195}. Furthermore, the D-diastereomer **8e**, having the opposite configuration, has the carboxyl in one rotamer form on the same side as the lactam carbonyl, while the other rotamer has the carboxyl group pointing toward the catalytic triad.

Compounds incorporating the larger and more polar thiohetereocycles (**11a–f**) afforded good activity, especially with the triazine ring which has been found to possess definite advantages in the antibiotic ceftriaxone.[25] The desirable effects of this leaving group are evident from their greater stability and excellent in vivo activity (**11e** vs **2**, **11f** vs **8f**, **11g** vs **3**). The enzyme inhibition for **11e** was much improved over that of **2** ($k_{obs}/[I] = 6200$ vs $300\ M^{-1}\ s^{-1}$), while that of **11f** was lower than that of **8f**, as would be expected for a much more polar compound. However, **11g** is apparently not a time-dependent inhibitor, giving a constant inhibition in the HLE inhibition assay. This result was similar to that seen for the simple benzyl esters as reported previously[13] and again underscores the role that different substituents on the cephalosporin ring can exert on the mechanism of inhibition. A possible explanation for the non-time-dependent inhibition is a much faster turnover rate for **11g**, thus being closer to a competitive substrate, and for this reason it was not pursued further.

In order to differentiate the best compounds and make an ultimate choice for a potential drug candidate, three compounds were chosen for in-depth evaluation. Compound **3** was chosen as the best from the C-3′ acetoxy series, especially in terms of the improved HLE activity and stability. Compound **11e** was the best C-3′-modified amino acid derivative while **11f**, which lacks a carboxylic acid group on the amide portion, was also considered potentially interesting. Repeated testing in the lung hemorrhage assay and further investigation of these three compounds confirmed their potent HLE activity as

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(24) Navia, M. A.; Mckeever, B. M.; Springer, J. P.; Lin, T.-Y.; Williams, H. R.; Fluder, E. M.; Dorn, C. P.; Hoogsteen, K. Structure of Human Neutrophil Elastase in Complex with a Peptide Chloromethyl Ketone Inhibitor at 1.84-A Resolution. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7–11.

(25) Angehrn, P.; Probst, P. J.; Reiner, R.; Then, R. L. Ro 13-9904, a Long-Acting Broad-Spectrum Cephalosporin: In Vitro and In Vivo Studies. *Antimicrob. Agents Chemother.* **1980**, *18*, 913–921.

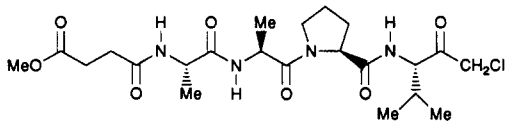
Table III. Biological Profile for **3**, **11e**, and **11f**

assay	3	11e	11f
	Lung Hemorrhage Assay[a]		
ED_{50} (μg/animal at −30 min), μg	5	10	20
T_{50} at 200 μg/animal, min	185	180	240
postdose at +20 min, μg (% inhibition)[b]	30 (70) ED_{50} = 15 μg	400 (86)	30 (93)
	RPA in the Rat[c]		
% inhibition at 250 μg/animal (dosed at +1.5 and +2.5 h)	87 ● 7 ED_{50} = 55 μg	82 ± 15	18 ± 21
	HLE Inhibition		
$k_{obs}/[I]$,[d] M^{-1} s^{-1} (SD)	3800 (800)	6200 (1100)	12800 (3400)
partition ratio[e]	2 ± 0.1	nd	3.8 ± 0.2
$T_{1/2}$ reactivation time at 37 °C,[e] h	9 ± 1.1	nd	6.5 ± 2.2
IC_{50} for inhibition of elastin solubilization,[f] μM	0.5–1	nd	2–4

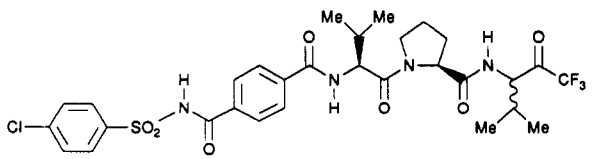
[a] See ref 16a, for methodology. The compounds were administered IT at the dose and time, prior to or after HLE. [b] Compound was administered 20 min after HLE and the inhibition was based on the ratio of hemorrhage at 3 h relative to that at the 20 min time point. In order to obtain comparable inhibition, 4000 μg of α_1-PI was required. [c] See ref 30 for methodology. [d] See refs 12 or 22 for methodology. [e] See ref 27 for methodology. [f] See ref 16a for methodology.

summarized in Table III. Of particular note were the 20-min postdose experiments in which these compounds were very effective at preventing further damage, while α_1-PI required 4000 μg/animal to give comparable results.[16b] As a result of this testing and in consideration of the problems encountered with the purification and crystallization of **11e** and **11f**, the L-proline derivative **3** was determined to possess the best overall chemical, physical, and biological properties for a topical aerosol drug candidate. It was specific for HLE-mediated damage, since no inhibition was seen for a similar thermolysin-induced hemorrhage,[16b] and was subsequently found to be negative in a preliminary Ames test, a P-450 induction assay, and our routine antibacterial screens.[26]

Some limited comparison of these compounds to some known peptide-based HLE inhibitors has also been done in our laboratories. The known, irreversible peptide chloromethyl ketone MeOSuc-AAPV-CH_2Cl[8e] was initially



MeOSuc-AAPV-CH_2Cl



21, ICI-200,880

used to validate the lung hemorrhage assay as previously reported.[16a,b] While MeOSuc-AAPV-CH_2Cl was less potent at the 30-min predose time (ED_{50} = 30 μg/animal), it appeared to show comparable duration in the lung assay (72 ± 18% at 200 μg at −4 h) and lung pharmacokinetics[16b] compared to **11f**. In addition, the mechanism-based, but reversible, peptide trifluoromethyl ketone inhibitor ICI-200,880 (**21**)[8a] was about equipotent to **3** at the 30-min predose protocol (ED_{50} = 5–10 μg/animal), but has been reported[8a] to have a remakable duration in the lung environment ($t_{1/2}$ = 10 h). A definitive experiment to evaluate the relative advantages and/or disadvantages[23] of a reversible vs an irreversible HLE inhibitor in a clinical setting has yet to be reported.

Table IV. Selectivity of **3** as an Inhibitor of HLE Compared to Other Serine Enzymes and Non-Serine Proteinases

enzyme	concn, mM	% inhibn at 10 min
HLE[a]	48	100 (K_i = 1.7 μM)
thrombin (human)[b]	48	0
plasmin (human)[b]	48	14[b]
cathepsin G (human)[c]	48	18[b]
pepsin[d]	48	0
acetylcholinesterase[e]	48	0
thermolysin[f]	48	0
papain[g]	48	0
complement cascade	120	6
clotting cascade	240 nM	−8

[a] HLE was assayed as in Table II. [b] Thrombin and plasmin were assayed as in Table II except using 0.2 mM Tosyl-Gly-Pro-Arg-*p*-nitroanilide as substrate. [c] Cathepsin G was assayed by monitoring the release of *p*-nitrophenol at 348 nm from *t*-Boc-Tyr-ONp in pH 6.5 PIPES buffer, 10% DMSO. There was no indication that this inhibition was time-dependent. [d] Pepsin was assayed by following the hydrolysis at 310 nm of 1 mM Phe-Gly-His-*p*-NO_2-Phe-Phe-Ala-OMe in 0.04 M formate buffer, pH 4.0. [e] Acetylcholinesterase was assayed by monitoring the decrease in absorbance at 420 nm caused by the hydrolysis of acetylcholine chloride (6 mg/mL) in the presence of *m*-nitrophenol (0.45 mg/mL) in 0.04 M phosphate, pH 7.8. [f] Thermolysin was assayed by monitoring the release of the fluorescent 7-amino-4-methylcoumarin (λ_{ex} 383, λ_{em} 455) from 0.2 mM Suc-Ala-Ala-PheAMC in pH 7.5 TES in the presence of excess leucine aminopeptidase. [g] Papain was assayed by following the release at 410 nm of *p*-nitroanilide from benzoyl-Arg-*p*-nitroanilide in pH 7.5 TES, 0.001 M EDTA, 0.005 M Cys, 10% DMSO.

Additional enzymatic work[27] showed that **3** was an extremely selective and effective mechanism-based inhibitor. Incubation of HLE with it required only 3 equiv to completely inactivate the enzyme. This 2 to 1 partition ratio is excellent when compared with the known β-lactamases, which have much higher ratios.[28] The rate of reactivation of the HLE–**3** complex was found to be 9 h at 37 °C, which essentially makes it a functionally irreversible inhibitor. This property of **3** should eliminate any inherent problem of a competitive, reversible inhibitor.[23] It was also found to be very selective for HLE, giving little or no inhibition of several other serine proteases or biological systems as shown in Table IV. Also, no intracellular inhibition of HLE was seen on incubation with neutrophils.[16a] The finding that these compounds can effectively inhibit the solubilization of elastin by HLE under conditions where α_1-PI is ineffective[16a] demonstrates a unique feature of a low molecular weight, irreversible inhibitor.[29]

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Subsequent biological testing continues to establish 3 as an effective HLE inhibitor in several environments.[6] Additional validation of the lung hemorrhage model for inhibition of HLE in vivo has been achieved by the recovery of the inactivated HLE–3 complex from the lung lavage fluid.[30] In a more relevant disease-oriented model, 3 was able to reduce the hemorrhage caused by endogenously generated elastase in an acute reverse passive Arthus (RPA) reaction in the rat with an ED_{50} of 55 μg/animal dosed at 1.5 and 2.5 h after initiation of the reaction.[31] While the in vivo blood stability of 3 in the rat was still low ($t_{1/2} \ll 10$ min), its effectiveness in competition with α_1-PI was shown even in this environment by inhibiting both the cleavage of fibrinogen ($IC_{50} = 14.4 \pm 3.5$ μM) and also reducing ($IC_{50} = 38.3 \pm 4.4$ μM) the amount of α1-PI–HLE complex generated when HLE is released from stimulated neutrophils.[32]

Conclusion

The continued modification of our previously reported cephalosporin leads has led to the identification of a series of C-2 amides having potent, topical HLE inhibitory activity in an IT hamster lung hemorrhage assay. The initially observed in vivo activity of 2, a poor HLE inhibitor in vitro, was optimized in terms of better chemical stability, improved efficacy and duration in the hamster lung hemorrhage assay, and increased enzyme potency. This work resulted in the selection of 3-(acetoxymethyl)-2-[(2(*S*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-dioxide (3) as a potent, time-dependent HLE inhibitor suitable for formulation as a topical aerosol. Its efficacy for possible use in pulmonary diseases has also been demonstrated in a variety of biological environments and models. The results of further development of 3 as a clinical candidate will be reported elsewhere. The extension of this work to other β-lactam structures to afford extremely potent, orally active HLE inhibitors is reported in this issue.[33]

Experimental Section

General Procedures. Proton NMR spectra were recorded on a Varian XL-200 instrument with tetramethylsilane as internal standard (δ scale). Elemental analyses were conducted by the Micro-Analytical Laboratory of Merck and Co. or Robertson Microlit Laboratories, Inc., and were within 0.4% of the calculated values except as noted. Analytical TLC was carried out on Analtech, Inc. silica gel GF 250-μm plates (visualized with UV or cerric sulfate) 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 a portion of water and/or sodium bicarbonate solution followed by a portion of brine. The organic layers were then combined, dried over sodium sulfate, and concentrated in vacuo on a rotary evaporator. IR and NMR spectra were consistent with the assigned structures of all new compounds and intermediates. The reported yields were generally the result of a single experiment and were not optimized.

Method A: Deesterification of 5 with TFA. 3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (6).[13] To a cooled solution of trifluoroacetic acid (TFA) (75 mL) and anisole (5 mL) at 0 °C was added *tert*-butyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (5)[13,21] (18 g, 0.052 mol) as a solid or in a minimum amount of CH_2Cl_2. The reaction was stirred at 0 °C until complete by TLC (1 h). The solution was then poured into a mixture of ice water (200 mL) and CH_2Cl_2 (200 mL), and the layers were separated. The organic layer was washed twice with water and each aqueous layer was sequentially back-extracted with an additional three portions of CH_2Cl_2. The combined organic layers were then extracted with 2 portions of saturated $NaHCO_3$ solution maintained at pH = 7–8 with the addition of more solid $NaHCO_3$. The combined aqueous layers were acidified with 2 N HCl in the presence of EtOAc. The layers were separated, and the organic layer was washed with brine. The aqueous layers were further extracted with another 3 portions of EtOAc. The organic layers were combined, dried over Na_2SO_4, and evaporated. The crude product 6 (12–14 g, 80–90%) was obtained as a thick oil and was used as soon as possible in the amidation reaction.

Method B. DCC/*N*-Hydroxysuccinimide Preparation of Δ³ Sulfides 7. 3-(Acetoxymethyl)-2-[[2(*S*)-(*tert*-butoxycarbonyl)pyrrolidino]carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene (7; R_1R_1 = –CH(*S*)(CO_2-*t*-Bu)-(CH_2)$_3$–). To an ice bath cooled solution of the crude acid 6 (11.5 g, 40 mmol) in dioxane (100 mL) were sequentially added *N*-hydroxysuccinimide (5.7 g, 50 mmol) and dicyclohexylcarbodiimide (DCC) (12.4 g, 60 mmol). The reaction was stirred at room temperature under N_2 for 30 min and then recooled in an ice bath. Triethylamine (TEA) (5.5 mL, 40 mmol) was added and the reaction was stirred at 0 °C for another 30 min. L-Proline *tert*-butyl ester[34] (14 g, 80 mmol) was then added all at once. After a further 2 h at room temperature, the reaction was diluted with ether (200 mL) and filtered and the filtrate was poured into ice water (200 mL) containing 2 N HCl (60 mL). The layers were separated, and the organic layer was washed with water, $NaHCO_3$ solution, and brine. After the aqueous layers were back-extracted with another portion of ether, the organic layers were pooled, dried over Na_2SO_4, and evaporated. The product was purified by preparative LC (45% EtOAc/hexanes) to give 10.5 g (60%) of 7 (R_1R_2 = –CH(*S*)(CO_2-*t*-Bu)(CH_2)$_3$–) as an oil. The product usually contained a small amount of the Δ² isomer and DCC byproduct. NMR ($CDCl_3$): δ 1.44 and 1.50 (2 s, 9 H, *t*-Bu), 1.8–2.2 (m, 4 H, CH_2CH_2), 2.05 and 2.08 (2 s, 3 H, OAc), 3.53 and 3.56 (2 s, 3 H, MeO), 3.6–3.8 and 3.9–4.1 (2 m, 2 H, NCH_2), 4.40 (m, 1 H, NCH), 4.58 and 4.84 (2 AB q, J = 12 and 16 Hz, 2 H, 3′-CH_2), 4.65 (s, 1 H, 6-H), 4.94 (s, 1 H, 7-H), 5.18 (br s, 1 H, 2-H), 6.52 and 6.55 (2 br s, 1 H, 4-H). Anal. ($C_{20}H_{28}N_2O_7S$) C, H, N.

Acidification of the aqueous layer and extraction with EtOAc as above afforded any unreacted acid 6 which could be reused.

Method C. Alternate DCC/1-Hydroxybenzotriazole Preparation of 7. A solution of 6 (27 g, 94 mmol) and 1-hydroxybenzotriazole hydrate (15.9 g, 118 mmol) in DMF (200 mL) was cooled in an ice bath, and DCC (39 g, 188 mmol) was added portionwise over 5 min. The reaction was stirred at room temperature for 1.5 h and then recooled in an ice bath. L-Proline *tert*-butyl ester[34] (28 g, 165 mmol) was added all at once and the reaction was stirred at room temperature for 2 h. Filtration, aqueous HCl/ether workup, and preparative LC as in method B afforded 29 g (70%) of 7 (R_1R_2 = –CH(*S*)(CO_2-*t*-Bu)(CH_2)$_3$–).

Method D. *m*-CPBA Oxidation of Sulfides 7. 3-(Acetoxymethyl)-2-[[2(*S*)-(*tert*-butoxycarbonyl)pyrrolidino]carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (8; R_1R_2 = –CH(*S*)(CO_2-*t*-Bu)(CH_2)$_3$–). To

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a mixture of 7 (R_1R_2 = –CH(*S*)(CO_2-*t*-Bu)(CH_2)$_3$–) (10.5 g, 24 mmol) in CH_2Cl_2 (700 mL) was added 85% *m*-chloroperbenzoic acid (*m*-CPBA) (14.7 g, 72 mmol). The reaction was stirred at room temperature for 16 h and was then poured into an aqueous $NaHCO_3$ solution containing sodium sulfite (2 g) to destroy any excess *m*-CPBA. The layers were separated, and the organic layer was washed with brine. After the aqueous layers were back-extracted with CH_2Cl_2, the pooled organic layers were dried over Na_2SO_4, and pyridine (5 drops) was added. The solution was stirred for 30 min to isomerize any Δ^3 product completely to Δ^2 product and concentrated in vacuo. The residue was purified by preparative LC (50% EtOAc/hexanes) to give 9.5 g (85%) of 8 (R_1R_2 = –CH(*S*)(CO_2-*t*-Bu)(CH_2)$_3$–). NMR ($CDCl_3$): δ 1.49 and 1.51 (2 s, 9 H, *t*-Bu), 1.9–2.3 (m, 4 H, CH_2CH_2), 2.07 and 2.09 (2 s, 3 H, OAc), 3.4 and 3.7 (2 m, 2 H, NCH_2), 3.57 and 3.60 (2 s, 3 H, MeO), 3.93 (AB q, J = 18 Hz, H, 3′-CH_2), 4.25 and 4.46 (2 dd, J = 8 and 2 Hz and J = 8 and 4 Hz, 1 H, NCH), 4.5–4.9 (2 AB q, J = 12 and 16 Hz, 2 H, 4-CH_2), 4.66 (d, J = 2 Hz, 1 H, 7-H), 5.25 (2 d, J = 2 Hz, 6 H). Anal. ($C_{20}H_{28}N_2O_9S$) C, H, N.

Method E. TFA Deblocking of *tert*-Butyl Esters 8. 3-(Acetoxymethyl)-2-[(2(*S*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (3). To an ice bath cooled solution of TFA (75 mL) and anisole (5 mL) was added 8 (R_1R_2 = –CH(*S*)(CO_2-*t*-Bu)(CH_2)$_3$–) (8.0 g, 17 mmol). The reaction was stirred for 1 h and then evaporated in vacuo at <30 °C. The residue was taken up twice in CH_2Cl_2 and evaporated to remove most of the TFA. The product was then flash chromatographed (80% EtOAc/hexanes, then 1% acetic acid/EtOAc) to afford 6.9 g (97%) of the product 3 as a white foam. NMR ($CDCl_3$): δ 2.08 and 2.11 (2 s, 3 H, $COCH_3$), 1.8–2.4 (m, 4 H, CH_2CH_2), 3.56 and 3.60 (2 s, 3 H, MeO), 3.4–4.0 (m, 2 H, NCH_2), 3.90 (AB q, 2 H, 4-CH_2), 4.4–5.0 (m, 3 H, NCH and 3′-CH_2), 4.90 (br s, 1 H, 6-H), 5.26 (br s, 1 H, 7-H). Anal. ($C_{16}H_{20}N_2O_9S$) C, H, N.

Preparation of 2,8a–j. Compounds 2 and 8a,b,f were previously prepared[13] and were remade as described here. Starting with the sulfide acid 6 and using method B with the appropriate amine or amino acid *tert*-butyl ester,[34] the corresponding sulfide *tert*-butyl esters 7 were prepared and oxidized as in method D. Deesterification with TFA as in method E gave the following new compounds as listed in Table II.

***N*-Methyl-*N*-[1(*S*)-carboxyethyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (8c; R_1 = Me, R_2 = CH(*S*)-(Me)CO_2H).** Partial NMR ($CDCl_3$): δ 1.44 (br d, J = 7 Hz, (*S*)Me), 4.42 and 4.44 (2 s, 3 H, NMe), 4.61 (br t, J = 7 Hz, NCH). Anal.[35] ($C_{19}H_{28}H_2O_9S$) C, H, N.

***N*-Methyl-*N*-[1(*R*)-carboxyethyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (8d; R_1 = Me, R_2 = CH(*R*)-(Me)CO_2H).** Partial NMR ($CDCl_3$): δ 1.44 (br d, J = 7 Hz, (*R*)Me), 4.41 and 4.43 (2 s, 3 H, NMe), 4.60 (br t, J = 7 Hz, NCH). Anal.[35] ($C_{19}H_{28}N_2O_9S$) C, H, N.

3-(Acetoxymethyl)-2-[(2(*R*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (8e; R_1R_2 = –CH(*R*)(CO_2H)(CH_2)$_3$–). Partial NMR ($CDCl_3$): δ 1.9–2.2 (m, 4 H, CH_2CH_2), 3.6–4.2 (part of m, CH and NCH_2). Anal. ($C_{16}H_{20}N_2O_9S$) C, H, N.

***N*-(Methylphenyl)-*N*-(carboxymethyl)-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (8g; R_1 = Bn, R_2 = CH_2CO_2H).** Partial NMR ($CDCl_3$): δ 3.5–3.8 (part of m, NCH_2CO), 4.86 (br s, 2 H, NCH_2Ph), 7.3–7.4 (m, 5 H, C_6H_5). No analysis available.

3-(Acetoxymethyl)-2-[(3(*R,S*)-hydroxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-dioxide (8h; R_1R_2 = –CH_2CHOHCH_2CH_2–) was obtained as a mixture of diastereomers. NMR ($CDCl_3$): δ 1.9–2.2 (m, 2 H, NCH_2CH_2), 2.12 (s, 3H, OAc), 3.4–4.2 (m, 7 H, $NCH_2CHOHCH_2CH_2$ and 3′-CH_2), 3.57 and 3.59 (2 s, 3 H, MeO), 4.4–4.8 (m, 2 H, 4-CH_2), 4.6 (m, 1 H, 7-H), 4.22 and 4.25 (m, 1 H, 6H). Anal. ($C_{15}H_{20}N_2O_8S$) C, H, N.

3-(Acetoxymethyl)-2-[(2(*S*)-carboxy-4(*R*)-hydroxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (8i; R_1R_2 = –CH((*R*)-CO_2H)CH_2CH(*R*-OH)CH_2–). Partial NMR ($CDCl_3$): δ 2.16 (m, 2 H, NCHCH_2), 3.26 (dd, J = 7 and 11 Hz, 1 H, NCH), 3.4–3.8 (part of m, NCH_2-CH). Anal. ($C_{16}H_{20}N_2O_{10}S$) C, H, N.

3-(Acetoxymethyl)-2-[(3(*R,S*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (8j; R_1R_2 = –CH_2CH(*R,S*-CO_2H)CH_2CH_2–). Partial NMR ($CDCl_3$): δ 2.20 (m, 2 H, CHCH_2CH_2), 3.2 (m, 1 H, $CHCO_2$), 3.3–4.0 (part of m, –NCH_2CHCH_2CH_2–). Anal. ($C_{16}H_{20}N_2O_9S$) C, H, N.

***N*-Methyl-*N*-[[[[2-(*tert*-butoxycarbonyl)ethyl]methylamino]carbonyl]methyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (8l; R_1 = Me, R_2 = $CH_2CONMeCH_2CH_2CO_2$-*t*-Bu).** To a solution of 2 (200 mg, 0.52 mmol) in dry dioxane (10 mL) was added *N*-hydroxysuccinimide (75 mg, 0.64 mmol) and DCC (160 mg, 0.78 mmol). The reaction was stirred at room temperature for 30 min before *N*-methyl-β-alanine *tert*-butyl ester (165 mg, 1.04 mmol) was added. The reaction was stirred for another 2 h and then diluted with ether and filtered. The filtrate was washed with 0.5 N HCl (2 mL) and brine. The aqueous layers were reextracted with EtOAc and the organic layers were combined, dried over Na_2SO_4, and evaporated. Flash chromatography (70–80% EtOAc/hexanes) of the residue gave 240 mg (87%) of 8l (R_1 = Me, R_2 = $CH_2CONMeCH_2CH_2CO_2$-*t*-Bu). Partial NMR ($CDCl_3$): δ 1.40 (2 s, 9 H, *t*-Bu), 2.4–2.6 (m, 2 H, CH_2CO_2), 2.8–3.1 (2 m, NMeCH_2CH_2), 2.88, 2.98, and 3.01 (m, 6 H, 2 NMe), 3.4–3.6 (m, 2 H, NCH_2CO).

***N*-Methyl-*N*-[[[(2-carboxyethyl)methylamino]carbonyl]methyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (8k; R_1 = Me, R_2 = $CH_2CONMeCH_2CH_2CO_2H$).** Using method E, 8l (R_1 = Me, R_2 = $CH_2CONMeCH_2CH_2CO_2$-*t*-Bu) (220 mg, 0.41 mmol) was deesterified with TFA to afford 150 mg (76%) of 8k after prep TLC (1% HOAc/EtOAc). Partial NMR (acetone-d_6): δ 2.6–3.1 (2 m, 4 H, CH_2CO_2 and NMeCH_2CH_2), 3.0–3.2 (6 br s, 6 H, 2 NMe), 3.4–3.8 (m, 2 H, NCH_2CO). Anal. ($C_{18}H_{25}N_3O_{10}S$·0.5TFA) C, H, N.

Method F. Preparation of 3′-Alcohols 9. 3-(Hydroxymethyl)-7α-methoxy-8-oxo-2-(pyrrolidinocarbonyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (9; R_1R_2 = –$(CH_2)_4$–). To a solution of 3-(acetoxymethyl)-7α-methoxy-8-oxo-2-(pyrrolidinocarbonyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-dioxide[13] (8f; R_1R_2 = –$(CH_2)_4$–) (2.3 g, 6.2 mmol) (prepared as in method B and D) in 2-propanol (20 mL) under nitrogen was added titanium isopropoxide (4.6 mL, 15.5 mmol) and the reaction was heated to 50 °C for 3 h. It was then poured into ice water (10 mL) containing 2 N HCl (10 mL) and was extracted with 3 portions of EtOAc. The organic layers were each washed with a portion of brine, combined, dried over Na_2SO_4, and evaporated. The residue was flash chromatographed (EtOAc) to afford 1.5 g (74%) of 9 (R_1R_2 = –$(CH_2)_4$–) as a white solid. NMR ($CDCl_3$): δ 1.8–2.0 (m, 4 H, CH_2CH_2), 3.2–3.6 (m, 4 H, $N(CH_2)_2$), 3.56 (s, 3 H, OMe), 3.6–4.2 (2 AB q, 4 H, 4-CH_2 and 3′-CH_2), 4.77 (br s, 1 H, 6-H), 5.19 (d, J = 2 Hz, 1 H, 7-H). Anal. ($C_{13}H_{18}N_2O_6S$) C, H, N.

Method G. Preparation of 3′-Chlorides 10 from 9. 7α-Methoxy-8-oxo-2-(pyrrolidinocarbonyl)-3-(chloromethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (10; R_1R_2 = –$(CH_2)_4$–). A solution of 9 (R_1R_2 = –$(CH_2)_4$–) (500 mg, 1.5 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C, and then pyridine (0.66 mL, 3.0 mmol) and thionyl chloride (0.22 mL, 3.0 mmol) were added. After the reaction was stirred for 30 min, it was poured into ice water containing 2 N HCl (1.5 mL) and extracted with 3 portions of CH_2Cl_2. The organic layers were washed with water and brine, dried over Na_2SO_4, and evaporated. Flash chromatography (70% EtOAc/hexanes) gave 200 mg (38%) of the reactive chloride 10 (R_1R_2 = –$(CH_2)_4$–) which was immediately used in the next step.

Method H. Preparation of 3′-Thio Derivatives 11 from 10. 7α-Methoxy-8-oxo-2-(pyrrolidinocarbonyl)-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (11f; R_1R_2 = –$(CH_2)_4$–, R_3 = $C_4H_4N_3O_2$). The above chloride 10 (R_1R_2 = –$(CH_2)_4$–) (200 mg, 0.57 mmol) was dissolved in acetone (4 mL), and a solution of 1,2,5,6-tetrahydro-5,6-dioxo-3-mercapto-2-

(35) A correct analysis was obtained for the *tert*-butyl ester precursor before deesterification. No analysis is available for the final acid product.

methyl-*as*-triazine[36] (82 mg, 0.52 mmol) and $NaHCO_3$ (84 mg, 1.0 mmol) in water (1.5 mL) was added. The reaction was stirred at room temperature for 16 h. The acetone was removed in vacuo, and the reaction was diluted with water (1 mL) and extracted with 3 portions of EtOAc. The aqueous layer was acidified with 2 N HCl and extracted three times with EtOAc. The organic layers were washed with a portion of brine, pooled, dried over Na_2SO_4, and evaporated to give 210 mg (85%). NMR ($CDCl_3$): δ 1.8–2.1 (m, 4 H, CH_2CH_2), 3.3–3.8 (m, 4 H, $N(CH_2)_2$), 3.58 (s, 3 H, MeO), 3.74 (s, 3 H, NMe), 3.9–4.5 (m, 4 H, 4-CH_2 and 3′-CH_2), 5.25 (d, J = 2 Hz, 1 H, 7-H), 5.35 (br s, 1 H, 6-H). Precipitation from CH_2Cl_2/Et_2O afforded **11f** as a noncrystalline monohydrate. Anal. ($C_{17}H_{21}N_5O_7S\cdot H_2O$) C, H, N.

Method I. Preparation of 3′-Alcohols 12 from 7. *N*-Methyl-*N*-[(*tert*-butoxycarbonyl)methyl]-3-(hydroxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2- and -3-ene-2-carboxamide (12; R_1 = Me, R_2 = CH_2CO_2-*t*-Bu). To a solution of *N*-methyl-*N*-[(*tert*-butoxycarbonyl)methyl]-3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxamide (7, R_1 = Me, R_2 = CH_2CO_2-*t*-Bu) (1.45 g, 3.5 mmol) (prepared as in method B) in 2-propanol (10 mL) was added titanium isopropoxide (1.05 mL, 3.5 mmol) and the reaction was heated to 50 °C. After 2 h the reaction was cooled and diluted with EtOAc and water. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic layers were washed with water and brine, combined, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (50–60% EtOAc/hexanes) to afford 0.48 g (37%) of **12** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu). This material was used directly in the next reaction.

Method J. Preparation of 3′-Chlorides 10 from 12. *N*-Methyl-*N*-[(*tert*-butoxycarbonyl)methyl]-3-(chloromethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (10; R_1 = Me, R_2 = CH_2CO_2-*t*-Bu). To a solution of **12** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu) (0.48 g, 1.3 mmol) in THF (5 mL) was added pyridine (0.35 mL, 0.43 mmol). The solution was cooled in an ice bath and thionyl chloride (0.15 mL, 1.9 mmol) was added. The reaction was stirred for 10 min and diluted with EtOAc and water. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic layers were washed with water, $NaHCO_3$ solution, and brine, combined, dried over Na_2SO_4, and concentrated. The residue was dissolved in CH_2Cl_2 (3 mL) and oxidized with *m*-CPBA (0.52 g, 3.0 mmol) as in method D. Flash chromatography (30–50% EtOAc/hexanes) afforded 87 mg (16%) of **10** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu). Anal. ($C_{16}H_{23}ClN_2O_7S$) C, H, N.

***N*-Methyl-*N*-[(*tert*-butoxycarbonyl)methyl]-7α-methoxy-8-oxo-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (11d; R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = $C_4H_4N_3O_2$).** A sample of **10** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu) (3.79 g, 8.97 mmol) (prepared as in method G or J) was reacted as in method H to afford 4.0 g (45%) of **11d** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = $C_4H_4N_3O_2$). NMR (acetone-d_6): δ 1.48 (s, 9 H, *t*-Bu), 3.06 (br s, 1 H, OH), 3.15 (s, 3 H, CONMe), 3.55 and 3.56 (2 s, 3 H, MeO), 3.73 and 3.75 (2 s, 3 H, SCNMe), 3.9–4.5 (m, 6 H, 4-CH_2, 3′-CH_2, NCH_2), 5.26 (d, J = 2 Hz, 1 H, 7-H), 5.38 (br s, 1 H, 6-H). Anal. ($C_{20}H_{27}N_5O_9S_2\cdot 0.5H_2O$) C, H, N.

Method K. TFA Deesterification of *tert*-Butyl Esters 11. *N*-Methyl-*N*-(carboxymethyl)-7α-methoxy-8-oxo-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (11e; R_1 = Me, R_2 = CH_2CO_2H, R_3 = $C_4H_4N_3O_2$). To a cooled solution of TFA (35 mL) and anisole (5 mL) was added **11d** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = $C_4H_4N_3O_2$) (3.7 g, 6.8 mmol). After 1 h the TFA was removed in vacuo and the residue was taken up in $NaHCO_3$ solution and washed with ether. The aqueous layer was acidified with 1.2 N HCl in the presence of EtOAc, the layers were separated, and the aqueous layer was reextracted with EtOAc. The organic layers were washed with water and brine, combined, dried over Na_2SO_4, and evaporated. The residue was crystallized with difficulty from EtOAc/ether to give 1.2 g (37%) of **11e** (R_2 = Me, R_2 = CH_2CO_2H, R_3 = $C_4H_4N_3O_2$). NMR (acetone-d_6): δ 3.10 (AB q, J = 10 Hz, 2 H, NCH_2CO), 3.19 and 3.55 (2 s, 3 H, CONMe), 3.58 and 3.59 (2 s, 3 H, SCNMe), 3.73 and 3.75 (2 s, 3 H, MeO), 3.8–4.5 (m, 4 H, 4-CH_2 and 3′-CH_2), 5.26 and 5.28 (2 br s, 1 H, 7-H), 5.39 and 5.46 (2 br s, 1 H, 6-H). The dipotassium salt was formed by addition of an equivalent amount of K_2CO_3 to an acetone/water solution of **11e**, concentration, and lyophilization. The residue was precipitated from acetone/acetonitrile to afford a hygroscopic solid. Anal. ($C_{16}H_{18}N_5O_9S_2K_2\cdot 4H_2O$) C, H, N.

Preparation of 11a–c,g,h. Using the same methods as for the preparation of **11e** or **11f**, the following compounds were prepared.

***N*-Methyl-*N*-(carboxymethyl)-7α-methoxy-8-oxo-3-[[(1-methyltetrazol-5-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide 5,5-Dioxide (11a; R_1 = Me, R_2 = CH_2CO_2H, R_3 = SCN_4-1-Me).** Partial NMR (acetone-d_6): δ 3.03 and 3.10 (2 s, CONMe), 4.0–4.4 (part of m, NCH_2CO), 4.04 (s, 3 H, CN_4Me). No analysis available.

***N*-Methyl-*N*-[(*t*-butoxycarbonyl)methyl]-7α-methoxy-8-oxo-3-[[[1-(carboxymethyl)tetrazol-5-yl]thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (11b; R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = SCN_4-1-CH_2CO_2H).** Partial NMR (acetone-d_6): δ 1.49 (s, 9 H, *t*-Bu), 3.03 and 3.08 (2 s, CONMe), 4.0–4.4 (part of m, NCH_2CO), 5.32 (s, 2 H, CN_4CH_2). Anal. ($C_{19}H_{26}N_6O_9S_2\cdot 0.7Et_2O$) C, H, N. Presence of Et_2O was confirmed by NMR.

***N*-Methyl-*N*-(carboxymethyl)-7α-methoxy-8-oxo-3-[(2-pyridylthio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (11c; R_1 = Me, R_2 = CH_2CO_2H, R_3 = SC_5H_4N).** Partial NMR (acetone-d_6): δ 3.05 and 3.15 (2 s, CONMe), 3.8–4.6 (part of M, NCH_2CO), 7.18 (m, 1 H, 3-H), 7.38 (m, 1 H, 5-H), 7.69 (dt, J = 2 and 8 Hz, 1 H, 4-H), 8.50 (br d, J = 3 Hz, 1 H, 6-H). Anal. ($C_{17}H_{19}N_3O_7S_2\cdot 0.75$TFA salt) C, H, N.

2-[(2(*S*)-Carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (11g; R_1, R_2 = CH(*S*-CO_2H)$(CH_2)_3$, R_3 = $C_4H_4N_3O_2$). Partial NMR (acetone-d_6): δ 1.8–2.4 (2 m, 4 H, CH_2CH_2), 3.5–3.7 (m, 2 H, NCH_2), 3.70 (s, 3 H, NMe), 4.1–4.5 (part of m, 1 H, NCH). Anal.[35] ($C_{22}H_{29}N_5O_9S_2$) C, H, N.

***N*-Methyl-*N*-(2-carboxyethyl)-7α-methoxy-8-oxo-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (11h; R_1 = Me, R_2 = $CH_2CH_2CO_2H$, R_3 = $C_4H_4N_3OS$).** Partial NMR (acetone-d_6): δ 2.85 (t, J = 6 Hz, 2 H, CH_2CO), 3.03 (t, J = 6 Hz, 2 H, NCH_2), 3.12 (s, 3 H, NMe), 3.73 (s, 3 H, SCNMe). Anal. ($C_{17}H_{21}N_5O_9S_2\cdot 1.5$AcOH) C, H, N. Presence of AcOH was confirmed by NMR.

Method L. Acylation of 3′-Alcohol 12. *N*-Methyl-*N*-[(*tert*-butoxycarbonyl)methyl]-3-[[[[(phenylmethyl)amino]carbonyl]oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (13; R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = $OCONHCH_2Ph$). To a solution of **12** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu) (300 mg, 0.80 mmol) (prepared as in method I) in CH_2Cl_2 (2 mL) was added benzyl isocyanate (0.35 mL, 2.8 mmol) and a catalytic amount of DMAP. The reaction was heated to reflux for 2 h and then diluted with CH_2Cl_2 and washed with $NaHCO_3$ solution, water, and brine. After the aqueous layers were reextracted with CH_2Cl_2, the combined layers were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (50% EtOAc/hexanes) to give 400 mg (98%) of the sulfide product as a mixture of isomers. This was used directly in the *m*-CPBA oxidation as in method D and after flash chromatography (50–60% EtOAc/hexanes) afforded 374 mg (88%) of **13** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = $OCONHCH_2Ph$). NMR (acetone-d_6): δ 1.47 (s, 9 H, *t*-Bu), 3.01 and 3.09 (2 s, 3 H, NMe), 3.56 and 3.58 (2 s, 3 H, MeO), 3.7–4.5 (m, 6 H, 4-CH_2, 3′-CH_2, NCH_2), 4.58 and 4.71 (2 br s, 2 H, CH_2Ph), 5.26 (d, J = 2 Hz, 1 H, 7-H), 5.38 (br s, 1 H, 6-H), 6.8–7.1 (2 br s, 1 H, NH), 7.2–7.4 (m, 5 H, C_6H_5).

***N*-Methyl-*N*-(carboxymethyl)-3-[[[[(phenylmethyl)amino]carbonyl]oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (13b; R_1 = Me, R_2 = CH_2CO_2H, R_3 = $OCONHCH_2Ph$).** The deesterification of **13** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu, R_3 = $OCONHCH_2Ph$) (175 mg, 0.33 mmol) was done as in method E,

(36) Rhone-Poulanc Industries S. A., U. K. Patent Appl. 2051788A, 1981; *Chem. Abstr.* 1981, *95*, 97816*t*.

affording 121 mg (77%) of **13b** (R_1 = Me, R_2 = CH_2CO_2H, R_3 = $OCONHCH_2Ph$) after flash chromatography (1% HOAc/EtOAc). NMR (acetone-d_6): δ 3.03 and 3.09 (2 br s, 3 H, NMe), 3.56 and 3.59 (2 s, 3 H, MeO), 3.7–4.5 (m, 6 H, 4-CH_2, 3′-CH_2, NCH_2), 4.6–4.9 (m, 2 H, CH_2Ph), 5.27 (d, J = 2 Hz, 1 H, 7-H), 5.39 and 5.47 (2 br s, 1 H, 6-H), 6.9–7.2 (2 br s, 1 H, NH), 7.2–7.45 (m, 5 H, C_6H_5). Anal. ($C_{20}H_{23}N_3O_9S\cdot0.5H_2O$) C, H, N.

Method M: Acylation of 3′-Alcohol 9. *N*-Methyl-*N*-(phenylmethyl)-3-[[[[4-(*tert*-butoxycarbonyl)phenyl]carbonyl]oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (13; R_1 = Me, R_2 = CH_2Ph, R_3 = OCC_6H_4-4-CO_2-*t*-Bu). To a solution of *N*-methyl-*N*-(phenylmethyl)-3-(hydroxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-dioxide (**9**; R_1 = Me, R_2 = CH_2Ph) (220 mg, 0.58 mmol) in CH_2Cl_2 (10 mL) was added pyridine (150 μL, 1.8 mmol) and 4-(*tert*-butoxycarbonyl)benzoyl chloride (300 mg, 1.2 mmol). The reaction was stirred for 1 h, diluted with CH_2Cl_2, and poured into ice water containing 1 mL of 2 N HCl. The layers were separated, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated. Flash chromatography (40–50% EtOAc/hexanes) afforded 230 mg (68%) of **13** (R_1 = Me, R_2 = CH_2Ph, R_3 = OCC_6H_4-4-CO_2-*t*-Bu). Partial NMR ($CDCl_3$): δ 1.62 (s, 9 H, *t*-Bu), 2.88 and 2.92 (2 s, 3 H, NMe), 4.7–4.9 (part of m, CH_2Ph), 7.15–7.4 (m, 5 H, C_5H_6), 7.9–8.1 (m, C_6H_4).

***N*-Methyl-*N*-(phenylmethyl)-3-[[[(4-carboxyphenyl)carbonyl]oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (13c; R_1 = Me, R_2 = CH_2Ph, R_3 = $OCPh$-4-CO_2H).** The TFA deesterification of **13** (R_1 = Me, R_2 = CH_2Ph, R_3 = OCC_6H_4-4-CO_2-*t*-Bu) (210 mg, 0.36 mmol) was done as in method E. Preparative TLC (1% AcOH/EtOAc) afforded 150 mg (80%) of **13c** (R_1 = Me, R_2 = CH_2Ph, R_3 = OCC_6H_4-4-CO_2H). Partial NMR (acetone-d_6): δ 2.94 and 2.98 (2 s, 3 H, NMe), 7.2–7.4 (m, 5 H, C_6H_5), 8.0–8.2 (m, 4 H, C_6H_4). Anal. ($C_{25}H_{24}N_2O_9S\cdot1.7TFA$) C, H, N.

***N*-Methyl-*N*-(carboxymethyl)-3-[[(phenylcarbonyl)oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (13a; R_1 = Me, R_2 = CH_2CO_2H, R_3 = OCOPh).** After **12** (R_1 = Me, R_2 = CH_2CO_2-*t*-Bu) was acylated with benzoyl chloride and oxidized as in method L, deesterification with TFA as in method E afforded **13a** (R_1 = Me, R_2 = CH_2CO_2H, R_3 = OCOPh). Partial NMR (1:1, $CDCl_3$/acetone-d_6): δ 2.99 and 3.02 (2 s, 3 H, NMe), 3.6–4.0 (m, 2 H, NCH_2), 7.2–7.4 (m, 5 H, C_6H_5). Anal. ($C_{19}H_{20}N_2O_9S$) C, H, N.

3-(Acetoxymethyl)-7α-methoxy-2-(morpholinocarbonyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5α-Oxide (14). To a 0 °C solution of 3-(acetoxymethyl)-7α-methoxy-2-(morpholinocarbonyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2- and -3-ene[13] (**7**; R_1R_2 = $(CH_2CH_2)_2O$) (prepared as in method B) (120 mg, 0.34 mmol) in CH_2Cl_2 (10 mL) was added *m*-CPBA (75 mg, 0.34 mmol). The reaction was stirred for 1 h and then a drop of pyridine was added. After 15 min the reaction was concentrated and preparative TLC of the residue (EtOAc) afforded 30 mg (25%) of the α-sulfoxide **14** as the lower R_f product. NMR ($CDCl_3$): δ 2.08 (s, 3 H, OAc), 3.22 (half of AB q, br, J = 18 Hz, 1 H, 4-αH), 3.4–4.0 (m, 9 H, 4-βH, $(CH_2CH_2)_2O$), 3.60 (s, 3 H, MeO), 4.43 (br s, 1 H, 6-H), 4.64 (br AB q, J = 20 Hz, 3′-CH_2), 5.03 (d, J = 2 Hz, 7-H). Anal. ($C_{15}H_{20}N_2O_7S$) C, H, N.

***N*-Methyl-*N*-(carboxymethyl)-3-(acetoxymethyl)-7α-ethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (16a).** By starting with *tert*-butyl 3-(acetoxymethyl)-7α-ethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate[12] (**15**) and using the same methods as for the preparation of **3**, **16a** was prepared. NMR ($CDCl_3$): δ 1.11 (t, J = 8 Hz, 3 H, CH_2CH_3), 1.8–2.1 (m, 2 H, CH_2CH_3), 2.12 (s, 3 H, OAc), 3.1–3.2 (m, 4 H, NMe and 7-H), 3.6–4.1 (m, 4 H, 4-CH_2 and 3′-CH_2), 4.5–4.9 (m, 3 H, NCH_2 and 6-H). Anal.[35] ($C_{19}H_{28}N_2O_8S$) H, N; C: calcd 51.34; found 51.82.

***N*-Methyl-*N*-(carboxymethyl)-7α-ethyl-8-oxo-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (16b).** By starting with **15** and using the same methods as for the preparation of **11e**, **16b** was prepared. NMR (acetone-d_6): δ 1.08 (2 t, J = 8 Hz, 3 H, CH_2CH_3), 1.8–2.1 (m, 2 H, CH_2CH_3), 3.0–3.2 (m, 4 H, NMe and 7-H), 3.5–4.5 (m, 6 H, NCH_2, 4-CH_2 and 3′-CH_2), 3.72 (s, 3 H, SCNMe). Anal.[35] ($C_{21}H_{29}N_5O_8S_2\cdot1.3H_2O$) C, H, N.

***N*-Methyl-*N*-(carboxymethyl)-3-(acetoxymethyl)-7α-ethyl-4-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide 5,5-Dioxide (18).** By starting with *tert*-butyl 3-(acetoxymethyl)-7α-ethyl-4-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate[15] (**17**) and using the same methods as for the preparation of **2** or **3**, **18** was prepared. NMR ($CDCl_3$): δ 2.10 and 2.16 (2 s, 3 H, OAc) 2.22 (br s, 3 H, 4-CH_3), 3.04 and 3.42 (2 s, 3 H, NMe), 3.58 (s, 3 H, MeO), 4.21 (AB q, J = 20 Hz, 2 H, 3′-CH_2), 4.5–5.0 (m, 3 H, 4-H, NCH_2), 5.26 and 5.30 (2 br s, 1 H, 7-H), 5.50 and 5.70 (2 br s, 1 H, 6-H). No analysis was obtained.

3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid 5,5-Dioxide (19b). The TFA-catalyzed deesterification was done as in method A, but without using anisole as an isobutylene trap. A portion of TFA (200 mL) was cooled to 10 °C and *tert*-butyl 3-(acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide[12,21] (**19a**) (50 g, 0.133 mol) was added slowly, under N_2, as a powder at such a rate that the temperature remained at 20–25 °C. After the reaction was complete by HPLC (1–3 h), the TFA was evaporated in vacuo under 30 °C. Portions of CH_2Cl_2 (100 mL) were added and evaporated to ~60 mL six times to remove most of the TFA. The residual oil contained 42.5 g of acid **19b** and 1.5 equiv of TFA as determined by acid titration.

3-(Acetoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carbonyl Chloride 5,5-Dioxide (19c). The above acid **19b** was diluted to 450 mL with CH_2Cl_2 and a catalytic amount of DMF (0.4 mL, 0.005 mol) was added. The mixture was cooled and maintained at 15–20 °C as oxalyl chloride (25.6 mL, 0.294 mol) was added over 15 min. Stirring was continued at this temperature until the reaction was complete by NMR. The reaction was concentrated in vacuo to 200 mL and again twice diluted with CH_2Cl_2 and evaporated to 200 mL. This solution of acid chloride **19c** was diluted to 360 mL with CH_2Cl_2 and cooled to −15 °C before immediate use in the next reaction.

3-(Acetoxymethyl)-2-[[2(*S*)-(*tert*-butoxycarbonyl)pyrrolidino]carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene 5,5-Dioxide (20). A solution of proline *tert*-butyl[34] ester (32.0 g, 0.187 mol) in CH_2Cl_2 (400 mL) was cooled to 0 °C under N_2, and triethylamine (26 mL, 0.187 mol) was added slowly while being further cooled to −15 °C. With continued cooling, the above solution of acid chloride **19c** was added as rapidly as possible while the temperature was maintained below 25 °C. After an additional 15 min, the reaction was washed twice with cold 1% v/v phosphoric acid (2 × 225 mL) and twice with cold 1% w/v monobasic potassium phosphate (2 × 225 mL) and then dried over Na_2SO_4. HPLC analysis of the final CH_2Cl_2 solution indicated 54.2 g (86%) of product **20**, which was used directly in the next reaction.

3-(Acetoxymethyl)-2-[(2(*S*)-carboxypyrrolidino)carbonyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 5,5-Dioxide (3). The above solution of **20** was concentrated to a thick oil (~0.115 mol), placed under N_2, and cooled to 0–5 °C. With slow mechanical stirring, TFA (200 mL) was added in one portion and maintained at 20–25 °C for 1–4 h. The reaction was then concentrated in vacuo at <30 °C to 75 mL and EtOAc (200 mL portions) was added and reconcentrated six times to remove most of the residual TFA. The residue was then eluted with EtOAc through a bed of silica gel (8–9 g/g of oil). The product fractions were concentrated to give 46.8 g (99%) of crude **3**.

Crystallization of 3. Crude **3** (40.0 g, 96 mmol) was slurried in EtOAc (1 L) and heated at reflux until it was in solution. Darco KB (10 g) was slowly added and the hot solution was aged for 30 min and filtered. The filtrate was concentrated in vacuo to ~280 mL. The solution was seeded, allowed to cool to 20–25 °C for 12 h, and aged another 24 h at 0–5 °C. The solid was filtered and dried to afford 34.0 g (85%) of **3**, mp 160–161 °C, which was microjet milled[20] to a mean particle size of 1.1 μm. Anal. ($C_{16}H_{20}N_2O_9S$) C, H, N.

Hydrolysis Studies in pH 8 MOPS Buffer. Samples of the test compound (3–5 mg) were dissolved at 1 mg/mL in MOPS buffer (adjusted to pH 8 with 5 N NaOH) and incubated in a constant temperature water bath at 25 °C in a capped vial.

Periodically, depending on the half-life, 50-μL aliquots were removed and quenched into 50 μL of 2% aqueous TFA. The amount of compound left was determined by HPLC analysis using a Zorbax ODS column (4.6 mm × 15 cm) eluted with 1% TFA/10% MeOH in acetonitrile/water. The integrated areas and time were fit to the first-order decay curve $F(t) = A/2^{(t/t_{1/2})}$, where $F(t)$ is the peak area at time t, A is the calculated initial area, and $t_{1/2}$ is the calculated half-life. The reported errors are the standard deviation of the experimental points.

X-ray Structure Determination for 3. $C_{16}H_{20}N_2O_9S$, M_r = 416.41, monoclinic, $P2_1$, a = 8.752 (5) Å, b = 11.294 (3) Å, c = 10.504 (4) Å, β = 113.07 (4)°, V = 955 A^3, Z = 2, D_x = 1.448 g cm^{-3}, monochromatized radiation λ(Cu K$_\alpha$) = 1.54184 A, μ = 1.94 mm^{-1}, $F(000)$ = 436, T = 296 K. Data were collected[37] on an Enraf-Nonius CAD4 diffractometer to a 2θ limit of 115° with 1356 observed, $I > 3\sigma(I)$, reflections out of 1491 measured. The structure was solved by direct methods using SHELXS-86[38] and refined using full-matrix least-squares on F. Data were collected for absorption effects.[39] Final agreement statistics are R = 0.039, w_R = 0.062, S = 2.86, (Δ/σ_{max}) = 0.003. Weighting scheme is $1/\sigma^2(F)$. Maximum peak height in final difference Fourier map 0.20 (4) eA^{-3} had no chemical significance. All calculations were performed on a Sun Microsystems computer using SDP-Plus[40] software.

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Supplementary Material Available: A listing of complete crystal data, experimental conditions, positional and thermal parameters for all atoms, selected interatomic distances and angles, selected weighted least-squares planes, and an ORTEP plot with the atomic numbering scheme (6 pages). Ordering information is given on any current masthead page.

(37) The diffractometer programs are those supplied by Enraf-Nonius for operating the CAD4F diffractometer with some local modifications and additions.

(38) Sheldrick, G. M. *SHELXS*-86. In *Crystallographic Computing 3*; Sheldrick, G. M., Kruger, C., Goddard, R., Eds.; Oxford University Press: 1985, pp 175–189.

(39) Empirical correction was based on the absorption surface method of Walker and Stuart; see: Walker, N.; Stuart, D. An Empirical Method for Correcting Diffractometer Data for Absorption Effects. *Acta Crystallogr.* **1983**, *A39*, 158–166. The maximum and minimum correction coefficients applied to F_o were 1.1757 and 0.6965, respectively.

(40) *Structure Determination Package Version 3.* Enraf-Nonius, Delft: The Netherlands, 1985.