Inhibition of Human Leukocyte Elastase. 3. Synthesis and Activity of 3'-Substituted Cephalosporins

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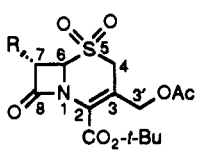
Several 3'-substituted cephalosporin sulfones were synthesized from 3-(hydroxymethyl)cephalosporin, which was prepared by $Ti(OiPr)_4$ hydrolysis of the corresponding acetate. A method was also developed to prepare a 3-vinylcephalosporin. Some of these compounds were found to be potent time-dependent inhibitors of human leukocyte elastase (HLE). The HLE inhibitory activity was correlated with σ_1 and it was concluded that the potency was determined by the electron-withdrawing ability as well as the size of the substituent. A mechanism for inhibition of HLE by cephalosporin sulfones is proposed.

Human leukocyte elastase (HLE, EC 3.4.21.37) has been implicated in a number of connective-tissue diseases.[1] It has been suggested that HLE is the principal destructive agent of structural proteins in these diseases, and inhibitors of HLE may be of therapeutic benefit.[2] A number of natural as well as synthetic inhibitors of HLE have been reported.[3] We recently demonstrated that cephalosporin antibiotics can be modified to produce time-dependent inhibitors of HLE.[4] Since then, we have begun a program to study the effect of substitution on the cephalosporin nucleus in order to find a potent and specific inhibitor of HLE. The preceding two papers discussed the effects of substitution at the 7-[5] and 2-position.[6] In this paper we report on the activity of the 3'-substituted cephalosporins. The 4-substituted analogues are being disclosed in another paper.[7]

From our initial work[4] it was established that the free carboxyl group of the cephalosporin nucleus had to be masked for activity against HLE. Also in contrast to the antibiotics, the enzyme preferred a small α-oriented substituent such as a methoxy or a chloro in the 7-position and oxidation of the sulfide to a sulfone resulted in substantial improvement in inhibitory activity. Thus, 1,1-dimethylethyl 3-[(acetyloxy)methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (**1a**) and the corresponding 7α-chloro compound (**1b**)



1a: R = OCH_3
b: R = Cl

were established as important early lead structures. Mechanistic studies with 1 have shown that the active site serine of HLE is acylated by the β-lactam and further modification of the acyl-enzyme is possible.[8] The large amount of work done in the cephalosporin antibiotics area has shown that the 3'-substituent has a strong influence on the reactivity of the β-lactam ring.[9] Thus the steric interaction with the enzyme as well as the electron-withdrawing ability and the leaving ability of the 3'-side chain may influence the activity of HLE.

Results and Discussion

Chemistry. A series of analogues of 1 were prepared where the 3'-substituent was varied while keeping the 7α-substituent as methoxy, the 2-substituent as a *t*-Bu ester, and the sulfur in the sulfone oxidation state. The readily available *tert*-butyl 7α-methoxycephalosporinate (**2**), prepared in two steps from the commercially available 7-aminocephalosporanic acid (7-ACA),[5] was used as a starting material for further modification. Our strategy for the preparation of 3'-substituted cephalosporins is outlined in Scheme I. The double bond of the thiazine ring can be readily isomerized upon heating with a mild base like triethylamine. At equilibrium 3-ene isomer **3** predominates in a 3/1 ratio, which is separated from **2** by chromatography. Treatment of **3** with titanium(IV) isopropoxide ($Ti(OiPr)_4$),[10] either at 50 °C for 2 h or overnight at room temperature, resulted in the hydrolysis of the acetate without affecting the β-lactam or the *t*-Bu ester and furnished alcohol **4** in 60–65% isolated yield after chromatography. This reaction is specific for 3-ene isomer **3**, since 2-ene isomer **1** gave γ-lactone **13a** as the only observed product. Ester derivatives of **3** other than *t*-Bu were not stable to $Ti(OiPr)_4$. When a benzyl ester was used in this reaction it was converted to an isopropyl ester.

3-(Hydroxymethyl)cephalosporin **4** was a key intermediate for further modifications. Treatment of **4** with thionyl chloride furnished a very reactive chloride **5** in 65%

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(2) Snider, G. L. *Drug. Dev. Res.* **1987**, *10*, 235.
(3) For a review, see: Trainor, D. A. *Trends Pharm. Sci.* **1987**, *8*, 303.
(4) Doherty, J. B.; Ashe, B. M.; Argenbright, L. W.; Barker, P. L.; Bonney, R. J.; Chandler, G. O.; Dahlgren, M. E.; Dorn, C. P.; Finke, P. E.; Firestone, R. A.; Fletcher, D.; Hagmann, W. K.; Mumford, R. A.; O'Grady, L.; Maycock, A. M.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Zimmermann, M. *Nature* **1986**, *322*, 192.
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(6) Finke, P. E.; Ashe, B. M.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; Shah, S. K.; Thompson, K. R.; Underwood, D. J.; Weston, H.; Zimmerman, M.; Doherty, J. B. *J. Med. Chem.*, second of three papers in this issue.
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(9) (a) Indelicato, J. M.; Norvilas, T. T.; Pfeiffer, R. R.; Wheeler, W. J.; Wilham, W. L. *J. Med. Chem.* **1974**, *17*, 523. (b) Narisada, M. *Pure Appl. Chem.* **1987**, *59*, 459.
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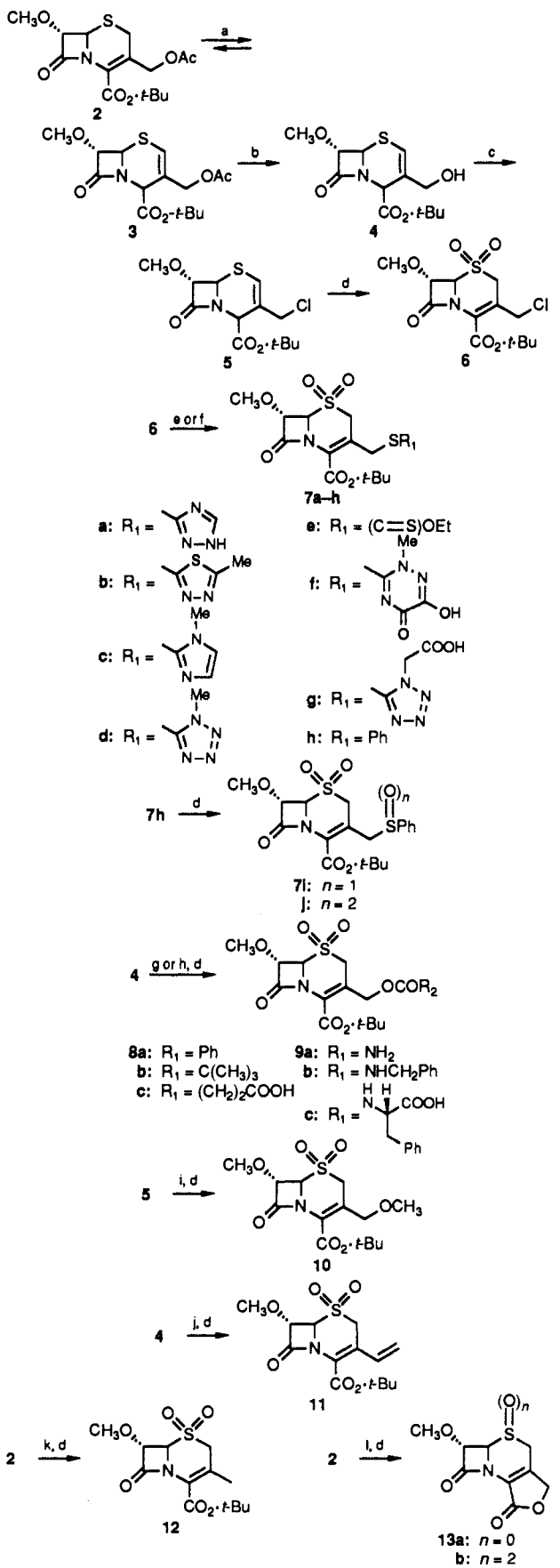
Scheme I[a]



[a] Reagents: (a) $Et_3N/CHCl_3$; (b) $Ti(OiPr)_4/i$-PrOH; (c) $SOCl_2$/pyridine/THF; (d) m-CPBA/CH_2Cl_2; (e) $R_1S^-K^+$/acetone–water; (f) R_1SH/base/DMF; (g) R_1COCl/pyridine/CH_2Cl_2; (h) carbonyldiimidazole/CH_2Cl_2; R_1H/DMAP/DMF; (i) MeOH; (j) Ph_3PHBr/MeCN; $CH_2O/Na_2CO_3/CH_2Cl_2$–water; (k) H_2/10% Pd–C/EtOH; (l) TFA; 2 N HCl/dioxane.

yield. Oxidation of **5** with 2 equiv of *m*-chloroperoxybenzoic acid (*m*-CPBA) was accompanied by a spontaneous rearrangement of the double bond to give 2-ene isomer **6**. It is known that the 2-ene isomer is thermodynamically more stable for cephem sulfoxide or sulfone, whereas the 3-ene isomer is favored for the sulfide.[11] The double bond isomerization and oxidation of sulfur make **6** much more stable than **5** to solvolysis. Displacement of the chloride in **6** by aryl mercaptans furnished thioethers. Some thioethers (**7a–e**) were prepared by reacting a salt of the mercaptan with **6**, but in other cases (**7f–h**) the mercaptan and **6** were allowed to react in the presence of a base. Thus the reaction occurred readily when **6** was stirred with thiophenol and *N,N*-diisopropylethylamine to give **7h**. Oxidation of **7h** with 1.5 equiv of *m*-CPBA gave a mixture of a sulfoxide (**7j**) and sulfone (**7i**), which were easily separated by chromatography.

Ester analogues **8a–c** were prepared by acylation of alcohol **4** followed by oxidation. Parent carbamate **9a** was synthesized by reaction of **4** with chlorosulfonyl isocyanate, followed by hydrolysis and oxidation with *m*-CPBA. Benzylcarbamate **9b** was prepared by reacting **4** with benzyl isocyanate and oxidation. A more complex acyl derivative **9c** was prepared by first reacting **4** with carbonyldiimidazole and treatment of the resulting acylimidazole with L-phenylalanine *p*-methoxybenzyl (PMB) ester.[12] Oxidation of the sulfide and selective removal of the PMB ester protecting group by brief treatment with cold trifluoroacetic acid gave **9c**. Ether **10** was prepared by solvolysis of **5** in methanol followed by oxidation with *m*-CPBA.

When **4** was treated with triphenylphosphine hydrobromide[13] in acetonitrile, a phosphonium salt was formed. Wittig reaction of the crude salt with aqueous formaldehyde in a two-phase system gave the 3-vinyl compound as a mixture of double bond isomers. Oxidation of this mixture to sulfone was accompanied by double bond isomerization to furnish **11**.

3-Methyl analogue **12** was prepared by hydrogenolysis of **2** following the procedure developed by Stedmann[14] and oxidation. Finally, lactone **13b** was prepared by following a literature procedure.[15]

Biochemical Activity. The HLE inhibitory activities of the synthesized compounds are listed in Table I. Initially the potency was measured as an IC_{50} (μM) value, the concentration of the inhibitor required to produce a 50% reduction in the activity of 1 μg/mL of HLE at 2 min after mixing. Upon longer incubation, most compounds showed a time-dependent inhibition. Therefore, in order to get a better understanding of this kinetic inactivation process and to estimate the relative potency of each compound, a second-order rate constant ($k_{obs}/[I]$, M^{-1} s^{-1}) was determined by following the inactivation of HLE by inhibitor for 15 min. For compounds **10** and **7j**, which did not show time-dependent inhibition, K_i was calculated from the initial velocity. These compounds appear to be simple competitive inhibitors, but they could be slow substrates or very inefficient inactivators.

The data in Table I show that the elastase inhibitory activity can be substantially improved by varying the 3′-

(11) Webber, J. A.; Van Heyningen, E. M.; Vasileff, R. T. *J. Am. Chem. Soc.* **1969**, *91*, 5674.
(12) Stelakatos, G. C.; Argyropoulos, N. *J. Chem. Soc. C* **1970**, 964.
(13) Hercouret, A.; Le Corre, M. *Tetrahedron Lett.* **1979**, 2145.
(14) Stedmann, R. J.; Swered, K.; Hoover, J. R. E. *J. Med. Chem.* **1964**, *7*, 117.
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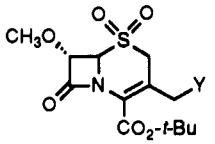
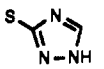
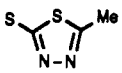
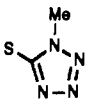
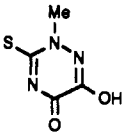
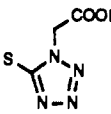
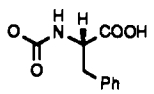
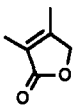
Table I. Elastase Inhibitory Activity of 3′-Substituted Cephalosporins



no.	Y	σ_I[a]	IC_{50},[b] μM	$k_{obs}/[I]$,[c] M^{-1} s^{-1} (SD)
1	OAc	0.38	1.33	12700 (2400)
6	Cl	0.47	0.43	14900 (400)
7a			2.40	3500 (100)
7b		0.47	2.23	7900 (1500)
7c			2.33	1880 (60)
7d		0.53	1.62	15000 (400)
7e	S(C=S)OEt	0.42	1.82	4300 (300)
7f			0.63	8600 (500)
7g			ND	3700 (300)
7h	SPh	0.31	0.94	ND
7i	SOPh	0.51	0.91	16800 (1600)
7j	SO_2Ph	0.56	0.44	K_i = 0.22 μM (0.002)
8a	OCOPh	0.43	ND	32500 (4000)
8b	$OCOC(CH_3)_3$		ND	15800 (60)
8c	$OCO(CH_2)_2COOH$		ND	13800 (300)
9a	$OCONH_2$		ND	10800 (1300)
9b	$OCONHCH_2Ph$		ND	15100 (200)
9c			0.13	63900 (4000)
10	OMe	0.27	1.00	K_i = 1.6 μM (0.07)
11	$=CH_2$		ND	6095 (20)
12	H	0.0	3.15	1630 (300)
13b			57.8	46 (14)

[a] Values taken from refs 16 and 17. [b] Method reported in ref 4. [c] Determined by method described in ref 5. ND = not determined.

substituent (Y). Compounds such as **8a** and **9c** with large substituents in this position have the best activity. These experimental results are consistent with the model we have proposed[5,6] for the binding of cephalosporin inhibitors to HLE. In this model, the β-lactam carbonyl is at the active site with the oxygen pointing toward the backbone NH of Ser-195 and Gly-193 ("oxyanion" hole), the OH of Ser-195 interacts with the carbonyl carbon, and the 7α-substituent occupies the S1 binding site.[5] The 3-substituent is in a relatively open area and the enzyme can easily accommodate a large group in this position. Thus the activity increases with the size of the substituent as seen from the series 1, **8b**, and **8a**. Unlike the 2-substituent, presence of an acidic (compounds **7f**, **7g**, and **8c**) or a basic (compounds **7a**, **7c**, **7d**, and **7e**) function in this position is tolerated by HLE. Thus, there does not seem to be specific hydrogen-bonding or electrostatic interaction between Y and the enzyme. The lack of activity of **13b** may indicate that the conformation where the 3′-substituent is in the plane and syn to the 2-substituent (cis-planer) is not favorable for activity.

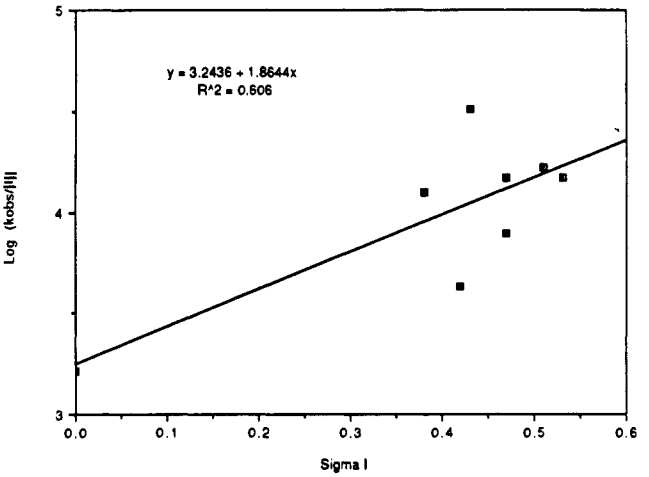


Figure 1. Plot of log ($k_{obs}/[I]$) vs σ_I.

Early mechanism studies[8] have shown that Ser-195 of HLE reacts with 1 to form an acyl–enzyme intermediate. From previous work[9] on the cephem antibiotics, the 3′-substituent is known to influence the reactivity of the β-lactam ring and it was expected that the electron-withdrawing ability of Y would affect the activity. Qualitatively, this seems to be true (compare compounds **7b** and **7d**, **10** and **6**). In order to more accurately assess the electronic effect of Y on HLE activity, the rate of inactivation ($k_{obs}/[I]$) was correlated with the inductive substituent constant σ_I,[16,17] and the results are shown in Figure 1. The least square line can be expressed as

$$\log (k_{obs}/[I]) = 1.864\sigma_I + 3.244 \qquad n = 8, r = 0.78$$

Thus the inductive electron-withdrawing effect can explain about 60% of the variance (r^2) in $k_{obs}/[I]$. It is interesting to note that, in Figure 1, the point farthest above the line represents **8a**, where Y is a large substituent, and the point farthest below the line represents **7e**, with a small substituent. If these two points are excluded from the regression, the best fit line

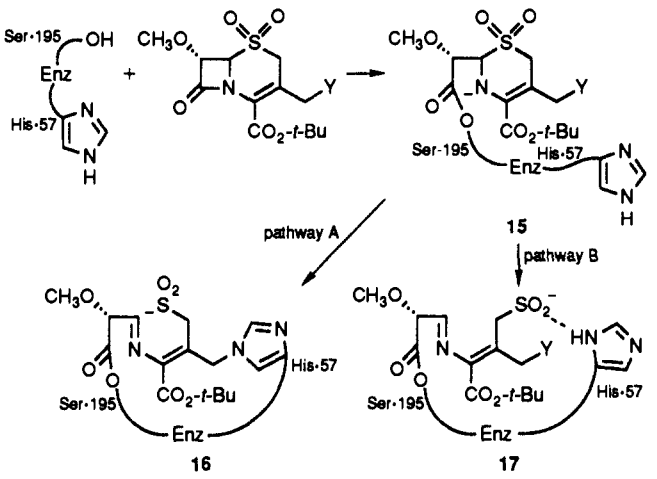
$$\log (k_{obs}/[i]) = 1.831\sigma_I + 3.244 \qquad n = 6, r = 0.95$$

is essentially unchanged but the correlation coefficient improves to 0.95. Thus, it seems that the inhibitory activity is determined by electronic as well as steric effects. This is quite different from the cephalosporin antibiotics where the in vitro antibacterial activity was found to be parabolically related to σ_I of the 3-substituent.[17a] However, the rate of alkaline hydrolysis of the same set of compounds was linearly correlated to σ_I.[17]

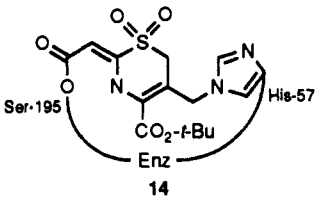
The activity is only slightly improved when Y is a very good leaving group such as chloro (compare 1 and 6). Thus the ability of Y as a leaving group has only a small effect on activity. Even more importantly, as seen from compounds 11 and 12, a leaving group is not required in order to have a time-dependent inhibitor. This observation has

(16) Charton, M. *Prog. Phys. Org. Chem.* **1987**, *16*, 287.
(17) (a) Boyd, D. B. *J. Med. Chem.* **1984**, *27*, 63. (b) Nishikawa, J.; Tori, K. *J. Med. Chem.* **1984**, *27*, 1657.

Scheme II



important implications for the mechanism of inactivation. A crystal structure (14) for a related serine protease,



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procine pancreatic elastase (PPE), inhibited by *tert*-butyl 7α-chlorocephalosporinate (**1b**) was reported from this laboratory and a "double-hit" mechanism for inhibition of PPE by this cephalosporin was proposed.[8] However, as we discussed in the first paper of this series, a different mechanism may operate for 7α-methoxycephalosporins[5] and it is outlined in Scheme II. After initial binding the hydroxyl of the Ser-195 reacts with the β-lactam carbonyl to form an acyl–enzyme intermediate **15**. Further modification of **15** can lead to either **16** or **17**. When Y is a good leaving group, pathway A is possible, where a second covalent bond is formed between the 3′-position of the inhibitor and His-57 of the enzyme, as depicted in **16**. This mechanism is analogous to that proposed for the inhibition of PPE by **1b**.[8] However, since a 3-methyl analogue (**11**) also leads to time-dependent inhibition, pathway A leading to **16** cannot be the only mechanism of inactivation. In such cases where Y is not a leaving group, pathway B may be operative, where the dihydrothiazine ring is opened to liberate a sulfinic acid. A salt bridge between the sulfinate and the enzyme (probably His-57) or a conformational change could then stabilize the inhibited species (**17**). Pathway B is similar to the mechanism proposed by Knowles[18] for the inactivation of β-lactamase by a penicillin sulfone. A detailed crystal structure for HLE inhibited by a 7α-methoxycephalosporin may be able to resolve this mechanistic question, but such data are not available at present.

A number of commercially important cephalosporin antibiotics have been developed by varying the 3′-substituent. In these cases the 3′-substituent not only enhances the antibacterial activity but also provides very desirable pharmacodynamic properties like cell-wall penetration and tissue distribution to the drug molecule.[19] The results reported here show that the 3′-substituent does have a strong effect on the in vitro HLE inhibitory activity. We are currently evaluating these compounds in animal models of tissue destruction to see whether the 3′-substituent has analogous beneficial effects on in vivo activity. The results of these investigations will be published separately.[20]

Experimental Section

General Procedures. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra of $CDCl_3$ solutions were recorded on a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts are reported as δ values relative to tetramethylsilane as internal standard. IR spectra of either neat liquids or dilute $CHCl_3$ solutions were obtained on a Perkin-Elmer 295 or a Perkin-Elmer 1310 spectrophotometer. Mass spectra were obtained with an LKB 9000 at an ionizing voltage of 70 eV. Flash chromatography was performed on silica gel (E. Merck, 0.04–0.063 mm). Thin-layer chromatography (TLC) and preparative thick-layer chromatography (prep TLC) were carried out on precoated Analtech silica gel GF plates. Visualization was done with UV light, iodine vapor, or ceric ammonium sulfate. Preparative liquid chromatography (prep LC) was performed on a Waters Prep LC500 instrument with silica gel (Prep Pak) columns. When elemental analyses are indicated only by symbols of the elements, the analytical results obtained for these elements are within 0.4% of the theoretical values.

1,1-Dimethylethyl 3-[(Acetyloxy)methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (3). A solution of 7.46 g (21.7 mmol) of 1,1-dimethylethyl 3-[(acetyloxy)methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (**2**)[5] in 90 mL of chloroform and 3.3 mL (23.6 mmol) of triethylamine was heated to reflux. After 3 h, NMR analysis of an aliquot showed that there was a mixture of 3-ene and 2-ene isomers in an approximate ratio of 3/1. The reaction mixture was concentrated in vacuo. The residue was purified by prep LC (solvent 25% EtOAc/hexane). The forecut of the partially resolved material was taken and evaporated to give 2.41 g (32%) of pure 3 as a yellow oil: NMR δ 1.50 (s, 9 H), 3.52 (s, 3 H), 4.6 (AB q, 2 H, J = 13 Hz), 4.65 (d, 1 H, J = 2 Hz), 5.05 (br s, 1 H), 6.45 (br s, 1 H). Collection of the remainder of the material and evaporation yielded 3.66 g (49%) of a 1/1 mixture of 2-ene and 3-ene isomers as a yellow oil which may be reused in the reaction.

1,1-Dimethylethyl 3-(Hydroxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (4). To a solution of 8.8 g (25.9 mmol) of 3 in 100 mL of 2-propanol was added 5.4 mL (18.1 mmol) of $Ti(OiPr)_4$.[10] The reaction mixture was heated to 50 °C under N_2 and monitored by TLC (50% EtOAc/hexane, starting material R_f 0.75, product R_f 0.5) until the starting material had just disappeared. The reaction mixture was then concentrated and the residue was partitioned between EtOAc and 1 N H_3PO_4. The aqueous layer was then back-washed with EtOAc. The organic layers were combined and washed with water and then saturated NaCl. The solution was dried and the filtrate was concentrated. The residue was purified by prep LC (solvent 33% EtOAc/hexane) to give 4.76 g (61%) of **4** as a light yellow oil which solidified on standing: NMR δ 1.50 (s, 9 H), 2.60 (br s, 1 H)8 3.53 (s, 3 H), 4.22 (br s, 2 H), 4.60 (d, 1 H, J = 1 Hz), 4.99 (d, 1 H, J = 1 Hz), 5.02 (s, 1 H) 6.32 (m, 1 H). Anal. ($C_{13}H_{19}NO_5S$·0.1H_2O) C, H, N.

1,1-Dimethylethyl 3-(Chloromethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (5). To a solution of 0.9 g (3 mmol) of **4** in 20 mL of THF was added 1 mL of pyridine. Thionyl chloride (0.5 mL, 6.8 mmol) was added dropwise over 5 min. After stirring of the reaction mixture for 0.5 h, it was poured into ice-cold water and extracted with EtOAc. The combined extract was washed with saturated $NaHCO_3$ and saturated NaCl and dried. The concentrated filtrate was purified

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by flash chromatography with 10% EtOAc/hexane to yield 0.626 g (65%) of **5** as a pale yellow solid: mp 85 °C; NMR δ 1.5 (s, 9 H), 3.53 (s, 3 H), 4.22 (AB q, 2 H, J = 11 Hz), 4.58 (s, 1 H), 4.96 (s, 1 H), 5.05 (br s, 1 H), 6.38 (br s, 1 H). Anal. ($C_{13}H_{18}ClNO_4S$) C, H, N.

1,1-Dimethylethyl 3-(Chloromethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (6). A solution of 0.4 g (85%, 2 mmol) of *m*-chloroperoxybenzoic acid (*m*-CPBA) in 3 mL of CH_2Cl_2 was added to a solution of 0.32 g (1 mmol) of **5** in 5 mL of CH_2Cl_2. After stirring of the solution overnight, it was poured into saturated $NaHCO_3$ containing excess Na_2SO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated NaCl and dried. The residue obtained after concentration was crystallized from 20% EtOAc/hexane to afford 0.301 g (86%) of **6**: NMR δ 1.53 (s, 9 H), 3.53 (s, 3 H), 3.87 (AB q, 2 H, J = 17 Hz), 4.33 (AB q, 2 H, J = 11 Hz), 4.62 (br s, 1 H), 5.07 (br s, 1 H). Anal. ($C_{13}H_{18}ClNO_6S·0.5H_2O$) C, H, N.

1,1-Dimethylethyl 7α-Methoxy-3-[[(1-methyltetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (7d). A solution of 35 mg (0.1 mmol) of **6** in 1 mL of acetone was added to a solution of 18 mg (0.1 mmol) of potassium 5-mercapto-1-methyltetrazole[21] in 0.1 mL of water and 1 mL of acetone. After stirring of the solution overnight, it was concentrated. The residue was partitioned between saturated $NaHCO_3$ and EtOAc. The EtOAc solution was washed with saturated NaCl and dried. The solid obtained after concentration of the filtrate was recrystallized from EtOAc/hexane to furnish 42 mg (97%) of **7d** as a white solid. NMR δ 1.59 (s, 9 H), 3.54 (s, 3 H), 3.91 (s, 3 H), 4.12 (br s, 2 H), 4.26 (AB q, 2 H, J = 14 Hz), 4.62 (br s, 1 H), 5.07 (d, 1 H, J = 2 Hz). Anal. ($C_{15}H_{21}N_5O_6S_2$) C, H, N.

The following compounds were prepared in an analogous manner.

1,1-Dimethylethyl 7α-methoxy-8-oxo-3-[(1*H*-1,2,4-triazol-3-ylthio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (7a): NMR δ 1.54 (s, 9 H), 3.52 (s, 3 H), 3.6–4.4 (m, 4 H), 4.61 (br s, 1 H), 5.04 (d, 1 H, J = 2 Hz), 8.07 (s, 1 H). Anal. ($C_{15}H_{20}N_4O_6S_2·H_2O$) C, H, N.

1,1-Dimethylethyl 7α-methoxy-3-[[(2-methyl-1,3,4-thiadiazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate 5,5-dioxide (7b): NMR δ 1.57 (s, 9 H), 2.73 (s, 3 H), 3.8–4.5 (m, 4 H), 4.63 (br s, 1 H), 5.07 (d, 1 H, J = 2 Hz). Anal. ($C_{16}H_{21}N_3O_6S_3·0.1C_4H_8O$) C, H, N. Presence of EtOAc was confirmed by NMR.

1,1-Dimethylethyl 7α-methoxy-3-[[(1-methylimidazol-2-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (7c): NMR δ 1.53 (s, 9 H), 3.55 (s, 3 H), 3.63 (s, 3 H), 3.6–4.5 (m, 4 H), 4.67 (br s, 1 H), 5.06 (d, 1 H, J = 2 Hz), 6.9 (br s, 1 H), 6.96 (br s, 1 H). Anal. ($C_{17}H_{23}N_3O_6S_2·0.5H_2O$) C, H, N.

1,1-Dimethylethyl 3-[[(ethoxythioxomethyl)thio]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (7e): NMR δ 1.41 (t, 3 H, J = 7 Hz), 1.57 (s, 9 H), 3.54 (s, 3 H), 3.6–4.5 (m, 4 H), 4.57 (br s, 1 H), 4.61 (q, 2 H, J = 7 Hz), 5.04 (d, 1 H, J = 2 Hz). Anal. ($C_{16}H_{23}NO_7S_3$) C, H, N.

1,1-Dimethylethyl 7α-Methoxy-3-[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-*as*-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (7f). A solution of 32 mg (0.2 mmol) of 1,2,5,6-tetrahydro-5,6-dioxo-3-mercapto-2-methyl-*as*-triazine[22] in 1 mL of water was prepared by adding 35 mg (0.42 mmol) of $NaHCO_3$. A solution of 70 mg (0.2 mmol) of **6** in 2 mL of acetone was added. After stirring of the reaction mixture overnight, it was concentrated. The residue was partitioned between saturated $NaHCO_3$ and ether. The ether layer was extracted with saturated $NaHCO_3$. The aqueous layers were combined and washed with ether. The aqueous layer was acidified to pH 2 in the presence of EtOAc with concentrated HCl. The layers were separated, and the aqueous layer was extracted with EtOAc. The EtOAc layer was washed with saturated NaCl and dried. The filtrate was concentrated and the residue was crystallized from EtOAc/ether to obtain 76 mg (80%) of **7f** as a light yellow solid: NMR δ 1.58 (s, 9 H), 3.58 (s, 3 H), 3.77 (s, 3 H), 3.8–4.5 (m, 5 H), 4.74 (br s, 1 H), 5.18 (br s, 1 H). Anal. ($C_{17}H_{22}N_4O_8S_2·0.5H_2O$) C, H. N.

1,1-Dimethylethyl 3-[[[1-(carboxymethyl)tetrazol-5-yl]-thio]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate 5,5-dioxide (7g) was prepared by a similar procedure: NMR δ 1.58 (s, 9 H), 3.56 (s, 3 H), 3.86 (d, 1 H, J = 18 Hz), 4.05 (d, 1 H, J = 14 Hz), 4.22 (d, 1 H, J = 14 Hz), 4.6 (d, 1 H, J = 14 Hz), 4.75 (br s, 1 H), 5.16 (d, 1 H, J = 2 Hz), 5.16 (AB q, 2 H, J = 18 Hz); MS m/e 476 (MH^+), 345, 273, 232, 217.

1,1-Dimethylethyl 7α-Methoxy-3-[(phenylsulfonyl)-methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (7j) and 1,1-Dimethylethyl 7α-Methoxy-3-[(phenylsulfinyl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (7i). To a solution of 0.85 g (2.42 mmol) of **6** in 20 mL of dimethylformamide (DMF) was added 1 mL (5.7 mmol) of diisopropylethylamine. After 5 min, 0.5 mL (4.8 mmol) of thiophenol was added. The solution was stirred under nitrogen for 1.5 h. The reaction mixture was poured into saturated $NaHCO_3$ and extracted with EtOAc. The EtOAc solution was washed with 1.2 N HCl and saturated NaCl and dried. The filtrate was concentrated and the residue was chromatographed on a flash column with 20% EtOAc/hexane to obtain 0.58 g (56%) of 1,1-dimethylethyl 7α-methoxy-3-[(phenylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (**7h**): NMR δ 1.45 (s, 9 H), 3.46 (s, 3 H), 3.58 (d, 1 H, J = 14 Hz), 3.62 (d, 1 H, J = 18 Hz), 4.08 (d, 1 H, J = 18 Hz), 4.18 (d, 1 H, J = 14 Hz), 4.4 (br s, 1 H), 5.02 (d, 1 H, J = 2 Hz), 7.1–7.4 (m, 5 H).

To a solution of 0.38 g (0.89 mmol) of **7h** in 5 mL of CH_2Cl_2 was added 0.28 g (1.37 mmol) of *m*-CPBA in 3 mL of CH_2Cl_2. After 1 h the reaction mixture was poured into saturated $NaHCO_3$ containing excess Na_2SO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated NaCl and dried. The filtrate was concentrated and the residue was purified by prep TLC using 50% EtOAc/hexane to obtain two products. The product obtained from the faster band was crystallized from CH_2Cl_2/hexane to yield 0.253 g (62%) of **7j** as a white solid: mp 131–132 °C; NMR δ 1.46 (s, 9 H), 3.52 (s, 3 H), 3.81 (d, 1 H, J = 14 Hz), 3.82 (d, 1 H, J = 18 Hz), 4.32 (d, 1 H, J = 18 Hz), 4.74 (d, 1 H, J = 14 Hz), 4.76 (br s, 1 H), 5.09 (d, 1 H, J = 2 Hz), 7.3–7.9 (m, 5 H). Anal. ($C_{19}H_{23}NO_8S_2$) C, H, N. The product from the slower band was triturated with CH_2Cl_2/hexane to furnish 84 mg (21%) of **7i** as a white solid: mp 128–129 °C; NMR δ 1.51 (s, 9 H), 3.53 (s, 3 H), 3.83 (br s, 2 H), 4.05 (br s, 2 H), 4.61 (br s, 1 H), 5.07 (d, 1 H, J = 2 Hz), 7.3–7.9 (m, 5 H). Anal. ($C_{19}H_{23}NO_7S_2$) C, H, N.

1,1-Dimethylethyl 3-[(Benzoyloxy)methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (8a). To a solution of 0.3 g (1 mmol) of **4** in 5 mL of CH_2Cl_2 were added 0.16 mL (2 mmol) of pyridine and 0.175 mL (1.5 mmol) of benzoyl chloride. After stirring for 4 h, the reaction mixture was concentrated and 2 mL of 30% EtOAc/hexane was added to the residue. The precipitated pyridine salt was filtered and the filtrate was chromatographed on a flash column (solvent 30% EtOAc/hexane) to obtain 0.42 g (100%) of 1,1-dimethylethyl 3-[(benzoyloxy)methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-3-ene-2-carboxylate: NMR δ 1.49 (s, 9 H), 3.54 (s, 3 H), 4.61 (s, 1 H), 4.89 (AB q, 2 H, J = 14), 5.0 (br s, 1 H), 5.04 (s, 1 H), 6.46 (br s, 1 H), 7.2–8.2 (m, 5 H). Oxidation of the above sulfide as described for the preparation of **6** furnished **8a**: NMR δ 1.56 (s, 9 H), 3.6 (s, 3 H), 3.81 (d, 1 H, J = 18 Hz), 4.12 (d, 1 H, J = 18 Hz), 4.77 (s, 1 H), 4.95 (d, 1 H, J = 14 Hz), 5.23 (s, 1 H), 5.39 (d, 1 H, J = 14 Hz), 7.4–8.4 (m, 5 H). Anal. ($C_{20}H_{23}NO_8S$) C, H, N.

The following compounds were prepared by a similar procedure.

1,1-Dimethyl 3-[[(dimethylpropionyl)oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (8b): NMR δ 1.22 (s, 9 H), 1.56 (s, 9 H), 3.58 (s, 3 H)8 3.65 (d, 1 H, J = 18 Hz), 3.96 (d, 1 H, J = 18 Hz), 4.70 (br s, 1 H), 4.73 (d, 1 H, J = 14 Hz), 5.05 (d, 1 H, J = 14 Hz), 5.17 (d, 1 H, J = 2 Hz). Anal. ($C_{18}H_{27}NO_8S$) C, H, N.

1,1-Dimethylethyl 3-[[(3-carboxypropionyl)oxy]-methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (8c): NMR δ 1.56 (s, 9 H), 2.7

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(m, 4 H), 3.57 (s, 3 H), 3.69 (d, 1 H, J = 18 Hz), 4.03 (d, 1 H, J = 18 Hz), 4.74 (d, 1 H, J = 14 Hz), 4.74 (br s, 1 H), 5.12 (d, 1 H, J = 14 Hz), 5.17 (d, 1 H, J = 2 Hz). Anal. ($C_{17}H_{23}NO_{10}S$) C, H, N.

1,1-Dimethylethyl 3-[[(Aminocarbonyl)oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (9a). To a solution of 0.1 g (0.33 mmol) of 4 in 1 mL of CH_2Cl_2 and 0.14 mL of Et_3N was added 0.1 mL (1.15 mmol) of chlorosulfonyl isocyanate. After 15 min, 2 mL of water was added. After 1 h the reaction mixture was diluted with CH_2Cl_2. It was washed with 1.2 N HCl and saturated NaCl and dried. The filtrate was concentrated to 2 mL and 0.15 g (0.73 mmol) of *m*-CPBA was added. The solution was stirred overnight and then poured into saturated $NaHCO_3$ containing 3 drops of saturated Na_2SO_3. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl and dried. The concentrated filtrate was chromatographed on a flash column using 50–75% EtOAc/hexane to isolate 18 mg (15%) of **9a**: NMR δ 1.56 (s, 9 H), 3.57 (s, 3 H), 3.87 (AB q, 2 H, J = 18 Hz), 4.69 (d, 1 H, J = 14 Hz), 4.7 (d, 1 H, J = 2 Hz), 4.8 (br s, 2 H), 5.03 (d, 1 H, J = 14 Hz), 5.17 (d, 1 H, J = 2 Hz). Anal. ($C_{14}H_{20}N_2O_8S \cdot 0.5H_2O$) C, H, N.

1,1-Dimethylethyl 3-[[[[(phenylmethyl)amino]carbonyl]oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (9b) was prepared by a similar procedure in 61% yield: NMR δ 1.55 (s, 9 H), 3.56 (s, 3 H), 3.71 (d, 1 H, J = 18 Hz), 3.98 (d, 1 H, J = 18 Hz), 4.36 (m, 2 H) 4.68 (s, 1 H), 4.71 (d, 1 H, J = 14 Hz), 5.05 (d, 1 H, J = 14 Hz), 5.1 (br s, 1 H), 5.16 (s, 1 H), 7.2–7.4 (m, 5 H). Anal. ($C_{21}H_{26}N_2O_8S$) C, H, N.

1,1-Dimethylethyl 3-(1-Carboxy-2-phenylethyl)-1-L-[[(aminocarbonyl)oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (9c). To a solution of 0.620 g (2.06 mmol) of 4 in 3 mL of CH_2Cl_2 was added 0.367 g (2.26 mmol) of 1,1′-carbonyldiimidazole. After stirring for 0.5 h the reaction mixture was concentrated. The residue was dissolved in 5 mL of DMF and 0.963 g (3 mmol) of L-phenylalanine 4-methoxybenzyl ester[12] was added. When all the solid had dissolved, 0.366 g (3 mmol) of 4-(dimethylamino)pyridine (DMAP) was added. The solution was stirred overnight. It was diluted with water and extracted with ether. The etheral extract was washed with water and saturated NaCl and dried. The filtrate was concentrated and the residue was purified by prep TLC using 50% EtOAc/hexane to isolate 0.752 g (58%) of 1,1-dimethylethyl 3[1-[[[(4-methoxyphenyl)methyl]oxy]carbonyl]-2-phenylethyl]-1-L-[[(aminocarbonyl)oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate as a yellow oil: NMR δ 1.48 (s, 9 H), 2.9–3.3 (m, 2 H), 3.52 (s, 3 H), 4.4–5.2 (m, 8 H), 6.3 (br s, 1 H), 6.7–7.4 (m, 9 H).

A solution of 0.752 g (1.2 mmol) of the above sulfide in 10 mL of CH_2Cl_2 was treated with 0.515 g (2.5 mmol) of *m*-CPBA. After stirring of the solution overnight, it was poured into saturated $NaHCO_3$ containing excess Na_2SO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated NaCl and dried. The residue obtained after concentration of the filtrate was purified by prep TLC using 50% EtOAc/hexane to obtain 0.734 g (93%) of 1,1-dimethylethyl 3-[1-[[[(4-methoxyphenyl)methyl]oxy]carbonyl]-2-phenylethyl]-1-L-[[(aminocarbonyl)oxy]methyl]-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide: NMR δ 1.56 (s, 9 H), 2.9–3.3 (m, 2 H), 3.58 (s, 3 H), 3.73 (s, 3 H), 3.6–4.0 (m, 2 H), 4.4–5.2 (m, 7 H), 6.8–7.4 (m, 9 H).

Cold trifluoroacetic acid (15 mL) and anisole (3 mL) were added to 0.734 g (1.14 mmol) of the sulfone obtained above. The flask was cooled in an ice bath and stirring was continued for 2 min after all the solids had dissolved. The solution was rapidly concentrated in vacuo. The residual yellow oil was purified by prep TLC using 50% EtOAc/hexane with 1% HOAc to obtain 0.156 g (26%) of **9c**: NMR δ 1.56 (s, 9 H), 2.9–3.3 (m, 2 H), 3.58 (s, 3 H), 3.78 (AB q, 2 H, J = 17 Hz), 4.6–5.3 (m, 4 H), 5.18 (d, 1 H, J = 2 Hz), 7.1–7.4 (m, 5 H). Anal. ($C_{23}H_{28}N_2O_{10}S$) C, H, N.

1,1-Dimethylethyl 3-(Methoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (10). A solution of 30 mg (0.09 mmol) of 4 in 1 mL of methanol was stirred at room temperature for 16 h. The solution was concentrated and the residue was chromatographed on a flash column with 20% EtOAc/hexane to obtain 21 mg of 1,1-dimethylethyl 3-(methoxymethyl)-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate: NMR δ 1.48 (s, 9 H), 3.25 and 3.39 (2 s, 3 H), 3.95 (AB q, 1.5 H, J = 11 Hz), 4.5–5.3 (m, 4 H), 6.23 (br s, 0.5 H). Following the procedure described for the preparation of **6**, 21 mg of the above sulfide was oxidized to obtain 11 mg (34%) of **10**: NMR δ 1.53 (s, 9 H), 3.27 (s, 3 H), 3.53 (s, 3 H) 3.72 (AB q, 2 H, J = 17 Hz), 4.14 (s, 2 H), 4.6 (br s, 1 H), 5.05 (d, 1 H, J = 2 Hz). Anal. ($C_{14}H_{21}NO_7S \cdot 0.5H_2O$) C, H, N.

1,1-Dimethylethyl 3-Ethenyl-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (11). To a solution of 0.6 g (2 mmol) of **4** in 10 mL of MeCN was added 0.686 g (2 mmol) of triphenylphosphine hydrobromide.[13] The solution was heated to reflux under N_2. After 1 h the solution was cooled and concentrated. The residue was triturated with ether. The white solid formed was filtered and washed with cold ether. The dry solid was dissolved in 10 mL of CH_2Cl_2 and 3 mL of formaldehyde (36% in water) was added. The aqueous layer was adjusted to pH 9 by adding a 10% Na_2CO_3 solution. After stirring for 2 h, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water and saturated NaCl and dried. The filtrate was concentrated and the residue was purified by flash chromatography using 20–30% EtOAc/hexane to furnish 0.144 g (25%) of 1,1-dimethylethyl 3-ethenyl-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate: NMR δ 1.51 (s, 9 H), 3.6 (s, 3 H), 3.9 (m, 1 H), 4.3–4.7 (m, 3 H), 5.08 (d, 1 H, J = 10 Hz), 5.31 (d, 1 H, J = 16 Hz), 6.51 (dd, 1 H, J = 16, 10 Hz). Following the procedure outlined for the preparation of **6**, 0.144 g of the above sulfide was oxidized to obtain 34 mg (22%) of **11**: NMR δ 1.52 (s, 9 H), 3.62 (s, 3 H), 4.02 (d, 1 H, J = 14 Hz), 4.41 (d, 1 H, J = 14 Hz), 4.9 (d, 1 H, J = 2 Hz), 5.24 (d, 1 H, J = 2 Hz), 5.49 (d, 1 H, J = 10 Hz), 5.71 (d, 1 H, J = 16 Hz), 6.58 (dd, 1 H, J = 16, 10 Hz). Anal. ($C_{14}H_{19}NO_6S \cdot 0.67H_2O$) C, H, N.

1,1-Dimethylethyl 7α-Methoxy-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-Dioxide (12). To a solution of 5 g (14 mmol) of **2** in 100 mL of ethanol was added 15 g of 10% palladium on carbon under a N_2 atmosphere. The solution was hydrogenated on a Parr apparatus for 3 h.[14] The reaction mixture was filtered and the catalyst was thoroughly washed with warm methanol. The filtrate and washings were combined and concentrated. The residue was purified by flash chromatography using 20% EtOAc/hexane to obtain 1.9 g (47%) of 1,1-dimethylethyl 7α-methoxy-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate: NMR δ 1.53 (s, 9 H), 2.01 (s, 3 H), 3.27 (AB q, 2 H, J = 18 Hz), 3.52 (s, 3 H), 4.4 (d, 1 H, J = 2 Hz), 4.6 (d, 1 H, J = 2 Hz). Following the procedure described for the preparation of **6**, 0.464 g (1.45 mmol) of 1,1-dimethylethyl 7α-methoxy-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was oxidized to obtain 0.393 g (76%) of **12**: NMR δ 1.53 (s, 9 H), 2.0 (s, 3 H), 3.6 (AB q, 2 H, J = 12 Hz), 4.54 (br s, 1 H), 5.05 (br s, 1 H). Anal. ($C_{13}H_{19}NO_6S$) C, H, N.

5a,6-Dihydro-6α-methoxy-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione 5,5-Dioxide (13b). Cold trifluoroacetic acid (2 mL) was added to 0.21 g (0.61 mmol) of **2**. The solution was cooled in an ice bath. After 0.5 h the solution was concentrated in vacuo. The residue was partitioned between water and CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated NaCl and dried. The filtrate was concentrated. The residue was dissolved in 2 mL of dioxane and 1 mL of 2N HCl was added.[15] After stirring overnight the reaction mixture was neutralized with saturated $NaHCO_3$. The solution was extracted with EtOAc. The EtOAc layer was washed with saturated NaCl and dried. The residue obtained after concentration of the filtrate was chromatographed by prep TLC (solvent 50% EtOAc/hexane) to isolate 52 mg (38%) of 5a,6-dihydro-6α-methoxy-3*H*,7*H*-azeto[2,1-*b*]-furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione (**13a**): NMR δ 3.56 (s, 3 H), 3.8 (m, 2 H), 4.58 (d, 1 H, J = 2 Hz), 4.67 (d, 1 H, J = 2 Hz), 4.88 (br s, 2 H). Following the procedure described for the preparation of **6**, 42 mg (0.18 mmol) of **13a** was oxidized to furnish 16 mg (33%) of **13b**: mp 242–243 °C dec; NMR δ 3.6 (s, 3 H), 4.54 (AB q, 2 H, J = 17 Hz), 5.06 (AB q, 2 H, J = 18 Hz), 5.26 (br s, 1 H), 5.34 (d, 1 H, J = 2 Hz). Anal. ($C_9H_9NO_6S$) C, H, N.

Registry No. 2, 95570-83-5; **3**, 95570-99-3; **4**, 116536-03-9; **5**,

95571-06-5; **6**, 95672-05-2; **7a**, 116561-60-5; **7b**, 116561-63-8; **7c**, 116561-62-7; **7d**, 116561-66-1; **7e**, 116561-61-6; **7f**, 116536-09-5; **7g**, 116536-10-8; **7h**, 95672-06-3; **7i**, 116561-67-2; **7j**, 95672-07-4; **8a**, 127792-62-5; **8a** (sulfide), 127792-51-2; **8b**, 127792-52-3; **8c**, 95672-02-9; **9a**, 127792-53-4; **9b**, 127792-54-5; **9c**, 127792-55-6; **9c** (sulfide; (*p*-methoxyphenyl)methyl ester), 127792-56-7; **9c** ((*p*-methoxyphenyl)methyl ester), 127792-57-8; **10** (sulfide), 95570-51-7; **10**, 95671-88-8; **11** (sulfide), 127792-58-9; **11**, 127792-59-0; **12** (sulfide), 95570-84-6; **12**, 95671-89-9; **13a**, 127792-60-3; **13b**, 127792-61-4; HS(C=S)OEt, 151-01-9; HSPh, 57658-36-3; L-$H_2NCH(CH_2Ph)C(O)OC_6H_4$-*p*-OMe, 47171-46-0; elastase, 9004-06-2; 5-mercapto-1-methyltetrazole, 13183-79-4; 1,2,5,6-tetrahydro-5,6-dioxo-3-mercapto-2-methyl-*as*-triazine, 58909-39-0; 5-mercapto-1,3,4-triazole, 3179-31-5; 2-methyl-5-mercapto-1,3,4-thiadiazole, 29490-19-5; 2-mercapto-1-methylimidazole, 60-56-0; 5-mercapto-1-tetrazolylacetic acid, 57658-36-3.

Aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum-(II) Complexes with Variable Substituents in the 2-Phenyl Ring. 1. Synthesis and Antitumor and Estrogenic Properties

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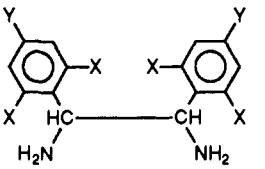
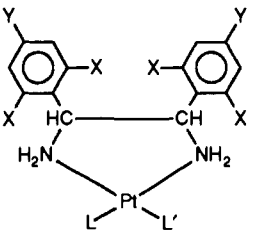
Erythro- and threo-configurated aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) complexes with variable substituents in the 2-phenyl ring (**2**-$PtSO_4$ to **9**-$PtSO_4$: H, 4-F, 3-OH, 4-OH, 2,6-F_2, 2,6-Cl_2, 2-F/4-OH, 2-Cl/4-OH) were synthesized and tested for estrogenic and antitumor activities. The ligands were obtained by a three-step reaction. The stilbenes were reacted with a mixture of IN_3 and NaN_3 to yield the respective 1,2-diazido-1,2-diphenylethanes. The subsequent reduction with $LiAlH_4$ led to the corresponding 1,2-diphenylethylenediamines. The (sulfato)platinum(II) complexes were synthesized by reaction of Ag_2SO_4 with the diiodo complexes, which had been obtained by coordination of the diamines with K_2PtI_4. Two complexes, *erythro*-**8**-$PtSO_4$ and *erythro*-**9**-$PtSO_4$, possess antitumor and estrogenic effects and are therefore of interest for the therapy of breast cancer.

[*meso*-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-1-$PtCl_2$) shows a strong and specific effect on estrogen receptor positive (ER^+) mammary carcinoma (MC) models, e.g. on the MXT, ER^+-MC of the mouse.[1,13] This effect is determined by the ligand *meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (*meso*-**1**) which possesses a marked estrogenic activity. The exchange of *meso*-**1** by its diastereomeric diamine *d,l*-**1** leads to a complex (*d,l*-1-$PtCl_2$) which, in contrast to *meso*-1-$PtCl_2$, shows neither MC-inhibiting nor estrogenic properties.[1] At equimolar dosage *meso*-1-$PtSO_4$, a water soluble derivative of *meso*-1-$PtCl_2$, is significantly more active on the 9,10-dimethyl-1,2-benzanthracene (DMBA) induced, hormone-dependent MC of the Sprague-Dawley (SD) rat than its ligand *meso*-**1** (0.5×10^{-5} M/kg, sc, three times per week, duration of therapy 4 weeks; % change of tumor area: *meso*-1-$PtSO_4$, −84%; *meso*-**1**, +129%; cisplatin, −38%; control, +420%). The complex *meso*-1-$PtSO_4$ is also superior to cisplatin,[1] which in the therapy of metastatic breast cancer does not lead to convincing results.[2] In the DMBA–MC experiment we found a 22-fold enrichment of *meso*-1-$PtSO_4$ in the tumor compared with the skeletal muscle.[1] These results support a mode of action which needs an intact ER system (i.e. an intact cytoplasm nucleus translocation process). According to this concept platinum complexes that contain an ER-affinic ligand are supposed to be accumulated in the nuclei of hormone-dependent breast cancer cells by the receptor system, thereby causing a stronger cytotoxic effect (due to the PtLL'–residue) than non-estrogenic platinum complexes. In contrast to the very promising results on the MXT, ER^+-MC *meso*-1-$PtCl_2$ does not cause an inhibition of the hormone-independent MXT-MC.[1] It cannot be excluded that the estrogenic properties of *meso*-1-$PtCl_2$ are partially responsible for its effect on the hormone-dependent MC, since high-dosed estrogens also evoke MC-inhibiting properties.[3]



1: X = Y = H
meso-**1**: X = Cl; Y = OH
d,l-1: X = Cl; Y = OH



1-PtLL': X,Y = H; L,L' = variable leaving groups
meso-1-$PtCl_2$: X = Cl; Y = OH; L,L' = Cl
d,l-1-$PtCl_2$: X = Cl; Y = OH; L,L' = Cl
meso-1-$PtSO_4$: X = Cl; Y = OH; L = OH_2^+; L' = $O–SO_3^-$

Platinum(II) complexes, which show marked activities not only on the ER positive MC but also on the ER negative MC, should cause a delay of the development of resistance, a well-known process in the therapy of breast cancer (e.g. with antiestrogens).[4,5]

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(5) The development of resistance in the endocrine therapy of the hormone-dependent breast cancer is often accompanied by a loss of ERs. In this process the resistant tumors possibly spring up from ER-negative subclones. They are only accessible to a cytotoxic chemotherapy.

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