Tetrahedron Vol 46, No 7, pp 2255–2262, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00
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MONOCYCLIC β-LACTAM INHIBITORS OF HUMAN LEUKOCYTE ELASTASE

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(*Received in USA* 29 *June* 1988)

Abstract - *Two types of monocyclic azetidinones have been synthesized that were designed to inactivate human leukocyte elastase (HLE) in three steps: first, acylation of active-site serine; second, creation of a powerful new electrophilic center; third, covalent capture of a nucleophile from the enzyme. Both types do irreversibly inactivate HLE, and structure-activity relationships are in accord with the proposed mechanism. In each series, one molecule of the most active compound inhibits one molecule of HLE in at least one out of every twenty encounters.*

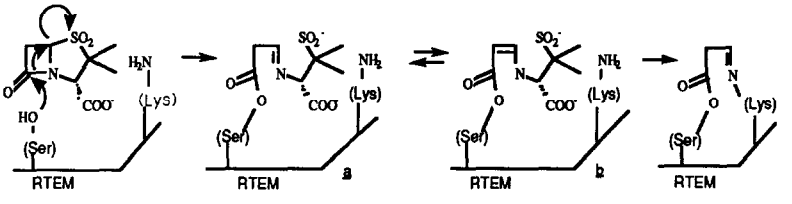
Human leukocyte elastase (HLE; EC 3.4.21.37) is a serine protease that has been implicated in the etiology of inflammatory diseases such as rheumatoid arthritis[1], emphysema[2], and others[3]. Therefore, inhibitors of this enzyme might be valuable anti-inflammatory agents.

Because of the possible similarity between HLE and β-lactamases, another class of serine proteases that can be irreversibly inhibited by certain β-lactams, it was suggested by Dr. M. Zimmerman of these laboratories that β-lactams might be capable of also inactivating HLE, and indeed early experiments bore this out[4].

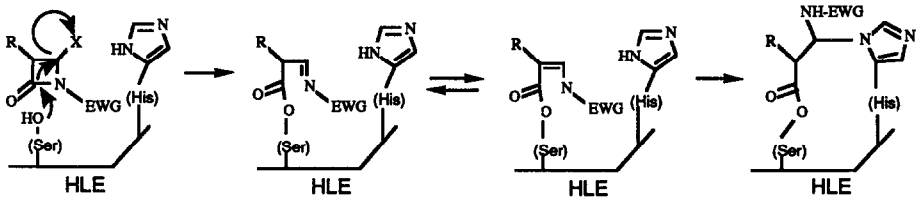
One portion of the Merck project involved sulfones of traditional bicyclic β-lactams[5], based on the reasoning of Knowles' group[6] that penicillanic acid sulfone inhibits RTEM β-lactamase in two stages, first the acylation of active-site serine by the β-lactam, followed by a second covalent modification in which a Schiff base, created by the elimination of RSO_2^- by the β-lactam's bonding electron pair released during acylation, exchanges its nitrogen for that of an active-site lysine, resulting in irreversible inactivation of the enzyme (Scheme I). A number of β-lactam sulfones were synthesized that did inhibit HLE in a manner suggestive of a dual-modification mechanism[7].

It occurred to us that it might not be necessary to retain the entire bicyclic β-lactam system in order to obtain a binary mechanism for inactivation of HLE. A monocyclic β-lactam could contain all the elements required for dual reactivity with the enzyme, such that the second reactive center is not created until after the first reaction. This attribute is desirable in order to prevent adventitious attack on the inhibitor before it comes together with the enzyme.

One way to achieve this goal is to have a leaving group X at position 4 of the β-lactam. Acylation of the enzyme (Ser195)[7] by the lactam would stimulate departure of the leaving group, creating a Schiff base that could accept a nucleophile (His57) from the enzyme at C-4. Since monocyclic β-lactams are less strained than bicyclic ones, activation by an electron-withdrawing group (EWG) on the nitrogen might be needed to achieve the initial acylation step, and would also provide a highly reactive imine as electrophile for the second covalent modification of the HLE (Scheme II).



Scheme I

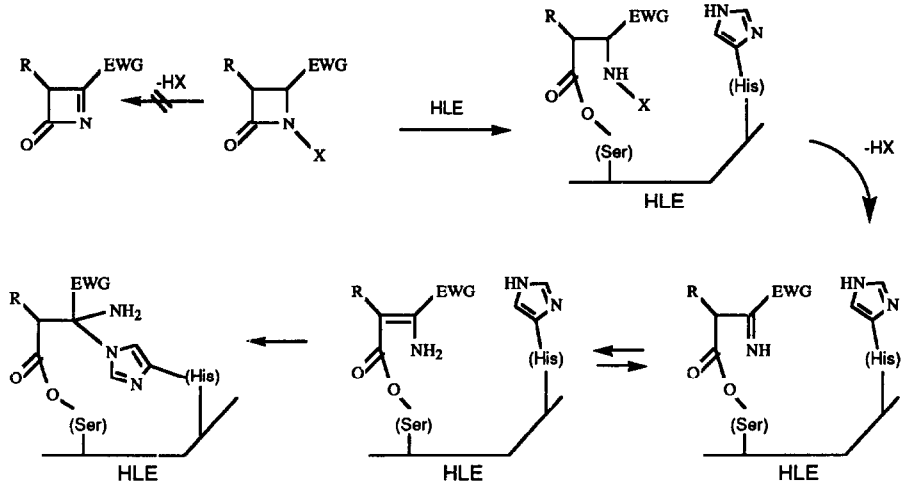


Scheme II

Another way to design a monocyclic β-lactam HLE inhibitor is to reverse the functions of the N- and 4-substituents, i.e., to have the leaving group on nitrogen such that it departs, *via* β-elimination, after acylation of the enzyme has occurred. An electron-withdrawing group is now required at C-4 in order to stimulate the elimination by acidifying the C-4 proton. This could create a Schiff base that is activated by the electron-withdrawing group to serve as electrophile for the second HLE modification. It is unlikely that HX would be eliminated prior to the first encounter with the enzyme that opens the lactam, because this would create two new SP^2 centers in the four membered ring, resulting in greatly increased I-strain (Scheme III).

We now wish to report that both classes of monocyclic β-lactams have furnished potent inhibitors of HLE. In both classes, structure-activity relationships support, although they do not yet prove, the mechanisms by which they were designed.

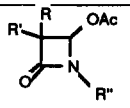
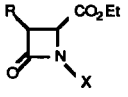
Of the first type (Class 1), six compounds are shown in the Table. Of particular interest are these observations: [1] Irreversible inhibition of HLE is obtained with many of these monocyclic β-lactams, some of which are very potent. [2] An acetyl group on nitrogen in place of H provides considerable, and sometimes very large, increases in activity. [3] It is known that HLE has a hydrophobic binding pocket near the active-site serine, because acylating inhibitors of other types[8], and also substrates[9], are greatly improved by having medium-sized alkyl groups next to the site of acylation. In line with Powers' observations[8,9], we also find that the presence of a small alkyl group at C-3 of the



Scheme III

β-lactam enhances the inhibitors potency, *viz.* H = Me < Et, n-Pr, n-Bu. A side chain of 2-4 carbons is optimum, just as it is in the best substrate known to date[10]. The data obtained with our assay procedure (see Experimental) represents a single observation made two minutes after mixing. In some cases, inactivation is incomplete at two minutes, so the reported IC_{50}'s are really $IC_{>50}$'s. Furthermore, since our compounds are all racemic, it is likely that the proper enantiomer will have the very low IC_{50} of 0.005 μg/mL (see Table 1). Under our test conditions, this concentration represents a ratio of only ten molecules of 3-n-propyl derivative (**5B**, MW = 213) per molecule of enzyme (MW = 30,000). Thus, each molecule of this compound inhibits a molecule of enzyme at least once in every twenty encounters.

TABLE 1. Inhibition of HLE by Monocyclic β-Lactams

Compound	R	R'	cis or trans	IC_{50}, mg/ml R"=H(A)	R"=Ac(B)	(X=H)(E)	IC_{50}, mg/ml X (F)	
				Class 1				
1	H	H	-	-	3			
2	CH_3	H	c+t	20	3			
3	CH_3	CH_3	-	20	0.5			
4	Et	H	c/t=1	15	0.1			
5	n-Pr	H	t	15	0.1[a]			
6	n-Bu	H	t	0.4	0.1			
				Class 2				
7	H[b]		-			-	PNBS	3
8	Et		t			3	PNBS	0.01
9	Et		c			1	PNBS	0.05
10	Et		t[c]			>20	PNBS	0.2, 0.6
11	Et		t			-	Ts	0.01
12	Vinyl		t			2	PNBS	0.5
13	Vinyl		c			10	PNBS	0.02
14	Vinyl		c			-	Benzoyl	0.1
15	n-Pr		c			2	PNBS	0.06
16	Allyl		t			2	-	-
17	Allyl		c			20	PNBS	0.05
18	2-Propenyl		t			>20	PNBS	1
19	2-Propenyl		c			5	PNBS	0.5
20	Ph		t			10	PNBS	4
21	Ph		c			>20	PNBS	3
22	Ph		c+t			-	SO_2CF_3	3
23	Ph		c+t			-	SCF_3	8

[a]0.01 after 10 min.
[b]Benzyl instead ofethyl ester
[c]Optically active; see text.

Inhibition of HLE by compounds of Class 1 peaks at about ten minutes, and the enzyme remains inactivated for several hours. After sixteen hours the HLE regains only about 10% of its initial activity, but this figure does not increase even after ten days. If hydroxylamine is added to the enzyme solution one minute after inhibitor has been added, enzyme activity is completely regenerated. This is consistent with reversal of acylation on serine (*i.e.*, a, Scheme I). However, if hydroxylamine is introduced ten minutes after inhibitor, the enzyme has been irreversably inactivated and enzyme activity is not regenerated. These observations are consistant with, although do not prove, the mechanism shown in Scheme II.

It should be noted that Knowles' has shown the enamine tautomer of the acyl-enzyme (a, Scheme I) to be the inactivating intermediate with RTEM β-lactamase[6]. We have no evidence to indicate which tautomeric form of our

acyl-enzyme is the inactivating species, except that in one example from Class 1 (**3B**, R=Me_2), the emanine tautomer cannot be formed and thus the inactivating intermediate must be the imine. The observation that the IC_{50} of this compound compares favorably with the better inhibitors of its class suggests that the imine form of the acyl-enzyme is at the very least a viable candidate for the inactivating species in this series of compounds.

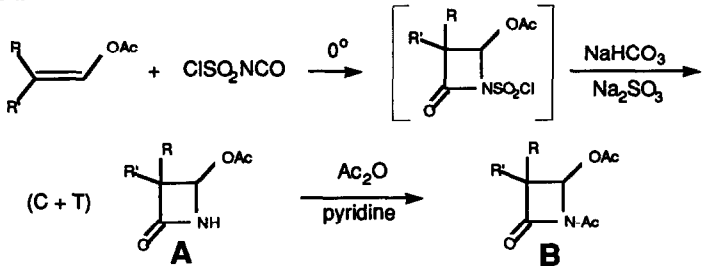
Of the second type of inhibitor (Class 2), seventeen compounds are shown in the Table. The leaving group is usually p-nitrobenzenesulfonate (PNBS), although others can serve in this capacity. Without it, activity is abolished or greatly reduced. This could reflect either activation of the β-lactam to acylation, or creation of a second electrophile, or both. One example of an activating group on the β-lactam nitrogen which is not a leaving group (benzoyl, **14F**) is shown in Table 1; this compound is five-fold less active an inhibitor than its analog with a PNBS leaving group (**13F**). In all cases, the electron-withdrawing group that activates the C-4 proton for elimination is a carboxylic ester. The structure-activity relationship for the 3-substituent is almost the same for Class 2 as it is for Class 1, i.e., a small alkyl group greatly enhances activity. It seems likely that in Class 2, as in Class 1, the optimum size is about propyl. Branching of the alkyl group is detrimental, presumably owing to poorer binding to the enzyme's hydrophobic pocket. For this series, the suggestion that one enantiomer will have more activity than the other has some experimental support, since one enantiomer of the ethyl derivative (**10F**) - apparently the wrong one - showed very low activity compared to the racemic form. Thus it may be assumed that the correct enantiomer of the most active compound will have IC_{50} = 0.005 μg/mL and that each molecule inhibits a molecule of enzyme at least once in every twenty encounters.

It is interesting to note that the optically active inhibitor was derived from the bioactive enantiomer of an intermediate in the synthesis of Thienamycin, a β-lactam antibiotic, indicating that HLE might want to see the opposite enantiomer from that which is active against bacterial cell-wall carboxypeptidase. There is a paradox, however, in that the best penicillin and cephalosporin derivatives that showed activity against HLE in the other part of the present program were all derived from natural sources. This contradiction is at present unresolved.

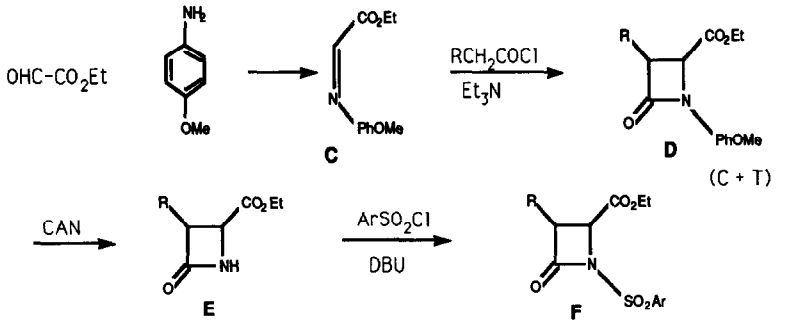
The kinetics of inhibition of HLE by compounds of Class 2 are the same as for Class 1, *i.e.*, enzyme activity is not regenerated after as long as ten days even with the addition of hydroxylamine. Colorimetric determination of *p*-nitrophenylsulfinic acid release (HX, Scheme III) by trapping with a diazo dye was inconclusive; some pNBS adduct, but not the predicted amount, was observed.

Synthesis

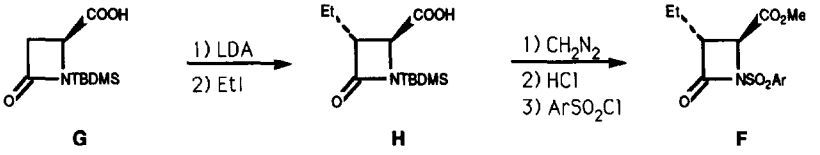
All but the first (see Experimental) of the compounds of Class 1 were prepared by the method of Clauss[11], followed by N-acylation.



The compounds of Class 2 were prepared by the method of Kronenthal[12], followed by N-sulfonylation. Cycloaddition of the acid chloride and imine gave a mixture of cis and trans azetidinones which were separated by chromatography. Hydrogenation of unsaturated R groups (vinyl, allyl) to saturated R was effected at this stage, followed by oxidative cleavage of the p-methoxyphenyl group with cerric ammonium nitrate (CAN) and then sulfonylation to the final products.



The optically active inhibitor was made from N-t-butyldimethylsilyl-4-carboxy-azetidinone, an optically pure intermediate in the synthesis of Thienamycin, whose sense of chirality at C-4 is the same as it is in the antibiotic. Alkylation at C-3 via the dianion is known to occur without epimerization at C-4.



Acknowledgements - We thank Drs. M. Zimmerman, T-Y Lin and J. B. Doherty for many helpful discussions, and Ms. M. J. Herman for typing the manuscript.

Experimental Section

All ^{1}H NMR spectra were run in $CDCl_3$, and are expressed in ppm downfield from TMS. Spectra were recorded on a Varian EM-390 spectrometer, except as noted. All reactions done under nitrogen employed a Firestone valve (Cat. no. 8766-12, Ace Glass Co., Vineland, NJ).

Assay Procedure

The procedure here outlined is essentially the same as the published one[14], with modifications as noted. The rate of hydrolysis of Suc-Ala-Ala-Pro-Ala-p-nitroanilide (0.2 mM) in 0.05 TES buffer containing 0.15 M NaCl and 10% DMSO, pH 7.5, 25°C, was measured at 410 nm 2 min after mixing with inhibitor and enzyme[15] (ca. 1 μg/mL). IC_{50} is the concentration of inhibitor that gives 50% inhibition. The only changes from the published procedure are that the substrate is the succinoyl instead of the BOC derivative, and NaCl has been added to the assay mixture.

1-Acetyl-4-acetoxyazetidinone (**1B**).

A mixture of 525 mg of 4-thiomethyl azetidinone[11] (4.5 mMol) and 18 mL Ac_2O was stirred 24 h at 125°C, evaporated *in vacuo* and flash chromatographed on a 50 mm silica gel column using 1:2 hexane-ethyl acetate, affording 331 mg impure material which was re-chromatographed on a 20 mm flash column using 9:1 chloroform-ethyl acetate. This gave 153 mg of a 1:1 mixture of product and N-acetyl-4-methylthioazetidinone, which was separated on a 10 mm Florisil column using 10:1 hexane-ethyl acetate, affording 61 mg (8%) product. ^{1}H NMR: 2.12 (s, 3H; 4-OAc), 2.37 (s, 3H; N-Ac), 3.0 (dd, J=16, 2, 1H; 3β-H), 3.4 (dd, J=16, 4, 1H; 3α-H), 6.4 (dd, J=4, 2, 1H; 4α-H).

3-Methyl-4-acetoxyazetidinone (**2A**).

1-Propenyl acetate, 1.0 g (10 mMol) in 5 mL dichloromethane was treated at 0°C under N_2 with 0.800 mL chlorosulfonylisocyanate (CSI). The mixture was stirred 2 h at 0°C, added dropwise to a mixture a 5 g of ice, 1.15 mL water, 2.82 g $NaHCO_3$ and 1.0 g Na_2SO_3, stirred 30 min, and extracted with 2 x 25mL dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to afford 155.4 mg (11%) crystalline product. ^{1}H NMR: 1.25 (d, J=8) and 1.35 (d, J=8) ratio ca. 3:1 (3H combined; 3-CH_3 cis + trans), 2.16 (s, 3H; OAc), 3.4 (m, 1H, 3-H), 5.28 (br s) and 5.84 (d, J=4) ratio ca. 1:3 (1H combined; 4-H cis + trans), 7.2 (br s, 1H; N-H).

1-Acetyl-3-methyl-4-acetoxyazetidinone (**2B**).

To 1 mL pyridine and 1 mL acetic anhydride was added 60 mg 3-methyl-4-acetoxy-azetidinone (0.42 mMol) under N_2, and the mixture was stirred 24 h at 100°C in a sealed tube. Volitiles were removed *in vacuo*, and the residue was chromatographed on Florisil with 3:1 hexane-ethyl acetate, affording 43 mg (56%) product, (Rf=0.25 on silica gel, same solvent, two close spots). ^{1}H NMR: 1.21 (d, J=8) and 1.40

(d, J=8) ratio ca. 3:1 (3H combined; 3-CH3 cis + trans), 2.15 (s, 3H; 4-OAc), 2.36 (s, 3H; N-Ac), 3.0-3.8 (m, 1H; 3-H cis + trans), 5.90 (d, J=2) and 6.40 (d, J=5) ratio ca. 1:3 (1H combined; 4-H).

3,3-Dimethyl-4-acetoxyazetidinone (3A).

Isopropenyl acetate, 22.8 g (0.20 mMol), was treated with 16 mL chlorosulfonyl-isocyanate in 50 mL of dichloromethane at 0°C under N_2 for 24 h with stirring. The mixture was added dropwise to a mixture of 20 mL of water, 90 g ice, 48 g $NaHCO_3$, and 16.6 g of Na_2SO_3, stirred 30 min, and extracted with 300 mL of dichloromethane. The organic phase was washed with water and brine, dried with $MgSO_4$, filtered and evaporated, leaving 27.75 g of crude product. This was chromatographed on a 50 mm flash column at 2.5 psi in ca. 120 g silica gel with 2L 4:1 chloroform-ethyl acetate followed by 2L 1:1 chloroform-ethyl acetate, affording 2.17 g product; (Rf=0.3 on tlc with 4:1 chloroform-ethyl acetate). ^{1}H NMR: 1.24 (s, 3H) and 1.37 (s, 3H; 3,3-Me_2), 2.16 (s, 3H; OAc). 5.55 (s, 1H; 4-H), 7.4 (br s, 1H; N-H).

1-Acetyl-3,3-dimethyl-4-acetoxyazetidinone (3B).

To 2 mL pyridine and 2 mL Ac_2O was added 283.3 mg 3,3-dimethyl-4-acetoxy- azetidinone (1.8 mmol), and the mixture was heated at 100°C under N_2 in a sealed tube for 36 h. Evaporation of volitiles *in vacuo* left an oil which was chromatographed on a 30 mm flash column, silica gel, with 4:1 chloroform-ethyl acetate, affording 27 mg (7.5%) product. ^{1}H NMR: 1.20 (s, 3H) and 1.40 (s, 3H; 3,3-Me_2), 2.15 (s, 3H; 4-OAc), 2.34 (s, 3H; N-Ac), 6.03 (s, 1H; 4-H). Some impurities were present owing to apparent instability on the silica gel.

3-Ethyl-4-acetoxyazetidinone (4A).

To 22.8 g (0.2 mol) 1-butenyl acetate in 50 mL dichloromethane was slowly added 16 mL chloro-sulfonylisocyanate, and the mixture was stirred 2 h at 0°C. This was then added dropwise to a mixture of 23 mL of water, 100 g of ice, 56.4 g of $NaHCO_3$, and 19.8 g Na_2SO_3, stirred at 0°C 30 min, and extracted with 2 x 100 mL dichloromethane. The organic phase was washed with brine, dried over $MgSO_4$, filtered, evaporated and chromatographed on Florisil with 3:1 hexane-ethyl acetate to provide 5.17 g (16.3%) product. ^{1}H NMR: 1.04 (t, J=7, 3H; $C\underline{H}_2CH_3$), 1.7 (m, 2H; $C\underline{H}_2CH_3$), 2.14 (s, 3H; 4-OAc), 3.2 (m, 1H; 3-H), 5.6 (d, J=1.5) and 5.9 (d, J=5) ratio ca. 1:2 (1H combined; 4-H cis + trans), 6.9 (br s, 1H; N-H). MS: 98. Anal: C, H, N.

1-Acetyl-3-ethyl-4-acetoxyazetidinone (4B).

A mixture of 33.3 mg (0.21 mMol) 3-ethyl-4-acetoxyazetidinone, 2 mL pyridine and 2 mL Ac_2O was heated at 100°C for 24 h, evaporated *in vacuo*, and the residue (23 mg) was purified by tlc on silica gel, developing with 1:1 hexane-ethyl acetate, yielding 7 mg (17%) product (Rf=0.8). ^{1}H NMR: 1.08 (t, J=7, 3H; $CH_2C\underline{H}_3$), 1.7 (m, 2H; $C\underline{H}_2CH_3$), 2.11 (s, 3H; 4-OAc), 2.36 (s, 3H; N-Ac), 3.0-3.6 (br m, 1H; 3-H), 6.05 (d, J=1.5) and 6.47 (d, J=5) ratio ca. 1:1 (1H combined; 4-H cis+trans). Anal: C, H, N.

Trans-3-n-propyl-4-acetoxyazetidinone (5A).

To 1.28 g 1-pentenyl acetate (10 mMol) in 5 mL dichloromethane was added 0.800 mL chlorosulfonylisocyanate, and the mixture was stirred 5 days at 0°C under N_2. The mixture was then added dropwise to a mixture of 1.15 mL water, 5 g ice, 2.82 g $NaHCO_3$ and 1.0 g Na_2SO_3, stirred 30 min, and extracted with 2 x 25 mL dichloromethane. The organic phase was washed with brine, dried with $MgSO_4$, filtered, evaporated and chromatographed on a 30 mm flash column of silica gel, using 3:1 hexane-ethyl acetate, affording 60 mg (3.4%) product (Rf=0.4). ^{1}H NMR: 0.95 (m) and 1.6 (m, 7H; n-propyl), 2.13 (s, 3H; OAc), 3.1 (m, 1H; 3-H), 5.52 (s, 1H; 4-H), 6.7 (br s, 1H; N-H). The trans configuration was assigned because of the low coupling constant to 4-H.

Trans-1-acetyl-3-n-propyl-4-acetoxyazetidinone (5B).

The previous product (56 mg, 0.33 mMol), 1 mL pyridine, and 1 mL Ac_2O were heated together at 100°C under N_2 in a sealed tube for 24 h. After volitives were removed *in vacuo*, the residue (54 mg) was flash chromatographed on a 10 mm silica gel column with 3:1 hexane-ethyl acetate, providing 16 mg (23%) product (Rf=0.5). ^{1}H NMR (200 MHz): 0.89 (t, J=7, 3H; $CH_2CH_2C\underline{H}_3$), 1.35 (m, 2H; $CH_2C\underline{H}_2CH_3$), 1.77 (m, 2H; $C\underline{H}_2CH_2CH_3$), 2.11 (s, 3H; OAc), 2.38 (s, 3H; N-Ac), 3.11 (dt, J=8, 2, 1H; 3-H), 6.12 (d, J=2, 1H; 4-H). Anal: C calc. 56.36, found 55.69; H, N.

Trans-3-n-butyl-4-actoxyazetidinone (6A).

1-Hexenyl acetate (14.2 g, 0.1 Mol) was treated dropwise in 50 mL dichloromethane with a solution of 8 mL chlorosulfonylisocyanate in 10 mL of dichloromethane at 0-5°C for 48 h. The mixture was slowly added to a mixture of 10 mL water, 45 g ice, 24 g $NaHCO_3$ and 8.3 g Na_2SO_3, stirring an additional 30 min at 0°C. The mixture was extracted with 2 x 100 mL dichloromethane, and the organic layers dried with $MgSO_4$, filtered, evaporated and flash chromatographed of a 50 mm silica gel column using 3:1 hexane-ethyl acetate, affording 167 mg (1%) product (Rf=0.2). ^{1}H NMR: 0.9-1.5 (m, 9H; n-butyl), 2.13 (s, 3H; OAc), 3.1 (m, 1H; 3-H), 5.5 (d, J=1.5, 1H; 4-H), 6.9 (br s, 1H; N-H).

Trans-1-acetyl-3-n-butyl-4-acetoxyazetidinone (6B).

To 154 mg (0.83 mMol) of the previous product were added 2 mL each pyridine and Ac_2O, and the mixture was heated in a sealed tube under N_2 20 h at 100°C. After removing volatiles *in vacuo*, the residue was flash chromatographed on a 20 mm silica gel column with 2:1 hexane-ethyl acetate, affording 81 mg (43%) product (Rf=0.4). ^{1}H NMR: 0.95 (m) and 1.5 (br m, 9H; n-butyl), 2.13 (s, 3H; OAc), 2.4 (s, 3H; N-Ac), 3.14 (m, 1H; 3-H), 6.13 (d, J=1.5, 1H; 4-H).

N-(Ethoxycarbonylmethylidine)-4-methoxyaniline (C).

A solution of ethyl glyoxylate hydrate (20.5 g, 171 mMol) in 200 mL dichloromethane was treated with anhydrous sodium sulfate (20 g) and cooled to 0°C under N_2. p-Anisidine (21.7 g, 171 mMol) was added and the solution was stirred for 2 h. Filtration through a pad of silica gel followed by evaporation gave 22 g (106 mMol) of an amber oil. ^{1}H NMR: 1.4 (t, J=7, 3H), 3.7 (s, 3H), 4.3 (q, J=7, 2H), 6.8 (d, J=9, 2H), 7.2 (d, J=9, 2H), 7.8 (s, 1H).

1-p-Methoxyphenyl-3-vinyl-4-ethoxycarbonylazetidinone.

A solution of C (10.8 g, 52 mMol) and triethylamine (7.3 mL, 52 mMol) in 100 mL dichloromethane was heated to reflux under N_2, while a solution of 20 mL (208 mMol) of crotonyl chloride in 50 mL dichloromethane was added over 15 min. After 30 min at reflux the solution was poured over an equal volumn of ice/1N HCl and washed. The organic layer was then washed with saturated aqueous bicarbonate solution, dried over sodium sulfate, filtered through a pad of silica gel and evaporated to yield semi-solid product. Trituration with ether/hexane yielded 4.8 g of crystalline cis azetidinone; mp 70-72°C. ^{1}H NMR: 1.2 (t, J=7, 3H), 3.7 (s, 3H), 4.2 (m, 3H), 4.6 (d, J=6, 1H), 5.2-5.9 (m, 3H), 6.8

(d, J=9, 2H), 7.2 (d, J=9, 2H). Chromatographic separation of the mother liquor yielded an additional 4.0 g of the cis azetidinone (B1, 32 mMol total, 62 % yield) as well as 3.5g (12.7 mMol, 24% yield of the oily trans azetidinone (B2). ^{1}H NMR: 1.25 (t, J=7, 3H), 3.70 (s, 3H), 3.9 (dd, J=8, 1, 1H), 4.2 (m, 3H), 5.2-6.2 (m, 3H), 6.8 (d, J=9, 2H), 7.2 (d, J=9, 2H).

1-p-Methoxyphenyl-3-isopropenyl-4-ethoxycarbonylazetidinone.

Prepared as above from **C** and 3,3-dimethylacryloyl chloride and chromatographed on silica gel yielding a mixture of isomeric (cis + trans) products. Recrystallization (pet ether/dichloromethane) gave pure cis azetidinone; mp 87-88°C. ^{1}H NMR: 1.2 (t, J=7, 3H), 1.8 (br s, 3H), 3.7 (s, 3H), 4.2 (m, 3H), 4.6 (d, J=6, 1H), 5.1 (br s, 2H), 6.7 (d, J=9, 2H), 7.2 (d, J=9, 2H). Impure trans azetidinone was recovered from the mother liquor.

1-p-Methoxyphenyl-3-phenyl-4-ethoxycarbonylazetidinone.

Prepared as above from **C** and phenylacetylchloride and chromatographed on silica gel affording the cis and trans azetidinones as oils. Cis isomer: ^{1}H NMR: 0.7 (t, J=7, 3H), 3.5 (m, 1H), 3.6 (q, J=7, 2H), 3.6 (s, 3H), 4.7 (s, 1H), 6.7 (d, J=9, 2H), 6.8-7.4 (m, 7H). Trans isomer: ^{1}H NMR: 1.2 (t, J=7, 3H), 3.7 (s, 3H), 4.1 (q, J=7, 2H), 4.5 (m, 2H), 6.8 (d, J=9, 2H), 7.0-7.4 (m, 7H).

1-p-Methoxyphenyl-3-allyl-4-ethoxycarbonylazetidinone.

Prepared as above from **C** and 4-pentenoyl chloride and chromatographed on silica gel giving a mixture of the cis and trans azetidinones. Recrystallization of the mixture from dichloromethane/hexane gave the pure cis isomer; mp 94-95°C. ^{1}H NMR: 1.2 (t, J=7, 3H), 2.5 (m, 2H), 3.6 (m, 1H), 3.7 (s, 3H), 4.2 (q, J=7, 2H), 4.5 (d, J=6, 1H), 5.0 (m, 2H), 5.5-5.9 (m, 1H), 6.7 (d, J=9, 2H), 7.1 (d, J=9, 2H). Impure trans isomer was recovered from the mother liquor.

Cis-1-p-Methoxyphenyl-3-propyl-4-ethoxycarbonylazetidinone.

Hydrogenation of 1-p-methoxyphenyl-3-allyl-4-ethoxycarbonylazetidinone over palladium/charcoal yielded the title compound as an oil. ^{1}H NMR: 0.9 (t, J=7, 3H), 1.2 (t, J=7, 3H), 1.5 (m, 4H), 3.5 (m, 1H), 3.7 (s, 3H), 4.2 (q, J=7, 2H), 4.5 (d, J=6, 1H), 6.7 (d, J=9, 2H), 7.1 (d, J=9, 2H).

3-Vinyl-4-ethoxycarbonylazetidinone (**12,13E**).

Prepared from 1-p-methoxyphenyl-3-vinyl-4-ethoxycarbonylazetidinone by the method of Kronenthal[12]; this material was sufficiently pure to use in the next step. Cis isomer (**13E**): ^{1}H NMR: 1.2 (t, J=7, 3H), 4.0-4.4 (m, 2H), 4.2 (q, J=7, 2H), 5.1-5.8 (m, 3H), 7.1 (br s, 1H). Trans isomer (**12E**): ^{1}H NMR: 1.2 (t, J=7, 3H), 3.8 (m, 1H), 3.9 (d, J=2.5, 1H), 4.2 (q, J=7, 2H), 5.1-5.4 (m, 2H), 5.7-6.1 (m, 1H), 6.9 (br s, 1H).

3-Isopropenyl-4-ethoxycarbonylazetidinone (**18,19E**).

Prepared as above from 1-p-methoxyphenyl-3-isopropenyl-4-ethoxycarbonyl-azetidinone. Cis isomer (**19E**): ^{1}H NMR: 1.2 (t, J=7, 3H), 1.7 (s, 3H), 4.1 (m, 4H), 4.3 (d, J=6, 1H), 4.9 (br s, 2H), 7.2 (br s, 1H). Trans isomer (**18E**): ^{1}H NMR: 1.3 (t, J=7, 3H), 1.8 (s, 3H), 3.8 (br s, 1H), 4.0 (d, J=3, 1H), 4.2 (q, J=7, 2H), 4.9 (m, 2H), 6.6 (br s, 1H).

3-Phenyl-4-ethoxycarbonylazetidinone (**20,21E**).

Prepared as above from 1-p-methoxyphenyl-3-phenyl-4-ethoxycarbonyl-azetidinone. Cis isomer (**21E**): ^{1}H NMR: 0.8 (t, J=7, 3H), 3.7 (q, J=7, 2H), 4.4 (d, J=6, 1H), 4.7 (d, J=6, 1H), 6.9 (br s, 1H). Trans isomer (**20E**): ^{1}H NMR: 1.2 (t, J=7, 3H), 4.0 (d, J=2, 1H), 4.2 (q, J=7, 1H), 4.3 (d, J=3, 1H), 6.8-7.3 (m, 6H).

3-Allyl-4-ethoxycarbonylazetidinone (**16,17E**).

Prepared as above from 1-p-methoxyphenyl-3-allyl-4-ethoxycorbonylazetidinone. Cis isomer (**17E**): ^{1}H NMR: 1.2 (t, J=7, 3H), 2.3 (m, 2H), 3.5 (m, 1H), 4.2 (m, 3H), 5.0 (m, 2H), 5.5-6.0 (m, 1H), 6.9 (br s, 1H). Trans isomer (**16E**): ^{1}H NMR: 1.2 (t, J=7, 3H), 2.5 (m, 2H), 3.3 (dt, J=7, 2, 1H), 3.8 (d, J=2, 1H), 4.2 (q, J=7, 2H), 5.0-5.2 (m, 2H), 5.5-6.0 (m, 1H), 6.8 (br s, 1H).

Cis-3-propyl-4-ethoxycarbonylazetidinone (**15E**).

Prepared as above from 1-p-methoxyphenyl-3-propyl-4-ethoxycarbonylazetidinone. ^{1}H NMR: 0.9 (t, J=7, 3H), 1.3 (t, J=7, 3H), 1.5 (m, 4H), 3.5 (m, 1H), 4.2 (m, 3H), 6.3 (br s, 1H).

3-Ethyl-4-ethoxycarbonylazetidinone (**8,9E**).

Hydrogenation of **16E** and **17E** over palladium/charcoal yielded the cis and trans ethylazetidinones, respectively. Cis isomer (**9E**): ^{1}H NMR: 1.0 (t, J=7, 3H), 1.3 (t, J=7, 3H), 1.6 (m, 2H), 3.4 (m, 1H), 4.2 (q, J=7, 2H), 4.6 (d, J=6, 1H), 6.8 (br s, 1H). Trans isomer (**8E**): ^{1}H NMR: 1.1 (t, J=7, 3H), 1.3 (t, J=7, 3H), 1.8 (m, 2H), 3.2 (dt, J=7, 2, 1H), 3.8 (d, J=2, 1H), 4.2 (q, J=7, 2H), 6.8 (br s, 1H).

1-p-Nitrobenzenesulfonyl-3-vinyl-4-ethoxycarbonylazetidinone (**12,13F**).

A solution of 280 mg (1.65 mMol) of **13E** in 2 mL of dichloromethane was cooled to 0°C under N_2 and 0.25 mL of DBU was added. A solution of 366 mg (1.65 mMol) of p-nitrobenzenesulfonyl chloride in 1 mL of dichloromethane was added and the solution was allowed to stir at room temp for 2 h. The solvent was then evaporated and the product (**13F**) was purified by flash chromatography on silica gel. ^{1}H NMR: 1.2 (t, J=7, 3H), 4.1 (m, 3H), 4.8 (s, 1H), 5.2-5.7 (s, 3H), 8.2 (d, J=8, 2H), 8.4 (d, J=8, 2H). Similarly prepared was the trans isomer (**12F**): ^{1}H NMR: 1.2 (t, J=7, 3H), 3.9-4.5 (m, 4H), 5.2-6.0 (m, 3H), 8.0 (d, J=8, 2H), 8.2 (d, J=8, 2H).

1-p-Nitrobenzenesulfonyl-3-isopropenyl-4-ethoxycarbonylazetidinone (**18,19F**).

Prepared as above from **18,19E**. Cis isomer (**19F**): ^{1}H NMR: 1.3 (t, J=7, 3H), 1.8 (s, 3H), 4.8 (s, 1H), 5.1 (s, 2H), 8.2 (d, J=8, 2H), 8.5 (d, J=8, 2H). Trans isomer (**18F**): ^{1}H NMR: 1.2 (t, J=7, 3H), 1.7 (s, 3H), 4.1 (m, 3H), 4.8 (s, 1H), 5.0 (s, 2H), 8.2 (d, J=8, 2H), 8.4 (d, J=8, 2H).

1-p-Nitrobenzenesulfonyl-3-phenyl-4-ethoxycarbonylazetidinone (**20,21F**).

Prepared as above from **20,21E**. Cis isomer (**21F**): ^{1}H NMR: 0.9 (t, J=7, 3H), 3.5-4.9 (m, 3H), 5.0 (s, 1H), 7.3 (br s, 5H), 8.2 (d, J=8, 2H), 8.4 (d, J=8, 2H). Trans isomer (**20F**): ^{1}H NMR: 1.3 (t, J=7, 3H), 4.3 (q, J=7, 2H), 4.4-5.0 (m, 2H), 7.2 (m, 5H), 8.2 (d, J=8, 2H), 8.4 (d, J=8, 2H).

Cis-1-p-nitrobenzenesulfonyl-3-allyl-4-ethoxycarbonylazetidinone (**17F**).

Prepared as above from **17E**. ^{1}H NMR: 1.2 (t, J=7, 3H), 2.3 (m, 2H), 3.7 (m, 1H), 4.1 (q, J=7, 2H), 4.7-5.0 (m, 2H), 5.4-5.8 (m, 1H), 8.2 (d, J=8, 2H), 8.4 (d, J=8, 2H).

Cis-1-p-nitrobenzenesulfonyl-3-propyl-4-ethoxycarbonylazetidinone (**15F**).

Prepared as above from **15E**. ^{1}H NMR: 0.8-1.8 (m, 10H), 3.5 (m, 1H), 4.0-4.6 (m, 6H), 8.1 (d, J=8, 2H), 8.3 (d, J=8, 2H).

1-p-Nitrobenzenesulfonyl-3-ethyl-4-ethoxycarbonylazetidinone (**9,9F**).

Prepared as above from L1. Cis isomer (**9F**): ^{1}H NMR: 1.0 (t, J=7, 3H), 1.3 (t, J=7, 3H), 1.5 (m, 2H), 3.5 (q, J=7, 1H), 4.2 (q, J=7, 2H), 4.8 (d, J=7, 1H), 8.2 (d, J=8, 2H), 8.3 (d, J=8, 2H). Anal: C, H, N. Trans isomer (**9F**): ^{1}H NMR: 1.0 (t, J=7, 3H), 1.2 (t, J=7, 3H), 1.8 (m, 2H), 3.1 (m, 1H), 4.2 (q, J=7, 2H), 4.3 (d, J=3, 1H), 8.2 (d, J=8, 2H), 8.4 (d, J=8, 2H). Anal: C, H, N.

Trans-1-p-toluenesulfonyl-3-ethyl-4-ethoxycarbonylazetidinone (**11F**).

Prepared as above from **8E** and p-toluenesulfonyl chloride. ^{1}H NMR: 0.9 (t, J=7, 3H), 1.2 (t, J=7, 3H), 1.7 (m, 2H), 2.4 (s, 3H), 3.1 (dd, J=7, 3, 3H), 4.1 (m, 3H), 7.2 (d, J=8, 2H), 7.8 (d, J=8, 2H). Anal: C, H, N.

1-t-Butyldimethylsilyl-3-R-ethyl-4-R-carboxyazetidinone (**H**).

A solution of 830 mg (3.63 mMol) of 4-R-carboxy-1-(t-butyldimethylsilyl)azetidinone (**G**) in 10 mL of dry THF was cooled to -78°C and treated with 7.26 mL of 1M lithium bis(trimethylsilyl)amide under N_2. The solution was allowed to warm to room temp and then cooled to -78°C and treated with 1.45 mL (18.15 mMol) of ethyl iodide. After stirring at room temp for 1 h the reaction mixture was poured over ice/1N HCl and extracted with dichloromethane and dried over Na_2SO_4. Filtration and evaporation gave 840 mg (3.27 mMol, 90% yield) of an amber oil. ^{1}H NMR: 0.1 (s, 3H), 0.3 (s, 3H), 1.0 (m, 12H), 1.8 (m, 2H), 3.2 (dt, J=7, 2, 1H), 3.7 (d, J=2, 1H), 10.7 (br s, 1H).

3-R-Ethyl-4-S-methoxycarbonylazetidinone.

The material from the above reaction (**H**) was dissolved in 15 mL of methanol and treated with an ethereal solution of approximately 15 mMol of diazomethane. After stirring at room temp for one h, 2 mL of concentrated hydrochloric acid was carefully added with ice bath cooling and the resulting solution was stirred for an additional hour. The solvent was evaporated and the residue was dissolved in dichloromethane and washed with water and brine. Drying (sodium sulfate) and evaporation gave product. ^{1}H NMR: 1.1 (t, J=7, 3H), 1.8 (m, 2H), 3.2 (dt, J=7, 2, 1H), 3.8 (s, 3H), 3.9 (d, J=2, 1H), 6.8 (br s, 1H).

1-p-Nitrobenzenesulfonyl-3-R-ethyl-4-S-methoxycarbonylazetidinone (**10F**).

Prepared as per **12,13F** above. ^{1}H NMR: 1.1 (t, J=7, 3H), 1.8 (m, 2H), 3.1 (m, 1H), 3.7 (s, 3H), 4.3 (d, J=4, 1H), 8.2 (d, J=9, 2H), 8.4 (d, J=9, 2H).

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