# Orally Active β-Lactam Inhibitors of Human Leukocyte Elastase-1. Activity of 3,3-Diethyl-2-azetidinones†

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A thorough analysis of the mechanism of inhibition of human leukocyte elastase (HLE) by a monocyclic β-lactam and the mechanism of β-lactam hydrolysis led to the preparation of potent and highly stable inhibitors of HLE. This work led to the identification of 4-[(4-carboxyphenyl)-oxy]-3,3-diethyl-1-[[(phenylmethyl)amino]carbonyl]-2-azetidinone (**2**) as the first orally active inhibitor of human leukocyte elastase (HLE). Analogs of **2** with different substituents on the urea N were synthesized and evaluated for their activity in vitro against HLE as well as in vivo in a hamster lung hemorrhage model. Compounds with a methyl or a methoxy group in the para position of the benzene ring were very potent in both assays. The results are discussed on the basis of the proposed model for the binding of this class of inhibitors to HLE and a possible mechanism of inhibition is presented.

Inhibitors of human leukocyte elastase (HLE, EC 3.4.21.37) are under active investigation in several laboratories.[1] HLE is a serine protease present in the azurophilic granules of human polymorphonuclear leukocytes (PMN) and is involved in the intracellular digestion of proteins after phagocytosis. In the extracellular environment, HLE is capable of degrading a variety of structural proteins, including elastin and collagen.[2] Normally, the potent destructive power of HLE is effectively controlled by its natural inhibitors $\alpha_1$-proteinase inhibitor ($\alpha_1$PI) and $\alpha_2$-macroglobulin ($\alpha_2$M) present in plasma,[3] as well as secretory leukocyte protease inhibitor (SLPI) on mucosal surfaces.[4] In diseases such as emphysema,[5a,b] cystic fibrosis,[5c] and rheumatoid arthritis[5d] it is believed that the balance between HLE and its inhibitor is disrupted and the unbound HLE causes destruction of connective tissue. Administration of an HLE inhibitor might be beneficial in such conditions. Some groups have attempted to provide additional amounts of natural inhibitors produced by recombinant methods[6] while we[7-11] and others[12] have concentrated on the design and development of novel low molecular weight inhibitors.

We have reported that cephalosporin sulfones are inhibitors of HLE[7] and appropriate modification of this nucleus resulted in very potent and selective inhibitors.[8-11] Some of these compounds were also shown to protect the

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

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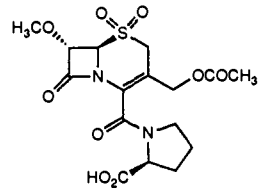
hamster lung from HLE-induced damage when administered intratracheally and one compound, L-658,758, was



L-658,758

selected for further development.[13,14] However, compounds of this class lack systemic activity, probably due to their poor stability in blood (half-life for degradation of L-658,758 in rat blood was <10 min).[14] We were interested in finding an orally active inhibitor of HLE because it might have therapeutic potential in systemic disease states. It was hoped that the insights gained about the mechanism of inhibition of HLE by cephem sulfones would prove applicable to other β-lactam nuclei. We thought that the monocyclic β-lactam nucleus (1) offered

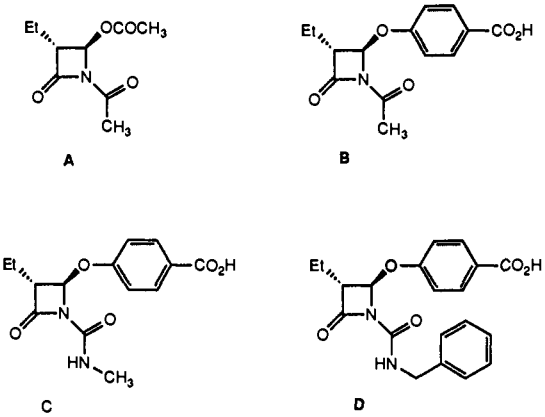
1

a structure which could be modified to improve HLE activity and hydrolytic stability, which may result in systemically active agents. When preliminary work showed that simple azetidinones did possess some inhibitory activity against HLE,[15,16] it was decided to study this class in detail. Results of this investigation, which led to the first orally active HLE inhibitors, are presented here.

## Chemistry

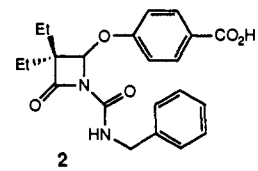
The instability of our cephalosporin-based HLE inhibitors in blood was thought to be a result of their high intrinsic reactivity. The half-life for hydrolysis ($T_{1/2}$) for the best cephalosporin elastase inhibitor, L-658,758, was 22 h at pH 8 at 25 °C, compared to more than 100 h for cephalothin, a commercially sold antibiotic.[14] Thus, the goal was to find a compound that would react with the enzyme after binding in the active site but would be sufficiently stable to hydrolysis by the nonspecific nucleophiles that it might encounter in blood. Therefore, as the lead structure was initially modified, both activity against HLE and stability at pH 8 were determined.

**Scheme I** [a]



[a] Compounds are racemic.

Previous work[15] from these laboratories suggested that the substituent at C-3 binds in the S1 pocket of the enzyme and the active site serine is in position to attack the β-lactam carbonyl. Therefore, we began by modifying other substituents on 3-ethylazetidinone A[15] ($T_{1/2}$ = 2 h, $IC_{50}$ = 0.1 mg/mL) as shown in Scheme I. The investigation of substitution at C-4 showed that a phenol at this position improves hydrolytic stability of the molecule while HLE activity is maintained.[17] A phenolic acid as present in B ($T_{1/2}$ = 8.6 h, $k_{obs}/I$ = 6680 $M^{-1}$ $s^{-1}$) was especially attractive because it can provide aqueous solubility which would be important for in vivo studies. It was also found that the *N*-methylurea substituent (as in C, $T_{1/2}$ > 80 h, $K_i$ = 16.5 μM) enhanced the stability by a factor of 10, but the enzymic activity was reduced. Fortunately, substitution of the methyl by a benzyl group (D, $T_{1/2}$ > 80 h, $k_{obs}/I$ = 2480 $M^{-1}$ $s^{-1}$) dramatically improved HLE activity, and the stability seen with C was retained. However, D was cleared rapidly from marmoset blood after an iv dose. When a second alkyl group was introduced at C-3, the compound was 2 orders of magnitude more stable to hydrolysis, and the in vitro activity ($k_{obs}/I$ = 1500 $M^{-1}$ $s^{-1}$) was only slightly reduced. Thus, when the 3,3-diethyl compound **2** was incubated in rat or human blood at 37



2

°C, no degradation was observed even after 12 h. Moreover, **2** showed activity in vivo (see below) after oral dosing in hamsters.

For the present study, **2** was chosen as a lead structure in order to find a more potent compound. First, the nitrogen substituent was modified while a 3,3-diethyl and a phenoxybenzoic acid were kept at C-4. This approach was quite attractive from the synthetic standpoint, because these racemic compounds can be quickly assembled from simple starting materials, as shown in Scheme II. In addition, it was hoped that the carboxylic acid might provide a useful handle to separate the two enantiomers,

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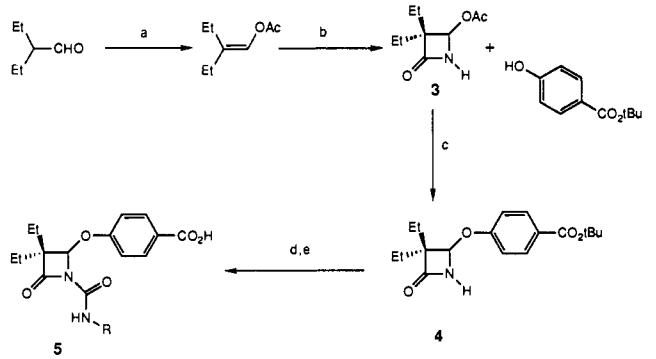
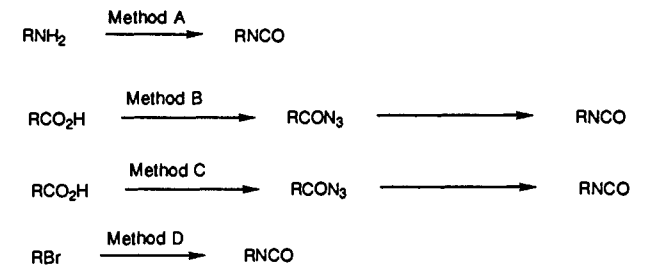
**Scheme II** [a]



[a] Reagents: (a) $Ac_2O$/NaOAc; (b) $ClSO_2NCO/CH_2Cl_2$; (c) NaOH/acetone–water; (d) $RNCO/Et_3N/DMAP/CH_2Cl_2$; (e) TFA/anisole.

**Scheme III** [a]



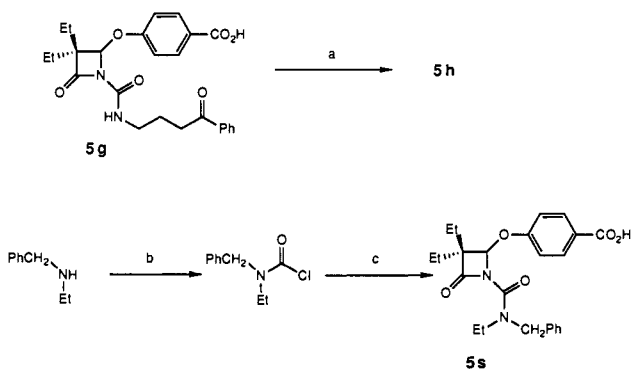
[a] Reagents: (method A) $COCl_2$/EtOAc; (method B) $(COCl)_2/CH_2Cl_2$; $NaN_3$/acetone–water; $CHCl_3$; (method C) $(PhO)_2PON_3/Et_3N/CH_2Cl_2$; (method D) NaNCO/DMF.

because it was clear from other work[18] done in parallel with this study that only one enantiomer was active against HLE.

The key intermediate for the work described here, 4-acetoxy-3,3-diethylazetidinone (**3**), was readily prepared by cycloaddition of chlorosulfonyl isocyanate to the enol acetate of 2-ethylbutyraldehyde in 35–45% yield. Since **3** decomposes on silica gel, rigorous purification was not practical, but it could be isolated as a low-melting solid after cooling of a hexane solution at −10 °C. Displacement of the acetoxy group in **3** by the sodium salt of *tert*-butyl 4-hydroxybenzoate in aqueous acetone furnished the ether **4** as a white solid. Reaction of **4** with an appropriate isocyanate in $CH_2Cl_2$ at 40 °C in the presence of triethylamine (TEA) gave the urea in very good yields. Removal of the *tert*-butyl ester by treatment with $CF_3CO_2H$ (TFA) gave the racemic acids **5** for biological evaluation.

The benzyl isocyanate used in the preparation of **2** and **7a** was commercially available. Other isocyanates required for acylation were prepared (Scheme III) by either reacting the corresponding amine with phosgene (method A) or by thermal decomposition of an acyl azide in a Curtius rearrangement (method B).[19] The acyl azide was prepared by reacting the appropriate acid chloride with $NaN_3$. In cases where the acid chloride was very unstable (**5f,g**) or where the molecule is sensitive to acid (**5n,o,p**), the azide was obtained by treating the acid with diphenyl phosphorazidate and TEA (method C).[20] The isocyanates required for **5i** and **5j** were prepared by displacement of the corresponding bromide with NaNCO (method D).[21]

**Scheme IV** [a]



[a] Reagents: (a) $NaBH_4$/EtOH; (b) $COCl_2$/EtOAc; (c) 4/$Et_3N$/DMAP/$CH_2Cl_2$.

Reduction of **5g** with $NaBH_4$ furnished the alcohol **5h** as a mixture of stereoisomers. The *N,N*-dialkyl urea analog **5s** was obtained by acylation of **4** with *N*-benzyl-*N*-ethylcarbamoyl chloride (Scheme IV). These analogs with modified substitution on the urea nitrogen are listed in Table I.

Next, the compounds (**6**, Scheme V) with substitution on the phenyl ring were prepared by analogous procedures and they are shown in Table II. Some analogs were prepared by further modifications of the initially formed urea. Thus, hydrogenation (Pd–C) of the nitro group in **6j** gave **6k**, and reductive alkylation with formaldehyde furnished the dimethylaniline **6l**. The 2-amino analog **6o** was similarly prepared. The hydroxy analogs **6m** and **6p** were obtained by hydrogenolysis (Pd–C) of the corresponding benzyl ethers. The isocyanate used in the synthesis of the 4-carboxy analog **6q** was obtained by in situ silylation of 4-(aminomethyl)benzoic acid and the reaction of the resulting trimethylsilyl ester with phosgene. The amide **6r** was prepared by reacting the acid chloride with $NH_4OH$ and removal of the *tert*-butyl group by TFA.

## Biochemical Results

The compounds were evaluated for their ability to inhibit HLE-catalyzed hydrolysis of MeO-Suc-Ala-Ala-Pro-Ala-pNA and were found to exhibit time-dependent inhibition. Therefore, second-order rate constants ($k_{obs}/I$, $M^{-1}$ $s^{-1}$), listed in Tables I and II, were determined by following the inactivation of the enzyme for 15 min as described[8,23] previously.

Starting with the benzylurea **2** ($k_{obs}/I$ = 1500 $M^{-1}$ $s^{-1}$), the elastase inhibitory activity increased as additional methylene groups were introduced between the N atom and the aromatic ring. The highest activity was seen for **5c** ($k_{obs}/I$ = 9200 $M^{-1}$ $s^{-1}$), which contained a four-carbon link. This compound was 6 times more potent than **2**; even **5e** with a $(CH_2)_6$ spacer was substantially more active than **2**. However, when an oxygen was inserted as in **5i** or **5j**, the activity was markedly reduced ($k_{obs}/I$ = 3500

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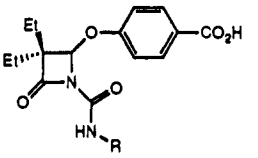
Table I. Modifications of the Nitrogen Substituent



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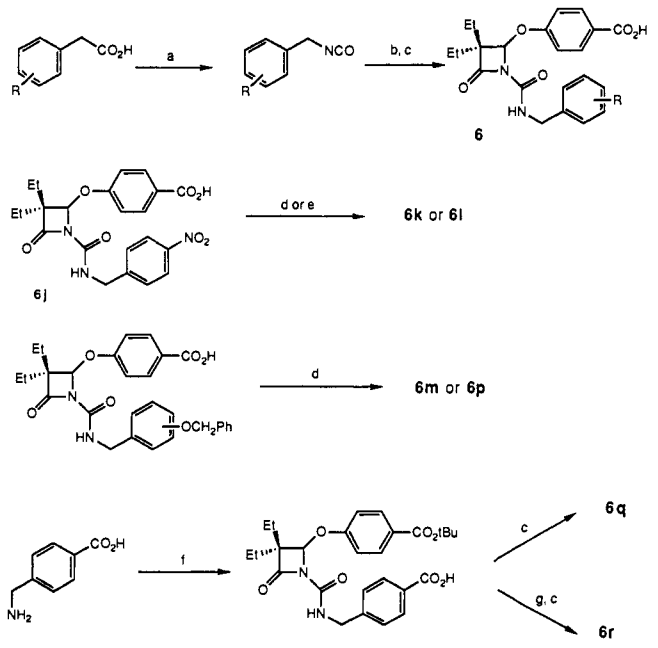
| compd | R | method[a] | $k_{obs}/I$[b] $M^{-1}$ $s^{-1}$ (SD) | lung hem.[c] % inhibn (SD) | mol formula[d] |
|---|---|---|---|---|---|
| **2** | $CH_2Ph$ | *e* | 1500 (200) | 56 (14) | $C_{22}H_{24}N_2O_5$ |
| **5a** | $(CH_2)_2Ph$ | A | 1300 (200) | 33 (19) | $C_{23}H_{26}N_2O_5 \cdot 0.25CH_2Cl_2$ |
| **5b** | $(CH_2)_3Ph$ | A | 4500 (100) | 12 (18) | $C_{24}H_{28}N_2O_5$ |
| **5c** | $(CH_2)_4Ph$ | A | 9200 (1600) | 35 (6) | $C_{25}H_{30}N_2O_5$ |
| **5d** | $(CH_2)_5Ph$ | B | 4800 (800) | 15 (33) | $C_{26}H_{32}N_2O_5$ |
| **5e** | $(CH_2)_6Ph$ | B | 5000 (700) | 14 (20) | $C_{27}H_{34}N_2O_5 \cdot 0.3H_2O$ |
| **5f** | $(CH_2)_2COPh$ | C | 2100 (100) | 8 (13) | $C_{24}H_{28}N_2O_6 \cdot 0.5H_2O$ |
| **5g** | $(CH_2)_3COPh$ | C | 2000 (200) | 0 (33) | $C_{25}H_{28}N_2O_6$ |
| **5h** | $(CH_2)_3CHOHPh$ | *e* | 5100 (500) | -22 (20) | $C_{25}H_{30}N_2O_6 \cdot 0.5H_2O$ |
| **5i** | $(CH_2)_3OPh$ | D | 3500 (400) | 40 (16) | $C_{24}H_{28}N_2O_6$ |
| **5j** | $(CH_2)_4OPh$ | D | 3400 (300) | 30 (17) | $C_{25}H_{30}N_2O_6$ |
| **5k** | $CH_2$(2-thioph) | C | 4900 (700) | 52 (16) | $C_{20}H_{22}N_2O_5S$ |
| **5l** | $CH_2$(3-thioph) | C | 1300 (60) | 53 (21) | |
| **5m** | $CH_2$(2-furan) | C | 5200 (200) | 58 (16) | $C_{20}H_{22}N_2O_6 \cdot 0.5H_2O$ |
| **5n** | $CH_2$(1-naphth) | B | 8400 (500) | | $C_{26}H_{26}N_2O_5$ |
| **5o** | $CH_2$(2-naphth) | B | 21300 (900) | 37 (20) | $C_{26}H_{26}N_2O_5$ |
| **5p** | $CH_2$(cyclohex) | A | 1400 (100) | 0 (34) | $C_{22}H_{30}N_2O_5 \cdot 0.5H_2O$ |
| **5q** | $nC_4H_9$ | *e* | 800 (20) | 45 (11) | |
| **5r** | $nC_9H_{19}$ | B | 8300 (400) | -71 (43) | $C_{24}H_{36}N_2O_5$ |
| **5s** | *f* | *e* | 2300 (100) | 34 (5) | $C_{24}H_{28}N_2O_5$ |

[a] See text for a description of the method of preparation. [b] Method reported in ref 8. [c] Percent inhibition in elastase-induced lung hemorrhage when the compound was dosed orally at 30 mg/kg 30 min before challenge by HLE. [d] Compounds were analyzed for C, H, and N. Values were within +/-0.4% of the theoretical value for the indicated molecular formula. [e] See Experimental Section. [f] See Scheme IV for structure.

**Scheme V** [a]




[a] Reagents: (a) Scheme II; (b) **4**/$Et_3N$/DMAP/$CH_2Cl_2$; (c) TFA/anisole; (d) $H_2$/Pd-C/EtOH; (e) $CH_2O$/$H_2$/Raney Ni/*t*-BuOH/HOAc; (f) $Et_2NSiMe_3$/$COCl_2$/toluene; (g) $(COCl)_2$/$CH_2Cl_2$; $NH_4OH$/$CH_2Cl_2$.

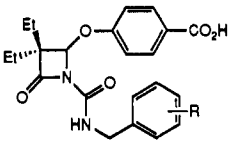
$M^{-1}$ $s^{-1}$). The presence of a hydroxyl (**5h**) or especially a carbonyl (**5g**) group in the chain also caused a loss of activity. Thus, it appears that this portion of the inhibitor may occupy a very hydrophobic region of the enzyme (see below).

Replacement of the phenyl ring by other aromatic rings was also investigated. Both the 2-furan and 2-thiophene analogs (**5m,k**) were more effective than **2**, but the 3-thiophene analog **51** was equipotent to **2**. This effect of positional isomers was more pronounced for the naphthyl compounds (**5n,o**), where the 2-naphthyl isomer **5o** with $k_{obs}/I$ = 21 300 $M^{-1}$ $s^{-1}$ was a very good inactivator of HLE. Reduction of the phenyl ring as in **5p** maintained activity. When the benzyl group was replaced by a small alkyl group as in **5q**, the potency was reduced, but **5r** with a larger alkyl group was more active. Replacement of the urea hydrogen by an ethyl group as in **5s** did not affect the activity.

The substitution on the phenyl ring (Table II) shows a similar trend. In general, compounds with large hydrophobic groups especially in the para position show very high activity. A compound with the large phenyl substituent, **6s** ($k_{obs}/I$ = 23 200 $M^{-1}$ $s^{-1}$), was at least 1 order of magnitude more active than **2**. While both *m*- and *p*-methyl substitution improved the in vitro potency, an *o*-methyl (compare **6c,d** to **6e**) caused loss of inhibitory activity. The effect of an *o*-amino (**6o**) or a hydroxy (**6p**) was not as detrimental, but the para isomers (**6k,6m**) were nonetheless more active. The effects of alkyl substituents were found to be additive. Thus, the 3,4-dimethyl analog **6u** was very active ($k_{obs}/I$ = 21 000 $M^{-1}$ $s^{-1}$), whereas in **6t** the effect of an *o*- and a *p*-methyl group balanced each other ($k_{obs}/I$ = 1500 $M^{-1}$ $s^{-1}$). The importance of positional isomers was again demonstrated for **6w** and **6x**, where **6w** was twice as active. Introduction of groups such as halogen (**6a,b**), methoxy (**6f**), acetyl (**6i**), or carboxamide (**6r**) in the para position also improved activity. The presence of an acid (**6q**) or an amine (**6k**) was tolerated, and even the dimethylamino analog **61** was quite potent. It appears that the enzyme can accommodate many functional groups in the region of the para position of the inhibitor.

The urea portion of the inhibitor very strongly influences activity, and the hydrophobic interactions appear to be more important than ionic or hydrogen bonds. The space, which is rather large, prefers large nonpolar groups such as a biphenyl or a naphthyl group, but it is interesting to

**Table II.** Effect of Substitution on the Phenyl Ring



6

| compd | R | method[a] | $k_{obs}/I$,[b] $M^{-1}$ $s^{-1}$ (SD) | lung hem.[c] % inhibn (SD) | mol formula[d] |
|---|---|---|---|---|---|
| **2** | H | *e* | 1500 (200) | 56 (14) | $C_{22}H_{24}N_2O_5$ |
| **6a** | 4-Cl | B | 3400 (500) | 29 (16) | $C_{22}H_{23}ClN_2O_5$ |
| **6b** | 4-F | B | 2300 (300) | 4 (7) | $C_{22}H_{23}FN_2O_5$ |
| **6c** | 4-Me | B | 4000 (500) | 86 (11) | $C_{23}H_{26}N_2O_5$ |
| **6d** | 3-Me | B | 7000 (300) | 47 (12) | $C_{23}H_{26}N_2O_5$ |
| **6e** | 2-Me | B | 700 (80) | 33 (27) | $C_{23}H_{26}N_2O_5 \cdot 0.5H_2O$ |
| **6f** | 4-OMe | B | 5200 (1000) | 60 (24) | $C_{23}H_{26}N_2O_6$ |
| **6g** | 4-SMe | | 9400 (300) | 28 (26) | $C_{23}H_{26}N_2O_5S \cdot CF_3CO_2H$ |
| **6h** | 4-Et | B | 8200 (900) | 46 (16) | $C_{24}H_{26}N_2O_5$ |
| **6i** | 4-COMe | B | 5900 (200) | 37 (18) | $C_{24}H_{26}N_2O_6$ |
| **6j** | 4-$NO_2$ | B | 5100 (60) | 22 (35) | $C_{22}H_{23}N_3O_7$ |
| **6k** | 4-$NH_2$ | *e* | 1800 (40) | 2 (20) | $C_{22}H_{25}N_3O_5 \cdot 0.4H_2O$ |
| **6l** | 4-NMe2 | *e* | 10000 (200) | 25 (20) | $C_{24}H_{29}N_3O_5 \cdot CF_3CO_2H$ |
| **6m** | 4-OH | *e* | 3000 (300) | −18 (27) | $C_{22}H_{24}N_2O_6$ |
| **6n** | 4-$OCH_2Ph$ | B | 12000 (1500) | −9 (24) | $C_{29}H_{30}N_2O_6 \cdot 0.25H_2O$ |
| **6o** | 2-$NH_2$ | *e* | 1200 (70) | 73 (5) | $C_{22}H_{25}N_3O_5 \cdot CF_3CO_2H$ |
| **6p** | 2-OH | *e* | 1500 (100) | −2 (43) | $C_{22}H_{24}N_2O_6$ |
| **6q** | 4-$CO_2H$ | *e* | 2200 (50) | 25 (130) | $C_{23}H_{24}N_2O_7$ |
| **6r** | 4-$CONH_2$ | *e* | 4300 (800) | 10 (25) | $C_{23}H_{25}N_3O_6 \cdot 0.5H_2O$ |
| **6s** | 4-Ph | B | 23200 (100) | 24 (10) | $C_{28}H_{28}N_2O_5$ |
| **6t** | 2,4-$Me_2$ | B | 1500 (40) | 45 (17) | $C_{24}H_{28}N_2O_5$ |
| **6u** | 3,4-$Me_2$ | B | 21000 (800) | 52 (10) | $C_{24}H_{28}N_2O_5 \cdot 0.5CF_3CO_2H$ |
| **6v** | 3,5-$Me_2$ | B | 14700 (1900) | 69 (16) | $C_{24}H_{28}N_2O_5 \cdot 0.4H_2O$ |
| **6w** | 4-OMe, 3-Me | B | 13100 (700) | 64 (17) | $C_{24}H_{28}N_2O_6 \cdot 0.7CF_3CO_2H$ |
| **6x** | 3-OMe, 4-Me | B | 6500 (200) | 76 (11) | $C_{24}H_{28}N_2O_6$ |
| **6y** | 3,4-$OCH_2O$ | A | 5000 (90) | 10 (22) | $C_{23}H_{24}N_2O_7$ |

[a] See text for a description of the method of preparation. [b] Method reported in ref 8. [c] Percent inhibition in elastase-induced lung hemorrhage when the compound was dosed orally at 30 mg/kg 30 min before challenge by HLE. [d] Compounds were analyzed for C, H, and N. Values were within +/−0.4% of the theoretical value for the indicated molecular formula. [e] See Experimental Section.

note that it can tolerate both acidic and basic functions as seen above.

## Biological Results

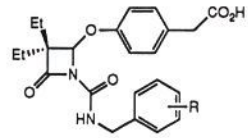
Since **2** was stable in blood for long periods, its in vivo activity was measured in an orally dosed hamster lung hemorrhage model. In this assay, which is a modification of our published procedure,[24] the hemorrhage was produced and quantitated as before by injecting 50 units of HLE into the trachea of anesthetized hamsters and by spectrophotometrically measuring the amount of hemoglobin in the lung lavage solution after 3 h. In the present assay, animals were dosed orally prior to instillation of elastase, and the percent inhibition was calculated by comparing the hemorrhage for the animals of the drug-treated group with the vehicle-only group. By varying the amount of the drug, one could obtain a dose response. Alternatively, the time between the oral dose and challenge by HLE could be changed to study time course of a compound. Initially, **2** was evaluated in this model at different doses and different times, and it was found to reproducibly give about 50% inhibition at a dose of 30 mg/kg administered 30 min prior to HLE. Therefore, this protocol was used for all the compounds and the percent inhibition from this screen is reported in Tables I and II.

Most of the compounds of this class (Tables I and II) do show oral activity, which confirmed our initial hypothesis about stability and potency. Unfortunately, in vivo activity did not improve as dramatically as enzyme inhibitory activity. Introduction of additional methylene groups between the phenyl ring and the urea N produced better HLE inhibitors, but the activity in hamsters was diminished (**5a–e**). Thus, in vivo activity depended on other factors, such as oral absorption, metabolism, and protein binding, in addition to elastase activity. It was thought that the increase in hydrophobicity with extra $CH_2$ groups could have affected these properties and thereby caused the reduction in activity. Therefore, more polar groups such as carbonyl (**5f,g**) and hydroxyl (**5h**) were introduced in the urea moiety, but both HLE and lung hemorrhage activities were diminished. The potency was restored for the ether analog **5i**. The reduction of the aromatic ring to a saturated ring (**5p**) or replacement by an alkyl group (**5r**) maintained elastase activity, but lung hemorrhage activity was lost. The lower in vivo activity of the 2-naphthyl analog **5o** cannot be due to poor oral absorption, because a significant amount of **5o** was detected in hamster blood after an oral dose of 30 mg/kg.[25] However, other pharmacokinetic properties like metabolism and tissue distribution might also be important for activity in the lung hemorrhage assay. The 2-thiophene (**5k**) and the 2-furan (**5m**) analogs are at least 3 times better at

(24) (a) Fletcher, D. S.; Osinga, D. G.; Hand, K. M.; Dellea, P. S.; Ashe, B. M.; Mumford, R. A.; Davies, P.; Hagmann, W. K.; Finke, P. E.; Doherty, J. B.; Bonney, R. J. A Comparison of alpha-1-Proteinase Inhibitor, Methoxysuccinyl-Ala-Ala-Pro-Val-Chloro-Methylketone, and Specific Beta-Lactam Inhibitors in Acute Model of Human PMN Elastase-Induced Lung Hemorrhage in the Hamster. *Am. Rev. Respir. Dis.* **1990**, *141*, 672–677. (b) Bonney, R. J.; Ashe, B.; Maycock, A.; Dellea, P.; Hand, K.; Osinga, D.; Fletcher, D.; Mumford, R.; Davies, P.; Frankenfield, D.; Nolan, T.; Schaeffer, L.; Hagmann, W.; Finke, P.; Shah, S.; Dorn, C.; Doherty, J. Pharmacological Profile of the Substituted Beta-Lactam L-659,286: A Member of a New Class of Human PMN Elastase Inhibitors. *J. Cell. Biochem.* **1989**, *39*, 47–53.

(25) Fletcher, D. S., unpublished results.

**Table III.** Activities of Phenylacetic Acids



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| compd | R | method[a] | $k_{obs}/I^b$ $M^{-1}$ $s^{-1}$ (SD) | lung heme.[c] % inhibn (SD) | mol formula[d] |
|---|---|---|---|---|---|
| **7a** | H | *e* | 3200 (300) | 71 (13) | |
| **7b** | 4-Me | B | 4500 (500) | 72 (19) | $C_{24}H_{28}N_2O_5$ |
| **7c** | 4-Ph | B | 29000 (5000) | 19 (18) | $C_{29}H_{30}N_2O_5$ |

[a] See text for a description of the method of preparation. [b] Method reported in ref 8. [c] Percent inhibition in elastase-induced lung hemorrhage when the compound was dosed orally at 30 mg/kg 30 min before challenge by HLE. [d] Compounds were analyzed for C, H, and N. Values were within +/−0.4% of the theoretical value for the indicated molecular formula. [e] See Experimental Section.

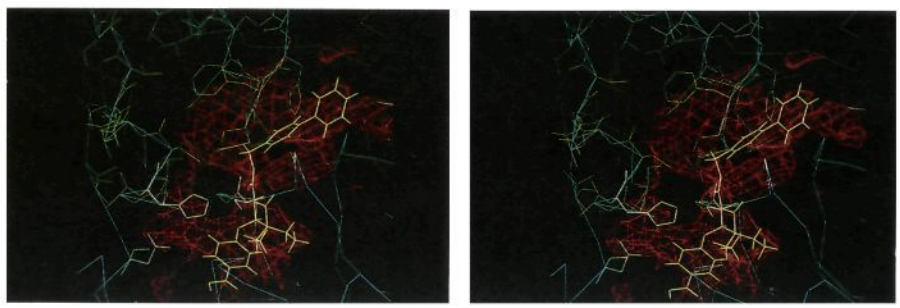


**Figure 1.** Displayed in this stereo figure is a model of the Michaelis complex of the inhibitor **6s** (shown in yellow) in the active site of HLE. The atomic coordinates of the enzyme come from the 1.8-Å-resolution X-ray structure of HLE inhibited with MeOSuccinyl-AAPA-$CH_2Cl$.[26] The position of $His^{57}$'s imidazole ring has been slightly adjusted to correspond to that found in other native serine proteases. The catalytic triad atoms of the enzyme, $Asp^{102}$, $His^{57}$, and $Ser^{195}$, are highlighted. Important features of this model are as follows: the β-lactam carbonyl oxygen is positioned in the "oxy-anion" hole and the carbonyl carbon is about 2.5 Å from the nucleophilic Oγ of $Ser^{195}$; the *gem*-dialkyl C-3 substituents are fully enclosed by the residues of the S1 pocket and "capped" with the side chain of $Phe^{192}$ (not shown); the 4-phenoxybenzoic acid at C-4 ("leaving group") occupies part of the S2 site with the acid functionality stretching into solution. Energy-optimized[27] models of the first tetrahedral intermediate along the pathway for inhibition of HLE by this class of compound indicate that rehybridization of the β-lactam ring does not affect the orientation from that shown. The red grid represents the hydrophobic character of HLE in the active site region as mapped using OPTIMOL.[27] Clearly the S1 pocket, the S2 site, and the prime sites are hydrophobic as also seen in the SAR. Residues $Phe^{41}$, $Cys^{42}$, and $Gly^{43}$ are the primary determinants of the hydrophobicity in the S1′ site. This can be seen as the large red region to the left of the benzylic position.

inhibiting HLE than the 3-thiophene **51**, but all are equipotent in vivo, which again demonstrates that many factors are responsible for oral activity.

We next studied the effect of substitution on the phenyl ring and these compounds are listed in Table II. These results were more encouraging. Methyl or methoxy groups at the 4-position improved inhibitory activity as well as potency in the lung hemorrhage assay. Since the 4-methyl analog **6c** exhibited 86% inhibition in the initial screen, it was studied in more detail. It was evaluated at 30, 10, and 3 mg/kg and an $ED_{50}$ = 10 mg/kg was estimated. A time course was also determined and the activity (88% inhibition) was unchanged even when **6c** was dosed 1 h before challenge by HLE. Compounds containing one methoxy and one methyl group (**6w,x**) or two methyl groups (**6u,v**) also had good activity. Consistent with our findings in other series, larger hydrophobic substituents found in **6n** and **6s** increased HLE activity but reduced in vivo activity. Aqueous solubility seems to be necessary for in vivo activity, but increasing solubility does not necessarily improve potency. Thus **6k,m,o–q**, which would be expected to be more soluble, are in fact less active in this assay. Compounds with neutral polar substituents (**6i,r**) were also less potent.

Next, the effect of modifying the C-4 substituent was studied briefly. It was hoped that replacement of the benzoic acid of **2** by a phenylacetic acid might improve pharmacokinetic properties. For this series, three ureas, containing a benzyl (**7a**), a 4-methylbenzyl (**7b**), and a 4-phenylbenzyl (**7c**) group, spanning the range of activities in the other class were selected. These analogs were synthesized by substituting *tert*-butyl 4-hydroxyphenylacetate for the 4-hydroxybenzoate in the displacement reaction followed by acylation as in the other series, and their activities are listed in Table III. All of them are somewhat more active against HLE than their benzoic acid counterparts (**2**, **6c**, and **6s**). In vivo both **7a** and **7b** had excellent activities very similar to their benzoic acid analogs **2** and **6c**. It was hoped that in this series the activity of the biphenyl analog **7c** would improve, but it was not better than **6s**.

## Discussion

A model for the binding of **6s** to HLE, using the atomic coordinates from the X-ray structure of the enzyme,[26] is shown in Figure 1. The energy for the HLE-**6s** complex was minimized using OPTIMOL.[27] In this model, the inhibitor is bound in the active site of the enzyme with the hydroxyl of $Ser^{195}$ in a position to interact with the β-lactam carbon and the carbonyl oxygen pointing toward the "oxy-anion hole" formed by the backbone amide NH of $Ser^{195}$

(26) 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-Å Resolution. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7–11.

and Gly[193]. The dialkyl substituent at C-3 is occupying the S1 pocket and the C-4 group points toward the S2 site. The substituent at N-1 occupies the hydrophobic prime site region of the enzyme. This model is consistent with the following trends observed in the inhibition of HLE.

(1) The residues closest to the benzyl group of the urea are Gly[43], Cys[42], and Phe[41]. Thus compounds with extra methylene groups between the urea N and the phenyl ring (**5a–c**) have stabilizing interactions with these hydrophobic residues, but introduction of more hydrophilic groups like carbonyl, alcohol or ether (**5f–j**) is disfavored and causes loss in HLE inhibitory activity.

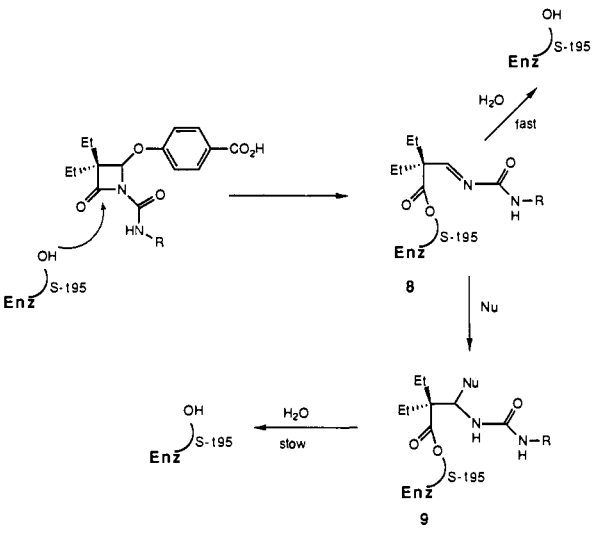
(2) This model indicates that the *o*-methyl group of **6e** will be either in close proximity to the carbonyl of Phe[41] or be in water; both of these orientations being unfavorable results in lower activity. However, either arrangement would be acceptable for the more polar hydroxy (**6p**) or an amine (**6o**) group, and the inhibitory activity is maintained.

(3) While the nonpolar substituents in the 3- and 4-positions of the benzene ring can fit in the hydrophobic pocket of the enzyme, the polar substituents can extend into the surrounding water with a single-bond rotation. Thus compounds with both acidic (**6q**) as well as basic groups (**6k,l**) in this position can bind to HLE without loosing solvation by water and they are tolerated.

A crystal structure of a related serine protease, porcine pancreatic elastase (PPE), inhibited by a peptide containing a 2-phenylethyl amide, has been reported.[28] In this structure the phenylethyl group reached out to the S2′ site, but the authors observed that there was substantial positional freedom for this group and strong hydrogen-bonding interactions were absent in this region. The results reported here strongly suggest that the benzyl group of our structure occupies a similar region of HLE, where many substituents are accommodated and the van der Waals' interactions are much more important than hydrogen bonds or other ionic interactions.

The time-dependent inactivation seen for these β-lactams may imply that after initial binding (Michaelis complex) they react further with HLE to inactivate the enzyme. A possible mechanism for inhibition by this class of compounds is outlined in Scheme VI. Attack by the hydroxyl of Ser[195] from the α-face forms a tetrahedral intermediate. Collapse of this intermediate by ring opening accompanied by or immediately followed by loss of the phenolic leaving group at C-4 would form an acyl enzyme 8 containing an imine. This intermediate can be deacylated, as occurs with substrates and other β-lactams,[29a] to regenerate active enzyme or it can react further to generate a more fully inactivated enzyme. This second inactivation step can be an addition of a nucleophile to give 9, which might further stabilize the inhibited species. This type of a "double-hit" inhibition has been confirmed crystallographically for PPE inhibited by a cephalosporin, a bicyclic β-lactam.[30] Alternatively, a

**Scheme VI**




conformational change in 8, which might make the carbonyl less accessible to an external nucleophile, could also explain the observed results. Irrespective of the precise nature of this step, the intermediate can be hydrolyzed and eventually the active enzyme is regenerated. The rate of this regeneration process can be measured experimentally. This mechanism predicts that the stability of the enzyme–inhibitor complexes may depend on the substitution at N-1 because it is present in the inhibited species, but the C-4 substituent should have no effect. The effect of the urea substituent on the rate of reactivation has been studied and will be reported separately.[29b]

## Conclusion

The results presented here clearly demonstrate that a careful analysis of the enzyme mechanism and β-lactam activation has led to more stable inhibitors of HLE with improved potencies. This has enabled us to identify a class of orally active inhibitors of HLE for the first time. The substituent at N-1 had a very dramatic effect on enzyme inhibition in vitro. The modification of the urea group also improved activity in the hamster lung hemorrhage assay, and this in vivo activity depended on many factors besides HLE activity. Further refinement of the lead structure in order to identify a highly potent, orally active compound suitable for clinical evaluation is in progress and the details of this investigation will be reported in the near future.

## Experimental Section

**General Procedures.** $^1$H NMR spectra of $CDCl_3$ solutions were recorded on 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 a LKB 9000 at an ionizing voltage of 70 eV. Flash chromatography was performed on silica gel 60 (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 sulfate. Preparative liquid chromatography (prep LC) was performed on a Waters Prep LC500 instrument with silica gel (Prep Pak) columns. Workup solutions were routinely dried by filtering through anhydrous $Na_2SO_4$, and the solvent

(27) OPTIMOL contains the MM2X and MMFF force fields, which differ from MM2 principally in that lone pairs on heteroatoms are not used and in that electrostatic interactions take place between atom-centered charges, allowing proper treatment of charged systems. MM2X has been parameterized for a wide range of functional groups but shares many parameters with MM2. OPTIMOL has been developed at Merck by T. A. Halgren and other members of Molecular Systems.

(28) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E., Jr.; Meyer, E. F.; Trainor, D. A. Crystal Structure of the Covalent Complex Formed by a Peptidyl α,α-Difluoro-β-keto Amide with Porcine Pancreatic Elastase at 1.78-Å Resolution. *J. Am. Chem. Soc.* **1989**, *111*, 3368–3374.

(29) (a) Knight, W. B.; Chabin, R.; Green, B., unpublished results. (b) Knight, W. B., unpublished results.

(30) Navia, M. A.; Springer, J. P.; Lin, T.-Y.; Williams, H. R.; Firestone, R. A.; Pisano, J. M.; Doherty, J. B.; Finke, P. E.; Hoogsteen, K. Crystallographic Study of a β-Lactam Inhibitor Complex with Elastase at 1.84 Å Resolution. *Nature* **1987**, *327*, 79–82.

was removed from the filtrate first on a rotary evaporator and finally under high vacuum. Elemental analyses were performed either by the Micro-Analytical Laboratory of Merck & Co. or by Robertson Microlit Laboratories, Inc. When elemental analyses are indicated only by symbols of elements, the analytical results obtained for these elements are within 0.4% of the theoretical values.

**1-Acetoxy-2-ethyl-1-butene.** A solution of 600 g (5.99 mol) of 2-ethylbutyraldehyde, 900 mL (8.15 mol) of acetic anhydride, and 61.5 g (0.75 mol) of sodium acetate was heated to reflux under a $N_2$ atmosphere. After 2 days the reaction mixture was poured into 1 L of $CH_2Cl_2$, 1 L of water, and 500 g of ice. The solution was neutralized by adding solid $Na_2CO_3$ (ca. 1 kg required, $CO_2$ evolution!). The layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$. The organic layer was washed with saturated NaCl and dried over $Na_2SO_4$ and the filtrate was concentrated. Distillation of the residue in vacuo furnished 464.5 g (55% yield) of 1-acetoxy-2-ethyl-1-butene: bp 65–90 °C (10 mm) [lit.[31] bp 98–101 °C (90 mm)].

**4-Acetoxy-3,3-diethyl-2-azetidinone (3).** A solution of 169 g (1.19 mol) of 1-acetoxy-2-ethyl-1-butene in 300 mL of $CH_2Cl_2$ was cooled in an ice-ethanol bath under $N_2$, and 200 g (1.41 mol) of chlorosulfonyl isocyanate (CSI) was added with an addition funnel.[32] The solution was allowed to warm to room temperature and stirred overnight. The next morning, the reaction mixture was diluted with ether and added to ice-cold $NaHCO_3$ solution containing $Na_2SO_3$. The solution was kept below 5 °C during addition. The solution was stirred with a mechanical stirrer for 2 h. The layers were separated, and the aqueous layer was extracted with ether. The combined extracts were washed with water and saturated NaCl and dried. The filtrate was concentrated in vacuo, and the dark oil obtained after the removal of the last traces of solvent under high vacuum was diluted with 100 mL of hexane. Upon cooling of this solution to −10 °C in a freezer for 2 days, a low-melting light yellow solid was obtained, which was filtered and washed with cold hexane to obtain 79.2 g (36% yield) of **3** sufficiently pure for use in the next reaction: NMR $\delta$ 0.99 (t, 3 H, $J$ = 7 Hz), 1.02 (t, 3 H, $J$ = 7 Hz), 1.72 (m, 4 H), 2.13 (s, 3 H), 5.58 (s, 1 H), 6.4 (br s, 1 H).

**1,1-Dimethylethyl 4-Hydroxybenzoate.** A pressure bottle containing a solution of 40 mL of concentrated $H_2SO_4$ in 400 mL of dioxane was cooled in an ice bath. To this solution were added 100 g (0.82 mol) of 4-hydroxybenzoic acid and 600 mL of isobutylene. The bottle was tightly capped and the cap was secured. After stirring the solution for 2 days the bottle was cooled in an ice bath and opened slowly, and the contents were poured into cold water containing 160 g of $NaHCO_3$ with stirring. The solution was extracted with ethyl acetate (EtOAc). The organic layer was washed with water (three times) and saturated NaCl and then dried. White crystals appeared in the residue after removal of solvent. The residue was diluted with ca. 500 mL of hexane and allowed to stand. The crystals were collected, washed with cold hexane, and dried to obtain 67.9 g (43% yield) of 1,1-dimethylethyl 4-hydroxybenzoate:[33] NMR $\delta$ 1.58 (s, 9 H), 6.84 (d, 2 H, $J$ = 8 Hz), 7.89 (d, 2 H, $J$ = 8 Hz).

**1,1-Dimethylethyl 4-hydroxyphenylacetate**[34] was prepared by a similar method: NMR $\delta$ 1.45 (s, 9 H), 3.46 (s, 2 H), 6.74 (d, 2 H, $J$ = 8 Hz), 7.11 (d, 2 H, $J$ = 8 Hz).

**3,3-Diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]phenyl]-oxy]-2-azetidinone (4).** To a solution of 5.82 g (30 mmol) of 1,1-dimethylethyl 4-hydroxybenzoate in 30 mL of acetone was added 30 mL (30 mmol) of 1 N NaOH. After stirring for 10 min, a solution of 5 g (27 mmol) of **3** in 20 mL of acetone was added. After stirring for 0.5 h, the reaction mixture was partitioned between water and ether. The aqueous layer was extracted with ether. The combined ether layer were washed with water and saturated NaCl and dried. The filtrate was concentrated and the residue was purified by chromatography using 20–50% ether/hexane to isolate 7.30 g (85% yield) of 4 as a white solid: NMR $\delta$ 1.03 (t, 3 H, $J$ = 7 Hz), 1.04 (t, 3 H, $J$ = 7 Hz), 1.58 (s, 9 H), 1.67–2.04 (m, 4 H), 5.41 (s, 1 H), 6.84 (d, 2 H, $J$ = 8 Hz), 7.93 (d, 2 H, $J$ = 8 Hz). Anal. ($C_{18}H_{25}NO_4$) C, H, N.

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[(3-phenylpropyl)amino]carbonyl]-2-azetidinone (5b, R = $(CH_2)_3Ph$) (Method A).** Ethyl acetate (EtOAc, 6 mL) was placed in a three-necked flask equipped with an addition funnel (without a side arm), a condenser, and a gas inlet tube, and the flask was heated in a 60 °C bath. Phosgene gas was passed through the solution and a solution of 0.28 mL (2 mmol) of 3-phenyl-1-propylamine in 5 mL of EtOAc was added at such a rate so that the white solid did not build up. After all the amine was added and the white solid dissolved, phosgene addition was stopped. After stirring for 5 min, the solution was heated in a 100 °C bath and EtOAc was removed by distillation. The residue was added to a solution of 0.25 g (0.78 mmol) of 4 in 2 mL of $CH_2Cl_2$, 1 mL of TEA, and 2 crystals of 4-(dimethylamino)pyridine (DMAP), and the solution was heated to reflux. After 2 h the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with $CH_2Cl_2$. The organic layer was washed with water, 1.2 N HCl, and saturated NaCl. The solution was dried and the filtrate was concentrated. The residue was purified by flash chromatography using a gradient of 10–20% EtOAc/hexane to yield 0.366 g (98% yield) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]-phenyl]oxy]-1-[[(3-phenylpropyl)amino]carbonyl]-2-azetidinone: (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.58 (s, 9 H), 1.66–2.1 (m, 6 H), 2.64 (t, 2 H, $J$ = 7 Hz), 3.32 (q, 2 H, $J$ = 7 Hz), 5.78 (s, 1 H), 6.6 (t, 1 H, $J$ = 6 Hz), 7.1–7.4 (m, 7 H), 8.02 (d, 2 H, $J$ = 8 Hz).

Cold trifluoroacetic acid (TFA, 2 mL) and 0.5 mL of anisole were added to 0.36 g of the urea obtained above. The solution was stirred at 0 °C for 1 h and then diluted with dichloroethane and concentrated in vacuo. The residue was purified on a flash column using a gradient of 30–40% EtOAc/hexane with 0.5% acetic acid (HOAc) to furnish 0.277 g (87% yield) of **5b** as a white solid: NMR $\delta$ 1.03 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.65–2.1 (m, 6 H), 2.66 (t, 2 H, $J$ = 7 Hz), 3.33 (q, 2 H, $J$ = 7 Hz), 5.8 (s, 1 H), 6.61 (t, 1 H, $J$ = 7 Hz), 7.1–7.4 (m, 7 H), 8.05 (d, 2 H, $J$ = 8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[(phenylmethyl)amino]carbonyl]-2-azetidinone (2)** was prepared by a similar procedure except that commercial benzyl isocyanate was used: NMR (acetone-$d_6$) $\delta$ 1.0 (t, 3 H, $J$ = 7 Hz), 1.08 (t, 3 H, $J$ = 7 Hz), 1.64-2.05 (m, 4 H), 4.5 (d, 2 H, $J$ = 6 Hz), 6.01 (s, 1 H), 7.2 (br, 1 H), 7.36 (d, 2 H, $J$ = 8 Hz), 8.02 (d, 2 H, $J$ = 8 Hz).

The following compounds were synthesized by analogous procedures.

**5a (R = $(CH_2)_2Ph$):** NMR $\delta$ 1.02 (t, 3 H, $J$ = 7 Hz), 1.04 (t, 3 H, $J$ = 7 Hz), 1.64–2.1 (m, 4 H), 2.88 (t, 2 H, $J$ = 7 Hz), 3.57 (m, 2 H), 5.78 (s, 1 H), 6.63 (br s, 1 H), 7.04–7.3 (m, 7 H), 8.04 (br s, 2 H).

**5c (R = $(CH_2)_4Ph$):** NMR $\delta$ 1.02 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.5–2.1 (m, 8 H), 2.65 (t, 2 H, $J$ = 7 Hz), 3.32 (q, 2 H, $J$ = 7 Hz), 5.58 (s, 1 H), 7.1–7.36 (m, 7 H), 8.04 (d, 2 H, $J$ = 8 Hz).

**5p (R = $CH_2$(cyclohexane)):** NMR $\delta$ 0.82–1.4 (m, 14 H), 1.44–2.1 (m, 7 H), 5.74 (s, 1 H), 7.23 (d, 2 H, $J$ = 8 Hz), 7.97 (d, 2 H, $J$ = 8 Hz).

**5q (R = $nC_4H_9$):** partial NMR (acetone-$d_6$) $\delta$ 0.94 (t, 3 H, $J$ = 7 Hz), 1.2–1.62 (m, 4 H), 4.1 (q, 2 H, $J$ = 7 Hz).

**6y (R = 3,4-$OCH_2O$):** NMR $\delta$ 1.03 (t, 3 H $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.64–2.0 (m, 4 H), 4.4 (d, 2 H, $J$ = 6 Hz), 5.81 (s, 1 H), 5.96 (s, 2 H), 6.72–6.84 (m, 4 H), 7.25 (d, 2 H, $J$ = 8 Hz), 8.06 (d,2 H, $J$ = 8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[(6-phenylhexyl)amino]carbonyl]-2-azetidinone (5e, R = $(CH_2)_6$-Ph). (Method B). Warning:** *Caution should be exercised in handling acyl azides because they are potentially explosive. We have used them in dilute solutions and the solutions were always kept cold during routine handling.* To a solution of 0.41 g (2 mmol) of 7-phenylheptanoic acid in 5 mL of $CH_2Cl_2$ containing 2 drops of dimethylformamide (DMF) was added 0.25 mL (2.9 mmol) of oxalyl chloride. Gas evolution stopped after 10 min, and the solution was stirred for another 15 min and then

(31) Casey, C. P.; Cyr, C. R.; Iron Carbonyl Catalyzed Isomerization of 3-Ethyl-1-pentene. Multiple Olefin Isomerizations via a $\pi$-Allyl Metal Hydride Intermediate. *J. Am. Chem. Soc.* **1973**, *95*, 2248–2253.

(32) This procedure is adapted: 4-Acetoxyazetidin-2-one: Synthesis of a Key Beta-Lactam Intermediate by a [2 + 2] cycloaddition Route. Mickel, S. J.; Hsiao, C.-N.; Miller, M. J. *Org. Syn.* **1987**, *65*, 135–139.

(33) Sarges, R. Ger. Pat. 1,912,416, 1969 (*Chem. Abstr.* **1970**, *72*, 66951*d*).

(34) Tomita, M.; Fujitani, K.; Aoyagi, Y.; Kajita, Y. Studies on the Alkaloids of Menispermaceous Plants. CCXLIV. Synthesis of dl-Cepharanthine. *Chem. Pharm. Bull.* **1968**, *16*, 217–226.

concentrated in vacuo. The residue was dissolved in 3 mL of dry acetone, and this solution was added dropwise to a solution of 0.3 g (4.6 mmol) of $NaN_3$ in 3 mL of water, the temperature being kept between 0 and 5 °C. After stirring of the reaction mixture at this temperature for 30 min, it was diluted with chloroform and washed with cold water and saturated NaCl. The chloroform solution was dried and concentrated to approximately 5 mL, the bath temperature being kept below 30 °C. This solution of acyl azide was heated to reflux. (Gas evolution!) After 30 min the reaction was complete as indicated by IR, where the azide band at 2140 $cm^{-1}$ was replaced by isocyanate at 2280 $cm^{-1}$. The solution was concentrated to give 0.4 g of residue, which was added to a solution of 0.25 g (0.78 mmol) of 4 in 2 mL of $CH_2Cl_2$ containing 0.5 mL (3.5 mmol) of TEA and 2 crystals of DMAP, and the solution was heated to reflux. After stirring overnight, the reaction mixture was diluted with $CH_2Cl_2$, washed with saturated $NaHCO_3$, water, 1.2 N HCl, and saturated NaCl, and dried. The filtrate was concentrated and the residue was chromatographed using 10–20% EtOAc/hexane to furnish 0.36g (88% yield) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]phenyl]oxy]-1[[(6-phenylhexyl)amino]carbonyl]-2-azetidinone: NMR δ 1.0 (t, 3 H, $J$ = 7 Hz), 1.04 (t, 3 H, $J$ = 7 Hz), 1.16–2.06 (m, 12 H), 1.58 (s, 9 H), 2.61 (t, 2 H, $J$ = 7 Hz), 3.28 (q, 2 H, $J$ = 7 Hz), 5.74 (s, 1 H), 6.53 (t, 1 H, $J$ = 7 Hz), 7.04–7.38 (m, 7 H), 7.96 (d, 2 H, $J$ = 8 Hz).

This ester was treated with cold TFA (2 mL) and 0.5 mL of anisole and the solution was cooled in an ice bath. After stirring for 1 h the reaction mixture was diluted with dichloroethane and concentrated. The residue was purified by prep TLC using 50% EtOAc/hexane with 1% HOAc to isolate 0.29 g of **5e**: NMR δ 1.02 (m, 6 H), 1.16–2.1 (m, 12 H), 2.61 (t, 3 H, $J$ = 7 Hz), 3.3 (m, 2 H), 5.8 (s, 1 H), 6.6 (br s, 1 H), 7.06–7.3 (m, 7 H), 8.01 (br s, 2 H).

The following compounds were also prepared by this method.

**5d (R = $(CH_2)_5Ph$)**: NMR δ 1.02 (t, 3 H, $J$ = 7 Hz). 1.06 (t, 3 H, $J$ = 7 Hz), 1.2–2.1 (m, 10 H), 2.62 (t, 2 H, $J$ = 7 Hz), 3.3 (q, 2 H, $J$ = 7 Hz), 5.78 (s, 1 H), 6.58 (t, 1 H, $J$ = 6 Hz), 7.1–7.3 (m, 7 H), 8.04 (d, 2 H, $J$ = 8 Hz).

**5n (R = $CH_2$(1-naphthalene))**: partial NMR δ 5.0 (d, 2 H, $J$ = 6 Hz), 7.34–8.1 (m, 7 H).

**5o (R = $CH_2$(2-naphthalene))**: partial NMR δ 4.48 (d, 2 H, $J$ = 6 Hz), 7.18–7.94 (m, 7 H).

**5r (R = $nC_9H_{19}$)**: partial NMR δ 0.8–2.1 (m, 17 H, 3.27 (q, 2 H, $J$ = 7 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[(4-oxo-4-phenylbutyl)amino]carbonyl]-2-azetidinone (5g, R = $(CH_2)_3COPh$) (Method C)**. To a solution of 0.384 g (2 mmol) of 4-benzoylbutyric acid in 5 mL of $CH_2Cl_2$ and 0.28 mL (2 mmol) of TEA was added 0.44 mL (2 mmol) of diphenyl phosphorazidate.[20] After stirring for 2.5 h, the yellow solution was heated in a 50 °C bath. After 2 h, 0.25 g (0.78 mmol) of 4, 0.3 mL (2.14 mmol) of TEA, and 2 crystals of DMAP were added. After 2 h at reflux temperature, the solution was allowed to stir at room temperature overnight. The reaction mixture was diluted with $CH_2Cl_2$, washed with saturated $NaHCO_3$, water, 1.2 N HCl, and saturated NaCl, and dried. The filtrate was concentrated and the residue was purified on a flash column using 10–20% EtOAc/hexane to furnish 0.356 g (89% yield) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]phenyl]oxy]-1-[[(4-oxo-4-phenylbutyl)amino]carbonyl]-2-azetidinone: NMR δ 1.0 (t, 3 H, $J$ = 7 Hz), 1.04 (t, 3 H, $J$ = 7 Hz), 1.59 (s, 9 H), 1.7–2.1 (m, 6 H), 3.02 (t, 2 H, $J$ = 7 Hz), 3.4 (q, 2 H, $J$ = 7 Hz), 5.7 (s, 1 H), 6.65 (t, 1 H, $J$ = 6 Hz), 7.18 (d, 2 H, $J$ = 8 Hz), 7.4–7.6 (m, 3 H), 7.9 (m, 4 H).

Cold TFA (2 mL) and 0.5 mL of anisole were added to this ester and the solution was cooled in an ice bath. After stirring for 1 h, the solution was diluted with 10 mL of dichloroethane and concentrated. The solid residue was recrystallized from ether. The solid was filtered, washed with cold ether, and dried to isolate 0.284 g (90% yield) of **5g**: NMR δ 1.04 (m, 6 H), 1.7–2.1 (m, 6 H), 3.04 (t, 2 H, $J$ = 7 Hz), 3.4 (q, 2 H, $J$ = 7 Hz), 5.8 (s, 1 H), 6.70 (t, 1 H, $J$ = 7 Hz), 7.15–7.6 (m, 5 H), 7.96 (d, 2 H, $J$ = 8 Hz), 8.04 (d, 2 H, $J$ = 8 Hz).

In an analogous manner the following compounds were synthesized.

**5f (R = $(CH_2)_2COPh$)**: NMR δ 1.04 (m, 6 H0, 1.66–2.0 (m, 4 H), 3.28 (t, 2 H, $J$ = 7 Hz), 3.74 (m, 2 H), 5.79 (s, 1 H), 7.1 (t, 1 H, $J$ = 6 Hz), 7.18–7.6 (m, 5 H), 7.96 (d, 2 H, $J$ = 8 Hz), 8.04 (d, 2 H, $J$ = 8 Hz).

**5k (R = $CH_2$(2-thiophene))**: partial NMR δ 4.69 (d, 2 H, $J$ = 6 Hz), 6.98 (m, 1 H), 7.28 (br s, 2 H).

**5l (R = $CH_2$(3-thiophene)**: partial NMR δ 4.5 (d, 2 H, $J$ = 7 Hz), 7.06 (m, 1 H), 7.16–7.4 (m, 2 H).

**5m (R = $CH_2$(2-furan)**: partial NMR δ 4.52 (d, 2 H, $J$ = 6 Hz), 6.3 (d, 1 H, $J$ = 8 Hz), 7.15–7.4 (m, 2 H).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[(4-hydroxy-4-phenylbutyl)amino]carbonyl]-2-azetidinone (5h, R = $(CH_2)_3$-CHOHPh)**. A suspension of 90 mg (0.2 mmol) of **5g** in 3 mL of ethanol was treated with 9 mg (0.23 mmol) of $NaBH_4$. Gas evolution was observed and the solid dissolved. The reaction was not complete after stirring for 0.5 h, so another 8 mg (0.2 mmol) of $NaBH_4$ was added. After an additional 0.5 h, the solution was quenched with 1.2 N HCl and extracted with EtOAc. The organic layer was washed with water and saturated NaCl and dried. The residue after concentration of the filtrate was purified by prep TLC using 50% EtOAc/hexane containing 1% HOAc to furnish 83 mg (92% yield) of **5h** as a white foam: NMR δ 1.04 (m, 6 H), 1.6–2.0 (m, 8 H), 3.36 (q, 2 H, $J$ = 7 Hz), 4.73 (t, 1 H, $J$ = 7 Hz), 5.80 (s, 1 H), 6.69 (t, 1 H, $J$ = 6 Hz), 7.1–7.36 (m, 7 H), 8.03 (d, 2 H, $J$ =8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[(4-phenoxybutyl)amino]carbonyl]-2-azetidinone (5j, R = $(CH_2)_4$-OPh) (Method D)**. To a suspension of 187 mg (2.88 mmol) of sodium cyanate in 3 mL of DMF was added 632 mg (2.76 mmol) of 1-bromo-4-phenoxybutane, and the mixture was heated in a 100 °C bath for 1 h.[21] The solution was cooled to 80 °C and 400 mg (1.25 mmol) of 4 was added. The reaction mixture was stirred at 80 °C overnight and then cooled and diluted with EtOAc. The EtOAc solution was washed with cold 1.2 N HCl, water, saturated $NaHCO_3$, and saturated NaCl and dried. The filtrate was concentrated and the residue was chromatographed on a flash column using 10–20% EtOAc/hexane to isolate 428 mg (67% yield) of 3,3-diethyl-4-[[4-[(1,1dimethylethoxy)carbonyl]-phenyl]oxy]-1-[[(4phenoxybutyl)amino]carbonyl]-2-azetidinone: NMR δ 1.0 (t, 3 H, $J$ = 7 Hz), 1.08 (t, 3 H, $J$ = 7 Hz), 1.59 (s, 9 H), 1.64–2.1 (m, 8 H), 3.38 (q, 2 H, $J$ = 7 Hz), 3.98 (t, 2 H, $J$ = 7 Hz), 5.74 (s, 1 H), 6.66 (t, 1 H, $J$ = 6 Hz), 6.72–7.4 (m, 7 H), 7.98 (d, 2 H, $J$ = 8 Hz). Deesterification as described for **5b** furnished 257 mg (73% yield) of **5j**: NMR δ 1.04 (t, 3 H, $J$ = 7 Hz), 1.07 (t, 3 H, $J$ = 7 Hz), 1.6–2.0 (m, 8 H), 3.4 (q, 2 H, $J$ = 7 Hz), 4.01 (t, 2 H, $J$ = 7 Hz), 5.8 (s, 1 H), 6.68 (t, 1 H, $J$ = 6 Hz), 6.72–7.3 (m, 7 H), 8.04 (d, 2 H, $J$ = 8 Hz).

In a similar manner **5i** (R = $(CH_2)_3OPh$) was synthesized: NMR δ 1.03 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.66–2.16 (m, 6 H), 3.36 (q, 2 H, $J$ = 7 Hz), 4.08 (t, 2 H, $J$ = 7 Hz), 5.81 (s, 1 H), 6.8–7.4 (m, 8 H), 8.05 (d, 2 H, $J$ = 8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-(*N*-ethyl-*N*-benzylcarbamoyl)-2-azetidinone (5s, R = N(Et)($CH_2Ph$))**. Phosgene gas was passed through 6 mL of EtOAc heated in a 60 °C bath. A solution of 0.3 mL (2 mmol) of *N*-ethylbenzylamine in 5 mL of EtOAc was added dropwise at such a rate so that the white solid did not build up. Phosgene addition was stopped after all the amine was added, and a clear solution was formed. EtOAc was removed by distillation, and the residue was added to a solution of 0.25 g (0.78 mmol) of 4 in 3 mL of $CH_2Cl_2$, 1 mL (7.14 mmol) of TEA, and a 3 crystals of DMAP. After heating of the solution to reflux for 16 h, it was diluted with $CH_2Cl_2$ and washed with saturated $NaHCO_3$, water, 1.2 N HCl, and saturated NaCl. The organic layer was dried and concentrated. The residue was chromatographed using 10–20% EtOAc/hexane to obtain (3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]-phenyl]oxy]-1-(*N*-benzyl-*N*-ethylcarbamoyl)-2-azetidinone: NMR δ 1.0 (m, 6 H), 1.12 (t, 3 H, $J$ = 7 Hz), 1.59 (s, 9 H), 1.65–2.0 (m, 4 H), 3.22 (br s, 1 H), 3.6 (m, 1 H), 4.47 (d, 1 H, $J$ = 16 Hz), 4.84 (br s, 1 H), 5.98 (s, 1 H), 7.06–7.38 (m, 7 H), 7.98 (d, 2 H, $J$ = 8 Hz). This ester was deblocked as described for **5b** to furnish 0.306 g (92% yield) of **5s**: NMR δ 1.02 (m, 6 H), 1.14 (t, 3 H, $J$ = 7 Hz), 1.64–2.0 (m, 4 H), 3.24 (br s, 1 H), 3.62 (m, 1 H), 4.48 (d, 1 H, $J$ = 16 Hz), 4.84 (br s, 1 H), 6.02 (s, 1 H), 7.04–7.4 (m, 7 H), 8.08 (d, 2 H, $J$ = 8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[[(4-methylphenyl)methyl]amino]carbonyl]-2-azetidinone (6c, R = 4-$CH_3$)**. 4-Methylbenzyl isocyanate was prepared as described in method B above. A solution of 0.32 g (1 mmol) of 4 in 5 mL

of $CH_2Cl_2$ containing 0.14 mL (1 mmol) of TEA and 2 crystals of DMAP was treated with 0.44 g (3 mmol) of this isocyanate. After stirring for 24 h, the solution was diluted, washed with 2% citric acid solution, water, and saturated NaCl, and then dried. The residue after removal of solvent was purified by chromatography using 20% ether/hexane to isolate 0.454 g (97% yield) of 3,3-diethyl-4-[[4-[(1,1dimethylethoxy)carbonyl]-phenyl]oxy]-1[[[(4-methylphenyl)methyl]amino]-carbonyl]-2-azetidinone: NMR δ 1.0 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.56 (s, 9 H), 1.6–2.1 (m, 4 H), 2.3 (s, 3 H), 4.43 (d, 2 H, $J$ = 6 Hz), 5.76 (s, 1 H), 6.9 (br, 1 H), 7.73 (m, 6 H), 8.05 (d, 2 H, $J$ = 8 Hz).

This ester was dissolved in 4 mL of TFA, and the solution was stirred in an ice bath for 1 h. The solution was concentrated and the residue was triturated with hexane. The white solid formed was filtered, washed with ether, and dried to furnish 0.27 g (73% yield) of **6c**: NMR δ 1.02 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.64–2.1 (m, 4 H), 2.34 (s, 3 H), 4.46 (d, 2 H, $J$ = 6 Hz), 5.79 (s, 1 H), 6.88 (t, 1 H, $J$ = 6 Hz), 7.06–7.3 (m, 6 H), 8.05 (d, 2 H, $J$ = 8 Hz).

Other compounds prepared by this procedure and their partial NMR are as follows: **6a** (R = 4 Cl) δ 7.26 (m, 4 H); **6b** (R = 4-F) δ 6.92–7.3 (m, 4 H); **6d** (R = 3-Me) δ 2.35 (s, 3 H), 7.06–7.4 (m, 4 H); **6e** (R = 2-Me) δ 2.37 (s, 3 H), 7.2 (m, 4 H); **6f** (R = 4-OMe) δ 3.8 (s, 3 H), 6.9 (d, 2 H, $J$ = 8 Hz), 7.25 (d, 2 H, $J$ = 8 Hz); **6g** (R = 4-SMe) δ 2.49 (s, 3 H), 7.18–7.3 (m, 4 H); **6h** (R = 4-Et) δ 1.24 (t, 3 H, $J$ = 7 Hz), 2.65 (q, 2 H, $J$ = 7 Hz), 7.2 (m, 4 H); **6i** (R = 4-COMe) δ 2.6 (s, 3 H), 7.34–8.0 (m, 4 H); **6j** (R = 4-$NO_2$) δ 7.48 (d, 2 H, $J$ = 8 Hz), 8.18 (d, 2 H, $J$ = 8 Hz); **6n** (R = 4-$OCH_2Ph$) δ 5.06 (s, 2 H), 6.8–7.46 (m, 9 H); **6t** (R = 2,4-$Me_2$) δ 2.31 (s, 6 H), 6.9–7.3 (m, 3 H); **6u** (R = 3,4-$Me_2$) δ 2.25 (s, 6 H), 7.0–7.3 (m, 3 H); **6w** (R = 4-OMe, 3-Me) δ 2.22 (s, 3 H), 3.82 (s, 3 H), 6.76–7.6 (m, 3 H); **6x** (R = 3-OMe, 4-Me) δ 2.19 (s, 3 H), 3.82 (s, 3 H), 6.76–7.18 (m, 3 H).

Reduction of **6j** in ethanol using $H_2$ and 10% Pd–C furnished **6k** (R = 4-$NH_2$): partial NMR δ 6.67 (d, 2 H, $J$ = 8 Hz), 7.13 (d, 2 H, $J$ = 8 Hz). **6o** (R = 2-$NH_2$) was similarly prepared: partial NMR δ 6.7-7.3 (m, 4 H).

Hydrogenation of **6n** on a Parr shaker using 10% Pd–C in ethanol gave **6m** (R = 4-OH): partial NMR δ 6.84 (d, 2 H, $J$ = 8 Hz), 7.15 (d, 2 H, J = 8 Hz). **6p** (R = 2-OH) was obtained by the same procedure: partial NMR δ 6.72–7.3 (m, 4 H).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[[[4-(dimethylamino)phenyl]methyl]amino]carbonyl]-2-azetidinone (6l, R = 4-$N(CH_3)_2$).** A solution of 0.295 g (0.6 mmol) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]phenyl]-oxy]-1-[[[(4-nitrophenyl)methyl]amino]carbonyl]-2-azetidinone in 10 mL of *tert*-butylalcohol, 1 mL of HOAc containing 0.5 mL of 37% formaldehyde, and 0.2 g of Raney nickel was hydrogenated at a Parr apparatus. After 1 h the solution was filtered, and the catalyst was washed with EtOAc. The filtrate was concentrated, and the residue after purification was treated with TFA as described for **6c** to isolate 0.203 g (76% yield) of **6l** after purification: partial NMR δ 2.98 (s, 3 H), 6.86 (d, 2 H, $J$ = 8 Hz), 7.24 (d, 2 H, $J$ = 8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[[(4-carboxyphenyl)methyl]amino]carbonyl]-2-azetidinone (6q, R = 4-$CO_2H$).** To 3.02 g (20 mmol) of 4-(aminomethyl)benzoic acid was added 11.4 mL (60 mmol) of *N,N*-diethyltrimethylsilylamine, and the solution was heated to reflux. After heating overnight, the solution was cooled, diluted with 20 mL of toluene, and added dropwise to 20 mL of phosgene (12.5%) in toluene in a three-necked flask equipped with a dry ice condenser, the temperature being kept between 5 and 7 °C. After the addition was complete, the flask was allowed to warm to room temperature during the next 2 h. The precipitated white solid was filtered under a $N_2$ atmosphere and the filtrate was concentrated to give 3.2 g of the isocyanate[22] as an oil. One-half of this oil (1.6 g) was added to a solution of 1 g (3.13 mmol) of 4 in 10 mL of $CH_2Cl_2$, 3 mL (21 mmol) of TEA, and 3 crystals of DMAP. After 4 h at reflux, the reaction mixture was poured into 1.2 N HCl, diluted with $CH_2Cl_2$, and centrifuged to separate the layers. The aqueous layer was extracted with $CH_2Cl_2$, and the organic layer was washed with water and saturated NaCl. The filtrate, after drying, was concentrated, and the residue was purified on a flash column using 20–30% EtOAc/hexane containing 0.5% HOAc to isolate 0.73 g (47% yield) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)-carbonyl]phenyl]oxy]-1-[[[(4-carboxyphenyl)methyl]-amino]carbonyl]-2-azetidinone: NMR δ 1.04 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.64–2.1 (m, 4 H), 4.57 (d, 2 H, $J$ = 6), 5.79 (s, 1 H), 7.06 (t, 1 H, $J$ = 6 Hz), 7.22 (d, 2 H, $J$ = 8 Hz), 7.42 (d, 2 H, $J$ = 8 Hz), 7.99 (d, 2 H, $J$ = 8 Hz), 8.08 (d, 2 H, $J$ = 8 Hz). Removal of the *tert*-butyl ester as described for **5b** furnished **6q**: partial NMR δ 7.22 (d, 2 H, $J$ = 8 Hz), 7.86 (d, 2 H, $J$ = 8 Hz).

**4-[(4-Carboxyphenyl)oxy]-3,3-diethyl-1-[[[(4-carbamoylphenyl)methyl]amino]carbonyl]-2-azetidinone (6r, R = 4-$CONH_2$).** A solution of 0.15 g (0.3 mmol) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]phenyl]oxy]-1-[[[(4-carboxyphenyl)methyl]amino]carbonyl]-2-azetidinone in 3 mL of $CH_2Cl_2$ and 1 drop of DMF was treated with 0.2 mL (2.3 mmol) of oxalyl chloride. After 0.5 h the solution was concentrated, the residue was dissolved in 3 mL of $CH_2Cl_2$, and 0.5 mL of $NH_4OH$ was added. The mixture was stirred for 0.5 h, diluted with $CH_2Cl_2$, and washed with saturated $NaHCO_3$, water, and saturated NaCl. The solution was dried and concentrated, and the residue was purified using 30–100% EtOAc/hexane to isolate 0.1 g (67% yield) of 3,3-diethyl-4-[[4-[(1,1-dimethylethoxy)carbonyl]phenyl]oxy]-1-[[[(4-carbamoylphenyl)methyl]amino]carbonyl]-2-azetidinone: NMR δ 1.02 (t, 3 H, $J$ = 7 Hz), 1.05 (t, 3 H, $J$ = 7 Hz), 1.59 (s, 9 H), 1.64–2.04 (m, 4 H), 4.52 (d, 2 H, $J$ = 6 Hz), 5.77 (s, 1 H), 6.2 (br s, 2 H), 7.02 (t, 1 H, $J$ = 6 Hz), 7.1 (d, 2 H, $J$ = 8 Hz), 7.36 (d, 2 H, $J$ = 8 Hz), 7.79 (d, 2 H, $J$ = 8 Hz), 7.97 (d, 2 H, $J$ = 8 Hz). Deesterification by TFA as described for **5b** gave 0.07 g (79% yield) of **6r**: partial NMR (DMSO) δ 7.36 (d, 2 H, $J$ = 8 Hz), 7.84 (d, 2 H, $J$ = 8 Hz).

**4-[[4-(Carboxymethyl)phenyl]oxy]-3,3-diethyl-1-[[[(4-methylphenyl)methyl]amino]carbonyl]-2-azetidinone (7b, R = 4-$CH_3$).** to a solution of 0.624 g (3 mmol) of *tert*-butyl 4-hydroxyphenylacetate in 3 mL of acetone was added 1.5 mL of 2 N NaOH. After stirring for 5 min, a solution of 0.555 g of **3** in 3 mL of acetone was added. The solution was stirred for 1 h and concentrated. The residue was partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was washed with water and saturated NaCl and dried. The residue after concentration was purified by chromatography using 10–30% EtOAc/hexane to obtain 0.88 g (88% yield) of 3,3-diethyl-4-[[4-[[(1,1-dimethylethoxy)carbonyl]methyl]phenyl]oxy]-2-azetidinone: NMR δ 1.04 (t, 3 H, $J$ = 7 Hz), 1.07 (t, 3 H, $J$ = 7 Hz), 1.44 (s, 9 H), 1.68–2.1 (m, 4 H), 3.49 (s, 2 H), 5.34 (s, 1 H), 6.5 (br s, 1 H), 6.82 (d, 2 H, $J$ = 8 Hz), 7.21 (d, 2 H, $J$ = 8 Hz).

A solution of 0.15 g (0.45 mmol) of the azetidinone in 2 mL of $CH_2Cl_2$ was treated with 0.25 g (1.7 mmol) of 4-methylbenzyl isocyanate as described for the preparation of **6c** above. After chromatographic purification, 0.175 g (81% yield) of 3,3-diethyl-4-[[4-[[(1,1-dimethylethoxy)carbonyl]methyl]phenyl]oxy]-1-[[[(4-methylphenyl)methyl]amino]carbonyl]-2-azetidinone was isolated: NMR δ 0.98 (t, 3 H, $J$ = 7 Hz), 1.05 (t, 3 H, $J$ = 7 Hz), 1.68–2.1 (m, 4 H), 2.35 (s, 3 H), 3.48 (s, 2 H), 4.45 (d, 2 H, $J$ = 6 Hz), 5.65 (s, 1 H), 6.86 (br t, 1 H), 7.08–7.3 (m, 8 H). This ester was treated with 2 mL of cold TFA and 0.5 mL of anisole. After stirring for 1 h at ice-bath temperature, the solution was diluted with dichloroethane and concentrated. The residue was chromatographed on prep TLC using 50% EtOAc/hexane containing 1% HOAc to isolate 0.144 g (93% yield) of **7b**: NMR δ 1.0 (t, 3 H, $J$ = 7 Hz), 1.06 (t, 3 H, $J$ = 7 Hz), 1.7–2.1 (m, 4 H), 2.34 (s, 3 H), 3.62 (s, 2 H), 4.48 (d, 2 H, $J$ = 6 Hz), 5.67 (s, 1 H), 6.89 (t, 1 H, $J$ = 6 Hz), 7.1–7.32 (m, 8 H).

By a similar procedure, the following compounds were synthesized.

**7a (R = H)**: NMR δ 1.02 (t, 3 H, $J$ = 7 Hz), 1.08 (t, 3 H, $J$ = 7 Hz), 1.7–2.1 (m, 4 H), 3.64 (s, 2 H), 4.51 (d, 2 H, $J$ = 6 Hz), 5.68 (s, 1 H), 6.94 (br, 1 H), 7.1–7.4 (m, 9 H).

**7c (R = 4-Ph)**: NMR δ 1.0 (t, 3 H, $J$ = 7 Hz), 1.07 (t, 3 H, $J$ = 7 Hz), 1.7-2.1 (m, 4 H), 3.61 (s, 2 H), 4.55 (d, 2 H, $J$ = 6 Hz), 5.68 (s, 1 H), 6.98 (t, 1 H, $J$ = 6 Hz), 7.1–7.7 (m, 13 H).

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