Nonpeptidic Inhibitors of Human Neutrophil Elastase. 7. Design, Synthesis, and *in Vitro* Activity of a Series of Pyridopyrimidine Trifluoromethyl Ketones†

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Received September 18, 1995⊗

Using molecular modeling and the information derived from X-ray crystal structures of human neutrophil elastase (HNE) and porcine pancreatic elastase (PPE) complexed to peptidic ligands, we have developed a new series of nonpeptidic inhibitors of HNE, the pyridopyrimidine trifluoromethyl ketones (TFMKs). These bicyclic inhibitors were designed to extend the concept of the related pyridone trifluoromethyl ketones by incorporating a rigidly positioned carbonyl group to participate in a hydrogen bonding interaction with the backbone NH groups of Gly-218 and Gly-219 of the enzyme. In addition, the pyrimidine ring serves as a scaffold to vector substituents toward the S_5-S_4 subsites of the enzyme's extended binding pocket. Furthermore, the heteroatoms of the pyrimidine ring generally increase the aqueous solubility of the pyridopyrimidines relative to pyridone TFMKs. Pyridopyrimidine TFMKs containing a 6-phenyl substituent afforded potent inhibitors of elastase, and several inhibitors from this class of compounds possessed aqueous solubilities of >0.1 mg/mL and K_i values of ≤ 10 nM.

Introduction

For over a quarter century, researchers in both industry and academics have pursued the identification of compounds which would prevent the tissue destruction caused by the proteolytic enzyme human neutrophil elastase (HNE, EC 3.4.21.37).[1] This search has resulted in the development of numerous strategies to inhibit HNE and led to the discovery of several classes of potent HNE inhibitors.[2] While no elastase inhibitor has yet been approved for commercial use, this class of compounds should afford extremely important therapeutic agents. HNE is one of the most promiscuous proteolytic enzymes in the body with the ability to degrade a variety of endogenous and exogenous proteins.[3] As a result, HNE is believed to play a role in either initiating or contributing to the pathological effects associated with pulmonary emphysema,[4] adult respiratory distress syndrome,[5] rheumatoid arthritis,[6] atherosclerosis,[7] cystic fibrosis,[8] and other inflammatory disorders.[9] Under normal homeostatic conditions, the destructive activity of elastase is controlled by a number of endogenous proteinase inhibitors. However, in the disease state, the balance between HNE and its inhibitors is shifted in favor of the enzyme. The search for elastase inhibitors has been aimed at discovering drugs which would supplement the body's elastase inhibitory capacity and restore the enzyme−inhibitor balance.

One of the first diseases associated with HNE was emphysema. The seminal studies by Laurell and Eriksson led to the hypothesis that emphysema was caused by the uncontrolled destruction of lung tissue as a result of a deficiency in serum levels of one the primary endogenous inhibitors of HNE, α_1-proteinase inhibitor.[10,11] Consequently, the initial development of HNE inhibitors was focused on the identification of compounds which possessed physiochemical properties suitable for administration intratracheally to treat inflammatory pulmonary diseases. Many different types of inhibitors have been evaluated for their ability to inhibit the pulmonary effects of HNE when administered intratracheally.[12] Several classes of these inhibitors have been shown to be particularly effective in inhibiting HNE-induced lung injury including fluorinated ketones,[13−15] α-ketoheterocycles,[16] boronic acids,[17−19] and β-lactams.[20−22]

The elastase inhibition program at ZENECA initially focused on the development of peptide-based electrophilic ketone inhibitors of HNE which would be suitable for aerosol administration. This effort culminated in the identification of three series of peptidyl ketones which afford unparalleled protection against the destructive effects of HNE following intratracheal administration: trifluoromethyl ketones (**A**, Figure 1),[11,12,23,24] α,α-difluro-β-keto amides (**B**),[25,26] and α-ketobenzoxazoles (**C**).[14,27,28] While intratracheal administration of drugs may be an acceptable, in some cases even preferred, method of treating pulmonary diseases such as emphysema and cystic fibrosis, oral dosing is the method of choice for treating most diseases, both pulmonary and nonpulmonary. This is due to the comparative ease of administration, which not only may improve effectiveness but also increases patient compliance. Unfortunately, the physiochemical properties of a compound which make it suitable for intratracheal administration generally render it ineffective following oral dosing. For example, diarylacylsulfonamide peptidyl trifluoromethyl ketones (e.g. **A**) have a long residence time in the lung following intratracheal administration as a result of their relative inability to cross the interstitium and penetrate the capillary membranes perfusing the lung. However, this property confers low bioavailability following oral administration as a result of poor absorption from the gastrointestinal tract. Peptides are often inactive following oral administration not only due to poor absorption but also as the result of extensive metabolism and/or rapid elimination. Therefore, large modifications to the structure of an

† For part 6 in this series see ref 34.
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⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1996.

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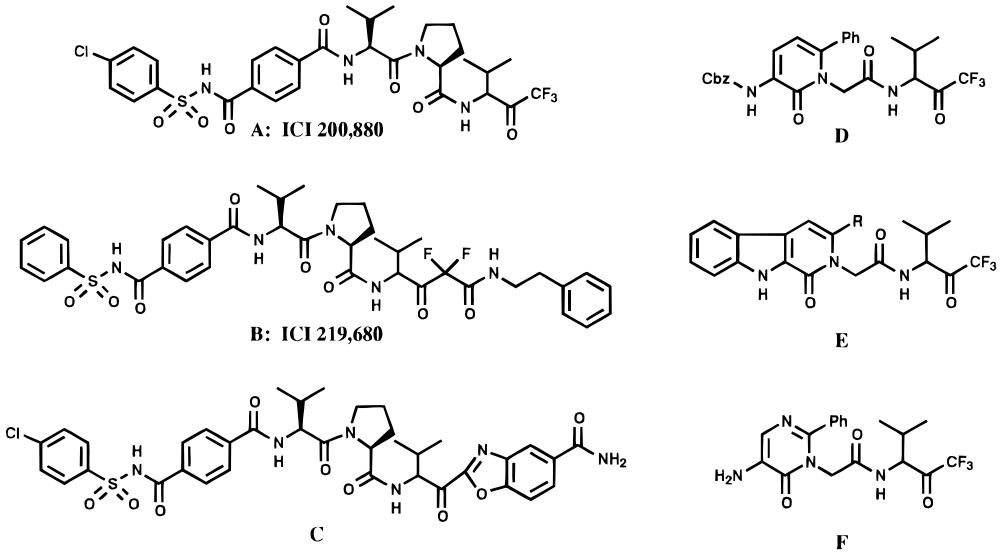


Figure 1. Peptidic and nonpeptidic inhibitors of HNE discovered at ZENECA Pharmaceuticals.

intratracheally active compound are generally required to change its profile such that it will be orally active.

Thus, while it has been possible to develop peptidic fluorinated ketones which possess oral activity,[29,30] we mounted a large effort to identify nonpeptidic compounds that would have an *in vivo* profile superior to that of peptidic inhibitors following oral administration. This work led to the discovery of the nonpeptidic, pyridone TFMKs (**D**, Figure 1).[31−33] Structure−activity studies within this series resulted in nonpeptidic TFMKs which not only displayed excellent *in vitro* potency (K_i <10 nM) but also possessed good *in vivo* activity following either intratracheal[34] or oral administration.[35] In addition, we were able to extend the concept of pyridone-based peptidomimetics to the design of the tricyclic β-carbolinone TFMKs (**E**).[36] However, when we began our investigation of pyridone TFMKs, the early members of this series were not active following oral administration. It was determined that these compounds were not well-absorbed from the gastrointestinal tract, which we ascribed to the fact that they possessed poor aqueous solubility. One approach taken to improve aqueous solubility was the incorporation of water solubilizing groups onto the 3-amino group of the pyridone ring.[35] A second approch was to incorporate solubilizing groups directly into the pyridone ring such as found in the pyrimidinone TFMKs (**F**).[37] The third strategy was to incorporate the solubilizing groups into a second heterocyclic ring. In this report we describe the *in vitro* structure−activity relationships of a new series of bicyclic pyridone-containing inhibitors, the pyridopyrimidine TFMKs.

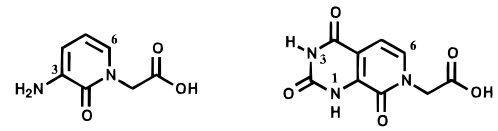
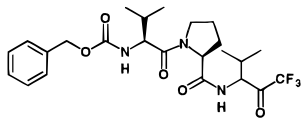
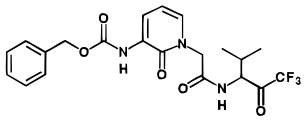
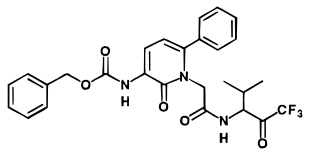


Figure 2. Pyridone and pyridopyrimidine dipeptide isosteres.

Table 1. Comparison of Peptidic and Pyridone TFMKs

Compound		K_i (nM)[a]
I		1.6[b]
J		280[c]
K		4.5[d]

[a]All inhibition constants in this report were determined for the inhibition of HNE-catalyzed hydroiysis of the synthetic substrate Meo-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as previously reported.[27] All compounds are a 1:1 mixture of epimers at the stereogenic center α to the ketone carbonyl group. [b]Reference 24. [c]Reference 32. [d]Reference 33.

Design Concepts

The pyridone TFMKs were designed via the modeling of peptidic inhibitors into the active site of HNE as previously described.[32] These studies suggested that the P_3 residue of the peptides could be cyclized onto the P_2 nitrogen atom and that the P_2 side chain could be eliminated. These modifications were incorporated into the pyridone-based P_3-P_2 dipeptide isostere 2-(3-amino-2-oxo-1,2-dihydro-1-pyridyl)acetic acid (**G**, Figure 2). Minimization of pyridone-containing TFMKs into the active site of HNE revealed that they retained the ability to participate in almost all of the key binding interactions with the enzyme as observed for peptidic inhibitors (Figure 3a,b). In particular, the 3-amino group and the carbonyl of the pyridone ring were able to form hydrogen bonds to Val-216 of the enzyme. Structure−activity studies with peptidic inhibitors have demonstrated that formation of these two hydrogen bonds is particularly important for maximizing potency.[2] Simple ring-unsubstituted pyridone TFMKs were significantly less potent than the corresponding peptidyl TFMKs with K_i values in the 1−0.1 μM range (Table 1, compare **I** vs **J**). Analysis of the pyridone-based inhibitors modeled into HNE revealed that these inhibitors were incompletely occupying the S_2 subsite as a result

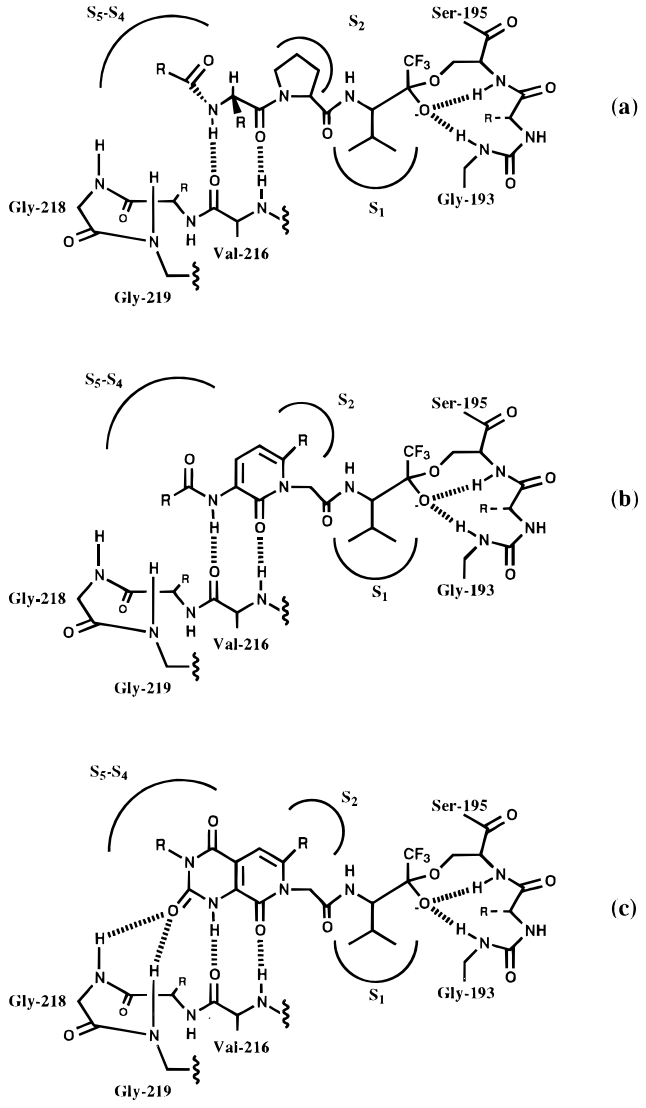


Figure 3. Binding interactions between HNE and (a) peptidic, (b) pyridone, and (c) pyridopyrimidine TFMKs.

of the elimination of the P_2 proline ring, and that substituents in the 5- or 6-positions of the pyridone ring should fill this binding pocket and restore activity. This proved to be the case, with 6-phenylpyridone TFMKs displaying potency comparable to the corresponding peptidic inhibitors (Table 1, compare **I** vs **K**).[33]

One of the advantages of the pyridone-based inhibitors over the tripeptides is that the pyridone TFMKs have only a single stereogenic center. Hence pyridone TFMKs which are epimeric at the stereogenic center α to the ketone carbonyl are a mixture of enantiomers rather than diastereomers, a property which could be of major significance with respect to both regulatory and manufacturing concerns. However, as a result of the fact that the 3-amino group of the pyridione is attached to a trigonal sp^2 center rather than a tetrahedral sp^3 center, the 3-amino group and its substituents are vectored away from the S_5-S_4 subsite and cannot readily take full advantage of the binding opportunities afforded by this hydrophobic pocket of the enzyme. On the other hand, static modeling of the pyridone inhibitors indicated that the 3-amino substituent is vectored toward a region of the enzyme that possesses two accessible NH groups. The hydrogen atoms of Gly-218 and Gly-219 point up away from the surface of the enzyme and appear to form a hydrogen-bonding pocket analogous to the oxyanion hole in the catalytic site (Figure 3b,c). It is possible that the carbonyl group of a 3-amido substituent could form hydrogen bonds with the NH groups of Gly-218 and Gly-219 and thereby increase the stability of the enzyme−inhibitor complex. However, to participate in this interaction, it would be necessary for the amide group to adopt an unfavorable cis conformation about the nitrogen/carbonyl bond. Thus, our static modeling studies suggested that the substituent on the 3-amino group of the pyridone TFNKs would not bind to either the S_5-S_4 subsite or the loop formed by Gly-218 and Gly-219. Supporting this hypothesis, aqueous molecular dynamics simulations indicated that a 3-amido carbonyl group and its substituents do not interact strongly with the enzyme surface, either through hydrophobic or hydrogen-bonding interactions.[35]

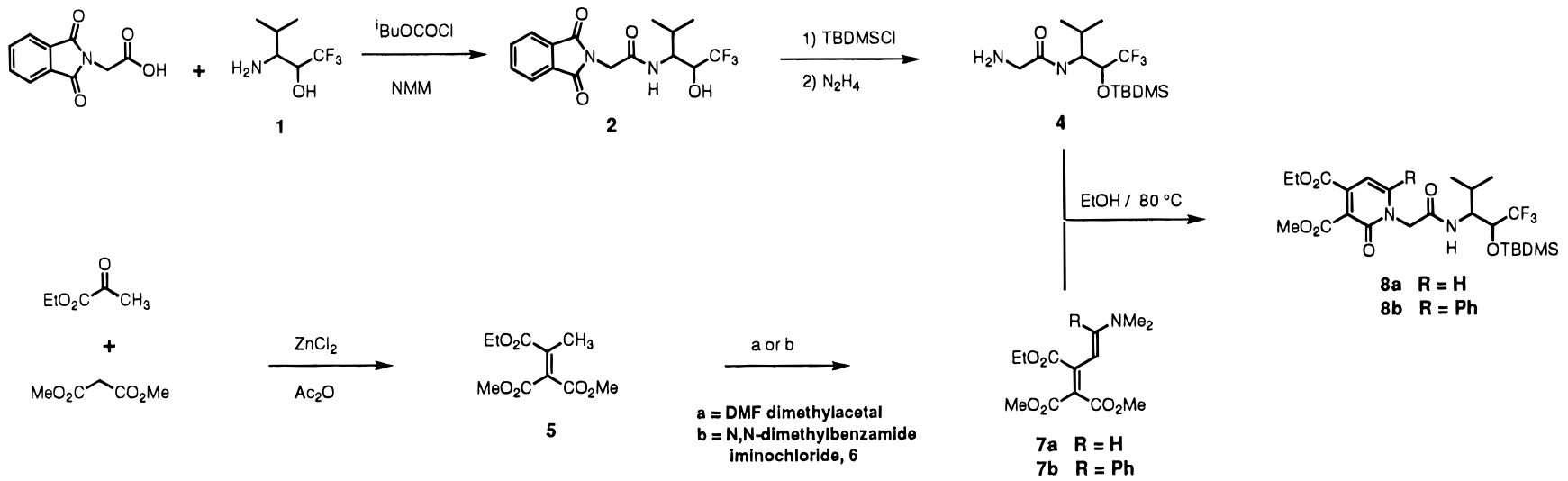
We initiated the design of bicyclic pyridone-based inhibitors both as a means of locking the 3-amido group into the desired cis conformation that would allow the carbonyl oxygen atom to form hydrogen bonds to Gly-218 and Gly-219 and as a means of directing a hydrophobic substituent toward the S_5-S_4 subsite. The pyridopyrimidine **H** (Figures 2 and 3c) appeared to satisfy these design goals. Static modeling of the pyridopyrimidine TFMKs in the active site of HNE indicated that not only could this ring system participate in the hydrogen-bonding interactions with Val-216 in a pyridone-like manner but that the carbonyl group in the 2-position (pyridopyrimidine numbering) could also form hydrogen bonds to the NH groups of both Gly-218 and Gly-219. Furthermore, appropriate substitutions off the nitrogen atom in the 3-position should be able to access the S_5-S_4 binding pocket. An additional perceived advantage of the pyridopyrimidine scaffold was that the heteroatoms at the 1-, 2-, 3-, and 4-positions of the pyrimidine ring would increase aqueous solubility and thereby might help to improve oral bioavailablitity.

Chemistry

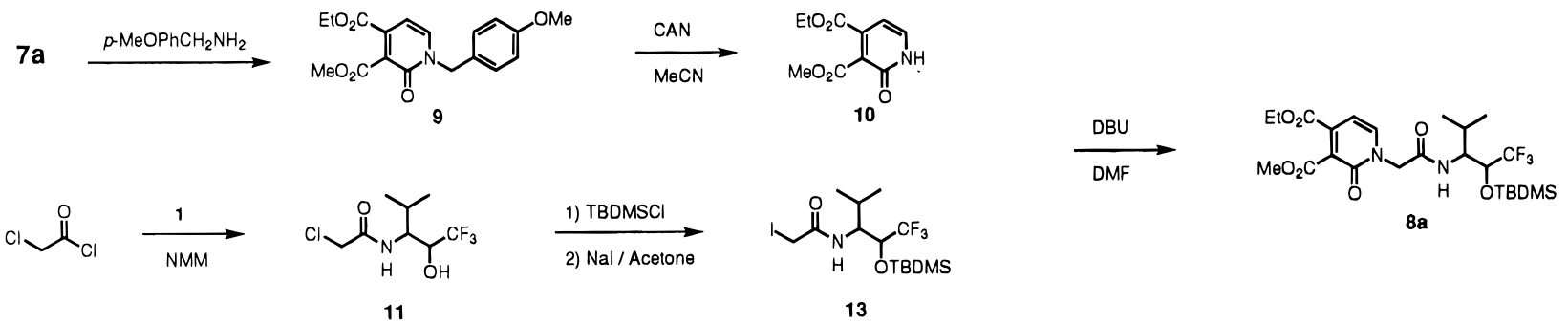
The strategy we developed for the preparation of the pyridopyrimidine bicyclic nucleus involves (a) construction of a 3,4-disubstituted pyridone (**8**, Scheme 1) and (b) cyclization of these substituents to form the bicyclic ring system (**19** and **20**, Scheme 2). The preferred procedure we used for the preparation of **8** is a modification of that reported by Borch et al.[38] (method A, Scheme 1). Mixed anhydride coupling of phthaloyl glycine with amino alcohol **1**[39] followed by hydroxyl group protection and hydrazinolysis afforded intermediate **4**. The other cyclization partner, enamine **7**, was prepared by alkylation of **5**[40] with either dimethylformamide dimethyl acetal to afford **7a** or with *N*,*N*-dimethylbenzamide imino chloride **6** in the presence of sodium hydride to give **7b**. Dienamine **7b** is somewhat unstable to the reaction conditions and requires rapid isolation. However, modest yields (50%) of highly pure **7b** could consistently be prepared. Dienamine **7a** was cyclized with amine **4** in refluxing ethanol to afford the 2-pyridone **8a** in good yield (70%). Cyclization of the phenyl-substituted dienamine **7b** with **4**, on the other hand, required more forcing conditions (DMF, 120 °C) and the yields were lower (40−50%). An alternative procedure used for the preparation of **8a** involved

Scheme 1. Methods A and B for the Synthesis of Intermediate **8**

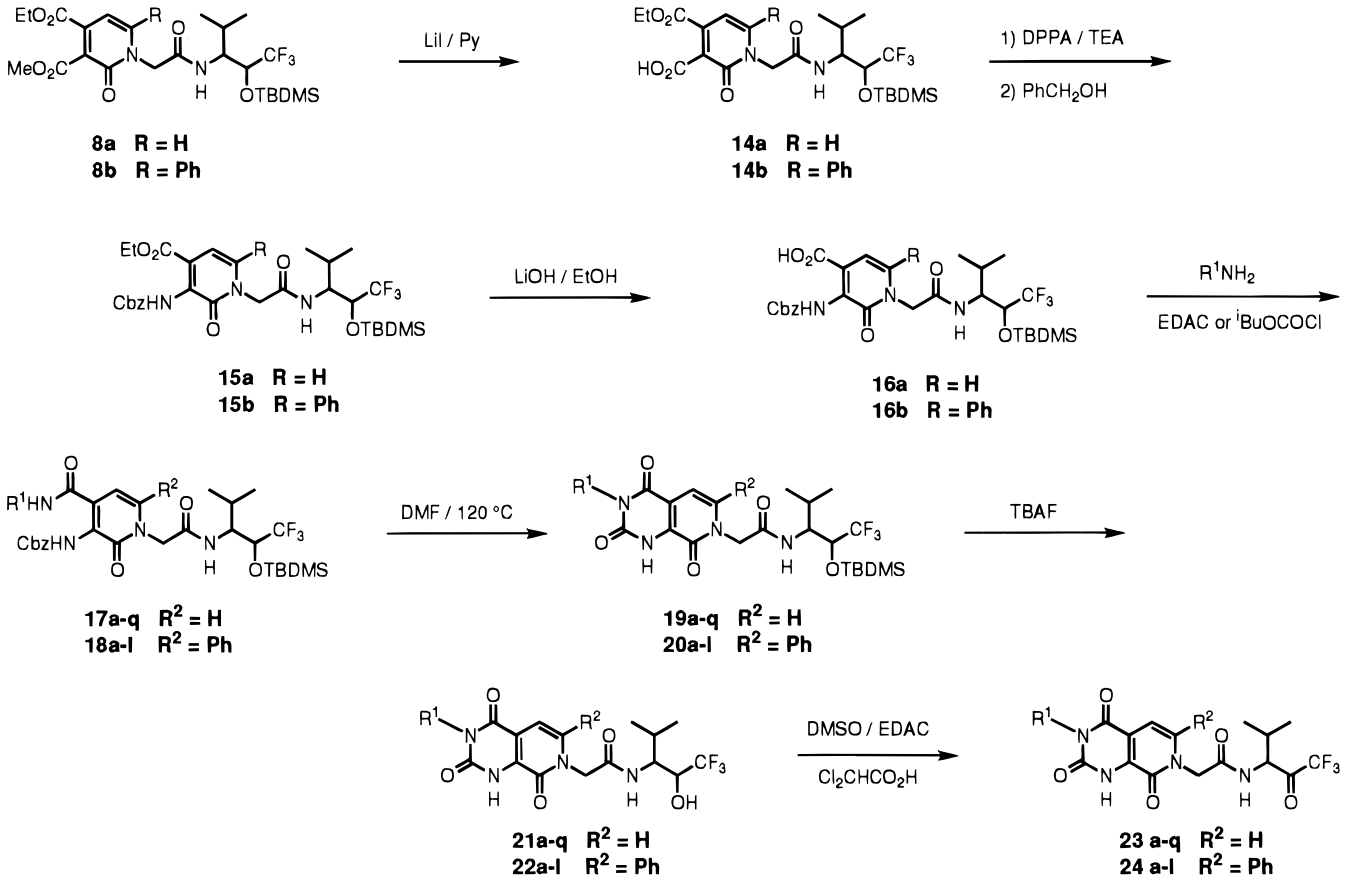
Method A



Method B



Scheme 2. Synthesis of Pyridopyrimidines **23** and **24**



cyclization of **7a** with *p*-methoxybenzylamine to afford the *N*-(methoxybenzyl)-2-pyridone **9** (method B, Scheme 1). Oxidative debenzylation afforded **10** which was *N*-alkylated with iodide **13** to give **8a**. This sequence generally gave lower overall yields than method A.

Introduction of the functionality required for formation of the bicyclic system hinged on selective dealkylation of the methyl ester **8** with LiI (Scheme 2). Curtius rearrangement of the monoacid **14** in the presence of benzyl alcohol introduced the 3-amino group. Hydrolysis of the ethyl ester **15** followed by carbodiimide or mixed anhydride-mediated amide formation set the stage for cyclization to the pyridopyrimidine bicyclic system which was effected by heating **17** or **18** in DMF. Removal of the silyl group and oxidation afforded the pyridopyrimidine TFMKs **23** and **24**.

Biological Evaluation

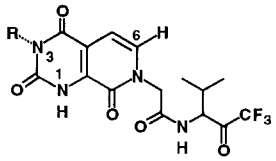
The compounds listed in Table 2 were tested *in vitro* for their ability to inhibit HNE activity. Inhibition constants (K_i values) were determined for the inhibition of the enzyme's ability to hydrolyze the synthetic substrate MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as previously described.[27]

Results and Discussion

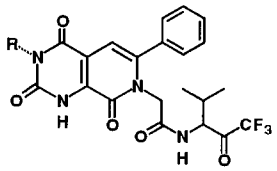
We have attempted to extend the discovery of nonpeptidic monocyclic pyridone-based TFMKs to bicyclic scaffolds and found that TFMKs containing a pyridopyrimidine ring afford potent inhibitors of HNE. The parent 3-unsubstituted analog **23a** had a K_i of 910 nM. Incorporation of lipophilic and aromatic groups in the 3-position increased activity significantly (Table 2, **23b**–**q**). Aromatic substituents afforded the best potency. The distance between the aromatic group and the pyrimidine ring is clearly important (compare **23c**, **23h**, and **23j**). We believe this results from the ability of the extended 3-substituents to partially occupy the S_5–S_4 subsites (see modeling discussion below). Indeed, the 3-phenyl derivative **23c** is slightly less potent than the 3-methyl compound **23b**, showing that simply increasing lipophilicity does not necessarily increase potency. Further support for the binding of the extended 3-substituents to a specific enzyme subsite (rather than a nonspecific hydrophobic interaction) comes from modifications that restrict the flexibility of the ethylene chain: both **23k** with a cyclopropyl group and **23l** with a carbonyl group in the two atom linker were significantly weaker than **23j**. That this decrease in potency is not due to either increased substitution or the incorporation of polar groups can be seen from a comparison with compounds **23m** and **23n**. In fact, **23n**, with a K_i of 16 nM, is the most potent of the compounds which are unsubstituted in the 6-position.

Modifications of the 3-substituent were not sufficient to push the potency of the pyridopyrimidine TFMKs into the <10 nM range. A similar phenomenon was observed with the monocyclic pyridone-based TFMKs where substituents on the 3-amino group did not afford nanomolar inhibitors when the 4-, 5-, and 6-positions were unsubstituted.[32] However, it was discovered that a 6-phenyl group was a unique substituent which afforded nanomolar pyridione TFMKs.[33] Neither 4- or 5-substituents nor other 6-substituents (e.g. methyl, benzyl, or phenethyl) were as effective as the 6-phenyl substituent. Molecular modeling studies suggested that

Table 2. *In Vitro* Inhibitory Activities of Pyridopyrimidine TFMKs



23



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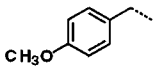
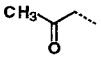
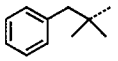
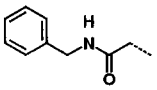
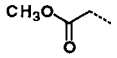
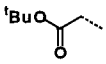
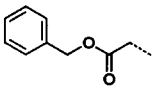
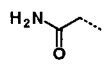
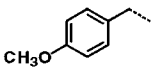
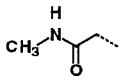
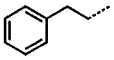
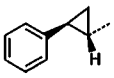
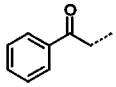
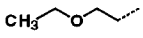
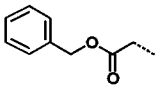
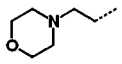
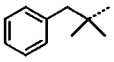
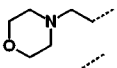
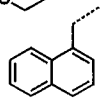
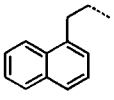
Compd	R	K_i (nM)[a]	Compd	R	K_i (nM)	Solubility[b] (mg/mL)	C log P[c]
23a	H	910 ± 140	**24a**	H	15 ± 4.0	0.22	1.59
23b	CH_3	620 ± 20	**24b**	CH_3	10 ± 2.6	0.044	1.99
23c	Ph	830 ± 210	**24c**		5.7± 1.5	0.23	3.47
23d		310 ± 140	**24d**		0.95 ± 0.38	ND[d]	4.79
23e		690 ± 70	**24e**		9.9 ± 2.3	ND	1.45
23f		490 ± 100					
23g		290 ± 90	**24f**		2.8 ± 1.4	ND	2.67
23h		160 ± 50	**24g**		15 ± 3.4	0.13	-0.35
23i		100 ± 20	**24h**		22 ± 4.0	0.10	-0.11
23j		42 ± 5.0	**24i**		19 ± 4.0	0.32	ND
23k		230 ± 60	**24j**		12 ± 6.0	0.42	0.64
23l		74 ± 24	**24k**		7.5 ± 0.7	0.008	1.63
23m		44 ± 18	**24l**		8.4 ± 1.1	0.30	1.42
23n		16 ± 4.0					
23o		330 ± 40					
23p		26 ± 5					
23q		21 ± 6.0					

[a] See footnotes in Table 1. [b] Solubility of compound in 0.01 M sodium phosphate buffer at pH = 7.4. [c] Calculated using CLOGP3 v3.4, MedChem Software, Daylight Chemical Information Systems, 2 Corporate Park, Suite 204, Irvine, CA 92714. [d] ND = not determined.

the 6-phenyl group was capable of filling the S_2 subsite, which was occupied by the pyrrolidine ring of the P_2 proline in our peptidic inhibitors. Thus the hydrophobic binding lost by removal of the proline is restored to the pyridone-based TFMKs by incorporation of a 6-phenyl group.

We applied the same tactic to the pyridopyrimidines and discovered that 6-phenylpyridopyrimidines are excellent inhibitors of HNE. As observed with the pyridone TFMKs, the 6-phenyl group affords an approximately 60-fold increase in potency over the unsubstituted analogs (compare **23a** vs **24a** and **23b** vs **24b**, Table 2). Several derivatives had K_i values <10 nM (**24c**−**f**, **2k,l**). As with the 6-unsubstituted analogs **23**, the potency of the 6-phenylpyridopyrimidine TFMKs is increased by substitutions at the 3-position, especially

Table 3. Characterization of Pyridopyrimidine TFMKs **23** and **24**

compd[a]	yield (%)[b]	MS (M + 1)	formula[c]
23a	26	389	$C_{15}H_{15}N_4F_3O_5 \cdot 0.5H_2O \cdot 0.5CH_3OH$
23b	50	403	$C_{16}H_{17}N_4F_3O_5 \cdot 0.65H_2O$
23c	37	465	$C_{21}H_{19}N_4F_3O_5 \cdot 0.7H_2O \cdot 0.5C_2H_5OH$
23d	24	445	$C_{18}H_{19}N_4F_3O_6 \cdot 0.65H_2O \cdot 0.2C_6H_{14}$
23e	83	447	$C_{17}H_{17}N_4F_3O_7 \cdot 1.4H_2O \cdot 0.5CH_3OH$
23f	53	461	$C_{18}H_{19}N_4F_3O_7$
23g	23	*d*	$C_{21}H_{25}N_4F_3O_7 \cdot 0.2C_6H_{14}$
23h	39	479	$C_{22}H_{21}N_4F_3O_5$
23i	49	509	$C_{23}H_{23}N_4F_3O_6$
23j	89	493	$C_{23}H_{23}N_4F_3O_5 \cdot 0.8H_2O$
23k	59	505	$C_{24}H_{23}N_4F_3O_5 \cdot 1.0H_2O \cdot 0.2C_6H_{14}$
23l	49	507	$C_{23}H_{21}N_4F_3O_6 \cdot 0.2H_2O \cdot 0.4C_2H_5OH$
23m	47	537	$C_{24}H_{23}N_4F_3O_7$
23n	83	521	$C_{25}H_{27}N_4F_3O_5$
23o	20	502	$C_{21}H_{26}N_5F_3O_6 \cdot 0.7H_2O$
23p	50	529	$C_{26}H_{23}N_4F_3O_5 \cdot 1.2H_2O$
23q	84	543	$C_{27}H_{25}N_4F_3O_5 \cdot 0.3H_2O \cdot 0.6CH_3OH$
24a	37	465	$C_{21}H_{19}N_4F_3O_5 \cdot 1.0H_2O \cdot 0.5CH_3OH$
24b	64	479	$C_{22}H_{21}N_4F_3O_5 \cdot 1.2H_2O$
24c	54	585	$C_{29}H_{27}N_4F_3O_6 \cdot 0.2CH_3OH$
24d	77	597	$C_{31}H_{31}N_4F_3O_5 \cdot 0.5H_2O$
24e	44	612	$C_{30}H_{28}N_5F_3O_6 \cdot 1.0H_2O \cdot 1.5CH_3OH$
24f	46	613	$C_{30}H_{27}N_4F_3O_7 \cdot 0.4H_2O \cdot 0.4CH_3OH$
24g	55	552	$C_{23}H_{22}N_5F_3O_6 \cdot 2.5H_2O$
24h	24	536	$C_{24}H_{24}N_5F_3O_6 \cdot 2.0H_2O \cdot 0.2C_6H_{14}$
24i	74	508	$C_{23}H_{24}N_5F_3O_5 \cdot 1.4H_2O \cdot 0.4C_2H_6OS$
24j	23	553	$C_{25}H_{27}N_4F_3O_7 \cdot 1.5H_2O$
24k	30	537	$C_{25}H_{27}N_4F_3O_6 \cdot 1.0H_2O$
24l	52	578	$C_{27}H_{30}N_5F_3O_6 \cdot 1.0H_2O$

[a] All compounds are approximately a 1:1 mixture of epimers at the stereogenic center α to the ketone carbonyl group. [b] Yields are for final synthetic step. All compounds were purified by flash chromatography on silica gel except **21e** and **21q** which were analytically pure following work up. [c] All elemental analyses for C, H, and N agree within ±0.4% of calculated values. [d] Base peak was 447 (M + 1 − isobutylene).

by 3-benzyl and 3-phenethyl derivatives (**24c**−**f**). Much of our efforts in the 6-phenyl pyridopyrimidine series was directed at introducing substituents in the 3-position which would impart aqueous solubility (>0.1 mg/mL) while retaining good *in vitro* potency ($K_i \leq 10$ nM). As can been seen in Table 2, many of the compounds we prepared satisfied these criteria (**23a**, **24c**, **24g**−**j**, and **24l**).

Molecular modeling studies have provided further support for the proposed binding interactions between the pyridopyrimidines and HNE. A model pyridopyrimidine, desdimethyl **24d**, was minimized in the extended binding pocket of HNE in a fashion analogous to that previously described for pyridone TFMKs.[32,33,35] Figure 4 is a view of the minimized enzyme−inhibitor complex with a solvent accessible surface applied to the enzyme. The inhibitor interacts with the enzyme in a substrate-like fashion occupying the S_3-S_1 subsites as depicted in Figure 3c. The carbonyl oxygen atom at the 8-position and the NH group in the 1-position form hydrogen bonds to Val-216 as observed in the crystal structures of pyridone TFMKs complexed to HNE.[35,37] The phenyl group in the 6-position is nestled over the top of the S_2 subsite of the enzyme located in the upper left quadrant of Figure 4. The carbonyl group in the 2-position is pointed directly into the pocket formed by the NH groups of Gly-218 and Gly-219 where it can form hydrogen bonds with these residues. In addition, the aromatic group of the 3-substituent is located in the mouth of the S_5-S_4 subsite. It can be seen that the 3-substituent is not vectored directly toward the S_5-S_4 binding pocket from the pyridopyrimidine scaffold but accesses this pocket by appropriate rotations about the methylene groups connecting the phenyl group to the bicyclic ring. This observation provides a possible explanation of why the 3-phenyl-substituted analog **23c** was no more potent than the methyl-substituted compound **23b** and why the phenethyl derivatives afford the best inhibition constants.

Our objective at the outset was the identification of a pyridone-based scaffold which would maintain the potency of the parent 3-aminopyridone TFMKs while affording increased aqueous solubility. These objectives have been realized with the series of pyridopyrimidine TFMKs. A comparison of pyridopyrimidine TFMKs with related pyridone TFMKs reveals that the two series of inhibitors have similar potencies against HNE. Many of the pyridopyrimidine TFMKs with a 6-phenyl group have K_i values in the nanomolar range, with one, **24d**, having a K_i of 0.95 nM. In fact, **24d** is one of only two pyridone-based TFMKs which has been reported with a subnanomolar K_i.[34,35] As part of our initial design concepts, we envisioned that the rigidly positioned 2-carbonyl group of the pyridopyrimidines would form a hydrogen-bonding interaction with the NH groups of Gly-218 and Gly-219 (Figure 3), and that this interaction would increase the potency of the pyridopyrimidine TFMKs relative to the pyridone TFMKs. As seen in Table 4, the pyridopyrimidine TFMKs are only marginally more potent, at best. However, the pyridopyrimidine TFMKs contain three additional heteroatoms capable of forming hydrogen bonds; these atoms should therefore be strongly solvated in aqueous solution. Upon binding, these atoms must be partially, if not completely, desolvated: a process which will contribute to a decrease in binding energy. A corresponding degree of desolvation is not required for the pyridone TFMKs. Thus, it is believed that the energetic penalty which must be paid for desolvation of the pyridopyrimidine TFMKs is compensated by the hydrogen-bonding interactions between the 2-carbonyl group and the enzyme such that the two series of inhibitors have similar inhibition constants.

It was anticipated that the array of heteroatoms present in the pyrimidine ring of the pyridopyrimidine TFMKs would confer improved aqueous solubility to this series of compounds relative to the pyridone TFMKs. This property was perceived to be important for obtaining oral bioavailability since early studies with the pyridone TFMKs indicated that their poor absorption from the gastrointestinal tract was at least in part the result of poor aqueous solubility.[35] Table 4 lists the aqueous solubility and *C* log *P* values for several pyridopyrimidine TFMKs and structurally related pyridone TFMKs. While it is inappropriate to make rigid comparisons of the properties of these two series because of their structural differences, the data in Table 4 suggest that the pyridopyrimidine TFMKs generally have a greater aqueous solubility and smaller *C* log *P* values than related pyridone TFMKs. Where these properties are similar, the pyridopyrimidine TFMKs have better *in vitro* potency.

One outlier in the above analysis is **24a** compared to **27**. However, **27** is the only pyridone which is not acylated on the 3-position aniline nitrogen atom. This nitrogen atom would be significantly protonated at pH

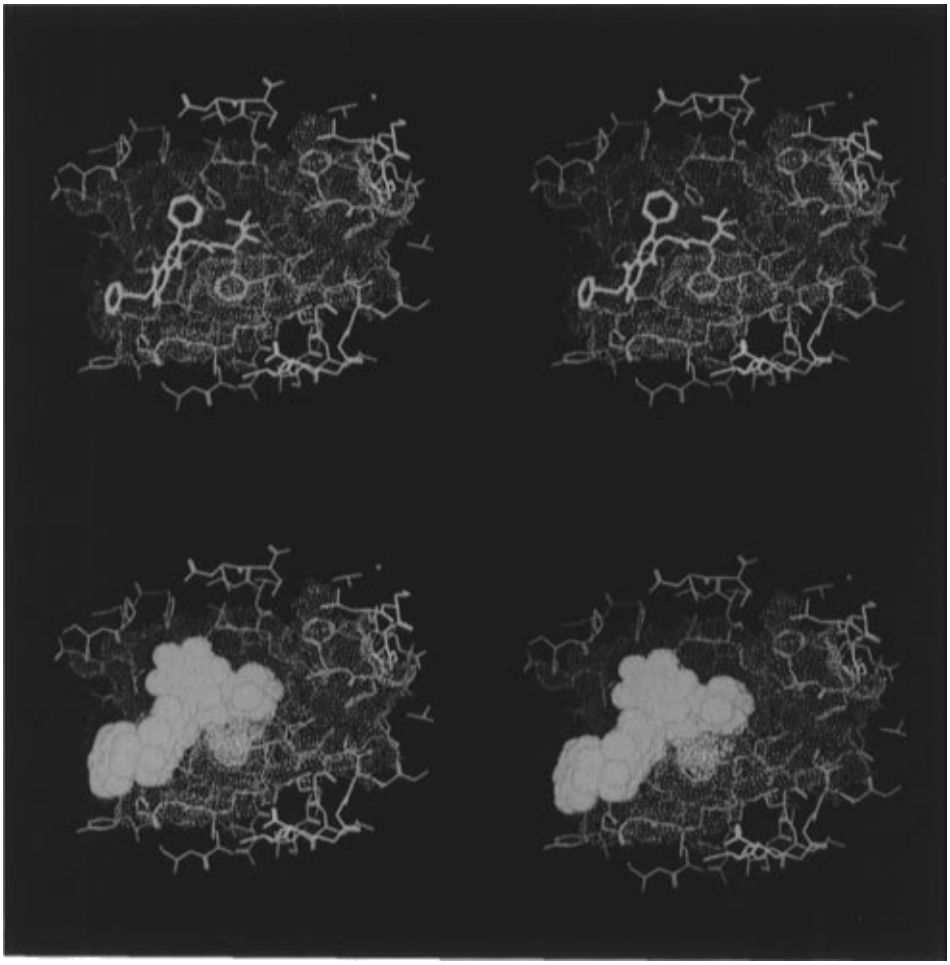


Figure 4. Two identical stereoviews of desmethyl **24d** energy minimized in the active site of HNE. The enzyme grid is the solvent accessible surface. Top view displays backbone atoms of inhibitor with the hydrogens masked. Inhibitor in bottom view is displayed with van der Waals surface. See refs 32, 33, and 35 for a description of modeling procedures.

7 and therefore should have increased aqueous solubility within the pyridone series. Indeed, it is the most soluble of the pyridones in Table 4. In contrast, the 3-position nitrogen atom in the pyridopyrimidines, whether or not it is acylated or alkylated, is nonbasic and not protonated at pH 7. Even so, **24a** is only 2-fold less soluble than **27**, further demonstrating the solubilizing power of the pyridopyrimidine ring system. Thus, the bicyclic pyridopyrimidine appears to afford a dipeptide mimetic which imparts good solubility/lipophilicity characteristics while allowing generation of potent inhibitors through appropriate substituents at the 3- and 6-positions. One compound, **24l**, has a particularly interesting profile with a K_i of 8.4 nM and an aqueous solubility of 0.3 mg/mL.

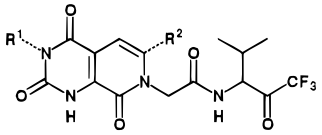
Summary

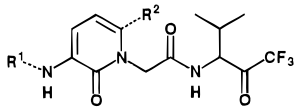
Monocyclic and tricyclic pyridone-based TFMKs have previously been shown to be potent inhibitors of HNE. When the proper substituents are incorporated into the monocyclic pyridones, *in vivo* activity following oral administration has been achieved. In this report, we have described a novel series of bicyclic pyridone-based TFMKs, the pyridopyrimidine TFMKs. This class of compounds has produced inhibitors with nanomolar and subnanomolar K_i values. This level of potency is believed to result from the ability of the carbonyl group in the 2-position to participate in a unique hydrogen-bonding interaction with Gly-218 and Gly-219 of the enzyme and from the ability of substituents in the 3-position to access the S_5-S_4 binding site. Furthermore, the array of heteroatoms present in the bicyclic ring system imparts increased solubility to the pyridopyrimidines such that this series of inhibitors has a significantly greater aqueous solubility than monocyclic pyridone TFMKs having similar potency and structure. It is therefore anticipated that, with the proper substituents in the 3-position, the pyridopyrimidine TFMKs will afford orally active inhibitors of HNE. In addition, this bicyclic dipeptide isostere should find wide applicability as a scaffold for the design of nonpeptidic inhibitors of other proteolytic enzymes.

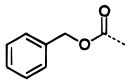
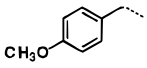
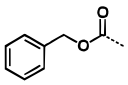
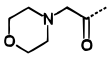
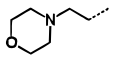
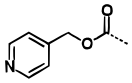
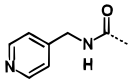
Experimental Section

General. Analytical samples were homogeneous by TLC and afforded spectroscopic results consistent with the assigned structures. Proton NMR spectra were obtained using either a Bruker WM-250 or AM-300 spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as internal standard. Mass spectra (MS) were recorded on a Kratos MS-80 instrument or a Finnigan MAT-60 operating in the chemical ionization (DCI) mode (only peaks $\geq$10% of the base peak are reported). Elemental analyses for carbon, hydrogen, and nitrogen were determined by the Analytical Section, ZENECA Pharmaceuticals, on a Perkin-Elmer 241 elemental analyzer and are within $\pm$0.4% of theory for the formulas given. Analytical thin-layer chromatography (TLC) was conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates was accomplished using UV light, phosphomolybdic acid−ethanol, and/or iodo-

Table 4. Comparison of Pyridopyrimidine and Pyridone TFMKs





Compd	R^1	R^2	K_i (nM)[a]	Solubility mg/mL	CLogP	Compd	R^1	R^2	K_i (nM)	Solubility mg/mL	CLogP
23a	H	H	910	>5	ND	**25**	H	H	2700[b]	ND	0.41
23h		H	160	0.73	1.46	**26**		H	280[b]	0.11	3.02
24a	H	Ph	15	0.22	1.59	**27**	H	Ph	62[c]	0.49[c]	2.51
24c		Ph	5.7	0.23	3.47	**28**		Ph	4.5[c]	0.0018[c]	5.12
						29		Ph	469[c]	0.023[c]	2.84
24l		Ph	8.4	0.30	1.42	**30**		Ph	8.0[c]	0.016[c]	3.64
						31		Ph	16[c]	0.14[c]	2.98

[a] See footnotes in Tables 1 and 2. [b] Reference 32. [c] Reference 35.

platinate charring. Flash chromatography was conducted on Kieselgel 60, 230−400 mesh (E. Merck, Darmstadt, West Germany). Solvents were either reagent or HPLC grade. Reactions were run at ambient temperature and under a nitrogen atmosphere unless otherwise noted. Solvent mixtures are expressed as volume:volume ratios. Solutions were evaporated under reduced pressure on a rotary evaporator. All starting materials were commercially available unless otherwise indicated.

2-Phthalimido-*N*-(3,3,3-trifluoro-2-hydroxy-1-isopropylpropyl)acetamide (2). A solution of *N*-phthaloylglycine (34.0 g, 162 mmol) and NMM (39.1 mL, 36.0 g, 356 mmol) in THF (680 mL) at −40 °C was treated dropwise with isobutyl chloroformate (22.1 mL, 23.2 g, 170 mmol) and stirred between −20 and −40 °C for 1.5 h. 3-Amino-1,1,1-trifluoro-4-methyl-2-pentanol hydrochloride[39] (**1**) (33.6 g, 162 mmol) was added, and the resulting mixture was allowed to warm to room temperature. The solution was stirred for 1.5 h, diluted with ethyl acetate, washed with 1 N HCl, 1 N NaOH, and brine, dried ($MgSO_4$), and evaporated to afford crude **2** as a white solid (47.2 g, 82%) which was used without further purification: TLC $R_f = 0.30$, chloroform/acetone (97:3); MS (DCI) $m/z = 359$ (M + 1, base), 341; ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.90 (6H, m), 1.83 (1H, heptet, $J = 6.7$ Hz), 3.84 (1H, t, $J = 7.3$ Hz), 4.14 (1H, q, $J = 6.2$ Hz), 4.26 (2H, q, $J = 13.9$ Hz), 7.89 (4H, m), 8.09 (1H, d, $J = 8.1$ Hz, NH).

2-Phthalimido-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (3). A solution of alcohol **2** (47.2 g, 132 mmol) and 2,6-lutidine (30.7 mL, 28.3 g, 264 mmol) in THF (600 mL) at 0 °C was treated dropwise with a solution of *tert*-butyldimethylsilyl trifluoromethanesulfonate (45.4 mL, 52.3 g, 198 mmol) in THF (50 mL) and was stirred for 16 h at room temperature. The resulting mixture was evaporated, taken up in ethyl acetate, washed with 1 N HCl, 1 N NaOH, and brine, dried ($MgSO_4$), and evaporated to afford crude **3** (65.3 g, >100%) as a white solid which was used without further purification: TLC $R_f = 0.54$ hexanes/acetone (2:1); MS (DCI) $m/z = 473$ (M + 1), 147, 134, 133 (base) 115, 108, 91, 89, 75, 73; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.08 (3H, s), 0.12 (3H, s), 0.86 (15H, m), 1.50 (1H, m), 3.81 (1H, t, $J = 8.3$ Hz), 4.17−4.50 (3H, m), 7.68 (1H, d, $J = 9.7$ Hz, NH), 7.86 (4H, m).

2-Amino-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (4). A refluxing solution of phthalimide **3** (62.3 g, 132 mmol) was treated with hydrazine hydrate (19.2 mL, 19.8 g, 396 mmol). The solution was refluxed for 3 h, acidified (pH 2) with 1 N HCl, refluxed for 1 h, cooled to 0 °C, filtered, and evaporated. The residue was taken up in ethyl acetate, washed with 1 N NaOH until the pH of the washes exceeded 8, then washed with brine, and evaporated. The crude amine was purified by flash chromatography on silica gel, eluting with a chloroform/methanol gradient (98:2, 95:5) to afford **4** (43.8 g, 81% from **1**) as a colorless oil: TLC $R_f = 0.68$, hexanes/acetone (3:2); MS (DCI) $m/z = 343$ (M + 1, base), 327, 285, 129; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.13 (3H, s), 0.16 (3H, s), 0.92 (12H, m), 0.98 (3H, d, $J = 6.6$ Hz), 1.81 (1H, m), 3.55 (1H, AB q, $J = 16.3$ Hz), 3.75 (1H, AB q, $J = 16.3$ Hz), 3.92 (1H, t, $J = 8.6$ Hz), 4.34 (1H, m), 7.92 (1H, d, $J = 9.7$ Hz, NH).

Dimethyl [2-(Ethoxycarbonyl)ethylidene]malonate (5). A suspension of anhydrous $ZnCl_2$ (258 g, 1.89 mol) in acetic anhydride was stirred for 2 h. The solution was decanted and treated dropwise with ethyl pyruvate (120 g, 1.03 mol) followed by dimethyl malonate (136 g, 1.03 mol). The resulting mixture was allowed to reflux for 2 h and the product isolated by vacuum distillation (bp 136 °C, 1 mmHg) to afford **5** (183 g, 77%) as a light yellow liquid: TLC $R_f = 0.25$, hexanes/acetone (9:1); MS (DCI) $m/z = 231$ (M + 1), 199 (base), 185, 167, 125; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.22 (3H, t, $J = 7.0$ Hz), 2.13 (3H, s), 3.72 (3H, s), 3.79 (3H, s), 4.19 (2H, q, $J = 7.0$ Hz).

(α-Chlorobenzylidine)dimethylammonium Chloride (6). A solution of *N*,*N*-dimethylbenzamide (20.0 g, 134 mmol) in CH_2Cl_2 (150 mL) was treated with oxalyl chloride (30.0 mL, 43.6 g, 344 mmol) and stirred at room temperature for 3 h. The solution was evaporated, diluted with hexanes, and stirred vigorously until a solid formed, and the solvent was decanted. The residue was triturated with hexanes and then ether and dried under vacuum to afford (α-chlorobenzylidine)dimethylammonium chloride **6** as a mositure-sensitive white solid which was stored under vacuum and used without further purification.

Dimethyl [3-(Dimethylamino)-2-(ethoxycarbonyl)-2-propenylidene]malonate (7a). A solution of **5** (38.0 g, 165 mmol) and *N*,*N*-dimethylformamide diethyl acetal (29.1 mL, 25.0 g, 170 mmol) in DMF was heated at 80 °C for 3 h and partitioned between brine and ethyl acetate. The ethyl acetate layer was washed with brine, and the combined aqueous washes were extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. The crude product was crystallized from CCl_4 (550 mL) to afford diene **7a** (37.5 g, 80%) as a yellow solid: TLC $R_f = 0.54$, chloroform/acetone (15:1); MS (DCI) $m/z = 286$ (M + 1), 285, 255, 254 (base); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.24 (3H, t, $J = 7.0$ Hz), 2.84 (3H, br s), 3.09 (3H, br s), 3.55 (3H, s), 3.67 (3H, s), 4.23 (2H, q, $J = 7.0$ Hz), 5.48 (1H, d, $J = 13$ Hz), 6.90 (1H, d, $J = 13$ Hz).

Dimethyl [2-(Ethoxycarbonyl)-3-(dimethylamino)-3-phenyl-2-propenylidene]malonate (7b). A suspension of hexanes-washed NaH (8.0 g, 60% oil dispersion, 20.0 mmol) in THF (300 mL) at 0 °C was treated with **5** (40.0 g, 174 mmol), stirred at room temperature for 4.5 h, cooled to 0 °C, and treated with a suspension of **6** (crude product isolated as described above) in THF (100 mL). The mixture was stirred at room temperature for 1 h. The resulting material was filtered through silica gel (1000 mL), eluting with ether until the filtrate ran colorless. The filtrate was evaporated, and the residue was triturated with ether and filtered to afford diene **7b** (13.1 g) as yellow crystals. The filtrate was evaporated and the residue purified by dry-column flash chromatography[41] on silica gel, eluting with a gradient of ether/hexanes (1:1, 100:0). The fractions containing the product were combined and evaporated on a rotorary evaporator without external heat until additional **7b** (8.5 g) crystallized as a yellow solid. Total yield was 21.6 g (45%): TLC $R_f = 0.29$, ether/hexanes (7:3); MS (DCI) $m/z = 362$ (M + 1), 330 (base), 316; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (3H, t, $J = 7.1$ Hz), 2.81 (6H, br s), 3.13 (2H, q, $J = 7.1$ Hz), 3.48 (3H, s), 3.71 (3H, s), 5.66 (1H, s), 7.15 (2H, m), 7.40 (3H, m).

2-[4-(Ethoxycarbonyl)-3-(methoxycarbonyl)-2-oxo-1,2-dihydro-1-pyridyl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (8a). A solution of **4** (30.1 g, 87.9 mmol) and **7a** (27.6 g, 96.7 mmol) in ethanol (140 mL) was heated at reflux for 16 h and evaporated. The residue was taken up in ethyl acetate, washed with 1 N HCl and brine, dried [50% (w/w) K_2CO_3/Na_2SO_4], and evaporated. The residue was taken up in ether, washed with 1 N HCl, and brine, dried [50% (w/w) K_2CO_3/Na_2SO_4], and evaporated to afford pyridone **8a** (34.5 g, 71%) as a light yellow solid: TLC $R_f = 0.68$, hexanes/acetone (1:1); MS (DCI) $m/z = 551$ (M + 1, base), 535, 519, 493, 266; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.13 (3H, s), 0.17 (3H, s), 0.95 (15H, m), 1.29 (3H, t, $J = 6.0$ Hz), 1.81 (1H, m), 3.78 (3H, s), 3.84 (1H, t, $J = 8.4$ Hz), 4.30 (3H, m), 4.59 (1H, d, $J = 13.1$ Hz), 4.89 (1H, d, $J = 13.1$ Hz), 6.59 (1H, d, $J = 5.8$ Hz), 7.88 (2H, m, CH, NH).

Alternative Procedure for Preparation of 8a (Method B, Scheme 1). A solution of pyridone **10** (6.2 g, 27.5 mmol) and iodide **13** (18.1 g, 40.0 mmol) in DMF (80 mL) was treated with DBU (6.47 mL, 6.59 g, 43.3 mmol) and stirred at room temperature for 24 h. The reaction mixture was diluted with ethyl acetate, washed with 1 N HCl, brine, 1 N NaOH, and brine, dried [50% (w/w) K_2CO_3/Na_2SO_4], and evaporated. The crude product was purified by flash chromatography eluting with acetone/hexanes (1:4) to afford **8a** (8.82 g, 58%) as a light yellow solid.

2-[4-(Ethoxycarbonyl)-3-(methoxycarbonyl)-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (8b). A mixture of the amine **4** (18 g, 70 mmol) and diene **7b** (18.0 g, 50.0 mmol) was heated under vacuum (1 mmHg) at 120 °C for 8 h, treated with additional **4** (6 g, 17.5 mmol), heated under vacuum at 120 °C for 16 h, and cooled to room temperature. The residue was taken up in ether, washed with 1 N HCl, 1 N NaOH, and brine, dried [50% (w/w) K_2CO_3/Na_2SO_4], and evaporated. The crude product was purified by flash chromatography eluting with a gradient of ether/hexanes (40:60, 50:50, 55:45, 60:40, 70:30, 100:0). The impure fractions were purified by a second flash chromatography eluting with a gradient of ether/hexanes (50:50, 55:45, 60:40, 70:30, 80:20, 100:0). The material from both chromatographies was combined to afford **8b** (14.9 g, 48%) as a solid: TLC $R_f = 0.41$, hexanes/acetone (7:3); MS (DCI) $m/z = 627$ (M + 1), 105, 97 (base), 79; ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.10 (3H, s), 0.11 (3H, s), 0.80 (3H, d, $J = 6.6$ Hz), 0.88 (9H, s), 0.93 (3H, d, $J = 6.8$ Hz), 1.77 (1H, m), 3.80 (1H, m), 3.82 (3H, s), 4.29 (4H, m), 4.67 (1H, m), 6.45 (1H, s), 7.50 (5H, m), 7.71 (1H, d, $J = 9.9$ Hz, NH).

4-(Ethoxycarbonyl)-1-(4-methoxybenzyl)-3-(methoxycarbonyl)-2-pyridone (9). A solution of diene **7a** (37.2 g, 130 mmol) in ethanol (186 mL) was treated dropwise with *p*-methoxybenzylamine (18.8 mL, 19.7 g, 143 mmol) and heated at reflux for 1.1 h. The solution was evaporated, and the residue was taken up in ethyl acetate, washed with 1 N HCl and brine, dried [50% (w/w) K_2CO_3/Na_2SO_4], and evaporated to afford **9** (45.6 g, >100%) as a brown gum which was used without further purification: TLC $R_f = 0.42$, chloroform/acetone (15:1); MS (DCI) $m/z = 346$ (M + 1, base), 121; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.25 (3H, t, $J = 7.0$ Hz), 3.72 (3H, s), 3.75 (3H, s), 4.26 (2H, q, $J = 7.0$ Hz), 5.07 (2H, s), 6.58 (1H, d, $J = 6.9$ Hz), 6.91 (2H, d, $J = 6.8$ Hz), 7.30 (2H, d, $J = 8.6$ Hz), 8.10 (1H, d, $J = 6.9$ Hz).

4-(Ethoxycarbonyl)-3-(methoxycarbonyl)-2-pyridone (10). A solution of **9** (24.0 g, 69.5 mmol) in CH_3CN/H_2O (275 mL, 10:1) was treated with cerric ammonium nitrate (190 g, 347 mmol) and stirred at room temperature for 0.5 h. The solution was diluted with chloroform (1 L) and filtered, and the organic filtrate was washed with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography on silica gel eluting with a gradient of chloroform/methanol (100:0, 95:5) to afford pyridone **10** (6.3 g, 40%) as a white solid: TLC $R_f = 0.49$, chloroform/methanol (9:1); MS (DCI) $m/z = 266$ (M + 1), 194 (base), 180, 166; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 1.28 (3H, t, $J = 7.1$ Hz), 3.78 (3H, s), 4.27 (2H, q, $J = 7.1$ Hz), 6.51 (1H, d, $J = 6.8$ Hz), 7.64 (1H, d, $J = 6.8$ Hz).

2-Chloro-*N*-(3,3,3-trifluoro-2-hydroxy-1-isopropylpropyl)acetamide (11). A solution of 3-amino-1,1,1-trifluoro-4-methyl-2-pentanol hydrochloride[39] (**1**) (10.0 g, 48.2 mmol) and NMM (10.9 mL, 10.0 g, 98.8 mmol) in THF (240 mL) at 0 °C was treated dropwise with a solution of chloroacetyl chloride (3.85 mL, 10.0 g, 48.2 mmol) in THF (20 mL). The solution was stirred at 0 °C for 3 h and evaporated. The residue was taken up in ethyl acetate, washed with 1 N HCl, 1 N NaOH, and brine, dried ($MgSO_4$), and evaporated to afford **11** (12.2 g, >100% as a yellow oil which was used without further purification: TLC $R_f = 0.75$, chloroform/methanol (95:5); MS (DCI) $m/z = 248$ (M + 1 for ^{35}Cl, base), 75; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.87 (3H, d, $J = 6.8$ Hz), 0.93 (3H, d, $J = 6.8$ Hz), 1.85 (1H, m), 3.87 (1H, m), 4.03−4.18 (1H, m), 4.12 (2H, s), 7.95 (1H, d, $J = 9.7$ Hz, NH).

2-Chloro-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (12). A solution of alcohol **11** (11.4 g, 46.0 mmol) and 2,6-lutidine (10.7 mL, 9.86 g, 92 mmol) in dichloromethane (46 mL) was treated dropwise with *tert*-butyldimethylsilyl trifluoromethanesulfonate (15.9 mL, 18.3 g, 69.0 mmol). The solution was stirred for 3 h at room temperature, diluted with ethyl acetate, washed with 1 N HCl, 1 N NaOH, and brine, dried ($MgSO_4$), and evaporated. The crude material was purified by flash chromatography eluting with a gradient of hexanes/ethyl acetate (100:0, 90:10, 80:20) to afford silyl ether **12** (10.3 g, 62%) as a white solid: TLC $R_f = 0.62$, hexanes/ethyl acetate (4:1); MS (DCI) $m/z = 362$ (M + 1 for ^{35}Cl, base), 346, 326, 304, 148; ^{1}H NMR

(300 MHz, DMSO-d_6) δ 0.13 (3H, s), 0.16 (3H, s), 0.87 (3H, d, J = 6.7 Hz), 0.91 (9H, s), 0.97 (3H, d, J = 6.6 Hz), 1.77 (1H, m), 3.84 (1H, t, J = 8.0 Hz), 4.18 (2H, s), 4.38 (1H, m), 7.56 (1H, d, J = 9.6 Hz, NH).

2-Iodo-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (13). A solution of NaI (8.45 g, 56.4 mmol) and chloride **12** (5.10 g, 14.1 mmol) in acetone (56 mL) was stirred at room temperature for 16 h and was partitioned between ethyl acetate and water. The organic layer was washed with saturated $Na_2S_2O_3$ and brine, dried ($MgSO_4$), and evaporated to afford iodide **13** (6.12 g, 96%) as a white solid: TLC R_f = 0.37, hexanes/ethyl acetate (9:1); MS (DCI) m/z = 454 (M + 1, base), 396, 326, 326, 240, 85; ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.13 (3H, s), 0.16 (3H, s), 0.89 (3H, m), 0.91 (9H, s), 0.96 (3H, d, J = 6.6 Hz), 1.77 (1H, m), 3.77 (3H, m), 4.30 (1H, m), 7.79 (1H, d, J = 9.7 Hz, NH).

General Procedure for the Preparation of Pyridones 14. 2-[3-Carboxy-4-(ethoxycarbonyl)-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (14b). A solution of **8b** (14.9 g, 22.4 mmol) and anhydrous LiI (7.49 g, 55.9 mmol) in pyridine (250 mL) was heated at reflux for 4 h, diluted with ether, washed with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. The crude product was purified by dry-column flash chromatography[41] eluting with chloroform/methanol (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 50:50) to afford acid **14b** (13.1 g, 96%) as a gray solid: TLC R_f = 0.55, chloroform/methanol/acetic acid (95:5:1); MS (DCI) m/z = 631 (M + 1), 123, 117, 97 (base), 91; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.10 (3H, s), 0.12 (3H, s), 0.82 (3H, d, J = 6.5 Hz), 0.88 (9H, s), 0.94 (3H, d, J = 6.5 Hz), 1.30 (3H, t, J = 7.2 Hz) 1.74 (1H, m), 3.82 (1H, t, J = 9.5 Hz), 4.34 (4H, m), 4.67 (1H, d, J = 15.8 Hz), 6.58 (1H, s), 7.55 (5H, m), 7.79 (1H, d, J = 9.7 Hz, NH).

General Procedure for the Preparation of Aminopyridones 15. 2-[3-[(Benzyloxycarbonyl)amino]-4-(ethoxycarbonyl)-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (15b). A solution of acid **14b** (13.1 g, 21.4 mmol), diphenyl phosphorazidate (6.91 mL, 8.82 g, 32.1 mmol), TEA (4.74 mL, 3.46 g, 34.2 mmol), and benzyl alcohol (3.77 mL, 3.94 g, 36.4 mmol) in *p*-dioxane (200 mL) was heated at reflux for 4 h and evaporated. The residue was taken up in ether, washed with 1 N HCl, brine, 1 N NaOH, and brine, dried [50% (w/w) K_2CO_3/Na_2SO_4], and evaporated. The crude product was purified by flash chromatography eluting with hexanes/ethyl acetate (85:15) to afford aminopyridone **15b** (12.8 g, 84%) as a tan semisolid: TLC R_f = 0.36, hexanes/ethyl acetate (7:3); MS (DCI) m/z = 718 (M + 1, base), 91; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.11 (3H, s), 0.13 (3H, s), 0.84 (3H, d, J = 6.5 Hz), 0.89 (9H, s), 0.95 (3H, d, J = 6.6 Hz), 1.20 (3H, t, J = 7.0 Hz), 1.77 (1H, m), 3.84 (1H, t, J = 9.7 Hz), 4.22 (4H, m), 4.65 (1H, m), 5.16 (2H, s), 6.32 (1H, s), 7.43 (10H, m), 7.69 (1H, d, J = 9.6 Hz, NH).

General Procedure for the Preparation of 4-Carboxypyridones 16. 2-(3-[(Benzyloxycarbonyl)amino]-4-carboxy-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl)-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (16b). A solution of ester **15b** (11.0 g, 15.3 mmol) in ethanol/water (270 mL, 100:35) at 0 °C was treated with LiOH·H_2O (0.674 g, 16.1 mmol), stirred for 4 h, treated with additional LiOH·H_2O (0.674 g, 16.1 mmol), stirred for 3 h, diluted with ether, washed with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. The crude material was purified by flash chromatography on silica gel eluting with a gradient of chloroform/methanol/acetic acid (100:0:0, 97.5:2.5:0, 95:5:0, 95:5:1) to afford acid **16b** (5.6 g, 53%) as an off-white foam. Additional material could be recovered by purification of the mixed fractions from the chromatography: TLC R_f = 0.30, chloroform/methanol (9:1); MS (DCI) m/z = 690 (M + 1), 672, 582, 92 (base); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.12 (3H, s), 0.14 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 0.90 (9H, s), 0.96 (3H, d, J = 6.5 Hz), 1.77 (1H, m), 3.85 (1H, t, J = 9.6 Hz), 4.30 (2H, m), 4.67 (1H, d, J = 15.4 Hz), 5.18 (2H, s), 6.32 (1H, s), 7.47 (10H, m), 7.70 (1H, d, J = 9.6 Hz, NH).

General Procedure for the Preparation of 3-Amidopyridopyrimidines 19 and 20 Using EDAC. 2-[3-(4-Methoxybenzyl)-2,4,8-trioxo-6-phenyl-1,2,3,4,7,8-hexahydropyrido[3,4-*d*]pyrimidin-7-yl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (20c). A solution of acid **16b** (1.50 g, 2.18 mmol), 4-methoxybenzylamine (0.57 mL, 597 mg, 4.35 mmol), and HOBt (588 mg, 4.35 mmol) in DMF (15 mL) was treated with EDAC (501 mg, 2.61 mmol) and stirred at room temperature for 16 h. The reaction mixture was dissolved in ethyl acetate, washed with 1 N HCl, brine, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated to afford **18c**. Crude **18c** was dissolved in DMF (15 mL), heated at 120 °C for 2.5 h, taken up in ethyl acetate, washed with 1 N HCl, brine, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography on silica gel eluting with hexanes/acetone (4:1) to afford **20c** (940 mg, 62%) as a light yellow foam: TLC R_f = 0.35, hexanes/acetone (4:1); MS (DCI) m/z = 701 (M + 1), 190, 169, 150, 121, 107, 97, 91, 79 (base); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.11 (3H, s), 0.12 (3H, s), 0.85 (3H, d, J = 8.3 Hz), 0.88 (9H, s), 0.95 (3H, d, J = 6.5 Hz), 1.77 (1H, m), 3.73 (3H, s), 3.85 (1H, t, J = 9.0 Hz), 4.25 (1H, m), 4.44 (1H, m), 4.65 (1H, m), 5.04 (2H, s), 6.50 (1H, s), 6.68 (2H, d, J = 8.6 Hz), 7.31 (1H, d, J = 8.6 Hz), 7.44 (5H, m), 7.66 (2H, d, J = 9.6 Hz, CH, NH).

General Procedure for the Preparation of 3-Amidopyridopyrimidines 19 and 20 Using Mixed Anhydride Coupling. 2-[3-[2-(4-Morpholino)ethyl]-2,4,8 trioxo-6-phenyl-1,2,3,4,7,8-hexahydropyrido[3,4-*d*]pyrimidin-7-yl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (20l). A solution of acid **16b** (603 mg, 0.875 mmol) and NMM (0.125 mL, 115 mg, 1.14 mmol) in THF (10 mL) at −40 °C was treated with isobutyl chloroformate (0.150 mL, 0.155 mg, 1.14 mmol) and stirred at −40 to −30 °C for 1 h. 4-(2-Aminoethyl)morpholine (0.150 mL, 0.148 g, 1.14 mmol) was added and the resulting mixture stirred at room temperature for 16 h. The reaction solution was taken up in ethyl acetate, washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. Crude **18l** was dissolved in DMF and heated at 120 °C for 16 h and evaporated. Purification by flash chromatography on silica gel eluting with a gradient of chloroform/methanol (97:3, 95:5) afforded **20l** (350 mg, 58%) as a white solid: TLC R_f = 0.47, chloroform/methanol (95:5); MS (DCI) m/z = 694 (M + 1), 231, 100, 74 (base); ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.07 (3H, s), 0.08 (3H, s), 0.80−0.91 (6H, m), 0.85 (9H, s), 1.73 (1H, m), 3.17 (2H, m), 3.48 (2H, m), 3.66 (4H, m), 3.98 (2H, m), 4.25 (2H, m), 4.44 (1H, m), 4.63 (1H, m), 6.46 (1H, s), 7.41 (5H, m), 7.68 (1H, d, J = 9.9 Hz, NH).

General Procedure for the Preparation of 3-Amidopyridopyrimidines 21 and 22 Using TBAF. 2-[3-(4-Methoxybenzyl)-2,4,8-trioxo-6-phenyl-1,2,3,4,7,8-hexahydropyrido[3,4-*d*]pyrimidin-7-yl]-*N*-(3,3,3-trifluoro-2-hydroxy-1-isopropylpropyl)acetamide (22c). A solution of **20c** (920 mg, 1.31 mmol) in THF (15 mL) was treated with TBAF (1.58 mL, 1.0 M, 1.58 mmol), stirred at room temperature for 2 h, and evaporated. The crude material was purified by flash chromatography eluting with a gradient of chloroform/methanol (97:3, 95:5) to afford **22c** (730 mg, 95%) as a white solid: TLC R_f = 0.20, chloroform/methanol (95:5); MS (DCI) m/z = 587 (M + 1), 416, 149, 121 (base); ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.84 (3H, d, J = 6.6 Hz), 0.91 (3H, d, J = 6.6 Hz), 1.78 (1H, m), 3.73 (3H, s), 3.87 (1H, t, J = 8.6 Hz), 4.10 (1H, m), 4.42 (1H, m), 4.56 (1H, m), 5.04 (2H, s), 6.48 (1H, s), 6.88 (2H, d, J = 8.7 Hz), 7.31 (2H, d, J = 8.6 Hz), 7.46 (5H, m), 7.94 (1H, d, J = 9.7 Hz, NH).

General Procedure for the Preparation of 3-Amidopyridopyrimidines 21 and 22 Using HF. 2-(2,4,8-Trioxo-6-phenyl-1,2,3,4,7,8-hexahydropyrido[3,4-*d*]pyrimidin-7-yl)-I-(3,3,3-trifluoro-2-hydroxy-1-isopropylpropyl)-acetamide (22a). A solution of **20a** (300 mg, 0.517 mmol) and aqueous HF (5.2 mL, 5%) in CH_3CN (46.8 mL) was stirred at room temperature for 16 h. Methanol was added to dissolve the precipatated solid, the mixture was preabsorbed onto silica gel (10 mL), and the product was isolated by flash chromatography on silica gel (100 mL) eluting with chloroform/

methanol (9:1) to afford **22a** (140 mg, 58%) as a solid: TLC R_f = 0.45, chloroform/methanol (9:1); MS (DCI) m/z = 467 (M + 1, base); ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.88 (6H, m), 1.78 (1H, heptet, J = 8.5 Hz), 3.85 (1H, m), 4.09 (1H, m), 4.23−4.60 (2H, m), 6.42 (1H, s), 7.47 (5H, m), 7.94 (1H, d, J = 9.4 Hz, NH).

General Procedure for the Preparation of Ketones 23 and 24. 2-[3-(4-Methoxybenzyl)-2,4,8-trioxo-6-phenyl-1,2,3,4,7,8-hexahydropyrido[3,4-*d*]pyrimidin-7-yl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (24c). A solution of alcohol **22c** (710 mg, 1.21 mmol) and EDAC (2.32 g, 12.1 mmol) in DMSO/toluene (30 mL, 1:1) at 0 °C was treated with 2,2-dichloroacetic acid (0.400 mL, 620 mg, 4.84 mmol). The reaction mixture was stirred at room temperature for 16 h and partitioned between ethyl acetate and 1 N HCl. The organic layer was washed with 1 N HCl, brine, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography on silica gel eluting with a gradient of chloroform/methanol (99:1, 97:3) to afford **24c** (380 mg, 54%) as a white solid: TLC R_f = 0.38, chloroform/methanol (97:3); MS (DCI) m/z = 585 (M + 1), 416, 149, 121 (base); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.81 (3H, d, J = 6.8 Hz), 0.96 (3H, d, J = 6.6 Hz), 2.26 (1H, m), 3.75 (3H, s), 4.10 (1H, d, J = 2.8 Hz), 4.59 (2H, m), 5.06 (2H, s), 6.52 (1H, s), 6.90 (2H, d, J = 8.6 Hz), 7.30 (2H, d, J = 8.6 Hz), 7.46 (5H, m). Anal. ($C_{29}H_{27}N_4F_3O_6 \cdot 0.CH_3OH$) C, H, N.

2-[3-(Carboxymethyl)-2,4,8-trioxo-6-phenyl-1,2,3,4,7,8-hexahydropyrido[3,4-*d*]pyrimidin-7-yl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (23e). A suspension of benzyl ester **23m** (160 mg, 0.298 mmol, prepared according the general precedure described above for ketones **23** and **24**), and 10% Pd−C (100 mg) in methanol (29 mL) was hydrogenated at 50 psi for 3 h, filtered, and evaporated to afford **23e** (110 mg, 83%) as an analytically pure white solid: TLC R_f = 0.15, chloroform/methanol (95:5); MS (DCI) m/z = 447 (M + 1, base), 429, 150; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.84−1.01 (6H, m), 2.25 (1H, m), 4.57 (2H, br s), 4.58−5.00 (3H, m), 6.62 (1H, m), 7.40 (1H, m), 7.93 (1H, d, J = 10 Hz, NH). Anal. ($C_{17}H_{17}N_4F_3O_7 \cdot 1.4H_2O \cdot 0.5CH_3OH$) C, H, N.

Acknowledgment. We thank Mr. Jim Hulsizer and Mr. Gary Moore for large-scale synthesis of intermediates and Dr. Prudence Bradley for the determination of aqueous solubilities.

References

(1) Abbreviations: HNE, human neutrophil elastase; PPE, porcine pancreatic elastase; Ac, acetyl; Cbz, benzyloxycarbonyl; TFMK, trifluoromethyl ketone; CAN, ceric ammonium nitrate; TEA, triethylamine; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; EDAC, 1-ethyl-[3-[3-(dimethylamino)propyl]]carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole monohydrate; TBAF, tetra-*n*-butylammonium fluoride; TFA, trifluoroacetic acid; DMF, dimethylformamide; NMM, *N*-methylmorpholine; DMP, Dess−Martin periodinane, 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one; MeO, methoxy; Suc, succinyl; *p*Na, *p*-nitroanilide; PBS, phosphate-buffered saline; it, intratracheal; DPPA, diphenyl phosphorazidate.

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JM950684Z