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The Synthesis of Lysine α-Ketoamide Thrombin Inhibitors via an Epoxy Amide Ring Opening.

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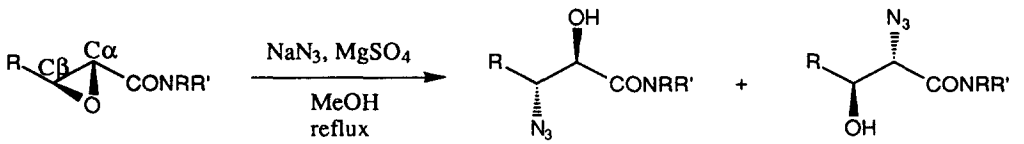
Abstract: We describe a novel route for the preparation of substituted α–ketoamides of lysine. These compounds, due to the presence of an electrophilic carbonyl, display submicromolar activity toward the enzyme thrombin.

Due to its complexity, the coagulation cascade offers a number of potential "targets" as therapeutic entry points for the preparation of anticoagulant agents; one of the most attractive of these is the final serine protease, thrombin. Recently,[1] researchers at Merck described a series of potent lysine α–ketoamides which were found to possess subnanomolar potency toward thrombin. We decided to replace the boronic acid in our very potent boropeptide series[2] with α-ketoamides to have a handle on extending our inhibitors into the S_1' domain of the thrombin active site, which cannot be achieved with boronic acids. Two of the most common methods found in the literature for the preparation of α–ketoamides involve the preparation of a precursor α-hydroxy acid from its corresponding aldehyde. The first method[1,3] requires the addition of a lithiated orthothioformate to the aldehyde, conversion to its α-hydroxy methyl ester via $HgCl_2/HgO$, and saponification to the α-hydroxy acid. The second method[4] involves the conversion of the aldehyde to its corresponding cyanohydrin with KCN, followed by acidic hydrolysis of the nitrile. During our efforts in preparing α–ketoamides, we sought an alternate methodology that avoided the use of these highly toxic, corrosive and malodorous reagents.

Numerous accounts exist in the literature pertaining to the preparation of α–amino alcohols via an epoxide ring opening utilizing either an amine or azide anion.[5] Typically, this procedure results in mixtures of regioisomers due to both C-α and C-β ring opening. However, Behrens and Sharpless[6] demonstrated (Scheme 1) that epoxy amides readily yield a regioselective C-β ring-opened product when $Mg(N_3)_2$ is utilized as the nucleophile.

Scheme 1



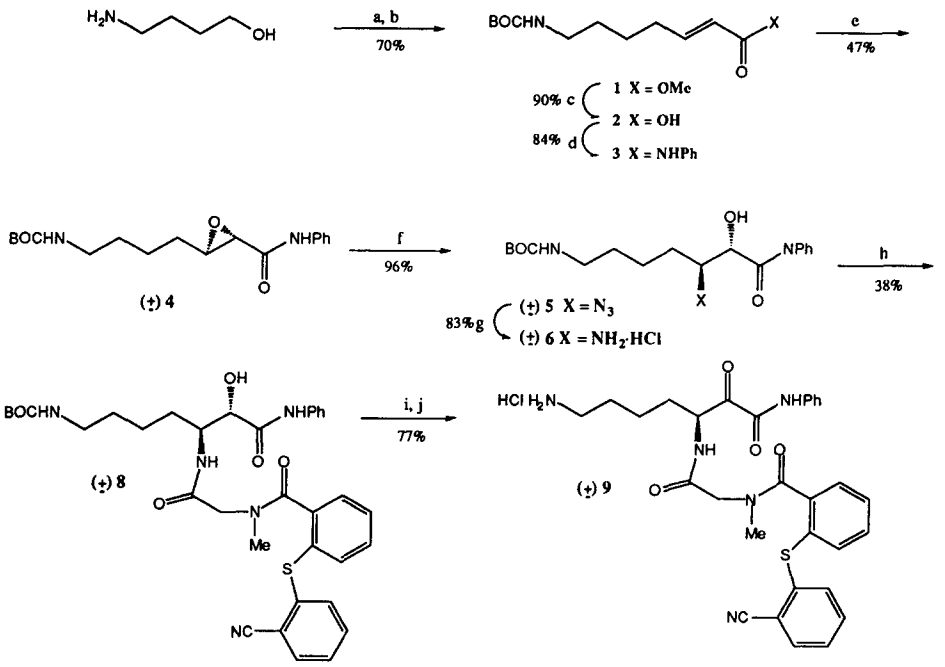
regioselectivity (Cβ:Cα)

10:1

The regioselectivity of the reaction is attributed to an intramolecular chelation between the epoxide oxygen and the amide carbonyl. We have successfully prepared a series of lysine α–ketoamides utilizing this procedure.

Our approach is shown in Scheme 2, as illustrated for the N-phenyl α–ketoamide **9**. 4-Amino-1-butanol was protected as its BOC derivative and smoothly homologated to the α,β-unsaturated ester **1**[7] in a one pot synthesis by way of an intermediate aldehyde. The ester **1** was hydrolyzed with LiOH to the corresponding acid **2**

Scheme 2



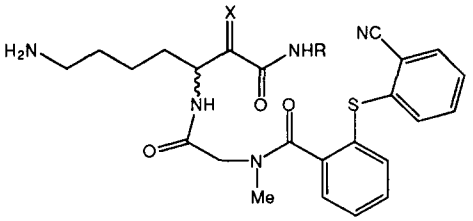
Reagents

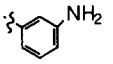
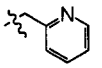
(a) $(BOC)_2O$, CH_2Cl_2. (b) $(ClCO)_2$, DMSO, TEA, $\phi_3P{=}CHCO_2Me$, CH_2Cl_2. (c) LiOH, MeOH/H_2O. (d) IBCF, NMM, $PhNH_2$, CH_2Cl_2. (e) t-BuOOH, n-BuLi, THF. (f) $MgSO_4$, NaN_3, MeOH. (g) H_2, Pd/C, MeOH, HCl. (h) EDC, HOBT, NMM, 7, Me_2NCOMe. (i) $(ClCO)_2$, DMSO, TEA, CH_2Cl_2. (j) TFA or 4M HCl in dioxane

and converted to the desired amide **3** via its mixed anhydride. Epoxidation of the α,β–unsaturated amide was easily accomplished with *tert*-butyl hydroperoxide anion to yield **4** as a racemate. Following Sharpless's procedure, we prepared the α–hydroxy azide **5** by refluxing a methanolic solution of **4** with an in situ ($MgSO_4/NaN_3$) preparation of $Mg(N_3)_2$. In accord with Sharpless's observations, we observed only negligible amounts of the C-α ring opened isomer during the conversion of **4** to **5**. Catalytic hydrogenation of the azide **5** over Pd/C resulted in its reduction to the corresponding amino alcohol **6**. While many methods exist for coupling of the amino alcohol **6** and fragment **7**[8], we found that a N,N- dimethylacetamide solution of EDC, HOBT and NMM gave the best results. Compound **8** was oxidized to the α–ketoamide under typical Swern conditions and deprotected with either 4M HCl/dioxane or TFA to give **9** as a pale yellow to tan solid.

Based on crystallographic and modeling studies, we designed and prepared a series of α–ketoamides outlined in Table 1. The phenyl ring of compounds **9** and **13** was intended to lie on a low ridge (Gly 193) in a shallow pocket at the thrombin S_1' site, while the nitrogen substituents of both the aniline **13** and the pyridine **14** were intended to form hydrogen bonds with two backbone carbonyl groups (Leu 40 and 41), the latter in its protonated form. Comparing the thrombin affinity[9] for **9** and **11**, it is clear that indeed the phenyl group improves the affinity. The aniline **13** is two fold less potent than **9**, implying either that steric problems occur or that the favorable hydrogen-bonded interactions of the amino group are counterbalanced by a larger desolvation penalty paid upon removal of the polar aniline moiety from solvent. The dramatic loss in activity of the pyridine compound **14**

Table 1



Compound	X	R	Ki (μM) Thrombin
9	O	Ph	0.055
10	OH, H	Me	>4.2
11	O	Me	0.570
12	OH, H	Ph	>4.2
13	O		0.120
14	O		7.4

is probably due to a less than optimal steric fit with the enzyme combined with a high desolvation penalty that is not compensated for by favorable interactions of either the neutral or protonated form of the pyridine moiety with the enzyme. As expected, the α-hydroxy amides **10** and **12**, were inactive toward both thrombin and trypsin due to their inability to covalently bind to serine 195.

We are currently utilizing this novel route for the preparation of optically active lysine α-ketoamides via Sharpless asymmetric epoxidation methodology, the details, including the crystallographic and modeling studies, will be described in a forthcoming paper.

Acknowledgment. The authors would like to thank Robert Knabb, Joe Luettgen and Susan Spitz for conducting the affinity assays.

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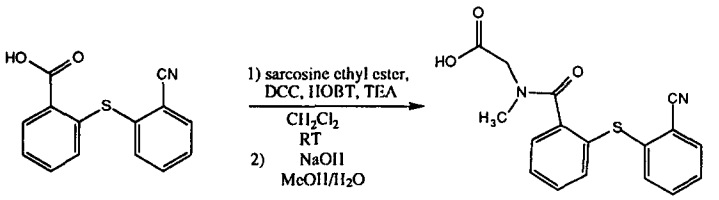
7. Satisfactory ^1H NMR and MS data were obtained for all new compounds described in this communication.

8. a) Unpublished results, this fragment is related to a series of conformationally restricted P3 residues found in reference 2. b) Fragment **7** was prepared in the following manner:



7

9. The inhibitory constant (K_i) assays were performed as described in Kettner, C.; Mersinger, L.; Knabb, R. *J. Biol. Chem.* **1990**, *265*, 18289-18297. Reported values are averages from multiple experiments.

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