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SYNTHESIS AND EVALUATION OF A β-LACTAM-CONTAINING DIPEPTIDE ANALOG AS A PROTEASE INHIBITOR

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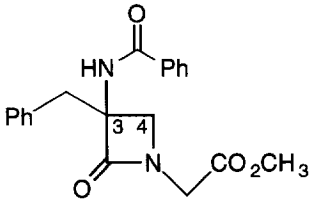
Abstract: A monocyclic 3-amino-β-lactam analog of the dipeptide Phe-Gly methyl ester has been synthesized as a potential protease inhibitor. This β-lactam is a non-time-dependent inhibitor of α-chymotrypsin, carboxypeptidase Y, and cathepsin G. Copyright © 1996 Elsevier Science Ltd

Potent inhibition of human leukocyte elastase,[1-5] cathepsin G,[6,7] and *E. coli* leader peptidase[8] by β-lactams has demonstrated the merit of this type of inhibitor for serine proteases. Many of the β-lactam inhibitors synthesized were thought to inhibit through a "double hit" mechanism, where an electrophile was unmasked at the active site upon cleavage of the lactam amide bond. This electrophile would then be attacked by a second active-site nucleophile. However, it was shown that complete inactivation of human leukocyte elastase by *trans*-4-(ethoxycarbonyl)-3-ethyl-1-[(4-nitrophenyl)sulfonyl]-azetidin-2-one, was simply due to the formation of a stable acyl-enzyme intermediate and not due to the action of a second active-site nucleophile.[9] This "single hit" mode of inhibition points to a simpler, more peptide-like β-lactam nucleus around which specific inhibitors of serine proteases could be built.

This communication is a report on the synthesis and initial evaluation of a C4 unsubstituted monocyclic 3-amino-β-lactam analog **1** of the dipeptide Phe-Gly methyl ester. Compound **1** has two major advantages as a protease inhibitor. Without C4 substitution, this compound more closely mimics natural peptide substrates, and it is primed for incorporation into longer peptide structures.

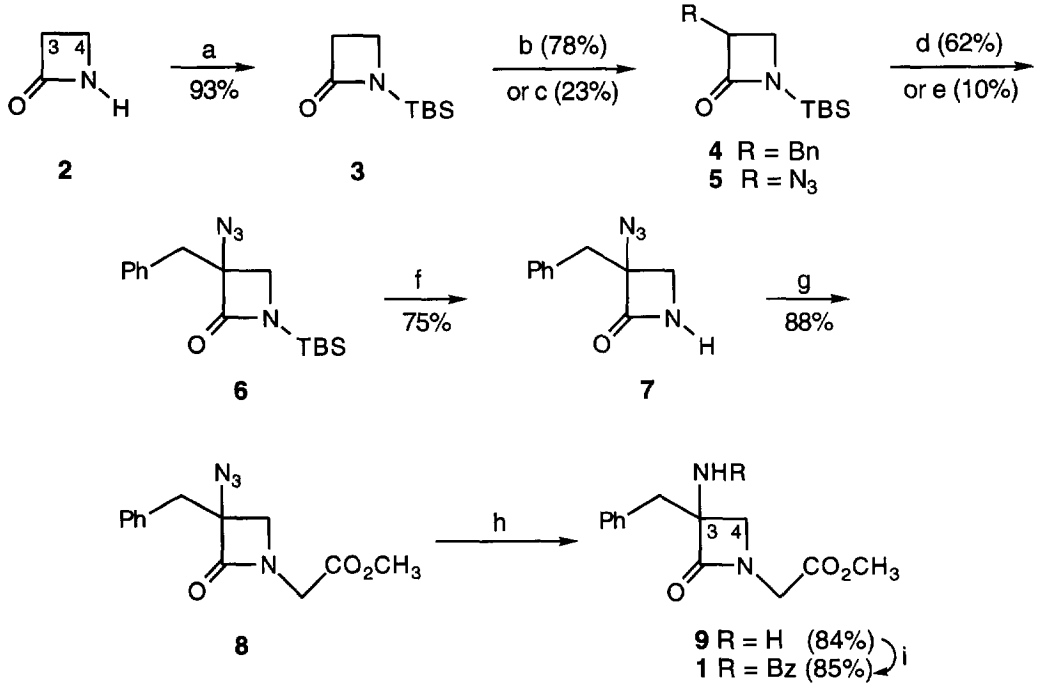


1

Chemistry: The synthesis of the targeted 3-benzamido-3-benzyl-2-azetidinone **1** from commercially available 2-azetidinone (**2**), employing an azide transfer as the key reaction, is shown in the **Scheme**.[10] Treatment of **2** with *tert*-butyldimethylsilyl chloride (TBSCl) in the presence of triethylamine provided *N*-silylated azetidinone **3** in 93% yield. Benzylation of the lithium enolate of **3** yielded 3-benzyl-2-azetidinone **4** in 78% yield. Reaction of the lithium enolate of **3** with tosyl azide (TsN_3) and trimethylsilyl chloride (TMSCl),

according to Kuhlein's protocol,[11] gave 3-azido-2-azetidinone **5** in an unoptimized yield of 23%. The order in which the substituents at C3 were introduced was of importance. When 3-benzyl-2-azetidinone **4** was treated with tosyl azide/TMSCl, 3-azido-3-benzyl-2-azetidinone **6** was obtained in 62% yield. However, benzylation of 3-azidoazetidinone **5** only gave 10% of **6**.[12] We also investigated 1,3,5-triisopropylbenzenesulfonyl azide,[13] which is a safer reagent than tosyl azide for the introduction of the 3-azido group. However, reaction of the lithium enolate of **4** with trisyl azide provided reaction product **6** only in 35%. A low yield was also obtained when we utilized Evans' protocol for the azide transfer reaction (potassium enolate of **4** and acetic acid instead of TMSCl).[13] Removal of the *N*-silyl group of **6** with potassium fluoride gave azetidinone **7** in 61% yield.[14] *N*-Alkylation of **7** with methyl bromoacetate in the presence of potassium *tert*-butoxide provided azetidinone **8** in 88% yield. The Phe-Gly methyl ester analog **9** was obtained in 84% yield by reduction of the 3-azido group of **8**. Finally, the target dipeptide analog **1** was obtained in 85% yield by benzoylation of **9** under Schotten-Baumann conditions.

Scheme



(a) TBSCl (1.2 equiv), Et_3N (1.5 equiv), 0 °C, 1 h; (b) LDA (1.2 equiv), THF, $PhCH_2Cl$ (1.2 equiv), -78 °C, 4 h; (c) LDA (1 equiv), THF, TsN_3 (1 equiv), TMSCl (1 equiv), -78 °C to 25 °C, 4 h; (d) LDA (1 equiv), THF, TsN_3 (1 equiv), TMSCl (1 equiv), -78 °C to 25 °C, 1 h; (e) LDA (1.2 equiv), THF, $PhCH_2Cl$ (1.2 equiv), -78 °C, 3 h; (f) i. KF/CaF_2 (calc. for 0.5 equiv of KF), MeOH, 0 °C, 1 h; ii AcOH; (g) $BrCH_2CO_2CH_3$ (1 equiv), *tert*-BuOK (1 equiv), THF, 0 °C to 25 °C, 4 h; (h) H_2 (1 atm), 5% Pd/C, isopropanol, 25 °C, 11 h; (i) benzoyl chloride (1 equiv), EtOAc/sat. $NaHCO_3$, 25 °C, 10 min.

Biological Evaluation: The Phe-Gly methyl ester analog **1** was screened against each of the following serine proteases: α-chymotrypsin,[15] cathepsin G,[15] elastase,[16] proteinase K,[17] and carboxypeptidase Y.[18] These enzymes were chosen because they are known to be "chymotryptic" in their specificity for the P1 position of peptide substrates. Assays were run in a microtitre plate in a final volume of 250 μL containing 0.05 to 0.1 M buffer (Tris pH 7.5 or pH 8.0, MES pH 6.2), sub-saturating substrate, and 0.05 to 1.2 mM **1**. The initial screening was a simple time-course in which enzyme was added to the assay solution containing 0.12 mM **1** and the appropriate substrate. Enzyme activity was followed on a Molecular Devices $Thermo_{Max}$ plate reader with a 414 nm filter. Enzymes displaying sensitivity to the β-lactam were further examined to determine if the inhibition was time-dependent. Carboxypeptidase Y, α-chymotrypsin, and cathepsin G showed initial sensitivity to **1**; however, none of the enzymes tested displayed time-dependent inhibition. The simple inhibition constants are given in the **Table**.

Table. Inhibition of chymotryptic serine proteases by 3-benzamido-3-benzyl-2-azetidenone **1**.

Enzyme	Inhibition Constant [19] (mM)
α-chymotrypsin	0.3
carboxypeptidase Y	0.3
cathepsin G	0.4
elastase	> 1.2
proteinase K	> 1.2

Summary: We have demonstrated that a simple β-lactam dipeptide mimic can be a non-time-dependent inhibitor of α-chymotrypsin, carboxypeptidase Y, and cathepsin G. The synthetic route employed allows for the introduction of a variety of substituents at the C3 and N1 positions of the β-lactam ring. This will be important for the design of more specific protease inhibitors. The synthetic scheme also provides the necessary framework for incorporation of the "dipeptide" β-lactam into longer peptide chains. The additional peptide structure may provide the binding energy necessary for enzyme catalyzed opening of the β-lactam ring and may produce potent time-dependent inhibitors.

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(15) Assay conditions: buffer = 0.1 M tris(hydroxymethyl)-aminomethane (Tris) pH 7.5; substrate = 0.1 M succinyl-alanyl-alanyl-prolyl-phenylalanyl-para-nitroanilide (Suc-AAPF-pNA).

(16) Assay conditions: buffer = 0.1 M tris(hydroxymethyl)-aminomethane (Tris) pH 8.0; substrate = 0.08 M succinyl-alanyl-alanyl-alanyl-para-nitroanilide (Suc-AAA-pNA).

(17) Assay conditions: buffer = 0.1 M tris(hydroxymethyl)-aminomethane (Tris) pH 8.0; 0.05 M $CaCl_2$; substrate = 0.1 M succinyl-alanyl-alanyl-prolyl-phenylalanyl-para-nitroanilide (Suc-AAPF-pNA).

(18) Assay conditions: buffer = 0.05 M 2-(N-morpholino)ethanesulfonic acid (MES) pH 6.2; substrate = 0.5 M *N*-benzoyl-tyrosyl-para-nitroanilide (BTNA).

(19) Concentration of **1** that produces one-half maximal enzyme activity.

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