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SYNTHESIS OF POTENT AND SELECTIVE INHIBITORS OF HUMAN PLASMA KALLIKREIN

Garry S. Garrett,[a] Sara J. McPhail,[a] Keith Tornheim,[b] Paul E. Correa,[a] and John M. McIver[a,*]

[a]*Corporate Research Division, Miami Valley Laboratories, The Procter & Gamble Company, P.O. Box 538707, Cincinnati, OH 45253-8707, U.S.A.*
[b]*Boston University School of Medicine, 80 E. Concord Street, Boston, MA 02118, U.S.A.*

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Abstract: The synthesis and in vitro enzyme inhibition profile of a series of novel trifluoromethylketone (TFMK) inhibitors of human plasma kallikrein (PK) are described. We have developed an efficient method for the construction of peptide TFMKs that provides the final product devoid of compromised stereochemical integrity. Many of these compounds are potent inhibitors of PK and exhibit reduced inhibition of tissue kallikrein (TK) and plasmin (HP).

As part of an ongoing program dedicated to the investigation of the potential utility of kallikrein inhibitors as therapeutic agents, we have synthesized a series of potent and selective inhibitors of PK. During a previous investigation of enzyme/inhibitor subsite interactions with peptide aldehyde inhibitors,[1] we found that tripeptides containing a variety of P2 and P3[2] substitution patterns provided potent inhibitors but little enzyme selectivity. The P1 subsite was not evaluated during that study and we felt that a judicious choice of structure at this subsite might provide the PK selectivity that we desired. We selected the *p*-guanidinophenylalanine residue to fill the S1 subsite on the enzyme surface in view of its synthetic accessibility and its incompatibility with the TK S1 subsite. Our attempt to model a P1 substituted *p*-guanidinophenylalanine residue into the TK S1 subsite[3] indicated that the planarity of the phenyl ring of the inhibitor must be sacrificed before a complementary binding interaction can be obtained. The compounds described in this report provide evidence that this subsite does indeed offer the opportunity for selectivity.

The well-described, potent inhibition of other serine proteases (i.e., elastase) by compounds containing the trifluoromethylketone (TFMK) functionality prompted our interest in synthesizing compounds containing this structural element.[4] The TFMK is thought to function much like an aldehyde in that they both form a tetrahedral intermediate with the active site serine and thus take advantage of transition state affinity.[5] Several methods exist for the construction of TFMK's and the literature has recently been extensively reviewed.[6] Peptide TFMK's present the additional problem of maintaining the stereochemical integrity of the carbon α to the fluoroketone[7] while ensuring the facile synthesis of the target.

The addition of the trifluoromethyl group to aldehydes via the action of fluoride anion on CF_3TMS has recently been described[8] and we were interested in the application of this reaction to the above stated goal of

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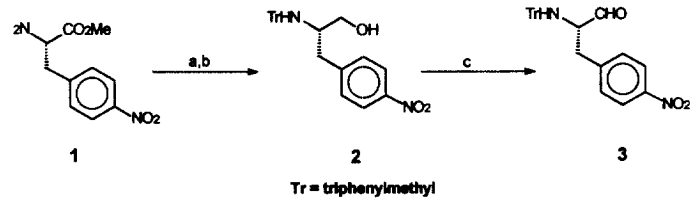
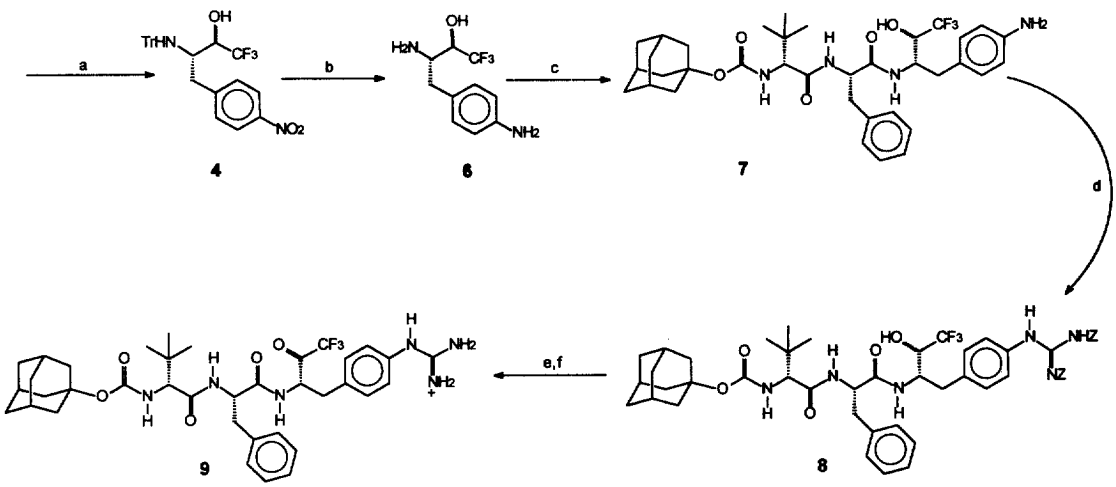


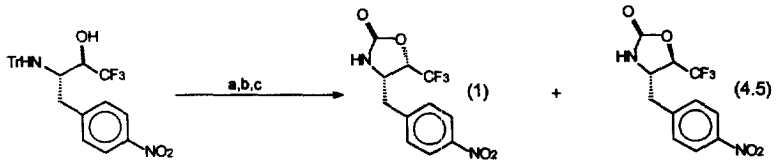
Scheme 1. *Reagents*: (a) TrCl, TEA, CH_2Cl_2; (b) DIBAL-H, THF, 61% (combined a+b); (c) DMSO, $(COCl)_2$, TEA, –78 °C, 89%.

stereochemical homogeneity. Initial attempts to add this reagent to amino acid aldehydes that were protected as the carbamate met with limited success.[9] However, if the carbamate protecting group is replaced with triphenylmethyl the reaction proceeds rapidly and cleanly. The requisite aldehyde **3** was prepared in a straightforward manner and is described in Scheme 1. Reaction of aldehyde **3** with CF_3TMS under theconditions described by Prakash[8] provided the desired addition product **4** as a mixture of diastereomers (4.5:1) in good yield (88%) (Scheme 2). The stereochemical outcome of this reaction was determined by separation of the diastereomers and independent conversion to the urethanes as outlined in Scheme 3. Analysis of the vicinal coupling constants of the ring protons[10] provided ample evidence to assign stereochemistry.



Scheme 2. *Reagents*: (a) CF_3TMS, n-$Bu_4N^+F^-$, THF, 88%; (b) $SnCl_2$, EtOH, 62%; (c) DECP, TEA, Adoc-d-*t*-Bug-Phe-OH (**10**), DMF, 66%; (d) $Hg(OAc)_2$, MeSC(NZ)NHZ, THF, 89%; (e) Dess–Martin Periodinane; (f) H_2, Pd-C, MeOH, AcOH, combined e+f, 50%.

Reduction of the nitro group of **4** is accomplished with $SnCl_2$ in EtOH. These conditions catalyze the concomitant removal of the trityl protecting group and provide the diamine **6** in a single operation (62% yield). With diamine **6** in hand, we attempted to selectively couple the aliphatic amine with the desired dipeptide coupling partner **10**[1] via the action of diethylcyanophosphonate (DECP). Our initial attempt in CH_2Cl_2 was



4 5a 5b

Scheme 3. *Reagents*: (a) HCl in dioxane, (b) silica gel chromatography separation of diastereomers; (c) phosgene, TEA, CH_2Cl_2, 100%.

unsuccessful, providing the product of coupling at both nitrogens. However, when DMF was used as solvent, this reaction could be carried out with complete chemoselectivity, providing the desired peptide **7** in good yield (66%). Successive reaction of the remaining aromatic amine with bis-Cbz-2-methyl-2-thiopseudourea[11] provided the desired guanylated aniline derivative **8**. When this reaction was run at room temperature no reaction occurred. Heating the reaction to 50 °C provides the desired product, although the reaction is lengthy (48 h) and capricious. However, we found that a catalytic amount of $Hg(OAc)_2$ ensures a rapid reaction at room temperature and provides a clean product in excellent yield (89%).[12]

Oxidation of **8** with the Dess–Martin periodinane[13] provided an excellent recovery of the TFMK. The subsequent hydrogenation to afford the final product **9** is performed on the crude oxidation product. Attempts to chromatograph the crude product on silica resulted in rapid loss of stereochemical integrity. The combined oxidation/deprotection of **8** affords **9** in 50% yield as a single diastereomer after reverse-phase HPLC purification. We observed no evidence of another diastereomer before or after chromatography. Interestingly, the final product **9** is far more resistant to racemization than we suspected. Exposure of **9** to pH 10 buffer results in equilibration to a 65:35 mixture of diastereomers in 12 h. If the pH is maintained at 7.4 the same equilibration ratio is achieved in 4 days. The R or (D) configuration is predominate when the compound is fully equilibrated.

Compound **9** is a potent, slow binding inhibitor of human PK (Figure 1). Evaluation of the progress curves by the method of Cha[14] revealed a value for kon of 0.068 min^{-1} μM^{-1}. The K_i was determined to be 0.002 μM from measurement of residual activity after a 3 h incubation of 1–2 μM concentration of inhibitor with high amounts of enzyme. The k_{off} ($K_i.k_{on}$) was determined to be 0.00014 min^{-1} and was calculated from the aforementioned values since it was too small to be determined accurately from a kobsd plot.[15]

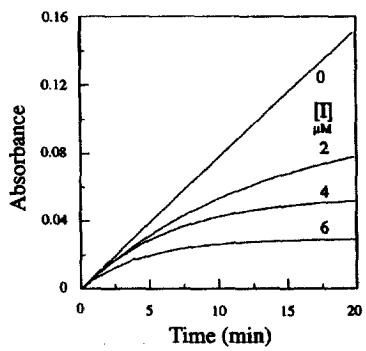
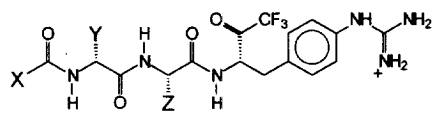


Figure 1. Slow-binding inhibition of compound **9**. K_{on} = 0.068 min^{-1} uM^{-1}, k_{off} = 0.00014 min^{-1}.

The incorporation of the *p*-guanidinophenylalanine P1 substituent affords a PK selectivity profile superior to any that we have previously observed.[1] All of our previous studies have included the arginine residue at the P1 subsite. Based on information gathered from those studies,[1] we have examined a series of inhibitors (Table 1) that were synthesized utilizing this methodology. Many of these compounds are quite potent and they all exhibit excellent selectivity vs. tissue kallikrein. This impressive selectivity profile should aid us in our quest to assess the roles of tissue vs. plasma kallikrein in pertinent animal models. The selectivity relative to plasmin was more variable. The 60-fold selectivity exhibited by compound **9** was not improved with substitution at P2 or P3 (i.e., **13** and **14**, respectively). As expected, removal of the benzyl sidechain from the P2 subsite abolished most of the binding.

Table 1. Selective Inhibition of Plasma Kallikrein



		K_i (μM)		
		Plasma Kallikrein (PK)	**Tissue Kallikrein (TK)**	**Plasmin (HP)**
9	X = adamantyloxy Y = *t*-butyl Z = benzyl	0.002	47.3	0.12
10	X = adamantyloxy Y = isopropyl Z = benzyl	0.005	191	0.08
11	X = *t*-butyloxy Y = benzyl Z = 2-naphthyl	0.089	223	0.060
12	X = morpholine Y = *t*-butyl Z = benzyl	0.028	>2500	0.56
13	X = adamantyloxy Y = *t*-butyl Z = 2-naphthyl	0.5	> 50	0.5
14	X = adamantyloxy Y = CH_2benzyl Z = benzyl	0.004	7	0.04
15	X = morpholine Y = *t*-butyl Z = H	15.5	>150	110

In summary, we have developed an efficient method for constructing peptide trifluoromethyl ketones wherein the stereochemical integrity of all chiral centers is uncompromised. The kallikrein inhibitors synthesized utilizing this methodology are quite potent and exhibit an impressive selectivity profile. Additional studies probing potential improvements in the observed selectivity and in the performance of these inhibitors in animal models of inflammation will be reported in due course.

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10. The vicinal ^1H-^1H couplings of the ring protons of the diastereomeric urethane derivatives depicted in Scheme 3 were determined after assignments were made utilizing coupling patterns and 2-D COSY experiments. The predominant isomer had a coupling constant of 4.4 Hz while the minor product possessed a larger constant (8.1 Hz) indicative of the cis substituted product.

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15. The following alternative procedure was used to determine the K_i. Relatively high concentrations of enzyme were first incubated with inhibitor in the absence of substrate for 1, 2, or 3 at 30 °C; substrate was then added and the enzymatic activity determined spectrophotometrically. The residual activity was compared with the control lacking inhibitor (which must be diluted 10-fold for assay). K_i was calculated from the residual activity (R); $K_i = [I]*R/(100 - R)$. There is no term for competition by substrate in this case, since inhibitor binding is in the absence of substrate. If k_{off} is low (which is the case when this assay must be used), then there is little displacement of I by S during the assay period (5 min). If there was a slow increase in rate, then the initial rate was used. koff was calculated using the value of k_{on} from the progress curve experiment: $k_{off} = K_i*k_{on}$.