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IRREVERSIBLE INHIBITIONS OF SERINE PROTEASES BY PEPTIDYL ALLYLIC HALIDE DERIVATIVES

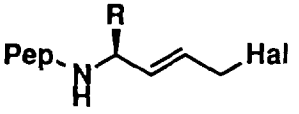
Tsuyoshi Ohba, Eitatsu Ikeda, Jun Wakayama and Hisashi Takei*

Interdisciplinary Graduate School of Science and Engineering,
Tokyo Institute of Technology
Nagatsuta 4259, Midoriku, Yokohama 226, Japan

Abstract. Peptidyl 4-amino-5-phenyl-2-pentenyl bromide (**7a**, **9a**, **10a**) and chloride derivatives (**7b**, **9b**, **10b**) were found to be active-site directed irreversible inhibitors of α-chymotrypsin but did not show any irreversible inhibitory activity toward porcine pancreatic elastase.

Serine proteases are concerned in numerous reactions *in vivo* and, therefore, selective and irreversible inhibitors are useful tools in the study of each serine protease mechanism and in the design of new inhibitors as drugs.[1,2,3]

Although a number of irreversible inhibitors containing electrophilic moiety in their structures such as peptidyl chloromethyl ketones[4,5,6], peptidylphosphonates[7,8], and alkyl isocyanates[9,10] have been reported, there is no report about the potency and the selectivity of allylic halide derivatives as an irreversible inhibitor of serine proteases, to the best of our knowledge. Since it has been known that amide bond resembles to carbon-carbon double bond in three dimentional structure[11,12], we designed peptidyl allylic halide derivatives as novel irreversible inhibitors of serine proteases.

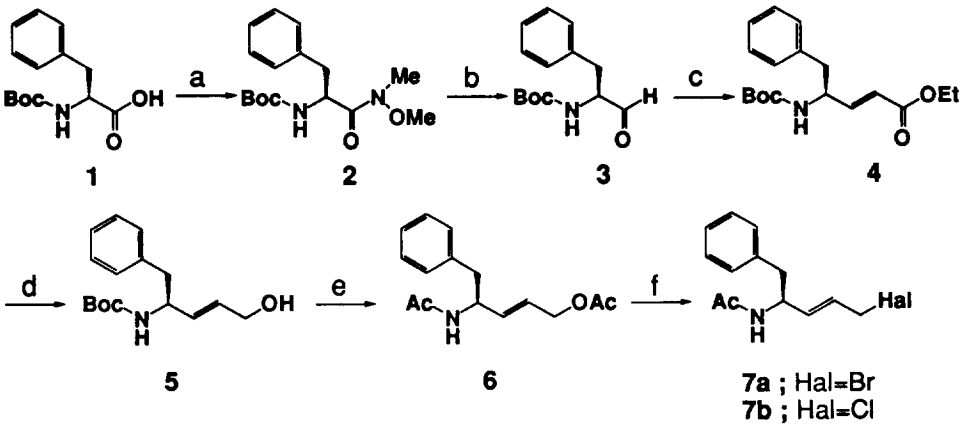


R = Amino acid side chain
Pep = Peptide chain or Protecting group
Hal = F, Cl or Br

Peptidyl allylic halide derivatives would be thought to show high affinity toward serine proteases and react with active-site nucleophiles. Although allylic fluorides are not so electrophilic species as allylic bromides or chlorides, we assumed that the hydrogen bonding between fluorine atom and amide proton in active-site would assist the displacement of fluorine atom[13,14]. We chose α-chymotrypsin, which favors peptide substrates possessing Trp, Tyr, and Phe side chains at position P_1, as our target enzyme and

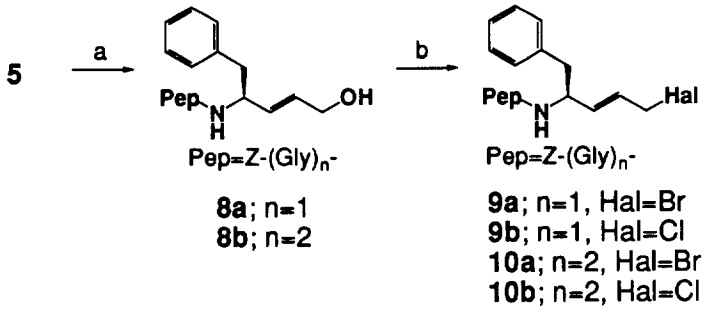
synthesized a series of peptidyl 4-amino-5-phenyl-2-pentenyl halide derivatives **7a-b, 9a-b, 10a-b, 12a-c** considering the substrate specificity of α-chymotrypsin.

Scheme 1



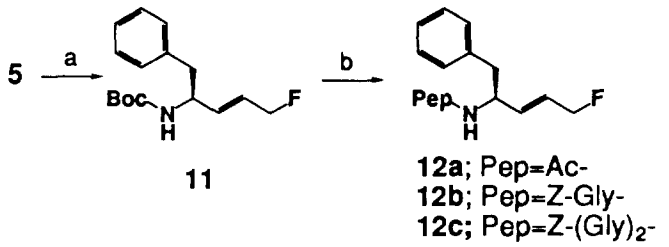
(a) EDC•HCl, HOBt, HCl•HN(OMe)Me, CH_2Cl_2 (92 %); (b) $LiAlH_4$, THF, 0 °C (95 %); (c) $(EtO)_2POCH_2CO_2Et$, NaH, THF, 0 °C (75 %); (d) DIBAL-H, BF_3•OEt_2, CH_2Cl_2 (80 %); (e) 1) 4 N-HCl/dioxane (quant.); 2) Ac_2O, NEt_3, CH_2Cl_2 (quant.); (f) 1) K_2CO_3aq., MeOH (quant.); 2) **7a**: PPh_3, CBr_4, CH_2Cl_2, 0 °C (24 %) / **7b**: PPh_3, CCl_4, THF, reflux (30 %)

Scheme 2



(a) 1) 4 N-HCl/dioxane (quant.), 2) Z-$(Gly)_n$-OH, EDC•HCl, HOBt, CH_2Cl_2 (**8a**: 59 %, **8b**: 76 %); (b) **9a, 10a**: PPh_3, CBr_4, CH_2Cl_2 (**9a**: 36 %, **10a**: 45 %) / **9b, 10b**: PPh_3, CCl_4, THF, reflux (**9b**: 51 %, **10b**: 39 %)

Scheme 3



(a) Et_2NSF_3, DME, -78 °C (37 %); (b) 1) 4 N-HCl/dioxane (quant.), 2) **12a**: Ac_2O, Pyridine (76 %); **12b**: Z-Gly-OH, EDC • HCl, DMAP, CH_2Cl_2-dioxane (91 %); **12c**: Z-Gly-Gly-OH, EDC•HCl, DMAP, CH_2Cl_2-dioxane (68 %)

All compounds were synthesized using known procedures via optically active common intermediate **5** [15], which was synthesized from compound **4** by the method of Morikawa et al.[16,17] as shown in Scheme 1[18]. A series of peptidyl allylic fluorides were synthesized as shown in Scheme 3. Fluorination of compound **5** was carried out with diethylaminosulfur trifluoride (DAST) in 1,2-dimethoxyethane[19].

First, we evaluated the inhibitory activity of a series of bromides **7a**, **9a**, **10a** and obtained the second-order rate constants (k_{obsd}/[I]) according to the literature procedures [8,20]. Incubation of compound **7a** with α-chymotrypsin resulted in a time-dependent loss of enzyme activity with k_{obsd}/[I] = 14 $M^{-1}s^{-1}$ ([I] = 101 μM). The inactivation assay of α-chymotrypsin by compound **7a** (101 μM) in the presence of the substrate (Suc-Ala-Ala-Pro-Phe-NA, 0.5 mM and 0.25 mM) resulted in a decrease in the inactivation rate (k_{obsd}/[I] = 3.5 M^{-1} s^{-1} and 5.0 $M^{-1}s^{-1}$, respectively) and dialysis of assay solution against buffer solution (pH 7.5, 24 h) at 4 °C did not restore any enzyme activity, indicating that compound **7a** is active-site directed. Furthermore, dipeptidyl and tripeptidyl derivatives **9a**, **10a** exhibited more potent inhibitory activity with k_{obsd}/[I] = 215 $M^{-1}s^{-1}$ ([I] = 27 μM) and 1238 $M^{-1}s^{-1}$ ([I] = 7 μM), respectively. The inactivation was selective because the bromides exhibited no inhibitory activity toward porcine pancreatic elastase (PPE). However, it was found that the bromides decompose gradually under the assay conditions.

Next, we investigated a series of chlorides **7b**, **9b** and **10b**. The chlorides are stable under the assay conditions and exhibited irreversible inhibitory activity toward α-chymotrypsin similarly to the bromides. Mono amino acid derivative **7b** exhibited rather weak inhibitory activity with k_{obsd}/[I] = 0.8 $M^{-1}s^{-1}$ ([I] = 476 μM), compared to corresponding the bromide derivative **7a**, and time-dependent loss of enzyme activity was not observed at low concentration (< 200 μM). However, dipeptidyl and tripeptidyl derivatives **9b**, **10b** exhibited significant improvement on the inactivation rate at low inhibitor concentrations with k_{obsd}/[I] value = 12 $M^{-1}s^{-1}$ ([I] = 28 μM) and 61 $M^{-1}s^{-1}$ ([I] = 17 μM), respectively. In addition, the inactivation was selective because both dipeptidyl and tripeptidyl chlorides exhibited no irreversible inhibitory activity toward PPE. These data indicates that the chemical reactivity of the chlorides is modest but the inhibitory activity of the chlorides can be improved by increasing the affinity toward enzyme. Therefore, the chlorides would be also selective and potent irreversible inhibitors of serine proteases as well as the bromides.

On the other hand, a series of peptidyl allylic fluorides exhibited no irreversible inhibitory activity toward α-chymotrypsin. Therefore, contrary to our speculation, the effect of the hydrogen bonding seems not to be expected for the displacement of fluorine atom in this case.

Table 1. Inactivation of Serine Proteases by Peptidyl Allylic Halide Derivatives

		enzyme			
		α-Chymotrypsin[a]		PP elastase[b]	
inhibitor	Hal	[I] ; inhibitor concentration (μM)	k_{obsd}/[I] ($M^{-1}s^{-1}$)	[I] ; inhibitor concentration (μM)	k_{obsd}/[I] ($M^{-1}s^{-1}$)
7a	Br	101	14	101	N. I.[c]
9a	Br	25	215	25	N. I.
10a	Br	7	1238	15	N. I.
7b	Cl	476	0.8	—	—
9b	Cl	28	12	600	N. I.
10b	Cl	17	61	97	N. I.
12a	F	13.5 mM	N. I.	—	—
12b	F	1.05 mM	N. I.	—	—
12c	F	4.4 mM	N. I.	—	—

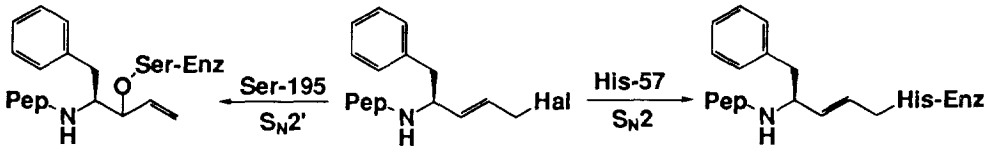
[a]α-Chymotrypsin (1.6 μM) was incubated in 500 μl of buffer (0.1 M sodium phosphate buffer, 0.5 M NaCl, 5 % Me_2SO, pH 7.8 at 25 °C) containing inhibitors. At various time intervals, 10 μl aliquots were withdrawned and assayed with 1500 μl of Suc-Ala-Ala-Pro-Phe-NA (0.5 mM, buffered as above) as a substrate (Suc=succinyl; NA=4-nitroanilide); [b]PP elastase (11 μM) was incubated in 500 μl of above buffer containing inhibitors. At various time intervals, 50 μl aliquots were withdrawned and assayed with 1950 μl of Suc-Ala-Ala-Ala-NA (0.7 mM, buffered as above) as a substrate; [c]no inactivation

In summary, a series of peptidyl 4-amino-5-phenyl-2-pentenyl bromide and chloride derivatives has been found to be selective irreversible inhibitors of α-chymotrypsin. From the kinetic data, the chlorides would be regarded as not classic affinity labels but quiescent affinity labels such as peptidyl acyloxymethylketones[21,22],

specific inhibitors of cysteine proteases. Since the inactivation rates of both the bromides and the chlorides were improved significantly by introducing simple peptide chain (such as Z-Gly- or Z-Gly-Gly-) into the amino group of mono amino acid derivatives, our compounds will be more potent inhibitors by introducing more suitable peptide chain for target enzyme. In addition, optically active peptidyl allylic bromides and chlorides can be prepared from α-amino acid without difficult procedures and, therefore, they would be useful irreversible inhibitors for the future enzyme studies.

Two inactivation mechanisms have been postulated as shown in Scheme 4. One is the reaction with Ser 195 by S_N2' fashion and the other with His 57 by S_N2 fashion. Studies leading to the proof of the precise mechanism and the extention of our design to other serine proteases are now in progress.

Scheme 4. Postulated Inactivation Mechanism by Peptidyl Allylic Bromides and Chlorides



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18. Abbreviations: Ac, acetyl ; Boc, *tert.*-butyloxycarbonyl ; DIBAL-H, diisobutylaluminium hydride ; DMAP, 4-dimethylaminopyridine ; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole ; NA, 4-nitroanilide ; Suc, succinyl ; THF, tetrahydrofuran ; Z, benzyloxycarbonyl
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