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SYNTHESIS OF A PEPTIDYL 2,2-DIFLUORO-3-AMINOPROPIONATE

Michael R. Angelastro, Philippe Bey, and Shujaath Mehdi and Norton P. Peet*

Marion Merrell Dow Research Institute
2110 East Galbraith Road
Cincinnati, Ohio 45215, USA

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Summary: Ethyl 3-(benzylamino)-2,2-difluoro-4-methylpentanoate (4) was prepared via a Reformatsky reaction of ethyl bromodifluoroacetate with the imine from isovaleraldehyde and benzylamine. N-Debenzylation of 4 gave amino ester 6, which was coupled to Boc·Ala·Ala·Pro·OH to provide a potential proteinase inhibitor.

Peptidyl α-keto esters are potent, competitive inhibitors of proteinases.[1] It is generally assumed that the ester group activates the ketone carbonyl group in these α-keto esters such that 1,2-addition of an enzyme nucleophile to the ketone carbonyl group occurs in the catalytic site of the enzyme to form a hemiketal.

However, in a recent X-ray diffraction study of α-chymotrypsin incubated with MeOSuc·Ala·Ala·Pro·Phe·CO_2CH_3, the catalytic serine hydroxyl group was shown to undergo transesterification with the ester of the inhibitor.[2] Thus, it seemed prudent to prepare compounds in which the ketone carbonyl unit of the inhibitor is replaced with a difluoromethylene unit. Such compounds would be useful in helping to determine whether serine proteinase inhibition via transesterification is an operative and/or universal mechanism. Moreover, these compounds would constitute a new potential class of proteinase inhibitors.

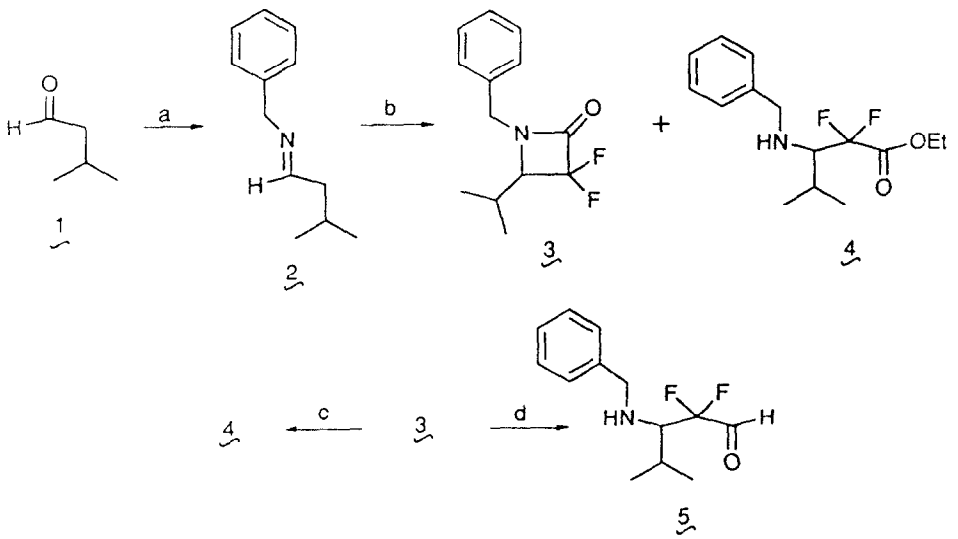
Retrosynthetic analysis suggested α,α-difluoroacetate enolates and imines as convenient precursors to 2,2-difluoro-3-aminopropionates. It has been previously shown that imines will react with appropriate nucleophiles to produce β-lactams. Thus, ketene silylated acetals,[3] ester enolate anions[4] and Reformatsky reagents[5] have been employed. Halodifluoroacetates have been previously used in Reformatsky reactions[6-8] with imines to give 3,3-difluoro-2-azetidinones, which were intermediates in the preparation of 2,2-difluoro-3-aminosugars.[6] 2,2-Difluoroketene silyl acetals when treated with imines afforded 2,2-difluoro-3-aminopropionates.[6]

The route chosen to access a valine-like α,α-difluoro-β-aminopropionate (4) is shown in Scheme I. Addition of the Reformatsky reagent derived from ethyl bromodifluoroacetate[9] to imine 2, which was prepared from isovaleraldehyde (1) and benzylamine, gave a mixture of lactam 3 (73%) and ester 4 (6%). Lactam 3 was converted to ester 4 in 80% yield by treatment with sodium ethoxide in ethanol.[10] Treatment of 3 with diisobutylaluminum hydride in tetrahydrofuran efficiently gave α,α-difluoroaldehyde 5, a transformation of interest since peptide aldehydes are proteinase inhibitors.[1c]

Removal of the N-benzyl protecting group from propionate 4 by hydrogenolysis gave the β-amino ester, which was conveniently isolated as the p-toluenesulfonic acid salt 6

(Scheme II). Coupling of **6** to Boc·Ala·Ala·Pro·OH[1a] using the mixed anhydride procedure provided α,α-difluoro ester **7**,[11] a potential inhibitor of elastase.

Scheme I



Although the N-protecting group on **7** is different from that on the α-keto ester used in the diffraction study, we have shown that HNE will accept a variety of N-protecting groups in a homologous series. Thus, as shown in Table I, the tetrapeptidyl trifluoromethylketones bearing methoxysuccinyl, t-butyloxycarbonyl, carbobenzyloxy and dansyl are all potent inhibitors of HNE.

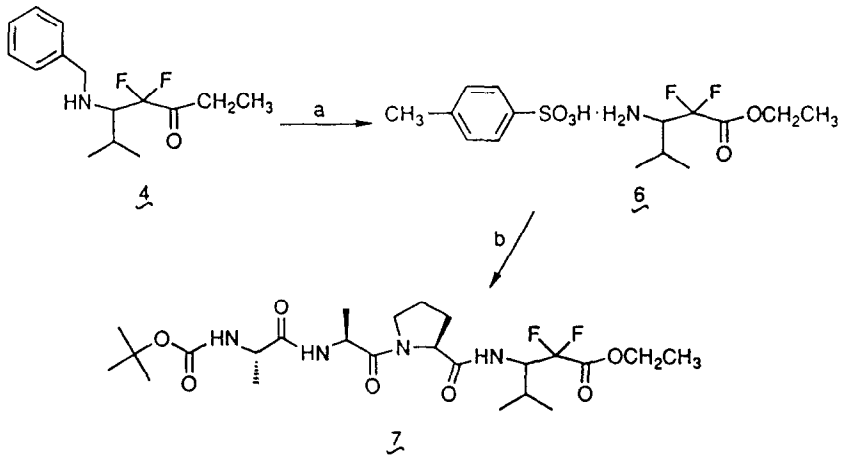
Table 1. Peptidyl Fluorinated Ketones as Elastase-Inhibitors

Cpd. No.	Structure	K_i,[a] μM human neutrophil elastase
-	MeO·Suc·Ala·Ala·Pro·Val·CO_2CH_3	0.20[b]
7	Boc·Ala·Ala·Pro·NHCH(iPro)CF_2CO_2Et	>300
-	MeO·Suc·Ala·Ala·Pro·Val·CF_3	0.014[b]
-	Boc·Ala·Ala·Pro·Val·CF_3	0.044[b]
-	Cbz·Ala·Ala·Pro·Val·CF_3	0.001[b]
-	Dan·Ala·Ala·Pro·Val·CF_3	0.010[b]

[a]Values are those of steady-state inhibition. [b]See reference 1a.

α,α-Difluoro ester 7 failed to inhibit human neutrophil elastase (HNE) in an in vitro assay at a concentration of 33 μM; the lower limit for the K_i was estimated to be 300 μM. In contrast, we have recently shown that MeO·Suc·Ala·Ala·Pro·Val·CO_2CH_3 inhibits HNE with a K_i of 0.20 μM.[1a,12] One of several possible explanations for this difference is that transesterification of the keto ester occurs in the active site of the enzyme in a

Scheme II



Reagents: a) 10% palladium on carbon, p-toluenesulfonic acid, ethanol; b) isobutyl chloroformate, N-methylmorpholine, BocAlaAlaProOH, CH_2Cl_2.

stepwise process involving initial attack of the active site serine hydroxyl group on the ketone carbonyl group. Since the difluoro ester 7 lacks the ketone, it would not participate in the initial addition of the enzyme nucleophile.

The synthesis of the peptidyl α,α-difluoro ester 7 described in Scheme II should be applicable to the preparation of a wide variety of related peptidyl esters which may be useful as inhibitors of other proteolytic enzymes.

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9. Prior to use the zinc was stirred briefly with 20% hydrochloric acid and collected, washed with water and acetone, and dried under high vacuum.

10. Scrupulously dry ethanol (freshly distilled from magnesium turnings) was necessary for this transformation.

11. For 7: ^{19}F NMR ($CDCl_3$, 282 MHz): major isomer (60%) δ -112.2 (dd, J=256 Hz, 11 Hz), -114.7 (dd, J=256 Hz, 19 Hz); minor isomer (40%) δ -113.4 (d, J=254 Hz), -115.6 (dd, J=254 Hz, 20 Hz); IR (KBr) 1770 cm^{-1} (α,α-difluoro ester C=O); MS (CI, CH_4) 535 ($M^+ + 1$), 563 ($M^+ + 29$), 575 ($M^+ + 41$); *Anal.* Calcd for $C_{24}H_{40}F_2N_4O_7$: C, 53.92; H, 7.54; N, 10.48. Found: C, 53.69; H, 7.59; N, 10.27.

12. In addition, we have recently shown that related α-keto esters and α-diketones inhibit human neutrophil elastase. See reference 1d.