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Stereoselective Synthesis and Inhibitor Properties towards Human Leucocyte Elastase of Chiral β-Peptidyl Trifluoromethyl Alcohols.

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Summary: The synthesis of chiral peptidyl trifluoromethyl alcohols **9** and **10** was performed from easily available epoxy ethers **3**, through different stereocontrol of the reduction of the azido ketone **4**. One stereoisomer in each pair of the diastereoisomeric peptidyl trifluoromethyl alcohols **9** and **10** behaved as reversible inhibitors of human leucocyte elastase, a serine protease implicated in inflammatory related diseases.

Fluorinated peptidyl ketones are effective transition state analogues of a variety of hydrolytic enzymes, in particular cysteine, serine and aspartyl proteases [1, 2, 3] Furthermore β-amino alcohols are key units of some described aspartyl protease inhibitors. However fluorinated β-peptidyl alcohols have not been studied to date due to the lack of any available chiral synthesis of fluorinated amino alcohols of type **1** and **2** (Fig. 1). We describe here an access to four stereoisomers of fluorinated β-peptidyl alcohols, thus allowing an investigation on to the influence of configuration on inhibitory properties. Preliminary studies have been carried out with human leucocyte elastase which is implicated in inflammatory related diseases, such as pulmonary emphysema,[4] rheumatoid arthritis[5] and artheriosclerosis

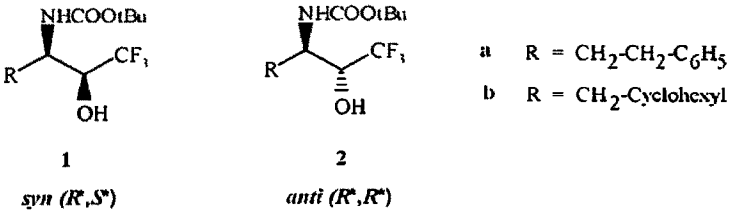
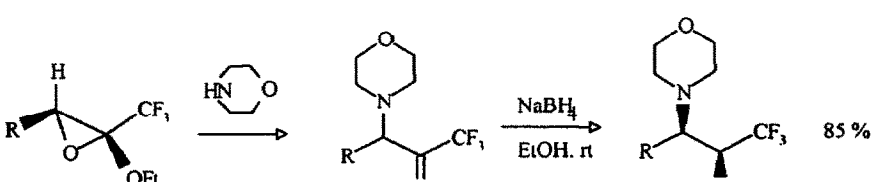


Figure 1

To the best of our knowledge, no general and stereoselective synthesis of such CF_3- or perfluoroalkyl aminoalcohols have been reported in the literature. In earlier reported methods for the preparation of CF_3-aminoalcohols - Henri condensation between a nitroalcane and fluoral,[2b-d,6] condensation of the dianion of carboxylic acid with fluoral followed by a Curtius rearrangement,[7] modified Dakin-West procedure,[8] trifluoromethylation using (trifluoromethyl)trimethylsilane of amino aldehyde,[2d] the relative configuration of the aminoalcohol moiety was not controlled. We previously described the first stereoselective preparation of the *syn* isomer of such amino alcohols[9] by reduction of α-amino trifluoromethyl ketones. Few months later, an access to a mixture 1:6 of two diastereoisomeric β-peptidyl alcohols was described by addition of a trifluoromethyl anion equivalent to an α-peptidyl aldehyde [10] We now report here an easy and versatile access to each of both stereoisomers of β-amino trifluoromethyl alcohols which can allow the access of corresponding homochiral β-peptidyl alcohols by coupling with a chiral aminoacid

Our previous approach was based on the nucleophilic opening of epoxy ethers **3**[11] by a secondary amine leading to the α-amino ketones **4**.[9] The further reduction by $NaBH_4$ or any other reagent follows the Felkin-Anh model[12] and provides the pure (*R*,S**) diastereoisomer **5** (Scheme 1)



3 (*R*,S**)

4

5 (*R*,S**)

R = CH_2-CH_2-C_6H_5

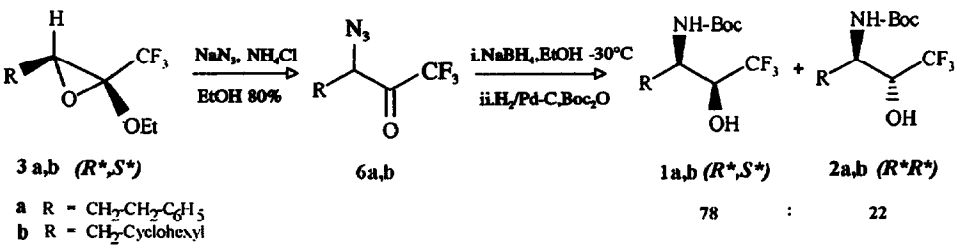
R = CH_2-Cyclohexyl

Scheme 1

This selectivity has been explained in terms of steric hindrance in the transition state, as often observed when the amino group is secondary.[13] In this respect, we assumed that the reduction of α-primary amino ketone could occur with the opposite stereoselectivity However all our attempts to react primary amines with epoxy ethers **3** failed, the degradation of products being faster than the nucleophilic opening of **3** We therefore pursued a similar approach by using an azide anion as nucleophile

Our attempts to achieve this reaction in non-protic medium failed. Epoxy ethers **3** have been found to be quite unreactive towards trimethylsilyl azide even in the presence of a catalyst: $Ti(O\textit{iso}\text{-}Pr)_4$[14,15] *iso*-$Pr_2NH/MeOH$,[16] BF_3 Et_2O, [17] $Bu_4N^+F^-$ in THF or DMF.[18] The use of lithium azide without additive or sodium azide with $Mg(OTf)_2$[19] in acetonitrile did not allow any nucleophilic addition either. The reaction finally succeeded only in protic solvent with sodium azide in EtOH (80 %) in the presence of NH_4Cl,[20] leading to the azido ketones **6** in 60 % yield (Scheme 2).[21a]

Reduction of the keto group in **6a,b** was performed with $NaBH_4$ directly on the crude product.[21b] The further catalytical reduction of the azido group (H_2, Pd-C) followed by in situ N-Boc protection afforded aminoalcohols **1** and **2** in 60 % overall yield from **6** (1 2 75.25). No significant improvement in diastereoselection was observed even when performing the reduction with $NaBH_4$ at a lower temperature (-30°C) (1:2, 78:22).



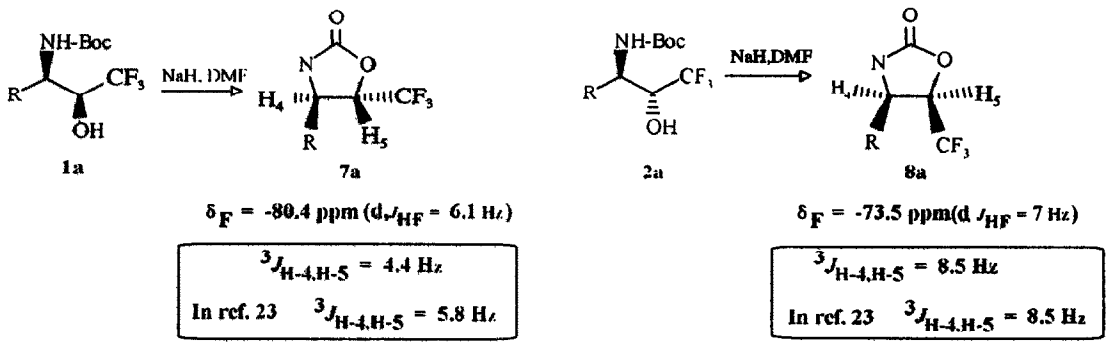
3 a,b ***(R*,S*)*** **6a,b** **1a,b** ***(R*,S*)*** **2a,b** ***(R*R*)***

a R = CH_2-CH_2-C_6H_5
b R = CH_2-Cyclohexyl

78 : 22

Scheme 2

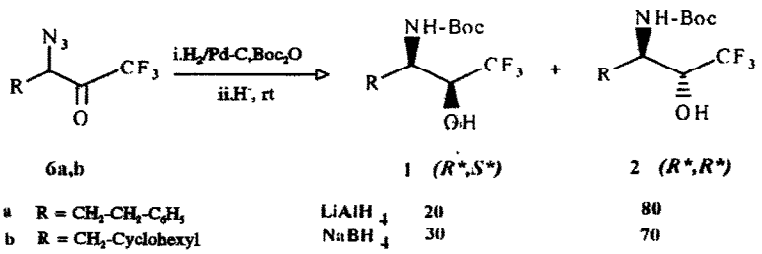
Treatment of the mixture stereoisomer **1a** and **2a** with NaH in DMF[22] provided the oxazolidinones **7a** and **8a**. ^1H NMR spectrum of the major product **7a** exhibits a coupling constant $^3J_{H\text{-}4,H\text{-}5}$ = 4.4 Hz, reflecting a *trans* relationship between H-4 and H-5,[23] and thus the (*R*,S**) *syn* configuration in **1a** This coupling constant is 8.5 Hz for the minor product **8a** (Scheme 3).



Scheme 3

The reduction of an α-azido trifluoromethylated ketone as well as that of a tertiary amino trifluoromethyl ketone affords the *syn* diastereoisomer. This surprising result suggests that steric hindrance of the α-substituent is no longer a dominent effect for the stereocontrol of trifluoromethyl ketone reduction unlike that of non-fluorinated ketones.[13,24]

Therefore in order to obtain the *anti* isomer, the reduction had to be performed under chelation control conditions (Fig. 2) and for this purpose, from 6 the reduction of the azido group had to be achieved first Effectively, when catalytical reduction (H_2, Pd-C) and N-Boc protection were performed before the reduction of the ketonic group, the reversed selectivity was observed (*anti*:*syn* 80 20 with $LiAlH_4$ and 70 30 with $NaBH_4$) (Scheme 4) All our attempts to improve the stereoselectivity (low temperature, other hydrides such as $Zn(BH_4)_2$, DIBAH, known to provide good diastereoselection[25]), have been uneffective



Scheme 4

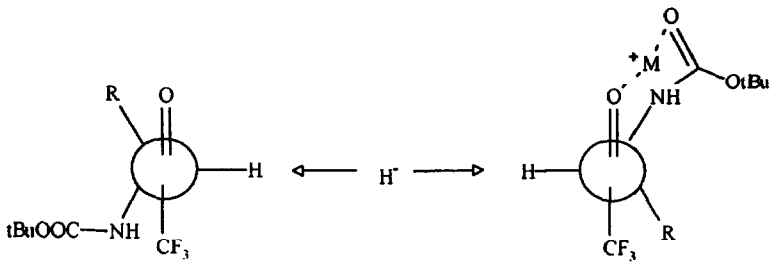
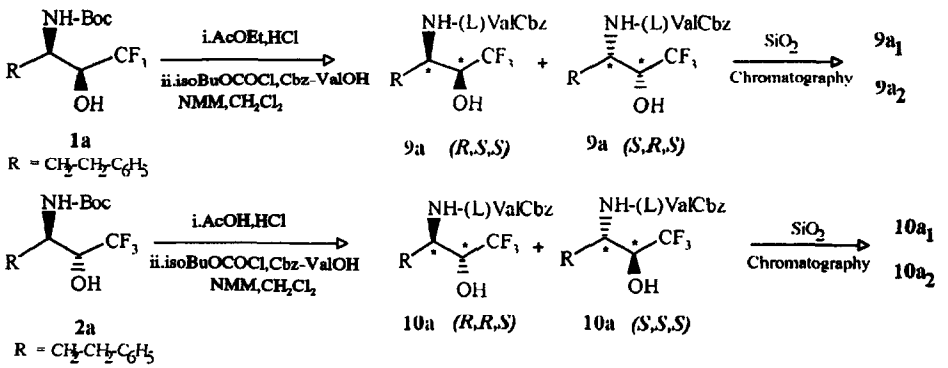


Figure 2

In order to prepare homochiral β-peptidyl trifluoromethyl alcohols, the separated amino alcohols **1a** and **2a** were coupled, after deprotection, with N-Cbz protected (*L*) valine under standard conditions (isobutyl chloroformiate)[2c] leading respectively to a pair of diastereoisomeric peptidyl alcohols **9a** and **10a** in 61 % yield. Diastereoisomers of **9a** and **10a** could be separated by SiO_2 chromatography leading to stereoisomers $\mathbf{9a_1}$, $\mathbf{9a_2}$, $\mathbf{10a_1}$, $\mathbf{10a_2}$ (Scheme 5).



Scheme 5

The less polar β-peptidyl trifluoromethyl alcohols $\mathbf{9a_1}$ (Rf = 0.4) and $\mathbf{10a_1}$ (Rf = 0.5) showed competive inhibition towards HLE as indicated by Dixon plots. The values of K_i estimated were 9.45 (±1.42)x10^{-4} M for $\mathbf{9a_1}$ and 1.26 (±0.20)x10^{-4} M for $\mathbf{10a_1}$. Interestingly, no inhibition effect was observed for the $\mathbf{9a_2}$ (Rf = 0.25) and $\mathbf{10a_2}$ (Rf = 0.3) isomers (Table 1).

Table 1: Optical Rotation and Inhibition Effects on Human Leucocyte Elastase of Peptidyl Trifluoromethyl alcohols **9a** and **10 a.**

	Rf	$[\alpha]_D$	K_i (10^{-4})
syn $9a_1$	0.4 (CH_2Cl_2/MeOH 98:2)	+ 0.7 (c = 0.865, MeOH)	9.45 (±1.42)
syn $9a_2$	0.25 (CH_2Cl_2/MeOH 98:2)	- 2.74 (c = 0.62, MeOH)	No inhibition
anti $10a_1$	0.5 (CH_2Cl_2/MeOH 96:4)	+ 8.4 (c = 0.925, MeOH)	1.26 (± 0.2)
anti $10a_2$	0.3 (CH_2Cl_2/MeOH 96:4)	- 14.5 (c = 0.935, MeOH)	No inhibition

Conclusion

From the same key intermediate azido fluorinated ketone **6** easily prepared in 3 steps from ethyl trifluoroacetate,[15,26] it is possible to prepare each isomer of chiral fluorinated β-peptidyl alcohols. A simple inversion in the sequence of reduction allows control of the formation of *syn* or *anti* diastereoisomers. This reported synthesis has potentially wide applications by possible variation, depending of the biological target, not only of the Rf group or the aminoacid but also of the R substituent. Clear effects of the configuration of carbons C-2 and C-3 on the inhibition properties has been demonstrated. Interpretation of these results requires the determination of the absolute configurations of C-2 and C-3 carbons which is under investigation Stereoselective inhibition may lead to new improved ways of designing inhibitors mimicking the transition state of the HLE catalysed hydrolysis of peptidic substrates.

Experimental

Melting points were observed on a Köfler apparatus, optical rotations measured (*c*, g /100 mL) on a Perkin-Elmer 241 apparatus, infrared spectra (ν cm^{-1}, $CHCl_3$) recorded on a Perkin-Elmer 841 spectrophotometer. ^{19}F NMR spectra were obtained at 84.6 MHz and ^{19}F chemical shifts (δ) are reported in ppm, negative upfield relative to internal $CFCl_3$. ^1H NMR and ^{13}C NMR chemical shifts (δ) are reported in ppm, positive downfield relative to internal Me_4Si, spectra were recorded in $CDCl_3$ or in CD_3OD (if it is specified), at 200 MHz (Bruker AC200) and 400 MHz (Bruker ARX 400). Analytical TLC were performed on Aluminum plates coated with silica gel containing a luminescer (254 nm) (Art. 5554 Merck). Column

chromatographies were performed on SiO_2 (70-230 or 230-400 Mesh Merck). Chromatograms were visualized under UV light and in I_2 vapor or with a spray of ninhydrine and heating GC analysis were performed on a 25 m SE30 capillary column. Elemental analyses were performed by the Service de Microanalyses of the Faculté de Pharmacie, Châtenay-Malabry.

Preparation of azido ketones 4: *General Procedure.*

Epoxy ether **3**[12](1.54 mmol) dissolved in aqueous ethanol 80 % (10 mL) was stirred with sodium azide (100 mg, 2 mol equiv.) and ammonium chloride (83 mg, 2 mol equiv.) for 7 h at room temperature (for **3a**) or 4 h at 40°C (for **3b**) The reaction was monitored by GC analysis and upon completion, the reaction mixture was poured into water (8 mL) and extracted with Et_2O The organic phase was dried ($MgSO_4$) and solvent was evaporated under reduced pressure to give the crude azido ketone as a yellow oil (60 % yield), which was directly used in the reduction step. In order to obtain spectrometric data the crude azido ketone **6** was chromatographied on SiO_2 (Petrol ether-Et_2O 75.25) to afford an unseparable mixture of mono- and diazido products **6** and **11**.[21]

1,1,1-Trifluoromethyl-3-azido-5-phenyl-pentane-2-one 6a: ^{19}F NMR δ -77.1 (s), ^{1}NMR δ 2-2.4 (m, 2 H), 2.6-3 (m, 2 H), 4.17 (dd, $^3J_1$ 9 3 Hz, $^3J_2$ 4 4 Hz, 1 H, CH-N_3), 7.3 (m, 5 H); ^{13}C NMR δ 29.0, 32.1, 62.2, 115.2 (q, $^1J_{CF}$ 292 4 Hz, CF_3) 126.7 to 128 7, 139, 188 3 (q, $^2J_{CF}$ 35 2 Hz, C=O); IR $\nu_{C=O}$ 1740 cm^{-1}, ν_{N3} 2100 cm^{-1}.
Diazidoadduct 11a: ^{19}F NMR δ - 82 7 (s); ^1H NMR δ 3 55 (dd, $^3J_1$ 11 2 Hz, $^3J_2$ 3 0 Hz, 1 H, CH-N_3), 7.3 (m, 5 H); ^{13}C NMR δ 28.0, 31.5, 63 9, 94.5 (q, $^2J_{CF}$ 31.3 Hz,); 122 ,4 (q, $^1J_{CF}$ 287.5)

1,1,1-Trifluoromethyl-3-azido-4-cyclohexyl-butan-2-one 6b: ^{19}F NMR δ -77.0 (s); ^{1}NMR δ 0.7 to 1 9 (m, 13 H), 4.23 (t, ^3J 7 Hz); ^{13}C NMR δ 24 6 to 36 6 , 60 5, 115 3 (q, $^1J_{CF}$ 292.6 Hz, CF_3), 189.1 (q, $^2J_{CF}$ 35.2 Hz, C=O), IR $\nu_{C=O}$ 1740 cm^{-1}, ν_{N3} 2100 cm^{-1}
Diazidoadduct 11b ^{19}F NMR δ - 82 3 (s); ^1H NMR δ 0 7 to 1.9 (m, 13 H), 3.64 (dd, ^3J 9 3 Hz, ^3J 4 5 Hz), ^{13}C NMR δ 62.1, 94.6 (q, $^2J_{CF}$ 31 3 Hz, C-CF_3), 122 5 (q, $^1J_{CF}$ 287 6 Hz, CF_3), IR ν_{OH} 3600, 3300.

General procedure for the preparation of N-Boc aminoalcohols* 1 (*Reduction of the azido ketone* 6 *with the syn selectivity).

Sodium borohydride (0.1 g, 5 mol equiv) was added to a solution of crude azido ketone **6** (0.15 g, 0.57 mmol) in EtOH (5 mL) at (-30°C) After 2 h (GC monitoring) a saturated NH_4Cl solution was slowly added, organic products were extracted (Et_2O), dried ($MgSO_4$) and evaporated to yield the corresponding azidoalcohols (mixture 78:22) (^{19}F NMR δ - 76.8 (d, $^3J_{HF}$ 7 Hz) (*major*) and - 77 6 (d $^3J_{HF}$ 7 Hz) (*minor*) A solution of this crude product (AcOEt, 8 mL) was introduced into a hydrogenation apparatus with Palladium/C 10 % (0.1 g) and Boc_2O (0.19 g, 1.5 equiv). The reaction was stirred for 7 h under a hydrogen pressure of 5 bars. Then the catalyst was filtered and washed with AcOEt. After evaporation of the solvent under reduced pressure, the crude product was chromatographied on SiO_2 (230-400 Mesh) (pentane/Et_2O 70:30) to give the protected aminoalcohols **1** (Rf 0.22) and **2** (Rf = 0 14) in a ratio 78:22 in 60 % yield.

General procedure for the preparation of N-Boc aminoalcohols* 2 *(Reduction of the azido ketones* 6 *with the anti selectivity).

Crude azido ketone (0.16 g, 0.6 mmol.) was dissolved in anhydrous Et_2O (7 mL) in a round bottom flask under argon, and $LiAlH_4$ (0.25 g, 10 equiv) was added After stirring at room temperature for 5 h, H_2O was added dropwise with continued stirring until hydrolysis was complete. After filtration, the organic layer was extracted with Et_2O and dried ($MgSO_4$). After evaporation of the solvent, the crude product was dissolved in dioxan (2 mL) and Boc_2O (0 13 g, 1.1 equiv) was added. The solution was stirred at room temperature for 24 h Dioxan was evaporated, the residue was dissolved in EtOAc, washed (brine) and dried ($MgSO_4$). After evaporation of the solvent, the crude product was chromatographied on SiO_2 (230-400 mesh) (pentane-Et_2O 70:30) to give the aminoalcohols **2** and **1** in a ratio 80:20 in 60 % yield.

1,1,1-Trifluoro-2-hydroxy-3-*t*butylcarboxyamino-5-phenylpentane (*R*,S) 1a:** Mp 86 °C; ^{19}F NMR δ - 77.7 (d, $^3J_{HF}$ 6.9 Hz); ^1H NMR δ 1 5 (s, 9 H, *t*-Bu), 2 0 (m, 2 H, CH_2), 2.8 (m, 2 H, CH_2), 3.63 (m, 1 H, H-3), 3.93 (ddq, $^3J_{H\text{-}2,H\text{-}3}$ 2 6 Hz, $^3J_{H\text{-}2,OH}$ 8 1, $^3J_{H\text{-}2,F}$ 6.3 Hz, 1 H, H-2), 4 8 (d, $^3J_{H\text{-}2,OH}$ 8.1, OH), 5 0 (d, $^3J_{H\text{-}2,NH}$ 7.3, 1 H, NH-Boc), 7 2 (m, 5 H); ^{13}C NMR δ 28 2 (*t*-Bu), 32 2 (C-4), 32.9 (C-5), 50 1(C-NHBoc), 71.7 (q, $^2J_{CF}$ 29 2 Hz, *C*-CF_3), 81 0 (*C*Me$_3$) , 123 9 (q, $^1J_{CF}$ 282.9 Hz, CF_3), 126.1, 128 3, 128.5, 140.9, 156.8 (C=O). Anal. Calcd for $C_{16}H_{22}NO_3F_3$, C, 57.65, H, 6 69, N, 4.2. Found C, 57.87; H, 6.86; N, 3.98.

1,1,1-Trifluoro-2-hydroxy-3-*t*butyloxycarbonylamino-4-cyclohexylbutane (*R*,S) 1b:** Mp 94°C; ^{19}F NMR δ - 77.5 (d, $^3J_{HF}$ 7 Hz), ^1H NMR δ 1 4 (s, 9H, *t*-Bu), 0 8 to 1.9 (m, 13 H, cyclohexyl-CH_2), 3.7 (m, 1H, H-3), 3.85 (m, 1H, H-2), 4 55 (1 H, OH), 4 8 (1H, NH), ^{13}C NMR δ 26 0, 26.2, 26.4, 28.2 (*C*Me$_3$), 32.6, 33.5, 34.0, 38.9, 47.7 (C-NH), 71 6 (q, $^2J_{CF}$ 29 5 Hz, *C*-CF_3), 80 2 (C-*t*Bu), 124.7 (q, $^1J_{CF}$ 283.5, *C*F_3), 156.5 (C=O); Anal. Calcd for $C_{15}H_{26}F_3NO_3$, C, 55.57, H, 8 05; N, 4 3. *Found: C, 57.47; H, 8.06; N, 4.19.*

1,1,1-Trifluoro-2-hydroxy-3-*t*butyloxycarbonylamino-5-phenylpentane (*R*,R) 2a:** Mp 123°C; ^{19}F NMR δ - 75 6 (d, $^3J_{HF}$ 6.9 Hz); ^1H NMR δ 1 5 (s, 9 H, *t*-Bu), 2.0 (m, 2 H, CH_2), 2 8 (m, 2 H, CH_2), 3 89 (m, 1 H, H-3), 4.05 (ddq, $^3J_{H\text{-}2,H\text{-}3}$ 1 93 Hz, $^3J_{H\text{-}2,OH}$ 6 8, $^3J_{H\text{-}2,F}$ 6.9 Hz, 1 H, H-2), 4 47 (d, $^3J_{H\text{-}2,OH}$ 6 8, OH), 5.0 (d, $^3J_{H\text{-}2,NH}$ 7 4, 1 H, NH-Boc), 7.2 (m, 5 H); ^{13}C NMR δ 28.3 (*t*-Bu), 31.6 (C-4 or C-5), 32 9 (C-4 or C-5), 52 2 (C-NHBoc), 73 3 (q, $^2J_{CF}$ 28 6 Hz, *C*-CF_3), 81 0 (O*C*Me$_3$), 125.0 (q, $^1J_{CF}$ 278.2 Hz, *C*F_3), 126.3, 128.5, 128.7, 140.8, 157 2 (*C*=O) Anal Calcd. for $C_{16}H_{22}F_3NO_3$, C, 57.65; H, 6.65; N, 4.2. Found: C, 57.78; H, 6.82; N, 4.05

1,1,1-Trifluoro-2-hydroxy-3-*t*butylcarboxyamino-4-cyclohexylbutane (*R*,R) 2b:** Mp 114°C; ^{19}F NMR δ - 75.3 (d, $^3J_{HF}$ 7 Hz), ^1H NMR δ. 1.45 (s, 9H, *t*-Bu), 0.8 to 1.9 (m, 13H, cyclohexyl-CH_2), 4.0 (m, 2H, H-2 and H-3), 4.65 (2 H, OH and NH); ^{13}C NMR δ 25 2, 25.9, 26.1, 28 1 (*t*Bu), 32.1, 33.8, 34.1, 50.0 (*C*-NH), 73.4 (q, $^2J_{CF}$ 28.7 Hz, *C*-CF_3), 80.7 (*C*Me$_3$), 124.6 (q, $^1J_{CF}$ 283.5, CF_3), 157.2 (*C*=O); Anal. Calcd for $C_{15}H_{26}F_3NO_3$: C, 55.37; H, 8.05; N, 4.3. Found: C, 55.45; H, 8.08; N, 4.21.

N-benzyloxycarbonyl-N-(3,3,3-trifluoromethyl-2-hydroxy-1-phenylethyl-propyl) *L*-valinamides 9 and 10. *General procedure*.

NBoc deprotection was achieved by stirring NBoc-aminoalcohols **1a** or **2a** with AcOEt/HCl (3 M, 1 mL/mmol.). TLC analysis (Petrol Ether/Et_2O 70 30) showed complete disappearance of the starting material spot after 6 h. Evaporation of solvent provided the crude hydrochloride of deprotected aminoalcohol **1a** or **2a** A solution of N-methylmorpholine (NMM) (58 µL, 1 1 equiv) and *Z*-(*L*)-valine (0.13 g, 1 1 equiv.) in CH_2Cl_2 (3 mL) was cooled to - 20°C, then *iso*Butyl chloroformiate (68 µL, 1.1 equiv.) was added. After 20 min., a solution of crude hydrochloride and of NMM (58 µL, 1 1 equiv.) in CH_2Cl_2 (2 mL) was added at - 20°C. The reaction mixture was stirred at - 20°C for 4 h, then allowed to warm to room temperature and stirred overnight. After extraction (AcOEt), the organic layer was washed with aqueous 10 % HCl solution , then with saturated $NaHCO_3$ aqueous solution and dried ($MgSO_4$) A chromatography on SiO_2 (CH_2Cl_2/MeOH 96:4) of **10a** gave successively the two stereoisomeric peptidylalcohols **10a$_1$** and **10a$_2$** as a white powder

A chromatography on SiO_2 (CH_2Cl_2/MeOH 98 2) of **9a** provided the two stereoisomers **9a$_1$** and **9a$_2$** as a white powder.

NMR data of the following peptidyl aminoalcohols were recorded in CD_3OD

9a$_1$ (***R,S,S or S,R,S***). Mp 72°C; Rf = 0 5 (CH_2Cl_2 MeOH 98·2), $[\alpha]_D$ = + 0.7 (MeOH, c = 0;865); ^{19}F NMR δ - 77.37 (d, $^3J_{FH}$ 7 5 Hz); ^1H NMR δ. 0.9 (d, *J* 6 7 Hz, 6H, CH_3 Val), 1.9 (m, 3H, $PhCH_2CH_2$ and $HCMe_2$), 2 5 (m, 2H, $PhCH_2$), 3.95 (m, $J_{H\text{-}2,H\text{-}3}$ 2 Hz, 1H, H-2), 3 82 (m, $^3J_{H\ Val}$ 6.6 Hz, $J_{H\text{-}3,\ H\text{-}2}$ 2 Hz, 2H, H-3 and H-Val), 5 1 (AB, *J* 8 Hz, 2H, $PhCH_2OCO$), 7 2 (m, 10 H, Ar); ^{13}C NMR δ 18.7 (CH_3 Val), 27.8 ($PhCH_2CH_2$), 32.0 (CMe_2), 32 2 ($PhCH_2$), 50 (C-3), 61.5 (C-NH Val), 71 (q, $^2J_{CF}$ 26 Hz, C-2), 71.5 ($PhCH_2OCO$), 124 3 (q, $^1J_{CF}$ 283.4 Hz, CF_3), 126 0, 128.2, 128.4, 140.7, 157.3 (C=O carbamate), 178.0 (C=O amide); Anal Calc. for $C_{24}H_{29}F_3N_2O_4$, C, 61,79, H, 6 26, N, 6 0 Found C, 61.61; H, 6 37; N, 6.05

E.e. (^{19}F NMR) > 99%

9a$_2$ (***S,R,S or R,S,S***) Mp 85°C; Rf: 0 3 (CH_2Cl_2:MeOH 98:2), $[\alpha]_D$ = - 2 74 (MeOH, c = 0.62), ^{19}F NMR δ - 77.30 (d, $^3J_{FH}$ 7.5 Hz), ^1H NMR δ. 0.95 (m, 6H, CH_3 Val), 1 9 and 2.1 (m, 3H, $PhCH_2CH_2$ and $HCMe_2$), 2.55 and 2 65 (m, 2H, $PhCH_2$), 3 95 (d, ^3J 7.1 Hz, H Val), 4 0 (m, $J_{H\text{-}2,H\text{-}3}$ 2 Hz, 1H, H-2), 4.25 (m, H-3), 5.1 (m, *J* 8 Hz, 2H, $PhCH_2OCO$), 7 2 (m, 10H, Ar); ^{13}C NMR δ 18.7 (CH_3 Val), 27 0 ($PhCH_2CH_2$), 31.0 (CMe_2), 32 0 ($PhCH_2$), 51 0 (C-3), 61 5 (C-NH Val), 69.0 (q, $^2J_{CF}$ 26.5 Hz, C-2), 71 5 ($PhCH_2OCO$), 123 (q, $^1J_{CF}$ 284 Hz, CF_3), 126 2, 128.0, 129.0, 140 0, 157 1 (C=O carbamate), 178.2 (C=O amide), Anal. Calc. for $C_{24}H_{29}F_3N_2O_4$, C, 61,79, H, 6.26; N, 6 0. Found C, 61.58; H, 6 36; N, 6.07

E.e. (^{19}F NMR) 95%

10a$_1$ (***R,R,S or S,S,S***) Mp 166°C; Rf = 0 4 (CH_2Cl_2.MeOH 96 4), $[\alpha]_D$ = + 8 4 (MeOH, c = 0.925); ^{19}F NMR δ. -72.55 (d, $^3J_{FH}$ 7.64 Hz), ^1H NMR δ. 0 9 (d, *J* 7Hz, 6H, CH_3 Val), 1.8 and 1.9 (m, 2H, $PhCH_2CH_2$), 2.1 (m, 1H, $HCMe_2$), 2.5 and 2.75 (m, 2H, $PhCH_2$), 3.90 (d, *J* 7 Hz, 1H, H Val), 3.95 (m, $J_{H\text{-}}$

2,H-3 4.9 Hz, $^3J_{HF}$ 7.6 Hz, 1H, H-2), 4.2 (m, $J_{H\text{-}3,H\text{-}2}$ 4.9 Hz, $J_{H\text{-}3,H\text{-}4}$ 10.8 Hz, 1H, H-3), 5.1 (s, 2H, PhCH_2OCO), 7.1 to 7.4 (m, 10H, Ar); ^{13}C NMR δ 20 1 (CH_3 Val), 32 5 (PhCH_2CH_2), 33.1 (CMe_2), 34.0 (PhCH_2), 50.1 (C-3), 62.0 (C-Val), 68.0 (PhCH_2OCO), 75.1 (C-2), 128, 130, 140, 142, 159 (C=O carbamate), 174.9 (C=O amide); Anal. Calc. for $C_{24}H_{29}F_3N_2O_4$, C, 61,79; H, 6.26; N, 6.0. Found: C, 61.57; H, 6.38; N, 6.03.
E.e. (^{19}F NMR) 92%

10a$_2$ ***(S,S,S or R,R,S)***: Mp 222°C; Rf = 0.25 (CH_2Cl_2 MeOH 96·4); $[\alpha]_D$ = - 14.5 (MeOH, c = 0 935), ^{19}F NMR δ. -72.5 (d, $^3J_{F\text{-}H}$ 7.6 Hz), ^{1}H NMR δ. 1 0 (m, 6H, CH_3 Val), 1.9 and 2 (m, 2H, PhCH$_2$CH_2), 2.1 (m, 1H, *H*CMe$_2$), 2.5 and 2.8 (m, 2H, PhCH_2), 3 90 (m, $J_{H\text{-}2,H\text{-}3}$ 5.4 Hz, $J_{H\text{-}Val}$, 7.4 Hz, $^3J_{HF}$ 7.6 Hz, 2 H, H-2 and H Val), 4.2 (m, $J_{H\text{-}3,H\text{-}2}$ 5 4 Hz, $J_{H\text{-}3,H\text{-}4}$ 10.8 Hz, 1 H, H-3), 5 2 (q., 2H, J_{AB} 12.5 Hz, PhCH_2OCO), 7.1 to 7 4 (m, 10 H, Ar); ^{13}C NMR δ 20 1 (CH_3 Val), 31 0 (CMe_2), 32.0 (PhCH_2CH_2), 33 1 (PhCH_2), 51.0 (C-3), 61.9 (C-NH Val), 68.0 (PhCH_2OCO), 72 1 (C-2), 126.0, 129.9, 139.1, 142 0, 158.1 (C=O carbamate), 175.0 (C=O amide), Anal. Calc for $C_{24}H_{29}F_3N_2O_4$, C, 61,79; H, 6.26, N, 6.0. Found: C, 61.61; H, 6.36; N, 6.08
E.e. (^{19}F NMR) 74%
*For **9** and **10**, attribution of signals and coupling constants have been determined by means of homonuclear and heteronuclear experiments and by means of gr hmqc and gr hmbc technics.*

Determination of stereochemistry of 1a and 2a by formation of 5-Trifluoromethyl-4-phenethyl oxazolidin-2-one 7a and 8a:

A solution of aminoalcohol **1a** or **2a** (45 mg, 0 135 mmol) in DMF (0 6 mL) was treated with NaH (6 mg, 1 mol equiv.) (60 % dispersion in oil) and stirred at room temperature for 7 h.. The resulting solution was diluted with water, extracted with Et_2O, dried (Na_2SO_4) and concentrated under vacuum. NMR data were recorded on the crude product
Oxazolidinone 7a: ^{19}F NMR δ - 80 4 (d, $^2J_{HF}$ 6 15 Hz); ^{1}H NMR δ 3 9 (td, $^3J_{H\text{-}4,H\text{-}5}$ 4 4 Hz, ^3J 6 5 Hz, H-4), 4.46 (qd, $^3J_{H\text{-}4,H\text{-}5}$ 4 4 Hz, $^2J_{HF}$ 7 Hz, H-5).

Oxazolidinone 8a: ^{19}F NMR δ - 73.5 (d, $^2J_{HF}$ 7 Hz), ^{1}H NMR δ 4.07 (td, $^3J_{H\text{-}4,H\text{-}5}$ 8.8 Hz, ^3J 5 Hz, H-4); 54.75 (qd, $^3J_{H\text{-}4,H\text{-}5}$ 8 5 Hz, $^2J_{HF}$ 7 Hz, H-5)

Enzyme assays for human leucocyte elastase.

Human leucocyte elastase (HLE) was purchased from Elastin Products Company (Owensville, USA). Enzyme molarity was based on active-site molarity determined using N-benzyloxycarbonyl-alanyl-alanyl-prolyl-azaalanyl-*p*-nitrophenylester.[27] The chromogenic substrate methoxysuccinyl-alanyl-alanyl-prolyl-valyl-*p*-nitroanilide (MeO-Suc-Ala-Ala-Pro-Val-*p*-NA) was obtained from Sigma. For the inhibitor-free assays and for inhibitor assays, the initial velocities v of the amidolytic activities of HLE (20 nM) towards MeO-Suc-Ala-Ala-Pro-Val-*p*-Na (5 to 50 μM) were determined at pH 8 0 and 25°C by continuous monitoring of the release of p-nitroaniline at 405 nm using a Lambda 5 Perkin Elmer UV-vis spectrophotometer equipped with a

thermostable holder. The total assay volume was 1 mL in the following buffer: 0 1 M Hepes, 0.5 M NaCl, 0 1 % (v/v) Tween 80, 2 % (v/v) DMSO. The reaction mixtures were incubated in the presence of 0 to 100 μM, 0 to 50 μM, 0 to 200 μM and 0 to 250 μM of $\mathbf{9a_1}$, $\mathbf{9a_2}$, $\mathbf{10a_1}$ and $\mathbf{10a_2}$ respectively. The double-reciprocal plots of v *vs.* substrate concentration gave straight lines with *x* intercepts of $-1/K'_M$, where K'_M (equal to $K_M\{1+[I]_o/K_i\}$) is the apparent Michaelis constant. The maximum velocity V_M and K'_M were estimated by iterative least-squares fits[28] to the equation for competitive inhibition $v = V_M.[S]_o/\{[S]_o + K'M.\}$ The constants K_i were determined from the linear plot of K'_M/V_M *vs* inhibitor concentration (least-squares analysis). Initial estimates of K_i were calculated from Dixon plots

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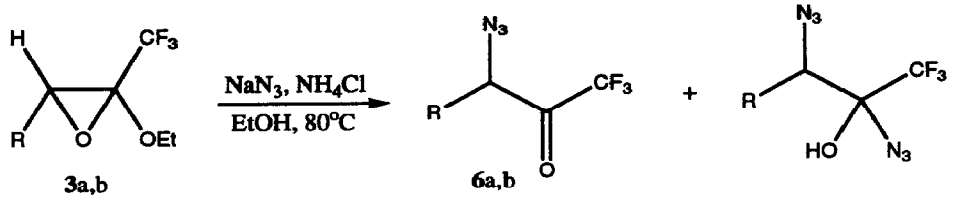

3a,b 6a,b

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