Pergamon

Tetrahedron, Vol. 52, No. 1, pp. 305-318, 1996
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0040-4020/96 $15.00 + 0.00

0040-4020(95)00896-9

Difluoromethyleneketone Retroamide, a Versatile Concept of Inactivation of Proteolytic Enzymes

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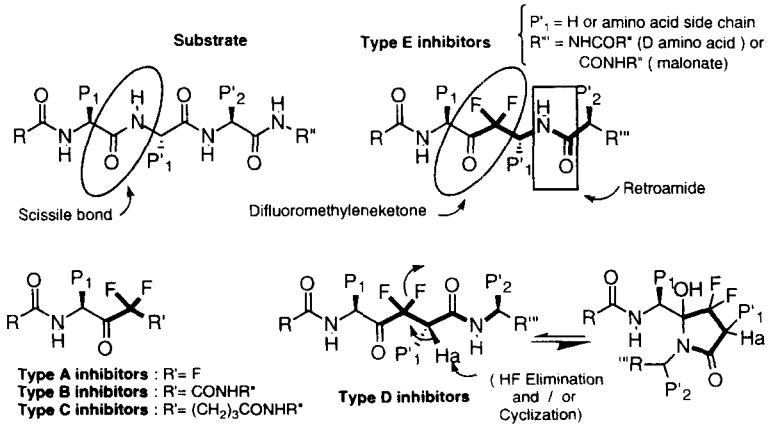
Abstract: The synthesis of difluoromethyleneketone retroamides is described. Several examples of application to aspartyl or seryl proteases illustrate the versatility of this inactivation concept.

Replacement in substrates of proteolytic enzymes of the scissile amide bond by polyfluoromethyl - or difluoromethyleneketones has generated a number of potent transition state type inhibitors[1]. The ability of difluoromethyleneketones to occupy additional binding sites on the leaving group side (S' subsites) as compared to for instance C-terminal trifluoromethylketones (Inhibitors of type A, fig. 1) represents an advantage in terms of potentially increased affinity and selectivity. We and others have proposed approaches to this kind of inactivators by making use of different "tricks" allowing extension of the backbone of the inhibitors towards the P' residues. Early on incorporation of " difluorostatones " building blocks (Inhibitors of type B, Figure 1) in inactivator sequences led to the discovery of efficient pepsin[2], renin[3], as well as elastase[4] and HIV-1 protease[5] inhibitors and more recently of Interleukin-1β converting enzyme inhibitors.[6] The introduction of difluoromethyleneketones through incorporation of a spacer group (Inhibitors of type C) has also been reported and applied to the inactivation of α-chymotrypsin[7a] and elastase.[7b] Only a few examples of true dipeptide isosteres[8a,b] (Inhibitors of type D) have been described due most probably to the difficulty of developping general and readily accessible chemistry. Moreover the potential reactivity of intermediates and of final structures certainly hampered their development. Elimination of HF and/or spontaneous cyclisation had for instance been observed for some difluoromethylene ketones of type D as shown figure 1.[9]

FIGURE 1



Difluoromethyleneketone Retroamides

These observations encouraged us to design an alternate way of mimicking true dipeptide analogues bearing difluoromethyleneketones. Reversal of the C-terminal amide bond adjacent to the difluoromethyleneketone in structures of type D generates inhibitors of type E (figure 1) that fulfill a number of criteria like:

- increased intrinsic stability, structures of type E being much less prone to spontaneous cyclization than for analogues of type D (figure 1).
- easy chemical access, inhibitors of type E being prepared by simple and flexible route, excluding HF elimination during synthesis (figure 1).
- maintained reactivity and potency towards target proteases as amply illustrated hereafter, structures of type E being obviously still able to form hydrates or covalent adducts with active site serine residues, mimicking thus the postulated transition states.[1]

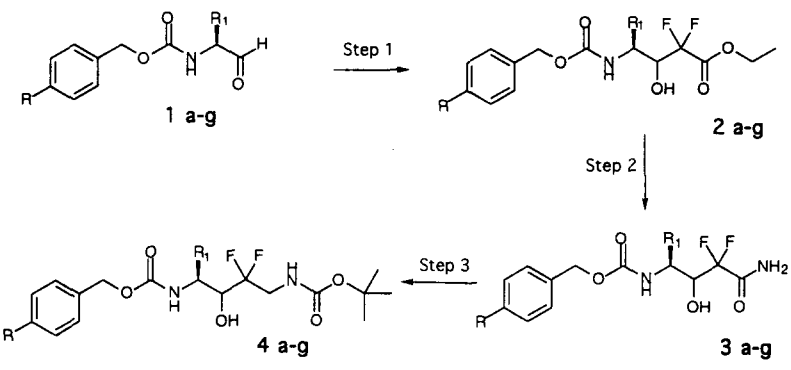
The difluoromethyleneketone retroamide concept (Inhibitors of type E, Figure 1) was tested initially on aspartic acid proteases[11] and finally extended to serine proteases.[10,12] This concept applies also to the inhibition of metalloproteases like for instance membrane bound aminopeptidase.[12] However, like for many other polyfluorinated ketones, it does not lead to potent inactivators of cysteine proteases(e.g. papain).[7a,13,14]

Synthesis

A convenient and versatile synthetic scheme had been developped giving access to a large number of difluoroketone dipeptide isosteres of the general structure XGly (figure 1, type E Inhibitors, P_1'=H) in decent yields. XY dipeptide isosteres (Figure1, P_1'= amino acid side chain) could be synthesized by a modification of the original scheme and introduction of the P_1' side chain in a non stereoselective manner.[15]

This strategy has since been improved as illustrated by the elegant synthesis of potent pseudo symmetrical HIV-1 protease inhibitors reported by H.L. Sham et al.[13]

SCHEME 1



R_1 (R=H) : a) iPr ; b) iBu ; c) $CH_2C_6H_5$; d) $CH_2C_6H_{11}$; e) $CH_2C_6H_4(4\text{-}NO_2)$
R_1 (R=NO_2) : f) $CH_2C_6H_4OCH_2C_6H_5$; g) $(CH_2)_4NHCbz$

The chemistry described in Scheme 1 is compatible with a number of hydrophobic or functionalized P_1 side chains. Orthogonally protected key intermediates **4** bearing hydrophobic "P_1" side chains have been easily obtained via a three steps synthetic sequence starting from appropriately substituted *N*-benzyloxycarbonyl-α-aminoaldehydes **1**. Reformatsky condensation followed by reaction of the difluorohydroxyesters **2** with ammonia yielded in good overall yields their corresponding primary amides **3** (Table 1). Reduction of the latter with borane dimethylsulfide complex and protection by reaction of the intermediate amine with di-*tert* -butyldicarbonate afforded the desired intermediates **4** in reasonable to good yields (Table1). ValylGly (**4a**), LeucylGly(**4b**), Cyclohexylalanylgly (**4d**), *p.* NO2Phenylalanylgly (**4e**), *O*-benzylTyrosylGly (**4f**) as well as CbzLysylGly (**4g**) analogues have been isolated as mixtures of isomers or as separated diastereoisomers in the case of PhenylalanylGly (**4c**).

The need for preparing "basically" substituted side chains led to reconsider the experimental conditions of the Reformatsky step in order to render the utilization of protected intermediates like nitro substituted aldehyde precursors more practical.

Table 1: Yields of Scheme 1

	R_1	Step 1[a] 2	Step 2 3	Step 3 4
a	$CH(CH_3)_2$	30	91	38
b	$CH_2CH(CH_3)_2$	50	88	70
c	$CH_2C_6H_5$	61	98	64
d	$CH_2C_6H_{11}$	47	97	60
e	$CH_2C_6H_4(4\text{-}NO_2)$	30[b](0[c],72[d])	95	61
f	$CH_2C_6H_4(4\text{-}OCH_2C_6H_5)$	46	57	50
g	$(CH_2)_4NHCbz$	52[b](0[c],72[d])	89	64

[a] One step, reflux temperature; [b] Two steps, reflux temperature; [c] One step, RT, US; [d] Two steps, RT, US.

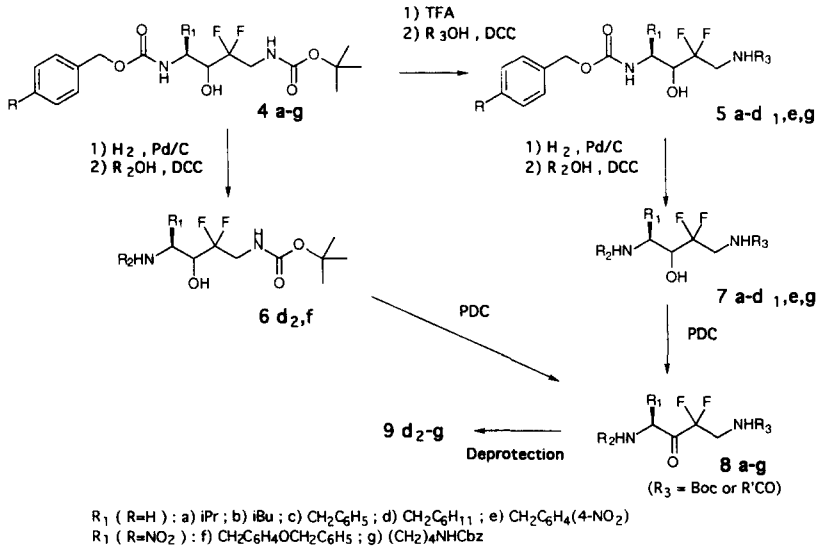
A two step procedure and ultrasonicating conditions allowed us to generate intermediates **2** in very good yields (Table 1). This procedure is remarkably efficient as examplified by entries **2e** and **2g** where under similar conditions (ultrasonication, RT) BUT IN ONE STEP instead of two subsequent steps the expected compounds are not formed at all. (Table 1).

Inhibitor Synthesis

Incorporation of key intermediates **4a-g** into pseudopeptide sequences was performed by sequential deprotection of the orthogonally protected difluorodiamines and coupling of the free amines with the appropriately *N*-protected amino acid residues or acids under standard conditions (dicyclohexylcarbodiimide coupling chemistry) (Scheme 2).

As shown in Tables 2 and 3 these conditions gave access to a number of test compound precursors. Final oxidation of the difluoroalcohol functionality of linear structures **6** and **7** to ketones **8** was performed using pyridinium dichromate (PDC) and molecular sieves[10] (Table 3). These alcohols could also be oxidized under Swern[17a] or modified Pfitzner-Moffatt[17b] conditions or using Dess-Martin periodinane.[18] A final deprotection step (cleavage of *tert*- butoxycarbonyl protecting groups by saturated solution of HCl in diethyl ether) was performed in order to access compounds **9d$_2$**, **e$_1$**, **e$_2$**, **g** and **f** (Scheme 2, Table 3).

SCHEME 2



R_1 (R=H) : a) iPr ; b) iBu ; c) $CH_2C_6H_5$; d) $CH_2C_6H_{11}$; e) $CH_2C_6H_4(4\text{-}NO_2)$
R_1 (R=NO_2) : f) $CH_2C_6H_4OCH_2C_6H_5$; g) $(CH_2)_4NHCbz$

Table 2: Yields of Scheme 2

	R_1	R_2	R_3	Yields
5a	$CH(CH_3)_2$	Cbz	CH_3CO	63-80
5b$_1$	$CH_2CH(CH_3)_2$	Cbz	$C_6H_5CH_2CO$-D-Val	50
5b$_2$	$CH_2CH(CH_3)_2$	Cbz	$C_6H_5CH_2NHCOCH(iPr)CO$	55
5b$_3$	$CH_2CH(CH_3)_2$	Cbz	$C_6H_5(CH_2)_3CH(iBu)CO$	54
5c	$CH_2C_6H_5$	Cbz	$(CH_3)_2CHCH_2CO$	60-77
5d$_1$	$CH_2C_6H_{11}$	Cbz	$(CH_3)_2CHCH_2CO$	55-72
5e	$CH_2C_6H_4(4\text{-}NO_2)$	Cbz	CH_3CO	83
5g	$(CH_2)_4NHCbz$	$(4\text{-}NO_2)Cbz$	CH_3CO	82
6d$_2$	$CH_2C_6H_{11}$	Iva-L-(OMe)Tyr-L-nVal	Boc	60-75
6f	$CH_2C_6H_4(4\text{-}OCH_2C_6H_5)$	Cbz-L-Val	Boc	48

As amply examplified hereafter, the final difluoromethyleneketone retroamides are chemically stable structures, displaying interesting competitive inhibitory activities towards their respective target proteases.

Renin Inhibitors

Several potential inhibitors of renin, a putative target for the treatment of hypertension, were synthesized from key intermediates **4b** or **4d**. A final oxidation step yielded homologous pseudopeptides **8b$_1$,b$_2$,b$_3$** and **d$_1$** (Figure 2) in acceptable to good overall yields (Tables 2 and 3).

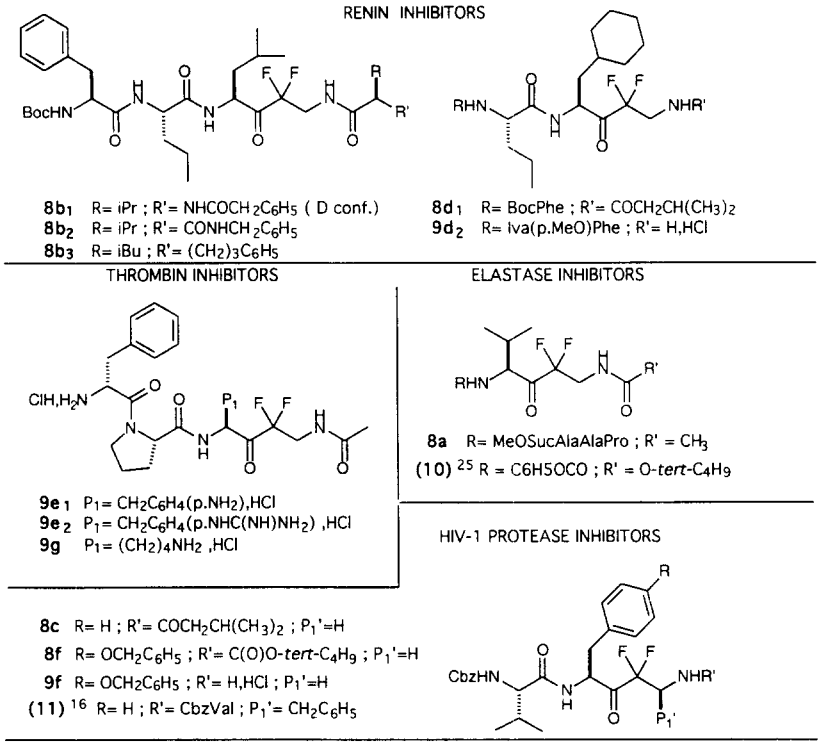
Tripeptide analogue **9d$_2$** (Figure 2), a water soluble renin inhibitor (IC_{50}= 0.016 μM) with extremely good inhibitory selectivity[19] was accessed via a four steps sequence from diaminoalcohol **4d** (Scheme 2, Table 3). Compounds **8b$_2$,b$_3$** and **d$_1$** display *in vitro* human plasma renin inhibitory activities(IC_{50}) of respectively 1.5 μM,1.45μM, and 0.0035 μM. Difluoroketoneretroamide **8b$_1$** bearing a D-Valine residue in order to compensate for the inversion of the adjacent amide bond at position P'_2[20], surprisingly demonstrated no renin inhibitory activity even at 50μM concentration, pointing out that the presence of two consecutive retroamide bonds is detrimental to the binding affinity of the inhibitor.[11]

Table 3: Yields of Scheme 2

	R_1	R_2	R_3	7	8	9
a	$CH(CH_3)_2$	MeOSucAlaAlaPro	CH_3CO	35-40	60	
b$_1$	$CH_2CH(CH_3)_2$	BocPhenVal	$C_6H_5CH_2CO$-D-Val	84	50	
b$_2$	$CH_2CH(CH_3)_2$	BocPhenVal	$C_6H_5CH_2NHCOCH(iPr)CO$	56	70	
b$_3$	$CH_2CH(CH_3)_2$	BocPhenVal	$C_6H_5(CH_2)_3CH(iBu)CO$	80	63	
c	$CH_2C_6H_5$	CbzVal	$(CH_3)_2CHCH_2CO$	50	62	
d$_1$	$CH_2C_6H_{11}$	BocPhenVal	$(CH_3)_2CHCH_2CO$	55-75	74-90	
d$_2$	$CH_2C_6H_{11}$	Iva-L-(OMe)Tyr-L-nVal	Boc[a]		68	68[a]
e$_1$	$CH_2C_6H_4(4\text{-}NHBoc)$[a]	[a] Boc-D-PhePro	CH_3CO	49	80	90[b]
e$_2$	$CH_2C_6H_4(4\text{-}NHBoc)$	[a] Cbz-DPhePro	CH_3CO	32[c]	70	84[d]
g	$(CH_2)_4NHCbz$	Boc-D-PhePro	CH_3CO	65	43[e]	73[b]
f	$CH_2C_6H_4(4\text{-}OCH_2C_6H_5)$	Cbz-L-Val	Boc[a]		54	59[a]

[a] HCl; [b] 2HCl; [c] $R_1 = CH_2C_6H_4(4\text{-}NHC(NH)NHCbz)$ (3 steps); [d] 3 HCl; [e] $R_1=(CH_2)_4NHBoc$.

FIGURE 2



HIV-1 Protease Inhibitors

The search for selective inhibitors of HIV-1 protease, an extremely appealing molecular target for the treatment of AIDS, has been the basis of an enormous medicinal chemistry effort worldwide.[21] Concepts developped earlier on for the inactivation of human renin have been extended to the inhibition of HIV-1 protease and evaluated.[22] Renin inhibitors like **8b$_2$** and **8d$_1$** were shown to be decent HIV-1 protease inhibitors with IC_{50} of 7 and 6 μM respectively. Freely water soluble inhibitor **9d$_2$** was totally inactive up to concentrations of 100μM, although a thirty fold improvement was achieved by modification of the P_1 and P_2 residues according to HIV-1 protease subsite selectivity[24] and afforded inhibitor **9f** (IC_{50}=3.2μM) (Figure 2). Linear inhibitors with greater resemblance to the gag-pol substrate cleavage sites like **8c** and more importantly **8f** (Figure 2) exhibit reasonable to good inhibitory potencies (IC_{50}=1.5 and 0.05 μM respectively).

Pseudosymmetrical inhibitor **11**[16] (Figure 2) demonstrates the utility and potential of our concept. The stereoselective introduction of a P'_1 side chain and the addition of a P'_2 residue results in a 500 fold increased potency.

Serine Protease Inhibition

A number of potent inhibitors of Human Leukocyte Elastase (HLE) and of Thrombin have been reported over the past ten years with potential utility in the treatment of lung emphysema or haemostasis and thrombosis respectively. A variety of electrophilic carbonyl derivatives capable of forming reversible tetrahedral adducts with the active site serine residue have been evaluated (e.g. inhibitors of type A or B).[4,23,25]

Extension of our concept to the inhibition of HLE yielded inhibitor **8a** (Figure 2) generated in 15-20 % overall yield from intermediate **4a**. The inhibitory potency of compound **8a** (Ki= 0.073μM) compares favorably with analogous statone type B structure (Ki=4.3μM)[4] or tifluoromethylketone type A structure (0.014 μM)[4].

Optimization of the inhibitor sequence led Bernstein et al.[25] to report recently inhibitor **10** (Figure 2) (Ki=0.39 nM).[20]

Application of the concept of difluoromethyleneketone retroamide to the inhibition of Thrombin led to the synthesis of compounds **9e$_1$,e$_2$** and **g** (Figure 2), potent competitive inactivators of this key blood clotting enzyme (Ki=0.36, 0.07, 0.28 μM respectively). The additional C-terminal retro acetamido functionality of inhibitor **9e$_2$** results in a 20 fold increase in potency *in vitro* when compared to its corresponding trifluoromethyl ketone. (Ki= 1.2μM).[26]

Conclusion

In conclusion, difluoromethyleneketone retroamide is a versatile concept of inhibition of proteolytic enzymes that applies to the inactivation of aspartyl, serine and metallo proteases. The easy access and flexible synthesis of key orthogonally protected synthons allows even incorporation of functionalized P_1 side chains. Several very potent inhibitors of proteases of potential therapeutic interest have been prepared. The extension of this general concept to newly discovered putative targets is currently under investigation.

Experimental Section

General procedures

All melting points were determined using a Büchi 535 apparatus and are uncorrected. ^{1}H NMR spectra were obtained from a Bruker AM-360. Chemical shifts are reported in δ(ppm) using TMS as a reference. Mass spectra were performed on a Finigan TSQ46 instrument operating in the desorption chemical ionisation mode (NH_3). IR spectra were obtained from a Bruker IFS66 apparatus. Microanalyses were determined using a Carlo Erba 1106 analyser. Column Chromatography was performed on silica gel (Merck $60F_{254}$). Solvents were dried by distillation over sodium/benzophenone (tetrahydrofuran, diethyl ether, toluene) and stored on 4Å molecular sieves. Triethylamine was stored over potassium hydroxide pellets. *Tert*-butyl alcohol was dried by distillation over calcium hydride.

General procedure for the preparation of aldehydes (1a-d).

***N*-Benzyloxycarbonyl-*L*-Valinal (1a).** A mixture of *N',O*-dimethyl-*N*-benzyloxycarbonyl-*L*-valine hydroxamate (13.25 g, 45 mmol) and lithium aluminium hydride (1.88 g, 49.5 mmol) in anhydrous diethyl ether (250 mL) was stirred at 0 °C for 1 h under nitrogen. Potassium hydrogenosulfate (1M, 90 mL) was added. The mixture was stirred for 0.5 h and extracted with diethyl ether (3 x 50 mL). The combined organic layers were washed with 3 N HCl (3 x 90 mL), water (1 x 50 mL), a saturated solution of sodium bicarbonate (1 x 50 mL) and brine (80 mL) and dried over anhydrous magnesium sulfate. Filtration and removal of the solvent *in vacuo* left 8.37 g of the expected aldehyde (79 % yield, colorless oil). TLC/Rf: 0.60 (ethyl acetate/cyclohexane 1:1; I_2). ^{1}H NMR ($CDCl_3$): δ 0.97 and 1.06 (two d, J_{HH} = 7 Hz, 6H); 2.33 (m, 1H); 4.30 (dd, 1H); 5.10 (s, 2H); 5.55 (br d, 1H); 7.25 (s, 5H); 9.55 (s, 1H).

***N*-Benzyloxycarbonyl-*L*-Leucinal (1b).** Prepared in 96 % yield from *N',O*-dimethyl-*N*-benzyloxycarbonyl-*L*-valine hydroxamate. Yellow oil. TLC/Rf: 0.55 (ethyl acetate/cyclohexane 1:1, I_2). ^{1}H NMR ($CDCl_3$): δ 0.95-1.05 (br d, J_{HH} = 6 Hz, 6H); 1.10-2.00 (m, 3H); 4.10-4.50 (m, 1H); 5.20 (s, 2H); 5.30 (br d, 1H); 7.37 (s, 5H); 9.65 (s, 1H).

***N*-Benzyloxycarbonyl-*L*-phenylalaninal (1c).** Prepared in 52 % yield (after recrystallisation) from *N',O*-dimethyl-*N*-benzyloxycarbonyl-*L*-phenylalanine hydroxamate. White solid. mp: 131 °C (diethyl ether/hexane). TLC/Rf: 0.45 (ethyl acetate/cyclohexane 1:1). ^{1}H NMR ($CDCl_3$): δ 3.10 (d, 2H); 4.45-4.55 (m, 1H); 5.20 (s, 2H); 5.45 (br d, 1H); 7.10-7.40 (m, 10H); 9.60 (s, 1H). Anal. calcd for $C_{17}H_{17}N_1O_3$: C, 72.07; H, 6.05; N, 4.94. Found: C, 72.73; H, 6.16; N, 5.08.

***N*-Benzyloxycarbonyl-*L*-cyclohexylalaninal (1d).** Prepared in 85 % yield from *N',O*-dimethyl-*N*-benzyloxycarbonyl-*L* cyclohexylalanine hydroxamate. Yellowish oil. TLC/Rf: 0.53 (ethyl acetate/cyclohexane, 1:1). ^{1}H NMR ($CDCl_3$): δ 0.80-2.05 (m, 13H), 4.30 (m, 1H), 5.10 (s, 2H), 5.40 (br d, 1H), 7.30 (s, 5H), 9.53 (s, 1H).

General procedure for the preparation of aldehydes (1e-g).

***(R,S)*-α-Benzyloxycarbonylamino-3-(4-nitro-phenyl)propanal (1e).** To a solution of *(R,S)*-α-benzyloxycarbonyl-amino-3-(4-nitrophenyl)propionic acid, methyl ester (3.58 g, 10 mmol) in anhydrous diethyl ether/toluene (1:2, 300 mL) was added dropwise under nitrogen at -78 °C a 1M solution of diisobutylaluminium hydride in hexane (20 mL, 2 equivalents). The mixture was stirred at - 78 °C for 10 min. A Rochel's solution (50 mL, saturated solution of potassium and sodium tartrate) was then added and the temperature was allowed to rise to room temperature. The solution was acidified by addition of a 1M aqueous solution of potassium hydrogenosulfate to pH3 and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with brine (50 mL) and dried over anhydrous magnesium sulfate. Filtration and removal of the solvent *in vacuo* afforded the crude aldehyde purified by flash chromatography (silica gel, ethyl acetate/cyclohexane 3:7). 2.35 g of the expected aldehyde (72 % yield) were obtained by crystallization of the oily product from ethyl acetate/pentane. White crystals. mp: 99.5-100 °C (ethyl acetate/pentane). TLC/Rf: 0.20 (ethyl acetate/cyclohexane 1:1); MS: MH^+ = 329; MNH_4^+ = 346; IR: CHO: 1729 cm^{-1} (C = 0); ^{1}H NMR ($CDCl_3$): δ 3.15 (dd, J_1 = 14.1 Hz, J_2 = 6.8 Hz, 1H), 3.35 (dd, J_1 = 14.1 Hz; J_2 = 6.2 Hz, 1H) 4.50-4.60 (m, 1H); 5.05-5.15 (m, 2H); 5.30 (d, J= 6.7 Hz, 1H); 7.20-7.40 (m, 7H); 8.10 (d, J = 8.7 Hz, 2H); 9.20 (s, 1H). Anal. calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53. Found: C, 62.46; H, 4.94; N, 8.62.

***(R,S)*-*N*-(4-Nitrobenzyloxycarbonyl)-*O*-benzyl-tyrosinal (1f).** Prepared in 51 % yield from *(R,S)*-*N*-(4-nitrobenzyl-oxycarbonyl)-*O*-benzyltyrosine, methyl ester. Colorless oil. TLC/Rf: 0.23 (ethyl acetate/cyclohexane, 1:1); ^{1}H NMR ($CDCl_3$): δ 3.20 (m, 2H); 4.45-4.60 (m, 1H); 5.05 (s, 2H); 5.18 (s, 2H); 5.50 (br d, 1H); 6.90 (d, J_{HH}= 8.5 Hz, 2H); 7.05 (d, J_{HH} = 8.5 Hz, 2H); 7.35-7.55 (m, 7H); 8.15 (d, J_{HH} = 8.5 Hz, 2H); 9.65 (s, 1H).

(*R*,*S*)-1-Formyl-1-(4-nitrobenzyl)-5-benzylpentylene dicarbamate (1g). Prepared in 77 % yield from *N*-α-(4-nitrobenzyloxycarbonyl)-*N*-ε-benzyloxycarbonyl lysine, methyl ester (using 3 equivalents of DIBAL). White crystals. mp: 81-82 °C (ethyl acetate/pentane); TLC/Rf: 0.10 (ethyl acetate/cyclohexane 1:1); IR: CHO: 1733cm^{-1} (C = 0);MS: MH^+ = 444; MNH_4^+ = 461; ^1H NMR ($CDCl_3$): δ 1.35-1.85 (m, 5H); 1.85-2.00 (m, 1H); 3.10-3.30 (m, 2H); 4.25-4.35 (m, 1H); 4.75-4.85 (m, 1H); 5.00-5.10 (m, 2H); 5.15 (dd, J = 13.2 Hz, 2H); 5.60 (d, J= 4.4 Hz, 1H); 7.35-7.40 (m, 5H); 7.55 (d, J = 8.5 Hz, 2H); 8.25 (d, J = 8.5 Hz, 2H); 9.55 (s, 1H). Anal. calcd for $C_{22}H_{25}N_3O_7$: C, 59.59; H, 5.68; N, 9.48. Found:C, 59.77; H, 5.54; N, 9.14.

General Procedure for the preparation of difluorohydroxyesters 2a-d and 2f.

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-5-methylhexanoic acid, ethyl ester (2a). A mixture of **1a** (8.30 g, 35 mmol) and ethyl bromodifluoroacetate (14.82 g, 75 mmol) in anhydrous tetrahydrofuran (90 mL) was added dropwise to a refluxing suspension of activated zinc wool (4.88 g, 75 matg) in anhydrous tetrahydrofuran (30 mL) under nitrogen. After the addition was complete, the solution was stirred 15 h at room temperature. Ethyl acetate (95 mL), 1M $KHSO_4$ (95 mL) and brine (95 mL) were successively added to the mixture. The organic layer was decanted. The aqueous phase was extracted with ethyl acetate (2 x 60 mL). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration, removal of the solvent *in vacuo* and purification by flash chromatography [silica gel, ethyl acetate/cyclohexane 1:9 (300 mL) then 2:8] yielded 4.10 g of the expected ester (32 % yield). TLC/Rf: 0.50 (ethyl acetate/cyclohexane, 1:1); MS: MH^+ = 360; ^1H NMR ($CDCl_3$): δ 0.95 and 0.97 (2 d, J_{HH} = 7 Hz, 6H); 1.30 (t, J_{HH} = 7.5 Hz, 3H); 2.00 (m, 1H); 3.55-4.33 (m) and 4.25 (q, J_{HH} = 7.5 Hz) (5H); 5.08 (s, 2H); 5.40 (br d, 1H); 7.30 (s, 5H).

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-6-methyl heptanoic acid, ethyl ester (2b). Prepared in 50 % yield from **1b**. Colorless oil; TLC/Rf: 0.57 (ethyl acetate/cyclohexane, 1:1); MS: MH^+ = 373; ^1H NMR ($CDCl_3$): δ 0.90-1.05 (br d, 6H); 1.05-1.90 (m, 6H); 3.80-4.40 (m, 5H); 5.05 (s, 2H); 5.20 (br d, 1H); 7,30 (s, 5H)

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-5-phenylpentanoic acid, ethyl ester (2c). Prepared in 61 % yield from **1c**. White solid. mp: 95-97 °C (ethyl acetate/pentane). MS: MH^+ = 408; TLC/Rf: 0.50 (ethyl acetate/cyclohexane 1:1); ^1H NMR ($CDCl_3$): δ 1.25 (t, J_{HH} = 7 Hz, 3H); 2.90 (br d, 2H); 3.85-4.40 (m, 5H); 5.00 (s, 2H); 5.45 (br d, J_{HH} = 9Hz, 1H); 7.25 and 7.30 (2s, 10H). Anal. calcd for $C_{21}H_{23}NO_5F_2$: C, 61.91; H, 5.69; N, 3.44. Found: C, 62.19; H, 5.75; N, 3.55.

4-Benzyloxycarbonylamino-5-cyclohexyl-2,2-difluoro-3-hydroxypentanoic acid, ethyl ester (2d). Prepared in 47 % yield from **1d**. Colorless oil; TLC/Rf: 0.57 (ethyl acetate/cyclohexane 1:1; UV). ^1H NMR ($CDCl_3$): δ 0.65-2.00 (m) and 1.30 (t, J_{HH} = 6 Hz) (16H); 3.75-4.45 (m) and 4.27 (q, J_{HH} = 6 Hz) (5H); 5.10 (s, 2H); 5.20 (d, J_{HH} = 9 Hz, 1H); 7.38 (s, 5H).

4-(4-Nitrobenzyloxycarbonylamino)-2,2-difluoro-3-hydroxy-5-(4-benzyloxyphenyl) pentanoic acid, ethyl ester (2f). Prepared in 46 % yield from **1f**. White solid. mp: 109-111°C. (ethyl acetate/pentane) TLC/Rf: 0.41 (ethyl acetate/cyclohexane 1:1); MS: MH^+ = 559; MNH_4^+ = 576; ^1H NMR ($CDCl_3$): δ 1.25 (t, J_{HH} = 7 Hz, 3H); 2.65-3.10 (m, 2H); 3.75-4.15 (m, 2H); 4.25 (q, J_{HH} = 7 Hz, 2H); 4.90-5.15 (m) and 5.05 (s) (4H); 6.35 (br d, J_{HH} = 8 Hz, 1H); 7.35-7.50 (m, 11H); 8.20 (d, 2H). Anal. calcd for $C_{28}H_{28}N_2O_8F_2$: C, 60.21; H, 5.05; N, 5.01. Found: C, 60.71; H, 5.01; N, 5.01.

General procedure for the preparation of difluorohydroxyesters 2e and 2g.

(*R*,*S*)-4-Benzyloxycarbonylamino-5-(4-nitrophenyl)-2,2-difluoro-3-hydroxypentanoic acid, ethyl ester (2e). A mixture of activated zinc powder (1.19 g, 18.35 mmol, 3 eq) and iodine (0.025 g 0.1 mmol) in anhydrous tetrahydrofuran (3 mL) is ultrasonicated for 15 minutes at room temperature under an argon atmosphere. A solution of ethyl bromodifluoroacetate [(3.78 g, 18.35 mmol, 3 eq)] in anhydrous tetrahydrofuran (3 mL) is added dropwise to the zinc suspension. After two more minutes, a solution of aldehyde **1e** (1 eq) in anhydrous tertrahydrofuran (3 mL) is added dropwise to the black solution of organozinc reagent. Ultrasonication is maintained for 35 minutes after completion of the addition. Hydrolysis (saturated aqueous ammonium chloride, 30 mL) and ethyl acetate extraction (2 x 100 mL) afford after usual work up and chromatography (silica gel, ethyl acetate/cyclohexane 3.7) the expected hydroester **2e** in 72 % yield. White solid. mp: 124.0-125.0 °C. (ethyl acetate/pentane) TLC/Rf: 0.30 (ethyl acetate/cyclohexane 1:1); IR: 1766 cm^{-1} (C = 0); MS: MH^+ = 453; MNH_4^+ = 470; ^1H NMR ($CDCl_3$): (6/4 mixture of two diastereoisomers) 1.15-1.45 (m, 3H); 2.95-3.25 (m, 2H); 3.60 (d, J_{major} = 6.0 Hz; J_{minor} = 5.1 Hz, 1H); 4.00-4.40 (m, 4H); 4.95 (d, J = 5.1 Hz); 5.00-5.10 (m, 2H); 5.15 (d, J = 5.8 Hz, major); 7.15-7.45 (m, 7H); 8.05-8.20 (m, 2H). Anal. calcd for $C_{21}H_{22}N_2O_7F_2$: C, 55.75; H, 4.90; N, 6.19. Found: C, 55.69; H, 4.79; N, 6.13.

8-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy 4-(*R*,*S*)-(4-nitrobenzyloxycarbonylamino) octanoic acid, ethyl ester (2g). Prepared in 72 % yield from aldehyde **1g**. Yellowish oil. TLC/Rf: 0.20 (ethyl acetate/cyclohexane 1:1); IR: 1759 cm^{-1} (C = 0); MS: MH^+ = 568; MNH_4^+ = 585; ^1H NMR ($CDCl_3$): (65/35 mixture of diastereoisomers) δ 1.10-2.00 (m, 9H); 3.10-3.35 (m, 2H); 3.85-4.00 (m, 1H); 4.00-4.15 (m, 1H major); 4.20 (ddd, J_1 = 18.0 Hz, J_2 = 7.0 Hz, J_3 = 4.0 Hz, 1H minor); 4.30 (q, J = 7.1 Hz, 2H); 4.75-4.90 (m, 1H); 5.10-5.25 (m, 5H); 5.30 (d, J = 8.5 Hz, minor) and 5.35 (d, J = 8.6 Hz, major) (1H); 7.30-7.45 (m, 5H); 7.50 (d, J = 8.5 Hz, 2H); 8.20 (d, J = 8.5 Hz, 2H).

General procedure for the preparation of amides 3a-g.

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-5-methylhexanamide (3a). A stream of dry ammonia was bubbled, at - 78 °C, through a solution of **2a** (1.45 g, 4 mmol) in anhydrous diethyl ether (20 mL). After saturation, the temperature was allowed to rise to room temperature with stirring for 15 additional hours. The excess ammonia was removed and the solvent was evaporated *in vacuo*, leaving 1.20 g of amide **3a**. (90 % yield). White solid. TLC/Rf: 0.19 (ethyl acetate/cyclohexane 1:1; UV and I_2); ^1H NMR ($CDCl_3$): δ 0.96 (d, J_{HH} = 7 Hz, 6H); 1.95 (m, 1H); 3.83 (m, 1H); 4.20-4.83 (m, 2H); 5.30 (s, 2H); 5.76 (br d, 1H); 6.93 and 7.13 (2 br s, 2H); 7.63 (s, 5H).

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-6-methylheptanamide (3b). Prepared in 88 % yield from ester **2b.** White solid. mp: 208 °C. TLC/Rf: 0.29 (ethyl acetate/cyclohexane 1:1); MS: MH^+ = 345; MNH_4^+ = 362; ^1H NMR (DMSO-d_6): δ 0.90-1.15 (br d, 6H); 1.35-1.95 (m, 3H); 3.80-4.40 (m, 3H); 5.25 (s, 2H); 6.85 (br d, 1H); 7.50 (s) and 7.50-8.30 (m) (7H). Anal. calcd for $C_{16}H_{22}N_2O_4F_2$: C, 55.81; H, 6.44; N, 8.13. Found: C, 54.94; H, 6.68; N, 8.06.

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-5-phenylpentanamide (3c). Prepared in 98 % yield from ester **2c**. White solid. mp: 254 °C. TLC/Rf: 0.29 (ethyl acetate); MS: MH^+ = 379; MNH_4^+ = 396; ^1H NMR (DMSO-d_6): 2.80-3.05 (m, 2H); 3.90-4.40 (m, 3H); 5.03 (s, 2H); 5.90 (br d, 1H); 6.75-7.15 (m), 7.25 (s) and 7.30 (s) (12H).

4-Benzyloxycarbonylamino-5-cyclohexyl-2,2-difluoro-3-hydroxypentanamide (3d). Prepared in 97 % yield from ester **2d**. White solid. mp: 124-125 °C. (ethyl acetate/pentane) TLC/Rf: 0.53 (ethyl acetate); MS: MH^+ = 385; MNH_4^+ = 402; ^1H NMR (DMSO-d_6): 0.60-1.90 (m, 13H); 3.80-4.05 (m, 2H); 5.05 (m, 2H); 5.85 (d, J_{HH} = 9 Hz, 1H); 6.65 (d, 1H); 7.35 (s, 5H); 7.95 (m, 2H).

4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-5-(4-nitrophenyl)pentanamide (3e). Prepared in 95 % yield from ester **3d**. White solid. mp: 230-232 °C. (ethyl acetate/pentane) TLC/Rf: 0.40 (ethyl acetate); MS: MH^+ = 424; MNH_4^+ = 441; ^1H NMR (DMSO-d_6): (65:35 mixture of diastereoisomers). δ 2.70-2.85 (m, major)and 2.85-2.95 (m, minor) (1H); 2.95-3.05 (m, minor) and 3.10-3.20 (m, major) (1H); 3.90-4.15 (m, 2H); 4.75-5.10 (m, 2H); 6.15 (d, J = 6.5 Hz minor) and 6.25 (d, J = 6.5 Hz, major) (1H); 7.00-7.55 (m, 10H); 8.05-8.15 (m, 2H). Anal. calcd for $C_{19}H_{19}N_3O_6F_2$: C, 53.90; H, 4.52; N, 9.93. Found: C, 54.27; H, 4.37; N, 10.04.

4-(4-Nitrobenzyloxycarbonylamino)-2,2-difluoro-3-hydroxy-5-(4-benzyloxyphenyl)pentanamide (3f). Prepared in 57 % yield from ester **2f**. White solid. mp: 207 °C; (ethyl acetate/pentane) TLC/Rf: 0.42 (ethyl acetate); MS: MH^+ = 530; MNH_4^+ = 547; ^1H NMR (DMSO-d_6): δ 2.40-3.10 (m, 2H); 3.80-4.18 (m, 2H); 4.80-5.20 (m, 4H); 6.05 (d, 1H); 6.85-7.05 (m, 2H); 7.05-7.25 (m, 2H); 7.25-7.50 (m, 7H); 7.80-8.05 (br d, 2H); 8.05-8.25 (m, 2H). Anal. calcd for $C_{26}H_{25}N_3O_7F_2$: C, 58.98; H, 4.76; N, 7.94. Found: C, 58.90; H, 4.81; N, 7.66.

8-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-4-(4-nitrobenzyloxycarbonylamino)octanamide (3g). Prepared in 89 % yield from ester **2g**. White solid. mp: 69-70 °C. (ethyl acetate/pentane)TLC/Rf: 0.25 (ethyl acetate); MS: MH^+ = 539; MNH_4^+ = 556; ^1H NMR (DMSO-d_6): δ 1.20-1.70 (m, 6H); 3.00-3.10 (m, 2H); 3.80-4.00 (m, 1H); 4.00-4.15 (m, 1H); 5.05-5.30 (m, 4H); 6.05 (d, J = 7.7 Hz, 1H); 6.95 (d, J = 9.4 Hz, 1H); 7.30 (t, J = 5.5 Hz, 1H); 7.35-7.50 (m, 5H); 7.70(d, J = 8.8 Hz, 2H); 7.95-8.15 (m, 2H); 8.35 (d, J = 8.7 Hz, 2H). Anal. calcd for $C_{24}H_{28}N_4O_8F_2$: C, 53.53; H, 5.24; N, 10.40. Found: C, 52.87; H, 5.35; N, 9.73.

General procedure for the preparation of carbamates 4a-g.

***N*^{4}-Benzyloxycarbonyl-*N*^{1}-*tert*-butoxycarbonyl-2,2-difluoro-3-hydroxy-5-methyl-1,4-hexanediamine (4a).** To a solution of **3a** (3.03 g, 9.2 mmol) in anhydrous tetrahydrofuran (50 mL), was added, under nitrogen a 10 M solution of $BH_3(CH_3)_2S$ (2 mL, 20 mmol). The mixture was heated at reflux for 4 h. After cooling to room temperature, methanol (25 mL) was added. The solvent was removed *in vacuo*. The residue was taken off in a saturated solution of hydrogen chloride in diethyl ether (20 mL) and the mixture was stirred for 0.45 h. The solvent was evaporated. The residue was taken off in saturated $NaHCO_3$ (15 mL), water (15 mL) and tetrahydrofuran (30 mL). Di-*tert*-butyl-dicarbonate (2.40 g) and sodium carbonate (1.46 g) were added and the mixture was stirred at room temperature for 15 h. Water (50 mL) was added and the mixture was extracted with diethyl ether (3 x 100 mL). The combined organic layers were dried over anhydrous magnesium sulfate. Filtration and removal of the solvent *in vacuo* left an oil which was purified by chromatography (silica gel, ethyl acetate/cyclohexane 1:9) yielding 2.10 g of the expected dicarbamate **4a** as a colorless oil (54 % yield). TLC/Rf: 0.50 (ethyl acetate/cyclohexane 1:1); MS: MH^+ = 417; MNH_4^+ = 434 . ^1H NMR ($CDCl_3$): δ 0.93 (d, J_{HH} = 7 Hz, 6H); 1.50 (s, 9H); 1.93 (m, 1H); 3.00-4.10 (m, 5H); 5.83 (s, 2H); 5.63 (br d, 1H); 7.30 (s, 5H).

***N*^{4}-Benzyloxycarbonyl-*N*^{1}-*tert*-butoxycarbonyl-2,2-difluoro-3-hydroxy-6-methyl-1,4-heptanediamine (4b).** Prepared in 60-70 % yield from amide **3b**. Colorless oil. TLC/Rf: 0.56 (ethyl acetate/cyclohexane 1:1). ^1H NMR ($CDCl_3$): δ 0.95-1.00 (2d, 6H); 1.25-1.70 (m) and 1.45 (s) (12H); 3.15-3.30 (m, 1H); 3.58 (d, J_{HH} = 9 Hz, 1H); 3.80-4.00 (m, 1H); 4.20 (m, 1H); 5.05 (m), 5.10 (s) and 5.15 (d) (4H); 7.35 (m, 5H).

***N*^{4}-Benzyloxycarbonyl *N*^{1}-*tert*-butoxycarbonyl-2,2-difluoro-3-hydroxy-5-phenylpentanediamine (4c).** Prepared in 64 % yield from amide **3c**. White solid. MS: MH^+ = 465; MNH_4^+ = 487. The two diastereoisomers of **4c** were separated by MPLC (silica gel, ethyl acetate/cyclohexane 1:9); 1st eluted diastereoisomer: Rf: 0.55 (ethyl acetate/cyclohexane 1:1). mp: 102-103 °C. (ethyl acetate/pentane). ^1H NMR ($CDCl_3$): δ 1.40 (s, 9H, *tert*-C_4H_9); 2.85-3.18 (m, 3H, -C-CH_2Ph and C$\underline{H}_A$NH); 3.05 (dd, 1H, J_{HF} = 24.05 Hz, J_{HH} = 4.6 Hz, -C$\underline{H}_B$ NH); 3.90 (m, 1H, C$\underline{H}$OH); 4.30 (m, 1H, -CH-); 4.87 (m, 2H, NH) and OH); 5.12 (s, 2H, -CH_2O-); 4.95 (d, 1H, N$\underline{H}CO_2CH_2$Ph); 7.15-7.38 (m, 10H, arom). ^{19}F NMR ($CDCl_3$, ext. C_6F_6): -123.05 [ddd, J(F_AF_B) = 256.8; J_{FHA} = 24.05; J_{FHB} = 3.63] and -115.25 [ddd, J(F_AF_B) = 256.8; J_{FHA} = 29.06; J_{FHB} = 11.61]. 2nd eluted diastereoisomer: Rf: 0.48 (ethyl acetate/cyclohexane 1:1); mp: 133-134 °C (ethyl acetate/pentane). ^1H NMR ($CDCl_3$): δ 1.45 (s, 9H, *tert*-C_4H_9); 2.87-3.15 (m, 2H, -C-CH_2Ph); 3.27 (m, 1H) and 3.82 (d, J_{HF} = 23.7 Hz, 2H, CH_2CF_2); 3.95 (m, 1H, C$\underline{H}$OH); 4.30 (m, 1H, -CH-); 4.85 (d, 1H), 4.95 (d) and 5.00 (s) (3H) (2NH, OH and CH_2O); 7.15-7.35 (m, 10H, arom). ^{19}F NMR ($CDCl_3$, ext. C_6F_6): -120.03 [ddd, J(F_AF_B) = 255 Hz, J_{FHA} = 23.7 Hz, J_{FHB} = 4 Hz] and -113.05 [ddd, J(F_AF_B) = 255 Hz, J_{FHA} = 27.7Hz, J_{FHB} = 2.7 Hz].

***N*^{4}-Benzyloxycarbonyl-*N*^{1}-*tert*-butoxycarbonyl-5-cyclohexyl-2,2-difluoro-3-hydroxy-1,4-pentanediamine (4d).** Prepared in 60 % yield from amide **3d**. mp: 109-111 °C (ethyl acetate/pentane). TLC/Rf: 0.50 (ethyl acetate/cyclohexane, 1:1); MS: MH^+ = 471; MNH_4^+ = 488; ^1H NMR ($CDCl_3$): δ 1.60-2.00(m) and 1.45 (s) (22H); 3.00-4.50 (m, 5H); 5.15 (s) and 5.10-5.35 (m) (4H); 7.35 (m, 5H). Anal. calcd for $C_{24}H_{36}O_5N_2F_2$: C, 61.26; H, 7.71; N, 5.95. Found: C, 60.99; H, 7.97; N, 5.79.

***N*^{4}-Benzyloxycarbonyl-*N*^{1}-*tert*-butoxycarbonyl-2,2-difluoro-3-hydroxy-5-(4-nitrophenyl)-1,4-pentane-diamine (4e).** Prepared in 61 % yield from amide **3e**. White crystals. mp: 100-104 °C (ethyl acetate/pentane). TLC/Rf: 0.60 (ethyl acetate); MS: MH^+ = 510; MNH_4^+ = 527; ^1H NMR (DMSO-d_6): δ 1.45 (s, 9H); 2.85 (dd, J_1 = J_2 = 13.5 Hz, 1H); 3.25 (dd, J_1 = 13.5 Hz, J_2 = 2.5 Hz, 1H); 3.50-3.70 (m, 2H); 3.75-3.90 (m, 1H); 4.05-4.15 (m, 1H); 4.85 (dd, J_1 = 12.8 Hz, 2H); 6.15 (d, J = 5.8 Hz, 1H); 7.00-7.60 (m, 8H); 8.20 (d, J= 8.5 Hz, 2H). Anal. calcd for $C_{24}H_{29}N_3O_7F_2$: C, 56.58; H, 5.74; N, 8.25. Found: C, 56.98; H, 5.62; N, 8.35.

N^4-(4-Nitrobenzyloxycarbonyl)-N^1-*tert*-butoxycarbonyl-2,2-difluoro-3-hydroxy-5-(4-benzyloxyphenyl)-1,4-pentane diamine (4f). Prepared in 50 % yield from amide **3f**. White solid. TLC/Rf: 0.41 (ethyl acetate/cyclohexane, 1:1); MS: MH^+ = 616; MNH_4^+ = 633; ^1H NMR (DMSO-d_6): δ 1.50 (s, 9H); 2.85 (m, 2H); 3.40-3.65 (m, 2H); 3.70-3.80 (m, 1H); 4.15-4.25 (m, 1H); 5.15-5.25 (m and s, 4H); 6.00 (m, 1H); 7.05 (d, 2H); 7.18-7.30 (m, 4H); 7.35-7.55 (m, 7H); 8.35 (d, 2H). Anal. calcd for $C_{31}H_{35}N_3O_8F_2$: C, 60.48; H, 5.73; N, 6.82. Found: C, 60.45; H, 5.70; N, 6.73.

N^8-Benzyloxycarbonyl-N^1-*tert*-butoxycarbonyl-2,2-difluoro-3-hydroxy-N^4-(4-nitrobenzyloxycarbonyl)-1,4,8-octanetriamine (4g). Prepared in 64 % yield from amide **3g**. Viscous oil. TLC/Rf: 0.15 (ethyl acetate/cyclohexane, 1:1). MS: MH^+ = 625; MNH_4^+ = 642; ^1H NMR ($CDCl_3$): (60-40 mixture of diastereoisomers) δ 1.20-1.90 (m, 15H); 3.10-3.30 (m, 3H); 3.60 (d, J_{HF} = 22.5 Hz, major) and 3.75 (d, J_{HF} = 27.0 Hz, minor) (1H); 3.80-4.15 (m, 2H); 4.70-4.90 (m, 1H); 4.95-5.35 (m, 7H); 7.35-7.40 (m, 5H); 7.40-7.55 (two d, J = 8.5 Hz, 2H); 8.20 (two d, J = 8.5 Hz, 2H). Anal. calcd for $C_{29}H_{38}N_4O_9F_2$: C, 55.76; H, 6.13; N, 8.97. Found: C, 56.03; H, 6.44; N, 8.72.

General procedure for the preparation of amides 5.

N^4-Benzyloxycarbonyl-5-cyclohexyl-2,2-difluoro-3-hydroxy-N^1-(3-methylbutanoyl)-1,4-pentanediamine ($5d_1$). Prepared in two steps from carbamate **4d** in 72 % overall yield.

Step a: **N^1-*tert*-butoxycarbonyl deprotection.** N^4-Benzyloxycarbonyl-5-cyclohexyl-2,2-difluoro-3-hydroxy-1,4-pentanediamine. A solution of **4d** (6g, 12.7 mmol) in trifluoroacetic acid (150 mL) was stirred at 0 °C for 1.5 h. The solvent was removed *in vacuo* and the residue was triturated with diethyl ether and evaporated to dryness (3 x 100 mL). The residue was dissolved in diethyl ether and the organic layer was washed with a saturated aqueous solution of sodium bicarbonate, brine and dried over anhydrous magnesium sulfate. Filtration and removal of the solvent *in vacuo* left a white solid. 4.25 g of the expected amine were isolated (90 % yield). mp: 88-89 °C (ethyl acetate/pentane). MS: MH^+ = 365; MNH_4^+ = 382; ^1H NMR ($CDCl_3$): δ 0.70-2.00 (m, 13H); 3.00 (bs, 3H); 3.17 (br t, J_{HF} = 15 Hz, 2H); 3.70-4.35 (m, 2H); 5.20 (s, 2H); 5.40 (d, J_{HH} = 9Hz, 1H); 7.40 (s, 5H).

Step b: **N^1-amino coupling.** To a solution of 3-methylbutanoic acid (1.055 g, 10.3 mmol) in anhydrous acetonitrile (50 mL) was added at - 20 °C under nitrogen *N*-methylmorpholine (1.09 g, 10.8 mmol) followed by isobutylchloroformate (1.41 g, 10.3 mmol). The mixture was stirred at -20 °C for 15 min. After that time, N^4-benzyloxycarbonyl-5-cyclohexyl-2,2-difluoro-3-hydroxy-1,4-pentanediamine (step a) (4.02 g, 10.8 mmol) in anhydrous dimethylformamide (5 mL) was added at -20 °C to the solution. The temperature was allowed to raise to room temperature and the mixture was stirred for 15 hrs. The solvent was removed *in vacuo*, and the crude residue was purified by column chromatography (MPLC, silica gel, ethyl acetate/cyclohexane 2:8 to 3:7). 3.70 g of the expected amide **$5d_1$** were isolated (79 % yield). White solid. TLC/Rf: 0.36 (ethyl acetate/cyclohexane 1:1). MS: MH^+ = 455; MNH_4^+ = 472; ^1H NMR ($CDCl_3$): δ 0.70-2.00 (m, 20H); 2.10 (br s, 2H); 3.00-4.40 (m, 4H); 5.15 (s, 2H); 5.30 (d, J_{HH} = 4Hz, 1H); 5.40 (d, J_{HH} = 9 Hz, 1H); 6.90 (t, J_{HH} = 6Hz, 1H); 7.40 (s, 5H).

N^1-Acetyl-N^4-benzyloxycarbonyl-2,2-difluoro-3-hydroxy-5-methyl-1,4-hexanediamine (5a). Prepared in two steps from carbamate **4a** and acetic anhydride in 66-80 % overall yield. Used in the next step without further purification. TLC/Rf: 0.42 (ethyl acetate); MS: MH^+ = 359; MNH_4^+ = 376; ^1H NMR ($CDCl_3$): δ 0.95 (br d, J_{HH} = 7 Hz, 6H); 1.60-2.05 (m) and 2.00 (br s) (4H); 3.00-4.20 (m, 5H); 5.10 (s, 2H); 5.25-5.55 (m, 1H); 5.70 (br d, 1H); 7.40 (s, 5H).

N^4-benzyloxycarbonyl-2,2-difluoro-3-hydroxy-6-methyl-N^1(*N*-phenylacetyl-*D*-valyl)-1,4-heptanediamine ($5b_1$). Prepared in two steps from carbamate **4b** and *N*-phenylacetyl-*D*-Valine in 50 % overall yield. White solid. mp: 201-202 °C (ethyl acetate/pentane). TLC/Rf: 0.35 (ethyl acetate); MS: MH^+ = 547; MNH_4^+ = 565; ^1H NMR ($CDCl_3$ + εCD_3OD): δ 0.75-2.00 (m, 16H); 3.30-4.40 (m, 8H); 5.15 (s, 2H); 5.65 (br d, 1H); 6.95 (br d, 1H); 7.50 (m, 10H); 7.95 (m, 1H).

N^1-(4-Benzyloxycarbonylamino-2,2-difluoro-3-hydroxy-6-methylheptyl)-2-(1-methylethyl)-N^3-phenylmethyl-1,3-propanediamide ($5b_2$). Prepared in two steps from carbamate **4b** and 3-benzylamino-2-(1-methylethyl)-3-oxopropanoic acid in 55 % overall yield. White solid. mp: 144-145 °C (ethyl acetate/pentane). TLC/Rf: 0.60 (ethyl acetate); MS: MH^+ = 548. ^1H NMR ($CDCl_3$ + CD_3OD): δ 0.70-2.50 (m, 16H); 2.90 (d, 1H); 3.40-4.55 (m, 7H); 5.15 (s, 2H); 5.90 (m, 1H); 7.40-7.45 (2s, 10H); 8.25 (m, 2H). Anal Calcd for $C_{29}H_{39}N_3O_5F_2$: C, 63.60; H, 7.18; N, 7.67. Found: C, 63.71; H, 7.10; N, 7.44.

N^4-Benzyloxycarbonyl-2,2-difluoro-3-hydroxy-6-methyl-N^1-[2-(1-methylpropyl)-4-phenylbutanoyl]-1,4-heptane diamine ($5b_3$). Prepared in two steps from carbamate **4b** and 2-(1-methylpropyl)-4-phenylbutanoic acyl chloride in 54 % overall yield; Colorless oil. TLC/Rf: 0.64 (ethyl acetate/cyclohexane, 1:1). MS: MH^+ = 547; MNH_4^+ = 564; ^1H NMR ($CDCl_3$): δ 0.70-2.20 (m, 23H); 2.40-2.60 (m, 2H); 2.90-4.40 (m, 4H); 4.70-5.40 (m) and 5.10 (s) (4H); 6.60-6.90 (m, 1H); 7.10-7.40 (m and s, 10H).

N^4-Benzyloxycarbonyl-N^1-(3-methylbutanoyl)-2,2-difluoro-3-hydroxy-5-phenyl-1,4-pentanediamine (5c). Prepared in two steps from carbamate **4c** and 3-methylbutanoic acid in 77% overall yield. White solid. mp: 175 °C (ethyl acetate/pentane). TLC/Rf: 0.31 (ethyl acetate/cyclohexane 1:1); MS: MH^+ = 449; MNH_4^+ = 466. ^1H NMR ($CDCl_3$ + CD_3OD): δ 0.95 (br d, 6H); 2.00-2.10 (m, 3H); 2.60-4.40 (m, 6H); 5.00 (s, 2H); 7.20-7.40 (m, 10H).

N^1-Acetyl-N^4-benzyloxycarbonyl-2,2-difluoro-3-hydroxy-5-(4-nitrophenyl)-1,4-pentanediamine (5e). Prepared in two steps from carbamate **4e** and acetic anhydride in 83% overall yield. White solid. mp: 162-163.5 °C (acetone/diethyl ether). TLC/Rf: 0.25 (ethyl acetate); MS: MH^+ = 452; MNH_4^+ = 469; ^1H NMR ($CDCl_3$/CD_3OD): (70/30 mixture of diastereoisomers) δ 1.90-2.00 (m, 3H); 2.80 (dd, J_1 = 14.2 Hz, J_2 = 11.0 Hz 1H major); 2.90-3.00 (m, 2H minor); 3.15 (dd, J_1 = 14.3 Hz, J_2 = 3.7 Hz, 1H major); 3.20-3.35 (m, 1H); 3.45-3.55 (dd, J_{HF} = 20.0 Hz, J_{HH} = 6.0 Hz, minor) and 3.65-3.85 (m, major) (1H); 3.80-4.05 (m, 1H); 4.10-4.20 (m, major) and 4.20-4.30 (m, minor) (1H); 4.80 (dd, J_{HH} = 12.3 Hz, major) and 4.95 (dd, J_{HH} = 12.4 Hz, minor) (2H); 7.00-7.15 (m, 7H); 7.95-8.05 (m, 2H). Anal. Calcd for $C_{21}H_{23}N_3O_6F_2$: C, 55.87; H, 5.14; N, 9.31. Found: C, 55.89; H, 5.04; N, 9.46.

N^1-Acetyl-N^8-benzyloxycarbonyl-2,2-difluoro-3-hydroxy-N^4-(4-nitrobenzyloxycarbonyl)-1,4,8-octanetriamine (5g). Prepared in two steps from carbamate **4g** and acetic anhydride in 82 % overall yield. Viscous oil. TLC/Rf: 0.30 (ethyl acetate); MS: MH^+ = 567; MNH_4^+ = 584; ^1H NMR ($CDCl_3$): (60/40 mixture of diastereoisomers) δ 1.30-1.90 (m, 6H);

2.05-2.10 (m, 3H); 3.05-3.25 (m, 3H); 3.55 (br d, J_{HF} = 23.5 Hz, major) and 3.60-3.75 (br d, J_{HF} = 25.5 Hz, minor) (1H); 3.90-4.30 (m, 2H); 4.75-4.95 (m, 1H); 5.05-5.30 (m, 5H); 5.40 (br d, J_{HH} = 9.4 Hz, 1H); 6.05 (br t, J_{HH} = 6.5 Hz, 1H); 7.25-7.45 (m, 5H); 7.50 (two d, J_{HH} = 8.5 Hz, 2H); 8.20 (two d, J_{HH} = 8.5 Hz, 2H). Anal. Calcd for $C_{26}H_{32}N_4O_8F_2$: C, 55.12; H, 5.69; N, 9.89. Found: C, 54.99; H, 6.00; N, 9.55.

N^1-*tert*-butoxycarbonyl-5-cyclohexyl-2,2-difluoro-3-hydroxy-N^4-(3-methylbutanoyl-*L*-*O*-methyltyrosyl-*L*-n-valyl)-1,4-pentanediamine ($6d_2$). Prepared in two steps from carbamate **4d** in 60 % overall yield.

Step a: N^4-benzyloxycarbonyl deprotection; A mixture of carbamate **4d** (3.60 g 7.9 mmol) in absolute ethanol (100 mL) and 10 % palladium on charcoal (1.30 g) was stirred at room temperature under 1 atmosphere of hydrogen gas for 12 hrs. Filtration and evaporation of the solvent *in vacuo* yielded 2.50 g of N^1-*tert*-butoxycarbonyl-5-cyclohexyl-2,2-difluoro-3-hydroxy-1,4-pentanediamine (quantitative yield). MS: MH^+ = 321; MNH_4^+ = 347; Anal. Calcd for $C_{16}H_{30}N_2O_2F_2$: C, 59.97; H, 9.44; N, 8.74. Found: C, 60.28; H, 9.49; N, 8.56.

Step b: N^4-amino coupling. To a solution of 3-methylbutanoyl-*L*-(0-methyltyrosyl)-*L*-n-valine (0.325g, 0.86 mmol) in anhydrous acetronitrile (15 mL) at -20 °C and under nitrogen were added *N*-methylmorpholine (0.090 g, 0.89 mmol) and isobutylchloroformate (0.117 g, 0.86 mmol). The mixture was stirred at -20 °C for 15 minutes. A solution of amine of step a (0.300 g, 0.89 mmol) in anhydrous acetonitrile (5 mL) was then added. The temperature was allowed to rise to room temperature and the mixture was stirred for 15 hrs. The solvent was removed *in vacuo* and the crude residue was purified by chromatography. (silica gel, ethyl acetate/cyclohexane gradient 2:8 to 1:1). 0.360 g of amide **$6d_2$** (61 % yield) were isolated. White solid. mp: 100-103 °C (ethyl acetate/pentane). TLC/Rf: 0.63 (ethyl acetate); MS: MH^+ = 697; ^1H NMR ($CDCl_3$): δ 0.45-2.25 (m) and 1.50 (s) (37H); 2.80-3.20 (m, 3H); 3.30-3.95 (m) and 3.70 (s) (8H); 4.30-4.75 (m, 2H); 4.80-5.50 (m, 2H); 6.80 (d, 2H); 7.10 (d, 2H); 7.35-7.80 (m, 1H); 8.05-8.30 (m, 1H). Anal. Calcd for $C_{36}H_{58}N_4O_7F_2$: C, 62.05; H, 8.39; N, 8.04. Found: C, 62.06; H, 8.30; N, 8.00.

N^4-(*N*-Benzyloxycarbonyl-*L*-valyl)-5-(4-benzyloxyphenyl)-N^1-tert-butoxycarbonyl-2,2-difluoro-3-hydroxy-1,4-pentanediamine (6f). Prepared in two steps from carbamate **4f** and *N*-benzyloxycarbonyl-*L*-valyl anhydride in 48 % overall yield.

Step a: N^4-(4-nitrobenzyloxycarbonyl)deprotection. To a solution of carbamate **4f** (0.220 g, 0.36 mmol) in absolute ethanol (10 mL) was added tin (II) chloride (0.406 g, 1. 8 mmol). The mixture was stirred at reflux temperature for 2 hrs. The temperature was then allowed to drop to room temperature. Ice cold water (10 mL) and aqueous sodium hydrogenocarbonate (in excess to neutralize) were added. The mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration and removal of the solvent in vacuo yielded the free N^4-amine (0.150 g) as a yellowish solid. Used in the next step without purification (95 % yield).

Step b: N^4-Coupling. To a solution of *N*-benzyloxycarbonyl-*L*-valyl anhydride (0.170 g, 0.35 mmol) in anhydrous methylene chloride (10 mL) was added the free amine of step a (0.150 g, 0.35 mmol) in anhydrous *N,N*-dimethylformamide (2 mL). The mixture was stirred at room temperature for 12 hrs. Removal of the solvent *in vacuo* and purification by chromatography (silica gel, ethyl acetate/cyclohexane, 2:8) afforded **6f** (0.126 g) in 54 % yield. White solid. mp: 146-147 °C (ethyl acetate/pentane). TLC/Rf: 0.41 (ethyl acetate/cyclohexane 1:1). MS: MH^+ = 670. ^1H NMR (DMSO-d_6): δ 0.55-0.85 (m, 6H); 1.50 (s, 9H); 1.75-1.95 (m, 1H); 2.65-3.05 (m, 2H); 3.50-3.95 (m, 4H); 4.20-4.30 (m, 1H); 5.10-5.25 (m, 4H); 5.95 (m, 1H); 6.95-7.25 (m, 6H); 7.30-7.55 (m, 10H); 7.90 and 8.10 (two d, 1H). Anal. Calcd. for $C_{36}H_{45}N_3O_7F_2$: C, 64.56; H, 6.77; N, 6.27. Found: C, 64.49; H, 6.87; N, 6.05.

General procedure for the preparation of amides (7). Two steps procedure similar to the one used to prepare **$6d_2$**.

N^1-Acetyl-2,2-difluoro-3-hydroxy-N^4-[(3-methoxycarbonyl-1-oxopropyl)-*L*-alanyl-*L*-alanyl-*L*-prolyl]-5-methyl-1H-hexanediamine 7a. Prepared in two steps from amide **5a** and (3-methoxycarbonyl-1-oxopropyl)-*L*-alanyl-*L*-alanyl-*L*-proline in 35-40 % yield. White foam. TLC/Rf: 0.19 (chloroform/methanol, 92:8); MS: MH^+ = 578; MNH_4^+ = 595; ^1H NMR ($CDCl_3$): δ 0.80-1.40 (m, 12H); 1.70-2.30 (m, 6H); 2.40-2.80 (m, 6H); 3.00-4.20 (m) and 3.70 (s) 13H; 6.95 (m), 7.20 (m), 7.50 (m) and 8.00 (m) (4H).

N^1-(3-Methyl-(*R*)-2-phenylmethylcarbonylaminobutanoyl)-2,2-difluoro-3-hydroxy-N^4-(*tert*-butoxy-carbonyl-*L*-phenylalanyl-*L*-n-valyl)-6-methyl-1,4-heptanediamine ($7b_1$). Prepared in two steps from amide **$5b_1$** and *tert*-butoxycarbonyl-*L*-phenylalanyl-*L*-n-valine in 84 % overall yield. TLC/Rf: 0.58 (ethyl acetate). ^1H NMR ($CDCl_3$): δ 0.65-2.30 (m, 32H); 2.70-4.50 (m, 12H) 5.10-5.50 (m, 2H); 6.90-7.50 (m, 12H); 7.90 (m, 1H).

N^1-[4-(*tert*-Butoxycarbonyl-*L*-phenylalanyl-*L*-n-valylamino)-2,2-difluoro-3-hydroxy-6-methylheptyl]-2-(1-methylethyl)-N^3-phenylmethyl-1,3-propanediamide ($7b_2$). Prepared in two steps from amide **$5b_2$** and *tert*-butoxycarbonyl-*L*-phenylalanyl-*L*-n-valine in 56 % overall yield. White solid. TLC/Rf: 0.56 (chloroform/methanol, 92:8); MS: MH^+ = 759; ^1H NMR ($CDCl_3$): δ 0.80-1.10 (m, 15H); 1.15-1.80 (m) and 1.40 (s) (16H); 2.20-2.35 (m, 1H); 2.80-3.15 (m, 3H); 3.30-3.70 (m, 2H); 3.80-4.60 (m, 7H); 4.80-5.20 (m, 2H); 6.60-6.80 (m, 2H); 7.10-7.35 (m, 11H). Anal. Calcd for $C_{40}H_{59}N_5O_7F_2$: C, 63.22; H, 7.83; N, 9.22. Found: C, 63.09; H, 7.85; N, 9.05.

N^4-(*tert*-Butoxycarbonyl-*L*-phenylalanyl-*L*-n-valyl)-2,2-difluoro-3-hydroxy-6-methyl-N^1-[2-(1-methylpropyl)-4-phenylbutanoyl]-1,4-heptanediamine ($7b_3$). Prepared in two steps fom amide **$5b_3$** and *tert*-butoxycarbonyl-*L*-phenylalanyl-*L*-n-valine in 80 % overall yield. TLC/Rf: 0.61 (chloroform/methanol, 92:8); MS: MH^+ = 759; MNH_4^+ = 776. ^1H NMR ($CDCl_3$ + CD_3OD): δ 0.75-2.30 (m) and 1.45 (s) (38H); 2.60-4.60 (m, 12H); 5.70 (m, 1H); 6.95-7.70 (m, 13H).

N^4-Benzyloxycarbonyl-*L*-valyl-2,2-difluoro-3-hydroxy-N^1-(3-methylbutanoyl)-5-phenyl-1,4-pentane-diamine (7c). Prepared in two steps from amide **5c** and *N*-benzyloxycarbonyl-*L*-valine in 50% overall yield. White solid. mp: 191-192 °C (ethyl acetate/pentane). TLC/Rf: 0.61 (ethyl acetate); MS: MH^+ = 548; MNH_4^+ = 565; ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.50-2.25 (m, 16H); 2.50-4.65 (m, 8H); 5.20(m, 2H); 6.80-7.50 (m, 12H); 8.00 (m, 1H).

N^4-(*tert*-Butoxycarbonyl-*L*-phenylalanyl-*L*-n-valyl)-5-cyclohexyl-2,2-difluoro-3-hydroxy-N^1-(3-methylbutanoyl)-1,4-pentane diamine ($7d_1$). Prepared in two steps from amide **$5d_1$** and *N-tert* -butoxycarbonyl-*L*-

phenylalanyl-*L*-n-valine in 55-75 % overall yield. White solid. TLC/Rf: 0.18 (ethyl acetate/cyclohexane, 1:1; MS: MH^+ = 667; MNH_4^+ = 684; ^1H NMR ($CDCl_3$ + CD_3OD): δ 0.70-2.00 (m), 1.45 (s) and 2.10 (br s) (31H); 2.70-4.50 (m, 8H); 7.40 (m, 5H).

(*N*-*tert*-Butoxycarbonyl-*D*-phenylalanyl)-*N*-[4-acetyl-amino-1-[[4-*tert*-butoxycarbonylaminophenyl]methyl]-3,3-difluoro-2-hydroxybutyl]-*L*-prolinamide ($7e_1$). Prepared in three steps from amide **5e**.

Step a. Reduction/protection of 4-nitrosubstituent. A mixture of amide **5e** (0.90 g, 2 mmol) and tin (II) chloride, dihydrate (3.15 g, 7 mmol, 7 eq) in absolute ethanol was stirred at reflux for 3 hrs. The mixture was then hydrolyzed by addition of ice cold water (60 mL), neutralized with sodium bicarbonate and reacted with di-*tert* -butyl dicarbonate (0.87 g, 4.0 mmol, 2 eq). The mixture was stirred at room temperature for 16 hrs. Extraction with ethyl acetate (200 mL), drying over anhydrous magnesium sulfate, filtration and evaporation of the solvent *in vacuo* left the crude product, purified by chromatography (siliga gel, ethyl acetate/cyclohexane 6:4). 0.68 g of N^1-acetyl-N^4-benzyloxycarbonyl-2,2-difluoro-3-hydroxy-5-(4-*tert* -butoxycarbonylamino-phenyl)-1,4-pentane diamine were isolated. White solid. mp: 147-150 °C (diethyl ether/pentane). TLC/Rf: 0.30 and 0.35 (ethyl acetate); MS: MH^+ = 522; MNH_4^+ = 539; Anal. Calcd for $C_{26}H_{33}N_3O_6F_2$: C, 59.88; H, 6.38; N, 8.06. Found: C, 59.94; H, 6.42; N, 8.00.

Steps b and c. The amide of step a is converted to $7e_1$, in two steps by a procedure similar to the one described for **6** d_2 by deprotection and coupling to *N*-*tert* -butoxycarbonyl-*D*-phenylalanyl-*L*-proline in 49 % overall yield (from **5e**).White solid. mp: 113-116 °C (ethyl acetate/pentane). TLC: Rf = 0.15 (ethyl acetate); MS: MH^+ = 732; MNH_4^+ = 749; ^1H NMR (DMSO-d6): (mixture of diastereoisomers) δ 1.10-1.80 (m, 22H); 1.85 (s) and 1.90 (s) (3H); 2.55-3.05 (m, 4H); 3.10-3.70 (m, 5H); 4.00-4.50 (m, 5H); 5.95 (d, J_{HH} = 7.5 Hz) and 6.00 (d, J_{HH} = 7.5 Hz) (1H); 7.00-7.50 (m, 11H); 8.00-8.20 (m, 1H); 9.15 (ls, 1H). Anal. Calcd for $C_{37}H_{51}N_5O_8F_2$: C, 59.62; H, 7.10; N, 9.40. Found: C, 59.79; H, 7.00; N, 9.32.

(*N*-Benzyloxycarbonyl-*D*-phenylalanyl)-*N*-[4-acetyl-amino-3,3-difluoro-2-hydroxy-1-[4((((benzyloxy-carbonylamino)benzyloxycarbonylimino)methyl)amino)phenyl]methyl]butyl-L-prolinamide($7e_2$). Prepared in three steps from amide **5e** in 32 % overall yield.

Steps a and b. Similar to steps a and b of compound $7e_1$, free amine being coupled to *N*-benzyloxycarbonyl-*D*-phenylalanyl-*L*-proline.

Step c. The *tert* -butoxycarbamate of step b (0.160 g, 0.21 mmol) was added to trifluoroacetic acid (10 mL) at 0 °C. The mixture was stirred at 0 °C for 1 hr and the solvent was removed *in vacuo*. The residue was taken up in ethyl acetate (50 mL) and the organic layer was washed with aqueous 5% potassium carbonate (2 x 10 mL). The solvent was removed *in vacuo* and the residue taken up in tetrahydrofuran (5 mL). ((Benzyloxycarbonylamino) (methylthio)methylene)benzylcarbamate (0.150 g, 0.42 mmol, 2 eq) was added to the mixture. After 48 hrs stirring at 35-40 °C, the solvent was removed *in vacuo*. The crude residue was purified by chromatography (silica gel, ethyl acetate). 0.160 g of expected protected urea $7e_2$ were isolated. White powder. TLC/Rf: 0.15 and 0.20 (ethyl acetate); MS (FAB): MH^+ = 977; Anal. Calcd for: $C_{52}H_{55}N_7O_{10}F_2$: C, 63.99; H, 5.68; N, 10.05. Found: C, 63.27; H, 5.62; N, 9.75.

***N*-tert-Butoxycarbonyl-*D*-phenylalanyl-*N*-[1-(3-acetylamino-2,2-difluoro-1-hydroxy-propyl)-5-benzyloxy-carbonylaminopentyl]-*L*-prolinamide (7 g).** Prepared in three steps from amide **5 g** and *tert* -butoxycarbonyl-*D*-phenylalanyl-*L*-proline in 65 % overall yield.

Step a. 4-nitrobenzyloxycarbonyl deprotection; Step b: α-aminoprotection; Step c: coupling; White solid. mp: 70-78 °C (ethyl acetate/pentane). TLC: Rf = 0.10 (ethyl acetate); MS: MH^+ = 732; MNH_4^+ = 749; ^1H NMR (CD_3OD): δ 1.20-2.10 (m, 22 H); 2.40-2.80 (m, 3H); 3.10-3.25 (m, 2H); 3.25-3.75 (m, 3H); 3.75-4.10 (m, 2H); 4.10-4.25 (m, 1H); 4.25-4.40 (m, 1H); 5.10-5.15 (m, 2H); 7.25-7.40 (m, 10H). Anal. Calcd for $C_{37}H_{51}N_5O_8F_2$: C, 60.72; H, 7.02; N, 9.57. Found: C, 60.42; H, 7.07; N, 9.25.

General procedure for the preparation of ketones 8

N^4-[*N*-(*tert*-Butoxycarbonyl)-*L*-phenylalanyl-*L*-n-valyl]-5-cyclohexyl-2,2-difluoro-N^1-(3-methylbutanoyl)-3-oxo-1,4-pentanediamine ($8d_1$). To a solution of $7d_1$ (0.118 g, 0.18 mmol) in anhydrous methylene chloride (8 mL) were added pyridinium dichromate (0.122 g, 0.32 mmol), molecular sieves powder (3 A, 0.220 g) and glacial acetic acid (10 µL). The mixture was stirred at room temperature for 15 h. The solvent was removed *in vacuo* and the crude residue was purified by chromatography (MPLC, silica gel, chloroform). 0.107 g of the expected ketone was isolated (90 % yield). TLC/Rf: 0.60 (methanol/chloroform 8:92) MS: MH^+= 665; MNH_4^+ = 682; NMR ($CDCl_3$): δ 0.75 - 2.00 (m), 1.45 (s) and 2.10 (br s) (38 H); 3.10 (m, 2H); 3.45 - 4.95 (m, 5 H); 5.05 (d, J_{HH} = 7 Hz, 1 H); 6.70 (d, J_{HH} = 7 Hz, 1 H); 6.80 (t,J_{HH} = 6 Hz, 1H); 7.10 (d, J_{HH} = 6Hz, 1H); 7.35 (m, 5H). Anal. calcd for $C_{35}H_{54}N_4O_6F_2$: C, 63.23; H, 8.19; N, 8.43. Found: C, 63.45, H, 8.42, N, 8.35.

N^1-Acetyl-2,2-difluoro-N^4-[(3-methoxycarbonyl-1-oxopropyl)-*L*-alanyl-*L*-alanyl-*L*-prolyl]-5-methyl-3-oxo-1,4-hexanediamine (8a). Prepared in 60 % yield from alcohol **7a.** White solid. mp: 124 °C (ethyl acetate/pentane). TLC/Rf: 0.23 (chloroform/methanol 92:8); MS: MH^+ = 576; MNH_4^+ = 593; ^1H NMR ($CDCl_3$): δ 0.80 - 1.00 (m,6H); 1.25-1.40 (m,6H); 1.85-2.15 (m) and 2.00 (s)(6H); 2.25-2.40 (m,2H); 2.45-2.55 (m, 2H); 2.65-2.75 (m, 2H); 3.50-3.75 (m) and 3.70 (s)(6H); 4.05-4.25 (m, 1H); 4.55-4.85 (m, 4H); 6.25-6.35 (m, 1H); 6.70-6.80 (m, 1H); 7.20-7.30 (m, 1H); 7.65-7.75 (m, 1H). Anal. Calcd for: $C_{25}H_{39}N_5O_8F_2$: C, 52.17; H, 6.83; N, 12.17. Found: C, 52.54; H,6.95; N, 11.58.

N^1-(3-Methyl-(*R*)-2-phenylmethylcarbonylaminobutanoyl)-2,2-difluoro-N^4-(*tert*-butoxycarbonyl-*L*-phenyl-alanyl-*L*-n-valyl)-6-methyl-3-oxo-1,4-heptanediamine ($8\ b_1$). Prepared in 50% yield from alcohol $7b_1$. White powder. TLC/Rf: 0.66 (chloroform/methanol 92:8). MS: MH^+ = 758; ^1H NMR (CD_3OD): δ 0.85-1.00 (m, 15H); 1.25-1.85 (m) and 1.35 (s) (16H); 2.00-2.10 (m, 1H); 2.75-2.85 (m, 1H); 3.05-3.15 (m, 1H); 3.50-4.10 (m, 4H); 4.20-4.45 (m, 3H); 4.85-5.00 (m, 1H); 7.15-7.35 (m, 10H). Anal. Calcd for $C_{40}H_{57}F_2N_5O_7$: C, 63.39; H, 7.58; N, 9.24. Found: C, 63.76; H, 7.93; N, 9.09.

N^1-[4-(*tert*-Butoxycarbonyl-*L*-phenylalanyl-*L*-n-valylamino)-2,2-difluoro-6-methyl-3oxoheptyl]-2-(1-methylethyl)-N^3-phenylmethyl-1,3-propanediamide ($8b_2$). Prepared in 70% yield from alcohol $7b_2$. White solid. TLC/Rf: 0.62 (chloroform/methanol 92:8); MS: MH^+ = 758; ^1H NMR (CD_3OD): δ 0.85-1.00 (m, 15H); 1.25-1.85 (m) and 1.35 (s) (16H); 2.20-2.35 (m, 1H); 2.85-2.95 (m, 2H); 3.05-3.15 (m, 1H); 3.75-4.00 (m, 2H); 4.25-4.45 (m) and 4.40 (br s) (4H); 4.85-5.00 (m, 1H); 7.15-7.35 (m, 10H). Anal. Calcd for $C_{40}H_{57}N_5O_7F_2$: C, 63.39; H, 7.58; N, 9.24; Found: C, 63.22; H, 7.67; N, 8.88.

N^4-(*tert*-Butoxycarbonyl-*L*-phenylalanyl-*L*-n-valyl)-2,2-difluoro-6-methyl-N^1-[2-(1-methylpropyl)-4-phenylbutanoyl]-3-oxo-1,4-heptanediamine (8b$_3$)
Prepared in 63 % yield from alcohol **7b$_3$**; White solid. mp: 141 °C (ethyl acetate/pentane). TLC/Rf: 0.50 (ethyl acetate/cyclohexane, 1:1); MS: MH^+ = 757; MNH_4^+ = 774. ^1H NMR ($CDCl_3$): 0.80-1.00 (m, 15 H); 1.00 - 1.85 (m) and 1.40 (s) (23H); 1.90-2.05 (m, 1H); 2.55-2.65 (m, 2H); 3.00-3.15 (m,2H); 3.55-3.85 (m, 1H); 4.00-4.40 (m, 3H); 4.65-4.75 (m, 1H); 4.85-4.95 (m, 1H); 6.40. (d ,1H); 6.65-6.85 (2 m, 2H); 7.15-7,35 (m, 10H). Anal. Calcd for $C_{42}H_{62}N_4O_6F_2$: C, 66.64; H, 8.25; N, 7.40. Found: 66.45; H, 8.42; N, 6.80.

N^4-Benzyloxycarbonyl-*L*-valyl-2,2-difluoro-N^1-(3-methylbutanoyl)-3-oxo-5-phenyl-1,4-pentanediamine (8c)
Prepared in 62 % yield from alcohol **7c**; White solid. mp: 151-152 °C (ethyl acetate/pentane). TLC/Rf: 0.29 (ethyl acetate/cyclohexane 1:1); MS: MH^+ = 546; MNH_4^+: 569; ^1H NMR ($CDCl_3$): δ 0.75-1.00 (m, 12H); 2.00-2.20 (m, 4H); 2.70-2.85 (m, 1H); 3.25-3.35 (dd, 1H); 3.55-3.75 (m, 1H); 3.90-4.00 (m,1H); 4.05-4.30 (m,1H); 5.00-5.15 (m,3H); 6.40-6.70 (m,2H); 7.10-7.20 (m,1H); 7.20-7.45 (m, 10H). Anal. Calcd for $C_{29}H_{37}N_3O_5F_2$: C, 63.84; H, 6.84; N, 7.70. Found: C, 63.97; H, 6.98; N, 7.55.

N^1-*tert*-Butoxycarbonyl-5-cyclohexyl-2,2-difluoro-N^4-(3-methylbutanoyl-*L*-*O*-methyltyrosyl-*L*-n-valyl)-3-oxo-1,4-pentanediamine (8d$_2$) Prepared in 68 % yield from alcohol **6d$_2$**. White solid. mp: 91-93 °C (ethyl acetate/pentane). TLC/Rf: 0.67 (ethyl acetate); MS: MH^+ = 695; ^1H NMR ($CDCl_3$): δ 0.80-1.10 (m,11 H); 1.10-1.90 (m) and 1.45 (s) (24H); 1.95-2.10 (m, 3H); 2.95-3.10 (m, 2H); 3.55-3.90 (m) and 3.80 (s) (5H); 4.30-4.45 (m, 1H); 4.55-4.65 (m, 1H); 4.80-4.90 (m, 1H); 5.25-5.35 (m, 1H); 5.85-5.95 (m,1H); 6.40-6.50 (m, 1H); 6.55-6.65 (m, 1H); 6.85 (m, 2H); 7.15 (m, 2H). Anal. Calcd for $C_{36}H_{56}N_4O_7F_2$: C, 62.23; H, 8.12; N, 8.06. Found: C, 62.31; H, 8.06; N, 8.16.

(*N*-*tert*-Butoxycarbonyl-*D*-phenylalanyl)-*N*-(4-acetylamino-1-[[4-*tert*-butoxycarbonylaminophenyl]methyl]-3,3-difluoro-2-oxobutyl]-*L*-prolinamide (8e$_1$). Prepared in 80 % yield from alcohol **7e$_1$**. White solid. mp: 100-104 °C (diethyl ether/pentane). TLC/Rf: 0.25 (ethyl acetate). MS: MH^+ = 730; MNH_4^+ = 747; ^1H NMR (DMSO-d_6): (55/45 mixture of diastereoisomers) δ 1.88 (m, 22H); 1.90 (s,minor) and 1.95 (s,major) (3H); 2.75-3.35 (m, 5H); 3.50-3.60 (m,1H); 3.60-3.90 (m, 2H); 4.25-4.45 (m, 1H); 4.45-4.55 (m, 1H); 4.95-5.10 (m, 1H); 7.40-7.50 (m, 10H); 8.10 (d, J_{HH} = 7.7 Hz, major) and 8.20 (d, J_{HH} = 7.5 Hz, minor) (1H); 8.25 (t, J_1=J_2=7.5 Hz, minor) and 8.35 (t, J_1=J_2=7.5 Hz,major) (1H); 9.35 (br s, 1H). Anal. Calcd for $C_{37}H_{49}N_5F_2O_8$, 0.5 H_2O: C, 60.15; H, 6.82; N, 9.48. Found: C, 60.09; H, 6.76; N, 9.45.

(*N*-Benzyloxycarbonyl-*D*-phenylalanyl)-*N*-[4-acetyl-amino-3,3-difluoro-1-[4((((benzyloxycarbonylamino) benzyloxycarbonylimino)methyl)amino)phenyl]methyl]-2-oxobutyl-L-prolinamide (8e$_2$). Prepared in 70 % yield from alcohol **7e$_2$**. White powder. mp: 84-86 °C (ethyl acetate/pentane). TLC/Rf: 0.25 and 0.20 (ethyl acetate); MS (FAB): MH^+ = 974; ^1H NMR (DMSO-d_6): (55/45 mixture of diastereoisomers) δ 1.40-1.90 (m, 4H); 1.90 (s, 3H); 2.75-3.30 (m, 5H); 3.55-3.70 (m, 1H); 3.70-4.00 (m, 2H); 4.20-4.35 (m, 1H); 4.45-4.55 (m, 1H); 4.90-5.40 (m, 7H); 7.10-7.65 (m, 24H); 7.80-8.00 (m, 1H); 8.05-8.20 (m, 1H); 8.25 (t, J_1=J_2 = 7.5 Hz, minor) and 8.35 (t, J_1=J_2=7.2 Hz, major) (1H); 10.05 (br s, 1H); 11.45 (br s, 1H); Anal. Calcd for $C_{52}H_{53}N_7O_{10}F_2$: C, 64.12; H, 5.48; N, 10.07. Found: C, 63.71; H, 5.51; N, 9.84.

***N*-*tert*-Butoxycarbonyl-*D*-phenylalanyl-*N*-[1-(3-acetylamino-2,2-difluoro-1-oxo-propyl)-5-*tert*-butoxycarbonylaminopentyl]-*L*-prolinamide (8 g).** Prepared in two steps from alcohol **7 g** in 43 % overall yield .
Step a. benzyloxycarbonyl deprotection/protection with di*tert* butyl dicarbonate .
Step b. oxidation. White powder. mp: 63-65 °C (ethyl acetate/pentane). TLC/Rf: 0.20 (ethyl acetate); MS: MH^+ = 696; MNH_4^+ = 713; ^1H NMR ($CDCl_3$): (55/45 mixture of diastereoisomers); δ 1.00-2.25 (m, 31H); 2.40-2.60 (m, 1H); 2.90-3.30 (m, 4H); 3.50-3.70 (m, 2H); 4.00-4.25 (m, 1H); 4.30-4.80 (m, 3H); 5.30 (m, 1H); 5.70 (m, 1H); 6.80-7.10 (m, 1H); 7.10-7.40 (m, 5H); 7.90 (m, major); 8.45 (m, minor) (1H). Anal. Calcd for $C_{34}H_{51}N_5O_8F_2$: C, 58.69; H, 7.39; N, 10.07. Found: C, 57.98; H, 7.40; N, 9.72.

N^4-(*N*-Benzyloxycarbonyl-*L*-valyl)-5-(4-benzyloxyphenyl)-N^1-*tert*-butoxycarbonyl-2,2-difluoro-3-oxo-1,4-pentanediamine (8f). Prepared in 54% yield from alcohol **6f.** White powder. TLC/Rf: 0.48 (ethyl acetate/cyclohexane, 1:1); MS: MH^+ = 668; MNH_4^+ = 685; ^1H NMR (DMSO-d_6): δ 0.70-0.80 (m, 3H); 0.85-0.95 (m, 3H); 1.48 (s, 9H); 1.80-2.05 (m, 1H); 2.75-2.90 (m, 1H); 3.05-3.15 (m, 1H); 3.55-3.85 (m, 2H); 3.95-4.05 (m, 1H); 4.95-5.05 (m) and 5.00-5.20 (m) (5H); 6.90-7.05 (m, 2H); 7.20-7.55 (m, 14H); 8.55-8.65 (m, 1H). Anal. Calcd for $C_{31}H_{43}N_3O_7F_2$: C, 64.75; H, 6.49; N, 6.29. Found: C, 64.56; H, 6.47; N, 6.22.

General procedure for the preparation of amines 9.
D-Phenylalanyl-*N*-[4-acetylamino-1-[[4-aminophenyl]methyl]-3,3-difluoro-2-oxobutyl]-*L*-prolinamide, bishydrochloride (9e$_1$). To a solution of **8e$_1$** (0.030 g, 0.04 mmol) in anhydrous diethyl ether (2 mL) at 0 °C was added a saturated solution of HCl in anhydrous diethyl ether (10 mL). The mixture was stirred for 18 hrs while the temperature was allowed to rise to room temperature. The white precipitate was decanted, washed with pentane, filtered off and dried *in vacuo*. 0.022 g of **8e$_1$** were isolated (90 % yield). White powder. TLC/Rf: 0.15 (acetic acid/n-butanol/water 2:6:2); MS: MH^+ = 530; MNH_4^+ = 547; ^1H NMR (D_2O): (55/45 mixture of diastereoisomers). δ 0.90-1.90 (m, 4H); 2.00-2.10 (2s,3H); 2.55-2.65 (m, 1H); 2.80 (ddd, J_{HH} = 12.6 Hz, 1H); 3.05-3.40 (m, 4H); 3.80-4.00 (m, 2H); 4.15 (dd, J_{HH1} = 8.4 Hz, J_{HH2} = 3.6 Hz, major) and 4.25 (dd, J_{HH1} = 8.6 Hz, J_{HH2} = 3.5 Hz, minor) (1H); 4.40-4.50 (m) and 4.55 (dd, J_{HH1} = 12.0 Hz, J_{HH2} = 3.5 Hz, minor) (2H); 7.20-7.50 (m, 9H). Anal. Calcd for $C_{27}H_{33}N_5F_2O_4$, 2HCl, 1.25 H_2O: C, 51.89; H, 6.05; N, 11.21. Found: C, 51.76; H, 6.43; N, 10.80.

5-Cyclohexyl-2,2-difluoro-N^4-(3-methylbutanoyl-*L*-*O*-methyltyrosyl-*L*-n-valyl)-3-oxo-1,4-pentanediamine,hydrochloride (9d$_2$). Prepared in 68 % yield from ketone **8d$_2$**. White solid. mp: 165-167 °C. TLC/Rf: 0.65 (acetic acid/n-butanol/water 2:6:2); MS: MH^+ = 595; ^1H NMR (DMSO-d_6): δ 0.75-2.05 (m, 29H); 2.70-2.85 (m, 1H); 2.95-3.05 (m, 1H); 3.60-3.85 (m) and 3.80 (s) (5H); 4.35-4.45 (m, 1H); 4.55 - 4.65 (m, 1H); 4.80-4.90 (m, 1H); 6.85-6.95 (m, 2H); 7.25-7.35 (m, 2H); 8.05-8.20 (m, 2H); 8.65-8.85 (m, 4H).

***D*-Phenylalanyl-*N*-[4-acetylamino-1-[[-4-[(aminoiminomethyl)amino]phenyl]methyl]-3,3-difluoro-2-oxobutyl]-*L*-prolinamide, trishydrochloride (9e$_2$).** Prepared in 84 % yield from ketone **8e$_2$**; White powder. TLC/Rf: 0.35 and 0.40 (acetonitrile/water 9:1 RP18); MS (FAB): MH^+ = 537; ^1H NMR (D_2O): (55/45 mixture of diastereoisomers); δ 1.00-1.90 (m, 4H); 2.00-2.15 (2s,3H); 2.55-2.70 (m, 1H); 2.70-2.85 (m, 1H); 3.05-3.25 (m, 2H); 3.25-3.40 (m,2H); 3.75-4.00 (m, 3H); 4.15-4.25 (m, major) and 4.25-4.35 (m, minor) (1H); 4.40-4.55 (m, 2H); 7.15-7.55 (m, 9H). Anal. Calcd for: $C_{28}H_{35}N_7O_4F_2$, 1.5 H_2O, 3HCl: C, 47.50; H, 5.84; N, 13.85. Found: C, 47.50; H, 5.88; N, 13.06.

***D*-Phenylalanyl-*N*-[1-(3-acetylamino-2,2-difluoro-1-oxopropyl)-5-aminopentyl]-*L*-prolinamide, bishydrochloride (9g).** Prepared in 73 % yield from ketone **8g**. White powder. TLC/Rf: 0.25 (acetic acid/n-butanol/water 2:6:2); MS: MH^+ = 496; ^1H NMR (D_2O): (1/10 mixture of ketone and hydrate of ketone); δ 1.20-2.00 (m, 10H): 2.00-2.10 (2s, 3H); 2.70-2.80 (m, 1H); 2.90-3.05 (m,2H); 3.10-3.20 (m,1H); 3.20-3.30 (m, 1H); 3.45-3.55 (m, 1H); 3.75-3.90 (m, 2H); 4.15-4.25 (m, 1H); 4.30-4.40 (m, 1H); 4.50-4.60 (m, 1H); 7.25-7.50 (m, 5H). Anal. Calcd for $C_{24}H_{35}N_5O_4F_2$, 2HCl, H_2O: c, 49.15; H, 6.70; N, 11.94. Found: C, 48.90; H, 6,93; N, 11.13.

***N*^{4}-(*N*-benzyloxycarbonyl-*L*-valyl)-5-(4-benzyloxyphenyl)-2,2-difluoro-3-oxo-1,4-pentanediamine, hydrochloride (9f)**

Prepared in 59 % yield from ketone **8 f;** White solid. TLC/Rf: 0.51 (acetic acid/n-butanol/water, 2:6:2); MS: MH^+ = 568; ^1H NMR (DMSO-d_6): δ 0.50-0.95 (m, 6H); 1.80-2.05 (m, 1H); 2.65-3.25 (m, 2H); 3.50-3.80 (m, 2H); 3.80-4.05 (m, 1H); 4.30-4.40 (m, Hα hydrate); 4.90-5.25 (m, 4H and Hα ketone); 6.80-7.55 (m, 14H); 8.45-8.70 (br s, 3H). Anal. Calcd for $C_{31}H_{36}N_3O_5F_2Cl$, H_2O: C, 59.85; H, 6.16; N, 6.75. Found: C: 59,57; H, 6.01; N, 6.61.

In vitro Assay of Enzyme Inhibition. Values of IC_{50} were determined under the following conditions: 1. **Renin**/endogeneous angiotensinogen/phosphate buffer pH 6.0/37 °C/radioimmuno-assay. 2. **HIV-1 protease** Protein source: recombinant enzyme (E. Coli); substrate: H-SerGlnAsnTyrProIleValNH2 (Km = 1mM); buffer: 0.1 M Mes-tri acetate, 0.2 M NaCl, pH 5.5-6.0 (EDTA, Phenylmethylsulfonylfluoride, DTT 1mM and 0.5% BSA), 37 °C; kinetic analysis: HPLC analysis of the two products. 3. **Human Leukocyte Elastase**: purified from purulent sputum. Spectrophotometric assay, substrate: MeOSucAlaAlaPro-p-nitroanilide (Sigma); buffer: 0.1 M NaCl, 0.01M N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES), 0.01 M Tris, 0.1 % polyethylene glycol 6000; pH: 8; 37 °C. 4. **Human plasma thrombin** (Sigma) activity was measured at 30 °C using Sarcosyl-prolyl-arginine (p)-nitroanilide as substrate in 0.1 M Tris buffer (pH 7.5). For rapid equilibrium inhibition, Ki-values were determined from a Dixon plot. In the case of slow establishment of the equilibrium ENZFITTER (Biosoft) kinetic analysis was used; the Ki-values were determined according to Williams and Morrison.

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(*Received* 13 *July* 1995)