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# Synthesis and Comparison of Tripeptidylfluoroalkane Thrombin Inhibitors

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**Abstract**—Fluorinated ketone thrombin inhibitors based on the peptide sequence methyl-(D)-Phe-Pro-Arg-$CF_2R$ were synthesized: MDL 73,446 (**1**, R = F); MDL 73,775 (**2**, R = $CF_3$); and MDL 75,579 (**3**, R = $CH_2CH_2CH_3$). These were shown to be highly effective, slow binding inhibitors of thrombin. Anticoagulant activity was dose-dependent with **3** > **2** > **1** at doubling thrombin time and APTT, respectively. Anticoagulant activity corresponded with efficacy in a platelet-dependent ($FeCl_3$-induced) rat carotid artery thrombosis model. Arterial occlusion was dose-dependently prolonged with **3** > **2** > **1** at doubling the occlusion time.

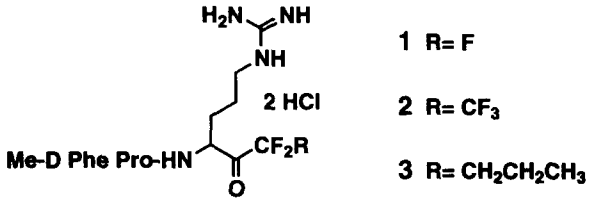
## Introduction

Blood vessel injury results in activation of the blood coagulation cascade and ultimately results in the generation of thrombin. This serine protease is the key enzyme that proteolytically converts circulating fibrinogen to insoluble fibrin. In addition, thrombin activates platelets exposing surface receptors to fibrinogen, thus promoting platelet aggregation. Thrombin therefore plays a central role in haemostasis and thrombosis and has become the focus for the development of new anticoagulant agents to treat thrombotic disorders. The critical role of thrombin makes it an ideal target for the design of synthetic anticoagulants.

Replacement of the scissile bond of thrombin substrate analogues by atom assemblies containing electrophilic carbonyl groups is a relatively new approach to proteinase inhibition effected through transition-state mimicry.[1] We,[2] and others,[3] have employed fluorinated ketones at the scissile site in inhibitor design. Among the various low molecular weight molecules that have demonstrated antithrombotic activity in animal models[4–9] peptide-like inhibitors based on the sequence D-phenylalanyl-prolyl-arginine have been shown to be effective.[4,8,10,11] Based on this optimal sequence, three differently fluorinated ketone tripeptide analogues **1–3** were synthesized and compared for thrombin inhibition, anticoagulant and antithrombotic activity.

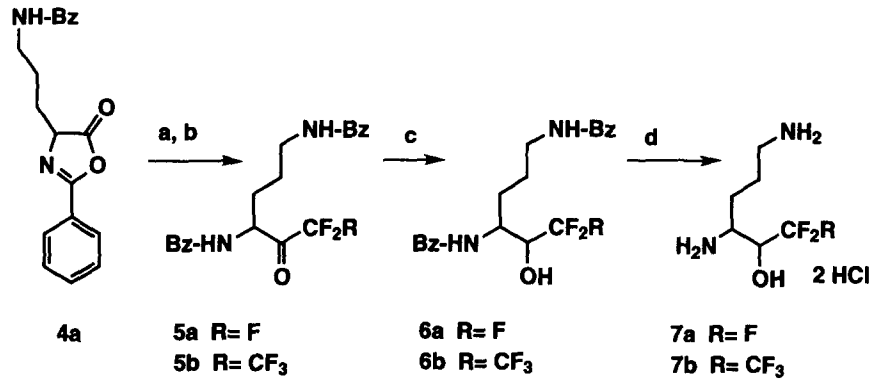
## Chemistry

All fluorinated ketone peptidyl inhibitors described for this study were synthesized in a convergent manner from the protected dipeptide *N*-methyl-D-Phe-ProOH[12]

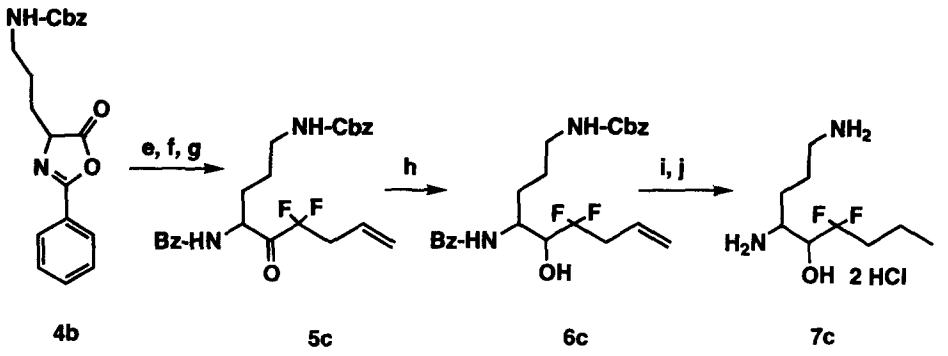



and fluorinated analogues of ornithine or arginine (Scheme 4). The fluorinated building blocks were prepared in a few steps starting with modified Dakin–West reactions of (a) δ-benzoyl protected ornithine azlactone **4a** and the anhydrides of trifluoroacetic acid (TFAA), or pentafluoropropionic acid (PFPAA)[2e,f] as depicted in Scheme 1; or (b) δ-Cbz-protected ornithine azlactone **4b** and 2,2-difluoro-4-pentenoyl chloride/triethylamine, followed by DMAP[2b] (Scheme 2). The reaction provided racemic ketones **5a–c** with 85, 79, and 72% yield. The highly reactive carbonyl function of the ketones was then 'protected' by conversion to the corresponding alcohols **6a–c** (91, 89, and 95%) by borohydride reduction. Benzoyl deprotection was achieved for the fluorinated amino alcohols by acid hydrolysis (12 N HCl, reflux) to give the diamino alcohols **7a** and **7b** (100%). Cbz-deprotection prior to benzamide hydrolysis afforded the difluorobutyl alcohol **7c** (100%) from **6c**.

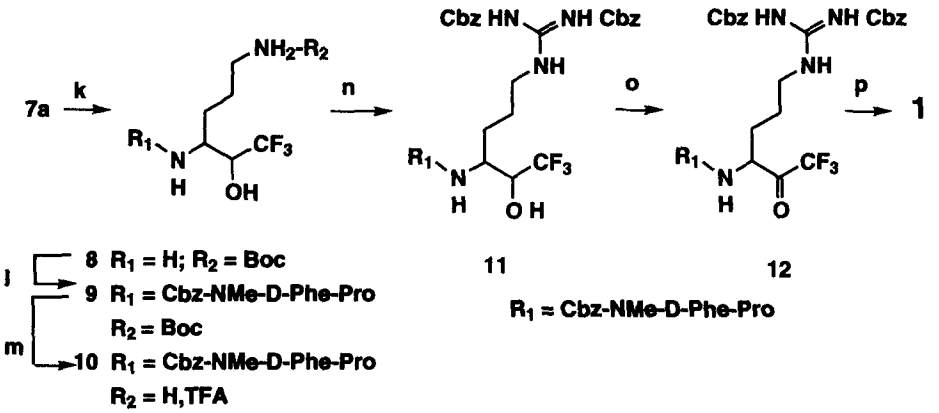
The diamino alcohols so obtained were then further processed to the desired tripeptide analogues through two distinct pathways (Schemes 3 and 4). Diamino-alcohol **7a** was ε-Boc protected to ornithine analogue **8** (47%) and the resulting amino alcohol coupled to Cbz-*N*-Methyl-D-Phe-ProOH[10] to give the tripeptide ornithine analogue **9** (81%). Deprotection (TFA/$CH_2Cl_2$, 84%) delivered amine **10**; ε-guanylation with bis-Boc-S-methyl-isothiourea[13] afforded the protected fluorinated




**Scheme 1.** (a) TFAA or PFPAA, rt; (b) $(CO_2H_2)_2$ anh. in THF; (c) $NaBH_4$, EtOH; (d) HCl 12 N, reflux.




**Scheme 2.** (e) 2,2-Difluoro-4-pentenoyl chloride, $NEt_3$, THF, 0 °C → rt; (f) DMAP, THF, rt; (g) $(CO_2H)_2$ anh. in THF; (h) $NaBH_4$, EtOH; (I) $H_2$, $Pd(OH)_2/C$ 10 %; (j) HCl 12 N, reflux.




**Scheme 3.** (k) $Boc_2O$, excess $NEt_3$, $THF/H_2O$; (l) Cbz-NMe-D-Phe-ProOH, DCC, HOBt, NMM, $CH_2Cl_2$; (m) TFA:$CH_2Cl_2$ (1:1), rt; (n) bis-Cbz-S-methyl-iso-thiourea, $NEt_3$, THF, 40 °C; (o) Swern oxidation; (p) $H_2$, cat. $Pd(OH)_2/C$ 10%, HCl 1 N.

arginyl tripeptide alcohol **11** (68%), which was oxidized (Swern conditions, 74%) to the fluorinated tripeptide-like ketone **12**. Final deprotection of all blocking groups delivered the trifluoromethyl ketone inhibitor **1** (MDL 73,446, 95% yield) in a completely hydrated form.

The diaminoalcohols **7b** and **7c** were first converted to the corresponding arginine analogues **17** and **18** (Scheme 3) by (a) trifluoroacetylation of the lateral amine, **13** and **14** (TFAA/TFA,[14] 95 and 85%) and (b) guanylation of the ω-amino group with bis-Boc-carboxamidino-pyrazol,[15] **15** and **16** (70 and 68%). Hydrolysis (LiOH, $THF/H_2O$, 88 and 95%) of the trifluoroacetamides afforded the protected arginine-like fluoromethyl alcohols **17** and **18**. Peptide coupling with Boc-*N*-Me-D-Phe-ProOH (DCC, HOBt, NMM; 82 and 90%) provided the fluorinated tripeptide alcohols **19** and **20**. The following Swern oxidation regenerated the original fluorinated ketone functionality and compounds **21** and **22** were isolated (73 and 71%) as ketones. Final deprotection of all protecting groups ($HCl_g/Et_2O$) and lyophilization of the material obtained gave the desired inhibitors **2** (MDL 73,775, 82%; ketone:hydrate ratio, 7:93) and **3** (MDL 75,579, 78%; ketone:hydrate ratio, 22:78). All three fluorinated ketones are composed of

an epimeric mixture of 50:50. Separation of the individual diastereomers was performed (reversed-phase HPLC); however optical integrity could be kept only under acidic conditions (pH 2–3). Rapid epimerization ($t_{1/2}$ *ca* 30 min) was observed ($^{19}F$ NMR) at 40 °C and at pH 7.8.[16]

*Thrombin inhibition and anticoagulant activity*

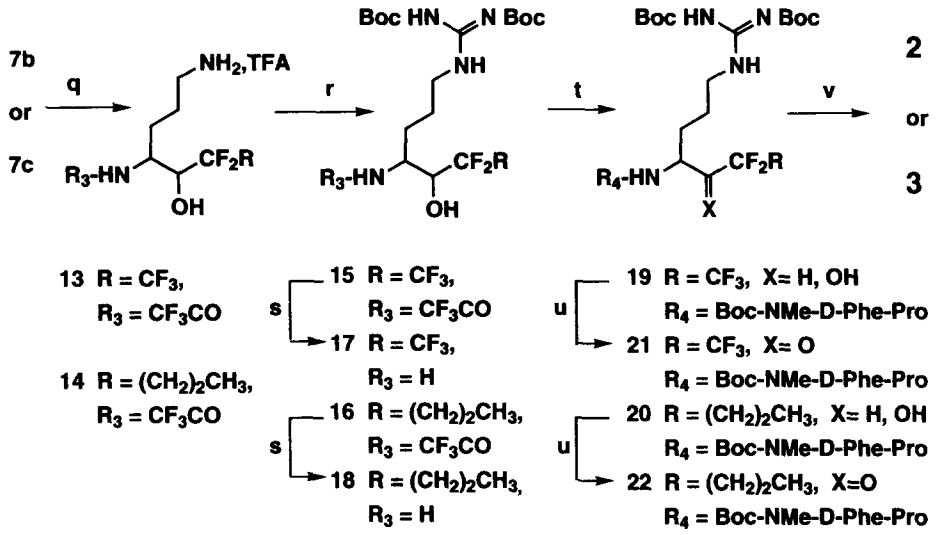
The kinetic evaluation of these tripeptidylfluoroketones demonstrates potent inhibition of human thrombin (Table 1). While the trifluoromethyl ketone **1** displayed 'irreversible'-like inactivation of thrombin only slow-tight binding kinetics were observed with the pentafluoroethyl **2** and difluorobutyl **3** analogues. Thus, **1** was expected to exhibit greater anticoagulant activity in human plasma. Surprisingly, **3** was found to be a more potent anticoagulant than **2** and **1** (Table 1). The tripeptidylfluoroketones dose-dependently prolonged clotting of human plasma in the activated partial thromboplastin time (APTT) and thrombin time (TT) assays. Inhibitor **3** doubled the aPTT at 0.7 μM, being approximately 4-fold more active than **2** and 300-fold more active than **1**. (The individual isomers did not exhibit the expected effect of greater activity *in vitro* since rapid epimerization to the original composition occurred during the evaluation of the activity. Therefore all further tests were run with the original mixture.)

*Antithrombotic activity of peptidylfluoroalkanes*

The anticoagulant effects in rat plasma of the tripeptidylfluoroketone analogues correspond with efficacy in a platelet-dependent[17] $FeCl_3$-induced rat carotid artery thrombosis model (Table 2). Inhibitor **3** dose-dependently (50–250 nmol $kg^{-1}$ $min^{-1}$) prolonged occlusion time and was antithrombotic at all doses tested (Fig. 1). Compound **3** prevented occlusion in all animals (five) at a rate of 250 nmol $kg^{-1}$ $min^{-1}$. Compound **2** dose-dependently (125–1000 nmol $kg^{-1}$ $min^{-1}$) prolonged occlusion and at a dose of 1000 nmol $kg^{-1}$ $min^{-1}$ prevented occlusion in all animals (six). The trifluoromethyl ketone **1** did not prolong occlusion time at a dose of 1000 nmol $kg^{-1}$ $min^{-1}$. The doses of inhibitors **2** and **3** required to double the control time to occlusion after $FeCl_3$ application were approximately 80 and 475 nmol $kg^{-1}$ $min^{-1}$, respectively (Table 2).

## Conclusion

Tripeptidylfluoroketone analogues (Me-D-Phe-Pro-Arg-$CF_2R$) are potent inhibitors of thrombin. Based on the kinetic data we expected **1** (MDL 73,446) to have better anticoagulant activity. Surprisingly, the results demonstrate that **3** (MDL 75,579) exhibits the greatest anticoagulant activity in human plasma. It is possible




**Scheme 4.** (q) TFAA/TFA, 40 °C; (r) bis-Boc-S-methyl-isothiourea, $NEt_3$, THF; (s) LiOH 1 N, THF/$H_2O$; (t) Boc-*N*-Me-D-Phe-ProOH, DCC, HOBt, NMM, $CH_2Cl_2$; (u) Swern oxidation; (v) HCl*g*/$Et_2O$.

**Table 1.** Comparison of peptidylfluoroketones in human plasma

| | Methyl-D-Phe-Pro-Arg-$CF_2R$ | | |
|---|---|---|---|
| | **1**, R = F | **2**, R = $CF_3$ | **3**, R = $CH_2CH_2CH_3$ |
| $K_i$, nM | < 1 | 80 | 5 |
| *Coagulation assays:* | | | |
| TT, $ID_2$ (μM)[a] | 119 | 2 | 0.2 |
| APTT, $ID_2$ (μM)[a] | 210 | 3 | 0.7 |

[a] $ID_2$: Concentration required to double the clotting time.

**Table 2.** Comparison of peptidylfluoroketones in rats

| | Methyl-(D)-Phe-Pro-Arg-$CF_2R$ | | |
|---|---|---|---|
| | **1** R = F | **2** R = $CF_3$ | **3** R = $CH_2CH_2CH_3$ |
| *Coagulation assays:* | | | |
| TT, $ID_2$ (μM)[a] | 47 | 4 | 0.2 |
| APTT, $ID_2$ (μM)[a] | 828 | 16 | 2 |
| *$FeCl_3$ arterial injury:* | | | |
| $ED_{2x}$ (nmol $kg^{-1}$ $min^{-1}$)[b] | > 1000 | 475 | 80 |

[a]$ID_2$: Concentration required to double the clotting time.
[b]$ED_{2x}$: Dose required to double the control time to occlusion.

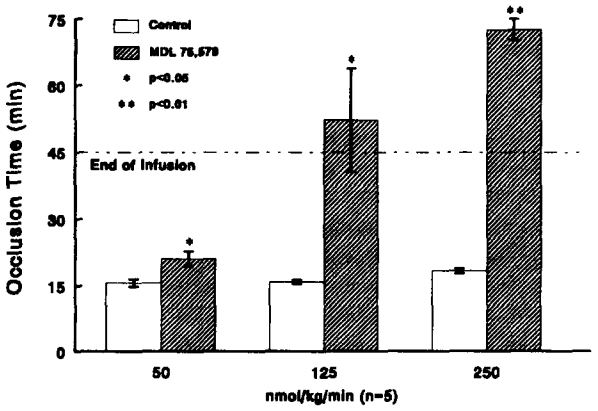



**Figure 1.** $FeCl_3$-induced rat artery thrombosis; prevention by MDL 75,579.

that the high carbonyl activity of the trifluoromethyl ketone MDL 73,446 (supported by the complete hydration of the ketone in aqueous solution) render this compound more reactive towards plasma constituents than do the corresponding pentafluoroethyl- and difluorobutyl ketones MDL 73,775 and MDL 75,579; a potential difference in metabolism and bioavailability could also be considered. However, there seems to be a correlation between the length of the ketone side-chain with anticoagulant activity. Anticoagulant activity of these tripeptidylfluoroketone analogues corresponds with antithrombotic efficacy in a platelet-dependent $FeCl_3$-induced rat arterial thrombosis model. The results of the present investigation show that the slow tight-binding active site inhibitor of thrombin, **3**, is a very effective anticoagulant and antithrombotic agent. MDL 75,579 demonstrates dose-dependent antithrombotic activity at all doses tested and is more effective than the other analogues in preventing occlusion in a rat thrombosis model.

Thrombin plays a critical role in triggering arterial thrombosis.[4] Specific inhibition of thrombin seems to be one of the best approaches for preventing thrombus growth. r-Hirudin has been shown to inhibit platelet deposition and thrombus formation on carotid arteries following balloon angioplasty.[18,19] Unlike heparin, which inhibits thrombin activity indirectly through antithrombin III, the major advantage of the specific thrombin blockers is their direct effect on thrombin.

This study provides evidence that in the $FeCl_3$-induced rat carotid artery injury model of thrombosis, thrombin plays a major thrombogenic role. We have tested the effects of MDL 75,579 as an anticoagulant and antithrombotic and demonstrated a correlation between both conditions. Clinically, plaque rupture or angioplasty are associated with severe arterial injury. In these situations, inhibition of locally generated thrombin might be the best strategy to prevent platelet-dependent arterial thrombosis. MDL 75,579, therefore, may be useful as a treatment for thrombosis.

## Experimental

### In vitro *assay of enzyme inhibition*

Human plasma thrombin (Sigma) activity was measured at 30 °C using Sarcosyl-prolyl-arginine *p*-nitroanilide as substrate[20] in 0.1 M Tris buffer (pH 7.5).[21] For rapid equilibrium inhibition, $K_i$ values were determined from a Dixon plot.[22] In the case of slow establishment of the equilibrium ENZFITTER (Biosoft) kinetic analysis was used; the $K_i$ values were determined according to Williams and Morrison.[23] $K_i$ values corresponding to a time-dependent inhibition were determined from a Kitz and Wilson plot.[24]

### *Antithrombotic activity*

Activated partial thromboplastin time (APTT) and thrombin times (TT) were measured semiautomatically using a MLA-Electra 750, MLA, Inc. (Pleasantville, NY). The concentration required for doubling the clotting time ($1D_2$) was calculated using simple linear regression. Rats were anesthetized with sodium pentobarbital (65 mg $kg^{-1}$, ip), a jugular vein cannulated for drug administration, and carotid arteries freed from surrounding tissue. The tracheae were cannulated (PE 240) and the rats placed on a heating pad (37 °C). In a well-defined technique,[17] occlusive carotid arterial thrombosis was detected by monitoring the rat carotid

arterial temperature with a thermistor probe (Yellow Springs Instruments, Model 427, Yellow Springs, OH) connected to Tele-Thermometer (YSI, series 400). Occlusive thrombosis occured with an abrupt drop in temperature of at least 2 °C and was continuously recorded on a strip chart recorder. Chemical injury to the exposed artery was accomplished by 5% $FeCl_3$ (in 1 N HCl). The time elapsed from application of the $FeCl_3$ solution until occlusive arterial thrombosis was indicated by an abrupt temperature fall on the strip chart. This time is termed the 'occlusion' time and is used for quantitation of antithrombotic effectiveness. The test was performed twice in each animal, first as a control in the right carotid artery, and, after treatment, in the left carotid artery. All results are expressed as mean values accompanied by the standard error (S.E.). The results were analyzed using the t-test. A $p$ value of $< 0.05$ was considered significant.

*General procedures*

All solvents were distilled prior to use. THF was distilled from $LiAlH_4$ and kept under an atmosphere of $N_2$. Diethylether was distilled from sodium-benzophenone-ketyl and kept under $N_2$. Melting points were determined on a Büchi melting point apparatus. Chemical shift indications refer to TMS references if not otherwise indicated. $^1H$ and $^{19}F$ NMR spectra were carried out on Bruker AC 200 and AM 360 spectrometers.

Abbreviations used: TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; THF, tetrahydrofuran; $Et_2O$, diethylether; AcOEt, ethylacetate; DMSO, dimethylsulfoxide; anh., anhydrous; PE petroleum ether (40–60 °C); PFPAA, pentafluoropropionic anhydride; PFPA, pentafluoropropionic acid; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; NMM, *N*-methylmorpholine.

*1,1,1-Trifluoro-(3,6-bis-benzamido)hexan-2-one hydrate (5a)*

A mixture of 4-(3-benzamidopropyl)-5(4*H*)-oxazolone, **4a** [6.44 g, 20 mmol, freshly prepared by the usual acetanhydride cyclization of α,δ-bis-benzoylamino-ornithine and TFAA (10 mL, 70 mmol)] was stirred under an atmosphere of $N_2$ for 24 h at room temperature. The acid formed and excess of TFAA were evaporated (0.1 Torr, $CO_2$-acetone traps). Last traces of the reagents were removed by this procedure on heating the residual oil to 60–70 °C (oil bath temperature). A solution of anhydrous oxalic acid (4.5 g, 50 mmol) in 20 mL of anhydrous THF was added to the residual red oil and the solution stirred until effervescence had completely stopped (24 h). The solvent was evaporated and the solid residue dissolved in $H_2O$/AcOEt (100 mL each). The organic layer was separated, the aqueous layer extracted with AcOEt (2 × 100 mL) and the combined organic phases washed with a saturated solution of $K_2CO_3$, water and brine. Drying of the organic layer ($MgSO_4$) and evaporation of solvent afforded 6.7 g (85%) of the trifluoromethyl ketone **5a** as a beige solid, consisting of 10% ketone and 90% hydrate form (see $^1H$ and $^{19}F$ NMR). $R_f$ = 0.6 (AcOEt:MeOH, 9:1). $^1H$ NMR ($CDCl_3$, DMSO-$d_6$) δ 8.3 (*m*, 1H, NH), 8.0 (*m*, 1H, NH), 7.9 (*m*, 4H, aroyl), 7.5 (*m*, 6H, aroyl), 4.9 (m) and 4.4 [badly resolved *t*, $J$ = 5 Hz, ratio 1:9, 1H, CHCO and $CHC(OH)_2$], 3.5 (*m*, 2H, $CH_2N$), 2.1 and 1.85–1.55 (2*m*, 4H, $2CH_2$). $^{19}F$ NMR ($CDCl_3$): δ 86.00 (*s*, $COCF_3$), 80.00 (*s*, $CF_3C(OH)_2$), ratio 1:9. UV (λ max): 268.4 (ε : 2385) and 225.7 (22795); $c$ = 54 μM in $CH_3CN$.

A small sample (100 mg) was allowed to crystallize from AcOEt/hexane to afford 90 mg of an analytically pure material. Anal. calcd for $C_{20}H_{21}F_3N_2O_4 \cdot H_2O$ (410.40): C, 58.53; H, 5.16; N, 6.83; found: C, 58.62; H, 5.22; N, 6.64. MS(CI) m/z = 393 $(M + H)^+$.

N,N'-*[1-(Pentafluoropropionyl)-1,4-butanediyl]-bis(benzamide) hydrate (5b)*

Pentafluoropropionic anhydride (11.9 mL, 19 g, 0.060 mol) was added under an atmosphere of $N_2$ to a well-stirred powder of 5(4*H*)-oxazolone **4a** (6.44 g, 0.020 mol). The resulting mixture was stirred at 40 °C for 16 h. Solvents were then evaporated at a final temperature of 55–60 °C (0.5–1 Torr, dry ice–acetone trap) for *ca* 6 h to give an orange oil. At this time 40 mL of a saturated solution of oxalic acid (15.0 g, 0.15 mol) in THF was added and the resulting orange solution stirred at 55 °C for 6 h when effervescence had totally stopped. The solvent was evaporated and the oily residue dissolved in AcOEt. This solution was stirred for 15 h at room temperature with a saturated solution of $KHCO_3$ (hydrolysis of some pentafluoropropionamide, which was formed as a side product during reaction). Phases were separated and the organic layer washed with $H_2O$, 1 N HCl and brine, dried ($MgSO_4$) and evaporated. The resulting orange oil was subjected to flash chromatography on $SiO_2$ (eluent AcOEt:PE, 1:3). Product containing fractions were evaporated to afford 7.1 g of the desired ketone **5b** (79%) as a white solid. $^1H$ NMR ($CDCl_3$): δ 8.1–7.8 (*m*, 4H, aryl), 7.7–7.4 (*m*, 7H, aryl, NH), 6.6 (*m*, 1H, NH), 5.3 (*m*, 1H, CHCO), 3.7 (broad *t*, 2H, $NCH_2$), 2.4–1.8 (*m*, 4H, $2CH_2$). $^{19}F$ NMR ($CDCl_3/C_6F_6$) δ 40.3 (*d*, $J$ = 7.5 Hz, $CF_2$), 80.0 (*s*, $CF_3$). MS ($CI/NH_3$) m/z = 443 $(M + H)^+$.

A small sample of **5b** (100 mg) was allowed to crystallize from AcOEt/PE to give analytically pure (80 mg) title compound. Anal. calcd for $C_{21}H_{19}O_3N_2F_5$ (442.39): C, 57.02; H, 4.33; N 6.33; found: C, 57.14; H, 4.23; N, 6.36.

N-*9-(Phenylmethoxycarbonyl)amino-6-benzamido-5-oxo-4,4-difluoro-1-nonene (5c)*

*2-Phenyl-[4-(3-phenylmethoxycarbonyl)aminopropyl]-5-(4H)-oxazolane (4b).*[25] A mixture of α-benzoyl-ω-Cbz-ornithine (30.6 g, 0.083 mol) and 200 mL of acetic anhydride was heated to 90 °C for 45 min under stirring.

The clear solution was allowed to cool to room temperature and solvents were evaporated (15 Torr, 40 °C, several times with $CCl_4$ as co-solvent). The oily residue was dissolved in anhydrous $Et_2O$ and the solution kept at 4 °C for 16 h. The resulting mixture was filtered and the filter-residue dried under vacuum to afford 28.2 g (97%) of the oxazolone **4b** as a white solid. $^1H$ NMR ($CDCl_3$) δ 8.0 (*d*, *J* = 8Hz, 2H, aroyl), 7.65–7.2 (*m*, 8H, aroyl, aryl), 5.1 (*s* with shoulder, 3H, aryl $CH_2$, NH), 4.4 (*m*, 1H, CH), 3.3 (*m*, 2H, $NCH_2$), 2.2–2.0 (*m*, 1H) and 1.95–1.6 (*m*, 3H, $CH_2$-$CH_2$). IR (KBr) 1820 $cm^{-1}$. Anal. calcd for $C_{20}H_{20}N_2O_4$ (352.39): C, 68.17; H, 5.72; N 7.95; found: C, 68.12; H, 5.80; N, 7.88.

*2,2-Difluoro-4-pentenoyl chloride.* Oxalylchloride (12.7 g, 0.1 mol) was slowly added to a stirred solution of 2,2-difluoro-4-pentenoic acid (13.0 g, 96 mmol) in 170 mL of anhydrous heptane. When the effervescence had stopped 2 drops of distilled DMF was added and the solution stirred for 2 h. Gentle heating (40–50 °C, for a short time period to avoid distillation) was applied to complete the reaction. An aliquot (0.5 mL) of the solution was taken for a $^{19}F$ NMR spectrum which indicated complete formation of the acid chloride. The acid chloride solution so prepared was used as such for the following reaction; it can, however, be stored in a freezer for several days. $^{19}F$ NMR (heptane, $C_6F_6$ as reference): δ 61.0 (*t*, *J* = 15 Hz).

*Intermediate O-difluoropentenoyl-oxazole.* The above prepared solution of 2,2-difluoro-4-pentenoyl chloride in heptane was added slowly under an atmosphere of $N_2$ to a cooled (0 °C) and well-stirred solution of the 5(4*H*)-oxazolone **4b** (28.0 g, 79.5 mmol) and $NEt_3$ (9.65 g, 95.4 mmol) in anhydrous THF (170 mL). The resulting mixture was stirred for 1 h at 0 °C, for 20 h at room temperature, and then filtered from $NEt_3$–HCl. The filtrate was evaporated under reduced pressure (0.1 Torr, $CO_2$-acetone trap to avoid contact with humidity) to give a yellow syrup. An aliquot (*ca* 20 mg) was taken for identification of the intermediate O-acyl-oxazole by $^{19}F$ and $^1H$ NMR. $^1H$ NMR ($CDCl_3$) δ 8.0 (*m*, 2H, aroyl), 7.6–7.2 (*m*, 8H, aroyl, aryl), 5.9–5.65 (*m*, 1H, CH), 5.5–5.0 (*m*, 5H, $CH_2$=, NH, aryl $CH_2$), 4.4 (*m*, *ca* 0.1–0.2H, $NCH_2$, $CF_2CH_2$), 2.5 (*t*, *J* = 5 Hz, 0.7–0.8H, part A of AB system of $CH_2$–C=C–oxazole), 2.2–1.6 (*m*, 3.1–3.2H, $CH_2$ + B part of AB system of $CH_2$–C=C–oxazole, -$CH_2$), 1.3 (*t*, *J* = 7 Hz, *ca* 1.5H-representing *ca* 10% of residual $NEt_3$). $^{19}F$ NMR ($CDCl_3$, $C_6F_6$) δ 55.33 (*t*, $J_{HF}$ = 15 Hz, $CF_2$-CO-oxazole), trace signal at 55.89 (*t*, $J_{HF}$ = 15 Hz, $CF_2$-acid or anhydride, *ca* 10% by integration).

A solution of 970 mg (8 mmol) of DMAP in 5 mL of anhydrous THF was added to the oxazole and the resulting viscous oil was stirred at room temperature for 16 h. A concentrated solution of anhydrous oxalic, (Aldrich, gold-label, 0.185 mol) in 50 mL of anhydrous THF was added to the syrup of the oxazolone and the resulting solution was stirred at 50 °C (bath temp.) for 60 h, when effervescence had completely stopped (*this operation should be performed in a well ventilated hood since CO is liberated*). The resulting mixture was dissolved in AcOEt and washed carefully with saturated solutions of sodium bicarbonate, 1 N HCl and brine. Drying of the organic solution ($MgSO_4$), filtration, and evaporation of the solvents afforded a yellow oil (33.0 g). Crystallization from AcOEt/pentane afforded 28.9 g (82%) of the desired difluoroketone analogue of ornithine **5c** as a white solid. $R_f$ = 0.5 (PE:AcOEt, 3:2). $^1H$ NMR ($CDCl_3$) δ 7.8 (*m*, 2H, aroyl), 7.6–7.4 (*m*, 3H, aroyl), 7.3 (*s*, 5H, aryl), 7.1 (*m*, 1H, NH), 5.9–5.1 (*m*, 1H, CH=), 5.35–5.15 (*m*, 2H, =$CH_2$), 5.1 (*s*, 2H, aryl $CH_2$), 4.95 (*m*, 1H, CHCO), 3.3 (*m*, 2H, $NCH_2$), 2.9 (*dt*, $J_{HF}$ = 15 Hz, $J_{HH}$ = 7 Hz, 2H, $CF_2CH_2$), 2.1 and 1.7 (2*m*, 4H, $CH_2$–$CH_2$). $^{19}F$ NMR ($CDCl_3$) δ ABX centered at 55.70: A 58.4 and B 53.0 ($J_{FA-FB}$ = 270 Hz, $J_{FA-HX}$ = $J_{FB-HX}$ = 15 Hz, $CF_2$). MS (CI/$NH_3$) m/z= 445 $(M + H)^+$. Anal. calcd for $C_{24}H_{26}F_2N_2O_4$ (444.48): C, 64.85; H, 5.90; N, 6.30; found: C, 64.56; H, 5.87; N, 6.07.

*1,1,1-Trifluoro-(3,6-bis-benzamido)-2-hydroxy-hexane (6a)*

$NaBH_4$ (280 mg, 7.5 mmol) was added to a stirred and cooled (0 °C) solution of the trifluoromethyl ketone **5a** (5.9 g, 15 mmol) in 150 mL of EtOH. The solution was allowed to warm to room temperature and stirred for 16 h. HCl (6 N) was carefully added (to a final pH of 1) and solvents evaporated to one third of the original volume. The solid residue was dissolved in water and the solution neutralized with a saturated solution of bicarbonate. Extraction of the aqueous solution with AcOEt, washing of the organic layer with water and brine, drying of the organic phase ($MgSO_4$), and evaporation of the solvent afforded 5.38 g (91%) of the trifluoromethyl alcohol **6a** as a white solid. The two diastereomers were present in a ratio of 60:40 (see $^{19}F$ NMR). $R_f$ = 0.6 (AcOEt). $^1H$ NMR (acetone-$d_6$, 90 MHz) δ 8.0 (*m*, 4H, aroyl), 7.5 (*m*, 6H, aroyl), 6.2 and 5.7 (2 m, 2H, 2NH), 4.7–4.0 (*m*, 1H, $CHCF_3$), 3.5 (*m*, 1H, CHN), 2.9 (*s*, 2H, $CH_2N$), 2.1–1.6 (*m*, 4H, $2CH_2$). $^{19}F$ NMR (acetone-$d_6$, $C_6F_6$, 88.4 MHz) δ 88.7 (*d*, *J* = 6 Hz) and 87.0 (*d*, *J* = 6 Hz); ratio 6:4. MS (CI) m/z = 395 $(M + H)^+$. Anal. calcd for $C_{20}H_{21}F_3N_2O_3$ (394.40): C, 60.76; H, 5.52; N, 7.05; found: C, 60.55; H, 5.55; N, 7.01.

*N,N'-[1-(2,2,3,3,3-Pentafluoro-1-hydroxypropyl)-1,4-butanediyl]-bis(benzamide) (6b)*

$NaBH_4$ (300 mg, 7.9 mmol) was added in one portion to a cooled (0 °C) and stirred solution of the pentafluoroethyl ketone **5b** (6.91 g, 15.6 mmol) in EtOH (90 mL). The mixture was allowed to warm up to room temperature and was further stirred (1 h). HCl (6 N) was added carefully until effervescence stopped. The solution was neutralized with solid $Na_2CO_3$ and EtOH evaporated. The resulting mixture was redissolved in AcOEt/$H_2O$ and the phases separated. The aqueous layer was extracted twice with AcOEt and the combined organic phases washed with $H_2O$ and brine. Drying ($MgSO_4$) and evaporation of solvents afforded a white solid, which was subjected to flash chroma-

tography on $SiO_2$ (eluents AcOEt:PE, 1:1 then 4:1). Product-containing fractions were evaporated to give 6.05 g (89%) of the desired alcohol **6b** as a white solid. $R_f$ = 0.45 (AcOEt:PE, 1:1); two badly separated spots for the two diastereoisomers. $^1$H NMR ($CDCl_3$, $CD_3OD$) δ 7.8–7.5 (*m*, 4H, aryl), 7.45–7.1 (*m*, 6H, aryl), 4.5 and 4.2 (2 *m*, 1H, CHOH, ratio 3:1), 3.5 (*m*, 4H, $2CH_2$). $^{19}$F NMR ($CDCl_3$, $CD_3OD$, $C_6F_6$) δ ABX system centered at 36.3; A: 40.3 ($J_{FA-FB}$ = 280 Hz, $J_{FA-HX}$ = 3 Hz), B: 32.3 ($J_{FB-FA}$ = 280 Hz, $J_{FB-HX}$ = 30 Hz, $CF_2$), 79.0 (*s*, $CF_3$) = diastereoisomer 1. ABX system centered at 35.3; A: 36.3; B: 33.3 (with equal coupling constants as mentioned above) = diastereoisomer 2; ratio 75:25. MS ($CI/NH_3$) m/z = 445 $(M + H)^+$. Anal. calcd for $C_{21}H_{21}O_3N_2F_5$ (444.40): C, 56.76; H, 4.76; N, 6.30; found: C, 56.94; H, 4.83; N, 6.29.

*N-9-(Phenylmethoxycarbonyl)amino-6-benzamido-5-hydroxy-4,4-difluoro-1-nonene (6c)*

Sodium borohydride (2.6 g, 68.5 mmol) was added to a cooled (0 °C) and stirred solution of the ketone **5c** (28.4 g, 63.9 mmol) in 2.7 L of EtOH in five portions. The solution was allowed to stir at room temperature for 4 h. 1 N HCl (65 mL) was carefully added to the mixture and the solution evaporated to one tenth of its original volume after complete addition. The mixture was dissolved in AcOEt and the solution washed with $H_2O$ and brine. The organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness. The solid residue was dissolved in a mixture of AcOEt/PE and left for 1 day at 4 °C. The crystals formed were collected by filtration and dried to give 27.12 g (95.3% yield) of the alcohol **6c** as a white solid. $R_f$ = 0.5 (PE:AcOEt, 1:1). $^1$H NMR ($CDCl_3$) : δ 7.8 (*m*, 2H, aroyl), 7.6–7.4 (*m* + *s*, 8H, aroyl, aryl), 6.9 (*m*, 1H, NH), 5.95–5.7 (*m*, 1H, –CH=), 5.3 (*m*, 2H, $=CH_2$) 5.1 (*s*, 2H, aryl $CH_2$), 4.4 (*m*, 1H, CHN), 4.0 and 3.9 (2 *m*, 1H, $CHCF_2$), 3.2 (*m*, 2H, $NCH_2$), 2.9–2.6 (*m*, 2H, $CF_2CH_2$), 1.8 (*m*, 2H) and 1.6 (*m*, 2H, $CH_2CH_2$). $^{19}$F NMR ($CDCl_3$) δ $ABX_2Y$ system centered at 53.9: A, 52.35 and B, 55.44 ($J_{FA-FB}$ = 270 Hz, $J_{FA-HX} = J_{FA-HY}$ = 17 Hz, $J_{FB-HX} = J_{FB-HY}$ = *ca* 1 Hz); 53.10 (badly resolved *dt*); the two diastereoisomers were present in a ratio of 80:20. MS (FAB) m/z = 447 $(M + H)^+$. Anal. calcd for $C_{24}H_{28}F_2N_2O_4$ (446.5): C, 64.56; H, 6.32; N, 6.27; found: C, 64.56; H, 6.45; N, 6.12.

*1,1,1-Trifluoro-2-hydroxy-3,6-diaminohexane dihydrochloride (7a)*

A solution of the above trifluoromethyl alcohol **6a** (3.94 g, 10 mmol) and 50 mL of HCl (12 N) was heated to reflux for 24 h. The solution was evaporated to dryness and the residue submitted to the above conditions for 24 h. After cooling to room temperature the solution was extracted with $Et_2O$ (3 × 100 mL) and the aqueous phase evaporated to dryness. The resulting oily residue was further dried under vacuum (0.1 Torr, 40 °C) to remove last traces of solvent. The semi-solid, hygroscopic material **7a** (2.6 g, 100%) was used as such for the following reaction. $^1$H NMR ($D_2O$) δ 4.0 (*m*, 1H, CHO), 3.2 (*m*, 1H, CHN), 2.7 (*m*, 2H, $CH_2N$), 1.4 (*m*, 4H, $2CH_2$). $^{19}$F NMR ($D_2O$, TFA) δ –1.0 (*d*, *J* = 12 Hz) and 1.1 (*d*, *J* = 12 Hz); ratio of the two diastereomers approximately 6:4. $R_f$ = 0.2 and 0.25 (BuOH:AcOH:$H_2O$ = 3:1:1).

*4,7-Diamino-1,1,1,2,2-pentafluoro-3-heptanol dihydrochloride (7b)*

A stirred solution of the above-prepared alcohol **6b** (5.91 g, 13.3 mmol) in 12 N HCl (200 mL) was heated under stirring to reflux while the progress of hydrolysis was followed by TLC (BuOH:$H_2O$:AcOH, 4:1:1). After 16 h solvents were evaporated and the oily residue subjected a second time to the above conditions. When the complete formation of the bisamino alcohol was indicated by TLC, the solution was cooled to room temperature and solvents evaporated. The oily residue was dissolved in $H_2O$ and the solution washed with $Et_2O$ (3 × 100 mL). The aqueous layer was evaporated to dryness to afford 3.98 g (quantitative yield) of the desired diamino alcohol **7b** as a brownish foam. $^1$H NMR ($D_2O$, 360 MHz) δ 4.7 and 4.6 (2 *d*, ratio 85:15, *J* = 20 Hz, 1H, $CHCF_2$), 3.6 (*m*, 1H, CHN), 3.1 (*m*, 2H, $NCH_2$), 2.0 (*m*, 4H, $2CH_2$). $^{19}$F NMR ($D_2O$, 338.8 MHz $C_6F_6$ ext. reference) δ 68.7 and 67.9 (2 *s*, $CF_3$, ratio 15:85) $F_{2AI}$: 30.0 and $F_{2AII}$: 30.7 (2 *d*, *J* = 51 Hz, $CF_{2A}$ both isomers), 21.7 and 19.5 (2 *d*, *J* = 51 Hz, $CF_{2B}$ both isomers), ratio 85:15.

*6,9-Diamino-5-hydroxy-4,4-difluorononane dihydrochloride (7c)*

A mixture of the protected alcohol **6c** (34.2 g, 0.077 mol), 800 mL of EtOH, 1.70 mL of 1 N HCl and 1.25 g of Pearlman's catalyst [$Pd(OH)_2$/C, 10%] was hydrogenated under a pressure of 3 bar for 16 h, when no further $H_2$-uptake was observed. Filtration from the catalyst and evaporation of solvents under reduced pressure (10 Torr, 30–35 °C) afforded 26.0 g of the intermediate benzamido alcohol as an oil. $R_f$ = 0.55 (EtOH:$NH_4OH$ 25% in $H_2O$, 4:1). $^1$H NMR ($CD_3OD$) δ 7.9 (*m*, 2H, aroyl), 7.5 (*m*, 3H, aroyl), 4.4 (*m*, 1H, CHN), 3.9 (*dt*, $J_{HF}$ = 17 Hz, $J_{HH}$ = 5 Hz, 1H, $CHCF_2$), 3.0 (*m*, 2H, $NCH_2$), 2.2–1.4 (2 *m*, 8H, $4CH_2$), 0.95 (*t*, *J* = 7 Hz, 3H, $CH_3$). $^{19}$F NMR ($CD_3OD$) δ $ABX_2Y$ system centered at 56.35: A, 57.35 and B, 55.45 ($J_{FA-FB}$ = 270 Hz, $J_{FA-HX} = J_{FA-HY}$ = 17 Hz, $J_{FB-HX} = J_{FB-HY}$ = 7 Hz). $ABX_2Y$ system centered at 54.62: A, 55.41 and B, 53.45 (coupling constants as above). The two AB systems were present in a ratio of 83:17. MS (ESI) m/z = 315.1 $(M + H)^+$. A solution of the above amino alcohol (26.0 g, 0.074 mol) in 500 mL of 12 N HCl was heated to reflux for 18 h and then allowed to cool to room temperature. The resulting mixture was filtered from some precipitated benzoic acid and the filtrate washed with $Et_2O$ (3 × 100 mL). The aqueous layer was heated to reflux for 1/4 h in the presence of charcoal. Filtration of the mixture and evaporation of the solution (10 Torr, 30–50 °C) afforded the title diaminoalcohol **7c** as a beige to brown solid (very hygroscopic): 21.7 g (100% yield), used as such for the following reaction.

$R_f$ = 0.36 and 0.23 (BuOH:AcOH:$H_2O$ = 7:2:1). $^1H$ NMR ($D_2O$) δ 4.0 and 3.9 (2 *t*, *J* = 4 Hz, 1H, $CHCF_2$), 3.5 (*m*, 1H, CHN), 2.9 (*m*, 2H, $NCH_2$), 2.0–1.5 (*m*, 6H, $3CH_2$), 1.3 (*m*, 2H, $CH_2$), 0.8 (*t*, *J* = 7 Hz, 3H, $CH_3$). $^{19}F$ NMR ($D_2O$): δ $ABX_2Y$ system centered at 54.47 : A, 55.20 and B, 53.73 (coupling constants as in compound **6c**). $ABX_2Y$ system centered at 53.10: A, 54.67 and B, 51.98 (coupling constants as in **6c**). The two diastereomers were present in a ratio of 82:18. MS (ESI) m/z = 211 $(M + H)^+$.

*1,1,1-Trifluoro-2-hydroxy-3-amino-6-((dimethylethoxy-carbonyl)amino)-hexane (8)*

A solution of di-*tert*-butyldicarbonate ($Boc_2O$, 2.18 g, 10 mmol) in 10 mL of THF was added dropwise to a cooled (0 °C) and well stirred solution of the di-aminoalcohol **7a** (2.60 g, 10 mmol) in THF/$H_2O$ (50 mL each). The solution was stirred at 0 °C for 1 h when TLC (AcOEt:AcOH, 96:4) displayed a new fast moving spot (bis-Boc derivative) with an $R_f$ = 0.9. Solvents were evaporated to one third of the original volume and the resulting solution acidified with solid citric acid and extracted with $Et_2O$. The aqueous layer was then basified with NaOH (pellets, final pH *ca* 13) and extracted exhaustively with $Et_2O$. The combined organic phases were washed with a saturated solution of $KHCO_3$, dried ($MgSO_4$) and solvents evaporated to afford 990 mg (47%) of the ε-Boc protected di-aminoalcohol **8** as a yellow oil. $R_f$ = 0.25 (AcOEt: AcOH, 96:4). $^1H$ NMR ($CDCl_3$, 90 MHz) δ 4.9–4.7 (*m*, 1H, NH), 4.0–3.5 (*m*, 1H, $CHCF_3$), 3.1 (*m*, 3H, CHN, $NCH_2$), 2.6 (broad *s*, 3H, OH, $NH_2$), 1.8–1.4 (*m*) and 1.5 (*s*, ε, 13H, $2CH_2$, *tert*-butyl). $^{19}F$ NMR ($CDCl_3$, $C_6F_6$, 88.4 MHz) δ 88.3 (*d*, *J* = 12 Hz) and 84.7 (*d*, *J* = 12 Hz, $CF_3$–CH); ratio of the two diastereomers *ca* 1:1. A small sample (100 mg) was allowed to crystallize (AcOEt/hexane) to give 50 mg of white crystals. Anal. calcd for $C_{11}H_{21}F_3N_2O_3$ (286.30): C, 46.15; H 7.39; N, 9.78; found: C, 46.25; H 7.62; N, 9.46.

*1,1,1-Trifluoro-2-hydroxy-3-(phenylmethoxycarbonyl-N-methyl-D-phenylalanyl)-prolinamide-6-dimethylethoxy-carbonylaminohexane 0.25 hydrate (9)*

Isobutylchloroformate (500 mg, 3.3 mmol) was added under an atmosphere of $N_2$ to a cooled (–10 °C) and well stirred solution of Cbz-*N*-Me-D-Phe-ProOH (1.23 g, 3 mmol) and NMM (335 mg, 0.33 mmol) in 15 mL of anhydrous THF. The resulting mixture was stirred at –10 °C for a further 30 min, when the Boc-protected diamino alcohol **8** was added. The mixture was stirred for 30 min at 0 °C and then stored at +4 °C for 16 h. THF was evaporated and the resulting oil dissolved in AcOEt. The solution was washed with saturated solutions of sodium bicarbonate, citric acid, water and brine, and dried ($MgSO_4$). Evaporation of solvents afforded a yellow oil (2.5 g) which was applied to flash chromatography on $SiO_2$ (230–400 mesh; eluents AcOEt:PE, 2:1). The pooled product containing fractions were evaporated to give 1.8 g (81%) of the fluorinated tripeptide alcohol **9** as a colourless foam. $R_f$ = 0.2 and 0.3 (AcOEt:PE, 1:1). $^1H$ NMR ($CD_3OD$, 360 MHz) δ 7.1–6.9 (*m*, 10H, 2 aryl), 5.1 (*m*, 2H, $CH_2O$), 4.9–3.3 (6 *m*, 4H, 4CH), 3.3–3.0 (*m*, 9H, $NCH_3$, $2CH_2N$, $CH_2$ aryl), 2.3–1.4 (*m*, 8H, $4CH_2$), 1.5 (*s*, 9H, *tert*-butyl). $^{19}F$ NMR ($CD_3OD$, $CF_3CO_2H$, 338.8 MHz) δ –0.3 and –0.2 (2 badly resolved *d*, *J* = 8 Hz), –0.4–0.6 (3 badly resolved *d*, *J* = 8 Hz), mixture of diastereomers and *cis/trans* isomers of Cbz-*N*-methyl-Phe. MS (EI) m/z = 678.9 $(M + H)^+$. Anal. calcd for $C_{34}H_{45}F_3N_4O_7 \cdot 0.25\ H_2O$ (683.26): C, 59.76; H, 6.78; N, 8.20; Found: C, 59.74; H, 6.70; N, 8.09.

*1,1,1-Trifluoro-2-hydroxy-3-(phenylmethoxy-carbonyl-N-methyl-D-phenylalanyl)-prolylamido-6-aminohexane trifluoroacetate hydrate (10)*

Trifluoroacetic acid (6 mL) was added to a stirred solution of the Boc-protected tripeptide analogue **9** (1.7 g, 2.5 mmol) in 6 mL of $CH_2Cl_2$. When effervescence had stopped the solution was evaporated to dryness (several times with $CH_2Cl_2$ as co-solvent) to afford a brownish oil. Precipitation with $Et_2O$/PE gave a beige solid, which gave after filtration and drying (0.1 Torr, 30 °C) 1.45 g of the desired ornithine analogue **10** (84%). $R_f$ = 0.7 (BuOH:AcOH:$H_2O$, 3:1:1). $^1H$ NMR ($CD_3OD$, 360 MHz) δ 7.4–7.2 (*m*, 10H, 2 aryl), 5.4–5.0 (*m*, 2H, $CH_2O$), 4.8–3.8 (4 *m*, 4H, 4CH), 3.5–3.0 (2 *m*, 9H, $2CH_2N$, $NCH_3$, $CH_2$ aryl), 2.3–1.7 (*m*, 8H, $4CH_2$). $^{19}F$ NMR ($CD_3OD$, $CF_3CO_2H$ ext. reference) δ –0.35 (*d*, *J* = 8 Hz), –0.6 (*m*), –0.7 (*d*, *J* = 8 Hz). Integration of this signals versus the signals of $CF_3CO_2H$ salt (–0.55, *s*) indicates a ratio of 1:1 for the two isomers. MS (ESI) m/z = 579.7 $(M + H)^+$. Anal. calcd for $C_{29}H_{37}F_3N_4O_5 \cdot CF_3CO_2H \cdot H_2O$ (710.68): C, 52.38; H, 5.63; N, 7.88; found C, 52.59; H, 5.60; N, 7.49.

N-*[(Phenylmethoxy)carbonyl]*-N-*methyl-D-phenylalanyl*-N-*[4-[N',N''-bis(phenylmethoxy)carbonyl]-aminoimino-methylamino]-1-[2,2,2-trifluoro-1-hydroxyethyl-butyl]-1-proline amide hemihydrate (11)*

A solution of the above prepared tripeptidyl trifluoromethyl alcohol **10** (1.39 g, 2 mmol), bis-Cbz-S-methyl-isothiourea (1.42 g, 4 mmol), and $NEt_3$ (1.25 g, 12 mmol) in 15 mL of anhydrous THF was stirred under an atmosphere of $N_2$ at 60 °C (bath temperature) for 72 h. The reaction flask was connected with a $KMnO_4$ trap to avoid pollution with the noxious gas produced. This trap was also used during evaporation of the solution. The residual oil was dissolved in AcOEt and the solution washed with $H_2O$, saturated solutions of sodium bicarbonate and citric acid. Drying ($MgSO_4$) and evaporation of solvents afforded an orange oil (2.5 g) which was applied to a flash chromatography column ($SiO_2$, 230–400 mesh, eluents AcOEt:PE, 1:1 to elute the guanylation reagent and then 3:1). Evaporation of the pooled product containing fractions afforded 1.2 g (68%) of the arginine analogue **11** as a colourless foam. $^1H$ NMR ($CDCl_3$, 360 MHz) δ 11.7 (*s*, 1H, NH), 8.3 (broad *s*, 1H, NH), 7.2 (*m*, 20H, 4 aryl), 5.6 (broad *s*, 1H, NH), 5.1 (*m*, 6H, $3CH_2O$), 4.7, 4.4 and 4.1 (3 *m*, 4H, 4CH), 3.6–2.9 (*m*, 9H, $3CH_2N$, $NCH_3$),

2.2–1.4 (*m*, 9H, $4CH_2$, OH). $^{19}F$ NMR ($CDCl_3$, $C_6F_6$, 338.8 MHz, $^1H$ broad-band decoupled) δ 87.5, 87.1, 87.0, and 86.9 (4 *s*, ratio 1:2:2:2). MS (FAB) m/z = 875.4 $(M + H)^+$. Anal. calcd for $C_{46}H_{51}F_3N_6O_9 \cdot 0.5H_2O$ (897.95): C, 61.53; H, 5.84; N, 9.36; Found: C, 6.53; H, 5.75; N, 9.27.

*N-[(Phenylmethoxy)carbonyl]-N-methyl-D-phenylalanyl-N-[4-[N',N''-bis(phenylmethoxy)carbonyl]-amino-iminomethylamino]-1-[2,2,2-trifluoroethanoylbutyl]-L-proline amide 1.5 hydrate (12)*

A solution of dimethylsulfoxide (304 mg, 3.9 mmol) in 5 mL of anhydrous $CH_2Cl_2$ was added slowly under an atmosphere of $N_2$ to a cooled (–65 °C) and stirred solution of oxalyl chloride (250 mg, 1.95 mmol) in 3 mL of $CH_2Cl_2$. After stirring of the solution for 5 min at –65 °C a solution of the alcohol **11** (1.2 g, 1.3 mmol) in 6 mL of $CH_2Cl_2$ was added. Stirring was continued while the reaction temperature was allowed to rise to –30 °C for 5 min and then adjusted to –55 °C for another 30 min. $NEt_3$ (660 mg, 6.5 mmol) was added slowly to keep the reaction trremperature at –55 °C. A solution of citric acid was added (5 mL) and the reaction mixture allowed to reach room temperature. Phases were separated and the aqueous layer extracted twice with $CH_2Cl_2$. The combined organic layers were washed with $H_2O$ and brine and dried ($MgSO_4$). Filtration and evaporation of the filtrate afforded a yellow foam (1.3 g) which was further purified by flash chromatography on $SiO_2$ (230–400 mesh, eluents AcOEt:PE, 1:1, then 2:1). The product containing fractions were combined and evaporated to yield 850 mg (74%) of the ketone **11** as a colourless foam. $R_f$ = 0.35 and 0.4 (AcOEt:PE, 1:1), 2 diastereomers. $^1H$ NMR ($CDCl_3$, 360 MHz) δ 11.7 (*s*, 1H, NH), 8.4 (*m*, 1H, NH), 7.3–7.1 (*m*, 20H, 4 aryl), 5.9 and 5.7 (2 *m*, 1H, NH), 5.1 (*m*, 7H, $3CH_2O$, NH), 4.8–3.8 (4 *m*, 4H, 4CH), 3.7–2.8 (*m*, 9H, $2CH_2N$ $CH_2$ aryl, $NCH_3$), 2.0–1.5 (*m*, 8H, $4CH_2$). $^{19}F$ NMR ($CDCl_3$, $C_6F_6$, 338.8 MHz) δ 90.5, 90.4 90.25 and 90.2 (4 *s*, $CF_3CO$), and 86.0, 85.95, 85.91, 85.88 and 85.70 [5 *s*, $CF_3$–$C(OH)_2$], diastereomers and *cis/trans* isomers around *N*-methyl Phe. Ratio of ketone:hydrate, 1:9. MS (FAB) m/z = 905.3 $(M + H)^+$. Anal. calcd for $C_{46}H_{49}F_3N_6O_9 \cdot 1.5H_2O$ (913.95): C, 60.45; H, 5.74; N, 9.20; found: C, 60.66; H, 5.59; N, 9.15.

*N-Methyl-D-phenylalanyl-N-[4-(aminoiminomethyl)-amino]-1-[(trifluoroethanoyl)-butyl]-L-proline amide dihydrochloride, trihydrate, MDL 73,446 (1),*

A solution of the ketone **12** (800 mg, 0.9 mmol), $Pd(OH)_2/C$ 10% (Pearlman's catalyst, *ca* 150 mg) and 3 mL of 1 N HCl in 30 mL of iso-propanal was hydrogenated (1 bar) for 48 h at room temperature. The mixture was filtered and the filtrate evaporated to dryness. The oily residue was dissolved in $H_2O$, filtered (Millipore® HVLP filter disk) and the filtrate lyophilized to yield 520 mg (95%) of the title compound as a colourless powder. $R_f$ = 0.6 ($BuOH:H_2O:AcOH$, 3:1:1). $^1H$ NMR ($D_2O$, TSP ext., 360 MHz) δ 7.5 (*m*, 3H, aryl), 7.4 (*m*, 2H aryl), 4.55 (*dd*, *J* = 9.8 and 5.4 Hz, 1H, CHPhe), 4.41 (*dd*) and 4.37 (*dd*, *J* = 9.8 and 5.4 Hz, 1H, α-CHO, both isomers), 4.22 (*m*) and 4.18 (*m*, 1H, α-CH-Arg, both isomers), 3.55 (*m*) and 3.49 (*m*, 1H, Hδ1 of Pro, both isomers), 3.38 and 3.35 (*dd*, *J* = 5.4 and 13.2 Hz, 1H, Hβ1-Phe, both isomers); 3.28 and 3.24 (2 *m*, 1H, Hδ-Arg, both isomers ), 3.15 and 3.14 (*dd*, *J* = 9.8 and 13.2 Hz, 1H, Hβ2-Phe, both isomers), 2.77 (*s*, 3H, $NCH_3$), 2.67 and 2.62 (*m*, 1H, H-δ-2, Pro, both isomers), 2.2–1.4 (5 *m*, 8H, $4CH_2$). $^{19}F$ NMR ($D_2O$, $CF_3CO_2H$, 338.8 MHz) δ –4.91 (*s*) and –5.08 (*s*), ratio 1:1 ($CF_3$). MS (FAB) m/z = 485 $(M + H)^+$. UV (λ max) 202.1 (ε = 17975), 194.0 (ε = 25920); c = 50 μM in $H_2O$. Anal. calcd for $C_{22}H_{31}F_3N_6O_3 \cdot 2HCl \cdot 3H_2O$ (611.49): C, 43.21; H, 5.93; N, 13.74; found: C, 42.89; H, 6.04; N, 13.37.

*4-Trifluoroacetylamino-7-amino-1,1,1,2,2-pentafluoro-3-heptanol trifluoroacetate (13)*

Trifluoroacetic anhydride (3.55 mL, 25 mmol) was added dropwise to a stirred solution of the diamino alcohol **7b** (3.3 g, 11 mmol) in 50 mL of trifluoroacetic acid. After 2 h stirring at room temperature, another 2.5 mL of TFAA was added to the solution and stirring continued for 10 h. The solution was evaporated to dryness, giving a brown oil. Trituration with $Et_2O$/PE afforded a brownish solid, which was filtered and washed with PE. Evaporation gave the title compound **13** (4.56 g, 95%), as a slightly coloured solid used as such for the following reaction. $^1H$ NMR ($D_2O$) δ 4.6 (*m*, 2H, 2CH), 3.1 (*m*, 2H, $NCH_2$), 2.0–1.7 (*m*, 4H, $2CH_2$). $^{19}F$ NMR ($D_2O$, reference $CF_3CO_2H$) δ ABX system centered at –49.00: A, –44.00 ($J_{FA-FB}$ = 280 Hz); B, –54.00 ($J_{FB-FA}$ = 280 Hz, $J_{FB-HX}$ = 30 Hz) = isomer 1; ABX centered at –49.33: A, 45.00; B, 40.67 (coupling constants as above) = isomer 2; ratio 4:1. MS ($CI/NH_3$) m/z = 333 $(M + H)^+$.

*9-Amino-6-trifluoroacetamido-5-hydroxy-4,4-difluorononane hydrochloride (14)*

Trifluoroacetic anhydride (17–65 mL, 0.125 mol) was added dropwise to a cooled (0 °C) and well-stirred solution of the bis-amino alcohol **7c** (14.2 g, 0.05 mol) in 150 mL of trifluoroacetic acid. After 2 h at room temperature the solution was heated to 40 °C (bath temp.) for 15 h. Solvents were evaporated (10 Torr, 40 °C) and the residual oil treated as above with TFAA and TFA (the $^1H$ NMR of an aliquot displayed residual starting material). This procedure was repeated a third time when no more starting material was displayed in the $^1H$ NMR spectrum (disappearance of the *m* at 3.5 ppm). The solution was evaporated to dryness and the residue dissolved in 1 N HCl to destroy the residual O-trifluoroacetyl ester. This was achieved by heating the solution for 2 h at 40 °C. Washing of the cooled solution with $Et_2O$ and evaporation of solvent afforded 14.6 g (85% yield) of the title compound as a beige solid. $R_f$ = 0.7 and 0.6 ($BuOH:AcOH:H_2O$, 3:1:1). $^1H$ NMR ($D_2O$) δ 4.0 (*m*, 1H, CHN), 3.75 and 3.65 (2 *t*, *J* = 5 Hz, 1H, $CHCF_2$), 2.8 (*m*, 2H, $NCH_2$), 1.9–1.2 (*m*, 8H,

$4CH_2$), 0.75 (*t*, $J$ = 7 Hz, 3H, $CH_3$). $^{19}F$ NMR ($D_2O$) δ 88.8 (several *s*, 3F, $CF_3$); $2ABX_2Y$ centers centered at 56.35 and 53.91 (2F, $CF_2$). The integration ratio of the two isomers was *ca* 80:20. All coupling constants are as indicated for the $^{19}F$ NMR spectrum of **6c**. MS (ESI) m/z = 348 $(M + H)^+$.

*N-[7-[N',N"-bis-(1,1-Dimethylethoxy)carbonyl]-aminoiminomethylamino]-N-(4-trifluoroacetylamino)-3-hydroxy-1,1,1,2,2-pentafluoroheptane hemihydrate (15)*

Bis-Boc-S-methylisothiourea[13] (7.3 g, 25 mmol) was added under an atmosphere of $N_2$ to a well-stirred solution of the TFA salt **13** (440 g, 10 mmol) and $NEt_3$ (3.5 mL, 25 mmol) in DMF (40 mL). The mixture was stirred at 40 °C for 60 h. Methanethiol was evaporated (a trap filled with an aqueous solution of $KMnO_4$/$Na_2CO_3$ was placed between the flask and the pump to avoid poisoning with the noxious gas), and $H_2O$ (200 mL) added to the solution. The solution was extracted with AcOEt (2 × 50 mL) and the combined organic phases washed with $H_2O$, saturated solutions of citric acid, $NaHCO_3$, and brine. Drying ($MgSO_4$) and evaporation of the solvent afforded an oil (8 g) which was subjected to flash chromatography on $SiO_2$ (eluents AcOEt:PE, 1:8, then 3:1). Product-containing fractions were evaporated to afford 4.02 g (70%) of the protected ω-guanidino-γ-amino alcohol **15** as a colourless foam. $^1H$ NMR ($CDCl_3$, 360 MHz) δ 11.20 (*s*, 1H, NH), 10.31 (*d*, $J$ = 10 Hz, 1H, NH–$COCF_3$), 9.73 (broad *s*, 1H, NH), 4.45 (*t*, $J$ = 9.5 Hz, 1H, CHN), 4.25 (*d*, $J$ = 22 Hz, $CHCF_2$), 3.75 (*m*, 1H, OH), 3.65 and 3.23 (2 *m*, 2H, $NCH_2$), 2.1 and 1.9 (2 m, 4H, $2CH_2$), 1.45 and 1.40 (2 *s*, 18H, 2 *tert*-butyl). $^{19}F$ NMR ($CDCl_3$, reference $C_6F_6$) δ ABX system centered at 35.50: A, 39.33, ($J_{FA-FB}$ = 277 Hz); B, 32.30 ($J_{FB-FA}$ = 277 Hz; $J_{FB-HX}$ = 22 Hz) = isomer 1; ABX system centered at 34.00: A, 40.05 ($J_{FA-FB}$) = 277 Hz); B, 27.5 ($J_{FB-FA}$ = 277 Hz, $J_{FB-HX}$ = 22 Hz) = isomer 2, ratio 4:1; 78.87 and 79.40 (2 *s*, ratio 4:1, $CF_3$); 86.12 and 86.53 (2 *s*, ratio 1:4, $CF_3CO$). Anal. calcd for $C_{20}H_{30}F_8N_4O_6 \cdot 0.5H_2O$ (574.47); C, 41.17; H, 5.36; N 9.60; found: C, 41.06; H, 5.15; N 9.57.

*N-9-[1-[3-[bis-[(1,1-Dimethylethoxy)carbonyl]amino]-methylene]amino]-6-trifluoroacetamido-5-hydroxy-4,4-difluorononane hemihydrate (16)*

A solution of the guanylation reagent bis-Boc-carboxamidino-1-pyrazol (12.1 g, 39 mmol) in 50 mL THF was added to a stirred solution of the TFA-protected amino alcohol **14** (12.1 g, 35.3 mmol) and $NEt_3$ (4.0 g, 39 mmol) in 200 mL of anhydrous THF. The pH of the solution was checked (pH 9) by dipping an aliquot of the solution on a wet pH paper. The solution was stirred at room temperature for 20 h. The solvent was evaporated and the residual oil dissolved in AcOEt. Washing of the solution with an aqueous solution of citric acid and brine, drying of the organic layer over $MgSO_4$, filtration, and evaporation of solvents afforded 22.5 g of crude title compound as an oil. Flash-chromatography of the oil ($SiO_2$, 230–400 mesh; eluents, PE:AcOEt, 9:1, then 8:2) and evaporation of the pooled product containing fractions afforded 13.2 g (68.1% yield) of the fully protected difluoro-arginine analogue **16** as an oil. $R_f$ = 0.35 (PE:AcOEt, 3:1). $^1H$ NMR ($CDCl_3$) δ 11.5 (*m*, 1H, NH), 9.4 (*d*, $J$ = 7.5 Hz, 1H, NH), 5.6 (*m*, 1H, NH), 4.4 (*m*, 1H, CHN) 3.85 (*ddd*, $J_{HF}$ = 17 Hz, $J_{HH}$ = 7Hz, $J_{HH}$ = 2 Hz, 1H, $CHCF_2$), 3.6 (*m*, 1H, OH), 3.45–3.1 (*m*, 2H, $NCH_2$), 2.1–1.4 (*m* + broad, 26H, 2 *tert*-butyl + $4CH_2$), 0.95 (*t*, $J$ = 7 Hz, 3H, $CH_3$). $^{19}F$ NMR ($CDCl_3$) δ 85.85–86.15 (4 *s*, 3F, $CF_3$), 2 $ABX_2Y$ systems centered at 52.48 and 50.06 (2F, $CF_2$). Coupling constants and relative chemical shifts as indicated for the $^{19}F$ NMR spectrum of **6c**. MS (ESI) m/z = 549 $(M + H)^+$. Anal. calcd for $C_{22}H_{37}F_5N_4O_6 \cdot 0.5H_2O$ (547.55): C, 47.39; H, 6.87; N 10.05; found C, 47.08; H, 6.61; N 9.95.

*N-[7-[N',N"-bis-(1,1-dimethylethoxy)carbonyl]-[(aminoiminomethyl)amino]-4-amino-3-hydroxy-1,1,1,2,2-pentafluoroheptane (17)*

A freshly prepared aqueous solution of LiOH (1 N, 7 mL) was added to a stirred solution of the trifluoroacetamide **15** (3.0 g, 5.2 mmol) in THF:$H_2O$ (9:1, 50 mL). The solution was stirred at room temperature for 20 h, when starting material had disappeared (TLC, AcOEt:PE, 1:5). THF was evaporated and the aqueous solution extracted with $Et_2O$ (4 × 50 mL). The combined extracts were washed with water and brine and dried ($MgSO_4$). Evaporation of the solvent afforded 2.18 g (88%) of the aminoalcohol **17** as a white solid. $^1H$ NMR ($CDCl_3$) δ 11.5 (*m*, 1H, NH), 8.4 (*m*, 1H, NH), 4.1 and 3.9 (2 *m*, 1H, $CHCF_2$), 3.5 and 3.2 (2 m, 3H, CHN, $NCH_2$), 2.5 (*m*, 2H, $NH_2$), 1.9–1.4 (*m*, 4H, $2CH_2$), 1.50 (*s*, 18H, 2 *tert*-butyl). $^{19}F$ NMR ($CDCl_3$) δ ABX system centered at 37.3: A, 43.67, ($J_{FA-FB}$ = 280 Hz); B, 30.9 ($J_{FB-FA}$ = 280 Hz, $J_{FB-HX}$ = 22 Hz, $CF_2$, isomer 1), 79.0 (*s*, $CF_3$) and ABX system centered at 39.0 (coupling constants as above), ($CF_2$ isomer 2), 79.5 (*s*, $CF_3$); ratio 4:1. MS (CI/$NH_3$) m/z = 479 $(M + H)^+$. Anal. calcd for $C_{18}H_{31}F_5N_4O_5$ (478.46); C, 45.19; H, 6.53; N, 11.71; found: C, 45.13; H, 6.44; N, 11.56.

*N-9-[1-[3-[bis-[(1,1-dimethylethoxy)carbonyl]amino]-methylene]amino]-6-amino-5-hydroxy-4,4-difluorononane (18)*

LiOH 1 N (36 mL, 36 mmol) was added slowly to a solution of the trifluoroacetamido arginine analogue **16** (13.2 g, 24.0 mmol) in 250 mL THF. The solution was stirred overnight at room temperature and 4 h at 40 °C. THF was evaporated and the residual solution extracted with $Et_2O$ (2 × 250 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated to afford 10.4 g of the fluorinated amino alcohol **18** as a white solid (95.5% yield). $R_f$ = 0.5 and 0.4 (BuOH:AcOH:$H_2O$, 3:1:1). $^1H$ NMR ($CDCl_3$) δ 11.4 (*m*, 1H, NHBoc), 8.3 (*m*, 1H, NH), 3.6 (*t*, $J$ = 5 Hz, 0.5H, CH–$CF_2$), 3.4 (*m*, 2.5H, $CHCF_2$, $NCH_2$), 2.9 (*m*, 1H, NCH), 2.0–1.0 (*m*, 26H, 2 *tert*-butyl, $4CH_2$), 0.90 and 0.80 (2 *t*, $J$ = 7 Hz, 3H, $CH_3$, ratio *ca* 8:2). $^{19}F$ NMR

($CDCl_3$) δ 2 $ABX_2Y$ systems centered at 53.9 and 49.8, coupling constants as indicated for **6c**. MS (ESI) m/z = 453.3 $(M + H)^+$. Anal. calcd for $C_{20}H_{38}F_2N_4O_5 \cdot 0.25H_2O$ (461.55): C, 52.00; H, 8.45; N, 12.19; found: C, 51.94; H, 8.20; N, 12.17.

*N-[(1,1-Dimethylethoxy)carbonyl]-N-methyl-D-phenylalanyl-N-[4-[N',N''-bis-(1,1-dimethylethoxy)carbonyl]-aminoiminomethylamino]-1-[3,3,3,2,2-pentafluoro-1-hydroxypropyl]-L-proline amide hydrate (19)*

Dicyclohexylcarbodiimide (970 mg, 4.7 mmol) was added to a stirred and cooled (0 °C) solution of Boc-*N*-methyl-D-Phe-Pro-OH (1.78 g, 4.7 mmol) and HOBt (711 mg, 4.7 mmol) in $CH_2Cl_2$ (50 mL). The mixture was stirred for 30 min at 0 °C, when the above-prepared amino alcohol **17** (2.10 g, 4.5 mmol) and NMM (0.52 mL, 4.7 mmol) were added. The resulting mixture was further stirred for 0.5 h at 0 °C and then allowed to warm up to room temperature. Stirring was continued for 16 h at room temperature. Filtration of the precipitated DCU and washing of the filtrate with saturated solutions of citric acid, $KHCO_3$, and brine was followed by drying ($MgSO_4$) and evaporation of the solvent to afford a viscous oil. Flash chromatography on $SiO_2$ (eluents AcOEt:PE, 1:1) and evaporation of the product-containing fractions afforded 3.09 g (82%) of the tripeptide alcohol **19** as a colourless oil. $^1H$ NMR ($CD_3OD$, 360 MHz) δ 7.2 (*m*, 5H, aryl), 5.1–4.9 (2 *m*, 1H, CH–Phe), 4.4–3.9 (*m*, 3H, CHPro, $CHCF_2$, CHN), 3.7–3.3 and 3.2–2.9 (2 *m*, 6H, $2NCH_2$, $CH_2$ aryl), 2.85–2.7 (5 *s*, 3H, $NCH_3$), 2.3–1.6 (*m*, 8H, $4CH_2$), 1.5–1.1 (6 *s*, 27H, 3 *tert*-butyl). $^{19}F$ NMR ($CD_3OD$, $C_6F_6$ ext. reference, 360 MHz) δ 3ABX-systems centered at 43.7 ($CF_2$, isomer 1 and 2; *cis/trans*-isomers) and 81.80, 81.55, 81.30 (3 *s*, $CF_3$ of different isomers). MS (FAB) m/z = 837 $(M + H)^+$. Anal. calcd for $C_{38}H_{57}F_5N_6O_9$ (836.90): C, 54.55; H, 6.86; N, 10.04; found: C, 54.64; H, 7.21; N, 10.14.

*L-Prolinamide [(1,1-dimethylethoxy)carbonyl]-N-methyl-D-phenylalanyl-N-[1-3[[bis-[[(1,1-dimethylethoxy)carbonyl]amino]methylene]-amino]propyl]-3,3-difluoro-2-hydroxyhexyl]hemihydrate (20)*

DCC (4.08 g, 19.8 mmol) was added to a cooled (0 °C) and stirred solution of Boc-*N*-Me-D-Phe-Pro-OH (7.95 g, 19.8 mmol) and HOBt (2.67 g, 19.8 mmol) in 250 mL of $CH_2Cl_2$. Precipitation of DCU started after *ca* 5 min and the mixture was stirred at 0 °C for a further 45 min. The difluoroamino alcohol **18** (8.8 g, 19.4 mmol) and NMM (2.03 mL, 19.8 mmol) were added and the mixture stirred at room temperature for 20 h. The mixture was filtered and the filtrate evaporated. The oily residue was dissolved in AcOEt (250 mL) and the solution washed with saturated solutions of citric acid, sodium bicarbonate, sodium chloride, and dried ($MgSO_4$). Filtration of the mixture and evaporation of the solvents afforded a yellow oil (16.9 g) which was applied to a column for flash chromatography ($SiO_2$, 230–240 mesh, 1 kg; eluents AcOEt:PE, 2:3 and then 1:1). Analytical HPLC was applied for purity control of the different fractions ($T_r$ = 8.5 min, isomer I; $T_r$ = 8.8 min, isomer II). Product containing fractions (40 fractions of 50 mL) were evaporated to afford 14.2 g (90.3%) of the desired tripeptide analogue **21** as a foam; $R_f$ = 0.25–0.3 (AcOEt:PE, 2:3). $^1H$ NMR (360 MHz, $CD_3OD$) δ 7.3 (*m*, 5H, $C_6H_5$), 5.1 (*m*, 1H, α-CH-phenylalanine), 4.5–4.0 (*m*, 2H, α-CH-proline, CH-OH), 3.9–3.3 and 3.2 (2 *m*, 10H, $CH_2$-phenyl, $CH_2$-N-proline, $CH_2$-N-gua, $CH_3N$, CH-N), 2.2–1.5 (*m*, 12H, $6CH_2$), 1.5–1.2 (3 *br s*, 27H, 3 *tert*-butyl), 0.9 (*dt*, 3H, *J* = 7 Hz, *J* = 2 Hz, $CH_3$). $^{19}F$ NMR (338.8 MHz, 1H-broad band decoupled) δ badly resolved AB systems centered at 55 ppm ($J_{FF}$ = 254 Hz, 2 diastereoisomers with their corresponding *cis/trans* isomers of Boc-*N*-methyl-D-phenylalanine and prolinamide). MS (FAB) m/z = 811 $(M + H)^+$. Anal. calcd for $C_{40}H_{64}F_2N_6O_9 \cdot 0.5H_2O$ (819.99): C, 58.59; H, 7.99; N, 10.26; found: C, 58.65; H, 7.84; N, 10.13.

*N-[1,1-(Dimethylethoxy)carbonyl]-N-methyl-D-phenylalanyl-N-[4-[N',N''-bis-(1,1-dimethylethoxy)carbonyl]-(aminoiminomethyl)amino]-1-[(2,2,3,3,3-pentafluoropropionyl)butyl]-L-proline amide hydrate (21)*

A 100-mL three-necked flask equipped with a magnetic stirring bar, thermometer, and a $N_2$-inlet was charged with a solution of oxalylchloride (0.52 mL, 5.7 mmol) in 5 mL of anhydrous $CH_2Cl_2$. After cooling the solution to –60 °C a solution of DMSO (1.2 mL, 14.3 mmol) in 10 mL of anh. $CH_2Cl_2$ was added at a rate to keep internal temperature at –55 °C. The mixture was stirred for 15 min at –55 °C, when the alcohol **19** (3.0 g, 3.58 mmol) in 20 mL of anh. $CH_2Cl_2$ was added dropwise. After complete addition the cooling bath was removed and stirring continued until the internal temperature reached –20 °C. Stirring was continued for *ca* 5 min and the solution cooled again to –55 °C. $NEt_3$ (2.5 mL, 17.8 mmol) was added at a rate to keep the internal temperature at –55 °C. After complete addition stirring was continued for 15 min at –55 °C and a saturated citric acid solution (10 mL) was added. The mixture was allowed to warm up to room temperature and 200 mL of $CH_2Cl_2$ was added. Phases were separated and the organic layer washed with $H_2O$, a saturated solution of $NaHCO_3$, and brine. Drying ($MgSO_4$) and evaporation of solvents gave a colourless oil (*ca* 3 g) which was subjected to flash chromatography on $SiO_2$ (eluent AcOEt:PE, 1:2, then 1:1, then 2:1). Product-containing fractions were evap-orated to obtain 2.2 g (73%) of pure pentafluoroethyl ketone **21** as a colourless foam. $^1H$ NMR ($CDCl_3$, 338.8 MHz) δ 11.5 (*m*, 1H, NH), 8.5 (*m*, 1H, NH), 7.8 (*m*, 1H, NH), 7.2 (*m*, 5H, aryl), 5.1–4.3 (*m*, 3H, α-CH-Phe, α-CH-Pro, α-CHCO), 3.7–3.1 and 3.1–2.6 (2 *m*, 9H, $2NCH_2$, $CH_2$-aryl, $NCH_3$), 2.2–1.6 (*m*, 8H, $4CH_2$), 1.5–1.2 (*m*, 27H, $9CH_3$). $^{19}F$ NMR ($CDCl_3$, 338.8 MHz) δ 40.33 and 40.19 (2 *s*, $CF_2CO$), ABX systems centered at 39.0 ($CF_2$), 80.0 (*s*, $CF_3$), 82.7 and 82.9 (2 *s*, $CF_3$), ratio 4:1. MS (ESI) m/z = 836.4 $(M + H)^+$. Anal. calcd for $C_{38}H_{55}O_9N_6F_5 \cdot 1.5H_2O$ (61.88): C, 52.90; H, 6.74; N, 9.85; found : C, 52.91; H, 6.78; N, 9.70.

*L-Prolinamide [(1,1-dimethylethoxy)carbonyl]-N-methyl-D-phenylalanyl-N-[1-3[[bis-[[(1,1-dimethylethoxy)carbonyl]amino]methylene]-amino]propyl]-3,3-difluoro-2-oxohexyl]hemihydrate (22)*

A solution of DMSO (anh., 2.31 g, 29.8 mmol) in 5 mL of $CH_2Cl_2$ was added under an atmosphere of $N_2$ to a cooled (–65 °C) and well-stirred solution of oxalylchloride (1.87 g, 14.8 mmol) in 10 mL of $CH_2Cl_2$. Stirring was continued for 15 min at –55 °C and the alcohol **20** (4.0 g, 4.93 mmol) in 25 mL of $CH_2Cl_2$ was added. While stirring the mixture was allowed to reach –40 °C for 30 min and cooled to –50 °C again. Stirring was continued for 30 min when $NEt_3$ (4.03 g, 40 mmol) was added at a rate to keep the temperature of the reaction mixture between –50 and –45 °C. Stirring was continued for 15 min and a solution of citric acid was added; the mixture was allowed to warm up to room temperature. Phases were separated and the aqueous phase extracted twice with $CH_2Cl_2$ (50 mL). The combined organic phases were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated to afford a colourless foam (4 g) which was applied to flash chromatography on $SiO_2$ (230–240 mesh, eluent AcOEt:PE, 2:3). Purity control of the different fractions was again performed by analytical HPLC ($T_r$ = 8.9 and 12.3 min, isomer I, hydrate and ketone form; $T_r$ = 9.2 and 13.3 min, isomer II, hydrate and ketone form). The product-containing fractions were pooled and solvents evaporated to give 2.82 g of the desired ketone **22** (70.7%) as a white foam; $R_f$ = 0.36 and 0.50 (AcOEt:PE, 2:3), two diastereoisomers, ratio *ca* 1:1. $^1H$ NMR (360 MHz): δ 11.50 (*d*, *J* = 5 Hz, 1H, NH-Boc), 9.40 (*d*, *J* = 6 Hz, 1H, NH), 7.60 and 7.2 (2 *d*, *J* = 5 Hz, NH-CHCO, 2 isomers), 5.0 and 4.5 (2 *m*, 3H, 3 α-CH), 3.6–2.7 (*m*, 9H, $2CH_2N$, $CH_2$-$C_6H_5$, $CH_3N$), 2.3–1.7 (*m*, 8H, $4CH_2$), 1.7–1.1 (*m*, 31H, 3 *tert*-butyl, $2CH_2$), 0.95 (*t*, *J* = 7 Hz, $CH_3$). $^{19}F$ NMR (338.8 MHz, 1H-broad band decoupled) δ 3 AB systems centered at: 55.22, 55.04 and 55.02, ratio 2:1:1 ($J_{FF}$ = 271 Hz). MS (FAB) m/z = 809.6 $(M + H)^+$. Anal. calcd for $C_{40}H_{62}F_2N_6O_9 \cdot 0.5H_2O$ (817.90): C, 58.74; H, 7.76; N 10.27; found: C, 59.13; H, 7.78; N, 10.28.

*N-Methyl-D-phenylalanyl-N-[4-(aminoiminomethyl)-amino]-1-[3,3,3,2,2-pentafluoro-1-oxopropyl]-L-prolinamide dihydrochloride hydrate MDL 73,775 (2)*

900 mg (1.07 mmol) of the above-prepared protected tripeptide derivative **21** was dissolved in 10 mL of anhydrous $Et_2O$. 50 mL of a saturated HCl gas/$Et_2O$ solution was added and the resulting solution stirred at room temperature for 48 h. PE (*ca* 100 mL) was added and the precipitate filtered under $N_2$. Drying of the filter residue (0.1 Torr, 40°C) afforded 600 mg of a white amorphous powder. The filtrate was then lyophilized to give 550 mg (82%) of the title compound as a white fluffy powder. $^1H$ NMR ($D_2O$) δ 7.55 (*m*, 3H, aryl), 7.30 (*m*, 2, aryl), 4.55 (*m*, 1H, CH-Phe), 4.38 (*m*, 1H, CH-Pro), 4.27 [*m*, 1H, CHN-$C(OH)_2$], 3.50 (*m*, 1H, $HCH_A$-Pro), 3.37 (*m*, 1H, $C_6H_5CH_A$), 3.25 (*m*, 2H, $NCH_2$-Gua), 3.15 (*m*, 1H, $C_6H_5CH_B$), 2.75 (*s*, broad, 3H, $CH_3N$), 2.73–2.63 (*m*, 1H, $NCH_B$-Pro), 2.2–1.4 (*m*, 8H, $4CH_2$). $^{19}F$ NMR ($D_2O$, $CF_3CO_2H$ ext. reference) δ –3.65 and –3.71 (2 *s*, ratio 45:55, 3F, $CF_3$), AB system centered at –47.80: A, –47.44 ($J_{FA-FB}$ = 281 Mz); B, –48.16 ($J_{FB-FA}$ = 281 Hz), AB system centered at –48.09: A, –47.64 ($J_{FA-FB}$ = 281 Hz); –48.53 ($J_{FB-FA}$ = 281 Hz), = $CF_2$ of the two diastereoisomers, ratio 45:55. MS (FAB) m/z = 535 $(M + H)^+$. Anal. calcd for $C_{23}H_{31}F_5N_6O_3 \cdot 2HCl \cdot H_2O$ (625.47): C, 44.17; H, 5.64; N, 13.44; found: C, 44.38; H, 5.55; N, 13.26.

*L-Prolinamide N-methyl-D-phenylalanyl-N-[1-[3-[(amino-iminomethyl)amino]propyl]-3,3-difluoro-2-oxohexyl]-di-hydrochloride 0.75 hydrate MDL 75,579 (3)*

A saturated solution of HCl gas in $Et_2O$ (~4 M, 250 mL) was added to a solution of the Boc-protected tripeptide ketone **22** (1.64 g, 2 mmol) in 5 mL of $Et_2O$ and the solution was stirred for 40 h at room temperature when a precipitate was formed. The precipitate was collected by filtration, washed with anhydrous $Et_2O$ (2 × 50 mL) and dried under vacuum to afford 1.1 g of the tripeptide analogue **1** as a white powder (hygroscopic); $R_f$ = 0.4–0.5 ($BuOH:H_2O:AcOH$, 3:1:1). This product was dissolved in 50 mL of water, washed 2 times with 50 mL of $Et_2O$ and filtered through a Millipore® filter unit (HVLP). Lyophilization of the filtrate gave 960 mg (78%) of the title ketone as a white solid. The product consists of 23% ketone and 77% of the hydrated form of the ketone. MS (FAB) m/z = 509 $(M + H)^+$. $^1H$ NMR (360 MHz, $D_2O$) δ 7.4 and 7.3 (2 *m*, 5H, $C_6H_5$), 4.9 (*m*, 0.2H, $CHCO-CF_2$) and 4.10 (*dd*, *J* = 13 Hz, *J* = 3 Hz, 0.8H, $CHC(OH)_2-CF_2$), 4.55 (*dd*, *J* = 9 Hz, *J* = 5 Hz, 1H, α-CH-phenylalanine), 4.35 (*m*, 1H, α-CH-proline), 3.5 and 2.6 (2m, 2H, $CH_2$-N-proline, HA and HB), ABX system centered at 3.25 ($J_{HA-HB}$ = 13 Hz, $J_{HA-HX}$ = 6 Hz), 3.3 (*m*, 2H, $CH_2$-N), 2.70 (*s*, 3H, $CH_3N$), 2.2–1.4 (*m*, 12H, $6CH_2$), 0.95 (2 *t*, 3H, *J* = 7 Hz, $CH_3$). $^{19}F$ NMR (338.8 MHz, $D_2O$, 1H-broad band decoupled, $CF_3CO_2H$) δ 2 AB systems for the two diastereomeric ketones centered at –30.66 and –30.64 ($J_{FA-FB}$ = 262 Hz). 2 AB systems for the ketone-hydrates centered at –36.50 and –37.03 ($J_{FF}$ = 248 Hz). Ratio of ketone isomers 50:50; ratio of hydrate isomers 53:47; ratio for ketone:hydrate 23:77. Anal. calcd for $C_{25}H_{38}F_2N_6O_3 \cdot 2HCl \cdot 0.75H_20$ (595.05): C, 50.46; H, 7.03; N, 14.12; found: C, 50.69; H, 7.04; N, 14.28.

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