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Fluorinated peptides incorporating a 4-fluoroproline residue as potential inhibitors of HIV protease

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Abstract

N-Fmoc-4-fluoro-L-proline methyl ester was prepared as an attractive synthon for both solid and solution phase peptide synthesis. Its use for the synthesis of Fmoc–Phe–Pro(F)–OMe and Fmoc–Pro(F)–Val–Val–OMe is presented. Direct fluorination with DAST of a 4-hydroxy proline residue incorporated into a peptide and elongation from the terminal amino group allowed preparation of the hexapeptide Boc–Ala–Ala–Phe–Pro(F)–Val–Val–OMe, analogous to the p17–p24 gag junction of structural HIV proteins. None of the fluoropeptides in the paper displayed anti-protease or anti-HIV activity.

Keywords: Monofluorinated proline; Fluorinated peptides; HIV-1 protease; Inhibitors

1. Introduction

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS) [1]. During the replication cycle of HIV, gag and gag–pol gene products are translated as polyproteins. These are subsequently processed by a virally encoded protease to yield structural proteins p17, p24, p9 and p7 of the virus core, together with essential viral enzymes including the protease itself (integrase, reverse transcriptase, protease) [2,3]. Inactivation of this protease results in the production of non-infectious virus and consequently inhibition of the spread of viral infection in susceptible cells. Inhibition of this enzyme provides an attractive therapeutic goal in the treatment of AIDS [4,5]. An interesting feature of the HIV protease is its specificity for the unusual Tyr/Phe–Pro bond (uncleaved by mammalian endopeptidases) and its ability to cleave at the N-terminus of proline. Several analogues of HIV protease substrates, in which the fissile Phe–Pro amide bond is substituted for non-hydrolyzable and isosteric moieties, have been reported. These groups mimic the tetrahedral transition state and some of them lead to in vitro and in vivo potent inhibitors of the HIV protease [6–11]. Our hypothesis was that substituting a fluorine atom for one hydrogen atom into a peptide substrate of the HIV protease was likely not to affect its recognition by the enzyme, the two atoms having approximately the same Van der Waals radius ($R_F = 1.35$ Å and $R_H = 1.20$ Å). On the other hand, the high electronegativity and the powerful electron withdrawing effect of a fluorine atom close to the peptide bond normally cleaved by the enzyme was expected to have a pronounced effect on the electron density of the fissile Phe–Pro bond. Thereby, the enzymatic hydrolysis mechanism may be disturbed, either during the first step of the formation of the tetrahedral intermediate by hydration of the fissile amide bond, or during its decomposition. We present here the synthesis and the biological evaluation of a series of peptides incorporating the 4-fluoro-L-proline residue and, in particular, of the Boc–Ala–Ala–Phe–Pro(F)–Val–Val–OMe hexapeptide **20**, which is an analogue of the p17–p24 gag junction, incorporating a 4-fluoro-L-proline residue at the Phe–Pro bond recognized by the HIV protease.

2. Results

Two pathways have been tested for the synthesis of Boc–Ala–Ala–Phe–Pro(F)–Val–Val–OMe **20**. The first (*pathway A*) is the incorporation of a 4-fluoroproline residue into a peptide, which can be performed at different stages of the peptide synthesis. The second (*pathway B*) is the direct fluorination of a 4-hydroxyproline residue incorporated into a peptide.

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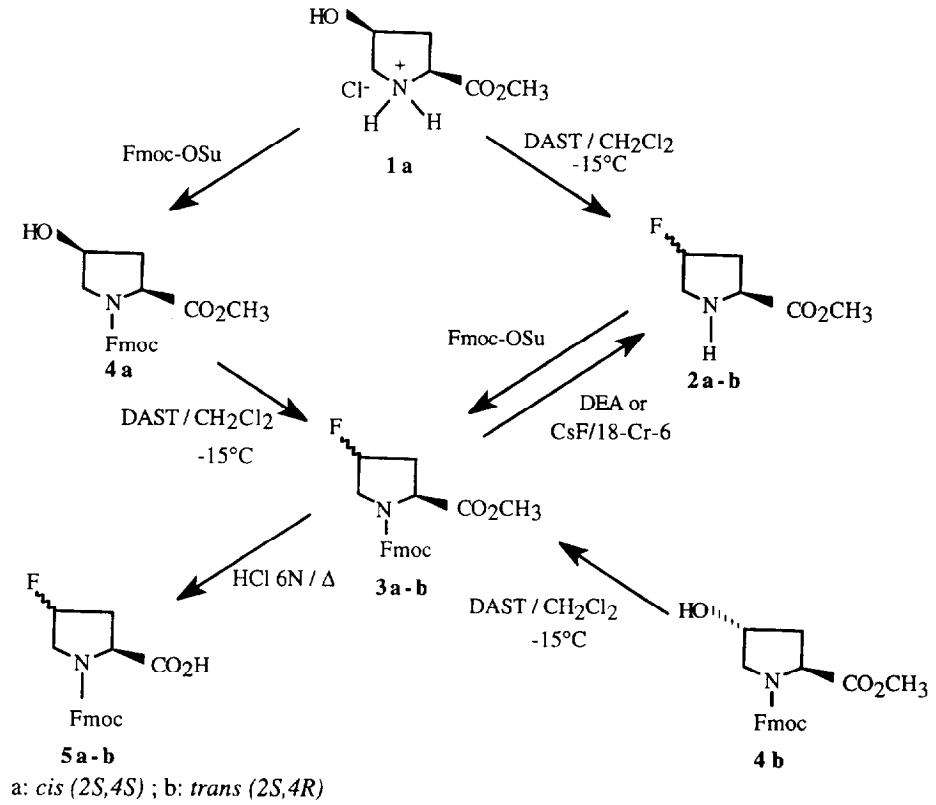


a: *cis (2S,4S)* ; b: *trans (2S,4R)*

Scheme 1. Synthesis of N-Fmoc-4-fluoro-L-proline derivatives.

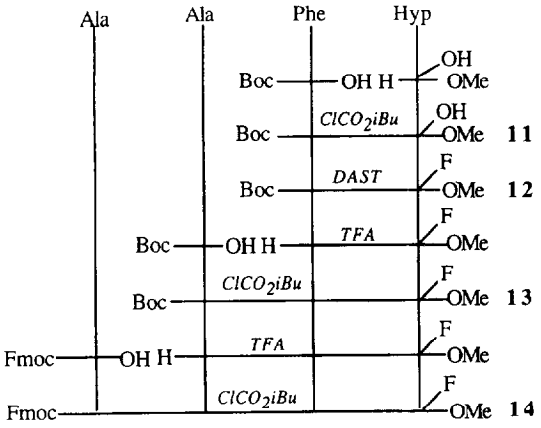
2.1. Pathway A

2.1.1. Synthesis of 4-fluoroproline derivatives

A number of studies have been reported for the syntheses of 4-fluoroproline derivatives. Starting from aspartic acid, Burger [12] has developed a stereoselective methodology leading to *trans*-4-fluoroproline but it is time consuming. Hudlicky [13] has obtained 4-fluoroproline diastereoisomers as byproducts during the preparation of 4-fluoroglutamic acid. The same author [13,14] described the synthesis of methyl 1-acetyl-4-fluoroprolinate, which cannot be used in peptide synthesis because of the inappropriate acetyl group. Gottlieb [15] reported the preparation of methyl N-benzyloxycarbonyl-4-fluoroprolinate, which cannot allow coupling at the carboxyl terminus: saponification (alkaline medium) or hydrolysis (harsh acidic conditions) of methyl ester would induce either the loss of a molecule of hydrofluoric acid or the deprotection of the amino function.

We selected and synthesized the methyl N-Fmoc-4-fluoro-L-prolinate **3**, as an interesting synthon in the field of solution and solid phase peptide synthesis: the 9-fluorenylmethoxycarbonyl group is acid resistant, and allows the acidolysis of the methyl ester. On the other hand, it can be removed smoothly (diethylamine or fluoride ion [16]) without HF elimination.

Compound **3** was prepared from the commercially available *cis*-methyl 4-hydroxy-L-prolinate (**1a**) and *trans*-N-Fmoc-4-hydroxy-L-proline, using diethylaminosulfur trifluoride (DAST) as fluorinating agent [17] (Scheme 1). Fluorination of compound **1a** in mild conditions (− 15 °C, CH_2Cl_2, 45 min), followed by protection of the amino function of the resulting **2** with 9-fluorenylmethyl-N-hydroxysuccinimide afforded **3** in good yield (70%). Cohen and Bergman [18] failed to obtain methyl 4-fluoroprolinate when CTT (1,1,1-trifluoro-2-chloroethyl-diethylamine) was used as the fluorinating reagent for the N-unprotected methyl 4-hydroxy-prolinate. When the amino group was methylated, fluorination occurred. Compounds **2** and **3** were obtained as a 1:1 mixture of diastereoisomers *cis* and *trans* (as determined from the ^{19}F signals measured at − 174.42 ppm and − 174.45 ppm for compound **2**, − 173.24 ppm and − 173.28 ppm for compound **3**. The corresponding configurations were not assigned). Hudlicky [13] has reported that fluorination with DAST (0 °C, CH_2Cl_2, 15 h) of the *trans* and *cis*-methyl 1-acetyl-4-hydroxy-L-prolinate is stereospecific: large excess of *cis* and *trans*-methyl 1-acetyl-4-fluoro-L-prolinate were obtained. In marked contrast, in our hands, the fluorination with DAST of the *cis*-methyl N-Fmoc-4-hydroxy-L-prolinate (**4 a**) (− 15 °C, CH_2Cl_2, 1 h) yielded compound **3** (70%) as a 1:1 diastereoisomeric mixture. Starting with the *trans* isomer (**4b**) (obtained by refluxing the commercialy *trans*-N-Fmoc 4-hydroxyproline in methanol with 10% HCl), the stereoselectivity of the DAST fluorination process was greatly improved giving a 1:2 mixture of the two diastereoisomers (configurations were not assigned). Our results are taken as a strong indication that fluorination with DAST does not occur via a simple S_N2 process and that the stereospecificity of the reactants is very much dependent



Scheme 2. Synthesis of the fluoro tetrapeptide **14**.

upon the amino protecting group. ^{19}F NMR shifts of the two diastereoisomers differ by 4 ppm when the amino function of the fluoroproline residue is acetylated [13] (*cis*: −174.1; *trans*: −178.3) and by only 0.04 ppm when it is protected by the Fmoc group (−173.24 and −173.28).

This difference could arise from the shielding effect of the bulky Fmoc group upon the fluorine atom in one of the diastereoisomers. Hydrolysis of compound **3** in refluxing 6 N HCl produced compound **5** in quantitative yield.

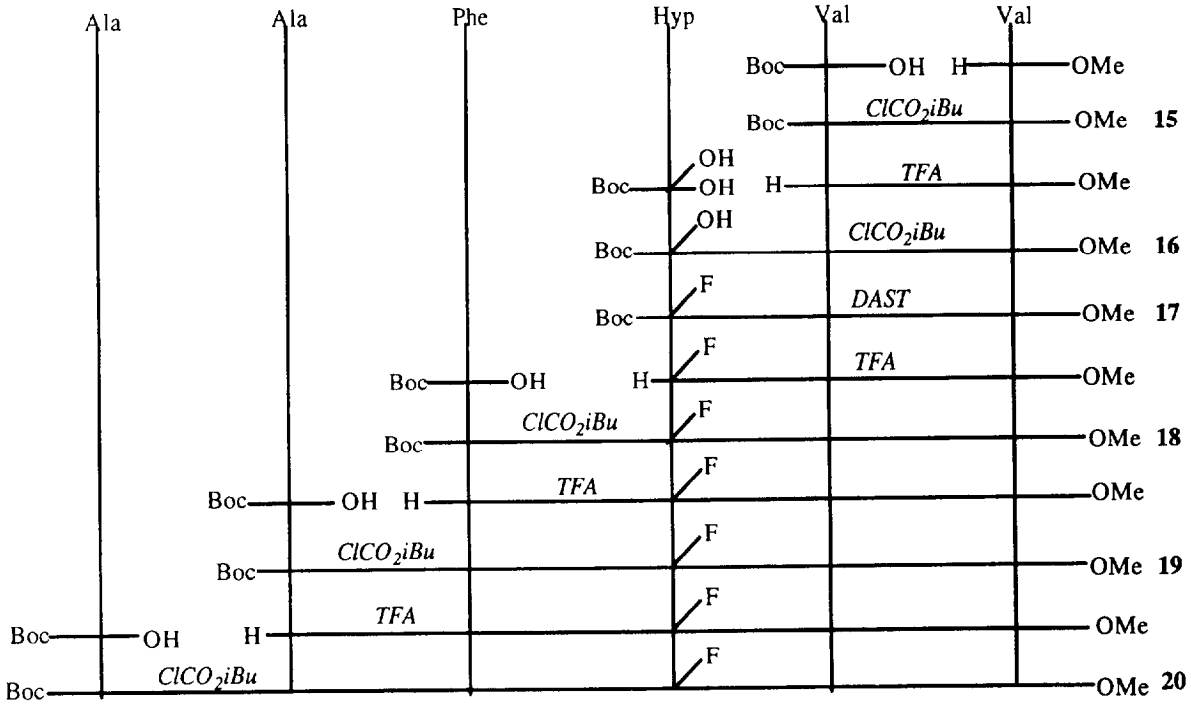
The dipeptide Fmoc–Phe–Pro(F)–OMe **7** was then obtained in 30% yield from compound **2** (mixture of diastereoisomers) via the isobutyl chloroformate procedure. The rather disappointing yield of the reaction is attributable to partial deprotection of the Fmoc group in the presence of the secondary amino group of proline, which can lead to diketopiperazine formation. Our attempts to prepare Fmoc–Phe–Pro(F)–OH **8** through acidolysis of the dipeptide ester **7** in 6 N HCl resulted in the rupture of the Phe–Pro(F) bond. The tripeptide Fmoc–Pro(F)–Val–Val–OMe **9** was, therefore, prepared from compound **5** (mixture of diastereoisomers) with the same coupling reagent (60% yield). Deprotection of the amino group in compound **9** either by diethylamine or cesium fluoride resulted in a mixture of compounds which led us to undertake the synthesis of the fluorohexapeptide via pathway B.

2.2. Pathway B

Pathway B, i.e. direct fluorination of a hydroxyproline residue already incorporated into a peptide and elongation from the terminal amino group, was performed starting from Boc–Phe–Hyp–OMe **11** (Scheme 2) and Boc–Hyp–Val–Val–OMe **16** (Scheme 3), which both include the *trans* isomer of the 4-hydroxy-L-proline residue.

The fluorination with DAST (−15 °C, CH_2Cl_2, 1 h) of peptides **11** and **16** afforded the fluoro compounds Boc–Phe–Pro(F)–OMe **12** and Boc–Pro(F)–Val–Val–OMe **17** in 70% yield, as a mixture of two diastereoisomers incorporating the *cis* and *trans*-4-fluoroproline residues. Elongation of compound **12** (Scheme 2) was performed from the terminal amino group, using trifluoroacetic acid as Boc deprotective agent (quantitative yield) and isobutyl chlorocarbonate as coupling reagent (70–80% yields). Acidolysis of the tetrapeptide **14** in refluxing 6 N HCl essentially led to the rupture of the Phe–Pro(F) bond.

Finally, the Boc–Ala–Ala–Phe–Pro(F)–Val–Val–OMe hexapeptide **20** was obtained following Scheme 3, via successive elongation of **17**, **18** and **19**, using for each step trifluoroacetic acid as Boc deprotective agent and isobutyl



Scheme 3. Synthesis of the fluoro hexapeptide **20**.

chlorocarbonate as coupling reagent. **20** was isolated as a mixture of diastereoisomers in a 40% overall yield.

The fluorohexapeptide **20** and all the **16–19** intermediates, taken as a mixture of diastereoisomers, were evaluated for their inhibitory effect on the HIV protease in vitro and in cell culture. None of them displayed any anti-protease or anti-HIV activity.

3. Experimental

^{1}H and ^{19}F NMR spectra were recorded on a Bruker WP200 (200 MHz) spectrometer, using solutions in deuterochloroform or deuterated methanol, and tetramethylsilane or $CFCl_3$ as internal standards. The NMR data given were for a mixture of diastereoisomers. The ^{19}F NMR spectra were proton decoupled. Purification of fluoro compounds was performed by semi-preparative reverse-phase C_{18}-HPLC, with acetonitrile–water as eluant. Elemental analyses were performed at Vernaison (France) by CNRS.

3.1. Methyl 4-fluoro-L-prolinate (2a–b)

To a suspension of **1a** (1 mmol) in CH_2Cl_2 was added DAST (1.1 mmol) at −15 °C. The mixture was stirred at −15 °C for 45 min and then allowed to warm to room temperature. At this time, 2 ml of methanol were added to quench the reaction. The solvent was evaporated in vacuo and the resulting oil dissolved in ethyl acetate and washed with 1 N hydrochloric acid solution. The aqueous layer was washed with ethyl acetate, neutralized (pH = 7–8) by addition of a 32% ammonia solution, and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$ and then evaporated (70% yield).

^{19}F NMR (CD_3OD) δ −174.42 and −174.45. ^{1}H NMR (CD_3OD) δ 2.65 (dm, 1H, J = 14.2 Hz, H_3); 2.5 (dd, J = 12.2 Hz, J = 6.1 Hz, 1H, $H_{3'}$); 3.07 (dd, J = 12.2 Hz, J = 6.1 Hz, 1H, H_5); 3.2 (dt, J = 12.2 Hz,1H, $H_{5'}$); 3.7 (s, 3H, OCH_3); 3.85 (dd, J = 12.2 Hz, J = 6.1 Hz, 1H, H_2); 4.4 (m, 1H, H_4). MS: m/z = 147 $[M]^+$.

3.2. Methyl N-Fmoc-4-hydroxy-L-prolinate (4a) and (4b)

3.2.1. Cis isomer (4a)

To compound (**1a**) (1 mmol) in CH_2Cl_2 was added Fmoc-OSu (1 mmol) and N-methyl morpholine (1 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C and then allowed to stand at room temperature for 12 h. The solvent was removed in vacuo and the residue extracted in ethyl acetate, washed with a 10% sodium hydrogencarbonate solution, a 10% citric acid solution and brine, then dried over $MgSO_4$. Evaporation of the solvent yielded an oil (95%) which was purified by silica gel chromatography using hexane and ethyl acetate as eluent.

^{1}H NMR ($CDCl_3$), δ 2.0 (m, 1H, H_3 Pro); 2.25 (m, 1H, $H_{3'}$ Pro); 3.55 (m, 2H, H_5 and $H_{5'}$ Pro); 3.55 and 3.65 (2s, 3H, OCH_3 of the two conformations); 4.3 (m, 5H, H_4 and H_2 Pro, CH and CH_2 Fmoc); 7.5 (m, 8H, H arom Fmoc). MS: m/z = 367 $[M]^+$.

3.2.2. Trans isomer (4b)

This was prepared by refluxing for 4 h a 10% HCl methanolic solution of the *trans* N-Fmoc-4-hydroxy-L-proline. The solvent was evaporated and the oily residue dissolved in ethyl acetate, washed with a 10% sodium hydrogencarbonate solution, then with brine and dried over $MgSO_4$. Evaporation of the solvent yielded (**4b**) in quasi quantitative yield, as an oil that did not crystallize.

^{1}H NMR data are identical with those of (**4b**), except for the methyl ester which gives a single signal (δ 3.65).

3.3. Methyl N-Fmoc-4-fluoro-L-prolinate (3a–b)

3.3.1. From 4a or 4b

To compound **4a** (**4b**) (1 mmol) in CH_2Cl_2 was added DAST (1.1 mmol) at −15 °C. The mixture was stirred at −15 °C for 1 h and then allowed to warm to room temperature. At this time, 2 ml of methanol were added to quench the reaction. The solvant was evaporated in vacuo and the resulting oil was dissolved in CH_2Cl_2, washed with a 10% sodium hydrogencarbonate solution, a 10% citric acid solution, brine and dried over $MgSO_4$. Evaporation of the solvent yielded a oily residue which was purified by silica gel chromatography using dichloromethane and ethyl acetate as eluent (70% yield).

3.3.2. From 2a–b

Fmoc-OSu (1.1 mmol) was added to a solution of **2a–b** (1 mmol) in CH_2Cl_2 at 0 °C and the mixture was stirred at this temperature for 1 h, then at room temperature for 12 h. Treatment was analogous to the previous one (90% yield).

Data for a mixture of **3a** and **3b:** Analysis: Calc. for $C_{21}H_{20}O_4NF$: C, 68.28; H, 5.46; N, 3.75; F, 5.14. Found: C, 68.20; H, 5.33; N, 3.75; F, 5.03%. ^{19}F NMR ($CDCl_3$) δ −173.24 and −173.28. ^{1}H NMR ($CDCl_3$) δ 2.35 (m, 2H, H_3 and $H_{3'}$); 3.7 (m, 2H, H_5 and $H_{5'}$); 3.7 and 3.8 (2s, 3H, OCH_3); 4.35 (m, 4H, H_2 Pro and (CH,CH_2)Fmoc); 5.15 (dm, J(^{1}H–^{19}F) = 52.2 Hz, 1H, H_4); 7.6 (m, 8H Fmoc). MS: m/z = 369 $[M]^+$.

3.4. N-Fmoc-4-fluoro-L-proline (5a–b)

30 ml of a 6 N HCl solution were added to a solution of 50 ml of dioxane and 10 mmol of **3a–b.** The mixture was heated under reflux for 2h. The solvent was then evaporated in vacuo, the residue dissolved in ethyl acetate, washed with water, brine and dried over $MgSO_4$. Evaporation of the solvent gave **5a–b** in quantitative yield as a white solid.

Data for a mixture of **5a** and **5b:** ^{19}F NMR ($CDCl_3$) δ −172.8 and −173.6. ^{1}H NMR ($CDCl_3$) δ 2.30 (m, 2H, H_3 and $H_{3'}$); 3.6 (m, 2H, H_5 and $H_{5'}$); 4.35 (m, 4H, H_2 Pro and

(CH, CH_2)Fmoc); 5.2 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4); 7.5 (m, 8H Fmoc). MS: $m/z = 355$ $[M]^+$.

3.5. General procedures for the syntheses of peptides

3.5.1. Isobutyl chloroformate-mediated coupling reactions

To a cold solution (− 15 °C) of N-protected amino acid (10 mmol) in dry THF was added N-methylmorpholine (10 mmol). Isobutyl chloroformate (10 mmol) was then carefully added while the temperature was maintained between − 10 °C and − 15 °C. The solution was stirred for 10 minutes at this temperature and then a solution of aminoester or peptide ester hydrochloride (10 mmol) in DMF was added, followed by NMM (10 mmol). The reaction was stirred for 2 h at 0 °C. The solvent was removed in vacuo and the residue extracted in ethyl acetate, washed with a 10% sodium hydrogen carbonate solution, a 10% citric acid solution and brine, then dried over MgSO4. Evaporation of the solvent yielded oil which was purified by silica gel chromatography using dichloromethane and ethyl acetate as eluent.

3.5.2. Removal of Boc protecting group

N-Boc peptide ester (1 mmol) was dissolved in 5 ml of CH_2Cl_2 at 0 °C. 5 ml of trifluoroacetic acid were then added. After 2 h, the solvent was removed in vacuo, the oily residue triturated with ethyl acetate until precipitation occured. The solid was filtered and used in the next step without further purification.

3.5.3. Removal of the Fmoc protecting group

3.5.3.1. With diethylamine

N-Fmoc amino acid or peptide ester (1 mmol) was dissolved in a solution containing 2 ml of CH_2Cl_2 and 2 ml of diethylamine. The reaction was monitored on TLC. Upon complete deprotection, the solvents were removed in vacuo. The residue was dissolved in ethyl acetate and washed with 1 N hydrochloric acid solution. The aqueous layer was washed with ethyl acetate, neutralized ($pH = 7–8$) by the addition of a 32% ammonia solution, and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$ and then evaporated.

3.5.3.2. With CsF/18-Crown-6

N-Fmoc amino acid or peptide ester (1 mmol) was dissolved in anhydrous DMF (1 ml mmol − 1), and 18-Crown-6 (4 mmol) was added followed by cesium fluoride (10 mmol). The reaction was monitored on TLC. Treatment was identical to the previous one.

3.6. Fmoc-Phe-Pro(F)-OMe (7) (mixture of diastereoisomers)

HPLC rt: 7.12 min ($CH_3CN–H_2O$ 70:30). ^{19}F NMR ($CDCl_3$) δ − 173.4 and − 174.2. ^1H NMR ($CDCl_3$) δ 2.3 (m, 2H, H_3 and $H_{3'}$ Pro); 3.15 (m, 2H, H_β Phe); 3.7 (m, 2H, H5 and $H_{5'}$ Pro); 3.6 and 3.7 (2s, 3H, OCH_3); 4.25 (m, 2H, CH_2 Fmoc); 4.65 (m, 2H, CH Fmoc and H_α Phe); 4.8 (dd, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro isomer 1); 5.25 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro isomer 2); 5.2 (m, 1H, H_2 Pro); 7.45 (m, 13H, H arom Phe and Fmoc). SM (FAB > 0): $m/z = 517$ $[M+1]^+$.

3.7. Fmoc-Pro(F)-Val-Val-OMe (9) (mixture of diastereoisomers)

^{19}F NMR ($CDCl_3$) δ − 173.2 and − 175.1. ^1H NMR ($CDCl_3$) δ 0.7 (2dd, 12H, $4CH_3$, Val_1 and Val_2); 2.25 (m, 4H, H_β Val_1 and Val_2, H_3 and $H_{3'}$ Pro); 3.7 and 3.8 (2s, 3H, OCH_3); 3.75 (m, 2H, H_5 and $H_{5'}$ Pro); 4.15 (m, 1H, CH Fmoc); 4.35 (m, 5H, H_α Val_1 and Val_2, H_2 Pro, CH_2 Fmoc); 5.15 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro); 7.6 (m, 8H, H arom Fmoc). SM (FAB > 0): $m/z = 567$ $[M+1]^+$.

3.8. Boc-Phe-Hyp-OMe (11)

^1H NMR ($CDCl_3$), δ 1.3 (s, 9H, Boc); 1.85 (m, 1H, H_3 Pro); 2.2 (m, 1H, $H_{3'}$ Hyp); 2.95 (m, 4H, H_β Phe, H_5 and $H_{5'}$ Hyp); 3.7 (s, 3H, OCH_3); 4.4 (s, 1H, H_4 Hyp); 4.55 (m, 2H, H_α Phe and H_2 Hyp); 5.4 (d, 1H, NH Phe); 7.2 (m, 5H, H arom Phe). SM (FAB > 0): $m/z = 393$ $[M+1]^+$.

3.9. Boc-Phe-Pro(F)-OMe (12) (mixture of diastereoisomers)

^{19}F NMR ($CDCl_3$) δ − 173.5 and − 174.6. ^1H NMR ($CDCl_3$) δ 1.4 (s, 9H, Boc); 2.2 (m, 2H, H_3 and $H_{3'}$ Pro); 2.9 (m, 2H, H_β Phe); 3.6 (m, 2H, H_5 and $H_{5'}$ Pro); 3.5 and 3.6 (2s, 3H, OCH_3 of two isomers); 4.4 (m, 1H, H_α Phe); 4.55 (dd, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro isomer 1); 4.95 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro isomer 2); 5.3 (m, 1H, H_2 Pro); 7.15 (m, 5H, H arom Phe). SM (FAB > 0): $m/z = 395$ $[M+1]^+$.

3.10. Boc-Ala-Phe-Pro(F)-OMe (13) (mixture of diastereoisomers)

^{19}F NMR ($CDCl_3$) δ − 173.6 and − 174.5. ^1H NMR ($CDCl_3$) δ 1.2 (d, 3H, H_β Ala); 1.4 (s, 9H, Boc); 2.3 (m, 2H, H_3 and $H_{3'}$ Pro); 3.0 (m, 2H, H_β Phe); 3.5 (m, 2H, H_5 and $H_{5'}$ Pro); 3.6 and 3.7 (2s, 3H, OCH_3 of the two isomers); 4.65 (m, 1H, H_α Phe); 4.75 (dd, 1H, H_2 Pro); 5.15 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro); 7.25 (m, 5H, H arom. Phe). SM (FAB > 0): $m/z = 537$ $[M+1]^+$.

3.11. Fmoc–Ala–Ala–Phe–Pro(F)–OMe (14) (mixture of diastereoisomers)

HPLC rt: 20.15 min ($CH_3CN–H_2O$ 50/50); ^{19}F NMR ($CDCl_3$) δ − 173.5 and − 174.6. ^1H NMR ($CDCl_3$) δ 1.3 (2 dd, 6H, H_β Ala_3 and Ala_4); 2.2 (m, 2H, H_3 and $H_{3'}$ Pro); 2.95 (m, 2H, H_β Phe); 2.95 (m, 2H, H_5 and $H_{5'}$ Pro); 3.65

and 3.7 (2s, 3H, OCH_3 of the two isomers); 4.15 (m, 1H, CH Fmoc); 4.4 (m, 2H, CH_2 Fmoc); 4.7 (m, 4H, H_α Ala_3 and Ala_4, H_α Phe, H_2 Pro); 5.15 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro); 7.4 (m, 13H, H arom. Phe and Fmoc). SM (FAB > 0): $m/z = 659$ $[M+1]^+$.

3.12. Boc–Hyp–Val–Val–OMe (16)

^1H NMR ($CDCl_3$) δ 1,0 (2 dd, 12H, CH_3 Val_1 and Val_2); 1,4 (d, 9H, Boc); 2,2 (m, 4H, H_β Val_1 and Val_2, H_3 and $H_{3'}$ Hyp); 3,45 (m, 2H, H_α Val_1 and Val_2); 3,7 (s, 3H, OCH_3); 4,35 (m, 4H, $H_{2'}$, $H_{4'}$, H_5 and $H_{5'}$ Hyp). SM (FAB > 0): $m/z = 444$ $[M+1]^+$.

3.13. Boc–Pro(F)–Val–Val–OMe (17) (mixture of diastereoisomers)

^{19}F NMR ($CDCl_3$) δ −173.2 and −175.1. ^1H NMR ($CDCl_3$) δ 0.7 (2 dd, 12H, CH_3 Val_1 and Val_2); 1.4 (d, 9H, Boc); 2.15 (m, 4H, H_β Val_1 and Val_2, H_3 and H_3' Pro); 3.7 (s, 3H, OCH_3); 3.75 (m, 2H, H_5 and $H_{5'}$ Pro); 4.3 (m, 3H, H_α Val_1 and Val_2, H_2 Pro); 5.15 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro). SM (FAB > 0): $m/z = 446$ $[M+1]^+$.

3.14. Boc–Phe–Pro(F)–Val–Val–OMe (18) (mixture of diastereoisomers)

Analysis Calc. for $C_{30}H_{45}O_7N_4F$: C, 60.78; H, 7.66; N, 9.46; F, 3.21. Found: C, 60.52; H, 7.59; N, 9.37; F, 3.46%. HPLC rt: 10.5 min ($CH_3CN–H_2O$ 60/40). ^{19}F NMR ($CDCl_3$) δ −174.2 and −174.9. ^1H NMR ($CDCl_3$) δ 0.9 (m, 12H, 4 CH_3 Val_1 and Val_2); 1.4 (d, 9H, Boc); 2.05 (m, 4H, $2H_\beta$ Val_1 and Val_2, H_3 and $H_{3'}$ Pro); 3.0 (m, 2H, H_β Phe); 3.75 (m, 2H, H_5 and $H_{5'}$ Pro); 3.7 (s, 3H, OCH_3); 4.3 (m, 1H, H_α Phe); 4.6 (m, 3H, H_α Val_1 and Val_2, H_2 Pro); 5.25 (dm, $J(^1H–^{19}F) = 52.2$ Hz, 1H, H_4 Pro); 7.25 (m, 5H, H arom. Phe). SM (FAB > 0): $m/z = 593$ $[M+1]^+$.

3.15. Boc–Ala–Phe–Pro(F)–Val–Val–OMe (19) (mixture of diastereoisomers)

^{19}F NMR ($CDCl_3$), δ: −173.2 and −174.5; ^1H NMR ($CDCl_3$) δ 0.8 (m, 12H, 4 CH_3 Val_1 and Val_2); 1.2 (dd, $J = 9$ Hz, 3H, CH_3 Ala); 1.4 (dd, 9H, Boc); 2.1 (m, 4H, H_β Val_1 and Val_2, H_3 and $H_{3'}$ Pro); 3.0 (m, 2H, H_β Phe); 3.75 (m, 2H, H_5 and $H_{5'}$ Pro); 3.7 (s, 3H, OCH_3); 4.3 (m, 1H, H_α Phe); 4.65 (m, 4H, H_α Val_1 and Val_2, H_2 Pro, H_α Ala); 5.1 (dm, 1H, $J(^1H–^{19}F) = 52.2$ Hz, H_4 Pro); 7.15 (m, 5 H, H arom. Phe). SM (FAB > 0): $m/z = 665$ $[M+1]^+$.

3.16. Boc–Ala–Ala–Phe–Pro–Val–Val–OMe (20) (mixture of diastereoisomers)

Analysis Calc. for $C_{36}H_{55}O_9N_6F$: C, 58.82; H, 7.55; N, 11.44; F, 2.59. Found: C, 58.77; H, 7.61; N, 11.35; F, 2.46%. HPLC rt: 13.5 min ($CH_3CN–H_2O$ 60/40). ^{19}F NMR ($CDCl_3$) δ −173.8 and −174.7. ^1H NMR ($CDCl_3$) δ 0.9 (m, 12H, $4CH_3$ Val_1 and Val_2); 1.1 (d, 3H, CH_3 Ala_5); 1.4 (m, 12H, CH_3 Ala_6 and Boc); 2.0 (m, 4H, H_β Val_1 and Val_2, H_3 and $H_{3'}$ Pro); 3.0 (m, 2H, H_β Phe); 3.55 (m, 2H, H_5 and $H_{5'}$ Pro); 3.7 (s, 3H, OCH_3); 4,5 (m, 6H, H_α Ala_5 and Ala_6, H_α Val_1 and Val_2, H_α Phe, H_2 Pro); 5.1 (dm, $J(^1H–^{19}F) = 52.2$ Hz, H_4 Pro); 7.2 (m, 5H, H arom. Phe). SM (FAB > 0): $m/z = 735$ $[M+1]^+$.

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