Pergamon

Tetrahedron 54 (1998) 6019–6028

TETRAHEDRON

Synthesis of RGD Analogues Containing α-Tfm-Arginine as Potential Fibrinogen Receptor Antagonists

Alma Dal Pozzo[a*], Laura Muzi[a], Maurizio Moroni[a], Riccardo Rondanin[a], Roberto de Castiglione[a], Pierfrancesco Bravo[b] and Matteo Zanda[b]

a- Istituto di Ricerche Chimiche e Biochimiche "G. Ronzoni", via G. Colombo 81, 20133 Milano, Italy

b- CNR, Dipartimento di Chimica del Politecnico, via Mancinelli 7, 20131 Milano, Italy

Received 23 February 1998; accepted 26 March 1998

Abstract The synthesis of two peptide mimetics of RGD, α-Tfm-Arg-Gly-Asp-Phe-NH_2 **9** and α-Tfm-Arg-Gly-Asp-NH-$(CH_2)_2$-C_6H_5 **13**, is described. The precursor of α-Tfm-ornithine was obtained in two synthetic steps from 2-N-Cbz-2-Tfm-hexanediacid-1-alkyl ester and introduced into the peptide chain by α-carboxy-group activation via oxazolone. The introduction of the guanidine residue led to the final peptides as mixtures of the two diastereomers. Configurationally pure peptides were obtained in good yields by RP-HPLC.

INTRODUCTION

The binding of fibrinogen to its platelet receptors GP IIb/IIIa is mediated by the sequence RGD (Arg-Gly-Asp), and represents a contributing factor in the platelet-mediated thrombus formation. Antagonists of this natural sequence constitute an attractive goal for an antithrombotic therapy. Thus, extensive efforts have been directed toward the discovery and synthesis of such agents [1-6]. Many classes of RGD peptide mimetics have been developed from systematic conformational search of constrained fibrinogen receptor antagonists with increased potency.

We pursued the aim to find a new approach leading to molecules with improved affinity and selectivity to the fibrinogen receptor by applying a strategy already suggested by other authors [7,8]. It is known that the incorporation of α,α-disubstituted amino acids into key positions of peptides is an useful approach to stabilize secondary structure [9]. On the other hand, α-trifluoromethyl-amino acids (Tfm-Xaa) containing peptides are a special class of peptide mimetics which could profoundly improve the pharmacodynamic and pharmacokinetic characteristics of the natural peptides. This type of substitution can influence, beside the three-dimensional conformation of the peptide, lipophilicity and proteolytic stability as well [10,11].

0040-4020/98/$19.00
PII: S0040-4020(98)00282-8

To verify this assumption, we started to synthesize two peptides containing α-Tfm-arginine at the N-terminal position, namely α-Tfm-Arg-Gly-Asp-Phe-NH_2 **9** and α-Tfm-Arg-Gly-Asp-NH-$(CH_2)_2$-C_6H_5 **13**, as described in the present article.

RESULTS AND DISCUSSION

In order to obtain the precursor of α-Tfm-arginine, we synthesized the 2-N-Cbz-2-Tfm-hexanediacid-1-alkyl ester **1**, following the method of the trifluoromethyl-containing building blocks already described by Burger et al.[7,12]. Amidation of ω-carboxy-group and hydrolysis of the ester **2** gave 2-N-Cbz-2-Tfm-hexanediacid-6-amide **3**.

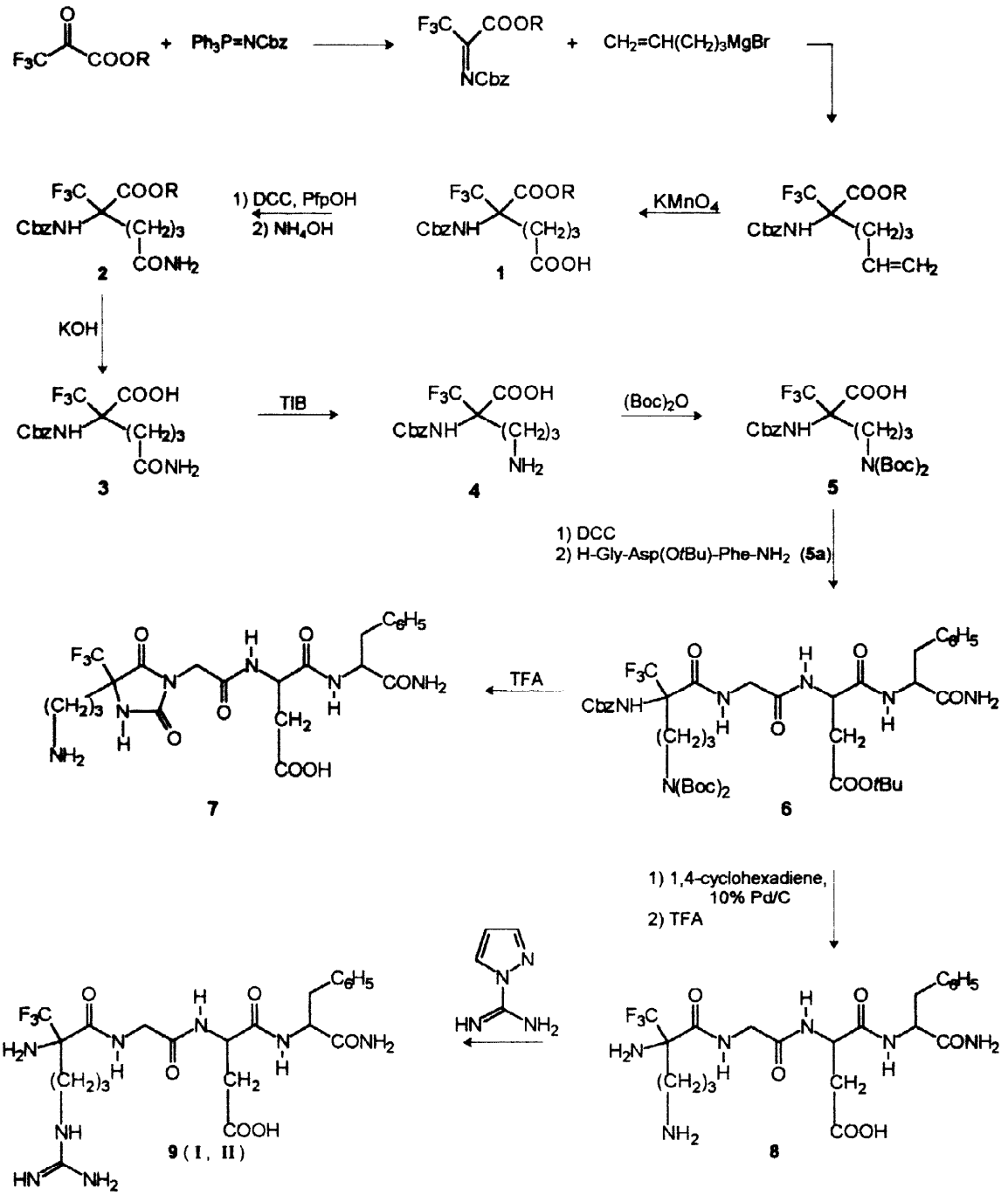
To obtain the final peptides **9** and **13**, two alternative synthetic pathways were followed, as illustrated in Scheme 1 and 2.

Synthesis of α-Tfm-Arg-Gly-Asp-Phe-NH_2 9 (Scheme 1).

The amide **3** was transformed into the corresponding amine **4** by Hoffman-type degradation with 1,1-bis-(trifluoroacetoxy)-iodobenzene (TIB) (78%). The amino group of Tfm-Orn **4** was then protected with 2 equiv. of $(Boc)_2O$, providing the $N(Boc)_2$ derivative **5** (81%).

At this stage, it is useful to recall that common solution phase strategies are not generally applicable to the introduction of Tfm-amino acids into peptides. In fact, the presence of a trifluoromethyl group in the α-position strongly influences the reactivity of both the amino and carboxylic functions. However, activation of the carboxylic group of **5** could be achieved via intramolecular formation of the corresponding intermediate 2-benzyloxy-4-trifluoromethyl-5-oxazolone, with DCC [13]. *In situ* addition of the tripeptide H-Gly-Asp(O*t*Bu)-Phe-NH_2 **5a** afforded the totally protected diastereomeric tetrapeptides **6** in 60% yield.

Preliminary attempts to cleave the protecting groups of **6** gave rise to an undesired side reaction. In fact, treament with TFA produced along with the desired cleavage of both $N(Boc)_2$ and *tert*-butyl ester functions the formation of a hydantoin ring between the NH of Gly and CO of Cbz group in Tfm-Orn. The structure of the resulting product **7** was unequivocally determined by mass spectrometry and ^{1}H-NMR. To overcome this problem, compound **6** was first submitted to hydrogenolysis of the Cbz function with 1,4-cyclohexadiene and Pd/C, then treated with TFA, producing the desired unprotected tetrapeptide **8** in 88% yield. The final guanylation step[14] was carried out upon treatment of **8** with 1-H-pyrazole-1-carboxamidine hydrochloride, which produced the diastereomeric α-Tfm-Arg-Gly-Asp-Phe-NH_2 **9** (91%). Separation and purification of diastereomers **I** and **II** were performed by RP-HPLC under the conditions described in the experimental section. The elemental analysis did not give satisfactory results due to the difficulty of removing quantitatively the trifluoroacetic acid.



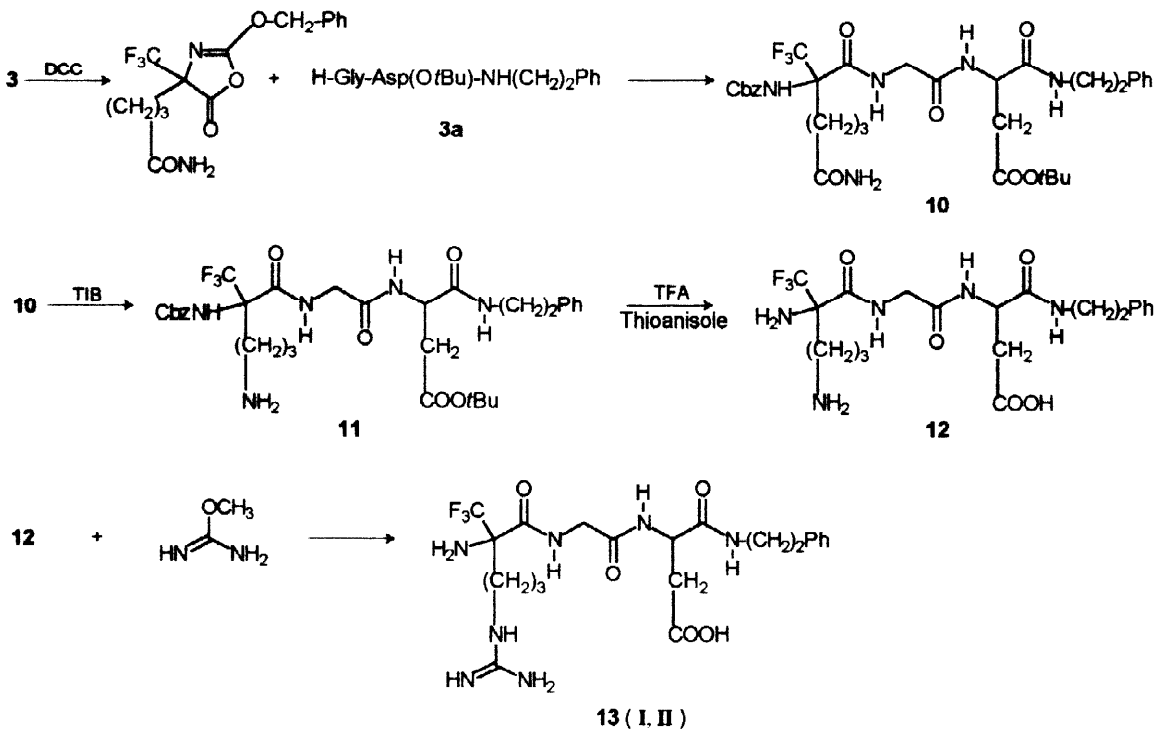
Scheme 1

Synthesis of α-Tfm-Arg-Gly-Asp-NH-$(CH_2)_2$-C_6H_5 13 (Scheme 2).

The amide **3** was directly introduced into the peptide chain upon treatment with DCC and subsequent coupling with the tripeptide H-Gly-Asp(O*t*Bu)-NH-$(CH_2)_2$-C_6H_5 **3a**, affording the tetrapeptide **10** (51%). Transformation of the primary amide function of **10** into the corresponding amine was chemoselectively achieved by treatment with TIB providing N-Cbz-α-Tfm-Orn-Gly-Asp(O*t*Bu)-NH-$(CH_2)_2$-C_6H_5 **11** in 82% yield.

Treatment of **11** with TFA/thioanisole produced the desired cleavage of both N-Cbz and O-*tert*-butyl groups yielding the unprotected tetrapeptide **12**. The crude **12** was directly treated with O-methyl-isourea sulphate which allowed for the guanylation of the terminal amino group. The final tetrapeptides α-Tfm-Arg-Gly-Asp-NH-$(CH_2)_2$-C_6H_5 **13**, **I** and **II** were isolated in optical pure form after RP-HPLC separation as TFA salts (75% from **11**). In order to avoid the inconvenience encountered with the previous peptide, TFA salts were converted into hydrobromide salts, obtaining better fitting elemental analyses.

In conclusion, we have presented the synthesis of two tetrapeptide mimics of RGD, incorporating Tfm-Arg, which are potential fibrinogen receptor antagonists. Evaluation of their biological activity as well as the synthesis of other Tfm-amino acid-containing peptides are presently underway.



Scheme 2

EXPERIMENTAL SECTION

Abbreviations.

Tfm = trifluoromethyl, Cbz = benzyloxycarbonyl, Boc = *tert*-butyloxycarbonyl, OtBu = *tert*-butyl ester, TFA = trifluoroacetic acid, Fmoc = 9-fluorenylmethyloxycarbonyl, OPfp = pentafluorophenyl ester, TEA = triethylamine, DCM = dichloromethane, TIB = 1,1-bis-(trifluoroacetoxy)-iodobenzene, DMF = N,N-dimethylformamide, DCC = N,N'-dicyclohexylcarbodiimide.

General.

Amino acids were purchased from Bachem Chem. Co. Switzerland; methyl or ethyl trifluoropyruvate were purchased from Fluorochem, Derbyshire, UK and from Apollo Scientific, Stockport, UK, respectively. Other reagents were purchased from Sigma Aldrich. N-Cbz-triphenyl-phosphimine was prepared in our laboratories according to the method described by Kricheldorf [15]. As necessary, solvents and bases were either dried over 3Å molecular sieves or freshly distilled. Thin layer chromatography (TLC) was routinely carried out to monitor reactions using Silica gel 60 plates F_{254}, Merck; precursor intermediates and peptide derivatives were located with UV light (254 nm), ninhydrin, Pancaldi reagent or I_2 vapours, according to their structure. Flash chromatography columns were filled with Silica gel 60, 230-400 mesh, Merck. Analytical and preparative liquid chromatography were carried out on a Waters 600E gradient HPLC system, UV detector at μ= 220 nm, with the following columns: Ultrasphere ODS (5μ, 10 x 250 mm) Beckmann and Novapack HRC 18 (60Å, 6μ, 19 x 300 mm) Waters. ^{1}H, ^{19}F, ^{13}C-NMR spectra were recorded on P.Elmer R 32 90 and Bruker spectrometers AC 300 or ARX 400. Chemical shift values (δ) are reported in ppm of the applied field. Me_4Si was used as internal standard for ^{1}H and ^{13}C nuclei, while C_6F_6 was used as external standard (δ_F = -162.90) for ^{19}F nucleus.

Mass spectra were registered on a Hitachi-Perkin Elmer system ZAB 2F instrument. In the case of the pseudopeptide **13**, only TLC, HPLC and ^{1}H-NMR are described; in fact, because of the great structural similarity with peptide **9**, intermediates and final products can be easily characterized by analogy.

Peptide synthesis.

The two peptides **5a** and **3a** were prepared in solution by stepwise coupling of Fmoc-amino acid derivatives under standard conditions. First step was the coupling of Fmoc-Asp(O*t*Bu)-OPfp with either H-Phe-NH_2·HCl (peptide **5a**) or phenylethylamine (peptide **3a**), in the presence of TEA. The obtained intermediates were deblocked with piperidine, purified by flash chromatography ($CHCl_3$/MeOH 93:7) and coupled with Fmoc-Gly-OPfp. After the final deprotection, the peptides were purified by flash chromatography with MeOH/EtOAc 50:50. Overall yield 35%.

2-N-Cbz-2-Tfm-Hexanediacid-1-ethyl (or methyl) ester 1.

1 was synthesized by methods already described in the literature[7]. The synthetic route proceeds through the addition of N-Cbz-triphenyl-phosphimine to the alkyl trifluoropyruvate (aza-Wittig reaction)[16], and subsequent introduction of the side chain with pentenemagnesium bromide[12]. Oxidation of the side chain[12] gave product **1**. For a representative example of the aza-Wittig reaction, N-Cbz-triphenyl-phosphimine (3.86 g, 9.4 mmol) was added under stirring to a solution of methyl trifluoropyruvate (1.467 g, 9.4 mmol) in 7 ml of anhydrous benzene at 0°C under N_2 atmosphere. After 5 min, the reaction was left at r. t. for about 1 h. On TLC (EtOAc/hexane 65:35) it was possible to follow the disappearance of the phosphimine and appearance of the product (Rf 0.7). At the end, the reaction mixture was directly added to the Grignard reagent. ^{1}H-NMR and ^{19}F-NMR data of this intermediate as well as of the product **1** were consistent with those reported in the literature.

2-N-Cbz-2-Tfm-Hexanediacid-1-ethyl ester-6-amide 2.

Compound **1** (2.624 g, 6.705 mmol), pyridine (541 ml, 6.705 mmol) and pentafluorophenol (1.355 g, 7.362 mmol) were dissolved in 50 ml of DCM and cooled to 0°C. DCC (1.660 g, 8.049 mmol) was added and, after 10 min, the reaction mixture was kept at r. t. under stirring for additional 2 h. Finally, the suspension was kept for 1 h at -20°C and filtered through a fritted-glass funnel. The filtrate was taken to dryness at reduced pressure. The residue was redissolved with 100 ml of dioxane and diluted with 30 ml of 25% ammonium hydroxide. The formation of the amide was followed on TLC with $CHCl_3$/MeOH 95:5 (Rf 0.25). After 30 min the reaction mixture was taken to dryness and the residue redissolved with 300 ml of DCM and washed with water (1 x 50 ml), 5% Na_2CO_3 (2 x 70 ml) and water until neutrality. After dehydration and evaporation of the solvent, 2.288 g of pure amide as a slightly yellow oil were obtained (yield 87.4%).

^{19}F-NMR ($CDCl_3$) δ -75.9; ^{1}H-NMR (CD_3OD) δ 7.35-7.28 (m, 5H, arom), 5.10-5.06 (2d, $\underline{CH_2}$-C_6H_5, J = 8.4 Hz), 4.23 (q, $\underline{CH_2}$-CH_3), 2.26-1.40 (m, 6H, $(CH_2)_3$ CO), 1.23 (t, CH_2-$\underline{CH_3}$, J = 7.1 Hz); ^{13}C-NMR ($CDCl_3$) δ 179.25, 166.4, 154.1, 136.0, 128.6, 128.3, 128.1, 123.95 (q, J = 288 Hz), 67.1, 65.95 (q, J = 28.8 Hz), 63.6, 34.9, 28.05, 19.0, 13.8; MS (EI, 70 eV) m/z (%) 391 (M^+ + 1, 33), 283 (28), 166 (21), 107 (35), 91 (100)

2-N-Cbz-2-Tfm-Hexanediacid-6-amide 3.

Compound **2** (2.288 g, 5.8604 mmol) was suspended in a solution of 0.5 N KOH (1.683 g of potassium hydroxide in 60 ml of MeOH/H_2O 7:3) and left at r. t. under stirring overnight. The disappearance of the ester was followed on TLC, $CHCl_3$/MeOH 90:10. At the end of the hydrolysis, the solution was passed through a column of resin (Dowex 50-X8, cationic exchanger) and evaporated to dryness. The residue obtained consisted of 1.722 g of a colorless thick oil (yield 81.8%).

^{19}F-NMR ($CDCl_3$) δ -75.9; ^{1}H-NMR (CD_3OD) δ 7.35-7.28 (m, 5H arom), 5.09 (s, $\underline{CH_2}$-C_6H_5), 2.48-2.10 (m, 2 CH_2), 1.64 (m, CH_2-$\underline{CH_2}$-CH_2); ^{13}C-NMR ($CDCl_3$) δ 178.2, 169.0, 154.4, 135.8, 128.6, 128.4, 128.1, 124.0 (q, J = 286.6 Hz), 67.2, 65.6 (q, J = 29.6 Hz), 34.5, 27.8, 19.2; MS (EI, 70 eV) m/z (%) 363 (M^+ + 1, 9), 319 (6), 255 (50), 212 (68), 166 (100), 121 (52), 108 (82), 91 (91).

Cbz-α-Tfm-Orn-OH·HCl 4.

The amide **3** (1.16 g, 3.2 mmol) was dissolved in 10 ml of acetonitrile and diluted with 4 ml of water. Freshly crystallized TIB (4 g, 9.3 mmol) was added and the solution left overnight at r. t. with stirring and in a subdued light. The reaction mixture was concentrated to remove the acetonitrile, diluted with 150 ml of 0.1 N HCl and washed with diethyl ether (3 x 70 ml). The organic layer was washed back with 0.1 N HCl (2 x 20 ml). The collected aqueous phase was evaporated under reduced pressure to give 928 mg of the amine hydrochloride (yield 78%).

^{19}F-NMR (DMSO-D) δ -68.23; ^{1}H-NMR (DMSO-D) δ 8.03 (br signal, NH_3^+), 7.37 (m, 5H arom), 5.05 (s, $\underline{CH_2}$-C_6H_5), 2.76 (m, CH_2-N), 2.00 (m, CH_2-C-CF_3), 1.75-1.62 (m, $\underline{CH_2}$-CH_2-N); MS (EI, 70 eV) m/z (%) 316 (M^+ - H_2O, 6), 209 (8), 182 (30), 166 (39), 138 (41), 137 (77), 91 (100).

Cbz-α-Tfm-Orn(Boc)$_2$ -OH **5.**

The amine **4** (928 mg, 2.5 mmol) was dissolved with 4 ml of dioxane and 5.5 ml of 0.5 N NaOH in an ice bath. Boc-anhydride (1.091 g, 5 mmol) was added dropwise and the pH adjusted to about 8. The reaction mixture was kept under stirring at r. t. for 1.5 h, adjusting the pH with 0.5 N NaOH when necessary. The disappearance of the amine was followed on TLC, $CHCl_3$/MeOH 70:30 + 1% AcOH. The reaction mixture was concentrated to about 1 ml and diluted with 5 ml water, cooled in an ice bath and acidified to pH 2 with $KHSO_4$, then immediately extracted with ethyl acetate (4 x 5 ml). The collected organic phase was washed back with 2 ml of brine and dried with Na_2SO_4. After evaporation 1.083 g of product **5** was obtained as an oil, with 81% yield.

^{19}F-NMR ($CDCl_3$) δ -76.1; ^{1}H-NMR ($CDCl_3$) δ 7.33 (m, 5H arom), 6.1 (s, NH-CO), 5.1 (s, $\underline{CH_2}$-C_6H_5), 3.18-2.72 (m, 2 CH_2), 2.18 (m, CH_2-$\underline{CH_2}$-CH_2),1.5 (2s, CH_3, Boc); MS (EI, 70 eV) m/z (%) 335 (M^+ + 3 - 2 Boc, 22), 182 (19), 137 (29), 108 (33), 91 (100).

Cbz-α-Tfm-Orn(Boc)$_2$-Gly-Asp(OtBu)-Phe-NH$_2$ **6.**

The protected amine **5** (812 mg, 1.52 mmol) was dissolved with 13 ml of a mixture of DMF/DCM 4:9 and DCC (380 mg, 1.82 mmol) was added with stirring. The disappearance of the substrate and the formation of the oxazolone was followed on TLC with $CHCl_3$/MeOH 70:30 + 1% AcOH. After 2 h the reaction was complete and the suspension was cooled to -20°C and filtered. The tripeptide **5a** (596.6 mg, 1.52 mmol) was directly added into the filtrate and kept at r. t. for 3 h with stirring. The consumption of the oxazolone (Rf 0.75) and formation of the peptide (Rf 0.25) were monitored by TLC with $CHCl_3$/MeOH 93:7. The reaction mixture was evaporated to dryness under vacuo and the residue was purified by flash-chromatography with $CHCl_3$/MeOH 93:7. The collected fractions after evaporation gave the pure peptide **6**, 822 mg, yield 60%.

^{1}H-NMR (DMSO-D) δ 8.4-7.13 (br, 6H amide), 7.29 (m, 2 C_6H_5), 5.19 (m, $\underline{CH_2}$, Cbz), 4.63 (q, CH, Asp), 4.46 (q, CH, Phe), 3.64 (d, CH_2, Gly), 3.10 (m, CH_2, Phe), 2.98 (m, CH_2-N), 2.79 (m, CH_2, Asp), 2.48 (m, CH_2-C-CF_3), 1.50 (m, CH_2-$\underline{CH_2}$-CH_2), 1.46 (2s, CH_3, Boc); MS (FAB 70 eV) m/z 809 (M^+ - Boc + 2).

5-Aminopropyl-5-Tfm-hydantoin,3-methylcarbonyl-Asp-Phe-NH$_2$ **7.**

200 mg of compound **6** (0.22 mmol) were dissolved with 18 ml of TFA and left at r.t. for 1h. The disappearance of the substrate was monitored by RP-HPLC with CH_3CN 40% in H_2O + 0.1% TFA, Rt 36.5 min; while the formation of product **7** with CH_3CN 16% in H_2O + 0.1% TFA, Rt 16 and 20 min (**I** and **II** isomers). The two diastereomers were collected together with 100% yield.

^{19}F-NMR (D2O) δ -71.96 (s, CF_3COOH), -73.20 and -73.23 (2s, CF_3, hydantoin); ^{1}H-NMR (D_2O) δ 7.40 (m, 5H arom), 4.68 (m, CH, Asp + Phe), 4.34 (q, CH_2, Gly), 3.10 (m, CH_2-N), 3.28-3.00 (m, CH_2, Phe), 2.86-2.80 (m, CH_2, Asp), 2.32-2.16 (m, CH_2-C-CF_3), 1.85-1.67 (m, CH_2-$\underline{CH_2}$-CH_2); MS (FAB 70 eV) m/z 545 (M^+ + 1).

α-Tfm-Orn-Gly-Asp-Phe-NH$_2$·2 TFA **8.**

To a solution of compound **6** (800 mg, 0.88 mmol) in 4 ml MeOH, 1,4-cyclohexadiene (824 μl, 8.8 mmol) and 800 mg of 10% Pd/C were added under N_2 atmosphere with vigorous stirring. After 1h at r.t. the reaction

mixture was diluted with MeOH up to 2 ml and centrifuged. After separation of the supernatant, the residue was washed with additional 2 ml of MeOH and the operation repeated several times. The collected supernatant fractions were taken to dryness and the residue was redissolved with 20 ml of TFA and left at r.t. for 1h with stirring. After removing TFA, the residue consisted of 583 mg of crude deprotected peptide. Yield 88%. A small amount of the latter residue was dissolved in MeOH and purified on semipreparative HPLC (16% CH_3CN in H_2O + 0.1 % TFA; Rt from 19.09 to 24.41 min) for spectrometric characterization.

^{19}F-NMR (DMSO-D) δ -69.58 (s, CF_3COOH), -71.20 (2s, CF_3, Orn); ^{1}H-NMR (DMSO-D + D_2O) δ 7.21 (m, 5H arom), 4.51 (q, CH, Asp), 4.38 (q, CH, Phe), 3.75 (m, CH_2, Gly), 3.05 (m, CH_2, Phe), 2.75 (m, CH_2-N), 2.61-2.41 (m, CH_2, Asp), 1.95-1.48 (m, CH_2-CH_2); MS (FAB, 70 eV) m/z 519 (M^+ + 1).

α-Tfm-Arg-Gly-Asp-Phe-NH_2·n TFA ***9.***

500 mg (0.67 mmol) of the peptide **8** were dissolved in 2.5 ml of MeOH under N_2 atmosphere. Diisopropylethylamine (686 ml, 4.02 mmol) and 1-H-pyrazole-1-carboxamidine·HCl (295 mg, 2.01 mmol) were added and the solution kept under stirring at r. t. for 2 h. The guanylation reaction was monitored by RP-HPLC (Ultrasphere column; flow rate 2 ml/min; mobile phase 16% CH_3CN in H_2O + 0.1% TFA). The two initial peaks at Rt 19.1 and 24.4 min disappeared gradually to give way to the two new twin peaks at Rt 20.3 and 25.5 min, corresponding to the diastereomeric forms of the peptide **9**. The two diastereomers were separately collected and lyophilized with a yield of 91%; according to their Rt, they were indicated as **I** (fast-moving) and **II** (slow-moving).

Isomer **I**: ^{19}F-NMR (DMSO-D) δ -69.6 (s, CF_3COOH), -71.0 (s, CF_3, Arg); ^{1}H-NMR (DMSO-D) δ 8.29 (t, NH, Gly, J= 5.3 Hz), 8.27 (d, NH, Asp, J= 8.0 Hz), 7.83 (d, NH, Phe, J= 8.7 Hz), 7.44 (t, NH, Arg, J= 6.0 Hz), 7.28-7.15 (m, 5H arom), 7.09 (s, CO-NH_2, Phe), 6.99 (br, NH_3^+, Arg), 4.54 (td, CH, Asp, J= 6.0 and 7.9 Hz), 4.37 (td, CH, Phe, J= 4.8 and 8.7 Hz), 3.78 and 3.72 (2dd, CH_2, Gly, J= 5.3 and 16.8 Hz), 3.09 (t, CH_2, Arg, J= 6.0 Hz), 3.04 and 2.82 (2 dd, CH_2, Phe, J= 4.8, 8.7 and 13.6 Hz), 2.65 and 2.41 (2 dd, CH_2, Asp, J= 6.0, 7.9 and 16.6 Hz), 1.94-1.45 (m, CH_2-CH_2); MS (FAB, 70 eV) m/z 561 (M^+ +1).

Isomer **II**: ^{19}F-NMR (DMSO-D) δ -69.4 (br, CF_3COOH), -71.1 (s, CF_3, Arg); ^{1}H-NMR (DMSO-D) δ 8.33 (t, NH, Gly, J= 5.5 Hz), 8.31 (d, NH, Asp, J= 7.8 Hz), 7.85 (d, NH, Phe, J= 8.8 Hz), 7.43 (br, NH, Arg), 7.28-7.15 (m, 5 H arom), 7.09 (s, CO-NH_2, Phe), 6.99 (br, NH_3^+, Arg), 4.54 (td, CH, Asp, J= 5.8 and 7.8 Hz), 4.37 (td, CH, Phe, J= 4.6 and 8.8 Hz), 3.79 and 3.70 (2 dd, CH_2, Gly, J= 5.5 and 16.5 Hz), 3.09 (m, CH_2, Arg), 3.02 and 2.84 (2 dd, CH_2, Phe, J= 4.6, 8.8 and 13.8 Hz), 2.66 and 2.41 (2 dd, CH_2, Asp, J= 5.8, 7.8 and 16.5 Hz), 1.95-1.45 (m, CH_2-CH_2); MS (FAB, 70 eV) m/z 561 (M^+ + 1); Elem.Anal.: for $C_{22}H_{31}F_3N_8O_6$·3 CF_3COOH·2 H_2O, MW 938.6, Calc.%: C 35.8, H 4.05, F 24.30, N 11.90, Found %: C 36.2, H 4.03, F 23.65, N 11.64.

Cbz-α-Tfm-Homo-Gln-Gly-Asp(OtBu)-NH-$(CH_2)_2$-C_6H_5 ***10.***

Compound **3** (958 mg, 2.64 mmol) was dissolved in 20 ml of a mixture of DMF/DCM 1:1.5, DCC (650 mg, 3.15 mmol) was added and the reaction mixture was left with stirring overnight. The formation of the oxazolone was monitored by TLC, $CHCl_3$/EtOAc 1:1 + 1% AcOH, by spraying with ninhydrin. After cooling

and filtering, the solution was taken to dryness under reduced pressure and the residue dissolved with 18 ml of DCM. The peptide H-Gly-Asp(OtBu)-NH-$(CH_2)_2$ -C_6H_5 **3a** (898 mg, 2.57 mmol) was added and the solution left at r. t. for 5 h. The consumption of oxazolone (Rf 0.71) and formation of the peptide (Rf 0.4) were monitored by TLC, $CHCl_3$/MeOH 8:2. After evaporation of the solvent, the residue was purified by flash-chromatography in two elution stages: I $CHCl_3$/MeOH 98:2; II $CHCl_3$/MeOH 90:10. The collected fractions gave 878 mg of the tripeptide **10**. Yield 51%.

^{1}H-NMR (DMSO-D) δ 8.56, 8.35, 8.07, 7.88, 7.62, 6.77 (NH amides), 7.35 (m, 10 H, arom.), 5.15 (m, CH_2-O), 4.65 (t, CH, Asp), 4-3.6 (m, CH_2, Gly), 3.25 (m, NH-$\underline{CH_2}$-CH_2), 2.75 (m, NH-CH_2-$\underline{CH_2}$), 2.5 (m, CH_2 Asp), 2.05 (m, CH_2-C-CF_3 + $\underline{CH_2}$-$CONH_2$), 1.6 (m, CH_2-$\underline{CH_2}$-CH_2), 1.45 (s, 9 H, OtBu).

Cbz-α-Tfm-Orn-Gly-Asp(OtBu)-NH-$(CH_2)_2$-C_6H_5·TFA ***11.***

Compound **10** (878 mg, 1.26 mmol) was dissolved in 9.6 ml of acetonitrile and 1.9 ml of water were added dropwise under stirring. The TIB reagent (1.092 g, 2.54 mmol) was added to the cloudy solution (which became clear) and left under stirring at r. t. in a subdued light. On TLC ($CHCl_3$/MeOH 80/20) it was possible to follow the consumption of the amide (Rf 0.55) and the formation of the amine (Rf 0.2). After 4 h the solution was concentrated to small volume, diluted to 20 ml with water and extracted with ether (5 x 10 ml). After evaporation of the water, the peptide **11** was recovered in the residue in 82 % yield.

α-TFM-Orn-Gly-Asp-NH-$(CH_2)_2$ -C_6H_5·2 TFA ***12.***

Compound **11** (800 mg, 1.024 mmol) and thioanisole (6.36 g, 51.2 mmol) were dissolved in 25 ml of TFA, left 1 h under stirring at r. t. and evaporated to dryness. The peptide **12** was identified by RP-HPLC as the typical twin peaks (due to the two diastereomeric forms) with Rt 23.05 and 24.13 min; mobile phase 12% CH_3CN in H_2O + 0.1% TFA.

α-Tfm-Arg-Gly-Asp-NH-$(CH_2)_2$ -C_6H_5·2 HBr ***13.***

The unpurified tripeptide **12** (720 mg, 1.024 mmol) was dissolved with 9 ml of water and O-methylisourea sulphate (1.763 g, 10.24 mmol) was added followed by 11.8 ml of 2N NaOH. PH 10 must be maintained during the reaction. The mixture was heated at 75°C for 1 h with stirring. The reactions kinetics was followed on HPLC with 12 % CH_3CN in H_2O + 0.1% TFA as the mobile phase: the two peaks at Rt 23.05 and 24.13 min disappeared gradually giving way to the new peaks at 25.7 and 27.8 min. After 1 h the reaction was complete. The two diastereomers were recovered as TFA salts with a purity ≥ 99%, in 75% yield. In order to convert the TFA salts into HBr salts, 100 mg of both diastereoisomers were dissolved in 2.77 ml of 0.1 N HBr and concentrated to a small volume under reduced pressure at a temperature of about 30°C, diluted with 5 ml of water and taken to dryness. The residue was redissolved with water and taken to dryness again, and the same operation repeated for several times. At the end, the products were dissolved with 5 ml of water and lyophilized to obtain 85 mg of each pure peptide hydrobromide.

^{1}H-NMR 90 MHz (D_2O) δ 7.3 (s, 5 H arom.), 4.65 (t, CH, Asp), 4.06 (d, CH_2, Gly), 3.5 (t, $\underline{CH_2}$-NH), 3.3 (t, $\underline{CH_2}$-NH-C=NH), 2.82 (t, $\underline{CH_2}$-C_6H_5), 2.78 (d, CH_2 Asp), 2.35 (m, CH_2-C-CF_3), 1.78 (m, CH_2-$\underline{CH_2}$-CH_2); Elem.

Anal.: for $C_{21}H_{30}F_3N_7O_5 \cdot 2$ HBr 2.5 H_2O, MW 724, Calc. %: C 34.78, H 5.10, Br 22.1, F 7.87, N 13.53, **Found %**: C 34.70, H 5.03, Br 22.9, F 8.20, N 13.52.

REFERENCES

1. Samanen, J.; Ali, F.; Romoff, T.; Calvo, R.; Sorenson, E.; Vasko, J.; Storer, B.; Berry, D.; Bennett, D.; Strohsacker, M.; Powers, D.; Stadel, J.; Nichols, A. *J. Med. Chem.* **1991**, *34*, 3114-3125.
2. Alig, L.; Edenhofer, A.; Hadváry, P.; Hürzeler, M.; Knopp, D.; Müller, M.; Steiner, B.; Trzeciak, A.; Weller, T. *J. Med. Chem.* **1992**, *35*, 4393-4407.
3. Weller, T.; Alig, L.; Beresini, M.; Blackburn, B.; Bunting, S.; Hadváry, P.; Hürzeler, M.; Knopp, D.; Levet-Trafit, B.; Lipari, M. T.; Modi, N. B.; Müller, M.; Refino, C. J.; Schmitt, M.; Schönholzer, P.; Weiss, S.; Steiner, B. *J. Med. Chem.* **1996**, *39*, 3139-3147.
4. McDowell, R. S.; Gadek, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 9245-9253.
5. Ali, F. E.; Bennett, D. B.; Calvo, R. R.; Elliott, J. D.; Hwang, S.; Ku, T. W.; Lago, M. A.; Nichols, A. J.; Romoff, T. T.; Shah, D. H.; Vasko, J. A.; Wong, A. S.; Yellin, T. O.; Yuan, C.; Samanen, J. M. *J. Med. Chem.* **1994**, *37*, 769-780.
6. McDowell, R. S.; Blackburn, B.K.; Gadek, T. R.; McGee, L. R.; Rawson, T.; Reynolds, M. E.; Robarge, K. D.; Somers, T. C.; Thorsett, E. D.; Tischler, M.; Webb II, R. R.; Venuti, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 5077-5083.
7. Sewald, N.; Burger, K. Synthesis of β-fluorine-containing Amino Acids. In *Fluorine-containing Amino Acids;* Kukhar', V.P.; Soloshonok, V. A., Eds.; John Wiley: New York, 1995, pp. 139-220.
8. Koksch, B.; Ullmann, D.; Jakubke, H.-D.; Sewald, N.; Burger, K. Proc. 23rd Eur. Pept. Symp., **1994**; Braga, Portugal, pp. 323-324.
9. Marshall, G. R.; Clarc, J. D.; Dunbar Jr.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. *Int. J. Pept. Protein Res.* **1988**, *32*, 544-555.
10. Koksch, B.; Sewald, N.; Jakubke, H.D. Synthesis and Incorporation of α-Trifluoromethyl-Substituted Amino Acids into Peptides. In *Biomedical Frontiers of Fluorine Chemistry* ; ACS Symp. Series 639, Chapt 3; Ojima, I.; McCarthy, J.R.; Welch, J.T., Eds.; Am. Chem. Soc.: Washington DC, 1996; pp. 42-58.
11. Koksch, B.; Sewald, N.; Hofmann, H.J.; Burger, K.; Jakubke, H.D. *J. Pept. Sci.* **1997**, *3*, 157-167.
12. Burger, K.; Gaa, K. *Chemiker Zeitung* **1990**, *114*, 101-104.
13. Burger, K.; Mütze, K.; Hollweck, W.; Koksch, B.; Kuhl, P.; Jakubke, H.-D.; Riede, J.; Schier, A. *J. prakt. Chem.* **1993**, *335*, 321-331.
14. Bernatowicz, M. S.; Youling, Wu; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497-2502.
15. Kricheldorf, H. R. *Synthesis* **1972**, 695-697.
16. Soloshonok, V. A.; Gerus, I. I.; Yagupol'skii, Yu I.; Kukhar', V. P. *Zh. Org. Khim.* **1987**, *23*, 2308-2313.