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Synthesis and Evaluation of RGD Peptidomimetics Aimed at Surface Bioderivatization of Polymer Substrates

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Abstract—Several RGD peptidomimetics have been prepared, in a convergent way, from the common *ortho*-aminotyrosine template (*O*-substituted with an anchorage-arm or a methyl group, and αN-substituted with a fluorine tag for XPS analysis), and various ω-aminoacid derivatives. The most flexible compounds have shown a biological activity similar to that of the peptide reference (RGDS) in the platelet aggregation test. The compound **16a** could be fitted (by modelisation) with DMP 728 and c(RGDfV), two cyclic peptides that are good ligands of integrins. The compound **16b** has been covalently fixed on the surface of a poly(ethylene terephthalate) membrane used as support for mammalian cell cultivation.

Introduction

The use of synthetic polymers in medical engineering and biotechnological applications has increased considerably in recent years.[1,2] For instance, polymer membranes are developed as supports for in vitro cultivations of mammalian cells used as models of biological barriers to investigate transport of nutrients or pharmacological agents.[3–5] Our interest in this field led us to perform surface modifications of the supports in view to improve the cellular adhesion and growth.[6] A first approach consisted in the introduction of functional motifs susceptible to increase the surface hydrophilicity and charge.[7–13] The chemically modified surfaces were further biocompatibilized by the covalent coupling of extracellular matrix (ECM) proteins.[6,14] Our current purpose is to replace proteins by stable synthetic biological signals that would similarly promote cell anchorage. Accordingly, our active biocompatibilization strategy of polymeric substrates relies on the covalent fixation of small molecules acting as integrin ligands onto the material surface.

Integrins[15] are heterodimeric transmembrane glycoproteins involved in cell–cell and cell–matrix interactions;[16] these cell surface receptors interact with the cytoskeleton and play a crucial role in the signal transduction processes.[17] Most of integrins bind to adhesive proteins displaying the Arg-Gly-Asp (RGD) sequence.[18] Platelets aggregation is mediated by the $\alpha_{IIb}\beta_3$ integrin, which natural ligand is the blood protein fibrinogen.[19] The related integrin $\alpha_v\beta_3$ of endothelial cells has been recognized as an important mediator of cellular adhesion and migration in the angiogenesis process;[20] in this case, the natural ligand is the ECM protein vitronectin. A lot of flexible linear peptides containing the RGD sequence bind to integrins, but non specifically.[21] On the other hand, cyclic RGD peptides appear more selective towards either $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ integrins.[22]

Over the last 10 years, peptidomimetics[23–25] of the RGD sequence have been developed as potential drugs,

Key words: RGD (Arg-Gly-Asp); peptidomimetic; integrin ligand; poly(ethylene terephthalate) surface; substratum bioderivatization.

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mainly in the field of orally available anticoagulants ($\alpha_{IIb}\beta_3$ antagonists) for the treatment of thromboembolic diseases.[26] Very recently, the search of $\alpha_v\beta_3$ antagonists has emerged in order to treat cancers[27] and other disorders in which neovascularization plays a critical role.[28]

We planned to functionalize our cell culture substrates with RGD peptidomimetics. In the previous literature, the grafting of RGD peptides was reported to significantly improve the cellular adhesion on various polymeric supports.[29–31] However the effect of non-peptide mimics was never considered, though such synthetic signals should be more stable under biological fluids, storage and sterilization conditions.

In this paper we report the preparation of several RGD peptidomimetics based on the *ortho*-amino-tyrosine template, and their evaluation in the classical platelet aggregation test. One molecule (**16b**) equipped with an anchorage-arm has been covalently fixed on a poly (ethylene terephthalate) (PET) microporous membrane used as cell cultivation support.

Results and Discussion

Synthesis

Several series of RGD peptidomimetics based on various rigid scaffolds[32,33] have been proposed as antiplatelet agents; for instance, molecules were constructed from benzodiazepine,[34] isoquinolone,[35] isoxazoline,[36] pyrazolopiperazinone,[37] or 3-(hydroxymethyl) benzamide[38] moieties. The situation appears totally different in the case of RGD mimics designed to promote adhesion phenomena; to our knowledge, only four types of molecules have been very recently disclosed, based on *p*-hydroxybenzamide,[27] piperazine,[39] carbohydrate,[40] or benzodiazepine[27,41] templates.

When starting this work, without reliable guide in hand, we decided to examine relatively flexible structures derived from (L)-tyrosine. The utilization of this template has been well exemplified by the Merck's scientists;[42,43] one compound (Aggrastat™, MK-383) is in phase III clinical trials.[44] The advantages of the tyrosine template and the designed structural features of our RGD peptidomimetics are summarized in the Figure 1: (a) the aromatic template is commercially available and already equipped with the first arm mimicking the D (Asp) residue; (b) the α-amino function, that has to be masked with a lipophilic group,[43] can be advantageously used for the fixation of a fluorine tag in view of the X-ray photoelectron spectroscopic (XPS) analysis of the final polymer substrates;[45,46] (c) the aromatic

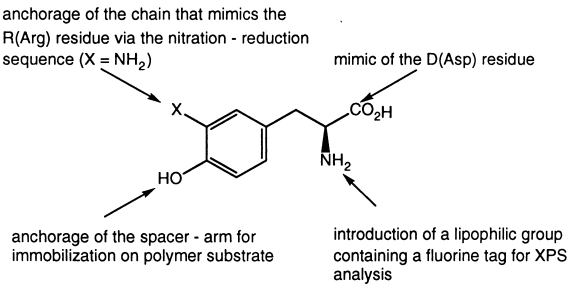


Figure 1. The tyrosine (X=H) template and its arrangement.

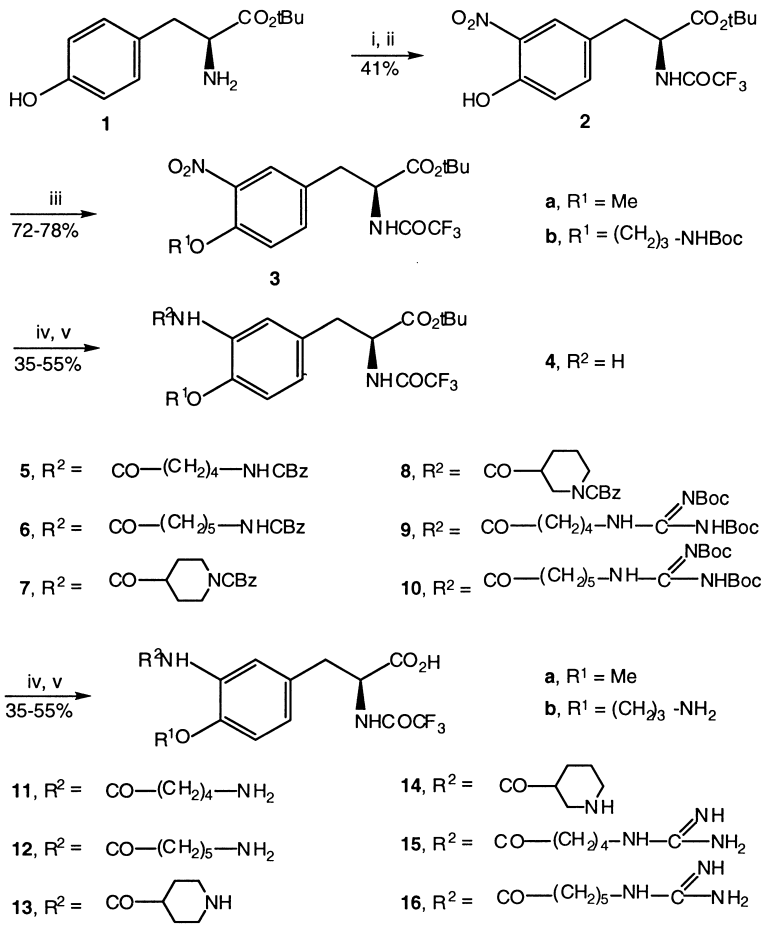
hydroxyl function can be used to anchor a second arm needed for immobilizing the molecule onto polymer substrates; (d) nitration of the aromatic ring, followed by reduction, offers the anchorage point of the third arm mimicking the R (Arg) residue; the distance separating the acidic and basic residues of the pharmacophore should be within 10–20 Å.[27,43,47]

The *N*-trifluoroacetyl group was initially chosen as fluorine tag for the XPS analysis of the surface modified polymer membranes. Therefore, in our synthetic plan, we have avoided using protective groups removable under basic conditions that could cleave our spectroscopic label; accordingly, we played exclusively with the classical tertio-butyl (^{t}Bu), tertio-butoxycarbonyl (Boc), and benzyloxycarbonyl (Cbz) groups.

(L)-Tyrosine tertio-butylester **1** was reacted with neat trifluoroacetic anhydride to give *N*-trifluoroacetyl-(L)-tyrosine tertio-butylester. This intermediate was directly treated with a solution of nitric acid in acetic acid at low temperature to furnish the mono-nitration product **2** isolated by column-chromatography (Scheme 1).

For chemoselectivity reasons, the *O*-alkylation of the phenol ring has to be performed before the reduction of the nitro group, and the subsequent functionalization of the resulting aniline. This Williamson reaction, planned to introduce the anchorage-arm for immobilization onto polymer membranes, was first examined with methyliodide. Thus, **2** was reacted with methyliodide, in refluxing acetonitrile, in the presence of powdered potassium carbonate and a crown ether as catalyst. The product **3a**, contaminated with a small amount of *N*-methylation product of the trifluoroacetamide residue, was purified by preparative medium pressure liquid chromatography (MPLC) in 72% yield. The *N*-protected anchorage-arm, *N*-(Boc)-3-bromopropylamine, could be similarly coupled to **2**, giving **3b** in 78% yield after purification (Scheme 1).

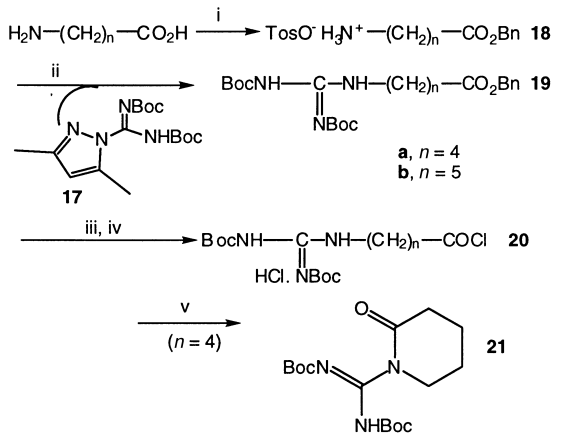
The aromatic ring functionalization with various arms mimicking the basic residue of Arg was systematically



Scheme 1. Synthesis of peptidomimetics (first family). Reagents and conditons: (i) $(CF_3CO)_2O$, 0°C, 2 h; (ii) HNO_3-HOAc, 10 °C, 1 h; (iii) a. CH_3I, CH_3CN, K_2CO_3, reflux, 48 h: b. BocNH-$(CH_2)_3$-Br, CH_3CN, K_2CO_3, reflux, 24 h; (iv) H_2, PtO_2, EtOH, 4–12h, 20 °C; (v) acid chloride, pyridine, CH_2Cl_2, 17 h, 20 °C; then column chromatography; (vi) Pd-C, EtOAc, 20 °C, 18 h; (vii) CF_3CO_2H, 2 h, 20 °C.

investigated, using the precursor **3a** in which the anchorage-arm is replaced with a simple methyl group. Reduction of **3a** by catalytic hydrogenation over platinium oxide furnished the key-intermediate aniline **4a** which was directly used without purification. The reactivity of this aniline in the peptide coupling reaction was examined, under various experimental conditions, using *N*-(Cbz)-5-aminovaleric acid as partner. Low yields of the anilide **5a** were obtained when the acid was activated in situ with diisopropylcarbodiimide/dimethylaminopyridine, dicyclohexyl-carbodiimide/*N*-hydroxysuccinimide, or *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate (HBTU). The best results were obtained when preforming the acid chloride with thionyl chloride or 1-chloro-*N*,*N*, 2-trimethylpropenylamine;[48] reaction of *N*-(Cbz)-5-aminovaleryl chloride with **4a** and triethylamine in dichloromethane at room temperature gave 45% yield of the coupling product **5a** after purification by column-chromatography. This strategy was thus selected for the coupling of *N*-(Cbz)-6-aminocaproic acid, *N*-(Cbz)-isonipecotic acid and *N*-(Cbz)-nipecotic acid. Reaction of the corresponding preformed acid chlorides with **4a** led, respectively, to the anilides **6a**, **7a**, and **8a** in 35–55% yields (Scheme 1).

Hydrogenolysis of the Cbz protective group of **5a** gave the free amine **11a** (*t*Bu ester) which was submitted to various guanidylation conditions. Unfortunately, all attempts to react **11a** with unprotected guanidylation reagents (i.e. aminoiminomethane sulfonic acid[49] and 1*H*-pyrazole-1-carboxamidine)[50] and with the protected reagent **17**, *N*,*N*′-ditertio-butoxycarbonyl-3,5-dimethylpyrazole-1-carboxamidine,[51] failed. Therefore, we considered a more convergent route towards **9a** (or **15a**) and **10a** (or **16a**), using *N*-guanidyl-aminoacid derivatives as partners in the peptide coupling with the aniline **4a**. The required acid chlorides **20** were obtained according to the Scheme 2.

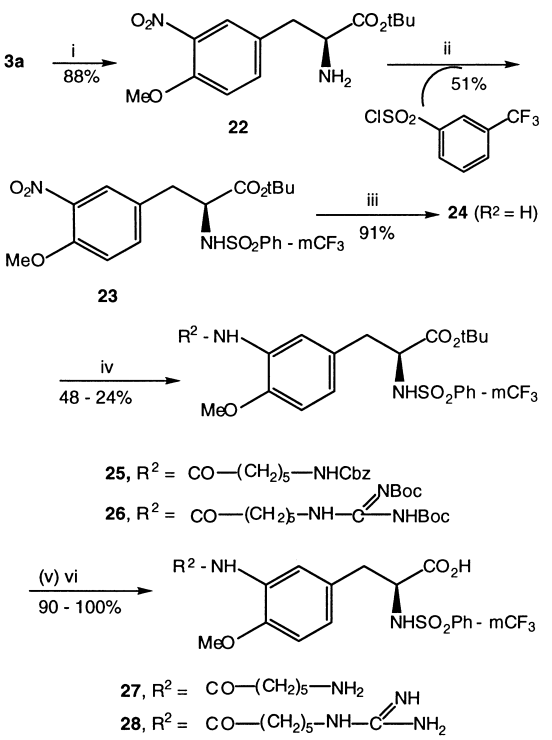


Scheme 2. Synthesis of acid chlorides. Reagents and conditions: (i) $PhCH_2OH$, pTos-OH, benzene, reflux, 3 h; (ii) Et_3N, CH_2Cl_2, 20 h, 20 °C; (iii) H_2, Pd-C, EtOH:EtOAc (1:1), 18 h, 20 °C; (iv) $SOCl_2$, CH_2Cl_2, 1 h, 30 min, reflux; (v) pridine, CH_2Cl_2, 20 °C.

5-Aminovaleric acid (**a**, $n=4$) and 6-aminocaproic acid (**b**, $n=5$) were esterified with benzyl alcohol under standard conditions (**18a**,**b**), then reacted with *N*,*N*′-ditertiobutoxycarbonyl-3,5-dimethylpyrazole-1-carboxamidine **17**[51] and triethylamine in dichloromethane. After preparative MPLC, compounds **19a**,**b** were quantitatively obtained. Cleavage of the benzyl ester by hydrogenolysis, followed by reaction with thionyl chloride gave the acid chlorides **20a**,**b**, which were not purified (Scheme 2). Reaction of **20b** ($n=5$) with the aniline key-intermediate **4a** in the presence of pyridine furnished the coupling product **10a** in 35% yield after purification by column-chromatography (Scheme 1). The lower homologue **9a** could not be obtained by similarly treating **4a** with the valeric derivative **20a** ($n=4$); in the presence of pyridine, the acid chloride **20a** cyclized intramolecularly very rapidly (**21**, Scheme 2). Thus, in our hands, the peptidomimetic **15a** (after deprotection of **9a**) was not accessible. Treatment of **10a** with trifluoroacetic acid produced the fully deprotected guanidyl derivative **16a**. *N*-Deprotection of the aminocaproic derivative **6a** and (iso)nipecotic derivatives **7a** and **8a** by hydrogenolysis, followed by treatment with trifluoroacetic acid to cleave the tertio-butyl ester, gave the unprotected compounds **12a**, **13a** and **14a**, respectively (Scheme 1).

All the compounds **6**–**8**, **10**, **12**–**14**, and **16** were fully characterized by the usual spectroscopic methods (see Experimental). The typical features in the ^1H NMR spectra of the tested compounds (unprotected derivatives in D_2O) are as follows: (a) the three aromatic protons of the tyrosine backbone give respectively a fine doublet at 7.3–7.5 δ ($J \sim 2$ Hz), a doublet of doublet at 7.1–7.2 δ ($J \sim 2$ Hz and 8.7 Hz) and a doublet at 7.0–7.1 δ ($J \sim 8.7$ Hz); (b) the proton of the tyrosine α-CH group is a doublet of doublet at 4.6–4.8 δ ($J \sim 5$ and 9.5 Hz); (c) the protons of the tyrosine β-CH_2 group appear to be non-equivalent, giving a ABX pattern centred at 2.95–3.00 δ and 3.25–3.30 δ (JAB ~ 14 Hz). In the ^{13}C NMR spectra (D_2O), (a) the carbon atom of the CF_3 XPS tag is visible at 118 ppm (Q); (b) the three substituted carbon atoms of the tyrosine aromatic ring give lines at 153 ppm, 131 ppm and 127 ppm, corresponding to carbons linked to oxygen, nitrogen and carbon respectively; (c) the α and β carbons of the aliphatic tyrosine chain appear at 57 ppm and 38 ppm, respectively.

In another set of experiments, we considered the *meta*-trifluoromethyl-benzenesulfonyl group (XPS tag) for masking the α-amino function of the tyrosine template. Due to the high acidity of the sulfonamide proton, the presence of this function is not compatible with the phenol ring etherification under the Williamson conditions. Accordingly, the sulfonylation of the α-amino function was performed after the tyrosine *O*-alkylation step. Thus, compound **3a** (see Scheme 1) was submitted



Scheme 3. Synthesis of peptidomimetics (second family). Reagents and conditions: (i) K_2CO_3, MeOH:H_2O (1:1), 17 h, 20 °C; (ii) Et_3N, CH_2Cl_2, 20 h, 20 °C; (iii) H_2, PtO_2, MeOH, 17 h 20 °C; (iv) acid chloride, pyridine, CH_2Cl_2, 20 h, 20 °C; then column chromatography; (v) H_2, Pd-C, EtOH:EtOAc (1:1), 18 h, 20 °C; (vi) CF_3CO_2H, 2 h 30 min, 20 °C.

to basic hydrolysis to furnish the free amine **22** which was directly treated with *meta*-trifluoromethyl-benzenesulfonyl chloride and triethylamine; the sulfonamide **23** was purified by flash-chromatography with medium yields (Scheme 3). Reduction of the aromatic nitro function by hydrogenation over platinium oxide gave the aniline key-intermediate **24**. This aniline was coupled with *N*-(Cbz)-6-aminocaproyl chloride and with (*N*,*N*′-diterbutoxycarbonyl)-6-guanidino-caproyl chloride **20b** as previously described; the corresponding anilides **25** and **26** were obtained in 48% and 24% yield after flash-chromatography. The final deprotections were realized by hydrogenolysis followed by treatment with trifluoroacetic acid to give **27**, and by treatment with trifluoroacetic acid to give **28** (Scheme 3). The NMR characteristics of compounds **25**–**28** are similar to those of the previous series bearing the trifluoroacetamide group (see Experimental): the proton of the α-CH group gives a doublet of doublet at 4 δ; the carbon of the CF_3 XPS tag is visible at 124 ppm (Q).

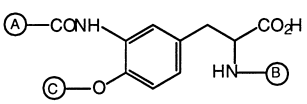
At last, we prepared a peptidomimetic fully equipped for the anchorage onto polymer substrates. Reduction of the aromatic nitro function of compound **3b** (bearing the *N*-protected aminopropyl arm) by catalytic hydrogenation over platinium oxide gave the key-intermediate aniline **4b** (Scheme 1). Coupling with the *N*-protected 6-guanidino-caproyl chloride **20b** in the presence of pyridine furnished the anilide **10b** in 51% yield after flash-chromatography. Complete deprotection of the ester, guanidino and amino functions was realized, as usual, by treatment with trifluoroacetic acid at room temperature; compound **16b** was quantitatively recovered (Scheme 1). In the ^1H NMR spectrum, the three methylene protons of the anchorage-arm give multiplets at 2.13 δ, 3.16 δ and 4.16 δ; the corresponding ^{13}C NMR lines are found at 28.9 ppm, 39.6 ppm and 68.5 ppm.

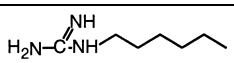
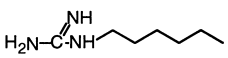
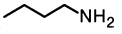
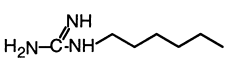
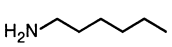
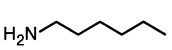
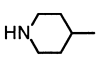
Biological evaluation (in solution)

The in vitro activity of the compounds listed in Table 1 was assayed in a standard test of platelet aggregation inhibition.[52] Human platelet rich plasma (PRP) was freshly prepared and platelet aggregation was measured by recording the velocity of light transmission change with an aggregometer. Serial dilutions of the inhibitors were added, and aggregation was induced by addition of adenosine diphosphate (ADP). Inhibition of platelet aggregation was determined by comparison of light transmittance values for the control (absence of inhibitor) and the samples. The IC_{50} was determined as the concentration necessary to inhibit the change in light transmittance by 50%. We used the tetrapeptide RGDS (Arg-Gly-Asp-Ser) as active reference compound: it inhibits ADP-mediated platelet aggregation with a IC_{50} value of about 100 μM.

We found that the 6-guanidino- (entries 1–3) and the 6-aminocaproic derivatives (entry 5) are inhibitors, while

Table 1. Biological evaluation in solution



Entry	Molecules Ⓐ	Ⓑ	Ⓒ	Compound	IC_{50} (μM) (reference)[a]
1		$COCF_3$	Me	**16a**	465 (129)
2		$COCF_3$		**16b**	320 (68)
3		SO_2PhCF_3	Me	**28**	85 (68)
4		$COCF_3$	Me	**12a**	unsoluble
5		SO_2PhCF_3	Me	**27**	125 (100)
6		$COCF_3$	Me	**13a**	> 1000
7		$COCF_3$	Me	**14a**	> 1000

[a]IC_{50} value (μM) of the RGDS tetrapeptide (Arg-Gly-Asp-Ser).

the (iso)nipecotic derivatives are inactive (entries 6 and 7). In the more active compounds, the α-amino function of the tyrosine template was masked with a hydrophobic arylsulfonyl group (entries 3 and 5). The presence of the anchorage-arm (for surface immobilization) did not perturb the activity (entry 2). The levels of activities were similar to that of the reference peptide (RGDS).

The flexible RGDS tetrapeptide can adopt many conformations allowing its binding to many integrins,[18,21] even though with moderate affinity. We could expect a similar behaviour in the case of the flexible peptidomimetic **16b**, for which we have recorded a significant affinity toward the $\alpha_{IIb}\beta_3$ integrin (fibrinogen receptor). The same level of activity toward the $\alpha_v\beta_3$ integrin (vitronectin receptor) should be sufficient to promote cell adhesion after immobilization of **16b** on the surface of culture substrates.

Modelisation

Two cyclic RGD peptides have been chosen as reference compounds in view to evaluate the conformational arrangement of the selected peptidomimetic **16a** (anchorage-arm replaced with a methyl group). The DMP 728 (Fig. 2) is a potent and selective $\alpha_{IIb}\beta_3$ integrin antagonist,[53,54] while the cyclo (Arg-Gly-Asp-D-Phe-Val) (c[RGDfV]) is a potent and selective $\alpha_v\beta_3$ integrin antagonist (Fig. 3).[55,56]

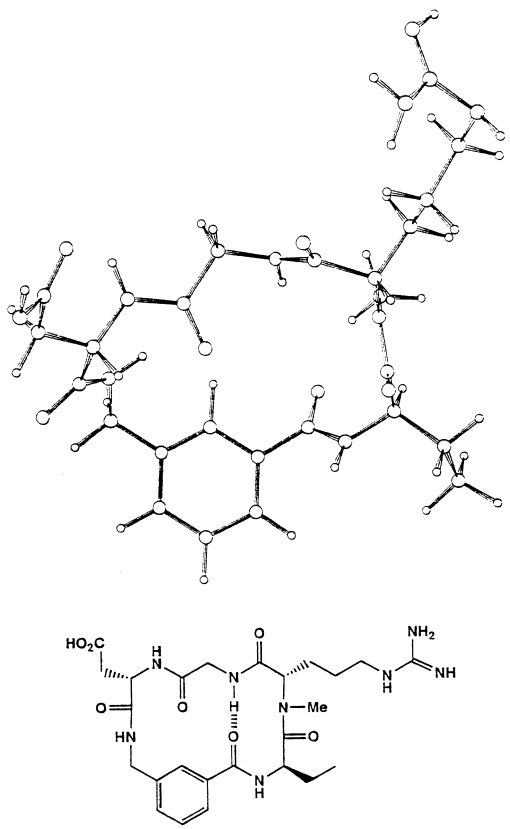


Figure 2. Cyclopeptide DMP728.

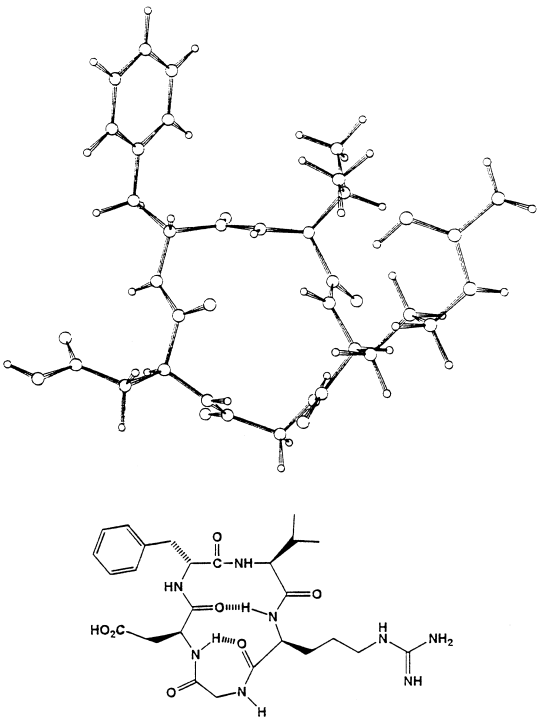


Figure 3. Cyclopeptide c(RGDfV).

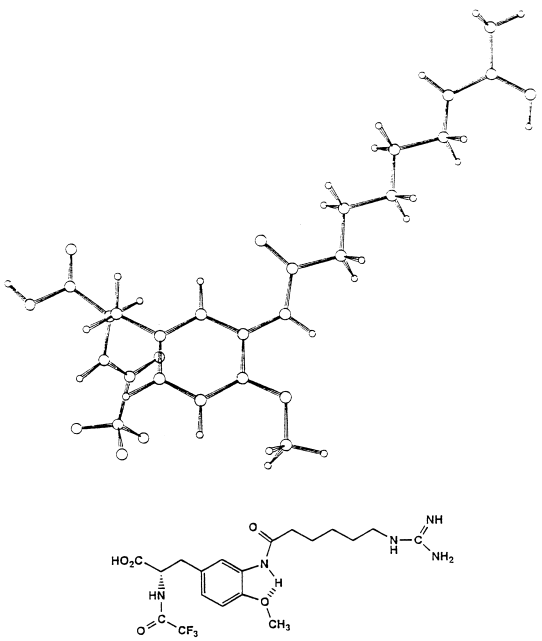


Figure 4. Peptidomimetic **16a**.

The references and compound **16a** (Fig. 4) were fully optimized at the approximate quantum chemistry level AM1; the molecules were studied as isolated neutral entities, and not as zwitterionic forms, in order to avoid internal self-folded conformations.

As previously pointed out in the literature,[27,41] the overall length of c[RGDfV] is shorter comparatively to the $\alpha_{IIb}\beta_3$ ligands, such as DMP 728. Indeed, the structure is stabilized by two hydrogen-bonds between the C=O...N-H group of Gly and the adjacent amide bonds (distances of 2.216 Å and 2.568 Å, respectively) (Fig. 3). With its flexible side-chains, the potential ligand **16a** displays terminal functions that can be fairly fitted onto the corresponding carboxyl- and guanidyl groups of both references (Figs 5 and 6).

Surface chemistry

Poly(ethylene terephthalate) (PET) is a synthetic aromatic polyester widely used as a biomaterial for making non-resorbable sutures, or performing prosthetic replacements in orthopedic surgery (tendons, ligaments, facial implants). Actually, the most successful medical applications of PET are in the area of cardiovascular surgery, including prosthetic heart valves and vascular grafts of large to medium diameter (Dacron).[1,2] Membranes made from PET films are also appropriate substrates for cultivating mammalian cells.[4,6] In our laboratory, we use microporous membranes obtained by a track-etching process which allows the preparation of 'capillary-pore' membranes[57] with very uniform, nearly perfectly round cylindrical pores. Such membranes are made from homogeneous 10–20 μm thick polymer film precursors in two steps, consisting of bombardment with heavy ions (Ar^{+9}) accelerated in a cyclotron, followed by immersion into an appropriate solution of reagents which preferentially etches the tracks, leading to the creation of pores.[58] The etching treatment of the PET creates functionalities (chain-endings) on the membrane open surface that could be used for immobilizing molecules of interest.

The surface displayed functions of PET membranes are carboxyl- and hydroxyl end-groups (Scheme 4). We have already demonstrated that the amount of carboxyl groups could be significantly increased by a surface oxidative treatment ($KMnO_4$ in 1.2 N H_2SO_4) which transforms native hydroxyl chain-ends into new carboxyl endings[7,9]; the resulting membrane was called PET-CO_2H (Scheme 4).

The surface reactivity of the PET-CO_2H membrane was assayed by the covalent coupling of ^{3}H-lysine followed by liquid scintillation counting (LSC) of the sample associated radioactivity.[7,9] For that purpose, the labeling reaction was conducted under mild conditions (water solution, near the physiological pH, room temperature) mimicking at best the conditions to be encountered in the covalent coupling of the peptidomimetic molecule **16b**. Thus, the PET membrane was activated by treatment with water soluble carbodiimide (0.1% WSC, 1 h, 20 °C, pH 3.5) and then incubated with ^{3}H-lysine (10^{-3} M solution, 2 h, 20 °C, pH 8); we found a value of about 35 pmol/cm^2 (open surface) of fixed label (corrected value, obtained by subtracting the adsorption contribution, see Experimental). From a previously established model of the PET surface[7], we calculated that this value corresponds to the functionalization of about 1.2% of the monomer units (see Experimental).

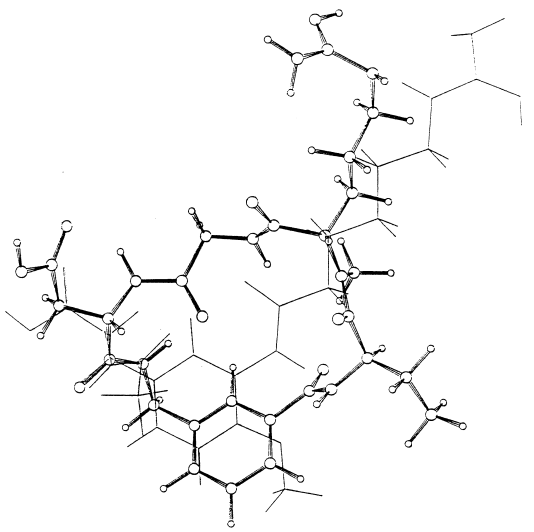


Figure 5. Superposition of **16a** and DMP728.

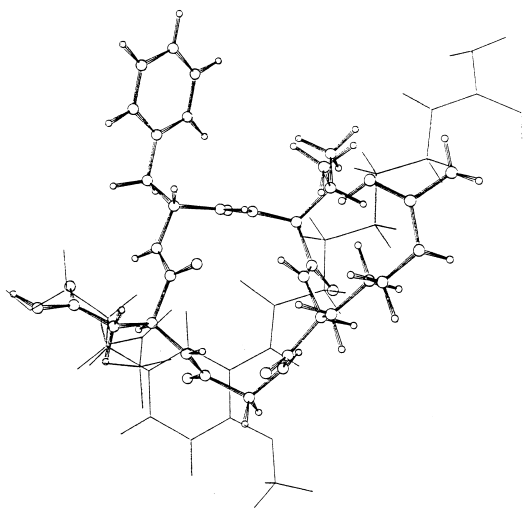
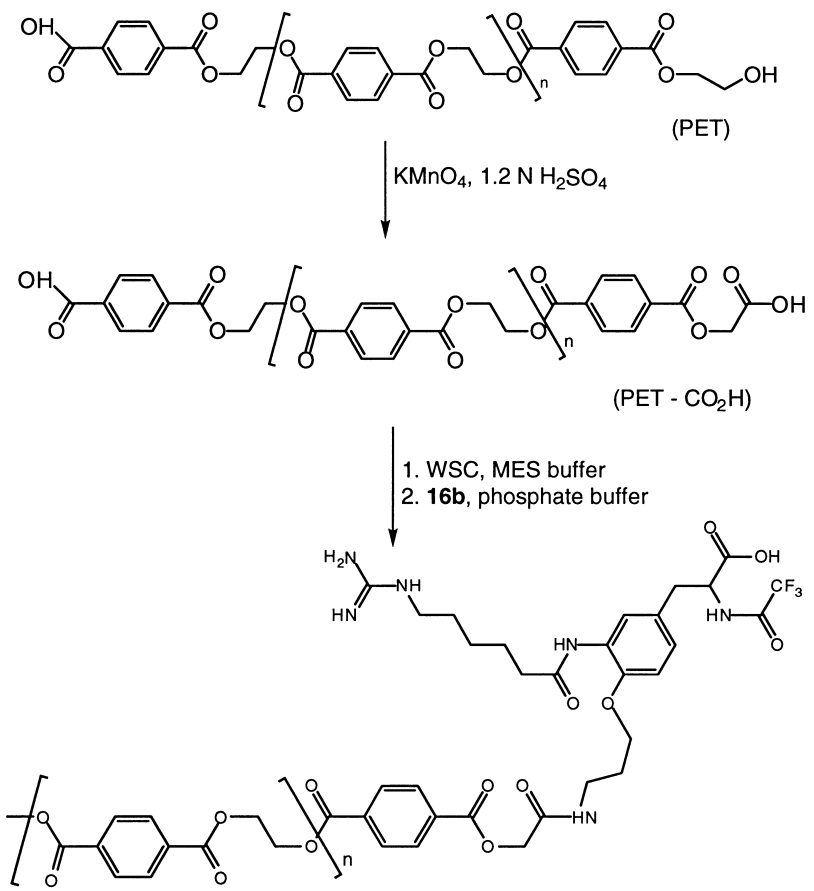


Figure 6. Superposition of **16a** and c(RGDfV).



Scheme 4. Covalent coupling of **16a** onto the PET membrane surface.

The RGD peptidomimetic **16b** was similarly coupled to the PET-CO_2H membrane activated by the pre-treatment with WSC (Scheme 4). A blank sample was prepared by immersing a non-activated PET-CO_2H membrane into the solution of **16b** in phosphate buffer (0.062%, or $\sim 10^{-3}$ M). The X-ray photoelectron spectroscopy (XPS) of the blank sample did not show the presence of fluorine atoms on the surface (thus, no detectable adsorption). However, the activated membrane fixed (most probably by covalent grafting) the RGD peptidomimetic as revealed by the presence of 0.24% of fluorine atoms (XPS analysis) in the atomic composition of the sample surface (sampling depth of about 50 Å). From the experimental F/C × 100 atomic ratio of 0.339, we calculated (see Experimental) that about 1.1% of the surface monomer units have fixed the biological signal. This value is in good agreement with the theoretical value based on the surface radiolabeling assay (1.2%). According to Massia and Hubbell,[59,60] a surface concentration of about 10 fmol/cm^2 of grafted natural peptides is large enough to improve the cell-adhesive properties of a biomaterial. Thus our PET substrate, displaying about 1% of synthetic 'RGD-like' signals (i.e. about 30 pmol/cm^2) should be a good candidate to promote the adhesion of anchorage-dependent cells.

Conclusion

Several RGD peptidomimetics have been constructed from the *ortho*-amino-tyrosine template. The synthetic strategy allowed to equip the structures with an anchorage-arm and a fluorine tag, as required for the surface immoblilization on polymer substrates, and the subsequent quantification of the amount of fixed biological signals by X-ray photoelectron spectroscopy.

The most flexible molecule **16** has been selected for coupling on the poly(ethylene terephthalate) membrane currently used as cell culture support. In solution, **16a**,**b** exhibited moderate activities, in the platelet aggregation test, that range the compounds at the level of the tetrapeptide RGDS; the presence of an anchorage-arm did

not perturb the biological response. The structure **16a** could be fairly fitted with two cyclic RGD peptides which are representative ligands of the fibrogen- and vitronectin receptors, respectively.

Using the wet-chemistry technique, we enriched the PET membrane surface with carboxyl functions. Their activation with WSC allowed to fix the peptidomimetic **16b** in good yield, as controlled by XPS: almost all the reactive CO_2H chain-endings assayed by radiolabeling and LSC have quenched the biological signal.

To our knowledge, the present work is the first report dealing with the covalent coupling of RGD peptidomimetics on the surface of PET membrane. The performances of such a modified support are currently examined in our laboratory by culturing different mammalian cell lines.

Experimental

Synthesis

The reagents (analytical grade) were purchased from Acros, Aldrich, or Fluka. The solvents were distilled, after drying as follows: acetonitrile, dichloromethane, dimethoxyethane, triethylamine and pyridine, over calcium hydride; tetrahydrofurane, over sodium; acetone, over drierite.

The thin-layer chromatographies were carried out on silica gel 60 plates F254 (Merck, 0.2 mm thick); visualization was effected with UV light, iodine vapor, a spray of ninhydrin in ethanol or a spray of potassium permanganate (3 g) and potassium carbonate (20 g) in aqueous acetic acid (1%, 300 mL). The column-chromatographies (under normal pressure) were carried out with Merck silica gel 60 of 70–230 mesh ASTM, and the flash-chromatographies, with Merck silica gel 60 of 230–400 mesh ASTM. The MPLC purifications were realized on a Prochrom equipment, with silica gel of 400 mesh ASTM, under a pressure of 40 bar and a flow of 160 mL/min.

The melting points were determined with an Electrothermal microscope and are uncorrected. The rotations ($\pm$ 0.1°) were determined on a Perkin–Elmer 241 MC polarimeter. The IR spectra were taken with a Perkin–Elmer 600 instrument or with a Bio-Rad FTS 135 instrument, and calibrated with polystyrene (1601 cm^{-1}). The ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 300 (at 300 MHz for proton and 75 MHz for carbon), or Bruker AM-500 spectrometers (at 500 MHz for proton and 125 MHz for carbon); the chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (internal standard), or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for the spectra recorded in D_2O. The atom numbering used for the description of the spectra is shown in the Figure 7; the attributions were established by selective decoupling experiments. The mass spectra were obtained on a Finnigan-MAT TSQ-70 instrument at 70 eV (electronic impact (EI) mode), or with a Xenon ION TECH 8 KV (fast atom bombardment (FAB) mode). The microanalyses were performed at the Christopher Ingold Laboratories of the University College, London. The HRMS were performed at the University of Liège (Belgium) on a VG-AutoSpec-Q equipment (Fisons Instruments, Manchester).

***N*-(Trifluoroacetyl)-*ortho*-nitro-l-tyrosine terbutylester (2).** L-Tyrosine *t*-butylester **1** (478 mg, 2.013 mmol) was

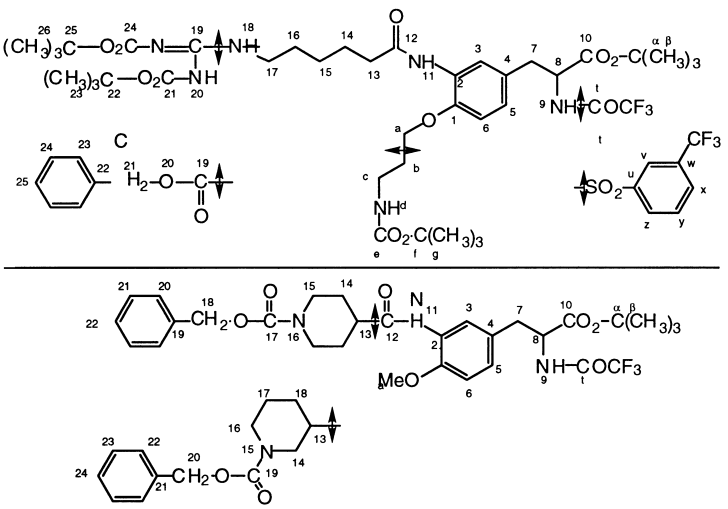


Figure 7. Atoms numbering used in the NMR spectra description.

dissolved, at 0 °C, in trifluoroacetic anhydride (1.5 mL, 2.23 g, 10.6 mmol, 5.27 equiv). After 30 min of stirring at room temperature, the mixture was concentrated under vacuum. The residue was dissolved in acetic acid (15 mL) and treated with concentrated nitric acid (115 μL, 2.4 mmol, 1.2 equiv) diluted in acetic acid (15 mL); the solution of HNO_3 was added dropwise, and the mixture was maintained at 10–14 °C during the addition. After 75 min of reaction (TLC control), the crude mixture was poured onto ice (400 g). The yellow precipitate was filtered off. The aqueous phase was extracted with ethyl acetate (4×150 mL). The organic phases were dried over $MgSO_4$, concentrated under vacuum and purified by column chromatography on silica gel, with CH_2Cl_2 as eluent, to give 0.307 g (yield: 41%) of **2**: R_f = 0.6; mp 85.4–87.6 °C; IR (KBr) ν 3358, 1755, 1709, 1550, 1161 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.48 (s, 9H, H-β), 3.13 (dd, J = 5.2 and 14.2 Hz, 1H, H-7), 3.25 (dd, J = 5.9 and 14.2 Hz, $CDCl_3$ 1H, H-7′), 4.70 (m, 1H, H-8), 6.96 (d, J = 6.4 Hz, 1H, NH-9), 7.11 (d, J = 9.0 Hz, 1H, H-6), 7.36 (dd, J = 9.0 and 2.1 Hz, 1H, H-5), 7.89 (d, J = 2.1 Hz, 1H, H-3), 10.5 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.86 (C-β), 35.87 (C-7), 53.70 (C-8), 84.40 (C-α), 115.35 (CF_3), 120.25 (C-6), 125.24 (C-3), 127.38 (C-4), 133.17 (C-2), 138.55 (C-5), 154.26 (C-1), 156.50 (*C*OCF_3), 168.51 (C-10); MS(EI) *m*/*e* 378; Anal. calcd for $C_{15}H_{17}F_3N_2O_6$ (378.3): C, 46.62; H, 4.52; N, 7.4. Found: C, 47.7; H, 4.42; N, 7.1.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-nitro-L-tyrosine terbutylester (3a).** The reaction was conducted under argon atmosphere. To a solution of **2** (727 mg, 1.92 mmol) in acetonitrile (150 mL) were added iodomethane (0.140 mL, 312 mg, 2.248 mmol, 1.17 equiv), potassium carbonate (268 mg, 1.94 mmol) and [18-*c*-6]crown-ether (54 mg, 0.203 mmol, 0.105 equiv). The mixture was refluxed, under stirring, during 48 h (TLC control). CH_3CN was removed under vacuum; the residue was dissolved in CH_2Cl_2 (100 mL) and washed with water (3×20 mL). Drying over $MgSO_4$, concentration and flash chromatography on silica gel (hexane:isopropanol, 9:1) gave 698 mg of **3a** contaminated with the methylation product of the trifluoroacetamide fraction (^1H NMR: 2.91 δ (s, 3H); ^{13}C NMR: 32.99 ppm; MS (FAB^+) *m*/*e* 407). The product was further purified by preparative MPLC on reverse phase (Novapak 4 μ; methanol:water, 3:2; 1 mL/min) to furnish 548 mg (yield: 72%) of **3a**: R_f (hexane:*i*PrOH, 9:1) = 0.4; IR (KBr) ν 3345, 2980, 1726, 1625, 1531 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 9H, H-β), 3.13 (dd, J = 5.2 and 14.2 Hz, 1H, H-7), 3.23 (dd, J = 6.4 and 14.2 Hz, 1H, H-7′), 3.94 (s, 3H, H-a), 4.68 (m, 1H, H-8), 6.98 (d, J = 7.3 Hz, 1H, NH-9), 7.03 (d, J = 8.6 Hz, 1H, H-6), 7.31 (dd, J = 2.1 and 8.6 Hz, 1H, H-5), 7.64 (d, J = 2.1 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.78 (C-β), 35.76 (C-7), 53.77 (C-8), 56.45 (C-a), 84.25 (C-α), 113.74 (C-6), 115.42 (CF3), 126.34 (C-3), 127.41 (C-4), 135.96 (C-5), 139.23 (C-2), 152.24 (C-1), 156.48 (*C*OCF_3), 168.51 (C-10); MS(FAB^-) *m*/*e* 391 (M-1), 335 (M-*t*Bu); Anal. calcd for $C_{16}H_{19}F_3N_2O_6$ (392.23): C, 48.98; H, 4.88; N, 7.14. Found: C, 48.91; H, 4.94; N, 6.77.

***N*-(Trifluoroacetyl)-*O*-[*N*-(terbutoxycarbonyl)-3-aminopropyl]-*ortho*-nitro-L-tyrosine terbutylester (3b).** *N*-(Terbutoxycarbonyl)-3-bromopropylamine was obtained by protection of 3-bromopropylamine as usual: a solution of 3-bromopropylamine hydrobromide (4.97 g, 22.28 mmol) in terbutanol–water (100–130 mL) was treated with diterbutyl dicarbonate (5.27 mL, 5.01 g, 22.28 mmol, 1 equiv) and sodium hydroxide (1.87 g, 46.89 mmol, 2.1 equiv) during 16 h at 20 °C. The mixture was extracted with pentane; the organic phase was washed with 10% $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated under vacuum to give 4.51 g (yield: 85%) of *N*-Boc-3-bromopropylamine: mp 33–34 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.38 (s, 9H), 1.99 (m, 2H), 3.21 (m, 2H), 3.38 (t, 2H), 4.83 (m, NH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.15, 30.61, 32.52, 38.75, 79.09, 155.78; MS(EI) *m*/*e* 239, 237.

To a solution of **2** (371 mg, 0.979 mmol) in CH_3CN (35 mL), under argon atmosphere, were added *N*-Boc-3-bromopropylamine (233 mg, 0.976 mmol, 1 equiv), potassium carbonate (149 mg, 1.077 mmol) and [18-*c*-6] crown-ether (0.269 mg). The mixture was refluxed, under stirring, during 22 h (TLC control), and then worked up as described for **3a**. Flash chromatography on silica gel (hexane:ethyl acetate, 4:1) furnished 343 mg of **3b** (yield: 78%): R_f = 0.1; mp 104.7–106.2 °C; IR ($CDCl_3$) ν 3423 (NH), 1719 (br, CO), 1534, 1262, 1169 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.43 (s, 9H, H-g), 1.48 (s, 9H, H-β), 2.05 (m, 2H, H-b), 3.14 (dd, J = 5.2 and 14.2 Hz, 1H, H-7), 3.25 (dd, J = 5.9 and 14.2 Hz, 1H, H-7′), 3.36 (m, 2H, H-c), 4.16 (t, J = 7.3 Hz, 2H, H-a), 4.69 (m, 1H, H-8), 5.06 (br s, NH-d), 6.94 (d, J = 7.3 Hz, NH-9), 7.03 (d, J = 8.6 Hz, 1H, H-6), 7.30 (dd, J = 2.1 and 8.6 Hz, 1H, H-5), 7.67 (d, J = 2.1 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.85 (C-g), 28.25 (C-β), 29.00 (C-b), 35.66 (C-7), 37.90 (C-c), 53.71 (C-8), 67.87 (C-a), 79.06 (C-f), 84.40 (C-α), 114.50 (C-6), 115.40 (*C*F_3), 126.50 (C-3), 127.40 (C-4), 135.16 (C-5), 139.06 (C-2), 151.57 (C-1), 156.05 (c-e), 156.40 (*C*OCF_3), 168.50 (C-10); Anal. calcd for $C_{23}H_{32}F_3N_3O_8$ (535.51): C, 51.58; H, 6.02; N, 7.84. Found: C, 51.60; H, 6.02; N, 7.78.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-amino-L-tyrosine terbutylester (4a).** A solution of **3a** (690 mg, 1.759 mmol) in methanol (12 mL) containing platinium IV oxide (40 mg, 0.177 mmol, 1 equiv) was placed in a Parr flask and shaked under hydrogen atmosphere (p = 40 psi) during 18 h at room temperature. After filtration and

evaporation of the methanol, the residue was dissolved in ethyl acetate, dried over $MgSO_4$ and concentrated under vacuum to give 558 mg of crude aniline **4a** (yield: 88%): R_f (hexane:ethyl acetate, 7:3) = 0.3; IR ($CHCl_3$) ν 3368, 3314, 2981, 2927, 1717, 1615, 1501, 1432, 1362 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 9H, H-β), 3.04 (m, 2H, H-7), 3.83 (s, 3H, H-a), 4.66 (dt, J = 6.1, 6.1 and 7.7 Hz, 1H, H-8), 6.45 (m, 2H, H-3 + H-5), 6.68 (d, J = 8.6 Hz, 1H, H-6), 6.76 (d, J = 7.7 Hz, NH-9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.86 (C-β), 36.47 (C-7), 53.83 (C-8), 55.40 (C-a), 83.22 (C-α), 110.28 (C-6), 115.52 (*C*F_3), 115.66 (C-3), 119.05 (C-5), 127.20 (C-4), 136.19 (C-2), 146.57 (C-1), 156.40 (*C*OCF_3), 168.98 (C-10); MS (FAB$^+$) *m/e* 363 (M + 1), 262, 307, 289.

***N*-(Trifluoroacetyl)-*O*-[*N*-(terbutoxycarbonyl)-3-amino-propyl]-*ortho*-amino-L-tyrosine terbutylester (4b).** A solution of **3b** (162 mg, 0.303 mmol) in methanol (10 mL) containing PtO_2 (4.4 mg, 0.019 mmol, 0.06 equiv) was hydrogenated (Parr apparatus, p = 50 psi) during 5 h at 20 °C. After filtration and concentration, the residue was dissolved in chloroform and dried over $MgSO_4$. Evaporation under vacuum furnished 125 mg of crude aniline **4b** (yield: 82%): ^1H NMR ($CDCl_3$, 500 MHz) δ 1.43 (s, 9H, H-g), 1.46 (s, 9H, H-β), 1.99 (m, 2H, H-b), 3.03 (m, 2H, H-7), 3.34 (m, 2H, H-c), 4.02 (t, J = 7.3 Hz, 2H, H-a), 4.66 (m, 1H, H-8), 4.73 (br s, NH-d), 6.40 (m, 2H, H-3 + H-5), 6.70 (d, J = 8.6 Hz, 1H, H-6), 6.77 (br s, NH-9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.88 (C-β), 28.28 (C-g), 29.65 (C-b), 36.46 (C-7), 37.83 (C-c), 53.81 (C-8), 65.96 (C-a), 78.80 (C-f), 83.25 (C-α), 111.48 (C-6), 115.40 (*C*F_3), 115.80 (C-3), 119.05 (C-5), 127.50 (C-4), 136.40 (C-2), 145.64 (C-1), 155.88 (C-e), 156.40 (*C*OCF_3), 168.97 (C-10).

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-[6-(*N*,*N*′-diterbutoxy-carbonyl)guanidino-caproyl]-amino-L-tyrosine terbutylester (10a).** To a solution of **4a** (514 mg, 1.419 mmol) in CH_2Cl_2 (10 mL) were added, successively, the acid chloride **20b** (612 mg, 1.561 mmol, 1.1 equiv) and pyridine (0.130 mL, 127 mg, 1.607 mmol, 1.13 equiv) in CH_2Cl_2 (10 mL). The mixture was stirred during 20 h at room temperature. Washing with 1.5 N HCl (2×20 mL), 10% $NaHCO_3$ (2×20 mL), and water (2×20 mL), drying over $MgSO_4$ and concentration gave crude **10a** which was purified by column-chromatography on silica gel (hexane:ether, 1:1) to furnish 355 mg of **10a** (yield: 35%): mp 53.7–54.6 °C; IR (KBr) ν 3338, 2980, 2935, 1724, 1641, 1619, 1535, 1483, 1420, 1369, 1334, 1228 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.44 (m, 2H, H-15), 1.46 (s, 9H, H-β), 1.48 (s, 9H, H-23), 1.49 (s, 9H, H-26), 1.62 (m, 2H, H-16), 1.74 (m, 2H, H-14), 2.38 (t, J = 7.3 Hz, 2H, H-13), 3.05 (dd, J = 6.0 and 14.2 Hz, 1H, H-7), 3.16 (dd, J = 6.0 and 14.2 Hz, 1H, H-7′), 3.41 (m, 2H, H-17), 3.85 (s, 3H, H-a), 4.67 (m, 1H, H-8), 6.77 (m, 2H, H-5 + H-6), 6.83 (s, 1H, NH-9), 7.71 (s, 1H, NH-11), 8.25 (s, 1H, H-3), 8.31 (m, 1H, NH-18), 11.50 (s, 1H, NH-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.15 (C-14), 26.29 (C-15), 27.81 (c-β), 27.95 (C-26), 28.18 (C-23), 28.66 (C-16), 36.71 (C-7), 37.63 (C-13), 40.61 (C-17), 53.87 (C-8), 55.61 (C-a), 79.06 (C-22), 82.93 (C-25), 83.49 (C-α), 109.75 (C-6), 115.52 (*C*F_3), 120.52 (C-3), 124.00 (C-5), 127.44 (C-4), 127.73 (C-2), 146.80 (C-1), 153.21 (C-24), 156.01 (C-21), 156.40 (*C*OCF_3), 163.51 (C-19), 168.94 (C-10), 170.61 (C-12); Anal. calcd for $C_{33}H_{50}F_3N_5O_9$ (717.78): C, 55.22; H, 7.02; N, 9.75. Found: C, 56.23; H, 7.31; N, 9.13.

***N*-(Trifluoroacetyl)-*O*-[*N*-terbutoxycarbonyl)-3-amino-propyl]-*ortho*-[6-(*N*,*N*′-diterbutoxycarbonyl)guanidino-caproyl]-amino-L-tyrosine terbutylester (10b).** To a solution of **4a** (874 mg, 1.728 mmol) and pyridine (411 mg, 5.192 mmol, 3 equiv) in CH_2Cl_2 (10 mL), was added the acid chloride **20b** (742 mg, 1.893 mmol, 1.09 equiv) in CH_2Cl_2 (10 mL). The mixture was stirred for 15 h at room temperature, under molecular sieves (4 Å). Washing with 1.5 N HCl (2×20 mL), 10% $NaHCO_3$ (2×20 mL), and brine (2×20 mL), drying over $MgSO_4$ and concentration gave crude **10b** which was flash-chromatographed (silica gel, hexane:isopropanol, 9:1) to furnish 750 mg of **10b** (yield: 51%): R_f = 0.3; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.41 (s, 9H, H-g), 1.44 (m, 2H, H-15), 1.47 (s, 9H, H-β), 1.49 (s, 9H, H-23), 1.50 (s, 9H, H-26), 1.62 (m, 2H, H-16), 1.76 (m, 2H, H-14), 1.97 (m, 2H, H-b), 2.46 (t, J = 7.3 Hz, 2H, H-13), 3.05 (dd, J = 6.0 and 14.2 Hz, 1H, H-7), 3.16 (dd, J = 5.2 and 14.2 Hz, 1H, H-7′), 3.35 (m, 2H, H-c), 3.41 (m, 2H, H-17), 4.07 (t, J = 7.0 Hz, 2H, H-a), 4.68 (m, 1H, H-8), 4.74 (m, 1H, NH-d), 6.75 (dd, J = 2.1 and 9.0 Hz, 1H, H-5), 6.79 (d, J = 9.0 Hz, 1H, H-6), 6.87 (d, J = 7.8 Hz, 1H, NH-9), 8.17 (s, 1H, NH-11), 8.22 (d, J = 2.1 Hz, 1H, H-3), 8.31(m, 1H, NH-18), 11.50 (s, 1H, NH-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.24 (C-14), 26.36 (C-15), 27.80 (C-β), 27.93 (C-26), 28.20 (C-23 and C-g), 28.75 (C-16), 29.58 (C-b), 36.68 (C-7), 36.86 (C-c), 37.37 (C-13), 40.65 (C-17), 53.87 (C-8), 65.23 (C-a), 79.05 (C-22), 79.28 (C-f), 82.89 (C-25), 83.45 (C-α), 111.18 (C-6), 115.52 (*C*F_3), 121.20 (C-3), 123.99 (C-5), 127.58 (C-4), 128.31 (C-2), 146.10 (C-1), 153.18 (C-24), 155.98 (C-21 and C-e), 156.30 (*C*OCF_3), 163.50 (C-19), 168.92 (C-10), 171.11 (C-12); Anal. calcd for $C_{40}H_{63}F_3N_6O_{11}$ (860.96): C, 55.8; H, 7.37; N, 9.76. Found: C, 55.64; H, 7.06; N, 9.06.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-[6-(benzyloxycarbonyl)-amino-caproyl]-amino-L-tyrosine terbutylester (6a).** *N*-(Benzyloxycarbonyl)-6-aminocaproyl chloride was prepared from *N*-(benzyloxycarbonyl)-6-aminocaproic acid (644 mg, 2.43 mmol) in CH_2Cl_2 (25 mL) treated with thionyl chloride (0.9 mL, 1.47 g, 12.3 mmol, 5 equiv) for 2 h at reflux; concentration under high vacuum gave the

crude acid chloride (625 mg, 91% yield). To a solution of **4a** (464 mg, 1.28 mmol) in CH_2Cl_2 (4 mL) were added successively *N*-(benzyloxycarbonyl)-6-aminocaproyl chloride (400 mg, 1.41 mmol, 1.1 equiv) and pyridine (303 mg, 3.83 mmol, 4 equiv) in CH_2Cl_2 (4 mL). The mixture was stirred for 20 h at 20 °C, then washed twice with 1.5 N HCl, 10% $NaHCO_3$ and water. Drying ($MgSO_4$), concentration and flash-chromatography ($CHCl_3$) gave 196 mg of **6a** (yield: 25%): $R_f = 0.2$; IR ($CHCl_3$) ν 3315, 2933, 1718, 1654, 1596, 1560, 1535, 1483 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.39 (m, 2H, H-15), 1.46 (s, 9H, H-β), 1.54 (m, 2H, H-16), 1.73 (m, 2H, H-14), 2.36 (t, $J = 7.3$ Hz, 2H, H-13), 3.04 (dd, $J = 6.1$ and 14.0 Hz, 1H, H-7), 3.16 (dd, $J = 5.8$ and 14.0 Hz, 1H, H-7′), 3.19 (m, 2H, H-17), 3.85 (s, 3H, H-a), 4.68 (dt, $J = 6.1$, 5.8 and 7.3 Hz, 1H, H-8), 4.84 (m, 1H, NH-18), 5.08 (s, 2H, H-21), 6.78 (m, 2H, H-5 + H-6), 6.89 (d, $J = 7.3$ Hz, 1H, NH-9), 7.27–7.37 (m, 5H, Ph), 7.71 (s, 1H, NH-11), 8.25 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.02 (C-14), 26.11 (C-15), 27.78 (c-β), 29.58 (C-16), 36.70 (C-7), 36.61 (C-13), 40.72 (C-17), 53.87 (C-8), 55.58 (C-a), 66.42 (C-21), 83.43 (C-α), 109.74 (C-6), 115.42 (*C*F_3), 120.48 (C-3), 124.02 (C-5), 127.46 (C-4), 127.66 (C-2), 127.90 (C-24 + C-25), 128.35 (C-23), 136.51 (C-22), 146.71 (C-1), 156.26 (C-19), 156.4 (*C*OCF_3), 168.95 (C-10), 170.61 (C-12); MS (FAB^+) *m/e* 510 (M + 1-Boc), 446, 136, 95, 91, 83, 69.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-[*N*-(benzyloxycarbonyl)-isonipecotyl]-amino-L-tyrosine terbutylester (7a).** *N*-(Benzyloxycarbonyl)-isonipecotyl chloride was prepared from *N*-(benzyloxycarbonyl)-isonipecotic acid (331 mg, 1.257 mmol) in CH_2Cl_2 (25 mL) treated with $SOCl_2$ (0.49 mL, 800 mg, 6.72 mmol, 5.3 equiv) for 1 h 30 min at reflux; concentration under high vacuum gave the crude acid chloride (343 mg, 97%). To a solution of **4a** (404 mg, 1.115 mmol) in CH_2Cl_2 (10 mL) were added successively *N*-(benzyloxycarbonyl)-isonipecotyl chloride (343 mg, 1.218 mmol, 1.09 equiv) in CH_2Cl_2 (10 mL), and pyridine (0.1 mL, 98 mg, 1.236 mmol, 1.11 equiv). The mixture was stirred for 16 h at 20 °C (TLC control), then washed with 1 N HCl (3×10 mL), 10% $NaHCO_3$ (3×10 mL), and brine (3×10 mL). Drying ($MgSO_4$), concentration and flash-chromatography (silica gel, hexane-isopropanol, 10:1) gave 380 mg of **7a** (yield: 56%): $R_f = 0.1$; mp 62.3–63.5 °C; IR (KBr) n 3422, 3309, 3083, 2944, 1717, 1696, 1675, 1597, 1536, 1369 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 9H, H-β), 1.74 (m, 2H, H-14), 1.90 (m, 2H, H-14′), 2.43 (m, 1H, H-13), 2.88 (m, 2H, H-15), 3.03 (dd, $J = 6.1$ and 14.0 Hz, 1H, H-7), 3.16 (dd, $J = 5.2$ and 14.0 Hz, 1H, H-7′), 3.85 (s, 3H, H-a), 4.23 (m, 2H, H-15′), 4.67 (m, 1H, H-8), 5.13 (s, 2H, H-18), 6.78 (m, 2H, H-5 + H-6), 7.03 (d, $J = 7.6$ Hz, 1H, NH-9), 7.27–7.37 (m, 5H, Ph), 7.81 (s, 1H, NH-11), 8.25 (d, $J = 2.1$ Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.66 (C-β), 28.22 (C-14), 28.36 (C-14′), 36.56 (C-7), 43.08 (C-15), 44.01 (C-13), 53.87 (C-8), 55.51 (C-a), 66.92 (C-18), 83.32 (C-α), 109.67 (C-6), 115.44 (*C*F_3), 120.50 (C-3), 124.16 (C-5), 127.33 (C-2), 127.48 (C-4), 127.64 (C-21), 127.77 (C-22), 128.25 (C-20), 136.51 (C-19), 146.83 (C-1), 154.96 (C-17), 156.26 (*C*OCF_3), 168.87 (C-10), 171.91 (C-12); HRMS (FAB^+): calcd for $C_{30}H_{36}F_3N_3O_7$: 607.2505. Found: 607.2528.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-[*N*-(benzyloxycarbonyl)-nipecotyl]-amino-L-tyrosine terbutylester (8a).** *N*-(Benzyloxycarbonyl)-nipecotyl chloride (335 mg, 95% yield) was prepared, as above, from *N*-(benzyloxycarbonyl)-nipecotic acid (330 mg, 1.25 mmol) and $SOCl_2$ (750 mg, 6.3 mmol, 5 equiv). To a solution of **4a** (384 mg, 1.06 mmol) and pyridine (0.1 mL, 98 mg, 1.236 mmol, 1.17 equiv) in CH_2Cl_2 (10 mL), was added *N*-(benzyloxycarbonyl)-nipecotyl chloride (335 mg, 1.19 mmol, 1.12 equiv) in CH_2Cl_2 (10 mL). The mixture was stirred for 17 h at 20 °C (TLC control), then worked-up as above. Flash-chromatography (silica gel, CH_2Cl_2:EtOAc, 9:1) gave 318 mg of **8a** (yield: 49%): mp 50–51 °C; IR (film) ν 3417, 2944, 2865, 1718, 1685, 1597, 1536, 1482, 1433, 1370, 1259, 1154 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 9H, H-b), 1.52 (m, 1H, H-17), 1.76 (m, 1H, H-18), 1.80 (m, 1H, H-17′), 2.02 (m, 1H, H-18′), 2.48 (m, 1H, H-13), 2.90 (m, 1H, H-16), 3.04 (dd, $J = 6.1$ and 14.0 Hz, 1H, H-7), 3.05 (m, 0.5 H, H-14), 3.15 (m, 0.5 H, H-14), 3.16 (dd, $J = 5.2$ and 14.0 Hz, 1H, H-7′), 3.84 (s, 3H, H-a), 4.10 (m, 1H, H-16′), 4.21 (m, 0.5 H, H-14′), 4.31 (m, 0.5 H, H-14′), 4.67 (m, 1H, H-8), 5.14 (s, 2H, H-20), 6.78 (m, 2H, H-5 + H-6), 6.81 (d, $J = 7.9$ Hz, 1H, NH-9), 7.28–7.38 (m, 5H, Ph), 7.81 (s, 1H, NH-11), 8.23 (m, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.18 (C-17), 27.65 (C-18), 27.82 (C-β), 36.78 (C-7), 44.14 (C-16), 44.52 (C-13), 46.36 (C-14), 53.90 (C-8), 55.63 (C-a), 67.12 (C-20), 83.56 (C-α), 109.81 (C-6), 115.44 (*C*F_3), 120.68 (C-3), 124.36 (C-5), 127.40 (C-4), 127.46 (C-2), 127.80 (C-23), 127.94 (C-24), 128.41 (C-22), 136.59 (C-21), 146.97 (C-1), 155.19 (C-19), 156.40 (*C*OCF_3), 168.95 (C-10), 170.85 (C-12); MS (FAB^+) *m/e* 608.5; Anal. calcd for $C_{30}H_{36}F_3N_3O_7$ (607.62): C, 59.3; H, 5.97; N, 6.91. Found: C, 58.72; H, 5.98; N, 6.64.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-(6-guanidino-caproyl)-amino-(L)-tyrosine (16a).** The Boc-protected precursor **10a** (76.5 mg, 0.106 mmol) was dissolved in trifluoroacetic acid (1.7 mL) and left for 2.5 h at 20 °C. After evaporation under vacuum, the residue was dissolved in water and washed with chloroform. The aqueous phase was freeze-dried to give 58 mg of **16a** (yield: 86%): mp 95.5–96.5 °C; IR (KBr) ν 3416, 1664, 1542, 1206, 1138 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 1.44 (m, 2H, H-15), 1.64 (m, 2H, H-16), 1.73 (m, 2H, H-14), 2.47 (t,

$J = 7.3$ Hz, 2H, H-13), 2.98 (dd, $J = 9.5$ and 14.0 Hz, 1H, H-7), 3.20 (t, $J = 7.3$ Hz, 2H, H-17), 3.30 (dd, $J = 5.2$ and 14.0 Hz, 1H, H-7′), 3.85 (s, 3H, H-a), 4.68 (dd, $J = 9.5$ and 5.2 Hz, 1H, H-8), 7.06 (d, $J = 8.6$ Hz, 1H, H-6), 7.15 (dd, $J = 2.1$ and 8.6 Hz, 1H, H-5), 7.44 (d, $J = 2.1$ Hz, 1H, H-3); ^{13}C NMR (D_2O, 125 MHz) δ 27.45 (C-14), 27.80 (C-15), 30.14 (C-16), 38.44 (C-13), 38.54 (C-7), 43.58 (C-17), 57.79 (C-8), 58.55 (C-a), 114,82 (C-6), 118.37 (*C*F_3), 127,71 (C-4), 128.04 (C-3), 130.41 (C-5), 131.58 (C-2), 153.40 (C-1), 159.33 (C-19), 160.85 (*C*OCF_3), 176.83 (C-12), 178.51 (C-10); HRMS (FAB$^+$): calcd for $C_{21}H_{27}F_6N_5O_7$: 462.1964. Found: 462.1971.

***N*-(Trifluoroacetyl)-*O*-(3-aminopropyl)-*ortho*-(6-guanidinocaproyl)-amino-L-tyrosine (16b).** The Boc-protected precursor **10b** (750 mg, 0.871 mmol) was dissolved in CF_3CO_2H (13.5 mL) and left for 2 h at 20 °C. Work up as above gave 616 mg of **16b** (yield: 97%): $[\alpha]_D^{20}$ +2.5° (*c* 1, H_2O); ^{1H} NMR (D_2O, 500 MHz) δ 1.44 (m, 2H, H-15), 1.63 (m, 2H, H-16), 1.71 (m, 2H, H-14), 2.13 (m, 2H, H-b), 2.45 (t, $J = 7.3$ Hz, 2H, H-13), 2.96 (m, 1H, H-7), 3.16 (m, 2H, H-c), 3.20 (t, $J = 7.3$ Hz, 2H, H-17), 3.28 (m, 1H, H-7′), 4.16 (t, $J = 7.3$ Hz, 2H, H-a), 4.68 (m, 1H, H-8), 7.04 (d, $J = 8.6$ Hz, 1H, H-6), 7.16 (m, 1H, H-5), 7.27 (m, 1H, H-3); ^{13}C NMR (D_2O, 125 MHz) δ 27.45 (C-14), 27.77 (C-15), 28.92 (C-b), 30.06 (C-16), 38.28 (C-7 + C-13), 39.65 (C-c), 43.45 (C-17), 57.62 (C-8), 68.46 (C-a), 115.89 (C-6), 118.37 (*C*F_3), 127.50 (C-4), 129.22 (C-3), 130.96 (C-5), 132.01 (C-2), 153.00 (C-1), 159.24 (C-19), 160.84 (*C*OCF_3), 176.61 (C-12), 178.50 (C-10); HRMS (FAB$^+$): calcd for $C_{21}H_{32}F_3N_6O_5$: 505.2386. Found: 505.2385.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-(6-amino-caproyl)-amino-L-tyrosine (12a).** A solution of **6a** (55 mg, 0.09 mmol) in EtOAc (5 mL)) was placed in a Parr flask and hydrogenated ($pH_2 = 40$ psi) in the presence of Palladium (10% on C, 2 mg, 0.21 equiv). The mixture was vigorously shaked during 72 h (fresh catalyst was added three times). After filtration and concentration, the residue was dissolved in $CHCl_3$, dried over $MgSO_4$ and concentrated under vacuum. The residue was dissolved in CF_3CO_2H (2 mL) and left for 2.5 h at 20 °C. After evaporation, the residue was dissolved in water and extracted with $CHCl_3$. The aqueous phase was freeze-dried to give 40 mg of **12a** (yield: 100%): IR (CD_3OD) ν 3419, 2947, 2361, 1683, 1541, 1490, 1207, 1143, 1029 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 1.47 (m, 2H, H-15), 1.71 (m, 4H, H-14 + H-16), 2.46 (t, $J = 7.3$ Hz, 2H, H-13), 2.93 (t, $J = 7.3$ Hz, 2H, H-17), 2.96 (dd, $J = 9.5$ and 14.2 Hz, 1H, H-7), 3.23 (dd, $J = 5.2$ and 14.2 Hz, 1H, H-7′), 3.84 (s, 3H, H-a), 4.61 (m, 1H, H-8), 6.91 (d, $J = 8.6$ Hz, 3H, H-6), 6.96 (dd, $J = 2.1$ and 8.6 Hz, 1H, H-5), 7.84 (d, $J = 2.1$ Hz, 1H, H-3); ^{13}C NMR (CD_3OD, 125 MHz) δ 26.16 (C-14), 26.88 (C-15), 28.28 (C-16), 37.23 (C-13), 37.32 (C-7), 40.57 (C-17), 55.93 (C-8), 56.33 (C-a), 111.77 (C-6), 117.34 (*C*F3), 124.41 (C-3), 126.89 (C-5), 127.99 (C-4), 130.15 (C-2), 150.63 (C-1), 158.71 (*C*OCF_3), 173.98 (C-10), 174.37 (C-12); HRMS (FAB$^+$): calcd for $C_{18}H_{24}F_3N_3O_5$: 420.1746. Found: 420.1749.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-(isonipecotyl)-amino-L-tyrosine (13a).** A solution of **7a** (130 mg, 0.214 mmol) in EtOAc:EtOH (1:1, 10 mL) was placed in a Parr flask and hydrogenated ($pH_2 = 40$ psi) in the presence of Palladium (10% on C; 23 mg, 0.021 mmol), during 18 h under vigorous shaking. After filtration and concentration under vacuum, the residue (93 mg) was dissolved in CF_3CO_2H (3 mL) and left for 2.5 h at 20 °C. Workup as above gave 100 mg (Yield: 100%) of **13a**: ^{1}H NMR (D_2O, 500 MHz) δ 1.94 (m, 2H, H-14), 2.16 (m, 2H, H-14′), 2.85 (tt, $J = 3.4$ and 11.9 Hz, 1H, H-13), 2.97 (dd, $J = 9.5$ and 14. Hz, 1H, H-7), 3.11 (m, 2H, H-15), 3.29 (dd, $J = 5.2$ and 14 Hz, 1H, H-7′), 3.53 (m, 2H, H-15′), 3.83 (s, 3H, H-a), 4.75 (m, 1H, H-8), 7.03 (d, $J = 8.8$ Hz, 1H, H-6), 7.13 (dd, $J = 2.1$ and 8.8 Hz, 1H, H-5), 7.44 (d, $J = 2.1$ Hz, 1H, H-3); ^{13}C NMR (D_2O, 125 MHz) ppm 27.55 (C-14), 27.63 (C-14′), 38.20 (C-7), 42.64 (C-13), 45.61 (C-15), 57.00 (C-8), 58.52 (C-a), 114.74 (C-6), 118.11 (*C*F_3), 127.53 (C-4), 127.76 (C-3), 130.39 (C-5), 131.17 (C-2), 153.30 (C-1), 160.96 (*C*OCF_3), 175.84 (C-12), 177.66 (C-10); HRMS: calcd for $C_{18}H_{22}F_3N_3O_5$: 418.1590. Found: 418.1603.

***N*-(Trifluoroacetyl)-*O*-methyl-*ortho*-(nipecotyl))-amino-L-tyrosine (14a).** A solution of **8a** (160 mg, 0.263 mmol) in EtOAc:-EtOH (1:1, 10 mL) was placed in a Parr flask and hydrogenated ($pH_2 = 40$ psi) in the presence of Palladium (10% on C; 28 mg, 0.026 mmol), under vigorous shaking, during 18 h. After filtration and concentration, the residue (111 mg) was dissolved in CF_3CO_2H (3.5 mL) and left for 2.5 h at 20 °C. Work up as above gave 120 mg (yield: 100%) of **14a**: ^{1}H NMR (D_2O, 500 MHz) δ 1.85 (m, 2H, H-17 + H-18), 1.99 (m, 1H, H-17′), 2.15 (m, 1H, H-18′), 2.97 (m, 1H, H-7), 3.03 (m, 1H, H-13), 3.11 (m, 1H, H-16), 3.29 (m, 2H, H-16′ + H-14), 3.29 (m, 1H, H-7′), 3.45 (m, 1H, H-14′), 3.83 (s, 3H, H-a), 4.75 (m, 1H, H-8), 7.03 (d, $J = 8.6$ Hz, 1H, H-6), 7.13 (dd, $J = 2.1$ and 8.6 Hz, 1H, H-5), 7.45 (d, $J = 2.1$ Hz, 1H, H-3); ^{13}C NMR (D_2O, 125 MHz) δ 23.07 (C-17), 28.25–28.30 (C-18), 38.07–38.13 (C-7), 41.76 (C-13), 46.41 (C-16), 47.22 (C-14), 56.91 (C-8), 58.52 (C-a), 114.76 (C-6), 118.13 (*C*F_3), 127.32 (C-4), 127.78–127.86 (C-3), 130.50–130.56 (C-5), 131.14 (C-2), 153.30–153.38 (C-1), 160.96 (*C*OCF_3), 175.78 (C-12), 176.11 (C-10); HRMS: calcd for $C_{18}H_{22}F_3N_3O_5$: 418.1590. Found: 418.1587.

***O*-Methyl-*ortho*-nitro-L-tyrosine terbutylester (22).** To a solution of **3a** (2.27 g, 5.785 mmol) in MeOH:H_2O (1:1,

100 mL), was added K_2CO_3 (4.207 g, 30.14 mmol). The mixture was stirred during 17 h at 20 °C, then concentrated under vacuum. Toluene was added and distilled. The residue was dissolved in CH_2Cl_2 and washed with water (3×). The organic phase was dried ($MgSO_4$), and concentrated under vacuum to give the crude amine **22** (1.504 g, 88% yield): R_f (SiO_2; CH_2Cl_2:EtOAc, 9:1) = 0.1; IR ($CHCl_3$) ν 3383, 2977, 2934, 1727, 1623, 1531, 1506, 1457, 1358, 1289, 1260, 1154, 1090, 1019 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 9H, H-β), 2.83 (dd, J = 7.3 and 14.2 Hz, 1H, H-7), 2.98 (dd, J = 5.5 and 14.2 Hz, 1H, H-7′), 3.56 (m, 1H, H-8), 3.93 (s, 3H, H-a), 7.01 (d, J = 8.6 Hz, 1H, H-6), 7.41 (dd, J = 2.1 and 8.6 Hz, 1H, H-5), 7.72 (d, J = 2.1 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.88 (C-β), 39.48 (C-7), 55.91 (C-8), 56.44 (C-a), 81.49 (C-α), 113.38 (C-6), 126.21 (C-3), 130.07 (C-4), 135.12 (C-5), 139.25 (C-2), 151.69 (C-1), 173.82 (C-10); MS (FAB^+) *m/e* 297, 241, 57.

***N*-(*meta*-Trifluoromethyl-benzenesulfonyl)-*O*-methyl-*ortho*-nitro-L-tyrosine terbutyl ester (23).** To a solution of **22** (1.454 g, 4.91 mmol) in CH_2Cl_2 (63 mL), were added successively Et_3N (0.75 mL, 0.546 mg, 5.397 mmol, 1.1 equiv) and *meta*-trifluoromethyl-benzenesulfonyl chloride (0.865 mL, 1.32 g, 5.397 mmol, 1.1 equiv). The mixture was stirred at 20 °C during 20 h, then washed with 1 N HCl (2×10 mL) and water (2×10 mL). After drying ($MgSO_4$), concentration and flash-chromatography (silica gel, $CHCl_3$), the sulfonamide **23** was recovered (1.26 g, 51% yield): IR ($CDCl_3$) ν 3650, 3273, 3076, 2982, 2937, 2846, 1733, 1624, 1575, 1534, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.22 (s, 9H, H-β), 2.93 (dd, J = 7.3 and 14.2 Hz, 1H, H-7), 3.04 (dd, J = 5.9 and 14.2 Hz, 1H, H-7), 3.87 (s, 3H, H-a), 4.05 (m, 1H, H-8), 5.75 (br s, 1H, NH-9), 6.95 (d, J = 8.6 Hz, 1H, H-6), 7.36 (dd, J = 2.1 and 8.6 Hz, 1H, H-5), 7.58 (d, J = 2.1 Hz, 1H, H-3), 7.58 (t, J = 8.0 Hz, 1H, H-y), 7.75 (d, J = 8.0 Hz, 1H, H-x), 7.93 (d, J = 8.0 Hz, 1H, H-z), 8.00 (s, 1H, H-v); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.38 (C-β), 37.68 (C-7), 56.24 (C-a), 56.85 (C-8), 83.43 (C-α), 113.46 (C-6), 122.91 (*C*F_3), 123.80 (C-v), 126.24 (C-3), 127.48 (C-4), 129.15 (C-x), 129.75 (C-y), 130.22 (C-z), 131.34 (C-w), 135.41 (C-5), 138.85 (C-u), 140.84 (C-2), 151.94 (C-1), 169.14 (C-10); MS (FAB^-) *m/e* 503 (M–1), 209; Anal. calcd for $C_{21}H_{23}F_3N_2O_7S$ (504.47): C, 49.99; H, 4.59; N, 5.55; S, 6.35. Found: C, 49.79; H, 4.50; N, 5.28; S, 6.09.

***N*-(*meta*-Trifluoromethyl-benzenesulfonyl)-*O*-methyl-*ortho*-amino-L-tyrosine terbutylester (24).** A solution of **23** (1.259 g, 2.495 mmol) in methanol (20 mL) was placed in a Parr flask and hydrogenatad (pH_2 = 40 psi) in the presence of platinium IV oxide (56.7 mg, 0.249 mmol, 0.1 equiv), under vigorous shaking, during 17 h at 20 °C. After filtration and concentration, the residue was dissolved in CH_2Cl_2 and dried over MgSO4. Concentration under vacuum gave 1.074 g of crude **23** (Yield: 91%): mp 40–41 °C; IR ($CHCl_3$) ν 3275, 2980, 1734, 1617, 1517, 1438, 1327, 1280, 1232, 1161, 1132, 1105 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (s, 9H, H-β), 2.90 (m, 2H, H-7), 3.80 (s, 3H, H-a), 4.06 (m, 1H, H-8), 5.44 (d, 1H, NH-9), 6.52 (d, 1H, H-5), 6.63 (d + s, 2H, H-6 + H-3), 7.58 (t, 1H, H-y), 7.76 (d, 1H, H-x), 7.93 (d, 1H, H-z), 8.04 (s, 1H, H-v); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.59 (C-β), 38.58 (C-7), 55.44 (C-a), 57.17 (C-8), 82.69 (C-α), 110.37 (C-6), 117.32 (C-3), 120.82 (C-5), 122.9 (*C*F_3), 124.04 (C-v), 127.53 (C-4), 129.0 (C-x), 129.64 (C-y), 130.42 (C-z), 131.4 (C-w), 133.56 (C-2), 141.3 (C-u), 147.13 (C-1), 169.61 (C-10); MS (FAB^+) *m/e* 475 (M + 1), 474, 419, 401, 373.

***N*-(*meta*-Trifluoromethyl-benzenesulfonyl)-*O*-methyl-*ortho*-[6-*N*,*N*′-(diterbutoxycarbonyl) guanidino-caproyl]-amino-L-tyrosine terbutylester (26).** To a solution of **24** (229 mg, 0.483 mmol) in CH_2Cl_2 (5 mL) were added the acid chloride **20b** (207 mg, 0.528 mmol, 1.09 equiv) and pyridine (0.117 mL, 114 mg, 1.447 mmol, 3 equiv) in CH_2Cl_2 (5 mL). The mixture was stirred at 20 °C for 20 h, then washed twice with 1.5 N HCl, and water. Drying ($MgSO_4$), concentration and flash chromatography (silica gel, CH_2Cl_2:EtOAc, 9:1) gave 92 mg (Yield: 24%) of **26**: IR ($CDCl_3$) ν 3332, 2978, 2930, 1723, 1641, 1617, 1537, 1481, 1161, 1134 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.27 (s, 9H, H-β), 1.44 (m, 2H, H-15), 1.48 (s, 9H, H-23), 1.49 (s, 9H, H-26), 1.62 (m, 2H, H-16), 1.74 (m, 2H, H-14), 2.37 (m, 2H, H-13), 2.86 (dd, J = 7.3 and 14.2 Hz, 1H, H-7), 3.00 (dd, J = 5.2 and 14.2 Hz, 1H, H-7′), 3.41 (m, 2H, H-17), 3.84 (s, 3H, H-a), 4.06 (m, 1H, H-8), 5.23 (d, J = 9.4 Hz, 1H, NH-9), 6.72 (d, J = 8.6 Hz, 1H, H-6), 6.82 (dd, J = 2.1 and 8.6 Hz, 1H, H-5), 7.55 (dd, J = 8.0 Hz, 1H, H-y), 7.67 (s, 1H, NH-11), 7.73 (d, J = 8.0 Hz, 1H, H-x), 7.94 (d, J = 8.0 Hz, 1H, H-z), 7.99 (s, 1H, H-v), 8.14 (d, J = 2.1 Hz, 1H, H-3), 8.31 (m, 1H, NH-18), 11.50 (s, 1H, NH-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.09 (C-14), 26.35 (C-15), 27.58 (C-β), 27.96 (C-26), 28.19 (C-23), 28.69 (C-16), 37.61 (C-13), 38.72 (C-7), 40.65 (C-17), 55.54 (C-a), 57.14 (C-8), 79.14 (C-22), 82.96 (C-25 + C-α), 109.60 (C-6), 120.50 (C-3), 122.91 (*C*F_3), 124.12 (C-v), 124.39 (C-5), 127.48 (C-4), 127.56 (C-2), 128.93 (C-x), 129.53 (C-y), 130.51 (C-z), 131.37 (C-w), 141.20 (C-u), 146.65 (C-1), 153.24 (C-24), 156.00 (C-21), 163.47 (C-19), 169.67 (C-10), 170.58 (C-12); MS (FAB^-) *m/e* 829 (M-1) 828, 711, 209; Anal. calcd for $C_{38}H_{54}F_3N_5O_{10}S$ (829.92): C, 54.99; H, 6.55 ; N, 8.43. Found: C, 55.10; H, 6.20; N, 8.16.

***N*-(*meta*-Trifluoromethyl-benzene sulfonyl)-*O*-methyl-*ortho*-[6-(benzyloxycarbonyl) amino-caproyl]-amino-L-tyrosine terbutylester (25).** To a solution of **24** (669 mg, 1.409 mmol) in CH_2Cl_2 (5 mL), were added *N*-(benzyloxycarbonyl) aminocaproyl chloride (400 mg, 1.409 mmol,

1 equiv) and pyridine (0.33 mL, 323 mg, 4.08 mmol, 2.89 equiv) in CH_2Cl_2 (5 mL). The mixture was stirred for 20 h at 20 °C, then worked-up as before to furnish 490 mg (yield: 48%) of **25**: IR (film) ν 3370, 2937, 1715, 1698, 1597, 1536, 1162 cm^{-1}; R_f (SiO_2; CH_2Cl_2:EtOAc, 9:1) = 0.1; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.25 (s, 9H, H-β), 1.37 (m, 2H, H-15), 1.52 (m, 2H, H-16), 1.70 (m, 2H, H-14), 2.34 (t, J = 7.3 Hz, 2H, H-13), 2.82 (dd, J = 7.3 and 14.2 Hz, 1H, H-7), 2.97 (dd, J = 5.6 and 14.2 Hz, 1H, H-7′), 3.18 (m, 2H, H-17), 3.80 (s, 3H, H-a), 4.05 (m, 1H, H-8), 4.96 (m, 1H, NH-18), 5.06 (m, 2H, H-21), 5.61 (d, J = 9.5 Hz, 1H, NH-9), 6.68 (d, J = 8.6 Hz, 1H, H-6), 6.80 (m, 1H, H-5), 7.27–7.37 (m, 5H, H-23 + H-24 + H-25), 7.51 (dd, J = 8.0 Hz, 1H, H-y), 7.67 (s, 1H, NH-11), 7.70 (d, J = 8.0 Hz, 1H, H-x), 7.90 (d, J = 8.0 Hz, 1H, H-z), 7.98 (s, 1H, H-v), 8.14 (d, J = 2.1 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.88 (C-14), 26.03 (C-15), 27.45 (C-β), 29.48 (C-16), 37.40 (C-13), 38.53 (C-7), 40.65 (C-17), 55.41 (C-a), 57.21 (C-8), 66.29 (C-21), 82.66 (C-α), 109.53 (C-6), 120.42 (C-3), 123.00 (CF_3), 123.89 (C-v), 124.30 (C-5), 127.30 (C-4), 127.58 (C-2), 127.80 (C-24 + C-25), 128.25 (C-23), 128.71 (C-x), 129.41 (C-y), 130.35 (C-z), 131.11 (C-w), 136.51 (C-22), 141.23 (C-u), 146.55 (C-1), 156.25 (C-19), 169.68 (C-10), 170.61 (C-12); HRMS: calcd for $C_{35}H_{42}F_3N_3O_8S$: 721.2645. Found: 721.2647.

***N*-(*meta*-Trifluoromethyl-benzenesulfonyl)-*O*-methyl-*ortho*-(6-guanidino-caproyl)-amino-L-tyrosine (28).** A solution of **26** (75 mg, 0.131 mmol) in trifluoroacetic acid (2 mL) was left for 2 h at 20 °C, then evaporated under vacuum. The residue was dissolved in water and extracted with $CHCl_3$. Freeze-drying of the aqueous phase gave 120 mg (yield ∼ 100%) of **28** (hydroscopic white powder): ^1H NMR (CD_3OD, 500 MHz) δ 1.46 (m, 2H, H-15), 1.64 (m, 2H, H-16), 1.74 (m, 2H, H-14), 2.45 (t, J = 7.3 Hz, 2H, H-13), 2.73 (dd, J = 9.5 and 14.0 Hz, 1H, H-7), 3.00 (dd, J = 4.6 and 14.0 Hz, 1H, H-7′), 3.18 (t, J = 7.3 Hz, 2H, H-17), 3.81 (s, 3H, H-a), 4.04 (m, 1H, H-8), 6.74 (d, J = 8.6 Hz, 1H, H-6), 6.83 (m, 1H, H-5), 7.55 (dd, J = 8.0 Hz, 1H, H-y), 7.73 (d, J = 2.1 Hz, 1H, H-3), 7.79 (d, J = 8.0 Hz, 1H, H-z), 7.81 (d, J = 8.0 Hz, 1H, H-x), 7.91 (s, 1H, H-v); ^{13}C NMR (CD_3OD, 125 MHz) δ 26.27 (C-14), 27.21 (C-15), 29.60 (C-16), 37.49 (C-13), 39.25 (C-7), 42.33 (C-17), 56.18 (C-a), 59.34 (C-8), 111.53 (C-6), 124.22 (C-3), 124.62 (C-v), 124.90 (CF_3), 126.91 (C-5), 127.85 (C-4), 129.69 (C-x), 129.80 (C-2), 131.06 (C-y), 131.46 (C-z), 132.02 (C-w), 143.83 (C-u), 150.22 (C-1), 158.68 (C-19), 174.33 (C-12), 174.58 (C-10); HRMS; calcd for $C_{26}H_{31}F_6N_5O_8S$: 574.1947. Found: 574.1950.

***N*-(*meta*-Trifluoromethyl-benzenesulfonyl)-*O*-methyl-*ortho*-(6-amino-caproyl)-amino-l-tyrosine (27).** A solution of **25** (112 mg, 0.154 mmol) in EtOH:EtOAc (50:50, 10 mL) was placed in a Parr flask and hydrogenated (pH_2 = 40 psi) in the presence of Palladium catalyst (10% on C, 4.5 mg, 0.042 mmol, 0.27 equiv) during 18 h at 20 °C, under vigorous shaking. Filtration and concentration under vacuum gave a residue which was dissolved in trifluoroacetic acid (6 mL) and left for 2.5 h at 20 °C. Work up as before furnished 88 mg (yield: 90%) of **27** (hygroscopic solid): $[\alpha]_D^{20}$ −7.5° (*c* 0.04; H_2O:CH_3CN, 20:80); IR (film) ν 3423, 2948, 2534, 1674, 1655, 1539, 1204, 1136 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 1.47 (m, 2H, H-15), 1.72 (m, 4H, H-14 + H-16), 2.46 (t, J = 7.3 Hz, 2H, H-13), 2.72 (dd, J = 9.5 and 14.0 Hz, 1H, H-7), 2.94 (m, 2H, H-17), 3.00 (dd, J = 5.2 and 14.0 Hz, 1H, H-7′), 3.81 (s, 3H, H-a), 4.05 (dd, J = 5.2 and 9.5 Hz, 1H, H-8), 6.74 (d, J = 8.6 Hz, 1H, H-6), 6.83 (dd, J = 2.1 and 8.6 Hz, 1H, H-5), 7.55 (dd, J = 8.0 Hz, 1H, H-y), 7.74 (d, J = 2.1 Hz, 1H, H-3), 7.78 (d, J = 8.0 Hz, 1H, H-x), 7.81 (d, J = 8.0 Hz, 1H, H-z), 7.91 (s, 1H, H-v); ^{13}C NMR (CD_3OD, 125 MHz) δ 26.16 (C-14), 26.93 (C-15), 28.31 (C-16), 37.32 (C-13), 39.22 (C-7), 40.57 (C-17), 56.19 (C-a), 59.27 (C-8), 111.55 (C-6), 124.16 (C-3), 124.64 (C-v), 124.90 (CF_3), 126.85 (C-5), 127.89 (C-4), 129.62 (C-x), 129.79 (C-2), 131.02 (C-y), 131.46 (C-z), 131.96 (C-w), 143.82 (C-u), 150.18 (C-1), 174.14 (C-10), 174.34 (C-12); HRMS; calcd for $C_{23}H_{28}F_3N_3O_6S$: 532.1729. Found: 532.1743.

Benzyl 6-amino-caproate *para*-toluene sulfonate (18b). A mixture of 6-aminocaproic acid (5 g, 38.11 mmol), benzyl alcohol (250 mL) and *para*-toluene sulfonic acid (8.38 g, 42.3 mmol, 1.1 equiv) in benzene (500 mL) was heated at 100 °C for 3 h (azeotropic distillation with a Dean–Stark equipment). The solution was cooled to 20 °C under argon atmosphere. After addition of ether (500 mL), the product was allowed to crystallize at −30 °C, during 3 days. Filtration, washing with ether and drying under vacuum gave 14.38 g (yield: 95%) of salt **18b**: mp 106–107 °C; IR (KBr) ν 3465, 2943, 2869, 3041, 1728, 1626, 1482, 1196, 1142 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.19 (m, 2H), 1.48 (m, 4H), 2.20 (t, J = 7.3 Hz, 2H), 2.31 (s, 3H), 2.74 (m, 2H), 5.04 (s, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.27–7.37 (m, 5H), 7.68 (br s, 3H), 7.72 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.17, 24.02, 25.65, 26.92, 33.71, 39.55, 66.02, 125.74, 128.04, 128.09, 128.44, 128.97, 135.91, 140.76, 141.02, 173.01; MS (FAB^+) *m*/*e* 222.

***N*,*N*′-Diterbutoxycarbonyl-3,5-dimethylpyrazole-1-carboxamidine (17).** To a solution of 3,5-dimethylpyrazole-1-carboxamidine nitrate (2 g, 9.74 mmol) and di-*t*-butyl dicarbonate (11.53 mL, 10.96 g, 48.71 mmol, 5 equiv) in dry THF (80 mL), was added, under argon atmosphere, sodium hydride (90% purity, 1.23 g, 48.71 mmol, 5 equiv). The mixture was stirred under reflux (80 °C) during 6 h. Ethanol was cautiously added dropwise, and the solution was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 and washed with water.

Drying over $MgSO_4$, concentration and flash chromatography gave 2.43 g (yield: 73%) of **17**: mp 100–101 °C; IR (KBr) ν 3322, 3111, 2937, 1761, 1694, 1656 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.50 (s, 9H), 1.52 (s, 9H), 2.21 (s, 3H), 2.55 (s, 3H), 5.92 (s, 1H), 9.05 (br s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.40, 15.09, 27.94, 80.49, 82.53, 111.24, 140.37, 144.00, 149.52, 150.32, 157.46; MS (EI) *m/e* 338 (M), 282, 254, 238, 182, 165, 138, 96, 57, 43; Anal. calcd for $C_{16}H_{26}N_4O_4$ (338.40): C, 56.78; H, 7.74; N, 16.55. Found: C, 56.66; H, 7.76; N, 16.47.

Benzyl 6-(*N*,*N*′-diterbutoxycarbonyl)-guanidino-caproate (19b). To a solution of **17** (2.5 g, 7.38 mmol) in CH_2Cl_2 (300 mL), were added **18b** (3.19 g, 8.12 mmol, 1.1 equiv) and triethylamine (1.2 mL, 873 mg, 8.63 mmol, 1.16 equiv) The mixture was stirred for 20 h at 20 °C, then washed successively with 1 N HCl, water, 10% $NaHCO_3$ and water (3×). Drying ($MgSO_4$), concentration and chromatography on preparative MPLC gave 3.40 g (yield: 98%) of **19b**: R_f (SiO_2; hexane:isopropanol, 2:1) = 0.2; IR (KBr) ν 3328, 2980, 1740, 1728, 1651, 1627, 1417, 1370, 1362, 1156 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.38 (m, 2H), 1.49 (s, 9H), 1.50 (s, 9H), 1.57 (m, 2H), 1.68 (m, 2H), 2.36 (t, J = 7.3 Hz, 2H), 3.39 (m, 2H), 5.11 (s, 2H), 7.20–7.40 (m, 5H), 8.30 (m, 1H), 11.50 (br s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.51, 26.26, 27.98, 28.20, 28.62, 34.01, 40.58, 66.05, 79.11, 82.93, 128.10, 128.44, 135.95, 153.24, 156.02, 163.54, 173.21; MS (EI) *m/e* 463, 351, 290, 202, 188, 161, 158, 145, 144, 130, 117, 108, 65; Anal. calcd for $C_{24}H_{37}N_3O_6$ (463.57): C, 62.18; H, 8.04; N, 9.06. Found: C, 62.44; H, 8.21; N, 8.96.

6-(*N*,*N*′-Diterbutoxycarbonyl)-guanidino-caproyl chloride (20b). A solution of **19b** (2.9 g, 6.25 mmol) in EtOH: EtOAc (1:1; 50 mL), placed in a Parr flask, was hydrogenated (pH_2 = 40 psi) in the presence of Palladium catalyst (10% on C; 102 mg, 0.957 mmol, 0.153 equiv), during 18 h at 20 °C (vigorous shaking). Filtration, concentration and drying under vacuum gave 2.21 g (yield: 94%) of 6-(*N*,*N*′-diterbutoxycarbonyl)-guanidino-caproic acid: mp 83.1–84.1 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.41 (m, 2H), 1.49 (s, 9H), 1.50 (s, 9H), 1.60 (m, 2H), 1.67 (m, 2H), 2.36 (t, J = 7.3 Hz, 2H), 3.43 (m, 2H), 8.40 (s, 1H), 11.50 (br s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.15, 26.11, 27.92, 28.12, 28.53, 33.64, 40.72, 79.51, 83.15, 153.12, 155.88, 162.88, 178.57; MS (EI) *m/e* 373 (M), 317, 261, 244, 188, 161, 130, 57; Anal. calcd for $C_{17}H_{31}N_3O_6$ (373.44): C, 54.67; H, 8.36; N, 11.25. Found: C, 54.75; H, 8.11; N, 10.81. A solution of this acid (0.6 g, 1.6 mmol) and thionyl chloride (0.6 mL, 978 mg, 8.22 mmol, 5.12 equiv) in CH_2Cl_2 (20 mL) was refluxed (50 °C) during 1.5 h, then concentrated under vacuum. The excess of $SOCl_2$ was removed by azeotropic distillation with toluene (3×) and $CHCl_3$ (3×). The acid chloride **20b** was dried under high vacuum (617 mg, 98% yield) and used without purification.

Biological evaluation (in solution)

The purity of the pepdidomimetics was controlled by HPLC before use, with the following conditions: column: Nucleosil C18, 5 μ, 25 cm; temperature: 25 °C; eluent: gradient from 20% CH_3CN + 0.015% TFA − 80% H_2O + 0.015% TFA to 80% CH_3CN + 0.015% TFA − 20% H_2O + 0.015%; flow rate: 1 mL/min; equipment: Beckman, System Gold, 126 P Solvent module, 168 Detector (Analis, Belgium). The retention times (area) were: **16b**: t = 5.43 min (98.8%); **16a**: t = 7.53 min (96.9%); **12a**: t = 6.88 min (100%); **13a**: t = 11.53 min (94.1%); **27**: t = 8.67 min (98.5%).

The phosphate buffered saline (PBS) solution (pH 7.3) was prepared from NaCl (4 g), KCl (0.1 g), KH_2PO_4 (0.1 g) and Na_2HPO_4, $2H_2O$ (0.71 g) dissolved in water (HPLC grade, 500 mL). The stock solutions of peptidomimetics contained 1 to 5 mg of product per milliliter of PBS buffer; if needed, 0.5% DMSO could be added for complete dissolution. The tested concentrations were within 10^{-6} to 10^{-1} M.

Blood was drawn from the antecubital vein of healthy adult volunteers, who denied taking any medication for the previous 15 days, into a plastic syringe containing one part of 3.8% trisodium citrate to nine parts of blood. Platelet rich plasma (PRP) was prepared by centrifuging the blood at 1500 *g* for 10 min at room temperature. The PRP was drawn off and the remaining blood was centrifuged at 6000 *g* for 20 min at room temperature to make platelet poor plasma (PPP). The PRP was adjusted with PPP to a count of 3×10^5 platelets/mL; platelet count was measured with a Coulter counter. 400 μL of the PRP preparation and 50 μL of the solution of peptidomimetic to be tested, or saline, were preincubated for 2 min at 37 °C in an aggregometer; 50 μL of 0.047 mM ADP were added, and the aggregation was monitored during 4 min. Results were calculated as follows: [observed % aggregation (antagonist)] divided by [maximum % aggregation (control)] equals the % of control. The % inhibition = 100 − % of control. Concentration–response curves were constructed and the IC_{50} were determined as the concentration of antagonist required to produce 50% inhibition of the response to the agonist. At least two determinations were made for each compound and the IC_{50} calculated by fitting to a four parameter equation (average standard error of ± 30%).

Modelisation

All the degrees of freedom describing the molecular geometry have been fully optimized at the approximate quantum chemistry level AM1,[61] using the minimization procedures available in Gaussian suite of programs.[62] In

the structures superpositions, the starting point was the fitting of the respective carboxyl functions.

Surface chemistry

The PET microporous membrane was manufactured by Whatman SA (Louvain-la Neuve, Belgium) by track-etching treatment of PET Mylar A film (Dupont de Nemours, Brussels) characterized by a thickness of 12 μm, a density of 1.39 g/cm^3 (ASTM D 1505-66), a melting point of 251 °C (ASTM D 3418-82), a M_n of 48,800 and a MW of 88,800. The membrane contained 1.45×10^6 pores/cm^2 (apparent surface) of 0.49 μm in diameter. For the surface modifications, disks of 13 mm in diameter were cut off the membrane; the open surface of a disk sample is 3.01 cm^2 (apparent surface and internal surface of the pores).

The phosphate buffer (pH 8.2) was prepared from $Na_2HPO_4.2H_2O$ (4.215 g) and $NaH_2PO_4.H_2O$ (0.2065 g) dissolved in water (250 mL, HPLC grade). The MES buffer (pH 3.5) was obtained from 2-(*N*-morpholino)-ethanesulfonic acid (MES, 5.331 g) dissolved in water (250 mL, HPLC grade). L-[4,5-^{3}H] lysine monohydrochloride in aqueaus solution was purchased from Amersham (Little Chalfont, UK); the labeling solution (10^{-3} M) was prepared as follows: to 250 μL of unlabeled lysine solution (0.1826 g/10 mL phosphate buffer) were added 187.5 μL of labeled lysine (as = 98 Ci/mmol); this solution was diluted to 25 mL with phosphate buffer. Water (HPLC grade) was obtained with a Milli-Q system (Millipore, Bedford, MA, USA).

Radioactivity measurement: the amount of labeled lysine fixed on the PET disks was measured by liquid scintillation counting (LSC) of the sample-associated radioactivity according to references 7–9, using a Tri-Carb 1600 TR liquid scintillation analyser (PACKARD).

XPS analysis: the surface chemical composition of the modified PET disks was determined by X-ray photoelectron spectroscopy according to references 10 and 11 and 45-46, using a SSI-X probe (SSX-100/206) spectrometer from Fisons (Surface Science Laboratories, Mountain View, CA).

PET membrane oxidation. The PET disks (1 sample per 10 mL of reactive solution) were immersed into an acidic solution of $KMnO_4$ (6 g, in 120 mL of 1.2 N H_2SO_4), and heated at 60 °C during 1 h, under shaking with an Edmund B hler stirrer (model KL-2). The PET disks were taken off the solution with tweezers and washed successively with 6 N HCl (1×20 min and 2×5 min; 10 mL per sample) and water (2×10 min; 10 mL per sample). The disks were drained over filter paper and air-dried. Oxidized PET is called PET- CO_2H.

Activation of PET-CO_2H. The PET-CO_2H disks (1 sample per 20 mL of reactive solution) were immersed into a solution of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (water soluble carbodiimide = WSC; 0.1 g) in 0.1 N MES buffer (100 mL), for 1 h at 20 °C, under shaking. The samples were taken off the solution and rinsed successively with 0.1 N MES buffer (1×10 min; 20 mL per sample) and water (2×10 min; 20 mL per sample). The activated PET-CO_2H disks were directly used for the radiolabeling and the coupling to the peptidomimetic.

Radiolabeling of PET-CO_2H. The activated PET- CO_2H disks were individually treated in small pyrex tubes containing 1.5 mL of the radioactive lysine* solution (10^{-3} M), during 2 h at 20 °C, under shaking. The disks were individually washed with 1.5 mL of phosphate buffer (pH 8, 1×10 min and 2×5 min), 1.5 mL of water (1×5 min), 1.5 mL of 0.005 M HCl (3×10 min) and 1.5 mL of water (1×5 min and 2×5 min). The samples were drained over filter paper and directly used for the radioactivity measurement; they were individually placed in 20 mL polyethylene vials containing 5 mL of aqualuma cocktail (Lumac, Basel, Switzerland). The blank samples (references for the counting of the non-specific fixation or adsorption of the radioactive label) were prepared according to the previous procedures, but, in the activation step, the carbodiimide was omitted.

LSC counting results (average of five different samples ± standard deviation): sample: 57.5 ± 3.8 pmol/cm^2 of open surface; blank: 22.9 ± 2.9 pmol/cm^2 of open surface; corrected value: 34.6 ± 3.3 pmol/cm^2 of open surface. Since a PET interface domain of 50 Å in depth contains about 2860 pmol of monomer units/cm^2, the value of 35 pmol/cm^2 corresponds to 1.22% of surface derivatization.[7]

Coupling of peptidomimetic to PET-CO_2H. The activated PET-CO_2H disks were individually treated in small pyrex tubes containing 1.5 mL of peptidomimetic solution (30.9 mg of **16b** in 50 mL of phosphate buffer), during 2 h at 20 °C, under shaking. The disks were individually washed as described for the radiolabelling, then air-dried and storred in the dark.

The blank samples (references for the XPS analysis of the peptidomimetic adsorption) were prepared according to the previous procedures, but, in the activation step, the carbodiimide was omitted.

XPS analysis results: sample: C_{1s}, 70.86%; O_{1s} 27.58%; N_{1s}, 1.32%; F_{1s}, 0.24%; blank; C_{1s}, 70.69%; O_{1s}, 28.96%; N_{1s}, 0.35%; F_{1s}, 0.00%. F/C × 100 atomic ratio: sample: 0.339; blank: 0.000 (no correction needed). Calculation of the percentage of surface derivatization: we considered a theoretical monomer unit consisting of

$[(PET)_x + (PET-RGD\ peptidomimetic)_y]$, i.e. $[(C_{10}H_8O_4)_x + (C_{31}H_{36}O_9N_6F_3)_y]$, where $x+y=1$. Thus, the F/C atomic ratio is $3x/[31x+10(1-x)]$. For F/C = 0.00339 (experimental value), $x = 0.01079$, i.e. 1.08% of modified units.

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