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-amino-tyrosine template (O-substituted with an anchorage-arm or a methyl group, and α-N-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. © 1998 Elsevier Science Ltd. All rights reserved.

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 biocompatible 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 biocompatibility 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 αIIbβ3 integrin, which natural ligand is the blood protein fibrinogen. 19 The related integrin αvβ3 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 αIIbβ3 or αvβ3 integrins. 22

Over the last 10 years, peptidomimetics 23–25 of the RGD sequence have been developed as potential drugs,
mainly in the field of orally available anticoagulants (\(\alpha_{1b}\beta_3\) antagonists) for the treatment of thromboembolic diseases. Very recently, the search of \(\alpha_{1b}\beta_3\) antagonists has emerged in order to treat cancers and other disorders in which neovascularization plays a critical role. 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. 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 have been proposed as antiplatelet agents; for instance, molecules were constructed from benzodiazepine, isoquinolone, isoxazoline, pyrazolopiperazinone, or 3-(hydroxymethyl) benzamide 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 \(\text{p-hydroxybenzamide}, \text{piperazine}, \text{carbohydrate}, \text{or benzoyloxycarbonyl}\) groups.

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; one compound (Aggrastat, \(\text{MK-383}\)) is in phase III clinical trials. 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 \(\alpha\)-amino function, that has to be masked with a lipophilic group, 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; (c) the aromatic 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 pharmophore should be within 10–20 Å.

The \(\text{N-trifluoroacetyl}\) 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-butyloxycarbonyl (Boc), and benzoyloxycarbonyl (Cbz) groups.

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

For chemoselectivity reasons, the \(\text{O-alkylation}\) 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 methyl iodide. Thus, 2 was reacted with methyl iodide, 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 \(\text{N-methylation product of the trifluoroacetamide residue}\), was purified by preparative medium pressure liquid chromatography (MPLC) in 72% yield. The \(\text{N-protected anchorage-arm, N-(Boc)-3-bromopropylamine}\), 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...
investigated, using the precursor 3a in which the anchorage-arm is replaced with a simple methyl group. Reduction of 3a by catalytic hydrogenation over platinum 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-\(N\),\(N\),\(N\),\(N\)0-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-amino-caproic 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 (tBu 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 \(1H\)-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 1. Synthesis of peptidomimetics (first family). Reagents and conditions: (i) (CF\(_3\)CO\(_2\))\(_2\)O, 0°C, 2 h; (ii) HNO\(_3\)-HOAc, 10°C, 1 h; (iii) a. CH\(_3\)I, CH\(_3\)CN, K\(_2\)CO\(_3\), reflux, 48 h; b. BocNH-(CH\(_2\))\(_3\)-Br, CH\(_3\)CN, K\(_2\)CO\(_3\), reflux, 24 h; (iv) H\(_2\), PtO\(_2\), EtOH, 4–12 h, 20°C; (v) acid chloride, pyridine, CH\(_2\)Cl\(_2\), 17 h, 20°C; then column chromatography; (vi) Pd-C, EtOAc, 20°C, 18 h; (vii) CF\(_3\)CO\(_2\)H, 2 h, 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 \(N, N\)-ditertio-butoxycarbonyl-3,5-dimethylpyrazole-1-carboxamidine \(17\) 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 \(\delta\) \((J \sim 2\) Hz), a doublet of doublet at 7.1–7.2 \(\delta\) \((J \sim 2\) Hz and 8.7 Hz) and a doublet at 7.0–7.1 \(\delta\) \((J \sim 8.7\) Hz); (b) the proton of the tyrosine \(\alpha\)-CH group is a doublet of doublet at 4.6–4.8 \(\delta\) \((J \sim 5\) and 9.5 Hz); (c) the protons of the tyrosine \(\beta\)-CH\(_2\) group appear to be non-equivalent, giving a ABX pattern centred at 2.95–3.00 \(\delta\) and 3.25–3.30 \(\delta\) \((JAB \sim 14\) 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 \(\alpha\) and \(\beta\) 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 \(\alpha\)-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 \(\alpha\)-amino function was performed after the tyrosine O-alkylation step. Thus, compound \(3a\) (see Scheme 1) was submitted
to basic hydrolysis to furnish the free amine 22 which was directly treated with meta-trifluoromethyl-benzene-sulfonyl 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 platinum 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 \( \alpha \)-CH group gives a doublet of doublet at 4 \( \delta \); 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 \(^1\)H NMR spectrum, the three methylene protons of the anchorage-arm give multiplets at 2.13 \( \delta \), 3.16 \( \delta \) and 4.16 \( \delta \); 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 \( \mu \)M.

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

<table>
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<th>Entry</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Compound</th>
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<tr>
<td>1</td>
<td></td>
<td>COCF(_3)</td>
<td>Me</td>
<td>16a</td>
<td>465 (129)</td>
</tr>
<tr>
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<td></td>
<td>COCF(_3)</td>
<td>NH(_2)</td>
<td>16b</td>
<td>320 (68)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>SO(_2)PhCF(_3)</td>
<td>Me</td>
<td>28</td>
<td>85 (68)</td>
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<tr>
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<td></td>
<td>COCF(_3)</td>
<td>Me</td>
<td>12a</td>
<td>unsoluble</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>SO(_2)PhCF(_3)</td>
<td>Me</td>
<td>27</td>
<td>125 (100)</td>
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<tr>
<td>6</td>
<td></td>
<td>COCF(_3)</td>
<td>Me</td>
<td>13a</td>
<td>&gt;1000</td>
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<td>COCF(_3)</td>
<td>Me</td>
<td>14a</td>
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\( a IC_{50} \) value (\( \mu M \)) of the RGDS tetrapeptide (Arg-Gly-Asp-Ser).
the (iso)nipecotic derivatives are inactive (entries 6 and 7). In the more active compounds, the \( \alpha \)-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, 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, while the cyclo (Arg-Gly-Asp-D-Phe-Val) (c[RGDfV]) is a potent and selective \( \alpha_v\beta_3 \) integrin antagonist (Fig. 3).
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,\textsuperscript{27,41} the overall length of \text{c[RGDfV]} is shorter comparatively to the \text{\textalpha\textbeta} 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)\textsuperscript{1,2}. Membranes made from PET films are also appropriate substrates for cultivating mammalian cells.\textsuperscript{4,6} In our laboratory, we use microporous membranes obtained by a track-etching process which allows the preparation of ‘capillary-pore’ membranes\textsuperscript{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\textsuperscript{+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.\textsuperscript{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\textsubscript{4} in 1.2 N H\textsubscript{2}SO\textsubscript{4}) which transforms native hydroxyl chain-ends into new carboxyl endings\textsuperscript{7,9}; the resulting membrane was called PET-CO\textsubscript{2}H (Scheme 4).

The surface reactivity of the PET-CO\textsubscript{2}H membrane was assayed by the covalent coupling of \textsuperscript{3}H-lysine followed by liquid scintillation counting (LSC) of the sample associated radioactivity.\textsuperscript{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 \textsuperscript{3}H-lysine (10^{-3} M solution, 2 h, 20 °C, pH 8); we found a value of about 35 pmol/cm\textsuperscript{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\textsuperscript{7}, we calculated that this value corresponds to the functionalization of about 1.2% of the monomer units (see Experimental).
The RGD peptidomimetic 16b was similarly coupled to the PET-CO₂H membrane activated by the pre-treatment with WSC (Scheme 4). A blank sample was prepared by immersing a non-activated PET-CO₂H membrane into the solution of 16b in phosphate buffer (0.062%, or \(10^{-3}\)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 \(\times 100\) 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, a surface concentration of about 10 fmol/cm² 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/cm²) 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 immobilization 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

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**Scheme 4.** Covalent coupling of 16a onto the PET membrane surface.
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 CO₂H 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 (± 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 ¹³C 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 D₂O. 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
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 HNO₃ 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 MgSO₄, concentrated under vacuum and purified by column chromatography on silica gel, with CH₂Cl₂ as eluent, to give 0.307 g (yield: 41%) of 2: Rf = 0.6; mp 85.4–87.6 °C; IR (KBr) v 3358, 1755, 1709, 1550, 1161 cm⁻¹; ¹H NMR (CDCl₃, 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, CDCl₃ 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); ¹³C NMR (CDCl₃, 125 MHz) δ 27.86 (C-β), 35.87 (C-7), 53.70 (C-8), 84.40 (C-α), 115.35 (CF₃), 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 (COF₃), 168.51 (C-10); MS (EI) m/e 378; Anal. calcd for C₁₇H₁₇F₃N₂O₆ (392.23): C, 48.98; H, 5.25; N. Found: C, 47.7; H, 4.42; N, 7.1.

**N-(Trifluoroacetyl)-O-[N-(terbutyloxycarbonyl)-3-aminopropyl]-ortho-nitro-t-tyrosine tertbutylster (3a).** The reaction was conducted under argon atmosphere. To a solution of 2 (727 mg, 1.92 mmol) in acetonitrile (150 mL) were added iodo-methane (0.140 mL, 312 mg, 2.248 mmol, 1.17 equiv), potassium carbonate (268 mg, 1.94 mmol) and [18-crown-6]crown-ether (54 mg, 0.203 mmol, 0.105 equiv). The mixture was refluxed, under stirring, during 48 h (TLC control). CH₂CN was removed under vacuum; the residue was dissolved in CH₂Cl₂ (100 mL) and washed with water (3×20 mL). Drying over MgSO₄, concentration and flash chromatography on silica gel (hexane:isopropanol, 9:1) gave 698 mg of 3a contaminated with the methylolation product of the trifluoroacetamide fraction (¹H NMR: 2.91 δ (s, 3H); ¹³C NMR: 32.99 ppm; MS (FAB⁺) m/e 407). The product was further purified by preparative MPLC on reverse phase (Novapak 472% of 2). 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 HNO₃ 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 MgSO₄, concentrated under vacuum and purified by column chromatography on silica gel, with CH₂Cl₂ as eluent, to give 0.307 g (yield: 41%) of 2: Rf = 0.6; mp 85.4–87.6 °C; IR (KBr) v 3358, 1755, 1709, 1550, 1161 cm⁻¹; ¹H NMR (CDCl₃, 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, CDCl₃ 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); ¹³C NMR (CDCl₃, 125 MHz) δ 27.86 (C-β), 35.87 (C-7), 53.70 (C-8), 84.40 (C-α), 115.35 (CF₃), 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 (COF₃), 168.51 (C-10); MS (EI) m/e 378; Anal. calcd for C₁₇H₁₇F₃N₂O₆ (392.23): C, 48.98; H, 5.25; N. Found: C, 47.7; H, 4.42; N, 7.1.

**N-(Trifluoroacetyl)-O-[N-(terbutyloxycarbonyl)-3-aminopropyl]-ortho-nitro-t-tyrosine tertbutylster (3b).** The reaction was conducted under argon atmosphere. To a solution of 2 (371 mg, 0.979 mmol) in CH₂CN (35 mL), under argon atmosphere, were added 1 equiv of NaBO₃ and 1 equiv of sodium hydroxide (1.87 g, 0.979 mmol) in CH₂CN (35 mL). The mixture was stirred, under argon atmosphere (p=40 psi) and shaked under hydrogen atmosphere (p=40 psi) was placed in a Parr flask and shaken 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$_3$) v 3368, 3214, 2981, 2927, 1717, 1615, 1432, 1362 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 1.46 (s, 9H, H-β), 3.04 (m, 2H, H-7), 3.83 (s, 3H, H-a), 4.66 (dd, $J$ = 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) C-1, 111.48 (C-6), 115.52 (C-3), 119.05 (C-5), 127.20 (C-4), 136.19 (C-2), 146.57 (C-1), 154.40 (COOC$_3$), 168.98 (C-10); MS (FAB+) $m/e$ 363 (M+1), 262, 307, 289.

$\text{N-}(\text{Trifluoroacetyl})-\text{O}-[\text{N-(tert-butoxy carbonyl)-3-amino-propyl}]-\text{ortho-amino-1-tyrosine terbutylester (4b).}$ A solution of 3b (162 mg, 0.303 mmol) in methanol (10 mL) containing PtO$_2$ (4.4 mg, 0.016 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%); $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 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-α), 4.66 (m, 1H, H-8), 4.73 (m, 1H, H-9); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 15.62 (C-9), 28.72 (C-17), 28.86 (C-26), 28.95 (C-23 and C-g), 29.58 (C-16), 36.71 (C-7), 37.63 (C-13), 40.65 (C-25), 78.80 (C-8), 82.93 (C-25), 111.48 (C-6), 115.40 (CF$_3$), 115.80 (C-3), 119.05 (C-5), 127.50 (C-4), 136.40 (C-2), 145.64 (C-1), 155.88 (C-α), 156.40 (COOC$_3$), 168.97 (C-10).

$\text{N-}(\text{Trifluoroacetyl})-\text{O}-[\text{N-(tert-butoxy carbonyl)-3-amino-propyl}]-\text{ortho-6-[N, N-dibutoxy carbonbyl]guanidino-caproyl-amino-1-tyrosine terbutylester (10a).}$ To a solution of 4a (874 mg, 1.728 mmol) and pyridine (411 mg, 5.192 mmol, 3 equiv) in CH$_2$Cl$_2$ (10 mL), was added the acid chloride 20b (742 mg, 1.893 mmol, 1.09 equiv) in CH$_2$Cl$_2$ (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; $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 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-α), 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) $\delta$ 15.57 (C-9), 29.58 (C-16), 36.68 (C-7), 36.86 (C-6), 37.37 (C-13), 40.65 (C-25), 53.87 (C-8), 65.23 (C-α), 79.05 (C-22), 79.28 (C-5), 82.89 (C-25), 83.45 (C-2), 111.18 (C-6), 115.52 (CF$_3$), 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-c), 156.30 (COOC$_3$), 163.50 (C-19), 168.92 (C-10), 171.11 (C-12); Anal. calcd for C$_{39}$H$_{53}$F$_{3}$N$_{6}$O$_{11}$: C, 55.8; H, 7.37; N, 9.76. Found: C, 55.64; H, 7.06; N, 9.06.
crude acid chloride (625 mg, 91% yield). To a solution of 4a (464 mg, 1.28 mmol) in CH₂Cl₂ (4 mL) were added successively N-(benzylxoycarbonyl)-6-aminocaproyl chloride (400 mg, 1.41 mmol, 1.1 equiv) and pyridine (303 mg, 3.83 mmol, 4 equiv) in CH₂Cl₂ (4 mL). The mixture was stirred for 20 h at 20 °C, then washed twice with 1.5 N HCl, 10% NaHCO₃ and water. Drying (MgSO₄), concentration and flash-chromatography (silica gel, CHCl₃) gave 196 mg of 6a (yield: 25%): Rf = 0.2; IR (CHCl₃) ν 3309, 3083, 2944, 1717, 1696, 1675, 1597, 1536, 1569 cm⁻¹; ¹H NMR (CDCl₃, 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-α), 4.68 (dt, J = 6.1 and 7.6 Hz, 1H, H-18), 5.08 (s, 2H, H-21), 6.78 (m, 2H, H-5 + H-6), 7.03 (d, J = 7.6 Hz, 1H, NH-9), 7.27–7.38 (m, 5H, Ph), 7.81 (s, 1H, NH-10), 8.02 (s, 2H, H-21), 8.78 (m, 2H, H-5 + H-6), 8.89 (d, J = 7.3 Hz, 1H, NH-9), 7.28–7.37 (m, 5H, 5H, Ph), 7.11 (s, 1H, NH-11), 8.25 (s, 2H, H-21), 8.78 (m, 2H, H-5 + H-6), 6.89 (d, J = 7.9 Hz, 1H, NH-9), 7.27–7.37 (m, 5H, 5H, Ph); ¹³C NMR (CDCl₃, 125 MHz) δ 25.02 (C-14), 26.11 (C-15), 27.78 (C-16), 36.78 (C-7), 44.14 (C-16), 44.52 (C-13), 46.36 (C-15), 53.87 (C-8), 55.63 (C-α), 67.12 (C-20), 83.56 (C-b), 109.74 (C-6), 115.44 (C-8), 127.40 (C-4), 127.94 (C-24), 136.51 (C-19), 154.96 (C-17), 156.26 (C-1), 156.40 (COF₃C₃), 168.87 (C-12); HRMS (FAB⁺): m/e 607.5; Anal. calcd for C₃₀H₃₆F₃N₃O₇: 607.625. Found: C, 58.72; H, 5.97; N, 6.64.

N-(Trifluoroacetyl)-O-methyl-ortho-[N-(benzylxoycarbonyl)-nipeotyl]-amino-L-tyrosine terbutylester (7a). N-(Benzyloxycarbonyl)-nipeotyl chloride was prepared from N-(benzylxoycarbonyl)-nipeotic acid (331 mg, 1.25 mmol) and SOCl₂ (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 CH₂Cl₂ (10 mL), was added N-(benzyloxycarbonyl)-nipeotyl chloride (335 mg, 1.19 mmol, 1.22 equiv) in CH₂Cl₂ (10 mL). The mixture was stirred for 17 h at 20 °C (TLC control), then worked-up as above. Flash-chromatography (silica gel, CH₂Cl₂:EtOAc, 9:1) gave 318 mg of 7a (yield: 49%): mp 50–51 °C; IR (film) ν 3471, 2944, 1718, 1685, 1597, 1536, 1482, 1433, 1370, 1259, 1154 cm⁻¹; ¹H NMR (CDCl₃, 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-α); ¹³C NMR (CDCl₃, 125 MHz) δ 24.18 (C-17), 27.65 (C-18), 27.82 (C-16), 36.78 (C-7), 44.14 (C-16), 44.52 (C-13), 46.36 (C-14), 53.90 (C-8), 55.63 (C-α), 67.12 (C-20), 83.56 (C-β), 109.81 (C-6), 115.44 (CF₃), 120.68 (C-3), 124.36 (C-5), 127.40 (C-12), 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 (COF₃C₃), 168.95 (C-10), 170.85 (C-12); MS (FAB⁺) m/e 608.5; Anal. calcd for C₃₀H₃₆F₃N₃O₇: 607.62; C, 59.3; H, 5.97; N, 6.61. Found: C, 58.72; H, 5.98; N, 6.64.

N-(Trifluoroacetyl)-O-methyl-ortho-[N-(benzylxoycarbonyl)-nipeotyl]-amino-L-tyrosine terbutylester (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 (D₂O, 500 MHz) δ 1.44 (m, 2H, H-15), 1.64 (m, 2H, H-16), 1.73 (m, 2H, H-14), 2.47 (t,
14.0 Hz, 1H, H-7), 7.27 (m, 1H, H-3); 13C NMR (D2O, 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 (Cf), 127.71 (C-14), 128.04 (C-3), 130.41 (C-5), 131.58 (C-2), 153.40 (C-1), 159.33 (C-19), 160.85 (COCf3), 176.83 (C-12), 178.51 (C-10); HRMS (FAB+) for C18H22F3N3O5: 462.1971. Found: 462.1964. Front: 462.1971.

N-(Trifluoroacetyl)-O-(3-aminoapropionic)-ortho-(6-guanidinocaproyl)-amino-L-tyrosine (16b). The Boc-protected precursor 10b (750 mg, 0.871 mmol) was dissolved in CF3CO2H (13.5 mL) and left for 2 h at 20 °C. Work up as above gave 616 mg of 16b (yield: 97%): [2]20° = +2.5° (c 1, H2O); 1H NMR (D2O, 500 MHz) δ 1.31 (d, J = 7.3 Hz, 2H, H-13), 2.98 (dd, J = 9.5 and 14.0 Hz, 1H, H-7), 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); 13C NMR (D2O, 125 MHz) δ 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 (CF3), 124.41 (C-3), 126.89 (C-5), 127.99 (C-4), 130.15 (C-2), 150.63 (C-1), 158.71 (COCf3), 173.98 (C-10), 174.37 (C-12); HRMS: calcd for C18H22F3N3O5: 462.1746. 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 (pH2 = 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 CF3CO2H (3 mL) and left for 2.5 h at 20 °C. Work up as above gave 100 mg (Yield: 100%) of 13a: 1H NMR (D2O, 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 14.0 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); 13C NMR (D2O, 125 MHz) ppm δ 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 (CF3), 127.53 (C-4), 127.76 (C-3), 130.39 (C-5), 131.17 (C-2), 153.30 (C-1), 160.96 (COCf3), 175.84 (C-12), 177.66 (C-10); HRMS: calcd for C18H22F3N3O5: 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 (pH2 = 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 CF3CO2H (3.5 mL) and left for 2.5 h at 20 °C. Work up as above gave 120 mg (yield: 100%) of 14a: 1H NMR (D2O, 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); 13C NMR (D2O, 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 (CF3), 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 (COCf3), 175.84 (C-12), 177.66 (C-10); HRMS: calcd for C18H22F3N3O5: 418.1590. Found: 418.1587.

O-Methyl-ortho-nitro-L-tyrosine tertbutylester (22). To a solution of 3a (2.27 g, 5.785 mmol) in MeOH:H2O (1:1,
To a solution of 22 (1.545 g, 4.91 mmol) in CH2Cl2 (63 mL), were added successively Et3N (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 (MgSO4), concentration and flash-chromatography (silica gel, CHCl3), the sulfonamide 23 was recovered (1.26 g, 51% yield): IR (CDCl3) ν 3383, 2977, 2934, 1727, 1623, 1517, 1457, 1358, 1289, 1260, 1154, 1090, 1019 cm⁻¹; 1H NMR (CDCl3, 500 MHz) δ 1.22 (s, 9H, H-β), 3.56 (m, 1H, H-8), 3.93 (s, 3H, H-α), 4.06 (d, J = 8.6 Hz, 1H, H-6), 5.23 (d, J = 4.9 Hz, 1H, NH-9), 6.23 (d, J = 8.0 Hz, 1H, H-z), 7.38 (s, 1H, H-β), 7.58 (d, J = 2.1 Hz, 1H, H-3), 7.58 (t, J = 8.0 Hz, 1H, H-2), 8.00 (s, 1H, H-ν); 13C NMR (CDCl3, 125 MHz) δ 27.38 (C-β), 37.68 (C-7), 56.24 (C-α), 56.85 (C-8), 83.43 (C-3), 113.46 (C-6), 122.91 (C-F), 123.80 (C-6), 124.26 (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-α), 140.84 (C-2), 151.94 (C-1), 169.14 (C-10); MS (FAB⁺) m/z 503 (M+1−), 209; Anal. calc'd for C23H23F3N3O4S (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-1-tyrosine terbutyl ester (24). A solution of 23 (1.259 g, 2.495 mmol) in methanol (20 mL) was placed in a Parr flask and hydrogenated (pH2 = 40 psi) in the presence of platinum 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 CH2Cl2 and dried over MgSO4. Concentration under vacuum gave 1.074 g of crude 23 (Yield: 91%); mp 40–41 °C; IR (CHCl3) ν 3275, 2980, 1734, 1617, 1517, 1438, 1327, 1280, 1232, 1161, 1132, 1105 cm⁻¹; 1H NMR (CDCl3, 300 MHz) δ 1.24 (s, 9H, H-β), 2.90 (m, 2H, H-7), 3.80 (s, 3H, H-α), 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); 13C NMR (CDCl3, 75 MHz) δ 27.59 (C-β), 38.58 (C-7), 55.44 (C-α), 57.17 (C-8), 82.69 (C-6), 110.37 (C-6), 117.32 (C-3), 120.82 (C-5), 122.9 (CF3), 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/z 475 (M+1), 474, 419, 401, 373.
1 equiv) and pyridine (0.33 mL, 323 mg, 4.08 mmol, 2.89 equiv) in CH₂Cl₂ (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; Rf (SiO₂; CH₂Cl₂:EtOAc, 9:1) = 0.1; ¹H NMR (CDCl₃, 500 MHz) δ 1.25 (3H, 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 = 7.7 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); ¹³C NMR (CDCl₃, 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-αa), 57.21 (C-8), 66.29 (C-21), 82.66 (C-a), 109.53 (C-6), 123.89 (C-FC₃), 132.39 (C-17), 124.30 (C-5), 127.30 (C-14), 127.58 (C-21), 128.70 (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 C₂₃H₂₅F₆N₅O₈S: 574.1947. Found: 574.1950.

-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 CHCl₃. Freeze-drying of the aqueous phase gave 120 mg (yield: 90%) of 28 hydroscopic white powder; ¹H NMR (CDCl₃, 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 = 8.4 Hz, 2H, H-2), 7.72–7.37 (m, 5H, H-8), 7.68 (br s, J = 8.4 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 13C NMR (CDCl₃, 125 MHz) δ 112.90 (C-5), 123.40 (C-3), 124.62 (C-1), 141.47 (C-18), 145.95 (C-17), 148.40 (C-16), 150.18 (C-1, 174.14 (C-10), 174.34 (C-12); HRMS: calcd for C₂₃H₂₅F₆N₅O₈S: 573.1272. Found: 573.1274.

Benzy1 6-amino-caproate para-toluene sulfonate (18b). A mixture of 6-aminoacaproic acid (5 g, 38.11 mmol), benzy1 alcohol (250 mL) and para-toluene sulfonic acid (8.38 g, 42.33 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 (CDCl₃, 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); ¹³C NMR (CDCl₃, 125 MHz) δ 112.17, 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-carboxamide (17). To a solution of 3,5-dimethylpyrazole-1-carboxamide nitrate (2 g, 9.74 mmol) and di-isobutyl 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 CH₂Cl₂ and washed with water.
Drying over MgSO₄, 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 (CDCl₃, 500 MHz) δ 1.50 (s, 9H), 2.12 (s, 3H), 3.11 (s, 3H), 5.68 (s, 1H), 9.05 (br s, 1H); ¹³C NMR (CDCl₃, 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 1592 (M), 128.44, 135.95, 153.24, 156.02, 163.54, 173.21; MS (EI) m/e 338 (M), 282, 254, 238, 182, 165, 138, 96, 57, 43; Anal. calcd for C₁₆H₂₆N₄O₄ (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’-diterbutoxy carbonyl)-guanidino-caproate (19b). To a solution of 17 (2.5 g, 7.38 mmol) in CH₂Cl₂ (300 mL), were added triethylamine (1.2 mL, 873 mg, 8.63 mmol, 1.16 equiv) and water (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% NaHCO₃ and water (3 ×). Drying (MgSO₄), concentration and chromatography on preparative MPLC gave 3.40 g (yield: 73%) of 19b: Rₛ (SiO₂; hexane:isopropanol, 2:1) = 0.2; IR (KBr) ν 3322, 3111, 2937, 1761, 1694, 1656 cm⁻¹; ¹H NMR (CDCl₃, 125 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); ¹³C NMR (CDCl₃, 500 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 28.20, 28.62, 34.01, 40.58, 66.05, 79.11, 82.93, 128.10, 128.44, 135.95, 153.24, 156.02, 156.34, 173.21; MS (EI) m/e 463, 351, 290, 202, 188, 161, 158, 145, 144, 130, 117, 108, 65; Anal. calcd for C₂₄H₃₇N₃O₆ (463.57): C, 62.18; H, 8.04; N, 9.06. Found: C, 62.18; H, 8.21; N, 8.96.

6-(N,N’-Diterbutoxy carbonyl)-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 (pH₂ = 40 psi) in the presence of Palladium during 18 h at 20 °C (vigorous shaking). Filtration, concentration and drying under vacuum gave 2.12 g (yield: 94%) of 6-(N,N’-diterbutoxy carbonyl)-guanidino-caproyl acid: mp 83.1–84.1 °C; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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 737 (M), 317, 261, 244, 188, 161, 130, 57; Anal. calcd for C₁₇H₃₁N₃O₆ (373.57): C, 64.67; H, 8.36; N, 11.25. Found: C, 64.57; 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 CH₂Cl₂ (20 mL) was refluxed (50 °C) during 1.5 h, then concentrated under vacuum. The excess of SOCl₂ was removed by azeotropic distillation with toluene (3 ×) and CHCl₃ (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 pepidomimetics was controlled by HPLC before use, with the following conditions: column: Nucleosil C18, 5 μ, 25 cm; temperature: 25 °C; eluent: gradient from 20% CH₃CN + 0.015% TFA–80% H₂O + 0.015% TFA to 80% CH₃CN + 0.015% TFA–20% H₂O + 0.015%; flow rate: 1 mL/min; equipment: Beckman, System Gold, 126 P Solvent module, 168 Detector (Analys, 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), KH₂PO₄ (0.1 g) and Na₂HPO₄.2H₂O (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 × 10⁵ 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 incubated 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 IC₅₀ 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 IC₅₀ 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, using the minimization procedures available in Gaussian suite of programs. In
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/cm³ (ASTM D 1505-66), a melting point of 251 °C (ASTM D 3418-82), a Mₙ of 48,800 and a MW of 88,800. The membrane contained 1.45×10⁶ pores/cm² (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 cm² (apparent surface and internal surface of the pores).

The phosphate buffer (pH 8.2) was prepared from Na₂HPO₄·2H₂O (4.215 g) and NaH₂PO₄·H₂O (0.2065 g) dissolved in water (250 mL, HPLC grade). The MES buffer (pH 5.3) was obtained from 2-(N-morpholino)-ethanesulfonic acid (MES, 5.331 g) dissolved in water (250 mL, HPLC grade). 1-[4,5-³H] lysine monohydrochloride in aqueous solution was purchased from Amersham (Little Chalfont, UK); the labeling solution (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 KMnO₄ (6 g, in 120 mL of 1.2 N H₂SO₄), 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- CO₂H.

Activation of PET-CO₂H. The PET-CO₂H disks (1 sample per 20 mL of reactive solution) were immersed into a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide 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-CO₂H disks were directly used for the radiolabeling and the coupling to the peptidomimetic.

Radiolabeling of PET-CO₂H. The activated PET-CO₂H 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 aquaaluma 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/cm² of open surface; blank: 22.9±2.9 pmol/cm² of open surface; corrected value: 34.6±3.3 pmol/cm² of open surface. Since a PET interface domain of 50 Å in depth contains about 2860 pmol of monomer units/cm², the value of 35 pmol/cm² corresponds to 1.22% of surface derivatization.⁷

Coupling of peptidomimetic to PET-CO₂H. The activated PET-CO₂H 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 stored 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: C₁₅₋₇0.86%; O₁₅, 27.58%; N₁₅, 1.32%; F₁₅, 0.24%; blank: C₁₅₋₇0.69%; O₁₅, 28.96%; N₁₅, 0.35%; F₁₅, 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

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[(PET)_x + (PET–RGD peptidomimetic)_y], i.e. [(C_20H_18O_8)_x + (C_31H_36O_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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The biological evaluations have been performed in the laboratory of hematology (UCL, Prof. Lavenne). The XPS analyses have been realized in the laboratory of chemistry at interfaces (UCL, Prof. P. Rouxhet). The LSC measurements have been done in the laboratory of cellular biochemistry (UCL, Prof. Y.-J. Schneider).

References