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Novel RGD-like molecules based on the tyrosine template: design, synthesis, and biological evaluation on isolated integrins $\alpha_V\beta_3/\alpha_{IIb}\beta_3$ and in cellular adhesion tests

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Abstract—RGD (Arg-Gly-Asp) peptidomimetics have been designed for covalent anchorage on biomaterials. The tyrosine template was thus equipped with (i) a basic side chain of various flexibility, (ii) an acidic side chain, which incorporated the XPS fluorine tag, and (iii) a spacer-arm terminated by a primary amine for surface grafting. The most active compounds showed IC_{50} values in the nanomolar range versus isolated human integrins $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$. Preincubation of CaCo2 cells with soluble peptidomimetics (**2** and **19a**) prevented cellular adhesion on culture plates coated with vitronectin. On the other hand, peptidomimetics (**19a** and **19b**) immobilized on a poly(ethylene)terephthalate membrane (PET) promoted CaCo2 cells adhesion. A modeling study at the ab initio level in MINI-1′ basis allowed to compare the various synthetic ligands of integrins and to propose novel pharmacophore structures.

1. Introduction

Modern clinical medicine relies increasingly upon man-made materials, called biomaterials, for providing implants, prostheses, and artificial organs.[1] Over the last 10 years, tailored substrates for tissue engineering emerged as an important field of biomaterials.[2] In this context, various supports were purposely surface modified with a view to promote mammalian cells adhesion.[3] We are interested in the biocompatibilization of poly(ethylene terephthalate) (PET) track-etched membranes by the covalent grafting of integrin ligands.[4] The preparation of long-term stable biomaterials required the use of synthetic biologically active compounds, for surface modification, instead of the natural adhesive proteins and derived peptides.[5] Accordingly, we considered nonpeptide mimics of the RGD (Arg-Gly-Asp) active sequence of the extracellular matrix (ECM) proteins. For our purpose, the designed molecules have to be equipped with a spacer-arm allowing further surface anchorage on the PET membranes used as cell culture substrates. In this paper, we report on the design, synthesis, and evaluation of novel RGD peptidomimetics based on the (L)-tyrosine template derivatized with three functionalized chains, respectively, the mimic of the Arg basic residue, the mimic of the Asp carboxyl function, and the spacer-arm. The presence of this last structural feature raised unexpected activities against isolated integrins.

2. Results and discussion

2.1. Design

Integrins are cell adhesion receptors intensively studied over the last years concerning their structures and functions.[6] They are considered as pharmaceutical targets in a number of therapeutic areas, especially those involved in thrombosis, cancer, and osteoporosis treatments.[7]

Keywords: RGD peptidomimetic; Tyrosine template; Integrin $\alpha_V\beta_3$; Integrin $\alpha_{IIb}\beta_3$; Cell adhesion.
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The integrin $\alpha_V\beta_3$ (called vitronectin receptor), is expressed on endothelial, smooth muscle, bone, and epithelial cells, and mediates cellular adhesion to various ECM and serum proteins via the RGD motive.[8] Constrained cyclic peptides involving this RGD sequence (Fig. 1) showed higher affinity and selectivity (for instance, versus the platelet integrin $\alpha_{IIb}\beta_3$) than flexible linear peptides;[9] they were thus used as reference templates for the design of nonpeptide RGD mimics. Indeed, the development of peptidomimetics constitutes actually a major strategy for the discovery of new leads (potential drugs) in medicinal chemistry.[10] A lot of works are devoted to the search of potent, selective, and orally bioavailable antagonists of integrin $\alpha_V\beta_3$.[11] In the field of 'intelligent' materials, RGD peptidomimetics were practically not considered for improving cell adhesive properties of substrates,[4] while both linear and cyclic RGD peptides were abundantly used.[3c] This could be due to the relatively hard synthetic investment required, which discourages the traditional materials chemist! However, the demands for application of RGD-like molecules in tissue engineering are less severe than in the therapeutic field since selectivity and ADME properties (adsorption–distribution–metabolism–excretion) should be of little or none importance. But, on the other hand, the molecules need an anchorage arm, which does not disturb their capacity of binding to integrin.

Several rigid scaffolds have been used to construct $\alpha_V\beta_3$ antagonists mimicking the RGD ligands.[12] We have selected the (L)-tyrosine template, initially exploited by the Merck's group for the synthesis of $\alpha_{IIb}\beta_3$ antagonists,[13] because the aromatic nucleus offers three points of functionalization: the α-aminoacid chain, the phenolic hydroxyl function, and its *ortho*-position after a sequence of nitration–reduction–acylation reactions.[14] The structural features required for the construction of our $\alpha_V\beta_3$ ligands from tyrosine are as follows: (i) a basic moiety (Arg mimic) fixed in *ortho*-position; (ii) a carboxylic function (Asp mimic), which is in fact the tyrosine acid; (iii) a lipophilic substituent fixed on the α-amino function via preferably a sulfonamide linkage as suggested by recent publications[11c,15]—this substituent will display a fluorine tag useful for quantitative analysis of the biomaterials surface by X-ray photoelectron spectroscopy (XPS);[4,14] (iv) a spacer-arm fixed on the aromatic hydroxyl function. The choice of the basic moiety resulted from molecular modeling and comparison with the reference cyclic peptide *c*[RGDfV]. We have previously shown that the peptidomimetic **1**[14] fulfilled our requirements, but due to the flexibility and length of the side chain bearing the basic function (guanidine), this molecule could be fitted also onto the cyclic peptide DMP728 (Figs. 1 and 2). Experimentally, **1** was demonstrated to be an inhibitor of platelet aggregation (antagonist of $\alpha_{IIb}\beta_3$).[14] We have now designed the peptidomimetic **2**, as better $\alpha_V\beta_3$ ligand, in which the guanidine function (Arg mimic) is fixed on the shorter and somewhat rigid isonipecotic motive. Also, the bulkiness of the lipophilic fluorine tag was increased. The geometry of compound **2** (with R = Me, in place of the spacer-arm) was fully optimized at the approximate quantum chemistry level AM1; the molecule was studied as an isolated neutral entity, and not as a zwitterionic form, in order to avoid internal self-folded conformations. The conformer of low energy (Fig. 3) could be fitted onto *c*[RGDfV]: the respective carboxyl and guanidyl functions superposed well. The fitting was definitively less good considering DMP728 as reference. Indeed, in structure **1**, the distance between the two pharmacophoric groups was of 13 bonds, but of 11 bonds only in structure **2**. This is in agreement with the previous works of several companies: transformation of $\alpha_{IIb}\beta_3$ antagonists into $\alpha_V\beta_3$ antagonists was achieved using compounds with a shorter distance between the carboxyl and guanidinyl groups and inserting a hydrophobic residue next to the carboxyl function.[16]

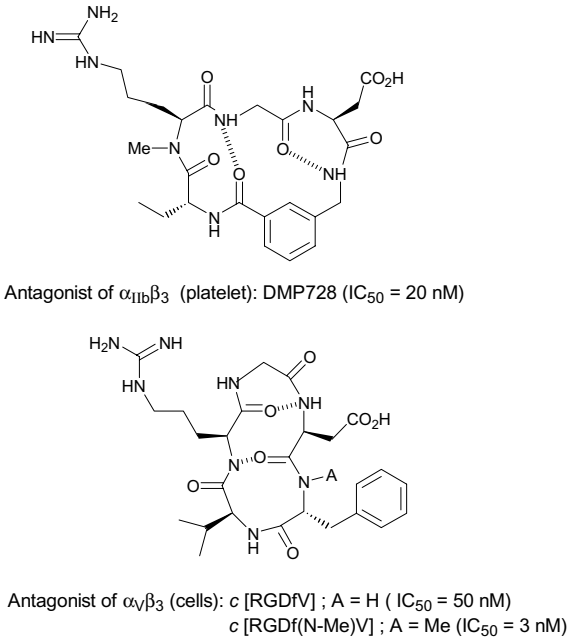


Figure 1. Cyclic peptides as integrin antagonists.

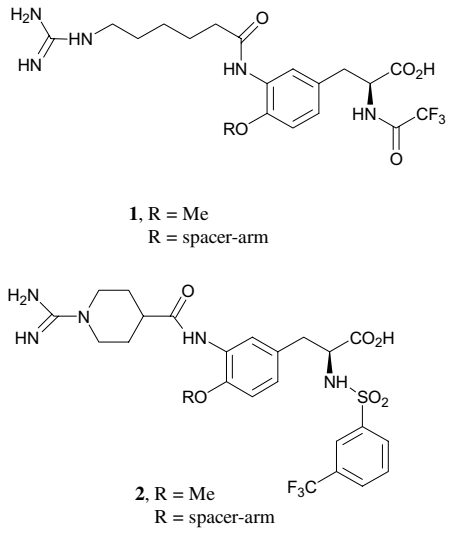


Figure 2. RGD peptidomimetics based on the tyrosine template.

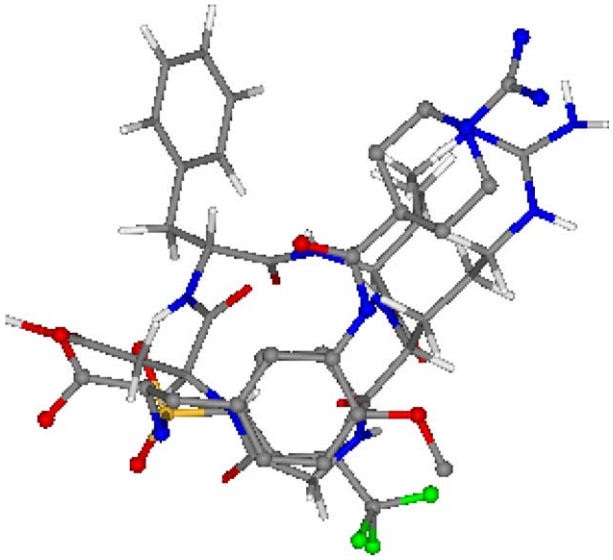
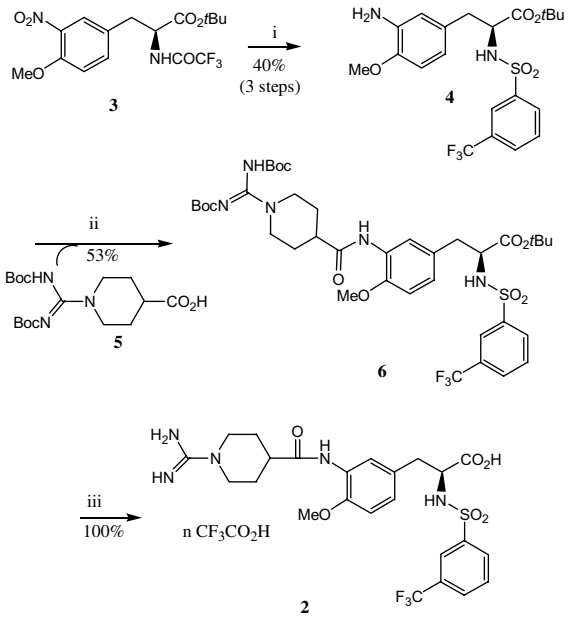


Figure 3. Fitting of compound **2** onto *c*[RGDfV]. Atoms coloring is as follows: C, gray; O, red; N, blue; F, green; S, yellow; H, white.



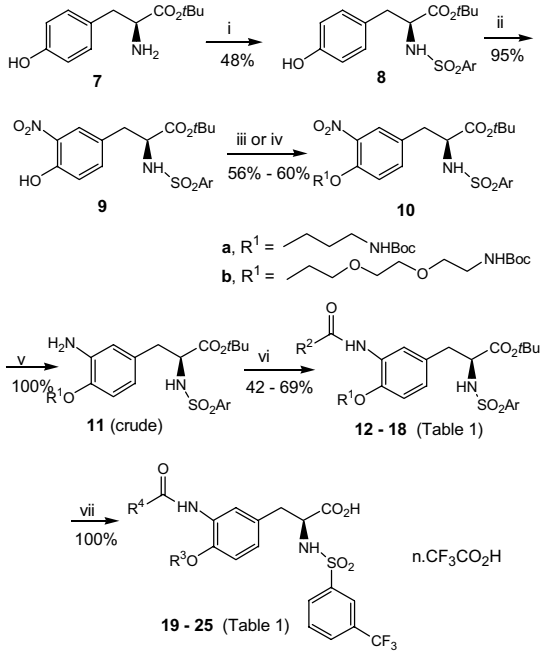
Scheme 1. Synthesis of the reference peptidomimetic (without spacer-arm). Reagents and conditions: (i) see Ref. 14; (ii) PYBOP, DIEA, DMF, 48 h, 20 °C; (iii) TFA–CH_2Cl_2 (1:1), 2 h, 20 °C.

2.2. Chemistry

The peptidomimetic devoid of spacer-arm was first synthetized (**2**, R = Me, Fig. 2); this molecule was required as reference for the biological evaluations. The starting material **3** was prepared in three steps from L-tyrosine *t*-butyl ester by known methods,[14] namely *N*-trifluoroacetylation (95%), aromatic nitration (90%), and *O*-methylation under modified Mitsunobu conditions (87%).[17] The key intermediate **4** was then obtained by trifluoroacetamide hydrolysis (90%), sulfonylation with *m*-trifluoromethyl-benzenesulfonyl chloride (56%), and reduction of the nitro function by catalytic hydrogenation (70%)[14] (Scheme 1).

After protection of isonipecotic acid as benzyl ester, the guanidine substituent was introduced by reaction with *N*,*N*′-bis-(*t*-butoxycarbonyl)-3,5-dimethylpyrazolyl-1-carboxamidine.[18] Hydrogenation over palladium on charcoal furnished the reagent **5** in an overall yield of 40% for three steps. This acid **5** was coupled to the tyrosine derivative **4** under activation by PYBOP.[19] The fully protected RGD mimic **6** was isolated in 53% yield after chromatographic purification; its structure was established by the usual spectroscopies (see Section 4). Lastly, the treatment with trifluoroacetic acid cleaved simultaneously all the protections to give compound **2** (100% yield) as TFA salt (Scheme 1).

We next considered the synthesis of the related peptidomimetics equipped with a spacer-arm. We selected a short linker (aminopropyl motive) and a longer hydrophilic linker (2-[2-(2-aminoethoxy)ethoxy]ethyl motive) on the basis of a previous study of PET membrane reactivity and covalent grafting of molecular probes with various linkers.[20] L-Tyrosine-*t*-butyl ester (**7**) was sulfonylated as usual (compound **8**) before aromatic nitration to furnish the intermediate **9** (Scheme 2). Etherification of the phenol moiety could be realized without competition with *N*-alkylation of the sulfonamide function, provided that the alkylation reagent is bulky enough;[14,17] it was not the case for the simple methylation, which requires the temporary NH_2 masking as trifluoroacetamide (see Scheme 1). Thus,



Scheme 2. Synthesis of peptidomimetics with spacer-arms. Reagents and conditions: (i) $ArSO_2Cl$, pyridine, CH_2Cl_2, 5 h, 20 °C; (ii) HNO_3 (1 equiv), HOAc, 16 °C; (iii) R^1Br (1 equiv), K_2CO_3, 18-crown-6, CH_3CN, reflux, 18 h; (iv) R^1OH (1 equiv), DIAD, dppe, THF, 24 h, 20 °C; (v) PtO_2 (5%), H_2 (30 psi), EtOAc, 20 °C, 18 h; (vi) PYBOP, DIEA, DMF, 48 h, 20 °C; (vii) TFA–CH_2Cl_2 (1:1), 2 h, 20 °C.

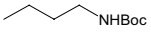
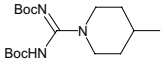
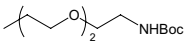
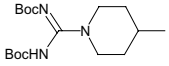
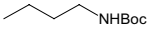
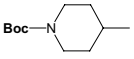
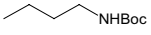
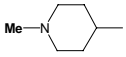
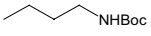
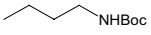
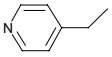
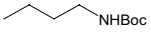
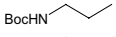
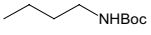
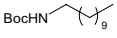
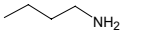
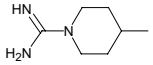
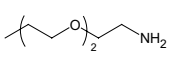
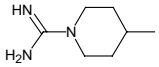
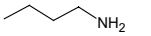
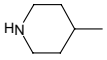
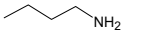
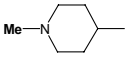
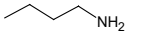
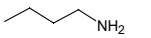
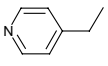
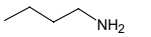
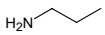
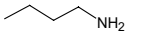
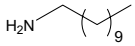
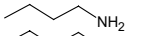
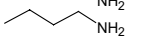
N-(*t*-butoxycarbonyl)-3-bromopropylamine[20] was coupled to **9** under Williamson conditions to give the ether **10a** in 56% yield after column chromatography. On the other hand, 2-[2-(2-*t*-butoxycarbonylaminoethoxy)ethoxy]ethanol[20] reacted well under modified Mitsunobu conditions;[17] pure compound **10b** could be isolated in 60% yield (Scheme 2). The next step was the nitro group reduction by hydrogenation: anilines **11a**,**b** were characterized in ^{1}H NMR by an important shielding of the aromatic proton in *ortho*-position with respect to NH_2 (from 7.56 ppm in **10** to 6.39 ppm in **11**). The crude anilines **11a**,**b** were reacted with acid **5** (see Scheme 1) in the presence of PYBOP as previously. Protected peptidomimetics **12a**,**b** (Table 1, entries 1 and 2) were recovered in, respectively, 48% and 42% yield, and well characterized by NMR spectroscopy (see Section 4); amine acylation provoked a significant deshielding of the *ortho* aromatic proton, which appeared at 8 ppm. As before, treatment with TFA quantitatively yielded the final compounds **19a** and **19b** (Table 1, entries 9 and 10).

We have also prepared related peptidomimetics with different basicity/lipophilicity of the Arg mimic residue. *N*-Boc isonipecotic acid, *N*-methyl isonipecotic acid,[21] isonicotinic acid and 4-pyridylacetic acid were coupled to the key intermediate **11a** (Scheme 2) to furnish, respectively, compounds **13a**, **14a**, **15a**, and **16a** in moderate yields (Table 1, entries 3–6), and the corresponding peptidomimetics **20a**, **21a**, **22a**, and **23a** after deprotection (Table 1, entries 11–14). The flexibility (conformational mobility) and length of the Arg mimic have been varied as well, with the synthesis of compounds **24a** and **25a** (from the precursors **17a** and **18a**) obtained by the coupling of 3-aminopropionic acid and 12-aminododecanoic acid, respectively (Table 1, entries 7–8 and 15–16).

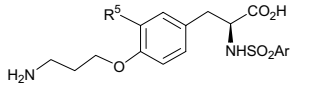
2.3. Biological evaluation

We have evaluated the ability of the lead compound **2** (no spacer-arm) to inhibit cellular adhesion in a compet-

Table 1. Structures of the side chains and yields of compounds

Entry	Compd	R^1/R^3	R^2/R^4	%
1	**12a**			48
2	**12b**			42
3	**13a**			53
4	**14a**			56
5	**15a**			52
6	**16a**			56
7	**17a**			45
8	**18a**			69
9	**19a**			Quantitative
10	**19b**			Quantitative
11	**20a**			Quantitative
12	**21a**			Quantitative
13	**22a**			Quantitative
14	**23a**			Quantitative
15	**24a**			Quantitative
16	**25a**			Quantitative
17	**26a**		—[a]	
18	**27a**		—[a]	

[a] Compounds with no acyl side chain; R_4CONH was thus replaced with NH_2 or NO_2.



itive test versus vitronectin, the natural ligand of integrin $\alpha_V\beta_3$. The assay was realized with CaCo2 cells (human epithelial cells from colon adenocarcinoma); they express the target integrin and are routinely cultivated in our laboratory.[4,22] Cells were preincubated in the presence of the synthetic integrin ligand **2** at different concentrations, and then inoculated on culture plates of polystyrene (PS) coated with vitronectin. After 15 min at 37 °C, the nonadherent cells were removed by suction and the adherent cells were assayed by a colorimetric enzymatic test based on their *N*-acetyl-β-D-glucosaminidase activity.[23] Incubation with 4-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (enzyme substrate) produced *p*-nitrophenolate detected at 405 nm. Results indicated that compound **2** is active at the level of 10^{-7}–10^{-6} M. At such concentration, the target integrin is filled up with the antagonist and, therefore, could not interact with the adhesive protein coating the culture plate. In the same experiment, RGDS peptide was active at the level of 10^{-6} M. At this stage, we concluded that our designed molecule **2** was well endowed with the required properties for substrate biocompatibilization. Since the spacer-arm could possibly disturb the activity, we similarly assayed compound **19a** (=**2** + aminopropyl linker). Surprisingly, this compound was more active than **2** (10^{-8}–10^{-7} M, Fig. 4). This result prompted us to examine more finely the effect of the spacer-arm.

Compounds listed in Table 2 were evaluated in a molecular binding test with purified human integrin $\alpha_V\beta_3$ in the presence of vitronectin ligand. The selectivity was controlled versus purified human integrin $\alpha_{IIb}\beta_3$, in the presence of fibrinogen ligand. Integrins were adsorbed on PS test plates. The synthetic antagonists (in various concentrations) were added in the presence of the natural ligands labeled with biotin. After incubation and washings, the amount of bound ligands was assayed with an antibody against biotin coupled to peroxidase and colorimetric reaction. Values are given in equivalent dose of compound producing 50% inhibition of natural ligand (IC_{50}). The reference compounds used in our tests were the most active cyclopeptide ($\alpha_V\beta_3$ antagonist)[9d] and the anti-platelet BIBU-52 ($\alpha_{IIb}\beta_3$ antagonist, Fradafiban).[24]

Results showed that the lead compound **2** (no spacer-arm) and compound **19b** equipped with the longer spacer-arm are active against $\alpha_V\beta_3$ at the micromolar level. As predicted from molecular modeling, **2** was less

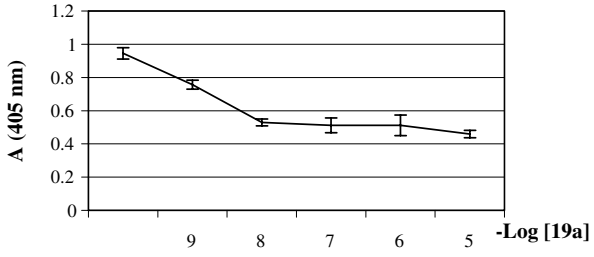


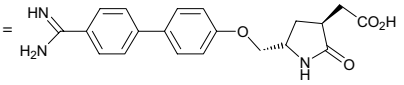
Figure 4. Inhibition of cellular adhesion by compound **19a** in solution.

Table 2. Activity of compounds against isolated integrins (IC_{50} values are given in nM)[a]

Entry	Compd	$\alpha_V\beta_3$	$\alpha_{IIb}\beta_3$
1	**2**	1312	6913
2	**19b**	765	5
3	**19a**	63	11
4	**20a**	55	16
5	**21a**	154	3.8
6	**22a**	114	27
7	**23a**	64	7.8
8	**24a**	73	28
9	**25a**	127	16
10	**26a**	41	20
11	**27a**	636	195
12	*c*[RGDf(*N*-Me)V]	0.4	—
13	BIBU-52	—	2.6

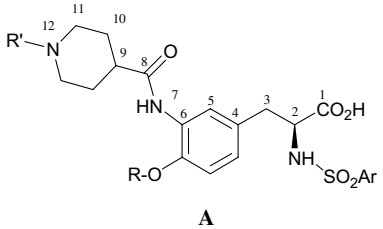
[a] Mean of at least two experiments with comparable results.

BIBU-52 =



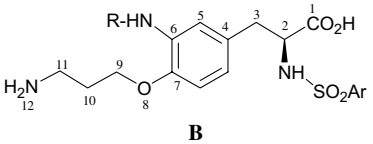
active against the platelet integrin, but not **19b**, which offers a second basic residue (Table 2, entries 1 and 2). All the other tested compounds **19a**–**26a** are characterized by the presence of the aminopropyl chain fixed on the phenol function. It appeared that this structural motive was in fact responsible for the activity, since all compounds were active at the nanomolar level against $\alpha_V\beta_3$ and, to a slightly higher extent, against $\alpha_{IIb}\beta_3$, but with a poor selectivity (Table 2, entries 3–10).

We speculated that our molecules could offer two possible Arg mimics, namely the isonipecotic basic function (**A**) and the aminopropyl residue (**B**, Fig. 5); in both cases, the acid and basic pharmacophores are separated with the same number of bonds. However, experimentally, structures **B** were found to be the most active ones, as well illustrated with the comparison between compounds **2** and **26a** (Table 2, entries 1 and 10), which



A

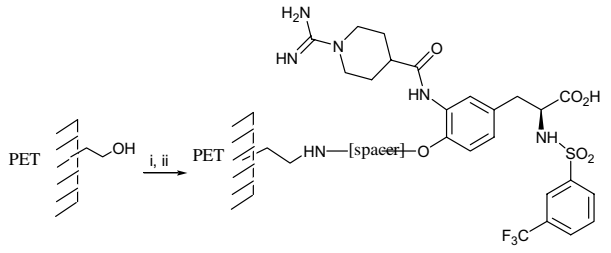
R = Me, **2**
R ≠ Me, **19b**



B

R = H, **26a**
R ≠ H, **19a - 25a**

Figure 5. Two possible Arg mimics (Ar = *m*CF_3–C_6H_4).



Scheme 3. Surface chemistry. Reagents and conditions: (i) TsCl, Pyridine, acetone, 60 °C; (ii) H_2N–[spacer]–'RGD', PBS–CH_3CN (1:1), 20 °C.

belong unambiguously to series **A** and **B**, respectively. The binding results are thus fully consistent with the previous cellular tests showing that **19a** was a better adhesion inhibitor, in solution, than **2**.

We next examined the adhesive effect induced by peptidomimetics **19a** (short spacer) and **19b** (long spacer) when they are immobilized on PET membrane. The compounds were covalently fixed on the material surface via the hydroxyl chain ends of the polymer according to known procedures.[20] After surface activation by tosylation, the polymer membrane was incubated with solutions of compounds **19a** and **19b**. Surface grafting should normally occur via the most nucleophilic basic function of the peptidomimetics, that is the primary amine of the propyl- and [2-(2-ethoxy)ethoxy]ethyl side chains (Scheme 3), thus displaying the structural features of the designed compound **2** for cell interaction (see Fig. 2). The amounts of grafted biologically active molecules were determined by XPS analysis, from the F/C atomic ratio, as previously described.[4] CaCo2 cells were cultivated on the native PET membrane and on the surface-modified membranes during 2 h at 37 °C in a synthetic medium devoid of added proteins. As reference for the most adhesive surface, we considered the support coated with a large amount of vitronectin (3 μg/cm^2). The adherent cells were fixed and stained, and the samples were examined with a phase contrast microscope. The percentages of surface occupancy were determined by image analysis (Table 3). Results clearly showed that immobilized peptidomimetics behaved as good to excellent promoters of cellular adhesion: 62% of the vitronectin performance could be reached with only 120 pmol/cm^2 of grafted **19b** compared to 3 μg/mL/cm^2 of coated protein. These results (and others) will be fully described and discussed in another publication.[25]

Table 3. CaCo2 cell adhesion on surface modified PET membranes

	Sample	Fixed 'RGD' (pmol/cm^2)	Surface occupancy (%)	Relative performance (%)
1	Native PET membrane		2 (±0.7)	6
2	PET coated with vitronectin		33.3 (±2.4)	100
3	PET grafted with **19a**	70	8.7 (±1.2)	26
4	PET grafted with **19b**	120	20.7 (±2.7)	62

2.4. Modeling

The unexpected results collected in the binding assays (see Table 2) with isolated integrins raised up questioning about our starting design. Not only the nature of the Arg mimic has to be considered, but also its anchorage position of the aromatic template. This has been examined again by molecular modeling taking into account the spacer-arm.

The geometry of compound **19a** was fully optimized at the approximate quantum chemistry level AM1 and also at the ab initio level in MINI-1′ basis. Remarkably, three energy minima have been located named I (folded), II (extended), and III (π–π stacked) (Fig. 6). Their relative energies are summarized in Table 4. As expected, the ab initio calculations gives a better description of the intramolecular interactions as conformations II and III are slightly more stable than I. The thermochemistry data are derived from the analytical frequency calculation at 1 atm and 298.15 K. In the compact III structure, the entropy term is lower than in the extended conformation thus inducing an inversion in the relative variation of free energy between the two conformers. In the folded conformer I, a hydrogen bond was formed between one proton of the aminopropyl

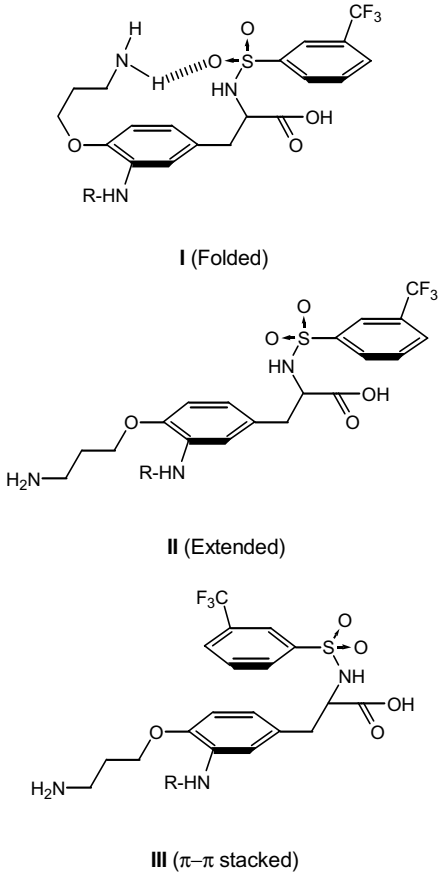


Figure 6. Optimized geometries of compound **19a**.

Table 4. Conformer relative energies of compound **19a** (see Fig. 6)

Conformer	AM1 ΔE	MINI-1′ ΔE	ΔG
I (folded)	0.00	0.00	0.00
II (extended)	3.31	−1.98	−3.96
III (π–π stacked)	3.32	−2.87	−3.56

Values are given in kcal mol^{-1}.

Table 5. Distances (Å) between the pharmacophoric groups, measured from the MINI-1′ conformers

Compd	Conformer	α *C*(acid)–*N*(guanidine)	α *C*(acid)–*N*(spacer)
19a	I (folded)	10.03, 12.17, 12.28	6.54
	II (extended)	9.45, 11.57, 11.70	8.63
	III (π–π stacked)	9.81, 11.78, 12.18	8.68
2	II (extended)	10.00, 11.74, 12.37	—
	III (π–π stacked)	10.27, 12.40, 12.53	—
26a	I (folded)	—	6.68
19b	I (folded)	10.17, 12.37, 12.38	11.04
	II (extended)	10.09, 12.27, 12.29	16.32
	c[RGDfV]	9.52, 10.05, 11.51	
	DMP728	10.50, 11.40, 11.60	

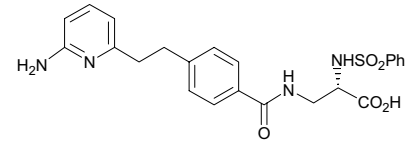
NH_2 and one oxygen of the sulfonamide SO_2 group. The distances between the carbon α to the acid and each of the nitrogens of the basic residues were measured on the MINI-1′ optimized structures, showing that a large range of values, between 6.5 and 12.3 Å (Table 5), could be covered depending on the basic residue and conformer considered. Similar treatment of the cyclic peptides *c*[RGDfV] and DMP728 gave distances of 9.5–11.6 Å. This could explain the fact that **19a** was almost equally recognized by integrins $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ (Table 2, entry 3). For comparison, compounds **2** (the less active against $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$; entry 1), **26a** (the most active against $\alpha_V\beta_3$, entry 10) and **19b** (the most active and selective against $\alpha_{IIb}\beta_3$; entry 2) were similarly analyzed (Table 5). In this last case, the long distance between the acid and the amine of the spacer-arm was not surprising since these functions are separated by 16 bonds compared to 11 bonds in all the other situations. This amine function most probably played the role of the basic pharmacophore for integrin $\alpha_{IIb}\beta_3$, but not for $\alpha_V\beta_3$. Beside this value, the other distances for **2**, **26a**, and **19b** were comprised between 8.2 and 12.5 Å, as for **19a**.

From the molecular modeling study, we concluded that activities and selectivities are hardly predictable in the case of relatively flexible structures displaying different conformers with almost equal probability, and that the Arg mimic could be fixed on the tyrosine aromatic template either in *ortho* of the hydroxyl group or on this function itself. Accordingly, permutation of the relative positions of the linker and the basic residue is now under investigation in our group.

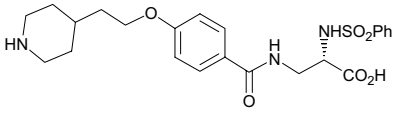
3. Conclusion

Usually, the design of RGD peptidomimetics as integrin antagonists considers a rigid template, which links a guanidine-type functionality and a carboxylic acid moiety. The selectivity of RGD-like compounds depends on the backbone conformations, the distances, and orientations of the charged side chains, and the presence of hydrophobic moieties flanking the Asp mimic. Vitronectin receptor (integrin $\alpha_V\beta_3$) shares the same β subunit as the fibrinogen receptor (integrin $\alpha_{IIb}\beta_3$). Due to the close relationship between these two receptors, they were often used as targets for selectivity studies. Recently, Fuston et al.[26] proposed a binding model for nonpeptide antagonists of integrin $\alpha_V\beta_3$, based on the crystal structure[27] of the extracellular segment of this integrin and docking experiments. They identified strong interactions between the basic nitrogen of antagonists (such as compound **27**) and the D150 residue of α_V subunit on the one hand, and between the ligands' carboxylic acid and the R214 residue of β_3 subunit, on the other hand. Moreover, a π–π stacking interaction was found between the aryl motive of sulfonamide group (if present) and the Y178 residue of α_V subunit. All the potent and selective compounds tested in this model were characterized by a distance of 12 bonds between their charged endings; when the molecules adopt an extended conformation, this corresponds to the most favorable interactions with $\alpha_V\beta_3$ receptor. As negative control, the authors showed that compound **28** (Fig. 7), displaying a distance of 15 bonds between the charged endings, cannot fit into their model. Experimentally, **28** is a potent and selective antagonist of platelet integrin $\alpha_{IIb}\beta_3$.

The compounds (**19a**–**24a**) we designed for materials surface modification, in view to promote cell adhesion, are characterized by one acid terminus and two basic termini, separated, respectively, by 11 bonds whatever the basic moiety being considered (see Fig. 5), except in compounds **19b**, **24a**, and **25a** showing distances of 11 bonds for one pair of endings, but of 16, 10, and 18 bonds, respectively, for the other one (see Table 1 for structures). The recorded activities against $\alpha_V\beta_3$ (IC_{50} values between 50 and 150 nM for **19a**–**25a**, see Table 2) could reasonably fit with the previous model, considering **26a** as the common pharmacophore. However, the lack of selectivity and the systematically higher activity against $\alpha_{IIb}\beta_3$ was unexpected, and



27 : IC_{50} = 0.08 nM ($\alpha_V\beta_3$)
IC_{50} = 1600 nM ($\alpha_{IIb}\beta_3$)



28 : IC_{50} = 10 nM ($\alpha_{IIb}\beta_3$)
IC_{50} > 1000 nM ($\alpha_V\beta_3$)

Figure 7. Molecules considered in the $\alpha_V\beta_3$ binding model of Feuston et al.[26]

unpredictable on the basis of the actual knowledge on integrin structures.[6c] Maybe, not only critical distances between charged endings have to be taken in account, but also the global charge of the potential ligands. The aniline (or anilide) function of compounds **26a** and **19a**–**25a** should also play a role in both receptor binding since the nitro derivative **27a** appeared about 10-fold less active (Table 2, entry 11). A nitro-aromatic template was rarely considered in the previous literature.[28] Our results contradicted the generally accepted dogma of '15-bond distance' between the basic and acidic residues for optimum recognition by integrin $\alpha_{IIb}\beta_3$: in our hands, a '11-bond distance' gave excellent results too. Amongst the novel potent $\alpha_{IIb}\beta_3$ antagonists disclosed in the present study (**21a**: $IC_{50} = 3.8$ nM; **19b**: $IC_{50} = 5$ nM), compound **19b** could be of interest for further development due to its selectivity versus $\alpha_V\beta_3$ (Table 2, entry 2); this molecule, structurally related to Tirofiban **29**,[29] displays the basic residue at the end of a triethylene glycol chain; to our knowledge, such a flexible, hydrophilic, structural motive has not been exploited in the previous works about RGD mimics.

Our compounds active against $\alpha_V\beta_3$ (**19a** to **26a**) are structurally related to the tyrosine-based antagonists discovered by the Merck company,[9,16] such as **30** (Fig. 8). Thus possible improvement of our design should involve (i) the one bond lengthening of the basic arm, (ii) the use of aminoheterocycles as basic residues,[11a,c,30] and (iii) the fixation of the polymer linker in *ortho*-position considering the phenol ring. Nevertheless, the actual RGD peptidomimetics **19a** and **19b**, with the polymer linker fixed on the phenol hydroxyl function, were found to be good to excellent promoters of CaCo2 cells adhesion after their grafting on PET culture substrates. Most probably, the surface displayed RGD mimic was structure **A**, and not the more active pharmacophore **B** according to the molecular binding assays (see Fig. 5). However, differences between molecular and cellular tests could be observed, since cellular adhesion is a highly complex phenomenon involving, notably, lateral clustering of integrins for signal transduction.[6,7f]

We have thus achieved our goal of PET biocompatibilization for in vitro cell culture by using small synthetic RGD-like molecules instead of ECM proteins (like vitronectin). Even if our system could be possibly further improved, application to materials used for bone regeneration could now be envisaged.[7f,31]

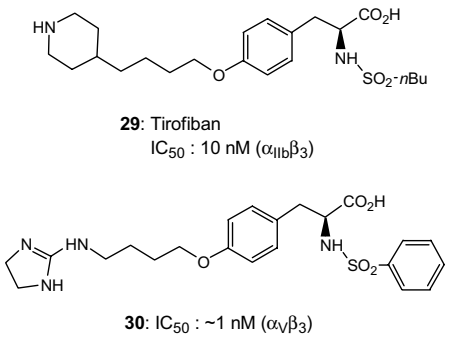


Figure 8. Integrin antagonists based on the tyrosine template.

4. Experimental

4.1. General

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

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 melting points were determined with an Electrothermal microscope and are uncorrected. The IR spectra were taken 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 200 (at 200 MHz for proton and 50 MHz for carbon), or Bruker AM-500 spectrometers (at 500 MHz for proton and 125 MHz for carbon); the chemical shifts are recorded 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 coupling constant values are given in Hertz. The attributions were established by selective decoupling experiments. The carbon of Aryl-CF_3 was not visible in the ^{13}C NMR spectra. The mass spectra were obtained on a Finnigan-MAT TSQ-70 instrument at 70 eV or with a Xenon ION TECH 8KV apparatus. The modes were EI (electronic impact), FAB (fast atom bombardment), APCI (atmospheric pressure chemical ionization), and ESI (electron-spray ionization). The microanalyses were performed at the Christopher Ingold Laboratories of the University College, London. The HRMS were performed at the University of Mons-Hainaut (Belgium) on a VG-AutoSpec-Q equipment (Fisons Instruments, Manchester).

4.2. Chemistry

4.2.1. *t*-Butyl *O*-methyl-*meta*-amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (4). This compound, prepared according to Ref. 14, was obtained as a colorless oil. R_f 0.3 (DCM/EA, 9:1); IR (film) ν 3275, 2980, 1734, 1617, 1517, 1438, 1327, 1280, 1232, 1161, 1132, 1105 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.06 (s, 1H), 7.95 (d, $J = 7.7$, 1H), 7.80 (d, $J = 8.1$, 1H), 7.60 (dd, $J = 8.1$; 7.7, 1H), 6.65 (d, $J = 8.1$, 1H), 6.52 (d, $J = 1.9$, 1H), 6.47 (dd, $J = 8.1$; 1.9, 1H), 5.30 (d, $J = 9.2$, 1H), 4.13 (m, 1H), 3.85 (br s, 2H), 3.80 (s,

3H), 2.91 (m, 2H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.86, 146.91, 141.53, 135.28, 131.26, 130.59, 129.78, 129.21, 127.65, 124.31, 120.03, 116.64, 110.47, 82.87, 57.38, 55.56, 38.89, 27.79; MS (FAB) *m/e* 475 ($C_{21}H_{25}O_5N_2F_3S$+1), 474, 419, 401.

4.2.2. *t*-Butyl *O*-methyl-*meta*-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl)guanidino-isonipecotyl]amino-*N*-[3-(trifluoromethyl)-phenylsulfonyl]-(L)-tyrosinate (6). To a suspension of acid **5** (430 mg, 1.16 mmol) and PYBOP (603 mg, 1 equiv) in DMF (18 mL), stirred under argon atmosphere, was added diisopropylethylamine (0.396 mL, 2 equiv). After complete dissolution, aniline **4** (550 mg, 1 equiv), in solution in DMF (2 mL), was added in one portion. The mixture was stirred for 2 days at room temperature. After dilution with ethyl acetate, the solution was washed with water and then with an aqueous solution of NH_4Cl. The organic layer was dried over $MgSO_4$, concentrated, and purified by column chromatography on silica gel to furnish **6** as a white solid. Yield: 528 mg (55%); mp 104.0–104.2 °C; R_f 0.2 (DCM/EA, 9:1); IR (KBr) *v* 3260, 2978, 1748, 1683, 1635, 1539, 1506, 1327, 1161, 1133, 954, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.17 (s, NH–C(O)guan), 8.14 (s, 1H), 7.99 (s, 1H), 7.95 (d, J = 8.0, 1H), 7.75 (s, NH–C(O) amide), 7.74 (d, J = 8.0, 1H), 7.56 (dd, J = 8.0; 8.0, 1H), 6.84 (d, J = 8.4, 1H), 6.73 (d, J = 8.4, 1H), 5.25 (d, J = 9.5, $NHSO_2$), 4.21 (m, 2H), 4.05 (ddd, J = 9.5; 7.0; 5.2, 1H), 3.85 (s, 3H), 3.10 (m, 2H), 3.0 (dd, J = 5.2; 13.6, 1H), 2.86 (dd, J = 7.0; 13.6, 1H), 2.52 (m, 1H), 1.97–1.91 (m, 4H), 1.49 (s, 18H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.58, 169.63, 154.91, 146.75, 141.11, 131.35, 130.51, 129.55, 128.96, 127.59, 127.17, 124.74, 124.10, 120.55, 109.6, 83.01, 57.11, 55.56, 46.40, 43.93, 38.67, 28.02, 27.57; MS (FAB) *m/e* (%) 828 (24, M+1), 628 (24), 572 (80), 318 (28), 149 (40), 136 (100), 77 (80), 57 (56); Anal. Calcd for $C_{38}H_{52}O_{10}N_5F_3S$: C, 55.13; H, 6.33; N, 8.46. Found: C, 54.92; H, 6.72; N, 8.59.

4.2.3. *O*-Methyl-*meta*-(*N*-guanidino-isonipecotyl)amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (2). Compound **6** (50 g, 0.06 mmol) was dissolved in an ice-cold 1:1 mixture (1 mL) of dichloromethane (DCM) and trifluoroacetic acid (TFA). The mixture was stirred for 2 h at 20 °C, then concentrated under vacuum. The residue was triturated in dry ether to furnish **2** (bis-trifluoroacetic salt) as a yellow gum. Yield: 48 mg (100%); ^{1}H NMR (DMSO, 500 MHz) δ 8.98 (s, 1H, NHCO), 8.58 (d, J = 9.1, 1H, $NHSO_2$), 7.89 (s, 1H), 7.85 (d, J = 8.2, 1H), 7.78 (d, J = 8.2, 1H), 7.75 (d, J = 2.1, 1H), 7.62 (dd, J = 8.2; 8.2, 1H), 7.43 (m, 3H, NH_2, NH), 6.79 (dd, J = 8.5; 2.1, 1H), 6.76 (d, J = 8.5, 1H), 3.88 (m, 2H), 3.87 (ddd, J = 10.0; 4.7; 9.1, 1H), 3.77 (s, 3H), 3.04 (m, 2H), 2.87 (m, 1H), 2.87 (dd, J = 14.0; 4.7, 1H), 2.60 (dd, J = 14.0; 10.0, 1H), 1.84 (m, 2H), 1.57 (m, 2H); ^{13}C NMR (DMSO, 125 MHz) δ 172.41, 172.09, 155.76, 148.10, 142.24, 130.25, 130.12, 129.31, 128.64, 128.25, 126.87, 124.90, 122.25, 121.73, 110.50, 57.81, 55.55, 44.80, 41.12, 37.31, 27.84; MS (ESI) *m/e* (%) 589 (44, M+ H_2O), 572 (100, M+1); HRMS: calcd for $C_{24}H_{29}N_5O_6F_3S$: 572.1791. Found: 572.1792.

4.2.4. *t*-Butyl *N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (8). To a solution of (L)-tyrosine *t*-butylester (2 g, 8.42 mmol) in CH_2Cl_2 (100 mL) were added, successively, pyridine (0.7 mL, 8.42 mmol, 1 equiv), and *meta*-trifluoromethyl-benzenesulfonyl chloride (1.35 mL, 8.42 mmol, 1 equiv). The mixture was stirred at room temperature for 5 h, then washed with brine and water. After drying ($MgSO_4$), concentration, and chromatography (silica gel), the sulfonamide **8** was recovered as a pale yellow solid. Yield: 1.79 g (48%); mp 95–95.2 °C; R_f 0.6 (DCM/EA, 9:1); IR (KBr) 3477, 3266, 3083, 2980, 2938, 2857, 2792, 1732, 1614, 1517, 1437, 1327, 1159 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.07 (s, 1H), 7.96 (d, J = 8.0, 1H), 7.80 (d, J = 8.0, 1H), 7.61 (dd, J = 8.0; 8.0, 1H), 7.00 (d, J = 8.5, 2H), 6.70 (d, J = 8.5, 2H), 5.28 (d, J = 9.4, 1H, $NHSO_2$), 5.15 (br s, 1H, OH), 4.12 (m, 1H), 2.97 (m, 2H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.9, 155.02, 141.44, 131.35, 130.83, 130.55, 129.88, 129.35, 127.04, 124.34, 115.50, 83.19, 57.40, 38.77, 27.78; MS (APCI) *m/e* (%) 444 (100, M−1), 388 (36), 342 (6), 209 (59); Anal. Calcd for $C_{20}H_{22}O_5NSF_3$: C, 53.93; H, 4.98; N, 3.14; S, 7.20. Found: C, 53.26; H, 4.92; N, 3.12; S, 7.12.

4.2.5. *t*-Butyl *meta*-nitro-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (9). To a solution of **8** (1.5 g, 3.37 mmol) in acetic acid (100 mL), nitric acid 90% (200 μL, 3.40 mmol) diluted in acetic acid (20 mL) was added dropwise. The mixture was maintained at 16–20 °C during the addition. After reactive consumption (TLC control), the crude mixture was poured onto ice. The aqueous phase was extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$), concentrated under vacuum, and purified by column chromatography on silica gel to give **9** as a yellow solid. Yield: 1.57 g (95%); R_f 0.8 (DCM/EA, 95:5); mp 80.4–80.6 °C; IR (KBr) *v* 3274, 2980, 2926, 2875, 1733, 1632, 1540, 1431, 1327, 1162, 1071 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 10.43 (s, 1H, OH), 8.03 (s, 1H), 7.97 (d, J = 7.7, 1H), 7.83 (d, J = 2.2, 1H), 7.79 (d, J = 7.8, 1H), 7.60 (dd, J = 7.7; 7.7, 1H), 7.43 (dd, J = 8.5; 2.2, 1H), 7.03 (d, J = 8.5, 1H), 5.43 (d, J = 9.2, 1H, $NHSO_2$), 4.09 (m, 1H), 3.09 (dd, J = 5.5; 14.1, 1H), 2.95 (dd, J = 6.8; 14.1, 1H), 1,26 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.44, 154.18, 141.32, 139.11, 133.27, 131.35, 130.54, 129.99, 129.35, 128.01, 125.54, 124.11, 120.17, 83.74, 57.18, 38.12, 27.69; MS (FAB) *m/e* (%) 489 (100, M−1), 306 (88), 199 (32), 153 (60). Anal. Calcd for $C_{20}H_{21}O_7N_2SF_3$: C, 48.98; H, 4.32; N, 5.71; S, 6.54. Found: C, 49.09; H, 4.17; N, 5.49; S, 6.36.

4.2.6. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-nitro-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (10a). To a solution of **9** (2.32 g, 4.8 mmol) in CH_3CN (180 mL), under argon atmosphere, were added N-Boc-3-bromopropylamine (1.13 g, 1 equiv), potassium carbonate (0.66 g), and [18-c-6] crown-ether (1.25 g). The mixture was refluxed, under stirring, for 22 h. CH_3CN was removed under vacuum; the residue was dissolved in CH_2Cl_2, and washed with 0.1 N HCl and water. Drying over $MgSO_4$, concentration, and chromatography on silica gel gave **10** as a yellow oil. Yield: 1.74 g (56%); R_f 0.55 (DCM/EA, 8:1); IR (film) *v* 3422,

3275, 2981, 1714, 1625, 1535, 1458, 1438, 1369, 1353, 1328, 1263, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.04 (s, 1H), 7.98 (d, $J = 8.0$, 1H), 7.81 (d, $J = 8.0$, 1H), 7.63 (dd, $J = 8.0$, 1H), 7.62 (d, $J = 2.1$, 1H), 7.40 (dd, $J = 2.1$; 8.6, 1H), 6.99 (d, $J = 8.6$, 1H), 5.30 (d, $J = 9.2$, 1H, $NHSO_2$), 5.07 (s, 1H, NHCO), 4.14 (t, $J = 7.3$, 2H), 4.07 (m, 1H), 3.37 (m, 2H), 3.09 (dd, $J = 5.8$; 14.0, 1H), 2.99 (dd, $J = 6.1$; 14.0, 1H), 2.04 (m, 2H), 1.44 (s, 9H), 1.21 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.01, 156.08, 151.45, 140.79, 139.09, 135.61, 131.68, 130.37, 129.85, 129.37, 127.54, 126.58, 124.09, 114.39, 83.82, 79.06, 67.85, 56.73, 38.03, 38.01, 29.03, 28.29, 27.59; MS (FAB) *m/e* (%) 646 (20, M−1), 546 (24), 490 (12), 386 (8), 209 (10); Anal. Calcd for $C_{28}H_{36}F_3N_3O_9S$: C, 51.93; H, 5.60; N, 6.49; S, 4.95. Found: C, 51.80; H, 5.80; N, 6.25; S, 4.78.

4.2.7. *t*-Butyl *O*-[2-(2-(2-(*N*-*t*-butoxycarbonyl)-2-aminoethoxy)ethoxy)ethyl]-*meta*-nitro-*N*-[3-(trifluoromethyl)-phenylsulfonyl]-(L)-tyrosinate (10b). To a solution of **9** (1 g, 2.4 mmol) in THF (6 mL), under argon atmosphere, were added 2-(2-(2-*t*-butoxycarbonyl)aminoethoxy) ethoxyethanol[20] (536 mg, 1.1 equiv) and bis(diphenylphosphino) ethane (1.22 g, 1.5 equiv). At 0 °C, diisopropyl azodicarboxylate (600 μL, 618 mg, 1.3 equiv) was added dropwise. The mixture was stirred for 20 h at room temperature. THF was removed under vacuum and the crude mixture was suspended in diethylether and left at −20 °C overnight. After filtration, the organic phase was concentrated, and purified by column chromatography on silica gel to give **10b** as a yellow oil. Yield: 1.04 g (60%); R_f 0.5 (DCM/EA, 4:1); IR (film) *v* 3299, 2980, 2933, 2884, 1711, 1620, 1533, 1327, 1164, 1103 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.03 (s, 1H), 7.96 (d, $J = 8.1$, 1H), 7.79 (d, $J = 8.1$, 1H), 7.50 (dd, $J = 8.1$; 8.1, 1H), 7.59 (d, $J = 2.1$, 1H), 7.37 (dd, $J = 8.8$; 2.1, 1H), 6.99 (d, $J = 8.8$, 1H), 5.75 (d, $J = 8$, 1H, $NHSO_2$), 5.07 (br s, NHCO), 4.23 (m, 2H), 4.06 (m, 1H), 3.89 (m, 2H), 3.73 (m, 2H), 3.63 (m, 2H), 3.53 (m, 2H), 3.28 (m, 2H), 3.06 (dd, $J = 5.9$; 13.8, 1H), 2.95 (dd, $J = 5.9$; 13.8, 1H), 1.42 (s, 9H), 1.22 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.28, 156.12, 151.62, 141.13, 135.57, 132.11, 131.45, 130.54, 130.03, 129.50, 128.07, 126.54, 124.23, 115.18, 83.82, 77.42, 71.13, 70.40, 70.29, 69.79, 69.36, 56.97, 40.43, 38.10, 28.45, 27.71; MS (FAB) *m/e* (%) 720 (28, M−1), 620 (48), 564 (20), 224 (20), 209 (100); Anal. Calcd for $C_{31}H_{42}F_3N_3O_{11}S{\cdot}H_2O$: C, 50.32; H, 5.95; N, 5.68; S, 4.33. Found: C, 50.10; H, 6.01; N, 5.95; S, 4.17.

4.3. General procedure for hydrogenation

In a Parr vessel, the nitro precursor dissolved in ethyl acetate (1 mmol/25 mL) was shaked under hydrogen atmosphere ($p = 40$ psi) for 18 h at room temperature in the presence of PtO_2 (20 mg/mmol nitro compd). After filtration and concentration, the residue (100% crude yield) was used in the next step without purification.

4.3.1. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (11a). White solid; mp 61–61.2 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.02 (s, 1H), 7.89 (d, $J = 8.0$, 1H), 7.75 (d, $J = 8.0$, 1H), 7.55 (dd, $J = 8.0$; 8.0, 1H), 6.58 (d, $J = 7.9$, 1H), 6.44 (s, 1H), 6.39 (d, $J = 7.9$, 1H), 5.75 (br d, 1H, $NHSO_2$), 4.91 (br s, NHCO), 4.05 (m, 1H), 3.96 (t, $J = 6.2$, 2H), 3.60 (br s, 2H, NH_2), 3.31 (m, 2H), 2.88 (dd, $J = 6.0$; 14.0, 1H), 2.80 (dd, $J = 6.0$; 14.0, 1H), 1.96 (t, $J = 6.2$, 2H), 1.42 (s, 9H), 1.22 (s, 9H), ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.97, 157.23, 146.82, 142.68, 137.59, 131.69, 130.89, 130.34, 129.05, 125.44, 120.48, 117.33, 112.74, 83.96, 80.20, 67.05, 58.46, 40.07, 38.10, 31.01, 29.62, 28.92; MS (APCI) *m/e* (%) 619 (25), 618 (40 M+1, $C_{28}H_{38}F_3N_3O_7S$), 519 (25), 518 (100), 462 (45), 338(18).

4.3.2. *t*-Butyl *O*-[2-(2-(2-(*N*-*t*-butoxycarbonyl)-2-aminoethoxy)ethoxy)ethyl]-*meta*-amino-*N*-[3-(trifluoromethyl)-phenylsulfonyl]-(L)-tyrosinate (11b). White gum; R_f 0.4 (DCM/EA, 7:3); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.02 (s, 1H), 7.89 (d, $J = 8.1$, 1H), 7.74 (d, $J = 8.1$, 1H), 7.54 (dd, $J = 8.1$; 8.1, 1H), 6.60 (d, $J = 7.8$, 1H), 6.44 (d, $J = 2.2$, 1H), 6.38 (dd, $J = 7.8$; 2.2, 1H), 5.57 (d, $J = 7.9$, 1H, $NHSO_2$), 5.10 (br s, NHCO), 4.07 (m, 2H), 4.04 (ddd, $J = 8.1$; 6.2; 5.8, 1H), 3.80 (m, 2H), 3.66 (m, 2H), 3.62 (m, 2H), 3.58 (br s, 2H, NH_2), 3.51 (m, 2H), 3.28 (m, 2H), 2.86 (dd, $J = 6.2$; 14.1, 1H), 2.82 (dd, $J = 5.9$; 14.1, 1H), 1.41 (s, 9H), 1.21 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.68, 155.90, 145.34, 141.30, 136.81, 131.26, 130.34, 129.61, 128.98, 128.22, 124.00, 119.03, 116.14, 112.56, 82.54, 79.04, 70.49, 70.12, 69.60, 68.18, 57.15, 40.25, 38.71, 28.26, 27.53; MS (APCI) *m/e* (%) 693 (32), 692 (100, M+1, $C_{31}H_{44}F_3N_3O_9S$), 636 (30), 592 (85), 536 (16).

4.4. General procedure for 11 coupling with acids

Acid of interest (1 equiv) and PYBOP (1 equiv) were suspended in DMF (10 mL/0.5 mmol) under argon atmosphere. Diisopropylethylamine (2 equiv) was added and the mixture was stirred until dissolution. Then aniline **11** (1 equiv) in DMF (5 mL/0.5 mmol) was added in one portion. The mixture was stirred for 2 days at 20 °C. After addition of ethyl acetate and washing with aqueous NH_4Cl and brine, the organic layer was dried over $MgSO_4$, and concentrated under vacuum. The crude anilide was purified by column chromatography on silica gel.

4.4.1. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-guanidino)isonipecotyl amino]-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (12a). Yield from 300 mg (0.486 mmol) of **11a**: 226 mg (48%); hygroscopic beige solid; mp 74.2–74.4 °C; R_f 0.3 (DCM/MeOH, 30:1); IR (KBr) *v* 3317, 2978, 1748, 1680, 1599, 1536, 1485, 1393, 1326, 1164, 1105, 954 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 10.3 ($NH_{gua}Boc$), 8.15 (ArNHCO), 8.11 (d, $J = 1.8$, 1H), 8.02 (s, 1H), 7.97 (d, $J = 8.4$, 1H), 7.74 (d, $J = 8.4$), 7.57 (dd, $J = 8.4$; 8.4, 1H), 6.82 (d, $J = 8.4$, 1H), 6.74 (d, $J = 8.4$, 1H), 5.18 (d, $J = 9.6$; 1H, $NHSO_2$), 4.72 ($NHCO_2tBu$), 4.17 (m, 2H), 4.08 (m, 2H), 4.07 (ddd, $J = 5.1$; 5.7; 9.5, 1H), 3.35 (m, 2H), 3.26 (m, 2H), 3.00 (dd, $J = 5.1$; 13.6, 1H), 2.9 (dd, $J = 7.0$; 13.6, 1H), 2.72 (m, 1H), 2.05 (m, 4H), 1.98 (m, 2H), 1.51 (s,

18H), 1.43 (s, 9H), 1.29 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.96, 169.61, 156.05, 146.1, 141.1, 131.35, 130.52, 129.58, 129.01, 127.65, 127.60, 124.73, 121.36, 111.00, 82.98, 79.34, 64.65, 57.08, 38.68, 36.70, 28.29, 27.97, 27.35; MS (FAB) *m/e* (%) 971 (88, M+1), 771 (88), 715 (44), 615 (20), 462 (32), 275 (96); Anal. Calcd for $C_{45}H_{65}F_3N_6O_{12}S\cdot 0.7H_2O$: C, 54.90; H, 6.75; N, 8.54; S, 3.25. Found: C, 54.41; H, 6.71; N, 8.02; S, 3.43.

4.4.2. *t*-Butyl *O*-[2-(2-(2-*N*-*t*-butoxycarbonyl)-2-aminoethoxy)ethoxyethyl]-*meta*-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylguanidino)isonipecotyl]amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (12b). Yield from 384 mg (0.555 mmol) of **11b**: 243 mg (42%); hygroscopic white solid; mp 65.2–65.4 °C; R_f 0.3 (DCM/EA, 1:1); IR (KBr) *v* 2987, 2927, 2851, 2373, 2325, 1651, 1317, 1260, 1227, 1146, 1118, 959 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, J = 1.8, 1H), 8.00 (s, 1H), 7.94 (d, 1H), 7.74 (d, 1H), 7.56 (dd, 1H), 6.82 (dd, J = 8.4; 1.8, 1H), 6.76 (d, J = 8.4, 1H), 5.02 (br s, 1H, NHBoc), 4.24 (m, 2H), 4.15 (m, 2H), 4.04 (dd, J = 5.2; 7.1, 1H), 3.83 (m, 2H), 3.68 (m, 2H), 3.63 (m, 2H), 3.53 (m, 2H), 3.29 (m, 2H), 3.07 (m, 2H), 3.00 (dd, J = 14.0; 5.2, 1H), 2.86 (dd, J = 14.0; 7.1, 1H), 2.54 (m, 1H), 1.96 (m, 2H), 1.89 (m, 2H), 1.48 (s, 18H), 1.40 (s, 9H), 1.24 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.86, 169.62, 155.85, 154.90, 146.12, 141.16, 131.29, 130.44, 129.52, 128.91, 128.46, 128.00, 124.76, 124.04, 121.10, 112.23, 82.90, 81.84, 79.40, 70.42, 70.14, 70.02, 69.35, 68.49, 57.06, 46.25, 43.13, 40.14, 38.70, 28.28, 28.25, 28.08, 27.95, 27.50; MS (APCI) *m/e* (%) 1045 (12, M+1), 945 (4), 871 (100), 845 (6), 728 (50); Anal. Calcd for $C_{48}H_{71}F_3N_6O_{14}S\cdot 3H_2O$: C, 52.41; H, 7.01; N, 7.64. Found: C, 52.22; H, 7.03; N, 7.53.

4.4.3. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-[*N*-(*t*-butoxycarbonyl)-isonipecotyl]amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (13a). Yield from 157 mg (0.25 mmol) of **11a**: 110 mg (53%); white solid; mp 60.4–60.6 °C; R_f 0.4 (DCM/MeOH, 30:1); IR (KBr) *v* 3357, 2979, 2924, 2868, 1742, 1682, 1591, 1537, 1420, 1362, 1325, 1248, 1154, 1127 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.17 (br s, 1H, $N_{ar}HCO$), 8.13 (d, J = 1.8, 1H), 8.01 (s, 1H), 7.96 (d, J = 7.9, 1H), 7.74 (d, J = 7.9, 1H), 7.57 (dd, J = 7.9; 7.9, 1H), 6.82 (d, J = 8.4, 1H), 6.74 (d, J = 8.4, 1H), 5.24 (d, J = 9.4, 1H, $NHSO_2$), 4.66 (br s, 1H, $NHCO_2$), 4.15 (m, 2H), 4.06 (ddd, J = 7.0; 5.2; 9.4, 1H), 4.06 (m, 2H), 3.34 (m, 2H), 3.00 (dd, J = 5.2; 14.1, 1H), 2.88 (dd, J = 7.0; 14.1, 1H), 2.82 (m, 2H), 2.57 (m, 1H), 1.96 (m, 2H), 1.90 (m, 2H), 1.72 (m, 2H), 1.47 (s, 9H), 1.43 (s, 9H), 1.26 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.72, 169.61, 156.00, 154.64, 146.04, 141.14, 131.39, 130.50, 129.56, 128.95, 127.7, 124.6, 124.11, 121.31, 110.94, 82.98, 79.48, 79.41, 64.88, 57.10, 43.85, 43.12, 38.71, 36.79, 29.57, 28.57, 28.44, 28.35, 28.28, 27.56; MS (APCI) *m/e* (%) 830 (41, M+1), 730 (50), 729 (100), 673 (95), 658 (20), 629 (14), 518 (20); Anal. Calcd for $C_{39}H_{55}F_3N_4O_{10}S$: C, 56.51; H, 6.69; N, 6.75; S, 3.87. Found: C, 56.00; H, 6.55; N, 6.33; S, 3.82.

4.4.4. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-[*N*-(methyl)isonipecotyl]amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (14a). Yield from 125 mg (0.202 mmol) of **11a**: 84 mg (56%); pale yellow solid; mp 84.2–84.6 °C; R_f 0.2 (DCM/MeOH, 95:5); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.22 (br s, ArNHCO), 7.99 (s, 1H), 7.96 (s, 1H), 7.93 (d, J = 8.0, 1H), 7.74 (d, J = 8.0, 1H), 7.56 (dd, J = 8.0; 8.0, 1H), 6.80 (d, J = 8.4, 1H), 6.72 (d, J = 8.4, 1H), 4.88 (br s, 1H, $NHSO_2$), 4.5 (br s, 1H, NHBoc), 4.05 (m, 1H), 4.03 (m, 1H), 3.44 (m, 2H), 3.31 (m, 2H), 2.98 (m, 2H), 2.97 (dd, J = 5.6; 13.6, 1H), 2.84 (dd, J = 7.0; 13.6, 1H), 2.78 (m, 1H), 2.77 (s, 3H), 2.10 (m, 4H), 1.98 (m, 2H), 1.4 (s, 9H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.24, 169.74, 156.39, 147.0, 141.02, 131.23, 130.43, 129.76, 129.06, 127.59, 126.90, 125.28, 123.84, 122.21, 111.26, 82.92, 79.23, 65.33, 57.41, 54.02, 44.43, 39.76, 38.43, 36.97, 29.26, 28.23, 27.49, 26.62; MS (FAB) *m/e* (%) 741 (70, M−1), 667 (95), 641 (25), 584 (20), 481 (18), 209 (100); Anal. Calcd for $C_{35}H_{49}O_8N_4SF_3$: C, 56.59; H, 6.65; N, 7.54. Found: C, 56.37; H, 6.96; N, 7.18.

4.4.5. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-(4-pyridylcarbonyl)amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (15a). Yield from 126 mg (0.202 mmol) of **11a**: 76 mg (52%); orange solid; mp 51.4–51.6 °C; R_f 0.3 (DCM/MeOH, 30:1); IR (KBr) *v* 3419, 3068, 2985, 2938, 2875, 1734, 1704, 1677, 1594, 1530, 1472, 1432, 1310, 1252, 1160 cm^{-1}, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.90 (s, 1H, ArNHCO), 8.83 (br s, 2H), 8.22 (d, J = 1.8, 1H), 8.02 (s, 1H), 7.98 (d, J = 7.9, 1H), 7.91 (br s, 2H), 7.74 (d, J = 7.9, 1H), 7.57 (dd, J = 7.9; 7.9, 1H), 6.92 (dd, J = 8.4; 1.8, 1H), 6.79 (d, J = 8.4, 1H), 5.43 (br d, 1H, $NHSO_2$), 4.70 (br s, 1H, $NHCO_2$), 4.11 (m, 1H), 4.10 (m, 2H), 3.39 (m, 2H), 3.05 (dd, J = 5.2; 14.1, 1H), 2.92 (dd, J = 7.0; 14.1; 1H), 2.01 (m, 2H), 1.39 (s, 9H), 1.28 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.57, 162.59, 155.98, 149.84, 146.72, 142.53, 141.16, 131.39, 130.49, 129.59, 128.97, 127.84, 126.93, 125.78, 124.1, 121.61, 121.28, 110.84, 83.02, 79.47, 65.18, 57.09, 38.67, 37.00, 29.68, 28.22, 27.58; MS (APCI) *m/e* (%) 724 (55, M+1), 723 (100), 667 (70), 623 (90), 567 (65); Anal. Calcd for $C_{34}H_{41}F_3N_4O_8S$: C, 56.50; H, 5.72; N, 7.75. Found: C, 56.27; H, 5.83; N, 8.01.

4.4.6. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-(4-pyridylacetyl)amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (16a). Yield from 200 mg (0.324 mmol) of **11a**: 137 mg (56%); hygroscopic yellow solid; mp 71.8–72.0 °C; R_f 0.25 (DCM/MeOH, 30:1); IR (KBr) *v* 3376, 3315, 3052, 2979, 2927, 2875, 1734, 1686, 1588, 1536, 1432, 1362, 1322, 1252, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.59 (br s, 2H), 8.34 (br s, 1H, ArNHCO), 8.07 (d, J = 1.8, 1H), 7.99 (s, 1H), 7.94 (d, J = 7.9, 1H), 7.72 (d, J = 7.9, 1H), 7.54 (dd, J = 7.9; 7.9, 1H), 7.39 (br s, 2H), 6.82 (dd, J = 1.8; 8.4, 1H), 6.70 (d, J = 8.4, 1H), 5.34 (d, J = 9.4, 1H, $NHSO_2$), 4.80 (br s, 1H, $NHCO_2$), 4.04 (ddd, J = 7.0; 5.2; 9.4, 1H), 3.97 (m, 2H), 3.83 (s, 2H), 3.23 (m, 2H), 2.99 (dd, J = 5.2; 14.0, 1H), 2.85 (dd, J = 7.0; 14.0, 1H), 1.84 (m, 2H), 1.43 (s, 9H), 1.23 (s, 9H); ^{13}C

NMR ($CDCl_3$, 125 MHz) δ 169.57, 167.13, 156.18, 149.46, 146.16, 144.71, 141.11, 131.31, 130.47, 129.58, 128.97, 127.67, 127.44, 125.08, 124.89, 124.08, 121.19, 111.02, 82.96, 79.41, 64.84, 57.08, 43.74, 38.67, 36.74, 29.52, 28.26, 27.53; MS (APCI) *m/e* (%) 738 (80, M+1), 737 (100), 681 (95), 625 (15), 518 (10), 258 (50); Anal. Calcd for $C_{35}H_{43}F_3N_4O_8S\cdot H_2O$: C, 55.64; H, 5.96; N, 7.42; S, 4.23. Found: C, 55.22; H, 5.99; N, 7.40; S, 3.96.

4.4.7. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-[3-(*N*-*t*-butoxycarbonyl)aminopropanoyl]amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (17a). Yield from 218 mg (0.353 mmol) of **11a**: 125 mg (45%); white foam; R_f 0.4 (DCM/EA, 5:1); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.22 (br s, 1H, ArNHCO), 8.09 (d, J = 1.8, 1H), 8.02 (s, 1H), 7.97 (d, J = 7.9, 1H), 7.75 (d, J = 7.9, 1H), 7.58 (dd, J = 7.9; 7.9, 1H), 6.82 (dd, J = 8.3; 1.8, 1H), 6.74 (d, J = 8.3, 1H), 5.33 (br s, 1H, NHBoc), 5.22 (d, J = 9.3, 1H, $NHSO_2$), 4.74 (br s, NHBoc), 4.07 (m, 1H), 4.07 (t, J = 7.3, 2H), 3.47 (m, J = 6.3; 6.0, 2H), 3.34 (m, J = 7.3; 6.0, 2H), 3.00 (dd, J = 5.2; 14.0, 1H), 2.88 (dd, J = 6.8; 14.0, 1H), 2.68 (t, J = 6.8, 2H), 1.97 (m, J = 7.3, 2H), 1.43 (s, 9H), 1.41 (s, 9H), 1.27 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.91, 169.55, 156.07, 155.89, 146.01, 141.13, 131.39, 130.47, 129.58, 128.95, 127.71, 127.62, 124.70, 124.10, 121.19, 111.10, 82.96, 79.40, 79.06, 65.13, 57.04, 38.71, 37.07, 36.86, 36.61, 29.55, 28.29, 28.27, 27.56; MS (APCI) *m/e* (%) 789 (14, M+1, $C_{36}H_{51}F_3N_4O_{10}S$), 689 (94), 633 (66), 589 (30), 533 (18).

4.4.8. *t*-Butyl *O*-[*N*-(*t*-butoxycarbonyl)-3-aminopropyl]-*meta*-[12-(*N*-*t*-butoxycarbonyl)aminododecanoyl amino]-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosinate (18a). Yield from 131 mg (0.212 mmol) of **11a**: 134 mg (69%); white foam; R_f 0.4 (DCM/MeOH, 20:1); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.14 (d, J = 1.8, 1H), 8.04 (br s, 1H, ArNHCO), 8.00 (s, 1H), 7.94 (d, J = 7.9, 1H), 7.74 (d, J = 7.9, 1H), 7.58 (dd, J = 7.9; 7.9, 1H), 6.80 (dd, J = 8.5; 1.8, 1H), 6.72 (d, J = 8.5, 1H), 5.26 (d, J = 9.1, $NHSO_2$), 4.70 (br s, 1H, NHBoc), 4.50 (br s, 1H, NHBoc), 4.07 (t, J = 7.3, 2H), 4.07 (m, 1H), 3.34 (m, 2H), 3.09 (m, 2H), 2.99 (dd, J = 14.1; 5.3, 1H), 2.87 (dd, J = 14.1; 6.8, 1H), 2.45 (t, J = 7.3, 2H), 1.97 (m, 2H), 1.68 (m, 2H), 1.45 (m, 2H), 1.43 (s, 18H), 1.36 (m, 2H), 1.30 (m, 2H), 1.27 (m, 10H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.45, 169.65, 155.92, 155.82, 145.88, 141.12, 131.29, 130.50, 129.56, 128.91, 127.97, 127.64, 124.32, 124.10, 121.04, 110.88, 82.89, 79.31, 78.87, 65.22, 57.09, 40.51, 38.70, 37.67, 36.94, 29.93, 29.58, 29.40, 29.38, 29.34, 29.28, 29.15, 29.11, 28.30, 28.24, 27.56, 26.68, 25.57; MS (APCI) *m/e* (%) 916 (5, M+1, $C_{45}H_{69}F_3N_4O_{10}S$), 815 (100), 759 (35), 715 (4), 659 (13).

4.5. General procedure for deprotection

In a 1:1 (v/v) mixture of CH_2Cl_2/trifluoroacetic acid (TFA) cooled at 0 °C, was added the protected compound (0.05 mmol/mL). The mixture was stirred at room temperature for 4 h. After concentration, the crude residue was extracted with cold diethylether, then dried under vacuum. The deprotected product was quantitatively recovered as *n*TFA salt (gum or foam).

4.5.1. *O*-(3-Aminopropyl)-*meta*-[*N*-(guanidino)isonipecotyl]amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (19a). ^1H NMR (D_2O, 500 MHz) δ 7.87 (s, 1H), 7.85 (d, J = 8.2, 1H), 7.76 (d, J = 8.2, 1H), 7.55 (dd, J = 8.2; 8.2, 1H), 7.26 (d, J = 2.1, 1H), 6.90 (dd, J = 8.5; 2.1, 1H), 6.76 (d, J = 8.5, 1H), 4.12 (dd, J = 4.1; 11.1, 1H), 4.09 (t, J = 7.5, 2H), 3.95 (m, 2H), 3.21 (m, 2H), 3.19 (m, 2H), 3.12 (dd, J = 4.1; 13.7, 1H), 2.83 (m, 1H), 2.69 (dd, J = 11.1; 13.7, 1H), 2.17 (m, 2H), 2.02 (m, 2H), 1.77 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 178.26, 177.67, 158.64, 151.77, 142.62, 133.16, 132.75, 132.65, 132.06, 131.36, 130.17, 127.59, 127.43, 125.90, 126.02, 115.15, 68.13, 60.40, 47.73, 44.55, 39.70, 39.29, 30.11, 29.17; MS (APCI) *m/e* (%) 616 (8, M+1), 615 (30), 283 (16), 282 (56); HRMS (ESI): calcd for $C_{26}H_{34}N_6O_6F_3S$: 615.2213. Found: 615.2238.

4.5.2. *O*-[2-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)]-*meta*-[*N*-(guanidino)isonipecotyl]amino-*N*-[3-(trifluoromethyl)-phenylsulfonyl]-(L)-tyrosine (19b). ^1H NMR (D_2O, 500 MHz) δ 7.86 (s, 1H), 7.83 (d, J = 8.4, 1H), 7.76 (d, J = 8.4, 1H), 7.54 (d, J = 1.8, 1H), 7.54 (dd, J = 8.4; 8.4, 1H), 6.85 (dd, J = 8.5; 1.8, 1H), 6.73 (d, J = 8.5, 1H), 4.17 (dd, J = 10.8; 4.2, 1H), 3.98 (m, 2H), 3.96 (m, 2H), 3.85 (m, 4H), 3.83 (m, 2H), 3.28 (m, 2H), 3.26 (m, 2H), 3.14 (dd, J = 4.2; 14.0, 1H), 2.92 (m, 1H), 2.72 (dd, J = 10.8; 14.0, 1H), 2.07 (m, 2H), 1.83 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ: 175.26, 175.00, 156.08, 148.09, 140.30, 130.53, 130.18, 130.01, 129.36, 128.62, 126.54, 125.63, 123.40, 123.31, 112.20, 69.88, 69.67, 69.24, 67.38, 66.56, 57.92, 45.19, 42.12, 39.25, 36.85, 27.60; MS (ESI) *m/e* (%) 690 (80, M+1), 689 (100); HRMS (ESI): calcd for $C_{29}H_{40}F_3N_6O_8S$: 689.2593. Found: 689.2580.

4.5.3. *O*-(3-Aminopropyl)-*meta*-(isonipecotyl)amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (20a). ^1H NMR (D_2O, 500 MHz) δ 7.92 (s, 1H), 7.90 (d, J = 7.9, 1H), 7.81 (d, J = 7.9, 1H), 7.62 (dd, J = 7.9; 7.9, 1H), 7.38 (d, J = 1.8, 1H), 6.95 (dd, J = 8.4; 1.8, 1H), 6.82 (d, J = 8.4, 1H), 4.20 (dd, J = 10.6; 4.6, 1H), 4.17 (m, 2H), 3.66 (m, 2H), 3.29 (m, 2H), 3.21 (m, 2H), 3.20 (dd, J = 14.3; 4.6, 1H), 2.96 (m, 1H), 2.76 (dd, J = 14.3; 10.6, 1H), 2.29 (m, 2H), 2.27 (m, 2H), 2.04 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 175.02, 174.66, 149.04, 140.08, 130.60, 130.30, 130.06, 129.48, 128.67, 127.49, 124.82, 124.74, 123.40, 112.46, 65.52, 57.79, 43.20, 40.25, 37.15, 36.71, 26.64, 25.26; MS (APCI) *m/e* (%) 575 (15), 574 (25, M+1), 573 (100), 283 (18), 282 (85); HRMS (ESI): calcd for $C_{25}H_{32}N_4O_6F_3S$: 573.1995. Found: 573.2018.

4.5.4. *O*-(3-Aminopropyl)-*meta*-[*N*-(methyl)isonipecotyl]amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (21a). ^1H NMR (D_2O, 500 MHz) δ 7.83 (s, 1H), 7.79 (d, J = 7.9, 1H), 7.70 (d, J = 7.9, 1H), 7.50 (dd, J = 7.9; 7.9, 1H), 7.21 (d, J = 2.0, 1H), 6.85 (dd, J = 8.4; 2.0, 1H), 6.71 (d, J = 8.4, 1H), 4.07 (dd, J = 4.1; 11.1, 1H), 4.04 (t, J = 7.1, 1H), 3.58 (m, 2H), 3.12 (t, J = 7.1,

2H), 3.08 (dd, $J = 4.1$; 13.7, 1H), 3.03 (m, 2H), 2.84 (s, 3H), 2.77 (tt, $J = 12.2$; 3.8, 1H), 2.63 (dd, $J = 11.1$; 13.7, 1H), 2.14 (m, 2H), 2.13 (m, 2H), 1.92 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 177.61, 177.08, 151.81, 142.61, 133.30, 132.79, 132.67, 132.12, 131.37, 130.30, 127.6, 127.31, 126.04, 115.19, 68.14, 60.40, 56.22, 45.91, 42.36, 39.70, 39.29, 29.19, 28.88; MS (APCI) *m/e* (%) 589 (8), 588 (25, M+1), 587 (100), 283 (8), 282 (40); HRMS (ESI): calcd for $C_{26}H_{34}N_4O_6F_3S$: 587.2151. Found: 587.2139.

4.5.5. *O*-(3-Aminopropyl)-*meta*-(4-pyridylcarbonyl)-amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (22a). ^{1}H NMR (D_2O, 500 MHz) δ 8.72 (d, $J = 6.4$, 2H), 8.00 (d, $J = 6.4$, 2H), 7.77 (s, 1H), 7.76 (d, $J = 8.0$, 1H), 7.60 (d, $J = 8.0$, 1H), 7.45 (dd, $J = 8.0$; 8.0, 1H), 7.25 (d, $J = 1.8$, 1H), 6.83 (dd, $J = 8.4$; 1.8, 1H), 6.70 (d, $J = 8.4$, 1H), 4.05 (dd, $J = 3.8$; 11.0, 1H), 4.05 (t, $J = 7.3$, 2H), 3.14 (t, $J = 7.3$, 2H), 3.05 (dd, $J = 3.8$; 13.7, 1H), 2.60 (dd, $J = 11.0$; 13.7, 1H), 2.13 (tt, $J = 7.3$; 7.3, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 177.59, 171.34, 159.13, 151.34, 143.49, 142.64, 133.08, 132.7, 132.6, 131.9, 131.32, 130.17, 130.90, 127.35, 126.95, 125.96, 115.05, 68.23, 60.34, 39.72, 39.23, 29.17; MS (APCI) *m/e* (%) 569 (8), 568 (25, M+1), 567 (100); HRMS (ESI): calcd for $C_{25}H_{26}N_4O_6F_3S$: 567.1525. Found: 567.1508.

4.5.6. *O*-(3-Aminopropyl)-*meta*-(4-pyridylacetyl) amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (23a). ^{1}H NMR (D_2O, 500 MHz) δ 8.87 (m, 2H), 8.16 (m, 2H), 7.88 (m, 1H), 7.87 (s, 1H), 7.76 (m, 1H), 7.57 (m, 1H), 7.41 (d, $J = 1.8$, 1H), 6.94 (dd, $J = 8.4$; 1.8, 1H), 6.81 (d, $J = 8.4$, 1H), 4.18 (dd, $J = 10.8$; 4.3, 1H), 4.16 (m, 2H), 3.31 (m, 2H), 3.16 (dd, $J = 14.1$; 4.3, 1H), 2.72 (dd, $J = 14.1$; 10.8, 1H), 2.30 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 174.97, 168.56, 156.53, 148.56, 140.87, 140.06, 130.39, 129.96, 129.32, 128.59, 128.30, 127.36, 124.79, 124.11, 123.28, 112.27, 65.57, 57.76, 41.96, 37.13, 36.64, 26.59; MS (APCI) *m/e* (%) 583 (10), 582 (30, M+1), 581 (100), 463 (8), 462 (45), 283 (22), 282 (100); HRMS (ESI): calcd for $C_{26}H_{28}N_4O_6F_3S$: 581.1682. Found: 581.1707.

4.5.7. *O*-(3-Aminopropyl)-*meta*-(3-aminopropanoyl)-amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (24a). ^{1}H NMR (D_2O, 500 MHz) δ 7.92 (s, 1H), 7.92 (d, $J = 8.0$, 1H), 7.81 (d, $J = 8.0$, 1H), 7.62 (dd, $J = 8.0$; 8.0, 1H), 7.38 (d, $J = 1.8$, 1H), 6.95 (dd, $J = 8.4$; 1.8, 1H), 6.82 (d, $J = 8.4$, 1H), 4.20 (dd, $J = 10.6$; 4.6, 1H), 4.17 (m, 2H), 3.86 (m, 2H), 3.29 (m, 2H), 3.21 (dd, $J = 14.3$; 4.6, 1H), 3.01 (m, 2H), 2.76 (dd, $J = 14.3$, 10.6, 1H), 2.27 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 175.02, 174.66, 149.04, 140.08, 130.60, 130.20, 130.06, 129.48, 128.67, 127.49, 124.82, 124.74, 123.40, 123.30, 112.46, 65.52, 57.79, 37.62, 37.22, 37.15, 36.71, 26.64; MS (ESI) *m/e* (%) 533 (100, M+1), 159 (4); HRMS (ESI): calcd for $C_{22}H_{28}N_4O_6F_3S$: 533.1682. Found: 533.1680.

4.5.8. *O*-(3-Aminopropyl)-*meta*-(12-aminododecanoyl)-amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (25a). ^{1}H NMR (CD_3 OD, 200 MHz) δ 7.84 (s, 1H), 7.77 (d, $J = 8.0$, 1H), 7.70 (d, $J = 8.0$, 1H), 7.49 (dd, $J = 8.0$; 8.0, 1H), 7.44 (br s, 1H), 6.80 (br d, $J = 8.4$, 1H), 6.70 (d, $J = 8.4$, 1H), 4.00 (m, 2H+1H), 3.18 (m, 2H), 3.07 (t, $J = 6.9$, 2H), 2.89 (dd, $J = 14.0$; 5.0, 1H), 2.70 (dd, $J = 14.0$; 10.0, 1H), 2.33 (t, $J = 6.9$, 2H), 2.07 (m, 2H), 1.55 (m, 4H), 1.25 (m, 14H); ^{13}C NMR (CD_3OD, 50 MHz) δ 174.17, 174.13, 161.50, 143.93, 134.0, 131.64, 131.19, 130.58, 129.90, 127.86, 126.01, 125.96, 120.18, 114.45, 66.96, 59.19, 39.28, 38.60, 37.85, 30.56, 30–25 (several lines); MS (ESI) *m/e* (%) 659 (100, M+1), 299 (14), 285 (15); HRMS (ESI): calcd for $C_{31}H_{46}N_4O_6F_3S$: 659.3090. Found: 659.3119.

4.5.9. *O*-(3-Aminopropyl)-*meta*-amino-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (26a). ^{1}H NMR (CD_3OD, 200 MHz) δ 7.99 (s, 1H), 7.94 (d, $J = 8.0$, 1H), 7.82 (d, $J = 8.0$, 1H), 7.66 (dd, $J = 8.0$; 8.0, 1H), 7.01 (s, 1H), 6.93 (m, 2H), 4.15 (t, $J = 7.0$, 2H), 4.05 (m, 1H), 3.18 (t, $J = 7$, 2H), 3.03 (dd, $J = 5.1$; 13.9, 1H), 2.81 (dd, $J = 9$; 13.9, 1H), 2.16 (m, 2H); ^{13}C NMR (CD_3OD, 50 MHz) δ 174.60, 150.46, 143.99, 131.77, 131.29, 130.63, 130.09, 128.15, 128.0, 126.17, 125.0, 124.90, 113.15, 66.81, 59.24, 39.32, 38.54, 26.94; MS (ESI) *m/e* (%) 462 (100, M+1), 209 (10); HRMS (ESI): calcd for $C_{19}H_{23}N_3O_5F_3S$: 462.1311. Found: 462.1298.

4.5.10. *O*-(3-Aminopropyl)-*meta*-nitro-*N*-[3-(trifluoromethyl)phenylsulfonyl]-(L)-tyrosine (27a). ^{1}H NMR (CD_3OD, 200 MHz) δ 8.01 (s, 1H), 7.95 (d, $J = 7.9$, 1H), 7.85 (d, $J = 7.9$, 1H), 7.72 (d, $J = 2.1$, 1H), 7.65 (dd, $J = 7.9$; 7.9, 1H), 7.45 (dd, $J = 2.2$; 6.8, 1H), 7.14 (d, $J = 6.8$, 1H), 4.33 (m, 2H), 4.15 (m, 1H), 3.29 (m, 2H), 3.10 (m, 1H), 2.83 (m, 1H), 2.20 (m, 2H); ^{13}C (CD_3OD, 50 MHz) δ 173.61, 152.20, 144.04, 140.51, 137.09, 131.63, 131.50, 131.39, 131.28, 130.12, 127.80, 124.88, 115.97, 68.69, 58.79, 39.18, 38.55, 28.10; MS (ESI) *m/e* (%) 492 (100, M+1), 271 (24); HRMS (ESI): calcd for $C_{19}H_{21}N_3O_7F_3S$: 492.1052. Found: 492.1049.

4.6. Biological evaluation

4.6.1. Binding assay. The $\alpha_v\beta_3$ integrin purified from human placenta was purchased from Chemicon International Inc., (Temecula, CA, USA). The $\alpha_{IIb}\beta_3$ integrin was purified from human platelets by affinity chromatography, as described in Ref. 32. Solutions containing the integrins, diluted to 500 ng/mL ($\alpha_v\beta_3$) or 1 μg/mL ($\alpha_{IIb}\beta_3$) in a Tris binding buffer (2 mM $CaCl_2$, 1 mM $MgCl_2$, and $MnCl_2$, pH 7.5), were transferred to 96-well Costar binding plates (100 μL per well). After a 1 h incubation at room temperature, the plates were incubated for another hour with a blocking solution of 1% albumin to prevent nonspecific binding. The physiological ligands vitronectin (for $\alpha_v\beta_3$) and fibrinogen (for $\alpha_{IIb}\beta_3$) were labeled with biotin. The plates were washed three times in binding buffer containing 0.1% albumin and adsorbed integrins were then incubated for 30 min with their corresponding soluble ligands in excess, in the binding buffer containing 0.1% albumin and in the presence of various concentrations of compounds. After several washings, the amount of bound ligands was indirectly estimated after the sequential incubation with an antibody against biotin coupled to alkaline phosphatase

and *para*-nitrophenyl phosphate for a readout at 405 nm in a colorimetric assay. The concentration of compound corresponding to 50% inhibition (IC_{50}) was inferred from the dose–response curves of at least two pooled experiments and used to compare the products.

4.6.2. Cellular assay. The CaCo2 cells from the American-Type Culture Collection (ATCC, Rockville, MD) were cultured at 37 °C in a water-saturated incubator with an atmosphere of 5% CO_2 in air, in 175 cm^3 flasks (Becton Dickinson, Lincoln Park, NJ) precoated for 2 h at 37 °C with type I collagen (30 μg/mL in PBS; Vitrogen 100, Collagen Corp., Palto Alto, CA). The cells were progressively adapted to S-BDM (synthetic basal defined medium) hormone-defined nutritive medium as described in Ref. 22.

The $\alpha_V\beta_3$ antagonist activity of peptidomimetics in solution has been evaluated in competition with vitronectin. 96-Well polystyrene plate (Falcon) was coated with human vitronectin (Sigma) dissolved at 10 μg/mL in PBS buffer containing Ca^{2+} and Mg^{2+} ions (PBS++: 16.86 g $Na_2HPO_4{\cdot}2H_2O$ and 0.826 g $NaH_2PO_4{\cdot}H_2O$ in 1 L Milli-Q water; addition of 0.132 g $CaCl_2{\cdot}2H_2O$, and 0.100 g $MgCl_2{\cdot}6H_2O$). The coated plate was conditioned overnight at 4 °C and then rinsed twice with PBS++ before use. CaCo2 cells (250,000 cells) in S-BDM medium were preincubated in the presence of a peptidomimetic (or RGDS peptide), at various concentrations, during 30 min at 37 °C (atmosphere of 5% CO_2 in air). The concentrations were from 10^{-3} to 10^{-9} M. The pretreated cells were inoculated into the coated wells at 50,000 cells/well in S-BDM medium. After 15 min at 37 °C, the medium was removed by suction and each well was gently rinsed with PBS++ to eliminate nonadherent cells. A solution of 4-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (Acros, 7.5 mM) and Triton X-100 (0.5%) was added in each well (60 μL/well). After 2 h of incubation at 37 °C, the enzymatic reaction (of cellular *N*-acetyl-β-D-glucosaminidase) was stopped by the addition of a solution of glycine (150 mM) and EDTA (5 mM) at 90 μL/well. Absorbance (*A*) due to the enzymatic formation of 4-nitrophenolate was measured at 405 nm with a Bio-Rad FTS 135 instrument. Results of Figure 4 are the average of three independent measurements (standard deviation indicated on the graph).

The promotion of cellular adhesion by grafted peptidomimetics has been evaluated with home-made culture inserts, as described elsewhere.[25] CaCo2 cells were seeded at 50,000 cells/cm^2 in the inserts (adhesion area = 5.3 cm^2/insert) with S-BDM (3 mL). After 2 h of incubation at 37 °C (atmosphere of 5% CO_2 in air), the culture medium was removed by suction and the inserts were washed three times with PBS (elimination of the nonadherent cells). The adherent cells were fixed with formaldehyde 5% (15 mL of a water solution of formaldehyde at 37% and 85 mL of PBS buffer) during 10 min at 4 °C. The substrates were then copiously rinsed with water (Milli-Q system). The fixed cells were stained (30 min at 20 °C) with aqueous solution of Coomasie Blue (0.25% w/v) + 45% (v/v) methanol + 9% (v/v) acetic acid. The staining solution was removed by suction and the inserts were washed successively with AcOH in methanol–water (7 mL AcOH + 10 mL MeOH + 83 mL Milli-Q water), and water (twice). The samples were mounted on glass slides and observed with a phase contrast microscope (Labovert, Leitz; original magnification of ×250 to ×400) equipped with a color video camera (JVC TK 1280E) coupled to a Macintosh AV 840 computer. The surface occupied by the cells was analyzed using the NIH 1.54 Image software. For each sample, three zones were analyzed, selected by systemic sampling on the culture area. The results given are the average of 2×3 measurements performed with two substrates similarly treated. The standard deviation is indicated in parentheses.

4.7. Theoretical[33]

All the calculations have been performed using the Gaussian 98 A7 suite of programs.[34] The starting geometries were sketched drawn by standard fragments and then completely optimized following all the $3N-6$ degrees of freedom.

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