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Bioorganic & Medicinal Chemistry Letters 18 (2008) 1084–1090
Bioorganic & Medicinal Chemistry Letters

A graftable LDV peptidomimetic: Design, synthesis and application to a blood filtration membrane

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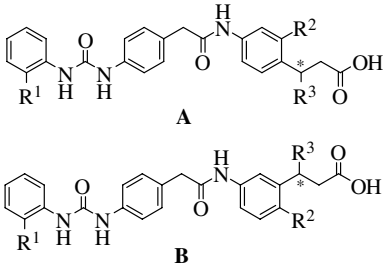
Received 3 December 2007; accepted 4 December 2007
Available online 8 December 2007

Abstract—A graftable LDV (Leu-Asp-Val) peptidomimetic molecule (**B-c**) has been prepared from 3-(5-amino-2-hydroxy)phenylpropionic acid, as $\alpha_4\beta_1$ (VLA-4) integrin ligand. For that purpose, the mechanism of 3-(4-azidophenyl)propionic acid rearrangement has been revisited. Activation of Durapore DVPP-hydrophilic membrane, by surface wet chemistry using triazine trifluoride, followed by covalent coupling of **B-c** produced a modified filter (0.8% of derivatisation from XPS analysis) with improved capacity of leukocyte retention.

The design of smart materials for medical devices is an actual challenge and most of the recent developments in this field aim at controlling the cellular adhesion phenomena.[1] Mammalian cells use transmembrane receptors, called integrins, to mediate their adhesion on the extracellular matrix (ECM).[2] Integrins are $\alpha_x\beta_y$ heterodimeric proteins that bind peptide ligands.[3] Of the 25 known integrins, about one-third bind to the RGD (Arg-Gly-Asp) ligand which is therefore frequently used for the preparation of surface-modified biomaterials.[4]

We are interested in the covalent surface grafting of non-peptide mimics of adhesive molecules as a general strategy for the biocompatibilisation of synthetic polymers.[5] We have previously developed RGD peptidomimetics as ligands of $\alpha_V\beta_3$ integrin; their coupling to a culture support made of poly(ethylene terephthalate) (PET) promoted $CaCo_2$ (epithelial cells) adhesion.[6] Now we are considering the adhesion of leukocyte cells on blood filtration membranes in view of potential applications for leukocyte depletion from blood products.[7] The $\alpha_4\beta_1$ (also called VLA-4, very late antigen-4) integrin is involved in the migration of mononuclear leukocytes to sites of inflammation.[8] This integrin, expressed on all leukocytes except platelets, is known to bind ECM fibronectin via the LDV (Leu-Asp-Val) sequence of an alternatively spliced segment (CS-1).[9] Due to the role of $\alpha_4\beta_1$ in the inflammatory response, the LDV motif became the starting point in the discovery of (non-peptide) small molecule antagonists.[10] Recently, compound **A-a** has been disclosed as a nanomolar active antagonist (Scheme 1).[11] We decided to synthesize a structurally close molecule, but equipped with a spacer-arm for grafting on a blood filter.

From the known antagonist **A-a**, we designed graftable molecules **A-c** ($n \geqslant 2$), easily accessible via the key intermediate **A-b**, featuring the following modifications (Scheme 1): (i) the substituent R^1 of the diphenylurea



Scheme 1. Design of graftable LDV peptidomimetics. Reagents: (a) R^1 = Me; R^2 = H; R^3 = Me: IC_{50} = 1.3 nM (**A**) and 2.5 μM (**B**) versus $\alpha_4\beta_1$ integrin. (b) $R^1 = CF_3$; R^2 = OH; R^3 = H. (c) $R^1 = CF_3$; $R^2 = O–(CH_2–CH_2–O)_n–CH_2–CH_2–NH_2$; R^3 = H.

Keywords: $\alpha_4\beta_1$ integrin ligand; LDV peptidomimetic; PVDF filter; Surface wet chemistry; Leukocyte depletion.
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doi:10.1016/j.bmcl.2007.12.006

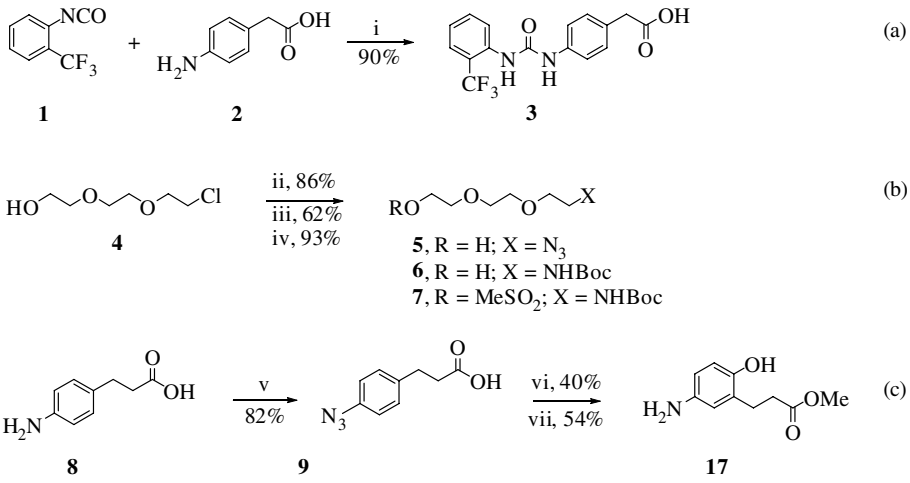
portion is a trifluoromethyl instead of a methyl group; this constitutes a spectroscopic tag for the X-ray photoelectron spectroscopy (XPS) analysis of the derivatised material surface[12]; (ii) the substituent R^2 being a hydroxyl group is introduced on the 3-(*p*-aminophenyl)-propionic acid portion; this represents the anchorage point for the fixation of a spacer-arm of polyethylene glycol (PEG) type[13] via an etherification reaction; (iii) the substituent R^3 (methyl group) of the propionic chain is suppressed; this simplifies the synthesis and avoids the control of a chiral centre which appears not too crucial for the antagonistic activity.[11a]

The choice of the position for the spacer-arm fixation on the LDV peptidomimetic has been inspired from literature data showing that structural variations on this aromatic ring maintain the biological activity.[11] Ethylene glycol oligomers are usually considered for the coupling of biologically active molecules on medical devices due to their hydrophilic character and repulsive effect versus non-specific adsorption of proteins.[14] Finally, we have selected an amine function as terminal group of the spacer-arm because this versatile function allows the grafting on polymer surfaces displaying either carboxyl or hydroxyl groups.[15]

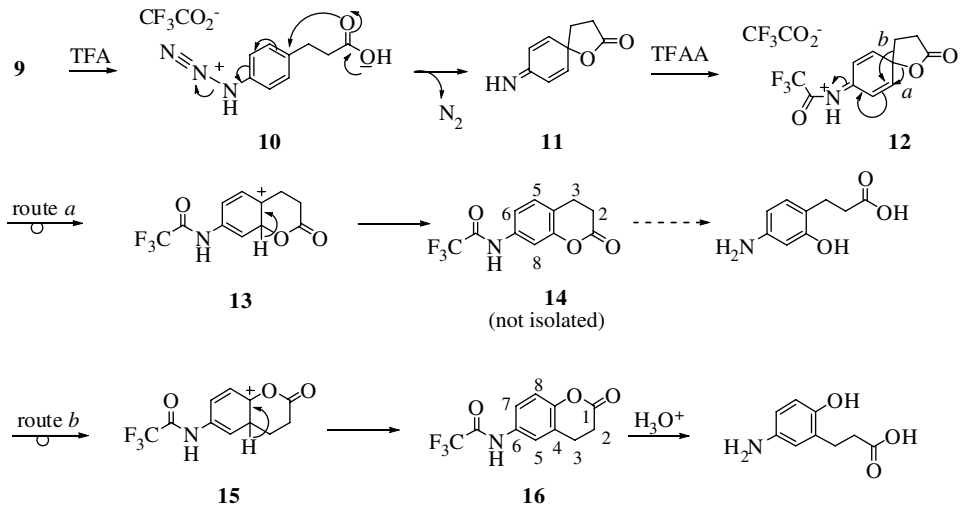
The convergent synthesis of the target molecule **A-c** (exemplified with $n = 2$) required the preparation of three building blocks (Scheme 2). 4-(3-(2-Trifluoromethyl)-phenylureido)-phenylacetic acid (**3**) was obtained by reaction of 2-(trifluoromethyl)-phenyl isocyanate (**1**) with 4-(amino-phenyl)-acetic acid (**2**) (Eq. a).[16] The spacer-arm **7**, N-protected and O-activated, was prepared in four steps from 2-(2-(2-chloroethoxy)-ethoxy)-ethanol (**4**); nucleophilic substitution with NaN_3 gave the azide **5**; reduction with PPh_3 and amine protection with Boc_2O furnished the carbamate **6**[17]; reaction with mesyl chloride led to **7** (Eq. b). We planned to prepare the third portion of the peptidomimetic, namely 3-(4-amino-2-hydroxy)-phenyl-propionic acid[18], from 3-(4-amino)-phenyl-propionic acid (**8**) by using the protocol of Abramovitch et al.[19] during their study of acid-catalysed decomposition of arylazide **9**, they reported an intramolecular nucleophilic trapping in *para* position with respect to the arylnitrenium function, by the carboxyl group leading to *ipso*-substitution and spirolactone formation (Scheme 3, compound **12**). This intermediate further rearranged into dihydrocoumarin **14** (route a) whose hydrolysis should give our target compound (this corresponds formally to the *ortho*-hydroxylation of **8**).

We prepared thus the azide **9** and submitted this compound to the Abramovitch conditions. Reaction with trifluoroacetic acid followed by addition of trifluoroacetic anhydride, aqueous work-up and chromatography afforded the coumarin **16** instead of **14** (Scheme 3)! In our hands,[20] the isolated product resulted from the rearrangement of the spirolactone **12** by C-migration (route b), and not O-migration (route a), leading to intermediate **15** whose positive charge is stabilized by mesomeric effect of the oxycarbonyl function. The structure of **16** was unambiguously confirmed by ^1H and ^{13}C NMR analysis.[21] HMBC experiment showed the coupling of H-3 with H-5, but not with H-7 or H-8; coupling of H-2 with C-4 was also visible (see numbering of **16** in Scheme 3). Strong acidic treatment of **16** deprotected the aniline function and opened the lactone ring; the resulting acid was esterified as usual to furnish **17**[22] (Scheme 2, Eq. c). This unexpected result forced us to reconsider our initial objective (Scheme 1, molecules of series A). The LDV peptidomimetic **B-a** is also described in the literature,[11a] but its antagonistic activity versus $\alpha_4\beta_1$ integrin has been reported in the micromolar range. Nevertheless, we decided to achieve the total synthesis of the graftable LDV peptidomimetic **B-c** via the key-intermediate **B-b** (Scheme 1, molecules of series B).

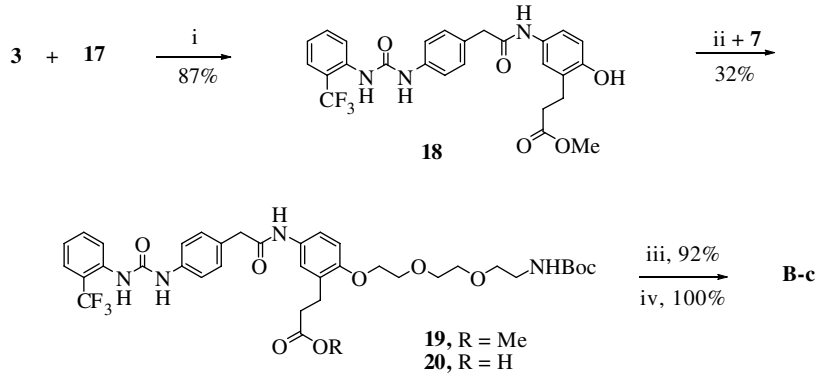
The coupling of synthons **3** and **17** in the presence of carbodiimides as activation agents afforded the anilide



Scheme 2. Synthesis of the three building blocks. Reagents and conditions: (i) THF, reflux, Et_3N (cat.), 2 h; (ii) NaN_3 (excess), NaI (cat.), H_2O, 50 °C, 48 h; (iii) a—Ph_3P, THF, 1 h, 20 °C; b—H_2O, 20 °C, 17 h; c—Boc_2O, CH_3CN, 1 N NaOH, 6 h, 20 °C; (iv) CH_3SO_2Cl, Et_3N, acetone, 20 °C, 4 h; (v) $NaNO_2$, NaN_3, HCl–H_2O, 0 °C, 1 h; (vi) a—TFA, 0 °C, 7 h; b—TFAA, 20 °C, 16 h; (vii) a—MeOH, 6 N HCl, reflux, 20 h; b—H_3PO_4 (cat.), MeOH, reflux, 12 h.



Scheme 3. Rearrangement product of 3-(4-azidophenyl)-propanoic acid.

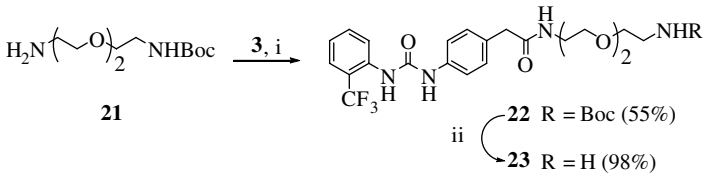


Scheme 4. Synthesis of a graftable LDV peptidomimetic. Reagents and conditions: (i) PyBOP, DMF, 2 h, 20 °C; (ii) NaH, DMF, 20–60 °C, 17 h; (iii) LiOH, CH_3CN–H_2O, 1 h, 20 °C; (iv) TFA, CH_2Cl_2, 1 h, 20 °C.

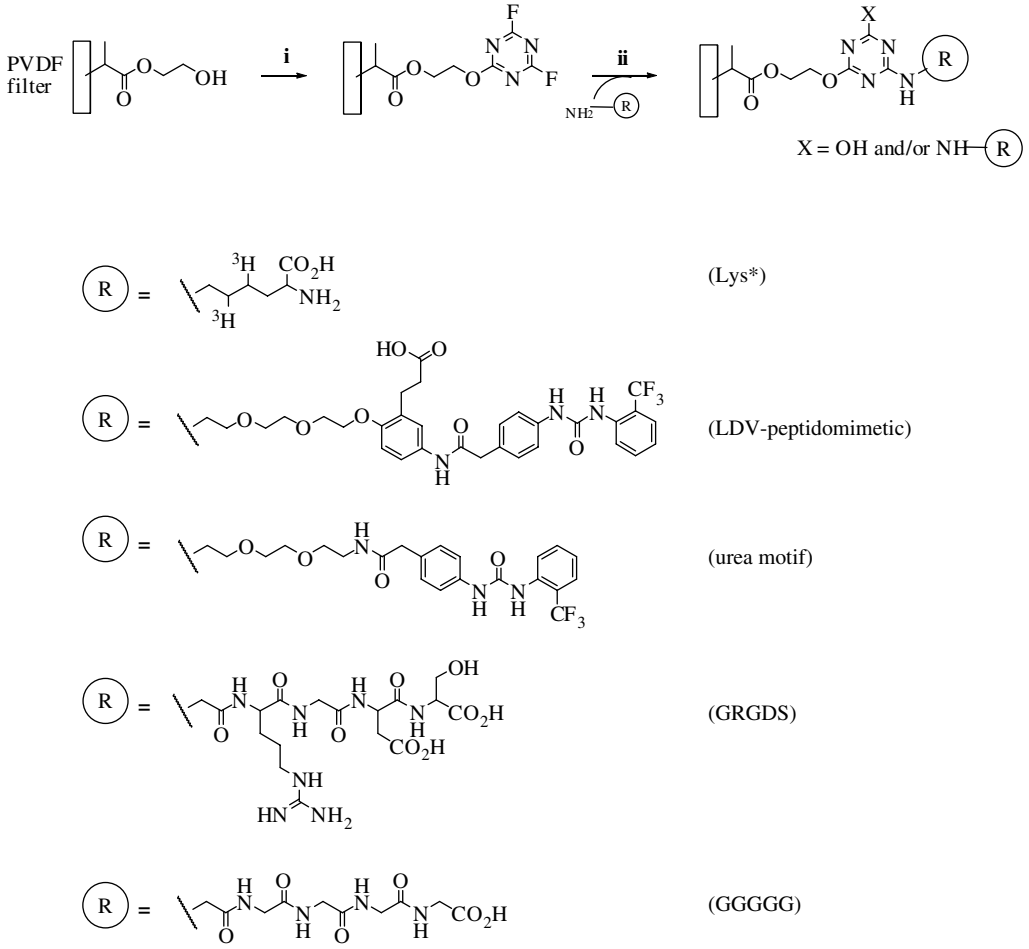
18 in poor yields (⩾25%); the use of BDDC (bis-(4-(2,2-dimethyl-1,3-dioxolyl)-methyl)-carbodiimide),[23] which makes easier the work-up and purification, did not significantly improve the yields. Finally, good results were obtained with PyBOP (benzotriazolyloxy-tris (pyrrolidino)-phosphonium hexafluorophosphate) as coupling agent[24] (Scheme 4). The next step was the coupling of the spacer-arm (**7**) to the peptidomimetic core (**18**). This was realized under the Williamson conditions of etherification. Compound **19** was isolated in modest yields after column chromatography. Applying the Mitsunobu conditions of etherification (phenol **18** + alcohol **6**), we did not obtain **19**. Lastly, saponification of the methyl ester (acid **20**) and TFA deprotection of Boc carbamate gave the target compound **B-c** ($n = 2$)[25] (Scheme 4).

A reference molecule **23** featuring the bis-aryl urea motif solely and the spacer-arm was prepared by conventional chemistry, as described in Scheme 5.[26] Indeed, this moiety is frequently found in the structures of various peptidomimetics.[27]

The biologically active molecules of interest were further covalently fixed to a blood filtration membrane made of poly(vinylidene difluoride) (PVDF). We used the commercial filter Durapore DVPP-hydrophilic from Millipore whose open surface displays hydroxyl functions resulting from a graft-copolymerization treatment performed by the manufacturer. We have previously developed a method of surface derivatisation of such a filter based on the reaction with cyanuryl fluoride by wet chemistry:[28] one fluorine atom of the cross-linking agent is substituted by the polymer hydroxyl group; the remaining fluorine atoms of the triazine motif are further substituted by the molecules via an amine function (Scheme 6). The efficiency of the method has been assayed by coupling a radioactive probe, namely [^{3}H]lysine:[28] under the conditions used to graft peptidomimetic or peptide molecules, we measured by LSC (liquid scintillation counting) the fixation of about 135 pmol of lysine per cm^2 of open surface. The grafting of LDV peptidomimetic (molecule **B-c**), urea (molecule **23**), GRGDS (Gly-Arg-Gly-Asp-Ser) and GGGGG (Gly_5) as reference peptides was realized according to Scheme 6.[29] The resulting surface-modified filters were analysed by XPS to determine their atomic composition (on the apparent surface). Results of Table 1 showed the presence of nitrogen atoms due to the cross-linking



Scheme 5. Synthesis of the graftable urea motif. Reagents and conditions: (i) DCC, THF/CH_2Cl_2 (5:1), 3 h, 50 °C; (ii) TFA/CH_2Cl_2 (1:1), 30 min, 20 °C and NaOH work-up.



Scheme 6. Coupling of molecules on PVDF hydrophilic membrane (with cyanuryl fluoride as cross-linking agent). Reagents and conditions: (i) $C_3N_3F_3$ (1.5%), EtOAc, 20 °C, 1 h; then washing twice with EtOAc; (ii) 10^{-3} M Lys* (or **B-c**, **23**, GRGDS, GGGGG) in phosphate buffer/acetone (9:1, v/v), 4 h, 37 °C; then several washings.

Table 1. XPS analysis of derivatised PVDF membrane

Entry	Sample	Atomic composition (%)				N/C × 100	Surface grafting (%)
		C1s	O1s	N1s	F1s		
1	Native filter	54.65	14.43	—	30.93	—	—
2	PVDF + **B-c**	56.69	11.18	0.97	31.15	1.71	0.8
3	PVDF + urea	56.56	10.07	1.21	32.16	2.10	1
4	PVDF + GRGDS	54.66	9.60	1.31	34.44	2.40	0.7
5	PVDF + GGGGG	57.60	12.90	⩽0.5	29.38	<0.87	<0.4

agent and the coupled ligands. In one case (entry 5), the measurement of N concentration was close to the XPS detection limit. The reproducibility of the filter derivatisation with **B-c** was excellent: two batches independently prepared gave similar N/C × 100 ratios of 1.7–1.8. The fluorine tag incorporated in our peptidomimetic

Table 2. Filtration of 'buffy coat' solutions

Entry	Filter	% Leukocyte in filtrate[a]
1	Native filter	9.74 (±6.10)
2	PVDF + **B-c**	1.37 (±0.75)
3	PVDF + urea	2.36 (±0.79)
4	PVDF + GRGDS	3.45 (±3.40)
5	PVDF + GGGGG	2.19 (±1.24)

[a] Mean of two independent experiments with $n = 4$.

molecule is not useful in the case of a PVDF membrane to determine the amount of grafted molecules from the *F*/*C* ratio (this tag has been used to measure the percentages of grafting on polyester membranes).[30] However, we could estimate the percentages of apparent surface derivatisation from the N/C atomic ratios: values ranging within 0.7–1% are calculated for the filters grafted with **B-c**, **23** and GRGDS, and a lower value of about 0.4% is estimated for GGGGG.[29]

The native Durapore filter and the modified filters were tested for leukocyte depletion. Filtration experiments were performed with 'buffy coats' (concentrated solutions of human leukocytes) as described elsewhere.[30] Leukocyte solutions were passed through the PVDF membranes and the number of cells recovered in the filtrates was determined by flow cytometry. Results of Table 2 showed that the native filter (entry 1) retains about 90% of leukocytes, most probably by steric and non-selective physico-chemical interactions.[31] All the modified filters showed improved performances by retaining between 96% and 99% of leukocytes, and the filter grafted with LDV peptidomimetic (entry 2) was clearly the most efficient one. From the statistical analysis,[32] it appeared significantly different from the native PVDF membrane (reference). Filters grafted with urea (entry 3) and GRGDS peptide (entry 4) were found less efficient and not significantly different from the reference. This latter result is quite surprising since RGD is usually considered as the 'universal adhesive-peptide sequence'.[33] Interestingly, GGGGG peptide used as negative control revealed in fact to be active for leucoreduction. Although the corresponding modified filter (entry 5) was less performant than the one grafted with LDV peptidomimetic, this result is statistically significant[32] compared to the reference (entry 1).

The improvement of leukocyte retention into the membranes is indicative of novel adhesive interactions with cells,[34] created most probably by the binding of integrins to the immobilized ligands. In the case of PVDF derivatised with LDV peptidomimetic (Table 2, entry 2), the involvement of $\alpha_4\beta_1$ integrin is assumed on the basis of the structural similarity of **B-c** with the known antagonists **A-a** and **B-a** (Scheme 1). For PVDF derivatised with GGGGG peptide (entry 5), other integrins could participate in the cell adhesion phenomenon, for instance receptors of the β_2 family. Those are expressed on leukocytes,[35] and LLG (Leu-Leu-Gly) containing peptides have been identified as their antagonists.[36] One can assume that a peptide devoid of functional residues, such as $(Gly)_5$, could play a similar role.

Anyway, leukocyte depletion by filtration is a complex, multi-parametric phenomenon[31]; our preliminary results in this field indicate that the use of surface-modified filters with LDV peptidomimetics could be a valuable strategy. To our knowledge, only one example of LDV peptide immobilization on a PET support to promote adhesion of umbilical cord blood $CD34^+$ cells has been reported in the previous literature.[37] On the other hand, the use of LDV peptidomimetics in the field of medical devices was not yet mentioned. Our work paves the route towards smart filters and new devices dedicated to the filtration of blood products.

Acknowledgments

This work has been supported by the FNRS (Fonds National de la Recherche Scientifique, Belgium) and the FRIA (Fonds pour la formation à la Recherche dans l'Industrie et l'Agriculture, Belgium). The authors thank the Unité de Chimie des Interfaces from UCL—Louvain-la-Neuve (Pr P. Rouxhet, Ir M. Genet) for access to the XPS facilities, and Dr. Anne des Rieux and the Ludwig Institute from UCL—Woluwé (Belgium) for the FACS measurements. The 'buffy coat' pockets were prepared at the Centre de transfusion de la Croix Rouge of Namur (Belgium) and kindly furnished by Dr. M. Bertrand. The authors acknowledge Dr. J.-L. Dewez (Baxter, Belgium) for stimulating discussions, V. Fievez for statistical analysis and S. Devouge for able assistance in preparing the manuscript. J.M.-B. is senior research associate of the FNRS.

Supplementary data

Supplementary information associated with this article can be found in the online version, at doi:10.1016/j.bmcl.2007.12.006.

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20. Experimental details are not available from Abramovitch publication. The authors only mentioned the recovery of lactone **14** in 15% yield, after treatment of β-(4-azidophenyl)-propanoic acid with TFA at 0 °C (catalysis with trifluoromethane sulfonic acid), then with TFAA, before aqueous work-up. No isomer **16** was detected, and much tar was formed. In fact, from our experiments, it appears that both regioisomers **14** and **16** are formed, but that **14** is more soluble in water than **16**. After aqueous basic work-up, only **16** could be extracted with an organic solvent.
21. *Rearrangement of 3-(4-azidophenyl)-propionic acid.* Acid **9** (275 mg) was added by small portions, during 5 h, to cold (0 °C) trifluoroacetic acid (13.5 mL), under stirring, in argon atmosphere. After a further 2 h at 0 °C, trifluoroacetic anhydride (3.8 mL) was added and the mixture was kept at room temperature overnight. After concentration under vacuum, the residue was dissolved in ethyl acetate (15 mL), washed with 1 M Na_2CO_3, water and brine (three times), dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (elution with CH_2Cl_2) gave 6-trifluoroacetamido-3,4-dihydrocoumarin (**16**) as a white solid (260 mg, 40% yield): mp 130 °C; R_F = 0.4 (CH_2Cl_2/CH_3CN, 19:1); IR ν = 1755, 1716 cm^{-1}; MS (APCI) *m/z* = 260 (100%, $C_{11}H_8O_3NF_3$ + 1); ^1H NMR (CD_3OD, 500 MHz, 50 °C) δ = 7.31 (d, *J* = 2.4 Hz, 1H), 7.29 (dd, *J* = 8.55 and 2.4 Hz, 1H), 6.79 (d, *J* = 8.55 Hz, 1H), 2.92 (m, 2H), 2.65 (m, 2H); ^{13}C NMR (acetone-d_6, 125 Hz) δ = 167.2, 154.5 (q, J_{C-F} = 45 Hz), 149.4, 132.1, 124, 120.5, 120.4, 116.6, 115.8 (q, J_{C-F} = 287 Hz), 28.2, 23.2.
22. *Preparation of ester 17.* Compound **16** (780 mg) was dissolved in methanol (30 mL) and 6 N HCl (20 mL), and refluxed for 20 h. After evaporation under vacuum, the residue was dissolved in methanol (20 mL), concentrated H_3PO_4 (3 drops) was added and the solution was refluxed for 12 h. After concentration, the residue was dissolved in EtOAc and washed with aqueous HCl (pH 1). The aqueous phase was separated, treated with Na_2CO_3 to reach pH 8 and extracted (three times) with EtOAc. The organic phases were collected and dried over $MgSO_4$. Concentration under vacuum gave a beige solid which was purified by precipitation from CH_2Cl_2, filtration and washing with ether to yield **17** as a white powder (294 mg, 50% yield): mp 131–133 °C; R_F = 0.57 (EtOAc); IR ν = 3350, 1724, 1167 cm^{-1}; MS (APCI) *m/z* = 196.0 (100%, $C_{10}H_{13}O_3N$ + 1); ^1H NMR ($CDCl_3$, 500 MHz) δ = 6.70 (d, *J* = 8.2 Hz, 1H), 6.48 (dd, *J* = 2.9 and 8.2 Hz, 1H), 6.46 (d, *J* = 2.9 Hz, 1H), 3.67 (s, 3H), 2.82 (t, *J* = 6.7 Hz, 2H), 2.69 (t, *J* = 6.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 175.7, 146.9, 139.7, 128.1, 118.0, 117.2, 115.1, 52.0, 34.9, 24.5.
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25. Protocols of Scheme 4 are provided as Supplementary Information. Structural characterization of **B-c**: ^1H NMR (acetone-d_6, 300 MHz) δ = 9.14 (br s, 1H, NH urea), 8.83 (br s, 1H, NH urea), 8.15 (m, 1H), 7.64 (m, 2H + NH amide), 7.52 (m, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.37 (br s, 1H), 7.27 (m + d, *J* = 8.3 Hz, 1H + 2H), 6.85 (m, 1H), 4.11 (t, *J* = 5.2 Hz, 2H), 3.81 (t, *J* = 5.2 Hz, 2H), 3.64 (t, *J* = 5.2 Hz, 2H), 3.58 (s + t, 2H + 2H), 3.48 (t, *J* = 5.2 Hz, 2H), 3.20 (m, 2H), 2.84 (t, *J* = 6.7 Hz, 2H), 2.57 (t, *J* = 6.7 Hz, 2H); ^{13}C NMR (acetone-d_6, 50 MHz)

δ = 173.1 (C=O amide), 168.4 (C=O acid), 152.6 (C_{Ar}–O), 151.9 (C=O urea), 137.9, 136.5, 132.2, 132.1, 129.8, 129.2, 129.1, 125.4, 124.6, 122.8, 121.1, 118.3, 118.1, 111.4, 70.0, 69.7, 69.3, 69.1, 67.6, 42.7, 39.7, 33.0, 25.6 (C_{Ar}–CF_3, not visible); HRMS (TOF-ES) = 633.2516 ($M+H^+$; M = $C_{31}H_{35}O_7N_4F_3$).

26. Protocols of Scheme 5 are provided as Supplementary Information. Structural characterization of **23**: IR ν = 1648, 1590, 1315, 1123 cm^{-1}; MS (APCI) m/z = 469 (100%; $C_{22}H_{27}O_4N_4F_3$ + 1); ^1H NMR ($CDCl_3$, 500 MHz) δ = 8.80 (s, NH urea, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.57 (s, NH urea, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.48 (dd, J = 8.3 and 7.9 Hz, 1H), 7.14 (d, J = 8.5 Hz, 2H), 7.11 (dd, J = 7.9 and 7.9 Hz, 1 H), 7.04 (d, J = 8.5 Hz, 2H), 6.69 (t, J = 5.4 Hz, NH amide), 3.55 (m, 4H), 3.50 (m, 4H), 3.49 (s, 2H), 3.43 (m, 2H), 3.42 (br s, 2H, NH_2 amine), 2.86 (t, J = 5.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ = 172.2 (C=O amide), 153.0 (C=O urea), 137.8, 136.5, 132.5, 129.5, 129.2, 125.8, 124.6, 123.0 (CF_3, q, J_{C-F} = 276 Hz), 122.9, 120.4, 119.8 (C–CF_3, q, J_{C-F} = 35 Hz), 72.9, 70.2, 69.9, 69.5, 42.8, 41.4, 39.30.
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29. Protocols of Scheme 6 are provided as Supplementary Information. The percentages of derivatisation are calculated as follows. The surface of native hydrophilic PVDF corresponds to $[(C_2F_2)_x + (C_6O_3)_y]$, where $x + y = 1$, as atomic formula (without hydrogen atoms since XPS cannot detect H); x and y values are determined from the experimental XPS values of Table 1, entry 1. About 14% of O and 30% of F gave a O/F ratio of 0.467. For $x = 0.75$ and $y = 0.25$, the calculated O/F ratio is $(3 \times 0.25)/(2 \times 0.75) = 0.75/1.50 = 0.5$, a value close to the experimental one. After activation with triazine and GRGDS grafting, the modified surface corresponds to $[(C_2F_2)_x + (C_6O_3)_{y'} + (C_{20}O_{10}N_{11})_z]$ as atomic formula (where $x + y' + z = 1$), considering an average of one peptide molecule grafted per molecule of triazine. For $x = 0.75$, $y' = 0.243$ and $z = 0.007$, the calculated N/C ratio is: $(11 \times 0.007)/[(2 \times 0.75) + (6 \times 0.243) + (20 \times 0.007)] = 0.077/3.098 = 0.0248$ (Experimental: N/C × 100 = 2.4; Table 1 entry 3). This corresponds to 0.7% of surface derivatisation. The modified surface with peptidomimetic molecule **B-c** is described by the atomic formula: $[(C_2F_2)_x + (C_6O_3)_{y'} + (C_{34}O_8N_7F_3)_z]$. For $x = 0.75$, $y' = 0.242$ and $z = 0.008$, the calculated N/C ratio is: $(7 \times 0.008)/[(2 \times 0.75) + (6 \times 0.242) + (34 \times 0.008)] = 0.056/3.224 = 0.0173$ (Experimental: N/C × 100 = 1.71; Table 1, entry 2). This corresponds to 0.8% of surface derivatisation. Similarly, the surface grafted with urea molecules **23** corresponds to the formula $[(C_2F_2)_x + (C_6O_3)_{y'} + (C_{25}O_5N_7F_3)_z]$ where $x = 0.75$, $y' = 0.24$ and $z = 0.01$, thus giving a rate of surface derivatisation of 1% (calculated N/C = 0.0219—Experimental N/C × 100 = 2.1; Table 1, entry 3), and the GGGGG coupled surface is described by the formula $[(C_2F_2)_x + (C_6O_3)_{y'} + (C_{13}O_7N_8)_z]$ where $x = 0.75$, $y' = 0.246$ and $z = 0.004$, thus giving a rate of surface derivatisation of 0.4% (calculated N/C = 0.010—Experimental N/C × 100 = 0.87; Table 1, entry 5).
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32. All data of Table 2 are expressed as means ± standard error (SEM). Data were tested for homogeneity of variances by the Levene's test. When homogeneous variances were confirmed, the data were analysed by one way ANOVA coupled with the Tukey–Kramer test to identify means with significant differences. Two groups of filters were found: group A containing the native membrane and the membranes grafted with GRGDS and urea, and group B containing the membranes grafted with GRGDS, urea, GGGGG and LDV peptidomimetic. Statistical analyses were performed using JMP6 software (SAS Institute, Cary, NC, USA). P-values <0.05 were considered significant.
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