Novel Peptide-Heterocycle Hybrids: Synthesis and Preliminary Studies on Calpain Inhibition*

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Dedicated to Professor Manuel Bernabé on the occasion of his 66th birthday.

Abstract: New peptidic compounds, having peptide chains linked to bi- and tricyclic heterocycles (peptide-heterocycle hybrids), have been synthesized. The heterocyclic components are derivatives of partially reduced isoquinoline and pyrido[1,2-*b*]isoquinoline bearing α,β-unsaturated carbonyl functionalities. The heterocyclic compounds have been used as acylating agents in coupling reactions with short *N*-unprotected peptides. Based on our interest on potential calpain inhibitors, we have used short (2–4 amino acids) peptides with hydrophobic amino acids of the two enantiomeric series. We report preliminary studies on the inhibition of calpain, with some compounds having IC_{50} values in the nanomolar range.

Keywords: aromatic compounds; calpain inhibitor; heterocycles; peptide-heterocyclic hybrids

Introduction

Promoted by their diverse biological activities, peptides and related compounds are the focus of an intense research activity.[1,2] Although many peptides possess biological activity, they are frequently useless as drugs due to problems of bioavailability and *in vivo* instability.

In order to circumvent these problems, many peptide analogues have been prepared. The three strategies that have been frequently used (sometimes in conjunction) are:

a) Substitution of the amide bond by a surrogate of it.[3]

b) Employment of a non-peptidic cyclic compound (usually heterocyclic) with restricted mobility to imitate the torsion angles of a fragment of the peptidic framework.[4]

c) Use of a readily available non-peptidic cyclic compound as scaffold where one or several peptide chains[5] are bonded, with the expectation that the non-peptide platform will restrict the conformational mobility of the peptidic fragments.[6]

All the three approaches have advantages and drawbacks.[7] Thus, the strategy **a)** gives compounds that are structurally closer to the peptide to mimic, but usually these compounds are very mobile from a conformational point of view.

The fact that the biological activity of many peptides is due to their secondary structural features has prompted a great deal of work on the synthesis of conformationally restricted peptidic compounds.[4,6] To achieve this objective, the approaches **b)** and **c)** are more useful. One advantage of the strategy **b)** is that it yields compounds more restricted than strategy **c)**, but an inconvenience is that the synthetic schemes are usually quite long and with low flexibility. The approach **b)** is very suitable to imitate turns.[8]

On the other hand, the tactic **c)** is synthetically more straightforward and with a higher synthetic versatility than the approach **b)**; and it can be used for the generation of molecular diversity through a combinatorial strategy.[9] A possible inconvenience of this strategy is to predict if the scaffold is going to induce a conformational bias at the peptidic fragment; but a preliminary analysis of the structural features of both the scaffold and its peptidic derivatives can assist the design. A requirement of this approach is that the non-peptidic scaffold must be readily available. Carbocycles,[10] steroids,[11] arenes,[12] carbohydrates,[13] and heterocycles[14] have been used as the non-peptidic platform.

In connection with our ongoing project on the synthesis of polyannular heterocycles and peptidomi-

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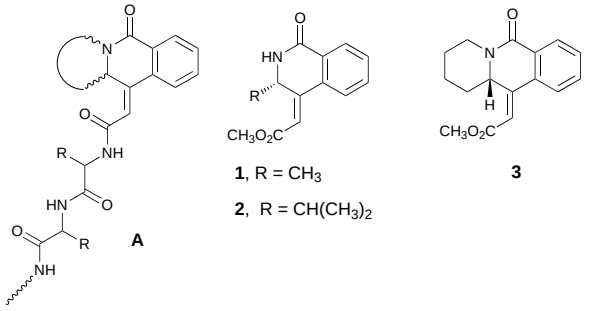


Figure 1. Structures of the target peptide-heterocycle hybrid (A) and chiral heterocycles (1 – 3) used as scaffolds.

metics, we have reported efficient syntheses of a variety of functionalized heterocycles[15] that can be used as platforms for the generation of peptidic compounds following the strategy **c)** indicated above. Our target molecules are peptide conjugates bearing peptidic fragments on a heterocyclic scaffold (peptide-heterocycle hybrids, such as **A**, Figure 1). As heterocyclic starting materials, we have chosen chiral isoquinoline (**1** and **2**) and pyrido[1,2-*b*]isoquinoline (**3**) derivatives.[15b,16] Some structural features of the target molecules are the presence of one aromatic ring and an electrophilic double bond. The aromatic ring is intended to influence the molecular and supramolecular structures (including the putative binding to some biological target).[17,18] On the other hand, the electrophilic conjugated double bond of **A** can be useful from both chemical (to obtain derivatives) and biological (to act as irreversible enzyme inhibitors) points of view.

As a biological application, we have investigated the inhibition of calpains. These enzymes are a family of cytosolic cysteine proteases[19] with a very active role, that includes the catalysis of the hydrolysis of a variety of proteins involved in signal transduction and cytoskeletal remodelling, as well as the participation in physiological processes, such as cell cycle regulation and apoptosis.[20] In mammals, the calpain family comprises several tissue-specific isoforms and two ubiquitous isoenzymes: the μ-calpain (or calpain I) and the *m*-calpain (or calpain II), that require micromolar and millimolar amounts, respectively, of Ca(II) for activation.[21] It has been claimed that the over-activation of the calpains causes several pathological disorders, including brain ischemia, multiple sclerosis, Alzheimer's disease, muscular dystrophy, and other degenerative diseases.[22]

The scarce reports on calpain inhibition have described synthetic inhibitors having electrophilic functionality (mostly activated carbonyl groups) and short peptidic chains with hydrophobic amino acids. A representative selection of inhibitors and their biological activity is depicted in Figure 2.[23]

On basis to the structure of the inhibitors of calpains, we reasoned that some peptide-heterocyclic hybrids of type **A** can be also inhibitors, provided that the peptidic fragments are those recognized by the enzyme. Our expectation was based on the electrophilic nature of the exocyclic olefin of **A**,[24] and the fact that their peptidic fragments are preferentially in extended conformations,[25] a structural feature that has been attributed to protease inhibitors.[19c,26]

Our assumption has been fulfilled, and in this paper we report the synthesis of a variety of novel peptide-heterocycle hybrids of general structure **A** (compounds **6** – **12** and **15** – **21**), bearing isoquinoline and pyrido[1,2-*b*]isoquinoline scaffolds; as well as their activities as calpain inhibitors.

Results and Discussion

The methyl esters of the isoquinolines **1** and **2** were hydrolyzed to the corresponding acids **4** and **5** in nearly quantitative yields (Scheme 1). From these acids, the hybrids **6** – **12** (Figure 3) were obtained in moderate to good yields.[27] All these hybrids, except **7**, were prepared by the mixed anhydride method, using isobutyl chloroformate as promoter,[28] and the corre-

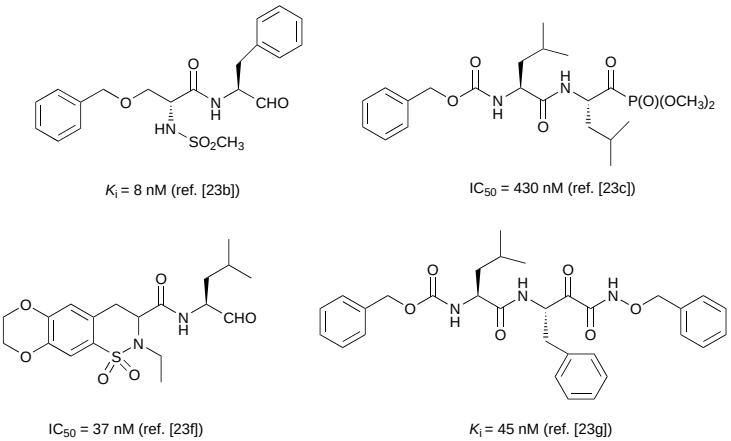
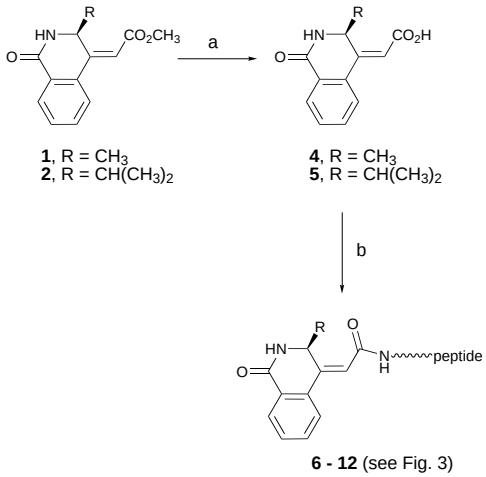


Figure 2. Structures of reported inhibitors of calpain I.



Scheme 1. (a) 1 M aqueous LiOH (2.0 mol equiv.), THF-H_2O, r.t., 4 hours (92% for **4**, 93% for **5**). (b) Synthesis of the hybrids **6** and **8-12**: (i) NMM (1.0 mol equiv.), *i*-BuOCOCl (1.0 mol equiv.), $CHCl_3$, 0 °C; (ii) any of the peptides **22**, or **24** − **27** (as trifluoroacetate salt, 1.1 mol equiv.), NMM (3 mol equiv.), $CHCl_3$, 0 °C to r.t. (45–88%, see Figure 3 and experimental section for details). For **7**: peptide **23** (as trifluoroacetate salt, 1.2 mol equiv.), EDC (1.2 mol equiv.), HOBT (1.2 mol equiv.), Et_3N (3 mol equiv.), DMAP (0.1 mol equiv.), $CHCl_3$, r.t. (65%).

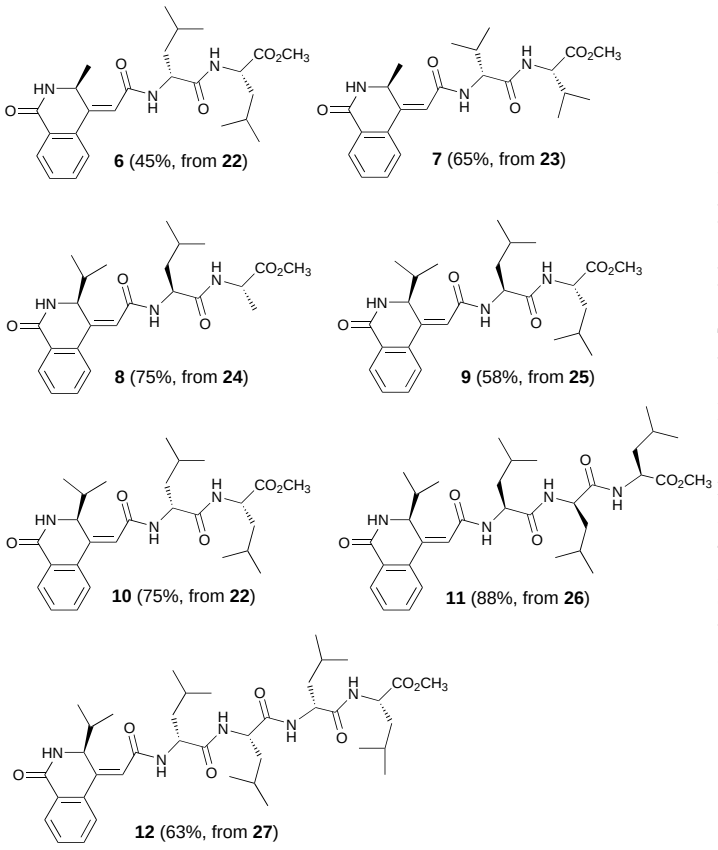


Figure 3. Structures of the peptide-heterocycle hybrids with isoquinoline scaffold prepared by the route depicted in Scheme 1. The isolated yields and the nucleophilic peptides used are indicated into brackets.

sponding *N*-deprotected amino acid as nucleophiles (Figure 4).[29] The divaline derivative **7** was synthesized from **4** and the sterically demanding dipeptide **23** using a combination of 1-hydroxybenzotriazole (HOBT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), and 4-(dimethylamino)pyridine (DMAP) as promoter.[30]

The methyl ester **3** was hydrolyzed to the acid **13** (Scheme 2), that was transformed to the peptide-heterocycle hybrids **15** − **18** (Figure 5) using the mixed-anhydride method. On the other hand, the acid **13** was converted to the pentafluorophenyl derivative **14** in high yield. This activated ester was transformed to the hybrids **19**–**21** (Figure 5) by reaction with the corresponding *N*-unprotected peptides in the presence of *N*-methylmorpholine.[27,31]

Some of the compounds reported in this paper have been essayed as calpain inhibitors. We have studied both calpain I and calpain II. Initially, we carried out exploratory experiments just to estimate qualitatively the activity. Some of the more active compounds have been further studied to determine the IC_{50} values. The results are collected in Table 1.

Although the results reported herein are still insufficient to obtain definitive conclusions, several features deserve commentary:

a) The IC_{50} values of the most active compounds (**11**, **12**, **15**, and **18**) are comparable to those of the most active compounds reported in the literature.[23]

b) A remarkable fact is that some compounds (**11**, **12**, and **18**) present a high biological selectivity between the two isoenzymes, which can be useful from a therapeutic point of view.[21]

c) The heterocyclic compounds alone (without a peptidic chain) are inactive (**5**, entry 1; and **13**, entry 12).

Table 1. Results on the inhibition of calpain I and calpain II by heterocycles and peptide-heterocycle hybrids.

Entry	Compound	Enzyme	IC_{50} (nM)
1	**5**	Calpain I	Inactive
2	**8**	Calpain I	Low activity
3	**8**	Calpain II	Inactive
4	**9**	Calpain I	Moderate activity
5	**9**	Calpain II	Moderate activity
6	**10**	Calpain I	Moderate activity
7	**10**	Calpain II	Moderate activity
8	**11**	Calpain I	45
9	**11**	Calpain II	361
10	**12**	Calpain I	201
11	**12**	Calpain II	10661
12	**13**	Calpain I	Inactive
13	**15**	Calpain I	140
14	**15**	Calpain II	170
15	**18**	Calpain I	130
16	**18**	Calpain II	618
17	**21**	Calpain I	Inactive
18	**21**	Calpain II	Low activity

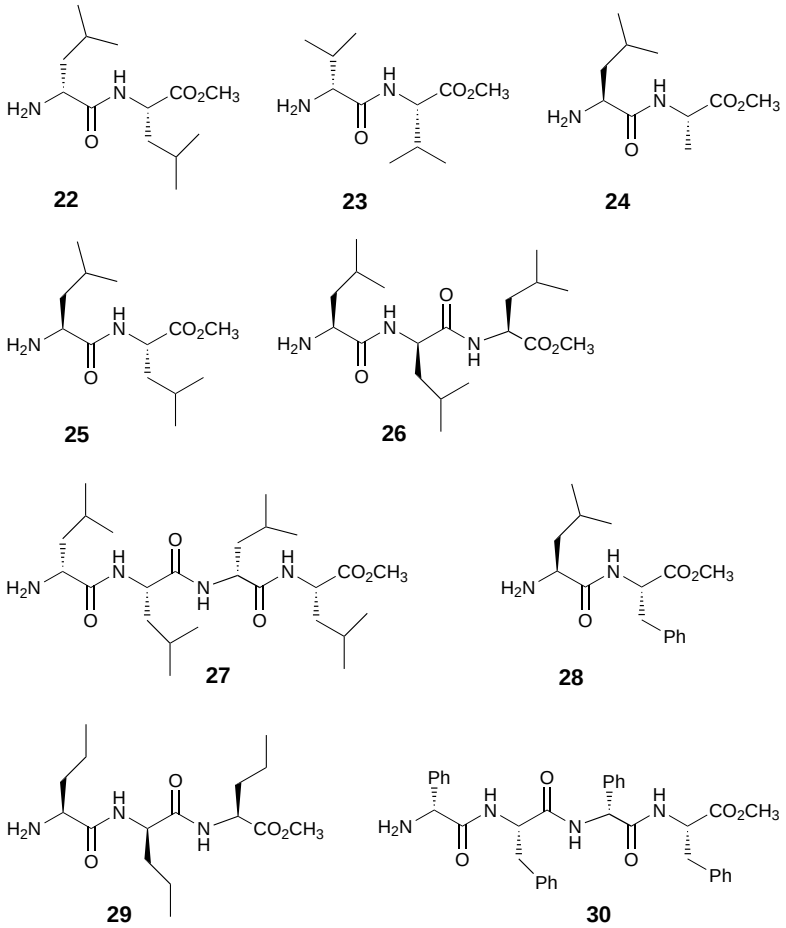
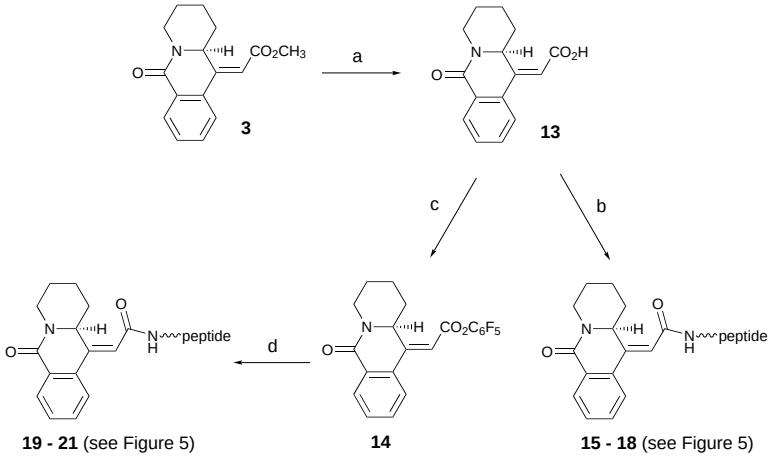


Figure 4. Structures of the nucleophilic peptides used in the synthesis of peptide-heterocyclic hybrids.



Scheme 2. (a) 1 M aqueous LiOH (2.0 mol equiv.), THF-H_2O, r.t, 4 hours (95%). (b) (i) NMM (1.0 mol equiv.), *i*-BuOCOCl (1.0 mol equiv.), $CHCl_3$, 0 °C; (ii) Peptide **22**, or **23**, or **25**, or **26** (as trifluoroacetate salt, 1.1 mol equiv.), NMM (3 mol equiv.), $CHCl_3$, 0 °C to r.t. (53–75%, see Figure 5 and experimental section for details). (c) C_6F_5OH (3.0 mol equiv.), DCC (1.6 mol equiv.), CH_2Cl_2, r.t., 7 hours (91%). (d) Peptide **28**, or **29**, or **30** (as trifluoroacetate salt, 1.5 mol equiv.), NMM (3.0 mol equiv.), DMAP (3 mol equiv.), $CHCl_3$, r.t., 72 hours (55–85%, see Figure 5 and experimental section for details).

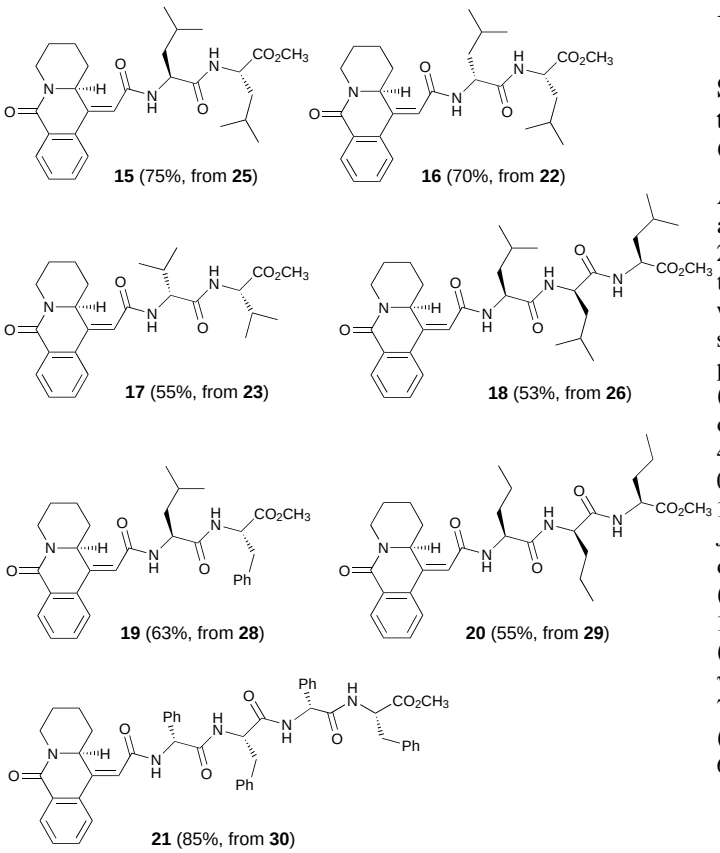


Figure 5. Structures of the peptide-heterocycle hybrids with pyrido[1,2-*b*]isoquinoline scaffold prepared by the route indicated in Scheme 2. The isolated yields and the nucleophilic peptides used are shown into brackets.

d) The fact that the hybrids **8** and **21** are much less active then the analogues with all-leucine peptidic chain suggests the strict requirement of hydrophobic residues on this kind of inhibitors.

e) Although not thoroughly studied, the isoquinoline derivatives require a longer peptidic chain than the corresponding pyrido[1,2-*b*]isoquinoline analogue (compare data from **9** and from **15**, entries 4, 5, 13, and 14).

Conclusions

The synthesis of novel peptide-conjugates bearing bicyclic and tricyclic heterocycles has been reported. Since the starting heterocycles are readily available,[15b] the method is useful for the synthesis of peptide conjugates on rigid functionalized scaffolds. Additionally, we have presented our preliminary results on the inhibition of calpain, finding that some of the hybrids of type **A** are potent inhibitors of this thiol-protease. Work is in progress to improve these results as well as to study the pharmacological properties of these and related compounds.

Experimental Section[32]

Synthesis of (*S*,*Z*)-2-(3-Methyl-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-ylidene)acetic Acid (4, L-Miq-OH)

A 1 M aqueous solution of LiOH (4.0 mL, 4.0 mmol) was added to a stirred solution of the methyl ester **1** (470 mg, 2.0 mmol) in a 5:3 THF-H_2O mixture (16 mL) at room temperature. The mixture was stirred for 24 hours, and then was treated with 5% aqueous HCl until pH 2. The organic solvent was removed at reduced pressure and the aqueous phase was thoroughly extracted with EtOAc. After drying ($MgSO_4$) and evaporation of the solvent, the crude acid **4** was obtained. It was purified by crystallization from EtOAc; yield: 402 mg (92%); white solid; mp $>230\,^{\circ}C$; $[\alpha]_D$: −206 (MeOH, *c* 0.7). ^1H NMR (300 MHz, DMSO-d_6): $\delta = 8.31$ (d, $J = 4.4$ Hz, 1H, NH), 7.97 (dd, $J_{8,7} = 7.3$, $J_{8,6} = 1.7$ Hz, 1H, H-8), 7.84 (dd, $J_{5,6} = 7.2$, $J_{5,7} = 1.7$ Hz, 1H, H-5), 7.65 – 7.55 (m, 2H), 6.38 (s, 1H, olefinic H), 5.47 (qd, $J_{3,Me} = 6.5$, $J_{3,NH} = 4.4$ Hz, 1H, H-3), 1.20 (d, $J = 6.6$ Hz, 3H, CH_3); ^{13}C NMR (50 MHz, DMSO-d_6): $\delta =$ 166.5 (s), 161.8 (s), 149.8 (s), 133.4 (s), 132.7 (d), 130.6 (d), 128.1 (s), 127.3 (d), 125.1 (d), 115.7 (d), 46.5 (d), 23.6 (q); IR (KBr): $\nu = 3436$, 1687, 1625, 1599, 1417, 1287, 1258, 1212, 963, 878, 773 cm^{-1}. MS (EI): $m/z = 217$ (M^+, 61), 202 (27), 172 (100), 131 (29), 101 (16); anal. calcd. for $C_{12}H_{11}NO_3$: C 66.35, H 5.10, N 6.45; found: C 66.59, H 5.08, N 6.40.

Synthesis of (*S*,*Z*)-2-(3-Isopropyl-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-ylidene)acetic Acid (5, L-Iiq-OH)

A 1 M aqueous solution of LiOH (7.8 mL, 7. 8 mmol) was added to a stirred solution of the methyl ester **2** (1.0 g, 3.86 mmol) in a 4: 3 THF-H_2O mixture (35 mL) at room temperature. The mixture was stirred overnight, and then was treated with 5% aqueous HCl until pH 2. The organic solvent was removed at reduced pressure and the aqueous phase was thoroughly extracted with EtOAc. After drying ($MgSO_4$) and evaporation of the solvent, the crude acid **5** was obtained. It was purified by crystallization from EtOAc; yield: 880 mg (93%); white solid; mp $>230\,^{\circ}C$; $[\alpha]_D$: −420 (MeOH, *c* 0.5). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.55$ (very broad s, CO_2H, NH), 8.11 (dd, $J_{8,7} = 7.5$, $J_{8,6} = 1.6$ Hz, 1H, H-8), 7.67 – 7.54 (m, 3H, H-5, H-6, H-7), 6.43 (s, 1H, olefinic H), 5.64 (m, 1H, H-3), 2.09 (m, 1H, $CHMe_2$), 1.06 (d, $J = 6.9$ Hz, 3H, CH_3), 0.83 (d, $J =$ 6.7 Hz, 3H, CH_3); ^1H NMR (300 MHz, DMSO-d_6): $\delta = 8.54$ (d, $J = 4.2$ Hz, 1H, NH), 7.92 (d, $J_{8,7} = 7.4$ Hz, 1H, H-8), 7.76 (d, $J_{5,6} = 7.4$ Hz, 1H, H-5), 7.63 – 7.53 (m, 2H, H-6, H-7), 6.38 (s, 1H, olefinic H), 5.21 (m, 1H, H-3), 1.62 (m, 1H, $CHMe_2$), 0.78 (d, $J = 6.8$ Hz, 3H, CH_3), 0.74 (d, $J = 6.5$ Hz, 3H, CH_3); ^{13}C NMR (50 MHz, DMSO-d_6): $\delta = 167.2$ (s), 163.0 (s), 149.5 (s), 135.5 (s), 133.1 (d), 130.9 (d), 129.1 (s), 127.5 (d), 124.8 (d), 118.0 (d), 55.6 (d), 35.6 (d), 19.7 (q), 18.7 (q); IR (KBr): $\nu = 3430$, 2965, 1674, 1645, 1597, 1444, 1295 cm^{-1}. MS (EI): $m/z = 245$ (M^+, 26), 202 (100), 184 (7), 158 (14), 131 (19), 103 (12); anal. calcd for $C_{14}H_{15}NO_3$: C 68.56, H 6.16 N 5.71; found: C 68.85, H 6.42, N 5.57.

Synthesis of the Peptide-Heterocycle Hybrid L-Miq-D-Leu-L-Leu-OMe (6) using the Mixed Anhydride Method

N-Methylmorpholine (NMM, 0.122 mL, 1.38 mmol) and isobutyl chloroformate (0.174 mL, 1.38 mmol) were sequentially added to a solution of the acid **4** (300 mg, 1.38 mmol) in $CHCl_3$ (14 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (TLC control, *ca.* 4 hours). Then, a solution of the trifluoroacetate salt of peptide **22** (565 mg, 1.52 mmol) and NMM (0.366 mL, 4.06 mmol) in $CHCl_3$ (14 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (*ca.* 48 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 4:1), to give **6**; yield: 284 mg (45%); white solid; mp 166 – 168 °C; $[\alpha]_{D:}$ −117 ($CHCl_3$, *c* 0.5). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.49 (d, *J* = 7.9 Hz, 1H, NH-L-Leu), 8.30 (d, *J* = 8.4 Hz, 1H, NH-D-Leu), 8.20 (d, *J* = 4.3 Hz, 1H, NH-L-Miq), 7.97 (m, 1H, H-8), 7.65 (m, 2H, H-5, H-6), 7.55 (m, 1H, H-7), 6.62 (s, 1H, olefinic H), 5.70 (m, 1H, H-3), 4.52 (m, 1H, CH_α-D-Leu), 4.29 (m, 1H, CH_α-L-Leu), 3.62 (s, 3H, CO_2CH_3), 1.64-1.42 (m, 6H, 2 × [CH_2-CH] from D- and L-Leu), 1.47 (d, *J* = 6.6 Hz, 3H, CH_3 at C-3), 0.89 (d, *J* = 6.2 Hz, 3H, CH_3), 0.88 (d, *J* = 6.2 Hz, 3H, CH_3), 0.88 (d, *J* = 6.2 Hz, 3H, CH_3), 0.83 (d, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 173.6 (s), 172.6 (s), 164.9 (s), 162.6 (s), 146.0 (s), 135.0 (s), 133.2 (d), 130.5 (d), 128.7 (s), 127.9 (d), 125.0 (d), 118.9 (d), 52.5 (q), 51.3 (d), 50.8 (d), 46.6 (t), 42.4 (t), 25.1 (d), 24.9 (d), 24.3 (q), 23.5 (2C, q), 22.6 (q), 21.7 (q); IR (KBr): ν = 3425, 3266, 2958, 1751, 1657, 1540 cm^{-1}; MS (ES, positive ionization mode): *m/z* = 458 ($M^+ + 1$); anal. calcd for $C_{25}H_{35}N_3O_5$: C 65.62, H 7.71, N 9.18; found: C 65.81, H 7.74, N 9.00.

Synthesis of the Peptide-Heterocycle Hybrid L-Miq-D-Val-L-Val-OMe (7) Promoted by EDC/HOBT

The trifluoroacetate salt of the dipeptide **23** (267 mg, 0.70 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC, 134 mg, 0.70 mmol), 1-hydroxybenzotriazole (HOBT, 92 mg, 0.68 mmol), Et_3N (230 μL, 1.74 mmol), and 4-(dimethylamino)pyridine (DMAP, 7 mg, 0.058 mmol) were sequentially added to a solution of the acid **4** (126 mg, 0.58 mmol) in $CHCl_3$ (3 mL). The mixture was stirred at room temperature for 24 hours. Then, water was added, and thoroughly extracted with $CHCl_3$. The organic phase was washed with brine and dried ($MgSO_4$). The solvent was removed at reduced pressure, and the crude product was chromatographed (hexane-EtOAc, 1:1) to give pure **7**; yield: 160 mg (65%); white solid; mp 156 – 159 °C. $[\alpha]_D$: −100 ($CHCl_3$, *c* 0.5); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.15 (m, 1H, H-8), 7.61-7.49 (m, 3H, H-5, H-6, H-7), 7.09 (br s, 1H, NH), 6.91 (br s, 1H, NH), 6.77 (br s, 1H, NH), 6.26 (s, 1H, olefinic H), 5.92 (m, 1H, H-3), 4.53 (dd, *J* = 8.4, 5.2 Hz, 1H, CH_α-D-Val), 4.40 (m, 1H, CH_α-L-Val), 3.69 (s, 3H, CO_2CH_3), 2.15 (m, 2H, 2 × $CHMe_2$), 1.37 (d, *J* = 6.6 Hz, 3H, CH_3 at C-3), 1.03 (d, *J* = 6.9 Hz, 3H, CH_3), 1.01 (d, *J* = 6.9 Hz, 3H, CH_3), 0.98 (d, *J* = 6.8 Hz, 3H, CH_3), 0.97 (d, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1 (s), 172.0 (s), 165.5 (s), 163.7 (s), 148.4 (s), 134.7 (s), 132.7 (d), 130.2 (d), 128.5 (s), 128.2 (d), 124.8 (d), 117.3 (d), 59.5 (d), 58.0 (d), 52.0 (q), 47.7 (d), 31.3 (d), 31.1 (d), 23.6 (q), 19.6 (q), 19.2 (q), 18.7 (q), 18.7 (q), 18.3 (q); IR (KBr): ν = 3428, 2963, 1743, 1654, 1530 cm^{-1}; MS (ES, positive ionization mode): *m/z* = 430 ($M^+ + 1$); anal. calcd. for $C_{23}H_{31}N_3O_5$: C 64.32, H 7.27, N 9.78; found: C 64.50, H 7.22, N 9.48.

Synthesis of the Peptide-Heterocycle Hybrid L-Iiq-L-Leu-L-Ala-OMe (8) using the Mixed Anhydride Method

NMM (0.130 mL, 1.46 mmol) and isobutyl chloroformate (0.184 mL, 1.46 mmol) were sequentially added to a solution of the acid **5** (360 mg, 1.46 mmol) in $CHCl_3$ (14 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (TLC evidence, *ca.* 3 hours). Then, a solution of the trifluoroacetate salt of peptide **24** (578 mg, 1.75 mmol) and NMM (0.390 mL, 4.38 mmol) in $CHCl_3$ (10 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (*ca.* 17 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 4:1), to give **8**; yield: 485 mg (75%; white solid; mp 217 – 219 °C; $[\alpha]_D$: −294 ($CHCl_3$, *c* 0.6). ^{1}H NMR (400 MHz, 303 K, $CDCl_3$): δ = 8.56 (d, *J* = 4.8 Hz, 1H, NH-L-Iiq), 7.94 (m, 1H, H-8), 7.67 (d, *J* = 6.8 Hz, 1H, NH-L-Ala), 7.34 – 7.22 (m, 4H, H-5, H-6, H-7, NH-L-Leu), 6.07 (s, 1H, olefinic H), 5.54 (dd, $J_{3\text{-exocyclic H}}$ = 7.1, $J_{3,NH}$ = 4.7 Hz, 1H, H-3), 4.77 (m, 1H, CH_α-L-Leu), 4.40 (m, 1H, CH_α-L-Ala), 3.69 (s, 3H, CO_2CH_3), 1.80 – 1.64 (m, 4H, [CH_2-CH] from L-Leu, $CHMe_2$), 1.25 (d, *J* = 7.1 Hz, 3H, CH_3-L-Ala), 0.99 (d, *J* = 6.2 Hz, 3H, CH_3-L-Leu), 0.97 (d, *J* = 6.2 Hz, 3H, CH_3-L-Leu), 0.88 (d, *J* = 7.0 Hz, 3H, CH_3), 0.84 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3 (s), 172.7 (s), 165.1 (s), 164.6 (s), 147.7 (s), 136.4 (s), 132.2 (d), 129.8 (d), 128.6 (s), 127.6 (d), 124.0 (d), 119.6 (d), 55.7 (d), 52.4 (q), 51.3 (d), 48.3 (d), 42.5 (t), 35.2 (d), 24.9 (d), 23.0 (q), 22.4 (q), 19.3 (q), 18.8 (q), 17.2 (q); IR (KBr): ν = 3430, 2959, 1747, 1652, 1533, 1209, 1163; IR ($CHCl_3$): ν = 3417, 3274, 1742, 1658; MS (EI): *m/z* = 443 (M^+, 3), 400 (23), 269 (44), 241 (12), 227 (100), 212 (55), 200 (12), 185 (35), 157 (13), 129 (18); anal. calcd. for $C_{24}H_{33}N_3O_5$: C 64.99, H 7.50, N 9.47; found: C 65.12, H 7.28, N 9.49.

Synthesis of the Peptide-Heterocycle Hybrid L-Iiq-L-Leu-L-Leu-OMe (9) using the Mixed Anhydride Method

NMM (0.102 mL, 1.16 mmol) and isobutyl chloroformate (0.146 mL, 1.16 mmol) were sequentially added to a solution of the acid **5** (284 mg, 1.16 mmol) in $CHCl_3$ (12 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (TLC evidence, *ca.* 3 hours). Then, a solution of the trifluoroacetate salt of peptide **25** (521 mg, 1.40 mmol) and NMM (0.310 mL, 3.48 mmol) in $CHCl_3$ (10 mL) was dropwise added at 0 °C. The

mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (TLC control, *ca.* 22 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 4:1), to give **9**; yield: 326 mg (58%; white solid; mp 213 – 215 °C; $[\alpha]_D$: −268 ($CHCl_3$, *c* 0.6). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.32 (d, *J* = 4.9 Hz, 1H, NH-L-Iiq), 8.02 (m, 1H, H-8), 7.71 (d, *J* = 7.3, 1H, NH-L-Leu[2]), 7.41 – 7.33 (m, 3H, H-5, H-6, H-7), 7.01 (d, *J* = 9.1 Hz, 1H, NH-L-Leu[1]), 6.11 (s, 1H, olefinic H), 5.67 (dd, $J_{3\text{-exocyclic H}}$ = 6.9, $J_{3,NH}$ = 5.1 Hz, 1H, H-3), 4.93 (m, 1H, CH_α-L-Leu[1]), 4.47 (m, 1H, CH_α-L-Leu[2]), 3.72 (s, 3H, CO_2CH_3), 1.87 – 1.52 (m, 7H, 2 × [CH_2-CH], $CHMe_2$), 1.04 (d, *J* = 5.6 Hz, 3H, CH_3-L-Leu), 1.03 (d, *J* = 5.5 Hz, 3H, CH_3-L-Leu), 0.94 (d, *J* = 6.8 Hz, 3H, CH_3-L-Leu), 0.90 (d, *J* = 6.7 Hz, 3H, CH_3-L-Leu), 0.75 (d, *J* = 6.0 Hz, 3H, CH_3), 0.70 (d, *J* = 5.9 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.4 (s), 172.7 (s), 164.7 (s), 164.4 (s), 147.9 (s), 136.0 (s), 132.7 (d), 129.8 (d), 128.5 (s), 127.7 (d), 123.7 (d), 118.9 (d), 55.7 (d), 52.3 (q), 51.4 (d), 51.0 (d), 42.9 (t), 40.6 (t), 35.2 (d), 24.8 (d), 24.6 (d), 22.9 (q), 22.7 (q), 22.4 (q), 21.7 (q), 19.3 (q), 18.6 (q); IR (KBr): ν = 3425, 2959, 1745, 1657, 1534, 1469, 1387, 1207, 1160 cm^{-1}; MS (EI): *m/z* = 485 (M^+, 2), 442 (19), 269 (37), 227 (100), 212 (44), 200 (9), 185 (31), 157 (11), 129 (14), 86 (13); anal. calcd. for $C_{27}H_{39}N_3O_5$: C 66.78, H 8.09, N 8.65; found: C 66.49, H 7.90, N 8.67.

Synthesis of the Peptide-Heterocycle Hybrid L-Iiq-D-Leu-L-Leu-OMe (10) using the Mixed Anhydride Method

NMM (0.110 mL, 1.26 mmol) and isobutyl chloroformate (0.160 mL, 1.26 mmol) were sequentially added to a solution of the acid **5** (306 mg, 1.26 mmol) in $CHCl_3$ (13 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (*ca.* 3 hours). Then, a solution of the trifluoroacetate salt of peptide **22** (521 mg, 1.40 mmol) and NMM (0.336 mL, 3.80 mmol) in $CHCl_3$ (12 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (*ca.* 19 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 4:1), to give **10**; yield:458 mg (75%); white solid; mp 197 – 199 °C; $[\alpha]_D$: −167 ($CHCl_3$, *c* 0.4); ^{1}H NMR (400 MHz, 303 K, $CDCl_3$): δ = 8.09 (d, $J_{8,7}$ = 7.1 Hz, 1H, H-8), 7.74 (d, *J* = 7.7 Hz, 1H, NH-L-Leu), 7.51 – 7.45 (m, 3H, H-5, H-6, H-7), 7.41 (d, *J* = 4.8 Hz, NH-L-Iiq), 6.85 (d, *J* = 7.7 Hz, 1H, NH-D-Leu), 6.27 (s, 1H, olefinic H), 5.69 (dd, $J_{3\text{-exocyclic H}}$ = 7.0, $J_{3,NH}$ = 5.0 Hz, 1H, H-3), 4.56 (m, 1H, CH_α-D-Leu), 4.54 (m, 1H, CH_α-L-Leu), 3.65 (s, 3H, CO_2CH_3), 1.84 (m, 1H, $CHMe_2$), 1.76 – 1.57 (m, 6H), 0.96 (d, *J* = 6.4 Hz, 3H, CH_3), 0.93 (d, *J* = 6.4 Hz, 3H, CH_3), 0.92 (d, *J* = 5.9 Hz, 3H, CH_3), 0.89 (d, *J* = 7.3 Hz, 3H, CH_3), 0.88 (d, *J* = 5.9 Hz, 3H, CH_3), 0.87 (d, *J* = 7.1 Hz, 3H, CH_3); ^{1}H NMR (300 MHz, 303 K, DMSO-d_6): δ = 8.45 (d, *J* = 8.2 Hz, 1H, NH-L-Leu), 8.37 (d, *J* = 4.7 Hz, NH-L-Iiq), 8.27 (d, *J* = 8.2 Hz, 1H, NH-D-Leu), 7.90 (d, $J_{8,7}$ = 7.7 Hz, 1H, H-8), 7.61 (m, 2H, H-5, H-6,), 7.50 (m, 1H, H-7), 6.62 (s, 1H, olefinic H), 5.47 (dd, $J_{3\text{-exocyclic H}}$ = 6.0, $J_{3,NH}$ = 4.7 Hz, 1H, H-3), 4.53 (m, 1H, CH_α-D-Leu), 4.26 (m, 1H, CH_α-L-Leu), 3.60 (s, 3H, CO_2CH_3), 1.60 – 1.43 (m, 7H, 2 × CH_2, 3 × $CHMe_2$), 0.88 (d, *J* = 6.5 Hz, 3H, CH_3-Leu), 0.87 (d, *J* = 6.6 Hz, 3H, CH_3-Leu), 0.86 (d, *J* = 6.5 Hz, 3H, CH_3-Leu), 0.81 (d, *J* = 6.1 Hz, 3H, CH_3-Leu), 0.78 (d, *J* = 6.8 Hz, 3H, CH_3), 0.69 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.6 (s), 173.0 (s), 165.4 (s), 164.4 (s), 147.4 (s), 136.5 (s), 132.8 (d), 129.7 (d), 128.7 (s), 127.7 (d), 124.2 (d), 119.7 (d), 55.6 (d), 52.3 (d), 52.1 (q), 51.2 (d), 41.4 (t), 40.9 (t), 35.0 (d), 24.9 (d, 2C), 23.1 (q), 22.9 (q), 22.0 (q), 21.6 (q), 19.6 (q), 18.6 (q); IR (KBr): ν = 3429, 2958, 1655; MS (EI): *m/z* = 485 (M^+, 4), 442 (14), 269 (30), 227 (100), 212 (44), 200 (14), 185 (32), 157 (11), 129 (14), 86 (14); anal. calcd. for $C_{27}H_{39}N_3O_5$: C 66.78, H 8.09, N 8.65; found: C 66.65, H 8.40, N 8.45.

Synthesis of the Peptide-Heterocycle Hybrid L-Iiq-L-Leu-D-Leu-L-Leu-OMe (11) using the Mixed Anhydride Method

NMM (0.082 mL, 0.92 mmol) and isobutyl chloroformate (0.116 mL, 0.92 mmol) were sequentially added to a solution of the acid **5** (224 mg, 0.92 mmol) in $CHCl_3$ (10 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (*ca.* 3 hours). Then, a solution of the trifluoroacetate salt of the tripeptide **26** (514 mg, 1.06 mmol) and NMM (0.246 mL, 2.76 mmol) in $CHCl_3$ (10 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (*ca.* 24 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 4:1), to give **11**; yield: 484 mg (88%); white solid; mp 126 – 128 °C; $[\alpha]_D$: −132 ($CHCl_3$, *c* 1.1); ^{1}H NMR (400 MHz, 303 K, $CDCl_3$): δ = 8.33 (d, *J* = 7.9 Hz, 1H, NH-D-Leu), 8.25 (d, *J* = 4.6 Hz, 1H, NH-L-Iiq), 7.92 (dd, $J_{8,7}$ = 6.6, $J_{8,6}$ = 1.9 Hz, 1H, H-8), 7.51-7.47 (m, 3H, H-5, H-6, H-7), 7.24 (d, *J* = 7.5 Hz, 1H, NH-L-Leu[3]), 6.83 (d, *J* = 8.6 Hz, 1H, NH-L-Leu[1]), 6.19 (s, 1H, olefinic H), 5.85 (dd, $J_{3,\text{exocyclic H}}$ = 7.9, $J_{3,NH}$ = 4.9 Hz, 1H, H-3), 5.02 (m, 1H, CH_α-L-Leu[1]), 4.42 (m, 1H, CH_α-D-Leu), 4.37 (m, 1H, CH_α-L-Leu[3]), 3.23 (s, 3H, CO_2CH_3), 1.85 – 1.61 (m, 7H), 1.45 (m, 1H), 1.35 (m, 1H), 1.21 (m, 1H), 1.01 (m, 12H), 0.95 (d, *J* = 5.5 Hz, 3H, CH_3), 0.94 (d, *J* = 6.8 Hz, 3H, CH_3), 0.90 (d, *J* = 6.7 Hz, 3H, CH_3), 0.85 (d, *J* = 6.0 Hz, 3H, CH_3), 0.80 (d, *J* = 7.0 Hz, 3H, CH_3), 0.78 (d, *J* = 6.6 Hz, 3H, CH_3), 0.72 (d, *J* = 6.5 Hz, 3H, CH_3), 0.70 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9 (s), 172.9 (s), 172.0 (s), 165.1 (s), 164.2 (s), 148.2 (s), 136.4 (s), 132.7 (d), 130.2 (d), 128.7 (s), 128.2 (d), 123.8 (d), 119.5 (d), 55.4 (d), 52.0 (q), 51.9 (d), 50.4 (d, 2C), 42.1 (t), 41.3 (t), 38.7 (t), 35.2 (d), 25.3 (d), 24.9 (d), 24.9 (d), 23.5 (q), 22.9 (q), 22.8 (q), 22.7 (q), 21.7 (q), 21.6 (q), 19.3 (q), 19.0 (q); IR (KBr): ν = 3425, 2959, 1745, 1657, 1534, 1469, 1387, 1207, 1160 cm^{-1}; MS (EI): *m/z* = 598 (M^+, 17), 555 (35), 410 (27), 341 (37), 313 (18), 297 (38), 269 (49), 227 (100), 212 (37), 200 (56), 185 (42), 158 (14), 146 (17), 129 (14), 86 (59); anal. calcd. for $C_{33}H_{50}N_4O_6$: C 66.19, H 8.42, N 9.36; found: C 65.89, H 8.53, N 9.12.

Synthesis of the Peptide-Heterocycle Hybrid L-Iiq-D-Leu-L-Leu-D-Leu-L-Leu-OMe (12) using the Mixed Anhydride Method

NMM (0.102 mL, 1.14 mmol) and isobutyl chloroformate (0.144 mL, 1.14 mmol) were sequentially added to a solution of the acid **5** (278 mg, 1.14 mmol) in $CHCl_3$ (12 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (TLC control, *ca.* 3 hours). Then, a solution of the trifluoroacetate salt of the tetrapeptide **27** (748 mg, 1.25 mmol) and NMM (0.306 mL, 3.42 mmol) in $CHCl_3$ (12 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (TLC control, *ca.* 18 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 4:1), to give **12**; yield: 510 mg (63%); white solid; mp 175 – 177 °C; $[\alpha]_D$: −89 ($CHCl_3$, *c* 0.6). ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.09 (dd, $J_{8,7}$ = 6.1, $J_{8,6}$ = 2.1 Hz, 1H, H-8), 7.53 – 7.44 (m, 3H, H-5, H-6, H-7), 6.98 (d, *J* = 8.2 Hz, 3H, 3 × NH), 6.89 (d, *J* = 8.0 Hz, 1H, NH), 6,79 (d, *J* = 4.5 Hz, 1H, NH-L-Iiq), 6.31 (s, 1H, olefinic H), 5.54 (dd, $J_{3,\text{exocyclic H}}$ = 6.3, $J_{3,NH}$ = 4.5 Hz, 1H, H-3), 4.56 (m, 2H, 2 × CH_α-Leu), 4.48 (m, 2H, 2 × CH_α-Leu), 3.67 (s, 3H, CO_2CH_3), 1.76 – 1.51 (m, 13H), 0.95 – 0.80 (m, 30H); ^{1}H NMR (300 MHz, 303 K, DMSO-d_6): δ = 8.36 (d, *J* = 4.6 Hz, 1H, NH-L-Iiq), 8.26 (d, *J* = 8.2 Hz, 1H, NH), 8.22 (d, *J* = 7.9 Hz, 1H, NH), 8.18 (d, *J* = 7.8 Hz, 1H, NH), 7.97 (d, *J* = 8.5 Hz, 1H, NH), 7.89 (d, *J* = 7.9 Hz, 1H, H-8), 7.59 (m, 2H, aromatic H), 7.51 (m, 1H, aromatic H), 6.60 (s, 1H, olefinic H), 5.41 (m, 1H, H-3), 4.47 (dt, *J* = 7.8, 7.3 Hz, 1H, CH_α), 4.39 – 4.20 (m, 3H, CH_α), 3.60 (s, 3H, CO_2CH_3), 1.66 (m, 1H), 1.60 – 1.39 (m, 12H), 0.88 – 0.76 (m, 27H), 0.65 (d, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.8 (s), 173.3 (s), 173.2 (s), 172.6 (s), 166.1 (s), 165.0 (s), 148.3 (s), 137.1 (s), 133.6 (d), 131.0 (d), 129.6 (s), 129.0 (d), 124.5 (d), 120.0 (d), 57.3 (d), 53.3 (2C, q, d), 52.8 (d), 52.5 (d), 51.8 (d), 42.3 (t), 42.1 (t), 41.7 (t), 41.1 (t), 36.3 (d), 25.9 (q, 4C), 23.8 (q, 4C), 23.1 (q, 2C), 22.9 (q, 2C), 20.4 (q), 19.0 (q); IR (KBr): ν = 3434, 2959, 1656, 1533 cm^{-1}; MS (EI): *m/z* = 711 (M^+, 17), 668 (10), 485 (48), 454 (31), 410 (19), 341 (42), 313 (29), 259 (26), 227 (100), 200 (58), 185 (33), 146 (14), 86 (72); anal. calcd. for $C_{39}H_{61}N_5O_7$: C 65.80, H 8.64, N 9.84; found: C 65.53, H 8.91, N 9.63.

Synthesis of (*S,Z*)-2-(6-Oxo-1,3,4,6,11,11a – hexahydro-2*H*-pyrido[1,2-*b*]isoquinolin-11-ylidene)acetic Acid (13, L-Piq-OH)

A 1 M aqueous solution of LiOH (15 mL, 15 mmol) was added to a stirred solution of the methyl ester **3** (2.02 g, 7.45 mmol) in a 1:1 THF-H_2O mixture (20 mL) at room temperature. The mixture was stirred for 4 hours, and then was treated with 5% aqueous HCl until pH 2. The organic solvent was removed at reduced pressure and the aqueous phase was thoroughly extracted with EtOAc. After drying ($MgSO_4$) and evaporation of the solvent, the crude acid **13** was obtained. It was purified by crystallization from EtOAc; yield: 1.82 g (95%); white solid; mp 183 – 185 °C. $[\alpha]_D$: −370 ($CHCl_3$, *c* 1.0). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.00 – 9.00 (very broad s, 1H, CO_2H), 8.28 (m, 1H, H-7), 7.70 (m, 1H, H-10), 7.55 (m, 2H, H-8, H-9), 6.41 (d, *J* = 1.2 Hz, 1H, olefinic H), 5.52 (broad d, *J* = 10.6 Hz, 1H, H-11a), 4.87 (broad d, *J* = 12.7 Hz, 1H, H-4_{eq}), 2.80 (distorted td, 1H, *J* = 12.7, 3.0 Hz, 1H, H-4_{ax}), 2.00 – 1.42 (m, 6H, 2 × H-1, 2 × H-2, 2 × H-3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.4 (s), 160.6 (s), 151.0 (s), 132.4 (s), 132.2 (d), 131.0 (d), 128.6 (d), 127.7 (s), 123.6 (d), 114.1 (d), 59.0 (d), 45.1 (t), 34.3 (t), 25.5 (t), 24.7 (t); IR (KBr): ν = 3400, 3100, 1690, 1610, 1595, 1570, 1550, 1470, 1430, 1370, 1290, 1260, 1170, 1155, 920, 855, 800, 750 cm^{-1}; MS (EI): *m/z* = 257 (M^+, 45), 212 (100), 198 (20), 184 (28), 155 (22), 129 (26), 115 (34), 101 (38), 77 (25), 75 (24), 63 (12); anal. calcd. for $C_{15}H_{15}NO_3$: C 70.02, H 5.88, N 5.44; found: C 70.25, H 6.02, N 5.67.

Synthesis of Pentafluorophenyl (*S,Z*)-2-(6-Oxo-1,3,4,6,11,11a – hexahydro-2*H*-pyrido[1,2-*b*]isoquinolin-11-ylidene)acetate (14, L-Piq-Opff)

C_6F_5OH (1.04 g, 5.7 mmol), dicyclohexylcarbodiimide (DCC, 625 mg, 3 mmol), and DMAP (50 mg, 0.40 mmol) were sequentially added to a solution of acid **13** (500 mg, 1.9 mmol) in CH_2Cl_2 (60 mL). The mixture was stirred at room temperature for 7 hours. Then, the solution was washed with 5% aqueous HCl, water, saturated aqueous $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was removed under vacuum and the crude residue was chromatographed (hexane-EtOAc, 4:1) to give pure pentafluorophenyl ester **14**; yield: 730 mg (91%); white solid; mp 147 – 149 °C; $[\alpha]_D$: −324 ($CHCl_3$, *c* 0.6). ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.33 (m, 1H, H-7), 7.82 (m, 1H, H-10), 7.63 (m, 2H, H-8, H-9), 6.66 (s, 1H, olefinic H), 5.43 (broad d, *J* = 11.0 Hz, 1H, H-11a), 4.89 (broad d, *J* = 13.1 Hz, 1H, H-4eq), 2.79 (distorted td, *J* = 12.8, 2.9 Hz, 1H, H-4_{ax}), 2.00 – 1.40 (m, 6H, 2 × H-1, 2 × H-2, 2 × H-3); IR (KBr): ν = 3380, 2900, 1720, 1620, 1595, 1570, 1490, 1350, 1205, 1085, 970 cm^{-1}; MS (ES, positive ionization mode): *m/z* = 446 (M^+ + Na); anal. calcd. for $C_{21}H_{14}F_5NO_3$: C 59.57, H 3.33, N 3.31; found: C 59.38, H 3.12, N 3.31.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-L-Leu-L-Leu-OMe (15) using the Mixed Anhydride Method

NMM (0.180 mL, 2.0 mmol) and isobutyl chloroformate (0.268 mL, 2.0 mmol) were sequentially added to a solution of the acid **13** (520 mg, 2.0 mmol) in $CHCl_3$ (40 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (TLC control, *ca.* 4 hours). Then, a solution of the trifluoroacetate salt of the peptide **25** (990 mg, 2.66 mmol) and NMM (0.540 mL, 6.0 mmol) in $CHCl_3$ (20 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (TLC control, *ca.* 18 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 3:2), to give **15**; yield: 745 mg (75%); white solid; mp 110 – 112 °C. $[\alpha]_D$: −229 ($CHCl_3$, *c* 1.1); ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.25 (m, 1H, H-7), 7.60 (m, 1H, H-10), 7.49 (m, 2H, H-8, H-9), 6.47 (d, *J* =

8.4 Hz, 1H, NH-L-Leu[1]), 6.40 (d, J = 8.2 Hz, 1H, NH-L-Leu[2]), 6.28 (d, J = 0.9 Hz, 1H, olefinic H), 5.62 (broad d, J = 10.2 Hz, 1H, H-11a), 4.81 (m, 1H, H-4_{eq}), 4.57 (m, 1H, CH_α-L-Leu[1]), 4.55 (m, 1H, CH_α-L-Leu[2]), 3.74 (s, 3H, CO_2CH_3), 2.72 (td, J = 12.7, 2.9 Hz, 1H, H-4_{ax}), 2.00 – 1.42 (m, 12H, 2 × H-1, 2 × H-2, 2 × H-3, 2 × [CH-CH_2]-Leu), 0.98 (d, J = 6.1 Hz, 3H, CH_3-L-Leu[1]), 0.96 (d, J = 6.0 Hz, 3H, CH_3-L-Leu[1]), 0.89 (d, J = 6.0 Hz, 3H, CH_3-L-Leu[2]), 0.86 (d, J = 5.9 Hz, 3H, CH_3-L-Leu[2]); ^{1}H NMR (300 MHz, 303 K, C_6D_6): δ = 8.63 (m, 1H, H-7), 7.90 (d, J = 8.4 Hz, 1H, NH-L-Leu[1]), 7.65 (d, J = 7.2 Hz, 1H, NH-L-Leu[2]), 7.54 (m, 1H, H-10), 7.16 (m, 2H, H-9, H-9), 6.48 (s, 1H, olefinic-H), 5.91 (broad d, J = 11.0 Hz, 1H, H-11a), 5.06 (m, 2H, CH_α-L-Leu[1], H-4_{eq}), 4.70 (m, 1H, CH_α-L-Leu[2]), 3.30 (s, 3H, CO_2CH_3), 2.58 (broad t, J = 12.2 Hz, 1H, H-4_{ax}), 2.10 – 1.20 (m, 12H, 2 × H-1, 2 × H-2, 2 × H-3, 2 × [CH-CH_2]-Leu), 0.98 (d, J = 6.0 Hz, 6H, 2 × CH_3-L-Leu[1]), 0.73 (d, J = 6.3 Hz, 3H, CH_3-L-Leu[2]), 0.67 (d, J = 6.2 Hz, 3H, CH_3-L-Leu[2]); ^{1}H NMR (300 MHz, 303 K, DMSO-d_6): δ = 8.53 (d, J = 8.3 Hz, 1H, NH-L-Leu[2]), 8.32 (d, J = 8.4 Hz, 1H, NH-L-Leu[1]), 8.05 (m, 1H, H-7), 7.73 (m, 1H, H-10), 7.74 (m, 1H, H-8), 7.54 (m, 1H, H-9), 6.74 (s, 1H, olefinic H), 5.71 (broad d, J = 11.6 Hz, 1H, H-11a), 4.60 (broad d, J = 11.1 Hz, 1H, H-4_{eq}), 4.51 (m, 1H, CH_α-L-Leu[1]), 4.28 (m, 1H, CH_α-L-Leu[2]), 3.61 (s, 3H, CO_2CH_3), 2.73 (td, J = 11.1, 2.1 Hz, H-4_{eq}), 1.82 – 1.23 (m, 12H, 2 × H-1, 2 × H-2, 2 × H-3, 2 × [CH-CH_2]-Leu), 0.90 (d, J = 6.5 Hz, 3H, CH_3), 0.88 (d, J = 6.6 Hz, 6H, 2 × CH_3), 0.83 (d, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, 303 K, $CDCl_3$): δ = 172.9 (s), 172.4 (s), 165.2 (s), 160.6 (s), 146.8 (s), 133.2 (s), 131.9 (d), 130.2 (d), 128.5 (d), 127.9 (s), 123.2 (d), 116.4 (d), 58.7 (d), 52.1 (q), 51.5 (d), 50.8 (d), 45.0 (t), 40.9 (t), 40.8 (t), 34.6 (t), 25.5 (t), 24.9 (t), 24.7 (d), 24.6 (d), 22.8 (q), 22.7 (q), 22.0 (q), 21.5 (q). IR (KBr): ν = 3400, 3240, 3020, 2920, 2890, 2830, 1725, 1615, 1525, 1415, 1365, 1355, 1260, 1240, 1200, 1180, 945, 775, 745 cm^{-1}; MS (EI): m/z = 497 (M^+, 16), 239 (30), 213 (20), 212 (100), 198 (18), 86 (7); anal. calcd. for $C_{28}H_{39}N_3O_5$: C 67.58, H 7.90, N 8.44; found: C 67.82, H 7.80, N 8.54.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-D-Leu-L-Leu-OMe (16) using the Mixed Anhydride Method

NMM (0.180 mL, 2.0 mmol) and isobutyl chloroformate (0.268 mL, 2.0 mmol) were sequentially added to a solution of the acid **13** (520 mg, 2.0 mmol) in $CHCl_3$ (40 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (*ca.* 4 hours). Then, a solution of the trifluoroacetate salt of the peptide **22** (930 mg, 2.50 mmol) and NMM (0.540 mL, 6.0 mmol) in $CHCl_3$ (20 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (TLC control, *ca.* 12 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 1:1), to give **16**; yield: 695 mg (70%); white solid; mp 190 – 192 °C; $[\alpha]_D$: −127 ($CHCl_3$, c 1.7). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.24 (m, 1H, H-7), 7.57 (m, 1H, H-10), 7.48 (m, 2H, H-8, H-9), 6.55 (d, J = 8.4 Hz, 1H, NH-D-Leu), 6.41 (d, J = 8.1 Hz, 1H, NH-L-Leu), 6.23 (d, J = 1.0 Hz, 1H, olefinic H), 5.69 (broad d, J = 11.2 Hz, 1H, H-11a), 4.83 (broad d, J = 12.9 Hz, 1H, H-4_{eq}), 4.58 (m, 2H, CH_α-D-Leu, CH_α-L-Leu), 3.69 (s, 3H, CO_2CH_3), 2.77 (broad t, J = 12.9 Hz, 1H, H-4_{ax}), 1.95 – 1.30 (m, 12H, 2 × H-1, 2 × H-2, 2 × H-3, 2 × [CH-CH_2]-Leu), 0.94 (m, 12H, 4 × CH_3); ^{1}H NMR (300 MHz, 303 K, DMSO-d_6); δ = 8.49 (d, J = 7.9 Hz, 1H, NH-L-Leu), 8.31 (d, J = 8.4 Hz, 1H, NH-D-Leu), 8.05 (m, 1H, H-7), 7.74 (m, 1H, H-10), 7.64 (m, 1H, H-8), 7.55 (m, 1H, H-9), 6.73 (s, 1H, olefinic H), 5.70 (broad d, J = 10.5 Hz, 1H, H-11a), 4.61 (broad d, J = 10.4 Hz, 1H, H-4_{eq}), 4.52 (m, 1H, CH_α-D-Leu), 4.28 (m, 1H, CH_α-L-Leu), 3.32 (s, 3H, CO_2CH_3), 2.73 (td, J = 10.3, 2.4 Hz, 1H, H-4_{ax}), 1.82 – 1.23 (m, 12H, 2 × H-1, 2 × H-2, 2 × H-3, 2 × [CH-CH_2]-Leu), 0.90 (d, J = 6.7 Hz, 3H, CH_3), 0.88 (d, J = 6.7 Hz, 6H, 2 × CH_3), 0.83 (d, J = 6.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, 303 K, $CDCl_3$): δ = 173.0 (s), 172.5 (s), 165.3 (s), 160.7 (s), 146.7 (s), 133.3 (s), 132.0 (d), 130.2 (d), 128.5 (d), 127.9 (s), 123.4 (d), 116.5 (d), 58.7 (d), 52.2 (q), 51.6 (d), 50.7 (d), 45.0 (t), 41.3 (t), 40.9 (t), 34.4 (t), 25.5 (t, 2C), 24.8 (d, 2C), 22.9 (q), 22.7 (q), 21.9 (q), 21.7 (q); IR (KBr): ν = 3400, 3240, 3030, 2920, 2900, 2830, 1735, 1630, 1610, 1580, 1525, 1450, 1415, 1365, 1260, 1180, 945, 855, 735 cm^{-1}; MS (EI): m/z = 497 (M^+, 7), 239 (19), 213 (19), 212 (100), 198 (11), 127 (8), 86 (14); anal. calcd. for $C_{28}H_{39}N_3O_5$: C 67.58, H 7.90, N 8.44; found: C 67.79, H 7.78, N 8.54.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-D-Val-L-Val-OMe (17) using the Mixed Anhydride Method

NMM (0.144 mL, 1.61 mmol) and isobutyl chloroformate (0.214 mL, 1.61 mmol) were sequentially added to a solution of the acid **13** (416 mg, 1.61 mmol) in $CHCl_3$ (30 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (*ca.* 4 hours). Then, a solution of the trifluoroacetate salt of the dipeptide **23** (720 mg, 2.09 mmol) and NMM (0.435 mL, 5.4 mmol) in $CHCl_3$ (15 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (TLC control, *ca.* 14 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 1:1), to give **17**; yield: 415 mg (55%); white solid; mp 195 – 197 °C; $[\alpha]_D$: −177 ($CHCl_3$, c 1.3). ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.25 (m, 1H, H-7), 7.60 (m, 1H, H-10), 7.51 (m, 2H, H-8, H-9), 6.37 (d, J = 8.6 Hz, 1H, NH-D-Val), 6.30 (d, J = 1.0 Hz, 1H, olefinic H), 6.29 (d, J = 8.5 Hz, 1H, NH-L-Val), 5.71 (broad d, J = 10.4 Hz, 1H, H-11), 4.83 (m, 1H, H-4_{eq}), 4.55 (dd, $J_{H\alpha\text{-}NH}$ = 8.5, $J_{H\alpha\text{-exocyclic H}}$ = 3.7 Hz, 1H, CH_α-L-Val), 4.43 (dd, $J_{H\alpha\text{-}NH}$ = 8.6, $J_{H\alpha\text{-exocyclic H}}$ = 6.2 Hz, 1H, CH_α-D-Val), 3.72 (s, 3H, CO_2CH_3), 2.78 (td, J = 12.7, 2.6 Hz, 1H, H-4_{ax}), 2.19 (m, 2H, 2 × $CHMe_2$), 1.98 – 1.35 (m, 6H, 2 × H-1, 2 × H-2, 2 × H-3), 1.01 (d, J = 6.8 Hz, 3H, CH_3-D-Val), 1.00 (d, J = 6.8 Hz, 3H, CH_3-D-Val), 0.96 (d, J = 6.8 Hz, 3H, CH_3-L-Val), 0.93 (d, J = 7.0 Hz, 3H, CH_3-L-Val); ^{1}H NMR (300 MHz, 303 K, DMSO-d_6): δ = 8.37 (d, J = 8.2 Hz, 1H, NH-L-Val), 8.20 (d, J = 9.0 Hz, 1H, NH-D-Val), 8.06 (m, 1H, H-7), 7.75 (m, 1H, H-10), 7.63 (m, 1H, H-8), 7.55 (m, 1H, H-9), 6.84 (s, 1H, olefinic H), 5.71 (broad d, J = 10.6 Hz, 1H, H-11a), 4.60 (broad d, J = 10.3 Hz, 1H, H-4_{eq}), 4.48 (dd, $J_{H\alpha\text{-}NH}$ = 9.0, $J_{H\alpha\text{-exocyclic H}}$ = 6.3 Hz, 1H, CH_α-D-Val), 4.19 (dd, $J_{H\alpha\text{-}NH}$ = 8.2,

$J_{H\alpha\text{-exocyclic H}} = 6.6$ Hz, 1H, CH_α-L-Val), 3.62 (s, 3H, CO_2CH_3), 2.72 (distorted td, $J = 10.6, 2.1$ Hz, 1H, H-4_{ax}), 2.04 (m, 2H, 2 × $CHMe_2$), 1.80 – 1.28 (m, 6H, 2 × H-1, 2 × H-2, 2 × H-3), 0.89 (d, $J = 7.1$ Hz, 3H, CH_3), 0.88 (d, $J = 6.4$ Hz, 3H, CH_3), 0.87 (d, $J = 6.7$ Hz, 3H, CH_3), 0.86 (d, $J = 7.2$ Hz, 3H, CH_3); ^{13}C NMR (75 MHz, 303 K, $CDCl_3$): δ = 171.9 (s), 171.5 (s), 165.0 (s), 160.6 (s), 146.9 (s), 133.3 (s), 131.9 (d), 130.2 (d), 128.5 (d), 128.0 (s), 123.4 (d), 116.6 (d), 58.7 (d), 58.4 (d), 57.3 (d), 52.1 (q), 45.0 (t), 34.3 (t), 31.3 (d), 31.1 (d), 25.5 (t), 24.8 (t), 19.3 (q), 19.0 (q), 18.2 (q), 17.8 (q); IR (KBr): ν = 3400, 3310, 3260, 3020, 2920, 2890, 2830, 1725, 1715, 1630, 1575, 1515, 1260, 1190, 945, 800, 750, 735 cm^{-1}. MS (EI): $m/z = 469$ (M^+, 8), 338 (3), 311 (3), 239 (15), 213 (20), 212 (100), 198 (11), 72 (12); anal. calcd. for $C_{26}H_{35}N_3O_5$: C 66.50, H 7.51, N 8.95; found: C 66.20, H 7.69, N 9.02.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-L-Leu-D-Leu-L-Leu-OMe (18) using the Mixed Anhydride Method

NMM (0.040 mL, 0.38 mmol) and isobutyl chloroformate (0.050 mL, 0.38 mmol) were sequentially added to a solution of the acid **13** (100 mg, 0.38 mmol) in $CHCl_3$ (10 mL) at 0 °C under argon. Stirring was maintained until no starting material remained and the mixed anhydride was formed (*ca.* 4 hours). Then, a solution of the trifluoroacetate salt of the tripeptide **26** (232 mg, 0.48 mmol) and NMM (0.114 mL, 1.42 mmol) in $CHCl_3$ (5 mL) was dropwise added at 0 °C. The mixture was allowed to warm-up slowly to room temperature, and stirred until all the starting material has reacted (TLC control, *ca.* 12 hours). Then, the organic phase was washed with water, 5% aqueous HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), and the solvent was removed under vacuum. The crude product was purified by flash chromatography (hexane-EtOAc, 1:1), to give **18**; yield: 122 mg (53%); white solid; mp 120 – 123 °C; $[\alpha]_D$: −173 ($CHCl_3$, *c* 0.7). ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.23 (m, 1H, H-7), 7.58 (m, 1H, H-10), 7.47 (m, 2H, H-8, H-9), 6.88 (d, $J = 8.8$ Hz, 1H, NH), 6.66 (d, $J = 8.4$ Hz, 1H, NH), 6.57 (d, $J = 7.9$ Hz, 1H, NH), 6.27 (s, 1H, olefinic H), 5.68 (broad d, $J = 10.4$ Hz, 1H, H-11a), 4.80 (dd, $J = 12.7, 2.3$ Hz, 1H, H-4_{eq}), 4.51 (m, 3H, 3 × CH_α-Leu), 3.53 (s, 3H, CO_2CH_3), 2.77 (td, $J = 12.6, 2.8$ Hz, 1H, H-4_{ax}), 2.00 – 1.40 (m, 15H, 2 × H-1, 2 × H-2, 2 × H-3, 3 × [CH-CH_2]), 0.96 (d, $J = 6.2$ Hz, 3H, CH_3), 0.94 (d, $J = 7.4$ Hz, 3H, CH_3), 0.91 (d, $J = 6.3$ Hz, 3H, CH_3), 0.88 (d, $J = 6.2$ Hz, 3H, CH_3), 0.83 (d, $J = 5.9$ Hz, 3H, CH_3), 0.80 (d, $J = 6.0$ Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.5 (s), 172.3 (s), 171.5 (s), 165.4 (s), 160.6 (s), 147.2 (s), 133.1 (s), 131.9 (d), 130.4 (d), 128.7 (d), 128.0 (s), 123.2 (d), 116.1 (d), 58.5 (d), 52.2 (q), 52.1 (d), 51.6 (d), 50.4 (d), 44.9 (t), 41.1 (t), 40.4 (t), 40.2 (t), 34.7 (t), 25.6 (t), 24.9 (t), 24.8 (d, 2C), 24.7 (d), 22.9 (q), 22.7 (q), 22.6 (q), 22.3 (q), 21.7 (q), 21.6 (q); IR (KBr): ν = 3402, 3297, 2960, 1747, 1655, 1521, 1261, 1098, 801 cm^{-1}; MS (CI, negative ionization mode): $m/z = 609$ ($M^+ - 1$); anal. calcd. for $C_{34}H_{50}N_4O_6$: C 66.86, H 8.25, N 9.17; found: C 66.98, H 8.13, N, 9.19.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-L-Leu-L-Phe-OMe (19) from the Active Ester 14

A solution of the trifluoroacetate of the dipeptide **28** (406 mg, 1.0 mmol) and NMM (0.200 mL, 2.1 mmol) in $CHCl_3$ (8 mL) was added to a solution of **14** (300 mg, 0.70 mmol) and DMAP (140 mg, 2.1 mmol) in $CHCl_3$ (4 mL). The mixture was stirred at room temperature for 19 hours. Then, the solution was washed with water, 5% aqueous HCl, water, saturated aqueous $NaHCO_3$, and water. The organic solvent was dried ($MgSO_4$) and removed under vacuum to give the crude product, that was purified by flash-chromatography (hexane-EtOAc, 2:3) to give **19**; yield: 244 mg (63%); white solid; mp 185 – 186 °C; $[\alpha]_D$: −188 ($CHCl_3$, *c* 0.5). ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.26 (m, 1H, H-7), 7.60 (m, 1H, H-10), 7.48 (m, 2H, H-8, H-9), 7.20 – 7.08 (m, 5H, C_6H_5), 6.64 (d, $J = 7.7$ Hz, 1H, NH), 6.49 (d, $J = 8.3$ Hz, 1H, NH), 6.27 (s, 1H, olefinic H), 5.58 (broad d, $J = 10.4$ Hz, 1H, H-11a), 4.84 (m, 2H, H-4_{eq}, CH_α-Phe), 4.52 (m, 1H, CH_α-Leu), 3.73 (s, 3H, CO_2CH_3), 3.14 (d, $J = 14.0$ Hz, 1H, CH-Ph), 3.03 (d, $J = 14.0$ Hz, 1H, CH-Ph), 2.68 (td, $J = 12.8, 3.2$ Hz, 1H, CH-4_{ax}), 1.97 – 1.41 (m, 9H, 2 × H-1, 2 × H-2, 2 × H-3, [CH-CH_2]-Leu), 0.95 (d, $J = 5.6$ Hz, 3H, CH_3), 0.93 (d, $J = 5.8$ Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.8 (s), 171.4 (s), 165.0 (s), 160.6 (s), 147.1 (s), 135.4 (s), 133.2 (s), 131.2 (s), 130.3 (d), 129.2 (d, 2C), 128.6 (d) 128.4 (d, 2C), 128.0 (d), 127.0 (d), 123.2 (d), 116.2 (d), 59.1 (d), 53.4 (d), 52.5 (q), 51.8 (d), 45.3 (t), 40.9 (t), 37.9 (t), 34.9 (t), 25.8 (t), 25.2 (t), 25.0 (d), 23.0 (q), 22.4 (q); IR (KBr): ν = 3435, 3275, 2957, 1751, 1654, 1535, 1261, 1097 cm^{-1}; MS (ES, positive ionization mode): $m/z = 532$ ($M^+ + 1$); anal. calcd. for $C_{31}H_{37}N_3O_5$: C 70.03, H 7.01, N 7.90; found: C 69.83, H 7.13, N 7.86.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-L-Nva-D-Nva-L-Nva-OMe (20) from the Active Ester 14

A solution of the trifluoroacetate of the tripeptide **29** (400 mg, 0.90 mmol) and NMM (0.180 mL, 1.8 mmol) in $CHCl_3$ (8 mL) was added to a solution of **14** (260 mg, 0.60 mmol) and DMAP (120 mg, 1.8 mmol) in $CHCl_3$ (4 mL). The mixture was stirred at room temperature for 14 hours. Then, the solution was washed with water, 5% aqueous HCl, water, saturated aqueous $NaHCO_3$, and water. The organic solvent was dried ($MgSO_4$) and removed under vacuum to give the crude product, that was purified by flash-chromatography (hexane-EtOAc, 2:3) to give **20**; yield: 187 mg (55%); white solid; mp 106 – 108 °C; $[\alpha]_D$: −178 ($CHCl_3$, *c* 0.48). ^{1}H NMR (300 MHz, 303 K, $CDCl_3$): δ = 8.22 (m, 1H, H-7), 7.57 (m, 1H, H-10), 7.45 (m, 2H, H-8, H-9), 6.92 (d, $J = 8.4$ Hz, 1H, NH), 6.76 (d, $J = 8.2$ Hz, 1H, NH), 6.71 (d, $J = 8.1$ Hz, 1H, NH), 6.29 (s, 1H, olefinic H), 5.63 (d, $J = 10.7$ Hz, 1H, H-11a), 4.80 (broad d, $J = 11.1$ Hz, 1H, H-4_{eq}), 4.50 (m, 3H, 3 × CH_α-Nval), 3.58 (s, 3H, CO_2CH_3), 2.75 (distorted td, $J = 12.8, 3.0$ Hz, 1H, H-4_{ax}), 2.00 – 1.50 (m, 12H, 2 × H-1, 2 × H-2, 2 × H-3, 3 × $CH_{2(\beta)}$-Nval), 1.50 – 1.20 (m, 6H, 3 × $CH_{2(\gamma)}$-Nval), 0.95 (t, $J = 7.1$ Hz, 3H, CH_3-Nval), 0.90 (t, $J = 7.4$ Hz, 3H, CH_3-Nval), 0.84 (t, $J = 7.4$ Hz, 3H, CH_3-Nval); ^{13}C NMR (75 MHz, $CDCl_3$): δ =173.0 (s), 171.9 (s), 171.1 (s), 165.1 (s), 160.6 (s), 146.9 (s), 133.3 (s), 131.9 (d), 130.3 (d), 128.7 (d), 128.1 (s), 123.2 (d), 116.5 (d), 58.7 (d), 53.3 (d), 52.9 (d), 52.2 (d), 51.8 (q), 44.9 (t), 34.6 (t), 34.3 (t), 34.1 (t, 2C), 25.6 (t), 24.9 (t), 18.8 (t), 18.7 (t), 18.6 (t), 13.8 (q), 13.7 (q), 13.5 (q); IR (KBr): ν = 3421, 3289, 2926, 1747, 1635, 1533, 1261, 1097 cm^{-1}: MS (CI,

positive ionization mode): $m/z = 569$ ($M^+ + 1$); anal. calcd. for $C_{31}H_{44}N_4O_6$: C 65.47, H 7.80, N 9.85; found: C 65.63, H 7.72, N 9.80.

Synthesis of the Peptide-Heterocycle Hybrid L-Piq-D-Phg-L-Phe-D-Phg-L-Phe-OMe (21) from the Active Ester 14

A solution of the trifluoroacetate of the tetrapeptide **30** (370 mg, 0.53 mmol) and NMM (0.070 mL, 0.69 mmol) in $CHCl_3$ (6 mL) was added to a solution of **14** (100 mg, 0.23 mmol) and DMAP (46 mg, 0.69 mmol) in $CHCl_3$ (3 mL). The mixture was stirred at room temperature for 18 hours. Then, the solution was washed with water, 5% aqueous HCl, water, saturated aqueous $NaHCO_3$, and water. The organic solvent was dried ($MgSO_4$) and removed under vacuum to give the crude product, that was purified by flash-chromatography (hexane-EtOAc, 2:3) to give **21**; yield: 167 mg (85%); white solid; mp 260–264 °C; $[\alpha]_D$: −156 ($CHCl_3$, *c* 0.3). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.89 (d, *J* = 8.4 Hz, 1H, NH), 8.87 (d, *J* = 8.2 Hz, 1H, NH), 8.84 (d, *J* = 8.4 Hz, 1H, NH), 8.77 (d, *J* = 8.4 Hz, 1H, NH), 8.06 (m, 1H, H-7), 7.76 (m, 1H, H-10), 7.64 (m, 1H, aromatic H), 7.55 (m, 1H, aromatic H), 7.23–7.05 (m, 20H, aromatic H), 6.93 (s, 1H, olefinic H), 5.73 (d, *J* = 8.2 Hz, 1H, CH_α-Phg), 5.64 (m, 1H, H-11a), 5.62 (d, *J* = 8.4 Hz, 1H, CH_α-Phg), 4.71 (m, 1H, CH_α-Phe or H-4$_{eq}$), 4.56 (m, 1H, CH_α-Phe or H-4$_{eq}$), 4.46 (m, 1H, CH_α-Phe or H-4$_{eq}$), 3.63 (s, 3H, CO_2CH_3), 2.91 (m, 4H, 2 × CH_2Ph), 2.71 (m, 1H, H-4$_{ax}$), 1.79–1.59 and 1.39–1.25 (m, 6H, 2 × H-1, 2 × H-2, 2 × H-3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.7 (s), 170.6 (s), 169.5 (s), 169.3 (s), 163.9 (s), 159.7 (s), 144.0 (s), 138.7 (s), 138.5 (s), 137.2 (s), 136.7 (s), 133.4 (s), 132.3 (d), 130.0 (d), 129.2 (d), 129.0 (d), 128.1 (s), 128.0 (d), 127.8 (d), 127.3 (d), 126.5 (d), 126.4 (d), 126.1 (d), 123.6 (d), 117.9 (d), 57.6 (d), 55.6 (d), 55.4 (d), 54.0 (d), 53.6 (d), 51.9 (q), 44.2 (t), 37.6 (t), 36.5 (t), 34.2 (t), 25.2 (t), 25.1 (t); IR (KBr): ν = 3428, 1641, 1496, 1214, 698 cm^{-1}; MS (ES, positive ionization mode): $m/z = 832$ ($M^+ + 1$); anal. calcd. for $C_{50}H_{49}N_5O_7$: C 72.18, H 5.94, N 8.42; found: C 71.97, H 6.07, N 8.56.

Enzyme-Inhibition Experiments.[33,34]

A solution of labelled casein, containing 0.1 mM NaN_3, in a 10 mM Tris-HCl buffer (pH 7.8) was sequentially treated with stock solutions of calpain [containing 20 mM imidazole-HCl buffer (pH 6.8), 1 mM EDTA, 1 mM EGTA, and 5 mM β-mercaptoethanol] and the corresponding inhibitor (in a 10:1 H_2O-DMSO solution). The experiments were carried out with variable amounts of substrate, enzyme, and inhibitor. In all the cases, the final volume of the reaction media was 2 mL by the addition of Tris-HCl buffer (pH 7.8). The mixture was stirred and the reaction was initiated by the addition of 20 mM $CaCl_2$ solution (20 μL). Fluorescence was continuously recorded at room temperature during 3 minutes.

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