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A New Conformationally Restricted Mimetic of Dipeptide EG – Synthesis of an Analogue of FEG

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Starting from the chiral pyrrolidin-2-one **2**, the carboxy group at C-4 underwent homologation, and subsequent removal of the 1-(4-methoxyphenyl)ethyl group gave lactam **6**. Alkylation of N-1 with benzyl bromoacetate led to **7**, a new conformationally restricted analogue of the dipeptide EG (Glu-Gly). The usefulness of **7** was demonstrated by its eventual conversion into **8**, an orthogonally protected analogue of bioactive tripeptide FEG. In order to provide the biological activity of the new mimetic **9**, available from **8** after the removal of the protecting groups, the conformational preference of **9** was ascertained by a detailed conformational analysis and a comparison with that of FEG (**1**).

Introduction

The incorporation of conformational constraints into biologically active peptides can provide them with very useful chemical and biological properties including a well-defined backbone conformation, specific topographical properties, high potency and selectivity at biological receptors, together with increased stability against enzymatic degradation. Thus, the synthesis of conformationally restricted amino acids and dipeptides and their application to both the general study of peptide behaviour and the preparation of drugs has received widespread attention.[1] In addition, recent developments in the field of protein engineering by site-directed mutagenesis[2] and total chemical synthesis[3] insure an important and continued role for peptide analogues in protein science and in drug discovery.

Within an ongoing research program aimed at preparing conformationally restricted analogues of amino acids or oligopeptides,[4] we hypothesized that mimetics of the salivary gland tripeptide FEG (Phe-Glu-Gly) (**1**)[5,6] and its enantiomer feG (*ent*-**1**),[5,7,8] could be of interest owing to the biological activity of the parent peptides. In fact, FEG was reported to display anti-hypotensive properties against anaphylactic shock,[5] together with a potent inhibition of intestinal anaphylaxis and inhibitory effects on inflammatory reactions.[9] On the other hand, feG has anti-inflammatory properties in allergic airways inflammation in Brown–Norway rats,[10] reduces endotoxin-provoked perturbation of intestinal motility and inflammation[11,12] and regulates leukocyte adhesion to the heart.[13,14]

Thus, at first we devised that compound **2**, having a (3*R*,4*S*) configuration, which was recently synthesized in our laboratory,[15] could be an attractive starting material for the preparation of a conformationally restricted dipeptide EG (Glu-Gly) suitable for the insertion in FEG, in order to change or improve its biological activity. In fact it is well known that insertion of a γ-lactam moiety into a peptide chain leading to severe conformational constrictions can give rise to significant changes in both the potency and biological activity.[16]

In addition, the diastereomer of **2** with the (3*S*,4*R*) configuration is also available,[15] so that the same synthetic route could be directed to obtain a mimetic of tripeptide feG *ent*-**1** which in turn displays interesting biological activity somewhat different from FEG.[10–14]

Results and Discussion

The ester **2**[15] was smoothly converted into the corresponding acid **3** in quantitative yield, which by reaction with methyl chloroformate gave the corresponding mixed anhydride which was directly treated with an ethereal solution of diazomethane to afford the diazo ketone **4** in moderate yield.

The homologation pathway was carried out by using an Arndt–Eistert rearrangement,[17] and the ester **5** was obtained in moderate yield with total retention at C-4, a sole diastereomer being recovered from the reaction mixture.

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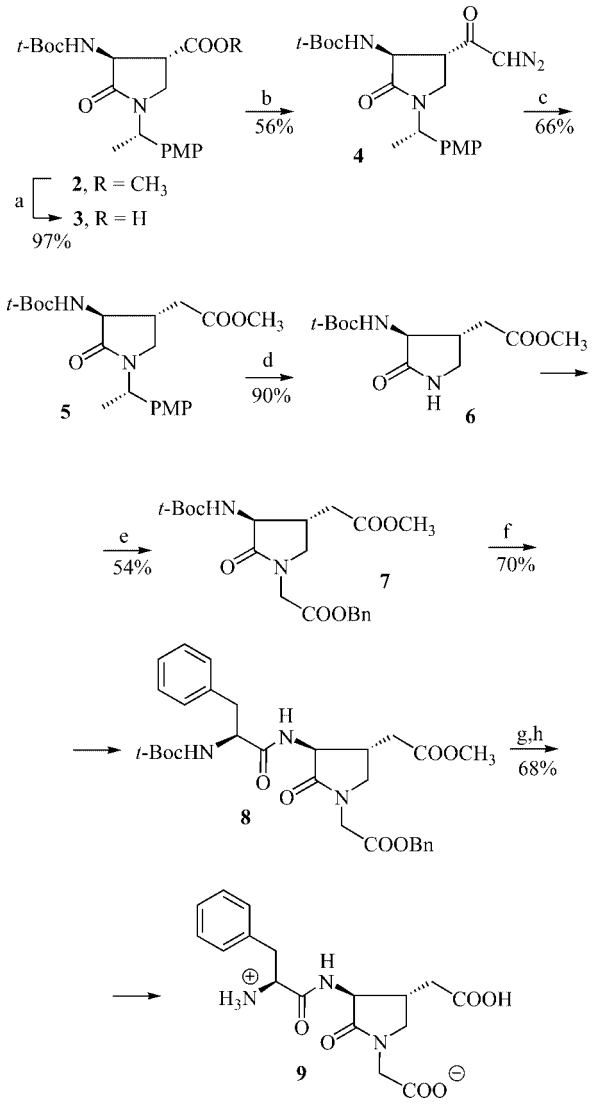
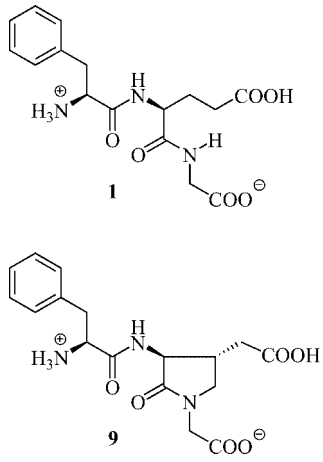
Removal of the chiral 1-(*p*-methoxyphenyl)ethyl group was easily accomplished by a reaction with CAN (cerium ammonium nitrate), to give the corresponding γ-lactam **6** in good yield. Once the required product **6** was in hand, it was alkylated with benzyl bromoacetate in dry THF, using NaH as the base, to give in good yield the chiral pyrrolidin-2-one **7**, a conformationally restricted analogue of dipeptide EG (Glu-Gly) (Scheme 1).



Scheme 1. (a) 1 M NaOH, then 1 M HCl; (b) $ClCOOCH_3$, Et_3N, DCM, 0 °C, then CH_2N_2, –10 °C; (c) PhCOOAg, Et_3N, MeOH, 0 °C; (d) CAN, CH_3CN/H_2O, room temp.; (e) NaH, THF, 0 °C, then $BrCH_2COOBn$, THF, 0 °C to room temp.; (f) TFA, DCM, room temp., then Et_3N, *t*Boc-Phe, EDCl, DCM, room temp.; (g) 2 M NaOH, followed by 1 M HCl; (h) TFA, then Dowex 50, 1 M NH_4OH.

The dipeptide EG is rather ubiquitous in peptide chains and in bioactive synthetic peptides[18] and the mimetic **7** could be useful for substitution in order to improve or change biological activity.

Thus, with the aim to test its usefulness, we prepared **9**, a conformationally restricted analogue of tripeptide FEG (**1**), which could be of interest owing to the biological activity of the parent peptide. At first, the *t*Boc protecting group in compound **7** was carefully removed by using TFA. Then, the corresponding trifluoroacetate salt underwent directly a reaction with Et_3N, followed by condensation with *t*Boc-Phe in the presence of *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDCl), to afford in moderate yield **8**, which was eventually deprotected to give **9**, a constrained analogue of tripeptide FEG (**1**) (Scheme 2).



Scheme 2. Structures of FEG (**1**) and of its constrained analogue **9**.

In order to ascertain the conformational preference of the fully deprotected analogue **9** with respect to FEG (**1**, Scheme 2), and to correlate the biological activity with the conformational behaviour, a detailed conformational analysis was performed. The MC search protocol was used together with the AMBER* force field[19] taking into account the dipolar interaction of the charged groups simulating the aqueous polar environment (GB/SA H_2O model).[20,21] In addition, a full exploration of the conformational potential energy surface (PES) of FEG (**1**) was carried out using the same protocol in order to locate all the possible stable orientations of the functional groups. In fact, the conformational behaviour of **7** was previously studied, and the most probable bioactive conformation for this peptide displays a strong interaction occurring between the glutamyl carboxyl group and the N-terminus of the peptide, together with an interaction between the aromatic ring and the terminal carboxyl group.[6,8]

Our results are in total agreement with these findings and prompted us to compare the conformational preferences of the two compounds identifying the most probable bioactive conformation of **1** having the following ϕ and ψ values (conformer number 1 at lowest energy): $\phi_1 = -174.0°$, $\psi_1 = 128.8°$; $\phi_2 = -137.3°$, $\psi_2 = 126.8°$; $\phi_3 = -179.7°$, $\psi_3 = -0.40°$.

Thus, from the cluster analysis of FEG conformers, we could divide the structures into two clusters by varying the central ϕ_2 and ψ_2 values (cluster value 0.4) although we noticed a very high flexibility of the lateral chains, in particular for the phenyl group of Phe (Figure 1).

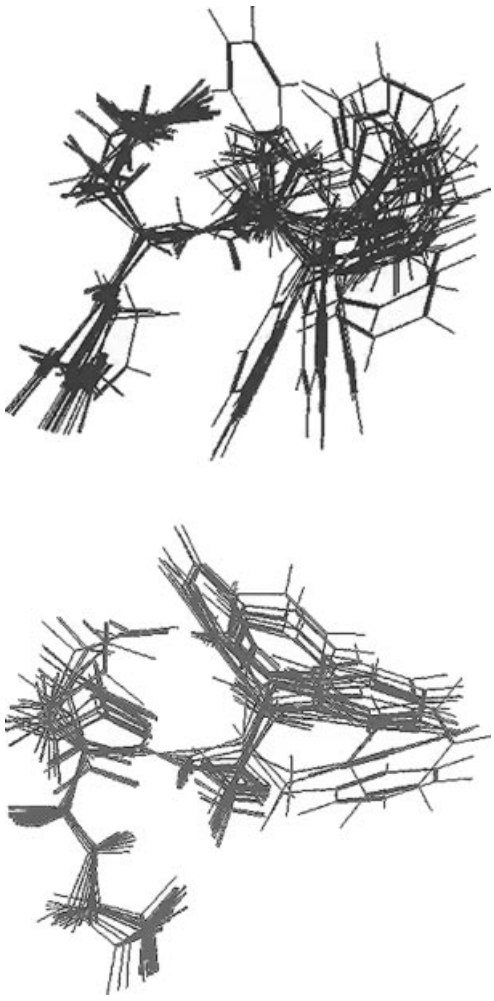


Figure 1. Cluster analysis of FEG (**1**). Cluster no. 1 (top) and no. 2 (bottom), with a cluster value of 0.4 for superimposing ϕ_2 and ψ_2.

On the contrary, a restriction of the conformational freedom was observed for the constrained mimetic **9**, leading to a smaller number of stable conformations (66 for **9** versus 155 for **1** within 6.0 kcal/mol). However, in all these conformations the required strong interaction between the N-terminal NH_3^+ group and the glutamyl carboxy group takes place, whereas the two remaining groups (Phe and the terminal carboxyl group) are instead kept at a distance owing to the constrained pyrrolidin-2-one ring mimicking Glu. In addition, from the cluster analysis we can divide all the conformations into two main clusters, which differ in the ϕ_2 and ψ_2 values (cluster value 0.2, cluster no. 1: $\phi_2 = -175.6°$, $\psi_2 = -138°$; cluster no. 2: $\phi_2 = -112.7°$, $\psi_2 = -137.9°$, Figure 2).

Particularly relevant is cluster no. 2 since by superimposing the representative structure of this cluster with the FEG lowest-energy conformer we could observe a high correspondence (Figure 3) since the N-terminus interacts with the carboxyl group of Glu through a hydrogen bond, whereas the phenyl ring interacts with the C=O group of the constrained mimetic ring instead of the terminal carboxy group.

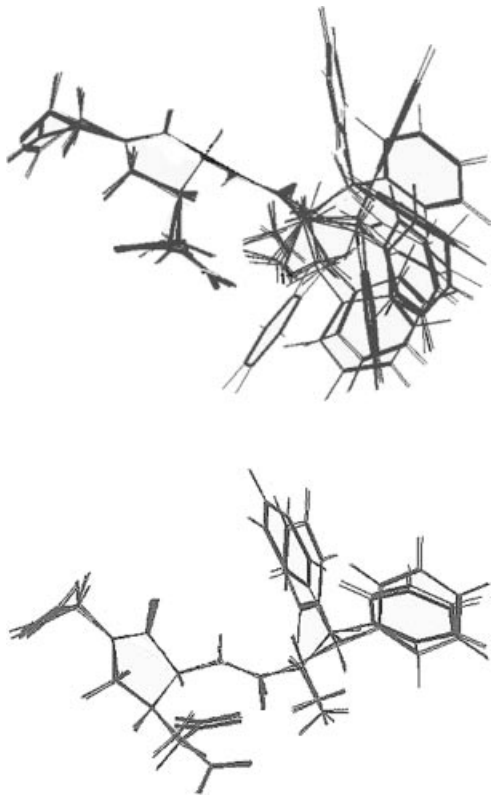


Figure 2. Cluster analysis of mimetic **9**. Cluster no. 1 (top) and no. 2 (bottom), with a cluster value of 0.2 for superimposing ϕ_2 and ψ_2.

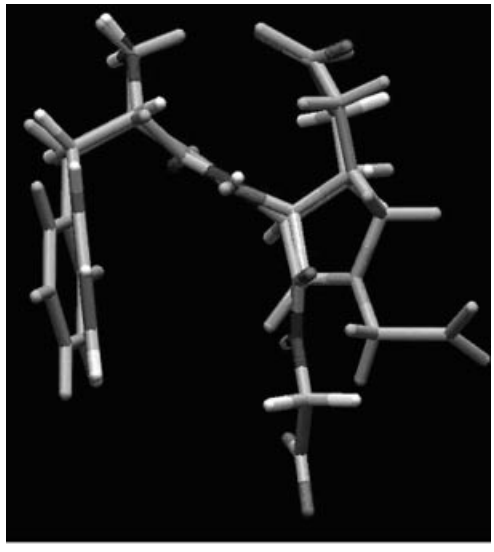


Figure 3. Superimposition of the FEG lowest-energy conformer and the most representative structure in cluster no. 2 of analogue **9**.

At present the role of each group in displaying the biological activity has not been well ascertained but it is only deduced from conformational analogies between FEG and some of its active (feG) and non-active analogues (weG).[5,6] Thus, the ability of compound **9** to inhibit rat intestinal

anaphylaxis was evaluated in comparison with that of FEG (**1**), according to the literature methods (Table 1).[5,6]

Table 1. Inhibition of intestinal anaphylaxis by FEG (**1**) and mimetic **9**.

Entry	Compound	A/BC[a,b]	*N*[c]
1	control	0.29 ± 0.06	4
2	FEG (**1**)	0.13 ± 0.02	4
3	**9**	0.22 ± 0.05	4

[a] Data are the mean ± S.D. for six independent experiments. [b] Albumin (A) induced contractile response relative to bethanechol chloride (BC) induced contractile response. [c] Number of rats.

A significant decrease in activity was observed for **9** compared to that of **1**, and this result suggests that the orientation of the functionalities observed in FEG by using MD simulations plays an important role in the bioactivity of the natural product, so that an effective mimetic of **1** must preserve both structural and conformational features of the parent tripeptide.

Conclusions

Starting from the chiral pyrrolidin-2-one **2**, the constrained analogue of EG (Glu-Gly) (**7**), was obtained, which was eventually converted into compound **9**, a mimetic of bioactive tripeptide FEG. The biological activity of **9** was significantly lower than that of the natural tripeptide owing to a different conformational behaviour. Thus, we are currently focusing on novel analogues of both FEG and its enantiomer, feG, with the aim to improve the therapeutic potential, and the results will be reported in due course.

Experimental Section

Methods: Melting points were measured with an Electrothermal IA 9000 apparatus and are uncorrected. IR spectra were recorded in $CHCl_3$ with a Nicolet Fourier Transform Infrared 20-SX spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 200 MHz and 50 MHz, respectively, with a Varian Gemini 200 spectrometer, using $CDCl_3$ as a solvent unless otherwise stated. Chemical shifts (δ) are reported in ppm relative to TMS, and coupling constants (J) are reported in Hz. Assignments were aided by decoupling and homonuclear two-dimensional experiments. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. The samples were analyzed with a liquid chromatography Agilent Technologies HP1100 equipped with a Zorbax Eclipse XDB-C8 Agilent Technologies column (flow rate 0.5 mL/min) and equipped with a diode-array UV detector (220 nm and 254 nm). Acetonitrile and methanol for HPLC were purchased from a commercial supplier. All the samples were prepared by diluting 1 mg in H_2O/acetonitrile (1:1, 5 mL) in pure acetonitrile or in pure methanol. The MSD1100 mass detector was utilized under the following conditions: mass range 100–2500 uma, positive scanning, energy of fragmentor 50 V, drying gas flow (nitrogen) 10.0 mL/min, nebulizer pressure 45 psig, drying gas temperature 350 °C, capillary voltage 4500 V. Column chromatography was performed with silica gel 60 (230–400 mesh). Compound **1** was synthesized according to ref.[15]

(3*R*,4*S*)-{4-(*tert*-Butoxycarbonylamino)-1-[(1*S*)-1-(4-methoxyphenyl)ethyl]-5-oxopyrrolidin-3-yl}carboxylic Acid (3): The ester **2**[15] (1.57 g; 4.0 mmol) was dissolved in methanol (5 mL) and then aqueous NaOH (1 M, 1.5 equiv, 6.0 mL) was added. After stirring the mixture at room temp. for 4 h, it was extracted with AcOEt (2 × 20 mL), the aqueous phase cooled to 0 °C, and 1 M HCl was added until the pH = 2. After extraction with AcOEt (2 × 20 mL), the solution was dried and the solvent eventually removed under reduced pressure, to give the acid **3** (1.47 g, 97% yield) as a white solid. M.p. 72–74 °C. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*Bu), 1.54 (d, J = 7.0 Hz, 3 H, CHCH_3), 3.09–3.35 (m, 2 H, 3-C*H* + 2-C*H*-pro-*R*), 3.41–3.51 (m, 1 H, 2-C*H*-pro-*S*), 3.80 (s, 3 H, OCH_3), 4.52 (dd, J = 6.1, J = 8.4 Hz, 1 H, 1 H, 4-C*H*), 5.46 (q, J = 7.0 Hz, 1 H, C*H*CH$_3$), 5.79 (d, J = 6.1 Hz, 1 H, NH), 6.88 (d, J = 8.6 Hz, 2 ArH), 7.25 (d, J = 8.6 Hz, 2 ArH), 11.54 (br. s, 1 H, COOH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.1, 28.1, 42.1, 43.9, 46.4, 50.2, 50.3, 55.3, 81.6, 114.1, 128.3, 130.1, 155.1, 159.3, 163.9, 174.6 ppm. $[\alpha]_D^{20}$ = –140.0 (c = 0.5, $CHCl_3$). MS-ESI: m/z = 379.4 [M + H]$^+$, 401.4 [M + Na]$^+$. $C_{19}H_{26}N_2O_6$ (378.42): calcd. C 60.30, H 6.93, N 7.40; found C 60.25, H 6.89, N 7.44.

(3*R*,4*S*)-{4-(*tert*-Butoxycarbonylamino)-1-[(1*S*)-1-(4-methoxyphenyl)ethyl]-5-oxopyrrolidin-3-yl} Diazomethyl Ketone (4): Methyl chloroformate (0.49 mL, 3.0 mmol) and Et_3N (0.28 mL, 3.0 mmol) were added at 0 °C to a solution of **3** (0.76 g, 2.0 mmol) in dry THF (15 mL). The reaction mixture was stirred at 0 °C for 40 min and then at room temp. for 30 min. The reaction mixture was cooled again to 0 °C, and an ethereal solution of CH_2N_2 (0.2 M, 20 mL) was added. After 14 h, excess CH_2N_2 was carefully destroyed by the dropwise addition of TFA/AcOEt (50:50) until N_2 evolution had ceased. The solvents were removed under reduced pressure, and the residue was purified by silica gel chromatography (cyclohexane/AcOEt, 50:50) to give the title product **4** (0.45 g, 56% yield) as a yellow oil. FTIR ($CHCl_3$): $\tilde{\nu}$ = 2207, 1667, 1650 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, *t*Bu), 1.49 (d, J = 7.1 Hz, 3 H, CHCH_3), 2.96–3.12 (m, 2 H, 3-C*H* and 2-C*H*-pro-*R*), 3.36–3.51 (m, 1 H, 2-C*H*-pro-*S*), 3.77 (s, 3 H, OCH_3), 4.36 (dd, J = 6.6, J = 8.7 Hz, 1 H, 4-C*H*), 5.32 (d, J = 6.6 Hz, 1 H, N*H*), 5.41 (q, J = 7.1 Hz, 1 H, C*H*CH$_3$), 5.55 (br. s, 1 H, C*H*N$_2$), 6.84 (d, J = 8.7 Hz, 2 ArH), 7.22 (d, J = 8.7 Hz, 2 ArH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.2, 28.2, 41.3, 49.2, 49.4, 55.3, 55.7, 56.4, 80.4, 114.0, 128.3, 131.2, 155.6, 159.1, 169.3, 192.1 ppm. $[\alpha]_D^{20}$ = –89.0 (c = 0.8, $CHCl_3$). MS-ESI: m/z = 403.4 [M + H]$^+$, 425.3 [M + Na]$^+$. $C_{20}H_{26}N_4O_5$ (402.45): calcd. C 59.69, H 6.51, N 13.92; found C 59.63, H 6.44, N 13.98.

Methyl (3*R*,4*S*)-{4-(*tert*-butoxycarbonylamino)-1-[(1*S*)-1-(4-methoxyphenyl)ethyl]-5-oxopyrrolidin-3-yl}acetate (5): The diazo ketone **4** (0.4 g, 1.0 mmol) was dissolved in a mixture of dry THF (20 mL) and MeOH (20 mL) and at 0 °C a solution of silver benzoate (30 mg, 0.1 mmol) in Et_3N (0.5 mL) was slowly added, and after 0.5 h the mixture was stirred at room temp. for 3 h. Water (10 mL) was then added, and the mixture was extracted with AcOEt (2 × 50 mL). After drying (Na_2SO_4) of the organic layer and removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography (cyclohexane/AcOEt, 4:6) to give the product **5** (0.27 g, 66% yield) as a colourless viscous oil. FTIR ($CHCl_3$): $\tilde{\nu}$ = 3345, 1742, 1685, 1665 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*Bu), 1.48 (d, J = 7.1 Hz, 3 H, CHCH_3), 2.25–2.52 (m, 2 H, CH_2COOMe), 2.90–2.98 (m, 1 H, 3-C*H*), 2.93 (dd, J = 9.2, J = 9.8 Hz, 1 H, 2-C*H*-pro-*R*), 3.21 (dd, J = 7.9, J = 9.8 Hz, 1 H, 2-C*H*-pro-*S*), 3.63 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 5.08 (dd, J = 7.0, J = 9.9 Hz, 1 H, 4-C*H*), 5.06 (d, J = 7.0 Hz, 1 H, NH), 5.41 (q, J = 7.1 Hz, 1 H, C*H*CH$_3$), 6.85 (d, J = 8.8 Hz, 2 ArH), 7.19 (d, J = 8.8 Hz, 2 ArH) ppm. ^{13}C NMR

(50 MHz, $CDCl_3$): δ = 16.2, 28.1, 35.9, 38.3, 44.2, 48.9, 51.5, 55.0, 57.3, 79.7, 113.8, 127.9, 131.3, 156.0, 158.8, 170.6, 172.0 ppm. $[\alpha]_D^{20}$ = –101.8 (*c* = 1.83, $CHCl_3$). MS-ESI: *m*/*z* = 407.4 $[M + H]^+$, 430.2 $[M + Na]^+$. $C_{21}H_{30}N_2O_6$ (406.47): calcd. C 62.05, H 7.44, N 6.89; found C 61.98, H 7.38, N 6.85.

Methyl (3*R*,4*S*)-[4-(*tert*-Butoxycarbonylamino)-5-oxopyrrolidin-3-yl]acetate (6): A solution of **5** (0.41 g, 1.0 mmol) in CH_3CN (5 mL) was treated at room temperature with CAN (1.1 g, 2.0 mmol) dissolved in H_2O (5 mL), and the reaction mixture was stirred for 1 h. The aqueous layer was extracted with AcOEt (3 × 25 mL), the organic layers were combined, washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a crude residue, which was purified by silica gel chromatography (cyclohexane/AcOEt, 3:7) to give **6** (0.25 g, 90% yield) as a low-melting solid. M.p. 36–38 °C. FTIR ($CHCl_3$): $\tilde{\nu}$ = 3350, 1743, 1684, 1665 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.44 (s, 9 H, *t*Bu), 2.48 (dd, *J* = 9.2, *J* = 15.8 Hz, 1 H, C*H*$_2$COOMe), 2.58–2.73 (m, 1 H, C*H*$_2$COOMe), 2.75–2.88 (m, 1 H, 3-C*H*), 3.04 (dd, *J* = 9.1, *J* = 9.5 Hz, 1 H, 2-C*H*-pro-*R*), 3.54–3.63 (m, 1 H, 2-C*H*-pro-*S*), 3.68 (s, 3 H, OC*H*$_3$), 4.02 (dd, *J* = 6.7, *J* = 9.9 Hz, 1 H, 4-C*H*), 5.04 (d, *J* = 6.7 Hz, 1 H, NH), 6.35 (br. s, 1 H, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.2, 35.9, 39.7, 44.7, 51.7, 56.3, 80.1, 156.3, 172.1, 172.2 ppm. $[\alpha]_D^{20}$ = –23.7 (*c* = 1.0, $CHCl_3$). MS-ESI: *m*/*z* = 273.2 $[M + H]^+$, 296.2 $[M + Na]^+$. $C_{12}H_{20}N_2O_5$ (272.30): calcd. C 52.93, H 7.40, N 10.29; found C 52.87, H 7.36, N 10.33.

Methyl (3*R*,4*S*)-[1-(Benzyloxycarbonylmethyl)-3-(*tert*-butoxycarbonylamino)-5-oxopyrrolidin-3-yl]acetate (7): To a solution containing **6** (0.27 g, 1.0 mmol) in dry THF (5 mL) under argon, NaH (50 mg of a 50% dispersion in oil, 1.01 mmol) was added, and the solution was stirred at 0 °C for 50 min. Benzyl bromoacetate (0.17 mL, 1.01 mmol) dissolved in dry THF (3 mL) was then added at 0 °C, and the reaction mixture was subsequently stirred at room temp. for 4 h. Water (5 mL) and AcOEt (40 mL) were added, and the mixture was extracted with AcOEt (2 × 50 mL). After drying (Na_2SO_4) of the organic layer and removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography (cyclohexane/AcOEt, 3:7) to give **7** (0.23 g, 54% yield) as a colourless oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*Bu), 2.47 (dd, *J* = 9.8, *J* = 15.8 Hz, 1 H, C*H*$_2$COOMe), 2.56–2.67 (m, 1 H, C*H*$_2$COOMe), 2.91–3.01 (m, 1 H, 3-C*H*), 3.21 (dd, *J* = 9.2, *J* = 9.4 Hz, 1 H, 2-C*H*-pro-*R*), 3.58 (dd, *J* = 7.9, *J* = 9.4 Hz, 1 H, 2-C*H*-pro-*S*), 3.66 (s, 3 H, OC*H*$_3$), 4.08 (dd, *J* = 6.7, *J* = 9.9 Hz, 1 H, 1 H, 4-C*H*), 4.10 (ABq, *J* = 17.6 Hz, 2 H, C*H*$_2$COOBn), 5.08 (d, *J* = 6.7 Hz, 1 H, NH) 5.14 (s, 2 H, COOC*H*$_2$Ph), 7.34 (m, 5 ArH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.2, 36.1, 38.5, 44.5, 49.8, 51.7, 56.6, 67.2, 80.1, 128.3, 128.5, 128.6, 135.0, 168.0, 172.1 ppm. $[\alpha]_D^{20}$ = –14.4 (*c* = 0.9, $CHCl_3$). MS-ESI: *m*/*z* = 421.4 $[M + H]^+$, 444.1 $[M + Na]^+$. $C_{21}H_{28}N_2O_7$ (420.46): calcd. C 59.99, H 6.71, N 6.66; found C 59.92, H 6.65, N 6.71.

Methyl (3*R*,4*S*)-{1-(Benzyloxycarbonylmethyl)-4-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanylamino]-5-oxopyrrolidin-3-yl}acetate (8): To a solution of **7** (210 mg, 0.5 mmol) in DCM (7 mL), TFA (0.6 mL) was added, and the clear solution was stirred at room temp. for 2 h. The volatiles were then removed under reduced pressure, and the residue was washed twice with diethyl ether. The raw trifluoroacetate salt obtained in a quantitative yield (217 mg, 0.5 mmol) was dissolved in DCM (10 mL) and then *t*Boc-L-Phe (131 mg, 0.5 mmol), TEA (70 μL, 0.6 mmol) and EDCl (113 mg, 0.6 mmol) were subsequently added, and the solution was stirred at room temp. for 12 h. Water (5 mL) was added, and the mixture was extracted with DCM (3 × 10 mL). After drying (Na_2SO_4) of the organic layer and removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography (AcOEt), to give **8** (199 mg, 70% yield) as a colourless viscous oil. FTIR ($CHCl_3$): $\tilde{\nu}$ = 3344, 1741, 1687, 1668 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.38 (s, 9 H, *t*Bu), 2.47 (dd, *J* = 9.7, *J* = 15.5 Hz, 1 H, C*H*$_2$COOMe), 2.56–2.66 (m, 1 H, C*H*$_2$COOMe), 2.83–3.16 (m, 3 H, 3-C*H* + L-Phe β-C*H*$_2$), 3.24 (dd, *J* = 8.9, *J* = 8.9 Hz, 1 H, 2-C*H*-pro-*R*), 3.61 (dd, *J* = 8.1, *J* = 8.9 Hz, 1 H, 2-C*H*-pro-*S*), 3.66 (s, 3 H, OC*H*$_3$), 4.16–4.27 (m, 1 H, L-Phe *α*-C*H*), 4.21 (ABq, *J* = 17.7 Hz, 2 H, C*H*$_2$COOBn), 4.37 (dd, *J* = 5.9, *J* = 7.1 Hz, 1 H, 4-C*H*), 5.03 (d, *J* = 6.9 Hz, 1 H, NH), 5.15 (s, 2 H, COOC*H*$_2$Ph), 6.57 (d, *J* = 5.9 Hz, 1 H, NH), 7.15–7.35 (m, 10 ArH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.2, 36.0, 37.7, 38.4, 44.5, 50.1, 51.7, 55.4, 55.5, 55.7, 67.2, 80.1, 80.2, 126.9, 128.4, 128.6, 128.7, 129.4, 135.1, 136.5, 155.2, 155.3, 168.0, 171.4, 172.0, 172.3 ppm. $[\alpha]_D^{20}$ = –21.5 (*c* = 0.1, $CHCl_3$). MS-ESI: *m*/*z* = 568.2 $[M + H]^+$, 590.2 $[M + Na]^+$. $C_{30}H_{37}N_3O_8$ (567.26): calcd. C 63.48, H 6.57, N 7.40; found C 63.55, H 6.51, N 7.35.

(3*R*,4*S*)-[1-(Carboxymethyl)-4-(L-phenylalanylamino)-5-oxopyrrolidin-3-yl]acetic Acid (9): Compound **8** (0.57 g, 1.0 mmol) dissolved in MeOH (1 mL) was added to NaOH (2 M, 3 mL), and the mixture was stirred at 0 °C for 4 h. The clear solution was extracted with AcOEt (2 × 10 mL), HCl (1 M, 3 mL) was added, and the mixture was extracted with AcOEt (3 × 10 mL). After drying (Na_2SO_4) of the organic layer and removal of the solvent, the residue was treated with TFA (2 mL), and the solution was stirred at room temp. for 24 h. Removal of the volatiles under reduced pressure gave an oil, which was dissolved in H_2O and subjected to an ion-exchange column (Dowex 50, elution with 1 M NH_4OH) to give the title product **9** as a white solid (0.24 g; 66% yield). M.p. 200–204 °C (dec). ^1H NMR (200 MHz, D_2O): δ = 2.47 (dd, *J* = 9.7, *J* = 15.5 Hz, 1 H, C*H*$_2$COOH), 2.56–2.66 (m, 1 H, C*H*$_2$COOH), 2.83–3.16 (m, 3 H, 3-C*H* + L-Phe β-C*H*$_2$), 3.24 (dd, *J* = 8.9, *J* = 8.9 Hz, 1 H, 2-C*H*-pro-*R*), 3.61 (dd, *J* = 8.1, *J* = 8.9 Hz, 1 H, 2-C*H*-pro-*S*), 4.16–4.27 (m, 1 H, L-Phe α-C*H*), 4.18 (ABq, *J* = 17.4 Hz, 2 H, NC*H*$_2$COOH), 4.41 (d, *J* = 5.7 Hz, 1 H, 4-C*H*), 4.97 (br. s, 5 H, N*H* and COO*H*), 7.24–7.49 (m, 5 ArH) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 28.2, 36.0, 37.7, 38.4, 44.5, 50.1, 51.7, 55.4, 55.5, 55.7, 67.2, 126.9, 128.4, 128.6, 128.7, 129.4, 135.1, 136.5, 155.2, 155.3, 166.2, 169.3, 171.0, 171.4 ppm. $[\alpha]_D^{20}$ = –14.6 (*c* = 0.2, H_2O). MS-ESI: *m*/*z* = 364.1 $[M + H]^+$, 386.2 $[M + Na]^+$. $C_{17}H_{21}N_3O_6$ (363.14): calcd. C 56.19, H 5.83, N 11.56; found C 56.13, H 5.89, N 11.49.

Computational Methods: All calculations were carried out with SGI Octane2 IRIX 6.5 workstations. Molecular mechanics calculations were performed using the implementation of the AMBER force field (AMBER*)[19] within the framework of Macromodel version 5.5.[19b] The torsional space of each molecule was randomly varied with the usage-directed Monte Carlo conformational search.[19c] For each search, at least 1000 starting structures for each variable torsion angle was generated and minimized until the gradient was less than 0.05 kJ/Å mol. Duplicate conformations and those with an energy in excess of 6.0 kcal/mol above the global minimum were discarded. The solvent effect was included by using the implicit water GB/SA solvation method,[20] to take into account polar solvent effects. The cluster analysis was performed within the Macromodel package using Xcluster.[21,22]

In Vitro Experiments: Male Sprague–Dawley (Harlan, Italy) rats weighing 170–180 g were sensitized to albumin from chicken egg white (A, 1 mg) and to pertussis toxin (50 ng, Sigma). Six to eight weeks after sensitisation, 1.5 cm sections were obtained from the terminal ileum and were mounted in 10 mL of organ baths containing Krebs–Henseleit buffer solution (118.9 mM NaCl, 4.6 mM KCl,

1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 10.1 mM glucose) under 1.0 g of tension. The contractions generated by albumin and [(*RS*)-2-carbamoyloxypropyl]-trimethylammonium chloride (bethanechol chloride, BC) were measured isometrically (Basile Mod. 7080, Italy) and recorded with a two-channel recorder (Basile Mod. 7070, Italy). The tissues were washed several times in Krebs–Henseleit solution and allowed to equilibrate for 15 min. Anti-anaphylactic properties of FEG (**1**) and its analogue **9** were determined by adding 10 μg of product to a bath and incubating for 10 min. After washing, the segments were treated with 1 mg of the A antigen and the corresponding contractile response was measured at peak contraction. The segments were washed again and peak contractile response was obtained by adding 10^{-5} M BC. Eventually, the mass of the muscle was measured, the tension was calculated in gram force per gram wet tissue, and the results are given by the ratio of A-induced contractile response relative to BC-induced contractile response (Table 1).

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[1] a) S. Hanessian, G. McNaughton-Smith, H. G. Lombart, W. D. Lubell, *Tetrahedron* **1997**, *53*, 12789–12854; b) S. Kotha, *Acc. Chem. Res.* **2003**, *36*, 342–351; c) "Synthesis of Peptides and Peptidomimetics", in *Houben-Weyl, Methods in Organic Chemistry* (Eds.: M. Goodman, A. Felix, L. Moroder, C. Toniolo), Thieme, Stuttgart, New York, **2003**, vol. E22c.

[2] a) J. A. Brannigan, A. J. Wilkinson, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 964–970; b) C. H. Collins, Y. Yokobayashi, D. Umeno, F. H. Arnold, *Curr. Opin. Biotechnol.* **2003**, *14*, 371–378; c) S. Lutz, W. M. Patrick, *Curr. Opin. Biotechnol.* **2004**, *15*, 291–297; d) Y. Lu, *Curr. Opin. Chem. Biol.* **2005**, *9*, 118–126.

[3] a) A. Loffet, *J. Pept. Sci.* **2002**, *8*, 1–7; b) V. J. Hruby, *Nat. Rev. Drug Discovery* **2002**, *1*, 847–858; c) M. G. Bursavich, D. H. Rich, *J. Med. Chem.* **2002**, *45*, 541–558.

[4] For recent papers, see: a) R. Galeazzi, G. Martelli, G. Mobbili, M. Orena, S. Rinaldi, *Tetrahedron: Asymmetry* **2003**, *14*, 3353–3358; b) R. Galeazzi, G. Martelli, G. Mobbili, M. Orena, S. Rinaldi, *Tetrahedron: Asymmetry* **2004**, *15*, 3249–3256; c) R. Galeazzi, G. Martelli, D. Natali, M. Orena, S. Rinaldi, *Tetrahedron: Asymmetry* **2005**, *16*, 1779–1787.

[5] R. Mathison, P. Lo, G. Moore, B. Scott, J. S. Davison, *Peptides* **1998**, *19*, 1037–1042.

[6] E. Metwally, J. M. Pires, G. J. Moore, D. A. Befus, J. S. Davison, R. Mathison, *Peptides* **2002**, *23*, 193–199.

[7] R. D. Mathison, J. S. Davison, E. Metwally, *Peptides* **2003**, *21*, 1221–1230.

[8] E. Metwally, H. A. Ismail, J. S. Davison, R. Mathison, *Biophys. J.* **2003**, *85*, 1503–1511.

[9] F. Turesin, A. Sadr, J. S. Davison, R. Mathison, *BMC Physiol.* **2002**, *2*, 13 (http://www.biomedcentral.com/1472–6793/2/13).

[10] R. E. Dery, M. Ulanova, L. Puttagunta, G. R. Stenton, D. James, S. Merani, R. Mathison, J. Davison, A. D. Befus, *Eur. J. Immunol.* **2004**, *34*, 3315–3325.

[11] R. D. Mathison, J. S. Davison, A. D. Befus, *Proc. West. Pharmacol. Soc.* **2001**, *44*, 157–158.

[12] R. Mathison, P. Lo, D. Tan, B. Scott, J. S. Davison, *Neurogastroenterol. Motil.* **2001**, *13*, 599–603.

[13] a) R. Mathison, R. Woodman, J. S. Davison, *Can. J. Physiol. Pharmacol.* **2001**, *79*, 785–792; b) F. Bao, S. M. John, Y. Chen, R. D. Mathison, L. C. Weaver, *Neuroscience* **2006**, *14*, 1011–1022.

[14] a) R. Mathison, A. D. Befus, J. S. Davison, R. C. Woodman, *BMC Immunol.* **2003**, *4*, 3 (http://www.biomedcentral.com/1471–2172/4/3); b) R. Mathison, *Curr. Immunol. Rev.* **2006**, *2*, 247–259; c) R. D. Mathison, J. S. Davison, *J. Inflammation* **2006**, *3*, 9 (http://www.journal-inflammation.com/content/3/1/9).

[15] R. Galeazzi, G. Martelli, M. Orena, S. Rinaldi, P. Sabatino, *Tetrahedron* **2005**, *61*, 5465–5473.

[16] a) J. Aubé, "Synthetic routes to lactam peptidomimetics", in *Advances in amino acid mimetics and peptidomimetics* (Ed.: A. Abell), JAI Press, Inc., Greenwich, CT, **1997**, vol. 1, pp. 193–232; b) R. M. Freidinger, *J. Med. Chem.* **2003**, *46*, 5553–5566; c) K. Dolbeare, G. F. Pontoriero, S. K. Gupta, R. K. Mishra, R. L. Johnson, *J. Med. Chem.* **2003**, *46*, 727–733; d) J. Podlech, D. Seebach, *Liebigs Ann.* **1995**, 1217–1228.

[17] See, for example: a) A. Gaucher, L. Dutot, O. Barbeau, W. Hamchaoui, M. Wakselman, J. P. Mazaleyrat, *Tetrahedron: Asymmetry* **2005**, *16*, 857–864; b) J. Spengler, J. Ruiz-Rodriguez, K. Burger, F. Albericio, *Tetrahedron Lett.* **2006**, *47*, 4557–4560.

[18] See, for relevant examples: a) H. T. Le, I. B. Lemaire, A. K. Gilbert, F. Jolicoeur, S. Lemaire, *J. Med. Chem.* **2003**, *46*, 3094–3101; b) H. R. Lijnen, B. Van Hoef, D. Collen, *Eur. J. Biochem.* **1987**, *162*, 351–356; c) D. Wernicke, C. Thiel, C. M. Duja-Isac, K. V. Essin, M. Spindler, D. J. R. Nunez, R. Plehm, N. Wessel, A. Hammes, R.-J. Edwards, A. Lippoldt, U. Zacharias, H. Strömer, S. Neubauer, M. J. Davies, I. Morano, L. Thierfelder, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.* **2004**, *287*, R685–R695.

[19] a) S. J. Weiner, P. A. Kollman, D. T. Nguyen, D. A. Case, *J. Comput. Chem.* **1986**, *7*, 230–252; b) F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467; c) J. M. Goodman, W. C. Still, *J. Comput. Chem.* **1991**, *12*, 1110–1117.

[20] W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.

[21] J. V. Ryzin, *Classification and clustering*, Academic Press, New York, **1977**.

[22] J. Zupan, *Algorithms for Chemists*, Wiley-VCH, New York, **1989**, chapter 7.

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