Synthesis and biological evaluation of glutathione-like tripeptides against *Trypanosoma cruzi*

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Dedicated to Professor Edmundo A. Rúveda on his 70th birthday and to Professor Roberto A. Rossi on his 60th birthday

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Abstract

Glutathionylspermidine synthase (GSS) is a crucial enzyme in the trypanothione biosynthetic pathway and is also an interesting molecular target for drug design. We synthesized a series of amides of a glutathione analogue (L-γ-Glu-L-Leu-Gly-NHR) where R are linear alkyl groups. All of these drugs exhibited marginal biological activity against the responsible agent of Chagas' disease, the protozoan *Trypanosoma cruzi*.

Keywords: *Trypanosoma cruzi*, American trypanosomiasis, glutathionylspermidine synthase, trypanothione

Introduction

There is considerable interest in developing novel chemotherapeutic approaches against American trypanosomiasis (Chagas' disease) based on unique features of the structure and metabolism of its etiological agent, the hemoflagellated protozoan *Trypanosoma cruzi*.[1–8] Chemotherapy for the treatment of Chagas' disease is still deficient,[9] because it is only based on two drugs empirically discovered, nifurtimox, now discontinued, and benznidazole. The studies of unique aspects of the biochemistry and physiology of *T. cruzi* have led to the recognition of specific molecular targets for drug design, among them, trypanothione biosynthesis arises as a specially attractive target.[7,10–13] It has been estimated that close to 18 million individuals are infected with *T. cruzi*.[14] In rural areas this disease is transmitted by reduviid bugs such as

Rhodnius prolixus and *Triatoma infestans* as a consequence of their blood-sucking activity.[15] *T. cruzi* has a complex life cycle possessing three main morphological forms: the dividing non-infective epimastigotes, the non-dividing highly infective trypomastigotes, and the intracellular amastigotes, the clinically more relevant form of the parasite.[16] Although nifurtimox and benznidazole are able to cure at least 50% of recent infections, they have important drawbacks such as selective drug sensitivity on different *T. cruzi* strains, serious side effects,[17] and long-term treatment.[18] In addition, gentian violet, the only drug available to prevent blood transmission of Chagas' disease, is carcinogenic in animals.[19]

All trypanosomatids have a particular thiol metabolism based on trypanothione (compound **1**, N^1, N^8-(bisglutathionyl)spermidine, $T[SH]_2$) and the NADPH-dependant flavoenzyme trypanothione reductase (TR),[20] which has their corresponding counterparts in mammals, the tripeptide glutathione (compound **2**, GSH) and glutathione reductase (GR) (Figure 1). GSH and GR are widespread in mammals and are responsible for controlling the cellular redox equilibrium and the oxidative stress as $T[SH]_2$ and TR do in trypanosomatids.[21] Trypanothione occurs exclusively in parasitic protozoa of the order Kinetoplastida.[21] The specific enzymes that catalyze the two ultimate steps of trypanothione biosynthesis are glutathionylspermidine synthase (GSS) and trypanothione synthase (TS) to form glutathionylspermidine (compound **3**) and trypanothione, respectively.[22] Bearing in mind that this mechanism of cell protection is present in several pathogenic trypanosomes such as *T. cruzi, T. brucei rhodesiense*, and *T. b. gambiense*, and *Leishmania spp.* this approach can be useful for the treatment of different trypanosomiasis and leishmaniasis. As trypanothione is a crucial metabolite for parasite survival, the general strategy to control parasite multiplication is the inhibition of trypanothione biosynthesis, specifically, at TS or GSS. Since these two enzymes are absent in the host, it is possible to predict the highly selective inhibitors of their enzymatic activity will have no effect against the mammalian cells. The mechanism of defense of trypanosomes is illustrated in Scheme 1.

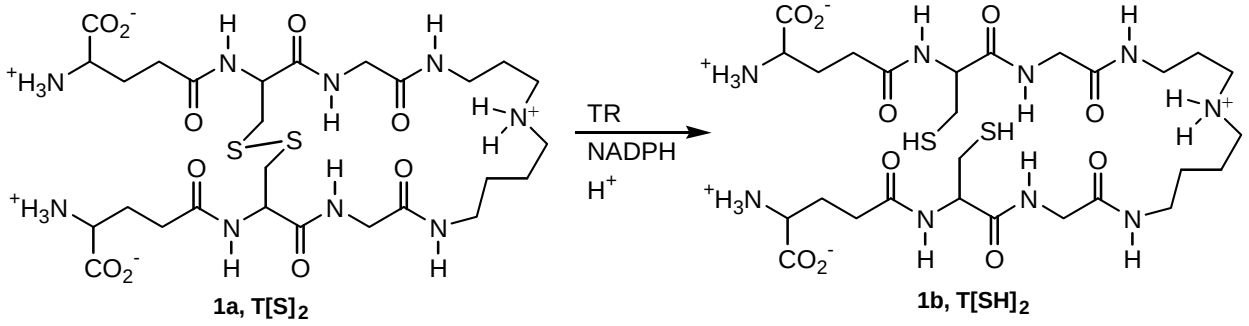
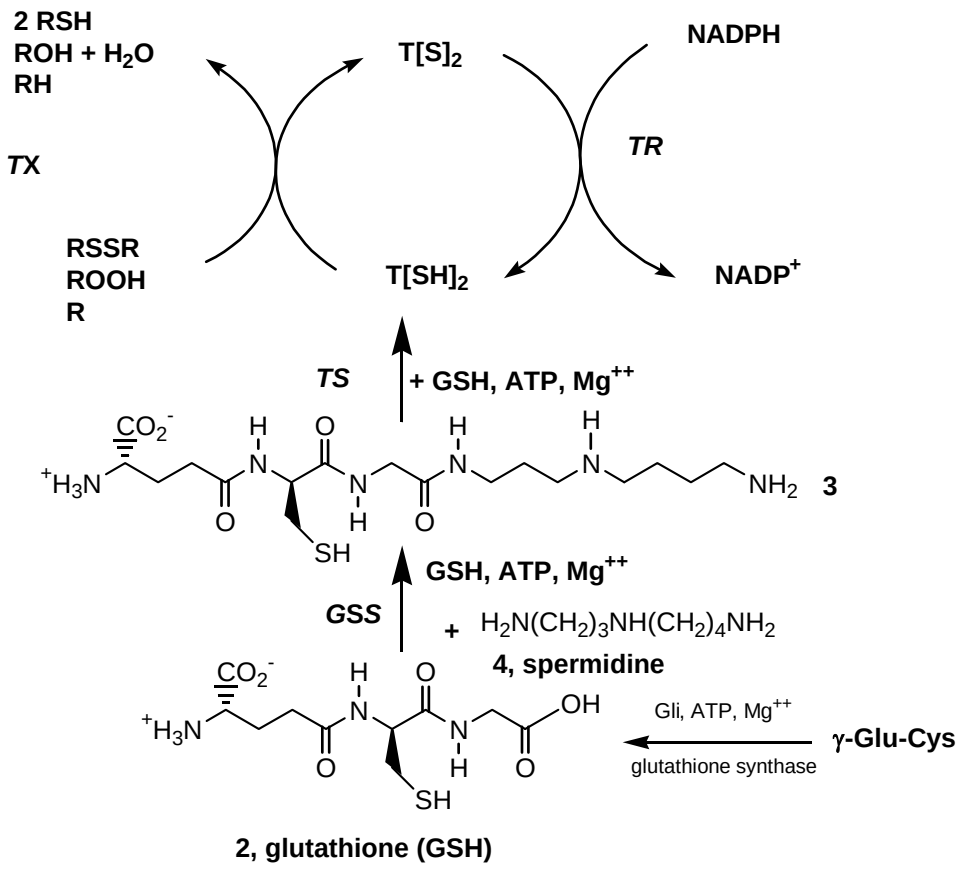


Figure 1. Oxidized and reduced form of trypanothione (compounds **1a** and **1b**, respectively).



Scheme 1. General scheme for trypanothione biosynthesis. TX = tryparedoxine reductase, TN = tryparedoxine peroxidase.

The crystal structure of TR has already been solved some years ago,[23–25] while the corresponding X-ray structure for GSS and TS are still unknown. The lack of information of the three-dimensional structure of the target proteins avoids a rational approach for drug design. Therefore, the design of inhibitors of the enzymatic activity for the mentioned ligases should be based on isosteric replacements or analogues of the transition state. However, the inhibition of the enzymatic activity of these enzymes has been studied and there are some data available that deal with inhibitors of GSS activity. For example, it has been found that analogues of glutathione in which the cisteine residue was replaced by isoleucine to form L-γ-Glu-L-Ile-Gly (compound **5**) or L-γ-Glu-L-Ile-L-Ala (compound **6**) exhibited a non-competitive inhibition with K_i at the low milimolar range.[26] Phosphonates and phosphinates are well known inhibitors of C:N-ligases.[27,28] This findings have led to the design of interesting phosphorous-containing drugs that behave as inhibitors of the enzymatic activity of GSS. For example, compound **7** exhibited a linear non-competitive inhibition with a *Ki* value of 60 μM against GSS from *Crithidia fasciculata*.[29] Moreover, when compounds structurally related to **7** are coupled with spermidine slow tight-binding inhibitors of GSS were obtained.[30,31] For instance, compound **8** exhibited a Ki value of 3.2 μM for binding to free enzyme (GSS from *Escherichia coli*), and 7.8 nM for binding to

enzyme-substrate complex E·I*, while compound **9** was much less potent than **8** showing inhibition constants of 6 μM and 14 μM for binding free enzyme and E·I*, respectively.[30,31] These compounds would act as inhibitors of the transition state at the active site of GSS. The chemical structure of drugs **7–9** is illustrated in Figure 2. The biological assays of **8** and **9** were performed on GSS from *Escherichia coli*.

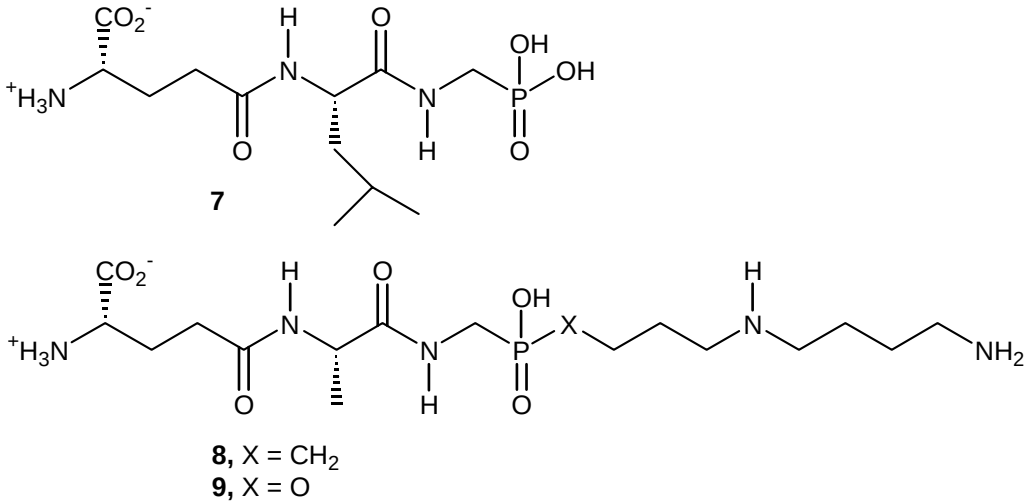


Figure 2. Chemical structure of inhibitors of the transition state at the active site of glutathionylspermidine synthase.

Very recently, we have prepared a new series of analogues of glutathione (L-γ-Glu-L-Leu-Gly), where the glycine carboxylic acid group has been substituted for other acidic groups such as tetrazole, hydroxamic acid, acylsulphonamide and boronic acid. Among this new family of drugs, the boronic acid derivative **10** was an effective inhibitor of the enzymatic activity of GSS with a K_i value of 81 μM and an IC50 value of 17 μM.[32] In addition, substitution of the glycine part on the same tripeptide lead to good inhibitors, specifically, when the glycine moiety was replaced by diaminopropionic acid to form **11**. This compound was a potent inhibitor of GSS activity with K_i = 7.2 μM.[33] The chemical structure of **10** and **11** is presented in Figure 3.

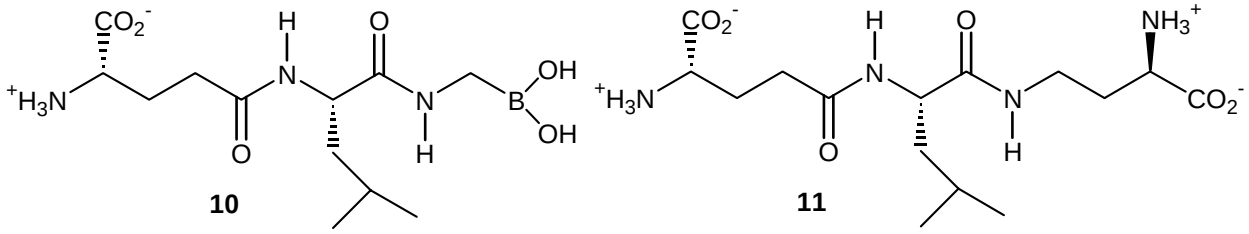


Figure 3. Chemical structures of boronic and diaminopropionic acids analogues of glutathione.

These results presented above together with the biological activity of glutathione derivatives reported by D'Silva *et al*[34,35] targeting trypanothione metabolism encouraged us to prepare analogues assuming that the optimum glutathione tripeptide derivative should be L-γ-Glu-L-Leu-Gly. Compound **12**, a protected tripeptide derivative of glutathione, was moderately potent against *T. brucei* (ED_{50} = 1.9 μM) but exhibited vanishing biological activity against *T. cruzi* (Figure 4).[34,35]

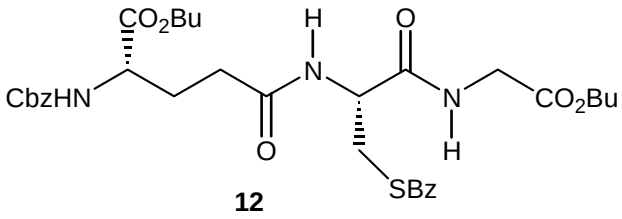


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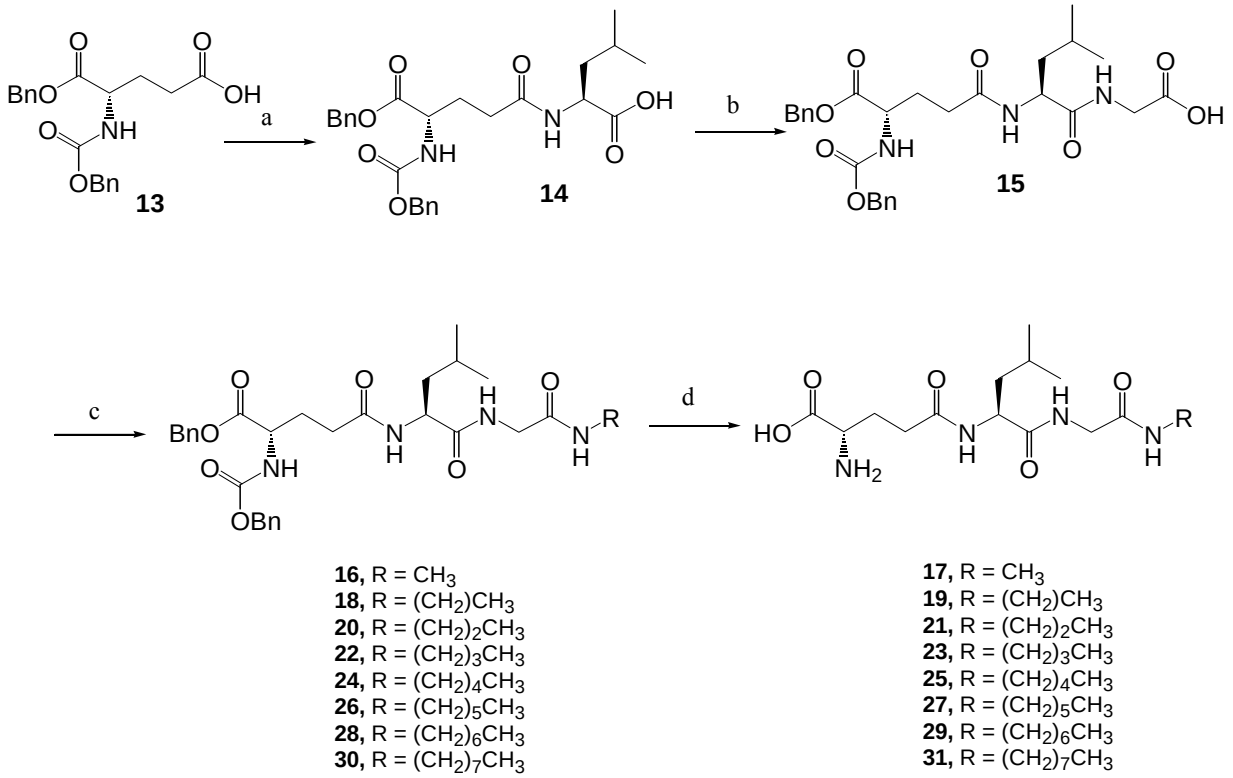
Figure 4. Chemical structure of a representative protected alkyl ester derivative of glutathione that presents efficient antiparasitic activity.

Results and Discussion

For the above reason a series of linear *N*-alkyl amides of glutathione were designed and synthesized motivated by the effectiveness of structurally related drugs. Thus, *N*-benzyloxycarbonyl-L-γ-glutamyl α-benzylester (compound **13**) was employed as starting material. Following a classical peptide synthesis, **13** was activated by treatment with dicyclohexylcarbodiimide in the presence of *N*-hydroxysuccinimide followed by addition of L-leucine to afford dipeptide **14**, which was activated in a similar way to be coupled with glycine to yield compound **15**. The preparation of compounds **14** and **15** has been reported but no experimental procedures nor spectroscopic data were depicted.[26,29] Tripeptide **15** was activated by treatment with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in the presence of *N*-hydroxysuccinimide. Once **15** was activated, the corresponding *N*-alkylamine was added to give the desired protected *N*-alkylamide derivative of glutathione. Benzyl ether cleavage was carried out by treatment with catalytic hydrogenation to afford the desired compounds as shown in Scheme 2.



Scheme 2. Reagents and Conditions: (a) i. *N*-hydroxysuccinimide, DCC, dioxane, rt, 16 h, ii. L-Leu, Et_3N, THF/H_2O, rt, 1 h, 84%; (b) i. *N*-hydroxysuccinimide, DCC, dioxane, rt, 16 h; ii. Gly, Et_3N, THF/H_2O, rt, 1 h, 84%; (c) *N*-hydroxysuccinimide, EDC.HCl, RNH_2, rt, 16 h, 65% for **16**, 73% for **18**, 55% for **20**, 51% for **22**, 57% for **24**, 51% for **26**, 57% for **28**, 62% yield for **30**; (d) H_2, Pd/C, rt, 2 h 100% for **17**, 99% yield for **19**, 99% for **21**, 97% for **23**, 98% for **25**, 98% for **27**, 100% yield, for **29**, 99% yield for **31**.

All of the target molecules as well as their synthetic intermediates, that is, compounds **16**–**31** were biologically evaluated against *T. cruzi* (epimastigotes). The free tripeptides (compounds **17**, **19**, **21**, **23**, **25**, **27**, **29**, and **31**) and the protected intermediates (compounds **16**, **18**, **20**, **22**, **24**, **26**, **28**, and **30**) were devoid of inhibitory action towards *T. cruzi* proliferation at concentrations up to 20 μg/ml. The lack of biological activity is extremely surprising bearing in mind the structural likeness between the tested drugs and the chemical structures of the lead drugs such as **7**–**12**. The marginal activity shown by all of these compounds might be attributable to the amidase activity of the target enzyme. This enzyme, in spite of being unique for trypanosomatids with a specific function of catalyzing the first of the two ultimate steps of trypanothione biosynthesis, would act as bifunctional synthetase/amidase.[36,37] The loss of the *N*-alkylamine moiety would be responsible for the marginal activity observed.

Work aimed at exploiting the potential usefulness of GSS as molecular target for drug design is currently being pursued at our laboratory, specifically as simplified analogues of compound **8**.

Experimental Section

General Procedures. The glassware used in air and/or moisture sensitive reactions was flame-dried and carried out under a dry argon atmosphere. Unless otherwise noted, chemicals were commercially available and used without further purification. Solvents were distilled before use. Tetrahydrofuran and 1,4-dioxane were distilled from sodium/benzophenone ketyl. Anhydrous *N*,*N*-dimethylformamide was used as supplied from Aldrich.

Nuclear magnetic resonance spectra were recorded using a Bruker AC-200 MHz or a Bruker AM-500 MHz spectrometers. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Coupling constants are reported in Hertz. ^{13}C-NMR spectra were fully decoupled. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet.

Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded using a Nicolet Magna 550 spectrometer.

Column chromatography was performed with E. Merck silica gel (Kieselgel 60, 230-400 mesh). Analytical thin layer chromatography was performed employing 0.2 mm coated commercial silica gel plates (E. Merck, DC-Aluminum sheets, Kieselgel 60 F_{254}).

Elemental analyses were conducted by Atlantic Microlab Inc., Norcross, Georgia. The results were within ± 0.4% of the theoretical values except where otherwise stated.

***N*-Benzyloxycarbonyl-L-glutamyl(α-benzylester)-L-leucine (14).** To a solution of *N*-benzyloxycarbonyl-L-γ-glutamyl α-benzylester (compound **13**; 5.0 g, 13.46 mmol) and *N*-hydroxysuccinimide (1.8 g, 16.16 mmol) in anhydrous 1,4-dioxane (100 ml) was added dicyclohexylcarbodiimide (4.17 g, 20.20 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The white precipitate of dicyclohexylurea was removed by filtration through a glass frit filter and the solvent was evaporated. The residue was dissolved in anhydrous tetrahydrofuran (60 ml), and this solution added dropwise to a stirred solution of L-leucine (2.12 g, 16.16 mmol) and triethylamine (2.25 ml, 16.16 mmol) in water (30 ml). The mixture was stirred at room temperature for 1 h. Then, the solvent was evaporated. Then, water (50 ml) was added and the pH was adjusted to 1.0 with a 5% aqueous solution of hydrochloric acid. The suspension was extracted with ethyl acetate (3 × 20 ml). The combined organic layers were washed with water (2 × 20 ml), dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by column chromatography (silica gel) employing a mixture of CH_2Cl_2-AcOH (99:1) to CH_2Cl_2-MeOH-AcOH (95:1:1) to afford 5.5 g (84% yield) of protected dipeptide **14** as a white solid. ^{1}H-NMR (200 MHz, DMSO-d_6) δ 0.81 (d, J = 6.3 Hz, 3H, CH_3Leu), 0.86 (d, J = 6.3 Hz, 3H, CH_3Leu), 1.34-1.68 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.69-1.89 (m, 1H, H_a-C(β)Glu), 1.89-2.11 (m, 1H, H_b-C(β)Glu), 2.11-2.32 (m, 2H, H_2-C(γ)Glu), 2.89-3.85 (broad s, 1H, COO*H*), 4.02-4.25 (m, 2H, C*H*COGlu, C*H*COLeu), 5.03 (s, 2H, OCH_2Ph), 5.13 (s, 2H, OCH_2Ph), 7.21-7.43 (m, 10H, Ph), 7.81 (d, J = 7.9 Hz, 1H, N*H*), 8.01 (d, J = 8.2 Hz, 1H, N*H*); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 21.6 (C_aH$_3$Leu), 22.7 (C_bH$_3$Leu), 24.7 (C(γ)Leu), 27.9 (C(γ)Glu), 32.0 (C(β)Glu), 40.8 (C(β)Leu), 51.1 (C(α)Leu), 53.6

(C(α)Glu), 66.9 (O*C*H$_2$Ph), 67.1 (O*C*H$_2$Ph), 127.9 (Ph), 128.0 (Ph), 128.2 (Ph), 128.3 (Ph), 128.4 (Ph), 135.1 (Ph), 136.0 (Ph), 156.4 (O*C*ONH), 171.8 (CO), 172.6 (CO), 176.4 (CO).

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycine (15).** This compopund was prepared following a similar procedure as depicted for **14** employing *N*-benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucine (2.64 g, 5.45 mmol), *N*-hydroxysuccinimide (0.78 g, 6.54 mmol), dicyclohexylcarbodiimide (1.69 g, 8.17 mmol) and glycine (0.49 g, 6.54 mmol). The product was purified by column chromatography (silica gel) eluting with a mixture of CH_2Cl_2-MeOH-AcOH (95:1:1) to CH_2Cl_2-MeOH-AcOH (90:10:1) to afford 1.36 g (80% yield) of tripeptide **15** as a white solid: mp 106–108 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 0.80 (d, *J* = 7.2 Hz, 3H, C*H*$_3$Leu), 0.84 (d, *J* = 7.2 Hz, 3H, C*H*$_3$Leu), 1.29-1.67 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.67-1.88 (m, 1H, *H*$_a$-C(β)Glu), 1.88-2.10 (m, 1H, *H*$_b$-C(β)Glu), 2.11-2.36 (m, 2H, *H*-C(γ)Glu), 2.87-3.96 (broad s, 1H, COO*H*), 3.57 (s, 2H, C*H*$_2$Gly), 4.00-4.17 (m, 1H, C*H*COLeu), 4.18-4.38 (m, 1H, C*H*COGlu), 5.03 (s, 2H, PhC*H*$_2$), 5.12 (s, 2H, PhC*H*$_2$), 7.21-7.48 (m, 10H, Ph), 7.83 (d, 1H, *J*=7.5 Hz, N*H*), 7.92 (m, 1H, N*H*), 8.07 (d, *J*=7.5 Hz, 1H, N*H*); ^{13}C-NMR (50 MHz, $CDCl_3$-CD_3OD (95:5)) δ 21.6 (*C*$_a$H$_3$Leu), 22.7 (*C*$_b$H$_3$Leu), 24.6 (C(γ)Leu), 27.1 (C(γ)Glu), 31.8 (C(β)Glu), 40.4 (C(β)Leu), 43.0 (*C*H$_2$Gly), 52.4 (C(α)Leu), 53.7 (C(α)Glu), 66.8 (O*C*H$_2$Ph), 67.0 (O*C*H$_2$Ph), 127.8 (Ph), 128.0 (Ph), 128.2 (Ph), 128.3 (Ph), 128.4 (Ph), 135.2 (Ph), 136.2 (Ph), 156.6 (O*C*ONH), 172.2 (CO), 173.6 (CO), 173.7 (CO), 177.3 (CO).

General procedure for the preparation of tripeptide amides

To a solution of compound **15** (100 mg, 0.185 mmol) and *N*-hydroxysuccinimide (0.554 mmol) in anhydrous *N*,*N*-dimethylformamide (5 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC; 177 mg, 0.92 mmol) was added at room temperature under argon atmosphere. The reaction mixture was stirred at room temperature overnight. Then, the corresponding amine* (0.22 mmol) was added and the mixture was stirred for 1 h. The mixture was partitioned between ethyl acetate (20 ml) and an aqueous saturated solution of sodium chloride (20 ml). The organic layer was washed with 5% hydrochloric acid (2 × 20 ml) and water (2 × 20 ml). The organic phase was dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography (silica gel) employing mixtures of CH_2Cl_2–*i*-PrOH ranging from 99:1 to 97:3 to afford the desired compounds.

* Methylamine and ethylamine were released *in situ* from their corresponding hydrochlorides by treatment with 10 equivalents of triethylamine.

General procedure for benzyl ether cleavage

A solution of the respective tripeptide *N*-benzyloxycarbonyl-L-glutamyl(α-benzylester) derivative (0.1 mmol) in methanol (10 ml) in the presence of 10% palladium on charcoal (catalyst) was treated with hydrogen at 3 atm. The reaction mixture was stirred at room temperature for 2 h. The mixture was filtered through a glass frit filter and the solvent was evaporated. The residue was purified by reverse phase column chromatography (C_{18}-silica gel) eluting with a mixture of MeOH-H_2O (1:1) to afford the desired target products.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-methylamide (16).** White solid; 65% yield; mp 168–170 °C; IR (KBr, cm^{-1}) 3309, 2959, 1747, 1697, 1639, 1545, 762, 699; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.89 (d, *J* = 6.0 Hz, 3H, C*H*$_3$Leu), 0.92 (d, *J* = 6.0 Hz, 3H, C*H*$_3$Leu), 1.44-1.80 (m, 3H, C*H*$_2$Leu, C*H*$(CH_3)_2$Leu), 1.80-2.04 (m, 1H, *H*$_a$-C(β)Glu), 2.12-2.36 (m, 3H, *H*$_b$-C(β)Glu, *H*-C(γ)Glu), 2.43 (d, *J* = 4.6 Hz, 3H, NHC*H*$_3$), 3.72 (dd, *J* = 16.6, 5.5 Hz, 1H, *H*$_a$Gly), 4.00 (dd, *J* = 16.6, 6.4 Hz, 1H, *H*$_b$Gly), 4.11-4.27 (m, 1H, C*H*COLeu), 4.27-4.44 (m, 1H, C*H*COGlu), 5.08 (s, 2H, C*H*$_2$Ph), 5.15 (s, 2H, C*H*$_2$Ph), 5.82 (d, *J* = 8.1 Hz, 1H, N*H*), 6.48 (d, *J* = 6.6 Hz, 1H, N*H*), 6.71 (d, *J* = 4.6 Hz, 1H, N*H*), 7.02-7.17 (m, 1H, N*H*), 7.34 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 21.9 (*C*$_a$H$_3$Leu), 22.8 (*C*$_b$H$_3$Leu), 24.7 (C(γ) Leu), 26.1 (NH-*C*H$_3$), 28.1 (C(γ) Glu), 31.7 (C(β) Glu), 40.5 (C(β) Leu), 42.9 (*C*H$_2$Gly), 52.4 (*C*HCOLeu), 53.4 (*C*HCOGlu), 67.0 (O*C*H$_2$Ph), 67.3 (O*C*H$_2$Ph), 128.0 (Ph), 128.1 (Ph), 128.2 (Ph), 128.4 (Ph), 128.6 (Ph), 135.0 (Ph), 136.1 (Ph), 156.4 (O*C*ONH), 169.5 (CO), 171.9 (CO), 172.7 (CO), 172.8 (CO). Anal. Calcd. for $C_{29}H_{38}N_4O_7$: C 62.80, H 6.91, N 10.10. Found: C 62.49, H 7.13.

L-γ-Glutamyl-L-leucylglycyl-*N*-methylamide (17). White solid; 100% yield; mp 115–117 °C; IR (KBr, cm^{-1}) 3287, 3075, 2962, 1659, 1542;^{1}H-NMR (200 MHz, CD_3OD) δ 0.92 (d, *J* = 6.6 Hz, 3H, C*H*$_3$Leu), 0.96 (d, *J* = 6.4 Hz, 3H, C*H*$_3$Leu), 1.54-1.77 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 2.09-2.24 (m, 2H, *H*-C(β)Glu), 2.54 (t, *J* = 7.0 Hz, 2H, *H*$_2$-C(γ)Glu), 2.73 (s, 3H, NHC*H*$_3$), 3.76 (m *AB*, 2H, C*H*$_2$Gly), 3.96 (t, *J* = 6.6 Hz 1H,), 4.28 (t, *J* = 7.2 Hz, 1H); ^{13}C-NMR (50 MHz, CD_3OD) δ 21.9 (*C*$_a$H$_3$Leu), 23.3 (*C*$_b$H$_3$Leu), 25.9 (C(γ)Leu), 26.3 (NH*C*H$_3$), 27.2 (C(γ)Glu), 32.2 (C(β) Glu), 41.3 (C(β)Leu), 43.4 (*C*H$_2$Gly), 54.0 (C(α)Leu), 172.0 (CO), 174.9 (CO), 176.1 (CO). Anal. Calcd. for $C_{14}H_{26}N_4O_5.2½H_2O$: C 44.79, H 6.98, N 14.93. Found: C 44.55, H 7.48, N 15.08.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-ethylamide (18).** White solid; 73% yield; mp 144–146 °C; IR (KBr, cm^{-1}) 3301, 2966, 1747, 1710, 1647, 1547, 756, 699; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.83-1.00 (m, 6H, C*H*$_3$Leu), 1.08 (t, *J* = 7.3 Hz, 3H, NHCH$_2$C*H*$_3$), 1.46-1.80 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.80-2.05 (m, 1H, *H*$_a$-C(β)Glu), 2.10-2.41 (m, 3H, *H*$_b$-C(β)Glu, *H*-C(γ)Glu), 3.08-3.35 (m, 2H, NHC*H*$_2$CH$_3$), 3.74 (dd, *J* = 16.6, 4.8 Hz, 1H, *H*$_a$Gly), 3.99 (dd, *J* = 16.6, 6.2 Hz, 1H, *H*$_b$Gly), 4.09-4.46 (m, 2H, C*H*COLeu, C*H*COGlu), 5.09 (s, 2H, C*H*$_2$Ph), 5.16 (s, 2H, C*H*$_2$Ph), 5.74 (d, *J* = 8.0 Hz, 1H, N*H*), 6.32 (d, *J* = 6.6 Hz, 1H), 6.45-6.61 (m, 1H, N*H*), 6.87-7.02 (m, 1H, N*H*), 7.33 (m, 10H, Ph); ^{13}C-NMR (50MHz, $CDCl_3$) δ 14.4 (NHCH$_2$*C*H$_3$), 21.9 (*C*$_a$H$_3$Leu), 22.8 (*C*$_b$H$_3$Leu), 24.7 ((C(γ)Leu)), 28.1 (C(γ)Glu), 31.8 (C(β)Glu), 34.3 (NH*C*H$_2$CH$_3$), 40.6 (C(β)Leu), 43.0 (*C*H$_2$Gly), 52.4 (*C*HCOLeu), 53.5 (*C*HCOGlu), 67.0 (Ph*C*H$_2$), 67.3 (Ph*C*H$_2$), 128.0 (Ph), 128.1 (Ph), 128.2 (Ph), 128.5 (Ph), 128.6 (Ph), 135.1 (Ph), 136.1 (Ph), 156.4 (O*C*ONH), 168.7 (CO), 171.9 (CO), 172.5 (CO), 172.7 (CO). Anal. Calcd. for $C_{30}H_{40}N_4O_7$: C 63.36, H 7.09, N 9.85. Found: C 63.10, H 7.06, N 9.71.

L-γ-Glutamyl-L-leucylglycyl-*N*-ethylamide (19). White solid; 99% yield; mp 119–120 °C; IR (KBr, cm^{-1}) 3287, 3078, 2964, 1658, 1551; ^{1}H-NMR (200 MHz, CD_3OD) δ 0.92 (d, *J* = 6.2 Hz, 3H, C*H*$_3$Leu), 1.12 (t, *J* = 7.1 Hz, 3H,), 1.53-1.77 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 2.09 (m, 2H, *H*-C(β) Glu), 2.52 (t, *J* = 6.8 Hz, 2H, *H*-C(γ) Glu), 3.17 (q, *J* = 7.0 Hz, 2H), 3.75 (m *AB*, 2H,

C*H*$_2$Gly), 3.80 (m, 1H, C*H*COLeu), , 4.23 (distorted t, *J* = 7.4 Hz, 1H); ^{13}C-NMR (50 MHz, CD_3OD) δ 14.8 (NHCH$_2$*C*H$_3$), 21.9 (*C*$_a$H$_3$Leu), 23.4 (*C*$_b$H$_3$Leu), 25.9 (C(γ)Leu), 27.7 (C(γ) Glu), 32.6 (C(β) Glu), 35.3 ((NH*C*H$_2$CH$_3$), 41.3 (C(β)Leu), 43.6 (*C*H$_2$Gly), 54.0 (*C*HCO, Leu), 55.2 (*C*HCOGlu), 171.2 (CO), 173.9 (CO), 175.4 (CO), 175.6(CO). Anal. Calcd. for $C_{15}H_{28}N_4O_5.2H_2O$: C 47.36, H 7.42, N 14.73. Found: C 47.79, H 7.84, N 14.14.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-(1-propyl)amide (20).** White solid; 55% yield; mp 172–174 °C; IR (KBr, cm^{-1}) 3294, 2966, 1740, 1695, 1633, 1547, 756, 706; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.78-0.98 (m, 9H, C*H*$_3$Leu, NH(CH$_2$)$_2$C*H*$_3$), 1.36-1.72 (m, 5H, NHCH$_2$C*H*$_2$CH$_2$, *H*-C(β)Leu, *H*-C(γ)Leu), 1.72-2.06 (m, 1H, *H*$_a$-C(β)Glu), 2.07-2.35 (m, 3H, *H*$_b$-C(β)Glu, *H*-C(γ)Glu), 3.08-3.22 (m, 2H, NHC*H*$_2$CH$_2$CH$_3$), 3.69-4.02 (m, 2H, C*H*$_2$Gly), 4.18-4.45 (m, 2H, C*H*COGlu, C*H*COLeu), 5.08 (s, 2H, PhC*H*$_2$), 5.15 (s, 2H, PhC*H*$_2$), 5.82 (d, *J* = 8.3 Hz, 1H, N*H*), 6.53 (d, *J* = 6.9 Hz, 1H, N*H*), 6.64 (t, *J* = 5,5 Hz, 1H, N*H*), 7.08 (t, *J* = 5.3 Hz, 1H, *N*H), 7.33 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 11.2 (NH(CH$_2$)$_2$*C*H$_3$), 21.8 (*C*$_a$H$_3$Leu), 22.4 (NHCH$_2$*C*H$_2$CH$_3$), 22.7 (*C*$_b$H$_3$Leu), 24.6 (*C*H(CH$_3$)$_2$Leu), 28.1 (C(γ)Glu), 31.8 (C(β)Glu), 40.6 (*C*H$_2$CH(CH$_3$)$_2$Leu), 41.1 (NH*C*H$_2$CH$_2$CH$_3$), 43.0 (*C*H$_2$Gly), 52.3 (*C*HCOLeu), 53.4 (*C*HCOGlu), 67.0 (Ph*C*H$_2$), 67.2 (Ph*C*H$_2$), 127.9 (Ph), 128.0 (Ph), 128.1 (Ph), 128.4 (Ph), 128.5 (Ph), 135.0 (Ph), 136.4 (Ph), 156.6 (O*C*ONH), 168.7 (CO), 171.7 (CO), 172.4 (CO), 172.6 (CO). Anal Calcd for $C_{31}H_{42}N_4O_7.½H_2O$: C 62.93, H 7.15, N 9.47. Found: C 62.80, H 7.27 N 9.47.

L-γ-Glutamyl-L-leucylglycyl-*N*-(1-propyl)amide (21). White solid; 99% yield; mp 125–127 °C; IR (KBr, cm^{-1}) 3297, 3075, 2963, 1658, 1543; ^{1}H-NMR (200 MHz, CD_3OD) δ 0.75-0.91 (m, 9H, C*H*$_3$Leu, NH(CH$_2$)$_2$C*H*$_3$), 1.33-1.70 (m, 5H, NHCH$_2$C*H*$_2$CH$_2$, *H*-C(β)Leu, *H*-C(γ)Leu), 1.88-2.16 (m, 2H, *H*-C(β)Glu), 2.42 (t, *J* = 7.0 Hz, 2H, *H*-C(γ)Glu), 3.05 (distorted t, *J* = 6.4 Hz, 2H, NHC*H*$_2$CH$_2$CH$_3$), 3.62 (t, *J* = 6.2 Hz, 1H, C*H*COLeu), 3.71 (m *AB*, 2H, C*H*$_2$Gly), 4.18 (distorted t, *J* = 7.3 Hz, 1H, C*H*COGlu); ^{13}C-NMR (50 MHz, MeOD) δ 11.7 (NH(CH$_2$)$_2$*C*H$_3$), 21.9 (C$_a$(δ)Leu), 23.4 (C$_b$(δ)Leu), 23.5 (NHCH$_2$*C*H$_2$CH$_3$), 25.9 (C(γ)Leu), 27.6 (C(γ)Glu), 32.5 (C(β)Glu), 41.3 (C(β)Leu), 42.2 (NH*C*H$_2$CH$_2$CH$_3$), 43.5 (C(α)Gly), 54.0 (C(α)Leu), 55.0 (C(α)Glu, 171.4 (CO), 173.5 (CO), 175.3 (CO), 175.6 (CO). Anal. Calcd. for $C_{16}H_{30}N_4O_5.1.7H_2O$: C 49.90, H 7.77, N 14.40. Found: C 49.54, H 8.20, N 14.18.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-(1-butyl)amide (22).** White solid; 51% yield; mp 148–150 °C; IR (KBr, cm^{-1}) 3302, 2959, 1754, 1701, 1635, 1544, 754, 701 ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.81-0.97 (m, 9H, C*H*$_3$Leu, NH(CH$_2$)$_3$C*H*$_3$), 1.18-1.75 (m, 7H, NHCH$_2$(C*H*$_2$)$_2$CH$_3$, *H*$_2$-C(β)Leu, *H*-C(γ)Leu), 1.82-2.03 (m, 1H, *H*$_a$-C(β)Glu), 2.09-2.34 (m, 3H, *H*$_b$-C(β)Glu, *H*-C(γ)Glu), 3.09-3.27 (m, 2H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.87 (m, 2H, C*H*$_2$Gly), 4.13-4.45 (m, 2H, C*H*CO Glu, C*H*CO Leu), 5.09 (s, 2H, PhC*H*$_2$), 5.16 (s, 2H, PhC*H*$_2$), 5.70 (d, *J* = 8.0 Hz,1H, N*H*), 6.37 (d, *J* = 6.6 Hz, 1H, N*H*), 5.51 (t, *J* = 5,5 Hz, 1H, N*H*), 6.93 (t, *J* = 5.5 Hz, 1H, *N*H), 7.34 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 13.7 (NH(CH$_2$)$_3$*C*H$_3$), 19.9 (NH(CH$_2$)$_2$*C*H$_2$CH$_3$), 21.9 (C$_a$(δ)Leu), 22.8 (C$_b$(δ)Leu), 24.7 (C(γ)Leu), 28.3 (C(γ)Glu), 31.3 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 31.9 (C(β)Glu), 39.3 (NH*C*H$_2$(CH$_2$)$_2$CH$_3$), 40.6 (C(β)Leu), 43.1 (C(α)Gly), 52.4 (C(α)Leu), 53.4 (C(α)Glu), 67.1 (Ph*C*H$_2$), 67.3 (Ph*C*H$_2$), 127.8 (Ph), 128.0

(Ph), 128.2 (Ph), 128.5 (Ph), 128.6 (Ph), 135.1 (Ph), 136.1 (Ph), 156.4 (NH-*C*O-O), 168.7 (CO), 171.8 (CO), 172.5 (CO), 172.6 (CO). Anal. Calcd. for $C_{32}H_{44}N_4O_7$: C 64.41, H 7.43. Found: C 64.53, H 7.53.

L-γ-Glutamyl-L-leucylglycyl-*N*-(1-butyl)amide (23). White solid; 97% yield; mp 126–128 °C; IR (KBr, cm^{-1}) 3298, 3086, 2964, 1659, 1542; ^{1}H-NMR (500 MHz, CD_3OD) δ 0.0.86-1.00 (m, 9H, C*H*$_3$Leu, NH$(CH_2)_3$C*H*$_3$), 1.27-1.39 (m, 2H, NH$(CH_2)_2$C*H*$_2$CH$_3$), 1.45-1.53 (NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.55-1.76 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.95-2.19 (m, 2H, *H*-C(β)Glu), 2.43-2.59 (m, 2H, *H*-C(γ)Glu), 3.11-3.25 (m, 2H, NHC*H*$_2(CH_2)_2CH_3$), 3.60-3.69 (bs, 1H, C*H*COLeu), 3.80 (m *AB*, 2H, C*H*$_2$Gly), 4.28 (distorted t, *J* = 7.2 Hz, 1H, C*H*-CO Glu); ^{13}C-NMR (50 MHz, CD_3OD) δ 14.1 (NH$(CH_2)_3$*C*H$_3$), 21.0 (NH$(CH_2)_2$*C*H$_2$CH$_3$), 21.8 (C$_a$(δ)Leu), 23.4 (C$_b$(δ)Leu) , 25.9 (C(γ)Leu), 27.7 (C(γ)Glu), 32.4 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 32.6 (C(β)Glu), 40.2 (NH*C*H$_2(CH_2)_2CH_3$), 41.3 (C(β)Leu), 43.6 (C(α)Gly), 54.0 (C(α)Leu), 171.3 (CO), 175.4 (CO), 173.8 (CO), 175.6 (CO). Anal. Calcd. for $C_{17}H_{32}N_4O_5.1.6H_2O$: C 50.89, H 8.04, N 13.96. Found: C 50.23, H 8.22, N 13.56.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-(1-pentyl)amide (24).** White solid; 57% yield; mp 185–187 °C; IR (KBr, cm^{-1}) 3302, 2960, 1736, 1701, 1632, 1550, 756, 708; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.1 Hz, 3H, NH$(CH_2)_3$C*H*$_3$), 0.89 (d, *J* = 6.1 Hz, 3H, C$_a$*H*$_3$Leu), 0.92 (d, *J* = 6.1 Hz, 3H, C$_b$*H*$_3$Leu), 1.20-1.32 (m, 4H, NH$(CH_2)_2$(C*H*$_2)_2$CH$_3$), 1.41-1.49 (m, 2H, NHCH$_2$C*H*$_2(CH_2)_2CH_3$), 1.50-1.72 (m, 3H, *H*$_2$-C(β)Leu, *H*-C(γ)Leu), 1.89-1.99 (m, 1H, *H*$_a$-C(β)Glu), 2.11-2.34 (m, 3H, *H*$_b$-C(β)Glu, *H*-C(γ) Glu), 3.84 (m, C*H*$_2$Gly), 4.23-4.43 (m, 2H, C*H*COGlu, C*H*COLeu), 5.08 (s, 2H, PhC*H*$_2$), 5.15 (s, 2H, PhC*H*$_2$), 5.83 (d, *J* = 7.5 Hz, 1H, N*H*), 6.53-6.68 (m, 2H, N*H*), 7.04-7.12 (broad s, 1H, N*H*), 7.32 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 13.9 (NH$(CH_2)_4$*C*H$_3$), 21.9 (C$_a$(δ)Leu), 22.3 (NH$(CH_2)_3$*C*H$_2$CH$_3$), 22.8 (C$_b$(δ)Leu), 24.8 (C(γ)Leu), 28.4 (C(γ)Glu), 28.9 (NHCH$_2$(*C*H$_2)_2$CH$_2$CH$_3$), 31.9 (C(β)Glu), 39.5 (NH*C*H$_2(CH_2)_3CH_3$), 40.5 (C(β)Leu), 43.2 (C(α)Gly), 52.5 (C(α)Leu), 53.4 (C(α)Glu), 67.2 (PhCH$_2$), 67.4 (Ph*C*H$_2$), 128.1 (Ph), 128.2 (Ph), 128.3 (Ph), 128.5 (Ph), 128.6 (Ph), 135.1 (Ph), 136.2 (Ph), 156.4 (NH*C*OO), 168.6 (CO), 171.8 (CO), 172.4 (CO), 172.5 (CO). Anal. Calcd. for $C_{33}H_{46}N_4O_7 \cdot 0.6\ H_2O$: C 63.36, H 7.09, N 9.85. Found: C 63.10, H 7.06, N 9.71.

L-γ-Glutamyl-L-leucylglycyl-*N*-(1-pentyl)amide (25). White solid; 98% yield; mp 163–165 °C; IR (KBr, cm^{-1}) 3309, 3097, 2966, 1654, 1543; ^{1}H-NMR (500 MHz, CD_3OD) δ 0.87-0.99 (m, 9H, C*H*$_3$Leu, NH$(CH_2)_4$C*H*$_3$), 1.26-1.37 (m, 4H, NH$(CH_2)_2$(C*H*$_2)_2$CH$_3$), 1.55-1.76 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.99-2.08 (m, 1H, *H*$_a$-C(β)Glu), 2.10-2.18 (m, 1H, *H*$_b$-C(β)Glu) 2.42-2.56 (m, 2H, *H*-C(γ)Glu), 3.10-3.24 (m, 2H, NHC*H*$_2(CH_2)_3CH_3$), 3.62 (t, *J* = 6.5 Hz, 1H, C*H*COLeu), 3.79 (m *AB*, 2H, C*H*$_2$Gly), 4.28 (dd, *J* = 8.9, 6.0 Hz, 1H, C*H*COGlu); ^{13}C-NMR (125 MHz, CD_3OD) δ 14.3 (NH$(CH_2)_4$*C*H$_3$), 21.8 (C$_a$(δ)Leu), 23.4 (NH$(CH_2)_3$*C*H$_2$CH$_3$, C$_b$(δ)Leu), 25.9 (C(γ)Leu), 27.8 (C(γ)Glu), 30.1 (NHCH$_2$(*C*H$_2)_2$CH$_2$CH$_3$), 32.7 (C(β)Glu), 40.4 (NH*C*H$_2(CH_2)_3CH_3$), 41.4 (C(β)Leu), 43.6 (C(α)Gly), 54.0 (C(α)Leu), 55.4 (C(α)Glu), 171.4 (CO), 175.4 (CO), 175.6 (CO). Anal. Calcd. for $C_{18}H_{34}N_4O_5.0.5H_2O$: C 54.66, H 8.60, N 14.17. Found: C 54.74, H 8.71, N 13.67.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-(1-hexyl)amide (26).** White solid; 59% yield; mp 170–172 °C; IR (film, cm^{-1}) 3301, 2931, 1739, 1704, 1647, 1547, 749, 699; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.99-0.80 (m, 9H, $NH(CH_2)_5CH_3$, CH_3Leu), 1.20-1.32 (m, 6H, $NH(CH_2)_2(CH_2)_3CH_3$), 1.38-1.75 (m, 5H, $NHCH_2CH_2(CH_2)_3CH_3$, *H*-C(β)Leu, *H*-C(γ)Leu), 1.81-2.03 (m, 1H, H_a-C(β)Glu), 2.13-2.33 (m, 3H, H_b-C(β)Glu, *H*-C(γ)Glu), 3.12-3.26 (m, 2H, $NHCH_2(CH_2)_4CH_3$), 3.87 (m, 2H, CH_2Gly), 4.12-4.46 (m, 2H, CHCOGlu, CHCOLeu), 5.09 (s, 2H, $PhCH_2$), 5.16 (s, 2H, $PhCH_2$), 5.71 (d, *J* = 8.1 Hz ,1H, N*H*), 6.31 (d, *J* = 6.2 Hz, 2H, N*H*), 6.88 (t, *J* = 5.5 Hz, 1H, N*H*), 7.37 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 14.0 ($NH(CH_2)_5CH_3$), 22.0 (C_a(δ)Leu), 22.5 ($NH(CH_2)_4CH_2CH_3$), 22.8 (C_b(δ)Leu), 24.7 (C(γ)Leu), 28.2 (C(γ)Glu), 29.2 ($NH(CH_2)_2CH_2CH_2CH_3$), 31.4 ($NHCH_2CH_2(CH_2)_3CH_3$), 31.9 (C(β)Glu), 39.6 ($NHCH_2(CH_2)_4CH_3$), 40.8 (C(β)Leu), 43.1 (C(α)Gly), 52.2 (C(α)Leu), 53.5 (C(α)Glu), 67.0 ($PhCH_2$), 67.3 ($PhCH_2$), 128.0 (Ph), 128.1 (Ph), 128.2 (Ph), 128.6 (Ph), 135.1 (Ph), 136.1 (Ph), 156.4 (NHCOO), 168.6 (CO), 171.8 (CO), 172.4 (CO), 172.7 (CO). Anal. Calcd. for $C_{34}H_{48}N_4O_7.0.3H_2O$: C 64.80, H 7.68, N 8.89. Found: C 64.77, H 7.67, N 8.75.

L-γ-Glutamyl-L-leucylglycyl-*N*-(1-hexyl)amide (27). White solid; 98% yield; mp 167–169 °C; IR (KBr, cm^{-1}) 3288, 3077, 2960, 1652, 1545; ^{1}H-NMR (200 MHz, CD_3OD) δ 0.87-1.04 (m, 9H, CH_3Leu, $NH(CH_2)_5CH_3$), 1.26-1.45 (m, 6H, $NH(CH_2)_2(CH_2)_3CH_3$), 1.45-1.82 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.99-2.29 (broad s, 2H, H_2-C(β)Glu) 2.45-2.62 (m, 2H, *H*-C(γ)Glu), 3.14-3.30 (m, 2H, $NHCH_2(CH_2)_4CH_3$), 3.62-3.76 (m, 1H, CHCOLeu), 3.83 (s, 2H, CH_2Gly), 4.28 (distorted t, *J* = 6.6 Hz, 1H, CHCOGlu); ^{13}C-NMR (125 MHz, CD_3OD) δ 14.3 ($NH(CH_2)_5CH_3$), 21.8 (C_aH_3Leu), 23.4 ($NH(CH_2)_4CH_2CH_3$),* 23.6 (C_bH_3Leu),* 25.9 (C(γ)Leu), 27.6 ($NH(CH_2)_3CH_2CH_2CH_3$), 27.7 (C(γ)Glu), 30.3 ($NH(CH_2)_2CH_2(CH_2)_2CH_3$), 32.6 ($NHCH_2CH_2(CH_2)_3CH_3$), 32.7 (C(β)Glu), 40.5 ($NHCH_2(CH_2)_4CH_3$), 41.4 (C(β) Leu), 43.6 (C(α) Gly), 54.0 (C(α) Leu), 55.3 (C(α) Glu, 171.3 (CO), 175.4 (CO), 175.6 (CO). Anal. Calcd. for $C_{19}H_{36}N_4O_5.1½H_2O$: C 53.38, H 8.49, N 13.11. Found: C 53.64 , H 8.79, N 12.79

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-(1-heptyl)amide (28).** White solid; 57% yield; mp 141–143 °C; IR (KBr, cm^{-1}) 3302, 2928, 1746, 1697, 1635, 1538, 762, 701; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.81-0.97 (m, 9H, $NH(CH_2)_6CH_3$, CH_3Leu), 1.20-1.32 (m, 8H, $NH(CH_2)_2(CH_2)_4CH_3$), 1.36-1.75 (m, 5H, $NHCH_2CH_2(CH_2)_4CH_3$, *H*-C(β)Leu, *H*-C(γ)Leu), 1.82-2.05 (m, 1H, H_a-C(β)Glu),2.09-2.35 (m, 3H, H_b-C(β)Glu, *H*-C(γ)Glu), 3.09-3.30 (m, 2H, $NHCH_2(CH_2)_5CH_3$), 3.87 (m, 2H, CH_2Gly), 4.15-4.45 (m, 2H, CHCOGlu, CHCOLeu), 5.10 (s, 2H, $PhCH_2$), 5.17 (s, 2H, $PhCH_2$), 5.73 (d, *J*=8.2 Hz, 1H, ,N*H*), 6.36 (d, *J* = 6.8 Hz, 1H, N*H*), 6.47 (t, *J* = 5.4 Hz, 1H, N*H*), 6.92 (t, *J* = 5.7 Hz, 1H, N*H*), 7.35 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 14.0 ($NH(CH_2)_6CH_3$), 22.0 (C_a(δ)Leu), 22.5 ($NH(CH_2)_5CH_2CH_3$), 22.8 (C_b(δ)Leu), 24.7 (C(γ)Leu), 26.8 ($NH(CH_2)_4CH_2CH_2CH_3$), 28.2 (C(γ)Glu), 28.9 ($NH(CH_2)_3CH_2(CH_2)_2CH_3$), 29.3 ($NH(CH_2)_2CH_2(CH_2)_3CH_3$), 31.7 ($NHCH_2CH_2(CH_2)_4CH_3$), 31.9 (C(β)Glu), 39.6 ($NHCH_2(CH_2)_5CH_3$), 40.8 (C(β)Leu), 43.0 (C(α)Gly), 52.2 (C(α)Leu), 53.5 (C(α)Glu), 67.0 ($PhCH_2$), 67.3 ($PhCH_2$), 128.0 (Ph), 128.1 (Ph), 128.2 (Ph), 128.5 (Ph), 128.6 (Ph), 135.1 (Ph), 136.1 (Ph), 156.4 (NHCOO), 168.7 (CO), 171.8 (CO), 172.4 (CO), 172.7 (CO). Anal. Calcd. for $C_{35}H_{50}N_4O_7$: C 65.81, H 7.89, N 8.77. Found: C 65.65, H 7.90, N 8.52.

L-γ-Glutamyl-L-leucylglycyl-*N*-(1-heptyl)amide (29). White solid; 100% yield, 171–173 °C; IR (KBr, cm^{-1}) 3311, 3078, 2934, 1640, 1543; ^{1}H-NMR (200 MHz, CD_3OD) δ 0.83-1.00 (m, 9H, C*H*$_3$Leu, NH$(CH_2)_6$C*H*$_3$), 1.23-1.37 (m, 8H, NH$(CH_2)_2(CH_2)_4CH_3$), 1.41-1.80 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.94-2.28 (m, 2H, *H*$_2$-C(β) Glu), 2.40-2.59 (m, 2H, *H*-C(γ)Glu), 3.08-3.27 (m, 2H, NHC*H*$_2(CH_2)_5CH_3$), 3.57-3.70 (m, 1H, C*H*-COLeu), 3.87 (s, 2H, C*H*$_2$Gly), 4.35 (distorted t, *J* = 4.6 Hz, 1H, C*H*COGlu); ^{13}C-NMR (50 MHz, CD_3OD) δ 14.4 (NH$(CH_2)_6$C*H*$_3$), 21.8 (C$_a$ (δ)Leu), 23.5 (NH$(CH_2)_5$C*H*$_2CH_3$),* 23.7 (C$_b$(δ)Leu),* 25.9 (C(γ)Leu), 27.8 (C(γ)Glu), 27.9 (NH$(CH_2)_4$C*H*$_2CH_2CH_3$), 30.1 (NH$(CH_2)_3$C*H*$_2(CH_2)_2CH_3$), 30.4 (NH$(CH_2)_2$C*H*$_2(CH_2)_3CH_3$), 32.6 (C(β)Glu), 40.5 (NHC*H*$_2(CH_2)_5CH_3$), 41.4 (C(β)Leu), 43.5 (C(α)Gly), 53.9 (C(α)Leu), 55.5 (C(α)Glu), 171.4 (CO), 175.4 (CO), 175.7 (CO). Anal. Calcd. for $C_{20}H_{38}N_4O_5.2H_2O$: C 53.32, H 8.50, N 12.44. Found: C 53.34, H 8.63, N 12.12.

***N*-Benzyloxycarbonyl-L-γ-glutamyl(α-benzylester)-L-leucylglycyl-*N*-(1-octyl)amide (30).** White solid; 62% yield; mp 152–154 °C; 3303, 2930, 1747, 1705, 1634, 1536, 754, 705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.81-0.97 (m, 9H, NH$(CH_2)_7$C*H*$_3$, C*H*$_3$Leu), 1.17-1.32 (m, 10H, NH$(CH_2)_2(CH_2)_5CH_3$), 1.38-1.80 (m, 5H, NHCH$_2$C*H*$_2(CH_2)_5CH_3$, *H*$_2$-C(β)Leu, *H*-C(γ)Leu), 1.83-2.07 (m, 1H, *H*$_a$-C(β)Glu), 2.10-2.34 (m, 3H, *H*$_b$-C(β)Glu, *H*-C(γ)Glu), 3.11-3.27 (m, 2H, NHC*H*$_2(CH_2)_6CH_3$), 3.87 (m, 2H, C*H*$_2$Gly), 4.17-4.47 (m, 2H, C*H*CO Glu, C*H*COLeu), 5.09 (s, 2H, PhC*H*$_2$), 5.17 (s, 2H, PhC*H*$_2$), 5.76 (d, *J*=7.8 Hz, 1H, N*H*), 6.41 (d, *J* = 6.1 Hz, 1H, N*H*), 6.49 (t, *J* = 5.7 Hz, 1H, N*H*), 6.90-7.02 (m, *J* = 5.7 Hz, 1H, N*H*), 7.35 (m, 10H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 14.0 (NH$(CH_2)_6$C*H*$_3$), 22.0 (C$_a$(δ)Leu), 22.7 (NH$(CH_2)_6$C*H*$_2CH_3$), 22.9 (C$_b$(δ)Leu), 24.8 (C(γ)Leu), 26.8 (NH$(CH_2)_5$C*H*$_2CH_2CH_3$), 28.3 (C(γ)Glu), 29.2 (NH$(CH_2)_4$C*H*$_2(CH_2)_2CH_3$), 29.3 (NH$(CH_2)_3$C*H*$_2(CH_2)_3CH_3$), 29.4 (NH$(CH_2)_2$C*H*$_2(CH_2)_4CH_3$), 31.8 (NHCH$_2$C*H*$_2(CH_2)_5CH_3$), 32.0 (C(β)Glu), 39.7 (NHC*H*$_2(CH_2)_6CH_3$), 41.0 (C(β)Leu), 43.1 (C(α)Gly), 52.3 (C(α)Leu), 53.6 (C(α)Glu), 67.1 (PhC*H*$_2$), 67.3 (PhC*H*$_2$), 128.0 (Ph), 128.2 (Ph), 128.3 (Ph), 128.5 (Ph), 128.6 (Ph), 135.2 (Ph), 136.2 (Ph), 156.5 (NHC*O*O), 168.7 (CO), 171.9 (CO), 172.5 (CO), 172.9 (CO). Anal. Calcd. for $C_{36}H_{52}N_4O_7$: C 66.23, H 8.03, N 8.58. Found: C 65.97, H 8.02, N 8.43.

L-γ-Glutamyl-L-leucylglycyl-*N*-(1-octyl)amide (31). White solid; 99% yield; mp 166–168 °C; IR (KBr, cm^{-1}) 3305, 3103, 2935, 1646, 1544; ^{1}H-NMR (200 MHz, CD_3OD) δ 0.85-1.05 (m, 9H, C*H*$_3$Leu, NH$(CH_2)_7$C*H*$_3$), 1.27-1.40 (m, 8H, NH$(CH_2)_2(CH_2)_5CH_3$), 1.45-1.84 (m, 3H, *H*-C(β)Leu, *H*-C(γ)Leu), 1.98-2.29 (broad s, 2H, *H*$_2$-C(β)Glu), 2.46-2.60 (m, 2H, *H*-C(γ)Glu), 3.13-3.30 (m, 2H, NHC*H*$_2(CH_2)_6CH_3$), 3.60-3.73 (m, 1H, C*H*COLeu), 3.83 (s, 2H, C*H*$_2$Gly), 4.29 (distorted t, *J* = 7.4 Hz,1H, C*H*COGlu); ^{13}C-NMR (50 MHz, CD_3OD) δ 14.5 (NH$(CH_2)_7$C*H*$_3$), 21.8 (C$_a$(δ)Leu), 23.5 (NH$(CH_2)_6$C*H*$_2CH_3$),* 23.7 (C$_b$(δ)Leu)*, 25.9 (C(γ)Leu), 27.8 (C(γ)Glu), 28.0 (NH$(CH_2)_5$C*H*$_2CH_2CH_3$), 30.4 (NH$(CH_2)_2$(C*H*$_2)_3(CH_2)_2CH_3$), 32.6 (C(β)Glu), 33.0 (NHCH$_2$C*H*$_2(CH_2)_5CH_3$), 40.5 (NHC*H*$_2(CH_2)$-CH_3), 41.3 (C(β)Leu), 43.5 (C(α)Gly), 53.9 (C(α)Leu), 55.3 (C(α)Glu), 171.4 (CO), 175.4 (CO), 175.6 (CO). Anal. Calcd. for $C_{21}H_{40}N_4O_5.1.2H_2O$: C 56.03, H 8.96, N 12.45. Found: C 55.86, H 9.30, N 12.45.

Drug screening

Biological assays on epimastigotes were performed as previously described.[38]

Trypanosoma cruzi epimastigotes (Y strain) were grown in 20 mL screw-cap tubes at 28 °C in a liquid medium containing brain-heart infusion (37 g/L), hemin chlorohydrate (20 mg/L) (dissolved in 50% triethanolamine) and 10% newborn calf serum. The initial inoculum contained 2-3 × 10^6 cells / mL (as determined by counting in a Neubauer chamber) in a final volume of 1 mL. The concentration of cells was determined by measuring the absorbance of the culture medium containing parasites at 600 nm against a blank with culture medium alone. Each drug was tested at five different concentrations (1, 2.5, 5, 10 and 20 μg/mL) each one in quadruplicate. Drugs were dissolved in ethanol. A control without drug was done with each group that was tested. To calculate percent inhibition, the following formula was used:

$$\text{Percent inhibition} = 100 - (\Delta A_d \times 100)/ \Delta A_c,$$

where ΔA_c and ΔA_d are the differences in the absorbance of control cultures and drug-treated cultures, respectively, at the beginning and at the end of the experiment. The maximum amount of solvent used (1% ethanol) did not have any significant effect on the epimastigotes growth. The values of IC_{50} were estimated by linear and polynomial regression.

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