Synthesis and Inhibitory Activities against Enkephalin Degrading Aminopeptidase of H-Trp(Nps)-Lys-OMe Analogues Bearing Chelating Groups

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With the aim of increasing the inhibitory potency of the analgesic dipeptide H-Trp(Nps)-Lys-OMe against enkephalin-degrading aminopeptidases, the following derivatives bearing chelating groups at the N-terminus have been synthesized: Ac-Trp(Nps)-Lys-OMe (**3**), $HS(CH_2)_nCO$-Trp(Nps)-Lys-OMe [n = 1 (**4**), n = 2 (**5**)], $MeOCO(CH_2)_n$-Trp(Nps)-Lys-OMe [n = 1 (**6**), n = 2 (**7**)] and analogues in which the N^{α}-amino group has been replaced by a methoxycarbonyl group (**8**) and a bidentate hydroxamate function (**9**), respectively. The inhibitory activities of all these compounds and the *S*-protected derivatives $EtNHCOS(CH_2)_nCO$-Trp(Nps)-Lys-OMe [n = 1 (**16**), n = (**17**)] against the mentioned enzyme, isolated from rat striatum, are compared with those of the parent dipeptide **2** and bestatin. All the new derivatives showed, in general, inhibitory potencies of the same order of magnitude as compound **2**.

Synthese von H-Trp(Nps)-Lys-OMe Analoga mit Chelatgruppen und deren Hemmung von Enkephalin-abbauenden Aminopeptidasen

Mit dem Ziel, eine Erhöhung der Hemmwirkung des analgetisch wirksamen Dipeptids H-Trp(Nps)-Lys-OMe gegenüber Enkephalin-abbauenden Aminopeptidasen zu erreichen, wurden die folgenden Verbindungen, einige mit Chelatgruppierungen, am "N-Terminus" hergestellt: Ac-Trp(Nps)-Lys-OMe (**3**), $HS(CH_2)_nCO$-Trp(Nps)-Lys-OMe [n = 1 (**4**), n = 2 (**5**)], $MeOCO(CH_2)_n$-Trp(Nps)-Lys-OMe [n = 1 (**6**), n = 2 (**7**)] und Analoga, bei denen die N^{α}-Aminogruppe durch eine Methoxycarbonylgruppe (**8**), beziehungsweise durch eine doppelzähnige Hydroxamatfunktion (**9**) ersetzt worden ist. Die Hemmung dieser Enzyme, isoliert aus Ratten-striatum, durch die neuen Verbindungen und durch die *S*-geschützten Derivate $EtNHCOS(CH_2)_nCO$-Trp(Nps)-Lys-OMe [n = 1 (**16**), n = 2 (**17**)], wird mit der des Bezugspeptids **2** und Bestatin verglichen. Alle neuen Verbindungen weisen im allgemeinen Hemmwirkungen mit, die in der gleichen Größenordnung wie die von Substanz **2** liegen.

We have reported[1,2] that intracerebroventricular administration of the synthetic dipeptide derivative H-Lys-Trp(Nps)-OMe (**1**) and the reverse sequence analogue H-Trp(Nps)-Lys-OMe (**2**) shows a naloxone-reversible antinociceptive effect comparable with that of the enkephalin analogue D-Ala^2-Met-enkephalinamide (DAME). Studies on the mechanism of action of these compounds appear to indicate that they not act directly on opioid receptors, but their antinociceptive effects could be possibly explained by a mixture of aminopeptidases (APs) inhibiting and Met-enkephalin-releasing properties[1]. Bestatin is the most frequently used APs inhibitor in enkephalin metabolism studies[3]. Other natural products showing APs inhibiting properties are amastatin[4] and actinonin[5]. The presence of the 3-amino-2-hydroxy acid residue in bestatin and amastatin is critical for binding to APs[4] while the inhibitory potency of actinonin seems to be related to the presence of the hydroxamate group[5]. Some synthetic APs inhibitors include thiobestatin[3] in which the C-2 hydroxyl group of bestatin has been replaced by a sulfhydryl group, amino acid hydroxamic acids[6,7], and α-mercapto ketones derived from amino acids[7].

Based on that enkephalin degrading AP has properties that suggest it to be a zinc metallopeptidase and with the aim of improving the moderate inhibitory potency of compound **2** against this enzyme, we have prepared a series of analogues bearing different zinc chelating groups (Scheme 1)*). This paper describes the synthesis of Ac-Trp(Nps)-Lys-OMe (**3**), *N*-mercaptoalkanoyl dipeptide derivatives **4** and **5**, *N*-carboxyalkyl analogues **6** and **7** and compounds **8** and **9** in which the Nα-amino group has been replaced by a methoxycarbonyl group and a bidentate hydroxamate function, respectively. The inhibitory activities of all these compounds and *S*-protected derivatives **16** and **17** against enkephalin degrading AP, isolated from rat striatum, are compared with those of compound **2** and bestatin.

Results and Discussion

The synthesis of compound **10**, common intermediate for the preparation of both *N*-acyl and *N*-alkyl derivatives **3-7**, was achieved in one step by treatment of Boc-Trp-Lys(Z)-OMe with *o*-nitrophenylsulfenyl chloride (Nps-Cl) in 2N HCl-MeOH. This acidic medium was employed for the sulfenylation[8] and *N*-deprotection reactions in order to minimize the *tert*-butylation reactions of the indole ring, which occur when trifluoroacetic acid is used for the removal of Boc protecting groups[9]. Treatment of **10** with Ac_2O in the presence of *N*,*N*-dimethylaminopyridine (DMAP) afforded the *N*-acetyl derivative **11**, which was deprotected

*) The C=O double bond of amide, ester, and ketone moieties has the capacity to chelate metallic ions, probably through an hydrated tetrahedral intermediate, as demonstrated in the case of carbonyl-derived inhibitors of ACE [*R.G. Almquist et al.*, J. Med. Chem. *28*, 1067 (1985)]. In this way, the CH_3CONH and the CO_2CH_3 groups, at the N-terminus of compounds **3** and **6-8**, can be considered as chelating groups. On the other hand, as all reported compounds are to be tested *in vivo* as antinociceptive agents, it is expected that, under physiological conditions, the methoxycarbonyl group of compounds **6-8** will be hydrolyzed to the corresponding CO_2H group, with higher chelating properties. This really happens with the C-terminal ester in the parent compound.

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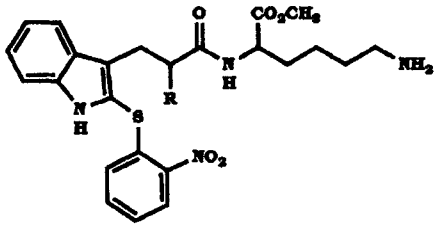


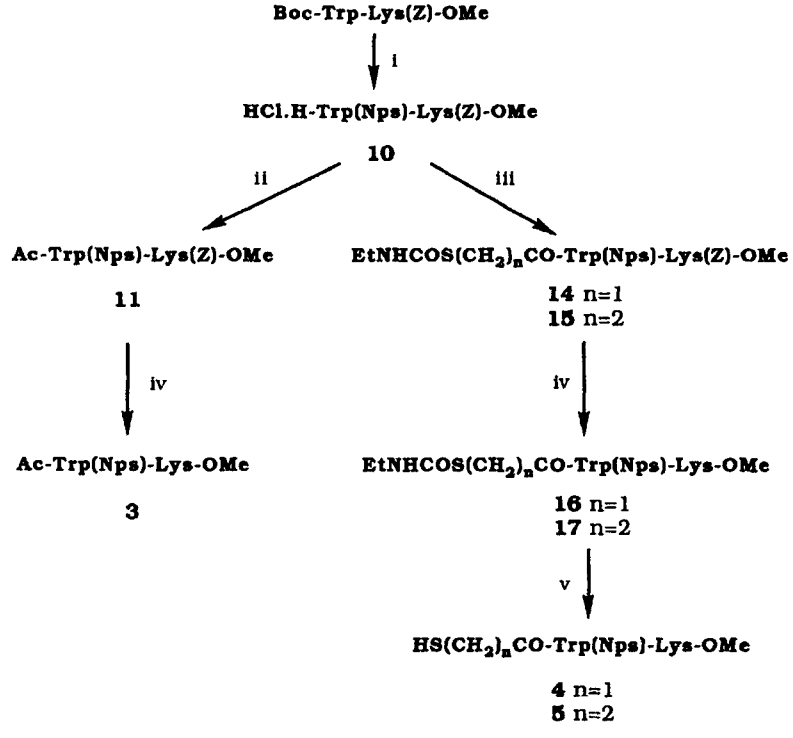
2: R = NH_2
3: R = $NHCOCH_3$
4: R = $NHCOCH_2SH$
5: R = $NHCO(CH_2)_2SH$
6: R = $NHCH_2CO_2CH_3$
7: R = $NH(CH_2)_2CO_2CH_3$
8: R = CO_2CH_3
9: R = CONHOH

H-Lys-Trp(NPS)-OMe **(1)**

with trimethylsilyliodide (ITMS)[10], to give the analogue **3**. N^{α}-mercaptoalkanoyl derivatives **4** and **5** were prepared by reaction of the dipeptide **10** with the *N*-hydroxysuccinimide active esters of *S*-ethylcarbamoylacetic acid **12** and *S*-ethylcarbamoylpropionic acid **13**, respectively, to give compounds **14** and **15**, followed by removal of the Z- and ethylcarbamoyl groups by successive treatment with ITMS and $NaOCH_3$[11] (Scheme 2). Synthetic intermediates **12** and **13** were prepared by reaction of mercaptoacetic and mercaptopropionic acids with ethyl isocyanate[11] and subsequent activation of the *S*-protected derivatives with HOSu-DCC. *N*-Alkylation of compound **10** with methyl bromoacetate or methyl bromopropionate, using triethylamine as a base, afforded the substituted derivatives **18** and **19**, which were treated with ITMS to give the N^{α}-carboxyalkyl derivatives **6** and **7** (Scheme 3).

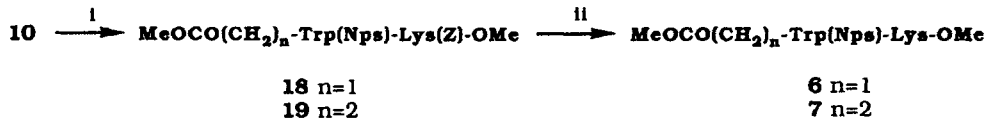
Compounds **8** and **9**, in which the N-terminal group has been replaced by a methoxycarbonyl and a hydroxamate group, respectively, were prepared from the propionic acid

Scheme 1



Reagents and conditions: i) 2N HCl/MeOH/Nps-Cl; ii) Ac_2O/DMAP; iii) $EtNHCOS(CH_2)nCO_2Su$ (n=1, 12; n=2, 13); iv) ITMS/CH_3CN; v) NaOMe/MeOH

Scheme 2

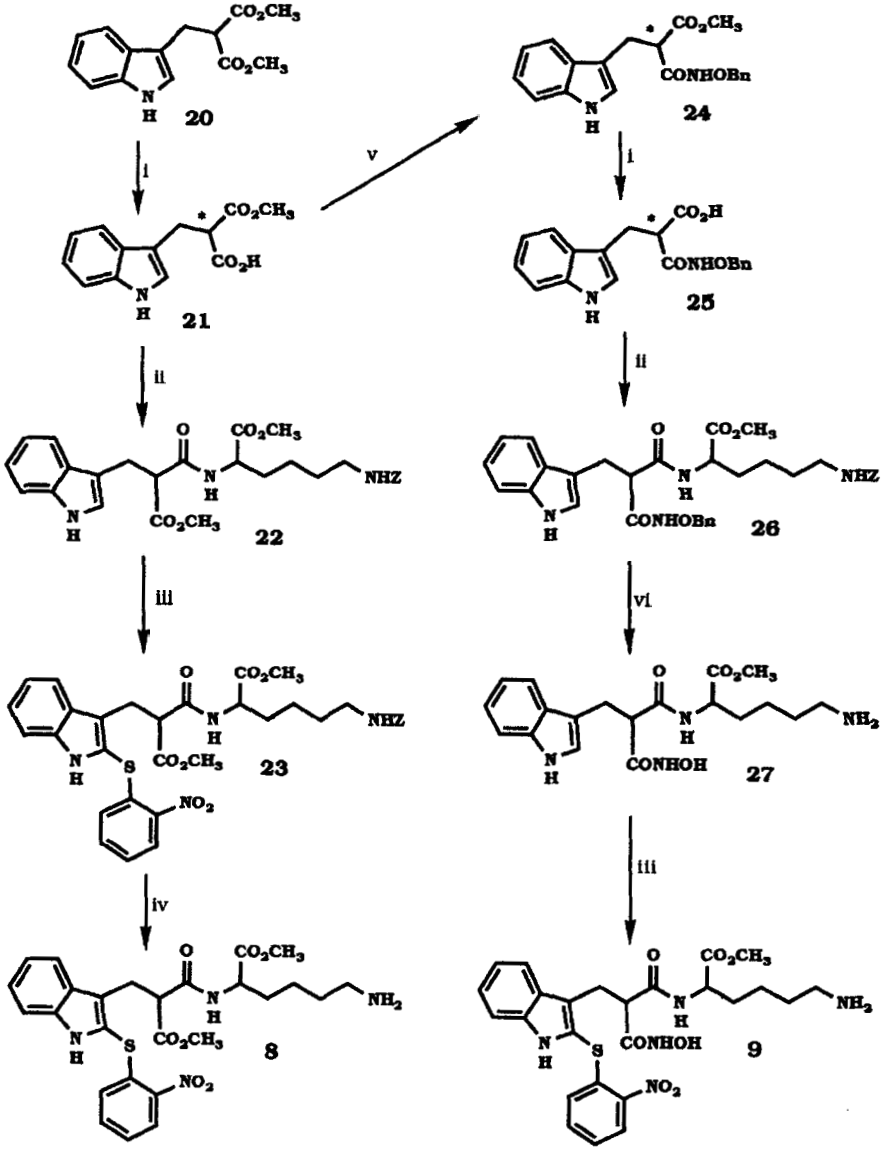


Reagents and conditions: i) $Br(CH_2)nCO_2CH_3$(n=1,2)/TEA; ii) ITMS/CH_3CN

Scheme 3

derivative **21**, obtained from its dimethyl ester **20**[12], as indicated in Scheme 4. Thus, compound **21** was coupled with H-Lys(Z)-OMe, employing the DCC/HOSu procedure, to give **22** as a mixture of two diastereoisomers due to the racemic malonic moiety. Reaction of **22** with Nps-Cl in acidic medium afforded the Trp(Nps)-containing analogue **23**, which on treatment with ITMS, as deblocking agent for the cleavage of the Z-group, provided the desired compound **8**. On the other hand, treatment of the common intermediate **21** with *O*-benzylhydroxylamine in the presence of DCC, gave the derivative **24**, which was saponified and coupled to H-Lys(Z)-OMe to provide **26**. Removal of the Z-group of **26** followed by sulfenylation of the resulting dipeptide analogue **27** afforded **9**. All compounds **8**, **9**, and **22-27** were obtained as mixtures of two diastereoisomers that could not be separated due to easy racemization of monoesters of 2-substituted malonic acid that prevents their resolution by classical methods[13]. Structural assignments of all the compounds here reported were made on the basis of their analytical and spectroscopic data.

The ^{1}H-NMR spectra of all the Trp(Nps)-containing compounds show a significant shielding of the phenyl H-6 ($\delta \equiv 6.6$ ppm) of the Nps group (Table 2). This shielding, which is identical to that previously observed in **2** and in all the related Trp(Nps) containing dipeptides[2,14,15], seems to be due to the adoption of a preferential conformation in which the phenyl and indole rings are not coplanar[15].



Reagents and conditions: i) NaOH/MeOH; ii) HOSu/DCC/HCl·H-Lys(Z)-OMe/TEA; iii) NPS-Cl/HCl; iv) ITMS/CH_3CN; v) HOSu/DCC/HCl·H_2NOBn/TEA; vi) H_2/Pd-C/EtOH

Scheme 4

Dipeptide derivatives **3-9** were evaluated as inhibitors of purified membrane-bound AP from rat brain[16], and the results compared with those of **2** and bestatin. As shown in Table 1, this series of dipeptide derivatives bearing chelating groups presented inhibitory potencies of the same order of magnitude as the model compound **2**, being in general modest inhibitors of enkephalin degrading AP when compared to bestatin. Although the necessity of a free amino group has been reported for other AP inhibitors for activity[17], this does not seem to be an important factor for the inhibitory potency of this series of compounds, as deduced from the activity of compound **2** and its acetyl derivative **3**. The low influence of the chelating moieties on the activity could be related to an inadequate position of these groups in the dipeptide molecule, that does not allow an efficient binding to the Zn ion present in the active site of the enzyme.

Table 1: Inhibition of enkephalin-degrading aminopeptidase from rat striatum by Trp(Nps)-Lys-OMe analogues **3-9**, **16**, and **17**

Compound	R	IC_{50}
2	NH_2	2.0×10^{-5}
3	$NHCOCH_3$	3.0×10^{-5}
4	$NHCOCH_2SH$	1.0×10^{-4}
5	$NHCOCH_2CH_2SH$	— [a]
6	$NHCH_2CO_2CH_3$	1.1×10^{-4}
7	$NHCH_2CH_2CO_2CH_3$	1.0×10^{-5}
8	CO_2CH_3	1.7×10^{-4}
9	CONHOH	3.0×10^{-5}
16	$NHCOCH_2SCONHEt$	6.0×10^{-5}
17	$NHCOCH_2CH_2SCONHEt$	1.3×10^{-4}
Bestatin	—	3.0×10^{-7}

Results are the mean of 4-5 experiments with 3-4 different concentrations of the inhibitor. S.E. were less than 10% of the mean.

[a] Not determined due to problems in the sample preparation.

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Experimental Part

Elemental analyses: Heraeus CHN-O-RAPID instrument.- Melting points: Kofler hot-stage apparatus and are uncorrected.- ^{1}H-NMR spectra: Varian EM-390, XL-300, and Brucker AM-200 spectrometers, TMS int. stand.- Analytical TCL: Al-sheets, 0.2 mm layer of silica gel 60 F_{254} (Merck).- Column chromatography: silica gel 60 (230-400 mesh) (Merck). Compounds were detected with UV light (254 nm) and ninhydrin spray.- Chemical shifts in δ (ppm).

Boc-Trp-Lys(Z)-OMe: prepared as described[18].

Methyl 3-(indole-3-yl)-2-methoxycarbonylpropionate: prepared as described[12].

*HCl · Trp(Nps)-Lys(Z)-OMe (**10**)*

Boc-Trp-Lys(Z)-OMe (2.3 g, 4.2 mmol) was dissolved in 2N HCl/MeOH solution (30 mL) and stirred at room temp. for 10 min. Then, NpsCl (0.81 g, 4.3 mmol) was added and stirring was continued for 1 h. After evaporation of the solvent the residue was purified by column chromatography (cc) using $CHCl_3$: MeOH 10:1: 2.2 g (88%), yellow foam.- $C_{32}H_{36}ClN_5OS$ (670.2) Calc. C 57.3 H 5.41 Cl 5.3 N 10.4 S 4.8 Found C 57.7 H 5.23 Cl 5.0 N 10.7 S 4.8.- ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.80 (s, 1H, NH^i), 8.22 (dd, J_1 = 8.1 Hz, J_2 = 1.3 Hz, 1H, Nps 3-H), 7.77-7.10 (m, 6H, indole, Nps

Table 2: ^{1}H-NMR data of compounds **3-9**, **16**, and **17** [DMSO-d_6, 300 MHz, δ (ppm)]

Compd.	R	3-Nps	6-Nps	α-Trp	β-Trp	α-Lys	ε-Lys	R
3	$NHCOCH_3$	8.27	6.64	4.62	3.19 3.03	4.22	2.73	1.63 (s, 3H, $COCH_3$)
4	$NHCOCH_2SH$	8.26	6.61	4.66	3.23 3.02	4.21	2.77	2.94 (m, 2H, $COCH_2SH$)
5	$NHCOCH_2CH_2SH$	8.24	6.62	4.63	3.32 3.01	4.19	2.74-2.56	2.74-2.56 (m, 4H, $COCH_2CH_2SH$) 2.33 (m, 2H, $COCH_2CH_2SH$)
6	$NHCH_2CO_2CH_3$	8.27	6.57	4.14	3.33	4.14	2.60	3.43 (s, 3H, CO_2CH_3) 3.88 (m, 2H, $CH_2CO_2CH_3$)
7	$NHCH_2CH_2CO_2CH_3$	8.27	6.57	4.17	3.26	4.17	2.70	3.40 (s, 3H, CO_2CH_3) 3.05 , 2.70 (2m, 4H, $CH_2CH_2CO_2CH_3$)
8 [a]	CO_2CH_3	8.26	6.65	3.82 3.81	3.26	4.17 4.02	2.71 2.69	3.49, 3.42 (2s, 6H, CO_2CH_3)
9 [a]	CONHOH	8.28	6.62	3.44 3.38	3.28	4.10 4.05	2.70 2.57	
16	$NHCOCH_2SCONHEt$	8.23	6.63	4.61	3.20	4.24	2.74	0.99 (t., 3H, $CONHCH_2CH_3$)
17	$NHCOCH_2SCONHEt$	8.29	6.64	4.63	3.09	4.21	2.76	1.00 (t, 3H, $CONHCH_2CH_3$) 2.27 (m, 2H, $COCH_2CH_2S$)

[a] Mixture of two diastereomers.

4- and 5-H), 7.30 (s, 5H, Z C_6H_5), 6.66 (dd, J_1 = 7.7 Hz, J_2 = 0.8 Hz, 1H, Nps 6-H), 5.04 (s, 2H, Z CH_2), 4.45 (m, 1H, Lys, α-CH), 3.75 (m, 1H, Trp α-CH), 3.67 (s, 3H, CO_2CH_3), 3.39 and 3.13 (2dd, J_1 = 13.7 Hz, J_2 = 5.7 and 8.2 Hz, 2H, Trp β-CH_2), 3.05 (m, 2H, Lys ε-CH_2), 1.70-0.93 (m, 6H, Lys β-, γ- and δ-CH_2).

Ac-Trp(Nps)-Lys(Z)-OMe (11)

To a solution of compound **10** (2 g, 2.9 mmol) in Ac_2O (20 mL), DMAP (0.37 g, 3.1 mmol) was added. After stirring at room temp. for 30 min, the mixture was poured into ice H_2O (50 mL) and extracted with $CHCl_3$ (2 x 50 mL). The org. extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by cc using $CHCl_3$:MeOH 8:1: 1.7 g (87%).- $C_{34}H_{37}N_5O_8S$ (675.8) Calc. C 60.4 H 5.52 N 10.4 S 4.7 Found C 60.2 H 5.51 N 10.3 S 4.4.- ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.38 (s, 1H, NH^i), 8.22 (dd, 1H, Nps 3-H), 7.70-7.08 (m, 6H, indole, Nps 4- and 5-H), 7.29 (s, 5H, Z C_6H_5), 6.67 (dd, 1H, Nps 6-H), 5.00 (s, 2H, Z CH_2), 4.68 (m, 1H, Trp α-CH), 4.44 (m, 1H, Lys α-CH), 3.66 (s, 3H, CO_2CH_3), 3.28 (m, 2H, Trp β-CH_2), 3.05 (m, 2H, Lys ε-CH_2), 1.75-0.98 (m, 6H, Lys β-, γ- and δ-CH_2).

(S-Ethylcarbamoyl)mercaptoacetic acid

To a solution of mercaptoacetic acid (2 mL, 2.8 mmol) in DMF (30 mL), ethyl isocyanate (2.4 mL, 3.0 mmol) was added. After stirring at room temp. for 3 days and evaporation of the solvent, the residue was recrystallized from Et_2O:hexane: 3.14 g (71%).- m.p. 111-112°C.- ^{1}H-NMR ($CDCl_3$, 90 MHz): 6.20 (m, 1H, NH), 3.70 (s, 2H, SCH_2CO), 3.32 (m, 2H, $C\underline{H}_2CH_3$), 1.16 (t, J = 7 Hz, 3H, $CH_2C\underline{H}_3$).

3-(S-Ethylcarbamoyl)mercaptopropionic acid

This compound was synthesized from 3-mercaptopropionic acid following the above procedure. Yield 2.8 g (56%).- ^{1}H-NMR ($CDCl_3$, 90 MHz): 6.20 (m, 1H, NH), 3.40-2.80 (m, 4H, $CH_3C\underline{H}_2NHCOSC\underline{H}_2$), 2.66 (t, J = 7.5 Hz, 2H, CH_2CO), 1.13 (t, J = 7 Hz, 3H, $C\underline{H}_3CH_2$).

General procedure for the synthesis of succinic esters 12 and 13

A solution of *S*-ethylcarbamoyl derivatives (6.1 mmol) in THF (30 mL) was treated with *N*-hydroxysuccinimide (6.7 mmol) and DCC (6.7 mmol). The mixture was stirred for 1 day, the DCU was filtered off and the solvent evaporated to dryness. The residues were recrystallized from EtOAc:hexane.

Succinyl (S-ethylcarbamoyl)mercaptoacetate (12)

Yield 77%.- m.p. 108-110°C.- ^{1}H-NMR ($CDCl_3$, 90 MHz): 5.70 (m, 1H, NH), 4.00 (s, 2H, SCH_2CO), 3.30 (m, 2H, $CH_3C\underline{H}_2$), 2.80 (s, 4H, $COCH_2CH_2CO$), 1.20 (t, 3H, $C\underline{H}_3CH_2$).

Succinyl (S-ethylcarbamoyl)mercaptopropionate (13)

Yield 83%.- m.p. 142-144°C.- ^{1}H-NMR ($CDCl_3$, 90 MHz): 5.56 (m, 1H, NH), 3.40-2.80 (m, 6H, SCH_2CH_2CO and $CH_3C\underline{H}_2$), 2.80 (s, 4H, $COCH_2CH_2CO$), 1.13 (t, J = 7 Hz, 3H, $C\underline{H}_3CH_2$).

N-[(S-ethylcarbamoyl)mercaptoalkanoyl] dipeptides

General procedure

To a solution of dipeptide derivative **10** (1.1 mmol) in THF (20 mL), triethylamine (1.1 mmol) and compounds **12** or **13** (1.1 mmol) were added. After stirring for 1 h, the solvent was eliminated and the residue was chromatographed on a silica gel column using $CHCl_3$:acetone 7:1. By this procedure the following compounds were obtained:

N-[(S-Ethylcarbamoyl)mercaptoacetyl]-Trp(Nps)-Lys(Z)-OMe (14)

Yield 73%.- $C_{37}H_{42}N_6O_9S_2$ (778.9) Calc. C 57.1 H 5.43 N 10.8 S 8.2 Found C 56.9 H 5.46 N 10.4 S 8.0.- ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.60 (s, 1H, NH^i), 8.22 (dd, 1H, Nps, 3-H), 7.70-7.10 (m, 6H, indole, Nps 4- and 5-H), 7.30 (s, 5H, Z C_6H_5), 6.67 (dd, 1H, Nps 6-H), 5.01 (s, 2H, Z CH_2), 4.65 (m, 1H, Trp α-CH), 4.46 (m, 1H, Lys α-CH), 3.70 (s, 3H, CO_2CH_3), 3.43-3.10 (m, 8H, Trp β-CH_2, Lys ε-CH_2, $COCH_2S$, $C\underline{H}_2CH_3$), 1.80-0.90 (m, 6H, Lys β-, γ- and δ-CH_2), 1.10 (t, 3H, $CH_2C\underline{H}_3$).

N-[(S-Ethylcarbamoyl)mercaptopropanoyl]-Trp(Nps)-Lys(Z)-OMe (15)

Yield 68%.- $C_{38}H_{44}N_6O_9S_2$ (792.9) Calc. C 57.6 H 5.59 N 10.6 S 8.1 Found C 57.3 H 5.70 N 10.3 S 8.0.- ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.61 (s, 1H, NH^i), 8.21 (dd, 1H, Nps, 3-H), 7.65-6.98 (m, 6H, indole, Nps 4- and 5-H), 7.25 (s, 5H, Z C_6H_5), 6.65 (dd, 1H, Nps 6-H), 5.05 (s, 2H, Z CH_2), 4.72 (m, 1H, Trp α-CH), 4.46 (m, 1H, Lys α-CH), 3.68 (s, 3H, CO_2CH_3), 3.34-2.98 (m, 8H, Trp β-CH_2, Lys ε-CH_2, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_3$), 2.43 (t, J = 6.9 Hz, 2H, $COC\underline{H}_2CH_2$), 1.90-1.20 (m, 6H, Lys β-, γ- and δ-CH_2), 1.09 (t, 3H, $CH_2C\underline{H}_3$).

N-carboxyalkyl dipeptides

General procedure

To a solution of compound **10** (4.8 mmol) and triethylamine (7.2 mmol) in MeOH (40 mL), methyl bromoacetate or methyl 3-bromopropionate (9.6 mmol) and NaI (9.6 mmol) were added. The reaction mixture was refluxed for 12 h and then evaporated to dryness. The residue was extracted with EtOAc (50 mL) and washed with H_2O (50 mL). The org. layer was dried (Na_2SO_4), filtered, and evaporated to give a residue, which was purified by cc using $CHCl_3$:MeOH 20:1.

The following compounds were obtained by this method:

N-(Methoxycarbonylmethyl)-Trp(Nps)-Lys(Z)-OMe (18)

Yield 78%.- $C_{35}H_{39}N_5O_9S$ (705.8) Calc. C 59.6 H 5.57 N 9.9 S 4.5 Found C 59.7 H 5.59 N 9.9 S 4.6.- ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.75 (s, 1H, NH^i), 8.24 (dd, 1H, Nps, 3-H), 7.70-7.10 (m, 6H, indole, Nps 4- and 5-H), 7.30 (s, 5H, Z C_6H_5), 6.66 (dd, 1H, Nps 6-H), 5.02 (s, 2H, Z CH_2), 4.43 (m, 1H, Lys α-CH), 3.54-3.22 (m, 5H, Trp α-CH and β-CH_2, $C\underline{H}_2CO_2CH_3$), 3.66 (s, 3H, CO_2CH_3), 3.58 (s, 3H, $CH_2CO_2C\underline{H}_3$), 3.05 (m, 2H, Lys ε-CH_2), 1.90-1.00 (m, 6H, Lys β-, γ- and δ-CH_2).

N-(2-Methoxycarbonylethyl)-Trp(Nps)-Lys(Z)-OMe (19)

Yield 87%.- $C_{36}H_{41}N_5O_9S$ (719.8) Calc. C 60.1 H 5.74 N 9.7 S 4.4 Found C 59.8 H 5.66 N 9.4 S 4.8.- ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.80 (s, 1H, NH^i), 8.23 (dd, 1H, Nps, 3-H), 7.72-7.12 (m, 6H, indole, Nps 4- and 5-H), 7.30 (s, 5H, Z C_6H_5), 6.66 (dd, 1H, Nps 6-H), 5.00 (s, 2H, Z CH_2), 4.38 (m, 1H, Lys α-CH), 3.66 (s, 3H, CO_2CH_3), 3.58 (s, 3H, $CH_2CH_2CO_2C\underline{H}_3$), 3.48 (m, 1H, Trp α-CH), 3.25 (m, 2H, Trp β-CH_2), 3.05 (m, 2H, Lys ε-CH_2), 2.76 (m, 2H, $CH_2C\underline{H}_2CO_2CH_3$), 2.32 (m, 2H, $C\underline{H}_2CH_2CO_2CH_3$), 1.80-0.90 (m, 6H, Lys β-, γ-, and δ-CH_2).

General procedure for the removal of the Z group

To a solution of the corresponding Z-protected dipeptide derivative (5 mmol) in dry acetonitrile, trimethylsilyliodide (5 mmol) was added at room temp. After 20 min of stirring, MeOH (3 mL) was added and the solvents were evaporated. The residue was purified by cc using $CHCl_3$:MeOH 6:1. By this method the following compounds were prepared:

Ac-Trp(Nps)-Lys-OMe (3)

Yield 78% (from **11**).- $C_{26}H_{31}N_4O_6S$ (527.6) Calc. C 59.2 H 5.92 N 10.6 S 6.1 Found C 59.4 H 5.61 N 10.2 S 5.9.

N-[(S-Ethylcarbamoyl)mercaptoacetyl]-Trp(Nps)-Lys-OMe (16)

Yield 82% (from **12**).- $C_{29}H_{36}N_5O_7S_2$ (630.8) Calc. C 55.2 H 5.75 N 11.1 S 10.2 Found C 55.5 H 5.89 N 10.9 S 9.9.

N-[3-(S-Ethylcarbamoyl)mercaptopropanoyl]-Trp(Nps)-Lys-OMe (17)

Yield 95% (from **13**).- $C_{30}H_{38}N_5O_7S_2$ (644.8) Calc. C 55.9 H 5.94 N 10.9 S 9.9 Found C 56.1 H 5.93 N 10.6 S 10.0.

N-(Methoxycarbonylmethyl)-Trp(Nps)-Lys-OMe (6)

Yield 92% (from **20**).- $C_{27}H_{33}N_4O_7S$ (557.6) Calc. C 58.15 H 5.96 N 10.0 S 5.7 Found C 58.4 H 5.82 N 10.0 S 6.0.

N-[2-(Methoxycarbonyl)ethyl]-Trp(Nps)-Lys-OMe (7)

Yield 90% (from **21**).- $C_{28}H_{35}N_4O_7S$ (571.7) Calc. C 58.8 H 6.17 N 9.8 S 5.6 Found C 58.5 H 6.18 N 9.5 S 5.9.

^{1}H-NMR data of all compounds so obtained are listed in Table 2.

Removal of the ethylcarbamoyl protecting group

N_2 was bubbled for 1 h through a solution of the dipeptides **16** or **17** (4.5 mmol) in MeOH (20 mL). Then, a 0.5 M $NaOCH_3$/MeOH solution (9.0 mmol) was added and stirring continued at room temp. under N_2 for 3 h. The mixture was acidified with AcOH to pH = 7 and the solvents evaporated. The residue was purified by cc using $CHCl_3$:MeOH 5:1 to give the following compounds:

N-(Mercaptoacetyl)-Trp(Nps)-Lys-OMe (4)

Yield 69% (from **14**).- $C_{26}H_{31}N_4O_6S_2$ (559.7) Calc. C 55.8 H 5.54 N 10.0 S 11.4 Found C 55.8 H 5.26 N 9.9 S 11.7.

N-(3-Mercaptopropanoyl)-Trp(Nps)-Lys-OMe (5)

Yield 65% (from **15**).- $C_{27}H_{33}N_4O_6S_2$ (573.7) Calc. C 56.5 H 5.80 N 9.8 S 11.2 Found C 56.2 H 5.64 N 9.4 S 11.0.

^{1}H-NMR data of **4** and **5**: Table 2.

3-(Indole-3-yl)-2(RS)-methoxycarbonylpropionic acid (21)

To a solution of compound **20** (10 g, 38 mmol) in MeOH (50 mL), 6N NaOH (6.3 mL, 38 mmol) was added at room temp. After 2 h of reaction, MeOH was evaporated, H_2O (50 mL) was added, the mixture acidified with N HCl to pH = 3 and extracted with EtOAc (2 x 50 mL). The org. layer was dried over Na_2SO_4 and evaporated to dryness. The product was purified by cc using $CHCl_3$:MeOH 6:1: 8.5 g (91%).- ^{1}H-NMR (DMSO-d_6, 90 MHz): 11.6 (s, 1H, NH), 7.70-6.90 (m, 5H, indole), 3.73 (t, J = 6.5 Hz, 1H, 2-H), 3.66 (s, 3H, CO_2CH_3), 3.40 (m, 2H, 3-H).

N-[3-(Indole-3-yl)-2(RS)-methoxycarbonylpropanoyl]-Lys(Z)-OMe (22)

A solution of compound **21** (1.36 g, 5.5 mmol), *N*-hydroxysuccinimide (0.63 g, 5.5 mmol), and DCC (1.13 g, 5.5 mmol) in THF was stirred at room temp. for 2 h. Then, HCl · Lys(Z)-OMe (1.82 g, 5.5 mmol) and triethylamine (0.42 mL, 5.5 mmol) were added. After 2 days of reaction, the DCU was filtered off and the solvent evaporated. The residue was extracted with EtOAc (50 mL) and washed with 10% citric acid, 10% $NaCO_3H$ and brine. The org. layer was dried over Na_2SO_4, evaporated and the product purified by cc using EtOAc:hexane 1:1: 1.76 g (61%).- $C_{28}H_{33}N_3O_7$ (523.6) Calc. C 64.2 H 6.35 N 8.0 Found C 64.6 H 5.41 N 7.9.- ^{1}H-NMR (DMSO-d_6, 200 MHz): 10.81 and 10.76 (2s, 1H, NH^i), 8.54 and 8.46 (2d, J = 7.1 and 7.2 Hz, 1H, CONH), 7.55-6.96 (m, 5H, indole), 7.33 (s, 5H, Z C_6H_5), 5.00 (s, 2H, Z CH_2), 4.16 and 4.05 (2m, 1H, Lys α-CH), 3.77 (t, J = 7.0 Hz, 1H, CHCONH), 3.60, 3.59, 3.58 and 3.56 (4s, 6H, CO_2CH_3), 3.16 (m, 2H, indole CH_2), 2.97 and 2.89 (2m, 2H, Lys ε-CH_2), 1.58-1.01 (m, 6H, Lys β-, γ- and δ-CH_2).

N-[3-[2-(o-Nitrophenylsulfenyl)indole-3-yl]-2(RS)-methoxycarbonylpropanoyl]-Lys(Z)-OMe (23)

Compound **22** (1 g, 2 mmol) was dissolved in 2N HCl/THF (30 mL) and Nps-Cl (0.6 g, 3 mmol) was added. After stirring at room temp. for 1 h, the solvent was eliminated and the residue was purified by cc using EtOAc:hexane 3:2: 1.2 g (91%).- $C_{34}H_{36}N_4O_9S$ (676.7) Calc. C 60.3 H 5.36 N 8.3 S 4.7 Found C 60.4 H 4.98 N 8.1 S 4.7.- ^{1}H-NMR (DMSO-d_6, 200 MHz): 11.44 (s, 1H, NH^i), 8.45 and 8.40 (2d, J = 7.3 and 7.1 Hz, 1H, CONH), 8.26 (d, 1H, Nps 3-H), 7.76-7.01 (m, 6H, indole, Nps 4- and 5-H), 7.32 (s, 5H, Z C_6H_5), 6.62 (d, 1H, Nps 6-H), 4.97 (s, 2H, Z CH_2), 4.16 and 4.00 (2m, 1H, Lys α-CH), 3.83 and 3.82 (2t, J = 7.2 Hz, 1H, CHCONH), 3.55, 3.52, 3.48 and 3.41 (4s, 6H, CO_2CH_3), 3.28 (m, 2H, indole CH_2), 2.93 and 2.81 (2m, 2H, Lys ε-CH_2), 1.60-1.00 (m, 6H, Lys β-, γ- and δ-CH_2).

N-[3-[2-(o-Nitrophenylsulfenyl)indole-3-yl]-2(RS)-methoxycarbonylpropanoyl]-Lys-OMe (8)

To a solution of compound **23** (0.5 g, 0.7 mmol) in acetonitrile (10 mL) trimethylsilyliodide (0.16 mL, 1.1 mmol) was added. After 20 min of reaction at room temp., MeOH (1 mL) was added and the solvent eliminated *in vacuo*. The residue was purified by cc using $CHCl_3$:MeOH 3:1: 0.31 g (86%).- $C_{26}H_{30}N_3O_7S$ (528.6) Calc. C 59.1 H 5.72 N 7.9 S 6.1 Found C 58.8 H 5.88 N 7.9 S 6.3.- ^{1}H-NMR data: Table 2.

Methyl 2-(RS)-(N-benzyloxycarbamoyl-3-(indole-3-yl)propanoate (24)

To a solution of compound **21** (2.2 g, 8.9 mmol) in THF (30 mL), *O*-benzylhydroxylamine hydrochloride (1.42 g, 8.9 mmol), triethylamine (1.23 g, 17.8 mmol) and dicyclohexylcarbodiimide (1.83 g, 8.9 mmol) were added at room temp. After 2 h, DCU was filtered off and the solvent evaporated. The residue was extracted with EtOAc (100 mL) and washed with water (2 x 50 mL). The org. layer was dried over Na_2SO_4, filtered and evaporated to give a syrup which was purified by cc using EtOAc:hexane 1:2: 2.4 g (85%).- $C_{20}H_{20}N_2O_4$ (352.4) Calc. C 68.2 H 5.72 N 7.9 Found C 67.9 H 5.81 N 7.6.- ^{1}H-NMR (DMSO-d_6, 200 MHz): 11.5 (s, 1H, NH^i), 7.54-6.97 (m, 10 H, indole, benzyl C_6H_5), 4.66 and 4.53 (2d, J = 11.0 Hz, 2H, benzyl CH_2), 3.61 (s, 3H, CO_2CH_3), 3.45 (dd, J_1 = 8.5 Hz, J_2 = 6.5 Hz, 1H, 2-H), 3.16 (m, 2H, 3-H).

2-(RS)-(N-Benzyloxy)carbamoyl-3-(indole-3-yl)propanoic acid (25)

To a solution of compound **24** (2 g, 5.6 mmol) in MeOH (50 mL), 6N NaOH (1.8 mL, 11.3 mmol) was added. The reaction mixture was stirred at room temp. for 20 h. The solution was concentrated *in vacuo*, dissolved in water (30 mL), and washed with EtOAc (2 x 30 mL). The aqueous layer was acidified with 6N HCl to pH 4 and extracted with EtOAc (2 x 50 mL). The org. extracts were dried (Na_2SO_4), filtered and evaporated: 1.7 g (94%) of crude **25** which was used in the following step without further purification.

N-[2(RS)-(N-Benzyloxy)carbamoyl-3-(indole-3-yl)propanoyl]-Lys(Z)-OMe (26)

To a solution of compound **25** (1.6 g, 4.9 mmol) in THF (30 mL), *N*-hydroxysuccinimide (0.57 g, 4.9 mmol) and DCC (1.02 g, 4.9 mmol) were

added. After 30 min of stirring, HCl · H-Lys(Z)-OMe (1.64 g, 4.9 mmol) and triethylamine (0.35 mL, 4.9 mmol) were added. The reaction mixture was stirred overnight at room temp. and then worked up as described for **22**. The obtained residue was purified by cc using $CHCl_3$:MeOH 10:1: 1.8 g (62%).- $C_{34}H_{38}N_4O_7$ (614.7) Calc. C 66.4 H 6.23 N 9.1 Found C 66.1 H 6.30 N 9.2.- ^{1}H-NMR (DMSO-d_6, 200 MHz): 11.97 (s, 1H, NH^i), 7.55-6.92 (m, 10 H, indole, benzyl C_6H_5), 7.32 (s, 5H, Z C_6H_5), 4.98 (s, 2H, Z CH_2), 4.62 (m, 2H, benzyl CH_2), 4.24 (m, 1H, Lys α-CH), 3.59 (s, 3H, CO_2CH_3), 3.36 (m, 1H, 2-H), 3.14 (m, 2H, 3-H), 2.94 (m, 2H, Lys ε-CH_2), 1.70-1.12 (m, 6H, Lys β-, γ- and δ-CH_2).

N-[2(RS)-(N-Hydroxy)carbamoyl-3-(indole-3-yl)propanoyl]-Lys-OMe (27)

Compound **26** (0.5 g, 0.8 mmol) in EtOH (80 mL) was hydrogenated at 20 psi and room temp. for 2 h in the presence of 6N HCl (0.2 mL) and 10% Pd-C (100 mg). The catalyst was filtered and the solvent evaporated to give a residue which was purified by cc using $CHCl_3$:MeOH 6:1: 0.17 g (49%).- $C_{19}H_{27}N_4O_5Cl$ (426.9) Calc. C 53.4 H 6.37 Cl 8.3 N 13.1 Found C 53.8 H 6.35 Cl 8.1 N 12.8.- ^{1}H-NMR (DMSO-d_6, 200 MHz): 10.88 (s, 1H, NH^i), 7.53-6.96 (m, 5H, indole), 4.20 (m, 1H, Lys α-CH), 3.62 (s, 3H, CO_2CH_3), 3.53 (t, J = 7.0 Hz, 1H, 2-H), 3.16 (m, 2H, 3-H), 2.73 (m, 2H, Lys ε-CH_2), 1.71-1.10 (m, 6H, Lys β-, γ- and δ-CH_2).

N-[2(RS)-(N-Hydroxy)carbamoyl-3-[2-(o-nitrophenylsulfenyl)indole-3-yl]propanoyl]-Lys-OMe (9)

To a solution of compound **27** (0.05 g, 0.11 mmol) in 2N HCl/MeOH (10 mL) Nps-Cl (0.026 g, 0.14 mmol) was added and the mixture was stirred for 30 min. The solvent was evaporated and the residue was purified by cc using $CHCl_3$:MeOH 6:1: 0.047 g (77%).- $C_{25}H_{30}ClN_5O_7S$ (580.1) Calc. C 51.8 H 5.21 Cl 6.1 N 12.1 S 5.5 Found C 51.8 H 5.41 Cl 5.9 N 12.0 S 5.7.- ^{1}H-NMR data: Table 2.

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