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TETRAHEDRON

Amino Acids and Peptides. LVI. Synthesis of pyrazinone ring-containing opioid mimetics and examination of their opioid receptor binding activity[1]

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Abstract

Cyclization of dipeptidyl chloromethyl ketones gave 6-(4-aminobutyl)-3-carboxyethyl-5-methyl-2(1*H*)-pyrazinone (**3**), 3-(4-aminobutyl)-6-carboxyethyl-5-methyl-2(1*H*)-pyrazinone (**6**), and 3,6-bis(4-aminobutyl)-5-methyl-2(1*H*)-pyrazinone (**15**). Using above pyrazinone derivatives, three opioid mimetics were prepared (III-V). Derivatives containing **3** and **6** demonstrated weak μ and δ-opioid receptor affinities ranging from 1.6 mM to 4.1 mM while the opioid mimetic containing derivative **15** displayed higher μ-opioid receptor binding affinity (K_i μ = 61 nM) and selectivity (K_i μ/K_i δ = 31).

Keywords: dipeptidyl chloromethyl ketone; cyclization; pyrazinone ring-containing amino acids; opioid mimetics;

INTRODUCTION

δ- μ- and κ-opioid receptors are located in the central nervous system and peripheral tissues such as mouse vas deferens, guinea pig ileum and rabbit jejunum. These receptors and their endogenous ligands, the enkephalins,[2] endorphins,[3] dynorphins,[4] and endomorphins[5] are primarily involved in the modulation and perception of pain. With the exception of the μ-receptor selective endomorphins, the mammalian endogenous ligands do not exhibit high selectivity for one receptor subtype over another. For this reason it is interesting to design ligands that demonstrate high affinity and selectivity for a specific receptor subtype in order to study the biological function unique to each receptor subtype and to understand the structure-activity relationships of the opioid system.

1:The customary L-configuration for amino acid residues is omitted. Abbreviations used in this report for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485-2489 (1966); **6**, 362-364 (1967); **11**, 1726-1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, dimethylformamide; DMSO, dimethylsulfoxide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; Z benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Fmoc, 9-fluornylmethyloxycarbonyl; OBu', *tert*-butyl ester; OSu, *N*-hydroxysuccinimide ester; DCC, *N,N'*-dicyclohexylcarbodiimide; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; DIEA, diisopropylethylamine.

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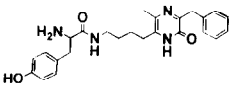
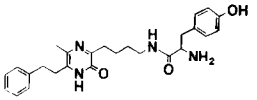
Recent interest has focused increasingly on cyclic dipeptide derivatives,[6] which are designed to substitute a dipeptide unit within a peptide chain, thus inducing certain conformational restrictions.[7] Such conformational constraints may alter (increase or decrease) the biological response,[8] induce receptor subtype selectivity,[9] cause antagonism,[10] or with an enzyme as target, give rise to inhibition.[11] Previously, a simple procedure for synthesis of 2(1*H*)-pyrazinone derivatives from dipeptidyl chloromethyl ketones was developed.[12] This novel method gave 2(1*H*)-pyrazinone derivatives containing substitutions with desired functional groups at positions 3 and 6 in high yield. By using the appropriate amino acids,[13] an amino and/or a carboxyl group can be easily introduced at position 3 or 6 of the pyrazinone ring. Several pyrazinone derivatives were previously reported using this method of synthesis [6]. Two examples are shown in Table 1. Compound **I** exhibited weak δ-opioid receptor binding

Table 1. Binding Activity of Compound I and II[6]

Compound		Receptor binding (K_i nM)		Binding selectivity
		δ	μ	K_i δ / K_i μ
I		332.7±14.6	3909±756	0.085
II		2165±334	55.8±10.8	38.8

affinity (K_i δ = 332.7nM) while compound **II** displayed elevated affinity and selectivity for the μ-opioid receptor (K_i μ = 55.8 nM; K_i δ/K_i μ = 38.8). The main difference between these compounds is the position of substitution of the tyrosylaminobutyl, β-phenylethyl, and benzyl groups on the pyrazinone rings. Based on this observation we designed and synthesized opioid mimetics **III-V** (Fig. 1) to investigate the relationship between the position of substitution on the pyrazinone ring and opioid receptor recognition.

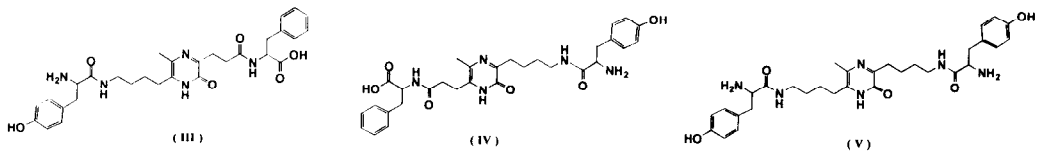


Figure 1. Structure of Compounds III-V

Results and Discussion

To construct compounds **III** and **IV**, two types of amino acids containing a pyrazinone ring were prepared according to Chart 1. After removal of the Boc group of the dipeptidyl

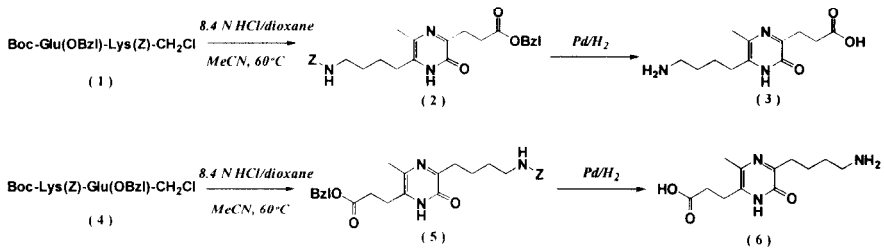


Chart 1. Synthetic Scheme for Pyrazinone Ring-Containing Amino Acids

chloromethyl ketones (**1** and **4**), the solution of the resultant amine hydrochloride in MeCN was stirred at 60 °C for 2 h, followed by hydrogenation over a Pd catalyst to give **3** and **6**. Compounds were identified using thin layer chromatography, HPLC, ^{1}H- and ^{13}C-NMR and elemental analysis.

The amino group was protected with Boc or Fmoc group as shown in Chart 2 by using $(Boc)_2O$ or Fmoc-OSu, respectively, to give compounds (**7**-**10**).

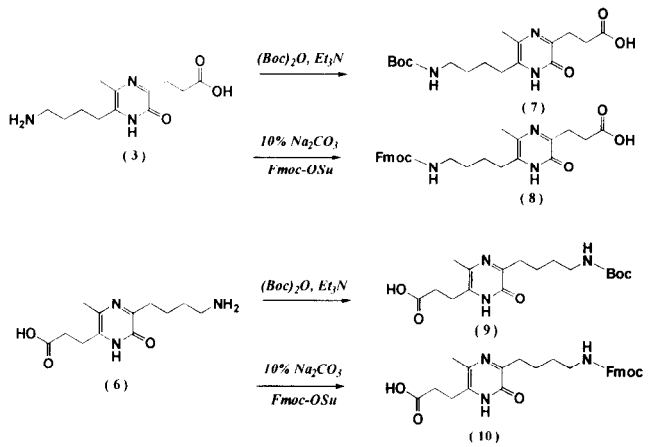


Chart 2. Synthesis of *N*-Protected Pyrazinone Ring-Containing Amino Acids

Opioid mimetics **III** and **IV** were prepared according to the Charts 3 and 4, respectively. As shown in Chart 3, the Fmoc-amino acid (**8**) was coupled with H-Phe-OBu^t by BOP reagent to give dipeptide (**11**). After removal of the Fmoc group, the resultant dipeptide amine was coupled with Boc-Tyr-OH by the BOP reagent to give the protected tripeptide (**12**). The Boc and Bu^t groups were removed by TFA-anisole. The product was lyophilized from water containing 1 N HCl to give peptide **III** as its hydrochloride.

As shown in Chart 4, the Fmoc-amino acid (**10**) was coupled with H-Phe-OBu^t by a BOP reagent to give a dipeptide (**13**). After removal of Fmoc group by 20% piperidine/DMF, the resultant dipeptide amine was coupled with Boc-Tyr-OH by a BOP reagent to give the protected tripeptide (**14**), which was treated with TFA-anisole and converted to the

corresponding hydrochloride by 1 N HCl to give compound **IV**. Analytical HPLC profiles of **III** and **IV** are shown in Fig. 2.

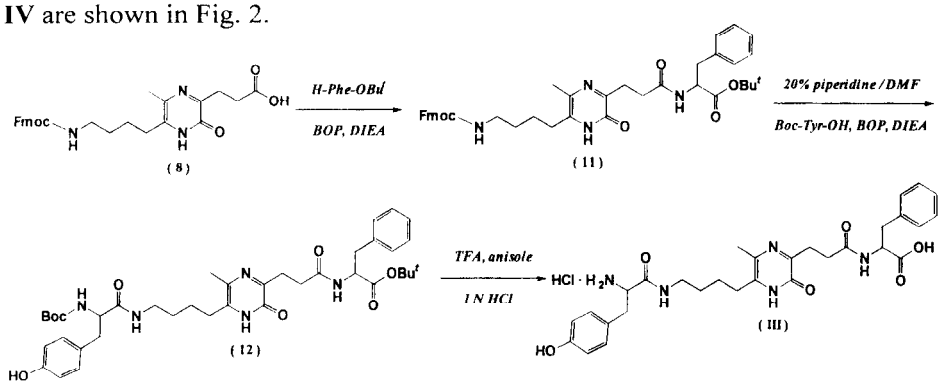


Chart 3. Synthetic Scheme for Compound III

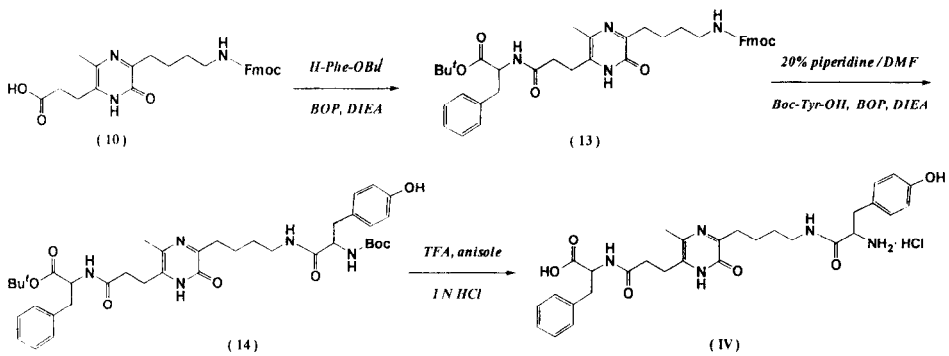


Chart 4. Synthetic Scheme for Compound IV

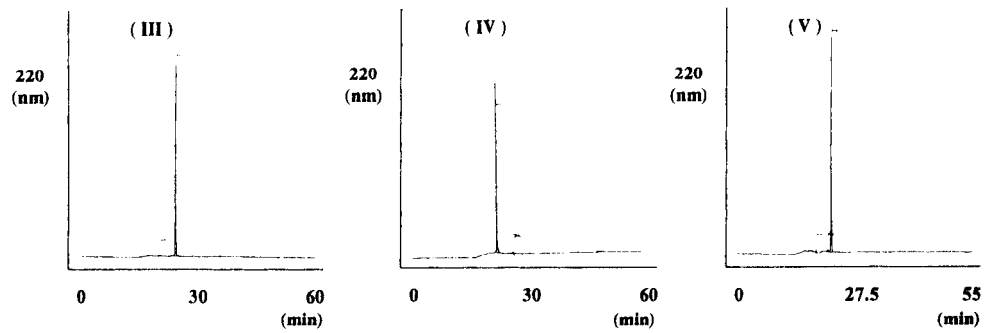


Figure 2. HPLC Profiles of Compounds III, IV and V

Chart 5, shows the coupling of 3,6-bis(4-aminobutyl)-2(1*H*)-pyrazinone (**15**) with Boc-Tyr-OH by BOP reagent to give compound (**16**) with a protection group. This was treated with TFA-anisole and converted to its hydrochloride. The analytical HPLC profile of **V** is shown in Fig. 2.

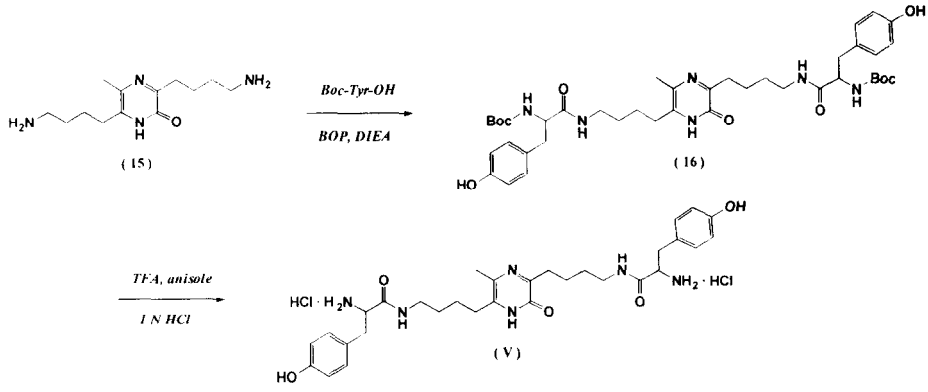
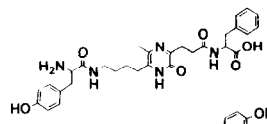
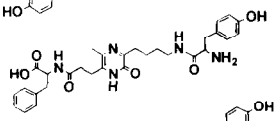
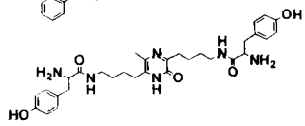


Chart 5. Synthetic Scheme for Compound V

Peptides **III** and **IV** containing pyrazinone derivatives **3** and **6**, respectively, demonstrated weak μ and δ-opioid receptor affinities ranging from 0.69 μM to 5.1 μM and rather δ-selectivity (Table 2). Although these peptide mimetics contained functional groups known to be important for opioid receptor interaction; namely the hydroxy group on tyrosine, the *N*-terminal amine and the presence of two aromatic rings, both compounds were almost inactive in the radiolabeled ligand-binding assay. Therefore, it could be deduced that the distance between the two aromatic rings in compounds **III** and **IV** was too large to manifest opioid receptor-binding activity. Peptide **V** containing derivative **15** displayed moderate μ-opioid

Table 2. Binding Activity of Compounds III, IV and V

Compound		Receptor binding (K_i nM)		Binding selectivity
		δ	μ	K_i δ / K_i μ
III		1877±299	5066±1166	0.37
IV		690.3±176	4277±100	0.16
V		2185±133	70.2±4.8	31

receptor binding affinity (K_i μ = 70 nM) and selectivity (K_i δ/ K_i μ = 31), indicating that compound **V** is μ-selective. Previously, it was reported that Tyr^4-enkephakin exhibited only 0.1% and 0.3% activity in GPI and MVD assay, respectively, compared with the parent molecule, enkephalin[15] and this compound was rather δ-selective, as enkephalin itself.[16]

Kondo *et al.*[17] prepared a cystamine-enkephalin dimer consisting of two molecules of [D-Ala^2, Leu^5]-enkephalin cross-linked at the C-terminal leucine with cystamine (NH_2-CH_2-CH_2-CH_2-S-)$_2$ and exmined its opioid receptor-binding activity. Their compound was almost five times more potent for δ-opioid receptors and four times more potent for μ-opioid receptors than the cystamine-enkephalin monomer and was rather δ-selective. The compound **V** exhibited quite a different opioid receptor-binding activity profile compared with Tyr^4-enkephlin and a cystamine-enkephalin dimer and increased μ-receptor-binding affinity between 56- to 72-fold relative to compounds **I**, **III**, and **IV**. However there was not a significant change in δ-opioid receptor affinity relative to the other derivatives. In addition, the μ-receptor binding profile, in both affinity and selectivity, was similar to that of opioid mimetic **II** (Table 1). These data revealed that opioid mimetics with aminobutyl or carboxyethyl at either position 3 or 6 of the pyrazinone ring did not influence opioid receptor affinity. However, the compound **V** which contained N^4-tyrosylaminobutyl groups at both positions 3 and 6 of the pyrazinone ring exhibited elevated μ-opioid receptor affinity. Therefore, it can be deduced that there is a possibility that compound **V** acts at different μ-opioid receptors. Due to the facile procedure for synthesis of pyrazinone ring-containing opioid mimetics, further studies involving the modification of the aromatic and carbonyl moieties may yield in derivatives with potential therapeutic application.

Experimental

General

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.). ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR spectra were recorded on a Bruker DPX-400 spectrometer. Chemical shifts are expressed as ppm downfield from tetramethylsilane, used as an internal standard (δ-value). The *J* values are given in Hz. The ^{13}C signals were assigned with the acid of distortionless enhancement by polarization transfer (DEPT) and 2D experiments, and multiplicities are indicated by p (primary), s (secondary), t (tertiary) or q (quaternary). Mass spectra were measured with a JEOL SX-102 mass spectrometer using the FAB technique. Waters model 600E was used for HPLC analysis. The solvents are as follows: A, 0.05% TFA in water; B, 0.05% TFA in CH_3CN. The retention time was reported as t_R. On TLC (Kieselgel G 60, Merck), Rf^1, Rf^2, Rf^3, Rf^4, Rf^5, Rf^6 and Rf^7 values refer to the systems of $CHCl_3$, MeOH and AcOH (90:8:2), $CHCl_3$, MeOH and water (8:3:1, lower phase), $CHCl_3$ and MeOH (19:1), AcOEt and hexane (1:1), AcOEt and hexane (2:1), *n*-BuOH, AcOH, pyridine and water (4:1:1:2) and *n*-BuOH, AcOH and water (4:1:5, upper phase), respectively.

Boc-Glu(OBzl)-CH_2Cl

To a solution of mixed anhydride [prepared from Boc-Glu(OBzl)-OH (5.80 g, 17.2 mmol), isobutyl chloroformate (2.25 ml, 17.2 mmol) and Et_3N (2.65 ml, 18.9 mmol) in the usual

manner] in THF (100 ml), diazomethane in ether (60 ml) [prepared from *p*-toluensulfonyl-*N*-methyl-*N*-nitrosoamide (14.7 g, 68.7 mol) in the usual manner] was added. The reaction mixture was stirred at 4 °C overnight. 6.7 N HCl/dioxane (6.42 ml, 43.0 mmol) was then added to the above solution under cooling with ice-salt and the reaction mixture was stirred at -15 °C for 2 h. The pH was adjusted to 8 with NMM. After removal of solvent, the residue was extracted with AcOEt. The extract was washed with water, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration, yield 3.50 g (55.0%), mp 56-58 °C, $[\alpha]_D^{25}$ -35.9° (*c*=1.0, MeOH), Rf^4 0.78. ^{1}H-NMR ($CDCl_3$) δ: 7.39-7.31 (5H, m, phenyl), 5.22 (1H, brd, *J=7.0*, NH), 5.12 (2H, s, $COCH_2Cl$), 4.55 (1H, m, α-CH), 2.51 (1H, dt, *J=17.0, 7.4*, Ha of γ-CH_2), 2.45 (1H, dt, *J=17.0, 6.8*, Hb of γ-CH_2), 2.22 (1H, m, Ha of β-CH_2), 1.88 (1H, ddt, *J=15.2, 7.4, 6.8* Hb of β-CH_2), 1.43 (9H, s, *t*-butyl) ^{13}C-NMR ($CDCl_3$) δ: 201.1($\underline{C}OCH_2Cl$), 172.6 (q, -$(CH_2)_2\underline{C}O_2CH_2$-Ph), 155.5 (q, carbonyl of Boc), 135.6 (q, C1 of phenyl), 128.6 (t, C3, C5 of phenyl), 128.4 (t, C4 of phenyl), 128.3 (t, C2, C6 of phenyl), 80.5 (q, *t*-butyl), 66.7 (s, benzyl), 56.6 (t, α-CH), 46.4 (s, $CO\underline{C}H_2Cl$), 29.9 (s, γ-CH_2), 28.3 (p, *t*-butyl), 26.3 (s, β-CH_2), *Anal.* Calcd for $C_{18}H_{24}ClNO_5$: C, 58.5; H, 6.54; N, 3.79. Found: C, 58.2; H, 6.45; N, 3.84.

Boc-Glu(OBzl)-Lys(Z)-CH_2Cl (1)

A mixed anhydride [prepared from Boc-Glu(OBzl)-OH (3.04 g, 9.00 mmol), isobutyl chloroformate (1.18 ml, 9.00 mmol) and NMM (0.990 ml, 9.00 mmol) in the usual manner] in THF (100 ml) was added to a solution of H-Lys(Z)-CH_2Cl · HCl [prepared from Boc-Lys(Z)-CH_2Cl[18] (3.10 g, 7.50 mmol) and 7.1 N HCl/dioxane (5.28 ml, 37.5 mmol) in the usual manner] in DMF (50 ml) containing NMM (0.825 ml, 7.50 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h and at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration, yield 2.58 g (54.4%), mp 105-108 °C, $[\alpha]_D^{25}$ -23.3° (*c*=1.0, MeOH), Rf^3 0.79. ^{1}H-NMR ($CDCl_3$) δ: 7.36-7.26 (10H, m, 2 x phenyl), 7.01 (1H, br, α-NH of Lys), 5.37 (1H, d, *J=7.6*, α-NH of Glu), 5.11 (2H, s, $C\underline{H}_2$-Ph), 5.10 (2H, s, $C\underline{H}_2$-Ph), 5.07 (1H, br, ε-NH of Lys), 4.70 (1H, m, α-CH of Lys), 4.22 (2H, s, $COCH_2Cl$), 4.18 (1H, br, α-CH of Glu), 3.16 (2H, m, ε-CH_2 of Lys), 2.48 (2H, m, γ-CH_2 of Glu), 2.10 and 1.91 (2H, m, β-CH_2 of Glu), 1.86 and 1.62 (2H, m, β-CH_2 of Lys), 1.50 (2H, m, δ-CH_2 of Lys), 1.41 (9H, s, *t*-butyl), 1.34 (2H, m, γ-CH_2 of Lys), ^{13}C-NMR ($CDCl_3$) δ: 200.8 ($\underline{C}OCH_2Cl$), 173.1 (q, -$(CH_2)_2\underline{C}O_2CH_2$-Ph), 172.1 (q, carbonyl of Boc), 156.7 (q, carbonyl of Z), 155.7 (q, α-carbonyl of Glu), 136.6 and 135.7 (q, 2 x phenyl), 128.6-128.1 (t, 2 x phenyl), 80.3 (q, *t*-butyl), 66.8 and 66.6 (s, 2 x $\underline{C}H_2$-Ph), 56.0 (t, α-CH of Lys), 53.6 (t, α-CH of Glu), 46.5 (s, $COCH_2Cl$), 40.0 (s, ω-CH_2 of Lys), 30.4 (s, γ-CH_2 of Glu), 30.2 (s, β-CH_2 of Lys), 29.3 (s, δ-CH_2 of Lys), 28.3 (p, *t*-butyl), 27.6 (s, β-CH_2 of Glu), 22.2 (s, γ-CH_2 of Lys), *Anal.* Calcd for $C_{32}H_{42}ClN_3O_8$: C, 60.8; H, 6.70; N, 6.65. Found: C, 60.7; H, 6.74; N, 6.81.

*Boc-Lys(Z)-Glu(OBzl)-CH_2Cl (**4**)*

A mixed anhydride [prepared from Boc-Lys(Z)-OH (1.14 g, 3.00 mmol), isobutyl chloroformate (0.393 ml, 3.00 mmol) and NMM (0.330 ml, 3.00 mmol) in the usual manner] in THF (30 ml) was added to a solution of H-Glu(OBzl)-CH_2Cl · HCl [prepared from Boc-Glu(OBzl)-CH_2Cl (0.925 g, 2.50 mmol) and 8.4 N HCl/dioxane (1.19 ml, 10.0mmol) in the usual manner] in DMF (10 ml) containing NMM (0.275 ml, 2.50 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h and at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration, yield 550 mg (34.8%), mp 79-81 °C, $[\alpha]_D^{25}$ -33.9° (*c*=0.5, MeOH), *Rf*[4] 0.40. ^{1}H-NMR ($CDCl_3$) δ: 7.40-7.25 (10H, m, 2 x phenyl), 7.10 (1H, d, *J=7.0,* α-NH of Glu), 5.19 (1H, d, *J=6.5*, α-NH of Lys), 5.10 (2H, s, C<u>H</u>$_2$-Ph), 5.10 and 5.07 (2H, ABq, *J=12.6*, C<u>H</u>$_2$-Ph), 4.97 (1H, t, *J=5.0*, ε-NH of Lys), 4.76 (1H, m, α-CH of Glu), 4.28 and 4.24 (2H, ABq, *J=15.9*, $COCH_2Cl$), 4.04 (1H, br, α-CH of Lys), 3.18 (2H, m, ε-CH_2 of Lys), 2.51-2.37 (2H, m, γ-CH_2 of Glu), 2.23 and 1.92 (2H, m, β-CH_2 of Glu), 1.80 and 1.61 (2H, m, β-CH_2 of Lys), 1.25 (2H, m, δ-CH_2 of Lys), 1.41 (9H, s, *t*-butyl), 1.37 (2H, m, γ-CH_2 of Lys), ^{13}C-NMR ($CDCl_3$) δ: 200.4 (<u>C</u>OCH_2Cl), 172.7 (q, -$(CH_2)_2$<u>C</u>O_2CH_2-Ph), 156.7 (q, carbonyl of Z), 155.7 (q, α-carbomyl of Lys), 136.6 and 135.6 (q, 2 x phenyl), 128.6-128.1 (t, 2 x phenyl), 80.3 (q, *t*-butyl), 66.8 and 66.7 (s, 2 x <u>C</u>H$_2$-Ph), 55.5 (t, α-CH of Glu), 54.4 (t, α-CH of Lys), 46.5 (s, CO<u>C</u>H_2Cl), 40.2 (s, ε-CH_2 of Lys), 31.3 (s, β-CH_2 of Lys), 29.9 (s, γ-CH_2 of Glu), 29.4 (s, δ-CH_2 of Lys), 28.3 (p, *t*-butyl), 25.7 (s, β-CH_2 of Glu), 22.4 (s, γ-CH_2 of Lys), *Anal.* Calcd for $C_{32}H_{42}ClN_3O_8$: C, 60.8; H, 6.70; N, 6.65. Found: C, 60.6; H, 6.71; N, 6.67.

Boc-Lys(Z)-Lys(Z)-CH_2Cl

A mixed anhydride [prepared from Boc-Lys(Z)-OH (2.19 g, 5.76 mmol), isobutyl chloroformate (0.754 ml, 5.76 mmol) and NMM (0.633 ml, 5.76 mmol) in the usual manner] in THF (60 ml) was added to a solution of H-Lys(Z)-CH_2Cl · HCl [prepared from Boc-Lys(Z)-CH_2Cl (1.98 g, 4.80 mmol) and 8.4 N HCl/dioxane (2.86 ml, 24.0 mmol) in the usual manner] in DMF (60 ml) containing NMM (0.528 ml, 4.80 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h and at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration, yield 1.98 g (59.5%), mp 127-129 °C, $[\alpha]_D^{25}$ -20.3° (*c*=0.5, $CHCl_3$), *Rf*[4] 0.32. ^{1}H-NMR ($CDCl_3$) δ: 7.37-7.27 (10H, m, 2 x phenyl), 5.30 (1H, br, α-NH, Lys), 5.11-5.05 (4H, m, 2 x C<u>H</u>$_2$-Ph), 4.52 (1H, m, α-CH, Lys), 4.22 (2H, s, $COCH_2Cl$), 4.14 (1H, m, α-CH, Lys), 3.25-3.10 (4H, m, 2 x ε-CH_2, Lys), 1.90-1.25 (12H, m, 2 x α-CH_2, 2 x β-CH_2 and 2 x γ-CH_2, Lys), 1.42 (9H, s, *t*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 200.9 (q, <u>C</u>OCH_2Cl), 172.6, 172.4, 156.63 and 156.61 (q, 4 x carbonyl), 136.7 and 136.6 (q, 2 x phenyl), 128.5-128.1 (t, 2 x phenyl), 80.1 (q, *t*-butyl), 66.7 and 66.6 (s, 2 x C<u>H</u>$_2$-Ph), 54.1 and 51.9 (t, 2

x α-CH, Lys), 46.6 (s, CO<u>C</u>H$_2$Cl), 40.4 (s, 2 x ε-CH$_2$, Lys), 32.0-29.2 (s, 2 x α-CH$_2$ and 2 x β-CH$_2$, Lys), 28.3 (p, *t*-butyl), 22.3 (s, γ-CH$_2$, Lys),*Anal.* Calcd for $C_{34}H_{47}ClN_4O_8$: C, 60.4; H, 7.01; N, 8.32. Found: C, 60.5; H, 7.01; N, 8.22.

6-(4-Benzyloxycarbonylaminobutyl)-3-(2-benzyloxycarbonylethyl)-5-methyl-2(1H)-pyrazinone (2)

A solution of H-Glu(OBzl)-Lys(Z)-CH$_2$Cl • HCl [prepared from Boc-Glu(OBzl)-Lys(Z)-CH$_2$Cl (2.00 g, 3.16 mol) and 7.0 N HCl/dioxane (4.51 ml, 31.6 mmol) in the usual manner] in CH$_3$CN (100 ml) was refluxed for 2 h. After removal of the solvent, the residue was extracted with CHCl$_3$ and the extract was washed with 0.1N HCl, 5% NaHCO$_3$, and water, dried over Na$_2$SO$_4$ and evaporated. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 1.17 g (77.5%), mp 103-105 °C, *Rf*[f] 0.62. ^{1}H-NMR (CDCl$_3$) δ: 13.2 (1H, br, NH), 7.36-7.23 (10H, m, 2 x phenyl), 5.70 (1H, brt, *J=6.0*, 6-(CH$_2$)$_4$N<u>H</u>-Z), 5.10 (2H, s, 3-(CH$_2$)$_2$CO$_2$C<u>H</u>$_2$-Ph), 5.06 (2H, s, 6-(CH$_2$)$_4$NHCO$_2$C<u>H</u>$_2$-Ph), 3.26 (2H, brq, *J*=6.0, 6-CH$_2$CH$_2$CH$_2$C<u>H</u>$_2$NH-Z), 3.08 (2H, t, *J*=7.1, 3-C<u>H</u>$_2$CH$_2$CO$_2$CH$_2$-Ph), 2.77 (2H, t, *J*=7.1, 3-CH$_2$C<u>H</u>$_2$CO$_2$CH$_2$-Ph), 2.51 (2H, brt, *J*=7.2, 6-C<u>H</u>$_2$CH$_2$CH$_2$CH$_2$NH-Z), 2.20 (3H, s, 5-CH$_3$), 1.67-1.59 (4H, m, 6-CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$CH$_2$NH-Z), ^{13}C-NMR (CDCl$_3$) δ: 173.1 (q, 3-(CH$_2$)$_2$<u>C</u>O$_2$CH$_2$-Ph), 157.6 (q, C-2), 156.7 (q, 6-(CH$_2$)$_4$NH<u>C</u>O$_2$CH$_2$-Ph), 153.3 (q, C-3), 136.7 (q, 6-C1''), 136.2 (q, 3-C1'), 134.6 (q, C-6), 129.5 (q, C-5), 128.5-128.0 (t, 2 x phenyl), 66.5 (s, 6-(CH$_2$)$_4$NHCO$_2$<u>C</u>H$_2$-Ph), 66.2 (s, 3-(CH$_2$)$_2$CO$_2$<u>C</u>H$_2$-Ph), 39.8 (s, 6-CH$_2$CH$_2$CH$_2$<u>C</u>H$_2$NH-Z), 30.4 (s, 3-CH$_2$<u>C</u>H$_2$CO$_2$CH$_2$-Ph), 29.2 (s, 6-<u>C</u>H$_2$CH$_2$CH$_2$CH$_2$NH-Z), 29.0 (s, 6-CH$_2$CH$_2$<u>C</u>H$_2$-CH$_2$NH-Z), 27.3 (s, 3-<u>C</u>H$_2$CH$_2$CO$_2$CH$_2$-Ph), 25.4 (s, 6-CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$NH-Z), 18.3 (p, 5-CH$_3$), *Anal.* Calcd for $C_{27}H_{31}N_3O_5$: C, 67.9; H, 6.54; N, 8.80. Found: C, 67.9; H, 6.53; N, 8.76.

3-(4-Benzyloxycarbonylaminobutyl)-6-(2-benzyloxycarbonylethyl)-5-methyl-2(1H)-pyrazinone (5)

The title compound was prepared from Boc-Lys(Z)-Glu(OBzl)-CH$_2$Cl (750 mg, 1.19 mmol), yield 456 mg (80.2%), mp 87-90 °C, *Rf*[f] 0.76. ^{1}H-NMR (CDCl$_3$) δ: 12.9 (1H, br, NH), 7.34-7.26 (10H, m, 2 x phenyl), 5.16 (1H, brt, *J=6.3*, 3-(CH$_2$)$_4$N<u>H</u>-Z), 5.10 (2H, s, 6-(CH$_2$)$_2$-CO$_2$C<u>H</u>$_2$-Ph), 5.07 (2H, s, 3-(CH$_2$)$_4$NHCO$_2$C<u>H</u>$_2$-Ph), 3.21 (2H, q, *J*=6.3, 3-CH$_2$CH$_2$CH$_2$C<u>H</u>$_2$-NH-Z), 2.84 (2H, t, *J*=7.3, 6-C<u>H</u>$_2$CH$_2$CO$_2$CH$_2$-Ph), 2.75 (2H, t, *J*=7.3, 6-CH$_2$C<u>H</u>$_2$CO$_2$CH$_2$-Ph), 2.72 (2H, t, *J*=7.5, 3-C<u>H</u>$_2$CH$_2$CH$_2$CH$_2$NH-Z), 2.25 (3H, s, 5-CH$_3$), 1.71 (2H, quint, *J*=7.5, 3-CH$_2$C<u>H</u>$_2$CH$_2$CH$_2$NH-Z), 1.54 (2H, quint, *J*=7.2, 3-CH$_2$CH$_2$C<u>H</u>$_2$CH$_2$NH-Z), ^{13}C-NMR (CDCl$_3$) δ: 172.0 (q, 6-(CH$_2$)$_2$<u>C</u>O$_2$CH$_2$-Ph), 157.3 (q, C-2), 156.4 (q, 3-(CH$_2$)$_4$NH<u>C</u>O$_2$CH$_2$-Ph+C-3), 136.8 (q, 3-C1'), 135.5 (q, 6-C1''), 132.6 (q, C-6), 129.7 (q, C-5), 128.6-128.0 (t, 2 x phenyl), 66.8 (s, 6-(CH$_2$)$_2$CO$_2$<u>C</u>H$_2$-Ph), 66.5 (s, 3-(CH$_2$)$_4$NHCO$_2$<u>C</u>H$_2$-Ph), 40.8 (s, 3-CH$_2$CH$_2$CH$_2$<u>C</u>H$_2$-NH-Z), 32.5 (s, 6-CH$_2$<u>C</u>H$_2$CO$_2$CH$_2$-Ph), 32.2 (s, 3-<u>C</u>H$_2$CH$_2$CH$_2$CH$_2$NH-Z), 29.3 (s, 3-CH$_2$CH$_2$<u>C</u>H$_2$CH$_2$NH-Z), 25.1 (s, 6-<u>C</u>H$_2$CH$_2$CO$_2$CH$_2$-Ph), 24.0 (s, 3-CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$NH-Z), 18.5 (p, 5-CH$_3$), *Anal.* Calcd for $C_{27}H_{31}N_3O_5$ • 0.25 H$_2$O: C, 67.3; H, 6.54; N, 8.72. Found: C, 67.3; H, 6.59; N, 8.78.

3,6-Bis(4-benzyloxycarbonylaminobutyl)-5-methyl-2(1H)-pyrazinone

The title compound was prepared from Boc-Lys(Z)-Lys(Z)-CH_2Cl (1.50 g, 2.16 mmol), yield 558 mg (49.6%), mp 158-160 °C, *Rf*[4] 0.63. ^{1}H-NMR ($CDCl_3$) δ: 13.1 (1H, br, NH), 7.35-7.27 (10H, m, 2 x phenyl), 5.65 (1H, m, 6-$(CH_2)_4$N<u>H</u>-Z), 5.10 (1H, m, 3-$(CH_2)_4$N<u>H</u>-Z), 5.06 (4H, s, 2 x -$(CH_2)_4NHCO_2C\underline{H}_2$-Ph), 3.27 (2H, q, *J*=6.2, 6-$CH_2CH_2CH_2C\underline{H}_2NH$-Z), 3.20 (2H, q, *J*=6.0, 3-$CH_2CH_2CH_2C\underline{H}_2NH$-Z), 2.75 (2H, t, *J*=7.4, 3-$C\underline{H}_2CH_2CH_2CH_2NH$-Z), 2.52 (2H, t, *J*=7.4, 6-$C\underline{H}_2CH_2CH_2CH_2NH$-Z), 2.26 (3H, s, 5-$CH_3$), 1.76-1.51 (8H, m, 2 x -$CH_2C\underline{H}_2C\underline{H}_2$-$CH_2NH$-Z), ^{13}C-NMR ($CDCl_3$) δ: 157.7 and 155.6 (q, C-2 and C-3), 156.7 and 156.5 (q, 2 x -$(CH_2)_4NH\underline{C}O_2CH_2$-Ph), 136.73 and 136.67 (q, C1' and C1"), 134.5 (q, C-6), 129.4 (q, C-5), 128.5-128.0 (t, 2 x phenyl), 66.61 and 66.55 (s, 2 x -$(CH_2)_2CO_2\underline{C}H_2$-Ph), 40.9 (s, 3-$CH_2CH_2CH_2\underline{C}H_2NH$-Z), 39.9 (s, 6-$CH_2CH_2CH_2\underline{C}H_2NH$-Z), 32.2 (s, 3-$\underline{C}H_2CH_2CH_2CH_2NH$-Z), 29.5 (s, 3-$CH_2CH_2\underline{C}H_2CH_2NH$-Z), 29.2 (s, 6-$\underline{C}H_2CH_2CH_2CH_2$-NH-Z), 29.1 (s, 6-$CH_2CH_2$-$\underline{C}H_2CH_2$-NH-Z), 25.4 (s, 6-$CH_2\underline{C}H_2CH_2CH_2NH$-Z), 24.0 (s, 3-$CH_2\underline{C}H_2CH_2CH_2NH$-Z), 18.4 (p, 5-$CH_3$), *Anal.* Calcd for $C_{29}H_{36}N_4O_5$: C, 66.9; H, 6.97; N, 10.8. Found: C, 66.8; H, 7.03; N, 10.7.

6-(4-Aminobutyl)-3-(2-carboxylethyl)-5-methyl-2(1H)-pyrazinone (3)

The title compound was prepared from 6-(4-Benzyloxycarbonylaminobutyl)-3-(2-benzyloxycarbonylethyl)-5-methyl-2(1*H*)-pyrazinone (1.00 g, 2.09 mmol) by catalytic hydrogenation in 60% AcOH (80 ml) over a Pd catalyst. After removal of Pd and solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 653 mg (99.7%), dec. 172-175 °C, *Rf*[6] 0.23, *Anal.* Calcd for $C_{14}H_{23}N_3O_5 \cdot 0.5$ AcOH $\cdot 0.8$ H_2O: C, 52.4; H, 7.65; N, 14.1. Found: C, 52.1; H, 7.35; N, 14.4.

3-(4-Aminobutyl)-6-(2-carboxylethyl)-5-methyl-2(1H)-pyrazinone (6)

The title compound was prepared from 3-(4-Benzyloxycarbonylaminobutyl)-6-(2-benzyloxycarbonylethyl)-5-methyl-2(1*H*)-pyrazinone (356 mg, 0.745 mmol), yield 200 mg (85.7%), dec. 151-153 °C, *Rf*[7] 0.32. *Anal.* Calcd for $C_{14}H_{23}N_3O_5 \cdot 0.75$ H_2O: C, 52.6; H, 7.59; N, 14.2. Found: C, 52.9; H, 7.33; N, 14.1.

3,6-Bis(4-aminobutyl)-5-methyl-2(1H)-pyrazinone (15)

The title compound was prepared from 3,6-Bis(4-aminobenzyloxycarbonylbutyl)-5-methyl-2(1*H*)-pyrazinone (350 mg, 0.672 mol), yield 240 mg (95.9 %), mp 170-178 °C, *Rf*[6] 0.11. *Anal.* Calcd for $C_{13}H_{24}N_4O \cdot 2$ AcOH $\cdot 0.5$ H_2O: C, 53.5; H, 8.72; N, 14.7. Found: C,53.4; H, 8.23; N, 14.6.

6-(4-t-Butyloxycarbonylaminobutyl)-3-(2-carboxyethyl)-5-methyl-2(1H)-pyrazinone (7)

To a solution of a 6-(4-Aminobutyl)-3-(2-carboxylethyl)-5-methyl-2(1*H*)-pyrazinone (100 mg, 0.319 mmol) in water (20 ml) containing triethylamine (0.112 ml, 0.798 mmol), a solution

of $(Boc)_2O$ (76.6 mg, 0.351 mmol) in dioxane (5 ml) was added in one portion at 0 °C and the reaction mixture stirred at room temperature for 4 h. After removal of the solvent, ether was added to the residue to give crystals, which were collected by filtration, and recrystallized from AcOEt and petroleum ether, yield 98.5 mg (87.4%), mp 124-126 °C, *Rf*[d] 0.53. ^{1}H-NMR ($CDCl_3$) δ: 5.13 (1H, br, 6-$(CH_2)_4$N<u>H</u>-Boc), 3.18 (2H, m, 6-$CH_2CH_2CH_2C\underline{H}_2$NH-Boc), 3.09 (2H, t, *J*=6.8, 3-$C\underline{H}_2CH_2CO_2H$), 2.79 (2H, t, *J*=6.8, 3-$CH_2C\underline{H}_2CO_2H$), 2.55 (2H, t, *J*=7.3, 6-$C\underline{H}_2CH_2CH_2CH_2$NH-Boc), 2.27 (3H, s, 5-$CH_3$), 1.66 (2H, m, 6-$CH_2C\underline{H}_2CH_2CH_2$NH-Boc), 1.57 (2H, m, 6-$CH_2CH_2C\underline{H}_2CH_2$NH-Boc), 1.41 (9H, s, *t*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 177.6 (q, 3-$(CH_2)_2\underline{C}O_2H$), 157.4 (q, C-2), 156.3 (q, 6-$(CH_2)_4NH\underline{C}O_2C(CH_3)_3$), 153.8 (q, C-3), 135.0 (q, C-5), 129.8 (q, C-6), 79.3 (q, *t*-butyl), 39.5 (s, 6-$CH_2CH_2CH_2\underline{C}H_2$NH-Boc), 31.2 (s, 3-$CH_2\underline{C}H_2$-$CO_2H$), 29.4 (s, 6-$\underline{C}H_2CH_2\underline{C}H_2CH_2$NH-Boc), 28.4 (p, *t*-butyl), 27.1 (s, 3-$\underline{C}H_2CH_2CO_2H$), 25.4 (s, 6-$CH_2\underline{C}H_2CH_2CH_2$NH-Boc), 18.3 (p, 5-$CH_3$), *Anal.* Calcd for $C_{17}H_{27}N_3O_5 \cdot 0.5\ H_2O$: C, 56.3; H, 7.78; N, 11.6. Found: C, 56.4; H, 7.60; N, 11.6.

*3-(4-t-Butyloxycarbonylaminobutyl)-6-(2-carboxyethyl)-5-methyl-2(1H)-pyrazinone (**9**)*

The title compound was prepared from 3-(4-Aminobutyl)-6-(2-carboxylethyl)-5-methyl-2(1*H*)-pyrazinone (50 mg, 0.160 mmol), yield 48.2 mg (85.2%), mp 166-169 °C, *Rf*[d] 0.40. ^{1}H-NMR ($CDCl_3$) δ: 3.06 (2H, t, *J*=7.1, 3-$CH_2CH_2CH_2C\underline{H}_2$NH-Boc), 2.82 (2H, t, *J*=7.6, 6-$CH_2C\underline{H}_2CO_2H$), 2.71 (2H, t, *J*=7.6, 3-$C\underline{H}_2CH_2CH_2CH_2$NH-Boc), 2.62 (2H, t, *J*=7.6, 6-$C\underline{H}_2CH_2CO_2H$), 2.29 (3H, s, 5-$CH_3$), 1.68 (2H, m, 3-$CH_2C\underline{H}_2CH_2CH_2$NH-Boc), 1.52 (2H, quint, *J*=7.1, 3-$CH_2CH_2C\underline{H}_2CH_2$NH-Boc), 1.42 (9H, s, *t*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 175.7 (q, 6-$(CH_2)_2\underline{C}O_2H$), 158.6 (q, 3-$(CH_2)_4NH\underline{C}O_2C(CH_3)_3$), 157.8 (q, C-3), 156.7 (q, C-2), 136.1 (q, C-6), 131.4 (q, C-5), 79.8 (q, *t*-butyl), 41.2 (s, 3-$CH_2CH_2CH_2\underline{C}H_2$NH-Boc), 33.3 (s, 6-$\underline{C}H_2CH_2$-$CO_2H$), 33.2 (s, 3-$\underline{C}H_2CH_2CH_2CH_2$NH-Boc), 30.7 (s, 3-$CH_2CH_2\underline{C}H_2CH_2$NH-Boc), 28.8 (p, *t*-butyl), 26.5 (s, 6-$CH_2\underline{C}H_2CO_2H$), 25.5 (s, 3-$CH_2\underline{C}H_2CH_2CH_2$NH-Boc), 18.4 (p, 5-$CH_3$), *Anal.* Calcd for $C_{17}H_{27}N_3O_5 \cdot 0.5\ H_2O$: C, 56.3; H, 7.78; N, 11.6. Found: C, 56.5; H, 7.57; N, 11.6.

*6-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-3-(2-carboxyethyl)-5-methyl-2(1H)-pyrazinone (**8**)*

6-(4-Aminobutyl)-3-(2-carboxylethyl)-5-methyl-2(1*H*)-pyrazinone (500 mg, 1.60 mmol) was dissolved in 10% Na_2CO_3 (25 ml) and cooled in an ice bath. To this mixture a solution of Fmoc-OSu (809 mg, 2.40 mmol) in ethyleneglycol dimethyl ether (50 ml) was added in one portion at 0 °C and the reaction mixture was stirred at room temperature for 4 hr. After the mixture was filtered and the pH of the solution was then adjusted to 8 with acetic acid. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration, yield 407 mg (53.5%), mp 156-159 °C, *Rf*[d] 0.60. ^{1}H-NMR ($CDCl_3$) δ: 7.66 (2H, d-like, *J*=7.4, 4,5-CH of Fmoc), 7.51 (2H, d-like, *J*=7.5, 1,8-CH of Fmoc), 7.30 (2H, t-like, *J*=7.4, 3,6-CH of Fmoc), 7.20 (2H, t-like, *J*=7.5, 2,7-CH of Fmoc), 5.67 (1H, br, 6-$(CH_2)_4N\underline{H}$-Fmoc), 4.36 (2H, d, *J*=6.8, -CH_2-fluorenyl), 4.11 (1H, t, *J*=6.8, 9-CH of Fmoc),

3.18 (2H, br, 6-$CH_2CH_2CH_2C\underline{H}_2$NH-Fmoc), 3.04 (2H, t, J=7.6, 3-$CH_2C\underline{H}_2CO_2H$), 2.75 (2H, t, J=7.6, 3-$C\underline{H}_2CH_2CO_2H$), 2.42 (2H, t, J=7.1, 6-$C\underline{H}_2CH_2CH_2CH_2$NH-Fmoc), 2.19 (3H, s, 5-CH_3), 1.55 (4H, m, 6-$CH_2C\underline{H}_2C\underline{H}_2CH_2$NH-Fmoc), ^{13}C-NMR ($CDCl_3$) δ: 177.7 (q, 3-$(CH_2)_2\underline{C}O_2H$), 157.3 (q, 6-$(CH_2)_4NH\underline{C}O_2CH_2$-fluorenyl), 156.7 (q, C-2), 153.7 (q, C-3), 143.9 (q, 1a-C of Fmoc), 141.3 (q, 4a-C of Fmoc), 134.9 and 129.9 (q, C-5 and C-6), 127.6 (t, C-3,6 of Fmoc), 127.0 (t, C-2,7 of Fmoc), 124.9 (t, C-1,8 of Fmoc), 119.9 (t, C-4,5 of Fmoc), 66.5 (s, -CH_2-fluorenyl), 47.3 (t, C-9 of Fmoc), 39.9 (s, 6-$CH_2CH_2CH_2\underline{C}H_2$NH-Fmoc), 31.2 (s, 3-$\underline{C}H_2CH_2CO_2H$), 29.3 (s, 6-$\underline{C}H_2CH_2CH_2CH_2$NH-Fmoc), 28.9 and 25.3 (s, 6-$CH_2\underline{C}H_2\underline{C}H_2CH_2$-NH-Fmoc), 27.1 (s, 3-$CH_2\underline{C}H_2CO_2H$), 18.3 (p, 5-$CH_3$), *Anal.* Calcd for $C_{27}H_{29}N_3O_5 \cdot H_2O$: C,65.7; H, 6.33; N, 8.51. Found: C, 65.8; H, 6.22; N, 8.41.

*3-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-6-(2-carboxyethyl)-5-methyl-2(1H)-pyrazinone (**10**)*

The title compound was prepared from 3-(4-Aminobutyl)-6-(2-carboxylethyl)-5-methyl-2(1*H*)-pyrazinone (350 mg, 1.12 mmol), yield 514 mg (96.5%), mp 106-110 °C, Rf^i 0.53.^{1}H-NMR (DMSO-d_6) δ: 7.88 (2H, d-like, J=7.4, 4,5-CH of Fmoc), 7.69 (2H, d-like, J=7.4, 1,8-CH of Fmoc), 7.41 (2H, t-like, J=7.4, 3,6-CH of Fmoc), 7.32 (2H, t-like, J=7.4, 2,7-CH of Fmoc), 7.28 (1H, t, J=5.6, 3-$(CH_2)_4N\underline{H}$-Fmoc), 4.29 (2H, d, J=6.9, -CH_2-fluorenyl), 4.21 (1H, t, J=6.9, 9-CH of Fmoc), 3.01 (2H, q, J=7.0, 3-$CH_2CH_2CH_2C\underline{H}_2$NH-Fmoc), 2.67 (2H, t, J=7.4, 6-$C\underline{H}_2CH_2CO_2H$), 2.59 (2H, t, J=7.4, 3-$C\underline{H}_2CH_2CH_2CH_2$NH-Fmoc), 2.52 (2H, t, J=7.4, 6-$CH_2C\underline{H}_2CO_2H$), 2.20 (3H, s, 5-$CH_3$), 1.58 (2H, m, 3-$CH_2C\underline{H}_2CH_2CH_2$NH-Fmoc), 1.45 (2H, m, 3-$CH_2CH_2C\underline{H}_2CH_2$NH-Fmoc), ^{13}C-NMR (DMSO-d_6) δ: 173.2 (q, 6-$(CH_2)_2\underline{C}O_2H$), 156.0 (q, 3-$(CH_2)_4NH\underline{C}O_2CH_2$-fluorenyl), 155.5 (q, C-2), 153.4 (q, C-3), 143.9 (q, 1a-C of Fmoc), 140.6 (q, 4a-C of Fmoc), 135.1 and 128.5 (q, C-5 and C-6), 127.5 (t, C-3,6 of Fmoc), 126.9 (t, C-2,7 of Fmoc), 125.1 (t, C-1,8 of Fmoc), 120.0 (t, C-4,5 of Fmoc), 65.1 (s, -CH_2-fluorenyl), 46.7 (t, C-9 of Fmoc), 40.1 (s, 3-$CH_2CH_2CH_2\underline{C}H_2$NH-Fmoc), 32.2 (s, 6-$CH_2\underline{C}H_2CO_2H$), 31.6 (s, 3-$\underline{C}H_2CH_2CH_2CH_2$NH-Fmoc), 29.2 (s, 3-$CH_2CH_2\underline{C}H_2CH_2$NH-Fmoc), 25.2 (s, 6-$\underline{C}H_2CH_2CO_2H$), 23.7 (s, 3-$CH_2\underline{C}H_2CH_2CH_2$NH-Fmoc), 18.3 (p, 5-$CH_3$), *Anal.* Calcd for $C_{27}H_{29}N_3O_5 \cdot 0.5\ H_2O$: C, 66.9; H, 6.20; N, 8.67. Found: C, 67.1; H, 6.00; N, 8.65.

*6-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-3-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1H)-pyrazinone (**11**)*

6-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-3-(2-carboxyethyl)-5-methyl-2(1*H*)-pyrazinone (400 mg, 0.841 mmol), H-Phe-OBu^t (224 mg, 1.01 mmol), BOP (447 mg, 1.01 mmol) and DIEA (0.173 ml, 1.01 mmol) were dissolved in DMF (40 ml) while cooling with ice-salt. The reaction mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt, and the extract washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration and purified by column chromatography ($CHCl_3$), yield 153 mg (26.8%), mp 151-153 °C, $[\alpha]_D^{25}$ -28.3° (c=0.5, $CHCl_3$), Rf^3 0.23. ^{1}H-

NMR ($CDCl_3$) δ: 7.68 (2H, d-like, *J*=7.5, 4,5-CH of Fmoc), 7.55 (2H, d-like, *J*=7.5, 1,8-CH of Fmoc), 7.31 (2H, t-like, *J*=7.4, 3,6-CH of Fmoc), 7.22-7.15 (5H, m, 2,7-CH of Fmoc and 3,4 and 5-CH of Ph), 7.10 (2H, d-like, *J*=8.0, 2,6-CH of Ph), 6.65 (1H, d, *J*=7.7, 3-$(CH_2)_2CONH$-), 6.02 (1H, t, *J*=6.0, 6-$(CH_2)_4NH$-Fmoc), 4.76 (1H, dt, *J*=7.7, 6.0, α-CH of Phe), 4.38 (2H, d, *J*=6.8, CH_2 of Fmoc), 4.14 (1H, t, *J*=6.8, 9-CH of Fmoc), 3.26 (2H, m, 6-$CH_2CH_2CH_2CH_2NH$-Fmoc), 3.06 and 2.64 (4H, m, 3-CH_2CH_2CO-), 3.05 (2H, d, *J*=6.0, β-CH_2 of Phe), 2.50 (2H, br, 6-$CH_2CH_2CH_2CH_2NH$-Fmoc), 2.20 (3H, s, 5-CH_3), 1.61 (4H, br, 6-$CH_2CH_2CH_2CH_2NH$-Fmoc), 1.37 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 172.2 (q, 3-$(CH_2)_2CO_2NH$-), 170.8 (q, carbonyl of Phe), 157.6 (q, C-2), 156.7 (q, 6-$(CH_2)_4NHCO_2$-), 153.7 (q, C-3), 144.1 (q, 1a-C of Fmoc), 141.3 (q, 4a-C of Fmoc), 136.4 (q, C-1 of Ph), 134.9 (q, C-6), 129.53 (q, C-5), 129.48 (t, 2,6-CH of Ph), 128.2 (t, C-2,7 of Fmoc) , 127.5 (t, C-3,6 of Fmoc), 127.0 (t, 3,5-CH of Ph), 126.8 (t, 4-CH of Ph), 125.0 (t, C-1,8 of Fmoc), 119.8 (t, C-4,5 of Fmoc), 82.1 (q, *tert*-butyl), 66.3 (s, CH_2 of Fmoc), 53.6 (t, α-CH of Phe), 47.4 (t, C-9 of Fmoc), 40.0 (s, 6-$CH_2CH_2CH_2CH_2NH$-Fmoc), 38.2 (s, β-CH_2 of Phe), 32.5 and 27.90 (s, 3-CH_2CH_2CONH-), 29.4 (s, 6-$CH_2CH_2CH_2$-CH_2NH-Fmoc), 29.0 and 25.6 (s, 6-$CH_2CH_2CH_2CH_2NH$-Fmoc), 27.94 (p, *tert*-butyl), 18.2 (p, 5-CH_3), *Anal.* Calcd for $C_{40}H_{46}N_4O_6$: C, 70.8; H, 6.83; N, 8.25. Found: C,70.5; H, 6.76; N, 8.08.

3-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-6-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1H)-pyrazinone (13)

The title compound was prepared from 3-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-6-(2-carboxyethyl)-5-methyl-2(1*H*)-pyrazinone (400 mg, 0.841 mmol), yield 380 mg (66.6%), mp 95-98 °C, $[\alpha]_D^{25}$ -30.0° (*c*=0.5, $CHCl_3$), Rf^j 0.65. ^{1}H-NMR ($CDCl_3$) δ: 7.74 (2H, d-like, *J*=7.5, 4,5-CH of Fmoc), 7.59 (2H, d-like, *J*=7.4, 1,8-CH of Fmoc), 7.37 (2H, t-like, *J*=7.5, 3,6-CH of Fmoc), 7.27 (2H, td, *J*=7.4, 1.0, 2,7-CH of Fmoc), 7.23-7.15 (3H, m, 3,4 and 5-CH of Ph), 7.08 (2H, d-like, *J*=8.2, 2,6-CH of Ph), 6.48 (1H, d, *J*=7.7, 6-$(CH_2)_2CONH$-), 5.25 (1H, t, *J*=6.0, 3-$(CH_2)_4NH$-Fmoc), 4.76 (2H, dt, *J*=7.7, 6.2, α-CH of Phe), 4.36 (2H, d, *J*=6.8, CH_2 of Fmoc), 4.19 (1H, t, *J*=6.8, 9-CH of Fmoc), 3.22 (2H, m, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 3.06 and 3.03 (2H, m,, β-CH_2 of Phe), 2.81 (2H, t, *J*=6.7, 6-CH_2CH_2CO-), 2.76 (2H, t, *J*=7.4,3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 2.57 (2H, m, 6-CH_2CH_2CO-), 2.26 (3H, s, 5-CH_3), 1.74 (2H, m, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 1.58 (2H, m, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 1.38 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 171.1 (q, 6-$(CH_2)_2CO_2NH$-), 170.9 (q, carbonyl of Phe), 156.6 and 156.2 (q, C-2 and C-3), 156.5 (q, 3-$(CH_2)_4NHCO_2$-fluorenyl), 144.1 (q, 1a-C of Fmoc), 141.3 (q, 4a-C of Fmoc), 136.1 (q, C-1 of Ph), 133.2 and 129.36 (q, C-5 and C-6), 129.39 (t, 2,6-CH of Ph), 128.4 (t, 3,5-CH of Ph), 127.6 (t, C-3,6 of Fmoc),127.01 (t, C-2,7 of Fmoc), 126.99 (t, 4-CH of Ph), 125.1 (t, C-1,8 of Fmoc), 119.9 (t, C-4,5 of Fmoc), 82.7 (q, *tert*-butyl), 66.5 (s, CH_2 of Fmoc), 53.6 (t, α-CH of Phe), 47.4 (t, C-9 of Fmoc), 40.8 (s, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 38.1 (s, β-CH_2 of Phe), 34.1 (s, 6-CH_2CH_2CONH-), 32.1 (s, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 29.3 (s, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 28.0 (p, *tert*-butyl), 25.3 (s, 6-CH_2CH_2CONH-), 23.9 (t, 3-$CH_2CH_2CH_2CH_2NH$-Fmoc), 18.6 (p, 5-CH_3), *Anal.* Calcd for $C_{40}H_{46}N_4O_6 \cdot 0.25\ H_2O$:

C, 70.3; H, 6.86; N, 8.20. Found: C, 70.3; H, 6.97; N, 7.96.

*6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-3-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1H)-pyrazinone (**12**)*

A solution of 6-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-3-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1*H*)-pyrazinone (200 mg, 0.295 mmol) in 20% piperidine/DMF (8 ml) was stored at room temperature for 1 h. After removal the solvent, the residue was dissolved in DMF (30 ml). To the above solution, Boc-Tyr-OH (99.5 mg, 0.354 mmol), BOP (157 mg, 0.354 mmol) and DIEA (60.6 μl, 0.354 mmol) were added while cooling with ice-salt. The reaction mixture was then stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt, and the extract washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration, yield 78.0 mg (36.7%), mp 93-95 °C, $[\alpha]_D^{25}$ -29.0° (*c*=0.5, $CHCl_3$), *Rf*^{1} 0.54. *Anal.* Calcd for $C_{39}H_{53}N_5O_8 \cdot 0.75$ AcOEt: C,64.2; H, 7.52; N, 8.92. Found: C, 64.2; H, 7.54; N, 8.57.

*3-[4-(N^{α}-Boc-Tyr)-aminobutyl]-6-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1H)-pyrazinone (**14**)*

The title compound was prepared from 3-[4-(9-Fluorenylmethoxycarbonyl)-aminobutyl]-6-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1*H*)-pyrazinone (150 mg, 0.221 mmol), yield 133 mg (83.6%), mp 98-102 °C, $[\alpha]_D^{25}$ -20.6° (*c*=0.5, $CHCl_3$), *Rf*^{1} 0.67. *Anal.* Calcd for $C_{39}H_{53}N_5O_8 \cdot$ 0.5 AcOEt: C,64.5; H, 7.47; N, 9.17. Found: C, 64.7; H, 7.44; N, 9.17.

*3,6-Bis[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-2(1H)-pyrazinone (**16**)*

3,6-Bis(4-aminobutyl)-5-methyl-2(1*H*)-pyrazinone (200 mg, 0.537 mmol), Boc-Tyr-OH (332 mg, 1.18 mmol), BOP (571 mg, 1.29 mmol) and DIEA (0.257 ml, 1.50 mmol) were dissolved in DMF (20 ml) while cooling with ice-salt. The reaction mixture was then stirred at room temperature for 4 h. After removal of the solvent, the residue was extracted with AcOEt, and the extract washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from AcOEt/ether, yield 184 mg (44.0 %), mp 130-135 °C, $[\alpha]_D^{25}$ +4.41° (*c*=0.5, MeOH), *Rf*^{2} 0.70. *Anal.* Calcd for $C_{41}H_{58}N_6O_9 \cdot 1.5\ H_2O$: C, 61.1; H, 7.58; N, 10.4. Found: C, 61.0; H, 7.49; N, 10.1.

*5-Methyl-3-[2-(Phe)-carbonylethyl]-6-(4-tyrosylaminobutyl)-2(1H)-pyrazinone Hydrochloride (**III**)*

A solution of 6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-3-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1*H*)-pyrazinone (30.0 mg, 42 μmol) in TFA (95.8 μl, 1.25 mmol) containing anisole (9.58 μl) was stored at room temperature for 1 h. Dry ether was added to the solution to form a precipitate, which was collected by filtration and lyophilized from water containing 1 N HCl

(41.7 μl, 417 μmol) to afford a fluffy powder, yield 22.0 mg (87.9 %), $[\alpha]_D^{25}$ -4.99° (*c*=0.2, MeOH), Rf_2 0.25, t_R 24.10 (min), FAB-MS *m/z*: 564 $(M+H)^+$. HPLC conditions: column, COSMOSIL C18 (4.6×250 mm); solvents, A : B (95 : 5) for 5 min, to A : B (60 : 40) in 15 min, to A : B (30 : 70) in 30 min, to A : B (10 : 90) in 10 min; flow rate, 1 ml/min; detection, 220nm.

5-Methyl-6-[2-(Phe)-carbonylethyl]-3-(4-tyrosylaminobutyl)-2(1H)-pyrazinone Hydrochloride (IV)

The title compound was prepared from 3-[4-(N^{α}-Boc-Tyr)-aminobutyl]-6-[2-(Phe-OBu^t)-carbonylethyl]-5-methyl-2(1*H*)-pyrazinone (50.0 mg, 70 μmol), yield 32.0mg (76.7%), $[\alpha]_D^{25}$ -2.28° (*c*=0.1, MeOH), Rf_2 0.30, t_R 23.74 (min), FAB-MS *m/z*: 564 $(M+H)^+$. HPLC conditions: column, COSMOSIL C18 (4.6×250 mm); solvents, A : B (95 : 5) for 5 min, to A : B (60 : 40) in 15 min, to A : B (30 : 70) in 30 min, to A : B (10 : 90) in 10 min; flow rate, 1 ml/min; detection, 220nm.

5-Methyl-3,6-bis(4-tyrosylaminobutyl)-2(1H)-pyrazinone Hydrochloride (V)

The title compound was prepared from 3,6-Bis[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone (100 mg, 128 μmol), yield 56.0 mg (67.1%), $[\alpha]_D^{25}$ +43.2° (*c*=0.5, MeOH), Rf^2 0.16, t_R 19.55 (min), TOF-MS *m/z*: 579.7 $(M+H)^+$. HPLC conditions: column, COSMOSIL C18 (4.6×250mm); solvents, A : B (95 : 5) for 5 min, to A : B (65 : 35) in 10 min, to A : B (50 : 50) in 30 min, to A : B (10 : 90) in 10 min; flow rate, 1 ml/min; detection, 220nm.

Radioligand Binding

Synaptosomal membranes were prepared from whole brains (minus cerebellum) from Sprague-Dawley rats. Brains were homogenized in 0.32 M sucrose, 10 mM HEPES, pH 7.5, and 50 μg/ml soybean trypsin inhibitor and obtained a P_2 fraction by differential centrifugation[19]. The synaptosomes were preincubated in 50 mM HEPES, pH 7.5, 100 mM NaCl, 0.1 mM GMP and soybean trypsin inhibitor to remove endogenous opioids[19,20]. The radioligand displacement for δ and μ receptors used 5.57 nM [^{3}H]DPDPE (NEN-DuPont) and 3.5 nM [^{3}H]DAGO (Amersham), respectively, under equilibrium conditions at 22 °C using 2 μM unlabeled ligand to suppress non-specific binding as described previously.[19-22] Each compound was assayed over three to four orders of magnitude in concentration and conducted in duplicate with 3 or more different membrane preparations. Affinity constants (K_i) were determined according to Cheng and Prusoff[23].

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