Amino Acids and Peptides. LII. Design and Synthesis of Opioid Mimetics Containing a Pyrazinone Ring and Examination of Their Opioid Receptor Binding Activity[1,2]

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An amino group was introduced to the 3 or 6 position of a pyrazinone ring by cyclization of dipeptidyl chloromethyl ketones. Boc-Tyr-OH was coupled with the amino function, followed by removal of the Boc group to give pyrazinone ring-containing tyrosine derivatives. Of the various tyrosine derivatives prepared, 5-methyl-6-β-phenethyl-3-tyrosylaminobutyl-2(1*H*)-pyrazinone exhibited strong binding to the μ-opioid receptor with a K_i value of 55.8 nM and to the δ-opioid receptor with a K_i value of 2165 nM and with a $K_i\mu/K_i\delta$ value of 0.026.

Key words 2(1*H*)-pyrazinone; simple synthetic procedure; amino-containing pyrazinone derivative; pyrazinone-containing tyrosine derivative; opioid-receptor binding activity

G-Protein coupled δ-, κ-, and μ-opioid receptors are located in the central nervous system and are generally involved in pain perception and modulation. The naturally occurring mammalian ligands for these receptors are the enkephalins,[3] endorphins,[4] dynorphins,[5] and the endomorphins.[6] Interestingly, with the exception of the endomorphins, these ligands exhibit only moderate selectivity for a given opioid-receptor subtype. For this reason, a common goal of researchers is to design receptor selective compounds that serve to unveil structure–activity relationships of the opioid system as well as the distinct biological functions of each opioid receptor subtype.

Receptor selectivity in opioid peptides has been attributed to a variety of causes, predominantly chemical contribution, conformation of the message and address domains[7—9] and net charge.[10] In the case of the enkephalins, H-Tyr-Gly-Gly-Phe-Leu-OH or H-Tyr-Gly-Gly-Phe-Met-OH, the message sequence is Tyr-Gly-Gly-Phe and the address sequence is Leu-OH or Met-OH. In the message sequence, the spacer residues Gly-Gly, play a significant role in orienting the biologically important Tyr and Phe residues for manifestation of opioid activity. It is well known that enkephalins are rapidly degraded by amino peptidases and/or enkephalinases. Therefore, enkephalin derivatives that are receptor selective and resist enzyme degradation are ideal. Our goal is to design opioid analogs by introduction of a pyrazinone ring into the enkephalin sequence to study the effects of altering the chain length of the message domain and modifying the address sequence. Previously, a facile and convenient synthetic procedure for preparation of 2(1*H*)-pyrazinone derivatives from dipeptidyl chloromethyl ketones was developed.[11] This novel method afforded 2(1*H*)-pyrazinone derivatives substituted at the 3 and 6 positions with the desired functional groups in high yield. Therefore, an amino and/or a carboxyl group can be easily introduced at position 3 or 6 of the pyrazinone ring by using the appropriate amino acid,[12] indicating that peptide mimetics containing a pyrazinone ring can be easily prepared. This paper describes the design and synthesis of a novel series of enkephalin analogs containing a pyrazinone ring, examination of their binding activity to opioid receptors and investigation of the relationship between structure and binding activity.

To investigate the role of the pyrazinone ring and the relationship of the phenyl ring of the Tyr residue to the other aromatic center, we designed the analogs shown in Fig. 1. The Gly-Gly sequence of enkephalin (a) corresponds to the aminobutyl moiety at position 6 of the pyrazinone in compound **2** and the Leu side chain corresponds to the isobutyl moiety at the 3 position. Compound **4**, without a phenolic hydroxyl group, was designed to study the role of the hydroxyl group in opioid receptor recognition.

As shown in Chart 1, compounds (**1**—**4**) were prepared starting from Boc-dipeptidyl chloromethyl ketones (R: methyl, isobutyl, benzyl). After removal of the Boc group,

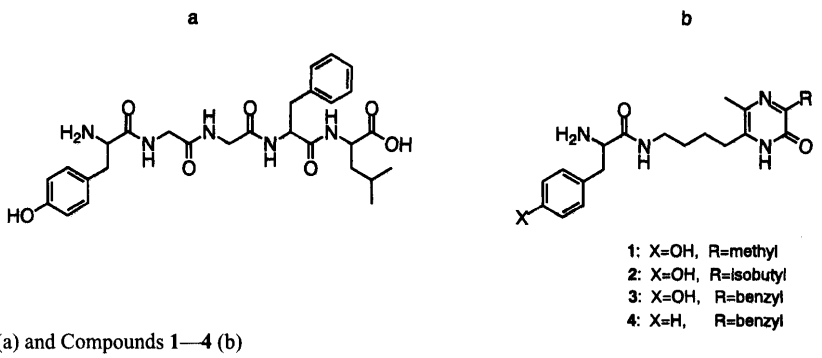

Fig. 1. Structures of LENK (a) and Compounds **1**—**4** (b)

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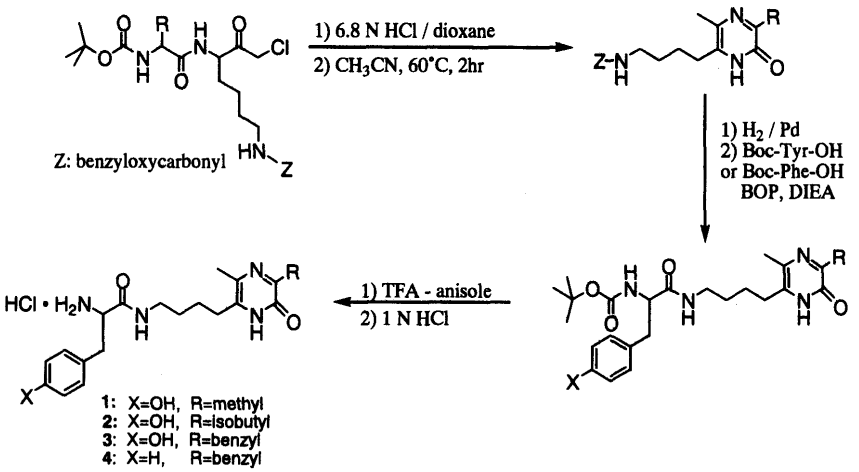


Chart 1. Synthetic Scheme for Compounds **1**—**4**

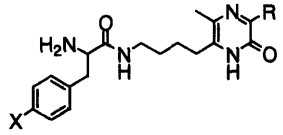
the resultant amine hydrochloride was converted to the corresponding pyrazinone derivative. The Z group was removed by hydrogenation over a Pd catalyst and the resultant amine was coupled with Boc-Tyr-OH using the BOP reagent to give a Boc-Tyr-pyrazinone derivative. The Boc group was removed with TFA and the resultant amine converted to its hydrochloride salt (**1**—**3**). In place of Boc-Tyr-OH, use of Boc-Phe-OH in the coupling with the pyrazinone derivative, and subsequent removal of the Boc group, yielded **4**. Binding activities of compounds (**1**—**4**) with opioid receptors were examined and the results are summarized in Table 1.

As shown in Table 1, compound **2** did not show affinity for either the δ- or μ-opioid receptor, indicating that the pyrazinone ring does not interact with opioid receptors like the aromatic ring of Phe in enkephalin. In contrast, compound **3** which contains a benzene ring at position 3 of the pyrazinone, exhibited weak affinity for the δ-opioid receptor with a K_i value of 332.7 nM. However, deletion of the phenolic hydroxyl group of Tyr from compound **3**, led to **4** and resulted in a loss of binding to both δ- and μ-opioid receptors. This result demonstrated that the aromatic ring of the phenol, the hydroxyl function on Tyr and the benzene ring at position 3 of the pyrazinone ring are required for manifestation of opioid receptor binding activity as was observed with opioid dipeptides.[13] Compound **3** may represent the message domain of enkephalin.

The effect of modifying the chain length between Tyr and the pyrazinone (Part A, Fig. 2) and between the pyrazinone and the second aromatic ring (Part B, Fig. 2) was next investigated. The synthetic scheme for the designed derivatives **5**—**8** is shown in Chart 2 and their receptor binding affinities are reported in Table 2. Deletion of the methylene moiety from the aminobutyl group at position 6 of **3** and from the benzyl group at position 3 of **3** yielded compounds **5** and **6**, respectively. The insertion of a methylene moiety to the benzyl group at position 3 of **3** gave compound **7**. Deletion of a methylene group from the aminobutyl group of **7** resulted in **8**. All of these derivatives (**5**—**8**) displayed decreased δ-receptor affinity, and only compounds (**5**, **7**, and **8**) displayed enhanced, albeit weak, μ-receptor affinity relative to **3**.

These results demonstrated that modification of the chain length of part A and/or part B negatively affected interaction with the δ-opioid receptor, presumably due to a change in the relative position of the phenol and phenyl rings in the three dimensional structure.

Table 1. Binding Activity of Compounds **1**—**4**



Compound	X	R	K_i (nM) δ		K_i (nM) μ		$K_i\mu/K_i\delta$
1	OH	Me	8670±710	(4)	4818±766	(4)	0.56
2	OH	Isobutyl	2376±281	(4)	5932±897	(4)	2.5
3	OH	Bzl	332.7±14.6	(5)	3909±756	(4)	11.7
4	H	Bzl	3366±164	(3)	9747±609	(3)	2.9

Displacement of [^{3}H]DAGO(μ selective) and [^{3}H]DPDPE (δ selective) from rat brain membrane synaptosomes. Values are mean±S.E.M. with number of independent experiments given in parenthesis.

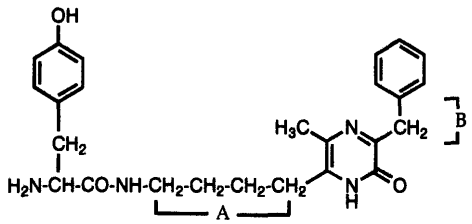


Fig. 2. Modifying Parts (A and B) of Compound **3**

The effect of exchange of the substituents at positions 3 and 6 of the pyrazinone ring on the binding activity with opioid receptors was also explored. Modification of the chain length of part C and/or part D (Fig. 3) led to compounds (**9**—**15**). Chart 3 outlines the synthetic scheme and Table 3 reports the receptor binding affinities. Compound **9** had a $K_i\delta$ value of 462 nM and $K_i\mu$ value of 438 nM, indicating that relocation of Tyr from position 6 to 3 on the pyrazinone ring gave different opioid activity profiles, relative to **3**. It is also possible that the pyrazinone ring plays the role of the address sequence due to the enhanced μ-opioid receptor affinity with these modifications. Reduction of the chain length of part D (Fig. 3) of **9** gave compounds (**10**—**12**). Higher μ-opioid receptor affinity was observed for both **10** and **11** than that of

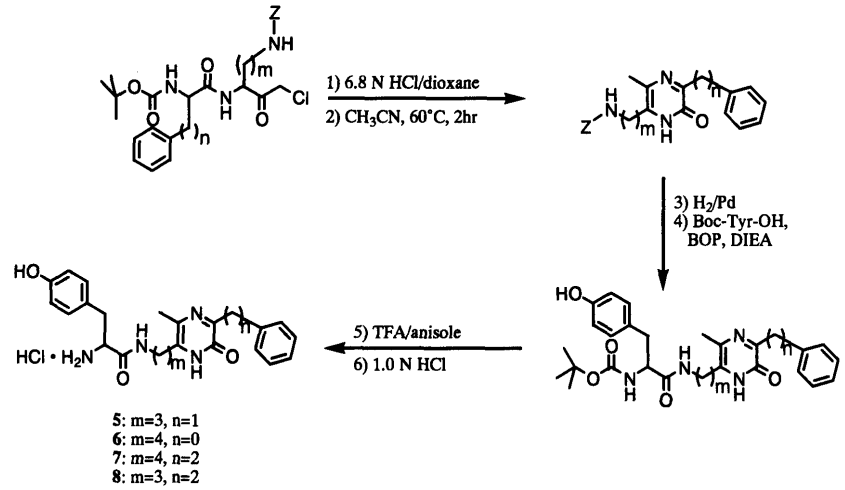
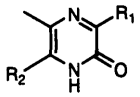


Chart 2. Synthetic Scheme for Compounds **5**—**8**

Table 2. Binding Activity of Compounds **5**—**8**



Compound	R_1	R_2	K_i (nM) δ	K_i (nM) μ	$K_i\mu/K_i\delta$
3	Bzl	H-Y-NH$(CH_2)_4$	332.7±14.6 (5)	3909±756 (4)	11.7
5	Bzl	H-Y-NH$(CH_2)_3$	3252±247 (4)	1165±215 (4)	0.36
6	Phenyl	H-Y-NH$(CH_2)_4$	2190±159 (4)	5336±682 (3)	2.4
7	β-Phenethyl	H-Y-NH$(CH_2)_4$	5646±1172 (5)	1513±160 (3)	0.2
8	β-Phenethyl	H-Y-NH$(CH_2)_3$	4794±274 (4)	1211±48 (3)	0.25

Displacement of [^{3}H]DAGO(μ selective) and [^{3}H]DPDPE (δ selective) from rat brain membrane synaptosomes. Values are the mean±S.E.M. with number of independent experiments given in parenthesis. Y: tyrosine.

3, but the affinity was still weak. On the other hand, **12** lost affinity for both δ- and μ-opioid receptors. Deletion of the methylene moiety from the benzyl group of **9** gave **13**. Insertion of a methylene moiety to the benzyl group of **9** gave **14**. Insertion of a methylene moiety to part C of **9** and deletion of the methylene moiety from part D of **9** yielded **15**. Amongst the derivatives, **14** (5-methyl-6-β-phenethyl-3-tyrosylaminobutyl-2(1*H*)-pyrazinone) exhibited the most potent μ-opioid receptor affinity ($K_i\mu$=55.8 nM) and the highest μ-opioid receptor selectivity ($K_i\mu/K_i\delta$ value=0.026). A β-phenethyl group at the 6 position of the pyrazinone in **14** either provides the optimal distance between the aromatic groups for μ-opioid receptor interaction or it provides additional flexibility for conforming to the structure of the μ-opioid receptor binding site.

Molecular modeling studies of compounds **3** and **14** were performed to investigate aromatic ring distances and flexibility. Conformational searches produced a diverse sample of low energy structures with aromatic ring distances varying from 6—14 nm for compounds **3** and **14**. The structural substitutions did not restrict the distances attainable by the aromatic rings for low energy orientations. The difference in energy (lowest to highest energy) between the conformers sampled for each derivative was approximately 8 kcal/mol. The lowest energy conformers had shorter ring distances measuring around 6 nm, while conformers with the larger ring distances displayed higher relative energies. Not surprisingly,

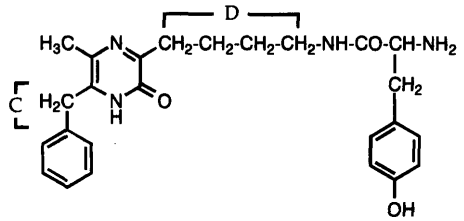


Fig. 3. Modifying Parts (C and D) of Compound **9**

the lowest energy structures, which had shorter ring to ring distances, superimposed best with the X-ray structures of μ selective *erythro*-5-methylmethadone (EMM)[14] and β-funaltrexamine (β-FNA).[15] Likewise the larger ring to ring distances superimposed best with two X-ray structures of [Leu^5]-enkephalin, the extended form, (LENK),[15] and the β-turn form of β-LENK[16] (Fig. 4). From this information, we cannot determine which conformation is most important for opioid receptor interaction. However, the models demonstrated that the aromatic rings can adopt a diverse range of distances with less than a 10 kcal/mol change in energy, even with the shortening of the pyrazinone side-chain from β-phenethyl group to a phenyl group. Superimposing the pharmacophoric elements, the amine, tyrosine aromatic ring and the other aromatic ring of the lowest energy structures of the δ-selective derivative **3** and the μ-selective derivative **14** gave almost a 2.0 nm root mean square deviation for both the folded

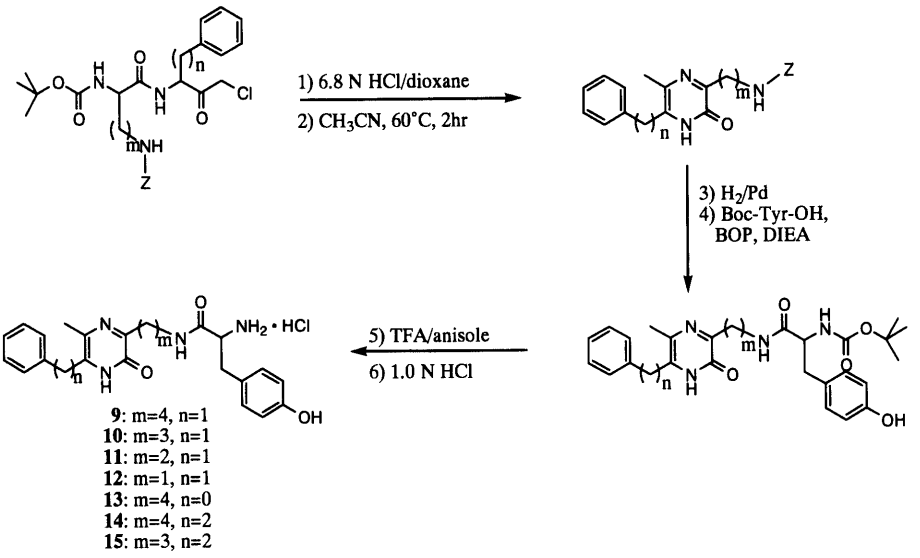
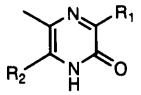


Chart 3. Synthetic Scheme for Compounds **9**—**15**

Table 3. Binding Activity of Compounds **9**—**15**



Compound	R_1	R_2	K_i (nM) δ	K_i (nM) μ	$K_i\mu/K_i\delta$
3	Bzl	H-Y-NH$(CH_2)_4$	332.7±14.6 (5)	3909±756 (4)	11.7
9	H-Y-NH$(CH_2)_4$	Bzl	462.4±62 (4)	438.3±52 (3)	0.95
10	H-Y-NH$(CH_2)_3$	Bzl	4930±1005 (4)	878.7±115 (3)	0.18
11	H-Y-NH$(CH_2)_2$	Bzl	985.2±147 (4)	813.2±58 (4)	0.82
12	H-Y-NHCH_2	Bzl	6518±1269 (5)	3275±126 (3)	0.6
13	H-Y-NH$(CH_2)_4$	Phenyl	1092±134 (4)	267.5±15 (4)	0.24
14	H-Y-NH$(CH_2)_4$	β-Phenethyl	2165±334 (4)	55.8±10.8 (4)	0.026
15	H-Y-NH$(CH_2)_3$	β-Phenethyl	6165±128 (4)	287.8±20 (4)	0.05

Displacement of [^{3}H]DAGO(μ selective) and [^{3}H]DPDPE (δ selective) from rat brain membrane synaptosomes. Values are the mean±S.E.M. with number of independent experiments given in parenthesis. Y: tyrosine.

and extended forms. One notable difference between these superimposed structures was the orientation of the carbonyl groups on the pyrazinone rings (Fig. 5). The position of the carbonyl may be an important factor for μ-opioid receptor interaction. This observation is supported by μ-selective D-isomer analogs of δ-selective opioid ligands. Dmt-Tic-NH_2,[17] Dmt-Tic-Ala-NH_2[17] and Tyr-Tic-Phe-Phe-OH[18] are selective for the δ-opioid receptor. Simply changing the chirality of L-Tic to the D-isomer changed receptor selectivity from δ to μ.[17,18] Both the L- and D-isomer ligands contain the basic pharmacophoric elements for receptor recognition, but the orientation of the carbonyl of the D-isomer may be a discriminating factor for μ-receptor interaction. It has been hypothesized, based on cyclic β-casomorphin analogs, that the orientation of the second aromatic ring distinguishes between δ- and μ-opioid receptors.[19] This hypothesis can be supported by the D-isomer compounds as well since the spatial orientation of the D-isomer aromatic ring may shift to a position that fits the μ-opioid receptor domain. However, the models of compounds **3** and **14** show a great deal of flexibility around the second aromatic ring and similar low energy conformations with a variety of orientations were accessible

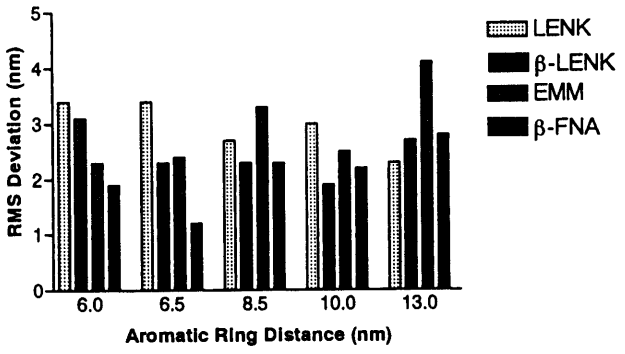


Fig. 4. Superposition of Compound **14** Low Energy Conformers with X-Ray Structures of Opioids

Superposition of five unique low energy conformers of **14** distinguishable by aromatic ring to ring distances ranging from 6 nm to 13 nm with X-ray structures of δ- and μ-opioid receptor ligands. LENK and β-LENK represent the extended and β-turn conformations of the δ-opioid receptor selective ligand, [Leu^5]enkephalin, respectively. EMM and β-FNA represent the μ-receptor selective opiates, *erythro*-5-methylmethadone and β-funaltrexamine. The superpositioning involves specific pharmacophore elements available for each substance regardless of the dissimilarity that exists between them. The root mean square (rms) is a highly valid means to compare the degree of fit between the chosen pharmacophores in each compound relative to the low energy conformers of **14**; *i.e.*, rms deviation is a measure of how well the pharmacophoric elements superimposed. Less than one nm rms deviation indicates a very similar orientation of atoms selected for superposition. The pharmacophoric elements selected for superposition were the aromatic hydroxyl group, both aromatic rings, a protonatable amine, and three atoms representing a hydrophobic spacer.

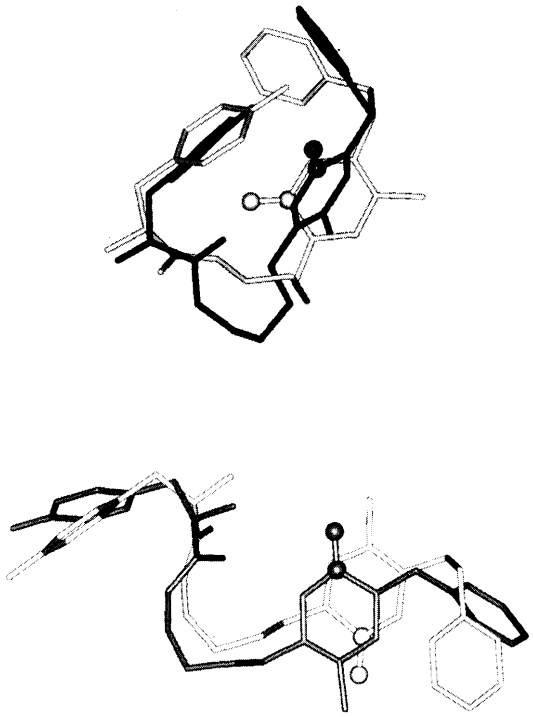


Fig. 5. Superposition of Low Energy Structures of Derivatives **3** and **14** [Folded (top) and Extended (bottom) Forms]

The δ-selective, **3** is shown as the dark color and the μ-selective **14** is shown in white. Hydrogen atoms are excluded. The carbonyl groups (−C=O) in the pyrazinone rings are shown with spheres. The lowest energy structure (top) and extended forms (bottom) gave 1.9 and 1.8 nm root mean square deviations, respectively.

to both derivatives. This suggests that for the pyrazinone analogs another factor may be involved in distinguishing between δ- and μ-opioid receptor binding domains. Compound **14**, in particular its pyrazinone substituent, may provide a framework suitable for further structure–activity studies to explore the role of the carbonyl and the second aromatic group in the μ-receptor interaction and differences between the μ- and δ-opioid receptor binding requirements.

Experimental

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 shift values 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 aid 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 and Rf^4 values refer to the systems of $CHCl_3$, MeOH and water (89 : 10 : 1), AcOEt and hexane (1 : 1), $CHCl_3$ and MeOH (19 : 1) and *n*-BuOH, AcOH and water (4 : 1 : 5, upper phase), respectively.

Boc-Orn(Z)-CH_2Cl To a solution of mixed anhydride [prepared from Boc-Orn(Z)-OH (12.0 g, 32.8 mmol), isobutyl chloroformate (4.3 ml, 32.8 mmol) and Et_3N (5.1 ml, 36.1 mmol) in the usual way] in THF (150 ml), diazomethane in ether (60 ml) [prepared from *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosoamide (35.1 g, 0.16 mol) in the usual way] was added. The reaction mixture was stirred at 4 °C overnight. 6.7 N HCl/dioxane (12.2 ml, 82.0 mmol) was then added to the above solution under cooling with ice-salt and the reaction mixture was stirred at −15 °C for 3 h. The pH of the solution was then adjusted to 8 with NMM. After removal of the 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 10.5 g (80.4%), mp 61—62 °C, $[\alpha]_D^{25}$ −25.5° (c=1.0, MeOH), Rf^2 0.68. *Anal.* Calcd for $C_{19}H_{27}ClN_2O_5$: C, 57.3; H, 6.80; N, 7.20. Found: C, 57.2; H, 6.82; N, 7.02.

Boc-DL-Hfe-CH_2Cl A solution of diazomethane [prepared from *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosoamide (11.5 g, 53.7 mmol) in the usual way] in ether (50 ml) was added to the mixed anhydride [prepared from Boc-DL-Hfe-OH (5.0 g, 17.9 mmol), isobutyl chloroformate (2.8 ml, 21.5 mmol) and NMM (2.9 ml, 26.9 mmol) in the usual way] in THF (60 ml) under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. To the solution, 6.7 N HCl/dioxane (18 ml, 120 mmol) was added under cooling with ice-salt. After 3 h at −15 °C, the pH of the solution was adjusted to 8 with NMM. The solvent was removed by evaporation and the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Petroleum ether and hexane were added to the residue to form crystals, which were collected by filtration, yield 3.8 g (67.3%), mp 68—70 °C. *Anal.* Calcd for $C_{16}H_{22}ClNO_3$: C, 61.6; H, 7.11; N, 4.49. Found: C, 61.9; H, 7.21; N, 4.56.

Boc-DL-Phg-CH_2Cl Yield 26.5%, mp 86—88 °C, Rfl 0.76. *Anal.* Calcd for $C_{14}H_{18}ClNO_3$: C, 59.3; H, 6.40; N, 4.94. Found: C, 59.2; H, 6.38; N, 4.99.

Boc-Xaa-Yaa-CH_2Cl, General Procedure for the Synthesis of Boc-Dipeptidyl Chloromethyl Ketone [Xaa and Yaa=Amino Acids] A mixed anhydride [prepared from Boc-XaaOH (4.68 mmol), isobutyl chloroformate (613 μl, 4.68 mmol) and NMM (510 μl, 4.68 mmol) in the usual way] in THF (50 ml) was added to a solution of H-Yaa-CH_2Cl·HCl [prepared from Boc-Yaa-CH_2Cl (3.6 mmol) and 7.5 N HCl/dioxane (4.8 ml, 36 mmol) in the usual way] in DMF (30 ml) containing NMM (510 μl, 4.68 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. Petroleum ether was added to the residue to form crystals, which were collected by filtration and recrystallized from EtOH. The yields, mps, $[\alpha]_D$ values, analytical data and Rf values are summarized in Table 4.

Boc-Lys(Z)-DL-Phg-CH_2Cl A solution of diazomethane [prepared from *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosoamide (2.7 g, 12.4 mmol) in the usual way] in ether (30 ml) was added to the mixed anhydride [prepared from Boc-Lys(Z)-DL-Phg-OH (2.20 g, 4.16 mmol), isobutyl chloroformate (545 μl, 4.16 mmol) and NMM (545 μl, 5.00 mmol) in the usual way] in THF (50 ml) under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. 7.5 N HCl/dioxane (1.7 ml, 12.8 mmol) was added to the solution at −15 °C and the reaction mixture was stirred at the same temperature for 3 h. The reaction mixture was diluted with water (50 ml) and extracted with AcOEt. The extract was washed with 5% $NaHCO_3$, and water, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column (2.0×20 cm) chromatography using 1% MeOH in $CHCl_3$. After removal of the solvent, ether was added to the residue to form an amorphous powder, yield 1.30 g (57.3%), Rf^1 0.50. *Anal.* Calcd for $C_{28}H_{36}ClN_3O_6$: C, 61.6; H, 6.65; N, 7.70. Found: C, 61.6; H, 6.67; N, 7.70.

6-(4-Benzyloxycarbonylaminobutyl)-3,5-dimethyl-2(1*H*)-pyrazinone A solution of H-Ala-Lys(Z)-CH_2Cl·HCl [prepared from Boc-Ala-Lys(Z)-CH_2Cl (1.0 g, 2.0 mmol) and 7.5 N HCl/dioxane (1.6 ml, 12.0 mmol) in the usual way] in CH_3CN (60 ml) and MeOH (10 ml) was refluxed for 2 h. After removal of the solvents, the residue was extracted with $CHCl_3$ and was washed with 0.1 N HCl, 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 EtOH, yield 280 mg (42.4%), mp 157—158 °C, Rf^1 0.51. ^{1}H-NMR ($CDCl_3$) δ: 13.3 (1H, br s, NH), 7.29 (5H, m, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 5.81 (1H, t-like, J=7.5, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 5.07 (2H, s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 3.31 (2H, q-like, J=6.0, 6-$CH_2CH_2CH_2CH_2$NH-Z) 2.55 (2H, t, J=6.9, 6-$CH_2CH_2CH_2CH_2$NH-Z), 2.39 (3H, s, 3-CH_3), 2.27 (3H, s, 5-CH_3), 1.72—1.62 (4H, m, 6-$CH_2CH_2CH_2$-CH_2NH-Z). ^{13}C-NMR ($CDCl_3$) δ: 158.0 (q, C-2), 156.6 (q, 6-$(CH_2)_4$-NHCO$_2$CH$_2$-Ph), 153.0 (q, C-3), 136.6 (q, 6-C1′), 134.4 (q, C-6), 129.4 (q, C-5), 128.4 (t), 128.0 (t), 127.9 (t), 66.5 (s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 39.5 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 29.0 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 28.6 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 25.3 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 19.7 (p, 3-CH_3), 18.3 (p, 5-CH_3). *Anal.* Calcd for $C_{18}H_{23}N_3O_3$: C, 65.6; H, 7.04; N, 12.8. Found: C, 65.7; H, 7.17; N, 12.7.

6-(4-Benzyloxycarbonylaminobutyl)-3-isobutyl-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Leu-Lys(Z)-CH_2Cl (1.0 g, 1.9 mmol), yield 280 mg (40.3%), mp 122—123 °C, Rf^1 0.42. ^{1}H-NMR ($CDCl_3$) δ: 13.3 (1H, br s, NH), 7.30 (5H, m, 6-$(CH_2)_4$NH-

Table 4. Yield, Melting Point, $[\alpha]_D$ Value, Elemental Analysis Data and TLC of Boc-Xaa-Yaa-CH_2Cl [Xaa, Yaa=Amino Acid]

Compound		Yield (%)	m.p. (°C)	$[\alpha]_D^{25}$ (MeOH) (°)	Formula	Elemental Calcd (Found)			TLC	
Xaa	Yaa					C	H	N	Rf^1	Rf^2
Ala	Lys(Z)	71.5	94—96	−47.9	$C_{23}H_{34}ClN_3O_6$	57.1 (57.2	7.08 7.24	8.68 8.72)	0.60	
Leu	Lys(Z)	44.8	125—126	−37.0	$C_{26}H_{40}ClN_3O_6$	59.4 (59.5	7.66 7.74	7.99 7.72)	0.78	
Phe	Lys(Z)	40.7	164—126	−27.7	$C_{29}H_{38}ClN_3O_6$	62.2 (62.3	6.84 6.96	7.50 7.61)	0.56	
Phe	Orn(Z)	40.0	154—157	−50.8	$C_{28}H_{36}ClN_3O_6$	61.6 (61.6	7.08 6.74	7.70 7.72)	0.56	
DL-Phg	Lys(Z)	59.2	135—138	−7.7	$C_{28}H_{36}ClN_3O_6$	61.6 (61.6	6.65 6.67	7.70 7.70)		0.60
DL-Hfe	Lys(Z)	10.5	122—124	−19.4	$C_{30}H_{40}ClN_3O_6$	62.8 (62.9	7.02 7.12	7.32 7.26)		0.57
DL-Hfe	Orn(Z)	47.9	124—126	−4.9	$C_{29}H_{38}ClN_3O_6$	61.8 (61.9	6.87 6.76	7.45 7.46)	0.80	
Lys(Z)	Phe	58.4	135—137	−50.8	$C_{29}H_{38}ClN_3O_6 \cdot 0.75H_2O$	65.2 (65.3	7.08 6.90	10.5 10.3)	0.30	
Orn(Z)	Phe	51.1	122—125	−60.4	$C_{28}H_{38}ClN_3O_6$	61.6 (61.5	6.65 6.62	7.70 7.81)		0.86
Dab(Z)[20]	Phe	46.3	121—125	−33.6	$C_{27}H_{34}ClN_3O_6$	61.0 (60.8	6.44 6.34	7.90 8.16)	0.63	
Dap(Z)[21]	Phe	66.1	178	−33.0	$C_{26}H_{32}ClN_3O_6$	60.3 (60.0	6.23 6.28	8.11 7.88)	0.50	
Lys(Z)	DL-Phg	25.7	Amorphous		$C_{28}H_{36}ClN_3O_6$	61.6 (62.8	6.64 6.65	7.70 7.79)	0.50	
Lys(Z)	DL-Hfe	31.4	99—103	−16.7	$C_{30}H_{40}ClN_3O_6$	61.8 (62.8	7.02 7.00	7.32 7.30)	0.63	
Orn(Z)	DL-Hfe	26.2	125—128	−2.8	$C_{29}H_{38}ClN_3O_6$	62.2 (62.1	9.84 6.84	7.50 7.29)	0.63	

CO_2CH_2-Ph), 5.63 (1H, t-like, J=6.2, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 5.08 (2H, s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 3.31 (2H, q-like, J=6.0, 6-$CH_2CH_2CH_2$-CH_2NH-Z) 2.62 (2H, d, J=7.2, 3-CH_2CH$(CH_3)_2$), 2.54 (2H, t, J=7.0, 6-$CH_2CH_2CH_2CH_2$NH-Z), 2.28 (3H, s, 5-CH_3), 2.17 (1H, m, 3-CH_2CH-$(CH_3)_2$), 1.72—1.62 (4H, m, 6-$CH_2CH_2CH_2CH_2$NH-Z), 0.92 (6H, d, J=6.7, 3-$CH_2CH(CH_3)_2$). ^{13}C-NMR ($CDCl_3$) δ: 158.0 (q, C-2), 156.6 (q, 6-$(CH_2)_4$-NHCO$_2$CH$_2$-Ph), 155.6 (q, C-3), 136.7 (q, 6-C1′), 134.3 (q, C-6), 129.5 (q, C-5), 128.4 (t), 128.04 (t), 127.99 (t), 66.5 (s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 41.6 (s, 3-CH_2CH$(CH_3)_2$), 39.7 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 29.1 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 28.9 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 27.1 (t, 3-CH_2-$CH(CH_3)_2$), 25.4 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 22.6 (p, 3-$CH_2CH(CH_3)_2$), 18.4 (p, 5-CH_3). *Anal.* Calcd for $C_{21}H_{29}N_3O_3$: C, 67.7; H, 7.90; N, 11.3. Found: C, 67.9; H, 7.87; N, 11.3.

3-Benzyl-6-(4-benzyloxycarbonylaminobutyl)-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Phe-Lys(Z)-CH_2Cl (1.2 g, 2.1 mmol), yield 540 mg (63.5%), mp 129—130 °C, Rf^1 0.51. ^{1}H-NMR ($CDCl_3$) δ: 13.4 (1H, br s, NH), 7.32—7.13 (10H, m, 3-CH_2-Ph+6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 6.16 (1H, br s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 5.08 (2H, s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 3.31 (2H, q-like, J=6.1, 6-$CH_2CH_2CH_2$-CH_2NH-Z) 2.49 (2H, t, J=7.5, 6-$CH_2CH_2CH_2CH_2$NH-Z), 2.27 (3H, s, 5-CH_3), 1.71—1.62 (4H, m, 6-$CH_2CH_2CH_2CH_2$NH-Z). ^{13}C-NMR ($CDCl_3$,) δ: 157.7 (q, C-2), 156.6 (q, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 154.1 (q, C-3), 138.0 (q, 3-C-1″), 136.7 (q, 6-C-1′), 135.3 (q, C-6), 129.8 (q, C-5), 129.1 (t, C-2″,6″), 128.4 (t), 128.2 (t, C-3″,5″), 128.03 (t), 128.01 (t), 126.3 (t, C-4″), 66.6 (s, 6-$(CH_2)_4$NHCO$_2$CH$_2$-Ph), 39.9 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 39.3 (s, CH_2-Ph), 29.1 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 28.9 (s, 6-$CH_2CH_2CH_2$-CH_2NH-Z), 25.4 (s, 6-$CH_2CH_2CH_2CH_2$NH-Z), 18.5 (p, 5-CH_3). *Anal.* Calcd for $C_{24}H_{27}N_3O_3 \cdot 0.25H_2O$: C, 70.5; H, 6.73; N, 10.3. Found: C, 70.3; H, 6.76; N, 10.3.

3-Benzyl-6-(3-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Phe-Orn(Z)-CH_2Cl (800 mg, 1.46 mmol), yield 243 mg (42.6%), mp 158—161 °C, Rf^3 0.26. ^{1}H-NMR ($CDCl_3$) δ: 13.6 (1H, br s, NH), 7.29-7.13 (10H, m, 3-CH_2-Ph+6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 6.16 (1H, br s, 6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 5.05 (2H, s, 6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 4.02 (2H, s, 3-CH_2-Ph), 3.18 (2H, q-like, J=5.8, 6-$CH_2CH_2CH_2$NH-Z), 2.56 (2H, t, J=7.0, 6-$CH_2CH_2CH_2$NH-Z), 2.27 (3H, s, 5-CH_3), 1.81 (2H, m, 6-$CH_2CH_2CH_2$NH-Z). ^{13}C-NMR ($CDCl_3$) δ: 157.5 (q, C-2), 156.7 (q, 6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 154.3 (q, C-3), 137.9 (q, 3-C-1″), 136.6 (q, 6-C-1′), 134.8 (q, C-6), 130.7 (q, C-5), 129.1 (t, 3-C-2″, 6″), 128.5 (t), 128.4 (t), 128.2 (t, 3-C-3″, 5″), 128.1 (t), 126.3 (t, 3-C-4″), 66.7 (s, 6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 39.1 (s, 6-$CH_2CH_2CH_2$NH-Z), 38.9 (s, 3-CH_2-Ph), 28.3 (s, 6-$CH_2CH_2CH_2$NH-Z), 26.4 (s, 6-$CH_2CH_2CH_2$NH-Z), 18.5 (p, 5-CH_3). *Anal.* Calcd for $C_{23}H_{25}N_3O_3$: C, 70.6; H, 6.44; N, 10.7. Found: C,70.4; H, 6.46; N, 10.7.

6-(4-Benzyloxycarbonylaminobutyl)-5-methyl-3-phenyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-DL-Phg-Lys(Z)-CH_2Cl (1.0 g, 1.80 mmol), yield 345.2 mg (49.0%), mp 185—186 °C, Rf^3 0.27. *Anal.* Calcd for $C_{23}H_{25}N_3O_3 \cdot 0.75H_2O$: C, 68.3; H, 6.33; N, 10.5. Found: C, 68.2; H, 6.60; N, 10.4.

6-(4-Benzyloxycarbonylaminobutyl)-5-methyl-3-β-phenethyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-DL-Hfe-Lys(Z)-CH_2Cl (1.0 g, 1.74 mmol), yield 309 mg (42.3%), mp 140—144 °C, Rf^1 0.50. ^{1}H-NMR ($CDCl_3$) δ: 13.0 (1H, br s, NH), 7.28—7.14 (10H, m, 3-CH_2CH_2-Ph+6-$(CH_2)_4$NHCO$_2$CH$_2$Ph), 5.36 (1H, br s, 6-$(CH_2)_4$NHCO$_2$-CH_2Ph), 5.09 (2H, s, 6-$(CH_2)_4$NHCO$_2$CH$_2$Ph), 3.25 (2H, q-like, J=6.1, 6-$CH_2CH_2CH_2CH_2$NH-Z), 3.07—3.01 (4H, m, 3-CH_2CH_2-Ph), 2.56 (2H, t-like, J=7.0, 6-$CH_2CH_2CH_2CH_2$NH-Z), 2.28 (3H, s, 5-CH_3), 1.68—1.61 (4H, m, 6-$CH_2CH_2CH_2CH_2$NH-Z). *Anal.* Calcd for $C_{25}H_{29}N_3O_3 \cdot 0.1H_2O$: C, 71.3; H, 6.99; N, 9.97. Found: C,71.3; H, 6.88; N, 9.87.

6-(3-Benzyloxycarbonylaminopropyl)-5-methyl-3-β-phenethyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-DL-Hfe-Orn(Z)-CH_2Cl (1.0 g, 1.87 mmol), yield 318.0 mg (43.9%), mp 131—137 °C, Rf^1 0.43. ^{1}H-NMR ($CDCl_3$) δ: 13.4 (1H, br s, NH), 7.29—7.10 (10H, m, 3-CH_2CH_2-Ph+6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 6.20 (1H, br s, 6-$(CH_2)_3$NHCO$_2$-CH_2Ph), 4.94 (2H, s, 6-$(CH_2)_3$NHCO$_2$CH$_2$Ph), 3.21 (2H, q-like, J=5.8, 6-$CH_2CH_2CH_2$NH-Z), 3.07 (2H, m, 3-CH_2CH_2-Ph), 2.94 (2H, m, 3-CH_2CH_2-Ph), 2.56 (2H, t, J=7.1, 6-$CH_2CH_2CH_2$NH-Z), 2.29 (3H, s, 5-CH_3), 1.85 (2H, m, 6-$CH_2CH_2CH_2$NH-Z). *Anal.* Calcd for $C_{24}H_{27}N_3O_3 \cdot 0.6H_2O$: C, 69.2; H, 6.83; N, 10.1. Found: C,69.3; H, 6.71; N, 10.1.

6-Benzyl-3-(4-benzyloxycarbonylaminobutyl)-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Lys(Z)-Phe-CH_2Cl (800 mg, 1.43 mmol), yield 360 mg (62.0%), mp 171—173 °C, Rf^3 0.35. ^{1}H-

NMR ($CDCl_3$) δ: 11.6 (1H, br s, NH), 7.35—7.21 (10H, m, 6-CH_2-Ph+3-$(CH_2)_4NHCO_2CH_2$Ph), 5.08 (2H, s, 3-$(CH_2)_4NHCO_2CH_2$Ph), 4.99 (1H, br s, 3-$(CH_2)_4NHCO_2CH_2$Ph), 3.86 (2H, s, 6-CH_2-Ph), 3.23 (2H, q-like, J=6.3, 3-$CH_2CH_2CH_2CH_2$NH-Z), 2.76 (2H, t, J=7.3, 3-$CH_2CH_2CH_2CH_2$NH-Z), 2.33 (3H, s, 5-CH_3), 1.73 (2H, m, 3-$CH_2CH_2CH_2CH_2$NH-Z), 1.61 (2H, m, 3-$CH_2CH_2CH_2CH_2$NH-Z). ^{13}C-NMR ($CDCl_3$) δ: 157.3 (q, C-2), 156.4 (q, 3-$(CH_2)_4NHCO_2CH_2$-Ph+C-3), 136.7 (q, 3-C-1″), 136.4 (q, 6-C-1″), 135.3 (q, C-6), 129.8 (q, C-5), 129.5 (t, 6-C-2″, 6″), 128.9 (t), 128.7 (t, 6-C-3″, 5″), 128.1 (t), 128.0 (t), 127.2 (t, 6-C-4″), 66.5 (s, 3-$(CH_2)_4NHCO_2CH_2$-Ph), 40.8 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 36.1 (s, 6-CH_2-Ph), 32.1 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 29.4 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 24.0 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 18.8 (p, 5-CH_3). *Anal.* Calcd for $C_{24}H_{27}N_3O_3\cdot 0.65H_2O$: C, 69.1; H, 6.84; N, 10.1. Found: C, 69.1; H, 6.58; N, 10.1.

6-Benzyl-3-(3-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Orn(Z)-Phe-CH_2Cl (700 mg, 1.28 mmol), yield 311 mg (61.2%), mp 175—178 °C, Rf^3 0.20. ^{1}H-NMR $CDCl_3$+CD_3OD) δ: 7.35—7.16 (10H, m, 6-CH_2-Ph+3-$(CH_2)_3NHCO_2CH_2$Ph), 5.07 (2H, s, 3-$(CH_2)_3NHCO_2CH_2$Ph), 3.87 (2H, s, 6-CH_2-Ph), 3.23 (2H, t, J=6.8, 3-$CH_2CH_2CH_2$NH-Z), 2.79 (2H, t, J=7.6, 3-$CH_2CH_2CH_2$NH-Z), 2.28 (3H, s, 5-CH_3), 1.90 (2H, m, 3-$CH_2CH_2CH_2$NH-Z). ^{13}C-NMR ($CDCl_3$) δ: 157.5 (q, C-2), 157.0 (q, 3-$(CH_2)_3NHCO_2CH_2$-Ph), 156.4 (q, C-3), 137.0 (q, 6-C-1″), 136.5 (q, 3-C-1′), 133.8 (q, C-6), 130.4 (q, C-5), 129.2 (t, 6-C-2″, 6″), 128.7 (t), 128.5 (t), 128.3 (t, 6-C-3″, 5″), 128.1 (t), 127.4 (t, 6-C-4″), 66.7 (s, 3-$(CH_2)_3NHCO_2CH_2$-Ph), 40.7 (s, 3-$CH_2CH_2CH_2$NH-Z), 36.1 (s, 6-CH_2-Ph), 30.1 (s, 3-$CH_2CH_2CH_2$NH-Z), 27.2 (s, 3-$CH_2CH_2CH_2$NH-Z), 18.5 (p, 5-CH_3). *Anal.* Calcd for $C_{23}H_{25}N_3O_3\cdot 0.5H_2O$: C, 69.0; H, 6.54; N, 10.5. Found: C,68.9; H, 6.27; N, 10.5.

6-Benzyl-3-(2-benzyloxycarbonylaminoethyl)-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Dab(Z)-Phe-CH_2Cl (800 mg, 1.50 mmol), yield 326.7 mg (57.7%), mp 199—201 °C, Rf^1 0.49. ^{1}H-NMR ($CDCl_3$+CD_3OD) δ: 7.33—7.17 (10H, m, 6-CH_2-Ph+3-$(CH_2)_2$-$NHCO_2CH_2$Ph), 5.05 (2H, s, 3-$(CH_2)_2NHCO_2CH_2$Ph), 3.85 (2H, s, 6-CH_2-Ph), 3.59 (2H, t, J=6.1, 3-CH_2CH_2NH-Z), 2.94 (2H, t, J=6.1, 3-CH_2-CH_2NH-Z), 2.30 (3H, s, 5-CH_3). *Anal.* Calcd for $C_{24}H_{27}N_3O_3$: C, 70.0; H, 6.14; N, 11.1. Found: C,69.9; H, 6.13; N, 11.0.

6-Benzyl-3-benzyloxycarbonylaminomethyl-5-methyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Dap(Z)-Phe-CH_2Cl (750 mg, 1.45 mmol), yield 243.9 mg (46.3%), mp 170—173 °C, Rf^1 0.42. ^{1}H-NMR ($CDCl_3$) δ: 12.6 (1H, br s, NH), 7.40—7.21 (10H, m, 6-CH_2-Ph + 3-$CH_2NHCO_2CH_2$Ph), 6.00 (1H, br s, 3-$CH_2NHCO_2CH_2$Ph), 5.15 (2H, s, 3-$CH_2NHCO_2CH_2$Ph), 3.89 (2H, s, 6-CH_2-Ph), 2.35 (3H, s, 5-CH_3), 1.62 (2H, s, 3-CH_2NH-Z). *Anal.* Calcd for $C_{21}H_{21}N_3O_3\cdot 0.1H_2O$: C, 69.1; H, 5.85; N, 11.5. Found: C,69.2; H, 5.74; N, 11.4.

3-(4-Benzyloxycarbonylaminobutyl)-5-methyl-6-phenyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Lys(Z)-DL-Phg-CH_2Cl (1.0 g, 1.80 mmol), yield 147.4 mg (20.9%), mp 149—150 °C, Rf^1 0.37. ^{1}H-NMR ($CDCl_3$) δ: 11.6 (1H, br s, NH), 7.49—7.42 (5H, m, 6-Ph), 7.35—7.30 (5H, m, 3-$(CH_2)_4NHCO_2CH_2$Ph), 6.16 (1H, br s, 3-$(CH_2)_4NHCO_2CH_2$Ph), 5.09 (2H, s, 3-$(CH_2)_4NHCO_2CH_2$Ph), 4.99 (2H, s, 3-$(CH_2)_4NHCO_2CH_2$Ph), 3.22 (2H, q-like, J=6.2, 3-$CH_2CH_2CH_2CH_2$NH-Z), 2.30 (3H, s, 5-CH3), 1.73 (2H, m, 3-$CH_2CH_2CH_2CH_2$NH-Z), 1.58 (2H, m, 3-$CH_2CH_2CH_2CH_2$NH-Z). ^{13}C-NMR ($CDCl_3$) δ: 157.7 (q, C-2), 156.43 (q, C-3), 156.38 (q, 3-$(CH_2)_4NHCO_2CH_2$Ph), 136.7 (q, 3-C-1′), 133.3 (q, C-6), 132.7 (q, 6-C-1″), 129.6 (t, 6-C-4″), 129.2 (q, C-5), 128.9 (t, 6-C-2″, 6″), 128.8 (t, 6-C-3″, 5″), 128.5 (t), 128.1 (t), 128.0 (t), 66.5 (s, 3-$(CH_2)_4NHCO_2CH_2$Ph), 40.7 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 32.3 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 29.5 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 28.3 (s, 3-$CH_2CH_2CH_2CH_2$NH-Z), 19.7 (p, 5-CH_3). *Anal.* Calcd for $C_{23}H_{25}N_3O_3\cdot H_2O$: C, 69.9; H, 6.48; N, 10.6. Found: C,69.9; H, 6.39; N, 10.7.

3-(4-Benzyloxycarbonylaminobutyl)-5-methyl-6-β-phenethyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Lys(Z)-DL-Hfe-CH_2Cl (800 mg, 1.39 mmol), yield 270 mg (46.3%), mp 158—161 °C, Rf^3 0.51. *Anal.* Calcd for $C_{25}H_{29}N_3O_3\cdot 0.4H_2O$: C, 70.4; H, 7.04; N, 9.85. Found: C,70.4; H, 6.87; N, 9.85

3-(4-Benzyloxycarbonylaminopropyl)-5-methyl-6-β-phenethyl-2(1*H*)-pyrazinone The title compound was prepared from Boc-Orn(Z)-DL-Hfe-CH_2Cl (950 mg, 1.70 mmol), yield 236.7 mg (36.0%), mp 153—154 °C, Rf^3 0.45. ^{1}H-NMR ($CDCl_3$) δ: 13.4 (1H, br s, NH), 7.33—716 (10H, m, 6-CH_2-CH_2-Ph+3-$(CH_2)_3NHCO_2CH_2$Ph), 5.51 (1H, br s, 3-$(CH_2)_3NHCO_2CH_2$Ph), 5.05 (2H, s, 3-$(CH_2)_3NHCO_2CH_2$Ph), 3.24 (2H, q, J=6.2, 3-$CH_2CH_2CH_2$NH-Z), 2.91—2.65 (4H, m, 6-CH_2CH_2Ph), 2.75 (2H, q-like, 3-$CH_2CH_2CH_2$NH-Z), 2.15 (3H, s, 5-CH_3), 1.96 (2H, m, 3-$CH_2CH_2CH_2$NH-Z). *Anal.* Calcd for $C_{25}H_{29}N_3O_3\cdot 0.2H_2O$: C, 70.5; H, 6.75; N, 10.3. Found: C,70.4; H, 6.56; N, 10.5

6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-3,5-dimethyl-2(1*H*)-pyrazinone 3,5-Dimethyl-6-aminobutyl-2(1*H*)-pyrazinone [prepared from 3,5-dimethyl-6-(4-benzyloxycarbonylaminobutyl)-2(1*H*)-pyrazinone (350 mg, 0.72 mmol) by catalytic hydrogenation], Boc-Tyr-OH (242 mg, 0.86 mmol), BOP (382 mg, 0.86 mmol) and DIEA (150 μl, 0.86 mmol) were dissolved in DMF (40 ml) 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 and recrystallized from EtOH, yield 311.0 mg (94.3%), mp 104—105 °C, $[\alpha]_D^{25}$ −34.9° (c=0.5, $CHCl_3$), Rf^1 0.36. *Anal.* Calcd for $C_{24}H_{34}N_4O_5\cdot H_2O$: C, 60.5; H, 7.61; N, 11.8. Found: C, 60.7; H, 7.56; N, 11.5.

6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-3-isobutyl-5-methyl-2(1*H*)-pyrazinone Yield 119.0 mg (25.3%), mp 103—106 °C, $[\alpha]_D^{25}$ −22.0° (c=0.5, $CHCl_3$), Rf^3 0.26. *Anal.* Calcd for $C_{27}H_{40}N_4O_5\cdot 0.9H_2O$: C, 62.8; H, 8.15; N, 10.8. Found: C, 62.8; H, 8.03; 10.7.

6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-3-benzyl-5-methyl-2(1*H*)-pyrazinone Yield 336.0 mg (44.9%), mp 140 °C, $[\alpha]_D^{25}$ −32.2° (c=0.5, $CHCl_3$), Rf^3 0.35. *Anal.* Calcd for $C_{30}H_{38}N_4O_5\cdot 0.4H_2O$: C, 66.5; H, 7.22; N, 10.3. Found: C, 66.5; H, 7.11; N, 10.4.

6-[4-(N^{α}-Boc-Phe)-aminobutyl]-3-benzyl-5-methyl-2(1*H*)-pyrazinone Yield 296.3 mg (69.9%), mp 123 °C, $[\alpha]_D^{25}$ +1.0° (c=0.5, MeOH), Rf^3 0.71. *Anal.* Calcd for $C_{30}H_{38}N_4O_4\cdot 0.5H_2O$: C, 68.3; H, 7.45; N, 10.6. Found: C, 68.2; H, 7.35; N, 10.7.

6-[3-(N^{α}-Boc-Tyr)-aminopropyl]-3-benzyl-5-methyl-2(1*H*)-pyrazinone Yield 73.6 mg (28.8%), mp 157—160 °C, $[\alpha]_D^{25}$ −29.5° (c=0.5, $CHCl_3$), Rf^1 0.35. *Anal.* Calcd for $C_{29}H_{36}N_4O_5\cdot 0.25H_2O$: C, 66.3; H, 7.00; N, 10.7. Found: C, 66.1; H, 6.93; N, 10.4.

6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-3-phenyl-2(1*H*)-pyrazinone Yield 69.4 mg (14.5%), mp 162—164 °C, $[\alpha]_D^{25}$ +4.5° (c=1.0, MeOH), Rf^1 0.30. *Anal.* Calcd for $C_{29}H_{36}N_4O_5\cdot 0.75H_2O$: C, 65.2; H, 7.08; N, 10.5. Found: C, 65.3; H, 6.90; N, 10.3.

6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-3-β-phenethyl-2(1*H*)-pyrazinone Yield 214.4 mg (65.1%), mp 89—94 °C, $[\alpha]_D^{25}$ +6.7° (c=1.0, MeOH), Rf^1 0.36. *Anal.* Calcd for $C_{31}H_{40}N_4O_5\cdot 0.75H_2O$: C, 66.2; H, 7.44; N, 9.97. Found: C, 66.4; H, 7.48; N, 9.88.

6-[3-(N^{α}-Boc-Tyr)-aminopropyl]-5-methyl-3-β-phenethyl-2(1*H*)-pyrazinone Yield 105.0 mg (37.3%), mp 148—150 °C, $[\alpha]_D^{25}$ +11.3° (c=1.0, MeOH), Rf^1 0.47. *Anal.* Calcd for $C_{30}H_{38}N_4O_5\cdot 0.7H_2O$: C, 65.8; H, 7.26; N, 10.2. Found: C, 65.7; H, 7.33; N, 10.3.

6-Benzyl-3-[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone Yield 158.7 mg (60.3%), mp 122—125 °C, $[\alpha]_D^{25}$ −31.5° (c=0.5, $CHCl_3$), Rf^1 0.44. *Anal.* Calcd for $C_{30}H_{38}N_4O_5\cdot 0.25H_2O$: C, 66.8; H, 7.20; N, 10.4.. Found: C, 66.8; H, 7.14; N, 10.4.

6-Benzyl-3-[3-(N^{α}-Boc-Tyr)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone Yield 93.6 mg (36.6%), mp 128—129 °C, $[\alpha]_D^{25}$ −26.5° (c=0.5, $CHCl_3$), Rf^1 0.39. *Anal.* Calcd for $C_{29}H_{36}N_4O_5\cdot 0.25H_2O$: C, 66.3; H, 7.00; N, 10.7. Found: C, 66.3; H, 6.90; N, 10.7.

6-Benzyl-3-[2-(N^{α}-Boc-Tyr)-aminoethyl]-5-methyl-2(1*H*)-pyrazinone Yield 314.4 mg (94.0%), mp 118—120 °C, $[\alpha]_D^{25}$ +24.2° (c=0.5, $CHCl_3$), Rf^1 0.40. *Anal.* Calcd for $C_{28}H_{34}N_4O_5\cdot 0.6H_2O$: C, 65.0; H, 6.86; N, 10.8. Found: C, 65.0; H, 6.88; N, 10.6.

6-Benzyl-3-[(N^{α}-Boc-Tyr)-aminomethyl]-5-methyl-2(1*H*)-pyrazinone Yield 196.2 mg (61.3%), mp 122—125 °C, $[\alpha]_D^{25}$ −46.7° (c=0.5, $CHCl_3$), Rf^1 0.39. *Anal.* Calcd for $C_{27}H_{32}N_4O_5\cdot 1.4H_2O$: C, 62.6; H, 6.77; N, 10.8. Found: C, 62.7; H, 6.61; N, 10.9.

3-[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-6-phenyl-2(1*H*)-pyrazinone Yield 77.0 mg (29.2%), mp 166—168 °C, $[\alpha]_D^{25}$ +2.4° (c=1.0, MeOH), Rf^1 0.32. *Anal.* Calcd for $C_{29}H_{36}N_4O_5\cdot 0.1H_2O$: C, 66.7; H, 6.98; N, 10.7. Found: C, 66.4; H, 6.69; N, 10.8.

3-[4-(N^{α}-Boc-Tyr)-aminobutyl]-5-methyl-6-β-phenethyl-2(1*H*)-pyrazinone Yield 164.8 mg (65.1%), mp 138—146 °C, $[\alpha]_D^{25}$ +2.7° (c=1.0, MeOH), Rf^1 0.51. *Anal.* Calcd for $C_{31}H_{40}N_4O_5\cdot 0.1H_2O$: C, 67.6; H, 7.36; N, 10.2. Found: C, 67.4; H, 7.30; N, 10.2.

3-[3-(N^{α}-Boc-Tyr)-aminopropyl]-5-methyl-6-β-phenethyl-2(1*H*)-pyrazinone Yield 132.8 mg (52.8%), mp 110—113 °C, $[\alpha]_D^{25}$ −1.7° (c=0.5, CHCl3), Rf^1 0.37. *Anal.* Calcd for $C_{30}H_{38}N_4O_5\cdot 0.3H_2O$: C, 66.7; H, 7.20; N, 10.2. Found: C, 66.8; H, 7.19; N, 10.2.

3,5-Dimethyl-6-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (1) A solution of 6-[4-(N^{α}-Boc-Tyr)-aminobutyl]-3,5-dimethyl-2(1*H*)-pyrazinone (100 mg, 0.22 mmol) in TFA (2.1 ml, 27.5 mmol) containing anisole (250 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 (220 μl, 0.22 mmol) to afford a fluffy powder, yield 38.9 mg (52.5%), $[\alpha]_D^{25}$ +15.9° (c=1.0, H_2O), Rf^4 0.28, t_R 17.14 (min), FAB-MS m/z: 359 $(M+H)^+$. HPLC conditions: column, COSMOSIL C18 (4.6×250 mm); solvents, A : B (90 : 10) for 5 min, to (80 : 10) in 5 min, to (60 : 40) in 10 min, (60 : 40) for 10 min, to initial conditions in 5 min; flow rate, 1 ml/min; detection, 210 nm.

3-Isobutyl-5-methyl-6-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (2) Yield 26.2 mg (43.0%), $[\alpha]_D^{25}$ +16.9° (c=1.0, H_2O), Rf^4 0.28, t_R 22.48 (min). FAB-MS m/z: 401 $(M+H)^+$.

3-Benzyl-5-methyl-6-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (3) Yield 65.0 mg (83.0%), $[\alpha]_D^{25}$ +15.4° (c=1.0, H_2O), Rf^4 0.29, t_R 24.13 (min). FAB-MS m/z: 435 $(M+H)^+$.

3-Benzyl-5-methyl-6-phenylalanylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (4) Yield 174.3 mg (98.2%), $[\alpha]_D^{25}$ +4.9° (c=0.5, H_2O), Rf^4 0.31, t_R 25.58 (min). FAB-MS m/z: 419 $(M+H)^+$.

3-Benzyl-5-methyl-6-tyrosylaminopropyl-2(1*H*)-pyrazinone Hydrochloride (5) Yield 32.7 mg (74.5%), $[\alpha]_D^{25}$ +20.7° (c=0.5, H_2O), Rf^4 0.37, t_R 24.99 (min). FAB-MS m/z: 421 $(M+H)^+$.

5-Methyl-3-phenyl-6-tyrosyaminobutyl-2(1*H*)-pyrazinone Hydrochloride (6) Yield 31.3 mg (79.6%), $[\alpha]_D^{25}$ +17.6° (c=0.5, H_2O), Rf^4 0.36, t_R 24.18 (min). FAB-MS m/z: 421 $(M+H)^+$.

5-Methyl-3-β-phenethyl-6-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (7) Yield 88.0 mg (88.3%), $[\alpha]_D^{25}$ +9.8° (c=0.1, H_2O), Rf^4 0.32, t_R 28.32 (min). FAB-MS m/z: 449 $(M+H)^+$.

5-Methyl-3-β-phenethyl-6-tyrosylaminopropyl-2(1*H*)-pyrazinone Hydrochloride (8) Yield 23.8 mg (62.8%), $[\alpha]_D^{25}$ +10.6° (c=0.5, H_2O), Rf^4 0.29, t_R 30.37 (min). FAB-MS m/z: 435 $(M+H)^+$.

6-Benzyl-5-methyl-3-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (9) Yield 23.4 mg (45.2%), $[\alpha]_D^{25}$ −9.1° (c=0.5, H_2O), Rf^4 0.34, t_R 24.35. FAB-MS m/z: 435 $(M+H)^+$.

6-Benzyl-5-methyl-3-tyrosylaminopropyl-2(1*H*)-pyrazinone Hydrochloride (10) Yield 40.4 mg (77.0%), $[\alpha]_D^{25}$ +7.6° (c=1.0, H_2O), Rf^4 0.34, t_R 24.02. FAB-MS m/z: 421 $(M+H)^+$.

6-Benzyl-5-methyl-3-tyrosylaminoethyl-2(1*H*)-pyrazinone Hydrochloride (11) Yield 38.7 mg (43.7%), $[\alpha]_D^{25}$ −5.7° (c=0.5, H_2O), Rf^4 0.38, t_R 22.76. FAB-MS m/z: 407 $(M+H)^+$.

6-Benzyl-5-methyl-3-tyrosylaminomethyl-2(1*H*)-pyrazinone Hydrochloride (12) Yield 69.4 mg (80.9%), $[\alpha]_D^{25}$ −36.2° (c=0.5, H_2O), Rf^4 0.34, t_R 23.99. FAB-MS m/z: 393 $(M+H)^+$.

5-Methyl-6-phenyl-3-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (13) Yield 20.5 mg (46.7%), $[\alpha]_D^{25}$ +8.2° (c=1.0, H_2O), Rf^4 0.29, t_R 22.90. FAB-MS m/z: 421 $(M+H)^+$.

5-Methyl-6-β-phenethyl-3-tyrosylaminobutyl-2(1*H*)-pyrazinone Hydrochloride (14) Yield 68.4 mg (78.4%), $[\alpha]_D^{25}$ +4.6° (c=0.5, H_2O), Rf^4 0.33, t_R 27.61 (min). FAB-MS m/z: 449 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 8.34 (1H, t, J=5.6 Hz, CO-NH), 8.15 (2H, br, NH_2), 7.30—7.18 (5H, m, phenyl protons), 7.01, 6.70 (each 2H, d-like, J=8.5 Hz, aromatic protons of tyrosine), 3.83 (1H, br d, α-proton of tyrosine), 3.13 (1H, ddt, J=19.0, 12.0, 5.6 Hz, one of 3-C$\underline{H}_2$CH$_2$CH$_2$CH$_2$-N), 3.04 (1H, ddt, J=19.0, 11.9, 5.4 Hz, one of 3-CH$_2$CH$_2$CH$_2$C$\underline{H}_2$-N), 2.92 (1H, dd, J=13.9, 6.9 Hz, one of the β-protons of tyrosine), 2.80 (1H, dd, J=13.9, 7.2 Hz, one of the β-protons of tyrosine), 2.81(2H, t, J=7.9 Hz, Ph-C$\underline{H}_2$CH$_2$-), 2.69 (2H, t, J=7.9 Hz, Ph-CH$_2$C$\underline{H}_2$-), 2.58 (2H, t, J=7.5 Hz, 3-C$\underline{H}_2$CH$_2$-CH$_2$CH$_2$-N), 1.55 (2H, q, J=7.9 Hz, 3-CH$_2$C$\underline{H}_2$CH$_2$CH$_2$-N), 1.39 (2H, m, 3-CH$_2$CH$_2$C$\underline{H}_2$CH$_2$-N).

5-Methyl-6-β-phenethyl-3-tyrosylaminopropyl-2(1*H*)-pyrazinone Hydrochloride (15) Yield 79.4 mg (87.3%), $[\alpha]_D^{25}$ +6.6° (c=0.5, H_2O), Rf^4 0.33, t_R 24.72 (min). FAB-MS m/z: 435 $(M+H)^+$.

Radioligand Binding Synaptosomal membranes were prepared from whole brains (minus cerebellum) from Sprague–Dawley rats. Brains were homogenized in 0.32 M sucrose, 1.0 mM HEPES, pH 7.5, and 50 μg/ml soybean trypsin inhibitor and the P_2 fraction obtained by differential centrifugation.[22] 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.[22,23] 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.[22—25] Each compound was assayed over three to four orders of magnitude in concentration and conducted in duplicate with 3 or more different membrane preparations; the n values (in parenthesis) reflect the number of repetitions with the data presented as the mean±S.E.M. of independent experiments (Tables 1—3). Affinity constants (K_i) were determined according to Cheng and Prusoff.[26] In each assay, 2 μM unlabeled peptide (DPDPE for δ and DAGO for μ) were included in separate duplicate tubes containing synaptosomes and the radioligand in the binding medium in each and every assay.

Molecular Modeling Methods All computations were performed using the Search-Compare program from BIOSYM/MSI (v.95). Compounds **3** and **14** were built using residue and atom fragments provided by the Builder module of BIOSYM/MSI (v. 95). Potentials and charges were assigned for the CFF91 force field. Variable torsion searching used values ranging from 0° to 360° in 120° increments about the C$^\alpha$Tyr–C$^\beta$Tyr (χ1), C$^\beta$Tyr–C$^\gamma$Tyr (χ2), N–C$^\alpha$, C$^\delta$-C$^\varepsilon$, and C$^\beta$Hfe–C$^\gamma$Hfe (**14**) bonds; 180° increments about the N-C′ (ωTyr) bonds; and 60° increments about the C$^\alpha$–C$^\beta$, C$^\beta$–C$^\gamma$, C$^\gamma$–C$^\delta$, C$^\alpha$phe–C$^\beta$phe, C$^\beta$phe–C$^\gamma$phe (**3**) and C$^\gamma$Hfe–C$^\varepsilon$Hfe (**14**) bonds. Generated structures were energy minimized using a conjugate gradient algorithm for 600 iterations or stopping with root mean square (rms) gradient of 0.01 kcal/mol-Å. Structures with energies within 0.5 kcal/mol of any other structure found, and also superimposed with 0.8 Å rms deviation or less, were considered duplicates and discarded. Conformers with energies greater than 20 kcal/mol relative to the lowest energy structure were not considered. Structures were arranged in clusters based on distances between the tyrosine and benzyl and β-phenethyl of analogues **3** and **14**, respectively. Lowest energy structures from each cluster were superimposed with the X-ray crystal structures of the extended form of [Leu^5]enkephalin (LENK),[15] the β-turn form of [Leu^5]enkephalin (β-LENK),[16] *erythro*-5-methylmethadone (EMM)[14] and β-funaltrexamine (β-FNA).[15]

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References and Notes

1) Part LI: Okada Y., Shintomi N., Kondo Y., Yokoi T., Joshi S., Li W., *Chem. Pharm. Bull.*, **45**, 1860—1864 (1997).
2) 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); *ibid*, **6**, 362—364 (1967); *ibid*, **11**, 1726—1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; Z benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; BOP, benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; DCHA, dicyclohexylamine; Dab, 2,4-diaminobutyric acid; Dap, 2,3-diaminopropionic acid; Hfe, homophenylalanine; Phg, phenylglycine; DIEA, diisopropylethylamine; Dmt, 2,6-dimethyltyrosine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
3) Hughes J., Smith T., Kosterlitz H. W., Fothergill L. A., Morgan B. A., Morris H. R., *Nature* (London), **258**, 577—588 (1975).
4) Ling N., Burgus, R., Guillemin R., *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3042—3046 (1976).
5) Goldstein A., Tachibana S., Lowney L. I., Hunkaoiller M., Hood L., *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 6666—6690 (1979).
6) Zadina J. E., Hackler L., Gee L. J., Kastin A. J., *Nature* (London), **386**, 499—502 (1997).
7) Gehrig C. A., Hruby V. J., *Med. Res. Rev.*, **9**, 343—367 (1989).
8) Schiller P. W., *The Peptides*, **6**, 220—256 (1984).
9) Temussi P. A., Picone D., Castiglione-Morelli M. A., Motta A., Tancredi T., *Biopolymers*, **28**, 91—102 (1989).
10) Schwyzer R., *Biochemistry*, **25**, 6335—6341 (1986).
11) Taguchi H., Yokoi T., Tsukatani M., Okada Y., *Terahedron*, **51**, 7361—7371 (1995).
12) Taguchi H., Yokoi T., Okada Y., *Chem. Pharm. Bull.*, **44**, 2037—2041 (1996).
13) Temussi P. A., Salvadori S., Amodeo P., Bianchi C., Guerrini G., Tomatis R., Lazarus H., Picone D., Tancredi T., *Biochem. Biophys. Res. Commun.*, **198**, 933—939 (1994).
14) Portoghese P. S., Poupaert J. H., Larson D. L., Groutas W. C., Meitzner, G. D., Swenson D. C., Smith G. D., Duax W. L., *J. Med. Chem.*, **25**, 684—688 (1992).
15) Griffin J. F., Langs D. A., Smith G. D., Blundell T. L., Tickle I. J., Bedarkar, S., *Proc. Nat. Acad. Sci. U.S.A.*, **83**, 3272—3276 (1986).
16) Smith G. D., Griffin, J. F., *Science*, **199**, 1214—1216 (1978).

17) Salvadori S., Attila M., Balboni G., Bianchi C., Bryant S. D., Crescenzi O., Guerrini R., Picone D., Tancredi T., Temussi P. A., Lazarus L. H., *Mol. Med.*, **1**, 678—689 (1995).
18) Schiller P. W., Nguyen T. M.-D., Weltrowska G., Wilkes B. C., Marsden B. J., Lemieux C., Chung, N. N., *Proc. Nat. Acad. Sci. U.S.A.*, **89**, 11871—11875 (1992).
19) Brandt W., Stoldt M., Schinke H., *J. Comp. Aid. Drug Des.*, **10**, 201—212 (1996).
20) Van Nispen J. W., Tesser G. I., Nivard R. J. F., *Int. J. Peptide Protein Res.*, **9**, 193—202 (1977).
21) Waki M., Kitajima Y., Izumiya N., *Synthesis*, **1981**, 266—268.
22) Lazarus L. H., Salvadori S., Santagada V., Tomatis R., Wilson W. E., *J. Med. Chem.*, **34**, 1350—1359 (1991).
23) Lazarus L. H., Salvadori S., Attila M., Grieco P., Bundy D. M., Wilson W. E., Tomatis R., *Peptides*, **14**, 21—28 (1993).
24) Salvadori S., Bianchi C., Lazarus L. H., Scaranari V., Attila M., Tomatis R., *J. Med. Chem.*, **35**, 4651—4657 (1992).
25) Salvadori S., Bryant S. D., Bianchi C., Balboni G., Scaranari V., Attila M., Lazarus L. H., *J. Med. Chem.*, **36**, 3748—3756 (1993).
26) Cheng Y.-C., Prusoff W. H., *Biochem. Pharmacol.*, **22**, 3099—3108 (1973).