Synthesis and Antinociceptive Activity of Orally Active Opioid Peptides: Improvement of Oral Bioavailability by Esterification

Tadashi OGAWA,[a] Mamoru ARAKI,[a] Tetsuhisa MIYAMAE,[a] Toru OKAYAMA,[a] Masaki HAGIWARA,[a] Shinobu SAKURADA,[b] and Tadanori MORIKAWA*,[a]

[a] *Research Institute, Daiichi Fine Chemical Co., Ltd.; 530 Chokeiji, Takaoka, Toyama 933–8511, Japan: and* [b] *Department of Physiology and Anatomy, Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai 981–8558, Japan.*
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To improve the oral bioavailability of a dermorphin tetrapeptide analog, N^{α}-1-iminoethyl-Tyr-D-MetO-Phe-MeβAla-OH (III),[1] which has a potent analgesic activity after oral administration, various derivatives were synthesized to increase lipophilicity by esterification of the C-terminal carboxyl group and/or acylation of the phenolic hydroxyl group on Tyr[1]. Antinociceptive activity was evaluated after subcutaneous or oral administration using the mouse tail pressure test. As a result, increased antinociceptive activity after oral administration as well as an improved ED_{50}(*p.o.*)/ED_{50}(s.c.) ratio, which is an indicator of oral bioavailability, were found for some compounds. With regard to the improvement of bioavailability, derivatives with acylation of the phenolic hydroxyl group on Tyr[1] showed better results than derivatives with esterification of the C-terminal carboxyl group. In particular, an ED_{50}(*p.o.*)/ED_{50}(s.c.) ratio equivalent to that of morphine was found for an acetylated derivative, N^{α}-1-iminoethyl-Tyr(COMe)-D-MetO-Phe-MeβAla-OH (7a), as well as for a methoxycarbonylated derivative, N^{α}-1-iminoethyl-Tyr(CO_2Me)-D-MetO-Phe-MeβAla-OH (7l).

Key words dermorphin tetrapeptide analog; oral bioavailability; lipophilicity; chemical modification

Dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2)[2] was originally isolated from the skin of a South American frog (*Phyllomedusa sauvagei*). It shows a potent affinity and selectivity towards opioid receptors exceeding that of morphine, especially for μ-opioid receptors, and possesses strong opioid activity. The smallest dermorphin sequence that demonstrates activity is the N-terminal tetrapeptide (Tyr-D-Ala-Phe-Gly).[3] A number of tetrapeptide analogs have already been synthesized and structure–activity relationship (SAR) studies have been performed on opioid activity, receptor affinity, and selectivity.[4,5] Since replacement of D-Ala with D-Arg at position 2 was found to lead to a dramatic increase of activity,[6] many [D-Arg^2]-dermorphin derivatives have been synthesized and intensive SAR studies have also been performed.[7—10] Using the tail pressure test in mice, we recently found that subcutaneous (s.c.) and oral (*p.o.*) administration of N^{α}-amidino-Tyr-D-Arg-Phe-MeβAla-OH (**I**: ADAMB), N^{α}-1-iminoethyl-Tyr-D-Arg-Phe-MeβAla-OH (**II**) or N^{α}-1-iminoethyl-Tyr-D-MetO-Phe-MeβAla-OH (**III**), achieved extremely strong and long-lasting antinociceptive activity after *p.o.* administration, which was superior to that of morphine.[11,12] Although constipation is a common side effect of morphine, compound **III** shows a weaker costive effect.[12] However, these compounds with a strong antinociceptive activity show low oral bioavailability, which has posed a problem.

Generally, peptide pharmaceuticals show very low intestinal absorbability after *p.o.* administration. This is thought to be due to the presence of many functional groups that form hydrogen bonds, such as amide bonds. It may also be due to the fact that membrane permeability is low for compounds with a high molecular weight (>M.W. 500), or because peptides are more susceptible to various hydrolytic enzymes in the intestines.[13—19] Accordingly, to improve the intestinal absorption of peptide pharmaceuticals, various absorption enhancers, peptidase inhibitors, and methods to alter molecular structure of peptides by chemical modification have been developed, and some success has been achieved.[20,21]

Dermorphin tetrapeptide analogues are known to be highly stable against enzymatic degradation.[22—24] Considering the stability of the compound itself, the low oral bioavailability of compounds **I**—**III** was inferred to be due to low intestinal absorption. Compounds **I**—**III** have a very high water solubility and we assumed that this was related to low intestinal absorption. This led to the assumption that intestinal absorption might be improved by modifying these peptides to increase lipophilicity. Here we report on improvement of the antinociceptive activity and oral bioavailability after s.c. and *p.o.* administration in mice using various analogues that were synthesized to have increased lipophilicity by esterification of the C-terminal carboxyl group or acylation of the phenolic hydroxyl group on Tyr[1] of compound **III** (Fig. 1).

Chemistry

All analogues were synthesized *via* the solution method using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) with 1-hydroxybenzotriazole (HOBt) as the coupling reagent.

Esterification of the C-Terminal Carboxyl Group C-Terminal ester derivatives were prepared through 3+1 condensation by coupling the N-terminal tripeptide segment

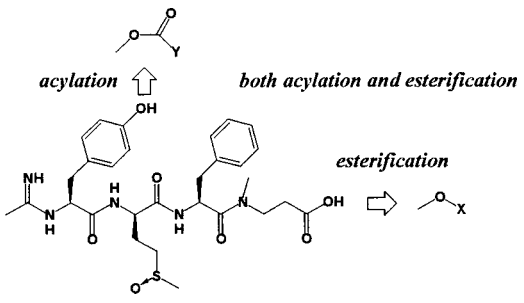

Fig. 1. Modification of the Chemical Structure of a [D-MetO^2]dermorphin Tetrapeptide Analogue (Compound **III**)

* To whom correspondence should be addressed. e-mail: morikawa@daiichi-fcj.co.jp

Boc-Tyr-D-MetO-Phe-OH (**1**) with H-MeβAla-OX (Chart 1). A fully protected tetrapeptide (**3a**—**u**) was treated with 90% trifluoroacetic acid (TFA) or 90% HCOOH to remove the Boc group, and then the N-terminus was 1-iminoethylated by treatment with ethyl acetimidate hydrochloride in the presence of triethylamine (TEA).

***O*-Acylation of the Hydroxy Group on Tyrosine** Derivatives with *O*-acylation of Tyr[1] were obtained *via* preparation of the tetrapeptide Boc-Tyr-D-MetO-Phe-MeβAla-OBu^t (**5**) and subsequent acylation of the phenol hydroxyl group with acid chloride; Y-COCl or carboxylic acid; Y-COOH (Chart 2). The fully protected tetrapeptide (**6a**—**p**) was treated with 90% TFA to remove the Boc group, and then the N-terminus was 1-iminoethylated.

Dual-esterification of Tyr[1] and the C-Terminus Analogues having both *O*-acylation and C-terminal esterification were prepared by condensation of the tripeptide Boc-Tyr-D-MetO-Phe-OH (**1**) with H-MeβAla-OX, followed by acylation with acid chloride; Y-COCl or carboxylic acid; Y-COOH (Chart 3). The fully protected tetrapeptide (**8a**—**g**) was treated with 90% TFA to remove the Boc group, and then the N-terminus was 1-iminoethylated.

All crude products were purified by reversed-phase flush column chromatography and eluted with aqueous CH_3CN containing 0.1% acetic acid to afford the purified products (>95% purity by HPLC), which were subsequently lyophilized to give the desired peptides in powder form. The structure of each product was confirmed from the ^{1}H-NMR and FAB mass spectra.

Antinociceptive Assay The synthesized compounds were tested for antinociceptive activity *in vivo* (Tables 1—3) using the tail pressure test in mice after subcutaneous (s.c.) or oral (*p.o.*) administration. The percentage of the maximum possible effect (% MPE) value was measured at fixed doses (1 mg/kg s.c. and 10 mg/kg *p.o.*) to initially evaluate the synthesized compounds. Then, analogues with a high activity underwent determination of the ED_{50} value by the method of Litchfield and Wilcoxon[25] to assess their antinociceptive activity. All values were calculated from data obtained at the time of peak effect after administration of either the peptides, or morphine. Furthermore, the ED_{50} dose ratio (*p.o.*/s.c.) was calculated and compared with that of morphine or compound **III** to assess oral bioavailability.

Results and Discussion

Esterification of the C-Terminal Carboxyl Group The carboxyl group located at the C-terminus of compound **III** is easily ionized, leading to reduced lipophilicity of the compound and substantially hindering gastrointestinal absorption. Therefore, it was expected that protection of this carboxyl group by esterification would improve bioavailability by preventing ionization and increasing lipophilicity. Since various esterases exist in the body, particularly in the intestines, it was anticipated that the appropriately esterified compound would be hydrolyzed and converted to the parent compound after intestinal absorption. Thus, esterification was expected to increase gastrointestinal absorption while maintaining a strong antinociceptive activity like that of the original compound. Accordingly, compounds **4a**—**u** were synthesized by esterification of the C-terminal carboxyl group of compound **III.**

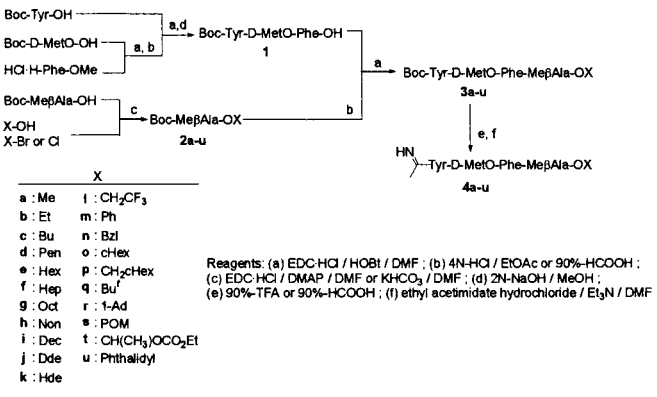

Chart 1. Synthesis of C-Terminal Esterified [D-MetO^2]dermorphin Tetrapeptide Analogues

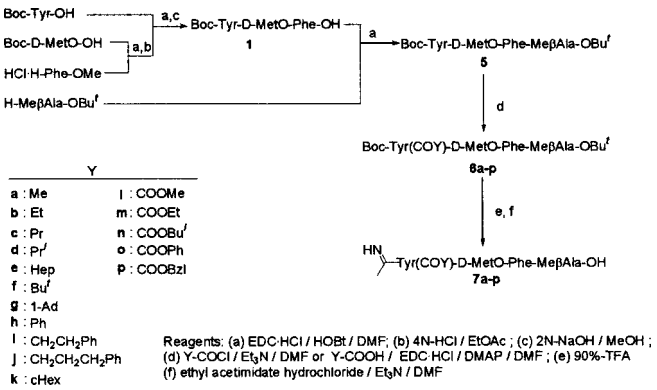

Chart 2. Synthesis of *O*-Acylated [D-MetO^2]dermorphin Terapeptide Analogues

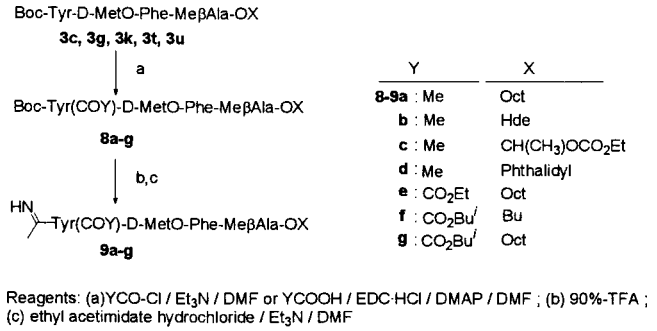

Chart 3. Synthesis of Dual-esterified [D-MetO^2]dermorphin Tetrapeptide Analogues

Table 1 shows a comparison of the antinociceptive activity of compounds **4a**—**u** with that of morphine. Relative oral bioavailability was also evaluated using the ED_{50} dose ratio after s.c. and *p.o.* administration. From comparison of the ED_{50} values, the antinociceptive activity of compounds **4a**—**u**, synthesized by esterification of the C-terminal carboxyl group of compound **III**, was lower than that of the parent compound after both s.c. and *p.o.* administration. However, the ED_{50} values obtained after s.c. administration of hexadecylated compound **4k** and compound **4t** (which has a 1′-ethoxycarbonyloxyethyl group[26] and is a successful example of a prodrug developed from a β-lactam antibiotic) were larger than that for compound **III**, although the values for

Table 1. Analgesic Activity of N^{α}-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OX

No.	X	MPE %		ED_{50} (mg/kg)[a,b]		
		s.c. 1 mg/kg	*p.o.* 10 mg/kg	s.c.	*p.o.*	*p.o.*/s.c.
Morphine		4.9	12.9	3.3	22.2	7
III	-H	100	96.2	0.08	3.1	39
4a	-Me	96.9	81.0	0.12	5.2	43
4b	-Et	100	93.6	0.27	5.6	21
4c	-Bu	100	58.4	0.23		
4d	-Pen	100	66.3	0.17		
4e	-Hex	100	79.8	0.22		
4f	-Hep	98.8	62.5	0.13		
4g	-Oct	100	80.8	0.24	6.0	25
4h	-Non	100	21.7	0.22		
4i	-Dec	100	56.1	0.25		
4j	-Dde	98.7	54.4	0.26		
4k	-Hde	78.1	95.8	0.38	3.6	10
4l	$-CH_2CF_3$	93.4	75.3	0.15	4.1	27
4m	-Ph	100	75.1	0.23		
4n	-Bzl	100	63.2	≦0.25		
4o	-cHex	100	37.6	0.20		
4p	$-CH_2$(cHex)	96.9	38.4	0.27		
4q	$-Bu^t$	91.0	44.5	0.36		
4r	-1-Ad	100	12.9	≦0.5		
4s	-POM	100	88.5	0.28	6.9	25
4t	$-CH(CH_3)OCO_2Et$	97.4	94.4	0.21	3.6	17
4u	-Phthalidyl	99.7	79.2	0.32	5.0	16

a) Data are given as the mean value for groups of 10 mice. *b*) ED_{50} values were calculated from data obtained at the time of peak effect after administration.

p.o. administration were about the same. The ED_{50}(*p.o.*)/ED_{50} (s.c.) ratio was 10 and 17, respectively, showing improvement compared with the ratio of 39 for parent compound **III**. Therefore, it seems likely that esterification attenuated the antinociceptive activity, but oral antinociceptive activity was maintained at a comparable level to that of compound **III** as a consequence of improved oral bioavailability. As other examples of prodrugs, compound **4s** with a pivaloyloxymethyl group[27] and compound **4u** with a phthalidyl group,[28] also showed the same trend, although these compounds had a lower antinociceptive activity for after s.c. or *p.o.* administration than **4t**.

***O*-Acylation of the Hydroxyl Group of Tyrosine** It is well known that esterification of the phenolic hydroxyl group in morphine-related compounds, such as heroin (synthesized by acetylation of the hydroxyl group of morphine), increases their lipophilicity and allows significantly improved permeation of the blood brain barrier (BBB), that leads to several-fold augmentation of analgesic activity.[29,30] There also is a phenolic hydroxyl group at Tyr^1 of compound **III**. This hydrophilic group was thought to increase water solubility and thus hinder gastrointestinal absorption. Therefore, as with esterification of the C-terminus, acylation of the hydroxyl group of Tyr was expected to improve lipophilicity and gastrointestinal absorption. Accordingly, we synthesized compounds with various acyl groups attached to the phenolic hydroxyl group of the Tyr^1 residue.

Table 2 shows the antinociceptive activity and ED_{50}(*p.o.*)/ED_{50}(s.c.) ratio of *O*-acylated compounds **7a**—**p**. From comparison of the dose-activity ratios, it is apparent that the oral bioavailability of these acylated compounds (**7a**—**p**) was clearly higher than that of the parent compound, which had a value of 39. In particular, the acetylated compound **7a** and the methoxycarbonylated compound **7l** had values of 9 and 5, respectively, which were close to the value for morphine. These compounds showed improved dose-activity ratios and exhibited analgesic activity after oral administration that was equal to or greater than that of compound **III**. This was assumed to be due to the improvement of oral bioavailability. On the other hand, activity was slightly decreased after s.c. administration. This was assumed to be due to increased lipophilicity of the compound because of acylation, which may have affected diffusion of the drug from the s.c. administration site, uptake into the blood, and binding with plasma proteins.

Dualesterification at the Hydroxy Group of Tyrosine and C-Terminal Carboxyl Group Esterification of the C-terminal carboxyl group or acylation of the Tyr^1 phenolic hydroxyl group improved the oral bioavailability of some compounds. Combining the two substituents that improved oral bioavailability was expected to have an augmented effect. Therefore, to achieve further improvement, we synthesized esterified and acylated compounds **9a**—**g**.

Table 3 shows the antinociceptive activity and ED_{50}(*p.o.*)/ED_{50}(s.c.) ratio of the esterified and acylated compounds **9a**—**g**. Contrary to our expectations, comparison of the dose-activity ratios revealed that the antinociceptive activity of the doubly modified compounds **9a**—**g** was not increased. However, the ED_{50} value obtained after s.c. administration of compound **9c**, with an acetylated phenolic hydroxyl at Tyr^1 and a 1′-ethoxycarbonyloxyethyl group at the C-terminal carboxyl group, was larger than that of compound **III**, but the ED_{50}(*p.o.*)/ED_{50}(s.c.) ratio was 11, showing an improvement from that of parent compound **III** (which was 39).

Comparison of the Duration of Antinociceptive Activity Figure 2 shows the time course of antinociceptive activity after s.c. administration of morphine and compound **III** in the mouse tail pressure test. Compound **III** (Fig. 2B) showed

Table 2. Analgesic Activity of N^{α}-Iminoethyl-Tyr(COY)-D-MetO-Phe-MeβAla-OH

No.	Y	MPE %		ED_{50} (mg/kg)[a,b]		
		s.c. 1 mg/kg	*p.o.* 10 mg/kg	s.c.	*p.o.*	*p.o.*/s.c.
Morphine		4.9	12.9	3.3	22.2	7
III	—	100	96.2	0.08	3.1	39
7a	-Me	100	94.4	0.14	1.2	9
7b	-Et	100	96.9	0.20	2.2	11
7c	-Pr	100	99.2	0.26	2.9	11
7d	-Pr^i	100	98.8	0.20	2.1	11
7e	-Hep	98.5	86.6	0.33	4.9	15
7f	-Bu^t	100	91.0	0.28	3.5	13
7g	-1-Ad	94.1	52.4	0.38		
7h	-Ph	96.5	95.2	0.21	3.3	16
7i	-$(CH_2)_2$Ph	100	91.1	0.25	3.0	12
7j	-$(CH_2)_3$Ph	98.0	89.6	0.30	3.5	12
7k	-cHex	89.1	76.9	0.21	4.4	21
7l	-OMe	100	99.1	0.25	1.3	5
7m	-OEt	91.5	93.8	0.25	4.3	17
7n	-OPr^i	100	94.7	0.21	3.5	17
7o	-OPh	96.3	98.9	0.21	4.3	20
7p	-OBzl	100	92.1	0.23	4.2	18

a) Data are given as the mean value for groups of 10 mice. *b*) ED_{50} values were calculated from data obtained at the time of peak effect after administration.

Table 3. Analgesic Activity of N^{α}-1-Iminoethyl-Tyr(COY)-D-MetO-Phe-MeβAla-OX

No.	Y	X	MPE %		ED_{50} (mg/kg)[a,b]		
			s.c. 1 mg/kg	*p.o.* 10 mg/kg	s.c.	*p.o.*	*p.o.*/s.c.
Morphine			4.9	12.9	3.3	22.2	7
III	—	-H	100	96.2	0.08	3.1	39
9a	-Me	-Oct	90.2	76.2	0.34	6.0	18
9b	-Me	-Hde	88.5	63.2			
9c	-Me	-$CH(CH_3)$OCOOEt	93.8	94.9	0.25	2.7	11
9d	-Me	-Phthalidyl	99.7	79.2	0.32	5.0	16
9e	-OEt	-Oct	83.7	38.1	0.50		
9f	-OBu^i	-Bu	100	60.4	0.22		
9g	-OBu^i	-Oct	44.0	30.4			

a) Data are given as the mean value for groups of 10 mice. *b*) ED_{50} values were calculated from data obtained at the time of peak effect after administration.

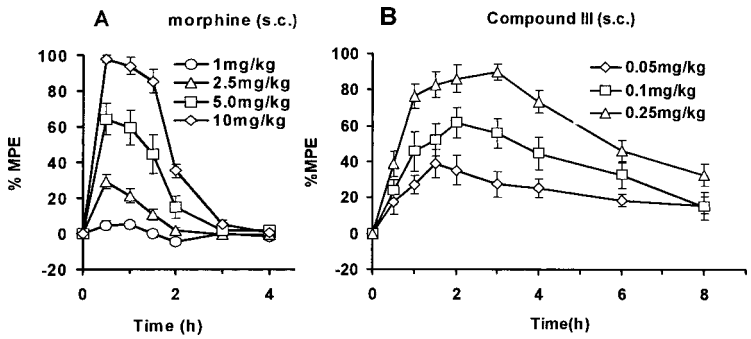

Fig. 2. Time Course of the Antinociceptive Effect of Subcutaneous Morphine (A) and Compound **III** (B) in the Mouse Tail Pressure Test

The doses used are shown in the figure. Data are given as the mean±S.E.M. for groups of 10 mice.

a higher dose-dependent antinociceptive activity than morphine (Fig. 2A). The duration of activity of compound **III** was over 8 h after administration of 0.25 mg/kg, but the activity of morphine ended within 4 h, even after administration of 10 mg/kg.

As can be seen in Fig. 3B, compound **III** showed a strong dose-dependent antinociceptive effect after *p.o.* administration. An antinociceptive effect equivalent to 30% of MPE was even maintained at 10 h after administration of 5.0 mg/kg, whereas the effect disappeared almost completely within 6 h after 80 mg/kg of morphine (Fig. 3A).

Figure 4 shows the time course of antinociceptive activity after s.c. administration of compound **4k** (Fig. 4A) and compound **7a** (Fig. 4B), both of which showed improved absorbability. The time course of activity for compound **4k** (Fig. 4A) and compound **7a** (Fig. 4B) resembled that of par-

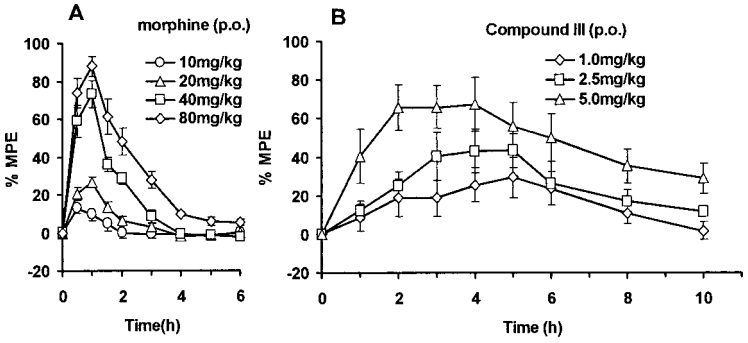

Fig. 3. Time Course of the Antinociceptive Effect of Oral Morphine (A) and Compound **III** (B) in the Mouse Tail Pressure Test

The doses used are shown in the figure. Data are given as the mean±S.E.M. for groups of 10 mice.

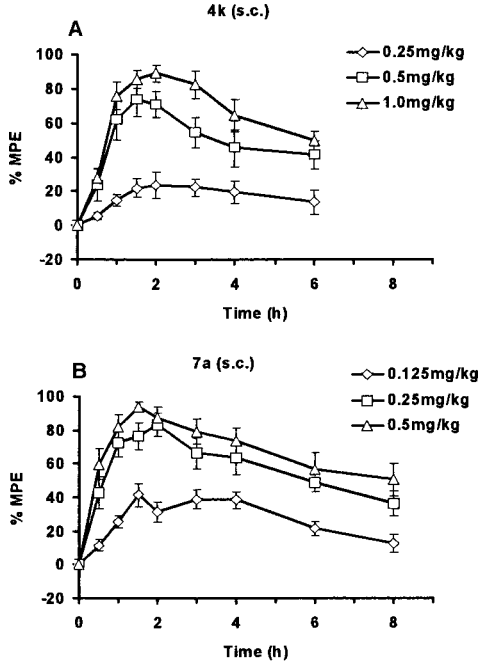

Fig. 4. Time Course of the Antinociceptive Effect of Subcutaneous **4k** (A) and **7a** (B) in the Mouse Tail Pressure Test

The doses used are shown in the figure. Data are given as the mean±S.E.M. for groups of 10 mice.

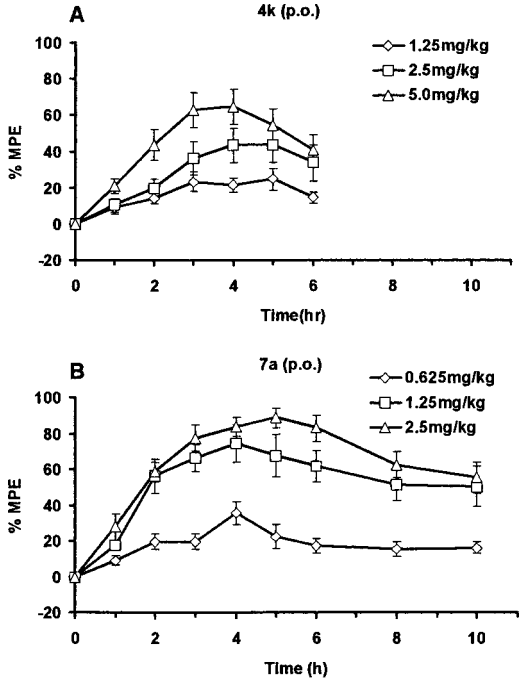

Fig. 5. Time Course of the Antinociceptive Effect of Oral **4k** (A) and **7a** (B) in the Mouse Tail Pressure Test

The doses used are shown in the figure. Data are given as the mean±S.E.M. for groups of 10 mice.

ent compound **III**. Although a time lag before the onset of antinociceptive activity and prolongation of its duration were expected judging from the time necessary for metabolism of ester and acyl derivatives involving the hydrolysis of ester and acyl groups, no such effects were seen.

Figure 5 shows the time course of antinociceptive activity after *p.o.* administration of compound **4k** (Fig. 5A) and compound **7a** (Fig. 5B). The peak antinociceptive effect of compound **III** after a dose of 5.0 mg/kg was around 3 h, whereas it was around 4 h for compound **4k** (Fig. 5A). The maximum %MPE for both compounds **III** and **4k** was 65%. The maximum %MPE after administration of compound **III** at 2.5 mg/kg was around 40%, whereas it was about 90% for compound **7a** (Fig. 5B), and clearly showed an increase of antinociceptive activity. Therefore, after *p.o.* administration, compound **7a** would show better absorption than compound **III**. In addition, the peak antinociceptive effect of compound **4k** and compound **7a** was seen around 5 h after administration of 2.5 mg/kg. Thus, the peak was later than for compound **III** and this was thought to be due to the time necessary for metabolism of the compounds, such as hydrolysis of the ester and acyl groups.

In conclusion, we synthesized various derivatives with improved lipophilicity based on N^{α}-1-iminoethyl-Tyr-D-MetO-Phe-MeβAla-OH (compound **III**), which has a strong antinociceptive activity, by esterification of the C-terminal carboxyl group and acylation of the phenolic hydroxyl group on Tyr^1 in order to improve intestinal absorption. We then investigated the improvement of antinociceptive activity and assessed the oral bioavailability of these compounds. As a result, an increase of antinociceptive activity after was seen *p.o.* administration of some of the compounds. Acylation of the phenolic hydroxyl group on Tyr^1 improved absorption more than esterification of the C-terminal carboxyl group. In particular, short chain substituents such as acetyl groups (compound **7a**) and methoxycarbonyl groups (compound **7l**) achieved an oral bioavailability equivalent to that of morphine. However, oral bioavailability was estimated using ED_{50}(*p.o.*)/ED_{50}(s.c.) ratios in this study. In the future, there

is the necessity of evaluating by the concentration measurement in blood, the membrane permeability assay using Caco-2 cell monolayer system, *etc*. On the other hand, when both esterification and acylation were performed (anticipating a synergistic effect), there was a significant decrease of antinociceptive activity after both s.c. and *p.o.* administration. In general, passive diffusion across membranes, is highly dependent on the molecular weight of a compound and if the molecular weight is too high, membrane permeability will decrease. Increasing the lipophilicity improves the BBB permeability and intestinal absorption of drugs. However, using large substituents may increase the molecular weight and reduce the water solubility, resulting in poor intestinal absorption. Some of the ester and acyl groups investigated in this study have a large enough ester moiety to markedly increase the molecular weight, so our failure to obtain the anticipated increase of absorption might be inevitable. Therefore, to achieve higher oral bioavailability, it will be necessary to use substituents with an appropriate balance between lipophilicity and water solubility, along with concepts such as the 'rule of 5'[31] or 'chemical delivery systems'.[32] The knowledge gained from this study about peptide chemical modifications and oral bioavailability will be useful for developing future oral peptidergic analgesics.

Experimental

Commercial amino acid derivatives, EDC·HCl and HOBt, were obtained from Kokusan Chemical Works, Ltd. (Tokyo, Japan) or Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Other reagents and solvents were purchased from Aldrich Chemicals (WI, U.S.A.). Thin-layer chromatography was performed on silica plates (0.25 mm; Merck, 60 F_{254}) with the following solvent systems: Rf_1 $CHCl_3$/MeOH (10 : 1, v/v); Rf_2 $CHCl_3$/MeOH/acetic acid (95 : 5 : 3, v/v/v); Rf_3 $CHCl_3$/MeOH/acetic acid (80 : 10 : 5, v/v/v); Rf_4 *n*-butanol/acetic acid/water/pyridine (15 : 3 : 10 : 12, v/v/v/v). Flush chromatography was performed on silica gel BW-300 from Fuji Silysia Chemical Ltd. (Aichi, Japan). Purification of the final products was achieved by flush chromatography using a reversed-phase silica gel Chromatorex ODS DM1020T from Fuji Silysia Chemical Ltd. (Aichi, Japan), which was eluted by a stepwise gradient of acetonitrile (starting from 1—5% and increasing stepwise by 1—2%) in 0.1 M acetic acid. Analytical high-pressure liquid chromatography was done on a Nucleosil 100 $5C_{18}$ column (4.6×150 mm) with an Agilent 1100 HPLC system. The products were analyzed using a linear gradient of 10—90% acetonitrile in 0.1% aqueous TFA over 20 min at a flow rate of 1 ml/min and UV detection at 230 and 280 nm. ^{1}H-NMR spectra were recorded with a JEOL JMN-AL300 (300 MHz) spectrometer, using tetramethylsilan (TMS) as the internal standard. Mass spectra (FAB-MS) were obtained with a JEOL mass spectrometer (model JMS-700).

Method A (Coupling Procedure 1) To a solution of a carboxy component (1.0 eq), the amino component (1.1 eq), HOBt (1.1 eq) in DMF, and TEA (1 eq if the amino component was in the protonated form) were added at 0 °C. Then, EDC·HCl (1.2 eq) was added to the solution at −10 °C. This mixture was stirred for 30 min at −10 °C and then stirred overnight at room temperature. Then the reaction mixture was diluted with EtOAc. The solution or suspension was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was crystallized from appropriate solvents or purified by flush column chromatography.

Method B (Coupling Procedure 2) To a solution of a carboxy component (1.0 eq), HOBt (1.1 eq) in DMF (2—5% v/w), EDC·HCl (1.2 eq) was added to the solution at −10 °C. This mixture was stirred for 30 min at −10 °C. Then the amino component (1.1 eq) in DMF (2—5% v/w) was added at −10 °C, followed by stirring overnight at room temperature. The reaction mixture was diluted with EtOAc. The solution or suspension was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was crystallized from appropriate solvents or purified by flush column chromatography.

Method C (Esterification) A mixture of the Boc-protected amino acid (1.0 eq), alcohol (1.1 eq), and DMAP (0.1 eq) in CH_2Cl_2 (2—5% v/w) was stirred at 0 °C. Then, EDC·HCl (1.2 eq) was added to the solution. This reaction mixture was stirred for 1 h at 0 °C and then stirred overnight at room temperature. The reaction mixture was subsequently concentrated *in vacuo*, and dissolved with EtOAc, after which the solution was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by flush column chromatography.

Method D (Deprotection 1) The Boc-protected amino acid or peptide was treated with 4 N-HCl/EtOAc (1—3% v/w) for 0.5—1 h at room temperature to remove the Boc group. Ether was added to the solution and the precipitated solid was separated by filtration and dried in a vacuum desiccator over solid NaOH.

Method E (Deprotection 2) The Boc protected amino acid *tert*-butyl ester or the N-Boc protected peptide with C-terminal *tert*-butyl ester was treated with 90%-HCOOH (1—3% v/w) for 3—5 h at room temperature to remove only the Boc group. The solvent was evaporated *in vacuo* at 20 °C. The residue was dissolved in EtOAc, then washed with sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was used for subsequent condensation without purification.

Method F (*O*-Acylation 1) To a solution of the protected peptide with a free hydroxy group of tyrosine (1.0 eq) in DMF (2—5% v/w), the acid chloride (1.1 eq) was added at 0 °C. Then TEA (1.2 eq) was added to the solution at 0 °C. This mixture was stirred for 1 h at 0 °C and then stirred overnight at room temperature. Then the reaction mixture was diluted with EtOAc, after which the solution or suspension was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was crystallized from appropriate solvents or purified by flush column chromatography.

Method G (*O*-Acylation 2) To a solution of the peptide with a free hydroxy group of tyrosine (1.0 eq) in DMF (2—5% v/w), Y-COOH (1.1 eq) and DMAP (0.1 eq) were added at 0 °C. Then EDC·HCl (1.2 eq) was added to the solution at 0 °C, followed by stirring for 1 h at 0 °C and then stirred overnight at room temperature. Subsequently, the reaction mixture was diluted with EtOAc, after which the solution or suspension was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was crystallized from appropriate solvents or purified by flush column chromatography.

Method H (1-Iminoethylation of the N-Terminus and Final Purification) The fully-protected peptide was treated with 90%–TFA (1—3% v/w) or 90%–HCOOH (1—3% v/w, if it was *tert*-butyl ester) for 1—5 h at room temperature to remove the Boc group. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in DMF (2—5% v/w), after which ethyl acetimidate hydrochloride (2.0 eq) and TEA (3.0 eq) were added. This solution was stirred at room temperature for 2—4 h and then the reaction mixture was concentrated *in vacuo*. The residue was purified by ODS chromatography with a stepwise gradient of acetonitrile (starting from 1—5% and increasing stepwise by 1—2%) in 0.1 N-acetic acid as the eluting solvent and then lyophilized to give a white powder.

Preparation of Boc-Tyr-D-MetO-Phe-OH (1) A mixture of Boc-D-MetO-OH (39.8 g, 150 mmol), HCl·H-Phe-OMe (32.4 g, 150 mmol) and HOBt (22.3 g, 165 mmol) in DMF (400 ml) was stirred at 0 °C. TEA (15.1 g, 150 mmol) was added, and then EDC·HCl (34.5 g, 180 mmol) was added to the solution at −10 °C. This mixture was stirred for 1 h at −10 °C and then stirred overnight at room temperature. Subsequently, the reaction mixture was diluted with EtOAc, and the solution was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was solidified from EtOAc and hexane to give Boc-D-MetO-Phe-OMe (55.9 g, 87%) as a white amorphous solid.

Boc-D-MetO-Phe-OMe (42.5 g, 100 mmol) was treated with 4 N-HCl/EtOAc (500 ml) for 30 min at room temperature to remove the Boc group. Ether was added to the solution and the precipitated solid was separated by filtration and dried in a vacuum desiccator over solid NaOH. This amine component was dissolved in DMF (750 ml) on an ice bath. To this solution was added Boc-Tyr-OH (30.9 g, 110 mmol) and HOBt (27 g, 200 mmol). Neutralization with TEA (12.1 g) was followed by addition of EDC·HCl (23.0 g, 120 mmol) at −10 °C. The reaction mixture was stirred for 1 h at the same temperature, and then stirred overnight at room temperature. Subsequently, the reaction mixture was diluted with EtOAc, and the solution was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was solidified from EtOAc and hexane to give Boc-Tyr-D-MetO-Phe-OMe (51.4 g, 87%) as a white amorphous solid.

To a solution of Boc-Tyr-D-MetO-Phe-OMe (48.0 g, 81.4 mmol) in MeOH (400 ml), 2 N-NaOH (62 ml) was added and the mixture was stirred at room

temperature for 2 h. Then the solution was diluted with water, evaporated *in vacuo* to remove the MeOH, and washed with Et_2O. The aqueous layer was acidified with 1 N-HCl and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Finally, the residue was solidified from EtOAc and hexane to give Boc-Tyr-D-MetO-Phe-OH (**1**), 40.1 g (86%) as a white amorphous solid. Rf_3 0.48. ^{1}H-NMR ($CDCl_3$) δ: 7.89—7.30 (1H, m), 7.30—7.10 (5H, m), 6.97 (2H, t, J=8.0 Hz), 6.72 (2H, t, J=7.7 Hz), 4.82 (1H, m), 4.62 (1H, m), 4.23 (1H, m), 3.17 (1H, m), 2.87 (2H, d, J=7.5 Hz), 2.72 (1H, m), 2.53 (2H, s), 2.50 (1H, s), 2.39 (2H, m), 2.10 (1H, br m), 1.60 (1H, br m), 1.39 (9H, s). FAB-MS m/z: 576 $(M+H)^+$.

Preparation of Boc-MeβAla-OMe (2a) According to method C, Boc-MeβAla-OH (1.0 g, 4.9 mmol) was reacted with MeOH (172 mg, 5.4 mmol). The crude compound was purified by flush column chromatography eluted with CH_2Cl_2/MeOH (50 : 1, v/v) to give **2a** (958 mg, 90%) as an oil. Rf_1 0.85. ^{1}H-NMR ($CDCl_3$) δ: 3.66 (3H, s), 3.50 (2H, t, J=6.8 Hz), 2.87 (3H, s), 2.54 (2H, t, J=6.9 Hz), 1.46 (9H, s). FAB-MS m/z: 218 $(M+H)^+$.

Compounds **2b**—**r** were prepared in the same manner (method C) from Boc-MeβAla-OH and the corresponding alcohol (X-OH), respectively.

Boc-MeβAla-OEt (2b) Using method C, Boc-MeβAla-OH (1.22 g, 6.0 mmol) was reacted with EtOH (304 mg, 6.60 mmol) to give **2b** (1.33 g, 96%). Rf_1 0.88. ^{1}H-NMR ($CDCl_3$) δ: 4.14 (2H, t, J=7.1 Hz), 3.50 (2H, t, J=6.8 Hz), 2.87 (3H, s), 2.54 (2H, t, J=6.9 Hz), 1.46 (9H, s), 1.26 (3H, t, J=7.1 Hz). FAB-MS m/z: 232 $(M+H)^+$.

Boc-MeβAla-O(Bu) (2c) Using method C, Boc-MeβAla-OH (1.22 g, 6.0 mmol) was reacted with 1-butanol (489 mg, 6.60 mmol) to give **2c** (1.50 g, 96%). Rf_1 0.90. ^{1}H-NMR ($CDCl_3$) δ; 4.08 (2H, t, J=6.6 Hz), 3.50 (2H, t, J=6.9 Hz), 2.87 (3H, s), 2.54 (2H, t, J=7.1 Hz), 1.62 (2H, m), 1.46 (9H, s), 1.38 (2H, m), 0.94 (3H, t, J=7.4 Hz). FAB-MS m/z: 260 $(M+H)^+$.

Boc-MeβAla-O(Pen) (2d) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with 1-Pentanol (970 mg, 11.0 mmol) to give **2d** (1.61 g, 98%). Rf_1 0.90. ^{1}H-NMR ($CDCl_3$) δ: 4.06 (2H, t, J=6.8 Hz), 3.50 (2H, t, J=6.8 Hz), 2.85 (3H, s), 2.51 (2H, t, J=6.8 Hz), 1.62 (2H, m), 1.46 (9H, s), 1.27 (4H, m), 0.87 (3H, t, J=6.8 Hz). FAB-MS m/z: 274 $(M+H)^+$.

Boc-MeβAla-O(Hex) (2e) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with 1-hexanol (1.12 g, 11.0 mmol) to give **2e** (2.84 g, 99%). Rf_1 0.90. ^{1}H-NMR ($CDCl_3$) δ: 4.07 (2H, t, J=6.8 Hz), 3.50 (2H, t, J=7.1 Hz), 2.87 (3H, s), 2.54 (2H, t, J=7.1 Hz), 1.62 (2H, m), 1.46 (9H, s), 1.31 (6H, br s), 0.89 (3H, t, J=6.8 Hz). FAB-MS m/z: 288 $(M+H)^+$.

Boc-MeβAla-O(Hep) (2f) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with 1-heptanol (1.28 g, 11.0 mmol) to give **2f** (2.65 g, 88%). Rf_1 0.91. ^{1}H-NMR ($CDCl_3$) δ: 4.07 (2H, t, J=6.8 Hz), 3.51 (2H, t, J=7.1 Hz), 2.86 (3H, s), 2.52 (2H, t, J=7.2 Hz), 1.61 (2H, m), 1.46 (9H, s), 1.31 (8H, br s), 0.87 (3H, t, J=6.9 Hz). FAB-MS m/z: 302 $(M+H)^+$.

Boc-MeβAla-O(Oct) (2g) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with octyl alcohol (1.43 g, 11.0 mmol) to give **2g** (2.90 g, 92%). Rf_1 0.92. ^{1}H-NMR ($CDCl_3$) δ: 4.08 (2H, t, J=6.8 Hz), 3.50 (2H, t, J=6.8 Hz), 2.85 (3H, s), 2.51 (2H, t, J=7.1 Hz), 1.62 (2H, m), 1.42 (9H, s), 1.26 (10H, br s), 0.88 (3H, t, J=6.8 Hz). FAB-MS m/z: 316 $(M+H)^+$.

Boc-MeβAla-O(Non) (2h) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with nonyl alcohol (1.59 g, 11.0 mmol) to give **2h** (2,90 g, 88%). Rf_1 0.91. ^{1}H-NMR ($CDCl_3$) δ: 4.06 (2H, t, J=6.9 Hz), 3.49 (2H, t, J=6.9 Hz), 2.85 (3H, s), 2.51 (2H, t, J=6.8 Hz), 1.61 (2H, t, J=6.6 Hz), 1.39 (9H, s), 1.26 (12H, br s), 0.88 (3H, t, J=6.8 Hz). FAB-MS m/z: 330 $(M+H)^+$.

Boc-MeβAla-O(Dec) (2i) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with decyl alcohol (1.74 g, 11.0 mmol) to give **2i** (3.12 g, 99%). Rf_1 0.92. ^{1}H-NMR ($CDCl_3$) δ: 4.07 (2H, t, J=6.9 Hz), 3.50 (2H, t, J=6.9 Hz), 2.87 (3H, s), 2.54 (2H, t, J=6.9 Hz), 1.62 (2H, m), 1.46 (9H, s), 1.27 (14H, br s), 0.88 (3H, t, J=6.8 Hz). FAB-MS m/z: 344 $(M+H)^+$.

Boc-MeβAla-O(Dde) (2j) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with dodecyl alcohol (2.05 g, 11.0 mmol) to give **2j** (3.51 g, 100%). Rf_1 0.93. ^{1}H-NMR ($CDCl_3$) δ: 4.07 (2H, t, J=6.8 Hz), 3.50 (2H, t, J=6.8 Hz), 2.87 (3H, s), 2.54 (2H, t, J=6.8 Hz), 1.62 (2H, m), 1.46 (9H, s), 1.26 (18H, br s), 0.88 (3H, t, J=6.6 Hz). FAB-MS m/z: 372 $(M+H)^+$.

Boc-MeβAla-O(Hde) (2k) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol), was reacted with 1-hexadecanol (2.67 g, 11.0 mmol) to give **2k** (3.04 g, 71%). Rf_1 0.92. ^{1}H-NMR ($CDCl_3$) δ: 4.06 (2H, t, J=6.8 Hz), 3.50 (2H, t, J=6.8 Hz), 2.85 (3H, s), 2.51 (2H, t, J=6.8 Hz), 1.24 (26H, m), 1.42 (9H, s), 0.88 (3H, t, J=6.8 Hz). FAB-MS m/z: 428 $(M+H)^+$.

Boc-MeβAla-OCH$_2$CF$_3$ (2l) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with 2,2,2-trifluoroethanol (1.10 g, 11.0 mmol) to give **2l** (2.78 g, 98 %). Rf_1 0.80. ^{1}H-NMR ($CDCl_3$) δ: 4.62 (2H, m), 3.50 (2H, m), 2.87 (3H, s), 2.52 (2H, t, J=6.8 Hz), 1.42 (9H, s). FAB-MS m/z: 286 $(M+H)^+$.

Boc-MeβAla-OPh (2m) Using method C, Boc-MeβAla-OH (1.22 g, 6.0 mmol) was reacted with phenol (678 mg, 7.2 mmol) to give **2m** (1.68 g, 100%). Rf_1 0.80. ^{1}H-NMR ($CDCl_3$) δ: 7.45—6.80 (5H, m), 3.63 (2H, br s), 2.94 (3H, s), 2.51 (2H, t, J=6.9 Hz), 1.48 (9H, s). FAB-MS m/z: 280 $(M+H)^+$.

Boc-MeβAla-OBzl (2n) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with benzyl alcohol (1.19 g, 11.0 mmol) to give **2n** (2.70 g, 92%). Rf_1 0.81. ^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, m), 5.10 (2H, m), 3.50 (2H, m), 2.87 (3H, s), 2.53 (2H, t, J=6.9 Hz), 1.46 (9H, s). FAB-MS m/z: 294 $(M+H)^+$.

Boc-MeβAla-OcHex (2o) Using method C, Boc-MeβAla-OH (1.22 g, 6.0 mmol) was reacted with cyclohexanol (721 mg, 7.2 mmol) to give **2o** (1.70 g, 100%). Rf_1 0.76. ^{1}H-NMR ($CDCl_3$) δ: 4.76 (1H, m), 3.49 (2H, m), 2.87 (3H, s), 2.52 (2H, t, J=6.8 Hz), 1.82 (2H, m), 1.72 (2H, m), 1.55—1.24 (6H, m), 1.46 (9H, s). FAB-MS m/z: 286 $(M+H)^+$.

Boc-MeβAla-OCH$_2$cHex (2p) Using method C, Boc-MeβAla-OH (1.22 g, 6.0 mmol) was reacted with cyclohexylmethanol (822 mg, 7.2 mmol) to give **2p** (1.80 g, 100%). Rf_1 0.79. ^{1}H-NMR ($CDCl_3$) δ: 3.89 (2H, d, J= 6.3 Hz), 3.50 (2H, t, J=6.9 Hz), 2.87 (3H, s), 2.54 (2H, t, J=6.6 Hz), 1.71 (6H, m), 1.46 (9H, s), 1.20 (3H, m), 0.96 (2H, m). FAB-MS m/z: 300 $(M+H)^+$.

Boc-MeβAla-OBu^t (2q) Using method C, Boc-MeβAla-OH (2.03 g, 10.0 mmol) was reacted with *tert*-butanol (815 mg, 11.0 mmol) to give **2q** (1.81 g, 70%). Rf_1 0.84. ^{1}H-NMR ($CDCl_3$) δ: 3.54 (2H, br s), 2.90 (3H, s), 2.51 (2H, t, J=6.9 Hz), 1.49 (s, 9H), 1.42 (s, 9H). FAB-MS m/z: 260 $(M+H)^+$.

Boc-MeβAla-O(1-Ad) (2r) Using method C, Boc-MeβAla-OH (4.06 g, 20.0 mmol) was reacted with 1-adamantanol (3.35 g, 22.0 mmol) to give **2r** (1.73 g, 26%). Rf_1 0.90. ^{1}H-NMR ($CDCl_3$) δ: 3.54 (2H, t, J=6.9 Hz), 2.95 (3H, s), 2.45 (2H, t, J=6.9 Hz), 2.16 (3H, br s), 2.10 (6H, br s), 1.66 (6H, br s), 1.47 (9H, s). FAB-MS m/z: 338 $(M+H)^+$.

Preparation of Boc-MeβAla-OPOM (2s) To a solution of Boc-MeβAla-OH (5.08 g, 25.0 mmol) in DMF (20 ml), chloromethyl pivalate (POM-Cl, 7.53 g, 50.0 mmol), TEA (8.09 g, 80.0 mmol), and NaI (0.5 g) were added at 0 °C. This mixture was stirred for 1 h at 0 °C and then stirred overnight at room temperature. After the reaction mixture was diluted with EtOAc, the solution was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by flush column chromatography eluted with hexane/EtOAc (2 : 1, v/v) to give **2s** (8.17 g, 100%). Rf_1 0.80. ^{1}H-NMR ($CDCl_3$) δ: 5.76 (2H, s), 3.52 (2H, t, J=6.9 Hz), 2.86 (3H, s), 2.58 (2H, t, J=6.9 Hz), 1.45 (9H, s), 1.20 (9H, s). FAB-MS m/z: 318 $(M+H)^+$.

Preparation of Boc-MeβAla-OCH(Me)OCOOEt (2t) To a solution of Boc-MeβAla-OH (2.0 g, 9.8 mmol) in DMF (30 ml), 1-bromoethyl ethyl carbonate[33] (2.12 g, 10.8 mmol) and $KHCO_3$ (1.2 g, 12.0 mmol) were added at 0 °C. This mixture was stirred overnight at 50 °C. Then the reaction mixture was diluted with EtOAc, and the solution was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by flush column chromatography eluted with CH_2Cl_2/MeOH (90 : 1, v/v) to give **2t** (3.0 g, 95%). Rf_1 0.77. ^{1}H-NMR ($CDCl_3$) δ: 6.77 (1H, q, J=5.4 Hz), 4.22 (2H, q, J=7.1 Hz), 3.50 (2H, br s), 2.87 (3H, s), 2.58 (2H, t, J=6.9 Hz), 1.52 (3H, d, J=5.7 Hz), 1.46 (9H, s), 1.32 (3H, t, J=7.2 Hz). FAB-MS m/z: 320 $(M+H)^+$.

Preparation of Boc-MeβAla-O(Phthalidyl) (2u) To a solution of Boc-MeβAla-OH (1.0 g, 4.93 mmol) in DMF (20 ml), bromophthalide[34] (2.3 g, 10.8 mmol) and $KHCO_3$ (1.18 g, 1.18 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then stirred overnight at room temperature. Subsequently, the reaction mixture was diluted with EtOAc, and the solution was washed with 1 N-HCl, sat. $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by flush column chromatography eluted with CH_2Cl_2/MeOH (90 : 1, v/v) to give **2u** (2.18 g, 66%). Rf_1 0.74. ^{1}H-NMR ($CDCl_3$) δ: 8.00—7.30 (5H, m), 3.56 (2H, t, J=6.9 Hz), 2.88 (3H, s), 2.68 (2H, t, J=7.1 Hz), 1.44 (9H, s). FAB-MS m/z: 336 $(M+H)^+$.

Preparation of Boc-Tyr-D-MetO-Phe-MeβAla-OMe (3a) Compound **1** (2.88 g, 5.0 mmol) and HCl·H-MeβAla-OMe, obtained from **2a** by deprotection using method D, were coupled (method A) to yield **3a** (2.76 g, 82%) as a white powder. Rf_3 0.50. ^{1}H-NMR ($CDCl_3$) δ: 7.30—7.10 (5H, m), 7.04 (2H, d, J=7.6 Hz), 6.80 (2H, m), 5.95—5.65 (1H, m), 5.04 (1H, m), 4.64 (1H, br s), 4.22 (1H, br s), 3.65 (4H, m), 3.35 (1H, br s), 2.96 (4H, m), 2.86

(1H, s), 2.76 (2H, s), 2.50 (5H, m), 2.22 (2H, br s), 1.94 (1H, br s), 1.80 (1H, br s), 1.42 (9H, s). FAB-MS *m/z*: 675 $(M+H)^+$.

Compounds **3b**—**p**, **3s**—**u** were prepared in the same manner from compound **1** and the corresponding Boc-MeβAla-OX (**2b**—**p**, **2s**—**u**), respectively.

Boc-Tyr-D-MetO-Phe-MeβAla-OEt (3b) A white powder (2.65 g, 77%). Rf_3 0.52. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.10 (5H, m), 7.03 (2H, d, J=7.8 Hz), 6.79 (2H, m), 5.90—5.64 (1H, m), 5.02 (1H, m), 4.67 (1H, br s), 4.23 (1H, br s), 4.10 (2H, q, J=7.1 Hz), 3.67 (1H, m), 3.38 (1H, br s), 2.98 (4H, m), 2.86 (1H, s), 2.77 (2H, s), 2.48 (5H, m), 2.20 (2H, br s), 1.92 (1H, br s), 1.78 (1H, br s), 1.41 (9H, s), 1.24 (3H, t, J=7.1 Hz). FAB-MS *m/z*: 689 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Bu) (3c) A white powder (2.69 g, 75%). Rf_3 0.58. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.15 (5H, m), 7.03 (2H, d, J=8.1 Hz), 6.80 (2H, m), 5.90—5.60 (1H, m), 5.00 (1H, m), 4.68 (1H, m), 4.24 (1H, m), 4.05 (2H, t, J=6.8 Hz), 3.70 (1H, m), 3.38 (1H, m), 3.01 (3H, m), 2.87 (1H, s), 2.78 (2H, s), 2.49 (6H, m), 2.27 (2H, br s), 1.91 (1H, br s), 1.78 (1H, br s), 1.59 (2H, m), 1.42 (9H, s), 0.93 (3H, t, J=7.2 Hz). FAB-MS *m/z*: 717 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Pen) (3d) A white powder (2.92 g, 80%). Rf_3 0.55. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.15 (5H, m), 7.03 (2H, d, J=8.1 Hz), 6.79 (2H, m), 5.90—5.60 (1H, m), 5.02 (1H, m), 4.66 (1H, m), 4.28 (1H, br s), 4.04 (2H, m), 3.70 (1H, m), 3.38 (2H, br s), 2.88 (4H, m), 2.88 (1H, s), 2.79 (2H, s), 2.65—2.38 (5H, m), 2.29 (2H, m), 2.06 (2H, m), 1.61 (2H, t, J=7.1 Hz), 1.39 (9H, s), 1.41 (4H, br s), 0.90 (3H, m). FAB-MS *m/z*: 731 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Hex) (3e) A white powder (3.09 g, 83%). Rf_3 0.54. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.10 (5H, m), 7.03 (2H, d, J=8.1 Hz), 6.78 (2H, dd, J=8.4, 4.8 Hz), 6.10—5.66 (1H, m), 5.02 (1H, m), 4.73 (1H, br s), 4.25 (1H, br s), 4.03 (2H, m), 3.74 (1H, m), 3.36 (1H, m), 3.01 (4H, m), 2.88 (1.5H, s), 2.79 (1.5H, s), 2.50 (5H, m), 2.20 (2H, br s), 1.90 (1H, br s), 1.60 (1H, t, J=7.2 Hz), 1.42 (9H, s), 1.30 (6H, br s), 0.88 (3H, m). FAB-MS *m/z*: 745 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Hep) (3f) A white powder (2.62 g, 69%). Rf_3 0.57. ^{1}H-NMR ($CDCl_3$) δ: 7.45—7.15 (5H, m), 7.02 (2H, d, J=8.4 Hz), 6.81 (2H, m), 5.90—5.60 (1H, m), 5.02 (1H, m), 4.65 (1H, m), 4.28 (1H, br s), 4.04 (2H, t, J=6.8 Hz), 3.70 (1H, m), 3.40 (2H, br s), 2.98 (4H, m), 2.87 (1H, s), 2.78 (2H, s), 2.65—2.43 (5H, m), 2.29 (2H, m), 2.05 (2H, m), 1.61 (2H, t, J=6.8 Hz), 1.39 (9H, s), 1.31 (8H, br s), 0.88 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 759 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Oct) (3g) A white powder (2.78 g, 72%). Rf_3 0.60. ^{1}H-NMR ($CDCl_3$) δ: 7.40—7.15 (5H, m), 7.02 (2H, d, J=8.4 Hz), 6.81 (2H, m), 5.88—5.60 (1H, m), 5.00 (1H, m), 4.64 (1H, m), 4.27 (1H, br s), 4.04 (2H, t, J=6.8 Hz), 3.70 (1H, m), 3.39 (2H, br s), 2.99 (4H, m), 2.86 (1H, s), 2.79 (2H, s), 2.65—2.40 (5H, m), 2.29 (2H, m), 2.07 (2H, m), 1.61 (2H, t, J=6.8 Hz), 1.39 (9H, s), 1.29 (10 br s), 0.88 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 773 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Non) (3h) A white powder (2.94 g, 75%). Rf_3 0.62. ^{1}H-NMR ($CDCl_3$) δ: 7.40—7.15 (5H, m), 7.03 (2H, d, J=8.4 Hz), 6.80 (2H, m), 5.88—5.58 (1H, m), 5.01 (1H, m), 4.65 (1H, m), 4.27 (1H, br s), 4.04 (2H, t, J=6.8 Hz), 3.70 (1H, m), 3.41 (2H, br s), 3.00 (4H, m), 2.87 (1H, s), 2.79 (2H, s), 2.65—2.44 (5H, m), 2.30 (2H, m), 2.08 (2H, m), 1.61 (2H, t, J=6.6 Hz), 1.39 (9H, s), 1.26 (12H, br s), 0.88 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 787 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Dec) (3i) A white powder (2.64 g, 66%). Rf_3 0.67. ^{1}H-NMR ($CDCl_3$) δ: 7.30—7.10 (5H, m), 7.03 (2H, d, J=7.5 Hz), 6.79 (2H, dd, J=8.3, 4.5 Hz), 6.10—5.73 (1H, m), 5.04 (1H, m), 4.72 (1H, br s), 4.24 (1H, br s), 4.03 (2H, t, J=6.6 Hz), 3.77 (1H, m), 3.36 (1H, m), 3.10 (2H, m), 2.88 (1.5H, s), 2.80 (1.5H, s), 2.50 (5H, m), 2.20 (2H, br s), 1.92 (1H, br s), 1.81 (1H, br s), 1.61 (2H, t, J=6.8 Hz), 1.42 (9H, s), 1.26 (14H, br s), 0.88 (3H, t, J=6.5 Hz). FAB-MS *m/z*: 802 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Dde) (3j) A white powder (2.57 g, 62%). Rf_3 0.69. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.15 (5H, m), 7.11 (2H, d, J=8.4 Hz), 6.91 (2H, m), 6.10—5.73 (1H, m), 5.03 (1H, m), 4.67 (1H, m), 4.29 (1H, br s), 4.04 (2H, m), 3.75 (1H, m), 3.39 (1H, m), 3.00 (4H, m), 2.88 (1H, s), 2.80 (2H, m), 2.60—2.35 (6H, m), 2.26 (1H, m), 2.06 (2H, m), 2.20 (2H, br s), 1.60 (2H, m), 1.81 (1H, br s), 1.61 (2H, t, J=6.8 Hz), 1.39 (9H, s), 1.26 (18H, br s), 0.89 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 830 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Hde) (3k) A white powder (2.79 g, 63%). Rf_3 0.70. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.15 (5H, m), 7.04 (2H, d, J=8.4 Hz), 6.85 (2H, m), 6.10—5.73 (1H, m), 5.01 (1H, m), 4.65 (1H, m), 4.28 (1H, br s), 4.05 (2H, m), 3.73 (1H, m), 3.40 (1H, m), 3.02 (4H, m), 2.89 (1H, s), 2.79 (2H, m), 2.60—2.35 (6H, m), 2.24 (1H, m), 2.05 (2H, m), 2.22 (2H, br s), 1.62 (2H, m), 1.80 (1H, br s), 1.61 (2H, t, J=6.8 Hz), 1.38 (9H, s), 1.26 (26H, br s), 0.88 (3H, t, J=6.9 Hz). FAB-MS *m/z*: 886 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OCH$_2$CF$_3$ (3l) A white powder (3.08 g, 83%). Rf_3 0.63. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.10 (5H, m), 7.03 (2H, d, J=7.8 Hz), 6.79 (2H, m), 5.90—5.64 (1H, m), 5.02 (1H, m), 4.67 (1H, m), 4.62 (2H, m), 4.23 (1H, m), 3.65 (1H, m), 3.36 (1H, m), 2.99 (4H, m), 2.85 (1H, s), 2.78 (2H, s), 2.46 (5H, m), 2.21 (2H, m), 1.93 (1H, br s), 1.78 (1H, m), 1.41 (9H, s), 1.23 (3H, t, J=7.2 Hz). FAB-MS *m/z*: 743 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OPh (3m) A white powder (2.54 g, 69%). Rf_3 0.46. ^{1}H-NMR ($CDCl_3$) δ: 7.31—6.94 (12H, m), 6.64 (2H, m), 5.72 (1H, br m), 5.10 (2H, m), 4.87 (1H, m), 4.52 (1H, m), 4.15 (1H, m), 3.63 (1H, m), 3.35 (1H, m), 3.05—2.74 (6H, m), 2.66 (2H, m), 2.45 (3H, m), 2.31—2.15 (2H, m), 1.89 (1H, m), 1.71 (1H, m), 1.41 (9H, s). FAB-MS *m/z*: 737 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OBzl (3n) A white powder (2.36 g, 63%). Rf_3 0.46. ^{1}H-NMR ($CDCl_3$) δ: 7.97—7.82 (2H, m), 7.34 (5H, m), 7.24 (3H, m), 7.16 (2H, m), 7.04 (2H, d, J=8.1 Hz), 6.77 (2H, m), 5.73 (1H, br m), 5.10 (2H, m), 4.87 (1H, m), 4.50 (1H, m), 4.14 (1H, m), 3.65 (1H, m), 3.51 (2H, s), 3.38 (2H, m), 3.05—2.65 (6H, m), 2.49 (5H, m), 1.96 (1H, m), 1.73 (1H, m), 1.40 (9H, s). FAB-MS *m/z*: 751 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OcHex (3o) A white powder (2.63 g, 71%). Rf_3 0.54. ^{1}H-NMR ($CDCl_3$) δ: 8.61 (1H, br m), 8.18—7.94 (1H, m), 7.43—7.33 (1H, m), 7.29—7.16 (5H, m), 7.04 (2H, d, J=8.1 Hz), 6.79 (2H, dd, J=8.4, 4.8 Hz), 6.08—5.98 (1H, m), 4.71 (2H, m), 4.26 (1H, br s), 3.72 (0.66H, m), 3.36 (1.33H, m), 2.98 (8H, m), 2.86 (1H, s), 2.77 (2H, s), 2.48 (3H, m), 2.42 (2H, m), 2.16 (2H, m), 1.90 (1H, m), 1.80 (3H, m), 1.70 (2H, m), 1.54 (1H, m), 1.41 (9H, s), 1.37 (5H, m). FAB-MS *m/z*: 743 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OCH$_2$cHex (3p) A white powder (2.65 g, 70%). Rf_3 0.61. ^{1}H-NMR ($CDCl_3$) δ: 8.50 (1H, br m), 8.14—7.90 (1H, m), 7.38 (1H, m), 7.29—7.16 (5H, m), 7.04 (2H, d, J=8.1 Hz), 6.79 (2H, dd, J=8.3, 5.0 Hz), 5.97—5.69 (1H, m), 5.00 (1H, m), 4.68 (1H, m), 4.26 (1H, m), 3.85 (2H, d, J=6.0 Hz), 3.71 (0.66H, m), 3.36 (1.33H, m), 3.12—2.70 (11H, m), 2.48—2.40 (5H, m), 2.19 (2H, m), 1.90 (1H, m), 1.70 (7H, m), 1.41 (9H, s), 1.20 (3H, m), 0.95 (2H, m). FAB-MS *m/z*: 757 $(MC+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OBu^t (3q) Compound **1** (2.88 g, 5.0 mmol) and H-MeβAla-OBu^t, obtained from **2q** by deprotection using method E, were coupled (method A) to yield **3q** (2.79 g, 78%) as a white powder. Rf_3 0.57. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.10 (5H, m), 7.03 (2H, d, J=8.1 Hz), 6.97 (2H, dd, J=8.4, 4.8 Hz), 6.12—5.76 (1H, m), 5.03 (1H, m), 4.73 (1H, m), 4.25 (1H, br s), 3.70 (1H, m), 3.36 (1H, m), 2.96 (4H, m), 2.88 (1.5H, s), 2.79 (1.5H, s), 2.48 (3H, m), 2.36 (2H, m), 2.17 (2H, m), 1.92 (1H, br s), 1.76 (1H, br s), 1.42 (18H, s). FAB-MS *m/z*: 717 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(1-Ad) (3r) Compound **1** (2.88 g, 5.0 mmol) and H-MeβAla-O(1-Ad), obtained from **2r** by deprotection using method E, were coupled (method A) to yield **3r** (2.50 g, 63%) as a white powder. Rf_3 0.62. ^{1}H-NMR ($CDCl_3$) δ: 7.30—7.10 (5H, m), 7.04 (2H, d, J=7.6 Hz), 6.80 (2H, m), 5.95—5.65 (1H, m), 5.04 (1H, m), 4.64 (1H, br s), 4.22 (1H, br s), 3.63 (1H, m), 3.33 (1H, m), 2.95 (4H, m), 2.87 (1H, s), 2.76 (2H, s), 2.50 (5H, m), 2.22 (2H, br s), 2.16 (3H, br s), 2.10 (6H, br s), 1.94 (1H, br s), 1.80 (1H, br s), 1.66 (6H, br s), 1.42 (9H, s). FAB-MS *m/z*: 795 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(POM) (3s) A white powder (2.75 g, 71%). Rf_3 0.64. ^{1}H-NMR ($CDCl_3$) δ: 7.30—7.10 (5H, m), 7.04 (2H, d, J=7.6 Hz), 6.80 (2H, m), 5.95—5.65 (3H, m), 5.04 (1H, m), 4.64 (1H, m), 4.22 (1H, m), 3.65 (1H, m), 3.35 (1H, br s), 2.96 (4H, m), 2.87 (1H, s), 2.76 (2H, s), 2.53 (5H, m), 2.25 (2H, m), 1.96 (1H, m), 1.80 (1H, br s), 1.42 (9H, s), 1.20 (9H, s). FAB-MS *m/z*: 775 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-OCH(Me)OCOOEt (3t) A white powder (2.52 g, 65%). Rf_3 0.62. ^{1}H-NMR ($CDCl_3$) δ: 8.60—8.47 (1H, m), 8.24—8.03 (2H, m), 7.28—7.17 (6H, m), 7.03 (2H, d, J=8.1 Hz), 6.80—6.70 (3H, m), 6.17—5.87 (1H, m), 5.04 (1H, m), 4.78 (1H, m), 4.27 (1H, br m), 4.25—4.08 (2H, m), 3.75 (1H, m), 3.36 (1H, m), 3.27 (1H, br m), 3.01 (2H, m), 2.94 (2H, m), 2.87 (1H, s), 2.82 (2H, s), 2.48 (5H, m), 2.24—2.08 (2H, m), 1.90 (1H, br m), 1.79 (1H, br m), 1.50 (3H, d, J=5.4 Hz), 1.41 (9H, s), 1.34—1.20 (3H, m). FAB-MS *m/z*: 777 $(M+H)^+$.

Boc-Tyr-D-MetO-Phe-MeβAla-O(Phthalidyl) (3u) A white powder (2.46 g, 62%). Rf_3 0.61. ^{1}H-NMR ($CDCl_3$) δ: 7.80—7.55 (4H, m), 7.35 (2H, d, J=5.4 Hz), 7.12 (5H, m), 6.97 (2H, m), 6.60 (2H, m), 4.50 (1H, m), 4.32 (2H, m), 3.60 (1H, m), 3.50 (1H, m), 3.20—2.65 (7H, m), 2.55 (2H, m), 2.45 (3H, m), 2.27 (2H, m), 1.83 (1H, m), 1.70 (1H, m), 1.42 (9H, s). FAB-MS *m/z*: 793 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OMe (4a)** As described in method H, compound **3a** (2.02 g, 3.0 mmol) was treated with 90%–TFA (30 ml) for 1 h at room temperature to remove the Boc group. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in DMF

(60 ml), and then ehtyl acetimidate hydrochloride (741 mg, 6.0 mmol) and TEA (1.25 ml, 9.0 mmol) were added. This solution was stirred at room temperature for 2 h. The crude peptide was purified by ODS chromatography with a stepwise gradient of acetonitrile (starting from 1% and increasing stepwise by 2%) in 0.1 N acetic acid as the eluting solvent, and then was lyophilized to give **4a** (867 mg, 47%) as a white amorphous powder. HPLC t_R (min): 8.39. Rf_4 0.66. ^{1}H-NMR (CD_3OD) δ: 7.21—7.10 (5H, m), 6.99 (2H, d, J=8.4 Hz), 6.64 (2H, d, J=8.4 Hz), 5.05 (0.5H, m), 4.93 (0.5H, m), 4.29 (2H, m), 3.56 (3H, s), 3.52 (1H, m), 3.40 (1H, m), 3.07—2.91 (3H, m), 2.87—2.93 (m, 2H), 2.87—2.76 (4H, m), 2.47 (3H, m), 2.40 (1H, m), 2.31 (1H, m), 2.20 (1H, m), 2.09 (3H, d, J=2.4 Hz), 1.87 (1H, m), 1.71 (1H, m). FAB-MS m/z: 616 $(M+H)^+$.

Compounds **4b**—**p**, **4s**—**u** were prepared in the same manner.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OEt (4b)** Compound **3b** (2.07 g, 3.0 mmol) was treated according to method H, yielding **4b** (661 mg, 35%) as a white powder. HPLC t_R (min): 9.92. Rf_4 0.66. ^{1}H-NMR (CD_3OD) δ: 7.20—7.10 (5H, m), 7.00 (2H, d, J=7.8 Hz), 6.74 (2H, m), 5.02 (0.5H, m), 4.94 (0.5H, m), 4.32 (2H, m), 4.08 (2H, q, J=7.2 Hz), 3.60 (1H, m), 3.38 (1H, m), 2.98 (4H, m), 2.86 (1H, s), 2.77 (2H, s), 2.48 (3H, m), 2.27 (1H, m), 2.19 (1H, m), 2.10 (3H, d, J=2.4 Hz), 1.90 (1H, m), 1.74 (1H, m), 1.24 (3H, t, J=7.2 Hz). FAB-MS m/z: 630 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Bu) (4c)** Compound **3c** (2.15 g, 3.0mmol) was treated according to method H, yielding **4c** (809 mg, 41%) as a white powder. HPLC t_R (min): 11.01. Rf_4 0.68. ^{1}H-NMR (CD_3OD) δ: 7.21—7.09 (5H, m), 6.99 (2H, d, J=7.8 Hz), 6.63 (2H, d, J=7.8 Hz), 5.01 (0.5H, m), 4.93 (0.5H, m), 4.36 (1H, m), 4.29 (1H, m), 3.97 (2H, t, J=7.2 Hz), 3.53 (1H, m), 3.37 (1H, m), 3.05—2.90 (3H, m), 2.87—2.75 (4H, m), 2.47 (3H, m), 2.40 (2H, m), 2.29 (1H, m), 2.21 (1H, m), 2.09 (3H, d, J=2.4 Hz), 1.85 (1H, m), 1.71 (1H, m), 1.51 (2H, m), 1.29 (2H, m), 0.84 (3H, t, J=7.4 Hz). FAB-MS m/z: 658 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Pen) (4d)** Compound **3d** (2.19 g, 3.0 mmol) was treated according to method H, yielding **4d** (705 mg, 35%) as a white powder. HPLC t_R (min): 11.99. Rf_4 0.69. ^{1}H-NMR (CD_3OD) δ: 7.22—7.11 (5H, m), 6.99 (2H, d, J=8.4 Hz), 6.63 (2H, d, J=8.7 Hz), 5.01 (0.5H, m), 4.90 (0.5H, m), 4.31 (2H, m), 3.96 (2H, t, J=6.6 Hz), 3.54 (1H, m), 3.37 (2H, m), 3.05—2.90 (3H, m), 2.87—2.76 (4H, m), 2.47 (3H, m), 2.41 (1H, m), 2.32—2.18 (3H, m), 2.09 (3H, m), 1.85 (1H, m), 1.68 (1H, m), 1.53 (2H, m), 1.23 (4H, m), 0.82 (3H, t, J=6.5 Hz). FAB-MS m/z: 672 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Hex) (4e)** Compound **3e** (2.23 g, 3.0 mmol) was treated according to method H, yielding **4e** (473 mg, 42%) as a white powder. HPLC t_R (min): 12.95. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.21—7.08 (5H, m), 6.98 (2H, d, J=8.4 Hz), 6.63 (2H, d, J=8.4 Hz), 5.02 (0.5H, m), 4.93 (0.5H, m), 4.28 (2H, m), 3.97 (2H, t, J=6.6 Hz), 3.54 (1H, m), 3.37 (2H, m), 3.06—2.90 (3H, m), 2.88—2.72 (4H, m), 2.47 (3H, m), 2.40 (1H, m), 2.32—2.18 (3H, m), 2.09 (3H, m), 1.83 (1H, m), 1.70 (1H, m), 1.53 (2H, m), 1.23 (6H, m), 0.81 (3H, t, J=6.8 Hz). FAB-MS m/z: 686 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Hep) (4f)** Compound **3f** (2.28 g, 3.0 mmol) was treated according to method H, yielding **4f** (777 mg, 37%) as a white powder. HPLC t_R (min): 13.81. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.21—7.08 (5H, m), 6.99 (2H, d, J=8.4 Hz), 6.63 (2H, d, J=8.4 Hz), 5.01 (0.5H, m), 4.93 (0.5H, m), 4.38—4.26 (2H, m), 3.96 (2H, t, J=6.8 Hz), 3.54 (1H, m), 3.35 (2H, m), 3.05—2.90 (3H, m), 2.88—2.73 (4H, m), 2.47 (3H, m), 2.39 (1H, m), 2.32—2.16 (3H, m), 2.09 (3H, m), 1.84 (1H, m), 1.70 (1H, m), 1.52 (2H, m), 1.22 (8H, m), 0.81 (3H, t, J=6.6 Hz). FAB-MS m/z: 700 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Oct) (4g)** Compound **3g** (2.32 g, 3.0 mmol) was treated according to method H, yielding **4g** (750 mg, 35%) as a white powder. HPLC t_R (min): 14.67. Rf_4 0.70. ^{1}H-NMR (CD_3OD) δ: 7.20—7.09 (5H, m), 7.00 (2H, d, J=8.1 Hz), 6.63 (2H, d, J=8.1 Hz), 5.04 (0.5H, m), 4.93 (0.5H, m), 4.31 (2H, m), 3.96 (2H, t, J=6.8 Hz), 3.56 (1H, m), 3.36 (2H, m), 3.10—2.75 (7H, m), 2.48 (3H, s), 2.35—2.15 (4H, m), 2.09 (3H, s), 1.86 (1H, m), 1.71 (1H, m), 1.52 (2H, m), 1.21 (10H, m), 0.80 (3H, t, J=6.6 Hz). FAB-MS m/z: 714 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Non) (4h)** Compound **3h** (2.36 g, 3.0 mmol) was treated according to method H, yielding **4h** (699 mg, 32%) as a white powder. HPLC t_R (min): 15.52. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.21—7.12 (5H, m), 7.00 (2H, d, J=7.5 Hz), 6.63 (2H, d, J=8.4 Hz), 5.03 (0.5H, m), 4.94 (0.5H, m), 4.30 (2H, m), 3.96 (2H, t, J=6.8 Hz), 3.55 (1H, m), 3.35 (1H, m), 3.07—2.90 (3H, m), 2.87—2.76 (4H, m), 2.47 (3H, m), 2.40 (1H, m), 2.35—2.17 (3H, m), 2.09 (3H, m), 1.86 (1H, m), 1.71 (1H, m), 1.52 (2H, m), 1.20 (12H, m), 0.80 (3H, t, J=6.8 Hz). FAB-MS m/z: 728 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Dec) (4i)** Compound **3i** (2.40 g, 3.0 mmol) was treated according to method H, yielding **4i** (334 mg, 15%) as a white powder. HPLC t_R (min): 16.54. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.27—7.19 (5H, m), 7.07 (2H, d, J=8.4 Hz), 6.72 (2H, d, J=8.4 Hz), 5.12 (0.5H, m), 5.04 (0.5H, m), 4.40 (2H, m), 4.06 (2H, t, J=6.8 Hz), 3.64 (1H, m), 3.46 (1H, m), 3.15—2.99 (3H, m), 2.97—2.81 (4H, m), 2.56 (3H, m), 2.48 (1H, m), 2.43—2.25 (3H, m), 2.18 (3H, m), 1.92 (1H, m), 1.77 (1H, m), 1.60 (2H, m), 1.29 (14H, m), 0.89 (3H, t, J=6.8 Hz). FAB-MS m/z: 742 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Dde) (4j)** Compound **3j** (2.49 g, 3.0 mmol) was treated according to method H, yielding **4j** (809 mg, 35%) as a white powder. HPLC t_R (min): 18.56. Rf_4 0.70. ^{1}H-NMR (CD_3OD) δ: 7.21—7.10 (5H, m), 6.99 (2H, d, J=7.5 Hz), 6.63 (2H, d, J=8.4 Hz), 5.02 (0.5H, m), 4.93 (0.5H, m), 4.32 (2H, m), 3.96 (2H, t, J=6.8 Hz), 3.57 (1H, m), 3.36 (1H, m), 3.06—2.90 (3H, m), 2.88—2.76 (4H, m), 2.47 (3H, m), 2.39 (1H, m), 2.34—2.16 (3H, m), 2.09 (3H, m), 1.86 (1H, m), 1.70 (1H, m), 1.53 (2H, m), 1.19 (18H, m), 0.80 (3H, t, J=6.8 Hz). FAB-MS m/z: 770 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Hde) (4k)** Compound **3k** (2.66 g, 3.0 mmol) was treated according to method H, yielding **4k** (818 mg, 33%) as a white powder. HPLC t_R (min): >20.0. Rf_4 0.73. ^{1}H-NMR (CD_3OD) δ: 7.28—7.19 (5H, m), 7.08 (2H, m), 6.73 (2H, m), 5.11 (0.5H, m), 5.03 (0.5H, m), 4.38 (2H, m), 4.06 (2H, m), 3.66 (1H, m), 3.46 (1H, m), 3.15—3.00 (3H, m), 2.97—2.77 (4H, m), 2.63 (1H, s), 2.56 (2H, s), 2.50—2.20 (4H, m), 2.19 (3H, m), 1.94 (1H, m), 1.81 (1H, m), 1.62 (1H, m), 1.28 (26H, m), 0.89 (3H, t, J=6.8 Hz). FAB-MS m/z: 827 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OCH$_2$CF$_3$ (4l)** Compound **3l** (2.23 g, 3.0 mmol) was treated according to method H, yielding **4l** (617 mg, 30%) as a white powder. HPLC t_R (min): 10.68. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.25—7.10 (5H, m), 6.99 (2H, m), 6.64 (2H, m), 5.13 (0.5H, m), 4.93 (0.5H, m), 4.30 (2H, m), 3.57 (1H, m), 3.33 (1H, m), 3.05—2.89 (3H, m), 2.87—2.74 (4H, m), 2.47 (3H, m), 2.29 (3H, m), 2.09 (3H, s), 1.87 (1H, m), 1.60 (1H, m). FAB-MS m/z: 686 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OPh (4m)** Compound **3m** (2.21 g, 3.0 mmol) was treated according to method H, yielding **4m** (691 mg, 34%) as a white powder. HPLC t_R (min): 10.83. Rf_4 0.66. ^{1}H-NMR (CD_3OD) δ: 7.31—6.94 (12H, m), 6.64 (2H, m), 5.08 (0.5H, m), 4.97 (0.5H, m), 4.32 (2H, m), 3.63 (1H, m), 3.55 (1H, m), 3.05—2.94 (3H, m), 2.93—2.74 (4H, m), 2.66 (2H, m), 2.45 (3H, m), 2.31—2.15 (2H, m), 2.06 (3H, m), 1.84 (1H, m), 1.71 (1H, m). FAB-MS m/z: 678 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OBzl (4n)** Compound **3n** (2.25 g, 3.0 mmol) was treated according to method H, yielding **4n** (789 mg, 38%) as a white powder. HPLC t_R (min): 11.32. Rf_4 0.68. ^{1}H-NMR (CD_3OD) δ: 7.28—7.22 (5H, m), 7.17—7.07 (5H, m), 6.98 (2H, m), 6.64 (2H, m), 5.01 (2H, s), 4.91 (1H, m), 4.49 (1H, m), 4.28 (1H, m), 3.54 (1H, m), 3.40 (1H, m), 3.05—2.86 (3H), 2.84—2.70 (4H, m), 2.51—2.40 (4H, m), 2.32—2.13 (2H, m), 2.06 (3H, m), 1.86 (1H, m), 1.69 (1H, m). FAB-MS m/z: 692 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OcHex (4o)** Compound **3o** (2.23 g, 3.0 mmol) was treated according to method H, yielding **4o** (451 mg, 22%) as a white powder. HPLC t_R (min): 11.86. Rf_4 0.70. ^{1}H-NMR (CD_3OD) δ: 7.21—7.11 (5H, m), 6.99 (2H, d, J=7.8 Hz), 6.63 (2H, m, J=8.1 Hz), 5.06 (0.5H, m), 4.93 (0.5H, m), 4.63 (1H, m), 4.29 (2H, m), 3.54 (1H, m), 3.38 (1H, m), 3.06—2.90 (3H, m), 2.87—2.76 (4H, m), 2.47 (3H, m), 2.38 (1H, m), 2.31—2.19 (3H, m), 2.09 (3H, m), 1.86 (1H, m), 1.73 (2H, m), 1.63 (2H, m), 1.45 (1H, m), 1.39—1.23 (6H, m). FAB-MS m/z: 684 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OCH$_2$cHex (4p)** Compound **3p** (2.27 g, 3.0 mmol) was treated according to method H, yielding **4p** (691 mg, 33%) as a white powder. HPLC t_R (min): 12.87. Rf_4 0.70. ^{1}H-NMR (CD_3OD) δ: 7.22—7.09 (5H, m), 6.98 (2H, d, J=8.4 Hz), 6.63 (2H, m, J=8.4 Hz), 5.03 (0.5H, m), 4.93 (0.5H, m), 4.29 (2H, m), 3.78 (2H, d, J=6.3 Hz), 3.54 (1H, m), 3.36 (1H, m), 3.06—2.90 (3H, m), 2.87—2.72 (4H, m), 2.48 (3H, m), 2.39 (1H, m), 2.31—2.17 (3H, m), 2.09 (3H, m), 1.86 (1H, m), 1.63 (6H, m), 1.15 (3H, m), 0.91 (2H, m). FAB-MS m/z: 698 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OBu^t (4q)** Compound **3q** (2.15 g, 3.0 mmol) was treated according to method H, yielding **4q** (592 mg, 30%) as a white powder. HPLC t_R (min): 10.76. Rf_4 0.69. ^{1}H-NMR (CD_3OD) δ: 7.28—7.17 (5H, m), 7.08 (2H, d, J=7.2 Hz), 6.72 (2H, d, J=8.4 Hz), 5.13 (0.5H, m), 5.03 (0.5H, m), 4.40 (2H, m), 3.59 (1H, m), 3.42 (1H, m), 3.15—3.00 (3H, m), 2.95—2.81 (4H, m), 2.56 (4H, m), 2.40 (2H, m), 2.32 (1H, m), 2.18 (3H, d, J=2.4 Hz), 1.96 (1H, m), 1.80 (1H, m), 1.42 (9H, s). FAB-MS m/z: 658 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(1-Ad) (4r)** Compound **3r** (2.38 g, 3.0 mmol) was treated according to method H, yielding **4r** (508 mg, 23%) as a white powder. HPLC t_R (min): 12.45. Rf_4 0.69. ^{1}H-NMR (CD_3OD) δ: 7.30—7.15 (5H, m), 7.04 (2H, d, J=7.6 Hz), 6.80 (2H, m), 5.12 (0.5H, m), 5.01 (0.5H, m), 4.42 (2H, m), 3.61 (1H, m), 3.39 (1H, m), 3.15—3.00 (3H, m), 2.95—2.81 (4H, m), 2.57 (4H, m), 2.38 (2H, m), 2.32 (1H, m), 2.19 (3H, d, J=2.4 Hz), 2.16 (3H, m), 2.10 (6H, m), 1.96 (1H, m), 1.80 (1H, m), 1.66 (6H, m). FAB-MS *m/z*: 736 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OPOM (4s)** Compound **3s** (2.32 g, 3.0 mmol) was treated according to method H, yielding **4s** (537 mg, 25%) as a white powder. HPLC t_R (min): 11.44. Rf_4 0.67. ^{1}H-NMR (CD_3OD) δ: 7.21—7.03 (7H, m), 6.98 (2H, m), 6.63 (2H, d, J=8.1 Hz), 5.63 (2H, m), 5.04—4.91 (1H, m), 4.28 (2H, m), 3.53 (1H, m), 3.40 (1H, m), 3.07—2.72 (7H, m), 2.48 (5H, m), 2.31—2.15 (2H, m), 2.10 (3H, d, J=2.4 Hz), 1.85 (1H , m), 1.71 (1H, m). FAB-MS *m/z*: 716 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-OCH(Me)OCOOEt (4t)** Compound **3t** (2.33 g, 3.0 mmol) was treated according to method H, yielding **4t** (431 mg, 20%) as a white powder. HPLC t_R (min): 10.74. Rf_4 0.67. ^{1}H-NMR (CD_3OD) δ:7.16 (5H, m), 6.98 (2H, d, J=8.4 z), 6.65 (3H, m), 5.04 (0.5H, m), 4.95 (0.5H, m), 4.51 (1H, m), 4.28 (2H, m), 4.06 (2H, m), 3.55 (1H,m), 3.41 (1H, m), 3.10—2.70 (7H, m), 2.46 (5H, m), 2.25 (2H, m), 2.09 (3H, s), 1.85 (2H, m), 1.69 (1H, m), 1.38 (3H, d, J=5.7 Hz), 1.18 (3H, m). FAB-MS *m/z*: 718 $(M+H)^+$.

***N*$^{\alpha}$-1-Iminoethyl-Tyr-D-MetO-Phe-MeβAla-O(Phthalidyl) (4u)** Compound **3u** (2.38 g, 3.0 mmol) was treated according to method H, yielding **4u** (594 mg, 27%) as a white powder. HPLC t_R (min): 10.15. Rf_4 0.66. ^{1}H-NMR (CD_3OD) δ: 7.81—7.55 (4H, m), 7.33 (2H, d, J=5.4 Hz), 7.14 (5H, m), 6.97 (2H, mb, 6.62 (2H, m), 4.95 (1H, m), 4.30 (2H, m), 3.61 (1H, m), 3.48 (1H, m), 3.10—2.70 (7H, m), 2.54 (2H, m), 2.46 (3H, m), 2.25 (2H, m), 2.09 (3H, m), 1.85 (1H, m), 1.71 (1H, m). FAB-MS *m/z*: 734 $(M+H)^+$.

Preparation of Boc-Tyr-D-MetO-Phe-MeδAla-OBu^t (5) Compound **1** (17.3 g, 30.0 mmol) and H-MeβAla-OBu^t, obtained from **2q** by deprotection using method E, were coupled (method A) to yield **5** (17.8 g, 83 %) as a white powder. Rf_1 0.46. ^{1}H-NMR ($CDCl_3$) δ: 8.45—7.90 (2H, m), 7.35—7.15 (5H m), 7.03 (2H, d, J=8.1 Hz), 6.79 (2H, dd, J=8.4, 4.8 Hz), 6.11—5.76 (1H, m), 5.03 (1H, m), 4.73 (1H, s), 4.25 (1H, m), 3.70 (1H, m), 3.36 (1H, m), 3.10—2.90 (4H, m), 2.88 (1.5H, s), 2.79 (1.5H, s), 2.48 (3H, m), 2.36 (2H, m), 2.16 (2H, br m), 1.92 (1H, br m), 1.76 (1H, br m), 1.42 (18H, s). FAB-MS *m/z*: 717 $(M+H)^+$.

Boc-Tyr(COMe)-D-MetO-Phe-MeβAla-OBu^t (6a) As described in method F, compound **5** (6.0 g, 8.37 mmol) was reacted with acetyl chloride (723 mg, 9.21 mmol) to yield **6a** (6.0 g, 95%) as a white powder. Rf_2 0.45. ^{1}H-NMR ($CDCl_3$) δ: 7.89 (1H, m), 7.68 (1H, m), 7.27—7.21 (7H, m), 7.01—6.97 (2H, m), 5.48 (1H, br s), 5.02 (1H, br s), 4.64 (1H, m), 4.38 (1H, br s), 3.07—2.96 (4H, m), 2.83—2.76 (3H, m), 2.53 (3H, s), 2.49 (3H, s), 2.26 (3H, s), 2.11—2.05 (2H, s), 1.43 (9H, s), 1.39 (9H, s). FAB-MS *m/z*: 759 $(M+H)^+$.

Compounds **6c**, **d**, **h**, **l**—**p** were prepared in the same manner from compound **5** and the corresponding YCO-Cl, respectively.

Boc-Tyr(COPr^n)-D-MetO-Phe-MeβAla-OBu^t (6c) Using method F, compound **5** (2.50 g, 3.49 mmol) was reacted with *n*-butyryl chloride (409 mg, 3.84 mmol) to give **6c** (1.38 g, 50%) as a white powder. Rf_3 0.68. ^{1}H-NMR ($CDCl_3$) δ: 7.75—7.49 (2H, m), 7.26 (7H, m), 7.00 (2H, dd, J=8.4, 2.1 Hz), 5.51—5.35 (1H, m), 5.01 (1H, m), 4.63 (1H, m), 4.30 (1H, m), 3.65 (0.5H, m), 3.39 (1.5H, m), 3.06 (1H, m), 2.99 (3H, m), 2.87 (1H, s), 2.78 (2H, s), 2.65—2.45 (8H, m), 2.36 (2H, m), 2.09 (4H, m), 1.76 (3H, m), 1.43 (9H, s), 1.40 (9H, s), 1.03 (3H, t, J=7.4 Hz). FAB-MS *m/z*: 787 $(M+H)^+$.

Boc-Tyr(COPr^i)-D-MetO-Phe-MeβAla-OBu^t (6d) Using method F, compound **5** (2.50 g, 3.49 mmol) was reacted with isobutyryl chloride (409 mg, 3.84 mmol) to give **6d** (1.79 g, 65%) as a white powder. Rf_2 0.39. ^{1}H-NMR ($CDCl_3$) δ: 7.78—7.48 (1H, m), 7.26 (7H, m), 7.00 (2H, dd, J=8.4, 2.4 Hz), 5.60—5.42 (1H, m), 5.02 (1H, m), 4.64 (1H, m), 4.31 (1H, br s), 3.67 (1H, m), 3.38 (1H, m), 3.06 (1H, m), 2.99 (3H, m), 2.87 (1H, s), 2.82—2.73 (3H, m), 2.59—2.44 (3H, m), 2.36 (2H, m), 2.27 (2H, m), 2.20—1.90 (2H, m), 1.43 (9H, s), 1.40 (9H, s), 1.29 (3H, t, J=6.9 Hz). FAB-MS *m/z*: 787 $(M+H)^+$.

Boc-Tyr(COPh)-D-MetO-Phe-MeβAla-OBu^t (6h) Using method F, compound **5** (2.15 g, 3.00 mmol) was reacted with benzoyl chloride (464 mg, 3.3 mmol) to give **6h** (2.28 g, 93%) as a white powder. Rf_1 0.58. ^{1}H-NMR ($CDCl_3$) δ: 8.16 (2H, d, J=7.5 Hz), 7.62 (1H, t, J=7.4 Hz), 7.49 (3H, m), 7.30—7.10 (8H, m), 5.32—5.22 (1H, m), 5.01 (1H, m), 4.56 (1H, m), 4.32 (1H, m), 3.64 (0.67H, m), 3.39 (1.33H, m), 3.11 (1H, m), 2.99 (3H, m), 2.85 (1H, s), 2.78 (2H, s), 2.59 (2H, m), 2.55 (2H, s), 2.47 (1H, m), 2.38 (2H, m), 2.14 (2H, m), 1.43 (9H, s), 1.42 (9H, s). FAB-MS *m/z*: 821 $(M+H)^+$.

Boc-Tyr(CO$_2$Me)-D-MetO-Phe-MeβAla-OBu^t (6l) Using method F, compound **5** (2.15 g, 3.00 mmol) was reacted with methyl chloroformate (311 mg, 3.3 mmol) to give **6l** (2.12 g, 91%) as a white powder. Rf_1 0.59. ^{1}H-NMR ($CDCl_3$) δ: 7.42 (2H, m), 7.27—7.17 (7H, m), 7.09 (2H, m), 5.32—5.20 (1H, m), 5.01 (1H, m), 4.54 (1H, m), 4.30 (1H, m), 3.87 (3H, s), 3.62 (0.67 H, m), 3.41 (1.33H, m), 3.08 (1H, m), 3.00 (3H, m), 2.85 (1H, s), 2.79 (2H, s), 2.58 (2H, m), 2.54 (2H, s), 2.46 (1H, s), 2.40 (2H, m), 2.03 (2H, m), 1.43 (9H, s), 1.40 (9H, s). FAB-MS *m/z*: 775 $(M+H)^+$.

Boc-Tyr(CO$_2$Et)-D-MetO-Phe-MeβAla-OBu^t (6m) Using method F, compound **5** (2.15 g, 3.00 mmol) was reacted with ethyl chloroformate (358 mg, 3.9 mmol) to give **6m** (2.12 g, 90%) as a white powder. Rf_1 0.57. ^{1}H-NMR ($CDCl_3$) δ: 7.84—7.66 (1H, m), 7.35—7.17 (8H, m), 7.09 (2H, m), 5.72—5.43 (1H, m), 5.02 (1H, m), 4.66 (1H, m), 4.33 (1H, m), 4.29 (2H, q, J=7.1 Hz), 3.66 (0.66H, m), 3.38 (1.33H, m), 3.16—2.90 (4H, m), 2.87 (1H, s), 2.64—2.51 (2H, m), 2.45 (3H, m), 2.40—2.32 (5H, m), 2.20—1.92 (2H, m), 1.43 (9H, m), 1.40 (9H, s), 1.38 (3H, t, J=6.9 Hz). FAB-MS *m/z*: 789 $(M+H)^+$.

Boc-Tyr(CO$_2$Bu^i)-D-MetO-Phe-MeβAla-OBu^t (6n) Using method F, compound **5** (1.79 g, 2.50 mmol) was reacted with isobutyl chloroformate (376 mg, 2.75 mmol) to give **6n** (1.85 g, 91%) as a white powder. Rf_1 0.58. ^{1}H-NMR ($CDCl_3$) δ: 7.93—7.74 (1H, m), 7.35—7.18 (8H, m), 7.09 (2H, m), 5.85—5.52 (1H, m), 5.13—5.01 (1H, m), 4.69 (1H, m), 4.34 (1H, m), 4.01 (2H, d, J=6.9 Hz), 3.70 (0.66H, m), 3.45 (0.33H, m), 3.37 (1H, m), 3.16—3.02 (2H, m), 2.96 (2H, m), 2.87 (1H, s), 2.80 (2H, s), 2.64—2.51 (2H, m), 2.45 (3H, m), 2.40—2.32 (3H, m), 2.20—1.92 (3H, m), 1.43 (9H, m), 1.40 (9H, s), 1.00 (6H, d, J=6.9 Hz). FAB-MS *m/z*: 817 $(M+H)^+$.

Boc-Tyr(CO$_2$Ph)-D-MetO-Phe-MeβAla-OBu^t (6o) Using method F, compound **5** (2.15 g, 3.00 mmol) was reacted with phenyl chloroformate (516 mg, 3.3 mmol) to give **6o** (2.34 g, 93%) as a white powder. Rf_1 0.60. ^{1}H-NMR ($CDCl_3$) δ: 7.96—7.73 (1H, m), 7.41 (2H, m), 7.30—7.15 (12H, m), 5.88—5.56 (1H, m), 5.13—5.00 (1H, m), 4.69 (1H, m), 4.35 (1H, m), 3.69 (0.66H, m), 3.47 (0.33H, m), 3.37 (1H, m), 3.10 (2H, m), 2.99 (2H, m), 2.87 (1H, s), 2.80 (2H, s), 2.57—2.32 (8H, m), 2.26—1.93 (3H, m), 1.43 (9H, m), 1.40 (9H, s). FAB-MS *m/z*: 837 $(M+H)^+$.

Boc-Tyr(CO$_2$Bzl)-D-MetO-Phe-MeβAla-OBu^t (6p) Using method F, compound **5** (2.15 g, 3.00 mmol) was reacted with benzyl chloroformate (563 mg, 3.3 mmol) to give **6p** (2.20 g, 86%) as a white powder. Rf_1 0.58. ^{1}H-NMR ($CDCl_3$) δ: 7.88—7.53 (1H, m), 7.45—7.34 (5H, m), 7.30—7.17 (7H, m), 7.10 (2H, dd, J=8.7, 2.4 Hz), 5.75—5.47 (1H, m), 5.24 (2H, s), 5.02 (1H, m), 4.64 (1H, m), 4.32 (1H, m), 3.67 (0.66H, m), 3.38 (1.33H, m), 3.08 (2H, m), 2.99 (2H, m), 2.86 (1H, s), 2.79 (2H, s), 2.58—2.42 (7H, m), 2.37 (2H, m), 2.19—1.94 (3H, m), 1.43 (9H, m), 1.39 (9H, s). FAB-MS *m/z*: 851 $(M+H)^+$.

Boc-Tyr(COEt)-D-MetO-Phe-MeβAla-OBu^t (6b) Using method G, compound **5** (3.58 g, 5.0 mmol) was reacted with propionic acid (407 mg, 5.5 mmol) to give **6b** (3.63 g, 94%) as a white powder. Rf_1 0.51. ^{1}H-NMR ($CDCl_3$) δ: 7.50 (1H, m), 7.24 (7H, m), 7.00 (2H, dd, J=8.6, 2.0 Hz), 5.30—5.24 (1H, m), 4.99 (1H, m), 4.57 (1H, m), 4.28 (1H, m), 3.63 (0.5H, m), 3.37 (1.5H, m), 3.08 (1H, m), 2.99 (3H, m), 2.86 (1.5H, s), 2.77 (1.5H, s), 2.60—2.53 (6H, m), 2.46—2.44 (1H, m), 2.37 (2H, m), 2.25—2.01 (2H, m), 1.92 (2H, br s), 1.43 (9H, s), 1.41 (9H, s), 1.24 (3H, t, J=7.5 Hz). FAB-MS *m/z*: 773 $(M+H)^+$.

Compounds **6e**—**g**, **i**—**k** were prepared in the same manner from compound **5** and the corresponding YCOOH, respectively.

Boc-Tyr(COHep)-D-MetO-Phe-MeβAla-OBu^t (6e) Using method G, compound **5** (4.00 g, 5.58 mmol) was reacted with octanoic acid (885 mg, 6.14 mmol) to give **6e** (4.25 g, 90%) as a white powder. Rf_2 0.54. ^{1}H-NMR ($CDCl_3$) δ: 7.52 (1H, m), 7.25 (7H, m), 7.02 (2H, m), 5.25—5.21 (1H, m), 4.96 (1H, m), 4.46 (1H, m), 4.21 (0.5H, m), 3.85—3.65 (1H, m), 3.25 (1H, m), 3.04 (2H, m), 2.93—2.89 (4H, m), 2.56—2.53 (5H, m), 2.37 (4H, m), 2.20 (3H, s), 2.10—2.80 (2H, m), 1.72 (2H, m), 1.43 (9H, s), 1.33 (8H, m), 0.92 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 843 $(M+H)^+$.

Boc-Tyr(COBu^t)-D-MetO-Phe-MeβAla-OBu^t (6f) Using method G, compound **5** (2.15 g, 3.00 mmol) was reacted with pivalic acid (337 mg, 3.3 mmol) to give **6f** (1.22 g, 51%) as a white powder. Rf_1 0.52. ^{1}H-NMR ($CDCl_3$) δ: 7.75—7.45 (1H, m), 7.31—7.18 (7H, m), 6.98 (2H, m), 5.55—5.32 (1H, m), 5.01 (1H, m), 4.59 (1H, m), 4.29 (1H, m), 3.63 (0.5H, m), 3.37 (1.5H, m), 3.08 (1H, m), 2.99 (3H, m), 2.87 (1.5H, s), 2.79 (1.5H, s), 2.61—2.51 (4H, m), 2.46—2.44 (1H, m), 2.37 (2H, m), 2.25—2.01 (2H, m), 1.92 (2H, br s), 1.43 (9H, s), 1.41 (9H, s), 1.33 (9H, s). FAB-MS *m/z*: 801 $(M+H)^+$.

Boc-Tyr(CO(1-Ad))-D-MetO-Phe-MeβAla-OBu^t (6g) Using method G, compound **5** (2.15 g, 3.0 mmol) was reacted with 1-adamantane carboxylic acid (594 mg, 3.30 mmol) to give **6g** (1.64 g, 62%) as a white pow-

der. Rf_1 0.43. ^{1}H-NMR ($CDCl_3$) δ: 7.48 (1H, m), 7.33—7.15 (7H, m), 6.99 (2H, m), 5.28—5.20 (1H, m), 4.96 (1H, m), 4.53 (1H, m), 4.27 (1H, m), 3.60 (0.5H, m), 3.34 (1.5H, m), 3.05 (1H, m), 2.97 (3H, m), 2.87 (1.5H, s), 2.77 (1.5H, s), 2.60—2.51 (4H, m), 2.46—2.44 (1H, m), 2.35 (2H, m), 2.28—2.03 (5H,m), 1.96 (10H, m), 1.87—1.76 (2H, m), 1.71 (8H, m), 1.42 (9H, s), 1.40 (9H, s). FAB-MS *m*/*z*: 879 $(M+H)^+$.

Boc-Tyr($COCH_2CH_2Ph$)-D-MetO-Phe-MeβAla-OBu^t (6i) Using method G, compound **5** (2.00 g, 2.79 mmol) was reacted with hydrocinnamic acid (461 mg, 3.07 mmol) to give **6i** (2.24 g, 95%) as a white powder. Rf_3 0.76. ^{1}H-NMR ($CDCl_3$) δ: 7.73—7.50 (2H, m), 7.34—7.17 (12H, m), 6.93 (2H, dd, *J*=8.6, 2.9 Hz), 5.57—5.37 (1H, m), 5.01 (1H, m), 4.62 (1H, m), 4.29 (1H, br s), 3.67 (1H, m), 3.36 (2H, m), 3.09—2.96 (6H, m), 2.88—2.83 (3H, m), 2.78 (2H, s), 2.58—2.45 (3H, m), 2.42—2.31 (4H, m), 2.17 (3H, s), 2.11—2.02 (2H, m), 1.43 (9H, s), 1.40 (9H, s), FAB-MS *m*/*z*: 849 $(M+H)^+$.

Boc-Tyr($COCH_2CH_2CH_2Ph$)-D-MetO-Phe-MeβAla-OBu^t (6j) Using method G, compound **5** (3.00 g, 4.18 mmol) was reacted with 4-phenylbutyric acid (756 mg, 4.60 mmol) to give **6j** (3.51 g, 97%) as a white powder. Rf_3 0.82. ^{1}H-NMR ($CDCl_3$, TMS) δ:7.62—6.69 (17H, m), 6.45—5.36 (1H, m), 5.01 (1H, m), 4.63 (1H, m), 4.29 (1H, m), 3.40—3.34 (4H, m), 3.10—2.94 (4H, m), 2.86—2.70 (3H, m), 2.59—2.40 (9H, m), 2.28—2.01 (6H, m), 1.43 (9H, s), 1.40 (9H, s). FAB-MS *m*/*z*: 863 $(M+H)^+$.

Boc-Tyr(COcHex)-D-MetO-Phe-MeβAla-OBu^t (6k) Using method G, compound **5** (2.00 g, 2.79 mmol) was reacted with cyclohexanecarboxylic acid (393 mg, 3.07 mmol) to give **6k** (2.18 g, 94%) as a white powder. Rf_3 0.86. ^{1}H-NMR ($CDCl_3$) δ: 7.76—7.40 (2H, m), 7.31—7.17 (7H, m), 6.98 (2H, dd, *J*=8.6, 2.3 Hz), 5.51—5.34 (1H, m), 5.02 (1H, m), 4.64 (1H, m), 4.30 (1H, br s), 3.65 (1H, m), 3.38 (1H, m), 3.06 (1H, m), 2.99 (3H, m), 2.86 (1H, s), 2.78 (2H, s), 2.60—2.25 (5H, m), 2.25—1.90 (3H, m), 1.79 (2H, m), 1.75—1.45 (3H, m), 1.43 (9H, s), 1.41 (9H, s), 1.38—1.22 (3H, m). FAB-MS *m*/*z*: 827 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COMe)-D-MetO-Phe-MeβAla-OH (7a) Compound **6a** (6.00 g, 7.91 mmol) was treated according to method H, yielding **7a** (2.14 g, 42%) as a white powder. HPLC t_R (min): 8.50. Rf_4 0.62. ^{1}H-NMR (CD_3OD) δ: 7.16 (7H, m), 6.96 (2H, m), 5.12 (0.5H, t, *J*=7.3 Hz), 4.93 (0.5H, t, *J*=7.3 Hz), 4.50—4.30 (2H, m), 3.51 (1H, m), 3.42 (1H, m), 3.15 (1H, m), 2.93 (2H, m), 2.85—2.78 (4H, m), 2.46 (1.5H, s), 2.44 (1.5H, s), 2.32 (2H, m), 2.16 (3H, s), 2.09 (3H, s), 2.00—1.55 (2H, m). FAB-MS *m*/*z*: 644 $(M+H)^+$.

Compounds **7b**—**o**, **7s**—**u** were prepared in the same manner.

N$^\alpha$-1-Iminoethyl-Tyr(COEt)-D-MetO-Phe-MeβAla-OH (7b) Compound **6b** (3.00 g, 3.88 mmol) was treated according to method H, yielding **7b** (561 mg, 22%) as a white powder. HPLC t_R (min): 9.37. Rf_4 0.64. ^{1}H-NMR (CD_3OD) δ: 7.19 (7H, m), 6.95 (2H, d, *J*=6.9 Hz), 5.15 (0.5H, t, *J*=7.3 Hz), 4.92 (0.5H, t, *J*=7.3 Hz), 4.49 (0.5H, m), 4.38 (1H, m), 4.31 (0.5H, m), 3.65 (1H, m), 3.26 (1H, m), 3.15 (1H, m), 2.95 (2H, m), 2.86—2.74 (4H, m), 2.53—2.43 (5H, m), 2.37—2.24 (3H, m), 2.09 (3H, s), 1.93 (1H, m), 1.77 (1H, m), 1.62 (1H, m), 1.11 (3H, t, *J*=7.5 Hz). FAB-MS *m*/*z*: 658 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COPr)-D-MetO-Phe-MeβAla-OH (7c) Compound **6c** (1.20 g, 1.52 mmol) was treated according to method H, yielding **7c** (440 mg, 43%) as a white powder. HPLC t_R (min): 10.72. Rf_4 0.67. ^{1}H-NMR (CD_3OD) δ: 7.25 (7H, m), 7.04 (2H, d, *J*=7.2 Hz), 5.25 (0.5H, m), 5.02 (0.5H, m), 4.56 (0.5H, m), 4.45 (1H, m), 4.40 (0.5H, m), 3.70 (1H, m), 3.42 (1H, m), 3.25 (1H, m), 3.04 (2H, m), 2.94 (1H, m), 2.88 (2H, m), 2.54 (3H, m), 2.39 (4h, m), 2.19 (3H, s), 2.10—1.80 (2H, m), 1.74 (2H, m), 1.03 (3H, t, *J*=7.5 Hz, 2H). FAB-MS *m*/*z*: 672 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COPr^i)-D-MetO-Phe-MeβAla-OH (7d) Compound **6d** (1.60 g, 2.03 mmol) was treated according to method H, yielding **7d** (464 mg, 34%) as a white powder. HPLC t_R (min): 11.03. Rf_4 0.63. ^{1}H-NMR (CD_3OD) δ: 7.25—7.12 (7H, m), 6.94 (2H, d, *J*=7.0 Hz), 5.25 (0.5H, t, *J*=6.4 Hz), 5.04 (0.5H, t, *J*=6.4 Hz), 4.57 (0.5H, t, *J*=8.4 Hz), 4.51—4.40 (1.5H, m), 3.74 (1H, m), 3.47—3.22 (2H, m), 3.06 (2H, m), 2.95 (1H, s), 2.93 (1H, s), 2.90 (3H, s), 2.87—2.77 (3H, m), 2.57 (2H, s), 2.54 (2H, s), 2.44—2.33 (4H, m), 2.20 (3H, s), 2.06—1.70 (3H, m), 1.30 (6H, d, *J*=7.0 Hz). FAB-MS *m*/*z*: 672 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COHep)-D-MetO-Phe-MeβAla-OH (7e) Compound **6e** (1.70 g, 2.02 mmol) was treated according to method H, yielding **7e** (82 mg, 5.6%) as a white powder. HPLC t_R (min): 13.94. Rf_4 0.65. ^{1}H-NMR (CD_3OD) δ: 7.26 (7H, m), 7.04 (2H, m), 5.25 (0.5H, t, *J*=6.4 Hz), 5.01 (0.5H, m), 4.56 (0.5H, m), 4.46 (1H, m), 4.41 (0.5H, m), 3.85—3.65 (1H, m), 3.25 (1H, m), 3.04 (2H, m), 2.93—2.89 (4H, m), 2.56—2.53 (5H, m), 2.37 (4H, m), 2.20 (3H, s), 2.10—2.80 (2H, m), 1.72 (2H, m), 1.33 (8H, m), 0.92 (3H, t, *J*=6.8 Hz). FAB-MS *m*/*z*: 728 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COBu^t)-D-MetO-Phe-MeβAla-OH (7f) Compound **6f** (1.10 g, 1.42 mmol) was treated according to method H, yielding **7f** (330 mg, 34%) as a white powder. HPLC t_R (min): 13.77. Rf_4 0.65. ^{1}H-NMR (CD_3OD) δ: 7.25 (7H, m), 7.04 (2H, d, *J*=7.2 Hz), 5.25 (0.5 H, m), 5.02 (0.5H, m), 4.56 (0.5H, m), 4.45 (1H, m), 4.40 (0.5H, m), 3.70 (1H, m), 3.42 (1H, m), 3.25 (1H, m), 2.94 (1H, m), 2.88 (2H, m), 2.54 (3H, m), 2.39 (4h, m), 2.19 (3H, s), 2.10—1.80 (2H, m), 1.41 (9H, s). FAB-MS *m*/*z*: 686 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(CO(1-Ad))-D-MetO-Phe-MeβAla-OH (7g) Compound **6g** (1.60 g, 1.82 mmol) was treated according to method H, yielding **7g** (487 mg, 35%) as a white powder. HPLC t_R (min): 14.31. Rf_4 0.63. ^{1}H-NMR (CD_3OD) δ: 7.24—7.08 (7H, m), 6.90 (2H, m), 5.13 (0.5H, m), 4.93 (0.5H, m), 4.44 (0.5H, m), 4.35 (1H, m), 4.30 (0.5H, m), 3.60 (1H, m), 3.32 (1H, m), 3.13 (1H, m), 2.95 (2H, m), 2.86—2.76 (4H, m), 2.46 (1.5H, s), 2.43 (1.5H, s), 2.36—2.17 (4H, m), 2.10 (3H, m), 1.95 (8H, br s), 1.87—1.76 (2H, m), 1.71 (8H, m). FAB-MS *m*/*z*: 764 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COPh)-D-MetO-Phe-MeβAla-OH (7h) Compound **6h** (2.00 g, 2.44 mmol) was treated according to method H, yielding **7h** (1.01 g, 59%) as a white powder. HPLC t_R (min): 12.11. Rf_4 0.60. ^{1}H-NMR (CD_3OD) δ: 8.05 (2H, m), 7.59 (1H, t, *J*=7.4 Hz), 7.45 (2H, t, *J*=7.5 Hz), 7.28 (2H, m), 7.16 (7H, m), 5.14 (0.5H, t, *J*=7.3 Hz), 4.92 (0.5H, m), 4.53 (0.5H, m), 4.45 (1H, m), 4.37 (1H, m), 3.57 (1H, m), 3.34 (1H, m), 3.19 (1H, m), 2.95 (2H, m), 2.84—2.75 (4H, ms), 2.47 (2H, s), 2.44 (1H, s), 2.30 (4H, m), 2.11 (3H, m), 2.00—1.60 (2H, m). FAB-MS *m*/*z*: 706 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr($COCH_2CH_2Ph$)-D-MetO-Phe-MeβAla-OH (7i) Compound **6i** (2.00 g, 2.36 mmol) was treated according to method H, yielding **7i** (778 mg, 45%) as a white powder. HPLC t_R (min): 12.67. Rf_4 0.59. ^{1}H-NMR (CD_3OD) δ: 7.28 (12H, m), 6.95 (2H, d, *J*=8.4 Hz), 5.23 (0.5H, t, *J*=6.4 Hz), 5.02 (0.5H, m), 4.53 (0.5H, m), 4.46 (1H, m), 4.39 (0.5H, m), 3.71 (1H, m), 3.41 (1H, m), 3.23 (1H, m), 3.07—3.00 (4H, m), 2.96—2.84 (6H, m), 2.60—2.25 (7H, m), 1H), 2.18 (3H, s), 2.10—1.60 (2H, m). FAB-MS *m*/*z*: 734 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr($COCH_2CH_2CH_2Ph$)-D-MetO-Phe-MeβAla-OH (7j) Compound **6j** (3.00 g, 3.48 mmol) was treated according to method H, yielding **7j** (177 mg, 6.8%) as a white powder. HPLC t_R (min): 13.91. Rf_4 0.65. ^{1}H-NMR (CD_3OD) δ: 7.26 (12H, m), 7.03 (2H, d, *J*=7.5 Hz), 5.24 (0.5H, t, *J*=6.4 Hz), 5.01 (0.5H, m), 4.56 (0.5H, m), 4.48 (1H, m), 4.38 (0.5H, m), 3.73 (1H, m), 3.35 (1H, m), 3.23 (1H, m), 3.04 (2H, m), 2.96—2.88 (4H, m), 2.72 (2H, t, *J*=7.5 Hz), 2.40 (4H, m), 2.19 (3H, s), 2.02 (2H, m), 1.91—1.60 (2H, m). FAB-MS *m*/*z*: 748 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(COcHex)-D-MetO-Phe-MeβAla-OH (7k) Compound **6k** (2.00 g, 2.42 mmol) was treated according to method H, yielding **7k** (430 mg, 25%) as a white powder. HPLC t_R (min): 14.38. Rf_4 0.65. ^{1}H-NMR (CD_3OD) δ: 7.25 (7H, m), 7.02 (2H, d, *J*=7.8 Hz), 5.24 (0.5H, m), 5.01 (0.5H, m), 4.57 (0.5H, m), 4.47 (1H, m), 4.38 (0.5H, m), 3.73 (1H, m), 3.36 (1H, m), 3.24 (1H, m), 3.04 (2H, m), 2.92—2.89 (4H, m), 2.62—2.52 (5H, m), 2.37 (4H, m), 2.19 (3H, s), 2.10—1.20 (13H, m). FAB-MS *m*/*z*: 712 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(CO_2CH_3)-D-MetO(RS)-Phe-MeβAla-OH (7l) Compound **6l** (2.00 g, 2.58 mmol) was treated according to method H, yielding **7l** (800 mg, 47%) as a white powder. HPLC t_R (min): 8.67. Rf_4 0.60. ^{1}H-NMR (CD_3OD) δ: 7.30—7.10 (7H, m), 7.04 (2H, dd, *J*=8.6, 1.7 Hz), 5.15 (0.5H, t, *J*=6.4 Hz), 4.93 (0.5H, m), 4.46 (0.5H, m), 4.38 (1H, m), 4.31 (0.5H, m), 3.76 (3H, s), 3.63 (1H, m), 3.30 (1H, m), 3.20 (1H, m), 2.93 (2H, m), 2.84—2.79 (4H, m), 2.46 (1.5H, s), 2.79 (1.5H, s), 2.28 (4H, m), 2.09 (3H, s), 2.00—1.55 (2H, m). FAB-MS *m*/*z*: 660 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(CO_2Et)-D-MetO-Phe-MeβAla-OH (7m) Compound **6m** (2.00 g, 2.54 mmol) was treated according to method H, yielding **7m** (700 mg, 41%) as a white powder. HPLC t_R (min): 9.46. Rf_4 0.63. ^{1}H-NMR (CD_3OD) δ: 7.25—7.02 (9H, m), 7.04 (2H, dd, *J*=8.6, 1.7 Hz), 5.13 (0.5H, m), 4.92 (0.5H, m), 4.45—4.28 (1.5H, m), 4.17 (2H, q, *J*=7.2 Hz), 3.91 (0.5H, m), 3.54 (1H, m), 3.36 (1H, m), 3.14 (1H, m), 3.20 (1H, m), 2.97—2.89 (3H, m), 2.86—2.74 (4H, m), 2.56—2.39 (4H, m), 2.36—2.17 (4H, m), 2.09 (3H, s), 2.00—1.55 (2H, m), 1.24 (3H, t, *J*=7.2 Hz). FAB-MS *m*/*z*: 674 $(M+H)^+$.

N$^\alpha$-1-Iminoethyl-Tyr(CO_2Bu^i)-D-MetO-Phe-MeβAla-OH (7n) Compound **6n** (1.80 g, 2.20 mmol) was treated according to method H, yielding **7n** (588 mg, 38%) as a white powder. HPLC t_R (min): 10.12. Rf_4 0.66. ^{1}H-NMR (CD_3OD) δ: 7.16 (7H, m), 6.94 (2H, d, *J*=7.0 Hz), 5.14 (0.5H, m), 4.92 (0.5H, m), 4.46 (0.5H, m), 4.38 (1H, m), 4.32 (0.5H, m), 3.63 (1H, m), 3.30 (1H, m), 3.15 (1H, m), 2.95 (2H, m), 2.95—2.65 (5H, m), 2.46 (1.5H, s), 2.43 (1.5H, s), 2.28 (4H, m), 2.09 (3H, s), 1.87 (1H, m), 1.74 (2H, m), 1.60 (1H, m), 1.19 (6H, d, *J*=6.9 Hz). FAB-MS *m*/*z*: 702 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(CO_2Ph)-D-MetO-Phe-MeβAla-OH (7o) Compound **6o** (2.00 g, 2.39 mmol) was treated according to method H, yielding **7o** (483 mg, 28%) as a white powder. HPLC t_R (min): 11.08. Rf_4 0.63. ^{1}H-NMR (CD_3OD) δ: 7.30—7.04 (9H, m), 6.80—6.70 (5H, m), 5.19 (0.5H, m), 5.04 (0.5H, m), 4.53—4.27 (1.5H, m), 3.98 (0.5H, m), 3.63—3.39 (2H, m), 3.15—2.94 (3H, m), 2.92—2.82 (4H, m), 2.63—2.50 (4H, m), 2.38 (3H, m), 2.17 (3H, m), 1.90 (1H, m), 1.75 (1H, m). FAB-MS *m/z*: 722 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(CO_2Bzl)-D-MetO-Phe-MeβAla-OH (7p) Compound **6p** (2.00 g, 2.35 mmol) was treated according to method H, yielding **7p** (415 mg, 24%) as a white powder.HPLC t_R (min): 11.59. Rf_4 0.64. ^{1}H-NMR (CD_3OD) δ: 7.32—7.02 (14H, m), 6.94 (2H, d, J=7.0 Hz), 5.15 (2H, s), 5.06 (0.5H, m), 4.93 (0.5H, m), 4.38—4.25 (2H, m), 3.55 (1H, m), 3.36 (1H, m), 3.15 (1H, m), 2.99—2.91 (3H, m), 2.85—2.73 (4H, m), 2.54—2.36 (4H, m), 2.29 (3H, m), 2.20 (1H, m), 2.09 (3H, s), 1.81 (1H, m), 1.66 (1H, m). FAB-MS *m/z*: 736 $(M+H)^+$.

Boc-Tyr(COMe)-D-MetO-Phe-MeβAla-O(Oct) (8a) As described in method F, compound **3g** (2.32 g, 3.0 mmol) was reacted with acetyl chloride (260 mg, 3.30 mmol) to yield **8a** (2.20 g, 90%) as a white powder. Rf_3 0.67. ^{1}H-NMR ($CDCl_3$) δ: 7.74—7.52 (1H, m), 7.22 (7H, m), 7.01 (2H, dd, J=8.4, 1.8 Hz), 5.58—5.31 (1H, m), 4.99 (1H, m), 4.58 (1H, m), 4.29 (1H, m), 4.04 (2H, t, J=6.8 Hz), 3.68 (1H, m), 3.40 (1H, m), 3.08 (1H, m), 2.99 (3H, m), 2.87 (1H, s), 2.78 (2H, s), 2.65—2.30 (7H, m), 2.27 (3H, s), 2.05 (2H, m), 1.61 (2H, t, J=7.2 Hz), 1.40 (9H, s), 1.29 (10H, m), 0.88 (3H, m). FAB-MS *m/z*: 815 $(M+H)^+$.

Boc-Tyr(COMe)-D-MetO-Phe-MeβAla-OHde (8b) As described in method F, compound **3k** (2.66 g, 3.0 mmol) was reacted with acetyl chloride (260 mg, 3.3 mmol) to yield **8b** (2.31 g, 83%) as a white powder. Rf_3 0.69. ^{1}H-NMR ($CDCl_3$) δ: 7.72—7.60 (1H, m), 7.22 (7H, m,), 7.00 (2H, dd, J=8.4, 1.8 Hz), 5.55—5.30 (1H, m), 4.97 (1H, m), 4.80 (1H, m), 4.30 (1H, m), 4.03 (2H, t, J=6.8 Hz), 3.66 (1H, m), 3.42 (1H, m), 3.06 (1H, m), 2.98 (3H, m), 2.88 (1H, s), 2.78 (2H, s), 2.65—2.30 (7H, m), 2.27 (3H, s), 2.05 (2H, m), 1.61 (2H, t, J=7.2 Hz), 1.40 (9H, s), 1.29 (26H, m), 0.88 (3H, m). FAB-MS *m/z*: 928 $(M+H)^+$.

Boc-Tyr(COMe)-D-MetO-Phe-MeβAla-OCH(Me)OCOOEt (8c) As described in method F, compound **3t** (2.33 g, 3.0 mmol) was reacted with acetyl chloride (260 mg, 3.3 mmol) to yield **8c** (2.09 g, 85%) as a white powder. Rf_3 0.66. ^{1}H-NMR ($CDCl_3$) δ: 7.74—7.52 (1H, m), 7.22 (7H, m), 7.01 (2H, dd, J=8.4, 1.8 Hz), 6.77 (1H, q, J=5.4 Hz), 5.60—5.35 (1H, m), 5.00 (1H, m), 4.55 (1H, m), 4.27 (1H, m), 4.22 (2H, q, J=7.1 Hz), 3.68 (1H, m), 3.40 (1H, m), 3.08 (1H, m), 2.99 (3H, m), 2.87 (1H, s), 2.78 (2H, s), 2.65—2.30 (7H, m), 2.27 (3H, s), 2.05 (2H, m), 1.52 (3H, d, J=5.7 Hz), 1.40 (9H, s), 1.32 (3H, t, J=7.2 Hz). FAB-MS *m/z*: 819 $(M+H)^+$.

Boc-Tyr(COMe)-D-MetO-Phe-MeβAla-O(Phthalidyl) (8d) As described in method F, compound **3u** (2.38 g, 3.0 mmol) was reacted with acetyl chloride (260 mg, 3.3 mmol) to yield **8d** (2.08 g, 83%) as a white powder. Rf_3 0.67. ^{1}H-NMR ($CDCl_3$) δ: 7.95—7.65 (4H, m), 7.43 (2H, d, J=6.6 Hz), 7.27 (7H, m), 7.05 (2H, m), 5.58—5.30 (1H, m), 4.99 (1H, m), 4.58 (1H, m), 4.29 (1H, m), 3.68 (1H, m), 3.40 (1H, m), 3.08 (1H, m), 2.99 (3H, m), 2.87 (1H, s), 2.78 (2H, s), 2.65—2.30 (7H, m), 2.27 (3H, s), 2.05 (2H, m), 1.85 (1H, m), 1.40 (9H, s). FAB-MS *m/z*: 835 $(M+H)^+$.

Boc-Tyr(CO_2Et)-D-MetO-Phe-MeβAla-O(Oct) (8e) As described in method F, compound **3g** (2.32 g, 3.0 mmol) was reacted with Ethyl chloroformate (357 mg, 3.3 mmol) to give **8e** (2.00 g, 79%) as a white powder. Rf_3 0.67. ^{1}H-NMR ($CDCl_3$) δ: 7.92—7.70 (1H, m), 7.30—7.18 (7H, m), 7.09 (2H, dd, J=8.4, 2.4 Hz), 5.78—5.45 (1H, m), 5.05 (1H, m), 4.66 (1H, m), 4.33 (1H, m), 4.29 (2H, q, J=7.2 Hz), 4.00 (2H, m), 3.72 (0.67H, m), 3.52 (0.33H, m), 3.38 (1H, m), 3.10 (2H, m), 2.97 (2H, m), 2.88 (1H, s), 2.80 (2H, s), 2.60—2.21 (8H, m), 2.01 (2H, m), 1.61 (2H, t, J=5.4 Hz), 1.40 (9H, s), 1.38 (3H, t, J=7.2 Hz), 1.28 (10H, m), 0.88 (3H, m). FAB-MS *m/z*: 845 $(M+H)^+$.

Boc-Tyr(CO_2Bu^i)-D-MetO-Phe-MeβAla-O(Bu) (8f) As described in method F, compound **3c** (2.15 g, 3.0 mmol) was reacted with Isobutyl chloroformate (451 mg, 3.30 mmol) to give **8f** (1.84 g, 75%) as a white powder. Rf_3 0.74. ^{1}H-NMR ($CDCl_3$) δ: 7.83—7.63 (1H, m), 7.30—7.17 (7H, m), 7.09 (2H, dd, J=8.4, 2.4 Hz), 5.69—5.38 (1H, m), 5.03 (1H, m), 4.66 (1H, m), 4.30 (1H, m), 4.08 (2H, t, J=6.6 Hz), 3.71 (0.67H, m), 3.49 (0.33H, m), 3.40 (1H, m), 3.09 (2H, m), 2.99 (2H, m), 2.87 (1H, s), 2.79 (2H, s), 2.60—2.21 (8H, m), 2.04 (4H, m), 1.62 (2H, m), 1.40 (9H, s), 1.35 (2H, m), 0.95 (3H, t, J=7.4 Hz). FAB-MS *m/z*: 817 $(M+H)^+$.

Boc-Tyr(CO_2Bu^i)-D-MetO-Phe-MeβAla-O(Oct) (8g) As described in method F, compound **3g** (2.32 g, 3.0 mmol) was reacted with Isobutyl chloroformate (450 mg, 3.3 mmol) to give **8g** (1.89 g, 72%) as a white powder. Rf_3 0.76. ^{1}H-NMR ($CDCl_3$) δ: 7.83—7.63 (1H, m), 7.30—7.17 (7H, m), 7.09 (2H, dd, J=8.4, 2.4 Hz), 5.69—5.38 (1H, m), 5.03 (1H, m), 4.66 (1H, m), 4.30 (1H, m), 4.02 (4H, m), 3.71 (0.67H, m), 3.49 (0.33H, m), 3.40 (1H, m), 3.09 (2H, m), 2.99 (2H, m), 2.87 (1H, s), 2.79 (2H, s), 2.60—2.21 (8H, m), 2.04 (4H, m), 1.61 (2H, t, J=5.4 Hz), 1.39 (9H, s), 1.28 (10H, m), 0.88 (3H, m). FAB-MS *m/z*: 873 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(COMe)-D-MetO-Phe-MeβAla-O(Oct) (9a) Compound **8a** (2.00 g, 2.45 mmol) was treated according to method H, yielding **9a** (464 mg, 25%) as a white powder. HPLC t_R (min): 15.89. Rf_4 0.70. ^{1}H-NMR (CD_3OD) δ: 7.35—7.20 (7H, m), 7.04 (2H, d, J=8.4 Hz), 5.15 (0.5H, m), 5.03 (0.5H, m), 4.53 (1H, m), 4.41 (1H, m), 4.03 (2H, t, J=6.8 Hz), 3.65 (1H, m), 3.42 (1H, m), 3.22 (1H, m), 3.10—2.97 (3H, m), 2.96—2.83 (4H, m), 2.57 (3H, m), 2.53—2.35 (4H, m), 2.25 (3H, s), 2.19 (3H, m), 1.95 (1H, m), 1.81 (1H, m), 1.62 (2H, m), 1.29 (10H, m), 0.89 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 756 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(COMe)-D-MetO-Phe-MeβAla-O(Hde) (9b) Compound **8b** (2.10 g, 2.26 mmol) was treated according to method H, yielding **9b** (433 mg, 22%) as a white powder. HPLC t_R (min): >20.0. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.32—7.20 (7H, m), 7.06 (2H, d, J=8.4 Hz), 5.14 (0.5H, m), 5.05 (0.5H, m), 4.53 (1H, m), 4.41 (1H, m), 4.05 (2H, t, J=6.8 Hz), 3.66 (1H, m), 3.44 (1H, m), 3.23 (1H, m), 3.10—2.99 (3H, m), 2.96—2.83 (4H, m), 2.57 (3H, m), 2.53—2.35 (4H, m), 2.25 (3H, s), 2.19 (3H, m), 1.96 (1H, m), 1.81 (1H, m), 1.61 (2H, m), 1.31 (26H, m), 0.89 (3H, t, J=6.8 Hz). FAB-MS *m/z*: 869 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(COMe)-D-MetO-Phe-MeβAla-OCH(Me)-OCOOEt (9c) Compound **8c** (2.00 g, 2.44 mmol) was treated according to method H, yielding **9c** (538 mg, 29%) as a white powder. HPLC t_R (min): 11.69. Rf_4 0.69. ^{1}H-NMR (CD_3OD) δ: 7.27 (7H, m), 7.06 (2H, d, J=8.4 Hz), 6.71 (1H, m), 5.05 (1H, m), 4.43 (1H, m), 4.16 (2H, m), 3.66 (1H, m), 3.48 (1H, m), 3.23 (1H, m), 3.10—2.80 (7H, m), 2.70—2.15 (4H, m), 2.55 (3H, s), 2.26 (3H, s), 2.19 (3H, s), 1.96 (1H, m), 1.81 (1H, m), 1.47 (3H, d, J=5.4 Hz), 1.24 (3H, m). FAB-MS *m/z*: 760 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(COMe)-D-MetO-Phe-MeβAla-O(Phthalidyl) (9d) Compound **8d** (2.00 g, 2.40 mmol) was treated according to method H, yielding **9d** (390 mg, 21%) as a white powder. HPLC t_R (min): 11.13. Rf_4 0.67. ^{1}H-NMR (CD_3OD) δ: 7.95—7.65 (4H, m), 7.43 (2H, d, J=6.6 Hz), 7.27 (7H, m), 7.05 (2H, m), 5.23 (0.5H, t, J=6.4 Hz), 5.06 (0.5H, t, J=6.4 Hz), 4.45 (2H, m), 3.80—3.40 (2H, m), 3.23 (1H, m), 3.10—2.80 (6H, m), 2.64 (2H, m), 2.54 (3H, m), 2.41 (2H, m), 2.25 (3H, s), 2.18 (3H, s), 1.97 (1H, m), 1.85 (1H, m). FAB-MS *m/z*: 776 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(CO_2Et)-D-MetO-Phe-MeβAla-O(Oct) (9e) Compound **8e** (1.90 g, 2.25 mmol) was treated according to method H, yielding **9e** (424 g, 24%) as a white powder. HPLC t_R (min): 16.18. Rf_4 0.72. ^{1}H-NMR (CD_3OD) δ: 7.23—7.10 (7H, m), 7.04 (2H, d, J=8.4 Hz), 5.03 (0.5H, m), 4.95 (0.5H, m), 4.39 (1H, m), 4.32 (1H, m), 4.18 (2H, q, J=7.2 Hz), 3.96 (2H, t, J=6.6 Hz), 3.55 (1H, m), 3.35 (1H, m), 3.15 (1H, m), 3.00—2.91 (3H, m), 2.88—2.72 (4H, m), 2.54—2.45 (3H, m), 2.39 (2H, m), 2.32 (2H, m), 2.09 (3H, m), 1.88 (1H, m), 1.72 (1H, m), 1.52 (2H, m), 1.24 (3H, t, J=7.2 Hz), 1.20 (10H, m), 0.80 (3H, t, J=6.9 Hz). FAB-MS *m/z*: 786 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(CO_2Bu^i)-D-MetO-Phe-MeβAla-O(Bu) (9f) Compound **8f** (1.80 g, 2.20 mmol) was treated according to method H, yielding **9f** (317 mg, 19%) as a white powder. HPLC t_R (min): 14.26. Rf_4 0.71. ^{1}H-NMR (CD_3OD) δ: 7.24—7.12 (7H, m), 7.05 (2H, d, J=8.4 Hz), 5.03 (0.5H, m), 4.95 (0.5H, m), 4.38 (1H, m), 4.32 (1H, m), 3.97 (2H, t, J=6.6 Hz), 3.91 (2H, d, J=6.6 Hz), 3.54 (1H, m), 3.37 (1H, m), 3.15 (1H, m), 3.00—2.91 (3H, m), 2.88—2.72 (4H, m), 2.54—2.45 (3H, m), 2.39 (2H, m), 2.32 (2H, m), 2.09 (3H, s), 1.89 (3H, m), 1.72 (1H, m), 1.51 (2H, m), 1.29 (2H, m), 0.89 (6H, d, J=6.6 Hz), 0.85 (3H, t, J=7.5 Hz). FAB-MS *m/z*: 758 $(M+H)^+$.

N^{α}-1-Iminoethyl-Tyr(CO_2Bu^i)-D-MetO-Phe-MeβAla-O(Oct) (9g) Compound **8g** (1.80 g, 2.06 mmol) was treated according to method H, yielding **9g** (436 mg, 26%) as a white powder. HPLC t_R (min): 17.12. Rf_4 0.73. ^{1}H-NMR (CD_3OD) δ: 7.33—7.19 (7H, m), 7.12 (2H, d, J=8.4 Hz), 5.13 (0.5H, m), 5.04 (0.5H, m), 4.53 (1H, m), 4.43 (1H, m), 4.05 (2H, t, J=6.6 Hz), 4.00 (2H, d, J=6.6 Hz), 3.64 (1H, m), 3.44 (1H, m), 3.24 (1H, m), 3.08—3.00 (3H, m), 2.96—2.82 (4H, m), 2.64—2.54 (3H, m), 2.48 (2H, m), 2.43 (2H, m), 2.18 (3H, m), 2.00 (3H, m), 1.81 (1H, m), 1.61 (2H, m), 1.30 (10H, m), 0.98 (6H, d, J=6.6 Hz), 0.89 (3H, t, J=6.0 Hz). FAB-MS *m/z*: 814 $(M+H)^+$.

Antinociceptive Assay Male ddY mice weighing 10—32 g were used. The animals were purchased from Japan SLC Inc. (Shizuoka, Japan) and were housed in cages (5—6 matched for weight) in a colony room. Animals were given standard food (MM-3, Japan SLC Inc.) and tap water *ad libitum*. The air-conditioned room was maintained at 23±2 °C and 55±20% relative humidity, with a standard 12-h light-dark cycle (lights on from 6:00 to 18:00). Antinociceptive activity was examined by the tail pressure test ac-

cording to the method of Sakurada *et al.*[35] with slight modification. In brief, mechanical pressure was applied to the base of the tail at 32 g/s using an automated tail-pressure unit (Ugo Basile, Italy). Biting or struggling behavior of the mice was used as an indication of the response threshold and only mice responding to a tail pressure of 100 to 300 g were selected for this experiment. The trial was terminated at a level of 500 g to prevent tissue damage to the tail. The mean±S.E.M. of the pressure was plotted. To obtain the dose–response curve, the dose was plotted against the percentage of the maximum possible effect (%MPE), which was calculated as follows: $\%MPE=(P_2-P_1/500-P_1)\times100$, where P_1 is the response pressure before drug administration (g) and P_2 is the response pressure after drug administration (g). The peptides were dissolved in saline (Fuso Chemical Industries, Osaka, Japan) for administration and saline alone was used as the control. ED_{50} values were obtained by the method of Litchfield and Wilcoxon[25] to compare the antinociceptive activity of the compounds. The values were calculated from data that were obtained at the time of peak effect after either peptide or morphine administration.

References and Notes

1) Symbols and abbreviations are in accordance with recommendations of IUPAC-IUB Joint Commission on Biochemical Nomenclature: Nomenclature and Symbolism for Amino Acids and Peptides. *Biochem. J.*, **219**, 345—373 (1984). The other abbreviations are as follows: AcOH, acetic acid; Ad, adamantyl; Bu, *n*-butyl; Bu^i, isobutyl; Bu^t, *tert*-butyl; Bzl, benzyl; Boc, *tert*-butyloxycarbonyl; cHex, cyclohexyl; Dec, decyl; Dde, dodecyl; DMF, *N*,*N*-dimethylformamide; EtOAc, ethyl acetate; Hde, hexadecyl; Hep, heptyl; Hex, hexyl; HOBt, 1-hydroxybenzotriazole; MeβAla, *N*-methyl-β-alanine; MetO, methionine (*R*,*S*)-sulfoxide; Non, nonyl; Oct, octyl; Pen, pentyl; Ph, phenyl; Pr, *n*-propyl; Pr^i, isopropyl; TEA, triethylamine; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride.
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