Studies on Analgesic Oligopeptides. VI.[1,2] Further Studies of Synthesis and Biological Properties of Tripeptide Alkylamides, Tyr–D-Arg–Phe–X

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Nine analogs based on a structure of Tyr–D-Arg–Phe–X (X = alkylamides or alkylhydrazide containing electron-withdrawing atoms or groups) were newly synthesized and their biological properties were examined by the opioid receptor binding properties of μ-, δ- and κ-receptors, guinea-pig ileum (GPI) assay and analgesic activity in the tail pinch test after subcutaneous administration in mice. Analogs with X = $NHCH_2CF_3$, Sar-ol, or $NH(CH_2)_2CN$ showed potent activities in the GPI and analgesic assays and high affinity for μ-receptor. An analog with X = taurinamide was found to possess 4-fold higher μ-receptor selectivity than that of [D-Ala², MePhe⁴, Gly-ol⁵]enkephalin (DAGO). The receptor binding properties of previously reported analogs [*Chem. Pharm. Bull.*, 33, 1528 (1985); *ibid.*, 33, 4865 (1985); *ibid.*, 36, 4834 (1988)] were also examined for overall discussion of the structure–activity relationships of this series of tripeptide amides.

Keywords D-Arg²-dermorphin analog; tripeptide alkylamide; μ-receptor selective analog; guinea-pig ileum assy; analgesic activity; opioid receptor binding; electron-withdrawing group

In a previous study on structure–analgesic activity relationships of a structure of Tyr–D-Arg–Phe–X or Tyr–D-Arg–Phe–NH–R, we revealed that the existence of electron-withdrawing oxygen atom(s) and a proper carbon chain length (C2–3) at the alkyl moiety (R) of Tyr–D-Arg–Phe–NH–R are important for potent analgesic activity.[1] To investigate more exactly the structural requirements for biological activity, nine tripeptide alkylamides (or hydrazide) incorporated various electron-withdrawing atoms (oxygen and fluorine), and groups in the alkylamine residue were newly synthesized and their biological properties were examined by the opioid receptor binding affinities for μ-, δ- and κ-receptors, guinea-pig ileum (GPI) assay and analgesic activity by the tail pinch test. In addition, the receptor binding properties of previously reported analogs[1,3] whose biological activities have only been defined on the basis of their analgesic activity were examined for overall discussion of the structure–activity

TABLE I. Analytical Data of Synthetic Intermediates

Compound	mp (°C)	$[\alpha]_D$[a] (°)	TLC[b] *Rf* (A)	Formula	Analysis (%) Found C	Found H	Found N	Calcd C	Calcd H	Calcd N
Boc–Phe–$NHCH_2CF_3$	70—72	+4.2	0.57	$C_{16}H_{21}F_3N_2O_3$	55.22	6.01	8.00	55.49	6.11	8.09
Boc–D-Arg(Tos)–Phe–$NHCH_2CF_3$	64—66	−13.6	0.58	$C_{29}H_{39}F_3N_6O_6S$	53.29	5.80	12.46	53.04	5.99	12.80
Boc–Tyr–D-Arg(Tos)–Phe–$NHCH_2CF_3$	153—155	−2.1	0.70	$C_{38}H_{48}F_3N_7O_8S$	55.42	5.66	11.59	55.68	5.90	11.96
Boc–Phe–$NHCH_2CF_2CF_3$	83—84	+1.7	0.56	$C_{17}H_{21}F_5N_2O_3$	51.82	5.20	7.08	51.52	5.34	7.08
Boc–D-Arg(Tos)–Phe–$NHCH_2CF_2CF_3$	94—96	−11.3	0.60	$C_{30}H_{39}F_5N_6O_6S$	50.69	5.53	11.52	50.99	5.56	11.89
Boc–Tyr–D-Arg(Tos)–Phe–$NHCH_2CF_2CF_3$	120—124	−5.7	0.71	$C_{39}H_{48}F_5N_7O_8S$	53.79	5.90	10.98	53.85	5.56	11.27
Boc–Phe–$NHNHCH_2CF_3$	159—160	−5.8	0.57	$C_{16}H_{22}F_3N_3O_3$	52.89	5.84	11.48	53.18	6.14	11.63
Boc–D-Arg(Tos)–Phe–$NHNHCH_2CF_3$	166—168	−11.6	0.55	$C_{29}H_{40}F_3N_7O_6S$	51.64	6.06	14.91	51.85	6.00	14.60
Boc–Tyr–D-Arg(Tos)–Phe–$NHNHCH_2CF_3$	196—197	−4.0	0.65	$C_{38}H_{49}F_3N_8O_8S$	54.65	6.21	13.33	54.67	5.92	13.42
Boc–Phe–Tau–NH_2	117—120	−5.0	0.51	$C_{16}H_{25}N_3O_5S$	51.60	7.20	14.22	51.73	6.78	14.31
Boc–D-Arg(Tos)–Phe–Tau–NH_2	95—98	−13.9	0.52	$C_{29}H_{43}N_7O_8S_2$	51.00	6.60	14.05	51.08	6.36	14.38
Boc–Tyr–D-Arg(Tos)–Phe–Tau–NH_2	125—130	−12.9	0.64	$C_{38}H_{52}N_8O_{10}S_2$	53.70	6.35	13.14	54.01	6.20	13.26
H–Phe–Sar-ol·HCl	48—50	+48.2	0.48	$C_{12}H_{18}N_2O_2 \cdot HCl$	48.52	6.98	9.24	48.82	6.83	9.49
Boc–D-Arg(NO_2)–Phe–Sar-ol	107—110	+5.3	0.36	$C_{23}H_{37}N_7O_7$	53.05	7.45	18.54	52.76	7.12	18.73
Boc–Tyr–D-Arg(NO_2)–Phe–Sar-ol	125—129	+18.2	0.55	$C_{32}H_{46}N_8O_9$	56.36	6.99	15.98	55.97	6.75	16.32
Boc–Phe–Asn–OBzl	110—113	−7.7	0.65	$C_{25}H_{31}N_3O_6$	63.89	6.62	9.04	63.95	6.65	8.95
Boc–D-Arg(NO_2)–Phe–Asn–OBzl	130—134	−14.6	0.56	$C_{31}H_{42}N_8O_9$	55.83	6.43	17.00	55.51	6.31	16.71
Boc–Tyr–D-Arg(NO_2)–Phe–Asn–OBzl	156—159	−5.4	0.72	$C_{40}H_{51}N_9O_{11}$	57.45	6.02	15.22	57.61	6.31	16.71
Boc–Phe–$NH(CH_2)_3OCH_3$	83—85	+1.0	0.50	$C_{18}H_{28}N_2O_4$	64.31	8.63	8.54	64.27	8.39	8.33
Boc–D-Arg(NO_2)–Phe–$NH(CH_2)_3OCH_3$	98—102	−10.9	0.39	$C_{24}H_{39}N_7O_7$	53.24	7.05	17.93	53.62	7.31	18.24
Boc–Tyr–D-Arg(NO_2)–Phe–$NH(CH_2)_3OCH_3$	107—111	+6.1	0.52	$C_{33}H_{48}N_8O_9$	56.33	6.68	15.61	56.56	6.90	15.99
Boc–Phe–$NH(CH_2)_4OH$	92—93	+3.7	0.50	$C_{18}H_{28}N_2O_4$	64.33	8.58	8.54	64.27	8.39	8.33
Boc–D-Arg(NO_2)–Phe–$NH(CH_2)_4OH$	63—65	−8.7	0.61	$C_{24}H_{39}N_7O_7$	53.28	7.03	17.89	53.62	7.31	18.24
Boc–Tyr–D-Arg(NO_2)–Phe–$NH(CH_2)_4OH$	108—114	+7.5	0.75	$C_{33}H_{48}N_8O_9$	56.60	7.03	15.64	56.56	6.90	15.99
Boc–Phe–$NH(CH_2)_2CN$	84—86	−1.0	0.55	$C_{17}H_{23}N_3O_3$	63.97	7.19	13.39	64.33	7.30	13.24
Boc–D-Arg(Tos)–Phe–$NH(CH_2)_2CN$	90—93	−15.9	0.48	$C_{30}H_{41}N_7O_6S$	57.66	6.42	15.33	57.40	6.58	15.62
Boc–Tyr–D-Arg(Tos)–Phe–$NH(CH_2)_2CN$	119—124	−13.6	0.60	$C_{39}H_{50}N_8O_8S$	59.20	6.75	14.56	59.22	6.37	14.17

a) Optical rotations were measured in DMF ($c = 1$) at 18—20 °C. *b*) After removal of N^a-Boc group by treatment with 4 N HCl/dioxane.

relationships of this series of tripeptides.

The analogs of Tyr–D-Arg–Phe–X with X = $NHCH_2CF_3$ (**1**), $NHCH_2CF_2CF_3$ (**2**), $NHNHCH_2CF_3$ (**3**), Tau-NH_2 (**4**), Sar-ol (**5**), Asn (**6**), $NH(CH_2)_3OCH_3$ (**7**), $NH(CH_2)_4OH$ (**8**) and $NH(CH_2)_2$ CN (**9**) were synthesized by conventional solution method in essentially the same manner as

TABLE II. Analytical Data of Synthetic Analogs

Analog No.	$[\alpha]_D$[a] (°)	TLC[b] *Rf* (A)	*Rf* (B)	Formula	Analysis (%) Found C	H	N	Calcd C	H	N
1	+36.4	0.40	0.78	$C_{26}H_{34}F_3N_7O_4 \cdot 2CH_3COOH \cdot 2H_2O$	49.96	6.50	13.34	49.93	6.42	13.58
2	+34.1	0.57	0.88	$C_{27}H_{34}F_5N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	50.14	6.22	14.06	50.21	5.81	14.13
3	+33.5	0.37	0.73	$C_{26}H_{35}F_3N_8O_4 \cdot 2CH_3COOH \cdot H_2O$	50.28	6.50	15.21	50.13	6.31	15.59
4	+32.4	0.28	0.76	$C_{26}H_{38}N_8O_6S \cdot 3CH_3COOH \cdot 2H_2O$	48.04	6.75	14.09 S: 3.83	47.63	6.75	13.89 S: 3.97
5	+47.0	0.33	0.73	$C_{27}H_{39}N_7O_5 \cdot 2CH_3COOH \cdot 2H_2O$	53.55	7.21	13.80	53.36	7.34	14.05
6	+22.1	0.28	0.77	$C_{28}H_{38}N_8O_7 \cdot 2CH_3COOH \cdot H_2O$	52.02	6.56	14.88	52.17	6.57	15.21
7	+37.2	0.45	0.84	$C_{28}H_{41}N_7O_5 \cdot 2CH_3COOH \cdot H_2O$	55.42	7.20	13.82	55.40	7.41	14.13
8	+35.9	0.39	0.83	$C_{28}H_{41}N_7O_5 \cdot 2CH_3COOH \cdot 2H_2O$	53.96	7.71	13.57	53.99	7.50	13.77
9	+39.7	0.33	0.71	$C_{27}H_{36}N_8O_4 \cdot 2CH_3COOH \cdot 3H_2O$	52.50	6.81	15.44	52.38	7.09	15.76

a) Optical rotations were measured in H_2O ($c = 0.5$) at 24 °C. *b*) See Experimental.

TABLE III. Receptor Binding Assay of New Synthetic Analogs

No.	Tyr–D-Arg–Phe–X X	[^{3}H] DAGO (μ) IC_{50} (nM)	Relative potency[a]	[^{3}H] DADLE (δ) IC_{50} (nM)	Relative potency[b]	IC_{50} (δ) / IC_{50} (μ)	[^{3}H] U-69593 (κ) IC_{50} (nM)
1	$NHCH_2CF_3$	5.4	52.9	63.0	16.7	11.7	7800.0
2	$NHCH_2CF_2CF_3$	21.0	13.3	200.0	5.3	9.5	10000<
3	$NHNHCH_2CF_3$	3.8	73.7	76.0	13.8	20.0	10000<
4	Tau–NH_2	3.7	75.7	320.0	3.3	86.5	10000<
5	Sar-ol	3.1	90.3	54.0	19.4	17.4	10000<
6	Asn	8.4	33.3	280.0	3.8	33.3	10000<
7	$NH(CH_2)_3OCH_3$	17.5	16.0	155.0	6.8	8.9	10000<
8	$NH(CH_2)_4OH$	37.0	7.6	40.0	26.3	1.1	10000<
9	$NH(CH_2)_2CN$	3.4	82.4	51.0	20.6	15.0	10000<
	DAGO	2.8	100	58.0	18.1	20.7	9400.0
	DADLE	45.0	6.2	10.5	100	0.2	8200.0
	U-69593	10000<	—	9000.0	—	—	7.4

a) Relative potencies to DAGO (DAGO = 100) on molar basis. *b*) Relative potencies to DADLE (DADLE = 100) on molar basis.

TABLE IV. Receptor Binding Assay of Previously Reported Analogs[a]

No.	Tyr–D-Arg–Phe–X X	[^{3}H] DAGO (μ) IC_{50} (nM)	Relative potency[b]	[^{3}H] DADLE (δ) IC_{50} (nM)	Relative potency[c]	IC_{50} (δ) / IC_{50} (μ)	[^{3}H] U-69593 (κ) IC_{50} (nM)
10	OH	220.0	1.3	1200.0	0.9	5.5	10000<
11	OCH_2CH_3	115.0	2.4	185.0	5.7	1.6	10000<
12	NH_2	63.0	4.4	650.0	1.6	10.3	9000.0
13	$NHCH_3$[e]	6.4	43.8	32.0	32.8	5.0	10000<
14	$N(CH_3)_2$[e]	9.2	30.4	19.5	53.8	2.1	10000<
15	$NHCH_2CH_3$	6.6	42.4	80.0	13.1	12.1	10000<
16	Gly[e]	5.0	56.0	40.0	26.3	8.0	10000<
17	Gly–OEt[e]	20.0	14.0	120.0	8.8	6.0	10000<
18	Gly–NH_2[e]	1.6	175.0	65.0	16.2	40.6	3100.0
19	Sar[e]	4.4	63.6	60.0	17.5	13.6	10000<
20	βAla[e]	2.4	117.0	33.0	31.8	13.8	10000<
21	Leu	285.0	1.0	730.0	1.4	2.6	10000<
22	Lys–NH_2[d]	18.0	15.6	1100.0	1.0	61.1	10000<
	DAGO	2.8	100	58.0	18	20.7	9400.0
	DADLE	45.0	6	10.5	100	0.2	8200.0
	U-69593	10000<	—	9000.0	—	—	7.4

a) See refs. 1 and 3. *b*) Relative potencies to DAGO (DAGO = 100) on molar basis. *c*) Relative potencies to DADLE (DADLE = 100) on molar basis. *d*) See ref. 6. *e*) Analog showing potent analgesia more than that of morphine after s.c. administration in mice (see refs. 1 and 3).

TABLE V. Opioid Activity on GPI Assay and Analgesic Activity in Tail Pinch Test after s.c. Injection in Mice

No.	Tyr–D-Arg–Phe–X X	GPI[a] (1×10^{-7} M)	Tail pinch test[a] (30 mg/kg, s.c.)
1	$NHCH_2CF_3$	++[b]	+
2	$NHCH_2CF_2CF_3$	+	−
3	$NHNHCH_2CF_3$	+	−
4	Tau–NH_2	+	+
5	Sar-ol	++[c]	++
6	Asn	+	−
7	$NH(CH_2)_3OCH_3$	+	+
8	$NH(CH_2)_4OH$	+	+
9	$NH(CH_2)_2CN$	++[d]	++
20	βAla	++[e]	ND

a) See Experimental. *b*) IC_{50}, $(4.2 \pm 0.8) \times 10^{-8}$ M. *c*) IC_{50}, $(2.7 \pm 0.7) \times 10^{-8}$ M. *d*) IC_{50}, $(3.9 \pm 1.9) \times 10^{-8}$ M. *e*) IC_{50}, $(2.5 \pm 0.7) \times 10^{-8}$ M.

previously reported.[1,3] Intermediates and their analytical data are shown in Table I. Final deprotection was done by catalytic hydrogenolysis or by treatment with a mixture of anhydrous HF and anisole.[4] The deblocked peptides were purified by ion-exchange chromatography on CM cellulose and, if necessary, partition chromatography on Sephadex G-25.[5] Physicochemical data of all synthetic peptides are shown in Table II.

Receptor binding data of newly synthesized and previously reported[1,3] analogs are shown in Tables III and IV, respectively, and the data of the new analogs in the GPI assay and analgesic activity in the tail pinch test after subcutaneous (s. c.) administration in mice are shown in Table V. In the binding assay, all new analogs showed affinity for μ- and δ-receptors and negligible affinity for κ-receptor. One fluorinated analog, **1**, showed high μ-receptor affinity, high potency in the GPI assay and moderate analgesic activity in the tail pinch test. Interestingly, fluorinated analogs **2** and **3** showed μ-receptor affinity and a moderate potency in the GPI assay, but the tail pinch test was negative at a dose of 30 mg/kg, s. c. Analogs **5** and **9** showed high μ-affinity, but their μ-selectivities were relatively low. Tripeptide alkylamides with short alkyl chains, **13**, **14** and **15**, showed high μ-receptor affinity. The prototype tripeptide, **10**, and its derivatives, **12** and **13**, showed very low affinity by more than one order of magnitude. These lines of evidence seem to suggest that the amide bond between Phe^3 and X^4 is of primary importance for high μ- and δ-affinities, but **21** containing the bulky hydrophobic group in X reduces the affinities in proportion to the analgesic activity.[3a] In general, derivatives with an acid amide moiety in X, **4**, **6**, **18** or **22**[6] showed higher μ-selectivity than that of DAGO used widely as a binding assay reagent. Activity of the binding for μ-receptor coincides with the analgesic activity, although there are a few exceptions, **2**, **3** and **6**.[1,3] In the GPI aasay, all new analogs tested possessed the ability to inhibit electrically induced contraction of guinea-pig ileum in a dose dependent manner. Analogs **1**, **5** and **9** showed very potent activity comparable to that of analog **20**.[1] Among the three, analogs **5** and **9** showed very potent and long-lasting analgesia over 3 h at a dose of 30 mg/kg, s. c. (data not shown).

It is of interest that the assay of analgesic activity by intracerebroventricular and intrathecal administration of analogs **13**, **14**, **18** and **20** showed that opioid receptor affinity in the brain and spinal cord of analogs **13** and **14** containing hydrophobic R are similar to morphine,[7] but that analogs **18** and **20** containing hydrophilic R possess higher affinity in the spinal cord.[8]

In conclusion, it was confirmed that tripeptide derivatives, Tyr–D-Arg–Phe–NHR, with electron-withdrawing groups in R showed potent opioid activities. Fluorine-containing analog **1** may be a useful reagent for the study of interaction with the receptor.[9] Analog **4** showing high and selective μ-receptor affinity may be a useful reagent for the receptor binding assay. The property of high affinity for μ-receptor of acid amide containing R may be good information to have in the design of derivatives having high affinity for μ-receptor.

Experimental

Melting points were determined with a Yanaco MP-S3 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 polarimeter. Thin layer chromatography (TLC) was carried out on silica gel plates (Merck, Kiesel gel $60F_{254}$, 5 × 10 cm) with the following solvent systems: *Rf*(A), 1-BuOH–AcOH–H_2O (4 : 1 : 5, upper phase); *Rf*(B), 1-BuOH–AcOH–pyridine–H_2O (15 : 3 : 10 : 12).

Peptide Synthesis All peptides were synthesized by classical solution method in the same manner as reported previously.[1,3] For example, Boc–Phe alkylamides were obtained by DCC-HOBt[10]-mediated coupling of Boc–Phe–OH with the corresponding alkylamines, followed by deprotection with 4 N HCl–dioxane. Subsequently, Boc–D-Arg (NO_2 or Tos)–OH and then Boc–Tyr–OH were coupled to give protected tripeptide alkylamides. Protected tetrapeptides were prepared in this way starting with H–Tau–NH_2[11] (**4**), H–Asn–OBzl[12] (**7**) and H–Gln–OBzl[13] (**8**). Intermediates and their analytical data are shown in Table I. Final deprotection was done by catalytic hydrogenolysis or treatment with HF–anisole (10%) mixture, and the peptides were purified on CM-cellulose and, if necessary, partition chromatography on Sephadex G-25[5] as described earlier.[1,3]

Opiate Receptor Binding Assay Crude synaptosomal fractions were prepared from rat decerebrated whole brain for μ and δ ligand bindings according to the method of Walker *et al.*[14] For κ ligand binding, membrane fraction was analogously prepared from guinea-pig whole brain. Briefly, brains were homogenized in a 10 mM Tris–sucrose buffer (pH 7.4) at 0 °C and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 31000 × *g* for 10 min and the pellets were suspended in a 50 mM Tris–HCl buffer (pH 7.4). After 15 min incubation at 25 °C, the suspension was centrifuged at 31000 × *g* for 15 min and the pellets were suspended in Tris buffer to a final volume of 18 ml/g of the brain (2 mg protein/ml).

[^{3}H]DAGO, [^{3}H] DADLE and [^{3}H]U-69593 were respectively used for determining relative affinities for μ-, δ- and κ-receptors. Binding assays were carried out by incubating an aliquot of the membrane fraction (600 μg of protein) in an assay containing 500 μg of bovine serum albumin (BSA), 50 μg of bacitracin, 5 μg of bestatin and 2 nM of each [^{3}H]ligand described above in a final volume of 500 μl (in 50 mM Tris–HCl buffer at pH 7.4). After 120 min at 25 °C, the reaction mixture was filtered over a Whatman GF/B filter soaked with 0.1% polyethyleneimine (μ and κ) or 0.5% BSA (δ) and the filter was washed twice with cold Tris–HCl buffer. Filters were counted in a Whatman liquid scintillation counter after overnight extraction with liquid scintillation fluid (3 ml each). Specific binding is the difference between total binding and that in the presence of excess (1 μM) unlabeled ligand. All assays were carried out in duplicate and the mean of the data was used in data analysis (the standard error of the replicates is approximately 5%). IC_{50} values were determined from log dose–displacement curves.

GPI Assay GPI assay was performed according to the method of Rang.[15] Longitudinal muscle strips were taken from male Hartley guinea-pigs weighing 300—500 g. The tissue was suspended in an organ bath containing Krebs–Henseleit solution that was continuously bubbled with 95% O_2–5% CO_2 at 37 °C. The tissue was then transmurally stimulated (0.1 Hz, 1 ms, 10 V). All drugs were dissolved in dist. H_2O. Atropine sulfate (3×10^{-7} M) was used as standard of 100% inhibition. The inhibitory effect of drugs on the electrically evoked contraction was scored as ++, 40—60

inhibition and +, less than 40% inhibition of the contractile response at a dose of 1×10^{-7} M. The IC_{50} of analogs (**1**, **5**, **9** and X=βAla as a reference sample) which showed high potency (++) was determined from a dose–response curve.

Analgesic Assay Analgesic activity was examined by the tail pinch test reported by Takagi *et al.*,[16] using male ddY strain mice (20—25 g). Mice were injected s.c. with a drug dissolved in saline at a dose of 30 mg/kg. Six or 8 mice were used for evaluation of each drug and the analgesic potency was scored as ++, 100% of animals used showed complete analgesia; +, more than 50% of animals used showed complete analgesia; −: partial analgesia or normal response. When the ratio of animals showing complete analgesia was less than 50%, the drugs were ranked as −.

References and Notes

1) Part V: K. Suzuki, H. Fujita, Y. Sasaki, M. Shiratori, S. Sakurada and K. Kisara, *Chem. Pharm. Bull.*, **36**, 4834 (1988).
2) Amino acids and peptides are of L-configuration unless otherwise noted. Abbreviations used are: Boc=*tert*-butoxycarbonyl, Tos=tosyl, NO_2=nitro, Sar-ol=2-methyaminoethanol, Tau-NH_2=taurineamide (2-amino-ethanesulfonamide), βAla=β-alanine, DAGO=[D-Ala^2, MePhe^4, Gly-ol^5]enkephalin, DADLE=[D-Ala^2, D-Leu^5]enkephalin, U-69593=(5,7,8)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide, DCC=dicyclohexylcarbodiimide, HOBt=1-hydroxybenztriazole, CM=carboxymethyl.
3) *a*) Y. Sasaki, M. Matsui, H. Fujita, M. Hosono, M. Tagichi, K. Suzuki, S. Sakurada, T. Sato, T. Sakurada and K. Kisara, *Chem. Pharm. Bull.*, **33**, 1528 (1985); *b*) K. Suzuki, H. Fujita, M. Matsui, Y. Sasaki, S. Sakurada, T. Sakurada and K. Kisara, *ibid.*, **33**, 4865 (1985).
4) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and M. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967).
5) D. Yamashiro, *Nature* (London), **201**, 76 (1963).
6) P. W. Schiller, T. M.-D. Nguyen, N. N. Chung and C. Lemieux, *J. Med. Chem.*, **32**, 698 (1989).
7) K. Chaki, S. Sakurada, T. Sakurada, K. Kisara and K. Suzuki, in preparation.
8) K. Chaki, S. Sakurada, T. Sakurada, T. Sato, S. Kawamura, K. Kisara, H. Watanabe and K. Suzuki, *Pharmacol. Biochem. Behav.*, **31**, 439 (1988).
9) K. Wurthrich, "NMR in Biological Research: Peptides and Proteins," North-Holland Publishing Company, Amsterdam, 1976.
10) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
11) H. McIlwain, *J. Chem. Soc.*, **1941**, 75.
12) G. Amiard and R. Heymes, *Bull. Soc. Chim. Fr.*, **1957**, 1373.
13) L. Zervas and C. Hamalidis, *J. Am. Chem. Soc.*, **87**, 99 (1965).
14) J. M. Walker, W. D. Bowen, S. T. Atkins, M. K. Hemstreet and D. H. Coy, *Peptides*, **8**, 869 (1987).
15) H. P. Rang, *Br. J. Pharmacol.*, **22**, 356 (1964).
16) H. Takagi, T. Inukai and M. Nakama, *Jpn. J. Pharmacol.*, **16**, 287 (1966).