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Studies on Peptides. CL.[1,2] Syntheses of [D-His^2]-Analogs of Enkephalin and Adrenorphin and Several [D-Arg^2]enkephalin Analogs

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[D-His^2]Leu-enkephalin and [D-His^2]adrenorphin were synthesized. In addition, eight [D-Arg^2]enkephalin-related peptides were synthesized. Their inhibitory effects on electrically stimulated myenteric plexus-longitudinal muscle preparations of guinea-pig ileum were examined.

Keywords——[D-His^2]enkephalin; [D-His^2]adrenorphin; [D-Arg^2]enkephalin analog; μ receptor; guinea-pig ileum assay; trifluoromethanesulfonic acid deprotection; methanesulfonic acid deprotection

Replacement of the Gly^2 residue of enkephalin[3] and related peptides by D-amino acids, such as D-Ala,[4] or D-Met,[5] or D-Met(O)[6] or D-Arg,[7,8] is known to bring about considerably higher analgesic activity. We therefore examined the biological activities of eight [D-Arg^2]enkephalin-related peptides, together with those of [D-His^2]-analogs of enkephalin and adrenorphin,[9] possessing different basicity from that of [D-Arg^2]-derivatives.

A [D-His^2]-analog of Leu-enkephalin (**1**) was synthesized by successive azide (Az) condensations[10] of Z(OMe)–D-His–$NHNH_2$ and Z(OMe)–Tyr–$NHNH_2$ with a TFA-treated sample of Z(OMe)–Gly–Phe–Leu–OBzl,[11] followed by removal of the two protecting groups from the resulting protected pentapeptide, *i.e.*, the Bzl group by catalytic hydrogenolysis, then the Z(OMe) group readily by TFA treatment. The deprotected peptide was purified by partition chromatography[12] on Sephadex G-10, followed by high-performance liquid chromatography (HPLC).

[D-His^2]adrenorphin (**2**) was similarly prepared by succesive Az condensations of Z(OMe)–D-His–$NHNH_2$ and Z(OMe)–Tyr–$NHNH_2$ with a TFA-treated sample of Z(OMe)–Gly–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH_2, followed by deprotection of the resulting protected octapeptide with 1 M TFMSA–thioanisole/TFA.[13] The above amino component was obtained by the Su condensation[14] of Z(OMe)–Gly–OH with a TFA-treated sample of Z(OMe)–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH_2, an intermediate of our previous synthesis of adrenorphin.[11] After incubation with dithiothreitol, the desired peptide was purified by partition chromatography on Sephadex G-10, followed by HPLC.

Next, we prepared five [D-Arg^2]enkephalin-related peptides, **3** to **7**. The C-terminal end of [D-Arg^2]Leu-enkephalin (in one instance, Met-enkephalin) was extended by Arg, Arg–Arg, Arg–Arg–Ile, and Arg–Arg–Ile–Arg, respectively, to examine the effects due to such basic extensions. These compounds can be classified as analogs of kyotorphin[15] or adrenorphin or the N-terminal portion of dynorphin.[16]

H–Tyr–D-Arg–Gly–Phe–Leu–Arg–OH (**3**) was prepared starting from H–Arg(NO_2)–OBzl. Z(OMe)–Leu–Arg(NO_2)–OBzl was prepared by the PCP procedure[17] and this, after TFA treatment, was condensed with a newly prepared dipeptide hydrazide, Boc–Gly–Phe–$NHNH_2$, *via* the Az to give Boc–Gly–Phe–Leu–Arg(NO_2)–OBzl. This tetrapeptide chain was elongated by two successive condensations of Z(OMe)–D-Arg(NO_2)–OH *via* the mixed anhydride (MA)[18] and Z(OMe)–Tyr(Bzl)–OH *via* the TCP ester[19] to give Z(OMe)–Tyr(Bzl)–D-Arg(NO_2)–Gly–Phe–Leu–Arg(NO_2)–OBzl, from which all protecting groups were removed by catalytic hydrogenolysis. The product (**3**) was purified by ion-exchange chromatography on CM cellulose, followed by gel-filtration on Sephadex G-10, as was done with other analogs.

Next, considering convenient procedures for the syntheses of analogous compounds, we decided to construct the common sequence, Tyr–D-Arg–Gly–Phe, by the Az condensations of two units, Boc–Gly–Phe–$NHNH_2$ and Z–Tyr–D-Arg(Mts)–$NHNH_2$. Only C-terminal portions were prepared for each analog. Thus, H–Tyr–D-Arg–Gly–Phe–Met–Arg–OH (**4**) was prepared starting from Z(OMe)–Met–Arg(Mts)–OH obtained by the TCP procedure. Z–Tyr–D-Arg(Mts)–Gly–Phe–Met–Arg(Mts)–OH thus obtained was treated with MSA[20] to afford **4**.

For the preparation of H–Tyr–D-Arg–Gly–Phe–Leu–Arg–Arg–OH (**5**), Z(OMe)–Leu–Arg(Mts)–$NHNH_2$, obtained by PCP condensation of Z(OMe)–Leu–OH and H-Arg(Mts)–OMe, followed by the usual hydrazine treatment, was condensed with H–Arg(Mts)–OH to give Z(OMe)–Leu–Arg(Mts)–Arg(Mts)–OH. Chain elongation of this tripeptide was carried out as described above, then all protecting groups employed were cleaved by 1 M TFMSA–thioanisole in TFA to give **5**. Next, Z(OMe)–Leu–Arg(Mts)–$NHNH_2$ obtained above was used to prepare the C-terminal portion of H–Tyr–D-Arg–Gly–Phe–Leu–Arg–Arg–Ile–OH (**6**). The necessary C-terminal tetrapeptide unit was obtained by Az condensation of the above hydrazide with a TFA-treated sample of Z(OMe)–Arg(Mts)–Ile–OBzl. Subsequent chain elongation and deprotection were carried out as described above to give **6**. Next, Z(OMe)–Arg(Mts)–Ile–OBzl obtained above was used to prepare H–Tyr–D-Arg–Gly–Phe–Leu–Arg–Arg–Ile–Arg–OH (**7**). This dipeptide ester was converted to the corresponding hydrazide, then condensed with H–Arg(Mts)–OH to give Z(OMe)–Arg(Mts)–Ile–Arg(Mts)–OH. The rest of the reactions were performed as described above to give **7**.

Next, three analogs of [D-Arg^2]Leu-enkephalin, H–Tyr–D-Arg–Gly–Phe–Leu–R [R = D-Arg–OH (**8**), Arg-ol (**9**), and Arg–NH_2 (**10**)], were prepared. For these syntheses, an available tripeptide, Boc–Gly–Phe–Leu–OH,[8] was converted to the corresponding hydrazide *via* the methyl ester. Z(OMe)–Gly–Phe–Leu–$NHNH_2$ thus obtained was used to prepare the respective C-terminal portions of these analogs. For the preparation of compound (**8**), this tripeptide hydrazide was condensed with H–D-Arg(Mts)–OH and the resulting protected tetrapeptide, after TFA treatment, was condensed with Z–Tyr–D-Arg(Mts)–$NHNH_2$. All protecting groups were cleaved from the resulting hexapeptide by 1 M TFMSA–thioanisole in TFA and the deprotected peptide was purified by ion-exchange chromatography on CM-cellulose, followed by gel-filtration on Sephadex G-10 as stated above. Using H–Arg(Mts)–NH_2, compound (**10**) was similarly prepared. For the preparation of compound (**9**), Boc–Gly–Phe–Leu–Arg(Mts)–ol was obtained by reduction of Boc–Gly–Phe–Leu–Arg(Mts)–OMe with $NaBH_4$.[21] Subsequent chain elongation and deprotection were carried out as described above.

In the present investigations, electrically stimulated myenteric plexus-longitudinal muscle preparations of guinea-pig ileum,[22] a typical μ receptor preparation,[23] were used to test the biological activities of synthetic peptides. The volume of bath fluid was 1.5 ml. In each preparation, the inhibitory effects of Met–enkephalin and a synthetic peptide were examined in turn, and the relative potency was calculated as shown in Table I. In this assay system, only

TABLE I. Relative Potencies of Inhibitory Effects of Synthetic Peptides on Guinea-Pig Ileum

		Relative potencies
1	H–Tyr–D-His–Gly–Phe–Leu–OH	0.17
2	H–Tyr–D-His–Gly–Phe–Met–Arg–Arg–Val–NH_2	2.04
3	H–Tyr–D-Arg–Gly–Phe–Leu–Arg–OH	3.30
4	H–Tyr–D-Arg–Gly–Phe–Met–Arg–OH	0.50
5	H–Tyr–D-Arg–Gly–Phe–Leu–Arg–Arg–OH	0.38
6	H–Tyr–D-Arg–Gly–Phe–Leu–Arg–Arg–Ile–OH	1.00
7	H–Tyr–D-Arg–Gly–Phe–Leu–Arg–Arg–Ile–Arg–OH	0.24
8	H–Tyr–D-Arg–Gly–Phe–Leu–D-Arg–OH	0.36
9	H–Tyr–D-Arg–Gly–Phe–Leu–Arg–ol	0.07
10	H–Tyr–D-Arg–Gly–Phe–Leu–Arg–NH_2	0.14
	H–Tyr–Gly–Gly–Phe–Leu–OH (Leu-enkephalin)	0.50
	H–Tyr–Gly–Gly–Phe–Met–OH (Met-enkephalin)	1.00

[D-His^2]adrenorphin and the analog **3** exhibited higher activities than Leu-enkephalin, but no enhancement of biological activity was observed in the other compounds. Further accumulation of experimental results seems necessary for a better understanding of the subtle relationship between opioid receptors and enkephalin.

Experimental

General experimental methods employed in this investigation are essentially the same as described in our previous synthesis of [D-Arg^2]enkephalin.[7] *Rf* values in thin layer chromatography (TLC), performed on silica gel (Kieselgel G, Merck), refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8 : 3 : 1), Rf_2 *n*-BuOH–AcOH–AcOEt–H_2O (1 : 1 : 1 : 1), and Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (4 : 1 : 1 : 2). HPLC was conducted with a Waters 204 compact model equipped with a Nucleosil 5C18 column (4 × 150 mm).

Synthesis of [D-His^2]Leu-Enkephalin

Z(OMe)-D-His-Gly-Phe-Leu-OBzl——The Az [prepared from 2.36 g (7.0 mmol) of Z(OMe)–D-His–$NHNH_2$] in DMF (25 ml) and Et_3N (1.82 ml, 13.0 mmol) were added to an ice-chilled solution of a TFA-treated sample of Z(OMe)–Gly–Phe–Leu–OBzl (3.50 g, 6.0 mmol) in DMF (30 ml), then the mixture was stirred at 4 °C overnight and the solvent was removed by evaporation. The residue was treated with AcOEt and the resulting powder was recrystallized from MeOH and ether; yield 2.30 g (54%), mp 100—105 °C, $[\alpha]_D^{15}$ −19.6° (c = 1.0, MeOH), Rf_1 0.74. *Anal.* Calcd for $C_{39}H_{46}N_6O_8 \cdot 2H_2O$: C, 61.40; H, 6.61; N, 11.02. Found: C, 61.63; H, 6.33; N, 11.44.

Z(OMe)-Tyr-D-His-Gly-Phe-Leu-OBzl——The Az [prepared from 0.82 g (2.3 mmol) of Z(OMe)–Tyr–$NHNH_2$] in DMF (5 ml) and Et_3N (0.59 ml, 4.2 mmol) were added to a TFA-treated sample of the above protected tetrapeptide (1.36 g, 1.9 mmol) in DMF (10 ml), then the mixture was stirred at 4 °C overnight and the solvent was removed by evaporation. The residue was treated with AcOEt and the resulting powder was recrystallized from MeOH and ether; yield 0.91 g (55%), mp 114—118 °C, $[\alpha]_D^{15}$ −16.1° (c = 1.0, MeOH), Rf_1 0.76. *Anal.* Calcd for $C_{48}H_{55}N_7O_{10} \cdot H_2O$: C, 63.49; H, 6.33; N, 10.80. Found: C, 63.55; H, 6.30; N, 11.16.

H-Tyr-D-His-Gly-Phe-Leu-OH (1)——The above protected pentapeptide (250 mg, 0.28 mmol) in MeOH (20 ml) was hydrogenated over a Pd catalyst for 5 h, then the catalyst was removed by filtration. The filtrate was concentrated and dry ether was added. The resulting powder was next treated with TFA (1.0 ml) in the presence of thioanisole (0.11 ml) in an ice-bath for 60 min. Dry ether was added and the resulting power was purified by partition chromatography on Sephadex G-10 (3.0 × 100 cm), equilibrated with the lower phase of *n*-BuOH–AcOH–H_2O (4 : 1 : 5). The column was eluted with the upper phase of the above solvent. The desired fractions (10 ml each, tube Nos. 40—60, monitored by measuring the ultraviolet (UV) absorption at 275 nm) were combined and the solvent was removed by evaporation. The residue was lyophilized to give a fluffy powder; yield 102 mg (57%). The sample (purity 97%, determined by analytical HPLC) was submitted to bioassay. For characterization, a part of the product (25 mg) was purified by repeated HPLC using isocratic elution with 15% MeCN in 0.1% TFA. The solvent of the desired eluate (retention time, 7.5 min) was evaporated off, and the residue was lyophilized. Yield 18 mg (71%), $[\alpha]_D^{15}$ −2.9° (c = 1.1, MeOH), Rf_1 0.21. Amino acid ratios in a 6 N HCl hydrolysate are listed in Table II, together with those of other peptides. *Anal.* Calcd for $C_{32}H_{41}N_7O_7 \cdot 2CF_3COOH \cdot 4H_2O$: C, 46.20; H, 5.49; N, 10.48. Found: C, 46.09; H,

TABLE II. Amino Acid Ratios in 6 N HCl Hydrolysates of Enkephalin Analogs

	Analogs	Tyr Y	Gly G	Phe F	Leu L	Met M	Val V	Ile I	His H	Arg R	Rec. %
1	H–(Y–H–G–F–L)–OH	0.88	1.00	0.94	0.93				0.88		87
2	H–(Y–H–G–F–M–R–R–V)–NH_2	0.89	1.00	0.98		0.82	0.97		0.94	1.93	72
3	H–(Y–R–G–F–L–R)–OH	1.02	1.00	0.95	1.05					1.97	87
4	H–(Y–R–G–F–M–R)–OH	0.97	1.00	1.04		0.95				1.94	84
5	H–(Y–R–G–F–L–R–R)–OH	0.94	1.00	1.03	1.04					2.88	85
6	H–(Y–R–G–F–L–R–R–I)–OH	1.03	1.00	1.00	0.96			0.97		2.96	80
7	H–(Y–R–G–F–L–R–R–I–R)–OH	0.89	1.00	0.96	1.03			0.93		4.03	82
8	H–(Y–R–G–F–L–R)–OH	0.92	1.00	0.98	0.96					2.07	84
9	H–(Y–R–G–F–L–R)–ol	0.93	1.00	0.97	0.93					1.08	79
10	H–(Y–R–G–F–L–R)–NH_2	0.93	1.00	1.07	0.95					1.88	82

Capital letters in parentheses indicate amino acids. Underlining indicates D-amino acids.

TABLE III. Characterization of Enkephalin Analogs, **3** and **4**

Compound	Proc.	Yield (%)	mp (°C)	$[\alpha]_D^{24}$ (DMF)	Formula	Analysis (%) Calcd (Found) C	H	N
Z(OMe)–(L–R)–OBzl	PCP	90	65—67	−14.3°	$C_{28}H_{38}N_6O_8$	57.32 (57.21)	6.53 6.46	14.33 14.01)
Boc–(G–F)–OBzl	DCC	69	80—82	−5.2°	$C_{23}H_{28}N_2O_5$	66.97 (67.36)	6.84 6.78	6.79 6.91)
Boc–(G–F)–$NHNH_2$	NH_2NH_2	85	180—185	−3.3°	$C_{16}H_{24}N_4O_4$	57.13 (57.48	7.19 7.36	16.66 16.51)
Boc–(G–F–L–R)–OBzl	Az	87	105—110	−12.5°	$C_{35}H_{50}N_8O_9$	57.83 (57.63	6.93 6.85	15.42 15.00)
Z(OMe)–(R–G–F–L–R)–OBzl	MA	90	104—107	−8.2°	$C_{45}H_{61}N_{13}O_{13}$ $\cdot 3H_2O$	51.66 (51.66	6.45 5.89	17.41 17.22)
Z(OMe)–(Y–R–G–F–L–R)–OBzl	TCP	97	105—110	−14.0°	$C_{61}H_{76}N_{14}O_{15}$ $\cdot H_2O$	57.99 (57.96	6.22 6.04	15.52 15.41)
H–(Y–R–G–F–L–R)–OH (**3**)	H_2–Pd	43		+18.8° 0.2 N AcOH	$C_{38}H_{58}N_{12}O_8$ $\cdot 3AcOH \cdot 3H_2O$	50.56 (50.79	7.33 6.98	16.08 16.23)
Z(OMe)–(M–R)–OH	TCP	75	88—92	−9.1°	$C_{29}H_{41}N_5O_8S_2$	53.44 (53.51	6.14 6.22	10.75 10.74)
Boc–(G–F–M–R)–OH	Az	87	127—132	−2.9°	$C_{36}H_{53}N_7O_9S_2$ $\cdot 0.5H_2O$	53.97 (53.77	6.80 6.61	12.24 12.16)
Z–(Y–R–G–F–M–R)–OH	Az	63	139—144	−11.6°	$C_{63}H_{82}N_{12}O_{14}S_3$	56.99 (56.56	6.23 6.22	12.66 12.35)
H–(Y–R–G–F–M–R)–OH (**4**)	MSA	34		+18.0° 0.2 N AcOH	$C_{37}H_{56}N_{12}O_8S$ $\cdot 3AcOH \cdot 3H_2O$	48.57 (48.05	7.02 6.61	15.81 15.74)

TABLE IV. Characterization of Enkephalin Analogs, **5** and **6**

Compound	Proc.	Yield (%)	mp (°C)	$[\alpha]_D^{24}$ (DMF)	Formula	Analysis (%) Calcd (Found) C	H	N
Z(OMe)-(L-R(m))-OMe	PCP	87	65—70	−11.2°	$C_{31}H_{45}N_5O_6S \cdot H_2O$	55.92 (55.97)	7.12 6.72	10.52 10.20)
Z(OMe)-(L-R(m))-$NHNH_2$	NH_2NH_2	91	95—97	−13.5°	$C_{30}H_{45}N_7O_7S \cdot 0.5H_2O$	54.86 (54.88	7.06 6.64	14.93 14.88)
Z(OMe)-(L-R(m)-R(m))-OH	Az	95	115—118	−2.4°	$C_{45}H_{65}N_9O_{11}S_2$	55.59 (56.16	6.74 6.76	12.97 12.93)
Z(OMe)-(G-F-R(m)-R(m))-OH	Az	92	142—146	−7.6°	$C_{52}H_{77}N_{11}O_{12}S_2 \cdot H_2O$	55.25 (55.20	7.04 6.97	13.63 13.27)
Z-(Y-R(m)-G-F-L-R(m)-R(m))-OH	Az	88	129—133	+1.0°	$C_{79}H_{106}N_{16}O_{17}S_3 \cdot 1.5H_2O$	56.64 (56.38	6.56 6.71	13.38 13.15)
H-(Y-R-G-F-L-R-R)-OH (**5**)	TFMSA	54		+8.3° (0.2 N AcOH)	$C_{44}H_{70}N_{16}O_9 \cdot 4AcOH \cdot 2H_2O$	50.23 (50.25	7.30 7.29	18.03 18.22)
Z(OMe)-(R(m)-I)-OBzl	DCC	83	45—50	−7.5°	$C_{37}H_{49}N_5O_8S$	61.39 (61.80	6.82 6.82	9.68 9.42)
Z(OMe)-(L-R(m)-R(m)-I)-OBzl	Az	72	110—115	−20.9°	$C_{58}H_{82}N_{10}O_{12}S \cdot H_2O$	58.36 (58.41	7.09 6.96	11.74 11.74)
Z(OMe)-(G-F-L-R(m)-R(m)-I)-OBzl	Az	84	126—131	−12.4°	$C_{65}H_{94}N_{12}O_{13}S_2 \cdot H_2O$	58.53 (58.52	7.26 7.01	12.60 12.69)
Z-(Y-R(m)-G-F-L-R(m)-R(m)-I)-OBzl	Az	97	203—207	−8.4°	$C_{92}H_{123}N_{17}O_{18}S_3$	58.83 (58.56	6.76 6.68	12.68 12.86)
H-(Y-R-G-F-L-R-R-I)-OH (**6**)	TFMSA	46		−3.0° (0.2 N AcOH)	$C_{50}H_{81}N_{17}O_{10} \cdot 4AcOH \cdot 2.5H_2O$	51.01 (50.77	7.53 7.38	17.44 17.20)

5.53; N, 10.52.

Synthesis of [D-His^2]adrenorphin

Z(OMe)-Gly-Phe-Met(O)-Arg(Mts)-Arg(Mts)-Val-NH_2——A mixture of Z(OMe)-Gly-OSu (3.10 g, 12.9 mmol), Et_3N (3.46 ml, 24.7 mmol) and a TFA-treated sample of Z(OMe)-Phe-Met(O)-Arg(Mts)-Arg(Mts)-Val-NH_2 (14.73 g, 11.8 mmol) in DMF (80 ml) was stirred at room temperature overnight. The solvent was removed by evaporation and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, and recrystallized from MeOH and ether; yield 8.36 g (54%), mp 170—171 °C, $[\alpha]_D^{19}$ −8.8° (c=0.9, DMF), Rf_1 0.64. *Anal.* Calcd for $C_{60}H_{85}N_{13}O_{14}S_3$: C, 55.07; H, 6.55; N, 13.92. Found: C, 54.78; H, 6.44; N, 13.97.

Z(OMe)-D-His-Gly-Phe-Met(O)-Arg(Mts)-Arg(Mts)-Val-NH_2——The Az [prepared from 1.26 g (3.8 mmol) of Z(OMe)-D-His-$NHNH_2$] in DMF (10 ml) and Et_3N (0.58 ml, 4.1 mmol) were added to an ice-chilled solution of a TFA-treated sample of the above hexapeptide amide (3.79 g, 2.9 mmol) in DMF (15 ml) containing Et_3N (0.4 ml, 2.9 mmol), and the solution was stirred at 4 °C overnight. After evaporation of the solvent, the residue was treated as stated above and precipitated from DMF with MeOH; yield 2.86 g (68%), mp 179—181 °C, $[\alpha]_D^{19}$ −7.9° (c=0.5, DMF), Rf_1 0.38. *Anal.* Calcd for $C_{66}H_{92}N_{16}O_{15}S_3 \cdot 2H_2O$: C, 53.49; H, 6.53; N, 15.10. Found: C, 53.59; H, 6.43; N, 14.78.

Z(OMe)-Tyr-D-His-Gly-Phe-Met(O)-Arg(Mts)-Arg(Mts)-Val-NH_2——The Az [prepared from 0.83 g (2.3 mmol) of Z(OMe)-Tyr-$NHNH_2$] in DMF (10 ml) and Et_3N (0.35 ml, 2.5 mmol) were added to an ice-chilled solution of a TFA-treated sample of the above heptapeptide amide (2.56 g, 1.8 mmol) in DMF (10 ml) containing

TABLE V. Characterization of Enkephalin Analogs, **7** and **8**

Compound	Proc.	Yield (%)	mp (°C)	$[\alpha]_D^{24}$ (DMF)	Formula	Analysis (%) Calcd (Found) C	H	N
Z(OMe)-(R-I)-NHNH$_2$	NH_2NH_2	93	135—138	+4.7°	$C_{30}H_{45}N_7O_7S$	55.62 (55.50)	7.00 6.97	15.14 14.99)
Z(OMe)-(R-I-R)-OH	Az	89	125—129	−13.6°	$C_{45}H_{65}N_9O_{11}S_2 \cdot 0.5H_2O$	55.08 (55.22	6.78 6.84	12.85 12.43)
Z(OMe)-(L-R-R-I-R)-OH	Az	91	132—136	−15.6°	$C_{66}H_{98}N_{14}O_{15}S \cdot 1.5H_2O$	54.64 (54.55	7.02 7.02	13.52 13.54)
Z(OMe)-(G-F-L-R-R-I-R)-OH	Az	81	155—159	−4.0°	$C_{73}H_{110}N_{16}O_{16}S_3 \cdot 2H_2O$	54.80 (54.71	7.18 6.98	14.01 14.04)
Z-(Y-R-G-F-L-R-R-I-R)-OH	Az	84	164—167	+0.9°	$C_{100}H_{139}N_{21}O_{21}S_4 \cdot 1.5H_2O$	56.48 (56.40	6.73 6.78	13.83 13.80)
H-(Y-R-G-F-L-R-R-I-R)-OH (**7**)	TFMSA	51		−11.3° 0.2 N AcOH	$C_{56}H_{93}N_{21}O_{11} \cdot 5AcOH \cdot 4H_2O$	49.27 (49.46	7.58 7.19	18.28 17.83)
Z(OMe)-(G-F-L)-NHNH$_2$	NH_2NH_2	83	137—142	−21.1°	$C_{26}H_{35}N_5O_6$	60.80 (60.38	6.87 6.78	13.64 13.46)
Z(OMe)-(G-F-L-R)-OH	Az	88	129—134	−9.6°	$C_{41}H_{55}N_7O_{10}S$	58.76 (59.04	6.62 6.68	11.70 11.53)
Z-(Y-R-G-F-L-R)-OH	Az	90	143—149	−11.3°	$C_{64}H_{84}N_{12}O_{14}S_2 \cdot 0.5H_2O$	58.29 (58.16	6.50 6.75	12.75 12.48)
H-(Y-R-G-F-L-R)-OH (**8**)	TFMSA	68		+12.0° 0.2 N AcOH	$C_{38}H_{58}N_{12}O_8 \cdot 2AcOH \cdot 2.5H_2O$	51.68 (52.05	7.33 7.33	17.22 17.44)

Et_3N (0.25 ml, 1.8 mmol), then the solution was stirred at 4 °C overnight and the solvent was removed by evaporation. The residue was purified as stated above; yield 1.52 g (53%), mp 167—169 °C, $[\alpha]_D^{19}$ −15.6° (c=0.5, DMF), Rf_1 0.45. *Anal.* Calcd for $C_{75}H_{101}N_{17}O_{17}S_3 \cdot 3.5H_2O$: C, 53.87; H, 6.51; N, 14.24. Found: C, 53.93; H, 6.34; N, 13.75.

H-Tyr-D-His-Gly-Phe-Met-Arg-Arg-Val-NH$_2$ (2)——The above protected octapeptide amide (100 mg, 62 μmol) was treated with 1 M TFMSA (1.9 ml) in the presence of *m*-cresol (0.1 ml, 15 eq) in an ice-bath for 2 h, then ether was added and the resulting powder was treated with Amberlite CG-4B (acetate form) for 30 min. The resin was removed by filtration, then the filtrate was incubated with dithiothreitol (0.48 g, 50 eq) at 37 °C overnight and the solvent was removed by lyophilization. The product was next purified by gel-filtration on Sephadex G-15 (3.2 × 104 cm) using 0.5 N AcOH as an eluant. The fractions corresponding to the front main peak (monitored by UV absorption measurement at 275 nm) were collected and the solvent was removed by lyophilization. The product was next purified by partition chromatography on Sephadex G-10 (2.5 × 83 cm) using the solvent system of *n*-BuOH–AcOH–H_2O (4:1:5) as stated above. The desired fractions (5.4 ml each, tube Nos. 104—110, monitored at UV 275 nm) were combined, then the solvent was removed by evaporation and the residue was lyophilized to give a fluffy powder; yield 32 mg (48%). The sample (purity 96%, determined by analytical HPLC) was submitted to bioassay. For characterization, the product was further purified by HPLC using isocratic elution with 20% MeCN in 0.1% TFA; yield 20 mg (64%), $[\alpha]_D^{20}$ −77.9° (c=0.2, MeOH), Rf_3 0.57. Amino acid ratios in a 6 N HCl hydrolysate are listed in Table II. *Anal.* Calcd for $C_{48}H_{73}N_{17}O_9S \cdot 4CF_3COOH \cdot 2.5H_2O$: C, 42.97; H, 5.28; N, 15.21. Found: C, 43.27; H, 5.58; N, 15.45.

Syntheses of [D-Arg^2]enkephalin Analogs

Eight [D-Arg^2]enkephalin analogs were synthesized as described in the text. Amino acid ratios in 6 N HCl hydrolysates of these compounds are listed in Table II. Physical constants and analytical data of these compounds are listed in Tables III to VI, together with those of their intermediates. Capital letters in parentheses indicate amino acids: Y=Tyr, H=His, R=Arg, G=Gly, F=Phe, L=Leu, I=Ile. Small letters at shoulders indicate protecting

TABLE VI. Characterization of Enkephalin Analogs, **9** and **11**

Compound	Proc.	Yield (%)	mp (°C)	$[\alpha]_D^{24}$ (DMF)	Formula	Analysis (%) Calcd (Found) C	H	N
Boc-(G-F-L-R(m))-OMe	Az	89	104—107	−22.2°	$C_{38}H_{57}N_7O_9S \cdot H_2O$	56.02 (56.76)	7.38 (7.00)	12.17 (12.32)
Boc-(G-F-L-R(m))-ol	$NaBH_4$	89	102—105	−17.3°	$C_{37}H_{57}N_7O_8S \cdot H_2O$	57.11 (57.29)	7.64 (7.31)	12.60 (12.46)
Z-(Y(m)-R-G-F-L-R(m))-ol	Az	93	135—139	−9.5°	$C_{64}H_{86}N_{12}O_{13}S_2 \cdot 2H_2O$	57.72 (57.70)	6.81 (6.38)	12.62 (12.25)
H-(Y-R-G-F-L-R)-ol (**9**)	TFMSA	33		+20.0° (0.2 N AcOH)	$C_{38}H_{60}N_{12}O_7 \cdot 3AcOH \cdot 2.5H_2O$	51.70 (51.69)	7.59 (7.32)	16.45 (16.36)
Boc-(G-F-L-R(m))-NH_2	NH_3	95	129—134	+2.0°	$C_{37}H_{56}N_8O_8S$	57.49 (57.03)	7.30 (7.21)	14.50 (14.31)
Z-(Y(m)-R-G-F-L-R(m))-NH_2	Az	92	139—142	−2.9°	$C_{64}H_{84}N_{12}O_{14}S_2 \cdot H_2O$	57.94 (57.87)	6.53 (6.80)	12.67 (13.03)
H-(Y-R-G-F-L-R)-NH_2 (**10**)	TFMSA	47		+15.8° (0.2 N AcOH)	$C_{32}H_{48}N_{12}O_6 \cdot 3AcOH \cdot H_2O$	50.99 (51.43)	6.98 (7.07)	18.78 (18.31)

groups.[2] Arrows indicate positions at which reactions took place.

References and Notes

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2) Unless otherwise stated, amino acids used in this report are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Bzl(or b) = benzyl, Mts(or m) = mesitylenesulfonyl, NO_2(or n) = nitro, Su = *N*-hydroxysuccinimidyl, TCP = 2,4,5-trichlorophenyl, PCP = pentachlorophenyl, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, DMF = dimethylformamide, MSA = methanesulfonic acid, CM = carboxymethyl, DCC = dicyclohexylcarbodiimide.
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