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Synthetic Studies on Enkephalin Analogs. III.[1,2] A Highly Potent Enkephalin Analog, H-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-CH_2CH_3

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Thirty-five tetrapeptide acyl-hydrazide analogs of enkephalin substituted at position 2 were synthesized. Substitution of D-Ala at position 2 of H–Tyr–D-Ala–Gly–Phe–NHNH–CO–R (R=lower alkyl), by D-Met(O), D-Gln, D-Glu(NH–CH_3) or D-Thr enhanced the analgesic potency, but substitution by D-Glu or Ser resulted in an analog with no antinociceptive activity. Among the analogs synthesized, the D-Met(O)-analog was the most potent and H–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–CH_2CH_3 exhibited analgesic activtiy four times more potent than that of morphine in mice following subcutaneous injection. Structure-activity relations for position 2 of the enkephalin-like tetrapeptide are discussed.

Keywords——enkephalin analog; analgesia; tetrapeptide acyl-hydrazide; morphine; methionine sulfoxide; structure-activity relations

In the course of synthetic studies on enkephalin analogs altered at position 5, we found a new type of tetrapeptide analog, H–Tyr–D-Ala–Gly–Phe–NHNH–CO–R (R=lower alkyl),[3] to be a potent analgesic upon intravenous or subcutaneous administration to mice. Subsequently, it occurred to us that replacement of the D-Ala residue at position 2 of the tetrapetide acyl-hydrazide by a suitable D-amino acid residue might further enhance the analgesic activity, because some structural modifications at position 2 in Met-enkephalin are known to be successful. For instance, two potent analogs of enkephalin, [D-Met^2, Pro^5]- and [D-Thr^2, Thz^5]-enkephalinamides, which were both obtained by double replacement of Gly at position 2 and Met at position 5 of Met-enkephalinamide, have been reported by Bajusz *et al.*[4] and Li *et al.*,[5] respectively. Also, Shaw and Turnbull recently reported high agonist potency of H–Tyr–D-Ser–Gly–Phe–Met–OMe[6] in the mouse vas deferens assay in comparison with D-Ala^2–Met-enkephalinamide.[7]

As part of a search for an analog with more potent activity and in order to identify the structural requirements of position 2 of the tetrapeptide acyl-hydrazide for analgesic activity, we synthesized thirty-five new analogs in which D-Ala at position 2 of the tetrapeptide[3] was replaced by other D-amino acid residues with a variety of side chains.

The newly synthesized tetrapeptides are shown in Fig. 1. All the analogs were synthesized by the solution method in a way similar to that described previously.[3] Starting from H–Phe–NHNH–CO–R (R=alkyl), Z(or Boc)–Tyr–D-X–Gly–Phe–NHNH–CO–R (X=amino acid residue) was prepared by the stepwise elongation method using HONB active esters[8] of protected amino acids, or by coupling to Z(or Boc)–Tyr–D-X–Gly–OH directly. An exception was Z–Tyr–D-Met(O)–Gly–Phe–NHNH–R_1 (R_1=H, CO–$CH_2CH_2CH_3$, CO–$CH_2CH_2CH_2CH_2$–CH_3), which was prepared by a route similar to Route B.[3] Analogs X, XI and XIX were synthesized as illustrated in Fig. 2, using Boc–Tyr–D-Glu(OBzl)–Gly–Phe–NHNH–CO–CH_2CH_3 (**21**). For the synthesis of the D-Met(O_2)-analog (XXII), we used Boc–D-Met(O_2)–OH (**40**), which was prepared by oxidation of Boc–D-Met–OH with aqueous H_2O_2. H–D-Met^R(O)–OH (**43**) and H–D-Met^S(O)–OH (**47**), which were used for the synthesis of the D-Met-(O)-analogs with a chiral S-atom, were prepared by stereospecific oxidation[9] and resolution from diastereoisomeric sulfoxides,[10] respectively. The optical purity of Boc–D-Met^R(O)–OH

H–Tyr-D-Leu–Gly–Phe–NHNH–CO–X_1
I, X_1=CH_3; II, X_1=$CH_2CH_2CH_3$; III, X_1=$OC(CH_3)_3$
H–Tyr–X_2–Gly–Phe–NHNH–CO–CH_3
IV, X_2=D-Nva; V, X_2=D-Phe; VI, X_2=D-Lys(Cl-Z); VII, X_2=D-Glu(OMe); VIII, X_2=D-Gln; XII, X_2=D-Asn; XIII, X_2=D-Ser; XIV, X_2=Ser; XV, X_2=D-Thr; XVI, X_2=D-Lys; XVII, X_2=D-Arg; XXII, X_2=D-Met(O_2)
H–Tyr–X_3–Gly–Phe–NHNH–CO–CH_2–CH_3
IX, X_3=D-Gln; X, X_3=D-Glu(NH–CH_3); XI, X_3=D-Glu($NHNH_2$); XVIII, X_3=D-His; XIX, X_3=D-Glu; XXI, X_3=D-Met; XXIII, X_3=D-Met$^+$(CH_3); XXIV, X_3=D-Met^R (O); XXV, X_3=D-Met^S(O)
H–Tyr-D-Met(O)–Gly–Phe–X_4
XX, X_4=NHNH–CO–CH_2CH_3; XXVI, X_4=NH_2; XXVII, X_4=NH–CH_2CH_3; XXVIII, X_4=$NHNH_2$; XXIX, X_4=NHNH–SO_2CH_3; XXX, X_4=NHNH–CO–CH_3; XXXI, X_4=NHNH–CO–$(CH_2)_2$–CH_3; XXXII, X_4=NHNH–CO–$(CH_2)_4$–CH_3; XXXIII, X_4=NHNH–CO–CH_2SCH_3
H–Tyr-D-Met(O)–Gly–MePhe–NHNH–CO–CH_2CH_3 (XXXIV)
H–MeTyr-D-Met(O)–Gly–Phe–NHNH–CO–CH_2CH_3 (XXXV)

Fig. 1. Structures of Tetrapeptide Analogs of Enkephalin

(**44**) and Boc–D-Met^S(O)–OH (**48**) was checked by measuring $[\alpha]_D$ and ^{1}H-nuclear magnetic resonance (NMR) spectra. For the synthesis of the analog XXXIV, H–MePhe–NHNH–CO–CH_2CH_3, which was prepared using Z–MePhe–OH,[11] was used, while Z–MeTyr(Bu^t)–OH[1] was used for the synthesis of the analog XXXV. The D-Met–analog (XXI) was obtained by reduction of the sulfoxide of the D-Met(O)–analog (XX) with thioglycolic acid.[12] The analog XXIII was obtained by S-methylation of the Met residue of the analog XXI with CH_3I in a similar manner to that described for the synthesis of S-methyl-glucagon.[13]

Deblocking of most of the protected peptides was carried out by hydrogenolysis over Pd-black or by TFA treatment. In the case of analogs XXVIII, XXXI, XXXII and XXXV, CH_3SO_3H[14] was used as a deblocking reagent and in the case of the analog XVII, anhydrous HF[15] was used. Crude peptide thus obtained was purified by Sephadex LH-20 column chromatography.

The synthetic route to the analog XX is illustrated in Fig. 3 as an example. All the synthetic analogs of enkephalin were chromatographically pure and gave the expected amino acid ratios. Physicochemical properties of intermediates and final products are listed in Tables I and II, respectively.

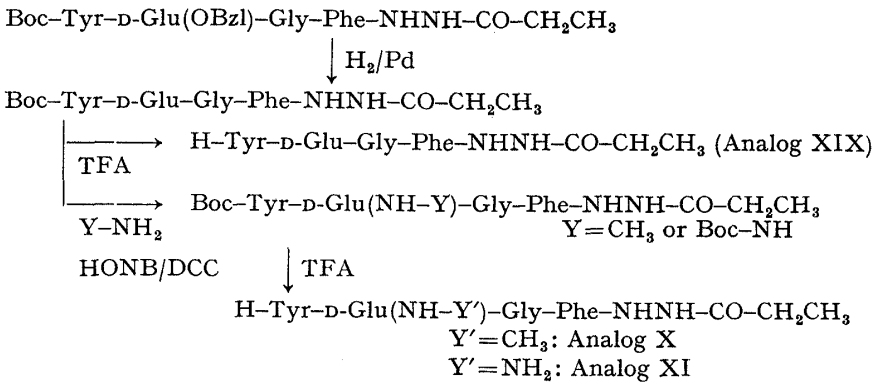

Fig. 2. Synthetic Routes to Analogs X, XI and XIX

The analgesic activities of synthetic tetrapeptides were measured in mice by the hot-plate test as described previously,[3] and compared with those of the D-Ala^2–analog[3] and morphine.

As shown in Table III, the substitution of D-Ala at position 2 of the tetrapeptide acylhydrazide by D-Met(O) enhanced the potency remarkably. That is, H–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–CH_2CH_3 (XX) was eight times more potent than the corresponding D-Ala–

TABLE I. Physicochemical Properties of Intermediates

Compound No.	Structure	mp (°C)	$[\alpha]_D$ in DMF (Temp., conc.)	TLC[a] Rf^1	Formula	Analysis (%) Found (Calcd) C	H	N	S
1	Z-D-Leu-Gly-OH	110—111	+24.3°[b] (27, 1.0)	0.42	$C_{16}H_{22}N_2O_5$	59.59 (59.61	6.85 6.88	8.74 8.69)	
2	Z-Tyr-D-Leu-Gly-OH	179—181	+45.2°[b] (27, 0.50)	0.38	$C_{25}H_{31}N_3O_7$	61.68 (61.84	6.51 6.44	8.64 8.66)	
3	Z-Tyr-D-Leu-Gly-Phe-NHNH-CO-CH_3	184—185	−13.4° (23, 0.50)	0.29	$C_{36}H_{44}N_6O_8$	62.52 (62.77	6.81 6.44	12.04 12.20)	
4	Z-Tyr-D-Leu-Gly-Phe-NHNH-CO-$CH_2CH_2CH_3$	178—179	−11.7° (27, 0.29)	0.51	$C_{38}H_{48}N_6O_8$	63.45 (63.67	6.70 6.75	11.68 11.73)	
5	Z-Tyr-D-Leu-Gly-Phe-NHNH-Boc	137—139	−11.4° (19, 0.50)	0.58	$C_{39}H_{50}N_6O_9$	62.89 (62.71	6.95 6.75	10.89 11.25)	
6	Z-Gly-Phe-NHNH-CO-CH_3	154—155	−0.9° (23, 0.50)	0.52	$C_{21}H_{24}N_4O_5$	61.09 (61.15	5.69 5.87	13.18 13.59)	
7	Z-D-Nva-Gly-Phe-NHNH-CO-CH_3	235—237	+3.4° (23, 0.45)	0.44	$C_{26}H_{33}N_5O_6$	61.33 (61.04	6.62 6.50	13.66 13.69)	
8	Z-Tyr-D-Nva-Gly-Phe-NHNH-CO-CH_3	210—211	−18.3° (23, 0.48)	0.38	$C_{35}H_{42}N_6O_8 \cdot 1/2H_2O$	61.39 (61.48	6.34 6.33	12.09 12.29)	
9	Boc-D-Phe-Gly-Phe-NHNH-CO-CH_3	208—209	+3.3° (23, 0.45)	0.51	$C_{27}H_{35}N_5O_6$	61.99 (61.70	6.78 6.71	13.20 13.33)	
10	Z-Tyr-D-Phe-Gly-Phe-NHNH-CO-CH_3	203—204	−13.0° (23, 0.44)	0.40	$C_{39}H_{42}N_6O_8 \cdot 1/2H_2O$	64.13 (64.00	5.78 5.92	11.52 11.48)	
11	Boc-D-Lys(Cl-Z)-Gly-Phe-NHNH-CO-CH_3	169—171	+4.7° (23, 0.47)	0.45	$C_{32}H_{43}ClN_6O_8$	56.87 (57.00	6.31 6.41	12.25[c] 12.46)	
12	Boc-Tyr-D-Lys(Cl-Z)-Gly-Phe-NHNH-CO-CH_3	196—198	−2.0° (23, 0.40)	0.46	$C_{41}H_{52}ClN_7O_{10} \cdot H_2O$	57.88 (57.56	5.92 6.12	11.27[d] 11.46)	
13	Boc-D-Glu(OMe)-Gly-Phe-NHNH-CO-CH_3	205—206	+2.8° (23, 0.42)	0.43	$C_{24}H_{35}N_5O_8$	55.47 (55.26	6.72 6.76	13.36 13.43)	
14	Z-Tyr-D-Glu(OMe)-Gly-Phe-NHNH-CO-CH_3	200—202	−14.8° (23, 0.44)	0.31	$C_{36}H_{42}N_6O_{10}$	59.54 (60.16	5.71 5.89	11.47 11.69)	
15	Boc-D-Gln-Gly-Phe-NHNH-CO-CH_3	198—199	−0.8° (23, 0.50)	0.19	$C_{23}H_{34}N_6O_7 \cdot H_2O$	52.75 (52.66	6.79 6.91	15.62 16.02)	
16	Z-Tyr-D-Gln-Gly-Phe-NHNH-CO-CH_3	209—210	−21.5° (23, 0.40)	0.11	$C_{35}H_{41}N_7O_9 \cdot 1/2H_2O$	58.42 (58.97	5.88 5.93	13.71 13.75)	

17	Z–Gly–Phe–NHNH–CO–CH_2CH_3	151—152	−1.2° (21, 0.50)	0.46	$C_{22}H_{26}N_4O_5$	62.25 (61.96	6.23 6.15	12.85 13.15)	
18	Boc–D-Gln–Gly–Phe–NHNH–CO–CH_2CH_3	170—171	−3.1° (25, 0.36)	0.25	$C_{24}H_{36}N_6O_7$	54.94 (55.37	6.96 6.97	15.33 16.15)	
19	Z–Tyr–D-Gln–Gly–Phe–NHNH–CO–CH_2CH_3	243—244	−23.7° (25, 0.40)	0.17	$C_{36}H_{43}N_7O_9 \cdot 1/2H_2O$	59.43 (59.49	6.02 6.10	13.24 13.49)	
20	Boc–D-Glu(OBzl)–Gly–Phe–NHNH–CO–CH_2CH_3	154—155	+2.5° (25, 0.36)	0.56	$C_{31}H_{41}N_5O_8$	60.89 (60.86	6.65 6.76	11.18 11.45)	
21	Boc–Tyr–D-Glu(OBzl)–Gly–Phe–NHNH–CO–CH_2CH_3	169—170	−5.0° (25, 0.40)	0.50	$C_{40}H_{50}N_6O_{10}$	61.91 (62.00	6.61 6.50	10.92 10.85)	
22	Boc–Tyr–D-Glu–Gly–Phe–NHNH–CO–CH_2CH_3	163—165	−7.7° (25, 0.44)	0.11	$C_{33}H_{44}N_6O_{10}$	57.64 (57.88	6.80 6.48	11.77 12.27)	
23	Boc–Tyr–D-Glu(NH–CH_3)–Gly–Phe–NHNH–CO–CH_2CH_3	182—183	−7.3° (24, 0.37)	0.24	$C_{34}H_{47}N_7O_9$	58.91 (58.52	7.05 6.79	13.76 14.05)	
24	Boc–Tyr–D-Glu(NHNH–Boc)–Gly–Phe–NHNH–CO–CH_2CH_3	169—171	−0.9° (24, 0.34)	0.33	$C_{38}H_{54}N_8O_{11}$	57.41 (57.13	7.02 6.81	14.15 14.03)	
25	Boc–D-Asn–Gly–Phe–NHNH–CO–CH_3	172—174	+1.3° (25, 0.38)	0.12	$C_{22}H_{32}N_6O_7$	53.97 (53.65	6.84 6.55	16.08 17.06)	
26	Z–Tyr–D-Asn–Gly–Phe–NHNH–CO–CH_3	210—211	−13.5° (22, 0.31)	0.14	$C_{34}H_{39}N_7O_9$	58.85 (59.20	5.65 5.69	13.36 14.02)	
27	Z–D-Ser–Gly–Phe–NHNH–CO–CH_3	168—169	+3.4° (23, 0.50)	0.25	$C_{24}H_{29}N_5O_7$	57.33 (57.72	5.95 5.85	13.50 14.02)	
28	Z–Tyr–D-Ser–Gly–Phe–NHNH–CO–CH_3	184—186	−18.3° (23, 0.30)	0.19	$C_{33}H_{38}N_6O_9$	59.37 (59.81	5.92 5.78	12.42 12.68)	
29	Z–Ser–Gly–Phe–NHNH–CO–CH_3	182—183	−4.2° (24, 0.38)	0.26	$C_{24}H_{29}N_5O_7$	57.47 (57.72	5.66 5.85	13.41 14.02)	
30	Z–Tyr–Ser–Gly–Phe–NHNH–CO–CH_3	223—224	−0.9° (24, 0.35)	0.20	$C_{33}H_{38}N_6O_9$	59.00 (59.81	5.64 5.78	12.36 12.68)	
31	Z–D-Thr–Gly–Phe–NHNH–CO–CH_3	193—195	+5.3° (25, 0.41)	0.41	$C_{25}H_{31}N_5O_7$	58.13 (58.47	6.13 6.08	13.25 13.64)	
32	Z–Tyr–D-Thr–Gly–Phe–NHNH–CO–CH_3	208—209	−19.0° (25, 0.50)	0.28	$C_{34}H_{40}N_6O_9 \cdot 1/2H_2O$	59.79 (59.55	6.11 6.03	12.40 12.25)	
33	Boc–D-Arg(Tos)–Gly–Phe–NHNH–CO–CH_3	144—145	+1.2° (25, 0.40)	0.19	$C_{31}H_{44}N_8O_8S$	53.87 (54.05	6.28 6.43	15.77 16.27	4.75 4.65)
34	Z–Tyr–D-Arg(Tos)-Gly–Phe–NHNH–CO–CH_3	154—156	−13.7° (25, 0.50)	0.25	$C_{43}H_{51}N_9O_{10}S \cdot 1/2H_2O$	57.67 (57.70	5.65 5.85	13.54 14.07	3.38 3.58)
35	Boc–D-His(Tos)–Gly–Phe–NHNH–CO–CH_2CH_3	178—179	−1.7° (23, 0.42)	0.48	$C_{32}H_{41}N_7O_8S$	56.61 (56.21	5.98 6.04	14.03 14.34	4.42 4.69)
36	Z–Tyr–D-His–Gly–Phe–NHNH–CO–CH_2CH_3	181—182	−10.5° (23, 0.38)	0.46[e]	$C_{37}H_{42}N_8O_8 \cdot H_2O$	59.42 (59.67	5.72 5.68	14.79 15.04)	

Compound No.	Structure	mp (°C)	$[\alpha]_D$ in DMF (Temp., conc.)	TLC[a] Rf^1	Formula	Analysis (%) Found (Calcd) C	H	N	S
37	Boc–D-Met(O)–OH	125—126	+7.2° (23, 0.50)	0.33	$C_{10}H_{19}NO_5S$	45.19 (45.26	7.52 7.21	5.50 5.27	11.75 12.08)
38	Boc–D-Met(O)–Gly–Phe–NHNH–CO–CH_2CH_3	121—122	0° (21, 0.50)	0.40	$C_{24}H_{37}N_5O_7S \cdot 1/2H_2O$	52.41 (52.53	6.99 6.97	12.46 12.76	5.61 5.84)
39	Boc–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–CH_2CH_3	134—135	0° (21, 0.50)	0.20	$C_{35}H_{46}N_6O_9S \cdot H_2O$	54.72 (54.98	6.82 6.71	11.16 11.66	4.43 4.44)
40	Boc–D-Met(O_2)–OH[f]	201	−16.5° (23, 0.18)	0.26	$C_{22}H_{42}N_2O_6S$	57.41 (57.11	9.25 9.14	6.10 6.05	6.98 6.93)
41	Boc–D-Met(O_2)–Gly–Phe–NHNH–CO–CH_3	198—199	−2.5° (23, 0.49)	0.25	$C_{23}H_{35}N_5O_8S$	51.30 (51.00	6.49 6.51	12.95 12.93	5.78 5.92)
42	Boc–Tyr–D-Met(O_2)–Gly–Phe–NHNH–CO–CH_3	176—177	−2.9° (23, 0.24)	0.23	$C_{32}H_{44}N_6O_{10}S \cdot 1/2H_2O$	53.83 (53.84	6.25 6.35	11.41 11.77	4.25 4.49)
43	H–D-Met^R(O)–OH	243—244	−45.8°[g] (23, 0.40)	0.30[h]	$C_5H_{11}NO_3S$	36.43 (36.34	6.50 6.71	8.17 8.47	19.49 19.41)
44	Boc–D-Met^R(O)–OH[i]	130—131	−30.2° (23, 0.50)	0.25	$C_{10}H_{19}NO_5S$	45.91 (45.26	7.52 7.21	5.61 5.27	11.53 12.08)
45	Boc–D-Met^R(O)–Gly–Phe–NHNH–CO–CH_2CH_3	140—141	−23.5° (25, 0.36)	0.23	$C_{24}H_{37}N_5O_7S \cdot 1/2H_2O$	52.66 (52.53	6.80 6.97	12.44 12.76	5.69 5.84)
46	Boc–Tyr–D-Met^R(O)–Gly–Phe–NHNH–CO–CH_2CH_3	164—165	−19.5° (25, 0.38)	0.18	$C_{33}H_{46}N_6O_9S \cdot H_2O$	54.36 (54.98	6.69 6.71	11.24 11.66	4.60 4.44)
47	H–D-Met^S(O)–OH	234—235	+73.0°[g] (22, 0.22)	0.30[h]	$C_5H_{11}NO_3S$	36.42 (36.34	6.81 6.71	8.38 8.47	19.66 19.41)
48	Boc–D-Met^S(O)–OH[j]	135—136	+51.4° (24, 0.38)	0.25	$C_{10}H_{19}NO_5S$	45.41 (45.26	7.44 7.21	5.15 5.27	12.12 12.08)
49	Boc–D-Met^S(O)–Gly–Phe–NHNH–CO–CH_2CH_3	133—134	+11.0° (22, 0.43)	0.21	$C_{24}H_{37}N_5O_7S$	53.45 (53.41	7.20 6.91	12.70 12.97	5.38 5.94)
50	Boc–Tyr–D-Met^S(O)–Gly–Phe–NHNH–CO–CH_2CH_3	155—157	+11.1° (22, 0.43)	0.20	$C_{33}H_{46}N_6O_9S \cdot 2H_2O$	53.47 (53.64	6.45 6.81	10.71 11.37	4.60 4.34)
51	Boc–Tyr–D-Met–Gly–OEt	121—122	+16.8° (22, 0.40)	0.62	$C_{23}H_{35}N_3O_7S$	55.61 (55.51	7.14 7.09	8.21 8.44	6.20 6.44)
52	Boc–Tyr–D-Met–Gly–OH	184—186	+13.3° (22, 0.46)	0.24	$C_{21}H_{31}N_3O_7S$	53.59 (53.71	6.64 6.65	8.72 8.94	6.92 6.83)
53	Boc–Tyr–D-Met(O)–Gly–OH	185	+1.8° (21, 0.50)	0.16	$C_{21}H_{31}N_3O_8S$	51.88 (51.93	6.41 6.43	8.67 8.66	6.02 6.60)

54	Boc–Tyr–D-Met(O)–Gly–Phe–NH_2	151—152	−7.1° (22, 0.30)	0.15	$C_{30}H_{41}N_5O_8S \cdot H_2O$	55.30 (55.45	6.59 6.66	10.66 10.78	4.79 4.93)
55	Z–Phe–NH–CH_2CH_3	138—140	−11.2° (24, 0.50)	0.70	$C_{19}H_{22}N_2O_3$	69.95 (69.91	6.93 6.79	8.69 8.58)	
56	Boc–Tyr–D-Met(O)–Gly–Phe–NH–CH_2CH_3	194—195	−6.6° (24, 0.50)	0.33	$C_{32}H_{45}N_5O_8S$	58.51 (58.24	6.99 6.87	10.25 10.61	4.49 4.86)
57	Z–Tyr–D-Met(O)–Gly–Phe–OEt	162—163	−3.2° (21, 0.37)	0.42	$C_{35}H_{42}N_4O_9S$	60.45 (60.50	6.38 6.09	8.07 8.06	4.37 4.61)
58	Z–Tyr–D-Met(O)–Gly–Phe–$NHNH_2$	182—184	−13.4° (21, 0.44)	0.10	$C_{33}H_{40}N_6O_8S$	58.19 (58.21	6.01 5.92	11.86 12.34	4.60 4.71)
59	Z–Phe–NHNH–SO_2–CH_3	167—168	−1.1° (24, 0.46)	0.67	$C_{18}H_{21}N_3O_5S$	55.55 (55.22	5.38 5.40	10.71 10.73	7.89 8.19)
60	Boc–Tyr–D-Met(O)–Gly–Phe–NHNH–SO_2–CH_3	151—153	+2.0° (24, 0.40)	0.17	$C_{31}H_{44}N_6O_{10}S_2 \cdot 1/2H_2O$	50.70 (50.73	6.33 6.17	11.07 11.45	8.44 8.74)
61	Boc–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–CH_3	174—176	−2.7° (22, 0.44)	0.13	$C_{32}H_{44}N_6O_9S$	56.14 (55.80	6.70 6.44	11.89 12.20	4.25 4.65)
62	Z–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–$CH_2CH_2CH_3$	161—163	−8.8° (21, 0.34)	0.27	$C_{37}H_{46}N_6O_9S$	57.76 (57.79	6.02 6.29	10.81 10.93	3.96 4.17)
63	Z–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–$(CH_2)_4$–CH_3	170—172	−9.1° (21, 0.48)	0.34	$C_{39}H_{50}N_6O_9S$	59.93 (60.13	6.90 6.47	10.63 10.79	4.08 4.11)
64	Boc–Phe–NHNH–CO–CH_2–S–CH_3	151—152	−18.6° (21, 0.50)	0.66	$C_{17}H_{25}N_3O_4S$	55.43 (55.55	7.11 6.86	11.29 11.44	8.33 8.73)
65	Boc–Tyr–D-Met(O)–Gly–Phe–NHNH–CO–CH_2S–CH_3	154—156	−2.0° (22, 0.46)	0.25	$C_{33}H_{46}N_6O_9S_2$	54.06 (53.93	6.92 6.31	11.04 11.43	8.06 8.72)
66	Boc–D-Met–Gly–OEt	55—56	+12.5° (21, 0.67)	0.70	$C_{14}H_{26}N_2O_5S$	50.88 (50.28	7.99 7.83	8.46 8.37	9.42 9.59)
67	Boc–D-Met–Gly–OH	124—125	+12.3° (21, 0.59)	0.41	$C_{12}H_{22}N_2O_5S$	47.57 (47.04	7.28 7.23	9.07 9.14	9.94 10.46)
68	Boc–D-Met(O)–Gly–MePhe–NHNH–CO–CH_2CH_3	116—117	−25.7° (21, 0.38)	0.20	$C_{25}H_{39}N_5O_7S \cdot H_2O$	52.17 (52.49	6.89 7.22	11.73 12.25	4.80 5.60)
69	Boc–Tyr–D-Met(O)–Gly–MePhe–NHNH–$COCH_2CH_3$	138—139	−21.3° (21, 0.55)	0.20	$C_{34}H_{48}N_6O_9S \cdot H_2O$	55.37 (55.72	6.74 6.60	10.97 11.46	4.16 4.36)
70	Z–MeTyr(Bu^t)–D-Met(O)–Gly–Phe–NHNH–CO–CH_2CH_3	142—144	−30.0° (21, 0.50)	0.46	$C_{41}H_{54}N_6O_9S$	60.88 (61.02	6.92 6.74	10.20 10.41	3.66 3.97)

a) See "Experimental." *b*) MeOH. *c*) Cl, Found 5.05; Calcd 5.26. *d*) Cl, Found 4.48; Calcd 4.15. *e*) *Rf*[3] *f*) DCHA salt, ^{1}H-NMR ($CDCl_3$) δ: 2.88 (3H, s, SO_2–C<u>H</u>$_3$).
g) H_2O. *h*) *Rf*[5]. *i*) ^{1}H-NMR ($CDCl_3$) δ: 2.66 (3H, s, SO–C<u>H</u>$_3$). *j*) ^{1}H-NMR ($CDCl_3$) δ: 2.73 (3H, s, SO–C<u>H</u>$_3$).

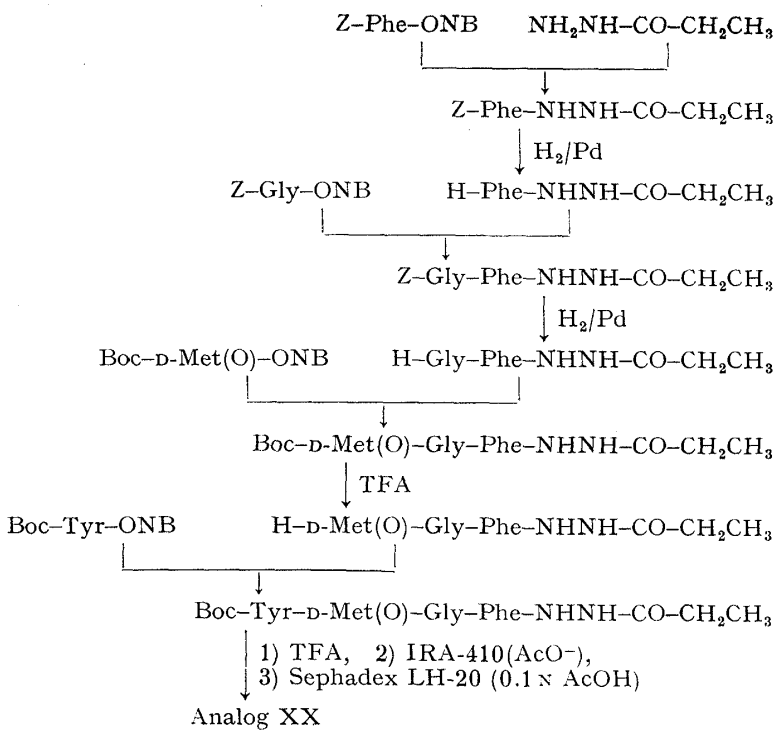

Fig. 3. Synthetic Route to Analog XX

Table II. Physicochemical Properties of Synthetic Analogs

Analog I:	$[\alpha]_D^{23}$ +26.7° (c=0.25, MeOH), Rf^3=0.41; Rf^4=0.63.[a] Amino acid analysis: Gly 1.00; Leu 1.05; Tyr 0.93; Phe 1.02 (82%)[b]
Analog II:	$[\alpha]_D^{27}$ +24.7° (c=0.28, MeOH), Rf^3=0.62; Rf^4=0.72. Amino acid analysis: Gly 1.00; Leu 0.97; Tyr 0.93; Phe 1.00 (88%).
Analog III:	$[\alpha]_D^{19}$ +16.3° (c=0.22, MeOH), Rf^3=0.66; Rf^4=0.79. Amino acid analysis: Gly 1.00; Leu 1.10; Tyr 1.02; Phe 1.07 (81%).
Analog IV:	$[\alpha]_D^{23}$ +22.6° (c=0.30, MeOH), Rf^3=0.44; Rf^4=0.62. Amino acid analysis: Gly 1.00; Nva 1.10; Tyr 0.99; Phe 1.07 (83%).
Analog V:	$[\alpha]_D^{23}$ −11.4° (c=0.35, MeOH), Rf^3=0.37; Rf^4=0.64. Amino acid analysis: Gly 1.00; Tyr 1.00; Phe 2.06 (91%).
Analog VI:	$[\alpha]_D^{23}$ +16.5° (c=0.26, MeOH), Rf^3=0.47; Rf^4=0.62. Amino acid analysis: Gly 1.00; Tyr 1.05; Phe 1.13; Lys 1.03 (88%).
Analog VII:	$[\alpha]_D^{23}$ +17.5° (c=0.38, MeOH), Rf^3=0.37; Rf^4=0.58. Amino acid analysis: Glu 1.03; Gly 1.00; Tyr 1.05; Phe 1.14 (80%). ^{1}H-NMR (D_2O), δ: 3.75 [3H, s, Glu(OCH_3)].
Analog VIII:	$[\alpha]_D^{23}$ +14.5° (c=0.35, MeOH), Rf^3=0.18; Rf^4=0.50. Amino acid analysis: Glu 1.09; Gly 1.00; Tyr 0.96; Phe 0.97 (81%).
Analog IX:	$[\alpha]_D^{25}$ +12.9° (c=0.28, MeOH), Rf^3=0.20; Rf^4=0.52. Amino acid analysis: Glu 1.08; Gly 1.00; Tyr 0.96; Phe 1.00 (82%).
Analog X:	$[\alpha]_D^{24}$ +14.5° (c=0.35, MeOH), Rf^3=0.23; Rf^4=0.58. Amino acid analysis: Glu 1.09; Gly 1.00; Tyr 0.94; Phe 0.98 (84%).
Analog XI:	$[\alpha]_D^{24}$ +13.3° (c=0.30, MeOH), Rf^3=0.18; Rf^4=0.51. Amino acid analysis: Glu 1.09; Gly 1.00; Tyr 0.94; Phe 1.02 (89%).
Analog XII:	$[\alpha]_D^{25}$ +2.5° (c=0.36, MeOH), Rf^3=0.17; Rf^4=0.50. Amino acid analysis: Asp 1.07; Gly 1.00; Tyr 1.04; Phe 1.02 (90%).
Analog XIII:	$[\alpha]_D^{23}$ +11.4° (c=0.31, MeOH), Rf^3=0.21; Rf^4=0.51. Amino acid analysis: Ser 0.93; Gly 1.00; Tyr 0.99; Phe 1.01 (83%).
Analog XIV:	$[\alpha]_D^{24}$ −10.0° (c=0.30, MeOH), Rf^3=0.20; Rf^4=0.51. Amino acid analysis: Ser 0.97; Gly 1.00; Tyr 0.98; Phe 1.02 (78%).
Analog XV:	$[\alpha]_D^{25}$ +12.0° (c=0.25, MeOH), Rf^3=0.24, Rf^4=0.58. Amino acid analysis: Thr 1.02; Gly 1.00; Tyr 0.98; Phe 1.04 (88%).

Analog XVI: $[\alpha]_D^{23}$ +26.1° (c=0.36, MeOH), Rf^3=0.05; Rf^4=0.46. Amino acid analysis: Gly 1.00; Tyr 1.09; Phe 1.09; Lys 1.09 (82%).

Analog XVII: $[\alpha]_D^{25}$ +22.4° (c=0.42, MeOH), Rf^3=0.07; Rf^4=0.44. Amino acid analysis: Gly 1.00; Tyr 0.90; Phe 0.98; Arg 1.04 (80%).

Analog XVIII: $[\alpha]_D^{25}$ −5.9° (c=0.27, MeOH), Rf^3=0.05; Rf^4=0.48. Amino acid analysis: Gly 1.00; Tyr 1.04; Phe 1.00; His 0.96 (85%).

Analog XIX: $[\alpha]_D^{25}$ −3.0° (c=0.36, MeOH), Rf^3=0.24; Rf^4=0.63. Amino acid analysis: Glu 1.02; Gly 1.00; Tyr 1.09; Phe 0.99 (83%).

Analog XX: $[\alpha]_D^{21}$ +24.3° (c=0.35, MeOH), Rf^3=0.19; Rf^4=0.48. Amino acid analysis:[c] Gly 1.00; Met 1.03; Tyr 1.07; Phe 1.05 (90%). ^{1}H-NMR (D_2O), δ: 2.65(3H, s, SO–C$\underline{H}_3$).

Analog XXI: $[\alpha]_D^{21}$ +20.0° (c=0.20, MeOH), Rf^3=0.46, Rf^4=0.68. Amino acid analysis: Gly 1.00; Met 0.77; Tyr 0.80; Phe 1.02 (85%). ^{1}H-NMR (D_2O), δ: 2.20 (3H, s, S–CH_3 of Met).

Analog XXII: $[\alpha]_D^{23}$ +19.0° (c=0.33, MeOH), Rf^3=0.22; Rf^4=0.63. Amino acid analysis: Gly 1.00; Tyr 0.95; Phe 1.05 (84%). ^{1}H-NMR (D_2O), δ: 3.09 [3H, s, SO_2–C$\underline{H}_3$ of Met(O_2)].

Analog XXIII: $[\alpha]_D^{24}$ +18.7° (c=0.15, MeOH), Rf^3=0.10; Rf^5=0.39. Amino acid analysis: Gly 1.00; Met 0.32; Tyr 0.81; Phe 1.00 (79%).

Analog XXIV: $[\alpha]_D^{25}$ −3.8° (c=0.40, MeOH), Rf^3=0.20; Rf^4=0.51. Amino acid analysis:[c] Gly 1.00; Met 0.97; Tyr 0.94; Phe 0.95 (84%).

Analog XXV: $[\alpha]_D^{22}$ +47.8° (c=0.29, MeOH), Rf^3=0.20; Rf^4=0.51. Amino acid analysis:[c] Gly 1.00; Met 0.96; Tyr 0.95; Phe 0.99 (79%.)

Analog XXVI: $[\alpha]_D^{22}$ +33.6° (c=0.50, MeOH), Rf^3=0.11; Rf^4=0.42. Amino acid analysis:[c] Gly 1.00; Met 0.98; Tyr 0.97; Phe 0.99 (80%).

Analog XXVII: $[\alpha]_D^{24}$ +30.9° (c=0.36, MeOH), Rf^3=0.24; Rf^4=0.44. Amino acid analysis: Gly 1.00; Met 0.34; Tyr 0.95; Phe 1.00 (81%).

Analog XXVIII: $[\alpha]_D^{21}$ +33.0° (c=0.34, MeOH), Rf^3=0.14; Rf^4=0.51. Amino acid analysis: Gly 1.00; Met 0.33; Tyr 1.00; Phe 1.03 (85%).

Analog XXIX: $[\alpha]_D^{24}$ +20.7° (c=0.29, MeOH), Rf^3=0.17; Rf^4=0.49. Amino acid analysis:[c] Gly 1.00; Met 0.97; Tyr 0.93; Phe 0.99 (89%).

Analog XXX: $[\alpha]_D^{22}$ +19.6° (c=0.27, MeOH), Rf^3=0.12; Rf^4=0.46. Amino acid analysis:[c] Gly 1.00; Met 0.97; Tyr 0.99; Phe 1.08 (88%).

Analog XXXI: $[\alpha]_D^{21}$ +23.7° (c=0.32, MeOH), Rf^3=0.20; Rf^4=0.52. Amino acid analysis: Gly 1.00; Met 0.32; Tyr 0.99; Phe 1.08 (78%).

Analog XXXII: $[\alpha]_D^{21}$ +25.7° (c=0.35, MeOH), Rf^3=0.45; Rf^4=0.71. Amino acid analysis: Gly 1.00; Met 0.33; Tyr 0.89; Phe 1.00 (89%).

Analog XXXIII: $[\alpha]_D^{22}$ +15.4° (c=0.33, MeOH), Rf^3=0.18; Rf^4=0.48. Amino acid analysis: Gly 1.00; Met 0.88; Tyr 0.19[d]; Phe 0.99 (84%).

Analog XXIV: $[\alpha]_D^{21}$ +4.1° (c=0.21, MeOH), Rf^3=0.18; Rf^4=0.49. Amino acid analysis:[c] Gly 1.00; Met 1.00; Tyr 0.90; MePhe 1.30 (88%). ^{1}H-NMR (D_2O), δ: 2.65 [3H, s, SO–C$\underline{H}_3$ of Met(O)], 2.95 (3H, s, N–C$\underline{H}_3$ of MePhe).

Analog XXXV: $[\alpha]_D^{21}$ +19.5° (c=0.20, MeOH), Rf^3=0.18; Rf^4=0.48. Amino acid analysis: Gly 1.00; Met 0.35; MeTyr 0.80; Phe 1.10 (84%). ^{1}H-NMR (D_2O), δ: 2.60 (3H, s, N–C$\underline{H}_3$ of MeTyr), 2.65 [3H, s, SO–C$\underline{H}_3$ of Met(O)].

a) Solvent systems used for TLC are described in "Experimental." *b*) Average recovery.
c) See ref. 20. *d*))See ref. 22.

analog and was as potent as FK-33-824,[16] surprisingly, without N-methylation of Phe at position 4. Substitution by D-Gln, D-Glu(NH–CH_3), or D-Thr also gave analogs with appreciably increased activity.

Replacement of D-Ala by a D-amino acid residue of basic character such as D-Glu(NHNH_2), D-Lys, D-Arg and D-Met$^+$(CH_3) resulted in analogs with almost equal activity. On the other hand, the analog XIX which possesses an acidic D-amino acid residue, D-Glu, at position 2 did not show any analgesic activity even at a subcutaneous dose of 10 mg/kg. Interestingly, the active D-Arg–analog resembles kyotorphin with the sequence H–Tyr–Arg–OH, which was reported recently by Takagi *et al.*[17] to be another morphine-like peptide in the brain.

The D-Leu^2–, D-Phe^2–, D-Lys(Cl–Z)^2-analogs were much less potent; two to five times less potent than the D-Ala–analog. The results agree with those obtained with pentapeptide

TABLE III. Analgesic Activities of Tetrapeptide Acyl-hydrazide Derivatives altered at Position 2 H–Tyr–X–Gly–Phe–NHNH–CO–Y

Compound No.	X	Y	Relative potency[a] *s.c.*
I	D-Leu	CH_3	0.25
II	D-Leu	$CH_2CH_2CH_3$	0.1
III	D-Leu	$OC(CH_3)_3$	0.01
IV	D-Nva	CH_3	0.5
V	D-Phe	CH_3	0.05
VI	D-Lys(Cl–Z)	CH_3	0.05
VII	D-Glu(OMe)	CH_3	0.1
VIII	D-Gln	CH_3	1.0
IX	D-Gln	CH_2CH_3	2.0
X	D-Glu($NHCH_3$)	CH_2CH_3	1.0
XI	D-Glu($NHNH_2$)	CH_2CH_3	0.5
XII	D-Asn	CH_3	0.25
XIII	D-Ser	CH_3	0.25
XIV	Ser	CH_3	<0.05
XV	D-Thr	CH_3	1.0
XVI	D-Lys	CH_3	0.25
XVII	D-Arg	CH_3	0.5
XVIII	D-His	CH_2CH_3	0.25
XIX	D-Glu	CH_2CH_3	<0.05
XX	D-Met(O)	CH_2CH_3	4.0
XXI	D-Met	CH_2CH_3	0.5
XXII	D-Met(O_2)	CH_3	0.5
XXIII	D-$Met^+(CH_3)$	CH_2CH_3	0.25
XXIV	D-Met^R(O)	CH_2CH_3	2.0
XXV	D-Met^S(O)	CH_2CH_3	4.0
	D-Ala	CH_2CH_3	0.5[b]

a) Morphine=1; minimum effective dose of morphine·HCl=0.5 mg/kg (weight/weight).
b) See ref. 3.

analogs in the stereospecific binding test relative to Met-enkephalin.[18] The reduced activity of the above analogs seems to be due to the bulky side chain at position 2, which might sterically hinder the peptide-receptor interaction. Replacement of D-Ser at position 2 of the analog XIII by its L-isomer gave an analog with no activity, as expected from the study of Terenius *et al.*[19] with Met-enkephalin.

The reason for the remarkably enhanced analgesic activity of the D-Met(O)-analog is not known at the present time. It may, however, be reasonable to consider that the side chain of the D-amino acid residue at position 2 not only protects the peptide from enzymatic degradation at the N-terminal part, but also provides an extra binding site for the receptor; according to Fig. 4, both the size and partially hydrophilic nature of the side chain are critical. The difference in activity between the D-Met^R(O)-analog and the D-Met^S(O)-analog indicates a steric contribution of the side chain, presumably when the peptide approaches the receptor.

As shown in Table IV, most of the D-Met(O)–analogs were more than four times as potent as the D-Ala–analog, and N-methylation of Phe at position 4 of the analog XX further in-

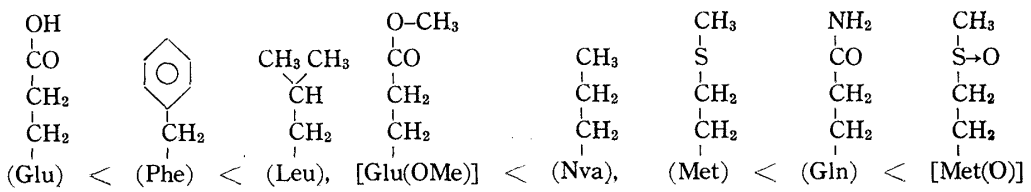

Fig. 4. Structure-activity Relationship in the Side Chain Moiety of D-Amino Acid at Position 2 of Tetrapeptide Acyl-hydrazide

creased the potency to yield a derivative (XXXIV) that was six times more active than morphine. The analog XXXIV might possess a prolonged and powerful analgesic activity in man, and clinical studies seem desirable.

TABLE IV. Analgesic Activities of D-Met(O)^2-analogs

Compound No.	H–Tyr–D-Met(O)–Gly–Phe–X X	Relative potency (morphine=1) hot-plate test in *s.c.*
XXVI	NH_2	1.0
XXVII	$NH\text{-}CH_2CH_3$	0.5
XXVIII	$NHNH_2$	2.0
XXIX	$NHNH\text{-}SO_2CH_3$	0.5
XXX	$NHNH\text{-}CO\text{-}CH_3$	2.0
XXXI	$NHNH\text{-}CO\text{-}CH_2CH_2CH_3$	1.0
XXXII	$NHNH\text{-}CO\text{-}CH_2CH_2CH_2CH_2CH_3$	0.5
XXXIII	$NHNH\text{-}CO\text{-}CH_2SCH_3$	4.0
XXXIV	H–Tyr–D-Met(O)–Gly–MePhe–$NHNH\text{-}COCH_2CH_3$	6.0
XXXV	H–MeTyr–D-Met(O)–Gly–Phe–$NHNH\text{-}COCH_2CH_3$	2.0
	H–Tyr–D-Ala–Gly–Phe–$NHNH\text{-}COCH_2CH_3$[a]	0.5
	H–Tyr–D-Ala–Gly–Phe–$NHNH\text{-}COCH_2CH_2CH_2CH_3$[a]	0.5
	H–Tyr–D-Ala–Gly–MePhe–Met(O)-ol[b]	4.0

a) See ref. 3. *b*) FK-33-824, see ref. 16.

Experimental

General experimental methods were essentially the same as described in the previous papers.[1,3] Thin layer chromatography was performed on silica gel (precoated silica gel plate $60F_{254}$, Merck). The solvents employed were: Rf^1, $CHCl_3$–MeOH–AcOH (9:1:0.5); Rf^2, $CHCl_3$–MeOH–AcOH (8:2:0.5); Rf^3, AcOEt–pyridine–AcOH–H_2O (60:20:6:11); Rf^4, *n*-butanol–AcOH–H_2O (4:1:1); Rf^5, AcOEt–*n*-butanol–AcOH–H_2O (1:1:1:1).

Z-Gly-Phe-NHNH-CO-CH$_3$ (**6**)——Z–Phe–$NHNH\text{-}CO\text{-}CH_3$[3] (42.4 g) was hydrogenated over Pd-black catalyst in MeOH (400 ml). The mixture was filtered to remove the catalyst and the filtrate was evaporated to dryness. The residue was dissolved in DMF (100 ml), and Z–Gly–ONB[8] (44.0 g) was added. The mixture was stirred for 10 h at room temperature and evaporated to dryness. The residue was treated with ether to give a powder, which was collected by filtration and crystallized from aqueous CH_3CN; yield, 45.2 g (91%), mp 154—155°C, $[\alpha]_D^{23}$ −0.9° (c=0.50, DMF), Rf^1=0.52. *Anal.* Calcd for $C_{21}H_{24}N_4O_5$: C, 61.15; H, 5.87; N, 13.59. Found: C, 61.09; H, 5.69; N, 13.18.

Z-D-Nva-Gly-Phe-NHNH-CO-CH$_3$ (**7**)——The compound **6** (5.0 g) was hydrogenated over Pd-black catalyst in MeOH (100 ml). The catalyst was filtered off and the filtrate was evaporated to dryness to give H–Gly–Phe–$NHNH\text{-}CO\text{-}CH_3$ as crystals (yield, 3.5 g). This compound (0.89 g) and Z–D-Nva–ONB [prepared from Z–D-Nva–OH (0.80 g) and HONB (0.63 g) by the DCC method] were dissolved in DMF (10 ml). The mixture was stirred for 10 h at room temperature and evaporated to dryness. The residue was treated with ether to give a powder, which was crystallized from CH_3CN; yield, 1.3 g (80%), mp 235—237°C, $[\alpha]_D^{23}$ +3.4° (c=0.45, DMF), Rf^1=0.44. *Anal.* Calcd for $C_{26}H_{33}N_5O_6$: C, 61.04; H, 6.50; N, 13.69. Found: C, 61.33; H, 6.62; N, 13.66.

Z-Tyr-D-Nva-Gly-Phe-NHNH-CO-CH$_3$ (**8**)——The compound **7** (0.82 g) was hydrogenated over Pd-black in MeOH (50 ml). The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in DMF (10 ml), and Z–Tyr–ONB (0.86 g) was added to the solution. The mixture was stirred for 10 h at room temperature and evaporated to dryness. The residue was treated with ether to give a powder, which was crystallized from CH_3CN; yield, 0.85 g (71%), mp 210—211°C, $[\alpha]_D^{23}$ −18.3° (c=0.48, DMF), Rf^1=0.38. *Anal.* Calcd for $C_{35}H_{42}N_6O_8 \cdot 1/2H_2O$: C, 61.48; H, 6.33; N, 12.29. Found: C, 61.39; H, 6.34; N, 12.09.

Z-Gly-Phe-NHNH-CO-CH$_2$CH$_3$ (**17**)——Z–Phe–$NHNH\text{-}CO\text{-}CH_2CH_3$[1] (2.2 g) was dissolved in MeOH (50 ml) and catalytic reduction was carried out over Pd-black. After removal of the catalyst by filtration, the MeOH was evaporated off and the residue was dissolved in DMF (10 ml). Z–Gly–ONB[8] (2.2 g) was added, and the mixture was stirred at room temperature overnight. The DMF was evaporated off and the residue was treated in the usual manner (extraction with AcOEt, followed by washing of the extract with water, drying over anhydr. Na_2SO_4 and evaporation to dryness). The resulting crystals were collected by filtration and recrystallized from AcOEt–CH_3CN; yield, 1.8 g (71%), mp 151—152°C, $[\alpha]_D^{21}$ −1.2° (c=0.50,

DMF), $Rf^1=0.46$. *Anal.* Calcd for $C_{22}H_{26}N_4O_5$: C, 61.96; H, 6.15; N, 13.14. Found: C, 62.25; H, 6.23; N, 12.85.

Boc-D-Met(O)-OH (37)——Boc-D-Met-OH (37.1 g) was dissolved in CH_3CN (200 ml), and 30% aqueous H_2O_2 (19 ml) was added at 0°C. The mixture was stirred for 8 h at room temperature, then *n*-butanol (300 ml) and 5% aqueous NaCl were added to the reaction mixture. The *n*-butanol layer was washed with water and evaporated to dryness. The residue was crystallized from ether; yield, 32.0 g (80%), mp 125—126°C, $[\alpha]_D^{23}+7.2°$ ($c=0.50$, DMF), $Rf^1=0.33$. *Anal.* Calcd for $C_{10}H_{19}NO_5S$: C, 45.26; H, 7.21; N, 5.27; S, 12.08. Found: C, 45.19; H, 7.52; N, 5.50; S, 11.75. ^{1}H-NMR ($CDCl_3$), δ: 2.66 and 2.77 (3H, s, SO–C$\underline{H}_3$).

Boc-D-Met(O)-Gly-Phe-NHNH-CO-CH_2CH_3 (38)——The compound **17** (1.0 g) was dissolved in MeOH (50 ml) and hydrogenated. After removal of the catalyst, the MeOH was evaporated off and the residue was dissolved in CH_3CN (20 ml). Boc-D-Met(O)–ONB [prepared from **37** (0.63 g)] was added to the solution, and the mixture was stirred at room temperature for 10 h. The reaction mixture was evaporated to dryness and the residue was extracted with *n*-butanol (50 ml). The extract was washed with H_2O. The *n*-butanol was evaporated off and the residue was crystallized from ether; yield, 1.1 g (88%), mp 121—122°C, $[\alpha]_D^{21}$ 0° ($c=0.50$, DMF), $Rf^1=0.40$. *Anal.* Calcd for $C_{24}H_{37}N_5O_7S\cdot 1/2H_2O$: C, 52.53; H, 6.97; N, 12.76; S, 5.84. Found: C, 52.41; H, 6.99; N, 12.46; S, 5.61.

Boc-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-CH_2CH_3 (39)——The compound **38** (0.82 g) was dissolved in TFA (10 ml) and the solution was allowed to stand at room temperature for 20 min. The TFA was evaporated off and the residue was treated with ether, then collected by filtration. The powder thus obtained was dissolved in DMF (10 ml), and Boc–Tyr–ONB (0.71 g) and TEA (0.3 ml) were added to the solution. After the mixture had been stirred at room temperature overnight, the DMF was evaporated off. The residue was treated with ether to give a powder, which was crystallized from ethanol–CH_3CN; yield, 0.75 g (72%), mp 134—135°C, $[\alpha]_D^{21}$ 0° ($c=0.50$, DMF), $Rf^1=0.20$. *Anal.* Calcd for $C_{33}H_{46}N_6O_9S\cdot H_2O$: C, 54.98; H, 6.71; N, 11.66; S, 4.44. Found: C, 54.72; H, 6.82; N, 11.16; S, 4.43.

Z-Tyr-D-Met(O)-Gly-Phe-OEt (57)——H–Phe–OEt·HCl (4.8 g) was dissolved in a mixture of CH_3CN (50 ml) and TEA (2.8 ml). Next, Z–Gly–ONB (7.4 g) was added, and the mixture was stirred at room temperature overnight. The solvent was evaporated off and the residue was extracted with AcOEt (200 ml), and treated in the usual manner. The resulting oil was dissolved in MeOH (100 ml) for catalytic reduction with Pd-black. After removal of the catalyst by filtration, the MeOH was evaporated and the residue was dissolved in CH_3CN (50 ml). Boc–D-Met–ONB [prepared from Boc–D-Met–OH (4.7 g) and HONB (3.7 g)] was added to the solution, and the whole was stirred at room temperature overnight. Then 1 N HCl (0.1 ml) and 30% aqueous H_2O_2 (2.2 ml) were added, and the mixture was allowed to stand at room temperature for 10 h. The solvent was evaporated and the residue was extracted with AcOEt (200 ml), followed by treatment in the usual manner. The resulting residue was dissolved in TFA (30 ml). The solution was allowed to stand at room temperature for 20 min, then evaporated to dryness. The residue was treated with ether to give a powder, which was dissolved in CH_3CN (60 ml). TEA (2.5 ml) and Z–Tyr–ONB (7.2 g) were added to the solution, and the mixture was stirred overnight. The solvent was evaporated and the residue was extracted with AcOEt (200 ml), followed by treatment in the usual manner. The resulting crystals were collected by filtration and recrystallized from AcOEt; yield, 6.9 g (total 50%), mp 162—163°C, $[\alpha]_D^{21}$ −3.2° ($c=0.37$, DMF), $Rf^1=0.42$. *Anal.* Calcd for $C_{35}H_{42}N_4O_9S$: C, 60.50; H, 6.09; N, 8.06; S, 4.61. Found: C, 60.45; H, 6.38; N, 8.07; S, 4.37.

Z-Tyr-D-Met(O)-Gly-Phe-$NHNH_2$ (58)——The compound **57** (6.0 g) was dissolved in a mixture of ethanol (20 ml) and DMF (2 ml), and $NH_2NH_2\cdot H_2O$ (2 ml) was added to the solution, which was then allowed to stand at room temperature for 2 d. The solvent was evaporated, and ether was added to the residue. The resulting crystals were boiled with CH_3CN as a washing procedure, and collected by filtration; yield, 5.0 g (85%), mp 182—184°C, $[\alpha]_D^{21}$ −13.4° ($c=0.44$, DMF), $Rf^1=0.10$. *Anal.* Calcd for $C_{33}H_{40}N_6O_8S$: C, 58.21; H, 5.92; N, 12.34; S, 4.71. Found: C, 58.19; H, 6.01; N, 11.86; S, 4.60.

Z-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-$CH_2CH_2CH_3$ (62)——The compound **58** (0.51 g) and *n*-butyric acid (0.08 ml) were dissolved in DMF (5 ml). HOBT (120 mg) and DCC (190 mg) were added at 0°C, and the mixture was stirred at 0°C for 5 h then at room temperature overnight. The DMF was evaporated and the residue was treated with ether to give a powder, which was crystallized from aqueous ethanol; yield, 410 mg (73%), mp 161—163°C, $[\alpha]_D^{21}$ −8.8° ($c=0.34$, DMF), $Rf^1=0.27$. *Anal.* Calcd for $C_{37}H_{46}N_6O_9S\cdot H_2O$: C, 57.79; H, 6.29; N, 10.93; S, 4.17. Found: C, 57.76; H, 6.02; N, 10.81; S, 3.96.

Z-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-$CH_2CH_2CH_2CH_2CH_3$ (63)——From **58** (0.51 g) and *n*-caproic acid (0.09 ml), the desired compound was obtained in a similar manner to that described for the synthesis of 62; yield, 430 mg (80%), mp 170—172°C, $[\alpha]_D^{21}$ −9.1° ($c=0.48$, DMF), $Rf^1=0.34$. *Anal.* Calcd for $C_{39}H_{50}N_6O_9S$: C, 60.13; H, 6.47; N, 10.79; S, 4.11. Found: C, 59.93; H, 6.90; N, 10.63; S, 4.08.

Boc-D-Met-Gly-OEt (66)——DCC (9.0 g) was added to a solution of Boc-D-Met–OH (10.0 g) and HONB (7.9 g) in THF (100 ml), and the solution was stirred at 0°C for 6 h. The DC-urea precipitate was filtered off and H–Gly–OEt·HCl (5.9 g) and TEA (5.6 g) were added to the solution, which was then stirred at room temperature overnight. The solvent was evaporated and the residue was extracted with AcOEt (100 ml), followed by treatment in the usual manner. The residue thus obtained was crystallized from AcOEt–pet. ether; yield, 9.8 g (74%), mp 55—56°C, $[\alpha]_D^{21}$ +12.5° ($c=0.67$, DMF), $Rf^1=0.70$. *Anal.* Calcd for $C_{14}H_{26}N_2O_5S$:

C, 50.28; H, 7.83; N, 8.37; S, 9.59. Found: C, 50.88; H, 7.99; N, 8.46; S, 9.42.

Boc-D-Met-Gly-OH (67)——A solution of **66** (4.7 g) in ethanol (20 ml) was treated with 1 N NaOH (16 ml) at 0°C. After the mixture had been stirred at room temperature for 1 h, the ethanol was evaporated. The residue, after addition of aqueous citric acid to neutralize it, was extracted with AcOEt (100 ml) and treated in the usual manner. The residue obtained was crystallized with pet.ether and recrystallized from AcOEt–pet.ether; yield, 3.8 g (71%), mp 124—125°C, $[\alpha]_D^{21}$ +12.3° (c=0.59, DMF), Rf^1=0.41. *Anal.* Calcd for $C_{12}H_{22}N_2O_5S$: C, 47.04; H, 7.23; N, 9.14; S, 10.46. Found: C, 47.57; H, 7.28; N, 9.07; S, 9.94.

Boc-D-Met(O)-Gly-MePhe-NHNH-CO-CH_2CH_3 (68)——Z-MePhe-OH[11] (4.7 g) was dissolved in MeOH (50 ml) containing 6 N HCl–dioxane (5 ml), and the solution was allowed to stand at room temperature overnight. The MeOH was evaporated and the residue was extracted with AcOEt (100 ml), followed by treatment in the usual manner. The resulting residue was dissolved in MeOH (50 ml), and to this was added $NH_2NH_2 \cdot H_2O$ (1.5 ml). The mixture was allowed to stand at room temperature for 3 d, then the MeOH was evaporated and the residue was extracted with AcOEt (100 ml), followed by treatment in the usual manner. The residue was dissolved in THF (20 ml), and propionic anhydride (1.4 ml) and pyridine (0.9 ml) were added at 0°C. After the mixture had been stirred at room temperature for 5 h, the THF was evaporated and the residue was extracted with AcOEt (100 ml), followed by treatment in the usual manner. The resulting oily product [Z–MePhe–NHNH–CO–CH_2CH_3 (2.3 g)] was dissolved in MeOH (50 ml) and hydrogenated over Pd-black. The catalyst was removed by filtration, the MeOH was evaporated and the residue was dissolved in DMF (10 ml). On the other hand, DCC (1.4 g) was added to a mixture of **67** (1.85 g) and HONB (1.2 g) in CH_3CN (20 ml) at 0°C, and the mixture was stirred at 0°C for 4 h. The DC-urea precipitate was filtered off, the filtrate was combined with the above-mentioned amine component and the mixture was stirred overnight. The solvent was evaporated and the residue was extracted with AcOEt (100 ml). The AcOEt solution was washed with H_2O and concentrated to 20 ml, then acetic acid (5 ml) and 30% aqueous H_2O_2 (0.5 ml) were added. After the mixture had been stirred for 5 h, it was extracted with *n*-butanol (50 ml). The extract was washed with H_2O. The *n*-butanol was evaporated and the residue was treated with ether to give a powder; yield, 1.9 g (58%), mp 116—117°C, $[\alpha]_D^{21}$ −25.7° (c=0.38, DMF), Rf^1=0.20. *Anal.* Calcd for $C_{25}H_{39}N_5O_7S \cdot H_2O$: C, 52.49; H, 7.22; N, 12.25; S, 5.60. Found: C, 52.17; H, 6.89; N, 11.73; S, 4.80.

Boc-Tyr-D-Met(O)-Gly-MePhe-NHNH-CO-CH_2CH_3 (69)——**68** (420 mg) was dissolved in TFA (4 ml) and the solution was allowed to stand at room temperature for 20 min. The TFA was evaporated and the residue was treated with ether to give a powder, which was collected by filtration and dried. The powder was dissolved in DMF (10 ml) and the solution was cooled to 0°C. Next, TEA (0.14 ml) and Boc–Tyr–ONB (330 mg) were added at 0°C, and the mixture was stirred at room temperature overnight. The DMF was evaporated and the residue was extracted with *n*-butanol (50 ml). The extract was washed with H_2O. The *n*-butanol was evaporated and the residue was treated with ether to give a powder, which was reprecipitated from MeOH–ether; yield, 310 mg (68%), mp 138—139°C, $[\alpha]_D^{21}$ −21.3° (c=0.55, DMF), Rf^1=0.20. *Anal.* Calcd for $C_{34}H_{48}N_6O_9S \cdot H_2O$: C, 55.72; H, 6.60; N, 11.46; S, 4.36. Found: C, 55.37; H, 6.74; N, 10.97; S, 3.19.

Synthesis of Tetrapeptide Analogs of Enkephalin. H-Tyr-D-Nva-Gly-Phe-NHNH-CO-CH_3 (IV)——The compound **8** (0.40 g) was dissolved in MeOH (50 ml) and hydrogenated over Pd-black. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in a small amount of 0.1 N aqueous acetic acid and applied to a column of Sephadex LH-20 (2.5×120 cm), which was eluted with the same solvent. The desired fractions (320—335 ml) were combined and lyophilized; yield, 160 mg, $[\alpha]_D^{23}$ +22.6° (c=0.30, MeOH), Rf^3=0.44; Rf^4=0.62. Amino acid analysis: Gly 1.00; Nva 1.10; Tyr 0.99; Phe 1.07 (average recovery 83%).

H-Tyr-D-Arg-Gly-Phe-NHNH-CO-CH_3 (XVII)——Boc-D-Arg(Tos)–Gly–Phe–NHNH–CO–CH_3 (**33**) and Z–Tyr-D-Arg(Tos)–Gly–Phe–NHNH–CO–CH_3 (**34**) were synthesized in a similar manner to that described for the synthesis of the compounds **7** and **8**, respectively (see Table I). The compound **34** (310 mg) was dissolved in anhydrous HF (*ca.* 6 ml) together with anisole (0.4 ml) at −40°C, and the mixture was stirred at 0°C for 50 min. Volatile compounds were evaporated, and the residue was dissolved in H_2O (20 ml). The solution was washed with ether and the aqueous layer was applied to a column (2×5 cm) of Amberlite IRA-410 (AcO^-). The passed solution and washings were combined and lyophilized. The powder obtained was dissolved in H_2O (2 ml) and applied to a column (2.5×120 cm) of Sephadex LH-20, which was eluted with 0.1 N aqueous acetic acid. The fractions (280—295 ml) were combined and lyophilized; yield, 150 mg, $[\alpha]_D^{25}$ +22.4° (c=0.42, MeOH), Rf^3=0.07; Rf^4=0.44. Amino acid analysis: Gly 1.00; Tyr 0.90; Phe 0.98; Arg 1.04 (average recovery 80%).

H-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-CH_2CH_3 (XX)——The compound **39** (0.40 g) was dissolved in TFA (4 ml), and the mixture was allowed to stand at room temperature for 20 min. The TFA was evaporated and the residue was treated with ether to give a powder, which was dried and dissolved in H_2O (30 ml). The solution was passed through a column (2×6 cm) of Amberlite IRA-410 (AcO^-). The eluate and washings were combined and lyophilized. The resulting powder was dissolved in a small amount of 0.1 N aqueous acetic acid and applied to a column (2.4×120 cm) of Sephadex LH-20, which was eluted with the same solvent. The fractions from 310 to 330 ml were collected and lyophilized; yield, 310 mg, $[\alpha]_D^{21}$ +24.3° (c=

0.35, MeOH), $Rf^3=0.19$; $Rf^4=0.48$. Amino acid analysis:[20] Gly 1.00; Met 1.03; Tyr 1.07; Phe 1.05 (average recovery 90%). ^{1}H-NMR (D_2O), δ: 1.15 (3H, t, CO–$CH_2C\underline{H}_3$), 2.35 (2H, q, CO–$C\underline{H}_2CH_3$), 2.65 [3H, s, SO–CH_3 of Met(O)].[21]

H-Tyr-D-Met-Gly-Phe-NHNH-CO-CH_2CH_3 (XXI)——The analog XX (100 mg) was treated with 4% aqueous thioglycolic acid (6 ml) at 50°C for 18 h, then applied to a column (2.5×120 cm) of Sephadex G-25, which was eluted with 30% aqueous acetic acid. The desired fractions (270—320 ml) were collected and lyophilized; yield, 80 mg, $[\alpha]_D^{21}$ +20.0° ($c=0.20$, MeOH), $Rf^3=0.46$; $Rf^4=0.68$. Amino acid analysis: Gly 1.00; Met 0.77; Tyr 0.80; Phe 1.02 (average recovery 85%). ^{1}H-NMR (D_2O), δ: 2.20 (3H, s, S–$C\underline{H}_3$ of Met).

H-Tyr-D-Met$^+$(CH_3)-Gly-Phe-NHNH-CO-CH_2CH_3 (XXIII)——The analog XXI (60 mg) was dissolved in 0.001 N HCl (5 ml), and to this was added CH_3I (0.3 ml) at 0°C. The mixture was stirred at room temperature overnight and evaporated at 10°C to a small volume. This was passed through a column of Amberlite IRA-410 (AcO^-, 2×3 cm). The eluate and washings were combined and lyophilized. The powder thus obtained was dissolved in a small amount of 0.1 N aqueous acetic acid and applied to a column (2.5×120 cm) of Sephadex LH-20, which was eluted with the same solvent. The fractions (270—285 ml) were combined and lyophilized; yield, 40 mg $[\alpha]_D^{24}$ +18.7° ($c=0.15$, MeOH), $Rf^3=0.10$; $Rf^5=0.39$. Amino acid analysis: Gly 1.00; Met 0.32; Tyr 0.81; Phe 1.00 (average recovery 79%).

H-Tyr-D-Met(O)-Gly-Phe-$NHNH_2$ (XXVIII)——The compound **58** (300 mg) was dissolved in methanesulfonic acid (3 ml) in the presence of anisole (0.3 ml). The solution was allowed to stand at room temperature for 30 min, then ether was added to the solution and the mixture was allowed to stand at −20°C for 30 min. After removal of the supernatant solution, the oily product was dissolved in H_2O (30 ml) and passed through a column (2×6 cm) of Amberlite IRA-410 (AcO^-). The eluate and washings were combined and lyophilized. The powder obtained was dissolved in a small amount of 0.1 N aqueous acetic acid. The solution was applied to a column (2.5×120 cm) of Sephadex LH-20, which was eluted with the same solvent. The fractions from 270 ml through 285 ml were combined and lyophilized; yield, 140 mg, $[\alpha]_D^{21}$ +33.0° ($c=0.34$, MeOH), $Rf^3=0.14$; $Rf^4=0.51$. Amino acid analysis: Gly 1.00; Met 0.33; Tyr 1.00; Phe 1.03 (average recovery 85%).

H-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-$CH_2CH_2CH_3$ (XXXI)——From **62** (300 mg), the desired compound was obtained in a similar manner to that described for the synthesis of the analog XXVIII; yield, 110 mg, $[\alpha]_D^{21}$ +23.7° ($c=0.32$, MeOH), $Rf^3=0.20$; $Rf^4=0.52$. Amino acid analysis: Gly 1.00; Met 0.32; Tyr 0.99; Phe 1.08 (average recovery 78%).

H-Tyr-D-Met(O)-Gly-Phe-NHNH-CO-$CH_2CH_2CH_2CH_2CH_3$ (XXXII)——From **63** (350 mg), the desired compound was synthesized in a similar manner to that described for the synthesis of the analog XXVIII; yield, 140 mg, $[\alpha]_D^{21}$ +25.7° ($c=0.35$, MeOH), $Rf^3=0.45$; $Rf^4=0.71$. Amino acid analysis: Gly 1.00; Met 0.33; Tyr 0.89; Phe 1.00 (average recovery 89%).

H-Tyr-D-Met(O)-Gly-MePhe-NHNH-CO-CH_2CH_3 (XXXIV)——The compound **69** (150 mg) was dissolved in TFA (2 ml) and allowed to stand at room temperature for 30 min. The TFA was evaporated and the residue was treated with ether to give a powder, which was collected by filtration and dried. The powder was dissolved in H_2O (20 ml) and passed through a column (2.5×12 cm) of Amberlite IRA-410 (AcO^-). The eluate and washings were combined and lyophilized. The resulting powder was dissolved in a small amount of 0.1 N aqueous acetic acid and applied to a column (2.5×120 cm) of Sephadex LH-20, which was eluted with the same solvent. The fractions from 290 ml through 315 ml were combined and lyophilized; yield, 60 mg, $[\alpha]_D^{21}$ +4.1° ($c=0.21$, MeOH), $Rf^3=0.18$; $Rf^4=0.49$. Amino acid analysis[20]: Gly 1.00; Met 1.00; Tyr 0.90; MePhe 1.30 (average recovery 88%). ^{1}H-NMR (D_2O), δ: 1.16 (3H, t, CO–$CH_2C\underline{H}_3$), 2.35 (2H, q, CO–$C\underline{H}_2CH_3$), 2.65 [3H, s, SO–$C\underline{H}_3$ of Met(O)], 2.95 (3H, s, N–$C\underline{H}_3$ of MePhe).

Analogs I, II, III, XXVI, XXVII, XXIX, XXX and XXXIII were synthesized by a route similar to Route A described previously,[3] using Z–Tyr–D-Leu–Gly–OH (**2**) or Boc–Tyr–D-Met(O)–Gly–OH (**53**) instead of Z–Tyr–D-Ala–Gly–OH.[3] Analogs V—XVI, XVIII, XIX, XXII, XXIV, XXV and XXXV were synthesized in the same way as described for the synthesis of the analog IV or XX, using the corresponding intermediates (Table I). Physicochemical properties of synthetic analogs of enkephalin are listed in Table II.

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References and Notes

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