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Studies on Analgesic Oligopeptides. V.[1,2] Structure–Activity Relationship of Tripeptide Alkylamides, Tyr–D-Arg–Phe–X

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Twenty-one analogs based on the structure Tyr–D-Arg–Phe–X (X = OH, alkyl ester, alkylamide or amino acid having a different carbon chain) were synthesized by the solution method and their analgesic activities were tested after subcutaneous (s. c.) administration in mice. Most tripeptide alkylamides showed no analgesia at a dose of 10 mg/kg, s. c. However, some tripeptide alkylamides having the hydroxyl group on the alkyl moiety showed greater activity than morphine. Introduction of the carboxyl group on the alkyl moiety also led to tetrapeptide analogs with potent analgesia, *e.g.*, the compound with X = β-alanine is 33 times more potent than morphine on a molar basis. These results suggest that proper carbon chain lengths and the presence of an oxygen atom at the fourth position are important for high analgesic activity in the series of D-Arg[2]-dermorphin analogs.

Keywords——D-Arg[2]-dermorphin analog; tripeptide alkylamide; peptide synthesis; analgesic activity; subcutaneous administration; mouse

Since the discovery of dermorphins (Tyr–D-Ala–Phe–Gly–Tyr–X–Ser–NH_2, X = Pro or Hyp),[3] a number of structure–activity studies have been done and it was found that amino-terminal small peptide derivatives retained the potent and long-lasting analgesic activity after subcutaneous (s. c.) administration. Among them, N-terminal tetrapeptide analogs with substitution of D-Met (O)[4] and D-Arg[5] for D-Ala at position 2 are highly potent analgesic derivatives. On the other hand, when given intracerebroventricularly, the N-terminal tripeptide amide, Tyr–D-Arg–Phe–NH_2, is the minimum sequence required for the analgesic activity.[6] There results prompted us to examine structural requirements for activity of the tripeptide amides in detail. In the present study, eighteen tripeptide and three tetrapeptide analogs were prepared and tested for analgesic activity after s. c. administration in mice.

All analogs were synthesized by the solution method using the WSCI–HOBt coupling method.[7] The analogs based on the structure Tyr–D-Arg–Phe–X with X = OH (**1**), OCH_3 (**2**), OCH_2CH_3 (**3**), $NHCH_3$ (**4**), $NHCH_2CH_3$ (**5**), NH $(CH_2)_3C_6H_5$ (PPA, **6**), NH–Ada (**7**), $NHCH_2CH{=}CH_2$ (**8**), $N(CH_3)_2$ (**9**), $N(CH_2CH_3)_2$ (**10**), $N(CH_2CH{=}CH_2)_2$ (**11**), Pip (**12**), Mor (**13**), $NH(CH_2)_2OH$ (**14**), $NH(CH_2)_3OH$ (**15**), $NHNH_2$ (**16**), $NHNHCOCH_3$ (**17**), $NHNHCOCH_2CH_3$ (**18**), $NH(CH_2)_2COOH$ (**19**), $NH(CH_2)_3COOH$ (**20**) and $NH(CH_2)_4COOH$ (**21**) were synthesized. Boc–D-Arg(NO_2 or Tos)–OH and Boc–Tyr–OH were coupled to Phe–R (R = alkyl esters or alkyl amides) in a stepwise manner to afford protected tripeptide esters or amides. Three tetrapeptide analogs were newly synthesized analogously starting with β-Ala–OBzl, γ-Abu–OBzl or δ-Ave–OBzl. Intermediate peptides and their physicochemical properties are summarized in Table I. The protected peptides were deprotected by catalytic hydrogenolysis in the presence of Pd–C or by using the TFMSA–thioanisole system[8] and the peptides were purified by column chromatography on CM-cellulose and/or partition chromatography on Sephadex G-25. Homogeneity of the peptides

TABLE I. Physicochemical Properties of Intermediates

	Structure	mp (°C)	$[\alpha]_D$ [a] (°)	Rf [1 b]	Formula	Analysis (%) Found (Calcd) C	H	N
22	Boc-D-Arg(NO_2)-Phe-OBzl	74—77	−8.2	0.58	$C_{27}H_{36}N_6O_7$	58.14 (58.26)	6.60 (6.52)	14.75 (15.10)
23	Boc-Tyr-D-Arg(NO_2)-Phe-OBzl	102—104	+12.8	0.76	$C_{36}H_{45}N_7O_9$	60.17 (60.07)	6.50 (6.30)	13.19 (13.62)
24	Boc-D-Arg(NO_2)-Phe-OMe	68—70	−3.1	0.36	$C_{21}H_{32}N_6O_7$	52.58 (52.49)	6.60 (6.71)	17.33 (17.49)
25	Boc-Tyr-D-Arg(NO_2)-Phe-OMe	110—114	+11.4	0.51	$C_{30}H_{41}N_7O_9$	56.34 (55.97)	6.59 (6.42)	14.98 (15.23)
26	Boc-D-Arg(NO_2)-Phe-OEt	66—68	−1.6	0.39	$C_{22}H_{34}N_6O_7$	53.29 (53.43)	6.88 (6.93)	16.69 (17.00)
27	Boc-Tyr-D-Arg(NO_2)-Phe-OEt	112—114	+15.2	0.53	$C_{31}H_{43}N_7O_9$	57.00 (56.60)	6.81 (6.59)	14.63 (14.91)
28	Z-Phe-$NHCH_3$	144—145	−3.7	0.48	$C_{18}H_{20}N_2O_3$	69.32 (69.21)	6.44 (6.45)	8.90 (8.97)
29	Boc-D-Arg(NO_2)-Phe-$NHCH_3$	110—113	−7.8	0.44	$C_{21}H_{33}N_7O_6$	53.01 (52.60)	6.80 (6.94)	20.04 (20.45)
30	Boc-Tyr-D-Arg(NO_2)-Phe-$NHCH_3$	112—114	+6.6	0.33	$C_{30}H_{42}N_8O_8$	56.55 (56.06)	6.94 (6.59)	17.21 (17.43)
31	Boc-Phe-NHC_2H_5	110—111	+8.1	0.56	$C_{16}H_{24}N_2O_3$	65.96 (65.72)	8.64 (8.27)	9.69 (9.58)
32	Boc-D-Arg(NO_2)-Phe-NHC_2H_5	97—99	−9.7	0.47	$C_{22}H_{35}N_7O_6$	53.52 (53.53)	6.90 (7.15)	19.66 (19.87)
33	Boc-Tyr-D-Arg(NO_2)-Phe-NHC_2H_5	140—142	+8.6	0.62	$C_{31}H_{44}N_8O_8$	57.05 (56.69)	6.72 (6.75)	16.87 (17.06)
34	Boc-Phe-NH-PPA	104—105	+3.9	0.63	$C_{23}H_{30}N_2O_3$	72.10 (72.22)	8.11 (7.91)	7.48 (7.32)
35	Boc-D-Arg(NO_2)-Phe-PPA	105—107	−3.1	0.53	$C_{29}H_{41}N_7O_6$	60.01 (59.67)	7.11 (7.08)	16.56 (16.80)
36	Boc-Tyr-D-Arg(NO_2)-Phe-NH-PPA	111—114	+8.4	0.56	$C_{38}H_{50}N_8O_8$	61.33 (61.11)	6.57 (6.75)	14.85 (15.00)
37	Boc-Phe-NH-Ada	62—63	+3.5	0.75	$C_{24}H_{34}N_2O_3$	72.49 (72.33)	8.58 (8.60)	7.32 (7.03)
38	Boc-D-Arg(NO_2)-Phe-NH-Ada	116—118	−6.9	0.67	$C_{30}H_{45}N_7O_6$	60.02 (60.08)	7.80 (7.56)	16.25 (16.35)
39	Boc-Tyr-D-Arg(NO_2)-Phe-NH-Ada	120—122	+8.3	0.82	$C_{39}H_{54}N_8O_8$	61.16 (61.40)	7.04 (7.13)	14.46 (14.69)
40	Boc-Phe-$NHCH_2CH=CH_2$	88—90	+2.5	0.54	$C_{17}H_{24}N_2O_3$	66.96 (67.08)	7.86 (7.95)	9.56 (9.20)
41	Boc-D-Arg(Tos)-Phe-$NHCH_2CH=CH_2$	97—99	−11.3	0.47	$C_{30}H_{42}N_6O_6S$	58.22 (58.61)	7.06 (6.89)	13.39 (13.67)
42	Boc-Tyr-D-Arg(Tos)-Phe-$NHCH_2CH=CH_2$	147—149	−3.3	0.62	$C_{39}H_{51}N_7O_8S$	60.07 (60.21)	6.94 (6.61)	12.42 (12.60)
43	H-Phe-$N(CH_2)_3\cdot HBr$	259—262	+57.5	0.42	$C_{11}H_{16}N_2O\cdot HBr\cdot H_2O$	45.02 (45.37)	6.40 (6.58)	9.27 (9.62)
44	Boc-D-Arg(NO_2)-Phe-$N(CH_3)_2$	115—118	+9.1	0.38	$C_{22}H_{35}N_7O_6$	53.83 (53.53)	7.24 (7.15)	19.58 (19.87)
45	Boc-Tyr-D-Arg(NO_2)-Phe-$N(CH_3)_2$	108—111	+27.4	0.56	$C_{31}H_{44}N_8O_8$	56.80 (56.69)	6.96 (6.75)	16.82 (17.06)
46	Boc-Phe-$N(C_2H_5)_2$	57—59	+3.5	0.42	$C_{18}H_{28}N_2O_3$	67.01 (67.47)	8.50 (8.81)	8.52 (8.74)
47	Boc-D-Arg(NO_2)-Phe-$N(C_2H_5)_2$	78—85	−1.9	0.36	$C_{24}H_{39}N_7O_6$	55.69 (55.26)	7.31 (7.54)	18.55 (18.80)
48	Boc-Tyr-D-Arg(NO_2)-Phe-$N(C_2H_5)_2$	108—112	+17.0	0.59	$C_{33}H_{48}N_8O_8$	57.54 (57.88)	6.82 (7.07)	15.89 (16.36)
49	Boc-Phe-$N(CH_2CH=CH_2)_3$	46—48	−10.1	0.61	$C_{20}H_{28}N_2O_3$	69.67 (69.74)	8.44 (8.19)	8.38 (8.13)
50	Boc-D-Arg(Tos)-Phe-$N(CH_2CH=CH_2)_2$	71—75	−1.3	0.50	$C_{33}H_{46}N_6O_6S$	60.31 (60.53)	7.31 (7.08)	12.61 (12.83)

TABLE I. (continued)

	Structure	mp (°C)	$[\alpha]_D$ [a] (°)	Rf [1 b]	Formula	Analysis (%) Found (Calcd) C	H	N
51	Boc–Tyr–D-Arg(Tos)–Phe–$N(CH_2CH=CH_2)_2$	123—127	+10.1	0.64	$C_{42}H_{55}N_7O_8S$	61.82 (61.67)	6.94 (6.78)	11.84 (11.99)
52	H–Phe–Pip·HCl	52—55	+40.2	0.45	$C_{14}H_{20}N_2O \cdot HCl \cdot H_2O$	58.41 (58.22)	7.79 (8.03)	9.42 (9.70)
53	Boc–D-Arg(NO_2)–Phe–Pip	96—98	+7.2	0.53	$C_{25}H_{39}N_7O_6$	56.42 (56.27)	7.56 (7.37)	18.22 (18.37)
54	Boc–Tyr–D-Arg(NO_2)–Phe–Pip	109—111	+25.4	0.67	$C_{34}H_{48}N_8O_8$	58.85 (58.61)	7.02 (6.94)	15.85 (16.08)
55	H–Phe–Mor·HCl	50—54	+39.6	0.48	$C_{13}H_{18}N_2O_2 \cdot HCl \cdot H_2O$	50.59 (50.90)	7.30 (7.56)	8.80 (9.13)
56	Boc–D-Arg(NO_2)–Phe–Mor	103—105	+6.1	0.36	$C_{24}H_{37}N_7O_7$	54.09 (53.82	7.05 6.96)	18.01 18.31)
57	Boc–Tyr–D-Arg(NO_2)–Phe–Mor	125—129	+28.4	0.60	$C_{33}H_{46}N_8O_9$	56.95 (56.72)	6.87 (6.64)	15.84 (16.04)
58	Z–Phe–$NH(CH_2)_2OH$	127—128	−3.8	0.52	$C_{19}H_{22}N_2O_4$	66.35 (66.65)	6.50 (6.48)	8.15 (8.18)
59	Boc–D-Arg(NO_2)–Phe–$NH(CH_2)_2OH$	103—107	−7.1	0.41	$C_{22}H_{35}N_7O_7$	51.64 (51.85)	7.09 (6.92)	18.89 (19.24)
60	Boc–Tyr–D-Arg(NO_2)–Phe–$NH(CH_2)_2OH$	135—140	+13.4	0.58	$C_{31}H_{44}N_8O_9$	55.14 (55.34)	6.83 (6.59)	16.36 (16.44)
61	Boc–Phe–$NH(CH_2)_3OH$	89—90	+4.1	0.51	$C_{17}H_{26}N_2O_4$	63.22 (63.33)	8.04 (8.13)	8.66 (8.69)
62	Boc–D-Arg(NO_2)–Phe–$NH(CH_2)_3OH$	90—94	−11.6	0.42	$C_{23}H_{37}N_7O_7$	53.13 (52.76)	7.34 (7.12)	18.48 (18.73)
63	Boc–Tyr–D-Arg(NO_2)–Phe–$NH(CH_2)_3OH$	125—128	+7.6	0.59	$C_{32}H_{46}N_8O_9$	56.11 (55.97)	6.52 (6.75)	15.97 (16.32)
64	Boc–Tyr–D-Arg(Tos)–Phe–$NHNH_2$	121—125	+10.6	0.59	$C_{36}H_{48}N_8O_8S$	57.56 (57.43)	6.40 (6.43)	14.61 (14.88)
65	Boc–D-Arg(NO_2)–Phe–$NHNHCOCH_3$	120—125	−11.2	0.30	$C_{22}H_{34}N_8O_7$	50.91 (50.57)	6.64 (6.56)	21.09 (21.45)
66	Boc–Tyr–D-Arg(NO_2)–Phe–$NHNHCOCH_3$	147—150	+1.1	0.52	$C_{31}H_{43}N_9O_9$	54.59 (54.30)	6.52 (6.32)	18.11 (18.39)
67	Boc–D-Arg(NO_2)–Phe–$NHNHCOC_2H_5$	135—138	−16.4	0.32	$C_{23}H_{36}N_8O_7$	51.75 (51.48)	6.60 (6.76)	20.41 (20.88)
68	Boc–Tyr–D-Arg(NO_2)–Phe–$NHNHCOC_2H_5$	155—162	−1.8	0.51	$C_{32}H_{45}N_9O_9$	54.70 (54.93)	6.19 (6.48)	17.68 (18.01)
69	Boc–Phe–β-Ala–OBzl[c]	89—90	+1.3	0.62	$C_{24}H_{30}N_2O_5$	67.47 (67.58)	7.18 (7.09)	6.55 (6.57)
70	Boc–D-Arg(NO_2)–Phe–β-Ala–OBzl	83—85	−13.5	0.53	$C_{30}H_{41}N_7O_8$	57.30 (57.40)	6.66 (6.58)	15.31 (15.62)
71	Boc–Tyr–D-Arg(NO_2)–β-Ala–OBzl	105—107	+3.1	0.72	$C_{39}H_{50}N_8O_{10}$	59.60 (59.23)	6.50 (6.37)	13.89 (14.17)
72	Boc–Phe–γ-Abu–OBzl	92—93	+4.7	0.61	$C_{25}H_{32}N_3O_5$	65.85 (66.06)	7.38 (7.10)	9.10 (9.25)
73	Boc–D-Arg(NO_2)–Phe–γ-Abu–OBzl	85—88	−7.9	0.55	$C_{31}H_{43}N_8O_8$	57.10 (56.78)	6.82 (6.61)	16.81 (17.09)
74	Boc–Tyr–D-Arg(NO_2)–Phe–γ-Abu–OBzl	104—107	+10.2	0.69	$C_{40}H_{52}N_8O_{10}$	60.07 (59.69)	6.76 (6.51)	13.44 (13.92)
75	Boc–Phe–δ-Ava–OBzl	86—87	+2.7	0.62	$C_{26}H_{34}N_2O_5$	68.57 (68.70)	7.60 (7.54)	6.31 (6.16)
76	Boc–D-Arg(NO_2)–Phe–δ-Ava–OBzl	76—81	−11.1	0.54	$C_{32}H_{45}N_7O_8$	59.03 (58.61)	7.14 (6.65)	15.29 (14.95)
77	Boc–Tyr–D-Arg(NO_2)–Phe–δ-Ava–OBzl	95—96	+10.8	0.68	$C_{41}H_{54}N_8O_{10}$	59.94 (60.13)	6.80 (6.65)	13.41 (13.68)

a) Optical rotations were measured in MeOH ($c=1$) at 18—26 °C. *b*) See Experimental. *c*) Lit. (ref. 9*b*): mp 95—96 C; $[\alpha]_D$ +4.7° ($c=1$, MeOH).

TABLE II. Physicochemical Properties of Synthetic Analogs

	$[\alpha]_D$[a] (°)	TLC[b] Rf^1	TLC[b] Rf^2	Formula	Analysis (%) Found (Calcd) C	H	N	FAB-MS m/z ($M^+ + 1$)	Amino acid ratio
1	+55.2	0.27	0.59	$C_{24}H_{32}N_6O_5 \cdot 2CH_3COOH \cdot H_2O$	54.34 (54.01)	6.78 (6.80)	13.26 (13.50)	—	Arg 1.02 Phe 1.00 Tyr 0.89
2	+35.3	0.40	0.72	$C_{25}H_{34}N_6O_5 \cdot 2CH_3COOH \cdot H_2O$	54.76 (54.70)	6.86 (6.97)	13.31 (13.20)	—	Arg 0.99 Phe 1.00 Tyr 0.86
3	+34.2	0.45	0.73	$C_{26}H_{36}N_6O_5 \cdot 2CH_3COOH \cdot H_2O$	55.54 (55.37)	6.99 (7.12)	12.80 (12.91)	—	Arg 0.95 Phe 1.00 Tyr 0.86
4	+39.5	0.33	0.69	$C_{25}H_{35}N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	54.55 (54.79)	6.99 (7.14)	15.54 (15.42)	—	Arg 1.03 Phe 1.00 Tyr 0.83
5	+35.9	0.36	0.66	$C_{26}H_{37}N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	55.88 (55.46)	7.02 (7.29)	14.79 (15.09)	498	Arg 0.96 Phe 1.00 Tyr 0.83
6	+32.1	0.46	0.68	$C_{33}H_{43}N_7O_4 \cdot 2CH_3COOH \cdot 2H_2O$	58.99 (58.64)	7.18 (7.31)	13.03 (12.94)	512	Arg 0.98 Phe 1.00 Tyr 0.83
7	+38.7	0.60	0.65	$C_{34}H_{47}N_7O_4 \cdot 2CH_3COOH \cdot 2H_2O$	57.76 (57.64)	7.58 (7.76)	12.53 (12.38)	602	Arg 1.02 Phe 1.00 Tyr 0.88
8	+38.6	0.66	0.74	$C_{27}H_{37}N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	55.92 (56.27)	7.01 (7.16)	14.45 (14.82)	618	Arg 1.01 Phe 1.00 Tyr 0.98
9	+55.5	0.39	0.64	$C_{26}H_{37}N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	55.20 (55.46)	7.41 (7.29)	15.11 (15.09)	512	Arg 0.98 Phe 1.00 Tyr 0.91
10	+45.4	0.45	0.76	$C_{28}H_{41}N_7O_4 \cdot 2CH_3COOH \cdot 2H_2O$	55.14 (55.24)	7.41 (7.68)	13.72 (14.09)	540	Arg 1.02 Phe 1.00 Tyr 0.97
11	+37.5	0.60	0.73	$C_{30}H_{41}N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	58.60 (58.19)	7.01 (7.32)	13.62 (13.97)	564	Arg 1.01 Phe 1.00 Tyr 0.88
12	+54.6	0.38	0.64	$C_{29}H_{41}N_7O_4 \cdot 2CH_3COOH \cdot H_2O$	57.08 (57.46)	7.16 (7.45)	14.17 (14.21)	552	Arg 1.02 Phe 1.00 Tyr 0.92
13	+55.1	0.32	0.60	$C_{28}H_{39}N_7O_5 \cdot 2CH_3COOH$	57.22 (57.05)	7.20 (7.03)	14.82 (14.55)	554	Arg 0.96 Phe 1.00 Tyr 0.93
14	+38.0	0.40	0.69	$C_{26}H_{37}N_7O_5 \cdot 2CH_3COOH \cdot 2H_2O$	52.95 (52.70)	7.41 (7.22)	14.14 (14.34)	528	Arg 1.02 Phe 1.00 Tyr 0.87
15	+40.9	0.36	0.66	$C_{27}H_{39}N_7O_5 \cdot 2CH_3COOH \cdot H_2O$	54.60 (54.77)	7.07 (7.27)	14.11 (14.42)	542	Arg 1.05 Phe 1.00 Tyr 0.90
16	+41.5	0.30	0.60	$C_{24}H_{34}N_8O_4 \cdot 3CH_3COOH \cdot 2H_2O$	50.55 (50.41)	6.82 (7.05)	15.35 (15.68)	—	Arg 1.01 Phe 1.00 Tyr 0.88
17	+28.6	0.29	0.59	$C_{26}H_{36}N_8O_5 \cdot 2CH_3COOH \cdot 2H_2O$	51.71 (51.72)	6.74 (6.94)	15.69 (16.08)	—	Arg 0.97 Phe 1.00 Tyr 0.90
18	+30.6	0.33	0.61	$C_{27}H_{38}N_8O_5 \cdot 2CH_3COOH \cdot H_2O$	53.41 (53.75)	6.72 (6.98)	15.88 (16.17)	—	Arg 1.03 Phe 1.00 Tyr 0.95
19	+28.5	0.30	0.59	$C_{27}H_{37}N_7O_6 \cdot 2CH_3COOH$	54.74 (55.10)	6.60 (6.71)	14.92 (14.51)	—	Arg 1.01 Phe 1.00 Tyr 0.94 β-Ala 1.10
20	+36.4	0.31	0.61	$C_{28}H_{39}N_7O_6 \cdot 2CH_3COOH \cdot H_2O$	54.21 (54.30)	7.08 (6.98)	13.74 (13.85)	—	Arg 1.04 Phe 1.00 Tyr 0.92 γ-Abu 1.15
21	+33.6	0.30	0.62	$C_{29}H_{41}N_7O_6 \cdot 2CH_3COOH \cdot H_2O$	54.60 (54.91)	7.30 (7.12)	13.80 (13.59)	—	Arg 0.95 Phe 1.00 Tyr 0.89 δ-Ave 1.12

a) Optical rotations were measured in H_2O ($c=1$) at 20—26 °C. *b*) See Experimental.

was checked by TLC. They were characterized by elemental analysis and amino acid analysis after acid hydrolysis. The tripeptide alkyl amides were also characterized by FAB-MS. Physicochemical properties of synthetic analogs are summarized in Table II.

Analgesic activity of the peptides was assessed by the tail pressure method after s.c. adminstration in mice and compared with that of morphine (Table III). The free tripeptide (**1**) and the esters (**2**, **3**) showed no significant activity at a dose of 10 mg/kg. Among a series of *N*-

TABLE III. Analgesic Activity of Synthetic Analogs after Subcutaneous Administration in Mice

	Tyr–D-Arg–Phe–X X	ED_{50}[a] (mg/kg, s.c.)	Relative potency[b]
	Morphine·HCl	6.2 (4.1—9.4)	1
1	OH	10<	—
2	OCH_3	10<	—
3	OCH_2CH_3	10<	—
	NH_2[c]	40<	—
4	$NHCH_3$	4.0 (2.6—6.1)	2.8
5	$NHCH_2CH_3$	10<	—
6	NH–PPA	10<	—
7	NH–Ada	10<	—
8	$NHCH_2CH=CH_2$	10<	—
9	$N(CH_3)_2$	2.0 (1.2—3.3)	5.7
10	$N(CH_2CH_3)_2$	10<	—
11	$N(CH_2CH=CH_2)_2$	10<	—
12	Pip	10<	—
13	Mor	10<	—
14	$NH(CH_2)_2OH$	3.4 (2.3—5.1)	3.5
15	$NH(CH_2)_3OH$	1.2 (0.3—4.5)	9.9
16	$NHNH_2$	10<	—
17	$NHNHCOCH_3$	10<	—
18	$NHNHCOCH_2CH_3$	10<	—
	$NHCH_2COOH$[d]	2.4 (1.5—4.0)	4.8
19	$NH(CH_2)_2COOH$	0.4 (0.3—0.7)	32.7
20	$NH(CH_2)_3COOH$	4.4 (2.7—7.3)	2.8
21	$NH(CH_2)_4COOH$	5.1 (3.3—7.8)	2.5

a) The 95% confidence limits are given in parentheses. *b*) The ED_{50} value of each peptide compared with that of morphine on a molar basis. *c*) Data cited from ref. 1. *d*) Data cited from ref. 5.

alkyl analogs, mono-(**4**) and dimethyl (**9**), and hydroxyethyl (**14**) and hydroxypropyl (**15**) amides showed greater activity than morphine on a molar basis. In contrast, analogs having a bulky alkyl group showed loss of activity in this assay system (**6**, **7**, **12**, **13**), suggesting that increased hydrophobicity at the alkyl amide moiety decrease the activity. It is noteworthy that in a series of dermorphin tetrapeptide alkyl amides these phenomena were reversed.[9] Mono and diallyl analogs (**8**, **11**) were shown to have no antagonistic action to morphine but to have potent opioid activity (4 times that of morphine in the guinea pig ileum assay).[10] However, in the present assay, both analogs were devoid of activity at doses up to 10 mg/kg, possibly due to difficulty in crossing the blood-brain barrier. The hydrazide type analogs (**16**, **17**, **18**) did not show activity although such a modification has been successfully applied to obtain potent enkephalin tetrapeptide analogs.[11] Interestingly, introduction of a hydroxyl or carboxyl group into the alkyl moiety led to very potent analogs (**14**, **15**, **19**, **20**, **21**) and the β-Ala analog (**19**) showed the highest activity found in this study. These lines of evidence seem to indicate that proper carbon chain lengths (C_2–C_3) and the presence of an oxygen atom at the alkyl moiety (R) of Tyr–D-Arg–Phe–NH–R are important for eliciting high analgesic activity. The hydroxyl or carboxyl oxygen atoms may contribute to hydrogen bonding in the active conformation of the peptide molecule *via* intramolecular or peptide-receptor interaction. However, this assumption can not explain the high activity of **4** and **9**. The discrepancy remains to be resolved.

Experimental

Melting points were determined with a Yanaco model MP-S3 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 polarimeter. Amino acid analyses were performed on a Hitachi model 835 amino acid analyzer after hydrolysis of peptides with 6 N HCl at 110 °C for 20 h. FAB-MS were measured with a JEOL JMS-DX303 instrument. TLC was performed on silica gel plates (Kieselgel GF_{254}, Merck) with the solvent systems of Rf^1, 1-BuOH–AcOH–H_2O (4 : 1 : 5, upper phase) and Rf^2, 1-BuOH–pyridine–AcOH–H_2O (15 : 10 : 3 : 12). The N^{α}-protecting group of intermediates was removed before TLC.

Phe–Alkyl Amides——Compound **28**: A solution of methylamine (40%) in H_2O (1.96 ml) was added to a solution of Z–Phe–OSu (1.0 g in THF (5 ml) and the resulting solution was stirred at room temperature for 2 d, then EtOAc (20 ml) was added. The extract was washed with 1 N AcOH, H_2O, dried over $MgSO_4$ and evaporated to dryness. The product was recrystallized from EtOAc–petroleum ether.

Compounds **31**, **34**, **37**, **40**, **43**, **46**, **49**, **58** and **66**: These compounds were obtained from Boc (or Z)–Phe–OH and the corresponding alkylamide by the WSCI–HOBt method as described below, and the Z group was removed by catalytic hydrogenation for further coupling.

Compounds **52** and **55**: Boc–Phe–ONB was coupled to piperidine and morpholine in THF and worked up in the same manner as described for the preparation of **28** to yield Boc–Phe–Pip and Boc–Phe–Mor, respectively. The Boc group was removed by treatment with 4 N HCl–dioxane for further coupling.

General Procedure of WSCI–HOBt Coupling Method——The carboxyl component (1 mmol), HOBt (1.2 mmol) and WSCI (1.1 mmol) were added to a solution of the amine component (1 mmol) in DMF (5 mmol) containing *N*-Me-morpholine (1.1 mmol) at 0 °C. The reaction mixture was stirred at 5 °C for 5—14 h. The solution was diluted with H_2O (40 ml) and extracted twice with EtOAc (20 ml × 2). The extract was washed with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O and dried over $MgSO_4$. After evaporation of the solvent, the resulting residue was triturated with ether or petroleum ether and the solid was collected and dried.

Removal of N^{α}-Boc Group——A protected peptide (1 mmol) was treated with 4 N HCl–dioxane (15 ml) at room temperature for 30 min. The solvent was evaporated off at below 40 °C, the residue was dissolved in dry dioxane and this solution was evaporated to dryness. This evaporation step was repeated 3 times, then the residue was further dried using a vacuum pump.

Boc–Tyr–D-Arg(Tos)–Phe–$NHNH_2$(64)——A solution of **24** (980 mg) in MeOH (5 ml) was treated with $NH_2NH_2 \cdot H_2O$ (1.26 ml). The solution was stirred at room temperature for 2 d and poured into H_2O (100 ml). The precipitate formed was washed, dried and then reprecipitated from MeOH–ether.

Boc–D-Arg(NO_2)–Phe–NHNHCOCH_3(65)——This compound was obtained from Boc–D-Arg(NO_2)–OH and H–Phe–NHNHCOCH_3[11a] by the WSCI–HOBt method. Boc–D-Arg(NO_2)–Phe–NHNHCOCH_2CH_3 was analogously obtained using H–Phe–NHNHCOCH_2CH_3.[11b]

Deblocking and Purification——Typically, the Boc group of a protected peptide (200 mg) was removed as described above and the resulting residue was dissolved in MeOH–H_2O (2 : 1, 15 ml) and hydrogenated over 10% Pd–C for 16 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The resulting residue was treated with Dowex 1 × 2 resin (AcOH form, 10 g) in H_2O (10 ml) for 15 min. After removal of the resin the solution was freeze-dried. The product was applied to a column (1.5 × 12 cm) of CM-cellulose, which was eluted with a linear gradient from H_2O (400 ml) in the mixing chamber to 0.35 M pyridine–acetate buffer (pH 5.2, 400 ml) in the reservoir. Fractions of 6 ml each were collected and the main fractions of Sakaguchi reaction positive eluates were pooled and lyophilized. If necessary, the product was further purified on a Sephadex G-25 column, which was pre-equilibrated and eluted with the upper layer of 1-BuOH–AcOH–H_2O (4 : 1 : 5). Appropriate Sakaguchi-positive eluates on TLC were pooled and lyophilized.

The Tos group on the Arg residue was removed as follows: protected peptide (200 mg) was dissolved in TFA (3 ml) containing *o*-cresol (0.1 ml) and thioanisole (1.2 ml). Then TFMSA (0.3 ml) was added and the mixture was stirred at 5 °C for 30 min and at room temperature for 90 min. Dry ether (60 ml) was added and the resulting oil was washed repeatedly with dry ether. The oily residue was treated with Dowex 1 × 2 resin and purified as described above. Physicochemical data for synthetic peptides are given in Table II.

Analgesic Assay——Male Std-ddy strain mice (20—25 g) were used. Mice were injected s. c. with a test compound dissolved in Ringer's solution. The analgesic effect was assessed by means of the tail pressure test as described previously.[12] Changes in responsive tail pressure were expressed as a percentage of possible effect (% MPE) as follows: % MPE = $(P_t - P_o/100 - P_o)$ where P_o is the pre-drug responsive pressure (mmHg) and P_t is the responsive pressure (mmHg) after drug administration. The ED_{50} values and 95% confidence limits were determined by the method of Litchfield and Wilcoxon.[13]

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References and Notes

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Bull., **33**, 4865 (1985).
2) Amino acids, peptides and their derivatives in this study are of L-configuration unless otherwise stated. Abbreviations used are: Boc = *tert*-butoxycarbonyl, Z = carbobenzoxy, Tos = tosyl, PPA = phenylpropyl, Ada = adamantyl, Pip = 1-piperidino, Mor = 4-morpholino, γ-Abu = γ-amino-*n*-butyric acid, δ-Ave = δ-aminovaleric acid, OSu = succinimide ester, ONB = 5-norbornene-2,3-dicarboximide ester, WSCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = 1-hydroxybenzotriazole, TFMSA = trifluoromethanesulfonic acid, DMF = dimethylformamide, EtOAc = ethyl acetate, THF = tetrahydrofuran, MeOH = methanol, CM = carboxymethyl, TLC = thin layer chromatography, FAB-MS = fast atom bombardment mass spectroscopy, TFA = trifluoroacetic acid.
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