ester), 119436-61-2; **37**, 119435-89-1; **37** (methyl ester), 119436-63-4; **38**, 6683-48-3; **39**, 119435-90-4; **39** (X = $PPh_3^+Br^-$), 119436-52-1; (±)-**40**, 119435-91-5; (±)-**40** (acid chloride), 119436-55-4; **41**, 119435-92-6; **42**, 119435-93-7; **42** (X = H, OMs), 119436-56-5; **43**, 27452-17-1; **44**, 92654-79-0; **45**, 119435-94-8; **45** (ethyl ester), 119436-58-7; **45** (acid chloride), 119436-59-8; **46**, 102296-82-2; (±)-**47**, 119435-95-9; (±)-**48**, 119435-96-0; (±)-**49**, 119435-97-1; **50**, 22824-31-3; **51**, 119435-98-2; **52**, 119435-99-3; **53**, 119436-00-9; **54**, 119436-01-0; **55**, 119436-02-1; **56**, 119436-03-2; (±)-**58**, 119436-04-3; (±)-**58** (methyl ester), 119436-64-5; (±)-**59**, 119454-60-3; (±)-**59** (methyl ester), 119436-65-6; (±)-**60**, 119436-05-4; (±)-**60** (methyl ester), 119436-66-7; (±)-**61**, 119436-06-5; (±)-**61** (methyl ester), 119436-67-8; (±)-**62**, 119436-07-6; (±)-**62** (methyl ester), 119436-68-9; (±)-**63**, 119436-08-7; (±)-**63** (methyl ester), 119436-69-0; (±)-**64**, 119436-09-8; (±)-**64** (methyl ester), 119436-70-3; (±)-**65**, 119436-10-1; (±)-**65** (methyl ester), 119436-71-4; (±)-**66**, 119436-11-2; (±)-**66** (methyl ester), 119436-72-5; (±)-**67**, 119436-12-3; (±)-**67** (methyl ester), 119436-73-6; (±)-**68**, 119436-13-4; (±)-**68** (methyl ester), 119436-74-7; (±)-**69**, 119436-14-5; (±)-**69** (methyl ester), 119436-75-8; **70**, 119436-15-6; **71** (regioisomer 1), 119436-16-7; **71** (regioisomer 2), 119436-76-9; **71** (regioisomer 1, methyl ester), 119436-77-0; **71** (regioisomer 2, methyl ester), 119436-78-1; $PhNH_2$, 62-53-3; 3-$MeC_6H_4NH_2$, 108-44-1; 3,4-$Et_2C_6H_3NH_2$, 54675-14-8; 3-$EtC_6H_4NH_2$, 587-02-0; 4-*i*-$PrC_6H_4NH_2$, 99-88-7; 3-*i*-$PrC_6H_4NH_2$, 5369-16-4; 2-*i*-$PrC_6H_4NH_2$, 643-28-7; 3-*t*-$BuC_6H_4NH_2$, 5369-19-7; 2,5-(*i*-Pr)$_2C_6H_3NH_2$, 91552-65-7; 2,4-(*i*-Pr)$_2C_6H_3NH_2$, 79069-41-3; 3,5-(*i*-Pr)$_2C_6H_3NH_2$, 7544-57-2; 3,4-(*i*-Pr)$_2C_6H_3NH_2$, 116233-13-7; 3-$PhC_6H_4NH_2$, 2243-47-2; 3-c-$C_6H_{11}C_6H_4NH_2$, 5369-21-1; 4-ONC_6H_4COOMe, 13170-28-0; *o*-$C_6H_4Et_2$, 135-01-3; 3,4-$Et_2C_6H_3COCH_3$, 102405-35-6; (±)-3,4-$Et_2C_6H_3CH(OH)CH_3$, 119436-17-8; (±)-3,4-$Et_2C_6H_3CHBrCH_3$, 119436-18-9; (±)-3,4-$Et_2C_6H_3CH(CH_3)PPh_3^+Br^-$, 119436-19-0; *p*-$OHCC_6H_4COOMe$, 1571-08-0; *m*-*t*-$BuC_6H_4Me$, 1075-38-3; *m*-*t*-$BuC_6H_4CH_2Br$, 102405-32-3; *m*-*t*-$BuC_6H_4CH_2PPh_3^+Br^-$, 119436-35-0; *p*-$MeOCOC_6H_4CH_2PPh_3^+Br^-$, 1253-46-9; *o*-$Me_2C_6H_4$, 95-47-6; 3,4-$Me_2C_6H_3COCH_3$, 3637-01-2; (±)-3,4-$Me_2C_6H_3CH(OH)CH_3$, 100646-15-9; (±)-3,4-$Me_2C_6H_3CHBrCH_3$, 119436-40-7; (±)-3,4-$Me_2C_6H_3CH(CH_3)PPh_3^+Br^-$, 119436-41-8; *o*-(*i*-Pr)$_2C_6H_4$, 577-55-9; 3,4-(*i*-Pr)$_2C_6H_3COCH_3$, 94291-81-3; (±)-3,4-(*i*-Pr)$_2C_6H_3CH(OH)CH_3$, 119436-43-0; (±)-3,4-(*i*-Pr)$_2C_6H_3CHBrCH_3$, 119436-44-1; (±)-3,4-(*i*-Pr)$_2C_6H_3CH(CH_3)PPh_3^+Br^-$, 119436-45-2; *p*-*t*-$BuC_6H_4Me$, 98-51-1; *p*-*t*-$BuC_6H_4CH_2Br$, 18880-00-7; *p*-*t*-$BuC_6H_4CH_2PPh_3^+Br^-$, 65413-33-4; *t*-BuPh, 98-06-6; *p*-*t*-$BuC_6H_4COCH_3$, 943-27-1; (±)-*p*-*t*-$BuC_6H_4CH(OH)CH_3$, 119479-30-0; (±)-*p*-*t*-$BuC_6H_4CHBrCH_3$, 119436-49-6; (±)-*p*-*t*-$BuC_6H_4CH(CH_3)PPh_3^+Br^-$, 119436-50-9; $PhCH_2Br$, 100-39-0; $PhCH_2PPh_3^+Br^-$, 1449-46-3; 4-$MeOCOC_6H_4CH_2CO_2H$, 22744-12-3; 4-$MeOCOC_6H_4COCO_2H$, 119436-62-3; 3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-amine, 109139-99-3; 3,4-dihydro-4,4-dimethyl-2*H*-1-benzothiopyran-6-amine, 119436-21-4; 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylamine, 92050-16-3; 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl trifluoromethyl ketone, 119436-37-2; ethyl (*E*)-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)acrylate, 119436-57-6.

Peripherally Acting Enkephalin Analogues. 2.[1] Polar Tri- and Tetrapeptides[2]

George W. Hardy,*[†] Lawrence A. Lowe,[†] Gail Mills,[†] Pang Yih Sang,[†] Dean S. A. Simpkin,[†] Rhonda L. Follenfant,[‡] Clare Shankley,[‡] and Terence W. Smith[‡]

Departments of Medicinal Chemistry and Pharmacology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U.K. BR3 3BS. *Received August 29, 1988*

The design, synthesis, and biological activity of a series of D-Arg^2-enkephalin-derived tetrapeptide amides and tripeptide aralkylamides are reported. These polar analogues were designed to be excluded from the central nervous system with their action thus limited to peripheral opioid receptors. The effects of the nature of the aromatic ring, aryl ring substitution, and aralkylamine chain length on activity were investigated; in a number of cases the N-terminal amino group of Tyr^1 was converted to a guanidino group to further increase hydrophilicity. The peptides were all synthesized by classical solution methodology. The opioid activity of the peptides was assessed in vitro on the guinea pig ileum and their antinociceptive activity was determined in vivo in chemically induced writhing models (peripheral activity) and in the hot-plate test (central activity), in rodents. That the analgesic effects were predominantly mediated in the periphery was demonstrated by antagonism of antinociception by the peripheral opioid antagonist *N*-methylnalorphine and by comparison of the activities in the writhing and hot-plate tests. As a class, the tetrapeptides were more potent than the tripeptides; N$^\alpha$-amidination generally increased activity. A number of compounds exhibited very potent opioid activity and had the desired pharmacological profile, indicating a high degree of peripheral selectivity.

As part of a research program designed to investigate the potential of peripherally selective opioids as analgesic agents, we have examined the effect of the introduction of polar substituents on the activities of a variety of classes of opioids in order to restrict their passage across the blood-brain barrier.[1,3] Although the primary site of action of analgesic opioids is in the CNS, recent evidence suggests that there is a significant peripheral component to this activity;[4] inhibition of the cough reflex by opioids has also been shown to be peripherally mediated.[5] The serious side effects of respiratory depression, tolerance, and addictive liability associated with opiates, such as morphine, are mediated in the CNS.[6] It might be expected, therefore, that an effective peripherally acting opioid analgesic agent would be free from these undesirable side effects.

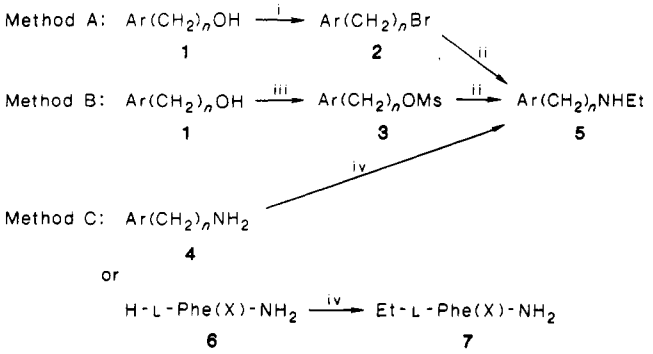
We have previously described the design and synthesis of a series of peripherally acting polar pentapeptide

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmacology.

(1) Part 1: Hardy, G. W.; Lowe, L. A.; Pang, Y. S.; Simpkin, D. S. A.; Follenfant, R. L.; Smith, T. W. *J. Med. Chem.* **1988**, *31*, 960.
(2) Abbreviations used: acetic acid (AA), 1-amidino-3,5-dimethylpyrazole acetate (ADMP), central nervous system (CNS), dicyclohexylcarbodiimide (DCCI), dicyclohexylurea (DCU), dimethylformamide (DMF), guinea pig ileum (GPI), 1-hydroxybenzotriazole (HOBt), high-performance liquid chromatography (HPLC), 4A molecular sieve (MS4A), *N*-methylmorpholine (NMM), phenyl-*p*-benzoquinone (PBQ), structure-activity relationship(s) (SAR), tetrahydrofuran (THF), subcutaneous (sc), oral (po). All amino acids are of the L configuration unless otherwise noted.
(3) (a) Smith, T. W.; Buchan, P.; Parsons, D. N.; Wilkinson, S. *Life Sci.* **1982**, *31*, 1205. (b) Hardy, G. W.; Doyle, P. M.; Smith, T. W. *Eur. J. Med. Chem.* **1987** *22*, 331. (c) Doyle, P. M. In preparation.

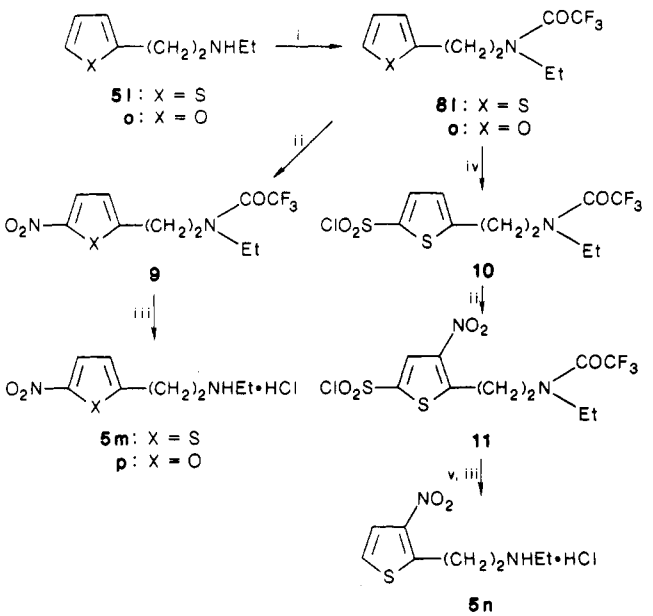
0022-2623/89/1832-1108$01.50/0

Scheme I. Synthesis of Aralkylethylamines and *N*-Ethylphenylalaninamides[a]



[a] Reagents: (i) 48% HBr, 110 °C; (ii) 33% $EtNH_2$–EtOH; (iii) MsCl, NEt_3, CH_2Cl_2, −5 °C; (iv) EtI, $NaHCO_3$, EtOH, 80 °C.

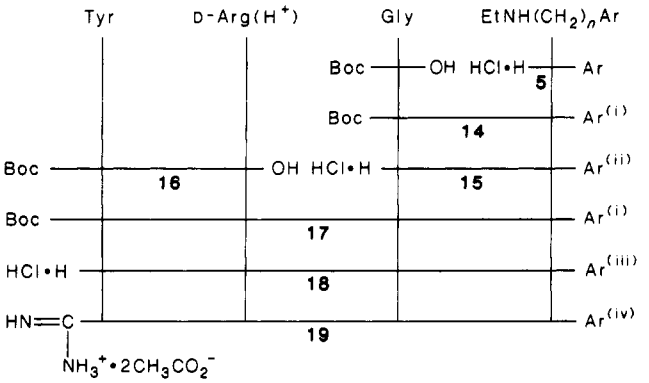
Scheme II. Synthesis of Thienyl and Furyl Derivatives[a]



[a] Reagents: (i) $(CF_3CO)_2O$, NEt_3, CH_2Cl_2, 0 °C; (ii) f HNO_3–Ac_2O, −20 °C; (iii) (a) NaOH, H_2O, dioxane, (b) HCl–dioxane; (iv) $ClSO_3H$, PCl_5; (v) 40% H_2SO_4, reflux, 1.5 h.

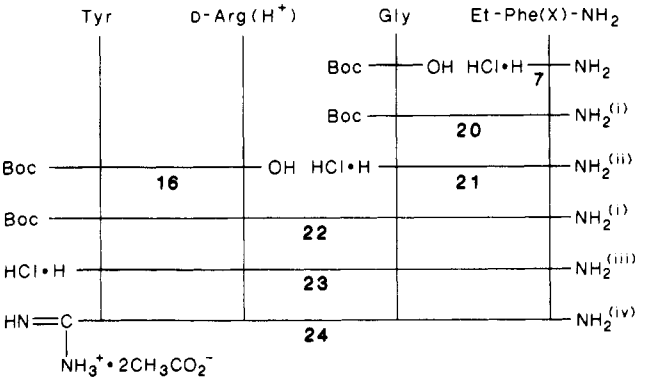
enkephalin analogues.[1] From a diverse group of polar [D-amino acid][2] substitutions in a potent centrally acting enkephalin analogue, the [D-Arg][2] compound BW 443C [H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2] was selected for further investigation. Detailed pharmacological studies served to support the proposed peripherally mediated analgesic[7] and antitussive[8] actions of this compound; other studies have also demonstrated a greatly reduced propensity to elicit the above-mentioned centrally mediated side effects.[9] Encouraged by these results, we have extended these studies, and in this paper we report the synthesis and opioid activities of two series of structurally simpler enkephalin analogues which might be expected to be more suitable as drug candidates.[10]

Scheme III. Synthesis of Tripeptides[a]



[a] Reagents: (i) DCCI, HOBt, NMM, DMF; (ii) 1 M HCl–HOAc; (iii) 1 M HCl–HOAc, anisole; (iv) ADMP, EtOH, DMF, NEt_3, 50 °C.

Scheme IV. Synthesis of Tetrapeptides[a]



[a] Reagents: (i) DCCI, HOBt, NMM, DMF; (ii) 1 M HCl–HOAc; (iii) 1 M HCl–HOAc, anisole; (iv) ADMP, EtOH, DMF, NEt_3, 50 °C.

Methods

Chemistry. The peptides described in this paper were all synthesized by classical methods in solution. The synthetic routes are exemplified in Schemes I–IV. Peptide couplings were mediated by DCCI–HOBt.[11] The strategy of minimal protection was used: the *tert*-butyloxycarbonyl group was used throughout for α-amino group protection; the guanidino group of D-arginine was protected through protonation; the phenolic group of tyrosine was left unprotected. The key intermediate N-terminal dipeptide Boc-Tyr-D-Arg-OH·HCl (**16**) was prepared as previously described.[1] In selected cases, the amino group of Tyr[1] in the parent peptides (**18** and **23**) was converted to a guanidino group to give the derivatives (**19** and **24**) by reaction with 1-amidino-3,5-dimethylpyrazole acetate.[12]

(4) (a) Bentley, G. A. Newton, S. H.; Starr, J. *Br. J. Pharmacol.* **1981**, *73*, 325. (b) Ferreira, S. H.; Nakamura, M. *Prostaglandins* **1979**, *18*, 191. (c) Rios, L.; Jacob, J. J. C. *Life Sci.* **1982**, *31*, 1209. (d) Rios, L.; Jacob, J. J. C. *Eur. J. Pharmacol.* **1983**, *96*, 277.

(5) Parsons, D. N.; Schneider, C.; Smith, T. W. *Br. J. Pharmacol. Proc. Suppl.* **1986**, *89*, 818P.

(6) Budd, K. In *International Encyclopaedia of Pharmacology and Therapeutics*; Williams, N. E., Wilkinson, H., Eds.; Pergammon: Oxford, 1983; Section 112, pp 51–63.

(7) (a) Follenfant, R. L.; Hardy, G. W.; Lowe, L. A.; Schneider, C.; Smith, T. W. *Br. J. Pharmacol.* **1988**, *93*, 85. (b) Follenfant, R. L.; Hardy, G. W.; Lowe, L. A.; Smith, T. W. *Br. J. Pharmacol. Proc. Suppl.* **1987**, *90*, 68P. (c) Lorenzetti, B. B.; Ferreira, S. H. *Br. J. Pharmacol. Proc. Suppl.* **1987**, *90*, 69P.

(8) (a) Adcock, J. J.; Allan, G.; Richardson, P. J.; Schneider, C.; Smith, T. W. *Br. J. Pharmacol. Proc. Suppl.* **1987**, *90*, 143P. (b) Adcock, J. J., Smith, T. W. *Br. J. Pharmacol. Proc. Suppl.* **1987**, *92*, 596P.

(9) Smith, T. W. Unpublished observations.

(10) Some of the work described herein was presented as a poster communication at the 19th European Peptide Symposium: Hardy, G. W.; Lowe, L. A.; Smith, T. W. In *Peptides 1986*; Theorodoropoulos, D., Ed.; de Gruyter: Berlin, 1987; pp 435–438.

(11) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

(12) (a) Bajusz, S.; Ronai, A. Z.; Szekely, J. I.; Miglecz, E.; Berzetei, I. *FEBS Lett.* **1980**, *110*, 85. (b) U.K. Pat. No. 2,053,925.

Table I. Analytical Data for Intermediate Aralkylethylamines [$EtNH(CH_2)_nAr$]

no.	Ar	n	formula	anal.	mp, °C	cryst solvent	method[a] (yield, %)
5a	$C_6H_4(4\text{-}NO_2)$	2	$C_{10}H_{14}N_2O_2\cdot HBr$	C,H,N,Br	213–215	EtOH–Et_2O	A (68)
5b	$C_6H_4(2\text{-}NO_2)$	2	$C_{10}H_{14}N_2O_2\cdot HBr$	C,H,N,Br	162–163	*i*-PrOH	A (43)
5c	$C_6H_4(4\text{-}F)$	2	$C_{10}H_{14}FN\cdot HCl$	C,H,N,F	173–174	*i*-PrOH–*i*-Pr_2O	C (24)
5d	$C_6H_4(2\text{-}F)$	2	$C_{10}H_{14}FN\cdot HBr$	C,H,N,Br	154–155	*i*-PrOH	A (47)
5e	$C_6H_4(4\text{-}Br)$	2	$C_{10}H_{14}BrN\cdot HCl$	C,H,N	223–225	dioxane	B (74)
5f	$C_6H_3(3,4\text{-}Cl_2)$	2	$C_{10}H_{13}Cl_2N\cdot HCl$	C,H,N,Cl	197–199	dioxane	B[b] (71)
5g	$C_6H_4(2\text{-}CF_3)$	2	$C_{11}H_{14}F_3N$[c]		173–175	dioxane	B (74)
5h	$C_6H_4(4\text{-}CN)$	2	$C_{11}H_{14}N_2\cdot HBr\cdot 0.15H_2O$	C,H,N	154–156	*i*-PrOH	B (14)
5i	$C_6H_4(4\text{-}SO_2NH_2)$	2	$C_{10}H_{16}N_2O_2S\cdot HBr$	C,H,N,S,Br	209–210	EtOH	A[b] (43)
5j	$1\text{-}C_{10}H_7$	2	$C_{14}H_{17}N\cdot HCl$	C,H,N,Cl		dioxane	B (85)
5k	$2\text{-}C_{10}H_7$	2	$C_{14}H_{17}N\cdot HCl$	C,H,N	220–223	dioxane	B (82)
5l	$2\text{-}C_4H_3S$	2	$C_8H_{13}NS\cdot HCl$	C,H,N,Cl	167–168 dec	dioxane	B (68)
5m	$2\text{-}C_4H_2S(5\text{-}NO_2)$	2	$C_8H_{12}N_2O_2S\cdot HCl$	C,H,N,Cl	178–180	dioxane	D[b] (18.5)
5n	$2\text{-}C_4H_2S(3\text{-}NO_2)$	2	$C_8H_{12}N_2O_2S\cdot HCl$	ND	ND	dioxane	D[b,d] (10)
5o	$2\text{-}C_4H_3O$	2	$C_8H_{13}NO\cdot HCl$	C,H,N,Cl	158	*i*-PrOH–*i*-Pr_2O	B[b] (51)
5p	$2\text{-}C_4H_2O(5\text{-}NO_2)$	2	$C_8H_{12}N_2O_3\cdot HCl$	C,H,N,Cl	185–187 dec	dioxane	D (22)
5r	$4\text{-}C_5H_5N$	2	$C_9H_{14}N_2\cdot 2HCl$	C,H,N,Cl	164–166	EtOH–Et_2O	F (8)
5s	$C_6H_4(4\text{-}NO_2)$	1	$C_9H_{12}N_2O_2\cdot HBr$	C,H,N,Br	200–203 dec	EtOH	A (51)
5t	$C_6H_4(2\text{-}NO_2)$	1	$C_9H_{12}N_2O_2\cdot HBr$	C,H,N,Br	218–220	EtOH	A (42)
5u	$C_6H_4(4\text{-}F)$	1	$C_9H_{12}FN\cdot HCl$	C,H,N,Cl	201–202	*i*-PrOH	A[e] (14)
5v	$C_6H_4(2\text{-}F)$	1	$C_9H_{12}FN\cdot HBr$	C,H,N,Br	165–167	*i*-PrOH	A (42)
5w	C_6F_5	1	$C_9H_8F_5N\cdot HCl$	C,H,N	200–201.5	dioxane	A (48)
5x	$C_6H_4(4\text{-}Ph)$	1	$C_{15}H_{17}N\cdot HCl$	C,H,N,Cl	264	EtOH	A[e] (46)
5y	$C_6H_4(4\text{-}NO_2)$	3	$C_{11}H_{16}N_2O_2\cdot HCl$	C,H,N,Cl	171–172	Et_2O (trit)	E (3.6)

[a] A, RBr + $EtNH_2$; B, ROMs + $EtNH_2$; C, RNH_2 + EtI (Scheme I); D, nitration of parent (Scheme II). (Overall yield of purified product, not optimized.) [b] See Experimental Section for preparation of starting material. [c] Free base mass spectrum found m/z 217. [d] Used crude in next stage. [e] Prepared from the benzyl chloride.

Table II. Analytical Data for Intermediates for Intermediate *N*-Alkylphenylalaninamides [H-L-(R)Phe(X)-NH_2]

no.	X	R	formula	anal.	mp, °C	cryst solvent	method[a] (yield, %)
7a	$4\text{-}NO_2$	Et	$C_{11}H_{15}N_3O_3$	C,H,N	ND	EtOAc	C (50)
7b	$4\text{-}NO_2$	Me	$C_{10}H_{13}N_3O_3$	C,H,N	146.5–147.5	EtOAc–Et_2O	*b* (39)
7c	4-F	Et	$C_{11}H_{15}FN_2O$	C,H,N	117.5–118.5	EtOAc–Et_2O	C (40)
7d	3-F	Et	$C_{11}H_{15}FN_2O$	C,H,N	ND	EtOAc	C (39)
7e	2-F	Et	$C_{11}H_{15}FN_2O$	C,H,N	107–109	EtOAc–Et_2O	C (46)
7f	4-Cl	Et	$C_{11}H_{15}ClN_2O$	C,H,N	131–133	Et_2O (trit)	C (41)
7g	$4\text{-}CF_3$	Et	$C_{12}H_{15}F_3N_2O$	C,H,N[c]	ND		C (51)
7h	$3,4\text{-}Cl_2$	Et	$C_{11}H_{14}Cl_2N_2O$	C,H,N	ND		C (60)

[a] C: H-L-Phe(X)-NH_2 + EtI (Scheme I). (Overall yield of purified product, not optimized.) [b] Prepared by ammonolysis of the corresponding methyl ester. [c] N: calcd, 10.76; found, 9.93.

Table III. Intermediate Glycinamide Isosteres

no.	structure	formula	anal.	mp, °C	cryst solvent	TLC R_f (solvent)	yield,[a] %
12	$H_2N(CH_2)_2N(Et)(CH_2)_2C_6H_4(4\text{-}NO_2)$	$C_{12}H_{19}N_3O_2\cdot 2HCl$	C,H,N,Cl	204–205	*i*-PrOH–EtOH–*i*-Pr_2O	0.21 (B), 0.45 (C), 0.15 (F)	49
13	$H_2N(CH_2)_2S(CH_2)_2C_6H_4(4\text{-}NO_2)$	$C_{10}H_{14}N_2O_2S\cdot HCl$	C,H,N,S,Cl	110–111	MeCN	0.70 (B), 0.64 (C), 0.47 (F)	35

[a] Overall yield of purified product, not optimized.

The aralkylamines (**5**, Table I) required for the synthesis of the tripeptides (**18**) were obtained by several routes (Scheme I). Where the aralkyl halide (**2**) was commercially available, this was converted directly to the corresponding ethylamine (**5**). Alternatively, available aralkyl alcohols (**1**) were converted via either the bromides (**2**) or the mesylates (**3**) to the required ethylamines (**5**). Compound **5c** was obtained by treatment of 2-(4-fluorophenyl)ethylamine (**4c**, Aldrich) with EtI–$NaHCO_3$. The resultant mixture of primary, secondary, and tertiary amines was separated chromatographically on silica gel. In certain cases, it was necessary to prepare the required substituted aralkyl intermediates from other available materials. Thus, 4-cyanophenethyl alcohol (**1h**) was prepared by a Sandmeyer reaction,[13] the 3,4-dichloro derivative (**1f**) was obtained by hydrolysis and reduction of the corresponding phenylacetonitrile,[14] and the bromide (**2i**) by chlorosulfonation of phenethyl bromide[15] and reaction with aqueous ammonia. The 4-pyridyl amine (**5r**) was prepared by addition of ethylamine to 4-vinylpyridine.[16] 2-(2-Furyl)ethanol (**1o**) was prepared by lithiation of furan and reaction with ethylene oxide.[17] Preparation of the nitro derivatives of the thienyl and furyl ethylamines (**5m**, **5n**, **5p**) is illustrated in Scheme II. 5-Nitration[18] (**9**) was achieved with fuming HNO_3–Ac_2O after protection of the secondary amino group by trifluoroacetylation (**8**). The 3-nitrothienyl derivative (**5n**) was obtained by nitration of the 5-chlorosulfonyl derivative (**10**), albeit in low yield as a result of the vigorous hydrolytic deprotection step.[19]

The substituted phenylalanines required for the synthesis of the tetrapeptides were either prepared by al-

(13) Uhlmann, E.; Pfleiderer, W. *Helv. Chim. Acta* **1981**, *64*, 1688.
(14) May, E. L.; Mosettig, G. *J. Org. Chem.* **1946**, *11*, 631.
(15) Inskeep, G. E.; Deanin, R. *J. Am. Chem. Soc.* **1947**, *69*, 2237.
(16) Brady, L. E.; Freifelder, M.; Stone, G. R. *J. Org. Chem.* **1961**, *26*, 4757.
(17) Chadwick, D. J.; Chambers, J.; Meakins, G. D.; Snowden, R. L. *J. Chem. Soc., Perkin Trans. 1* **1975**, *5*, 523.
(18) (a) Butter, A. R.; Hendry, J. B. *J. Chem. Soc., Ser. B* **1971**, 102. (b) Sice, J. *J. Am. Chem. Soc.* **1953**, *75*, 3697.
(19) Gronowitz, S.; Ander, I. *Acta Chem. Scand.* **1975**, *B29*, 513.

Table IV. Analytical Data for Intermediate Glycinamides [H-Gly-N(Et)$(CH_2)_n$Ar]

no.	Ar	n	formula	anal.	mp, °C	cryst solvent	TLC R_f (solvent)	yield,[a] %
14c[b]	C_6H_4(4-F)	2	$C_{17}H_{25}FN_2O_3$	C,H,N	84–85	EtOH–H_2O	0.70 (A), 0.85 (B), 0.83 (C)	78
14i[b]	C_6H_4(4-SO_2NH_2)	2	$C_{17}H_{27}N_3O_5S$	C,H,N,S	111	EtOAc	0.34 (A), 0.81 (B)	59
15a	C_6H_4(4-NO_2)	2	$C_{12}H_{17}N_3O_3$·HCl	C,H,N	119–120	MeOH–*i*-PrOH	0.45 (B), 0.64 (C), 0.38 (F)	76
15b	C_6H_4(2-NO_2)	2	$C_{12}H_{17}N_3O_3$·HCl	C,H,N	173–176	*i*-PrOH–EtOH	0.63 (B), 0.74 (C), 0.40 (F)	79
15d	C_6H_4(2-F)	2	$C_{12}H_{17}FN_2O$·HCl	C,H,N	122–124	*i*-PrOH–*i*-Pr_2O	0.47 (B), 0.77 (C), 0.36 (F)	66
15e	C_6H_4(4-Br)	2	$C_{12}H_{17}BrN_2O$·HCl	C,H,N	136–137	*i*-PrOH–*i*-Pr_2O	0.66 (B), 0.74 (C), 0.44 (F)	65
15f	C_6H_3(3,4-Cl_2)	2	$C_{12}H_{16}Cl_2N_2O$·HCl	ND	ND	gum	0.58 (C), 0.93 (E)	48
15g	C_6H_4(2-CF_3)	2	$C_{13}H_{17}F_3N_2O$·HCl·0.5H_2O	C,H,N	ND	*i*-PrOH–*i*-Pr_2O	0.68 (C), 0.45 (F)	64
15h	C_6H_4(4-CN)	2	$C_{13}H_{17}N_3O$·HCl	C,H,N	185–187	*i*-PrOH	0.38 (B), 0.82 (C), 0.45 (F)	85
15j	1-$C_{10}H_7$	2	$C_{16}H_{20}N_2O$·HCl	ND	ND	ND	0.52 (B), 0.52 (F)	83
15k	2-$C_{10}H_7$	2	$C_{16}H_{20}N_2O$·HCl	C,H,N	153–153.5	*i*-PrOH	0.55 (B), 0.80 (C), 0.55 (F)	73
15l	2-C_4H_3S	2	$C_{10}H_{16}N_2OS$·HCl·H_2O	C,H,Cl[c]	ND	FD[d]	0.65 (C), 0.40 (F)	73
15m	2-C_4H_2S(5-NO_2)	2	$C_{10}H_{15}N_3O_3S$·HCl	C,H,N,S,Cl	ND	FD[d]	0.32 (B), 0.32 (F)	88
15n	2-C_4H_2S(3-NO_2)	2	$C_{10}H_{15}N_3O_3S$·HCl	C,H,N	ND	FD[d]	0.53 (B), 0.30 (F)	61
15o	2-C_4H_3O	2	$C_{10}H_{16}N_2O_2$·HCl	ND	ND	gum	0.50 (B), 0.42 (F)	78
15p	2-C_4H_2O(5-NO_2)	2	$C_{10}H_{15}N_3O_4$·HCl·0.25H_2O	C,H,N	ND	FD[d]	0.43 (B), 0.65 (C), 0.33 (F)	84
15q	2-C_5H_5N	2	$C_{11}H_{18}N_3O$·2HCl	C,H,N,Cl	ND	FD[d]	ND	77
15r	4-C_5H_5N	2	$C_{11}H_{18}N_3O$.2HCl	C,H,N	ND	FD[d]	0.13 (B), 0.60 (C)	46
15s	C_6H_4(4-NO_2)	1	$C_{11}H_{15}N_3O_3$·HCl	C,H,N,Cl	ND	FD[d]	ND	79
15t	C_6H_4(2-NO_2)	1	$C_{11}H_{15}N_3O_3$·HCl	C,H,N,Cl	157–158	*i*-PrOH	0.33 (F)	66
15u	C_6H_4(4-F)	1	$C_{11}H_{15}FN_2O$·HCl·H_2O	C,H,N,Cl	ND	FD[d]	0.35 (B), 0.50 (C), 0.41 (F)	79
15v	C_6H_4(2-F)	1	$C_{11}H_{15}FN_2O$.HCl	C,H,N,Cl	112–114	*i*-PrOH–*i*-Pr_2O	0.50 (F), 0.28 (G)	88
15x	C_6H_4(4-Ph)	1	$C_{17}H_{20}N_2O$·HCl	ND	ND	trit Et_2O	0.45 (B), 0.57 (C), 0.46 (F)	92
15y	C_6H_4(NO_2)	3	$C_{17}H_{19}N_3O_3$·HCl·0.75H_2O	C,H,N,Cl	ND	FD[d]	0.60 (B), 0.88 (C), 0.44 (F)	67

[a] Overall yield of purified product from relevant ethylamine (Table I), not optimized. [b] N^{α}-Boc compound. [c] N: calcd, 10.50; found, 9.92. [d] Freeze-dried amorphous powder.

Table V. Analytical Data for Intermediate Dipeptide Amides [H-Gly-L-(R)Phe(X)-NH_2]

no.	X	R	formula	anal.	cryst solvent	TLC R_f (solvent)	yield,[a] %
21a	4-NO_2	Et	$C_{13}H_{18}N_4O_4$·HCl	C,H,N	MeOH–Et_2O	0.20 (B), 0.74 (D), 0.29 (F)	79
21b	4-NO_2	Me	$C_{12}H_{16}N_4O_4$·HCl	C,H,N[b]	FD[c]	0.20 (B), 0.29 (C), 0.43 (F)	76
21c	4-F	Et	$C_{13}H_{18}FN_3O_2$·HCl	C,H,N[d]	FD[c]	0.33 (B), 0.40 (C), 0.49 (F)	55
21d	3-F	Et	$C_{13}H_{18}FN_3O_2$·HCl	C,H,N,Cl	FD[c]	ND	65
21e	2-F	Et	$C_{13}H_{18}FN_3O_2$·HCl	C,H,N	FD[c]	0.24 (B), 0.25 (C), 0.42 (F)	62
21f	4-Cl	Et	$C_{13}H_{18}ClN_3O_2$·HCl·0.6H_2O	C,H,N	Et_2O[e]	0.30 (B), 0.37 (F)	58
20g[f]	4-CF_3	Et	$C_{19}H_{26}F_3N_3O_4$	C,H,N	Et_2O[e]	0.50 (A), 0.88 (C), 0.78 (F)	50

[a] Overall yield from relevant *N*-alkylphenylalaninamide (Table II), not optimized. [b] N: calcd, 17.69; found, 16.97. [c] Freeze-dried amorphous powder. [d] N: calcd, 13.84; found, 13.39. [e] Triturated. [f] N^{α}-Boc compound.

kylation of diethyl acetamidomalonate by using standard methodology[20,28c] or, when available, purchased, from commercial sources. In either case, the amino acids were resolved enzymically via hog acylase I (*N*-chloroacetyl derivatives)[21] or carboxypeptidase A (*N*-trifluoroacetyl derivatives).[22] The requisite amides (**6**, Scheme I) were synthesized from the N^{α}-Boc derivatives by amination of the mixed anhydride prepared from isobutyl chloroformate and subsequent N^{α}-deprotection. The N^{α}-ethyl derivatives (**7**, Table II) were then obtained by alkylation of **6** with EtI–$NaHCO_3$.[31]

Two intermediates containing isosteric replacements for the Gly^3 amide bond were also prepared (**12**, **13**, Table III). The former was obtained by reduction of the glycinamide (**15a**, Table IV) with $LiAlH_4$ and the latter by alkylation of cysteamine with 4-nitrophenethyl bromide.

The tripeptide amides were synthesized as shown in Scheme III. Glycinamides (**15**, Table IV) were obtained by DCCI–HOBt mediated coupling of Boc-Gly-OH to the appropriate aralkylethylamine (**5**) followed by acidolytic deprotection. Subsequent coupling to Boc-Tyr-D-Arg-OH·HCl (**16**) and deprotection yielded the parent tripeptides (**18**, Table VI). In the majority of cases these tripeptides were converted to the N^{α}-amidino derivatives (**19**, Table VI) as described above. As an example, the synthesis of **19v** is described in the Experimental Section.

Tetrapeptide amides were obtained via 2 + 2 couplings of the glycyl dipeptides (**21**, Table V) with Boc-Tyr-D-Arg-OH·HCl (**16**) as shown in Scheme IV. Removal of the N^{α}-protecting group yielded the parent tetrapeptide amides (**23**), two of which were converted to the *N*-amidino derivatives (**24**, Table VII). As an example, the synthesis of **23a** is described in the Experimental Section.

For comparative purposes the D-Met^2 peptides (**18bb**, **23i**) were prepared by using the same methodology, but with substitution of Boc-Tyr-D-Met-OH (**25**) for **16** (Schemes III and IV).

Each of the analogues was purified by ion-exchange chromatography, followed in some cases by preparative reverse-phase HPLC; final products were isolated by lyophilization. In a series of pentapeptides, 3–4% of the L-Arg^2 isomer was detected by TLC and HPLC following similar fragment coupling of Boc-Tyr-D-Arg-OH·HCl to a glycyl peptide.[1] A similar degree of racemization could occur in the present study although there was no evidence for this by TLC or HPLC. The peptides were generally characterized by optical rotation, TLC in several solvent systems, and elemental analysis; the data are presented in Tables VI and VII.

Pharmacology. The in vitro biological activity of the polar tri- and tetrapeptides (**18**, **19**, **23**, **24**) was evaluated on the isolated guinea pig ileum by determination of the inhibition of electrically induced contractions. The opioid

(20) Barrett, G. C. *Chemistry and Biochemistry of the Amino Acids*; Chapman and Hall: London, 1985; Chapter 8, pp 246–296.
(21) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Chapter 9, pp 715–759.
(22) (a) Turk, J.; Panse, G. T.; Marshall, G. R. *J. Org. Chem.* **1975**, *40*, 953. (b) Sarda, N.; Grouiller, A.; Pacheco, H. *Tetrahedron Lett.* **1976**, 271.

Table VI. Analytical Data and Biological Activities of Polar Tripeptide Amide Enkephalin Analogues [Y-L-Tyr-D-Arg-Gly-N(Et)$(CH_2)_n$Ar]

no.	Y[a]	Ar	n	formula[b]	anal.	$[\alpha]_D$, deg (c, t °C)[c]	TLC	yield,[d] %	GPI[e]	PBQ writhing[f] sc	PBQ writhing[f] po	AA writhing[f] sc	dose[g] ratio	hot plate[f] sc
18a	H	C_6H_4(4-NO_2)	2	$C_{27}H_{38}N_8O_6{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+33.7 (1.0, 22)	0.67 (D), 0.22 (F)	68	48.6	17.5	43.5	3.1	4.5	0 (100)
18b	H	C_6H_4(2-NO_2)	2	$C_{27}H_{38}N_8O_6{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N	ND	0.63 (D), 0.27 (F)	42	118	31.0	0 (50)	2.7	6.4	0 (20)
18c	H	C_6H_4(4-F)	2	$C_{27}H_{38}FN_7O_4{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+35.0 (1.0, 25)	0.66 (D), 0.24 (E), 0.82 (F)	65	20.5	1.6	30.8	0.14	4.5	12.3
18d	H	C_6H_4(2-F)	2	$C_{27}H_{38}FN_7O_4{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	ND	0.65 (D), 0.26 (F)	60	22.0	13.7	0 (50)	0.3	13.3	0 (10)
18e	H	C_6H_4(4-Br)	2	$C_{27}H_{38}BrN_7O_4{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	+31.4 (1.0, 24.5)	0.20 (F), 0.11 (H)	60	<100	10 (1.0)	0 (20)	0 (100)		0 (100)
18f	H	C_6H_3(3,4-Cl_2)	2	$C_{27}H_{37}Cl_2N_7O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	+31.1 (1.0, 25)	0.64 (B), 0.44 (F)	57	12.5	4.7	0 (20)	0 (20)	0.5	ca. 100
18g	H	C_6H_4(2-CF_3)	2	$C_{28}H_{38}F_3N_7O_4{\cdot}1.75CH_3CO_2H{\cdot}H_2O$	C,H,N	+33.1 (1.0, 25)	0.66 (E), 0.43 (F)	69	72.1	33 (10)	0 (20)			
18h	H	C_6H_4(4-CN)	2	$C_{28}H_{38}N_8O_4{\cdot}2CH_3CO_2H{\cdot}1.25H_2O$	C,H,N	ND	0.20 (F)	51	1800			2.2	1.4	
18i	H	C_6H_4(4-SO_2NH_2)	2	$C_{27}H_{40}N_8O_6S{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N,S	+23.3 (1.0, 27)	0.50 (E), 0.25 (F)	44	11000			0 (100)		
18j	H	1-$C_{10}H_7$	2	$C_{31}H_{41}N_7O_4{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N	+31.8 (1.0, 25)	0.69 (E), 0.42 (F)	57	75.9	1.4	31 (20)			0 (50)
18k	H	2-$C_{10}H_7$	2	$C_{31}H_{41}N_7O_4{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	+30.9 (1.0, 25)	0.36 (D), 0.61 (E), 0.43 (F)	75		27 (1)	0 (20)			
18l	H	2-C_4H_3S	2	$C_{25}H_{37}N_7O_4S{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N,S	+35.8 (1.0, 25)	0.16 (F)	62		0 (1)	21 (20)			
18m	H	2-C_4H_2S(5-NO_2)	2	$C_{25}H_{36}N_8O_6S{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	ND	0.60 (B), 0.16 (F)	55	217			1.7	6.2	
18n	H	2-C_4H_2S(3-NO_2)	2	$C_{25}H_{36}N_8O_6S{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	ND	0.69 (B), 0.19 (F)	39	146					
18o	H	2-C_4H_3O	2	$C_{25}H_{37}N_7O_5{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	ND	0.61 (B), 0.61 (F)	50		0 (1)	0 (20)			
18p	H	2-C_4H_2O(5-NO_2)	2	$C_{25}H_{36}N_8O_7{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	ND	0.75 (B), 0.48 (F)	38		0 (1)	0 (20)			
18q	H	2-C_5H_5N	2	$C_{26}H_{38}N_8O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	+32.5 (1.0, 25)	0.65 (D), 0.40 (E), 0.04 (F)	23	510					
18r	H	4-C_5H_5N	2	$C_{26}H_{38}N_8O_4{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N	+32.0 (1.0, 25)	0.61 (E), 0.04 (F)	46	569			0 (3)		
18s	H	C_6H_4(4-NO_2)	1	$C_{26}H_{36}N_8O_6{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	+34.8 (1.0, 25)	0.64 (E), 0.32 (F)	43	9.7	0 (10)	0 (50)	0.02	2.8	0 (50)
18t	H	C_6H_4(2-NO_2)	1	$C_{26}H_{36}N_8O_6{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+36.3 (1.0, 25)	0.63 (E), 0.35 (F)	26	522	60.3		6.1	2.8	0 (50)
18u	H	C_6H_4(4-F)	1	$C_{26}H_{36}FN_7O_4{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+37.4 (1.0, 25)	0.65 (E), 0.38 (F)	51	63.8	10.4		0.09	3.9	0 (20)
18v	H	C_6H_4(2-F)	1	$C_{26}H_{36}FN_7O_4{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+38.7 (1.0, 25)	0.65 (E), 0.42 (F)	84	325	31.1		1.1	8.0	0 (50)
18w[h]	H	C_6F_5	1	$C_{26}H_{32}F_5N_7O_4{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	ND	0.49 (B), 0.45 (F)	36	820					
18x	H	C_6H_4(4-Ph)	1	$C_{32}H_{41}N_7O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	+32.4 (1.0, 23.5)	0.50 (F), 0.14 (H)	40		13 (1)	0 (20)			
18y	H	C_6H_4(4-NO_2)	3	$C_{28}H_{40}N_8O_6{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	ND	0.73 (D), 0.58 (E), 0.34 (F)	34	1800			0 (30)		
18z[i]	H	C_6H_4(4-NO_2)	2	$C_{27}H_{40}N_8O_5{\cdot}1.7CH_3CO_2H{\cdot}H_2O$	C,H,N	+31.2 (1.0, 22)	0.65 (E), 0.10 (F)	42	25% @ 7400					
18aa[j]	H	C_6H_4(4-NO_2)	2	$C_{25}H_{37}N_7O_5S{\cdot}1.7CH_3CO_2H{\cdot}H_2O$	C,H,N	+34.4 (1.0, 23)	0.65 (E), 0.44 (F)	52	4200					
18bb[k]	H	C_6H_4(4-NO_2)	2	$C_{26}H_{35}N_5O_6S{\cdot}CH_3CO_2H$	C,H,N,S	+40.8 (1.0, 26.5)	0.97 (E), 0.61 (F)	57	41			0.87	1.1	
19a	Am	C_6H_4(4-NO_2)	2	$C_{28}H_{40}N_{10}O_6{\cdot}2CH_3CO_2H{\cdot}1.25H_2O$	C,H,N	+23.9 (1.0, 21)	0.84 (D), 0.24 (E), 0.35 (F)	80	30.4	0.24	10	0.15	9.6	19.2
19b	Am	C_6H_4(2-NO_2)	2	$C_{28}H_{40}N_{10}O_6{\cdot}2CH_3CO_2H{\cdot}2.5H_2O$	C,H,N	+17.0 (1.0, 26)[l]	0.83 (D), 0.44 (F)	64	1.2	1.1	76.1	0.03	8.6	52.9
19c	Am	C_6H_4(4-F)	2	$C_{28}H_{40}FN_9O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	ND	ND	70	6.8	0.08	5.3	0.02	2.4	20.0
19d	Am	C_6H_4(2-F)	2	$C_{28}H_{40}FN_9O_4{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+20.7 (1.0, 26)	0.83 (D), 0.45 (F)	64	24.6	0.9	ca. 50	0.17	9.8	22.2
19e	Am	C_6H_4(4-Br)	2	$C_{28}H_{40}BrN_9O_4{\cdot}2CH_2CO_2H{\cdot}1.5H_2O$	C,H,N	+22.9 (1.0, 24.5)	0.16 (E), 0.40 (F)	36	216	3.4	15 (20)	0.35	1.5	0 (10)
19f	Am	C_6H_3(3,4-Cl_2)	2	$C_{28}H_{39}Cl_2N_9O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	+23.7 (1.0, 24.5)	0.16 (E), 0.41 (F)	77	<100	0.2	14 (20)	0.62	1.0	0 (50)
19g	Am	C_6H_4(2-CF_3)	2	$C_{29}H_{40}F_3N_9O_4{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N	+22.5 (1.0, 21.5)	0.15 (E), 0.43 (F)	78	28.4	40 (10)	0 (20)			
19i	Am	C_6H_4(4-SO_2NH_2)	2	$C_{28}H_{42}N_{10}O_6S{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N,S	ND	0.04 (E), 0.26 (F)	62	4900			0 (30)		
19j	Am	1-$C_{10}H_7$	2	$C_{32}H_{43}N_9O_4{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N	+24.0 (0.36, 25)	0.16 (E), 0.43 (F)	79	30.0	1.4	0 (20)			0 (50)
19k	Am	2-$C_{10}H_7$	2	$C_{32}H_{43}N_9O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	+24.6 (0.5, 25)	0.44 (D), 0.38 (F)	54		11 (1)	0 (20)			
19l	Am	2-C_4H_3S	2	$C_{26}H_{39}N_9O_4S{\cdot}2CH_3CO_2H{\cdot}1.5H_2O$	C,H,N,S	+24.4 (1.0, 23.5)	0.09 (E), 0.38 (F)	79	9.3	0.44	15 (20)			22.9
19m	Am	2-C_4H_2S(5-NO_2)	2	$C_{26}H_{38}N_{10}O_6S{\cdot}2CH_3CO_2H{\cdot}2.5H_2O$	C,H,N	ND	0.10 (D), 0.39 (F)	78	25.2	2.0	7 (20)			
19o	Am	2-C_4H_3O	2	$C_{26}H_{39}N_9O_5{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	+25.6 (1.0, 25)	0.15 (E), 0.37 (F)	80	10.9	0.32	10 (20)			
19p	Am	2-C_4H_2O(5-NO_2)	2	$C_{26}H_{38}N_{10}O_6{\cdot}2CH_3CO_2H{\cdot}2.5H_2O$	C,H,N	+24.1 (1.0, 24.5)	ND	54		17 (1)	15 (20)			
19s	Am	C_6H_4(4-NO_2)	1	$C_{27}H_{38}N_{10}O_6{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+26.4 (1.0, 25)	0.79 (D), 0.38 (F)	66	470	7.8		0.9	4.6	
19t	Am	C_6H_4(2-NO_2)	1	$C_{27}H_{38}N_{10}O_6{\cdot}2CH_3CO_2H{\cdot}1.2H_2O$	C,H,N	+26.6 (1.0, 25)	0.37 (F)	76	244	4.0	14 (20)	1.1	11.0	0 (50)
19u	Am	C_6H_4(4-F)	1	$C_{27}H_{38}FN_9O_4{\cdot}2.2CH_3CO_2H{\cdot}2H_2O$	C,H,N	ND	0.62 (E), 0.34 (F)	54	46	1.7	12 (20)	0.32	6.4	0 (50)
19v	Am	C_6H_4(2-F)	1	$C_{27}H_{38}FN_9O_4{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	+28.2 (1.0, 25)	0.43 (B), 0.42 (F)	66	20.6	0.43	16 (20)	0.18	7.8	14.6
19x	Am	C_6H_4(4-Ph)	1	$C_{33}H_{43}N_9O_4{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N[m]	+23.8 (0.9, 25)	0.20 (E), 0.43 (F)	64	324	2.3	0 (20)			

[a] Am = N^{α}-amidino peptide. [b] Acetic acid level confirmed by NMR. [c] Determined in MeOH. [d] Overall yield of purified product from relevant glycinamide (Table IV). [e] Guinea pig ileum, ED_{50}, nM. [f] ED_{50}/mg kg^{-1} or percent inhibition (dose/mg) at 30 min, after dosing. [g] Dose ratio in the presence/absence of *N*-methylnalorphine (11.5 mg kg^{-1}) in AA writhing. [h] Prepared by a 3 + 1 coupling. [i] Reduced Gly isostere derived from **12**, Table III. [j] Thia Gly isostere derived from **13**, Table III. [k] D-Met^2 peptide. [l] Determined in MeOH–H_2O (3:1 v/v). [m] N: calcd, 16.04; found, 15.28.

Table VII. Analytical Data and Biological Activities of Polar Tetrapeptide Amide Enkephalin Analogues [Y-L-Tyr-D-Arg-Gly-(R)Phe(X)-NH_2]

no.	Y	R	X	formula[a]	anal.	$[\alpha]_D$, deg (c, t °C)[b]	TLC	yield,[c] %	GPI[d]	PBQ writhing[e] sc	PBQ writhing[e] po	AA writhing[e] sc	dose[f] ratio	hot plate[e] sc
23a	H	Et	4-NO_2	$C_{28}H_{39}N_9O_7{\cdot}2CH_3CO_2H{\cdot}H_2O$	C,H,N	−40.9 (1.0, 23)	0.79 (D), 0.30 (E), 0.12 (F)	44	5.2	0.06	13.9	0.05	3.4	0.52
23b	H	Me	4-NO_2	$C_{27}H_{37}N_9O_7{\cdot}2CH_3CO_2H{\cdot}0.25H_2O$	C,H,N	+32.0 (1.0, 25)	0.52 (D), 0.23 (F)	59	36.5			0.25	2.5	
23c	H	Et	4-F	$C_{28}H_{39}FN_8O_5{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	−7.5 (1.0, 25)	0.64 (D), 0.34 (F)	50	2.2	0.13	7.9	0.0006	2.3	1.6
23d	H	Et	3-F	$C_{28}H_{39}FN_8O_5{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	−3.9 (1.0, 25)	0.81 (E), 0.36 (F)	73	25	0.08		0.04	2.2	1.7
23e	H	Et	2-F	$C_{28}H_{39}FN_8O_5{\cdot}2CH_3CO_2H{\cdot}0.8H_2O$	C,H,N	−11.1 (1.0, 25)	0.68 (D), 0.33 (F)	53	0.39	0.15	5.9	0.0009	3.9	1.95
23f	H	Et	4-Cl	$C_{28}H_{39}ClN_8O_5{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	−31.1 (1.0, 25)	0.41 (F)	47	0.64			0.03	1.3	
23g	H	Et	4-CF_3	$C_{29}H_{39}F_3N_8O_5{\cdot}2CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	−27.7 (0.85, 25)	0.80 (D), 0.41 (F)	37	6.25			0.13	2.9	16.9
23h	H	Et	3,4-Cl_2	$C_{28}H_{38}Cl_2N_8O_5{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	+5.0 (1.0, 25)	0.21 (F), 0.10 (H)	34	0.79	1.79	29.2	0.008	9.7	38.2
23i[g]	H	Et	4-NO_2	$C_{27}H_{36}N_6O_7S{\cdot}CH_3CO_2H{\cdot}0.5H_2O$	C,H,N	−27.9 (1.0, 25)	0.37 (B), 0.61 (D), 0.39 (F)	42	1.2			0.0015	0.8	
24a	Am[h]	Et	4-NO_2	$C_{29}H_{41}N_{11}O_7{\cdot}2CH_3CO_2H{\cdot}2H_2O$	C,H,N	−47.2 (1.0, 22)[i]	0.18 (D), 0.15 (F)	70	3.9	0.005	1.9	0.009	5.1	0.34
24c	Am[h]	Et	4-F	$C_{29}H_{41}FN_{10}O_5{\cdot}2CH_3CO_2H{\cdot}2.5H_2O$	C,H,N	−12.8 (1.0, 22)	0.75 (D), 0.44 (F)	56	0.09	0.04	25.6	0.002	6.5	0.9

[a] Acetic acid level confirmed by NMR. [b] Determined in MeOH. [c] Overall yield of purified product from relevant dipeptide amide (Table V), not optimized. [d] Guinea-pig Ileum, ED_{50}/nM. [e] ED_{50}/mg kg^{-1} or % inhibition (dose/mg) at 30 min, after dosing. [f] Dose ratio in the presence/absence of *N*-methylnalorphine (11.5 mg kg^{-1}) in AA writhing. [g] D-Met[2] peptide. [h] N^{α}-amidino-peptide. [i] Determined in MeOH–H_2O (4:1, v/v).

nature of this inhibition was confirmed by its reversal in the presence of naloxone (1 μg mL^{-1}). In vivo antinociceptive activity was determined in mice by using writhing and hotplate assays; the peripheral nature of the peptide analogues was investigated in writhing assays with the use of the peripherally acting opioid antagonist *N*-methylnalorphine and by comparison of antinociceptive effects in the writhing and hot-plate assays.

Chemically induced writhing assays were carried out with intraperitoneal injections of either of two irritants, phenyl-*p*-benzoquinone (PBQ) and acetic acid (AA). Writhing assays with these irritants have been used to investigate the antinociceptive effects of nonsteroidal antiinflammatory drugs[23] and peripherally acting opioids.[3a,7a,24] In the previous studies the peripheral nature of the antinociceptive effects of *N*-methylmorphine[3a] and BW 443C[7a] was demonstrated by antagonism of these effects by *N*-methylnalorphine. The centrally mediated antinociceptive effects of morphine were not antagonized by *N*-methylnalorphine. Antagonism by *N*-methylnalorphine is expressed by means of dose ratio, which is defined as the ratio of the doses of agonist required to produce an equiactive effect in the presence and absence of the antagonist. A dose ratio of unity, therefore, signifies no antagonism and a high ratio expresses significant antagonism and hence indicates a peripherally mediated effect.

Activity was also determined in the hot-plate assay. In contrast with the writhing assays, such heat-induced noxious assays detect only centrally acting opioids.[25] A peripheral mode of action is indicated by a high potency in the writhing tests coupled with a low potency in the hot-plate test.

Subcutaneous activity of the peptide analogues was determined in all assays and oral activity in the PBQ-induced writhing assay.

Discussion

We have previously described[1] the synthesis and pharmacology of the peripherally acting polar opioid pentapeptide BW 443C [H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2]. The results obtained from pharmacological[7] and clinical[26] studies with this enkephalin analogue have encouraged us to investigate a series of structurally simpler compounds[10] in which the D-Arg[2] residue of BW 443C was retained while a variety of structural modifications were applied to the carboxyl terminal of the parent pentapeptide.

The peptides described in this paper fall essentially into two classes: a group of tetrapeptide amides obtained by deletion of the C-terminal Pro-NH_2 residue of BW 443C and a series of tripeptide aralkylamides in which additional simplification is achieved by further elimination of the carboxamide and, hence the chiral center, of the Phe(4-NO_2)[4] residue of BW 443C.

Numerous studies have been reported which deal with truncated enkephalin analogues,[27] although to our

(23) (a) Hendershot, L. C.; Forsaith, J. *J. Pharmacol. Exp. Ther.* **1959**, *125*, 237. (b) Koster, R.; Anderson, M.; de Beer, E. J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1959**, *18*, 412.

(24) Smith, T. W.; Follenfant, R. L.; Ferreira, S. H. *Int. J. Tiss. Reac.* **1985**, *7*, 61.

(25) Tyres, M. *Br. J. Pharmacol.* **1980**, *69*, 503.

(26) (a) Posner, J.; Dean, K.; Jeal, S.; Moody, G.; Peck, A. W.; Rutter, G.; Telekes, A. *Br. J. Clin. Pharmacol.* **1987**, *24*, 266P. (b) Cohen, A. F.; Harkin, N.; Posner, J. *Br. J. Clin. Pharmacol.* **1987**, *24*, 267P. (c) Posner, J.; Moody, S. G.; Peck, A. W.; Rutter, G.; Telekes, A. *Br. J. Clin. Pharmacol.* **1987**, *25*, 120P. (d) Posner, J.; Dean, K.; Jeal, S.; Moody, S. G.; Peck, A. W.; Rutter, G.; Telekes, A. *Eur. J. Clin. Pharmacol.* **1988**, *34*, 67.

knowledge, no such compound has been indicated as having a selectively peripheral mode of action. For example, it has been shown[27a] that the synthetic tripeptide aralkylamide syndyphalin-33 [H-Tyr-D-Met(O)-Gly-N-(Me)-CH_2CH_2Ph] exhibits potent opioid activity in vivo and prolonged analgesic action after sc administration in mice, while in a series of tetrapeptide enkephalin analogues, it has been demonstrated that N-alkylation of the Phe^4 residue increases analgesic efficacy and that the *N*-ethyl substituent is optimal.[27b] In the light of these reports and the results of our earlier investigations,[28] we have sought to produce polar analogues having the desired peripheral opiate activity. At the same time the effects of further variation of the aryl substituent at Phe^4, the nature of the aromatic ring and the aralkyl chain length have also been investigated. In an attempt to further simplify the structure, two examples of isosteric amide bond replacement at Gly^3 were also prepared (**18z**, **18aa**).

The analytical data and the biological activities of the polar tri- and tetrapeptide enkephalin analogues are presented in Tables VI and VII, respectively. Generally, the GPI and/or the ED_{50}s (sc) in the PBQ-induced writhing assay were taken as a primary indication of useful activity, and only those compounds showing promise were subjected to further evaluation in the AA writhing and hot-plate assays. As a measure of peripheral versus central activity, the dose ratio in the presence and absence of the peripheral opioid antagonist *N*-methylnalorphine was determined in the AA writhing assay, as previously described.[1] Comparison of the ED_{50}s in the writhing (peripheral) and hot-plate (central) assays also provides evidence for a predominantly peripheral mode of action. In agreement with previous observations,[29] there is generally poor correlation between potencies determined in the in vitro and the in vivo assays. This is in marked contrast with the close agreement reported for classical opiates.[30]

As a class, the tetrapeptides exhibit greater antinociceptive potencies than the tripeptides in the writhing assays; e.g. compare **18a**/**19a**, Table VI with **23a**/**24a**, Table VII. Some observations on the effects of each type of structural variation on activity are made below.

Aromatic Substitution. The choice of substituent was essentially limited to those known from previous work[10,28,31] to be associated with the retention of opioid activity (generally electron withdrawing, small or planar), except in cases where an increase in hydrophilicity was sought. The bulk of the work was carried out in the tripeptide series of reasons for synthetic ease. An examination of the effects on activity of ring substitution in the tripeptide phenylalkylamide series (Table VI) reveals that the F[27b] and NO_2[28b] (**18**/**19a–d**) substituents confer high levels of analgesic potency while bulkier groups such as Br, CF_3, CN (**18**/**19e**, **18**/**19g**, **18h**) reduce activity in all assays. More dramatically, the sulfonamido substituent, chosen as an example of a hydrophilic group, essentially abolishes opioid activity (**18i**, **19i**). The 3,4-Cl_2 substitution pattern (**18f**, **19f**) which would be expected to increase lipophilicity, results in a reduction of dose ratio, suggesting a comparatively greater degree of CNS penetration, as might be expected: for both compounds, however, the writhing/hot-plate potencies are still consistent with a predominantly peripheral effect. Within the tetrapeptide series (Table VII) a number of the analogues show extremely high activity in the AA writhing test (e.g. **23c**, **23e**, <1 μg kg^{-1}) but are less potent in the PBQ assay. For all of these compounds there is a lack of correlation between their activities in the two writhing assays. The significance of this observation is currently unknown but it renders interpretation of the effects of the aryl substituents on activity difficult in this series. However, high potency is again associated with F and NO_2 substitution. In this case the dose ratio is unexpectedly high for the Phe(3,4-Cl_2) analogue (**23h**) for reasons which remain unclear. These results essentially agree with previous observations[28,31] and demonstrate that SAR for aromatic substitution in these polar analogues do not diverge from those determined for more lipophilic analogues.

Aryl Ring Replacement. A number of analogues with aryl ring replacements were prepared to investigate the effects of variations in bulk and lipophilicity (naphthyl, pyridyl) and aromatic character (thienyl, furyl). No advantage in activity is observed over the parent tripeptides when the phenyl ring is replaced with an alternative aromatic ring (**18j–r**, Table VI); the 1-naphthyl derivative (**18j**) retains reasonable activity in the PBQ writhing assay. Higher activities are seen for the corresponding N^{α}-amidino compounds (**19j–m,o,p**), which were studied in more detail. In the case of the thienyl and furyl derivatives (**19l**, **19o**), nitration decreases activity (**19m**, **19p**), in contrast with the result found for the phenyl compounds.[28,31] The incorporation of a pyridyl ring (**18q**, **18r**), to further increase polarity, markedly reduces activity on the GPI and parallels the effect of the sulfonamido substituent (**18i**/**19i**) discussed above.

Aralkyl Chain Length. A limited study of the effect of varying the aralkyl chain length was undertaken. Opioid activity was reduced, but maintained at a reasonable level when the chain length was reduced to a single methylene group as demonstrated by the activities of the substituted benzylamides (**18s–v**). Only preliminary studies were conducted with compounds wherein the number of methylene groups was increased to three (**18y**).

N^{α}-Amidination. We and others have previously shown that conversion of the α-amino group of Tyr^1 to a guanidino group has a beneficial effect on activity[1,10,12] and

(27) (a) Kiso, Y.; Miyazaki, T.; Moritoki, H.; Takei, M.; Nakamura, H. *FEBS Lett.* **1981**, *136*, 101. (b) Shuman, R. T.; Gesellchen, P. D.; Smithwick, E. L.; Frederickson, R. C. A. In *Peptides: Synthesis, Structure, Function*; Rich, D. H., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; pp 617–620. (c) Chipkin, R. E.; Morris, D. H.; English, M. G.; Rosamond, J. D.; Stammer, C. H.; York, E. J.; Stewart, J. M. *Life Sci.* **1981**, *28*, 1517. (d) Vavrek, R. J.; Hsi, L.-H.; York, E. J.; Hall, M. E.; Stewart, J. M. *Peptides* **1981**, *2*, 303. (e) Shinagawa, S.; Fujino, M.; Ishii, H.; Kawai, K. *Chem. Pharm. Bull.* **1981**, *29(12)*, 3630, 3639, 3646. (f) Vavrek, R. J.; York, E. J.; Stewart, J. M. In *Peptides: Synthesis, Structure, Function*; Rich, D. H., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; pp 629–631. (g) Casiano, F. M.; Cumiskey, W. R.; Gordon, T. D.; Hansen, P. E.; McKay, F. C.; Morgan, B. A. In *Peptides: Structure and Function*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 311–314.

(28) (a) Beddell, C. R.; Clark, R. B.; Hardy, G. W.; Lowe, L. A.; Ubatuba, F. B.; Vane, J. R.; Wilkinson, S.; Chang, K.-J.; Cuatrecasas, P.; Miller, R. J. *Proc. R. Soc. London, B* **1977**, *198*, 249. (b) Smith, T. W.; Wilkinson, S. In *The Chemical Regulation of Biological Mechanisms*; Creighton, A. M., Turner, S., Eds.; Special Publication No. 42; The Royal Society of Chemistry: London, 1982; pp 231–254. (c) Wilkinson, S. U.S. Patent 4 244 944 and 4 254 106.

(29) (a) Dutta, A. S.; Gormley, J. J.; Hayward, C. F.; Morley, J. S.; Shaw, J. S.; Stacey, G. J.; Turnbull, M. T. *Life Sci.* **1977**, *21*, 559. (b) Ronai, A. Z.; Berzetei, I. P.; Szekely, J. I.; Miglecz, E.; Kurgyis, J.; Bajusz, S. *Eur. J. Pharmacol.* **1981**, *69*, 263.

(30) Kosterlitz, H. W.; Waterfield, A. A. *Annu. Rev. Pharmacol.* **1975**, *15*, 26.

(31) Gesellchen, P. D.; Frederickson, R. C. A.; Tafur, S.; Smiley, D. In *Peptides: Synthesis, Structure, Function*; Rich, D. H., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; pp 621–624.

peripheral selectivity[1] in enkephalin analogues. Consideration of the biological data obtained for the tripeptides (Table VI) confirms the useful effect of N^{α}-amidination for these truncated analogues. The majority of the peptides thus modified show increased antinociceptive potency and peripheral selectivity as indicated by their dose ratios. For example, the activities of the N^{α}-amidino derivatives of the thienyl and furyl compounds (**19l,m,o,p**) are much improved over the parent peptides (**18l,m,o,p**); the activities of the N^{α}-amidino benzylamides (**19s–v**) compare favorably with those of the corresponding parent phenethyl compounds (**18a–d**); thus amidination compensates for the effect of reduction in chain length on activity. For the two examples in the tetrapeptide series, the effect of amidination on opioid activity is less clear-cut but both show an increased dose ratio (compare **23a/24a**, **23c/24c**), again indicating a higher degree of peripheral selectivity for the guanidino peptides compared with the corresponding parent peptides.

Glycine Isosteres. Replacement of the Gly^3 residue in the tripeptide **18a** by the reduced isostere **12**, to create a third basic center in the molecule and hence further increase hydrophilicity, results in a dramatic loss in activity (**18z**, Table VI). Similarly, substitution by the thia analogue **13** to give **18aa** also destroys activity. These results are in line with early SAR for enkephalin peptides which established that very little structural modification was tolerated at $Gly^{3,28}$ although this residue may be deleted with retention of activity.[27c-e,g]

The most potent compounds in this study are found in the tetrapeptide series. However, extended time course studies of one example, **24a**, in the hot-plate model revealed a U-shaped dependence of the ED_{50} on time after dosing, i.e. the ED_{50} values decrease over a period of 1–2 h after dosing prior to rising again. Such observations exactly parallel the effects which were seen with the prototype pentapeptide BW 443C and which have been reported in a detailed pharmacological study.[7a] A possible explanation for these effects is that such metabolically stable analogues, despite their polarity, gradually accumulate in the CNS after dosing, and are only slowly eliminated. In contrast, representative analogues from the tripeptide series (e.g. **19a**) exhibit a simple dependence of ED_{50} on time after dosing, larger doses being required to achieve equiactive effects at increased times after dosing; such behavior is consistent with there being no slow penetration into the CNS, possibly as a result of their greater metabolic lability. The high dose ratios found with the peripheral antagonist coupled with the low potencies observed in the hot-plate model as compared with the writhing models support the contention that these compounds act in the periphery. Additional evidence for this may be seen in the comparison of the dose ratios obtained for the D-Met^2 peptides **18bb** and **23i** with those of the corresponding D-Arg^2 peptides **18a** and **23a**. A dose ratio close to unity for the former compounds indicates little or no antagonism by *N*-methylnalorphine and implies a significant central contribution to the activity of the D-Met^2 peptides which is absent for the D-Arg^2 peptides.

Although a number of compounds were identified which exhibit oral activity in the PBQ writhing assay at <10 mg kg^{-1} (**18f**, **19a**, **19c**, **19m**, **23c**, **23e**, **24a**), it is apparent from a comparison of the ED_{50} values obtained after sc and po administration that these analogues are less effective after oral dosing. Such results are not unexpected since the increase in polarity engendered to impede CNS penetration may be expected to have a similar effect on absorption from the gastrointestinal tract. Recent studies[32] suggest that alternative routes of administration of peptides may prove advantageous in order to circumvent their poor absorption after oral administration.

In conclusion, the studies described in this paper have successfully identified two series of simplified enkephalin analogues whose pharmacological profiles are indicative of restricted access to the CNS and hence whose opioid activity is expressed preferentially in the periphery. The tetrapeptides, although more potent as opioids, possibly accumulate slowly in the CNS: if this is the case, they may thus evoke the undesired centrally mediated side effects at extended times after dosing. In contrast, the tripeptides, particularly the N^{α}-amidino derivatives **19a–d**, exhibit the desired pharmacological profile and are worthy of more detailed study with a view to the development of a selectively peripheral opioid analgesic agent free from detrimental side effects.

Experimental Section

Melting points were determined on a Gallenkamp apparatus and are uncorrected. Optical rotations were determined on a Thorn-NPL Automatic Polarimeter (type 243). Proton magnetic resonance spectra were recorded on Brucker WH 90, AM 200-SY, or AM 360 instruments. All compounds described had NMR spectra consistent with their structure. FAB mass spectra were recorded on a VG ZAB 1F or a Kratos MS 50 TC instrument.

TLC was performed on 0.25 mm thickness silica gel plates (Merck, silica gel 60, F-254); the following solvent systems were used: (A) $CHCl_3$–MeOH, 8:1 v/v; (B) $CHCl_3$–MeOH–32% HOAc, 120:90:5 v/v; (C) $CHCl_3$–MeOH–"0.88"NH_4OH, 120:90:5 v/v; (D) $CHCl_3$–MeOH–32% HOAc, 120:90:40 v/v; (E) $CHCl_3$–MeOH–"0.88"NH_4OH, 120:90:40 v/v; (F) *n*-BuOH–HOAc–H_2O, 3:1:1 v/v; (G) CH_2Cl_2–MeOH, 95:5 v/v; (H) *sec*-BuOH–3% NH_4OH, 100:44 v/v. Compounds were visualized by viewing under ultraviolet light and/or by spraying with ninhydrin, *t*-BuOCl/starch–KI or Sakaguchi reagent. Ion-exchange chromatography was performed with either (carboxymethyl)cellulose (Whatman CM52) or CM-Sepharose Fast Flow (Pharmacia) eluted with a linear gradient of aqueous NH_4OAc, pH 5.1 (0.005–0.5 M), unless otherwise stated; fractions were collected with an LKB automated fraction collector. Analytical reverse-phase HPLC was carried out with a Waters ALC with Du Pont Zorbax C8 prepacked columns. Reverse-phase desalting of peptides was performed on preparative-scale HPLC columns packed with a C18 stationary phase; samples were applied in aqueous solution, the column was washed with water, and the products were eluted with 5% HOAc–MeOH.[33]

Solutions in organic solvents were dried over anhydrous $MgSO_4$. Solvents were evaporated on a Büchi Rotavapor with a water bath temperature <40 °C at water pump pressure except for DMF when high vacuum was required. Isobutyl chloroformate, anisole, NMM, and DCCI were redistilled before use; THF was distilled from CaH_2 and stored over MS4A; HPLC grade DMF (Romil) was stored over prebaked MS4A for 24 h before use; HOBt was recrystallized from water and thoroughly dried before use. 2-, 3-, and 4-fluoro-D,L-phenylalanines were purchased from Koch Light. Hog renal acylase I and carboxypeptidase A were obtained from Sigma. Precursors for synthesis were purchased as follows: 4-fluorobenzyl chloride (Janssen), 2-[2-(trifluoromethyl)-phenyl]ethanol (Lonza), 2-(4-aminophenyl)ethanol hydrochloride (Kodak), *N*-ethyl(2-pyridyl)methylamine hydrochloride (Bader); all others were available from Aldrich or Fluka.

General Methods for the Preparation of the Required *N*-Ethyl Compounds. Method A. *N*-Ethyl-*N*-(2-fluorobenzyl)amine Hydrobromide (5v). A solution of $EtNH_2$ in EtOH (33%, 250 mL) was cooled in ice and 2-fluorobenzyl bromide (25 g; 132 mmol) added with stirring. After an initial exotherm, the mixture was heated to gentle reflux for 1.5 h. The solvent was evaporated and the crude product crystallized twice from

(32) Disposition and Delivery of Peptide Drugs, Proceedings of the FIP Satellite Symposium, Leiden, Netherlands, 1987; *Pharm. Weekbl., Sci. Ed.* **1988**, *10(1)*.
(33) Bohlen, P.; Castillo, F.; Guillemin, R. *Int. J. Pept. Protein Res.* **1980**, *16*, 306.

i-PrOH. Yield 12.85 g (41.6%). Table I.

Method B. *N*-Ethyl-2-(4-bromophenyl)ethylamine Hydrochloride (5e). A stirred solution of 4-bromophenethyl alcohol (Aldrich; 10 g, 50 mmol) and NEt_3 (7.6 g, 75 mmol) in CH_2Cl_2 (100 mL) was cooled to 0 °C. Methanesulfonyl chloride (6.3 g, 55 mmol) was added dropwise such that the temperature did not rise above 5 °C. The reaction mixture was stirred at 0 °C for 1 h and then washed with water (2 × 50 mL). After drying, the solvent was evaporated to leave an oil, which soon crystallized. The solid was triturated with petroleum ether (bp 60–80 °C), filtered off, and dried to give the pure mesylate, **3e**. Yield 12.8 g (92%), mp 69–70 °C. Anal. ($C_9H_{11}BrSO_3$) C, H, Br, S. A portion of the mesylate (5.0 g, 17.91 mmol) was dissolved in a solution of $EtNH_2$ in EtOH (33%, 25 mL) and heated under reflux for 4 h. The solvent was evaporated and the residue dissolved in 5% aqueous citric acid (50 mL) and washed with CH_2Cl_2 (3 × 25 mL). The aqueous phase was adjusted to pH 11 with 10 M NaOH and the product extracted into CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with water (2 × 25 mL), filtered through phase-separating paper, and dried. After evaporation of the solvent, the residual gum was dissolved in dioxane (50 mL) and treated with 3.7 M HCl–dioxane (5 mL) with stirring. After 1 h the precipitated HCl salt was filtered off and dried. Yield 3.81 g (80.4%). Table I.

Method C. N^1-Ethyl-L-4-nitrophenylalaninamide (7a). A mixture of L-Phe(4-NO_2)-NH_2·HCl, **6a** (8.95 g, 36.5 mmol), $NaHCO_3$ (12.25 g, 146 mmol), and EtI (5.69 g, 36.5 mmol) in EtOH (170 mL) was heated to reflux with stirring for 6.5 h. The reaction was monitored by analytical HPLC. A further portion of EtI (2.8 g, 18 mmol) was added and heating continued for a total of 24 h. The solvent was evaporated and the residue distributed between water (100 mL) and EtOAc (800 mL). The organic phase was further washed with water (2 × 100 mL), dried, and concentrated to yield a solid (8.28 g). Analytical HPLC showed this to consist of mainly the desired product contaminated with the starting amine and also the N^1,N^1-diethyl compound. The pure product was obtained by chromatography on a silica "Lobar" column (Merck), eluting with MeOH–CH_2Cl_2 (5:95 v/v). Yield 4.76 g (55%). Table II.

Method D. *N*-Ethyl-2-(5-nitro-2-thienyl)ethylamine Hydrochloride (5m). $(CF_3CO)_2O$ (1.1 g, 5.23 mmol) was added dropwise over 3 min to a stirred mixture of *N*-ethyl-2-(2-thienyl)ethylamine hydrochloride (**5l**) (1.0 g, 5.22 mmol) and NEt_3 (1.06 g, 10.5 mmol) in CH_2Cl_2 (15 mL) cooled to 0 °C. After stirring for 1 h at this temperature, the mixture was washed successively with 10-mL portions of water, 5% aqueous citric acid, 5% aqueous $NaHCO_3$, and finally water. Drying and evaporation of the solvent gave the *N*-trifluoroacetyl compound **8l** as a mobile oil. Yield 1.05 g (80%); R_f (A) 0.76, R_f (B) 0.85; *m/z* 251. A solution of this compound in Ac_2O (6 mL) was cooled to −20 °C and a mixture of fuming nitric acid (0.6 mL) in Ac_2O (4 mL) at −20 °C was added dropwise over 1 h.[18] The resultant orange solution was poured onto ice (60 g) and 10 M NaOH (15 mL) was added cautiously with vigorous stirring. The pH was adjusted to 6 and the orange oil was extracted into Et_2O (2 × 40 mL). The combined extracts were washed with water (2 × 20 mL), dried, and evaporated. The resulting oil was purified by chromatography on silica with CH_2Cl_2 as eluent to yield **9l**, 0.7 g (59%), *m/z* 296. This product (0.7 g, 2.36 mmol) was dissolved in dioxane (5 mL) and 2 M NaOH (1.3 mL, 2.6 mmol) added. The mixture was stirred vigorously for 30 min and then 1 M HCl (2.6 mL, 2.6 mmol) was added. The mixture was evaporated and the residue dissolved in 1 M HCl (20 mL) and washed with Et_2O (15 mL) and EtOAc (15 mL). The pH was adjusted to 10 with 10 M NaOH and the precipitated oil extracted into CH_2Cl_2 (3 × 15 mL). The combined extracts were washed with water (2 × 15 mL), dried, and evaporated to leave the crude product as a dark oil, yield 0.37 g. Purification by chromatography on silica eluting with $CHCl_3$–MeOH (8:1 v/v) gave the product as a green oil, 0.35 g (1.75 mmol, 74%). This was dissolved in dioxane (8 mL) and 3.7 M HCl–dioxane (1 mL, 3.7 mmol) added with stirring. The precipitated salt was filtered off and recrystallized from *i*-PrOH (20 mL)–*i*-Pr_2O (5 mL) to give the title compound as a beige solid. Yield 0.296 g (53%). Table I.

N^2-Ethyl-N^2-[2-(3-nitrothienyl)ethyl]glycinamide Hydrochloride (15n). The *N*-(trifluoroacetyl)amine **8l** (3.0 g, 12 mmol) was chlorosulfonated in the 5-position with $ClSO_3H$–PCl_5 by the method described,[19] to give **10**, yield 3.47 g (83%). This material was nitrated in the 3-position by the method described for the parent **8l** above, to give **11**, yield 3.43 g (95%). Hydrolysis of this compound was achieved by reflux (139 °C) in a mixture of concentrated H_2SO_4–H_2O (8 mL + 10 mL) for 1.5 h. The hot solution was poured onto crushed ice (200 g) and the solution made basic with 10 M NaOH (35 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with water (2 × 50 mL) and brine (50 mL), dried, and evaporated to leave an orange oil, yield 65 mg (13%). The product was converted to the hydrochloride by dissolution in EtOH (3 mL), treatment with 3.7 M HCl–dioxane (0.5 mL), and evaporation, to give **5n** (Table I), yield 78 mg (100%). Acylation of the product with Boc-Gly-OH by the DCCI–HOBt method as exemplified for **18v** and subsequent removal of the Boc group with 1 M HCl–HOAc and lyophilization from water gave the title compound as an amorphous powder, yield 70 mg, Table IV.

Method E. *N*-Ethyl-3-(4-nitrophenyl)propylamine Hydrochloride (5y). A stirred mixture of 3-(4-nitrophenyl)butanoic acid (Wellcome, Dartford; 16.7 g, 80 mmol), NEt_3 (10.5 g, 104 mmol), diphenyl phosphorazidate (27.5 g, 100 mmol), and *t*-BuOH (300 mL) was heated under reflux for 5 h. The solvent was evaporated and the residue taken up to Et_2O–EtOAc (3:1 v/v, 250 mL) and washed with 100-mL portions of water, 5% $NaHCO_3$, water, 5% citric acid, water, and brine. After filtration and drying, evaporation of the solvents left an oil. Crystallization, twice, from *i*-Pr_2O gave *N*-(*tert*-butyloxycarbonyl)-3-(4-nitrophenyl)propylamine, 3.92 g (17.5%), mp 85 °C. Anal. ($C_{14}H_{20}N_2O_4$) C, H, N. A portion of this material (2.0 g, 7.14 mmol) was dissolved in CF_3CO_2H (10 mL) to remove the Boc group. After 15 min the acid was evaporated and the oily residue triturated with Et_2O. The amine was further converted to **5y** by using method C above. Table I.

Method F. 4-(*N*-Ethyl-2-aminoethyl)pyridine Hydrochloride (5r). HOAc (28.6 g, 476 mmol) was added cautiously to a stirred mixture of 4-vinylpyridine (50 g, 476 mmol) and $EtNH_2$–EtOH (33%, 28 mL, 476 mmol). When the exotherm had subsided, the mixture was heated on a steam bath for 24 h. The reddish mixture was concentrated to half volume in vacuo (bath 60 °C), diluted with water (100 mL), and made strongly basic with 10 M NaOH. The precipitated oil was extracted into CH_2Cl_2 (3 × 250 mL), and the combined extracts were washed with water (100 mL), treated with charcoal, dried, and evaporated to leave a brown oil, 35 g. Vacuum distillation yielded a fraction rich (NMR) in the desired product (bp 86–136 °C, 8.6 g). To a solution of this material in dry Et_2O (250 mL) was added dropwise 4.5 M HCl–dioxane (26 mL). The precipitated salt was filtered off and recrystallized from EtOH–Et_2O (100 mL each) to give the title compound. Yield 8.5 g (8%). Table I.

2-(3,4-Dichlorophenyl)ethanol (1f). A solution of ethyl 3,4-dichlorophenylacetate (6.0 g, 25.8 mmol) [obtained by hydrolysis of 3,4-dichlorophenylacetonitrile as described[14] and esterification] in dry ether (40 mL) was added to a stirred solution of $LiAlH_4$ (0.6 g, 15.8 mmol) in dry ether (50 mL) at a rate to maintain gentle reflux. Reflux was continued for 15 min after the addition was complete, and then EtOAc (5 mL) was added with caution, followed after a further 15 min by 5% H_2SO_4 (50 mL). The organic layer was separated and the acid extracted further with ether (3 × 30 mL). The combined organic phases were washed with water (2 × 30 mL) and brine (30 mL) and dried. Evaporation gave the title compound (yield 5.02 g, *m/z* 232, 236 for $C_8H_8OCl_2$) sufficiently pure for conversion to the required ethylamine (**5f**) by method B (Table I).

2-[4-(Aminosulfonyl)phenyl]ethyl Bromide (2i). 2-[4-(Chlorosulfonyl)phenyl]ethyl bromide[15] (30 g, 105 mmol) was shaken with NH_4OH (sp gr 0.88, 250 mL). A vigorous exothermic reaction ensued with deposition of a white solid. The solid was collected (32 g) and recrystallized from EtOH (250 mL) to give the required product in two crops. Yield 21.5 g (77%), mp 186–187 °C. Anal. ($C_8H_{10}BrNO_2S$) C, H, Br, N, S.

Example Synthesis of a Tripeptide. N^1-(*tert*-Butyloxycarbonyl)-N^2-ethyl-N^2-(2-fluorobenzyl)glycinamide (14v). A solution of **5v** (21.2 g, 90.6 mmol), Boc-Gly-OH (15.85 g, 90.6 mmol), HOBt (24.5 g, 181.2 mmol), and NEt_3 (12.53 mL, 90.6 mmol) in DMF was cooled in ice and treated with DCCI (20.53

g, 99.6 mmol). The reaction mixture was stirred at ambient temperature for 48 h. The suspension was filtered and the filtrate concentrated in vacuo to an oil. The oil was distributed between EtOAc (600 mL) and 10% aqueous Na_2CO_3 (300 mL). The organic layer was filtered to remove DCU and washed successively with 250-mL portions of 10% aqueous Na_2CO_3 (twice), half-saturated aqueous NaCl (once), 5% aqueous citric acid (twice), and finally half-saturated aqueous NaCl (once). The EtOAc solution was dried and refrigerated. A further small amount of DCU was filtered off and the filtrate evaporated to leave a clear oil. Yield 27.76 g (98%). TLC indicated that a trace of DCU was still present, but the product was used in the next stage without further purification.

N^2-Ethyl-N^2-(2-fluorobenzyl)glycinamide Hydrochloride (15v). To a stirred solution of **14v** (27.5 g, ca. 88 mmol) in HOAc (250 mL) was added 2 M HCl–HOAc (250 mL). The mixture was stirred at ambient temperature for 60 min and then concentrated in vacuo. The resulting oil was dissolved in water (500 mL) and filtered to remove DCU. The solution was evaporated to dryness and the residue dried by reconcentration from EtOH (twice). The crude product was crystallized from a mixture of *i*-PrOH (150 mL) and *i*-Pr_2O (600 mL). Yield 19.4 g (89%). Table IV.

N^1-(L-Tyrosyl-D-arginyl)-N^2-ethyl-N^2-(2-fluorobenzyl)-glycinamide (18v). A solution of compound **15v** (6.66 g, 27 mmol), Boc-Tyr-D-Arg-OH·HCl (**16**)[1] (12.78 g, 27 mmol), HOBt (7.29 g, 54 mmol), and NMM (2.73 g, 27 mmol) in DMF (80 mL) was cooled with stirring to −5 °C. DCCI (5.55 g, 27 mmol) was added and the reaction mixture was stirred at 4 °C for 48 h. The mixture was warmed to 20 °C and treated with *N,N*-dimethylethylenediamine (0.55 mL, 5 mmol) for 1.5 h. DCU was removed by filtration and the filtrate was concentrated in vacuo. The residue was distributed between EtOAc (300 mL) and 5% aqueous citric acid (600 mL). The aqueous layer was separated, washed with EtOAc (2 × 300 mL), and adjusted to pH 6 by the addition of solid K_2CO_3. The precipitated crude product was extracted into EtOAc–*n*-BuOH (4:1 v/v; 3 × 300 mL). The combined extracts were filtered through phase-separating paper and evaporated to dryness. The resultant solid **17v** was reevaporated twice from dry EtOH. Yield 14.4 g (85%). The product was dissolved in a mixture of HOAc (175 mL) and anisole (55 mL) and the stirred solution was treated with 2 M HCl–HOAc (230 mL) for 30 min at ambient temperature. The reaction mixture was concentrated and the residual gum was dissolved in a mixture of water (250 mL) and Et_2O (100 mL). The aqueous layer was washed again with Et_2O and then freeze-dried to give the crude product. Yield 12.92 g (100%). A portion of this material (5 g) was purified by ion-exchange chromatography on a CM-Sepharose column (5 × 50 cm) eluted with a linear concentration gradient of NH_4OAc buffer at pH 6.5 (8 L). Fractions containing pure product were identified by analytical HPLC, combined, and reverse-phase desalted to give the pure tripeptide amide. Yield 4.21 g (84%). Table VI.

N^1-(N^α-Amidino-L-tyrosyl-D-arginyl)-N^2-ethyl-N^2-(2-fluorobenzyl)glycinamide (19v). Peptide **18v** (7.17 g, 11.0 mmol) was dissolved in a mixture of DMF (20 mL) and EtOH (35 mL). 1-Amidino-3,5-dimethylpyrazole acetate (3.55 g, 18 mmol) and NEt_3 (2.42 g, 24 mmol) were added, and the mixture was stirred at 65 °C for 7.5 h and then at ambient temperature overnight. The solvents were evaporated, and the residue was distributed between water (100 mL) and EtOAc (100 mL). The aqueous solution of the crude product was applied in two batches to a column (5 × 50 cm) of CM-Sepharose. Gradient elution and desalting were carried out as described for **18v**. Yield 4.76 g (66%). Table VI.

Example Synthesis of Tetrapeptide. *N*-(*tert*-Butyloxycarbonyl)-L-4-nitrophenylalaninamide. A solution of Boc-L-Phe(4-NO_2)-OH (12.12 g, 39.1 mmol) and NMM (3.95 g, 39.1 mmol) in DMF (70 mL) was cooled to −15 °C and treated with isobutyl chloroformate (5.34 g, 39.1 mmol). The mixture was stirred vigorously for 5 min and then dry ammonia gas was bubbled into the mixture for 1 h while the temperature was maintained at −15 °C. Excess ammonia was removed in a stream of N_2 and the mixture was concentrated in vacuo to leave a solid. The crude product was dissolved in EtOAc (900 mL) and washed with 5% aqueous citric acid (2 × 100 mL), water (150 mL), 5% aqueous $NaHCO_3$ (2 × 100 mL), and finally water (150 mL). Drying and evaporation of the solvent gave a white solid which was triturated with EtOAc–petroleum ether (bp 60–80 °C), filtered off, and dried. Yield 11.60 g (96%), mp 194–195 °C dec; R_f (B) 0.83. Anal. ($C_{14}H_{19}N_3O_5$) C, H, N.

L-4-Nitrophenylalaninamide Hydrochloride (6a). A solution of the above amide (11.6 g, 37.5 mmol) and anisole (100 mL) in HOAc (170 mL) was treated with 2 M HCl–HOAc (270 mL) and the mixture was stirred at ambient temperature for 30 min. After removal of the solvents in vacuo, the resultant solid was dissolved in water (250 mL) and the solution was washed with Et_2O (3 × 50 mL). The product was isolated by lyophilization and dried over P_2O_5. Yield 8.96 g (97%), mp 242–244 °C dec; R_f (B) 0.35, R_f (C) 0.57, R_f (F) 0.41. Anal. ($C_9H_{11}N_3O_3$·HCl) C, H, N.

N^1-[*N*-(*tert*-Butyloxycarbonyl)glycyl]-N^1-ethyl-L-4-nitrophenylalaninamide (20a). A solution of Boc-Gly-OH (28.82 g, 165 mmol) and HOBt (44.5 g, 330 mmol) in DMF (280 mL) was cooled in ice and treated with DCCI (33.93 g, 165 mmol). The mixture was stirred in ice for 45 min and then **7a** (see method C, above) (26.0 g, 110 mmol) was added. After stirring at ambient temperature for 24 h, the precipitated DCU was filtered off and the filtrate concentrated in vacuo. The residual gum was distributed between EtOAc (800 mL) and water (180 mL), and the organic phase was further washed with 5% aqueous citric acid (3 × 180 mL), water (2 × 180 mL), 5% aqueous $NaHCO_3$ (4 × 180 mL), and water (2 × 180 mL). Drying and concentration gave the product as an amorphous solid. Yield 32.9 g (76%). TLC revealed the presence of a trace of DCU in the product. This material was used directly in the next stage.

N^1-Ethyl-N^1-glycyl-L-4-nitrophenylalaninamide Hydrochloride (21a). 20a (9.21 g, 23.3 mmol) was dissolved in a mixture of anisole (65 mL) and HOAc (90 mL) and treated with 2 M HCl–HOAc (155 mL). The mixture was stirred at ambient temperature for 45 min and then concentrated in vacuo to yield a solid, which was triturated with dry Et_2O. The solid was filtered off, dissolved in hot MeOH (130 mL), filtered, and diluted slowly with dry Et_2O (200 mL). After stirring overnight at 4 °C, the product was filtered off, washed with MeOH–Et_2O and Et_2O, and dried. Yield 6.11 g (79%). Table V.

N^1-Ethyl-N^1-(L-tyrosyl-D-arginylglycyl)-L-4-nitrophenylalaninamide Diacetate (23a). Boc-L-Tyr-D-Arg-OH·HCl (**16**) (12.7 g, 25.8 mmol) was coupled to amino compound **21a** (8.54 g, 25.8 mmol) by the DCCI–HOBt method as exemplified for **18v**. The reaction was allowed to proceed for 48 h at 5 °C. After filtration to remove DCU and concentration of the filtrate, the crude tetrapeptide was distributed between EtOAc (400 mL) and water (400 mL). The aqueous layer was adjusted to pH 6.5 and extracted with EtOAc–*n*-BuOH (5:1 v/v; 4 × 300 mL). The combined extracts were washed successively with water (400 mL), 5% Na_2CO_3-saturated NaCl (1:1 v/v; 4 × 400 mL), and saturated NaCl (2 × 200 mL). The solvents were evaporated, and the residue was reconcentrated once from water and twice from EtOH to leave an off-white powder. Yield 16.1 g (81%). The protected tetrapeptide was dissolved in a mixture of HOAc (225 mL) and anisole (75 mL) and treated with 2 M HCl–HOAc (300 mL). The reaction mixture was stirred at ambient temperature for 45 min and concentrated in vacuo, and the residue was triturated thoroughly with dry Et_2O. The crude product (16.8 g) was purified on a CM52 column (9 × 50 cm) with a 20-L linear concentration gradient of NH_4OAc at pH 5.1. Fractions containing pure product were identified by analytical HPLC and pooled. Buffer was removed by repeated lyophilization (thrice). The peptide was obtained as the diacetate. Yield 8.15 g (49%). Table VII.

N^1-Ethyl-N^1-[2-(4-nitrophenyl)ethyl]ethylenediamine Dihydrochloride (12). The glycinamide hydrochloride **15a** (0.5 g, 1.7 mmol) was converted to the base by washing a $CHCl_3$ suspension twice with 5 M NaOH. The base was dissolved in dry THF (10 mL) and treated dropwise over 15 min with 1 M BH_3–THF complex (3.5 mL, 3.5 mmol) at 0 °C under N_2. The mixture was heated to reflux for 1 h and allowed to cool. HCl (6 M, 425 μL) was added and the solution concentrated to remove THF. Water (7 mL) was added and the solution was made strongly basic (pH ca. 14) with 10 M NaOH, saturated with NaCl, and extracted with CH_2Cl_2 (10 × 5 mL). The combined extracts were washed with brine (10 mL), dried, and evaporated to leave a yellow gum (0.4 g). The gum was dissolved in dioxane (20 mL)

and 3.7 M HCl–dioxane (0.92 mL, 3.4 mmol) added to the stirred solution. The precipitated gum was dissolved in hot *i*-PrOH–EtOH (30 mL each) and the solution diluted with *i*-Pr_2O (40 mL). On cooling the title compound crystallized and was filtered off and dried. Yield 0.26 g (49%). Table III.

***S*-[2-(4-Nitrophenyl)ethyl]cysteamine Hydrochloride (13).** 4-Nitrophenethyl bromide (2.3 g, 10 mmol) was added to a stirred solution of cysteamine hydrochloride (1.14 g, 10 mmol) and NEt_3 (2.02 g, 20 mmol) in EtOH (10 mL). The mixture was stirred at ambient temperature for 2 days and then evaporated. The residue was dissolved in 2 M HCl (30 mL) and washed with Et_2O (25 mL) and EtOAc (3 × 25 mL). The aqueous phase was basified to pH 14 with 10 M NaOH and extracted with CH_2Cl_2 (2 × 25 mL). TLC indicated that the required product was present in the EtOAc and CH_2Cl_2 extracts. The EtOAc extract was washed with 1 M NaOH (3 × 10 mL) and brine (25 mL) and then combined with the CH_2Cl_2 extract. Drying and concentration gave ca. 1.5 g of a yellow oil. The oil was dissolved in dioxane (45 mL) and the stirred solution was treated with 4 M HCl–dioxane (10 mL). The precipitated salt was collected by filtration and recrystallized twice from MeCN (50 mL and 30 mL). Yield 0.91 g (34.6%). Table III.

***N*-(*tert*-Butyloxycarbonyl)-L-tyrosyl-D-methionine (25).** A solution of Boc-L-Tyr-OH (7.8 g, 27.8 mmol) and HOBt (7.50 g, 55.5 mmol) in DMF (25 mL) was cooled to −15 °C and treated with DCCI (5.72 g, 27.8 mmol). The reaction mixture was stirred at −15 °C for 10 min and then H-D-Met-OMe (4.52 g, 27.8 mmol) was added and the mixture was stirred at 4 °C overnight. The DCU was removed by filtration and the DMF evaporated in vacuo. The crude product was dissolved in EtOAc (450 mL) and washed with 10% aqueous citric acid (3 × 50 mL), half-saturated NaCl (50 mL), 10% aqueous $NaHCO_3$ (3 × 50 mL), and half-saturated NaCl (2 × 50 mL). The solution was dried and evaporated to leave a solid, which was crystallized from EtOAc–petroleum ether. Yield 9.94 g (84%); R_f (B) 0.93, R_f (C) 0.85, contaminated with DCU. The product was dissolved in MeOH (100 mL) and water (30 mL) and saponified at pH 12 by addition of 1 M NaOH with a pH stat. When the hydrolysis was complete, the MeOH was removed by evaporation in vacuo and insoluble material (DCU) was removed by filtration. The filtrate was cooled in ice and the pH adjusted to 2 by careful addition of 1 M HCl. The crude product was extracted into EtOAc (2 × 250 mL), and the combined extracts were washed with water (50 mL), dried, and concentrated to give an oily foam, which solidified on trituration with Et_2O. The product was filtered off, washed with Et_2O, and dried. Yield 7.77 g (81%), mp 76 °C dec; R_f (B) 0.78, R_f (C) 0.65. Anal. ($C_{19}H_{28}N_2O_6S$) C, H, N.

Pharmacological Methods. A. Isolated Guinea Pig Ileum. Segments of the terminal portion of the ileum of guinea pigs (300–350 g) were suspended in a 20-mL organ bath under 1 g tension and bathed in Krebs bicarbonate solution gassed with 95% O_2 and 5% CO_2. Contractions were induced by coaxial stimulation of the ileum with pulses at 0.1 Hz, 0.5-ms duration, and at supramaximal voltage and recorded by means of isometric transducers. Dose–response curves were constructed allowing 15-min washout between doses.

B. Writhing Assays. (a) Groups of five to six female Charles River mice of the CD1 strain were injected intraperitoneally with phenyl-*p*-benzoquinone (PBQ) at 2.5 mg kg^{-1} in a dose volume of 10 mL kg^{-1}. The irritant induced a syndrome (writhing) characterized by a series of abdominal constrictions and/or hind-limb extensions, which were counted for a 2.5-min period commencing 10 min after PBQ injection. Vehicle or drugs were administered either subcutaneously or orally via a blunt-ended intragastric needle, in a dose volume of 10 mL kg^{-1}, 30 min prior to PBQ. Antinociceptive activity was assessed in terms of an ED_{50} and determined by linear regression. The ED_{50} was defined as that dose of drug which induced a 50% reduction in the number of writhes obtained compared to vehicle administration alone.

(b) Groups of five to six male Tuck mice of the TFW strain were injected intraperitoneally with acetic acid (0.6%) in a dose volume of 25 mL kg^{-1}. Writhes were counted for a 5-min period commencing 15 min after acetic acid injection. *N*-Methylnalorphine (11.5 mg kg^{-1}) was administered intraperitoneally 20 min prior to the analogues, which were administered subcutaneously, both antagonist and test compounds being given in a dose volume of 10 mL kg^{-1}. Antinociceptive activity was determined as above. Dose ratios were determined as the shift of the parallel regression lines in the absence and presence of *N*-methylnalorphine.

C. Hot-Plate Assays. Groups of five to six male Hacking and Churchill mice of the CFLP strain were used. Each mouse was placed on a copper surface maintained at 55 °C and observed for signs of discomfort such as licking/shaking of the paw or jumping. A cut off time of 30-s exposure was used to prevent tissue damage. Drugs were administered subcutaneously in a dose volume of 10 mL kg^{-1}. ED_{50}s were defined as that dose of drug which increased the latency of response 2-fold compared to vehicle and were determined by parallel-line probit analysis.

Antiinflammatory Activity of a Series of Substituted 2,3-Dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-diones

James J. Kaminski,* Daniel M. Solomon,* David J. Conn, Shing-Chun Wong, P. J. S. Chiu, Tobias Massa, Marvin I. Siegel, and Arthur S. Watnick

Pharmaceutical Research Division, Schering-Plough Corporation, Bloomfield, New Jersey 07003. *Received August 10, 1988*

A series of substituted analogues based on the novel 2,3-dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione ring system have been synthesized and shown to exhibit antiinflammatory activity in the adjuvant-induced arthritis rat model (AAR). The activity exhibited by the pyrimidopurinediones in this model of chronic inflammation is comparable to that of their previously studied 2-oxo congeners, the 6-hydroxypyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-triones, the best of which show potency levels approximately equal to that of naproxen. On the basis of its potency in the AAR assay, 9-benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(3-methyl-2-butenyl)pyrimido-[2,1-*f*]purine-4,8(1*H*,9*H*)-dione was selected for further evaluation and found to exhibit cyclooxygenase inhibitory activity in the in vitro rat neutrophil model. With respect to side-effect liability, this prenylated derivative has been shown to be devoid of gastric ulcer inducing potential, as well as the ocular toxicity observed previously with the 2-oxo series.

In the course of investigating a series of 6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones, which are atypical nonsteroidal antiinflammatory agents,[1] the reduction of a member of the series, containing an ester side

0022-2623/89/1832-1118$01.50/0