# Conformationally Restricted Deltorphin Analogues[†,‡]

Peter W. Schiller,* Grazyna Weltrowska, Thi M.-D. Nguyen, Brian C. Wilkes, Nga N. Chung, and Carole Lemieux

*Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal,[§] 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7*

*Received May 29, 1992*

Conformationally restricted deltorphin analogues were synthesized either through incorporation of cyclic phenylalanine analogues in position 2 or 3 of the peptide sequence or through various side chain-to-side chain cyclizations. Compounds were tested in $\mu$-, $\delta$-, and $\kappa$-receptor selective binding assays and in the guinea pig ileum (GPI) and mouse vas deferens (MVD) bioassays. Replacement of Phe$^3$ in [D-Ala$^2$]deltorphin I with 2-aminoindan-2-carboxylic acid (Aic) or L- or D-2-aminotetralin-2-carboxylic acid (Atc) resulted in agonist compounds which retained the high $\delta$ receptor selectivity of the parent peptide. Substitution of a tetrahydroisoquinoline-3-carboxylic acid (Tic) residue in the 2-position of [D-Ala$^2$]deltorphin I and of [Phe$^4$,Nle$^6$]deltorphin produced a partial $\delta$ agonist, H-Tyr-Tic-Phe-Asp-Val-Val-Gly-$NH_2$, and a pure $\delta$ antagonist, H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-$NH_2$, respectively. The latter antagonist displayed high $\delta$ selectivity ($K_i^{\mu}/K_i^{\delta}$ = 502) and was a potent antagonist against selective $\delta$ agonists in the MVD assay ($K_e \simeq$ 10 nM). Various [D-Ala$^2$]-deltorphin I analogues cyclized between the side chains of Orn (or Lys) and Asp (or Glu) residues substituted in positions 2 and 4, 4 and 7, and 2 and 7 were essentially nonselective. Comparison with corresponding N-terminal tetrapeptide analogues revealed that the C-terminal tripeptide segment in the deltorphin heptapeptides made a crucial contribution to $\delta$ affinity and $\delta$ selectivity in the case of the agonist peptides but not in the case of the antagonist.

The development of potent and stable agonists and antagonists with high specificity for each of the three major opioid receptor classes ($\mu$, $\delta$, $\kappa$) continues to be an important goal in opioid pharmacology, even though substantial progress has been made in recent years (for recent reviews, see refs 3 and 4). The development of highly selective $\delta$ ligands with agonist or antagonist properties represents a particular challenge. Linear analogues of [Leu$^5$]-enkephalin with selectivity for $\delta$ opioid receptors include the hexapeptides H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH (DSLET), H-Tyr-D-Thr-Gly-Phe-Leu-Thr-OH (DTLET), and H-Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu)-OH (BUBU).[5] Among various prepared cyclic opioid peptide analogues, the pentapeptides H-Tyr-D-Pen-Gly-Phe-Pen-OH (DPLPE), H-Tyr-D-Pen-Gly-Phe-D-Pen-OH (DPDPE), and H-Tyr-D-Pen-Gly-*p*-ClPhe-D-Pen-OH,[6,7] and the tetrapeptide H-Tyr-D-Cys-Phe-D-Pen-OH[8] showed markedly improved $\delta$ selectivity. The most potent and most selective $\delta$ agonists reported to date are three heptapeptides, H-Tyr-D-Met-Phe-His-Leu-Met-Asp-$NH_2$ (deltorphin), H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-$NH_2$ ([D-Ala$^2$]deltorphin I), and H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-$NH_2$ ([D-Ala$^2$]-deltorphin II), which have been isolated from frog skin extracts.[9,10] The use of various design principles has led to the development of several compounds with $\delta$ antagonist properties, including the enkephalin analogue *N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174864),[11] the potent non-

† This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday.

‡ Presented in part at the International Narcotics Research Conference, Noordwijkerhout, The Netherlands, July 8-13, 1990 (see ref 2).

§ Affiliated to the University of Montreal.

(1) Symbols and abbreviations are in accordance with recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature: Nomenclature and Symbolism for Amino Acids and Peptides. *Biochem. J.* **1984**, *219*, 345-373. The following other abbreviations were used: Aic, 2-aminoindan-2-carboxylic acid; Atc, 2-aminotetralin-2-carboxylic acid; Boc, *tert*-butoxycarbonyl; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BUBU, H-Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu)-OH; DAMGO, H-Tyr-D-Ala-Gly-N$^{\alpha}$MePhe-Gly-ol; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DPDPE, H-Tyr-D-Pen-Gly-Phe-D-Pen-OH; DPLPE, H-Tyr-D-Pen-Gly-Phe-Pen-OH; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; DTLET, H-Tyr-D-Thr-Gly-Phe-Leu-Thr-OH; Fmoc, (fluoren-9-ylmethoxy)carbonyl; GPI, guinea pig ileum; HOBt, 1-hydroxybenzotriazole; MVD, mouse vas deferens; Nle, norleucine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIP, H-Tyr-Tic-Phe-OH; TIP-$NH_2$, H-Tyr-Tic-Phe-$NH_2$; TIPP, H-Tyr-Tic-Phe-Phe-OH; TFA, trifluoroacetic acid; U69,593, (5$\alpha$,7$\alpha$,8$\beta$)-(-)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide.

(2) Schiller, P. W.; Nguyen, T. M.-D.; Weltrowska, G.; Lemieux, C.; Chung N. N. Development of [D-Ala$^2$]deltorphin I Analogs with Extraordinary Delta Receptor Selectivity. In *New Leads in Opioid Research*; van Ree, J. M., Mulder, A. H., Wiegant, V. M., van Wimersma Greidanus, T. B., Eds.; Excerpta Medica: Amsterdam, 1990; pp 288-290.

(3) Hruby, V. J.; Gehrig, C. A. Recent Developments in the Design of Receptor Specific Opioid Peptides. *Med. Res. Rev.* **1989**, *9*, 343-401.

(4) Schiller, P. W. Development of Receptor-Specific Opioid Peptide Analogs. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier Science Publ.: Amsterdam, 1991; Vol. 28, pp 301-340.

(5) Gacel, G.; Daugé, V.; Breuzé, P.; Delay-Goyet, P.; Roques, B. P. Development of Conformationally Constrained Linear Peptides Exhibiting a High Affinity and Pronounced Selectivity for $\delta$ Opioid Receptors. *J. Med. Chem.* **1988**, *31*, 1891-1897.

(6) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Bis-penicillamine Enkephalins Possess Highly Improved Specificity Toward $\delta$ Opioid Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871-5874.

(7) Thôt, G.; Kramer, T. H.; Knapp, R.; Lui, G.; Davis, P.; Burks, T. F.; Yamamura, H. I.; Hruby, V. J. [D-Pen$^2$,D-Pen$^5$]enkephalin Analogues with Increased Affinity and Selectivity for $\delta$ Opioid Receptors. *J. Med. Chem.* **1990**, *33*, 249-253.

(8) Mosberg, H. I.; Omnaas, J. R.; Smith, C. B.; Medzihradsky, F. Cyclic Disulfide- and Dithioether-Containing Tetrapeptides: Development of a Ligand with Enhanced *Delta* Opioid Receptor Selectivity and Potency. *Life Sci.* **1988**, *43*, 1013-1020.

(9) Erspamer, V.; Melchiorri, P.; Falconieri-Erspamer, G.; Negri, L.; Corsi, R.; Severini, C.; Barra, D.; Simmaco, M.; Kreil, G. Deltorphins: A Family of Naturally Occurring Peptides with High Affinity and Selectivity for $\delta$ Opioid Binding Sites. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5188-5192.

(10) Amiche, M.; Sagan, S.; Mor, A.; Delfour, A.; Nicolas, P. Dermenkephalin (Tyr-D-Met-Phe-His-Leu-Met-Asp-$NH_2$): A Potent and Fully Specific Agonist for the $\delta$ Opioid Receptor. *Mol. Pharmacol.* **1989**, *35*, 774-779.

(11) Cotton, R.; Giles, M. G.; Miller, L.; Shaw, J. S.; Timms, D. ICI 174864: A Highly Selective Antagonist for the Opioid $\delta$-Receptor. *Eur. J. Pharmacol.* **1984**, *97*, 331-332.

0022-2623/92/1835-3956$03.00/0 © 1992 American Chemical Society

peptide antagonist naltrindole,[12] and the recently discovered, highly δ-selective tetrapeptide H-Tyr-Tic-Phe-Phe-OH (TIPP).[13]

Linear opioid peptides such as the deltorphins are structurally flexible molecules and need to be conformationally restricted in order to obtain insight into their bioactive conformation or to try to establish structural (conformational) relationships with other opioid peptides. Conformational restriction of a peptide can be achieved either locally through incorporation of conformational constraints at a particular amino acid residue or more globally through peptide cyclizations. Various types of cyclizations of opioid peptides resulted in a number of compounds with high selectivity toward either μ or δ receptors (for reviews, see refs 3 and 4). More recently, conformational restriction of phenylalanine residues in opioid peptides has been shown to have drastic effects on receptor selectivity and signal transduction. Thus, substitution of Phe[3] in the relatively nonselective cyclic dermorphin analogue H-Tyr-D-Orn-Phe-Glu-$NH_2$ with 2-aminoindan-2-carboxylic acid (Aic) or 2-aminotetralin-2-carboxylic acid (Atc) produced μ-selective agonists[14] and opioid tri- and tetrapeptide analogues containing a tetrahydroisoquinoline-3-carboxylic acid (Tic) residue in the 2-position of the peptide sequence turned out to be potent and very selective δ antagonists.[13]

In the present paper we describe novel deltorphin analogues that either resulted from substitution of an Aic, Atc, or Tic residue at the 2- or 3-position, or were obtained through peptide cyclization between the side chains of Orn (or Lys) and Asp (or Glu) residues that had been substituted in various positions of the peptide sequence. We show that some of the Aic[3] and Atc[3] analogues displayed extraordinary δ receptor affinity and δ selectivity and that substitution of a Tic residue in position 2 of deltorphin-related peptides in one case produced a potent, highly selective δ antagonist and in another case resulted in a partial δ agonist. Finally, we report that none of the prepared cyclic deltorphin analogues showed high δ selectivity, primarily as a consequence of their decreased affinity for δ opioid receptors.

**Chemistry.** The linear deltorphin analogues (1–7, 12, 13) were prepared by the usual solid-phase techniques with $N^{\alpha}$-Boc-protected amino acids and with dicyclohexylcarbodiimide (DCC)/hydroxybenzotriazole (HOBt) as coupling agents. Aic and Atc were prepared by a modified version of the Strecker synthesis[15,16] through conversion of 2-indanone or 2-tetralone to the corresponding spirohydantoins and subsequent hydrolysis with 40% alkali, as described.[14] Atc was obtained in racemic form and incorporated as such into the peptide structure. The resulting diastereoisomeric peptides were isolated separately by semipreparative HPLC. Tic was obtained through condensation of phenylalanine with paraformaldehyde as reported elsewhere.[14]

The cyclic deltorphin analogues were prepared by the solid-phase method on a *p*-methylbenzhydrylamine resin according to a scheme described elsewhere.[17] Linear peptide segments were put together as described above, and the peptide segments to be cyclized were assembled by using $N^{\alpha}$-Fmoc amino acids with Boc and *tert*-butyl protection, respectively, for the side chains of the Orn (Lys) and Asp (Glu) residues engaged in the peptide ring formation. The latter protecting groups were removed by treatment with TFA, and side chain-to-side chain cyclization of the still resin-bound peptide was then performed by reaction with DCC/HOBt or with the BOP [(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate] reagent.[18] The time required for amide bond formation to be complete varied from 12 to 48 h. After the cyclization step, the N-terminal Fmoc protecting group was removed and the peptide chains were completed by adding the N-terminal exocyclic residue(s). In the case of analogue 11, the side chain of the endocyclic Asp[4] residue had to be protected with the TFA-resistant benzyl group.

Peptides were cleaved from the resin by HF/anisole treatment in the usual manner. Crude products were purified by gel filtration on Sephadex G-25 and by reversed-phase chromatography. In the case of the cyclic analogues cyclodimerization[17] and cyclooligomerization did occur to some extent. Separation of the cyclic monomers from the cyclic dimers and oligomers was easily achieved by reversed-phase chromatography. The desired cyclic monomer was the predominant component (60–90%) in the crude reaction products of analogues 8–11, whereas cyclodimerization was extensive (~60%) in the case of analogue 7.

**Receptor Binding Assays and in Vitro Bioassays.** Binding affinities for μ and δ opioid receptors were determined by displacing, respectively, [$^3$H]DAMGO and [$^3$H]DSLET from rat brain membrane binding sites, and κ opioid receptor affinities were measured by displacement of [$^3$H]U69,593 from guinea pig brain membrane binding sites. For the determination of their in vitro opioid activities, analogues were tested in bioassays based on inhibition of electrically evoked contractions of the guinea pig ileum (GPI) and of the mouse vas deferens (MVD). The GPI assay is usually considered as being representative for μ receptor interactions, even though the ileum does also contain κ receptors. κ receptor interactions in the GPI assay are indicated by relatively high $K_e$ values for naloxone as antagonist (20–30 nM),[19] in contrast to the low $K_e$ values (1–2 nM) observed with μ ligands.[20] In the

(12) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. Application of the Message-Address Concept in the Design of Highly Potent and Selective Non-Peptide δ Opioid Receptor Antagonists. *J. Med. Chem.* 1988, *31*, 281–282.

(13) Schiller, P. W.; Nguyen, T. M.-D.; Weltrowska, G.; Wilkes, B. C.; Marsden, B. J.; Lemieux, C.; Chung, N. N. Differential Stereochemical Requirements of μ Versus δ Opioid Receptors for Ligand Binding and Signal Transduction: Development of a New Class of Potent and Highly δ-Selective Peptide Antagonists. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

(14) Schiller, P. W.; Weltrowska, G.; Nguyen, T. M.-D.; Lemieux, C.; Chung, N. N.; Marsden, B. J.; Wilkes, B. C. Conformational Restriction of the Phenylalanine Residue in a Cyclic Opioid Peptide Analogue: Effects on Receptor Selectivity and Stereospecificity. *J. Med. Chem.* 1991, *34*, 3125–3132.

(15) Cannon, J. G.; O'Donnell, J. P.; Rosazza, J. P.; Hoppin, C. R. Rigid Amino Acids Related to α-Methyldopa. *J. Med. Chem.* 1974, *17*, 565–568.

(16) Pinder, R. M.; Buther, B. H.; Buxton, D. A.; Howells, D. J. 2-Aminoindan-2-carboxylic Acids. Potential Tyrosine Hydrolase Inhibitors. *J. Med. Chem.* 1971, *14*, 892–893.

(17) Schiller, P. W.; Nguyen, T. M.-D.; Miller, J. Synthesis of Side-Chain to Side-Chain Cyclized Peptide Analogs on Solid Supports. *Int. J. Pept. Protein Res.* 1985, *25*, 171–178.

(18) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Peptide Coupling Reagents. VI. 1-Benzotriazol-N-oxytrisdimethylaminophosphonium hexafluorophosphate (B.O.P.) *Tetrahedron Lett.* 1975, 1219–1222.

(19) Chavkin, C.; James, I. F.; Goldstein, A. Dynorphin is a Specific Endogenous Ligand of the κ Opioid Receptor. *Science (Washington, D.C.)* 1982, *215*, 413–415.

(20) Lord, J. A. H.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. Endogenous Opioid Peptides: Multiple Agonists and Receptors. *Nature (London)* 1977, *267*, 495–499.

**Table I.** Receptor Binding Assays of Deltorphin Analogues

| no. | compd | [$^3$H]DAMGO | | [$^3$H]DSLET | | |
|---|---|---|---|---|---|---|
| | | $K_i^{\mu}$, nM[a] | rel potency[b] | $K_i^{\delta}$, nM[a] | rel potency[b] | $K_i^{\mu}/K_i^{\delta}$ |
| 1 | H-Tyr-D-Ala-Aic-Asp-Val-Val-Gly-NH$_2$ | 207 ± 32 | 0.0456 ± 0.0070 | 4.96 ± 0.97 | 0.510 ± 0.100 | 41.7 |
| 2 | H-Tyr-D-Ala-(D or L)-Atc-Asp-Val-Val-Gly-NH$_2$ (I) | 671 ± 52 | 0.0141 ± 0.0011 | 5.36 ± 0.71 | 0.472 ± 0.063 | 125 |
| 3 | H-Tyr-D-Ala-(D or L)-Atc-Asp-Val-Val-Gly-NH$_2$ (II) | 1410 ± 190 | 0.00669 ± 0.00090 | 6.52 ± 0.44 | 0.388 ± 0.026 | 216 |
| 4 | H-Tyr-D-Ala-Tic-Asp-Val-Val-Gly-NH$_2$ | 15200 ± 2100 | 0.000620 ± 0.000086 | 4960 ± 320 | 0.000510 ± 0.000033 | 3.06 |
| 5 | H-Tyr-Tic-Phe-Asp-Val-Val-Gly-NH$_2$ | 9230 ± 650 | 0.00102 ± 0.00007 | 6.49 ± 1.00 | 0.390 ± 0.060 | 1420 |
| 6 | H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-NH$_2$ | 4150 ± 840 | 0.00227 ± 0.00046 | 8.27 ± 1.52 | 0.306 ± 0.056 | 502 |
| 7 | H-Tyr-D-Orn-Phe-Asp-Val-Val-Gly-NH$_2$ | 143 ± 11 | 0.0659 ± 0.0051 | 290 ± 17 | 0.00872 ± 0.00051 | 0.493 |
| 7a | H-Tyr-D-Orn-Phe-Asp-NH$_2$ | 10.4 ± 3.7 | 0.907 ± 0.032 | 2220 ± 58 | 0.00114 ± 0.00003 | 0.00468 |
| 8 | H-Tyr-D-Lys-Phe-Glu-Val-Val-Gly-NH$_2$ | 17.5 ± 4.5 | 0.539 ± 0.139 | 3.66 ± 0.57 | 0.691 ± 0.108 | 4.78 |
| 9 | H-Tyr-D-Ala-Phe-Asp-Val-Val-Orn-NH$_2$ | 338 ± 79 | 0.0279 ± 0.0065 | 562 ± 30 | 0.00450 ± 0.00024 | 0.601 |
| 10 | H-Tyr-D-Ala-Phe-Glu-Val-Val-Lys-NH$_2$ | 955 ± 19 | 0.00987 ± 0.00020 | 724 ± 165 | 0.00349 ± 0.00080 | 1.32 |
| 11 | H-Tyr-D-Lys-Phe-Asp-Val-Val-Glu-NH$_2$ | 891 ± 26 | 0.0106 ± 0.0003 | 248 ± 29 | 0.0102 ± 0.0012 | 3.59 |
| 12 | H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH$_2$ (deltorphin) | 415 ± 37 | 0.0227 ± 0.0020 | 2.13 ± 0.62 | 1.19 ± 0.35 | 195 |
| 13 | H-Tyr-D-Nle-Phe-His-Leu-Nle-Asp-NH$_2$ | 117 ± 6 | 0.0806 ± 0.0041 | 0.665 ± 0.208 | 3.80 ± 1.19 | 176 |
| 14 | H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH$_2$ ([D-Ala$^2$]deltorphin I) | 262 ± 6 | 0.0360 ± 0.0008 | 1.44 ± 0.28 | 1.76 ± 0.34 | 182 |
| 14a | H-Tyr-D-Ala-D-Phe-Asp-Val-Val-Gly-NH$_2$ | 8650 ± 1420 | 0.00109 ± 0.00018 | 67.2 ± 6.0 | 0.0376 ± 0.0034 | 129 |
| 15 | [Leu$^5$]enkephalin | 9.43 ± 2.07 | 1 | 2.53 ± 0.35 | 1 | 3.73 |

[a] Mean of three determinations ± SEM. [b] Potency relative to [Leu$^5$]enkephalin.

**Table II.** Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD) Assay of Deltophin Analogues

| no. | compd | GPI | | MVD | | GPI/MVD |
|---|---|---|---|---|---|---|
| | | IC50, nM[a] | rel potency[b] | IC50, nm[a] | rel potency[b] | IC50 ratio |
| 1 | H-Tyr-D-Ala-Aic-Asp-Val-Val-Gly-NH$_2$ | 290 ± 52 | 0.848 ± 0.152 | 0.0803 ± 0.0090 | 142 ± 16 | 3610 |
| 2 | H-Tyr-D-Ala-(D or L)-Atc-Asp-Val-Val-Gly-NH$_2$ (I) | 1820 ± 90 | 0.135 ± 0.007 | 0.115 ± 0.012 | 99.1 ± 10.3 | 15800 |
| 3 | H-Tyr-D-Ala-(D or L)-Atc-Asp-Val-Val-Gly-NH$_2$ (II) | 1380 ± 160 | 0.178 ± 0.021 | 0.163 ± 0.012 | 69.9 ± 5.1 | 8470 |
| 4 | H-Tyr-D-Ala-Tic-Asp-Val-Val-Gly-NH$_2$ | 14500 ± 2500 | 0.0170 ± 0.0029 | 481 ± 45 | 0.0237 ± 0.0022 | 30.1 |
| 5 | H-Tyr-Tic-Phe-Asp-Val-Val-Gly-NH$_2$ | >65000 | <0.00378 | 22.8 ± 2.9[c] | 0.500 ± 0.064 | >2850 |
| 6 | H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-NH$_2$ | >10000 | <0.0246 | >10000 (antagonist) | | |
| 7 | H-Tyr-D-Orn-Phe-Asp-Val-Val-Gly-NH$_2$ | 4420 ± 940 | 0.0557 ± 0.0118 | 98.3 ± 9.3 | 0.116 ± 0.011 | 45.0 |
| 7a | H-Tyr-D-Orn-Phe-Asp-NH$_2$ | 36.2 ± 3.7 | 6.80 ± 0.69 | 3880 ± 840 | 0.00294 ± 0.00064 | 0.00933 |
| 8 | H-Tyr-D-Lys-Phe-Glu-Val-Val-Gly-NH$_2$ | 102 ± 19 | 2.41 ± 0.45 | 0.467 ± 0.059 | 24.4 ± 3.1 | 218 |
| 9 | H-Tyr-D-Ala-Phe-Asp-Val-Val-Orn-NH$_2$ | 251 ± 25 | 0.980 ± 0.098 | 90.5 ± 8.6 | 0.126 ± 0.012 | 2.77 |
| 10 | H-Tyr-D-Ala-Phe-Glu-Val-Val-Lys-NH$_2$ | 3880 ± 390 | 0.0634 ± 0.0064 | 919 ± 82 | 0.0124 ± 0.0011 | 4.22 |
| 11 | H-Tyr-D-Lys-Phe-Asp-Val-Val-Glu-NH$_2$ | 380 ± 60 | 0.647 ± 0.102 | 17.3 ± 1.6 | 0.659 ± 0.061 | 22.0 |
| 12 | H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH$_2$ (deltorphin) | 281 ± 29 | 0.876 ± 0.091 | 0.695 ± 0.089 | 16.4 ± 2.1 | 404 |
| 13 | H-Tyr-D-Nle-Phe-His-Leu-Nle-Asp-NH$_2$ | 241 ± 24 | 1.02 ± 0.10 | 0.712 ± 0.067 | 16.0 ± 1.5 | 338 |
| 14 | H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH$_2$ ([D-Ala$^2$]deltorphin I) | 854 ± 133 | 0.288 ± 0.045 | 0.147 ± 0.019 | 77.6 ± 10.0 | 5810 |
| 14a | H-Tyr-D-Ala-D-Phe-Asp-Val-Val-Gly-NH$_2$ | 11900 ± 3160 | 0.0207 ± 0.0055 | 42.7 ± 6.7 | 0.267 ± 0.042 | 279 |
| 15 | [Leu$^5$]enkephalin | 246 ± 39 | 1 | 11.4 ± 1.1 | 1 | 21.6 |

[a] Mean of three determinations ± SEM. [b] Potency relative to [Leu$^5$]enkephalin. [c] Partial agonist (IC25; maximal inhibition of contractions was 50%).

MVD assay opioid effects are primarily mediated by $\delta$ receptors, even though $\mu$ and $\kappa$ receptors also exist in this tissue.

## Results and Discussion

Substitution of the conformationally restricted Aic residue in the 3-position of [D-Ala$^2$]deltorphin I resulted in a compound (1) which in the opioid receptor binding assays showed about the same $\mu$ receptor affinity as the parent peptide 14 but three times lower affinity for $\delta$ receptors (Table I). Interestingly, the Aic$^3$ analogue was nearly twice as potent as the native parent peptide in the $\delta$ receptor-representative MVD assay and about 3 times as potent in the $\mu$ receptor-representative GPI assay (Table II). These results indicate that analogue 1 retains high $\delta$ receptor affinity and is only slightly less $\delta$-selective than [D-Ala$^2$]deltorphin I. Both diastereoisomers of H-Tyr-D-Ala-(D or L)-Atc-Asp-Val-Val-Gly-NH$_2$ (compounds 2 and 3) had about the same $\delta$ receptor affinity as the Aic$^3$ analogue (1) but 3–7 times lower affinity for $\mu$ receptors. In comparison with [D-Ala$^2$]deltorphin I, analogues 2 and 3 showed about 4 times lower $\delta$ receptor affinity in the receptor binding assay but about the same high $\delta$ receptor selectivity. In the GPI assay each of the two Atc$^3$-containing diastereoisomeric peptides was about half as potent as parent peptide 14, while showing nearly the same

high potency as 14 in the MVD assay. Consequently, the IC50(GPI)/IC50(MVD) ratios of analogues 2 and 3 were about 2–3 times higher than that of [D-Ala[2]]deltorphin I, and therefore, these compounds rank among the most selective $\delta$ agonists reported to date. The observation that the Aic[3] and Atc[3] analogues displayed somewhat higher potency in the MVD assay than was expected on the basis of their $\delta$ receptor affinities determined in the binding assay can be explained with a relatively better receptor access of the more lipophilic compounds 1–3 as compared to native [D-Ala[2]]deltorphin I. Alternatively, these discrepancies may also be due to the fact that central and peripheral $\delta$ opioid receptors may have somewhat different structural requirements for ligand interactions. Enzymatic degradation can be excluded as a factor affecting the potency relationships of these compounds, since the natural deltorphins have been shown to be quite stable against enzymatic degradation in the assay systems used in the present study[21,22] and since the presence of the conformationally restricted Aic and Atc amino acid residues in the 3-position of the peptide sequence should make these analogues even more resistant to enzymolysis. It was interesting to note that both diastereoisomers of H-Tyr-D-Ala-(D or L)-Atc-Asp-Val-Val-Gly-$NH_2$ had very similar potencies in all assay systems, in sharp contrast to the observation that the parent peptide [D-Ala[2]]deltorphin I (14) displayed 13–47 times higher potency in these assays than its D-Phe[3]-analogue (14a). This loss of stereospecificity as a consequence of conformational restriction at the Phe[3] residue may be due to a different process or mode of receptor binding of the D-Atc[3] analogue as compared to the D-Phe[3] analogue (see ref 14). The deltorphin analogue containing Tic in place of Phe[3] (compound 4) showed poor affinity for both $\mu$ and $\delta$ receptors and had very weak agonist activity in the GPI and MVD bioassays.

The replacement of the Phe residue in [D-Ala[2]]-deltorphin I with an Aic, Atc, or Tic residue produces severe constraints on both side-chain rotational mobility and backbone flexibility at the 3-position residue. In the case of the Aic[3] analogue side chain torsional angles are limited to values of $\chi_1 = -80°$, $\chi_2 = -20°$ and $\chi_1 = -160°$, $\chi_2 = +20°$, whereas the backbone torsional angles preferentially assume values of $\phi = -50°$, $\psi = -50°$ and $\phi = +50°$, $\psi = +50°$. The side-chain conformations possible for the L-Atc and the D-Atc residue are $t$ or $g^-$ and $t$ or $g^+$, respectively, and the limitations on the $\psi$, $\phi$ angles are the same as in the case of Aic. The $\phi$ angle of the Tic residue is limited to values around −90°, and the two possible side-chain conformations for this residue are $g^+$ and $g^-$. It is interesting to point out that substitution of these same conformationally restricted phenylalanine derivatives for Phe[3] in the cyclic opioid peptide analogue H-Tyr-D-Orn-Phe-Glu-$NH_2$ produced parallel effects on potency at the $\mu$ receptor, insofar as the Aic[3], D-Atc[3], and L-Atc[3] cyclopeptides were potent $\mu$ agonists and the Tic[3] analogue again showed poor activity.[14] Furthermore, an almost complete loss of stereospecificity had also been observed with the cyclic D- versus L-Atc[3] analogues in comparison with the corresponding cyclic D- versus L-Phe[3] analogues. This parallel behavior suggests that the $\mu$ and $\delta$ receptor *sub*sites interacting with the Phe[3] side chain of opioid peptides must have very similar structural (conformational) requirements.

Substitution of a Tic residue in the 2-position of deltorphins had most interesting effects on receptor selectivity and on the intrinsic activity ("efficacy") at the $\delta$ receptor. A first example is the [D-Ala[2]]deltorphin I analogue H-Tyr-Tic-Phe-Asp-Val-Val-Gly-$NH_2$ (5), which retained good $\delta$ receptor affinity and showed extraordinary $\delta$ receptor selectivity ($K_i^{\mu}/K_i^{\delta} = 1420$), being about 8 times more $\delta$-selective than [D-Ala[2]]deltorphin I (Table I). This compound was virtually inactive in the GPI assay and in the MVD assay was a partial agonist able to inhibit the electrically evoked vas contractions to a maximal extent of only 50% (Table II). This partial agonist effect could be reversed with the $\delta$ antagonists naltrindole and TIPP, indicating that it was mediated by $\delta$ receptors. Since the tripeptides H-Tyr-Tic-Phe-OH (TIP) and H-Tyr-Tic-Phe-$NH_2$ (TIP-$NH_2$) are both pure $\delta$ antagonists,[13] this result indicates that the C-terminal extension with the tetrapeptide segment -Asp-Val-Val-Gly-$NH_2$ partially restored agonist activity. The potent and highly selective $\delta$ antagonist H-Tyr-Tic-Phe-Phe-OH (TIPP)[13] shows some structural resemblance to deltorphin (12), which contains a Phe and a His residue in the 3- and 4-position, respectively. Therefore, it was of interest to substitute the TIPP tetrapeptide segment for the N-terminal tetrapeptide segment contained in deltorphin. This substitution was carried out with the Nle[6] analogue of deltorphin, since substitution of Nle for Met in both position 2 and position 6 has no appreciable effect on the activity profile (compound 13) and has the advantage of eliminating the problem of methionine oxidation. The resulting analogue, H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-$NH_2$ (6) retained good $\delta$ affinity but bound very poorly to $\mu$ receptors and, therefore, was very $\delta$-selective ($K_i^{\mu}/K_i^{\delta} = 502$). In both the GPI and the MVD assay this compound showed no agonist activity at concentrations up to 10 $\mu$M. However, in the MVD assay analogue 6 was a potent $\delta$ antagonist against the $\delta$ agonists DPDPE and [D-Ala[2]]-deltorphin I with respective $K_e$ values of 10.8 ± 3.1 and 10.4 ± 2.3 nM. No antagonist effect was observed with this compound in the GPI assay at a concentration as high as 10 $\mu$M. In comparison with the tetrapeptide antagonist TIPP, analogue 6 had about 6 times lower $\delta$ affinity, was about 3 times less $\delta$-selective, and was about 2.5 times less potent as antagonist in the MVD assay. Thus, C-terminal extension of the TIPP tetrapeptide with the C-terminal tripeptide segment of [Nle[6]]deltorphin slightly decreased rather than increased $\delta$ receptor affinity and $\delta$ selectivity. This finding is in contrast to the observation that the C-terminal tripeptide segment plays a crucial role in determining the high $\delta$ affinity and $\delta$ selectivity of deltorphins, since the N-terminal tetrapeptide segment of deltorphin has been shown to have weak affinity for $\delta$ receptors and to be $\mu$- rather than $\delta$-selective.[23] Analogue 6 is about 15 times less potent as $\delta$ antagonist than naltrindole[12] but, on the other hand, has better $\delta$ selectivity and no $\mu$ antagonist properties.

Substitution of the D-Ala[2] residue in [D-Ala[2]]deltorphin I with a D-Orn residue and amide bond formation between

(21) Sagan, S.; Corbett, A. D.; Amiche, M.; Delfour, A.; Nicolas, P.; Kosterlitz, H. W. Opioid Activity of Dermenkephalin Analogues in the Guinea-Pig Myenteric Plexus and the Hamster Vas Deferens. *Br. J. Pharmacol.* 1991, *104*, 428–432.

(22) Dupin, S.; Tafani, J.-A. M.; Mazarguil, H.; Zajac, J.-M. [^{125}I]-[D-Ala[2]]Deltorphin-I: A High Affinity, Delta-Selective Opioid Receptor Ligand. *Peptides* 1991, *12*, 825–830.

(23) Lazarus, L. H.; Salvadori, S.; Tomatis, R.; Wilson, W. E. Opioid Receptor Selectivity Reversal in Deltorphin Tetrapeptide Analogues. *Biochem. Biophys. Res. Commun.* 1991, *178*, 110–115.

the D-Orn[2] and Asp[4] side-chain groups resulted in a cyclic lactam analogue (compound 7), which in comparison with the linear parent peptide had comparable $\mu$ receptor affinity but 200 times lower affinity for $\delta$ receptors and, thus, was essentially nonselective (Table I). Previously, it had been observed that the corresponding cyclic tetrapeptide amide H-Tyr-D-Orn-Phe-Asp-$NH_2$ (7a) was hightly $\mu$-selective ($K_i^\delta/K_i^\mu$ = 213) (Table I).[24] Comparison of 7 and 7a revealed that C-terminal extension of the cyclic tetrapeptide with the -Val-Val-Gly-$NH_2$ segment resulted in a 14-fold decrease in $\mu$ affinity and an 8-fold increase in $\delta$ affinity, indicating that, as in the case of the native linear deltorphin, the C-terminal tripeptide ("address" segment) is responsible for shifting the $\mu/\delta$ selectivity ratio in favor of the $\delta$ receptor. Expansion of the 13-membered ring structure in 7 to a 15-membered one (compound 8) produced an 8-fold increase in $\mu$ affinity and an 80-fold enhancement of $\delta$ affinity. Analogue 8 had quite high $\delta$ affinity ($K_i^\delta = 3.66 \pm 0.57$ nM) but only slight preference for $\delta$ receptors over $\mu$ receptors. In the GPI and MVD bioassays, analogues 7 and 8 were both full agonists and showed potency relationships similar to those observed in the receptor binding assays. Two [D-Ala[2]]deltorphin analogues containing a cyclic tetrapeptide segment at the C-terminus, H-Tyr-D-Ala-Phe-Asp-Val-Val-Orn-$NH_2$ (9) and H-Tyr-D-Ala-Phe-Glu-Val-Val-Lys-$NH_2$ (10), showed weak affinity for $\mu$ and $\delta$ receptors, were only slightly $\delta$-selective, and displayed weak potency in the MVD assay. A similar lack of $\delta$ affinity and $\delta$ selectivity as well as relatively low potency in the MVD assay were also observed with the cyclic lactam analogue H-Tyr-D-Lys-Phe-Asp-Val-Val-Glu-$NH_2$ (11).

In summary, none of the cyclic lactam analogues 7–11 retained the high $\delta$ affinity and $\delta$ selectivity of [D-Ala[2]]-deltorphin I. On the basis of a recently performed NMR study, it has been proposed that the conformation of [D-Ala[2]]deltorphin I in DMSO features a type II′ $\beta$-turn stabilized by a Tyr[1]-CO←HN-Asp[4] hydrogen bond and a type I $\beta$-turn stabilized by a Phe[3]-CO←HN-Val[6] hydrogen bond.[25] Inspection of the conformational possibilities of the cyclic peptides described here revealed that a type II′ $\beta$-turn is not possible with cyclic analogues 7 and 8, whereas analogues 9 and 10 can accommodate the proposed C-terminal type I $\beta$-turn. The formation of both turns is possible with analogue 11. However, because the opioid activity profiles of the cyclic analogues described here are different from that of [D-Ala[2]]deltorphin I, this analysis does not permit an unambiguous assessment of the proposed conformational model in terms of its relevance to the bioactive conformation.

None of the deltorphin analogues displayed significant affinity for $\kappa$ opioid receptors ($K_i^\kappa > 1$ $\mu$M). The lack of $\kappa$ receptor interactions was also indicated by the low $K_e$ values (1–2 nM) for naloxone as antagonist that were observed with all compounds in the GPI assay.

(24) Schiller, P. W.; Nguyen, T. M.-D.; Maziak, L. A.; Lemieux, C. A Novel Cyclic Opioid Peptide Analog Showing High Preference for $\mu$-Receptors. *Biochem. Biophys. Res. Commun.* 1985, *127*, 558–564.

(25) Balboni, G.; Marastoni, M.; Picone, D.; Salvadori, S.; Tancredi, T.; Temussi, P. A.; Tomatis, R. New Features of the $\delta$ Opioid Receptor: Conformational Properties of Deltorphin I Analogues. *Biochem. Biophys. Res. Commun.* 1990, *169*, 617–622.

## Conclusions

In the present paper we describe the effect on the opioid activity profile of deltorphin-related peptides produced either by local conformational constraints introduced at the 2- and 3-position residues or by more global conformational restriction resulting from various peptide cyclizations. Substitution of an Aic or of an L- or D-Atc residue for Phe[3] in [D-Ala[2]]deltorphin I resulted in agonist compounds that retained the high $\delta$ receptor selectivity of the parent peptide. Analogues containing a Tic residue in the 2-position of the peptide sequence showed even higher $\delta$ selectivity and either antagonist or partial agonist properties at the $\delta$ receptor. In comparison with the tetrapeptide antagonist H-Tyr-Tic-Phe-Phe-OH (TIPP), the deltorphin related antagonist H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-$NH_2$ (6) showed about 6 times lower $\delta$ affinity, 3 times lower $\delta$ selectivity, and 2.5 times lower $\delta$ antagonist potency in the MVD assay, indicating that the C-terminal tripeptide segment did not contribute to the $\delta$ characteristics of the peptide. None of the cyclic deltorphin analogues displayed the high $\delta$ selectivity observed with the native, linear deltorphins. It was interesting to note, however, that C-terminal extension of the $\mu$-selective cyclic tetrapeptide analogue H-Tyr-D-Orn-Phe-Asp-$NH_2$ with the C-terminal [D-Ala[2]]deltorphin I tripeptide segment -Val-Val-Gly-$NH_2$ reduced $\mu$ affinity and increased $\delta$ affinity quite drastically. The observation that the C-terminal tripeptide segment plays a differential role in contributing to the $\delta$ affinity and $\delta$ selectivity of deltorphin-related peptides with agonist properties versus those with antagonist properties suggests a difference in the mode of receptor binding between agonists and antagonists.

## Experimental Section

**General Methods.** Precoated plates (silica gel G, 250 $\mu$m, Analtech, Newark, DE) were used for ascending TLC in the following solvent systems (all v/v): (I) $n$-BuOH/AcOH/$H_2O$ (4/1/5, organic phase) and (II) $n$-BuOH/pyridine/AcOH/$H_2O$ (15/10/3/12). Reversed-phase HPLC was performed on a Varian VISTA 5500 liquid chromatograph, utilizing a Vydac $C_{18}$ RP column (25 cm × 10 mm). For amino acid analyses peptides (0.2 mg) were hydrolyzed in 6 N HCl (0.5 mL) containing a small amount of phenol for 24 h at 110 °C in deaerated tubes. Hydrolysates were analyzed on a Beckman Model 121C amino acid analyzer equipped with a Model 126 Data System integrator. Molecular weights of peptides were determined by FAB mass spectrometry on an MS-50 HMTCTA mass spectrometer interfaced to a DS-90 data system (Drs. M. Evans and M. Bertrand, Department of Chemistry, University of Montreal).

**Solid-Phase Synthesis and Purification of Linear and Cyclic Peptide Analogues.** Aic, (D,L)-Atc, and Tic were synthesized as elsewhere described in detail.[14] Other Boc- and Fmoc-amino acids were purchased from BioChem Pharma Inc., Laval, Quebec, Canada, and from Bachem Bioscience, Philadelphia, PA.

Peptide synthesis was performed by the manual solid-phase technique using a $p$-methylbenzhydrylamine resin (1% cross-linked, 100–200 mesh, 0.2 mmol/g of titratable amine) obtained from United States Biochemical Corp., Cleveland, OH. Linear peptides were assembled using Boc-protected amino acids and dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as coupling agents. Side-chain protection was as follows: benzyl (Asp), tosyl (His), Boc (Tyr). The following steps were performed in each cycle: (1) addition of Boc amino acid in $CH_2Cl_2$ (2.5 equiv), (2) addition of HOBt (2.5 equiv), (3) addition of DCC (2.5 equiv) and mixing for 2–3 h, (4) washing with $CH_2Cl_2$ (3 × 1 min), (5) washing with EtOH (1 min), (6) monitoring

completion of the reaction with the ninhydrin test,[26] (7) Boc deprotection with 50% (v/v) TFA in $CH_2Cl_2$ (30 min), (8) washing with $CH_2Cl_2$ (5 × 1 min), (9) neutralization with DIEA in $CH_2Cl_2$ (2 × 5 min), (10) washing with $CH_2Cl_2$ (5 × 1 min). Double coupling was necessary to complete coupling of the Tyr residue to the Tic residue in the case of analogues **5** and **6**.

In the case of the analogues containing cyclic peptide structures the exocyclic C-terminal and N-terminal peptide segments were assembled using Boc amino acids according to the protocol described above. The cyclic peptide segments were synthesized according to a protection scheme based on the use of Fmoc amino acids[17] by performing the following steps in each cycle: (1) addition of Fmoc amino acid (2.5 equiv) in $CH_2Cl_2$/DMF (85/15, v/v), (2) addition of HOBt (2.5 equiv), (3) addition of DCC (2.5 equiv) and mixing for 2–3 h, (4) washing with $CH_2Cl_2$ (3 × 1 min), (5) washing with EtOH (1 min), (6) monitoring completion of the reaction with the ninhydrin test, (7) Fmoc deprotection with 50% (v/v) piperidine in $CH_2Cl_2$ (30 min), (8) washing with DMF (3 × 1 min), (9) washing with EtOH (3 × 1 min), (10) washing with $CH_2Cl_2$ (3 × 1 min). After coupling of the last Fmoc amino acid, Fmoc protection of the N-terminal amino group was retained and the side chains of the Orn (Lys) and Asp (Glu) residues to be linked were deprotected by treatment with 50% (v/v) TFA in $CH_2Cl_2$ (30 min). Following neutralization with 10% (v/v) DIEA in $CH_2Cl_2$ (2 × 10 min) and washing with $CH_2Cl_2$ (3 × 1 min) and DMF (3 × 1 min), cyclization was carried out in DMF containing 1.5% (v/v) DIEA at room temperature by addition of HOBt/DCC (2.5 equiv) for 12–48 h. Completion of the ring-closure reaction was monitored with the ninhydrin test. If necessary, the reaction was continued and brought to completion by addition of the BOP coupling reagent (2 equiv) and DIEA (3 equiv), again using DMF as solvent. Cyclization was usually complete after 12–48 h. After performance of the cyclization step, the N-terminal Fmoc group was removed as usual and washing of the resin was carried out as described above. Subsequently, the peptide chain was extended at the N-terminus as required. After final deprotection with 50% (v/v) TFA in $CH_2Cl_2$ (30 min), the resin was washed with $CH_2Cl_2$ (3 × 1 min) and EtOH (3 × 1 min) and was dried in a desiccator. Peptides were cleaved from the resin by treatment with HF for 90 min at 0 °C and for 15 min at room temperature (20 mL of HF plus 1 mL of anisole/g of resin). After evaporation of the HF, the resin was extracted three times with $Et_2O$ and, subsequently, three times with 7% AcOH. The crude peptide was then obtained in solid form through lyophilization of the acetic acid extract.

Peptides were purified by gel filtration on a Sephadex-G-25 column in 0.5 N AcOH followed by reversed-phase chromatography on an octadecasilyl silica column,[27] with a linear gradient of 0–80% MeOH in 1% TFA. If necessary, further purification to homogeneity was performed by semipreparative HPLC [20–80% MeOH (linear gradient) in 0.1% TFA]. Semipreparative HPLC under the same conditions was also used to separate the two diastereoisomers of the Atc[3] analogue. Final products were obtained as lyophilisates. Homogeneity of the peptides was established by TLC and by HPLC under conditions identical with those described above. All peptides were at least 98% pure as judged from the HPLC elution profiles. Analytical data are presented in Table III (Supplementary Material).

**Receptor Binding Assays and Bioassays.** Opioid receptor binding studies were performed as described in detail elsewhere.[28] Binding affinities for $\mu$ and $\delta$ receptors were determined by displacing, respectively, [$^3$H]DAMGO (New England Nuclear) and [$^3$H]DSLET (Amersham) from rat brain membrane preparations, and $\kappa$ opioid receptor affinities were measured by displacement of [$^3$H]U69,593 (New England Nuclear) from guinea pig brain membranes. Incubations were performed for 2 h at 0 °C with [$^3$H]DAMGO, [$^3$H]DSLET, and [$^3$H]U69,593 at respective concentrations of 0.72, 0.78, and 0.80 nM. IC50 values were determined from log dose–displacement curves, and $K_i$ values were calculated from the obtained IC50 values by means of the equation of Cheng and Prusoff,[29] using values of 1.3, 2.6, and 2.9 nM for the dissociation constants of [$^3$H]DAMGO, [$^3$H]-DSLET, and [$^3$H]U69,593, respectively.

The GPI[30] and MVD[31] bioassays were carried out as reported in detail elsewhere.[28,32] A log dose–response curve was determined with [Leu[5]]enkephalin as standard for each ileum and vas preparation and IC50 values of the compounds being tested were normalized according to a published procedure.[33] $K_e$ values for naloxone or for the antagonist H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-$NH_2$ (**6**) were determined from the ratio of IC50 values obtained in the presence and absence of a fixed antagonist concentration.[34]

**Acknowledgment.** This work was supported by operating grants from the Medical Research Council of Canada (Grant MT-5655), The Heart and Stroke Foundation of Quebec, and the National Institute on Drug Abuse (DA-04443). Naloxone hydrochloride was a gift from Endo Laboratories, Inc. We thank Drs. M. Evans and M. Bertrand, Department of Chemistry, University of Montreal, for the performance of the FAB mass spectroscopic determinations. Thanks are also due to H. Zalatan for typing the manuscript.

**Supplementary Material Available:** Table III, listing analytical data of deltorphin analogues (1 page). Ordering information is given on any current masthead page.

**Registry No.** 1, 143293-95-2; 2, 143293-96-3; 3, 143344-20-1; 4, 143293-97-4; 5, 143293-98-5; 6, 143293-99-6; 7, 143294-00-2; 7a, 96382-72-8; 8, 143294-01-3; 9, 143294-02-4; 10, 143294-03-5; 11, 143294-04-6; 12, 119975-64-3; 13, 143294-05-7; 14, 122752-15-2; 14a, 143294-06-8; 15, 58822-25-6.

(26) Kaiser, E.; Colescott, R. L.; Bossinger, D. C.; Cook, P. I. Color Test for the Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides. *Anal. Biochem.* **1970**, *34*, 595–598.

(27) Böhlen, P.; Castillo, F.; Ling, N.; Guillemin, R. Purification of Peptides: An Efficient Procedure for the Separation of Peptides from Amino Acids and Salt. *Int. J. Pept. Protein Res.* **1980**, *16*, 306–310.

(28) Schiller, P. W.; Lipton, A.; Horrobin, D. F.; Bodanszky, M. Unsulfated C-terminal 7-Peptide of Cholecystokinin: A New Ligand of the Opiate Receptor. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 1332–1338.

(29) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition Constant ($K_I$) and the Concentration of Inhibitor which Causes 50 Per Cent Inhibition ($I_{50}$) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3102.

(30) Paton, W. D. M. The Action of Morphine and Related Substances on Contraction and on Acetylcholine Output of Coaxially Stimulated Guinea-Pig Ileum. *Br. J. Pharmacol.* **1957**, *12*, 119–127.

(31) Henderson, G.; Hughes, J.; Kosterlitz, H. W. A New Example of a Morphine Sensitive Neuroeffector Junction. *Br. J. Pharmacol.* **1972**, *46*, 764–766.

(32) DiMaio, J.; Nguyen, T. M.-D.; Lemieux, C.; Schiller, P. W. Synthesis and Pharmacological Characterization In Vitro of Cyclic Enkephalin Analogues: Effects of Conformational Constraints on Opiate Receptor Selectivity. *J. Med. Chem.* **1982**, *25*, 1432–1438.

(33) Waterfield, A. A.; Leslie, F. M.; Lord, J. A. H.; Ling, N.; Kosterlitz, H. W. Opioid Activities of Fragments of $\beta$-Endorphin and of its Leucine[65]-Analogue. Comparison of the Binding Properties of Methionine- and Leucine-Enkephalin. *Eur. J. Pharmacol.* **1979**, *58*, 11–18.

(34) Kosterlitz, H. W.; Watt, A. J. Kinetic Parameters of Narcotic Agonists and Antagonists with Particular Reference to N-Allylnoroxymorphone (naloxone). *Br. J. Pharmacol.* **1968**, *33*, 266–276.