Design and Synthesis of Somatostatin Analogues with Topographical Properties That Lead to Highly Potent and Specific μ Opioid Receptor Antagonists with Greatly Reduced Binding at Somatostatin Receptors

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A series of conformationally restricted, cyclic octapeptides containing a conformationally stable tetrapeptide sequence related to somatostatin, -Tyr-D-Trp-Lys-Thr-, as a template, were designed and synthesized with the goal of developing highly potent and selective μ opioid antagonists with minimal or no somatostatin-like activity. Three distinct structures of the peptide became targets of chemical modifications and constraints; the N- and C-terminal amino acids and the cyclic 20-membered ring moiety. Based on the conformational analysis of active and inactive analogues of the parent peptide D-Phe[1]-Cys[2]-Tyr[3]-D-Trp[4]-Lys[5]-Thr[6]-Pen[7]-Thr[8]-NH_2, CTP (Kazmierski, W.; Hruby, V. J. *Tetrahedron* **1988**, *44*, 697–710), we designed analogues to include the tetrahydroisoquinolinecarboxylate (Tic) moiety as the N-terminal amino acid instead of D-Phe, since Tic can exist only as a gauche (−) or a gauche (+) conformer. In this series, the following peptides were synthesized and pharmacologically evaluated: D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (TCTP), D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (TCTOP), and D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (TCTAP). In rat brain membrane opioid radioligand binding assays, all three peptides displayed high affinity for μ opioid receptors (IC_{50} = 1.2, 1.4, 1.2 nM, respectively), and exceptional μ vs δ opioid receptor selectivity: 7770, 11 396, and 1060, respectively. TCTOP and TCTAP also possess exceptional μ vs somatostatin receptor selectivity: 14 574 and 28 613, respectively. In the peripheral in vitro GPI bioassay, TCTP, TCTOP, and TCTAP were highly effective antagonists of the potent μ opioid receptor agonist PL017, with pA_2 = 8.69 for TCTAP, 8.10 for TCTP, and 7.38 for TCTOP. Our results show that a 10-fold higher affinity and selectivity for μ opioid receptors (in both central and peripheral studies) over δ and somatostatin receptor was gained as a result of the D-Tic[1] substitution. These three peptides, TCTP, TCTOP, and TCTAP, are the most potent and selective μ opioid antagonists known. CTP has been shown to possess prolonged biological action, much longer than that of naloxone. This renders these analogues potentially useful ligands for investigating the physiological functions of the μ opioid receptor. Analogues of TCTP in which the 20-membered disulfide ring was contracted by deletion of D-Trp[4], and/or Lys[5], and/or Thr[6] led to compounds with greatly reduced potency at the μ opioid receptor. Furthermore, modification of the Thr[8] residue with Val, Ser, Asn, and Asp or by simple deletion of the residue led to analogues with greatly reduced potency and reduced μ opioid receptor selectivity.

The cyclic tetradecapeptide somatostatin, H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH (SS-14), is a regulatory hormone that is distributed throughout the central nervous system, gastrointenstinal tract, and pancreas. Studies of its ability to inhibit growth hormone, glucagon, insulin, and gastrin release have shown that fragments of the native hormone, including the essential pharmacophore Phe[7]-Trp[8]-Lys[9]-Thr[10], carries the full binding and transduction message of somatostatin.[2,3]

Further work has indicated that substitution of D-Trp[8] for L-Trp[8] increases the potency of the hormone. Veber and co-workers have developed a class of cyclic somatostatin-related hexapeptides, among which *cyclo*(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe) is a potent inhibitor of growth hormone, insulin, and glucagon release.[4] The cyclic octapeptide, D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol), SMS-201995, synthesized by Bauer et al.[5] is also an exceptionally potent inhibitor of growth hormone release.

In addition to its well-established role as a regulating hormone,[6] somatostatin has also been observed to have some neurotransmitter-like properties. These would include localization in presynaptic terminals,[7] effects on spontaneous neuronal discharge following microiontophoretic application,[8] and behavioral effects after CNS administration.[9] Somatostatin also appears to bind weakly to CNS opioid receptors. High concentrations of somatostatin can inhibit binding of naloxone and DADLE ([D-Ala[2],D-Leu[5]]enkephalin) to rat brain homogenates and, in addition, SS-14 can give rise to an in vivo analgesic response in mice.[10,11] Moreover, SMS-201995, a somatostatin analogue, also exhibits quite good affinity for opioid receptors[12] and μ opioid receptor selectivity.

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). All optically active amino acids are of the L variety unless otherwise stated. Other abbreviations include the following: DPDPE, [D-Pen[2],D-Pen[5]]enkephalin; PL017, [MePhe[3],D-Pro[4]]morphiceptin; ICI 174,864, *N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH; 2-ClZ, 2-chlorobenzyloxycarbonyl; DCM, dichloromethane; HOBt, *N*-hydroxybenzotriazole; Tos, tosyl; N^{α}-Boc, N^{α}-*tert*-butyloxycarbonyl; Pen, penicillamine; Pgl, phenylglycine; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; FAB-MS, fast atom bombardment mass spectrometry; CGP23,996, des-Ala[1],Gly[2]-[desamino-Cys[3],Tyr[11]]-3,14-dicarbasomatostatin; Tic, tetrahydroisoquinolinecarboxylate.

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Table I. Binding Affinities and Selectivities of CTP Analogues in Competition with [^{3}H]CTOP, [^{3}H]DPDPE, and [^{125}I]CGP23,996 in Receptor Binding to Rat Brain Membranes

peptide	IC_{50}, nM: binding vs [^{3}H]CTOP	IC_{50}, nM: binding vs [^{3}H]DPDPE	IC_{50}, nM: binding vs [^{125}I]-CGP23,996	μ/δ selectivity	μ/somatostatin selectivity
1, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2, CTP	3.7 ± 0.8	1153 ± 116	1462 ± 114	312	395
2, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2, CTOP	4.3 ± 0.8	5598 ± 317	47704 ± 3112	1301	11094
3, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2, CTAP	2.1 ± 0.3	5314 ± 278	8452 ± 285	2530	4025
4, D-Tyr-Cys-Phe-Asn-Pen-Thr-NH_2	7468 ± 297	≥10000	≥100000	≥1.3	≥1.3
5, D-Phe-Cys-Tyr-Asn-Pen-Thr-NH_2	17% inhibn at 10000	≥10000	≥100000	ND	ND
6, D-Phe-Cys-Tyr-D-Trp-Thr-D-Pen-Thr-NH_2	10000	7822 ± 4372	ND	ND	ND
7, D-Phe-Cys-Tyr-D-Trp-Lys-D-Pen-Thr-NH_2	18% inhibn at 10000	≥10000	6103 ± 848	ND	ND
8, D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2, TCTP	1.2 ± 0.0	9324 ± 546	949 ± 170	7770	791
9, D-Tic-Cys-Tyr-D-Orn-Thr-Pen-Thr-NH_2, TCTOP	1.4 ± 0.2	15954 ± 3582	20403 ± 1363	11396	14574
10, D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2, TCTAP	1.2 ± 0.2	1274 ± 78	34336 ± 2241	1060	28613
11, D-N-Me-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2	284 ± 36	≥10000	8054 ± 1620	≥35	28
12, Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2	278.7 ± 0.5	5352 ± 503	19408 ± 6738	19	70
13, D-Trp-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2	6.3 ± 0.5	≥10000	1599 ± 15	≥1587	254
14, D-Tic-Cys-Tyr-D-Trp-Lys-Pen-Thr-NH_2	9408 ± 695	≥10000	13610 ± 3726	≥1.0	1.5
15, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-NH_2	187 ± 40	≥10000	47273 ± 4811	≥53	253
16, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-NH_2	115 ± 10.2	≥10000	1942 ± 13	≥87	17
17, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Asn-NH_2	130 ± 17.1	≥10000	1801 ± 413	≥77	6.3
18, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Asp-NH_2	3467 ± 84.5	≥10000	8300 ± 4597	≥2.9	2.4
19, D-Tic-Cys-Tyr-D-Typ-Lys-Thr-Pen-Val-NH_2	46.0 ± 11.0	2122 ± 13	1992 ± 115	46	43
20, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2	20.0 ± 4.7	≥10000	1888 ± 238	≥490	94
21, D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2	7.8 ± 2.0	3828 ± 126	1499 ± 131	491	192

Utilizing various structural considerations in conjunction with conformational constraints,[13-15] we have modified the structure of somatostatin to enhance its affinity for the μ opioid receptor, while at the same time decreasing its affinity for somatostatin receptor with which native somatostatin interacts with high affinity, and in the process converted this hormone to an opioid antagonist. We have previously prepared a series of compounds, with our most selective μ opioid receptor ligand D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2, CTP.[16,17] This class of compounds was found to be extremely stable to biodegradation conditions and do not appear to cross the blood–brain barrier.[18] These characteristics make CTP a very convenient ligand for studies of the physiological and pharmacological importance of the μ opioid receptor. Recently, utilizing ^{1}H NMR and computational techniques, we have analyzed the conformational properties of potent and nonpotent analogues of CTP.[19-21] The results have stimulated us to design and synthesize even more selective and potent μ opioid receptor antagonists.

In the present work, we report the design, synthesis, and *in vitro* biological activity of 18 new analogues of CTP. These studies have led to a new class of opioid peptides that are among the most potent and selective μ opioid antagonists known and that have very little somatostatin-like activity. Several of these analogues are further conformationally constrained and provide new insights into the topographical structural requirements of the μ opioid receptor in the brain and in peripheral tissues.

Results

The peptides were synthesized by the solid-phase method using general procedures previously developed in our laboratory for this class of peptides.[16,17,22] The peptides were purified by gel filtration chromatography followed by reversed-phase high-pressure liquid chromatography (RP-HPLC). The structure and purity of the peptides was assessed by thin-layer chromatography, amino acid analysis, fast-atom-bombardment mass spectrometry, ^{1}H NMR, and analytical RP-HPLC. In the design of new analogues, we have focused our structural modifications on distinct moieties of the parent peptide, CTP, which would provide specific structural, conformational, and/or topographical modifications. Binding data of somatostatin

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Table II. Pharmacological Characterization of Selected Somatostatin Analogues in GPI Assays

peptide	GPI agonism[a]	GPI antagonism[b]	pA_2[c]
1, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2	no	yes	7.10 ± 0.17
2, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2	no	yes	6.37 ± 0.07
3, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2	no	yes	7.12 ± 0.08
8, D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2	no	yes	8.10 ± 0.29
9, D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2	no	yes	7.38 ± 0.03
10, D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2	no	yes	8.69 ± 0.25
13, D-Trp-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2	yes	ND[d]	ND[d]
19, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Val-NH_2	no	yes	6.59 ± 0.13
20, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2	no	yes	6.24 ± 0.11

[a] Intrinsic agonist activity in GPI assay. [b] Antagonism of PL017-induced inhibition of electrically induced contractions of GPI. [c] Negative logarithm of antagonist concentration that requires doubling of agonist concentration to induce original agonist response. [d] Not determined.

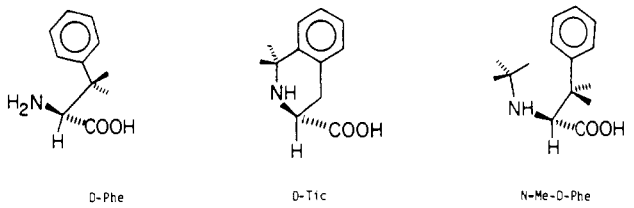

Figure 1. Structures of amino acids used on N-terminal position of somatostatin analogues.

analogues to rat brain μ and δ opioid and somatostatin membrane receptors were examined and the results are presented in Table I. Peripheral in vitro opioid antagonist properties for those compounds that exhibited strong opioid antagonistic properties are shown in Table II for the guinea pig ileum assay.

Compounds **1–3**, CTP, CTOP, and CTAP, respectively, have been synthesized previously[16,17] and are included in both Tables I and II for comparison, as slightly modified receptor binding protocols were used in this study than in previous studies[16,17] (see the Experimental Section). First, an effort was made to explore the possibility of obtaining active antagonist analogues of CTP, but with a reduced ring size from 20-membered to 14-membered and 17-membered. Peptides D-Tyr-Cys-Phe-Asn-Pen-Thr-NH_2 (**4**) and D-Phe-Cys-Tyr-Asn-Pen-Thr-NH_2 (**5**) are truncated somatostatin analogues with 14-membered rings. They showed weak receptor binding activities (Table I). Analogues D-Phe-Cys-Tyr-D-Trp-Thr-D-Pen-Thr-NH_2 (**6**) and D-Phe-Cys-Tyr-D-Trp-Lys-D-Pen-Thr-NH_2 (**7**), which contain 17-membered rings and a D-penicillamine residue, also showed little affinity to opioid or stomatostatin receptors (Table I).

Next, we examined the importance of the N-terminal amino acid, in regular 20-membered ring analogues, for opioid receptor recognition. In these studies we have used a conformationally and topographically constrained residue, tetrahydroisoquinolinecarboxylic acid (Tic) (Figure 1). Analogues of CTP containing this residue, including D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (**8**, TCTP), D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (**9**, TCTOP), and D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (**10**, TCTAP), all exhibited significantly increased affinity for the μ opioid receptor and decreased affinity for the δ opioid receptor, in comparison with CTP, CTOP, and CTAP, respectively (Table I). However, there was no major influence of this new moiety on peptide binding to somatostatin receptors. Thus, introduction of the conformationally constrained tetrahydroisoquinolinecarboxylate instead of phenylalanine results in about a 2–3-fold increase in μ opioid receptor binding in **8–10** and an 8- and 3-fold decrease in δ opioid receptor binding in **8** and **9** as well as substantially greater μ vs somatostatin receptor selectivity by peptides **8–10**. For example, D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (TCTP, **8**) showed an increased affinity for μ receptors (3 times relative to CTP) and lower affinity (8 times) for δ opioid receptors with an overall μ vs δ selectivity of 7770 compared with about 312 for CTP (Table I) in the binding assays, and its antagonistic potency in the guinea pig ileum assay was 10 times greater than that of CTP (Table II). Similarly, D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (TCTOP, **9**) was found to have higher affinity for μ receptors (3 times with respect to CTOP) as well as higher μ vs δ selectivity (9 times, Table I). Remarkably, TCTOP is also 10 times more potent on peripheral μ opioid receptors when compared to CTOP (Table II).

In the peripheral opioid assay system (Table II), D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (TCTAP, **10**) is definitely the most potent μ opioid receptor antagonist yet obtained, with $pA_2 = 8.69$ (compared with $pA_2 = 7.12$ for CTAP, **3**), followed by TCTOP (**9**) with $pA_2 = 7.38$ (vs 6.37 for CTOP, **2**) and TCTP (**8**) with $pA_2 = 8.10$ (vs 7.10 for CTP, **1**). Replacement of Lys^5 by Orn and Arg has been found to be deleterious for the affinity of somatastatin analogues for somatostatin receptors in the cyclic hexapeptides[23] as well as octapeptides.[16] Indeed, rat brain receptor binding for TCTOP (**9**) exhibits a 21-fold decrease of affinity for rat brain somatostatin receptor relatively to TCTP (**8**). Affinity of TCTAP (**10**) for somatostatin receptors is 35 times lower than that of TCTP.

When the structurally, but not conformationally,[11] similar amino acid D-*N*-Me-Phe^1 (Figure 1) is substituted for D-Tic^1 in CTOP, the peptide D-*N*-MePhe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (**11**) is obtained and, perhaps surprisingly, it exhibits a dramatic loss of affinity for the μ opioid receptor (approximately 200-fold with respect to analogue **9**), while conserving equal affinity to δ opioid and somatostatin receptors compared to **9**.

The only nonapeptide in this series, Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (**12**), in which Gly is attached to TCTOP (**9**), also exhibited a sharp decrease of affinity for the μ opioid receptor (about 200-fold with respect to **2**), but a modest increase of affinity to the δ opioid receptor (3 times with respect to **9**) occurs.

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The importance of N-terminal position in these cyclic octapeptides for binding, and possibly transduction, at opioid receptors was further demonstrated by the synthesis of D-Trp-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (**13**). Rat brain binding studies (Table I) suggest that **13** is a quite potent (IC_{50} = 6.3 nM) and selective (≥1599) ligand for the μ opioid receptor. However, unlike other compounds in this class, **13** has some activity in the GPI assay at low concentrations ($ED_{50} \cong 300$ nM). As this effect is not reversible with naloxone and ICI 174,864 (data not shown), peptide **13** may provide unique opportunities to investigate the relationships of opioid and other receptor types.

Analogue **14** is another truncated analogue of potent TCTP (**8**), in which the endocyclic threonine has been deleted with respect to **8**. This causes a dramatic decrease in affinity (about 7800 with respect to **8**) for the μ opioid receptor.

We next examined the importance of the C-terminal Thr-NH_2 residue for opioid and somatostatin receptors. Analogue **15**, which is identical with the potent analogue CTOP (**2**) except for the deletion of the exocyclic Thr^8 residue, exhibited greatly reduced affinity (43 times lower than **2**) to μ opioid receptors as well as to δ opioid receptors, while it conserved low affinity (of the same order of magnitude as **2**) for somatostatin receptors. In the same manner, **16**, an analogue of **15** with Lys^5 substituting Orn^5 as in **1**, showed greatly reduced affinity at the μ (approximately 31-fold, relative to **1**) as well as the δ opioid receptor but not at the somatostatin receptor.

Since a carboxamide C-terminal group greatly improves the ligand's affinity and selectivity for the μ opioid receptor relative to a carboxylate terminal,[16] we prepared the "double carboxamide" analogue D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Asn-NH_2 (**17**) to determine whether an increased "concentration" of carboxamide groups would enhance the binding. **17** exhibited lower affinity (by a factor of 10 or more) in all receptor bioassays. As expected, however, an even sharper decrease of affinity to the μ opioid receptor was found for the analogue with a mixed carboxylic acid–carboxamide terminal, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Asp-NH_2 (**18**, Table I).

The SAR of analogues **15**–**18** (Table I) suggested that the Thr^8 residue plays a very important role in binding of these analogues to μ receptors. Thus we have further examined the relative importance of the hydrophobic β-methyl and hydrophilic β-hydroxyl moieties of threonine for interaction with the μ receptor by preparing the analogues D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Val-NH_2 (**19**) and D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2 (**20**) in which the β-OH group of Thr^8 has been replaced by a methyl group or in which the β-methyl group had been replaced by a hydrogen, respectively. In the peripheral guinea pig ileum assay, both peptides were found to be moderately potent μ antagonists, with **19** being more potent than **20** (Table II). In the rat brain binding assay, **20** was more potent and selective for μ opioid receptors than **19**. Finally, D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2 (**21**) was prepared to examine the effect of the $D\text{-}Tic^1$ in the Ser^8 analogue. The substantial (3-fold) increase of affinity of **21** for μ opioid receptors was consistent with our previous experience.

Discussion

Three distinct structural and/or conformational properties of the octapeptide analogue **1** had been examined with respect to their significance for μ and δ opioid receptor interactions: (1) the importance of ring size was examined by its reduction; (2) the importance of the side-chain conformation of the N-terminal D-Phe to opioid receptor potency and selectivity was examined by the design and incorporation of the conformationally restricted amino acid $D\text{-}Tic^1$; and (3) the importance of the C-terminal Thr^8 residue to μ opioid receptor potency and selectivity was examined by deleting the residue or by structurally modifying it.

Analogues with reduced size from the 20-membered ring were examined by preparing the 14-membered ring compounds **4** and **5**. This ring size is comparable to the one characterizing the δ-selective disulfide-containing enkephalins of Mosberg et al.[24-26] which contained a Pen^5 residue and those of Schiller et al.[27] which did not contain a Pen^5 residue and were somewhat μ receptor selective. In addition, 17-membered ring analogues **6** and **7** were made by deleting Lys^5 or Thr^6, respectively.

Peptides **4**–**7** all showed a dramatic decrease in affinity for both types of opioid receptors compared to the 20-membered ring peptides **1**–**3**. NMR investigations[20,21] revealed that the potent analogue CTP (**1**) is characterized by a type II′ β-turn of the core tetrapeptide -Tyr-D-Trp-Lys-Thr- with a hydrogen bond between the Tyr^3 (CO) and the Thr^6 (N*H*), and conformational analysis of the inactive analogue CTDP,[20] D-Phe-Cys-Tyr-D-Trp-Lys-Thr-D-Pen-Thr-NH_2, revealed that replacement of $L\text{-}Pen^7$ (in **1**) with $D\text{-}Pen^7$ (in CTDP) is associated with a change in the helicity of the disulfide bond, being negative for CTP (**1**) and positive for CTDP.[20] The low affinity of both **6** and **7** for the μ opioid receptors, therefore, may be due to both the ring size reduction and the presence of $D\text{-}Pen^7$ in these molecules. Since a difference in the dihedral disulfide angle in CTDP can cause an alteration of the relative spatial disposition of the exocyclic amino acids in $D\text{-}Phe^1$ and Thr^8, the large decrease in affinity for μ receptors observed for CTDP suggested that both N- and C-terminal positions might be important pharmacophores for interaction with μ opioid receptors, and most of the studies in the paper have concentrated on these positions.

The single substitution of D-phenylglycine[1] instead of D-phenylalanine[1] in **1** to give D-Pgl-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (PCTP) caused a dramatic decrease (about 100-fold) in its affinity for μ and a modest (about 2.5-fold) increase in binding to δ opioid receptors relative to CTP, as measured in the rat brain binding assay.[16] To examine the effect of this change on peptide conformation in relationship to the observed pharmacological properties, extensive conformational studies of this compound were made by utilizing a variety of 1D and 2D ^1H NMR techniques.[19] PCTP was characterized to possess a similar backbone conformation (type II′ β-turn and negative disulfide chirality) and overall topology (side-chain populations) as the parent CTP.[19] Thus we concluded that exchange of $D\text{-}Phe^1$ by $D\text{-}Pgl^1$ resulted in a more compact

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Table III. Analytical Characteristics of Somatostatin Analogues

peptide	thin-layer chromatography[a] R_f values I	II	III	IV	HPLC[b] K' values	FAB-MS $[M + H]_{obsd}$	$[M + H]_{calcd}$
4	0.47	0.72	0.79	0.75	0.0	775	775
5	0.45	0.67	0.80	0.75	0.4	775	775
6	0.63	0.79	0.84	0.81	1.7	949	949
7	0.36	0.60	0.77	0.72	0.7	976	976
8	0.45	0.68	0.77	0.74	4.8	1088	1088
9	0.44	0.69	0.77	0.74	3.5	1075	1075
10	0.47	0.58	0.78	0.75	4.8	1116	1116
11	0.43	0.69	0.77	0.73	3.5	1077	1077
12	0.38	0.68	0.74	0.71	3.2	1131	1131
13	0.45	0.65	0.77	0.73	7.9	1115	1115
14	0.37	0.64	0.76	0.73	1.5	988	988
15	0.50	0.74	0.78	0.75	3.0	961	961
16	0.36	0.68	0.72	0.73	2.9	976	976
17	0.39	0.65	0.76	0.72	2.7	1090	1090
18	0.37	0.66	0.71	0.67	1.8	1089	1089
19	0.63	0.70	0.79	0.75	6.4	1086	1086
20	0.44	0.66	0.77	0.74	3.2	1062	1062
21	0.43	0.67	0.77	0.74	4.1	1075	1075

[a] Silica gel GF 250 μm (Analtech) glass plates were used. The following solvent system has been applied: (I) *n*-BuOH/AcOH/H_2O, 4/1/5 (v/v/v); (II) *i*-PrOH/NH_3/H_2O, 3/1/1 (v/v/v); (III) *n*-BuOH/AcOH/H_2O/Py, 6/1/5/6 (v/v/v/v); (IV) *n*-BuOH/AcOH/H_2O/Py 15/3/10/12 (v/v/v/v). [b] Vydac 218 TP C_{18} column (25 cm × 4.5 mm), 0.1% TFA/CH_3CN 80/20, flow rate 2.5 mL/min, monitored at λ = 214 nM.

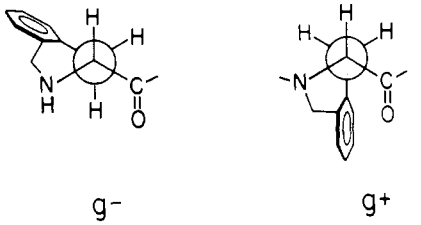

Figure 2. Two discrete conformations for the side chain of tetrahydroisoquinoline carboxylic acid (Tic).

conformation in that the distance between the aromatic rings of the N-terminal amino acid in D-Pgl[1] and Tyr[3] was greatly reduced. Apparently this conformation is not compatible with strong interaction with the μ opioid receptor.

In CTP, there is a large participation of the g(−) side chain rotamer for the D-Phe[1] residue[20] which leads to a more extended overall conformation. This suggested that a further increase (via conformational constraint) of the g^- population of the amino acid in the 1-position should increase μ opioid receptor affinity and selectivity. The tetrahydroisoquinoline carboxylic acid (Tic, Figure 1) would appear to be an excellent amino acid residue to test this hypothesis since only two discrete side chain conformations are possible for this residue (Figure 2). Furthermore it is clear from model building that only the g(−) conformer would correspond to an extended conformation in the somatostatin analogue such as CTP, while the g(+) conformer would lead to a rather folded conformation in the same peptide. It should be emphasized that operationally D-Tic is a product of insertion of a methylene unit bridging the α-N and the 2′-phenyl carbon of D-Phe. Thus we synthesized D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (TCTP, **8**), D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (TCTOP, **9**), and D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (TCTAP, **10**). As previously discussed, vide supra, approximately a 10-fold increase of μ opioid antagonist potency was observed in the peripheral (GPI) assay system on replacement of D-Phe[1] by D-Tic[1], and these results were consistent with the binding data. Furthermore, moderately higher affinity for μ and lower affinity for δ receptors caused a significant increase of the μ vs δ selectivity for these analogues (except for TCTAP).

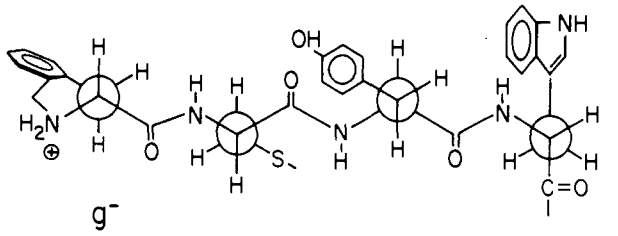

Figure 3. Newman projection of the partial structure of TCTP (**8**) emphasizing mutual spatial relationship of aromatic side chains of D-Tic and Tyr. Note extended conformation of the peptide.

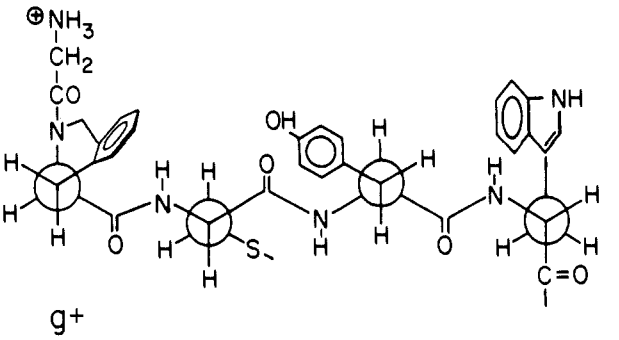

Figure 4. Newman projection of the partial structure of **12** emphasizing spatial relationship of aromatic side chains of D-Tic and Tyr. Note folded conformation of the peptide.

To account for the higher μ opioid receptor affinity and selectivity of D-Tic analogues **8**, **9**, and **10** compared with **1**, **2**, and **3**, we have examined the conformational properties of TCTP (**8**).[19] These studies have shown that the side chain of the D-Tic[1] residue exists exclusively in the gauche(−) conformation, resulting in an extended conformation of the peptide (Figure 3).

To test this hypothesis further, two other peptides were synthesized: D-*N*-Me-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (**11**) and Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (**12**). Both **11** and **12** exhibited very low binding affinity for the central μ opioid receptors. It is important to note that in **11**, *N*-Me-D-Phe can be viewed as a product of C_{CH_2}–C_{ortho} bond disconnection of D-Tic (Figure 1). This is confirmed by the very similar hydrophobic properties of both **9** and **11** (Table (III). Fur-

thermore, ^{1}H NMR investigations of **11** indicated a large participation of trans and gauche(+) side chain rotamers of D-*N*-MePhe.[19] As previously discussed, the g^+ side chain conformation of the N-terminal amino acid results in a relatively folded conformation of the peptide (Figure 4). All of these modifications at the N-terminal position have no effect on the topology and conformation of the central tetrapeptide of the cyclic moiety. That is, in all investigated cases (PCTP, TCTP (**8**), **11**, and **12**), the peptides are characterized by a type II′ β-turn of the Tyr-D-Trp-Xxx-Thr moiety, where Xxx = Lys or Orn, and a negative chirality of the disulfide bond. The compact conformation of PCTP, **11** and **12** makes them topographically very similar, and thus they display similar pharmacological properies (Tables I and II) with reduced μ receptor potency and selectivity. In contrast, the extended conformation found for TCTP makes it more compatible with the μ but not the δ opioid receptor. Thus, these results suggest one of the important topographical features of μ selective opioid ligands, namely an extended conformation, which requires an increased distance between the aromatic pharmacophores of Tyr^3 and the amino acid residue in the 1-position.

We also examined the C-terminal position, which was suggested by results of conformational investigations of CTDP to be another important binding element of these cyclic octapeptides. This was confirmed by the very low affinity for μ opioid receptors of peptides in which C-terminal threonine was deleted (**15**, **16**) or substituted by amino acids featuring carboxylic (**18**) or carboxamide (**17**) side-chain groups. These results suggest that the hydrophobic (methyl) or hydrophilic (hydroxyl) groups of threonine may be important binding elements at the μ opioid receptor. However, peptides in which these structural features have been segregated by means of substituting Val (**19**) or Ser (**20**, **21**) for Thr^8 did not show the expected dissociation of their pharmacological properties. Indeed, peptides **19** and **21** are less potent antagonists, with lower affinity for μ opioid receptors than TCTP (**8**). These results suggest that Thr^8 may possess hydrophobic properties that enable optimal transport of peptides through the water/lipid systems. Ser^8- or Val^8-containing analogues may be either too hydrophilic (partial absorption by water phase) or too hydrophobic (partial absorption by lipid phase), respectively, for high potency. However, it is also possible that a direct effect on the interaction of the analogue with the μ receptor binding pocket may account for the reduced potency of these analogues.

Experimental Section

General Methods. Synthesis of CTP (**1**), CTAP (**2**), and CTOP (**3**) were accomplished as previously reported.[16,17] Peptides **4**–**21** were prepared by solid-phase synthetic techniques[28,29] with a Vega (Tucson, AZ) Model 250 or 1000 peptide synthesizer. Amino acids either were purchased from Bachem (Torrance, CA) or were prepared by literature methods.[29] Carboxamide peptides were synthesized with a *p*-methylbenzhydrylamine (pMBHA) resin which was prepared by literature methods[30] (substitution 1.0 mM/g of resin). A 1.5 M excess of preformed symmetrical anhydrides or 3 M excess of hydroxybenzotriazole active esters was used for coupling reactions, which were monitored by ninhydrin[31] or chloranil tests.[32] Purity of the final product was assessed by TLC in four different solvents, HPLC, FAB-MS, amino acid analysis, and ^{1}H NMR. In all cases, when the amino acid composition of peptides are reporting, values in brackets refer to the number of residues of a given type in a molecule.

D-Tyr-Cys-Phe-Asn-Pen-Thr-NH$_2$ (**4**). N^α-Boc-Thr(*O*-Bzl) was coupled to 0.5 mM of pMBHA resin by using the active ester method, followed by deprotection by 50% TFA in dichloromethane (DCM) (2% anisole added) for 2 and 20 min. Stepwise coupling and deprotection of N^α-Boc-Pen(*S*-4-MeBzl), N^α-Boc-Asn, N^α-Boc-Phe, N^α-Boc-Cys(*S*-4-MeBzl) and N^α-Boc-D-Tyr(*O*-2,6-Cl$_2$Bzl) was then accomplished by standard methods of solid-phase synthesis.[16,17,22]

For N^α-Boc-Asn coupling an active ester derivative was applied to avoid nitrile formation. After coupling of the last amino acid, the N^α-Boc protecting group was removed, the amino acid was neutralized with diisopropylethylamine, and the resulting D-Tyr(*O*-2,6-Cl$_2$Bzl)-Cys(*S*-4-MeBzl)-Phe-Asn-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl)-pMBHA peptide resin was dried in vacuo.

Cleavage of all side protecting groups as well as peptide from the resin was achieved with liquid HF (approximately 15 mL) with addition of anisole (1 mL), followed by stirring for 40 min at 0 °C. After evaporation of HF and anisole at 0 °C, the product was washed with ethyl ether (3 × 20 mL) and extracted with 10% aqueous HOAc (3 × 20 mL) followed by glacial HOAc (2 × 20 min); both fractions were lyophilized separately.

Next, the linear peptide was cyclized by dissolving in 1.5 L of water (pH adjusted with aqueous ammonia to 8.5) and oxidized with 0.01 M $K_3Fe(CN)_6$ until the yellow color persisted for 20 min. After the reaction was terminated, the pH was adjusted to 4.5 with AcOH, excess ferro- and ferricyanides were removed by 15 mL of Amberlite IRA-45 (mesh 15–60, Cl^- form), the mixture stirred for 1 h and filtered, and the solution concentrated in vacuo and lyophilized. Gel filtration on 100 × 2.5 cm Sephadex G-15 with 5% (v/v) aqueous HOAc was generally sufficient. Final purification has been achieved by RP-HPLC with a gradient of 10–30% acetonitrile and 0.1% aqueous TFA and a Vydac C_{18} column. Total yield of **4** was 12%. Amino acid analysis was performed on either a Beckman Model 120C or a Beckman 7300 amino acid analyzer, after acid hydrolysis in sealed tubes with 4 M methanesulfonic acid for 24 h. Amino acid analysis: Asn 0.95 (1.0), Thr 1.04 (1.00), D-Tyr 0.98 (1.00), Phe 1.03 (1.00). The structure was confirmed by ^{1}H NMR assignments. Integration: D-Tyr(Ar) 4 H (1.00), Cys(α) 0.97 H (1.00), Pen(α) 0.98 H (1.00). the TLC, analytical HPLC, and FAB-MS data are given in Table III.

D-Phe-Cys-Tyr-Asn-Pen-Thr-NH$_2$ (**5**). The protected peptide resin of **5** was prepared by sequentially coupling N^α-Boc-Pen(*S*-4-pMeBzl), N^α-Boc-Asn-ONP, N^α-Boc-Tyr(*O*-2,6-Cl$_2$Bzl), N^α-Boc-Cys(*S*-4-pMeBzl) and N^α-Boc-D-Phe to (H)-Thr(*O*-Bzl)-pMBHA, resulting in D-Phe-Cys(*S*-4-pMeBzl)-Tyr(*O*-2,6-Cl$_2$Bzl)-Asn-Pen-(*S*-4-pMeBzl)-Thr(*O*-Bzl) peptide resin.

The workup was identical with that described for **4**. The yield was 13%. Amino acid analysis: D-Phe 0.98 (1.0), Tyr 0.97 (1.0), Asn 1.01 (1.00), Thr 0.98 (1.00). Additionally, a ^{1}H NMR spectrum confirmed the amino acid constitution and relative abundance: Tyr(Ar) 4 H (1.00), Cys(α), 0.98 H (1.00), Pen(γ) 6.3 H (1.00). The TLC, HPLC, and FAB-MS data are presented in Table III.

D-Phe-Cys-Tyr-D-Trp-Thr-D-Pen-Thr-NH$_2$ (**6**). The protected peptide resin of **6**, D-Phe-Cys(*S*-4-MeBzl)-Tyr-(*O*-2,6-Cl$_2$Bzl)-D-Trp-Thr(*O*-Bzl)-D-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl) was obtained by sequential coupling of N^α-Boc-Thr(*O*-Bzl), N^α-Boc-D-Pen(*S*-4-MeBzl), N^α-Boc-Thr(*O*-Bzl), N^α-Boc-D-Trp, N^α-Boc-Tyr(*O*-2,6-Cl$_2$Bzl), N^α-Boc-Cys(*S*-4-MeBzl), and N^α-Boc-D-Phe to pMBHA resin.

Workup was as for **4** to give the title peptide **6**. The yield was 16%. Amino acid analysis: D-Phe 0.98 (1.00), Tyr 1.01 (1.00), Thr 1.91 (2.00). The structure of **6** was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), Cys(α) 1.10 H (1.00), Pen(α) 1.09 H (1.00), D-Trp(C5H) 0.98 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

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D-Phe-Cys-Tyr-D-Trp-Lys-D-Pen-Thr-NH_2 (7). The same scheme as above was applied, except that N^{α}-Boc-Lys(N^{ϵ}-2-ClZ) was coupled instead of N^{α}-Boc-Thr(*O*-Bzl) in the 5-position, resulting in D-Phe-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-D-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl) on the resin.

Workup was as for **4** to give the title peptide **7**. The yield was 16%. Amino acid analysis: Thr 1.05 (1.00), Tyr 0.98 (1.00), D-Phe 0.95 (1.00), Lys 1.05 (1.00).

The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), Cys(α) 0.97 H (1.00), Pen(α) 0.99 H (1.00), D-Trp(Ar C4H) 1.00 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (TCTP, 8). D-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (D-Tic) was prepared by condensation of D-Phe and formaldehyde[33] with a yield of 78.5%; the melting point, 309–311 °C, was determined with a Thomas-Hoover capillary apparatus and is uncorrected. N^{α}-Boc-D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (N^{α}-Boc-D-Tic) was prepared by a standard α-amino acid protection scheme[29] in a yield of 63.2%; mp 121–122 °C; $[\alpha]^{25}_D$ −18.1° (*c* 1.0, CH_3OH); ^{1}H NMR (250 MHz, [$^2H_6$]DMSO) of N^{α}-Boc-D-Tic 13.0 ppm (br, ^{1}H, acid), 7.13 (m, 4 H, aromat), 4.84 (m, 0.5 H, $C^{\alpha}H$), 4.5 (m, 2.5 H, 0.5 $C^{\alpha}H$ + NCH_2), 3.1 (m, 2 H, $C^{\beta}H_2$), 1.39 and 1.46 (both singlets, 9 H, *t*-Bu); TLC of N^{α}-Boc-D-Tic R_f 0.83 (AcOH/*n*-BuOH/Py/H_2O, 20/10/3/5, Baker silica gel plates), R_f 0.64 ($CHCl_3$/MeOH/AcOH, 94/4/2, Baker silica gel plates).

Subsequent coupling and deprotecting of N^{α}-Boc-Thr(*O*-Bzl), N^{α}-Boc-Pen(*S*-4-MeBzl), N^{α}-Boc-Thr(*O*-Bzl), N^{α}-Boc-Lys(N^{ϵ}-2-ClZ), N^{α}-Boc-D-Trp, N^{α}-Boc-Tyr(*O*-2,6-Cl_2-Bzl), N^{α}-Boc-Cys(*S*-4-MeBzl), and N^{α}-Boc-D-Tic gave D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2-Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl)-pMBHA peptide resin. Workup as for **4** gave the title peptide **8**. The yield was 15%. Amino acid analysis: Lys 1.00 (1.00), Tyr 1.00 (1.00), Thr 1.92 (2.00). The structure was confirmed by extensive 1D and 2D ^{1}H NMR analysis,[19] integration: Tyr(Ar) 4.00 H (1.00), Pen(α) 1.08 H (1.00), Cys(α) 1.06 H (1.00), D-Tic(β-CH_2) 2.13 H (2.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (TCTOP, 9). The title compound was synthesized in analogy to TCTP (**8**), except that N^{α}-Boc-Orn(N^{δ}-Z) was used instead of N^{α}-Boc-Lys(N^{ϵ}-2-ClZ) in the coupling scheme to give D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Orn(N^{δ}-Z)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl) peptide resin. The workup as for **4** gave the title peptide **9**. The yield was 17%. Amino acid analysis: Tyr 1.05 (1.00), Thr, 1.93 (2.00), Orn 0.99 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), D-Trp(Ar C4H) 0.97 (1.00), Cys(α) 1.03 (1.00), Pen(α) 1.08 (1.00), D-Tic (β-CH_2) 2.16 (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (TCTAP, 10). The title compound was prepared the same way as TCTP (**8**), except that N^{α}-Boc-Arg(N^{G}-Tos) was coupled instead of N^{α}-Boc-Lys(N^{ϵ}-2-ClZ) in the coupling scheme to give D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Arg(N^{G}-Tos)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl)-resin. The workup as for **4** gave the title peptide **10**. The yield was 13%. Amino acid analysis: Tyr 1.02 (1.00), Arg 0.96 (1.00), Thr 1.90 (2.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), D-Trp(Ar C4H), 1.12 H (1.00), Pen(α) 1.10 H (1.00), Cys(α) 1.03 H (1.00), D-Tic(β-CH_2) 2.08 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-*N*-Me-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (11). N^{α}-Boc-D-*N*-Me-Phe was synthesized as described previously.[34] The title peptide was synthesized the same way as TCTOP (**9**), except N^{α}-Boc-D-*N*-Me was used instead of N^{α}-Boc-D-Tic in the coupling scheme. The workup as for **4** gave the title peptide **11**. The yield was 18%. Amino acid analysis: Tyr 0.99 (1.00), Orn 1.00 (1.00), Thr 1.98 (2.00). The structure was confirmed by extensive 1D and 2D ^{1}H NMR analysis,[9] integration: D-*N*-Me-Phe(α) 1.00 H (1.00), Cys(α) 1.00 H (1.00), D-Trp(Ar C4H) 0.98 H (1.00), Pen(α) 1.00 H (1.00), Tyr (4H Ar) 4.00 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (12). The title compound **12** was obtained in a manner analogous to TCTOP (**9**), except that, after coupling of N^{α}-Boc-D-Tic and deprotection, N^{α}-Boc-Gly was coupled and deprotected. The extent of this reaction was monitored by the chloranil test.[30] The protected peptide resin obtained, Gly-D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Orn(N^{δ}-Z)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl)-resin underwent similar workup as **4**, giving the title peptide **12**. The yield was 11%. Amino acid analysis: Gly 1.01 (1.00), Tyr 0.96 (1.00), Orn 1.04 (1.00), Thr 1.98 (2.00). The structure was confirmed by 1D and 2D ^{1}H NMR analysis,[24] integration: Tyr (4H Ar) 4.00 H (1.00), Cys(α) 1.00 H (1.00), Pen(α) 1.03 H (1.00), D-Trp(C4H Ar) 0.98 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Trp-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (13). The title compound was synthesized in a manner analogous to that for **8**, except N^{α}-Boc-D-Trp was used instead of N^{α}-Boc-D-Tic in the coupling scheme, giving D-Trp-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Thr-(*O*-Bzl) peptide resin. The workup as for **4** gave the title peptide **13**. The yield was 16.7%. Amino acid analysis: Thr 1.95 (2.00), Tyr 0.97 (1.00), Lys 1.03 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), Cys(α) 0.94 H (1.00), Pen(α) 1.03 H (1.00), D-Trp(β-CH_2) 1.88 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Tic-Cys-Tyr-D-Trp-Lys-Pen-Thr-NH_2 (14). The protected peptide resin of **14** was obtained as for TCTP (**8**) except for N^{α}-Boc-Lys(N^{ϵ}-2-ClZ) was coupled to Pen(*S*-4-pMeBzl)-Thr(*O*-Bzl)-resin, without coupling of N^{α}-Boc-Thr(*O*-Bzl). The obtained D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Lys-(N^{ϵ}-2-ClZ)-Pen(*S*-4-MeBzl)-Thr(*O*-Bzl)-resin was worked up as **4** to give **14**. The yield was 13%. Amino acid analysis: Lys 1.01 (1.00), Tyr 1.03 (1.00), Thr 0.93 (1.00). The structure was confirmed by ^{1}H NMR analysis: Tyr(Ar) 4.00 H (1.00), Cys(α) 1.03 H (1.00), Pen(α) 1.05 H (1.00), D-Tic(Ar) 4.20 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-NH_2 (15). The peptide resin of D-Phe-Cys-(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2-Bzl)-D-Trp-Orn(N^{δ}-Z)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl) was obtained by similar coupling scheme as for TCTOP, **9**, except that N^{α}-Boc-D-Phe was used instead of N^{α}-Boc-D-Tic, as well as that N^{α}-Boc-Pen(*S*-4-MeBzl) was directly coupled to the resin without coupling of N^{α}-Boc-Thr(*O*-Bzl). The workup as for **4** gave the title peptide **15**. The yield was 14.8%. Amino acid analysis: D-Phe 0.99 (1.00), Tyr 0.99 (1.00), Thr 0.95 (1.00), Orn 1.01 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), D-Trp(C4H) 0.93 H (1.00), Cys(α) 0.98 H (1.00), Pen(α) 1.00 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-NH_2 (16). This peptide was synthesized as **15**, except N^{α}-Boc-Lys(N^{ϵ}-2-ClZ) was used instead of N^{α}-Boc-Orn(N^{δ}-Z) in the coupling scheme, giving D-Phe-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2-Bzl)-D-Trp-Lys-(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-resin. The workup as for **4** gave the title peptide **16**. The yield was 16.3%. Amino acid analysis: Tyr 1.02 (1.00), D-Phe 0.98 (1.00), Lys 1.05 (1.00), Thr 0.95 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), Pen(γ) 6.40 H (1.00), Cys(α) 0.97 H (1.00), D-Trp(C4H) 0.94 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Asn-NH_2 (17). The title compound was synthesized similarly to the synthetic scheme for 1,[16] except that N^{α}-Boc-Asn was coupled to the resin, instead of N^{α}-Boc-Thr(*O*-Bzl), giving D-Phe-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2-Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Asn-resin. The workup as for **4** gave the title peptide **17**. The yield was 15%. Amino acid analysis: Asn 1.02 (1.00), Thr 0.99 (1.00), Tyr 1.02 (1.00), Lys 1.02 (1.00), D-Phe 1.00 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Lys(α) 1.00 H (1.00), Pen(γ) 6.00 H (1.00), D-Trp(Ar C4H) 0.99 H (1.00), Thr(γ)

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3.10 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Asp-NH_2 (18). This peptide was synthesized according to the synthetic scheme of **17**, except that N^{α}-Boc-Asp(β-Bzl) was used instead of N^{α}-Boc-Asn, giving D-Phe-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2-Bzl)-D-Trp-Lys-(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Asp(β-Bzl)-resin. The workup as for 4 gave the title peptide **18**. The yield was 13.4%. Amino acid analysis: Lys 0.99 (1.00), D-Phe 1.00 (1.00), Tyr 1.01 (1.00), Thr 0.95 (1.00), Asp 1.01 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), D-Trp(Ar) 5.30 H (1.00), Cys(α) 0.95 H (1.00), Pen(α) 1.08 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Val-NH_2 (19). The title peptide was synthesized as TCTP (**8**), except that N^{α}-Boc-Val was coupled to the resin instead of N^{α}-Boc-Thr(*O*-Bzl) in the original scheme, yielding D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Val-resin. The workup as for 4 gave the title peptide **19**. The yield was 12.7%. Amino acid analysis: Lys 1.06 (1.00), Tyr 0.94 (1.00), Thr 0.92 (1.00), Val 0.98 (1.00). The structure was confirmed by ^{1}H NMR analysis, Tyr(Ar) 4.00 H (1.00), D-Tic (β-CH_2) 2.09 H (1.00), Pen(α) 1.05 H (1.00), Cys(α) 0.97 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2 (20). The title peptide **20** was synthesized like **17**, except that N^{α}-Boc-Ser(*O*-Bzl) was used instead of N^{α}-Boc-Asn in the coupling scheme, giving D-Phe-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Ser(*O*-Bzl)-resin. The workup as for 4 gave the title peptide **20**. The yield was 16.3%. Amino acid analysis: Lys 1.02 (1.00), D-Phe 0.98 (1.00), Tyr 1.04 (1.00), Ser 0.95 (1.00), Thr 0.94 (1.00). The structure was confirmed by ^{1}H NMR analysis, integration: Tyr(Ar) 4.00 H (1.00), Pen(α) 0.97 H (1.00), Cys(α) 1.05 H (1.00), D-Trp(C4H) 0.97 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Ser-NH_2 (21). This peptide was synthesized similarly to **19**, except that N^{α}-Boc-Ser(*O*-Bzl) was coupled to the resin instead of N^{α}-Boc-Thr(*O*-Bzl), giving D-Tic-Cys(*S*-4-MeBzl)-Tyr(*O*-2,6-Cl_2Bzl)-D-Trp-Lys(N^{ϵ}-2-ClZ)-Thr(*O*-Bzl)-Pen(*S*-4-MeBzl)-Ser(*O*-Bzl)-resin. The workup as for **4** gave the title compound **21**. The yield was 14.8%. Amino acid analysis: Thr 0.95 (1.00), Ser 0.95 (1.00), Tyr 1.00 (1.00), Lys 1.08 (1.00). The structure was confirmed by ^{1}H NMR analysis: Tyr(Ar) 4.00 H (1.00), D-Trp(C4H) 0.97 H (1.00), Cys(α) 0.92 H (1.00), Pen(α) 0.94 H (1.00). The TLC, HPLC, and FAB-MS data are given in Table III.

Biological Assays. Antagonist Activity Determination in the Guinea Pig Ileum (GPI). Male Hartley guinea pigs (200–500 g), were sacrificed, and the distal small bowel was removed. The longitudinal muscle–myenteric plexus of the nonterminal ileum was removed and cut into 1–2-cm segments. Segments were suspended in 20-mL organ baths containing warmed (37 °C) Krebs–bicarbonate buffer, which was continuously bubbled wth 95% O_2/5% CO_2. Tissues were attached to isometric force transducers, equilibrated for 10 min, stretched to 1 g tension (L_0), and allowed to equilibrate for another 10 min. Tissues were then stimulated transmurally between platinum plate electrodes at 0.1 Hz, supramaximal voltage, and 0.4-ms pulses. Stimulated twitch contractions were recorded on multichannel recorders. Each analogue was tested for its ability to inhibit electrically stimulated contractions (i.e., tested for agonist activity) and to antagonize the inhibitory effects of PL017 (μ agonist) in the GPI (tested for antagonist activity). Multiple agonist dose–response curves were obtained in the absence and in the presence of multiple concentrations of antagonistic compounds for calculation of pA_2 values.[35]

Radioreceptor Binding Assays. Adult male Sprague–Dawley rats (200–250 g) were sacrificed and the brains were immediately removed and placed on ice. Whole brain minus cerebellum was homogenized with a Polytron homogenizer (Brinkman, setting 5, 15 s). The homogenate was preincubated at 25 °C for 30 min to remove endogenous opioids and centrifuged two times at 43000*g* for 10 min before use in the radioreceptor binding assay.

[^{3}H]DPDPE (33.6 Ci/mmol, New England Nuclear, Boston, MA) and [^{3}H]CTOP (84.2 Ci/mmol, New England Nuclear, Boston, MA) binding was measured by a rapid filtration technique. A 100-μL aliquot of rat brain homogenate (0.5% final) was incubated with either 1.0 nM [^{3}H]DPDPE or 0.5 nM [^{3}H]CTOP in a total volume of 1 mL of 50 mM Tris-HCl (pH 7.4 at 25 °C) containing 5 mM $MgCl_2$, bovine serum albumin (1 mg/mL) and phenylmethanesulfonyl fluoride (100 μM). All binding measurements were done in duplicate, and the binding displaced by 1 μM naloxone hydrochloride was defined as specific tissue binding. Steady-state binding experiments were carried out at 25 °C for 120 min. The binding reaction was terminted by rapid filtration of samples through GF/B Whatman glass fiber filter strips pretreated with 0.1% polyethylenamine solution with a Brandel cell harvester: this was followed immediately by three rapid washes with 4-mL aliquots of ice-cold saline solution. Filters were removed and allowed to dry before the filter bound radioactivity was assayed by liquid scintillation spectrophotometry.

The data were analyzed by using nonlinear least-squares regression analysis on the Apple II$^+$ computer. Programs were generously provided by SHM Research Corp., Tucson, AZ.

The relative binding affinity of the analogues for somatostatin binding sites was measured by competitive inhibition studies using [^{125}I]CGP 23,996 prepared as described by Czernik and Petrac.[36] Binding assays were carried out with membranes prepared from whole brains of adult male Sprague–Dawley rats at a final tissue concentration of 1.0%. The assay buffer consisted of 50 mM Tris (pH 7.4) containing 2.0 mg/mL BSA, 20 μg/mL bacitracin, and 5 mM magnesium chloride. Analogues were tested over a concentration range of 100–0.3 μM against 0.2 nM [^{125}I]CGP 23,996 in a final incubate volume of 1.0 μL. Incubation was continued over a period of 1 h at 37 °C before being terminated by rapid filtration through Whatman GF/B filter soaked in 5 mg/μL BSA to prevent filter binding. The filtrates were washed three times with 4.0-mL volumes of ice-cold saline, and the retained radioactivity was measured with a Packard Auto-Gamma 5650 gamma counter having an efficiency of about 70%.

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Registry No. 1, 103335-28-0; **2**, 103429-31-8; **3**, 103429-32-9; **4**, 115962-12-4; 4 (reduced), 115981-83-4; **5**, 115962-13-5; **6**, 115962-14-6; **7**, 115962-15-7; **8**, 115981-69-6; **9**, 115981-70-9; **10**, 115981-71-0; 11, 115962-16-8; **12**, 115962-17-9; **13**, 115962-18-0; **14**, 115981-72-1; **15**, 115981-73-2; **16**, 115962-19-1; **17**, 115962-20-4; **18**, 115962-21-5; **19**, 115981-74-3; **20**, 115981-75-4; **21**, 115981-76-5; BOC-Thr(Bzl)-OH, 15260-10-3; BOC-Pen(4-MeBzl)-OH, 104323-41-3; BOC-Asn-OH, 7536-55-2; BOC-Phe-OH, 13734-34-4; BOC-Cys(4-MeBzl)-OH, 61925-77-7; BOC-D-Tyr(2,6-Cl_2Bzl)-OH, 69541-62-4; BOC-Asn-ONP, 4587-33-1; BOC-D-Phe-OH, 18942-49-9; BOC-D-Pen(4-MeBzl)-OH, 115962-34-0; BOC-D-Trp-OH, 5241-64-5; BOC-Lys(2-Clz)-OH, 54613-99-9; BOC-Orn(Z)-OH, 2480-93-5; BOC-Arg(Tox)-OH, 13836-37-8; BOC-D-*N*-Me-Phe-OH, 85466-66-6; BOC-Gly-OH, 4530-20-5; BOC-Asp(OBzl)-OH, 7536-58-5; BOC-Val-OH, 13734-41-3; BOC-Ser(Bzl)-OH, 23680-31-1; BOC-D-Tic-OH, 115962-35-1; H-D-Tic-OH, 103733-65-9; H-D-Phe-OH, 673-06-3; BOC-Tyr(2,6-Cl_2Bzl)-OH, 40298-71-3.

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