morning period are expressed as mean (±SEM) dose required to suppress intakes approximately 50% compared to vehicle, as determined by least squares regression analysis.

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Registry No. 1, 112882-78-7; 2, 112882-74-3; 3, 141982-54-9; 4, 127902-33-4; 5, 141982-55-0; 6, 141982-56-1; 7, 127902-34-5; 8, 141982-57-2; 9, 142003-24-5; 10, 142003-25-6; 11, 141982-58-3; 12, 141982-59-4; 13, 141982-60-7; 14, 141982-61-8; 15, 141982-62-9; 16, 141982-63-0; 17, 142003-26-7; 18, 141982-64-1; 19, 141982-65-2; 20, 6667-38-5; 21, 15368-43-1; 22, 104161-05-9; 23, 141982-66-3; 24, 142003-27-8; 25, 142184-11-0; 26, 142036-60-0; 27, 131451-30-4; 28, 141408-45-9; 29, 142003-28-9; 30, 141982-67-4; 31, 141982-69-6; 32, 141982-70-9; 33, 131450-72-1; 34, 134676-15-6; 35, 134675-58-4; 36, 141982-71-0; H-Nle-Asp-Phe-NH_2, 15373-76-9; Boc-Trp-OSu, 3392-11-8; Boc-Nle-OH, 6404-28-0; TSOH·H-Gly-OBn, 1738-76-7; Fmoc-Asp(OBn)-OH, 86060-84-6; H-Phe-NH_2, 5241-58-7; TFA·H-Asp⇄OBn)-Phe-NH_2, 60058-91-5; HCl·Asp(OBn)-(*N*-Me)Phe-NH_2, 131450-72-1; H-(NMe)Phe-NH_2, 17193-30-5; Boc-3PP-OH, 123724-22-1; *d,l*-β-phenylserine, 69-96-5; di-*t*-butyl dicarbonate, 24424-99-5; amylase, 9000-92-4; 3-(4-hydroxyphenyl)propionic acid, 501-97-3; (*R*)-3-[(*tert*-butyloxycarbonyl)amino]-2-oxo-1-pyrrolidineacetic acid, 78444-90-3.

Analogs of the δ Opioid Receptor Selective Cyclic Peptide [2-D-Penicillamine,5-D-penicillamine]-enkephalin: 2′,6′-Dimethyltyrosine and Gly³-Phe⁴ Amide Bond Isostere Substitutions

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In order to develop systemically-active opioid peptides, the δ-selective, opioid pentapeptide [D-Pen²,D-Pen⁵]-enkephalin (DPDPE) was modified by esterification and by substitution of 2′,6′-dimethyltyrosine for tyrosine to yield 4. Compound 4 was on the order of 8- and 800-fold more active than DPDPE in both δ and μ opioid radioligand binding assays, respectively, in rat neural membrane suspensions. Compound 4 was considerably more potent than DPDPE at inhibiting contractions of electrically-stimulated mouse vas deferens in vitro, and this effect was very sensitive to naltrindole, a δ-selective opioid antagonist. These observations can be taken as indication that 4 exerts its effects through δ opioid receptors. This interpretation is supported by the finding that the EC_{50} value of 4 derived in the smooth muscle assay is very similar to that derived in NG108-15 neuroblastoma cells, a preparation devoid of μ receptors. Unlike DPDPE, 4 exhibited significant, naloxone-sensitive, antinociceptive activity when administered systemically, as measured by inhibition of phenylbenzquinone-induced stretching in mice (ED_{50} = 2.1 mg/kg). Compound 4 also displayed significant antinociceptive activity following systemic administration as measured by its action in mice to increase latencies for tail withdrawal from radiant heat (ED_{50} = 50 mg/kg). Compound 4 did not produce morphine-like discriminative stimulus effects in rats trained to discriminate 3.0 mg/kg morphine from vehicle at doses ranging from 30 to 120 mg/kg. This observation can be interpreted as indication that within this dosage range there is an absence of morphine-like subjective effects. Physical dependence, however, could be induced in mice at higher doses of 4 under a progressively-graded, 4-day dose regimen. Congeners of 4 with amide bond surrogates for the Gly-Phe amide bond (oxymethylene, trans-double bond, and bismethylene isosteres) in the cyclic core of DPDPE were prepared in an attempt to increase the antinociceptive activity of 4. While some of the congeners were active in the in vitro assays, they did not display significant antinociceptive activity following systemic administration. The preparation of all the compounds was accomplished by solution-phase methods. The mechanisms which might underlie the biological and systemic activity of 4 are discussed.

Introduction

The discovery of endogenous pentapeptide brain opioids, the enkephalins,[1] led to much excitement in the arena of the medicinal chemistry of pain.[2,3] The activity was fueled by the possibility that analogs of these relatively simple linear peptides might yield safer analgesics than narcotic alkaloids typified by morphine. While many enkephalin congeners have been described, only a handful have undergone clinical trials. None of these has yet advanced into the clinician's armamentarium against pain.[4-6] An important result of this work was the recognition that a δ opioid receptor type, along with the μ and κ opioid receptor types, could mediate analgesia in mammals.[7-10] Evidence is emerging that analgesia elicited through δ opioid receptors is associated with a more benign side effect profile than analgesia elicited through other opioid receptors.[11]

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§ Cellular Pharmacology Laboratory.

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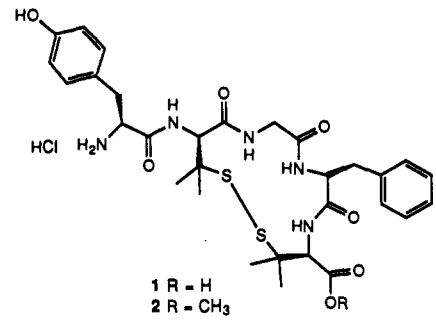
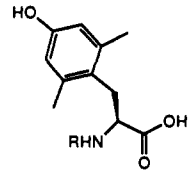
Although several δ-selective peptides have been synthesized and characterized, their utility as analgesics for human pain is limited by their lack of systemic activity. The best known of these is [D-Pen^2,D-Pen^5]-enkephalin (DPDPE, 1),[12] a δ opioid cyclic pentapeptide agonist having selectivity on the order of 100-fold for δ receptors over μ receptors in radioligand binding assays. Substitution of para-halogenated phenylalanine for phenylalanine in 1 has resulted in even more selective δ receptor ligands.[13]

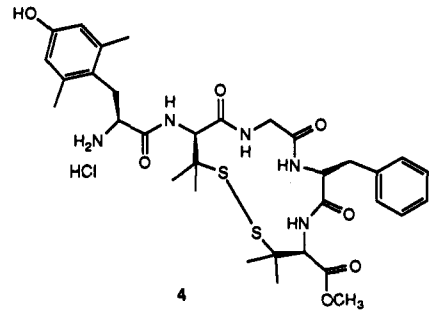
Recently, we initiated a program to develop systemically active, δ-selective analgesics, including analogs of 1. It has been reported that replacement of the N-terminal tyrosine by 2′,6′-dimethyl-L-tyrosine (DMT) (3) in diverse series of enkephalin analogs yielded compounds with significantly enhanced potency and antinociceptive activity.[14] When we introduced this modification into 2, the methyl ester of 1, the resulting compound 4 showed promising systemic activity (vide infra). There have been many attempts to improve the activity of peptide hormones and drugs by replacing a peptide bond with a non-amide function. Analogs containing amide bond surrogates have frequently been utilized to investigate aspects of peptide structure and function, including rotational freedom in the backbone, intra- and intermolecular hydrogen-bond patterns, and modifications of local and total polarity and hydrophobicity, as well as oral bioavailability.[15,16] Our series seemed to offer an excellent opportunity for such modifications. In a medium to large ring, substitution for one peptide bond would not be expected to drastically perturb molecular conformation. Thus, we could expect to preserve biological activity and improve in vivo stability.



1 R = H
2 R = CH_3



3 R = H
8 R = Boc



4

Herein we describe the preparation and pharmacological activities of compounds **5A** and **5B**, **6A** and **6B**, and **7A** and **7B**, which contain the oxymethylene, trans-double bond, and bismethylene isosteres for the Gly-Phe amide bond in the cyclic core of 1. The compounds are notable as these particular isosteres for the Gly-Phe amide bond have hitherto not appeared in the literature.[15-20]

The Gly-Phe amide bond was chosen for manipulation because it is the most susceptible to proteolysis in the natural enkephalins. DPDPE has a very special structure from the standpoint of enzymatic degradation. Configurationally, the amino acids alternate L, D, Gly, L, D. Since D-amino acids tend to stabilize both the amino and carboxyl linkages to that amino acid, inspection of DPDPE suggests that, in vivo, by far the most labile amide bond is that between glycine and phenylalanine. The particular peptide mimetics (CH_2O, *trans*-CH=CH, CH_2CH_2) were selected for several reasons. They avoid introduction of highly charged and chemically reactive groups (e.g., CH_2NH, $COCH_2$) which might be expected to disrupt in-

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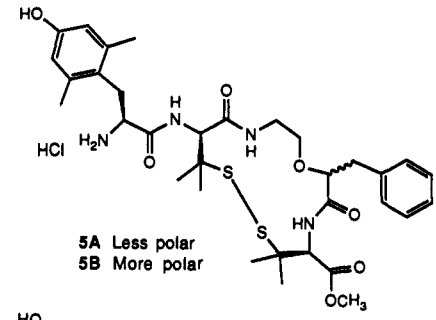
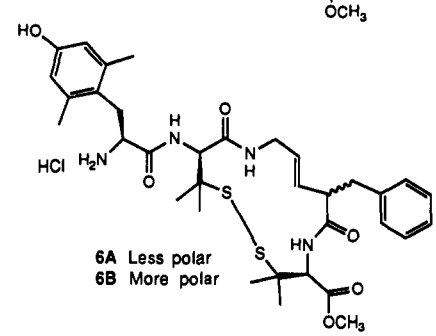
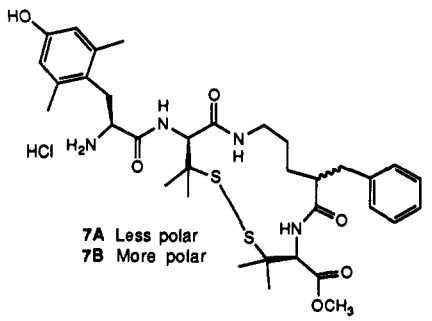






teraction between ligand and the receptor. Also, the trans-double bond is the preferred mimetic on steric grounds, since a peptide bond normally adopts the trans-configuration.

Chemistry

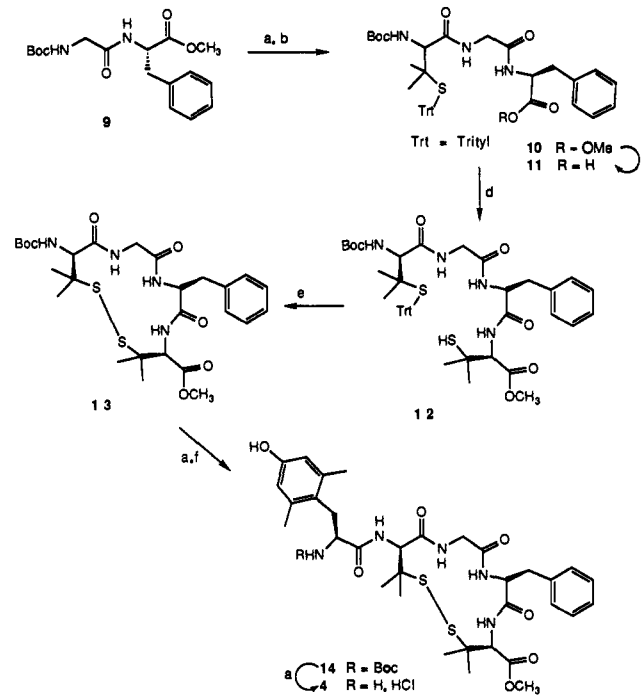
The unnatural amino acid L-2',6'-dimethyltyrosine (3)[14] used in this study showed an enantiomeric excess of 95%–96% by chiral HPLC analysis. A mixed-anhydride coupling procedure was used for forming the amide bonds. Deprotection of the Boc-protected amino group was carried out using anhydrous HCl in CH_2Cl_2–dioxane. The amine-HCl salt was used in the next step without prior purification.

Scheme I outlines the preparation of 4. Boc-Gly-L-Phe-OMe (9) was N-deprotected and coupled to *N*-Boc-S-trityl-D-Pen to give 10, which was treated with lithium hydroxide to cleave the methyl ester group. The resulting acid 11 was reacted with D-Pen-OMe to give the tetrapeptide 12. A dilute solution of 12 in 80% aqueous acetic acid, when treated with an equally dilute solution of iodine in 80% aqueous acetic acid, smoothly cyclized to give the cyclic tetrapeptide derivative 13. Removal of the Boc group in 13, followed by coupling to 8 and deprotection, yielded 4, the target compound.

Analogs **5A** and **5B**, **6A** and **6B**, and **7A** and **7B** were prepared by procedures similar to those used for the preparation of 4. The required isosteres for the Gly-Phe amide bond were prepared in racemic form as described below.

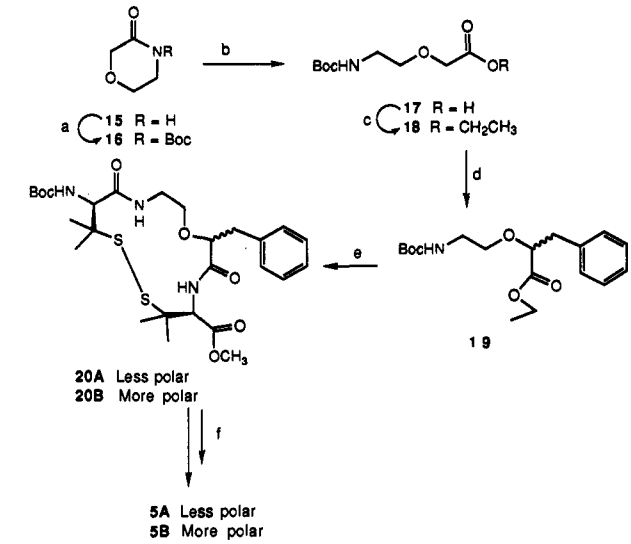
Recently two general methods for preparing oxymethylene isosteres of peptide bonds have been described.[17] We were unable to adapt these methods for preparation of the oxymethylene isostere of the Gly-Phe

Scheme I[a]



[a] Reagents: (a) 3.5 N HCl, dichloromethane/dioxane; (b) *N*-Boc-S-Trt-D-PenOH, NMM (*N*-methylmorpholine), isobutyl chloroformate (IBCF); (c) LiOH; (d) (i) NMM, (ii) IBCF, (iii) D-Pen-OMe; (e) I_2 in 80% aqueous acetic acid; (f) 8, NMM, IBCF then N-deprotected 13.

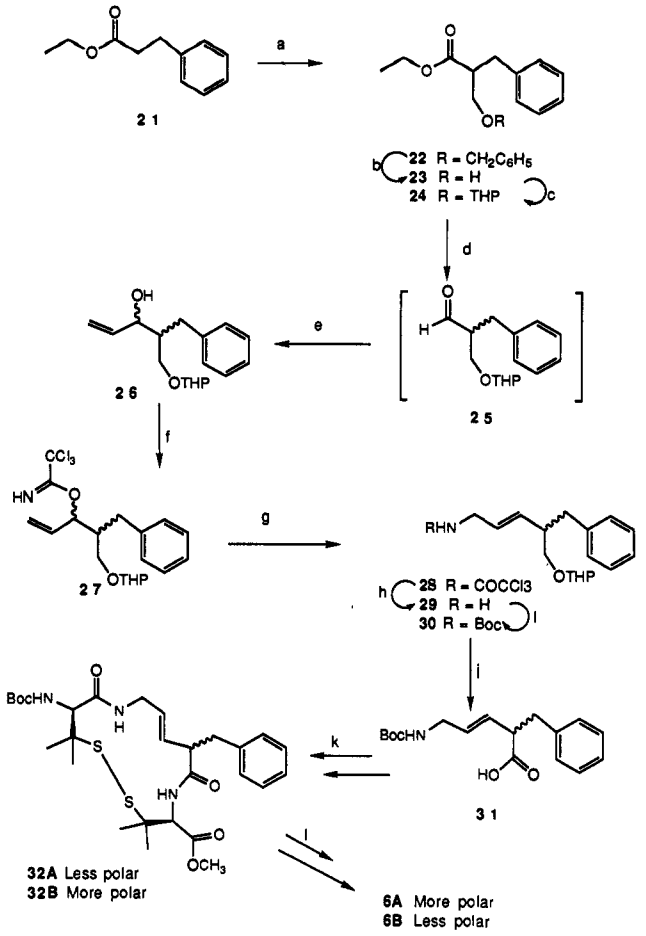
Scheme II[a]



[a] Reagents: (a) (i) *n*-butyllithium, (ii) $(Boc)_2O$; (b) LiOH; (c) ethyl iodide, K_2CO_3, DMF; (d) (i) lithium bis(trimethylsilylamide), (ii) benzyl bromide; (e and f) see Experimental Section.

amide bond. Scheme II shows the sequence of reactions that gave 19, the required derivative of the isostere. We originally planned to alkylate 16 with benzyl bromide and open the lactam ring with methoxide; however, this could not be done, since the lithium enolate of 16 gave only the product of dialkylation on treatment with benzyl bromide. Treatment of 16 with (ostensibly dry) lithium ethoxide, followed by workup, gave mainly the corresponding acid 17 with only a trace of the expected ester 18. Enolate alkylation of 18, obtained by esterification of 17 with benzyl bromide, gave a 3:1 mixture of the dialkylated (less polar) and monoalkylated esters. These were separated by chromatography to give Boc-Glyψ[CH_2O]Phe-OEt (19).

Scheme III[a]

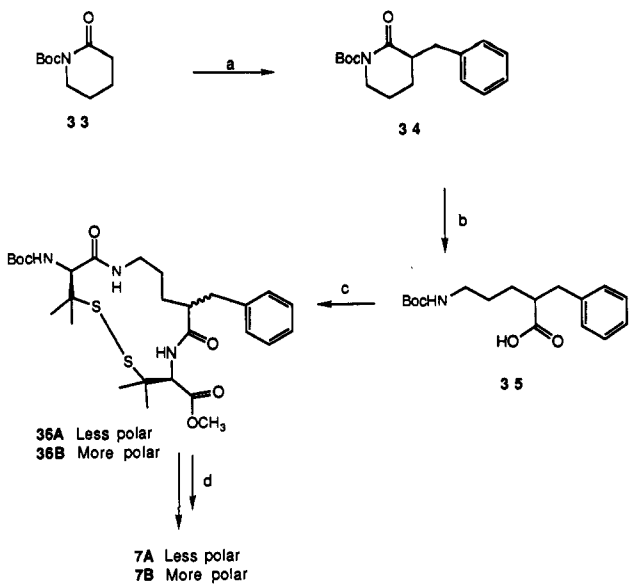


[a] Reagents: (a) (i) lithium diisopropylamide, (ii) benzyl chloromethyl ether; (b) H_2/Pd–C; (c) dihydropyran, pyridinium *p*-toluenesulfonate; (d) diisobutylaluminum hydride; (e) vinylmagnesium bromide; (f) trichloroacetonitrile, NaH; (g) xylene, reflux; (h) 6 N NaOH; (i) $(Boc)_2O$/trace 4-(dimethylamino)pyridine; (j) Jones reagent; (k and l) see Experimental Section.

Preparation of the trans-double bond isostere of the Gly-Phe amide bond is outlined in Scheme III. The application of Julia olefin synthesis for the preparation of compounds similar to **30** in optically active form has been reported.[20] We used Overman's method for 1,3-transpositioning of alcohol and amine functions to obtain **30**.[21] Treatment of the lithium enolate of ethyl hydrocinnamate (**21**) with benzyl chloromethyl ether gave **22**, which was hydrogenolyzed to the hydroxymethyl compound **23**. The hydroxyl group of **23** was protected as the tetrahydropyranyl ether **24**. This material was then treated successively with DIBAL and vinylmagnesium bromide to produce allyl alcohol **26**. Compound **26** was transformed to the allyl amine **29** following Overman's procedure.[21] After protection of the amino function with a *tert*-butyloxycarbonyl group, the resulting compound **30** was subjected to Jones oxidation to generate Boc-Glyψ(*trans*-CH=CH)Phe-OH (**31**).

Scheme IV shows the preparation of bismethylene isostere **35**. Alkylation of the enolate derived from Boc-lactam **33** with benzyl bromide gave a mixture of the mono- and dialkylated products. The monoalkylated product **34** (more polar) was separated from the mixture by chromatography. The lactam ring of **34** was opened with lithium hydroxide to give Boc-Glyψ[CH_2CH_2]Phe-OH (**35**).

Scheme IV[a]



[a] Reagents: (a) (i) lithium bis(trimethylsilylamide), (ii) benzyl bromide; (b) LiOH; (c and d) see Experimental Section.

Table I. Potencies of DPDPE (1), DPDPE-OMe (2), DMT-DPDPE-OMe (4), and the Gly-Phe Amide Bond Isosteres of the Latter in μ ([^{3}H]DAMPGO) and δ ([^{3}H]DSLET) Opioid Radioligand Binding Assays

compd	[^{3}H]DAMPGO[a]	[^{3}H]DSLET[a]	μ/δ[b]
4	0.9 ± 0.2	0.9 ± 0.3	1
2	9.5 ± 0.2	2.5 ± 0.8	4
5A	6.5 ± 1.8	43 ± 11	0.15
5B	150 ± 28	91 ± 36	1.7
6A	32 ± 8	45 ± 7.0	0.7
6B	1.1 ± 0.25	2.3 ± 0.8	0.5
7A	176 ± 32	79 ± 14	2.3
7B	11 ± 2.3	21 ± 5	0.5
DPDPE (1)	700 ± 250	7 ± 2	100

[a] Average K_i ± SEM (nM) from at least three experiments. [b] Ratio of μ to δ K_i values, i.e., a measure of receptor selectivity.

Results and Discussion

Opioid binding affinities of the analogs were measured in rat forebrain membrane suspensions by their competition for specific binding sites for the μ selective radioligand [^{3}H]DAMPGO (Tyr-D-Ala-Gly-MePhe-Gly-ol) and the δ selective radioligand [^{3}H]DSLET (Tyr-D-Ser-Gly-Phe-Leu-Thr). Compound **4** showed the highest affinity at both μ and δ receptors (Table I). Relative to **1**, it was about 8-fold more active at the δ receptor and 800-fold more active at the μ receptor. Thus, while the binding affinities of **4** to μ and δ opioid receptors were increased relative to DPDPE, δ selectivity was lost due to a marked increase in μ potency. The methyl ester of DPDPE (**2**) was 3-fold more active than **1** at δ opioid binding sites, while its μ affinity was increased on the order of 70-fold. The analogues containing the amide bond isosteres were less active than **4**. Of the compounds which contained the trans-double bond, **6A** and the more polar **6B**, only the latter displayed affinity comparable with that of **4** in the radioligand binding assays, but not the functional assays (see below). Both isomers of the oxymethylene analog, **5A** and **5B**, were considerably less active than **4**, although the former was moderately active, and exhibited on the order of a 6-fold preference for μ receptors (Table I). Of the bismethylene analogs, **7A** and **7B**, only the latter (more

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Table II. Activities of DPDPE (1), DPDPE-OMe (2), DMT-DPDPE-OMe (4), and the Gly-Phe Amide Bond Isosteres of the Latter in Inhibiting Electrically-Induced Contractions of Mouse Vas Deferens

compd	EC_{50} (nM)[a]	compd	EC_{50} (nM)[a]
4	0.65 ± 0.03	6B	27.42 ± 5.70
2	10.5 ± 2.1	7A	>1000
5A	143 ± 17.53	7B	>1000
5B	>1000	DPDPE (1)	5.2 ± 1.2
6A	>1000		

[a] Concentration of compound that inhibited electrically-induced contractions of the mouse vas deferens by 50%. The method used was that of Smith et al.[23] except that tyrosine, ascorbic acid, and disodium edetate were omitted from the buffer. All assays were run at 37 °C. Values are means ±SD from two or three experiments (except DPDPE which represents an average from eight determinations).

polar) showed moderate, but roughly equal, affinity at μ and δ receptors.

The analogs were also examined for their functional activity in vitro, by their ability to inhibit electrically-induced contractions of the mouse vas deferens (MVD). In relative agreement with the radioligand binding data, **4** showed the highest activity—about 7 times better than that of DPDPE (Table II). Of the other analogs, only **5A** and **6B** showed activity in this assay, with greater activity residing in the latter. For the most part, the results in the smooth muscle assay are consistent with the radioligand binding data, although **7B** was inactive in the MVD but showed moderate affinity in the radioligand binding assays. It is possible that this compound is an antagonist, or a weak partial agonist, or that the δ receptors in brain and MVD differ subtly from one another.

In order to determine which receptors mediated the agonist actions of **1**, **2**, and **4** in the MVD, we examined their sensitivities to naltrindole, a δ-selective antagonist.[22] This compound appears as a potent and selective δ antagonist in the MVD preparation. The effects of **4** were much more sensitive to the δ-selective antagonist naltrindole compared with naltrexone. The dose–response curve obtained for **4** was shifted to the right over 10-fold by 10 nM naltrindole while 100 nM of naltrexone resulted in only a 2.2-fold shift. Further studies using standard methods[23] revealed that the pA_2 value for naltrindole against **4** was 9.8 ± 0.97. The pA_2 value for DPDPE and **2** were 8.8 and 9.6, respectively. The sensitivity of **4** to the δ-selective antagonist naltrindole indicates that **4** exerts a major portion of its actions through δ opioid receptors; however, it remained possible that the apparent greater potency of **4** compared to **2** and DPDPE in the MVD was due to a significant interaction with μ receptors. For example, in our hands the EC_{50} value in the MVD for DAMPGO—a relatively pure and potent μ agonist—was 38 ± 19 nM, a value which is comparable to that of DPDPE. We felt, therefore, that it would be useful to examine the activities of **1**, **2**, and **4** in inhibiting PGE_1-stimulated cAMP formation in NG108-15 cells. These cells contain δ receptors which are coupled to adenylate cyclase by an inhibitory guanine nucleotide regulatory protein. As far as is known, they contain no μ receptors, a notion we have confirmed in our own laboratory. We found, using standard methods,[24] that the EC_{50} values of **1**, **2**, and **4** in this assay were remarkably similar to the EC_{50} values obtained in the MVD (Table III). On the other hand DAMPGO was inactive in this preparation. These findings support our interpretation that the DMT moiety, not the methyl ester function, underlies the enhanced activity of **4** compared with DPDPE, and that this enhanced efficacy is δ opioid in nature.

In the writhing assay, the ED_{50} value for **4** following subcutaneous administration was 2.1 mg/kg (Table IV). The antinociceptive response was sensitive to the opioid antagonist naloxone; a dose of 1 mg/kg completely reversed the antinociceptive effect of **4**. Aside from **4**, none of the

Table III. Activities of DPDPE (1), DPDPE-OMe (2), and DMT-DPDPE-OMe (4) in Inhibiting cAMP Formation in NG108-15 Cells

compd	EC_{50} (nM)[a]	compd	EC_{50} (nM)[a]
4	1.1 ± 0.5	DPDPE (1)	18 ± 4
2	21 ± 11	DAMPGO	>3000

[a] Concentration of compound that inhibited PGE_1-stimulated cAMP formation by 50%. Values represent means ±SD from two separation determinations. The method used was one similar to that of Law et al.,[24] except that in the present studies radioimmunoassay methods were used to measure cAMP levels, rather than incorporation of radiolabeled substrate.

Table IV. Antinociceptive Effects of DPDPE (1), DPDPE-OMe (2), and DMT-DPDPE-OMe (4)

	writhing	tail flick	hot plate
DPDPE	*a*	ND[d]	ND
2	*a*	ND	ND
4	2.1 ± 1.5[b]	40 ± 15[b]	*c*

[a] Inactive at 30 mg/kg, the highest dose tested. [b] EC_{50} ± SE in mg/kg (sc), determined at 30 min postinjection. [c] Elicited consistent increases in average latencies to jump at 30 and 100 mg/kg at 10, 30, 60, and 120 min postinjection. The increases in the latencies were statistically significant ($p < 0.05$) at some, but not all, of the time points at a given dose. We were, therefore, unable to calculate statistically reliable EC_{50} values for the weak antinociceptive effect in the hot-plate test. [d] ND = not determined.

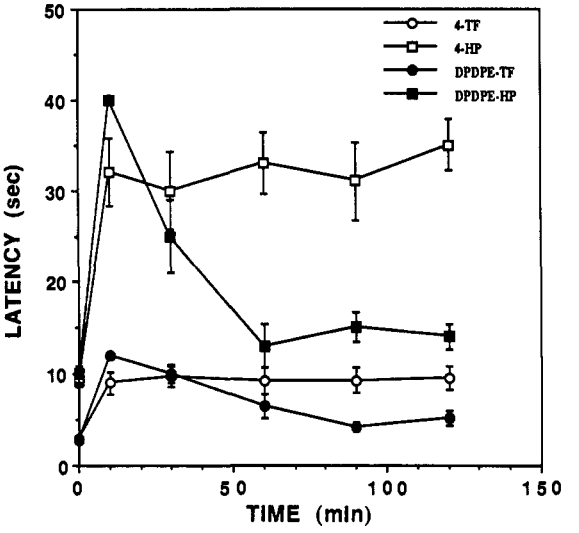

Figure 1. Effects of treatment with 10 μg of **4** or DPDPE administered intracerebroventricularly on tail-flick and hot-plate analgesic response latencies measured in the mouse at different times following administration. The control latencies were 2.8 ± 0.1 and 9.7 ± 0.7 s for the tail flick and hot plate tests, respectively. TF = tail-flick responses. HP = hot-plate responses.

(22) Portoghese, P. M.; 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 antagonist. *J. Med. Chem.* **1988**, *31*, 281–284.

(23) Smith, C. B.; Bennet-Kelly, L.; Woods, J. H. Comparison of Selective opiate receptor antagonists on the isolated mouse vas deferens. *Neuropeptides* **1984**, *5*, 161–164.

(24) Law, P. Y.; Hom, D. S.; Loh, H. H. Opioid receptor down regulation and desensitization in neuroblastoma X glioma hybrid cells are two separate cellular adaptation processes. *Mol. Pharmacol.* **1983**, *24*, 413–424.

analogs, including DPDPE and 2, were active enough for calculation of ED_{50} values in this test, and they received no further testing. The effects of 4 were dose-dependent in the tail-flick test following subcutaneous administration; the ED_{50} value was 40 mg/kg, although the dose–response curve was rather shallow (results not shown). In addition, 4 elicited potent and long-lasting antinociceptive effects in both the tail flick and hot plate tests following intracerebroventricular (icv) administration (Figure 1). We interpreted the latter finding as consistent with the in vitro data that 4 was a potent δ opioid agonist and sought to determine its abuse liability at doses which elicited analgesia following systemic administration.

Compound 4 was tested in rats which had been trained to discriminate 3.0 mg/kg sc morphine from water vehicle to determine whether it could produce discriminative stimulus (subjective) effects similar to those of morphine. This test is one preclinical predictor of abuse liability, and those drugs having morphine-like discriminative stimulus effects generally have a morphine-like potential for abuse. When the 3.0 mg/kg training dose of morphine was tested, 99% of all responses emitted by the group were made on the morphine-designated lever. When water, morphine's vehicle, or the cyclodextrin vehicle used for compound 4 was tested, 0.3 and 7% of the group's responses occurred on the morphine-lever, respectively. When compound 4 was tested from 30 to 120 mg/kg, no more than 17% of the group's responses occurred on the morphine-designated lever within the dose range tested, an observation which indicates a lack of morphine-like discriminative stimulus effects. Response rates were not markedly affected by compound 4; even at the highest dose tested (120 mg/kg), rates were only reduced to 1.34 responses/s compared to 1.71 responses/s when vehicle was administered.

Another preclinical predictor of abuse liability of a drug is whether it can induce physical dependence. When compound 4 was tested in mice, the opiate antagonist naloxone was able to precipitate withdrawal-like jumping in three of the five mice tested. The two mice not exhibiting jumping following the naloxone challenge appeared moribund before and after naloxone administration. These results indicate that compound 4 can induce physical dependence in mice when administered at very high doses.

In summary of the discrimination and dependence results, although the drug discrimination data in rats suggests that compound 4 may be free of morphine-like subjective effects up to 120 mg/kg, the results obtained in mice suggest that compound 4 does have a potential to induce physical dependence and would likely have a degree of abuse liability. One possible explanation of why compound 4 did not produce morphine-like discriminative stimulus effects, although it was able to induce physical dependence, may have been because the doses tested in the rat discrimination study were lower than those administered in the mouse dependence study. Because of solubility limitations and because high doses of compound 4 produced localized lesions at the site of subcutaneous injection, only doses up to 120 mg/kg were tested during the rat discrimination studies.

Since 4 showed promising systemic activity, we attempted to enhance its antinociceptive actions by modifying the potentially vulnerable Gly-Phe bond, as discussed in the Introduction. All of the modifications, however, led to the abolition of antinociceptive activity. Consistent with the in vivo findings, the structural modifications to 4, for the most part, adversely affected δ-like binding activity and functional activity in the MVD in vitro, as discussed above. Though compounds containing isosteric replacements for the Gly-Phe amide bond did show activity in the in vitro assays (**6B** and **5B**) they lacked in vivo activity, a finding which indicates that the elements of the amide bond are crucial for systemic antinociceptive activity.

The question arises as to the mechanism underlying the systemic antinociceptive activity of 4. The presence of the ester moiety in place of the carboxylic acid group in DPDPE had relatively little effect on activity as measured by the smooth muscle assays, although in the radioligand binding assays, this modification resulted in marked enhancement of μ affinity (Tables II and I, respectively). On the other hand, the substitution of DMT for Tyr enhanced the δ-like agonist activity of DPDPE in the radioligand binding, smooth muscle, and NG108-15 assay (Tables I–III, respectively). Furthermore, the agonist action of 4 in the smooth muscle assay was sensitive to naltrindole. These data are consistent with the interpretation that enhanced δ opioid intrinsic efficacy underlies the systemic activity of 4. Finally, the interpretation that 4 acts via a δ opioid mechanism in producing antinociception following systemic administration is consistent with the lack of morphine-like discriminative effects at doses of up to 120 mg/kg sc. However, other explanations can also be invoked to account for the systemic activity of 4. One mechanism could be that the DMT moiety alters the pharmacokinetics of DPDPE by mechanisms involving metabolism or biodistribution. A very recent report demonstrates that DPDPE is metabolically stable;[25a,b] therefore, greater metabolic stability alone is unlikely to underlie the systemic activity of 4. Alternatively, it may be that the modification of the tyrosyl group with 2,6-dimethyltyrosine improves the central bioavailability of 4 by a route not involving metabolism. The poor central nervous system (CNS) bioavailability of DPDPE most likely is the cause for its lack of systemic activity at the doses tested.[25a] It may be that the DMT group enhances penetration into the CNS and thus affords systemic antinociceptive activity. Finally, it may be that 4 elicits its antinociceptive activity by activation of μ receptors as well as δ receptors given that its affinity for [^{3}H]DAMPGO binding sites was considerably enhanced over DPDPE.

In summary, the results taken together demonstrate that 4 has enhanced δ opioid intrinsic efficacy in vitro and significant systemic activity in two different antinociceptive paradigms. This systemic activity is in contrast to DPDPE and its methyl ester, both of which are inactive. Furthermore, it appears that introduction of the DMT moiety in place of tyrosine in DPDPE is the essential structural feature which imparts systemic activity to 4.

Experimental Section

Details for peptide bond formation by mixed-anhydride coupling and oxidative cyclization to form the disulfide bond have been given only in the case of the pentapeptide 4. NMR spectra were determined in $CDCl_3$ (unless specified) on a GE 300-MHz instrument and are reported in δ units downfield from tetramethylsilane internal reference. The target compounds (HCL salts) on HPLC using solvents containing trifluoroacetic acid were converted to the trifluoroacetate salts. The NMR spectra were recorded for these trifluoroacetate salts of the target compounds on a Varian VXR 400-MHz instrument. These trifluoroacetate

(25) (a) Weber, S. J.; Greene, D. L.; Sharma, S. D.; Yamamura, H. I.; Kramer, T. H.; Burks, T. F.; Hruby, V. J.; Hersh, L. B.; Davis, T. P. Distribution and analgesia of [^{3}H][D-Pen^2,D-Pen^5]Enkephalin and two halogenated analogs after intravenous administration. *J. Pharmacol. Exp. Ther.* **1991**, *259*, 1109–1117. (b) We were unaware of this recent report on the central bioavailability and metabolic stability of DPDPE at the time the rationale for the isosteric substitutions was developed.

salts contained a small amount of the corresponding hydrochloride salts. The biological testing were carried out on the hydrochloride salts. The methyl groups of *t*-Boc and of D-Pen moieties in the ^{1}H NMR spectra of peptides did not appear as simple singlets, but a complex pattern showing proper relative integration was observed. These have been reported here as "complex"; the notation "bs" refers to broad singlet. Mass spectral data were obtained by using a Finnigan MAT-8430, TSQ-70 or 4500 double-focusing high-resolution mass spectrometer. Microanalysis were performed by the Searle Physical Methodology Department. Yields refer to chromatographically homogeneous products. No effort was made to optimize the yields in any reaction. Column chromatography was performed according to the procedure of Still[26] using Merck silica gel 60 (230–400 mesh). TLC was performed on precoated silica gel plastic plates Polygram SIL G/UV 254 from Macherey-Nagel Co. The solvent systems used were (A) ethyl acetate/hexane 3/17, (B) ethyl acetate/hexane 3/7, (C) ethyl acetate/hexane 1/1, (D) ethyl acetate/hexane 3/2, (E) ethyl acetate/hexane 13/7, (F) ethyl acetate/hexane 4/1. The plates were developed with the following reagents: (1) iodine, (2) phosphomolybdic acid, and (3) ninhydrin. The TLC spots were also visualized by UV light. Analytical HPLC was performed on a reverse-phase Supelco LC-18 column and/or on Zorbax-Rx column (length = 25 cm; diameter = 0.46 cm; detection at 230 nm; flow rate = 1.0 mL/min); t_R refers to retention time in minutes.

***N*-[*N*-[*N*-[(1,1-Dimethylethoxy)carbonyl]-3-[(triphenylmethyl)thio]-D-valyl]glycyl]-L-phenylalanine, Methyl Ester (*N*-Boc-*S*-trityl-D-Pen-Gly-Phe-OMe) (10).** A solution of Boc-Gly-Phe-OMe (9) (9.78 g, 29.1 mmol) in a mixture of 25 mL each of CH_2Cl_2 and anhydrous HCl in dioxane (7 N) was allowed to stand at room temperature for 15 min. The volatiles were removed in vacuo. The residue was stirred in DMF (20 mL) with 1-methylmorpholine (3.2 mL, 29.1 mmol) at 5 °C for 5 min to give a suspension which was used in the coupling reaction.

To a stirred solution of *N*-Boc-S-trityl-D-Pen-OH (14.3 g, 29.2 mmol) in THF (100 mL) at −23 °C was added 1-methylmorpholine (NMM) (3.2 mL, 29.1 mmol) followed by isobutyl chloroformate (IBCF) (3.8 mL, 29.3 mmol). After 45 min, the above-mentioned suspension was added. The mixture was allowed to warm to room temperature and stirring continued for 16 h. The mixture was concentrated in vacuo to remove most of the THF and the residue was extracted with ethyl acetate. The extract was washed successively with 0.5 N $KHSO_4$, saturated $NaHCO_3$, water, and half-saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography using 50% ethyl acetate in hexane as eluant. Appropriate fractions were pooled and concentrated to give 12.8 g (62%) of 10 as a white solid. TLC: R_f (C) 0.67, (D) 0.72. ^{1}H NMR: δ 1.17 (s, 3 H), 1.25 (s, 3 H), 1.47 (s, 9 H), 2.58 (bs, 1 H), 3.02 (dd, J = 14, 9 Hz, 1 H), 3.18 (dd, J = 14, 5 Hz, 1 H), 3.62 (s, 3 H), 3.72–3.86 (complex, 2 H), 4.72 (dt, J = 9.9, 5 Hz, 1 H), 5.39 (bs, 1 H), 5.83 (broad t, J = 5 Hz, 1 H), 7.09 (d, J = 8 Hz, 1 H), 7.16–7.33 (complex, 14 H), 7.64 (d, J = 8 Hz, 6 H).

***N*-[*N*-[(1,1-Dimethylethoxy)carbonyl]-3-[(triphenylmethyl)thio]-D-valyl]glycyl]-L-phenylalanine (*N*-Boc-*S*-trityl-D-Pen-Gly-Phe-OH) (11).** To a stirred solution of 10 (12.8 g, 18 mmol) in THF (55 mL) at 0 °C was added 0.5 N LiOH (55 mL). After 30 min the mixture was concentrated in vacuo to remove most of the THF. The residue was acidified with 0.5 N $KHSO_4$ (60 mL) and extracted with ethyl acetate. The extract was washed with water and half-saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo to give 11.3 g (90%) of 11 as a white solid. ^{1}H NMR: δ 1.11 (s, 3 H), 1.16 (s, 3 H), 1.45 (s, 9 H), 2.80 (d, J = 3 Hz, 1 H), 2.99 (dd, J = 14, 9 Hz, 1 H), 3.73 (dd, J = 17, 5 Hz, 1 H), 3.85 (dd, J = 17, 5 Hz, 1 H), 4.62 (m, 1 H), 5.52 (d, J = 4 Hz), 6.31 (broad t, J = 5 Hz, 1 H), 7.15–7.30 (complex, 14 H), 7.62 (d, J = 7 Hz, 6 H).

***N*-[*N*-[*N*-[*N*-[(1,1-Dimethylethoxy)carbonyl]-3-[(triphenylmethyl)thio]-D-valyl]glycyl]-L-phenylalanyl]-3-mercapto-D-valine, Methyl Ester (*N*-Boc-*S*-trityl-D-Pen-Gly-Phe-D-Pen-OMe) (12).** To a stirred solution of 11 (11.3 g, 16.3 mmol) in THF (100 mL) at −23 °C was added NMM (1.8 mL, 16.4 mmol) followed by IBCF (2.1 mL, 16.2 mmol). After 45 min, a solution of D-Pen-OMe [obtained from D-Pen-OMe·HCl (3.25 g, 16.3 mmol) and NMM (1.8 mL, 16.3 mmol) in DMF (20 mL)] was added. The mixture was allowed to warm to room temperature, stirred for 16 h, and worked up as described in the preparation of 10 to give 14 g (98%) of 12 as a white solid. This material showed a single spot on TLC and was used immediately in the next reaction. TLC: R_f (E) 0.64. ^{1}H NMR: δ 1.21 (s, 3 H), 1.27 (s, 3 H), 1.31 (s, 3 H), 1.40 (s, 3 H), 1.46 (s, 9 H), 2.02–2.1 (m, 2 H), 3.03 (dd, J = 14, 10 Hz, 1 H), 3.33 (dd, J = 14, 4 Hz, 1 H), 3.57 (dd, J = 17, 7 Hz, 1 H), 3.69 (s, 3 H), 3.88 (dd, J = 17, 7 Hz, 1 H), 4.57 (d, J = 9 Hz, 1 H), 4.63 (m, 1 H), 5.39 (bs, 1 H), 5.60 (m, 1 H), 7.07 (d, J = 8 Hz, 1 H), 7.18–7.38 (complex, 14 H), 7.55 (d, J = 7 Hz, 1 H), 7.66 (d, J = 8 Hz, 6 H).

Methyl 13α-[[(1,1-Dimethylethoxy)carbonyl]amino]-3,3,14,14-tetramethyl-6,9,12-trioxo-7β-(phenylmethyl)-1,2-dithia-5,8,11-triazacyclotetradecane-(4*R*)-4α-carboxylate (*N*-Boc-D-Pen-Gly-Phe-D-Pen-OMe) (13). To a well stirred solution of iodine (4.54 g, 17.7 mmol) in 80% aqueous acetic acid (2.9 L) was added, over 1 h, a solution of 12 (14 g, 16.7 mmol) in 80% aqueous acetic acid (2.9 L). The mixture was further stirred for 20 min and 0.25 M Na_2SO_3 was added in drops to remove the iodine color. To this was added 1 N NaOH (18 mL). The mixture was concentrated in vacuo (bath temperature = 30 °C). The residue was taken up in ethyl acetate and washed with 0.25 N Na_2SO_3 (to remove the iodine color), water, and half-saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was stirred in ether (500 mL) and the white solid was collected by filtration to give 7.2 g (72%) of 13. TLC: R_f (E) 0.44, (F) 0.63. ^{1}H NMR ($CDCl_3/CD_3OD$): δ 1.33 (s, 3 H), 1.43 (s, 12 H), 1.45 (s, 3 H), 1.57 (s, 3 H), 2.96 (dd, J = 14, 10 Hz, 1 H), 3.30 (dd, J = 14, 4 Hz, 1 H), 3.46 (m, 1 H), 3.77 (s, 3 H), 4.19–4.38 (complex, 2 H), 4.63 (m, 1 H), 4.67 (d, J = 8 Hz, 1 H), 5.58 (d, J = 10 Hz, 1 H), 6.91 (bs, 1 H), 7.01 (d, J = 7 Hz, 1 H), 7.21–7.40 (complex, 6 H). HRMS (CI): m/e 597.2419 (M + H) calcd for $C_{27}H_{40}N_4O_7S_2$ 597.2420.

Methyl 13α-[[2(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]amino]-3,3,14,14-tetramethyl-6,9,12-trioxo-7β-(phenylmethyl)-1,2-dithia-5,8,11-triazacyclotetradecane-(4*R*)-4α-carboxylate (*N*-Boc-2′,6′-dimethyl-Tyr-D-Pen-Gly-Phe-D-Pen-OMe) (14). As described in the procedure used for the conversion of 9 to 10, cyclic peptide 13 (7.07 g, 11.86 mmol) was treated with anhydrous HCl to remove the Boc protecting group and treated with the mixed anhydride obtained from *N*-Boc-2′,6′-dimethyl-L-tyrosine (8) (3.67 g, 11.86 mmol). The crude product was chromatographed using 4% methanol in CH_2Cl_2 to give 7.5 g (80%) of 14 as a white solid. TLC: R_f (E) 0.36, (F) 0.58. ^{1}H NMR ($CDCl_3/CD_3OD$): δ 0.9–1.53 (complex, 21 H), 2.29 (s, 6 H), 2.82–2.94 (m, 2 H), 3.10 (dd, J = 13, 8 Hz, 1 H), 3.32 (dd, J = 14, 4 Hz, 1 H), 3.58 (broad d, J = 15 Hz, 1 H), 3.8 (s, 3 H), 3.75–3.85 (broad m, 1 H), 4.11 (m, 1 H), 4.45 (s, 1 H), 4.53 (m, 1 H), 4.77 (m, 1 H), 5.57 (bs, 1 H), 6.50 (s, 2 H), 7.2–7.35 (m, 6 H), 7.47 (d, J = 8.5 Hz, 1 H). Anal. ($C_{38}H_{53}N_5O_9S_2\cdot H_2O$): C, H, N, S.

Methyl 13α-[[2(*S*)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]amino]-3,3,14,14-tetramethyl-6,9,12-trioxo-7β-(phenylmethyl)-1,2-dithia-5,8,11-triazacyclotetradecane-(4*R*)-4α-carboxylate Hydrochloride (2′,6′-Me$_2$-Tyr-D-Pen-Gly-Phe-D-Pen-OMe, HCl) (4). A solution of 7.4 g of 14 in 30 mL each of CH_2Cl_2 and 7 N anhydrous HCl in dioxane was allowed to stand at room temperature for 15 min. The mixture was concentrated in vacuo and the residue was triturated with ether, filtered, and washed several times with ether to give 6.8 g of 4 as a white solid. ^{1}H NMR (DMSO-d_6): δ 0.68 (s, 3 H), 1.23 (s, 3 H), 1.27 (s, 3 H), 1.30 (s, 3 H), 2.21 (s, 6 H), 2.82 (m, 2 H), 3.06 (m, 2 H), 3.27 (after D_2O exchange) (d, J = 15 Hz, 1 H), 3.66 (s, 3 H), 4.16 (m, 1 H), 4.28–4.34 (m, 2 H), 4.38 (d, J = 8 Hz, 1 H), 4.42 (d, J = 9 Hz, 1 H), 6.37 (s, 2 H), 7.19–7.30 (m, 5 H), 7.41 (d, J = 7.5 Hz, 1 H), 8.37 (bs, 3 H), 8.51 (d, J = 9 Hz, 1 H), 8.64 (dd, J = 7, 4 Hz, 1 H), 8.82 (d, J = 7.5 Hz, 1 H), 9.04 (s, 1 H). HRMS (EI): calcd for $C_{33}H_{45}N_5O_7S_2$ m/z 687.2760, found

(26) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separation with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.

687.2729. HPLC: t_R = 8.22 (solvent, methanol/water/trifluoroacetic acid 30/70/0.1), t_R = 26.34 [Zorbax-Rx column; solvent, acetonitrile/TEAP (triethylamine phosphate buffer, pH 3.5) 1/3]. Anal. ($C_{33}H_{45}N_5O_7S_2$·HCl·H_2O): C, H, N, Cl, S.

Methyl 13α-[[2(*S*)-Amino-3-(4-hydroxyphenyl)-1-oxopropyl]amino]-3,3,14,14-tetramethyl-6,9,12-trioxo-7β-(phenylmethyl)-1,2-dithia-5,8,11-triazacyclotetradecane-(4*R*)-4α-carboxylate Hydrochloride (Tyr-D-Pen-Gly-Phe-D-Pen-OMe, HCl) (2). Compound **2** was obtained as a white solid using the procedure described for the preparation of 4 with the exception that Boc-Tyr was substituted for 8. ^{1}H NMR (DMSO-d_6): δ 0.90 (s, 3 H), 1.29 (s, 3 H), 1.32 (s, 3 H), 1.34 (s, 3 H), 2.80 (dd, J = 14, 8.5 Hz, 1 H), 2.83 (dd, J = 14, 11 Hz, 1 H), 2.96 (dd, J = 14, 6.5 Hz, 1 H), 3.08 (dd, J = 14, 4 Hz, 1 H), 3.31 (m, 1 H), 3.67 (s, 3 H), 4.27 (m, 1 H), 4.31 (ddd, J = 11, 8, 4 Hz, 1 H), 4.33 (dd, J = 8.5, 6.5 Hz, 1 H), 4.39 (d, J = 8 Hz, 1 H), 4.54 (d, J = 9 Hz, 1 H), 6.70 (d, J = 8.5 Hz, 2 H), 7.11 (d, J = 8.5 Hz, 2 H), 7.20 (m, 1 H), 7.26 (m, 4 H), 7.40 (d, J = 8 Hz, 1 H), 8.10 (bs, 3 H), 8.70 (dd, J = 6.5, 4.5 Hz, 1 H), 8.77 (d, J = 9 Hz, 1 H), 8.86 (d, J = 8 Hz, 1 H), 9.37 (s, 1 H). HRMS (EI): calcd for $C_{31}H_{41}N_5O_7S_2$ m/z 659.2447, found 659.2420. HPLC: t_R = 22.0. [Zorbax-Rx column; solvent, acetonitrile/TEAP 1/3]. Anal. ($C_{31}H_{41}N_5O_7S_2$·HCl·H_2O): C, H, N, Cl, S.

1,1-Dimethylethyl 3-Oxo-4-morpholinecarboxylate (16). To a stirred solution of 3-oxo-4-morpholine[27] (15) (24.18 g, 0.24 mol) in THF (700 mL) at −78 °C was added a hexane solution of *n*-BuLi (100 mL, 2.5 M) over 5 min. After 1 h, di-*tert*-butyl dicarbonate (52.2 g, 0.24 mol) in THF (50 mL) was added over 5 min. After stirring for 3 h, excess saturated NH_4Cl was added. The organic phase was washed with saturated NH_4Cl, dried over $MgSO_4$ and concentrated in vacuo to give 48 g (100%) of 16 as a pale yellow liquid, which was used without purification. TLC: R_f (A) 0.57, (B) 0.73. ^{1}H NMR: δ 1.55 (s, 9 H), 3.75 (t, J = 5 Hz, 2 H), 3.9 (t, J = 5 Hz, 2 H), 4.2 (s, 2 H).

[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethoxy]acetic Acid (17). To a stirred solution of 16 (48 g, 0.24 mol) in THF (250 mL) at −10 °C was added a 1 M solution of LiOH (250 mL). After 3 h, most of the THF was removed in vacuo and the residue extracted with ether. The aqueous phase was acidified with 0.5 N $KHSO_4$ to pH = 3, and extracted with ether–CH_2Cl_2. The organic extract was dried and concentrated in vacuo to give 38 g (73%) of 17 as a colorless thick liquid. TLC: R_f (C) 0.33, (D) 0.52. ^{1}H NMR: δ 1.46 (s, 9 H), 3.37 (q, J = 4.5 Hz, 2 H), 3.64 (t, J = 4.5 Hz, 2 H), 4.14 (s, 2 H), 5.09 (bs, 1 H), 8.3 (bs, 1 H). HRMS (CI): m/e 220.1176 (M + H) calcd for $C_9H_{17}NO_5$ 220.1185.

Ethyl [2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethoxy]acetate (18). To a stirred solution of 17 (38.28 g, 0.175 mol) in DMF (250 mL) at 0 °C was added K_2CO_3 (25 g) followed by ethyl iodide (14 mL, 0.175 mol). The mixture was allowed to warm to room temperature and stirred for 48 h. The mixture was filtered and the filtrate shaken with water and ether. The aqueous phase was extracted with ether. The combined organic phase was dried and concentrated in vacuo to give 40 g (93%) of 18 as a pale yellow liquid. TLC: R_f (A) 0.18, (B) 0.40. ^{1}H NMR: δ 1.3 (t, J = 7 Hz, 3 H), 1.45 (s, 9 H), 3.35 (q, J = 5 Hz, 2 H), 3.62 (t, J = 5 Hz, 2 H), 4.09 (s, 2 H), 4.23 (q, J = 7 Hz, 2 H), 5.13 (bs, 1 H). HRMS (CI): m/e 248.1516 (M + H) calcd for $C_{11}H_{21}NO_5$ 248.1498.

Ethyl α-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethoxy]benzenepropanoate (Boc-Glyψ(CH_2O)Phe-OEt) (19). To a stirred 1 M THF solution of lithium bis(trimethylsilyl)amide (LHMDS) (350 mL, 0.35 mol) at −78 °C was added using a cannula a cold (−78 °C) solution of 18 (40 g, 0.162 mol) in THF (50 mL) over 5 min. After 45 min, benzyl bromide (19.3 mL, 0.162 mol) was added neat. After 1 h, excess saturated NH_4Cl was added. The mixture was extracted with ether and the organic phase was dried and concentrated. The residue was flash chromatographed using 20% ethyl acetate in hexane. Appropriate fractions were pooled to give 20 g (29%) of the dialkylated product [Less polar. TLC: R_f (A) 0.33, (B) 0.66; ^{1}H NMR: δ 1.17 (t, J = 7 Hz, 3 H), 1.5 (s, 9 H), 3.09 (d, J = 15 Hz, 2 H), 3.17 (d, J = 15 Hz, 2 H), 3.42 (q, J = 5 Hz, 2 H), 3.74 (t, J = 5 Hz, 2 H), 4.03 (q, J = 7 Hz, 2 H), 5.15 (bs, 1 H), 7.20–7.40 (m, 10 H). HRMS (CI): m/e 428.2419 (M + H) calcd for $C_{25}H_{33}NO_5$ 428.2437] and 11 g (20%) of the monoalkylated product 19 [More polar. TLC: R_f (A) 0.23, (B) 0.57. ^{1}H NMR: δ 1.25 (t, J = 7 Hz, 3 H), 1.42 (s, 9 H), 2.96 (dd, J = 13, 9 Hz, 1 H), 3.07 (dd, J = 13, 4.5 Hz, 1 H), 3.21 (q, J = 5 Hz, 2 H), 3.35 (dt, J = 9, 5 Hz, 1 H), 3.61 (dt, J = 9, 5 Hz, 1 H), 4.01 (dd, J = 9, 4.5 Hz, 1 H), 4.20 (q, J = 7 Hz, 2 H), 4.75 (bs, 1 H), 7.2–7.39 (m, 5 H). HRMS (CI): m/e 338.1961 (M + H) calcd for $C_{18}H_{27}NO_5$ 338.1967. Anal. ($C_{18}H_{27}NO_5$): C, H, N] as thick liquids.

Methyl 10α-[[(1,1-Dimethylethoxy)carbonyl]amino]-6,6,9,9-tetramethyl-3,11-dioxo-2-(phenylmethyl)-1-oxa-7,8-dithia-4,12-diazacyclotetradecane-(5*R*)-5α-carboxylate (*N*-Box-D-Pen-Glyψ(CH_2O)Phe-D-Pen-OMe) (20A and 20B). Following the procedures identical to the ones used in the conversion of **9** to **13**, compound **19** was converted to a 1:1 mixture of the cyclic tetrapeptides (the yield in the cyclization reaction was 68%) **20A** (less polar) and **20B** (more polar), which were separated by chromatography using 60% ethyl acetate in hexane as eluant. For **20A**. ^{1}H NMR: δ 1.3, 1.41, 1.45, 1.5, 1.6 (complex pattern broad singlets, 21 H), 2.85 (dd, J = 14, 10 Hz, 1 H), 3.15 (dd, J = 14, 2.5 Hz, 1 H), 3.23 (m, 1 H), 3.5–3.72 (m, 3 H), 3.79 (s, 3 H), 3.98 (dd, J = 10, 2.5 Hz, 1 H), 4.24 (m, 1 H), 4.80 (d, J = 10 Hz, 1 H), 5.48 (bs, 1 H), 6.73 (t, J = 5 Hz, 1 H), 7.20–7.33 (complex, 5 H), 7.71 (d, J = 8 Hz, 1 H). MS (EI): m/e 583 (M^+). For **20B**. ^{1}H NMR: δ 1.17, 1.30, 1.41–1.44 (complex pattern broad singlets, 21 H), 2.97 (dd, J = 14, 7 Hz, 1 H), 3.23 (dd, J = 14, 3 Hz, 1 H), 3.38 (m, 1 H), 3.52 (bs, 2 H), 3.77 (s, 3 H), 3.88 (m, 1 H), 4.05 (dd, J = 7, 3 Hz, 1 H), 4.15 (d, J = 7.5 Hz, 1 H), 4.38 (d, J = 6 Hz, 1 H), 5.51 (bs, 1 H), 6.21 (bs, 1 H), 7.18–7.32 (complex, 5 H), 7.92 (m, 1 H). MS (EI): m/e 583 (M^+). Anal. (mixture of **20A** and **20B**) ($C_{27}H_{41}N_3O_7S_2$·H_2O): C, H, N, S.

Methyl 10α-[[2(*S*)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]amino]-6,6,9,9-tetramethyl-3,11-dioxo-2-(phenylmethyl)-1-oxa-7,8-dithia-4,12-diazacyclotetradecane-(5*R*)-5α-carboxylate Hydrochloride (2′,6′-Me_2-Tyr-D-Pen-Glyψ(CH_2O)Phe-D-Pen-OMe, HCl) (5A and 5B). Conversion of **20A** to **5A** involved an identical procedure used in the conversion of **13** to **4**. Yield: 80%. ^{1}H NMR (DMSO-d_6): V 0.78 (s, 3 H), 1.11 (s, 3 H), 1.33 (s, 3 H), 1.39 (s, 3 H), 2.17 (s, 6 H), 2.78–2.85 (complex, 1 H), 3.0–3.09 (complex, 2 H), 3.14 (bs, 2 H), 3.42 (broad d, J = 9.1 Hz, 1 H), 3.59 (m, 1 H), 3.65 (s, 3 H), 4.08–4.15 (complex, 3 H), 4.49 (d, J = 8.6 Hz, 1 H), 6.37 (s, 2 H), 7.18–7.28 (complex, 5 H), 7.55 (d, J = 8.8 Hz, 1 H), 7.99 (m, 1 H), 8.35 (bs, 3 H), 8.42 (d, J = 7.3 Hz, 1 H), 9.06 (s, 1 H). HRMS (EI): calcd for $C_{33}H_{46}N_4O_7S_2$ m/z 674.2808, found 674.2792. HPLC: t_R = 37.9 min (solvent, methanol/water/trifluoroacetic acid 50/50/0.1), t_R = 9.17 min (solvent, methanol/water/trifluoroacetic acid 70/30/0.1). Anal. ($C_{33}H_{46}N_4O_7S_2$·HCl·1.5H_2O): C, H, N, Cl, S.

Conversion of **20B** to **5B** involved an identical procedure used in the conversion of **13** to **4**. Yield: 75%. ^{1}H NMR (DMSO-d_6): δ 0.69 (s, 3 H), 1.10 (s, 3 H), 1.16 (s, 3 H), 1.28 (s, 3 H), 2.19 (s, 6 H), 2.80 (bs, 1 H), 2.82 (dd, J = 14, 6.7 Hz, 1 H), 3.02 (dd, J = 14, 4.5 Hz, 1 H), 3.04 (bs, 2 H), 3.59 (s, 3 H), 3.82 (t, J = 8.8 Hz, 1 H), 4.08 (t, J = 6.0 Hz, 1 H), 4.21 (d, J = 7.5 Hz, 1 H), 4.26 (d, J = 7.9 Hz, 1 H), 6.36 (s, 2 H), 7.16–7.26 (complex, 5 H), 8.05 (d, J = 7.5 Hz, 1 H), 8.20 (broad t, J = 4.5 Hz, 1 H), 8.32 (broad d, J = 7 Hz, 1 H), 8.35 (bs, 3 H), 9.05 (s, 1 H). HRMS (EI): calcd for $C_{33}H_{46}N_4O_7S_2$ m/z 674.2808, found 674.2795. HPLC: t_R = 30.1 min (solvent, methanol/water/trifluoroacetic acid 50/50/0.1), t_R = 12.20 min (solvent, methanol/water/trifluoroacetic acid 70/30/0.1). Anal. ($C_{33}H_{46}N_4O_7S_2$·1.1HCl·1.5H_2O): C, H, N, Cl, S.

Ethyl α-[(Phenylmethoxy)methyl]benzenepropanoate (22). Ethyl hydrocinnamate (26.7 g, 0.15 mol) was added neat to a stirred solution of lithium diisopropylamide (100 mL, 1.5 M cyclohexane solution, 0.15 mol) in THF (250 mL) at −78 °C. After 30 min HMPA (26.9 g, 0.15 mol) was added and stirring continued for another 15 min. Then benzyl chloromethyl ether (23.5 g, 0.15 mol) was added to the reaction mixture. After 20 min, the dry ice cooling bath was replaced with an ice bath. After 10 min, excess saturated NH_4Cl was added. The organic phase was separated, dried, and concentrated in vacuo. The residue was distilled in vacuo. The fraction with bp 200–210 °C (0.05 mm) was collected to give 25 g (56%) of **22** as a colorless liquid. TLC: R_f (A) 0.49,

(27) Vieles, MM. P.; Seguin, J. Syntheses de morpholones-3 et d'-oxazolidone-2. *Bull. Soc. Chim. Fr.* **1953**, 287–289.

(B) 0.70. ^{1}H NMR: δ 1.19 (t, J = 7 Hz, 3 H), 2.8–3.1 (m, 3 H), 3.5–3.7 (m, 2 H), 4.10 (q, J = 7 Hz, 2 H), 4.5 (s, 2 H), 7.0–7.5 (m, 10 H).

Ethyl α-(Hydroxymethyl)benzenepropanoate (23). A 14.8-g of sample of **22** was hydrogenated in ethanol (200 mL) over 4% Pd–C under 5 psi of hydrogen pressure. The catalyst was removed by filtration after the uptake of hydrogen ceased, and the filtrate concentrated to give 9.2 g (89%) of **23** as a colorless thick liquid. The product was shown to be pure by TLC [R_f (A) 0.20, (B) 0.33]. ^{1}H NMR: δ 1.21 (t, J = 7 Hz, 3 H), 2.18 (bs, 1 H), 2.78–3.09 (m, 3 H), 3.66–3.81 (m, 2 H), 4.15 (q, J = 7 Hz, 2 H), 7.13–7.33 (m, 15 H). HRMS (CI): m/e 209.1169 (M + H) calcd for $C_{12}H_{16}O_3$ 209.1177.

Ethyl α-[[(Tetrahydro-2*H*-pyran-2-yl)oxy]methyl]-benzenepropanoate (24). A mixture of **23** (5.82 g), pyridinium p-toluenesulfonate (0.29 g), and dihydropyran (2.89 mL) in CH_2Cl_2 (100 mL) was allowed to stir at room temperature for 4 h. The mixture was taken up in ether and washed with saturated $NaHCO_3$, dried, and concentrated in vacuo. The residue was distilled in vacuo. The fraction boiling at 140 °C (0.2 mm) was collected to give 7 g (85%) of **24** as a colorless liquid. Though the TLC of this material seemed to indicate a homogeneous substance, it was a mixture of stereoisomers as shown by NMR. TLC: R_f (A) 0.56, (B) 0.72. ^{1}H NMR: δ 1.18 (m, 3 H), 1.40–1.90 (m, 6 H), 2.80–3.05 (m, 3 H), 3.44–3.62 (m, 2 H), 3.75–3.97 (m, 2 H), 4.05–4.15 (m, 2 H), 4.60 (m, 1 H), 7.14–7.33 (m, 5 H). HRMS (CI): m/e 293.1759 (M + H) calcd for $C_{17}H_{22}O_4$ 293.1765.

α-Ethenyl-β-[[(tetrahydro-2*H*-pyran-2-yl)oxy]methyl]-benzenepropanol (26). To a stirred solution of **24** (10 g, 34.2 mmol) in ether (250 mL) at −78 °C was added a 1 M toluene solution of DIBAL (35 mL, 35 mmol) using a syringe pump at the rate of 1.6 mL/min. Stirring was continued for another 45 min. Then a 1 M THF solution of vinylmagnesium bromide (40 mL, 40 mmol) was added rapidly to the reaction mixture. The mixture was warmed to 0 °C over 30 min. Then excess saturated NH_4Cl was added. The mixture was filtered through Celite. The organic phase was dried and concentrated. The residue was chromatographed using 25% ethyl acetate in hexane as eluant to give 6.7 g (71%) of **26** as a thick liquid. Though the TLC of this material seemed to indicate a homogeneous substance, it was a mixture of stereoisomers as shown by NMR. TLC: R_f (A) 0.26, (B) 0.53. ^{1}H NMR: δ 1.4–4.6 (several multiplets, 16 H), 5.15–5.40 (m, 2 H), 5.86–6.03 (m, 1 H), 7.1–7.3 (m, 5 H). HRMS (CI): m/e 277.1791 (M + H) calcd for $C_{17}H_{24}O_3$ 277.1803.

Trichloro-*N*-[4-(phenylmethyl)-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]-2(*E*)-pentenyl]acetamide (28). A 60% mineral oil suspension of NaH (126 mg, 5.25 mmol) was washed with hexane and suspended in ether (100 mL) at 0 °C. To this stirred suspension was added **26** (6.69 g, 24 mmol) in ether (30 mL). After 30 min, trichloroacetonitrile (2.5 mL, 25 mmol) was added neat and the cooling bath removed. After 1 h, the solvents were removed in vacuo. The residue was shaken with hexane (100 mL) containing methanol (125 mL) and filtered (gravity). The filtrate was concentrated in vacuo to give the imidate **27** which was found to be pure on ^{1}H NMR analysis. This material was taken up in xylene (400 mL) and refluxed for 2 h. The solvent was removed in vacuo. A solution of the residue in 20% ethyl acetate in hexane was filtered through a short column of silica gel. The filtrate was concentrated to give 8.3 g (81%) of **28** as a thick gum. TLC: R_f (A) 0.33, (B) 0.54. ^{1}H NMR: δ 1.45–1.90 (m, 6 H), 2.53–2.73 (m, 2 H), 2.82–2.95 (m, 1 H), 3.30–3.38 (m, 1 H), 3.45–3.53 (m, 1 H), 3.68–3.75 (m, 1 H), 3.79–3.90 (m, 3 H), 4.54–4.58 (m, 1 H), 5.40 (dt, J = 15, 6 Hz, 0.5 H), 5.41 (dt, J = 15, 6 Hz, 0.5 H), 5.6–5.72 (m, 1 H), 6.52 (bs, 1 H), 7.13 (d, J = 7.5 Hz, 2 H), 7.18 (t, J = 7.5 Hz, 1 H), 7.27 (t, J = 7.5 Hz, 2 H).

4-(Phenylmethyl)-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]-2-(*E*)-pentenamine (29). A mixture of **28** (12.3 g, 29 mmol) and 6 N NaOH (148 mL) in 95% ethanol (155 mL) was stirred at room temperature for 48 h. The mixture was concentrated in vacuo to remove most of the ethanol and the residue was extracted with ether. The ether extract was dried and concentrated in vacuo. The residue was chromatographed using 1/1 ethyl acetate in hexane containing 2% triethylamine to give 5.3 g (66%) of **29** as a thick pale yellow oil. TLC (solvent 2% triethylamine in ethyl acetate/hexane 1/1): R_f 0.45. ^{1}H NMR: δ 1.31 (s, 2 H), 1.42–1.90 (m, 6 H), 2.54–2.68 (m, 2 H), 2.81–2.97 (m, 1 H), 3.2 (d, J = 4 Hz, 2 H), 3.42 (m, 1 H), 3.49 (m, 1 H), 3.69 (m, 1 h), 3.85 (m, 1 H), 4.56 (m, 1 H), 5.42–5.59 (m, 2 H), 7.13–7.29 (m, 5 H).

1,1-Dimethylethyl [4-(Phenylmethyl)-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]-2(*E*)-pentenyl]carbamate (30). A solution of **29** (5.22 g, 19 mmol) and di-*tert*-butyl dicarbonate (4.2 g, 19 mmol) in $CHCl_3$ (100 mL) containing a trace of 4-(dimethylamino)pyridine was allowed stand at room temperature for 16 h. The solvent was removed in vacuo and the residue was taken up in ether. The ether extract was washed with water, dried, and concentrated. An ether solution of the residue was filtered through a short column of silica gel and the filtrate concentrated to give 6.56 g (92%) of **30** as a yellowish gum. TLC: R_f (A) 0.59. ^{1}H NMR: δ 1.43 (s, 9 H), 1.49–1.90 (m, 6 H), 2.53–2.67 (m, 2 H), 2.78–2.94 (m, 1 H), 3.31 (dd, J = 10, 5 Hz, 1 H), 3.49 (m, 1 H), 3.61–3.71 (m, 2 H), 3.83 (m, 1 H), 4.38 (bs, 1 H), 4.55 (m, 1 H), 5.38 (dt, J = 15, 7 Hz, 1 H), 5.54 (m, 1 H), 7.12–7.29 (m, 5 H).

α-[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-1(*E*)-propenyl]benzenepropanoic Acid (Boc-Glyψ(*trans*-CH=CH)Phe-OH) (31). To a cold (5 °C) solution of 5.8 g of CrO_3 in water (30 mL) was added concentated H_2SO_4 (5 mL). Thus prepared Jones reagent was cooled to 0 °C and added to a stirred solution of **30** (6.52 g) in acetone (350 mL) at 0 °C. The mixture was stirred for 3 h and added to excess water and ether. The aqueous phase was separated and extracted five times with ether. The combined organic extract was concentrated in vacuo to a volume of 100 mL and extracted with 10% NaOH (200 mL) in five portions. The organic extract was saved (extract A). The combined basic extract was neutralized with 1 N HCl and extracted with ether. This extract was dried and concentrated to give 1.58 g (60%, based on unrecovered **30**) of acid **31** as a pale yellow oil. [The original neutral extract (extract A) was concentrated and the residue chromatographed to give 3.25 g of **30**.] TLC: R_f (C) 0.47, (D) 0.53. ^{1}H NMR: δ 1.43 (s, 9 H), 2.81 (dd, J = 13, 8 Hz, 1 H), 3.11 (dd, J = 13, 8 Hz, 1 H), 3.30 (q, J = 8 Hz, 1 H), 3.54–3.69 (m, 2 H), 4.58 (bs, 1 H), 5.47 (dt, J = 15, 5 Hz, 1 H), 5.63 (dd, J = 15, 8 Hz, 1 H), 7.13–7.29 (m, 5 H), 10.5 (bs, 1 H). Anal. ($C_{17}H_{23}NO_4$): C, H, N.

Methyl 13α-[[(1,1-Dimethylethoxy)carbonyl]amino]-3,3,14,14-tetramethyl-6,12-dioxo-7-(phenylmethyl)-1,2-dithia-5,11-diazacyclotetradec-8-ene-(4*R*)-4α-carboxylate (*N*-Boc-D-Pen-Glyψ(*trans*-CH=CH)Phe-D-Pen-OMe) (32A and 32B). Acid **31** was coupled to D-Pen-OMe using the mixed-anhydride procedure described earlier. The crude produce was treated with 4 N HCl in CH_2Cl_2/dioxane to remove the Boc protecting group. The resulting amine hydrochloride was reacted with *N*-Boc-S-trityl-D-Pen by the mixed-anhydride coupling procedure. The product obtained in this reaction was found to be impure. However treatment of this crude material with iodine in 80% aqueous acetic acid as described for the preparation of **13** and chromatography of the crude product using 40% ethyl acetate in hexane gave **32A** (less polar) and **32B** (more polar) in equal amounts (combined overall yield from **31** was 10.5%). For **32A**. ^{1}H NMR: δ 1.28–1.5 (complex pattern, 21 H), 2.80 (m, 1 H), 3.26 (m, 2 H), 3.57–3.97 (m, 2 H), 3.71 (s, 3 H), 4.18 (d, J = 9 Hz, 1 H), 4.62 (d, J = 8 Hz, 1 H), 5.56 (d, J = 8 Hz, 1 H), 5.74 (dd, J = 15, 8 Hz, 1 H), 5.85 (dt, J = 15, 5, 5 Hz, 1 H), 6.72 (bs, 1 H), 6.92 (bs, 1 H), 7.1–7.3 (m, 5 H). MS (EI): m/e 579 (M^+). For **32B**. ^{1}H NMR: δ 1.3–1.48 (complex pattern, 21 H), 2.8 (m, 1 H), 3.18–3.31 (m, 2 H), 3.68–3.89 (m, 2 H), 3.73 (s, 3 H), 4.15 (d, J = 8 Hz, 1 H), 4.65 (d, J = 8 Hz, 1 H), 5.42 (d, J = 9 Hz, 1 H), 5.70–5.83 (m, 2 H), 6.52–6.66 (m, 2 H), 7.12–7.28 (m, 5 H). MS (EI): m/e 579 (M^+).

Methyl 13α-[[2(*S*)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]amino]-3,3,14,14-tetramethyl-6,12-dioxo-7-(phenylmethyl)-1,2-dithia-5,11-diazacyclotetradec-8-ene-(4*R*)-4α-carboxylate Hydrochloride (2′,6′-Me$_2$-Tyr-D-Pen-Glyψ(*trans*-CH=CH)Phe-D-Pen-OMe, HCl) (6A and 6B). Conversion of **32A** to **6A** involved an identical procedure used in the conversion of **13** to **4**. Yield: 99%. ^{1}H NMR (DMSO-d_6): δ 0.88 (s, 3 H), 0.90 (s, 3 H), 1.23 (s, 3 H), 1.24 (s, 3 H), 2.19 (s, 6 H), 2.64 (dd, J = 14, 6.9 Hz, 1 H), 2.72–2.78 (complex, 1 H), 2.96–3.05 (complex, 2 H), 3.17 (m, 1 H), 3.54 (q, J = 7.5 Hz, 1 H), 3.59 (s, 3 H), 3.74 (m, 1 H), 4.11 (m, 1 H), 4.20 (d, J = 9.2 Hz, 1 H), 4.41 (d, J = 8.8 Hz, 1 H), 5.50 (dd, J = 15.6, 7.9 Hz, 1 H), 5.96 (ddd, J = 15.6, 8.0, 5.5 Hz, 1 H), 6.36 (s, 2 H),

7.13–7.26 (complex, 5 H), 8.18 (d, J = 8.7 Hz, 1 H), 8.28 (d, J = 8.8 Hz, 1 H), 8.40 (broad t, J = 5.5 Hz, 1 H), 9.04 (s, 1 H). HRMS (EI): calcd for $C_{34}H_{46}N_4O_6S_2$ m/z 670.2859, found 670.2847. HPLC: t_R = 30.7 min (solvent, methanol/water/trifluoroacetic acid 50/50/0.1), t_R = 9.28 min (solvent, methanol/water/trifluoroacetic acid 70/30/0.1). Anal. ($C_{34}H_{46}N_4O_6S_2$·1.25HCl·2H_2O): C, H, N.

Conversion of **32B** to **6B** involved an identical procedure used in the conversion of **13** to **4**. Yield 75%. ^{1}H NMR (DMSO-d_6): δ 0.78 (s, 3 H), 0.96 (s, 3 H), 1.30 (s, 3 H), 1.31 (s, 3 H), 2.19 (s, 6 H), 2.74–2.83 (complex, 2 H), 3.01–3.08 (complex, 2 H), 3.21–3.31 (complex, 2 H), 3.56 (s, 3 H), 3.92 (m, 1 H), 4.18 (d, J = 7.4 Hz, 1 H), 4.2 (m, 1 H), 4.25 (d, J = 8.9 Hz, 1 H), 5.63 (ddd, J = 16, 7.5, 4.0 Hz, 1 H), 5.79 (dd, J = 16, 8.3 Hz, 1 H), 6.36 (s, 2 H), 7.13–7.28 (complex, 5 H), 8.24–8.54 (complex, 5 H), 9.05 (s, 1 H). HRMS (EI): calcd for $C_{34}H_{46}N_4O_6S_2$ m/z 670.2859, found 670.2858. HPLC: t_R = 34.8 min (solvent, methanol/water/trifluoroacetic acid 50/50/0.1), t_R = 9.40 min (solvent, methanol/water/trifluoroacetic acid 70/30/0.1). Anal. ($C_{34}H_{46}N_4O_6S_2$·1.25HCl·2H_2O): C, H, N: calcd, 7.45; found, 6.90.

1,1-Dimethylethyl 2-Oxo-3-(phenylmethyl)-1-piperidinecarboxylate (34). To a stirred 1 M pentane solution of LHMDS (100 mL, 0.1 mol) in THF (500 mL) at −78 °C was added 1,1-dimethylethyl 2-oxo-1-piperidinecarboxylate (**33**) (18.8 g, 0.095 mol) in THF (50 mL). After 15 min, benzyl bromide (11.2 mL, 0.095 mole) was added rapidly. Stirring was continued for 2 h, and then the temperature was allowed to raise to 0 °C over 2 h. Excess saturated NH_4Cl was added to the reaction mixture, and the layers were separated. The organic phase was washed with half-saturated NaCl, dried, and concentrated. The residue was chromatographed using 15% ethyl acetate in hexane to give, in the order of elution, dialkylated product [TLC: R_f (A) 0.51, (B) 0.73. ^{1}H NMR: δ 1.35 (m, 2 H), 1.54 (s, 9 H), 1.71 (m, 2 H), 2.64 (d, J = 13 Hz, 2 H), 3.17 (t, J = 6 Hz, 2 H), 3.39 (d, J = 13 Hz, 2 H), 7.15–7.30 (complex, 10 H). HRMS: m/e 380.2234 (M + H) calcd for $C_{24}H_{29}NO_3$ 380.2225] followed by 22 g (80%) of **34** as a thick liquid. TLC: R_f (A) 0.39, (B) 0.64. ^{1}H NMR: δ 1.31–1.95 (m, 4 H), 1.55 (s, 9 H), 2.63 (m, 2 H), 3.43 (m, 1 H), 3.58 (dt, J = 11, 6 Hz, 1 H), 3.72 (ddd, J = 11, 7, 5 Hz, 1 H), 7.2–7.35 (m, 5 H). HRMS (CI): m/e 290.1768 (M + H) calcd for $C_{17}H_{23}NO_3$ 290.1756.

α-[3-[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]-benzenepropanoic Acid (Boc-Glyψ(CH_2CH_2)Phe-OH) (35). To a stirred solution of **34** (8 g, 27.6 mmol) in THF (125 mL) at room temperature was added a 0.5 M solution of LiOH (80 mL). Stirring was continued for 16 h, and the solvent was removed in vacuo. The residue was taken up in water and acidified with 20% acetic acid and extracted with ethyl acetate. The organic phase was dried and concentrated in vacuo to give **35** (8 g, 94%) as a thick gum. TLD: R_f (C) 0.55, (D) 0.66. ^{1}H NMR: δ 1.43 (s, 9 H), 1.43–1.72 (complex, 4 H), 2.62–2.79 (complex, 2 H), 2.93–3.15 (complex, 3 H), 4.53 (m, 1 H), 7.16–7.32 (complex, 5 H). HRMS (CI): m/e 308.1877 (M + H) calcd for $C_{17}H_{25}NO_4$ 308.1882. Anal. ($C_{17}H_{25}NO_4$): C, H. N.

Methyl 13α-[[(1,1-Dimethylethoxy)carbonyl]amino]-3,3,14,14-tetramethyl-6,12-dioxo-7-(phenylmethyl)-1,2-dithia-5,11-diazacyclotetradecane-(4*R*)-4α-carboxylate (*N*-Boc-D-Pen-Glyψ(CH_2CH_2)Phe-D-Pen-OMe) (36A and 36B). Employing the protocol used to convert **31** to **32A** and **32B**, acid **35** was converted to a 1/1.2 mixture of the cyclic tetrapeptides **36A** (less polar) and **36B** (more polar), which were separated by chromatography using 40% ethyl acetate in hexane. Combined overall yield of these cyclic peptides from **35** was 25%. For **36A**. ^{1}H NMR: δ 0.85–1.95 (complex, 25 H), 2.40–3.88 [complex, 8 H, 3.62 (s, 3 H)], 4.17 (d, J = 9 Hz, 1 H), 4.66 (s, J = 9 Hz), 5.65 (d, J = 9 Hz, 1 H), 6.62 (d, J = 9 Hz), 7.00 (bs, 1 H), 7.1–7.3 (m, 5 H). MS (EI): m/e 581 (M^+). MS (CI): m/e 582 (M^+ + 1). **36B**, ^{1}H NMR: δ 0.85–1.9 (complex, 25 H), 2.6–3.85 [complex, 8 H, 3.78 (s, 3 H)], 4.25 (d, J = 8 Hz, 1 H), 4.61 (d, J = 8 Hz, 1 H), 5.62 (d, J = 8 Hz), 6.60 (d, J = 8 Hz), 6.87 (bs, 1 H), 7.0–7.3 (m, 5 H). MS (EI): m/e 581 (M^+). MS (CI): m/e 582 (M^+ + 1).

Methyl 13α-[[2(*S*)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]amino]-3,3,14,14-tetramethyl-6,12-dioxo-7-(phenylmethyl)-1,2-dithia-5,11-diazacyclotetradecane-4(*R*),4α-carboxylate Hydrochloride (2′,6′-Me_2-Tyr-D-Pen-Glyψ(CH_2CH_2)Phe-D-Pen-OMe, HCl) (7A and 7B). Conversion of **36A** to **7A** involved an identical procedure used in the conversion of **13** to **4**. Yield: 80%. ^{1}H NMR (DMSO-d_6): δ 0.82 (s, 3 H), 1.02 (s, 3 H), 1.22 (s, 3 H), 1.23 (s, 3 H), 1.36–1.62 (complex, 3 H), 1.76 (m, 1 H), 2.19 (s, 6 H), 2.46–2.54 (complex, 2 H), 2.71–2.78 (complex, 2 H), 2.89 (dd, J = 14, 8.8 Hz, 1 H), 3.00 (dd, J = 14, 10 Hz, 1 H), 3.40 (m, 1 H), 3.54 (s, 3 H), 4.11 (m, 1 H), 4.18 (d, J = 9.0 Hz, 1 H), 4.43 (d, J = 9.5 Hz, 1 H), 6.36 (s, 2 H), 7.11–7.24 (complex, 5 H), 7.80–8.40 (bs, 2 H), 8.09 (d, J = 9 Hz, 1 H), 8.19–8.24 (complex, 2 H), 9.03 (s, 1 H). HRMS (EI): calcd for $C_{34}H_{46}N_4O_6S_2$ m/z 672.3016, found 672.3035. HPLC: t_R = 32.1 min (solvent, methanol/water/trifluoroacetic acid 50/50/0.1), t_R = 21.18 min (solvent, methanol/water/trifluoroacetic acid 60/40/0.1). Anal. ($C_{34}H_{48}N_4O_6S_2$·HCl·1.25H_2O): C, H, N, S. Cl, calcd: 4.84; found, 5.42.

Conversion of **36B** to **7B** involved an identical procedure used in the conversion of **13** to **4**. Yield: 50%. ^{1}H NMR (DMSO-d_6): δ 0.79 (s, 3 H), 0.89 (s, 3 H), 1.30 (s, 3 H), 1.36 (s, 3 H), 1.43 (m, 2 H), 1.62 (m, 1 H), 1.76 (m, 1 H), 2.20 (s, 6 H), 2.51–2.62 (complex, 2 H), 2.71–2.83 (complex, 2 H), 2.94 (dd, J = 13, 9.0 Hz, 1 H), 3.05 (dd, J= 14, 11 Hz, 1 H), 3.30 (bs, 1 H), 3.45 (s, 3 H), 3.92 (d, J = 8.0 Hz, 1 H), 4.15 (m, 1 H), 4.26 (d, J = 9.0 Hz, 1 H), 6.37 (s, 2 H), 7.12–7.26 (complex, 5 H), 7.80 (d, J = 7.5 Hz, 1 H), 8.27 (m, 1 H), 8.12–8.42 (complex, 3 H), 8.45 (d, J = 9 Hz, 1 H), 9.04 (s, 1 H). HRMS (EI): calcd for $C_{34}H_{46}N_4O_6S$ m/z 672.3016, found 672.2992. HPLC: t_R = 44.6 min (solvent, methanol/water/trifluoroacetic acid, 50/50/0.1), t_R = 25.29 min (solvent, methanol/water/trifluoroacetic acid, 60/40/0.1). Anal. ($C_{34}H_{48}N_4O_6S_2$·HCl·1.5H_2O): C, H, N, S. Cl, calcd: 4.81; found, 5.34.

Pharmacological Methods. Binding Assays. Male Sprague-Dawley albino rats (150–300 g) were stunned and decapitated. Their forebrains (minus the cerebellum and associated hindbrain) were quickly removed and rinsed in ice-cold 50 mM Tris buffer, pH 7.4, and homogenized in 20 volumes of buffer with a Polytron (Brinkman) at setting 6 for 30 s. The membranes were washed by centrifugation for 20 min at 30000g, followed by resuspension to twice the original volume. The homogenate was incubated at 25 °C for 1 h followed by centrifugation as above. The final pellet was resuspended to 10 mg of protein/mL (assuming 6% of wet weight is protein) and 4-mL aliquots were rapidly frozen in liquid N_2. The concentrated homogenates were stored for 6 months with no loss in activity. In some instances the membrane homogenates used were prepared as above but were obtained from Analytical Biological Services Inc. (Wilmington, DE). The purchased membranes appeared identical in the radioligand binding assays to our in-house preparation.

After thawing, the membranes were diluted 1/4 and to each well of a microtiter plate were added 100 μL of tissue, 50 μL of [^{3}H]DSLET (2 nM final, specific activity = 42 Ci/mmol from Du Pont/NEN) or [^{3}H]DAMPGO (4 nM final, specific activity = 30–45 Ci/mmol from Du Pont/NEN), and 50 μL of drugs. Nonspecific binding was measured in the presence of 10 μM levorphan. Following incubation for 60–90 min at 25 °C, samples were filtered under reduced pressure through GF/B-type filters with the aid of either a Brandel or Skatron harvester. The filters were washed with ice-cold buffer and specific binding was measured by either a Packard CA1900 or an LKB Betaplate counter. Under these conditions, the K_d for [^{3}H]DSLET is 2.0 nM with a B_{max} of 100 fmol/mg protein. The K_d for [^{3}H]DAMPGO was 1.3 nM with a B_{max} of 200 fmol/mg protein.

Writhing Assay. Male Charles River albino mice (CD-1/HAM/1LR) weighing between 20 and 30 g were used. Twenty five minutes after subcutaneous or intragastric administration of the test compound (0.1 mL/10 g of body weight), 0.025% (w/v) phenylbenzoquinone was injected intraperitoneally (0.1 mL/g of body weight). Five minutes later each mouse was placed in a large glass beaker and the number of writhes that occurred in the subsequent 10 min was recorded. A writhe consisted of a dorsoflexion of the back, extension of the hind limbs and strong contraction of the abdominal musculature. The test compound was considered to have produced analgesia in a mouse if the number of writhes elicited by phenylbenzoquinone was equal to or less than half the median number of writhes recorded for the saline-treated group that day. The results were expressed as the number of mice (out of 10 possible) in which the test compound

produced analgesia. The test compound was rated active if the number of writhes in the drug treatment group was significantly less than the number of writhes in the saline treatment group as determined by a one-way analysis of variance. If the initial test dose of 10 mg/kg inhibited writhing in greater than seven out of 10 mice, the effect of additional doses was evaluated and an ED_{50} value was calculated using a maximum likelihood function.

Tail-Flick and Hot-Plate Tests. Male albino mice (Charles River Laboratory, Portage, MI; CD-1/HAM/ICR) 20–30 g were used for testing. Testing occurred during the light segment of a 12 h light/12 h dark cycle. Tail-flick (TF) and hot-plate (HP) tests were administered to each mouse. In the TF test, each animal's tail was blackened and then a high-intensity beam of light (IITC, Model 33TF; Woodland Hills, CA) was focused on the tail and the time that elapsed between light onset and the reflexive removal of the tail was measured to the nearest $^1/_{10}$ s via photosensors (i.e., tail flick latency; TFL). The average TFL of two successive tail flick determinations was used for data analysis. Immediately following the second TF determination during each trial the mice were placed on a 55 ± 0.5 °C copper hot plate (IITC, Model 35-D; Woodland Hills, CA) and confined with a 600-mL glass beaker. The interval between placement on the hot plate and either a jump or hindpaw lick (i.e., hot plate latency; HPL) was measured to the nearest $^1/_{10}$ s. Tests were terminated after a maximum of 12 s during the TF test or 40 s during the HP tests to prevent tissue damage.

Prior to drug or vehicle administration, mice were tested in the HP and TF (i.e., time = 0) and only those animals with TFL ≤ 4.0 s or HPL ≤ 15 s were used for subsequent testing. TFL's and HPL's were redetermined at 10, 30, 60, 90 (sc and icv), and 120 (icv only) min following an injection. Groups of 10 mice were subcutaneously administered either 0 (vehicle), 10, 30, or 100 mg/kg of compound 4 in a volume equivalent to 10 mL/kg. Alternatively, 10 μg 4 or vehicle was administered intracerebroventricularly in a volume of 5 μL. A repeated analysis of variance was conducted on TFL and HPL scores to determine if an effect of group treatment was significant ($p > 0.05$); if there was a significant main effort following this analysis, contrast t-tests at each time interval were performed to compare groups at equivalent time intervals.

Drug-Discrimination Studies. Six adult male Long-Evans hooded rats (Charles River Breeding Labs, Portage, MI) trained previously to discriminate between sc injections of morphine and water were maintained at 85% of their ad libitum body weights. Training and testing sessions occurred in commercially-available operant conditioning chambers (BRS/LVE, Inc., Laurel, MD; Model SEC 002 CFS) equipped with two response levers, a white house light, and a food hopper into which 45 mg food pellets (BIO-SERV, Inc., Frenchtown, NJ; #0021) could be delivered. During daily (Mon) 15-min training sessions a food pellet was delivered following the completion of every 10 lever presses on the lever designated correct for that day. The lever designated correct during a training session depended upon whether 3.0 mg/kg morphine sulfate or water had been injected prior to start of the session. Morphine- and water-training sessions alternated following a two monthly alternating sequence (M = morphine and W = water: (1) MWWMW, WMMWW, WMWMM, MWMWM and (2) WMMWW, MWMWM, MWWMM, WMWMW). For half the rats the left lever, and for the other half the right lever, was designated the morphine-correct lever. Responses on the incorrect lever did not have a scheduled consequence but were recorded. Test sessions occurred on Tuesday and Friday and only if a rat completed the first 10 responses on the correct lever during the immediately preceding water- and morphine-training sessions. Test sessions were identical to training sessions except completions of every 10 lever presses on either lever resulted in pellet delivery. Compound 4 was tested at 0 (i.e., vehicle; $N = 6$), 30 ($N = 6$), 60 ($N = 5$), 90 ($N = 5$), and 120 ($N = 3$) mg/kg. Different numbers of rats were tested at each dose because some rats died before completing the experiment. Morphine was dissolved in sterile water. 4 was dissolved in 14.25% w/v (2-hydroxypropyl)-β-cyclodextrin (RBI Inc., Wayland, MA; H-107) in water. All injections of morphine and water were administered 30 min prior to the start of the session. All injections of 4 and vehicle were administered 90 min prior to start of the session. Preliminary analgesic studies in rats suggested that these injection times for morphine and 4 would likely arrange the time of peak effect for these drugs to occur during testing. Injections were administered sc into the back of the rat in a volume of 1 mL/kg. A drug would be considered to have substituted for morphine if it produced an average of >90% responding on the morphine-designated lever.

Physical-Dependence Studies. Five adult male CD-1 mice (Charles River Breeding Labs, Portage, MI), weighing between 22 and 28 g at the time of testing, served as subjects. The mice had ad libitum access to food and water and were maintained on a 12 h light/12 h dark cycle. All injections and testing occurred during the light cycle. Compound 4 was injected twice daily (at ca. 0900 and 1600 h) at 210, 280, and 375 mg/kg sc on consecutive days 1, 2, and 3, respectively. On day 4 the mice received a morning injection of 375 mg/kg and 4 h later were challenged with a 10 mg/kg sc dose of naloxone. For 30 min following the naloxone injection, the mice were continuously observed and any instance of jumping (all four paws off the floor) was recorded. Morphine-dependent mice will normally engage in vigorous jumping when challenged with naloxone, and naloxone-precipitated jumping was used to infer opiatelike dependence in the present study.

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