Peptide Mimetics of Thyrotropin-Releasing Hormone Based on a Cyclohexane Framework: Design, Synthesis, and Cognition-Enhancing Properties

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The design and synthesis of peptide mimetics of thyrotropin-releasing hormone (TRH) in which the peptide backbone is entirely replaced by a cyclohexane framework are described. The cis-1,3,5-trisubstituted ring was expected to permit key pharmacophoric groups to adopt conformations consistent with proposed bioactive conformations of the peptide. Compounds were synthesized by a stereoselective synthesis starting from L-glutamic acid. In a behavioral model of cognition in which TRH is active, the mimetics are potent, active compounds, exhibiting oral activity. One analog (**26**, (1S,3R,5(2S),5S)-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-5-[(1H-imidazol-5-yl)methyl]cyclohexaneacetamide) was radiolabeled for binding studies and evaluated in other binding assays and pharmacological tests. Competition binding of **26** vs [³H]MeTRH to rat brain slices suggests a two-site model for ligand binding with IC₅₀'s of 1 μ M and 3 mM. Direct binding of [³H]-**26** shows a biphasic curve with IC₅₀'s of 80 and 49 μ M, respectively. Further studies would be needed to establish a link between the novel binding site(s) and the behavioral activity of **26** and TRH analogs.

The design of nonpeptide molecules that mimic the interaction of peptide ligands with their biological receptors is the principal goal of peptide mimetics research. The approach is based on the analysis of structure-activity relationships (SAR) of peptide analogs, peptide bond surrogates, and constrained peptides together with structural studies, including X-ray crystallography, NMR, and conformational analysis. When the three-dimensional pharmacophore of a peptide can be defined, rational steps can be followed in the transition to a nonpeptide molecule. The methodology to perform peptide to nonpeptide transformations is still in the early stages of development and is the subject of intensive investigation.¹⁻⁴

We are pursuing an approach to replace the peptide backbone of selected peptides by a nonpeptide framework that carries peptide side-chain groups in a lowenergy conformation coincident with the proposed bioactive conformation of the peptide.^{2,4} In general, if a peptide can be shown to interact with its receptor principally via its side-chain functional groups, then backbone replacement should not compromise receptor interaction. Similar approaches have been pursued by Hirschmann and colleagues for their design of somatostatin mimetics based on a glucose scaffolding.³ Several groups have also obtained potent nonpeptide mimetics of the Arg-Asp-Gly sequence (RGD mimetics) that inhibit GpIIb/IIIa binding to fibrinogen receptors.⁵ We have applied a backbone replacement approach to peptide mimetics for several targets,⁴ each selected because there are well-founded hypotheses concerning the orientation and role of the pharmacophoric sidechain substituents and the bioactive conformations of the peptide. In this paper, we describe our efforts to design and synthesize mimetics of the neuroendocrine

peptide thyrotropin releasing hormone (TRH, pGlu-His-ProNH $_2$) using a cyclohexane framework to replace the peptide backbone.



Thyrotropin-releasing hormone elicits a wide range of biological responses. It functions as a neuroendocrine hormone by increasing thyrotropin-stimulating hormone (TSH) leading to an elevation of thyroid hormone levels. TRH binds to high- and low-affinity receptors labeled by [³H]TRH and [³H]MeTRH in discrete brain regions.⁶ In addition to its hormonal activity, TRH has pronounced central nervous system (CNS) effects. The effect of TRH on some CNS measures appears to be related to its ability to facilitate cholinergic and monoaminergic neurotransmission,7 independently of its hormonal activity.8 The ability of TRH and TRH peptide analogs to enhance cognitive performance in behavioral models in animals⁹ suggests its potential to treat cognitive disorders, including those associated with Alzheimer's disease. These CNS effects are not observed when TRH is administered orally because the compound, like most peptides, is rapidly metabolized. This limitation has been addressed through the preparation of numerous degradation-resistant TRH analogs,¹⁰ some of which have been studied clinically. However, with the exception of Nva²- and Nle²-TRH analogs¹¹ and TA-0910,¹² endocrine side effects of TRH analogs are observed at doses below or similar to those which produce CNS effects. While endocrine activity correlates well with high-affinity [³H]MeTRH receptor

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Figure 1. X-ray (open bonds) and proposed bioactive (solid bonds) conformations of TRH.

binding,¹³ there is little correlation between high-affinity binding of TRH analogs *in vitro* and inhibition of haloperidol catalepsy *in vivo*,¹⁴ stimulation of phosphoinositide turnover *in vitro*,¹⁵ or activity in behavioral models of cognition. Although there is no direct evidence for TRH receptor subtypes associated with these individual activities, the results are difficult to rationalize solely on the basis of tissue specificity or compartmentalization of certain analogs. These findings suggest the possibility of identifying TRH analogs or mimetics that are selective for one or more of these properties and which might prove useful in identifying novel receptor subtypes. The existence of low-affinity TRH receptors which bind CNS-selective analogs (e.g., Nva²- and Nle²-TRH) has previously been suggested.¹⁶

Design

Application of a peptide mimetics approach to TRH is facilitated by the abundance of structure-activity data from peptide analogs¹⁷ and by the fact that crystal^{18,19} and solution structures²⁰ of TRH and TRH peptide analogs have been determined. In addition, models have been proposed for the pharmacophore and bioactive conformation(s) of TRH and TRH analogs. In Marshall's lab, Moore²¹ proposed the lactam moiety of the pyroglutamyl residue, the histidine imidazole ring, and the carboxamide of the terminal prolinamide as pharmacophoric groups on the basis of activities in TSH release and high-affinity receptor binding. Studies of CNS effects of TRH analogs²² suggest a similar pharmacophore but with a wider tolerance for variants at the His² position. In the X-ray and proposed bioactive conformations of TRH, these key groups are oriented to form a Y- or propeller-shaped structure, and there is evidence from crystal structures that the Y-shaped conformations are more closely associated with CNS effects.²³ For the purposes of our mimetics design, we adopted the Moore and Marshall model in which the peptide backbone approximates the X-ray structure of TRH. However, in our model, we considered alternative conformations of the imidazole side chain in which the terminal carboxamide is not locked in a hydrogenbonded interaction as in the crystal but is free to adopt conformations consistent with solution NMR studies. This model (Figure 1) is analogous to that proposed by Marshall and colleagues^{24,25} and is similar to several of the Y-shaped conformations suggested by Stezowski et $al.^{23}$

In our design of a TRH mimetic, we recognized that the spatial orientation of the pharmacophore could be approximated by replacement of the peptide backbone of TRH with a cis-1,3,5-trisubstituted cyclohexane framework. The cyclohexane was chosen because SAR studies had not implicated any backbone hydrogen bonds as pharmacophores and because we felt that a scaffolding composed of methylenes would contribute only hydrophobic character, without adding other specific binding interactions that could have been present, for example, with aromatic ring-, sugar-, or amide-containing templates. The replacement of the peptide backbone by the cyclohexane ring is also intended to make the molecule more like traditional drug compounds in terms of lipophilicity, improving the prospects for oral bioavailability, enzymatic stability, and penetration of the blood-brain barrier. Despite the greater synthetic challenge presented by the cyclohexane system, it represents a good test of the backbone replacement strategy and allows comparison of the activity of the designed, all-cis skeleton with other isomers.

Structure 1 retains the lactam, carboxamide, and imidazole pharmacophoric groups hypothesized to be required for activity and permits orientations consistent with the proposed bioactive conformation (Figure 2). At the same time, the cyclohexane ring system partially constrains the structure at its center while permitting conformational mobility in the side chains. Maintaining side-chain flexibility in a first-generation mimetic may be important because the model is hypothetical, not being based on the actual receptor-bound conformation.^{1a} In addition, variations in the side-chain conformations have been observed for TRH and TRH analogs in both crystal structures²³ and solution. Overall, the flexibility of 1 and TRH is similar, each having six rotatable bonds. Another feature of our design is that the mimetic with the cyclohexane framework lies within the volume occupied by the peptide backbone and side chains. This factor is potentially quite important, since a bulky framework or scaffolding external to the volume of the peptide could interfere with binding. Because the designed compound fits a chiral template, the cyclohexane mimetic is homochiral. In compound 1, the configuration at the four asymmetric centers is 1S, 3R, 5(2S), 5Sas depicted in Figure 2. In addition to compound 1, we also decided to prepare the analog 2 in which a methyl group replaces the imidazolylmethyl side chain to mimic the CNS-selective Nva²- and Nle²-TRH analogs of Szirtes.¹¹ Other variations of this substituent are reported in a separate paper.²⁶

Synthesis

Our approach to the synthesis of cyclohexane TRH mimetic 1 or 2 was to perform a stepwise attachment



Figure 2. Cyclohexane mimetic of TRH (solid bonds) superimposed on proposed bioactive (open bonds) conformation of TRH. The conformation of the mimetic was generated using the Multifit procedures of SYBYL (Tripos Assoc., St. Louis, MO) to match the pharmacophoric groups to the TRH template followed by minimization using the Tripos force field.

of functionalized side chains to the dione **3**, a compound having its single asymmetric center derived from Lglutamic acid. The key steps in realizing a stereocontrolled synthesis were the transformation of **3** into the separable diastereomeric ethoxycyclohexenones **11** and **12**, elaboration of side chains, and stereoselective hydrogenations of olefinic intermediates to establish the relative stereochemistry at ring junctures. The relative configurations of intermediates which were critical to the stereochemical outcome (e.g., **2**, **12**, **18**) were established by X-ray crystallography.



The synthesis of dione 3 proceeded from L-glutamic acid as illustrated in Scheme 1. L-Glutamic acid was benzylated using NaBH₄ and benzaldehyde, cyclized by heating in aqueous HCl, and esterified to give the lactam methyl ester 4. Reduction of 4 with NaBH₄ in MeOH/t-BuOH afforded the hydroxymethyl derivative 5 as a pure enantiomer after one recrystallization as judged by chromatography and ¹H-NMR of the derived (-)-MTPA ester.²⁷ Conversion of **5** to the iodide **6** via the tosylate and subsequent coupling with excess vinylmagnesium bromide/Li2CuCl4 gave the allyl compound 7. Ozonolysis of 7 with reductive workup gave aldehyde 8 which was treated with the ylide 9 to give the acrylate 10. Michael addition of ethyl acetoacetate, Dieckmann cyclization, and hydrolysis-decarboxylation steps afforded dione 3. Treatment of 3 with ethanol and p-TsOH afforded the ethoxycyclohexenone mixture of diastereoisomers 11 and 12 in a ca. 1:1 ratio. Chromatographic separation of the isomers and recrystallization of 12 made it possible to obtain pure diastereoisomers. In addition, the isomers could be equilibrated to the ca. 1:1 mixture by resubjecting them to the

etherification conditions, providing a means to recycle an unwanted diastereoisomer. However, since our synthesis was intended to prepare compounds with either a methyl (e.g., 2) or imidazolylmethyl substituent (e.g., 1), we decided to use isomer 11 to prepare the methyl series (Scheme 2) and isomer 12 to synthesize the imidazolylmethyl series (Scheme 3). These two routes differ conceptually in the order of introduction of the substituents.

For the methyl series (Scheme 2), ethoxycyclohexenone 11 was treated with methyllithium in 1:1 ether: THF followed by 50% HOAc to afford dienone 13. Peterson olefination²⁸ with ethyl (trimethylsilyl)acetate gave the dienic ester 14 as a 3:2 Z:E isomeric mixture. Catalytic hydrogenation (Pd/C, EtOH) of the mixture afforded the desired all-cis isomer 15 (1R,3R,5S,5(2S)) together with ca. 15% of other diastereoisomers. Chromatography and recrystallization of the final product obtained by hydrolysis of the ester and conversion to the amide with ethyl chloroformate/NH₃ gave isomerically homogeneous N-benzyl amide 16. N-Debenzylation of 16 to produce 2 was accomplished by reduction with Na/NH₃. The stereochemistry of product 2 was again confirmed by X-ray crystallography.

For the preparation of the imidazolylmethyl series (Scheme 3), the less polar, crystalline ethoxycyclohexenone 12 was used as the starting material and the acetic acid side chain was introduced first. Thus treatment of 12 with ethyl (trimethylsilyl)acetate/LDA followed by acidic rearrangement (6 N HCl) gave the enone ester 17. Catalytic hydrogenation of 17 over 10% Pd/C yielded the keto ester 18 as the cis isomer depicted, together with ca. 3-6% of the trans isomer. Recrystallization provided stereochemically homogeneous keto ester 18, whose stereochemistry was confirmed by X-ray crystallography. The imidazolylmethyl group was introduced in a two-step procedure. Thus a directed Aldol condensation²⁹ using the benzylimine/enamine 19 derived from diethyl phosphonoacetaldehyde gave the imine 20. Immediate treatment of crude 20 with tosylmethyl isocyanide following the van Leusen procedure³⁰ with benzylamine as the base to prevent amine exchange gave 21 as an E/Z olefin mixture. The product was isolated by chromatography with facile separation of the E- and Z-isomers. However, for the purpose of the synthesis, the isomers were recombined, hydrolyzed to the acid 22, and converted to the amide 23 as described for the methyl series.

Catalytic hydrogenation of the double bond in ester 21 (followed by amidation) or direct hydrogenation of

Scheme 1



Scheme 2



amide 23 gave different ratios of the all-cis isomer 24 and the trans isomer 25 depending on the catalyst used. Ester 21 afforded a 2:8 ratio of cis/trans with 10% Pd/ C. This outcome is different from the case of the methyl series (e.g., $14 \rightarrow 15$) where the predominant product is cis. A similar mixture was produced by Pd/C reduction of amide 23. However, Ra-Ni yielded a 9:1 ratio favoring the desired all-cis isomer, regardless of the E/Zstereochemistry of the exocyclic double bond. One possible explanation is that for Pd, the large benzylimidazole group hinders approach of the *re* face to the catalyst surface, leading to the trans, 3R-product. By contrast, the amide may be complexed with nickel on one face prior to reduction from the opposite side. For efficient preparation of 24, amide 23 was reduced with Ra-Ni, whereas for preparation of 25, ester 21 was reduced over 10% Pd/C and the product subsequently converted to the amide. The isomerically pure amides 24 and 25 were obtained by chromatography, and stereochemical assignments were based on two-dimensional ¹H-NMR. Monodebenzylation of the imidazole group was achieved by a second catalytic hydrogenation at 50 °C/50 psi over 10% Pd/C to give compounds 26 and 27. Subsequent Na/NH₃ reduction gave the corresponding debenzylated products 1 and its isomer 28.

Pharmacology

Because of our primary interest in TRH mimetics as agents to treat cognitive disorders, their activity was first assessed in an animal model of cognitive dysfunction. Since no animal correlate of Alzheimer's disease is known, we chose to evaluate the effects of compounds in C57BL/10 mice, a strain that has been shown to be deficient in a spatial memory task³¹ potentially related to a reduction in hippocampal cell density. Performance in this mouse strain has been shown to be enhanced by classes of compounds with memory facilitation effects, including cholinergic³²⁻³³ and nootropic³⁴ agents as well as TRH and TRH analogs. The activity of compounds shown in Schemes 2 and 3 was determined in these mice using the Morris water maze test.³⁵ This task requires an animal to attend to spatial cues in order to locate the position of a hidden platform. Active compounds should reduce the time required to locate the platform. Mean latencies for TRH and the TRH mimetic compounds tested are listed in Table 1.

As shown in Table 1, TRH reduced mean latencies over a dose range from 0.003 to 3 mg/kg ip. At the peak active dose of 0.3 mg/kg, mice were able to locate the platform in approximately one-half the time of controls. The U-shaped dose—response curve (Figure 3) is typical for compounds affecting memory tasks in animals. The





cyclohexane-based mimetics were active in the Morris water maze test procedure. In the methyl series, for example, compound 2 was active at 0.1 mg/kg ip, in the same range as the peak active dose for TRH. Its *N*-benzyl precursor **16** was more potent than **2** or TRH. with significant activity from 0.003 to 0.1 mg/kg ip. Compound 16 also exhibited oral activity from 0.03 to 0.1 mg/kg. The imidazole series produced highly potent compounds which were active by both ip and oral routes. The all-cis mimetic 1 was active from 0.01 to 1 mg/kg ip, while its N-benzylpyrrolidinyl precursor 26 was highly active over a wide dose range (0.0003-3 mg/kg ip) and produced a robust reduction of latencies (see Figure 4). The *oral* potency of **26** in this animal model was virtually indistinguishable from its activity ip, demonstrating the excellent bioactivity by the oral route. We propose that this oral activity can be attributed in large part to the cyclohexane replacement of the peptide backbone.

The dose-response curves for **26** and its diastereoisomer **27**, which differs in chirality at the imidazolylmethyl group attachment to the cyclohexane ring, are compared in Figure 4. Peak activity occurs at ca. 10fold lower dose and is of a greater magnitude (up to 2-fold greater decrease in latency) for the all-cis diastereomer **26** than for its isomer **27**. Under the same conditions, mimetic **1** was more potent than its isomer **28**. This evidence of stereoselectivity supports the idea of a specific ligand-receptor interaction.

On the basis of the results for the methyl and imidazolylmethyl compounds and the isomers described, it would appear that the CNS activity of the mimetics does not *require* the presence of an imidazole substituent, but the potency is improved when it is present. The analysis of the activity of the diastereoisomers indicates that precise orientation of the imidazole substituent relative to the cyclohexane ring is not an absolute requirement for activity but does affect potency and efficacy. This result is also supported by the activity of other compounds having phenyl, 2-imidazolyl, and 1-methyl-4-imidazolyl groups in place of the 4-imidazolyl group²⁶ and by the analogous activity and stereoseletive potency of diastereoisomers of compounds 1, **28**, **26**, and **27** with all ring centers inverted.³⁶

While the TRH mimetics exhibited more potent and robust effects than TRH in the behavioral test, they were unlike TRH in other respects. If the cyclohexane compounds mimicked TRH in all respects, activity would be expected in endocrine TSH release assays and analeptic effects should be observed in reversal of pentobarbital sleeping time tests. In addition, a potent, classical TRH mimetic should bind to the high-affinity TRH receptor in rat brain membrane homogenate binding assays with nanomolar potency. None of these effects were observed for the cyclohexane mimetics. Thus, the methyl-substituted analogs 2 and 16, as well as diastereomeric mixtures (1 + 28) and (26 + 27) in the imidazolylmethyl series, were devoid of TRH-like increase in spontaneous TSH release in cultured rat pituitary cells up to 10^{-5} M, whereas TRH caused an increased release of TSH of 300-500% of control with an ED_{50} of 2 nM.³⁷ In this sense, the cyclohexane compounds, regardless of whether they possess an imidazolylmethyl group or an alkyl chain, resemble the CNS-selective Nva²-TRH (RGH 2202) or the Tanabe compound TA-0910.38 The cyclohexane mimetics also are similar to RGH 2202 in that they do not exhibit binding to high-affinity TRH receptors up to 10^{-5} M

Table 1. Effects of TRH and TRH Mimetics on Performance in
the Morris Water Maze Test^a

	dose	$\text{mean} \pm \text{SEM}$		dose	$mean \pm SEM$
compd	(mg/kg)	latency (s)	compd	(mg/kg)	latency (s)
TRH (ip)	vehicle	51.53 ± 0.64	26 (ip)	vehicle	50.71 ± 0.46
•	0.001	50.59 ± 0.94	•	0.00003	$62.15 \pm 0.65^{**}$
	0.003	$43.08 \pm 0.67^{**}$		0.0003	$33.29 \pm 0.52^{**}$
	0.01	$37.97 \pm 0.79^{**}$		0.003	$22.24 \pm 1.02^{**}$
	0.03	$32.69 \pm 0.74^{**}$		0.03	$15.65 \pm 0.45^{**}$
	0.1	$25.76 \pm 2.20^{**}$		0.3	$17.62 \pm 0.60^{**}$
	0.3	$20.85 \pm 0.53^{**}$		3.0	$31.62 \pm 0.96^{**}$
	1.0	$28.87 \pm 0.74^{**}$			
	3.0	$33.57 \pm 0.90^{**}$			
	10.0	$46.00 \pm 2.15^{**}$			
1 (ip)	Vehicle	49.89 ± 1.16	26 (po)	Veh	51.20 ± 0.35
	0.001	47.62 ± 0.93		0.00003	$46.67 \pm 0.49^{**}$
	0.01	$38.72 \pm 1.07^{**}$		0.0003	$30.90 \pm 0.38^{**}$
	0.1	$45.06 \pm 1.29^{**}$		0.003	$35.23 \pm 0.88^{**}$
	1.0	$25.23 \pm 0.91^{**}$		0.03	$21.10 \pm 0.63^{**}$
2 (ip)	vehicle	48.10 ± 2.21	27 (po)	vehicle	51.39 ± 0.32
	0.01	42.77 ± 3.29		0.00003	51.70 ± 0.69
	0.03	37.86 ± 2.43		0.0003	$42.81 \pm 0.47^{**}$
	0.1	$30.08 \pm 6.49^{**}$		0.003	$37.01 \pm 0.59^{**}$
	0.3	43.13 ± 4.21		0.03	$28.87 \pm 1.07^{**}$
				0.3	$27.59 \pm 0.71^{**}$
				3.0	$43.72 \pm 0.47^{**}$
16 (ip)	vehicle	48.92 ± 1.66	28 (ip)	vehicle	51.00 ± 0.49
	0.001	47.48 ± 3.83		0.01	57.12 ± 0.96
	0.003	$36.26 \pm 2.82^{**}$		0.1	$46.09 \pm 1.02^{**}$
	0.01	$22.15 \pm 2.68^{**}$		1.0	$24.19 \pm 0.77^{**}$
	0.03	$26.49 \pm 2.92^{**}$			
	0.1	$26.87 \pm 5.03^{**}$			
10()	0.3	46.83 ± 3.08			
16 (po)	ven	50.64 ± 2.28			
	0.003	42.20 ± 0.30			
	0.01	30.88 ± 8.20			
	0.03	$32.87 \pm 3.34^{\circ}$			
	0.1	24.00 ± 3.00^{mm}			
	0.3	40.03 ± 2.07			
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Compound Dose (mg/kg) ip

Figure 3. Effects of TRH on mean latency $(\pm SEM)$ of mice in the Morris water maze procedure to locate the escape platform. Vehicle or TRH was administered ip 30 min prior to the four-trial session.

when [³H]MeTRH is employed as the radioligand. Because of its potency and synthetic accessibility, the all-cis benzyl derivative **26** (Ro 24-9975) was selected for further pharmacological and biochemical evaluation. Compound **26** was active in reversing scopolamineinduced deficits in mice (like TRH) but, unlike TRH, did not reverse pentobarbital-induced sleep in mice (0.001-1 mg/kg ip). Compound **26** did not compete with [³H]-MeTRH in the rat brain homogenate membrane receptor binding assay³⁹ (IC₅₀ > 10⁻⁵ M) and was devoid of significant receptor binding in a panel of diverse recep-



Compound Dose (mg/kg) ip

Figure 4. Effects of TRH mimetic diastereoisomeric compounds 26 (solid bars) and 27 (hashed bars) on mean latency $(\pm \text{SEM})$ of mice in the Morris water maze procedure to locate the escape platform. Vehicle, compound 26, or compound 27 was administered ip 30 min prior to the four-trial session.



Figure 5. [³H]MeTRH vs compound **26**. Competition analysis of mimetic **26** inhibition of [³H]MeTRH binding exhibited a two-site model of ligand binding. The IC₅₀'s for the high- and low-affinity sites are 1 μ M and 3 mM, respectively. The proportion of high-affinity sites was 30%, while low-affinity sites account for the remaining 70% of sites. $R^2 = 0.939$.

tor binding assays (NovaScreen, see the supporting information).

These unexpected results suggested either that **26** exhibits cognitive effects by an unknown mechanism unrelated to TRH or that TRH and **26** exhibit activity in behavioral tests by interacting with the same, unknown mechanism. In either case, additional biochemical studies would be required to establish a mechanistic relationship to TRH.

As a first step, the binding characteristics of **26** to rat brain slices have been studied using [³H]MeTRH as radioligand under conditions established for analysis of TRH binding by Manaker et al.⁶ As illustrated in Figure 5, compound **26** competes for binding sites labeled with [³H]MeTRH with a biphasic binding curve with high- and low-affinity sites of 1 μ M and 3 mM, respectively. Since millimolar concentrations of **26** (mol wt. 408) would not be reached in mice dosed at levels which produced behavioral effects, the 3 mM site is probably not relevant to the cognitive effects.

In a second experiment, $[{}^{3}H]$ -26 was employed a radioligand. ($[{}^{3}H]$ -26 was readily prepared by iodination of 26 followed by catalytic reduction with ${}^{3}H_{2}$ over 10% Pd/C.) Binding studies, as shown in Figure 6, showed



Figure 6. [³H]-**26** vs compound **26**. Self-competition analysis using [³H]-**26** demonstrated a biphasic displacement curve with IC₅₀'s of high- and low-affinity sites of 80 μ M and 49 mM, respectively. High-affinity sites comprise 65% of binding sites, and the remaining 35% represent low-affinity sites. $R^2 = 0.884$.

specific and saturable biphasic binding with high- and low-affinity sites (80 μ M and 49 mM, respectively). Binding to these sites can be competed by excess (1 mM) of TRH, MeTRH, and TA-0910 (data not shown). The lower affinty site (49 mM) again is inconsistent with the pharmacologically active doses required to produce behavioral activity in mice. Kinetic analysis (data not shown) of [³H]-**26** binding indicates rapid association and dissociation, consistent with the observed binding affinities.

On the basis of this biochemical data, it is clear that 26 interacts with a different binding site than the nanomolar endocrine TRH receptor or that a metabolite (data not obtained) is responsible for receptor binding. The binding is characteristic of a receptor with an affinity in the low-micromolar range. Evidence from other studies supporting the existence of low-affinity TRH receptors remains controversial. Some TRH analogs, such as CG 3703 and DN-1417, elicit potent TRHlike CNS effects following administration yet bind TRH receptors with relatively low affinity.⁴⁰ A property of these compounds is their apparent CNS selectivity, as they demonstrate limited activation of pituitary TSH release. On the basis of these data, it has been hypothesized that the low affinities observed for CG 3703 and DN-1417 for the classical TRH receptor are directly correlated with their decreased ability to stimulate TSH release, i.e., to bind to classical high-affinity TRH receptors.^{16,41}

The relationship of the behavioral pharmacology of the cyclohexane TRH mimetics to the low-micromolar binding site observed in the present study is unclear, and further studies would be required to establish a definitive link between this site and the cognitive effects of TRH analogs or **26**. It is hoped that the cyclohexane mimetics may provide a stimulus to further study of the behavioral and pharmacological effects of TRH and TRH receptors.⁴²

Another question which remains open in this study is why the cyclohexane mimetics fail to interact with the endocrine, high-affinity TRH receptor. One possiblity is that the backbone replacement has eliminated one or more carbonyl or NH groups that may not have been recognized as essential parts of the pharmacophore. This possiblity suggested the synthesis of the

piperidine TRH mimetic 29, in which the pGlu carboxamide is restored to the structure. This compound was prepared as a mixture of diastereoisomers and found to be devoid of activity in either the behavioral or binding assay.⁴³ A second possibility is that the conformation which could bind to the TRH high-affinity endocrine receptor is not available to the cyclohexane mimetic. This is difficult to evaluate because of the inherent flexiblity of both TRH and the cyclohexane mimetics. Nevertheless, the result that the *N*-benzyl compound 26 is as active as the NH analog 1 suggests that these two compounds must be able to adopt the same conformation and that this conformation is associated with cognitive effects. N-Methylation of TRH peptides does reduce high-affinity binding and endocrine effects (TSH release) dramatically.²¹ This observation suggested that the N-benzyl group in 26 might select against the high-affinity binding/endocrine conformation. To have a proper reference for this hypothesis, we prepared N-benzyl TRH 30^{43} and determined that it was active in the Morris water maze test (1.0-10 mg/)kg ip) but only weakly active (ca. 2.8 μ M) in binding to the high-affinity endocrine receptor.44



Taken together, the results with N-benzyl TRH and piperidine **29** support a hypothesis that the conformation, rather than an additional pharmacophore, is the determinant of cognitive activity and that binding to the micromolar affinity binding site in rat brain slices is distinct from, and probably unrelated to, binding to the high-affinity endocrine TRH receptor. Furthermore, the imidazole NH on TRH and TRH analogs appears to be an essential pharmacophore for high-affinity binding and endocrine activity but is not essential for cognitive effects in the Morris water maze model.

Experimental Section

General. Melting points were determined on a Büchi Meltemp apparatus and are uncorrected. ¹H-NMR spectra were obtained using Varian XL-200 and XL-400 instruments. Infrared spectra were measured using a Digilab FTS-15E Fourier transform infrared spectrophotometer. FAB mass spectra were recorded using a VG-7070HF spectrometer. Thin layer chromatography was carried out on Merck silica gel GF254 plates and column chromatography on EM reagents silica gel 60 (70-230 or 230-400 (flash) mesh). Liquid chromatography was performed on Waters Prep 500 and Delta-Prep instruments using the columns and eluants indicated.

(S)-5-Oxo-1-(phenylmethyl)-2-pyrrolidinecarboxylic Acid Methyl Ester (4). L-Glutamic acid, monosodium salt (250 g, 1.33 mol) was added at room temperature to a solution of NaOH (53.6 g, 1.34 mol) in 550 mL of water. To the resulting solution was added benzaldehyde (142.3 g, 1.34 mol). The mixture was stirred and cooled to 10 °C, and NaBH₄ (15.2 g, 0.409 mol) was added in portions, keeping the temperature at 10–15 °C. The mixture was stirred for 30 min, and another portion of benzaldehyde (7.5 g, 0.07 mol) was added. After 10 min, a second portion of NaBH₄ (3.7 g, 0.10 mol) was added as before. The mixture was then allowed to stir at room temperature overnight. The solution was washed with CH₂Cl₂ (250 mL, discarded) and acidified to pH 3 with 6 N HCl. The paste, consisting of N-(phenylmethyl)-L-glutamic acid, was diluted with 500 mL of H₂O and heated to reflux overnight. The resulting solution was cooled to room temperature and extracted with chloroform. The combined extracts were washed with brine, dried over Na_2SO_4 , and concentrated on a rotary evaporator to give 160 g (55% yield) of (S)-5-oxo-1-(phenylmethyl)-2-pyrrolidinecarboxylic acid as a white solid. The crude acid (160 g, 0.73 mol) was dissolved in 300 mL of toluene and 550 mL of methanol. To the solution was added 9 mL of concentrated H_2SO_4 , and the solution was heated to reflux overnight. The solution was cooled in an ice bath and neutralized to pH 5 with 25% NaOH followed by adding 50 mL of saturated NaHCO₃ to bring the solution to pH 7. The methanol was removed on a rotary evaporator, and the residue was diluted with 500 mL of water and extracted with CH₂Cl₂. The combined extracts were washed with brine and dried over MgSO₄. Evaporation of the solvent afforded 132 g of the known⁴⁵ methyl ester 4 as an oil which was >95% pure by NMR: ¹H-NMR (CDCl₃) δ 2.0–2.6 (m, 4 H, CH₂'s), 3.68 (s, 3 H, CH₃), 3.95 (dd, 1 H, J = 4, 9 Hz, H-4), 4.02 and 5.03 (AB, 2 H, $J_{gem} = 16$ Hz, CH₂Ph), 7.2-7.4 (m, 5 H, arom).

(S)-5-(Hydroxymethyl)-1-(phenylmethyl)-2-pyrrolidinone (5). A solution of the ester 4 (132 g, 0.556 mol) in 600 mL of t-BuOH was cooled to ca. 18 °C. Sodium borohydride (41 g, 1.12 mol) was added in one portion. To the suspension was added 425 mL of methanol over 45 min. The reaction mixture was kept at 15 °C during the addition, during which time hydrogen gas evolved. After the addition, the mixture was kept at 20 °C until hydrogen evolution had subsided and was allowed to stand at room temperature overnight. The solvents were removed on a rotary evaporator, and the residue was dissolved in 1 L of water and the pH adjusted to pH 7.5 with 2 N HCl. The mixture was extracted with CH_2Cl_2 , and the combined extracts were washed with 2 N HCl and brine and dried over Na₂SO₄. Evaporation of the solvent afforded 96.3 g of crude 5, which was recrystallized once from toluene to give 89.5 g (77% yield) of pyrrolidinone 5 as a white solid: mp 82–84 °C (toluene); ¹H-NMR (CDCl₃) δ 1.92 and 1.94 (dd, J = 6.5 Hz, 1 H, OH), 1.96–2.11 (m, 2 H, CH₂), 2.36–2.61 (m, 2 H, COCH₂), 3.48-3.78.(m, 3 H, CH₂O and CH), 4.29 and 4.81 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 7.26–7.35 (m, 5 H, phenyl); $[\alpha]^{25}_{D} = +116^{\circ}$ (c 1.07, methanol). Anal. (C₁₂H₁₅NO₂) C,H,N.

The optical purity of the (S)-pyrrolidinone **5** was established by formation of the ester derived from (S)-(-)- α -methoxy- α -(trifluoromethyl)phenylacetic acid ((-)-MTPA) following the Mosher procedure.²⁷ A single diastereomeric ester, as judged by ¹H-NMR and TLC, was obtained and could readily be differentiated from the diastereomeric mixture (by the doubling of the CH₂N and CH₂O signals in the ¹H-NMR) produced when racemic 5-(hydroxymethyl)-1-(phenylmethyl)-2-pyrrolidinone was used to form the (-)-MTPA ester.

(S)-5-(Iodomethyl)-1-(phenylmethyl)-2-pyrrolidi**none (6).** To a solution of the alcohol 5 (143.7 g, 0.70 mol) in 3.5 L of CH₂Cl₂ were added 4-(dimethylamino)pyridine (94.1 g, 0.77 mol), and p-toluenesulfonyl chloride (133.5 g, 0.70 mol). The solution was stirred at room temperature overnight and then washed with cold 1 N HCl (600 mL), saturated sodium bicarbonate (500 mL), and brine and dried over sodium sulfate (Na_2SO_4) . Evaporation of the solvent gave the crude tosylate (244.6 g, 97.2% yield) as a white solid, mp 79-80 °C (ether), which was refluxed overnight with sodium iodide (305 g, 2.03 mol) in 3 L of acetone. The suspension was cooled to 10 $^\circ\mathrm{C}$ and filtered. The salts were rinsed with three 250-mL portions of acetone, and the acetone washes and filtrate were concentrated on a rotary evaporator to a thick slurry. Methylene chloride (1.5 L) was added, and the white precipitate was filtered off and washed with CH_2Cl_2 . The filtrate was dried over MgSO₄, filtered through a pad of silica gel, and concentrated on a rotary evaporator to give 196.8 g (92% yield crude) of iodide **6** as an off-white solid. A sample recrystallized from cyclohexane had mp 92–94 °C: ¹H-NMR (CDCl₃) δ 1.7–2.75 (m, 4 H, CH₂), 3.18-3.28 (m, 2 H, CH₂I), 3.42 (m, 1 H, NCH),

3.98 and 4.05 (AB, 2 H, J_{gem} = 15 Hz, CH₂Ph), 7.20 (m, 5 H, phenyl); [α]²⁵_D = +12.11° (*c* 0.35, methanol). Anal. (C₁₂H₁₄NOI) C,H,N.

(S)-1-(Phenylmethyl)-5-(2-propenyl)-2-pyrrolidinone (7). To a solution of crude iodide 6 (31.5 g, 0.10 mol) in 250 mL of anhydrous THF was added a solution of Li₂CuCl₄ (7.5 mL of a 0.1 M solution in THF; prepared from anhydrous lithium chloride (85 mg) and anhydrous cupric chloride (134.5 mg) in 10 mL of THF). The solution was cooled to -78 °C and vinylmagnesium bromide (200 mL of a 1 M solution in THF) was added over 25 min. After stirring for 1 h at -78 °C, a second portion of vinylmagnesium bromide (200 mL of a 1 M solution) was added as before. After stirring for another 1 h, a third portion of vinylmagnesium chloride (200 mL of a 1 M solution) was added as before, and the mixture was stirred at -78 °C overnight. The cold solution was then poured onto 1.5 kg of ice and acidified with 100 mL of 6 N HCl. The mixture was extracted with CH₂Cl₂, washed with saturated NaHCO₃ and brine, and dried over MgSO4. The solution was concentrated on a rotary evaporator to give 23.8 g of crude propenylpyrrolidinone 7 as a brown oil. The crude product was chromatographed on 700 g of silica gel, eluting with 50% EtOAc in hexanes to give 14.2 g (66% yield) of the pure 7 as a colorless oil: 1H-NMR (CDCl₃) & 1.7-2.75 (m, 6 H, CH₂'s), 3.51 (m, 1 H, NCH), 4.99 and 5.02 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 5.0-5.2 (m, 2 H, vinyl H), 5.4-5.9 (m, 1 H, vinyl H), 7.25-7.40 (m, 5 H, phenyl); $[\alpha]^{20}_{D} = +31.87^{\circ}$ (c 1.0, MeOH).

(S)-5-Oxo-1-(phenylmethyl)-2-pyrrolidineacetaldehyde (8). A solution of propenylpyrrolidinone 7 (44.8 g, 0.208 mol) in 800 mL of 1:1 methanol:CH₂Cl₂ was cooled to -78 °C and ozonized using a Welsbach ozonizer for 6.5 h, approximately 1 h longer than the time required to observe a light blue color of ozone in the solution. Excess ozone was flushed out of the system with oxygen, and the solution was treated with methyl sulfide (80 mL). The solution was then allowed to come to room temperature and stand overnight. The solvents were removed on a rotary evaporator, and the residual liquid was dissolved in 700 mL of CH₂Cl₂, washed with water, and dried over Na₂SO₄. Evaporation of the solvent afforded 43.0 g of crude aldehyde 8. Chromatography on silica gel (1 kg) eluting with 4% methanol in CH_2Cl_2 afforded 38 g (84.4% yield) of the pure aldehyde 8: ¹H-NMR (CDCl₃) δ 1.60-2.60 $(m, 4 H, CH_2), 2.77$ (one-half of ABX, 1 H, $J_{gem} = 18 Hz, J_{vic} =$ 4 Hz, CH₂CHO), 3.98 (m, 1 H, NCH), 4.10 and 4.82 (AB, 2 H, J_{gem} = 15 Hz, CH₂Ph), 7.15-7.40 (m, 5 H, phenyl), 9.65 (s, 1 H, CHO).

(E)-4-[2(R)-5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]-2butenoic Acid Ethyl Ester (10). To a solution of aldehyde 8 (38 g, 0.175 mol) in 700 mL of toluene was added phosphorane 9 (73.0 g, 0.21 mol), and the mixture was heated to 90°C for 5.5 h. The solvent was removed on a rotary evaporator, and the residue was slurried with 50 mL of EtOAc. The bulk of the precipitate of triphenylphosphine oxide was removed by filtration, and the filter cake was washed with 150 mL of 1:1 EtOAc:hexane. The solution was concentrated on a rotary evaporator, mixed with 200 mL of 3:1 hexane:EtOAc, and allowed to stand at room temperature for 72 h. The crystalline triphenylphosphine oxide was filtered off, and the residual oil (63 g) was chromatographed on 2 kg of silica gel, eluting with EtOAc to give 48 g (95.6% yield) of butenoate 10 as an oil: ¹H-NMR (CDCl₃) δ 1.30 (t, J = 5.5 Hz, CH₃), 1.60–2.50 (m, 6 H, CH₂'s), 3.60 (m, 1 H, NCH), 3.96 and 4.99 (AB, $J_{gem} = 15$ Hz, CH₂Ph), 4.15 (q, J = 5.5 Hz, 2 H, OCH₂), 5.85 (d, J = 16Hz, 1 H, C-2 vinyl H), 6.74 and 6.80 (dt, J = 8, 16 Hz, 1 H, C-3 vinyl H), 7.20–7.40 (m, 5 H, phenyl); $[\alpha]^{25}_{D} = -20^{\circ}$ (c 1.0, MeOH); HRMS (EI) calcd for C17H21NO3 287.1521, obsd 287.1523

(S)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-1,3-cyclohexanedione (3). Sodium metal (7.82 g, 0.34 g-atom) was dissolved in 1.1 L of ethanol. Ethyl acetoacetate (44.2 g, 0.34 mol) and butenoate 10 (66.0 g, 0.23 mol) were added, and the solution was refluxed and stirred overnight. The solvent was removed on a rotary evaporator, and the residue was dissolved in 500 mL of water. The solution was washed with CH_2Cl_2 (discarded), acidified to pH 1 with 6 N HCl, and extracted with CH_2Cl_2 . The combined extracts were washed with water and dried over Na_2SO_4 . The solvent was removed on a rotary evaporator to give 70 g of the intermediate diketo ester as an oil. Potassium hydroxide (128.8 g (2.3 mol)) was dissolved in 2.0 L of ethanol. To the solution was added 85.5 g (0.23 mol) of crude diketo ester (from this and a similar run on a smaller scale), and the solution was heated to reflux for 3 h. The ethanol was removed on a rotary evaporator, and to the residue was added 1200 mL of concentrated HCl. The mixture was heated at 85 °C for 3 h. The bulk of the aqueous HCl was removed on a rotary evaporator, and the residue was dissolved in 500 mL of water and made alkaline with Na₂CO₃. The solution was washed (discarded) with 200 mL of CH₂Cl₂, and the aqueous phase was acidified to pH 1 with 2 N HCl. The mixture (gummy residue) was extracted with $3 \times 300 \text{ mL}$ of CH₂Cl₂, and the combined extracts were washed with water and dried over NaSO₄. The solvent was removed on a rotary evaporator to give 60.0 g (87% yield) of cyclohexanedione 3 as a white solid: mp 159-161 °C (EtOAc); ⁱH-NMR (CDCl₃) (9:1 keto:enol forms) δ 1.38 (ddd, 1 H, J_{vic} = 4, 11 Hz, J = 14 Hz, CH of CH₂), 1.60-2.71 (m, 10 H, 4 CH₂, CH of CH₂, CH), 3.37 (s, 10/9 H, CH₂ of keto), 3.46 (m, 1 H, NCH), 3.94 and 4.96 (AB, 8/9 H, $J_{gem} = 15$ Hz, CH₂ of enol), 3.95 and 4.99 (AB, 10/9 H, $J_{gem} = 15$ Hz, CH₂ of keto), 5.45 (s, 4/9 H, -CH of enol), 7.19 (br d, 2 H, J_{ortho} = 7 Hz, arom), 7.30 (t, 1 H, J_{ortho} = 7 Hz, arom), 7.32 (t, 2 H, J_{ortho} = 7 Hz, arom); IR (CHCl₃) 1730, 1710, 1600, 702 cm⁻¹; MS (EI) m/e 299 (M⁺); $[\alpha]^{25}_{D} =$ +44.97° (c 1.04, methanol). Anal. (C18H21NO3) C,H,N

(5S,1R)-5-[(3-Ethoxy-5-oxo-3-cyclohexen-1-yl)methyl]-1-(phenylmethyl)-2-pyrrolidinone (11) and (5S,1S)-5-[(3-Ethoxy-5-oxo-3-cyclohexen-1-yl)methyl]-1-(phenylmethyl)-2-pyrrolidinone (12). A solution of cyclohexanedione 3 (60 g, 0.20 mol) and p-toluenesulfonic acid monohydrate (3.8 g, 0.02 mol) in 600 mL of ethanol and 1200 mL of toluene was stirred and refluxed for 1.5 h. The solvent was removed on a rotary evaporator and the residue dissolved in CH₂Cl₂. The CH₂Cl₂ solution was washed with saturated NaHCO₃ solution and brine and dried over Na₂SO₄. The solvent was removed on a rotary evaporator to give 57.6 g of a crude oil. The crude product was chromatographed on silica gel (800 g) eluting with 2-4% methanol in EtOAc to give a 1:1 mixture of diastereomeric products: the (5S,1R)-ethoxy enone 11 and the (5S,1S)ethoxy enone 12 (37.5 g, 57% yield). A total of 52.5 g of mixture (from this and a similar preparation) was chromatographed using a Waters Prep 500 high-pressure liquid chromatograph, eluting with 1:24:25 methanol:EtOAc:hexane and recovering and rechromatographing the mixed fractions. A total of 24.3 g (25.0% yield) of the more polar, 5S,1Rdiastereomer 11 as an oil and 23.9 g (24.6% yield) of the less polar, solid, 5S,1S-diastereomer 12 was obtained.

For the 5S,1R-diastereomer 11: TLC (ethyl acetate: methanol, 95:5) $R_f = 0.4$; $[\alpha]^{25}_{D} = 0.0^{\circ}$ (methanol, $c \ 1.0$); ¹H-NMR (CDCl₃) $\delta \ 1.35$ (t, J = 6 Hz, 3 H, CH₃), 1.50–2.50 (m, 11 H, CH₂ and CH), 3.46 (m, 1 H, CHN), 3.90 (q, J = 6 Hz, 2 H, CH₂CH₃), 3.95 and 5.05 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 5.35 (s, 1 H, vinyl H), 7.24–7.34 (m, 5 H, phenyl); MS (EI) m/e 327 (M⁺); IR (CHCl₃) 1672 cm⁻¹ (C=O). Anal. (C₂₀H₂₅NO₃) C,H,N.

For the 5S,1S-diastereomer 12: mp 92–93 °C; TLC (ethyl acetate:methanol, 95:5) $R_f = 0.5$; $[\alpha]^{25}_D = +74.61^{\circ}$ (methanol, $c \ 1.0$); ¹H-NMR (CDCl₃) $\delta \ 1.35$ (t, J = 6 Hz, 3 H, CH₃), 1.50–2.50 (m, 11 H, CH₂ and CH), 3.46 (m, 1 H, CHN), 3.90 (q, J = 6 Hz, 2 H, CH_2CH_3), 3.96 and 5.00 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 5.35 (s, 1 H, vinyl H), 7.24–7.34 (m, 5 H, phenyl); MS (EI) m/e 327 (M⁺); IR (CHCl₃) 1672 cm⁻¹ (C=O). Anal. (C₂₀H₂₅NO₃) C,H,N.

For an X-ray analysis, crystals were grown from EtOAc/ MeOH. The stereochemistry was determined to be 5S,1S for 12 with final discrepancy indices of R = 0.040 and $R_w = 0.057$. Absolute stereochemistry is inferred from the synthesis starting from L-glutamic acid. Details of the crystal structure are provided in the supporting information.

(5S,1R)-5-[(3-Methyl-5-oxo-3-cyclohexen-1-yl)methyl]-1-(phenylmethyl)-2-pyrrolidinone (13). To a solution of (5S,1R)-ethoxy enone 11 (3.94 g, 12.0 mmol) in 150 mL of 1:1 THF:ether was added a solution of methyllithium (41 mL, 1.4 M in ether, 57.4 mmol) at -78 °C over 10 min. The mixture was stirred for 30 min at -78 °C and the reaction guenched by the addition of 50 mL of 50% aqueous acetic acid. The mixture was allowed to warm to room temperature and was extracted with CH₂Cl₂. The combined extracts were washed with saturated NaHCO₃, dried over Na₂SO₄, and concentrated on a rotary evaporator to give 4.05 g of a crude oil. Chromatography of the crude product on silica gel (100 g) eluting with 1% methanol in EtOAc afforded 1.85 g (52% yield) of (5S,1R)keto ester 13 as an oil. A sample was evaporatively distilled at 200-210 °C/0.05 Torr for analysis: ¹H-NMR (CDCl₃) & 1.94 $(s, 3 H, CH_3), 1.35-2.55 (m, 11 H, CH_2 and CH), 3.45 (m, 1 H,$ NCH), 3.95 and 4.98 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 5.87 (s, 1 H, vinyl H), 7.50 (m, 5 H, phenyl); MS (EI) m/e 297 (M⁺); $[\alpha]^{25}_{D} = +59.86$ ° (c 0.2155, methanol); CD (ethanol) 326 ($[\Theta]$ = +1425, max), 283 ([Θ] = -42, min.), 238 ([Θ] = +13 620, infl), 218 ($[\Theta] = +50\ 100$, max), 212 nm ($[\Theta] = +39\ 820^\circ$, min.). Anal. (C₁₉H₂₃NO₂) C,H,N.

(5S-(5S))-[3-Methyl-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-2-cyclohexen-1-ylidene]acetic Acid Ethyl Ester (14). To a solution of diisopropylamine (1.50 g, 14.8 mmol) in 20 mL of THF at -30 °C was added *n*-butyllithium (6.0 mL, 2.5 M, 15.0 mmol), and the solution was stirred for 30 min at -30 °C and then cooled to -50 °C. A solution of ethyl (trimethylsilyl)acetate (2.40 g, 15.0 mmol) in 20 mL of tetrahydrofuran was added over 10 min, and the solution was stirred at -40 to -50 °C for 1 h. A solution of keto ester 13 (1.85 g, 6.23 mmol) in 20 mL of THF was added at $-50 \text{ }^{\circ}\text{C}$ over 10 min, and the solution was allowed to warm to $-20~^\circ\mathrm{C}$ over 30 min. The cold solution was poured into ice water and the mixture extracted with CH_2Cl_2 . The combined extracts were washed with brine and dried over MgSO₄, and the solvent was removed on a rotary evaporator to give 2.55 g of crude product as an oil. Chromatography on silica gel (100 g) eluting with 1% methanol in EtOAc afforded 1.78 g of the dienic ester 14 (3:2 mixture of Z/E isomers) as an oil. A sample was evaporatively distilled at 200-210 °C/0.05 Torr for analysis: ¹H-NMR (CDCl₃) δ 1.25 (2t (Z/E), 3 H, OCH₂CH₃), 1.60 and 1.70 (2s (Z/E), CH₃), 1.20-2.50 (m, 12 H, CH₂ and CH), 3.45 and 3.58 (2m (Z/E), NCH), 3.90, 5.00 and 3.99, 4.90 (2AB (Z/E)))E), J_{gem} = 15 Hz, 2 H, CH₂Ph), 4.08 and 4.15 (2q, J = 6 Hz, 2 H, OCH_2CH_3), 5.30 and 5.50 (2s (Z/E), 1 H, ring vinyl H), 5.90 and 7.25 (2s (Z/E), 1 H, chain vinyl H), 7.20-7.4 (m, 5 H, phenyl); MS (EI) m/e 367 (M⁺); CD (ethanol) 276 ([Θ] = +13 125, max), 232 ($[\Theta]$ = +1495, max), 217 ($[\Theta]$ = +33 643, max), 213 ($[\Theta] = +30$ 321, min.), 198 nm ($[\Theta] = +45$ 690°, max). Anal. (C₂₃H₂₉NO₃) C,H,N.

(1R,3R,5S,5(2S))-3-Methyl-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]cyclohexaneacetic Acid Ethyl Ester (15). Dienic ester 14 (1.72 g, 4.67 mmol) was hydrogenated in 80 mL of ethanol at 50 psi over 10% Pd/C for 5 h. The catalyst was filtered off, and the solvent was removed on a rotary evaporator to give 1.47 g of saturated ester 15 mainly as the 1R, 3R, 5S, 5(2S)-isomer containing ca. 15% of two other diastereomers by NMR. A sample was evaporatively distilled at 200–210 °C/0.25 Torr for analysis: ¹H-NMR (CDCl₃) δ 0.4 (q, J = 10 Hz, 1 H, axial CH), 0.55 (m, 2 H, 2 axial CH), 0.84 $(\hat{d}, J = 6.6 \text{ Hz}, 0.45 \text{ H}, \text{CH}_3 \text{ of minor diastereomer}), 0.86 (d, J)$ = 6.6 Hz, 2.1 H, CH₃ of major diastereomer), 0.96 (d, J = 6.6Hz, 0.45 H, CH₃ of minor diastereomer), 1.20 (t, J = 6 Hz, 3 H, OCH₂CH₃), 1.20–2.50 (m, 14 H, CH₂ and CH), 3.45 (m, 1 H, CHN), 4.10 (q, J = 6 Hz, OCH₂CH₃), 3.95 and 4.96 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 7.20–7.35 (m, 5 H, phenyl); MS $(\bar{E}I) m/e 371 (M^+); CD (ethanol) 264 ([\Theta] = +118, max), 261$ $([\Theta] = +65, \text{ min.}), 258 ([\Theta] = +191, \text{ max}), 254 ([\Theta] = +121,$ min.), 233 ($[\Theta] = -1032$, max), 217 ($[\Theta] = +32475$, max), 211 $([\Theta] = +28\ 070, \text{ min.}), 195\ \text{nm}\ ([\Theta] = +46\ 787^\circ, \text{ max})$

(1R,3R,5S,5(2S))-3-Methyl-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]cyclohexaneacetamide (16). A mixture of the crude ester 15 (1.4 g, 3.77 mmol) and KOH (2.0 g, 35 mmol) in 40 mL of ethanol was heated to reflux for 30 min and cooled, and the solvent was removed on a rotary evaporator. The residue was dissolved in water, acidified to pH 1 with 2 N HCl, and extracted with CH₂Cl₂. The combined extracts were washed with brine, dried over Na₂SO₄, and evaporated to give 1.31 g of the corresponding acid (1.31 g). The crude acid (1.31 g, 3.82 mmol) was dissolved in 15 mL of CHCl₃ and cooled to -30 °C. To the solution was added dropwise ethyl chloroformate (2.06 g, 19.1 mmol) in 15 mL of CHCl₃ followed by triethylamine (1.93 g, 19.1 mmol) in 10 mL of CHCl₃. After the addition was complete, the mixture was allowed to warm over 1 h to 0 °C. Ammonia was then bubbled into the solution for 20 min. The mixture was stirred for 1 h at 0 °C and then allowed to warm to room temperature. The precipitate (NH_4Cl) was filtered off, and the filtrate was washed with 2 N HCl, saturated NaHCO₃, and brine and dried over Na₂SO₄. The solvent was removed on a rotary evaporator to give 2.05 g of crude monobenzyl cyclohexaneacetamide 16 which was chromatographed on silica gel (80 g) eluting with 5% methanol in ethyl acetate to give 1.05 g (81.3%) of **16**. Recrystallization from tetrahydrofuran:ether (1:3) at -20 °C and then from tetrahydrofuran:ether (1:2) at room temperature afforded 0.23 g of cyclohexaneacetamide 16 as a single diastereomer, mp 90-92 °C. An additional 0.35 g was obtained from the mother liquors by HPLC (Waters Prep 500 column, 4% methanol, 48% ethyl acetate, 48% hexane): ¹H-NMR (CDCl₃) δ 0.4 (q, J = 10 Hz, 1 H, axial ring CH), 0.5-0.62 (m, 2 H, axial ring CH), 0.86 $(d, J = 6.5 Hz, 3 H, CH_3), 1.15-2.55 (m, 14 H, CH_2 and CH),$ 3.47 (m, 1 H, NCH), 3.94 and 4.97 (AB, J_{gem} = 15 Hz, CH₂Ph), 5.30 (br d, J = 15 Hz, 2 H, CONH₂), 7.20–7.35 (m, 5 H, phenyl); MS (EI) m/e 342 (M⁺); HRMS (FAB) calcd for $C_{21}H_{30}N_2O_2$ (M + H) 342.2307, obsd (M + H) 342.2297; CD (ethanol) 264 ($[\Theta] = +115$, max), 261 ($[\Theta] = +52$, min.), 258 $([\Theta] = +190, \max), 255 ([\Theta] = +107, \min), 252 ([\Theta] = +164,$ max), 233 ($[\Theta] = -1850$, max), 216 ($[\Theta] = +41$ 667, max), 211 $([\Theta] = +36\ 980, \text{min.}), 196\ \text{nm} ([\Theta] = +57\ 812^\circ, \text{max}).$ Anal. $(C_{21}H_{30}N_2O_2)$ C,H,N.

(1R,3R,5S,5(2S))-3-Methyl-5-[(5-oxo-2-pyrrolidinyl)methyl]cyclohexaneacetamide (2). A solution of the (1R, 3R, 5S, 5(2S))-3-methylcyclohexaneacetamide 16 (0.58 g, 1.70 mmol) in 10 mL of THF was placed in a flask equipped with a dry ice condenser, and ammonia (10 mL) was condensed into the solution. To the solution at reflux was added sodium metal (0.39 g, 17.0 mg-atom) in small pieces. The resulting blue solution was stirred for 30 min and the reaction quenched by the addition of solid NH₄Cl. The dry ice condenser was removed, and the ammonia was allowed to evaporate. The mixture was then diluted with CH₂Cl₂ and filtered. The filtrate was concentrated on a rotary evaporator, and the residue was chromatographed on silica gel (15 g) eluting with 5-10% methanol in EtOAc to give 0.275 g (64.1% yield) of the cyclohexaneacetamide 2 as a white solid: mp 190-192 °C (ethanol); ¹H-NMR (CDCl₃) δ 0.4–0.6 (m, 3 H, axial ring H), $0.90 (d, J = 6 Hz, 3 H, CH_3), 1.3-2.20 (m, 14 H, CH_2 and CH),$ 3.77 (m, 1 H, CHN), 5.45 and 5.80 (2s, 2 H, CONH₂), 6.40 (s, 1 H, NH); IR (CHCl₃) 1688 cm⁻¹; MS (EI) m/e 252 (M⁺); CD (ethanol) 215 ($[\Theta] = -11.120$, max), 195 nm ($[\Theta] = +21.685^{\circ}$, max). Anal. $(C_{14}H_{24}N_2O_2)$ C,H,N.

The structure and stereochemistry were confirmed through an X-ray crystallographic analysis. The final discrepancy indices are R = 0.038 and $R_w = 0.043$. Details are provided in the supporting information.

(5S-5(2S))-3-Oxo-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-1-cyclohexene-1-acetic Acid Ethyl Ester (17). To a solution of diisopropylamine (16.2 g, 160 mmol) in 480 mL of ether was added at -50 °C a solution of n-butyllithium (64.8 mL, 2.5 M, in hexane, 160 mmol), and the mixture was stirred for 30 min at -20 to -30 °C. A solution of ethyl (trimethylsilyl)acetate (25.6 g, 160 mmol) in 240 mL of THF was added at -50 °C, and the mixture was stirred at -30 to -40 °C for 1 h. To the solution was added ethoxy enone 12 (21 g, 64 mmol) in 240 mL of dry THF at -50 °C. Following the addition, the mixture was allowed to warm slowly over 1 h to 0° C, stirred for 1 h at 0° C, and poured onto ice water. The mixture was extracted with CH₂Cl₂, and the solvent was removed on a rotary evaporator. The residual material was dissolved in 330 mL of THF, 330 mL of 6 N HCl was added, and the mixture was stirred for 1 h at room temperature. The mixture was extracted with CH₂Cl₂, and the extracts were washed with saturated $NaHCO_3$ and brine and dried over Na₂SO₄. The solvent was removed on a rotary evaporator to give 15.2 g of crude enone ester 17 which was chromatographed on silica gel (30 g) eluting with 1% methanol in ethyl acetate to give 12.0 g (51% yield) of the purified enone ester 17. A sample was evaporatively distilled at 230–240 °C/0.25 Torr for analysis: ¹H-NMR (CDCl₃) δ 1.26 (t, J = 7 Hz, CH₃), 1.25–2.60 (m, 11 H, CH₂ and CH), 3.14 and 3.15 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂CO), 3.47 (m, 1 H, NCH), 3.97 and 5.00 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 4.15 (q, J = 7 Hz, 2 H, OCH₂), 7.20–7.40 (m, 5 H, phenyl); MS (EI) m/e 369 (M⁺). Anal. (C₂₂H₂₇NO₄) C,H,N.

 $(1S, 5S, 5(2S)) \hbox{-} 3 \hbox{-} 0xo \hbox{-} 5 \hbox{-} [[5 \hbox{-} 0xo \hbox{-} 1 \hbox{-} (phenylmethyl) \hbox{-} 2 \hbox{-} pyr \hbox{-} 2 \hbox{-} (phenylmethyl) \hbox{-} 2 \hbox{-} pyr \hbox{-} 2 \hbox{-} (phenylmethyl) \hbox{-} 2 \hbox{-} pyr \hbox{-} 2 \hbox{$ rolidinyl]methyl]-cyclohexaneacetic Acid Ethyl Ester (18). A solution of enone ester 17 (16.4 g, 44.6 mmol) in 500 mL of ethanol was hydrogenated over 10% Pd/C (1.6 g) at 50 psi for 3 h. The mixture was filtered to remove the catalyst, and the solvent was removed on a rotary evaporator. The oily product was dissolved in CH₂Cl₂, the solution was dried over Na₂SO₄, and the solvent was removed on a rotary evaporator to give 14.5 g (87.5% yield) of cyclohexaneacetic acid ethyl ester 18 containing approximately 5% of a trans isomer by NMR. The product oil was recrystallized twice from 1:1 ethyl acetate: hexane to give 7.8 g of pure 18 (>97% cis isomer), mp 65-70°C. X-ray crystallographic analysis (see the supporting information) confirmed the structural assignments, and because L-glutamic acid was the origin of the pyrrolidinone ring, the absolute stereochemistry was also confirmed. Final discrepancy indices are R = 0.057 and $R_w = 0.068$. A summary of the crystal data is provided in the supporting information: ¹H-NMR (CDCl₃) δ 0.95 (m, 1 H, axial ring H), 1.25 (t, J = 7 Hz, 3 H, CH₃), 1.6-2.6 (m, 13 H, CH₂ and CH), 3.45 (m, 1 H, NCH), 3.97 and 4.98 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 4.12 (q, J = 7 Hz, 2 H, OCH₂), 7.20–7.40 (m, 5 H, phenyl); MS (EI) m/e 371 (M^+) ; $[\alpha]^{25}_{D} = +16.88^{\circ}$ (c 0.9359, methanol). Anal. $(C_{22}H_{29}-$ NO₄) C,H,N.

[2-[(Phenylmethyl)amino]ethenyl]phosphonic Acid Diethyl Ester (19). Diethyl phosphonoacetaldehyde diethyl acetal (50.0 g, 0.2 mol) in 160 mL of 2.5% HCl in water was heated to reflux for 45 min. The solution was cooled to room temperature and extracted with CH₂Cl₂. The combined extracts were washed with saturated NaHCO3 and dried over Na₂SO₄. The solvent was removed on a rotary evaporator, and the residue (34.5 g) of diethyl phosphonoacetaldehyde was used without purification. The crude diethyl phosphonoacetaldehyde (34.5 g) was dissolved in 140 mL of methanol and cooled to 0 °C, and benzylamine (23 g, 0.216 mol) was added. The mixture was stirred for 1 h at 0 °C, the solvent was removed on a rotary evaporator, and the residue (50 g) was chromatographed on alumina III, eluting with 1:1 ethyl acetate:hexane to give enaminophosphonate **19** (38.6 g, 71.4% yield) as an oil. A sample was distilled, bp 90-92 °C/0.5 Torr: ¹H-NMR $(\text{CDCl}_3) \delta 1.26 \text{ (t, } J = 7 \text{ Hz, } 6 \text{ H, CH}_3\text{'s}\text{)}, 3.92-4.10 \text{ (m, 5 H, } 1.00 \text{ H})$ OCH_2 , CHP), 4.19 (d, J = 5.5 Hz, 2 H, CH₂Ph), 5.35 (br s, 1 H, NH), 7.14-7.35 (m, 1 H, vinyl H), 7.22-7.35 (m, 6 H, Ph); HRMS (EI) calcd for $C_{13}H_{20}NO_3P$ (M⁺) 269.1181, obsd 269.1183.

 $(E) \cdot (1S, 5R, 5(2S)) \cdot 5 \cdot [[5 \cdot Oxo \cdot 1 \cdot (phenylmethyl) \cdot 2 \cdot pyr \cdot 1]$ rolidinyl]methyl]-3-[[1-(phenylmethyl)-1H-imidazol-5-yl]methylenelcyclohexaneacetic Acid Ethyl Ester (21E) and (Z)-(1S,5R,5(2S))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1H-imidazol-5-yl]methylene]cyclohexaneacetic Acid Ethyl Ester (21Z). To a solution of diisopropylamine (3.6 g, 36 mmol) in 150 mL of ether was added a solution of n-butyllithium in hexane (14.4 mL, 2.5 M, 36 mmol) at -30 °C. The mixture was stirred for 30 min at -30 °C, and a solution of phosphonate 19 (9.6 g, 36 mmol) in 60 mL of THF was added at -40 °C. The solution was stirred for 0.5 h at -15 to -20 °C and then cooled to -40°C. To the solution was added a solution of keto ester 18 (6.6 g, 18 mmol) in 90 mL of THF at -40 °C. The mixture was stirred for 15 min at -40 °C and then for 2.5 h at 0 °C and subsequently poured into ice water. The organic layer was separated, the aqueous layer was extracted with CH₂Cl₂, and the solvent was removed on a rotary evaporator. The residue was dissolved in 400 mL of CH_2Cl_2 , the solution was dried over Na₂SO₄, and the solvent was removed on a rotary evaporator to give crude imine 20:46 ¹H-NMR (CDCl₃) δ 0.65–1.00 (m, 2 H, axial ring H), 1.15-1.35 (2t, 3 H, CH₃), 3.50 (m, 1 H, NCH), 3.95-4.20 (m, 4, CH₂CO), 4.00 and 5.00 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph), 5.87 and 6.05 (2br d, 1 H, vinyl H), 7.20-7.40, (m, 6 H, phenyl and imine H), 8.34 (br d, ca. 0.5 H, aldehyde vinyl H), 9.96 (2d, J = 7.5 Hz, CHO (E/Z)).

The total crude imine was dissolved in 210 mL of methanol, and benzylamine (3.84 g, 36 mmol) was added along with 9 g of 3 Å molecular sieves. The mixture was stirred for 1 h at room temperature; then (4-tolylsulfonyl)methyl isocyanide (TOSMIC; 7.2 g, 36 mmol) was added, and the mixture was allowed to stir at room temperature overnight. The mixture was filtered to remove the sieves, and the filtrate was concentrated on a rotary evaporator and then dissolved in 300 mL of CH₂Cl₂. The solution was washed with 2 N HCl and saturated NaHCO₃, and dried over anhydrous MgSO₄. The solvent was removed on a rotary evaporator, and the residue was chromatographed on silica gel (700 g) eluting with 2.5-5% methanol in CH_2Cl_2 to afford a total of 8.5 g of imidazole ester 21 as the pure Z-isomer 21Z (3.9 g), the E-isomer 21E(3.6 g), and mixed fractions (1.0 g). The E and Z assignments and analytical data were made at the stage of the corresponding amides 23 (vide infra).

For 21Z: ¹H-NMR (CDCl₃) δ 0.67 (q, J = 12.3 Hz, 1 H, axial H of ring CH₂), 1.23 (t, J = 7.4 Hz, 3 H, OCH₃), 1.15–2.28 (m, 12 H, 2 CH, 4 CH₂, 2 CH of CH₂), 2.41 (m, 2 H, ring CH₂CO), 2.91 (br d, 1 H, $J_{gem} = 12.6$ Hz, equatorial H of ring CH₂), 8.44 (br m, 1 H, NCH), 3.94 and 4.96 (AB, 2 H, $J_{gem} = 14.8$ Hz, CH₂Ph on lactam), 4.11 (q, 2 H, J = 7.4 Hz, OCH₂), 5.05 (s, 2 H, CH₂Ph on imidazole), 5.72 (s, 1 H, vinyl H), 6.97 and 7.40 (2s, 2 H, imidazole H's), 7.0–7.4 (m, 10 H, phenyl H's).

For 21E: ¹H-NMR (CDCl₃) δ 0.66 (br q, J = 11.5 Hz, 1 H, axial H of ring CH₂), 1.23 (t, J = 7.4 Hz, 3 H, OCH₃), 1.10– 2.60 (m, 12 H, 2 CH, 4 CH₂, 2 CH of CH₂), 2.40 (m, 2 H, ring CH₂CO), 2.79 (br d, 1 H, $J_{gem} = 12.6$ Hz, equatorial H of ring CH₂), 3.39 (br m, 1 H, NCH), 3.93 and 4.94 (AB, 2 H, $J_{gem} =$ 14.8 Hz, CH₂Ph on lactam), 4.10 (q, 2 H, J = 7.4 Hz, OCH₂), 5.03 (s, 2 H, CH₂Ph on imidazole), 5.75 (s, 1 H, vinyl H), 6.90 and 7.50 (2s, 2 H, imidazole H's), 7.0–7.4 (m, 10 H, phenyl H's).

(E)-(1S,5R,5(2S))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1H-imidazol-5-yl]methylene]cyclohexaneacetamide (23E). The imidazole ester 21E (7.4 g, 14 mmol) was heated to 75 °C in 145 mL of ethanol with KOH (4.0 g, 70 mmol) for 30 min. The mixture was concentrated on a rotary evaporator and diluted with 50 mL of water. The solution was washed twice with CH₂Cl₂ (discarded) and acidified to pH 3.0-3.5 with 6 N HCl. The gummy residue was extracted with CH₂Cl₂, the combined extracts were dried over Na₂SO₄, and the solvent was removed on a rotary evaporator to give 6 g (86% crude yield) of the crude acetic acid 22E. The crude acid was dissolved in 325 mL of CHCl₃, cooled to -40 °C, and treated with 6.5 g (60 mmol) of ethyl chloroformate followed by triethylamine (6.0 g, 60.0 mmol). The mixture was stirred, allowed to warm slowly to 0 °C, and maintained at 0 °C for 30 min, and then gaseous ammonia was bubbled into the solution for 20 min at 0 °C. The cooling bath was removed, and the white suspension was stirred for 2 h at room temperature. The mixture was filtered to remove the NH₄Cl precipitate and concentrated on a rotary evaporator. The residue was chromatographed on silica gel (400 g), eluting with 5-10% MeOH in CH₂Cl₂. The fractions were concentrated, and the residue was dissolved in CH₂Cl₂, washed with water, and dried over Na₂SO₄ to give 3.78 g (54% overall yield from ester 21E) of the dibenzyl cyclohexane-acetamide 23E as a white solid: mp 60-70 °C; $[\alpha]^{25}_{D} =$ -28.04° (c 1%, MeOH); IR (KBr) 3525, 3405, 1675, 702 cm⁻¹; ¹H-NMR (CDCl₃) δ 0.66 (q, 1 H, J = 12.3 Hz, axial CH of ring CH₂), 1.21 (m, 1 H, CH), 1.25 (br m, 1 H, CH), 1.48 (t, 1 H, J = 12.3 Hz, axial CH of ring CH₂), 1.53-1.88 (m, 6 H, CH₂, 4 CH of CH₂), 2.08 (m, 2 H, CH₂CO), 2.34 (m, 1 H, CH of CH₂), 2.38 (ddd, 1 H, $J_{vic} = 6.8$, 9.6 Hz, $J_{gem} = 17.2$ Hz, CH of ring CH₂CO), 2.49 (ddd, 1 H, $J_{vic} = 6.6$, 9.6 Hz, $J_{gem} = 17.2$ Hz, CH of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, $J_{gem} = 13.4$ Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial of ring CH₂CO), 2.82 (br d, CH of ring CH₂), 3.42 (m, 1 H, NCH), 3.95 and 4.94 (AB, 2 H, $J_{gem} = 15.3$ Hz, CH₂Ph on lactam), 5.04 (s, 2 H, CH₂Ph on imidazole), 5.34 and 5.41 (2s, 2 H, CONH₂), 5.76 (s, 1 H, vinyl H), 6.90 and 7.49 (2s, 2 H, imidazole H's), 7.03 (d, 2 H, phenyl H's), 7.20 (d, 2 H, phenyl H's), 7.22–7.35 (m, 6 H, phenyl H's). Irradiation of the vinyl proton at 5.76 ppm resulted in an observed NOE for the ring proton at C-2 at 2.32 ppm, confirming the assignment of the *E* stereochemistry. HRMS (EI) m/e (M⁺) calcd for C₃₁H₃₆N₄O₂ 496.2838, obsd 496.2823.

(Z)-(1S,5R,5(2S))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1H-imidazol-5-yl]methylene]cyclohexaneacetamide (23Z). Following the above procedure for the Z-isomer 21Z (5.3 g, 10 mmol), there was obtained 2.9 g (58% yield from 21Z). A sample was recrystallized from EtOAc to give the pure product: mp 160-161 °C; $[\alpha]^{25}_{D}$ -45.31° (c 1%, MeOH); IR (KBr) 3525, 3410, 701 cm⁻¹; MS (EI) m/e 496 (M⁺); ¹H-NMR (CDCl₃) δ 0.68 (q, 1 H, J = 12.3 Hz, axial CH of ring CH₂), 1.26 (m, 1 H, CH), 1.38 (br m, 1 H, CH), 1.48 (t, 1 H, J = 12.3 Hz, axial CH of ring CH₂), 1.57-1.67 (m, 2 H, 2 CH of CH₂), 1.67-1.83 (m, 3 H, CH₂, CH of CH₂), 2.00-2.18 (m, 4 H, CH₂CO), 2.34 (m, 1 H, CH of CH₂), 2.38 (ddd, 1 H, $J_{\rm vic} = 6.8$, 9.6 Hz, $J_{\rm gem} = 17.2$ Hz, CH of CH₂CO, 2 CH of CH₂), 2.43 (m, 2 H, ring CH₂CO), 2.94 (br d, 1 H, $J_{gem} = 13.0$ Hz, equatorial CH of ring CH₂), 3.46 (m, 1 H, NCH), 3.95 and 4.95 (AB, 2H, $J_{gem} = 15.0$ Hz, CH₂Ph on lactam), 5.05 (s, 2 H, CH₂Ph on imidazole), 5.54 and 5.56 (2br s, 2 H, CONH₂), 5.72 (s, 1 H, vinyl H), 6.97 and 7.47 (2s. 2 H, imidazole H's), 7.03 (d, 2 H, phenyl H's), 7.20 (d, 2 H, phenyl H's), 7.22-7.36 (m, 6 H, phenyl H's). Anal ($C_{31}H_{36}N_4O_2$) C.H.N.

(1S,3R,5(2S),5S)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1H-imidazol-5-yl]methyl]cyclohexaneacetamide (24). To a solution of the unsaturated imidazole 23E (1.0 g, 2.0 mmol) in 100 mL of methanol containing 2% NH3 was added 10 g of Raney Ni (Aldrich; wet slurry in H₂O, washed twice with H₂O and MeOH). The mixture was hydrogenated at 50 psi in a Parr shaker at room temperature for 48 h. The catalyst was filtered off and the methanol removed on a rotary evaporator to give 620 mg (62% crude yield) of a mixture (88:4:8 ratio) of 24, the more polar 3S-isomer 25, and unreacted 23E. The crude product was rereduced over 10% Pd/C (200 mg) to reduce the remaining 23E. Concentration of the methanol afforded 578 mg of a mixture of 24, 25, and some of the debenzylated compound 26 (see below) in a ratio of 85.8:8.6:5.6 by reversephase HPLC. The product mixture was chromatographed on silica gel (dry column) eluting with CHCl₃:MeOH:HOAc (100: 20:1). The fractions containing 24 were combined and concentrated on a rotary evaporator. Water was added followed by NH₄OH until alkaline, and the material was extracted with CH_2Cl_2 . The combined extracts were washed with brine and dried over Na₂SO₄. The solvent was removed on a rotary evaporator to afford 380 mg (38%) of pure 24: mp 75 °C (softens above 63 °C); ¹H-NMR (CDCl₃) δ 0.36–0.41 (m, 1 H, axial ring H), 0.43-0.58 (m, 2 H, axial ring H), 3.42 (br s, 1 H, NCH), 3.91 and 4.93 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph on lactam), 5.03 (s, 2 H, CH₂Ph on imidazole), 5.30 (d, J = 12Hz, 2 H, CONH₂), 6.80 and 7.46 (2s, 2 H, imidazole H's), 7.00-7.40 (m, 10 H, phenyls); MS (EI) 498 (M⁺); HRMS (EI) calcd for $C_{31}H_{38}N_4O_2(M^+)$ 498.2995, obsd (M⁺) 498.2990; CD (ethanol) 258 ($[\Theta] = +80$, max), 233 ($[\Theta] = -2960$, max), 216 ($[\Theta]$ = +38 400, max), 213 ($[\Theta]$ = +33 600, max), 200 nm ($[\Theta]$ = +56 000°, max).

(1S,3S,5(2S),5S)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1H-imidazol-5-yl]methyl]cyclohexaneacetamide (25). Unsaturated imidazole ester 21E (600 mg, 1.1 mmol) in 15 mL of MeOH was hydrogenated over 10% Pd/C (300 mg) at 1 atm overnight at room temperature. The catalyst was removed by filtration and the solvent removed to afford the saturated ester product 21 (600 mg) as a mixture of isomers in a 6:1 ratio by NMR. The crude ester was dissolved in 15 mL of MeOH, KOH (280 mg, 5 mmol) was added, and the mixture was heated at 75 °C for 30 min. The solvent was removed on a rotary evaporator and the residue diluted with 10 mL of H₂O and washed with CH₂-Cl₂ (discarded). The aqueous solution was acidified to pH 3-3.5 with 6 N HCl and the mixture extracted with CH_2Cl_2 . Extracts were dried over Na₂SO₄ and concentrated on a rotary evaporator to afford 432 mg of crude acid 21. The crude acid 21 (432 mg, ca. 0.84 mmol) was dissolved in 40 mL of CHCl₃, cooled to -40 °C, and treated with ethyl chloroformate (454 mg, 4.2 mmol) followed by triethylamine (424 mg, 4.2 mmol).

The mixture was stirred and allowed to warm gradually over 1 h to 0 °C and then stirred for 30 min. Ammonia gas was bubbled into the solution at 0 °C for 20 min, the cooling bath was removed, and the mixture was stirred for 2 h at room temperature. The white suspension was filtered to remove the NH4Cl, and the filtrate was washed with H2O, dried over Na_2SO_4 , and concentrated on a rotary evaporator to give the crude amide 24. The crude product was chromatographed on silica gel (30 g, dry column) eluting with the lower phase of a mixture prepared by shaking CHCl₃, MeOH, H₂O, and HOAc in a ratio of 9:3:1:0.6. The chromatography fractions were combined, concentrated on a rotary evaporator, diluted with H₂O, made alkaline with NH₄OH, and extracted with CH₂Cl₂. The extracts were dried over Na₂SO₄ and concentrated on a rotary evaporator to afford 230 mg of an amide mixture, an 86:14 ratio of the more polar isomer 24 to the less polar isomer 25. Further purification by HPLC (Waters Delta Prep 3000 instrument) on a Waters Delta Pak C_{18} 5 μ m 3.5 mm x 15 cm column eluting with a gradient of H_2O (0.1% CF₃COOH)/ CH₃CN (0.1% CF₃COOH) yielded the isomer 25 (99.7% purity by HPLC): mp 72 °C (softens above 56 °C); ¹H-NMR (CDCl₃) δ 0.36–0.46 (m, 1 H, axial ring H), 1.00–1.15 (m, 3 H), 3.42 (br s, 1 H, NCH), 3.92 and 4.92 (AB, $J_{gem} = 15$ Hz, 2 H, CH₂Ph on lactam), 5.03 (s, 2 H, CH₂Ph on imidazole), 5.30 (br s, 2 H, CONH₂), 6.79 and 7.44 (2s, 2 H, imidazole H's), 7.00-7.40 (m, 10 H, phenyls); MS (EI) 498 (M⁺); HRMS (EI) calcd for $C_{31}H_{38}\dot{N}_4O_2~(M^+)$ 498.2995, obsd (M~+~H) 498.2965; CD (ethanol) 258 ($[\Theta] = -56$, min.), 232 ($[\Theta] = -2360$, max), 216 $([\Theta] = +36\ 800, \text{max}), 213\ ([\Theta] = +34\ 400, \text{max}), 202\ \text{nm}\ ([\Theta]$ $= +57\ 600^{\circ}, \ max).$

(1S, 3R, 5(2S), 5S)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-5-[(1H-imidazol-5-yl)methyl]cyclohexaneacetamide (26). A solution of the less polar amide 24 (360 mg, 0.7 mmol) in 35 mL of MeOH was reduced at 50 psi of hydrogen at 50 °C over 10% Pd/C (360 mg) in a Fisher-Porter bottle with magnetic stirring overnight. The mixture was cooled and filtered to remove the catalyst, and the solvent was removed on a rotary evaporator. The residue was chromatographed on silica gel $(15 \, \overline{g}, dry \, column)$ eluting with the lower phase of a mixture prepared by shaking CHCl₃, MeOH, H₂O, and HOAc in a ratio of 9:3:1:0.6. The chromatography fractions were combined, concentrated on a rotary evaporator, diluted with H_2O , made alkaline with NH_4OH , and extracted with CH₂Cl₂. The extracts were dried over Na₂SO₄ and concentrated on a rotary evaporator to afford 146 mg (51% yield) of the monobenzyl amide 26: ¹H-NMR (CDCl₃) δ 0.37-0.47 (m, 1 H, axial ring H), 0.56-0.68 (m, 2 H, axial ring H), $3.44 \text{ (m, 1 H, NCH)}, 3.92 \text{ and } 4.95 \text{ (AB, } J_{gem} = 15 \text{ Hz, CH}_2\text{Ph}),$ 5.32 and 5.41 (br s, 2 H, amide H), 6.74 (s, 1 H, imidazole ring H), 7.54 (s, 1 H, imidazole ring H), 7.18–7.35 (m, 5 H, phenyl); MS (EI) m/e 408 (M⁺); HRMŠ (EI) calcd for $C_{24}H_{32}N_4O_2$ (M⁺) 408.2525, obsd (M⁺) 408.2519; CD (ethanol) 233 ([Θ] = -1660, max), 217 ($[\Theta] = +39\ 200$, max), 211 ($[\Theta] = +34\ 400$, min.), 199 nm ([Θ] = +59 200°, max).

(1S,3S,5(2S),5S)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-5-[(1H-imidazol-5-yl)methyl]cyclohexaneacetamide (27). In the same manner as described for the preparation of 26, reduction of the more polar diastereomer 25 (430 mg, containing ca. 4% of 24) over 400 mg of 10% Pd/C afforded 210 mg (96.5%) of monobenzyl amide 27. NMR indicated an isomeric purity of 57%: ¹H-NMR (CDCl₃) δ 0.48– 0.58 (m, 1 H, axial ring H), 0.88–0.95 (m, 1 H), 3.46 (m, 1 H, NCH), 3.92 and 4.98 (AB, $J_{gem} = 15$ Hz, CH₂Ph), 5.39 and 5.62 (br s, 2 H, amide H), 6.75 (s, 1 H, imidazole ring H), 7.53 (s, 1 H, imidazole ring H), 7.20–7.35 (m, 5 H, phenyl); MS (EI) m/e408 (M⁺); HRMS (EI) calcd for C₂₄H₃₂N₄O₂ (M⁺) 408.2525, obsd (M⁺) 408.2512; CD (ethanol) 233 ([Θ] = -1700, max), 217 ([Θ] = +38 400, max), 211 ([Θ] = +35 200, min.), 201 nm ([Θ] = +55 200°, max).

(1S,3S,5(2S),5R)-3-[(1H-Imidazol-5-yl)methyl]-5-[(5-oxo-2-pyrrolidinyl)methyl]cyclohexaneacetamide (28). To a solution of monobenzyl amide 26 (122 mg, 0.3 mmol) in 5 mL of THF was added liquid ammonia (5 mL). To the solution was added sodium metal (69 mg, 3.0 mg-atom). The blue solution was stirred under reflux at -33 °C for 30 min and the reaction quenched with solid ammonium chloride. The

ammonia was allowed to evaporate, and the residual paste was slurried with methanol and filtered. The methanol was removed on a rotary evaporator, and the residue was chromatographed on a C₁₈ 120 Å silica gel column, eluting with 1:1 H₂O:MeOH. Removal of the solvent on a rotary evaporator gave 67 mg of **28** (70% yield): mp 122–128 °C; ¹H-NMR (CDCl₃ + DMSO-d₆) δ 0.57, 0.63, and 0.67 (3q, 3 H, 3 axial CH of ring CH₂), 1.32, 1.43, 1.55–1.92, 2.07, and 2.14–2.33 (5m, 1 H, 2 H, 6 H, 2 H, and 3 H, respectively, 3 CH, 3 CH₂, 3 CH of CH₂), 2.45 and 2.50 (2dd, 2 H, $J_{vic} = 7.3$, 6.0 Hz, $J_{gem} = 16.2$ Hz, ring CH₂CO), 3.69 (m, 1 H, NCH), 5.82 and 6.46 (2s, 2 H, CONH₂), 6.70 and 7.48 (2s, 2 H, imidazole H's); [a]²⁵_D = +10.42° (c 1%, MeOH); HRMS (FAB) calcd for C₁₇H₂₆N₄O₂ (M⁺ + H) 319.2134, obsd 319.2143.

(1S,3S,5(2S),5S)-3-[(1H-Imidazol-5-yl)methyl]-5-[(5-oxo-2-pyrrolidinyl)methyl]cyclohexaneacetamide (1). In the manner described for the preparation of 28, a solution of monobenzyl amide 27 (80 mg) was debenzylated with Na/NH₃ to afford 35 mg (56% yield) of 1: mp ca. 212 °C; ¹H-NMR (DMSO-*d*₆) δ 0.54 (q, 1 H, *J* = 12 Hz, axial CH of ring CH₂), 0.87, 1.03, 1.12, 1.32, 1.43–1.62, 1.62–1.77, and 1.77–2.20 (7m, 1 H, 1 H, 1 H, 3 H, 2 H, and 7 H, respectively, 3 CH, 6 CH₂, CH of CH₂), 2.46–2.55 (m, 1 H, CH of ring CH₂CO), 2.61 (dd, 1 H, *J*_{vic} = 9.0 Hz, *J*_{gem} = 15.0 Hz, CH of ring CH₂CO), 3.52 (m, 1 H, NCH), 6.70 and 7.24 (2s, 2 H, CONH₂), 6.77 and 7.53 (2s, 2 H, imidazole H's), 7.93 (s, 1 H, NH of lactam), 11.9 (br, 1 H, NH on imidazole); [α]²⁵_D = +15.37° (c 1%, MeOH); HRMS (FAB) calcd for C₁₇H₂₆N₄O₂ (M⁺ + H) 319.2134, obsd 319.2134.

Biological Test Procedures. Morris Water Maze. The test animals were male C57BL/10 mice, weighing 17-21 g at the time of testing. Mice were housed in groups of 10 and had ad libitum access to food and water. Mice of this strain appear to be deficient in learning the water maze task and are therefore suitable for use in drug evaluation.³¹ The Morris water maze task requires an animal to attend to spatial cues in order to locate the position of a hidden platform submerged underwater.^{35b} The maze consisted of a $60 \text{ cm} \times 60 \text{ cm} \times 60$ cm transparent Plexiglas chamber filled to a depth of 30 cm, leaving 30 cm of wall extending up from the water surface. The water was made opaque by the addition of powdered milk. The water temperature was maintained at 20 °C. Both distal cues (i.e., standard room objects) and proximal cues (that is, $20~\text{cm}\times22~\text{cm}$ unique black and white patterns pasted to the center of each of the four walls of the maze) were used. The submerged platform, 8×8 cm, 1 cm below the water's surface was positioned near one of the four corners of the maze.

Each animal was given four consecutive trials (maximum of 2 min/trial and a 10-s intertrial interval) to locate the position of the hidden platform. On each trial the animal was placed into the water at the opposite corner to that of the submerged platform. Between trials animals were removed from the water and placed on a dry surface under a heat lamp before the start of the next trial. The time required for each animal to locate the platform on each of the four trials was recorded (latency). The mean total latency of the four trials was used as the score for a given animal.

The test compounds and TRH (thyrotropin-releasing hormone, pGlu-His-ProNH₂), were dissolved in saline or 5% acacia and administered by the intraperitoneal or oral route 30 min prior to the start of the first trial. Mice received 10 mL/kg of body weight. A group of mice in each of the experiments with test compounds received a 0.1 mg/kg dose of TRH and thus served as a positive control treatment condition. The overall statistical significance for each experiment was computed on mean total time to locate the submerged platform, using a oneway analysis of variance. If an *F* ratio that was significant at p < 0.05 was obtained in the overall analysis, a statistical evaluation of each of the individual drug treatment groups versus the vehicle-treated group was performed using a Dunnett *t*-test. A p < 0.05 was considered significant.

Receptor Binding Assays. Rats were decapitated and the brains removed and immediately frozen on dry ice. Coronal sections 20 μ m thick were cut, mounted on chrome-alum subbed slides, and stored at -20 °C with dessicant until assayed for TRH receptors. Slides containing brain sections

were warmed to room temperature and preincubated for 10 min in slide containers containing 50 mM Tris-HCl, 5 mM $MgCl_2,$ and 0.25% BSA, all at $p\bar{H}$ 7.4. Sections were then chilled to 4 °C and allowed to air dry. Slides were then incubated with 400 μ L of 30 μ M bacitracin and 10 nM [³H]-MeTRH or [³H]-26. Nonspecific binding is defined as the binding of [³H]MeTRH (or [³H]-26) in the presence of 10 μ M MeTRH (or 26, respectively). Specific binding under these conditions is typically 75-85%. For competition studies, sections were also incubated with compound 26 at concentrations ranging from 100 nM to 10 mM.

Sections were incubated with radioligand for 2 h at 4 °C followed by four washes in ice cold buffer for 15 s each. For scintillation counting, sections were wiped off the slides with Whatman glass fiber filter disks, placed in vials with 5 mL of Liquiscint, and counted at 60% efficiency. Values for total and nonspecific binding were carried out according to methods previously described⁴⁷ and statistical comparisons made using ANOVA followed by Newman-Keuls post hoc analysis for significant F-tests.

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Supporting Information Available: Tables 1-13 listing final atomic parameters, final anisotropic thermal parameters, bond distances, bond angles, and X-ray refinement details for compounds 2, 12, and 18, Figures 1-3 showing corresponding ORTEP plots of the determined crystal structures, and the NovaScreen panel of receptor binding assays for compound 26 (Tables 14 and 15) (17 pages). Ordering information is given on any current masthead page.

References

- (a) Farmer, P. S. In *Drug Design*; Ariëns, E. J., Ed.; Academic Press: New York, 1980; Vol X, pp 119-143. (b) Hirschmann, R. Medicinal Chemistry in the Golden Age of Biology: Lessons from Steroid and Peptide Research. Angew. Chem., Int. Ed. Engl. 1991, 30, 1278-1301. (c) Gante, J. Peptidomimetics-Tailored Enzyme Inhibitors. Angew. Chem., Int. Ed. Engl. 1994, 33, 1699 - 1720.
- (2) Olson, G. L.; Voss, M. E.; Hill, D. E.; Kahn, M.; Madison, V.; Cook, C. M. Design and Synthesis of a Protein β -Turn Mimetic. J. Am. Chem. Soc. 1990, 112, 323-333.
- (3) Nicolaou, K. C.; Salvino, J. M.; Raynor, K.; Pietranico, S.; Reisine, T.; Freidinger, R. M.; Hirschmann, R. Design and Synthesis of
- T.; Freidinger, R. M.; Hirschmann, R. Design and Synthesis of a Peptidomimetic Employing β-D-Glucose for Scaffolding. J. Am. Chem. Soc. 1993, 115, 12550-12586.
 (4) (a) Sarabu, R.; Lovey, K. M.; Madison, V. S.; Fry, D. C.; Greeley, D. N.; Cook, C. M.; Olson, G. L. Design, Synthesis, and 3-Dimensional Structural Characterization of a Constrained Ω-Loop excised from Interleukin-1a. Tetrahedron 1993, 49, 3629-3640. (b) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusjecki, V. K.; Sarabur, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. Concepts and Progress in the Development of Peptide Mimetics J. Med. Chem. 1993. in the Development of Peptide Mimetics J. Med. Chem. 1993, (5) (a) Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Pierschbacher, M. D. Design and Synthesis of Novel Cyclic
- RGD-Containing Peptides as Highly Potent and Selective Inte-grin $\alpha_{IIb}\beta_3$ Antagonists. J. Med. Chem. **1994**, 37, 1–8 and references therein. (b) See footnotes 22–26 in ref 4b. (c) Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.;

Shakespeare, W. C.; Smith, A. B., III. Nonpeptidal Peptidomimetics with β -D-Glucose Scaffolding. A Partial Somatostatin Agonist Bearing a CloseStructural Relationship to a Potent, Selective Substance P Antagonist. J. Am. Chem. Soc. 1992, 114, 9217.

- (6) Manaker, S.; Winokur, A.; Rostene, W. H.; Rainbow, T. C. Autoradiographic Localization of Thyrotropin-Releasing Hormone Receptors in the Rat Central Nervous System. J. Neurosci. 1985, 5, 167-174.
- (a) Yarbrough, G. Thyrotropin Releasing Hormone and CNS (7)Cholinergic Neurons. Life Sci. 1983, 33, 111-118. (b) Yarbrough, G. TRH Potentiates Excitatory Actions of Acetylcholine on Cerebral Cortical Neurons. Nature 1976, 263, 523-524. (c) Okada, M. Effect of a New Thyrotropin Releasing Hormone Analog, YM-14673, on the In Vivo Release of Acetylcholine as Measured by Intracerebral Dialysis in Rats. J. Neurochem. 1991, 56, 1544-1547. (d) Horita, A.; Carino, M. A.; Lai, H. Pharmacology of Thyrotropin Releasing Hormone. Annu. Rev. Pharmacol. Toxicol. 1986, 26, 311–332. (8) Stwertka, S. A.; Vincent, G. P.; Gamzu, E. R.; MacNeil, D. A.;
- Vederese, A. TRH Protection Against Memory Retrieval Deficits Is Independent of Endocrine Effects. Pharmacol. Biochem. Behav. 1991, 41, 145-152. (a) Yarbrough, G. G.; Pomara, M. The Therapeutic Potential of
- Thyrotropin Releasing Hormone (TRH) in Alzheimer's Disease (AD). Prog. Neuro-Psychopharmacol. Biol. Psychiatry 1985, 9, 285-289. (b) Miyamoto, M.; Yamazaki, N.; Nagaoka, A.; Nigawa, Y. Effects of TRH and its Analog, DN-1417, on Memory Impairment in Animal Models. Ann. N.Y. Acad. Sci. 1989, 508-510.
 (10) Metcalf, G. Regulatory Peptides as a Source of New Drugs-the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospects for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Clincal Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which was Designed to the Prospect for Analogues of TRH which
- Clincal Prospects for Analogues of TRH which Are Resistant to
- Metabolic Degradation. Brain Res. **1982**, 257, 389–408. (11) Szirtes, T.; Kisfaludy, L.; Pálosi, É.; Balaspiri, L.; Szporny, L. Tripeptide Amides and Pharmaceutical Compositions Containing Them. Ger. Offen. DE3024313 (1/29/81), 1981.
- (a) Maeda, H.; Suzuki, M.; Sugano, H.; Yamamura, M.; Ishida, (12)R. Synthesis and Central Nervous System Actions of Thyrotropin-Releasing Hormone Analogs Containing a 1-Substituted 2-Oxoimidazolidine Moiety. Int. J. Pept. Protein Res. 1989, 33, 403-411. (b) Maeda, H.; Suzuki, M.; Sugano, H.; Yamamura, M.; Ishida, R. Synthesis and Central Nervous System Actions of Thyrotropin-Releasing Hormone Analogs Containing a 1-Oxo-1,2,3,4-Tetrahydroisoquinoline Moiety. Chem. Pharm. Bull. 1988, 36, 190-201.
- (13) (a) Sharif, N. A. Diverse Roles of Thyrotropin Releasing Hormone in Brain, Pituitary, and Spinal Function. Trends Pharmacol. Sci. **1985**, 119–122. (b) Veber, D. F.; Holly, F. W.; Varga, S. L.; Hirschmann, R.; Nutt, R.; Lotti, V. J.; Porter, C. C. The Dissociation of Hormonal and CNS Effects in Analogs of TRH.
- Dissociation of Territoria and CAS Effects in Analogs of Territoria Pept., Proc. Eur. Pept., Symp., 14th, 1976 1976, 453-461.
 (14) (a) Alamo, C.; Vallejo, M.; Cuenca, E. Effect of TRH on the Catalepsy Induced by Various Neuroleptics. Arch. Pharmacol. Toxicol. 1982, 8, 151-156. (b) Faakkari, P.; Feuerstein, G. Antagonism of Dermorphin-induced Catalepsy with Naloxone, TRH-analog CG3703 and the Benzodiazepine Antagonist, Ro 15-
- 1788. Neuropharmacology 1988, 27, 1007-1012.
 (15) (a) Sharif, N. A.; To, Z.; Whiting, R. L. First Pharmacological Characterization of TRH Receptors Linked to Phosphoinositide Hydrolysis in GH3 Cells Using Agonist Specificity of TRH Analogs. Biochem. Biophys. Res. Commun. 1989, 161, 1306-1311. (b) McDermott, A. M.; Dickinson, S. L.; Wilkin, G. P. Thyrotropin Releasing Hormone (TRH) and a Degradation Stabilized Analogue (RX77368) Stimulate Phosphoinositide Turnover in Cultured Astrocytes in a Regionally Specific Manner. Neurochem. Int. 1992, 20, 307-313.
- (16) Vonhof, S.; Feuerstein, G. Z.; Cohen, L. A.; Labroo, V. M. Norvaline²-TRH: Binding to TRH Receptors in Rat Brain Homogenates. Eur. J. Pharmacol. 1990, 180, 1-12.
 (a) Hinkle, P. M.; Woroch, E. L.; Tashjian, G. H. Receptor-
- (17)Binding Affinities and Biological Activities Analogs of Thyrotro-pin-Releasing Hormone in Prolactin-Producing Pituitary Cells in Culture. J. Biol. Chem. 1974, 249, 3085-3090. (b) Goren, H. J.; Bauce, L. G.; Vale, W. Forces and Structural Limitations of Binding of Thyrotrophin-Releasing Factor to the Thyrotrophin-Releasing Receptor: the Pyroglutamic Acid Moiety. *Mol. Phar-macol.* **1977**, *13*, 606-614.
- (18)Kamiya, K.; Takamoto, M.; Wada, Y.; Fujino, M.; Nishikawa, M. Molecular Conformation of Thyrotropin Releasing Hormone from the X-ray Analysis of its Tartrate. J. Chem. Soc., Chem.
- Commun. 1980, 438-439. Szirtes T.; Kisfaludy, L.; Palosi, E.; Szporny, L. Synthesis of (19)Szirtes T.; Kistaludy, L.; Palosi, E.; Szporny, L. Synthesis of Thyrotropin Releasing Hormone Analogs. 1. Complete Dissocia-tion of Central Nervous System Effects from Thyrotropin Releasing Activity. J. Med. Chem. 1984, 27, 741-745. Vicar, J.; Abillon, E.; Toma, F.; Piriou, F.; Lintner, K.; Bláha, K.; Fromageot, P.; Fermandjian, S. Two Conformations of TRH in Solution. FEBS Lett. 1979, 97, 275-278.
- (20)
- (21)Moore, M. L. Probing the Thyroliberin Receptor. Ph.D. Thesis, Washington University, St. Louis, MO, 1978; pp 23-28.

- System Activity. J. Med. Chem. 1986, 29, 1654-1658.
 (23) Stezowski, J. J.; Eckle, E. Structural Properties of TRH Analogs: Probing Structure-CNS Activity Relationships at the Molecular Level. J. Med. Chem. 1985, 28, 125-137.
- (24) Font, J. Computer-Assisted Drug Design and the Receptor-Bound Conformation of Thyrotropin Releasing Hormone (TRH); Design and Synthetic Approaches Towards Rigid TRH Analogs. Thesis, Washington University, St. Louis, MO, 1986.
- (25) For a recent report on progress toward synthesis of a constrained analog based on this hypothesis, see: Moeller, K. D.; Rothfus, S. L. Tetrahedron Lett. 1992, 33, 2913-2916.
 (26) Bös, M.; Olson, G. L.; Vincent, G. P. J. An "Umpolung" Route to a set of the set of
- (26) Bös, M.; Olson, G. L.; Vincent, G. P. J. An "Umpolung" Route to Peptide Mimetics of Thyrotropin Releasing Hormone Based on a Cyclohexane Framework. *Helv. Chim. Acta* **1994**, 77, 463– 469.
- (27) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. J. Org. Chem. 1969, 34, 2543-2549.
- (28) Ager, D. J. The Peterson Olefination Reaction. Org. React. 1990, 38, 1-223.
- (29) Wittig, G.; Reiff, H. New Methods of Preparative Organic Chemistry. VI. Directed Aldol Condensations. Angew. Chem., Int. Ed. Engl. 1968, 7, 7-14.
- (30) Van Leusen, A. M.; Schaart, F. J.; Van Leusen, D. Chemistry of Sulfonylmethyl isocyanides. 18. Synthesis of 1-Isocyano-1-tosyl-1-alkenes and their Use in the Preparation of Imidazoles. *Recl. Trav. Chim. Pays-Bas* 1979, 98, 258-262.
- (31) Symons, J. P.; Davis, R. E.; Marriott, J. G. Water-Maze Learning and Effects of Cholinergic Drugs in Mice Strains with High and Low Hippocampal Pyramidal Cell Counts. *Life Sci.* 1988, 42, 375-383.
- (32) Vincent, G. P.; Sepinwall, J. AF102, a Novel M₁ Agonist, Enhanced Spatial Learning in C57BL/10 Mice with a Long Duration of Action. Brain Res. 1992, 597, 264-268.
- (33) Vincent, G. P.; Pietrusiak, N.; Rumennik, L.; Sepinwall, J. The Effects of Galanthamine, an Acetylcholinesterase Inhibitor, on Learning in Mice and Monkeys. Soc. Neurosci. Abstr. 1988, 14, 58.
- (34) Pietrusiak, N.; Vincent, G. P.; Harney, R.; Sepinwall, J. Use of Proximal vs. Distal Cues in a Water Maze Task is Strain Dependent: Aniracetam (Ro 13-5057) Reverses Distal Cue Deficits. Soc. Neurosci. Abstr. 1986, 12, 705.
- (35) (a) Morris, R. Development of a Water Maze Procedure for Studying Spatial Learning in the Rat. J. Neurosci. Methods 1984, 11, 47-60. (b) Morris, R. G. M. Spatial Localization Does Not Require the Presence of Local Cues. Learning Motivation 1981, 12, 239-260.
- (36) The side chains of such compounds can be superimposed on their inverted diastereoisomers because the relative stereochemistry is essentially different only in the cyclohexane ring puckering. Olson, G. L.; Chiang, E.; Vincent, G. P.; Sepinwall, J. Unpublished results.

- (37) Edelmann, A. Unpublished results.
- (38) Suzuki, M.; Sugano, H.; Matsumoto, K.; Yamamura, M.; Ishida, R. Synthesis and Central Nervous System Actions of Thyrotropin Releasing Hormone Analog Containing a Dihydroorotic Acid Moiety. J. Med. Chem. 1990, 33, 2130-2137.
- (39) Simasko, S. M.; Horita, A. Characterization and distribution fo [³H]-(3MeHis2) thryrotropin releasing hormone receptors in rat brain. *Life Sci.* **1982**, *30*, 1793-1799.
- (40) (a) Griffiths, E. C.; Kelly, J. A.; Ashcroft, A.; Ward, D. J.; Robson, B. Comparative Metabolism and Conformation of TRH and its Analogs. In *Thyrotropin Releasing Hormone: Biomedical Significance*; Metcalf, G., Jackson, I. M. D., Eds.; New York Academy of Sciences: New York, 1989; p 217. (b) Miyamoto, M.; Fukuda, N.; Narumi, S.; Nagai, Y.; Saji, Y.; Nagawa, Y. Gamma.-Butyrolactone-Gamma.-carbonyl-histidyl-prolinamide Citrate-(DN-1417): a Novel TRH Analog with Potent Effects on the Central Nervous System. *Life Sci.* 1981, 28, 861-869.
- (41) Grant, G.; Vale, W.; Guillemin, R. Characteristics of the Pituitary Binding Sites for Thyrotropin-Releasing Factor. *Endocrinology* 1973, 92, 1629–1633.
- (42) One referee has suggested that the cyclohexane compounds could bind to different sites than TRH on the TRH receptor, functioning as "effector molecules". In this concept, the observed activity of the compounds could be due to modulation of the functional performance of the native ligand. While this idea has some merit, it is difficult to reconcile with the structural homology of the cyclohexane compounds to TRH, the parallel SAR of the cyclohexane compounds and TRH peptide analogs, and the ability of the mimetics to compete for TRH in binding assays. Nevertheless, alternatives to the direct binding interaction need to be considered to explain the high *in vivo* potency of the compounds and may stimulate a different way of thinking about approaches to selectively enhance the CNS activities of TRH.
- (43) Olson, G. L.; Jiang, N.; Chiang, E.; Jones, M. E.; Vincent, G. P.; Sepinwall, J. Unpublished results.
- (44) Schoch, P. Unpublished results. In the same assay, TRH has an $\rm IC_{50}$ value of 18.5 nM.
- (45) Campaigne, E.; Matthews, D. P. Reaction of N-Benzylpyroglutamic Acid with Phenyl Grignard. J. Heterocycl. Chem. 1975, 12, 391-392.
- (46) If the crude imine 20 was chromatographed on silica gel, eluting with 1% methanol in EtOAc, the product also contained ca. 30– 50% of the corresponding aldehyde. This mixture could also be used directly in the subsequent TOSMIC reaction, since benzylamine was employed as the base in that step, permitting *in situ* formation of the imine from the aldehyde.
- (47) (a) Unnerstall, J. R.; Niehoff, D. L.; Kuhar, M. J.; Palacios, J. In Vitro Labeling Receptor Autoradiography: Loss of Label During Ethanol Dehydration and Preparative Procedures. J. Neurosci. Methods 1982, 6, 59-73. (b) Rainbow, T. C.; Bleisch, W. V.; Biegon, A.; McEwen, B. S. J. Neurosci. Methods. 1982, 6, 127-138.

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