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

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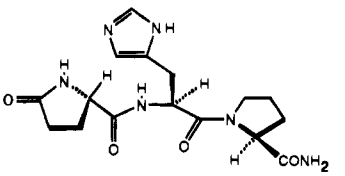
Received October 17, 1994®

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**, (1*S*,3*R*,5(2*S*),5*S*)-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]-methyl]-5-[(1*H*-imidazol-5-yl)methyl]cyclohexaneacetamide) was radiolabeled for binding studies and evaluated in other binding assays and pharmacological tests. Competition binding of **26** vs [^{3}H]MeTRH to rat brain slices suggests a two-site model for ligand binding with IC_{50}'s of 1 μM and 3 mM. Direct binding of [^{3}H]-**26** shows a biphasic curve with IC_{50}'s of 80 and 49 μM, 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 low-energy 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 side-chain 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.



TRH

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 [^{3}H]TRH and [^{3}H]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 animals[9] 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 Nva^2- and Nle^2-TRH analogs[11] 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 [^{3}H]MeTRH receptor

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® Abstract published in *Advance ACS Abstracts*, June 15, 1995.

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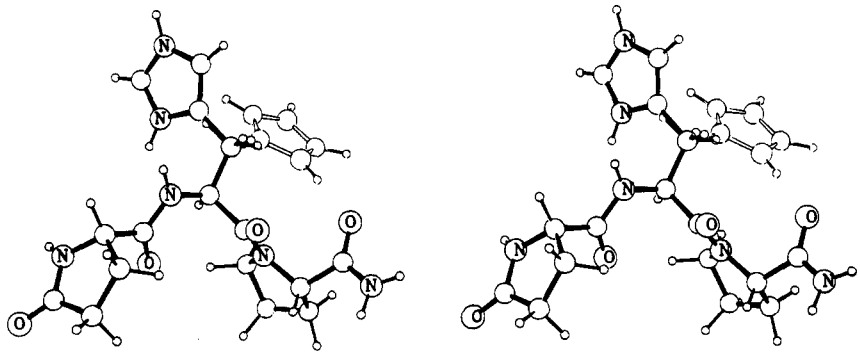


Figure 1. X-ray (open bonds) and proposed bioactive (solid bonds) conformations of TRH.

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., Nva^2- and Nle^2-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 analogs[17] and by the fact that crystal[18,19] and solution structures[20] 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, Moore[21] 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 analogs[22] suggest a similar pharmacophore but with a wider tolerance for variants at the His^2 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 hydrogen-bonded 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 structures[23] 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 1*S*,3*R*,5(2*S*),5*S* as 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 Nva^2- and Nle^2-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

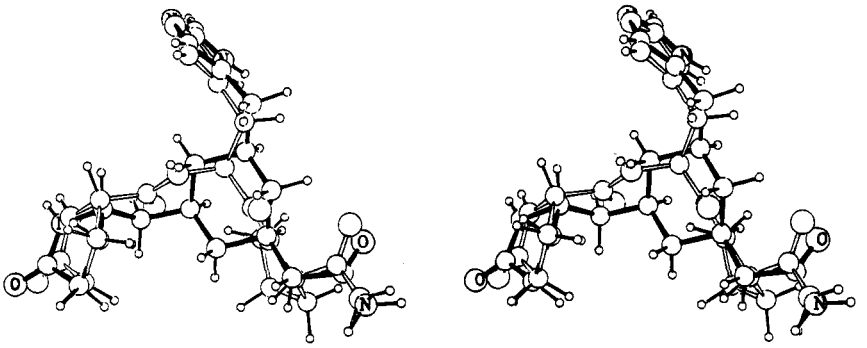
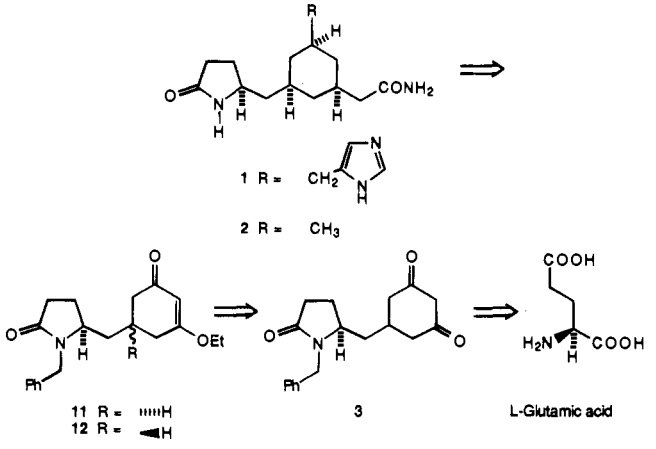


Figure 2. Cyclohexane mimetic of TRH (solid bonds) superimposed on proposed bioactive (open bonds) conformation of TRH. The conformation of the mimetic was generated using the Multifit procedures of SYBYL (Tripos Assoc., St. Louis, MO) to match the pharmacophoric groups to the TRH template followed by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

of functionalized side chains to the dione **3**, a compound having its single asymmetric center derived from L-glutamic 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.



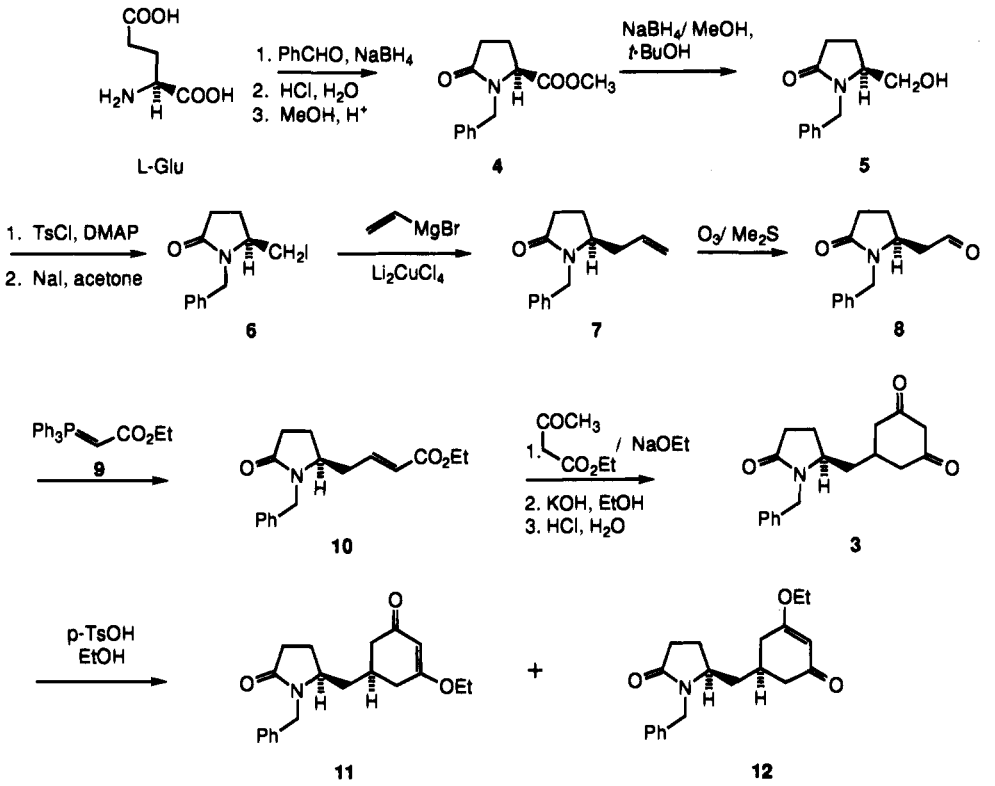
The synthesis of dione **3** proceeded from L-glutamic acid as illustrated in Scheme 1. L-Glutamic acid was benzylated using $NaBH_4$ and benzaldehyde, cyclized by heating in aqueous HCl, and esterified to give the lactam methyl ester **4**. Reduction of **4** with $NaBH_4$ in MeOH/*t*-BuOH afforded the hydroxymethyl derivative **5** as a pure enantiomer after one recrystallization as judged by chromatography and ^{1}H-NMR of the derived (−)-MTPA ester.[27] Conversion of **5** to the iodide **6** via the tosylate and subsequent coupling with excess vinylmagnesium bromide/Li_2CuCl_4 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 olefination[28] 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** (1*R*,3*R*,5*S*,5(2*S*)) 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/NH_3 gave isomerically homogeneous *N*-benzyl amide **16**. N-Debenzylation of **16** to produce **2** was accomplished by reduction with Na/NH_3. The stereochemistry of product **2** was again confirmed by X-ray crystallography.

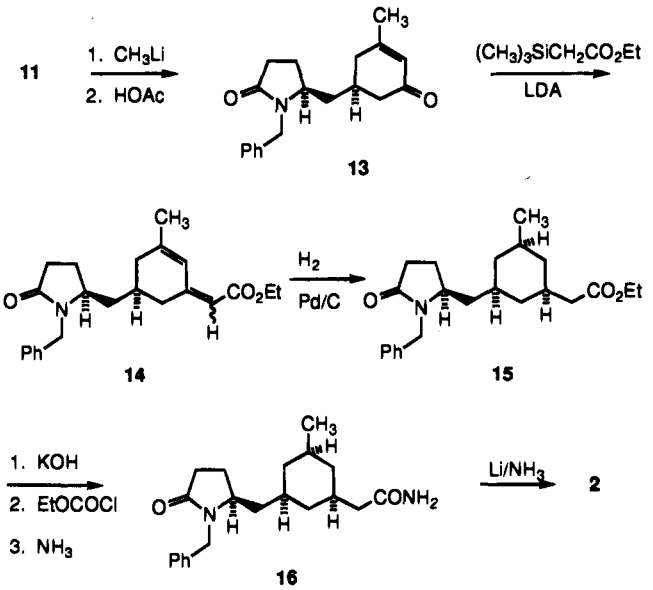
For the preparation of the imidazolylmethyl series (Scheme 3), the less polar, crystalline ethoxycyclohexenone **12** was used as the starting material and the acetic acid side chain was introduced first. Thus treatment of **12** with ethyl (trimethylsilyl)acetate/LDA followed by acidic rearrangement (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 condensation[29] 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 procedure[30] 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



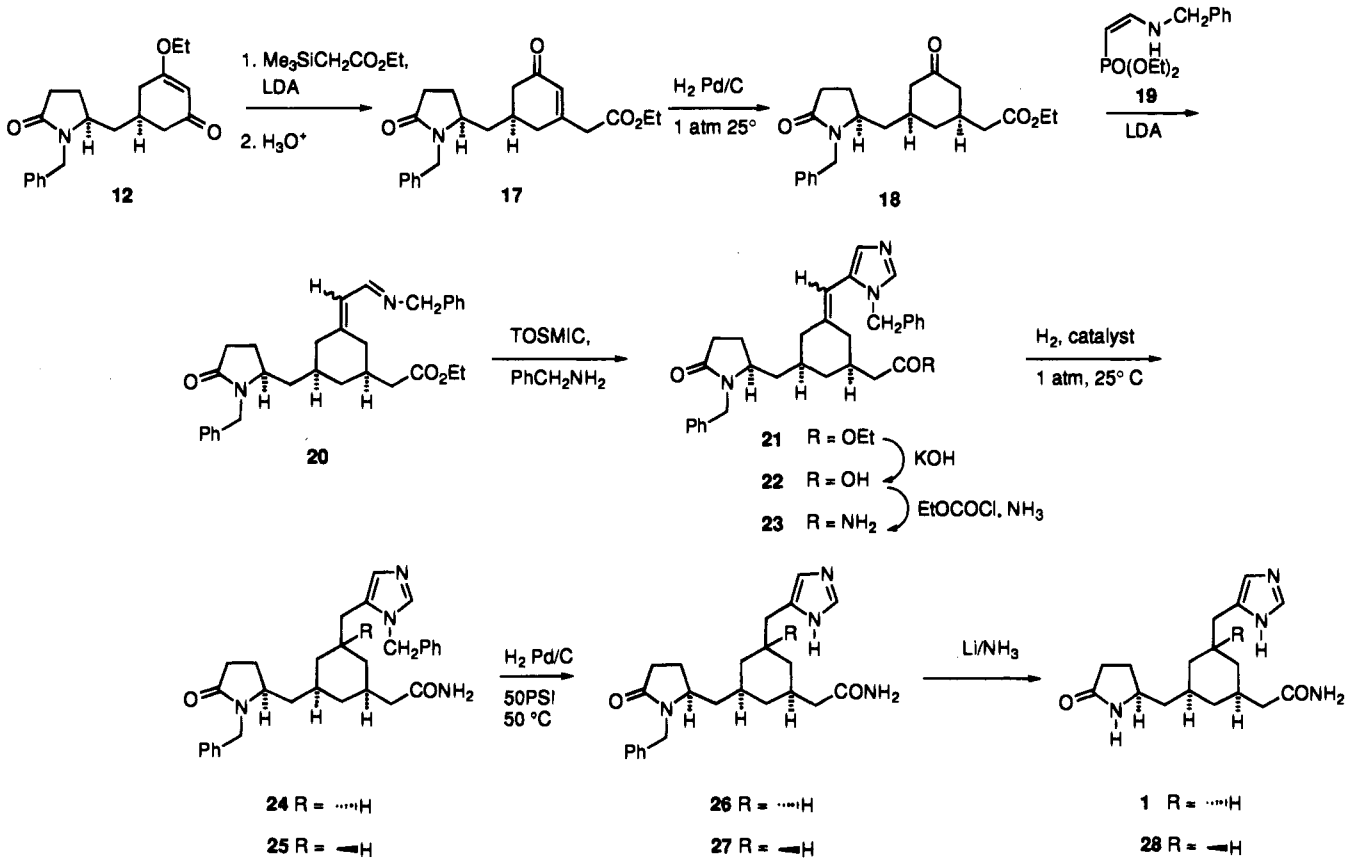
amide **23** gave different ratios of the all-cis isomer **24** and the trans isomer **25** depending on the catalyst used. Ester **21** afforded a 2:8 ratio of cis/trans with 10% Pd/C. This outcome is different from the case of the methyl series (e.g., **14** → **15**) where the predominant product 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*/*Z* stereochemistry 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, 3*R*-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 ^{1}H-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/NH_3 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 task[31] 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 cholinergic[32-33] and nootropic[34] 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

Scheme 3



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. 10-fold 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 group[26] 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 Nva^2-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]

compd	dose (mg/kg)	mean ± SEM latency (s)	compd	dose (mg/kg)	mean ± SEM 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 ± 0.65**
	0.003	43.08 ± 0.67**		0.0003	33.29 ± 0.52**
	0.01	37.97 ± 0.79**		0.003	22.24 ± 1.02**
	0.03	32.69 ± 0.74**		0.03	15.65 ± 0.45**
	0.1	25.76 ± 2.20**		0.3	17.62 ± 0.60**
	0.3	20.85 ± 0.53**		3.0	31.62 ± 0.96**
	1.0	28.87 ± 0.74**			
	3.0	33.57 ± 0.90**			
	10.0	46.00 ± 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 ± 0.49**
	0.01	38.72 ± 1.07**		0.0003	30.90 ± 0.38**
	0.1	45.06 ± 1.29**		0.003	35.23 ± 0.88**
	1.0	25.23 ± 0.91**		0.03	21.10 ± 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 ± 0.47**
	0.1	30.08 ± 6.49**		0.003	37.01 ± 0.59**
	0.3	43.13 ±4.21		0.03	28.87 ± 1.07**
				0.3	27.59 ± 0.71**
				3.0	43.72 ± 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 ± 2.82**		0.1	46.09 ± 1.02**
	0.01	22.15 ± 2.68**		1.0	24.19 ± 0.77**
	0.03	26.49 ± 2.92**			
	0.1	26.87 ± 5.03**			
	0.3	46.83 ± 3.08			
16 (po)	Veh	50.64 ± 2.28			
	0.003	42.20 ± 6.35			
	0.01	35.88 ± 8.25			
	0.03	32.87 ± 3.34*			
	0.1	24.55 ± 3.55**			
	0.3	46.53 ± 2.67			

[a] * = $p < 0.05$. ** = $p < 0.01$.

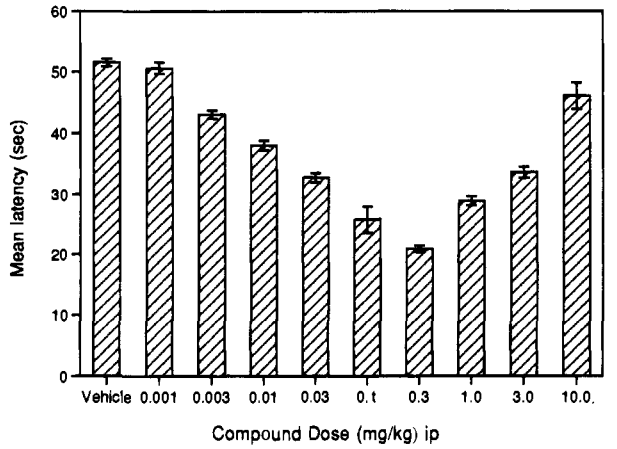


Figure 3. Effects of TRH on mean latency (±SEM) of mice in the Morris water maze procedure to locate the escape platform. Vehicle or TRH was administered ip 30 min prior to the four-trial session.

when [^{3}H]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 scopolamine-induced 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 [^{3}H]-MeTRH in the rat brain homogenate membrane receptor binding assay[39] ($IC_{50} > 10^{-5}$ M) and was devoid of significant receptor binding in a panel of diverse receptor binding assays (NovaScreen, see the supporting information).

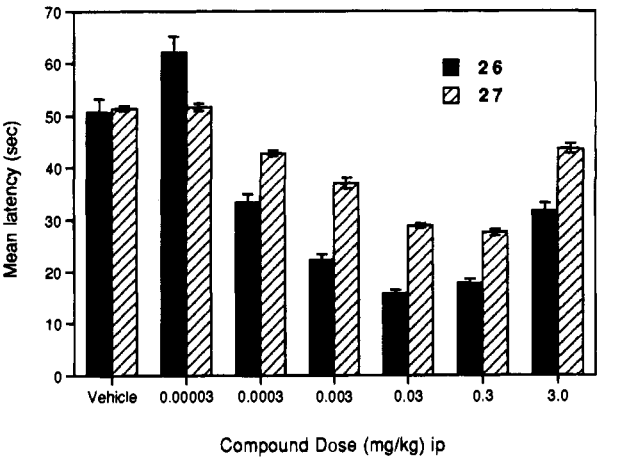


Figure 4. Effects of TRH mimetic diastereoisomeric compounds **26** (solid bars) and **27** (hashed bars) on mean latency (±SEM) of mice in the Morris water maze procedure to locate the escape platform. Vehicle, compound **26**, or compound **27** was administered ip 30 min prior to the four-trial session.

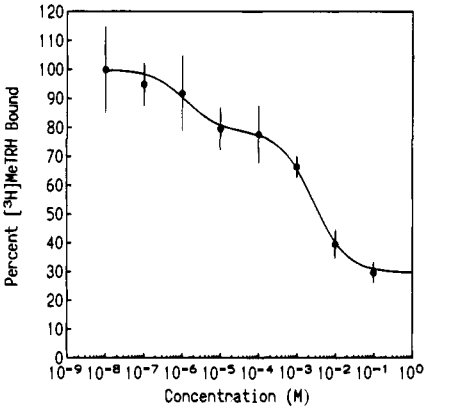


Figure 5. [^{3}H]MeTRH vs compound **26**. Competition analysis of mimetic **26** inhibition of [^{3}H]MeTRH binding exhibited a two-site model of ligand binding. The IC_{50}'s for the high- and low-affinity sites are 1 μM and 3 mM, respectively. The proportion of high-affinity sites was 30%, while low-affinity sites account for the remaining 70% of sites. $R^2 = 0.939$.

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 [^{3}H]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 [^{3}H]MeTRH with a biphasic binding curve with high- and low-affinity sites of 1 μM 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 $^3H_2$ over 10% Pd/C.) Binding studies, as shown in Figure 6, showed

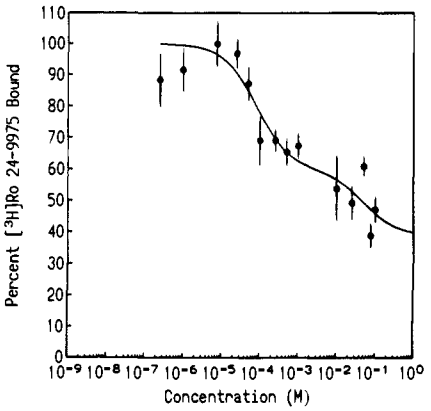


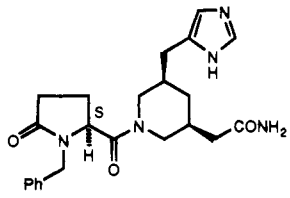
Figure 6. [^{3}H]-**26** vs compound **26**. Self-competition analysis using [^{3}H]-**26** demonstrated a biphasic displacement curve with IC_{50}'s of high- and low-affinity sites of 80 μM and 49 mM, respectively. High-affinity sites comprise 65% of binding sites, and the remaining 35% represent low-affinity sites. R^2 = 0.884.

specific and saturable biphasic binding with high- and low-affinity sites (80 μM 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 [^{3}H]-**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 TRH-like 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 μM) in binding to the high-affinity endocrine receptor.[44]



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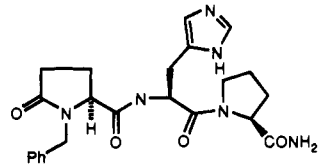

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Taken together, the results with *N*-benzyl TRH and piperidine **29** support a hypothesis that the conformation, rather than an additional pharmacophore, is the determinant of cognitive activity and that binding to the micromolar affinity binding 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. ^{1}H-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 $NaBH_4$ (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 $NaBH_4$ (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 CH_2Cl_2 (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 H_2O 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 $NaHCO_3$ 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 CH_2Cl_2. The combined extracts were washed with brine and dried over $MgSO_4$. Evaporation of the solvent afforded 132 g of the known[45] methyl ester **4** as an oil which was >95% pure by NMR: ^{1}H-NMR ($CDCl_3$) δ 2.0–2.6 (m, 4 H, CH_2's), 3.68 (s, 3 H, CH_3), 3.95 (dd, 1 H, J = 4, 9 Hz, H-4), 4.02 and 5.03 (AB, 2 H, J_{gem} = 16 Hz, CH_2Ph), 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 Na_2SO_4. 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); ^{1}H-NMR ($CDCl_3$) δ 1.92 and 1.94 (dd, J = 6.5 Hz, 1 H, OH), 1.96–2.11 (m, 2 H, CH_2), 2.36–2.61 (m, 2 H, $COCH_2$), 3.48–3.78.(m, 3 H, CH_2O and CH), 4.29 and 4.81 (AB, J_{gem} = 15 Hz, 2 H, CH_2Ph), 7.26–7.35 (m, 5 H, phenyl); $[\alpha]^{25}_D$ = +116° (*c* 1.07, methanol). Anal. ($C_{12}H_{15}NO_2$) 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 ^{1}H-NMR and TLC, was obtained and could readily be differentiated from the diastereomeric mixture (by the doubling of the CH_2N and CH_2O signals in the ^{1}H-NMR) produced when racemic 5-(hydroxymethyl)-1-(phenylmethyl)-2-pyrrolidinone was used to form the (−)-MTPA ester.

(*S*)-5-(Iodomethyl)-1-(phenylmethyl)-2-pyrrolidinone (6). To a solution of the alcohol **5** (143.7 g, 0.70 mol) in 3.5 L of CH_2Cl_2 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 °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 $MgSO_4$, 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: ^{1}H-NMR ($CDCl_3$) δ 1.7–2.75 (m, 4 H, CH_2), 3.18–3.28 (m, 2 H, CH_2I), 3.42 (m, 1 H, NCH), 3.98 and 4.05 (AB, 2 H, J_{gem}= 15 Hz, CH_2Ph), 7.20 (m, 5 H, phenyl); $[\alpha]^{25}_D$ = +12.11° (*c* 0.35, methanol). Anal. ($C_{12}H_{14}NOI$) 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 Li_2CuCl_4 (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 CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. 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: ^{1}H-NMR ($CDCl_3$) δ 1.7–2.75 (m, 6 H, CH_2's), 3.51 (m, 1 H, NCH), 4.99 and 5.02 (AB, J_{gem} = 15 Hz, 2 H, CH_2Ph), 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° (*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:CH_2Cl_2 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 CH_2Cl_2, washed with water, and dried over Na_2SO_4. 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**: ^{1}H-NMR ($CDCl_3$) 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, CH_2CHO), 3.98 (m, 1 H, NCH), 4.10 and 4.82 (AB, 2 H, J_{gem}= 15 Hz, CH_2Ph), 7.15–7.40 (m, 5 H, phenyl), 9.65 (s, 1 H, CHO).

(*E*)-4-[2(*R*)-5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]-2-butenoic 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: ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, J = 5.5 Hz, CH_3), 1.60–2.50 (m, 6 H, CH_2's), 3.60 (m, 1 H, NCH), 3.96 and 4.99 (AB, J_{gem}= 15 Hz, CH_2Ph), 4.15 (q, J = 5.5 Hz, 2 H, OCH_2), 5.85 (d, J = 16 Hz, 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° (*c* 1.0, MeOH); HRMS (EI) calcd for $C_{17}H_{21}NO_3$ 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 Na_2CO_3. The solution was washed (discarded) with 200 mL of CH_2Cl_2, and the aqueous phase was acidified to pH 1 with 2 N HCl. The mixture (gummy residue) was extracted with 3 × 300 mL of CH_2Cl_2, and the combined extracts were washed with water and dried over $NaSO_4$. 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); ^{1}H-NMR ($CDCl_3$) (9:1 keto:enol forms) δ 1.38 (ddd, 1 H, J_{vic} = 4, 11 Hz, J = 14 Hz, CH of CH_2), 1.60–2.71 (m, 10 H, 4 CH_2, CH of CH_2, CH), 3.37 (s, 10/9 H, CH_2 of keto), 3.46 (m, 1 H, NCH), 3.94 and 4.96 (AB, 8/9 H, J_{gem} = 15 Hz, CH_2 of enol), 3.95 and 4.99 (AB, 10/9 H, J_{gem} = 15 Hz, CH_2 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 ($CHCl_3$) 1730, 1710, 1600, 702 cm^{-1}; MS (EI) m/e 299 (M^+); $[\alpha]^{25}_D$ = +44.97° (*c* 1.04, methanol). Anal. ($C_{18}H_{21}NO_3$) C,H,N.

(5*S*,1*R*)-5-[(3-Ethoxy-5-oxo-3-cyclohexen-1-yl)methyl]-1-(phenylmethyl)-2-pyrrolidinone (11) and (5*S*,1*S*)-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 CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4. 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 (5*S*,1*R*)-ethoxy enone **11** and the (5*S*,1*S*)-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, 5*S*,1*R*-diastereomer **11** as an oil and 23.9 g (24.6% yield) of the less polar, solid, 5*S*,1*S*-diastereomer **12** was obtained.

For the 5*S*,1*R*-diastereomer 11: TLC (ethyl acetate:methanol, 95:5) R_f = 0.4; $[\alpha]^{25}_D$ = 0.0° (methanol, *c* 1.0); ^{1}H-NMR ($CDCl_3$) δ 1.35 (t, J = 6 Hz, 3 H, CH_3), 1.50–2.50 (m, 11 H, CH_2 and CH), 3.46 (m, 1 H, CHN), 3.90 (q, J = 6 Hz, 2 H, CH_2CH_3), 3.95 and 5.05 (AB, J_{gem}= 15 Hz, 2 H, CH_2Ph), 5.35 (s, 1 H, vinyl H), 7.24–7.34 (m, 5 H, phenyl); MS (EI) m/e 327 (M^+); IR ($CHCl_3$) 1672 cm^{-1} (C=O). Anal. ($C_{20}H_{25}NO_3$) C,H,N.

For the 5*S*,1*S*-diastereomer 12: mp 92–93 °C; TLC (ethyl acetate:methanol, 95:5) R_f = 0.5; $[\alpha]^{25}_D$ = +74.61° (methanol, *c* 1.0); ^{1}H-NMR ($CDCl_3$) δ 1.35 (t, J = 6 Hz, 3 H, CH_3), 1.50–2.50 (m, 11 H, CH_2 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, CH_2Ph), 5.35 (s, 1 H, vinyl H), 7.24–7.34 (m, 5 H, phenyl); MS (EI) m/e 327 (M^+); IR ($CHCl_3$) 1672 cm^{-1} (C=O). Anal. ($C_{20}H_{25}NO_3$) C,H,N.

For an X-ray analysis, crystals were grown from EtOAc/MeOH. The stereochemistry was determined to be 5*S*,1*S* 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.

(5*S*,1*R*)-5-[(3-Methyl-5-oxo-3-cyclohexen-1-yl)methyl]-1-(phenylmethyl)-2-pyrrolidinone (13). To a solution of (5*S*,1*R*)-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 quenched by the addition of 50 mL of 50% aqueous acetic acid. The mixture was allowed to warm to room temperature and was extracted with CH_2Cl_2. The combined extracts were washed with saturated $NaHCO_3$, dried over Na_2SO_4, 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 (5*S*,1*R*)-keto ester **13** as an oil. A sample was evaporatively distilled at 200–210 °C/0.05 Torr for analysis: ^{1}H-NMR ($CDCl_3$) δ 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, CH_2Ph), 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 ([Θ] = +1425, max), 283 ([Θ] = −42, min.), 238 ([Θ] = +13 620, infl), 218 ([Θ] = +50 100, max), 212 nm ([Θ] = +39 820°, min.). Anal. ($C_{19}H_{23}NO_2$) C,H,N.

(5*S*-(5*S*))-[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 °C over 10 min, and the solution was allowed to warm to −20 °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 $MgSO_4$, 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: ^{1}H-NMR ($CDCl_3$) δ 1.25 (2t (*Z*/*E*), 3 H, OCH_2CH_3), 1.60 and 1.70 (2s (*Z*/*E*), CH_3), 1.20–2.50 (m, 12 H, CH_2 and CH), 3.45 and 3.58 (2m (*Z*/*E*), NCH), 3.90, 5.00 and 3.99, 4.90 (2AB (*Z*/*E*), J_{gem}= 15 Hz, 2 H, CH_2Ph), 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 ([Θ] = +1495, max), 217 ([Θ] = +33 643, max), 213 ([Θ] = +30 321, min.), 198 nm ([Θ] = +45 690°, max). Anal. ($C_{23}H_{29}NO_3$) C,H,N.

(1*R*,3*R*,5*S*,5(2*S*))-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 1*R*,3*R*,5*S*,5(2*S*)-isomer containing ca. 15% of two other diastereomers by NMR. A sample was evaporatively distilled at 200–210 °C/0.25 Torr for analysis: ^{1}H-NMR ($CDCl_3$) δ 0.4 (q, J = 10 Hz, 1 H, axial CH), 0.55 (m, 2 H, 2 axial CH), 0.84 (d, J = 6.6 Hz, 0.45 H, CH_3 of minor diastereomer), 0.86 (d, J = 6.6 Hz, 2.1 H, CH_3 of major diastereomer), 0.96 (d, J = 6.6 Hz, 0.45 H, CH_3 of minor diastereomer), 1.20 (t, J = 6 Hz, 3 H, OCH_2CH_3), 1.20–2.50 (m, 14 H, CH_2 and CH), 3.45 (m, 1 H, CHN), 4.10 (q, J = 6 Hz, OCH_2CH_3), 3.95 and 4.96 (AB, J_{gem}= 15 Hz, 2 H, CH_2Ph), 7.20–7.35 (m, 5 H, phenyl); MS (EI) m/e 371 (M^+); CD (ethanol) 264 ([Θ] = +118, max), 261 ([Θ] = +65, min.), 258 ([Θ] = +191, max), 254 ([Θ] = +121, min.), 233 ([Θ] = −1032, max), 217 ([Θ] = +32 475, max), 211 ([Θ] = +28 070, min.), 195 nm ([Θ] = +46 787°, max).

(1*R*,3*R*,5*S*,5(2*S*))-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 CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, 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 $CHCl_3$ and

cooled to −30 °C. To the solution was added dropwise ethyl chloroformate (2.06 g, 19.1 mmol) in 15 mL of $CHCl_3$ followed by triethylamine (1.93 g, 19.1 mmol) in 10 mL of $CHCl_3$. 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 $NaHCO_3$, and brine and dried over Na_2SO_4. 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): ^{1}H-NMR ($CDCl_3$) 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, CH_2Ph), 5.30 (br d, J = 15 Hz, 2 H, $CONH_2$), 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 ([Θ] = +115, max), 261 ([Θ] = +52, min.), 258 ([Θ] = +190, max), 255 ([Θ] = +107, min), 252 ([Θ] = +164, max), 233 ([Θ] = −1850, max), 216 ([Θ] = +41 667, max), 211 ([Θ] = +36 980, min.), 196 nm ([Θ] = +57 812°, max). Anal. ($C_{21}H_{30}N_2O_2$) C,H,N.

(1*R*,3*R*,5*S*,5(2*S*))-3-Methyl-5-[(5-oxo-2-pyrrolidinyl)-methyl]cyclohexaneacetamide (2). A solution of the (1*R*,3*R*,5*S*,5(2*S*))-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 NH_4Cl. The dry ice condenser was removed, and the ammonia was allowed to evaporate. The mixture was then diluted with CH_2Cl_2 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); ^{1}H-NMR ($CDCl_3$) 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, $CONH_2$), 6.40 (s, 1 H, NH); IR ($CHCl_3$) 1688 cm^{-1}; MS (EI) m/e 252 (M^+); CD (ethanol) 215 ([Θ] = −11.120, max), 195 nm ([Θ] = +21 685°, 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.

(5*S*-5(2*S*))-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 CH_2Cl_2, 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 CH_2Cl_2, and the extracts were washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. 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: ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, J = 7 Hz, CH_3), 1.25−2.60 (m, 11 H, CH_2 and CH), 3.14 and 3.15 (AB, J_{gem} = 15 Hz, 2 H, CH_2CO), 3.47 (m, 1 H, NCH), 3.97 and 5.00 (AB, J_{gem} = 15 Hz, 2 H, CH_2Ph), 4.15 (q, J = 7 Hz, 2 H, OCH_2), 7.20−7.40 (m, 5 H, phenyl); MS (EI) m/e 369 (M^+). Anal. ($C_{22}H_{27}NO_4$) C,H,N.

(1*S*,5*S*,5(2*S*))-3-Oxo-5-[[5-oxo-1-(phenylmethyl)-2-pyrrolidinyl]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 CH_2Cl_2, the solution was dried over Na_2SO_4, 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: ^{1}H-NMR ($CDCl_3$) δ 0.95 (m, 1 H, axial ring H), 1.25 (t, J = 7 Hz, 3 H, CH_3), 1.6−2.6 (m, 13 H, CH_2 and CH), 3.45 (m, 1 H, NCH), 3.97 and 4.98 (AB, J_{gem} = 15 Hz, 2 H, CH_2Ph), 4.12 (q, J = 7 Hz, 2 H, OCH_2), 7.20−7.40 (m, 5 H, phenyl); MS (EI) m/e 371 (M^+); $[\alpha]^{25}_D$ = +16.88° (*c* 0.9359, methanol). Anal. ($C_{22}H_{29}NO_4$) 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 CH_2Cl_2. The combined extracts were washed with saturated $NaHCO_3$ and dried over Na_2SO_4. 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: ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, J = 7 Hz, 6 H, CH_3's), 3.92−4.10 (m, 5 H, OCH_2, CHP), 4.19 (d, J = 5.5 Hz, 2 H, CH_2Ph), 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)*-(1*S*,5*R*,5(2*S*))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1*H*-imidazol-5-yl]-methylene]cyclohexaneacetic Acid Ethyl Ester (21E) and *(Z)*-(1*S*,5*R*,5(2*S*))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1*H*-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 CH_2Cl_2, 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 Na_2SO_4, and the solvent was removed on a rotary evaporator to give crude imine **20**:[46] ^{1}H-NMR ($CDCl_3$) δ 0.65−1.00 (m, 2 H, axial ring H), 1.15−1.35 (2t, 3 H, CH_3), 3.50 (m, 1 H, NCH), 3.95−4.20 (m, 4, CH_2CO), 4.00 and 5.00 (AB, J_{gem} = 15 Hz, 2 H, CH_2Ph), 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 CH_2Cl_2. The solution was washed with 2 N HCl and saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$. 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: ^{1}H-NMR ($CDCl_3$) δ 0.67 (q, J = 12.3 Hz, 1 H, axial H of ring CH_2), 1.23 (t, J = 7.4 Hz, 3 H, OCH_3), 1.15–2.28 (m, 12 H, 2 CH, 4 CH_2, 2 CH of CH_2), 2.41 (m, 2 H, ring CH_2CO), 2.91 (br d, 1 H, J_{gem} = 12.6 Hz, equatorial H of ring CH_2), 8.44 (br m, 1 H, NCH), 3.94 and 4.96 (AB, 2 H, J_{gem} = 14.8 Hz, CH_2Ph on lactam), 4.11 (q, 2 H, J = 7.4 Hz, OCH_2), 5.05 (s, 2 H, CH_2Ph 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: ^{1}H-NMR ($CDCl_3$) δ 0.66 (br q, J = 11.5 Hz, 1 H, axial H of ring CH_2), 1.23 (t, J = 7.4 Hz, 3 H, OCH_3), 1.10–2.60 (m, 12 H, 2 CH, 4 CH_2, 2 CH of CH_2), 2.40 (m, 2 H, ring CH_2CO), 2.79 (br d, 1 H, J_{gem} = 12.6 Hz, equatorial H of ring CH_2), 3.39 (br m, 1 H, NCH), 3.93 and 4.94 (AB, 2 H, J_{gem} = 14.8 Hz, CH_2Ph on lactam), 4.10 (q, 2 H, J = 7.4 Hz, OCH_2), 5.03 (s, 2 H, CH_2Ph 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)*-(1*S*,5*R*,5(2*S*))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1*H*-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 CH_2Cl_2 (discarded) and acidified to pH 3.0–3.5 with 6 N HCl. The gummy residue was extracted with CH_2Cl_2, the combined extracts were dried over Na_2SO_4, 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 $CHCl_3$, 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 NH_4Cl precipitate and concentrated on a rotary evaporator. The residue was chromatographed on silica gel (400 g), eluting with 5–10% MeOH in CH_2Cl_2. The fractions were concentrated, and the residue was dissolved in CH_2Cl_2, washed with water, and dried over Na_2SO_4 to give 3.78 g (54% overall yield from ester **21E**) of the dibenzyl cyclohexaneacetamide **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}; ^{1}H-NMR ($CDCl_3$) δ 0.66 (q, 1 H, J = 12.3 Hz, axial CH of ring CH_2), 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 CH_2), 1.53–1.88 (m, 6 H, CH_2, 4 CH of CH_2), 2.08 (m, 2 H, CH_2CO), 2.34 (m, 1 H, CH of CH_2), 2.38 (ddd, 1 H, J_{vic} = 6.8, 9.6 Hz, J_{gem} = 17.2 Hz, CH of ring CH_2CO), 2.49 (ddd, 1 H, J_{vic} = 6.6, 9.6 Hz, J_{gem} = 17.2 Hz, CH of ring CH_2CO), 2.82 (br d, 1 H, J_{gem} = 13.4 Hz, equatorial CH of ring CH_2), 3.42 (m, 1 H, NCH), 3.95 and 4.94 (AB, 2 H, J_{gem} = 15.3 Hz, CH_2Ph on lactam), 5.04 (s, 2 H, CH_2Ph on imidazole), 5.34 and 5.41 (2s, 2 H, $CONH_2$), 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 $C_{31}H_{36}N_4O_2$ 496.2838, obsd 496.2823.

***(Z)*-(1*S*,5*R*,5(2*S*))-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1*H*-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^+); ^{1}H-NMR ($CDCl_3$) δ 0.68 (q, 1 H, J = 12.3 Hz, axial CH of ring CH_2), 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 CH_2), 1.57–1.67 (m, 2 H, 2 CH of CH_2), 1.67–1.83 (m, 3 H, CH_2, CH of CH_2), 2.00–2.18 (m, 4 H, CH_2CO), 2.34 (m, 1 H, CH of CH_2), 2.38 (ddd, 1 H, J_{vic} = 6.8, 9.6 Hz, J_{gem} = 17.2 Hz, CH of CH_2CO, 2 CH of CH_2), 2.43 (m, 2 H, ring CH_2CO), 2.94 (br d, 1 H, J_{gem} = 13.0 Hz, equatorial CH of ring CH_2), 3.46 (m, 1 H, NCH), 3.95 and 4.95 (AB, 2H, J_{gem} = 15.0 Hz, CH_2Ph on lactam), 5.05 (s, 2 H, CH_2Ph on imidazole), 5.54 and 5.56 (2br s, 2 H, $CONH_2$), 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.

(1*S*,3*R*,5(2*S*),5*S*)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1*H*-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% NH_3 was added 10 g of Raney Ni (Aldrich; wet slurry in H_2O, washed twice with H_2O 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 3*S*-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 reverse-phase HPLC. The product mixture was chromatographed on silica gel (dry column) eluting with $CHCl_3$:MeOH:HOAc (100:20:1). The fractions containing **24** were combined and concentrated on a rotary evaporator. Water was added followed by NH_4OH until alkaline, and the material was extracted with CH_2Cl_2. The combined extracts were washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to afford 380 mg (38%) of pure **24**: mp 75 °C (softens above 63 °C); ^{1}H-NMR ($CDCl_3$) 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, CH_2Ph on lactam), 5.03 (s, 2 H, CH_2Ph on imidazole), 5.30 (d, J = 12 Hz, 2 H, $CONH_2$), 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 ([Θ] = +80, max), 233 ([Θ] = −2960, max), 216 ([Θ] = +38 400, max), 213 ([Θ] = +33 600, max), 200 nm ([Θ] = +56 000°, max).

(1*S*,3*S*,5(2*S*),5*S*)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-3-[[1-(phenylmethyl)-1*H*-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 H_2O and washed with CH_2Cl_2 (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 Na_2SO_4 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 $CHCl_3$, 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 NH_4Cl, and the filtrate was washed with H_2O, 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 $CHCl_3$, MeOH, H_2O, 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 CH_2Cl_2. The extracts were dried over Na_2SO_4 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 μm 3.5 mm x 15 cm column eluting with a gradient of H_2O (0.1% CF_3COOH)/CH_3CN (0.1% CF_3COOH) yielded the isomer **25** (99.7% purity by HPLC): mp 72 °C (softens above 56 °C); ^{1}H-NMR ($CDCl_3$) 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, CH_2Ph on lactam), 5.03 (s, 2 H, CH_2Ph on imidazole), 5.30 (br s, 2 H, $CONH_2$), 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 $C_{31}H_{38}N_4O_2$ (M^+) 498.2995, obsd (M + H) 498.2965; CD (ethanol) 258 ([Θ] = −56, min.), 232 ([Θ] = −2360, max), 216 ([Θ] = +36 800, max), 213 ([Θ] = +34 400, max), 202 nm ([Θ] = +57 600°, max).

(1*S*,3*R*,5(2*S*),5*S*)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-5-[(1*H*-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 g, dry column) eluting with the lower phase of a mixture prepared by shaking $CHCl_3$, MeOH, H_2O, 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 CH_2Cl_2. The extracts were dried over Na_2SO_4 and concentrated on a rotary evaporator to afford 146 mg (51% yield) of the monobenzyl amide **26**: ^{1}H-NMR ($CDCl_3$) δ 0.37–0.47 (m, 1 H, axial ring H), 0.56–0.68 (m, 2 H, axial ring H), 3.44 (m, 1 H, NCH), 3.92 and 4.95 (AB, J_{gem} = 15 Hz, CH_2Ph), 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^+); HRMS (EI) calcd for $C_{24}H_{32}N_4O_2$ (M^+) 408.2525, obsd (M^+) 408.2519; CD (ethanol) 233 ([Θ] = −1660, max), 217 ([Θ] = +39 200, max), 211 ([Θ] = +34 400, min.), 199 nm ([Θ] = +59 200°, max).

(1*S*,3*S*,5(2*S*),5*S*)-5-[[5-Oxo-1-(phenylmethyl)-2-pyrrolidinyl]methyl]-5-[(1*H*-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%: ^{1}H-NMR ($CDCl_3$) 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, CH_2Ph), 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/e* 408 (M^+); HRMS (EI) calcd for $C_{24}H_{32}N_4O_2$ (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).

(1*S*,3*S*,5(2*S*),5*R*)-3-[(1*H*-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 C_{18} 120 Å silica gel column, eluting with 1:1 H_2O:MeOH. Removal of the solvent on a rotary evaporator gave 67 mg of **28** (70% yield): mp 122–128 °C; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ 0.57, 0.63, and 0.67 (3q, 3 H, 3 axial CH of ring CH_2), 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 CH_2, 3 CH of CH_2), 2.45 and 2.50 (2dd, 2 H, J_{vic} = 7.3, 6.0 Hz, J_{gem} = 16.2 Hz, ring CH_2CO), 3.69 (m, 1 H, NCH), 5.82 and 6.46 (2s, 2 H, $CONH_2$), 6.70 and 7.48 (2s, 2 H, imidazole H's); $[\alpha]^{25}_D$ = +10.42° (*c* 1%, MeOH); HRMS (FAB) calcd for $C_{17}H_{26}N_4O_2$ (M^+ + H) 319.2134, obsd 319.2143.

(1*S*,3*S*,5(2*S*),5*S*)-3-[(1*H*-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/NH_3 to afford 35 mg (56% yield) of 1: mp ca. 212 °C; ^{1}H-NMR (DMSO-d_6) δ 0.54 (q, 1 H, *J* = 12 Hz, axial CH of ring CH_2), 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, 1 H, 3 H, 2 H, and 7 H, respectively, 3 CH, 6 CH_2, CH of CH_2), 2.46–2.55 (m, 1 H, CH of ring CH_2CO), 2.61 (dd, 1 H, J_{vic} = 9.0 Hz, J_{gem} = 15.0 Hz, CH of ring CH_2-CO), 3.52 (m, 1 H, NCH), 6.70 and 7.24 (2s, 2 H, $CONH_2$), 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); $[\alpha]^{25}_D$ = +15.37° (*c* 1%, MeOH); HRMS (FAB) calcd for $C_{17}H_{26}N_4O_2$ (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 cm × 60 cm × 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 cm × 22 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-ProNH_2), 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 one-way 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 μm 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 pH 7.4. Sections were then chilled to 4 °C and allowed to air dry. Slides were then incubated with 400 μL of 30 μM bacitracin and 10 nM [^{3}H]-MeTRH or [^{3}H]-**26**. Nonspecific binding is defined as the binding of [^{3}H]MeTRH (or [^{3}H]-**26**) in the presence of 10 μM 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 described[47] and statistical comparisons made using ANOVA followed by Newman–Keuls *post hoc* analysis for significant *F*-tests.

Acknowledgment. We thank members of our Physical Chemistry Department for obtaining spectral and microanalytical data, especially Mr. Louis J. Todaro and Ms. Ann-Marie Chiu for performing the X-ray crystallography analyses and Dr. Thomas J. Williams and Mr. Gino J. Sasso for assistance with NMR studies. We are also grateful to Ms. Dawn Johnson for performing the Morris water maze test procedure, Dr. Andreas Edelmann of F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland, for providing information on endocrine responses, and Dr. Thomas Zebovitz for supplying quantities of intermediates. The encouragement and support of Dr. Manfred Weigele and the helpful comments of Professor Ralph Hirschmann are gratefully acknowledged.

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.

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JM940685B