A Novel 3-Substituted Benzazepinone Growth Hormone Secretagogue (L-692,429)

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The 3-substituted benzazepinone, L-692,429 (compound 1), is the prototype compound of a novel class of compounds that stimulate release of growth hormone (GH). The molecule evolved from efforts to identify a non-peptide mimic of the growth hormone-releasing hexapeptide, GHRP-6. Compound 1 is prepared by sequential attachment of dimethyl-β-alanine and 2′-biphenylyltetrazole side chains to a chiral 3-aminobenzolactam nucleus. Comparison of the biological activity of 1 with the corresponding six- and eight-membered lactam analogs shows the seven-membered benzazepinone skeleton to be preferred. Molecular modeling of the structurally diverse GH secretagogues, L-692,429 and GHRP-6, was performed.

Introduction

Growth hormone (GH) is a 191-amino acid peptide (MW 21 500) which is the primary hormone responsible for growth in mammals. Since its first clinical use in 1959, GH therapy has been evaluated in a variety of patient populations, particularly GH-deficient children. Historically, human GH (hGH) was obtained from the pituitary glands of cadavers. In addition to limited supply, cadaver-derived GH had the associated risk of contamination with other disease-causing agents.[1] More recently, modern recombinant DNA technology has been successful in providing an adequate supply of pure GH, and recombinant human GH (rhGH) was approved for use in humans in the mid-1980s. With the increased availability of rhGH, potential indications for GH therapy have been extended to diverse therapeutic areas. For example, recent clinical evidence suggests that GH treatment in aged subjects partially reverses some of the effects of aging.[2] Despite the expanded use of the drug and the relatively abundant supply, treatment with rhGH still remains expensive, with annual costs in excess of $20 000.[3] Coupled with the inconvenience of chronic administration by subcutaneous injection, alternatives to GH treatment have been sought. The strategy of stimulating secretion of endogenous GH by activation of the physiological GH release mechanism has been recently pursued as an attractive alternative to treatment with exogenous rhGH. The approach is clinically appropriate since, in the majority of cases, GH deficiency results from hypothalamic dysfunction rather than inadequate pituitary GH content.[4]

GH is synthesized and stored in the pituitary gland from which it is released episodically into the peripheral circulation through the action of the hypothalamic hormones, growth hormone-releasing hormone (GRF), which stimulates GH release and somatostatin, which is inhibitory. Human GRF, a 44-amino acid C-amidated peptide, was originally isolated in 1982 from a patient suffering from acromegaly.[5] The structure–activity relationships of GRF and its analogs have been studied extensively,[4] and several clinical trials have been undertaken to evaluate its efficacy as a GH secretagogue.[6] In addition to GRF, structurally unrelated peptides that stimulate GH release have also been described.[7] These growth hormone releasing peptides (GHRP's) act directly on the pituitary gland to release GH *via* a mechanism independent of GRF. From this series evolved a hexapeptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6), which was identified as a potent GH secretagogue that also demonstrated activity *in vivo*.[8] GHRP-6 has also been extensively evaluated in animal models and in humans and is efficacious for release of GH.[4] Despite excellent activity and specificity, the peptidyl nature of both GRF and GHRP-6 limits their clinical potential.

An alternative strategy to stimulate production of endogenous GH is to block the action of somatostatin, and although a variety of somatostatin receptor ligands have been reported,[9] such an approach is limited due to the diverse physiological actions of the neuropeptide itself. In addition to its action on the pituitary, somatostatin receptors located in the brain and gastrointestinal tract play important roles in modulating locomotor activity, cognitive function, and gastric acid secretion.[10]

The demonstrated efficacy of GHRP-6 in releasing GH *in vivo* supports the hypothesis that stimulation of endogenous GH by a relatively small molecule presents a viable alternative to treatment with rhGH. We recently reported the results of our efforts to identify a *non-peptidyl* mimic of GHRP-6 that culminated in the discovery of a novel series of compounds, the benzolactam secretagogues.[11] A lead compound, the biphenylcarboxylic acid 2 (L-158,077), identified through directed screening of non-peptidyl templates, stimulated GH release from rat primary pituitary cells in a dose-dependent manner (Figure 1). Replacement of the 2′-carboxylic acid of 2 with a tetrazole gave the more potent analog 3 (L-158,432), and further resolution of the racemic center at C-3 afforded the individual antipodes, 1 (L-692,429) and 4 (L-692,428). The 3*R* enantiomer 1 is the first example of a specific, non-peptidyl GH secretagogue that has been shown to be well tolerated and highly active in stimulating GH release in humans.[12]

Herein we report the preparation and biological activities of the benzolactam GH secretagogue 1 and closely related analogs.

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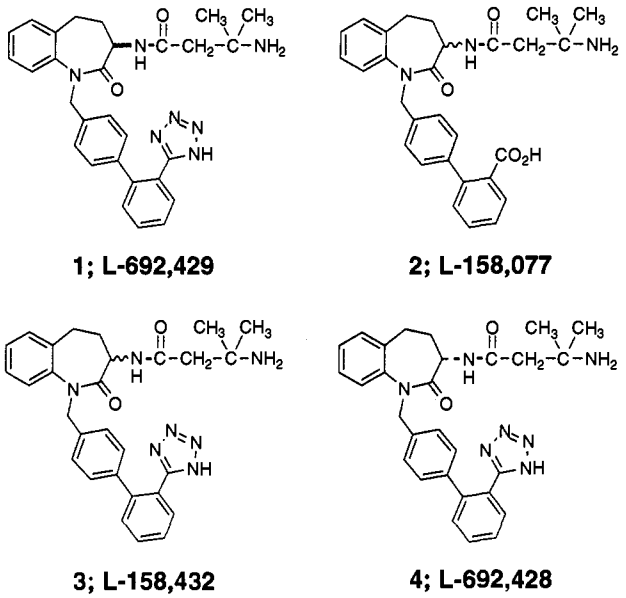
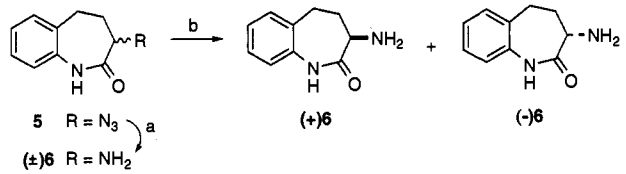


Figure 1. Structures of benzolactam growth hormone secretagogues.

Scheme 1[a]



[a] Reagents: (a) H_2 (40 psi), CH_3OH, 5% Pt–C (10 w/w %), 25 °C, 4.5 h; (b) fractional crystallization of tartaric acid salts.

Chemistry

Benzolactam GH secretagogues were prepared by stepwise attachment of dimethyl-β-alanine and 2′-substituted biphenyl side chains to a 3-aminobenzolactam nucleus. As shown in Scheme 1, racemic amino lactam (±)-**6** was prepared by catalytic hydrogenation of the known[13] 3-azido derivative **5**. Resolution of (±)-**6** was achieved by fractional crystallization of the diastereomeric tartaric acid salts. The dextrorotatory isomer (+)-**6**, $[\alpha]_D$ +455° (c = 1.0, CH_3OH), was obtained from the crystalline D-tartrate salt by neutralization of an aqueous solution followed by extractive isolation. Provisional assignment of absolute configuration was made by analogy to similar compounds reported previously.[13] Further proof was obtained from an X-ray structure determination of the crystalline urea **7** derived from reaction of the levorotatory isomer (−)-**6** with (*R*)-naphthylethyl isocyanate. Examination of the X-ray structure of **7** confirms the correlation of the 3*S* configuration with the levorotatory isomer (Figure 2).

A convenient route to the dimethyl-β-alanine amino acid side chain fragment **10**, protected as the *N*-*tert*-butoxycarbonyl (BOC) derivative, is outlined in Scheme 2. The sequence proceeds through β-lactam **8**, obtained from cycloaddition of isobutylene and chlorosulfonyl isocyanate[14] followed by reductive cleavage of the residual chlorosulfonyl group. Protection as the *tert*-butoxycarbonyl derivative **9** followed by saponification with aqueous lithium hydroxide provided acid **10**.

The 2′-substituted biphenylylmethyl bromides were assembled as illustrated in Scheme 3. The 2′-*tert*-butoxycarbonyl derivative **13** was constructed by a Pd(0)-mediated cross-coupling reaction of *tert*-butyl 2-bromobenzoate **11** and *p*-tolylboronic acid **12** using the conditions of Suzuki.[15] Conversion to the bromide **14** was carried out by free-radical bromination with *N*-bromosuccinimide (NBS) in refluxing carbon tetrachloride in the presence of azoisobisbutyronitrile (AIBN). The 2′-tetrazole derivative **15**, prepared by the method of Shumann,[16] was also converted to the corresponding bromide **16** by reaction with NBS/AIBN.

Compound **2** was prepared by sequential attachment of amino acid and biphenyl side chains to the aminobenzolactam template as described in Scheme 4. Coupling of BOC-protected dimethyl-β-alanine **10** with amino lactam (±)-**6** using (benzotriazol-1-yloxy)tris(dimethylamino)-phosphorium hexafluorophosphate (BOP) gave (±)-**17** in high yield. Resolved compounds (+)-**17** and (−)-**17** were also prepared from the corresponding aminolactams (+)-**6** and (−)-**6** and acid **10** by the same procedure. Treatment

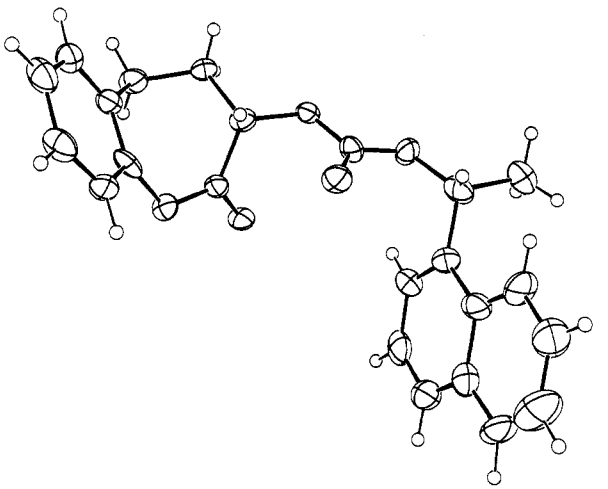
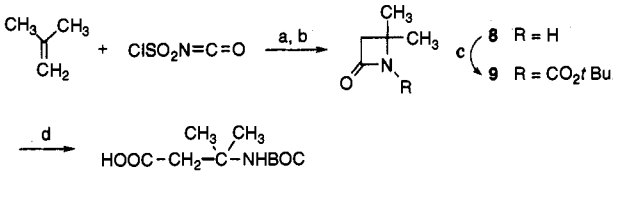


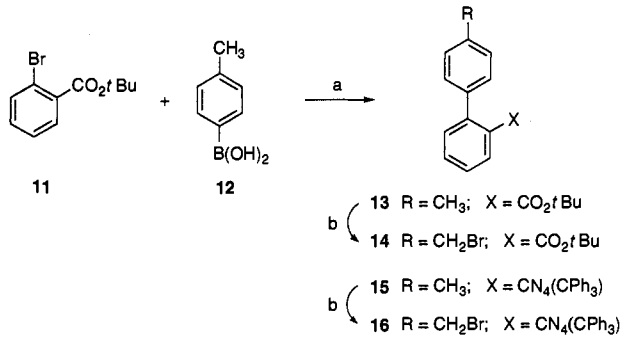
Figure 2. A perspective view (ORTEP) of urea **7**. Atomic ellipsoids are drawn at the 20% probability level except for the hydrogen atoms which are an arbitrary size. One water molecule and one acetone molecule have been deleted for clarity.

Scheme 2[a]



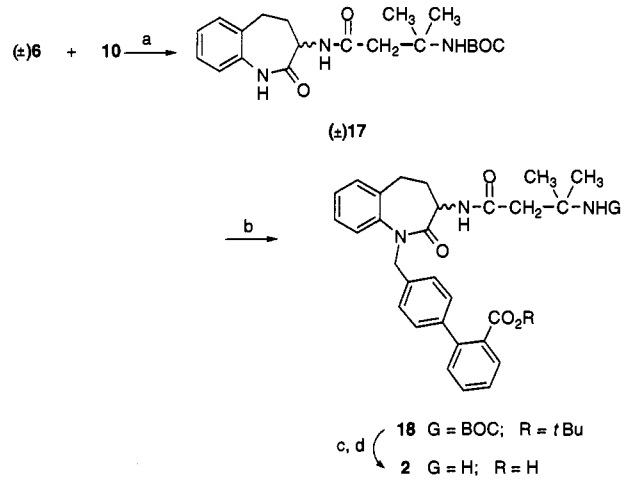
[a] Reagents: (a) Et_2O, −65 → −5 °C, 3 h; (b) aqueous Na_2SO_3, $NaHCO_3$, pH 7, 30 min; (c) di-*tert*-butyl dicarbonate, Et_3N, DMAP, CH_2Cl_2, 14 h; (d) aqueous LiOH, THF, 0–5 °C; aqueous $NaHSO_4$.

Scheme 3[a]



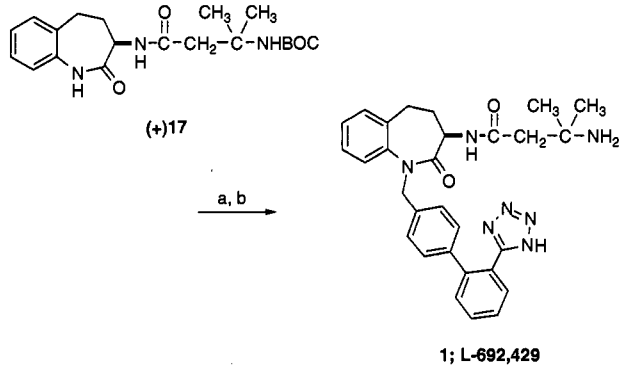
[a] Reagents: (a) 3 mol % $Pd(PPh_3)_4$, Na_2CO_3, ethanol, toluene, reflux, 5 h; (b) *N*-bromosuccinimide, 2 mol % AIBN, CCl_4, reflux, 4.5 h.

Scheme 4[a]



[a] Reagents: (a) BOP, $(i\text{-Pr})_2NEt$, CH_2Cl_2, 25 °C, 2 h; (b) NaH, DMF, 0 °C, 15 min; bromide 14/DMF, 0–25 °C, 16 h; (c) 6 N HCl, CH_3COOH, 50 °C, 3 h; (d) CH_3OH, propylene oxide.

Scheme 5[a]



[a] Reagents: (a) NaH, DMF, 0 °C, 15 min; bromide 16/DMF, 0–25 °C, 1 h; (b) 9 N HCl, CH_3OH, hexane, 16 h; crystallize from H_2O.

of lactam (±)-17 with sodium hydride in DMF followed by reaction with bromide 14 gave the alkylated product 18 efficiently. Simultaneous removal of both BOC and *tert*-butyl protecting groups was carried out by treatment with 6 N hydrochloric acid. The crude hydrochloride salt thus obtained was treated with propylene oxide in methanol to precipitate the free base of compound 2 (L-158,077).

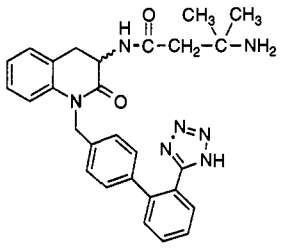
The synthesis of the tetrazole analog compound 1 (L-692,429) proceeds through an analogous route as shown in Scheme 5. Lactam (+)-17, prepared from the 3(*R*)-amino lactam (+)-6 by the procedure described in Scheme 4, is alkylated with bromide 16 using sodium hydride in dimethylformamide. Removal of both BOC and trityl protecting groups was carried out by partitioning between 9 N hydrochloric acid and hexane. The hydrochloride salt crystallized from the aqueous layer and gave compound 1 as a stable dihydrate, melting point 210 °C. The 3*S* enantiomer 4 was prepared by the same route starting from the 3(*S*)-amino lactam (−)-6. The racemic compound 3 (L-158,432) was also prepared by essentially the same route.

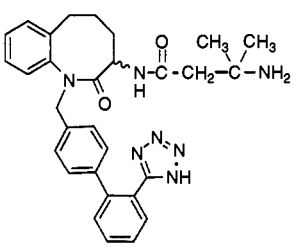
Effect of Ring Size. In order to assess the effect of ring size on the *in vitro* activity of the benzolactam secretagogues, the corresponding six- and eight-membered lactam analogs were prepared. The six-membered analog 19 was prepared from the known[17] 3-amino-1,2,3,4-tetrahydroquinolin-2-one according to the procedures described above. The eight-membered homolog, 20, was constructed in a similar fashion from 3-azido-3,4,5,6-tetrahydro-1-benzazocin-2(1*H*)-one.[13]

Table 1. Growth Hormone Secretagogues—Activity in Vitro[a]

compd	ED_{50}, nM[b]	compd	ED_{50}, nM[b]
1	60 ± 7 (n = 5)	19	2000[d]
2	3200 ± 400 (n = 5)	20	8000[d]
3	160 ± 40 (n = 5)	GHRP-6	10 ± 2 (n = 6)
4	weakly active[c] (n = 3)	GRF	0.47 ± 0.09 (n = 6)

[a] Rat pituitary cell assay. [b] Mean ED_{50} values (±SE) calculated from n replicate determinations. [c] 20 μg/mL. [d] Average of duplicate determinations.





Structure–Activity Relationships. The growth hormone releasing activities of the GH secretagogues discussed are presented in Table 1. The lead structure, compound 2, stimulates GH release from rat primary pituitary cells in a dose-dependent manner with an ED_{50} value of 3.2 μM. Although a relatively weak GH secretagogue, compound 2 shows specificity for GH; other pituitary hormones remain unaffected.[18] Development of this lead initially focused on modification of the carboxylic acid with the goal of replacing it with a functional group of similar acidity. The tetrazole group, a heterocycle with comparable acidity, was selected as replacement for the carboxyl. Thus, substitution of the carboxylic acid of 2 with a tetrazole gave compound 3 which shows an ED_{50} of 160 nM as a racemate. In addition to the 20-fold improvement in intrinsic potency, compound 3 retains specificity for GH release.[18] Compound 3 is a zwitterionic molecule with pK_a's of 4.6 and 9.2.[19] Substitution of a carboxylic acid with a tetrazole has also resulted in improved binding affinity of structurally similar angiotensin II receptor antagonists.[20,21]

Evaluation of the enantiomers of 3 was carried out to identify the bioactive configuration at C-3 of the benzazepinone ring. It was found that the 3*S* compound 4 released GH weakly at 20 μg/mL while the 3*R* enantiomer 1 (L-692,429) stimulated GH release from rat primary pituitary cells in a time- and dose-dependent manner with an ED_{50} value of 60 nM.[22] It is noteworthy that the *R* configuration of the non-peptidyl secretagogue 1 corresponds to that of a D-amino acid. The presence of D-amino acid residues is an essential structural feature in the peptidyl secretagogue GHRP-6.[23]

As shown in Table 1, the GH releasing activity in this series is also dependent upon the ring size of the benzolactam template. Comparison of the ED_{50}'s of the corresponding six- and eight-membered analogs 19 and 20 reveals activities 10–50-fold less than the seven-membered compound 3. Presumably, the seven-membered lactam provides a preferred template to position the dimethyl-β-alanine and biphenylyltetrazole appendages for optimal receptor binding.

Comparison of Peptide and Non-peptidyl GH Secretagogues. Molecular Overlays. To probe similarity

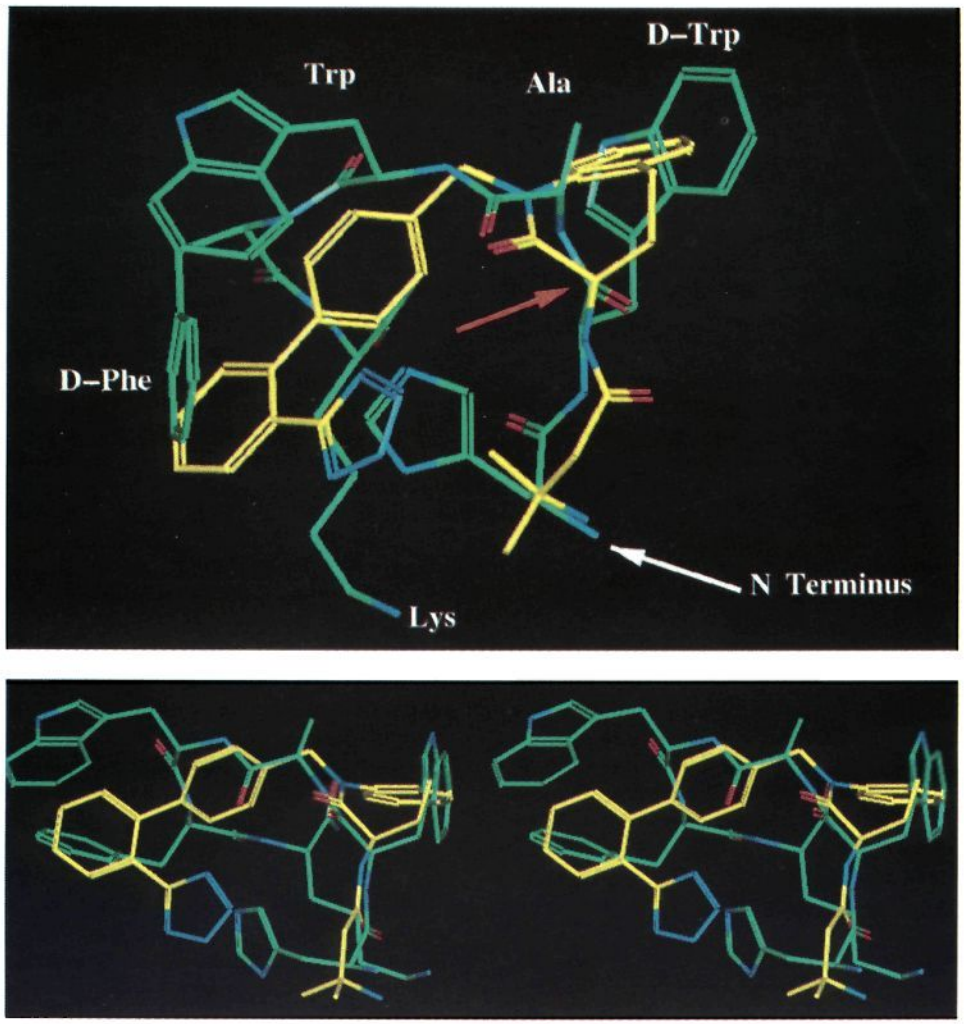


Figure 3. (a, Top) Superposition of the peptidyl GH secretagogue, GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) shown in green, and the non-peptidyl mimic, compound 1 (L-692,429; yellow), using *SEAL*.[24] (b, Bottom) Further refinement of the overlay subject to the constraints described in the text.

between the three-dimensional structures of compound 1 (L-692,429) and GHRP-6, molecular modeling techniques were employed. Our initial approach was to employ *SEAL*[24] to automatically generate superpositions of the two secretagogues with minimal user bias. Using QUANTA/CHARMm,[25] 200 conformers of GHRP-6 were generated and minimized with a distance-dependent dielectric model. A low-energy set of 50 was selected for further analysis. Conformations of compound 1 were generated using distance geometry[26] and optimized with Merck's in-house force field, MM2X (see the Experimental Section). Nine low-energy, maximally dissimilar structures were selected for comparison to GHRP-6. After assigning MM2X charges to all molecules, *SEAL* was employed to scan and rank 45 000 overlays of GHRP-6 and compound 1 based on steric and electrostatic fields. From the top-scoring superpositions, several sets most consistent with the structure–activity relationships of the two secretagogues were selected and subjected to more refined searching using *SEAL*. The highest scoring aligned pair is shown in Figure 3a. Since both molecules are highly flexible, the limited set of conformers used in the analysis was not capable of achieving complete atom-to-atom superposition. A further refinement was generated (Figure 3b) by constraining the functional group mappings suggested from the initial *SEAL* overlay. The calculated energy difference between the native conformers derived from the *SEAL* overlay (Figure 3a) and the final conformers in Figure 3b is minimal. In fact, the refined structure of compound 1 was 0.4 kcal/mol lower in energy than the starting structure, and GHRP-6 was 3.5 kcal/mol lower, due to the establishment of an intramolecular hydrogen bond in the refined peptide geometry in Figure 3b.

A common feature of the *SEAL*-generated alignments is the superposition of the amino group of 1 and the N-terminus of GHRP-6 (1.1 Å).[27] As shown in Figure 3b, the carbonyl unit of the D-Trp residue of GHRP-6 maps to the carbonyl of the benzolactam ring (1.2 Å). The benzolactam nucleus aligns fairly well with the D-Trp residue of the peptidyl secretagogue and almost complete superposition of the asymmetric center at C-3 of compound 1 with the D-Trp α-carbon is apparent (0.4 Å, red arrow). Here again the correlation of the 3*R* configuration of compound 1 with the stereochemistry of a D-amino acid is particularly relevant. The bridging phenyl group of the biphenyl unit functions as a rigid scaffold, placing the terminal phenyl ring in a region occupied by the D-Phe (1.3 Å) and L-Trp (3.0 Å) residues of the peptide. This alignment suggests the possible existence of a region on

Table 2. Comparison of Activity in Vitro—L-692,429 *vs* GHRP-6

L-692,429		GHRP-6
60 ± 7	ED_{50} (nM)[a]	10 ± 2
synergy	+GRF	synergy
no effect	+GHRP-6 or L-692,429	no effect
antagonism	+His-D-Trp-D-Lys-Trp-D-Phe-Lys-NH_2	antagonism
inhibitory	+somatostatin	inhibitory

[a] Rat pituitary cell assay.

the receptor suited for specific recognition of aromatic rings. This hypothesis is also supported by the known[28] preference for aromatic amino acids at residues 4 and 5 of GHRP-6. The proximity of the tetrazole of compound 1 to the imidazole ring of the histidine residue of GHRP-6 (1.0 Å) is clearly evident. However, alternative mappings for the tetrazole were also identified, either as an indole mimic of the L-Trp side chain, or as the amide backbone unit of D-Phe (data not shown). Examination of Figure 3b reveals that no element of compound 1 correlates with the lysine residue of GHRP-6. This is not surprising in the context of the known structure–activity relationships of GHRP-6 that indicate that although the C-terminal lysine contributes significantly to activity *in vivo*, it has little effect on intrinsic activity *in vitro*.[28] These correlations suggest that the receptor may not require an acidic element at all, and the observed preference for tetrazole over carboxy is due to its diffuse, nondirectional charge distribution and higher pK_a.

While computer modeling has provided a convenient means to visualize and compare molecules in three dimensions, this type of analysis is limited since it does not account for binding properties of the secretagogues with the receptor. Such ligand–receptor interactions will undoubtedly affect the bioactive conformation of each secretagogue. However, in the absence of such information, qualitative comparison of these two structurally dissimilar molecules in this manner has been successful in generating a working hypothesis for the benzolactam secretagogues and has also revealed some provocative structural analogies between the two series.

Biological Evaluation. The *in vitro* GH releasing properties of both compound 1 (L-692,429) and GHRP-6 have recently been reported in detail[22] and are summarized in Table 2. These studies support the hypothesis that compound 1 is a mimic of the peptidyl secretagogue GHRP-6. Both secretagogues stimulate GH release from rat pituitary cells in a dose- and time-dependent manner. The activity of either secretagogue in the presence of the other, or to added GRF, is also identical. For example, cells stimulated by maximal concentrations of either GHRP-6 or compound 1 do not respond to further treatment with the other secretagogue, yet remain fully responsive to GRF. Furthermore, the magnitude of GH release achieved by combinations of compound 1 and GRF is indicative of a synergistic, rather than simply additive, response. Analogous synergism of GHRP-6 and GRF has also been described.[29] The peptide His-D-Trp-D-Lys-Trp-D-Phe-Lys-NH_2, which has been shown to block GH release by GHRP-6,[29] has a comparable antagonistic effect on GH release stimulated by compound 1. Somatostatin completely inhibits GH release by either secretagogue.

Aside from the difference in intrinsic potency, the GH-releasing properties *in vitro* of compound 1 closely parallel those observed for the peptidyl secretagogue GHRP-6. These results provide additional support for the relationship of compound 1 as a non-peptidyl mimic of GHRP-6.

Conclusions

Compound 1 (L-692,429) is the prototype compound of a novel class of non-peptidyl GH secretagogues. It stimulates GH release *in vitro* with an ED_{50} value of 60 nM while maintaining high specificity for GH. The *in vitro* GH-releasing properties of compound 1 mirror those of GHRP-6, and a comparison of the two structures by molecular modeling lends further support to the hypothesis that compound 1 is a non-peptidyl mimic of the peptidyl secretagogue GHRP-6. Compound 1 also exhibits efficacy and specificity in animal models.[30] Clinical evaluation of compound 1 in humans shows it to be well tolerated and highly active for release of GH.[12] Compound 1 is the first example of a non-peptidyl agonist of GH release that demonstrates the degree of biological specificity required for consideration as a clinical alternative for GH. Additional details of the structure–activity relationships of the benzolactam GH secretagogues will be presented in the future.

Experimental Section

Chemistry. General Methods. ^1H NMR spectra were recorded on Varian XL series spectrometers at the indicated field strengths. Low-resolution mass spectral analyses were obtained with a LKB 9000 at an ionizing voltage of 70 eV. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter at ambient temperature. Reagents, solvents, and drying agents were obtained from commercial sources and used without further purification or drying unless stated otherwise. GHRP-6 was obtained from BACHEM Bioscience, Inc., Philadelphia, PA, custom synthesis lot ZF892. Rat GRF was obtained from Sigma Chemical Co., St. Louis, MO. Preparative medium-pressure liquid chromatography was carried out with Lobar LiChroprep Si60 (E. Merck, 40–63μm) prepacked columns. Preparative HPLC was carried out on a Waters PrepLC System 500A equipped with a refractive index detector and two 55–105-μm, 125-Å silica gel PrepPaks in series. Preparative reverse-phase HPLC was performed with a Dynamax-60Å 8-μm C18 column (21.4-mm i.d. × 25-cm length) with UV detection at 280 nm. Elemental analyses were performed by the Analytical Services Department of the Merck Research Laboratories, Rahway, NJ, or by Robertson Microlit Laboratories, Inc., Madison, NJ.

3-Amino-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one [(±)-6]. A solution of 9.22 g (45.6 mmol) of 3-azido-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one[13] (5) in 30 mL of methanol was hydrogenated at ambient temperature and 40 psi in the presence of 1.0 g of 5% platinum on carbon for 4.5 h. Celite was added and the mixture filtered through a pad of Celite. The filtrate was concentrated *in vacuo* and aged at room temperature for 16 h which resulted in formation of crystals. The material was isolated by filtration and dried *in vacuo* to afford 4.18 g (23.7 mmol, 52%) of (±)-6. The mother liquors were diluted to 100 mL with methanol, treated with 2 g of charcoal, and filtered through Celite, and the filtrate was concentrated *in vacuo* to approximately 15 mL. A second crop of crystals formed and was isolated as described above to give an additional 2.02 g (11.5 mmol, 25%) of product. Another recycling of the mother liquors afforded a third crop containing 0.88 g of product (5.0 mmol, 11%). A total of 7.08 g (40.2 mmol, 88%) of (±)-6 was thus obtained. FAB-MS: calculated for $C_{10}H_{12}N_2O$ 176, found 177 (M + H, 100).

3(*R*)-Amino-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one [(+)-6] and 3(*S*)-Amino-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one [(−)-6]. A suspension of 2.37 g (13.5 mmol) of (±)-6 and 2.02 g (13.5 mmol) of L-tartaric acid in 40 mL of ethanol was gently heated. Solids were completely dissolved by dropwise addition of water (~5 mL) to the warm suspension. The solution was cooled to room temperature and aged overnight. The solid that formed was removed by filtration, washed with ethanol/diethyl ether (1:1), and dried *in vacuo* to give 1.75 g of crude L-tartrate salt. The mother liquors were evaporated to dryness *in vacuo*

and redissolved in 40 mL of water and the pH adjusted to 10–11 by the addition of solid potassium carbonate. The mixture was extracted with chloroform (6 × 20 mL), the combined extracts were washed with water and saturated aqueous sodium chloride, dried over anhydrous potassium carbonate, and filtered, and solvents were removed *in vacuo* to afford 1.29 g (7.33 mmol) of partially enriched 3(*R*)-amine (+)-6.

The original 1.75-g batch of L-tartrate salt was recrystallized twice from aqueous ethanol to give 1.03 g (3.17 mmol, 24%) of purified L-tartrate salt, $[\alpha]_D$ −212° (c = 1.0, H_2O). This material was dissolved in 20 mL of water and the pH adjusted to 10–11 by the addition of solid potassium carbonate. The mixture was extracted with chloroform; combined extracts were washed with water and brine, dried over anhydrous potassium carbonate, and filtered, and solvents were removed *in vacuo* to afford 0.522 g (2.96 mmol, 22% from **5**) of the 3(*S*)-amine (−)-6, $[\alpha]_D$ −446° (c = 1.0, CH_3OH).

The remaining 1.29 g (7.33 mmol) of partially enriched 3(*R*)-amine (+)-6 was treated with 1.10 g (7.33 mmol) of D-tartaric acid as described above and the resulting salt recrystallized twice from aqueous ethanol to afford 1.20 g (3.69 mmol, 28%) of purified D-tartrate salt, $[\alpha]_D$ +214° (c = 1.0, H_2O). Liberation of the free amine by the method described above gave 0.629 g (3.57 mmol, 26% from (±)-6) of the 3(*R*)-amine (+)-6, $[\alpha]_D$ +455° (c = 1.0, CH_3OH). ^1H NMR (400 MHz, $CDCl_3$ + 1 drop CD_3OD): δ 1.74 (br s, 2H), 1.88 (m, 1H), 2.48 (m, 1H), 2.61 (dd, *J* = 8, 14 Hz, 1H), 2.87 (m, 1H), 3.38 (dd, *J* = 8, 11 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 7.11 (m, 1H), 7.19 (m, 2H), 7.9 (br s, 1H).

3(*S*)-[[[[(*R*)-Naphthylethyl]amino]carbonyl]amino]-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one (7) and 3(*R*)-[[[[(*R*)-Naphthylethyl]amino]carbonyl]amino]-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one. To a solution of 20 mg (0.11 mmol) of (±)-6 in 2 mL of CH_2Cl_2 was added 20 μL (22 mg, 0.11 mmol, 1 equiv) of (*R*)-naphthylethyl isocyanate (Aldrich) and the mixture stirred at room temperature for 16 h. Solvent was removed under vacuum, and the residue was purified by preparative thin-layer chromatography on two 20-cm × 20-cm, 250-μm E. Merck silica plates eluting with ethyl acetate/hexane (2:1). Two bands were isolated; the faster moving band afforded 20 mg (0.054 mmol, 47%) of **7** following extractive isolation with ether. The lower band yielded 18 mg (0.048 mmol, 42%) of material. ^1H NMR (200 MHz, CD_3OD) less polar diastereomer (**7**): δ 1.49 (d, *J* = 8 Hz, 3H), 1.93 (m, 1H), 2.4–2.9 (m, 3H), 4.27 (dd, *J* = 8, 12 Hz, 1H), 5.55 (q, *J* = 8 Hz, 1H), 6.99 (dd, *J* = 2, 8 Hz, 1H), 7.05–7.27 (m, 3H), 7.35–7.55 (m, 4H), 7.72 (dd, *J* = 2, 8 Hz, 1H), 7.81 (dd, *J* = 2, 8 Hz, 1H), 8.05 (dd, *J* = 2, 8 Hz, 1H). More polar diastereomer: 1.49 (d, *J* = 8 Hz, 3H), 1.90 (m, 1H), 2.3–2.9 (m, 3H), 4.26 (dd, *J* = 8, 12 Hz, 1H), 5.56 (q, 1H), 7.02 (dd, *J* = 2, 8 Hz, 1H), 7.05–7.25 (m, 3H), 7.35–7.50 (m, 4H), 7.71 (d, *J* = 8 Hz, 1H), 7.80 (dd, *J* = 2, 8 Hz, 1H), 8.03 (d, *J* = 8 Hz, 1H). FAB-MS: calculated for $C_{23}H_{23}N_3O_2$ 373, found less polar diastereomer **7** 374 (M + H, 70), more polar diastereomer 374 (M + H, 66).

Correlation of absolute configuration was achieved as follows. A solution of 10 mg (0.057 mmol) of the levorotatory amine (−)-6 in 2 mL of CH_2Cl_2 was treated with 10 μL (11 mg, 0.057 mmol, 1 equiv) of (*R*)-naphthylethyl isocyanate and the mixture stirred at room temperature for 2 h. Solvent was removed under vacuum and the residue purified by preparative thin-layer chromatography on two 20-cm × 20-cm 250-μm E. Merck silica plates eluting with ethyl acetate/hexane (2:1) to give 20 mg (0.054 mmol, 94%) of product. Comparison of the urea derived from (−)-6 with the less polar of the two distereomeric ureas obtained from (±)-6 shows them to be one and the same by 200-MHz ^1H NMR and analytical tlc mobility on silica (ethyl acetate/hexane, 1:1).

4,4-Dimethylazetidin-2-one (8). A three-neck, 3-L round-bottom flask equipped with a magnetic stirrer, thermometer, cold finger condenser, and nitrogen bubbler was charged with 1 L of ether. The flask was cooled to −65 °C, and isobutylene (500–600 mL) was condensed into it. The cold finger condenser was replaced with a dropping funnel, and 200 mL (325 g, 2.30 mol) of chlorosulfonyl isocyanate was added dropwise over 1.5 h. The mixture was maintained at −65 °C for 1.5 h and then the dry ice/acetone cooling bath replaced with methanol/ice. The internal temperature slowly increased to −5 °C at which time the reaction initiated and the internal temperature rose to 15 °C with evolution of gas. The internal temperature remained at 15 °C for several minutes then dropped back down to −5 °C, and the mixture was stirred at −5 °C for 1 h. The methanol/ice bath was removed and the reaction mixture warmed to room temperature and stirred overnight.

The reaction mixture was transferred to a three-neck, 12-L round-bottom flask fitted with a mechanical stirrer and diluted with 2 L of ether. The well-stirred reaction mixture was treated with 2 L of saturated aqueous sodium sulfite. After 1 h, an additional 1 L of saturated aqueous sodium sulfite was added followed by sufficient solid sodium bicarbonate to adjust the pH to approximately 7. The mixture was stirred another 30 min, and then the layers were allowed to separate. The ether layer was removed and the aqueous layer reextracted with ether (2 × 1 L). The combined ether extracts were washed once with 500 mL of saturated aqueous sodium bicarbonate and once with 500 mL of saturated aqueous sodium chloride. The ether layer was removed, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to give 33.0 g of a pale yellow oil. The aqueous layer was made basic by the addition of solid sodium bicarbonate and further extracted with ether (3 × 1 L). The combined ether extracts were washed and dried as described above, combined with the original 33.0 g of pale yellow oil, and concentrated *in vacuo* to give 67.7 g of **8**. Further extraction of the aqueous layer with methylene chloride (4 × 1 L) and washing and drying as before gave an additional 74.1 g of **8**. Still further extraction of the aqueous layer with methylene chloride (4 × 1 L) gave an additional 21.9 g of **8**. The combined product (163.7 g, 1.65 mol, 72%) was used without purification. ^1H NMR (200 MHz, $CDCl_3$): δ 1.45 (s, 6H), 2.75 (d, *J* = 3 Hz, 2H), 5.9 (br s, 1H).

***N*-(*tert*-Butoxycarbonyl)-4,4-dimethylazetidin-2-one (9).** A 5-L, three-neck round-bottom flask equipped with a magnetic stirrer, thermometer, nitrogen bubbler, and addition funnel was charged with 88.2 g (0.89 mol) of **8**, 800 mL of CH_2Cl_2, 150 mL of triethylamine (1.08 mol), and 10.9 g (0.089 mol) of DMAP. To the stirred solution at room temperature was added dropwise over 15 min a solution of 235 g (1.08 mol) of di-*tert*-butyl dicarbonate in 300 mL of methylene chloride. The reaction mixture was stirred at room temperature overnight, diluted with 1 L of methylene chloride, and washed with 500 mL of saturated aqueous ammonium chloride, 500 mL of water, and 500 mL of saturated aqueous sodium chloride. The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to afford 180.3 g of crude **9** as an orange solid. The material was used in the next step without purification. ^1H NMR (200 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.54 (s, 6H), 2.77 (s, 2H).

3-[(*tert*-Butoxycarbonyl)amino]-3-methylbutanoic Acid (10). A 3-L, three-neck round-bottom flask equipped with a magnetic stirrer, thermometer, nitrogen bubbler, and addition funnel was charged with 180.3 g (0.89 mol) of **9** dissolved in 1 L of THF. The solution was cooled to 0–5 °C and treated dropwise with 890 mL of 1.0 M aqueous lithium hydroxide over 30 min. The reaction mixture was stirred at 0–5 °C for 2 h and then diluted with 1 L of ether and 1 L of water. The layers were allowed to separate, and the aqueous layer was reextracted with an additional 1 L of ether. The aqueous layer was acidified by the addition of 1 L of saturated aqueous sodium bisulfate and then extracted with ether (1 × 1 L and 2 × 500 mL). The combined organic layer and ether extracts were washed with 500 mL of saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to give 173 g of a yellow oil that solidified upon standing. The material was slurried with warm hexane, filtered, and dried under high vacuum to afford 168.5 g (0.775 mol, 87%) of **10** as a white solid. ^1H NMR (200 MHz, $CDCl_3$): δ 1.39 (s, 6H), 1.44 (s, 9H), 2.72 (s, 2H). FAB-MS: calculated for $C_{10}H_{19}NO_4$ 217, found 218 (M + H, 54).

***tert*-Butyl 2-Bromobenzoate (11).** Via the procedure of Ohta,[31] 100 g (0.50 mol) of 2-bromobenzoic acid dissolved in 500 mL of DMF was treated with 80.66 g (0.50 mol, 1.0 equiv) of *N,N'*-carbonyldiimidazole, the mixture was heated at 40 °C for 1 h, then 93.8 mL (73.7 g, 1.0 mol, 2.0 equiv) of *tert*-butyl alcohol and 75.7 mL (77.1 g, 0.50 mol, 1.0 equiv) of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) were added, and the resulting solution was heated at 40 °C for 24 h and at 50 °C for 16 h. The mixture

was cooled to room temperature, diluted with 500 mL of hexane/ether (1:1), and washed with 10% hydrochloric acid (3×), water (2×), 10% potassium carbonate (2×), and brine. The organic layer was removed, dried over anhydrous sodium sulfate, and then filtered, and solvents were removed *in vacuo*. Vacuum distillation gave 55.32 g (0.22 mol, 44%) of **11** as a colorless liquid, $bp_{0.1mm}$ 76–81 °C.

***tert*-Butyl 4′-Methylbiphenyl-2-carboxylate (13).** Via the procedure of Suzuki,[15] 2.06 g (1.78 mmol, 0.03 equiv) of tetrakis(triphenylphosphine)palladium in a 250-mL round-bottom flask under a nitrogen atmosphere was treated sequentially with 15.29 g (59.49 mmol) of **11** dissolved in 120 mL of toluene, 60 mL of 2 M aqueous sodium carbonate, and 8.90 g (65.4 mmol, 1.1 equiv) of *p*-tolylboronic acid dissolved in 30 mL of ethanol. The mixture was heated at reflux (~100 °C) under nitrogen for 4 h, then cooled, and quenched by the addition of 2 mL of 30% hydrogen peroxide and stirred for another hour. The reaction mixture was partitioned between water and ether; the ether layer was removed and the aqueous layer reextracted with several portions of ether. The combined extracts were washed with brine, dried over anhydrous sodium sulfate, and filtered, and solvents were removed *in vacuo*. Purification by preparative HPLC, eluting with hexane/ethyl acetate (23:1), afforded 15.13 g (56.46 mmol, 95%) of **13** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (s, 9H), 2.38 (s, 3H), 7.19 (s, 4H), 7.31 (dt, J = 1.5, 7.5 Hz, 1H), 7.36 (dd, J = 1.5, 7.5 Hz, 1H), 7.45 (dt, J = 1.5, 7.5 Hz, 1H), 7.73 (dd, J = 1.5, 7.5 Hz, 1H).

***tert*-Butyl 4′-(Bromomethyl)biphenyl-2-carboxylate (14).** To a solution of 13.78 g (51.42 mmol) of **13** in 800 mL of carbon tetrachloride was added 9.426 g (52.96 mmol, 1.03 equiv) of recrystallized NBS and 200 mg (1.3 mmol, 0.02 equiv) of azoisobisbutyronitrile (AIBN). The mixture was heated at reflux for 2.5 h at which time additional NBS (0.92 g, 5.2 mmol, 0.1 equiv) and AIBN (25 mg) were added and the mixture refluxed for another 2 h. Heating was discontinued and the mixture refrigerated overnight at 0–5 °C. The mixture was filtered to remove succinimide and the filtrate concentrated to a minimum volume under vacuum, redissolved in 20 mL of hexanes, and aged at room temperature overnight. The crystalline product was removed by filtration, washed with cold hexane, and air-dried to give 12.90 g (37.1 mmol, 72%) of bromide **14** as a white solid. NMR indicates the presence of trace amounts of starting material and dibromide product. The mother liquors were evaporated to dryness under vacuum and purified by medium-pressure liquid chromatography on silica, eluting with hexanes/ethyl acetate (39:1), to give an additional 1.662 g (4.79 mmol, 9.3%) of **14** and 1.472 g (5.49 mmol, 11%) of recovered **13**. Total product yield was 14.56 g (41.93 mmol, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (s, 9H), 2.38 (s, trace of unreacted **13**), 4.53 (s, 2H), 6.69 (s, trace of dibromide), 7.2–7.5 (m, 7H), 7.78 (d, J = 8 Hz, 1H).

***N*-(Triphenylmethyl)-5-[2-[4′-(bromomethyl)biphenylyl]]-tetrazole (16).** A solution of 3.15 g (6.65 mmol) of **15**[16] in 25 mL of CH_2Cl_2 was treated with 1.29 g (7.25 mmol, 1.1 equiv) of NBS, 80 mg (0.5 mmol, 0.07 equiv) of AIBN, 200 mg of sodium acetate, and 200 mg of acetic acid. The mixture was heated at reflux for 16 h, cooled, and washed with saturated aqueous sodium bicarbonate. The organic layer was removed, dried over sodium sulfate, filtered, and concentrated to a minimum volume by atmospheric distillation. Methyl *tert*-butyl ether was added and distillation continued until almost all the methylene chloride was removed and the total volume was reduced to about 12 mL. Hexanes (12 mL) were added, and the mixture was aged at room temperature for 2 h. The product was isolated by filtration, washed with hexanes, and then dried under vacuum at 50 °C to give 2.81 g (5.04 mmol, 76%) of **16**. ^{1}H NMR (200 MHz, $CDCl_3$); δ 4.38 (s, 2H), 6.9–8.0 (m, 23H). NMR indicates presence of approximately 1% of the starting material **15** and 7% of the dibromo derivative.

3-[(*tert*-Butoxycarbonyl)amino]-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepin-3-yl]butanamide [(±)-17]. A solution of 450 mg (2.55 mmol) of amine (±)-**6** and 555 mg (2.56 mmol, 1 equiv) of acid **10** in 3 mL of CH_2Cl_2 at room temperature was treated with 0.89 mL of diisopropylethylamine (0.66 g, 5.1 mmol, 2 equiv) followed by 1.69 g of BOP (3.82 mmol, 1.5 equiv). After 2 h at room temperature, the mixture was concentrated under vacuum to a minimum volume, redissolved in 50 mL of ethyl acetate, and washed with 10% aqueous citric acid (2×), saturated aqueous sodium bicarbonate (2×), and saturated aqueous sodium chloride. The organic layer was removed, dried over anhydrous magnesium sulfate, and filtered, and the solvents were removed *in vacuo*. The residue was purified by medium-pressure liquid chromatography on silica, eluting with ethyl acetate/hexanes (2:1), to afford 836 mg (2.22 mmol, 87%) of (±)-**17** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (s, 6H), 1.41 (s, 9H), 1.90 (m, 1H), 2.44 (d, J = 15 Hz, 1H), 2.55 (d, J = 15 Hz, 1H), 2.65 (m, 1H), 2.77 (m, 1H), 2.92 (m, 1H), 4.52 (m, 1H), 5.21 (br s, 1H), 6.63 (d, J = 7 Hz, 1H), 6.97 (d, J = 8 Hz, 1H), 7.10–7.30 (m, 3H), 7.34 (br s, 1H). FAB-MS: calculated for $C_{20}H_{29}N_3O_4$ 375, found 376 (M + H, 45); 276 (M − BOC, 100).

***tert*-Butyl 4′-[[3-[[3-*tert*-Butoxycarbonyl)amino]-3-methyl-1-oxobutyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepin-1-yl]methyl][1,1′-biphenyl]-2-carboxylate (18).** To a solution of 307 mg (0.82 mmol) of (±)-**17** in 2 mL of DMF at 0 °C under nitrogen was added 34 mg of 60% sodium hydride oil dispersion (21 mg NaH, 0.86 mmol, 1.05 equiv). After 15 min, a solution of 312 mg (0.90 mmol, 1.1 equiv) of **14** in 1 mL of DMF was added, and the mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was added to 100 mL of ethyl acetate and washed with 5% aqueous citric acid (2×), 5% aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic layer was removed, dried over anhydrous magnesium sulfate, and filtered, and the solvents were removed *in vacuo*. Purification by medium-pressure liquid chromatography, eluting with ethyl acetate/hexanes (2:1), afforded 501 mg (0.78 mmol, 95%) of **18** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (s, 9H), 1.34 (s, 6H), 1.40 (s, 9H), 1.86 (m, 1H), 2.42 (m, 1H), 2.42 (d, J = 14 Hz, 1H), 2.52 (d, J = 14 Hz, 1H), 2.62 (m, 2H), 4.52 (m, 1H), 4.82 (d, J = 15 Hz, 1H), 5.31 (s, 1H), 5.35 (d, J = 15 Hz, 1H), 6.68 (d, J = 7 Hz, 1H), 7.1–7.3 (m, 9H), 7.34 (m, 1H), 7.44 (m, 1H), 7.72 (d, J = 8 Hz, 1H). FAB-MS: calculated for $C_{38}H_{47}N_3O_6$ 641, found 642 (M + H, 15); 542 (M − BOC, 93).

4′-[[3-[(3-Amino-3-methyl-1-oxobutyl)amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepin-1-yl]methyl][1,1′-biphenyl]-2-carboxylic Acid (2, L-158,077). A solution of 500 mg (0.78 mmol) of **18** in 2 mL of glacial acetic acid was treated with 2 mL of 6 N hydrochloric acid, and the mixture was heated at 50 °C for 3 h. The mixture was concentrated under vacuum to a minimum volume, the residue was redissolved in 3 mL of distilled water and filtered, and the filtrate was lyophilized. The crusty solid was redissolved in 2 mL of methanol and treated dropwise with stirring with 5 mL of propylene oxide. The mixture was stirred at room temperature for 5 h and then filtered. The filtercake was washed with ether, air-dried, and then dried under vacuum to give 278 mg (0.57 mmol, 73%) of **2** as a white powder. ^{1}H NMR (300 MHz, 0.5 mL of D_2O + 10 μL of CF_3COOH): δ 1.43 (s, 3H), 1.47 (s, 3H), 2.10 (m, 1H), 2.29 (m, 1H), 2.32 (m, 2H), 2.66 (m, 2H), 4.28 (dd, J = 7, 11 Hz, 1H), 4.70 (d, J = 14 Hz, 1H), 5.29 (d, J = 14 Hz, 1H), 6.92 (m, 1H), 7.0–7.4 (m, 10H), 7.70 (m, 1H). FAB-MS: calculated for $C_{29}H_{31}N_3O_4$ 485, found 486 (M + H, 100). Anal. ($C_{29}H_{31}N_3O_4$) C, H, N.

3-[(*tert*-Butoxycarbonyl)amino]-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl][1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazepin-3-yl]butanamide. To a solution of 341 mg (0.91 mmol) of (±)-**17** in 2 mL of DMF at room temperature under nitrogen was added 44 mg of 60% sodium hydride oil dispersion (26 mg of NaH, 1.1 mmol, 1.2 equiv). After 20 min, a solution of 660 mg (1.2 mmol, 1.3 equiv) of bromide **16** in 2 mL of DMF was added and the mixture stirred for 2 h. The reaction mixture was added to 100 mL of ethyl acetate and washed with 5% aqueous citric acid (2×), saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic layer was removed, dried over anhydrous magnesium sulfate, and filtered, and solvents were removed *in vacuo*. Purification by medium-pressure liquid chromatography, eluting with ethyl acetate/hexanes (1:1), afforded 698 mg (0.82 mmol, 90%) of the product as an off-white, crusty foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (s, 3H), 1.34 (s, 3H), 1.40 (s, 9H), 1.77 (m, 1H), 2.3–2.6 (m, 5H), 4.46 (m, 1H), 4.68 (d, J = 15 Hz, 1H), 5.11 (d, J = 15 Hz, 1H), 5.35 (br s, 1H), 6.65 (d, J = 7 Hz, 1H), 6.9–7.5 (m, 26H), 7.85 (m, 1H).

3-Amino-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazepin-3-yl]butanamide, Trifluoroacetate (3, L-158,432). A solution of 698 mg (0.82 mmol) of 3-[(*tert*-butoxycarbonyl)amino]-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1-[[2′-[*N*-(triphenylmethyl)-tetrazol-5-yl][1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazepin-3-yl]butanamide in 5 mL of glacial acetic acid was treated with 0.5 mL of 6 N hydrochloric acid and the mixture stirred at room temperature for 14 h. All volatiles were removed under vacuum, and the residue was purified by preparative reverse-phase HPLC, eluting with methanol/0.1% aqueous trifluoroacetic acid (linear gradient: 60% methanol increased to 80% methanol over 10 min; retention time approximately 8 min) to afford 490 mg (0.79 mmol, 96%) of **3** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23 (s, 3H), 1.25 (s, 3H), 2.00–2.15 (m, 2H), 2.35–2.65 (m, 4H), 4.26 (m, 1H), 4.87 (d, J = 15 Hz, 1H), 5.14 (d, J = 15 Hz, 1H), 7.00 (d, J = 8 Hz, 2H), 7.12 (d, J = 8 Hz, 2H), 7.23 (m, 2H), 7.34 (m, 2H), 7.53 (m, 2H), 7.64 (m, 2H), 7.88 (br s, 3H), 8.69 (d, J = 7 Hz, 1H). FAB-MS: calculated for $C_{29}H_{31}N_7O_2$ 509, found 510 (M + H, 100). Anal. ($C_{29}H_{31}N_7O_2$·1.4CF_3COOH) C, H, N.

3-[(*tert*-Butoxycarbonyl)amino]-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepin-3(*R*)-yl]butanamide [(+)-17]. To a solution of 8.70 g (49.4 mmol) of the 3(*R*)-amino (+)-**6** in 100 mL of CH_2Cl_2 at room temperature were added 10.73 g (49.4 mmol, 1.0 equiv) of acid **10** and 13.8 mL of triethylamine (10.0 g, 99 mmol, 2 equiv). The flask was immersed in an ambient temperature water bath and treated with 26 g (59 mmol, 1.2 equiv) of BOP all at once. After 2 h at room temperature, the mixture was diluted into 300 mL of ethyl acetate and washed with 5% aqueous citric acid (3×), saturated aqueous sodium bicarbonate (2×), and saturated aqueous sodium chloride. The organic layer was removed, dried over anhydrous magnesium sulfate, and filtered, and solvents were removed *in vacuo*. The residue was redissolved in 50 mL of ethyl acetate/hexanes (4:1) and filtered and the filtrate purified by preparative HPLC, eluting with ethyl acetate/hexanes (4:1) to afford 17.42 g (46.4 mmol, 94%) of (+)-17. ^{1}H NMR (200 MHz, $CDCl_3$ + 1 drop CD_3OD): δ 1.34 (s, 6H), 1.40 (s, 9H), 1.93 (m, 1H), 2.44 (d, J = 14 Hz, 1H), 2.54 (d, J = 14 Hz, 1H), 2.6–3.0 (m, 3H), 4.48 (dd, J = 7, 12 Hz, 1H), 6.96 (d, J = 8 Hz, 1H), 7.1–7.3 (m, 3H). FAB-MS: calculated for $C_{20}H_{29}N_3O_4$ 375, found 376 (M + H, 43); 276 (M − BOC, 100).

3-[(*tert*-Butoxycarbonyl)amino]-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl][1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazepin-3(*R*)-yl]butanamide. A solution of 17.42 g (46.40 mmol) of (+)-17 in 25 mL of DMF at 0 °C under nitrogen was treated with 2.41 g of 60% sodium hydride oil dispersion (1.45 g NaH, 60.3 mmol, 1.3 equiv). After 15 min, a solution of 33.6 g (60.3 mmol, 1.3 equiv) of bromide **16** in 50 mL of DMF was added over 1 min and the mixture allowed to warm to room temperature and stirred for 1 h. The reaction mixture was added to 600 mL of ethyl acetate/hexanes (1:1) and washed with water (2×) and saturated aqueous sodium chloride (2×). The organic layer was removed, dried over anhydrous magnesium sulfate, and filtered, and solvents were removed *in vacuo*. Purification by preparative HPLC, eluting with ethyl acetate/hexanes (1:1), afforded 37.22 g (43.68 mmol, 94%) of the product as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (s, 3H), 1.39 (s, 3H), 1.68 (m, 1H), 2.2–2.5 (m, 5H), 4.44 (m, 1H), 4.67 (d, J = 14 Hz, 1H), 5.06 (s, 2H), 5.12 (d, J = 14 Hz, 1H), 5.63 (br s, 1H), 6.65 (d, J = 8 Hz, 1H), 6.9–7.5 (m, 31H), 7.85 (m, 1H).

3-Amino-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazepin-3(*R*)-yl]butanamide, Hydrochloride Dihydrate (1, L-692,429). A solution of 37.22 g (43.68 mmol) of (+)-17 in 150 mL of methanol at room temperature was stirred vigorously while 140 mL of 9 N hydrochloric acid was added dropwise over 1 h. The mixture was stirred vigorously for 16 h and then treated with 50 mL of hexanes, and stirring continued for 1 h. Stirring was discontinued, and the layers were allowed to separate. The upper hexane layer was carefully removed by pipet, another 50 mL of hexanes was added, and the mixture was stirred vigorously for 10 min. The top layer was again removed by pipet, the lower, aqueous layer was removed and filtered, and the filtrate was aged at 4 °C for 17 h. A first crop of crystals formed and was isolated by filtration and air-dried to give 21.18 g (36.4 mmol, 83%) of **1** as a fine white powder. The mother liquors were concentrated under vacuum to approximately 30 mL, filtered, seeded with the first crop, and aged overnight at 4 °C. A second crop was deposited which was isolated by filtration and air-dried giving 1.88 g (3.23 mmol, 7%) of L-692,429. The combined yield of compound **1** was 23.06 g (39.62 mmol, 90.6%). Both crops were identical by 200-MHz ^{1}H NMR, FAB-MS, and optical rotation. ^{1}H NMR (200 MHz, CD_3OD): δ 1.33 (s, 3H), 1.37 (s, 3H), 2.0–2.6 (m, 6H), 4.35 (dd, J = 7, 11 Hz, 1H), 4.86 (d, J = 15 Hz, 1H), 5.20 (d, J = 15 Hz, 1H), 7.00 (d, J = 8 Hz, 2H), 7.15–7.35 (m, 6H), 7.45–7.70 (m, 4H). FAB-MS: calculated for $C_{29}H_{31}N_7O_2$ 509, found 510 (M + H, 100). $[\alpha]_D$ +112° (c 1.05, CH_3OH). Anal. ($C_{29}H_{31}N_7O_2$·HCl·2H_2O) C, H, N, Cl.

3-Amino-3-methyl-*N*-[2,3,4,5-tetrahydro-2-oxo-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazepin-3(*S*)-yl]butanamide, Hydrochloride Dihydrate (4, L-692,428). The 3*S* enantiomer **4** was prepared from 3(*S*)-amino-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one [(−)-**6**] by the methods described above for the synthesis of compound **1**. $[\alpha]_D$ −112° (c 1.01, CH_3OH). Anal. ($C_{29}H_{31}N_7O_2$·HCl·2H_2O) C, H, N, Cl.

3-Amino-3-methyl-*N*-[1,2,3,4-tetrahydro-2-oxo-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-1-quinolin-3-yl]butanamide, Trifluoroacetate (19). **19** was prepared from 3-amino-1,2,3,4-tetrahydroquinolin-2-one[17] by the procedures described above for the synthesis of **3**. ^{1}H NMR (400 MHz, CD_3OD): δ 1.43 (s, 3H), 1.45 (s, 3H), 2.61 (m, 2H), 3.12 (m, 2H), 4.78 (dd, J = 5, 12 Hz, 1H), 5.10 (d, J = 16 Hz, 1H), 5.33 (d, J = 16 Hz, 1H), 7.05 (m, 4H), 7.24 (m, 4H), 7.54 (m, 2H), 7.65 (m, 2H). FAB-MS: calculated for $C_{29}H_{29}N_7O_2$ 495, found 496 (M + H, 100). Anal. ($C_{28}H_{29}N_7O_2$·1.3CF_3COOH·2.3H_2O) C, H, N.

3-Amino-3-methyl-*N*-[1,2,3,4,5,6-hexahydro-2-oxo-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-1-benzazocin-3-yl]butanamide, Trifluoroacetate (20). **20** was prepared from 3-azido-3,4,5,6-tetrahydro-1-benzazocin-2(1*H*)-one[13] by the procedures described above for the synthesis of **3**. ^{1}H NMR (400 MHz, CD_3OD): δ 1.32 (s, 3H), 1.35 (s, 3H), 1.44 (m, 1H), 1.80 (m, 3H), 2.08 (m, 1H), 2.40–2.58 (m, 3H), 4.02 (dd, J = 2, 11 Hz, 1H), 4.66 (d, J = 14 Hz, 1H), 5.21 (d, J = 14 Hz, 1H), 7.01 (d, J = 8 Hz, 2H), 7.13 (d, J = 8 Hz, 2H), 7.22–7.36 (m, 4H), 7.55 (m, 2H), 7.65 (m, 2H). FAB-MS: calculated for $C_{30}H_{33}N_7O_2$ 523, found 524 (M + H, 100). Anal. ($C_{30}H_{33}N_7O_2$·1.3CF_3-$COOH$·1.5H_2O) C, H, N.

Single-Crystal X-ray Diffraction Determination of the Absolute Stereochemistry of 3(*S*)-[[[[(*R*)-Naphthylethyl]-amino]carbonyl]amino]-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one (7). Colorless, prismatic crystals of compound **7** were grown from an acetone/hexane mixture (~2:1). The crystal chosen for data collection (approximate dimensions 0.22 × 0.10 × 0.37 mm) was mounted in a nonspecific orientation on an Enraf-Nonius CAD4F diffractometer.

The crystal data and experimental conditions are as follows: molecular formula = $C_{29}H_{31}N_3O_4$, M_r = 449.55, monoclinic space group $P2_1$, a = 11.068(1) Å, b = 7.6877(7) Å, c = 14.769(2) Å, β = 105.378(9)°, V = 1211.7 Å^3, Z = 2, D_{calc} = 1.232 g cm^{-3}, μ(Cu Kα) = 0.64 mm^{-1}, F(000) = 480, T = 296 K. Data were collected[32] with Cu Kα monochromatized radiation (λ = 1.541 84 Å) to a 2θ limit of 144°, yielding 2716 measured reflections. Scan type is ω with a range of 0.70 + 0.14 tan(θ)° and a variable speed of 10.1–2.5 deg min^{-1}. The data set was corrected for Lorentz, polarization, and background effects. Monitoring standard reflections (three every 60 min of exposure time) showed no decay correction necessary. Averaging equivalent reflections (R_{int} = 0.040) gave a unique data set of 2597 reflections with 1481 observed data at the $I \geq 3\sigma(I)$ level. No absorption correction was calculated. The structure was solved using SHELXS-86[33] and refined[34] using full-matrix least-squares on F with a weighting scheme of $1/\sigma^2(F)$. The final agreement statistics for 285 variables are R = 0.096, R_w = 0.095, S = 6.30, $(\Delta/\sigma)_{max}$ = 0.9. The maximum peak in the final difference Fourier is 0.45(8) e Å^{-3}.

Compound **7** crystallizes with one water molecule and one disordered acetone molecule in the asymmetric unit. The disorder was partially modeled using half-occupancy carbon atoms. Hydrogen atoms for **7** were included when they could be located in a difference Fourier map or reliably calculated and these were refined using a "riding" model and fixed thermal parameters.

Molecular Modeling Studies. Default *SEAL* parameters were used, limiting an increase in the steric weighting to 10. This was determined to yield an appropriate balance between the steric and electrostatic alignment terms for molecules with formal charges, such as compound 1 and GHRP-6. Structures were minimized using the MM2X force field which is derived, in large part, from the MM2 force field.[35] Parameters for the non-bonded, out-of-plane, and stretch–bend interactions are taken directly from MM2 without change. For intramolecular interactions, the force field includes all the type of interactions that are in MM2, and the functional form of interaction is identical with that in MM2 with one exception: the electrostatic term is represented by Coulomb's law acting on atom-centered point charges. These atomic charges q are derived from bond dipole moments $q = 1/4.803\Sigma_i\mu_i r_{i0}$ where μ is the bond dipole moment, r_0 is the reference bond length, and the sum extends over all the bonds for a given atom. MM2X does not use lone pairs on aliphatic amines, alcohols, and ether oxygens, and carboxylic acid and ester oxygens. The parameter differences are due primarily to the need to compensate for the elimination of lone pairs on oxygen, reproduction of gas-phase dimerization energies of acids and amides, and incorporation of parameters for aromatic carbons.

Rat Pituitary Cell Assay. Cell Culture. Wistar male rats (150–200 g) were obtained from Charles River Laboratories (Wilmington, MA). Rats were maintained at a constant temperature (25 °C) on a 14-h light, 10-h dark cycle. Rat chow and water were available *ad libitum*. Animals were sacrificed by decapitation and anterior lobes of the pituitary quickly removed. Rat pituitary cells were isolated from pituitaries by enzymatic digestion with 0.2% collagenase and 0.2% hyaluronidase in Hank's Balanced Salt Solution as described previously.[29] For culture, cells were suspended in culture medium and adjusted to a final concentration of 1.5×10^5 cells/mL; 1.0 mL of this suspension was placed in each well of a 24-well tray (Costar; Cambridge, MA). Cells were maintained in a humidified 5% CO_2–95% air atmosphere at 37 °C for 3–4 days. The culture medium consisted of Dulbecco's Modified Eagle's Medium containing 0.37% $NaHCO_3$, 10% horse serum, 2.5% fetal bovine serum, 1% nonessential amino acids, 1% glutamine, 1% nystatin, and 0.1% gentamycin.

Experiments for GH Release. On the day of an experiment, cells were washed twice 90 min prior to and once more immediately before the start of the experiment with the above culture medium containing 25 mM HEPES, pH 7.4. Stock solutions of 2 mg/mL of the test agents were prepared in dimethyl sulfoxide, and serial dilutions in the culture medium were made from the stock solutions. The final concentration of DMSO in the assay medium was 1%. GH release was initiated by adding 1 mL of fresh medium containing test agents to each well in quadruplicate. Incubation was carried out at 37 °C for 15 min. After incubation, medium was removed and centrifuged at 2000*g* for 15 min to remove any cellular material. The supernatant fluid was removed and assayed for GH content.

Radioimmunoassays. Rat GH in culture medium was measured by a double antibody RIA procedure using materials obtained from Dr. A. F. Parlow (Harbor-UCLA Medical Center, Torrance, CA) and expressed in terms of the standard rat GH RP-2.

Data Analyses. ED_{50} values for test compounds were computed by fitting a four-parameter logistic function to the dose–response curve.[36] Least-squares estimates of the four-parameter logistic function coefficients and their variances were derived using the iterative algorithm described by Bates and Watts.[37]

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Supplementary Material Available: Tables of crystallographic coordinates, thermal parameters, and geometric quantities of urea 7 and a table of atomic coordinates of the alignment shown in Figure 3b (17 pages). Ordering information is given on any current masthead page.

References

(1) Brown, P.; Preece, M. A.; Will, R. G. "Friendly fire" in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. *Lancet* **1992**, *340*, 24–27.
(2) Jørgensen, J. O. L.; Christiansen, J. S. Growth hormone therapy. Brave new senescence: GH in adults. *Lancet* **1993**, *341*, 1247–1248.
(3) Lehrman, S. Challenge to growth hormone trial. *Nature* **1993**, *364*, 179.
(4) Schoen, W. R.; Wyvratt, M. J., Jr.; Smith, R. G. Growth hormone secretagogues. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: San Diego, CA, 1993; Vol. 28, Chapter 19.
(5) Thorner, M. O. On the discovery of growth hormone-releasing hormone. *Acta Pædiatr. Suppl.* **1993**, *388*, 2–7.
(6) Low, L. C. K. Growth hormone-releasing hormone: clinical studies and therapeutic aspects. *Neuroendocrinology* **1991**, *53* (Suppl. 1), 37–40.
(7) Bowers, C. Y.; Chang, J.; Momany, F.; Folkers, K. In *Molecular Endocrinology*; MacIntyre, I., Ed.; Elsevier/North Holland: Amsterdam, 1977; p 287.
(8) Bowers, C. Y. GH releasing peptides – structure and kinetics. *J. Pediatr. Endocrinol.* **1993**, *6*, 21–31.
(9) Raynor, K.; Murphy, W. A.; Coy, D. H.; Taylor, J. E.; Moreau, J.-P.; Yasuda, K.; Bell, G. I.; Reisine, T. Cloned somatostatin receptors: identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. *Mol. Pharmacol.* **1993**, *43*, 838–844.
(10) Bell, G. I.; Reisine, T. Molecular biology of somatostatin receptors. *Trends Neurosci.* **1993**, *16*, 34–38.
(11) Smith, R. G.; Cheng, K.; Schoen, W. R.; Pong, S.-S.; Hickey, G.; Jacks, T.; Butler, B.; Chan, W. W.-S.; Chaung, L.-Y. P.; Judith, F.; Taylor, J.; Wyvratt, M. J.; Fisher, M. H. A novel non-peptidyl growth hormone secretagogue. *Science* **1993**, *260*, 1640–1643.
(12) Gertz, B. J.; Barrett, J. S.; Eisenhandler, R.; Krupa, D. A.; Wittreich, J. M.; Seibold, J. R.; Schneider, S. H. Growth hormone response in man to L-692,429, a novel nonpeptide mimic of growth hormone releasing peptide. *J. Clin. Endocrinol. Metab.* **1993**, *77*, 1393–1397.
(13) Watthey, J. W. H.; Stanton, J. L.; Desai, M.; Babiarz, J. E.; Finn, B. M. Synthesis and biological properties of (carboxyalkyl)amino-substituted bicyclic lactam inhibitors of angiotensin converting enzyme. *J. Med. Chem.* **1985**, *28*, 1511–1516.
(14) Graf, v. R. Umsetzungen mit *N*-carbonyl-sulfamidsäurechlorid, III. (Reactions of *N*-chlorosulfonyl isocyanate, III.) *Chem. Ber.* **1963**, *96*, 111–157.
(15) Miyaura, N.; Yanagi, T.; Suzuki, A. The palladium-catalyzed cross-coupling reaction of phenylboronic acid with haloarenes in the presence of bases. *Synth. Commun.* **1981**, *11*, 513–519.
(16) Shumann, R. F.; King, A. O.; Anderson, R. K. Ortho-lithiation process for the synthesis of 2-substituted 1-(tetrazol-5-yl)benzenes. U.S. Pat. 5,039,814, 1991.
(17) Davis, A. L.; Lloyd, R.; Fletcher, J.; Bayliss, L.; McCord, T. J. The syntheses and biological activities of *o*-aminophenylalanine and related compounds. *Arch. Biochem. Biophys.* **1963**, *102*, 48–51.
(18) Cheng, K. Unpublished results.
(19) pK_a's were determined by direct titration in 50% aqueous methanol.
(20) Carini, D. J.; Duncia, J. V.; Aldrich, P. E.; Chiu, A. T.; Johnson, A. L.; Pierce, M. E.; Price, W. A.; Santella, J. B.; Wells, G. J.; Wexler, R. R.; Wong, P. C.; Yoo, S.-E.; Timmermans, B. M. W. M. Nonpeptide angiotensin II receptor antagonists: The discovery of a series of *N*-(biphenylmethyl)imidazoles as potent, orally active antihypertensives. *J. Med. Chem.* **1991**, *34*, 2525–2547.
(21) Benzimidazole-based angiotensin II receptor antagonists containing the 2′-substituted biphenyl element have also been reported wherein 2′-carboxy and 2′-tetrazole derivatives have *comparable* binding affinities. Kubo, K.; Inada, Y.; Sugiura, Y.; Ojima, M.; Itoh, K.; Furukawa, Y.; Nishikawa, K.; Naka, T. Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazoles. *J. Med. Chem.* **1993**, *36*, 1772–1784.
(22) Cheng, K.; Chan, W. W.-S.; Butler, B.; Wei, L.; Schoen, W. R.; Wyvratt, M. J.; Fisher, M. H.; Smith, R. G. Stimulation of growth hormone release from rat primary pituitary cells by L-692,429, a novel non-peptidyl growth hormone secretagogue. *Endocrinology* **1993**, *132*, 2729–2731.
(23) Momany, F. A.; Bowers, C. Y.; Reynolds, G. A.; Hong, A.; Newlander, K. Conformational energy studies and *in vitro* and *in vivo* activity data on growth hormone-releasing peptides. *Endocrinology* **1984**, *114*, 1531–1536.
(24) Kearsley, S.; Smith, G. An alternative method for the alignment of molecular structures: Maximizing electrostatic and steric overlap. *Tetrahedron Comput. Methodol.* **1992**, *3*, 615–633.
(25) QUANTA/CHARMm, Polygen Corp., 200 Fifth Avenue, Waltham, MA 02254. (1986, 1987, 1988, 1991).
(26) Crippen, G. M.; Havel, T. F. Stable calculation of coordinates from distance information. *Acta Crystallogr.* **1978**, *A34*, 282–284.

(27) Distance measurements are derived from the alignment of GHRP-6 and compound 1 shown in Figure 3b. A table of atomic coordinates of the alignment is included in the supplementary material.
(28) Bowers, C. Y.; Momany, F. A.; Reynolds, G. A.; Hong, A. On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* **1984**, *114*, 1537–1545.
(29) Cheng, K.; Chan, W. W.-S.; Barreto, A.; Convey, E. M.; Smith, R. G. The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 on growth hormone (GH)-releasing factor-stimulated GH release and intracellular adenosine 3′,5′-monophosphate accumulation in rat primary pituitary cell culture. *Endocrinology* **1989**, *124*, 2791–2798.
(30) Hickey, G.; Jacks, J.; Judith, F.; Taylor, J.; Schoen, W. R.; Krupa, D.; Cunningham, P.; Clark, J.; Smith, R. Efficacy and specificity of L-692,429, a novel nonpeptidyl growth hormone secretagogue in beagles. *Endocrinology* **1994**, *134*, 695–701.
(31) Ohta, S.; Shimabayashi, A.; Aono, M.; Okamoto, M. A general convenient one-pot procedure for the conversion of carboxylic acids into their *t*-butyl esters which is also applicable to aliphatic carboxylic acids. *Synthesis* **1982**, 833–834.
(32) The diffractometer control programs are those supplied by Enraf-Nonius for operating the CAD4F diffractometer.
(33) Sheldrick, G. M. Phase annealing in *SHELX*-90; direct methods for larger structures. *Acta Crystallogr.* **1990**, *A46*, 467–473.
(34) *Structure Determination Package Version 3.* Enraf-Nonius, Delft. The Netherlands (1985). Implemented on a Sun Microsystems workstation.
(35) Allinger, N. L.; Yuh, Y. H. MM2. *QCPE* **1981**, *13*, 395.
(36) Rodbard, D.; Frazier, G. R. Statistical Analysis of Radioligand Assay Data. *Methods Enzymol.* **1975**, *37*, 3–22.
(37) Bates, D.; Watts, D. *Nonlinear Regression Analysis and its Applications*; John Wiley and Sons: New York, 1988.