Novel Orally Active Growth Hormone Secretagogues

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A novel class of growth hormone-releasing compounds with a molecular weight in the range from 500 to 650 has been discovered. The aim of this study was to obtain growth hormone secretagogues with oral bioavailability. By a rational approach we were able to reduce the size of the lead compound ipamorelin (**4**) and simultaneously to reduce hydrogen-bonding potential by incorporation of backbone isosters while retaining in vivo potency in swine. A rat pituitary assay was used for screening of all compounds and to evaluate which compounds should be tested further for in vivo potency in swine and oral bioavailability, f_{po}, in dogs. Most of the tested compounds had f_{po} in the range of 10−55%. In vivo potency in swine after iv dosing is reported, and ED_{50} was found to be 30 nmol/kg of body weight for the most potent compound.

Introduction

The release of growth hormone (GH) from somatotrophs in the pituitary gland is known to be controlled via two separate receptor systems triggered by somatostatin and GHRH (growth hormone-releasing hormone). GH release is probably influenced by a third, separate mechanism as well—a growth hormone secretagogue (GHS) pathway (also known as the GHRP pathway). The mechanism of action, for the compounds now known as GHSs, implies binding to one or more receptor(s) different from those used by somatostatin or GHRH. Recently, Howard et al. disclosed the isolation of a cDNA encoding a putative GHS receptor[1] that binds GHSs with high affinity, but so far there are no reports on an endogenous ligand.

The field of GHSs originates from Momany and Bowers work[2] on enkephalines in the 1970s which led to potent and specific compounds such as the heptapeptide GHRP-1 (**3**) and hexapeptide GHRP-6. A substantial level of worldwide interest[3] has now been reached. This process has been driven not only by an increase in the understanding of the varied effects of GH itself and the potential clinical applications but also by the discovery of novel small molecules, which mimic the action of Momany and Bowers' original peptides.

At present, a number of studies[4] suggest benefits of using GH in elderly patients with osteoporosis, wasting conditions, and recovery, i.e., following major surgery. And there is reason to believe that orally administered GHS therapy could substitute direct GH replacement by parenteral routes and at the same time produce a GH concentration profile that mimics the natural pulsatile pattern better.[5] Considering the nature of the conditions that may be treated with GHS in the future, it seems unlikely that patients will accept daily injections. As most of these conditions are not life-threatening, it is of great importance to find safe, orally active substances that offer the convenience of oral administration and that have no or few adverse effects.

The first nonpeptide GHS, L-692,429 (**1**), was discovered by scientists from Merck[6] after a directed screening program. This compound had low oral bioavailability, but the same group later discovered orally active compounds of which the prototype is MK-677 (**2**).[7]

Results

Our program took a different route and was an attempt directly to reduce the size of GHRP-1 (**3**) in a rational, stepwise manner with the aim of retaining activity and achieving oral bioavailability (Scheme 1). The initial part of our program was based on classical peptide chemistry and has been reported elsewhere.[8] Major achievements during this program were the elimination of the central Ala-Trp sequence from GHRP-1 leading to the very potent clinical candidate ipamorelin (**4**) (NNC 26-0161) and the substitution of the N-terminal Aib-His residues with 3-(aminomethyl)-benzoic acid leading to **5** (NNC 26-0235). This was achieved without loss of in vitro or in vivo potency. Removal of the C-terminal Lys residue from **5** gave us the first compound (**6**) with oral bioavailability (f_{po} = 20% in rats), but with a more than 100-fold loss of activity in the rat pituitary in vitro assay (EC_{50} from 2.0 nM for **5** to 265 nM for **6**). Yet, compound **6** served as a lead compound at later stages of our program where our task was to improve the potency without losing oral bioavailability.

Starting from **6**, our strategy was to maintain or reduce the number of potential hydrogen-bonding sites and to keep the molecular weight as low as possible in order to achieve high oral bioavailability. This strategy was based on the suggestions provided by other groups,[9]

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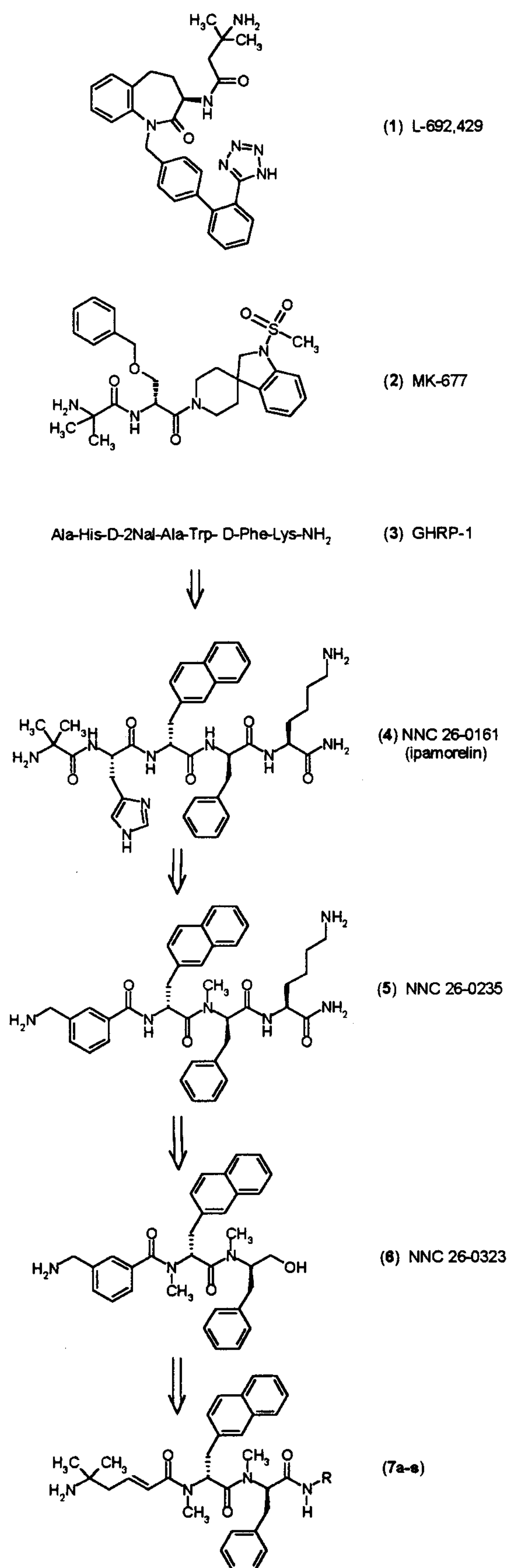
Scheme 1



although peptide drug absorption across membranes still is a relatively poorly understood process. According to this strategy, we consistently employed N-methylated amino acids in order to reduce desolvation energy and thereby maximize the chance of absorption across the intestinal membrane. Previous results in our peptide program indicated that shielding of the N-terminal nitrogen atom by the bulk of two methyl groups was particularly advantageous. Furthermore, 3-(aminomethyl)benzoic acid[10] constitutes a very rigid mimic of an Aib-His dipeptide unit, leading us to believe that substitution of the *m*-phenylene with an allyl unit was attractive in order to achieve more flexibility. These considerations led to the synthesis and incorporation of 5-amino-5-methyl-2-hexenoic acid as an N-terminal dipeptide mimic in a series of novel modified tripeptides, **7a**−**s**. Indeed, when this substitution was carried out on **6** along with a simple modification in the C-terminal, we obtained compounds that were generally very much improved with respect to in vitro potency. The first compound in this series (**7a**), was 15 times more potent than **6**. After the discovery of **7a**, a series of close analogues was prepared and screened for oral bioavailability in beagle dogs and for GH-releasing properties in swine after iv administration.

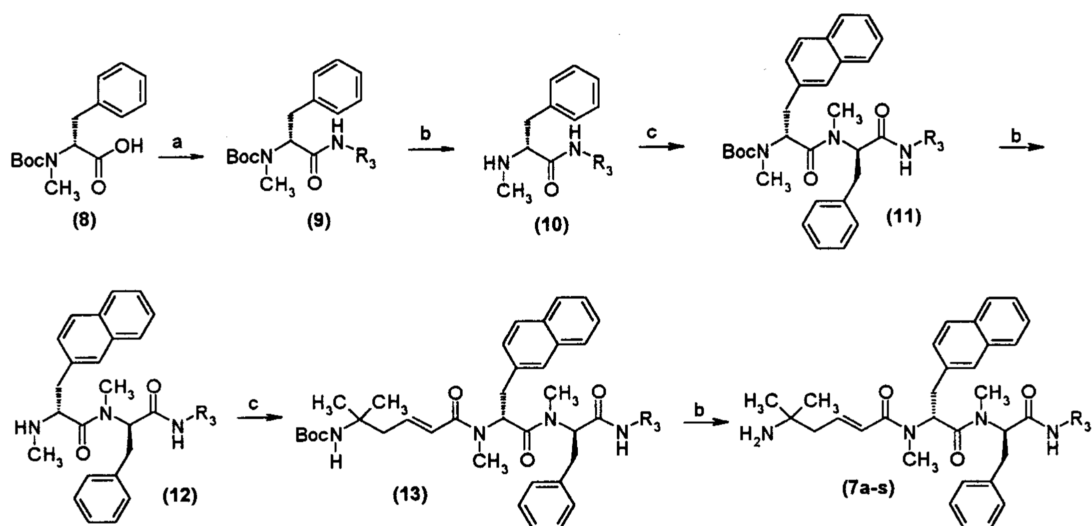
Chemistry

The synthesis of compounds **7a**−**s** (Scheme 2, Table 1) was based on commercially available Boc-protected amino acids (with exception of the N-terminal) which were *N*-methylated in THF with methyl iodide in the presence of sodium hydride. This procedure[11] allows virtually racemization-free N-methylation without concomitant esterification of the carboxylic acid. Attachment of a C-terminal amine proceeded as HOBt-mediated couplings in almost quantitative yield. All peptide couplings on secondary amines were performed as activated esters with 7-azahydroxybenzotriazole[12] (HOAt) and EDAC as activating agents. In general, couplings were relatively slow[13] and required prolonged reaction times to go to completion.

Yields in all steps were good, except for the final deprotection. In all cases studied (**7a**−**s**) the molecules showed pronounced tendency to cleave between the Nal and Phe residues[14] during treatment with TFA. This cleavage was clearly induced by the anhydrous and very acidic conditions. Minimizing this undesired cleavage required that the Boc-protected precursors were exposed to TFA for a very short time. Already after 7 min in 50% TFA in methylene chloride at room temperature we observed substantial cleavage between Nal and Phe residues. Fast neutralization was essential. However, once the compounds were purified, they showed good stability even during prolonged storage in aqueous solution at pH 4−5.

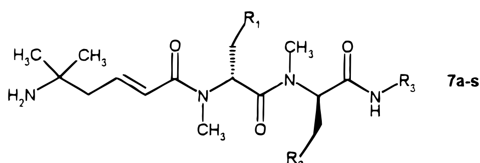
(2*E*)-5-(*tert*-Butoxycarbonylamino)-5-methylhex-2-enoic acid (**18**) was used as a general N-terminal and was unknown in the literature. The acid was prepared in four reaction steps from the known[6] 3-(*tert*-butoxycarbonylamino)-3-methylbutanoic acid (**14**) (Scheme 3). Compound **14** was converted to 3-(*tert*-butoxycarbonylamino)-3-methylbutanol (**15**) by reduction with lithium borohydride after in situ formation of an anhydride with ethyl chloroformate. Alcohol **15** was oxidized under Swern conditions to the aldehyde **16** which underwent smooth two-carbon elongation by a Horner−Emmons reaction with triethyl phosphonoacetate in THF. Fi-

Scheme 2[a]



[a] (a) HOBt, EDAC, R_3NH_2; (b) TFA; (c) AA, HOAt, EDAC.

Table 1. Prepared Compounds



compd	R_1	R_2	R_3
7a	2-naphthyl	phenyl	methyl
7b	2-naphthyl	phenyl	(2*S*)-2-hydroxypropyl
7c	2-naphthyl	phenyl	(2-tetrahydrofuranyl)methyl
7d	2-naphthyl	phenyl	cyclopropylmethyl
7e	benzyloxy	phenyl	methyl
7f	1-naphthyl	phenyl	methyl
7g	2-naphthyl	4-fluorophenyl	methyl
7h	3-benzothienyl	phenyl	methyl
7i	1-naphthyl	phenyl	(2-tetrahydrofuranyl)methyl
7j	2-naphthyl	2-thienyl	methyl
7k	4-biphenylyl	phenyl	methyl
7l	2-naphthyl	4-iodophenyl	methyl
7m	2-naphthyl	3,4-difluorophenyl	methyl
7n	2-naphthyl	phenyl	ethyl
7o	2-naphthyl	phenyl	(2*R*)-2-hydroxypropyl
7p	2-naphthyl	2-thienyl	(2*S*)-2-hydroxypropyl
7q	4-biphenylyl	4-fluorophenyl	(2*R*)-2-hydroxypropyl
7r	4-biphenylyl	2-thienyl	(2*S*)-2-hydroxypropyl
7s	2-naphthyl	3,4-difluorophenyl	(2*S*)-2-hydroxypropyl

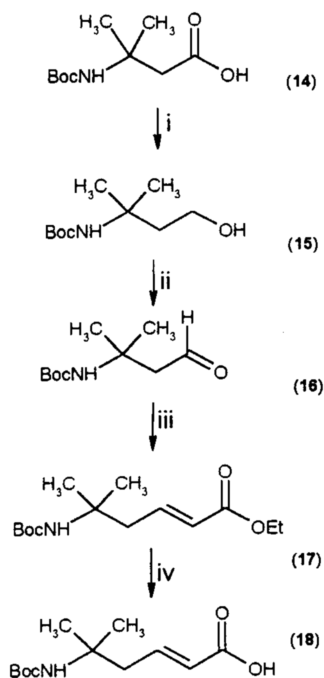
nally saponification with lithium hydroxide gave **18** in good overall yield.

Biology

For our screening strategy, the classical rat pituitary cell assay was chosen as the primary assay in order to narrow the focus to compounds with superior GH-releasing properties. The more potent compounds within a series were chosen for further evaluation. The next step was divided into two parts. The first was a test for oral bioavailability. Beagle dogs were chosen for this purpose, due to their convenient temperament during administration. The GH release was also measured, but this was not the primary objective of the test. The oral bioavailability was calculated as the total area under the plasma concentration versus time curve following po. administration divided by the area following iv administration, appropriately corrected for dose. The second test was an in vivo test for GH release in swine after iv dosing. A single dose per compound (50 nmol/kg of body weight (BW)) was given iv. We favor a swine model for GH release because this species has better resemblance to humans with respect to physiology in general and endocrinology in particular, than other common laboratory animals such as rat and dog. On the basis of our previous experience, measurements of pGH release following a 50 nmol/kg BW iv dose allowed us to make a crude selection of compounds for further evaluation. Results from this test are tabulated as C_{max} (peak pGH levels at the given dose). Compounds which simultaneously showed a relatively high f_{po} and C_{max} at the given dose were selected for further investigation.

The screening data are collected in Table 2. All compounds were active in the rat pituitary assay with

Scheme 3[a]



[a] (i) $LiBH_4$; (ii) oxalyl chloride DMSO; (iii) Homer–Emmons; (iv) LiOH.

Table 2. Results of in Vitro and in Vivo Screening of GH Secretagogues[a]

	rat pit assay		swine,	f_{po}, oral
compd	EC_{50} (nM)	E_{max} (% of GHRP-6)	GH C_{max} (ng of pGH/mL)	bioavail, beagle dog (%)
GHRP-6	1.7	100		
7a	18	110		
7b	8.9	130	26	25
7c	16	100	9	17
7d	1.8	105	14	24
7e	5.0	125	0.4	11
7f	24	115	12	
7g	8.0	90	30*	49
7h	35	80		
7i	21	110		
7j	2.0	110	55**	15
7k	3.0	120		0
7l	20	110	0.4	26
7m	17	85	6	54
7n	13	105		14
7o	9.0	105	9	0
7p	45	120		
7q	6.0	90	24	10
7r	1.5	110	56	0
7s	5.0	100	5	

[a] Dose–response curves of GH release from rat pituitary cells in culture were constructed as described in the Experimental Section, and the potency and efficacy were calculated from these. Results are showed as means. To obtain GH C_{max} values four swine were dosed with 50 nmol/kg BW of the test compounds; results shown as mean. To obtain the f_{po} values four dogs were dosed orally with 2.5 mg/kg BW of the test compounds and iv with 0.5 mg/kg BW; results shown as mean. *Exatrapolated from a Hill plot using 5, 25, 270 nmol/kg BW. **Exatrapolated from a Hill plot using 2, 240, 560 nmol/kg BW.

a factor of 30 between the most and least potent compound. We chose the most potent of the compounds and tested them for oral bioavailability and GH release. We found two compounds, **7b**,**g**, which at the same time were systemically available following oral administration and were among the more potent with respect to GH release.

Table 3. Oral Bioavailability and GH Release in Beagle Dogs[a]

dose (mg/kg BW)	C_{max} (nmol/L)	f_{po} (%)	C_{max} for GH (ng/mL)
	7b		
0.5	nd	nd	1.8 ± 1.1
2.5	260 ± 61	21 ± 4	6.6 ± 5.1
10	2318 ± 1266	41 ± 16	42.5 ± 9.9*'**
	7g		
0.5	99 ± 32	53 ± 23	2.7 ± 3.4
2.5	247 ± 106	55 ± 41	4.5 ± 3.3
10	3031 ± 917*,**	138 ± 64	12.0 ± 8.4

[a] Results are shown as mean ± SEM ($n = 4$). nd, not detectable; *$p < 0.05$ vs 0.5 mg/kg BW; **$p < 0.05$ vs 2.5 mg/kg BW.

Table 4. Potency and Efficacy of Different GH Secretagogues in Swine after iv Administration[a]

compd	potency ED_{50} (nmol/kg BW)	E_{max} (ng of pGH/mL)
GHRP-6	3.9 ± 1.4	74 ± 7
ipamorelin	2.3 ± 0.03	65 ± 0.2
7b	31 ± 8*,**	96 ± 5*,**
7g	129 ± 42*,**	86 ± 7**

[a] The values were calculated from dose–response curves based on mean plasma GH concentrations obtained after iv administration of test compounds in a dose range of 10–10000 nmol/kg. Each value is given as mean ± SEM, ($n = 6$). *$p < 0.05$ vs GHRP-6; **$p < 0.05$ vs ipamorelin.

The two compounds chosen for further biological study were evaluated in a pharmacokinetic and pharmacodynamic model using three ascending oral doses (0.5, 2.5, and 10 mg/kg BW) and a single intravenous dose (0.5 mg/kg BW) in fasted beagle dogs ($n = 4$). Table 3 summarizes the phamacokinetic parameters that were calculated from these experiments. In particular both compounds showed dose-dependent oral bioavailability. Compound **7b** showed an increase in f_{po} from 21% to 41% with increasing doses (the 0.5 mg/kg BW dose could not be detected), whereas f_{po} for compound **7g** increased from 55% to 138%, eventually attributed to enterohepatic circulation or a saturation phenomenon of elimination pathways. Plasma elimination half-lives were 1.3 h for **7b** and 1.1 h for **7g**.

An investigation of the dose dependency of GH release for **7b**,**g** in swine after iv dosing is summarized in Table 4 and compared to the values found for ipamorelin and GHRP-6.

We also wanted to investigate the molecular site of action of **7b**,**g** and to compare it with that of GHRP-6. For this purpose the effect of GHS and GHRH antagonists were tested, and the data are summarized in Table 5. We used the peptide (GHS) antagonist[15] His-D-Trp-D-Lys-Trp-D-Phe-Lys-NH_2, the nonpeptide (GHS) antagonist[16] L-692,400, and [D-Arg[1],D-Phe[5],D-Trp[7,9],Leu[11]]-substance P.[17,18] Thus, GH release induced by all three compounds was inhibited by GHS antagonists but not by a GHRH receptor antagonist. For a comparison, the GHRH(1–29)NH_2-induced GH release was potently inhibited by the GHRH antagonist[19] [N-acetyl-Tyr[1],D-Arg[2]]-hGHRH(1–29), whereas the GHS antagonists had no effect. The data clearly suggest a similar site of action of **7b**,**g** and GHRP-6.

Discussion

The antagonist data summarized in Table 5 indicate that **7b**,**g** and GHRP-6 mediate their effects through the same receptor, which is distinct from the GHRH recep-

Table 5. Effect of Different GHS and GHRH Antagonists on the Stimulatory Effect of **7b** (50 nM), **7g** (50 nM), and GHRP-6 (10 nM) on the GH Release from Rat Pituitary Cells in Vitro

	IC_{50} (μM)			
compd	D-Lys[3]-GHRP-6 (**19**)	L-692,400 (**20**)	D-Arg[1]D-Phe[5]D-Trp[7,9],Leu[11] (**21**), substance P antagonist	*N*-acetyl-Tyr[1]-D-Arg[2](1−29)GHRH-NH_2 (**22**)
7b	8 ± 4	11 ± 3	0.14 ± 0.08	>10
7g	9 ± 4	14 ± 5	0.049 ± 0.02	>10
GHRP-6	8 ± 2	10 ± 3	0.038 ± 0.015	>10

[a] Results are shown as mean ± SEM ($n = 4$). No significant differences were observed ($p > 0.05$) between the compounds.

tor. Clearly, we are dealing with true peptide mimetics of Momany and Bowers' original peptides.

The series of compounds presented above were all prepared by classical peptide methods and were readily applicable for scaleup. In the C-terminal domain we used D-Phe (or isosters) coupled to commercially available amines. Simple amines caused no problems during peptide couplings. We encountered less clean reactions and slightly lower yields when the amine was 1-amino-2-propanol, probably due to esterification of the hydroxy group as a side reaction during acylation of the hindered amines. (*S*)-1-Amino-2-propanol was introduced as a C-terminal because there were reports in the literature[20] indicating that the N- and C-termini might be situated closely together in the active conformation and that alkylation of the N-terminal with 2-hydroxypropyl was advantageous.[21] The employment of 5-amino-5-methylhex-2-enoic acid as a general N-terminal highlights the use of this building block as a potentially useful dipeptide isoster that seems particularly advantageous[22] as a substitute for Aib-containing peptides. Compared to 3-(aminomethyl)benzoic acid, it is clear that 5-amino-5-methylhex-2-enoic acid resembles a peptide backbone more closely in terms of flexibility and steric bulk. Our choice to make consistent use of 5-amino-5-methylhex-2-enoic acid as an N-terminal residue, D-2-Nal (or isosters) as the core, and D-Phe (or isosters) as the C-terminal inherently allows for relatively limited structural variation, and focus of this study was indeed on the importance of the C-terminal domain. This is reflected in the fact that all compounds are active in the lower nanomolar range in the rat pituitary assay. Our experience is that the rat pituitary assay is useful as a primary, crude test to select compounds with good GH-releasing properties and reasonably predictive for in vivo potency. We prefer that compounds show potency in the range below 20 nM before we initiate further investigation. Due to limited structural variations, relatively few compounds were rejected on the basis of the rat pituitary assay.

The oral bioavailability in the dog turned out to be a most difficult parameter to rationalize. Despite the similarity within the series, oral bioavailability varied considerably. Within the series presented we were unable to correlate oral bioavailability to any physicochemical or structural parameter. All compounds have approximately the same molecular weight and closely related structure. Scientists from Pfizer[23] have presented a "rule of five" for prediction of oral absorption. According to their experience compounds have a poor chance of being orally bioavailable if the molecular weight exceeds 500 and if there are more than 5 H-bond donors or more than 10 H-bond acceptors. Furthermore, calculated log *P* values should not exceed certain values that are dependent on the method of calculation: maximum of 4.15 for MlogP and maximum of 5.0 for ClogP. In our case, none of the "rules" could differentiate compounds. Poor absorption is predicted if the molecular weight is higher than 500. However, all our molecules are above 500 with the heaviest molecule being **7l** (MW: 656), and this compound has an oral bioavailability of 26%. The calculated log *P* values varied between 2.72 for **7e** and 4.60 for **7l** using ClogP calculations and between 1.64 for **7e** and 3.37 for **7d** using MlogP. This is well within the limits of the "rules of five". Furthermore, all compounds had good solubility in water, leading us to believe that the oral bioavailability in this series is determined by very subtle interactions during passage of the intestine wall as well as quite unpredictable levels of first-pass elimination.

The two compounds that were chosen for further investigation were each representatives for two subclasses within our series: **7g** for those that had simple aliphatic C-termini and **7b** for those with a 2-hydroxypropyl group in the C-terminal. There was a tendency for the former ones to have higher oral bioavailability on the average and for the latter ones to be more active in the in vivo model. The unpredictability of the oral bioavailability and the importance of the C-terminal domain were highlighted by the difference in bioavailability between the *S*-isomer **7b** (25%) and the corresponding *R*-isomer **7o** (0%).

With regard to in vivo potency we observed high potency in those cases where R_2 (Table 2) was either thienyl or 4-fluorophenyl instead of phenyl, leading us to believe that these side chains produced a better fit to the receptor. Also the use of 4-biphenylyl as R_1 instead of 2-naphthyl was an acceptable substitution in contrast to 1-naphthyl or 3-benzothienyl. However, substitutions that had proven beneficial on the potency parameter were not always additive, and their influence on the oral bioavailability was unpredictable. One such example was compound **7r** which contained a substitution pattern that we prior to synthesis predicted optimal for high in vivo potency based on previous SAR. Indeed, this compound was the most potent we observed in this series (C_{max} = 56 ng/mL) but turned out to have no oral bioavailability at all.

From a comparison of **7b**,**g** with the original leads GHRP-6 and ipamorelin (Table 4), it is clear that potency has been lost with regard to the GH-releasing effect. GHRP-6 is approximately 8 times more potent than **7b**. However, the much reduced size of our compounds and the fact that the compounds are orally bioavailable are improvements that we believe will make up for the loss in potency.

In conclusion, we have identified a novel class of GH secretagogues for which oral bioavailability is the rule rather than the exception. This class of compounds has been developed directly from hexapeptide leads via

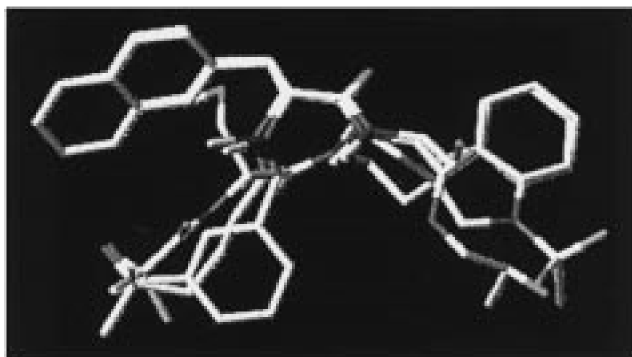


Figure 1. Overlap of **6**, **7b**, and MK-677.

ipamorelin. The synthetic strategy has concentrated on replacement of backbone amide bonds with amide isosters with less hydrogen-bonding capacity. The final outcome was compounds with one amide substituted to a trans double bond and two amides substituted to N-methylated amides. Also a reduction in size has been achieved. Throughout, the optimization process has been stepwise and guided by rational considerations. Nevertheless, the resulting tripeptides bear structural resemblance with MK-677. In both cases a shielded amine and two aromatic fragments are present[24] and found to be important. To substantiate the similarity of our molecules to MK-677, we have perfomed a molecular modeling study on the overlap of **6**, **7b**, and MK-677 based on the assumption that these molecules bind to the same receptor. A pharmacophore model was derived from Catalyst[25] in HipHop mode, and refined overlays of the three molecules were generated by distance geometry calculations (DGEOM95[26]) based on the Catalyst model and were followed by energy optimizations by a SYBYL multifit[27] approach. The result is shown in Figure 1 and indicates that the molecules readily overlap each other despite their differences. The phenyl ring of the benzylserine moiety of MK-677 could be overlapped with the outer ring in the naphthylalanines in **6** and **7b**, while the phenyl rings in the phenylalanines overlapped the phenyl ring in the spiropiperidine. Also the N-terminal amines overlapped readily even with substantial difference in length and rigidity. The relatively low potency of **6** compared to MK-677 may be due to a less optimal or missing pharmacophore in the C-terminal. It is also noteworthy that the hydroxy group in **7b** is capable of reaching the same place in space as the sulfonamide of MK-677, perhaps serving as a mimic of this pharmacophore.

As a result of this investigation we have shown that a direct stepwise method for size reduction of heptapeptides is viable and can result in orally active tripeptides. Although the resulting substances are somewhat less potent than the lead ipamorelin, we believe that the potency is in a range that is satisfactory and allows for further examination and development.

Experimental Section

Amino acids were purchased from Synthetec. HRMS was performed at Odense University using a Varian M311 apparatus. PDMS spectra were run on a Bioion 1100 instrument (Upsala, Sweeden). NMR spectra were obtained at 400 MHz on a Bruker instrument.

HPLC Methods. The RP-HPLC analysis was performed using UV detection at 214, 254, 276, and 301 nm on a Vydac 218TP54 4.6-mm × 250-mm 5-μm C-18 silica column (The Separations Group, Hesperia), which was eluted at 1 mL/min at 42 °C. Two different elution conditions were used. Method A1: The column was equilibrated with 5% acetonitrile in a buffer consisting of 0.1 M ammonium sulfate, which was adjusted to pH 2.5 with 4 M sulfuric acid. After injection the sample was eluted by a gradient of 5−60% acetonitrile in the same buffer during 50 min. Method B1: The column was equilibrated with 5% acetonitrile/0.1% TFA/water and eluted by a gradient of 5% acetonitrile/0.1% TFA/water to 60% acetonitrile/0.1% TFA/water during 50 min.

Synthesis: 3-Hydroxy-1,1-dimethylpropylcarbamic Acid *tert*-Butyl Ester (15). At 0 °C, ethyl chloroformate (1.10 mL, 11.5 mmol) was added dropwise to a solution of 3-(*tert*-butoxycarbonylamino)-3-methylbutanoic acid[6] (**14**) (2.50 g, 11.5 mmol) and triethylamine (1.92 mL, 13.8 mmol) in tetrahydrofuran (10 mL). The solution was stirred for 40 min at 0 °C. The formed precipitate was filtered off and washed with tetrahydrofuran (20 mL). The liquid was immediately cooled to 0 °C. A 2 M solution of lithium borohydride in tetrahydrofuran (14.4 mL, 28.8 mmol) was added dropwise. The solution was stirred at 0 °C for 2 h and then warmed to room temperature over a period of 4 h. The solution was again cooled to 0 °C and methanol (5 mL) was added dropwise; 1 N hydrochloric acid (100 mL) was added. The solution was extracted with ethyl acetate (2 × 100, 3 × 50 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (100 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was chromatographed on silica (110 g) with ethyl acetate/heptane (1:2) as eluent to give 1.84 g (79%) of **15**. ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 6H); 1.44 (s, 9H); 1.88 (t, 2H); 1.94 (br, 1H); 3.75 (q, 2H); 4.98 (br, 1H).

3-(*tert*-Butoxycarbonylamino)-3-methylbutanal (16). DMSO (1.22 mL, 17.2 mmol) was added to a solution of oxalyl chloride (1.1 mL, 12.9 mmol) at −78 °C in dichloromethane (15 mL). The mixture was stirred for 15 min at −78 °C. A solution of **15** (1.75 g, 8.6 mmol) in dichloromethane (10 mL) was added dropwise over a period of 15 min. The solution was stirred at −78 °C for another 15 min. Triethylamine (6.0 mL, 43 mmol) was added. The solution was stirred at −78 °C for 5 min and then warmed to room temperature. The solution was diluted with dichloromethane (100 mL) and extracted with 1 N hydrochloric acid (100 mL). The aqueous phase was extracted with dichloromethane (50 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (100 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by column chromatography on silica (140 g) with ethyl acetate/heptane (1:3) to give 1.10 g (63%) of **16**. ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 6H); 1.45 (s, 9H); 2.85 (d, 2H); 4.73 (br, 1H); 9.80 (t, 1H).

Ethyl (2*E*)-5-(*tert*-Butoxycarbonylamino)-5-methylhex-2-enoate (17). Triethyl phosphonoacetate (1.96 mL, 9.8 mmol) was dissolved in tetrahydrofuran (30 mL). Potassium *tert*-butoxide (1.10 g, 9.8 mmol) was added. The solution was stirred for 40 min at room temperature. A solution of **16** (1.10 g, 5.5 mmol) in tetrahydrofuran (6 mL) was added. The solution was stirred at room temperature for 75 min. It was diluted with ethyl acetate (100 mL) and 1 N hydrochloric acid (100 mL). The phases were separated. The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with saturated sodium hydrogen carbonate solution (60 mL) and dried over magnesium sulfate. The solvent was removed in vacuo. The crude product was purified by column chromatography on silica (90 g) with ethyl acetate/hepatane (1:4) to give 1.27 g (79%) of **17**. ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 6H); 1.30 (t, 3H); 1.46 (s, 9H); 2.62 (d, 2H); 4.27 (q, 2H); 4.42 (br, 1H); 5.88 (d, 1H); 6.94 (td, 1H).

(2*E*)-5-(*tert*-Butoxycarbonylamino)-5-methylhex-2-enoic Acid (18). Compound **17** (1.233 g, 4.54 mmol) was dissolved in dioxane (20 mL). Lithium hydroxide (0.120 g, 5.00 mmol) was added as a solid. Water (10 mL) was added, until a clear solution was obtained. The solution was stirred for 16 h at

room temperature. The solution was diluted with water (70 mL) and extracted with *tert*-butyl methyl ether (2 × 100 mL). The aqueous phase was acidified with 1 N sodium hydrogen sulfate solution (pH = 1) and was extracted with *tert*-butylmethyl ether (3 × 70 mL). The organic phases were combined and dried over magnesium sulfate. The solvent was removed in vacuo to give 1.05 g (95%) of **18**. The crude product was used for further syntheses. An analytical sample was obtained by recrystallization from heptane. ^{1}H NMR (DMSO-d_6): δ 1.15 (s, 6H); 1.35 (s, 9H); 2.53 (d, 2H); 5.75 (d, 1H); 6.57 (br, 1H); 6.75 (td, 1H); 12.15 (s, 1H). Anal. ($C_{12}H_{21}NO_4$) C, H, N.

Procedure for Compound 7a (Compounds 7b–s were prepared in the same way): *N*-Methyl-*N*-((*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamic Acid *tert*-Butyl Ester (9a). *N*-(*tert*-Butoxycarbonyl)-*N*-methyl-D-phenylalanine (**8**) (1.22 g, 4.4 mmol), 1-hydroxybenzotriazole hydrate (0.59 g, 4.4 mmol), and EDAC (1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimid hydrochloride) (0.88 g, 4.6 mmol) were dissolved in methylene chloride (25 mL) and stirred for 15 min. Methylamine (0.51 g of a 40% solution in methanol, 6.6 mmol) was added, and the mixture was stirred overnight. Methylene chloride (80 mL) and water (100 mL) were added, and the phases were separated. The organic phase was washed with aqueous sodium hydrogen carbonate (20 mL, saturated), aqueous sodium hydrogen sulfate (50 mL, 10%), and water (50 mL). The organic phase was dried (magnesium sulfate), and the solvent was removed in vacuo to afford 1.27 g (99%) of **9a** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.25, 1.35 (two s, 9H); 2.73–2.94 (m, 7H); 3.30–3.50 (m, 1H); 4.68, 4.90 (two m, 1H); 5.90, 6.12 (two s (br); 1H); 7.12–7.25 (m, 5H) (mixture of rotamers).

(*R*)-*N*-Methyl-2-(methylamino)-3-phenylpropionamide (10a). Compound **9a** (1.39 g, 7.23 mmol) was dissolved in a mixture of trifluoroacetic acid (10 mL) and methylene chloride (10 mL) and stirred for 1 h. The volatiles were removed in vacuo, and the residue was stirred with aqueous sodium hydrogen carbonate (100 mL, saturated, pH adjusted to 9.5 with sodium carbonate) and extracted with methylene chloride (3 × 100 mL). The organic phase was dried (magnesium sulfate), and the solvent was removed in vacuo to afford 810 mg (89%) of **10a** as a white solid. ^{1}H NMR ($CDCl_3$): δ 2.1 (s (br), 3H); 2.32 (s, 3H); 2.77 (dd, 1H); 2.81 (s, 3H); 2.82 (s, 3H); 3.21 (dd, 1H); 3.32 (dd, 1H); 7.12 (s (br), 1H); 7.20–7.34 (m, 5H).

***N*-Methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)carbamic Acid *tert*-Butyl Ester (11a).** (*R*)-(*tert*-Butoxycarbonyl)-*N*-methylamino-3-(2-naphthyl)propionic acid (548 mg, 1.66 mmol) was dissolved in methylene chloride (5 mL), and 1-hydroxy-7-azabenzotriazole (HOAt) (227 mg, 1.66 mmol, dissolved in DMF (2 mL)) was added. 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (351 mg, 1.83 mmol) was added, and the solution was stirred for 15 min. Compound **10a** (320 mg, 1.66 mmol) (dissolved in methylene chloride (4 mL)) and diisopropylethylamine (0.28 mL, 1.66 mmol) were added, and the mixture was stirred overnight. Methylene chloride (50 mL) was added, and the organic phase was washed with water (100 mL), sodium hydrogen sulfate (50 mL, 5%), and sodium hydrogen carbonate (50 mL, saturated). The organic phase was dried (magnesium sulfate), and the solvent was removed in vacuo. The residue was chromatographed (silica, 2 × 45 cm) using ethyl acetate/methylene chloride (1:1) as eluent to afford 604 mg (72%) of **11a**. ^{1}H NMR ($CDCl_3$): δ 1.05, 1.31, 1.56 (three s, 9H); 2.28–3.37 (m, 13H); 5.04, 5.17, 5.29, 5.48 (four dd, 2H); 7.05–7.79 (m, 12H); (mixture of rotamers).

(2*R*)-*N*-Methyl-2-(methylamino)-*N*-((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)-3-(2-naphthyl)propionamide (12a). Compound **11a** (600 mg, 1.19 mmol) was stirred in trifluoroacetic acid/methylene chloride (1:1, 5 mL) for 10 min, and the volatiles were removed in vacuo. The residue was dissolved in methanol (2 mL) and mixed with sodium hydrogen carbonate (10 mL) and ethyl acetate (15 mL). The organic phase was separated and dried (magnesium sulfate) to afford 420 mg (87%) of **12a**. ^{1}H NMR ($CDCl_3$): δ 1.69 (s, 3H); 2.08 (d, 3H); 2.54 (s, 3H); 2.76 (dd, 1H); 2.92 (dd, 1H), 3.12 (dd, 1H), 3.31 (dd, 1H); 3.72 (dd, 1H), 4.95 (q (br), 1H); 5.50 (dd, 1H) (selected values, mixture of rotamers).

(3*E*)-1,1-Dimethyl-4-(*N*-methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)carbamoyl)but-3-enylcarbamic Acid *tert*-Butyl Ester (13a). Compound **18** (200 mg, 0.82 mmol), 1-hydroxy-7-azabenzotriazole (112 mg, 0.82 mmol), and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (173 mg, 0.90 mmol) were dissolved in a mixture of methylene chloride (10 mL) and DMF (1 mL) and stirred for 15 min. Compound **12a** (332 mg, 0.82 mmol) dissolved in methylene chloride (5 mL) and diisopropylethylamine (0.14 mL) were added, and the mixture was stirred overnight under a nitrogen atmosphere. The mixture was diluted with methylene chloride (50 mL) and washed with water (50 mL), sodium hydrogen carbonate (30 mL, saturated), and sodium hydrogen sulfate (30 mL, 5%). The phases were separated, and the organic phase was dried with magnesium sulfate and evaporated in vacuo. The residue was chromatographed (silica, 2 × 40 cm) to afford 450 mg (88%) of **13a**. ^{1}H NMR ($CDCl_3$): (selected values) δ 1.20, 1.22, 1.24, 1.30, 1.41, 1.55 (six s, 15H); 4.30, 4.40 (two s (br), 1H); 5.08, 5.18, 5.32, 5.60, 5.87 (five dd, 2H); 6.05 (dd, 1H); 6.75 (m, 1H) (mixture of rotamers).

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-Methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (7a). Compound **13a** (403 mg, 0.63 mmol) was stirred in a mixture of trifluoroacetic acid (4 mL) and methylene chloride (4 mL) for 7 min. The mixture was poured onto a stirred mixture of ice (10 g), sodium hydrogen carbonate (6 g), and water (10 mL). Methylene chloride (50 mL) was added, and the organic phase was dried and evaporated. The crude product was chromatograped on silica (200 g) using a mixture of methylene chloride, absolute ethanol, and ammonia (25% in water) (80/18/2) to afford 140 mg (41%) of **7a** as a white solidified foam. ^{1}H NMR ($CDCl_3$): δ 1.05, 1.10, 1.15, 1.16 (four s, 6H); 2.07 (s (br), 3H); 5.12, 5.32, 5.40, 5.60, 5.91 (five dd, 2H); 6.05, 6.14 (two d, 1H); 6.80 (m, 1H) (mixture of rotamers, selected peaks). ^{1}H NMR (DMSO at 160 °C): δ 1.08 (s, 6H); 2.17 (d, 2H); 2.59 (d, 3H); 2.76 (s, 3H); 2.87 (s, 3H); 2.92 (dd, 1H); 3.02 (dd, 1H); 3.21 (dd, 1H); 3.25 (dd, 1H); 5.11 (dd, 1H); 5.59 (dd, 1H); 6.10 (d, 1H); (dt, 1H); 7.13 (m, 1H), 7.18 (m, 2H); 7.19 (m, 2H); 7.19 (m, 2H); 7.31 (ab-pattern, 1H); 7.42 (m, 1H); 7.44 (m, 1H); (s, 1H); 7.74 (ab-pattern, 1H); 7.77 (m, 1H); 7.81 (m, 1H) (full spectrum, no rotamers). HPLC: t_R = 29.02 min (99% pure) (method A1). HPLC: t_R = 33.45 min (method B1). ESMS: m/z = 529 (100%) $(M + H)^+$. EIMS: m/z = 528 (5%, M^+), 470 (9), 279 (30), 184 (79), 58 (100). HRMS: calculated for $C_{32}H_{40}N_4O_3$, m/z = 528.3100; found, m/z = 528.3070.

The compounds listed below were all prepared by the general procedure above. The purity (determined by HPLC) was generally in the 95–100% range. In general compounds were colorless and noncrystalline.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-((1*R*)-1-(((1*R*)-1-((2*S*)-2-Hydroxypropylcarbamoyl)-2-phenylethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)-*N*-methylamide (7b). Prepared according to the general procedure without protection of the hydroxyl group. ^{1}H NMR ($CDCl_3$) (selected peaks, mixture of rotamers): δ 3.90 (m, 1H); 5.55 (dd, 1H); 5.58 (d, 1H). HPLC: t_R = 29.03 min (method A1). HPLC: t_R = 32.60 min (method B1). PDMS: m/z = 573.5 (100%) $(M + H)^+$. EIMS: m/z = 572 (3%, M^+), 514 (8), 279 (38), 184 (95), 58 (100). HRMS: calculated for $C_{34}H_{44}N_4O_5$, m/z = 572.3363; found, m/z = 572.3382.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-methyl-*N*-((1*R*)-1-(methyl((1*R*)-2-phenyl-1-(((tetrahydrofuran-2-yl)-methyl)carbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethylamide (7c). ^{1}H NMR ($CDCl_3$) (selected peaks, mixture of rotamers): δ 1.06, 1.09 (two d, 6H); 2.78 (d, 2H); 5.25–5.62 (m, 2H); 6.05 (m, 1H). HPLC: t_R = 33.65 min

(method A1). HPLC: t_R = 36.02 min (method B1). EIMS: *m/z* = 598 (2%, M^+), 540 (5), 439 (11), 184 (100), 58 (78). HRMS: calculated for $C_{36}H_{46}N_4O_4$, *m/z* = 598.3519; found, *m/z* = 598.3552.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-((1*R*)-1-(((1*R*)-1-((Cyclopropylmethyl)carbamoyl)-2-phenylethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)-*N*-methylamide (7d). ^1H NMR ($CDCl_3$) (selected peaks, mixture of rotamers): δ 0.08−0.20 (m, 2H); 1.05; 1.15 (two s, 6H); 6.02, 6.05 (two d, 1H). HPLC: t_R = 35.7 min (method A1). HPLC: t_R = 37.28 min (method B1). EIMS: *m/z* = 568 (8%, M^+), 510 (10), 439 (12), 279 (51), 184 (90), 58 (100). HRMS: calculated for $C_{35}H_{44}N_4O_3$, *m/z* = 568.3413; found, *m/z* = 568.3442.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-((1*R*)-2-benzyloxy)-1-(*N*-methyl-*N*-((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)ethyl)-*N*-methylamide (7e). ^1H NMR ($CDCl_3$) (selected data for major rotamer): δ 1.27 (s, 3H); 1.28 (s, 3H); 2.84 (d, 3H); 2.95 (s, 3H); 3.08 (s, 3H); 4.32 (d, 1H); 4.40 (d, 1H); 5.12 (dd, 1H); 6.34 (d, *J* = 18 Hz, 1H). ESMS: *m/z* 509.7 (M + H)$^+$. HPLC: t_R = 23.45 min (method A1). HPLC: t_R = 31.71 min (method B1). EIMS: *m/z* = 508 (1%, M^+), 260 (64), 259 (55), 164 (43), 58 (100). HRMS: calculated for $C_{29}H_{40}N_4O_4$, *m/z* = 508.3050; found, *m/z* = 508.3055.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-Methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(1-naphthyl)ethyl)amide (7f). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.22 (s, 3H); 1.23 (s, 3H); 2.82 (d, 3H); 2.92 (s, 3H); 3.08 (s, 3H); 5.22 (dd, 1H); 5.92 (dd, 1H); 6.12 (d, 1H). ESMS: *m/z* 529.2 (M + H)$^+$. HPLC: t_R = 30.90 min (method A1). HRMS: calculated for $C_{32}H_{40}N_4O_3$, *m/z* = 528.3100; found, *m/z* = 528.3101.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid ((1*R*)-1-(((1*R*)-2-(4-Fluorophenyl)-1-(methylcarbamoyl)ethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)methylamide (7g). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.15 (s, 6H); 2.14 (d, 3H); 2.73 (s, 3H); 3.09 (s, 3H); 5.23 (dd, 1H); 5.90 (dd, 1H); 6.12 (dd, 1H). PDMS: *m/z* 547.4 (M + H)$^+$. HPLC: t_R = 32.05 min (method A1). HPLC: t_R = 34.18 min (method B1). EIMS: *m/z* = 546 (10%, M^+), 488 (19), 279 (48), 184 (81), 58 (100). HRMS: calculated for $C_{32}H_{39}N_4FO_3$, *m/z* = 546.3006; found, *m/z* = 546.3026.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid ((1*R*)-2-(Benzo[*b*]thiophene-3-yl)-1-(methyl((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)ethyl)methylamide (7h). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.25 (s, 6H); 2.47 (s, 3H); 2.75 (d, 3H); 2.96 (s, 3H); 4.98 (dd, 1H); 5.89 (dd, 1H); 6.10 (d, 1H). PDMS: *m/z* 535.7 (M + H)$^+$. HPLC: t_R = 30.87 min (method A1). HPLC: t_R = 33.03 min (method B1). EIMS: *m/z* = 534 (11%, M^+), 476 (17), 320 (32), 231 (40), 190 (36), 187 (42), 58 (100). HRMS: calculated for $C_{30}H_{38}N_4O_3S$, *m/z* = 534.2665; found, *m/z* = 534.2619.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid Methyl((1*R*)-1-(methyl((1*R*)-2-phenyl-1-(((tetrahydrofuran-2-yl)methyl)carbamoyl)ethyl)carbamoyl)-2-(1-naphthyl)ethyl)amide (7i). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.25 (s, 6H); 2.25 (d, 2H); 2.08 (s, 3H); 2.89 (d, 3H); 3.18 (s, 3H); 5.90 (dd, 1H); 6.65 (d, 1H). PDMS: *m/z* 599.8 (M + H)$^+$. HPLC: t_R = 33.50 (method A1). HPLC: t_R = 35.83 min (method B1). HRMS: calculated for $C_{36}H_{46}N_4O_4$, *m/z* = 598.3519; found, *m/z* = 598.3512.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-Methyl-*N*-((1*R*)-1-(*N*-methyl-*N*-((1*R*)-1-(methylcarbamoyl)-2-(thiophene-2-yl)ethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (7j). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 2.31 (d, 3H); 2.63 (s, 3H); 2.91 (s, 3H); 5.18 (dd, 1H); 5.55 (dd, 1H); 6.19 (d, 1H). HPLC: t_R = 30.3 min (method A1). HPLC: t_R = 32.93 min (method B1). PDMS: *m/z* 534.8 (M + H)$^+$. HRMS: calculated for $C_{30}H_{38}N_4O_3S$, *m/z* = 534.2665; found, *m/z* = 534.2619.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid ((1*R*)-2-(Biphenyl-4-yl)-1-(methyl((1*R*)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)ethyl)methylamide (7k). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.18 (s, 6H); 2.75 (d, 3H); 2.78 (s, 3H); 2.97 (s, 3H); 5.45 (dd, 1H); 5.75 (dd, 1H); 6.08 (d, *J* = 17 Hz, 1H). ESMS: *m/z* 555.8 (M + H)$^+$. HPLC: t_R = 34.45 min (method A1). HPLC: t_R = 36.55 min (method B1). HRMS: calculated for $C_{34}H_{42}N_4O_3$, *m/z* = 554.3257; found, *m/z* = 554.3275.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-((1*R*)-1-(*N*-((1*R*)-2-(4-Iodophenyl)-1-(methylcarbamoyl)ethyl)-*N*-methylcarbamoyl)-2-(2-naphthyl)ethyl)-*N*-methylamide (7l). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.15 (s, 6H); 2.09 (d, 3H); 2.69 (s, 3H); 2.70 (s, 3H); 5.24 (dd, 1H); 5.90 (dd, 1H); 6.18 (d, 1H). HPLC: t_R = 35.25 min (method A1). HPLC: t_R = 37.55 min (method B1). PDMS: *m/z* = 655.7 (M + H)$^+$. HRMS: calculated for $C_{32}H_{39}N_4O_3I$, *m/z* = 654.2067; found, *m/z* = 654.2101.

5-(Methylamino)hex-2-enoic Acid ((1*R*)-1-(((1*R*)-2-(3,4-Difluorophenyl)-1-(methylcarbamoyl)ethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)methylamide (7m). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.22 (s, 6H); 2.10 (d, 3H); 2.71 (s, 3H); 2.85 (s, 3H); 5.22 (dd, 1H); 5.86 (dd, 1H); 6.17 (d, 1H). HPLC: t_R = 33.18 min (method A1). HPLC: t_R = 35.47 min (method B1). PDMS: *m/z* 566.0 (M + H)$^+$.

5-(Methylamino)hex-2-enoic Acid ((1*R*)-1-(((1*R*)-2-Phenyl-1-(ethylcarbamoyl)ethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)methylamide (7n). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 0.61 (t, 3H); 1.05 (s, 6H); 1.98 (s, 3H); 2.24 (s, 3H); 2.97 (d, 3H); 5.57 (dd, 1H); 5.85 (dd, 1H); 6.07 (d, 1H). HPLC: t_R = 33.0 min (method A1). HPLC: t_R = 35.27 min (method B1). PDMS: *m/z* 544.0 (M + H)$^+$. HRMS: calculated for $C_{33}H_{42}N_4O_3$, *m/z* = 542.3257; found, *m/z* = 542.3296.

5-Amino-5-methylhex-2-enoic Acid ((1*R*)-1-(((1*R*)-1-((2*S*)-2-Hydroxypropylcarbamoyl)-2-(2-thienyl)ethyl)-methylcarbamoyl)-2-(2-naphthyl)ethyl)methylamide (7o). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.10 (d, 3H); 1.14 (s, 3H); 1.15 (s, 3H); 2.18 (d, 2H); 2.95 (s, 3H); 3.05 (s, 3H); 5.28 (dd, 1H); 5.72 (dd, 1H); 6.07 (d, 1H). HPLC: t_R = 30.4 min (method A1). HPLC: t_R = 32.68 min (method B1). PDMS: *m/z* 580.0 (M + H)$^+$. HRMS: calculated for $C_{34}H_{44}N_4O_5$, *m/z* = 572.3363; found, *m/z* = 572.3387.

5-Amino-5-methylhex-2-enoic Acid ((1*R*)-1-(((1*R*)-1-((2*R*)-2-Hydroxypropylcarbamoyl)-2-phenylethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)methylamide (7p). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.09 (d, 3H); 1.19 (s, 6H); 2.97 (s, 3H); 2.99 (s, 3H); 5.15 (dd, 1H); 5.53 (dd, 1H); 6.12 (d, 1H). HPLC: r_t= 31.8 min (method A1). HPLC: t_R = 33.28 min (method B1). PDMS: *m/z* 573.7 (M + H)$^+$.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-((1*R*)-2-(Biphenyl-4-yl)-1-(*N*-((1*R*)-2-(4-fluorophenyl)-1-((2*R*)-2-hydroxypropylcarbamoyl)ethyl)-*N*-methylcarbamoyl)-ethyl)-*N*-methylamide (7q). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.08 (d, 3H); 1.30 (s, 3H); 1.37 (s, 3H); 2.91 (s, 3H); 3.04 (s, 3H); 5.15 (dd, 1H); 5.57 (dd, 1H); 6.38 (d, 1H); 6.70 (m, 1H). HPLC: t_R = 35.08 min (method A1). HPLC: t_R = 37.18 min (method B1).

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-((1*R*)-2-(Biphenyl-4-yl)-1-(*N*-((1*R*)-1-((2*S*)-2-hydroxypropylcarbamoyl)-2-(2-thienyl)ethyl)-*N*-methylcarbamoyl)ethyl)-*N*-methylamide (7r). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.07 (d, 3H); 1.36 (s, 3H); 1.39 (s, 3H); 2.81 (s, 3H); 3.05 (s, 3H); 5.21 (dd, 1H); 5.55 (dd, 1H); 6.36 (d, 1H); 6.69 (m, 1H). HPLC: t_R = 33.92 min (method A1). HPLC: t_R = 35.93 min (method B1).

5-Amino-5-methylhex-2-enoic Acid ((1*R*)-1-(((1*R*)-1-((2*S*)-2-Hydroxypropylcarbamoyl)-2-(3,4-difluorophenyl)ethyl)methylcarbamoyl)-2-(2-naphthyl)ethyl)-methylamide (7s). ^1H NMR ($CDCl_3$) (selected peaks for major rotamer): δ 1.01 (t, 3H); 1.10 (s, 6H); 2.74 (s, 3H); 3.04 (s, 3H); 5.08 (dd, 1H); 5.56 (dd, 1H); 6.07 (d, 1H). HPLC: t_R = 32.9 min (method A1). PDMS: *m/z* 610.3 (M + H)$^+$.

Biological Methods: Pharmacokinetics, Oral Bioavailability. Oral bioavailability studies were conducted in male and female beagle dogs. The dogs were fasted overnight

prior to dosing. Diet was withheld for at least 3 h postdosing. A 1-week washout period separated peroral (po) and intravenous (iv) dosing. The compounds were administered in a vehicle of citrate/phosphate buffer, pH 5.0. For po administration the dogs received a dose of 2.5 mg/kg of body weight via gavage (three doses for **7b**,**g**). For iv administration the dogs received a dose of 0.5 mg/kg of body weight as a bolus in a hind leg vein. EDTA blood samples were drawn from a front leg vein at intervals up to 6 h after dosing. Blood samples were placed on an ice−water bath immediately after sampling. Plasma was separated by centrifugation and stored frozen pending analysis. An HPLC assay with UV detection and solid-phase extraction was developed for each compound. Analytical C8 columns and disposable C3 extraction columns were used. The oral bioavailability was calculated as the total area under the plasma concentration versus time curve following po administration divided by the area following iv administration, appropriately corrected for dose (eq 1):

$$f = \frac{\mathrm{AUC}_{\mathrm{po}} \cdot \mathrm{dose}_{\mathrm{iv}}}{\mathrm{AUC}_{\mathrm{iv}} \cdot \mathrm{dose}_{\mathrm{po}}} \quad (1)$$

In Vivo Characterization in Conscious Swine. For the initial screening, four female 30−40-kg Danish slaughter swine of the breed Landrace Yorkshire cross were used for each GH secretagogue. The swine were housed at least 1 week prior to experiments. Prior to experiments it was tested that the swine used had similar basal GH levels. Indwelling jugular catheters were inserted and fixed under general halothane anesthesia at day 0. The test compounds were administered as 50 nmol/kg iv bolus injections with at least 48-h intervals between compounds. Each group of swine was used to test a maximum of five different compounds. The test compounds were dissolved in phosphate/citrate buffer diluted in saline containing 0.5% porcine serum albumin. Blood samples were drawn from the jugularis catheter at frequent intervals from 1 h prior to stimulation until 3 h poststimulation. For a more detailed characterization of **7b**,**g**, the same setup was used except six animals were used for each compound. The two compounds were administered as single iv injections in six increasing doses with 72-h intervals between doses (10, 30, 100, 300, 1000, and 10000 nmol/kg). Plasma was analyzed for porcine GH (pGH) by ELISA. The basal GH level, for the individual swine, was calculated as the average of the three GH values obtained prior to stimulation. Peak hormone levels ($C_{\max}$) adjusted for basal level, obtained following administration of test compounds, were used to characterize the hormone response of individual swine. In single-dose experiments $C_{\max}$ was used directly. In multidose experiments dose−response curves were constructed using $C_{\max}$ GH plasma concentrations. Fitting to the Hill equation or hyperbolic Michaelis−Menten equation was performed by nonlinear regression using the Prism software (GraphPad). Using the efficacy ($E_{\max}$ values) of the individual compounds, the potency (ED_{50} values) was calculated as the dose inducing half-maximal stimulation, i.e., the increase in plasma GH from basal. The results were tested for normal probability distribution by Shapiro−Wilk test. All statistical comparisons were performed using Prism and SAS software. When appropriate, the data are given as means ± SEM.

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