Journal of
Medicinal
Chemistry



Volume 41, Number 14 July 2, 1998

## Communications to the Editor

# 1-[2(*R*)-(2-Amino-2-methylpropionylamino)-3-(1*H*-indol-3-yl)propionyl]-3-benzylpiperidine-3(*S*)-carboxylic Acid Ethyl Ester (L-163,540): A Potent, Orally Bioavailable, and Short-Duration Growth Hormone Secretagogue

Lihu Yang,*,† Greg Morriello,† Arthur A. Patchett,† Kwan Leung,‡ Tom Jacks,§ Kang Cheng,§ Klaus D. Schleim,§ William Feeney,§ Wanda W.-S. Chan,§ Shuet-Hing L. Chiu,‡ and Roy G. Smith§

*Merck Research Laboratories, Rahway, New Jersey 07065*

*Received January 13, 1998*

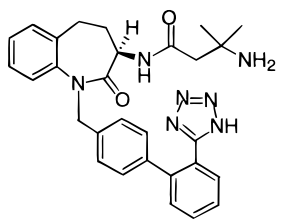
Growth hormone (GH), which is secreted by the pituitary gland in a pulsatile pattern, has important functions such as to promote growth and enhance anabolic processes.[1] The clinical use of recombinant human GH (rhGH) has demonstrated its therapeutic benefit of promoting height growth in GH-deficient children[2] and reversing some of the effects of aging in the elderly.[3] The high cost and inconvenience associated with the subcutaneous administration of rhGH as well as the accompanying side effects hinder its widespread applications. Side effects such as carpal tunnel syndrome and fluid retention may be, in part, the result of bolus administration. The recent discovery of a group of small oligopeptides, such as GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-$NH_2$),[4] which, when administered subcutaneously or by infusion, promote the pituitary to release GH in the more physiologically relevant pulsatile pattern, has led to intense efforts to identify their small molecule mimetics.[5] Two classes of peptidomimetic growth hormone secretagogues (GHS), benzolactam biphenyltetrazoles (**1**)[6] and 4-spiropiperidines (**2**),[7] have been reported. The growth hormone-releasing peptides (GHRPs) and their mimetics were found to act through a common second messenger pathway distinct from that transduced by endogenous growth hormone releasing hormone (GHRH). They act through a recently cloned G-protein-coupled receptor (GHSr).[8] It has been suggested that the GHS are agonists of an unidentified natural hormone, which together with somatostatin and GHRH control the pulsatile release of GH.

A 4-spiropiperidine class of secretagogues has been extensively investigated, which ultimately resulted in the clinical candidate **2** (MK-0677, L-163,191).[9] **2** has a long duration of action and has been administered orally once a day in clinical studies. Chronic dosing of **2** results in some down regulation of GH release with maintenance of elevated IGF-1 (insulin-like growth factor-1) levels.[10] On the other hand, short-acting GH secretagogues such as **1** (L-692,429), or **2** given every other day, cause little GH down regulation and no sustained IGF-1 elevation. It has been suggested that elevated IGF-1 levels are needed for good clinical efficacy. However, results of a recent clinical study indicated that the short-acting secretagogue GHRP-2 (D-Ala-D-$\beta$Nal-Ala-Trp-D-Phe-Lys-$NH_2$) administered twice or three times daily intranasally to GH-deficient and idiopathic short stature children increased their growth velocity in the absence of significantly elevated IGF-1.[11] To permit finer control over IGF-1 and GH levels, we desired to synthesize a secretagogue with a shorter duration of action than **2**. Previously, the Merck group has reported **3** (L-163,833) whose short duration of action resulted from the facile ester hydrolysis and the fast clearance rate of the resulting acid. Its oral bioavailability (accounting both **3** and its major metabolite acid) was modest in dogs (11.8%) and rats (4.5%).[12] Herein we report the identification of 1-[2(*R*)-(2-amino-2-methylpropionylamino)-3-(1*H*-indol-3-yl)propionyl]-3-benzylpiperidine-3(*S*)-carboxylic acid ethyl ester (**4**, L-163,540), as a short-acting GHS with excellent potency and oral bioavailability. At 0.5 mg/kg orally once daily in beagles for 7 days, **4** caused a mean GH release which was similar on days 1, 4, and 7 accompanied by nonsustained IGF-1 elevations after each administration.

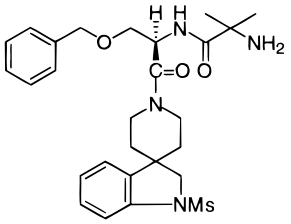
The preparation of **4** has been carried out in nine steps in 17% overall yield from the commercially available (±)-ethyl nipecotate, *N*-Boc-D-tryptophan, and *N*-Boc-aminoisobutyric acid as described in Scheme 1.

† Medicinal Chemistry.
‡ Drug Metabolism.
§ Biochemistry & Physiology.

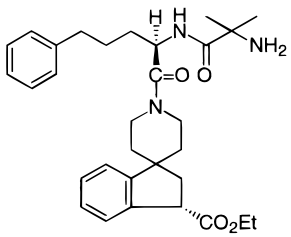
S0022-2623(98)00019-3 CCC: $15.00 
Published on Web 06/16/1998



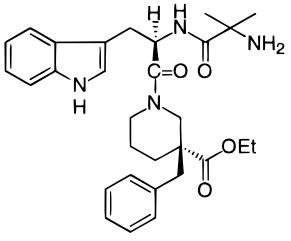
**1 (L-692,429)**



**2 (L-163,191, MK-0677)**



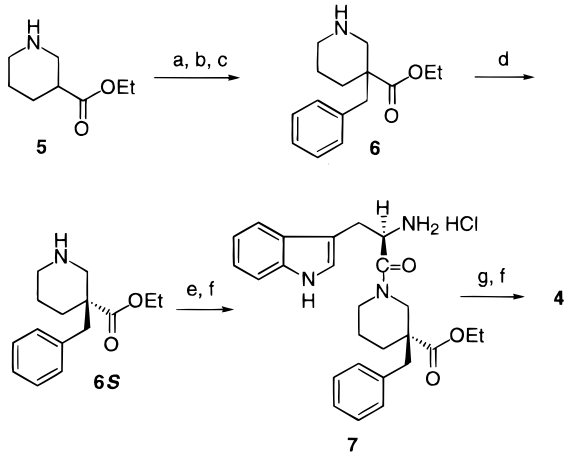
**3 (L-163,833)**



**4 (L-163,540)**

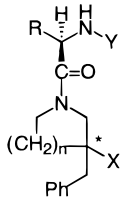
Treatment of (±)-ethyl nipecotate (**5**) with di-*tert*-butyl dicarbonate ($Boc_2O$) gave the *N*-Boc-protected material. Alkylation with benzyl bromide and lithium bis(trimethylsilyl)amide (LiHMDS) followed by removing the Boc protecting group afforded the racemic ethyl 3-benzylnipecotate (**6**). Resolution was accomplished by crystallization with L-tartaric acid in 4:1 acetone/water. The absolute stereochemistry was assigned to be *S*, based on the X-ray crystallographic analysis of the crystalline salt as well as the final product **4**. The incorporation of D-Trp and 2-aminoisobutyric acid (Aib) was carried out by successive 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole (HOBT) coupling of the Boc amino acid in dichloromethane (DCM) followed by HCl removal of the Boc protecting group. **4** was isolated as a white crystalline hydrochloride salt monohydrate. It has a melting point of 158−160 °C and solubility of 11.6 mg/mL in distilled water. The *R*-isomer **4a** was prepared similarly starting with the ethyl (*R*)-3-benzylnipecotate. The acid **4b** was prepared by direct saponification of **4**. The amide **4c** was synthesized from ethyl (*S*)-3-benzylnipecotamide, which in turn was prepared from **6***S* through Boc protection, saponification, ethyl amide formation, and Boc deprotection. Analogues **4d**−**h** were prepared similarly using the easily accessible starting Boc amino acids.

**Scheme 1.** Synthesis of **4**[a]



[a] Reagents: (a) $Boc_2O$/DCM; (b) LiHMDS, BnBr, THF, −78−0 °C; (c) HCl/EtOAc, $NaOH/H_2O$−DCM; (d) L-tartaric acid, acetone/water (4:1), $NaOH/H_2O$−DCM; (e) Boc-D-Trp-OH, HOBT, EDC, DCM; (f) HCl/EtOAc; (g) Boc-Aib-OH, HOBT, EDC, DCM.

**Table 1.** GHS Potency in the Pituitary Cell Assay[a]



| entry | *n* | R | X | * isomer | Y | $EC_{50}$ (nM) |
|---|---|---|---|---|---|---|
| **4** | 2 | 3-indolylmethyl | $CO_2Et$ | *S* | Aib | 1.6 ± 0.5 (*n* = 11) |
| **4a** | 2 | 3-indolylmethyl | $CO_2Et$ | *R* | Aib | 22 |
| **4b** | 2 | 3-indolylmethyl | $CO_2H$ | *S* | Aib | 190 |
| **4c** | 2 | 3-indolylmethyl | CONHEt | *S* | Aib | 0.8 ± 0.4 (*n* = 3) |
| **4d** | 2 | 3-phenylpropyl | $CO_2Et$ | *S* | Aib | 8 |
| **4e** | 2 | benzyloxymethyl | $CO_2Et$ | *S* | Aib | 20 |
| **4f** | 2 | 3-indolylmethyl | $CO_2Et$ | *S* | D-Ala | 6 |
| **4g** | 1 | 3-indolylmethyl | $CO_2Et$ | *RS* | Aib | >50 |
| **4h** | 3 | 3-indolylmethyl | $CO_2Et$ | *RS* | Aib | >200 |

[a] $EC_{50}$ values are for half-maximal release of GH in the rat pituitary cell assay, normalized against standard **1** (60 nM).

The compounds were evaluated in vitro for the stimulation of GH release from rat pituitary cells according to the published protocol,[13] and the results are summarized in Table 1. **4** is from a structurally unique class of growth hormone secretagogues with a nonspiro, 3,3-disubstituted piperidine as the "privileged structure".[7,14] Introduction of the ester and benzyl groups in the piperidine 3-position produces a new center of asymmetry, and as expected, the resultant diastereomers differ in potency with the *R*-epimer **4a** being less potent ($EC_{50}$ = 22 nM). Hydrolysis of the ester yields a poorly active carboxylic acid (**4b**; $EC_{50}$ = 190 nM). Conversion of the ethyl ester of **4** to the ethyl carboxamide (**4c**; $EC_{50}$ = 0.8 nM) results in a slight increase of GH-releasing activity. In common with the other classes of secretagogues, Aib was the best amino acid side chain.[7,15] However, the structure−activity relationship (SAR) around **4** differs from that of **2**. Thus, the phenylpropyl analogue **4d** ($EC_{50}$ = 8 nM) is distinctly less potent in the in vitro GH release assay than **4** ($EC_{50}$ = 1.6 nM), and the benzyloxymethyl analogue has even poorer activity (**4e**; $EC_{50}$ = 20 nM). All of these side chains provide equivalent or slightly better GH release activities in the **2** series. There are also differences with respect to SAR at the amino terminus. For example, whereas the D-Ala analogue of **2** is only weakly active ($EC_{50}$ = 80 nM), this substitution in **4** retains an excellent level of GH release activity (**4f**; $EC_{50}$ = 6 nM). Finally, replacing the piperidine with pyrrolidine (**4g**) or hexahydro-1*H*-azepine (**4h**) caused a dramatic drop in the in vitro potency.

In a series of in vitro studies, **4** was shown to have the same mechanism of action as the GHRPs and small molecule mimetics **1** and **2**. It blocks the binding of [$^{35}$S]-**2** to rat pituitary membranes in a dose-dependent manner with an $IC_{50}$ value similar to that of **2**. The in vitro specificity of **4** was evaluated in over 100 receptor

and enzyme assays in which no significant binding was observed at 10 $\mu$M. These included opiate, neurokinin, adrenergic, somatostatin, cholecystokinin, bradykinin, vasopressin, and benzodiazepine assays.

**4** was active when administered iv to rats (0.025 mg/kg) and beagles (0.005 mg/kg). When administered orally to beagles at doses of 0.25, 0.50, and 1.0 mg/kg, **4** increased GH levels with mean peaks of 10, 38, and 99 ng/mL versus 4 ng/mL for placebo. Despite its higher GH peaks, **4** elicited smaller GH areas under the curve compared to **2** reflecting its short duration of action. It was active for only 2 h at the 0.5 mg/kg oral dose, whereas **2** at the same dose maintained elevations in GH up to 8 h. When **4** was administered daily at 0.50 mg/kg for 7 days, the GH responses on days 1, 4, and 7 were similar. IGF-1 increases of 21−27% occurred at 8 h after dosing on each of the sampling days, but the pretreatment levels on days 4 and 7 were not increased over day 1 baseline levels. Modestly elevated cortisol responses (2-fold) were similar on these days. These results are in contrast to results with **2** at the same dosage where both the GH and cortisol responses were decreased on days 4 and 7 relative to the first day of treatment.

Pharmacokinetics of **4** were studied in male Sprague−Dawley rats and male beagle dogs. After an oral dose of 26.3 mg/kg to rats, $C_{max}$ was 182.4 ng/mL that occurred between 15 and 60 min. Bioavailability was 12.1%. At an iv dose of 5.5 mg/kg, $Cl_p$, $V_{dss}$, and $t_{1/2}$ in rats were 206 mL/min/kg, 2.7 L/kg, and 0.4 h. Following oral administration of 3.8 mg/kg **4** to dogs, the average $C_{max}$ was 560 ng/mL with a $T_{max}$ that ranged between 15 and 30 min. Bioavailability was 29%. After iv dosing of 0.46 mg/kg, $Cl_p$, $V_{dss}$, and $t_{1/2}$ of **4** were 23.0 mL/min/kg, 1.3 L/kg, and 1.2 h.

In summary, we report here a potent, short-duration GH secretagogue that also has excellent oral bioavailability. The discovery provided a very useful tool for long-term efficacy studies of a short-acting secretagogue.

**Acknowledgment.** We would like to thank Amy Bernick for mass spectrometry support, Dr. Richard Ball and Ms. Nancy Tsou for X-ray crystallography, and Dr. Gerard Kieczykowski of the Basic Chemistry Preparation Laboratory for large-scale synthesis of key intermediates.

**Supporting Information Available:** Full experimental details for the synthesis of compound **4** (4 pages). Ordering information is given on any current masthead page.

## References

(1) For a review on GH, see: Strobl, J. S.; Thomas, M. J. Human Growth Hormone. *Pharmacol. Rev.* **1994**, *46*, 1−34.

(2) Blethen, S. L.; Baptista, J.; Kuntze, J.; Foley, T.; LaFranchi, S.; Johanson, A. Adult Height In Growth Hormone (GH)-Deficient Children Treated With Biosynthetic GH. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 418−420.

(3) Bouillanne, O.; Rainfray, M.; Tissandier, O.; Nasr, A.; Lahlou, A.; Cnockaert, X.; Piette, F. Growth Hormone Therapy In Elderly People: An Age-Delaying Drug? *Fundam. Clin. Pharmacol.* **1996**, *10*, 416−430.

(4) Momany, F. A.; Bowers, C. Y.; Reynolds, G. A.; Chang, D.; Hong, A.; Newlander, K. Design, Synthesis, And Biologoical Activity Of Peptides Which Release Growth Hormone In Vitro. *Endocrinology* **1981**, *108*, 31−39.

(5) For a recent review on GHRP, see: Ghigo, E.; Arvat, E.; Muccioli, G.; Camanni, F. Growth Hormone-Releasing Peptides. *Eur. J. Endocrinol.* **1997**, *136*, 445−460. For recent reviews on GHS, see: (a) DeVita, R. J.; Wyvratt, M. J. Benzolactam Growth hormone Secretagogues. *Drugs Future* **1996**, *21*, 273−281. (b) Nargund, R. P.; Van der Ploeg, L. H. T. Growth Hormone Secretagogues. *Ann. Rep. Med. Chem.* **1997**, *32*, 221−230.

(6) (a) 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 Nonpeptidyl Growth Hormone Secretagogue. *Science* **1993**, *260*, 1640−1643. (b) Schoen, W. R.; Pisano, J. M.; Prendergast, K.; Wyvratt, M. J.; Fisher, M. H.; Cheng, K.; Chan, W.-S.; Butler, B.; Smith, R. G.; Ball, R. G. A Novel 3-Substituted Benzazepinone Growth Hormone Secretagogue (L-692,429). *J. Med. Chem.* **1994**, *37*, 897−906.

(7) Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M. H.; Barakat, K. H.; Johnston, D. B. R.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Hickey, G.; Jacks, T.; Schleim, K.; Pong, S.-S.; Chaung, L.-Y. P.; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S.-H. L.; Smith, R. G. Design And Biological Activities Of L-163,-191 (MK-0677): A Potent, Orally Active Growth Hormone Secretagogue. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7001−7005.

(8) (a) Pong, S. S.; Chaung, L. Y.; Dean, D. C.; Nargund, R. P.; Patchett, A. A.; Smith, R. G. Identification Of A New G-Protein-Linked Receptor For Growth Hormone Secretagogues. *Mol. Endocrinol.* **1996**, *10*, 57−61. (b) Howard, A. D.; Feighner, S. D.; Cully, D. F.; Arena, J. P.; Liberator, P. A.; Rosenblum, C. I.; Hamelin, M.; Hreniuk, D. L.; Palyha, O. C.; Anderson, J.; Paress, P. S.; Diaz, C.; Chou, M.; Liu, K. K.; McKee, K. K.; Pong, S.-S.; Chaung, L.-Y.; Elbrecht, A.; Dashkevicz, M.; Heavens, R.; Rigby, M.; Sirinathsinghji, D. J. S.; Dean, D. C.; Melillo, D. G.; Patchett, A. A.; Nargund, R.; Griffin, P. R.; DeMartino, J. A.; Gupta, S. K.; Schaeffer, J. M.; Smith, R. G.; Van Der Ploeg, L. H. T. A Receptor In Pituitary And Hypothalamus That Functions In Growth Hormone Release. *Science* **1996**, *273*, 974−977.

(9) (a) Chen, M.-H.; Steiner, M. G.; Patchett, A. A.; Cheng, K.; Wei, L.; Chan, W. W.-S.; Butler, B.; Jacks, T. M.; Smith, R. G. Analogues Of The Orally Active Growth Hormone Secretagogue L-162,752. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2163−2168. (b) Nargund, R. P.; Chen, M.-H.; Johnston, D. B. R.; Barakat, K. J.; Tata, J. R.; Cheng, K.; Jacks, T. M.; Chan, W. W.-S.; Butler, B.; Hickey, G. J.; Smith, R. G.; Patchett, A. A. Peptidomimetic Growth Hormone Secretagogues: Synthesis And Biological Activities Of Analogues Varied At The Indole Unit Of The Prototypical Spiropiperidine L-162,752. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1731−1736. (c) Tata, J. R.; Nargund, R. J.; Murphy, M. M.; Johnston, D. B. R.; Patchett, A. A.; Cheng, K.; Wei, L.; Chan, W. W.-S.; Butler, B.; Jacks, T. M.; Hickey, G.; Smith, R. G. The Synthesis And Activity Of Spiroindane Growth Hormone Secretagogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 663−668. (d) Yang, L.; Morriello, G.; Prendergast, K.; Cheng, K.; Jacks, T.; Chan, W. W.-S.; Schleim, K. D.; Smith, R. G.; Patchett, A. A. Potent 3-spiropiperidine growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 107−112.

(10) (a) Jacks, T.; Smith, R.; Judith, F.; Schleim, K.; Frazier, E.; Chen, H.; Krupa, D.; Hora, D., Jr.; Nargund, R.; Patchett, A.; Hickey, G. MK-0677, A Potent, Novel, Orally Active Growth Hormone (GH) Secretagogue: GH, Insulin-Like Growth Factor I, And Other Hormonal Responses In Beagles. *Endocrinology* **1996**, *137*, 5284−5289. (b) Hickey, G. J.; Jacks, T. M.; Schleim, K. D.; Frazier, E.; Chen, H. Y.; Krupa, D.; Feeney, W.; Nargund, R. P.; Patchett, A. A.; Smith, R. G. Repeat Administration Of The GH Secretagogue MK-0677 Increases And Maintains Elevated IGF-I Levels In Beagles. *J. Endocrinol.* **1997**, *152*, 183−192.

(11) Pihoker, C.; Badger, T. M.; Reynolds, G. A.; Bowers, C. Y. Treatment Effects Of Intranasal Growth Hormone Releasing Peptide-2 In Children With Short Stature. *J. Endocrinol.* **1997**, *155*, 79−86.

(12) Tata, J. R.; Lu, Z.; Jacks, T. M.; Schleim, K. D.; Cheng, K.; Wei, L.; Chan, W. W.-S.; Butler, B.; Tsou, N.; Leung, K.; Chiu, S.-H. L.; Hickey, G.; Smith, R. G.; Patchett, A. A. The Design And Synthesis Of Orally Active Short Duration Spiroindane Growth Hormone Secretagogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2319−2324.

(13) 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-NH2 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.

(14) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. Methods For Drug Discovery: Development Of Potent, Selective, Orally Effective Cholecystokinin Antagonists. *J. Med. Chem.* **1988**, *31*, 2235−2246.

(15) Detailed SAR studies will be reported in due course.

JM9800191