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<p>(21) International Application Number: PCT/US90/05842 (22) International Filing Date: 11 October 1990 (11.10.90) (30) Priority data: 421,245 13 October 1989 (13.10.89) US (71) Applicant: THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND [US/US]; 1430 Tulane Avenue, New Orleans, LA 70115 (US). (72) Inventors: COY, David, H. ; 4319 Perrier Street, New Orleans, LA 70115 (US). MOREAU, Jacques-Pierre ; 159 Westboro Road, Upton, MA 01568 (US).</p>		<p>(74) Agent: CLARK, Paul, T.; Fish & Richardson, One Financial Center, Suite 2500, Boston, MA 02111-2658 (US). (81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report.</i></p>
<p>(54) Title: THERAPEUTIC DECAPEPTIDES</p>		
<div style="text-align: center; padding: 20px;"> $\text{N-Ac-A}^1\text{-A}^2\text{-A}^3\text{-Ser}^4\text{-A}^5\text{-A}^6\text{-A}^7\text{-A}^8\text{-A}^9\text{-A}^{10}$ <p>(I)</p> </div>		
<p>(57) Abstract</p>		
<p>A decapeptide of the formula (I), wherein each A¹, A², and A³, independently, is D-β-Nal, D-p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl); A⁵ is p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl); A⁶ is D-Lys, D-Arg, β-Nal, D-β-Nal, D-Trp, D-p-X-Phe (where X is halogen, H, NH₂, NO₂, or C₁₋₃ alkyl) or D-Lys-ε-NH-R (where R is H, a branched or straight chain or cyclo C₁-C₁₀ alkyl group, or an aryl group); A⁷ is p-X-Phe (where X is halogen, H, NH₂, NO₂, HO, C₂F₅, or C₁₋₃ alkyl), cyclohexylala, or Trp; A⁸ is Arg, Lys, or Lys-ε-NH-R (where R is H, a branched or straight chain or cyclo C₁-C₁₀ alkyl group, or an aryl group); A⁹ is Pro; A¹⁰ is D-Ala-NH₂, Gly-NH₂, D-Ser, or D-Ser-NH₂; provided that at least one of A² or A³ must be D-Phe or D-Tyr; and further provided that at least one of A⁶ and A⁸ must be the following: A⁶ must be D-Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group); A⁸ must be Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group), or a pharmaceutically acceptable salt thereof.</p>		

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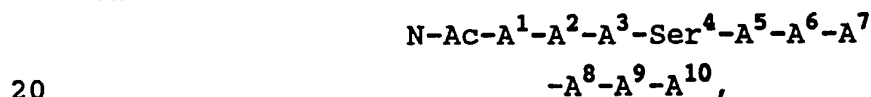
THERAPEUTIC DECAPEPTIDESBackground of the Invention

This invention relates to therapeutic peptides.

5 A number of luteinizing hormone releasing hormone (LH-RH) analogs have been described which inhibit the release of LH-RH, a peptide hormone having the formula pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂. These analogs are called LH-RH antagonists. For example, Coy et
 10 al. U.S. Patent No. 4,431,635, hereby incorporated by reference, describes LH-RH analogs having the general formula X-R¹-R²-R³-Ser-Tyr-R⁴-Leu-Arg-Pro-R⁵-NH₂, in which X can be Ac; R¹ and R⁴, independently, can be D-Trp or D-p-X-Phe, where X is a halogen or methyl group; R² can be
 15 D-p-X-Phe; R³ can be D-Trp; and R⁵ can be Gly or D-Ala.

Summary of the Invention

In general, the invention features a decapeptide of the formula:



20 wherein each A¹, A², and A³, independently, is D-β-Nal, D-p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl, e.g., methyl, ethyl, or n-propyl); A⁵ is p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl); A⁶ is
 25 D-Lys, D-Arg, β-Nal, D-β-Nal, D-Trp, D-p-X-Phe (where X is halogen, H, NH₂, NO₂, or C₁₋₃ alkyl) or D-Lys-ε-NH-R (where R is H, a branched or straight chain or cyclo C₁-C₁₀ alkyl group, e.g., methyl, ethyl, isopropyl, heptyl, butyl, or cyclopentyl, or an aryl group, e.g., benzyl, p-Cl-benzyl, or
 30 CH₂-naphthyl); A⁷ is p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, C₂F₅, or C₁₋₃ alkyl), cyclohexylAlanine, or Trp; A⁸ is Arg, Lys, or Lys-ε-NH-R (where R is H, a branched or

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straight chain or cyclo C₁-C₁₀ alkyl group, e.g., methyl, ethyl, isopropyl, heptyl, butyl, or cyclopentyl, or an aryl group, e.g., benzyl, p-Cl-benzyl, or CH₂-naphthyl); A⁹ is Pro; and A¹⁰ is D-Ala-NH₂, Gly-NH₂, D-Ser, or D-Ser-NH₂; provided that at least one of A² or A³ must be D-Phe or D-Tyr; and further provided that at least one of A⁶ and A⁸ must be the following: A⁶ must be D-Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group); A⁸ must be Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group), or a pharmaceutically acceptable salt thereof. (β-Nal refers to β-naphthylalanine; where no L- or D- designation is given herein, the L-isomer is intended; N-Ac refers to the N-acetyl protecting group, i.e., an acetyl group attached to a terminal amino acid residue on the amine nitrogen; halogen refers to fluoro, chloro, or bromo.)

Preferred decapeptides include those wherein A⁶ is D-Lys-ε-NH-R (where R is cyclo C₁₋₁₀ alkyl group); wherein A⁸ is Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group).

Other preferred decapeptides are wherein A¹-A¹⁰ is
 20 N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-
 D-(cyclopentyl)Lys-Phe-Arg-Pro-D-Ala-NH₂;
 N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-
 D-(cyclopentyl)Lys-Phe-(cyclopentyl)Lys-Pro-D-Ala-NH₂.

The invention also features decapeptides of the following formulae:

25 N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-
 (isopropyl)D-Lys-Pro-D-Ala-NH₂;
 N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-
 D-(benzyl)Lys-Phe-Arg-Pro-D-Ala-NH₂;
 30 N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-
 D-(Cl-benzyl)Lys-Phe-Arg-Pro-D-Ala-NH₂;

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N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-
 D-(heptyl)Lys-Phe-Arg-Pro-D-Ala-NH₂;
 N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-
 Phe-(t-butylmethyl)Lys-Pro-D-Ala-NH₂;
 5 N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-
 (4-methylbenzyl)Lys-Pro-D-Ala-NH₂;
 N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-
 (benzyl)Lys-Pro-D-Ala-NH₂;
 N-acetyl-D- β -Nal-D-p-Cl-Phe-D-Trp-Ser-Tyr-
 10 D-p-NH₂-Phe-Phe-(isopropyl)Lys-Pro-D-Ala-NH₂;
 N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-(heptyl)Lys-
 Phe-(heptyl)Lys-Pro-D-Ala-NH₂;
 N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-
 D-(1-butylpentyl)Lys-Phe-(1-butylpentyl)Lys-Pro-D-Ala-NH₂.

15 In other preferred embodiments, a therapeutically
 effective amount of a therapeutic decapeptide of the
 invention and a pharmaceutically acceptable carrier
 substance or salt, e.g., magnesium carbonate or lactose,
 together form a therapeutic composition for inhibiting the
 20 release of sex hormones, particularly LH, induced by LH-RH.
 This composition can be in the form of a pill, tablet,
 capsule, liquid, or sustained release tablet for oral
 administration; a liquid spray for nasal administration; or
 a liquid for parenteral (intravenous, intramuscular, and
 25 subcutaneous) topical, vaginal, rectal, buccal (including
 sublingual), or intraperitoneal administration.

As used herein, the term "pharmaceutically
 acceptable" carrier substance or salts refers to a carrier
 or salt that retain the desired biological activity of the
 30 parent compound and do not impart any undesired
 toxicological effects. Examples of salts are (a) acid
 addition salts formed with inorganic acids, for example,

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hydrochloric acid, hydrobromic acid, sulfuric acid,
phosphoric acid, nitric acid and the like; and salts formed
with organic acids such as, for example, acetic acid, oxalic
acid, tartaric acid, succinic acid, malic acid, ascorbic
5 acid, benzoic acid, tannic acid, pamoic acid, alginic acid,
polyglutamic acid, naphthalenesulfonic acids,
naphthalenedisulfonic acids, polygalacturonic acid; (b) base
addition salts formed with polyvalent metal cations such as
zinc, calcium, bismuth, barium, magnesium, aluminum, copper,
10 cobalt, nickel, cadmium, and the like; or with an organic
cation formed from N,N'-dibenzylethylene-diamine or
ethylenediamine; or (c) combinations, of (a) and (b), e.g.,
a zinc tannate salt and the like. The most suitable route
will depend upon the use, particular active ingredient, and
15 the subject involved.

Another preferred form for administration is an
injectable suspension of the peptide with a bioerodible,
biocompatible polymer matrix capable of effecting sustained
release of the peptide. Other suitable forms are
20 peptide/polymer implants, transdermal patches, transmucosal
patches (vaginal), and nasal spray, and compositions usable
with iontophoretic techniques.

The decapeptides of the invention are active in
inhibiting the LH-RH induced release of LH, and exhibit a
25 long duration of activity, thus minimizing the amount and
frequency of dosages. Furthermore, manufacture is
relatively simple and inexpensive. In addition, the
peptides have the advantage of being able to be
administered orally, a property owing to their high
30 lipophilicity and their ability to withstand enzymatic
degradation by peptidase.

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The peptides of the invention have D-X-Phe or D-Tyr at at least one of positions A² or A³. D-X-Phe, D-Trp, or D-Tyr at position A³ have been found to be modifications of particular importance in terms of activity, while D-Phe at position A² provides further cost reduction, compared to D-p-X-Phe at A², without significant comparative loss of activity. The presence of D-Lys-ε-NH-R at position A⁶ and Lys-ε-NH-R at position A⁸ when R is an alkyl or aryl group lessen the irritant effect of the decapeptide. Presumably, this is due to an observed decrease in histamine-releasing activity. It is further believed that the presence of Arg at position A⁵ and D-Tyr at position A⁶, known as the Hodgen modification, also decreases histamine-releasing activity. p-X-Phe at position A⁷ is also particularly advantageous in terms of activity.

It has also been discovered that LH-RH antagonists in general, and the above-described decapeptides in particular, can be used to treat immunosuppressed patients when administered as described above. The antagonists rejuvenate the thymus, which then produces T-cells to replace T-cells lost as a result of the immunodeficiency.

The peptide antagonists of the invention can be used to treat some forms of hormone dependent cancers including breast, prostate, and ovary. Some benign conditions resulting from an overproduction of sex hormone can benefit from treatment with a peptide of the invention; e.g., benign prostatic hyperplasia, endometriosis, or lyomas. It has also been discovered that the LHRH antagonists of the invention can exhibit a direct antitumoral effect in human mammary cancer cell lines (e.g. MCF-7). Other uses include but are not limited to the following: female contraception; ovulation prevention or

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delay; pregnancy termination in domestic animals and pets;
induction of parturition; synchronization of ovulation;
estrus suppression; growth promotion in female animals;
luteolysis, menses induction; therapy for premenstrual
5 syndrome; therapy for precocious puberty; therapy for
uterine leiomyoma; early, first trimester abortifacient;
therapy for endometriosis; therapy for mammary tumors and
cysts; therapy for polycystic ovary syndrome/disease;
therapy for uterine carcinoma; therapy for benign prostatic
10 hypertrophy and for prostatic carcinoma; male contraception;
therapy for diseases which result from excessive gonadal
hormone production in either sex; functional castration in
male food producing animals; suppression of proestrous
bloody discharge in dogs; diagnostic utilities, such as
15 predisposition to osteoporosis; prevention of ovarian
hyperstimulation; preservation of fertility in case of
chemotherapy or irradiation; and other uses as set forth in
Vickery. B.H., Endocrine Reviews, 7:115 (1986), which is
fully incorporated by reference herein.

20 Other features and advantages of the invention will
be apparent from the following description of the preferred
embodiments thereof, and from the claims.

Description of the Preferred Embodiments

25 Before describing the structure, synthesis,
testing, and use of preferred embodiments of the invention,
we first describe the drawings.

Drawings

30 Fig. 1 is a growth curve of the MXT mouse mammary
tumor in response to the agonist BIM-21003 and the
antagonist BIM-21009.

Fig. 2 is a growth curve of the MXT mouse mammary
tumor in response to different doses of the antagonist
BIM-21009.

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Fig. 3 is a growth curve of the MXT mouse mammary tumor in response to different doses of BIM-21009.

Fig. 4 is a growth curve of the MT/W9A-S mammary adenocarcinoma in response to BIM-21009.

5 Fig. 5 is a growth curve of the DU145/01 human prostate tumor in response to BIM-21009.

Fig. 6 is a growth curve of the Dunning R3327 prostate tumor in rats in response to BIM-21009.

10 Fig. 7 is a growth curve of the 2PR-121D(1)/S rat prostate tumor in response to BIM-21003 and BIM-21009.

Fig. 8 is a growth curve of the 2PR-121D(1)/R rat prostate adenocarcinoma in response to BIM-21009.

Structure

15 The decapeptides of the invention have the general formula recited in the Summary of the Invention above. They all have an acetyl group at the amino terminal end in addition to Ser at position 4. Substitution of non-natural substituents at positions other than A², A³, and A⁷ can be used to modify the properties of the compound, and will not
20 prevent the A², A³, and/or A⁷ substituents from providing their beneficial effects.

The decapeptides can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic
25 acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the
30 hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis

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Example 1

The synthesis of N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-Arg-Pro-D-Ala follows.

Other decapeptides of the invention can be prepared
5 by making appropriate modifications of the following synthetic method.

The first step is the preparation of N-Acetyl-D- β -Nal-D-Phe-D-Phe-benzyl-Ser-Tyr-D-tosyl-Arg-Phe-tosyl-Arg-Pro-D-Ala-benzydrylamine-resin, as
10 follows.

Benzydrylamine-polystyrene resin (Bachem, Inc.) (1.00 g, 0.3 mmole) in the chloride ion form is placed in the reaction vessel of a Beckman 990B peptide synthesizer programmed to perform the following reaction cycle: (a)
15 CH₂Cl₂; (b) 33% trifluoroacetic acid in CH₂Cl₂ (2 times for 1 and 25 min each); (c) CH₂Cl₂; (d) ethanol; (e) CH₂Cl₂; (f) triethylamine in CHCl₃; and (g) CH₂Cl₂.

The neutralized resin is stirred with alpha-t-butoxycarbonyl (Boc)-D-Ala and diisopropylcarbodiimide (1.5
20 mmole) in CH₂Cl₂ for 1 hour and the resulting amino acid resin is then cycled through steps (a) to (g) in the above wash program. The following amino acids (1.5 mmole) are then coupled successively by the same procedure: Boc-Pro,
Boc-Tosyl-Arg, Boc-Phe, Boc-Tosyl-D-Arg, Boc-Tyr,
25 Boc-benzyl-Ser, Boc-D-Phe, and Boc-D- β -Nal.

After removal of the N-terminal Boc group, the peptide-benzydrylamine resin is neutralized and acetylated by treatment with 5% acetic acid in CH₂Cl₂. The completed resin is then washed with CH₃OH and air dried.

30 From the above resin is prepared N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-Arg-Pro-D-Ala, as follows.

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A mixture of the above decapeptide resin (1.85 g, 0.5 mmole) and a solution of 4 ml anisole, 100 mg dithiothreitol, and 36 ml hydrogen fluoride is stirred at 0°C for 45 minutes. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen, after which the free peptide is precipitated and washed with ether.

The peptide is then dissolved in a minimum volume of 50% acetic acid and eluted on a column (2.5 X 100 mm) of Sephadex G-25. Fractions containing a major component, as determined by u.v. absorption and thin layer chromatography (tlc), are pooled and evaporated to a small volume in vacuo. This solution is applied to a column (2.5 X 50 cm) of octadecylsilane-silica (Whatman LRP-1, 15-20 um mesh size) which is eluted with a linear gradient of 15-50% acetonitrile in 20% acetic acid in water. Fractions are examined by tlc and analytical high performance liquid chromatography (hplc) and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 117 mg of the product as a white, fluffy powder.

This material is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the decapeptide.

N-Ac-D- β -Nal-D-p-Cl-Phe-D-Trp-Ser-Phe-D-Arg-Leu-Arg-Pro-D-Ala was prepared according to the synthesis described above, substituting Boc-D-p-Cl-Phe for Boc-D-Phe at position A², Boc-D-Trp for Boc-D-Phe at position A³, Boc-Phe for Boc-Tyr at position A⁵, and Boc-Leu for Boc-Phe at A⁷.

N-Ac-D- β -Nal-D-p-Cl-Phe-D-Tyr-Ser-Phe-D-Arg-Leu-Arg-Pro-D-Ala was prepared according to the synthesis described above, substituting Boc-D-p-Cl-Phe for Boc-D-Phe at position A², Boc-D-Tyr for Boc-D-Phe at position A³,

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Boc-Phe for Boc-Tyr at position A⁵, and Boc-Leu for Boc-Phe at position A⁷.

N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-Phe-D-Arg-Leu-Arg-Pro-D-Ala was prepared according to the synthesis described above, substituting Boc-Phe for Boc-Tyr at A⁵ and Boc-Leu for Boc-Phe at A⁷.

Example 2

To synthesize peptides featuring D-Lys- ϵ -NH-R at position A⁶ or Lys- ϵ -NH-R at position A⁸, where R is an alkyl or aryl group, there is used the method described in Coy, et al., U.S. Patent application S.N. 879,348, filed June 27, 1986 and assigned to the same assignee as this application, hereby incorporated by reference. In general, the synthesis involves reacting a carbonyl-containing compound, e.g., acetone or formaldehyde, with a resin-bound polypeptide featuring a Lys or D-Lys subunit in the presence of sodium cyanoborohydride. The carbonyl-containing compound reacts with the free ϵ -NH₂ group on the side chain of the Lys or D-Lys subunit; reaction with acetone produces an ϵ -N-isopropyl moiety, whereas reaction with formaldehyde produces an ϵ -N-methyl moiety.

The synthesis of Ac-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-(isopropyl)Lys-Phe-(isopropyl)Lys-Pro-D-Ala-NH₂ follows.

Ac-D- β -Nal-D-Phe-D-Phe-Ser(Bzl)-Tyr-D-Lys (FMOC)-Phe-D-Lys (FMOC)-Pro-D-Ala-benzhydrylamine resin (BzL= benzyl; FMOC=fluorenylmethyloxycarbonyl) was prepared by standard methods in a Beckman 990B automatic peptide synthesizer using 33% TFA (trifluoroacetic acid) for removal of the α -BOC protecting groups. The ϵ -FMOC protecting groups on the Lys residues are completely stable to these acidic conditions, and to subsequent

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neutralization steps with 10% triethylamine in chloroform. The resin was then treated with 50ml of a 50% solution of piperidine in DMF (dimethylformamide) for about 12h to remove the Fmoc protecting groups from the Lys residues.

5 To react the free ϵ -amino groups of the Lys residues, the resin (0.25 mmole) was mixed with 5ml of acetone, and 1 mmole of sodium cyanoborohydride in DMF/1% acetic acid added. The resin mixture was then stirred until it was negative to ninhydrin reaction (about 3h); the
10 negative ninhydrin reaction indicated that the free ϵ -amino groups had been converted to N-isopropyl amino groups.

The resin was then cleaved from the support by treatment with HF/anisole and purified under standard conditions to yield the desired polypeptide.

15 Ac-D-Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(isopropyl)-Phe-Arg-Pro-D-Ala-amide; N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-(cyclopentyl)Lys-Phe-Arg-Pro-D-Ala-NH₂; and N-acD-(cyclopentyl)Lys-Phe-(cyclopentyl)Lys-Pro-D-Ala-NH₂ are prepared in an analogous fashion using appropriate
20 modifications of the above-described procedure.

Testing

A decapeptide of the invention can be tested for its ability to reduce the size of one or more of the following hormone-dependant tumors: the human MCF-7 mammary tumor, the
25 murine MXT or rat MT/W9A-S papillary ductal tumor, and the human DU/145 or rat Dunning R3327 prostate adenocarcinoma. The BIM-2000 code numbers used in the Tables and Figures herein refer to the following decapeptides:

30 BIM-21003: p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH₂

BIM-21006: p-Glu-His-Trp-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH₂

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- BIM-21009: N-Ac-D- β -Nal-D-P-Cl-Phe-D-Phe-Ser-Tyr
-D-Arg-Phe-Arg-Pro-D-Ala-NH₂
- BIM-21011: N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-
Arg-Phe-Arg-Pro-D-Ala-NH₂
- 5 BIM-21023: N-Ac-D- β -Nal-D-p-Cl-Phe-D-Trp-Ser-Tyr
D-Arg-Phe-Arg-Pro-D-Ala-NH₂
- BIM-21024: N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-
Phe-D-Arg-Leu-Arg-Pro-D-Ala-NH₂
- BIM-21025: N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-
10 Tyr-D-Lys(benzyl)-Phe-Arg-Pro-
D-Ala-NH₂
- BIM-21026: N-Ac-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-
D-Lys(cyclopentyl)-Phe-
Lys(cyclopentyl)-Pro-D-Ala-NH₂
- 15 BIM-23014C: D-Nal-Cys-Tyr-D-Trp-Lys-Val-
Cys-Thr-NH₂.
- BIM-21032: N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-
D-(cyclopentyl)Lys-Phe-Arg-
Pro-D-Ala-NH₂;

20 Human MCF-7 Mammary Tumor

Female CDF-1 mice were implanted with a human mammary tumor (MCF-7) underneath the kidney capsules on day 0. On day 1, animals were injected subcutaneously with BIM-21009, once a day for 6 days. The negative control group received vehicle only and the positive control was ovariectomized on day 0.

Table 1 shows the results of experiment 1, in which daily subcutaneous injections of BIM-21009 (25ug) over a period of 6 days decreased the size of the tumor to 28% (unless otherwise stated, percentage results are relative to the vehicle-treated control). This effect was similar to that obtained by ovariectomy (-33%). Table 2 shows the

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results of experiment 2, in which daily subcutaneous injections of BIM-21009 (10ug) over a period of 6 days decreased the size of the tumor to 28% of the size of the vehicle-treated control tumor. Even at a dose of 1 ug, BIM-21009 reduced the size of the tumor to 53% of the size of the control tumor. These effects were weaker than the effect of ovariectomy on tumor size (tumor reduction to 5% of control size). Table 2 also shows that, at a high dosage, the LHRH superagonists BIM-21003 and BIM-21006 do not desensitize mouse pituitary LHRH receptors. Table 3 shows the results of experiment 3, in which daily subcutaneous injections (25 ug) of the LHRH antagonists BIM-21023, BIM-21024, BIM-21025, and BIM-21026 over a period of 6 days resulted in a decreased tumor size ranging from 29%, 11%, 12%, and 8%, respectively, of the control size. A control peptide which is a somatostatin octapeptide analog, Somatuline (BIM-23014C), at doses of 2 ug, 10 ug, or 50 ug, reduced tumor size to 45%, 32%, and 10%, respectively. The peptide BIM-21032 may also be tested in this system as shown above.

Murine MXT Mammary Papillary Ductal Carcinoma
or Rat MT/W9A-S Adenocarcinoma

Female mice or rats were implanted subcutaneously with either murine MXT mammary ductal papillary carcinoma or rat MT/W9A-S mammary adeno-carcinoma, respectively, on day 0 and injected once daily subcutaneously with various doses of BIM-21009 from day 1 to day 37. The negative control groups received vehicle only and the positive one was ovariectomized 5 days prior to implanting the tumor.

Table 4 and Fig. 1 show that BIM-21009 induced a highly significant tumor growth inhibitory effect (-95%), on MXT estrogen sensitive mammary carcinoma in mice which was better than the effect of ovariectomy on tumor growth

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(-84%). Fig. 1 also shows the stimulatory effect of the agonist, BIM-21003, on tumor growth since mice pituitary LHRH receptors are not desensitized by LHRH superagonist as opposed to rats or humans. Table 5 shows that BIM-21009 induced a marked reduction in uterus weight (-77%) comparable to that of ovariectomized animals (-85%). Ovaries and pituitary weights were also significantly reduced (-47% and -35%, respectively). Fig. 2 shows that BIM-21009 at a dose of 25 ug significantly reduced tumor size, but not to the extent of castrate control, and demonstrates that the dose of BIM-21009 is proportionate to the size of reduction of the tumor. Fig. 3 shows that the estrogen sensitive MXT murine mammary tumor escapes the growth inhibitory effects of castration, and that BIM-21009 at a dose of 50 ug reduces tumor size and thus retards that escape. Fig. 4 shows that estrogen sensitive rat mammary adenocarcinoma MT/W9A escapes the effects of ovariectomy and that a very high dose of BIM-21009 (250 ug) reduces tumor size and thus retards that escape.

20 Human DU/145 or Rat Dunning R3327 Prostate Adenocarcinoma

Athymic nude male mice were implanted supercutaneously with xenografts of the human prostate tumor DU/145 and male Copenhagen rats were implanted subcutaneously with the Dunning rat prostate tumor R3327. Negative control groups received vehicle only and positive controls were castrated on day 3 or day 99 post-implant, for the human or rat tumor, respectively. Plasma testosterone levels were determined in the animals at time of sacrifice. The results in Fig. 5 shows that, by day 33 post-implant, BIM-21009 at a dose of 12.5 ug daily for 30 days reduced the average human prostate tumor size significantly, even though the tumor was not responsive to castration. The results in Table 6 and Fig. 6 show that BIM-21009,

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administered first on day 99 post-implant and then daily, subcutaneously, for 68 days to animals bearing well established growing Dunning R3327 prostate tumors, inhibited tumor growth by 41% at the highest dose tested (20 ug/rat).
5 This effect was weaker than that of castration (-71%). Plasma testosterone levels in these two groups was nil, whereas at a lower dose (5 ug/rat), hormone levels were similar to that observed in the vehicle treated groups (0.87 ng/ml and 0.71 ng/ml, respectively); no significant effect
10 on tumor growth was seen at this dose.

The results in Table 7 show that BIM-21009 at a dose of 20 ug reduced the weight of the whole prostate, the ventral prostate, and the testes (-85%, -53%, and -68%, respectively). The results in Table 8 show that, when
15 BIM-21009 was first administered earlier to Copenhagen rats bearing the R3327 prostate tumor, i.e., at 15 days rather than at 99 days post-implant (control castration was also performed 15 days post-implant), tumor size was reduced to a greater extent than the castrate control at 96 days
20 post-implant. In Figs. 7 and 8, BIM-21009 was administered daily for 10 to 30 days post-tumor implant. The results show that the androgen sensitive (2PR-121D(1)/S) and androgen resistant (2PR-121D(1)/R) noble rat prostate tumors both respond to BIM-21009, at a dose of 50 ug, by reduction
25 in tumor size. This reduction is of a greater degree than that observed for the castrate control.

Use

When administered to a mammal (e.g., orally, intravenously, parenterally, nasally, or by suppository),
30 the decapeptides are effective in inhibiting the release of LH induced by LH-RH.

The decapeptides of the invention can be used for the treatment of precocious puberty, hormone dependent

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tumors (e.g., malignant and benign prostatic, mammary, ovarian and testicular tumors), hirsutism, acne, amenorrhea (e.g., secondary amenorrhea), endometriosis, and ovarian and mammary cystic diseases; the particular decapeptide
5 described above is particularly effective in preventing the growth of mammary tumors. The decapeptides can also be used to regulate human menopausal gonadotropin luteinizing hormone (LH) and follicle-stimulating hormone (FSH) during perimenopausal and postmenopausal periods in women. The
10 decapeptides can also be used as female contraceptives and as an abortifacient.

In general, for the uses herein above described, the decapeptides can be in amounts between about 0.001 and 5 mg/kg body weight. Preferably, for human therapy, the
15 active ingredient will be administered in the range of from about 0.01 to about 1 mg/kg/day, preferably 25-250 mcg/kg/day; and for animal therapy, the active ingredient will be administered in the range of from about 0.1 to 1 mg/kg/day. This administration may be accomplished by a
20 single administration, by distribution over several applications or by slow release in order to achieve the most effective results. Most preferably, for the interruption of heat or prevention of pregnancy in animals, the dose will be in the range of from about 1 to 10 mg/kg,
25 administered as a single dose.

The exact dose and regimen for administration of these compounds and compositions will necessarily be dependent upon the needs of the individual subject being treated, the type of treatment, and the degree of
30 affliction or need. In general, parenteral administration requires lower dosage than other methods of administration which are more dependent upon absorption.

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The decapeptides, and LH-RH antagonists in general, can also be used to treat immunosuppressed patients since chemical castration may restore the thymus and thus stimulate the immune system. Examples of additional LH-RH antagonists are described in Coy, U.S. Pat. No. 4,647,653, hereby incorporated by reference, and Coy et al., U.S. Pat. No. 4,431,635, previously incorporated by reference. The LH-RH antagonists rejuvenate the thymus when administered as described above.

10 The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example, as described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA., 1970. Formulations for parenteral administration may contain as common excipients 15 sterile water or saline, alkylene glycols such as propylene glycol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Formulations for vaginal or rectal administration, 20 e.g., suppositories, may contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for nasal administration may be solid and contain as excipients, for example, lactose or dextran, or may be aqueous or oily solutions for administration in the 25 form of nasal drops or metered spray. For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like.

Nasal administration of the instant nona- and 30 decapeptides is particularly preferred. The absorption across the nasal mucous membrane is enhanced by surfactant acids, such as for example, glycocholic acid, cholic acid, taurocholic acid, cholanic acid, ethocholic acid,

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desoxycholic acid, chenodesoxycholic acid, dehydrocholic acid, and glycodeoxy-cholic acid.

One or more surfactant acids or salts, but preferably a single pharmaceutically acceptable acid salt, can be added to the LHRH antagonist in solution or powder formulation. Suitable pharmaceutically acceptable surfactant salts will be those salts which retain the phenomenon of enhanced peptide absorption, as well as the compound's surfactant characteristics, and which are not deleterious to the subject or otherwise contraindicated. The amount of surfactant used for the practice of this invention will be some amount which increases the absorption of LHRH peptides over that of other surfactants which also may enhance peptide absorption to a certain degree. It has been found that such an amount is often in the range between 0.2 and 15%, more often 0.2 to 5 percent by weight/volume of the solution. It is preferred that the surfactant be present in an amount between about 0.5 to 4 percent by weight volume, conveniently about 1 percent by weight volume, preferably about 2 percent by weight volume.

Other embodiments are within the following claims.

Table 1

EFFECT OF BM-21009 ON MCF-7 HUMAN
MAMMARY CARCINOMA GROWTH IN MICE

<u>Group</u>	<u>Daily Dose</u> <u>per mouse</u>	<u>Change In</u> <u>Tumor Size (mm)</u>	<u>% Variation</u>
Vehicle	--	0.60±0.54	--
ovariectomized	--	-0.20±0.46 ^{**}	-33.33
BIM-21009	25µg	-0.17±0.75 [*]	-28.33

* P <0.05

** P <0.01

Table 2

TESTING OF LH-RH AGONISTS AND ANTAGONISTS AGAINST THE
TRANSPLANTATION ESTABLISHED MCF-7 HUMAN BREAST TUMOR
IN THE 6-DAY SUBRENAL CAPSULE ASSAY

Group No.	No. Animals	Treatment ¹	Average Delta Tumor Size ² (Mean ± S.D.)	% Test/Control	FBW ³ /IBW
1	10	Intact-Control, 0.2 ml/inj.	2.00 ± 1.47	--	0.97
2.	9	Ovexed (Day 0) Vehicle Control	0.10 ± 0.80	5	0.99
3.	10	BIM-21003, 100 µg/inj.	2.44 ± 0.98	122	1.04
4.	10	BIM-21006, 100 µg/inj.	1.25 ± 1.39	63	0.99
5.	10	BIM-21009, 10 µg/inj.	0.56 ± 0.85*	28	0.99
6.	10	BIM-21009, 1µg/inj.	1.06 ± 0.83	53	0.98
7.	10	BIM-21011, 15µg/inj.	0.069 ± 0.61*	34	0.99
8.	10	BIM-21011, 10µg/inj.	1.44 ± 1.21	72	0.99
9.	10	BIM-21011, 1µg/inj.	1.65 ± 0.71	83	0.98

1 Control and test compounds were administered s.c. on QDO-5 schedule

2 Change in tumor size (Day 6 - Day 0) in ocular micrometer units (omu). Significance of difference from intact control: * p < 0.05.

3 FBW/IBW = Final Body Weight / Initial Body Weight

Table 3

COMPARATIVE ACTIVITY OF SOMATULINE (BIM-23014C) AND
LH-RH ANTAGONISTS (BIM-21000 TESTED AGAINST THE
TRANSPLANTATION ESTABLISHED MCF-7 HUMAN
BREAST TUMOR IN THE 6-DAY SUBRENAL CAPSULE ASSAY

Group No.	No. Animals	Treatment ¹	Average Delta Tumor Size ² (Mean ± S.D.)	%Test/Control	FBW ³ /IBW
1	10	Intact - Control, 0.2 ml/inj.	3.65 ± 2.06	--	1.02
2	9	Ovexed (Day 0) Control, 0.2 ml/inj.	1.44 ± 0.55**	40	0.99
3	10	BIM-23014C, 50 µg/inj.	0.35 ± 0.78***	10	0.98
4.	10	BIM-23014C, 10 µg/inj.	1.15 ± 0.95**	32	0.99
5.	10	BIM-23014C, 2 µg/inj.	1.65 ± 1.00*	45	1.00
6.	10	BIM-21023, 25 µg/inj.	1.05 ± 1.13**	29	1.01
7.	10	BIM-21024, 25 µg/inj.	0.04 ± 0.77***	11	1.00
8.	10	BIM-21025, 25 µg/inj.	0.45 ± 0.76***	12	0.97
9.	10	BIM-21026, 25 µg/inj.	0.30 ± 0.46***	8	1.01

1 Vehicle Control and BIM-23014C groups were administered s.c. on QD0-5, b.i.d. schedule. All other test materials were administered s.c. on a QD0-5 schedule

2 Change in tumor size (Day 6 - Day 0) in ocular micrometer units (omu).
Significance of difference from intact control: * p < 0.05; ** p < 0.01;
*** p < 0.001.

3 FBW/IBW = Final Body Weight / Initial Body Weight

Table 4

EFFECT OF BM-21009 ON MXT MAMMARY CARCINOMA
GROWTH IN MICE

<u>Group</u>	<u>Daily Dose per mouse</u>	<u>Tumor Size (mm)</u>		<u>Tumor Increase</u>	<u>% Variation</u>
		<u>Day 14</u>	<u>Day 38</u>		
Vehicle	--	2.2±0.64	10.9±1.63	8.7	--
ovariectomized	--	0	1.4±0.70 ^{***}	1.4	-83.91
BM-21009	25µg	1.8±0.66	2.2±0.64 ^{***}	0.4	-95.40

*** P < 0.001

Table 5

EFFECT OF BM-21009 ON ENDOCRINE ORGANS
OF MXT TUMOR BEARING MICE

Group	Organ Weights (mg)			
	<u>Pituitary</u>	<u>Adrenals</u>	<u>Uteri</u>	<u>Ovaries</u>
Vehicle	2.31±0.23	8.10±0.54	76.97±11.40	15.46±0.74
<u>ovariectomized</u>	2.13±0.14	6.78±0.15*	11.25±0.67***	--
% Variation	-7.79	-16.30	-85.38	
<u>BM-21009</u>				
25µg/day	1.50±0.08	8.90±0.27	17.69±1.00***	8.14±0.58***
% Variation	-35.06	0	-77.02	-47.35

* P < 0.05
 ** P < 0.01
 *** P < 0.001

EFFECT OF BM-21009 ON DUNNING R3327 PROSTATE
ADENOCARCINOMA GROWTH IN RATS

<u>Group</u>	<u>Daily Dose per Rat</u>	<u>Tumor Size (mm)</u>		<u>Tumor Increase</u>	<u>% Variation</u>
		<u>Day 99</u>	<u>Day 165</u>		
Vehicle	--	11.80±2.33	34.00±5.05	22.20	--
Castrate	--	15.40±1.66	21.80±2.09	6.40	-71.17
BM-21009	20µg	11.00±2.51	24.10±2.22	13.100	-40.99
	5µg	11.20±1.29	28.90±3.48	17.70	-20.27

Table 7

EFFECT OF BM-21009 ON ENDOCRINE ORGANS IN
R3327 PROSTATE TUMOR BEARING RATS

<u>Organ Weights</u>	<u>Treated Groups</u>			
	<u>Vehicle</u>	<u>Castrate</u>	<u>BM-21009</u> <u>20µg</u>	<u>BM-21009</u> <u>5µg</u>
Whole Prostate (mg)	265±48	29±4	41±19	245±31
% Variation	--	-89.06	-84.53	-7.55
Ventral Prostate (mg)	55±17	15±3	26±8	64±9
% Variation	--	-72.73	-52.73	+16.36
Testes (g)	2.03±0.17	--	0.65±0.43	1.8±0.1
% Variation	--	--	-67.98	-11.33
Adrenals (mg)	34±2	29±4	33±1	32±2
% Variation	--	-14.71	-2.94	-5.88
Pituitary (mg)	7.3±0.6	7.2±0.9	6.5±0.2	7.0±0.8
% Variation	--	-1.37	-10.96	-4.11

R-3327-H PROSTATE TUMOR
TUMOR SIZE 96 DAYS POST TUMOR IMPLANTATION
RX BIM-21009 INITIATED 15 DAYS POST IMPLANTATION
CASTRATION ON DAY 15 POST IMPLANTATION

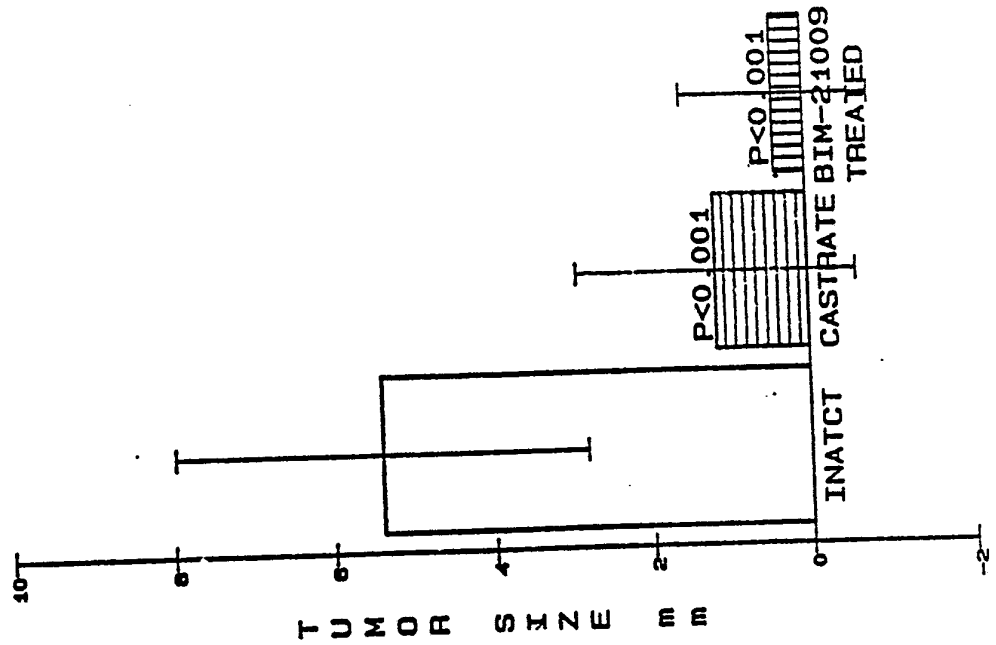
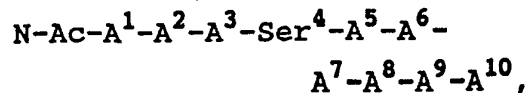


Table 8

Claims

1. A decapeptide of the formula:



5 wherein each A¹, A², and A³, independently, is D-β-Nal, D-p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl); A⁵ is p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl); A⁶ is D-Lys, D-Arg, β-Nal, D-β-Nal, D-Trp, D-p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, or C₁₋₃ alkyl) or D-Lys-ε-NH-R (where R is H, a branched or straight
10 chain or cyclo C₁-C₁₀ alkyl group, or an aryl group); A⁷ is p-X-Phe (where X is halogen, H, NH₂, NO₂, OH, C₂F₅, or C₁₋₃ alkyl), cyclohexylalanine, or Trp; A⁸ is Arg, Lys, or Lys-ε-NH-R (where R is H, a branched or straight chain or
15 cyclo C₁-C₁₀ alkyl group, or an aryl group); A⁹ is Pro; and A¹⁰ is D-Ala-NH₂, Gly-NH₂, D-Ser, or D-Ser-NH₂; provided that at least one of A² or A³ must be D-Phe or D-Tyr; and further provided that at least one of A⁶ and A⁸ must be the following: A⁶ is D-Lys-ε-NH-R (where R is cyclo C₁-C₁₀
20 alkyl group); A⁸ is Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group), or a pharmaceutically acceptable salt thereof.

2. The decapeptide of claim 1 wherein A⁶ is D-Lys-ε-NH-R (where R is cyclo C₁₋₁₀ alkyl group).

3. The decapeptide of claim 1 or 2 wherein A⁸ is
25 Lys-ε-NH-R (where R is cyclo C₁-C₁₀ alkyl group).

4. The decapeptide of claim 2 wherein A¹-A¹⁰ is
N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(cyclo-pentyl)-
Phe-Arg-Pro-D-Ala-NH₂.
5. The decapeptide of claim 2 or 3 wherein A¹-A¹⁰
5 is N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(cyclopentyl)-
Phe-Lys(cyclopentyl)-Pro-D-Ala-NH₂.
6. A decapeptide of the formula:
N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-
(isopropyl)D-Lys-Pro-D-Ala-NH₂.
- 10 7. A decapeptide of the formula:
N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(benzyl)-
Phe-Arg-Pro-D-Ala-NH₂.
8. A decapeptide of the formula:
N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(Cl-benzyl)-
15 Phe-Arg-Pro-D-Ala-NH₂.
9. A decapeptide of the formula:
N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(heptyl)-
Phe-Arg-Pro-D-Ala-NH₂.
10. A decapeptide of the formula:
20 N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-
Lys (t-butylmethyl)-Pro-D-Ala-NH₂.
11. A decapeptide of the formula:
N-acetyl-D-β-Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-Lys
(4-methyl-benzyl)-Pro-D-Ala-NH₂.

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12. A decapeptide of the formula:

N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-Lys
(benzyl)-Pro-D-Ala-NH₂.

13. A decapeptide of the formula:

5 N-acetyl-D- β -Nal-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-p-NH₂-
Phe-Phe-(isopropyl)Lys-Pro-D-Ala-NH₂.

14. A decapeptide of the formula:

N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(heptyl)-Phe-Lys
(heptyl)-Pro-D-Ala-NH₂.

10 15. A decapeptide of the formula:

N-acetyl-D- β -Nal-D-Phe-D-Phe-Ser-Tyr-D-Lys(1-butylpentyl)-
Phe-Lys(1-butylpentyl)-Arg-Pro-D-Ala-NH₂.

15 16. A therapeutic composition for inhibiting the
LH-RH induced release of sex hormones comprising a
therapeutically effective amount of the decapeptide of
claim 1 together with a pharmaceutically acceptable carrier
substance.

20 17. A method of treating a mammal in need of
inhibition of LH-RH induced release of sex hormones
comprising administering to said mammal a therapeutically
effective amount of the decapeptide of claim 1.

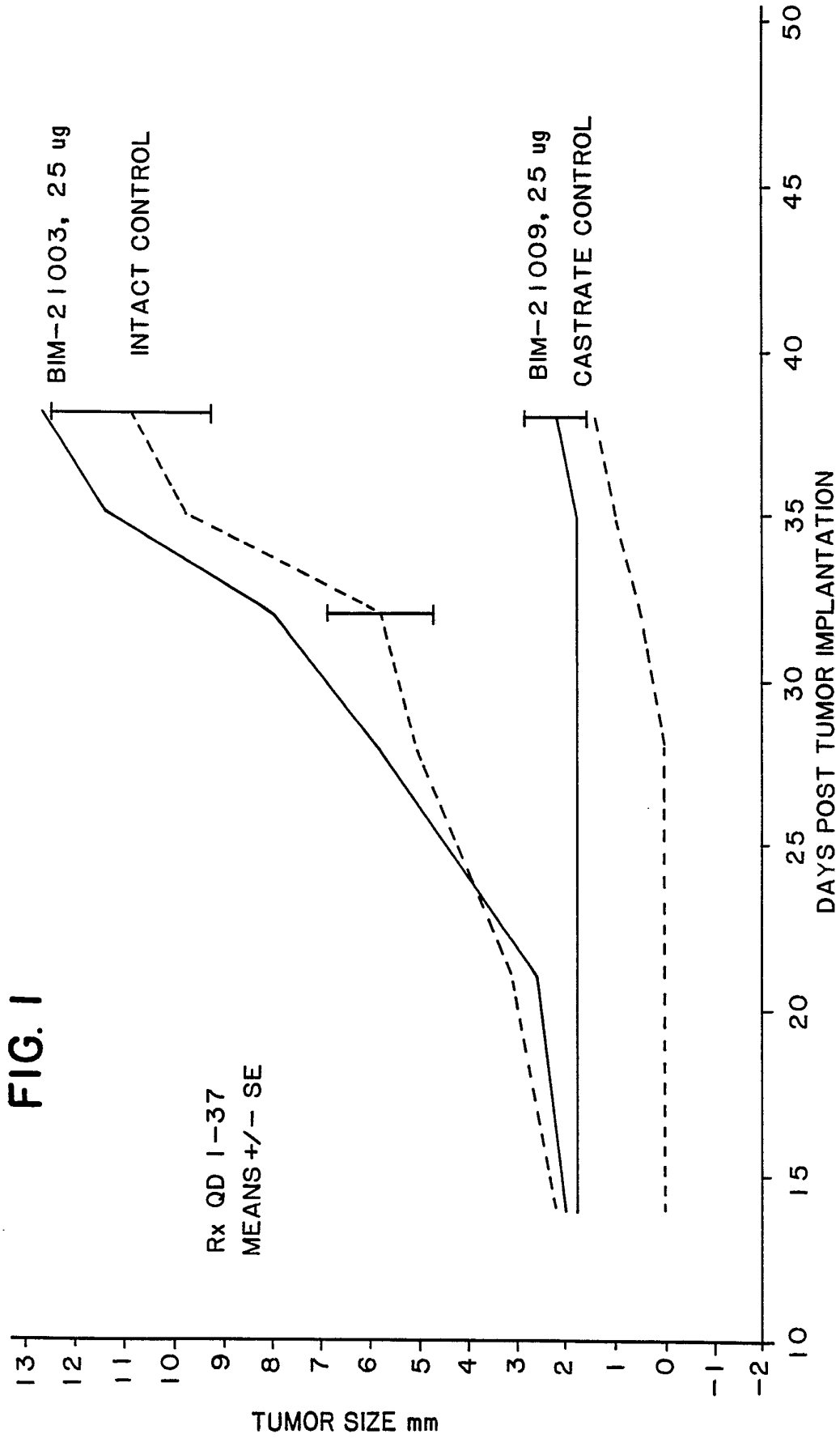
25 18. The composition of claim 16 wherein said
composition is in the form of a pill, tablet, capsule,
liquid, or sustained release tablet for oral administration
to a patient in need of said decapeptide.

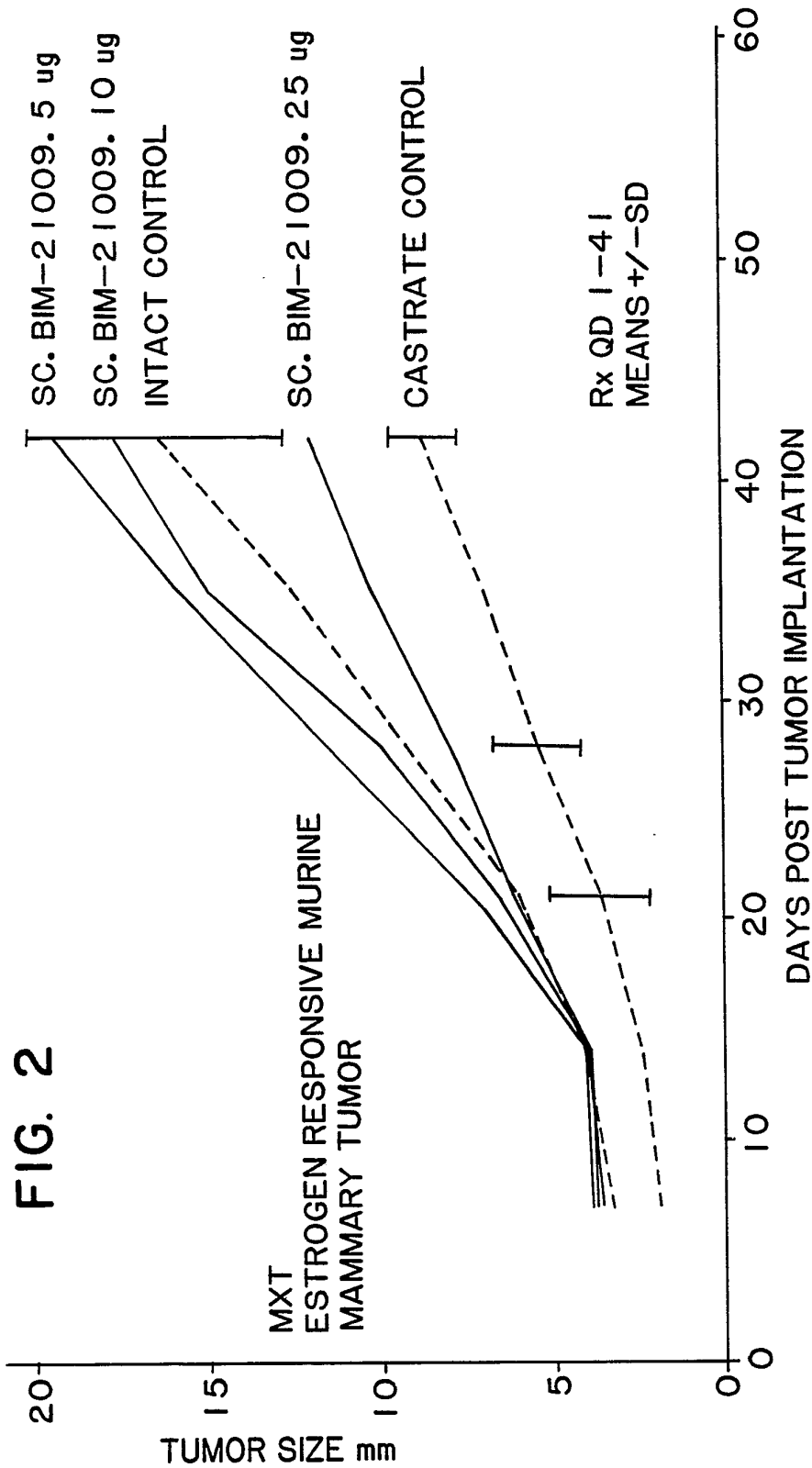
19. The composition of claim 16 wherein said composition is in the form of a liquid capable of being administered intravenously, subcutaneously, parenterally, topically, or intraperitoneally to a patient in need of said
5 decapeptide.

20. The composition of claim 16 wherein said composition is in the form of an injectible suspension comprising said decapeptide and a bioerodible, biocompatible polymer matrix capable of effecting sustained release of
10 said decapeptide.

21. The composition of claim 16 wherein said composition is in the form of a decapeptide/bioerodible, biocompatible implant.

22. The composition of claim 16 wherein said
15 composition is a transdermal patch or transmucosal patch, or a nasal spray.





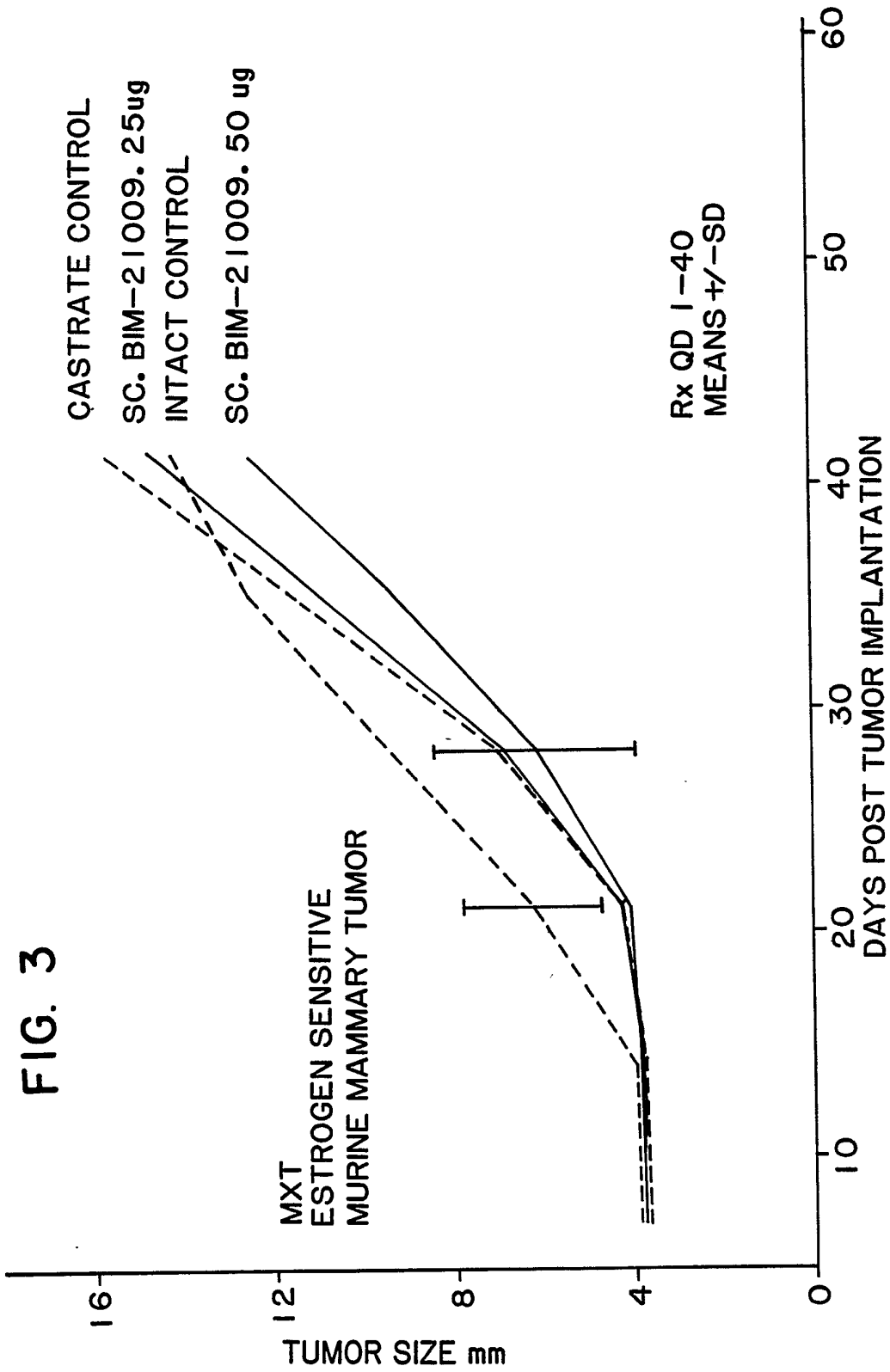
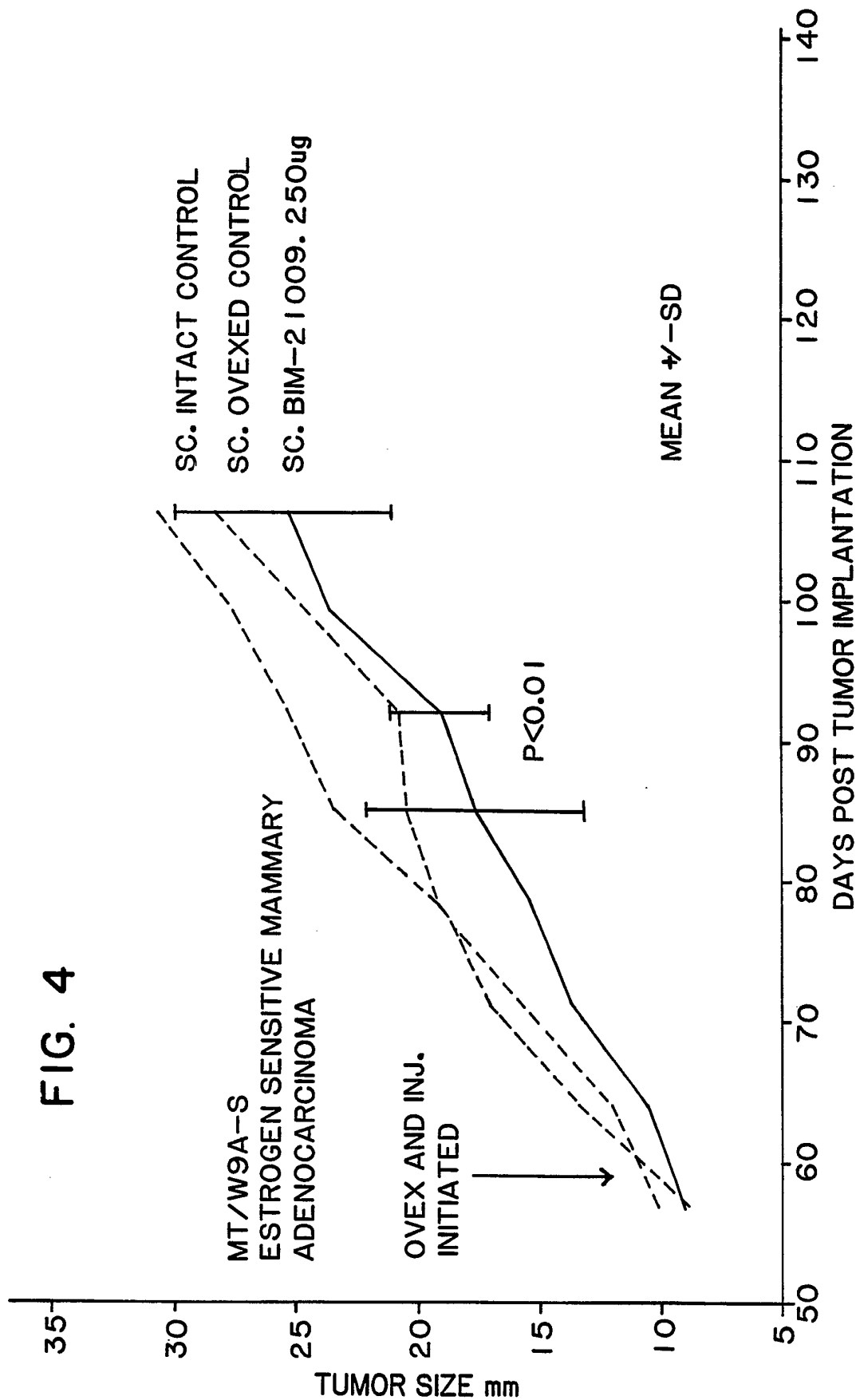
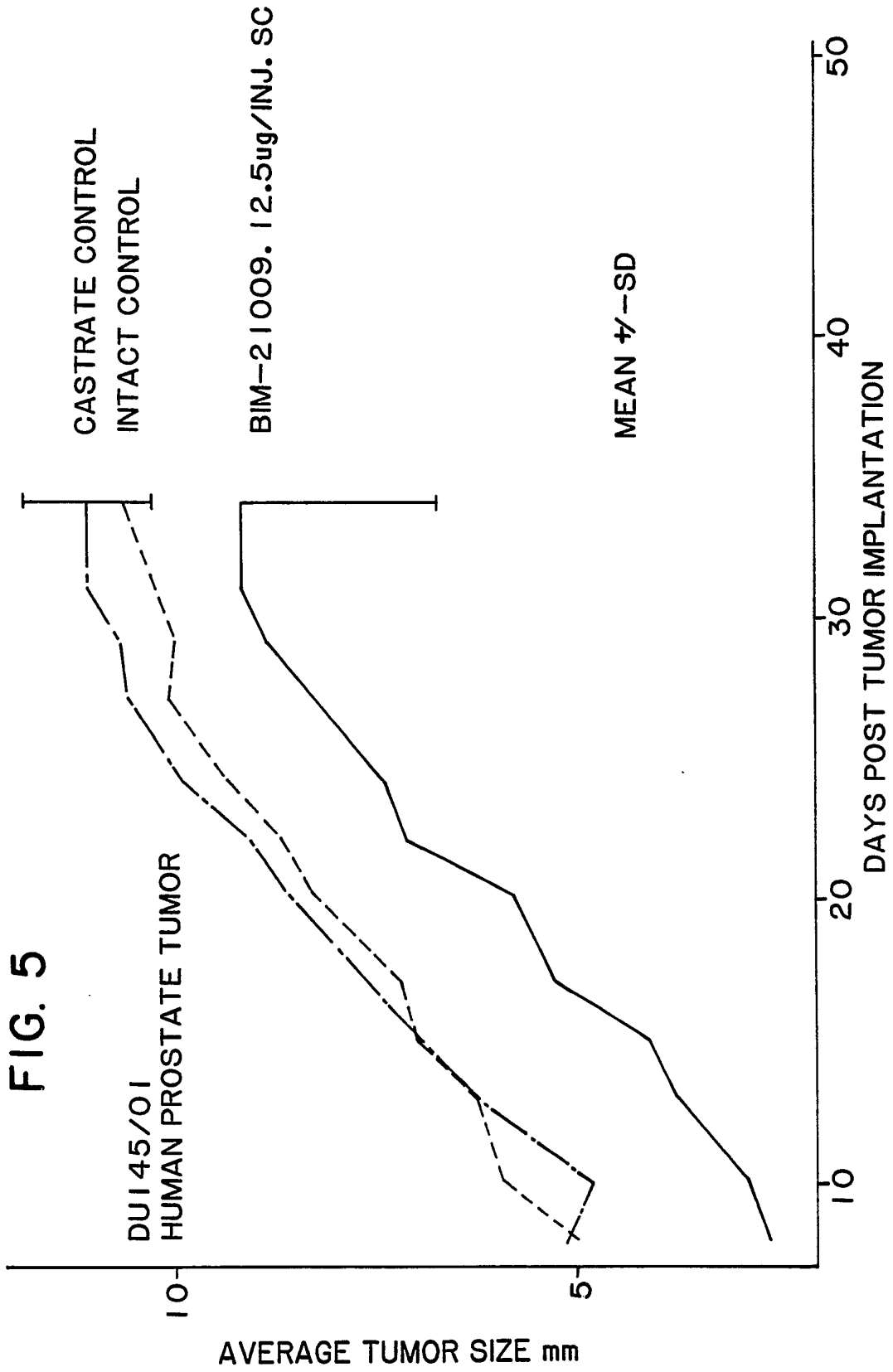


FIG. 4





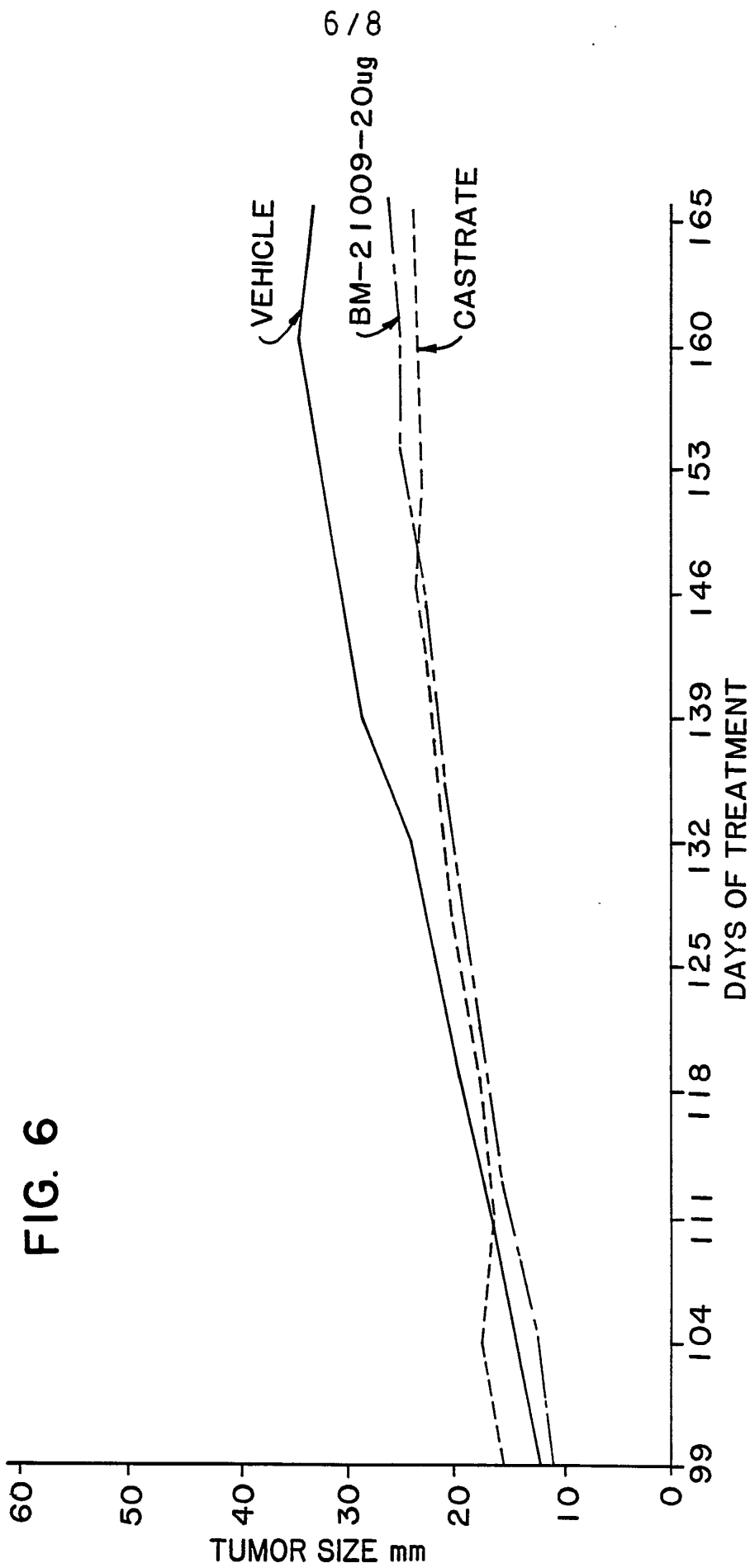
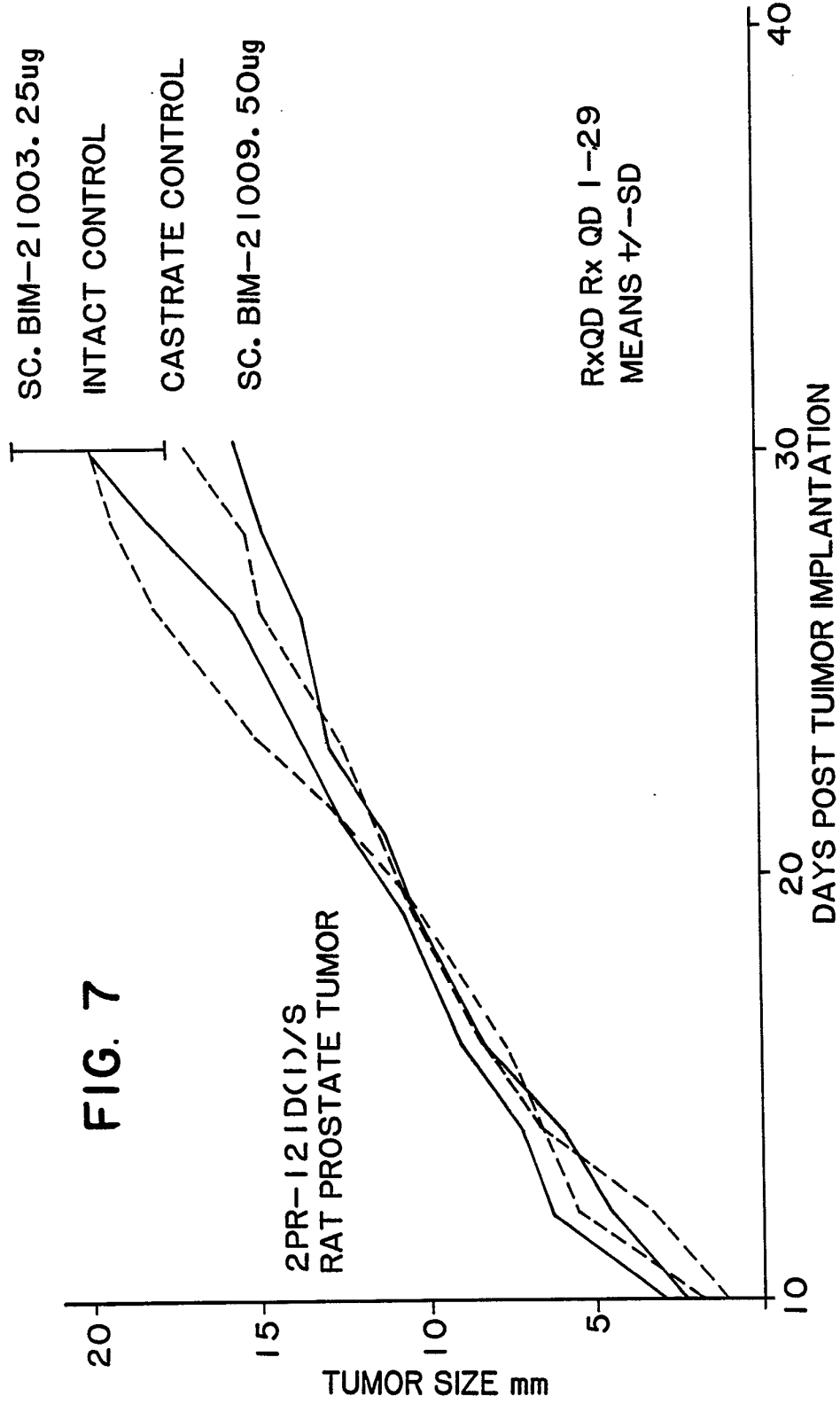
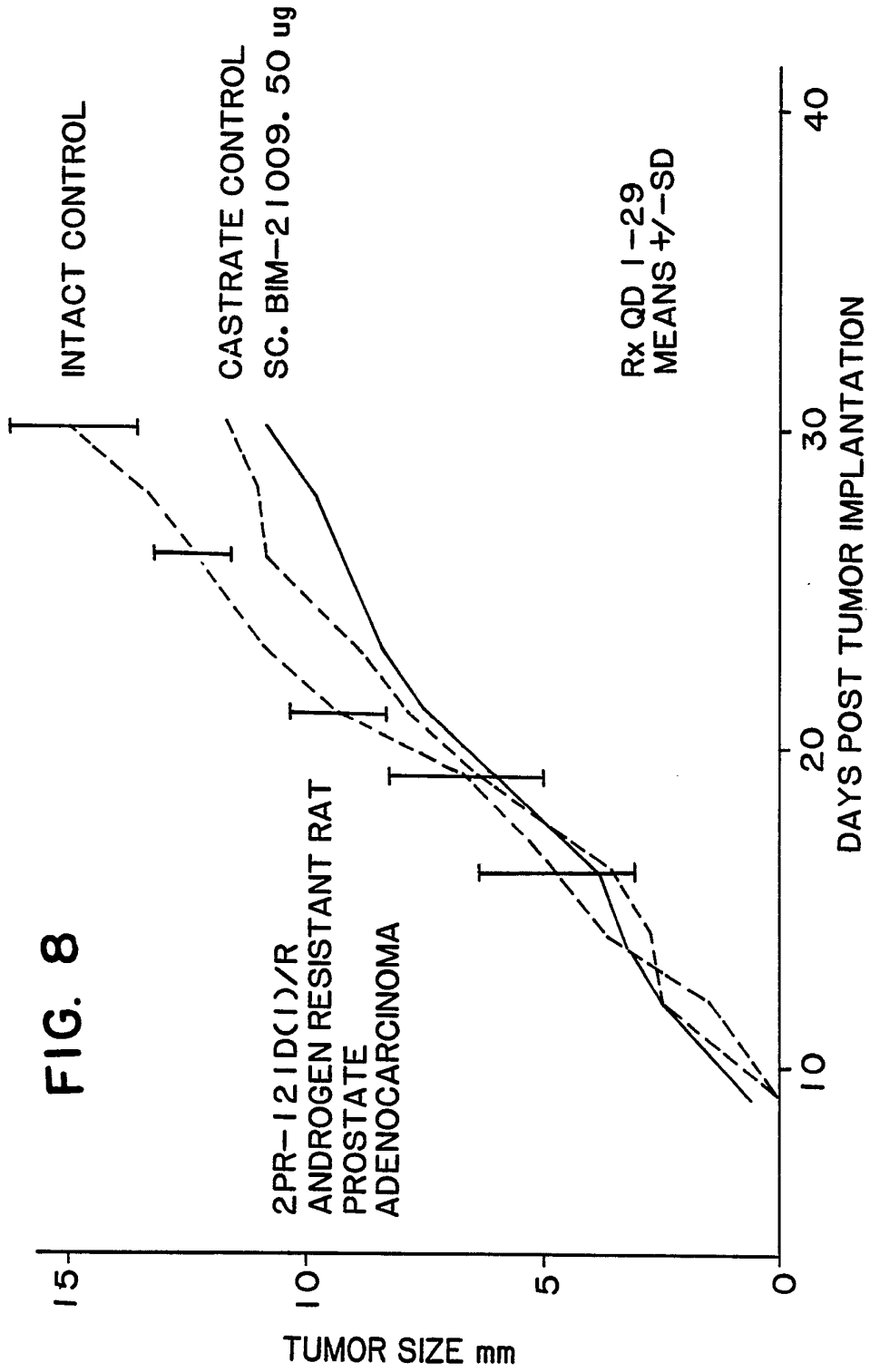


FIG. 6





SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05842

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(5): A61K 37/02; C07K 7/06 USCL: 514/15, 800, 885; 530/328		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System ¹	Classification Symbols	
US	514/15, 800,885 530/328	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
A	US,A, 4,851,385, (Roeske), 25 JULY 1989, see entire document.	
X	US,A, 4,866,160, (Coy et al), 12 September 1989, see col. 1 lines 30-68, col. 2, lines 1-10.	1-22
<p>* Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²		Date of Mailing of this International Search Report ²
16 NOVEMBER 1990		08 FEB 1991
International Searching Authority ¹		Signature of Authorized Officer ²⁰
ISA/US		<i>Sandra Marshall</i> SANDRA MARSHALL