TITLE OF THE INVENTION
POLYMORPHIC FORMS OF A GROWTH HORMONE SECRETAGOGUE

BACKGROUND OF THE INVENTION

Growth hormone, which is secreted from the pituitary, stimulates growth of all tissues of the body that are capable of growing. In addition, growth hormone is known to have the following basic effects on the metabolic processes of the body: (1) Increased rate of protein synthesis in all cells of the body; (2) Decreased rate of carbohydrate utilization in cells of the body; (3) Increased mobilization of free fatty acids and use of fatty acids for energy. A deficiency in growth hormone secretion can result in various medical disorders, such as dwarfism.

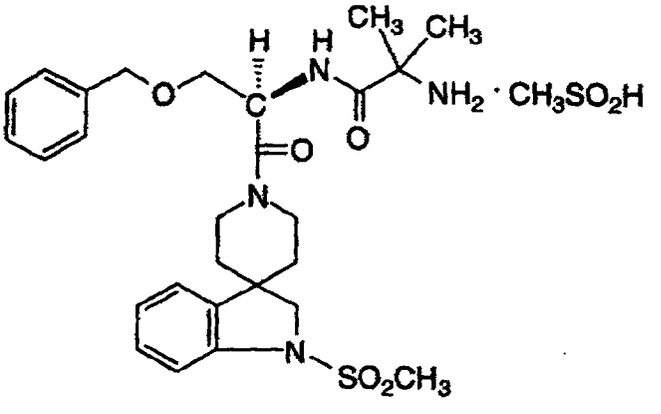
Various ways are known to release growth hormone. For example, chemicals such as arginine, L-3,4-dihydroxyphenyl-alanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth hormone to be released from the pituitary by acting in some fashion on the hypothalamus perhaps either to decrease somatostatin secretion or to increase the secretion of the known secretagogue growth hormone releasing factor (GRF) or an unknown endogenous growth hormone-releasing hormone or all of these.

In cases where increased levels of growth hormone were desired, the problem was generally solved by providing exogenous growth hormone or by administering GRF or a peptidal compound which stimulated growth hormone production and/or release. In either case the peptidyl nature of the compound necessitated that it be administered by injection. Initially the source of growth hormone was the extraction of the pituitary glands of cadavers. This resulted in a very expensive product and carried with it the risk that a disease associated with the source of the pituitary gland could be transmitted to the recipient of the growth hormone. Recombinant growth

hormone has become available which, while no longer carrying any risk of disease transmission, is still a very expensive product which must be given by injection or by a nasal spray. Other compounds have been developed which stimulate the release of endogenous growth hormone.

In particular, certain spiro compounds are disclosed in U.S. Patent No. 5,536,716, PCT Patent Publication WO 94/13696 and Proc. Natl. Acad. Sci. USA, 92, 7001-7005 (July 1995) as being non-peptidal growth hormone secretagogues. These compounds have the ability to stimulate the release of natural or endogenous growth hormone and thus may be used to treat conditions which require the stimulation of growth hormone production or secretion such as in humans with a deficiency of natural growth hormone or in animals used for food or wool production where the stimulation of growth hormone will result in a larger, more productive animal.

Among the preferred compounds disclosed therein is spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which has the structure:



U.S. Patent No. 5,536,716 and PCT Patent Publication WO 94/13696 disclose methods for preparing this compound (see Examples 18, 19 and 55). In particular, Example 55 states that the compound prepared by recrystallization from ethyl acetate-ethanol-water had a melting point of "166-168°C". This compound was subsequently

identified as being of the polymorphic form designated "Form II" herein. Proc. Natl. Acad. Sci. USA, 92, 7001-7005 (July 1995) notes that the compound isolated as a monohydrate had a melting point of 168-170°C, but only discloses very general methods for preparing the compound and does not disclose how the compound was crystallized.

Morphological forms of pharmaceutical compounds may be of interest to those involved in the development of a suitable dosage form because if the morphological form is not held constant during clinical and stability studies, the exact dosage used or measured may not be comparable from one lot to the next. Once a pharmaceutical compound is produces for use, it is important to recognize the morphological form delivered in each dosage form to assure that the production process use the same form and that the same amount of drug is included in each dosage. Therefore, it is imperative to assure that either a single morphological form or some known combination of morphological forms is present. In addition, certain morphological forms may exhibit enhanced thermodynamic or hydroscopic stability and may be more suitable than other morphological forms for inclusion in pharmaceutical formulations. As used herein, a "polymorphic form" of a chemical compound is the same chemical entity, but in a different crystalline arrangement.

SUMMARY OF THE INVENTION

The present invention is concerned with polymorphic forms of the compound: N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate, as well as processes for the preparation of such polymorphic forms.

The present invention is also concerned with pharmaceutical formulations comprising these polymorphic forms as an active ingredient and the use of these polymorphic forms and their formulations in the treatment of certain disorders.

The polymorphic forms of this invention are growth hormone secretagogue that are useful in food animals to promote their

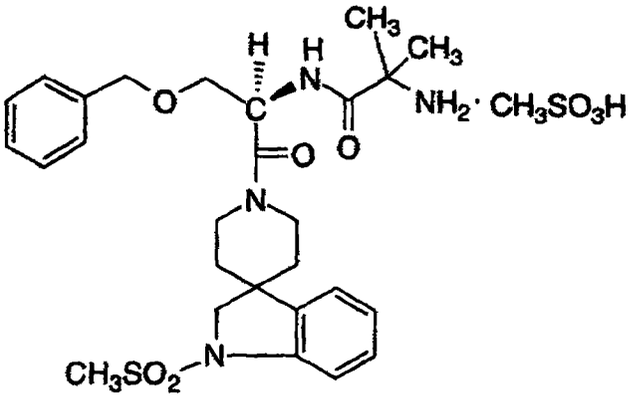
growth thereby rendering the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, and to treat medical conditions which are improved by the anabolic effects of growth hormone.

These polymorphic forms have advantages over the other known forms of N-[1(R)-[(1,2-dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in terms of thermodynamic stability and suitability for inclusion in pharmaceutical formulations.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel polymorphic forms of the compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate and the processes for the preparation of these polymorphic forms.

The compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate has the structure:



and is a growth hormone secretagogue which induces the release of growth hormone in humans and animals. This property can be utilized

to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, and to treat medical conditions which are improved by the anabolic effects of growth hormone.

These particular polymorphic forms (herein designated "Form I", "Form II", "Form III", "Form IV", "Form V", "Form VI", "Form VII","Form VIII","Form IX","Form X",) have superior properties over other crystalline forms of the compound in that they are more suitable for inclusion in pharmaceutical formulations. A preferred crystalline form for pharmaceutical development is Form I based on its thermodynamic stability and non-hygroscopic properties. Another preferred crystalline form for pharmaceutical development is Form IV based on its formulation properties, particularly with respect to compression for tablet preparation. Form IV has been found to have a higher bulk density than other forms.

The present invention is also concerned with a process for the preparation of Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

treating a solution of the free base of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide in ethyl acetate containing ethanol (about 8 volume %) with methanesulfonic acid (about 1.1 equivalents) at approximately 50°C, heating to approximately 55°C, and cooling to approximately 45°C.

Optionally, the temperature subsequently may be raised to approximately 51°C where it is maintained for 2-24 hours.

The present invention is further concerned with an alternate process for the preparation of Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

alternatively adding to a solution of the free base of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide in ethyl acetate containing ethanol (about 8 volume %) at approximately 50-55°C,

methanesulfonic acid (about 1.1 equivalents), and

Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methyl-propanamide methanesulfonate (wherein the relative order of addition is not critical),

followed by heating at approximately 55°C for approximately 2-15 hours, cooling to approximately 25-30°C, and aging for approximately 2-3 hours.

The present invention is further concerned with an alternate process for the preparation of Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

stirring a solution of Form II of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in isopropanol at approximately 25°C for about 2-24 hours.

The present invention is also concerned with a process for the preparation of Form II of N-[1(R)-[(1,2-dihydro-1-methane-sulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenyl-methyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

treating a solution of the free base of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide in ethyl acetate containing ethanol (about 8 volume %) with methanesulfonic acid (about 1.1 equivalents) at approximately 50°C, heating to approximately 55°C, and cooling to ambient temperature.

The present invention is further concerned with a process for the preparation of Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

dissolving N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate of optional morphological composition in a solution of ethanol/water (preferably 25:75 v/v);

evaporating the solvent from the solution, preferably at a temperature of 40°C;

grinding the resultant solid to a fine powder; and

exposing the fine powder to a relative humidity of approximately 75%.

The present invention is further concerned with an alternate process for the preparation of Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

recrystallization of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate of optional morphological composition from a solution of ethylacetate/ethanol/water (preferably 24.8/1.6/1.95 v/v/v).

The present invention is further concerned with an alternate process for the preparation of Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

exposing Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide

methanesulfonate to a relative humidity of greater than about 75%, more preferably a relative humidity of about 88%, at ambient temperature for a sufficient time.

The present invention is further concerned with an alternate process for the preparation of Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

isolation from a slurry of Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in isopropyl acetate/ethanol (90:10 v/v) containing approximately 2.8 wt% water at approximately 25°C.

The present invention is further concerned with a process for the preparation of Form V of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

exposing Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate to below 30% relative humidity at ambient temperature.

The present invention is further concerned with a process for the preparation of Form VI of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

drying Form V of N-[1(R)-[(1,2-dihydro-1-methane-sulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenyl-methyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in the absence of moisture at approximately room temperature, such as under an atmosphere of sieve dried nitrogen at approximately 25°C.

The present invention is further concerned with a process for the preparation of Form VII of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

isolation from a slurry of Form I or Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in isopropyl acetate/ethanol (90:10 v/v) containing approximately 1.5 wt% water.

The present invention is further concerned with a process for the preparation of Form VIII of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

dehydrating Form VII of N-[1(R)-[(1,2-dihydro-1-methane-sulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenyl-methyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate under a dry inert gas, such as dry nitrogen, for a sufficient time.

The present invention is further concerned with a process for the preparation of Form IX of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

preparing a solution of N-[1(R)-[(1,2-dihydro-1-methane-sulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenyl-methyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate of optional morphological composition in water, followed by isolating the solid formed by controlled evaporation at 20% relative humidity at approximately room temperature.

The present invention is further concerned with a process for the preparation of Form X of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-

(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

drying Form IX of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate at ambient room temperature and humidity for a sufficient time.

The present invention is further concerned with an alternate process for the preparation of Form X of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate which comprises:

exposing Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate to 100% relative humidity for approximately 1 to 4 days.

Similarly, the present invention is also directed to a process for the preparation of morphologically homogeneous N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate comprising any of the processes mentioned herein.

The compounds of this invention, the novel polymorphic forms of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate, are growth hormone secretagogues that are useful in food animals to promote their growth thereby rendering the production of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, and to treat medical conditions which are improved by the anabolic effects of growth hormone. Accordingly, the present invention is further concerned with pharmaceutical formulations comprising a polymorphic form as an active ingredient, and the use of this polymorphic form and its formulations in the treatment of certain disorders.

Differential Scanning Calorimeteric Cell [DSC]

The DSC curve for Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate at 10 °C/min in an open cup under nitrogen flow exhibits a single endotherm, due to melting, with a peak temperature of about 180°C and an extrapolated onset temperature (melting point) of about 170°C with an asociated heat of approximately 53 J/g.

The DSC curve for Form II of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate at 10 °C/min in an open cup under under nitrogen flow exhibits a single endotherm, due to melting, with a peak temperature of about 174°C and an extrapolated onset temperature (melting point) of about 165°C with an asociated heat of approximately 37 J/g.

The DSC curve for Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate at 10 °C/min in an open cup under under nitrogen flow exhibits a water loss endotherm at about 45°C followed by an endotherm with a peak temperature of about 134°C and an extrapolated onset temperature (melting point) of about 129°C, due to melting of Form VI, with an asociated heat of approximately 23 J/g.

DSC Data [samples are heated at a rate of 10 °C/min under a nitrogen atmosphere (extrapolated onset temperature)]:

Form I:	170°C (melting endotherm)
Form II:	165°C (melting endotherm)
Form VI:	129°C (melting endotherm)

Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is a relatively anhydrous polymorph characterized by the following properties: a melting point of 169°C and solubility in isopropanol of 4.6 mg/mL.

Form II of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is an anhydrous polymorph characterized by the following properties: a melting point of 158°C and solubility in isopropanol of 12.3 mg/mL.

Form III of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is a hydrate characterized by the following properties: a water loss endotherm at a peak temperature of 46°C, followed by a minor melting/decomposition endotherm with an extrapolated onset temperature of 123°C.

Form IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is a hydrate characterized by the following properties:

a water loss endotherm at a peak temperature of 45°C, followed by a melting/decomposition endotherm with an extrapolated onset temperature of 129°C (presumably due to the melting/ decomposition of Form VI).

Form IV N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate appears to be a hygroscopic hydrate containing 3.5 moles of water per mole of

N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate.

Form V N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate appears to be a hygroscopic hydrate containing 1 moles of water per mole of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate.

Form VI N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate appears to be an anhydrous polymorph and is characterized by a melting point of 129°C.

Form VII of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is a hydrate characterized by the following properties: a broad water loss endotherm at a peak temperature of 60°C, followed by a melting/decomposition endotherm with an extrapolated onset temperature of 144°C (presumably due to the melting/decomposition of Form VIII).

Form VIII N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is an anhydrous polymorph characterized by a melting point of 144°C.

Form X of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-

oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is characterized by a broad water loss endotherm at a peak temperature of 49°C.

X-Ray Powder Diffraction (XRPD)

X-Ray powder diffraction studies are widely used to elucidate molecular structures, crystallinity and polymorphism. X-ray powder diffraction (XRPD) patterns were collected using a Philips APD3720 Automated Powder Diffraction instrument with copper Kα radiation. Measurements were made from 2° to 40° (2 theta) with the sample maintained at ambient room temperature.

Form I was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 6.5, 14.7, 16.9, 17.1, 17.9, 19.5, 21.1, 21.7, and 22.0° (2 theta).

Form II was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 4.8, 11.8, 17.5, 19.4, 21.6, 21.9, 22.5, and 22.7° (2 theta).

Form III was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 13.8, 14.1, 18.0, 18.8, 19.5, 20.1, 20.6, 21.8, and 25.7° (2 theta).

Form IV was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 16.0, 16.2, 18.3, 20.1, 21.0, and 24.2° (2 theta).

Form V was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 14.8, 17.1, 17.6, 19.0, 19.1, 19.4, 20.6, 21.5, and 21.8° (2 theta).

Form VI was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 9.8, 14.0, 14.8, 17.1, 17.6, 19.0, 19.5, 20.6, and 21.6° (2 theta).

Form VII was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 9.1, 11.3, 17.1, 17.4, 20.0, 22.1, and 24.5° (2 theta).

Form VIII was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 11.5, 11.6, 18.1, 19.6, 22.5, 24.7, and 24.8° (2 theta).

Form IX was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 8.0, 12.1, 15.3, 15.8, 19.6, 19.7, 21.1, 22.3, and 23.7° (2 theta).

Form X was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 15.5, 15.8, 18.0, 18.4, 18.6, 19.4, 20.7, 20,8, 23.9, and 24.8° (2 theta).

These XRPD patterns confirm that all of Forms I - X are distinct crystalline forms.

Microscopy

Examination of the polymorphic forms was conducted at 100X magnification under plain and polarized light. Form I and Form II were needle shaped particles. Both Form I and Form II appeared birefringent under polarized light.

Hygroscopicity

The total volatiles content (as established by TGA analysis) of solid samples of Forms I, II, III and IV upon exposure to various controlled humidities is tabulated below. It was found that Form I possessed 0.79 wt % water; Form II possessed 0.56 wt % water; Form III possessed 4.5 - 5.0 wt % water; and Form IV possessed 9.5 - 10.0 wt % water.

Hygroscopicity was assessed by storing the solid compound in constant relative humidity chambers. A comparison of anhydrous Forms I and II at room temperature indicates Form II is hygroscopic and exhibits a large increase in moisture starting at 65% RH. No substantial moisture increase is exhibited by Form I except when stored above 76% RH. The results are tabulated below in Tables 1 and 2.

TABLE 1

Room Temperature (48 Hrs)

	Form I	Form II
% RH	% Gain/Loss	% Gain/Loss
0	-0.02	+0.08
11	-0.05	+0.02
33	-	+0.33
47	+0.21	+0.39
65	+0.37	>10.0[a]
76	+0.12	>10.0[a]
100	>12.0	>10.0[a,b]

([a] = Sample becomes a gummy semi-solid)

([b] = Converts to Form IV upon exposure to ambient humidity)

TABLE 2

Room Temperature (96 Hrs)

	Form I
% RH	% Gain/Loss
7	+0.4
22	+0.3
47	+0.5
68	+0.6
88	+12.9*
100	+23.9*

(* = deliquescent material)

Hydrated forms III and IV of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate were also evaluated by storage in relative humidity chambers for 48 hrs at room temperature. Form III shows an increase in moisture after 48-hr storage at 33% RH. Form IV, although not gaining an appreciable amount of moisture until storage at 100% RH, loses its water of hydration when stored at 11% RH or below. The results are tabulated below in Table 3.

TABLE 3

Room Temperature (48 Hrs)

	Form III	Form IV
% RH	% Gain/Loss	% Gain/Loss
0	-	-7.8
11	-0.15	-4.9
33	+2.23	+0.3
47	+3.99	+0.7
66	-	+1.4
76	+3.76	+1.5
100	-	+5.3

This data indicates that Form I is relatively anhydrous.

Solubility

The solubility of Form I in distilled water at room temperature is >100 mg/mL. The aqueous solubility (RT) of Form II in buffered solutions (pH 4-9) is >100 mg/mL. The solubility of Form I in ethanol/water mixtures is shown below:

% Ethanol/H_2O	Solubility (mg/mL)
25/75	>100
50/50	>100
75/25	>90
100% ethanol	>90

Thermal Stability - Neat Compound

The solid state stability of neat compound was assessed by storage of the drug in glass screw-cap vials in the dark. Samples were assayed by HPLC and the parent compound was quantitatively analyzed. The isocratic method that was employed is outlined below:

Column: Beckman Ultrasphere ODS (250x4.6 mm, 5μ)
Mobile Phase: 0.1% TEA, pH 4.0 with H_3PO_4:Acetonitrile (65:35)
Flow Rate: 1.0 mL/min
Detection Wavelength: 228 nm
Run Time: 14 minutes
Column Temperature: Ambient
Injection Volume: 20 μL of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (50 μg/mL)

The results shown below for Forms I and II were calculated as weight percent.

Form I

	% Inital	
℃	6 Wks	17 Wks
40	100.1	98.7
60	100.7	101.3
80	100.7	99.4

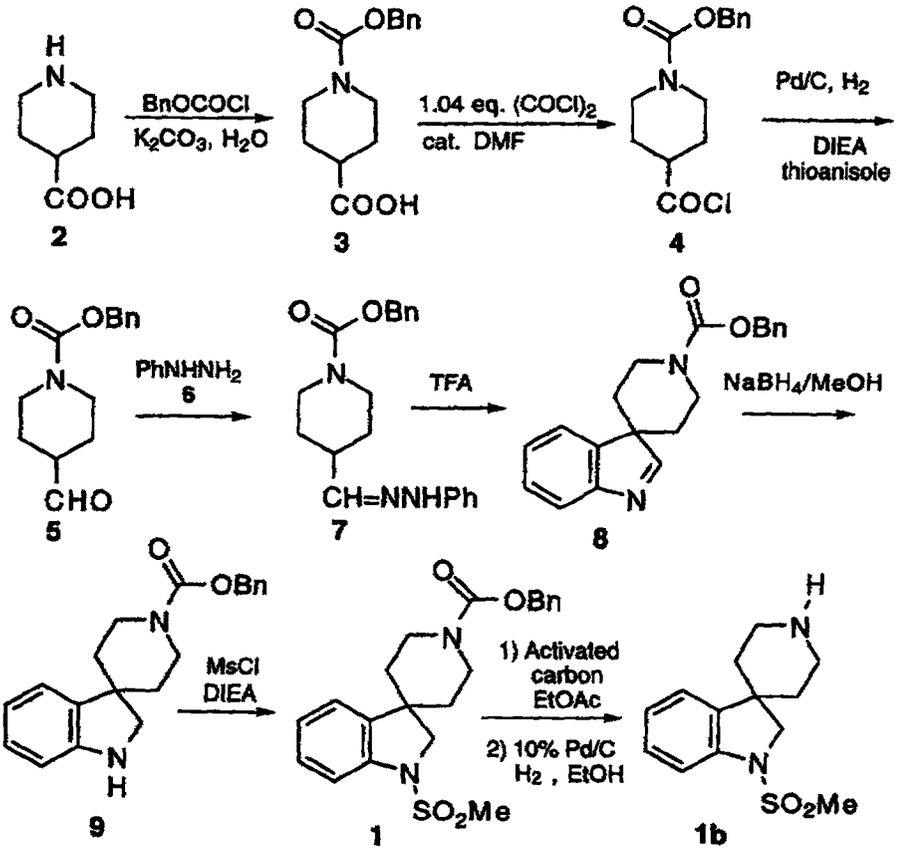
Form II

	% Initial					
℃	1 Wk	2 Wks	4 Wks	8 Wks	12 Wks	24 Wks
40	99.7	100.7	99.5	--	100.1	100.1
60	99.4	99.6	100.3	100.0	101.0	100.8
80	99.2	100.3	99.2	99.8	100.4	--

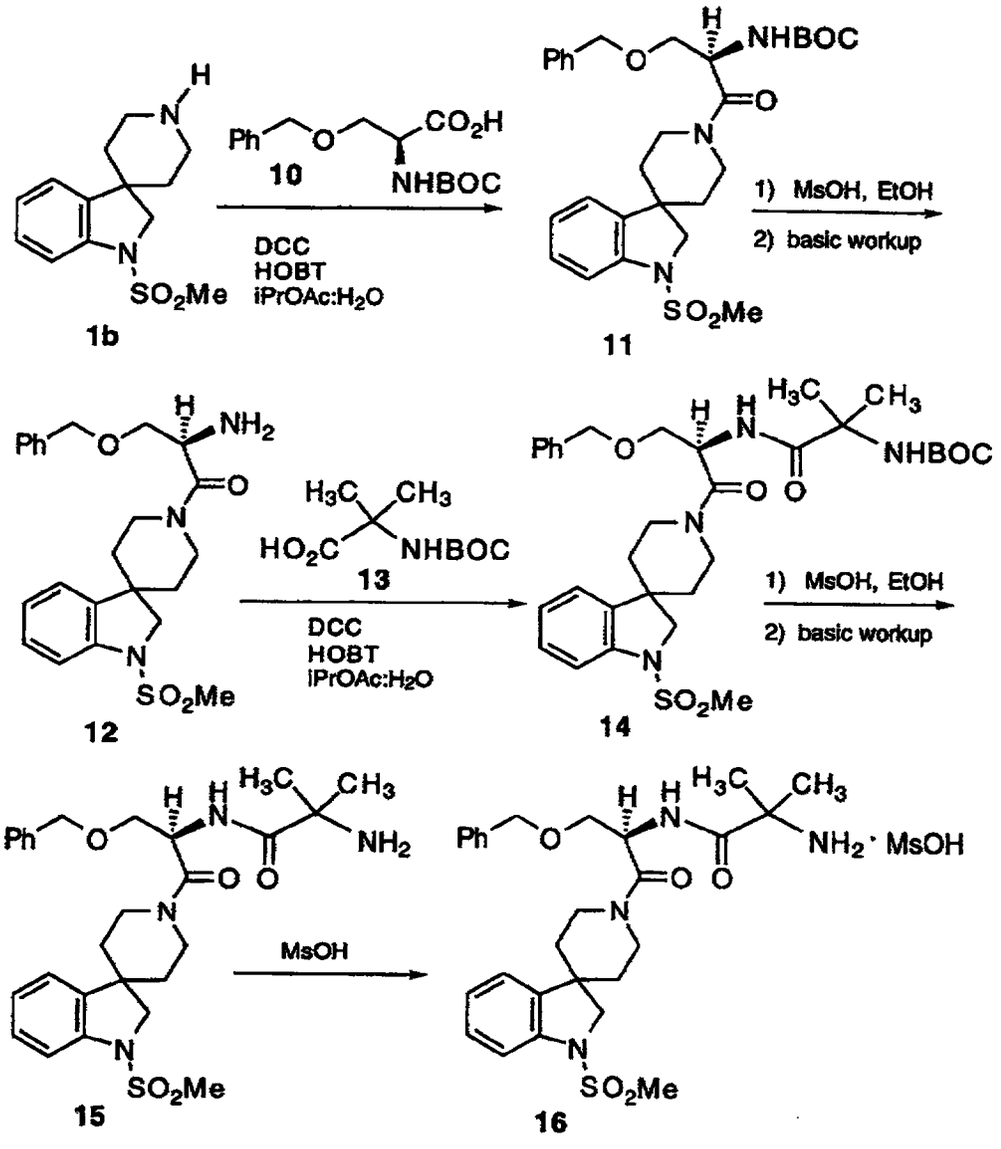
These results indicate that neat solid Form I and Form II had good thermal stability.

The processes to prepare the subject compound are outlined as follows:

SCHEME I:



SCHEME I (CONT'D)



As depicted in Scheme I, the CBZ-Spiroindoline 1 is treated with Darco (20% by weight) prior to hydrogenation. The hydrogenation is carried out in ethanol at 65°C over 10% Pd/C with vigorous stirring.

A solution of 1b in isopropyl acetate and water is coupled with commercially available N-BOC-O-benzyl-D-serine in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). After filtration of the dicyclohexylurea (DCU) side product, the 2-phase filtrate is separated and the organic layer is washed successively with 1M aqueous sodium hydroxide solution, 0.5M aqueous hydrochloric acid and finally saturated aqueous sodium hydrogen carbonate. Improved results in this coupling are achieved when a solution of the free amino in iPrOAc/H_2O is treated with DCC, HOBT followed by addition of the amino acid at ambient temperature and followed by reaction for 3-5 hrs The batch is then concentrated *in vacuo* and the solvent is switched from isopropyl acetate to ethanol. This solvent switch generally proceeds swiftly by "feeding and bleeding" 3x batch volumes to remove isopropyl acetate..

The BOC-group of 11 is removed by treatment with methanesulfonic acid (MsOH) (3 eq) in ethanol at 35-40°C. Partitioning between isopropyl acetate and aqueous 1M sodium hydroxide solution affords 12.

The coupling of 12 with N-BOC-α-aminoisobutyric acid is best conducted in a two-phase solvent system, isopropyl acetate/water (1:1) in the presence of DCC and HOBt (1.1 eq. each). Removal of the DCU by filtration, separation of the layers and washing the organic layer successively with 1M aqueous sodium hydroxide, 0.5M aqueous hydrochloric acid and saturated aqueous sodium hydrogen carbonate affords 14.

The mixture is solvent switched to ethanol for the subsequent methanesulfonic acid cleavage of the Boc group. Deprotection of 14 is more difficult than that of 11 and requires a concentrated solution of ethanol/methanesulfonic acid and heating to 35-40°C. After extractive workup (EtOAc-NaOH), the free amine 15 is

isolated. The organic layer is washed well with 1N NaOH to ensure complete removal of methanesulfonic acid.

The ethyl acetate solution of the free base 15 is concentrated to low bulk *in vacuo* and is azeotroped dry (KF <500 $mgml^{-1}$) by "feeding and bleeding" 2x batch volumes of 90/10, ethyl acetate/ethanol followed by 2x batch volumes of ethyl acetate. The resulting dry, slightly hazy solution of the free base 15 in ethyl acetate is treated with Darco G-60 (25 weight %) at room temperature for about 10 hours. Removal of the Darco by filtration with a filtration agent gives the free base 15.

Formation of the methanesulfonic acid salt 16 from 15 is carried out in EtOAc with 1.1 eq of MsOH at about 50°C. The free base 15 is treated with 8 volume % of EtOH and 1 eq of H_2O and heated to 55°C until complete dissolution. Cooling to ambient temperature and stirring the resulting slurry for 4 hours gives crystalline material of 16 designated as crystal Form II [solubility in IPA = 12 mg/mL].

The conversion of Form II to Form I is accomplished where the salt is formed in EtOAc-EtOH as above, but instead of cooling the initial solution of the salt (at 55°C) to ambient temperature, it is cooled to 45°C. Crystals should start appearing at that temperature and the slurry should become thicker with time. The temperature is then raised to 51°C and the slurry is aged overnight. Complete conversion to Form I of 16 should be expected.

Preferably, the conversion of Form II to Form I is achieved by adding seed crystals of Form I to a solution of the free base in EtOAc-EtOH at 50-55°C followed by aging. Accordingly, the free base 15 may be treated with 1.1 equivs. of methanesulfonic acid in 8% ethanol in ethyl acetate at 50-55°C. The batch is then seeded with approximately 2% by weight of Form I of the methanesulfonate salt 16, and then aged at 55°C overnight. The batch is cooled to room temperature and aged for approximately 2-3 hours. The product is isolated by filtration at room temperature under a nitrogen atmosphere, dried at 35°C *in vacuo* and sieved to give the methanesulfonate salt 16.

The methanesulfonic acid salt 16 may also be formed by alternating the stepwise addition of MsOH (1.1 eq) and seed crystals of Form I to a solution of the free base in EtOAc-EtOH at about 50°C, wherein the order of addition of the MsOH and the seed is not critical.

The utility of the polymorphic compounds of the present invention as growth hormone secretagogues may be demonstrated by methodology known in the art, such as an assay described by Smith , et al., Science, 260, 1640-1643 (1993) (see text of Figure 2 therein). In particular, all of the polymorphic forms of the present invention had activity as growth hormone secretagogues in the aforementioned assay. Such a result is indicative of the intrinsic activity of the polymorphic forms of the present invention as growth hormone secretagogues.

The growth hormone releasing compounds of the present invention are useful *in vitro* as unique tools for understanding how growth hormone secretion is regulated at the pituitary level. This includes use in the evaluation of many factors thought or known to influence growth hormone secretion such as age, sex, nutritional factors, glucose, amino acids, fatty acids, as well as fasting and non-fasting states. In addition, the compounds of this invention can be used in the evaluation of how other hormones modify growth hormone releasing activity. For example, it has already been established that somatostatin inhibits growth hormone release. Other hormones that are important and in need of study as to their effect on growth hormone release include the gonadal hormones, e.g., testosterone, estradiol, and progesterone; the adrenal hormones, e.g., cortisol and other corticoids, epinephrine and norepinephrine; the pancreatic and gastrointestinal hormones, e.g., insulin, glucagon, gastrin, secretin; the vasoactive peptides, e.g., bombesin, the neurokinins; and the thyroid hormones, e.g., thyroxine and triiodothyronine. The compounds of the present invention may also be employed to investigate the possible negative or positive feedback effects of some of the pituitary hormones, e.g., growth hormone and endorphin peptides, on the pituitary to modify growth hormone release. Of particular scientific importance is the use

of these compounds to elucidate the subcellular mechanisms mediating the release of growth hormone.

The compounds of the present invention may be administered to animals, including man, to release growth hormone *in vivo*. For example, the compounds can be administered to commercially important animals such as swine, cattle, sheep and the like to accelerate and increase their rate and extent of growth, to improve feed efficiency and to increase milk production in such animals. In addition, these compounds can be administered to humans *in vivo* as a diagnostic tool to directly determine whether the pituitary is capable of releasing growth hormone. For example, the compounds of the present invention can be administered *in vivo* to children. Serum samples taken before and after such administration can be assayed for growth hormone. Comparison of the amounts of growth hormone in each of these samples would be a means for directly determining the ability of the patient's pituitary to release growth hormone.

Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of the present invention in association with a pharmaceutical carrier or diluent. Optionally, the active ingredient of the pharmaceutical compositions can comprise an anabolic agent in addition to at least one of the compounds of the present invention or another composition which exhibits a different activity, e.g., an antibiotic growth permittant or an agent to treat osteoporosis or in combination with a corticosteroid to minimize the catabolic side effects or with other pharmaceutically active materials wherein the combination enhances efficacy and minimizes side effects.

Growth promoting and anabolic agents include, but are not limited to TRH, diethylstilbesterol, estrogens, β-agonists, theophylline, anabolic steroids, enkephalins, E series prostaglandins, compounds disclosed in U.S. Patent No. 3,239,345, e.g., zeranol, and compounds disclosed in U.S. Patent No. 4,036,979, e.g., sulbenox or peptides disclosed in U.S. Patent No. 4,411,890.

A still further use of the compounds of this invention is in combination with other growth hormone secretagogues such as the growth hormone releasing peptides GHRP-6, GHRP-1 as described in U.S. Patent Nos. 4,411,890 and publications WO 89/07110, WO 89/07111 and B-HT920 as well as hexarelin and GHRP-2 as described in WO 93/04081 or growth hormone releasing hormone (GHRH, also designated GRF) and its analogs or growth hormone and its analogs or somatomedins including IGF-1 and IGF-2 or α-adrenergic agonists such as clonidine or serotonin 5HTID agonists such as sumitriptan or agents which inhibit somatostatin or its release such as physostigmine and pyridostigmine. In particular, the compounds of this invention may be used in combination with growth hormone releasing factor, an analog of growth hormone releasing factor, IGF-1, or IGF-2. For example, a compound of the present invention may be used in combination with IGF-1 for the treatment or prevention of obesity. In addition, a compound of this invention may be employed in conjunction with retinoic acid to improve the condition of musculature and skin that results from intrinsic aging.

The present invention is further directed to a method for the manufacture of a medicament for stimulating the release of growth hormone in humans and animals comprising combining a polymorphic form of the present invention with a pharmaceutical carrier or diluent.

As is well known to those skilled in the art, the known and potential uses of growth hormone are varied and multitudinous. The administration of the compounds of this invention for purposes of stimulating the release of endogenous growth hormone can have the same effects or uses as growth hormone itself. These varied uses of the present compounds thus may be summarized as follows: stimulating growth hormone release in elderly humans; treating growth hormone deficient adults; prevention of catabolic side effects of glucocorticoids; treatment of osteoporosis; stimulation of the immune system, acceleration of wound healing; accelerating bone fracture repair; treatment of growth retardation; treating acute or chronic renal failure or insufficiency; treatment of physiological short stature, including

growth hormone deficient children; treating short stature associated with chronic illness; treatment of obesity and growth retardation associated with obesity; treating growth retardation associated with Prader-Willi syndrome and Turner's syndrome; accelerating the recovery and reducing hospitalization of burn patients or following major surgery such as gastrointestinal surgery; treatment of intrauterine growth retardation, and skeletal dysplasia, treatment of peripheral neuropathies; replacement of growth hormone in stressed patients; treatment of osteochondrody-splasias, Noonans syndrome, schizophrenia, depression, Alzheimer's disease, delayed wound healing, and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of protein catabolic response after a major operation; treating malabsorption syndromes; reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; accelerating weight gain and protein accretion in patients on TPN (total parenteral nutrition); treatment of hyperinsulinemia including nesidioblastosis; adjuvant treatment for ovulation induction and to prevent and treat gastric and duodenal ulcers; to stimulate thymic development and prevent the age-related decline of thymic function; adjunctive therapy for patients on chronic hemodialysis; treatment of immunosuppressed patients and to enhance antibody response following vaccination; increasing the total lymphocyte count of a human, in particular, increasing the T4/T8-cell ratio in a human with a depressed T4/T8-cell ratio resulting, for example, from physical trauma, such as closed head injury, or from infection, such as bacterial or viral infection, especially infection with the human immunodeficiency virus; improvement in muscle strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling, and cartilage growth; stimulation of the immune system in companion animals and treatment of disorders of aging in companion animals; growth promotant in livestock; and stimulation of wool growth in sheep. Further, the instant compounds are useful for increasing feed efficiency, promoting growth, increasing milk production and improving the carcass quality of

livestock. Likewise, the instant compounds are useful in a method of treatment of diseases or conditions which are benefited by the anabolic effects of enhanced growth hormone levels that comprises the administration of an instant compound.

In particular, the instant compounds are useful in the prevention or treatment of a condition selected from the group consisting of: osteoporosis; catabolic illness; immune deficiency, including that in individuals with a depressed T4/T8 cell ratio; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and protein loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or burns, in a patient in need thereof.

In addition, the instant compounds may be useful in the treatment of illnesses induced or facilitated by corticotropin releasing factor or stress- and anxiety-related disorders, including stress-induced depression and headache, abdominal bowel syndrome, immune suppression, HIV infections, Alzheimer's disease, gastrointestinal disease, anorexia nervosa, hemorrhagic stress, drug and alcohol withdrawal symptoms, drug addiction, and fertility problems.

It will be known to those skilled in the art that there are numerous compounds now being used in an effort to treat the diseases or therapeutic indications enumerated above. Combinations of these therapeutic agents some of which have also been mentioned above with the growth hormone secretagogues of this invention will bring additional, complementary, and often synergistic properties to enhance the growth promotant, anabolic and desirable properties of these various therapeutic agents. In these combinations, the therapeutic agents and the growth hormone secretagogues of this invention may be independently present in dose ranges from one one-hundredth to one times the dose levels which are effective when these compounds and secretagogues are used singly.

Combined therapy to inhibit bone resorption, prevent osteoporosis and enhance the healing of bone fractures can be illustrated

by combinations of bisphosphonates and the growth hormone secretagogues of this invention. The use of bisphosphonates for these utilities has been reviewed, for example, by Hamdy, N.A.T., Role of Bisphosphonates in Metabolic Bone Diseases, Trends in Endocrinol. Metab., 4, 19-25 (1993). Bisphosphonates with these utilities include alendronate, tiludronate, dimethyl-APD, risedronate, etidronate, YM-175, clodronate, pamidronate, and BM-210995. According to their potency, oral daily dosage levels of the bisphosphonate of between 0.1 mg and 5 g and daily dosage levels of the growth hormone secretagogues of this invention of between 0.01 mg/kg to 20 mg/kg of body weight are administered to patients to obtain effective treatment of osteoporosis.

In the case of alendronate daily oral dosage levels of 0.1 mg to 50 mg are combined for effective osteoporosis therapy with 0.01 mg/kg to 20 mg/kg of the growth hormone secretagogues of this invention. Osteoporosis and other bone disorders may also be treated with compounds of this invention in combination with calcitonin, estrogens, raloxifene and calcium supplements such as calcium citrate.

Anabolic effects especially in the treatment of geriatric male patients are obtained with compounds of this invention in combination with anabolic steroids such as oxymetholone, methyltesterone, fluoxymesterone and stanozolol.

The compounds of this invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual, or topical routes of administration and can be formulated in dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage

forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax.

Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

The compound of this invention may be administered to patients (animals and humans) in need of such treatment in dosages that will provide optimal pharmaceutical efficacy. It will be appreciated that the dose required for use in any particular application will vary from patient to patient, not only with the particular compound or composition selected, but also with the route of administration, the nature of the condition being treated, the age and condition of the patient, concurrent medication or special diets then being followed by the patient, and other

factors which those skilled in the art will recognize, with the appropriate dosage ultimately being at the discretion of the attendant physician.

The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. Generally, dosage levels of between 0.0001 to 10 mg/kg. of body weight daily are administered to patients and animals, e.g., mammals, to obtain effective release of growth hormone. Preferably, the dosage level will be about 0.001 to about 25 mg/kg per day; more preferably about 0.01 to about 10 mg/kg per day.

Methods for preparing the polymorphic forms of the present invention are illustrated in the following Examples. The following examples are given for the purpose of illustrating the present invention and shall not be construed as being limitations on the scope or spirit of the instant invention.

EXAMPLE 1

N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide

Step A: 1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdine]hydrochloride

To a solution of 1.20 g (5.8mmol) of 1'-methyl-1,2-dihydro-spiro[3H-indole-3,4'-piperdine] (prepared as described by H. Ong, et al., J. Med. Chem., 23, 981-986 (1983)) in 20 mL of dry dichloromethane at 0°C was added triethylamine (0.90 mL; 6.4 mmol) and methanesulfonyl chloride (0.49 mL; 6.35 mmol) and stirred for 30 min. The reaction mixture was poured into 15 mL of saturated aqueous sodium bicarbonate solution and extracted with dichloromethane (2X10 mL). The combined organics were washed with brine (20 mL), dried over anhydrous potassium carbonate, filtered and the solvent removed under reduced pressure to yield 1.44 g of the methanesulfonamide derivative as pale yellow oil which was used without purification.

To a solution of above crude product in 20 mL of dry 1,2-dichloroethane at 0°C was added 1.0 mL (9.30 mmol) of 1-chloroethyl chloroformate, and then stirred at RT for 30 min and finally at reflux for 1h. The reaction mixture was concentrated to approximately one third of the volume and then diluted with 20 mL of dry methanol and refluxed for 1.5h. The reaction was cooled to RT and concentrated to approximately one half of the volume. The precipitate was filtered and washed with a small volume of cold methanol. This yielded 1.0 g of the piperidine HCl salt as a white solid. The filtrate was concentrated and a small volume of methanol was added followed by ether. The precipitated material was once again filtered, washed with cold methanol, and dried. This gave an additional 0.49 g of the desired product. Total yield 1.49 g (70%).

^{1}H NMR ($CDCl_3$, 200MHz) δ 7.43-7.20 (m, 3H), 7.10 (dd, 1H), 3.98 (bs, 2H), 3.55-3.40 (bd, 2H), 3.35-3.10 (m, 2H), 2.99 (s, 3H), 2.15 (t, 2H), 2.00 (t, 2H).

Step B: N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-[(1,1-dimethylethoxy)carbonyl]amino-2-methyl-propanamide

To 0.35g (1.15 mmol) of (2R)-2-[(1,1-dimethylethoxy)-carbonyl]amino-3-[2-(phenylmethyloxy)ethyl]-1-propanoic acid in 13 mL of dichloromethane was added 1,2-dihydro-1-methanesulfonylspiro-[3H-indole-3,4'-piperdine] hydrochloride (0.325 g; 1.07 mmol), 0.18 mL (1.63 mmol) of N-methylmorpholine, 0.159 g (1.18 mmol) of 1-hydroxybenztriazole(HOBT) and stirred for 15 min. EDC (0.31 g; 1.62 mol) was added and stirring was continued for 1h. An additional 60 µL of N-methylmorpholine was added and stirred for 45 min. The reaction mixture was poured into 5 mL of water and the organic layer was separated. The organic layer was washed with 5 mL of 0.5N aqueous hydrochloric acid and 5 mL of saturated aqueous sodium bicarbonate solution. The combined organics were dried over anhydrous magnesium sulfate, and concentrated to yield 0.627 g of the product as a yellow foam which was used without purification.

To a 0.627 g (1.07 mmol) of the above product in 5 mL of dichloromethane was added 1.0 mL of trifluoroacetic acid and stirred at RT for 75 min. An additional 1.00 mL of trifluoroacetic acid was added and stirred for 10 min. The reaction mixture was concentrated, diluted with 5.0 mL of dichloromethane and carefully basified by pouring into 10 mL of 10% aqueous sodium carbonate solution. The organic layer was separated and the aqueous layer was further extracted with 2X15 mL of dichloromethane. The combined organics were washed with 5 mL of water, dried over potassium carbonate, filtered and concentrated to give the 0.486 g of the amine as a light yellow foam which was used without purification.

To 0.486 g (1.01 mmol) of the amine and 10 mL of dichloromethane was added 0.26g (1.28 mmol) of 2-[(1,1-dimethyl-ethoxy)carbonyl]amino-2-methyl-propanoic acid, 0.173 g (1.28 mmol) of 1-hydroxybenztriazole (HOBT) and EDC (0.245 g; 1.28 mol) and stirried at RT overnight. The reaction mixture was poured into 5.0 mL of water and the organic layer was separated. The aqueous layer was back extracted with 5 mL of dichloromethane. The combined organics were washed with 5.0 mL of 0.5N aqueous hydrochloric acid, 5 mL of saturated aqueous sodium bicarbonate solution dried over anhydrous magnesium sulfate, and concentrated to yield 0.751 g of the crude product as a yellow foam. A solution of this crude product in dichloromethane was chromatographed on 25 g of silica gel and eluted first with hexanes/acetone/dichloromethane (70/25/5) and then with hexanes/acetone/dichloromethane (65/30/5). This gave 0.63 g of the title compound as a white solid.

^{1}H NMR ($CDCl_3$, 400MHz) Compound exists as a 3:2 mixture of rotamers δ 7.40-7.10 (m, 6H), 7.06 (d, 1/3H), 7.02 (t, 1/3H), 6.90 (t, 1/3H), 6.55 (d, 1/3H), 5.15 (m, 1H), 4.95 (bs, 1H), 4.63 (bd, 1/3H), 4.57-4.40 (m, 2 2/3 H), 4.10 (bd, 1/3H), 4.00 (bd, 1/3H), 3.82 (t, 1H), 3.78-3.62 (m, 2H), 3.60-3.50 (m, 1H), 3.04 (q, 1H), 2.87 (s, 1H), 2.86 (s, 2H), 2.80-2.60 (m, 1H), 1.90 (bs, 1H), 2.85-2.75 (m, 1H), 1.82-1.60 (m, 3H), 1.55-1.45 (m, 1H), 1.45 (s, 4H), 1.42 (s, 2H), 1.39 (s, 9H).

Step C: N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide hydrochloride

To 0.637 g (0.101 mmol) of the intermediate from Step B in 5 mL of dichloromethane was added 2.5 mL of trifluoroacetic acid and stirred at RT for 30 min. The reaction mixture was concentrated to an oil, taken up in 10 mL of ethyl acetate and washed with 8 mL of 10% aqueous sodium carbonate solution. The aqueous layer was further extracted with 5 mL of ethyl acetate. The combined organics were washed with 10 mL of water, dried over magnesium sulfate, filtered and concentrated to give the 0.512 g of the free base as a white foam.

To 0.512 g of the free base in 5 mL of ethyl acetate at 0°C was added 0.2 mL of saturated hydrochloric acid in ethyl acetate and stirred for 1.5 h. The white precipitate was filtered under nitrogen, washed with ether, and dried to give 0.50 g of the title compound as a white solid

^{1}H NMR (400MHz, CD_3OD) Compound exists as 3:2 mixture of rotamers. δ 7.40-7.28 (m, 4H), 7.25-7.17 (m, 2H), 7.08 (t, 1/3H), 7.00 (t, 1/3H), 6.80 (d, 1/3H), 5.16 (ddd, 1H), 4.60-4.42 (m, 3H), 4.05 (t, 1H), 3.90 (bs, 2H), 3.83-3.70 (m, 2H), 3.30-3.15 (m, 1H0, 2.97 (s, 1H), 2.95 (s, 2H), 2.90-2.78 (m, 1H), 1.96 (t, 1/3H), 1.85-1.65 (m, 4H), 1.63 (s, 2H), 1.60 (s, 4H).

EXAMPLE 2

N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl) carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide

Step A: (2R)-[[[-2-(1,1-dimethylethoxy)carbonyl]amino]-2,2-dimethyl-1-oxoethyl]amino-2-(phenylmethoxy)ethyl]-1-propanoic acid allyl ester

Prepared from (2R)-2-[(1,1-dimethylethoxy)carbonyl]-amino-3-(phenylmethyloxy)ethyl-propanoic acid and allyl alcohol by carrying out the coupling reaction in CH_2Cl_2 in the presence of EDC and DMAP.

^{1}H NMR (400MHz, $CDCl_3$) δ 7.25 (s, 5H), 5.8 (m, 1H), 5.2 (dd, 2H), 5.0 (bs, 1H), 4.7 (m, 1H), 4.6 (m, 2H), 4.4 (dd, 2H), 3.9 (dd, 1H), 3.6 (dd, 1H), 1.45 (d, 6H), 1.39 (s, 9H).

Step B: (2R)-[[[-2-(1,1-dimethylethoxy)carbonyl]amino]-2,2-dimethyl-1-oxoethyl]amino-2-(phenylmethyloxy)ethyl)-1-propanoic acid

To a stirred solution of the crude intermediate obtained in Step A (6.7 g, 15.9 mmol), tetrakis (triphenylphosphine)-palladium (1.8 g, 0.1 eq) and, triphenyl phosphine (1.25 g, 0.3 eq) was added a solution

of potassium-2-ethyl hexanoate (35 mL, 0.5M solution in EtOAc). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1h and then diluted with ether (100 mL) and poured into ice-water. The organic layer was seperated and the aqueous fraction was acidified with citric acid (20%), then extracted with EtOAc. The EtOAc extracts were washed with brine, dried over magnesium sulfate, filtered and evaporated to give the title compound as a solid.

^{1}H NMR (400Hz, CD_3OD) δ 7.3 (s, 5H), 4.7 (m, 1H), 4.5 (s, 2H), 4.0 (m, 1H), 3.6 (m, 1H), 1.4 (d, 6H), 1.3 (s, 9H).

Step C: N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-[(1,1-dimethyl-ethoxy)carbonyl]amino-2-methyl-propanamide

To a solution of 1.0 g (3.44 mmol) of 1-methanesulfonyl-spiro[indoline-3,4'-piperidine] hydrochloride, 1.44 g (3.78 mmol) of (2R)-[[-2-(1,1-dimethylethoxy)carbonyl)amino]-2,2-dimethyl-1-oxo-ethyl]-amino-2-(phenylmethyloxy)ethyl)-1-propanoic acid, N-methyl morpholine (0.58 mL; 5.20 mmol), and 1-hydroxybenztriazole (HOBT) (0.58 g; 3.78 mmol), in 50 mL of dichloromethane was added EDC (1.03 g; 5.20 mmol) and stirred at RT for 16h. The reaction mixture was diluted with an additional 50 mL of dichloromethane and washed with aqueous sodium bicarbonate solution (50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated. Flash chromatography (50 g silica gel) of the crude oily residue gave 2.148 g (90%) of the desired material as a colorless foam.

^{1}H NMR ($CDCl_3$, 400MHz) Compound exists as a 3:2 mixture of rotamers δ 7.40-7.10 (m, 6H), 7.06 (d, 1/3H), 7.02 (t, 1/3H), 6.90 (t, 1/3H), 6.55 (d, 1/3H), 5.15 (m, 1H), 4.95 (bs, 1H), 4.63 (bd, 1/3H), 4.57-4.40 (m, 2 2/3 H), 4.10 (bd, 1/3H), 4.00 (bd, 1/3H), 3.82 (t, 1H), 3.78-3.62 (m, 2H), 3.60-3.50 (m, 1H), 3.04 (q, 1H), 2.87 (s, 1H), 2.86 (s, 2H), 2.80-2.60 (m, 1H), 1.90 (bs, 1H), 2.85-2.75 (m, 1H), 1.82-1.60 (m, 3H), 1.55-1.45 (m, 1H), 1.45 (s, 4H), 1.42 (s, 2H), 1.39 (s, 9H).

Step D: N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide hydrochloride

To a solution of 2.148 g (3.41 mmol) of the intermediate from Step C in 10 mL of dichloromethane was added 5 mL of trifluoroacetic acid and stirred for 1h. The reaction mixture was concentrated and basified with 100 mL of 5% aqueous sodium carbonate solution and extracted with dichloromethane (3X50 mL). The combined organics were washed with brine (50 mL), dried over anhydrous potassium carbonate, filtered, and concentrated to yield a colorless foam. To a solution of the foam in 25 mL of ethyl acetate at 0°C was added 4 mL of 1M solution of hydrochloric acid in ethyl acetate. The precipitate was filtered and washed first with ethyl acetate and then with ethyl acetate-ether (1:1), dried to yield 1.79 g (93%) of the title compound as a colorless solid.

^{1}H NMR (400MHz, CD_3OD) Compound exists as 3:2 mixture of rotamers. δ 7.40-7.28 (m, 4H), 7.25-7.17 (m, 2H), 7.08 (t, 1/3H), 7.00 (t, 1/3H), 6.80 (d, 1/3H), 5.16 (ddd, 1H), 4.60-4.42 (m, 3H), 4.05 (t, 1H), 3.90 (bs, 2H), 3.83-3.70 (m, 2H), 3.30-3.15 (m, 1H0, 2.97 (s, 1H), 2.95 (s, 2H), 2.90-2.78 (m, 1H), 1.96 (t, 1/3H), 1.85-1.65 (m, 4H), 1.63 (s, 2H), 1.60 (s, 4H).

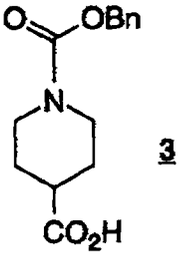
EXAMPLE 3

N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)ethyl]-2-amino-2-methylpropanamide mesylate

This compound was prepared by the treating the free base obtained in Example 5, Step D, with methane sulfonic acid. The title compound was obtained by recrystallizing it from ethyl acetate-ethanol-water. m.p. = 166°-168°C. This sample was subsequently identified as being polymorphic Form II. It was characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 4.7,

11.6, 17.4, 19.2, and 21.6° (2 theta). It was further characterized by a DSC at 10 °C/min in an open cup under under nitrogen flow and exhibited a single endotherm, due to melting, with a peak temperature of about 174°C and an extrapolated onset temperature (melting point) of about 165°C with an asociated heat of approximately 37 J/g.

EXAMPLE 4



3

Isonipecotic acid-*N*-benzyl carbamate (3)

Materials:

Isonipecotic acid (2) T.C.I.	4.02 kg (31.1 mol)
Benzyl chloroformate (Schweitzerhall)	6.91 kg (40.5 mol)
K_2CO_3	10.1 kg (72.9 mol)
Water	40.2 L

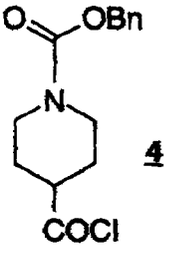
Isonipecotic acid (2) and K_2CO_3 were dissolved in 40.2 L of water in a 100 L 4 neck flask with mechanical stirring under N_2 and the solution was cooled to 10°C. Benzyl chloroformate was added, maintaining the temperature between 9 and 14°C, and the mixture was warmed up to 22°C after the addition was complete and aged for 58 h. The addition was completed in 4 h at which point the pH was 9.0. After aging for 58 h there was no change in the pH.

The reaction mixture was transferred to a 200 L extractor and washed with 3 × 13 kg (15 L) of IPAC and 1 × 12 L of EtOAc. The aqueous layer was extracted with 8 L of toluene. After the washes the benzyl alcohol content was reduced from 3.8% to 1.4% by HPLC analysis. HPLC analytical: Dupont Zorbax 25 cm RXC8 column with

1.5 mL/min flow and detection at 254 nm; isocratic mixture with 35% MeCN, 65% of 0.1% aqueous H_3PO_4; retention times: **3** = 6.9 min, benzyl alcohol = 3.3 min, toluene = 17.3 min.

The aqueous phase was acidified with 37% aqueous HCl to pH 1.8. Carbon dioxide was evolved during the addition of HCl, but gas evolution was easily controlled. The addition of HCl took <1 h and required 10 L of conc. HCl. The aqueous phase was extracted with 3 × 6.6 L of toluene. The toluene extracts were dried with 2 kg of sodium sulfate and filtered through a pad of Solka-floc™. The combined filtrates weighed 17.8 kg. The crude yield of carbamate **3** was 7.89 kg (97%) (as obtained by evaporation of weighed aliquots of the filtrates to dryness). The filtrates were transferred through a 10 μ inline filter to a 100 L flask. The extracts were concentrated at 10 mbar at <25°C to a volume of 18 L. The final concentration of carbamate **3** was 440 g/L. The concentration of the toluene filtrate served to azeotropically remove final traces of water (final KF = 170mg/L). The product was 99.1 area % pure with 0.9 area % benzyl alcohol as the only impurity.

EXAMPLE 5



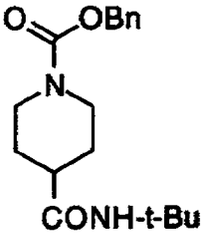
4

Isonipecotic acid chloride-*N*-benzyl carbamate (**4**)

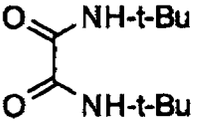
Materials:

Isonipecotic acid *N*-benzyl carbamate (**3**) in toluene. (MW = 263.30)	7.89 kg (30.0 mol) in 17.9 L
Oxalyl chloride (MW = 126.93)	3.94 kg (31.0 mol)
DMF (MW = 73.10)	10 mL
Toluene	12 L

To the toluene solution of benzyl carbamate **3** from the preceding step was added 5 mL of DMF and 10 L of toluene. The oxalyl chloride was added over a period of 20 min. The reaction mixture was aged for 16 h at 18°C under a slow stream of nitrogen. HPLC analysis of the reaction mixture showed that 1.3% of the carboxylic acid **3** still remained unreacted. The reaction mixture was warmed to 26°C, and 5 mL of DMF were added. The mixture was aged for 2.5 h. A 1.0 mL aliquot of the reaction mixture was quenched with 5.0 mL of tert-butylamine and analyzed after evaporation by HPLC: 25 cm Dupont Zorbax RXC8 column at 50°C with 1 mL/min flow and detection at 220 nm; isocratic 42% MeCN, 58% of 0.1% aqueous H_3PO_4. This method showed that <0.05% of the acid **3** remained (as judged by **A**) and showed >3 area % **B** (>1 mol% $(COCl)_2$).



A



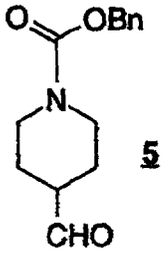
B

The mixture was concentrated at 10 mbar and a temperature of 20-25°C until 5 L of solvent had been removed.

The typical HPLC profile of concentrated toluene solution after t-$BuNH_2$ quench described above is as follows:

Retention time (min)	Area %	Identity
2.1	<0.5%	carboxylic acid **3**
7.8	<0.5%	benzyl chloride
11.0	>99%	Cbz-t-butylcarboxamide **A**
12.1	NA	toluene
12.7	<0.5%	ditert-butyloxamide **B**

EXAMPLE 6



5

Piperidine-4-carboxaldehyde-1-benzyl carbamate (**5**)

Materials:

Isonipecotic acid chloride *N*-benzyl carbamate (**4**)	3.38 kg (12.0 mol)
in toluene (MW = 281.74)	in 5.54 kg
DIEA (KF = 18 mg/L)	1.55 kg (15.0 mol)
10% Pd/C (KF < 20 mg/g)	101 g
thioanisole (MW = 124.21, d = 1.058)	0.56 g

The DIEA and thioanisole were added to the solution of (**4**) in toluene from the previous step and the catalyst was suspended in this mixture. The mixture was immediately placed into the 5 gal autoclave and hydrogenated at 20°C and 40 psi of H_2. After 18 h the reaction had taken up 70% the theoretical amount of hydrogen and HPLC analysis of an aliquot that was quenched with tert-butylamine indicated that 14.2 area % of acid chloride **2** remained. HPLC conditions same as above. Retention time: **5** = 8.1 min.

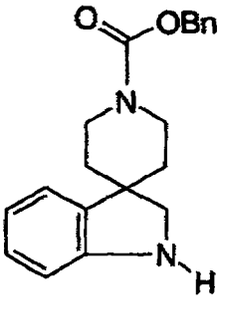
A second charge of catalyst (101 g) and thioanisole (0.54 g) were added as a slurry in 1375 mL toluene to the hydrogenator. After 23 h HPLC analysis of an aliquot that was quenched with tert-butylamine indicated that 1.8 area % of acid chloride **2** remained. The mixture was purged with nitrogen and the catalyst and precipitated DIEA•HCl were removed by filtration through Solka-floc™. The filter cake was washed with 10 L of toluene. The filtrates were transferred through a 10 μ inline filter to a 50 L extractor and washed with 2 × 7.2 L of 1 M aqueous HCl and 2 × 7.2 L of water. The mixture was

concentrated at 10 mbar and a temperature of 25-30°C until 5 L of residue remained.

Retention time (min)	Area %	Identity
2.1	<2	carboxylic acid **3**
6.6	<1	dimer **21**
8.1	>95	aldehyde **5**

The assay yield of aldehyde **3** was 94% by HPLC analysis.

EXAMPLE 7



9

CBZ-Spiroindoline (**9**)

Materials:

Piperidine-4-carboxaldehyde-1-benzyl carbamate (**5**) in toluene solution	1.71 kg (6.89 mol) in 21.4 kg
Phenylhydrazine	900 mL, 981 g (9.15 mol)
Trifluoroacetic acid (TFA)	2.20 L, 3.26 kg (28.6 mol)
$NaBH_4$	300 g, (7.93 mol)
Toluene	34.4 kg
MeCN	7.0 L
MeOH	7.0 L

The crude aldehyde **5** solution from the previous step was transferred through a 10 μ inline filter to a 100 L reactor equipped with Teflon coated copper coils for cooling or heating and a mechanical

stirrer. Toluene (34.4 kg) and MeCN (7 L) were added, and the resulting solution was cooled to 0°C. Phenylhydrazine was added in portions and the temperature was maintained at -1 to 3°C while nitrogen was continuously bubbled through the reaction mixture.

The phenylhydrazine was added until TLC and HPLC analysis indicated complete consumption of the aldehyde **5** and the appearance of a slight excess (<5%) of phenylhydrazine. TLC conditions: Silica, E. Merck Kieselgel G60 F254 0.25 mm; diethyl ether/pentane (4/1); and developing agent 0.5% ceric sulfate, 14% ammonium molybdate in 10% aqueous sulfuric acid then heat; Rf: aldehyde **5** = 0.52, phenylhydrazone **7** = 0.61, phenylhydrazine **6** = 0.21.

HPLC conditions: 25 cm Dupont Zorbax RXC8 column at 30°C with 1.0 mL/min flow and detection at 254 nm; gradient schedule:

Time (min)	acetonitrile:water
0	57:43
10	65:35
15	75:25
18	75:25

retention times: phenylhydrazine **6** = 4.5 min, toluene = 7.2 min, phenylhydrazone **7** = 11.4 min.

The reaction mixture was aged for 30 min at 0-2°C, and TFA was added maintaining the temperature between 2 and 7°C. The reaction mixture was warmed to 50°C over 30 min, and maintained for 17 h. The nitrogen sparge through the reaction mixture was stopped and a slow stream of nitrogen was maintained over the reaction mixture. During the first hour at 5°C the color gradually darkened to a deep green, and a relatively small amount of a white crystalline precipitate (ammonium trifluoroacetate) formed. After 17 h HPLC analysis (same conditions as above) indicated that the reaction mixture contained 91.6 area % indolenine **8** and 1.5% of unreacted phenylhydrazone remained. Aging the mixture for longer periods of time did not increase the assay yield of indolenine **8**.

The reaction mixture was cooled to 12°C, and 7.0 L of MeOH was added. $NaBH_4$ was added in small (<20 g) portions maintaining the temperature below 15°C. The addition took 30 min. Moderate hydrogen evolution was observed during the addition, but it was easily controlled and there was virtually no frothing. Near the end of the addition the color rapidly changed from green to brown and then bright orange. A small amount (<200 mL) of a heavier phase had separated (presumably aqueous salts). HPLC analysis (conditions as before) indicated that all of the indolenine **8** had been consumed (90.4 area % CBZ-indoline **9**); retention times: indolenine **8** = 7.5 min, indoline **9** = 8.2 min. TLC: ethyl ether as solvent, ceric sulfate-ammonium molybdate stain or 1% anisaldehyde stain; retention factors: indolenine **8** = 0.18, CBZ-indoline **9** = 0.33.

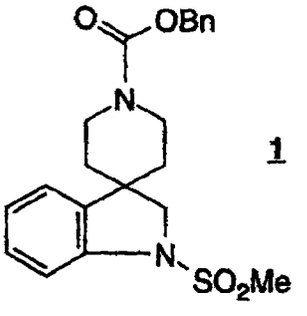
The color change from green to orange corresponds very closely to reaction end point. The quantity of $NaBH_4$ required to complete the reaction is heavily dependent on the temperature and rate of addition of $NaBH_4$, but the yield and quality of the product is virtually unaffected provided that the reaction is complete. The reaction mixture was cooled to 5°C over a period of 30 min. Then 8 L of 3% aqueous NH_4OH (8 L) were added to bring the pH of the aqueous phase to 7.4, the mixture was agitated, and allowed to settle. The temperature rose to 15°C. The cloudy yellow lower aqueous phase was separated. The organic phase was washed with 4 L of 3% aqueous NH_4OH, 2 × 4 L of water, and 2 × 4 L of brine. The weight of the organic phase after the washings was 53.5 kg, and the assay yield was 94%.

The washed toluene solution was combined with the washed organic phases of two other similarly processed reactions. The total aldehyde used in the three reactions was 5.06 kg, (20.5 mol). The total weight of CBZ-indoline **9** assayed in the combined organic phases was 5.91 kg, (18.3 mol, 90% assay yield). The combined organic phases were dried with 5 kg of sodium sulfate, treated with 250 g of Darco G60 carbon for 30 min, and filtered through Solka-floc™. The filtrates were vacuum concentrated at 10 mbar at <25°C until the residue was

near dryness. The solvent switch was completed by slowly bleeding in 30 L of IPAC and reconcentrating to 14 L at 200 mbar at 50-60°C. The mixture was heated to reflux in order to obtain a clear homogeneous deep orange solution. ^{1}H NMR analysis indicated that the solution contained ca. 6 mol% of residual toluene after solvent switch.

The solution was cooled to 68°C and seeded with 4 g of crystalline CBZ-indoline **9**. The solution was allowed to gradually cool to 26°C over 6 h and aged for 9 h at 20-26°C. The slurry was cooled to 2°C over 1 h and aged at 2°C for 1h. The product was isolated by filtration, and the filter cake was washed with 2 × 2 L of 5°C IPAC and 2 × 2 L of 5°C MTBE. The product was dried in the vacuum oven at 30°C under a nitrogen bleed to give 4.37 kg (74%) of the title compound **9** as a light tan crystalline powder. HPLC analysis of the product indicated 99.5 area % purity. The mother liquor (11 L) and the washes contained 1.15 kg (19%) of additional product **9** and ca 3% of Cbz-isonipecotic acid phenylhydrazide (retention time = 4.8 min).

EXAMPLE 8



1

CBZ-Spiroindoline-methanesulfonamide (**1**)

Materials:

CBZ-Spiroindoline (**9**)	1.69 kg (5.23 mol)
Methanesulfonyl chloride	599 g (5.23 mol)
Et_3N (KF = 151)	635 g (6.27 mol)
THF (KF = 41)	12 L

A 22 L flask was charged with the solid CBZ-spiroindoline **9** and then 11.5 L of THF and the Et_3N were transferred into the flask through a 10 μ inline filter. The resulting homogenous solution was cooled to 0°C. A 1 L dropping funnel was charged with the methanesulfonyl chloride and 500 mL of THF. The solution of the MsCl in THF was added to the reaction mixture maintaining the temperature between 0 and 4°C. The addition took 5 h and was exothermic. A white precipitate, presumably triethylammonium hydrochloride formed during the addition. HPLC analysis indicated that the reaction was complete at the end of the addition (**9** was undetectable).

HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 254 nm. Gradient Schedule:

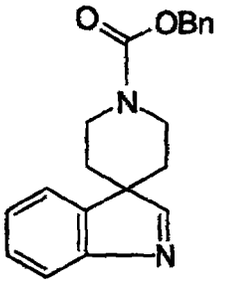
Time (min)	0.1% aq. H_3PO_4:MeCN
0	70:30
3	70:30
12	20:80
25	20:80

Retention times: **9** = 7.6 min, **1** = 13.6 min.

After the addition was complete the reaction mixture was warmed to 18°C and aged for 16 h. There was no change in the appearance of the reaction mixture, and HPLC profile between the end of the addition and after the 16 h age. The reaction mixture was slowly transferred over 1h into a vigorously stirred solution of 30 L of water and 200 mL of 37% aqueous HCl in a 50 L flask. The temperature in the 50 L flask rose from 22 to 28°C. The product separated as a pale tan gummy solid which changed to a granular solid. The aqueous suspension was cooled to 22°C and aged for 1 h. The suspension was filtered, and the filter cake was washed with 2 × 4 L of MeOH/water (50/50). HPLC analysis indicated that <0.1% of the CBZ-Spiroindoline-methanesulfonamide**1** was in the mother liquors.

The filter cake was washed with 4 L of MeOH/water (50/50) to which 50 mL of 28% aqueous NH_4OH had been added. The filter cake was washed with 2 × 4 L of MeOH/water (50/50), and the solid was dried in the vacuum oven at 50°C under a nitrogen bleed to give 2.03 kg (97%) of the title product **1** as an off-white powder. HPLC analysis of the solids indicated 93.7 area % **1**.

EXAMPLE 9



8

Optional Procedure for Isolation of Intermediate CBZ-Spiroindolenine (**8**)

Materials:

Piperidine-4-carboxaldehyde-1-benzyl carbamate (**5**)	12.37 g (0.050 mol)
Phenylhydrazine	5.41 g (0.050 mol)
Trifluoroacetic acid (TFA)	11.56 mL,17.10 g (0.150 mol)
Methylene chloride	500 mL

The CBZ-aldehyde **5** was dissolved in dichloromethane in a 1 L flask equipped with Teflon coated magnetic stirring bar. The resulting solution was cooled to 0°C. Phenylhydrazine was added via a weighed syringe over 5 min and the temperature was maintained at -1 to 3°C while nitrogen was continuously bubbled through the reaction mixture. TLC and HPLC analysis indicated complete consumption of the CBZ-aldehyde **5** and the appearance of a slight excess (<2%) of phenylhydrazine. TLC conditions: Silica, E. Merck Kieselgel G60 F254

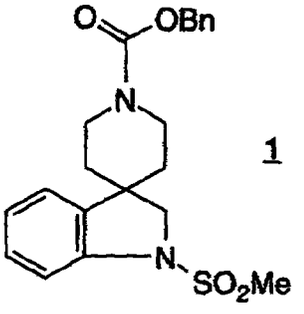
0.25 mm; diethyl ether/pentane (4/1); and developing agent 0.5% ceric sulfate, 14% ammonium molybdate in 10% aqueous sulfuric acid then heat; Rf: aldehyde **5** = 0.52, phenylhydrazone **7** = 0.61, phenylhydrazine **6** = 0.21. HPLC conditions: 25 cm Dupont Zorbax RXC8 column at 30°C with 1.0 mL/min flow and detection at 254 nm; gradient schedule:

Time (min)	acetonitrile:water
0	57:43
10	65:35
15	75:25
18	75:25

retention times: phenylhydrazine **6** = 4.5 min, toluene = 7.2 min, phenylhydrazone **7** = 11.4 min.

The reaction mixture was aged for 10 min at 0-2°C, and TFA was added by syringe maintaining the temperature between 2 and 7°C. The reaction mixture was warmed to 35°C over 30 min, and maintained for 17 h. The nitrogen sparge through the reaction mixture was stopped and a slow stream of nitrogen was maintained over the reaction mixture. During the first hour at 35°C the color gradually darkened to a rosy pink then to a deep green, and a relatively small amount of a white crystalline precipitate (ammonium trifluoroacetate) formed. After aging for 17 h HPLC analysis (same conditions as above) indicated that the reaction mixture contained 93 area % indolenine **8** and <0.5% of unreacted phenylhydrazone remained. Aging the mixture for longer periods of time did not increase the assay yield of indolenine **8**. The reaction mixture was cooled to 10°C, and a mixture containing 60 mL 28-30% ammonium hydroxide, 90 mL water and 150 g crushed ice was added with good stirring. The color of the mixture changed to a salmon color. The organic phase was separated and washed twice with 400 mL water then 100 mL saturated aqueous NaCl. The organic phase was dried over magnesium sulfate and filtered through a plug of 5 g of silica. The filtrate was evaporated to give 15.84 g (99%) of indolenine **8** as a pale orange oil.

EXAMPLE 10



1

Procedure for the Preparation of CBZ-Spiroindoline-methanesulfonamide (1) without Isolation of Intermediate CBZ-Spiroindoline (9)

Step 1: CBZ-Spiroindoline (9)

Materials:

Piperidine-4-carboxaldehyde-1-benzyl carbamate (5)	49.5 g (0.20 mol)
Phenylhydrazine (Aldrich)	23.7 g (0.22 mol)
Trifluoroacetic acid (TFA)	75.4 g (0.66 mol)
Toluene (KF < 250 mg/L)	654 mL
MeCN (KF < 250 mg/L)	13.3 mL
$NaBH_4$	11.3 g, (0.30 mol)
Toluene	20 mL
MeOH	50 mL

A 2% (by volume) solution of MeCN in toluene was made up using 654 mL of toluene and 13.3 mL of MeCN. In a 2 L 3 neck flask equipped with a mechanical stirrer 617 ml of the above solution were degassed by passing a fine stream of nitrogen through the solution for 5 min. Phenylhydrazine and TFA were added to the mixture while still degassing.

The CBZ-aldehyde 5 was dissolved in the rest of the solution prepared above (50 mL) and degassed by bubbling nitrogen through the solution while in the addition funnel. The solution in the flask was heated to 35°C, and the aldehyde solution was slowly added to the phenylhydrazine-TFA over 2 h. The mixture was aged at 35°C for 16h.

HPLC conditions: 25 cm Dupont Zorbax RXC8 column at 50°C with 1 mL/min flow and detection at 220 nm; isocratic 55% MeCN, 45% of 0.1% aqueous H_3PO_4. Typical HPLC profile after 16 h age:

Retention time (min)	Area %	Identity
1.6	0.1-0.5	phenylhydrazine **6**
4.1	<0.1	dimer **21**
4.7	<0.1	aldehyde **5**
5.0	NA	spiroindoline **9**
6.3	NA	toluene
6.9	97	spiroindolenine **8**
10.3	<0.2	phenylhydrazone **7**
	2-3 tot.	other impurities <0.2% ea.

The mixture was cooled to -10°C and MeOH was added. A suspension of sodium borohydride in 20 mL toluene was added in small portions (1 mL) over 30 min taking care that the temperature did not exceed -2°C.

Area %	Identity
0.1-1	phenylhydrazine **6**
85-90	CBZ-spiroindoline **9**
<0.1	CBZ-spiroindolenine **8**
10-15 tot.	other impurities (<3% ea.)

The temperature was raised to 10°C over 1h, and 6% aqueous ammonia (200 mL) was added. The mixture was agitated for 10 min, allowed to settle for another 10 min, and the lower aqueous phase was drawn off. Acetonitrile (20 mL) and MeOH (20 mL) were

added to the organic phase and it was washed with 150 mL of 15% brine. The organic phase was found to contain a 92% assay yield of CBZ-spiroindoline **9**.

Step 2: CBZ-Spiroindoline-methanesulfonamide (**1**)

Materials:

CBZ-Spiroindoline (**9**) (MW = 322.51)	(0.184 mol)
Methanesulfonyl chloride	21.1 g (0.184 mol)
DIEA (KF = 150 mg/L)	29.7 g, 40.1 mL (0.230 mol)
THF (KF = 41 mg/L)	150 mL

The crude solution of CBZ-spiroindoline **9** solution from Step 1 above was concentrated in a 1L 3 neck flask (60-70°C, 150-200 Torr) until 250 g of residue remained. The THF and DIEA were added, and the resulting homogenous solution was cooled to 0°C. A 125 mL dropping funnel was charged with the methanesulfonyl chloride and 50 mL of THF. The solution of MsCl in THF was added over 2 h to the reaction mixture maintaining the temperature between 0 and 4°C and the mixture was aged for 2 h at 5-8°C. The addition was slightly exothermic. A white precipitate, presumably DIEA-hydrochloride, formed during the addition. HPLC conditions were the same as above. HPLC analysis indicated that the reaction was complete 1 h after the end of the addition (**9** was undetectable) and the assay yield was 94% from **9**. Retention time: **1** = 7.8 min. Typical HPLC profile of reaction mixture after 2 h age:

Area %	Identity
<0.1	CBZ-spiroindoline **9**
90-92	CBZ-sulfonamide **1**
8-10 tot.	other impurities (<2% ea.)

The mixture was warmed to 20°C, and 200 mL of 1M aqueous HCl was added. The mixture was warmed to 50°C, and the

aqueous phase was separated. The organic phase was washed sequentialy with 100 mL water, 100 mL 5% aqueous sodium bicarbonate, and 100 mL water. The organic phase was transferred to a 1 L 3 neck flask equipped for mechanical stirring and distillation. The mixture (ca 400 mL) was distilled at atmospheric pressure until 150 mL of distillate had been collected. The head temperature reached 107°C; the pot temperature was 110°C. The distillation was continued with continuous addition of n-propanol at such a rate as to maintain a constant volume (ca 350 mL) in the pot. The distillation was stopped when a total of 525 mL of n-PrOH had been added and a total of 800 mL of distillate had been collected.

The temperature of both the head and pot rose from 94°C to 98°C during the solvent switch. Toluene and n-PrOH form an azeotrope boiling at 97.2°C composed of 47.5% toluene and 52.5% n-PrOH. The mixture was allowed to cool gradually to 20°C over 3h and aged for 12 h. The mother liquor was found to contain 2% toluene and 4 mg/mL of sulfonamide. The solubility of the sulfonamide in various mixtures of toluene and n-PrOH has been determined by HPLC assay:

%toluene in n-PrOH	solubility of 1 in mg/mL
0	2.36
5	3.02
10	4.23
20	7.51
25	10.3

The crystalline slurry was filtered and washed with 3 x 100 mL of n-PrOH. The product was dried in a vacuum oven at 50°C with a nitrogen bleed for 16 h to furnish 65.5g (82 % from aldehyde **5**) of **6** as a tan solid with 93.5 wt% purity.

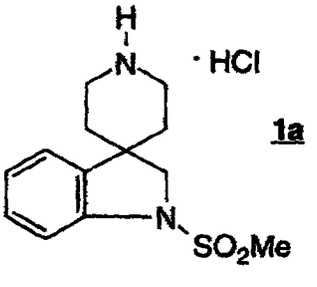
Typical HPLC profile of solid:

Area %	Identity
<0.1	CBZ-spiroindoline **9**
>99	CBZ-sulfonamide **1**

<1 tot. other impurities (<0.2% ea.)

For additional purification, a 40.0 g sample of the n-PrOH crystallized sulfonamide was dissolved in 134 mL of EtOAc at 60°C and treated with 8.0 g of Darco G-60 carbon for 1 h at 60°C. After the addition of 2.0 g Solkafloc™, the slurry was filtered through a pad of 4.0 g Solkafloc™, and the pad was washed with 90 mLof EtOAc at 60°C. Prior to the addition of the carbon the solution was a brown color. The filtration proceeded well without plugging to give a golden yellow filtrate. The filtrate was distilled at atmospheric pressure in a 500 mL flask (pot temperature 80-85°C) until 100 g (100 mL) of residue remained. This solution was allowed to cool to 35°C over 3 h. Over a 1h period, 116 mL of cyclohexane was added with good agitation at 35°C. The mixture was cooled to 20°C over 1 h and aged at 20°C for 12 h. At 35°C much of the sulfonamide has crystallized out and the mixture was thick. Addition of cyclohexane at 20°C makes agitation difficult. After the aging period, the supernatant was found to contain 2.5 mg 1/g. The crystalline slurry was filtered and the cake was washed with 77 mL of 2:1 cyclohexane-EtOAc and 2 x 77 mL of cyclohexane. The product was dried in a vacuum oven at 50°C with a nitrogen bleed for 16 h to furnish 34.2 g of 1 (MW = 400.3) as a white crystalline solid (85 % recovery from crude 1, 70 % from 5 with >99.9 wt % purity).

EXAMPLE 11



1a

HCl Salt of Spiroindoline-methanesulfonamide (**1a**)

Materials:

CBZ-spiroindoline-methanesulfonamide (1)	941 g (2.35 mol)
Pearlman's catalyst 20% $Pd(OH)_2/C$	188 g
THF	8 L
MeOH	7 L

The catalyst was suspended in 7 L of MeOH and transferred into the 5 gal autoclave followed by the solution of 1 in 8 L of THF. The mixture was hydrogenolyzed at 25°C at 80 psi of H_2. After 2.5 h the temperature was raised to 35°C over 30 min.

HPLC analysis indicated complete consumption of Cbz-spiroindoline-methanesulfonamide. HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 254 nm. Gradient Schedule:

Time (min)	0.1% aq. H_3PO_4:MeCN
0	70:30
3	70:30
12	20:80
25	20:80

retention times: Spiroindoline = 7.6 min, Cbz-spiroindoline-methanesulfonamide = 13.6 min.

The mixture was purged with nitrogen and the catalyst was removed by filtration through Solka-floc™ while still warm. The catalyst was washed with 4 L of THF and 2 L of MeOH. The pale yellow filtrates were concentrated to a thick oil at 10 mbar and <25°C. The solvent switch was completed by slowly bleeding in 15 L of EtOAc and reconcentrating to dryness. The residue solidified to a hard off-white mass. MeOH (1.5 L) was added and the mixture was heated to 70°C to give a homogenous solution. While the solution was at 70°C, 10.5 L of EtOAc at 20°C was added. The temperature fell to 40°C, and the mixture remained homogenous.

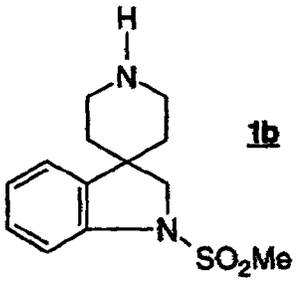
Subsequent experiments suggested that it is more convenient to solvent switch the MeOH-THF filtrates to MeOH,

concentrate to the desired volume, and then add the EtOAc. This avoids the solidification of the residue upon concentration of the EtOAc solution.

Hydrogen chloride diluted with about an equal volume of nitrogen was passed into the solution. The temperature rose to 60°C over the course of 15 min, and a white precipitate of the hydrochloride salt formed. Diluting the HCl with nitrogen only avoids the reaction mixture sucking back and may not be necessary.

The mixture was cooled in an ice bath, and the hydrogen chloride addition was continued for 1h. The temperature gradually fell to 20°C. The suspension was aged for 2 h while the temperature was lowered to 10°C. The crystalline product was isolated by filtration, and the filter cake was washed with 3 L of EtOAc. It was dried in the vacuum oven at 35°C to give 1.18 kg (86%) of the title product **1a** as an off-white crystalline solid of >99.5 area % purity by HPLC analysis. HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 230 nm; isocratic 35% MeCN, 65% of 0.1% aqueous ammonium acetate. Retention time: **1a** = 5.4 min.

EXAMPLE 12



1b

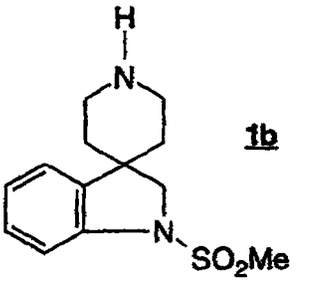
Spiroindoline-methanesulfonamide (Free base form) (**1b**)

A 250 mL aliquot of the filtrate from the Cbz-hydrogenolysis containing 4.67 g of **1b** (free base) was concentrated to ca 10 mL. The residue was dissolved in 20 mL of EtOAc and the solution was reconcentrated to ca 10 mL. This was repeated once more, and 10 mL of EtOAc was added to the residue. A crystalline precipitate began to form. MTBE (20 mL) was added in one portion. Additional

crystalline solid precipitated, but the supernatent still contained a substantial quantity of dissolved product which did not precipitate on standing. Hexanes (70 mL) were added dropwise over 2 h to the mixture with vigorous stirring. The slow addition of the hexanes is neccessary to avoid the oiling out of the amine.

The agitated mixture was aged for 1h and filtered. The filter cake was washed with 20 mL of 1:1 MTBE-hexanes and then with 20 mL of hexanes. The product was dried under a stream of nitrogen to give 3.86 g (82%) of the free amine of **1b** as an off white crystalline solid of >99.5 area % purity. HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 230 nm; isocratic 35% MeCN, 65% of 0.1% aqueous ammonium acetate. Retention time: **1b** = 5.4 min.

EXAMPLE 13A



1b

Spiroindoline-methanesulfonamide (Free base form) (**1b**)

Materials:

CBZ-Spiroindoline-sulfonamide (**1**)	833.5 gr (2.08 mol)
$Pd(OH)_2/C$ (20% weight of $Pd(OH)_2$)	124.5 (15%)
THF	6.5 L
MeOH	19.5 L
NH_4OH (conc)	60 mL

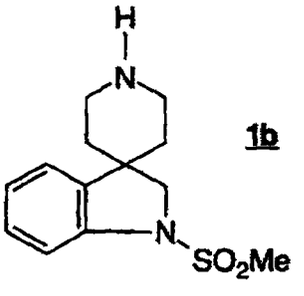
The hydrogenation was run three (3) times due to equipment limitations; this procedure refers to a single run. The CBZ

spiroindoline sulfonamide1 was dissolved in THF (6.5 L, KF = 53 μg/μL) and then MeOH (KF=18 μg/mL, 4L) was added followed by addition of the catalyst and the slurry was transferred to a 5 gal autoclave. The remainder of the MeOH (2.5 L) was used for rinsing. The mixture was heated to 40°C at 50 psi for 24 hours. The catalyst loading and reaction time are a function of the purity of starting material 1. This material was unique requiring ≥ 15% catalyst and long reaction time. Purer batches of spiroindoline required only 5% of catalyst and 4-6 hrs reaction time.

Upon completion (<0.1 A% 1 by LC) the mixture was filtered thru Solka Floc™ and the carbon cake washed with MeOH (13 L) containing NH_4OH (0.5%, 60 mL). The combined filtrates (assay shows 1587 g of spiroindoline amine **1b**) were concentrated in vacuo and the resulting solids were partitioned between 40 L (of toluene:THF (3:1) and 0.5N NaOH (18 L). Although the layers separated easily a heavy precipitate could be seen in the aqueous layer. The aqueous suspension was thus extracted with CH_2Cl_2 (15 L). The aqueous and organic layer separated slowly. Prior to CH_2Cl_2 addition THF was added to the aqueous layer along with enough NaCl to saturate the layer. However dissolution of the product was not achieved which necessitated the use of CH_2Cl_2.

The combined toluene, THF and CH_2Cl_2 layers were combined and concentrated in the batch concentrator. The residue was flushed with 7 L of CH_3CN. Finally 10 L of CH_3CN were added and the solution stood overnight under N_2 atmosphere.

EXAMPLE 13B



1b

Spiroindoline-methanesulfonamide (Free base form) (**1b**)

Materials:

CBZ-Spiroindoline-sulfonamide (1)	3 kg (7.49 mol)
Darco G-60	600 g
Ethyl Acetate	36 L
Absolute Ethanol	189 L
10% Pd/C	450 g
Ammonia Solution	500 ml
Solka Floc™	2.5 kg
Isopropyl Acetate	65 L

A mixture of CBZ-spiroindoline (**1**) (1 kg) and Darco G-60 (200 g) in ethyl acetate (9 L) was stirred and heated at 60-65°C under a nitrogen atmosphere for 8 hours. The Darco was removed by filtration at 60-65°C, the solid washed with hot ethyl acetate (3 L) and the filtrate and washings combined. LC wt/wt assay confirmed negligible loss to the Darco. The ethyl acetate solution was evaporated to dryness *in vacuo* using a 20 L Buchi apparatus and then flushed with absolute ethanol (2 x 5 L). This material was then slurried in absolute ethanol (8 L) warmed to 65-70°C and placed in the 20 L autoclave. The batch was rinsed in with absolute ethanol (1 L). A slurry of 10% Palladium on charcoal (75 g, 7.5% by weight) in absolute ethanol (750 ml) was then added to the autoclave and rinsed in with a further portion of absolute ethanol (250 ml).

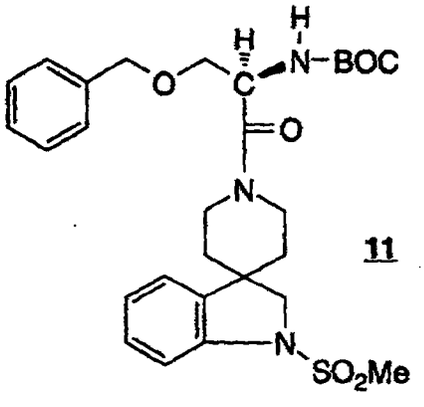
The batch was hydrogenated at 65°C with vigorous stirring under 40 psi hydrogen pressure for 3 hours, a second portion of 10% palladium on charcoal (75 g) was added, the batch was hydrogenated for a further 2 hours and then sealed overnight. The batch was transferred (still hot, 60-65°C) to a 20 L Buchi apparatus and degassed *in vacuo* to remove formic acid by "feeding and bleeding" absolute ethanol (18 L total).

This procedure was repeated twice more and the three batches were combined in a 10 gallon glass-lined vessel and the

combined batch was degassed again by the addition and distillation (*in vacuo*) of absolute ethanol (2 x 10 L). Solka floc™ (0.5 kg) was added to the batch and rinsed in with ethanol (10 L). An Estrella filter was loaded with Solkafloc™ (2 kg) as a slurry in ethanol (20 L). The resulting mixture was warmed to 60-65°C and then transferred at this temperature *via* heated filter using pump to two tared stainless-steel bins. The initial vessel, the filter, the pump and the lines were rinsed with a hot (60-65°C) mixture of aqueous ammonia (500 ml) in absolute ethanol (25 L). The filtrate and washings were combined in the two stainless-steel bins.

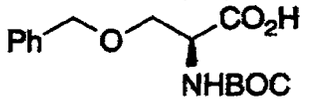
The batch was then transferred to a vessel using an in-line filter containing a 10 micron cartridge, and then concentrated *in vacuo* to low bulk (~15 L). The ethanol was replaced by isopropyl acetate by the "feeding and bleeding" of 3x batch volumes of isopropyl acetate (45 L total), while maintaining a batch volume of ~15 L. The solvent switch, when complete, contained <1% residual ethanol by GC. The batch was then diluted to ~33 L by the addition of isopropyl acetate (20 L), and this solution of spiroindoline-amine **1b** (1.855 kg by LC analysis) in isopropyl acetate was used for the next stage of the process.

EXAMPLE 14A



11

Boc-O-Benzylserine Spiroindoline (**11**)

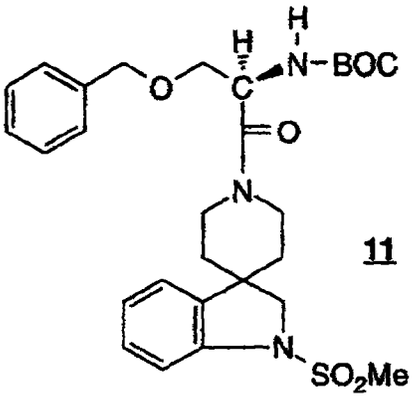
Materials:	
Spiroindoline-amine (**1b**)	1587 g (5.966 moles)
Amino acid (**10**) 	1938 g (6.563 moles)
DCC	1334.5 g (6.563 moles)
HOBT	884 g (6.563 moles)
CH_3CN	25 L
0.5N NaOH	18 L
0.5N HCl	18 L
$NaHCO_3$ sat.	18 L
iPrOAc	28 L

The spiroindoline-amine**1b** in CH_3CN or iPrOAc:H_2O (25 L) at ambient temperature under N_2 was treated in sequence with HOBT (884 g; 1.1 eq) as a solid, DCC (1334.5 g, 1.1 eq) as the melt (heating in hot water at 60°C for ca. 1 hr) and finally the amino acid **10** (1938 g) as the solid. The mixture was stirred for 3 hr upon which time heavy precipitation of DCU occurred and LC analysis showed ca. 0.5 A% of amine **1b** remaining. IPAc (9 L) was added, the slurry was filtered through Solka Floc™ and the cake was washed with IPAc (19 L). The combined organic solution was washed in sequence with 0.5N NaOH (18 L), 0.5N HCl (18 L) and saturated $NaHCO_3$ (18 L). A final water wash at this point resulted in an emulsion and was thus eliminated.

The organic layer was concentrated in vacuo and the residue was dissolved in MeOH or EtOH (10 L final volume). Assay yield 3026 gr (89%).

The use of alternative peptide coupling agents such as carbonyldiimidazole or formation of mixed anhydrides, such as sec-butyl carbonate, gave inferior yields of **11** and/or **14** with a high degree of epimerization in the case of the former compound. Other peptide coupling reagents were prohibitively expensive.

EXAMPLE 14B



11

Boc-O-Benzylserine Spiroindoline (11)

Materials:

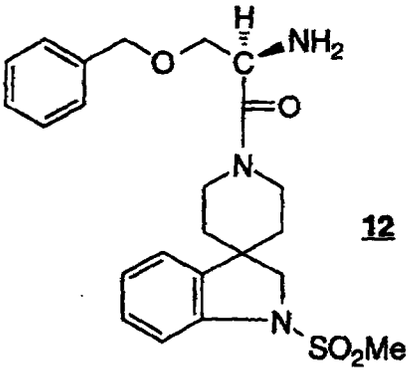
Spiroindoline-amine (1b)	1.855 kg (6.96 mol)
Isopropyl acetate	29 L
Dicyclohexylcarbodiimide (DCC)	1.58 kg (7.65 mol)
1-Hydroxybenzotriazole (HOBt)	1.03 kg (7.62 mol)
N-Boc-O-benzyl-D-Serine	2.26 kg (7.65 mol)
1M Aqueous sodium hydroxide	26 L
0.5M Aqueous hydrochloric acid	26 L
Satd. Aqueous sodium hydrogen carbonate	26 L
Absolute Ethanol	50 L

Water (20 L) was added to a stirred solution of the spiroindoline-amine**1b** (1.855 kg) in isopropyl acetate (33 L) in a reaction vessel. The following chemicals were then added sequentially at room temperature under a nitrogen atmosphere: DCC (1.58 kg, 1.1 equivs.), HOBt (1.03 kg, 1.1. equivs.) and finally N-Boc-O-benzyl-D-Serine (2.26 kg, 1.1 equivs.). The reagents were rinsed in with isopropyl acetate (7 L). The batch was stirred at room temperature under nitrogen atmosphere for 5 hours when LC showed the ratio of product/starting material to be 99.4/0.6. The mixture was then filtered through an Estrella filter using cloth and cardboard only and utilizing a

pump into another vessel. The sending vessel was rinsed with isopropyl acetate (22 L) and this was used to rinse the filter, the pump and the lines into the receiving vessel. The 2-phase mixture in the vessel was stirred for 10 minutes and then allowed to settle for 15 minutes. The lower aqueous layer was separated off and the organic solution was left to stand at room temperature overnight.

The next day, the organic solution was washed with 1M aqueous sodium hydroxide solution (26 L) then 0.5M aqueous hydrochloric acid (26 L) and finally saturated aqueous sodium hydrogen carbonate (26 L). LC analysis gave an assay yield of 3.787 kg, 93% overall yield from 7.49 moles (3 kg) of starting CBZ-spiroindoline (1). The batch was concentrated *in vacuo* (internal temperature = 13-15°C. jacket temperature = 40°C, Vacuum = 29") to low bulk (~15 L) and solvent switched to ethanol by "feeding and bleeding" ethanol (50 L) whilst maintaining the volume at ~15 L. GC showed <1% isopropyl acetate remaining. This solution was used for the next stage of the process.

EXAMPLE 15A



12

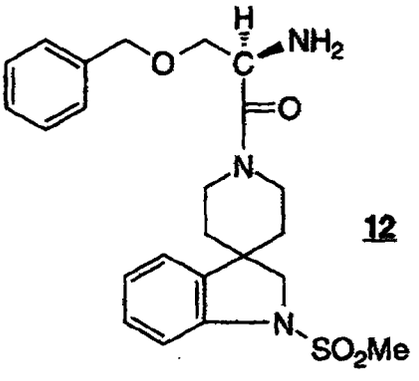
O-Benzylserine Spiroindoline (free base form) (12)

Materials:

Boc-O-Benzylserine Spiroindoline (**11**)	3026 g (5.57 moles)
Methane sulfonic acid (MsOH)	1.16 L (17.9 moles)
MeOH	10 L
iPrOAc	24 L
0.5 N NaOH	35 L

The Boc-O-benzylserine spiroindoline **11** in 10 L of MeOH (or EtOH) was treated with neat MsOH (1.16 L) added over ca. 30-40 min, (initial temperature 16°C, final temperature 28°C). The dark red solution was aged overnight under N_2. The mixture was then pumped into a 100 L extractor containing 24 L iPrOAc and 35 L 0.5 N NaOH. The pH of the aqueous layer was 7. NaOH (6M) was added until pH $\geq$ 10.5. As the pH increased the color changed from red to yellow. The layers were separated and the organic layer (24 L) was shown by NMR to contain 13 mole % of MeOH in iPrOAc [5 volume %]. LC assay 2.48 kg.

EXAMPLE 15B



12

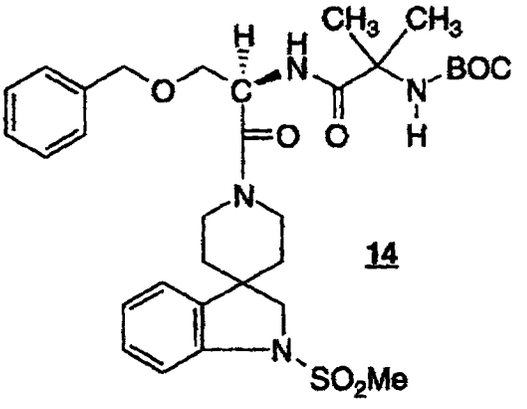
O-Benzylserine Spiroindoline (free base form) (**12**)

Materials:

Boc-O-Benzylserine Spiroindoline (11)	3.787 kg (6.96 mol)
Methanesulphonic acid	2.006 kg (20.87mol)
Isopropyl acetate	38 L
1M Aqueous sodium hydroxide	16 L
50% Aqueous sodium hydroxide	1.6 L

Methanesulphonic acid (2.006 kg, 1.355 L, ~3 equivs.) was added to the stirred solution of Boc-O-benzylserine spiroindoline (11) (3.787 kg) in ethanol (total volume ~15 L) in a reaction vessel. The batch was warmed to 35-40°C. After 7 hours, LC showed the absence of starting material and the reaction was allowed to cool to room temperature overnight. The next day, water (44 L) was added to the batch with stirring. The batch was cooled to ~5°, stirred for 30 minutes and then filtered through an in-line filter (loaded with a 10μ cartridge) into a bin. The batch was then sucked back into the vessel. A water rinse (10 L) was used to rinse the vessel and lines into the bin and this was used to then rinse back into the vessel. Isopropyl acetate (38 L) was added followed by a 1M aqueous sodium hydroxide (16 L). The batch was cooled to 10-15°C, the pH of the lower aqueous layer was confirmed as ~7 and 50% aqueous sodium hydroxide solution was added (1.6 L) (pH >10). The batch was stirred at 10-15°C for 25 minutes and then allowed to settle for 10-15 minutes. The lower aqueous layer was separated (78.1 kg). LC assay indicated 28.4 g of 12 (0.85% of theory) contained in the aqueous liquors. Volume of the organic solution = 51 L. LC assay indicated 3.057 kg, 92% overall yield from 3 kg, 7.49 moles of CBZ-spiroindoline sulfonamide (1). This solution was used for the next stage.

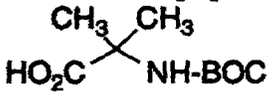
EXAMPLE 16A



14

Boc-Aminoisobutyryl O-Benzylserine Spiroindoline (14)

Materials:

Spiroindoline amine (12)	2481 g (5.57 moles)
amino acid peptide (13) 	1247.1 g (6.16 moles)
DCC	1266.7 g (6.16 moles)
HOBT	827 g (6.16 moles)
IPAc	52 L
H_2O	37 L
0.5N NaOH	36 L
0.5N HCl	36 L
Sat. $NaHCO_3$	36 L

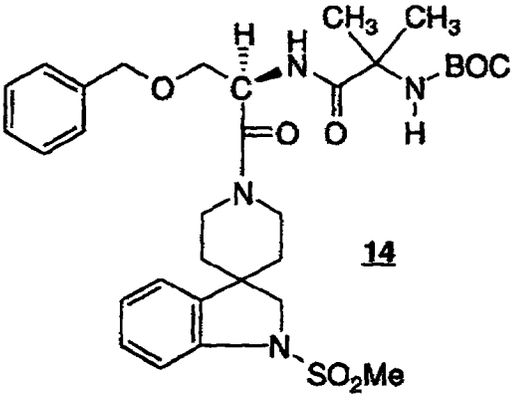
The solution of the amine **12** in IPAc was diluted to a total volume of 39 L with IPAc and 37 L of H_2O was added. The biphasic mixture was then treated in sequence with HOBT (827 g) as a solid, DCC (1266.7 g) as a melt, and amino acid **13** at ambient temperature under nitrogen. The reaction mixture was stirred for 2 h upon which time LC analysis indicated dissappearance of the starting material **12** (<0.3 A%). The mixture was filtered through Solka Floc™ and the

solids were washed with 13 L of IPAc. The material may be stored at this point as a biphasic mixture overnight.

The mixture was transferred to a 100 L extractor, the aqueous layer was separated and the organic layer was washed successively with 36 L of 0.5N NaOH, 0.5N HCl and saturated $NaHCO_3$. Assay yield 3160 g (81% from spiroindoline $\pm$ 5% for volume measurement error). The solution was concentrated to a small volume and was flushed with ethanol (2 x 4 L). If desired, the inermediate compound **14** may be isolated by adding water to crystalize it out.

The use of alternative peptide coupling agents such as carbonyldiimidazole or formation of mixed anhydrides, such as sec-butyl carbonate, gave inferior yields of **14** with a high degree of epimerization. Other peptide coupling reagents were prohibitively expensive.

EXAMPLE 16B



14

Boc-Aminoisobutyryl O-Benzylserine Spiroindoline (**14**)

Materials:

Spiroindoline amine (**12**)	3.057 kg (6.89 mol)
Dicyclohexylcarbodiimide (DCC)	1.56 kg (7.56 mol)
1-Hydroxybenzotriazole (HOBt)	1.02 kg (7.55 mol)
Boc-2-Aminoisobutyric acid (**13**)	1.54 kg (7.58 mol)
Isopropyl acetate	32 L
1M Aqueous sodium hydroxide	38 L
0.5M Aqueous hydrochloric acid	38 L
Satd. aqueous sodium hydrogen carbonate	38 L
Absolute ethanol	45 L

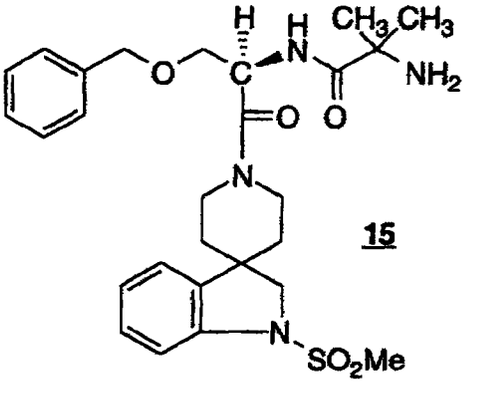
Water (49 L) was added to the stirred solution of the spiroindoline amine **12** (3.057 kg) in isopropyl acetate (total volume ~51 L) in a reaction vessel at room temperature under a nitrogen atmosphere. The following chemicals were then added sequentially: DCC (1.56 kg, ~1.1 equivs.), HOBt (1.02 kg, ~1.1 equivs.) and finally, N-Boc-2-aminoisobutyric acid **13** (1.54 kg, ~1.1 equivs.). The mixture was stirred vigorously at room temperature for 2 hours when LC showed the reaction to be complete. The mixture was filtered to to another vessel *via* an Estrella filter using a pump. Isopropyl acetate (22 L) was used to rinse vessel, the filter , the pump and the lines into the receiving vessel. The 2-phase mixture was then stirred for 5 minutes and the layers were allowed to separate. The lower aqueous layer was separated without incident (weight of aqueous liquors = 51.1 kg). The organic solution was then washed sequentially with 1M aqueous sodium hydroxide (38 L), 0.5M aqueous hydrochloric acid (38 L) and finally, saturated aqueous sodium hydrogen carbonate (38 L) without incident.

The organic solution was then transferred using a pump *via* an in-line filter (containing a 10μ cartridge) to another vessel for the solvent switch to ethanol. The vessel was rinsed with isopropyl acetate (10 L) and this was used to rinse the pump , the filter and the lines into the receiving vessel. The filtrate and washings were combined. Total volume = 75 L (by dipstick). LC assay gave 4.395 kg of Boc-

aminoisobutyryl O-benzylserine spiroindoline (**14**), i.e. 93% overall from 7.49 moles of starting CBZ-spiroindoline sulfonamide (**1**).

The batch was concentrated *in vacuo* to low bulk (~15 L) and the isopropyl acetate switched to ethanol by "feeding and bleeding" absolute ethanol (45 L total). At the end of the solvent switch, GC showed <1% isopropyl acetate remaining. This solution (25 L) containing 4.395 kg of **14** was used for the next stage. If desired, the inermediate compound **14** may be isolated by adding water to crystalize it out.

EXAMPLE 17A



15

Aminoisobutyryl O-Benzylserine Spiroindoline (**15**)

Materials:

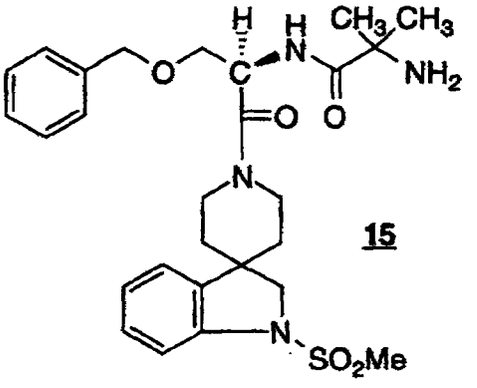
Boc Spiroindoline (**14**)	3160 g (5.03 moles)
Methanesulfonic acid (MsOH)	979 mL (15.1 moles)
EtOH	6.2 L
H_2O	30 L
1N NaOH	11 L
EtOAc	26 L
Darco 60 activated carbon	1 Kg

The Boc spiroindoline **14** was dissolved in 6.2 L of EtOH and treated with MsOH (979 mL). The temperature rose from 20 to 30°C and the reaction was allowed to proceed overnight. After 12

hours at 20°C there was still 15 A% of starting material left so the mixture was heated to 35°C for 6 hours. Upon completion (<0.1 A% 14) the reaction was cooled to 20°C and 30 L of H_2O were added and the solution was filtered through a glass funnel with a polypropylene filter to filter off residual DCU. The mixture was transferred to a 100 L extractor and 26 L of EtOAc were added. The aqueous layer was basified via addition of chilled 1N NaOH (11 L) and 1 L of 50% NaOH. Addition of ice was required to keep the temperature below 14°C. Higher temperatures resulted in significant emulsion problems.

The organic layer was distilled at 50°C at ca. 21" of Hg until KF <1000 µg/mL. Lower KF's result in more efficient carbon treatments and better recovery at the salt formation step. KF's of 160 µg/mL were achieved at the 700 g scale. The solution was diluted with ethyl acetate to a total volume of 31 L (LC assay 2.40 kg). Activated carbon (Darco G-60) was added and the mixture was stirred for 24 h. The mixture was filtered through Solka Floc™ and the filter cake was washed with ethyl acetate (16 L), assay 2.34 Kg.

EXAMPLE 17B



15

Aminoisobutyryl O-Benzylserine Spiroindoline (15)

Materials:	
Boc Spiroindoline (**14**)	4.395 kg (6.99 mol)
Methanesulfonic acid	2.017 kg (20.99 mol)
Ethyl acetate	185 L
1M Aqueous sodium hydroxide	16 L
50% Aqueous sodium hydroxide	2.6 L
Darco G-60	900 g
Solka Floc™	2.5 kg

Methanesulfonic acid (2.017 kg, 1.36 L, ~3 equivs.) was added to the stirred solution of the Boc spiroindoline **14** (4.395 kg) in ethanol (total volume ~25 L) in a reaction vessel at room temperature. The batch was warmed to 35-40°C, and stirred overnight. On the next day, the batch contained ~1.1 A% of starting material and so the reaction was continued for a further 4 hours, then LC showed ratio of product/ starting material to be 99.6/0.4. The batch was concentrated *in vacuo* to ~15 L volume and then diluted with water (44 L). The batch was cooled to 5°C, stirred for 30 minutes and then filtered through a Sparkler in-line filter (containing a 10μ cartridge) using a pump to another vessel to remove a small amount of residual DCU.

The vessel, the pump, the filter and the lines were rinsed with water (10 L), and this was added to the vessel. Ethyl acetate (36 L) was added to the vessel and the stirred mixture was cooled to 10°C. A solution of cold (5-10°C) 1M aqueous sodium hydroxide solution (16 L) and cold (5-10°C) 50% aqueous sodium hydroxide solution (2.6 L) were added at 10°C and the temperature rose to 14°C. The resulting mixture was stirred for 15 minutes at <14°C and then the lower aqueous layer separated off.

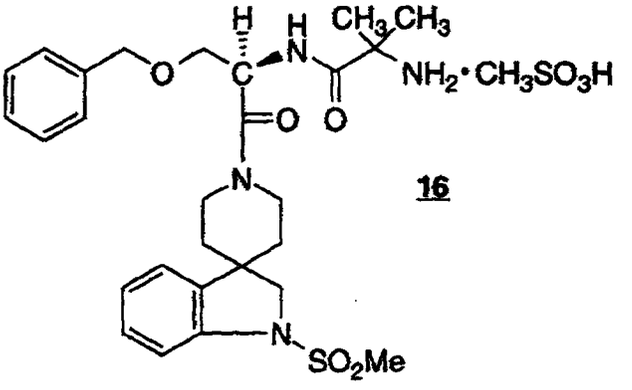
The batch was concentrated *in vacuo* to ~20 L volume and then a mixture of ethyl acetate (35 L) and ethanol (5 L) was fed in while maintaining the volume at ~20 L. At the end of this distillation the KF was 9160 $mgml^{-1}$. The batch was solvent switched to ethyl acetate by "feeding and bleeding" ethyl acetate (40 L total). At the end of this

distillation, KF was 446 $mgml^{-1}$. The batch was diluted with ethyl acetate (10 L).

Darco G-60 (900 g) was added to the hazy mixture. This was rinsed in with ethyl acetate (6 L). This mixture was stirred at room temperature overnight. Next day, Solka Floc™ (0.5 kg) was added to the stirred batch in the vessel and then Solka Floc™ (2.0 kg) was stirred in a little ethyl acetate and loaded into an Estrella filter . The excess solvent was pumped away through a Sparkler in-line filter containing a 10μ cartridge. The slurry was transferred from the vessel through a filter using a pump and then through another filter to 2 x 40 L stainless steel bins. Visual inspection showed the liquors to be clear and clean. The vessel was rinsed with ethyl acetate (22 L) and this was used to rinse through the route outlined above to the stainless steel cans. The contents of both cans was transferred into a reaction vessel and the solution was mixed thoroughly.

The batch (58 L) had a KF of 2950 $mgml^{-1}$ and so was redried by concentrating *in vacuo* to 20-25 L volume. The batch was diluted to 46 L volume (dipstick) by the addition of ethyl acetate (25 L). The KF was 363 $mgml^{-1}$. The batch was diluted to 62 L volume by the addition of ethyl acetate (17 L) and was used for the final stage of the process.

EXAMPLE 18A



16

Spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide Methanesulfonate (**16**)

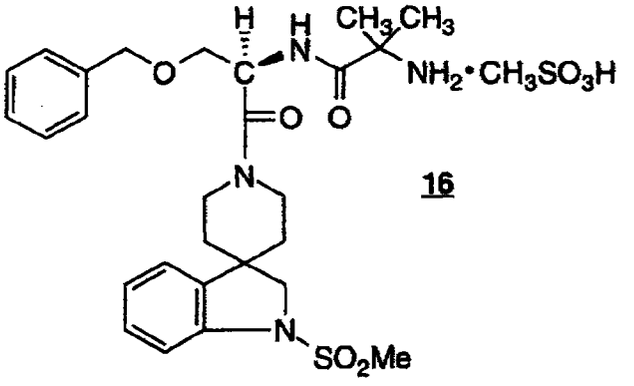
Materials:

Amine (**15**)	2340 g (4.43 moles)
Methane sulfonic acid (MsOH)	316 mL (4.88 moles)
EtOAc	60 L
EtOH	4.8 L
8% EtOH in EtOAc	20 L

The volume of the solution of **15** from the previous step was adjusted to 60 L with ethyl acetate and EtOH (4.8 L) was added. The MsOH (316 mL) was added in 3 L of EtOAc at 45°C. To the deep red homogeneous solution was added 496 g of the title compound Form I seed (10% seed based on the weight of the free amine was employed). The temperature rose to ca. 48°C and the reaction was aged at 52°C for 1.5 hours. Analysis indicated complete conversion to the title compound (Form I). (At less than 10% seed longer age (> 3 hours) was required). The slurry was allowed to cool to 20°C overnight and was filtered in a centrifuge under N_2. The cake was washed with 20 L of 8% EtOH in EtOAc. N_2 is essential during filtration because the wet crystals are very hygroscopic. The batch was dried at 35°C under vacuum to afford 2.7Kg (56% overall yield) of the title compound (Form I) (99.9 A% purity; < 0.1% enantiomer).

The conversion of Form II to Form I is also accomplished where the salt is formed in EtOAc-EtOH by addition of MsOH as above and the initial solution of the salt (at 55°C) is cooled to 45°C. Crystals start appearing at that temperature and the slurry becomes thicker with time. The temperature is then raised to 51°C and the slurry is aged overnight. Complete conversion to Form I of **16** should be expected. This procedure may also be employed to prepare seed crystals of Form I of **16**.

EXAMPLE 18B



16

Spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide Methanesulfonate (**16**)

Materials:

Amine (**15**)	3.1 kg (5.86 mol)
Methanesulfonic acid	620 g (6.45 mol)
Ethyl acetate	37 L
Absolute ethanol	8.7 L
Spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (Form I)	70 g (0.11 mol)

Absolute ethanol (6.4 L) was added to the solution of the amine (**15**) (3.1 kg) in ethyl acetate (total volume ~62 L) in a reacttion

vessel. The batch was warmed to 50°C and a solution of methanesulfonic acid (620 g, 412 ml, 1.1 equivs.) in ethyl acetate (11 L) was added over ~5 minutes at 50-54°C. The batch was seeded with spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (Form I) (70 g) and the resulting slurry was stirred and heated at 55°C under nitrogen atmosphere overnight.

The next day, the slurry was cooled to 15-20°C, held for 2 hours and then dropped to the 50 cm polypropylene filter under nitrogen atmosphere. The solid product was washed with a mixture of absolute ethanol (2.3 L) in ethyl acetate (26 L). The white, solid product was dug off and dried in an Apex oven *in vacuo* at 35°C for an appropriate time (approx. two days). The dried spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (3.352 kg) was sieved using a Jackson-Crockatt sieve to give 3.347 kg (including seed, 70 g) } yield = 3.277 kg.

HPLC Conditions:

LC Retention times on Zorbax RX-C8 (4.6 mm x 25 cm), λ = 210 nm, flow rate = 1.5 ml/min.

Compound **1**: 60:40 CH_3CN-H_2O (1% H_3PO_4) RT = 5.0 min
Compound **1b**: 35:65 CH_3CN-H_2O (0.1 w % NH_4OAc) RT = 6.2 min.
Compound **10**: 60:40 CH_3CN-H_2O (0.1 H_3PO_4) RT = 2.9 min.
Compound **11**: 60:40 CH_3CN-H_2O (0.1% H_3PO_4) RT = 5.4 min.
Compound **12**: 40:60 CH_3CN-H_2O [pH 5.25 NaH_2PO_4 (6.9 g/L of H_2O) (adjust pH with NaOH)] RT = 5.6 min
Compound **14**: 60:40% CH_3CN-H_2O (0.1% H_3PO_4) RT = 4.65 min
Compound **15**: 40:60% CH_3CN-H_2O [pH = 5.25 NaH_2PO_4 (6.9 g/L of H_2O)] adjust pH with NaOH)RT = 4.9 min

LC Retention times on Zorbax RX-C8 (4.6 mm x 25 cm), λ = 210 nm, flow rate = 1.2 ml/min, column temperature = 48°C
Solvent A = 0.05% Phosphoric acid + 0.01% Triethylamine in water
Solvent B = Acetonitrile
Gradient system:

Time	% A	% B
0 min	95	5
35 min	10	90
38 min	95	5
40 min	95	5

	Retention time (mins)
Compound **1**	25.2
Compound **1b**	8.5
Compound **10**	20.5
Compound **11**	26.3
Compound **12**	14.8
Compound **14**	25.6
Compound **15**	15.7

EXAMPLE 19

Preparation of Form I of N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)-ethyl]-2-amino-2-methylpropanamide methanesulfonate

The conversion of Form II to Form I may be accomplished by the procedure of Example 18A where the salt is formed in EtOAc-EtOH by addition of MsOH and the initial solution of the salt (at 55°C) is cooled to 45°C. Crystals should start appearing at that temperature and the slurry should become thicker with time. The temperature is then raised to 51°C and the slurry is aged overnight. Complete conversion to Form I should be expected.

EXAMPLE 20

Preparation of Form I of N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)-ethyl]-2-amino-2-methylpropanamide methanesulfonate

The conversion of Form II to Form I is accomplished by stirring a solution of Form II of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in isopropanol at approximately 25°C for about 2-24 hours.

EXAMPLE 21

Preparation of Form IV of N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)-ethyl]-2-amino-2-methylpropanamide methanesulfonate

A sample of 8.4 g N-[1(R)-[(1,2-dihydro-1-methane-sulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenyl-methyloxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate of

optional morphological form is dissolved in a mixture of 24.8 ml ethyl acetate, 1.6 ml ethanol and 1.95 ml water with stirring at 42°C. The solvent is evaporated from the solution at a temperature of 40°C, the resultant solid is ground in a morter to a fine powder and the fine powder is exposed to a relative humidity of approximately 75% to give the title Form IV.

EXAMPLE 22

Preparation of Form IV of N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)-ethyl]-2-amino-2-methylpropanamide methanesulfonate

A sample of N-[1(R)-[(1,2-dihydro-1-methane-sulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenyl-methyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate of optional morphological form is recrystallized from a solution of ethylacetate/ethanol/water (24.8/1.6/1.95 v/v/v) to give the title Form IV.

EXAMPLE 23

Preparation of Form IV of N-[1(R)-[(1,2-Dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyloxy)-ethyl]-2-amino-2-methylpropanamide methanesulfonate

A slurry of Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in isopropyl acetate/ethanol (90:10 v/v) containing approximately 2.8 wt% water is stirred at approximately 25°C overnight and the resultant solid is isolated.

While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. For example, effective dosages other than the particular dosages as set forth herein above may be applicable as a consequence of variations in the responsiveness of the mammal being treated for any of the indications with the compound of the invention indicated above. Likewise, the specific pharmacological responses observed may vary according to and depending upon the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.



WHAT IS CLAIMED IS:

1. A polymorphic form of the compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate designated Form I.

2. The polymorphic form of Claim 1 characterized by an endotherm of melting with an extrapolated onset temperature of about 170°C when heated in a differential scanning calorimetric cell at a rate of 10°C/min under a nitrogen atmosphere.

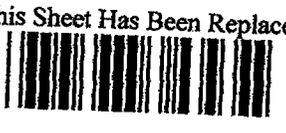
3. The polymorphic form of Claim 1 characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 6.5, 14.7, 16.9, 17.1, 17.9, 19.5, 21.1, 21.7, and 22.0° (2 theta).

4. A polymorphic form of the compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate designated Form IV.

5. The polymorphic form of Claim 4 characterized by a water loss endotherm at a temperature of about 45°C followed by an endotherm with an extrapolated onset temperature of about 129°C when heated in a differential scanning calorimetric cell at a rate of 10°C/min under a nitrogen atmosphere.

6. The polymorphic form of Claim 4 which contains approximately 3.5 moles of water per mole of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED

7. The polymorphic form of Claim 4 characterized by an X-ray powder diffraction pattern with principal reflections at approximately: 16.0, 16.2, 18.3, 20.1, 21.0, and 24.2° (2 theta).

8. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of the polymorphic form of Claim 1.

9. A pharmaceutical composition useful for the treatment of osteoporosis which comprises a combination of a bisphosphonate compound and the polymorphic form of Claim 1.

10. The pharmaceutical composition of Claim 9 wherein the bisphosphonate compound is alendronate.

11. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of the polymorphic form of Claim 4.

12. A pharmaceutical composition useful for the treatment of osteoporosis which comprises a combination of a bisphosphonate compound and the polymorphic form of Claim 4.

13. The pharmaceutical composition of Claim 12 wherein the bisphosphonate compound is alendronate.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

14. A method for increasing levels of endogenous growth hormone in a human or an animal which comprises administering to such human or animal an effective amount of the polymorphic form of Claim 1.

15. A method for increasing levels of endogenous growth hormone in a human or an animal which comprises administering to such human or animal an effective amount of the polymorphic form of Claim 1 and an additional growth hormone secretagogue.

16. The method of Claim 15 wherein the additional growth hormone secretagogue is selected from the group consisting of: growth hormone releasing peptide GHRP-6; growth hormone releasing peptide GHRP-2; growth hormone releasing peptide GHRP-1; B-HT920; growth hormone releasing factor; an analog of growth hormone releasing factor; IGF-1 and IGF-2.

17. A method for increasing feed efficiency, promoting growth, increasing milk production and improving the carcass quality of livestock which comprises administering to such livestock an effective amount of the polymorphic form of Claim 1.

18. A method of treating or preventing a condition selected from the group consisting of: osteoporosis; catabolic illness; immune deficiency, including that in individuals with a depressed T4/T8 cell ratio; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and protein loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or burns, in a patient in need thereof which comprises the administration to the patient of an effective amount of the polymorphic form of Claim 1.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

19. A method for the treatment of osteoporosis which comprises administering to a patient with osteoporosis a combination of a bisphosphonate compound and the polymorphic form of Claim 1.

20. The method of Claim 19 wherein the bisphosphonate compound is alendronate.

21. A method for increasing levels of endogenous growth hormone in a human or an animal which comprises administering to such human or animal an effective amount of the polymorphic form of Claim 4.

22. A method for increasing levels of endogenous growth hormone in a human or an animal which comprises administering to such human or animal an effective amount of the polymorphic form of Claim 1 and an additional growth hormone secretagogue.

23. The method of Claim 22 wherein the additional growth hormone secretagogue is selected from the group consisting of: growth hormone releasing peptide GHRP-6; growth hormone releasing peptide GHRP-2; growth hormone releasing peptide GHRP-1; B-HT920; growth hormone releasing factor; an analog of growth hormone releasing factor; IGF-1 and IGF-2.

24. A method for increasing feed efficiency, promoting growth, increasing milk production and improving the carcass quality of livestock which comprises administering to such livestock an effective amount of the polymorphic form of Claim 4.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

25. A method of treating or preventing a condition selected from the group consisting of: osteoporosis; catabolic illness; immune deficiency, including that in individuals with a depressed T4/T8 cell ratio; hip fracture; musculoskeletal impairment in the elderly; growth hormone deficiency in adults or in children; obesity; cachexia and protein loss due to chronic illness such as AIDS or cancer; and treating patients recovering from major surgery, wounds or burns, in a patient in need thereof which comprises the administration to the patient of an effective amount of the polymorphic form of Claim 4.

26. A method for the treatment of osteoporosis which comprises administering to a patient with osteoporosis a combination of a bisphosphonate compound and the polymorphic form of Claim 4.

27. The method of Claim 26 wherein the bisphosphonate compound is alendronate.

28. A process for the preparation of the polymorphic form of Claim 1 designated Form I which comprises:

stirring a solution of Form II of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate in isopropanol at approximately 25°C for about 2-24 hours.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED