Exhibit 10

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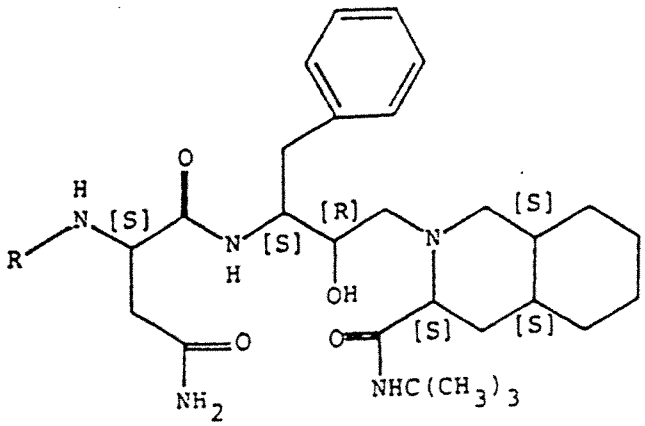
(54) Amino acid derivatives.

(57) Compounds of the general formula



I

wherein R represents benzyloxycarbonyl or 2-quinolylcarbonyl,
and their pharmaceutically acceptable acid addition salts inhibit proteases of viral origin and can be used as medicaments for the treatment or prophylaxis of viral infections.

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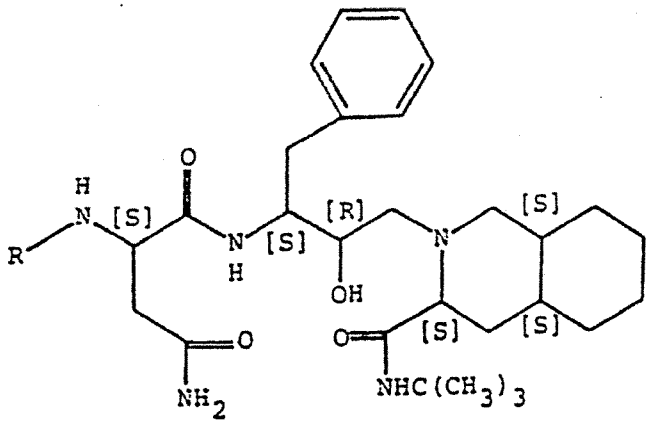
AMINO ACID DERIVATIVES

The present invention is concerned with amino acid derivatives.

The amino acid derivatives provided by the present invention are compounds of the general formula



I

wherein R represents benzyloxycarbonyl or 2-quinolylcarbonyl.

and pharmaceutically acceptable acid addition salts thereof.

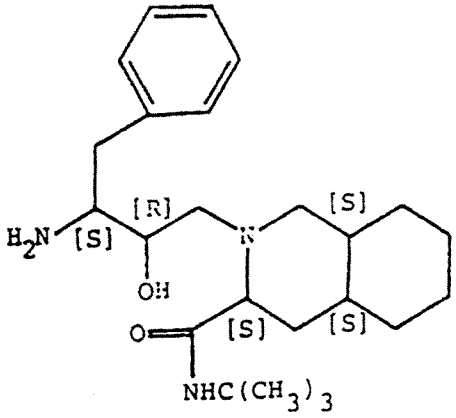
The compounds of formula I and their pharmaceutically acceptable acid addition salts are novel and possess valuable pharmacological properties. In particular, they inhibit proteases of viral origin and can be used in the prophylaxis or treatment of viral infections, particularly of infections caused by HIV and other retroid viruses.

Objects of the present invention are the compounds of formula I and their aforementioned salts per se and for use as therapeutically active substances, a process for the manufacture of said compounds and salts, intermediates used in said process, medicaments containing said compounds and salts, the use of said compounds and salts in the control or prevention of illnesses, especially in the treatment or prophylaxis of viral infections, and the use of said compounds and salts for the manufacture of medicaments for the treatment or prophylaxis of viral infections.

The pharmaceutically acceptable acid addition salts of the compounds of formula I are salts formed with inorganic acids, for example hydrohalic acids such as hydrochloric acid or hydrobromic acid, sulphuric acid, nitric acid, phosphoric acid etc, or with organic acids, for example acetic acid, citric acid, maleic acid, fumaric acid, tartaric acid, methanesulphonic acid, p-toluenesulphonic acid etc.

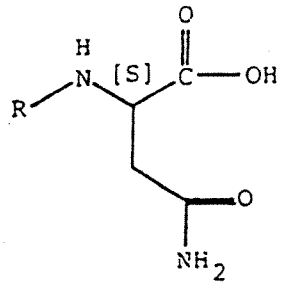
According to the process provided by the present invention, the compounds of formula I hereinbefore and their pharmaceutically acceptable acid addition salts are manufactured by

(a) reacting 2-[(3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N--tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3-(S)-carboxamide of the formula



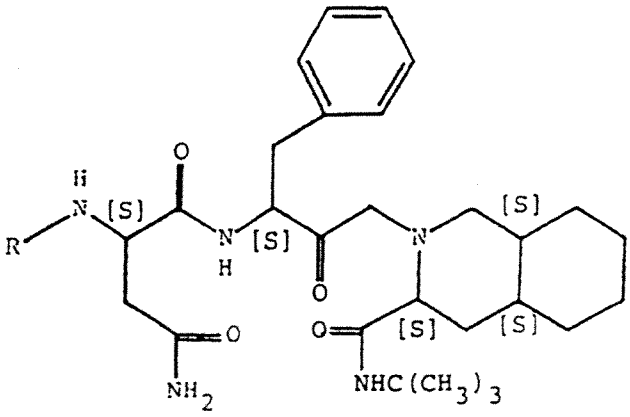
II

with an acid of the general formula



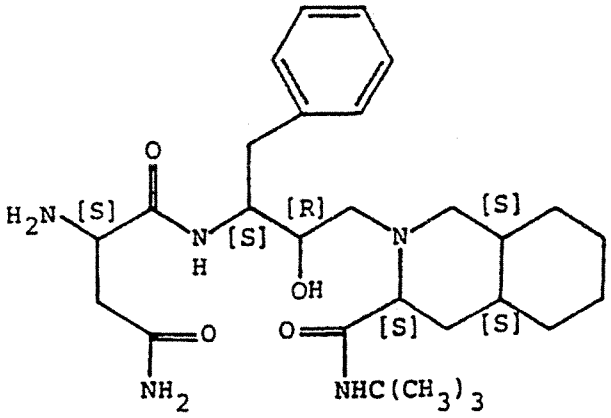
III

wherein R has the significance given earlier,
or a reactive derivative thereof, or
(b) reducing a compound of the general formula



IV

wherein R has the significance given earlier,
and separating the desired 2(R)-hydroxy isomer from the mixture obtained, or
(c) reacting 2-[3(S)-[(L-asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



V

with an agent yielding the benzyloxycarbonyl or 2-quinolylcarbonyl group, and

(d) if desired, converting a compound of formula I obtained into a pharmaceutically acceptable acid addition salt.

The reaction of a compound of formula II with an acid of formula III in accordance with embodiment (a) of the process can be carried out in accordance with methods known per se in peptide chemistry. Thus, when an acid of formula III is used, the reaction is preferably carried out in the presence of a condensation agent such as hydroxybenzotriazole and dicyclohexylcarbodiimide. This reaction is conveniently carried out in an inert organic solvent such as an ether (e.g. diethyl ether, tetrahydrofuran etc) or dimethylformamide at a low temperature, suitably at about -10°C to +5°C and especially at about 0°C. Suitable reactive derivatives of acids of formula III which can be used are, for example, the corresponding acid halides (e.g. acid chlorides), acid anhydrides, mixed anhydrides, activated esters etc. When a reactive derivative is used, the reaction is conveniently carried out in an inert organic solvent such as a halogenated aliphatic hydrocarbon (e.g. dichloromethane etc) or an ether (e.g. diethyl ether, tetrahydrofuran etc) and, where appropriate, in the presence or an organic base (e.g. N-ethylmorpholine, diisopropylethylamine etc) at a low temperature, suitably at about -10°C to +5°C and especially at about 0°C.

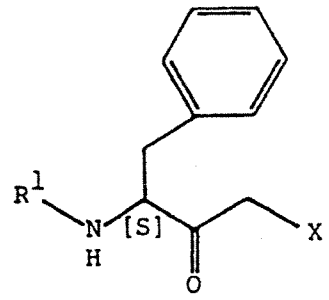
The reduction of a compound of formula IV in accordance with embodiment (b) of the process can be carried out according to methods known per se for the reduction of a carbonyl group to a hydroxy group. Thus, for example, the reduction can be carried out using a complex metal hydride such as an alkali metal borohydride, especially sodium borohydride, in an appropriate organic solvent such as an alkanol (e.g. methanol, ethanol, propanol, isopropanol etc). Conveniently, the reduction is carried out at about room temperature. The separation of the desired 2(R)-hydroxy isomer from the mixture obtained can be performed according to conventional methods, e.g. by chromatography and the like.

In accordance with embodiment (c) of the process, the suitable agent yielding the benzyloxycarbonyl group is benzyl chloroformate. Suitable agents which yield the 2-quinolylcarbonyl group are the corresponding acid or reactive derivatives thereof such as the corresponding acid halides (e.g. acid chloride), acid anhydride, mixed anhydrides, activated esters etc. The reaction of a compound of formula V with the aforementioned agents is carried out in the same manner as that described earlier in connection with embodiment (a) of the process.

The conversion of a compound of formula I into a pharmaceutically acceptable acid addition salt in accordance with embodiment (d) of the process can be carried out by treating such a compound in a conventional manner with an inorganic acid, for example a hydrohalic acid such as hydrochloric acid or hydrobromic acid, sulphuric acid, nitric acid, phosphoric acid etc, or with an organic acid such as acetic acid, citric acid, maleic acid, fumaric acid, tartaric acid, methanesulphonic acid, p-toluenesulphonic acid etc.

The compound of formula II which is used as starting material in embodiment (a) of the process is novel and also forms an object of the present invention.

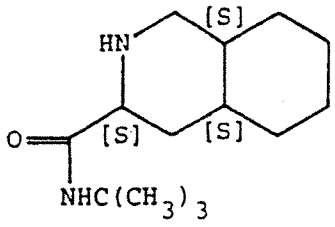
The compound of formula II can be prepared, for example, by reacting a compound of the general formula



VI

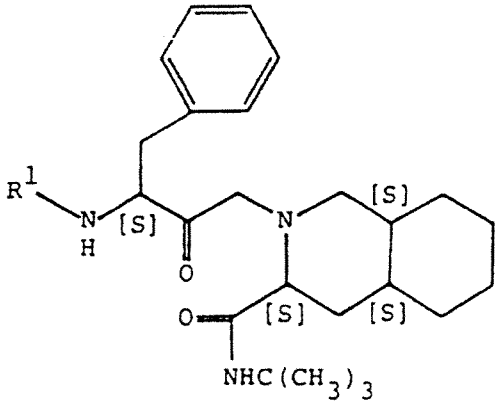
wherein R^1 represents a amino-protecting group (e.g. tert.butoxycarbonyl or benzyloxycarbonyl) and X represents a chlorine or bromine atom.

with N-tert.butyl-decahydro-(4aS,8aS)isoquinoline-3(S)-carboxamide of the formula



VII

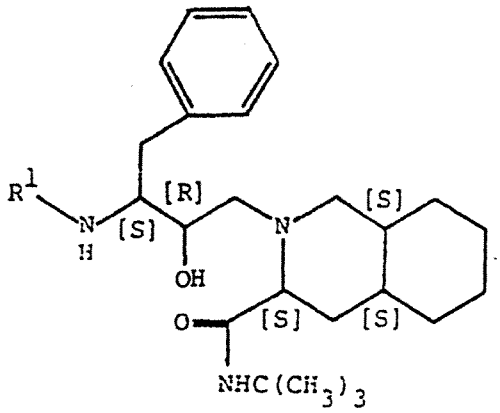
and reducing the resulting compound of the general formula



VIII

wherein R^1 has the significance given earlier,

separating the desired 2(R)-hydroxy isomer from the mixture obtained and cleaving off the group R^1 from the resulting compound of the general formula



IX

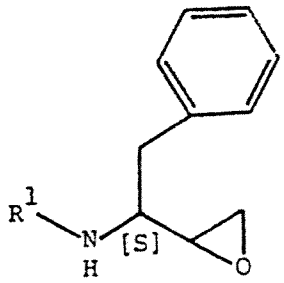
wherein R^1 has the significance given earlier,
to give a compound of formula II.

The reaction of a compound of formula VI, preferably one in which R^1 represents benzyloxycarbonyl, with a compound of formula VII can be carried out in a known manner: for example, in an inert organic solvent such as a halogenated aliphatic hydrocarbon (e.g. dichloromethane etc) and in the presence of a base (e.g. a trialkylamine such as triethylamine etc), conveniently at about room temperature.

The reduction of a compound of formula VIII to give a compound of formula IX and the subsequent separation of the desired 2(R)-hydroxy isomer can be carried out as described earlier in connection with embodiment (b) of the process of the invention, i.e. the reduction of a compound of formula IV and the separation of the desired 2(R)-hydroxy isomer from the mixture obtained.

The cleavage of the group R^1 from a compound of formula IX can also be carried out in a known manner; for example, using a strong inorganic acid such as a hydrohalic acid or a strong organic acid (e.g. trifluoroacetic acid etc), conveniently at about 0 °C to about room temperature. Alternatively, a hydrogenolytically-cleavable amino-protecting group R^1 can be cleaved off using hydrogen in the presence of a noble-metal catalyst (e.g. a palladium catalyst such as palladium-on-carbon) in an organic solvent or solvent mixture which is inert under the reaction conditions (e.g. an alkanol such as ethanol, isopropanol etc, an alkanecarboxylic acid ester such as ethyl acetate, etc) and conveniently at about room temperature.

A further method for the preparation of the compound of formula II comprises firstly reacting a compound of the general formula



X

wherein R^1 has the significance given earlier,
with the compound of formula VII hereinbefore, conveniently in an inert organic solvent such as an alkanol (e.g. methanol etc), dimethylformamide or the like and at an elevated temperature, conveniently at about 60 °C to about 120 °C, and then cleaving off the group R^1 in the reaction product (a compound of formula IX hereinbefore) as described earlier.

The compounds of formula IV which are used as starting materials in embodiment (b) of the process can be prepared, for example, by cleaving off the amino-protecting group R^1 from a compound of formula VIII and reacting the product with an acid of formula III or a reactive derivative thereof. This reaction can be

carried out in an analogous manner to that described earlier in connection with embodiment (a) of the process.

The compound of formula V which is used as starting material in embodiments (c) of the process is novel and forms a further object of the present invention.

The compound of formula V can be prepared, for example, by cleaving off the benzyloxycarbonyl group R from the compound of formula I in which R represents benzyloxycarbonyl or the tert.butoxycarbonyl group form a compound corresponding to formula I but in which R represents tert.butoxycarbonyl. This latter compound can be prepared, for example, by reacting the compound of formula II with N-(tert.butoxycarbonyl)-L-asparagine in accordance with embodiment (a) of the process. The above cleavage is carried out in a manner analogous to that described earlier in connection with the cleavage of the group R^1 from a compound of formula VIII.

The starting materials of formula III and their reactive derivatives as well as the compounds of formulae VI, VII and X hereinbefore, insofar as they are not known compounds or analogues of known compounds, can be prepared in a similar manner to the known compounds or as described in the Examples hereinafter or in analogy thereto. Moreover, the agents used in embodiment (c) of the process are generally known compounds.

As mentioned earlier, the compounds of formula I and their pharmaceutically acceptable acid addition salts inhibit proteases of viral origin and are useful in the treatment or prophylaxis of viral infections, particularly of infections caused by HIV and other retroid viruses.

The in vitro inhibition of HIV protease by the compounds provided by the present invention can be demonstrated by means of the following test:

HIV protease was expressed in E. coli and partially purified from soluble extracts of the bacterium by ammonium sulphate fractionation (0-30%). Protease activity was assayed using the protected hexapeptide succinyl-Ser-Leu-Asn-Tyr-Pro-Ile isobutylamide (S^1) or the protected heptapeptide succinyl-Val-Ser-Gln-Asn-Phe-Pro-Ile isobutylamide (S^2) as the substrate. Cleavage of the substrate was quantified by measuring the production of H-Pro-Ile isobutylamide by the spectrophotometric assay of N-terminal proline.

1.25 mM of substrate were dissolved in 125 mM of citrate buffer (pH 5.5) containing 0.125 mg.ml of Tween 20. 10 µl of a solution of various concentrations of the test compound (dissolved in methanol or dimethyl sulphoxide and diluted with water containing 0.1% Tween 20) and 10 µl of protease were added to 80 µl of the above buffered substrate. Digestion was carried out at 37° C for a fixed period of time and was terminated by the addition of 1 ml of colour reagent [30 g/ml of isatin and 1.5 mg.ml of 2-(4-chlorobenzoyl)-benzoic acid in 10% acetone in ethanol (vol./vol.)]. The solution was heated in a water bath and then the pigmented residues were re-dissolved in 1 ml of 1% pyrogallol in 33% water in acetone (wt./vol.). The optical density of the solution was measured spectrophotometrically at 599 nm. The formation of H-Pro-Ile isobutylamide in the presence of the test compound was compared with controls and the concentration of test compound required to give 50% inhibition (I_{50}) was determined by means of a graph plotted from the various concentrations of test compound used.

The in vitro antiviral activity of the compounds of formula I can be demonstrated in the assay described below:

Activity against HIV:

This assay uses HTLV-III (strain RF) grown in C8166 cells (a human CD4$^+$ T lymphoblastoid line) using RPMI 1640 medium with bicarbonate buffer, antibiotics and 10% foetal bovine serum.

A suspension of cells is infected with ten times the TCD_{50} of virus and adsorption allowed to proceed for 90 minutes at 37° C. The cells are washed three times with medium. The test is carried out in 6 ml tissue culture tubes, each tube containing 2 x 10^5 infected cells in 1.5 ml of medium. Test compounds are dissolved in either aqueous medium or dimethyl sulphoxide, according to solubility, and a 15 µl solution of the substance added. The cultures are incubated at 37° C for 72 hours in a humidified atmosphere containing 5% carbon dioxide in air. The cultures are then centrifuged and an aliquot of the supernatant solubilized with Nonidet P40 and subjected to an antigen capture assay which uses a primary antiserum with particular reactivity against the viral protein 24 and a horseradish peroxidase detection system. Colour generation is measured spectrophotometrically and plotted against the concentration of test substance. The concentration that produces 50% protection is determined (I_{50}).

A cytotoxicity assay based on dye uptake and metabolism or radio-labelled amino acid incorporation is run alongside the above assay in order to determine antiviral selectivity.

The results obtained in the foregoing tests using the compounds of formula I as the test compound are compiled in the following Table.

Table

Compound I R	I_{50} Inhibition of HIV protease (µM) S^1	I_{50} Inhibition of HIV protease (µM) S^2	Activity against HIV (nM)
Benzyloxycarbonyl	≤ 0.024	≤ 0.0027	20
2-Quinolylcarbonyl	≤ 0.033	≤ 0.00037	2

The compounds of formula I and their pharmaceutically acceptable acid addition salts can be used as medicaments (e.g. in the form of pharmaceutical preparations). The pharmaceutical preparations can be administered enterally such as orally (e.g. in the form of tablets, coated tablets, dragees, hard and soft gelatine capsules, solutions, emulsions or suspensions), nasally (e.g. in the form of nasal sprays) or rectally (e.g. in the form of suppositories). However, the administration can also be effected parenterally such as intramuscularly or intravenously (e.g. in the form of injection solutions).

For the manufacture of tablets, coated tablets, dragees and hard gelatine capsules the compounds of formula I and their pharmaceutically acceptable acid addition salts can be processed with pharmaceutically inert, inorganic or organic excipients. Lactose, maize starch or derivatives thereof, talc, stearic acid or its salts etc can be used, for example, as such excipients for tablets, dragees and hard gelatine capsules.

Suitable excipients for soft gelatine capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols etc.

Suitable excipients for the manufacture of solutions and syrups are, for example, water, polyols, saccharose, invert sugar, glucose etc.

Suitable excipients for injection solutions are, for example, water, alcohols, polyols, glycerol, vegetable oils etc.

Suitable excipients for suppositories are, for example, natural or hardened oils, waxes, fats, semi-liquid or liquid polyols etc.

Moreover, the pharmaceutical preparations can contain preserving agents, solubilizers, viscosity-increasing substances, stabilizing agents, wetting agents, emulsifying agents, sweetening agents, colouring agents, flavouring agents, salts for varying the osmotic pressure, buffers, coating agents or antioxidants. They can also contain still other therapeutically valuable substances.

In accordance with the invention the compounds of formula I and their pharmaceutically acceptable acid addition salts can be used in the treatment or prophylaxis of viral infections, particularly of retroviral infections. The dosage can vary within wide limits and will, of course, be fitted to the individual requirements in each particular case. In general, in the case of oral administration there should suffice a daily dosage of about 3 mg to about 3 g, preferably about 10 mg to about 1 g (e.g. approximately 300 mg per person), divided in preferably 1-3 unit doses, which can, for example, be of the same amount. It will, however, be appreciated that the upper limit given above can be exceeded when this is found to be indicated.

The following Examples illustrate the present invention.

Example 1

A solution of 561 mg of 2-[3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide and 372 mg of N-(benzyloxycarbonyl)-L-asparagine in 20 ml of dry tetrahydrofuran was cooled in an ice/salt mixture. 189 mg of hydroxybenzotriazole, 161 mg of N-ethylmorpholine and 317 mg of dicyclohexylcarbodiimide were added and the mixture was stirred for 16 hours. The mixture was then diluted with ethyl acetate and filtered. The filtrate was washed with aqueous sodium bicarbonate solution and sodium chloride solution. The solvent was removed by evaporation and the

residue was chromatographed on silica gel using dichloromethane/ methanol (9:1) for the elution to give 434 mg of 2-[3(S)-[[N-(benzyloxycarbonyl)-L-asparaginyl]amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide as a white solid from methanol/diethyl ether; MS: m e 650 $[M+H]^+$; NMR: δ (d_4 CN_3OH, 400 MNz):

7.33 (5N, m, $PhCH_2O$), 7.25 (2N, m), 7.18 (2N, m), 7.09 (IH, m), 5.05 (2N, s, $PhCH_2O$), 4.42 (IH, dd, Asn α J = 7.8, 6.1), 4.22 (IH, m, $-CH_2CHCH(OH)-$ J = 10.7, about 4, about 4), 3.85 (IH, m, $-CHCH(OH)CH_2-$ J = 8.0, 6.2, about 4), 3.02 (IH, dd, $PhCH(N)CHJ$ = -13.9, about 4), 3.02 (IH, dd, I_{eq} J = -12.0, small), 2.69 (IH, dd, $PhCH(H)CH-$ J = -13.9, 10.7), 2.63 (IH, dd, $-CH(OH)CH(H)N-$ J = -12.6, 8.0), 2.62 (IH, dd, $H3_{ax}$ J = about 11, small), 2.57 (IH, dd, Asn B_1 J = -15.2, 6.1), 2.38 (IH, dd, Asn B_2 J = -15.2, 7.8), 2.19 (IH, dd, $-CH(OH)CH(H)N-$ J = -12.6, 6.2), 2.17 (IH, dd, I_{ax} J = -12.0, 3.2), 2.07 (IH, m, $H4_{ax}$ J = -12.7, about 11, about 11.5), 1.78 (IH, m, H4a J_{4a-4ax} = about 11.5, J_{4a-4eq} = small, J_{4a-8a} = small), 1.63 (IH, m, H8a J_{8a-1ax} = 3.2, J_{8a-1eq} = small, J_{8a-4a} = small), 1.35 (IH, m, $H4_{eq}$ J = -12.7, small, small), 1.30 (9H, s, t-butyl), 2.0-1.2 (8N, m).

The 2[3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide used as the starting material was prepared as follows:

(i) A suspension of 12.676 g (71.6 mmol) of 1,2,3,4-tetrahydro-3(S)-isoquinolinecarboxylic acid (Chem. Pharm. Bull. 1983, 31, 312) in 200 ml of 90% acetic acid was hydrogenated at 80° C and under 140 atmospheres pressure over 5% rhodium-on-carbon for 24 hours. The mixture was left to cool to room temperature and the catalyst was then filtered off. The filtrate was evaporated to give a gum which was dissolved in 10 ml of ethyl acetate and added slowly to 100 ml of vigorously stirred diisopropyl ether. A resinous precipitate was produced. The supernatant liquors were removed by decantation and the precipitate was extracted with hot ethyl acetate. This hot solution was poured into a vigorously stirred mixture of 150 ml of diethyl ether/diisopropyl ether (1:1) to give a pale grey solid which was collected by filtration, washed with diethyl ether and dried. There were obtained 5.209 g of a mixture of decahydroisoquinoline-3(S)-carboxylic acids consisting of predominantly (about 65%) the 4aS,8aS isomer together with the 4aR,8aR isomer (about 25%) and about 10% of the trans isomers; MS: m.e 184 $[M+H]^+$.

(ii) 9.036 g (49.4 mmol) of the foregoing mixture of decahydroisoquinoline-3(S)-carboxylic acids were dissolved in 50 ml (50 mmol) of IM sodium hydroxide solution and the resulting solution was cooled to 0° C. 7.40 ml (51.87 mmol) of benzyl chloroformate and 58.7 ml (58.7 mmol) of IM sodium hydroxide solution were added dropwise over a period of 1 hour while maintaining a temperature of 0-5° C by cooling. The mixture was then stirred for a further 2 hours, during which time the mixture was allowed to warm to room temperature. 100 ml of diethyl ether were added and the mixture was filtered, whereby the insoluble R,R-isomer was removed. The aqueous layer of the filtrate was separated and adjusted to pH 1.5-2 by the addition of concentrated hydrochloric acid, whereby an oil precipitated. The mixture was extracted twice with 100 ml of ethyl acetate each time. The combined organic extracts were washed with water, dried over anhydrous sodium sulphate and evaporated to give an oil. This oil was dissolved in 35 ml of ethyl acetate and 2.85 ml (25 mmol) of cyclohexylamine were added. The white precipitate was collected by filtration to give, after several fractional recrystallizations from methanol/ethyl acetate, 2.38 g of the cyclohexylamine salt of 2-(benzyloxycarbonyl)-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxylic acid; MS: m/e 318 $[M+H]^+$.

(iii) 2.334 g of the cyclohexylamine salt of 2-(benzyloxycarbonyl)-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxylic acid were partitioned between 50 ml of ethyl acetate and 50 ml of 10% citric acid solution. The organic phase was separated, washed with water, filtered and evaporated to give 1.87 g of 2-(benzyloxycarbonyl)-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxylic acid in the form of a colourless gum; MS: m/e 318 $[M+H]$.

(iv) A solution of 0.634 g (2.0 mmol) of 2-(benzyloxycarbonyl)-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxylic acid in 6 ml of dimethoxyethane was treated with 0.23 g (2.0 mmol) of N-hydroxysuccinimide and 0.412 g (2.0 mmol) of dicyclohexylcarbodiimide. The mixture was stirred at room temperature for 18 hours. The mixture was filtered and the filtrate was evaporated to give 0.879 g of the N-hydroxysuccinimide ester of the foregoing acid in the form of a pale yellow oil. A solution of 0.828 g (2.0 mmol) of the foregoing N-hydroxysuccinimide ester in 5 ml of dichloromethane was stirred, cooled to 0° C and treated with 0.219 g (3.0 mmol) of tert.butylamine. The mixture was stirred at 0° C for 2 hours and then at room temperature for 4.5 hours. The mixture was then washed with 2M hydrochloric acid, sodium carbonate solution and sodium chloride solution, dried over anhydrous magnesium sulphate and evaporated. The residue was dissolved in 20 ml of diethyl ether and filtered. The filtrate was evaporated to give 0.712 g of 2-(benzyloxycarbonyl)-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide in the form of a white solid; MS: m/e 373 $[M+H]^+$.

(v) A solution of 0.689 g (1.85 mmol) of 2-(benzyloxycarbonyl)-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S) -carboxamide in 20 ml of ethanol was hydrogenated in the presence of 0.01 g of 10% palladium-on-carbon at room temperature and under atmospheric pressure for 18 hours. The catalyst was removed by filtration and the solvent was removed by evaporation to give in quantitative yield N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide as a clear oil; MS: m/e 239 $[M+H]^+$, which was used in the next step without further purification.

(vi) A solution of 440 mg of N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide and 549 mg of 3(S)(benzyloxyformamido)-1,2(S)-epoxy-4-phenylbutane in 6 ml of ethanol was stirred at 60° C for 7 hours. A further 54 mg of 3(S)-(benzyloxyformamido)-1,2(S)-epoxy-4-phenylbutane were added and the solution was stirred at 20° C for 16 hours. The solvent was removed by evaporation and the residue was chromatographed on silica gel using diethyl ether/n-hexane/methanol (47.5:47.5:5) for the elution to give 771 mg of 2-[3(S)-(benzyloxyformamido)-2(R)-hydroxy-4-phenylbutyl-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide as a white solid; MS: m/e 536 $[M+H]^+$.

(vii) A solution of 747 mg of 2-[3(S)-(benzyloxyformamido)-2(R)-hydroxy-4-phenylbutyl-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide in 40 ml of ethanol was hydrogenated over 10% palladium-on-carbon at 20° C and under atmospheric pressure for 5 hours. The catalyst was removed by filtration and the filtrate was evaporated to give 561 mg of 2-[3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide as a buff coloured solid which was used in the next step without further purification.

Example 2

A solution of 154 mg of 2-[3(S)-[(L-asparaginyl)-amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide and 52 mg of quinaldic acid in 6 ml of dry tetrahydrofuran was coold in an ice/salt mixture. 41 mg of hydroxybenzotriazole, 35 mg of N-ethylmorpholine and 68 mg of dicyclohexylcarbodiimide were added and the mixture was stirred for 64 hours. The mixture was diluted with ethyl acetate and filtered. The filtrate was washed with aqueous sodium bicarbonate solution and with sodium chloride solution and then evaporated. The residue was chromatographed on silica gel using dichloromethane/ methanol (9:1) for the elution to give 50 mg of N-tert.butyl-decahydro-2-[2(R)hydroxy-4-phenyl-3(S)-[[N(2-quinolylcarbonyl)-L-asparaginyl]amino]-butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide as a white solid; MS: m/e 671 $[M+H]^+$; NMR: δ (d_4 CH_3OH, 400 MHz): 8.52 (lH, m), 8.18 (lH, m), 8.14 (lH, m), 8.02 (lH, m), 7.84 (lH, m), 7.69 (lH, m), 7.18 (2H, m), 6.90 (2H, m), 6.72 (lH, m), 4.93 (lH, dd, Asn αCH J = 6.6, 6.8), 4.27 (lH, m, $-CH_2CHCH(OH)-$ J = 3.8, 3.8, 11.0), 3.89 (lH, m, $-CHCH(OH)CH_2-$ J = 7.2, 6.4, 3.8), 3.06 (lH, dd, HI_{eq} J = -12.0, 3.0), 3.02 (lH, dd, PhCH(H)CH- J = -14.0, 3.8), 2.77 (lH, dd, Asn β_1 J = -15.6, 6.6), 2.68 (lH, dd, Asn β_2 J = -15.6, 6.8), 2.68 (lH, dd, PhCH(H)CH- J = -14.0, 11.0), about 2.68 (lH, dd, -CN(OH)CH(H)N- J = -12.0, 7.2), 2.63 (lH, dd, $H3_{ax}$ J = 11.0, 2.2), 2.22 (lH, dd, -CH(OH)CH(H)N- J = -12.0, 6.4), 2.18 (lH, dd, HI_{ax} J = -12.0, 2.2), 2.06 (lH, m, $H4_{ax}$ J = -11.0, 11.0, 11.0), 1.78 (lH, m, 4a J_{4a-4ax} = 11.0, J_{4a-4eq} = about 4, J_{4a-8a} = about 4), 1.65 (lH. m, 8a J_{8a-1ax} = 2.2 J_{8a-1eq} = 3.0 J_{8a-4a} = about 4), 1.37 (lH, m, $H4_{eq}$ J = -11.0, 2.2, about 4), 1.30 (9H, s, t-butyl), 2.0-1.2 (8H, m).

The 2-[3(S)-[(L-asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide used as the starting material was prepared as follows:

A solution of 195 mg of 2-[3(S)-[[N-(benzyloxycarbonyl)-L-asparaginyl]amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide in 20 ml of ethanol was hydrogenated at room temperature and atmospheric pressure for 18 hours over 10 mg of 10% palladium-on-charcoal. The catalyst was filtered off and the filtrate was evaporated under reduced pressure to give 154 mg of 2-[3(S)-[(L-asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide which was used in the next step without further purification.

Example 3

A solution of 287 mg of N-(2-quinolylcarbonyl)-L-asparagine and 401 mg of 2-[3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide [prepared as described in Example 1 (i)-(vii)] in 3 ml of tetrahydrofuran was cooled to -10° C and 163 mg of 3-hydroxy-1,2,3-benzotriazin-4(3H)-one and 220 mg of dicyclohexylcarbodiimide were added. The mixture was stirred at -10° C for 2 hours and at 20° C for 16 hours, then diluted with ethyl acetate and filtered. The filtrate was washed with saturated sodium bicarbonate solution and saturated sodium chloride solution and then evaporated. The residue was chromatographed on silica gel using 4% (by volume) methanol in dich-

loromethane for the elution to give 537 mg of N-tert.butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]-butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide which was identical with the product obtained in the first paragraph of Example 2.

The N-(2-quinolylcarbonyl)-L-asparagine used as the starting material was prepared as follows:

A mixture of 540 mg of quinaldic acid succinamide ester and 300 mg of L-asparagine monohydrate in 2 ml of dimethylformamide was stirred at 20 °C for 96 hours. The solvent was removed by evaporation to give a white solid residue which was stirred vigorously in 10 ml of dichloromethane, filtered off and washed with dichloromethane. There were thus obtained 431 mg of N-(2-quinolylcarbonyl)-L-asparagine as a white solid: MS: m/e 288[M+H]$^+$.

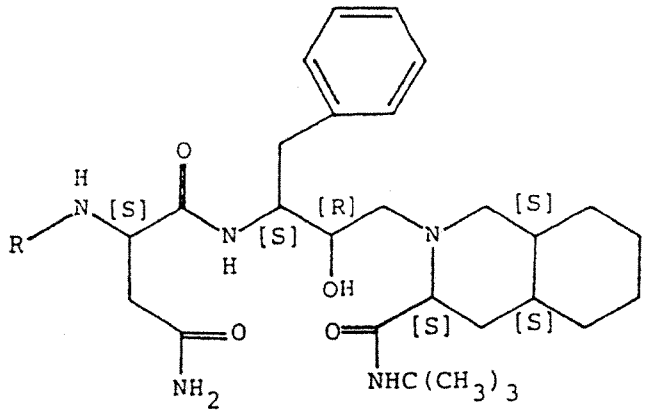
The following Example illustrates the manufacture of a pharmaceutical preparation containing a compound of formula I or a pharmaceutically acceptable acid addition salt thereof as the active ingredient:

Example A

An aqueous solution of the active ingredient is filtered sterile and mixed while warming with a sterile gelatine solution, which contains phenol as a preserving agent, using amounts such that 1 00 ml of the resulting solution contains 3.0 mg of active ingredient, 150.0 mg of gelatine, 4.7 mg of phenol and distilled water ad 1.0 ml. The mixture is filled into vials of 1.0 ml capacity under aseptic conditions.

Claims

1. Amino acid derivatives of the general formula



I

wherein R represents benzyloxycarbonyl or 2-quinolylcarbonyl,
and pharmaceutically acceptable acid addition salts thereof.

2. N-tert.Butyl-decahydro-2[2(R)-hydroxy-4-phenyl-3(S)-[[N(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide.

3. 2-[3(S)-Amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide.

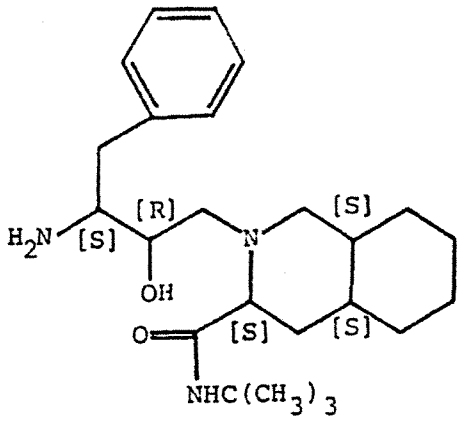
4. 2-[3(S)-[(L-Asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide.

5. An amino acid derivative according to claim 1 or claim 2 for use as a therapeutically active substance.

6. An amino acid derivative according to claim 1 or claim 2 for use in the treatment or prophylaxis of viral infections.

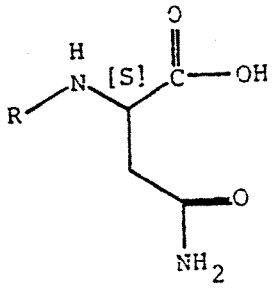
7. A process for the manufacture of an amino acid derivative in accordance with claim 1 or claim 2, which process comprises

(a) reacting 2-[(3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



II

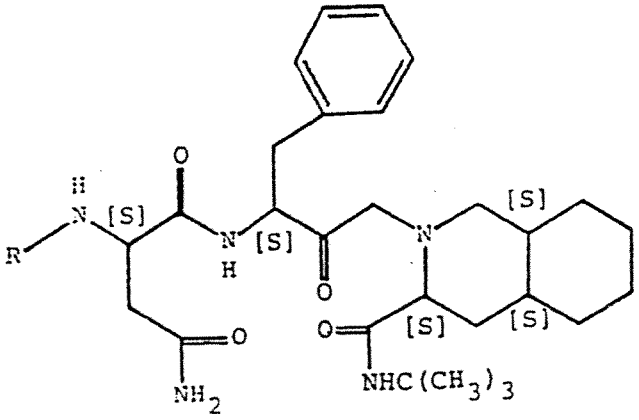
with an acid of the general formula



III

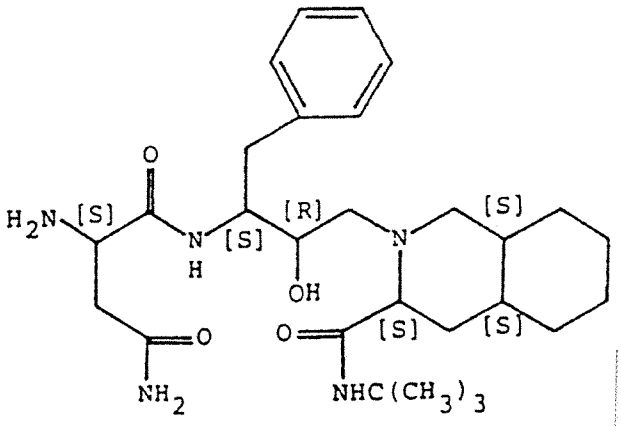
wherein R has the significance given earlier,
or a reactive derivative thereof, or

(b) reducing a compound of the general formula



IV

wherein R has the significance given earlier,
and separating the desired 2(R)-hydroxy isomer from the mixture obtained, or
(c) reacting 2-[3(S)-[(L-asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



V

with an agent yielding the benzyloxycarbonyl or 2-quinolylcarbonyl group, and
(d) if desired, converting a compound of formula I obtained into a pharmaceutically acceptable acid addition salt.

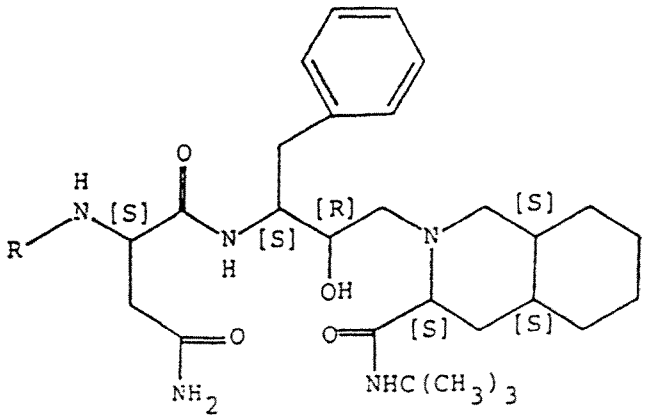
8. A medicament containing an amino acid derivative according to claim 1 or claim 2 and a therapeutically inert excipient.

9. A medicament for the treatment or prophylaxis of viral infections, containing an amino acid derivative according to claim 1 or claim 2 and a therapeutically inert excipient.

10. The use of an amino acid derivative according to claim 1 or claim 2 for the manufacture of a medicament for the treatment or prophylaxis of viral infections.

Claims for the following Contracting State: Greece

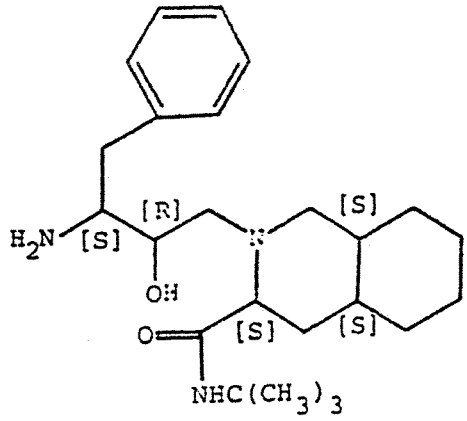
1. A process for the manufacture of an amino acid derivative of the general formula



I

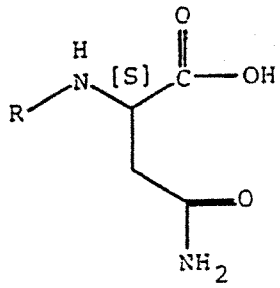
wherein R represents benzyloxycarbonyl or 2-quinolylcarbonyl.

or a pharmaceutically acceptable acid addition salt thereof, which process comprises
(a) reacting 2-[(3(S)-amino-2-(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



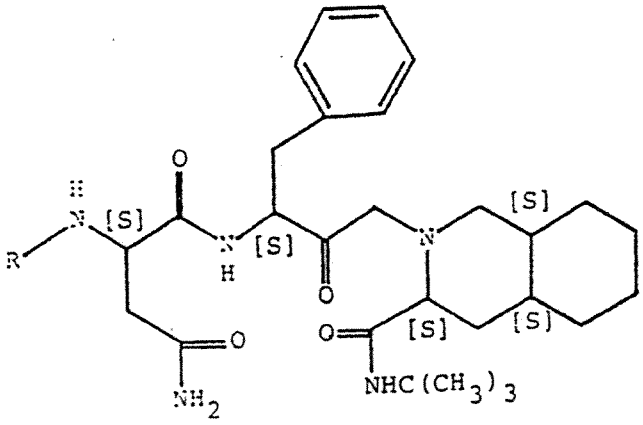
II

with an acid of the general formula



III

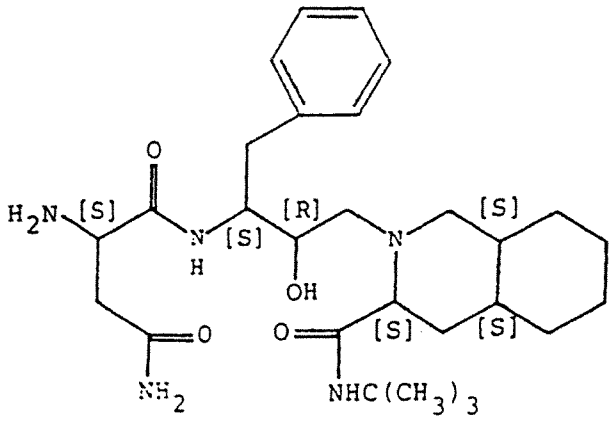
wherein R has the significance given earlier,
or a reactive derivative thereof, or
(b) reducing a compound of the general formula



IV

wherein R has the significance given earlier,

and separating the desired 2(R)-hydroxy isomer from the mixture obtained, or
(c) reacting 2[3(S)-[(L-asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



V

with an agent yielding the benzyloxycarbonyl or 2-quinolylcarbonyl group, and
(d) if desired, converting a compound of formula I obtained into a pharmaceutically acceptable acid addition salt.

2. A process according to claim 1 wherein N-tert.butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide is prepared.

3. A process for the manufacture of a medicament, particularly to be used in the treatment or prophylaxis of viral infections, which process comprises bringing a compound of formula I set forth in claim 1 or a pharmaceutically acceptable acid addition salt thereof into a galenical dosage form.

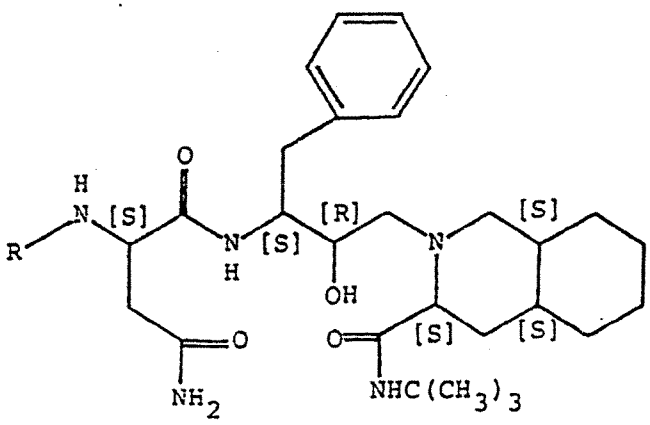
4. The use of a compound of formula I set forth in claim 1 or a pharmaceutically acceptable acid addition salt thereof for the manufacture of a medicament for the treatment or prophylaxis of viral infections.

5. 2-[3(S)-Amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide.

6. 2-[3(S)-[(L-Asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide.

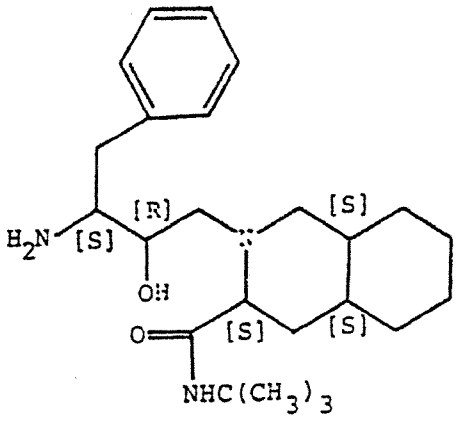
Claims for the following Contracting State: Spain

1. A process for the manufacture of an amino acid derivative of the general formula



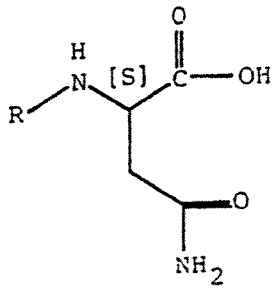
I

wherein R represents benzyloxycarbonyl or 2-quinolylcarbonyl,
or a pharmaceutically acceptable acid addition salt thereof, which process comprises
(a) reacting 2-[(3(S)-amino-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



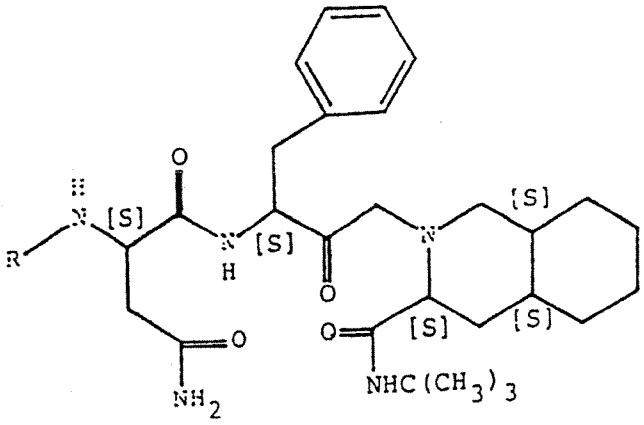
II

with an acid of the general formula



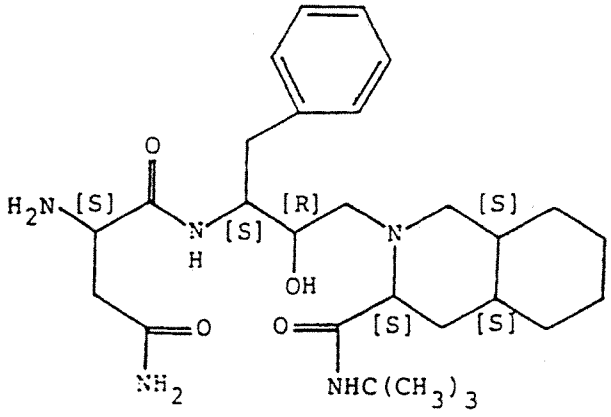
III

wherein R has the significance given earlier,
or a reactive derivative thereof, or
(b) reducing a compound of the general formula



IV

wherein R has the significance given earlier,
and separating the desired 2(R)-hydroxy isomer from the mixture obtained, or
(c) reacting 2-[3(S)-[(L-asparaginyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-tert.butyl-decahydro-(4aS,8aS)-isoquinoline-3(S)-carboxamide of the formula



V

with an agent yielding the benzyloxycarbonyl or 2-quinolylcarbonyl group, and
(d) if desired, converting a compound of formula I obtained into a pharmaceutically acceptable acid addition salt.

2. A process according to claim 1 wherein N-tert.butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide is prepared.

3. A process for the manufacture of a medicament, particularly to be used in the treatment or prophylaxis of viral infections, which process comprises bringing a compound of formula I set forth in claim 1 or a pharmaceutically acceptable acid addition salt thereof into a galenical dosage form.

4. The use of a compound of formula I set forth in claim 1 or a pharmaceutically acceptable acid addition salt thereof for the manufacture of a medicament for the treatment or prophylaxis of viral infections.