

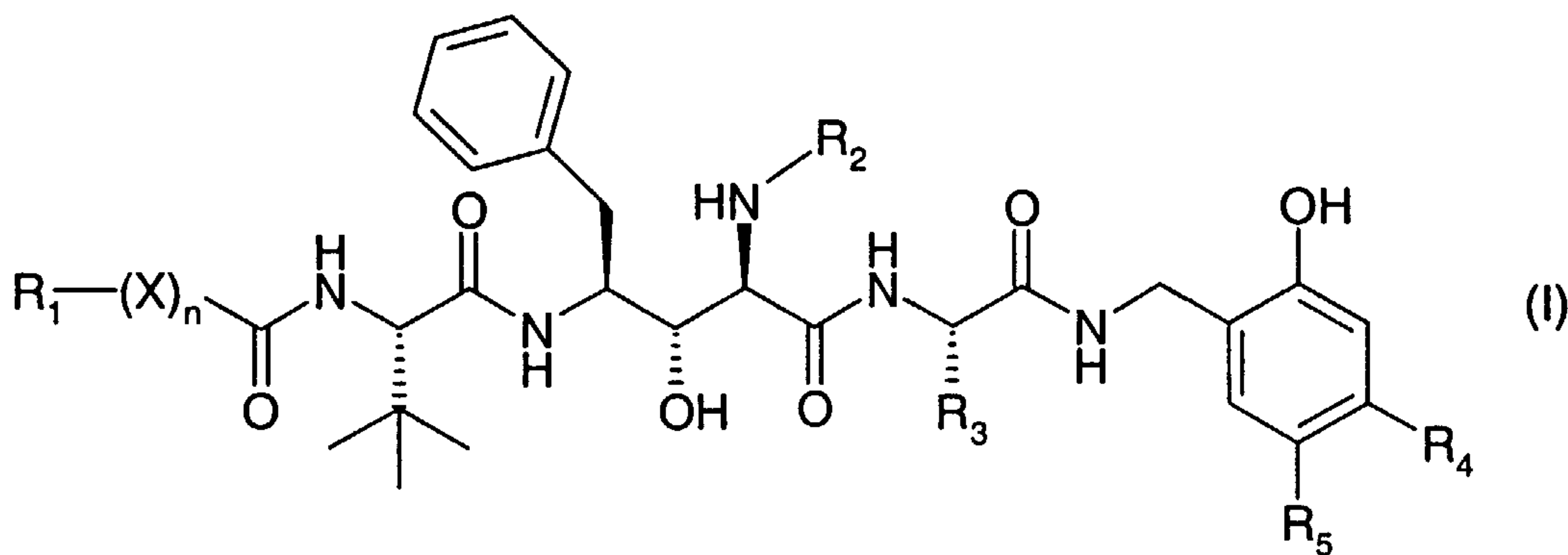


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(54) Titre : DERIVES DE L'AMIDE DE L'ACIDE 2-AMINO-3-HYDROXY-4-TERT-LEUCYL-AMINO-5-PHENYL-PENTANOIQUE

(54) Title: 2-AMINO-3-HYDROXY-4-TERT-LEUCYL-AMINO-5-PHENYL-PENTANOIC ACID AMIDE DERIVATIVES



(57) Abrégé/Abstract:

The invention relates to compounds of formula (I), wherein the substituents and symbols are defined as indicated in the description, to processes for the preparation thereof, to medicaments comprising those compounds, and to the use thereof in the preparation of pharmaceutical compositions for the therapeutic treatment of warm-blooded animals, including humans.

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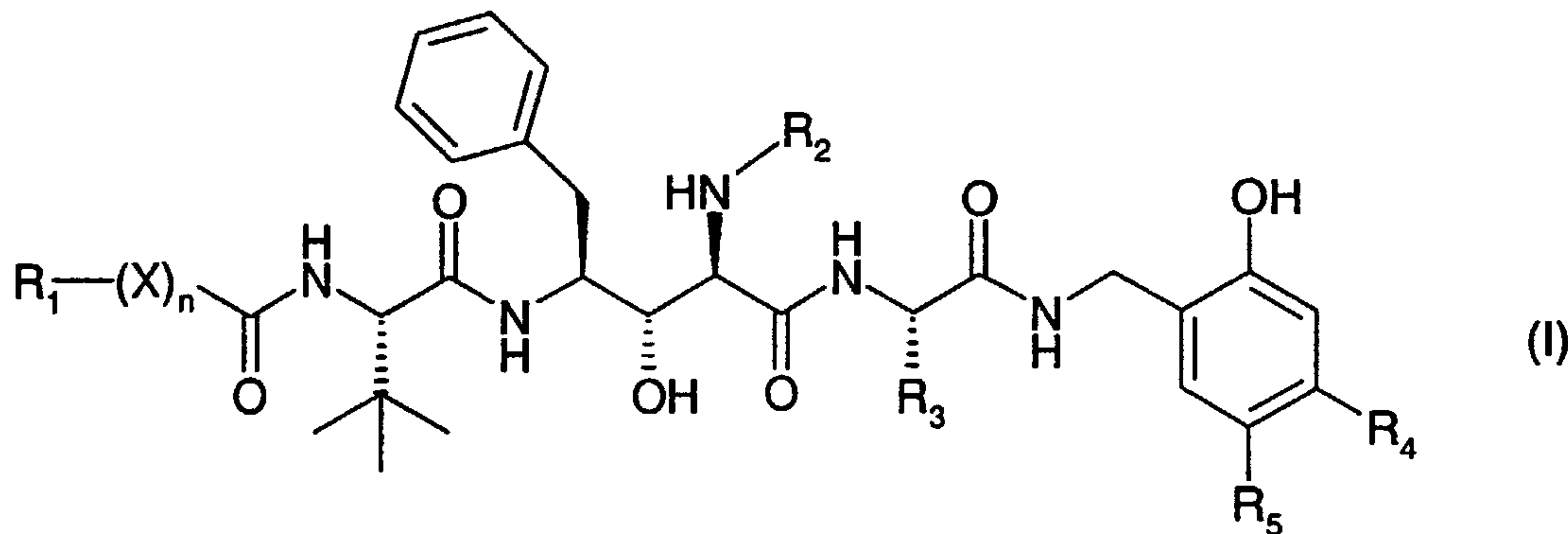
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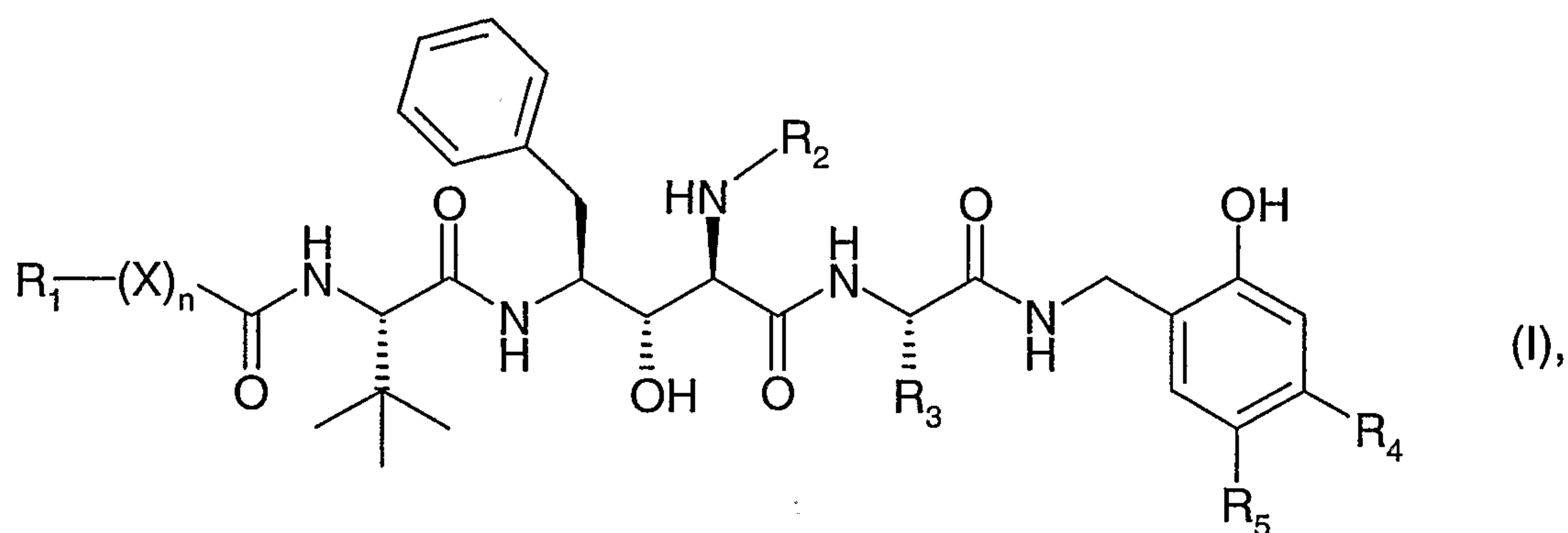
(57) Abstract: The invention relates to compounds of formula (I), wherein the substituents and symbols are defined as indicated in the description, to processes for the preparation thereof, to medicaments comprising those compounds, and to the use thereof in the preparation of pharmaceutical compositions for the therapeutic treatment of warm-blooded animals, including humans.

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2-amino-3-hydroxy-4-*tert*-leucyl-amino-5-phenyl-pentanoic acid amide derivatives

The invention relates to 2-amino-3-hydroxy-4-*tert*-leucyl-amino-5-phenyl-pentanoic acid amide derivatives, to processes for the preparation thereof, to medicaments comprising those compounds, and to the use thereof in the preparation of pharmaceutical compositions for the therapeutic treatment of warm-blooded animals, including humans.

The invention relates to compounds of formula I



wherein

n is 0 or 1;

R_1 and R_2 are independently of the other an aliphatic radical, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical, each radical having not more than 20 carbon atoms;

R_3 is hydrogen, oxa-alkyl, an aliphatic radical or a radical with up to 20 carbon atoms of the formula $-(Y)_m-R_6$, wherein Y is alkyl, m is 0 or 1 and R_6 is an unsubstituted or substituted monocyclic radical with 5 or 6 ring members containing up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur, wherein said monocyclic radical can also be fused to a benzo ring;

R_4 and R_5 are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-

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carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R_4 and R_5 have up to 12 carbon atoms, with the proviso that R_4 and R_5 are not both hydrogen if n is 1, R_1 is benzyl or *tert*-butyl, R_2 is benzyl or 4-methoxy-benzyl, R_3 is isopropyl and X is oxygen and that R_4 is not methoxy if n is 0 or 1, R_2 is 4-methoxy-benzyl, R_3 is isopropyl or *tert*-butyl, R_5 is hydrogen and X is oxygen; and X is nitrogen, oxygen or sulfur; or salts thereof.

Within the context of the present Application, the general terms used hereinbefore and hereinafter preferably have the following meanings:

n is 0 or 1, preferably 0.

An aliphatic radical has up to 12 carbon atoms, preferably up to 7 carbon atoms, most preferably up to 4 carbon atoms, and is such an unsubstituted or substituted aliphatic hydrocarbon radical, that is to say such an unsubstituted or substituted alkynyl, alkenyl or preferably alkyl radical, one or more substituents preferably being independently selected from the group consisting of free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; guanidino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro.

An aliphatic radical R_1 is preferably lower alkyl, such as especially *tert*-butyl.

An aliphatic radical R_3 is preferably unsubstituted lower alkyl or lower alkyl substituted by hydroxy, carboxy, amino, carbamoyl, guanidino, mercapto or alkyl-thio, most preferably a side chain of the amino acids alanine, leucine, isoleucine, serine, threonine, cysteine, methionine, asparagine, glutamine, aspartate, glutamate, lysine or arginine, especially valine.

An aliphatic radical R_4 is preferably methoxy.

An aromatic radical R_1 or R_2 has not more than 20 carbon atoms, especially not more than 12 carbon atoms, and is unsubstituted or substituted, preferably in each case unsubstituted or substituted phenyl or naphthyl, especially 1-naphthyl, one or more substituents preferably

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being independently selected from the group consisting of an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro, more preferably being selected from alkyl, e.g. methyl, ethyl or propyl; alkoxy, e.g. methoxy or ethoxy; di-substituted amino, e.g. dimethylamino; halogen, e.g. chloro or bromo; and halogen-alkyl, e.g. trifluoromethyl.

In an aromatic-aliphatic radical R_1 or R_2 having not more than 20 carbon atoms the aromatic moiety is as defined above and the aliphatic moiety is preferably lower alkyl, such as especially C_1 - C_2 alkyl, which is substituted preferably as defined for the aromatic radical or preferably unsubstituted. An aromatic-aliphatic radical R_1 is preferably benzyl or naphthalen-1-ylmethyl. An aromatic-aliphatic radical R_2 is preferably benzyl substituted in the benzene moiety by 1-5, preferably by 1-3 methoxy groups; benzyl substituted in the benzene moiety, preferably in position 4, by a dimethyl-amino group; or naphthalen-1-ylmethyl. Most preferably an aromatic-aliphatic radical R_2 is 2,3,4- or 3,4,5-trimethoxybenzyl.

A cycloaliphatic radical R_1 or R_2 has up to 20, especially up to 10 carbon atoms, is mono- or poly-cyclic and is substituted preferably as defined for the aromatic radical or preferably unsubstituted, for example such a cycloalkyl radical, especially such a 5- or 6-membered cycloalkyl radical, such as preferably cyclohexyl.

In a cycloaliphatic-aliphatic radical R_1 or R_2 having not more than 20 carbon atoms the cycloaliphatic moiety is as defined above and the aliphatic moiety is preferably lower alkyl, such as especially C_1 - C_2 alkyl, which is substituted preferably as defined for the aromatic radical or preferably unsubstituted, for example cyclohexyl-methyl.

A heterocyclic radical R_1 or R_2 contains up to 20 carbon atoms, generally up to 12 carbon atoms, and is substituted preferably as defined for the aromatic radical or unsubstituted and is preferably a saturated or unsaturated monocyclic radical having 5 or 6 ring members and 1 to 3 hetero atoms which are preferably selected from the group consisting of nitrogen, oxygen and sulfur, for example, thienyl or pyridyl, or a bi- or tri-cyclic radical wherein, for example, a benzene radical is fused to the mentioned monocyclic radical, especially, for example, indolyl, such as 5-indolyl, or chinolyl, such as 8-chinolyl.

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In a heterocyclic-aliphatic radical R_1 or R_2 having not more than 20 carbon atoms the heterocyclic moiety is as defined above and the aliphatic moiety is preferably lower alkyl, such as especially C_1 - C_2 alkyl, which is substituted preferably as defined for the aromatic radical or preferably unsubstituted. A heterocyclic-aliphatic radical R_1 or R_2 is for example indolyl-methyl, especially 5-indolyl-methyl, or chinolyl-methyl, especially 8-chinolyl-methyl.

Oxa-alkyl R_3 is a radical of the formula $-G(-O-CH_2-CH_2)_t-R_7$, in which G and R_7 are alkyl, preferably lower alkyl, and t is 1 to 3, preferably 2, and is especially 2-(1,4-dioxa-hexyl)-ethyl.

In a radical of the formula $-(Y)_m-R_6$ having up to 20 carbon atoms, Y is alkyl, preferably lower alkyl, m is 0 or 1 and the radical R_6 is a saturated or unsaturated monocyclic radical having 5 or 6 ring members and up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur and alternatively containing a fused benzo ring, such a radical being substituted preferably as defined for the aromatic radical or preferably unsubstituted. A radical R_6 is preferably bound to Y via a ring carbon atom and is for example an unsubstituted or substituted member selected from the group consisting of cyclopentyl, cyclohexyl, cyclopentadienyl, phenyl, pyrrolidyl, pyrazolidyl, imidazolidyl, tetrahydrofuryl, piperidyl, piperazinyl, morpholinyl, pyrrolyl, pyrazolyl, imidazolyl, furyl, thienyl, pyridyl, pyrazinyl, pyridazinyl, pyrimidinyl, indenyl, naphthyl, indolyl and chinolyl.

Most preferably a radical of the formula $-(Y)_m-R_6$ is piperidyl, especially 4-piperidyl, piperazin-ethyl, especially piperazin-1-ylethyl, morpholinyl-ethyl, especially morpholin-4-ylethyl, pyridyl-methyl, such as 2-, 3- or 4-pyridyl-methyl, or a side chain of the amino acids phenylalanine, tyrosine, tryptophane or histidine.

X is preferably oxygen (-O-).

Alkyl is preferably lower alkyl.

The prefix "lower" denotes a radical having up to and including 7, preferably up to and including 4, carbon atoms.

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Lower alkyl is, for example, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, isopentyl, neopentyl, *n*-hexyl or *n*-heptyl, preferably isobutyl, *sec*-butyl, *tert*-butyl, isopropyl, ethyl or methyl, most preferably isobutyl, ethyl or methyl.

Etherified hydroxy is, for example, alkoxy, especially lower alkoxy, such as ethoxy or methoxy. Esterified hydroxy is preferably hydroxy esterified by an organic carboxylic acid, such as alkanolic acid, or a mineral acid, such as a hydrohalic acid, for example lower alkanoyloxy or especially halogen, such as iodine or especially fluorine, chlorine or bromine.

Esterified carboxy is, for example, alkoxy-carbonyl, especially lower alkoxy-carbonyl, such as e.g. methoxy-carbonyl.

Alkanoyl is, for example, alkyl-carbonyl, especially lower alkyl-carbonyl, such as e.g. acetyl.

Mono- or di-substituted amino is, for example, *N*-alkylamino or *N,N*-dialkylamino, especially *N*-lower alkylamino or lower *N,N*-di-lower alkylamino, such as e.g. *N*-methylamino or *N,N*-dimethylamino.

Halogen is fluorine, chlorine, bromine or iodine, preferably fluorine, chlorine or bromine.

The structure of formula I as shown above indicates the absolute configuration.

Salt-forming groups in a compound of formula I are groups or radicals having basic or acidic properties. Compounds having at least one basic group or at least one basic radical, for example a free amino group, a pyrazinyl radical or a pyridyl radical, may form acid addition salts, for example with inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxy-benzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic

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sulfonic acids, for example benzene-, p-toluene- or naphthalene-2-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed.

Compounds of formula I having acidic groups, for example a free carboxy group in the radical R₁₀, may form metal or ammonium salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, for example triethylamine or tri-(2-hydroxyethyl)-amine, or heterocyclic bases, for example N-ethyl-piperidine or N,N'-dimethyl-piperazine.

Compounds of formula I having both acidic and basic groups can form internal salts.

For the purposes of isolation or purification, as well as in the case of compounds that are used further as intermediates, it is also possible to use pharmaceutically unacceptable salts. Only pharmaceutically acceptable, non-toxic salts are used for therapeutic purposes, however, and those salts are therefore preferred.

Owing to the close relationship between the novel compounds in free form and in the form of their salts, including those salts that can be used as intermediates, for example in the purification of the novel compounds or for the identification thereof, hereinbefore and hereinafter any reference to the free compounds should be understood as including the corresponding salts, where appropriate and expedient.

The compounds of formula I have valuable pharmacological properties and can be used, for example, as drugs to treat proliferative diseases.

The compounds of formula I inhibit the proteasome activity. It is known that proteins targeted for the degradation by the multicatalytic proteasome complex have *inter alia* functions in the cell-cycle control (e.g. cyclins, p21, p27) and apoptosis (e.g. p53) (Rolfe, M., Chiu, I.M. and Pagano, M., The ubiquitin-mediated proteolytic pathway as therapeutic area, J. Mol. Med. 75, 1997, 5-17). Inhibitors of the proteasome are therefore suitable for the treatment of proliferative diseases which respond to the inhibition of the proteasome activity. Proliferative diseases like psoriasis and tumors, in particular solid tumors like colon tumor, breast tumor, lung tumor and prostate tumor can be mentioned here.

Inhibition of 20S Proteasome

The compounds of formula I inhibit the 20S proteasome.

The multicatalytic proteasome complex is responsible for the ATP-dependent proteolysis of most cellular proteins. Although the 20S proteasome contains the proteolytic core, it cannot degrade proteins *in vivo* unless it is complexed with 19S caps, at either end of its structure, which itself contains multiple ATPase activities. This larger structure is known as the 26S proteasome and will rapidly degrade proteins that have been targeted for the degradation by the addition of multiple molecules of the 8.5 kDa polypeptide ubiquitin. As mentioned above, proteins targeted for proteasomal degradation have functions in the cell-cycle. The compounds of formula I are therefore highly suitable for the treatment of diseases which respond to inhibition of the activity of the 20S proteasome, which is the case for the proliferative diseases mentioned above.

The inhibition of the chymotrypsin-like activity of the 20S proteasome can be demonstrated by the following experiment. It is based on the hydrolysis of the fluorogenic peptide Suc-LLVY-AMC (Succinyl-leucine-leucine-valine-tyrosine-7-amino-4-methyl-coumarin), which is cleaved exclusively at the Y-AMC bond by the 20S proteasome. Hydrolysis of this peptide is accompanied by an increase in fluorescence intensity [λ_{ex} (excitation wavelength): 355 nm; λ_{em} (emission wavelength): 460 nm] due to release of the internally quenched 2-aminobenzoyl fluorescence that accompanies diffusion apart of the hydrolysis product Suc-LLVY.

2 μl of a 1 mM solution of a test compound in DMSO (dimethylsulfoxide, 20 μM final concentration in the well) are preincubated for 60 min at room temperature in black 96-well microtiter plates together with a mixture of 1 μl purified human placenta 20S proteasome (Diabetes Forschungsinstitut, Düsseldorf, Germany; about 100 ng proteasome, depending on the proteasome preparation) and 47 μl buffer-Ca [5 mM CaCl_2 , 20 mM Tris/HCl (Tris-(hydroxymethyl)-amino-methane hydrochloride) pH 8.0]. 3 μl of a 2.67 mM solution of Suc-LLVY-AMC (Bachem, Switzerland) in DMSO (80 μM final concentration in the well) and 47 μl buffer-Ca are mixed and added. The resulting mixture is incubated for 3 - 24 h at 37 °C. If

desired, proteolysis of the substrate can be stopped by addition of 50 μ l stop solution (100 mM monochloroacetic acid, 130 mM NaOH, 100 mM acetic acid, pH = 4.3). The fluorescence is monitored with a FLUOROSKAN ASCENTTM microtiter plate reader.

To each measure series two control experiments have to be carried out:

- 1.) 0% value: 2 μ l DMSO are used in the above described assay instead of 2 μ l of a 1 mM solution of a test compound in DMSO and 48 μ l buffer-Ca instead of a mixture of 47 μ l buffer-Ca and 1 μ l purified proteasome.
- 2.) 100% value: in the above described assay 2 μ l DMSO are used instead of 2 μ l of a 1 mM solution of the test compound in DMSO.

Calculation

$$\% \text{ remaining activity} = \frac{(\text{value with compound} - 0\% \text{ value})}{(100\% \text{ value} - 0\% \text{ value})} \cdot 100\%$$

The IC₅₀ value is defined as that concentration of a compound at which the remaining activity is 50%. Compounds of formula I exhibit an IC₅₀ value for the inhibition of the chymotrypsin-like activity of the 20S proteasome in the range of between 1 nM and 1 μ M, especially between 2 nM and 200 nM.

The antiproliferative activity of the compounds of formula I can be demonstrated *in vitro* against e.g. the human breast carcinoma cell line MDA-MB435 (HTB-129) obtained from the American Type Culture Collection (ATCC, Rockville, USA). Routinely the 'CellTiter96TM' proliferation assay (Promega, Madison MI) is done following the procedure recommended by the supplier. This assay is performed in 96well plates prepared for tissue culture. Cells are seeded in a density of 2.5x10⁴ per well in 50 μ l complete MEM [minimal essential medium] (Gibco-LifeTechnologies) supplemented with 10% fetal calf serum, 100 U/ml PenStrep, 1 mM sodium pyruvate, 4 mM L-Glutamine, 20 mM HEPES and Non Essential Amino Acids. Cells are incubated for 24 hours at 37 °C in humidified atmosphere equilibrated with 5% CO₂. Test compounds are added to the cell supernatant as serial dilutions in 50 μ l MEM-complete per well. Cells are incubated for at least 48 hours at 37 °C in humidified atmosphere equilibrated with 5% CO₂. The Tetrazolium dye is added to the cell supernatant in a volume of 15 μ l per well and cell cultures are incubated for 4 hours.

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Thereafter, the reaction is stopped by addition of 100 μ l of stopsolution per well and the plates are incubated for one additional hour. The conversion of the tetrazolium dye by metabolically active cells yields soluble formazan. The absorbance of the blue colored cell supernatant is proportional to the amount of viable cells. The absorbance is monitored at the wavelength of 550 and 630 nm using a microtiter plate reader (Dynatech MR5000). Serial dilutions of compound are prepared by first diluting the 10 mM compound stock solution (in DMSO) to a 60 μ M test solution in MEM-complete followed by nine successive 1:3 dilutions in MEM-complete. Wells containing MEM-complete serve as negative control, background (0%). Wells containing cells and MEM / 0.6% DMSO serve as positive control, (100%).

Calculation of the % remaining activity is done as described above for the inhibition of the chymotrypsin-like activity of the 20S proteasome. The IC_{50} value is defined as that concentration of a compound at which the remaining activity is 50%.

Compounds of formula I exhibit an IC_{50} value for the antiproliferative activity in the range of between 20 nM and 2 μ M, especially between 50 nM and 1 μ M.

The antitumoral action of the compounds of formula I can also be demonstrated *in vivo*:

In vivo evaluation of antitumor action in nude mice using human tumor xenografts

Female or male BALB/c *nu/nu* (Novartis animal farm, Sisseln, Switzerland or Bomholtgaard, Copenhagen, Denmark) mice with subcutaneously transplanted human tumors are used for the evaluation of the antitumor action of the compounds of formula I against cell lines originating from the four tumor types, breast tumor: MCF-7; lung tumor: NCI H596; colon tumor: HCT 116; and prostate tumor: PC 3.

Materials:

Human colon carcinoma HCT 116 (ATCC CCL 247), human squamous cell lung carcinoma NCI H596 (ATCC HTB 178), estrogen-dependent breast carcinoma MCF-7 (ATCC HTB 22), and the human prostate cancer PC 3 cell line are obtained from the American Type Culture Collection (ATCC, Rockville, USA). The cells are cultured at 37 °C in a 5 % v/v CO_2 and 80 % relative humidity atmosphere in the following media: NCI H 596: RPMI 1640, 20 % v/v

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FBS (fetal bovine serum), 1% w/v glutamine; HCT-116: McCoy's 5A, 10% v/v FBS, 1% w/v glutamine; PC 3: RPMI 1640, 20% v/v FBS, 1% w/v glutamine; MCF-7, RPMI 1640, 20% v/v FBS, 1% w/v glutamine. All of these cell lines are adherent and can be released by rinsing with Hank's balanced salt and treatment with 0.25% w/v trypsin. All of these cells are prepared as master cell stocks (in culture media supplemented to contain 20% v/v FBS and 7% v/v DMSO) and stored at -125 °C (liquid nitrogen vapors). Working cell stocks are prepared from the master stocks by thawing and expanding the cells over three passages, which are then distributed to vials and frozen. Viability (trypan blue exclusion test using 0.5% w/v trypan blue) prior to freezing is > 90% for all cell lines.

Establishing solid tumors in nude mice:

Mice are kept under controlled conditions [Optimal Hygienic Conditions, (OHC)] with free access to sterile food and water. Tumors are established after subcutaneous injection of cells (a minimum of 2×10^6 cells in 100 μ l PBS (phosphate buffered saline) or medium) in carrier mice (4-8 mice per cell line). Injections are made s.c. in the left flank of the mouse mid-way between the tail and head. The resulting tumors are serially passaged for a minimum of three consecutive transplantations prior to start of treatment. Tumors are transplanted when the tumor reaches a volume of 800 to 1000 mm^3 .

Transplanting solid tumors in nude mice:

Donor mice are anesthetized (Forene®, Abbott, Switzerland) and killed by cervical dislocation. The skin is disinfected and the tumor removed by dissection. The outer edges of the tumor mass is removed using a scalpel, and the resulting mass is trimmed into pieces of about 3-4 mm in height. Sections of 3-4 mm^2 are cut and placed into sterile 0.9% w/v NaCl. Sections of tumor that are necrotic are not used.

Tumor fragments are implanted s.c. into the left flank of the recipient mice. Recipient mice are anesthetized (Forene®, Abbott, Switzerland) and the skin on the entire back and sides of the mice is disinfected. The skin 0.5 to 1 cm above the tail is raised and a single 1 to 1.5 cm incision is made. Tumor sections are pushed into a 13-gauge trocar needle. The trocar needle is pushed into the opening of the skin, and advanced under the skin to a point mid-way between the head and the tail. The tumor fragment is deposited by advancing the trocar. The wound is sutured using one or two metal clips.

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In the case of estrogen-dependent breast tumors, estrogen pellets (17 β -estradiol, 5 mg/pellet giving a sustained release over 60 days are obtained from Innovative Research of America, Sarasota, USA), are placed subcutaneously in the other flank.

The tumors are allowed to increase until the size is 100 to 150 mm³ before treatment is begun. The tumors are then measured and placed into groups (normally n = 6 to 8) that are balanced according to the mean size and range of tumor volumes. Groups are randomly assigned to treatment groups.

Preparation and application of test compounds:

Stock solutions of 40 mg/ml of compound are dissolved in 100 % DMSO and stirred at room temperature until a clear solution is obtained. Prior to each administration, 10 % Tween 80 (FLUKA, Buchs, Switzerland) is added to the stock solution and then diluted 1:20 (v/v) with sterile 0.9 % w/v NaCl or water. Solutions and dilutions are prepared daily prior to application. Applications are given 7 days a week (p.o., i.p., s.c. or i.v.). The volumes of application are: p.o., 25 ml/kg; i.p., 25 ml/kg; s.c., 10 ml/kg; i.v., 10 ml/kg.

Measurement of tumor volumes:

Tumor growth is monitored once, twice or three times weekly (depending on the growth rate of the tumor line) and 24 hours after the last treatment by measuring perpendicular diameters. Calipers capable of determining mm distances are used. Tumor volumes are calculated according to the formula $L \times D^2 \times \pi/6$ (L: length; D: diameter). Antitumor activity is expressed as T/C % (mean increase of tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100). Tumor regression (%) represents the smallest mean tumor volume compared to the mean tumor volume at the start of treatment. Delta (Δ) tumor volumes compared the change in tumor volume during the duration of the experiment (volume on the last treatment day - volume on the first treatment day). Any animals in which the tumor reaches a size exceeding approximately 1500 to 2000 mm³ are sacrificed.

Additional measurements:

Body weights and survival data are also collected. Delta (Δ) body weights are calculated as an indication of tolerability to treatment (weight on the last treatment day - weight on the first treatment day). Statistically significant body weight loss, or mortalities, are considered

indicators of poor tolerability to treatment. Additionally, mice are monitored once or twice daily for general health.

Statistical analyses:

The basic approach for statistical analyses is to use tests for multiple comparisons to judge the statistical significance of differences between treatment groups, and differences within a group to determine if treatment induced stable disease or tumor regressions. As subcutaneous tumor volumes are not always normally distributed, differences in the subcutaneous tumor volumes between treatment groups is determined using the non-parametric Kruskal-Wallis one way ANOVA test on ranked data and the statistical significance of differences between treatment groups as compared to control groups determined using the Dunnett test. Pair wise comparisons between all groups is performed using the Student-Newman-Keuls (SNK) method. If only two groups are compared, the Rank sum test is used. Animal body weights are normally distributed, and changes in body weights within a group are analyzed by paired t-tests and between group differences are analyzed by a One-Way ANOVA and pair-wise comparisons made using the Tukey test. For all tests the level of significance is set at $p < 0.05$.

Another *in vivo* test to determine the antitumoral action of the compounds of formula I is the following hollow fiber assay:

Hollow Fiber Assay: evaluation of antitumor action in nude mice

In this assay four different human tumors encapsulated in hollow fibers are implantated subcutaneously and/or intraperitoneally into nude mice (athymic female outbred nude mice (Ncr nu/nu)). Animals are then treated with a test compound formulated in an appropriate vehicle, while control animals are treated with the vehicle alone. At the end of the experiment fibers are retrieved, and the number of viable cells is measured using a metabolic assay [MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Activity of the test compound is measured by comparing the growth of cells in the experimental animals (T) to the growth of cells in the control animals treated with the vehicle alone (C), and is expressed as %T/C.

Materials:

Human colon carcinomas SW 620 and LS 174T, and breast carcinoma MDA-MB-435S are obtained from the American Type Culture Collection (ATCC, Rockville, USA). The colon carcinoma MIP 101, expressing high levels of pgp-1, was originally established from a patient at the Dana Farber Cancer Institute, Boston, USA. All cell lines are grown according to standard tissue culture techniques in RPMI 1640 containing the following additives: 5% FBS (Fetal Bovine Serum), 5 mg/ml insulin, 5 mg/ml transferrin, 5 ng/ml selenous acid, 1 nM β -estradiol, 1 nM testosterone.

Design of the assay:

The human solid tumor cell lines are encapsulated in PVDF (polyvinylidene fluoride) hollow fibers that are permeable to molecules < 500,000 Daltons and that have an inner diameter of 1 mm (Biopore, Spectrum Medical, CA, USA). After encapsulation and prior to implantation into animals, cells are allowed to attach to the inner surface of the fiber by incubation in the tissue culture media for 24 hours. Four hollow fibers are then implanted into one animal intraperitoneally or subcutaneously. In the experimental setup four to six animals are used per group, and the experiment consists of a minimum of three groups:

1. "Time 0" group
2. Control (placebo) group
3. "Treated" (tested compound) group.

Treatment starts 24 hours after implantation of the fibers. Animals are treated once daily, at days 1, 3, and 5. At the same time when the treatment starts, the animals from "Time 0" group are sacrificed, fibers are retrieved, and the number of viable cells at the beginning of the experiment is determined (T_0). At day 7 after the implantation all animals are sacrificed, fibers are retrieved, and the number of viable cells is determined for the Control (C_v), and for the "Treated" (T_v) groups. %T/C is then calculated according to the formula:

$$\%T/C = (T_v - T_0) / (C_v - T_0) \times 100\%$$

Encapsulation of tumor cells in hollow fibers:

Fibers are cut into desired length and soaked for at least 72 h in 70% Ethanol. Afterwards, fibers and instruments for the encapsulation are sterilized. The human solid tumor cell line grown in tissue culture is trypsinized, suspended in a small amount of tissue culture media and transferred into the fibers using a syringe. The fibers are heat sealed and incubated at

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37 °C for 24 h in an atmosphere comprising 5% CO₂. The fibers are then implanted subcutaneously and/or intraperitoneally into nude mice.

Determination of viable cells in hollow fibers:

1 g MTT is added to 200 ml PBS (phosphate-buffered saline), stirred for 20 min and filtered (0.22 micron filter). 10 ml of this solution are mixed with 40 ml RPMI 1640 with additives to give the MTT working solution. The retrieved fibers are incubated in 5 ml RPMI 1640 for 30 min at 37 °C in 5% CO₂ for stabilization. 0.5 ml of MTT working solution are added to each well of the sample plate. The plates are incubated at 37 °C in 5% CO₂ for 4 h. The MTT is aspirated out of each well in the sample plate. 2 ml of 2.5% aqueous protamine sulfate solution is added to each well of the sample plate. The plates are incubated at 4 °C for 24h. The protamine sulfate is aspirated out of each well in the sample plate. 2 ml of protamine sulfate are added to each well and the plates are incubated at 4 °C for further 2-4 h. Each fiber is transferred to a well in a 24 well plate. The fibers are cut so that they lie on the bottom of the wells and dried overnight. 250 µl of DMSO are added to each well. The plates are placed on an orbital shaker for 4 h with a cover to protect the MTT from light. 150 µl of each sample are transferred to the appropriate well in a 96 well plate. The plates are read at 540 nm using DMSO as the blanking well.

The bioavailability after oral administration of compounds of formula I can be shown e.g. in the following test:

For peroral administration, a solution of the test substance (25 mg/ml) in a suitable solvent such as Cremophor RH40® / Maisine® / propylene glycol / ethanol (38/32/15/15) is prepared. Female Balb/c mice are fasted for 24 hours prior to the start, and throughout the experiment water is given ad libitum. At various times following drug administration, blood samples are obtained by sacrificing animals under anaesthesia by cutting the vena jugularis, followed by cervical dislocation. Samples are collected in heparinized tubes (typically 0.4-0.6 ml). For sample analysis solid phase extraction and HPLC are used. Drug concentration in the samples is calculated by least-squares linear regression analysis of the peak area ratio (inhibitor/internal standard) of spiked blood standards versus concentration. From the concentration versus time data, the "Area Under the Curve" (AUC) value is calculated by the trapezoidal rule.

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Preference is given to compounds of formula I, wherein

n is 0 or 1;

R₁ has not more than 20 carbon atoms and is a member selected from the group consisting of lower alkyl, cycloalkyl-lower alkyl, phenyl-lower alkyl, naphthyl-lower alkyl, and chinolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of hydroxy, lower alkoxy, lower alkanoyloxy, halogen, carboxy, lower alkoxy-carbonyl, formyl, lower alkanoyl, amino, *N*-lower alkylamino, *N,N*-di-lower alkylamino, mercapto, sulfo, lower alkyl-thio, carbamoyl, *N*-lower alkyl-carbamoyl, *N,N*-di-lower alkyl-carbamoyl, cyano, nitro and lower alkyl that can itself be substituted by said radicals;

R₂ has not more than 20 carbon atoms and is a member selected from the group consisting of phenyl-lower alkyl, naphthyl-lower alkyl, chinolyl-lower alkyl, indolyl-lower alkyl and *N*-lower alkyl-indolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of hydroxy, lower alkoxy, lower alkanoyloxy, halogen, carboxy, lower alkoxy-carbonyl, formyl, lower alkanoyl, amino, *N*-lower alkylamino, *N,N*-di-lower alkylamino, mercapto, sulfo, lower alkyl-thio, carbamoyl, *N*-lower alkyl-carbamoyl, *N,N*-di-lower alkyl-carbamoyl, cyano, nitro and lower alkyl that can itself be substituted by said radicals;

R₃ is hydrogen, a radical of the formula -G(-O-CH₂-CH₂)_t-R₇, in which G and R₇ are lower alkyl and t is 1 to 3, unsubstituted lower alkyl or lower alkyl substituted by hydroxy, carboxy, amino, carbamoyl, guanidino, mercapto or lower alkyl-thio and having not more than 12 carbon atoms, or a radical of the formula -(Y)_m-R₆, wherein Y is lower alkyl, m is 0 or 1 and R₆ is phenyl, hydroxy-phenyl, imidazolyl, indolyl, piperidyl, piperazinyl, morpholinyl or pyridyl; R₄ and R₅ are independently selected from the group consisting of hydrogen, hydroxy, lower alkoxy, lower alkanoyloxy, halogen, carboxy, lower alkoxy-carbonyl, formyl, lower alkanoyl, amino, *N*-lower alkylamino, *N,N*-di-lower alkylamino, mercapto, sulfo, lower alkyl-thio, carbamoyl, *N*-lower alkyl-carbamoyl, *N,N*-di-lower alkyl-carbamoyl, cyano, nitro and lower alkyl that can itself be substituted by said radicals, wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ and R₅ are not both hydrogen if n is 1, R₁ is benzyl or *tert*-butyl, R₂ is benzyl or 4-methoxy-benzyl, R₃ is isopropyl and X is oxygen and that R₄ is not methoxy if n is 0 or 1, R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl, R₅ is hydrogen and X is oxygen; and X is nitrogen, oxygen or sulfur;

or a salt thereof.

Preference is given especially to compounds of formula I, wherein

n is 0 or 1;

R₁ has not more than 20 carbon atoms and is a member selected from the group consisting of lower alkyl, cycloalkyl-lower alkyl, phenyl-lower alkyl, naphthyl-lower alkyl, and chinolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro;

R₂ has not more than 20 carbon atoms and is a member selected from the group consisting of phenyl-lower alkyl, naphthyl-lower alkyl, chinolyl-lower alkyl, indolyl-lower alkyl and *N*-lower alkyl-indolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro;

R₃ is hydrogen, oxa-alkyl, an aliphatic radical or a radical with up to 20 carbon atoms of the formula $-(Y)_m-R_6$, wherein Y is alkyl, m is 0 or 1 and R₆ is an unsubstituted or substituted monocyclic radical with 5 or 6 ring members containing up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur, wherein said monocyclic radical can also be fused to a benzo ring;

R₄ and R₅ are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ and R₅ are not both hydrogen if n is 1, R₁ is benzyl or *tert*-butyl, R₂ is benzyl or 4-methoxy-benzyl and R₃ is isopropyl and that R₄ is not methoxy if R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl and R₅ is hydrogen; and

X is oxygen;

or a salt thereof.

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Special preference is given to compounds of formula I, wherein

n is 0 or 1;

R₁ and R₂ are independently of the other an aliphatic radical, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical, each radical having not more than 20 carbon atoms;

R₃ is hydrogen, a radical of the formula -G(-O-CH₂-CH₂)_t-R₇, in which G and R₇ are lower alkyl and t is 1 to 3, unsubstituted lower alkyl or lower alkyl substituted by hydroxy, carboxy, amino, carbamoyl, guanidino, mercapto or lower alkyl-thio and having not more than 12 carbon atoms, or a radical of the formula -(Y)_m-R₆, wherein Y is lower alkyl, m is 0 or 1 and R₆ is phenyl, hydroxy-phenyl, imidazolyl, indolyl, piperidyl, piperazinyl, morpholinyl or pyridyl; R₄ and R₅ are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; N-alkyl-carbamoyl; N,N-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ and R₅ are not both hydrogen if n is 1, R₁ is benzyl or *tert*-butyl, R₂ is benzyl or 4-methoxy-benzyl and R₃ is isopropyl and that R₄ is not methoxy if R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl and R₅ is hydrogen; and

X is oxygen;

or a salt thereof.

Special preference is given especially to compounds of formula I, wherein

n is 0;

R₁ and R₂ are independently of the other an aliphatic radical, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical, each radical having not more than 20 carbon atoms;

R₃ is hydrogen, oxa-alkyl, an aliphatic radical or a radical with up to 20 carbon atoms of the formula -(Y)_m-R₆, wherein Y is alkyl, m is 0 or 1 and R₆ is an unsubstituted or substituted monocyclic radical with 5 or 6 ring members containing up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur, wherein said monocyclic radical can also be fused to a benzo ring; and

R₄ and R₅ are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl;

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unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R_4 and R_5 have up to 12 carbon atoms, with the proviso that R_4 is not methoxy if n is 0, R_1 is 1-naphthyl or 2-(3-aminophenyl)-ethyl, R_2 is 4-methoxy-benzyl, R_3 is isopropyl and R_5 is hydrogen;
or a salt thereof.

Preference is given above all to compounds of formula I, wherein
 n is 0 or 1;

R_1 is lower alkyl, phenyl-lower alkyl or naphthyl-lower alkyl;

R_2 is mono-, di-, or tri-lower alkoxy-phenyl-lower alkyl, *N,N*-di-lower alkyl-amino-phenyl-lower alkyl or naphthyl-lower alkyl;

R_3 is lower alkyl;

R_4 and R_5 are independently selected from the group consisting of hydrogen, lower alkoxy or halogen with the proviso that R_4 and R_5 are not both hydrogen if n is 1, R_1 is benzyl or *tert*-butyl, R_2 is 4-methoxy-benzyl and R_3 is isopropyl and that R_4 is not methoxy if R_2 is 4-methoxy-benzyl, R_3 is isopropyl or *tert*-butyl and R_5 is hydrogen; and

X is oxygen;

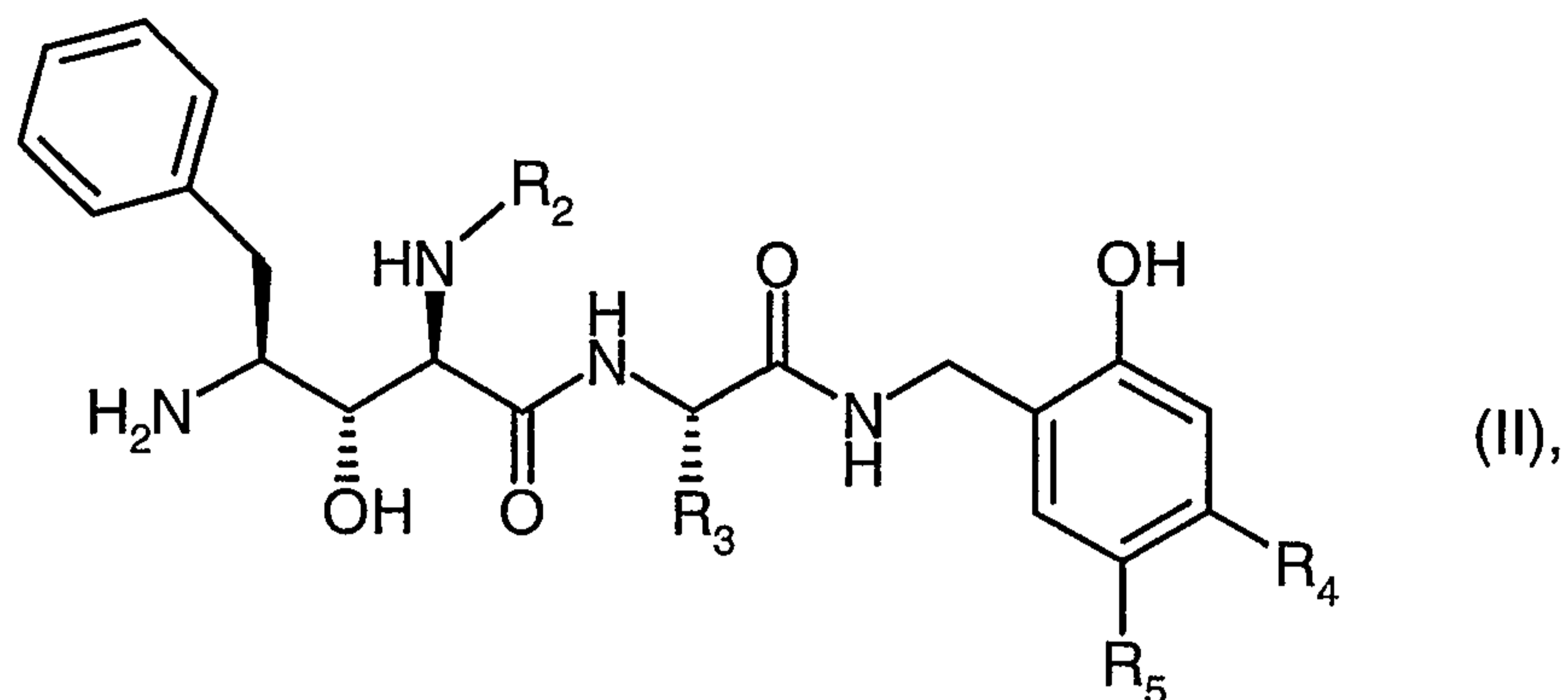
or a salt thereof.

Most especially preferred are the compounds of formula I described in the Examples and pharmaceutically acceptable salts thereof.

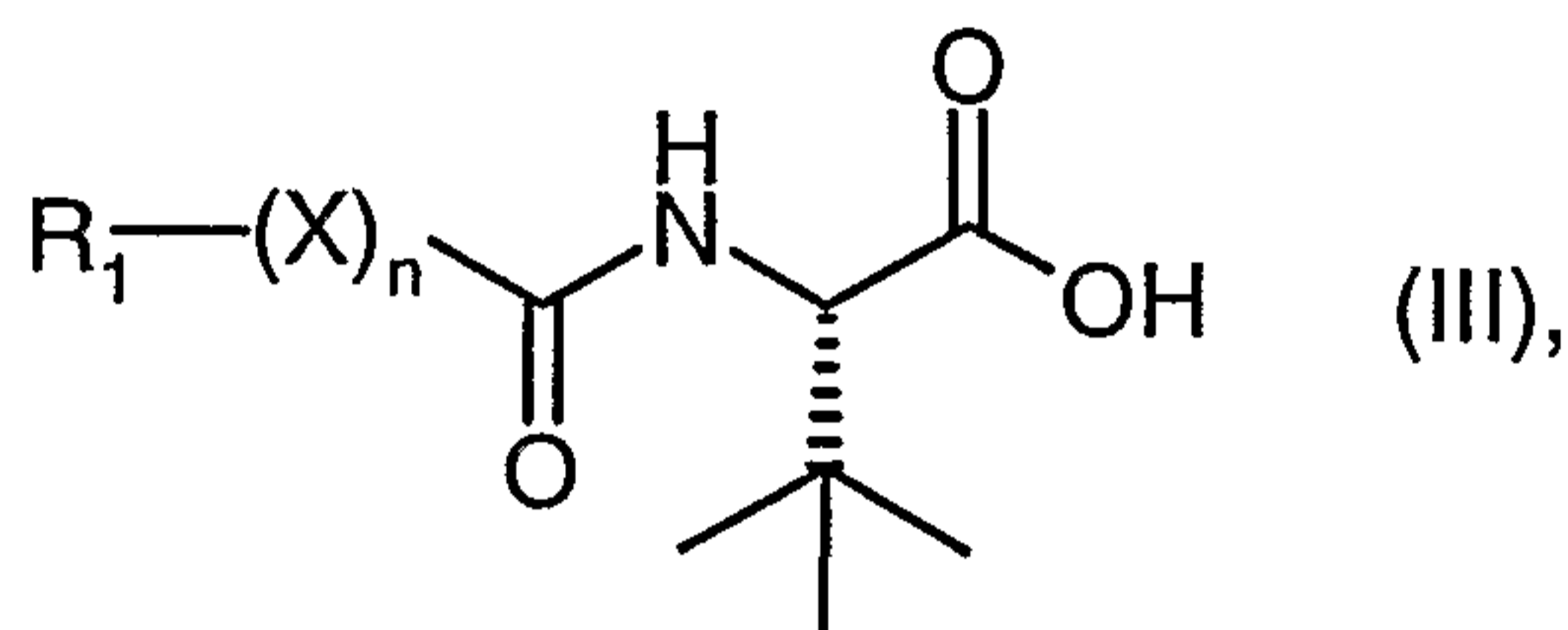
The compounds of formula I or salts thereof are prepared in accordance with processes known per se. The processes according to the invention are as follows:

a) a compound of formula II

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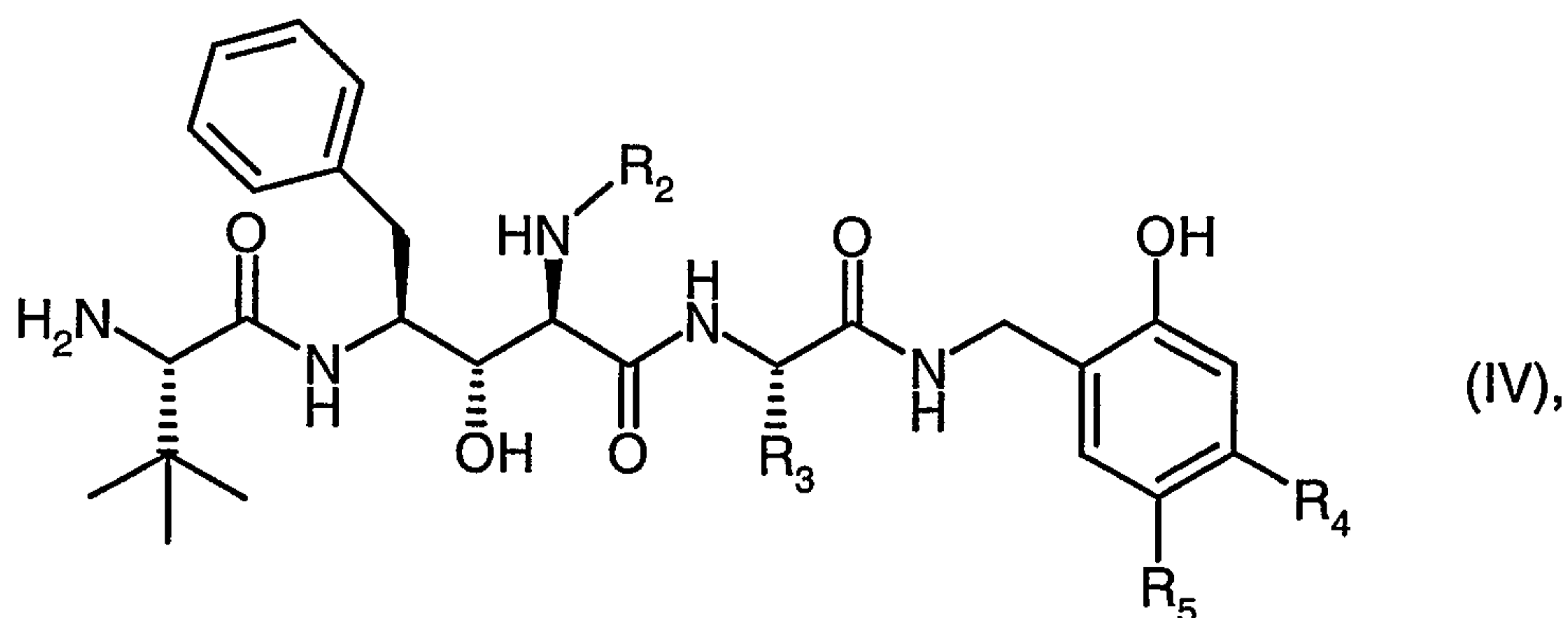


wherein R₂, R₃, R₄ and R₅ are as defined above under formula I, functional groups present in a compound of formula II, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a salt of such a compound, is reacted with an acid of formula III



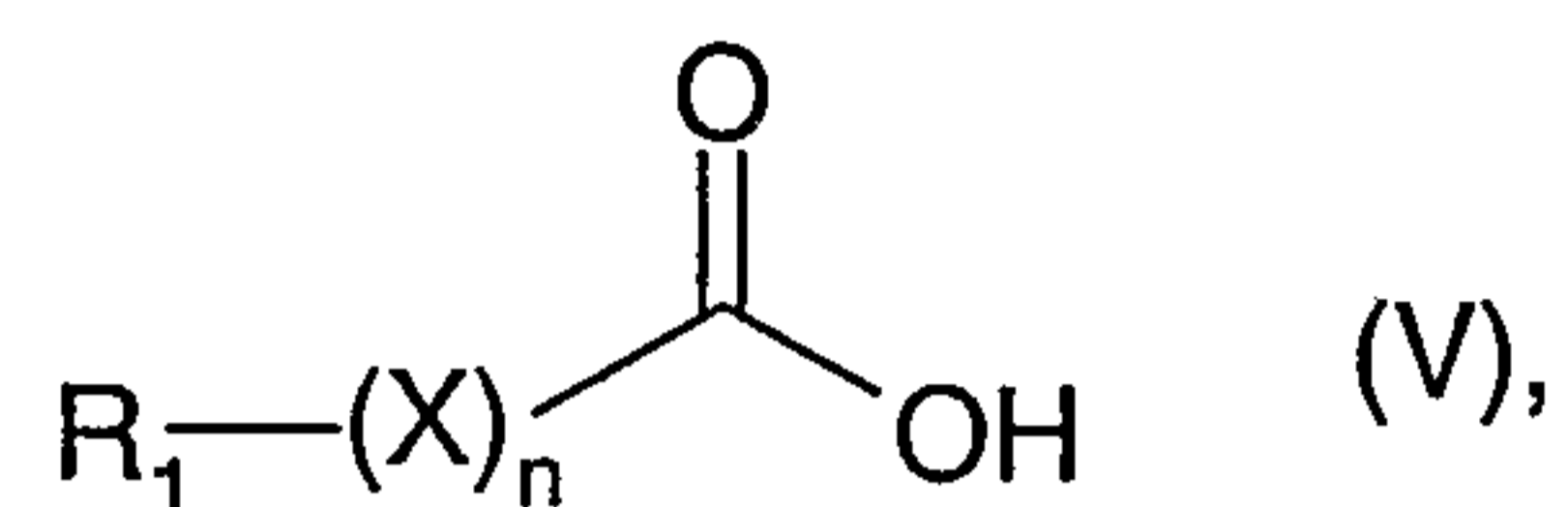
wherein n, R₁ and X are as defined above under formula I, functional groups present in a compound of formula III, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a suitable reactive acid derivative of such a compound, and any protecting groups present are removed, or

b) a compound of formula IV



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wherein R_2 , R_3 , R_4 and R_5 are as defined above under formula I, functional groups present in a compound of formula IV, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a salt of such a compound, is reacted with an acid of formula V



wherein n , R_1 and X are as defined above under formula I, functional groups present in a compound of formula V, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a suitable reactive acid derivative of such a compound, and any protecting groups present are removed, and, if desired, a compound of formula I obtained by process a) or b) is converted into another compound of formula I, an obtained free compound of formula I is converted into a salt, or an obtained salt of a compound of formula I is converted into another salt or into its free form.

General notes:

The end products of formula I may contain substituents that can also be used as protecting groups in starting materials for the preparation of other end products of formula I. Thus, within the scope of this text, only a readily removable group that is not a constituent of the particular desired end product of formula I is designated a "protecting group", unless the context indicates otherwise.

Protecting groups, and the manner in which they are introduced and removed are described, for example, in "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973, and in "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/1, Georg-Thieme-Verlag, Stuttgart 1974 and in Theodora W. Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York 1981. A characteristic of protecting groups is that they can be removed readily, i.e. without the occurrence of undesired secondary reactions, for example by solvolysis, reduction, photolysis or alternatively under physiological conditions.

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Process a:

The reaction is carried out by dissolving a compound of formula III in a suitable solvent, for example *N,N*-dimethylformamide, *N,N*-dimethylacetamide or *N*-methyl-2-pyrrolidone and by the addition of a suitable base, for example triethylamine, diisopropylethylamine or *N*-methylmorpholine and a suitable coupling agent, for example dicyclohexylcarbodiimide / 1-hydroxybenzotriazole (DCC/HOBT); *O*-(1,2-Dihydro-2-oxo-1-pyridyl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TPTU) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). For review of other coupling agents, see e.g. Klauser; Bodansky, *Synthesis* **1972**, 453-463. The reaction mixture is stirred at a temperature of between approximately -10 °C and room temperature for 0.5 to 1 hour, a compound of formula II is added and stirred for approximately 16 hours at the same temperature to yield a compound of formula I.

Process b:

The reaction is carried out under the conditions described for process a.

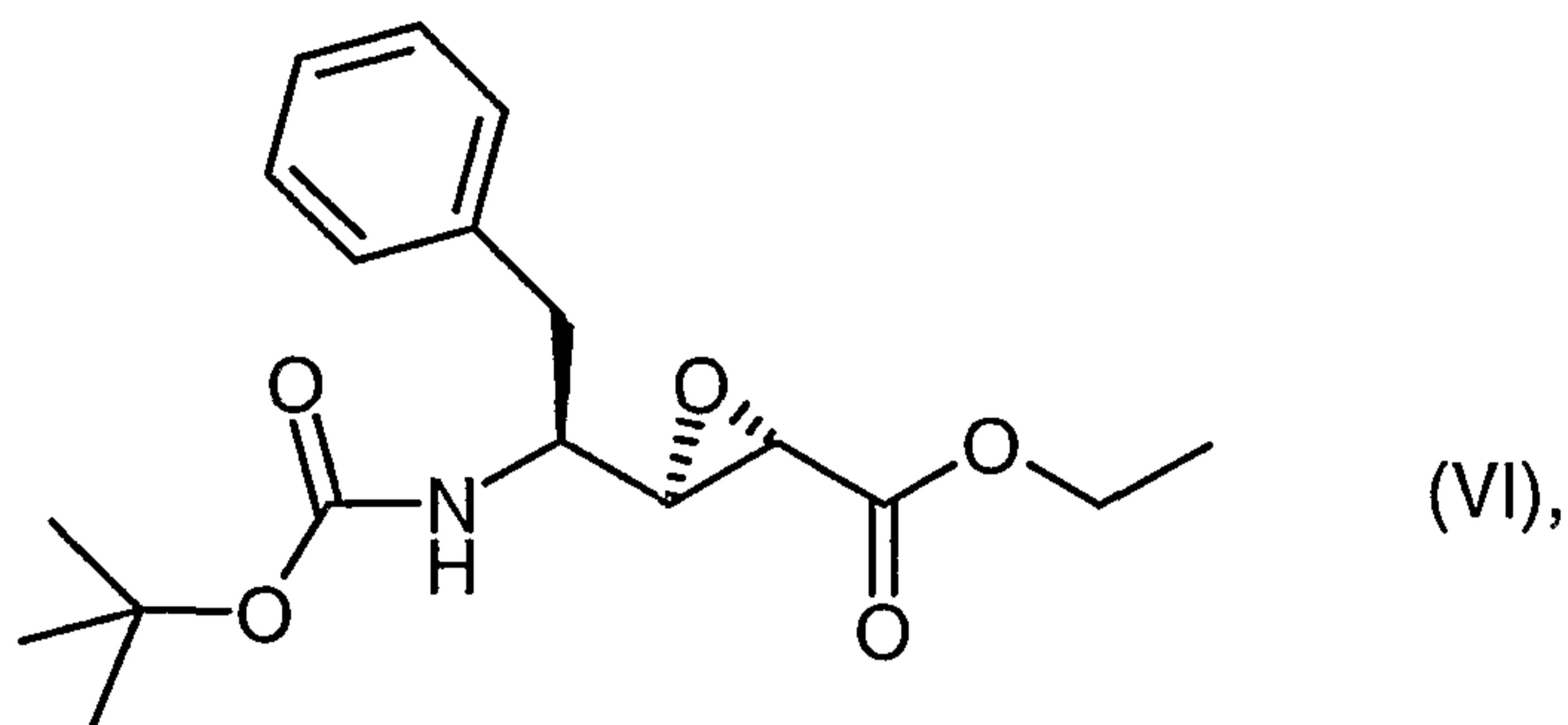
New starting materials and/or transients, as well as processes for the preparation thereof, are likewise the subject of this invention. In the preferred embodiment, such starting materials are used and reaction conditions so selected as to enable the preferred compounds to be obtained.

The starting materials of formulae II - V are known, capable of being prepared according to known processes, or commercially obtainable; in particular, they can be prepared using processes as described in the Examples.

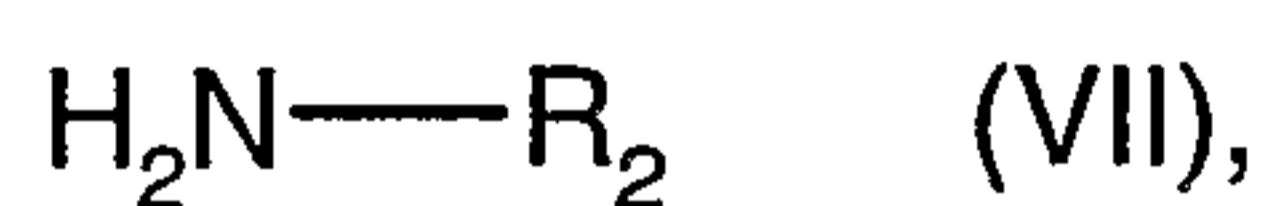
A compound of formula II, wherein the substituents are as defined above under formula I, is obtainable for example by the following reaction sequence:

a compound of formula VI

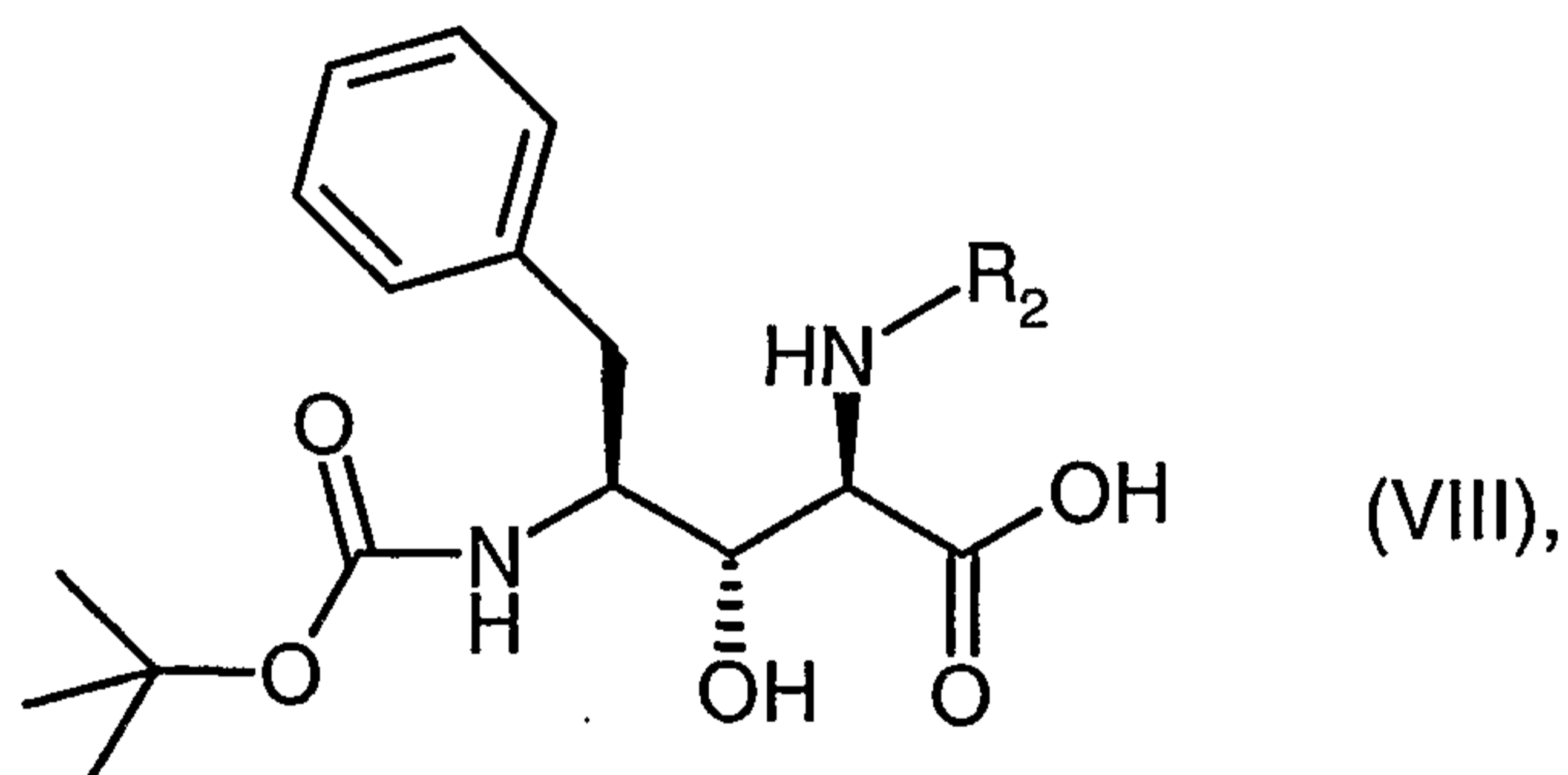
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is reacted with a compound of formula VII

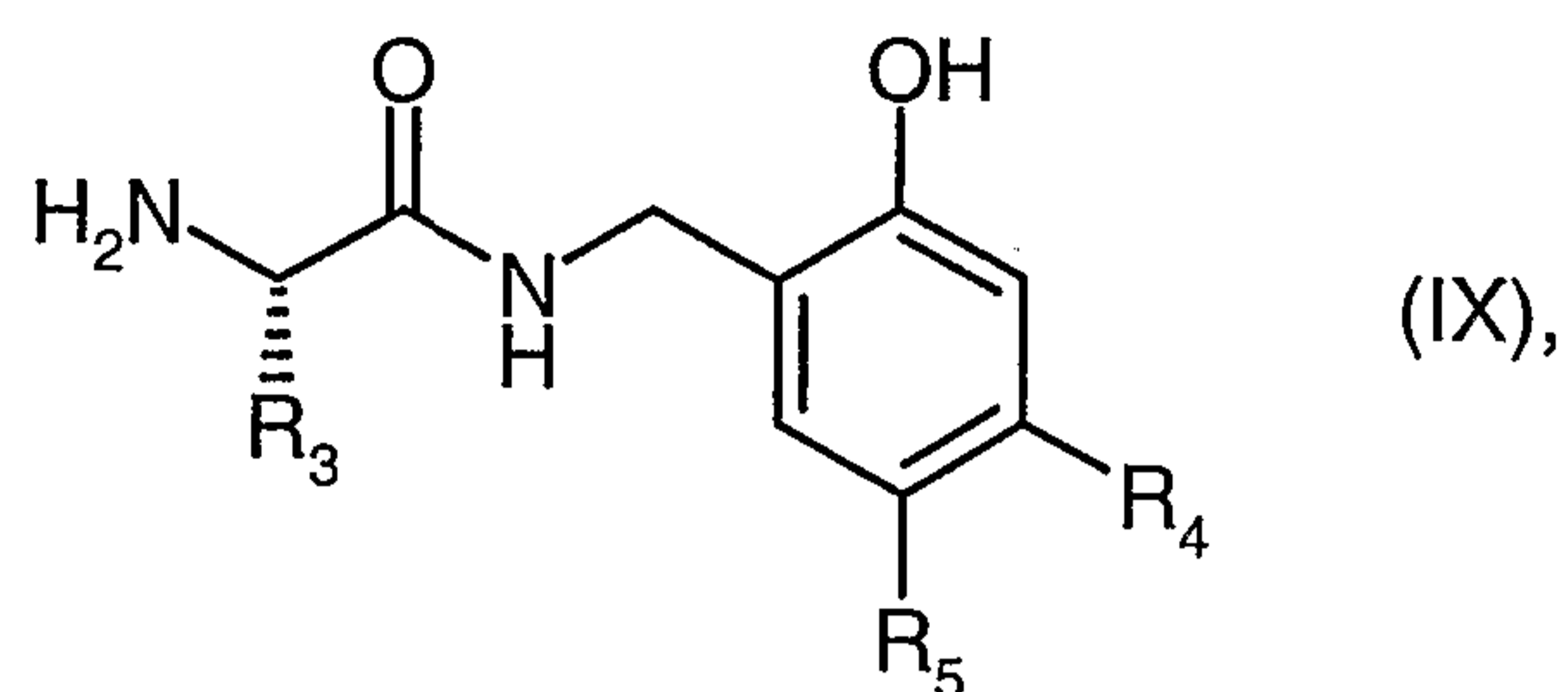


wherein R_2 is as defined above under formula I. The reaction is carried out by heating the reactants of formulae VI and VII for several hours, for example for from 4 to 6 hours, in the presence of an inert solvent, for example ethanol or isopropanol, at a temperature of approximately 60-90 °C. Hydrolysis of the ethyl ester group of the obtained product yields a compound of formula VIII

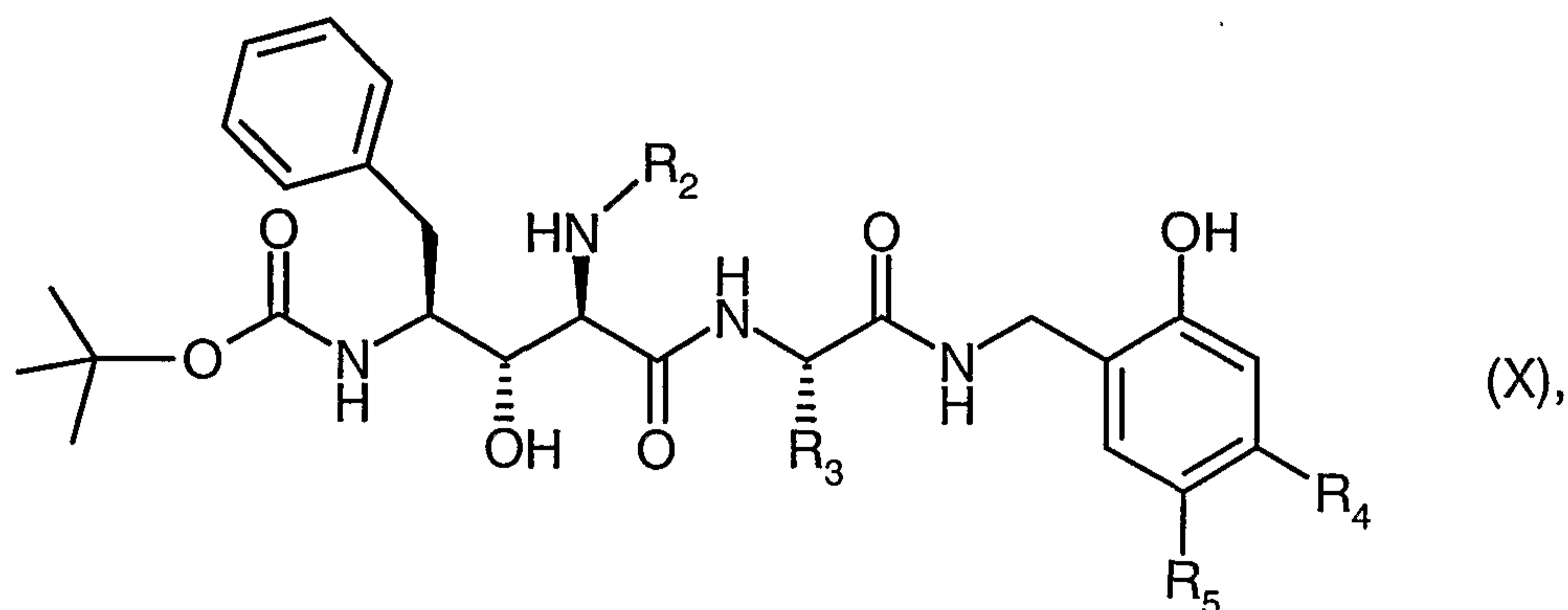


wherein R_2 is as defined above under formula I. A compound of formula VIII is then reacted with a compound of formula IX

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wherein the substituents are as defined above under formula I, to obtain a compound of formula X

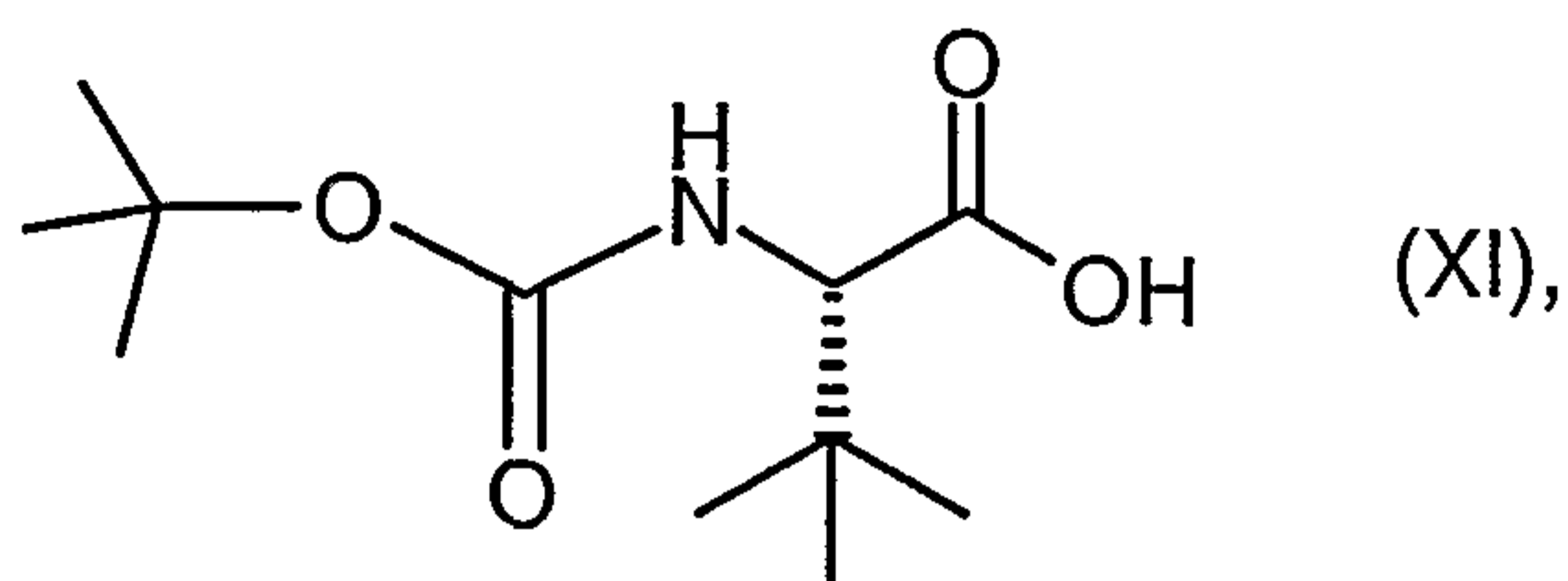


wherein the substituents are as defined above under formula I. The reaction is carried out as described in process a. From this the corresponding compound of formula II is prepared by removing the *N*-terminal *tert*-butoxycarbonyl protecting group in known manner, for example under the conditions described in Th. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis", Wiley, New York 1991 (2nd ed., pp. 328-330).

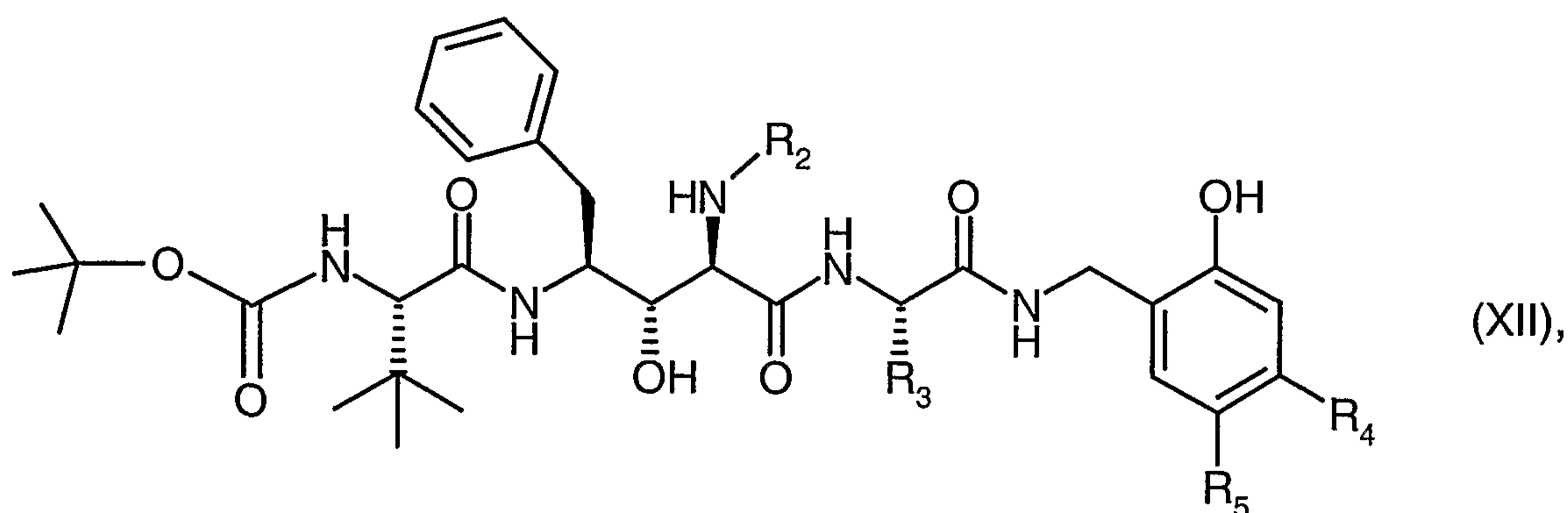
A compound of formula IV, wherein the substituents are as defined above under formula I, is obtainable for example by the following reaction sequence:

a compound of formula II, wherein the substituents are as defined above under formula I, is reacted with a compound of formula XI

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to obtain a compound of formula XII



wherein the substituents are as defined above under formula I. Compounds of formula XII correspond to compounds of formula I, wherein n is 1, R_1 is benzyl and X is oxygen. The reaction is carried out as described in process a. Removal of the *N*-terminal *tert*-butoxycarbonyl protecting group from a compound of formula XII in known manner (see above) yields a compound of formula IV.

The invention relates in particular to a method of treating warm-blooded animals, especially humans, suffering from a proliferative disease, especially a tumor disease and in particular such a disease which responds to inhibition of the multicatalytic proteasome complex, which method comprises administering, to warm-blooded animals requiring such treatment, an amount of a compound of formula I that is effective in inhibiting tumors, to the use of a compound of formula I for such treatment, or to the use of a compound of formula I for the preparation of a pharmaceutical composition for such treatment. The invention relates also to the use of a compound of formula I in the inhibition of the multicatalytic proteasome complex in warm-blooded animals, in particular humans. Effective doses, for example daily doses of approximately 0.05 g to about 10 g, preferably about 0.1 g to about 5 g, for example about 0.15 g to 1.5 g, of a compound of formula I are administered to a warm-

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blooded animal of approximately 70 kg body weight according to species, age, individual condition, mode of administration and the individual syndrome.

The invention relates also to pharmaceutical compositions comprising an effective amount, especially an amount effective in the prevention or therapy of one of the above-mentioned diseases, of the active ingredient together with pharmaceutically acceptable carriers that are suitable for topical, enteral, for example oral or rectal, or parenteral administration, and may be inorganic or organic, solid or liquid. For oral administration there are used especially tablets or gelatin capsules comprising the active ingredient together with diluents, for example lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycerol, and/or lubricants, for example silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Tablets may also comprise binders, for example magnesium aluminium silicate, starches, such as corn, wheat or rice starch, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and, if desired, disintegrators, for example starches, agar, alginic acid or a salt thereof, such as sodium alginate, and/or effervescent mixtures, or adsorbents, dyes, flavourings and sweeteners. The pharmacologically active compounds of the present invention can also be used in the form of parenterally administrable compositions or in the form of infusion solutions. Such solutions are preferably isotonic aqueous solutions or suspensions, which, for example in the case of lyophilised compositions that comprise the active ingredient alone or together with a carrier, for example mannitol, can be prepared before use. The pharmaceutical compositions may be sterilised and/or may comprise excipients, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers. The present pharmaceutical compositions which, if desired, may comprise further pharmacologically active substances, such as antibiotics, are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, confectioning, dissolving or lyophilising processes. The concentrations of active ingredient(s) will, of course, vary depending e.g. on the compound of formula I employed, the treatment desired and the nature of the form. The present pharmaceutical compositions comprise approximately from 1 % to 100 %, especially from approximately 1 % to approximately 20 %, active ingredient(s).

Compositions for oral administration can for example be obtained by formulating a compound of formula I with a carrier medium comprising a hydrophilic phase, a lipophilic

phase and a surfactant. Preferably the composition is in the form of a "microemulsion concentrate" or "emulsion concentrate", in particular of the type providing o/w (oil-in-water) microemulsions or emulsions. However, the composition may be in the form of a microemulsion or an emulsion which additionally contains an aqueous phase, preferably water. A "microemulsion concentrate" is a formulation which spontaneously forms a microemulsion in an aqueous medium, for example in water or in the gastric juices after oral application. A microemulsion is a non-opaque or substantially non-opaque colloidal dispersion that is formed spontaneously or substantially spontaneously when its components are brought into contact. A microemulsion is thermodynamically stable. An "emulsion concentrate" is a formulation which spontaneously forms an emulsion in an aqueous medium, for example in water or in the gastric juices, after oral application. The emulsion formed is opaque and thermodynamically stable. The lipophilic phase may comprise about 10 to 85 % by weight of the carrier medium; the surfactant may comprise about 5 to 80 % by weight of the carrier medium; and the hydrophilic phase may comprise about 10 to 50 % by weight of the carrier medium.

The hydrophilic phase may be selected from e.g. Transcutol® ($C_2H_5-[O-(CH_2)_2]_2-OH$), Glycofurol® (also known as tetrahydrofurfuryl alcohol polyethylene glycol ether) and 1,2-propylene glycol, or mixtures thereof, and is preferably 1,2-propylene glycol. It may include further hydrophilic co-components, for example C_1-C_5 alkanols.

Preferred lipophilic phase components are medium chain fatty acid triglycerides, mixed mono-, di-, tri-glycerides, and transesterified ethoxylated vegetable oils.

Examples of suitable surfactants are:

i) reaction products of a natural or hydrogenated castor oil and ethylene oxide. The oils available under the trade name Cremophor® are especially suitable. Particularly suitable are Cremophor RH 40® and Cremophor RH 60®. Also suitable are polyethyleneglycol castor oils such as that available under the trade name Cremophor EL®. Similar or identical products which may also be used are available under the trade names Nikkol®, Mapeg®, Incrocas® and Tagat®.

ii) Polyoxyethylene-sorbitan-fatty acid esters, for example esters of the type known and commercially available under the trade name Tween®.

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iii) Polyoxyethylene fatty acid esters, for example polyoxyethylene stearic acid esters of the type known and commercially available under the trade name Myrj®.

iv) Polyoxyethylene-polyoxypropylene co-polymers and block co-polymers, e.g. of the type known and commercially available under the trade names Pluronic®, Emkalyx® and Poloxamer®, especially preferred are Pluronic F68® and Poloxamer 188®.

v) Dioctylsulfosuccinate or di-[2-ethylhexyl]-succinate.

vi) Phospholipids, in particular lecithins.

vii) Propylene glycol mono- and di-fatty acid esters.

The surfactant selected preferably has an HLB (hydrophilic/lipophilic balance) of at least 10.

Full physical characteristics of the products referred to herein by trade name can be obtained e.g. from H.P. Fiedler, "Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und Angrenzende Gebiete", Editio Cantor, D-7960 Aulendorf, Germany, 3rd revised and expanded edition (1989).

The pharmaceutical compositions may also include further additives or ingredients, for example antioxidants. They exhibit especially advantageous properties when administered orally, for example in terms of consistency and high level of bioavailability obtained in standard bioavailability trials. Pharmacokinetic parameters, for example absorption and blood levels, also become surprisingly more predictable and problems in administration with erratic absorption may be eliminated or reduced. Additionally, the pharmaceutical composition is effective with tenside materials, for example bile salts, present in the gastro-intestinal tract.

The pharmaceutical compositions for oral use are preferably compounded in unit dosage form, for example by filling them into orally administrable capsule shells. The capsule shells may be soft or hard gelatine capsule shells. However, if desired the pharmaceutical compositions may be in a drink solution form and may include water or any other aqueous system, to provide emulsion or microemulsion systems suitable for drinking.

The present invention relates in particular to the use of compounds of formula I in the manufacture of a pharmaceutical composition for the treatment of a proliferative disease, e.g. of a solid tumor, and their use for the treatment of such a proliferative disease.

The following Examples illustrate the invention but do not limit the invention in any way.

Abbreviations:

brine	saturated sodium chloride solution
DIEA	<i>N</i> -Ethyl-diisopropylamine
DMF	<i>N,N</i> -Dimethylformamide
d.t.	decomposition temperature
equiv.	equivalent(s)
ES-MS	electron spray mass spectrometry
h	hour(s)
Me	methyl
min	minute(s)
m.p.	melting point
temp.	temperature
THF	tetrahydrofuran
TFA	trifluoroacetic acid
TPTU	<i>O</i> -(1,2-Dihydro-2-oxo-1-pyridyl)- <i>N,N,N',N'</i> -tetramethyluronium tetrafluoroborate
t _R	retention time

Example 1: ((S)-1-((1S,2R,3R)-1-Benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-[(naphthalen-1-ylmethyl)-amino]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester

N-Benzyloxycarbonyl-*L-tert*-leucine (1.05 equiv.) is dissolved in *N,N*-dimethylacetamide, and diisopropylethylamine (3.1 equiv.) and TPTU (1.05 equiv.) are added. The resulting mixture is stirred at room temperature for 0.5 - 1 h and then (2R,3R,4S)-4-amino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide (1.0 equiv.) is added. After 16 h at room temperature, ethyl acetate is added, the mixture is washed with a 5% NaHCO₃ solution and brine. The organic layer is dried, the solvent is evaporated and the residue is chromatographed on silicagel (solvent: dichloromethane-methanol). The title compound is obtained: ES-MS: 847.0; single peak at t_R= 7.73 min (System E).

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The starting material is obtained as follows:

Step 1.1: To the solution of 3.02 g (9 mmol) of (2S,3R)-3-((S)-1-*tert*-butoxycarbonylamino-2-phenyl-ethyl)-oxirane-2-carboxylic acid ethyl ester* in 23 ml of ethanol 2.6 ml (18 mmol) of 1-naphthyl-methylamine is added and stirred for 4.75 h at 70-80 °C. After removal of the solvent under reduced pressure, the crude product is purified by means of column chromatography on silicagel (solvent: hexanes-ethylacetate 3:1) to yield (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid ethyl ester; ES-MS: 493.0, single peak at $t_R = 11.18$ min (System A).

* The synthesis of this compound is described by Dieter Scholz et al. in *Journal of Medicinal Chemistry* **1994**, Vol. **37**, No.19, pp. 3079-3089.

Step 1.2: To the solution of 3.20 g (6.5 mmol) (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid ethyl ester in 30 ml of absolute THF is added 7.8 ml of 1M LiOH solution within 10 min and stirred for 5 h at room temperature. After completion, the reaction mixture was adjusted to pH 4.0 with 1N HCl. The precipitated product was filtered, washed repeatedly with water and dried to obtain (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid; m.p. 225-227 °C, single peak at $t_R = 9.68$ min (System A).

Step 1.3: To the suspension of 558 mg (1.2 mmol) of (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid in 10 ml of absolute DMF at room temperature is added 392 mg (1.32 mmol) of TPTU (Aldrich 36,572-6) and 0.82 ml (4.8 mmol) of DIEA (Fluka 03340). After 1 h 416 mg (1.44 mmol) of (S)-2-amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butamide** is added at 0 °C and the reaction mixture is stirred for 1h at 0-5 °C, then 1 h at room temperature. After completion, the mixture is poured onto ice water, extracted into ethyl acetate and the organic layer is subsequently being washed with water and saline. After filtration from MgSO₄ and evaporation the crude product was purified by means of column chromatography on silicagel (solvent: hexanes-ethylacetate 1:2) to yield {(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzyl)carbonyl]-2-methyl-propylcarbonyl]-3-[(naphthalen-1-ylmethyl)-amino]-propyl}-carbamic acid *tert*-butyl ester; m.p. 95-98 °C, single peak at $t_R = 11.54$ min (System A).

** The synthesis of this compound is described by A. Billich et al. in *Antiviral Chemistry & Chemotherapy* **1995**, Vol.6, No.5, pp. 327-336.

Step 1.4: (2R,3R,4S)-4-Amino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide
The N-terminal *tert*-butoxycarbonyl protecting group is removed by treatment of {(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl-carbamoyl]-3-[(naphthalen-1-ylmethyl)-amino]-propyl}-carbamic acid *tert*-butyl ester with a 4 N solution of HCl in dioxane using a protocol known in the art. After evaporation of the solvent, the crude compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness. The compound is used without further purification; ES-MS: 600.1; single peak at $t_R = 7.31$ min (System G).

Example 2: [(S)-1-[(1S,2R,3R)-1-Benzyl-3-(4-dimethylamino-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl]-2,2-dimethyl-propyl]-carbamic acid benzyl ester

The title compound is obtained starting from *N*-benzyloxycarbonyl-L-*tert*-leucine and (2R,3R,4S)-4-amino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 840.0, single peak at $t_R = 6.87$ min (System E).

The starting material is obtained as follows:

Step 2.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 4-*N*-dimethyl-benzylamine as a reagent; ES-MS: 486.0, single peak at $t_R = 8.35$ min (System A).

Step 2.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-butoxy-carbonylamino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-

phenyl-pentanoic acid ethyl ester as the starting material; m.p. 202-204 °C, single peak at $t_R = 6.90$ min (System A).

Step 2.3: {(1S,2R,3R)-1-Benzyl-3-(4-dimethylamino-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl}-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-butoxycarbonylamino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (S)-2-Amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butyramide as described in Example 1, Step 1.3; m.p. 89-91 °C, single peak at $t_R = 10.21$ min (System A).

Step 2.4: (2R,3R,4S)-4-Amino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide
The title compound is obtained from {(1S,2R,3R)-1-benzyl-3-(4-dimethylamino-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl}-carbamic acid *tert*-butyl ester as described in Example 1, Step 1.4; ES-MS: 593.0; single peak at $t_R = 6.03$ min (System G).

Example 3: {(S)-1-[(1S,2R,3R)-1-Benzyl-3-[(S)-1-(5-bromo-2-hydroxy-benzyl-carbamoyl)-2-methyl-propylcarbamoyl]-2-hydroxy-3-(4-methoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L*-*tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-2-(4-methoxy-benzylamino)-5-phenyl-pentanoic acid [(S)-1-(5-bromo-2-hydroxy-benzylcarbamoyl)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 875.9, single peak at $t_R = 6.74$ min (System D).

Example 4: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(3-methoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L*-*tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-2-(3-methoxy-benzylamino)-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 827.1, single peak at $t_R = 8.35$ min (System E).

The starting material is obtained as follows:

Step 4.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-2-(3-methoxy-benzylamino)-5-phenyl-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 3-methoxy-benzylamine as a reagent; ES-MS: 473, single peak at $t_R = 5.49$ min (System C).

Step 4.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-2-(3-methoxy-benzylamino)-5-phenyl-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-2-(3-methoxy-benzylamino)-5-phenyl-pentanoic acid ethyl ester as the starting material; d.t. 210 °C, single peak at $t_R = 4.80$ min (System C).

Step 4.3: [(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propylcarbamoyle]-3-(3-methoxy-benzylamino)-propyl]-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-2-(3-methoxy-benzylamino)-5-phenyl-pentanoic acid and (S)-2-Amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butamide as described in Example 1, Step 1.3; ES-MS: 679.0, single peak at $t_R = 5.89$ min (System C).

Step 4.4: (2R,3R,4S)-4-Amino-3-hydroxy-2-(3-methoxy-benzylamino)-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide
The title compound is obtained from [(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propylcarbamoyle]-3-(3-methoxy-benzylamino)-propyl]-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 580.1; single peak at $t_R = 6.69$ min (System G).

Example 5: [(S)-1-[(1S,2R,3R)-1-Benzyl-3-(2,4-dimethoxy-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propylcarbamoyle]-propylcarbamoyle]-2,2-dimethyl-propyl]-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L*-*tert*-leucine and (2R,3R,4S)-4-amino-2-(2,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 857.1, single peak at $t_R = 7.46$ min (System E).

The starting material is obtained as follows:

Step 5.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-2-(2,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 2,4,-dimethoxy-benzylamine as a reagent; ES-MS: 503.0, single peak at $t_R=$ 5.57 min (System C).

Step 5.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-2-(2,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-butoxycarbonylamino-2-(2,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid ethyl ester as the starting material; d.t. 221 °C, single peak at $t_R=$ 4.92 min (System C).

Step 5.3: {(1S,2R,3R)-1-Benzyl-3-(2,4-dimethoxy-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propylcarbonyl]-propyl}-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-2-(2,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (S)-2-amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butyramide as described in Example 1, Step 1.3; ES-MS: 709.0, single peak at $t_R=$ 5.89 min (System C).

Step 5.4: (2R,3R,4S)-4-Amino-2-(2,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propyl]-amide
The title compound is obtained from {(1S,2R,3R)-1-benzyl-3-(2,4-dimethoxy-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propylcarbonyl]-propyl}-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 610.1; single peak at $t_R=$ 7.45 min (System G).

Example 6: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propylcarbonyl]-3-(3,4,5-trimethoxy-benzylamino)-propylcarbonyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propyl]-amide as described in Example 1. Purification of the crude compound is performed by semi-preparative HPLC. The purified

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compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness; ES-MS: 887.0, single peak at t_R= 7.41 min (System F).

The starting material is obtained as follows:

Step 6.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxybenzylamino)-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 3,4,5-trimethoxybenzylamine as a reagent; ES-MS: 533.0, single peak at t_R= 5.34 min (System C).

Step 6.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxybenzylamino)-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxybenzylamino)-pentanoic acid ethyl ester as the starting material; d.t. 206.8 °C, single peak at t_R= 4.66 min (System C).

Step 6.3: {(1S,2R,3R)-1-Benzyl-3-(3,4,5-trimethoxybenzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxybenzylcarbonyl)-2-methyl-propylcarbonyl]-propyl}-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-butoxycarbonylamino-2-(3,4,5-trimethoxybenzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (S)-2-amino-*N*-(2-hydroxy-4-methoxybenzyl)-3-methyl-butyramide as described in Example 1, Step 1.3; ES-MS: 739.0, single peak at t_R= 5.69 min (System C).

Step 6.4: (2R,3R,4S)-4-Amino-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxybenzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxybenzylcarbonyl)-2-methyl-propyl]-amide
The title compound is obtained from [(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxybenzylcarbonyl)-2-methyl-propylcarbonyl]-3-(3,4,5-trimethoxybenzylamino)-propyl]-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 640.2; single peak at t_R= 6.44 min (System H).

Example 7: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxybenzylcarbonyl)-2-methyl-propylcarbonyl]-3-(2,3,4-trimethoxybenzylamino)-propylcarbonyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L*-*tert*-leucine and (2*R*,3*R*,4*S*)-4-amino-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(*S*)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide as described in Example 1. The crude compound is purified by semi-preparative HPLC. The purified compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness; ES-MS: 887.0, single peak at $t_R = 7.18$ min (System E).

The starting material is obtained as follows:

Step 7.1: (2*R*,3*R*,4*S*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 2,3,4-trimethoxybenzylamine as a reagent; ES-MS: 533.0, single peak at $t_R = 13.17$ min (System B).

Step 7.2: (2*R*,3*R*,4*S*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2*R*,3*R*,4*S*)-4-*tert*-butoxycarbonylamino-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid ethyl ester as the starting material; d.t. 207 °C, single peak at $t_R = 11.69$ min (System B).

Step 7.3: {(1*S*,2*R*,3*R*)-1-Benzyl-3-(2,3,4-trimethoxy-benzylamino)-2-hydroxy-3-[(*S*)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl}-carbamic acid *tert*-butyl ester is prepared from (2*R*,3*S*,4*S*)-4-*tert*-butoxycarbonylamino-2-(2,3,4-trimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (*S*)-2-amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butyramide as described in Example 1, Step 1.3; ES-MS: 739.0, single peak at $t_R = 5.90$ min (System C).

Step 7.4: (2*R*,3*R*,4*S*)-4-Amino-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(*S*)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide
The title compound is obtained from [(1*S*,2*R*,3*R*)-1-benzyl-2-hydroxy-3-[(*S*)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,3,4-trimethoxy-benzylamino)-propyl]-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 640.1; single peak at $t_R = 6.53$ min (System H).

Example 8: ((S)-1-((1S,2R,3R)-1-Benzyl-3-(3,4-dimethoxy-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L*-*tert*-leucine and (2R,3R,4S)-4-amino-2-(3,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 857.1, single peak at $t_R = 8.25$ min (System E).

The starting material is obtained as follows:

Step 8.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4-dimethoxy-benzylamino)-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 3,4-dimethoxybenzylamine as a reagent; ES-MS: 503.0, single peak at $t_R = 5.22$ min (System C).

Step 8.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4-dimethoxy-benzylamino)-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4-dimethoxy-benzylamino)-pentanoic acid ethyl ester as the starting material; d.t. 222 °C, single peak at $t_R = 4.54$ min (System C).

Step 8.3: ((1S,2R,3R)-1-Benzyl-3-(3,4-dimethoxy-benzyl amino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl)-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-butoxycarbonylamino-2-(3,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (S)-2-amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butylamide as described in Example 1, Step 1.3; m.p. 143.6 °C, single peak at $t_R = 5.57$ min (System C).

Step 8.4: (2R,3R,4S)-4-Amino-2-(3,4-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide
The title compound is obtained from ((1S,2R,3R)-1-benzyl-3-(3,4-dimethoxy-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl)-

carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 610.1; single peak at t_R = 6.61 min (System G).

Example 9: ((S)-1-((1S,2R,3R)-1-Benzyl-3-(3,5-dimethoxy-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propylcarbonyl)-propylcarbonyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-amino-2-(3,5-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 857.1, single peak at t_R = 7.61 min (System E).

The starting material is obtained as follows:

Step 9.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,5-dimethoxy-benzylamino)-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 3,5-dimethoxybenzylamine as a reagent; ES-MS: 503.0, single peak at t_R = 5.82 min (System C).

Step 9.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,4-dimethoxy-benzylamino)-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-5-phenyl-2-(3,5-dimethoxy-benzylamino)-pentanoic acid ethyl ester as the starting material; d.t. 206 °C, single peak at t_R = 4.92 min (System C).

Step 9.3: ((1S,2R,3R)-1-Benzyl-3-(3,5-dimethoxy-benzyl amino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propylcarbonyl)-propyl)-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-butoxycarbonylamino-2-(3,5-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (S)-2-amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butyramide as described in Example 1, Step 1.3; m.p. 138.2 °C, single peak at t_R = 5.87 min (System C).

Step 9.4: (2R,3R,4S)-4-Amino-2-(3,5-dimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbonyl)-2-methyl-propyl]-amide

The title compound is obtained from {(1S,2R,3R)-1-benzyl-3-(3,5-dimethoxy-benzylamino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl}-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 609.1; single peak at $t_R=7.07$ min (System G).

Example 10: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester

The title compound is obtained from *N*-benzyloxycarbonyl-*L*-*tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide as described in Example 1; ES-MS: 887.0, single peak at $t_R=7.28$ min (System E).

The starting material is obtained as follows:

Step 10.1: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid ethyl ester is prepared as described in Example 1, Step 1.1, but using 2,4,5-trimethoxybenzylamine as a reagent; ES-MS: 533.0, single peak at $t_R=5.33$ min (System C).

Step 10.2: (2R,3R,4S)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid is prepared as described in Example 1, Step 1.2, but using (2R,3R,4S)-4-*tert*-butoxycarbonylamino-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid ethyl ester as the starting material; m.p. 206 °C, single peak at $t_R=11.25$ min (System B).

Step 10.3: {(1S,2R,3R)-1-Benzyl-3-(2,4,5-trimethoxy-benzyl amino)-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propyl}-carbamic acid *tert*-butyl ester is prepared from (2R,3R,4S)-4-*tert*-butoxycarbonylamino-2-(2,4,5-trimethoxy-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid and (S)-2-amino-*N*-(2-hydroxy-4-methoxy-benzyl)-3-methyl-butyramide as described in Example 1, Step 1.3; ES-MS: 739.0, single peak at $t_R=5.66$ min (System C).

Step 10.4: (2R,3R,4S)-4-Amino-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzyl-amino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained from [(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,4,5-trimethoxy-benzylamino)-propyl]-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 639.1; single peak at $t_R = 7.33$ min (System G).

Example 11: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(3,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester

The title compound is obtained starting from *N-tert*-butoxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-Amino-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide (described in Example 6), and using the coupling conditions described in Example 1. The crude compound is purified by semi-preparative HPLC. The purified compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness; ES-MS: 853.1, single peak at $t_R = 7.28$ min (System F).

Example 12: (2R,3R,4S)-4-[(S)-3,3-Dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from 1-naphthylacetic acid (Fluka, Buchs, Switzerland) and (2R,3R,4S)-4-((S)-2-amino-3,3-dimethyl-butyrylamino)-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide, and using the coupling conditions described in Example 1. The crude compound is purified by semi-preparative HPLC. The purified compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness; ES-MS: 921.0, single peak at $t_R = 7.56$ min (System F).

The starting material is obtained as follows:

Step 12.1: (2R,3R,4S)-4-((S)-2-Amino-3,3-dimethyl-butyrylamino)-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from {(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(3,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester (Example 11) as described in Example 1; ES-MS: 753.1; single peak at t_R = 7.56 min (System F).

Example 13: (2R,3R,4S)-4-[(S)-3,3-Dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from 1-naphthylacetic acid (Fluka, Buchs, Switzerland) and (2R,3R,4S)-4-((S)-2-amino-3,3-dimethyl-butyrylamino)-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide, and using the coupling conditions described in Example 1. The crude compound is purified by semi-preparative HPLC. The purified compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness; ES-MS: 921.0, single peak at t_R = 7.71 min (System F).

The starting material is obtained as follows:

Step 13.1: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,3,4-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester

The title compound is obtained starting from *N-tert*-butoxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide (described in Example 7), and using the coupling conditions described in Example 1. The crude compound is purified by semi-preparative HPLC. The purified compound is taken up in ethyl acetate and washed several times with 5% NaHCO₃ and then brine. The organic phase is dried over MgSO₄ and the solvent is evaporated to dryness; ES-MS: 853.1, single peak at t_R = 7.34 min (System F).

Step 13.2: (2R,3R,4S)-4-((S)-2-Amino-3,3-dimethyl-butyrylamino)-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from {(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,3,4-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 753.1; single peak at t_R = 5.58 min (System F).

Example 14: (2R,3R,4S)-4-[(S)-3,3-Dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained from 1-naphthylacetic acid (Fluka, Buchs, Switzerland) and (2R,3R,4S)-4-((S)-2-amino-3,3-dimethyl-butyrylamino)-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide, and using the coupling conditions described in Example 1; ES-MS: 921.1, single peak at t_R = 7.47 min (System E).

The starting material is obtained as follows:

Step 14.1: {(S)-1-[(1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester

The title compound is obtained starting from *N-tert*-butoxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide (described in Example 10), and using coupling conditions described in Example 1; ES-MS: 853.1, single peak at t_R = 7.29 min (System E).

Step 14.2: (2R,3R,4S)-4-((S)-2-Amino-3,3-dimethyl-butyrylamino)-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from {(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 753.1 single peak at t_R = 5.71 min (System E).

Example 15: (2R,3R,4S)-4-[(S)-3,3-Dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from 1-naphthylacetic acid (Fluka, Buchs, Switzerland) and (2R,3R,4S)-4-((S)-2-amino-3,3-dimethyl-butyrylamino)-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide, and using the coupling conditions described in Example 1; ES-MS: 880.5, single peak at $t_R = 8.83$ min (System E).

The starting material is obtained as follows:

Step 15.1: ((S)-1-((1S,2R,3R)-1-Benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-[(naphthalen-1-ylmethyl)-amino]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid *tert*-butyl ester

The title compound is obtained starting from *N-tert*-butoxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-amino-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide (described in Example 1), and using the coupling conditions described in Example 1; ES-MS: 813.4, single peak at $t_R = 8.94$ min (System E).

Step 15.2: (2R,3R,4S)-4-((S)-2-Amino-3,3-dimethyl-butyrylamino)-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from ((S)-1-((1S,2R,3R)-1-benzyl-2-hydroxy-3-[(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-[(naphthalen-1-ylmethyl)-amino]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 713.4; single peak at $t_R = 7.56$ min (System E).

Example 16: (2R,3R,4S)-2-(4-Dimethylamino-benzylamino)-4-[(S)-3,3-dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from 1-naphthylacetic acid (Fluka, Buchs, Switzerland) and (2R,3R,4S)-4-((S)-2-amino-3,3-dimethyl-butyrylamino)-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-

benzylcarbamoyl)-2-methyl-propyl]-amide, and using the coupling conditions described in Example 1; ES-MS: 874.5, single peak at t_R = 8.23 min (System E).

The starting material is obtained as follows:

Step 16.1: ((S)-1-((1S,2R,3R)-1-Benzyl-3-(4-dimethylamino-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid *tert*-butyl ester

The title compound is obtained starting from *N-tert*-butoxycarbonyl-*L-tert*-leucine and (2R,3R,4S)-4-amino-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide (described in Example 2), and using the coupling conditions described in Example 1; ES-MS: 806.4, single peak at t_R = 8.36 min (System E).

Step 16.2: (2R,3R,4S)-4-((S)-2-Amino-3,3-dimethyl-butyrylamino)-2-(4-dimethylamino-benzylamino)-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propyl]-amide

The title compound is obtained starting from ((S)-1-((1S,2R,3R)-1-benzyl-3-(4-dimethylamino-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid *tert*-butyl ester as described in Example 1; ES-MS: 706.4; single peak at t_R = 7.07 min (System E).

Analytical HPLC conditions:

Sys-tem	Gradient	Column	Flow rate	Temp.
(A)	20-100% CH ₃ CN in 13min + 5min 100% CH ₃ CN (0.1% TFA); detection at 215 nm	Nucleosil C-18 Dimension: 4.6 x 250 mm	1.0 ml/min	30 °C
(B)	20-100% CH ₃ CN in 13min + 5min 100% CH ₃ CN (0.1% TFA); detection at 215 nm	Nucleosil C-18 Dimension: 4.6 x 250 mm	1.0 ml/min	30 °C

(C)	20-100% CH ₃ CN in 7min + 2min 100% CH ₃ CN (0.1% TFA); detection at 215 nm	Nucleosil 100-3 C-18 HD Dimension: 4.0 x 125 mm	1.0 ml/min	30 °C
(D)	20-100% CH ₃ CN in 7min + 3min 100% CH ₃ CN (0.1% TFA); detection at 215 nm	Nucleosil 100-3 C18 HD (125 x 4.6 mm)	1.0 ml/min	30 °C
(E)	linear gradient over 7 min of MeCN/0.09% TFA and H ₂ O/0.1% TFA from 1:49 to 1:0 and 3 min at 1:0; detection at 215 nm	Nucleosil C ₁₈ -column (250 x 4.6 mm, 5 μm, 100 Å)	2.0 ml/min	Room temp.
(F)	linear gradient over 7 min of MeCN/0.09% TFA and H ₂ O/0.1% TFA from 1:49 to 1:0 and 3 min at 1:0; detection at 215 nm	Nucleosil C _{18AB} -column (125 x 3 mm, 3 μm, 120 Å)	1.15 ml/min	Room temp.
(G)	linear gradient over 10 min of MeCN/0.09% TFA and H ₂ O/0.1% TFA from 1:49 to 1:0; detection at 215 nm	Nucleosil C ₁₈ -column (250 x 4 mm, 5 μm, 100 Å)	2.0 ml/min	Room temp.
(H)	linear gradient over 10 min of MeCN/0.09% TFA and H ₂ O/0.1% TFA from 1:49 to 1:0; detection at 215 nm	Nucleosil C _{18AB} -column (125 x 3 mm, 3 μm, 120 Å)	1.15 ml/min	Room temp.

Example 17: Inhibition of the chymotrypsin-like activity of the 20S proteasome

Exemplary IC₅₀ values determined according to the test described above for the compounds of formula I are given below (Table 1).

Table 1

Example	IC ₅₀ [μM]		Example	IC ₅₀ [μM]
1	0.26		9	0.19
2	0.6		10	0.2
3	0.8		11	1.04
4	1.5		12	0.011
5	1.6		13	0.018
6	0.05		14	0.029
7	0.1		15	0.15
8	0.15		16	0.16

Example 18: Composition for oral Application

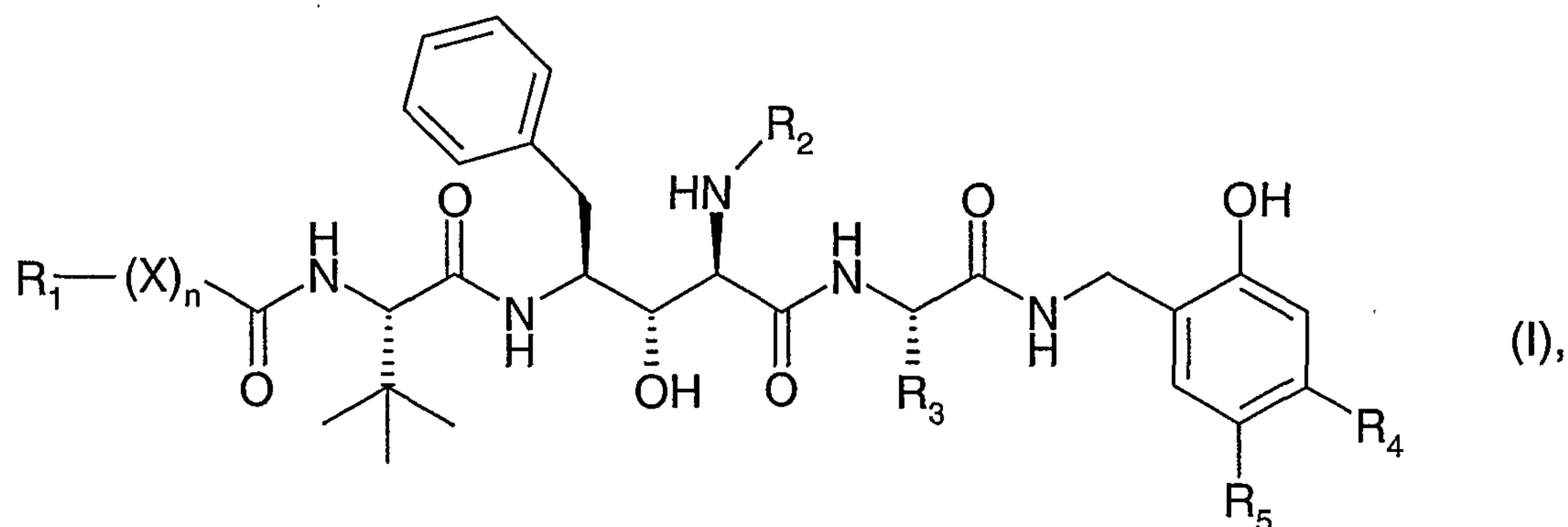
20.0 g of a solution for oral application can be prepared as follows (% means weight ingredient / total weight solution):

Cremophor RH 40®	9.6 g (48 %),
cornoil-mono-di-tri-glycerides	5.8 g (29 %),
propylene glycol	3.8 g (19 %),
compound of formula I	0.8 g (4 %).

The solution is kept at room temperature until use.

What is claimed is:

1. A compound of formula I



wherein

n is 0 or 1;

R_1 and R_2 are independently of the other an aliphatic radical, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical, each radical having not more than 20 carbon atoms;

R_3 is hydrogen, oxa-alkyl, an aliphatic radical or a radical with up to 20 carbon atoms of the formula $-(Y)_m-R_6$, wherein Y is alkyl, m is 0 or 1 and R_6 is an unsubstituted or substituted monocyclic radical with 5 or 6 ring members containing up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur, wherein said monocyclic radical can also be fused to a benzo ring;

R_4 and R_5 are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R_4 and R_5 have up to 12 carbon atoms, with the proviso that R_4 and R_5 are not both hydrogen if n is 1, R_1 is benzyl or *tert*-butyl, R_2 is benzyl or 4-methoxy-benzyl, R_3 is isopropyl and X is oxygen and that R_4 is not methoxy if n is 0 or 1, R_2 is 4-methoxy-benzyl, R_3 is isopropyl or *tert*-butyl, R_5 is hydrogen and X is oxygen; and

X is nitrogen, oxygen or sulfur;

or a salt thereof.

2. A compound of formula I according to claim 1, wherein

n is 0 or 1;

R₁ has not more than 20 carbon atoms and is a member selected from the group consisting of lower alkyl, cycloalkyl-lower alkyl, phenyl-lower alkyl, naphthyl-lower alkyl, and chinolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of hydroxy, lower alkoxy, lower alkanoyloxy, halogen, carboxy, lower alkoxy-carbonyl, formyl, lower alkanoyl, amino, *N*-lower alkylamino, *N,N*-di-lower alkylamino, mercapto, sulfo, lower alkyl-thio, carbamoyl, *N*-lower alkyl-carbamoyl, *N,N*-di-lower alkyl-carbamoyl, cyano, nitro and lower alkyl that can itself be substituted by said radicals;

R₂ has not more than 20 carbon atoms and is a member selected from the group consisting of phenyl-lower alkyl, naphthyl-lower alkyl, chinolyl-lower alkyl, indolyl-lower alkyl and *N*-lower alkyl-indolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of hydroxy, lower alkoxy, lower alkanoyloxy, halogen, carboxy, lower alkoxy-carbonyl, formyl, lower alkanoyl, amino, *N*-lower alkylamino, *N,N*-di-lower alkylamino, mercapto, sulfo, lower alkyl-thio, carbamoyl, *N*-lower alkyl-carbamoyl, *N,N*-di-lower alkyl-carbamoyl, cyano, nitro and lower alkyl that can itself be substituted by said radicals;

R₃ is hydrogen, a radical of the formula -G(-O-CH₂-CH₂)_t-R₇, in which G and R₇ are lower alkyl and t is 1 to 3, unsubstituted lower alkyl or lower alkyl substituted by hydroxy, carboxy, amino, carbamoyl, guanidino, mercapto or lower alkyl-thio and having not more than 12 carbon atoms, or a radical of the formula -(Y)_m-R₆, wherein Y is lower alkyl, m is 0 or 1 and R₆ is phenyl, hydroxy-phenyl, imidazolyl, indolyl, piperidyl, piperazinyl, morpholinyl or pyridyl; R₄ and R₅ are independently selected from the group consisting of hydrogen, hydroxy, lower alkoxy, lower alkanoyloxy, halogen, carboxy, lower alkoxy-carbonyl, formyl, lower alkanoyl, amino, *N*-lower alkylamino, *N,N*-di-lower alkylamino, mercapto, sulfo, lower alkyl-thio, carbamoyl, *N*-lower alkyl-carbamoyl, *N,N*-di-lower alkyl-carbamoyl, cyano, nitro and lower alkyl that can itself be substituted by said radicals, wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ and R₅ are not both hydrogen if

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n is 1, R₁ is benzyl or *tert*-butyl, R₂ is benzyl or 4-methoxy-benzyl, R₃ is isopropyl and X is oxygen and that R₄ is not methoxy if n is 0 or 1, R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl, R₅ is hydrogen and X is oxygen; and
X is nitrogen, oxygen or sulfur;
or a salt thereof.

3. A compound of formula I according to claim 1, wherein

n is 0 or 1;

R₁ has not more than 20 carbon atoms and is a member selected from the group consisting of lower alkyl, cycloalkyl-lower alkyl, phenyl-lower alkyl, naphthyl-lower alkyl, and chinolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro;

R₂ has not more than 20 carbon atoms and is a member selected from the group consisting of phenyl-lower alkyl, naphthyl-lower alkyl, chinolyl-lower alkyl, indolyl-lower alkyl and *N*-lower alkyl-indolyl-lower alkyl, the members being unsubstituted or substituted by one or more radicals independently selected from the group consisting of an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro;

R₃ is hydrogen, oxa-alkyl, an aliphatic radical or a radical with up to 20 carbon atoms of the formula -(Y)_m-R₆, wherein Y is alkyl, m is 0 or 1 and R₆ is an unsubstituted or substituted monocyclic radical with 5 or 6 ring members containing up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur, wherein said monocyclic radical can also be fused to a benzo ring;

R₄ and R₅ are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ and R₅ are not both hydrogen if

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n is 1, R₁ is benzyl or *tert*-butyl, R₂ is benzyl or 4-methoxy-benzyl and R₃ is isopropyl and that R₄ is not methoxy if R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl and R₅ is hydrogen; and
X is oxygen;
or a salt thereof.

4. A compound of formula I according to claim 1, wherein

n is 0 or 1;

R₁ and R₂ are independently of the other an aliphatic radical, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical, each radical having not more than 20 carbon atoms;

R₃ is hydrogen, a radical of the formula -G(-O-CH₂-CH₂)_t-R₇, in which G and R₇ are lower alkyl and t is 1 to 3, unsubstituted lower alkyl or lower alkyl substituted by hydroxy, carboxy, amino, carbamoyl, guanidino, mercapto or lower alkyl-thio and having not more than 12 carbon atoms, or a radical of the formula -(Y)_m-R₆, wherein Y is lower alkyl, m is 0 or 1 and R₆ is phenyl, hydroxy-phenyl, imidazolyl, indolyl, piperidyl, piperazinyl, morpholinyl or pyridyl;
R₄ and R₅ are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ and R₅ are not both hydrogen if n is 1, R₁ is benzyl or *tert*-butyl, R₂ is benzyl or 4-methoxy-benzyl and R₃ is isopropyl and that R₄ is not methoxy if R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl and R₅ is hydrogen; and
X is oxygen;
or a salt thereof.

5. A compound of formula I according to claim 1, wherein

n is 0;

R₁ and R₂ are independently of the other an aliphatic radical, or an aromatic, aromatic-aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, heterocyclic or heterocyclic-aliphatic radical, each radical having not more than 20 carbon atoms;

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R₃ is hydrogen, oxa-alkyl, an aliphatic radical or a radical with up to 20 carbon atoms of the formula -(Y)_m-R₆, wherein Y is alkyl, m is 0 or 1 and R₆ is an unsubstituted or substituted monocyclic radical with 5 or 6 ring members containing up to 3 hetero atoms selected from the group consisting of nitrogen, oxygen and sulfur, wherein said monocyclic radical can also be fused to a benzo ring; and

R₄ and R₅ are independently selected from the group consisting of hydrogen; an aliphatic radical; free, etherified or esterified hydroxy; free or esterified carboxy; formyl; alkanoyl; unsubstituted, mono- or di-substituted amino; mercapto; sulfo; alkyl-thio; carbamoyl; *N*-alkyl-carbamoyl; *N,N*-di-alkyl-carbamoyl; cyano and nitro; wherein carbon containing radicals R₄ and R₅ have up to 12 carbon atoms, with the proviso that R₄ is not methoxy if n is 0, R₁ is 1-naphthyl or 2-(3-aminophenyl)-ethyl, R₂ is 4-methoxy-benzyl, R₃ is isopropyl and R₅ is hydrogen;

or a salt thereof.

6. A compound of formula I according to claim 1, wherein

n is 0 or 1;

R₁ is lower alkyl, phenyl-lower alkyl or naphthyl-lower alkyl;

R₂ is mono-, di-, or tri-lower alkoxy-phenyl-lower alkyl, *N,N*-di-lower alkyl-amino-phenyl-lower alkyl or naphthyl-lower alkyl;

R₃ is lower alkyl;

R₄ and R₅ are independently selected from the group consisting of hydrogen, lower alkoxy or halogen with the proviso that R₄ and R₅ are not both hydrogen if n is 1, R₁ is benzyl or *tert*-butyl, R₂ is 4-methoxy-benzyl and R₃ is isopropyl and that R₄ is not methoxy if R₂ is 4-methoxy-benzyl, R₃ is isopropyl or *tert*-butyl and R₅ is hydrogen; and

X is oxygen;

or a salt thereof.

7. A compound of formula I according to any one of claims 1 to 6, wherein

if one of the radicals R₄ or R₅ is hydrogen, the other is as defined in any one of claims 1 to

6, with the exception of hydrogen, and if R₄ is methoxy, R₂ is as defined in any one of

claims 1 to 6, with the exception of benzyl monosubstituted at position 4 with halogen, alkyl, alkoxy or hydroxyalkoxy;

or a salt thereof.

8. A compound of formula I according to claim 1 selected from the group consisting of

((S)-1-((1S,2R,3R)-1-benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-[(naphthalen-1-ylmethyl)-amino]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester,

((S)-1-((1S,2R,3R)-1-benzyl-3-(4-dimethylamino-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester,

{(S)-1-[(1S,2R,3R)-1-benzyl-3-((S)-1-(5-bromo-2-hydroxy-benzyl-carbamoyl)-2-methyl-propylcarbamoyl]-2-hydroxy-3-(4-methoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester,

{(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(3-methoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester,

((S)-1-((1S,2R,3R)-1-benzyl-3-(2,4-dimethoxy-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester,

{(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(3,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester,

{(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,3,4-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester,

((S)-1-((1S,2R,3R)-1-benzyl-3-(3,4-dimethoxy-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester,

((S)-1-((1S,2R,3R)-1-benzyl-3-(3,5-dimethoxy-benzylamino)-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-propylcarbamoyl)-2,2-dimethyl-propyl)-carbamic acid benzyl ester,

{(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(2,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid benzyl ester,

{(S)-1-[(1S,2R,3R)-1-benzyl-2-hydroxy-3-((S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyl)-2-methyl-propylcarbamoyl]-3-(3,4,5-trimethoxy-benzylamino)-propylcarbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester,

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(2R,3R,4S)-4-[(S)-3,3-dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-2-(3,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide,

(2R,3R,4S)-4-[(S)-3,3-dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-2-(2,3,4-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide,

(2R,3R,4S)-4-[(S)-3,3-dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-2-(2,4,5-trimethoxy-benzylamino)-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide,

(2R,3R,4S)-4-[(S)-3,3-dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-2-[(naphthalen-1-ylmethyl)-amino]-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide,

(2R,3R,4S)-2-(4-dimethylamino-benzylamino)-4-[(S)-3,3-dimethyl-2-(2-naphthalen-1-yl-acetylamino)-butyrylamino]-3-hydroxy-5-phenyl-pentanoic acid [(S)-1-(2-hydroxy-4-methoxy-benzylcarbamoyle)-2-methyl-propyl]-amide,

and pharmaceutically acceptable salts thereof.

9. A pharmaceutically acceptable salt of a compound of formula I according to any one of claims 1-7.

10. A compound of formula I according to any one of claims 1 to 8 or a pharmaceutically acceptable salt of such a compound for use in a method for the therapeutic treatment of the human or animal body.

11. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula I according to any one of claims 1 to 8 or a pharmaceutically acceptable salt of such a compound together with a pharmaceutical carrier.

12. A pharmaceutical composition for the treatment of proliferative diseases in warm-blooded animals, including humans, comprising, in a dose effective against the disease, a compound of formula I according to any one of claims 1 to 8, or a pharmaceutically acceptable salt of such a compound, together with a pharmaceutical carrier.

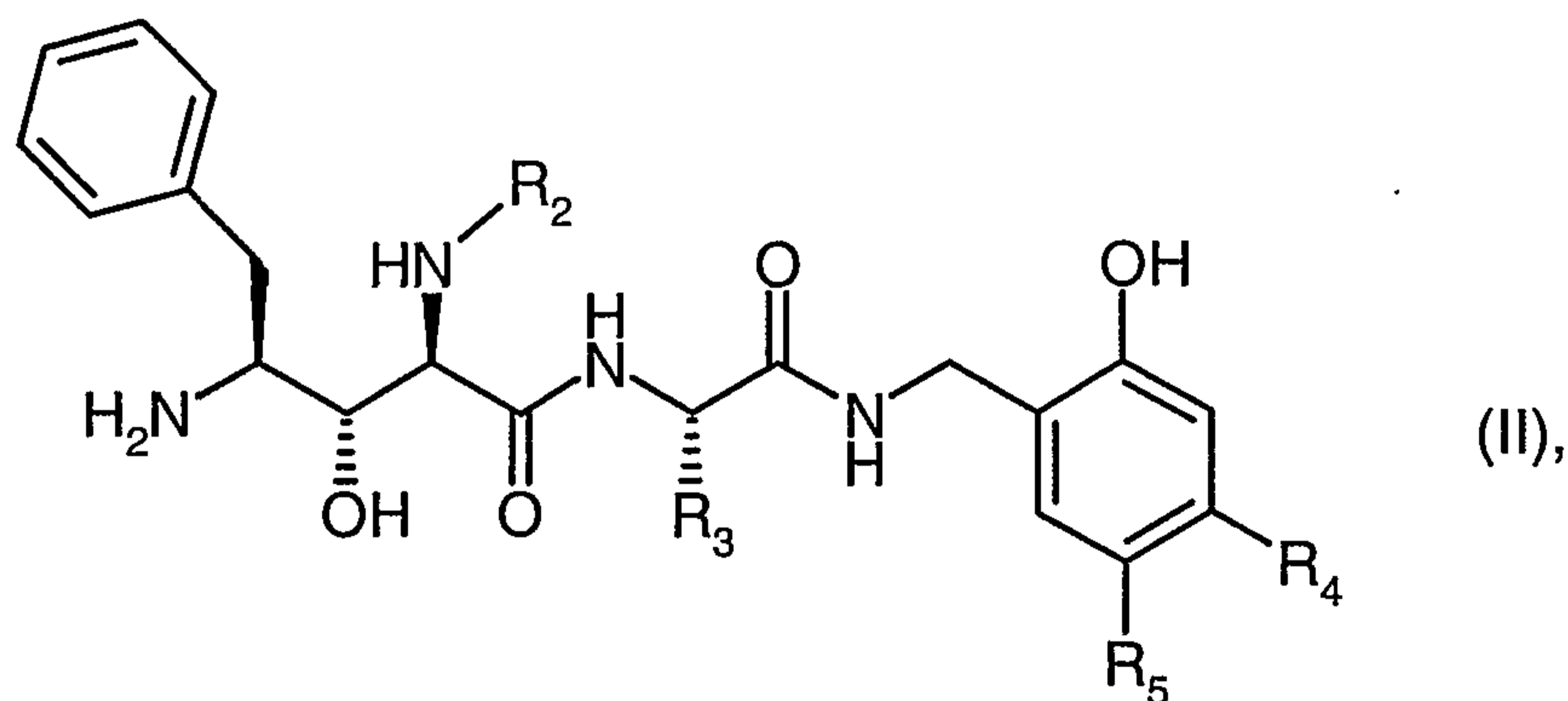
13. The use of a compound of formula I according to any one of claims 1 to 8 or a pharmaceutically acceptable salt of such a compound for the preparation of pharmaceutical compositions for use in the treatment of proliferative diseases.

14. The use of a compound of formula I according to any one of claims 1 to 8 or a pharmaceutically acceptable salt of such a compound in the treatment of proliferative diseases.

15. A method of treating warm-blooded animals, including humans, which comprises administering to such a warm-blooded animal suffering from a proliferative disease a dose, effective against tumours, of a compound of formula I according to any one of claims 1 to 8 or of a pharmaceutically acceptable salt of such a compound.

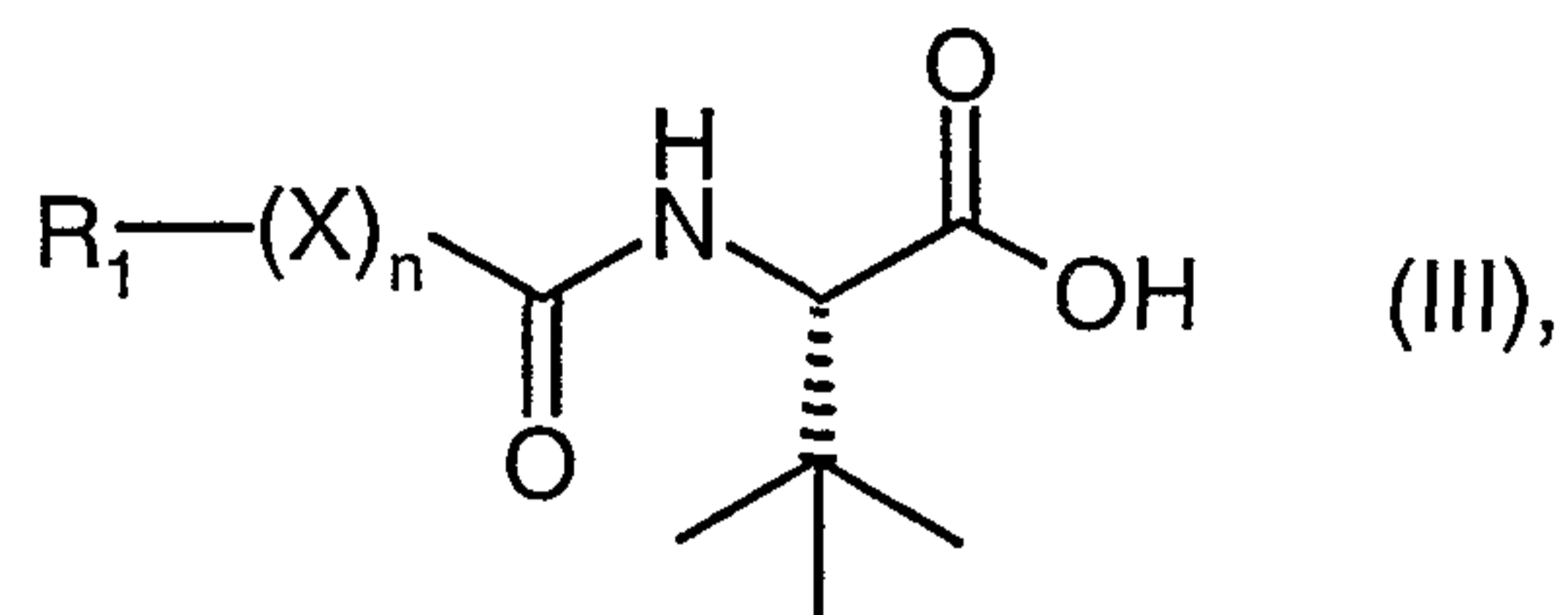
16. A process for the preparation of a compound of formula I according to claim 1 or of a salt of such a compound, characterized in that

a) a compound of formula II



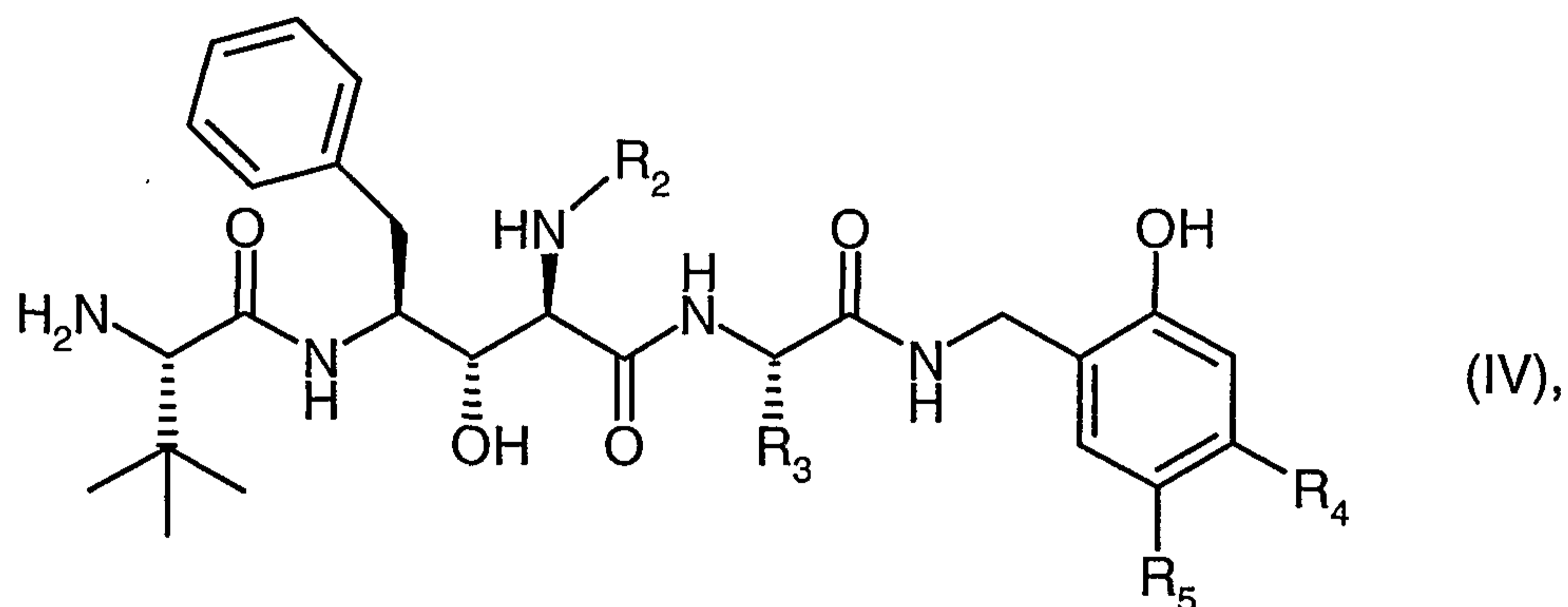
wherein R₂, R₃, R₄ and R₅ are as defined for compounds of formula I according to claim 1, functional groups present in a compound of formula II, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a salt of such a compound, is reacted with an acid of formula III

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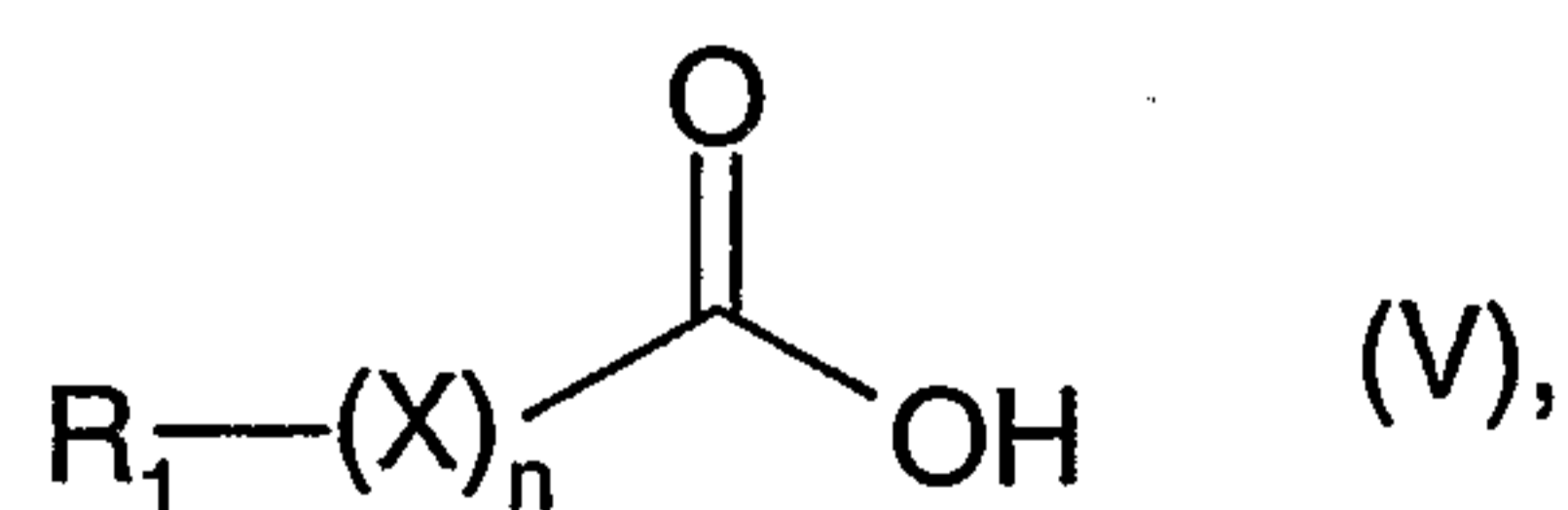


wherein n , R_1 and X are as defined for compounds of formula I according to claim 1, functional groups present in a compound of formula III, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a suitable reactive acid derivative of such a compound, and any protecting groups present are removed, or

b) a compound of formula IV



wherein R_2 , R_3 , R_4 and R_5 are as defined for compounds of formula I according to claim 1, functional groups present in a compound of formula IV, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting groups, or a salt of such a compound, is reacted with an acid of formula V



wherein n , R_1 and X are as defined for compounds of formula I according to claim 1, functional groups present in a compound of formula V, with the exception of the groups participating in the reaction, being protected if necessary by readily removable protecting

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groups, or a suitable reactive acid derivative of such a compound, and any protecting groups present are removed,

and, if desired, a compound of formula I obtained by process a) or b) is converted into another compound of formula I, an obtained free compound of formula I is converted into a salt, or an obtained salt of a compound of formula I is converted into another salt or into its free form.

