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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Sustained-Release Preparation

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Sustained-Release Preparation

Field of the Invention

This invention relates to a microcapsule containing an amorphous water-soluble 2-piperazinone-1-acetic acid compound or salt thereof and a method of preparing it.

Background of the Invention

On sustained-release microcapsules of various low-molecular water-soluble drugs, many reports have been made [e.g. JPA S57(1982)-118512, J. Pharm. Sci., 75, 750-755 (1986)]. Most of the microcapsules so far reported have the following drawbacks: (1) in the manufacturing process, the amount of the water-soluble drug leaked to the outer aqueous phase is relatively large to invite a relatively low entrapment ratio of the drug, and (2) the resulting microcapsules are generally porous and cause a relatively large initial drug release. Thus, at the present stage, no drugs of sustained-release over a sufficiently desirable long period have not yet been successfully prepared.

On the other hand, in recent years, novel peptides or low-molecular compounds having excellent cell-adhesion regulating or inhibiting actions have been found and are expected as therapeutic agents of various diseases. For example, compounds having GPIIb/IIIa antagonistic activity remarkably inhibit platelet aggregation or suppress the metastasis of tumor cells, which are expected as clinically useful drugs. (Sci., 233, 467-469 (1986); Sci., 238, 1132-1134 (1987); Proc. Natl. Acad. Sci. USA, 87, 2471-2475(1990)]. As examples of such compounds, linear or cyclic peptides containing the amino acid sequence, -Arg-Gly-Asp-(RGD) have been known [e.g. J. Biol. Chem., 262, 17294-17298 (1987); JPA H2(1990)-174797]. And, non-peptide compounds having an anti-thrombotic activity are disclosed in JPA H4(1992)-264068 and EPA No.505868, in

which having 4- to 7-membered cyclic alkyleneimino such as pyrrolidine ring and compounds having e.g. piperidine ring are respectively described. Further, compounds having piperidinone ring, which have cell-adhesion inhibiting activity, are disclosed in EPA No.529858.

These known compounds are not satisfactory from the viewpoints of the potency of their activity, undesirable side effects (e.g. prolonging bleeding time), absorbability, stability or durability of the action. Circumstances being such as above, for clinical application of these compounds, there are problems still to be solved.

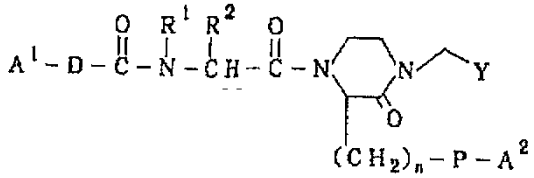
Recently, novel 2-piperazinone-1-acetic acid derivatives were synthesized, which were found to possess, based on the chemical structural characteristic feature, a potent platelet aggregation inhibiting activity and, at the same time, are safely administrable, i.e. slight in undesirable side effects such as prolongation of bleeding time. These compounds are expected to apply to a variety of circulatory diseases (e.g. thrombosis, transient cerebral ischemic attack, myocardial infarction, cerebral infarction, peripheral obstruction and arteriosclerotic obliteration), tumors, inflammatory diseases, or prevention of reobstruction and restenosis of coronary arteries after PTCA (percutaneous transluminal coronary angioplasty), prevention of reobstruction and restenosis after surgical operation for coronary artery bypass and secondary prophylaxis after re-opening of infarction. Especially, for patients of chronic diseases, administration of drugs for a long period is required. While preparations of sustained-release for a long period are desired, no report on sustained-release microcapsules of the above-mentioned novel compounds has been found.

Exploitation of a method of preparing sustained-release microcapsules which are high in entrapping ratio of a 2-piperazinone-1-acetic acid compound and less in initial release of the drug is expected.

Summary of the Invention

The present inventors have diligently studied for solving the above-mentioned problems to find that a microcapsule comprising an amorphous water-soluble 2-piperazinone-1-acetic acid compound and a polymer has a high entrapment of the said compound with a relatively less initial release thereof. Further diligent studies based on this finding have reached the accomplishment of the present invention.

Namely, the present invention is to provide a microcapsule comprising an amorphous water-soluble 2-piperazinone-1-acetic acid compound, which is a compound of the formula (I):



(I)

wherein A^1 and A^2 independently are a proton-accepting group or a group convertible into a proton-accepting group; D is a spacer having a 2- to 6-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain); R^1 is a hydrogen atom or a hydrocarbon group; R^2 is a hydrogen atom or a residual group formed by removing $-CH(NH_2)COOH$ from an α-amino acid, or R^1 and R^2 may be combined to form a 5- or 6-membered ring; P is a spacer having a 1- to 10-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position,

counted as 2- or 3-atomic chain); Y is an optionally esterified or amidated carboxyl group; and n denotes an integer of 0 to 8, or salt thereof, [hereinafter sometimes simply referred to as the compound (I)] and a polymer.

The present invention also provides a microcapsule which is prepared by dispersing, in an aqueous phase, a dispersion of an amorphous water-soluble 2-piperazinone-1-acetic acid compound which is a compound of the formula (I) or a salt thereof in a solution of a polymer in an organic solvent to prepare an s/o/w type emulsion and subjecting the emulsion to in-water drying.

The present invention is also to provide a method of preparing a microcapsule, which comprises dispersing, in an aqueous phase, a dispersion of an amorphous water-soluble 2-piperazinone-1-acetic acid compound which is a compound of the formula (I) or a salt thereof in a solution of a polymer in an organic solvent to prepare an s/o/w type emulsion and subjecting the emulsion to in-water drying.

Detailed Description of the Invention

The abbreviations of amino acids, peptides, protecting groups or the like used in this specification are based on those established by IUPAC-IUB Commission on Biochemical Nomenclature or those commonly used in the relevant fields. When optical isomers of amino acids are present, they are L-isomers unless otherwise specified.

The term "microcapsule" used in this specification includes microspheres, microcapsules, microparticles, nanospheres and nanocapsules.

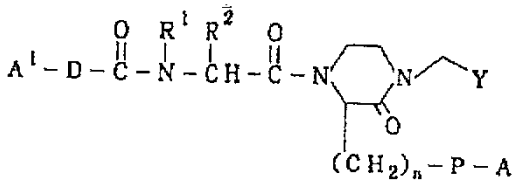
The term "s/o/w type emulsion" used in this specification means a solid/oil/water (solid phase in oil in water type). The "s" phase means a solid phase including microparticles and an aqueous phase in the

form of gel.

The present invention has made it possible to prepare a sustained-release microcapsule which contains a high content of the water-soluble compound (I) with a relatively small initial release thereof.

The amorphous compound (I) employed in the present invention is soluble in water, which means that the solubility of the compound (I) in water is not less than about 1 g/100 ml at 20°C. Preferably, the compound (I) is a one which is readily soluble in water. The term "readily soluble in water" means that the water-solubility of the compound (I) is, in general, not less than about 5 g/100 ml at 20°C.

As described above, the compound (I) of this invention is (1) a compound, whose characteristic feature in the chemical structure lies in having proton-accepting groups respectively at terminals of substituents at 3- and 4-positions on the piperazine ring, represented by the formula (I):



(I)

wherein A^1 and A^2 independently are a proton-accepting group or a group convertible into a proton-accepting group; D is a spacer having a 2- to 6-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain); R^1 is a hydrogen atom or a hydrocarbon group; R^2 is a hydrogen atom or a residual group formed by removing $-CH(NH_2)COOH$ from an α-amino acid, or R^1 and R^2 may be combined to form a 5- or 6-membered ring; P is a spacer having a 1- to 10-atomic chain optionally bonded through a hetero-atom and/or a

5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain); Y is an optionally esterified or amidated carboxyl group; and n denotes an integer of 0 to 8, or a salt thereof.

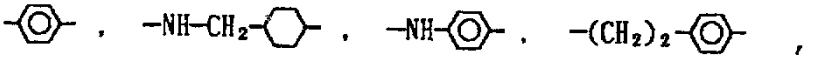
Especially, the following compounds are preferable, namely, (2) a compound as described in (1) above, wherein A^1 and A^2 independently are an optionally substituted amino, amidino or guanidino group or a group convertible to them,

(3) a compound as described in (1) above, wherein A^1 and A^2 independently are an optionally substituted oxadiazolyl or thiadiazolyl group,

(4) a compound as described in (1) above, wherein A^1 and A^2 independently are (1) an amidino or guanidino group which may be substituted with C_{2-6} alkoxycarbonyl, or (2) an amino group which may be substituted with an oxadiazolyl group which may be substituted with oxo or C_{1-4} alkyl which may be substituted with halogen,

(5) a compound as described in (1) above, wherein A^1 and A^2 independently are an unsubstituted amino, amidino or guanidino group,

(6) a compound as described in (1) above, wherein D is group of the formula:



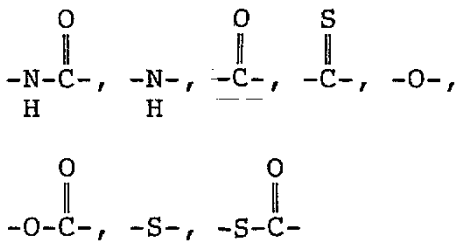
(7) a compound as described in (1) above, wherein R^1 is a hydrogen atom,

(8) a compound as described in (1) above, wherein R^2 is a hydrogen atom or a C_{1-4} alkyl group substituted with phenyl optionally substituted with C_{1-4} alkoxy,

(9) a compound as described in (1) above, wherein P is a group of the formula:

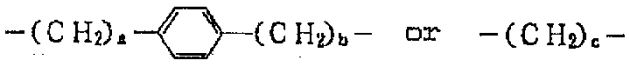
-Z-B-

in which Z is



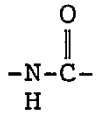
in which either bond may be bonded to B, or a bond; and B is

(i)



in which a is an integer of 0 to 2, b is an integer of 0 to 2 and c is an integer of 1 to 5, or (ii) a bond, excepting the case where Z and B both are a bond,

(10) a compound as described in (9) above, wherein Z is



in which either bond may be bonded to B,

(11) a compound as described in (9) above , wherein B is

or $-(CH_2)_d-$ in which d is an integer of 1 to 4,

(12) a compound as described in (1) above, wherein Y is a carboxyl group or a C_{1-6} alkoxy-carbonyl group,

(13) a compound as described in (1) above, wherein n is an integer of 1 to 4,

(14) a compound as described in (1) above, wherein n is 2 or 3,

(15) a compound as described in (1) above, wherein A^1

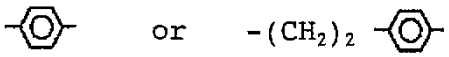
and A^2 independently are

1) an amidino or guanidino group optionally substituted with C_{2-8} alkoxycarbonyloxy,

2) an amino group optionally substituted with oxadiozolyl optionally substituted with oxo or C_{1-4} alkyl optionally substituted with halogen, or

3) an oxadiazolyl group optionally substituted with oxo or C_{1-4} alkyl optionally substituted with halogen,

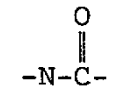
D is a group of the formula:

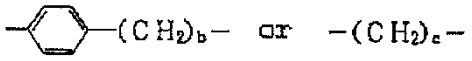


R^1 is a hydrogen atom,

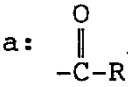
R^2 is a hydrogen atom or a C_{1-4} alkyl group substituted with phenyl optionally substituted with C_{1-4} alkoxy,

P is a group of the formula: -Z-B-

wherein Z is  a bond or  , and

B is 

in which b is 0 or 1, and c is an integer of 1 to 5,

Y is a group of the formula: 

wherein R^7 is 1) hydroxy group, 2) a C_{1-8} alkoxy or C_{2-12} alkenyloxy group which may be substituted with C_{1-4} alkoxy-carbonyl or 5-methyl-2-oxo-1,3-dioxolen-4-yl, or

3) a group of the formula: $-OCH(R^{7a})OCOR^8$ in which R^{7a} is a hydrogen atom or a C_{1-6} alkyl group, and R^8 is a C_{1-6} alkyl group or a C_{5-7} cycloalkyloxy group, and

n is an integer of 1 to 4,

(16) a compound as described in (1) above, wherein A^1 and A^2 are independently

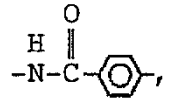
1) an amidino or guanidino group optionally substituted with methoxycarbonyl or

2) an amino group optionally substituted with 5-oxo-1,2,4-oxodiazol-3-yl or 5-trifluoromethyl-1,2,4-oxadiazol-3-yl,

D is  or

R^1 is a hydrogen atom,

R^2 is a hydrogen atom or p-methoxybenzyl,

P is  ,

Y is a carboxyl group and

n is 2 or 3, and

(17) a compound as described in (1) above, wherein A^1 and A^2 are independently an unsubstituted amino, amidino or guanidino group and R^2 is a hydrogen atom.

In the above formula (I), A^1 and A^2 independently are a proton-accepting group or a group convertible into a proton-accepting group.

In the above formula (I), the proton-accepting group means a group which accepts proton from a relevant group, namely a Brønsted base as exemplified by a group containing nitrogen atom capable of being positively charged. Specific examples of the proton-accepting group include optionally substituted amino, amidino and guanidino groups. Preferable examples of the proton-accepting group include unsubstituted amino, amidino and guanidino groups, or secondary or tertiary amino groups (especially ethylamino), amidino or guanidino groups substituted with a C_{1-4} alkyl group.

Examples of the substituents of optionally substituted amino, amidino and guanidino groups include chain-like or cyclic hydrocarbon groups such as C_{1-6} alkyl groups (e.g. methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl), C_{2-6}

alkenyl groups (e.g. vinyl, allyl, isopropenyl, butenyl, isobutenyl and sec-butenyl), C_{2-6} alkynyl groups (e.g. propargyl, ethynyl, butynyl and 1-hexynyl), C_{3-6} cycloalkyl groups (e.g. cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl), C_{6-14} aryl groups (e.g. phenyl, tolyl, xylyl, 1-naphthyl, 2-naphthyl, biphenyl, 2-indenyl and 2-anthryl, especially phenyl group), and C_{7-16} aralkyl groups (e.g. benzyl, phenethyl, diphenylmethyl, triphenylmethyl, 1-naphthylmethyl, 2-naphthylmethyl, 2-diphenylethyl, 3-phenylpropyl, 4-phenylbutyl and 5-phenylpentyl, especially benzyl group); C_{1-4} alkyl groups (e.g. methyl) substituted with carbamoyloxy optionally substituted with C_{1-4} alkyl (e.g. N,N-dimethylaminocarbonyloxy), C_{2-5} alkanoyloxy (e.g. pivaloyloxy) or a 5- or 6-membered heterocyclic group (e.g. a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, such as 2- or 3-thienyl, 2- or 3-furyl, 1-, 2- or 3-pyrrolyl, 1-, 2- or 3-pyrrolidinyl, 2-, 4- or 5-oxazolyl, 2-, 4- or 5-thiazolyl, 3-, 4- or 5-pyrazolyl, 2-, 4- or 5-imidazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl and 1H- or 2H-tetrazolyl, a 6-membered cyclic group, preferably pyrrolidin-1-yl and morpholino, containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, such as 2-, 3- or 4-pyridyl, N-oxido-2-, 3- or 4-pyridyl, 2-, 4- or 5-pyrimidinyl, N-oxido-2-, 4- or 5-pyrimidinyl, thiomorpholinyl, morpholinyl, piperidinyl, pyranyl, thiopyranyl, 1,4-oxazinyl, 1,4-thiadinyl, 1,3-thiadinyl, piperazinyl, triazinyl, 3- or 4-pyridazinyl, pyrazinyl, N-oxido-3- or 4-pyridazinyl); C_{2-8} alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, pentyloxycarbonyl, n-

hexyloxycarbonyl and n-octyloxycarbonyl); C_{1-8} alkylaminocarbonyl (e.g. n-hexylaminocarbonyl and n-octylaminocarbonyl); C_{2-8} alkoxycarbonyloxy (e.g. methoxycarbonyloxy, ethoxycarbonyloxy, propoxycarbonyloxy, butoxycarbonyloxy, pentyloxyoxycarbonyloxy, n-hexyloxycarbonyloxy and n-octyloxycarbonyloxy, preferably methoxycarbonyloxy); and 5- or 6-membered heterocyclic groups (e.g. a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, such as 2- or 3-thienyl, 2- or 3-furyl, 1-, 2- or 3-pyrrolyl, 1-, 2- or 3-pyrrolidinyl, 2-, 4- or 5-oxazolyl, 2-, 4- or 5-thiazolyl, 3-, 4- or 5-pyrazolyl, 2-, 4- or 5-imidazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl and 1H- or 2H-tetrazolyl, a 6-membered cyclic group, preferably e.g. tetrahydrofuran-2-yl, containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, such as 2-, 3- or 4-pyridyl, N-oxido-2-, 3- or 4-pyridyl, 2-, 4- or 5-pyrimidinyl, N-oxido-2-, 4- or 5-pyrimidinyl, thiomorpholinyl, morpholinyl, piperidinyl, pyranyl, thiopyranyl, 1,4-oxazinyl, 1,4-thiadinyl, 1,3-thiadinyl, piperazinyl, triazinyl, 3- or 4-pyridazinyl, pyrazinyl, N-oxido-3- or 4-pyridazinyl). And, in the case where two or more substituents of the amino, amidino or guanidino group exist, they may be combined to form a 5- or 6-membered heterocyclic group (e.g. pyrrolidine, piperidine, morpholine or imidazoline).

Preferable groups convertible into proton-accepting groups include groups which convert into proton-accepting groups in a living body and can accept physiologically active free proton. Examples of these groups include amidoxime groups optionally having substituents on oxygen atom (specific examples of the substituents include lower (C_{1-4}) alkyl (e.g. methyl,

ethyl, propyl), acyl (e.g. C_{2-5} alkanoyl (e.g. pivaloyl) and benzoyl), lower (C_{1-4}) alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), lower (C_{1-4}) alkylthiocarbonyl (e.g. methylthiocarbonyl, ethylthiocarbonyl), acyloxycarbonyl (e.g. C_{2-5} alkanoyloxycarbonyl (e.g. pivaloyloxycarbonyl) and benzoyloxycarbonyl), optionally substituted C_{6-12} aryloxycarbonyl (e.g. phenoxycarbonyl) or C_{7-14} aralkyloxycarbonyl (e.g. benzyloxycarbonyl) (specific examples of the substituents include cyano, nitro, amino, lower (C_{1-4}) alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), lower (C_{1-4}) alkyl (e.g. methyl, ethyl, propyl), lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy, propoxy), mono- and di- lower (C_{1-4}) alkylamino (e.g. methylamino, ethylamino, propylamino, dimethylamino), hydroxy, amido and lower (C_{1-4}) alkylthio (e.g. methylthio, ethylthio), optionally substituted C_{6-12} aryl-carbonyl groups (e.g. phenylcarbonyl) (specific examples of the substituents include lower (C_{1-4}) alkyl (e.g. methyl, ethyl, propyl), lower (C_{1-4}) alkenyl (e.g. vinyl, allyl) or lower (C_{1-4}) alkynyl (e.g. ethynyl), or optionally substituted carbamoyl groups (specific examples of the substituents include cyano, nitro, amino, lower (C_{1-4}) alkoxy carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), lower (C_{1-4}) alkyl (e.g. methyl, ethyl, propyl), lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy, propoxy), mono- and di- lower (C_{1-4}) alkylamino (e.g. methylamino, ethylamino, propylamino, dimethylamino), hydroxy, amido and lower (C_{1-4}) alkylthio (e.g. methylthio, ethylthio), and optionally substituted oxadiazolyl or thiadiazolyl groups (examples of the substituents include oxo, thioxo, hydroxy, amino, mono- and di- lower (C_{1-4}) alkylamino (e.g. methylamino, ethylamino, propylamino,

dimethylamino), halogen (e.g. fluoro, bromo, chloro), cyano, azido, lower (C_{1-4}) alkyl optionally substituted with halogen (e.g. trifluoromethyl), lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy, propoxy), lower (C_{1-4}) alkylthio (e.g. methylthio, ethylthio), lower (C_{1-4}) alkoxy carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), mono- or di- lower (C_{1-4}) alkylamino (e.g. methylamino, ethylamino, propylamino, dimethylamino), lower (C_{1-4}) alkylcarbamoyl (e.g. methylcarbamoyl, ethylcarbamoyl), C_{6-12} aryl (e.g. phenyl) groups optionally having a substituent (specific examples the substituents include cyano, nitro, amino, lower (C_{1-4}) alkoxy carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), lower (C_{1-4}) alkyl (e.g. methyl, ethyl, propyl), lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy, propoxy), mono- and di- lower (C_{1-4}) alkylamino (e.g. methylamino, ethylamino, propylamino, dimethylamino), hydroxy, amido and lower (C_{1-4}) alkylthio (e.g. methylthio, ethylthio), or C_{7-14} aralkyl groups (e.g. benzyl) optionally having a substituent (specific examples of the substituents include cyano, nitro, amino, lower (C_{1-4}) alkoxy carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), lower (C_{1-4}) alkyl (e.g. methyl, ethyl, propyl), lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy, propoxy), mono- and di- lower (C_{1-4}) alkylamino (e.g. methylamino, ethylamino, propylamino, dimethylamino), hydroxy, amido or lower (C_{1-4}) alkylthio (e.g. methylthio, ethylthio)), and among the optionally substituted oxadiazolyl or thiazolyl groups, 1,2,4-oxadiazol-3-yl or 1,2,4-thiadiazol-3-yl groups optionally having a substituent respectively are preferable. And, in the case where the substituent is oxo or thioxo, the groups may take either keto- or enol-form.

Among the optionally substituted C_{6-12} aryloxycarbonyl or C_{7-14} aralkyloxycarbonyl groups, optionally substituted carbamoyl groups, optionally substituted C_{6-12} aryl groups or optionally substituted C_{7-14} aralkyl groups as the above substituent of the amidoxime, oxadiazolyl and thiadiazolyl group, are preferable those respectively substituted with cyano, nitro, lower (C_{1-4}) alkoxy-carbonyl or lower (C_{1-4}) alkoxy.

Among the optionally substituted C_{6-12} aryl-carbonyl groups as the above substituent of the amidoxime group, are preferable those substituted with hydrogen atom or lower (C_{1-4}) alkyl.

More specific examples of the groups convertible into proton-accepting groups include 5-oxo-1,2,4-oxadiazol-3-yl group, 5-oxo-1,2,4-thiadiazol-3-yl group, 5-thioxo-1,2,4-oxadiazol-3-yl group, 5-thioxo-1,2,4-thiadiazol-3-yl group, 4-methyl-5-oxo-1,2,4-oxadiazol-3-yl group, 4-ethyl-5-oxo-1,2,4-oxadiazol-3-yl group, 4-propyl-5-oxo-1,2,4-oxadiazol-3-yl group, 1,2,4-oxadiazol-3-yl group, 5-ethoxycarbonyl-1,2,4-oxadiazol-3-yl group, 5-carbamoyl-1,2,4-oxadiazol-3-yl group, 5-cyano-1,2,4-oxadiazol-3-yl group, 5-trifluoromethyl-1,2,4-oxadiazol-3-yl group, 5-phenyl-1,2,4-oxadiazol-3-yl group, 5-amino-1,2,4-oxadiazol-3-yl group, 5-propylamino-1,2,4-oxadiazol-3-yl group, 5-methylthio-1,2,4-oxadiazol-3-yl group, 5-azido-1,2,4-oxadiazol-3-yl group, amino (hydroxy) imino group, amino (methoxycarbonyloxy) imino group, amino (ethoxycarbonyloxy) imino group, amino (n-propyloxycarbonyloxy) imino group, amino (benzyloxycarbonyloxy) imino group, amino (p-nitrobenzyloxycarbonyloxy) imino group, amino (p-nitrophenyloxycarbonyloxy) imino group, amino (p-nitrobenzoyloxycarbonyloxy) imino group, amino (methoxy) imino group, amino (carbamoyloxy) imino

group, amino (methylcarbamoyloxy) imino group, amino (ethylcarbamoyloxy) imino group, amino (n-propylcarbamoyloxy) imino group and amino (n-butylcarbamoyloxy) imino group.

Among them, are preferable 5-oxo-1,2,4-oxadiazol-3-yl group, 5-oxo-1,2,4-thiadiazol-3-yl group, 5-ethoxycarbonyl-1,2,4-oxadiazol-3-yl group, 5-cyano-1,2,4-oxadiazol-3-yl group, 5-trifluoromethyl-1,2,4-oxadiazol-3-yl group, amino (methoxycarbonyloxy) imino group, amino (carbonyloxy) imino group, amino (methylcarbamoyloxy) imino group and amino (ethylcarbamoyloxy) imino group.

Preferable example of A^1 and A^2 include (1) amidino and guanidino groups which may be substituted with C_{2-8} alkoxycarbonyloxy, and (2) amino groups which may be substituted with oxadiazolyl group which may be substituted with oxo or C_{1-4} alkyl which may be substituted with halogen, and are unsubstituted amino, amidino or guanidino groups are more preferable.

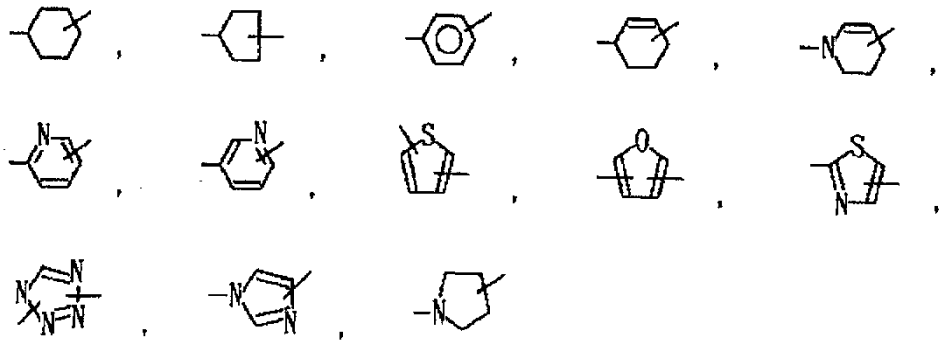
And, the compound (I), wherein A^1 or A^2 are a group convertible into a proton-accepting group, or a salt thereof can be advantageously used as an orally administrable preparation.

In the above formula (I), D is a spacer having a 2- to 6-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain).

The spacer of D means a linear interval between A^1 and $\overset{O}{\overset{\|}{C}}$, and means having a interval which is lined with 2 to 6 atoms between them in the present invention.

In the above formula (I), examples of hetero-atoms in the spacer having a 2- to 6-atomic chain (2- to 6-membered chain) optionally bonded through a hetero-atom

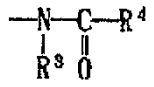
and/or a 5- or 6-membered ring include N, O and S. And, the 5- or 6-membered ring may be carbocyclic one or a heterocyclic one containing 1 to 4 hetero-atoms selected from N, O and S or a saturated ring or an unsaturated ring such as aromatic ring. Examples of such 5- or 6-membered ring include the following;



And, the above-mentioned 5- or 6-membered ring is preferably such one as having no bond at the adjacent position on the ring. The above-mentioned 5- or 6-membered ring is preferably such one as having a bond at the second or third position to one another on the ring. Usually, even the ring is saturated or unsaturated, it is regarded as 2- to 3-atomic chain (2- to 3-membered chain), and a group having a 2- to 6-atomic chain as D itself is preferable. As the hetero-atom existing in the spacer shown by D, nitrogen is preferable above all, and, D bonded to a group shown by A^1, such as amidino group existing through -NH- group, is especially preferable. And, the above-mentioned 5- or 6-membered ring may be bonded to the adjacent amidino group directly or to a group shown by A^1 such as amidino group through -NH- group, and further to a group shown by A^1 such as amidino group through methylene chain.

And, D may be such one as the adjacent carbonyl group is bonded directly to the above-mentioned 5- or 6-membered ring, or bonded through methylene chain or

bonded through a hetero atom. The methylene chain in D may be substituted with a group of the formula



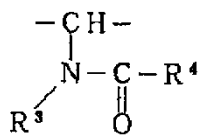
wherein R^3 is a hydrogen atom or a lower (C_{1-4}) alkyl group optionally substituted with an optionally substituted phenyl group; and R^4 is a lower (C_{1-4}) alkyl group optionally substituted with an optionally substituted phenyl group, an optionally substituted phenyl group or benzyloxy group.

Examples of substituents of the optionally substituted phenyl group as the substituent to the lower (C_{1-4}) alkyl group of R^3 or R^4 include lower (C_{1-4}) alkyl (e.g. methyl, ethyl), lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy), halogen (e.g. fluoro, chloro, bromo), and hydroxyl group.

Example of the lower (C_{1-4}) alkyl group of R^3 or R^4 include methyl and ethyl.

Preferable typical groups shown by D include those of the formula

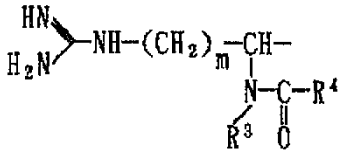
wherein h and i each is 0 or 1; m and k each is 0, 1 or 2; and E is the above-mentioned 5- or 6-membered ring, especially cyclohexane ring, benzene ring, piperidine or a group of the formula



As E, 5- or 6-membered ring is especially preferable. And, as h, 0 or 1, as m, 0, 1 or 2, and as

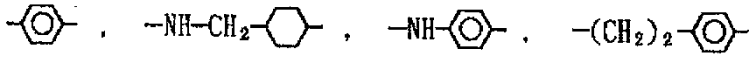
k, 0 are respectively preferable. Among 5- or 6-membered rings shown by E, benzene ring and cyclohexane ring are preferable, and benzene ring is especially preferable.

In the above-mentioned formula (I), groups of the formula



wherein R^3, R^4 and m are of the same meaning as defined above, are substituted groups derived from arginine or homoarginine.

As D, groups of the formula



(among others, above all  and  , especially)

are especially preferable.
(in these groups, either of the bonds may be bonded to A^1)

In the above formula (I), R^1 is a hydrogen atom or a hydrocarbon group.

As the hydrocarbon shown by R^1, mention is made of chain-like or cyclic hydrocarbon groups including C_{1-6} alkyl groups (e.g. methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl), C_{2-6} alkenyl groups (e.g. vinyl, allyl, isopropenyl, butenyl, isobutenyl and sec-butenyl), C_{2-6} alkynyl groups (e.g.propargyl, ethynyl, butynyl and 1-hexynyl), C_{3-6} cycloalkyl groups (e.g. cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl), C_{6-14} aryl groups (e.g.

phenyl, tolyl, xylyl, 1-naphthyl, 2-naphthyl, biphenyl, 2-indenyl and 2-anthryl, especially phenyl group), and C_{7-16} aralkyl groups (e.g. benzyl, phenethyl, diphenylmethyl, triphenylmethyl, 1-naphthylmethyl, 2-naphthylmethyl, 2-diphenylethyl, 3-phenylpropyl, 4-phenylbutyl and 5-phenylpentyl, especially benzyl group), and as R^1, are preferable hydrogen, lower (C_{1-4}) alkyl or benzyl (especially hydrogen).

In the above formula (I), R^2 is a hydrogen atom or a residual group formed by removing $-CH(NH_2)COOH$ from an α-amino acid.

As the group shown by R^2, any of the residual groups formed by removing $-CH(NH_2)COOH$ from an α-amino acid can be mentioned. And, R^1 and R^2 may be combined to form a 5- or 6-membered ring. Preferable examples of such 5- or 6-membered ring include rings as shown below,

Usually, preferable examples of R^2 include residual groups of essential amino acids. Especially preferable examples of R^2 include a hydrogen atom, lower (C_{1-4}) alkyl groups, lower (C_{1-4}) alkyl groups substituted with an optionally substituted phenyl group, lower (C_{1-4}) alkyl groups substituted with hydroxyl group and lower (C_{1-4}) alkyl groups substituted with carbamoyl group. More specifically, hydrogen, methyl, isopropyl, sec-butyl, isobutyl, hydroxylmethyl, benzyl, p-hydroxybenzyl, p-methoxybenzyl, carbamoylmethyl and carbamoylethyl are mentioned as typical examples.

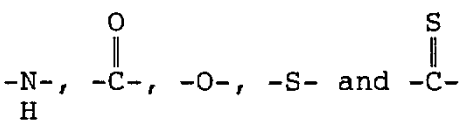
As substituents optionally substituted on the

benzene ring of optionally substituted phenyl group as the substitutent of the lower (C_{1-4}) alkyl of the above R^2, mention is made of, for example, lower (C_{1-4}) alkyl groups (e.g. methyl, ethyl, propyl, isopropyl, n-butyl and sec-butyl), lower (C_{1-4}) alkoxy groups (e.g. methoxy and ethoxy), halogen (e.g. chlorine, fluorine and bromine) and hydroxyl group, and the lower (C_{1-4}) alkoxy group is preferable.

As the group or atom shown by R^2, hydrogen atom or C_{1-4} alkyl group substituted with phenyl group optionally substituted with C_{1-4} alkoxy are preferable, p-hydroxybenzyl, p-methoxybenzyl or hydrogen atom (more preferably p-methoxybenzyl or hydrogen atoms especially hydrogen atom) are more preferable.

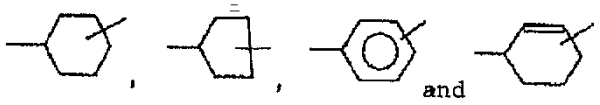
In the above-mentioned formula (I), n is an integer of 0 to 8 (preferably 1 to 4 especially 2 or 3).

In the above formula (I), P is a spacer having a 1- to 10-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain). The spacer of P means a linear interval between $(CH_2)_n$ and A^2, and means having a interval which is lined with 1 to 10 atoms between them in the present invention. As the spacer having 1- to 10-atomic chains (1- to 10-membered chain) optionally bonded through hetero-atoms and/or a 5- or 6-membered ring, mention is made of a divalent hydrocarbon group optionally bonded through 1 to 4 (preferable 1 or 2) groups selected from



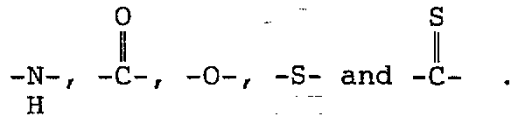
and/or a 5- or 6-membered ring (the 5- or 6-membered

ring may be a carbocyclic one or a heterocyclic one containing 1 to 4 hetero-atoms selected from N, O and S, which may be saturated ring or unsaturated one such as aromatic ring; as the carbocyclic one, for example,



are mentioned, and benzene ring and cyclohexane ring are preferable, and especially benzene ring is preferable; as the heterocyclic ring, a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from, for example, oxygen atom, sulfur atom and nitrogen atom, as exemplified by 2- or 3-thienyl, 2- or 3-furyl, 1-, 2- or 3-pyrrolyl, 1-, 2- or 3-pyrrolidinyl, 2-, 4- or 5-oxazolyl, 2-, 4- or 5-thiazolyl, 3-, 4- or 5-pyrazolyl, 2-, 4- or 5-imidazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, and 1H- or 2H-tetrazolyl, and, a 6-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, as exemplified by 2-, 3- or 4-pyridyl, N-oxido-2-, 3- or 4-pyridyl, 2-, 4- or 5-pyrimidinyl, N-oxido-2-, 4- or 5-pyrimidinyl, thiomorpholinyl, morpholinyl, piperidinyl, pyranyl, thiopyranyl, 1,4-oxazinyl, 1,4-thiazinyl, 1,3-thiazinyl, piperazinyl, triazinyl, 2- or 4-pyridazinyl, pyrazinyl, and N-oxido-3- or 4-pyridazinyl, and piperazine or piperidine is preferable).

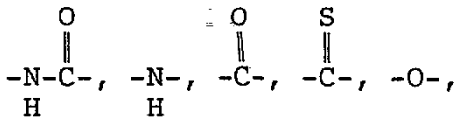
As more preferable spacer having 1- to 10-atomic chains optionally bonded through hetero-atoms and/or a 5- or 6-membered ring, mention is made of a divalent hydrocarbon group optionally bonded through 1 to 4 (preferably 1 or 2) groups selected from

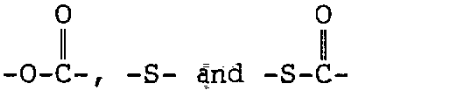


And, in the above-mentioned formula (I), P is groups represented by, for example, the formula,

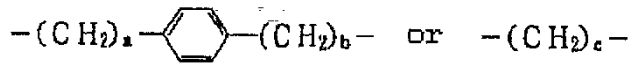
$$-Z-B-$$

wherein Z is a one selected from



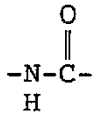


(either bond may be bonded to B) or a bond, and B is a group



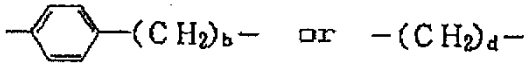
(a and b are an integer of 0 to 2 (preferably 0 or 1), and c is an integer of 1 to 5) or a bond (excepting the case where Z and B are both bonds).

Among the groups shown by the above Z, those represented by



(either of the bonds may be bonded to B) are preferable.

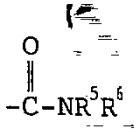
Among the groups shown by the above B, those represented by



wherein b is an integer of 0 to 2 (preferably 0 or 1), and d is an integer of 1 to 4, are preferable. Further preferable groups shown by the above B include

or $-(CH_2)_d-$ wherein d is an integer or 1 to 4.

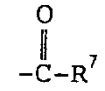
Preferable examples of the optionally amidated carboxyl group shown by Y include groups represented by the formula



wherein R^5 and R^6 independently are hydrogen, a lower (C_{1-6}) alkyl group (e.g. methyl, ethyl, propyl, butyl and hexyl), a C_{2-8} alkenyl group (e.g. allyl, 2-butenyl and 3-pentenyl), a lower (C_{1-4}) alkyl group (e.g. pyridylmethyl) substituted with a 5- or 6-membered heterocyclic group (e.g. a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from, for example, oxygen atom, sulfur atom and nitrogen atom, as exemplified by 2- or 3-thienyl, 2- or 3-furyl, 1-, 2- or 3-pyrrolyl, 1-, 2- or 3-pyrrolidinyl, 2-, 4- or 5-oxazolyl, 2-, 4- or 5-thiazolyl, 3-, 4- or 5-pyrazolyl, 2-, 4- or 5-imidazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1H- or 2H-tetrazolyl, and, a 6-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, as exemplified by 2-, 3- or 4-pyridyl, N-oxido-2-, 3- or 4-pyridyl, 2-, 4- or 5-pyrimidinyl, N-oxido-3-, 4- or 5-pyrimidinyl, thiomorpholinyl, morpholinyl, piperidinyl, pyranyl, thiopyranyl, 1,4-oxazinyl, 1,4-thiazinyl, 1,3-thiazinyl, piperazinyl, triazinyl, 2- or 4-pyridazinyl, pyrazinyl, and N-oxido-

3- or 4-pyridazinyl, preferably pyridyl) or a C_{6-12} aralkyl group (e.g. benzyl, phenethyl and phenyl propyl), and, the aryl groups in the aralkyl group may be unsubstituted or optionally substituted with one or two substituents as exemplified by nitro, halogen (chlorine, fluorine and bromine), lower (C_{1-4}) alkyl groups (e.g. methyl and ethyl) and lower (C_{1-4}) alkoxy groups (e.g. methoxy, ethoxy and propoxy).

Preferable examples of optionally esterified carboxyl groups shown by Y include groups of the formula



wherein R^7 is 1) hydroxyl group, 2) an optionally substituted alkoxy, alkenyloxy or benzyloxy group (e.g. lower (C_{1-8}) alkoxy (e.g. methoxy, ethoxy, propoxy), lower (C_{2-12}) alkenyloxy (e.g. vinyloxy, allyloxy) or benzyloxy group which may be substituted with hydroxyl group, optionally substituted amino (e.g. amino, N-lower (C_{1-4}) alkylamino (e.g. methylamino), N,N-di-lower (C_{1-4}) alkylamino (e.g. dimethylamino), piperidino and morpholino), halogen (e.g. chloro, fluoro, bromo), lower (C_{1-6}) alkoxy (e.g. methoxy, ethoxy), lower (C_{1-6}) alkylthio (e.g. methylthio, ethylthio), lower (C_{1-4}) alkoxy-carbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isobutyloxycarbonyl), or optionally substituted dioxolenyl (e.g. 5-methyl-2-oxo-1,3-dioxolen-4-yl)) or 3) a group of the formula $-OCH(R^{7a})OCOR^8$ in which R^{7a} is hydrogen, a straight-chain or branched lower (C_{1-6}) alkyl group (e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, isopentyl and neopentyl), or a C_{5-7} cycloalkyl group (e.g. cyclopentyl, cyclohexyl

and cycloheptyl), and R^8 is i) a straight-chain or branched lower (C_{1-6}) alkyl group (e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, isopentyl and neopentyl), ii) a lower (C_{2-8}) alkenyl group (e.g. vinyl, propenyl, allyl and isopropenyl), iii) a C_{5-7} cycloalkyl group (e.g. cyclopentyl, cyclohexyl and cyclobutyl), iv) a lower (C_{1-3}) alkyl group substituted with C_{5-7} cycloalkyl (e.g. cyclopentyl, cyclohexyl and cycloheptyl) or optionally substituted C_{6-12} aryl such as phenyl (e.g. benzyl, p-chlorobenzyl, phenethyl, cyclopentylmethyl and cyclohexylmethyl), v) a lower (C_{2-3}) alkenyl group substituted with C_{5-7} cycloalkyl (e.g. cyclopentyl, cyclohexyl and cycloheptyl) or optionally substituted C_{6-12} aryl such as phenyl (e.g. cinnamyl having alkenyl moiety such as vinyl, propenyl, allyl or isopropenyl), vi) an optionally substituted aryl groups such as optionally substituted phenyl group (e.g. phenyl, p-tolyl and naphthyl), vii) a straight-chain or branched lower (C_{1-6}) alkoxy group (e.g. methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, t-butoxy, n-pentyloxy, isopentyloxy and neopentyloxy), viii) a straight-chain or branched lower (C_{1-6}) alkenyloxy group (e.g. allyloxy and isobutenyloxy), ix) a C_{5-7} cycloalkyloxy group (e.g. cyclopentyloxy, cyclohexyloxy and cycloheptyloxy), x) a lower (C_{1-3}) alkoxy group substituted with C_{5-7} cycloalkyl groups (e.g. cyclopentyl, cyclohexyl and cycloheptyl) or optionally substituted C_{6-12} aryl such as phenyl (e.g. benzyloxy, phenethyloxy, cyclopentylmethyloxy and cyclohexylmethyloxy, having alkoxy moiety such as methoxy, ethoxy, n-propoxy or isopropoxy), xi) a lower (C_{2-3}) alkenyloxy group substituted with C_{5-7} cycloalkyl groups (e.g. cyclopentyl, cyclohexyl and cycloheptyl) or optionally substituted C_{6-12} aryl such as phenyl

(e.g. cinnamyloxy having alkenyloxy moiety such as vinyloxy, propenyloxy, allyloxy or isopropenyloxy), xii) an optionally substituted C_{6-12} aryloxy group such as an optionally substituted phenoxy group (e.g. phenoxy, p-nitrophenoxy and naphthoxy).

In the above formula, when the substituent R^8 includes an optionally substituted C_{6-12} aryl group, the C_{6-12} aryl group is exemplified by phenyl and naphthyl (preferably phenyl), and, as the substituents of the C_{6-12} aryl group, mention is made of, for example, nitro, halogen (e.g. chlorine, fluorine and bromine), lower (C_{1-4}) alkyl (e.g. methyl, ethyl, propyl) and lower (C_{1-4}) alkoxy (e.g. methoxy, ethoxy, propoxy), and, among them, unsubstituted phenyl is preferably used.

Preferable examples of Y are a carboxyl group and a lower (C_{1-4}) alkoxy-carbonyl group (e.g. carboxyl, ethoxycarbonyl), and a carboxyl group is more preferable.

The compounds of the formula (I) include the compound wherein A^1 and A^2 are

(1) an amino, amidino or guanidino group which may be substituted with C_{1-6} alkyl; C_{2-6} alkenyl; C_{2-6} alkynyl; C_{3-6} cycloalkyl; C_{6-14} aryl; C_{7-16} aralkyl; C_{1-4} alkyl substituted with carbamoyloxy optionally substituted with C_{1-4} alkyl, C_{2-5} alkanoyloxy or a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, or a 6-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom; C_{2-8} alkoxycarbonyl; C_{1-8} alkylaminocarbonyl; C_{2-8} alkoxycarbonyloxy; a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen

atom, or a 6-membered cyclic group, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, in the case where two or more substituents of the amino, amidino or guanidino group exist, they may be combined to form a 5- or 6-membered heterocyclic group,

(2) an amidoxime group which may be substituted on the oxygen atom with C_{1-4} alkyl; C_{2-5} alkanoyl; benzoyl; C_{1-4} alkoxycarbonyl; C_{1-4} alkylthiocarbonyl; C_{2-5} alkanoyloxycarbonyl; benzoyloxycarbonyl; C_{6-12} aryloxycarbonyl or C_{7-14} aralkyloxycarbonyl which may be substituted with cyano, nitro, amino, C_{1-4} alkoxycarbonyl, C_{1-4} alkyl, C_{1-4} alkoxy, mono- or di- C_{1-4} alkylamino, hydroxy, amido or C_{1-4} alkylthio; C_{6-12} aryl-carbonyl which may be substituted with C_{1-4} alkyl, C_{2-4} alkenyl or C_{2-4} alkynyl; carbamoyl which may be substituted with cyano, nitro, amino, C_{1-4} alkoxy-carbonyl, C_{1-4} alkyl, C_{1-4} alkoxy, mono- or di- C_{1-4} alkylamino, hydroxy, amido or C_{1-4} alkylthio or

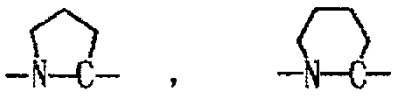
(3) an oxadiazolyl or thiadiazolyl group which may be substituted with oxo; thioxo; hydroxy; amino; mono- or di- C_{1-4} alkylamino; halogen; cyano; azido; C_{1-4} alkyl optionally substituted with halogen; C_{1-4} alkoxy; C_{1-4} alkylthio; C_{1-4} alkoxy-carbonyl; C_{1-4} alkylcarbamoyl; C_{6-12} aryl optionally substituted with cyano, nitro, amino, C_{1-4} alkoxy-carbonyl, C_{1-4} alkyl, C_{1-4} alkoxy, hydroxy, amido or C_{1-4} alkylthio; or C_{7-14} aralkyl optionally substituted with cyano, nitro, amino, C_{1-4} alkoxy-carbonyl, C_{1-4} alkyl, C_{1-4} alkoxy, mono- or di- C_{1-4} alkylamino, hydroxy, amido or C_{1-4} alkylthio,

D is a 2- to 6- membered chain optionally bonded through a hetero-atom and/or a 5- or 6- membered carbocyclic ring or the 5- or 6- membered heterocyclic ring containing 1 to 4 hetero-atoms selected from N, O and S, provided that the 5- or 6-membered carbocyclic

ring or the 5- or 6-membered heterocyclic ring containing 1 to 4 hetero-atoms selected from N, O and S is, depending on its bonding position, counted as 2- or 3- membered chain,

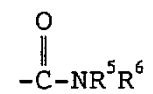
R^1 is a hydrogen atom, a C_{1-6} alkyl group, a C_{2-6} alkenyl group, a C_{2-6} alkynyl group, a C_{3-6} cycloalkyl group, a C_{6-14} aryl group or a C_{7-16} aralkyl group,

R^2 is a hydrogen atom; a C_{1-4} alkyl group; a C_{1-4} alkyl group substituted with phenyl which may be substituted with C_{1-4} alkyl, C_{1-4} alkoxy, halogen or hydroxy; a C_{1-4} alkyl group substituted with hydroxy; or a C_{1-4} alkyl group substituted with carbamoyl, or R^1 and R^2 may be combined to form:



P is a 1- to 10-membered chain optionally bonded through a hetero atom and/or a 5- or 6-membered carbocylic ring or a 5- or 6-membered heterocyclic ring containing 1 to 4 hetero-atoms selected from N, O and S, provided that the 5- or 6-membered carbocylic ring or the 5- or 6-membered heterocyclic ring containing 1 to 4 hetero-atoms selected from N, O and S is, depending on its bonding position, counted as 2- or 3-membered chain,

Y is a group of the formula:

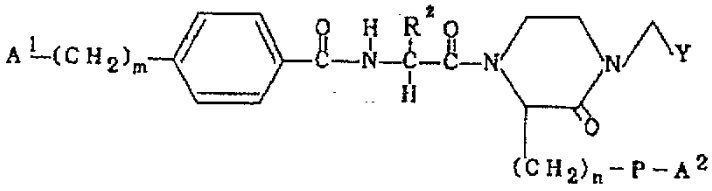


wherein R^5 and R^6 independently are hydrogen, a C_{1-6} alkyl group; a C_{2-8} alkenyl group; a C_{1-4} alkyl group substituted with a 5-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected

from, oxygen atom, sulfur atom and nitrogen atom, or, a 6-membered cyclic group containing, besides carbon atoms, 1 to 4 hetero-atoms selected from oxygen atom, sulfur atom and nitrogen atom, or a C_{6-12} aralkyl group which may be substituted with nitro, halogen, C_{1-4} alkyl or C_{1-4} alkoxy, or, a group of the formula:

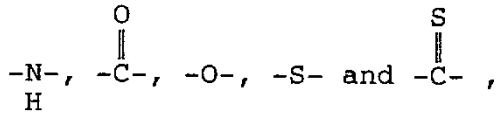
wherein R^7 is 1) hydroxyl group, 2) a C_{1-8} alkoxy, C_{2-12} alkenyloxy or benzyloxy group which may substituted with hydroxyl, amino, N-C_{1-4} alkylamino, N,N-di-C_{1-4} alkylamino, piperidino, morpholino, halogen, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-4} alkoxy-carbonyl, or 5-methyl-2-oxo-1,3-dioxolen-4-yl or 3) a group of the formula: $-OCH(R^{7a})OCOR^8$ in which R^{7a} is hydrogen, a C_{1-6} alkyl group or a C_{5-7} cycloalkyl group, and R^8 is i) a C_{1-6} alkyl group, ii) a C_{2-8} alkenyl group, iii) a C_{5-7} cycloalkyl, iv) C_{1-3} alkyl group substituted with C_{5-7} cycloalkyl or C_{6-12} aryl optionally substituted with nitro, halogen, C_{1-4} alkyl or C_{1-4} alkoxy, v) a C_{2-3} alkenyl group substituted with C_{5-7} cycloalkyl or C_{6-12} aryl, vi) a C_{6-12} aryl optionally substituted with nitro, halogen, C_{1-4} alkyl or C_{1-4} alkoxy, vii) a C_{1-6} alkoxy group, viii) a C_{2-6} alkenyloxy group, ix) a C_{5-7} cycloalkyloxy group, x) a C_{1-3} alkoxy group substituted with C_{5-7} cycloalkyl or C_{6-12} aryl optionally substituted with nitro, halogen, C_{1-4} alkyl or C_{1-4} alkoxy, xi) a C_{2-3} alkenyloxy group substituted with C_{5-7} cycloalkyl or C_{6-12} aryl optionally substituted with nitro, halogen, C_{1-4} alkyl or C_{1-4} alkoxy , or xii) a C_{6-12} aryloxy group optionally substituted with nitro, halogen, C_{1-4} alkyl or C_{1-4} alkoxy, and n is an integer of 0 to 8.

Among the compounds represented by the above-mentioned formula (I) or their salts, the compounds (Ia) of the formula



(Ia)

wherein A^1 and A^2 independently are an optionally substituted amino, amidino or guanidino group, an amidoxime group optionally having a substituent on the oxygen atom, or an optionally substituted oxadiazolyl or thiadiazolyl group, R^2 is hydrogen, a lower (C_{1-4}) alkyl group, a lower (C_{1-4}) alkyl group substituted with an optionally substituted phenyl group, a lower (C_{1-4}) alkyl group substituted with hydroxyl group or a lower (C_{1-4}) alkyl group substituted with carbamoyl group, P is a divalent hydrocarbon optionally bonded through 1 to 4 groups selected from

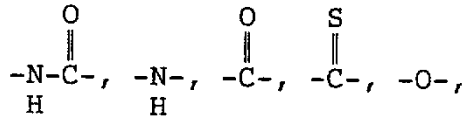


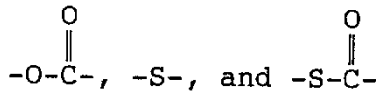
Y is an optionally esterified or amidated carboxyl group, m is an integer of 0 to 2, and n is an integer of 0 to 8, and their salts are preferable.

More preferable examples of the above-mentioned compounds (Ia) and their salts include compounds (Ia) wherein

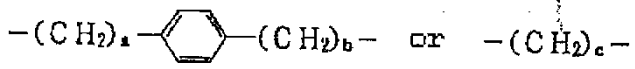
A^1 and A^2 independently are an unsubstituted amino, amidino or guanidino group, or an optionally substituted 1,2,4-oxadiazol-3-yl or 1,2,4-thiadiazol-3-

yl group,
R^2 is p-hydroxybenzyl, p-methoxybenzyl or hydrogen atom,
P is a group of the formula, -Z-B-
in which Z is a group selected from



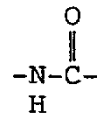


(either of the bonds of them may bonded to B) or a bond, and
B is

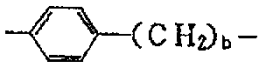


(a and b each is an integer of 0 to 2 (preferably 0 or 1), and c is an integer of 1 to 5) or a bond (excepting the case where Z and B both are a bond)],
Y is an optionally esterified or amidated carboxyl group,
m is an integer of 0 to 2, and
n is an integer of 1 to 4,
and their salts.

Furthermore preferable examples of the above-mentioned compounds (Ia) and their salts include compounds (Ia) wherein
A^1 and A^2 independently are unsubstituted amino, amidino or guanidino group, or an optionally substituted 1,2,4-oxadiazol-3-yl or 1,2,4-thiadiazol-3-yl group,
R^2 is p-hydroxybenzyl, p-methoxybenzyl or hydrogen atom, P is a group of the formula -Z-B-
in which Z is



B is a group of the formula



(b is an integer of 0 to 2 (preferably 0 or 1))],
Y is an optionally esterified or amidated carboxyl group,
m is an integer of 0 to 2, and
n is an integer of 1 to 4,
or their salts.

Preferable examples of the compound (I) and their salts include compounds (I) wherein A^1 and A^2 independently are

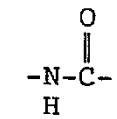
(1) an amidino or guanidino group optionally substituted with C_{2-8} alkoxycarbonyloxy,

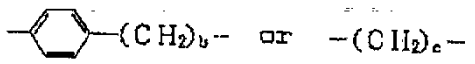
(2) an amino group optionally substituted with oxadiazolyl optionally substituted with oxo or C_{1-4} alkyl optionally substituted with halogen, or

(3) an oxadiazolyl group optionally substituted with oxo or C_{1-4} alkyl optionally substituted with halogen,

D is a group of the formula:

R^1 is a hydrogen atom,
R^2 is a hydrogen atom or a C_{1-4} alkyl group substituted with phenyl optionally substituted with C_{1-4} alkoxy,
P is a group of the formula: -Z-B-

wherein Z is  a bond or  , and

B is 

in which b is 0 or 1, and c is an integer of 1 to 5,

Y is a group of the formula: 

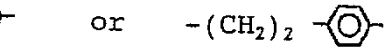
wherein R^7 is 1) hydroxy group, 2) a C_{1-8} alkoxy or C_{2-12} alkenyloxy group which may be substituted with C_{1-4} alkoxy-carbonyl or 5-methyl-2-oxo-1,3-dioxolen-4-yl, or 3) a group of the formula: $-OCH(R^{7a})OCOR^8$ in which R^{7a} is a hydrogen atom or a C_{1-6} alkyl group, and R^8 is a C_{1-6} alkyl group or a C_{5-7} cycloalkyloxy group, and

n is an integer of 1 to 4.

More preferable examples of the compound (I) and their salts include compounds (I) wherein A^1 and A^2 are independently

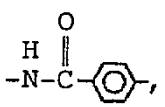
(1) an amidino or guanidino group optionally substituted with methoxycarbonyloxy or

(2) an amino group optionally substituted with 5-oxo-1,2,4-oxodiazol-3-yl or 5-trifluoromethyl-1,2,4-oxadiazol-3-yl,

D is  or 

R^1 is a hydrogen atom,

R^2 is a hydrogen atom or p-methoxybenzyl,

P is  ,

Y is a carboxyl group and

n is 2 or 3.

In the case where the compound of this invention is used as an orally administrable agent, desirable examples of optionally esterified carboxyl groups shown

by Y include methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl, propoxycarbonyl, pivaloyloxymethoxycarbonyl, 1-(cyclohexylcarbonyloxy)ethoxycarbonyl, 5-methyl-2-oxo-1,3-dioxolen-4-ylmethoxycarbonyl, acetoxymethyloxycarbonyl, propionyloxymethoxycarbonyl, n-butyloxymethoxycarbonyl, isobutyloxymethoxycarbonyl, 1-(ethoxycarbonyloxy)ethoxycarbonyl, 1-(acetyloxy)ethoxycarbonyl, 1-(isobutyloxy)ethoxycarbonyl, 2-(isobutyloxycarbonyl)-2-propylidenethoxycarbonyl and (3-phthalidylidene)ethoxycarbonyl.

The compounds of this invention have one or more asymmetric carbons in the molecule, and both R-configurated ones and S-configurated ones relative to these asymmetric carbons are included in the present invention.

Examples of the salts of the compounds (I) and (Ia) to be used in this invention include pharmaceutically acceptable salt such as inorganic acid salts such as hydrochloride, hydrobromide, sulfate, nitrate and phosphate, organic acid salts such as acetate, tartrate, citrate, fumarate, maleate, toluenesulfonate and methanesulfonate, metal salts such as sodium salt, potassium salt, calcium salt and aluminum salt, and salts with a base such as triethylamine salt, guanidine salt, ammonium salt, hydrazine salt, quinine salt and cinchonine salt.

The compounds (I) and (Ia) and their salts may be hydrates or not hydrates.

Specific examples of preferable compounds include 4-(4-amidinobenzoyl)aminoacetyl-3-[3-(4-amidinobenzoyl)aminopropyl]-2-oxopyperazine-1-acetic acid, 4-(4-amidinobenzoyl)aminoacetyl-3-[4-(4-amidinobenzoyl)aminobutyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoyl)aminoacetyl-3-[2-(4-

amidinobenzoyl)aminoethyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoyl)aminoacetyl-3-[2-(4-amidinophenylaminocarbonyl)ethyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoyl)aminoacetyl-3-[3-(4-amidinophenylaminocarbonyl)propyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoyl)aminoacetyl-3-[4-(4-amidinophenylaminocarbonyl)butyl]-2-oxopiperazine-1-acetic acid, 4-(4-guanidinobenzoyl)aminoacetyl-3-[2-(4-guanidinobenzoylamino)ethyl]-2-oxopiperazine-1-acetic acid, 4-(4-guanidinobenzoyl)aminoacetyl-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid, 4-(4-guanidinobenzoyl)aminoacetyl-3-[4-(4-guanidinobenzoylamino)butyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoylamino)acetyl-3-[2-(4-guanidinobenzoylamino)ethyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoylamino)acetyl-3-[4-(4-guanidinobenzoylamino)butyl]-2-oxopiperazine-1-acetic acid, 4-[4-(2-aminoethyl)benzoylamino]acetyl-3-[2-(4-amidinobenzoylamino)ethyl]-2-oxopiperazine-1-acetic acid, 4-[4-(2-aminoethyl)benzoylamino]-3-[3-(4-amidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid, and 4-[4-(2-aminoethyl)benzoylamino]acetyl-3-[4-(4-amidinobenzoylamino)butyl]-2-oxopiperazine-1-acetic acid, 4-(4-amidinobenzoylamino)acetyl-3-[3-(4-guanidinobutanoylamino)]propyl-2-oxopiperazine-1-acetic acid,

(S,S)-[3-[3-(4-guanidinobenzoylamino)propyl]-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl-[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxopiperazin-1-yl]acetic acid,

(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-

amino]propyl]piperazin-1-yl]acetic acid,
(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazin-1-yl]acetic acid or
(S,S)-4-[2-(4-guanidinobenzoyl)amino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoyl)aminopropyl]-2-oxopiperazine-1-acetic acid, or a salt thereof, more preferably,
(S)-4-(4-amidinobenzoyl)aminoacetyl-3-{3-(4-amidinobenzoyl)amino}propyl-2-oxopiperazine-1-acetic acid[trifluoroacetate of this compound may be hereinafter referred to as Compound B],
(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid[hydrochloride of this compound may be hereinafter referred to as Compound A],
(S)-4-(4-amidinobenzoylamino)acetyl-3-[2-(4-guanidinobenzoylamino)]ethyl-2-oxopiperazine-1-acetic acid[hydrochloride of this compound may be hereinafter referred to as Compound D],
(S)-4-[4-(2-aminoethyl)benzoylamino]acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid[trifluoroacetate of this compound may be hereinafter referred to as Compound C],
(S)-4-(4-amidinobenzoylamino)acetyl-3-[3-(4-guanidinobutanoylamino)]propyl-2-oxopiperazine-1-acetic acid [hydrochloride of this compound may be hereinafter referred to as Compound E],
(S,S)-[3-[3-(4-guanidinobenzoylamino)propyl]-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl-[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxopiperazin-1-yl]acetic acid,
(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-

ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazin-1-yl]acetic acid, (S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazin-1-yl]acetic acid or (S,S)-4-[2-(4-guanidinobenzoyl)amino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoyl)aminopropyl]-2-oxopiperazine-1-acetic acid, or a salt thereof, further more preferably, (S)-4-(4-amidinobenzoyl)aminoacetyl-3-{3-(4-amidinobenzoyl)amino}propyl-2-oxopiperazine-1-acetic acid, (S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid, (S)-4-(4-amidinobenzoylamino)acetyl-3-[2-(4-guanidinobenzoylamino)]ethyl-2-oxopiperazine-1-acetic acid or (S)-4-[4-(2-aminoethyl)benzoylamino]acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid trifluoroacetate, or a salt thereof.

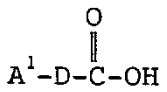
The most preferable example is (S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid or a salt thereof (a pharmaceutically acceptable salt thereof), more preferably

(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid or a pharmaceutically acceptable acid addition salt thereof, especially preferably (S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic

acid hydrochloride.

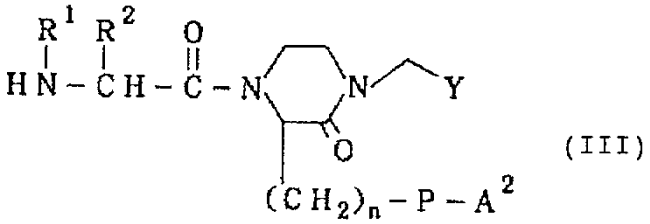
And, another preferable example is 4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid or a salt thereof.

The compounds (I) and (Ia) of this invention can be produced by, for example, methods as described below, namely, by reacting a compound (II) of the formula



(II)

wherein each symbol is of the same meaning as defined above or, a reactive derivative thereof, or a salt thereof, with a compound (III) of the formula



(III)

wherein each symbol is of the same meaning as defined above, or a salt thereof.

Examples of the salt of the compound (II) or (III) include inorganic acid salts such as hydrochloride, hydrobromide, sulfate, nitrate and phosphate, organic acid salts such as acetate, tartrate, citrate, fumarate, maleate, toluenesulfonate and methanesulfonate, metal salts such as sodium salt, potassium salt, calcium salt and aluminum salt, and salts with a base such as triethylamine salt, guanidine salt, ammonium salt, hydrazine salt, quinine salt and cinchonine salt, which are pharmaceutically acceptable ones.

Examples of the reactive derivative of the

compound (II) include compounds (II) of the formula

wherein A^1 is of the same meaning as defined above, and W is halogen (preferably chlorine) or corresponding acid halides, azides, active esters (esters with alcohol such as pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethylalcohol, paranitrophenol, N-hydroxy-5-norbornene-2,3-dicarboxyimide, N-hydroxysuccinimide, N-hydroxyphthalimide, and N-hydroxybenztriazole).

The condensation reaction as the methods for producing the compounds (I) and (Ia) of this invention can be carried out by an amide-linkage formation reaction in a conventional peptide synthesis, for example, the method using active ester, mixed acid anhydride or acid chloride.

For example, the condensation reaction between the compound (II) and the compound (III) can be conducted by subjecting the compound (II) to condensation with a phenol such as 2,4,5-trichlorophenol, pentachlorophenol, 2-nitrophenol or 4-nitrophenol or an N-hydroxy compound such as N-succinimide, N-hydroxy-5-norbornen-endo-2,3-dicarboxyimide, 1-hydroxybenztriazole or N-hydroxypiperidine in the presence of a reagent such as dicyclohexylcarbodiimide to convert into an active ester thereof, followed by condensation. Alternatively, the compound (II) is allowed to react with isobutyl chloroformate to give a mixed acid anhydride, which is then subjected to condensation.

The condensation between the compound (II) or a reactive derivative thereof and the compound (III) can also be performed by using singly a peptide-formation

reagent such as dicyclohexylcarbodiimide, N,N'-carbonyldiimidazole, diphenylphosphoryl azide or diethyl cyanophosphate.

In said condensation reaction, the amidino group, guanidino group or amino group present in the compound (II), a reactive derivative thereof or the compound (III) are preferably present as the salt of an inorganic acid (e.g. hydrogen chloride, sulfuric acid, nitric acid or hydrobromic acid) or protected with tert-butoxycarbonyl group or benzyloxycarbonyl group.

And, in said condensation reaction, the carboxyl group present in the compound (II), a reactive derivative thereof or the compound (III) is desirably present as the salt of an inorganic acid (e.g. hydrogen chloride, sulfuric acid, nitric acid or hydrobromic acid) or protected with methyl, ethyl, benzyl or tert-butyl group.

And, in said condensation reaction, the hydroxyl group present in the compound (II) a reactive derivative thereof or the compound (III) is desirably present as the salt of an inorganic acid (e.g. hydrogen chloride, sulfuric acid, nitric acid or hydrobromic acid) or protected with benzyl or tert-butyl group.

Any of the above-mentioned condensation reactions can be promoted by the addition of preferably an organic base (e.g. triethylamine, N-methylpiperidine, 4-N,N-dimethylaminopyridine) or an inorganic base (sodium hydrogencarbonate, sodium carbonate, potassium carbonate). The reaction temperature ranges usually from -20 to +50°C, preferably from 0°C to about +30°C. The reaction time varies depending on kinds of the solvents (including mixing ratio in the case of a mixed solvent) and reaction temperature, which ranges usually from one minute to 72 hours, preferably from about 15 minutes to 5 hours. Examples of solvents usually employed include water, dioxane, tetrahydrofuran,

acetonitrile, pyridine, N,N-dimethylformamide, dimethyl sulfoxide, N-methylpyrrolidone, chloroform and methylene chloride, and these can be used singly or as a mixture.

The protective group of the carboxyl group contained in the product of the final method (benzyl group or tert-butyl group, which is the protective group of the carboxyl group of Y in the general formula (I)) can be removed by a per se known method. For example, a compound having a benzyl ester group can be converted to a carboxylic acid derivative by subjecting the compound to hydrogenation in the presence of a precious metal catalyst such as palladium or platinum, and a compound having a tert-butyl ester group can be converted to a carboxylic acid derivative by processing the compound with an acid such as trifluoroacetic acid or hydrogen chloride.

The protective group of the amino group contained in the product in the final method (tert-butoxycarbonyl group or benzyloxycarbonyl group, which is the protective group of the amino group of X' in the below reaction schema) can be removed by a per se known method. For example, the tert-butoxycarbonyl group can be readily removed by processing the compound containing the group with an acid such as trifluoroacetic acid or hydrogen chloride in an organic solvent (e.g. methanol, ethanol, ethyl acetate and dioxane). And, the benzyloxycarbonyl group can be removed by subjecting the compound containing the group to catalytic reduction in the presence of a metal such as platinum, palladium or Raney's nickel or a mixture of such metal and an optional carrier.

While salts of the compound (I) can be obtained by the reaction for producing the compound (I) itself, they can be produced also by adding, upon necessity, an acid, alkali or base.

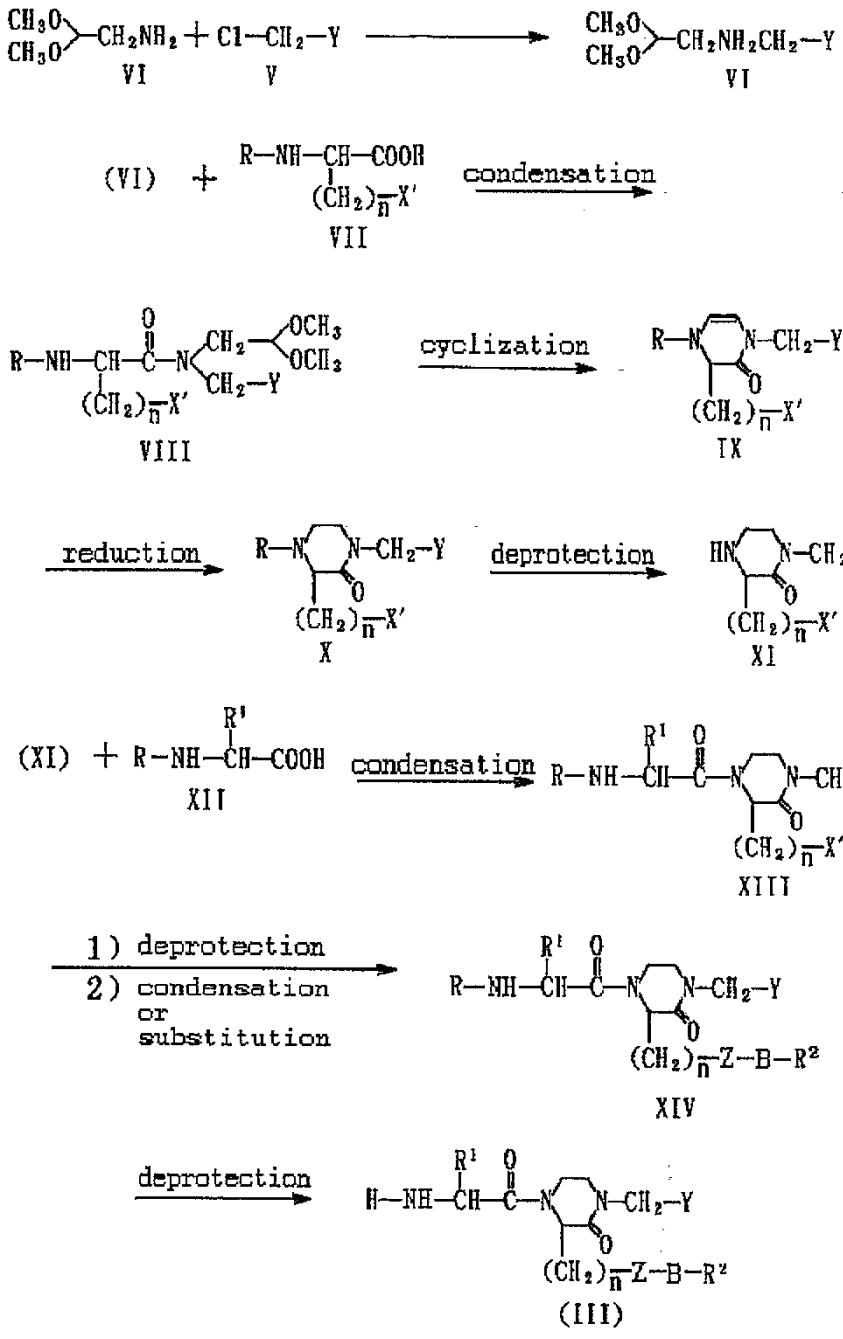
Thus-obtained compound (I) to be used in this invention can be isolated from the reaction mixture by a conventional separation and purification means such as extraction, concentration, neutralization, filtration, recrystalization, column chromatography and thin-layer chromatography.

In the compound (I), at least two stereoisomers can be present. These individual isomers or a mixture thereof are included in the scope of the present invention. And, it is also possible to produce these isomers individually.

By conducting the reaction as described using a single isomer of the compound (III), a single optical isomer of the compound (I) can be obtained.

And, when the product is a mixture of two or more isomers, it can be separated into respective isomers by a conventional separation method, for example, a method of causing formation of a salt with an optically active acid (e.g. camphor sulfonic acid, tartaric acid and dibenzoyl tartaric acid), an optically active base (e.g. cinchonine, cinchonidine, quinine, quinidine and α-methylbenzylamine), or various chromatographic means or fractional recrystallization.

The starting compounds (II) and (III) in the present invention are per se known compounds, or can be produced in a manner analogous to per se known methods. While the compound (III) can be produced by a method analogous to per se known methods, it can also be produced by the methods shown by the following reaction scheme.



In the above reaction formulae, R is an amino-protective group, and stands for benzyloxycarbonyl group or tert-butoxycarbonyl group. X' stands for a protected amino group (as the protective group, use is made of, for example, benzyloxycarbonyl group and tert-butoxycarbonyl group), a protected carboxyl group (as the protective group, use is made of, for example, methyl, ethyl, benzyl and tert-butyl group), a protected hydroxyl group (as the protective group, use is made of, for example, benzyl group and tert-butyl group) or a protected mercapto group (as the protective group, use is made of, for example, benzyl group and trityl group). Y stands for a protected carboxyl group (as the protective group, use is made of, for example, benzyl or tert-butyl group).

The method of producing the compound (III) shown by the above reaction scheme is explained in further detail. The reaction for obtaining the compound (VI) by reacting the compound (IV) with the compound (V) is a conventional alkylation of amino group. More specifically stating, the compound (IV) is allowed to react with the compound (V) usually at a temperature ranging from 0 to 100°C for a period ranging from about 15 minutes to 5 hours in the presence of a base (e.g. an inorganic base such as sodium carbonate, potassium carbonate, potassium hydrogencarbonate or cesium fluoride, or an organic base such as triethylamine, pyridine or 4-N,N-dimethylaminopyridine) to give the compound (VI). As the reaction solvent, mention is made of an organic solvent such as acetonitrile, N,N-dimethylformamide, tetrahydrofuran, toluene and methylene chloride.

The subsequent reaction of producing the compound (VIII) by subjecting the compound (VI) to condensation with the compound (VII) is a conventional peptide-linkage reaction, which can be conducted under

substantially the same reaction conditions as those for the condensation reaction of the compound (II) with the compound (III).

Cyclization of the compound (VIII) into the compound (IX) is a cyclization reaction with an acid catalyst. As the catalyst, use is made of, for example, p-toluenesulfonic acid, camphorsulfonic acid and methanesulfonic acid. The compound (IX) can be produced by conducting the reaction usually in a solvent such as toluene, benzene, ethyl acetate or 1,2-dichloroethane at a temperature ranging from 0 to 100°C, preferably from 30 to 80°C.

The subsequent reaction for reducing the compound (IX) to the compound (X) can be conducted by catalytic reduction using, as a catalyst, a metal such as platinum, palladium or Raney nickel, or a mixture of them with an optional carrier, or a reduction using a metallic hydride, for example, sodium borohydride. The above reactions are conducted usually in an organic solvent (e.g. methanol, ethanol, dioxane and ethyl acetate), and the reaction temperature ranges, in general, preferably from about -20 to about 100°C. This reaction can be conducted under normal pressure or under elevated pressure. When R is benzyloxycarbonyl group, the reaction of removing the protective group of R proceeds simultaneously to obtain the compound (XI).

Reactions for removing protective groups in (X) to (XI) and (XVI) to (III) are conventional reactions for removing protective groups of amino groups, and, in the case where R stands for a benzyloxycarbonyl group, the protective group can be removed by catalytic reduction using, as the catalyst, a metal such as platinum, palladium or Raney nickel or a mixture of the metal with an optional carrier. And, when R stands for tert-butoxycarbonyl group, the protective group can be easily removed by the use of an acid such as

trifluoroacetic acid or hydrogen chloride in an organic solvent such as methanol, ethanol, ethyl acetate or dioxane.

The condensation reaction of the compound (XI) with the compound (XII) is an amide-linkage formation reaction, which can be conducted in substantially the same manner as in the condensation of the compound (II) with the compound (III).

The reaction for converting the compound (XIII) to the compound (XIV) can be conducted usually in two steps, i.e. deprotection and condensation or substitution reaction. In the case where X' is a protected amino group, the amino group is deprotected under substantially the same conditions as in the conversion of the compound (X) into the compound (XI), which is then condensed with a corresponding carboxylic acid under substantially the same conditions as in the condensation of the compound (II) with the compound (III), or subjected to substitution reaction with a corresponding halogenide under substantially the same conditions as in the reaction employed for the substitution reaction of the compound (IV) and the compound (V). When X' is a protected carboxyl group, the protecting group can be removed by a per se known method. For example, the protective group is methyl or ethyl ester, it can be removed by allowing a base such as sodium hydroxide, potassium hydroxide or lithium hydroxide to act in an organic solvent such as methanol ethanol, tetrahydrofuran and dioxane. And, a compound having a benzyl ester group, the compound can be converted into a carboxylic acid derivative by subjecting to hydrogenation in the presence of a precious metal catalyst such as palladium and platinum, and a compound having a tert-butyl ester group can be converted into a carboxylic acid derivative by processing with an acid such as trifluoroacetic acid or

hydrogen chloride. Thus-obtained carboxylic acid can be led to the compound (XIV) by condensing with a corresponding amine or hydroxy compound by the method employed for the condensation of the compound (II) with the compound (III).

In the above-mentioned methods of producing the compound (I) and its intermediates, compounds to be employed for the reactions may, unless undesirable effects are brought about, be in the form of a salt with, for example, an inorganic acid such as hydrochloride, hydrobromide, sulfate, nitrate or phosphate, an organic acid such as acetate, tartrate, citrate, fumarate, maleate, toluenesulfonate or methanesulfonate, a metal salt such as sodium salt, potassium salt or aluminum salt, or a salt with a base such as triethylamine salt, guanidine salt, ammonium salt, hydrazine salt or quinine salt.

When the compound (I) is obtained in the free form by the above-mentioned production method, it can be converted to a salt thereof by a conventional method, and when the compound (I) is obtained as a salt, it can be converted to the compound (I) by a conventional method.

The compounds (I) (including their salts and hydrates) are low in toxicity and are used safely, which inhibit both the binding of fibrinogen, fibronectin and von Willebrand factor to the fibrinogen receptor of blood platelets (Glycoprotein IIb/IIIa) and the binding thereof and other adhesive proteins, such as vitronectin, collagen and laminin, to the corresponding receptors on the surface of various types of cells.

While the amount of the above-mentioned amorphous water-soluble compound (I) to be employed varies with, for example, kinds of the compound (I) and desired pharmacological effects and duration, it ranges, in

terms of the concentration in the solution of a polymer in an organic solvent, from about 0.001% to 90% (w/w), more preferably from about 0.01% to 80% (w/w), especially preferably from about 0.01% to 70% (w/w).

The said amorphous water-soluble compound (I) is used in the form of microparticles. The average particle size of the microparticles ranges, in general, less than 30 μm, usually from about 1 nm to about 10 μm, preferably less than 5 μm, more preferably from about 1 nm to about 1 μm.

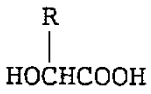
The polymer to be employed in the present invention is a hardly water-soluble or water insoluble polymer having biocompatibility. Examples of the polymer are biodegradable polymers and more specifically include poly fatty acid ester (e.g. polylactic acid, polyglycolic acid, polycitric acid, polymalic acid and polylactic acid caprolactone), poly-α-cyanoacrylic acid ester, poly-β-hydroxybutyric acid, polyalkylene oxalate (e.g. polytrimethylene oxalate and polytetramethylene oxalate), poly ortho-ester, poly ortho-carbonate or other polycarbonate (e.g. polyethylene carbonate and polypropylene carbonate), polyamino acid (e.g. poly-γ-benzyl-L-glutamic acid, poly-L-alanine and poly-γ-methyl-L-glutamic acid) and hyaluronic acid ester. Furthermore, other polymers having biocompatibility are exemplified by polystyrene, polymethacrylic acid, copolymers of acrylic acid, polyamino acid, dextran stearate, ethyl cellulose, maleic anhydride copolymers, ethylene-vinylacetate copolymers, polyvinylacetate and polyacrylamide.

These polymers may optionally be used singly or as a copolymer of two or more of them or as a simple mixture of them or in the form of their salts.

Among these polymers, biodegradable ones are preferable especially when they are used as injectable preparations. In the case of, for example, lactic

acid.glycolic acid copolymer (polymer) (PLGA), the biodegradability of the biodegradable polymer is defined as the percentage (w/w) of water-soluble low-molecular weight fragments degraded from PLGA relative to PLGA, and it should be not less than 10% in three months after subcutaneous or intramuscular administration, preferably not less than 80% in one year after subcutaneous or intramuscular administration. The said biodegradable polymer is preferably polyester. Preferred specific examples of the said biodegradable polymers include polymers or copolymers of hydroxycarboxylic acids or mixtures thereof.

While the hydroxycarboxylic acids are not necessarily specific ones, hydroxycarboxylic acids of the formula



wherein R represents hydrogen or an alkyl group are mentioned as preferable examples.

Preferable examples of the alkyl group represented by R in the above-mentioned formula include C_{1-8} straight-chain or branched alkyl groups (e.g. methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, heptyl and octyl). Among them, C_{1-3} straight-chain or branched alkyl groups are especially preferable.

Preferred examples of the above-mentioned hydroxycarboxylic acids include glycolic acid, lactic acid, hydroxybutyric acid (e.g. 2-hydroxybutyric acid), 2'-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxycaproic acid and 2-hydroxycaprylic acid. Among them, especially, glycolic acid, lactic acid, 2-hydroxybutyric acid, 2-hydroxy-3-methylbutyric acid and 2-hydroxycaproic acid are preferable. And, glycolic

acid, lactic acid and 2-hydroxybutyric acid are especially preferable. When these hydroxycarboxylic acids exist as D-isomers, L-isomers and D,L-isomers, any one of them may optionally be used, but, preferably D,L-isomers.

The copolymers may be any of random, block and graft ones. Among these glycolic acid copolymers, those whose biodegradability is relatively rapid and the release period when used singly is not longer than one month are preferred. Especially, lactic acid·glycolic acid copolymers or homopolymers (hereinafter, including copolymers and homopolymers of the respective acids, referred to briefly as copolymers) or hydroxybutyric acid·glycolic acid copolymers are preferable.

The polymer to be employed in the present invention can be synthesized without causing any problems by common synthetic methods [cf. e.g. JPA S61(1986)-28521].

The weight-average molecular weight of the polymer to be employed in the present invention ranges preferably from about 2000 to about 800000, more preferably from about 5000 to about 200000.

When lactic acid·glycolic acid copolymer (polymer) is used as the above-mentioned polymer, the molar ratio of lactic acid/glycolic acid ranges preferably from about 100/0 to about 25/75, more preferably from about 100/0 to about 50/50. The weight-average molecular weight of lactic acid·glycolic acid copolymer ranges from about 5000 to about 30000, more preferably from about 5000 to about 20000.

When hydroxybutyric acid·glycolic acid copolymer (polymer) (e.g. 2-hydroxybutyric acid·glycolic acid copolymer) is used as the above-mentioned polymer, the molar ratio of hydroxybutyric acid/glycolic acid ranges preferably from about 100/0 to about 25/75, more

preferably from about 100/0 to about 50/50. Especially, the molar ratio of 2-hydroxybutyric acid/glycolic acid ranges preferably from about 60/40 to about 30/70. The weight-average molecular weight of hydroxybutyric acid·glycolic acid copolymer ranges from about 5000 to about 25000, more preferably from about 5000 to about 20000.

When butyric acid·glycolic acid copolymer is used as the above-mentioned polymer, the molar ratio of butyric acid/glycolic acid ranges preferably from about 100/0 to about 25/75.

When a mixture of polylactic acid (A) and glycolic acid·2-hydroxybutyric acid copolymer (B), for example, is used as the above polymer, the mixing ratio shown by (A)/(B) ranges from about 10/90 to about 90/10 (by weight), preferably from about 25/75 to about 75/25 (by weight).

The weight-average molecular weight of polylactic acid ranges preferably from about 5000 to about 30000, more preferably from about 6000 to about 20000.

The molecular weight used herein means a molecular weight in terms of the molecular weight of polystyrene determined by gel permeation chromatography (GPC) using polystyrene as the standard material. The determination was carried out using GPC column KF 804L x 2 (manufactured by Showa Denko K.K. Japan) and RI monitor L-3300 (Hitachi, Japan) and using chloroform as the mobile phase. In the present specification, more specifically, the weight-average molecular weight is based on polystyrene, obtained by gel permeation chromatography (GPC) with 9 polystyrenes as reference substances with weight-average molecular weights of 120,000, 52,000, 22,000, 9,200, 5,050, 2,950, 1,050, 580 and 162, respectively.

The polydispersity of the said polymer is defined as the value of weight average molecular weight /

number average molecular weight, which ranges, in general, from 1 to 3.5, preferably from 1.5 to 2.5.

The amount of the polymer to be used depends upon, for example, the degree of the pharmacological activity of the physiologically active substance, release rate and release period of the said substance. For example, the polymer is used as the microcapsule base in an amount of about 0.2 to about 10000 times (by weight), preferably about about 1 to about 1000 times (by weight) relative to the weight of the physiologically active substance.

The concentration of the polymer in the oil phase is selected from the range of about 0.5 to about 90% (W/W), preferably about 2 to about 60% (W/W).

In order to inhibit the initial release of the water-soluble drug from the microcapsule, it is advantageous to add a basic substance or an oil and fat to the solution of this polymer in an organic solvent. Examples of the basic substance include basic amino acids such as L-arginine, N-methylglucamine and L-lysine. Among these, L-arginine or N-methylglucamine is preferred. Examples of the oil and fat include vitamin E, medium chain triglycerides (miglyols), cholesterol and phospholipids. The concentration of the basic substance in the solution of a polymer in an organic solvent ranges from about 0.01% to about 20 % (W/W), preferably from about 0.1% to about 5% (W/W), more preferably from about 0.1% to about 3% (W/W). The concentration of the oil and fat in the solution of a polymer in an organic solvent ranges from about 0.01% to about 30% (W/W), preferably from about 0.1 to about 20% (W/W), more preferably from about 0.2% to about 10% (W/W).

In the present invention, it is preferable to allow an osmotic pressure adjustor to be contained in the aqueous phase. Any osmotic pressure adjustor can

be employed so long as it produces osmotic pressure in an aqueous solution thereof.

Examples of the osmotic pressure adjustors include water-soluble polyhydric alcohols, water-soluble monovalent alcohols, water-soluble inorganic materials (e.g. inorganic salts), water-soluble monosaccharides, disaccharides, oligosaccharides and polysaccharides or their derivatives, water-soluble organic acids or salts thereof, water-soluble amino acids, water-soluble peptides, proteins or their derivatives. Among them, water-soluble polyhydric alcohols, water-soluble inorganic materials, water-soluble monosaccharides, disaccharides, oligosaccharides and polysaccharides or their derivatives, water-soluble organic acids or their salts. Furthermore, salts, water-soluble polyhydric alcohols and water-soluble inorganic materials are especially preferable.

Examples of the above-mentioned water-soluble inorganic salts include halogenated alkali metals such as potassium chloride, sodium chloride, potassium bromide, sodium bromide, potassium iodide and sodium iodide, halogenated alkaline earth metals such as calcium chloride and magnesium chloride, alkaline metal sulfates such as sodium sulfate and potassium sulfate, alkaline earth metal sulfates such as magnesium sulfate and calcium sulfate, alkali metal phosphates such as potassium dihydrogenphosphate, dipotassium hydrogenphosphate, potassium phosphate, sodium dihydrogenphosphate, disodium hydrogenphosphate and sodium phosphate. Among them, sodium chloride is especially preferred.

Examples of the above-mentioned polyhydric alcohols include dihydric alcohols such as glycerin, pentahydric alcohols such as arabitol, xylitol and adonitol, and hexahydric alcohols such as mannitol and sorbitol. Among these, hexahydric alcohols are

preferred.

Examples of the above-mentioned water-soluble monohydric alcohols include methanol, ethanol and isopropyl alcohol. Among these, ethanol is preferred.

Examples of the above-mentioned water-soluble monosaccharides include pentoses such as arabinose, xylose, ribose and 2-deoxyribose, and hexoses such as glucose, fructose, galactose, mannose, sorbose, rhamnose and fucose. Among these, hexoses are preferred.

Examples of the above-mentioned water-soluble disaccharides include maltose, cellobiose, α-trehalose, lactose and sucrose. Among these lactose and sucrose are preferred.

Examples of the above-mentioned water-soluble oligosaccharides include trisaccharides such as maltotriose and raffinose, and tetrasaccharides such as stachyose. Among these, trisaccharides are preferred.

Examples of the above-mentioned water-soluble polysaccharides include glucans such as cellulose, starch and glycogen, galacturonans such as pectic acid, mannuronans such as alginic acid, fructans such as inulin and levan, N-acetylglycosamine polymers such as chitin, xylans such as xylan of rice straw, and diheteroglucans such as mannan, glucomannan, galactomannan, hyaluronic acid, chondroitin sulfate and heparin. Among these, glucans and diheteroglucans are preferred.

Examples of the derivatives of the above-mentioned water-soluble monosaccharides, disaccharides, oligosaccharides and polysaccharides include glucosamine, galactosamine, glucuronic acid and galacturonic acid.

Examples of the above-mentioned water-soluble organic acids or their salts include citric acid, tartaric acid, malic acid, and their alkali metal salts

(e.g. sodium salts and potassium salts).

Examples of the above-mentioned water-soluble amino acids include neutral amino acids such as glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, proline, hydroxyproline, cysteine and methionine, acidic amino acids such as aspartic acid and glutamic acid, and basic amino acids such as lysine, arginine and histidine. Salts of these water-soluble amino acids with acids (e.g. hydrochloric acid, sulfuric acid and phosphoric acid) or alkalis (e.g. alkali metals such as sodium and potassium) are also used optionally.

Examples of the water-soluble peptides, proteins or their derivatives include casein, globulin, prolamins, albumin, gelatin, protamine and histone.

These osmotic pressure adjustors can be used alone or as a mixture of two or more of them. When the osmotic pressure adjustor is a non-ionic material, the concentration of the osmotic pressure adjustor in the outer aqueous phase ranges from about 0.001% to about 60 % (W/W), preferably from about 0.01 to about 40% (W/W), more preferably from about 0.05 to about 30 % (W/W). When the osmotic pressure adjustor is an ionic material, it is used in a concentration calculated by dividing the above-mentioned concentration by the total ionic valency. The concentration of the osmotic pressure adjustor to be added is not necessarily below their solubility, and a part of it may be left in the state of dispersion.

The microcapsules of the present invention can be prepared by, for example, an s/o/w type in-water drying process.

Initially, an amorphous water-soluble compound (I) is dispersed in a solution of a polymer in a water-insoluble organic solvent, then the resulting dispersion is mixed well to give an s/o type emulsion.

In this emulsion, the compound (I) is dispersed substantially homogeneously in the polymer solution.

When the water-soluble compound (I) is available in amorphous state, it can be used as it is. Even when it is available in crystalline form, it can be used after making it amorphous. The amorphous water-soluble compound (I) is preferably prepared by subjecting its aqueous solution, preferably its dilute aqueous solution to a rapid drying process such as freeze-drying or spray-drying. As described above, the amorphous water-soluble compound (I) is used preferably in the form of microparticles, and the average particle size of the compound (I) ranges generally from about 1 nm to about 30 μm, preferably from about 1 nm to about 5 μm. When the compound (I) is available in the form of microparticles, it can be used as it is. When it is not available in the form of microparticles, it can be used after pulverizing it to microparticles by conventional methods (e.g. jet mill method, atomization or ball mill method).

As the above-mentioned water-insoluble solvent, any one can be used so long as it dissolves the polymer and is insoluble in water. Examples of the water-insoluble solvent include halogenated hydrocarbons (e.g. dichloromethane, chloroform, dichlorohexane, chloroethane, dichloroethane, trichloroethane and carbon tetrachloride), esters (e.g. ethyl acetate), ethers (e.g. ethyl ether), aromatic hydrocarbons (e.g. benzene and toluene) and hydrocarbons (e.g. n-pentane and n-hexane).

The emulsification of the above-mentioned s/o type emulsion can be carried out by a conventional dispersion technique, as exemplified by intermittent shaking, mixing by means of a mixer such as propeller-type stirrer or turbine-type stirrer, colloid mill operation, mechanical homogenization and

ultrasonication. In this case, it is advantageous to use, when desired, the water-insoluble solvent in combination with a water-soluble solvent. As the said water-soluble solvent, any one can be employed so long as it is soluble in water and miscible with the above-mentioned water-insoluble solvent. Specific examples of the water-soluble solvent include alcohols (e.g. methanol, ethanol, propyl alcohol and isopropyl alcohol), acetone and acetonitrile. In the said s/o type emulsion, it is preferred to disperse more finely pulverized compound (I) having an average particle size ranging generally from about 1 nm to about 30 μm, preferable from about 1 nm to about 5 μm, most preferably about 1 nm to about 1 μm.

Subsequently, the s/o type emulsion thus prepared is subjected to in-water drying in an aqueous phase. Preferably, an osmotic pressure adjustor is allowed to be contained in the aqueous phase in the above-mentioned concentration. More specifically, the oil phase is added to the second aqueous phase containing the osmotic pressure adjustor to form an s/o/w type emulsion, followed by removing the solvent in the oil phase to prepare microcapsules.

To the outer aqueous phase in the s/o/w type in-water drying method, an emulsifying agent may optionally be added. As the emulsifying agent, any one can be used so long as it generally forms a stable o/w type emulsion. Specific examples of the emulsifying agent include anionic surfactants (e.g. sodium oleate, sodium stearate and sodium laurylsulfate), nonionic surfactants (e.g. polyoxyethylenesorbitan fatty acid ester [e.g. Tween 60, Tween 80 (Atlas Powder Co.)], polyoxyethylene castor oil derivatives [e.g. HCO-60, HCO-50 (Nikko Chemicals, Japan)] or polyvinyl pyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin and gelatin. These emulsifying

agents can be used singly or in combination of any ones of them. They are used in a concentration appropriately selected from the range of about 0.01% to about 20% (W/W), more preferably about 0.05% to about 10% (W/W).

For removing the solvent in the oil phase, a conventional method is employed. The removal of the solvent is conducted by, while reducing the pressure gradually, stirring the emulsion with a propeller-type stirrer or a magnetic stirrer, or, by using a rotary evaporator while controlling the vacuum extent. In this case, the time required for removing the solvent can be shortened by gradually warming the s/o/w type emulsion for the purpose of removing the solvent more completely at the time when the solidification of the polymer has proceeded to some extent and the loss of the compound (I) caused by its release from the internal phase has decreased. Alternatively, in the case where the thickening and solidification of the polymer is intended to conduct by a method other than that based on temperature, the solvent may be removed by merely leaving the s/o/w type emulsion to stand with stirring, or by warming the emulsion, or by spraying e.g. nitrogen gas. This step of removing the solvent is important and greatly influences the surface structure of microcapsules that controls the release of the compound (I). For example, rapid removal of the solvent produces many and larger pores on the surface to thereby increase the releasing rate of the compound (I).

The microcapsules thus prepared are collected by centrifugation or filtration. Then, the compound (I) and the substances that the compound (I) retains, which are attached onto the surface of the microcapsules are washed off with distilled water repeatedly several times. Then, depending on necessity, water in the

microcapsules and the solvent in the microcapsule preparation are removed more completely.

The microcapsules thus prepared are screened, when necessary after light pulverization, to remove those which are too large. The size of microcapsules varies with the desired degree of prolonged release, and, when the microcapsules are used as a suspension, the size is not specifically restricted so long as it falls in the range satisfying the dispersibility and needle-pass requirements. For example, the average diameter ranges preferably from about 0.5 to 400 μm, more preferably from about 2 to 200 μm.

The microcapsules prepared by the method of this invention can be administered, orally or parenteraly, as they are or in the various dose form. For example, the microcapsules can be administered in the form of injections or implants intramuscularly, subcutaneously, or into blood vessels, organs or joint cavities or foci of tumors and the like. They can also be administered after molding into various preparations, or can be used as raw materials in the production of such preparations.

The above-mentioned preparations include injections, orally administrable preparations (e.g. powders, granules, capsules and tablets), nasal preparations, suppositories (e.g. rectal suppositories and vaginal suppositories).

For example, when the microcapsules of this invention are processed into injections, they are dispersed in an aqueous vehicle together with, for example, a dispersing agent [e.g. Tween 80, HCO 60 (manufactured by Nikko Chemicals), carboxymethyl cellulose and sodium alginate], a preservative (e.g. methylparaben, benzyl alcohol and chlorobutanol) and an isotonication agent (e.g. sodium chloride, glycerin, sorbitol and glucose) to prepare an aqueous suspension,

or, they are dispersed in a vegetable oil such as olive oil, sesame oil, peanut oil, cotton seed oil and corn oil, or in propylene glycol to prepare an oily suspension, thus sustained-release injections being prepared.

To the above-mentioned sustained-release injections, an excipient (e.g. mannitol, sorbitol, lactose and glucose) is further added as the suspending agent to cause redipersion, which is then solidified by freeze-drying or spray-drying. Thus-solidified preparation is used by adding distilled water for injection or an adequate dispersing agent spontaneously. In this way, more stable sustained-release injections can be prepared.

The microcapsules of this invention can be processed into, for example, tablets by a method analogous to conventional methods. For example, to the microcapsules are added an excipient (e.g. lactose, crystalline cellulose, sucrose and starch such as corn starch), a disintegrant (e.g. starch such as corn starch, cross carmellose sodium, carboxymethyl starch sodium and calcium carbonate), a binder (e.g. crystalline cellulose, gum arabic dextrin, carboxymethyl cellulose, polyvinyl pyrrolidone and hydroxypropyl cellulose) or a lubricant (e.g. talc, magnesium stearate and polyethylene glycol 6000), then the mixture is subjected to compression molding.

For preparing the microcapsules of this invention into a composition for nasal administration, they are processed into solid, semi-solid or liquid preparations by conventional methods. For example, the solid composition for nasal administration can be prepared as a powdery composition from the microcapsules as they are or together with, for example, an excipient (e.g. glucose, mannitol, starch and microcrystalline cellulose) and a thickener (e.g. natural gum, cellulose

derivatives and polyacrylates). The above-mentioned liquid composition can be prepared as an oily or aqueous suspension by substantially the same manner as in the case of preparing injections. The semi-solid composition for nasal administration is preferably an aqueous or oily gel preparation of an ointment. In any of the above cases, pH adjustors (e.g. carbonic acid, phosphoric acid, citric acid, hydrochloric acid and sodium hydroxide) and preservatives (e.g. p-hydroxybenzoic acid esters, chlorobutanol and chlorobutanol and benzalkonium chloride) may optionally be supplemented.

For preparing the microcapsules of this invention into a suppository, an oily or aqueous solid, semi-solid or liquid suppository can be prepared by a per se known method. As the oleagenous bases for the above-mentioned composition, any one can be employed so long as it does not dissolve the microcapsules, as exemplified by higher fatty acid glycerides [cacao butter, Witepsol (Dynamit-Nobel, Germany)], medium chain triglycerides [e.g. Miglyol (Dynamit-Nobel, Germany)] or vegetable oil (e.g. sesame oil, soybean oil and cotton seed oil). The aqueous bases are exemplified by polyethylene glycol and propylene glycol, and the aqueous gel bases are exemplified by natural gum, cellulose derivatives, vinyl polymers and polyacrylates.

Since the microcapsules of this invention release a given amount of the drug over a long period, they exhibit a constant efficacy with low toxicity, thus being expected as a safe and highly effective sustained-release preparation. For example, even in the case where a bleeding tendency is feared as a side-effect brought about by their antithrombotic activity, use of the microcapsules of this invention serves to maintain non-toxic (i.e. free of any side-effect) and

effective concentration over a long period. Therefore, as mentioned above, since the compound (I) inhibits both the binding of fibrinogen, fibronectin and von Willebrand factor to the fibrinogen receptor of blood platelets (Glycoprotein (GP) IIb/IIIa) and the binding thereof and other adhesive proteins, such as vitronectin collagen and laminin, to the corresponding receptos on the surface of various types of cells and prevents the development of thrombus, the microcapsules of the present invention can be used for treatment or prophylaxis of diseases such as angina pectoris, unstable angina, acute myocardial infarction, Kawasaki disease, acute or chronic heart failure, transient ischemic attack (TIA), cerebral apoplexy, cerebral ischemic disturbance in acute phase of cerebral thrombosis, dissecting aneurysm of the aorta, cerebral vasospasm after subarachnoid hemorrhage, acute or chronic renal disease (e.g. acute or chronic renal disease due to overagglutination such as snake venom and immunopathy), chronic and acute glomerulonephritis, diabetic nephropathy and nerve disturbance, nephrotic syndrome, liver diseases, pulmonary embolism, bronchial asthma, pulmonary edema, adult respiratory distress syndrome (ARDS), arteriosclerotic obliteration, peripheral arterial obstruction, deep vein thrombosis, vibration disease, peripheral obstruction complicated with diabetes mellitus, thrombotic thrombocytopenic purpura (TTP), disseminated intravascular coagulation (DIC), sepsis, surgical or infective shock, postoperative and post-delivery trauma, premature separation of placenta, incompatible blood transfusion, systemic lupus erythematosus, Raynaud's disease, inflammations, arteriosclerosis, hemolytic uremic syndrome, symmetric peripheral necrosis, bedsore and hemorrhoids in mammals including humans (e.g. mouse, rat, guinea pig, dog, rabbit and human). And, the

microcapsules of this invention can be used for preventing thrombosis due to coronary bypass surgical operation, surgical operation for pump oxygenator, atrial fibrillation or fracture of hip joint, prosthetic valve replacement, artificial blood vessel and organs, or preventing thrombocytopenia during artificial dialysis, and further for secondary prophylaxis of myocardial infarction. The preventing thrombocytopenia during artificial dialysis also means preventing coagulation or non-washable blood in shunt of extracorporeal dialysis.

Further, the microcapsules of this invention can be used for coronary thrombolytic therapy (e.g. enhancing the action of thrombolytic agent such as tissue plasminogen activator (TPA)) and for preventing reobstruction, for preventing reobstruction and restenosis of coronary arteries after PTCA (percutaneous transluminal coronary angioplasty) or stent-indwelling and atherectomy, for preventing reobstruction and restenosis after surgical operation for coronary artery bypass, for preventing ischemic complication (e.g. myocardial infarction, death) after PTCA or coronary thrombolytic therapy, and, besides the compound (I) inhibits metastasis of tumors and can be used as an antitumor agent.

Especially, the microcapsules of the present invention are useful for the prophylaxis or treatment of thrombosis, angina pectoris, unstable angina or ischemic complication, reobstruction or restenosis after percutaneous transluminal coronary angioplasty or coronary thrombolytic therapy. The dosage of the microcapsule of the present invention for controlling or preventing the diseases referred to hereinbefore can vary within a wide range and can, of course, be adjusted to suit the individual circumstances in each particular case.

While the dosage of the sustained-release preparation of this invention varies with the types and contents of the compound (I) as the principal ingredient, dosage forms, duration of the release of the drug, subject animals (mammals, e.g. mouse, rat, horse, cow and human) and purposes of administration, it is sufficient if only the principal ingredient is contained in an effective amount. For example, When administered orally to a patient of unstable angina, or, ischemic complication or reobstruction of coronary or restenosis of coronary after PTCA or coronary thrombolytic therapy, the unit dosage for an adult (body weight: 50 kg) is adequately selected from the range of about 1 mg to about 10 g, preferably about 10 mg to 2 g, of the microcapsules such that the dose per day of the compound (I) ranges from about 1 mg to 500 mg preferably about 10 mg to 200 mg. When administered non-orally to a patient of transient ischemic attack (TIA), unstable angina, or, ischemic complication or reobstruction of coronary or restenosis of coronary after PTCA or coronary thrombolytic therapy, in the case of administration of the above-mentioned injection, the volume of the suspension can be appropriately selected from the range of about 0.1 to 5 ml, preferably about 0.5 to 3 ml such that the dose per day in terms of the compound (I) is about 0.05 to 50 mg, preferably about 1 to 20 mg/kg per day for an adult (50 kg).

Thus, pharmaceutical compositions can be prepared as the microcapsule which comprises the water soluble compound (I) in an effective therapeutic amount that is larger than a usual unit dose and a biocompatible polymer, which is capable of releasing the compound (I) sustainedly over a long period.

The microcapsules of this invention have, for example, the following characteristic features:

(1) An amorphous water-soluble medicinal substance or drug can be entrapped into the microcapsule more efficiently and in a larger amount than the corresponding medicinal substance in a crystalline form.

(2) The initial release of the medicinal substance after administration of the microcapsule can be reduced, whereby side-effects such as bleeding are suppressed.

(3) By using the microcapsules containing the medicinal substance in a high concentration, the total administration amount as a pharmaceutical composition can be reduced, thus serving to alleviate the pain or topical irritation at the site of subcutaneous administration.

Examples

The following experimental example, working examples and reference examples illustrate the present invention in further detail but are not to be construed to limit the scope thereof. In the working examples, all the percents (%) are indicated as weight/weight % unless otherwise specified.

Experimental Example

By following Working Example 1, using compound A in a crystalline form instead of amorphous compound A, the microcapsules containing crystalline compound A were obtained.

The ratio of the drug entrapped in the microcapsules and the initial release of one day were as shown below, compared with the microcapsules of Working Example 1.

Drug Form	Ratio of Entrapped Drug	Initial Release
Crystalline	67 %	46 %
Amorphous	78 %	9 %

From the results, it was shown that the amorphous drug is entrapped in the microcapsules in a larger amount and the initial release thereof is much more reduced than crystalline drug.

Working Example 1

The fine powdery amorphous compound A (450 mg) prepared by freeze-drying was dispersed in a solution of 4.05 g of lactic acid·glycolic acid copolymer (lactic acid/glycolic acid = 75/25, weight average molecular weight calculated as polystyrene = 10200) in 4 ml of methylene chloride. Thus-dispersed compound A was pulverized by using Polytron, (Chinematica, Smitzerland) to microparticles, which was emulsified by using a homogenizer in 800 ml of a 0.1% aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 2.7% of sodium chloride to give an s/o/w type emulsion. The emulsion was slowly stirred for 3

hours with a conventional propeller-type stirrer. After hardening of microcapsules with evaporation of methylene chloride, the microcapsules were collected by centrifugation and washed with purified water. The microcapsules thus collected were freeze-dried for a whole day and night to give a powdery product.

The ratio of the drug entrapped in the microcapsule and the releasability of the drug in vitro were determined to find that the drug entrapment was 78% and the initial release of one day was 9%.

Working Example 2

s/o/w method: The fine powdery compound A (60 mg) prepared by freeze-drying was dispersed in a solution of 1.94 g of a lactic acid·glycolic acid copolymer (lactic acid/glycolic acid = 75/25, weight average molecular weight calculated as polystyrene = 10200) in 2 ml of methylene chloride. Thus-dispersed compound A was pulverized to microparticles by using Polytron, which was emulsified by using a homogenizer in 800 ml of a 0.1% aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 2.7% of sodium chloride. The s/o/w type emulsion thus-prepared was subjected to substantially the same procedure as in Working Example 1 to prepare microcapsules containing the compound A.

w/o/w method: The compound A (60 mg) was dissolved in 1 ml of a 1% aqueous solution of acetic acid, which was mixed with a solution of 1.94 g of the above-mentioned lactic acid·glycolic acid copolymer (lactic acid/glycolic acid = 75/25, weight average molecular weight calculated as polystyrene = 10200) in 2 ml of methylene chloride. The compound in the mixture was pulverized to microparticles to give a w/o type emulsion. The w/o type emulsion was emulsified with a homogenizer in 800 ml of a 0.1 % aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 2.7% of sodium chloride. The w/o/w type emulsion thus-obtained

was subjected to substantially the same procedure as in Working Example 1 to prepare microcapsules containing the compound A.

The releasabilities in vitro of the microcapsules prepared in the above-mentioned s/o/w type and w/o/w type were determined to find that the initial releases of one day were respectively 16% and 33%. In the microcapsules prepared by the s/o/w method of this invention, control of the initial release was possible.

Working Example 3

The finely pulverized compound B prepared by freeze-drying (450 mg) was dispersed in a solution of 3.96 g of a lactic acid·glycolic acid copolymer (lactic acid / glycolic acid = 50/50, weight average molecular weight calculated as polystyrene = 9200) in 4 ml of methylene chloride in which L-arginine (90 mg) was previously dissolved. Thus-dispersed compound was pulverized to microparticles with Polytron. The microparticles were emulsified, using a homogenizer, in 800 ml of a 0.2% aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 2.7% of sodium chloride. Thus-prepared s/o/w type emulsion was subjected to substantially the same procedure as in Working Example 1 to prepare microcapsules containing the compound B.

Working Example 4

The fine powdery compound C (150 mg) prepared by spray-drying was dispersed in a solution of 4.26 g of lactic acid·glycolic acid copolymer (lactic acid/glycolic acid = 50/50, weight average molecular weight calculated as polystyrene = 8000) in 4.5 ml of methylene chloride. Thus-dispersed compound C was pulverized to microparticles by using Polytron, which was emulsified by using a homogenizer in 800 ml of a 0.2% aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 0.9% of sodium chloride to give an s/o/w type emulsion. The emulsion was slowly stirred

for 3 hours with a conventional propeller-type stirrer. After hardening of microcapsules with evaporation of methylene chloride, the microcapsules were collected by centrifugation and washed with purified water. The microcapsules thus collected were freeze-dried, together with mannitol, for a whole day and night to give a powdery product.

Working Example 5

The fine powdery compound D (300 mg) prepared by freeze-drying was dispersed in a solution of 4.20 g of a hydroxybutyric acid·glycolic acid copolymer (hydroxybutyric acid/glycolic acid = 75/25, weight average molecular weight calculated as polystyrene = 12000) in 5 ml of methylene chloride. Thus-dispersed compound D was pulverized to microparticles by using Polytron, which was emulsified by using a homogenizer in 1000 ml of a 0.2% aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 1.8% of sodium chloride. The s/o/w type emulsion thus-prepared was subjected to substantially the same procedure as in Working Example 4 to prepare microcapsules containing the compound D.

Working Example 6

The finely pulverized compound E prepared by freeze-drying (200 mg) was dispersed in a solution of 3.70 g of a lactic acid·glycolic acid copolymer (lactic acid / glycolic acid = 90/10, weight average molecular weight calculated as polystyrene = 8400) in 4 ml of methylene chloride in which N-methylglucamine (100 mg) was previously dissolved. Thus-dispersed compound was pulverized to microparticles with Polytron. The microparticles were emulsified, using a homogenizer, in 800 ml of a 0.1% aqueous solution, cooled at 15°C, of polyvinyl alcohol containing 2.7% of sodium chloride. Thus-prepared s/o/w type emulsion was subjected to substantially the same procedure as in Working Example

4 to prepare microcapsules containing the compound E.

Reference Example 1

(S)-3-(3-t-Butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester oxalate

In 54.6 cc of acetone were dissolved (2,2-dimethoxyethyl)aminoacetic acid t-butyl ester (6.0 g, 27.7 mmol) and N-Z-Orn(Boc)-OH (10.0 g, 27.7 mmol). To the solution was added, at 15°C under stirring, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (5.6 g, 29.2 mmol). The mixture was stirred for one hour at room temperature, and concentrated under reduced pressure. The concentrate was dissolved in ethyl acetate, and washed with a 5% aqueous solution of potassium hydrogensulfate and a saturated aqueous solution of sodium hydrogencarbonate. The organic layer was concentrated under reduced pressure to give a pale yellow oily substance. This oily substance and p-toluenesulfonic acid 1.0 hydrate (1.04 g, 5.46 mmol) were dissolved in 137 cc of toluene, and the solution was stirred for two hours at 70°C. The reaction mixture was poured into a saturated aqueous solution of sodium hydrogencarbonate. The mixture was subjected to extraction with ethyl acetate. The organic layer was concentrated under reduced pressure, and purified by means of a silica gel column chromatography (hexane/ethyl acetate=3/2) to give 8.3 g of a pale yellow oily substance. This oily substance (8.3 g, 16.5 mmol) was dissolved in 166 cc of ethyl acetate, to which was added 1.7 g of 10% Pd-C, and then the mixture was stirred for two hours under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was dissolved in 16.6 cc of methanol. To the solution was added oxalic acid 2.0 hydrate (2.1 g, 16.5 mmol), and the mixture was concentrated under reduced pressure. Resulting crystalline product was washed

with ethyl acetate to afford 5.1 g (66.8%) of the titled compound as white crystals.

Specific optical rotation: $[\alpha]_D$ -29.3° (c=0.73, H_2O)

m.p.: 181°C

Elemental Analysis for $C_{18}H_{33}N_3O_5 \cdot (CO_2H)_2$ (461.511):

Calcd.: C, 52.05; H, 7.64; N, 9.10

Found : C, 51.98; H, 7.61; N, 9.20.

Reference Example 2

(S)-4-Benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester

In a saturated aqueous solution of sodium hydrogencarbonate was dissolved (S)-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester oxalate (1.6 g, 3.47 mmol). The solution was subjected to extraction with ethyl acetate, and the extract solution was concentrated under reduced pressure. The concentrate and N-Z-Gly-OH (0.87 g, 4.16 mmol) were dissolved in 16.0 cc of acetone. To the solution was added, at 15°C under stirring, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.87 g, 4.51 mmol). The mixture was stirred for one hour at room temperature, and the reaction mixture was concentrated under reduced pressure. The concentrate was washed with a 5% aqueous solution of potassium hydrogensulfate and a saturated aqueous solution of sodium hydrogencarbonate. The organic layer was concentrated under reduced pressure, and the concentrate was purified by means of a silica gel column chromatography (ethyl acetate) to afford 1.95 g (100%) of the titled compound as a colorless amorphous powdery product.

IR ν max cm^{-1}: 3360, 2970, 2930, 1713, 1650, 1513, 1448, 1363, 1246, 1158, 1045, 964, 848, 744, 695

NMR($CDCl_3$) δ: 1.43(9H,s), 1.46(9H,s), 1.50-2.20(4H,m), 3.02-4.28(10H,m), 4.52-4.80(1H,m),

5.01(1H,dd,J=8.8,4.6Hz), 5.13(2H,s), 5.64-5.86(1H,m), 7.37(5H,s)

Reference Example 3

(S)-4-(4-Amidinobenzoylamino)acetyl-3-(3-aminopropyl)-2-oxopiperazine-1-acetic acid trifluoroacetate

In 13.4 cc of methanol was dissolved (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonyl-aminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester (1.34 g, 2.38 mmol). To the solution was added 0.54 g of 10% Pd-C, and the mixture was stirred for 30 minutes under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate and sodium hydrogencarbonate (0.4 g, 4.76 mmol) were dissolved in a mixture of 26.8 cc of water and 13.4 cc of 1,4-dioxane. To the solution was added, at room temperature under stirring, 4-amidinobenzoyl chloride hydrochloride (0.68 g, 3.09 mmol). The mixture was stirred for three hours, then pH of the reaction mixture was adjusted to 4 with 1N HCl, which was concentrated to dryness. The concentrate was dissolved in 3.75 cc of trifluoroacetic acid, and the solution was stirred for 2 hours at room temperature. The reaction mixture was concentrated under reduced pressure, which was purified by means of a CHP-20 (Mitsubishi Chemical Industries, Ltd.) column chromatography (water) to afford 1.0 g (63.3%) of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D$ +35.4° (c=0.75, MeOH)

Elemental Analysis for $C_{19}H_{26}N_6O_5 \cdot 2CF_3CO_2H \cdot H_2O$ (664.515):

Calcd.: C, 41.57; H, 4.55; N, 12.65

Found : C, 41.86; H, 4.50; N, 12.60.

Reference Example 4

(S)-4-(4-Amidinobenzoyl)aminoacetyl-3-{3-(4-amidinobenzoyl)amino}propyl-2-oxopiperazine-1-acetic

acid trifluoroacetate (Compound B)

In a mixture of 5.0 cc of water and 2.5 cc of 1,4-dioxane were dissolved (S)-4-(4-amidinobenzoylamino)acetyl-3-(3-aminopropyl)-2-oxo-piperazine-1-acetic acid trifluoroacetate (0.5 g, 0.94 mmol) and sodium hydrogencarbonate (0.32 g, 3.76 mmol). To the solution was added, at room temperature under stirring, 4-amidinobenzoyl chloride hydrochloride (0.22 g, 0.99 mmol). The mixture was stirred for two hours, whose pH was adjusted to 4 with 1N HCl, followed by concentration under reduced pressure. The concentrate was purified by means of a CHP-20 column chromatography (H_2O Right → 5% CH_3CN) to afford 0.34 g (50.7%) of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D$ +41.9° (c=0.73, MeOH)

Elemental Analysis for $C_{27}H_{32}N_8O_6 \cdot CF_3CO_2H \cdot 2H_2O$ (714.653):

Calcd.: C, 48.74; H, 5.22; N, 15.68

Found : C, 48.52; H, 5.22; N, 15.57.

Reference Example 5

(S)-3-(4-t-Butoxycarbonylaminobutyl)-2-oxopiperazine-1-acetic acid t-butyl ester oxalate

In substantially the same manner as in Reference Example 1, the titled compound was synthesized by using N-Lys(Boc)-OH.

Specific optical rotation: $[\alpha]_D$ -29.0° (c=1.02, DMSO)

m.p.: 170-172°C

Elemental Analysis for $C_{19}H_{35}N_3O_5 \cdot (CO_2H)_2$ (475.540):

Calcd.: C, 53.04; H, 7.84; N, 8.84

Found : C, 52.75; H, 7.65; N, 8.66.

Reference Example 6

(S)-4-Benzyloxycarbonylaminoacetyl-3-(4-t-butoxycarbonylaminobutyl)-2-oxopiperazine-1-acetic acid t-butyl ester

In substantially the same manner as in Reference Example 2, the titled compound was synthesized by using

(S)-3-(4-t-butoxycarbonylaminobutyl)-2-oxopiperazine-1-acetic acid t-butyl ester oxalate.

IR ν max cm^{-1}: 3400, 2990, 2945, 1713, 1657, 1520, 1458, 1368, 1253, 1166, 1070, 745, 700

NMR($CDCl_3$) δ: 1.42(9H,s), 1.46(9H,s), 1.18-2.12(6H,m), 2.92-4.28(10H,m), 4.48-4.84(1H,m), 5.02(1H,dd,J=8.6,4.8Hz), 5.13(2H,s), 5.60-5.88(1H,m), 7.36(5H,s)

Reference Example 7

(S)-4-(4-Amidinobenzoylamino)acetyl-3-(4-aminobutyl)-2-oxopiperazine-1-acetic acid trifluoroacetate

In substantially the same manner as in Working Example 1, the titled compound was synthesized by using (S)-[4-benzyloxycarbonylaminoacetyl-3-(4-t-butoxycarbonylamino-butyl)-2-oxopiperazin-1-yl)-acetic acid t-butyl ester.

Specific optical rotation: $[\alpha]_D$ +46.8° (c=1.01, H_2O)

Elemental Analysis for $C_{20}H_{28}N_6O_5 \cdot 1.7CF_3CO_2H \cdot 2H_2O$ (662.394):

Calcd.: C, 42.43; H, 5.13; N, 12.69

Found : C, 42.53; H, 4.88; N, 12.78.

Reference Example 8

(S)-4-(4-Amidinobenzoylamino)acetyl-3-{4-(4-amidinobenzoylamino)butyl}-2-oxopiperazine-1-acetic acid monotrifluoroacetate monohydrochloride

In substantially the same manner as in Working Example 2, the titled compound was synthesized by using (S)-4-(4-amidinobenzoylamino)acetyl-3-(4-aminobutyl)-2-oxopiperazine-1-acetic acid trifluoroacetate.

Specific optical rotation: $[\alpha]_D$ +44.3° (c=1.01, H_2O)

Elemental Analysis for $C_{28}H_{34}N_8O_6 \cdot CF_3CO_2H \cdot HCl \cdot 3H_2O$ (783.157):

Calcd.: C, 46.01; H, 5.41; N, 14.31

Found : C, 46.23; H, 5.22; N, 14.54.

Reference Example 9

(S,S)-4-{2-Benzyloxycarbonylamino-3-(4-

methoxyphenyl)propionyl}-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester

In substantially the same manner as in Reference Example 2, the titled compound was synthesized by using N-Z-Tyr(OMe)-OH.

IR ν max cm^{-1}: 3360, 2975, 2925, 1710, 1643, 1512, 1448, 1360, 1245, 1152, 1033, 743, 696

NMR($CDCl_3$) δ: 1.41(9H,s), 1.44(9H,s), 1.30-2.10(3H,m), 2.20-2.44(1H,m), 2.80-3.84(10H,m), 3.77(3H,s), 4.23(1H,d,J=17.2Hz), 4.50-4.85(1H,m), 4.93(1H,dd,J=6.2,7.0Hz), 5.09(2H,dd,J=12.0,16.4Hz), 5.67(1H,d,J=8.8Hz), 6.80(2H,d,J=8.8Hz), 7.09(2H,d,J=8.8Hz), 7.35(5H,s)

Reference Example 10

(S,S)-4-{2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)-propionyl}-3-(3-aminopropyl)-2-oxopiperazine-1-acetic acid trifluoroacetate

In substantially the same manner as in Working Example 1, the titled compound was synthesized by using (S,S)-4-{2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl}-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester.

Specific optical rotation: $[\alpha]_D$ +78.2° (c=0.62, H_2O)

Elemental Analysis for $C_{27}H_{34}N_6O_6 \cdot CF_3CO_2H \cdot 3H_2O$ (706.672):

Calcd.: C, 49.29; H, 5.85; N, 11.89

Found : C, 49.53; H, 5.68; N, 11.90.

Reference Example 11

(S,S)-4-{2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)-propionyl}-3-{3-(4-amidinobenzoylamino)propyl}-2-oxopiperazine-1-acetic acid trifluoroacetate

In substantially the same manner as in Working Example 2, the titled compound was synthesized by using (S,S)-4-{2-(4-amidinobenzoylamino)-3-(4-methoxyphenyl)propionyl}-3-(3-aminopropyl)-2-

oxopiperazine-1-acetic acid trifluoroacetate.
Specific optical rotation: $[\alpha]_D$ +52.8° (c=0.76, MeOH)
Elemental Analysis for $C_{35}H_{40}N_8O_7 \cdot CF_3CO_2H \cdot 3H_2O$ (852.820):
Calcd.: C, 52.11; H, 5.55; N, 13.14
Found : C, 52.27; H, 5.50; N, 13.26.

Reference Example 12

(S,S)-4-{2-Benzyloxycarbonylamino-3-(4-methoxyphenyl)-propionyl}-3-(4-t-butoxycarbonylaminobutyl)-2-oxopiperazine-1-acetic acid t-butyl ester

In substantially the same manner as in Reference Example 2, the titled compound was synthesized by using (S)-3-(4-t-butoxycarbonylaminobutyl)-2-oxopiperazine-1-acetic acid t-butyl ester oxalate and N-Z-Tyr(OMe)-OH.
IR ν max cm^{-1}: 3345, 2975, 2930, 1712, 1646, 1512, 1447, 1364, 1244, 1155, 1034, 743, 696
NMR($CDCl_3$) δ: 1.43(9H,s), 1.44(9H,s), 1.00-2.45(6H,m), 2.80-3.90(10H,m), 3.78(3H,s), 4.23(1H,d,J=17.4Hz), 4.70-5.10(2H,m), 5.10(2H,d,J=2.4Hz), 5.74(1H,d,J=8.8Hz), 6.81(2H,d,J=8.6Hz), 7.10(2H,d,J=8.6Hz), 7.35(5H,s)

Reference Example 13

(S,S)-4-{2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)propionyl}-3-(4-aminobutyl)-2-oxopiperazine-1-acetic acid trifluoroacetate

In substantially the same manner as in Reference Example 3, the titled compound was synthesized by using (S,S)-[4-{2-benzyloxycarbonylamino-3-(4-methoxyphenyl)-propionyl}-3-(4-t-butoxycarbonylaminobutyl)-2-oxopiperazin-1-yl]-acetic acid t-butyl ester.
Specific optical rotation: $[\alpha]_D$ +53.1° (c=0.64, MeOH)
Elemental Analysis for $C_{28}H_{36}N_6O_6 \cdot CF_3CO_2H \cdot 3H_2O$ (720.699):
Calcd.: C, 50.00; H, 6.01; N, 11.66
Found : C, 49.87; H, 5.77; N, 11.45.

Reference Example 14

(S,S)-4-{2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)propionyl}-3-{4-(4-

amidinobenzoylamino)butyl}-2-oxopiperazine-1-acetic acid hydrochloride

In substantially the same manner as in Reference Example 4, the titled compound was synthesized by using (S,S)-4-{2-(4-amidinobenzoylamino)-3-(4-methoxyphenyl)propionyl}-3-(4-aminobutyl)-2-oxopiperazine-1-acetic acid trifluoroacetate.

Specific optical rotation: $[\alpha]_D$ +54.5° (c=0.88, H_2O)

Elemental Analysis for $C_{36}H_{42}N_8O_7 \cdot HCl \cdot 6H_2O$ (843.329):

Calcd.: C, 51.27; H, 6.57; N, 13.29

Found : C, 51.24; H, 6.37; N, 13.26.

Reference Example 15

(S)-4-Benzyloxycarbonylaminoacetyl-3-{3-(6-t-butoxycarbonylaminohexanoylamino)propyl}-2-oxopiperazine-1-acetic acid

In 3.0 cc of trifluoroacetic acid was dissolved (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester (0.6 g, 1.07 mmol) produced in Reference Example 2. The solution was stirred for 30 minutes at room temperature, which was concentrated under reduced pressure. In 2.1 cc of DMF were dissolved 6-t-butoxyaminocaproic acid (0.26 g, 1.12 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.22 g, 1.14 mmol) and 1-hydroxybenzotriazole (0.15 g, 1.12 mmol). The solution was stirred for one hour, to which was added 2.1 cc of a DMF solution of the concentrate obtained above and triethylamine (0.3 cc, 2.14 mmol). The mixture was stirred for 4 hours at room temperature. The reaction mixture was diluted with ethyl acetate, which was washed with a 5% aqueous solution of potassium hydrogensulfate and a saturated aqueous solution of sodium hydrogencarbonate. The organic layer was concentrated under reduced pressure, which was purified by means of a silica gel column chromatography (ethyl

acetate/methanol/acetic acid=20/10/0.6) to afford 0.42 g (y. 63.3%) of the titled compound as a colorless amorphous powdery product.

IR ν max cm^{-1}: 3320, 2930, 1643, 1533, 1448, 1203, 1173, 1046

NMR($CDCl_3$) δ: 1.42(9H,s), 1.20-2.09(10H,m), 2.17(2H,t,J=7.3Hz), 2.92-4.20(12H,m), 4.80-4.98(1H,m), 5.11(2H,s), 7.22-7.44(5H,m)

Reference Example 16

(S)-4-(4-Amidinobenzoylamino)acetyl-3-{3-(6-aminohexanoylamino)propyl}-2-oxopiperazine-1-acetic acid trifluoroacetate

In 8.4 cc of methanol was dissolved (S)-4-benzyloxycarbonylaminoacetyl-3-{3-(6-t-butoxycarbonylaminohexanoylamino)propyl}-2-oxopiperazine-1-acetic acid (0.42g, 0.68 mmol). To the solution was added 0.17 g of 10% Pd-C, and the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate and sodium hydrogencarbonate (0.18 g, 2.14 mmol) were dissolved in a mixture of 8.4 cc of water and 4.2 cc of 1,4-dioxane. To the solution was added, while stirring at room temperature, 4-amidinobenzoyl chloride hydrochloride (0.20 g, 0.93 mmol). The mixture was stirred for one hour, then the pH of the reaction mixture was adjusted to 4 with 1N HCl, followed by concentration to dryness. The concentrate was dissolved in 4.3 cc of trifluoroacetic acid, and the solution was stirred for one hour at room temperature. The reaction mixture was concentrated under reduced pressure, which was purified by means of a CHP-20 column chromatography (water → 5% CH_3CN) to afford 0.26 g (y. 55%) of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D$ +42.7° (c=0.99, MeOH)

Elemental Analysis for $C_{25}H_{37}N_7O_6 \cdot 1.1CF_3CO_2H \cdot 2H_2O$

(693.067):

Calcd.: C, 47.14; H, 6.12; N, 14.15

Found : C, 47.30; H, 5.82; N, 14.40.

Reference Example 17

(S)-4-Benzyloxycarbonylaminoacetyl-3-{3-(5-t-butoxycarbonylaminopentanoylamino)propyl}-2-oxopiperazine-1-acetic acid

In substantially the same manner as in Reference Example 15, the titled compound was synthesized by using 5-t-butoxyaminovaleric acid.

IR ν max cm^{-1}: 3370, 2940, 1650, 1533, 1455, 1254, 1170, 1050

NMR($CDCl_3$) δ: 1.42(9H,s), 1.28-2.08(8H,m), 2.18(2H,t,J=7.0Hz), 3.03(2H,t,J=6.8Hz), 3.10-4.20(10H,m), 4.82-5.00(1H,m), 5.11(2H,s), 7.22-7.52(5H,m)

Reference Example 18

(S)-4-(4-Amidinobenzoylamino)acetyl-3-{3-(5-aminopentanoylamino)propyl}-2-oxopiperazine-1-acetic acid trifluoroacetate

In substantially the same manner as in Reference Example 16, the titled compound was synthesized by using (S)-4-benzyloxycarbonylaminoacetyl-3-{3-(5-t-butoxycarbonylaminopentanoylamino)propyl}-2-oxopiperazine-1-acetic acid.

Specific optical rotation: $[\alpha]_D$ +46.0° (c=1.01, MeOH)

Elemental Analysis for $C_{24}H_{35}N_7O_6 \cdot CF_3CO_2H \cdot 2.5H_2O$ (676.646):

Calcd.: C, 46.15; H, 6.11; N, 14.49

Found : C, 46.43; H, 6.15; N, 14.20.

Reference Example 19

(S)-4-Benzyloxycarbonylaminoacetyl-3-{3-(4-t-butoxycarbonylaminobutanoylamino)propyl}-2-oxopiperazine-1-acetic acid

In substantially the same manner as in Reference Example 7, the titled compound was synthesized by using

4-t-butoxyaminobutyric acid.

IR ν max cm^{-1}: 3350, 2930, 1642, 1530, 1452, 1252, 1170, 1050

NMR($CDCl_3$) δ: 1.42(9H,s), 1.30-2.10(4H,m), 1.73(2H,t,J=7.2Hz), 2.18(2H,t,J=7.5Hz), 3.04(2H,t,J=6.8Hz), 3.10-4.20(10H,m), 4.83-4.97(1H,m), 5.11(2H,s), 7.22-7.50(5H,m)

Reference Example 20

(S)-4-(4-Amidinobenzoylamino)acetyl-3-{3-(4-aminobutanoylamino)propyl}-2-oxopiperazine-1-acetic acid trifluoroacetate

In substantially the same manner as in Reference Example 16, the titled compound was synthesized by using (S)-4-benzyloxycarbonylaminoacetyl-3-{3-(4-t-butoxycarbonylaminobutanoylamino)propyl}-2-oxopiperazine-1-acetic acid.

Specific optical rotation: $[\alpha]_D$ +47.9° (c=1.00, H_2O)

Elemental Analysis for $C_{23}H_{33}N_7O_6 \cdot 1.5CF_3CO_2H \cdot 2H_2O$ (710.623):

Calcd.: C, 43.95; H, 5.46; N, 13.80

Found : C, 44.23; H, 5.63; N, 13.52.

Reference Example 21

(S,S)-4-{2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)propionyl}-3-(4-guanidinobutyl)-2-oxopiperazine-1-acetic acid hydrochloride

In 2.0 cc of trifluoroacetic acid was dissolved (S,S)-4-{2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl}-3-(4-t-butoxycarbonylaminobutyl)-2-oxopiperazine-1-acetic acid t-butyl ester (0.6 g, 0.86 mmol). The solution was stirred for one hour at room temperature, and the reaction mixture was concentrated under reduced pressure. An aqueous solution (5.6 cc) of the concentrate and sodium hydrogencarbonate (0.22 g, 2.57 mmol) was added to 5.6 cc of an aqueous solution of S-methylisothiourea sulfate (0.48 g, 1.71 mmol) and 2N

NaOH (0.86 cc, 1.71 mmol). The mixture was stirred for 14 hours at room temperature. Resulting precipitates were collected by filtration, washed with water and dried. This solid product was dissolved in 5.8 cc of methanol, to which was added 0.12 g of 10% Pd-C, and the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was purified by means of a CHP-20 column chromatography (water → 5%CH_3CN → 10%CH_3CN) to afford (S,S)-4-{2-amino-3-(4-methoxyphenyl)propionyl}-3-(4-guanidinobutyl)-2-oxopiperazine-1-acetic acid. This intermediate (0.16 g, 0.36 mmol) and sodium hydrogencarbonate (0.09 g, 1.07 mmol) were dissolved in a mixture of 3.2 cc of water and 1.6 cc of 1,4-dioxane. To the solution was added, under stirring at room temperature, 4-amidinobenzoylchloride hydrochloride (0.10 g, 0.46 mmol). The mixture was stirred for 1.5 hour, then, pH of the reaction mixture was adjusted to 4, followed by concentration under reduced pressure. The concentrate was purified by means of a CHP-20 column chromatography (water → 5%CH_3CN) to afford 0.16 g (y. 27.3%) of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D$ +62.7° (c=0.99, MeOH)

Elemental Analysis for $C_{29}H_{38}N_8O_6 \cdot HCl \cdot 3H_2O$ (685.176):

Calcd.: C, 50.84; H, 6.62; N, 16.35

Found : C, 50.76; H, 6.47; N, 16.11.

Reference Example 22

(S)-4-(4-Amidinobenzoylamino)acetyl-3-{3-(4-guanidinobutanoylamino)propyl}-2-oxopiperazine-1-acetic acid hydrochloride (Compound E)

In 6.6 cc of trifluoroacetic acid was dissolved (S)-4-benzyloxycarbonylaminoacetyl-3-{3-(4-t-butoxycarbonylaminobutanoylamino)propyl}-2-oxopiperazine-1-acetic acid (0.33 g, 0.56 mmol)

produced in Reference Example 16. The solution was stirred for one hour at room temperature, then the reaction mixture was concentrated under reduced pressure. An aqueous solution (3.3 cc) of the concentrate and sodium hydrogencarbonate (0.14 g, 1.68 mmol) was added to 3.3 cc of an aqueous solution of S-methyl isothiourea sulfate (0.93 g, 3.35 mmol) and 2N NaOH (1.68 cc, 3.35 mmol). The mixture was stirred for 14 hours at room temperature. The reaction mixture was concentrated under reduced pressure. The concentrate was purified by means of a CHP-20 column chromatography (H_2O → 5%CH_3CN → 10%CH_3CN → 15%CH_3CN) to give (S)-[4-(benzyloxycarbonylamino)-acetyl-3-{3-(4-guanidino-butylamino)-propyl}-2-oxopiperazin-1-yl]-acetic acid. This intermediate was dissolved in 6.0 cc of methanol, to which was added 0.30 g of 10%Pd-C, and the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate and sodium hydrogencarbonate (0.19 g, 2.23 mmol) were dissolved in a mixture of 6.0 cc of water and 3.0 cc of 1,4-dioxane. To the solution was added, while stirring at room temperature, 4-amidinobenzoylchloride hydrochloride (0.16 g, 0.73 mmol). The mixture was stirred for one hour, whose pH was adjusted to 4 with 1N HCl, followed by concentration under reduced pressure. The concentrate was purified by means of a CHP-20 column chromatography (H_2O) to afford 0.09 g (y.24.7%) of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D$ +48.4° (c=0.96, H_2O)

Elemental Analysis for $C_{24}H_{35}N_9O_6 \cdot 2HCl \cdot 3.5H_2O$ (681.572):

Calcd.: C, 42.29; H, 6.51; N, 18.50

Found : C, 42.34; H, 6.59; N, 18.28.

Reference Example 23

4-(N-Benzyloxycarbonyl)glycyl-1-t-butoxycarbonylmethyl-

2-oxopiperazine-3-acetic acid

In a mixture of 5 ml of water and 5 ml of methanol was dissolved 1.46 g of 4-(N-benzyloxycarbonyl)glycyl-1-t-butoxycarbonylmethyl-2-oxopiperazine-3-acetic acid methyl ester. To the solution was added 190 mg of lithium hydroxide monohydrate at 0°C in the course of five minutes. The mixture was stirred for one hour at the same temperature, then for further one hour at room temperature. With a 5% aqueous solution of potassium hydrogensulfate, pH of the reaction mixture was adjusted to 7. The reaction mixture was concentrated under reduced pressure to eliminate methanol. To the concentrate was further added 5% potassium hydrogensulfate to adjust the pH to 3, which was subjected to extraction with ethyl acetate. The extract solution was dried over anhydrous magnesium sulfate, followed by concentration under reduced pressure to afford 1.1 g of the titled compound as a colorless oily product.

NMR($CDCl_3$) δ: 1.452(9H,s), 2.80-4.65(10H,m), 5.10(2H,s), 5.82(1H,m), 6.03(1H,m), 7.33(5H,s)

IR ν max' cm^{-1}: 3000, 1730, 1660, 1465, 1370, 1230, 1160.

Reference Example 24

3-(4-Amidinophenyl)aminocarbonylmethyl-4-(N-benzyloxycarbonyl)glycyl-2-oxopiperazine-1-acetic acid t-butyl ester

In 5 ml of pyridine were dissolved 820 mg of 4-(N-benzyloxycarbonyl)glycyl-1-t-butoxycarbonylmethyl-2-oxopiperazine-3-acetic acid produced in Reference Example 5 and 370 mg of 4-aminobenzamidine dihydrochloride. To the solution were added 370 mg of dicyclohexyl carbodiimide and 10 mg of 4-dimethylaminopyridine. The mixture was stirred for 24 hours at room temperature. Insolubles were filtered off, and the filtrate was concentrated under

reduced pressure to give a crude product, which was dissolved in a 1% aqueous solution of hydrochloric acid. The solution was subjected to a CHP-20 column chromatography. Fractions eluted with 5% acetonitrile-water were collected and freeze-dried to afford 550 mg of the titled compound as a colorless powdery product.
NMR($DMSO_{d-6}$) δ: 1.42(9H,s), 2.83-4.44(13H,m), 5.02(2H,s), 7.34(5H,s), 7.78-7.82(4H,m), 9.03-9.25(3H,m)
IR ν max' cm^{-1}: 3325, 1730, 1680, 1640, 1480, 1365, 1260, 1155.

Reference Example 25

(S)-4-[N-(4-Amidinobenzoylamino)acetyl]-3-(4-amidinophenyl)aminocarbonylmethyl-2-oxopiperazine-1-acetic acid

In 15 ml of methanol was dissolved 930 mg of 3-(4-amidinophenyl)aminocarbonylmethyl-4-(N-benzyloxy-carbonyl)glycyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 12. To the solution was added 100 mg of 10%Pd-C, and the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to give an oily substance. The oily substance and 350 mg of sodium hydrogencarbonate were dissolved in a mixture of 25 ml of water and 15 ml of dioxane. To the solution was added, while stirring vigorously at room temperature, 307 mg of 4-amidinobenzoic acid in the course of 5 minutes. The reaction mixture was concentrated to give a crude product, which was dissolved in 5 ml of dichloromethane. To the solution was added 5 ml of trifluoroacetic acid at room temperature, and the mixture was stirred for one hour. The reaction mixture was concentrated under reduced pressure to give a crude product, which was purified by means of a CHP-20 column chromatography to afford 490 mg of the titled compound

as a colorless powdery product.

Specific optical rotation: $[\alpha]_D^{23}$ +57.5° (c=0.9, H_2O)

Elemental Analysis for $C_{25}H_{28}N_8O_6 \cdot CF_3CO_2H \cdot 2.7H_2O$:

Calcd.: C, 46.41; H, 4.96; N, 16.04

Found : C, 46.56; H, 4.80; N, 15.84.

Reference Example 26

(S)-4-(4-Guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid hydrochloride (Compound A)

In 4.9 ml of trifluoroacetic acid was dissolved 0.7 g of (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2. The solution was stirred for one hour at room temperature. The reaction mixture was concentrated under reduced pressure, and then subjected to azeotropic distillation with toluene several times. The residue was subjected to a CHP-20 (Mitsubishi Chemical Industries, Ltd.) column chromatography. Fractions eluted with 20% acetonitrile/water were combined and concentrated to give (S)-4-benzyloxycarbonylaminoacetyl-3-(3-amino)propyl-2-oxopiperazine-1-acetic acid as a crude product. This crude product was dissolved in 12.0 ml of methanol, to which was added 250 mg of 10%Pd-C, and then the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate and 836 mg of sodium hydrogencarbonate were dissolved in a mixture of 7.0 ml of 1,4-dioxane and 14.0 ml of water. To the solution was added, while stirring at room temperature, 1.27 g of 4-guanidinobenzoic acid N-hydroxy-5-norbornene-2,3-dicarboxylic acid imidoester hydrochloride. The mixture was stirred for one hour, then pH of the reaction mixture was adjusted to 3 to 4 with 1N hydrochloric acid, followed by concentration

under reduced pressure. The concentrate was subjected to CHP-20 column chromatography (eluted with 5% CH_3CN/H_2O). Relevant fractions were combined and freeze-dried to afford 0.48 g of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +56.3° (c=1.017, H_2O)

Elemental Analysis for $C_{27}H_{34}N_{10}O_6 \cdot 1.0HCl \cdot 3.5H_2O$:

Calcd.: C, 46.72; H, 6.10; N, 20.18

Found : C, 46.56; H, 6.17; N, 20.05.

Reference Example 27

(S)-3-(2-t-Butoxycarbonylamino)ethyl-2-oxopiperazine-1-acetic acid t-butyl ester oxalate

In 200 ml of acetonitrile were dissolved 26 g of (S)-N^2-benzyloxycarbonyl-N^4-t-butoxycarbonyl-2,4-diaminobutanoic acid and 15.5 g of N-(2,2-dimethoxyethyl)glycine t-butyl ester. To the solution was added, while stirring at room temperature, 19 g of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The mixture was stirred for further two hours at the same temperature. The reaction mixture was then concentrated to leave an oily substance, which was dissolved in ethyl acetate. The solution was washed with a 5% aqueous solution of potassium hydrogensulfate and, then, with a saturated aqueous solution of sodium hydrogencarbonate. The solution was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The concentrate was dissolved in 500 ml of toluene, to which was added 1.4 g of p-toluenesulfonic acid. The mixture was stirred for 3 hours at 70°C, which was cooled to room temperature and washed with a saturated aqueous solution of sodium hydrogencarbonate. The mixture was dried over anhydrous magnesium sulfate, which was then concentrated under reduced pressure. The concentrate was dissolved in 500 ml of methanol, to which was added 10 g of 10%Pd-C. The mixture was stirred for 10 hours

at room temperature under hydrogen atmosphere. The catalyst was filtered off. To the filtrate was added 6.4 g of oxalic acid, and the mixture was concentrated under reduced pressure to give a crude crystalline product. This crude product was recrystallized from methanol/ethyl acetate to afford 9.5 g of the titled compound as colorless crystals.

m.p.: 165-169°C

Elemental Analysis for $C_{17}H_{31}N_3O_5 \cdot (CO_2H)_2$:

Calcd.: C, 51.00; H, 7.43; N, 9.39

Found : C, 50.78; H, 7.59; N, 9.14.

Reference Example 28

(S)-4-(Benzyloxycarbonylamino)acetyl-3-(2-t-butoxycarbonylamino)ethyl-2-oxopiperazine-1-acetic acid t-butyl ester

In 20 ml of dichloromethane was suspended 900 mg of (S)-3-(2-t-butoxycarbonylaminoethyl)-2-oxopiperazine-1-acetic acid t-butyl ester oxalate produced in Reference Example 12. To the suspension was added 20 ml of a saturated aqueous solution of sodium hydrogencarbonate, and the mixture was vigorously stirred for 10 minutes. The organic layer was separated and dried over anhydrous magnesium sulfate, to which were added 420 mg of N-benzyloxycarbonyl glycine and 500 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The mixture was stirred for 2 hours at room temperature. The reaction mixture was concentrated under reduced pressure. The concentrate was dissolved in ethyl acetate, which was washed with 5% aqueous solution of potassium hydrogensulfate and a saturated aqueous solution of sodium hydrogencarbonate. The concentrate was dried over anhydrous magnesium sulfate, which was concentrated under reduced pressure. The concentrate was purified by means of a silica gel chromatography (eluent: ethyl acetate-hexane = 3:1) to afford 1.05 g

of the titled compound as a colorless oily product.
IR ν max cm^{-1}: 3450, 1705, 1655, 1640, 1500, 1450, 1360, 1240, 1160
NMR($CDCl_3$) δ: 1.43(9H,s), 1.46(9H,s), 2.05-2.33(1H,m), 2.73-2.95(1H,m), 3.15-4.20(10H,m), 5.05(1H,dd,J=3Hz), 5.13(2H,s), 5.30(1H,brs), 5.83(1H,brs), 7.36(5H,s).

Reference Example 29

(S)-4-(4-Amidinobenzoylamino)acetyl-3-[2-(4-guanidinobenzoylamino)]ethyl-2-oxopiperazine-1-acetic acid hydrochloride (Compound D)

In 5 ml of trifluoroacetic acid was dissolved 550 mg of (S)-4-(N-benzyloxycarbonylamino)acetyl-3-(2-t-butoxycarbonylamino)ethyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 28. The solution was stirred for one hour at room temperature. The reaction mixture was concentrated to give an oily substance. This oily substance and 400 mg of sodium hydrogencarbonate were dissolved in a mixture of 25 ml of water and 25 ml of dioxane. To the solution was added, while stirring at room temperature, 250 mg of 4-guanidinobenzoyl chloride hydrochloride. The reaction mixture was adjusted to pH 7 with 1N HCl, to which was added 100 mg of 10%Pd-C. The mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off. To the filtrate were added 30 ml of dioxane and 400 mg of sodium hydrogencarbonate. To the mixture was added, while stirring vigorously, 230 mg of 4-amidinobenzoic acid hydrochloride. The reaction mixture was adjusted to pH 3 with 1N HCl, which was concentrated under reduced pressure to half of its initial volume. The concentrate was purified by means of a CHP-20 column (5% acetonitrile/water) to afford 250 mg of the titled compound as a colorless amorphous solid product.

Specific optical rotation: $[\alpha]_D^{20}$ +26.112° (c=0.450, MeOH)

Elemental Analysis for $C_{26}H_{31}N_9O_6 \cdot HCl \cdot 5H_2O$:

Calcd.: C, 45.12; H, 6.12; N, 18.21

Found : C, 45.61; H, 6.06; N, 18.22.

Reference Example 30

(S)-4-Benzyloxycarbonylaminoacetyl-3-t-butoxycarbonylaminomethyl-2-oxopiperazine-1-acetic acid t-butyl ester

In substantially the same manner as in Reference Examples 1 and 2, the titled compound was produced as a colorless oily product by using (S)-N^2-benzyloxycarbonyl-N^3-t-butoxycarbonyl-2,3-diaminopropanoic acid.

H^1-NMR($CDCl_3$) δ: 1.38(9H,s), 1.47(9H,s), 3.19-4.20(10H,m), 4.90-5.05(2H,m), 5.13(2H,s), 5.82(1H,brs), 7.36(5H,s).

Reference Example 31

(S)-4-(4-Amidinobenzoylamino)acetyl-3-aminomethyl-2-oxopiperazine-1-acetic acid dihydrochloride

The titled compound was produced as a colorless amorphous powdery product by subjecting (S)-4-benzyloxycarbonylaminoacetyl-3-t-butoxycarbonylaminomethyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 30 to substantially the same procedure as in Working Example 1.

Specific optical rotation: $[\alpha]_D^{20}$ +44.9° (c=0.655, MeOH)

Elemental Analysis for $C_{17}H_{22}N_6O_5 \cdot 2HCl \cdot 4H_2O$:

Calcd.: C, 38.14; H, 6.02; N, 15.70

Found : C, 38.11; H, 5.65; N, 15.70.

Working Example 32

(S)-4-(4-Amidinobenzoylamino)acetyl-3-(4-amidinobenzoylamino)methyl-2-oxopiperazine-1-acetic acid hydrochloride

(S)-4-(4-Amidinobenzoylamino)acetyl-3-aminomethyl-2-oxopiperazine-1-acetic acid dihydrochloride produced in Working Example 17 was subjected to substantially

the same procedure as in Reference Example 4 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +60.2° (c=0.535, MeOH)

Elemental Analysis for $C_{25}H_{28}N_8O_6 \cdot HCl \cdot 3H_2O$:

Calcd.: C, 47.89; H, 5.63; N, 17.87

Found : C, 47.63; H, 5.36; N, 17.81.

Reference Example 33

(S)-4-(4-Amidinobenzoylamino)acetyl-3-[2-(4-amidinobenzoylamino)]ethyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(S)-4-(Benzyloxycarbonylamino)acetyl-3-(2-t-butoxy-carbonylamino)ethyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 28 was subjected to substantially the same procedure as in Reference Example 3 and 4 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +30.299° (c=0.470, H_2O)

Elemental Analysis for $C_{26}H_{30}N_8O_6 \cdot CF_3CO_2H \cdot 3H_2O$:

Calcd.: C, 46.80; H, 5.19; N, 15.59

Found : C, 46.67; H, 4.99; N, 15.39.

Reference Example 34

(S)-4-(4-Guanidinobenzoylamino)acetyl-3-[2-(4-guanidinobenzoylamino)]ethyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(S)-4-(benzyloxycarbonylamino)acetyl-3-(2-t-butoxy-carbonylamino)ethyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 28 was subjected to substantially the same procedure as in Working Example 15 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +35.207° (c=0.650, H_2O)

Elemental Analysis for $C_{26}H_{32}N_{10}O_6 \cdot CF_3CO_2H \cdot 3H_2O$:

Calcd.: C, 44.92; H, 5.25; N, 18.71

Found : C, 44.95; H, 5.54; N, 18.69.

Reference Example 35

(R)-4-(4-Amidinobenzoylamino)acetyl-3-(3-amino)propyl-2-oxopiperazine-1-acetic acid trifluoroacetic acid

Z-D-Orn(Boc)-OH was subjected to substantially the same procedure as in Reference Examples 1, 2 and 3 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ -35.6° (c=0.519, MeOH)

Elemental Analysis for $C_{19}H_{26}N_6O_5 \cdot 2CF_3CO_2H \cdot 1.5H_2O$:

Calcd.: C, 41.02; H, 4.64; N, 12.48

Found : C, 41.16; H, 4.47; N, 12.60.

Reference Example 36

(R)-4-(4-Amidinobenzoylamino)acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(R)-4-(4-Amidinobenzoylamino)acetyl-3-(3-amino)-propyl-2-oxopiperazine-1-acetic acid trifluoroacetic acid produced in Working Example 21 was subjected to substantially the same procedure as in Reference Example 4 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ -41.6° (c=0.495, MeOH)

Elemental Analysis for $C_{27}H_{32}N_8O_6 \cdot CF_3CO_2H \cdot 4H_2O$:

Calcd.: C, 46.40; H, 5.51; N, 14.93

Found : C, 46.66; H, 5.20; N, 14.90.

Reference Example 37

(S)-4-(4-Amidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(S)-4-(Benzyloxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2 was subjected to substantially the same procedure as in Working Example 18 to afford the titled compound as a

colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +48.6° (c=1.017, H_2O)

Elemental Analysis for $C_{27}H_{33}N_9O_6 \cdot 1.1CF_3CO_2H \cdot 1.5H_2O$:

Calcd.: C, 48.00; H, 5.30; N, 16.97

Found : C, 47.91; H, 5.11; N, 17.22.

Reference Example 38

(S)-4-(4-Amidinobenzoylamino)acetyl-3-(4-amidinophenylaminocarbonyl)ethyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(S)-4-Benzyloxycarbonylaminoacetyl-1-t-butoxycarbonylmethyl-2-oxopiperazine-3-propanoic acid methyl ester was subjected to substantially the same procedure as in Reference Example 23, 24 and 25 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +59.625° (c=0.360, H_2O)

Elemental Analysis for $C_{26}H_{30}N_8O_6 \cdot CF_3CO_2H \cdot 4H_2O$:

Calcd.: C, 45.65; H, 5.34; N, 15.21

Found : C, 45.70; H, 5.10; N, 14.91.

Reference Example 39

(S)-4-(4-Amidinobenzoylamino)acetyl-3-(4-amidinomethylbenzoylamino)methyl-2-oxopiperazine-1-acetic acid dihydrochloride

(S)-4-Benzyloxycarbonylaminoacetyl-3-t-butoxycarbonylaminomethyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 30, N-hydroxysuccinimide active ester of 4-amidinomethyl benzoic acid hydrochloride and 4-amidinobenzoyl chloride hydrochloride were subjected to substantially the same procedure as in Reference Example 29 to afford the titled compound as a colorless amorphous powdery product.

Elemental Analysis for $C_{26}H_{30}N_8O_6 \cdot 2HCl \cdot 4.5H_2O$:

Calcd.: C, 44.32; H, 5.87; N, 15.90

Found : C, 44.23; H, 5.74; N, 15.88.

Reference Example 40

(S)-4-(4-Amidinobenzoylamino)acetyl-3-(4-guanidinomethylbenzoylamino)methyl-2-oxopiperazine-1-acetic acid dihydrochloride

(S)-4-Benzyloxycarbonylaminoacetyl-3-t-butoxycarbonylaminomethyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 30, N-hydroxysuccinimide active ester of 4-guanidinomethyl benzoic acid hydrochloride and 4-amidinobenzoyl chloride hydrochloride were subjected to substantially the same procedure as in Reference Example 29 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +47.2° (c=0.553, H_2O)

Elemental Analysis for $C_{26}H_{31}N_9O_6 \cdot 2HCl \cdot 3H_2O$:

Calcd.: C, 45.09; H, 5.67; N, 18.20

Found : C, 45.32; H, 5.55; N, 18.10.

Reference Example 41

(S,S)-4-[2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)]propionyl-3-[3-(6-aminohexanoylamino)]propyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(S,S)-4-{2-Benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl}-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 9 was subjected to substantially the same procedure as in Reference Example 15 and 16 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +57.3° (c=0.678, MeOH)

Elemental Analysis for $C_{33}H_{45}N_7O_7 \cdot CF_3CO_2H \cdot 2.5H_2O$:

Calcd.: C, 51.85; H, 6.34; N, 12.09

Found : C, 52.02; H, 6.25; N, 12.04.

Reference Example 42

(S,S)-4-[2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)]propionyl-3-[4-(2-

aminoacetylamino)]butyl-2-oxopiperazine-1-acetic acid trifluoroacetate

(S,S)-4-[2-Benzyloxycarbonylamino-3-(4-methoxyphenyl)]propionyl-3-(4-t-butoxycarbonylamino)butyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 14 and N-t-butoxycarbonyl glycine were subjected to substantially the same procedure as in Reference Example 15 and Reference Example 16 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +59.8° (c=0.644, MeOH)

Elemental Analysis for $C_{30}H_{39}N_7O_7 \cdot CF_3CO_2H \cdot 2.5H_2O$:

Calcd.: C, 50.00; H, 5.90; N, 12.75

Found : C, 49.95; H, 5.72; N, 12.87.

Reference Example 43

4-(Amino-hydroxyimino)benzoic acid methyl ester

In 200 ml of methanol were dissolved 16.5 g of 4-cyanobenzoic acid methyl ester and 7.2 g of hydroxylamine hydrochloride. To the solution was added 8.82 g of sodium hydrogencarbonate at room temperature. The mixture was heated for 3 hours under reflux. The reaction mixture was cooled, to which was added 400 ml of water. Resulting crystalline precipitate was collected by filtration, which was washed with water and ether, followed by drying under reduced pressure to afford 16.1 g of the titled compound as colorless needles.

m.p.: 170-172°C

Elemental Analysis for $C_9H_{10}N_2O_3$:

Calcd.: C, 55.67; H, 5.19; N, 14.43

Found : C, 55.57; H, 5.22; N, 14.39.

Reference Example 44

4-(2,5-Dihydro-5-oxo-1,2,4-oxadiazol-3-yl)benzoic acid

In 30 ml of dioxane were suspended 5.83 g of 4-(amino-hydroxyimino)benzoic acid methyl ester produced in Reference Example 43 and 6 g of N,N'-

carbonyldiimidazole, which was stirred for 30 minutes at 110°C. The reaction mixture was concentrated to dryness. The concentrate was dissolved in water, which was adjusted to pH 4 with acetic acid. Then, resulting crystals were collected by filtration and dissolved in 60 ml of 2N NaOH. The solution was stirred overnight at room temperature. To the reaction mixture was added acetic acid to adjust its pH to 4. Resulting crystalline precipitate was collected by filtration and washed with water, followed by recrystallization from dimethylformamide/ethyl acetate to afford 4.3 g of the titled compound as a colorless crystalline product.

m.p.: not lower than 300°C

Elemental Analysis for $C_9H_6N_2O_4$:

Calcd.: C, 52.44; H, 2.93; N, 13.59

Found: C, 52.14; H, 3.29; N, 13.89.

Reference Example 45

(S)-4-[4-(2,5-Dihydro-5-oxo-1,2,4-oxadiazol-3-yl)benzoylamino]-acetyl-3-{3-[4-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)benzoylamino]}propyl-2-oxopiperazine-1-acetic acid ammonium salt

In 50 ml of methanol was dissolved 1 g of (S)-4-(benzyloxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)-propyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2. To the solution was added 0.2 g of 10%Pd-C, and the mixture was stirred for one hour under hydrogen streams. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. To the concentrate was

added 4-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)benzoic acid produced in Reference Example 16. The mixture was dissolved in 20 ml of dimethylformamide. To the solution was added 0.36 g of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (hereinafter referred to as WSC), and the mixture was stirred for 3 hours at room temperature. The reaction mixture was concentrated

under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (eluted with ethyl acetate - 25% methanol / ethyl acetate) to give an oily product. This product was dissolved in 6 ml of trifluoroacetic acid, which was stirred for 2 hours at room temperature. The reaction mixture was concentrated under reduced pressure. The concentrate was dissolved in 8 ml of dimethylformamide, to which was added 1.25 ml of triethylamine. To the mixture was added a dimethylformamide solution of the active ester prepared from 0.33 g of 4-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl) benzoic acid, 0.23 g of N-hydroxysuccinimide and 0.42 g of dicyclohexyl carbodiimide. The mixture was stirred for 3 hours at room temperature. Insolubles were filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was dissolved in water, to which was added acetic acid to adjust the pH to 4. Then, resulting precipitate was collected by filtration, which was dissolved in water. To the solution was added ammoniacal water to adjust the pH to 8, which was subjected to an XAD-2 column. Fractions eluted with 10% acetonitrile/water were combined and freeze-dried to afford 0.114 g of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +49.9° (c=0.522, MeOH)

Elemental Analysis for $C_{29}H_{31}N_9O_{10}\cdot 3.5H_2O$:

Calcd.: C, 47.80; H, 5.26; N, 17.30

Found : C, 47.87; H, 5.12; N, 17.81.

Reference Example 46

4-Cyanobenzoic acid t-butyl ester

In 612 ml of methylene chloride were suspended 45.0 g of 4-cyanobenzoic acid and 3.1 ml of conc. sulfuric acid. To the suspension was added, while stirring at 0°C, 310 ml of isobutene. The mixture was stirred for 13 days. The reaction mixture was

neutralized with a saturated aqueous solution of sodium hydrogencarbonate, which was subjected to extraction with ethyl acetate. The organic layer was concentrated under reduced pressure. Resulting precipitate was collected by filtration and washed with hexane. The filtrate and the washing were combined, which was concentrated under reduced pressure. The concentrate was purified by means of a silica gel chromatography (hexane/ethyl acetate=10/1), followed by crystallization from methylene chloride/petroleum ether to afford 43.1 g of the titled compound as a white crystalline product.

NMR($CDCl_3$) δ: 1.61(9H,s), 7.72(2H,d,J=8.8Hz), 8.08(2H,d,J=8.8Hz)

Reference Example 47

4-(Amino-hydroxyimino)methyl-benzoic acid t-butyl ester

In a mixture of 21.2 ml of t-butanol and 2.1 ml of water were dissolved 4.3 g of 4-cyanobenzoic t-butyl ester, 1.84 g of hydroxylamine hydrochloride and 2.31 g of sodium hydrogencarbonate. The solution was stirred for 2 hours at 80°C. To the reaction mixture was added water, and the mixture was subjected to extraction with ethyl acetate. The organic layer was concentrated under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (hexane/ethyl acetate=1/1), followed by crystallization from hexane to afford 4.41 g of the titled compound as colorless needles.

m.p.: 153-155°C

Elemental Analysis for $C_{12}H_{16}N_2O_3$:

Calcd.: C, 61.00; H, 6.83; N, 11.86

Found : C, 61.03; H, 6.70; N, 11.90.

Reference Example 48

4-(Amino-methoxycarbonyloxyiminomethyl)benzoic acid t-butyl ester

In 8.46 ml of 1,4-dioxane were dissolved 1.0 g of

4-(amino-hydroxyimino)methyl-benzoic acid t-butyl ester and 292 mg of potassium carbonate. To the solution was added, while stirring at 0°C, 343 μL of methyl chloroformate. The mixture was stirred for one hour at room temperature. To the reaction mixture was added water. Resulting crystalline precipitate was collected by filtration and washed with water to afford 1.22 g of the titled compound as a white crystalline product.

m.p.: 157-159°C

Elemental Analysis for $C_{14}H_{18}N_2O_5$:

Calcd.: C, 57.14; H, 6.16; N, 9.52

Found : C, 56.98; H, 6.21; N, 9.30.

Reference Example 49

4-(Amino-methoxycarbonyloxyiminomethyl)benzoic acid trifluoroacetate

In 4.0 ml of trifluoroacetic acid was dissolved 1.0 g of 4-(amino-methoxycarbonyloxyiminomethyl)benzoic acid t-butyl ester. The solution was stirred for one hour at room temperature. The reaction mixture was concentrated under reduced pressure. The concentrate was subjected to azeotropic distillation with toluene to afford 0.80 g of the titled compound as a colorless amorphous powdery product.

Elemental Analysis for $C_{10}H_{10}N_2O_5 \cdot CF_3CO_2H$(352.2233):

Calcd.: C, 40.92; H, 3.15; N, 7.95

Found : C, 41.21; H, 2.98; N, 7.96.

Reference Example 50

(S)-4-[4-(Amino-methoxycarbonyloxyiminomethyl)benzoylamino]acetyl-3-{3-[4-(amino-methoxycarbonyloxyiminomethyl)-benzoylamino]}propyl-2-oxopiperazine-1-acetic acid

(S)-4-(Benzyloxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2 and 4-(amino-methoxycarbonyloxyiminomethyl)benzoic acid trifluoroacetate produced in Reference Example 20 were

subjected to substantially the same procedure as in Reference Example 26 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +50.5° (c=1.018, MeOH)

Elemental Analysis for $C_{31}H_{36}N_8O_{12}\cdot 2H_2O$:

Calcd.: C, 49.73; H, 5.38; N, 14.97

Found : C, 49.54; H, 5.19; N, 14.87.

Reference Example 51

(S)-4-(4-Amidinobenzoylamino)acetyl-3-{3-[4-(amino-methxoycarbonyloxyiminomethyl)benzoylamino]}propyl-2-oxopiperazine-1-acetic acid hydrochloride

(S)-4-(Benzyloxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2, 4-(amino-methoxycarbonyloxyiminomethyl)benzoic acid trifluoroacetate produced in Reference Example 49 and 4-amidinobenzoic acid were subjected to substantially the same procedure as in Reference Example 29 to afford the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +47.5° (c=1.00, H_2O)

Elemental Analysis for $C_{29}H_{34}N_8O_9\cdot HCl\cdot 3H_2O$:

Calcd.: C, 47.77; H, 5.67; N, 15.37

Found : C, 47.51; H, 5.68; N, 15.27.

Reference Example 52

(S)-3-[3-(4-Amidinobenzoylamino)]propyl-4-benzyloxycarbonylaminoacetyl-2-oxopiperazine-1-acetic acid

In 6.8 ml of trifluoroacetic acid was dissolved 1.35 g of (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2. The solution was stirred for one hour at room temperature, which was then concentrated under reduced pressure. The concentrate was dissolved in a mixture of 20 ml of water and 10 ml of dioxane. To the

solution were added 806 mg of sodium hydrogencarbonate and then 683 mg of 4-amidinobenzoyl chloride hydrochloride. The mixture was stirred vigorously for 30 minutes. The reaction mixture was concentrated to give a crude product, which was purified by means of a CHP-20 column (eluted with 20% acetonitrile/water) to afford 1.0 g of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +106.6° (c=0.478, 0.1N HCl)

Elemental Analysis for $C_{27}H_{32}N_6O_7 \cdot 2H_2O$:

Calcd.: C, 55.09; H, 6.16; N, 14.28

Found : C, 55.36; H, 6.10; N, 14.35.

Reference Example 53

(S)-4-[4-(2-Aminoethyl)benzoylamino]acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid trifluoroacetate (Compound C)

In 20 ml of methanol was dissolved 300 mg of (S)-3-[3-(4-amidinobenzoylamino)]propyl-4-benzyloxycarbonylaminoacetyl-2-oxopiperazine-1-acetic acid produced in Reference Example 21. To the solution was added 120 mg of 10%Pd-C, and the mixture was stirred for one hour at room temperature in hydrogen streams. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to give an oily product, which was dissolved in 5 ml of dimethylformamide. To the solution was added 5 ml of activated-ester solution in dimethylformamide which was prepared from 94 mg of N-hydroxysuccinimide and 173 mg of 4-(2-t-butoxycarbonylaminoethyl)benzoic acid in the presence of 167 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The mixture was stirred for two hours at room temperature. The reaction mixture was concentrated to give an oily product, which was dissolved in 7 ml of trifluoroacetic acid. The solution was stirred for one hour at room

temperature. The reaction mixture was concentrated under reduced pressure to give a crude product, which was purified by means of a CHP-20 column (eluted with 10% acetonitrile/water) to afford 110 mg of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +41.7° (c=1.018, MeOH)

Elemental Analysis for $C_{28}H_{35}N_7O_6 \cdot 1.1CF_3CO_2H \cdot 4H_2O$:

Calcd.: C, 47.53; H, 5.82; N, 12.85

Found : C, 47.64; H, 5.60; N, 12.72.

Reference Example 54

(S)-4-(4-Amidinobenzoylamino)acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid hydrochloride

In 5 ml of 0.5N hydrochloric acid was dissolved 1 g of (S)-4-(4-amidinobenzoylamino)acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid trifluoroacetate produced in Reference Example 4. The solution was stirred for 5 minutes at 0°C, which was allowed to be adsorbed on a CHP-20 column. The column was washed with water until the eluate showed neutral pH. The column was then subjected to elution with 10% acetonitrile/water. Fractions of the eluate were combined and freeze-dried to afford 0.7 g of the titled compound as a colorless amorphous powdery product.

Specific optical rotation: +51.3° (c=1.018, H_2O)

Elemental Analysis for $C_{27}H_{32}N_8O_6 \cdot HCl \cdot 5H_2O$:

Calcd.: C, 46.92; H, 6.27; N, 16.21

Found : C, 47.13; H, 6.14; N, 16.23.

Reference Example 55

N-(4-t-butoxycarbonylphenyl)-N'-ethoxycarbonyl thiourea

In 150 ml of isopropyl ether was dissolved 13.51 g of 4-amino-benzoic acid t-butyl ester. To the solution was added, while stirring at room temperature, 9.83 g of ethoxycarbonyl isothiocyanate. The mixture was stirred for two hours, then the resulting crystalline

precipitate was collected by filtration, followed by recrystallization from isopropyl ether to give 21.83 g of the title compound as colorless needles.

m.p.: 119-120°C

Elemental Analysis for $C_{15}H_{20}N_2O_4S$:

Calcd.: C, 55.54; H, 6.21; N, 8.64

Found : C, 55.56; H, 6.06; N, 8.65.

Reference Example 56

N-(4-butoxycarbonylphenyl)-N'-ethoxycarbonyl-S-methyl isothiourea

In 80 ml of tetrahydrofuran was dissolved 21.7 g of N-(4-t-butoxycarbonylphenyl)-N'-ethoxycarbonyl thiourea produced in Reference Example 22. To the solution was added, while stirring on an ice-bath, 2.68 g of 60% oil sodium hydride which was previously washed with hexane. To the mixture was added dropwise a solution of 9.5 g of methyl iodide in 30 ml of hexane. Then, the mixture was stirred for one hour under the same conditions. The reaction mixture was concentrated under reduced pressure, which was dissolved in ethyl acetate. The solution was washed with water, which was then concentrated under reduced pressure. The concentrate was recrystallized from hexane to give 20 g of the titled compound as colorless needles.

m.p.: 67-68°C

Elemental Analysis for $C_{16}H_{22}N_2O_4S$

Calcd.: C, 56.78; H, 6.55; N, 8.28

Found : C, 56.63; H, 6.31; N, 8.15.

Reference Example 57

3-(4-t-Butoxycarbonylphenylamino)-1,2,4-oxadiazolin-4H-5-one

In 350 ml of methanol were dissolved 22.8 g of N-(4-butoxycarbonylphenyl)-N'-ethoxycarbonyl-S-methyl isothiourea produced in Reference Example 56 and 14 g of hydroxylamine hydrochloride. To the solution was added dropwise, while stirring on an ice-bath, 18 g of

triethylamine. The mixture was stirred for further 14 hours at room temperature. The reaction mixture was concentrated under reduced pressure. The concentrate was dissolved in ethyl acetate, and the solution was washed with 1N hydrochloric acid. The organic layer was concentrated under reduced pressure to give a crude product, which was recrystallized from ethyl acetate - hexane to afford 7.8 g of the title compound as colorless prisms.

m.p.: 271-272°C (decomp.)

Elemental Analysis for $C_{13}H_{15}N_3O_4 \cdot 1/10H_2O$:

Calcd.: C, 55.95; H, 5.49; N, 15.06

Found : C, 55.81; H, 5.47; N, 15.05.

Reference Example 58

3-(4-Carboxyphenylamino)-1,2,4-oxazolin-4H-5-one

In 70 ml of 1N NaOH was dissolved 7.7 g of 3-(4-t-butoxycarbonylphenylamino)-1,2,4-oxadiazolin-4H-5-one produced in Reference Example 24. The solution was stirred for 1.5 hour at 115 °C. The reaction mixture was cooled, which was neutralized with 2N HCl. The resulting precipitate was subjected to extraction with ethyl acetate. The extract solution was concentrated under reduced pressure to give a crude crystalline product, which was washed with ethyl acetate to afford 5.36 g of the title compound as yellow crystals.

m.p.: 272-273°C (decomp.)

Elemental Analysis for $C_9H_7N_3O_4$

Calcd.: C, 47.58; H, 3.40; N, 18.50

Found : C, 47.76; H, 3.39; N, 18.57.

Reference Example 59

4-Carboxyphenyl cyanamide

In 180 ml of tetrahydrofuran was dissolved 17.12 g of 4-amino(N-hydroxyimino)methylbenzoic acid methyl ester. To the solution was added 12.12 g of triethylamine. To the mixture was added dropwise, on an ice-bath, 12.65 g of methanesulfonyl chloride. The

mixture was stirred for one hour under the same conditions, followed by concentration under reduced pressure. To the concentrate was added methanol. The resulting crystalline precipitate was collected by filtration, which was dissolved in 100 ml of methanol. To the solution was added, while stirring at room temperature, 100 g of water containing 12 g of sodium hydroxide. Methanol was distilled off under reduced pressure. To the residue was added 700 ml of water. To the mixture was added, while stirring at room temperature, 80 ml of 4N HCl. The resulting crystalline precipitate was collected by filtration to give 13.12 g of the title compound as a colorless crystalline product.

m.p.: not lower than 300°C

Elemental Analysis for $C_8H_6N_2O_2$

Calcd.: C, 59.26; H, 3.73; N, 17.28

Found : C, 58.97; H, 3.82; N, 17.04.

Reference Example 60

N-(4-carboxyphenyl)-N'-hydroxyguanidine

In 150 ml of methanol was dissolved 6.56 g of 4-carboxyphenyl cyanamide produced in Reference Example 26. To the solution were added, while stirring at room temperature, 6.1 g of hydroxylamine hydrochloride and 8.88 g of triethylamine. The mixture was stirred for two hours. The resulting crystalline precipitate was collected by filtration to afford 4.45 g of the title compound as a colorless crystalline product.

m.p.: 200-202°C (decomp.)

Elemental Analysis for $C_8H_9N_3O_3$

Calcd.: C, 48.78; H, 4.71; N, 21.33

Found : C, 48.55; H, 4.69; N, 21.09.

Reference Example 61

3-(4-Carboxyphenylamino)-5-trifluoromethyl-1,2,4-oxadiazole

In 100 ml of tetrahydrofuran was dissolved 4.0 g

of N-(4-carboxyphenyl)-N'-hydroxyguanidine produced in Reference Example 60. To the solution was added, while stirring at 0°C, 6.75 g of anhydrous trifluoroacetic acid. The mixture was stirred for 1.5 hour under the same conditions, followed by concentration under reduced pressure. To the concentrate was added water. The resulting crystalline product was collected by filtration, which was recrystallized from ethyl acetate - hexane to afford 3.5 g of the title compound as a colorless crystalline product.

m.p.: 244-246°C

Elemental Analysis for $C_{10}H_6N_3O_3F_3$:

Calcd.: C, 43.97; H, 2.21; N, 15.38

Found : C, 44.06; H, 2.31; N, 15.28.

Reference Example 62

4-t-Butoxycarbonyl benzaldoxime

In 100 ml of methanol were dissolved 20.5 g of 4-cyanobenzoic acid t-butyl ester and 13.9 g of hydroxylamine. To the solution was added, while stirring at room temperature, 128 g of triethylamine, and the mixture was stirred for one hour at 85°C. The reaction mixture was concentrated under reduced pressure. The concentrate was dissolved in ethyl acetate, and the solution was washed with water. The organic layer was concentrated under reduced pressure to give a crude product, which was recrystallized from isopropyl ether to afford 11.45 g of the title compound as a colorless crystalline product.

m.p.: 113-114°C

Elemental Analysis for $C_{12}H_{16}N_2O_3 \cdot 1/10H_2O$:

Calcd.: C, 60.54; H, 6.86; N, 11.77

Found : C, 60.77; H, 6.79; N, 11.57.

Reference Example 63

4-t-Butoxycarbonyl phenyl cyanamide

In 150 ml of ethyl acetate was dissolved 16.3 g of 4-t-butoxycarbonyl benzaldoxime produced in Reference

Example 62. To the solution was added 13.7 ml of triethylamine. To the mixture was added dropwise, on an ice-bath, 9.92 g of methanesulfonyl chloride. The mixture was stirred for 0.5 hour under the same conditions. The reaction mixture was washed with water. The organic layer was concentrated under reduced pressure to leave an oily product. The oily product was dissolved in 150 ml of tetrahydrofuran, to which was added, while stirring at room temperature, 75 ml of 2N NaOH, followed by stirring for 0.5 hour. Tetrahydrofuran was then distilled off under reduced pressure. The residual solution was neutralized with 2N HCl, which was then subjected to extraction with ethyl acetate, followed by concentration under reduced pressure. The concentrate was recrystallized from hexane - isopropyl ether to afford the title compound as colorless crystals.

m.p.: 94-95°C

Elemental Analysis for $C_{12}H_{14}N_2O_2 \cdot 1/10H_2O$:

Calcd.: C, 65.50; H, 6.50; N, 12.73

Found : C, 65.51; H, 6.51; N, 12.52.

Reference Example 64

N-(4-t-butoxycarbonylphenyl)-N'-methoxycarbonyloxyguanidine

In 120 ml of methanol were dissolved 8.72 g of 4-t-butoxycarbonylphenyl cyanamide produced in Reference Example 30 and 5.56 g of hydroxylamine hydrochloride. To the solution was added dropwise 8.80 g of triethylamine at -25°C. The reaction mixture was then warmed up to room temperature and concentrated under reduced pressure. The concentrate was dissolved in ethyl acetate to which was washed with water. To the organic layer were added, at -10°C, 3.26 g of pyridine and 3.78 g of methyl chlorocarbonate. The temperature of the reaction mixture was reverted to room temperature. Then, the reaction mixture was washed

with water, and the organic layer was concentrated under reduced pressure to give a crude product. The crude product was recrystallized from isopropyl ether to afford 9.39 g of the title compound as colorless crystals.

m.p.: 122-126°C

Elemental Analysis for $C_{14}H_{19}N_3O_5$

Calcd.: C, 54.36; H, 6.19; N, 13.58

Found : C, 54.29; H, 6.02; N, 13.41.

Reference Example 65

N-(4-carboxyphenyl)-N'-methoxycarbonyloxyguanidine

In 25 ml of trifluoroacetic acid was dissolved 9.2 g of N-(4-t-butoxycarbonylphenyl)-N'-methoxycarbonyloxyguanidine produced in Reference Example 64. The solution was stirred for two hours at room temperature. The reaction mixture was concentrated under reduced pressure, to which was added 100 ml of water. To the mixture was added sodium hydrogencarbonate to adjust the pH to 6. The resulting crystalline precipitate was collected by filtration, followed by recrystallization from tetrahydrofuran-ethyl acetate to afford 4.53 g of the title compound as a colorless crystalline product.

m.p.: 174-175°C

Elemental Analysis for $C_{10}H_{11}N_3O_5$

Calcd.: C, 47.43; H, 4.38; N, 16.59

Found : C, 47.17; H, 4.33; N, 16.44.

Reference Example 66

(S)-2-oxo-4-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoyl]aminoacetyl-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoyl]aminopropylpiperazine-1-acetic acid

In 5 ml of trifluoroacetic acid was dissolved 500 mg of (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester. The solution was stirred for one

hour at room temperature. The reaction mixture was concentrated under reduced pressure to leave an oily product, which was dissolved in 10 ml of methanol. To the solution was added 10 mg of 10% palladium-carbon. The mixture was stirred for one hour at room temperature under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave a crude product of (S)-4-aminoacetyl-3-aminopropyl-2-oxopiperazine-1-acetic acid. This product was dissolved in a mixture of 10 ml of water and 10 ml of dioxane. To the solution was added 400 mg of sodium hydrogencarbonate. Subsequently, 420 mg of 3-(4-carboxyphenylamino)-1,2,4-oxadiazolin-4H-5-one produced in Reference Example 25 and 250 mg of N-hydroxysuccinimide were dissolved in 5 ml of dimethylformamide. To the solution was added 450 mg of dicyclohexyl carbodiimide. The mixture was stirred for 3 hours at room temperature, followed by concentration under reduced pressure to leave an oily substance. This substance was dissolved in 5 ml of dioxane, which was added to the solution of (S)-4-aminoacetyl-3-aminopropyl-2-oxopiperazine-1-acetic acid prepared as above. The mixture was stirred for 6 hours at room temperature. The reaction mixture was neutralized with 1N HCl, which was then concentrated under reduced pressure to give a crude product, followed by purification by means of a sephadex LH-20 column to afford 230 mg of the title compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ 51.9° (C=0.27, DMSO)

Elemental Analysis for $C_{29}H_{30}N_{10}O_{10}\cdot H_2O$:

Calcd.: C, 50.00; H, 4.63; N, 20.11

Found : C, 49.79; H, 4.91; N, 19.96.

Reference Example 67

(S)-2-oxo-4-[4-(5-trifluoromethyl[1,2,4]-oxadiazol-3-ylamino)benzoyl]aminoacetyl-3-[4-(5-

trifluoromethyl[1,2,4]-oxadiazol-3-ylamino)benzoyl]propylpiperazine-1-acetic acid

Using (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2 and 3-(4-carboxyphenylamino)-5-trifluoromethyl-1,2,4-oxadiazole produced in Reference Example 61, the title compound was produced as a colorless amorphous powdery product by substantially the same procedure as in Reference Example 66.

Specific optical rotation: $[\alpha]_D^{20}$ 39.7° (C=0.25, DMSO)

Elemental Analysis for $C_{31}H_{28}N_{10}O_8F_6 \cdot H_2O$:

Calcd.: C, 46.51; H, 3.78; N, 17.49

Found : C, 46.44; H, 3.97; N, 17.26.

Reference Example 68

(S)-4-[4-(N-methoxycarbonyloxyguanidino)benzoylaminoacetyl]-3-[3-(N-methoxycarbonyloxyguanidino)benzoylamino]propyl-2-oxopiperazine-1-acetic acid

Employing (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester produced in Reference Example 2 and N-(4-carboxyphenyl)-N'-methoxycarbonyloxyguanidine produced in Reference Example 32, the title compound was produced as a colorless amorphous powdery product by substantially the same procedure as in Reference Example 66.

Specific optical rotation: $[\alpha]_D^{20}$ 31.20° (C=0.28, DMSO)

Elemental Analysis for $C_{31}H_{38}N_{10}O_{12} \cdot 2H_2O$:

Calcd.: C, 47.81; H, 5.44; N, 17.99

Found : C, 47.63; H, 5.71; N, 17.83.

Reference Example 69

(S)-4-(N-t-butoxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid

In 10 ml of trifluoroacetic acid was dissolved 1.5

g of (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester. The solution was stirred for one hour at room temperature. The reaction mixture was concentrated under reduced pressure to leave an oily substance, which was dissolved in a mixture of 10 ml of water and 10 ml of dioxane. To the solution were added 400 mg of sodium hydrogencarbonate and 700 mg of di-t-butyl dicarbonate. The mixture was stirred for 3 hours at room temperature. Dioxane was distilled off under reduced pressure to leave an aqueous solution, which was washed with ethyl acetate, followed by adjusting the pH to 3 with the addition of potassium hydrogensulfate. The reaction mixture was subjected to extraction with ethyl acetate. The extract solution was dried over anhydrous magnesium sulfate, followed by concentration under reduced pressure to leave 1.2 g of the title compound as colorless crystals.

m.p.: 107-109°C

Elemental Analysis for $C_{21}H_{36}N_4O_8$

Calcd.: C, 53.38; H, 7.68; N, 11.86

Found : C, 53.35; H, 7.73; N, 11.95.

Reference Example 70

(S)-4-(N-benzyloxycarbonylamino)acetyl-3-(3-benzyloxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid

In 10 ml of trifluoroacetic acid was dissolved 2.0 g of (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester. The solution was stirred for one hour at room temperature. The reaction mixture was concentrated under reduced pressure to leave an oily substance, which was dissolved in a mixture of 10 ml of water and 10 ml of dioxane. To the solution were added 600 mg of sodium hydrogencarbonate and 550 mg of carbobenzoxy chloride. The mixture was stirred for one

hour at room temperature. Dioxane was distilled off under reduced pressure to leave an aqueous solution, which was washed with ethyl acetate. To the aqueous solution was added potassium hydrogencarbonate to adjust the pH to 3.5, followed by extraction with ethyl acetate. The extract solution was dried over anhydrous magnesium sulfate, which was then concentrated under reduced pressure to afford 1.5 g of the title compound as a colorless amorphous powdery product.

Elemental Analysis for $C_{27}H_{32}N_4O_8$

Calcd.: C, 59.99; H, 5.97; N, 10.36

Found : C, 60.13; H, 5.87; N, 10.22.

Reference Example 71

(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid pivaloyloxymethyl ester dihydrochloride

In 5 ml of dimethylformamide were dissolved 500 mg of (S)-4-(N-benzyloxycarbonylamino)acetyl-3-(3-benzyloxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid produced in Reference Example 70, 128 mg of potassium carbonate and 463 mg of potassium iodide. To the solution was added, at room temperature, 420 mg of pivaloyloxy methyl chloride. The mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated under reduced pressure to leave an oily substance, which was dissolved in ethyl acetate. The solution was washed with 10% aqueous solution of potassium hydrogen sulfate and a saturated aqueous solution of sodium hydrogencarbonate, followed by concentration under reduced pressure. The concentrate was dissolved in 10 ml of methanol, to which was added 100 mg of 10% palladium-carbon. The mixture was stirred for one hour under hydrogen atmosphere. Then, the catalyst was filtered off, and the filtrate was concentrated to leave an oily substance. The oily substance was dissolved in a mixture of 20 ml each of

water and dioxane. To the solution were added 400 mg of sodium hydrogencarbonate and 750 mg of 4-guanidinobenzoic acid 3,5-dioxo-4-azatricyclo[5,2,1,0 2,6]deca-8-en-4-ylester hydrochloride. The mixture was stirred for 3 hours at room temperature. Dioxane was distilled off under reduced pressure to leave an aqueous solution, to which was added hydrochloric acid to adjust the pH to 5, followed by purifying by means of a CHP-20 column to afford 240 mg of the title compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ 56.23° (C=0.27, H_2O)

Elemental Analysis for $C_{33}H_{44}N_{10}O_8 \cdot 2HCl \cdot H_2O$:

Calcd.: C, 49.56; H, 6.05; N, 17.51

Found : C, 49.31; H, 6.33; N, 17.24.

Reference Example 72

(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid 1-(cyclohexyloxycarbonyloxy)ethyl ester dihydrochloride

Employing (S)-4-(N-benzyloxycarbonylamino)acetyl-3-(3-benzyloxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid produced in Reference Example 70 and 1-(cyclohexyloxycarbonyloxy)ethyl chloride, the title compound was produced as a colorless amorphous powdery product by substantially the same procedure as in Reference Example 71.

Specific optical rotation: $[\alpha]_D^{20}$ 52.5° (C=0.50, H_2O)

Elemental Analysis for $C_{36}H_{48}N_{10}O_9 \cdot 2HCl \cdot 3H_2O$:

Calcd.: C, 48.49; H, 6.33; N, 15.71

Found : C, 48.35; H, 6.33; N, 15.52.

Reference Example 73

(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid 5-methyl-2-oxo-1,3-dioxolen-4-ylmethyl ester dihydrochloride

In 5 ml of dimethylformamide were dissolved 300 mg

of (S)-4-(N-t-butoxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid produced in Reference Example 69 and 62 mg of sodium hydrogencarbonate. To the solution was added 115 mg of 5-methyl-2-oxo-1,3-dioxolen-4-ylmethyl bromide, and the mixture was stirred for 5 hours at room temperature. The reaction mixture was concentrated under reduced pressure to leave an oily substance, which was dissolved in ethyl acetate. The solution was washed with a 10% aqueous solution of sodium hydrogencarbonate and a saturated aqueous solution of sodium hydrogencarbonate, followed by concentration under reduced pressure. The concentrate was dissolved in 5 ml of trifluoroacetic acid and stirred for one hour at room temperature, followed by concentration under reduced pressure to leave an oily substance. The oily substance was dissolved in 20 ml each of water and dioxane, to which were added 400 mg of sodium hydrogencarbonate and 500 mg of 4-guanidinobenzoic acid 3,5-dioxo-4-azatricyclo[5,2,2,0 2,6]deca-8-en-4-yl ester hydrochloride. The mixture was stirred for 3 hours at room temperature. Dioxane was distilled off under reduced pressure to leave an aqueous solution, to which was added 1N HCl to adjust the pH to 3.5, followed by purification by means of a CHP-20 column to afford 115 mg of the title compound as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ 43.7° (C=1.0, MeOH)

Elemental Analysis for $C_{32}H_{38}N_{10}O_9 \cdot 2HCl \cdot 3H_2O$:

Calcd.: C, 46.10; H, 5.56; N, 16.80

Found : C, 46.43; H, 5.41; N, 16.58.

Reference Example 74

(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid 2-(isobutyloxycarbonyl)-2-propylidene ethyl ester di-trifluoroacetate

Employing (S)-4-(N-t-butoxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid produced in Reference Example 33 and 2-(isobutyloxycarbonyl)-2-propylidene ethyl bromide, the title compound was produced as a colorless amorphous powdery product by substantially the same procedure as in Reference Example 73.

Specific optical rotation: $[\alpha]_D^{20}$ 47.34° (C=0.48, H_2O)

Elemental Analysis for $C_{37}H_{50}N_{10}O_8 \cdot 2CF_3CO_2H \cdot 2H_2O$:

Calcd.: C, 47.95; H, 5.50; N, 13.64

Found : C, 48.05; H, 5.51; N, 13.54.

Reference Example 75

(S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid ethyl ester dihydrochloride

Employing (S)-4-(N-t-butoxycarbonylamino)acetyl-3-(3-t-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid produced in Reference Example 69 and ethyl iodide, the titled compound was produced as a colorless amorphous powdery product by substantially the same procedure as in Reference Example 73.

Specific optical rotation: $[\alpha]_D^{20}$ 49.30° (C=0.47, H_2O)

Elemental Analysis for $C_{29}H_{38}N_{10}O_6 \cdot 2HCl \cdot 2H_2O$:

Calcd.: C, 47.61; H, 6.06; N, 19.14

Found : C, 47.29; H, 6.35; N, 18.88.

Reference Example 76

(S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-tert-butoxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid tert-butyl ester

(another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-tert-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid tert-butyl ester)

In 50 ml of water was dissolved 4.2 g of (S)-[3-(3-tert-butoxycarbonylamino)propyl-2-oxopiperazin-1-

yl]acetic acid tert-butyl ester·oxalate (another name: (S)-3-(3-tert-butoxycarbonylamino)propyl-2-oxopiperazine-1-acetic acid tert-butyl ester·oxalate) produced in Reference Example 3. To the solution was added 2.3 g of $NaHCO_3$. The mixture was subjected to extraction twice with 50 ml each portion of dichloromethane. The extract solution was dried (Na_2SO_4), followed by concentration under reduced pressure. To the concentrate was added 3 g of Z-Tyr(OMe)-OH, which was dissolved in 150 ml of dichloromethane. To the solution was added 1.92 g of WSC, which was stirred for two hours at room temperature. Dichloromethane was distilled off under reduced pressure, and the residue was subjected to extraction with ethyl acetate. The ethyl acetate layer was washed with a 3% aqueous solution of $KHSO_4$ and a saturated aqueous solution of $NaHCO_3$, which was dried (Na_2SO_4), followed by concentration under reduced pressure. The concentrate was purified by means of a silica gel chromatography (Hexane/AcOEt=1:2-AcOEt) to give 5.88 g of the title compound.

^{1}H-NMR($CDCl_3$) δ: 1.35-2.10(4H,m), 1.41(9H,s), 1.46(9H,s), 2.30(1H,m), 2.80-3.85(7H,m), 3.41(1H,d,J=17.4Hz), 3.78(3H,s), 4.24(1H,d,J=17.4Hz), 4.75(2H,m), 4.94(1H,t,J=6.5Hz), 5.10(2H,q,J=12.4Hz), 5.69(1H,d,J=8.2Hz), 6.80(2H,d,J=8.6Hz), 7.09(2H,d,J=8.6Hz), 7.35(5H,s).

Reference Example 77

(S,S)-[3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]]acetic acid

(another name: (S,S)-3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid)

In 20 ml of toluene was suspended 5.7 g of (S,S)-

[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-tert-butoxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-tert-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid tert-butyl ester) produced in Reference Example 76. The suspension was stirred under ice-cooling, to which was then added 20 ml of trifluoroacetic acid. The mixture was stirred for two hours at room temperature, to which was added toluene, followed by concentration under reduced pressure. The concentrate was dissolved in 30 ml of water, whose pH was adjusted to 5 with a conc. aqueous ammonia, followed by purification by means of an XAD-2 column chromatography (eluting with $H_2O \rightarrow 50\%CH_3CN$ water) to afford 4.3 g of the title compound.

^{1}H-NMR(CD_3OD) δ: 1.40-2.10(4H,m), 2.32(1H,m), 2.80-4.00(7H,m), 3.16(1H,d,J=16.5Hz), 3.77(3H,s), 4.61-4.85(2H,m), 4.72(1H,d,J=16.5Hz), 5.05(2H,q,J=12.3Hz), 6.82(2H,d,J=8.4Hz), 7.11(2H,d,J=8.4Hz), 7.32(5H,s).

Reference Example 78

(S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid

(another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid)

In 100 ml of a 50% aqueous solution of dioxane was dissolved 3.8 g of (S,S)-[3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid (another name: (S,S)-3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid)

produced in Reference Example 77. To the solution was added 1.52 g of $NaHCO_3$, to which was added dropwise, under ice-cooling, 1.24 ml of Z-chloride. The mixture was stirred for 1.5 hour at room temperature. Dioxane was distilled off. To the residue was added a 3% aqueous solution of $KHSO_4$ to adjust the pH to 2. The mixture was subjected to extraction with ethyl acetate. The extract solution was washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4), followed by concentration under reduced pressure. To the concentrate was added ether. The mixture was subjected to decantation twice to afford 4 g of the title compound.

^{1}H-NMR($CDCl_3$) δ: 1.40-2.05(4H,m), 2.22(1H,m), 2.75(9H,m), 3.74(3H,s), 4.65-5.20(6H,m), 5.52(1H,t,J=5.5Hz), 5.94(1H,d,J=8.6Hz), 6.78(2H,d,J=8.6Hz), 7.05(2H,d,J=8.6Hz), 7.31(10H,s).

Reference Example 79

(S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid tert-butyl ester)

In 50 ml of dichloromethane were dissolved 1.7 g of (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid) produced in Reference Example 78, 2 ml of tert-butanol and 1.6 g of 4-dimethylaminopyridine. To the solution was then added 0.6 g of WSC, and the

mixture was stirred for 24 hours at room temperature. Dichloromethane was distilled off, and the residue was subjected to extraction with ethyl acetate. The ethyl acetate layer was washed with water and a saturated aqueous saline solution, which was then dried (Na_2SO_4), followed by concentration under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (AcOEt), followed by crystallization from ether/hexane to afford 1.02 g of the title compound as colorless crystals.

m.p.: 138-140°C

Specific optical rotation: $[\alpha]_D^{20}$ +49.7° (C=0.431, MeOH)

Elemental Analysis for $C_{39}H_{48}N_4O_9$(716.832):

Calcd.: C, 65.35; H, 6.75; N, 7.82

Found : C, 65.17; H, 6.69; N, 7.91.

Reference Example 80

(S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride

(another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid hydrochloride)

In 50 ml of a 50% aqueous solution of dioxane was dissolved 0.5 g of (S,S)-[3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid (another name: (S,S)-3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid) produced in Reference Example 78. To the solution was added 0.24 g of $NaHCO_3$, to which was then added 0.377 g of 4-guanidinobenzoic acid 3,5-dioxo-4-azatricyclo[5,2,1,0 2,6]deca-8-en-4-yl ester hydrochloride. The mixture was stirred for two hours at room temperature. The pH of the reaction mixture

was adjusted to 3 with 1N-HCl, followed by distilling off dioxane. The residue was purified by means of a column chromatography (eluting with H_2O → 10% aqueous solution of CH_3CN → a 20% aqueous solution of CH_3CN → a 50% aqueous solution of CH_3CN) to afford 0.43 g of the title compound.

^{1}H-NMR(CD_3OD) δ: 1.50-2.10(4H,m), 2.45(1H,m), 2.80-4.25(9H,m), 3.77(3H,s), 4.60-5.00(2H,m), 5.00(2H,s), 6.83(2H,d,J=8.5Hz), 7.13(2H,d,J=8.5Hz), 7.30(5H,s), 7.35(2H,d,J=8.5Hz), 7.92(2H,d,J=8.5Hz).

Reference Example 81

(S,S)-[3-[3-(4-guanidinobenzoylamino)propyl]-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl-[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride (another name: (S,S)-3-[3-(4-guanidinobenzoylamino)-propyl]-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl-[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxopiperazine-1-acetic acid hydrochloride)

In 40 ml of methanol was dissolved (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid hydrochloride) produced in Reference Example 80. To the solution was added 0.2 g of 10%Pd-C. The mixture was subjected to catalytic reduction for two hours at room temperature. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure, which was dissolved in 50 ml of a 50% aqueous solution of dioxane. To the solution was added dropwise, while maintaining the pH at alkaline side, a dioxane solution of the acid chloride prepared from 4-(5-trifluoromethyl-[1,2,4]oxadiazol-3-ylamino)benzoic acid and oxazolyl chloride. The mixture was stirred

for 30 minutes at room temperature. The pH of the reaction mixture was adjusted to 3 with 1N-HCl, then the reaction mixture was concentrated to dryness. The concentrate was purified by means of a silica gel chromatography (AcOEt:AcOH:H_2O=8:1:1), to which was added ether to give 0.17 g of the title compound as a colorless powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +42.8° (C=0.94, DMSO)

Elemental Analysis for $C_{37}H_{39}N_{10}O_8F_3 \cdot HCl \cdot 0.1Et_2O$ (852.649):

Calcd.: C, 52.68; H, 5.08; N, 16.43

Found : C, 52.62; H, 5.01; N, 16.58.

Reference Example 82

(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazin-1-yl]acetic acid tert-butyl ester

(another name: (S,S)-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazine-1-acetic acid tert-butyl ester)

In 50 ml of methanol was dissolved 0.54 g of (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)-propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid tert-butyl ester) produced in Reference Example 79. To the solution was added 0.25 g of 10%Pd-C. The mixture was subjected to catalytic reduction for two hours. The catalyst was filtered off, and the filtrate was concentrated to

dryness under reduced pressure. To the concentrate were added 0.41 g of 4(5-trifluoromethyl-[1,2,4]oxadiazol-3-ylamino)benzoic acid and 0.1 g of 4-dimethyl aminopyridine. The mixture was dissolved in 30 ml of acetonitrile. To the solution was added 0.39 g of WSC, and the mixture was stirred for 20 hours. Acetonitrile was distilled off, and the residue was subjected to extraction with ethyl acetate. The extract solution was washed with a 3% aqueous solution of $KHSO_4$ and a saturated aqueous solution of NaCl, which was dried (Na_2SO_4), followed by concentration under reduced pressure. The concentrate was purified by means of a silica gel chromatography (AcOEt) to afford 0.44 g of the title compound.

^{1}H NMR(CD_3OD) δ: 1.45(9H,s), 1.50-2.10(4H,m), 2.47(1H,m), 2.95-4.20(9H,m), 3.77(3H,s), 4.80-5.20(2H,m), 6.86(2H,d,J=8.6Hz), 7.20(2H,d,J=8.6Hz), 7.41(2H,d,J=8.8Hz), 7.42(2H,d,J=8.8Hz), 7.72(2H,d,J=8.8Hz), 7.77(2H,d,J=8.8Hz).

Reference Example 83

(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazin-1-yl]acetic acid (another name: (S,S)-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazine-1-acetic acid)

In 6 ml of trifluoroacetic acid was dissolved, under ice-cooling, 0.44 g of (S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazin-1-yl]acetic acid tert-butyl

ester (another name: (S,S)-4-[3-(4-methoxyphenyl)-2-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[3-[4-(5-trifluoromethyl[1,2,4]oxadiazol-3-ylaminobenzoyl-amino]propyl]piperazine-1-acetic acid tert-butyl ester) produced in Reference Example 82. The solution was stirred for two hours at room temperature. The reaction mixture was added to toluene, which was twice concentrated to dryness under reduced pressure. The concentrate was dissolved in a small volume of ethyl acetate. To the solution was added ether to give 0.38 g of the title compound as a powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +0.7° (C=1.043, DMSO)

Elemental Analysis for $C_{39}H_{36}N_{10}O_9F_6 \cdot 2H_2O \cdot 0.2AcOEt$ (956.419):

Calcd.: C, 49.08; H, 4.38; N, 14.64

Found : C, 50.17; H, 4.17; N, 14.35.

Reference Example 84

(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazine-1-acetic acid tert-butyl ester)

In 50 ml of methanol was dissolved 0.54 g of (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl)acetic acid tert-butyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)-propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid tert-butyl ester) produced

in Reference Example 79. To the solution was added 0.25 g of 10%Pd-C. The mixture was subjected to catalytic reduction for two hours. The catalyst was filtered off, and the filtrate was concentrated to dryness under reduced pressure. To the concentrate was added 0.33 g of 4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoic acid. The mixture was dissolved in 10 ml of N,N-dimethylformamide, and the solution was stirred under ice-cooling. N,N-dimethylformamide was distilled off under reduced pressure. To the residue was added water, whose pH was adjusted to 2 with a 3% aqueous solution of $KHSO_4$. The solution was subjected to extraction with ethyl acetate containing a small volume of N,N-dimethylformamide. The extract solution was washed with a saturated aqueous solution of NaCl, which was dried (Na_2SO_4), followed by concentration under reduced pressure. The concentrate was purified by means of a silica gel chromatography (AcOEt → AcOEt/AcOH/H_2O=8:1:1) to afford 0.52 g of the title compound.

^{1}H-NMR(CD_3OD) δ: 1.44(9H,s), 1.50-2.10(4H,m), 2.48(1H,m), 2.90-4.20(9H,m), 3.75(3H,s), 4.80-5.20(2H,m), 6.84(2H,d,J=8.4Hz), 7.18(2H,d,J=8.4Hz), 7.33(2H,d,J=8.8Hz), 7.35(2H,d,J=8.8Hz), 7.71(2H,d,J=8.8Hz), 7.74(2H,d,J=8.8Hz).

Reference Example 85

(S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazin-1-yl]acetic acid

(another name: (S,S)-4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazine-1-acetic acid)

In 6 ml of trifluoroacetic acid was dissolved, under ice-cooling, 0.62 g of (S,S)-[4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[3-(4-methoxyphenyl)-2-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]propionyl]-2-oxo-3-[4-(5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-ylamino)benzoylamino]-propyl]piperazine-1-acetic acid tert-butyl ester) produced in Reference Example 84. The solution was stirred for two hours at room temperature. To the reaction mixture was added toluene. The mixture was twice concentrated to dryness under reduced pressure. The concentrate was dissolved in a small volume of methanol, to which was then added ethyl acetate to afford 0.43 g of the title compound as a powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +7.5° (C=0.983, DMSO)

Elemental Analysis for $C_{37}H_{38}N_{10}O_{11}\cdot 1.5H_2O\cdot 0.5AcOEt$ (869.846):

Calcd.: C, 53.85; H, 5.21; N, 16.10

Found : C, 53.71; H, 5.05; N, 15.97.

Reference Example 86

(S,S)-[4-[2-[4-(3-methoxycarbonyloxyguanidino)benzoylamino-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-oxyguanidino)benzoyl]amino]propyl-2-oxopiperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[2-[4-(3-methoxycarbonyloxyguanidino)benzoylamino-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-oxyguanidino)benzoyl]amino]propyl-2-oxopiperazine-1-acetic acid tert-butyl ester)

In 50 ml of methanol was dissolved 0.5 g of (S,S)-

[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl-propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenylpropionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid tert-butyl ester) produced in Reference Example 79. To the solution was added 0.25 g of 10%Pd-C, and the mixture was subjected to catalytic reduction for two hours. The catalyst was filtered off, and the filtrate was concentrated to dryness under reduced pressure. To the concentrate was added 0.38 g of 4-(3-methoxycarbonyloxyguanidino)benzoic acid. The mixture was dissolved in 10 ml of N,N-dimethylformamide. The solution was stirred under ice-cooling, to which was then added 0.21 ml of triethylamine. To the mixture was further added 0.25 g of diethyl cyanophosphate, followed by stirring for one hour under ice-cooling. To the reaction mixture was added 1 ml of acetic acid. The mixture was subjected to distillation under reduced pressure. The residue was purified by means of a silica gel chromatography (AcOEt → AcOEt/AcOH/H_2O=18:1:1) to afford 0.51 g of the title compound.

^{1}H-NMR(CD_3OD) δ: 1.44(9H,s), 1.50-2.10(4H,m), 2.48(1H,m), 2.90-4.20(9H,m), 3.76(3H,s), 3.84(6H,s), 4.80-5.20(2H,m), 6.84(2H,d,J=8.6Hz), 7.18(2H,d,J=8.6Hz), 7.33(4H,d,J=8.6Hz), 7.67(4H,d,J=8.6Hz).

Reference Example 87

(S,S)-[4-[2-[4-(3-methoxycarbonyloxyguanidino)benzoylamino-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-oxyguanidino)benzoyl]amino]propyl-2-oxopiperazin-1-yl]acetic acid

(another name: (S,S)-4-[2-[4-(3-

methoxycarbonyloxyguanidino)benzoylamino-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-oxyguanidino)benzoyl]amino]propyl-2-oxopiperazine-1-acetic acid)

In 6 ml of trifluoroacetic acid was dissolved, under ice-cooling, 0.51 g of (S,S)-[4-[2-[4-(3-methoxycarbonyloxyguanidino)benzoylamino-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-oxyguanidino)benzoyl]amino]propyl-2-oxopiperazin-1-yl]acetic acid tert-butyl ester (another name: (S,S)-4-[2-[4-(3-methoxycarbonyloxyguanidino)benzoylamino-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-oxyguanidino)benzoyl]amino]propyl-2-oxopiperazine-1-acetic acid tert-butyl ester) produced in Reference Example 86. The solution was stirred for two hours at room temperature. To the reaction mixture was added toluene, which was twice subjected to concentration to dryness under reduced pressure. The concentrate was dissolved in a 50% aqueous methanol, which was purified by means of a CHP-20 column chromatography (H_2O → 20% aqueous methanol → 50% aqueous methanol → 75% aqueous methanol) to afford 0.2 g of the title compound as a powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +9.7° (C=1.04, DMSO)

Elemental Analysis for $C_{39}H_{46}N_{10}O_{13} \cdot 0.5H_2O$ (871.862):

Calcd.: C, 53.73; H, 5.43; N, 16.07

Found : C, 53.76; H, 5.46; N, 16.09.

Reference Example 88

(S,S)-4-[2-(4-guanidinobenzoyl)amino-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoyl)aminopropyl]-2-oxopiperazine-1-acetic acid hydrochloride

In 5 ml of methanol was dissolved 250 mg of (S,S)-3-(3-aminopropyl)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid produced in Reference Example 77. To the solution was

added 100 mg of 10%Pd-C, and the mixture was stirred for one hour at room temperature in hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave an oily substance. The oily substance was dissolved in a mixture of 10 ml of dioxane and 10 ml of water. To the solution were added 210 mg of sodium hydrogencarbonate and 450 mg of 4-guanidinobenzoic acid 3,5-dioxo-4-azatricyclo[5,2,1,0 2,6]deca-8-en-4-ylester. The mixture was stirred for one hour at room temperature. The pH of the reaction mixture was adjusted to 3 with 1N HCl, then dioxane was distilled off under reduced pressure. The remaining aqueous solution was subjected to a CHP-20 column. The fraction eluted with 10% acetonitrile/water was freeze-dried to afford 130 mg of the title compound as an amorphous powdery product.

Elemental Analysis for $C_{35}H_{42}N_{10}O_7 \cdot 2H_2O$:

Calcd.: C, 53.40; H, 6.02; N, 17.79

Found : C, 53.11; H, 5.86; N, 18.06.

Reference Example 89

(S)-[3-[3-(4-amidinobenzoylamino)propyl]-4-[[4-(iminomethoxycarbonylaminomethyl)benzoylamino]acetyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride (another name: (S)-3-[3-(4-amidinobenzoylamino)propyl]-4-[[4-(iminomethoxycarbonylaminomethyl)benzoylamino]-acetyl]-2-oxopiperazine-1-acetic acid hydrochloride)

In a mixture of 1,4-dioxane (2.0 ml) and H_2O (2.0 ml) was dissolved (S)-[4-[(4-amidinobenzoylamino)acetyl]-3-[3-(4-amidinobenzoyl-amino)propyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride (another name: (S)-4-[(4-amidinobenzoylamino)acetyl]-3-[3-(4-amidinobenzoyl-amino)propyl]-2-oxopiperazine-1-acetic acid hydrochloride) (0.17 g, 0.29 mmol) produced in Reference Example 4. To the solution was gradually

added, under ice-cooling, a 2N aqueous solution of sodium hydroxide (0.46 ml, 0.91 mmol). To the mixture was then added gradually chlorocarbonic acid methyl ester (0.053 ml, 0.69 mmol), which was stirred for 30 minutes. The reaction mixture was adjusted to pH 3 with a 1N HCl, which was concentrated under reduced pressure. The concentrate was purified by means of a column chromatography (CHP-20, H_2O-5%CH_3CNaq-10%CH_3CNaq-15%CH_3CNaq), which was led to hydrochloride with 1N HCl to afford the title compound (0.20 g, 91%) as a colorless powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +49.7° (C=0.984, MeOH)

Elemental Analysis for $C_{29}H_{34}N_8O_8 \cdot 2.0HCl \cdot 2.5H_2O \cdot 1.0MeOH$ (772.640):

Calcd.: C, 46.64; H, 5.87; N, 14.50

Found : C, 46.34; H, 5.62; N, 14.26.

Reference Example 90

(S,S)-[4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-guanidino)benzoylamino]propyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride

(another name: (S,S)-4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-3-[3-[4-(3-methoxycarbonyl-guanidino)benzoylamino]propyl]-2-oxopiperazine-1-acetic acid hydrochloride)

In a mixture of 1,4-dioxane (5.2 ml) and H_2O (5.2 ml) was dissolved (S,S)-[4-[2-(4-guanidinobenzoylamino)-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazin-1-yl]acetic acid (another name: (S,S)-4-[2-(4-guanidinobenzoylamino)-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid) (0.52 g, 0.73 mmol) produced in Reference Example 88. To the solution were added, under ice-

cooling, a 2N aqueous solution of sodium hydroxide (4.35 ml, 8.70 mmol) and chlorocarbonic acid methyl ester (0.55 ml, 7.25 mmol) while keeping the pH range of the reaction system at not higher than 10. The mixture was stirred for 30 minutes, whose pH was adjusted to 7 with 1N HCl, followed by concentration under reduced pressure. The concentrate was dissolved in H_2O (5.0 ml), to which was added, under ice-cooling, lithium hydroxide (0.20 g, 4.78 mmol). The mixture was stirred for two hours at 0°C, to which was added 1N HCl to adjust the pH to 3, followed by concentration under reduced pressure. The concentrate was purified by means of a column chromatography [(CHP-20, 10%CH_3CNaq-15%CH_3CNaq-20%CH_3CNaq-25%CH_3CNaq) and (LH-20, H_2O)] to afford the title compound (0.28 g, 39%).

Specific optical rotation: $[\alpha]_D^{20}$ +64.4° (C=1.041, MeOH)

Elemental Analysis for $C_{39}H_{46}N_{10}O_{11}\cdot 2.0HCl\cdot 4.5H_2O$ (984.845):

Calcd.: C, 47.56; H, 5.83; N, 14.22

Found : C, 47.40; H, 5.55; N, 14.33.

Reference Example 91

(S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid 1-cyclohexyloxycarbonyloxy ethyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid 1-cyclohexyloxycarbonyloxy ethyl ester)

In DMF (5.8 ml) were dissolved (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid) (0.58 g, 0.88 mmol) produced in Reference

Example 78 and triethylamine (0.49 ml, 3.52 mmol). To the solution were added, while stirring at room temperature, carbonic acid 1-chloroethyl ester cyclohexyl ester (0.73 g, 3.52 mmol) and potassium iodide (0.58 g, 3.52 mmol). The mixture was stirred for 38 hours at room temperature, which was then poured into water. To the mixture was added ethyl acetate, and the mixture was shaken for extraction. The organic layer was dried over anhydrous magnesium sulfate, followed by concentration under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (hexane/ethyl acetate = 2/5) to afford the title compound (0.43 g, 59%) as a colorless amorphous powdery product.

IR ν max cm^{-1}: 3410, 2930, 1755, 1710, 1645, 1510, 1450, 1240, 1075

NMR(CD_3OD) δ: 1.10-2.10(14H,m), 1.52(3H,d,J=5.4Hz), 2.80-5.20(15H,m), 3.77(3H,s), 5.07(2H,s), 5.09(2H,s), 5.64(1H,d,J=7.8Hz), 6.67-6.87(2H,m), 7.08(2H,d,J=8.4Hz), 7.33(10H,s).

Reference Example 92

(S,S)-[4-[2-(4-guanidinobenzoylamino)-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazin-1-yl]acetic acid 1-cyclohexyloxycarbonyloxyethyl ester (another name: (S,S)-4-[2-(4-guanidinobenzoylamino)-3-(4-methoxyphenyl)propionyl]-3-[3-(4-guanidinobenzoylamino)propyl]-2-oxopiperazine-1-acetic acid 1-cyclohexyloxycarbonyloxyethyl ester)

In methanol (8.6 ml) were dissolved (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazin-1-yl]acetic acid 1-cyclohexyloxycarbonyloxyethyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-benzyloxycarbonylaminopropyl)-2-oxopiperazine-1-acetic

acid 1-cyclohexyloxycarbonyloxyethyl ester) (0.43 g, 0.52 mmol) produced in Reference Example 91 and acetic acid (0.062 ml, 1.09 mmol). To this solution was added 10%Pd-C (0.17 g), and the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was dissolved in a mixture of 1,4-dioxane (4.3 ml) and H_2O (8.6 ml). To the solution were added, while stirring at room temperature, sodium hydrogencarbonate (0.22 g, 2.59 mmol) and 4-guanidinobenzoic acid N-hydroxy-5-norbornene-2,3-dicarboxyimide ester (0.43 g, 1.14 mmol). One hour later, the pH of the reaction system was adjusted to 3 with 1N HCl, followed by concentration under reduced pressure. The concentrate was purified by means of a column chromatography [(CHP-20, 10%CH_3CNaq-15%CH_3CNaq-20%CH_3CNaq) and (LH-20, H_2O)] to afford the title compound (0.073 g, 14%) as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +63.4° (C=1.009, MeOH)

Elemental Analysis for $C_{44}H_{56}N_{10}O_{10} \cdot 2.0HCl \cdot 3.0H_2O$ (1011.957):

Calcd.: C, 52.22; H, 6.37; N, 13.84

Found : C, 52.38; H, 6.07; N, 13.81.

Reference Example 93

(S,S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester

(another name: (S,S)-3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid t-butyl ester)

In methanol (6.6 ml) was dissolved (S,S)-[4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazin-1-

yl]acetic acid t-butyl ester (another name: (S,S)-4-[2-benzyloxycarbonylamino-3-(4-methoxyphenyl)propionyl]-3-(3-t-butoxycarbonylaminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester) (0.66 g, 0.97 mmol) produced in Reference Example 9. To the solution was added 10%Pd-C (0.26 g), and the mixture was stirred for one hour under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was dissolved in a mixture of 1,4-dioxane (6.6 ml) and H_2O (6.6 ml). To the solution were added, at room temperature, 4-guanidinobenzoic acid N-hydroxy-5-norbornene-2,3-dicarboximide ester (0.55 g, 1.45 mmol) and sodium hydrogencarbonate (0.12 g, 1.45 mmol). One hour later, the pH of the reaction system was adjusted with 1N HCl, and the reaction mixture was concentrated under reduced pressure. The concentrate was purified by means of a column chromatography (CHP-20, H_2O-5%CH_3CNaq-10%CH_3CNaq-15%CH_3CNaq-25%CH_3CNaq-30%CH_3CNaq) to afford (S,S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[2-(4-guanidinobenzoylamino)-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S,S)-3-(3-t-butoxycarbonylaminopropyl)-4-[2-(4-guanidinobenzoylamino)-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid t-butyl ester) (0.50 g, 73%) as a colorless amorphous powdery product. This product was dissolved in 1,4-dioxane (5.0 ml), to which were added, while stirring at 0°C and keeping the pH of the reaction system at 10 or below, 2N NaOH (2.46 ml, 4.93 mmol) and chlorocarbonic acid methyl ester (0.27 ml, 3.52 mmol). The mixture was stirred for 30 minutes at 0°C, whose pH was adjusted to 3 with 1N HCl, followed by shaking together with ethyl acetate for extraction. The organic layer was dried over anhydrous magnesium sulfate, followed by concentration under reduced pressure. The concentrate was purified by

means of a silica gel column chromatography (hexane/ethyl acetate = 1/10) to afford the title compound (0.42 g, 72%) as a colorless amorphous powdery product.

IR ν max cm^{-1} (KBr): 3400, 2970, 1730, 1640, 1510, 1490, 1435, 1362, 1245, 1155, 1025, 948

NMR(CD_3OD) δ: 1.39(9H,s), 1.46(9H,s), 1.20-1.65(2H,m), 1.65-2.06(2H,m), 2.41-2.64(1H,m), 2.88-4.18(7H,m), 3.46(2H,s), 3.59(1H,d,J=17.2Hz), 3.72(3H,s), 3.77(3H,s), 4.08(1H,d,J=17.2Hz), 4.78-4.97(1H,m), 5.11(1H,dd,J=6.2,9.2Hz), 6.85(2H,d,J=8.4Hz), 7.19(2H,d,J=8.4Hz), 7.30(2H,d,J=8.4Hz), 7.84(2H,d,J=8.4Hz).

Reference Example 94

(S,S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester

(another name: (S,S)-3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid t-butyl ester)

In a mixture of methanol (4.1 ml) and H_2O (0.41 ml) was dissolved (S,S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(1,3-dimethoxy-carbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)-propionyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S,S)-3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(1,3-dimethoxy-carbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)-propionyl]-2-oxopiperazine-1-acetic acid t-butyl ester) (0.41 g, 0.50 mmol) produced in Reference Example 93. To the solution was added, under ice-cooling, lithium hydroxide·1.0 hydrate (22.9 mg, 0.55 mmol). The mixture was stirred for 30 minutes at 0°C, followed by adjusting the pH to 4 with 1N HCl. The reaction

mixture was concentrated under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (ethyl acetate/methanol = 10/1) to afford the title compound (0.36 g, 95%) as a colorless amorphous powdery product.

IR ν max cm^{-1} (KBr): 3400, 2970, 1733, 1640, 1508, 1435, 1360, 1240, 1150

NMR(CD_3OD) δ: 1.39(9H,s), 1.46(9H,s), 1.20-2.05(4H,m), 2.42-2.64(1H,m), 2.84-4.20(9H,m), 3.68(3H,s), 3.77(3H,s), 4.80-5.00(1H,m), 5.10(1H,dd,J=9.0,6.4Hz), 6.84(2H,d,J=8.8Hz), 7.18(2H,d,J=8.8Hz), 7.45(2H,d,J=8.8Hz), 7.82(2H,d,J=8.8Hz).

Reference Example 95

(S,S)-[3-[3-(4-guanidinobenzoylamino)propyl]-4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride

(another name: (S,S)-3-[3-(4-guanidinobenzoylamino)propyl]-4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid hydrochloride)

In methylene chloride (2.0 ml) was dissolved (S,S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(3-methoxycarbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S,S)-3-(3-t-butoxycarbonylaminopropyl)-4-[2-[4-(3-methoxy-carbonylguanidino)benzoylamino]-3-(4-methoxyphenyl)propionyl]-2-oxopiperazine-1-acetic acid t-butyl ester) (0.35 g, 0.46 mmol) produced in Reference Example 94. To the solution was added, while stirring at room temperature, trifluoroacetic acid (2.0 ml). Two hours later, the reaction mixture was concentrated under reduced pressure. The concentrate was dissolved in a mixture of 1,4-dioxane (3.5 ml) and

H_2O (7.0 ml). To this solution were added, at room temperature, 4-guanidinobenzoic acid N-hydroxy-5-norbornene-2,3-dicarboximide ester (0.19 g, 0.50 mmol) and sodium hydrogencarbonate (0.19 g, 2.28 mmol). One hour later, the pH of the reaction system was adjusted to 2 with a 1N aqueous solution of hydrochloric acid. The reaction mixture was concentrated under reduced pressure. The concentrate was purified by means of a column chromatography (CHP-20, H_2O-5%CH_3CNaq-10%CH_3CNaq-15%CH_3CNaq-20%CH_3CNaq-25%CH_3CNaq), which was processed with a 1N aqueous solution of hydrochloric acid to lead to the corresponding hydrochloride, i.e. the title compound (0.29 g, 69%) as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +69.9° (C=1.025, MeOH)

Elemental Analysis for $C_{37}H_{44}N_{10}O_9 \cdot 2.0HCl \cdot 4.0H_2O$ (917.801):

Calcd.: C, 48.42; H, 5.93; N, 15.26

Found : C, 48.30; H, 5.78; N, 15.20.

Reference Example 96

(S)-[4-[[4-(3-methoxycarbonylguanidino)-benzoylamino]acetyl]-3-[3-[4-(3-methoxycarbonylguanidino)benzoylamino]propyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride (another name: (S)-4-[[4-(3-methoxycarbonylguanidino)benzoylamino]acetyl]-3-[3-[4-(3-methoxycarbonylguanidino)benzoylamino]propyl]-2-oxopiperazine-1-acetic acid hydrochloride)

In a mixture of 1,4-dioxane (3.0 ml) and H_2O (3.0 ml) was dissolved (S)-[4-[(4-guanidinobenzoylamino)acetyl]-3-[3-(4-guanidino-benzoylamino)propyl]-2-oxopiperazin-1-yl]acetic acid hydrochloride (another name: (S)-4-[(4-guanidinobenzoylamino)acetyl]-3-[3-(4-guanidino-benzoylamino)propyl]-2-oxopiperazine-1-acetic acid hydrochloride) (0.3 g, 0.51 mmol) produced in Reference

Example 26. To the solution were added gradually, under stirring at 0°C while keeping the pH at 10 or below, a 2N aqueous solution of sodium hydroxide (2.60 ml, 5.10 mmol) and chlorocarbonic acid methyl ester (0.31 ml, 4.00 mmol). The reaction mixture was stirred for 10 minutes at 0°C, whose pH was adjusted to 4 with a 1N aqueous solution of hydrochloric acid, followed by shaking together with ethyl acetate for extraction. The organic layer was dried over anhydrous magnesium sulfate, followed by concentration under reduced pressure. The concentrate was purified by means of a column chromatography (CHP-20, 10% CH_3CNaq-15%CH_3CNaq-20%CH_3CNaq-25%CH_3CNaq-35%CH_3CNaq) to give (S)-[4-[[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]acetyl]-3-[3-[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]-propyl]-2-oxopiperazin-1-yl]acetic acid (another name: (S)-4-[[4-(1,3-dimethoxycarbonylguanidino)-benzoylamino]acetyl]-3-[3-[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]-propyl]-2-oxopiperazine-1-acetic acid) (0.26 g, 62%) as a colorless amorphous powdery product. This product (0.26 g, 0.31 mmol) was dissolved in a mixture of methanol (2.6 ml) and H_2O (0.26 ml). To the solution was added, under ice-cooling, lithium hydroxide·1.0hydrate (42 mg, 1.00 mmol). One hour later, the reaction system was adjusted to pH 4 with a 1N aqueous solution of hydrochloric acid, followed by concentration under reduced pressure. The concentrate was purified by means of a column chromatography [(CHP-20, 5%CH_3CNaq-10%CH_3CNaq-15%CH_3CNaq-20%CH_3CNaq) and (LH-20, H_2O)] to afford the title compound (0.13 g, 58%) as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +48.2° (C=1.043, MeOH)

Elemental Analysis for $C_{31}H_{38}N_{10}O_{10}\cdot 1.0HCl\cdot 3.0H_2O$ (801.211):

Calcd.: C, 46.47; H, 5.66; N, 17.48

Found : C, 46.30; H, 5.38; N, 17.35.

Reference Example 97

(S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[(4-guanidinobenzoylamino)acetyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-3-(3-t-butoxycarbonylaminopropyl)-4-[(4-guanidinobenzoyl-amino)acetyl]-2-oxopiperazine-1-acetic acid t-butyl ester)

In ethyl acetate (7.0 ml) was dissolved (S)-[4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonyl-aminopropyl)-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-4-benzyloxycarbonylaminoacetyl-3-(3-t-butoxycarbonyl-aminopropyl)-2-oxopiperazine-1-acetic acid t-butyl ester) (0.70 g, 1.24 mmol) produced in Reference Example 2. To the solution was added 10%Pd-C (0.21 g), which was stirred for one hour at room temperature under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was dissolved in a mixture of 1,4-dioxane (7.0 ml) and H_2O (7.0 ml). To the solution was added, at room temperature, 4-guanidinobenzoic acid N-hydroxy-5-norbornene-2,3-dicarboximide ester (0.56 g, 1.49 mmol). One hour later, the reaction system was adjusted to pH 4 with a 1N aqueous solution of hydrochloric acid, which was concentrated under reduced pressure. The concentrate was purified by means of a column chromatography (CHP-20, H_2O-5%CH_3CNaq-10%CH_3CNaq-15%CH_3CNaq-20%CH_3CNaq) to afford the title compound (0.70 g, 96%) as a colorless amorphous powdery product.

IR ν max cm^{-1} (KBr): 3320, 2970, 2920, 1730, 1640, 1560, 1500, 1445, 1360, 1250, 1155

NMR(CD_3OD) δ: 1.42(9H,s), 1.48(9H,s), 1.02-2.17(4H,m), 2.90-3.20(2H,m), 3.36-4.64(6H,m), 4.00(1H,d,J=17.5Hz), 4.12(1H,d,J=17.5Hz), 4.82-5.03(1H,m),

7.38(2H,d,J=8.6Hz), 7.97(2H,d,J=8.6Hz).

Reference Example 98

(S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]acetyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]-acetyl]-2-oxopiperazine-1-acetic acid t-butyl ester)

In a mixture of 1,4-dioxane (7.0 ml) and H_2O (7.0 ml) was dissolved (S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[(4-guanidinobenzoyl-amino)acetyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-3-(3-t-butoxycarbonylaminopropyl)-4-[(4-guanidinobenzoyl-amino)acetyl]-2-oxopiperazine-1-acetic acid t-butyl ester) (0.70 g, 1.19 mmol) produced in Reference Example 97. To the solution were added gradually, under stirring at 0°C while keeping the pH of the reaction system at 10 or below, a 2N aqueous solution of sodium hydroxide (4.20 ml, 8.33 mmol) and chlorocarbonic acid methyl ester (0.46 ml, 5.94 mmol). The mixture was stirred for 30 minutes at 0°C, then the reaction system was adjusted to pH 4 with a 1N aqueous solution of hydrochloric acid, which was shaken together with ethyl acetate for extraction. The organic layer was dried over anhydrous magnesium sulfate, which was concentrated under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (ethyl acetate/methanol = 13/1) to afford the title compound (0.67 g, 80%) as a colorless amorphous powdery product.

IR ν max cm^{-1} (KBr): 3380, 2970, 1730, 1640, 1490, 1433, 1362, 1250, 1155

NMR(CD_3OD) δ: 1.42(9H,s), 1.48(9H,s), 1.30-2.15(4H,m), 2.98-3.20(2H,m), 3.45(3H,s), 3.72(3H,s), 3.34-4.70(8H,m), 4.85-5.05(1H,m), 7.32(2H,d,J=8.5Hz),

7.91(2H,d,J=8.5Hz).

Reference Example 99

(S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(3-methoxycarbonylguanidino)benzoylamino]acetyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(3-methoxycarbonylguanidino)benzoylamino]acetyl]-2-oxopiperazine-1-acetic acid t-butyl ester)

In a mixture of methanol (6.7 ml) and H_2O (0.67 ml) was dissolved (S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]acetyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(1,3-dimethoxycarbonylguanidino)benzoylamino]acetyl]-2-oxopiperazine-1-acetic acid t-butyl ester) (0.67 g, 0.95 mmol) produced in Reference Example 98. To the solution was added, under ice-cooling, lithium hydroxide·1.0 hydrate (45.8 mg, 1.09 mmol). The mixture was stirred for 30 minutes at 0°C, and the reaction mixture was adjusted to pH 4 with a 1N aqueous solution of hydrochloric acid, followed by concentration under reduced pressure. The concentrate was purified by means of a silica gel column chromatography (ethyl acetate/methanol = 10/1-5/1) to afford the title compound (0.44 g, 72%) as a colorless amorphous powdery product.

IR ν max cm^{-1} (KBr): 3390, 2970, 2925, 1730, 1640, 1525, 1435, 1360, 1240, 1155

NMR(CD_3OD) δ: 1.42(9H,s), 1.47(9H,s), 1.20-2.14(4H,m), 2.96-3.18(2H,m), 3.68(3H,s), 3.98(1H,d,J=17.4Hz), 4.12(1H,d,J=17.4Hz), 3.22-4.66(6H,m), 4.82-5.04(1H,m), 7.45(2H,d,J=8.6Hz), 7.86(2H,d,J=8.6Hz).

Reference Example 100

(S)-[3-[3-(4-guanidinobenzoylamino)propyl]-4-[[4-(3-methoxycarbonylguanidino)benzoylamino]acetyl]-2-oxopiperazin-1-yl]acetic acid trifluoroacetate

(another name: (S)-3-[3-(4-guanidinobenzoylamino)-propyl]-4-[[4-(3-methoxycarbonylguanidino)-benzoylamino]acetyl]-2-oxopiperazine-1-acetic acid trifluoroacetate)

In methylene chloride (4.4 ml) was dissolved (S)-[3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(3-methoxy-carbonylguanidino)benzoylamino]acetyl]-2-oxopiperazin-1-yl]acetic acid t-butyl ester (another name: (S)-3-(3-t-butoxycarbonylaminopropyl)-4-[[4-(3-methoxy-carbonylguanidino)benzoylamino]acetyl]-2-oxopiperazine-1-acetic acid t-butyl ester) (0.44 g, 0.68 mmol) produced in Reference Example 99. To the solution was added, while stirring at room temperature, trifluoroacetic acid (4.4 ml). One hour later, the reaction system was concentrated under reduced pressure. The concentrate was dissolved in a mixture of 1,4-dioxane (4.4 ml) and H_2O (4.4 ml). To this solution were added, at room temperature, 4-guanidinobenzoic acid N-hydroxy-5-norbornene-2,3-dicarboximide ester (0.31 g, 0.82 mmol) and sodium hydrogencarbonate (0.29 g, 3.40 mmol). One hour later, the reaction system was adjusted to pH 2 with a 1N aqueous solution of hydrochloric acid, which was concentrated under reduced pressure. The concentrate was purified by means of a column chromatography [(CHP-20, H_2O-5%CH_3CNaq-10%CH_3CNaq-15%CH_3CNaq) and (LH-20, H_2O)] to afford the title compound (0.18 g, 32%) as a colorless amorphous powdery product.

Specific optical rotation: $[\alpha]_D^{20}$ +46.9° (C=0.976, MeOH)

Elemental Analysis for $C_{29}H_{36}N_{10}O_8 \cdot 1.0CF_3CO_2H \cdot 3.0H_2O$ (820.737):

Calcd.: C, 45.37; H, 5.28; N, 17.07

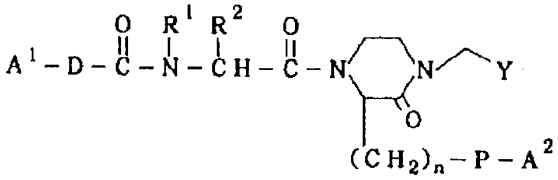
Found : C, 45.42; H, 5.08; N, 16.92.

The present invention provides, by dispersing and atomizing an amorphous water-soluble 2-piperazinone-1-

acetic acid compound in a polymer solution, a sustained-release microcapsule containing the compound in a high concentration and reduced in the initial drug release. Furthermore, use of this microcapsule can reduce undesirable side effects such as hemorrhage for a long period caused by a large amount of initial release of the above compound which is useful as, for example, the prophylaxis or treatment of thrombosis, angina pectoris, unstable angina or ischemic complication, reobstruction or restenosis after percutaneous transluminal coronary angioplasty or coronary thrombolytic therapy.

Claims

1. A microcapsule comprising (i) an amorphous water-soluble 2-piperazinone-1-acetic acid compound of the formula (I):



(I)

wherein A^1 and A^2 independently are a proton-accepting group or a group convertible into a proton-accepting group; D is a spacer having a 2- to 6-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain); R^1 is a hydrogen atom or a hydrocarbon group; R^2 is a hydrogen atom or a residual group formed by removing $-CH(NH_2)COOH$ from an α-amino acid, or R^1 and R^2 may be combined to form a 5- or 6-membered ring; P is a spacer having a 1- to 10-atomic chain optionally bonded through a hetero-atom and/or a 5- or 6-membered ring (provided that the 5- or 6-membered ring is, depending on its bonding position, counted as 2- or 3-atomic chain); Y is an optionally esterified or amidated carboxyl group; and n denotes an integer of 0 to 8 or salt thereof, and (ii) a polymer.

2. A microcapsule of Claim 1, which is a sustained-release microcapsule.

3. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound or salt thereof is dispersed in the polymer.

4. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound or salt thereof is readily soluble in water.

5. A microcapsule of Claim 1, wherein the water-solubility of the 2-piperazinone-1-acetic acid compound

or salt thereof is not less than about 1 g/100 ml at 20 C°.

6. A microcapsule of Claim 1, wherein the average particle size of the 2-piperazinone-1-acetic acid compound or salt thereof is not more than about 30 μm.

7. A microcapsule of Claim 1, wherein the average particle size of the 2-piperazinone-1-acetic acid compound or salt thereof is not more than about 5 μm.

8. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound is (S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid.

9. A microcapsule of Claim 1, which comprises (S)-4-(4-guanidinobenzoylamino)acetyl-3-[3-(4-guanidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid hydrochloride.

10. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound is (S)-4-(4-amidinobenzoyl)aminoacetyl-3-{3-(4-amidinobenzoyl)amino}propyl-2-oxopiperazine-1-acetic acid.

11. A microcapsule of Claim 1, which comprises (S)-4-(4-amidinobenzoyl)aminoacetyl-3-{3-(4-amidinobenzoyl)amino}propyl-2-oxopiperazine-1-acetic acid trifluoroacetate.

12. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound is (S)-4-[4-(2-aminoethyl)benzoylamino)acetyl-3-[3-(4-amidinobenzoylamino)]propyl-2-oxopiperazine-1-acetic acid.

13. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound is (S)-4-(4-amidinobenzoylamino)acetyl-3-[2-(4-guanidinobenzoylamino)]ethyl-2-oxopiperazine-1-acetic acid.

14. A microcapsule of Claim 1, wherein the 2-piperazinone-1-acetic acid compound is (S)-4-(4-amidinobenzoyl)aminoacetyl-3-[3-(4-guanidinobutanoylamino)]propyl-2-oxopiperazine-1-acetic acid.

15. A microcapsule of Claim 1, wherein the polymer is a biodegradable polymer.

16. A microcapsule of Claim 15, wherein the biodegradable polymer is a polyester.

17. A microcapsule of Claim 16, wherein the polyester is a lactic acid/glycolic acid copolymer or homopolymer.

18. A microcapsule of Claim 17, wherein the molar ratio of lactic acid/glycolic acid of the copolymer or homopolymer is about 100/0 to about 25/75.

19. A microcapsule of Claim 17, wherein the weight average molecular weight of the lactic acid/glycolic acid copolymer or homopolymer is about 5000 to about 30000.

20. A microcapsule of Claim 16, wherein the polyester is hydroxybutyric acid/glycolic acid copolymer or homopolymer.

21. A microcapsule of Claim 20, wherein the molar ratio of hydroxybutyric acid/glycolic acid of the copolymer or homopolymer is about 100/0 to about 25/75.

22. A microcapsule of Claim 20, wherein the weight-average molecular weight of the hydroxybutyric acid/glycolic acid copolymer or homopolymer is about 5000 to about 25000.

23. A microcapsule of Claim 1, which is used for the prophylaxis or treatment of diseases in the circulatory system.

24. A microcapsule of Claim 1, which is used for the prophylaxis or treatment of thrombosis, angina pectoris, unstable angina or ischemic complication, reobstruction or restenosis after percutaneous transluminal coronary angioplasty or coronary thrombolytic therapy.

25. A microcapsule which is produced by dispersing, in an aqueous phase, a dispersion of an amorphous water-soluble 2-piperazinone-1-acetic acid compound of the

formula (I) or salt thereof as defined in Claim 1, in a solution of a polymer in an organic solvent to obtain an s/o/w type emulsion, and then, subjecting the obtained emulsion to in-water drying.

26. A microcapsule of Claim 25, wherein the concentration of the 2-piperazinone-1-acetic acid compound or salt thereof in the solution of a polymer in an organic solvent is about 0.01 to about 70% (w/w).

27. A microcapsule of Claim 25, wherein the solution of a polymer in an organic solvent further contains a basic substance.

28. A microcapsule of Claim 27, wherein the basic substance is a basic amino acid.

29. A microcapsule of Claim 27, wherein the basic substance is L-arginine, L-lysine or N-methylglucamine.

30. A microcapsule of Claim 25, wherein the concentration of the basic substance in the solution of a polymer in an organic solvent is about 0.1 to about 3% (w/w).

31. A microcapsule of Claim 25, wherein the aqueous phase further contains an osmotic pressure adjustor.

32. A microcapsule of Claim 31, wherein the osmotic pressure adjustor is a sodium chloride.

33. A method of producing a microcapsule, which comprises dispersing, in an aqueous phase, a dispersion of an amorphous water-soluble 2-piperazinone-1-acetic acid compound of the formula (I) or salt thereof as defined in Claim 1, in a solution of a polymer in an organic solvent to obtain an s/o/w type emulsion, and then, subjecting the obtained emulsion to in-water drying.

34. Use of an amorphous water-soluble 2-piperazinone-1-acetic acid compound of the formula (I) or salt thereof as defined in Claim 1 for manufacture of a microcapsule of Claim 1.

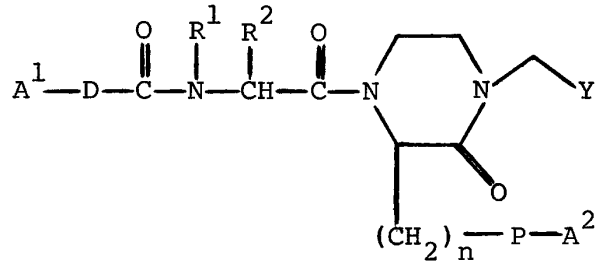
35. Use of a microcapsule of Claim 1 for a medicine

for preventing or treating thrombosis, angina pectoris, unstable angina or ischemic complication, reobstruction or restenosis after percutaneous transluminal coronary angioplasty or coronary thrombolytic therapy.

36. A method for preventing or treating thrombosis, angina pectoris, unstable angina or ischemic complication, reobstruction or restenosis after percutaneous transluminal coronary angioplasty or coronary thrombolytic therapy in a mammal which comprises administering an effective amount of a microcapsule of Claim 1 to said mammal.

37. A sustained-release microcapsule comprising:

(i) a pharmaceutically effective amount of microparticles of a physiologically active amorphous water-soluble 2-piperazinone-1-acetic acid compound of the formula:

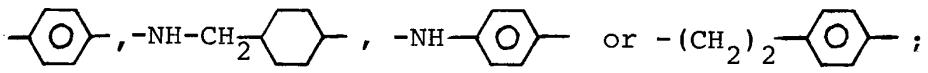


(I)

(wherein:

A^1 and A^2 are independently (1) an amidino or guanidino group which may be substituted with C_{2-8} alkoxycarbonyl, (2) an amino group which may be substituted with an oxadiazolyl group which may further be substituted with oxo or C_{1-4} alkyl which may still further be substituted with halogen or (3) an oxadiazolyl or thiadiazolyl group which may be substituted with oxo or C_{1-4} alkyl which may still further be substituted with halogen;

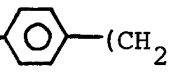
D is a group of the formula:



R^1 is a hydrogen atom;

R^2 is a hydrogen atom or a C_{1-4} alkyl group substituted with phenyl which may further be substituted with C_{1-4} alkoxy;

P is a group of the formula -Z-B- [in which Z is

-NHCO-, -NH-, -CO-, -CS-, -O-, -OCO-, -S-, -SCO- or a bond and may be bonded to B in either direction and B is $-(CH_2)_a$  $-(CH_2)_b-$, $-(CH_2)_c-$ or a bond, in which a is an integer of 0 to 2, b is an integer of 0 to 2, c is an integer of 1 to 5, provided that both Z and B are not a bond simultaneously];

Y is a group of the formula: $-COR^7$ [in which R^7 is 1) a hydroxyl group, 2) a C_{1-8} alkoxy or C_{2-12} alkenyloxy group which may be substituted with C_{1-4} alkoxycarbonyl or 5-methyl-2-oxo-1,3-dioxolen-4-yl or 3) a group of the formula: $-OCH(R^{7a})OCOR^8$ in which R^{7a} is a hydrogen atom or a C_{1-6} alkyl group and R^8 is a C_{1-6} alkyl group or a C_{5-7} cycloalkyloxy group]; and

n is an integer of 0 to 8) or a pharmaceutically acceptable salt thereof, and

(ii) a hardly water-soluble or water-insoluble biodegradable polymer in an amount of 0.2 to 10,000 times by weight relative to the 2-piperazinone-1-acetic acid compound or salt thereof.

38. A method of producing the sustained-release microcapsule as defined in claim 37, which comprises:

(A) providing a dispersion of the amorphous water-soluble 2-piperazinone-1-acetic acid compound of the formula (I) or salt thereof as defined in claim 37, dispersed in a solution of the hardly water-soluble or water-insoluble

biodegradable polymer in an organic water-insoluble solvent which dissolves the biodegradable polymer, the dispersion having a concentration of the polymer of 0.5 to 90%(w/w);

(B) mixing the dispersion made in step (A) into an aqueous phase so as to form an s/o/w type emulsion; and

(C) subjecting the s/o/w type emulsion to an in-water drying to remove the organic solvent.

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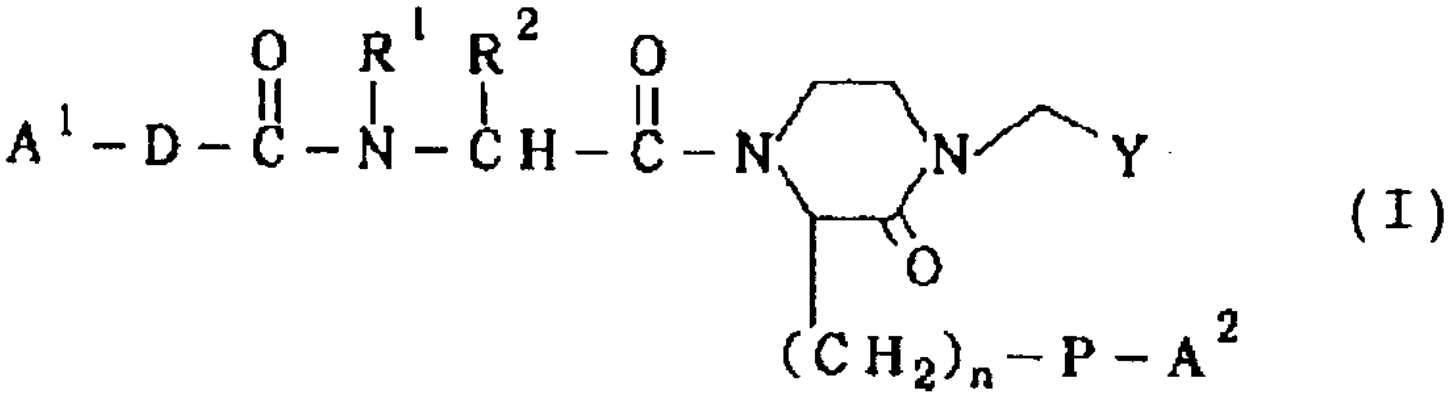
Abstract

A microcapsule containing an amorphous water-soluble 2-piperazinone-1-acetic acid compound or salt thereof and a polymer and a method of preparing said microcapsule, which comprises dispersing in an aqueous phase a dispersion of the amorphous water-soluble 2-piperazinone-1-acetic acid compound or salt thereof in a solution of a polymer in an organic solvent to give an s/o/w type emulsion and subjecting the emulsion to in-water drying.

The sustained-release microcapsule which is advantageous in entrapping 2-piperazinone-1-acetic acid compound or the salt thereof as a drug in a high concentration, and in the reduced initial release of the drug, thereby reducing undesirable side effects of the drug.



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