PROTEASE INHIBITOR CONJUGATES

This invention relates to inhibitors of serine proteases, e.g. thrombin, and conjugates of such inhibitors used as thrombus imaging agents.

Thrombin and other serine proteases and inhibitors therefor are discussed in WO 94/20526, the disclosure of which is incorporated herein by reference.

It has now been found that peptidic and pseudo-peptidic trypsin-like serine protease inhibitors which are transition state analogues and which comprise an amino group which can be modified with a chelating group without interfering with or preventing their binding to thrombin, provide novel compounds having interesting properties.

By transition state analogues are meant compounds as defined by A.J. Barrett et al. in Proteinase Inhibitors, Ed. Elsevier Amsterdam, 1986, p. 57.

The term "pseudo-peptide" or "pseudo-peptidic" as used herein refers to a peptide isostere in which the peptide bond between 2 amino acid residues, whether natural or unnatural, is replaced with any isosteric group, e.g. $-CH_2-NH-$. The pseudo-peptide may comprise one or more such isosteric bonds.

According to the invention, there is provided a trypsin-like serine protease inhibiting peptide or

pseudo-peptide bearing a chelating group capable of complexing a detectable element, the chelating group being linked to an amino group of the peptide or pseudo-peptide such that the chelating group does not interfere with or prevent the binding of the peptide or pseudo-peptide to thrombin.

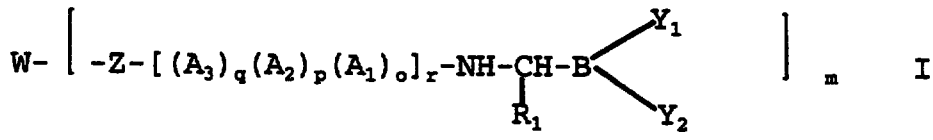
These compounds are referred to thereafter as compounds of the invention.

The chelating group is linked by a covalent bond to the amino group of the peptide or pseudo-peptide. The chelating group is preferably attached to the N-terminal amino group of the peptide or pseudo-peptide.

The chelating group may be attached either directly or indirectly, e.g. by means of a spacer group, to the amino group of the peptide or pseudo-peptide. Preferably the chelating group is coupled to the peptide or pseudo-peptide or to the peptide-spacer or (pseudo-peptide)-spacer moiety by an amide bond.

Preferred compounds of the invention are those comprising a C-terminal boronic acid derivative residue, particularly a C-terminal boronic acid derivative of lysine, ornithine, arginine or an aliphatic amino alcohol. More preferred are those comprising additionally at least one unnatural α-amino acid having a hydrophobic side chain.

In one embodiment the present invention provides a compound of formula I



I

wherein

Y_1 and Y_2, independently, are OH or F or, taken together, form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising 1 to about 20 carbon atoms and, optionally, a heteroatom which can be N, S, or O;

R_1 is a substituted alkyl selected from the group consisting of $-(CH_2)_z-X$, $-CH(CH_3)-(CH_2)_2-X$, $-CH_2-CH(CH_3)-CH_2-X$, $-(CH_2)_2-CH(CH_3)-X$, and $-(CH_2)_2-C(CH_3)_2-X$, where X is $-NH_2$, $-NH-C(NH)-NH_2$ or $-S-C(NH)-NH_2$, and z is 3 to 5;

m is 1 or 2;

r, o, p and q are, independently, either 1 or 0;

A_1, A_2 and A_3, independently, are amino acids of L- or D-configuration selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val;

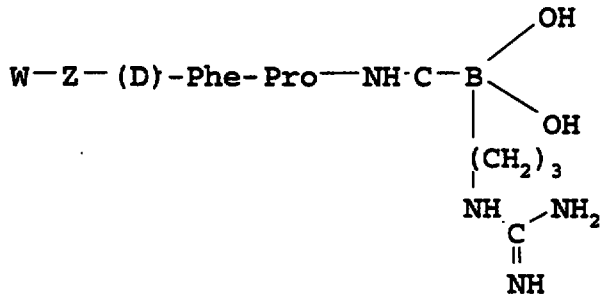
W is a chelating group attached to the N-terminal amino group of the compound, and

Z is a direct bond or a spacer

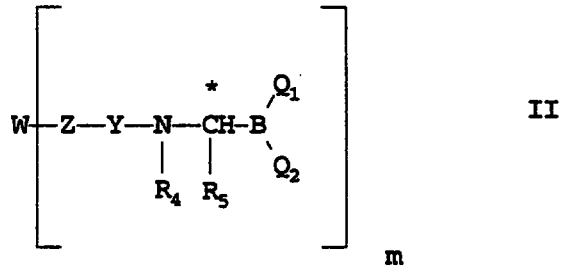
in free form or in salt form.

Preferred significances for A_1, A_2, A_3, R_1, Y_1, Y_2, p, q, r and o are as disclosed in EP EP-A2-293,881, the disclosure of which is incorporated herein by reference.

A particularly preferred compound of formula I is



In a further or alternative embodiment the invention provides a compound of formula II



II

wherein W, Z and m are as defined above,

Y is a sequence of n amino acids selected such that the n+1 amino acid peptide Y-Lys or Y-Arg has an affinity for the active site of a trypsin-like protease, where n is an integer from 1 to 10 preferably 1 to 4, and in which at least one amino acid is an unnatural amino acid having a hydrophobic side chain;

Q_1 and Q_2, which may be the same or different, are selected from -OH, COR_2, $-CONR_2R_3$, $-NR_2R_3$, and $-OR_6$, or Q_1 and Q_2 taken together form a diol residue, and wherein R_2, R_3 and R_6, which may be the same or different, are C_{1-10}alkyl, C_{6-10}aryl, C_{6-10}aralkyl, or phenyl substituted by up to three groups selected from C_{1-4}alkyl, halogen and C_{1-4}alkoxy;

R_4 is hydrogen or C_{1-10}alkyl;

R_5 is a group $-Z_1-X_1$,

wherein Z_1 is $-(CH_2)_s-$; $-CH(CH_3)-(CH_2)_2-$, $-CH_2-CH(CH_3)-CH_2-$, $-(CH_2)_2-CH(CH_3)-$, $-(CH_2)_2-C(CH_3)_2-$, $-CH(CH_3)-(CH_2)_3-$, $-CH_2-CH(CH_3)-(CH_2)_2-$, $-(CH_2)_2-CH(CH_3)-CH_2-$, $-(CH_2)_3-C(CH_3)_2-$, $-(CH_2)_2-C(CH_3)_2-CH_2-$, $(CH_2)_3-CH(CH_3)-$, $-(CH_2)_3-CH(CH_3)-CH_2-$, C_{6-10}aryl, or C_{6-10}aralkyl

wherein s is 2, 3, 4 or 5;

and wherein X_1 is $-NH_2$, $-NH-C(NH)-NH_2$, $-S-C(NH)-NH_2$, $-N_3$, C_{1-4}alkoxy, C_{1-4}alkylthio, $-Si(CH_3)_3$, OH, SH, NR_7R_8, or phenyl, optionally substituted by up to three groups selected from halogen, OH and C_{1-4}alkoxy, or $C(NH)R_{10}$;

wherein R_7 is H or C_{1-10}alkyl; R_8 is C_{1-10}alkyl, $-CO-R_9$ or $CS-R_9$; R_{10} is C_{1-3}alkyl or $N(CH_3)_2$, or $NR_{11}R_{12}$;

wherein R_9 is H or C_{1-10}alkyl, C_{1-10}alkoxy, C_{6-10}aryl, C_{6-10}aralkyl and R_{11} and R_{12} (which may be the same or different) are H or C_{1-10}alkyl;

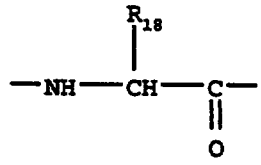
or R_4 and R_5 together form a trimethylene group,

and the asymmetric carbon atom marked * may have the D- or L-configuration, or represent any mixture of these.

Preferred significances for Y, R_4, R_5, Q_1 and Q_2 are as indicated below and also as disclosed in EP-A-471,651 and WO 94/20526, the disclosure of which is incorporated herein by reference.

By an unnatural amino acid is meant any amino acid other than D- or L- Ala, Arg, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val.

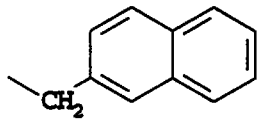
Preferred unnatural amino acids are of formula III

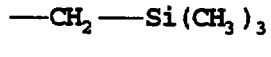


III

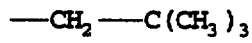
in which R_{18} is a hydrophobic group. Preferred hydrophobic groups consist of a methylene group linked to an aromatic group optionally substituted by a polar group, to an alicyclic group having at least two rings and no polar substituents, or to a tert.butyl or trimethylsilyl group. Preferably R_{18} is R_{18}' where R_{18}' is a group of formulae c), d), e), f), g), h) or i)

c)

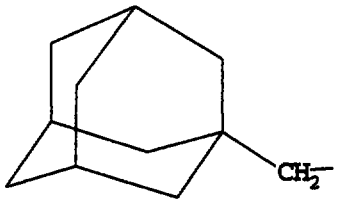




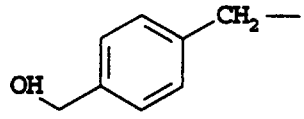
d)



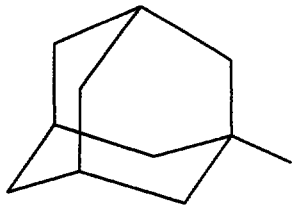
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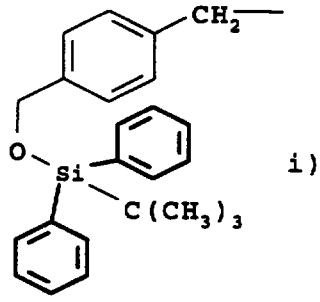
e)



h)



g)

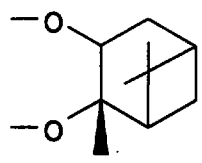


i)

The unnatural amino acids of formula III may be in D- or

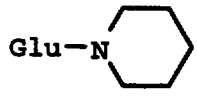
L-form or any mixture of these, but are preferably in D-form.

Particularly preferred compounds of formula II are those compounds in which Q_1 and Q_2 taken together represent the group OPin of formula a).



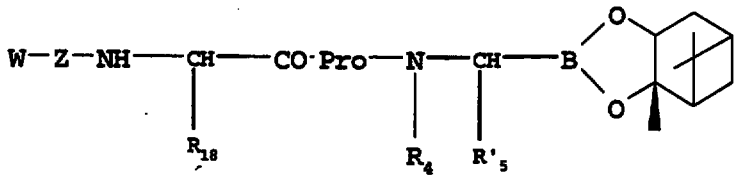
a)

The amino acids constituting Y are α-amino acids which may be selected from the L-amino acids naturally occurring in proteins, their corresponding enantiomeric D-amino acids or chemically modified alpha-amino acids such as glutamic acid gamma-piperidide:



or pipecolic acid (Pip), provided that at least one amino acid is an unnatural amino acid having a hydrophobic side chain.

More preferred compounds of formula II are those in which Y is a sequence of two amino acids, of which the N-terminal amino acid is the unnatural amino acid and the other amino acid is L-proline (Pro), e.g. a compound of formula IIa



IIa

in which W, Z, R_4 and R_{18} are as defined above, and R'_5 is $-(CH_2)_{s'}-X_1'$,

in which X_1' is $-NH_2$, $-NH-C(NH)-NH_2$, $-N_3$, OH $-Si(CH_3)_3$, phenyl, monochloro-phenyl, dimethylamino-phenyl, $-NH-C(O)-CH_3$, $-NHC(O)H$, $-NHC(S)-NHCH_3$, $-NH-C(O)-CH(CH_3)_2$, $-NH-C(O)-CH_2-CH_3$, $-NH-C(O)-CH_2-Cl$ or $-NH-C(O)-OCH_3$, and s' is 2,3 or 4.

Suitable chelating groups e.g. as W are physiologically acceptable chelating groups capable of complexing a detectable element.

Preferably the chelating group has a substantially hydrophilic character. Examples of chelating groups include iminodicarboxylic groups or polyaminopolycarboxylic groups, e.g. those derived from non cyclic ligands such as ethylene diaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), ethylene glycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), N,N'-bis(hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) and triethylenetetramine hexaacetic acid (TTHA); those derived from substituted-EDTA or -DTPA, such as p-isothiocyanato-benzyl-EDTA or -DTPA, p-amino-benzyl-DTPA or ω-amino-C_{1-4}alkylene-DTPA; those derived from macrocyclic ligands, e.g. 1,4,7,10-tetra azacyclo-dodecane-N,N',N",N'''-tetraacetic acid (DOTA), 1,4,7,10-tetra-azacyclotridecane-1,4,7,10-tetraacetic acid (TITRA) and 1,4,8,11-tetraazacyclotetradecane -N,N',N",N'''-tetraacetic acid (TETA); those derived from N-substituted or C-substituted macrocyclic amines as mentioned above including also cyclames, such as 1,4,8,11-tetraazacyclotetradecane (TETRA), and as disclosed in EP 304,780 A1 and in WO 89/01476-A.

Chelating groups particularly suitable for Tc labelling may be any of the known chelating groups capable of

complexing ^{99m}Tc, e.g. as disclosed in EP-A-279,417, DOS 41 28 183 or WO 92/05154, the disclosures of which are incorporated herein by reference. These complexing groups may be linked to the peptide or pseudo-peptide via a group of formula $-(R_{20})_{n'}-R_{21}$ wherein n' is 0 or 1, R_{20} is C_{1-6}alkylene, $-O-C_{1-6}$alkylene or $-NH-C_{1-6}$alkylene and R_{21} is -NCS, a carboxy group or a functional derivative thereof, e.g. acid halide, anhydride or hydrazide, optionally through a spacer group.

Preferred chelating groups are radicals of formula (j)

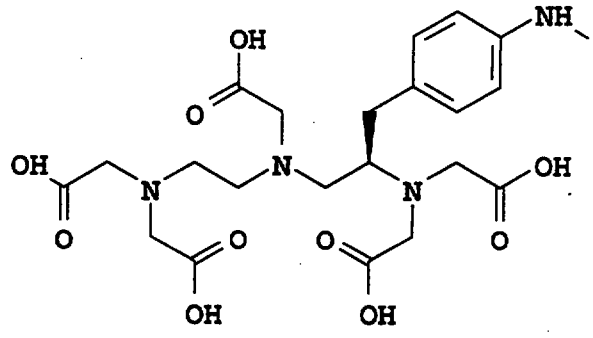
$$W'-CH_2-X_a-NH- \qquad (j)$$

wherein

W' is a chelating group derived from a non-cyclic or cyclic polyamino-polyacetic acid or anhydride, and

X_a is phenylene or C_{1-3}alkylene.

Groups derived from DTPA or substituted DTPA, e.g.



are preferred as chelating groups.

Suitable spacer groups, e.g. as Z, include groups of formula (k)

$$-R_{22}-R_{23}-CO- \qquad (k)$$

wherein

R_{22} is a divalent residue derived from a functional moiety capable of covalently reacting with the chelating agent, and

R_{23} is C_{1-11}alkylene optionally interrupted by one or more heteroatoms or residues selected from O, S, CO, -NH-, -NHCO-, N(C_{1-4}alkyl)-CO- and -N(C_{1-4}alkyl)-, C_{2-11}alkenylene or -CH(R_{24})- wherein R_{24} is a residue attached to a natural or unnatural α-amino acid, e.g. hydrogen, C_{1-11}alkyl, benzyl, optionally substituted benzyl, naphthyl-methyl, pyridyl-methyl.

In the compounds of formulae I and II, m is preferably 1.

The compounds of the invention may exist in free or salt form. Salts include acid addition salts, e.g. with organic acids, polymeric acids or inorganic acids; for example, hydrochlorides and acetates, and salt forms obtainable with the carboxylic acid groups present in the chelating group, e.g. alkali metal salts such as sodium or potassium, or substituted or unsubstituted ammonium salts.

The present invention also includes a process for the production of the compounds of the invention. They may be produced by analogy to known methods.

The compounds of the invention may be produced for example as follows:

a) removing at least one protecting group which is present in a trypsin-like serine protease inhibiting peptide of the invention bearing a protecting group;

b) linking together by an amide bond two peptide fragments each of them containing at least one amino acid in protected or unprotected form and one of them containing the chelating group, wherein the amide bond is such that the desired amino acid sequence is obtained, and then optionally effecting step a) above;

c) linking together a chelating agent and the desired peptide in protected or unprotected form in such a way that the chelating group is attached to the desired amino group of the peptide, and then optionally effecting step a) above; or

d) removing or converting a functional group of an unprotected or a protected peptide bearing a chelating group so that the desired unprotected or protected peptide bearing a chelating group is obtained and if appropriate carrying out step a) above,

and recovering the compound of the invention thus obtained in free form or in salt form.

The above reactions may be effected analogously to known methods, e.g. as described in the following examples, in particular process steps a) and c) above. When the chelating group is attached by an amide bond, this may be carried out analogously to the methods used for amide formation. Where desired, in these reactions, protecting groups which are suitable for use in peptides or for the desired chelating groups may be used for functional groups which do not participate in the reaction. The term protecting group may also include a polymer resin having functional groups.

When it is desired to attach the chelating group to the N-terminal amino group of a peptide or peptide fragment used as starting material, and which comprises one or more side chain amino groups, these side chain amino groups are conveniently protected with a protecting group, e.g. as used in peptide chemistry.

The peptide fragment bearing the chelating group and used in step b) above may be prepared by reacting the peptide fragment comprising at least one amino acid in protected or unprotected form with the chelating agent. The reaction may be performed by analogy with step c).

The chelating agent used in process step c) may be known or prepared analogously to known procedures. The compound used is such that it allows the introduction of the desired chelating group on the peptide, e.g. a polyaminopolycarboxylic acid as disclosed, a salt or anhydride thereof.

In the above process, amino-acids, peptide fragments or peptides used as starting materials, which have the chelating group attached through a spacer group to the peptide, e.g. a radical of formula (j) as defined above, may be prepared by reacting the corresponding amino-acids or peptides free from spacer group in conventional manner with a corresponding spacer-yielding compound, such as an acid or reactive acid derivative comprising the spacer group. For example, an acid of formula R'_{22}-R_{23}-COOH or a reactive acid derivative thereof such as an active ester, wherein R'_{22} is a functional moiety capable of covalently reacting with the chelating agent may be used. Examples of active ester groups or carboxy activating groups are e.g. 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, succinimidyl or

1-hydroxy-benzotriazolyl.

Alternatively the chelating agent may be reacted first with a spacer group-yielding compound, in order to bear the spacer group and then be reacted in conventional manner with the peptide, peptide fragment or amino-acid.

The peptides or peptide fragments used as starting materials in process steps b), c) and d) may be prepared e.g. as disclosed in EP-A2-293,881, EP-A2-471,561, or WO 94/20526.

The compounds of the invention may be purified in conventional manner, e.g. by chromatography. Preferably the compounds of the invention contain less than 5% by weight of peptides free of chelating groups.

In accordance with the present invention it has now been found that the compounds of the invention exhibit pharmacological activity and are therefore pharmaceutically useful.

The compounds of the invention have trypsin-like serine protease inhibiting properties, as indicated in the test methods descibed in WO 94/20526

The compounds of the invention are useful as inhibitors of trypsin-like serine proteases and may be employed and administered as described in WO 94/20526.

The compounds of the invention may be administered in free form or in pharmaceutically acceptable salt form. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing, the present invention further provides:

a) a compound of the invention or a pharmaceutically acceptable salt thereof for use as a pharmaceutical;

b) a method of preventing or treating disorders caused by an excess of one or more trypsin-like serine protease in a subject in need of such a treatment, which method comprises administering to said subject an effective amount of a compound of the invention or a pharmaceutically acceptable salt thereof;

c) a pharmaceutical composition comprising a compound of the invention in free form or in pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier.

According to a further embodiment of the invention, the compounds of the invention can be complexed with a detectable element.

Accordingly, the present invention also provides the compounds of the invention as defined above which are complexed with a detectable element (hereinafter referred to as chelates of the invention), in free form or in salt form, their preparation and their use as diagnostic agents.

By detectable element is meant any element, preferably a metal ion which exhibits a property detectable in diagnostic techniques, e.g. a metal ion which emits a detectable radiation or a metal ion which is capable of

influencing NMR relaxation properties.

Suitable detectable metal ions include for example paramagnetic ions, e.g. Gd^{3+}, Fe^{3+}, Mn^{2+} and Cr^{2+}, fluorescent metal ions, e.g. Eu^{3+}, and radionuclides, e.g. γ-emitting radionuclides, positron-emitting radionuclides e.g. ^{68}Ga.

Suitable γ-emitting radionuclides include those which are useful in diagnostic techniques. The γ-emitting radionuclides advantageously have a half-life of from 1 hour to 40 days, preferably from 5 hours to 4 days, more preferably from 5 hours to 3 days and preferably should exhibit a single γ-emission, preferably in the range of 100-200 KeV. Examples are radionuclides derived from Gallium, Indium, Technetium, Ytterbium, Rhenium and Thallium e.g. ^{67}Ga, ^{111}In, ^{99m}Tc, ^{169}Yb.

The chelates of the invention may be prepared by reacting the compound with a corresponding detectable element yielding compound, e.g. a metal salt, preferably a water-soluble salt. The reaction may be carried out by analogy with known methods, e.g. as disclosed in Perrin, Organic Ligand, Chemical Data Series 22. NY Pergamon Press (1982); in Krejcarit and Tucker, Biophys. Biochem. Res. Com. 77: 581 (1977) and in Wagner and Welch, J. Nucl. Med. 20: 428 (1979).

Preferably the complexing of a compound of the invention is effected at a pH at which it is stable.

Alternatively the detectable element may also be provided to the solution as a complex with an intermediate chelating agent, e.g. a chelating agent which forms a chelate complex that renders the element soluble at the physiological pH of the complex but is

less thermodynamically stable than the chelate. Example of such an intermediate chelating agent is 4,5-dihydroxy-1,3-benzene-disulfonic acid (Tiron). In such a process, the detectable element exchanges the ligand.

The above mentioned reactions may be effected analogously to known methods. The above mentioned reactions are conveniently effected under conditions avoiding trace metal contamination. Preferably distilled de-ionized water, ultrapure reagents, no carrier-added radioactivity etc. are used to reduce the effects of trace metal.

The chelates of the invention may exist e.g. in free or salt form. Salts include acid addition salts with e.g. organic acids, polymeric acids or inorganic acids, for example hydrochlorides and acetates, and salt forms obtainable with the carboxylic acid groups present in the molecule which do not participate to the chelate formation.

The chelates of the invention and their pharmaceutical acceptable salts exhibit diagnostic activity and are therefore useful as imaging agents, e.g. visualisation of thrombi. They exhibit significant serum stability and binding affinity to thrombin, as indicated in standard in vitro tests. The compound of Example 4 has a half life of 210 hours in human serum, when tested in vitro.

Accordingly, the present invention also provides a method for in vivo detection of a thrombus in a subject which comprises a) administering a chelate of the invention to said subject and b) recording the localisation of the thrombus targeted by said chelate.

The chelates of the invention for use as an imaging agent in the above method may be administered parenterally, preferably intravenously, e.g. in the form of injectable solutions or suspensions, preferably in a single injection. The appropriate dosage will of course vary depending upon, for example, the compound and the type of detectable element used. A suitable dose to be injected is in the range to enable imaging by photoscanning procedures known in the art. A chelate of the invention may advantageously be administered in a dose comprising 0.1 to 10 mCi of a stably chelated radionuclide.

In animals an indicated dosage range may be of from 0.1 to 1 μg/kg of a compound of the invention labeled with 0.02 to 0.5 mCi γ-emitting radionuclide. In larger animals, for example humans, an indicated dosage range may be of from 1 to 20 μg compound labeled e.g. with 3 to 10 mCi γ-emitting radionuclide, preferably 2 to 5 mCi.

The localisation of a thrombus with the chelates may be followed by the corresponding imaging techniques, e.g. using nuclear medicine imaging instrumentation, for example a scanner, a conventional gamma camera to obtain planar images, a rotating γ-camera to perform Single Photon Emission Computer Tomography (SPECT) or suitable instrumentation to perform Photon Emission Tomography (PET), each preferably computer assisted.

The chelates of the invention may be administered in free form or in pharmaceutically acceptable salt form. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

The chelates of the invention are particularly useful in detecting thrombi formation resulting from vascular thrombotic disorders caused by myocardial infarction, artheriosclerosis, stroke, disseminated intravascular coagulation, peri- and post-operative to surgery, vascular recanalisations, immobility, atrial fibrillation and heart insufficiency.

The chelates of the invention for use in the method of the present invention may preferably be prepared shortly before administration to a subject, i.e. the radiolabeling with the desired detectable metal ion, may be performed shortly before administration.

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising a chelate according to the invention in free or in pharmaceutically acceptable salt form, together with one or more pharmaceutically acceptable carriers or diluents therefor.

Such a composition may be manufactured in conventional manner.

A composition according to the invention may also be presented in separate package with instructions for mixing the compound with the metal ion and for the administration of the resulting chelate. It may also be presented in twin-pack form, that is, as a single package containing separate unit dosages of the compound and the detectable metal ion with instructions for mixing them and for administration of the chelate. A diluent or carrier may be present in the unit dosage forms.

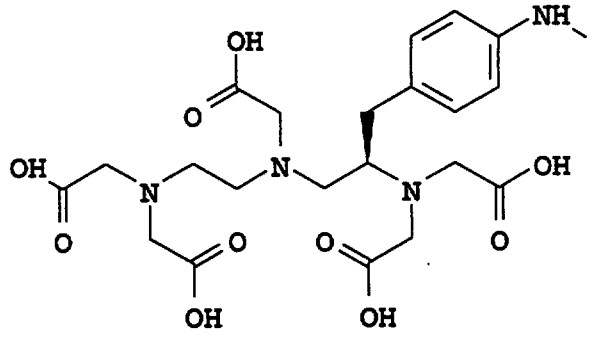
In the following examples, all temperatures are in ° C

and $[\alpha]_D^{20}$- values are uncorrected.

The following abbreviations are employed:

Bzl = benzyl
Z = benzyloxycarbonyl
Boc = t-butyloxycarbonyl
Ac = acetyl
MeOH = methyl alcohol
EtOH = ethyl alcohol
EtOAc = ethyl acetate
DCC = dicyclohexylcarbodiimide
HONSu = N-hydroxy-succinimide
OPin = pinanediol
TFA = trifluoroacetic acid
THF = tetrahydrofuran
DMF = dimethyl formamide
Np = p-nitrophenyl
TLC = thin layer chromatography
Baa = -NH-CH-($CH_2CH_2CH_2Br$)B-
TMSal = trimethylsilylalanine
BoroLys = -NH-CH-(CH_2-CH_2-CH_2-CH_2-NH_2)B-

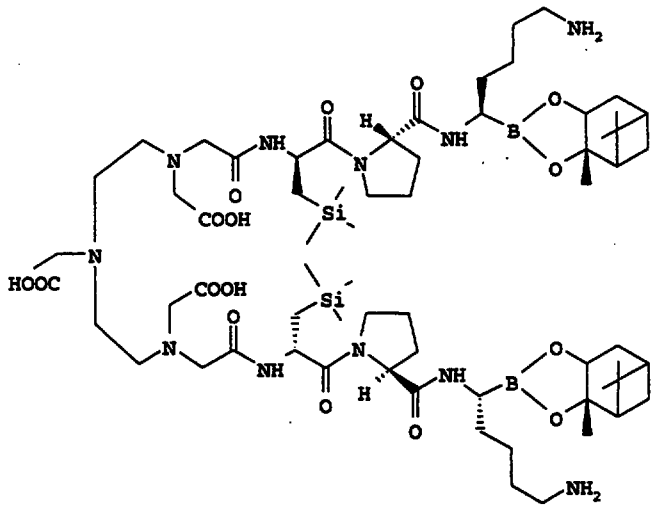
DTPA-(p-NH-Bzl) =



(t-Bu)-DTPA-(p-NH Bzl) = t-butyl ester (4x) of DTPA-(p-NH-Bzl)

EXAMPLE 1

DTPA-(D-TMSal-Pro-BoroLys-OPin)$_2$



a) DTPA-(D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin)$_2$

H-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin(trifluoroacetate) (428.4 mg, 0.5 mMol) is dissolved in dioxane and is treated with 5.0 ml saturated aqueous $NaHCO_3$ and DTPA-anhydride (0.268 g, 0.75 mMol). The mixture is stirred for 0.5 h at room temperature. Acetic acid (35.0 ml) is added and the mixture is concentrated in vacuo at 40°. The resulting residue is treated with 50 ml EtOAc and H_2O. The aqueous layer is extracted with EtOAc and the combined organic layers are washed with H_2O. After drying over Na_2SO_4 and concentration in vacuo the residue is chromatographed on a 4.0 x 25 cm HPLC-column RP18 (10 µm), H_2O/MeOH/acetic acid. The desired product is obtained as a white foam, MS: 1842 $(M + H)^+$.

b) DTPA-(D-TMSal-Pro-BoroLys-OPin)$_2$

DTPA-(D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin)$_2$(1.92 g, 1.042 mMol) of step a) is treated with 5.0 ml piperidine in DMF (20 % solution) at room temperature

for 15 min. The resulting suspension is diluted with 50 ml of ether and the desired product precipitates as a crude material. This material is purified over Dowex (1-X4, 200-400 mesh), EtOH/H_2O (1:1) and 10 % AcOH. After removal of the solvent the desired product is obtained as a white foam and is crystallized from EtOH/ether to give the title compound as an amorphous white solid. MS: 1398 $(M + H)^+$; $[\alpha]_D$ = -72.0° (c = 0.35 in MeOH).

H-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin(trifluoroacetate), used as starting material, may be prepared as follows:

A) Boc-D-TMSal-Pro-BoroLys-OPin

may be prepared as disclosed in Example 5 of EP-A-471,651.

B) Boc-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin

Boc-D-TMSal-Pro-BoroLys-OPin(620.7 mg,1.0 mMol) and Fmoc-OSu (337.3 mg, 1.0 mMol) in dioxane are treated at room temperature with saturated aqueous $NaHCO_3$ solution. After 45 min, the reaction mixture is diluted with EtOAc and is washed with water and brine. After drying over Na_2SO_4 and concentration in vacuo, the title compound is used without further purification in the next step.

C) H-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin (trifluoroacetate)

The product of step B) (2.36 g, 2.8 mMol) is treated with 8.5 ml TFA/H_2O (95:5) and 0.85 ml anisol. After 1 h at room temperature, the reaction mixture is concentrated in vacuo and the resulting oil is chromatographed on a 3.2 x 25 cm HPLC column RP18 (10 µm), H_2O/MeOH/TFA. The desired product is obtained as a white foam, MS: 743 $(M + H)^+$

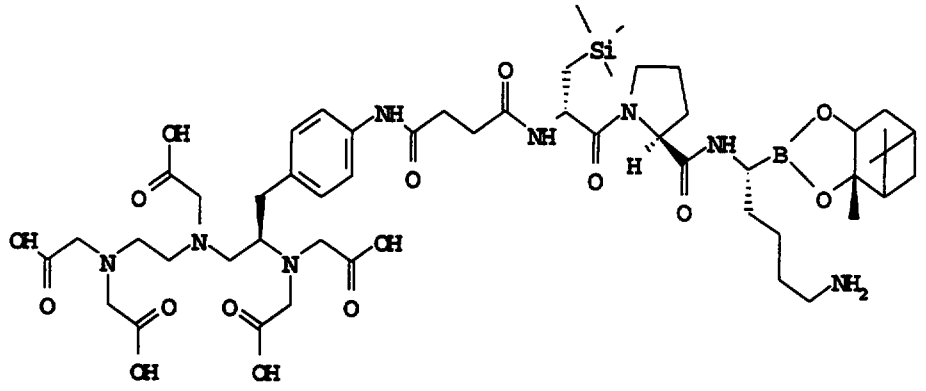
EXAMPLE 2: ^{111}In labeled compound of Example 1

1 mg of compound of Example 1 is dissolved in 5 ml 0.01 M acetic acid. The resulting solution is passed through a 0.22 µ Millex-GV filter and dispensed in 0.1 ml portions and stored at -20°C. $^{111}InCl_3$ (Amersham, 1 mCi/100 µl) is prediluted in an equal volume of 0.5 M sodium acetate and labeling is carried out by mixing the ligand with the $InCl_3$ solution and gentle homogenisation at room temperature.

HEPES buffer, pH 7.4, is then added to make a 10^{-6}M solution.

EXAMPLE 3:

DTPA-(p-NH-Bzl)-CO(CH$_2$)$_2$CO-D-TMSal-Pro-BoroLys-OPin



a) **HOOC(CH$_2$)$_2$CO-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin**

H-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin (trifluoro-acetate of Example 1, step C) (0.857 g,1.0 mMol) is dissolved in dioxane and is treated at room temperature with saturated aqueous $NaHCO_3$ solution and succinic anhydride (0.11 g, 1.10 mMol). After 45 min, the reaction mixture is diluted with EtOAc and is washed

with aqueous $NaHSO_4$-solution (1 M) and brine. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. The resulting crude product is chromatographed on a 3.2 x 25 cm HPLC-column RP18 (10 µm) H_2O/MeOH. The desired product is obtained as a white foam, MS: 843 $(M + H)^+$.

b) **(t.Bu)-DTPA-(p-NH-Bzl)-CO(CH$_2$)$_2$CO-D-TMSal-Pro-Boro(ε-N-Fmoc)-Lys-OPin**

The product of step a) (623 mg, 0.739 mMol) in THF is cooled to 0° and is treated with N-methyl morpholine (0.081 ml, 0.739 mMol and pivaloyl chloride (0.095 ml, 0.776 mMol) to form the mixed anhydride. After stirring for 1 h at 0°, the reaction mixture is treated with (^{t}Bu)-DTPA-(p-NH_2-Bzl) (575 mg, 0.739 mMol) (as described in Bioconjugate Chem. 1991, 2(3), 180-186) and additional N-methyl morpholine (0.081 ml, 0.739 mMol). The reaction mixture is stirred for 15 h at room temperature, and then diluted with EtOAc and washed with aqueous citric acid solution (1 M), aqueous saturated $NaHCO_3$-solution and brine. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. The resulting oil is chromatographed on a 3.2 x 25 cm HPLC-column RP18 (10 µm), H_2O/MeOH/TFA. The desired product is obtained as a white foam, MS: 1603 $(M + H)^+$.

c) **DTPA-(p-NH-Bzl)-CO(CH$_2$)$_2$CO-D-TMSal-Pro-Boro(ε-N-Fmoc)Lys-OPin**

The product of step b) (576 mg, 0.296 mMol) is saponified by treatment with TFA/H_2O (95:5) in the presence of anisol/ethanedithiol (1:1) for 7 h at room temperature. Excess TFA is removed in vacuo and the resulting residue is treated with ether to precipitate the desired product. After filtration and drying under reduced pressure, the title compound is obtained as an amorphous solid foam of the trifluoroacetate MS: 1323

$(M + H)^+$.

d DTPA-(p-NH-Bzl)-CO(CH$_2$)$_2$CO-D-TMSal-Pro-BoroLys-OPin

The product of step c) (326 mg, 0.2463 mMol) is treated with 2.0 ml piperidine in DMF (20 % solution) at room temperature for 15 min. The resulting mixture is diluted with ether and the desired product precipitates as a white solid. This material is purified over Dowex (1-X4, 200-400 mesh), EtOH/H_2O (1:1) and 10 % AcOH. After removal of the solvent the title compound is obtained as a white lyophilisate (from H_2O) MS: 1101 $(M + H)^+$, $[\alpha]_D$ = -31.8° (c = 0.27 in MeOH).

EXAMPLE 4: ^{111}In labeled compound of Example 3

The ^{111}In labeled compound is prepared by using the same procedure as disclosed in Example 2.

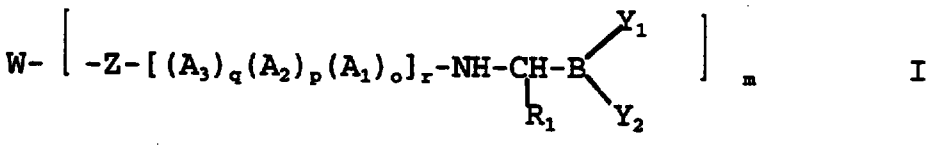
CLAIMS

1. A compound which is a trypsin-like serine protease inhibiting peptide or pseudo-peptide bearing a chelating group which is capable of complexing a detectable element, the chelating group being linked to an amino group of the peptide or pseudo-peptide such that the chelating group does not interfere with or prevent the binding of the peptide or pseudo-peptide to thrombin.

2. A compound which is a peptide or pseudo-peptide according to claim 1, in which the chelating group is attached to the N-terminal amino group of the peptide or pseudo-peptide.

3. A compound which is a peptide or pseudo-peptide according to claim 1 or 2, in which the chelating group is attached by means of a spacer group to the amino group of the peptide or pseudo-peptide.

4. A compound of formula I



I

wherein Y_1 and Y_2, independently, are OH or F or, taken together, form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising 1 to about 20 carbon atoms and, optionally, a heteroatom which can be N, S, or O;

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R_1 is a substituted alkyl selected from the group consisting of $-(CH_2)_z-X$, $-CH(CH_3)-(CH_2)_2-X$, $-CH_2-CH(CH_3)-CH_2-X$, $-(CH_2)_2-CH(CH_3)-X$, and $-(CH_2)_2-C(CH_3)_2-X$,

where X is $-NH_2$, $-NH-C(NH)-NH_2$ or $-S-C(NH)-NH_2$, and z is 3 to 5;

m is 1 or 2;

r, o, p and q are, independently, either 1 or 0;

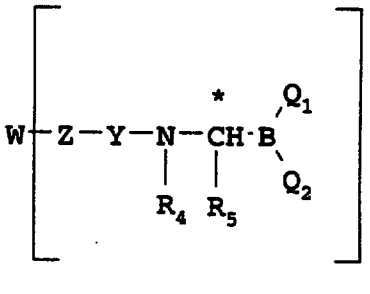
A_1, A_2 and A_3, independently, are amino acids of L- or D-configuration selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val;

W is a chelating group attached to the N-terminal amino group of the compound, and

Z is a direct bond or a spacer;

in free form or in salt form.

5. A compound of formula II



II

wherein W, Z and are as defined above,

Y is a sequence of n amino acids selected such

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that the n+1 amino acid peptide Y-Lys or Y-Arg has an affinity for the active site of a trypsin-like protease, where n is an integer from 1 to 10 preferably 1 to 4, and in which at least one amino acid is an unnatural amino acid having a hydrophobic side chain;

Q_1 and Q_2, which may be the same or different, are selected from -OH, COR_2, $-CONR_2R_3$, $-NR_2R_3$, and $-OR_6$, or Q_1 and Q_2 taken together form a diol residue, and wherein R_2, R_3 and R_6, which may be the same or different, are C_{1-10}alkyl, C_{6-10}aryl, C_{6-10}aralkyl, or phenyl substituted by up to three groups selected from C_{1-4}alkyl, halogen and C_{1-4}alkoxy;

R_4 is hydrogen or C_{1-10}alkyl;

R_5 is a group $-Z_1-X_1$,

wherein Z_1 is $-(CH_2)_s-$; $-CH(CH_3)-(CH_2)_2-$, $-CH_2-CH(CH_3)-CH_2-$, $-(CH_2)_2-CH(CH_3)-$, $-(CH_2)_2-C(CH_3)_2-$, $-CH(CH_3)-(CH_2)_3-$, $-CH_2-CH(CH_3)-(CH_2)_2-$, $-(CH_2)_2-CH(CH_3)-CH_2-$, $-(CH_2)_3-C(CH_3)_2-$, $-(CH_2)_2-C(CH_3)_2-CH_2-$, $(CH_2)_3-CH(CH_3)-$, $-(CH_2)_3-CH(CH_3)-CH_2-$, C_{6-10}aryl, or C_{6-10}aralkyl

wherein s is 2, 3, 4 or 5;

and wherein X_1 is $-NH_2$, $-NH-C(NH)-NH_2$, $-S-C(NH)-NH_2$, $-N_3$, C_{1-4}alkoxy, C_{1-4}alkylthio, $-Si(CH_3)_3$, OH, SH, NR_7R_8, or phenyl, optionally substituted by up to three groups selected from halogen, OH and C_{1-4}alkoxy, or $C(NH)R_{10}$;

wherein R_7 is H or C_{1-10}alkyl; R_8 is C_{1-10}alkyl, $-CO-R_9$ or $CS-R_9$; R_{10} is C_{1-3}alkyl or $N(CH_3)_2$, or $NR_{11}R_{12}$;

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wherein R_9 is H or C_{1-10}alkyl, C_{1-10}alkoxy, C_{6-10}aryl, C_{6-10}aralkyl and R_{11} and R_{12} (which may be the same or different) are H or C_{1-10}alkyl;

or R_4 and R_5 together form a trimethylene group,

and the asymmetric carbon atom marked * may have the D- or L-configuration, or represent any mixture of these.

6. A compound according to any of the preceding claims in which the chelating group is an iminodicarboxylic acid group or a polyaminopolycarboxylic acid group.

7. A compound according to any of the preceding claims in which the chelating group is a radical of formula (c)

$$W'\text{-}CH_2\text{-}X_a\text{-}NH\text{-} \qquad (c)$$

wherein
W' is a chelating group derived from a non-cyclic or cyclic polyamino-polyacetic acid or anhydride, and

X_a is phenylene or C_{1-3}alkylene.

8. A compound according to any one of claims 3-8 comprising a spacing group of formula (d)

$$-R_{22}\text{-}R_{23}\text{-}CO\text{-} \qquad (d)$$

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wherein

R_{22} is a divalent residue derived from a functional moiety capable of covalently reacting with the chelating agent, and

R_{23} is C_{1-11}alkylene optionally interrupted by one or more heteroatoms or residues selected from O, S, CO, -NH-, -NHCO-, N(C_{1-4}alkyl)-CO- and -N(C_{1-4}alkyl)-, C_{2-11}alkenylene or -CH(R_{14})- wherein R_{14} is a residue attached to a natural or unnatural α-amino acid, e.g. hydrogen, C_{1-11}alkyl, benzyl, optionally substituted benzyl, naphthyl-methyl, pyridyl-methyl.

9. A process for the production of a compound according to any of the preceding claims comprising

a) removing at least one protecting group which is present in a trypsin-like serine protease inhibiting peptide of the invention bearing a protecting group;

b) linking together by an amide bond two peptide fragments each of them containing at least one amino acid in protected or unprotected form and one of them containing the chelating group, wherein the amide bond is such that the desired amino acid sequence is obtained, and optionally effecting step a);

c) linking together a chelating agent and the desired peptide in protected or unprotected form such that the chelating group is fixed on the desired amino group of the peptide, and optionally effecting step a), or

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d) removing or converting a functional group of an unprotected or a protected peptide bearing a chelating group so that the desired unprotected or protected peptide bearing a chelating group is obtained and if appropriate effecting step a),

and recovering the compound thus obtained in free form or salt form.

10. The therapeutic use of a compound according to any one of claims 1-8.

11. A chelate comprising a compound according to any one of claims 1-8 complexed with a detectable element.

12. The diagnostic use of a chelate according to claim 11.

13. A pharmaceutical composition comprising a compound according to any one of claims 1-8, or a chelate according to claim 11, together with a pharmaceutically acceptable carrier or diluent.

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