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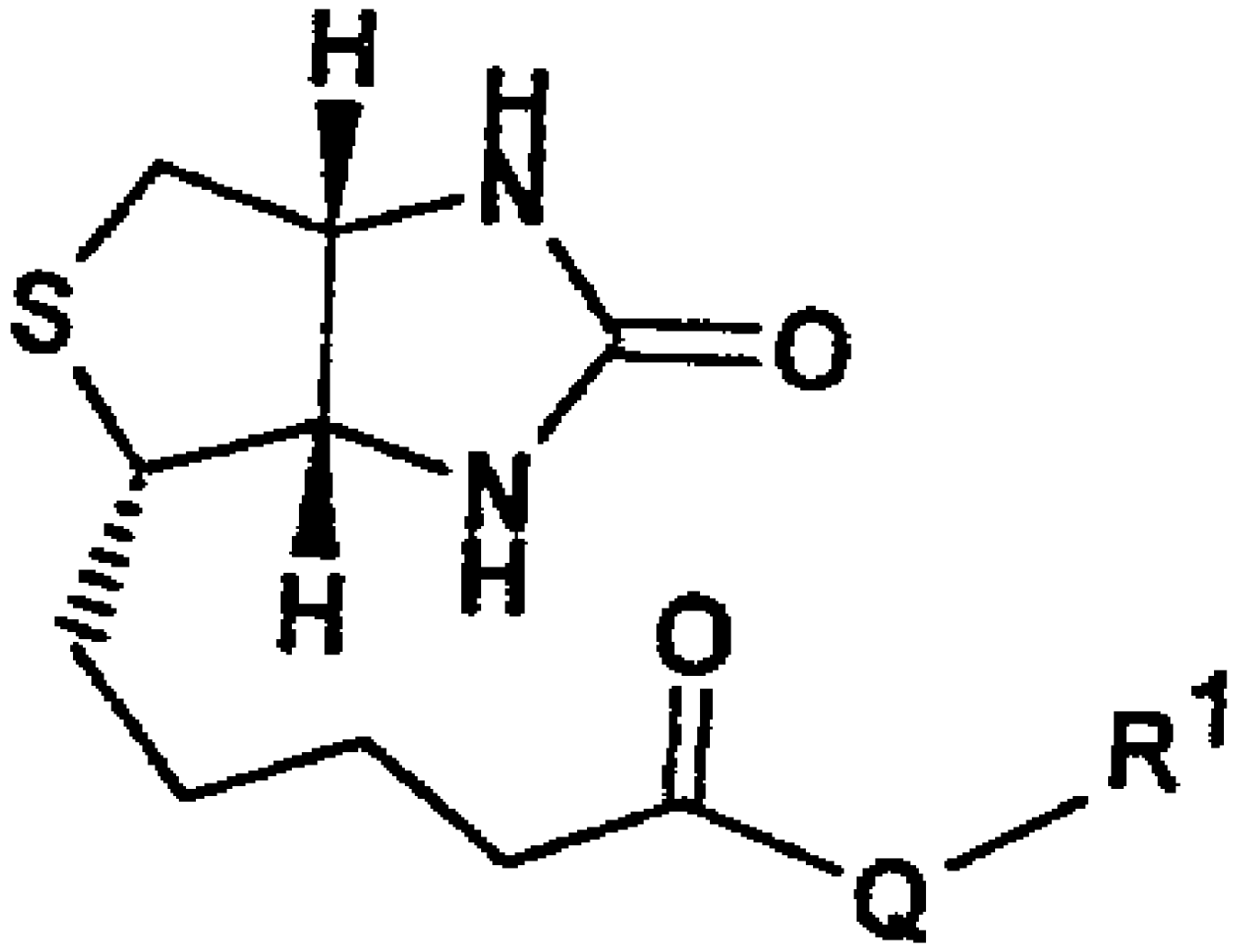
(54) Titre : DERIVES DE BIOTINE
(54) Title: BIOTINDERIVATE



I

(57) Abrégé/Abstract:
Biotin compounds of the formula I (see formula I) is which Q is absent, -NH-$(CH_2)_n$-CO or -NH-$(CH_2)_n$-NH- and R^1 is X-Arg-Gly-Asp-Y, A-Cys(R^2)-B-U or cyclo-(Arg-Gly-Asp-Z), where Z is bonded in the side chain to Q or, if Q is absent, to biotin, and A, B, U, X, Y, Z and n have the meanings stated is Claim 1, and their salts can be used as integrin inhibitors, in particular for the prophylaxis and treatment of disorders of the circulation, for thrombosis, myocardial infarct, coronary heart diseases, arteriosclerosis, angiogenic disorders and in tumour therapy.

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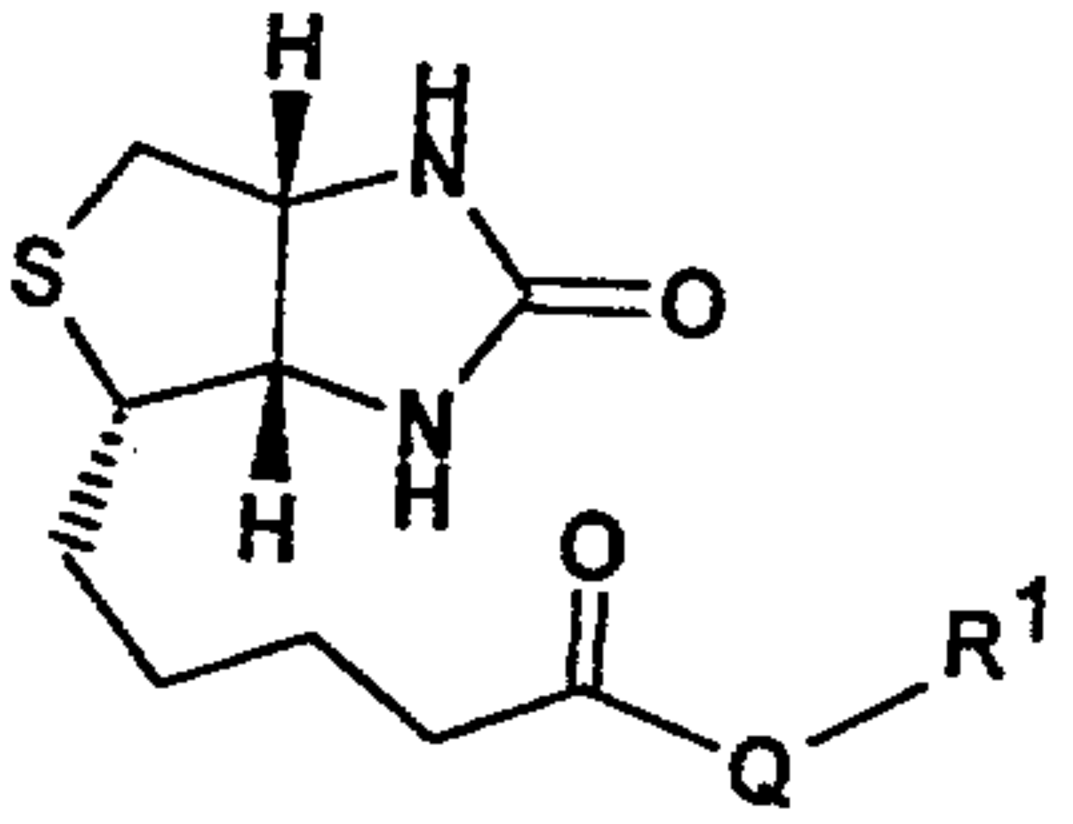
Abstract

Biotin compounds of the formula I



I

in which

Q is absent, -NH-$(CH_2)_n$-CO or -NH-$(CH_2)_n$-NH- and

R^1 is X-Arg-Gly-Asp-Y, A-Cys(R^2)-B-U or cyclo-(Arg-Gly-Asp-Z), where Z is bonded in the side chain to Q or, if Q is absent, to biotin,

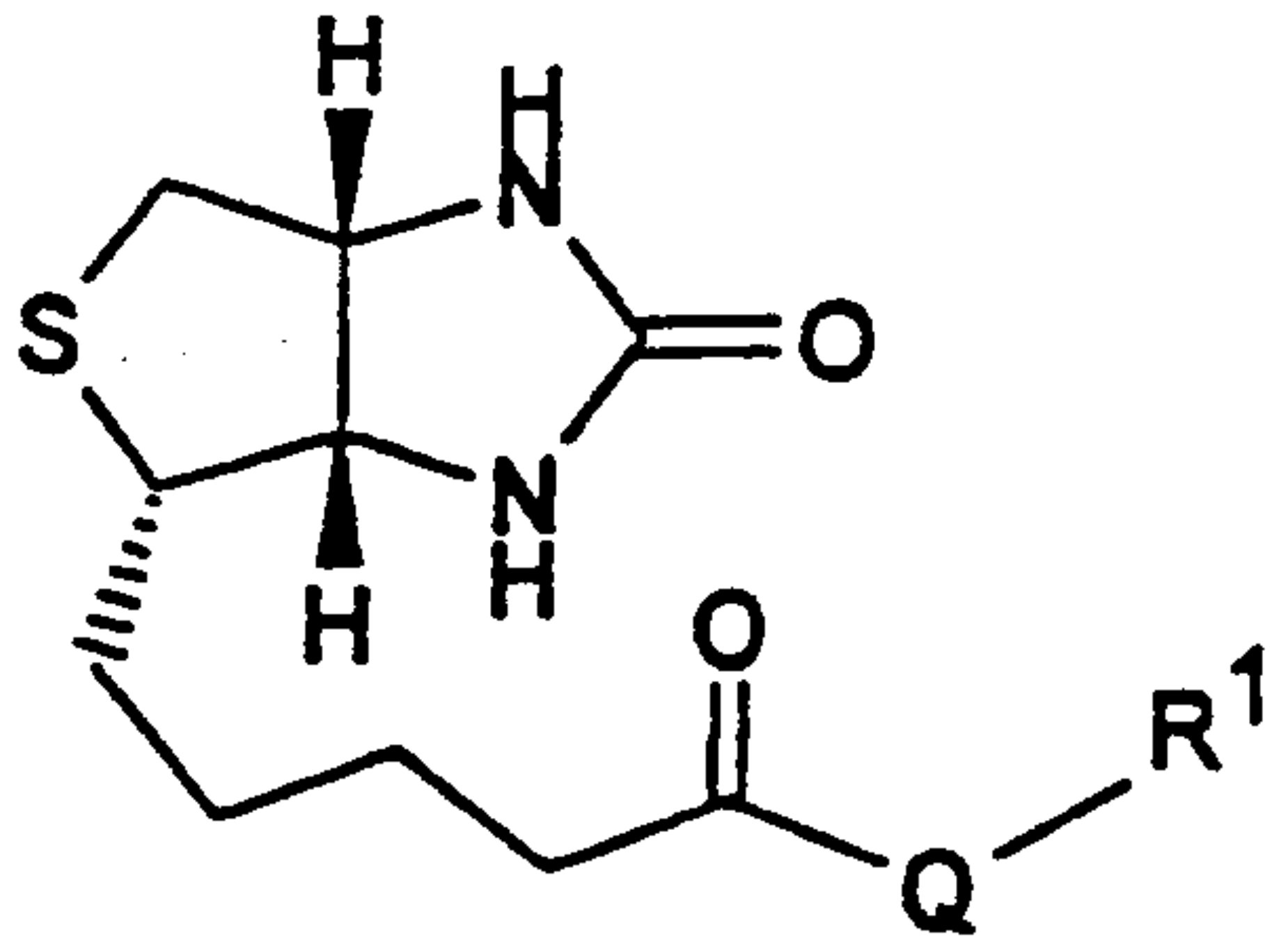
and A, B, U, X, Y, Z and n have the meanings stated in Claim 1,

and their salts

can be used as integrin inhibitors, in particular for the prophylaxis and treatment of disorders of the circulation, for thrombosis, myocardial infarct, coronary heart diseases, arteriosclerosis, angiogenic disorders and in tumour therapy.

Biotinderivate

The invention relates to biotin compounds of the formula I



I

in which

Q is absent, $-NH-(CH_2)_n-CO-$ or $-NH-(CH_2)_n-NH-$,

R^1 is X-Arg-Gly-Asp-Y, A-Cys(R^2)-B-U or cyclo-(Arg-Gly-Asp-Z), where Z is bonded in the side chain to Q or, if Q is absent, to biotin,

X and Y are each, independently of one another, an amino-acid residue or a di-, tri-, tetra- or pentapeptide residue, where the amino acids are selected, independently of one another, from a group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, 4-Hal-Phe, His, homo-Phe, Ile, Leu, Lys, Met, Nle, Phe, Phg, Pro, Ser, Thr, Trp, Tyr or Val,

and the said amino acids can also be derivatized,

A is absent, Asp or a peptide fragment selected from a group consisting of Ala-Asp, Thr-Ala-Asp, Lys-Thr-Ala-Asp, Lys-Thr-Ala-Asn, Lys-Thr-Gly-Asp, Lys-Ala-Ala-Asp, Arg-Thr-Ala-Asp, Ser-Ala-Asp, Gln-Ser-Ala-Asp, Glp-Ser-Ala-Asp, Gly-Lys-Thr-Ala-Asp, Asn-Gly-Lys-Thr-Ala-Asp, Ile-Ser-Ala-Gly, Arg-Ser-Ala-Gly, Cys-Asn-Gly-Lys-Thr-Ala-Asp,

Tyr-Cys-Asn-Gly-Lys-Thr-Ala-Asp,
Asp-Tyr-Cys-Asn-Gly-Lys-Thr-Ala-Asp,
Asp-Asp-Tyr-Cys-Asn-Gly-Lys-Thr-Ala-Asp,
Gly-Lys-Thr-Cys(Trt)-Asp,
Met-Asp-Asp-Tyr-Cys-Asn-Gly-Lys-Thr-Ala-Asp,
Asp-Met-Asp-Asp-Tyr-Cys-Asn-Gly-Lys-Thr-Ala-Asp,

B is absent, OH, Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Orn, Phe, 4-Hal-Phe, Pro, Ser, Thr, Trp, Tyr, Val or an N-methylated derivative of the said amino-acid residues, or a peptide fragment selected from a group consisting of Pro-Arg, Pro-Arg-Asn,
Pro-Arg-Asn-Pro, Pro-Arg-Asn-Pro-His,
Pro-Arg-Asn-Pro-His-Lys,
Pro-Arg-Asn-Pro-His-Lys-Gly,
Pro-Arg-Asn-Pro-His-Lys-Gly-Pro,
Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala,
Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr,

where, if R^1 is A-Cys(R^2)-B-U, only one of the radicals A or B can be absent,

R^2 is H, alkyl with 1-6 C atoms, Trt, Dpm or Bzl,

U is OH, OR^9, NH_2, NHR^9 or $N(R^9)_2$

Z is in each case, independently of one another, an amino-acid residue or a di-, tri- or tetrapeptide residue, where the amino acids thereof can also be selected, independently of one another, from a group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val or M,

where the said amino acids can also be derivatized, and the amino-acid residues can be linked together in the manner of a peptide via the α-amino and α-carboxyl groups, with M always being present,

M is $NH(R^8)-CH(R^3)-COOH$,

R^3 is $-R^5-R^4$, $-R^6-R^4$, $-R^7-R^4$,

R^4 is OH, NH_2, SH or COOH,

R^5 is alkylene with 1-6 C atoms,

R^6 is alkylenephenyl with 7-14 C atoms,

R^7 is alkylenephenylalkylene with 8-14 C atoms,

R^8 is H, alkyl with 1-6 C atoms or alkylenephenyl with 7-12 C atoms,

R^9 is alkyl with 1-6 C atoms,

Hal is F, Cl, Br or I and

n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10,

where both the D and the L forms are included if radicals of optically active amino acids and amino-acid derivatives are present, and the salts thereof.

Similar compounds of biotinylated peptides are described, for example, in WO 9415956 (biotinylated endothelin receptor antagonists), in WO 9413313 (biotinylated LHRH antagonists) or WO 9418325 (biotinylated necrosis factor).

The biotinylation of peptides during solid-phase synthesis on a resin for the purpose of improving the possibility of purification is described by T.J. Lobl et al. in Anal. Biochem. 170, 502 (1988). Similar compounds of cyclic and linear peptides are disclosed in

DE 43 10 643, DE 43 36 758, EP 0 406 428 and WO 89/05150.

The invention was based on the object of finding novel compounds with valuable properties, especially those which can be used to produce pharmaceuticals.

It has been found that the compounds of the formula I and their salts have, while being well tolerated, very valuable pharmacological properties. In particular, they act as integrin inhibitors, and they inhibit in particular the interactions of the α_v, β_3 or β_5 integrin receptors with ligands, such as, for example, the binding of fibrinogen to the β_3 integrin receptor. The compounds show particular activity in the case of the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_1$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$. This effect can be detected, for example, by the method described by J.W. Smith et al. in J. Biol. Chem. 265, 12267-12271 (1990).
The dependence of the beginning of angiogenesis on the interaction between vascular integrins and extracellular matrix proteins is described by P.C. Brooks, R.A. Clark and D.A. Cheresh in Science 264, 569-71 (1994).

The possibility of inhibiting this interaction and thus of initiating apoptosis (programmed cell death) of angiogenic vascular cells by a cyclic peptide is described by P.C. Brooks, A.M. Montgomery, M. Rosenfeld, R.A. Reisfeld, T.-Hu, G. Klier and D.A. Cheresh in Cell 79, 1157-64 (1994).

Compounds of the formula I which block the interaction of integrin receptors and ligands, such as, for example, of fibrinogen on the fibrinogen receptor (glycoprotein IIb/IIIa), prevent as GPIIb/IIIa antagonists the spread of tumour cells by metastasis. This is proved by the following observations:
The spread of tumour cells from a local tumour into the vascular system takes place by the formation of microaggregates (microthrombi) by interaction of the tumour cells with blood platelets. The tumour cells are

shielded by the protection in the microaggregate and are not recognized by the cells of the immune system.

The microaggregates may become attached to vessel walls, which facilitates further penetration of tumour cells into the tissue. Since the formation of microthrombi is mediated by fibrinogen binding to the fibrinogen receptors on activated blood platelets, the GPIIa/IIIb antagonists can be regarded as effective inhibitors of metastasis.

The compounds of the formula I can be employed as pharmaceutical active substances in human and veterinary medicine, in particular for the prophylaxis and/or therapy of thrombosis, myocardial infarct, arteriosclerosis, inflammations, stroke, angina pectoris, oncoses, osteolytic diseases such as osteoporosis, pathologically angiogenic disorders such as, for example, inflammations, ophthalmological disorders, diabetic retinopathy, macular degeneration, myopia, ocular histoplasmosis, rheumatoid arthritis, osteoarthritis, rubeotic glaucoma, ulcerative colitis, Crohn's disease, atherosclerosis, psoriasis, restenosis after angioplasty, viral infection, bacterial infection, fungal infection, for acute kidney failure and for wound healing to support the healing processes.

The compounds of the formula I can be employed as antimicrobially active substances in operations where biomaterials, implants, catheters or cardiac pacemakers are inserted. In this case, they act as antiseptics. The efficacy of the antimicrobial activity can be demonstrated by the method described by P. Valentin-Weigund et al. in Infection and Immunity, 2851-2855 (1988).

Since the compounds of the formula I are inhibitors of fibrinogen binding and thus ligands of the fibrinogen receptors on blood platelets, they can be used as diagnostic aids for detecting and locating thrombi in the vascular system in vivo, because the biotinyl radical is a UV-detectable radical.

The compounds of the formula I can, as inhibitors of fibrinogen binding, also be used as effective aids for studying the metabolism of blood platelets in various stages of activation or of intracellular signal mechanisms of the fibrinogen receptor. The detectable unit of the "biotin label" makes it possible to investigate the said mechanisms after binding to the receptor.

The abbreviations of amino-acid residues employed hereinbefore and hereinafter represent the radicals of the following amino acids:

Abu	4-Aminobutyric acid
Aha	6-Aminohexanoic acid, 6-aminocaproic acid
Ala	Alanine
Asn	Asparagine
Asp	Aspartic acid
Arg	Arginine
Cys	Cysteine
Dab	2,4-Diaminobutyric acid
Dap	2,3-Diaminopropionic acid
Gln	Glutamine
Glp	Pyroglutamic acid
Glu	Glutamic acid
Gly	Glycine
His	Histidine
homo-Phe	homo-Phenylalanine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Meth	Methionine
Nle	Norleucine
Orn	Ornithine
Phe	Phenylalanine
Phg	Phenylglycine
4-Hal-Phe	4-Halo-phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine

Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

and

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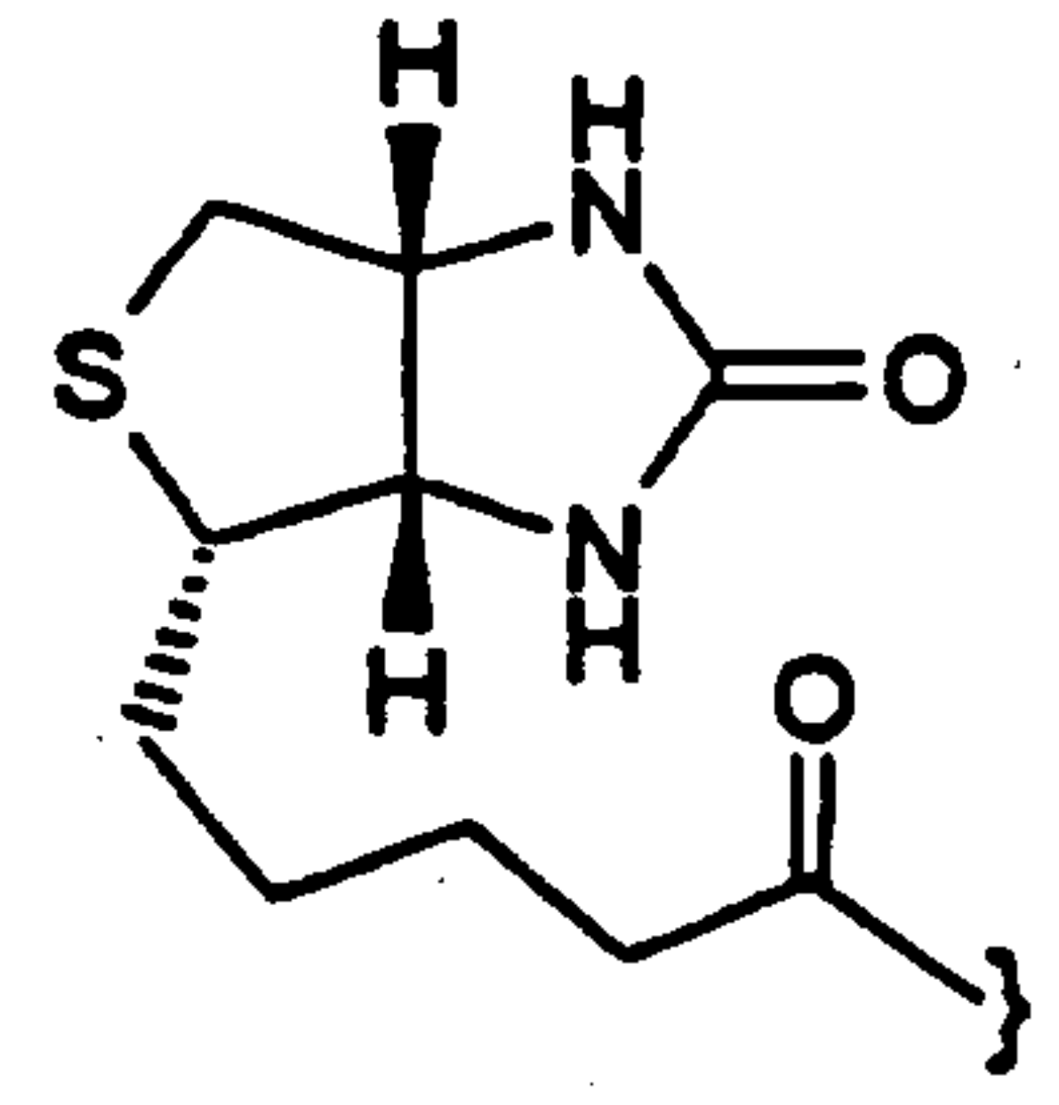


Further meanings are the following:

BOC	tert-Butoxycarbonyl
CBZ	Benzyloxycarbonyl
DCCI	Dicyclohexylcarbodiimide
DMF	Dimethylformamide
Et	Ethyl
Fmoc	9-Fluorenylmethoxycarbonyl
HOBt	1-Hydroxybenzotriazole
Me	Methyl
MBHA	4-Methylbenzhydrylamine
Mtr	4-Methoxy-2,3,6-trimethylphenylsulfonyl
OBut	tert-Butyl ester
OMe	Methyl ester
OEt	Ethyl ester
POA	Phenoxyacetyl
TFA	Trifluoroacetic acid
Trt	Trityl (triphenylmethyl).

When the abovementioned amino acids can occur in several enantiomeric forms, all these forms, and mixtures thereof (for example the D and the L forms), are also included hereinbefore and hereinafter, for example as constituent of the compounds of the formula I. Furthermore, the amino acids can be provided with appropriate protective groups which are known per se, for example as constituent of compounds of the formula I.

The compounds according to the invention also include so-called prodrug derivatives, that is to say compounds of the formula I which have been modified, for example, with alkyl or acyl groups, sugars or oligopeptides and which are rapidly cleaved in the body to the active compounds according to the invention.

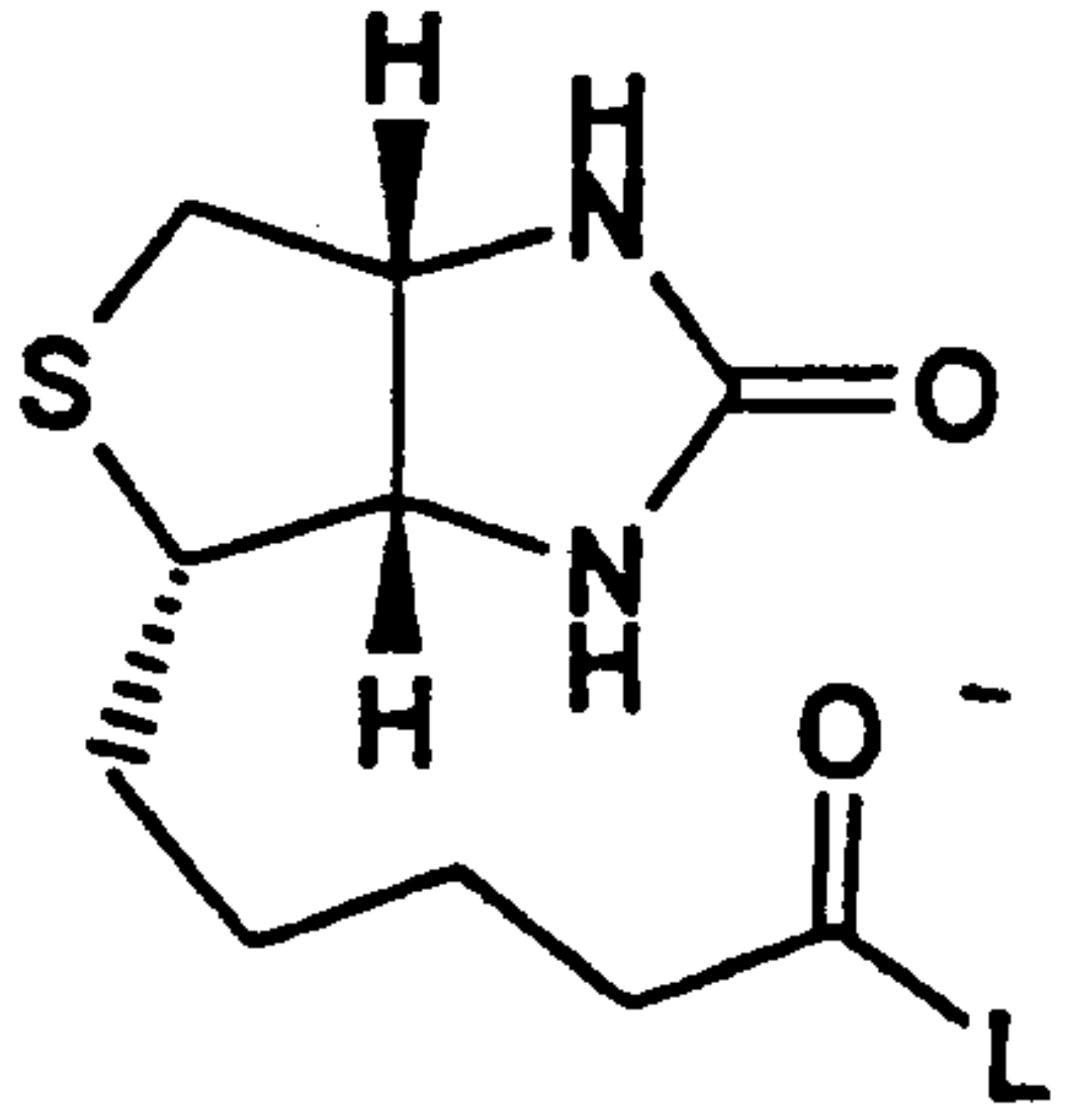
The invention furthermore relates to a process for the preparation of compounds of the formula I according to Claim 1, and the salts thereof, characterized in that

(a) a compound of the formula II

$$H\text{-}Q\text{-}R^1 \qquad II$$

in which

Q and R^1 have the meaning stated in Claim 1, is reacted in an acylation reaction with a compound of the formula III



III

in which

L is Cl, Br, I or a free or reactively functionally modified OH group,

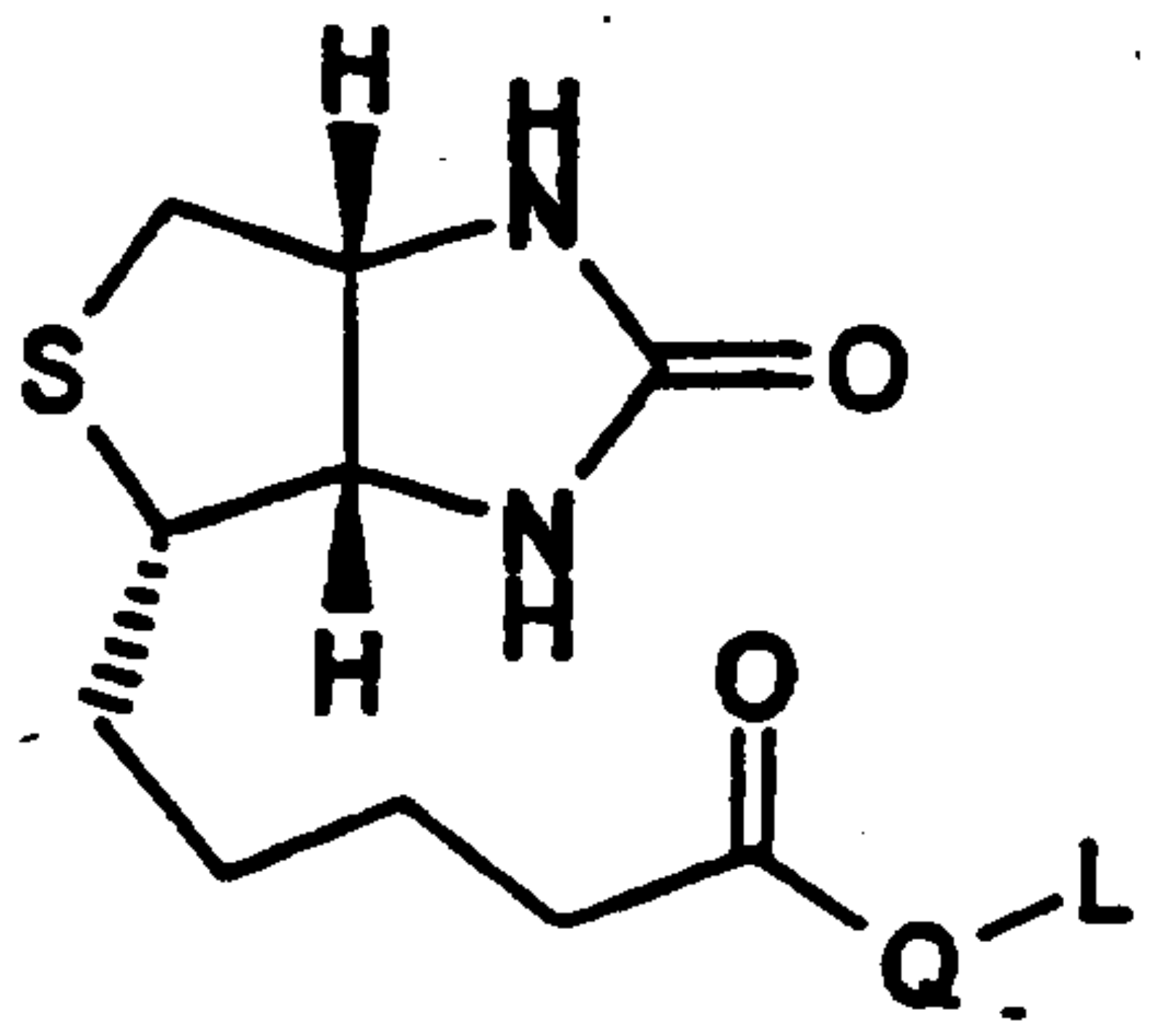
or

b) a compound of the formula IV

$$H\text{-}R^1 \qquad IV$$

in which

R^1 has the meaning stated in Claim 1, is reacted in an acylation reaction with a compound of the formula V



V

in which

Q has the meaning stated in Claim 1, and
L is H, Cl, Br, I or a free or reactively functionally modified OH group,

or

c) they are liberated from one of their **functional derivatives by treatment with a solvolysing or hydrogenolysing agent,**

and/or in that a basic or acidic compound of the formula I is converted by treatment with an acid or base into one of the salts thereof.

Hereinbefore and hereinafter, the radicals Q, R^1 and L have the meanings stated for formulae I, II and III unless expressly stated otherwise.

In the above formulae, alkyl is preferably methyl, ethyl, isopropyl or tert-butyl.

Alkylene is preferably methylene, ethylene, propylene, butylene, pentylene or hexylene. Alkylenephenyl is preferably benzyl or phenethyl. Alkylenephenylalkylene is preferably 4-methylenebenzyl or 4-ethylenebenzyl.

The radical $-R^6-R^4$ is preferably 2-, 3- or 4-hydroxybenzyl, 2-, 3- or 4-aminobenzyl, 2-, 3- or 4-mercaptobenzyl, 2-, 3- or 4-carboxybenzyl, furthermore preferably 2-, 3- or 4-hydroxyphenethyl, 2-, 3- or 4-aminophenethyl, 2-, 3- or 4-mercaptophenethyl or 2-, 3- or 4-carboxyphenethyl.

Q is preferably 6-aminohexanoic acid (6-aminocaproic acid) or is absent.

M is preferably Dap, Ser, Cys, Asp, D-Asp, Dab, homoserine, homocysteine, Glu, D-Glu, Thr, Orn, Lys, D-Lys, 4-aminomethyl-Phe or 4-aminomethyl-D-Phe.

X is preferably Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val, furthermore preferably Lys-Gly, Lys-Ala, Lys-β-Ala, Tyr-Gly, Tyr-Ala, Tyr-β-Ala, Phe-Gly, Phe-Ala, Phe-β-Ala, Tyr-Gly-Gly, Phe-Gly-Gly, Lys-Gly-Gly, Tyr-Gly-Ala, Phe-Gly-Ala, Lys-Gly-Ala, Arg-Gly-Asp, Lys-Gly-Gly-Gly, Tyr-Gly-Gly-Gly, Phe-Gly-Gly-Gly, Lys-Gly-Gly-Ala, Tyr-Gly-Gly-Ala, Phe-Gly-Gly-Ala, Lys-Gly-Gly-β-Ala, Tyr-Gly-Gly-β-Ala, Phe-Gly-Gly-β-Ala, furthermore also Lys-Gly-Gly-Gly-Gly, Tyr-Gly-Gly-Gly-Gly, Phe-Gly-Gly-Gly-Gly, Lys-Gly-Gly-Ala-Gly, Tyr-Gly-Gly-Ala-Gly, Phe-Gly-Gly-Ala-Gly, Lys-Gly-Gly-β-Ala-Gly, Tyr-Gly-Gly-β-Ala-Gly or Phe-Gly-Gly-β-Ala-Gly.

Y is preferably Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val, furthermore preferably Tyr-Ala, Tyr-Asn, Tyr-Asp, Tyr-Arg, Tyr-Cys, Tyr-Gln, Tyr-Glu, Tyr-Gly, Tyr-His, Tyr-Ile, Tyr-Leu, Tyr-Lys, Try-Met, Tyr-Phe, Tyr-Pro, Tyr-Ser, Tyr-Thr, Tyr-Trp, Tyr-Tyr,Tyr-Val, Phe-Ala, Phe-Asn, Phe-Asp, Phe-Arg, Phe-Cys, Phe-Gln, Phe-Glu, Phe-Gly, Phe-His, Phe-Ile, Phe-Leu, Phe-Lys, Phe-Met, Phe-Phe, Phe-Pro, Phe-Ser, Phe-Thr, Phe-Trp, Phe-Tyr, Phe-Val, Trp-Ala, Trp-Asn, Trp-Asp, Trp-Arg, Trp-Cys, Trp-Gln, Trp-Glu, Trp-Gly, Trp-His, Trp-Ile, Trp-Leu, Trp-Lys, Trp-Met, Trp-Phe, Trp-Pro, Trp-Ser, Trp-Thr, Trp-Trp, Trp-Tyr, Trp-Val, Asp-Ala, Asp-Asn, Asp-Asp, Asp-Arg, Asp-Cys, Asp-Gln, Asp-Glu, Asp-Gly, Asp-His, Asp-Ile, Asp-Leu, Asp-Lys, Asp-Met, Asp-Phe, Asp-Pro, Asp-Ser, Asp-Thr, Asp-Trp, Asp-Tyr, Asp-Val, Ser-Pro-Lys, Tyr-Pro-Lys, Phe-Pro-Lys, Trp-Pro-Lys, Asp-Pro-Lys, Ser-Gly-Lys, Tyr-Gly-Lys, Phe-Gly-Lys, Trp-Gly-Lys, Asp-Gly-Lys, Ser-Ala-Lys, Tyr-Ala-Lys, Phe-Ala-Lys, Trp-Ala-Lys, Asp-Ala-Lys, Ser-Pro-Ala, Ser-Leu-Lys, Tyr-Leu-Lys, Phe-Leu-Lys, Trp-Leu-Lys, Asp-Leu-Lys, Ser-Ile-Lys, Tyr-Ile-Lys, Phe-Ile-Lys, Trp-Ile-Lys, Asp-Ile-Lys, Ser-Pro-Ala-Ser, Tyr-Pro-Ala-Ser, Phe-Pro-Ala-Ser, Trp-Pro-Ala-Ser, Asp-Pro-Ala-Ser, Ser-Gly-Ala-Ser, Tyr-Gly-Ala-Ser, Phe-Gly-Ala-Ser, Trp-Gly-Ala-Ser, Asp-Gly-Ala-Ser, Ser-Ala-Ala-Ser, Tyr-Ala-Ala-Ser, Phe-Ala-Ala-Ser, Trp-Ala-Ala-Ser, Asp-Ala-Ala-Ser, Ser-Val-Ala-Ser, Tyr-Val-Ala-Ser, Phe-Val-Ala-Ser, Trp-Val-Ala-Ser, Asp-Val-Ala-Ser, Ser-Leu-Ala-Ser, Tyr-Leu-Ala-Ser, Phe-Leu-Ala-Ser, Trp-Leu-Ala-Ser, Asp-Leu-Ala-Ser, Ser-Ile-Ala-Ser, Tyr-Ile-Ala-Ser, Phe-Ile-Ala-Ser, Trp-Ile-Ala-Ser, Asp-Ile-Ala-Ser, furthermore also Ser-Pro-Ala-Ser-Ser,Tyr-Pro-Ala-Ser-Ser, Phe-Pro-Ala-Ser-Ser, Trp-Pro-Ala-Ser-Ser, Asp-Pro-Ala-Ser-Ser, Ser-Gly-Ala-Ser-Ser, Tyr-Gly-Ala-Ser-Ser, Phe-Gly-Ala-Ser-Ser, Trp-Gly-Ala-Ser-Ser, Asp-Gly-Ala-Ser-Ser, Ser-Ala-Ala-Ser-Ser, Tyr-Ala-Ala-Ser-Ser, Phe-Ala-Ala-Ser-Ser, Trp-Ala-Ala-Ser-Ser, Asp-Ala-Ala-Ser-Ser, Ser-Val-Ala-Ser-Ser, Tyr-Val-Ala-Ser-Ser, Phe-Val-Ala-Ser-Ser, Trp-Val-Ala-Ser-Ser, Asp-Val-Ala-Ser-Ser, Ser-Leu-Ala-Ser-Ser, Tyr-Leu-Ala-Ser-Ser, Phe-Leu-Ala-Ser-Ser, Trp-Leu-Ala-Ser-Ser, Asp-Leu-Ala-Ser-Ser, Ser-Ile-Ala-Ser-Ser, Tyr-Ile-Ala-Ser-Ser, Phe-Ile-Ala-Ser-Ser, Trp-Ile-Ala-Ser-Ser or Asp-Ile-Ala-Ser-Ser.

The amino acids and amino-acid residues mentioned in the meanings for X, Y and Z can also be derivatized, with the N-methyl, N-ethyl, N-propyl, N-benzyl or C_α-methyl derivatives being preferred.

Additionally preferred are derivatives of Asp and Glu, in particular the methyl, ethyl, propyl, butyl, tert-butyl, neopentyl or benzyl esters of the side-chain carboxyl groups, furthermore also derivatives of Arg which can be substituted on the -NH-C(=NH)-NH_2 group by an acetyl, benzoyl, methoxycarbonyl or ethoxycarbonyl radical.

Furthermore, the amino acids and amino-acid residues mentioned in the meanings for X and Y can be provided with appropriate protective groups known per se.

Z is preferably M, furthermore preferably D-Phe-M, D-Trp-M, D-Tyr-M, D-Phe-Lys, D-Phe-D-Lys, D-Trp-Lys, D-Trp-D-Lys, D-Tyr-Lys, D-Tyr-D-Lys, D-Phe-Orn, D-Phe-Dab, D-Phe-Dap, D-Phe-D-Orn, D-Phe-D-Dab, D-Phe-D-Dap, D-Phe-4-aminomethyl-Phe, D-Phe-4-aminomethyl-D-Phe, D-Trp-4-aminomethyl-Phe, D-Trp-4-aminomethyl-D-Phe, D-Tyr-4-aminomethyl-Phe, D-Tyr-4-aminomethyl-D-Phe, D-Phe-Asp, D-Phe-D-Asp, D-Trp-Asp, D-Trp-D-Asp, D-Tyr-Asp, D-Tyr-D-Asp, D-Phe-Cys, D-Phe-D-Cys, D-Trp-Cys, D-Trp-D-Cys, D-Tyr-Cys, D-Tyr-D-Cys, Phe-D-Lys, Trp-D-Lys, Tyr-D-Lys, Phe-Orn, Phe-Dab, Phe-Dap, Trp-Orn, Trp-Dab, Trp-Dap, Tyr-Orn, Tyr-Dab, Tyr-Dap, Phe-4-aminomethyl-D-Phe, Trp-4-aminomethyl-D-Phe, Tyr-4-aminomethyl-D-Phe, Phe-D-Asp, Trp-D-Asp, Tyr-D-Asp, Phe-D-Cys, Trp-D-Cys, Tyr-D-Cys, D-Phe-Lys-Gly, D-Phe-M-Gly, D-Trp-Lys-Gly, D-Trp-M-Gly, D-Tyr-Lys-Gly, D-Tyr-M-Gly, D-Phe-Val-Lys, D-Phe-Gly-Lys, D-Phe-Ala-Lys, D-Phe-Ile-Lys, D-Phe-Leu-Lys, D-Trp-Val-Lys, D-Trp-Gly-Lys, D-Trp-Ala-Lys, D-Trp-Ile-Lys, D-Trp-Leu-Lys, D-Tyr-Val-Lys, D-Tyr-Gly-Lys, D-Tyr-Ala-Lys, D-Tyr-Ile-Lys, D-Tyr-Leu-Lys, furthermore also M-Pro-Ala-Ser-Ser.

The compounds of the formula I may have one or more chiral centres and therefore occur in various stereoisomeric forms. Formula I embraces all these forms.

Accordingly, the invention relates in particular to those compounds of the formula I in which at least one of the said radicals has one of the meanings indicated above as preferred. Some preferred groups of compounds can be represented by the following part-formulae Ia to If which correspond to the formula I and in which the undefined radicals have the meanings stated for formula I, but in which

in Ia Q is absent and
R^1 is X-Arg-Gly-Asp-Y;

in Ib Q is $-NH-(CH_2)_5-CO-$ and
R^1 is X-Arg-Gly-Asp-Y;

in Ic Q is $-NH-(CH_2)_5-CO-$ and
R^1 is cyclo-(Arg-Gly-Asp-Z);

in Id Q is $-NH-(CH_2)_5-CO-$ and
R^1 is cyclo-(Arg-Gly-Asp-M);

in Ie Q is $-NH-(CH_2)_5-CO-$ and
R^1 is A-Cys(R^2)-B;

and

in If Q is $-NH-(CH_2)_n-CO-$,
R^1 is X-Arg-Gly-Asp-Y and
n is 1, 2, 3, 4, 5 or 6.

The compounds of the formula I, and the starting materials to prepare them, are moreover prepared by methods known per se, as described in the literature (for example in the standard works such as Houben-Weyl, Methoden der organischen Chemie [Methods of organic chemistry], Georg-Thieme-Verlag, Stuttgart), specifically under reaction conditions which are known and suitable for the said reactions. It is moreover possible to make use of variants which are known per se but which are not mentioned here in detail.

The starting materials can, if required, also be formed in situ so that they are not isolated from the reaction mixture but immediately converted further into the compounds of the formula I.

Compounds of the formula I can preferably be obtained by reacting compounds of the formula II with compounds of the formula III.

The compounds of the formula II and III are known as a rule. If they are unknown, they can be prepared by methods known per se.

The radical -CO-L in the compounds of the formula III is a preactivated carboxylic acid, preferably a carbonyl halide, symmetrical or mixed anhydride or an active ester. Radicals of this type for activating the carboxyl group in typical acylation reactions are described in the literature (for example in the standard works such as Houben-Weyl, Methoden der organischen Chemie, Georg-Thieme-Verlag, Stuttgart). Activated esters are preferably formed in situ, for example by adding HOBt or N-hydroxysuccinimide.
L is preferably H, Cl, Br or -ON-succinimide.

The reaction takes place, as a rule, in an inert solvent in the presence of an acid-binding agent, preferably an organic base such as triethylamine, dimethylaniline, pyridine or quinoline or an excess of the carboxyl component of the formula III.
It may also be beneficial to add an alkali metal or alkaline earth metal hydroxide, carbonate or bicarbonate or another salt of a weak acid of the alkali metals or alkaline earth metals, preferably of potassium, sodium, calcium or caesium.
The reaction time depends on the conditions used and is between a few minutes and 14 days, and the reaction temperature is between about -30° and 140°, normally between -10° and 90°, in particular between about 0° and about 70°.

Examples of suitable inert solvents are hydrocarbons such as hexane, petroleum ether, benzene, toluene or xylene; chlorinated hydrocarbons such as trichloroethylene, 1,2-dichloroethane, tetrachloromethane, chloroform or dichloromethane; alcohols such as methanol, ethanol, isopropanol, n-propanol, n-butanol or tert-butanol; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran (THF) or dioxane; glycol ethers such as ethylene glycol monomethyl or monoethyl ether (methylglycol or ethylglycol), ethylene glycol dimethyl ether (diglyme); ketones such as acetone or butanone; amides such as acetamide, dimethylacetamide or dimethylformamide (DMF); nitriles such as acetonitrile; sulfoxides such as dimethyl sulfoxide (DMSO); carbon disulfide; carboxylic acids such as formic acid or acetic acid; nitro compounds such as nitromethane or nitrobenzene; esters such as ethyl acetate, water or mixtures of the solvents mentioned.

Compounds of the formula I can furthermore be obtained by reacting compounds of the formula IV with compounds of the formula V. The starting compounds of the formula IV and V are, as a rule, known. If they are not known, they can be prepared by methods known per se.

The radical -CO-L in the compounds of the formula V is a preactivated carboxylic acid, preferably a carbonyl halide, symmetrical or mixed anhydride or an active ester. Radicals of this type for activating the carboxyl group in typical acylation reactions are described in the literature (for example in the standard works such as Houben-Weyl, Methoden der organischen Chemie, Georg-Thieme-Verlag, Stuttgart). L is preferably Cl, Br or -ON-succinimide.

Reaction of compounds of the formula IV with compounds of the formula V takes place under the same conditions relating to the reaction time, temperature and solvent as described for the reaction of the compounds of the formula II with compounds of the formula III.

Linear open-chain compounds of the formula I in which

R^1 is X-Arg-Gly-Asp-Y or A-Cys(R^2)-B

can furthermore be obtained by coupling, in the last step of the solid-phase synthesis, biotin in the same cycle as a normal N-terminally protected amino acid as last component, and cleaving the biotin-peptide off the resin under normal conditions.

The solid-phase synthesis, cleavage off and purification are carried out as described by A. Jonczyk and J. Meienhofer in Peptides, Proc. 8th Am. Pept. Symp., Eds. V. Hruby and D.H. Rich, Pierce Comp. III, p. 73-77 (1983) or in analogy to the techniques described in Angew. Chem, 104, 375-391 (1992).

Open-chain linear compounds of the formulae II and IV can moreover be prepared by conventional methods of amino acid and peptide synthesis as described, for example, in the standard works and patent applications mentioned, for example also by the solid-phase synthesis of Merrifield (B.F. Gysin and R.B. Merrifield, J. Am. Chem. Soc. 94, 3102 ff. (1972)).

Cyclic compounds of the formula II and IV in which R^1 is cyclo-(Arg-Gly-Asp-Z) can be prepared by cyclizing the linear compounds as described, for example, in DE 43 10 643 or in Houben-Weyl, l.c., Volume 15/II, pages 1 to 806 (1974).

The compounds of the formula I can furthermore be obtained by liberating them from their functional derivatives by solvolysis, in particular hydrolysis, or by hydrogenolysis.

Preferred starting materials for the solvolysis or hydrogenolysis are those which comprise in place of one or more free amino and/or hydroxyl groups corresponding protected amino and/or hydroxyl groups, preferably those which have in place of an H atom which is connected to an N atom an amino protective group, for example those which correspond to the formula I but comprise in place of an NH_2 group an NHR′ group (in which R′ is an amino protective group, for example BOC or CBZ).

Further preferred starting materials are those which have in place of the H atom of a hydroxyl group a hydroxyl protective group, for example those which correspond to the formula I but comprise in place of a hydroxyphenyl group an R″O-phenyl group (in which R″ is a hydroxyl protective group).

It is also possible for several - identical or different - protected amino and/or hydroxyl groups to be present in the molecule of the starting material. If the protective groups which are present differ from one another, they can in many cases be cleaved off selectively.

The term "amino protective group" is generally known and refers to groups which are suitable for protecting (blocking) an amino group from chemical reactions but which can easily be removed after the required chemical reaction has been carried out elsewhere in the molecule. Typical groups of this type are, in particular, unsubstituted or substituted acyl, aryl, aralkoxymethyl or aralkyl groups. Since the amino protective groups are removed after the required reaction (or sequence of reactions), their nature and size are not otherwise critical; however, those with 1-20, in particular 1-8 C atoms are preferred. The term "acyl group" is to be interpreted in its widest sense in connection with the present process. It includes acyl groups derived from aliphatic, araliphatic, aromatic or heterocyclic carboxylic acids or sulfonic acids, and, in particular, alkoxycarbonyl, aryloxycarbonyl and, especially, aralkoxycarbonyl groups. Examples of acyl groups of these types are alkanoyl such as acetyl, propionyl, butyryl; aralkanoyl such as phenylacetyl; aroyl such as benzoyl or toluyl; aryloxyalkanoyl such as POA; alkoxycarbonyl such as methoxycarbonyl, ethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, BOC, 2-iodoethoxycarbonyl; aralkyloxycarbonyl such as CBZ ("carbobenzoxy"), 4-methoxybenzyloxycarbonyl, FMOC; arylsulfonyl such as Mtr. Preferred amino protective groups are BOC and Mtr, also CBZ, Fmoc, benzyl and acetyl.

The term "hydroxyl protective group" is likewise generally known and refers to groups which are suitable for protecting a hydroxyl group from chemical reactions but which can easily be removed after the required chemical reaction has been carried out elsewhere in the molecule. Typical groups of this type are the abovementioned unsubstituted or substituted aryl, aralkyl or acyl groups, as well as alkyl groups. The nature and size of the hydroxyl protective groups is not critical because they are removed again after the required chemical reaction or sequence of reactions; groups with 1-20, in particular 1-10, C atoms are preferred. Examples of hydroxyl protective groups include benzyl, p-nitrobenzoyl, p-toluenesulfonyl, tert-butyl and acetyl, with benzyl and tert-butyl being particularly preferred. The COOH groups in aspartic acid and glutamic acid are preferably protected in the form of their tert-butyl esters (for example Asp(O But)).

The liberation of the compounds of the formula I from their functional derivatives takes place, depending on the protective group used, for example with strong acids, preferably with TFA or perchloric acid, but also with other strong inorganic acids such as hydrochloric acid or sulfuric acid, strong organic carboxylic acids such as trichloroacetic acid or sulfonic acids such as benzene- or p-toluenesulfonic acid. The presence of an additional inert solvent is possible but not always necessary. Suitable and preferred inert solvents are organic, for example carboxylic acids such as acetic acid, ethers such as tetrahydrofuran or dioxane, amides such as DMF, halogenated hydrocarbons such as dichloromethane, also alcohols such as methanol, ethanol or isopropanol, and water. Also suitable are mixtures of the abovementioned solvents. TFA is preferably used in excess without addition of another solvent, perchloric acid in the form of a mixture of acetic acid and 70% perchloric acid in the ratio 9:1. The reaction temperatures for the cleavage are preferably between about = [sic] and about 50°, preferably between 15 and 30° (room temperature).

The groups BOC, OBut and Mtr can be cleaved off, for example, preferably with TFA in dichloromethane or with approximately 3 to 5N HCl in dioxane at 15-30°, the FMOC group with an approximately 5 to 50% solution of dimethylamine. Diethylamine or piperidine in DMF at 15-30°.

The trityl group is employed to protect the amino acids histidine, asparagine, glutamine and cysteine. The cleavage off takes place, depending on the required final product, with TFA/10% thiophenol, in which case the trityl group is cleaved off all the amino acids mentioned, and when TFA/anisole or TFA/thioanisole is used, only the trityl group on His, Asn and Gln is cleaved off, whereas it remains on the Cys side chain.

Protective groups which can be removed by hydrogenolysis (for example CBZ or benzyl) can be cleaved off, for example, by treatment with hydrogen in the presence of a catalyst (for example of a noble metal catalyst such as palladium, preferably on a support such as carbon). Suitable solvents in this case are those indicated above, especially, for example, alcohols such as methanol or ethanol or amides such as DMF. The hydrogenolysis is, as a rule, carried out at temperatures between about 0 and 100° and under pressures between about 1 and 200 bar, preferably at 20-30° and 1-10 bar. Hydrogenolysis of the CBZ group takes place, for example, well on 5 to 10% Pd/C in methanol or with ammomium [sic] formate (in place of hydrogen) on Pd/C in methanol/DMF at 20-30°.

A base of the formula I can be converted with an acid into the relevant acid addition salt, for example by reacting equivalent amounts of the base and the acid in an inert solvent such as ethanol and subsequently evaporating. Acids particularly suitable for this reaction are those which provide physiologically acceptable salts. Thus, it is possible to use inorganic acids, for example sulfuric acid, nitric acid, hydrohalic acids such as hydrochloric acid or hydrobromic acid, phosphoric acids such as orthophosphoric acid, sulfamic acid, also organic acids, in particular aliphatic, alicyclic, araliphatic, aromatic or heterocyclic monobasic or polybasic carboxylic, sulfonic or sulfuric acids, for example formic acid, acetic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, citric acid, gluconic acid, ascorbic acid, nicotinic acid, isonicotinic acid, methane- or ethanesulfonic acid, ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalenemono- and -disulfonic acids, laurylsulfuric acid. Salts with physiologically unacceptable acids, for example picrates, can be used to isolate and/or purify the compounds of the formula I.

On the other hand, an acid of the formula I can be converted by reaction with a base into one of its physiologically acceptable metal or ammonium salt. Particularly suitable salts in this connection are the sodium, potassium, magnesium, calcium and ammonium salts, also substituted ammonium salts, for example the dimethyl-, diethyl- or diisopropylammonium salts, monoethanol-, diethanol- or diisopropylammonium salts, cyclohexyl, dicyclohexylammonium salts, dibenzylethylenediammonium salts, furthermore, for example, salts with arginine or lysine.

The invention furthermore relates to the use of the compounds of the formula I and/or their physiologically acceptable salts for producing pharmaceutical preparations, in particular by non-chemical means. For this purpose they can be converted into a suitable dosage form together with at least one solid, liquid and/or semiliquid vehicle or ancillary substance and, where appropriate, in combination with one or more other active substances.

The invention furthermore relates to pharmaceutical preparations comprising at least one compound of the formula I and/or one of its physiologically acceptable salts.

These preparations can be used as pharmaceuticals in human or veterinary medicine. Suitable vehicles are organic or inorganic substances which are suitable for enteral (for example oral), parenteral, topical administration or for administration in the form of an inhalation spray and which do not react with the novel compounds, for example water, vegetable oils, benzyl alcohols, alkylene glycols, polyethylene glycols, glycerol triacetate, gelatin, carbohydrates such as lactose or starch, magnesium stearate, talc, petrolatum. Used for oral administration are, in particular, tablets, pills, coated tablets, capsules, powders, granules, syrups, solutions or drops, for rectal administration are suppositories, for parenteral administration are solutions, preferably oily or aqueous solutions, furthermore suspensions, emulsions or implants, for topical administration are ointments, creams or dusting powders. The novel compounds can also be lyophilized and the resulting lyophilizates used, for example, to produce injection products. The indicated preparations can be sterilized and/or comprise ancillary substances such as lubricants, preservatives, stabilizers and/or wetting agents, emulsifiers, salts to influence the osmotic pressure, buffer substances, colorants, flavourings and/or several other active substances, for example one or more vitamins. The sprays which can be used for administration as inhalation spray comprise the active substance either dissolved or suspended in a propellant gas or mixture of propellant gases (for example CO_2 or chlorofluorocarbons). In this case, the active substance is preferably used in micronized form, it being possible for one or more additional physiologically tolerated solvents to be present, for example ethanol. Inhalation solutions can be administered using conventional inhalers.

The compounds of the formula I and their physiologically acceptable salts can be used as integrin inhibitors for controlling diseases, in particular pathologically angiogenic disorders, thromboses, myocardial infarct, coronary heart diseases, arteriosclerosis, tumours, osteoporosis, inflammations and infections.

For this purpose, the substances according to the invention can be administered as a rule in analogy to other known peptides which are commercially available, but in particular in analogy to the compounds described in US-A-4 472 305, preferably in doses between about 0.05 and 500 mg, in particular between 0.05 and 100 mg, per dosage unit. The daily dose is preferably between about 0.01 and 2 mg/kg of bodyweight. The specific dose for each patient depends, however, on a wide variety of factors, for example on the activity of the specific compound employed, on the age, bodyweight, general state of health, sex, on the diet, on the time and route of administration, on the rate of excretion, medicinal substance combination and severity of the particular disorder for which the therapy is applied. Parenteral administration is preferred.

The novel compounds of the formula I can furthermore be used in analytical biology and molecular biology. This entails utilization of the ability of the biotinyl radical to form a complex with the glycoprotein avidin.

Uses of the biotin-avidin complex are disclosed in E.A. Bayer and M. Wilchek in Methods of Biochemical Analysis 26, 1-45 (1980) (Lit. 1).

The novel compounds of the formula I can be used as integrin ligands for producing columns for affinity chromatography to prepare pure integrins. The complex of an avidin-derivatized support material, for example Sepharose, and the novel compounds of the formula I is formed by methods known per se, as described, for example, in Lit. 1.

For this reason, no further details of this method are given at this point, and reference is made to the corresponding literature, for example Lit. 1.

Suitable polymeric support materials are the polymeric solid phases which are known in peptide chemistry and preferably have hydrophilic properties, for example crosslinked polysaccharides such as cellulose, Sepharose or Sephadex^R, acrylamides, polymers based on polyethylene glycol or tentacular polymers^R.

The novel compounds of the formula I can also be used as diagnostic markers for anti-biotin antibody reactions in ELISA-type assays and in FACS (Fluorescence Activated Cell Sorter) analysis.

The use of antibiotin antibodies for detecting biotin is disclosed by M. Berger, Biochemistry 14, 2338-2342 (1975). The use of immunoglobulin IgG derivatized with biotin in an enzyme immunoassay (ELISA) is described by U. Holmkov-Nielsen et al. in Journal of Chromatography, 297, 225-233 (1984).

J. Gao and S.J. Shattil describe in J. Immunol. Methods 181, 55-64 (1995) an ELISA test which detects substances which inhibit integrin $\alpha_{IIb}\beta_{III}$ activation. In this case, biotinylated fibrinogen is employed for detection.

The use of flow cytometry in clinical cytodiagnosis is described by G. Schmitz and G. Rothe in DG Klinische Chemie Mitteilungen 24 (1993) No. 1, page 1-14.

The compounds of the formula I can furthermore be employed in force field microscopy (atomic force microscopy AFM) to measure the strength of ligand-receptor interactions.

Ligand preferably means a complex of avidin and the novel compounds of the formula I.

Receptor preferably means an integrin receptor.

E.-L. Florin et al. describe measurements of adhesion forces between an avidin-functionalized force field microscope and biotinylated agarose in Science 264, 415-417 (1994).

All temperatures are stated in °C hereinbefore and hereinafter. In the following examples "usual working up" means: if necessary, water is added, if necessary, depending on the constitution of the final product, the pH is adjusted to between 2 and 10, extraction is carried out with ethyl acetate or dichloromethane, the organic phase is separated off, dried over sodium sulfate and evaporated, and purification is carried out by chromatography on silica gel and/or by crystallization. Rf values on silica gel; mobile phase: ethyl acetate/methanol 9:1.

RT = retention time (minutes) on HPLC in the following systems:

[A]
Column: Nucleosil™ 7C18 250 x 4 mm
Eluent A: 0.1% TFA in water
Eluent B: 0.1% TFA in acetonitrile
Flow rate: 1 ml/min
Gradient: 20-50% B/30 min

[B]
50-minute gradient 0-80% 2-propanol in water with 0.3% TFA at 1 ml/min on a Lichrosorb^{R} RP Select B (7 μm) 250 x 4 mm column

[C]
Column: Lichrospher™ (5 μm) 100RP8 125 x 4 mm
Eluent A: 0.01 M Na phosphate pH 7.0
Eluent B: 0.005 M Na phosphate pH 7.0/60% by volume 2-propanol
Flow rate: 0.7 ml/min
Gradient: 1-99% B/50 min.

Mass spectrometry (MS): EI (electron impact ionization) M^{+}
FAB (Fast Atom Bombardment) (M+H)$^{+}$

DMPP-resin represents 4-(2',4'-dimethoxyphenylhydroxymethyl)phenoxy-resin [sic], a super acid-labile resin which permits the synthesis of side-chain protected peptides.

Example 1

0.6 g of Fmoc-Lys(Boc)-OH is dissolved in 100 ml of dichloromethane, 1.2 equivalents of DMPP-resin, 1.4 equivalents of HOBt and 1.4 equivalents of DCCI are added, and the mixture is stirred at room temperature for 12 hours. Removal of the solvent results in Fmoc-Lys(Boc)-DMPP-resin. In a peptide synthesizer, Fmoc-Pro-OH is condensed with H-Lys(BOC)-DMPP-resin [liberated from Fmoc-Lys(BOC)-DMPP-resin with piperidine/DMF (20%)] by employing a three-fold excess of the protected proline. The coupling is carried out with DCCI/HOBt at room temperature. Fmoc-Pro-Lys(BOC)-DMPP-resin is obtained. Analogously, subsequent cleavage off of the Fmoc protective group and consecutive couplings with Fmoc-Ser(But)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Gly-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Gly-OH and Bit-OH under reaction conditions repeated for each coupling

- Liberation of the α-amino group with piperidine/DMF (20%)
- Washing with dimethylacetamide
- Reaction with the Fmoc-amino acid or Bit-OH

Bit-Gly-Gly-Gly-Arg(Mtr)-Gly-Asp(OBut)-Ser(But)-Pro-Lys(BOC)-DMPP-resin.
The resin is washed with $CF_3SO_3H/CH_2Cl_2/H_2O$ to result in Bit-Gly-Gly-Gly-Arg(Mtr)-Gly-Asp(OBut)-Ser(But)-Pro-Lys(BOC)-OH.
The protective groups are cleaved off with 2N HCl in dioxane, the solvent if removed, the residue is taken up in TFA/CH_2Cl_2 and precipitated with Et_2O, and then purification is carried out by RP-HPLC.
Bit-Gly-Gly-Gly-Arg-Gly-Asp-Ser-Pro-Lys-OH x 2 TFA;

RT [B] = 12.14 is obtained; FAB 1056.

Example 2

In analogy to Example 1, consecutive couplings of the DMPP-resin with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Gly-OH and Bit-OH result in:

Bit-Gly-Gly-Gly-Lys(BOC)-Thr(But)-Ala-Asp(OBut)-Cys(Trt)-Pro-DMPP-resin.
Cleavage off from the resin, cleavage off of the protective groups and purification result in
Bit-Gly-Gly-Gly-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-OH x2 TFA;
RT [B] 27.6; FAB 1273.

The following are obtained analogously by condensing the DMPP-resin

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ala-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Ala-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Arg(Mtr)-OH and Bit-OH:

Bit-Arg-Thr-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH and Bit-OH:

Bit-Ser-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Gln(Trt)-OH and Bit-OH:

Bit-Gln-Ser-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Glp-OH and Bit-OH:

Bit-Glp-Ser-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Ile-OH and Bit-OH:

Bit-Ile-Ser-Ala-Gly-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Arg(Mtr)-OH and Bit-OH:

Bit-Arg-Ser-Ala-Gly-Cys(Trt)-Pro-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, FMOC-Asp(OBut)-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Gly-Gly-Asp-Cys(Trt)-Pro-OH;

with Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-OH;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol;

Bit-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:

Bit-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH,
Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Gly-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol;
Bit-Gly-Lys-Thr-Cys-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

b) when the protetive groups are cleaved off with TFA and 10% thioanisole:
Bit-Gly-Lys-Thr-Cys(Trt)-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

with Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:
Bit-Lys-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-OH;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:
Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-OH;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:

Bit-Lys-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

with Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Gly-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:

Bit-Gly-Lys-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-OH;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:

Bit-Gly-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-OH;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH and Bit-OH:

Bit-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

Bit-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-OH;

with Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

Bit-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-OH;

with Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-OH;

with Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-OH;

with Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-OH;

with Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-OH;

with Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH and Bit-OH:

Bit-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-OH;

with Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

Bit-Ala-Asp-Cys(Trt)-Pro-Arg-OH;

with Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, and Bit-OH:

Bit-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-OH;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-OH;

with Fmoc-NMeAla-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-NMeAla-OH;

Example 3

1.7 g of (+)-biotinyl-N-succinimidyl ester, which is inexpensive, and 0.5 g of triethylamine are added to a solution of 3.05 g of cyclo-(Arg-Gly-Asp-D-Phe-Lys) [obtainable by cyclizing H-Arg(Mtr)-Gly-Asp(OBut)-D-Phe-Lys(BOC)-OH to cyclo-(Arg(Mtr)-Gly-Asp(OBut)-D-Phe-Lys(BOC)) and subsequently cleaving off the protective groups] in 100 ml of dichloromethane. The mixture is stirred at room temperature for 5 hours, and the usual working up results in cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit)) x TFA; RT [B] 11.32; FAB 830.

Example 4

In analogy to Example 3, cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit-Aha)) x TFA; RT [C] 23.67; FAB 943, is obtained from 3.05 g of cyclo-(Arg-Gly-Asp-D-Phe-Lys) and 2.3 g of N-succinimidyl (+)-biotinyl-6-aminocaproate ("A"), which can be purchased inexpensively, and 0.5 g of triethylamine.

There are obtained analogously from "A" and the following cyclic compounds

Cyclo-(Arg-Gly-Asp-D-Trp-Lys)
Cyclo-(Arg-Gly-Asp-D-Tyr-Lys)

Cyclo-(Arg-Gly-Asp-D-Phe-D-Lys)

Cyclo-(Arg-Gly-Asp-D-Phe-Cys)
Cyclo-(Arg-Gly-Asp-D-Phe-Dab)
Cyclo-(Arg-Gly-Asp-D-Trp-D-Cys)
Cyclo-(Arg-Gly-Asp-D-Tyr-D-Cys)
Cyclo-(Arg-Gly-Asp-Phe-D-Lys)
Cyclo-(Arg-Gly-Asp-Trp-D-Lys)
Cyclo-(Arg-Gly-Asp-Tyr-D-Lys)
Cyclo-(Arg-Gly-Asp-Phe-D-Cys)
Cyclo-(Arg-Gly-Asp-Phe-Dab)
Cyclo-(Arg-Gly-Asp-Trp-D-Cys)
Cyclo-(Arg-Gly-Asp-Tyr-D-Cys)
Cyclo-(Arg-Gly-Asp-D-Trp-Orn)
Cyclo-(Arg-Gly-Asp-D-Tyr-Orn)
Cyclo-(Arg-Gly-Asp-D-Phe-Orn)
Cyclo-(Arg-Gly-Asp-D-Trp-D-Orn)
Cyclo-(Arg-Gly-Asp-D-Tyr-D-Orn)
Cyclo-(Arg-Gly-Asp-D-Phe-D-Orn)
Cyclo-(Arg-Gly-Asp-D-Trp-Dab)
Cyclo-(Arg-Gly-Asp-D-Tyr-Dab)
Cyclo-(Arg-Gly-Asp-D-Trp-Dap)
Cyclo-(Arg-Gly-Asp-D-Tyr-Dap)
Cyclo-(Arg-Gly-Asp-D-Phe-Dap)
Cyclo-(Arg-Gly-Asp-D-Trp-D-Dap)
Cyclo-(Arg-Gly-Asp-D-Tyr-D-Dap)
Cyclo-(Arg-Gly-Asp-D-Phe-D-Dap)

the following compounds

Cyclo-(Arg-Gly-Asp-D-Trp-Lys(N$^{\varepsilon}$-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-Lys(N$^{\varepsilon}$-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-D-Lys(N$^{\varepsilon}$-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-Cys(S-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-Dab(N$^{\gamma}$-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Trp-D-Cys(S-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-D-Cys(S-Bit-Aha))

Cyclo-(Arg-Gly-Asp-Phe-D-Lys(N^{ε}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-Trp-D-Lys(N^{ε}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-Tyr-D-Lys(N^{ε}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-Phe-D-Cys(S-Bit-Aha))
Cyclo-(Arg-Gly-Asp-Phe-Dab(N^{γ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-Trp-D-Cys(S-Bit-Aha))
Cyclo-(Arg-Gly-Asp-Tyr-D-Cys(S-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Trp-Orn(N^{δ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-Orn(N^{δ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-Orn(N^{δ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Trp-D-Orn(N^{δ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-D-Orn(N^{δ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-D-Orn(N^{δ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Trp-Dab(N^{γ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-Dab(N^{γ}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Trp-Dap(N^{β}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-Dap(N^{β}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-Dap(N^{β}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Trp-D-Dap(N^{β}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Tyr-D-Dap(N^{β}-Bit-Aha))
Cyclo-(Arg-Gly-Asp-D-Phe-D-Dap(N^{β}-Bit-Aha))

Example 5

6 g of Boc-Aha N-succinimidyl ester are added to a solution of 3.05 g of cyclo-(Arg-Gly-Asp-D-Phe-Lys) in 40 ml of 5% aqueous $NaHCO_3$ and 40 ml of THF. Stirring for 4 hours and the usual working up result in cyclo-(Arg-Gly-Asp-D-Phe-Lys(BOC-Aha)); RT [C] 27.7; FAB 817. Cleavage off of the BOC group in HCl/dioxane and the usual working up result in cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Aha)) x 2 TFA; RT [C] 14.76; FAB 717.
In analogy to Example 1, subsequent reaction with (+)-biotinyl-N-succinimidyl [sic] ester results in cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit-Aha)) x 2 TFA; RT [C] 23.67; FAB 943.

Example 6

In analogy to Example 4 there is obtained from cyclo-(Arg-Gly-Asp-D-Phe-Lys-Gly) [obtainable by cyclizing H-Arg(Mtr)-Gly-Asp(OBut)-D-Phe-Lys(BOC)-Gly-OH to cyclo-(Arg(Mtr)-Gly-Asp(OBut)-D-Phe-Lys(BOC)-Gly) and subsequently cleaving off the protective groups] and N-succinimidyl (+)-biotinyl-6-aminocaproate cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit-Aha)-Gly) x TFA; RT [A] 10.97; FAB 1000.

There is obtained analogously from cyclo-(Arg-Gly-Asp-D-Phe-Val-Lys) [obtainable by cyclizing H-Arg(Mtr)-Gly-Asp(OBut)-D-Phe-Val-Lys(BOC)-OH to cyclo-(Arg(Mtr)-Gly-Asp(OBut)-D-Phe-Val-Lys(BOC)) and subsequently cleaving off the protective groups] and N-succinimidyl (+)-biotinyl-6-aminocaproate cyclo-(Arg-Gly-Asp-D-Phe-Val-Lys(N^{ε}-Bit-Aha)) x TFA; RT [A] 16.11; FAB 1042.

There is obtained analogously from cyclo-(Arg-Gly-Asp-D-Phe-N-Me-Lys) and N-succinimidyl (+)-biotinyl-6-aminocaproate cyclo-(Arg-Gly-Asp-D-Phe-N-Me-Lys(N^{ε}-Bit-Aha)).

Example 7

In analogy to Example 1, successive couplings onto an MBHA-resin with the addition of 1.4 equivalents of HOBt and 1.4 equivalents of DCCI with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Gly-OH, Fmoc-Gly-OH and Bit-OH result in:

Bit-Gly-Gly-Gly-Lys(BOC)-Thr(But)-Ala-Asp(OBut)-Cys(Trt)-Pro-MBHA-resin.

Cleavage off from the resin with TFA, cleavage off of the protective groups with piperidine/DMF and purification result in

Bit-Gly-Gly-Gly-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-NH_2

There are obtained analogously by condensation of the MBHA-resin with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ala-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Ala-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Arg(Mtr)-OH and Bit-OH:

Bit-Arg-Thr-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH and Bit-OH:

Bit-Ser-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Gln(Trt)-OH, and Bit-OH:

Bit-Gln-Ser-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Glp-OH, and Bit-OH:

Bit-Glp-Ser-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Ile-OH and Bit-OH:

Bit-Ile-Ser-Ala-Gly-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Ser(But)-OH, Fmoc-Arg(Mtr)-OH and Bit-OH:

Bit-Arg-Ser-Ala-Gly-Cys(Trt)-Pro-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, and Bit-OH:

Bit-Lys-Gly-Gly-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-NH_2;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:
Bit-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH_2;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:
Bit-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH_2;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Gly-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:
Bit-Gly-Lys-Thr-Cys-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH_2;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:
Bit-Gly-Lys-Thr-Cys(Trt)-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH_2;

with Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:
Bit-Lys-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-NH$_2$;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:
Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-NH$_2$;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, FMoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:
Bit-Lys-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-NH$_2$;

b) when the protective groups are cleaved off with TFA and 10% thioanisole:
Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH$_2$;

with Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH, Fmoc-Gly-OH and Bit-OH:

a) when the protective groups are cleaved off with TFA and 10% thiophenol:
Bit-Gly-Lys-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-NH$_2$;

a) when the protective groups are cleaved off with TFA and 10% thioanisole:
Bit-Gly-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-Gly-NH$_2$;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH and Bit-OH:

Bit-Thr-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH_2;

with Fmoc-Thr(But)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

Bit-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-Pro-Ala-Thr-NH_2;

with Fmoc-Gly-OH, Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

Bit-Ala-Asp-Cys-Pro-Arg-Asn-Pro-His-Lys-Gly-NH_2;

with Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-NH_2;

with Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-NH_2;

with Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-NH_2;

with Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-NH_2;

with Fmoc-Lys(BOC)-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH and Bit-OH:

Bit-Asp-Cys(Trt)-Pro-Arg-Asn-Pro-His-Lys-NH_2;

with Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH and Bit-OH:

Bit-Ala-Asp-Cys(Trt)-Pro-Arg-NH_2;

with Fmoc-Arg(Mtr)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH and Bit-OH:

Bit-Thr-Ala-Asp-Cys(Trt)-Pro-Arg-NH_2;

with Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-NH_2;

with Fmoc-NMeAla-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ala-OH, Fmoc-Thr(But)-OH, Fmoc-Lys(BOC)-OH and Bit-OH:

Bit-Lys-Thr-Ala-Asp-Cys(Trt)-NMeAla-NH_2;

Example 8

Production of a material suitable for affinity chromatography to purify integrins:

The activation of Sepharose™ takes place as described in Lit. 1, page 14. Then 20 mg of avidin in 20 ml of 0.1 M sodium bicarbonate solution are added to 10 g of activated Sepharose™.

The suspension is stirred at 4° for 12 hours and then washed. The material is then suspended in water with a few crystals of sodium azide.

The avidin complex with the biotinylated compounds according to the invention, for example cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit)) x TFA, is formed by dissolving 1.1 equivalent of peptide in sodium acetate buffer, adding the solution to the suspension of avidin-Sepharose and stirring at 4° for 10 hours. Excess peptide is removed by washing.

The following examples relate to pharmaceutical preparations:

Example A: Vials

A solution of 100 g of an active substance of the formula I and 5 g of disodium hydrogen phosphate in 3 l of double-distilled water are [sic] adjusted to pH 6.5 with 2 N hydrochloric acid, sterilized by filtration, dispensed into vials and lyophilized and sealed under sterile conditions. Each vial comprises 5 mg of active substance.

Example B: Suppositories

A mixture of 20 g of an active substance of the formula I with 100 g of soya lecithin and 1400 g of cocoa butter is melted, poured into moulds and left to cool. Each suppository comprises 20 mg of active substance.

Example C: Solution

A solution is prepared from 1 g of an active substance of the formula I, 9.38 g of $NaH_2PO_4 \cdot 2\ H_2O$, 28.48 g of $Na_2HPO_4 \cdot 12\ H_2O$ and 0.1 g of benzalkonium chloride in 940 ml of double-distilled water. The pH is adjusted to 6.8, the volume is made up to 1 l and the solution is radiation-sterilized. This solution can be used in the form of eye drops.

Example D: Ointment

500 mg of an active substance of the formula I are mixed with 99.5 g of petrolatum under aseptic conditions.

Example E: Tablets

A mixture of 1 kg of active substance of the formula I, 4 kg of lactose, 1.2 kg of potato starch, 0.2 kg of talc and 0.1 kg of magnesium stearate is compressed to tablets in a conventional way so that each tablet comprises 10 mg of active substance.

Example F: Coated tablets

Tablets are compressed in analogy to Example E and are then provided in a conventional way with a coating of sucrose, potato starch, talc, tragacanth and colorant.

Example G: Capsules

2 kg of active substance of the formula I are packed into hard gelatin capsules in a conventional way so that each capsule comprises 20 mg of the active substance.

Example H: Ampoules

A solution of 1 kg of active substance of the formula I in 60 l of double-distilled water is sterilized by filtration, dispensed into ampoules and lyophilized and sealed under sterile conditions. Each ampoule comprises 10 mg of active substance.

Example I: Inhalation spray

14 g of active substance of the formula I are dissolved in 10 l of isotonic NaCl solution, and the solution is used to fill commercial spray vessels with a pump mechanism. The solution can be sprayed into the mouth or nose. One puff (about 0.1 ml) corresponds to a dose of about 0.14 mg.

CLAIMS:

1. Bit-Gly-Gly-Gly-Arg-Gly-Asp-Ser-Pro-Lys-OH; or a salt thereof.

2. Bit-Gly-Gly-Gly-Lys-Thr-Ala-Asp-Cys(Trt)-Pro-OH; or a salt thereof.

3. cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit)); or a salt thereof.

4. cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Aha)); or a salt thereof.

5. cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit-Aha)-Gly); or a salt thereof.

6. cyclo-(Arg-Gly-Asp-D-Phe-Val-Lys(N^{ε}-Bit-Aha)); or a salt thereof.

7. cyclo-(Arg-Gly-Asp-D-Phe-N-Me-Lys(N^{ε}-Bit-Aha)); or a salt thereof.

8. cyclo-(Arg-Gly-Asp-D-Phe-Lys(N^{ε}-Bit-Aha)); or a salt thereof.

9. cyclo-(Arg-Gly-Asp-D-Phe-Lys(BOC-Aha)); or a salt thereof.

10. A pharmaceutical composition comprising a compound according to any one of claims 1 to 9 or a physiologically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent.

11. A pharmaceutical composition according to claim 10 for treatment of a pathologically angiogenic disorder, thrombosis, myocardial infarction, a coronary heart disease,

arteriosclerosis, a tumour, osteoporosis, inflammation or an infection.

12. A pharmaceutical composition according to claim 10 for inhibition of an integrin.

13. A compound according to any one of claims 1 to 9, or a physiologically acceptable salt thereof for treatment of a pathologically angiogenic disorder, thrombosis, myocardial infarction, a coronary heart disease, arteriosclerosis, a tumour, osteoporosis, inflammation or an infection.

14. A compound according to any one of claims 1 to 9, or a physiologically acceptable salt thereof for inhibition of an integrin.

15. A use of a compound according to any one of claims 1 to 9, or a physiologically acceptable salt thereof for treatment of a pathologically angiogenic disorder, thrombosis, myocardial infarction, a coronary heart disease, arteriosclerosis, a tumour, osteoporosis, inflammation or an infection.

16. A use of a compound according to any one of claims 1 to 9, or a physiologically acceptable salt thereof for inhibition of an integrin.

17. A use of a compound according to any one of claims 1 to 9, or a physiologically acceptable salt thereof in manufacture of a medicament for treatment of a pathologically angiogenic disorder, thrombosis, myocardial infarction, a coronary heart disease, arteriosclerosis, a tumour, osteoporosis, inflammation or an infection.

18. A use of a compound according to any one of claims 1 to 9, or a physiologically acceptable salt thereof

in manufacture of a medicament for inhibition of an integrin.

19. Use of a compound or salt according to any one of claims 1 to 9 for purifying an integrin by affinity chromatography.

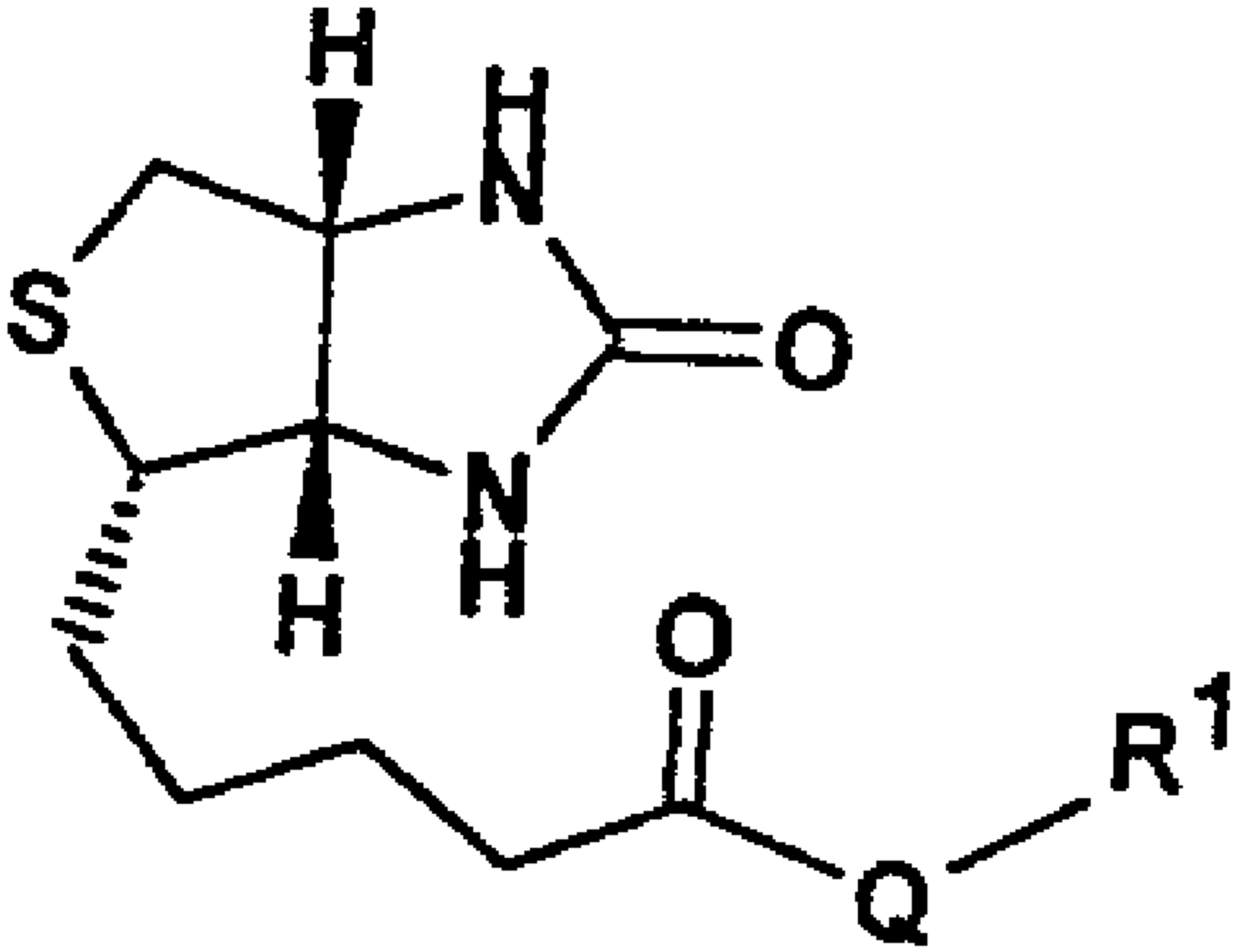
20. Use of a compound or salt according to any one of claims 1 to 9 as a diagnostic marker for an anti-biotin antibody reaction in an ELISA-type assay and FACS analysis.

21. Use of a compound or salt according to any one of claims 1 to 9 in atomic force microscopy to measure the strength of a ligand-receptor interaction.

FETHERSTONHAUGH & CO.

OTTAWA, CANADA

PATENT AGENTS



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