Form P.8

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

(To be lodged in duplicate)

PUBLICATION PARTICULARS AND ABSTRACT
(Section 32(3)(a) - Regulations 22(1)(g) and 31)

REFERENCE : P22494ZA00

OFFICIAL APPLICATION NO.			LODGING DATE		ACCEPTANCE DATE	
21	01	20016972	22/23	19 February 2000	43	7.8.2002

INTERNATIONAL CLASSIFICATION	
51	A61K, A61P, C07K

NOT FOR PUBLICATION

CLASSIFIED BY :

FULL NAME(S) OF APPLICANT(S)

71	AVENTIS PHARMA DEUTSCHLAND GMBH

FULL NAME(S) OF INVENTOR(S)

72	STILZ, Hans, Ulrich GERL, Martin FLYNN, Gary, A. STANKOVA, Magda BINNIE, Robert, A.

EARLIEST PRIORITY CLAIMED	COUNTRY		NUMBER		DATE	
NOTE : The country must be indicated by its International Abbreviation - see Schedule 4 of the Regulations	33	DE	31	EP 99103869.6	32	1 March 1999

TITLE OF INVENTION

54	LOW MOLECULAR WEIGHT PEPTIDE DERIVATIVES AS INHIBITORS OF THE LAMININ/NIDOGEN INTERACTION

57	ABSTRACT (NOT MORE THAN 150 WORDS)	NUMBER OF PAGES	104

FOR ABSTRACT SEE THE NEXT SHEET

20016972

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 14/78, 5/10, 5/08, A61K 38/39, A61P 19/00

A1

(11) International Publication Number: WO 00/52051

(43) International Publication Date: 8 September 2000 (08.09.00)

(21) International Application Number: PCT/EP00/01386

(22) International Filing Date: 19 February 2000 (19.02.00)

(30) Priority Data:
99103869.6 1 March 1999 (01.03.99) EP

(71) Applicant: AVENTIS PHARMA DEUTSCHLAND GMBH [DE/DE]; Brüningstrasse 50, D-65929 Frankfurt (DE).

(72) Inventors: STILZ, Hans, Ulrich; Johannesallee 18, D-65929 Frankfurt (DE). GERL, Martin; Zur Steinritz 48, D-65527 Niedernhausen (DE). FLYNN, Gary, A.; 9750 N Cliff View Place, Oro Valey, AZ 85737 (US). STANKOVA, Magda; 7045 N. Montebella Road, Tucson, AZ 85704 (US). BINNIE, Robert, A.; 8945 North Shadow Moutain Drive, Tucson, AZ 85737 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: LOW MOLECULAR WEIGHT PEPTIDE DERIVATIVES AS INHIBITORS OF THE LAMININ/NIDOGEN INTERACTION

(57) Abstract

Object of the present invention are low molecular weight peptide derivatives which are able to act as inhibitors of the interaction between laminin and nidogen (laminin/nidogen interaction), a process for their preparation, pharmaceutical compositions prepared therefrom and their use for preparing pharmaceuticals and for identifying inhibitors of the laminin/nidogen interaction.

Low molecular weight peptide derivatives as inhibitors of the laminin/nidogen interaction

Object of the present invention are low molecular weight peptide derivatives which are able to act as inhibitors of the interaction between laminin and nidogen (laminin/nidogen interaction), a process for their preparation, pharmaceutical compositions prepared therefrom and their use for preparing pharmaceuticals and for identifying inhibitors of the laminin/nidogen interaction.

The association of laminin (an 800 kDa glycoprotein) and nidogen (a 160 kDa glycoprotein) is regarded as a crucial biomolecular mechanism in the synthesis and stabilization of basement membranes (Mayer, U. and Timpl, R. (1994) in: Extracellular Matrix Assembly and Structure (P.D. Yurchenco, D. Birk and R.P. Mecham, Ed.) S. 389 - 416, Academic Press, Orlando, FL). The ability of nidogen to form ternary complexes with all main constituents of the basement membrane such as, for example, γ1-containing laminin isoforms (for nomenclature see: Burgeson, R.E.; Chiquet, M.; Deutzmann, R.; Ekblom, P.; Engel, J.; Kleinmann, H.; Martin, G. R.; Meneguzzi, G.; Paulsson M.; Sanes, J.; Timpl, R.; Tryggvasson, K.; Yamada, Y.; Yurchenco, P.D. (1994) Matrix Biology 14; 209 - 211), collagen IV, perlecan and fibulin, and the association structures of each of them, means that it assumes the function of a linker which connects together, spatially organizes and stabilizes the independent macrostructures (Fox, J.W.; Mayer, U.; Nischt, R.; Aumailley, M.; Reinhardt, D.; Wiedemann, H.; Mann, K.; Timpl, R.; Krieg, T.; Engel, J.; and Chu, M.-L. (1991) EMBO J. 10, 3137 - 3146).

Basement membranes are highly specialized extracellular structures which are attributed with important functions in the control of cell and tissue functions, tissue architecture, tissue interactions, cell growth, cell transformation, cell migration and in tissue-specific gene expression (Adams, J.C. and Watt, F.M. (1993) Development 117, 1183 - 1198).Experiments with polyclonal antilaminin antibodies have provided clear evidence of the central function of the laminin/nidogen interaction in the synthesis of a functional basement membrane. The described antibodies were obtained by immunizing rabbits with laminin P1 or with the recombinantly produced nidogen-binding domain of laminin (γ1 III 3-5). The antibodies concentrated by affinity chromatography on laminin P1 or laminin γ1 III 3-5 matrices showed complete inhibition of the laminin/nidogen association in inhibition assays. However, this is based on steric blockade of the access of nidogen to laminin by the antibodies, whose binding regions are located in the vicinity of the nidogen-binding sequences of laminin (Mayer, U.; Nischt, R.; Pöschl, E.; Mann, K.; Fukuda, K.; Gerl, M.; Yamada, Y.; Timpl, R. (1993) EMBO J. 12; 1879 - 1885).

In embryonic organ cultures, the described antibodies inhibited both the development of renal tubules, the formation of pulmonary alveoli and the morphogenesis of the embryonic salivary gland. These three models are representative of ontogenesis programs which depend on unimpeded synthesis of new basement membrane (Ekblom, P.; Ekblom, M.; Fecker, L.; Klein, G.; Zhang, H.-Y.; Kadoya, Y.; Chu, M.-L.; Mayer, U.; Timpl, R. (1994) Development 120; 2003 - 2014).

Antibodies directed against the laminin γ1 chain sequence region which is essential for nidogen binding are likewise able to inhibit the laminin/nidogen association. The inhibition is, however, competitive, in contrast to the antilaminin antibodies described above, because they compete directly with the nidogen for the binding site on laminin (WO 98/31709).

A monoclonal antibody of the IgM subclass (antilaminin P1 A6/2/4 – DSM ACC2327; see WO 98/31709) inhibits the laminin/nidogen interaction in vitro with an IC50 of 30 nM. Like the polyclonal antilaminin antibody preparation described above, it prevents the morphogenesis of the embryonic salivary gland in organ culture. This underlines the specificity of the laminin/nidogen interaction, and the importance of the LE-4 module and of the identified sequence region in the laminin γ1 III 4 domain in this interaction.

The nidogen binding domain of laminin has been unambiguously identified and characterized in terms of its location, sequence and its spatial structure (X-ray crystal structure and NMR structure) (Gerl, M.; Mann, K.; Aumailley, M.; Timpl, R. (1991) Eur. J. Biochem. 202; 167 - 174. Mayer, U.; Nischt, R.; Pöschl, E.; Mann, K.; Fukuda, K.; Gerl, M.; Yamada, Y.; Timpl, R. (1993) EMBO J. 12; 1879 - 1885. Baumgartner, R.; Czisch, M.; Mayer, U.; Pöschl, E.; Huber, R.; Timpl, R.; Holak, T.A. (1996) J. Mol. Biol. 257; 658 - 668. Stetefeld, J.; Mayer, U.; Timpl, R.; Huber, R. (1996) J. Mol. Biol. 257; 644 - 657). It is located in an "LE module" (laminin type epidermal growth factor-like) of the short arm of the γ1 chain of laminin, in the domain γ1 III 4. "LE modules" are structural motifs of 50-60 amino acids which have a complex folding pattern, analogous to EGF, with 4 disulfide bridges (Bairoch, A.; (1995) Nomenclature of extracellular domains. The SWISS-PROT Protein sequence data bank. release 310. Engel, J. (1989) FEBS Letters 251; 1 - 7).

High-affinity binding of nidogen to the complementary laminin domain has been detected for laminin P1 from the EHS tumor of mice, laminin 2 and laminin 4 from human placenta and laminin from drosophila. The cause of this species-overlapping binding specificity is the extremely large identity of sequences present in the γ1 III 4 domain for the species investigated. It is 97% between human and mouse, 61% between mouse and drosophila and, astonishingly, 51% between mouse and Caenorhabditis elegans when the whole domain is taken into account (Pikkarinen, T.; Kallunki, T.; Tryggvasson, K. (1987) J. Biol. Chem. 263; 6751 - 6758. Chi, H.-C.; Hui, C.-F. (1989) J. Biol. Chem. 264; 1543 – 1550. Wilson, R. et al.(1994) Nature 368: 32-38. Pöschl, E.; Mayer, U.; Stetefeld, J.; Baumgartner, R.; Holak, T.A.; Huber, R.; Timpl, R. (1996) EMBO J. 15: 5154-5159).

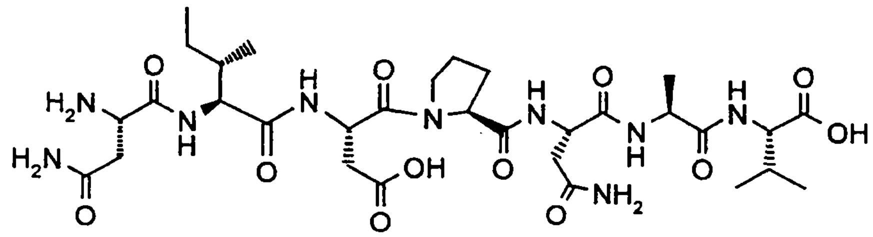
Besides the dependency of nidogen binding on an intact three-dimensional structure, unambiguous sequence regions located in the S-S stabilized loops a and c of the domain γ1 III 4 have been identified. Five essential amino acids have been identified, four located inside a section of 7 amino acids in loop a, and a tyrosine side-chain in loop c (Mann, K.; Deutzmann, R.; Timpl, R. (1988) Eur. J. Biochem. 178; 71 - 80).

Synthetic peptides which can be derived from the appropriate regions of the γ1 III 4 domain and are able to inhibit completely the laminin/nidogen binding in specific binding assays have been disclosed by J.W. Fox and R. Timpl (US 5,493,008).

The high-affinity binding to the laminin binding site of nidogen is thought to require an interaction with a tyrosine or histidine from a loop (loop c) adjacent to the actual binding sequence. This aromatic interaction was postulated as a precondition for inhibition in the IC50 range < 500 nM on the basis of the 3D structure of the laminin γ1 III 3-5 and as a result of the structure/function relations described in the US patent number 5,493,008. The question of whether loop c interacts directly with the nidogen, or whether it makes a contribution to stabilizing the suitable spatial structure of the NIDPNAV sequence region remained unclarified, however (Pöschl, E.; Fox, J.W.; Block, D.; Mayer, U.; Timpl, R, (1994) EMBO J. 13; 3741 – 3747. Baumgartner, R.; Czisch, M.; Mayer, U.; Pöschl, E.; Huber, R.; Timpl, R.; Holak, T.A. (1996) J. Mol. Biol. 257; 658 - 668. Stetefeld, J.; Mayer, U.; Timpl, R.; Huber, R. (1996) J. Mol. Biol. 257; 644 – 657).

The laminin/nidogen interaction is influenced by a strong conformational component (Mayer, U.; Nischt, R.; Pöschl, E.; Mann, K.; Fukuda, K.; Gerl, M.; Yamada, Y.; Timpl, R. (1993) EMBO J. 12; 1879 - 1885. Mann, K.; Deutzmann, R.; Timpl, R. (1988) Eur. J. Biochem. 178; 71 - 80). The synthetic peptides which can be derived from the nidogen binding site of laminin are not able to form a disulfide linkage pattern as is present in LE modules, but they show an activity in inhibition assays which is about 400 – 10,000-fold weaker than that of intact laminin P1 or laminin γ1 III 3-5 (Pöschl, E.; Fox, J.W.; Block, D.; Mayer, U.; Timpl, R, (1994) EMBO J. 13; 3741 - 3747. J.W. Fox and R. Timpl; US 5,493,008). This decline in activity is not unusual, since it is known that peptides may assume a myriad of different conformations in aqueous solution and that only a certain percentage of peptides is to be found in the biologically active conformation. The most active peptide described to date (IC50 of 22 nM) has a molecular weight of about 2700 Da (≅ about 50% of an LE module). It comprises an intact S-S loop which presumably stabilizes the structure of the essential NIDPNAV sequence region (Pöschl, E.; Fox, J.W.; Block, D.; Mayer, U.; Timpl, R, (1994) EMBO J. 13; 3741 - 3747. J.W. Fox and R. Timpl; US 5,493,008).

The chemical formula of the sequence NIDPNAV (Asn-Ile-Asp-Pro-Asn-Ala-Val) is as follows:



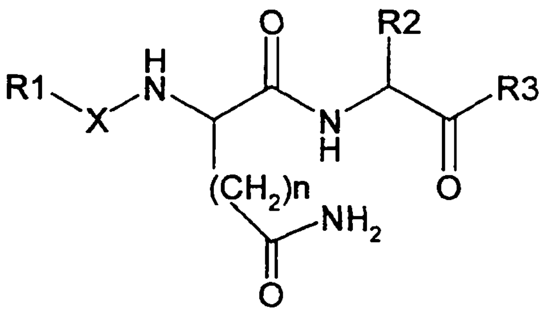
Inhibitors of the laminin/nidogen interaction should be suitable for preparing pharmaceuticals for diseases which are related to an increased or unwanted synthesis of basement membranes.

Such diseases are e.g. all types of late complications of diabetes which are accompanied by thickening of basement membranes (especially in the kidney, eye, vascular system), hepatic fibrosis, especially alcoholic hepatic fibrosis, characterized by synthesis of a continuous basement membrane in the sinusoids and a capillarization caused thereby, all fibroses (chronic or iatrogenic) in which an increased synthesis of basement membrane or components of the basement membrane can be observed (kidney, lung, skin), atherosclerosis characterized by a limitation of the regulation of lipid metabolism, which may be caused inter alia by impaired filtration of lipoproteins through the partly capillarized liver sinusoids (the pathological changes in the vascular system which can be observed with atherosclerosis may also in part be attributed to modifications of the composition and structure of the basement membranes in the vessels), diseases in which angiogenesis contributes to a deterioration in the clinical picture, for example cancers in which neovascularization is required for tumor growth, diabetic retinopathy, retrolental fibroplasia, disorders with a strong inflammatory component (for example rheumatoid arthritis, osteoarthritis, vasculitis), hemangiomas, psoriasis, and many others.

The use of peptides like those described in US patent number US 5,493,008 as medicine is however limited to a considerable extent because of their conformational flexibility, their instability to proteases and their poor bioavailability and pharmacodynamics (Milner-White, E.J. (1989) Trends Pharmacol. Sci. 10; 70 - 74. Verber, D.F.; Freidinger, R.M.; (1985) Trends Neurosci. 8; 392 - 396. Hruby, V.J. (1994) in: Peptides, Proc. Thirteenth American Peptide Symposium; (Hodges, R.S.; Smith, J.A.; Ed.) S. 3 - 17; ESCOM: Leiden, Netherlands).

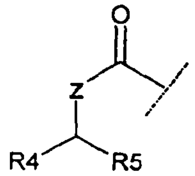
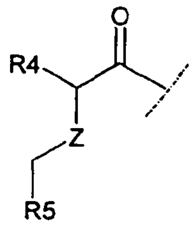
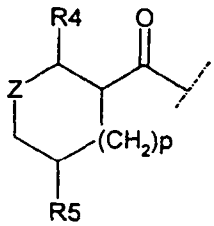
The object of this application was thus to find low molecular weight peptide derivatives which are able to interact specifically with the laminin binding site of nidogen and to inhibit competitively the association between laminin and nidogen at low concentration.

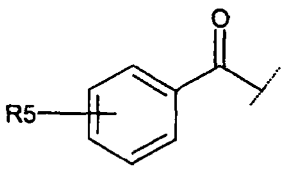
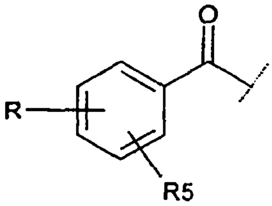
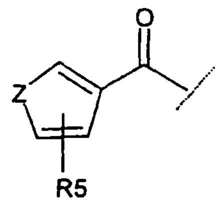
Therefore, the object of the present invention is a compound of the formula I wherein

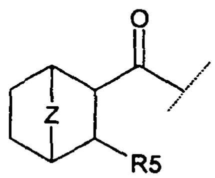
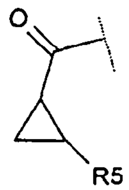


(I)

R1 is a group of one of the following formulae

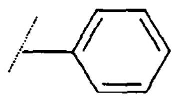
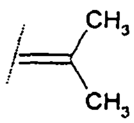
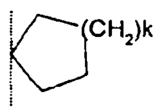

or

or

or


or

or


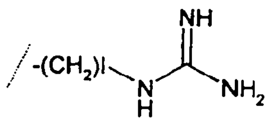
or

or


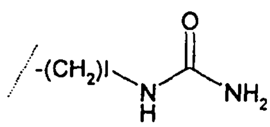
wherein

R4 means -A, $-NH_2$, -NHR, $-NR_2$, A_2, -NHR1,


or

or


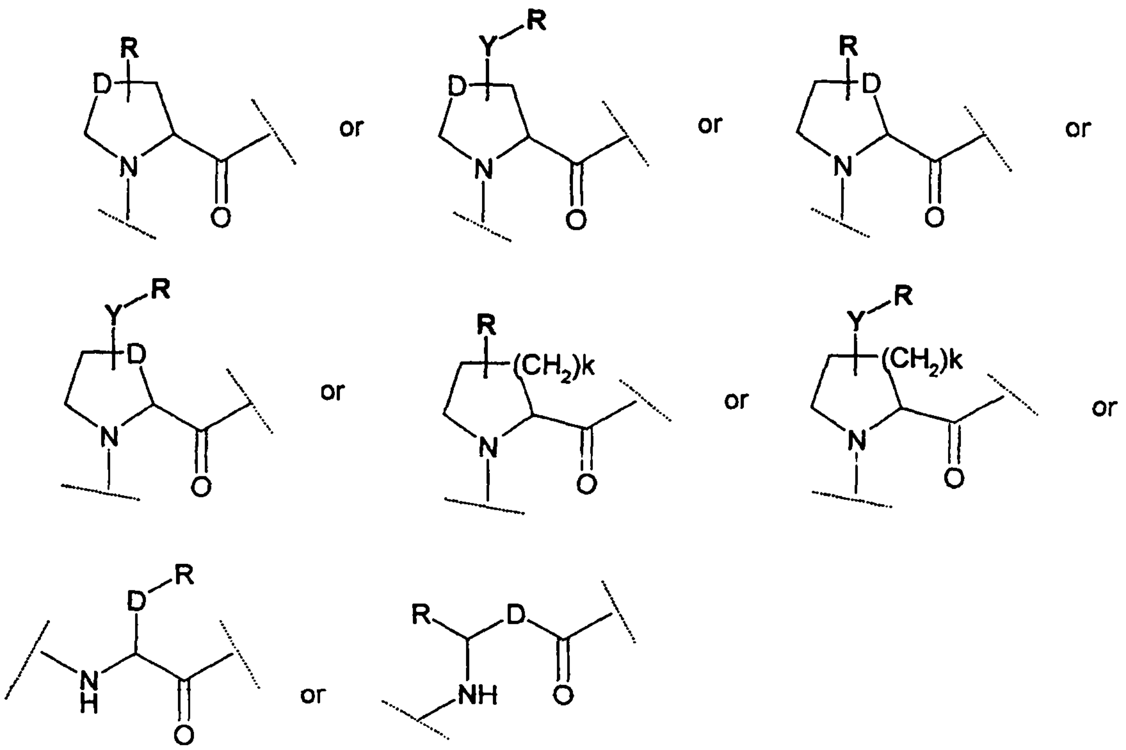
and R5 means $-(CH_2)_lCOOA$, $-(CH_2)_lCONH_2$, $-(CH_2)_lNH_2$ or $-(CH_2)_l-SO_3H$,


or



and X is a group of one of the following formulae

wherein



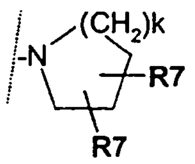
Y means O, S, -N(A)-CO- or $-(CH_2)_r-$,

D means $(CH_2)_r$, O, S, NH, NR, $(CH_2)_r$-O, $(CH_2)_r$-S, $(CH_2)_r$-NH or $(CH_2)_r$NR and

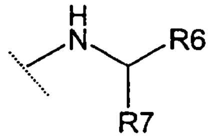
R2 means -A, -E-OH, -E-COOH or $-E-CONH_2$,

wherein E means a linear or branched C_1-C_{10}-alkyl chain, which is unsubstituted or substituted by -A, $-(CH_2)_m$-OH, $-(CH_2)_m$-COOH, $-(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group,

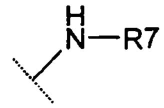
or E means C_5-C_{10}-cycloalkyl, which is unsubstituted or substituted by -A, $-(CH_2)_m$-OH, $-(CH_2)_m$-COOH, $-(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group, and R3 is a group of one of the following formulae



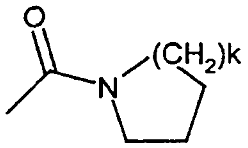
or



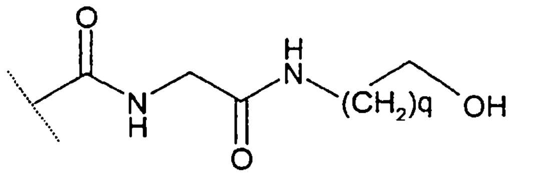
or



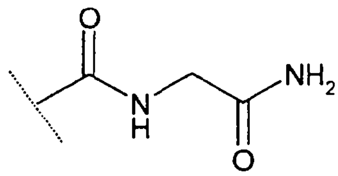
wherein R6 means -H, -COOH, $-CONH_2$, -CONHR, $-CONR_2$, $-CH_2OH$ or



or



or



and wherein R7 means a linear or branched C_1-C_{10}-alkyl group, which is unsubstituted or substituted by -A, $-(CH_2)_m$-OH, $-(CH_2)_m$-COOH, $-(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group,

or R7 means a C_5-C_{10}-cycloalkyl group, which is unsubstituted or substituted by -A, $-(CH_2)_m$-OH, $-(CH_2)_m$-COOH, $-(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group,

and R means branched or unbranched C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkinyl, C_5-C_{10}-cycloalkyl, Het or Ar which are optionally substituted by one ore more halogen, C_1-C_6-alkyloxy, branched or unbranched C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkinyl or

C_5-C_{10}-cycloalkyl groups or by -C_1-C_6-alkyl-Het, -C_1-C_6-alkyl-Ar, -O-C_1-C_6-alkyl-Het, -O-C_1-C_6-alkyl-Ar, Het or by Ar, wherein

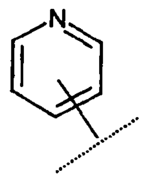
Het means	a monocyclic or bicyclic, 5- up to 10-membered aromatic or non-aromatic ring containing 1 or 2 equal or different hetero-atoms as members of said ring, selected from the group consisting of nitrogen, oxygen and sulfur, which is unsubstituted or substituted by one or more hydroxy or carboxy groups, and wherein
Ar means	a monocyclic or bicyclic 5- up to 10-membered aromatic ring which is unsubstituted or substituted by one or more hydroxy or carboxy groups, and
Z means	$(CH_2)_m$, O, S, NH, NR, N-C(O)-R or NSO_2R,
A means	H or C_1-C_4-alkyl and
l, m and r	are integers from 0 to 3,
n and k	are integers from 1 to 2,
p	is an integer from 0 to 1 and
q	is an integer from 1 to 3,

in all its stereoisomeric forms and mixtures thereof in all ratios including all its physiologically tolerable salts.

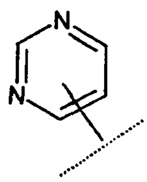
Physiologically tolerable salts are for example salts of inorganic and organic acids, e.g. hydrochloric acid, sulfuric acid, acetic acid, citric acid or p-toluenesulfonic acid, or salts of inorganic and organic bases, such as NH_4OH, NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$, diethanolamine or ethylenediamine, or salts of amino acids, such as arginine, lysine, lysyl-lysine or glutamic acid.

One preferred embodiment of the present invention is a compound of formula I wherein n is 1.

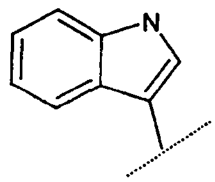
A further preferred embodiment is a compound of formula I wherein R in group X means Het or Ar which are optionally substituted by -C_1-C_6-alkyl-Het, -C_1-C_6-alkyl-Ar, -O-C_1-C_6-alkyl-Het, -O-C_1-C_6-alkyl-Ar, Het or by Ar. More preferably, R in group X means Het. For example Het means



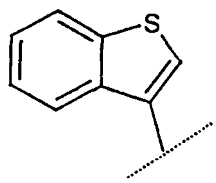
or



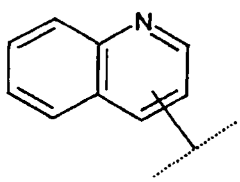
or



or

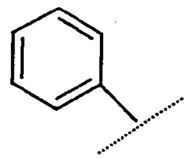


or

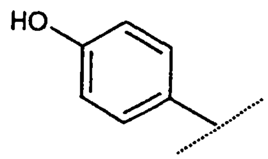


A preferred embodiment of the present invention is also compound of formula I wherein R in group X means Ar which is optionally substituted by -C_1-C_6-alkyl-Ar, -O-C_1-C_6-alkyl-Ar or by Ar. Preferably R in group X means Ar.

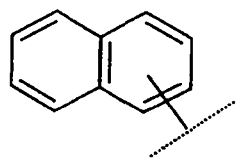
For example Ar means



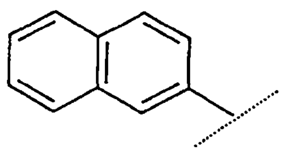
or



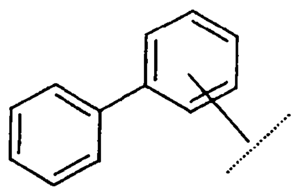
or



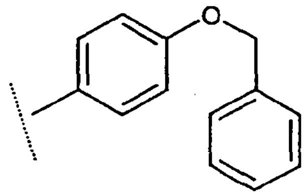
or



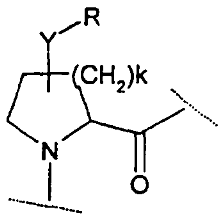
A preferred embodiment is also a compound of formula I wherein R in group X means



or

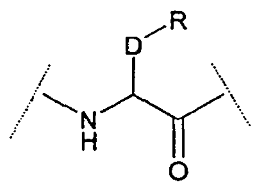


In the compound of formula I X is preferably a group of the following formula:



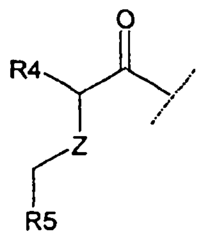
Preferably, Y means $-(CH_2)_r$, wherein r is preferably 0 or 1 and k is preferably 1 or 2.

A further preferred embodiment of the present invention is a compound of formula I wherein X is a group of the following formula

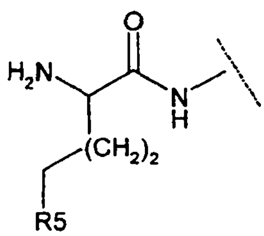


wherein D preferably means $-(CH_2)_r$-, wherein r is 0 or 1.

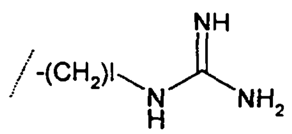
An also preferred embodiment of the present invention compound of formula I wherein R1 is a group of the following formula



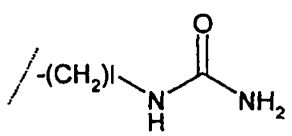
wherein Z means preferably $(CH_2)_m$ and m is 0 or 1. Preferably, R5 means $-(CH_2)_l$-COOA, wherein A means preferably H, or R5 means $-(CH_2)_l-COONH_2$., wherein l is 0. Preferably, R4 means -NH2 or -A, wherein A preferably means H, or preferably, R4 means -NHR1, wherein -NHR1 preferably means



and wherein R5 of -NHR1 preferably means

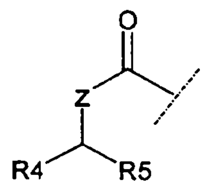


and l is preferably 0, or R5 of -NHR1 preferably means



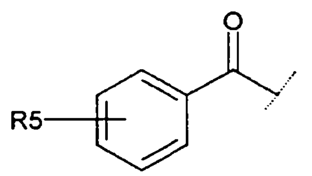
and l is preferably 0, or R5 of -NHR1 means preferably $(CH_2)_l$-NH_2 and l is preferably 0.

A further preferred embodiment of the present invention is a compound A compound of formula I wherein R1 is a group of the following formula



wherein Z means preferably $-(CH_2)_m-$.and m is preferably 1 and wherein R4 preferably means $-NH_2$, and R5 preferably means $-(CH_2)_l$-COOA, wherein l is preferably 0 and wherein A preferably means H.

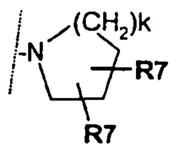
A further preferred embodiment of the present invention is a compound A compound of formula I wherein R1 is a group of the following formula



wherein R5 preferably means $-(CH_2)_l$-COOA, wherein l is preferably 0 and A preferably means H.

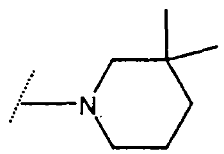
A further preferred embodiment of the present invention is a compound of formula I wherein R2 means A and A preferably means $-CH_3$, or wherein R2 means -E-COOH, preferably $-CH_2$-COOH, or wherein R2 means -E-OH, preferably $-CH_2$-OH.

A further preferred embodiment of the present invention is a compound of formula I wherein R3 is a group of the following formula

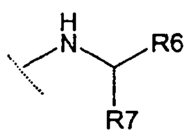


wherein k is preferably 2.

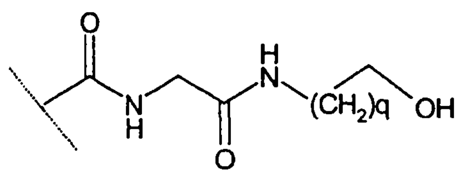
A further preferred embodiment of the present invention is a compound of formula I wherein R3 is a group of the following formula



A further preferred embodiment of the present invention is a compound of formula I wherein R3 is a group of the following formula

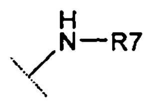


wherein R7 is preferably a branched C_1-C_{10}-alkyl group, preferably $-CH(CH_3)_2$, $-C(CH_3)_3$, $-CH(CH_3)CH_2-CH_3$ or $-CH_2-CH(CH_3)_2$, and wherein R6 preferably means -H, -COOH, $-CONH_2$, $-CH_2OH$, $-CON(CH_3)_2$ or, more preferably, wherein R6 means



wherein q is preferably 2.

A further preferred embodiment of the present invention is a compound of formula I wherein R3 is a group of the following formula



wherein R7 preferably means $-CH(CH(CH_3)_2)_2$ or $-CH_2C(CH_3)_3$.

The compounds according to the present invention are unnatural (i.e. naturally not occuring), low molecular weight peptide derivatives which are able to inhibit the laminin/nidogen interaction in the nM concentration range. Surprisingly, the low molecular weight structures which have been found are capable of high-affinity binding to the laminin binding site of nidogen without this requiring an interaction with a tyrosine or histidine from a loop (loop c) adjacent to the actual binding sequence.

It is all the more surprising that the low molecular weight peptide derivatives, with molecular weights between 550 and 800 Da, described in the present invention show inhibition of the same order of magnitude compared to the most active peptide described to date (IC50 of 22 nM) having a molecular weight of about 2700 Da (≅ about 50% of an LE module) and comprising an intact S-S loop which presumably stabilizes the structure of the essential NIDPNAV sequence region (J.W. Fox and R. Timpl; US 5,493,008).

The object was achieved by specifically synthesizing, on the basis of structure/function relationships and the published three-dimensional structure of the nidogen binding site, peptide derivatives on resin supports. The building blocks for the peptide syntheses were varied in accordance with suitable criteria to ensure a wide structural diversity and the integration of unnatural building blocks. A suitable, sensitive screening assay was used to test and compare the resulting peptide derivatives for inhibitory activity after they had been cleaved off the support resin.

The compounds according to the present invention can be used for preparing a pharmaceutical for the treatment of a disease which is related to an increased or unwanted synthesis of basement membranes.

Therefore, possible areas of therapeutic use of the present peptide derivatives and/or the physiologically tolerable salts thereof are:

1. All types of late complications of diabetes which are accompanied by thickening of basement membranes (especially in the kidney, eye, vascular system).

2. Hepatic fibrosis, especially alcoholic hepatic fibrosis, characterized by synthesis of a continuous basement membrane in the sinusoids and a capillarization caused thereby.

3. All fibroses (chronic or iatrogenic) in which an increased synthesis of basement membrane or components of the basement membrane can be observed (kidney, lung, skin).

4. Atherosclerosis characterized by a limitation of the regulation of lipid metabolism, which may be caused inter alia by impaired filtration of lipoproteins through the partly capillarized liver sinusoids. The pathological changes in the vascular system which can be observed with atherosclerosis may also in part be attributed to modifications of the composition and structure of the basement membranes in the vessels.

5. Diseases in which angiogenesis contributes to a deterioration in the clinical picture, for example cancers in which neovascularization is required for tumor growth, diabetic retinopathy, retrolental fibroplasia, disorders with a strong

inflammatory component (for example rheumatoid arthritis, osteoarthritis, vasculitis), hemangiomas, psoriasis, and many others.

Thus, the compounds according to the present invention and/or their respective physiologically tolerable salts are suitable for use as a pharmaceutical. Therefore, a further object of the present invention is a pharmaceutical composition containing at least one compound according to the present invention and/or its physiologically tolerable salts.

The compounds of the formula I and their physiologically tolerable salts and derivatives can be administered according to the invention to animals, preferably to mammals, and in particular to humans, as pharmaceuticals for therapy or prophylaxis. They can be administered per se, in mixtures with one another or in the form of pharmaceutical preparations which permit enteral or parenteral administration and which as active constituent contain an efficacious dose of at least one compound of the formula I and/or its physiologically tolerable salts and derivatives in addition to customary pharmaceutically innocuous excipients and/or additives.

The pharmaceuticals can be administered systemically or locally. They can be administered, for example, in the form of pills, tablets, film-coated tablets, sugar-coated tablets, granules, hard and soft gelatin capsules, powders, solutions, syrups, emulsions, suspensions or in other pharmaceutical forms. However, administration can also be carried out vaginally or rectally, for example in the form of suppositories, or parenterally or by implantation, for example in the form of injection solutions or infusion solutions, microcapsules or rods, or topically or percutaneously, for example in the form of ointments, solutions or tinctures, or in another way, for example in the form of nasal sprays or aerosol mixtures or as inhalable dry powder preparations. If solutions are parenterally administered they can be aministered, for example, intravenously, intramuscularly, subcutaneously, intraarticularly, intrasynovially or in another manner, e.g. by inhalation of wet aerosols or dry powder preparations.

The pharmaceutical preparations according to the invention are prepared in a manner known per se, it being possible to use pharmaceutically inert inorganic and/or organic excipients in addition to the compound(s) of the formula I and/or its/their physiologically tolerable salts and derivatives. For the preparation of pills, tablets, sugar-coated tablets and hard gelatin capsules, it is possible to use, for example, lactose, cornstarch or derivatives thereof, talc, stearic acid or its salts etc. Excipients for soft gelatin capsules and suppositories are, for example, fats, waxes, semisolid and liquid polyols, polyethylene glycols, natural or hardened oils etc. Suitable excipients for the preparation of solutions, for example injection solutions, or of emulsions or syrups are, for example, water, alcohols, glycerol, diols, polyols, sucrose, invert sugar, glucose, vegetable oils etc. Suitable excipients for microcapsules, implants or rods are, for example, copolymers of glycolic acid and lactic acid. The pharmaceutical preparations normally contain approximately 0.5 to 90% by weight of the compounds of the formula I and/or their physiologically tolerable salts and derivatives.

In addition to the active compounds and excipients, the pharmaceutical preparations can additionally contain auxiliaries or additives, such as, for example, fillers, disintegrants, binders, lubricants, wetting agents, stabilizers, emulsifiers, preservatives, sweeteners, colorants, flavorings or aromatizers, thickeners, diluents, buffer substances, solvents or solubilizers, means for achieving a depot effect, salts for altering the osmotic pressure, coating agents or antioxidants. They can also contain two or more compounds of the formula I and/or their physiologically tolerable salts and derivatives. Furthermore, they can also contain one or more other therapeutically or prophylactically active substances in addition to at least one compound of the formula I and/or its physiologically tolerable salts and derivatives. The pharmaceutical preparations normally contain 0.2 to 500 mg, preferably 1 to 100 mg, of active compound of the formula I and/or its physiologically tolerable salts and derivatives per dose.

If the compounds of the formula I or pharmaceutical preparations containing them are administered as aerosols, for example as nasal aerosols or by wet aerosols or dry powder inhalation, this can be effected, for example, using a spray, an atomizer, a pump atomizer, an inhalation apparatus, a metered inhaler or a dry powder inhaler, respectively. Pharmaceutical forms for administration of the compounds of the formula I as an aerosol can be prepared by the process well known to the person skilled in the art. For their preparation, for example, solutions or dispersions of the compounds of the formula I in water, water-alcohol mixtures or suitable saline solutions using customary additives, for example benzyl alcohol or other suitable preservatives, absorption enhancers for increasing the bioavailability, solubilizers, dispersants and others, and, if appropriate, customary propellants, for example chlorofluorohydrocarbons and/or fluorohydrocarbons are suitable, whereas dry powder preparations of the compounds of the formula I and/or their physiologically tolerable salts may be obtained by freeze drying or preferably spray drying aqueous solutions of the compounds of the formula I and/or their physiologically tolerable salts and of suitable water soluble additives, such as sugars or sugar derivatives and amino acids.

The dose when using the compounds of the formula I can vary within wide limits, and as customary it is to be tailored to the individual conditions in each individual case, as is known to the physician. It depends, for example, on the nature and severity of the disease to be treated, on the compound employed or whether an acute or chronic disease state is treated or prophylaxis is conducted or on whether further active compounds are administered in addition to the compounds of the formula I. In general, in the case of oral administration, a daily dose of approximately 0.01 to 100 mg/kg, preferably 0.1 to 10 mg/kg, in particular 0.3 to 2 mg/kg (in each case per kg of body weight) is appropriate in an adult to achieve effective results. In the case of intravenous administration, the daily dose is in general approximately 0.01 to 50 mg/kg, preferably 0.01 to 10 mg/kg of body weight. In particular when relatively large amounts are administered, the daily dose can be divided into a number, for example

2, 3 or 4, of part administrations. If appropriate, depending on individual behavior, it may be necessary to deviate upward or downward from the indicated daily dose.

Furthermore, the compounds of the formula I and their salts according to the present invention can be used as intermediates for the preparation of other compounds, in particular of other pharmaceutical active compounds which are obtainable from compounds of the formula I, for example, by modification or introduction of radicals or functional groups, for example by esterification, reduction, oxidation or other conversions of functional groups.

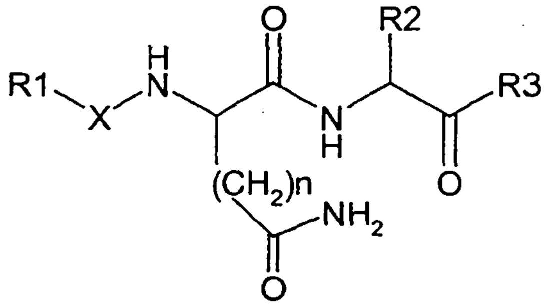
The peptide derivatives according to the present invention thus found can on the one hand be used directly as therapeutic agent, but they can also form the basis for related structures, which are also suitable for use as therapeutic agent for treating diseases relating to an increased or unwanted synthesis of basement membranes.

A further object of the present invention is a method for identifying a compound that inhibits the interaction of laminin and nidogen wherein the compound according to the present invention is used as a competetive inhibitor. This method may further comprise the formulation of the compound identified in a pharmaceutical acceptable form.

It is also an object of the present invention to provide a method for producing a pharmaceutical composition comprising the identification of a compound that inhibits the interaction of laminin and nidogen wherein the compound according to the present invention is used as a competetive inhibitor and furthermore mixing the compound identified and/or its physiologically tolerable salts with a pharmaceutical acceptable carrier.

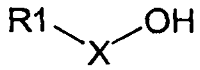
It is also an object of the present invention to provide a method for preparing the compound of the formula I according to the present invention.

The compound of formula I



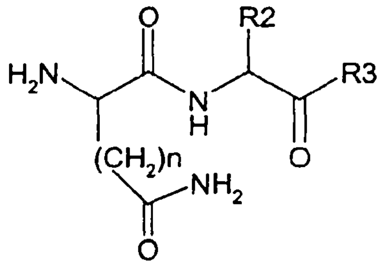
(I)

according to the present invention is prepared by a fragment condensation of a compound of formula II



(II)

with a compound of formula III



(III)

wherein the variables R1, X, n, R2 and R3 have the above-mentioned meanings and whereby the compounds of formulae II and III may be protected at the functional groups defined above by usual protecting groups known in peptide chemistry (see for example Houben-Weyl, Methoden der Organischen Chemie, vol. 15/1 and 15/2, Georg Thieme Verlag, Stuttgart, 1974). Suitable condensation methods are well known in the art (Houben-Weyl, Methoden der Organischen Chemie, vol. 15/1 and

15/2, Georg Thieme Verlag, Stuttgart, 1974). Suitable condensation agents or coupling reagents are for example carbonyl-diimidazoles, carbodiimides, such as di-cyclohexyl-carbodiimide or di-isopropyl-carbodiimide, or O-((cyano(ethoxycarbonyl)methylene)-amino)-N,N,N',N'-tetra-methyl-uronium-tetrafluoro-borate (TOTU) or pyro-phosphoric acid anhydride (PPA). The condensation reactions are carried out under standard conditions. As a rule, it is necessary during peptide condensation to protect amino groups which are not intended to be involved in the coupling reaction by protecting groups which are easily removed under conditions different to the conditions under which coupling occurs. The same applies for the carboxy groups not involved in the coupling reaction, which are preferably protected as C_1-C_6-alkyl esters, benzyl esters or tert-butyl esters during the coupling reaction. A protection of the amino groups is not necessary in case the amino groups are still present in the form of amino group precursors, e.g. in form of nitro or cyano groups. The amino groups are then formed by a hydration step subsequent to the condensation reaction. After the condensation step the protecting groups are removed by known suitable methods, e.g. benzyloxy-carbonyl and benzyl groups can be removed by hydration in benzyl esters; protecting groups of the tert-butyl type are in general cleaved under acidic conditions; the 9-fluorenylmethyloxycarbonyl residue is removed by secondary amines.

The preparation of the compound of the formula I according to the present invention may also be performed by stepwise addition of the respective components, e.g. natural, unnatural amino acids and their derivatives, on a solid phase, whereby the components may be added in various different sequences.

It may also be advantageous in order to produce the compound of formula I not to directly couple the compounds of formulae I and II by a fragment condensation but to couple their respective suitable precursors in order to obtain an intermediate which can be transferred into the compound of the formula I e.g. by derivatization.

The above described method for introducing functional groups not directly, but by the way of their respective precursors into the molecule in order to obtain intermediates from which the final product can easily be obtained by transforming the precursor groups into the respective functional groups subsequently to a condensation reaction may also be applied for different parts of the molecule of the compound of formula I, e.g. for the side chain of the compound of formula, I R1- or R1-X-, respectively.

Examples

The abbreviations have the following meanings:

Agents and solvents:

AcOH	acetic acid
aq	aqueous
BSA	bovine serum albumin
DCC	N,N'-dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
Et_2O	Diethylether
EtOAc	Ethylethanoate (acetic acid ethylester)
EtOH	ethanol
Fmoc-OSucc	Fmoc-O-succinimide
HOBT	1-hydroxybenzotriazole
KHMDS	potassiumhexamethyldisilazide
n-Buli	n-butyl-lithium
MeOH	methanol
MTBE	methyl tert-butyl ether

TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMEDA	tetramethylethylendiamine
TMSCl	trimethylsilyl chloride
TOTU	O-((cyano(ethoxycarbonyl)methylene)amino)-N,N,N',N'-tetramethyluronium tetrafluoroborate
$TrisN_3$	trisilyl azide

Chemical groups:

Me	methyl	CH_3-
Et	ethyl	CH_3-CH_2-
nPr	n-propyl	$CH_3CH_2CH_2$-
iPr	isopropyl	$(CH_3)_2CH$-
nBu	n-butyl	$CH_3CH_2CH_2CH_2$-
iBu	isobutyl	$(CH_3)_2CHCH_2$-
tBu	tert-butyl	$(CH_3)_3C$-
Ph	phenyl	C_6H_5-
Fmoc	9-fluorenylmethoxycarbonyl	
Z	benzyloxycarbonyl	C_6H_5-CH_2-O-CO-
BOC	tert-butyloxycarbonyl	$(CH_3)_3C$-O-CO-

1. Screening of a library of inhibitors of Laminin/Nidogen interaction

The library was designed to find smaller, more potent and more metabolically stable peptides related to the previously known heptapeptide NIDPNAV (Pöschl, E.; Fox, J.W.; Block, D.; Mayer, U.; Timpl, R, (1994) EMBO J. 13; 3741 – 3747. Pöschl, E.; Mayer, U.; Stetefeld, J.; Baumgartner, R.; Holak, T.A.; Huber, R.; Timpl, R. (1996)

EMBO J. 15: 5154-5159. Baumgartner, R.; Czisch, M.; Mayer, U.; Pöschl, E.; Huber, R.; Timpl, R.; Holak, T.A. (1996) J. Mol. Biol. 257; 658 – 668). The library was synthesized and screened as three sublibraries; pentamer, hexamer and heptamer. Following is a description of the screening strategy for the pentamer sublibrary. The method is representative of the methods employed for the other two sublibraries, except that the hexamers were screened in the first step at about 50 beads per well and the heptamers were screened at about 100 beads per well.

1.1 Screening of the pentamer library.

The pentamer library contained 2,160 different compounds.

1) About 8,800 individual beads were suspended in 0.1% HCl and distributed into seven filter bottom 96 well microtiter plates at approximately fourteen beads per well.

2) The beads were washed twice with 200 µl de-ionized water, then 50 µl of 500 mM HEPES, pH 7.0 was added. The linker used in the library releases one aliquot of compound when the pH is increased to 7.0, and this cleavage step was allowed to proceed overnight.

3) The plates were stacked on top of U-bottom filter plates and centrifuged. The mixtures of compounds released from the beads were collected in the bottom plate, while the corresponding beads remain in the original filter plate.

4) 25 µl DMSO per well was added to the beads to wash remaining free compound from the beads, and the plates were centrifuged again to separate the compounds in solution from the beads. The resulting stock was presumably 27 µM per compound in 333 mM HEPES, 33% DMSO.

5) The compound stocks were preincubated with nidogen (10 µl compound stock to 90 µl nidogen solution) and the assay was performed as described in the attached

protocol, yielding a final screening concentration of 2.7 μM per compound.

6) In the 25 assay wells where reproducible inhibition of ≥ 62% occurred, the corresponding beads from the original filter plates were suspended in 0.05% HCl, 0.1% Tween-20 and pipetted into five new filter plates at 1 bead per well. Two

control beads with the parent compound on the same linker were added to each plate as controls.

7) The beads were washed twice with 200 μl de-ionized water , then 25 μl of 50 mM NaOH was added to each well. The linker used in the library releases the second equimolar aliquot of compound when the pH is increased from 7.0 to 10.0 or more. This cleavage step was allowed to proceed for 3 hours.

8) The plates were stacked on top of U-bottom filter plates and centrifuged. The compounds released from the beads were collected in the bottom plate, while the corresponding beads remained in the original filter plate.

9) The beads were washed with 20 μl of 50 mM HEPES (initial pH 7.0) with 50 mM HCl added, and the solution was centrifuged into the lower plate and combined with the first releasate.

10) The beads were washed a third time with 25 μl DMSO, which was allowed to equilibrate with the beads for 10 minutes before centrifugation.

11) The resulting releasates were assayed at 1/10th volume, as in Step #5.

12) Solutions which inhibited as well or better than the control beads (about 50% inhibition) were considered hits. 23 hit beads were recovered, with the other two potential hit wells being explainable by additive weak inhibitors in single wells.

13) Hit solutions were subjected to mass spectrometry to determine the molecular weights.

14) The corresponding individual hit beads were subjected to Edman degradation to determine peptide sequences.

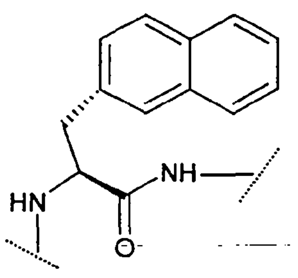
15) The combined MS and Edman data was analyzed to identify the hit compound structures.

The structures and frequency of their recovery are shown below. G-Hopa = glycine hydroxypropyl amide, the linker remnant.

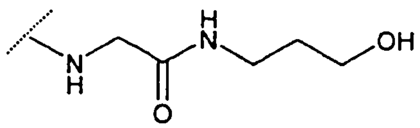
Frequency							IC50, μM
6	D	Nal2	N	D	V	G-Hopa	0.43
4	D	Nal2	N	A	V	G-Hopa	0.37
4	D	Nal2	N	D	I	G-Hopa	0.64
4	D	Nal2	N	S	V	G-Hopa	0.49
3	D	Nal2	N	S	I	G-Hopa	0.81
2	D	Nal2	N	A	I	G-Hopa	0.47

Legende:

Nal2 = L-3-(2-naphthyl)-alanyl:



G-Hopa = glycine-3-hydroxypropylamide:



D = Asp (aspartyl), P = Pro (prolyl), N = Asn (asparaginyl), A = Ala (alanyl), V = Val (valinyl), S = Ser (seryl), I = Ile (isoleucyl).

1.2 Procedures: Preparation of the peptide library

Peptide libraries were synthesized by a split/mix synthesis approach (Lam, K. S., Salmon, S. E., Hersh, E. M., Hruby, V. J., Kazmierski, W. M., and Knapp, R. J. (1991) Nature 354, 82; Furka, A., Sebestyen, F., Asgedom, M., and Dibo, G. (1991) Int. J. Pept. Protein Res. 37, 487) using standard solid-phase peptide Fmoc chemistry (Stewart, J. M., and Young, J. D. (1984) Solid Phase Peptide Synthesis. Pierce Chemical Co., Rockford, IL.; Atherton, E., and Sheppard, R. C. (1989) Solid Phase Peptide Synthesis. IRL Press Oxford). Each resin bead was exposed to only a single activated amino acid at each coupling cycle. Therefore, at the completion of the library synthesis, each resin bead expresses only one peptide entity. Since it is not possible to test all compounds separately, we have built the same structure on each resin bead in two copies via differentially cleavable linker, fig. 1 (Kocis, P., Krchnak, V., and Lebl, M. (1993) Tetr.Lett. 34, 7251; Lebl, M., Krchnak, V., Salmon, S.E., and Lam, K. S. (1994) A Companion to Methods in Enzymolog 6, 381). Release of the peptide from the resin bead can then be carried out in sequential steps using different mechanism of cleavage. Release of the first part of peptide as a hydroxypropylamide is performed in buffer at pH 7-9. The release of the second part of the peptide is achieved by the use of higher pH (Scheme 1).

In the peptide libraries, polyethylene glycol-grafted polystyrene beads or TentaGel®S NH2 were used. In fact, any resin beads that are compatible with peptide synthesis and screening under aqueous conditions are adequate.

Penta-, hexa-, and heptamer library were prepared with one fixed position (L-asparagine). Glycine hydroxypropylamide on C-terminus is a part of a linker:

H-X4X3-Asn-X2X1-Gly-NH (CH2)3OH (2,160 peptides)
H-X5X4X3-Asn-X2X1-Gly-NH (CH2)3OH (25,920 peptides)
H-X6X5X4X3-Asn-X2X1-Gly-NH (CH2)3OH (311,040 peptides)
X1: N-Fmoc-L-amino acids (9) used in the first randomization: Valine, isoleucine, threonine, phenylalanine, ß(2-naphthyl)alanine, 2-azetidinecarboxylic acid, proline, cyclohexylglycine, phenylglycine.

X2: N-Fmoc-L-amino acids (4) used in the second randomization: Alanine, glycine, serine, aspartic acid.

X3=X5=X6: N-Fmoc-L-amino acids (12) used in the third, fifth and sixth randomization: Pipecolic acid, ß(2-naphthyl)alanine, glutamic acid, lysine, 2-azetidinecarboxylic acid, threonine, proline, asparagine, isoleucine, 3,5-diiodotyrosine, citrulline, arginine.

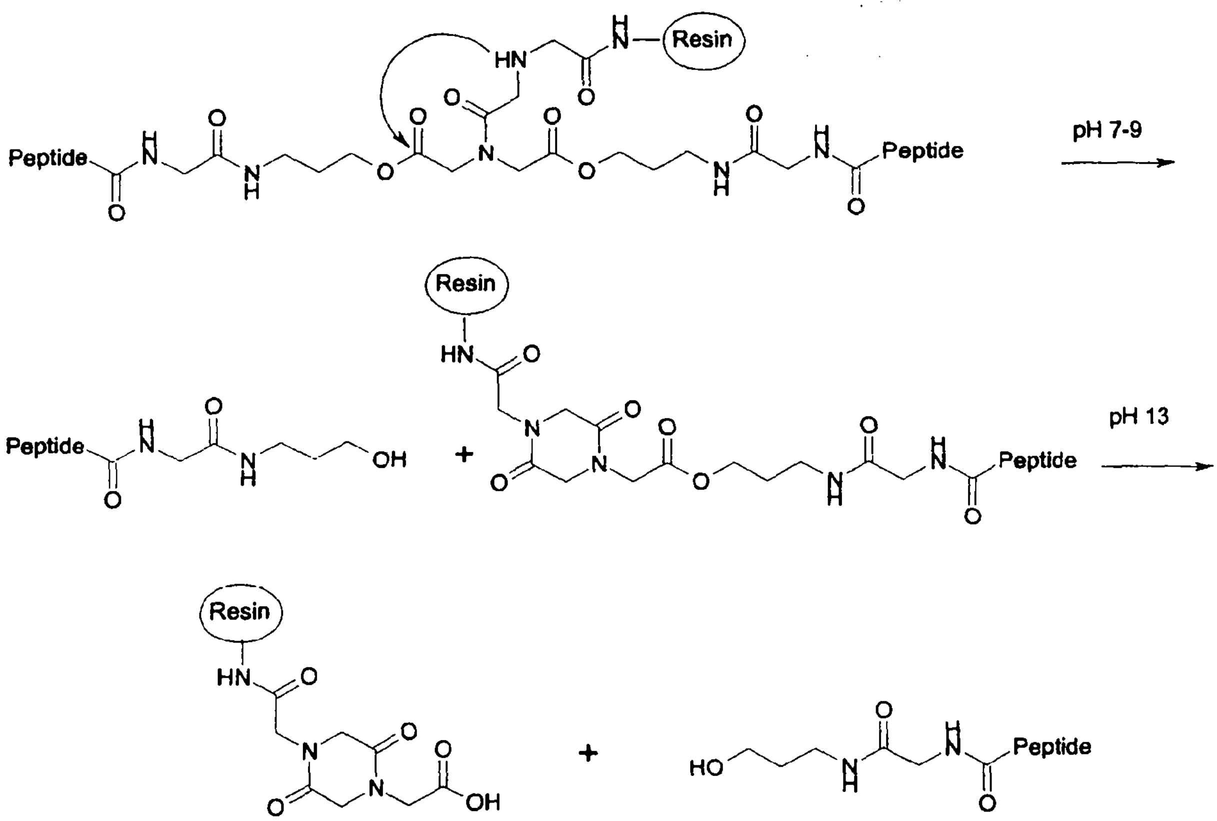
X4: N-Fmoc-L-amino acids (5) used in the fourth randomization: Aspartic acid, glutamic acid, 2-aminoadipic acid, O-sulfate tyrosine, γ-carboxyglutamic acid.

Resin (PEG-PS·HCl, Millipore®, 20 g, loading 0.58 mmol/g, 220 μm average particle size) was swollen in N,N-dimethylformamide for 2 hours and then neutralized with 10% N,N-diisopropylethylamine in dichloromethane. Resin was washed with dichloromethane and N,N-dimethylformamide. Linker (Fig.1, 3 eq) was coupled using 1,3-diisopropylcarbodiimide and 1-hydroxybenzotriazole (3 eq each) in N,N-dimethylformamide at room temperature for 12 hours. The reaction was monitored by bromophenol blue method (Krchnak, V., Vagner, J., Safar, P., and Lebl, M. (1988) Collec.Czech.Cem. Commun.53, 2542). Completion of the coupling was then determined by a ninhydrin test (Kaiser, E., Colescott, R. L., Bossinger, C. D., and Cook, P.I. (1969) Anal. Biochem. 34, 595). After washing with N,N-dimethylformamide, Fmoc protecting group was removed with 50 % piperidine in N,N-dimethylformamide for 15 min. Resin was then washed with N,N-dimethylformamide and the amount of released fulvene-piperidine adduct was quantitated by UV spectrometry (302 nm). A stable level of resin loading (mmol/g) determined in this manner throughout the library synthesis served as one of the quality control measures.

The resin was divided into 9 equal portions. Nine Fmoc-protected amino acids (X1) were then added separately into each of the resin aliquot and coupled by described procedure for 2 hours. The resin was then pooled in a cylindrical glass vessel fitted with a frit at the bottom. Dry nitrogen was bubbled through for mixing of the resin. Fmoc protecting group was removed as described above.

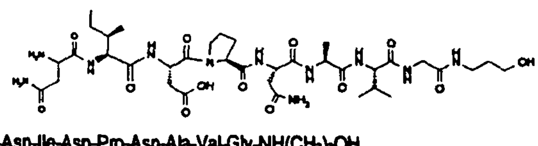
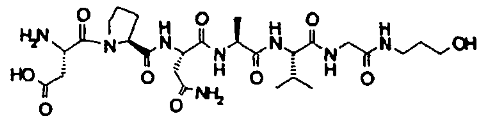
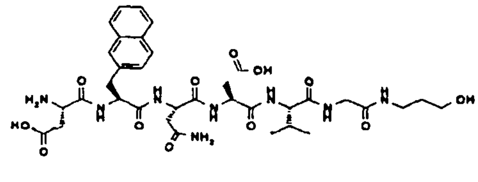
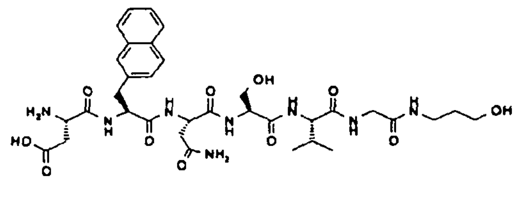
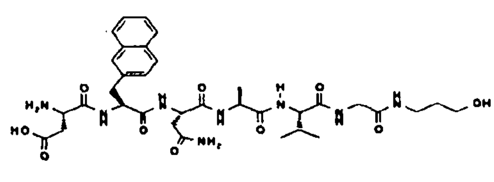
The resin was divided into 4 equal portions. Four Fmoc-protected amino acids (X2) were then added separately into each of the resin aliquot and coupled using the same coupling protocol. Fmoc protecting group was removed and resin loading was determined. In next cycle, L-asparagine was coupled by described procedure. The resin was then divided into aliquots for another cycle of coupling. After all the randomization steps were completed, the Fmoc group was removed and the side chain protecting groups were cleaved with a mixture of trifluoroacetic acid (82.5%), anisole (5%), water (5%), thioanisole (5%), ethanedithiole (2.5%) during 2,5 hours. The resin was then washed with trifluoroacetic acid, dichloromethane, N,N-dimethylformamide and methanole. The libraries were stored dried at 4°C.

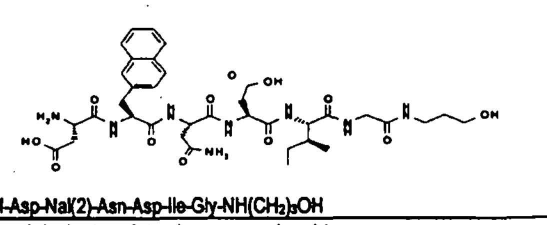
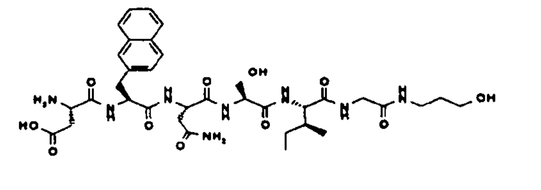
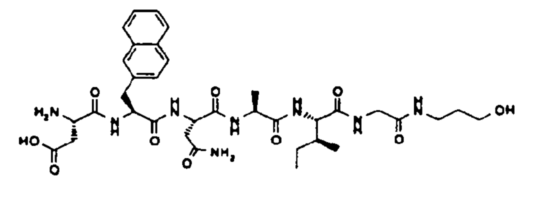
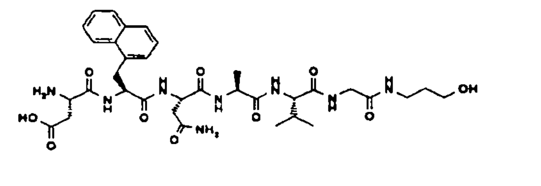
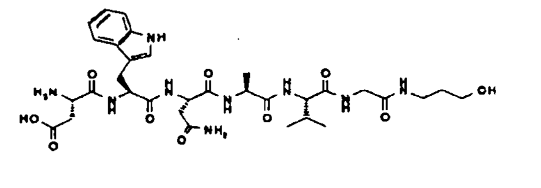
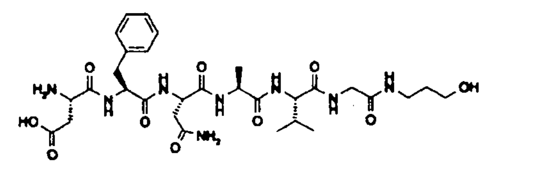
To verify the quality of the library, several randomly chosen beads were submitted for sequencing by Edman degradation and mass spectrometric techniques.

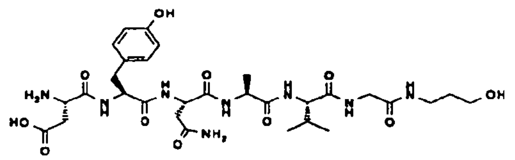
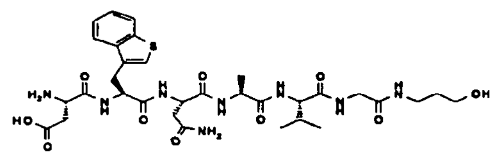
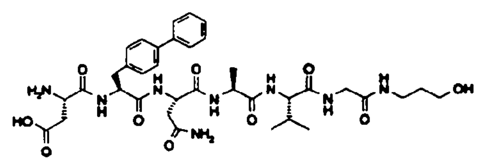
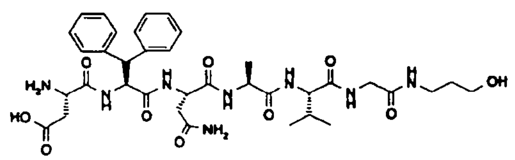
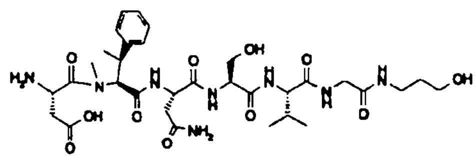
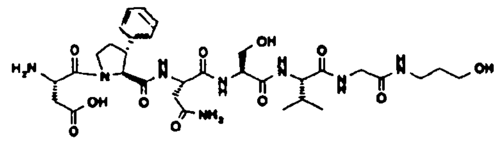

Resin
HN
H
N
O
O
Peptide
H
N
O
N
H
O
O
O
N
O
O
N
H
O
H
N
Peptide
O
pH 7-9
Resin
HN
O
Peptide
H
N
O
N
H
OH
+
N
O
O
N
O
O
N
H
O
H
N
Peptide
O
pH 13
Resin
HN
O
N
O
O
N
O
OH
+
HO
N
H
O
H
N
Peptide
O

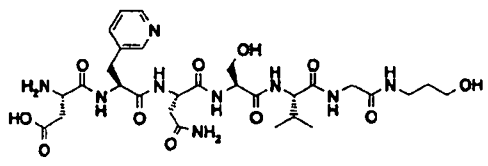
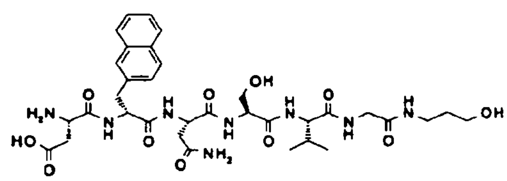
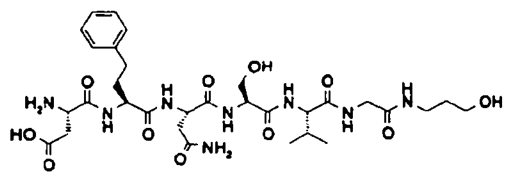
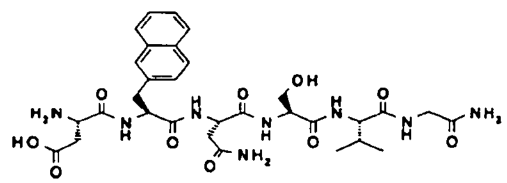
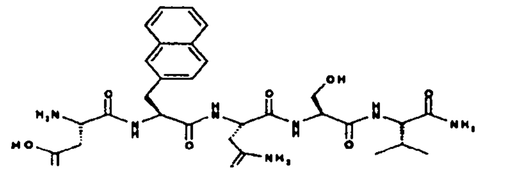
Scheme 1

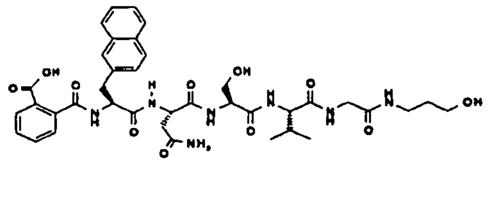
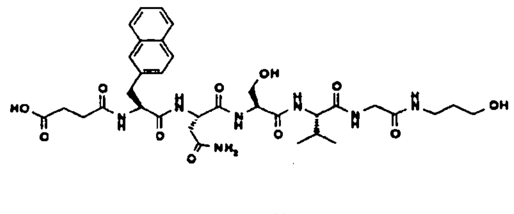
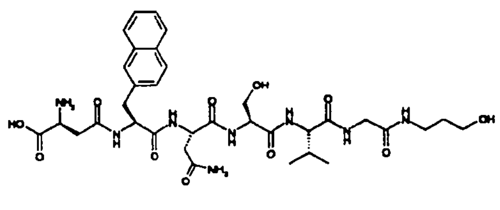
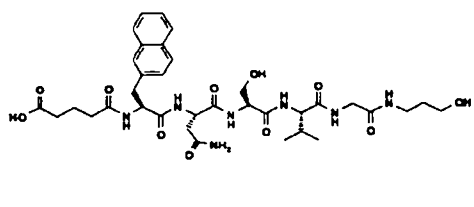
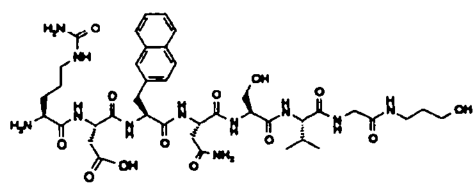
1.3 Results (see also figures 2-12)

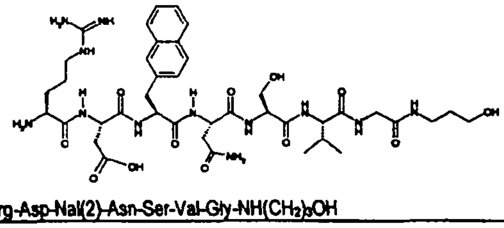
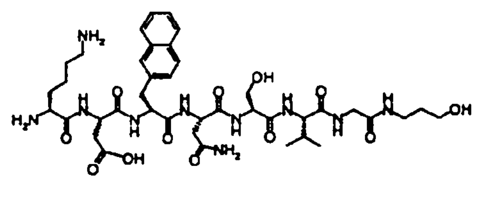
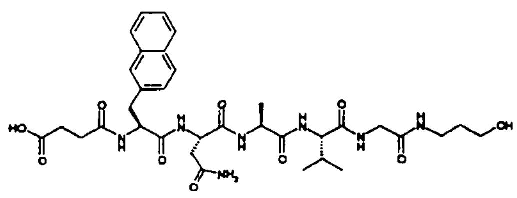
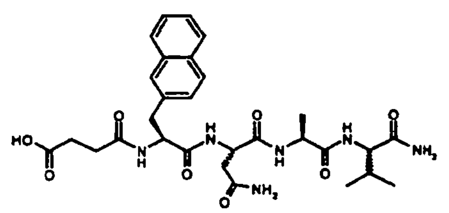
No		Mw	Purity	IC_{50} [µM]	Structure
1	Control	855	>95%	3,9	 H-Asn-Ile-Asp-Pro-Asn-Ala-Val-Gly-NH(CH$_2$)$_3$OH
	L-Asparaginyl-L-isoleucyl-L-aspartyl-L-prolyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
2	Control	628	>95%	7,7	 H-Asp-Pro-Asn-Ala-Val-Gly-NH(CH$_2$)$_3$OH
	L-Aspartyl-L-prolyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
3		772	>95%	0,51	 H-Asp-Nal(2)-Asn-Asp-Val-Gly-NH(CH$_2$)$_3$OH
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-aspartyl-L-valyl-glycine-3-hydroxypropylamide				
4		744	>95%	0,38	 H-Asp-Nal(2)-Asn-Ser-Val-Gly-NH(CH$_2$)$_3$OH
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
5		728	>95%	0,75	 H-Asp-Nal(2)-Asn-Ala-Val-Gly-NH(CH$_2$)$_3$OH
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				

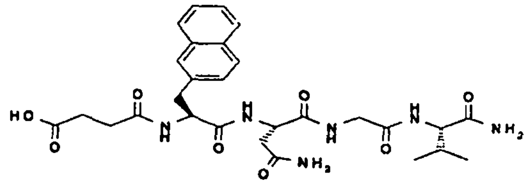
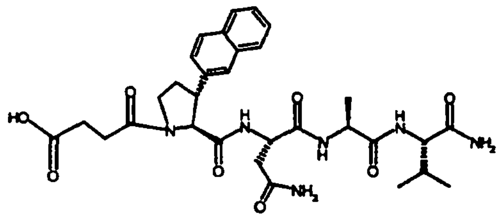
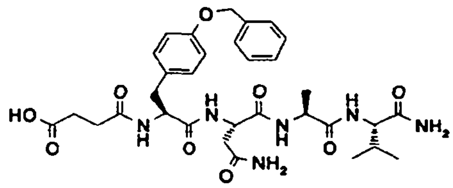
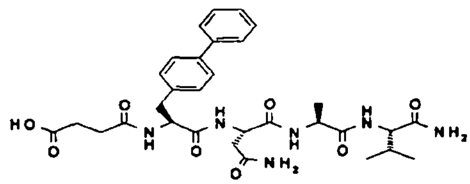
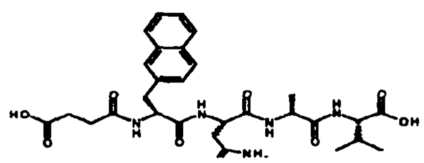
6		786	>95%	1,38	 H-Asp-Nal(2)-Asn-Asp-Ile-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-aspartyl-L-valyl-glycine-3-hydroxypropylamide				
7		758	>95%	0,6	 H-Asp-Nal(2)-Asn-Ser-Ile-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-isoleucyl-glycine-3-hydroxypropylamide				
8		742	>95%	0,7	 H-Asp-Nal(2)-Asn-Ala-Ile-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-alanyl-L-isoleucyl-glycine-3-hydroxypropylamide				
9		728	>95%	8,25	 H-Asp-Nal(1)-Asn-Ala-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-3-(1-naphthyl)alanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
10		717	>95%	8,57	 H-Asp-Trp-Asn-Ala-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-tryptophanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
11		678	>95%	3,38	 H-Asp-Phe-Asn-Ala-Val-Gly-NH(CH₂)₃OH

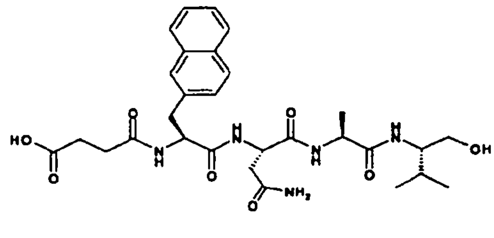
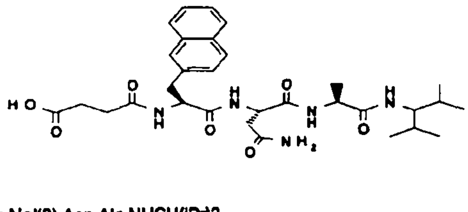
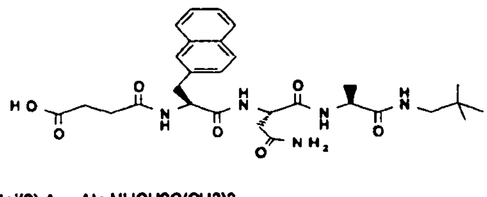
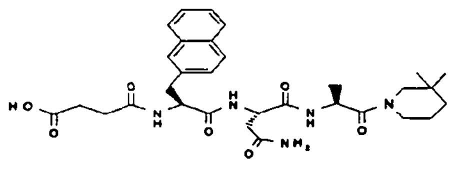
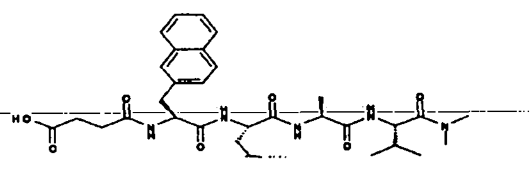
	L-Aspartyl-L-phenylalanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
12		694	>95%	3,79	 H-Asp-Tyr-Asn-Ala-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-tyrosyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
13		734	>95%	7,03	 H-Asp-Ala[3-(3-Benzothienyl)]-Asn-Ala-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-3-(3-benzothienyl)alanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
14		754	>95%	0,94	 H-Asp-Ala[3-(4-Biphenyl)]-Asn-Ala-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-3-(4-biphenyl)alanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
15		754	>95%	26,3	 H-Asp-Ala(3,3-Diphenyl)-Asn-Ala-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-(3,3-diphenyl)alanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
16		720	50-75%	4,28	 H-Asp-Pro[(3S)-Phenyl]-Asn-Ser-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-(3S)-phenylprolyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
17		720	50-75%	2,27	

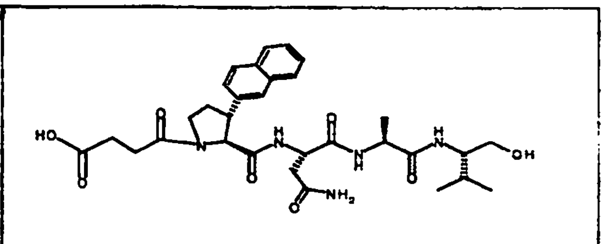
					H-Asp-Pro[(3R)-Phenyl]-Asn-Ser-Val-Gly-NH(CH₂)₃OH
	L-Aspartyl-L-(3R)-phenylprolyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
18		695	50-75%	25	 H-Asp-Ala[3-(3-pyridyl)]-Asn-Ser-Val-Gly-NH(CH2)3OH
	L-Aspartyl-L-3-(3-pyridyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
19		744	75-95%	25	 H-Asp-nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	L-Aspartyl-D-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
20		708	50-75%	32,5	 H-Asp-Hof-Asn-Ser-Val-Gly-NH(CH2)3OH
	L-Aspartyl-L-homophenylalanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
21		686	75-95%	0,34	 H-Asp-Nal(2)-Asn-Ser-Val-Gly-NH2
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-amide				
22		629	75-95%	0,18	

					H-Asp-Nal(2)-Asn-Ser-Val-NH2
	L-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valine-amide				
23		777	50-75%	1,49	 Phthalyl-Nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	Phthaloyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
24	See Example 2.3	729	>95%	0,39	 Suc-Nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	Succinyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
25		744	75-95%	0,23	 H-βAsp-Nal(2)-Asn-Ser-Val-Gly-NH(CH$_2$)$_3$OH
	L-β-Aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
26		743	75-95%	0,45	 Glutaryl-Nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	Glutaryl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
27		901	>95%	0,44	

					H-Cit-Asp-Nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	L-Citrulyl-L-aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
28		900	75-95%	0,15	 H-Arg-Asp-Nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	L-Arginyl-L-aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
29		872	>95%	0,24	 H-Lys-Asp-Nal(2)-Asn-Ser-Val-Gly-NH(CH2)3OH
	L-Lysyl-L-aspartyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-seryl-L-valyl-glycine-3-hydroxypropylamide				
30		713	>95%	0,25	 Suc-Nal(2)-Asn-Ala-Val-Gly-NH(CH2)3OH
	Succinyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-alanyl-L-valyl-glycine-3-hydroxypropylamide				
31	see example 3.1	598	>95%	0,19	 Suc-Nal(2)-Asn-Ala-Val-NH2
	Succinyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-alanyl-L-valine-amide				
	Succinyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-L-alanyl-L-2-tert.butyl-glycine-amide				

33		584	>95%	0,72	 Suc-Nal(2)-Asn-Gly-Val-NH2
	Succinyl-L-3-(2-naphthyl)alanyl-L-asparaginyl-glycinyl-L-valine-amide				
34	See Example 2.2	624	>95%	0,027	 Suc-Pro[(3R)-2-Naphthyl]-Asn-Ala-Val-NH2
	Succinyl-L-(3R)-(2-naphthyl)prolyl-L-asparaginyl-L-alanyl-L-valine-amide				
35		654	>95%	5,02	 Suc-Tyr(Bzl)-Asn-Ala-Val-NH2
	Succinyl-L-O-benzyl-tyrosyl-L-asparaginyl-L-alanyl-L-valine-amide				
36		624	>95%	2,83	 Suc-Ala[3-(4-Biphenyl)]-Asn-Ala-Val-NH2
	Succinyl-L-3-(4-biphenyl)alanyl-L-asparaginyl-L-alanyl-L-valine-amide				
37		599	>95%	0,83	 Suc-Nal(2)-Asn-Ala-Val-OH
	Succinyl-L-3-(2-Naphthyl)alanyl-L-asparaginyl-L-alanyl-L-valine				

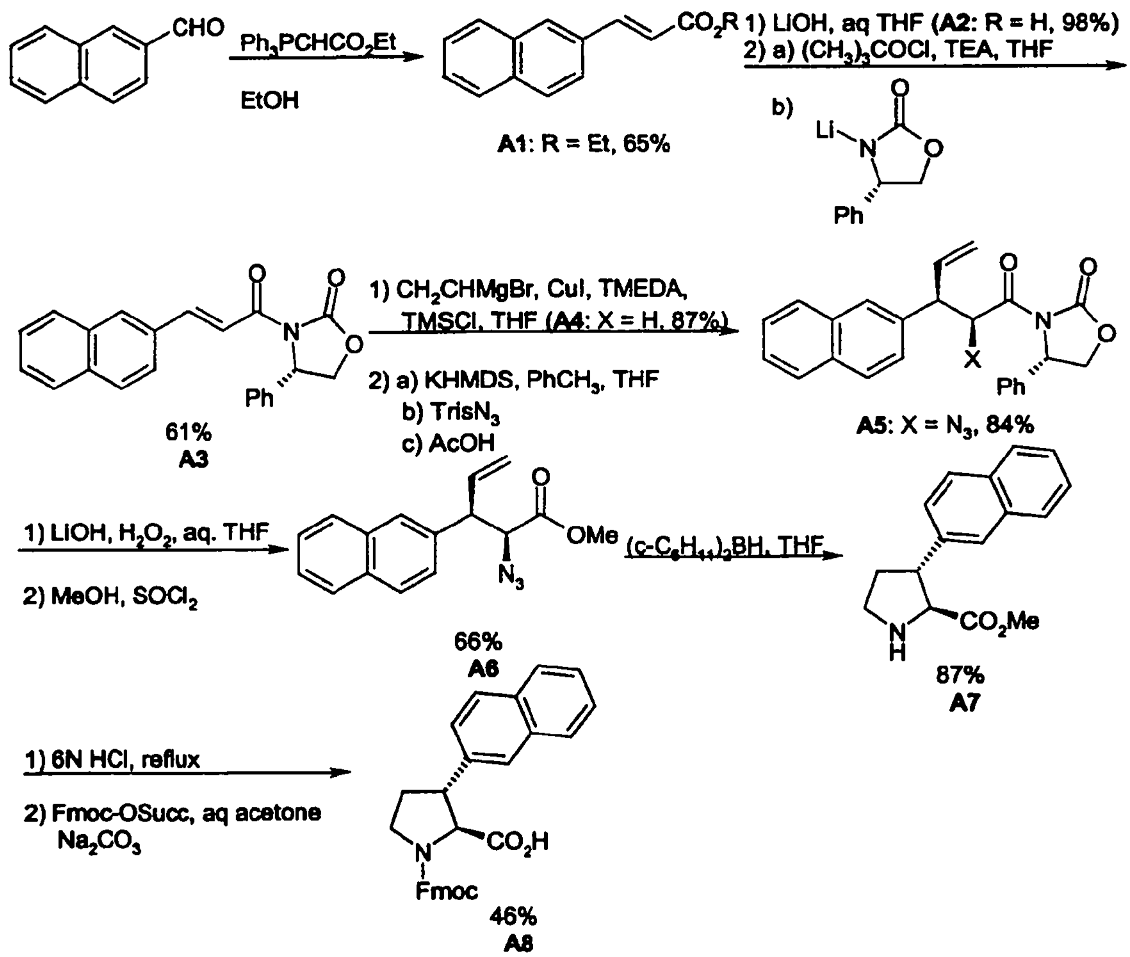
38		585	>95%	0,26	 Suc-Nal(2)-Asn-Ala-Val-ol
	Succinyl-L-3-(2-Naphthyl)alanyl-L-asparaginyl-L-alaninyl-L-valinol				
39		597	>95%	1,5	 Suc-Nal(2)-Asn-Ala-NHCH(iPr)2
	Succinyl-L-3-(2-Naphthyl)alanyl-L-asparaginyl-L-alanine-2,4-dimethylpentylamide				
40		569	>95%	1,16	 Suc-Nal(2)-Asn-Ala-NHCH2C(CH3)3
	Succinyl-L-3-(2-Naphthyl)alanyl-L-asparaginyl-L-alanine-neopentylamide				
41		595	>95%	8,17	 Suc-Nal(2)-Asn-Ala-3,3-dimethylpiperidine
	Succinyl-L-3-(2-Naphthyl)alanyl-L-asparaginyl-L-alanine-3,3-dimethylpiperidinylamide				
42		626	75-95%	0,24	 Suc-Nal(2)-Asn-Ala-Val-N(CH3)2
	Succinyl-L-3-(2-Naphthyl)alanyl-L-asparaginyl-L-alanyl-L-valine-dimethylamide				

43		611	>95%	0,026	 Suc-Pro[(3R)-2-Naphthyl]-Asn-Ala-Val-ol
	Succinyl-L-(3R)-(2-naphthyl)prolyl-L-asparaginyl-L-alanyl-(2S)-amino-3-methyl-1-butanol				

2. Large scale synthesis

2.1 Synthesis of N-Fmoc-trans-3-(2′-naphthyl)-L-proline (A8)

Summary: N-Fmoc-trans-3-(2′-naphthyl)-L-proline (A8) was prepared in 10 steps:



2.1.1 Ethyl trans-3-(2′-naphthyl)-propenoate (A1)

To a stirred solution of 2-naphthaldehyde (7.8 g, 50 mmol) in 50 mL ethanol was added (carbethoxymethylene)triphenylphosphorane (18.3 g, 52.5 mmol). A slight exotherm was noted. A precipitate formed while the mixture stirred overnight. The reaction mixture was diluted with Et_2O (500 mL) and washed with 1 M H_3PO_4 (2 x 100 mL), saturated $NaHCO_3$ (1 x 100 mL), water (100 mL), and brine (100 mL). The organic fraction was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was passed through a SiO_2 plug eluting with 9:1 hexane:EtOAc. After concentration in vacuo, a near quantitative yield of the product as an 85:15 mixture of geometric isomers (favoring trans, nmr) was recovered. The material was recrystallized from hexane/EtOAc (rich in hexane) to recover 4.5 g of the desired product as a 97:3 mixture of isomers (nmr). The mother liquor was concentrated and recrystallized as before to recover an additional 2.9 g (total 7.4 g, 33 mmol, 65% yield). NMR ($CDCl_3$) δ 7.93 (s, 1 H); 7.88-7.83 (c, 4 H); 7.67 (dd, 1 H, J = 1.6, 8.6 Hz); 7.53-7.50 (c, 2 H); 6.55 (d, 1 H, J = 16.0 Hz); 4.30 (q, 2 H, J = 7.1 Hz); 1.42 (t, 3 H, J = 7.1 Hz).

2.1.2 trans-3-(2′-Naphthyl)-propenoic acid (A2)

To a solution of ester A1 (4.24 g, 18.8 mmol) in THF (75 mL) was added $LiOH \cdot H_2O$ (2.36 g, 56.3 mmol) in water (19 mL). The initially heterogenous mixture was stirred vigorously overnight and became homogenous. The reaction mixture was acidified with concentrated HCl (pH ≈ 2) and a precipitate formed. The heterogenous mixture was transferred to a separatory funnel and extracted with EtOAc (3 x 150 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo to recover the carboxylic acid as a white solid (3.66 g, 98% yield). NMR ($CDCl_3$) δ 7.97 (d, 1 H, J = 15.7 Hz); 7.90 (d, 1 H, J = 15.3 Hz); 7.90-7.83 (c, 3 H); 7.70 (dd, 1 H, J = 1.6, 8.6 Hz); 7.57-7.50 (c, 2 H); 6.58 (d, 1 H, J = 16.0 Hz).

2.1.3 trans-(4S)-3-(3′-(2″-Naphthyl)-propenoyl)-4-phenyl-2-oxazolidinone (A3)

A solution of carboxylic acid A2 (3.66 g, 18.5 mmol) and triethylamine (1.87 g, 2.56 mL, 18.5 mmol) in anhydrous THF (74 mL) was cooled to -78 ° C. Pivaloyl chloride (2.35 g, 2.40 mL, 19.4 mmol) was added over two minutes accompanied by formation of a white precipitate. After 10 minutes, the flask was placed in a 0 ° C bath for a duration of 10 minutes after which the flask cooled back to -78 ° C for 1.5h. In a separate flask the oxazolidione derived from L-phenylglycinol (3.31 g, 20.3 mmol) in anhydrous THF (74 mL) was cooled to -78 ° C. A solution of n-BuLi (1.6 M in hexane, 11.6 mL, 18.5 mmol) was added and stirring continued for about 1 h accompanied by the metalated oxazolidinone precipitating from the THF/hexane solution. The mixed anhydride was added via cannula to the metallated oxazolidinone and the reaction mixture placed in a 0 ° bath. After 1 h the bath was removed and the mixture warmed to room temperature overnight. The reaction was quenched with 50 mL saturated NH_4Cl. THF was removed under reduced pressure and, after transfer to a separatory funnel, the mixture was extracted with CH_2Cl_2 (3 x 75 mL). The combined organic fractions were washed with 1 M NaOH (2 x 50 mL), dried ($MgSO_4$) and concentrated. The residue was recrystallized from EtOAc/hexane to recover a white solid (3.87 g, 11.2 mmol, 61 % yield). NMR ($CDCl_3$) δ 8.05 (d, 1 H, J = 15.7 Hz); 7.94 (d, 1 H, J = 15.4 Hz); 7.87-7.81 (c, 3 H); 7.76 (dd, 1 H, J = 1.5, 8.6 Hz); 7.53-7.47 (c, 2 H); 7.41-7.34 (c, 5 H); 5.58 (dd, 1 H, J = 8.7, 3.9 Hz); 4.76 (t, 1 H, J = 8.7 Hz); 4.33 (dd, 1 H, J = 8.8, 3.9 Hz).

2.1.4 (3′R4S)-3-(3′-(2″-Naphthyl)-4′-pentenoyl)-4-phenyl-2-oxazolidinone (A4)

To a solution of CuI (3.96 g, 20.9 mmol) and TMEDA (2.66 g, 3.46 mL, 22.9 mmol) in anhydrous THF (92 mL) at -78 ° C was added vinylmagnesium bromide (1.0 M in THF, 20.9 mL, 20.9 mmol). The mixture was stirred for 15 minutes. In a separate flask trimethylsilyl chloride (5.69 g, 6.64 mL, 52.2 mmol) was added to a solution of unsaturated imide A3 (3.87 g, 11.3 mmol) in anhydrous THF (42 mL). Owing to insolubility of the imide, the septum of the flask containing the cuprate reagent was removed and the slurried imide added in one portion rinsing quickly with a small amount of THF. The bath temperature was raised to -30 ° C and stirring continued for 1 h. The reaction mixture was poured into 250 mL of a 3:2 mixture of saturated NH_4Cl:concentrated NH_4OH. The layers were separated and the aqueous fraction extracted with EtOAc (3 x 200 mL). The combined organic fractions were washed sequentially with saturated NH_4Cl (1 x 100 mL) and water (1 x 100 mL). The organic fraction was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by passage through a plug of SiO_2 eluting with 4:1 hexane:EtOAc. The eluant was concentrated in vacuo to recover a white solid (3.64 g, 9.81 mmol, 87% yield). NMR ($CDCl_3$) δ 7.87-7.82 (c, 3 H); 7.72 (s, 1 H); 7.54-7.27 (c, 8 H); 6.11 (ddd, 1 H, J = 6.7, 10.4, 17.0 Hz); 5.34 (dd, 1 H, J = 8.6, 3.5 Hz); 5.10 (d, 1 H, J = 8.2 Hz); 5.08 (d, 1 H, J = 17.2 Hz); 4.56 (t, 1 H, J = 8.8 Hz); 4.26 (dd, 1 H, J = 8.8, 3.5 Hz); 4.16 (ddd, 1 H, J = 8.1, 7.0, 6.9 Hz); 3.68 (dd, 1 H, J = 8.4, 16.5 Hz); 3.50 (dd, 1 H, J = 6.5, 16.5 Hz).

2.1.5 (2′S3′R4S)-3-(2′-Azido-3′-(2″-naphthyl)-4′-pentenoyl)-4-phenyl-2-oxazolidinone (A5)

Potassium hexamethyldisilazide (0.5 M in toluene, 25.5 mL, 12.8 mmol) was added in one portion to anhydrous THF (34 mL) at -78 ° C. Imide A4 (3.64 g, 9.81 mmol) was slurried in THF (34 mL) and added via cannula, rinsing with THF (2 x 11 mL) to complete the transfer. After 30 min, trisylazide (4.40 g, 14.2 mmol) was dissolved in THF (34 mL), cooled to -78 ° C, and added via cannula. Thirty minutes later, AcOH (1.41 g, 1.34 mL, 23.4 mmol) was added to quench the reaction. The mixture was stirred at room temperature overnight. The mixture was partitioned between CH_2Cl_2 (300 mL) and dilute brine (150 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (3 x 150 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure.The residue was purified by flash chromatography to recover the product (3.41 g, 8.28 mmol, 84 % yield). NMR ($CDCl_3$) δ 7.85-7.82 (c, 3 H); 7.72 (s, 1 H); 7.53-7.47 (c, 2 H); 7.42 (dd, 1 H, J = 1.7, 8.5 Hz); 7.37-7.31 (c, 3 H); 7.18-7.15 (c, 2 H); 6.28 (ddd, 1 H, J = 8.2, 10.2, 17.1 Hz); 5.63 (d, 1 H, J = 10.2 Hz); 5.37 (d, 1 H, J = 17.0 Hz); 5.34 (d, 1 H, J = 10.2 Hz);

4.83 (dd, 1 H, J = 3.0, 8.3 Hz); 4.14 (t, 1 H, J = 7.2 Hz); 4.07 (dd, 1 H, J = 9.3, 17.9 Hz); 3.94 (dd, 1 H, J = 3.0, 5.8 Hz); 3.68 (t, 1 H, J = 8.6 Hz).

2.1.6 Methyl (2S3R)-2-Azido-3-(2′-naphthyl)-4-pentenoate (A6)

To a solution of imide A5 (3.41 g, 8.28 mmol) in THF (62 mL) was added water (21 mL), 35% H_2O_2 (2.7 mL), and $LiOH{\cdot}H_2O$ (695 mg, 16.6 mmol). After 2 hours Na_2SO_3 (4.17 g, 33.1 mmol) was added as a solution in water (41 mL). The mixture was stirred for 15 minutes and THF removed under reduced pressure. The aqueous solution was acidified with HCl and extracted with EtOAc (2 x 150 mL). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was passed through a SiO_2 plug column eluting with 1:1 hexane:EtOAc to recover, after concentration, a white solid that was presumably a mixture of the carboxylic acid and chiral auxiliary. Recrystallization from hexane/EtOAc yielded the chiral auxiliary as needles. The mother liquor was concentrated and carried on to the esterification step. The residue containing the crude carboxylic acid was dissolved in anhydrous MeOH (46 mL) and cooled to 0 ° C. Thionyl chloride (1.18 g, 725 µL, 9.94 mmol) was added and, after 10 minutes, the mixture heated at reflux for 2 hours. Water (1.0 mL) was added to the mixture, stirred for 10 minutes, and the contents of the flask concentrated under reduced pressure. The residue was partitioned between EtOAc (150 mL) and brine (100 mL). The layers were separated and the organic fraction was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (19:1 hexane:EtOAc) to recover the methyl ester (1.54 g, 5.48 mmol, 66% yield). NMR ($CDCl_3$) δ 7.84-7.80 (c, 3 H); 7.71 (s, 1 H); 7.50-7.46 (c, 2 H); 7.39 (dd, 1 H, J = 1.8, 8.5 Hz); 6.23 (ddd, 1 H, J = 8.3, 10.9, 17.6 Hz); 5.30 (d, 1 H, J = 9.9 Hz); 5.28 (d, 1 H, J = 17.7 Hz); 4.22 (d, 1 H, J = 7.5 Hz); 4.06 (t, 1 H, J = 7.9 Hz).

2.1.7 trans-3-(2′-naphthyl)-L-proline methyl ester (A7)

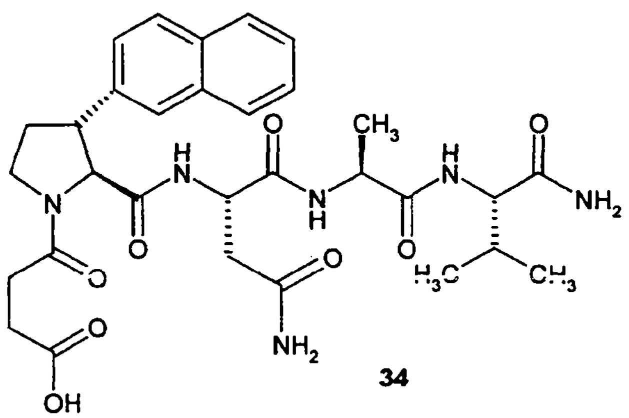
Borane-methyl sulfide complex (2.0 M in THF, 6.57 mL, 13.1 mmol) was diluted with anhydrous THF (26 mL) and cooled to 0 ° C. Cyclohexene (2.16 g, 2.66 mL, 26.3 mmol) was added cautiously via syringe. After 30 minutes a white precipitate had formed. Stirring was continued for three hours. The contents of the flask were concentrated in vacuo. The reagent was slurried in CH_2Cl_2 (36 mL) and cooled to 0 ° C. Vinyl azide A6 (1.23 g, 4.38 mmol) was dissolved in CH_2Cl_2 (9 mL) and added via cannula. The reaction mixture became pale yellow and gas evolution was evident. The mixture was warmed to room temperature overnight. Added MeOH (26 mL) and stirred for an additional 15 minutes. The mixture was concentrated under reduced pressure. The residue was taken up in Et_2O (25 mL) and extracted with 0.1 M HCl (5 x 25 mL). The aqueous extracts were basicified with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 100 mL). The organic extracts were dried ($MgSO_4$) and concentrated in vacuo to recover the cyclized product along with some dicyclohexyl borane derived contaminants (974 mg, 3.82 mmol, 87% yield of crude material). NMR ($CDCl_3$) δ 7.84-7.78 (c, 3 H); 7.71 (s, 1 H); 7.49-7.41 (c, 3 H); 3.91 (d, 1 H, J = 6.9 Hz); 3.69 (s, 3 H); 3.63 (m, 1 H), 3.48 (dd, 1 H, J = 8.2, 15.4 Hz); 3.27 (d, 1 H, J = 7.8 Hz); 3.25 (d, 1 H, J = 7.8 Hz); 2.33 (m, 1 H), 2.09 (m, 1 H).

2.1.8 N-Fmoc-trans-3-(2′-naphthyl)-L-proline (A8)

510 mg (2 mmol) of methyl ester (A7) in 12 ml of 6N HCl are heated 100°C for 10 hours. The reaction solution is concentrated under reduced pressure and the solid residue is suspended in 15 ml of acetone. The suspension is adjusted to pH 9-10 using 2N Na_2CO_3 solution. 742 mg (2.2 mmol) of Fmoc-O-succinimide are then added slowly. The pH is subsequently adjusted to 9-10 and the mixture is stirred at room temperature for 4 hours and then allowed to stand at room temperature overnight. The pH is subsequently adjusted to 2 using conc. HCl, and the mixture is admixed with ethyl acetate. 560 mg of the precipitated product are filtered off with suction. The aqueous phase is extracted three times with ethyl acetate and subsequently admixed with methylene chloride. This gives a further 185 mg of product as a precipitate. Yield:745 mg (80.4%). NMR (d6-DMSO) δ 7.95-

7.80 (c, 6 H); 7.68 (d, 1 H, J = 7.3 Hz); 7.60 (d, 1 H, J = 7.4 Hz); 7.50-7.34 (c, 6 H); 7.25 (m, 1 H), 4.39-4.15 (c, 4 H); 3.70-3.48 (c, 3 H); 2.29 (m, 1 H); 2.14 (m, 1 H).

2.2 N-Succinyl-trans-3-(2′-naphthyl)-L-prolyl-L-asparaginyl-L-alaninyl-L-valine-amide (34)



34

2.2.1 N-Fmoc-trans-3-(2'-naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide (B1)

463.5 mg (1 mmol) of *N*-Fmoc-trans-3-(2'-naphthyl)-*L*-proline (A8), 338 mg of H-Asn-Ala-Val-NH_2 hydrochloride (prepared according to customary methods of peptide chemistry) and 135 mg of HOBT are dissolved in 20 ml of DMF. At 0°C, 0.13 ml of N-ethylmorpholine and 220 mg of DCC are added. The mixture is stirred at 0°C for 1 hour and then at room temperature for 3 hours and is subsequently allowed to stand at room temperature overnight. The precipitate is filtered off with suction and the solution is concentrated under high vacuum. The residue is partitioned between pentanol and $NaHCO_3$ solution. The pentanol phase is washed with $KHSO_4$ solution and H_2O/NaCl solution. The precipitate is filtered off with suction and thoroughly triturated with diethyl ether. This gives 473 mg of product. The pentanol phase is dried using Na_2SO_4 and concentrated. The residue is triturated twice with diethyl ether. This gives another 257 mg of product.
Yield: 730 mg (97.7%).

2.2.2 trans-3-(2'-Naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide (B2).

248 mg (0.332 mmol) of *N*-Fmoc-trans-3-(2'-naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide B1 are taken up in 5 ml of DMF. 0.35 ml (3.32 mmol) of diethylamine are added and the mixture is stirred at room temperature for 15 minutes. The mixture is filtered off with suction through a clarifying layer and concentrated under high vacuum. The solid residue is triturated with diethyl ether and filtered off with suction.

Yield: 141 mg (81%).

2.2.3 Methyl tert-butyl succinate (B3).

Under argon, 13.2 g (100 mmol) of monomethyl succinate are suspended in 500 ml of methylene chloride. Over a period of 30 minutes, 12.9 ml (150 mmol) of oxalyl chloride are added dropwise, and the mixture is subsequently stirred at room temperature for 6 hours. After approximately 3.5 hours, a clear solution results. 300 ml of tert-butanol are subsequently added dropwise. The mixture is then allowed to stand at room temperature for 21 hours, and the clear solution is concentrated. The residue is dissolved in ethyl acetate and washed with H_2O, $NaHCO_3$ solution and H_2O. The solution is dried with Na_2SO_4 and concentrated.

Yield: 21.6 g (crude oil-like product).

2.2.4 Mono-tert-butyl succinate (B4)

9.4 g (50 mmol) of methyl tert-butyl succinate (B3) are dissolved in 115 ml of 1,4-dioxane. 110 ml of 0.5N NaOH are subsequently added. The mixture is allowed to stand at room temperature, and product precipitates out. The mixture is allowed to stand at room temperature over the weekend and is subsequently concentrated. The aqueous solution is extracted using diethyl ether. The aqueous phase is cooled to 0°C and acidified to pH 4 using cold 2N H_2SO_4. The mixture is subsequently extracted five times using diethyl ether. The organic phases are combined, washed with H_2O, dried with Na_2SO_4 and concentrated. Yield: 5.62 g of an oil (64.5%).

2.2.5 N-tert-*B*utyl-succinyl-trans-3-(2'-naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide (B5)

262 mg (0.5 mmol) of *trans*-3-(2'-naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide (B2), 87.1 mg (0.5 mmol) of mono-tert-butyl succinate (B4) and 67.5 mg of HOBt are dissolved in 5 ml of DMF. At 0°C, 110 mg of DCC are added and the mixture is stirred at 0°C for 1 hour and then at room temperature for 2 hours and allowed to stand at room temperature overnight. The precipitate is filtered off with suction and the filtrate is concentrated under high vacuum. The residue is triturated with $NaHCO_3$ solution, filtered off with suction, washed with H_2O and dried in a desiccator.

Yield: 169 mg (49.6%).

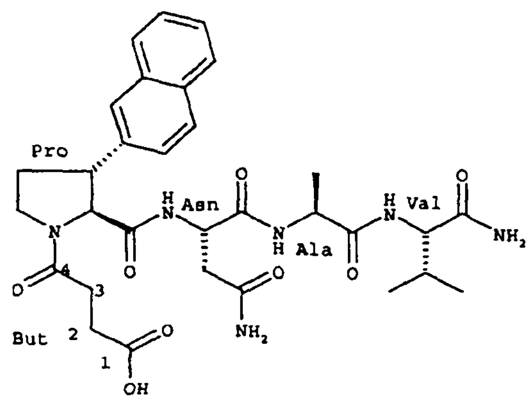
2.2.6 N-Succinyl-trans-3-(2'-naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide (34)

316 mg of *N*-tert-butyl-succinyl-*trans*-3-(2'-naphthyl)-*L*-proline-L-asparagine-L-alanine-L-valine-amide (B5) are dissolved in 2 ml of 90% strength trifluoroacetic acid and allowed to stand at room temperature for 1 hour. The mixture is subsequently filtered through a clarifying layer and concentrated. The residue is triturated with diethyl ether and filtered off with suction. This gives 159 mg of crude product. For purification, the substance is chromatographed over Sephadex® LH20 using a butanol/glacial acetic acid/water mixture.

Yield: 27.5 mg (9.5%).

m/z: 625.298949 $(M+H)^+$ (high resolution mass spectrum).

NMR data of compound 34:

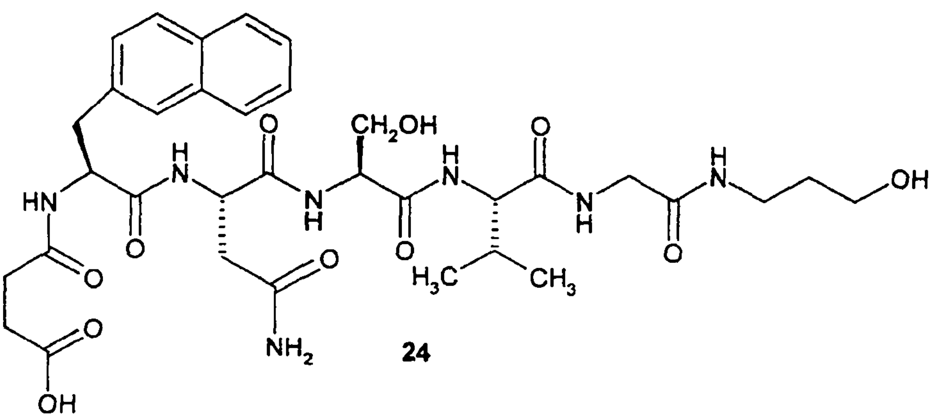


Chemical shifts of compound 34 in DMSO at 300 K:

See formula above	^{1}H		^{13}C	
	trans	cis	trans	cis
But-1	-	-	173.88	173.92
But-2	2.54/2.46	2.61/2.20	28.64	28.37
But-3	2.70/2.54	2.56/2.39	28.80	28.80
But-4	-	-	170.67	170.22
Pro-α	4.39	4.68	66.18	65.46
Pro-C'	-	-	171.03	170.94
Pro-β	3.55	3.68	47.42	49.45
Pro-γ	2.40/2.16	2.33/1.94	32.02	30.41
Pro-δ	3.81/3.72	3.59/3.53	46.12	45.40
Nap-1	-	-	138.79	139.55
Nap-2	7.76	7.78	125.14	124.79
Nap-2a	-	-	132.97	132.97
Nap-3	7.87	7.87	127.66	127.66
Nap-4	7.49	7.49	126.03	126.03
Nap-5	7.48	7.48	125.63	125.63
Nap-6	7.88	7.88	127.33	127.33
Nap-6a	-	-	131.98	131.98
Nap-7	7.87	7.89	127.97	127.97
Nap-8	7.45	7.46	125.96	125.96
Asn-NH	8.31	8.50	-	-
Asn-α	4.44	4.64	50.13	49.79
Asn-C'	-	-	170.58	170.29
Asn-β	2.64/2.46	2.57/2.45	36.96	36.25
Asn-γ-C'	-	-	171.73	171.44
Asn-δ-NH2	7.41/6.93	7.33/6.93	-	-
Ala-NH	7.74	8.02	-	-

Ala-α	4.19	4.27	48.71	48.46
Ala-C'	-	-	171.84	171.78
Ala-β	1.22	1.19	17.49	18.04
Val-NH	7.48	7.70	-	-
Val-α	4.04	4.08	57.69	57.57
Val-C'	-	-	172.77	172.73
Val-β	1.97	1.97	30.08	30.29
Val-γ	0.82	0.86	19.24	19.27
Val-γ'	0.82	0.84	17.89	17.97
Val-NH2	7.15/6.99	7.27/7.00	-	-

2.3 N-Succinyl-L-(2-naphtyl)alaninly-L-asparaginyl-L-serinyl-L-valinyl-glycine-3-hydroxypropylamide (24)



24

2.3.1 Benzyloxycarbonyl-glycine-(3-propanol)amide (C1)

627 g (30 mmol) of Gly-OH, 2.45 ml of 3-amino-1-propanol and 4.05 g of HOBt are dissolved in 60 ml of DMF. At 0°C, 6.6 g of DCC are added. The mixture is stirred at 0°C for 1 hour and at room temperature for 3 hours and allowed to stand at room temperature overnight. The precipitate is filtered off with suction and the filtrate is

concentrated under high vacuum. The residue is partitioned between ethyl acetate and $NaHCO_3$ solution. The organic phase is then washed with $NaHCO_3$ solution and H_2O/NaCl, dried using Na_2SO_4 and concentrated. The residue is triturated with diethyl ether.

Yield: 7.05 g (88.2%).

2.3.2 Benzyloxycarbonyl-glycine-(3-propanol tert-butyl ester)amide (C2)

7 g (26.28 mmol) of benzyloxycarbonyl-glycine-(3-propanol)amide (C1) are dissolved in 60 ml of dioxane. At low temperature (liquid CO_2), 6 ml of H_2SO_4 are added slowly. Subsequently, 60 ml of condensed isobutylene are added. The mixture is shaken in an autoclave at room temperature and a nitrogen pressure of approximately 20 bar for 3 days. The mixture is then admixed with diethyl ether and extracted three times with 2N Na_2CO_3 solution. The aqueous solution is washed with diethyl ether. The organic phases are combined, washed with water, dried with Na_2SO_4 and concentrated.

Yield: 7.98 g (94.2%).

2.3.3 Glycine-(3-propanol tert-butyl ester)amide hydrochloride (C3)

7.98 g (24.75 mmol) of benzyloxycarbonyl-glycine-(3-propanol tert-butyl ester)amide (C2) are dissolved in 80 ml of MeOH, admixed with Pd/carbon and hydrogenated on an autotitrator using methanolic HCl and H_2. The catalyst is subsequently filtered off with suction and the filtrate is concentrated. The residue is dried under high vacuum.

Yield: 4.7 g (84.5%).

2.3.4 Benzyloxycarbonyl-L-valine-glycine-(3-propanol tert-butyl ester)amide (C4)

5.13 g (20.43 mmol) of benzyloxycarbonyl-Val-OH, 4.59 g (20.43 mmol) of glycine-(3-propanol tert-butyl ester)amide hydrochloride (C3) and 2.75 g of HOBt are dissolved in 60 ml of DMF. At 0°C, 2.65 ml of N-ethylmorpholine and 4.5 g of DCC are added. The mixture is stirred at 0°C for 1 hour and then at room temperature for 2 hours. The mixture is allowed to stand at room temperature overnight and then concentrated under high vacuum. The residue is partitioned between glacial acetic acid and $NaHCO_3$ solution. The glacial acetic acid phase is then washed with $NaHCO_3$ solution, $KHSO_4$ solution and H_2O/NaCl, dried using Na_2SO_4 and concentrated. The solid residue is triturated with diethyl ether and filtered off with suction.

Yield: 7.32 g (85%).

2.3.5 L-Valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C5)

7.29 g (17.3 mmol) of benzyloxycarbonyl-L-valine-glycine-(3-propanol tert-butyl ester)amide (C4) are dissolved in 90 ml of MeOH, admixed with Pd/carbon and hydrogenated on an autotitrator using methanolic HCl. The catalyst is subsequently filtered off with suction and the filtrate is concentrated. The residue (amorphous) is dried under high vacuum, triturated with diethyl ether and filtered off with suction.

Yield: 5.22 g (93.2%).

2.3.6 Benzyloxycarbonyl-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide (C6)

5.46 g (18.5 mmol) of Z-Ser(But)OH, 6 g (18.5 mmol) of L-valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C5) and 2.5 g of HOBt are dissolved in 60 ml of DMF. At 0°C, 2.4 ml of N-ethylmorpholine and 4.07 g of DCC are added. The mixture is stirred at 0°C for 1 hour and at room temperature for 3 hours. The mixture is allowed to stand at room temperature overnight and then concentrated under high vacuum. The solid residue is partitioned between glacial acetic acid and $NaHCO_3$ solution. The glacial acetic acid phase is washed with $NaHCO_3$ solution, $KHSO_4$ solution and H_2O/NaCl, dried using Na_2SO_4 and concentrated. The residue is triturated with diethyl ether and filtered off with suction.

Yield: 9.74 g (93.2%).

2.3.7 L-Serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester) amide hydrochloride (C7)

9.74 g (17.25 mmol) of benzyloxycarbonyl-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide (C6) are dissolved in approximately 100 ml of MeOH, admixed with Pd/carbon and hydrogenated on an autotitrator using methanolic HCl. The catalyst is subsequently filtered off with suction and the filtrate is concentrated. The residue (amorphous) is dried under high vacuum and subsequently triturated with diethyl ether and filtered off with suction.

Yield: 8.02 g (99.6%).

2.3.8 Benzyloxycarbonyl-L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide (C8)

4.53 g (17 mmol) of Z-Asn-OH, 7.94 g of L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C7) and 2.3 g of HOBt are dissolved in 60 ml of DMF. At 0°C, 2.21 ml of N-ethylmorpholine and 3.74 g of DCC are added. The mixture is stirred at 0°C for 1 hour and at room temperature for 3 hours and then concentrated under high vacuum. The residue is partitioned between pentanol and $NaHCO_3$ solution. The pentanol phase is washed with $NaHCO_3$ solution, $KHSO_4$ solution and H_2O/NaCl, dried over Na_2SO_4 and filtered off with suction, and the filtrate is concentrated under high vacuum. The residue is triturated with diethyl ether, cooled and filtered off with suction. The product is dried in a desiccator over P_2O_5.

Yield: 10.8 g (93.6%).

2.3.9 L-Asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C9)

10.8 g (15.9 mmol) of benzyloxycarbonyl-L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide (C8) are dissolved in approximately 160 ml of warm MeOH, admixed with Pd/carbon and hydrogenated on an autotitrator using methanolic HCl. The catalyst is subsequently filtered off with suction and the filtrate is concentrated. The amorphous residue is dried under high vacuum, triturated with diethyl ether, cooled and filtered off with suction.

Yield: 8.96 g (97%).

2.3.10 Benzyloxycarbonyl-L-2-naphthylalanine-L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide (C10)

5.24 g (15 mmol) of benzyloxycarbonyl-2-Nal-OH, 8.72 g (15 mmol) of L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C9) and 2.04 g of HOBt are dissolved in 60 ml of DMF. At 0°C, 1.95 ml of N-ethylmorpholine and 3.3 g of DCC are added. The mixture is stirred at 0°C for 1 hour and at room temperature for 3 hours. The mixture is then allowed to stand at room temperature overnight, diluted with DMF and heated slightly. The precipitate is subsequently filtered off with suction and the filtrate is concentrated under high vacuum. The residue is triturated with $NaHCO_3$ solution and filtered off with suction and is then triturated with $KHSO_4$ solution, filtered off with suction, triturated with H_2O, filtered off with suction and washed with H_2O and dried in a desiccator over P_2O_5.

Yield: 13.25 g (>99%).

2.3.11 L-2-Naphthylalanine-L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C11)

8.85 g (10.1 mmol) of benzyloxycarbonyl-L-2-naphthylalanine-L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide (C10) are partly dissolved in 270 ml of MeOH, admixed with Pd/carbon and hydrogenated on an autotitrator using methanolic HCl. The suspension is diluted with DMF. After approximately 6 hours, the mixture is concentrated to half its original volume. All the material dissolves. The mixture is allowed to stand at room temperature overnight. The catalyst is subsequently filtered off with suction and the filtrate is diluted with the same amount of MeOH, admixed with new catalysts (Pd/carbon) and hydrogenated further on the autotitrator. After 7 hours, the mixture is allowed to stand at room temperature overnight. The mixture is subsequently hydrogenated for another 4 hours, the catalyst is filtered off with suction and the filtrate is concentrated. The residue (amorphous) is dried under high vacuum and subsequently triturated with diethyl ether and filtered off with suction.

Yield: 7.56 g (96.2%).

2.3.12 N-tert-Butyl-succinyl-*L*-2-naphthylalanine-L-asparagine-L-serine-L-valine-glycine-(3-propanol)amide (C12)

523 mg (3 mmol) of L-2-naphthylalanine-L-asparagine-L-serine(tert-butyl ester)-L-valine-glycine-(3-propanol tert-butyl ester)amide hydrochloride (C11), 2.33 g of mono-tert-butyl succinate (B4) and 405 mg of HOBt are dissolved in 20 ml of DMF. At 0°C, 0.39 ml of N-ethylmorpholine and 660 mg of DCC are added. The mixture is stirred at 0°C for 1 hour, at room temperature for 2 hours and then allowed to stand at room temperature overnight. The mixture is concentrated under high vacuum and the solid residue is triturated with $NaHCO_3$ solution and filtered off with suction. The product is subsequently triturated with $KHSO_4$ solution and filtered off with suction, washed with H_2O and dried in a desiccator over P_2O_5.

Yield: 3.04 g (crude product).

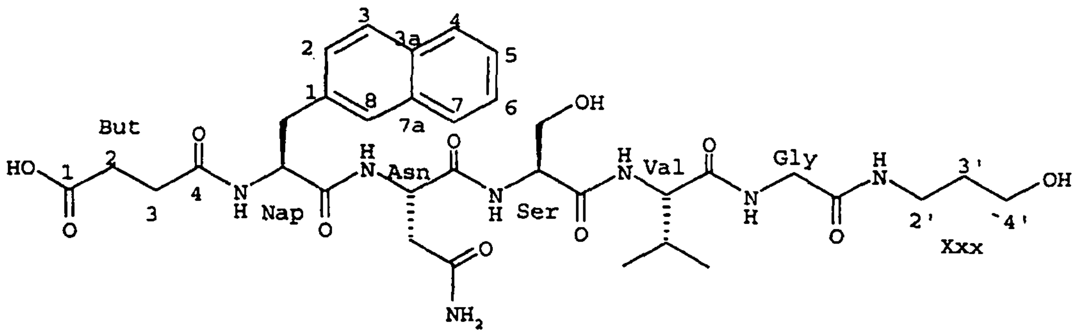
2.3.13 N-Succinyl-L-2-naphthylalanine-L-asparagine-L-serine-L-valine-glycine-(3-propanol)amide (24)

3 g (crude product) of *N*-tert-butyl-succinyl-*L*-2-naphthylalanine-L-asparagine-L-serine-L-valine-glycine-(3-propanol)amide (C12) are dissolved in 30 ml of 90% strength trifluoroacetic acid and allowed to stand at room temperature for 1 hour. The mixture is subsequently concentrated and the residue is triturated with diethyl ether and filtered off with suction. This gives 2.6 g of crude product. For purification, 250 mg of crude product are dissolved in warm glacial acetic acid and chromatographed over Sephadex LH20 using a butanol/glacial acetic acid/water mixture.

Yield: 103 mg

m/z: 730.341246 $(M+H)^+$ (high resolution mass spectrum).

NMR data of compound 24:



Chemical shifts of compound 24 in DMSO at 300 K:

See formula above	^{1}H	^{13}C
But-1	-	173.71
But-2	2.29	28.99
But-3	2.32/2.26	29.91
But-4	-	171.15
Nap-NH	8.19	-
Nap-α	4.60	53.92
Nap-C'	-	171.20
Nap-β	3.19/2.91	37.56
Nap-1	-	135.62
Nap-2	7.42	127.85
Nap-3	7.80	127.30
Nap-3a	-	131.75
Nap-4	7.85	127.38
Nap-5	7.44	125.31
Nap-6	7.47	125.83

Nap-7	7.82	127.38
Nap-7a	-	132.91
Nap-8	7.73	127.38
Asn-NH	8.35	-
Asn-α	4.60	49.73
Asn-C'	-	170.92
Asn-β	2.60/2.49	37.03
Asn-γ-C'	-	171.73
Asn-δ-NH2	7.44/6.99	-
Ser-NH	7.89	-
Ser-α	4.33	55.15
Ser-C'	-	170.13
Ser-β	3.66/3.56	61.52
Ser-OH	4.93	-
Val-NH	7.82	-
Val-α	4.10	58.37
Val-C'	-	171.02
Val-β	2.04	29.91
Val-γ	0.86	19.17
Val-γ'	0.86	18.11
Gly-NH	8.06	-
Gly-α	3.65	41.97
Gly-C'	-	168.45
Xxx-NH	7.63	-
Xxx-2'	3.10	35.78
Xxx-3'	1.54	32.24
Xxx-4'	3.40	58.33
Xxx-4'-OH	4.40	-

3. Inhibition of laminin/nidogen interaction and biological activity

Unless expressly stated, the chemicals used were purchased from Merck (Darmstadt), Sigma (Munich) or Riedel de Haën (Seelze).

The isolation of laminin P1 from human placenta, human nidogen from transfected HEK-293 cells and mouse laminin γ1 III 3-5 from HEK-293 cells is described in WO 98/31709.

Example 3.1 Inhibition assays – inhibition of laminin/nidogen binding with the peptide derivatives found

3.1.1. HTS screening assay (highly sensitive assay variant):

Time-Resolved Fluorescence Assay

Coating of test tubes

Microtiter plates (for example FluoroNunc®) were coated with 75 µl of a 0.1 µg/ml solution of laminin P1 (in 0.159 g of Na_2CO_3, 0.293 g of $NaHCO_3$, 0.02 g of NaN_3/liter, pH 9.2) at room temperature for 1 hour. The solution was then tipped off, and free binding sites were blocked by incubation with 0.5% BSA (in 7.9 g of NaCl, 1.2 g of Na_2HPO_4, 0.31 g of KCl, 0.23 g of NaH_2PO_4, 0.04% Tween 20/liter, pH 7.2) at room temperature for 0.5 hour. Completion of the blocking reaction was followed by decantation of the solution and washing once with 250 µl of washing buffer (PBS/0.04% Tween).

Sequential inhibition

In parallel with the coating, a preincubation of 85-100 µl of a 0.25 nM nidogen solution (recombinantly produced human nidogen) with inhibitor or standard was carried out in a separate reaction vessel (1 hour at room temperature in 7.9 g of NaCl, 1.2 g of Na_2HPO_4, 0.31 g of KCl, 0.23 g of NaH_2PO_4, 0.04% Tween 20/liter, 0.5% BSA, pH 7.2).

75 µl of the preincubation (nidogen + inhibitor or standard) were transferred into the coated wells of the microtiter plate and incubated at room temperature for 1 hour. This was followed by washing twice with PBS/0.04% Tween.
Detection of the bound nidogen took place by incubation (at room temperature) for 1 hour with 75 µl of a specific antibody preparation obtained from yolks of eggs from a chicken immunized with human nidogen. The IgY fraction was used in a dilution of 1:500 in PBS/0.04% Tween. The complex of nidogen and specifically bound antibodies was, after washing twice with PBS/0.04% Tween, detected by adding anti-chicken IgY-biotin (75 µl of a 1:2500 dilution; Promega, Madison, WI 53711, 608-274-4330). An incubation time of 1 hour and washing twice with PBS/0.04% Tween were followed for this purpose by incubation with streptavidin-europium (Wallac; 1 hour at room temperature) and washing twice with PBS/0.04% Tween. It was finally possible, after adding 100 µl of enhancement solution (Wallac) and shaking for 5 minutes, to measure a fluorescence signal in a Victor multilabel counter using the europium protocol. The relation between the amount of bound nidogen in the solutions with inhibitor and that of nidogen without added inhibitor was found.

3.1.2. Three-day equilibrium assay

Selected inhibitors were investigated for inhibitory activity in this assay variant. The assay is described in US patent number US 5,493,008.

The following table compares IC50 values of selected substances with the results of the HTS screening assay. It is clear that the 3-day assay gives slightly lower measured values and, as expected, is more sensitive than the screening assay. However, it is also clear from the comparison that inhibitory structures can be identified reliably with the screening assay developed by us.

Table 2: Characterization of specific inhibitors of the laminin/nidogen association: IC50 values (µM) in the various assay variants

Structure	HTS assay	3-day equilibrium assay
NIDPNAV	3.9	1.2
DPNAV	7.7	5.0
Compound 24	0.36	0.09
Compound 31	0.19	0.085

Example 3.2 (hypothetical)

Testing the biological activity of the peptide derivatives

Several models which are described in detail in the literature can be used to test the biological activity of the peptide derivatives.

Some representative ones are mentioned below:

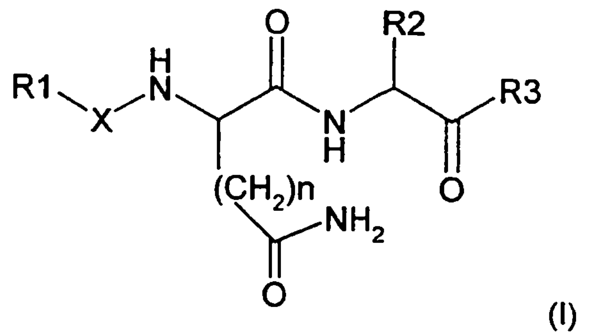
Formation of tubuli in cultures of embryonic kidneys.
Grobstein, C.; (1956) Exp. Cell Res. 10: 424-440.
Ekblom, P. et al. (1994) Development 120: 2003-2014
Branching morphology in embryonic lungs.
Ekblom, P. et al. (1994) Development 120: 2003-2014
Branching morphology in embryonic salivary glands.
Grobstein, C. (1953) J. Exp. Zool.124: 383-413
Kadoya, Y. et al. (1997) Development 124: 683-691
Basement membrane assembly in a organotypic skin culture.
Smola, H.; Stark, H.-J.; Thiekötter, G.; Mirancea, N.; Krieg, T.; Fusenig, N.E. (1998) Exp. Cell Res. 239: 399-410
Reconstitution of hydra from disintegrated cells.
Yang, Y.G.; Mayura, K.; Spainhour, C.B.; Edwards Jr., J.F.; Phillips, T.D. (1993) Toxicology 85: 179-198

Thickening of basement membranes in hydra after culturing at increased glucose concentration.
Zhang, X.; Huff, J.K.; Hudson, B.G.; Sarras Jr.; M.P. (1990) Diabetologia 33: 704-707

All types of quantitative angiogenesis assays summarized in a review article by Jain, R.K. et al. in Nature Medicine (1997) Vol. 3, No. 11, for example:
Induction of Haemangiomes in mice by implantation of cells from spontaneous hemangioendotheliomes.
O'Reilly, M.S.; Brem, M.S.; Folkman, J. (1995) J. Pediatr. Surg. 30:2; 325-329
Growth of micro-vessels in a serum-free culture of rat aorta.
Nicosia, R.F.; Ottinetti, A. (1990) Lab. Invest Vol.63, No. 1, 115-122
Formation of capillaries of endothelic cells on micro-carriers after imbedding into a fibrin gel.
Nehls, V.; Drenckhahn, D. (1995) Microvascular Research 50: 311-322.

Claims

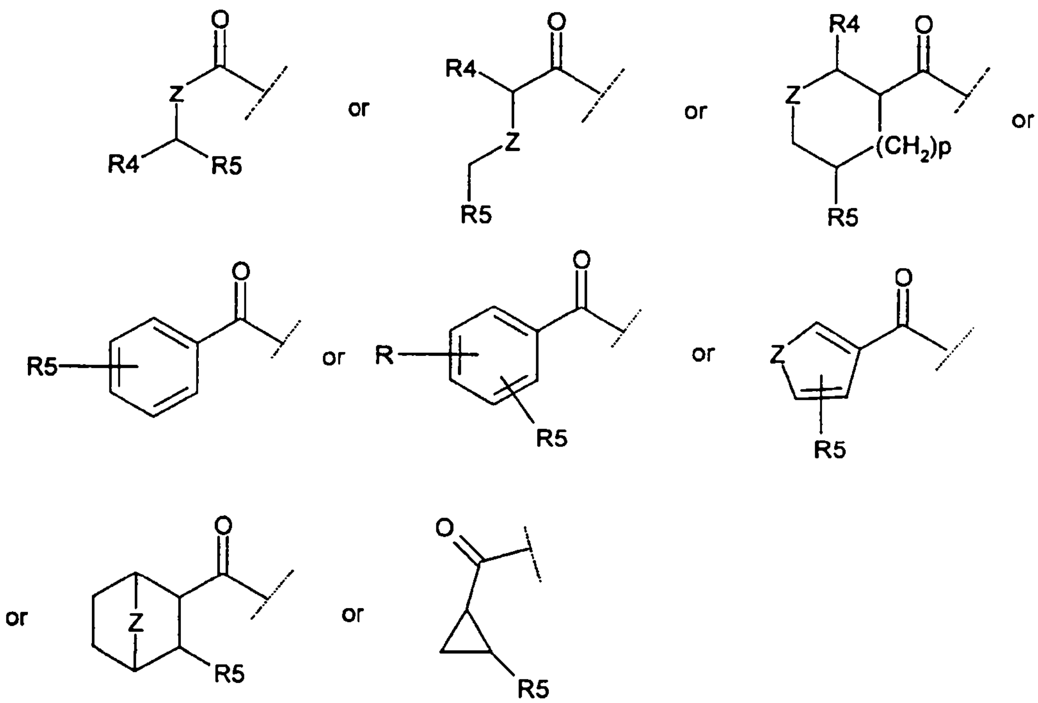
1. A compound of formula I



(I)

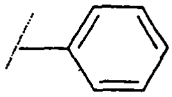
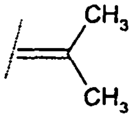
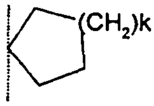
wherein

R1 is a group of one of the following formulae

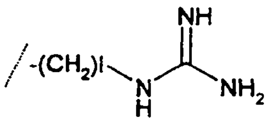


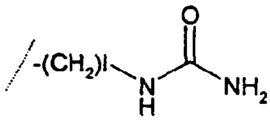
wherein

R4 means -A, $-NH_2$, -NHR, $-NR_2$, A_2, -NHR1,

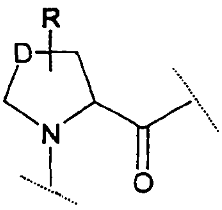
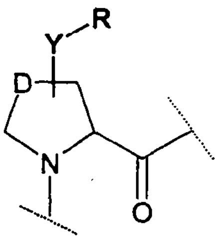
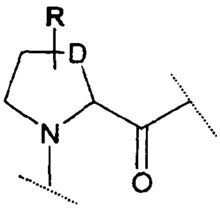
 or  or 

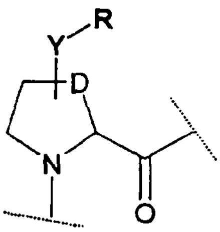
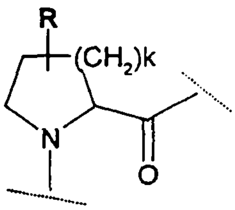
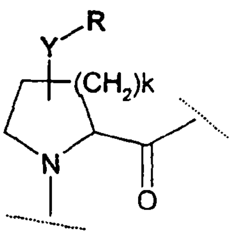
and R5 means $-(CH_2)_lCOOA$, $-(CH_2)_lCONH_2$, $-(CH_2)_lNH_2$ or $-(CH_2)_l-SO_3H$,

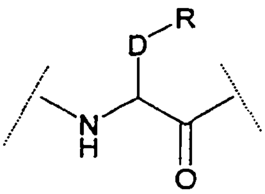
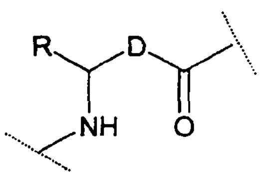
 or



and X is a group of one of the following formulae

 or  or  or

 or  or  or

 or 

wherein

Y means O, S, -N(A)-CO- or $-(CH_2)_r-$,

D means $(CH_2)_r$, O, S, NH, NR, $(CH_2)_r$-O, $(CH_2)_r$-S, $(CH_2)_r$-NH or

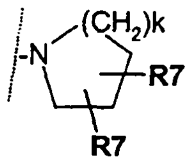
$(CH_2)_rNR$ and

R2 means -A, -E-OH, -E-COOH or -E-$CONH_2$,

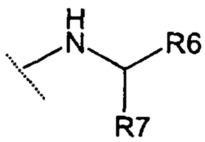
wherein E means a linear or branched C_1-C_{10}-alkyl chain, which is unsubstituted or substituted by -A, -$(CH_2)_m$-OH, -$(CH_2)_m$-COOH, -$(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group,

or E means C_5-C_{10}-cycloalkyl, which is unsubstituted or substituted by -A, -$(CH_2)_m$-OH, -$(CH_2)_m$-COOH, -$(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group,

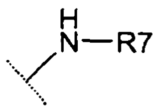
and R3 is a group of one of the following formulae



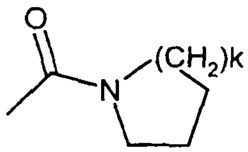
or



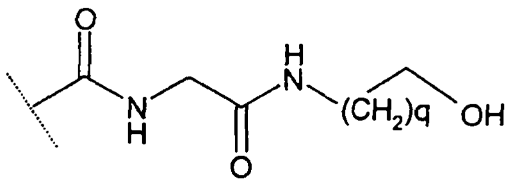
or



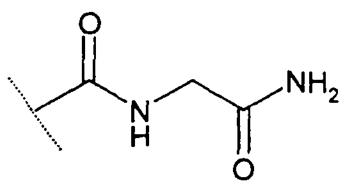
wherein R6 means -H, -COOH, -$CONH_2$, -CONHR, -$CONR_2$, -CH_2OH or



or



or



and wherein R7 means a linear or branched C_1-C_{10}-alkyl group, which is unsubstituted or substituted by -A, -$(CH_2)_m$-OH, -$(CH_2)_m$-COOH, -$(CH_2)_m$-C(O)NA_2 or by a

	C_5-C_{10}-cycloalkyl group,
or R7 means	a C_5-C_{10}-cycloalkyl group, which is unsubstituted or substituted by -A, -$(CH_2)_m$-OH, -$(CH_2)_m$-COOH, -$(CH_2)_m$-C(O)NA_2 or by a C_5-C_{10}-cycloalkyl group,
and R means	branched or unbranched C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkinyl, C_5-C_{10}-cycloalkyl, Het or Ar which are optionally substituted by one ore more halogen, C_1-C_6-alkyloxy, branched or unbranched C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkinyl or C_5-C_{10}-cycloalkyl groups or by -C_1-C_6-alkyl-Het, -C_1-C_6-alkyl-Ar, -O-C_1-C_6-alkyl-Het, -O-C_1-C_6-alkyl-Ar, Het or by Ar, wherein
Het means	a monocyclic or bicyclic, 5- up to 10-membered aromatic or non-aromatic ring containing 1 or 2 equal or different hetero-atoms as members of said ring, selected from the group consisting of nitrogen, oxygen and sulfur, which is unsubstituted or substituted by one or more hydroxy or carboxy groups, and wherein
Ar means	a monocyclic or bicyclic 5- up to 10-membered aromatic ring which is unsubstituted or substituted by one or more hydroxy or carboxy groups, and
Z means	$(CH_2)_m$, O, S, NH, NR, N-C(O)-R or NSO_2R,
A means	H or C_1-C_4-alkyl and
l, m and r	are integers from 0 to 3,
n and k	are integers from 1 to 2,
p	is an integer from 0 to 1 and
q	is an integer from 1 to 3,

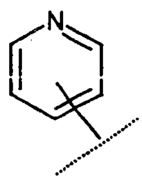
in all its stereoisomeric forms and mixtures thereof in all ratios, including their physiologically tolerable salts.

2. A compound of formula I as claimed in claim 1 wherein n is 1.

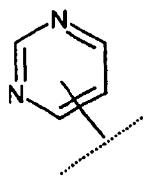
3. A compound of formula I as claimed in claims 1 or 2 wherein R in group X means Het or Ar which are optionally substituted by $-C_1$-C_6-alkyl-Het, $-C_1$-C_6-alkyl-Ar, -O-C_1-C_6-alkyl-Het, -O-C_1-C_6-alkyl-Ar, Het or by Ar.

4. A compound of formula I as claimed in claim 3 wherein R in group X means Het.

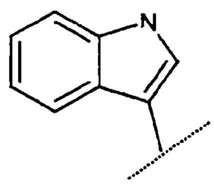
5. A compound of formula I as claimed in claim 4 wherein Het means



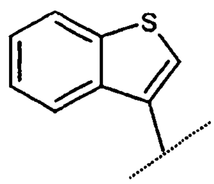
6. A compound of formula I as claimed in claim 4 wherein Het means



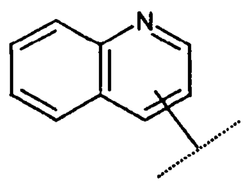
7. A compound of formula I as claimed in claim 4 wherein Het means



8. A compound of formula I as claimed in claim 4 wherein Het means



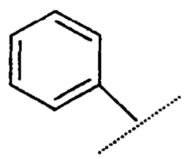
9. A compound of formula I as claimed in claim 4 wherein Het means



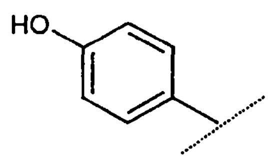
10. A compound of formula I as claimed in claim 3 wherein R in group X means Ar which is optionally substituted by -C_1-C_6-alkyl-Ar, -O-C_1-C_6-alkyl-Ar or by Ar.

11. A compound of formula I as claimed in claim 10 wherein R in group X means Ar.

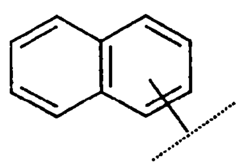
12. A compound of formula I as claimed in claim 11 wherein Ar means



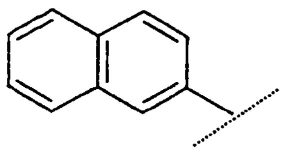
13. A compound of formula I as claimed in claim 11 wherein Ar means



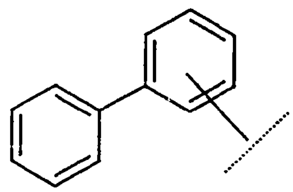
14. A compound of formula I as claimed in claim 11 wherein Ar means



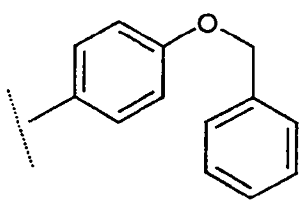
15. A compound of formula I as claimed in claim 11 wherein Ar means



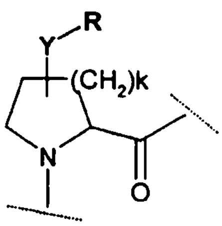
16. A compound of formula I as claimed in claim 10 wherein R in group X means



17. A compound of formula I as claimed in claim 10 wherein R in group X means



18. A compound of formula I as claimed in one or more of claims 1 to 17 wherein X is a group of the following formula



19. A compound of formula I as claimed in claim 18, wherein Y means $-(CH_2)_r-$.

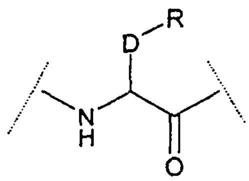
20. A compound of formula I as claimed in claim 19, wherein r is 1.

21. A compound of formula I as claimed in claim 19, wherein r is 0.

22. A compound of formula I as claimed in claims 18 to 21, wherein k is 2.

23. A compound of formula I as claimed in claims 18 to 21, wherein k is 1.

24. A compound of formula I as claimed in one or more of claims 1 to 17 wherein X is a group of the following formula

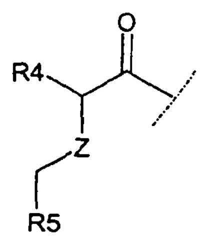


25. A compound of formula I as claimed in claim 24 wherein D means $-(CH_2)_r-$.

26. A compound of formula I as claimed in claim 24, wherein r is 0.

27. A compound of formula I as claimed in claim 24, wherein r is 1.

28. A compound of formula I as claimed in one or more of claims 1 to 27 wherein R1 is a group of the following formula



29. A compound of formula I as claimed in claim 28 wherein Z means $(CH_2)_m$.

30. A compound of formula I as claimed in claim 29 wherein m is 0.

31. A compound of formula I as claimed in claim 29 wherein m is 1.

32. A compound of formula I as claimed in one or more of claims 28 to 31 wherein R5 means $-(CH_2)_l$-COOA.

33. A compound of formula I as claimed in claim 32 wherein A means H.

34. A compound of formula I as claimed in one or more of claims 28 to 31 wherein R5 means $-(CH_2)_l-COONH_2$.

35. A compound of formula I as claimed in one or more of claims 31 to 34 wherein l is 0.

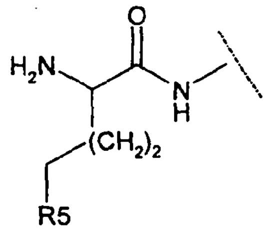
36. A compound of formula I as claimed in one or more of claims 28 to 35 wherein R4 means -NH2

37. A compound of formula I as claimed in one or more of claims 28 to 35 wherein R4 means -A.

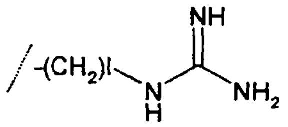
38. A compound of formula I as claimed in claim 37 wherein A means H.

39. A compound of formula I as claimed in one or more of claims 28 to 35 wherein R4 means -NHR1.

40. A compound of formula I as claimed in claim 39 wherein -NHR1 means

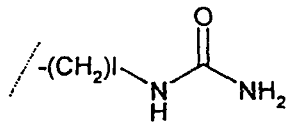


41. A compound of formula I as claimed in claim 40 wherein R5 of -NHR1 means



and l is 0.

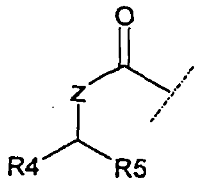
42. A compound of formula I as claimed in claim 40 wherein R5 of -NHR1 means



and l is 0.

43. A compound of formula I as claimed in claim 40 wherein R5 of -NHR1 means $(CH_2)_l$-NH_2 and l is 0.

44. A compound of formula I as claimed in one or more of claims 1 to 27 wherein R1 is a group of the following formula



45. A compound of formula I as claimed in claim 44 wherein Z means $-(CH_2)_m-$.

46. A compound of formula I as claimed in claim 45 wherein m is 1.

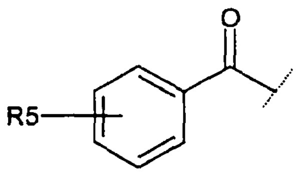
47. A compound of formula I as claimed in one or more of claims 44 to 46 wherein R4 means $-NH_2$.

48. A compound of formula I as claimed in one or more of claims 44 to 47 wherein R5 means $-(CH_2)_l-COOA$.

49. A compound of formula I as claimed in claim 48 wherein l is 0.

50. A compound of formula I as claimed in claims 48 or 49 wherein A means H.

51. A compound of formula I as claimed in one or more of claims 1 to 27 wherein R1 is a group of the following formula



52. A compound of formula I as claimed in claim 51 wherein R5 means $-(CH_2)_l-COOA$.

53. A compound of formula I as claimed in claim 52 wherein l is 0 and A means H.

54. A compound of formula I as claimed in one or more of claims 1 to 53 wherein R2 means A.

55. A compound of formula I as claimed in claim 54 wherein R2 means $-CH_3$.

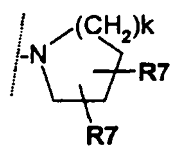
56. A compound of formula I as claimed in one or more of claims 1 to 53 wherein R2 means -E-COOH.

57. A compound of formula I as claimed in claim 56 wherein R2 means $-CH_2$-COOH.

58. A compound of formula I as claimed in one or more of claims 1 to 53 wherein R2 means -E-OH.

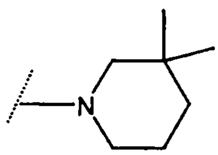
59. A compound of formula I as claimed in claim 58 wherein R2 means $-CH_2$-OH.

60. A compound of formula I as claimed in one or more of claims 1 to 59 wherein R3 is a group of the following formula

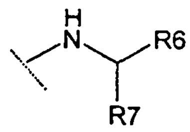


61. A compound of formula I as claimed in claim 60 wherein k is 2.

62. A compound of formula I as claimed in one or more of claims 1 to 59 wherein R3 means



63. A compound of formula I as claimed in one or more of claims 1 to 59 wherein R3 is a group of the following formula



64. A compound of formula I as claimed in claim 63 wherein R7 is a branched C_1-C_{10}-alkyl group.

65. A compound of formula I as claimed in claim 64 wherein R7 means $-CH(CH_3)_2$.

66. A compound of formula I as claimed in claim 64 wherein R7 means $-C(CH_3)_3$.

67. A compound of formula I as claimed in claim 64 wherein R7 means $-CH(CH_3)CH_2-CH_3$.

68. A compound of formula I as claimed in claim 64 wherein R7 means $-CH_2-CH(CH_3)_2$.

69. A compound of formula I as claimed in one or more of claims 63 to 68 wherein R6 means -H.

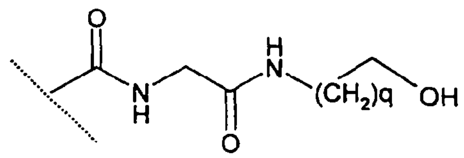
70. A compound of formula I as claimed in one or more of claims 63 to 68 wherein R6 means -COOH.

71. A compound of formula I as claimed in one or more of claims 63 to 68 wherein R6 means $-CONH_2$.

72. A compound of formula I as claimed in one or more of claims 63 to 68 wherein R6 means $-CH_2OH$.

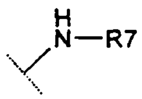
73. A compound of formula I as claimed in one or more of claims 63 to 68 wherein R6 means $-CON(CH_3)_2$.

74. A compound of formula I as claimed in one or more of claims 63 to 68 wherein R6 means



75. A compound of formula I as claimed in claim 74 wherein q is 2.

76. A compound of formula I as claimed in one or more of claims 1 to 59 wherein R3 is a group of the following formula



77. A compound of formula I as claimed in claim 76 wherein R7 means $-CH(CH(CH_3)_2)_2$.

78. A compound of formula I as claimed in claim 76 wherein R7 means $-CH_2C(CH_3)_3$.

79. A pharmaceutical composition containing at least one compound according to one or more of the preceding claims and/or its physiologically tolerable salts.

80. A compound according to one or more of claims 1 to 78 and/or its physiologically tolerable salts for use as a pharmaceutical .

81. A use of a compound according to one or more of claims 1 to 78 and/or the physiologically tolerable salts thereof for preparing a pharmaceutical for the treatment of a disease which is related to an increased or unwanted synthesis of basement membranes.

82. The use as claimed in claim 81 wherein the disease are late complications of diabetes mellitus.

83. The use as claimed in claim 81 wherein the disease is a fibrosis accompanied by an increased synthesis of basement membranes or their components.

84. The use as claimed in claim 83 wherein the disease is a fibrosis of the liver.

85. The use as claimed in claim 81 wherein the disease is atherosclerosis.

86. The use as claimed in claim 81 wherein the disease is cancer.

87. The use as claimed in claim 81 wherein the disease is diabetic retinopathia.

88. The use as claimed in claim 81 wherein the disease is fibroplasia retrolentalis.

89. The use as claimed in claim 81 wherein the disease is related to a strong inflammatory component.

90. The use as claimed in claim 89 wherein the disease is rheumatoic arthritis.

91. The use as claimed in claim 89 wherein the disease is osteoarthritis.

92. The use as claimed in claim 89 wherein the disease is vasculitis.

93. The use as claimed in claim 81 wherein the disease is related to haemangiomes.

94. The use as claimed in claim 81 wherein the disease is psoriasis.

95. A method for identifying a compound that inhibits the interaction of laminin and nidogen wherein the compound according to claims 1 to 79 is used as a competetive inhibitor.

96. The method of claim 95 further comprising formulating the compound identified in a pharmaceutical acceptable form.

97. A method for producing a pharmaceutical composition comprising the method according to claim 95 and furthermore mixing the compound identified and/or its physiologically tolerable salt with a pharmaceutical acceptable carrier.

Figure 1:

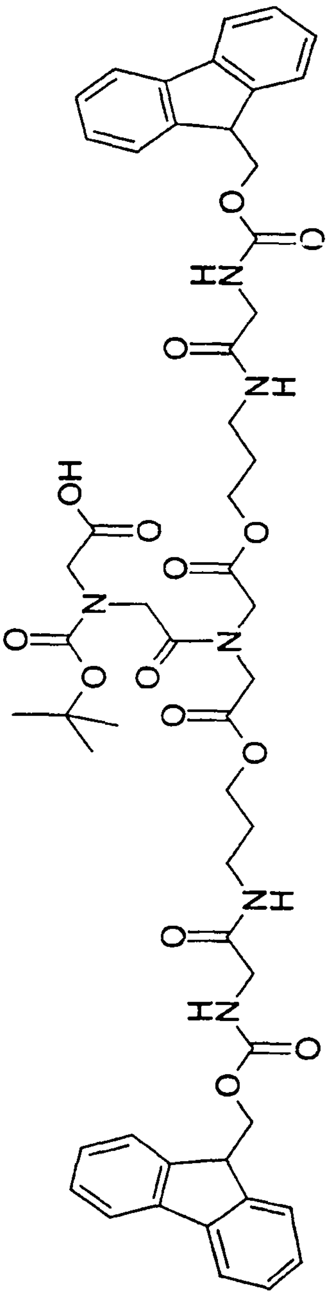
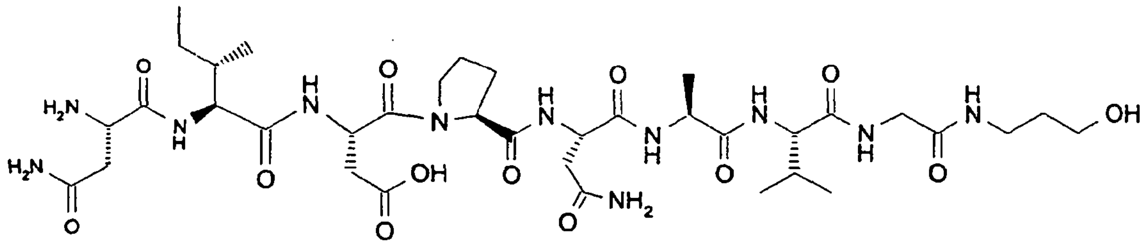
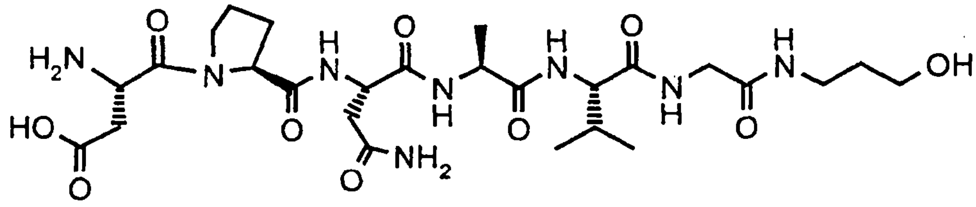


Figure 2: Compounds 1 to 4

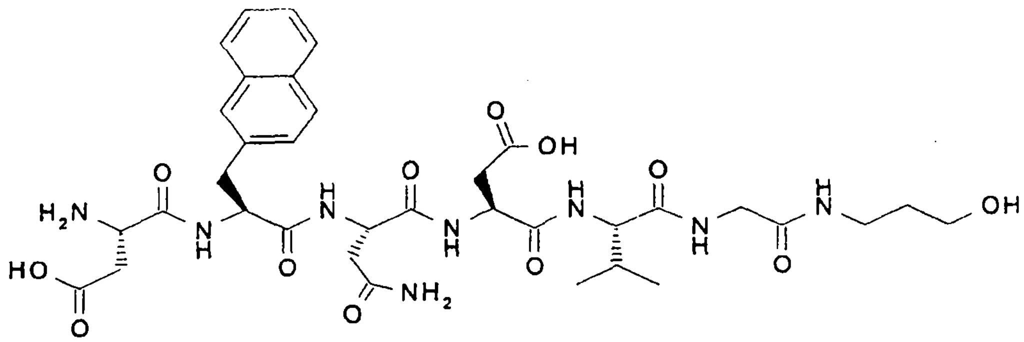
Compound 1 (Control)



Compound 2 (Control)



Compound 3



Compound 4

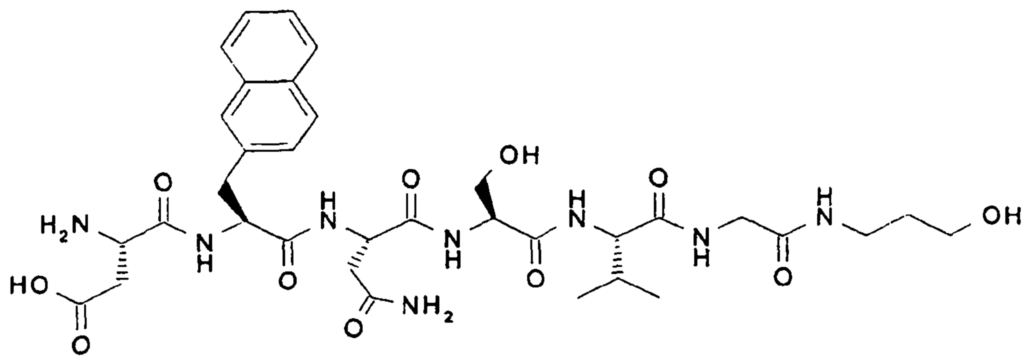
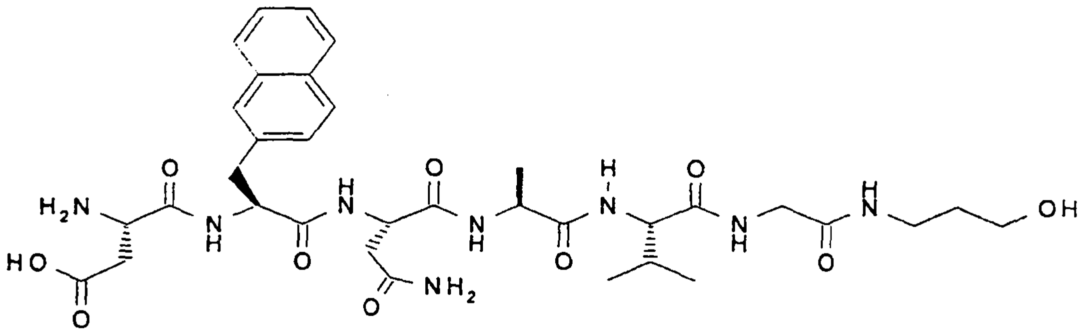
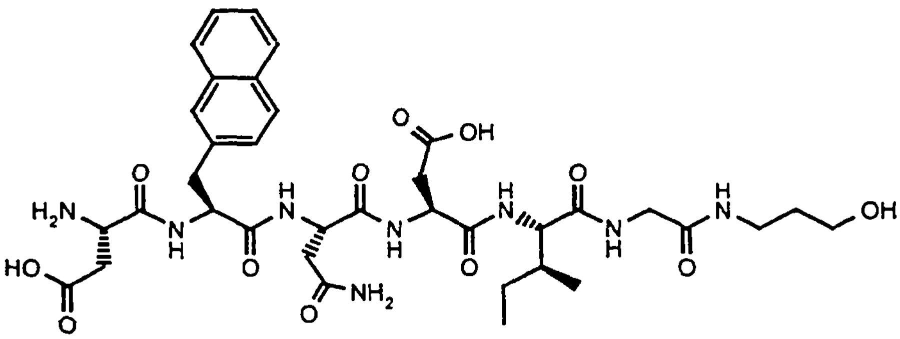


Figure 3: Compounds 5 to 8

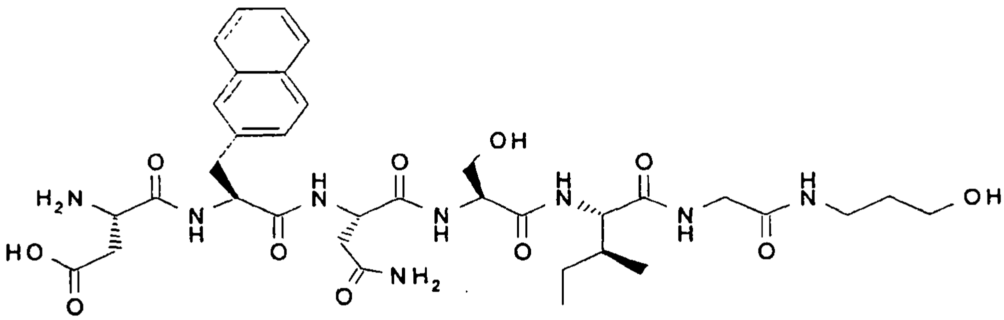
Compound 5



Compound 6



Compound 7



Compound 8

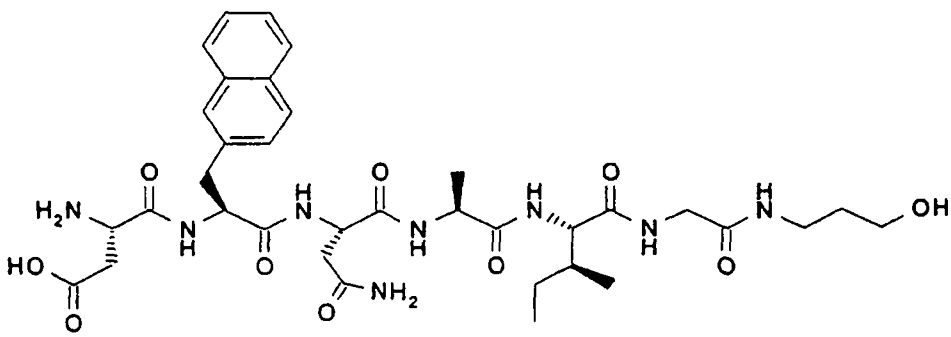
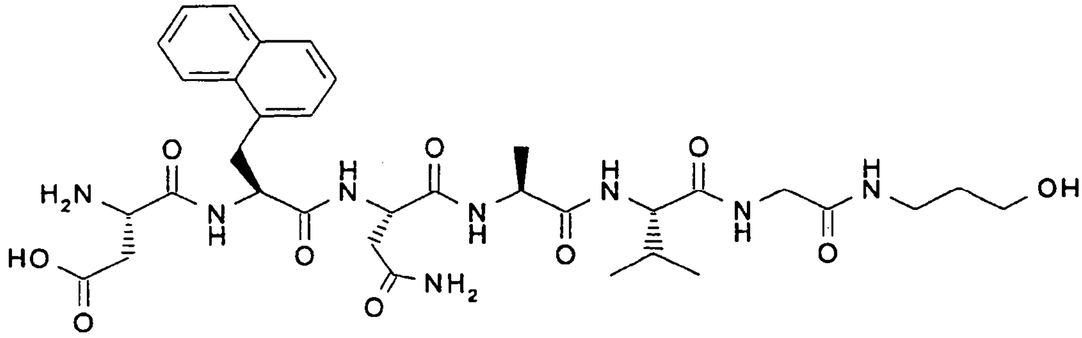
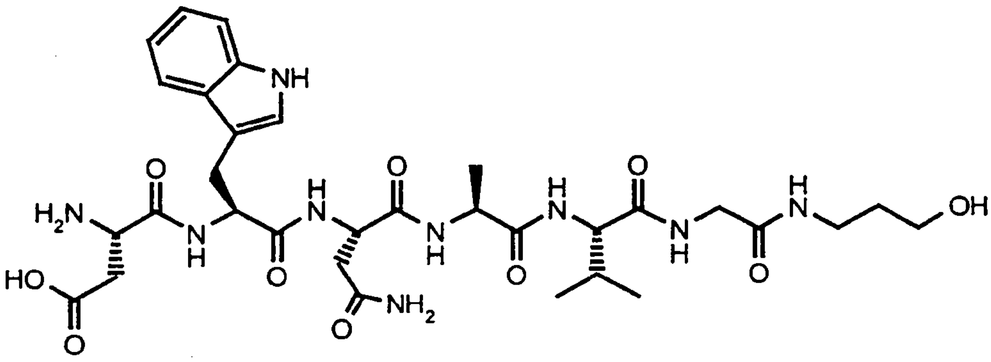


Figure 4: Compounds 9 to 12

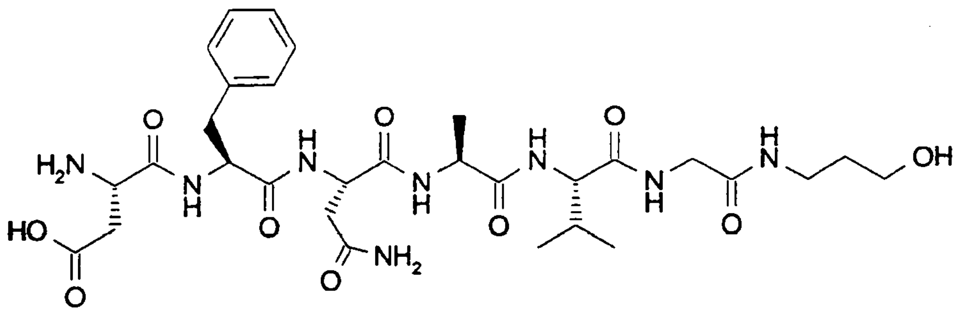
Compound 9



Compound 10



Compound 11



Compound 12

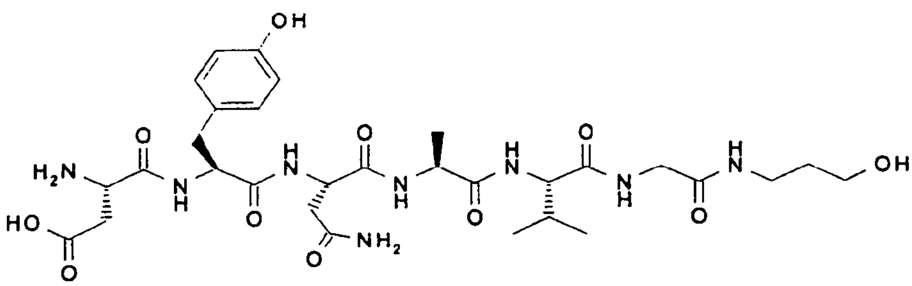
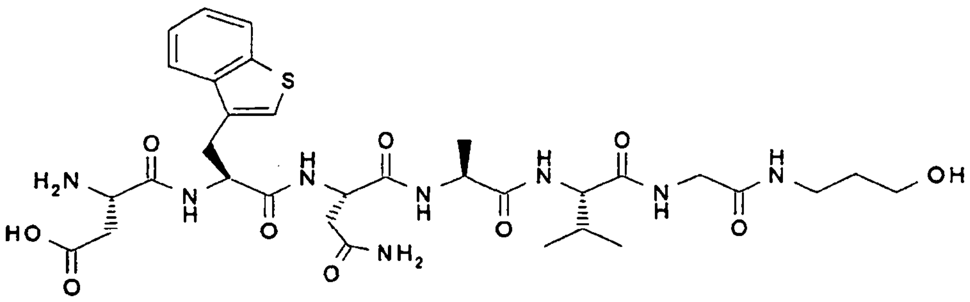
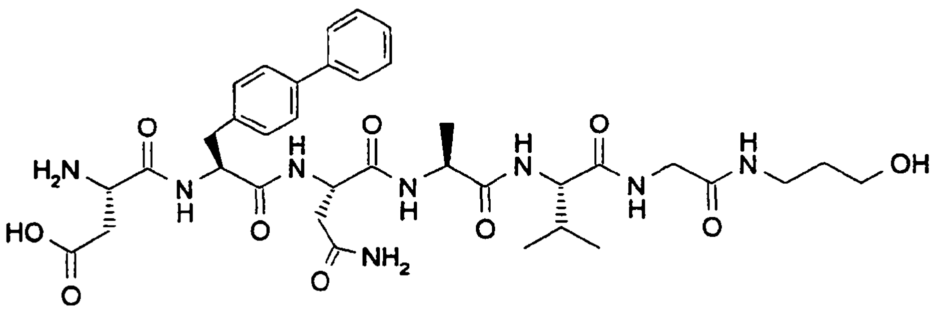


Figure 5: Compounds 13 to 16

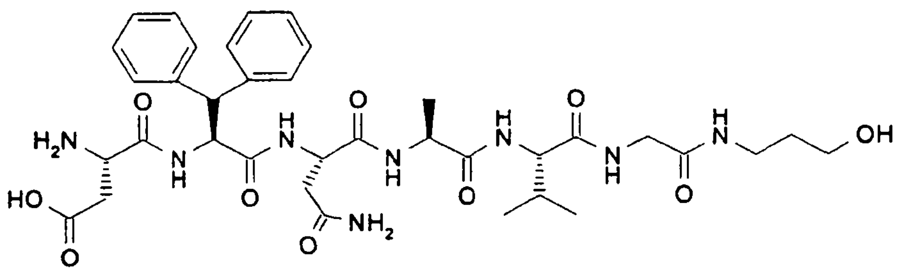
Compound 13



Compound 14



Compound 15



Compound 16

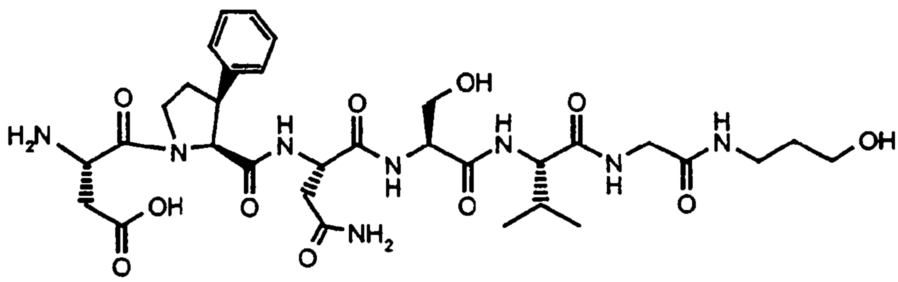
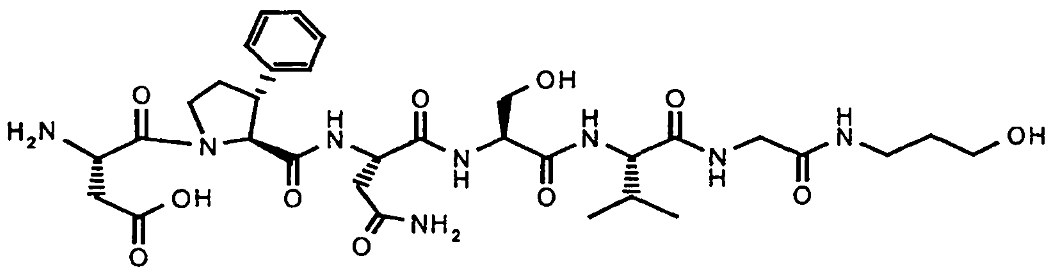
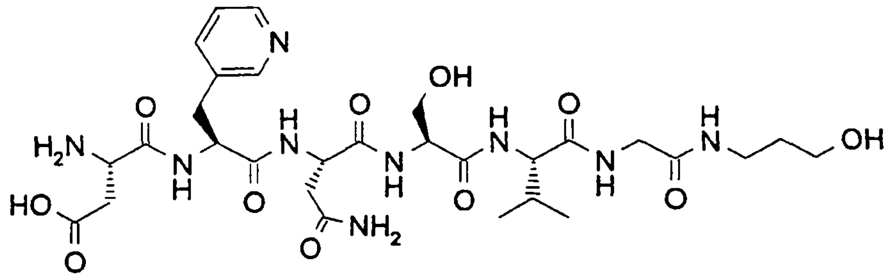


Figure 6: Compounds 17 to 20

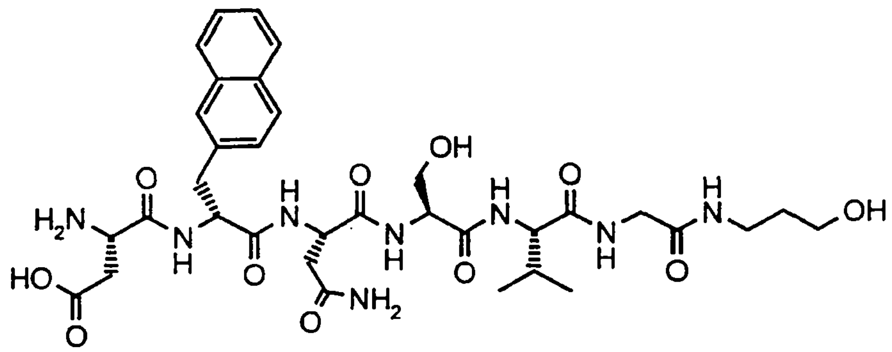
Compound 17



Compound 18



Compound 19



Compound 20

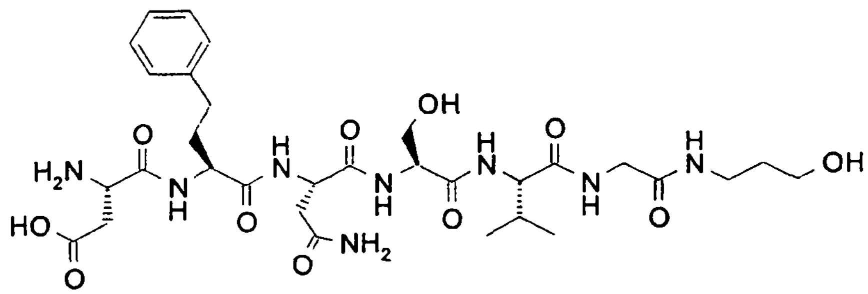
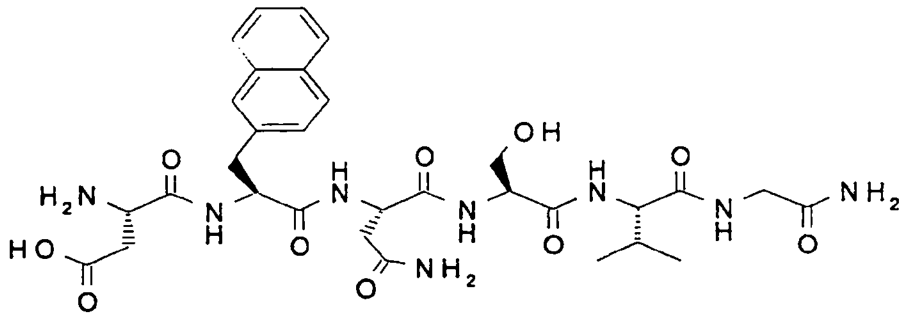
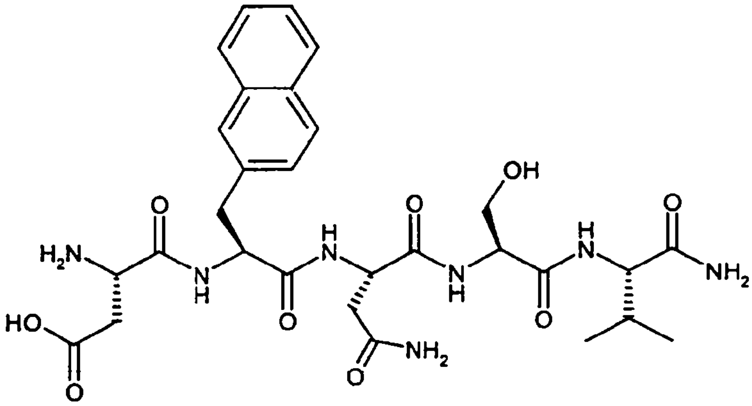


Figure 7: Compounds 21 to 24

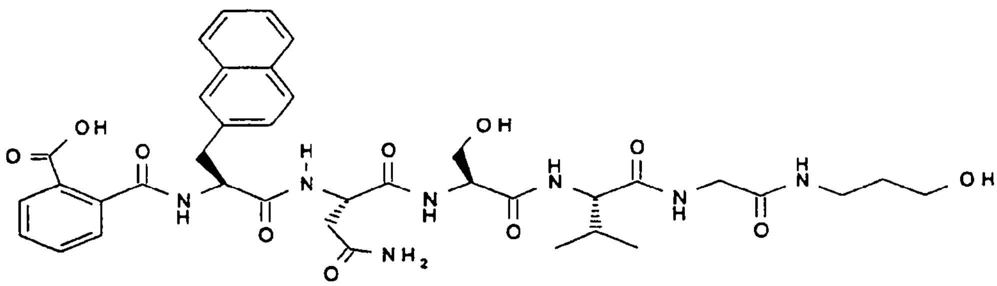
Compound 21



Compound 22



Compound 23



Compound 24

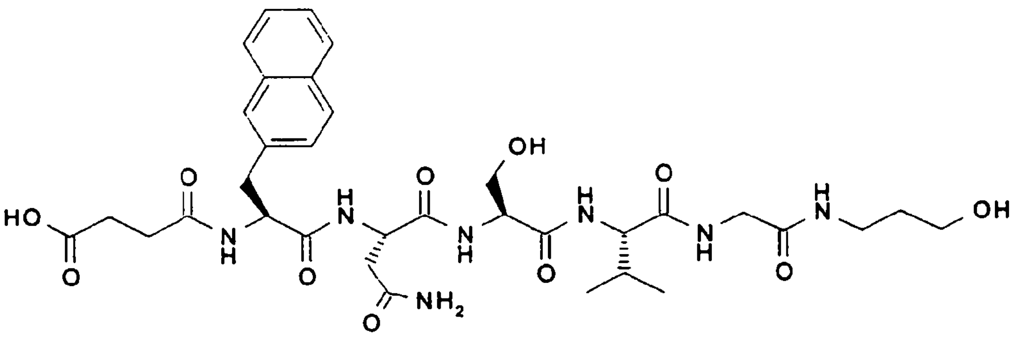
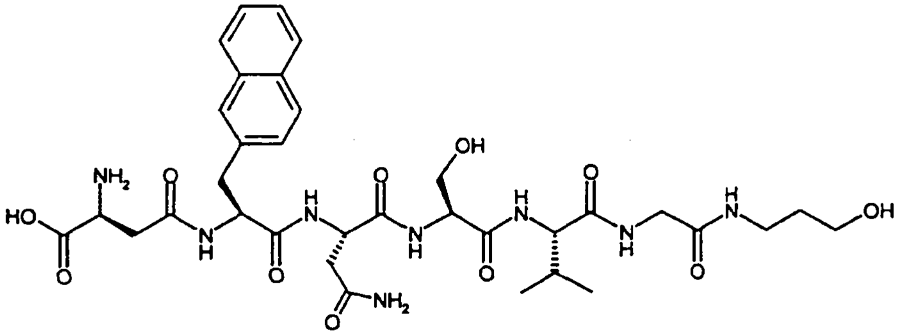
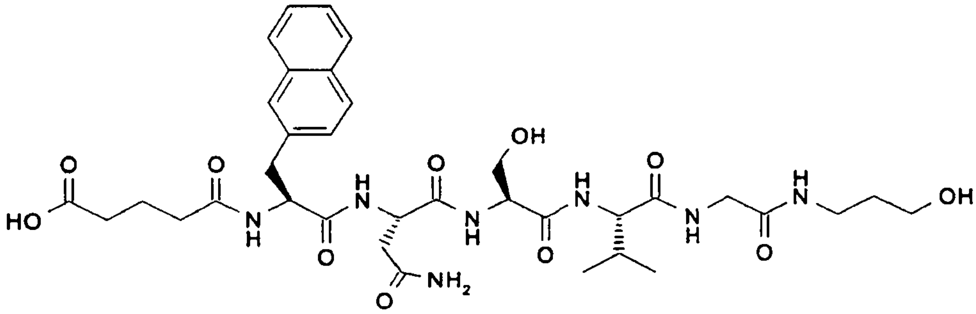


Figure 8: Compounds 25 to 28

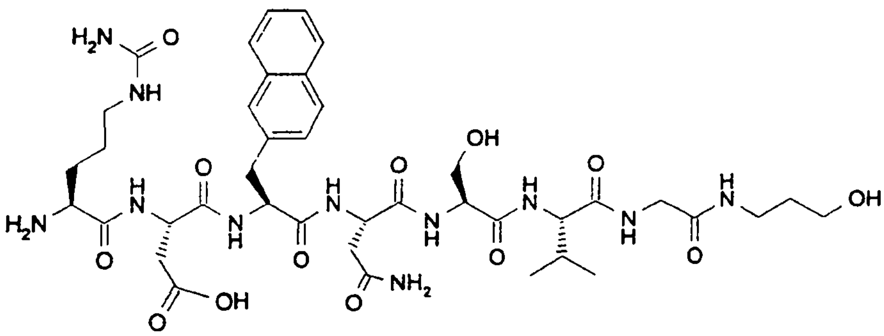
Compound 25



Compound 26



Compound 27



Compound 28

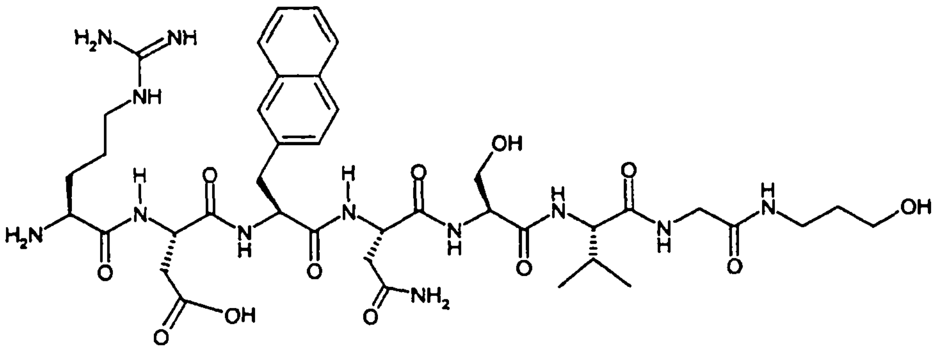
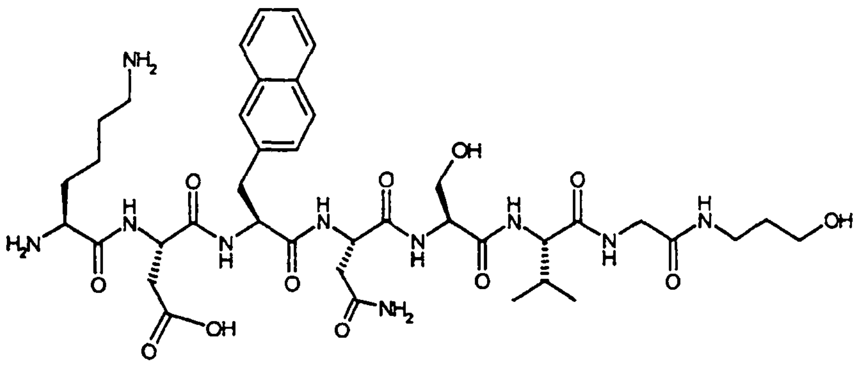
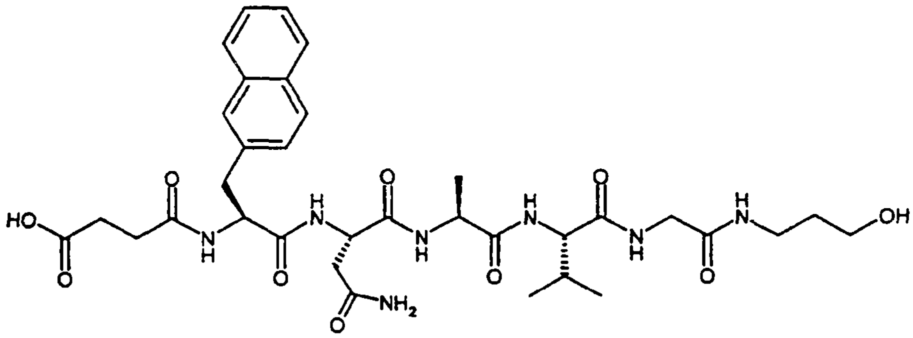


Figure 9: Compounds 29 to 32

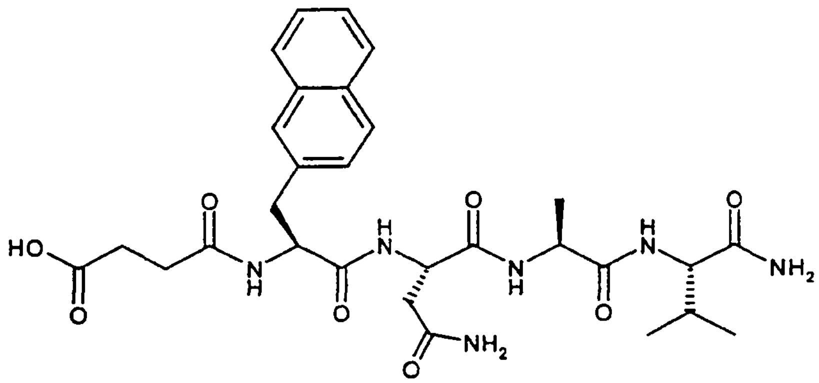
Compound 29



Compound 30



Compound 31



Compound 32

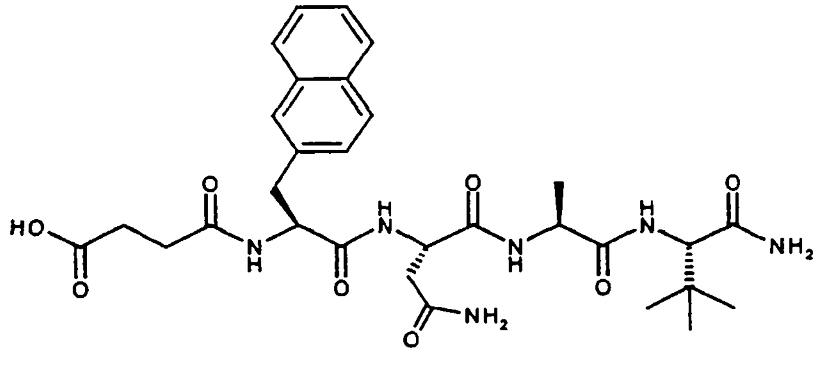
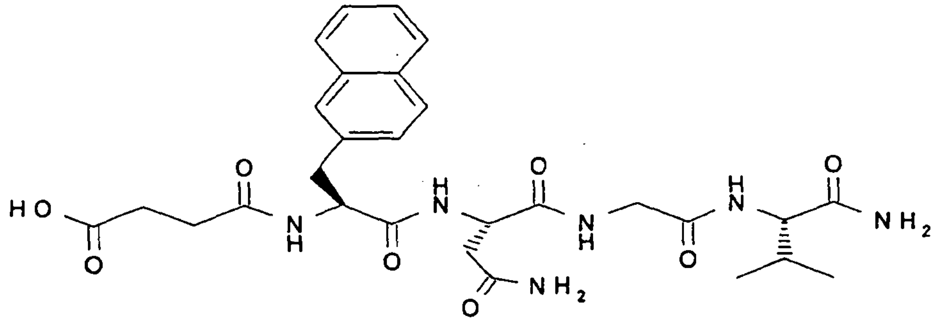
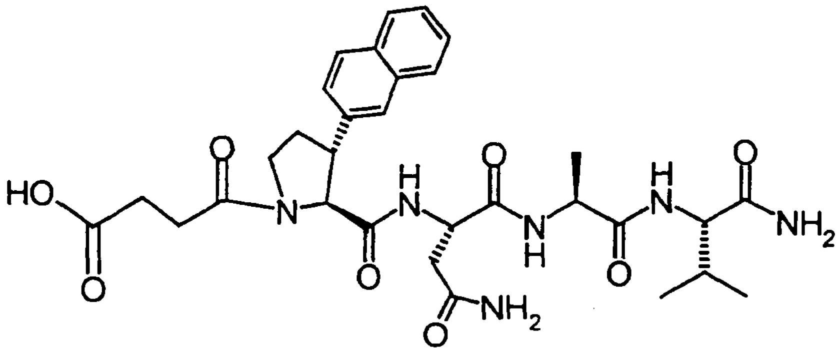


Figure 10: Compounds 33 to 36

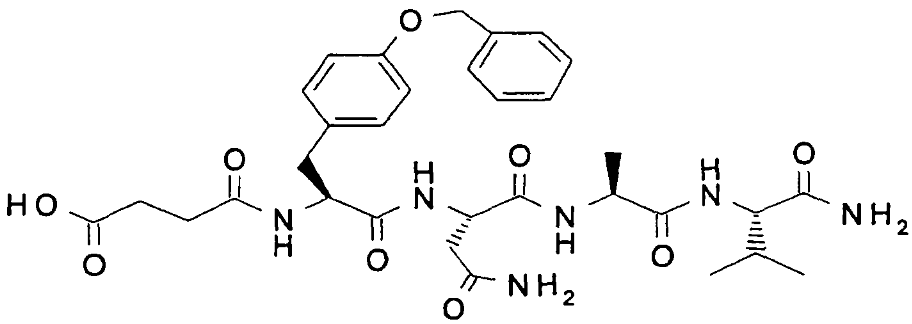
Compound 33



Compound 34



Compound 35



Compound 36

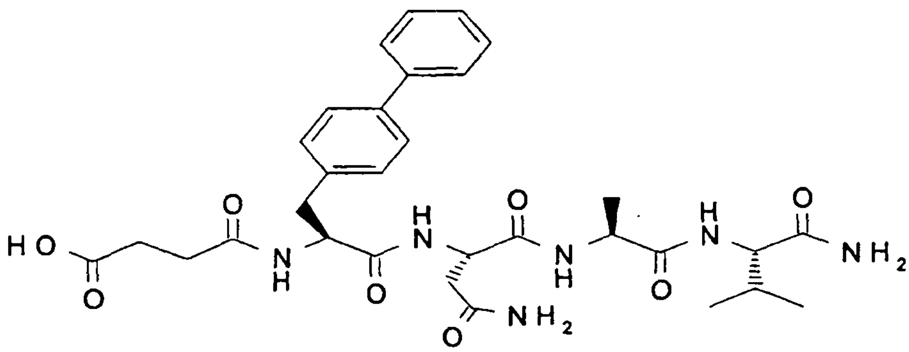
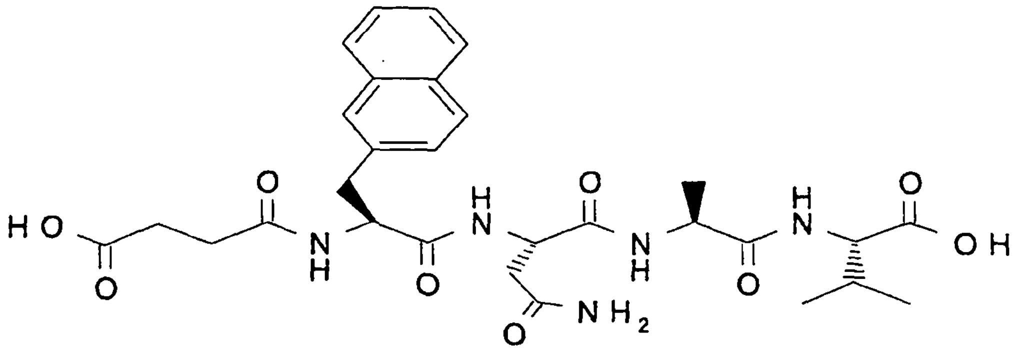
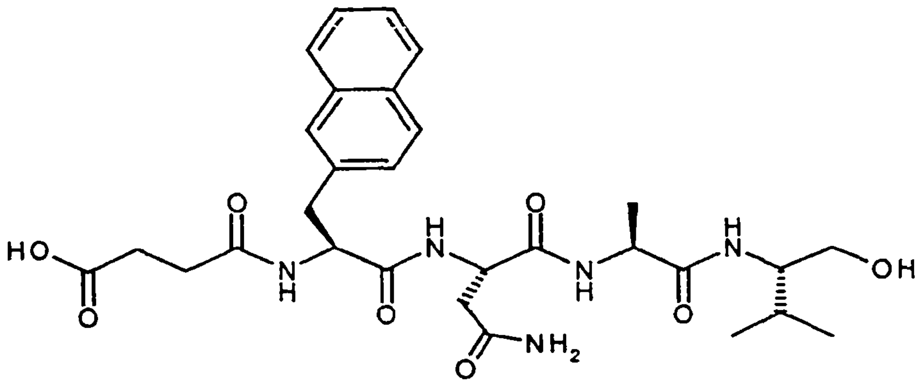


Figure 11: Compounds 37 to 40

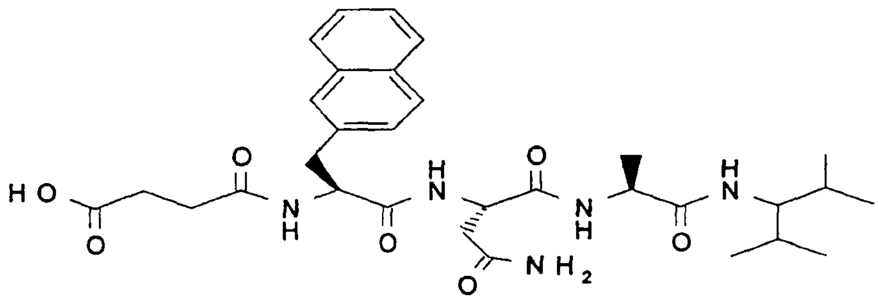
Compound 37



Compound 38



Compound 39



Compound 40

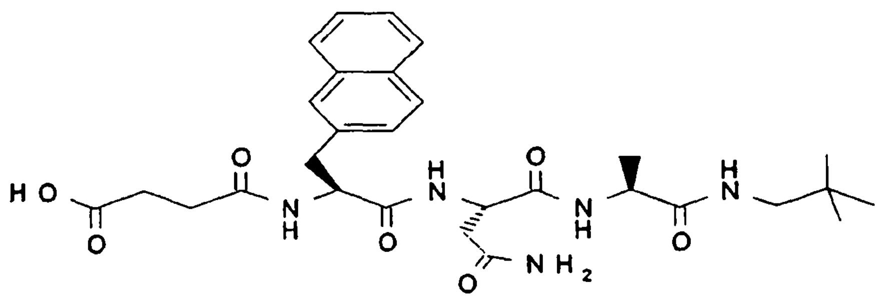
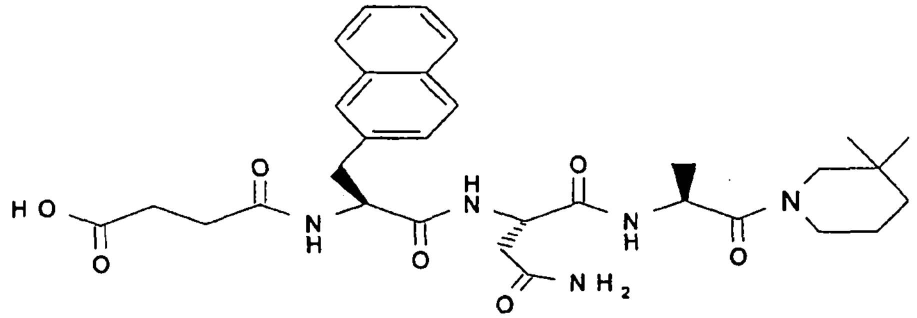
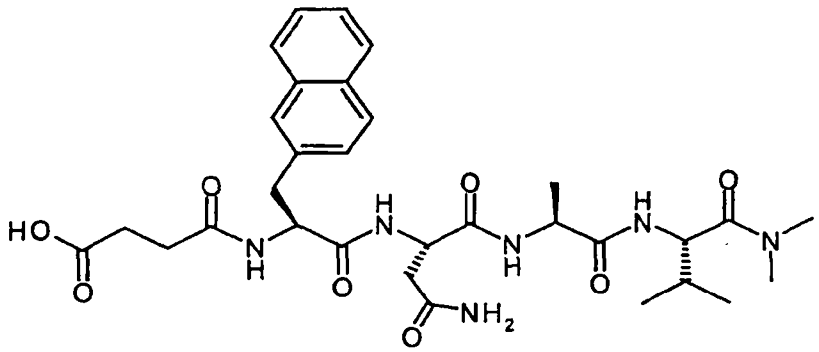


Figure 12: Compounds 41 to 43

Compound 41



Compound 42



Compound 43