FRAGMENTS OF VIRUS PROTEIN 2 OR 3 OF POLYOMA VIRUS AS VEHICLES FOR ACTIVE SUBSTANCES

The invention relates to a synthetic biologically active molecule and to a method for the preparation thereof.

Chen, X.S., Stehle, T. and Harrison, S.C. (1998): Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry, The EMBO Journal, Vol. 17, No. 12, pp. 3233-3240 describe the interactions responsible for anchoring virus proteins VP2 and VP3 of polyoma virus to virus protein 1. According to said publication, the anchoring takes place in the region of the C-terminal end of VP2 or VP3.

US 4,950,599 discloses that the polyoma virus is suitable for transporting active substances into cells. Furthermore, DE 196 18 797 A1 discloses that a capsomer derived from polyoma virus is suited to transporting molecular material into cells.

EP 0 259 149 A2 discloses using the rotavirus internal capsid protein VP6 as immunological carrier molecule and as a vaccine for stimulating the immune response to rotavirus infections. In this connection, immunogenic peptides are bound to VP6 via peptide-peptide interactions which are not defined in any detail. VP6 here does not form a structural capsomer but on the contrary displays a distinctive structural polymorphism. VP6 is present as a monomer or in oligomeric form. Although oligomeric VP6 can form particles, these particles are not capsids or capsomers but unstructured carrier proteins.

Redmond, M.J. et al. (1991): Rotavirus particles function as immunological carriers for the delivery of peptides from infectious agents and endogenous proteins, Mol. Immunol. 28, 269-278 describe the use of rotavirus internal capsid protein VP6 as transport particle. In this connection, VP6 is bound to immunogenic peptides or proteins via a binding protein derived from the peptide sequence of the rotaviral protein VP4. An antigen coupled to the peptide sequence derived from VP4 is located on the outside of the transport particle and therefore is not protected from degradation.

GB 22 57 431 A describes the use of a chimeric protein which is derived from the envelope protein of phage MS-2. This protein can form capsids. Antigenic peptides or the like coupled thereto are bound to the outside of the capsid. Spontaneous assembling of the chimeric protein during expression in E. coli carries a high risk of contamination by bacterial DNA or proteins.

DE 43 35 025 A1 discloses an endosomolytically active virus-like particle which has been modified with membrane-active peptides on its outer surface. The preparation of said particle is complicated.

It is the object of the invention to remove the disadvantages according to the prior art. In particular it is intended to provide a simple possibility of specifically associating active substances with polyoma virus VP1.

This object is achieved by the features of claims 1, 9 and 19. Expedient embodiments result from the features of claims 2 to 8, 10 to 18 and 20 to 24.

The description makes use of the following definitions:

Derived amino acid sequence: amino acid sequence which is unchanged compared with the amino acid sequence from which it is derived, or which differs therefrom by amino acid exchanges, insertions or deletions.

C-terminal end: region or area at the C terminals.

Synthetic molecule: artificially prepared molecule.

Coupling or attaching: covalent or noncovalent binding. Noncovalent binding may be carried out, for example, via a chelate bond.

Genetic engineering: technique which includes methods for introducing defined nucleic acids into cells.

In accordance with the invention there is provided a synthetic biologically active molecule in accordance with the features of claim 1.

The proposed synthetic biologically active molecule makes it possible in a simple manner to specifically associate active substances with polyoma virus VP1. This leads to the formation of a structured capsomer. By using said capsomer it is possible to prepare in a simple manner capsids as universal carriers for active substances.

Advantageously, the amino acid sequence (A1) comprises from 10 to 55, preferably from 28 to 38, amino acids. Limitation to a relatively short amino acid sequence reduces the cost of and simplifies the preparation of the synthetic biologically active molecule.

Expediently, the amino acid sequence at least in some sections corresponds to the VP2 sequence from amino acid position 250 to 319, preferably from amino acid position 260 to 300 and particularly preferably from amino acid position 287 to 297. Said amino acid sequence ensures secure anchoring to VP1.

In the synthetic biologically active molecule, the amino acid sequence (A1) preferably has amino acids in the sequence below:

Trp Met Leu Pro Leu Ile Leu Gly Leu Tyr Gly
1 5 10

The active substance is preferably bound to the amino acid sequence (A1) via a linker. This linker may be composed of at least one amino acid, a peptide, protein, lipid or the like. The active substance may be selected from the following group: nucleic acid, oligonucleotide, protein, peptide, peptidic substance, PNA, modifications of said substances and low-molecular weight pharmaceutically active substances. Particularly suitable are those active substances which couple to the amino acid sequence via one of the reactive groups mentioned below.

The synthetic biologically active molecule may be present coupled to an amino acid sequence derived from polyoma virus VP1 and/or may be an ingredient of a medicament.

In further accordance with the invention, a method for preparing the synthetic biologically active molecule of the invention is provided for, which method has the following steps:

a) providing an amino acid sequence (A1) derived from the C-terminal end of virus protein 2 (VP2) or 3 (VP3) of polyoma virus, with the amino acid sequence (A1) having a coupling agent and

b) binding the active substance to the amino acid sequence (A1) via the coupling agent.

The coupling agent may have as amino acid glycine, cysteine or glycine bound via lysine. The coupling agent is advantageously a further, preferably synthetically prepared amino acid sequence (A2) bound to the N- or C-terminal end of amino acid sequence (A1).

The synthetic biologically active molecule may be prepared, at least partly, by genetic engineering. In this connection, the amino acid sequence (A1), the further amino acid sequence (A2) and the active substance may be prepared completely or partially by genetic engineering. The further amino acid sequence (A2) expediently has glycines and/or amino acids with functional side groups, it being possible for the functional side groups to be selected from the following group: amino, sulfhydryl, carboxyl, hydroxyl, guanidinium, phenyl, indole and imidazole radical.

The coupling agent may be a reactive group bound to the C- or N-terminal end of amino acid sequence (A1) via an amino acid, preferably glycine, cysteine, or glycine bound via lysine. This may have one of the following components: amino acid with monobromoacetyl radical, amino acid with monochloroacetyl radical, amino acid with 3-nitro-2-pyridinesulfenyl radical (Npys). The proposed reactive groups can be used universally. They are suitable for coupling to a multiplicity of active substances.

It has proved particularly advantageous to bind the active substance to amino acid sequence (A1) or to the further amino acid sequence (A2) via a thioether or disulfide bridge. In practice, this kind of bond can readily be prepared. Of course, the use of other reactive groups is also conceivable. Suitable groups are, for example, N-succinimidyl bromoacetate or N-succinimidyl 3-(2-pyridylthio)propionate (SPDP).

The active substance may be bound to amino acid sequence (A1) or the further amino acid sequence (A2) via a linker. The linker may be composed of at least one amino acid, a peptide, protein, lipid, or the like.

In further accordance with the invention, a method for preparing the synthetic biologically active molecule of the invention is provided for, which method has the following steps:

aa) synthesizing an amino acid sequence (A1) derived from the C-terminal end of virus protein 2 (VP2) or 3 (VP3) of polyoma virus and

bb) coupling and synthesizing an active substance, namely a peptide, to the amino acid sequence (A1),

wherein the steps under aa and under bb are carried out by means of peptide synthesis or by means of genetic engineering methods.

In step bb) the amino acid sequence (A1) is extended by the active substance. The elongation and the attaching of the active substance are carried out by repeatedly attaching amino acid residues. This method can be carried out particularly easily.

Further advantageous embodiments relating to both aforementioned methods can be found in the subclaims.

Examples:

A. Synthesis and purification of the peptides:

Peptides are synthesized by simultaneous multiple peptides synthesis (Schnorrenberg, G. and Gerhardt, H. (1989) Tetrahedron 45, 7759) in a peptide synthesizer (type: PSSM-8 from SHIMADZU, Japan) using the 9-fluorenylmethoxycarbonyl (Fmoc)/tert. butyl (But) strategy according to Sheppard (Atherton, E. and Sheppard, R.C. (1989) "Solid phase peptide synthesis - a practical approach" IRL Press, Oxford). The coupling reactions are carried out with in each case 6 equivalents of Fmoc-protected amino acid/1-hydroxybenzotriazole (HOBt)/12 equivalents of n-methylmorpholine using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) on a polymeric carrier resin (type: Tentagel S Trityl resin, RAPP Polymere, Tübingen, Germany) with a load of 2 mmol/g of resin. The peptides contain a C-terminal COOH group.

The following protective groups are used in the synthesis: Cys (Trt), Arg (Pbf), Ser (But), Thr (But), Asp (OBut), Glu, (OBut), Asn (Trt), Gln (Trt), Lys (Boc), His (Trt), Trp (Boc), where Trt: trityl, But: t-butyl, OBut: t-butyl ester, Boc: t-butyloxycarbonyl and Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl.

All protective groups are removed using trifluoroacetic acid (TFA)/thioanisole/thiocresol (95:2.5:2.5) at room temperature over 3 h with the addition of 3% triisopropylsilane and subsequent addition of 10% trimethylchlorosilane for 1 h. After lyophilization,

the peptides are present in the form of their trifluoroacetic acid salts.

The peptides are purified by means of preparative HPLC on a Bischoff Polyencap 300 separating column, 10 µm, 250x16 mm, using a gradient of from 0.05% trifluoroacetic acid in water (eluent A) to 0.05% trifluoroacetic acid in 80% acetonitrile/water (eluent B).

Alternatively, a Vydac separating column of type 218 TP 101522 (10-15 µm, 250x22 mm) with a gradient of 43-73% eluent B in 30 min at a flow rate of 15 ml/min was used.

By means of peptide synthesis, the following amino acid sequence is synthesized, for example:

Trp Met Leu Pro Leu Ile Leu Gly Leu Tyr Gly
1 5 10

In this sequence, the reactive group is bound to the N-terminal end of the amino acid sequence via an amino acid, preferably via glycine. The reactive group may be composed of monochloroacetylglycine. Alternatively, it is also possible to attach a monobromoacetyl radical.

Coupling of monochloroacetylglycine in solid phase synthesis:

Equal equivalents of monochloroacetylglycine and 1-hydroxybenzotriazole (HOBt) are dissolved in dimethylformamide (DMF), mixed with an equal equivalent of N,N'-diisopropylcarbodiimide (DIC) and added to the peptide resin. Compared with the loaded peptide resin, monochloroacetylglycine is present in excess. The reaction is carried out with occasional stirring and should last for at least 1 h.

The reactive group facilitates covalent binding of the biologically active molecule prepared in this way to an active substance, for example a peptide, which has a free SH group. The reaction of the SH group with the chlorine atom of the monochloroacetyl group results in the formation of a stable thioether compound according to the following equation:

protein-SH + Cl-CH_2-CO-Gly peptide $\xrightarrow{-HCL}$ protein-S-CH_2-CO-Gly peptide

Conjugate formation between a monochloroacetyl-modified anchor peptide and a peptide having a terminal cysteine:

A monochloroacetyl-modified anchor peptide is used in excess of the peptide to be conjugated. The reaction is carried out in 0.1 M $NaHCO_3$ between [sic] pH 7-8 at room temperature. In the case of poor solubility of the peptide or the anchor in aqueous solution, the conjugate formation is carried out in 4 M guanidine hydrochloride, pH 8.0 (Lindner, W. and Robey, F.A. (1987) Int. J. Pept. Protein Res. 30, 794-800). Alternatively, the proportion of organic solvent, for example DMSO, in the reaction mixture can be increased. In order to avoid unwanted by-products, water-soluble phosphines may be added as reducing agent.

Optionally it is also possible to carry out the conjugation reaction under the following conditions. The monochloroacetylated anchor peptide and a peptide having a terminal SH group are incubated at room temperature in 1-methyl-2-pyrrolidone in the presence of about 10-fold excess of diisopropylethylamine and an approx. 5-fold excess of tributylphosphine. After the reaction, H_2O is subsequently added and the product is precipitated by the addition of ether and purified by

gel filtration (Defoort, J.P., Nardelli, B., Huang, W. and Tam, J.P. (1992) Int. J. Protein Res. 40, 214-221).

Optionally the conjugation reaction may also be carried out as follows. The peptide containing the SH group is dissolved in 0.2 M phosphate buffer, 10 mM EDTA, pH 7.4. To this mixture, the monochloroacetyl-modified anchor peptide dissolved in dimethylformamide is added. After the reaction, purification is carried out by gel filtration or RP-HPLC (Zhang, L. and Tam, J.P. (1997) J. Am. Chem. Soc. 119, 2363-2370).

Isolated VP1 pentamers are prepared by expressing VP1 as a recombinant protein with an N-terminal 6 x histidine affinity tag (=His tag) in E. coli. The protein is purified via Ni-NTA affinity chromatography. The His tag is removed by treatment with a factor Xa. The protein is analyzed in an SDS-PAGE gel with subsequent Coomassie staining.

This starting material (VP1 protein in 20 mM Hepes, pH 7.3, 1 mM EDTA, 200 mN NaCl, 5% glycerol) is concentrated in a centricon 100 (Amincon [sic]) and separated via FPLC gel filtration (Superdex 200) with an elution buffer (50 mM Tris, 0.15 M NaCl, 5 mM EDTA, pH 8.5) into the high-molecular weight capsid fraction and the pentamer subunits (molecular weight: about 225 kD). Both fractions are concentrated in centricon 100. Iodoacetamide (SIGMA) is added to the pentamer-containing solution in a 10-fold molar excess, in order to block potentially reactive SH groups. The reaction is carried out at room temperature for 2 hours. The modified pentamer fraction is separated from excess iodoacetamide via gel filtration. VP1-specific monoclonal antibodies are adsorbed with the aid of VP1-specific antibodies and an affinity matrix (protein A support from BIO-RAD). The antibody-coated matrix is

used to precipitate the purified pentamer fraction. In a further incubation step, the anchor sequence is added to the pentamer matrix. The samples are analyzed in an SDS polyacrylamide gel (12.5%).

Conjugate formation between an Npys-modified anchor peptide and a peptide having a terminal cysteine:

In addition to the reaction between a monochloro- or monobromoacetylated anchor peptide and a peptide having a terminal cysteine with formation of a thioether, the conjugate between anchor peptide and peptide sequence may optionally also be formed via the 3-nitro-2-pyridinesulfenyl (=Npys) group on a terminal cysteine of the anchor and an SH group of the peptide to be coupled. To this end, an Npys-modified cysteine instead of a monochloroacetylated glycine is coupled N-terminally to the anchor sequence. Said Npys-modified cysteine is an "activated disulfide" which is capable of reacting with thiols such as, for example, cysteines, to form an unsymmetric disulfide. This results in the removal of 3-nitro-2-thiopyridone whose UV maximum at 329 nm permits studying the kinetics of the reaction between the two compounds by spectrometry.

The following conditions are chosen for the reaction (Albericio, F., Andreu, D., Giralt, E., Navalpotro, C., Pedroso, E., Ponsati, B. and Ruiz-Gayo, M. (1989) Int. J. Peptide Res. 34, 124-128). The Npys-modified peptide is added to the peptide to be coupled having a terminal SH group and dissolved in 0.1 M sodium acetate, 0.1 M sodium chloride, pH 4.5, and the pH is then adjusted to 5.0 followed by incubation with stirring for at least 12 h. The pH is then adjusted to 7.0 by adding 1 N NaOH followed by another incubation for 3 h. After the reaction, the mixture is dialyzed against 10 mN $NaHCO_3$.

The optimal pH range of the reaction is between 4.5 and 7.0. These conditions ensure minimization of unwanted side reactions such as, for example, formation of symmetric disulfides between the peptide molecules to be coupled or removal of the Npys group. The Npys-modified peptide should be present in the reaction in excess over the peptide to be conjugated (Albericio, F., Andreu, D., Giralt, E., Navalpotro, C., Pedroso, E., Ponsati, B. and Ruiz-Gayo, M. (1989) Int. J. Peptide Res. 34, 124-128).

Examples of the invention are illustrated in the following sequence listings:

Sequence listing 1 depicts an amino acid sequence derived from polyoma virus VP2, position 287-297. It serves in the synthetic biologically active molecule as anchor for anchoring the active substance to VP1.

Sequence listing 2 depicts a first exemplary embodiment of a synthetic biologically active molecule. The HIV-1-derived peptide sequence corresponds to positions 1-21; the attached amino acid sequence acting as anchor occupies positions 22-33. It is derived from polyoma virus VP2.

Sequence listing 3 depicts another example of an amino acid sequence suitable as anchor.

Sequence listing 4 depicts the polyoma virus VP2 sequence. This shows the sequences between positions 250 and 300, which are suitable as anchors.

Sequence listings 5 and 6 show further synthetic biologically active molecules. They may be coupled with polyoma virus VP1 and then, for treatment of an HIV infection, be introduced into the infected cells.

Patent claims

1. A synthetic biologically active molecule composed of an active substance and an amino acid sequence (Al) derived from the C-terminal end of virus protein 2 (VP2) or 3 (VP3) of polyoma virus, wherein the active substance is bound to the amino acid sequence (Al) so that the active substance can be associated with the virus protein 1 (VP1) of the polyoma virus by means of the amino acid sequence (Al) with the formation of a structured capsomer, and wherein the synthetic biologically active molecule is not VP2 or VP3.

2. A synthetic biologically active molecule as claimed in claim 1, wherein the amino acid sequence (Al) comprises from 10 to 55, preferably from 28 to 38, amino acids.

3. A synthetic biologically active molecule as claimed in any of the preceding claims, wherein the amino acid sequence (Al) at least in some sections corresponds to the VP2 sequence from amino acid position 250 to 319, preferably from amino acid position 260 to 300 and particularly preferably from amino acid position 287 to 297.

4. A synthetic biologically active molecule as claimed in any of the preceding claims, wherein the amino acid sequence (Al) has amino acids in the sequence below:

Trp Met Leu Pro Leu Ile Leu Gly Leu Tyr Gly
1 5 10

5. A synthetic biologically active molecule as claimed in any of the preceding claims, wherein the active substance is bound to the amino acid sequence (Al) via a linker.

6. A synthetic biologically active molecule as claimed in any of the preceding claims, wherein the active substance is selected from the following group: nucleic acid, oligonucleotide, protein, peptide, peptidic substance, PNA, modifications of said substances and

low-molecular-weight pharmaceutically active substances.

7. A synthetic biologically active molecule as claimed in any of the preceding claims, coupled to an amino acid sequence derived from polyoma virus VP1.

8. A medicament having a synthetic biologically active molecule as claimed in any of the preceding claims.

9. A method for preparing the synthetic biologically active molecule as claimed in any of the preceding claims, having the following steps:

a) providing an amino acid sequence (A1) derived from the C-terminal end of virus protein 2 (VP2) or 3 (VP3) of polyoma virus, with the amino acid sequence (A1) having a coupling agent and

b) binding the active substance to the amino acid sequence (A1) via the coupling agent.

10. The method as claimed in claim 9, wherein the amino acid in the means for coupling is glycine, cysteine or glycine bound via lysine.

11. The method as claimed in claim 9 or 10, wherein the means for coupling is a further, preferably synthetically prepared amino acid sequence (A2) bound to the N- or C-terminal end of the amino acid sequence (A1).

12. The method as claimed in any of claims 9 to 11, wherein the synthetic biologically active molecule is prepared, at least partly, by genetic engineering.

13. The method as claimed in any of claims 9 to 12, wherein the further amino acid sequence (A2) has glycines and/or amino acids with functional side groups.

14. The method as claimed in claim 13, wherein the functional side groups are selected from the following group: amino, sulfhydryl, carboxyl, hydroxyl, guanidinium, phenyl, indole and imidazole radicals.

15. The method as claimed in any of claims 9 to 14, wherein the means for coupling is a reactive group bound to the C- or N-terminal end of the amino acid sequence (A1) via an amino acid, preferably glycine, cysteine or glycine bound via lysine.

16. The method as claimed in claim 15, wherein the reactive group has one of the following components: amino acid with monobromoacetyl radical, amino acid with monochloroacetyl radical, amino acid with 3-nitro-2-pyridinesulfenyl radical (Npys).

17. The method as claimed in any of claims 9 to 16, wherein the active substance is bound to the amino acid sequence (A1) or to the further amino acid sequence (A2) via a thioether or disulfide bridge.

18. The method as claimed in any of claims 9 to 17, wherein the active substance is bound to the amino acid sequence (A1) or the further amino acid sequence (A2) via a linker.

19. A method for preparing the synthetic biologically active molecule as claimed in claim 1, having the following steps:

aa) synthesizing an amino acid sequence (A1) derived from the C-terminal end of virus protein 2 (VP2) or 3 (VP3) of polyoma virus and

bb) coupling and synthesizing an active substance, namely a peptide, to the amino acid sequence (A1),

wherein the steps under aa and under bb are carried out by means of peptide synthesis or by means of genetic engineering methods.

20. The method as claimed in any of claims 9 to 19, wherein the amino acid sequence (A1) comprises from 10 to 55, preferably from 28 to 38, amino acids.

21. The method as claimed in any of claims 9 to 20, wherein the amino acid sequence (A1) at least in some sections corresponds to the VP2 sequence from amino acid position 250 to 319, preferably from amino acid position 260 to 300 and particularly preferably from amino acid position 287 to 297.

22. The method as claimed in any of claims 9 to 21, wherein the amino acid sequence (A1) has amino acids in the sequence below:

Trp Met Leu Pro Leu Ile Leu Gly Leu Tyr Gly
1 5 10

23. The method as claimed in any of claims 9 to 18 and 20 to 22, wherein the active substance is selected from the following group: nucleic acid, oligonucleotide, protein, peptide, PNA, peptidic substance and modifications of said substances.

24. The method as claimed in any of claims 9 to 23, wherein the synthetic biologically active molecule is

coupled to an amino acid sequence derived from polyoma virus VP1.

SEQUENCE LISTING 1

<110> november AG

<120> Synthetic amino acid molecule

<130> VP2 minimal sequence

<140>

<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 11
<212> PRT
<213> Polyoma virus sp.

<300>

<302> Sequence derived from VP2 position 282-297
<303> EMBO J.
<304> 1998
<305> 17/12
<306> 3233-3240
<308> J02288/EMBL
<309> 1995-08-22

<400> 1

Trp Met Leu Pro Leu Ile Leu Gly Leu Tyr Gly
1 5 10

SEQUENCE LISTING 2

<110> november AG

<120> Synthetic amino acid molecule

<130> Ex. of VP2 anchor and active substance peptide

<140>
<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 33
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: pos. 1-21 RT from HIV-1; 22-33 VP2 sequence from polyoma

<400> 1

Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
1 5 10 15

Tyr Asp Pro Ser Lys Pro Asp Trp Met Leu Pro Leu Ile Leu Gly Leu
20 25 30

Tyr

SEQUENCE LISTING 3

<110> november AG

<120> Synthetic amino acid molecule

<130> VP2 anchor pos. 263-296

<140>

<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 34
<212> PRT
<213> Polyoma virus sp.

<300>

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<303> EMBO J.
<304> 1998
<305> 17/12
<306> 3233-3240
<308> J02288/EMBL
<309> 1995-08-22

<400> 1

Gln Asp Glu Ser Gly Glu Val Ile Lys Phe Tyr Gln Ala Gln Val Val
1 5 10 15
Ser His Gln Arg Val Thr Pro Asp Trp Met Leu Pro Leu Ile Leu Gly
20 25 30
Leu Tyr

SEQUENCE LISTING 4

<110> november AG

<120> Synthetic amino acid molecule

<130> VP2 ProtSeq polyoma sp.

<140>

<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1

<211> 319

<212> PRT

<213> Polyoma virus sp.

<300>

<302> VP2 (capsid protein), gene locus PLY2CG, complete genome of polyoma strains a2 and a3 with accession number J02288 of European Molecular Biology Laboratory (EMBL, Outstation Eur. Bioinform. Inst.)

<303> EMBO J.

<304> 1998

<305> 17/12

<306> 3233-3240

<308> J02288/EMBL

<309> 1995-08-22

<400> 1

Met Gly Ala Ala Leu Thr Ile Leu Val Asp Leu Ile Glu Gly Leu Ala
1 5 10 15

Glu Val Ser Thr Leu Thr Gly Leu Ser Ala Glu Ala Ile Leu Ser Gly
20 25 30

Glu Ala Leu Ala Ala Leu Asp Gly Glu Ile Thr Ala Leu Thr Leu Glu
35 40 45

Gly Val Met Ser Ser Glu Thr Ala Leu Ala Thr Met Gly Ile Ser Glu
50 55 60

Glu Val Tyr Gly Phe Val Ser Thr Val Pro Val Phe Val Ser Arg Thr
65 70 75 80

Ala Gly Ala Ile Trp Leu Met Gln Thr Val Gln Gly Ala Ser Thr Ile
85 90 95

Ser Leu Gly Ile Gln Arg Tyr Leu His Asn Glu Glu Val Pro Thr Val
100 105 110

Asn Arg Asn Met Ala Leu Ile Pro Trp Arg Asp Pro Ala Leu Leu Asp
115 120 125

Ile Tyr Phe Pro Gly Val Asn Gln Phe Ala His Ala Leu Asn Val Val
130 135 140

His Asp Trp Gly His Gly Leu Leu His Ser Val Gly Arg Tyr Val Trp
145 150 155 160

Gln Met Val Val Gln Glu Thr Gln His Arg Leu Glu Gly Ala Val Arg
165 170 175

Glu Leu Thr Val Arg Gln Thr His Thr Phe Leu Asp Gly Leu Ala Arg
180 185 190

Leu Leu Glu Asn Thr Arg Trp Val Val Ser Asn Ala Pro Gln Ser Ala
195 200 205

Ile Asp Ala Ile Asn Arg Gly Ala Ser Ser Ala Ser Ser Gly Tyr Ser
210 215 220

Ser Leu Ser Asp Tyr Tyr Arg Gln Leu Gly Leu Asn Pro Pro Gln Arg
225 230 235 240

Arg Ala Leu Phe Asn Arg Ile Glu Gly Ser Met Gly Asn Gly Gly Pro
245 250 255

Thr Pro Ala Ala His Ile Gln Asp Glu Ser Gly Glu Val Ile Lys Phe
260 265 270

Tyr Gln Ala Gln Val Val Ser His Gln Arg Val Thr Pro Asp Trp Met
275 280 285

Leu Pro Leu Ile Leu Gly Leu Tyr Gly Asp Ile Thr Pro Thr Trp Ala
290 295 300

Thr Val Ile Glu Glu Asp Gly Pro Gln Lys Lys Lys Arg Arg Leu
305 310 315

SEQUENCE LISTING 5

<110> november AG

<120> Synthetic amino acid molecule

<130> Active substance 1 RT/part sequence from HIV

<140>
<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 21
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: pos. 1-20 RT from HIV-1 (accession number AJ006287) and an additional C-terminal Cys

<400> 1
Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
1 5 10 15

Tyr Asp Pro Ser Cys
20

SEQUENCE LISTING 6

<110> november AG

<120> Synthetic amino acid molecule

<130> Active substance 2 GAG/part sequence from HIV

<140>
<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 17
<212> PRT

<213> Human immunodeficiency virus type 1

<220>

<223> Part sequence of GAG from HIV-1 (accession number AJ006287)

<400> 1

Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr
1 5 10 15

Cys