



(86) Date de dépôt PCT/PCT Filing Date: 1991/12/13
(87) Date publication PCT/PCT Publication Date: 1992/06/25
(45) Date de délivrance/Issue Date: 2011/02/15
(85) Entrée phase nationale/National Entry: 1992/07/23
(86) N° demande PCT/PCT Application No.: EP 1991/002409
(87) N° publication PCT/PCT Publication No.: 1992/010514
(30) Priorité/Priority: 1990/12/14 (EP90124241.2)

(51) Cl.Int./Int.Cl. *C07K 7/08* (2006.01),
C07K 14/18 (2006.01), *G01N 33/576* (2006.01),
A61K 39/00 (2006.01)

(72) Inventeurs/Inventors:
DELEYS, ROBERT J., BE;
POLLET, DIRK, BE;
MAERTENS, GEERT, BE;
VAN HEUVERSWIJN, HUGO, BE

(73) Propriétaire/Owner:
INNOGENETICS N.V., BE

(74) Agent: SMART & BIGGAR

(54) Titre : ANTIGENES SYNTHETIQUES POUR LE DEPISTAGE D'ANTICORPS DU VIRUS DE L'HEPATITE C
(54) Title: SYNTHETIC ANTIGENS FOR THE DETECTION OF ANTIBODIES TO HEPATITIS C VIRUS

(57) **Abrégé/Abstract:**

Peptide sequences are provided which are capable of mimicking proteins encoded by HCV for use as reagents for screening of blood and blood products for prior exposure to HCV. The peptides are at least 5 amino acids long and can be used in various specific assays for the detection of antibodies to HCV, for the detection of HCV antigens, or as immunogens.





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07K 7/08, G01N 33/576 A61K 39/29, C07K 7/10	A3	(11) International Publication Number: WO 92/10514 (43) International Publication Date: 25 June 1992 (25.06.92)
(21) International Application Number: PCT/EP91/02409 (22) International Filing Date: 13 December 1991 (13.12.91) (30) Priority data: 90124241.2 14 December 1990 (14.12.90) EP <i>(34) Countries for which the regional or international application was filed:</i> DE et al. (71) Applicant (for all designated States except US): INNOGENETICS N.V. [BE/BE]; Industriepark Zwijnaarde 7, Box 4, B-9710 Ghent (BE). (72) Inventors; and (75) Inventors/Applicants (for US only) : DELEYS, Robert, J. [US/BE]; Diepekanten 20, B-1850 Grimbergen (BE). POLLET, Dirk [BE/BE]; Stokerijstraat 50, B-2110 Wijnegem (BE). MAERTENS, Geert [BE/BE]; Zilverparrenstraat 64, B-8310 Brugge (BE). VAN HEUVERS-WIJN, Hugo [BE/BE]; Colmanstraat 62, B-9270 Laarne (BE).		(74) Agents: DOST, Wolfgang et al.; Galileiplatz 1. Postfach 86 06 20, D-8000 München 86 (DE). (81) Designated States: AU, BR, CA, HU, JP, KR, US. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 20 August 1992 (20.08.92)
(54) Title: SYNTHETIC ANTIGENS FOR THE DETECTION OF ANTIBODIES TO HEPATITIS C VIRUS (57) Abstract Peptide sequences are provided which are capable of mimicking proteins encoded by HCV for use as reagents for screening of blood and blood products for prior exposure to HCV. The peptides are at least 5 amino acids long and can be used in various specific assays for the detection of antibodies to HCV, for the detection of HCV antigens, or as immunogens.		

2074370

SYNTHETIC ANTIGENS FOR THE DETECTION OF ANTIBODIES
TO HEPATITIS C VIRUS

5

The implementation of systematic testing for hepatitis B virus (HBV) has been instrumental in eliminating this virus from the blood supply. Nevertheless, a significant number of post-transfusion hepatitis (PTH) cases still occur. These cases are generally attributable to non-A, non-B
10 hepatitis (NANBH) virus(es), the diagnosis of which is usually made by exclusion of other viral markers.

The etiological agent responsible for a large proportion of these cases has recently been cloned (Choo, Q-L et al. Science (1988) 244:359-362)
15 and a first-generation antibody test developed (Kuo, G. et al. Science (1989) 244:362-364). The agent has been identified as a positive-stranded RNA virus, and the sequence of its genome has been partially determined. Studies suggest that this virus, referred to subsequently as hepatitis C virus (HCV), may be related to flaviviruses and pestiviruses.
20 A portion of the genome of an HCV isolated from a chimpanzee (HCV_{CDC/CHI}) is disclosed in EPO 88310922.5. The coding sequences disclosed in this document do not include sequences originating from the 5'-end of the viral genome which code for putative structural proteins. Recently however, sequences derived from this region of the HCV
25 genome have been published (Okamoto, H. et al., Japan J. Exp. Med. 60:167-177, 1990.). The amino acid sequences encoded by the Japanese clone HC-J1 were combined with the HCV_{CDC/CHI} sequences in a region where the two sequences overlap to generate the composite sequence depicted in Figure 1. Specifically, the two sequences were
30 joined at glycine₄₅₁. It should be emphasized that the numbering

SUBSTITUTE SHEET

2074370

system used for the HCV amino acid sequence is not intended to be absolute since the existence of variant HCV strains harboring deletions or insertions is highly probable. Sequences corresponding to the 5' end of the HCV genome have also recently been disclosed in EPO
5 90302866.0.

In order to detect potential carriers of HCV, it is necessary to have access to large amounts of viral proteins. In the case of HCV, there is currently no known method for culturing the virus, which precludes the
10 use of virus-infected cultures as a source of viral antigens. The current first-generation antibody test makes use of a fusion protein containing a sequence of 363 amino acids encoded by the HCV genome. It was found that antibodies to this protein could be detected in 75 to 85% of chronic NANBH patients. In contrast, only approximately 15% of those
15 patients who were in the acute phase of the disease, had antibodies which recognized this fusion protein (Kuo, G. et al. Science (1989) 244:362-364). The absence of suitable confirmatory tests, however, makes it difficult to verify these statistics. The seeming similarity between the HCV genome and that of flaviviruses makes it possible to predict the
20 location of epitopes which are likely to be of diagnostic value. An analysis of the HCV genome reveals the presence of a continuous long open reading frame. Viral RNA is presumably translated into a long polyprotein which is subsequently cleaved by cellular and/or viral proteases. By analogy with, for example, Dengue virus, the viral
25 structural proteins are presumed to be derived from the amino-terminal third of the viral polyprotein. At the present time, the precise sites at which the polyprotein is cleaved can only be surmised. Nevertheless, the structural proteins are likely to contain epitopes which would be useful for diagnostic purposes, both for the detection of antibodies as well as
30 for raising antibodies which could subsequently be used for the detection

SUBSTITUTE SHEET

- 2a -

of viral antigens. Similarly, domains of nonstructural proteins are also expected to contain epitopes of diagnostic value, even though these proteins are not found as structural components of virus particles.

5 Summary of the Invention

In one aspect of the present invention there is provided a peptide selected from:

(a) the group of amino acid sequences consisting of:

(1)

10 (I) Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X,

(20)

(7)

15 (II) Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X,

(26)

(8)

(18)

(IIA) Y-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Z-X,

(13)

20 (III) Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Z-X,

(32)

(37)

25 (IV) Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Z-X,

(56)

(49)

(V) Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Z-X,

30

(68)

- 2b -

(61)

(VI) Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Z-X, and

(80)

5

(73)

(VII) Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Z-X,

(92)

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

(b) the variants of each of the above peptides (I) to (VII), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (I) to (VII) provided that said variant peptides are capable of providing for immunological competition with at least one strain of HCV; wherein the variant consisting of the sequence: Met-Ser-Thr-Asn-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln, is excluded; and

(c) fragments of peptides (I) to (VII) having at least 6 amino acids of any of the peptide sequences 1-20, 7-26, 8-18, 13-32, 37-56, 49-68, 61-80 and 73-92 as defined in part (a); and said fragments being capable of providing for immunological competition with at least one strain of HCV; and provided that said peptides are different from the following list of peptides:

(1) Met-Ser-Thr-Asn-Pro-Lys-Pro-Gln-X₁-Lys, wherein X₁ represents Arg,

30

(2) Pro-Lys-Pro-Gln-X₁-Lys-X₂-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln, wherein X₁ represents Arg, and X₂ represents Thr,

(3) Gln-Asp-Val-Lys-Phe-Pro.

- 2c -

- (4) Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly,
 (5) Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg,
 (6) Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-
 Glu-Arg-Ser,
 5 (7) Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser,
 (8) Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile,
 (9) Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg,
 (10) Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-
 Asn-Arg-Arg-Pro-Gln, and
 10 (11) Gln-Arg-Lys-Thr-Lys-Arg.

In another aspect of the present invention there is provided a peptide composition comprising a peptide described herein, and further comprising at least one peptide selected from:

- 15 (a) the group of amino acid sequences consisting of:
 (1688)
 (VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-
 Glu-Phe-Asp-Glu-Z-X,
 (1707)
 20 (1694)
 (IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-
 Glu-Cys-Ser-Gln-Z-X,
 (1713)
 (1706)
 25 (X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-
 Met-Met-Leu-Ala-Z-X,
 (1725)
 (1712)
 (XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-
 30 Phe-Lys-Gln-Lys-Z-X,
 (1731)

- 2d -

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X,

(1737)

5

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X,

(1743)

(1730)

10

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

(1749)

(2263)

15

(XV) Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Z-X,

(2282)

(2275)

(XVI) Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Z-X,

20

(2294)

(2287)

(XVII) Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Z-X,

(2306)

25

(2299)

(XVIII) Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Z-X, and

(2318)

(2311)

30

(XIX) Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Z-X,

(2330)

- 2e -

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

- (b) the variants of each of the above peptides (VIII) to (XIX), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (VIII) to (XIX) provided that said variant peptides are capable of providing for immunological competition with at least one strain of HCV; and
- (c) fragments of peptides (VIII) to (XIX) having at least 6 amino acids of any of the peptide sequences 1688-1707, 1694-1713, 1706-1725, 1712-1731, 1718-1737, 1724-1743, 1730-1749, 2263-2282, 2275-2294, 2287-2306, 2299-2318 and 2311-2330, as defined in part (a), and said fragments being capable of providing for immunological competition with at least one strain of HCV.

In another aspect of the present invention there is provided use of a peptide or peptide composition described herein, for the incorporation into an immunoassay for detecting the presence of antibodies to Hepatitis C virus present in a body fluid.

In another aspect of the present invention there is provided a method for the *in vitro* detection of antibodies to Hepatitis C virus present in a body fluid comprising at least the steps of: (a) contacting body fluid of a person to be diagnosed with the peptide or peptide composition according to any one of claims 1 to 9; and, (b) detecting the immunological complex formed between said antibodies and the peptide(s) used.

In another aspect of the present invention there is provided a kit for the detection of anti-hepatitis C virus antibodies in a body fluid, comprising at least the following components:

- a peptide or peptide composition described herein.

- 2f -

- the means for detecting an immunological complex formed between said peptides and said antibodies.

In another aspect of the present invention there is provided use of a peptide or peptide
5 composition described herein, for incorporation into a vaccine composition against HCV.

In another aspect of the present invention there is provided a peptide or peptide composition described herein, for raising antibodies against HCV.

10 In another aspect of the present invention there is provided a peptide or peptide composition obtained by cyclizing any of the peptides described herein, or by coupling together two peptides described herein.

In another aspect of the present invention there is provided a peptide selected from:

15 (1688)

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X,

(1707)

(1694)

20 (IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X,

(1713)

(1706)

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Z-X,

(1725)

(1712)

25 (XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X,

(1731)

- 2g -

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X,

(1737)

5

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X,

(1743)

(1730)

10

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

(1749)

15

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions.

20

In another aspect of the present invention there is provided a process for preparing a peptide selected from:

(1688)

25

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X,

(1707)

(1694)

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X,

30

(1713)

- 2h -

(1706)

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Z-X,

(1725)

5

(1712)

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X,

(1731)

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X,

(1737)

(1724)

10

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X,

(1743)

(1730)

15

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

(1749)

20

25

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions, with said peptides being synthesized in solution or on a solid support employing t-butyloxycarbonyl- or 9-fluorenylmethoxy-carbonyl-protected activated amino acids.

In another aspect of the present invention there is provided a process for preparing a peptide

composition comprising at least two peptides selected from:

- 2i -

(1688)

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X,

(1707)

5

(1694)

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X,

(1713)

(1706)

10

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Z-X,

(1725)

(1712)

15

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X,

(1731)

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X,

(1737)

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X,

20

(1743)

(1730)

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

(1749)

25

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino

- 2j -

acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions, with said peptides being synthesized in solution or on a solid support employing t-butyloxycarbonyl- or 9-fluorenylmethoxy-carbonyl-protected activated amino acids and with said at least
 5 two peptides being mixed.

In another aspect of the present invention there is provided a process for the preparation of a kit for the detection of anti-hepatitis C virus antibodies in a body fluid, comprising
 10 gathering at least the following components:

- a peptide described herein,
- a means for detecting an immunological complex formed between said peptides and said antibodies.

15 In another aspect of the present invention there is provided a process for the preparation of a peptide composition described herein, wherein said peptides are such as obtained by synthesizing a peptides described herein directly on an oligo-lysine core in which both the alpha as well as the epsilon-amino groups of lysines are used as growth points for the peptides.

20

In another aspect of the present invention there is provided a peptide selected from:

(a) the group of amino acid sequences consisting of:

(2263)

(XV) Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-
 25 Arg-Arg-Phe-Ala-Z-X,

(2282)

(2275)

(XVI) Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-
 Pro-Asp-Tyr-Asn-Z-X,

30

(2294)

- 2k -

(2287)

(XVII) Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Z-X,

(2306)

5

(2299)

(XVIII) Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Z-X, and

(2318)

(2311)

10

(XIX) Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Z-X,

(2330)

15

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

20

(b) the variants of each of the above peptides (XV) to (XIX), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (XV) to (XIX) provided that said variant peptides are capable of providing for immunological competition with at least one strain of HCV; and

25

(c) fragments of peptides (XV) to (XIX) having at least 6 amino acids of any of the peptide sequences 2263-2282, 2275-2294, 2287-2306, 2299-2318 and 2311-2330 as defined in part (a); and said fragments being capable of providing for immunological competition with at least one strain of HCV, and provided that said peptides are

30

different from the following list of peptides: Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg (2265-2280) and Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg (2280-2300)

- 21 -

In another aspect of the present invention there is provided a peptide composition comprising a peptide described herein, and further comprising at least one peptide selected from:

5 (a) the group of amino acid sequences consisting of:

(1)

(I) Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X,

(20)

10

(7)

(II) Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X,

(26)

(8)

(18)

15

(IIA) Y-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Z-X,

(13)

(III) Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Z-X,

(32)

20

(37)

(IV) Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Z-X,

(56)

(49)

25

(V) Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Z-X,

(68)

(61)

(VI) Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Z-X,

30

(80)

- 2m -

(73)

(VII) Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-
Glu-Gly-Cys-Gly-Z-X,

(92)

5

(1688)

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-
Glu-Phe-Asp-Glu-Z-X,

(1707)

(1694)

10

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-
Glu-Cys-Ser-Gln-Z-X,

(1713)

(1706)

15

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-
Met-Met-Leu-Ala-Z-X,

(1725)

(1712)

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-
Phe-Lys-Gln-Lys-Z-X,

20

(1731)

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-
Gly-Leu-Leu-Gln-Z-X,

(1737)

25

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-
Ser-Arg-Gln-Ala-Z-X, and

(1743)

(1730)

30

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-
Ile-Ala-Pro-Ala-Z-X,

(1749)

- 2n -

- wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated; Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids; and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;
- (b) the variants of each of the above peptides (I) to (XIV), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (I) to (XIV) provided that said variant peptides are capable of providing for immunological competition with at least one strain of HCV; and
- (c) fragments of peptides (I) to (XIV) having at least 6 amino acids of any of the peptide sequences 1-20, 7-26, 8-18, 13-32, 37-56, 49-68, 61-80, 73-92, 1688-1707, 1694-1713, 1706-1725, 1712-1731, 1718-1737, 1724-1743 and 1730-1749, as defined in part (a), and said fragments being capable of providing for immunological competition with at least one strain of HCV, provided that the combinations containing at least one peptide selected from the group of amino acid sequences consisting of I, II, IIA, III, IV, V, VI, VII and at least one peptide selected from the group of amino acid sequences consisting of VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX are excluded.

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (XV) Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (XVI) Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

- 2o -

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (XVII) Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

5

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (XVIII) Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

10

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (XIX) Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

15

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XV) Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala.

20 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XVI) Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn.

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XVII) Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr.

25 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XVIII) Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro.

30

In another aspect of the present invention there is provided a peptide consisting of the amino

- 2p -

acid sequence: (XIX) Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys.

In another aspect of the present invention there is provided a peptide selected from the group consisting of: (a) a peptide consisting of a combination of at least two of amino acid sequences: II, III, V, IX and XVIII; (b) a peptide consisting of a combination of at least two of amino acid sequences: I, II, V, IX, XI, XVI and XVIII; (c) a peptide consisting of a combination of at least two of amino acid sequences: II, III, IV, V, VIII, XI, XVI and XVIII; (d) a peptide consisting of a combination of at least two of amino acid sequences: II, IX and XVIII; (e) a peptide consisting of a combination of at least two of amino acid sequences: II, III, IV and V; (f) a peptide consisting of a combination of at least two of amino acid sequences: VIII, IX, XI, XIII and XIV; and (g) a peptide consisting of a combination of at least two of amino acid sequences: XV, XVI, XVII, XVIII and XIX; wherein the amino acid sequences I, II, III, IV, V are as defined herein, and wherein the amino acid sequences VIII, IX, XI, XIII, XIV, XV, XVI, XVII, XVIII, and XIX are as defined herein.

In another aspect of the present invention there is provided a peptide consisting of a combination of at least two peptides selected from the group consisting of:

- (XV) Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala;
- (XVI) Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn;
- (XVII) Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr;
- (XVIII) Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro; and
- (XIX) Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys.

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (VIII) Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-

- 2q -

Arg-Glu-Phe-Asp-Glu, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (VIII) Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu.

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (IX) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (IX) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln.

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (X) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala.

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XI) Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys.

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XII) Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln.

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (XIII) Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

- 2r -

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (XIII) Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala.

- 5 In another aspect of the present invention there is provided a peptide comprising a linker arm which is 1 to 60 amino acids, and two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides having amino acid sequences VIII, IX, X, XI, XII, XIII, and XIV, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV;
10 wherein the amino acid sequences VIII, IX, X, XI, XII, XIII and XIV are as defined herein.

- In another aspect of the present invention there is provided a peptide comprising two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides consisting of amino acid sequences VIII, IX, X, XI, XII, XIII, and
15 XIV, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV; wherein the amino acid sequences VIII, IX, X, XI, XII, XIII and XIV are as defined herein.

- In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (I) Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-
20 Arg-Arg-Pro-Gln, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

- In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (II) Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-
25 Val-Lys-Phe-Pro-Gly, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

- In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (III) Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-
30 Gly-Gln-Ile-Val-Gly; and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

- 2s -

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (IV) Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser; and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

5

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (V) Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

10

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (VI) Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

15

In another aspect of the present invention there is provided a peptide comprising the amino acid sequence: (VII) Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly, and a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

20

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (I) Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln.

25 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (II) Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly.

30 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (III) Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly.

- 2t -

In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (IV) Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser.

- 5 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (V) Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val.

- 10 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (VI) Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly.

- 15 In another aspect of the present invention there is provided a peptide consisting of the amino acid sequence: (VII) Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly.

- In another aspect of the present invention there is provided A peptide comprising a linker arm which is 1 to 60 amino acids, and two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides having amino acid sequences I, II, III, IV, V, VI, and VII, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV; and wherein the amino acid sequences I, II, III, IV, V, VI and VII are as defined herein.
- 20

- In another aspect of the present invention there is provided A peptide comprising two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides consisting of amino acid sequences I, II, III, IV, V, VI, and VII, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV; wherein the amino acid sequences I, II, III, IV, V, VI, and VII are as defined herein.
- 25

- 3 -

5 Brief Description of the Drawings

Figure 1 shows the amino acid sequence of the composite HCV_{HC-J1/CDC/CHI}

10 Figure 2 shows the antibody binding to individual peptides and
various mixtures in an ELISA assay

Description of the Specific Embodiments

15 It is known that RNA viruses frequently exhibit a high rate of
spontaneous mutation and, as such, it is to be expected that no two
HCV isolates will be completely identical, even when derived from the
same individual. For the purpose of this disclosure, a virus is considered
to be the same or equivalent to HCV if it exhibits a global homology
20 of 60 percent or more with the HCV_{HC-J1/CDC/CHI} composite sequence
at the nucleic acid level and 70 percent at the amino acid level.

Peptides are described which immunologically mimic proteins encoded by
HCV. In order to accommodate strain-to-strain variations in sequence,
25 conservative as well as non-conservative amino acid substitutions may be
made. These will generally account for less than 35 percent of a specific
sequence. It may be desirable in cases where a peptide corresponds to
a region in the HCV polypeptide which is highly polymorphic, to vary
one or more of the amino acids so as to better mimic the different
30 epitopes of different viral strains.

2074370

The peptides of interest will include at least five, sometimes six, sometimes eight, sometimes twelve, usually fewer than about fifty, more usually fewer than about thirty-five, and preferably fewer than about twenty-five amino acids included within the sequence encoded by the HCV genome. In each instance, the peptide will preferably be as small as possible while still maintaining substantially all of the sensitivity of the larger peptide. It may also be desirable in certain instances to join two or more peptides together in one peptide structure.

- 10 It should be understood that the peptides described need not be identical to any particular HCV sequence, so long as the subject compounds are capable of providing for immunological competition with at least one strain of HCV. The peptides may therefore be subject to insertions, deletions, and conservative or non-conservative amino acid substitutions
15 where such changes might provide for certain advantages in their use.

Substitutions which are considered conservative are those in which the chemical nature of the substitute is similar to that of the original amino acid. Combinations of amino acids which could be considered
20 conservative are Gly, Ala; Asp, Glu; Asn, Gln; Val, Ile, Leu; Ser, Thr; Lys, Arg; and Phe, Tyr.

Furthermore, additional amino acids or chemical groups may be added to the amino- or carboxyl terminus for the purpose of creating a "linker
25 arm" by which the peptide can conveniently be attached to a carrier. The linker arm will be at least one amino acid and may be as many as 60 amino acids but will most frequently be 1 to 10 amino acids. The nature of the attachment to a solid phase or carrier need not be covalent.

SUBSTITUTE SHEET

2074370

Natural amino acids such as cysteine, lysine, tyrosine, glutamic acid, or aspartic acid may be added to either the amino- or carboxyl terminus to provide functional groups for coupling to a solid phase or a carrier. However, other chemical groups such as, for example, biotin and thioglycolic acid, may be added to the termini which will endow the peptides with desired chemical or physical properties. The termini of the peptides may also be modified, for example, by N-terminal acetylation or terminal carboxy-amidation. The peptides of interest are described in relation to the composite amino acid sequence shown in Figure 1. The amino acid sequences are given in the conventional and universally accepted three-letter code. In addition to the amino acids shown, other groups are defined as follows: Y is, for example, NH_2 , one or more N-terminal amino acids, or other moieties added to facilitate coupling. Y may itself be modified by, for example, acetylation. Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking. X is intended to represent OH, NH_2 , or a linkage involving either of these two groups.

Peptide I corresponds to amino acids 1 to 20 and has the following amino acid sequences:

(I)

Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X.

Peptide II corresponds to amino acids 7 to 26 and has the amino acid sequence:

(II)

Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X.

SUBSTITUTE SHEET

2074370

Of particular interest is the oligopeptide IIA:

(IIA)

Y-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Z-X.

5

Peptide III corresponds to amino acids 13 to 32 and has the sequence:

(III)

Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gln-Ile-Val-Gly-Z-X.

10

Peptide IV corresponds to amino acid 37 to 56 and has the sequences:

(IV)

Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Z-X.

15

Peptide V corresponds to amino acids 49 to 68 and has the sequence:

(V)

Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Z-X.

20

Peptide VI corresponds to amino acid 61 to 80 and has the following sequence:

(VI)

Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Z-X.

25

Peptide VII corresponds to amino acids 73 to 92 and has the sequence:

(VII)

Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Z-X.

30

SUBSTITUTE SHEET

Peptide VIII corresponds to amino acids 1688 to 1707 and has the sequence:

(VIII)

Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-
5 Asp-Glu-Z-X.

Peptide IX corresponds to amino acids 1694 to 1713 and has the sequence:

(IX)

10 Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-
Cys-Ser-Gln-Z-X.

Peptide X corresponds to amino acids 1706 to 1725 and has the sequence:

15 (X)

Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-
Met-Leu-Ala-Z-X.

Peptide XI corresponds to amino acids 1712 to 1731 and has the
20 sequence:

(XI)

Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-
Lys-Gln-Lys-Z-X.

25 Peptide XII corresponds to amino acids 1718 to 1737 and has the sequence:

(XII)

Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-
Leu-Leu-Gln-Z-X.

SUBSTITUTE SHEET

2074370

Peptide XIII corresponds to amino acids 1724 to 1743 and has the sequence:

(XIII)

Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-
5 Arg-Gln-Ala-Z-X.

Peptide XIV corresponds to amino acids 1730 to 1749 and has the sequence:

(XIV)

10 Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-
Ala-Pro-Ala-Z-X.

Peptide XV corresponds to amino acids 2263 to 2282 and has the sequence:

15 (XV)

Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-
Arg-Phe-Ala-Z-X.

20 Peptide XVI corresponds to amino acids 2275 to 2294 and has the sequence:

(XVI)

Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-
Asp-Tyr-Asn-Z-X.

25

Peptide XVII corresponds to amino acids 2287 to 2306 and has the sequence:

(XVII)

Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-
30 Pro-Asp-Tyr-Z-X.

SUBSTITUTE SHEET

2074370

Peptide XVIII corresponds to amino acids 2299 to 2318 and has the sequence:

(XVIII)

Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-
5 Leu-Pro-Pro-Z-X.

Peptide XIX corresponds to amino acids 2311 to 2330 and has the sequence:

(XIX)

10 Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-
Lys-Lys-Z-X.

Of particular interest is the use of the mercapto-group of cysteines or thioglycolic acids used for acylating terminal amino groups for cyclizing
15 the peptides or coupling two peptides together. The cyclization or coupling may occur via a single bond or may be accomplished using thiol-specific reagents to form a molecular bridge.

The peptides may be coupled to a soluble carrier for the purpose of
20 either raising antibodies or facilitating the adsorption of the peptides to a solid phase. The nature of the carrier should be such that it has a molecular weight greater than 5000 and should not be recognized by antibodies in human serum. Generally, the carrier will be a protein. Proteins which are frequently used as carriers are keyhole limpet
25 hemocyanin, bovine gamma globulin, bovine serum albumin, and poly-L-lysine.

There are many well described techniques for coupling peptides to carriers. The linkage may occur at the N-terminus, C-terminus or at an
30 internal site in the peptide. The peptide may also be derivatized for

SUBSTITUTE SHEET

2074370

coupling. Detailed descriptions of a wide variety of coupling procedures are given, for example, in Van Regenmortel, M.H.V., Briand, J.P., Muller, S., and Plaué, S., Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 19, Synthetic Polypeptides as Antigens, Elsevier Press,
5 Amsterdam, New York, Oxford, 1988.

The peptides may also be synthesized directly on an oligo-lysine core in which both the alpha as well as the epsilon-amino groups of lysines are used as growth points for the peptides. The number of lysines
10 comprising the core is preferably 3 or 7. Additionally, a cysteine may be included near or at the C-terminus of the complex to facilitate the formation of homo- or heterodimers. The use of this technique has been amply illustrated for hepatitis B antigens (Tam, J.P., and Lu, Y-A., Proc. Natl. Acad. Sci. USA (1989) 86:9084-9088) as well as for a variety of
15 other antigens (see Tam, J.P., Multiple Antigen Peptide System: A Novel Design for Synthetic Peptide Vaccine and Immunoassay, in Synthetic Peptides, Approaches to Biological Problems, Tam, J.P., and Kaiser, E.T., ed. Alan R. Liss Inc., New York, 1989).

20 Depending on their intended use, the peptides may be either labeled or unlabeled. Labels which may be employed may be of any type, such as enzymatic, chemical, fluorescent, luminescent, or radioactive. In addition, the peptides may be modified for binding to surfaces or solid phases, such as, for example, microtiter plates, nylon membranes, glass or plastic
25 beads, and chromatographic supports such as cellulose, silica, or agarose. The methods by which peptides can be attached or bound to solid support or surface are well known to those versed in the art.

Of particular interest is the use of mixtures of peptides for the detection
30 of antibodies specific for hepatitis C virus. Mixtures of peptides which

SUBSTITUTE SHEET

2074370

are considered particularly advantageous are:

- A. II, III, V, IX, and XVIII
- B. I, II, V, IX, XI, XVI, and XVIII
- 5 C. II, III, IV, V, VIII, XI, XVI, and XVIII
- D. II, IX, and XVIII
- E. II, III, IV, and V
- F. VIII, IX, XI, XIII, and XIV
- G. XV, XVI, XVII, XVIII, and XIX

10

Antibodies which recognize the peptides can be detected in a variety of ways. A preferred method of detection is the enzyme-linked immunosorbant assay (ELISA) in which a peptide or mixture of peptides is bound to a solid support. In most cases, this will be a microtiter plate but may in principle be any sort of insoluble solid phase. A suitable dilution or dilutions of serum or other body fluid to be tested is brought into contact with the solid phase to which the peptide is bound. The incubation is carried out for a time necessary to allow the binding reaction to occur. Subsequently, unbound components are removed by washing the solid phase. The detection of immune complexes is achieved using antibodies which specifically bind to human immunoglobulins, and which have been labeled with an enzyme, preferably but not limited to either horseradish peroxidase, alkaline phosphatase, or beta-galactosidase, which is capable of converting a colorless or nearly colorless substrate or co-substrate into a highly colored product or a product capable of forming a colored complex with a chromogen. Alternatively, the detection system may employ an enzyme which, in the presence of the proper substrate(s), emits light. The amount of product formed is detected either visually, spectrophotometrically, electrochemically, or luminometrically, and is

20
25
30**SUBSTITUTE SHEET**

2074370

compared to a similarly treated control. The detection system may also employ radioactively labeled antibodies, in which case the amount of immune complex is quantified by scintillation counting or gamma counting.

5

Other detection systems which may be used include those based on the use of protein A derived from Staphylococcus aureus Cowan strain I, protein G from group C Staphylococcus sp. (strain 26RP66), or systems which make use of the high affinity biotin-avidin or streptavidin binding
10 reaction.

15

Antibodies raised to carrier-bound peptides can also be used in conjunction with labeled peptides for the detection of antibodies present in serum or other body fluids by competition assay. In this case, antibodies raised to carrier-bound peptides are attached to a solid support which may be, for example, a plastic bead or a plastic tube. Labeled peptide is then mixed with suitable dilutions of the fluid to be tested and this mixture is subsequently brought into contact with the antibody bound to the solid support. After a suitable incubation period, the solid
20 support is washed and the amount of labeled peptide is quantified. A reduction in the amount of label bound to the solid support is indicative of the presence of antibodies in the original sample. By the same token, the peptide may also be bound to the solid support. Labeled antibody may then be allowed to compete with antibody present in the sample
25 under conditions in which the amount of peptide is limiting. As in the previous example, a reduction in the measured signal is indicative of the presence of antibodies in the sample tested.

Another preferred method of antibody detection is the homogeneous
30 immunoassay. There are many possible variations in the design of such

SUBSTITUTE SHEET

2074370

assays. By way of example, numerous possible configurations for homogeneous enzyme immunoassays and methods by which they may be performed are given in Tijssen, P., Practice and Theory of Enzyme Immunoassays, Elsevier Press, Amersham, Oxford, New York, 1985.

5 Detection systems which may be employed include those based on enzyme channeling, bioluminescence, allosteric activation and allosteric inhibition. Methods employing liposome-entrapped enzymes or coenzymes may also be used (see Pinnaduwege, P. and Huang, L., Clin. Chem. (1988) 34/2: 268-272, and Ullman, E.F. et al., Clin. Chem. (1987) 33/9: 10 1579-1584 for examples).

The synthesis of the peptides can be achieved in solution or on a solid support. Synthesis protocols generally employ the use t-butyloxycarbonyl- or 9-fluorenylmethoxy-carbonyl-protected activated amino acids. The

15 procedures for carrying out the syntheses, the types of side-chain protection, and the cleavage methods are amply described in, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2nd Edition, Pierce Chemical Company, 1984; and Atherton and Sheppard, Solid Phase Peptide Synthesis, IRL Press, 1989.

20

Experimental

1. Peptide Synthesis

25 All of the peptides described were synthesized on Pepsyn K polyamide-Kieselguhr resin (Milligen, Novato, California) which had been functionalized with ethylenediamine and onto which the acid-labile linker 4-(alpha-Fmoc-amino-2',4'-dimethoxybenzyl) phenoxyacetic acid had been coupled (Rink, Tetrahedron Lett. (1987) 28:3787). t-Butyl-based side-

30 chain protection and Fmoc alpha-amino-protection was used. The

SUBSTITUTE SHEET

2074370

- 14 -

guanidino-group of arginine was protected by the 2,2,5,7,8-pentamethylchroman-6-sulfonyl moiety. The imidazole group of histidine was protected by either t-Boc or trityl and the sulfhydryl group of cysteine was protected by a trityl group. Couplings were carried out using performed O-pentafluorophenyl esters except in the case of arginine where diisopropylcarbodiimide-mediated hydroxybenzotriazole ester formation was employed. Except for peptide I, all peptides were N-acetylated using acetic anhydride. All syntheses were carried out on a Milligen 9050 PepSynthesizer (Novato, California) using continuous flow procedures. Following cleavage with trifluoroacetic acid in the presence of scavengers and extraction with diethylether, all peptides were analyzed by C₁₈-reverse phase chromatography.

II. Detection of Antibodies to Hepatitis C Virus

A. Use of peptides bound to a nylon membrane.

Peptides were dissolved in a suitable buffer to make a concentrated stock solution which was then further diluted in phosphate-buffered saline (PBS) or sodium carbonate buffer, pH 9.6 to make working solutions. The peptides were applied as lines on a nylon membrane (Pall, Portsmouth, United Kingdom), after which the membrane was treated with casein to block unoccupied binding sites. The membrane was subsequently cut into strips perpendicular to the direction of the peptide lines. Each strip was then incubated with a serum sample diluted 1 to 100, obtained from an HCV-infected individual. Antibody binding was detected by incubating the strips with goat anti-human immunoglobulin antibodies conjugated to the enzyme alkaline phosphatase. After removing unbound conjugate by

SUBSTITUTE SHEET

2074370

washing, a substrate solution containing 5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium was added.

5 Positive reactions are visible as colored lines corresponding to the positions of the peptides which are specifically recognized. The reaction patterns of thirty-six different sera are tabulated in Table 1. The results shown in Table 1 are further summarized in Table 2.

10

B. Use of peptides in an enzyme-linked immunosorbent assay (ELISA).

15 Peptide stock solutions were diluted in sodium carbonate buffer, pH 9.6 and used to coat microtiter plates at a peptide concentration of 2 micrograms per milliliter. A mixture consisting of peptides II, III, V, IX, and XVIII was also used to coat plates. Following coating, the plates were blocked with casein. Fifteen HCV-antibody-positive sera and control sera from seven uninfected blood donors were diluted 1 to 20 and incubated in wells of the peptide-coated plates.

20 Antibody binding was detected by incubating the plates with goat anti-human immunoglobulin antibodies conjugated to the enzyme horseradish peroxidase. Following removal of unbound conjugate by washing, a solution containing H_2O_2 and 3,3',5,5'-tetramethylbenzidine was added. Reactions were stopped after a

25 suitable interval by addition of sulfuric acid. Positive reactions gave rise to a yellow color which was quantified using a conventional microtiter plate reader. The results of these determinations are tabulated in Table 3. To correct for any aspecific binding which could be attributable to the physical or chemical properties of the

30 peptides themselves, a cut-off value was determined for each peptide

SUBSTITUTE SHEET

2074370

individually. This cut-off absorbance value was calculated as the average optical density of the negative samples plus 0.200. Samples giving absorbance values higher than the cut-off values are considered positive. The results for the fifteen positive serum samples are further summarized in Table 4.

While it is evident that some of the peptides are recognized by a large percentage of sera from HCV-infected individuals, it is also clear that no single peptide is recognized by all sera. In contrast, the peptide mixture was recognized by all fifteen sera and, for six of the fifteen sera, the optical densities obtained were equal to or higher than those obtained for any of the peptides individually. These results serve to illustrate the advantages of using mixtures of peptides for the detection of anti-HCV antibodies.

C. Binding of antibodies in sera from HCV-infected patients to various individual peptides and peptide mixtures in an ELISA.

Five peptides were used individually and in seven different combinations to coat microtiter plates. The plates were subsequently incubated with dilutions of fifteen HCV antibody-positive sera in order to evaluate the relative merits of using mixtures as compared to individual peptides for antibody detection. The mixtures used and the results obtained are shown in Figure 2.

In general, the mixtures functioned better than individual peptides. This was particularly evident for mixture 12 (peptides I, III, V, IX, and XVIII) which was recognized by all twelve of the sera tested. These results underscore the advantages of using mixtures of peptides

SUBSTITUTE SHEET

in diagnostic tests for the detection of antibodies to HCV.

5 D. Use of a mixture of peptides in an ELISA assay for the detection of anti-HCV antibodies.

10 A mixture of peptides II, III, V, IX, and XVIII was prepared and used to coat microtiter plates according to the same procedure used to test the individual peptides. A total of forty-nine sera were tested from patients with clinically diagnosed but undifferentiated chronic non A non B hepatitis as well as forty-nine sera from healthy blood donors. Detection of antibody binding was accomplished using goat anti-human immunoglobulin antibodies conjugated to horseradish peroxidase. The resulting optical density
15 values are given in Table 5. These results indicate that the mixture of peptides is not recognized by antibodies in sera from healthy donors (0/49 reactives) but is recognized by a large proportion (41/49, or 84%) of the sera from patients with chronic NANBH. These results demonstrate that the peptides described can be used
20 effectively as mixtures for the diagnosis of HCV infection.

25 E. Detection of anti-HCV antibodies in sera from patients with acute NANB infection using individual peptides bound to nylon membranes and a mixture of peptides in an ELISA assay, and comparison with a commercially available kit.

Peptides were applied to nylon membranes or mixed and used to coat microtiter plates as previously described. The peptide mixture consisted of peptides II, III, V, IX, and XVIII. Sera obtained from
30 twenty-nine patients with acute non-A, non-B hepatitis were then

SUBSTITUTE SHEET

2074370

tested for the presence of antibodies to hepatitis C virus. These same sera were also evaluated using a commercially available kit (Ortho, Emeryville, CA, USA).

5 The results of this comparative study are given in Table 6. In order to be able to compare the peptide-based ELISA with the commercially available kit, the results for both tests are also expressed as signal to noise ratios (S/N) which were calculated by dividing the measured optical density obtained for each sample by
10 the cut-off value. A signal-to-noise ratio greater or equal to 1.0 is taken to represent a positive reaction. For the commercially available kit, the cut-off value was calculated according to the manufacturer's instructions. The cut-off value for the peptide-based ELISA was calculated as the average optical density of five negative
15 samples plus 0.200.

The scale used to evaluate antibody recognition of nylon-bound peptides was the same as that given in Table 1. Of the twenty-nine samples tested, twenty-five (86%) were positive in the peptide-based
20 ELISA and recognized one or more nylon-bound peptides. In contrast, only fourteen of the twenty-nine sera scored positive in the commercially available ELISA. These results serve to illustrate the advantages of using peptide mixtures for the detection of anti-HIV antibodies as well as the need to include in the mixtures peptides
25 which contain amino acid sequences derived from different regions of the HCV polyprotein.

SUBSTITUTE SHEET

Table 1. Recognition of peptides bound to nylon membranes by sera from persons infected by HCV.

PEPTIDE									
Serum nr.	I	II	III	IV	V	VI	VII	VIII	IX
1			3	1				1	
2									
3	1	0.5	2	1		0.5		2	0.5
4									
6			2	1	0.5				
7	0.5	1	2	1	0.5			3	2
8	0.5	1	3	1	1		1	1	
10		1	0.5					3	1
13	0.5	0.5	2		0.5				
15				0.5				2	1
16	2	1	0.5	0.5	1	0.5		2	0.5
18	1	1	3	0.5		2	0.5		
23	0.5		1	1			0.5		
24	1	0.5	2	1	0.5	0.5	0.5	2	
25			1	0.5				2	0.5
26								1	
27	0.5	0.5	1					3	2
29		0.5	3	2	1	1	0.5		
30		0.5	0.5	1	1	0.5			
31		1	0.5						
32		1	2						
33								0.5	
34		1	1	1				3	1
35	1	1	2	1	1	1	0.5		
36	1		2	1		1			
37	1	1							
44		1	2	1	0.5				
46		0.5	2	0.5	0.5		0.5	2	
47		0.5		0.5		0.5		1	
48	1	2		2			0.5	2	
49		1	1	0.5	0.5	0.5			
50		1	2	1				2	0.5
51			2	0.5	0.5		0.5		
52			2	0.5			0.5		
54			2		0.5	0.5	1	0.5	
56	ND	ND	ND	ND	ND	ND	ND	2	

Blank: no reaction; 0.5: weakly positive; 1: clearly positive; 2: strong reaction; 3: intense reaction; ND: not determined

SUBSTITUTE SHEET

2074370

20

TABLE 1 continued

X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
	0.5					2	2	1	1
	0.5		1	2		2	1		
1	0.5								
			1						
						2			
2	1		1		2		0.5	1	1
2	1		1			1	1	0.5	
1				0.5	2	2		2	2
1			1	0.5		0.5			
0.5				1		0.5			
1					2	2	1	2	
						1	0.5		
1	0.5		0.5		0.5				
						1			
0.5	2		1	1		2			
						0.5			
						1	2	1	0.5
2	1		1	1	2	2	1	1	1
0.5	0.5				0.5				
1	0.5				1	1			
1	0.5							0.5	
						1	1	0.5	
					2		0.5		
								1	1
			0.5		1	1		1	0.5
0.5	0.5		0.5				1		
1	1		1			1	1	0.5	0.5
						1	1	0.5	
0.5									
1	1		1			1	1	1	
	0.5	1	2	1					

SUBSTITUTE SHEET

Table 2. Summary of antibody binding to nylon-bound HCV peptides
by sera from infected patients.

5			
	Peptide	No. reactive sera	% reactive sera
10	I	13/35	37
	II	22/35	63
	III	27/35	77
	IV	24/35	69
	V	14/35	40
15	VI	11/35	31
	VII	11/35	31
	VIII	19/36	53
	IX	9/36	25
	X	17/36	47
20	XI	15/36	42
	XII	1/36	3
	XIII	13/36	36
	XIV	7/36	19
	XV	9/36	25
25	XVI	20/36	56
	XVII	14/36	39
	XVIII	14/36	39
	XIX	8/36	22

SUBSTITUTE SHEET

2074370

Table 3. Comparison of Individual Peptides in an ELISA Assay for the Detection of Antibodies to HCV.

sample ident	peptide									IX
	I	II	III	IV	V	VI	VII	VIII		
1	0.786	1.119	1.284	0.265	0.042	0.04	0.05	0.571	0.659	
2	0.044	0.039	0.11	0.041	0.037	0.038	0.039	0.479	0.78	
3	0.815	0.944	0.825	0.399	0.654	0.487	0.32	0.705	0.965	
7	1.122	1.23	0.588	0.682	0.659	0.182	0.107	0.907	1.42	
8	1.155	1.159	1.2	0.508	1.272	0.433	0.623	0.61	0.863	
10	1.089	1.236	1.083	0.044	0.508	0.042	0.073	1.49	1.529	
11	0.048	0.051	0.476	0.052	0.119	0.039	0.1	0.634	0.711	
15	0.224	0.602	0.813	0.093	0.068	0.077	0.147	0.807	1.225	
23	0.62	0.8	0.924	0.568	0.759	0.442	0.683	0.089	0.121	
24	1.042	1.132	1.026	0.518	0.916	0.302	0.253	1.013	1.364	
49	0.624	0.73	0.884	0.171	0.372	0.055	0.04	0.084	0.064	
13	0.76	0.857	0.815	0.087	0.422	0.098	0.045	0.473	0.489	
31	0.84	1.114	0.445	0.672	0.046	0.041	0.042	0.184	0.15	
47	1.303	1.53	1.236	0.751	0.83	0.629	0.073	0.545	0.739	
56	1.169	1.301	1.364	1.269	1.374	0.85	1.066	1.45	1.523	
bd A28	0.054	0.043	0.139	0.045	0.135	0.042	0.041	0.086	0.115	
bd A169	0.041	0.042	0.134	0.044	0.038	0.04	0.041	0.061	0.07	
bd A170	0.04	0.044	0.117	0.04	0.036	0.04	0.04	0.081	0.05	
bd A171	0.041	0.046	0.148	0.043	0.037	0.045	0.045	0.077	0.065	
bd A166	0.047	0.046	0.124	0.044	0.038	0.042	0.041	0.056	0.066	
bd A165	0.041	0.046	0.123	0.043	0.035	0.051	0.042	0.051	0.091	
AVG	0.044	0.045	0.131	0.043	0.053	0.043	0.042	0.069	0.076	
STD	0.005	0.002	0.011	0.002	0.037	0.004	0.002	0.013	0.021	
cut off	0.109	0.101	0.214	0.099	0.214	0.105	0.098	0.158	0.189	

SUBSTITUTE SHEET

2074370

TABLE 3 continued

X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
0.048	0.04	0.043	0.068	0.044	0.041	1.063	0.956	1.383	1.346
0.169	0.563	0.039	0.042	0.515	0.039	0.64	0.319	0.154	0.49
0.468	0.668	0.041	0.093	0.341	0.043	0.292	0.038	0.046	0.038
0.663	0.646	0.041	0.235	0.068	0.575	0.042	0.041	0.872	1.271
0.752	1.175	0.046	0.42	0.102	0.068	0.552	0.671	0.417	0.058
0.689	0.834	0.041	0.044	0.314	0.793	0.886	0.037	1.335	1.356
0.199	0.967	0.125	0.454	0.088	0.111	0.274	0.093	0.838	0.065
0.315	0.688	0.046	0.154	0.202	0.065	0.372	0.097	0.155	0.077
0.422	0.896	0.041	0.049	0.101	0.068	0.311	0.038	0.052	0.05
0.236	0.397	0.054	0.123	0.076	0.051	0.418	0.053	0.1	0.085
0.209	0.731	0.044	0.113	0.039	0.044	0.299	0.038	0.192	0.041
0.529	0.735	0.043	0.044	0.186	0.043	0.086	0.037	0.066	0.04
0.255	0.69	0.041	0.04	0.061	0.136	0.292	0.038	0.224	0.501
0.044	0.041	0.041	0.041	0.498	0.04	0.268	0.042	1.288	1.206
0.079	1.069	0.058	0.568	0.038	0.039	0.218	0.036	0.087	0.039
0.044	0.042	0.044	0.052	0.043	0.043	0.307	0.042	0.045	0.061
0.043	0.042	0.041	0.04	0.041	0.041	0.255	0.038	0.056	0.042
0.04	0.039	0.04	0.038	0.038	0.144	0.292	0.036	0.058	0.039
0.043	0.041	0.043	0.039	0.04	0.045	0.286	0.037	0.05	0.04
0.041	0.041	0.042	0.04	0.041	0.041	0.207	0.039	0.046	0.041
0.041	0.04	0.042	0.039	0.043	0.039	0.253	0.034	0.06	0.098
0.042	0.041	0.042	0.041	0.041	0.059	0.267	0.038	0.053	0.054
0.001	0.001	0.001	0.005	0.002	0.038	0.033	0.002	0.006	0.021
0.095	0.094	0.095	0.106	0.097	0.223	0.416	0.084	0.121	0.167

SUBSTITUTE SHEET

2074370

Table 4 Summary of antibody-binding to individual peptides in an ELISA assay.

5	Peptide	No. reactive sera	% reactive sera
10	I	13	87
	II	13	87
	III	14	93
	IV	10	67
	V	10	67
15	VI	7	47
	VII	8	53
	VIII	13	87
	IX	12	80
	X	13	87
20	XI	13	87
	XII	1	7
	XIII	7	47
	XIV	8	53
	XV	2	13
25	XVI	5	33
	XVII	4	27
	XVIII	10	67
	XIX	6	40
30			

SUBSTITUTE SHEET

2074370

Table 5 Use of a peptide mixture for the detection of antibodies to HCV in sera from chronic NANBH patients and comparison to sera from healthy blood donors.

5				
	Chronic NANB Sera		Control Sera	
10	Serum nr.	Optical Density	Serum nr.	Optical Density
	101	0.041	1	0.049
	102	1.387	2	0.047
15	103	1.578	3	0.049
	104	1.804	4	0.046
	105	1.393	5	0.049
	107	1.604	6	0.045
	108	1.148	7	0.043
20	109	1.714	8	0.053
	110	1.692	9	0.049
	112	0.919	10	0.047
	113	1.454	11	0.060
	114	0.936	12	0.044
25	115	0.041	13	0.049
	116	1.636	14	0.051
	118	1.242	15	0.056
	119	1.568	16	0.050
	120	1.290	17	0.049
30	121	1.541	18	0.055
	122	1.422	19	0.054
	123	1.493	20	0.058

SUBSTITUTE SHEET

2074370

	124	1.666	21	0.050
	125	1.644	22	0.044
	126	1.409	23	0.043
	127	1.625	24	0.045
5	128	1.061	25	0.046
	129	1.553	26	0.049
	130	1.709	27	0.050
	131	0.041	28	0.047
	132	0.044	29	0.050
10	133	1.648	30	0.053
	134	0.043	31	0.051
	135	1.268	32	0.053
	136	1.480	33	0.055
	138	0.628	34	0.064
15	139	0.042	35	0.063
	140	0.040	36	0.057
	141	0.039	38	0.048
	142	1.659	39	0.045
	143	1.457	40	0.046
20	144	0.722	41	0.046
	145	1.256	42	0.051
	146	0.373	43	0.057
	147	1.732	44	0.050
	148	1.089	45	0.050
25	149	1.606	46	0.045
	150	1.725	47	0.041
	151	1.449	48	0.064
	154	1.639	49	0.040
	155	1.775	50	0.036

SUBSTITUTE SHEET

2074370

Table 6. Comparison of anti-HCV antibody detection by nylon-bound peptides, a peptide-based ELISA, and a commercially available kit.

Serum nr.	Nylon-bound peptides										XIV	XV	XVI	XVII
	I	II	III	IV	V	VI	VIII	XI	XII	XIII				
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0
194	0	0	0	0	3	0	0	0.5	0.5	0.5	1	0.5	3	1
195	1	2	2	1	2	0.5	0.5	0.5	0.5	0.5	1	1	2	0
196	1	2	2	1	2	0	0.5	0.5	0.5	0.5	1	1	2	0
197	1	2	2	2	2	0	0	0	0	0	1	2	0	1
198	1	2	2	2	0.5	0	0	2	0	0	0	0	0	0
211	0.5	1	1	0.5	0.5	0	0	0	0	0	0	0	0	0
213	0	0	0	0	1	0	0	0	0	0	0	0	0	0
214	0	0	0	0	1	0	0	0	0	0	0	0	0	0
215	0	0	0	0	1	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0
217	0	0	0	0	1	0	0.5	1	0.5	0.5	0.5	0.5	0.5	1
219	0.5	1	1	1	2	1	0.5	1	0.5	0.5	0.5	0.5	0.5	1
220	0.5	1	1	1	2	1	0.5	1	0.5	0.5	0	0	0	0
221	0	0	0	0	0.5	0	0	0	0	0.5	0.5	0.5	0.5	0
222	1	1	1	1	1	0	1	2	3	0.5	0.5	0.5	0.5	0
223	1	1	1	1	1	0	1	3	3	0.5	0.5	0.5	0.5	0
224	1	1	1	2	1	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
225	0	0	0	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
226	0.5	0	0	0	0	0	2	3	3	2	0.5	0.5	0.5	0
227	0	0	0	0	0	0	2	2	2	2	0.5	0.5	0.5	0
229	0.5	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0
234	0.5	0.5	0.5	0.5	0.5	0	0	0	0	0	0	0	0	0
235	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0
236	0	0	0	0	1	0	0	0	0	0	0	0	0	0
237	0	0	0	0	1	0	0	0	0	0	0	0	0	0
238	0	0	0	0	1	0	1	1	1	1	0	0	0	0

0: no reaction; 0.5: weakly positive; 1: clearly positive;
2: strong reaction; 3: intense reaction;

SUBSTITUTE SHEET

2074370

Table 6 continued

Serum nr.	Optical density Peptide ELISA		Optical density Commercial ELISA	
	S/N		S/N	
191	0.045	0.18	0.295	0.47
192	0.042	0.17	0.289	0.46
193	0.039	0.16	0.197	0.32
194	0.044	0.18	0.183	0.29
195	1.692	6.77	3.000*	4.82*
196	1.569	6.28	0.386	0.62
197	1.523	6.09	0.447	0.72
198	1.578	6.31	0.354	0.57
211	1.606	6.42	3.000*	4.82*
213	0.369	1.48	0.127	0.20
214	0.444	1.78	0.101	0.16
215	0.637	2.55	0.101	0.16
216	0.812	3.25	0.092	0.15
217	1.320	5.28	0.875	1.40
219	1.547	6.19	3.000*	4.82*
220	1.536	6.14	3.000*	4.82*
221	1.428	5.71	0.327	0.52
222	1.362	5.45	3.000*	4.82*
223	1.316	5.26	3.000*	4.82*
224	1.304	5.22	3.000*	4.82*
225	1.178	4.71	2.398	3.85
226	1.256	5.14	3.000*	4.82*
227	1.335	5.34	3.000*	4.82*
229	1.409	5.60	3.000*	4.82*
234	1.421	5.92	3.000*	4.82*
235	0.351	1.40	0.257	0.41
236	0.475	1.90	0.245	0.39
237	1.134	4.54	0.351	0.56
238	1.056	4.38	1.074	1.72

Cut-off: 0.250 Cut-off: 0.623

* O.D. exceeded 3.000 and was out of range. The values given are therefore minimum values.

SUBSTITUTE SHEET

CLAIMS:

1. A peptide selected from:

5 (a) the group of amino acid sequences consisting of:

(1)

(I) Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X,

10 (20)

(7)

(II) Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X,

15 (26)

(8)

(18)

(IIA) Y-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Z-X,

20 (13)

(III) Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Z-X,

(32)

25 (37)

(IV) Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Z-X,

(56)

30

(49)

(V) Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Z-X,

5

(68)

(61)

(VI) Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Z-X, and

10

(80)

(73)

(VII) Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Z-X,

15

(92)

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated;

20

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids;

25

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

30

- (b) the variants of each of the above peptides (I) to (VII), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (I) to (VII) provided that said variant peptides are capable

of providing for immunological competition with at least one strain of HCV;
wherein the variant consisting of the sequence:

Met-Ser-Thr-Asn-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-
Pro-Gln, is excluded; and

- (c) fragments of peptides (I) to (VII) having at least 6 amino acids of any of the peptide
sequences 1-20, 7-26, 8-18, 13-32, 37-56, 49-68, 61-80 and 73-92 as defined in part
(a);

and said fragments being capable of providing for immunological
competition with at least one strain of HCV;

and provided that said peptides are different from any of those in the
following list of peptides:

- (1) Met-Ser-Thr-Asn-Pro-Lys-Pro-Gln-X₁-Lys, wherein X₁
represents Arg,
- (2) Pro-Lys-Pro-Gln-X₁-Lys-X₂-Lys-Arg-Asn-Thr-Asn-Arg-Arg-
Pro-Gln, wherein X₁ represents Arg, and X₂ represents Thr,
- (3) Gln-Asp-Val-Lys-Phe-Pro,
- (4) Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly,
- (5) Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg,
- (6) Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-
Thr-Ser-Glu-Arg-Ser,
- (7) Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser,
- (8) Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile,
- (9) Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg,
- (10) Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-
Asn-Thr-Asn-Arg-Arg-Pro-Gln, and
- (11) Gln-Arg-Lys-Thr-Lys-Arg.

2. A peptide composition comprising the peptide according to claim 1, and further comprising at least one peptide selected from:

(a) the group of amino acid sequences consisting of:

5

(1688)

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X,

(1707)

10

(1694)

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X,

(1713)

15

(1706)

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Z-X,

(1725)

20

(1712)

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X,

(1731)

25

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X,

(1737)

30

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X,

5

(1743)

(1730)

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

10

(1749)

(2263)

(XV) Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Z-X,

15

(2282)

(2275)

(XVI) Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Z-X,

20

(2294)

(2287)

(XVII) Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Z-X,

25

(2306)

(2299)

(XVIII) Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Z-X, and

30

(2318)

(2311)

(XIX) Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Z-X,

(2330)

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated;

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids;

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

(b) the variants of each of the above peptides (VIII) to (XIX), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (VIII) to (XIX) provided that said variant peptides are capable of providing for immunological competition with at least one strain of HCV; and

(c) fragments of peptides (VIII) to (XIX) having at least 6 amino acids of any of the peptide sequences 1688-1707, 1694-1713, 1706-1725, 1712-1731, 1718-1737, 1724-1743, 1730-1749, 2263-2282, 2275-2294, 2287-2306, 2299-2318 and 2311-2330, as defined in part (a),

and said fragments being capable of providing for immunological competition with at least one strain of HCV.

3. A peptide composition containing at least one of the following mixtures of peptides as defined in claim 1 or 2:

(a) peptides II, III, V, IX, and XVIII;

5

(b) peptides I, II, V, IX, XI, XVI, and XVIII;

(c) peptides II, III, IV, V, VIII, XI, XVI, and XVIII;

10 (d) peptides II, IX and XVIII; and

(e) peptides II, III, IV, and V.

4. The peptide or peptide composition according to any one of claims 1 to 3, wherein
15 said peptides are coupled N-terminally, C-terminally or internally to a carrier molecule for the purpose of raising antibodies or facilitating the adsorption of said peptides to a solid phase.

5. The peptide or peptide composition according to any one of claims 1 to 4, wherein
20 said peptides contain a detectable label.

6. The peptide or peptide composition according to any one of claims 1 to 5, wherein Y is H, or 1 to 10 amino acids and Z is a bond or 1 to 10 amino acids.

25 7. The peptide or peptide composition according to any one of claims 1 to 6, wherein Y is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

8. The peptide or peptide composition according to any one of claims 1 to 7, wherein Z
30 is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

9. The peptide or peptide composition according to any one of claims 1 to 5, wherein the group for coupling the peptide to a solid phase or carrier is selected from the group consisting of biotin and thioglycolic acid.

5 10. Use of the peptide or peptide composition according to any one of claims 1 to 9, for the incorporation into an immunoassay for detecting the presence of antibodies to Hepatitis C virus present in a body fluid.

11. A method for the *in vitro* detection of antibodies to Hepatitis C virus present in a
10 body fluid comprising at least the steps of:

(a) contacting body fluid of a person to be diagnosed with the peptide or peptide composition according to any one of claims 1 to 9, and,

15 (b) detecting the immunological complex formed between said antibodies and the peptide(s) used.

12. A kit for the detection of anti-hepatitis C virus antibodies in a body fluid, comprising at least the following components:

- 20 - the peptide or peptide composition according to any one of claims 1 to 9,
- means for detecting an immunological complex formed between said peptides and said antibodies.

25 13. The method according to claim 11, wherein said peptides are applied as lines on a nylon membrane.

14. The kit according to claim 12, wherein said peptides are applied as lines on a nylon membrane.

30

15. The kit according to claim 12, wherein said peptides, separately or in combination, are used to coat the wells of microtiter plates.

16. Use of the peptide or peptide composition according to any one of claims 1 to 9, for incorporation into a vaccine composition against HCV.

17. The peptide or peptide composition according to any one of claims 1 to 9, for raising
5 antibodies against HCV.

18. A peptide or peptide composition obtained by cyclizing any of the peptides of claims 1 to 2, or by coupling together two peptides of claims 1 to 2.

10 19. The peptide or peptide composition according to any one of claims 1 to 9, wherein said peptides are such as obtained by synthesizing the peptides of claims 1 to 2 directly on an oligo-lysine core in which both the alpha as well as the epsilon-amino groups of lysines are used as growth points for the peptides.

15 20. A peptide selected from:

(1688)

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-
Glu-Phe-Asp-Glu-Z-X,

20 (1707)

(1694)

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-
Glu-Cys-Ser-Gln-Z-X,

25 (1713)

(1706)

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-
Met-Met-Leu-Ala-Z-X,

(1725)

(1712)

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X,

(1731)

5

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X,

(1737)

10

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X, and

(1743)

15

(1730)

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

(1749)

20

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated;

25

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids;

30

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions.

21. The peptide according to claim 20, wherein said peptides are coupled N-terminally, C-terminally or internally to a carrier molecule for the purpose of raising antibodies or facilitating the adsorption of said peptides to a solid phase.

5 22. The peptide according to claim 20 or 21, wherein said peptides contain a detectable label.

23. The peptide according to any one of claims 20 to 22, wherein Y is H, or 1 to 10 amino acids and Z is a bond or 1 to 10 amino acids.

10

24. The peptide according to any one of claims 20 to 23, wherein Y is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

15 25. The peptide according to any one of claims 20 to 24, wherein Z is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

20 26. The peptide according to any one of claims 20 to 22, wherein the group for coupling the peptide to a solid phase or carrier is selected from the group consisting of biotin and thioglycolic acid.

25 27. Use of the peptide according to any one of claims 20 to 26, for the incorporation into an immunoassay for detecting the presence of antibodies to Hepatitis C virus present in a body fluid.

28. A method for the *in vitro* detection of antibodies to Hepatitis C virus present in a body fluid comprising at least the steps of:

30 (a) contacting body fluid of a person to be diagnosed with the peptide according to any one of claims 20 to 26, and,

(b) detecting an immunological complex formed between said antibodies and the peptide(s) used.

29. A kit for the detection of anti-hepatitis C virus antibodies in a body fluid,
5 comprising at least the following components:

- the peptide according to any of claims 20 to 26,
- a means for detecting an immunological complex formed between said
10 peptides and said antibodies.

30. The method according to claim 28, wherein said peptides are applied as lines on a nylon membrane.

15 31. The kit according to claim 29, wherein said peptides are applied as lines on a nylon membrane.

32. The kit according to claim 29, wherein said peptides, separately or in combination, are used to coat the wells of microtiter plates.

20 33. Use of the peptide according to any one of claims 20 to 26, for incorporation into a vaccine composition against HCV.

25 34. The peptide according to any one of claims 20 to 26, for raising antibodies against HCV.

35. A peptide or peptide composition obtained by cyclizing any of the peptides of claim 20 or by coupling together two peptides of claim 20.

30 36. The peptide according to any one of claims 20 to 26, wherein said peptides are such as obtained by synthesizing the peptides of claim 20 directly on an oligo-lysine core in which both the alpha as well as the epsilon-amino groups of lysines are used as growth joints for the peptides.

37. A process for preparing a peptide selected from:

(1688)

5 (VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-
Glu-Phe-Asp-Glu-Z-X,
(1707)

(1694)

10 (IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-
Glu-Cys-Ser-Gln-Z-X,
(1713)

(1706)

15 (X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-
Met-Met-Leu-Ala-Z-X,
(1725)

(1712)

20 (XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-
Phe-Lys-Gln-Lys-Z-X,
(1731)

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-
Gly-Leu-Leu-Gln-Z-X,
(1737)

(1724)

25 (XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-
Ser-Arg-Gln-Ala-Z-X, and
(1743)

(1730)

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X,

(1749)

5

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated;

10

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids;

15

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions, with said peptides being synthesized in solution or on a solid support employing t-butyloxycarbonyl- or 9-fluorenylmethoxy-carbonyl-protected activated amino acids.

20

38. A process for preparing a peptide composition comprising at least two peptides selected from:

(1688)

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X,

25

(1707)

(1694)

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X,

30

(1713)

(1706)

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-
Met-Met-Leu-Ala-Z-X,

5

(1725)

(1712)

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-
Phe-Lys-Gln-Lys-Z-X,

10

(1731)

(1718)

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-
Gly-Leu-Leu-Gln-Z-X,

(1737)

(1724)

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-
Ser-Arg-Gln-Ala-Z-X, and

15

(1743)

(1730)

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-
Ile-Ala-Pro-Ala-Z-X,

20

(1749)

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10
amino acids, a linker arm by which the peptide can be attached to a carrier or solid
phase comprising at least one amino acid and as many as 60 or is acetylated;

25

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be
attached to a carrier or solid phase comprising at least one amino acid and as many
as 60 amino acids;

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions, with said peptides being synthesized in solution or on a solid support employing t-butyloxycarbonyl- or 9-fluorenylmethoxy-carbonyl-protected activated amino acids and with said at least two peptides being mixed.

39. The process according to claim 37 or 38, further characterized in that said peptides are coupled N-terminally, C-terminally or internally to a carrier molecule for the purpose of raising antibodies or facilitating the adsorption of said peptides to a solid phase.

40. The process according to any one of claims 37 to 39, wherein said peptides contain a detectable label added to said peptide.

41. The process according to any one of claims 37 to 40, wherein Y is H, or 1 to 10 amino acids and Z is a bond or 1 to 10 amino acids.

42. The process according to any one of claims 37 to 41, wherein Y is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

43. The process according to any one of claims 37 to 42, wherein Z is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

44. The process according to any one of claims 37 to 40, wherein the group for coupling the peptide to a solid phase or carrier is selected from the group consisting of biotin and thioglycolic acid.

45. A process for the preparation of a kit for the detection of anti-hepatitis C virus antibodies in a body fluid, comprising gathering at least the following components:

- the peptide according to claim 20,
- a means for detecting an immunological complex formed between said peptides and said antibodies.

5

46. The process for the preparation of a kit according to claim 45, wherein said peptides are applied as lines on a nylon membrane.

47. The process for the preparation of a kit according to claim 45, wherein said peptides,
10 separately or in combination, are used to coat the wells of microtiter plates.

48. The process according to any one of claims 37 to 44, for incorporation into a vaccine composition against HCV.

15 49. The process according to any one of claims 37 to 44, for raising antibodies against HCV.

50. A process according to any one of claims 37 to 44, wherein said peptides are such as obtained by synthesizing the peptides of claim 20 directly on an oligo-lysine core in which
20 both the alpha as well as the epsilon-amino groups of lysines are used as growth points for the peptides.

51. A peptide selected from:

25 (a) the group of amino acid sequences consisting of:

(2263)

(XV) Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Z-X,

30

(2282)

(2275)

(XVI) Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Z-X,

(2294)

5

(2287)

(XVII) Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Z-X,

(2306)

10

(2299)

(XVIII) Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Z-X, and

(2318)

15

(2311)

(XIX) Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Z-X,

(2330)

20

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated;

25

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids;

30

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

(b) the variants of each of the above peptides (XV) to (XIX), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (XV) to (XIX) provided that said variant peptides are
 5 capable of providing for immunological competition with at least one strain of HCV; and

(c) fragments of peptides (XV) to (XIX) having at least 6 amino acids of any of the peptide sequences 2263-2282, 2275-2294, 2287-2306, 2299-2318 and 2311-2330 as defined in part (a); and said fragments being capable of providing for immunological competition
 10 with at least one strain of HCV, and provided that said peptides are different from the following list of peptides:

Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg (2265-2280),
 and

15 Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg (2280-2290).

52. A peptide composition comprising the peptide according to claim 51, and further comprising at least one peptide selected from:

20 (a) the group of amino acid sequences consisting of:

(1)

(I) Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X,

25 (20)

(7)

(II) Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X,

30 (26)

(8)

(18)

(IIA) Y-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Z-X,

(13)

5 (III) Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-
Gln-Ile-Val-Gly-Z-X,

(32)

(37)

10 (IV) Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-
Ser-Glu-Arg-Ser-Z-X,

(56)

(49)

15 (V) Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-
Ile-Pro-Lys-Val-Z-X,

(68)

(61)

20 (VI) Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-
Ala-Gln-Pro-Gly-Z-X,

(80)

(73)

25 (VII) Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-
Glu-Gly-Cys-Gly-Z-X,

(92)

(1688)

30 (VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-
Glu-Phe-Asp-Glu-Z-X,

(1707)

(1694)

5 (IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-
Glu-Cys-Ser-Gln-Z-X,

(1713)

(1706)

10 (X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-
Met-Met-Leu-Ala-Z-X,

(1725)

(1712)

15 (XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-
Phe-Lys-Gln-Lys-Z-X,

(1731)

(1718)

20 (XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-
Gly-Leu-Leu-Gln-Z-X,

(1737)

(1724)

25 (XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-
Ser-Arg-Gln-Ala-Z-X, and

(1743)

(1730)

30 (XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-
Ile-Ala-Pro-Ala-Z-X,

(1749)

wherein Y is H, a group for coupling the peptide to a solid phase or a carrier, 1 to 10 amino acids, a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 or is acetylated;

5

Z is a bond, 1 to 10 amino acids, or a linker arm by which the peptide can be attached to a carrier or solid phase comprising at least one amino acid and as many as 60 amino acids;

10

and X is NH₂, OH or a linkage involving either of these two groups; and provided that when Y or Z-X are (an) amino acid(s), they are different from any naturally occurring HCV flanking regions;

(b) the variants of each of the above peptides (I) to (XIV), with said variants presenting conservative as well as non-conservative amino acid substitutions accommodating for less than 35% strain-to-strain variation in HCV sequences with respect to each of the amino acid sequences (I) to (XIV) provided that said variant peptides are capable of providing for immunological competition with at least one strain of HCV; and

(c) fragments of peptides (I) to (XIV) having at least 6 amino acids of any of the peptide sequences 1-20, 7-26, 8-18, 13-32, 37-56, 49-68, 61-80, 73-92, 1688-1707, 1694-1713, 1706-1725, 1712-1731, 1718-1737, 1724-1743 and 1730-1749, as defined in part (a), and said fragments being capable of providing for immunological competition with at least one strain of HCV, provided that the combinations containing at least one peptide selected from the group of amino acid sequences consisting of I, II, IIA, III, IV, V, VI, VII and at least one peptide selected from the group of amino acid sequences consisting of VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX are excluded.

53. A peptide composition containing the following mixture of peptides as defined in claim 51 or 52:

30

(f) VIII, IX, XI, XIII, and XIX; and

(g) XV, XVI, XVII, XVIII, and XIX.

54. The peptide composition or peptide according to any one of claims 51 to 53, wherein said peptides are coupled N-terminally, C-terminally or internally to a carrier molecule for the purpose of raising antibodies or facilitating the adsorption of said peptides to a solid phase.

55. The peptide composition or peptide according to any one of claims 51 to 54, wherein said peptides contain a detectable label.

56. The peptide or peptide composition according to any one of claims 51 to 55, wherein Y is H, or 1 to 10 amino acids and Z is a bond or 1 to 10 amino acids.

57. The peptide or peptide composition according to any one of claims 51 to 56, wherein Y is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

58. The peptide or peptide composition according to any one of claims 51 to 57, wherein Z is an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamic acid and aspartic acid.

59. The peptide or peptide composition according to any one of claims 51 to 55, wherein the group for coupling the peptide to a solid phase or carrier is selected from the group consisting of biotin and thioglycolic acid.

60. Use of the peptide or peptide composition according to any one of claims 51 to 59, for the incorporation into an immunoassay for detecting the presence of antibodies to Hepatitis C virus present in a body fluid.

61. A method for the *in vitro* detection of antibodies to Hepatitis C virus present in a body fluid comprising at least the steps of:

(a) contacting said body fluid of a person to be diagnosed with the peptide composition or peptide according to any one of claims 51 to 59, and,

(b) detecting an immunological complex formed between said antibodies and the peptide(s) used.

62. A kit for the detection of anti-hepatitis C virus antibodies in a body fluid, comprising at least the following components:

- 10 - the peptide composition or peptide according to any one of claims 51 to 59,
- a means for detecting an immunological complex formed between said peptides and said antibodies.

15 63. The method according to claim 61, wherein said peptides are applied as lines on a nylon membrane.

64. The kit according to claim 62, wherein said peptides are applied as lines on a nylon membrane.

20

65. The kit according to claim 62, wherein said peptides, separately or in combination, are used to coat the wells of microtiter plates.

66. Use of the peptide or peptide composition according to any one of claims 51 to 59, for incorporation into a vaccine composition against HCV.

25

67. The peptide or peptide composition according to any one of claims 51 to 59, for raising antibodies against HCV.

30 68. A peptide or peptide composition obtained by cyclizing any of the peptides of claims 51 to 52 or by coupling together two peptides of claims 51 to 52.

69. The peptide or peptide composition according to any one of claims 51 to 59, wherein said peptides are such as obtained by synthesizing the peptides of claims 51 or 52 directly on an oligo-lysine core in which both the alpha as well as the epsilon-amino groups of lysines are used as growth points for the peptides.

5

70. The method according to any one of claims 11, 28 or 61, wherein said body fluid is serum or plasma.

71. A peptide comprising the amino acid sequence:

10

(XV) Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala, and

15

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

72. A peptide comprising the amino acid sequence:

20

(XVI) Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn, and

25

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

73. A peptide comprising the amino acid sequence:

30

(XVII) Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

74. A peptide comprising the amino acid sequence:

(XVIII) Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-
 5 Leu-Pro-Pro, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

10 75. A peptide comprising the amino acid sequence:

(XIX) Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-
 Arg-Lys-Lys, and

15 a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

76. A peptide consisting of the amino acid sequence:

20 (XV) Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala.

77. A peptide consisting of the amino acid sequence:

25 (XVI) Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn.

78. A peptide consisting of the amino acid sequence:

30 (XVII) Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr.

79. A peptide consisting of the amino acid sequence:

(XVIII)Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro.

5 80. A peptide consisting of the amino acid sequence:

(XIX) Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys.

10 81. The peptide according to any one of claims 71 to 80 wherein said peptide is cyclic.

82. A peptide selected from the group consisting of:

15 (a) a peptide consisting of a combination of at least two of amino acid sequences: II, III, V, IX and XVIII;

(b) a peptide consisting of a combination of at least two of amino acid sequences: I, II, V, IX, XI, XVI and XVIII;

20 (c) a peptide consisting of a combination of at least two of amino acid sequences: II, III, IV, V, VIII, XI, XVI and XVIII;

(d) a peptide consisting of a combination of at least two of amino acid sequences: II, IX and XVIII;

25 (e) a peptide consisting of a combination of at least two of amino acid sequences: II, III, IV and V;

30 (f) a peptide consisting of a combination of at least two of amino acid sequences: VIII, IX, XI, XIII and XIV; and

(g) a peptide consisting of a combination of at least two of amino acid sequences: XV, XVI, XVII, XVIII and XIX;

wherein the amino acid sequences I, II, III, IV, V are defined as in claim 1, and wherein the amino acid sequences VIII, IX, XI, XIII, XIV, XV, XVI, XVII, XVIII, and XIX are defined as in claim 2.

5

83. A peptide consisting of a combination of at least two peptides selected from the group consisting of:

10

(XV) Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala;

(XVI) Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn;

15

(XVII) Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr;

(XVIII) Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro; and

20

(XIX) Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys.

84. A peptide comprising the amino acid sequence:

25

(VIII) Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu, and

30

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

85. A peptide consisting of the amino acid sequence:

(VIII) Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu.

86. A peptide comprising the amino acid sequence:

5

(IX) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

10

87. A peptide consisting of the amino acid sequence:

(IX) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln.

15

88. A peptide consisting of the amino acid sequence:

(X) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala.

20

89. A peptide consisting of the amino acid sequence:

(XI) Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys.

25

90. A peptide consisting of the amino acid sequence:

(XII) Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln.

30

91. A peptide comprising the amino acid sequence:

(XIII) Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

92. A peptide consisting of the amino acid sequence:

(XIII) Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala.

93. The peptide according to any one of claims 84 to 92, wherein said peptide is coupled N-terminally, C-terminally or internally to a carrier molecule.

94. The peptide according to any one of claims 84 to 92, wherein said peptide contains a detectable label.

95. The peptide according to any one of claims 84 to 92, wherein said peptide has on its amino terminus an H, or one or more chemical linking groups, and has on its carboxy terminus an NH₂, or one or more chemical linking groups.

96. The peptide according to any one of claims 84 to 92, wherein said peptide is cyclic and is immunoreactive with HCV antibodies.

97. The peptide of any one of claims 84 to 92 wherein said peptide is cyclic.

98. A peptide comprising a linker arm which is 1 to 60 amino acids, and two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides having amino acid sequences VIII, IX, X, XI, XII, XIII, and XIV, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV; wherein the amino acid sequences VIII, IX, X, XI, XII, XIII and XIV are defined as in claim 2.

99. A peptide comprising two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides consisting of amino acid sequences VIII, IX, X, XI, XII, XIII, and XIV, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV;
 5 wherein the amino acid sequences VIII, IX, X, XI, XII, XIII and XIV are defined as in claim 2.

100. A peptide comprising the amino acid sequence:

10 (I) Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

15

101. A peptide comprising the amino acid sequence:

(II) Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly, and

20

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

102. A peptide comprising the amino acid sequence:

25

(III) Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly; and

30

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

103. A peptide comprising the amino acid sequence:

(IV) Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser; and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

104. A peptide comprising the amino acid sequence:

(V) Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

105. A peptide comprising the amino acid sequence:

(VI) Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

106. A peptide comprising the amino acid sequence:

(VII) Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly, and

a linker arm which is 1 to 60 amino acids and are different from any naturally occurring HCV flanking regions.

107. A peptide consisting of the amino acid sequence:

- (I) Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln.

108. A peptide consisting of the amino acid sequence:

5

- (II) Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly.

109. A peptide consisting of the amino acid sequence:

10

- (III) Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly.

110. A peptide consisting of the amino acid sequence:

15

- (IV) Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser.

111. A peptide consisting of the amino acid sequence:

20

- (V) Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val.

112. A peptide consisting of the amino acid sequence:

25

- (VI) Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly.

113. A peptide consisting of the amino acid sequence:

30

- (VII) Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly.

114. The peptide according to any one of claims 100 to 113, wherein said peptide is coupled N-terminally, C-terminally or internally to a carrier molecule.

115. The peptide according to any one of claims 91 to 97, wherein said peptide contains a
5 detectable label.

116. The peptide of any one of claims 100 to 113, wherein said peptide is cyclic.

117. The peptide of any one of claims 100 to 113, wherein said peptide is cyclic and is
10 immunoreactive with HCV antibodies.

118. The peptide of any one of claims 100 to 113, wherein said peptide has on its amino terminus an H, or one or more chemical linking groups, and has on its carboxy terminus an NH₂, or one or more chemical linking groups.

119. A peptide comprising a linker arm which is 1 to 60 amino acids, and two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides having amino acid sequences I, II, III, IV, V, VI, and VII, wherein said two or more peptides joined together is capable of providing immunological competition with at
15
20 least one strain of HCV; and wherein the amino acid sequences I, II, III, IV, V, VI and VII are defined as in claim 1.

120. A peptide comprising two or more peptides joined together, said two or more peptides being selected from the group consisting of peptides consisting of amino acid
25 sequences I, II, III, IV, V, VI, and VII, wherein said two or more peptides joined together is capable of providing immunological competition with at least one strain of HCV; wherein the amino acid sequences I, II, III, IV, V, VI, and VII are defined as in claim 1.

121. The method according to any one of claims 13, 30 and 63 wherein said nylon
30 membrane is cut into strips perpendicular to the direction of the peptide lines, thus allowing each strip to be incubated with an appropriately diluted serum sample from an individual.

122. The kit according to any one of claims 14, 31 and 64 wherein said nylon membrane is cut into strips perpendicular to the direction of the peptide lines, thus allowing each strip to be incubated with an appropriately diluted serum sample from an individual.

- 5 123. The process according to claim 45 wherein said nylon membrane is cut into strips perpendicular to the direction of the peptide lines, thus allowing each strip to be incubated with an appropriately diluted serum sample from an individual.

Fig. 1

Amino Acid Sequence of the Composite HCV_{HC-J1/CDC/CHI}

					5						10					15
1	Met	Ser	Thr	Ile	Pro	Lys	Pro	Gln	Arg	Lys	Thr	Lys	Arg	Asn	Thr	
16	Asn	Arg	Arg	Pro	Gln	Asp	Val	Lys	Phe	Pro	Gly	Gly	Gly	Gln	Ile	
31	Val	Gly	Gly	Val	Tyr	Leu	Leu	Pro	Arg	Arg	Gly	Pro	Arg	Leu	Gly	
46	Val	Arg	Ala	Thr	Arg	Lys	Thr	Ser	Glu	Arg	Ser	Gln	Pro	Arg	Gly	
61	Arg	Arg	Gln	Pro	Ile	Pro	Lys	Val	Arg	Arg	Pro	Glu	Gly	Arg	Thr	
76	Trp	Ala	Gln	Pro	Gly	Tyr	Pro	Trp	Pro	Leu	Tyr	Gly	Asn	Glu	Gly	
91	Cys	Gly	Trp	Ala	Gly	Trp	Leu	Leu	Ser	Pro	Arg	Gly	Ser	Arg	Pro	
106	Ser	Trp	Gly	Pro	Thr	Asp	Pro	Arg	Arg	Arg	Ser	Arg	Asn	Leu	Gly	
121	Lys	Val	Ile	Asp	Thr	Leu	Thr	Cys	Gly	Phe	Ala	Asp	Leu	Met	Gly	
136	Tyr	Ile	Pro	Leu	Val	Gly	Ala	Pro	Leu	Gly	Gly	Ala	Ala	Arg	Ala	
151	Leu	Ala	His	Gly	Val	Arg	Val	Leu	Glu	Asp	Gly	Val	Asn	Tyr	Ala	
166	Thr	Gly	Asn	Leu	Pro	Gly	Cys	Ser	Phe	Ser	Ile	Phe	Leu	Leu	Ala	
181	Leu	Leu	Ser	Cys	Leu	Thr	Val	Pro	Ala	Ser	Ala	Tyr	Gln	Val	Arg	
196	Asn	Ser	Thr	Gly	Leu	Tyr	His	Val	Thr	Asn	Asp	Cys	Pro	Asn	Ser	
211	Ser	Ile	Val	Tyr	Glu	Ala	His	Asp	Ala	Ile	Leu	His	Thr	Pro	Gly	
226	Cys	Val	Pro	Cys	Val	Arg	Glu	Gly	Asn	Val	Ser	Arg	Cys	Trp	Val	
241	Ala	Met	Thr	Pro	Thr	Val	Ala	Thr	Arg	Asp	Gly	Lys	Leu	Pro	Ala	
256	Thr	Gln	Leu	Arg	Arg	His	Ile	Asp	Leu	Leu	Val	Gly	Ser	Ala	Thr	
271	Leu	Cys	Ser	Ala	Leu	Tyr	Val	Gly	Asp	Leu	Cys	Gly	Ser	Val	Phe	
286	Leu	Ile	Gly	Gln	Leu	Phe	Thr	Phe	Ser	Pro	Arg	Arg	His	Trp	Thr	
301	Thr	Gln	Gly	Cys	Asn	Cys	Ser	Ile	Tyr	Pro	Gly	His	Ile	Thr	Gly	
316	His	Arg	Met	Ala	Trp	Asp	Met	Met	Met	Asn	Trp	Ser	Pro	Thr	Ala	
331	Ala	Leu	Val	Met	Ala	Gln	Leu	Leu	Arg	Ile	Pro	Gln	Ala	Ile	Leu	
346	Asp	Met	Ile	Ala	Gly	Ala	His	Trp	Gly	Val	Leu	Ala	Gly	Ile	Ala	
361	Tyr	Phe	Ser	Met	Val	Gly	Asn	Trp	Ala	Lys	Val	Leu	Val	Val	Leu	
376	Leu	Leu	Phe	Ala	Gly	Val	Asp	Ala	Glu	Thr	Ile	Val	Ser	Gly	Gly	
391	Gln	Ala	Ala	Arg	Ala	Met	Ser	Gly	Leu	Val	Ser	Leu	Phe	Thr	Pro	
406	Gly	Ala	Lys	Gln	Asn	Ile	Gln	Leu	Ile	Asn	Thr	Asn	Gly	Ser	Trp	
421	His	Ile	Asn	Ser	Thr	Ala	Leu	Asn	Cys	Asn	Glu	Ser	Leu	Asn	Thr	
436	Gly	Trp	Leu	Ala	Gly	Leu	Ile	Tyr	Gln	His	Lys	Phe	Asn	Ser	Ser	
451	Gly	Cys	Pro	Glu	Arg	Leu	Ala	Ser	Cys	Arg	Pro	Leu	Thr	Asp	Phe	
466	Asp	Gln	Gly	Trp	Gly	Pro	Ile	Ser	Tyr	Ala	Asn	Gly	Ser	Gly	Pro	
481	Asp	Gln	Arg	Pro	Tyr	Cys	Trp	His	Tyr	Pro	Pro	Lys	Pro	Cys	Gly	
496	Ile	Val	Pro	Ala	Lys	Ser	Val	Cys	Gly	Pro	Val	Tyr	Cys	Phe	Thr	
511	Pro	Ser	Pro	Val	Val	Val	Gly	Thr	Thr	Asp	Arg	Ser	Gly	Ala	Pro	
526	Thr	Tyr	Ser	Trp	Gly	Glu	Asn	Asp	Thr	Asp	Val	Phe	Val	Leu	Asn	
541	Asn	Thr	Arg	Pro	Pro	Leu	Gly	Asn	Trp	Phe	Gly	Cys	Thr	Trp	Met	
556	Asn	Ser	Thr	Gly	Phe	Thr	Lys	Val	Cys	Gly	Ala	Pro	Pro	Cys	Val	
571	Ile	Gly	Gly	Ala	Gly	Asn	Asn	Thr	Leu	His	Cys	Pro	Thr	Asp	Cys	
586	Phe	Arg	Lys	His	Pro	Asp	Ala	Thr	Tyr	Ser	Arg	Cys	Gly	Ser	Gly	
601	Pro	Trp	Ile	Thr	Pro	Arg	Cys	Leu	Val	Asp	Tyr	Pro	Tyr	Arg	Leu	
616	Trp	His	Tyr	Pro	Cys	Thr	Ile	Asn	Tyr	Thr	Ile	Phe	Lys	Ile	Arg	
631	Met	Tyr	Val	Gly	Gly	Val	Glu	His	Arg	Leu	Glu	Ala	Ala	Cys	Asn	
646	Trp	Thr	Arg	Gly	Glu	Arg	Cys	Asp	Leu	Glu	Asp	Arg	Asp	Arg	Ser	
661	Glu	Leu	Ser	Pro	Leu	Leu	Leu	Thr	Thr	Thr	Gln	Trp	Gln	Val	Leu	
676	Pro	Cys	Ser	Phe	Thr	Thr	Leu	Pro	Ala	Leu	Ser	Thr	Gly	Leu	Ile	
691	His	Leu	His	Gln	Asn	Ile	Val	Asp	Val	Gln	Tyr	Leu	Tyr	Gly	Val	

SUBSTITUTE SHEET

2/8

2074370

Fig. 1

Continued

706	Gly	Ser	Ser	Ile	Ala	Ser	Trp	Ala	Ile	Lys	Trp	Glu	Tyr	Val	Val
721	Leu	Leu	Phe	Leu	Leu	Leu	Ala	Asp	Ala	Arg	Val	Cys	Ser	Cys	Leu
736	Trp	Met	Met	Leu	Leu	Ile	Ser	Gln	Ala	Glu	Ala	Ala	Leu	Glu	Asn
751	Leu	Val	Ile	Leu	Asn	Ala	Ala	Ser	Leu	Ala	Gly	Thr	His	Gly	Leu
766	Val	Ser	Phe	Leu	Val	Phe	Phe	Cys	Phe	Ala	Trp	Tyr	Leu	Lys	Gly
781	Lys	Trp	Val	Pro	Gly	Ala	Val	Tyr	Thr	Phe	Tyr	Gly	Met	Trp	Pro
796	Leu	Leu	Leu	Leu	Leu	Leu	Ala	Leu	Pro	Gln	Arg	Ala	Tyr	Ala	Leu
811	Asp	Thr	Glu	Val	Ala	Ala	Ser	Cys	Gly	Gly	Val	Val	Leu	Val	Gly
826	Leu	Met	Ala	Leu	Thr	Leu	Ser	Pro	Tyr	Tyr	Lys	Arg	Tyr	Ile	Ser
841	Trp	Cys	Leu	Trp	Trp	Leu	Gln	Tyr	Phe	Leu	Thr	Arg	Val	Glu	Ala
856	Gln	Leu	His	Val	Trp	Ile	Pro	Pro	Leu	Asn	Val	Arg	Gly	Gly	Arg
871	Asp	Ala	Val	Ile	Leu	Leu	Met	Cys	Ala	Val	His	Pro	Thr	Leu	Val
886	Phe	Asp	Ile	Thr	Lys	Leu	Leu	Leu	Ala	Val	Phe	Gly	Pro	Leu	Trp
901	Ile	Leu	Asp	Ala	Ser	Leu	Leu	Lys	Val	Pro	Tyr	Phe	Val	Arg	Val
916	Gln	Gly	Leu	Leu	Arg	Phe	Cys	Ala	Leu	Ala	Arg	Lys	Met	Ile	Gly
931	Gly	His	Tyr	Val	Gln	Met	Val	Ile	Ile	Lys	Leu	Gly	Ala	Leu	Thr
946	Gly	Thr	Tyr	Val	Tyr	Asn	His	Leu	Thr	Pro	Leu	Arg	Asp	Trp	Ala
961	His	Asn	Gly	Leu	Arg	Asp	Leu	Ala	Val	Ala	Val	Glu	Pro	Val	Val
976	Phe	Ser	Gln	Met	Glu	Thr	Lys	Leu	Ile	Thr	Trp	Gly	Ala	Asp	Thr
991	Ala	Ala	Cys	Gly	Asp	Ile	Ile	Asn	Gly	Leu	Pro	Val	Ser	Ala	Arg
1006	Arg	Gly	Arg	Glu	Ile	Leu	Leu	Gly	Pro	Ala	Asp	Gly	Met	Val	Ser
1021	Lys	Gly	Trp	Arg	Leu	Leu	Ala	Pro	Ile	Thr	Ala	Tyr	Ala	Gln	Gln
1036	Thr	Arg	Gly	Leu	Leu	Gly	Cys	Ile	Ile	Thr	Ser	Leu	Thr	Gly	Arg
1051	Asp	Lys	Asn	Gln	Val	Glu	Gly	Glu	Val	Gln	Ile	Val	Ser	Thr	Ala
1066	Ala	Gln	Thr	Phe	Leu	Ala	Thr	Cys	Ile	Asn	Gly	Val	Cys	Trp	Thr
1081	Val	Tyr	His	Gly	Ala	Gly	Thr	Arg	Thr	Ile	Ala	Ser	Pro	Lys	Gly
1096	Pro	Val	Ile	Gln	Met	Tyr	Thr	Asn	Val	Asp	Gln	Asp	Leu	Val	Gly
1111	Trp	Pro	Ala	Pro	Gln	Gly	Ser	Arg	Ser	Leu	Thr	Pro	Cys	Thr	Cys
1126	Gly	Ser	Ser	Asp	Leu	Tyr	Leu	Val	Thr	Arg	His	Ala	Asp	Val	Ile
1141	Pro	Val	Arg	Arg	Arg	Gly	Asp	Ser	Arg	Gly	Ser	Leu	Leu	Ser	Pro
1156	Arg	Pro	Ile	Ser	Tyr	Leu	Lys	Gly	Ser	Ser	Gly	Gly	Pro	Leu	Leu
1171	Cys	Pro	Ala	Gly	His	Ala	Val	Gly	Ile	Phe	Arg	Ala	Ala	Val	Cys
1186	Thr	Arg	Gly	Val	Ala	Lys	Ala	Val	Asp	Phe	Ile	Pro	Val	Glu	Asn
1201	Leu	Glu	Thr	Thr	Met	Arg	Ser	Pro	Val	Phe	Trp	Asp	Asn	Ser	Ser
1216	Pro	Pro	Val	Val	Pro	Gln	Ser	Phe	Gln	Val	Ala	His	Leu	His	Ala
1231	Pro	Thr	Gly	Ser	Gly	Lys	Ser	Thr	Lys	Val	Pro	Ala	Ala	Tyr	Ala
1246	Ala	Gln	Gly	Tyr	Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala
1261	Thr	Leu	Gly	Phe	Gly	Ala	Tyr	Met	Ser	Lys	Ala	His	Gly	Ile	Asp
1276	Pro	Asn	Ile	Arg	Thr	Gly	Val	Arg	Thr	Ile	Thr	Thr	Gly	Ser	Pro
1291	Ile	Thr	Tyr	Ser	Thr	Tyr	Gly	Lys	Phe	Leu	Ala	Asp	Gly	Gly	Cys
1306	Ser	Gly	Gly	Ala	Tyr	Asp	Ile	Ile	Ile	Cys	Asp	Glu	Cys	His	Ser
1321	Thr	Asp	Ala	Thr	Ser	Ile	Leu	Gly	Ile	Gly	Thr	Val	Leu	Asp	Gln
1336	Ala	Glu	Thr	Ala	Gly	Ala	Arg	Leu	Val	Val	Leu	Ala	Thr	Ala	Thr
1351	Pro	Pro	Gly	Ser	Val	Thr	Val	Pro	His	Pro	Asn	Ile	Glu	Glu	Val
1366	Ala	Leu	Ser	Thr	Thr	Gly	Glu	Ile	Pro	Phe	Tyr	Gly	Lys	Ala	Ile
1381	Pro	Leu	Glu	Val	Ile	Lys	Gly	Gly	Arg	His	Leu	Ile	Phe	Cys	His
1396	Ser	Lys	Lys	Lys	Cys	Asp	Glu	Leu	Ala	Ala	Lys	Leu	Val	Ala	Leu
1411	Gly	Ile	Asn	Ala	Val	Ala	Tyr	Tyr	Arg	Gly	Leu	Asp	Val	Ser	Val
1426	Ile	Pro	Thr	Ser	Gly	Asp	Val	Val	Val	Val	Ala	Thr	Asp	Ala	Leu
1441	Met	Thr	Gly	Tyr	Thr	Gly	Asp	Phe	Asp	Ser	Val	Ile	Asp	Cys	Asn
1456	Thr	Cys	Val	Thr	Gln	Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe
1471	Thr	Ile	Glu	Thr	Ile	Thr	Leu	Pro	Gln	Asp	Ala	Val	Ser	Arg	Thr
1486	Gln	Arg	Arg	Gly	Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg
1501	Phe	Val	Ala	Pro	Gly	Glu	Arg	Pro	Ser	Gly	Met	Phe	Asp	Ser	Ser
1516	Val	Leu	Cys	Glu	Cys	Tyr	Asp	Ala	Gly	Cys	Ala	Trp	Tyr	Glu	Leu

SUBSTITUTE SHEET

3/8

2074370

Fig. 1

Continued

1531	Thr	Pro	Ala	Glu	Thr	Thr	Val	Arg	Leu	Arg	Ala	Tyr	Met	Asn	Thr
1546	Pro	Gly	Leu	Pro	Val	Cys	Gln	Asp	His	Leu	Glu	Phe	Trp	Glu	Gly
1561	Val	Phe	Thr	Gly	Leu	Thr	His	Ile	Asp	Ala	His	Phe	Leu	Ser	Gln
1576	Thr	Lys	Gly	Ser	Gly	Glu	Asn	Leu	Pro	Tyr	Leu	Val	Ala	Tyr	Gln
1591	Ala	Thr	Val	Cys	Ala	Arg	Ala	Gln	Ala	Pro	Pro	Pro	Ser	Trp	Asp
1606	Gln	Met	Trp	Lys	Cys	Leu	Ile	Arg	Leu	Lys	Pro	Thr	Leu	His	Gly
1621	Pro	Thr	Pro	Leu	Leu	Tyr	Arg	Leu	Gly	Ala	Val	Gln	Asn	Glu	Ile
1636	Thr	Leu	Thr	His	Pro	Val	Thr	Lys	Tyr	Ile	Met	Thr	Cys	Met	Ser
1651	Ala	Asp	Leu	Glu	Val	Val	Thr	Ser	Thr	Trp	Val	Leu	Val	Gly	Gly
1666	Val	Leu	Ala	Ala	Leu	Ala	Ala	Tyr	Cys	Leu	Ser	Thr	Gly	Cys	Val
1681	Val	Ile	Val	Gly	Arg	Val	Val	Leu	Ser	Gly	Lys	Pro	Ala	Ile	Ile
1696	Pro	Asp	Arg	Glu	Val	Leu	Tyr	Arg	Glu	Phe	Asp	Glu	Met	Glu	Glu
1711	Cys	Ser	Gln	His	Leu	Pro	Tyr	Ile	Glu	Gln	Gly	Met	Met	Leu	Ala
1726	Glu	Gln	Phe	Lys	Gln	Lys	Ala	Leu	Gly	Leu	Leu	Gln	Thr	Ala	Ser
1741	Arg	Gln	Ala	Glu	Val	Ile	Ala	Pro	Ala	Val	Gln	Thr	Asn	Trp	Gln
1756	Lys	Leu	Glu	Thr	Phe	Trp	Ala	Lys	His	Met	Trp	Asn	Phe	Ile	Ser
1771	Gly	Ile	Gln	Tyr	Leu	Ala	Gly	Leu	Ser	Thr	Leu	Pro	Gly	Asn	Pro
1786	Ala	Ile	Ala	Ser	Leu	Met	Ala	Phe	Thr	Ala	Ala	Val	Thr	Ser	Pro
1801	Leu	Thr	Thr	Ser	Gln	Thr	Leu	Leu	Phe	Asn	Ile	Leu	Gly	Gly	Trp
1816	Val	Ala	Ala	Gln	Leu	Ala	Ala	Pro	Gly	Ala	Ala	Thr	Ala	Phe	Val
1831	Gly	Ala	Gly	Leu	Ala	Gly	Ala	Ala	Ile	Gly	Ser	Val	Gly	Leu	Gly
1846	Lys	Val	Leu	Ile	Asp	Ile	Leu	Ala	Gly	Tyr	Gly	Ala	Gly	Val	Ala
1861	Gly	Ala	Leu	Val	Ala	Phe	Lys	Ile	Met	Ser	Gly	Glu	Val	Pro	Ser
1876	Thr	Glu	Asp	Leu	Val	Asn	Leu	Leu	Pro	Ala	Ile	Leu	Ser	Pro	Gly
1891	Ala	Leu	Val	Val	Gly	Val	Val	Cys	Ala	Ala	Ile	Leu	Arg	Arg	His
1906	Val	Gly	Pro	Gly	Glu	Gly	Ala	Val	Gln	Trp	Met	Asn	Arg	Leu	Ile
1921	Ala	Phe	Ala	Ser	Arg	Gly	Asn	His	Val	Ser	Pro	Thr	His	Tyr	Val
1936	Pro	Glu	Ser	Asp	Ala	Ala	Ala	Arg	Val	Thr	Ala	Ile	Leu	Ser	Ser
1951	Leu	Thr	Val	Thr	Gln	Leu	Leu	Arg	Arg	Leu	His	Gln	Trp	Ile	Ser
1966	Ser	Glu	Cys	Thr	Thr	Pro	Cys	Ser	Gly	Ser	Trp	Leu	Arg	Asp	Ile
1981	Trp	Asp	Trp	Ile	Cys	Glu	Val	Leu	Ser	Asp	Phe	Lys	Thr	Trp	Leu
1996	Lys	Ala	Lys	Leu	Met	Pro	Gln	Leu	Pro	Gly	Ile	Pro	Phe	Val	Ser
2011	Cys	Gln	Arg	Gly	Tyr	Lys	Gly	Val	Trp	Arg	Val	Asp	Gly	Ile	Met
2026	His	Thr	Arg	Cys	His	Cys	Gly	Ala	Glu	Ile	Thr	Gly	His	Val	Lys
2041	Asn	Gly	Thr	Met	Arg	Ile	Val	Gly	Pro	Arg	Thr	Cys	Arg	Asn	Met
2056	Trp	Ser	Gly	Thr	Phe	Pro	Ile	Asn	Ala	Tyr	Thr	Thr	Gly	Pro	Cys
2071	Thr	Arg	Leu	Pro	Ala	Pro	Asn	Tyr	Thr	Phe	Ala	Leu	Trp	Arg	Val
2086	Ser	Ala	Glu	Glu	Tyr	Val	Glu	Ile	Arg	Gln	Val	Gly	Asp	Phe	His
2101	Tyr	Val	Thr	Gly	Met	Thr	Thr	Asp	Asn	Leu	Lys	Cys	Pro	Cys	Gln
2116	Val	Pro	Ser	Pro	Glu	Phe	Phe	Thr	Glu	Leu	Asp	Gly	Val	Arg	Leu
2131	His	Arg	Phe	Ala	Pro	Pro	Cys	Lys	Pro	Leu	Leu	Arg	Glu	Glu	Val
2146	Ser	Phe	Arg	Val	Gly	Leu	His	Glu	Tyr	Pro	Val	Gly	Ser	Gln	Leu
2161	Pro	Cys	Glu	Pro	Glu	Pro	Asp	Val	Ala	Val	Leu	Thr	Ser	Met	Leu
2176	Thr	Asp	Pro	Ser	His	Ile	Thr	Ala	Glu	Ala	Ala	Gly	Arg	Arg	Leu
2191	Ala	Arg	Gly	Ser	Pro	Pro	Ser	Val	Ala	Ser	Ser	Ser	Ala	Ser	Gln
2206	Leu	Ser	Ala	Pro	Ser	Leu	Lys	Ala	Thr	Cys	Thr	Ala	Asn	His	Asp
2221	Ser	Pro	Asp	Ala	Glu	Leu	Ile	Glu	Ala	Asn	Leu	Leu	Trp	Arg	Gln
2236	Glu	Met	Gly	Gly	Asn	Ile	Thr	Arg	Val	Glu	Ser	Glu	Asn	Lys	Val
2251	Val	Ile	Leu	Asp	Ser	Phe	Asp	Pro	Leu	Val	Ala	Glu	Glu	Asp	Glu
2266	Arg	Glu	Ile	Ser	Val	Pro	Ala	Glu	Ile	Leu	Arg	Lys	Ser	Arg	Arg
2281	Phe	Ala	Gln	Ala	Leu	Pro	Val	Trp	Ala	Arg	Pro	Asp	Tyr	Asn	Pro
2296	Pro	Leu	Val	Glu	Thr	Trp	Lys	Lys	Pro	Asp	Tyr	Glu	Pro	Pro	Val
2311	Val	His	Gly	Cys	Pro	Leu	Pro	Pro	Pro	Lys	Ser	Pro	Pro	Val	Pro
2326	Pro	Pro	Arg	Lys	Lys	Arg	Thr	Val	Val	Leu	Thr	Glu	Ser	Thr	Leu
2341	Ser	Thr	Ala	Leu	Ala	Glu	Leu	Ala	Thr	Arg	Ser	Phe	Gly	Ser	Ser

SUBSTITUTE SHEET

4/8

2074370

Fig. 1

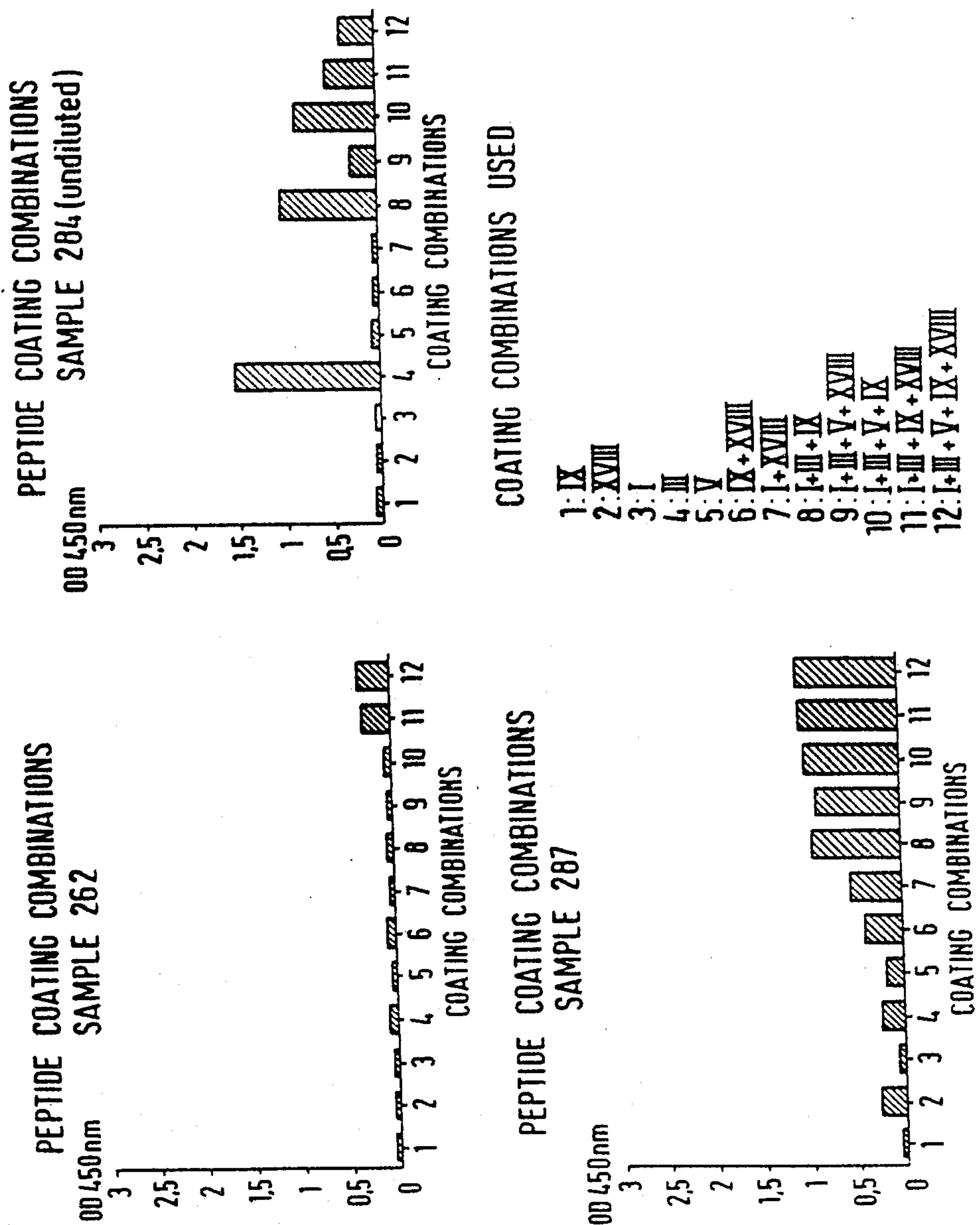
Continued

2356	Ser	Thr	Ser	Gly	Ile	Thr	Gly	Asp	Asn	Thr	Thr	Thr	Ser	Ser	Glu
2371	Pro	Ala	Pro	Ser	Gly	Cys	Pro	Pro	Asp	Ser	Asp	Ala	Glu	Ser	Tyr
2386	Ser	Ser	Met	Pro	Pro	Leu	Glu	Gly	Glu	Pro	Gly	Asp	Pro	Asp	Leu
2401	Ser	Asp	Gly	Ser	Trp	Ser	Thr	Val	Ser	Ser	Glu	Ala	Asn	Ala	Glu
2416	Asp	Val	Val	Cys	Cys	Ser	Met	Ser	Tyr	Ser	Trp	Thr	Gly	Ala	Cys
2431	Val	Thr	Pro	Cys	Ala	Ala	Glu	Glu	Gln	Lys	Leu	Pro	Ile	Asn	Ala
2446	Leu	Ser	Asn	Ser	Leu	Leu	Arg	His	His	Asn	Leu	Val	Tyr	Ser	Thr
2461	Thr	Ser	Arg	Ser	Ala	Cys	Gln	Arg	Gln	Lys	Lys	Val	Thr	Phe	Asp
2476	Arg	Leu	Gln	Val	Leu	Asp	Ser	His	Tyr	Gln	Asp	Val	Leu	Lys	Glu
2491	Val	Lys	Ala	Ala	Ala	Ser	Lys	Val	Lys	Ala	Asn	Leu	Leu	Ser	Val
2506	Glu	Glu	Ala	Cys	Ser	Leu	Thr	Pro	Pro	His	Ser	Ala	Lys	Ser	Lys
2521	Phe	Gly	Tyr	Gly	Ala	Lys	Asp	Val	Arg	Cys	His	Ala	Arg	Lys	Ala
2536	Val	Thr	His	Ile	Asn	Ser	Val	Trp	Lys	Asp	Leu	Leu	Glu	Asp	Asn
2551	Val	Thr	Pro	Ile	Asp	Thr	Thr	Ile	Met	Ala	Lys	Asn	Glu	Val	Phe
2566	Cys	Val	Gln	Pro	Glu	Lys	Gly	Gly	Arg	Lys	Pro	Ala	Arg	Leu	Ile
2581	Val	Phe	Pro	Asp	Leu	Gly	Val	Arg	Val	Cys	Glu	Lys	Met	Ala	Leu
2596	Tyr	Asp	Val	Val	Thr	Lys	Leu	Pro	Leu	Ala	Val	Met	Gly	Ser	Ser
2611	Tyr	Gly	Phe	Gln	Tyr	Ser	Pro	Gly	Gln	Arg	Val	Glu	Phe	Leu	Val
2626	Gln	Ala	Trp	Lys	Ser	Lys	Lys	Thr	Pro	Met	Gly	Phe	Ser	Tyr	Asp
2641	Thr	Arg	Cys	Phe	Asp	Ser	Thr	Val	Thr	Glu	Ser	Asp	Ile	Arg	Thr
2656	Glu	Glu	Ala	Ile	Tyr	Gln	Cys	Cys	Asp	Leu	Asp	Pro	Gln	Ala	Arg
2671	Val	Ala	Ile	Lys	Ser	Leu	Thr	Glu	Arg	Leu	Tyr	Val	Gly	Gly	Pro
2686	Leu	Thr	Asn	Ser	Arg	Gly	Glu	Asn	Cys	Gly	Tyr	Arg	Arg	Cys	Arg
2701	Ala	Ser	Gly	Val	Leu	Thr	Thr	Ser	Cys	Gly	Asn	Thr	Leu	Thr	Cys
2716	Tyr	Ile	Lys	Ala	Arg	Ala	Ala	Cys	Arg	Ala	Ala	Gly	Leu	Gln	Asp
2731	Cys	Thr	Met	Leu	Val	Cys	Gly	Asp	Asp	Leu	Val	Val	Ile	Cys	Glu
2746	Ser	Ala	Gly	Val	Gln	Glu	Asp	Ala	Ala	Ser	Leu	Arg	Ala	Phe	Thr
2761	Glu	Ala	Met	Thr	Arg	Tyr	Ser	Ala	Pro	Pro	Gly	Asp	Pro	Pro	Gln
2776	Pro	Glu	Tyr	Asp	Leu	Glu	Leu	Ile	Thr	Ser	Cys	Ser	Ser	Asn	Val
2791	Ser	Val	Ala	His	Asp	Gly	Ala	Gly	Lys	Arg	Val	Tyr	Tyr	Leu	Thr
2806	Arg	Asp	Pro	Thr	Thr	Pro	Leu	Ala	Arg	Ala	Ala	Trp	Glu	Thr	Ala
2821	Arg	His	Thr	Pro	Val	Asn	Ser	Trp	Leu	Gly	Asn	Ile	Ile	Met	Phe
2836	Ala	Pro	Thr	Leu	Trp	Ala	Arg	Met	Ile	Leu	Met	Thr	His	Phe	Phe
2851	Ser	Val	Leu	Ile	Ala	Arg	Asp	Gln	Leu	Glu	Gln	Ala	Leu	Asp	Cys
2866	Glu	Ile	Tyr	Gly	Ala	Cys	Tyr	Ser	Ile	Glu	Pro	Leu	Asp	Leu	Pro
2881	Pro	Ile	Ile	Gln	Arg	Leu	Gly	Cys	Pro	Glu	Arg	Leu	Ala	Ser	

SUBSTITUTE SHEET

Fig. 2

Antibody binding to individual peptides and various mixtures in an ELISA assay.



SUBSTITUTE SHEET

Fig. 2
Continued

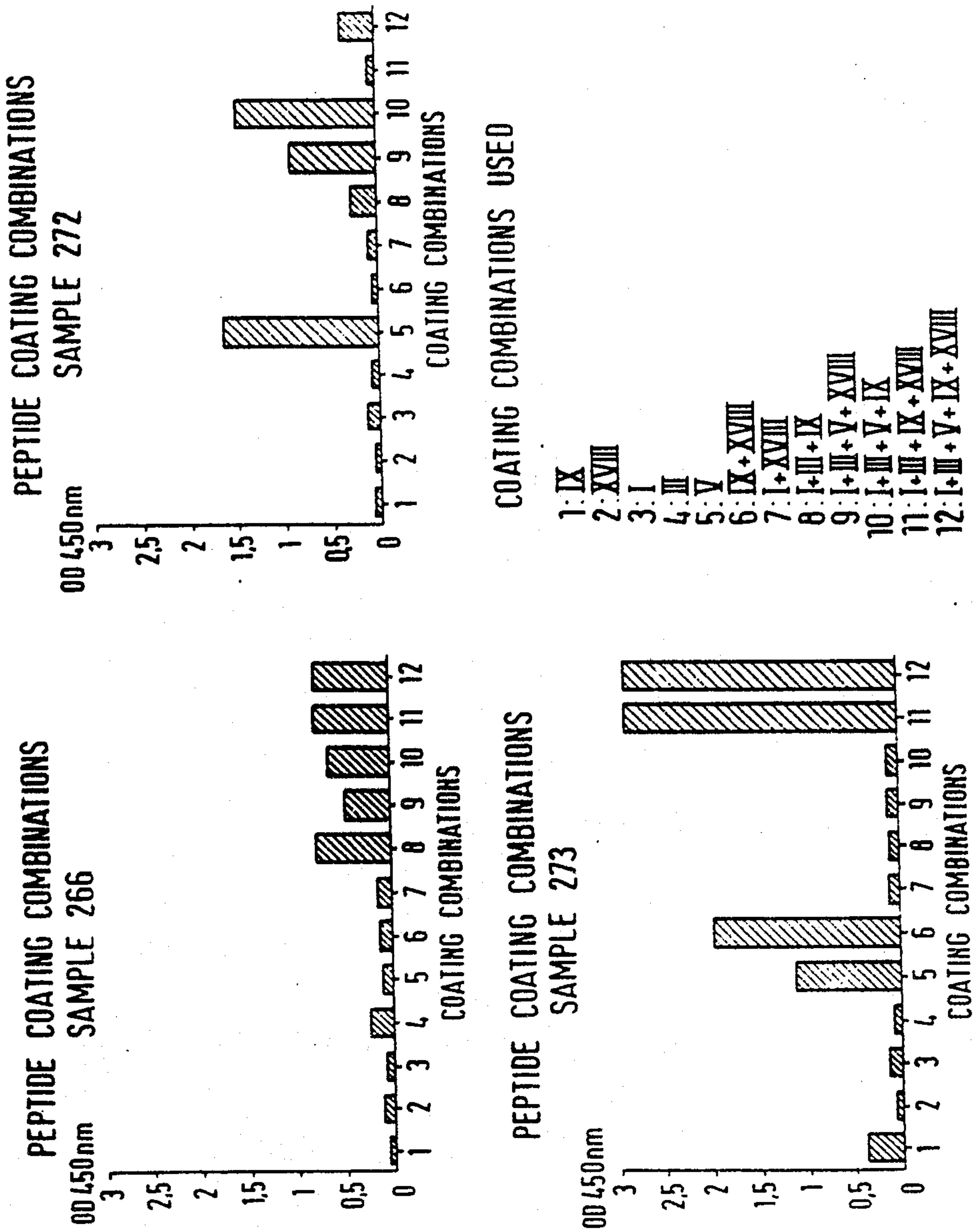


Fig. 2
Continued

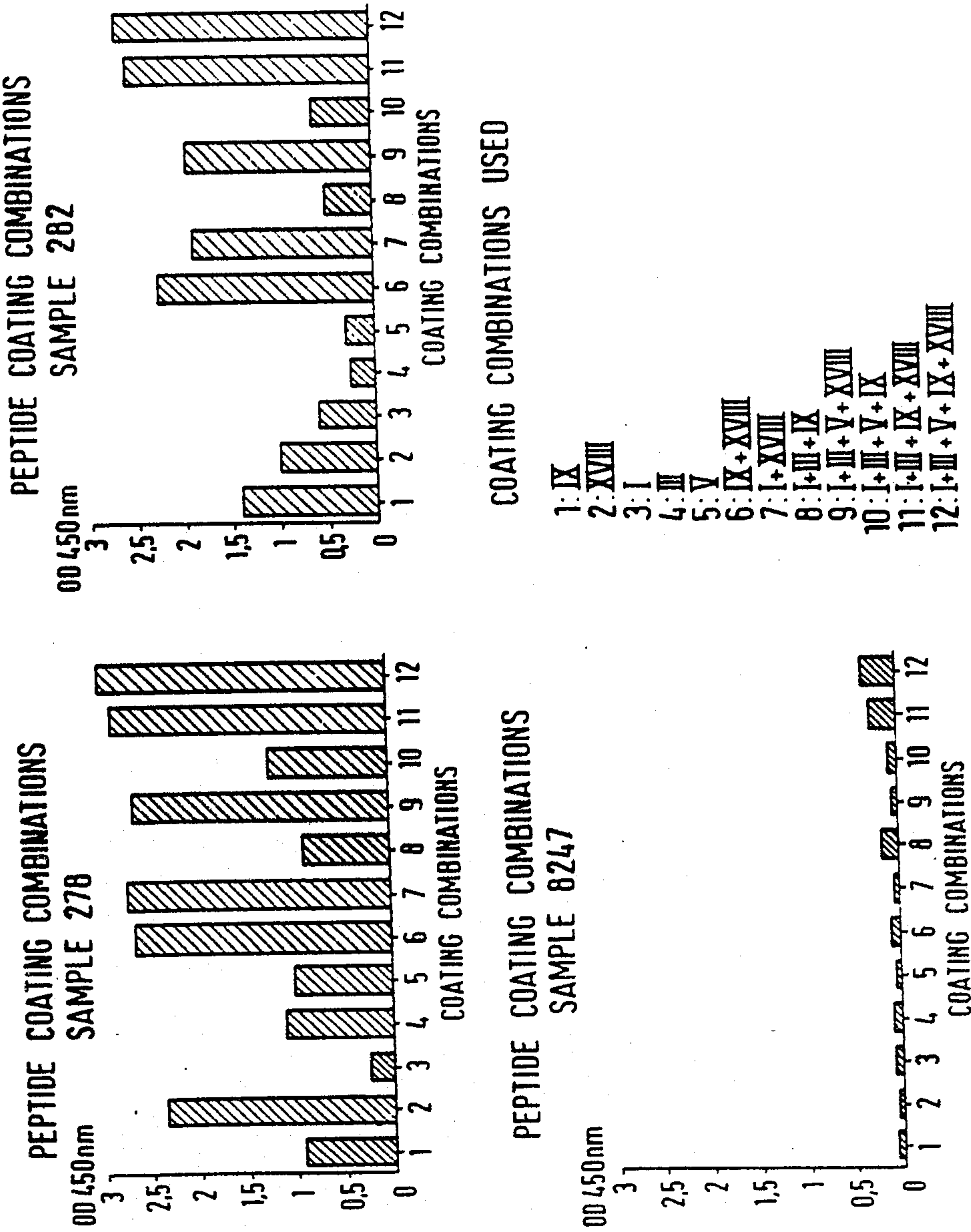
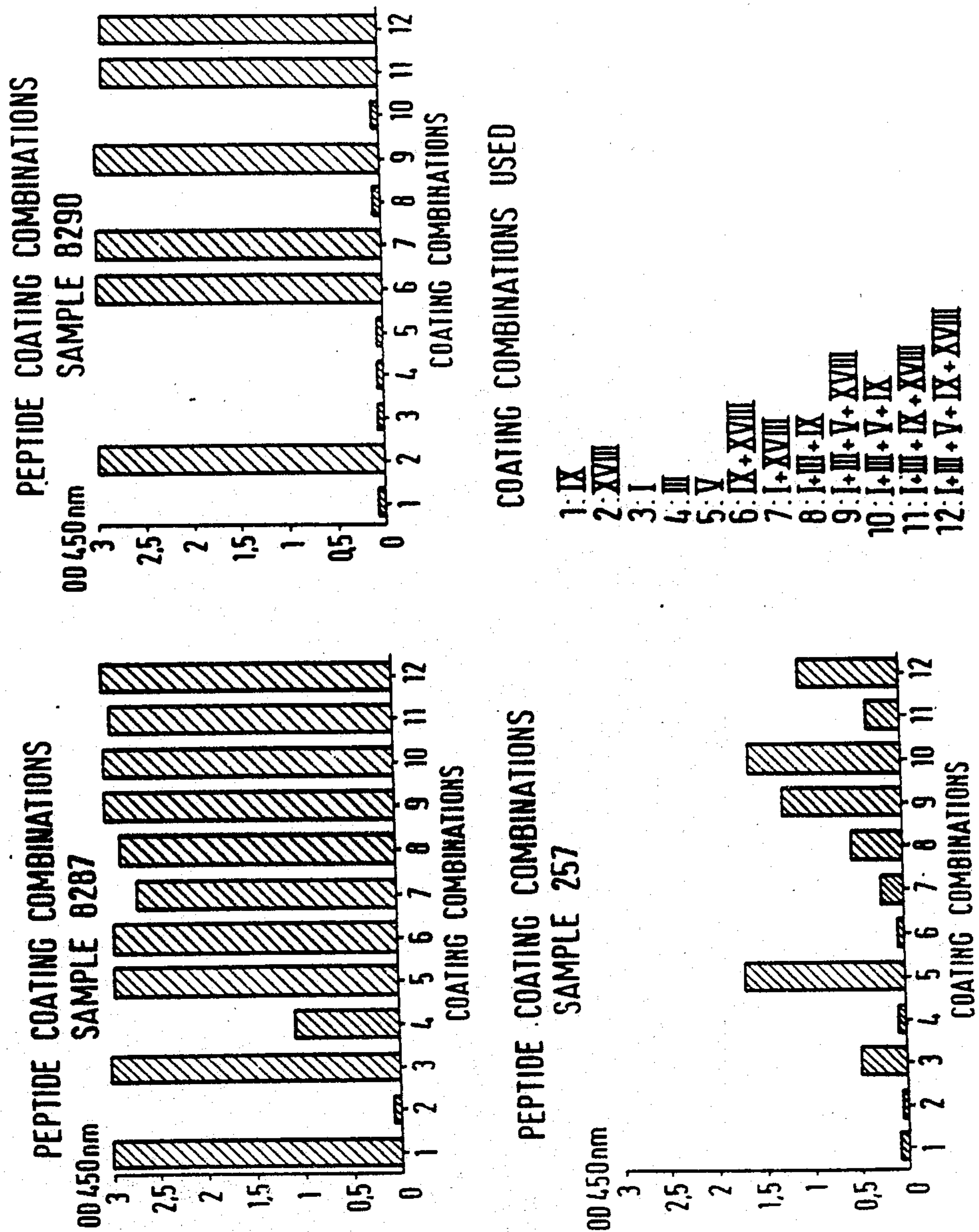


Fig. 2
Continued



SUBSTITUTE SHEET