Description

This invention relates to the synthesis of peptides, and to immobilized peptide-solid support preparations.

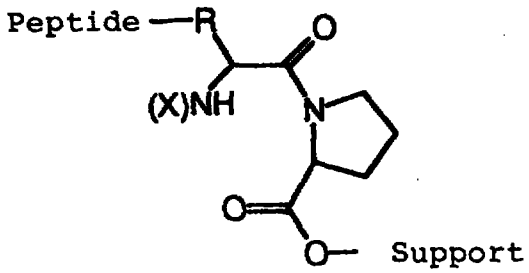
In International Patent Application PCT/AU84/00039 there is disclosed a method for the simultaneous synthesis of large numbers of peptides. This method is based on the solid phase synthesis of peptides onto polyethylene rods or pins as solid supports. In that patent application, it is pointed out that the technique would be useful for systematically determining the continuous antigenic determinants of various antigens by synthesizing all of the overlapping peptides which could be made from the sequence of the antigen and then testing for the ability of the peptide to bind to a binding entity such as an antibody. In effect, there is disclosed a method for a systematic and very rapid determination of the continuous B cell epitopes of an antigen.

Subsequent research using this method has led to further developments which enhance the usefulness of peptides synthesized on inert rods or similar supports. These further developments relate to modifications to the earlier invention which extend its use in systematic studies in the field of immunology. The modifications of the earlier invention also allow for harvesting of the peptide from the solid support.

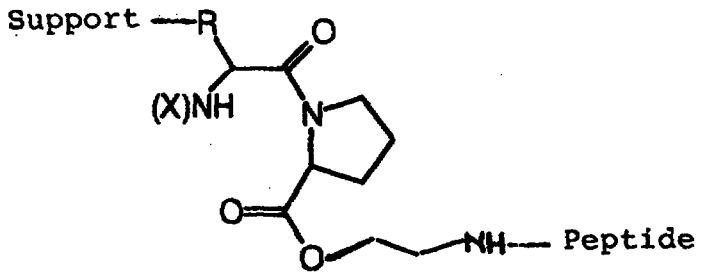
The present invention relates to the synthesis of peptides on solid supports, to immobilized peptide-solid support preparations and to the cleavage of immobilized peptide from a rod or pin or other solid support on which it has been synthesised. Cleavage is achieved by introducing a cleavable link into the peptide-solid support preparation between the support and the peptide. The use of cleavable ester links is well-established in solid-phase peptide synthesis and is described for example in Neurath et al. The Peptides, third edition, Volume II, Academic Press, New York, 1976. In the present invention a link provides the site where cleavage will occur after the synthesis and the deprotection of sidechains of the peptides has been carried out. It will be appreciated that such special groups need to be selected with care. Many of the techniques developed for cleavage in conventional peptide synthesis will be unsuitable for use in this system. Either they are too dangerous to be used except by the most highly skilled technicians (an example is the traditional use of hydrogen fluoride as a cleavage agent) or they require extensive purification to remove unused reactant and byproducts of reaction, or both. Ideally the final peptide containing solutions should be able to be used directly in a variety of biological tests without purification and without any residual toxicity or containing any other component which might interfere in a test.

In accordance with the present invention, a cleavable link is added during the synthesis between the solid support and the desired peptide, preferably between the solid support and the carboxy terminus of the peptide.

Specifically the present invention comprises an immobilized peptide-solid support preparation which comprises a compound of the general formula:-



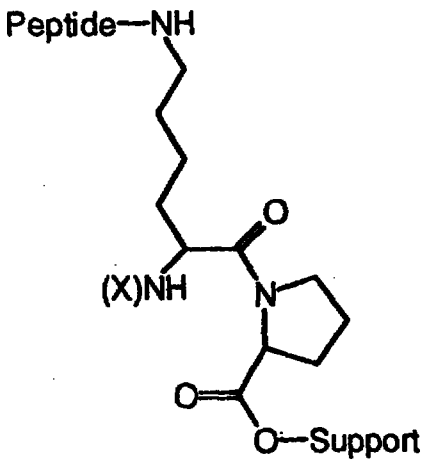
or of the general formula:-



wherein X is a protecting group and R is an amino acid side chain having a functional group.

This aspect of the present invention includes the use of an ester link, a proline residue and a bifunctional amino acid residue as one such suitable cleavable link, and the chemistry to exploit this cleavable link takes advantage of a particularly vexatious problem in peptide synthesis. Basically, when proline is synthesized at the carboxy-terminus of a peptide being built up on a solid support, and another amino acid having a protected α-amino acid group is then synthesized to it, following the removal of the α-amino protecting group and neutralization, the proline will spontaneously cyclize to form diketopiperazine and cleave from the solid support. This reaction is illustrated in Figure 1a.

The present invention takes advantage of the reaction illustrated in Figure 1a, by initially adding proline to the solid support via an ester link. Where the solid support is a treated polyethylene rod or pin as disclosed in prior International Patent Application No. PCT/AU84/00039, the rod or pin preferably has spacer or linker residues, such as β-alanine or hexamethylene diamine residues, added before synthesis of the peptide thereon in accordance with the present invention. Then a bifunctional amino acid is added to the proline. An example of such a bifunctional amino acid which would be compatible with the chemistry of the Fmoc method of peptide synthesis is α-BOC,ϵ-Fmoc lysine, where BOC and Fmoc are the protecting groups t-butyloxycarbonyl and 9-fluorenyl-methyloxycarbonyl, respectively. A variation of this bifunctional amino acid which is compatible with the BOC chemistry of peptide synthesis is α-Z,ϵ-BOC lysine, where Z is the protecting group benzyloxycarbonyl. It will be noted that in both cases the usual protecting group, Fmoc and BOC respectively, is protecting the ϵ-amino group rather than the α-amino group, and, furthermore, the protecting group which normally protects the ϵ-amino group (BOC and Z, respectively) is now protecting the α-amino group. After addition of this amino acid the synthesis of the peptide proceeds in the usual manner to give a compound of the formula:-



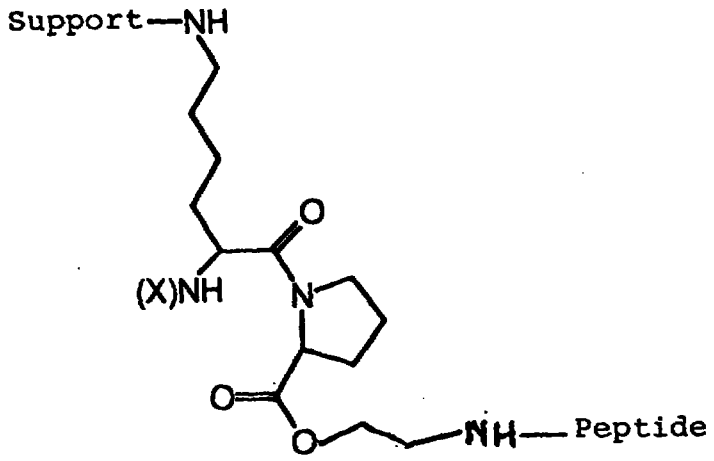
wherein X is as defined above.

The peptide will now be attached to the ϵ-amino group of the lysine instead of the usual α-amino group. After deprotection of the sidechains, the α-amino group of the lysine next to the proline will be in the form of the H_3N^- ion which can be neutralized under very mild basic conditions. When neutral, the proline will

spontaneously cyclize and cleave from the solid support as illustrated in Figure 1a. This will leave the peptide in solution with the group -NH-$(CH_2)_4$-diketopiperazine attached at its carboxy terminus.

Suitable compounds which can be used to form the ester link between the proline residue and the spacer or linker residues on the solid support include, but are not restricted to, the following and their derivatives: N-acetyl serine, HO-CH_2-CH(NH.Ac)-COOH; glycolic acid, HO-CH_2-COOH; lactic acid, HO-CH-(CH_3)-COOH; and p-(hydroxymethyl) benzoic acid, HO-CH_2-C_6H_4-COOH.

Alternatively, the bifunctional amino acid may be attached to the solid support (through the linker residues) before the proline. Again, where the bifunctional amino acid is lysine the attachment is carried out through the ϵ-amino acid to allow the α-amino acid to take part in the cyclization step at the end of synthesis. This is then reacted with proline which has had its carboxy group esterified, for example with Fmoc ethanolamine or serine. The peptide synthesis is then carried out as usual to produce a compound of the formula:-



wherein X is as defined above.

After side chain deprotection of the peptide, the α-amino group on the lysine attached directly to the solid support is gently neutralized and the proline moiety undergoes cyclization and cleavage of the peptide. In this variation the diketopiperazine remains on the solid support and the peptide has a group derived from the esterifying group at its carboxy terminus, for example, the group -NH-$(CH_2)_2$-OH where the esterifying group is ethanolamine. The reaction is illustrated in Figure 1b.

In some circumstances it may be undesirable to have the relatively large diketopiperazine moiety at the carboxy-terminal end of the cleaved peptide as shown in Figure 1a. In another embodiment of this aspect of the invention, the cleavable link comprises an ester link between the carboxy group of the terminal amino acid and the linker residues on the solid support as described above. Suitable compounds for forming such an ester link have been described above. After synthesis of the desired peptide and side-chain deprotection, the peptide can be cleaved from the solid support at the ester link. For example, saponification with base will cleave peptide with a -COOH group at its carboxy terminus, and the resulting peptide solutions would simply need to be neutralized before biological testing could be carried out. An alternative cleavage method is to use methylamine in ethanol and/or water. This would result in cleaved peptides whose carboxy terminal group was -CO-NH-CH_3. This method has the advantage that the cleavage agent is volatile and would be removed from the peptide solution on lyophilization. The peptides could then be dissolved in any desired buffer for use in biological testing.

Those skilled in the art will appreciate that the cleavable link and the cleavage chemistry can be modified to yield different end groups as desired on the carboxy-terminal of the cleaved peptides.

Where polyethylene rods or pins as described in the International Patent Application referred to above are used as the solid support, the quantity of polypeptide synthesized on each rod or pin may be of the order of 100 nmole (about 0.07 mg for a hexapeptide). This amount is sufficient to carry out systematic studies in a large number of systems. These include, for example, studies of T-cell epitopes, hormone studies in which the peptides are used as agonists or antagonists of hormones, enzyme inhibition and tests for potential new antibiotics.

The peptides synthesised in accordance with the invention can also be incorporated in other molecules. For instance, if cleavage is carried out in the presence of an activated carrier (such as keyhole limpet

haemocyanin - a protein well known to those skilled in the art to induce excellent immunological responses in laboratory animals) then a set of antibody sera can be made by injecting the peptide-activated carrier protein preparations (with or without the use an adjuvant such as Freund's Adjuvant) into animals and subsequently collecting serum from them. In this way, a large number of antisera can be produced which can then be used in any of the usual immunological tests, eg., neutralization and binding assays. The same process can be used to combine the peptide with a monoclonal antibody. For instance, the step of conjugation of a peptide which mimics an epitope with a monoclonal antibody which specifically reacts with human erythrocytes (red blood corpuscles or hRBC) produces a modified monoclonal antibody which can be used as a diagnostic agent specifically for binding entities which react specifically with the epitope. In use in a diagnostic test, the patient's hRBC become coated with the peptide when mixed with the modified monoclonal antibody. If the patient has antibodies which bind to the epitope then his/her hRBC will clump together because the antibodies will bind with the peptide mimic of the epitope (this gives an indication of previous exposure to the epitope) which can be easily recognised by relatively untrained technicians. Obviously, the peptide combined with such a monoclonal antibody will be selected so that it is specific for a particular condition, eg., HIV-1 (human immuno-suppressant virus, type 1) or HBsAg (human Hepatitis B virus, surface antigen).

Further features of the present invention are illustrated, by way of example only, in the following Examples.

EXAMPLE 1

Sets of rods were synthesized with the following peptide: α-Z,ϵ-DNP lysyl-proline attached to serine spacers by the synthesis method described in detail in International Patent Application PCT/AU84/00039. DNP is the dinitrophenyl group and is a chromophore by which the rate of cleavage of the peptide could be measured. The rods were then exposed to 0.05% NaOH solution and the optical density measured at 405 nm. Results of this experiment are given in Table 1 and demonstrate that the peptide does cleave from the rods under relatively mild conditions, i.e. unbuffered 0.05% NaOH solution.

TABLE 1

RATE OF PEPTIDE CLEAVAGE			
Time	Optical density		% complete
	Mean	sd	
0 min	0.064	0.002	0%
5 min	0.138	0.016	23%
15 min	0.284	0.007	47%
30 min	0.420	0.015	70%
60 min	0.569	0.079	95%
90 min	0.579	na	97%
120 min	0.600	0.046	100%

The above results demonstrate that larger peptides synthesised on the rods with the lysyl-proline cleavage group can similarly be removed from the rods under relatively mild conditions.

EXAMPLE 2

All overlapping decapeptide sequences which could be made from the MPB70 protein of Mycobacterium bovis BCG were synthesized on rods with a cleavable link. This protein is a major component of BCG and is known to stimulate T-cells. Each peptide was cleaved from its rod and individually tested for its ability to cause proliferation in a T-cell preparation. This was measured by the production of gamma-interferon which is released when T-cells are stimulated. The sequence of the protein is:-

```
  1  GDLVGPGCAE YAAANPTGPA SVQGMSQDPV AVAASNNPEL
 41  TTLTAALSGQ LNPQVNLVDT LNSGQYTVFA RTNAAFSKLP
 81  ASTIDELKTN SSLLTSILTY HVVAGQTSPA NVVGTRQTLQ
121  GASVTVTGQG NSLKVGNADV VCGGVSTANA TVYMIDSVLM
161  PPA
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The rods were grafted and BOC-hexamethylene diamine coupled to them using the methods described in International Patent Application PCT/AU84/0039. Then Fmoc-serine(t-butyl) is coupled to the rod using the usual Fmoc- chemistry. The serine was deprotected using piperidine/dimethyl formimide (DMF) to remove the Fmoc-group in the usual way. The α-amino group of the serine was then acetylated by standing in an acetic anhydride solution consisting of 40 ml triethylene, 80 ml acetic anhydride in 200 ml DMF for 90 min. The rods were then washed three times in methanol and allowed to dry. The t-butyl group on the serine side chain was then removed with trifluoroacetic acid (TFA) for 50 mins allowed to dry in air and placed under vacuum. Then BOC-proline was coupled to the free -OH group of the serine. The symmetrical anhydride of proline was prepared. 3.48 g of BOC-proline-OH was added to 1.67 g of N,N'dicyclohexylcarbodiimide (DCC) in 100 ml of dichloromethane (DCM) and allowed to react for 20 min at room temperature. The material was filtered and evaporated to dryness. The residue was dissolved in 150 ml of DMF and just before contacting the rods, 1.5 g of dimethylaminopyridine (DMAP) and 157 mg DCC added to the solution. The rods were allowed to react for 24 hours to allow the esterification reaction to go to completion. They were then washed in methanol. α-BOC lysine (ϵ-Fmoc) was then coupled to the proline using the usual Fmoc chemistry. After coupling and washing, the Fmoc protecting group was removed from the ϵ-amino group of the lysine in the usual way (piperidine in DMF). Then the amino acids of the peptides were coupled using the usual Fmoc chemistry. After synthesis was completed, the sidechains were deprotected with a solution of TFA:phenol:ethanedithiol (95:2.5:2.5). The rods were then washed with TFA for 5 mins and given two washes in methanol for 1 min. They were then left to stand in phosphate buffered saline (pH 5.0) for 48 hours. Each rod was then allowed to stand in cleavage solution for 4 hours with occasional agitation. The cleavage solution was 0.08% NaOH, 0.01% phenol red (as an indicator) in deaerated distilled water. Each rod was placed in 150 μl dispensed into a polystyrene microtitre plate. The rods were then removed from the plates and 20 μl of phosphate buffered saline (pH 7.2) added to each well of the plate. The peptide solutions were stored frozen until used.

The results obtained in the T-cell proliferation test are illustrated in Figure 2. Three regions in the sequence identified as significant stimulators of T cells were:

^{20}SVQGMSQDPVA

^{72}TNAAFSKLPASTID

^{150}ATVYMIDSVLMP

All peptides from length 9-mer to 12-mer of the MPB70 sequence around these regions were synthesized on rods and then cleaved in the same manner as described above. These peptide preparations were again tested for their ability to stimulate T-cell proliferation. The results are given in Figure 3. These results demonstrate that T-cell proliferation can be reproducibly stimulated by peptides synthesized using this invention. It also illustrates that large numbers of peptides can be used in such tests to systematically study variants of the stimulatory peptide.

EXAMPLE 3

The test peptide VQAAIDYING, well known to those skilled in the art (corresponding to residues 65-74 of the acyl carrier protein of Escherichia coli), was synthesized on pins. A labile ester link was introduced by synthesising a separate compound, Fmoc-Pro-Lact-OH.

Triethylamine (7 ml, equivalent to 50 mmol) and 10 g of phenacyl bromide (= 50 mmol) were added to a stirring solution of L-lactic acid (5.4 g of an 85% aqueous solution, equivalent to 50 mmol). After 40 h at room temperature, the reaction mixture was extracted with 100 ml of hot water. The organic phase was then sequentially washed with the following aqueous solutions: 10% citric acid, 7% sodium bicarbonate and finally with saturated NaCl solution (brine). The organic phase was then dried. Evaporation of the solvent yielded a gum which crystallized on standing. The solid was triturated with 50 ml of ether and collected by filtration. The 3.47 g yield of phenacyl lactate (Lac-OPa) was 68% of the theoretical for the step.

Fmoc-proline was then coupled to the phenacyl lactate. 2.06 g (10 mmol) of dicyclohexylcarbodiimide was added to a 4°C solution of Fmoc-Pro-OH (3.37 g, 10 mmol), phenacyl lactate (2.08 g, 10 mmol) and dimethylaminopyridine (240 mg, 2 mmol) in 50 ml of dichloromethane. The reaction mixture was filtered after 22 h and the filtrate evaporated. The resulting gum was dissolved in 100 ml ethyl acetate and washed sequentially with the following aqueous solutions: 10% citric acid, twice in 4% sodium hydroxide, 10% citric acid and finally, brine. The organic solution was evaporated yielding the product as 4.36 g of a yellow gum. This material was used without further purification.

The Fmoc-Pro-Lac-OPa was then converted to the dicyclohexylamine (DCHA) salt. The crude product was dissolved in 30 ml of ethyl acetate and 100 ml of glacial acetic acid and 30 ml of water were added. 6 g of zinc was added to the solution and the suspension stirred for 16 h at room temperature and then filtered. The gum obtained upon evaporation of the filtrate was partitioned between 150 ml of ethyl acetate and 200 ml of water. The organic phase was sequentially washed with the following aqueous solutions: brine, 10% citric acid, brine. The pale yellow oil obtained after evaporation was dissolved in 50 ml of ether. A solution of 2.00 g dicyclohexylamine (11 mmol) in 50 ml of light petroleum ether (40°-60°C fraction) was slowly added to the ether solution and a white precipitate formed which became crystalline on standing. This was collected by filtration and washed with 50 ml of ether three times and finally air dried. The yield of 4.46 g Fmoc-Pro-Lac-OH.DCHA obtained was 95% of the theoretical. This compound is stable and can be stored until required for use.

However, before covalently coupling to the pins, it must be converted to the free acid. Obviously, the amount used will depend of the number of pins to be coupled. A typical procedure is as follows. 20.0 g of Fmoc-Pro-Lac-OH.DCHA (equivalent to 34.02 mmol) was partitioned between 300 ml of ethyl acetate and 500 ml of 10% aqueous citric acid. The organic phase was then washed sequentially with the following aqueous solutions: 200 ml 10% citric acid and 200 ml of brine. The solution was then dried with anhydrous sodium sulphate and finally filtered and evaporated to yield a clear gum. This was dissolved in 1008 ml of dimethylformamide together with hydroxybenzotriazole (9.135 g, equivalent to 67.4 mmol) and 7.56 g DCC (36.5 mmol) and 150 µl of the solution dispensed into each well of 63 reaction trays. Pins were immersed into the solution for 16 h in sealed bags at 20°C. After coupling, the pins were washed for 15 min with methanol and air dried. Any unreacted amine groups on the pin were capped by acetylation with acetic anhydride. After washing, the pins were dried and stored until required. In this way, 6048 pins were coupled. It will be appreciated that the coupling the Fmoc-Pro-Lac-OH moeity to the pin uses the standard chemistry of solid phase peptide synthesis.

Using the usual Fmoc-chemistry steps, α-Boc lysine (ϵ-Fmoc) is coupled to the pins. In the example being cited, Fmoc-β alanine was then coupled to the growing peptide to act as a spacer entity between the desired peptide and the large diketopiperazine group of the cleaved peptide. The remaining amino acids of the sequence were then coupled to the growing peptide using the usual Fmoc-chemistry methods of peptide synthesis. The peptides were side-chain deprotected with trifluoroacetic acid:phenol:ethandithiol (95:2.5:2.5 v/w/v) for 6 h at room temperature. The pins were then air dried for 15 mins. They were then sonicated in 0.1% HCl in methanol/water (1:1) for 15 mins and then washed in pH 3 citrate-phosphate buffer for 5 h. The peptides were cleaved by immersing them into pH 7, 0.1M phosphate buffer (150 µl per well) for 16 h with gentle agitation under a nitrogen atmosphere at room temperature.

Figure 4 shows the HPLC analysis of the peptide solution and demonstrates that the test peptide, one which is notoriously difficult to synthesize, was prepared with better than 90% purity. This figure also shows the mass spectrograph of the peptide solution and shows that the desired peptide was indeed synthesised. The cleaved peptide solutions from the hydrolysate of a single pin were also subjected to amino acid analysis and yielded the typical result shown in Table 2.

TABLE 2

AMINO ACID ANALYSIS OF Ac-VQAAIDYING-β-cyclo(KP)		
Amino acid	RELATIVE Found	MOLAR RATIO Expected
Aspartic acid (D)	1.62	2
Glutamic acid (E)	0.78	1
Glycine (G)	0.52	1
β-alanine (β)	1.41	1
Alanine (A)	2.08	2
Proline (P)	1.37	1
Tyrosine (Y)	1.14	1
Valine (V)	1.02	1
Isoleucine (I)	2.22	2
Lysine (K)	0.83	1

This example illustrates that implementation of the invention yields the anticipated peptide with excellent purity, and also that reagents which simplify the routine synthesis of many different peptides can easily be made. Similar results (with appropriate modifications to the weights of the reagent to account for the different molecular weights) have been obtained replacing lactic acid with glycolic acid and p-(hydroxymethyl) benzoic acid in forming the labile ester link.

EXAMPLE 4

Every overlapping decapeptide which could be made from the sequence of the β-chain of human choriogonadotrophin (hCG) were synthesised using serine as the ester link as described in Example 2. The sequence of hCG is:

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  1 SKEPLRPRCR PINATLAVEK EGCPVCITVN TTICAGYCPT
 41 MTRVLQGVLP ALPQVVCNYR DVRFESIRLP GCPRGVNPVV
 81 SYAVALSCQC ALCRRSTTDC GGPKDHPLTC DDPRFQDSSS
121 SKAPPPSLPS PSRLPGPSDT PILPQ
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80 ml of concentrated tetanus toxoid (1190 Lf/ml and protein concentration of 8.74 mg/ml) was dialyzed against pH 6.66 0.1M phosphate buffer. 222 mg of 6-maleimido caproic acid N-hydroxysuccinimide ester (MCS) was dissolved in 6 ml of dimethylformamide (DMF). To the dialyzed tetanus toxoid solution was added, with continuous stirring at room temperature, 1.5 ml of the MCS solution. 15 mins later a further 1.5 ml of this solution was added. 15 mins later again, the remaining MCS solution was added. Stirring continued for a further hour. The resulting solution was then dialyzed overnight against pH 7.2, 0.1M phosphate buffer to which sodium ethylene-diamine-tetra-acetic acid (EDTA) to a final concentration of 10mM had been added. This resulting solution was diluted to 47% of its original strength with pH 7.2, 0.1M phosphate buffer to give a final concentration of MCS groups of 1.083 μmol/ml. Peptides were cleaved from the pins with 150 μl/pin of this solution and directly coupled to MCS-activated tetanus toxoid.

Mice were then vaccinated with the peptide/tetanus toxoid preparations. The peptide preparations were emulsified in Complete Freund's Adjuvant and were each injected by the intraperitoneal route into pairs of mice. A booster dose of the appropriate peptide preparation in Incomplete Freund's Adjuvant was given 17 days later. Blood samples were taken 21 days after the second inoculation.

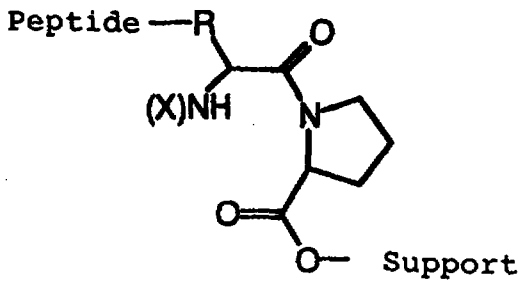
Figure 5 shows result of testing the blood sample against the peptide with which the mice were vaccinated. The sera were diluted 1/100000 for testing. Thus, sample 30 is a pool of the sera from the two mice which had been vaccinated with the peptide NTTICAGYCP coupled to tetanus toxoid, and was found to react very strongly with the peptide NTTICAGYCP.

This illustrates the use of the invention in determining regions of a sequence which become potential candidates for a synthetic peptide vaccine.

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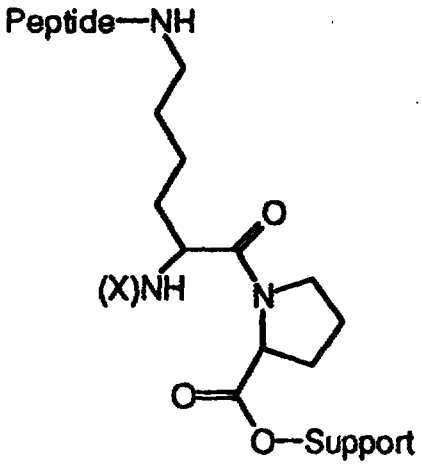
Claims

1. An immobilized peptide-solid support preparation which comprises a compound of the general formula:-



wherein X is a protecting group and R is an amino acid side chain having a functional group.

2. An immobilized peptide-solid support preparation as claimed in claim 1 which comprises a compound of the general formula:-

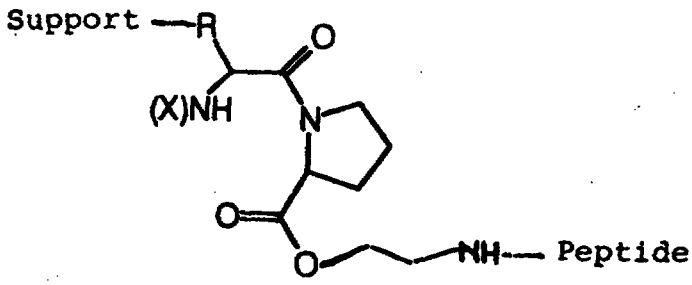


3. A preparation as claimed in claim 1 or 2 wherein the proline residue is linked to said solid support by an ester link.

4. A preparation as claimed in claim 3 wherein said ester link is formed from N-acetyl serine, glycolic acid, lactic acid or p-hydroxymethyl benzoic acid.

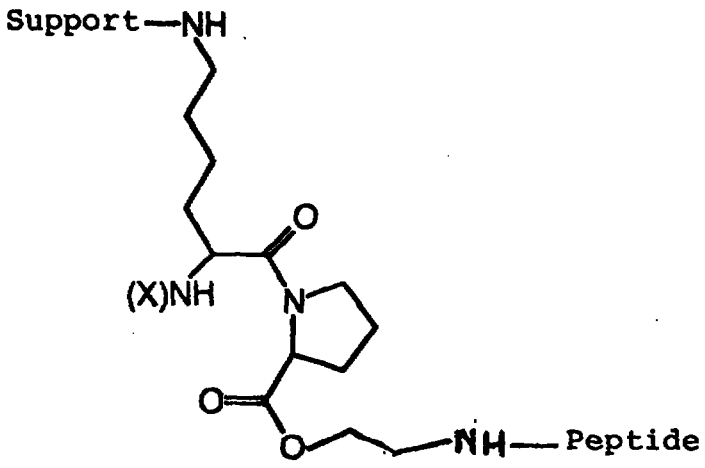
* C L M S *

5. An immobilized peptide-solid support preparation which comprises a compound of the general formula:-



wherein X is a protecting group and R is an amino acid side chain having a functional group.

6. An immobilized peptide-solid support preparation as claimed in claim 5 which comprises a compound of the general formula:-



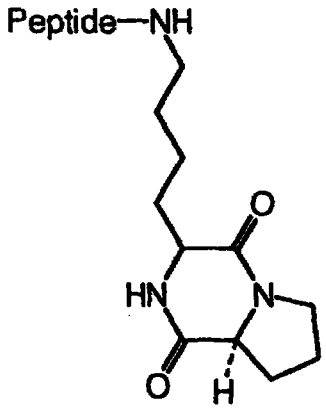
7. A preparation as claimed in any preceding claim wherein the linkage to said solid support further includes linker or spacer residues.

8. A preparation as claimed in claim 7 wherein said linker or spacer residues are β-alanine or hexamethylene diamine residues.

9. A preparation as claimed in any preceding claim wherein X is a t-butyloxycarbonyl protecting group.

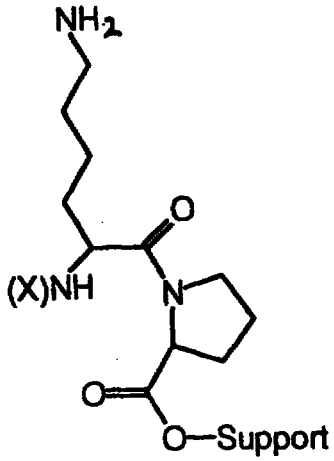
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10. A compound of the formula:-



11. A method of preparing a peptide comprising the steps of:-

(1) providing a support of the formula:-



wherein X is a protecting group,

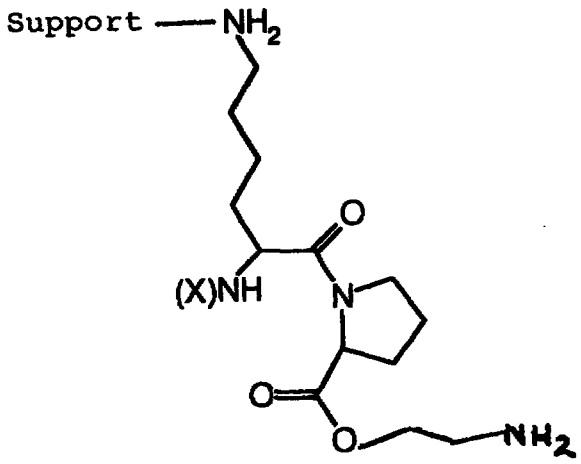
(2) synthesising a peptide attached to the ϵ-amino group of lysine

(3) removing X to provide a deprotected α-amino group of lysine and

(4) treating said deprotected α-amino group with a mild base.

12. A method of preparing a peptide comprising the steps of:-

(1) providing a support of the formula:-



wherein X is a protecting group,

(2) synthesising a peptide attached to the free amino group

(3) removing X to provide a deprotected α-amino group of lysine and

(4) treating said deprotected α-amino group with a mild base.

13. A method as claimed in claim 11 wherein the proline residue is linked to said solid support by an ester link.

14. A method as claimed in claim 13 wherein said ester link is formed from N-acetyl serine, glycolic acid, lactic acid or p-hydroxymethyl benzoic acid.

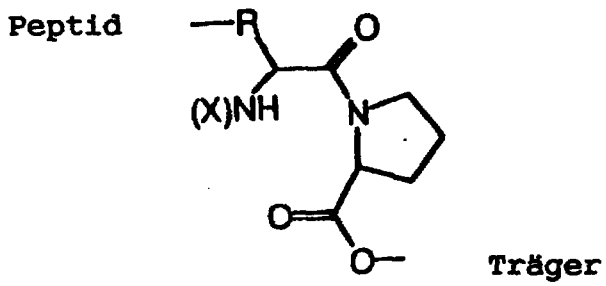
15. A method as claimed in any one of claims 11 to 14 wherein the lysine or proline residue is linked to the support via linker or spacer residues.

16. A method as claimed in claim 15 wherein said linker or spacer residues are β-alanine or hexamethylene diamine residues.

17. A method as claimed in any one of claims 11 to 16 wherein X is a t-butyloxycarbonyl protecting group.

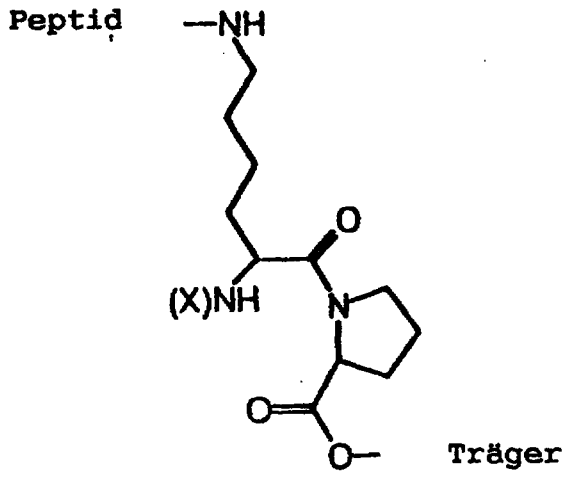
Patentansprüche

1. Aus einem immobilisierten Peptid und einem festen Träger bestehendes Präparat, das eine Verbindung der allgemeinen Formel



enthält, wobei X eine Schutzgruppe und R eine Aminosäureseitenkette mit einer funktionellen Gruppe bedeuten.

2. Aus einem immobilisierten Peptid und einem festen Träger bestehendes Präparat nach Anspruch 1, das eine Verbindung der allgemeinen Formel

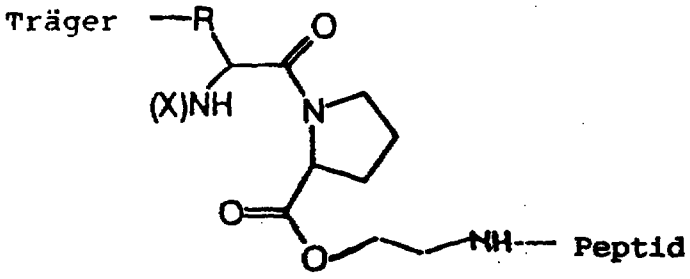


enthält.

3. Präparat nach Anspruch 1 oder 2, bei dem der Prolinrest mit dem festen Träger über eine Esterbindung verknüpft ist.

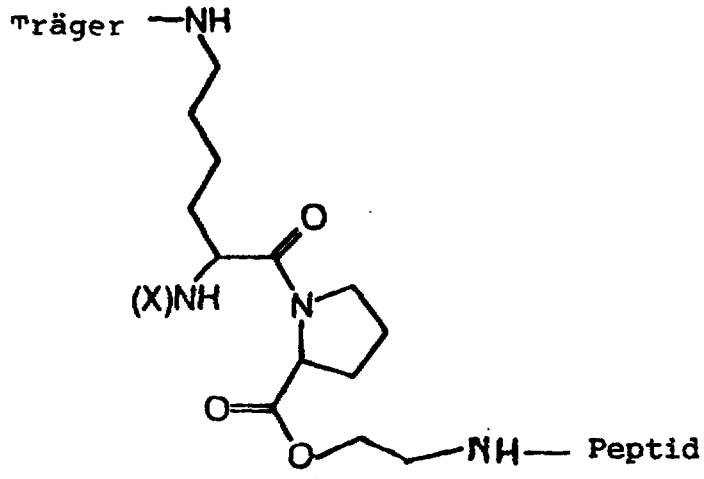
4. Präparat nach Anspruch 3, bei dem die Esterbindung gebildet wird aus N-Acetylserin, Glykol-, Milch- oder p-Hydroxymethylbenzoesäure.

5. Aus einem immobilisierten Peptid und einem festen Träger bestehendes Präparat, das eine Verbindung der allgemeinen Formel



enthält, wobei X eine Schutzgruppe und R eine Aminosäurenseitenkette mit einer funktionellen Gruppe bedeuten.

6. Ein aus einem immobilisierten Peptid und einem festen Träger bestehendes Präparat nach Anspruch 5, das eine Verbindung der allgemeinen Formel



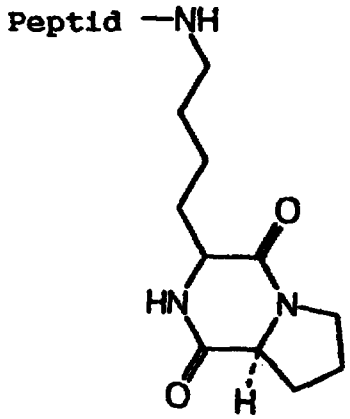
enthält.

7. Präparat nach einem der vorhergehenden Ansprüche, wobei die Bindung an den festen Träger außerdem noch einen Linker- oder Spacer-Rest umfaßt.

8. Präparat nach Anspruch 7, bei dem die Linker- oder Spacer-Reste β-Alanin- oder Hexamethylendiaminreste sind.

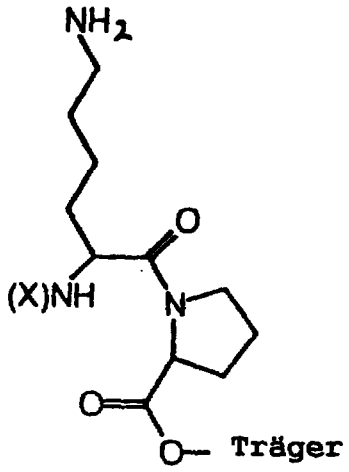
9. Präparat nach einem der vorhergehenden Ansprüche, wobei X eine t-Butyloxycarbonyl-Schutzgruppe ist.

10. Verbindung der Formel



11. Verfahren zur Herstellung eines Peptids, das die folgenden Stufen umfaßt:

(1) Bereitstellung eines Trägers der Formel



worin X eine Schutzgruppe bedeutet,

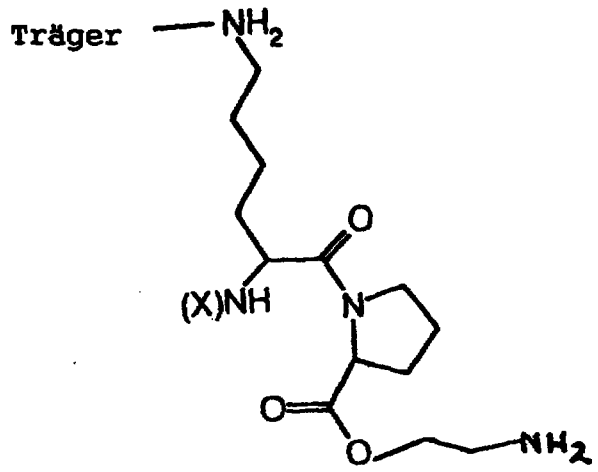
(2) Synthese eines mit der ϵ-Aminogruppe von Lysin verknüpften Peptids,

(3) Abspaltung von X zur Erzielung einer von der Schutzgruppe befreiten α-Aminogruppe von Lysin und

(4) Behandlung der von der Schutzgruppe befreiten α-Aminogruppe mit einer schwachen Base.

12. Verfahren zur Herstellung eines Peptids, das die folgenden Stufen umfaßt:

(1) Bereitstellung eines Trägers der Formel



worin X eine Schutzgruppe bedeutet,

(2) Synthese eines mit der freien Aminogruppe verknüpften Peptids,

(3) Abspaltung von X zur Erzielung einer von der Schutzgruppe befreiten α-Aminogruppe von Lysin und

(4) Behandlung der von der Schutzgruppe befreiten α-Aminogruppe mit einer schwachen Base.

13. Verfahren nach Anspruch 11, bei dem der Prolinrest mit dem festen Träger über eine Esterbindung verknüpft ist.

14. Verfahren nach Anspruch 13 bei dem die Esterbindung gebildet wird aus N-Acetylserin, Glykol-, Milch- oder p-Hydroxymethylbenzoesäure.

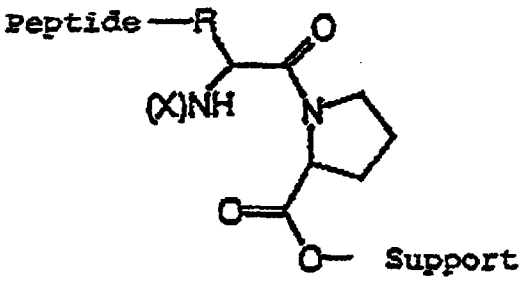
15. Verfahren nach einem der vorhergehenden Ansprüche, wobei der Lysin- oder Prolinrest mit dem Träger über Linker- oder Spacer-Reste verknüpft ist.

16. Verfahren nach Anspruch 15 bei dem die Linker- oder Spacer-Reste β-Alanin- oder Hexamethylendiaminreste sind.

17. Verfahren nach einem der Ansprüche 11-16, wobei X eine t-Butyloxycarbonyl-Schutzgruppe ist.

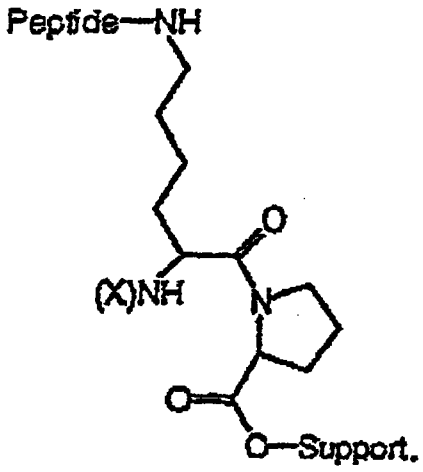
Revendications

1. Préparation à base de peptide immobilisé-support solide, comprenant un composé répondant à la formule générale :



où X est un groupe protecteur, et R est une chaîne latérale d'amino-acides ayant un groupe fonctionnel.

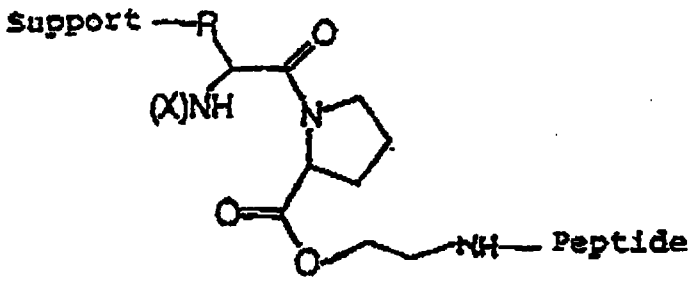
2. Préparation à base de peptide immobilisé-support solide telle que revendiquée dans la revendication 1, comprenant un composé répondant à la formule générale :



3. Préparation telle que revendiquée dans la revendication 1 ou 2, où le résidu de proline est lié audit support solide par une liaison ester.

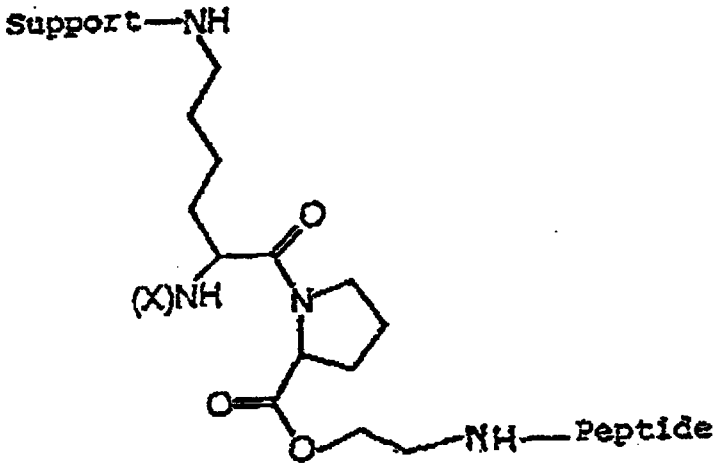
4. Préparation telle que revendiquée dans la revendication 3, où ladite liaison ester est formée de N-acétylsérine, d'acide glycolique, d'acide lactique ou d'acide p-hydroxyméthylbenzoïque.

5. Préparation à base de peptide immobilisé-support solide, comprenant un composé répondant à la formule générale :



où X est un groupe protecteur, et R est une chaîne latérale d'amino-acides ayant un groupe fonctionnel.

6. Préparation à base de peptide immobilisé-support solide telle que revendiquée dans la revendication 5, comprenant un composé répondant à la formule générale :

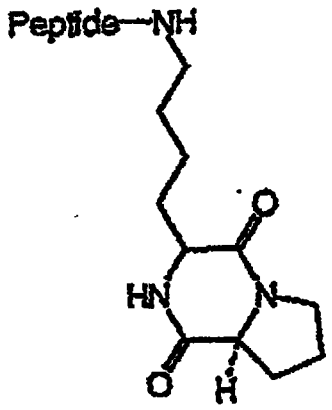


7. Préparation telle que revendiquée dans l'une quelconque des revendications précédentes, où la liaison avec ledit support solide comprend, en outre, des résidus de liaison ou d'espacement.

8. Préparation telle que revendiquée dans la revendication 7, dans laquelle lesdits résidus de liaison ou d'espacement sont des résidus de β-alanine ou d'hexaméthylènediamine.

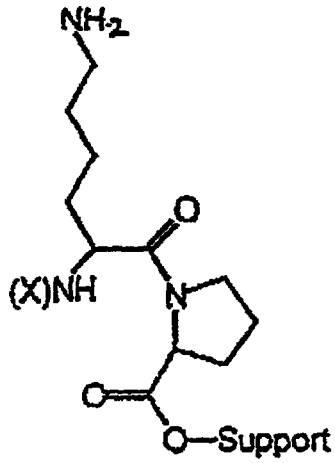
9. Préparation telle que revendiquée dans l'une quelconque des revendications précédentes, où X est un groupe protecteur t-butyloxycarbonyle.

10. Composé répondant à la formule :



11. Procédé de préparation d'un peptide, comprenant les étapes consistant à :

(1) fournir un support répondant à la formule :



où X est un groupe protecteur,

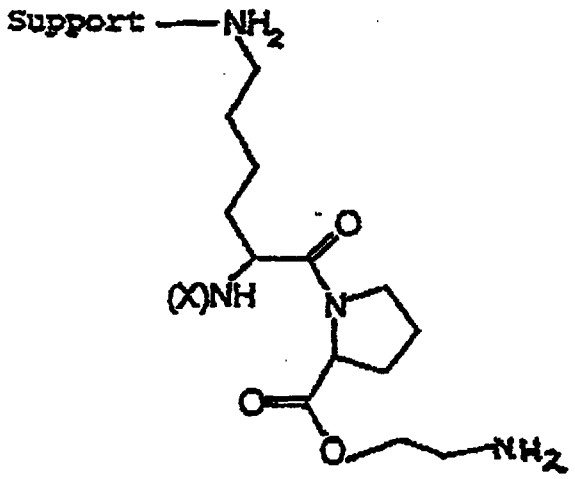
(2) synthétiser un peptide attaché au groupe ε-amino de lysine,

(3) enlever X pour fournir un groupe α-amino déprotégé de lysine, et

(4) traiter ledit groupe α-amino déprotégé avec une base douce.

12. Procédé de préparation d'un peptide, comprenant les étapes consistant à :

(1) fournir un support répondant à la formule :



où X est un groupe protecteur,
(2) synthétiser un peptide attaché au groupe amino libre,
(3) enlever X pour fournir un groupe α-amino déprotégé de lysine, et
(4) traiter ledit groupe α-amino déprotégé avec une base douce.

13. Procédé tel que revendiqué dans la revendication 11, où le résidu de proline est lié audit support solide par une liaison ester.

14. Procédé tel que revendiqué dans la revendication 13, où ladite liaison ester est formée de N-acétyl-sérine, d'acide glycolique, d'acide lactique ou d'acide p-hydroxyméthylbenzoïque.

15. Procédé tel que revendiqué dans l'une quelconque des revendications 11 à 14, où le résidu de lysine ou de proline est lié au support par l'intermédiaire de résidus de liaison ou d'espacement.

16. Procédé tel que revendiqué dans la revendication 15, où lesdits résidus de liaison ou d'espacement sont des résidus de β-alanine ou d'hexaméthylènediamine.

17. Procédé tel que revendiqué dans l'une quelconque des revendications 11 à 16, où X est un groupe protecteur t-butyloxycarbonyle.