TRIALKYSILYL ESTERS OF AMINO ACIDS AND THEIR USE IN THE SYNTHESIS OF PEPTIDES

A novel procedure is described for synthesising peptides in which successive amino acids are attached to an incipient polypeptide chain by addition of a carboxy-protected amino acid to the carboxy end of said chain. The procedure involves the use of silyl esters of amino acids, many of which are novel and form a further aspect of the invention.
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TRIALKYLSILYL ESTERS OF AMINO ACIDS AND THEIR USE IN THE SYNTHESIS OF PEPTIDES

This invention relates to novel procedures for synthesising peptides and to novel reagents for use in these procedures. The invention also provides kits of reagents for use in carrying out the procedures of the invention.

In recent years there has been an escalating need for synthetic peptides in a wide variety of applications, including the development of synthetic peptide vaccines, the detailed study of antigen-antibody interactions, the preparation of analogues of biologically active peptides, the optimisation of peptide antigens of clinical diagnostic utility, the mapping of protein products of specific genes and the study of conformational parameters. In a majority of these studies, the limiting factor has been the availability and cost of desired peptides. Clearly, these studies would be greatly facilitated if synthetic methods were available that would permit the synthesis of peptides rapidly and in a cost efficient manner. Similarly for commercial exploitation, a protocol suitable for large scale operation is required.

Peptides are linear polymers derived from amino acids and generally have the formula

\[ H_N.A^1.CO.NH.A^2.CO.NH.A^3.CO.NH.A^4.CO.A^{n-1}.CO.NH.A_n.COOH \]  

(I)

Where \( A^1, A^2, A^3 \ldots A^n \) are the residues of the amino acids making up the peptide.

Such amino acids may be represented by the formulae

\[ H_N.A^1.COOH, \quad H_N.A^2.COOH \ldots \text{ etc} \]  

(II)

Peptides may additionally include sub-units derived from imino acids (also termed "heterocyclic amino acids") such as, for example, proline. These sub-units may be represented by the formula

\[ .NKA.CO. \]  

(III)

wherein NKA represents a heterocyclic group.
The corresponding amino acids have the formula

\[ \text{HNXCOOH} \]  
(IV)

An important class of amino acids are the \( \alpha \)-amino acids that form the sub-units of proteins. These amino acids, which in nature are of the L-configuration, have been described as "naturally occurring amino acids".

Essentially all proteins occurring in nature, whether from unicellular prokaryotic microorganisms or from higher forms of life i.e. eukaryotes, are constructed from the same set of twenty \( \alpha \)-amino acids. Nineteen of these amino acids can be depicted as having the structure

\[ \text{H}_2\text{NCHR COOH} \]  
(V)

where CHR may be referred to as the amino acid residue and R as the side chain. In glycine, the only amino acid of the series which is not optically active, \( R \) is hydrogen. Proline cannot be represented by formula (V) as it is an imino acid of formula (IV) and its residue forms part of a pyrrolidine ring.

Generally, the side chain \( R \) may be a hydrocarbyl group, for example an an alkyl or aryl group, as in alanine, valine, leucine, isoleucine, methionine and phenylalanine. The residue \( R \) may contain polar, but non-ionizable groups, as in asparagine, glutamine, threonine and serine, or ionizable groups, such as in aspartic acid, glutamic acid, lysine, arginine, histidine, tyrosine, tryptophan and cysteine.

Peptides may be synthesised by two distinct routes, biological and chemical. This invention is concerned with the second route.

Many naturally occurring pharmacologically active peptides or protein hormones, including insulin, gastrin, oxytocin, vasopresin and bradykinin have been synthesized by chemical means.

However a fundamental problem in the chemical synthesis of peptides is that the groups which react to form the desired peptide bonds are likely to enter into unwanted side reactions with other functional groups. These other functional groups may be groups in the amino acid reactants other than those forming the desired peptide bond or they may be functional groups of reagents.
Consequently, most known procedures for synthesising peptides require the use of protective or blocking groups to prevent such sensitive functional groups from reacting with the peptide-forming reagents. Thus, the addition of each amino acid in the synthesis of a peptide chain requires several steps to attach and then remove protective or blocking groups, in addition to the steps actually involved in forming the peptide bond.

The expedient of utilizing blocking groups was first conceived by Emil Fisher, and the classical approach for the synthesis of peptides of defined structure was developed by Bergmann and coworkers. In the Bergmann procedure, the amino group of the N-terminal amino acid precursor is blocked or protected, typically by reaction with benzylxoycarbonyl chloride in the presence of alkali (Schotten-Baumann reaction):

\[
\text{C}_6\text{H}_5\text{CH}_2\text{OOC}\text{Cl} + \text{H}_2\text{NCHR'COOH} \rightarrow \text{C}_6\text{H}_5\text{CH}_2\text{OCONHRCHR'COOH} \quad (1)
\]

The next amino acid precursor is added and couples with the N-terminal benzylxoycarbonyl-protected amino acid precursor:

\[
\text{VII} + \text{H}_2\text{NCHR'COOH} \rightarrow \text{C}_6\text{H}_5\text{CH}_2\text{OCONHRCHR'CONHR'COOH} \quad (2)
\]

This sequence of steps is repeated to prepare tri- or higher peptides. Since the procedure is effected in solution, it is necessary to separate intermediate peptide precursors from by-products, unreacted reagents, etc. In the last step, the terminal \text{C}_6\text{H}_5\text{CH}_2\text{COO} blocking group is cleaved by catalytic hydrogenation (toluene and carbon dioxide are formed as by-products) or by use of HBr/acetic acid to yield the desired peptide.

Such chemical methods of peptide synthesis need to take account of three important factors. First in order to synthesise a desired polypeptide, the synthesis must be conducted in a step-wise fashion with desired amino acid residues attached to an incipient peptide chain sequentially in the desired order.
Secondly in order to form a peptide bond
\[ -A^1\text{CO.NH}_2^- \quad \text{(IX)} \]
by linking
\[ -A^1\text{COOH} \quad \text{to} \quad \text{NH}_2A^2^- \quad \text{(X)} \]
the carboxyl group .COOH needs to be converted to an active species .COε where ε is a leaving group. In this active species, the carbonyl group is activated and is susceptible to nucleophilic attack by the free amino group of the entity NH$_2$A$^2$-.

Thirdly, any reactive groups (other than the -NH$_2$ group and -COε group) which might enter into undesired side reactions need to be protected from attack by other active species.

Techniques have been established which take account of these factors, the most important being the solid state methods devised by Merrifield and Sheppard. In this regard solid-phase peptide synthesis has been thoroughly reviewed by Merrifield (1969, 1973), Meienhofen (11973) and Erickson and Merrifield (1976). Procedures described since then, with varying points of emphasis, include those of Atherton (1979), Sheppard (1977) and Marnett et al., (1976).

The key ideas and features of the solid phase principle are fourfold:

1) The peptide is synthesised while it is covalently attached to a polymeric support. This allows ready separation of the product from by-products.
2) Reactions of the polymer-supported peptide chains can be driven to completion through the use of excess reagents.
3) Mechanical losses are avoided by retaining the peptide polymer beads in a single reaction vessel throughout the synthesis.
4) Physical manipulations are amenable to automation.

The Merrifield strategy utilises an N-protected amino acid
\[ \text{HOOC.A.NHProt} \quad \text{(XI)} \]
which is converted to an activated form
\[ \varepsilon\text{CO.A.NHProt} \quad \text{(XII)} \]
Traditionally tertiary butoxycarbonyl (tBoc) has been the protective group of choice. In the Merrifield procedure, the initial (C-terminal) amino acid NH₂Aⁿ⁺COOH is covalently bonded to a resin via its carboxyl group. This may be achieved by reacting a resin having free -CH₂Cl groups with a protected amino acid caesium salt

\[ \text{CsO.CO.Aⁿ⁻NHProt (XII)} \]

to form resin particles having the protected nth amino acid attached, i.e.

\[ \text{Resin.CH₂OCO.Aⁿ⁺NHProt (XIII)} \]

In the next step (or "cycle") the protecting group Prot is removed, leaving a free amino group

\[ \text{Resin.CH₂CO.Aⁿ⁻NH₂ (XIV)} \]

and the resin particles, with the nth amino acid attached, are reacted with the next (n-1)th activated protected amino acid

\[ \varepsilon \text{CO.Aⁿ⁻¹NHProt (XII')} \]

which is to form the (n-1)th amino acid of the desired peptide, i.e.

\[ \text{Resin.CH₂OCO.Aⁿ⁻NH₂ + \varepsilon \text{CO.Aⁿ⁻¹NHProt} \rightarrow Resin.CH₂OCO.Aⁿ⁻NHCOAⁿ⁻¹NHProt. (XV)} \]

The sequence of steps is repeated until the desired resin-bound peptide is formed. The peptide is then cleaved from the resin and de-protected.

As indicated, in solid phase synthetic peptide chemistry, tertiary butoxy oxycarbonyl (tBoc) has been the protective group of choice. However, this protective group requires the use of strong acids in both deprotection and final cleavage. Furthermore, such use of acidic conditions has necessitated the use of acid-stable protecting moieties (e.g. benzyl groups) for protecting the side chains of amino acids such as the basic amino acids (Lys, His and Arg).

In more detail, in the solid phase procedure for the synthesis of peptides developed by Merrifield, the peptide chain is built amino acid-by-amino acid starting with the one intended to be the carboxyl or C-terminal residue of the peptide chain.
This C-terminal amino acid is covalently bonded via its -COOH group to an insoluble resin support. A chloromethylated polystyrene resin is most often used because the benzyl ester group formed with the C-terminal amino acid may be readily cleaved.

\[
t\text{-Boc-}\text{NHCHR}COOH + \text{ClCH}_2\text{-Resin} \rightarrow t\text{-Boc-}\text{NHCHR}COOCH\text{-Resin}
\]

(XVI) \hspace{2cm} (XVII) \hspace{2cm} (XVIII)

The next amino acid to be used in the peptide chain being formed, after having its amino group blocked with a t-butyloxy carbonyl (t-Boc) group, is activated with a coupling activator, such as dicyclohexyl-dicarboxdiimide (DCC), and coupled to the deprotected amino group of the amino acid attached to the resin - (H\text{₂NCHR}COOCH\text{₂-Resin XVIII})

\[
(t\text{-Bu-OCO})\text{₂O} + \text{H}_2\text{NCHR'COOH} \rightarrow t\text{-Boc-}\text{NHCHR'}COOH
\]

(XIX) \hspace{2cm} (XX) \hspace{2cm} (XXI)

The t-Boc group is then removed by treatment with trifluoroacetic acid, and after neutralisation the growing peptide chain attached to the resin is ready for addition of the next amino acid precursor. This sequence is repeated until a peptide chain having the required structure is synthesized. Having the growing peptide chain attached to resin particles large enough to be separated from a liquid phase by filtration, simplifies removal of excess reagents and washing of the resin particles in the many repetitious steps involved, and makes the procedure more convenient for the synthesis of larger peptides and proteins. When the peptide chain is complete, it is cleaved from its resin support by a reaction that does not affect the peptide linkages. Typically, hydrogen fluoride is utilized for cleaving the peptide from the resin.

Recent years have seen the introduction of fluorenylmethoxy carbonyl (Fmoc) as protective group for the activated protected amino acid $\epsilon\text{COA}_n\text{Prot}$. The most significant difference in the Fmoc approach is that the protecting group may be removed by base, typically using piperidine.
However, in principle, there is a great similarity between the Fmoc and tBoc approaches. Each cycle of the synthesis involves deprotection of the last attached amino acid residue followed by the coupling of the next activated amino acid. In both Fmoc and tBoc approaches, protected amino acid symmetrical anhydrides have generally been favoured as the active species. N-hydroxybenzotriazole derivatives and pentafluorophenyl esters of amino acids may also be used. The reason for favouring symmetrical anhydrides is mainly due to their greater reactivity compared to the corresponding amino acid active esters. However the necessity to form Fmoc symmetrical anhydrides immediately prior to use has prevented this activation protocol from being implemented in automated instruments. This is also true of automated peptide synthesis based on tBoc amino acids.

Available methods of peptide synthesis have a number of important disadvantages which severely limit their applicability, particularly in automated systems.

First, the tBoc and the Fmoc systems require the use of respectively stringent acidic conditions and basic conditions in order to remove the tBoc and Fmoc protective groups. Thus in the tBoc system, strong acids such as trifluoroacetic acid have to be used and bases such as, for example, piperidine in DMF, are required in the Fmoc system. The use of trifluoroacetic acid in order to remove the tBoc protective group is particularly troublesome as the repeated contacting of the resin particles at each cycle with the trifluoroacetic acid reagent causes degradation of the resin.

Secondly, in both the tBoc and Fmoc systems repeated washing cycles are required to remove acidic and basic reagents used.

Thirdly the use of certain activated protected amino acids (symmetrical anhydrides) prevents the ready application of the tBoc and Fmoc procedures to automated systems. Such systems require the use of reagents which are relatively stable because supplies of all the reagents necessary to synthesise a desired peptide need to be stored for relatively long periods. However, activated protected amino acids are generally unstable and susceptible to decomposition in the presence of even trace amounts of water and many commercially available activated protected amino acids are prohibitively expensive.
Fourthly the tBoc and Fmoc procedures require the use of an excess of the activated protected amino acid reagent in each cycle in order to ensure that the N-terminal amino acid residues of the resin-bound peptide chain react to completion with said reagent. Thus, for example, when a symmetrical anhydride is used as the activated protected amino acid reagent, the reagent needs to be present in a mole ratio of at least 4 moles of reagent per mole of N-terminal amino residues. As each mole of reagent is formed of two moles of amino acid, this represents a requirement for an 8x excess of amino acid starting material.

The present invention overcomes the problems associated with prior art solid state peptide synthesis procedures by binding the first amino acid of the desired peptide to a resin via its amino group, activating the carboxyl group of the thus-coupled amino acid and utilising a carboxy-protected amino acid reagent to produce a peptide bond by nucleophilic attack on the activated carboxy group of the bound amino acid. Subsequent amino acids are coupled in a similar manner.

In the above procedure, reactive side chains of the carboxy-protected amino acid may be protected (if necessary) by known procedures.

The carboxy-protective groups utilised in accordance with the invention are O-silyl ester groups. Apart from incidental exceptions referred to below, amino acids having O-silyl ester protective groups are novel and form a further aspect of the invention.

As indicated, certain silylated amino acids have been described in recent years, particularly for the derivatisation of carboxy moieties of N-acetyl amino acids for gas chromatography (Early et al, 1987).

Also in the 1960's Birkof er et al, Angew. Chem. 77, 414 (1965); Kricheldorf et al, Liebig's Ann. Chem., 763, 17-38 (1972); Rühmann et al., Liebig's Ann. Chem. 683, 211 (1965) describe trimethylchlorosilane derivatives of amino acids and peptides. In a typical experiment (Birkof er 1960, for example), an amino acid was heated at 130-140°C in excess of hexamethyldisilane containing a few drops of conc. H$_2$SO$_4$ until a solution was obtained. It was then cooled and benzene added followed by triethylamine and trimethylsilyl chloride and the mixture kept at room temperature for 12-16 hours.
The solid obtained was filtered off and the filtrate evaporated and distilled under reduced pressure to give the desired N-trimethyl silyl amino acid trimethyl silyl ester.

Furthermore, Barlos et al, J. Org. Chem. 47, 1324 (1982) postulated formation of trimethyl-silyl esters of amino acids as intermediates in the synthesis of N-trityl amino acids. However, no definitive evidence of the existence, preparation or isolation of such structural intermediates was presented.

As indicated, the present invention, in its process aspects, is based on the realisation that distinct advantages ensue from the synthesis of peptides by a procedure in which an initial amino acid forming the N-terminus of the desired peptide forms the starting point for the step-wise synthetic route, the carboxyl group of this amino acid is activated and the activated carboxy group is reacted with the free amino group of a carboxy-protected amino acid.
According to one aspect thereof, the present invention provides a process for producing a peptide of the formula

\[
\text{H}-\underbrace{\text{NH.A.CO}}_{n+x+1}\text{-OH} \tag{XXIV}
\]

which comprises the steps of

(A) reacting a solid phase reactant comprising a solid support-bound amino acid or a solid support-bound peptide, said solid phase reactant having the formula

\[
\text{Support - NH.A.CO}_n\text{-OH} \tag{XXV}
\]

with a carboxyl group activating agent to form an activated solid phase reactant of the formula

\[
\text{Support - NH.A.CO}_n\text{-}c \tag{XXVI}
\]

(B) reacting the activated solid phase reactant from step (A) with a carboxy-protected amino acid of formula

\[
\text{H.NH.A.CO.Prot} \tag{XXVII}
\]

to form a chain-extended product of formula

\[
\text{Support - NH.A.CO}_{n+1}\text{-0.Prot} \tag{XXVIII}
\]

(C) removing said protective group Prot,

(D) optionally repeating said steps (A), (B) and (C) x times.
and (E) cleaving the resulting peptide of formula

\[ H\left[\text{NH.A.CO}\right]_{n+x+1} \text{OH} \]  (XXIX)

from the support

wherein \( n \) is a positive integer,
\( x \) is 0 or a positive integer,
\( \epsilon \) is a leaving group,
Prot is a silyl group,
each A, which may be the same or different, either represents the residue of an amino acid or the structure \( \text{NH.A} \) is the residue \( \text{N</A> of an imino acid } \text{HN</ACOH} \)

and wherein any reactive side chains on residues A are protected and are subsequently deprotected and said steps (A) and (B) are carried out successively or in a single operation.

When carried out in a single operation, the process of the invention comprises the following steps:

(a) reacting a solid phase reactant comprising a solid support-bound amino acid or a solid support-bound peptide, said solid phase reactant having the formula

\[ \text{Support} \left[\text{NH.A.CO}\right]_{n} \text{OH} \]  (XXV)

simultaneously with a carboxyl group activating agent and with carboxy-protected amino acid of formula

\[ \text{H.NH.A.CO.Prot} \]  (XXVII)

to form a chain-extended product of formula

\[ \text{Support} \left[\text{NH.A.CO}\right]_{n+1} \text{Prot} \]  (XXVIII)

(b) removing said protective group Prot,
(c) optionally repeating said steps (A), (B) and (C) \( x \) times,
and (d) cleaving from the support the resulting peptide of formula

\[
\text{H} \frac{\text{NH.A.CO}}{\text{n+x+1}} \text{-OH} \quad (\text{XXIX})
\]

\(n, x, \epsilon, \text{Prot and A} \) being as previously defined and wherein any reactive
side chains on residue A are protected and are subsequently deprotected.

Preferably said solid phase reactant

\[
\begin{array}{c}
\text{Support} \\
\text{NH.A.CO} \\
\text{OH}
\end{array} \quad (\text{XXX}).
\]

\(n\) is 1, is produced by reacting a carboxy protected amino acid of
formula

\[
\text{H.NH.A.CO.OProt}
\]

with a support material having groups capable of forming a covalent bond
with the amino or imino group of the carboxy protected amino acid, and
removing said protective group Prot.

In the above reaction schemes, the amino acid residues A may be
derived from a wide range of amino acids, which is not restricted to the
amino acids commonly occurring in natural proteins.

Such amino acids may be represented by the general formula

\[
\text{H.N} \frac{\text{COOH}}{0}
\]

\(n\) represents either the group \(\text{NHB}\) wherein B represents a
saturated or unsaturated \(C_{1-10}\) hydrocarbyl group, optionally
substituted by one or more substituents selected from hydroxy, oxo, thio,
\(C_{1-4}\) alkylthio, \(C_{1-4}\) alkoxy, carboxy, acetamido or \(-\text{NR}_{4}\text{R}_{5}\) (wherein
\(R_{4}\) and \(R_{5}\), which may be the same or different represent \(C_{1-4}\) alkyl
groups), or \(n\) represents a heterocyclic ring containing 4-7 ring
atoms selected from N, C and O.
Thus the invention includes the production of peptides derived from amino acids in one or more of the following classes:

(i) the twenty L-α-amino acids glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, asparagine, glutamic acid, glutamine, lysine, histidine, arginine, phenylalanine, tyrosine, tryptophan, cysteine, methionine and proline,

(ii) D-analogues of amino acids in class (i),

(iii) dehydro derivatives of amino acids in class (i),

(iv) amino acids selected from the following:
- hydroxylysine, thyroxine, hydroxyproline, β-alanine,
- γ-aminobutyric acid, homoserine and statine.

In general, where the amino acid is an α-amino acid of formula H₂N.CH₂.CO₂H, R may be defined as being selected from C₁-10 hydrocarbyl groups optionally substituted by one or more substituents selected from hydroxy, thio, C₁-4 alkylthio, C₁-4 alkoxy, carboxy, acetamido, guandidyl, 3-indolyl and 2-imidazolyl.

The aforementioned hydrocarbyl groups include branched and straight chain alkyl groups (which preferably contain from 1 to 6 carbon atoms) and aryl groups, particularly phenyl groups, which may be unsubstituted or substituted by one or more moieties selected from C₁-4 alkoxy, nitro and halogen.

The groups of the support which are capable of forming covalent bonds with the amino or imino groups of the carboxy protected amino acid are preferably activated carboxy groups of formula -CO₂E or activated oxycarbonyl groups of formula -OCO₂E (E is as defined above).

Examples of suitable silyl groups are those having the formula

\[ \text{Si}(R₁,R₂,R₃) \] (XXXI)

wherein R₁, R₂ and R₃, which may be the same or different represent saturated or unsaturated hydrocarbyl groups containing 1 to 20 carbon atoms, which may be unsubstituted or substituted by one or more groups selected from C₄-alkoxy, nitro, tri(C₁-4 alkyl)silyl and halogen.

The aforementioned hydrocarbyl groups include branched and straight chain alkyl and alkenyl groups (which preferably contain from 1 to 6 carbon atoms) and aryl groups, particularly phenyl groups.
Preferably said silyl groups have the formula
\[
\text{Si}(R_1^1, R_2^1, R_3^1)
\]  
(XXXI)

wherein \( R_1^1, R_2 \) and \( R_3^1 \), which may be the same or different represent C\(_{1-20}\) alkyl groups. The alkyl groups may be straight and branched (and a combination of both may be present). Examples include methyl, ethyl, n-propyl, n-butyl, sec-butyl, t-butyl and dodecyl.

The carboxy protected amino acids XXVII may be formed from the corresponding amino acid or protected amino acid by converting a free carboxyl group to a silyl ester group using known silylating agents. Such agents may generally have the structure \( X-\text{Si}(R_1 R_2 R_3) \) where \( R_1^1 \), \( R_2 \) and \( R_3^1 \) are as defined above and \( X \) is a leaving group.

Examples of suitable leaving groups \( X \) include

(i) Cl, Br and I (as in trimethylsilyl chloride)

(ii) alkoxy, e.g. ethoxy and methoxy (as in trimethylethoxy silane)

(iii) secondary amino, for example di-C\(_{1-4}\)-alkylamino. A typical secondary amino group is dimethylamino (as in N-trimethylsilyldimethyl amine)

(iv) disilazano (as in hexamethyl disilazane)

The leaving group \( \epsilon \) should be selected according to known criteria such that the carbon atom to which it is attached is activated sufficiently that it can undergo nucleophilic attack by a lone electron pair on the free amino or imino group of the carboxy protected amino or imino acid. Preferably group \( \epsilon \) should be what is termed "good leaving group".

Particularly effective leaving groups \( \epsilon \) are strongly electron withdrawing.

Also it is desirable that the reaction(s) which introduce leaving group \( \epsilon \) minimise racemisation and that also the activated intermediate containing \( \epsilon \) should not be susceptible to racemisation.

Examples of leaving groups \( \epsilon \) are groups having the structure
\[
R^5-\text{NH}-C-N-R^6
\]  
(a)

where \( R^5 \) and \( R^6 \) which may be the same or different represent C\(_{1-10}\) hydrocarbyl groups. In a well studied example of such groups \( R^5 \) and \( R^6 \) are both cyclohexyl.
Other leaving groups include pentafluorophenoxy, i.e.

(b)

and the group

(c)

Compounds having leaving groups (a) may be produced by reacting a compound having a carboxyl-function with a corresponding carbodiimide.

Compounds having leaving groups (b) and (c) may be made from compounds containing leaving groups (a) by reaction with pentafluorophenol and 1-hydroxybenzotriazole.

In other words, the groups -COE represent carboxyl groups activated by known procedures, for example by reaction with a diimide such as for example, dicyclohexylcarbodiimide, by formation of a hydroxybenzotriazole ester or pentafluorophenyl ester or by using benzotriazole-1-yl-oxy-tris-(dimethyl-amino)phosphonium hexafluorophosphate.

As indicated carboxyl activation and addition of the carboxy-protected amino acid may be carried out successively or simultaneously. Thus:

(i) the carboxyl group can be activated prior to addition of protected amino acid, e.g. carboxyl activated by formation of the hydroxybenzotriazole ester or pentafluorophenyl ester. The "activated" resin is then washed and the protected amino acid then added or

(ii) the "activation" step (A) and the "addition of amino acid step" - Step (B) can be achieved in one step using e.g. benzotriazole-1-yl-oxy-tris(dimethyl-amino)phosphonium hexafluorophosphate (known as BOP or Castro's reagent), base and protected amino acid (stoichiometry 1:2:1).
The invention may also be used to synthesise peptides in the liquid phase, thus according to a further aspect of the invention there is provided a process for producing a peptide of the formula

\[ \text{H} \left[ -\text{NH.A.CO} - \text{OH} \right]_{n+x+1} \]  

(XXIX)

which comprises the steps of

(A) reacting a carboxy-activated reactant comprising an \( \text{NH}_2 \)-protected amino acid or \( N \)-terminal \( \text{NH}_2 \)-protected peptide, said carboxy-activated reacting having the formula

\[ \text{Prot'} \left[ -\text{NH.A.CO} \right] \epsilon_n \]  

(XXXII)

wherein \text{Prot'} represents an \(-\text{NH}_2\) protecting group with a carboxy protected amino acid of formula

\[ \text{H.NH.A.CO.OProt} \]

wherein \text{Prot} is a silyl group to form a chain-extended product of formula

\[ \text{Prot'} \left[ -\text{NH.A.CO} \right]_n \text{O.Prot} \]  

(XXXIII)

(B) removing said protecting group \text{Prot}

(C) optionally repeating steps (A) and (B) \( x \) times and
(D) removing the -NH₂ protecting groups Prot',

wherein n is a positive integer,
x is 0 or a positive integer,
ε is an leaving group,
each A, which may be the same or different, either represents the residue of an amino acid or the structure NH₂A is the residue NₐA of an imino acid HNₐACOOH,

and wherein any reactive side chains on residues A are protected and are subsequently deprotected. The protective groups Prot' are preferably t-butoxycarbonyl (tBoc), fluorenylmethoxycarbonyl (Fmoc) or triphenylmethyl (trityl or Trt) groups.

In the above procedures, the silyl groups are preferably of the formulae specified above, i.e. of the formula

\[ Si(R₁,R₂,R₃) \]

(XXXIII)

wherein R₁, R₂ and R₃, which may be the same or different represent hydrocarbyl groups containing 1 to 10 carbon atoms, preferably of the formula

\[ Si(R₁,R₂,R₃) \]

(XXXIII)

wherein R₁, R₂ and R₃, which may be the same or different represent C₁₋₄ alkyl groups.

Certain of the silyl esters suitable for use in accordance with the process aspects of the invention are novel. These include the following:

(A) silyl esters of an L-amino acids selected from L-valine, L-isoleucine, L-serine, L-threonine, L-aspartic acid, L-asparagine, L-lysine and L-methionine, said ester being substantially free of the corresponding D-isomer;
(B) silyl esters of a protected amino acid being:

(a) an O-protected amino acid selected from O-protected serine, O-protected threonine and O-protected tyrosine.

(b) α- and ω-carboxy-protected amino acid selected from ω-carboxy protected glutamic acid and β-carboxy protected aspartic acid;

(c) ε-amino protected lysine and guanino-protected arginine;

(d) (indo)-protected tryptophan ("(indo)-protected" indicates protection of the indole nitrogen)

(e) thio-protected cysteine; and

(f) imidazole-protected histidine;

Examples of classes (d), (e) and (f) include N-formyl tryptophan, 4-methoxy benzyl cysteine, and dinitrophenyl histidine.

(C) silyl esters of an α-NH₂-protected amino acids selected from α-amino protected glycine, α-amino protected alanine, α-amino protected valine, α-amino protected leucine, α-amino protected isoleucine, α-amino protected serine, α-amino protected threonine, α-amino protected aspartic acid, α-amino protected asparagine, α-amino protected glutamic acid, α-amino protected glutamine, α-amino protected lysine, α-amino protected histidine, α-amino protected arginine, α-amino protected phenylalanine, α-amino protected tyrosine, α-amino protected tryptophan, α-amino protected cysteine, α-amino protected methionine and α-amino protected proline, characterised in that said α-NH₂-protected group is other than a silyl group; and

(D) trialkyl silyl esters of amino acids of the formula

\[ H.NH.A.CO.O.Si(R_1R_2R_3) \]  

(XXXIV)

wherein \( R_1, R_2 \) and \( R_3 \), which may be the same or different represent alkyl groups having 1-20 carbon atoms, and H.NH.A.CO.O is the residue of an α-amino acid selected from glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, asparagine, glutamic acid, glutamine, lysine, histidine, arginine, phenylalanine, tyrosine, tryptophan, cysteine, methionine and proline, with the proviso that where the amino acid is other than lysine, aspartic acid or glutamic acid, at least one of \( R_1, R_2 \) and \( R_3 \) is other than methyl.
Where the novel silyl esters of the invention possess protective
groups of the amino function such groups are preferably t-butoxycarbonyl
(tBoc) groups, fluorenylmethoxycarbonyl (Fmoc) groups or triphenylmethyl
(Trt) groups.

In classes (B) and (C) above, the amino-protecting groups are
preferably t-butoxycarbonyl, fluorenylmethoxycarbonyl or triphenylmethyl
groups.

Further aspects of the invention include (a) the use of silyl
esters of amino acids in peptide synthetic procedures and (b) kits
comprising such esters, particularly kits of reagents for use in the
synthesis of peptides comprising supplies of silyl esters of amino acids,
and a supply of a reagent for activating carboxyl groups to convert them
to activated carboxyl groups capable of reacting with amino groups to
form peptide bonds.

The solid phase procedure for the synthesis of a peptide of
predetermined structure by the process of the present invention typically
involves the following steps:

(a) covalently bonding a trialkylsilyl ester of an alpha-amino acid
to an insoluble resin via the amino group of the amino acid;

(b) cleaving the trialkylsilyl ester;

(c) activating the carboxylic acid group formed by cleavage of the
atrialkylsilyl ester;

(d) coupling a trialkylsilyl ester of an alpha-amino acid via its
amino group to the activated carboxylic acid group;

(e) repeating steps (b), (c), and (d) in sequence until a peptide chain
of the predetermined structure has been formed; and

(f) cleaving the peptide chain from the resin, and cleaving the
atrialkylsilyl ester.

Although the process aspect of the invention described above is
directed to solid state synthesis of peptides utilizing a solid phase
reactant comprising a support, use of the preferred carboxyl protective
groups (silyl groups) in liquid phase systems is possible.
The liquid phase procedure for the synthesis of a peptide of predetermined structure by the process of the present invention typically involves the following steps:

(a) activating the carboxylic acid group of an N-protected alpha-amino acid;

(b) coupling the amino group of a trialkylsilyl ester of an alpha-amino acid to the activated carboxylic acid group of the N-protected amino acid;

(c) cleaving the trialkylsilyl ester;

(d) activating the carboxylic acid group resulting from cleavage of the trialkylsilyl ester;

(e) coupling the amino group of a trialkylsilyl ester of an alpha-amino acid to the activated carboxylic acid group;

(f) repeating steps (c), (d), and (e) in sequence until a peptide chain of the predetermined structure has been formed and

(g) cleaving the trialkylsilyl ester group and the N-protecting group.

Both the liquid phase and the solid phase procedures for the synthesis of peptides of the present invention are more convenient than the prior art procedures. The overall synthesis requires less time, requires less steps and uses lesser amounts of milder reactants under milder reaction conditions.
General Methods of Preparation

Trialkylsilyl esters of amino acids are most conveniently prepared according to the invention by the reaction of a trialkylsilyl chloride with an N-blocked amino acid. Many N-blocked amino acids may be obtained commercially, however in general they may be prepared in the well known manner from the corresponding amino acid. Thus, for example a t-Boc amino acid may be produced by reacting an amino acid with di-t-butyl dicarbonate.

The blocking group may be any conventional amine blocking group such as t-butoxycarbonyl, fluorenlymethoxycarbonyl, phenylacetyl, acetoacetyl, N-benzylidene, benzyl, benzyl, t-amlyoxycarbonyl, benzyloxycarbonyl, p-toluenesulfonyl, chorooacetyl, carbamyl triphenylmethylyl and the like, which can be readily cleaved, at the appropriate time, by acid or base, by hydrogenation or by enzymatic action.

As indicated, in preparing the silyl esters of amino acids in accordance with the invention, it is necessary first to block the amino group or groups. Preferably this is achieved by introducing a t-butoxycarbonyl (t-Boc) group by reaction with di-t-butyl dicarbonate.

An appropriate procedure is to dissolve the amino acid in aqueous dimethyl formamide or dioxan (2:1 vol. ratio of solvent:water) and add an equivalent amount of triethylamine (one mole/carboxyl group).

Then more than one equivalent of di-t-butyl dicarbonate is added with stirring for 1/2 - 2 hours at room temperature. The same volume of water is added and the mixture extracted with diethyl ether to remove unreacted reagent.

The reaction mixture is then acidified to pH 3 with citric acid or 1N HCl and extracted 2 x with ethyl acetate. The organic phase is then evaporated to isolate the t-Boc-protected amino acid.

For amino acids with more than one amino groups the individual amino groups generally require different blocking groups so that only the amino group destined to form the peptide bond is subsequently de-blocked.
Thus for lysine, the ε-amino group is preferably blocked with a benzylxoycarbonyl group prior to blocking the α-amino group with t-Boc. This may be achieved by forming a copper complex with the α-NH₂ and COOH groups, reacting with benzylxoycarbonyl chloride (Z-chloride) and then decomplexing and reacting with t-butyl dicarbonate as described above.

A similar procedure may be used for arginine, except that the guanidino group is blocked with a p-toluene sulphonyl group.

Amino acids having additional carboxyl groups are preferably in the form of their benzyl esters.

The hydroxyl groups of serine and threonine can be blocked, if necessary, by formation of benzyl esters and the hydroxyl group of tyrosine by formation of the 2-bromobenzylxoycarbonyl derivatives.

Similarly, the indolyl nitrogen of tryptophan may be blocked by formylation and the thio group of cysteine by 4-methylbenzylolation. Histidine may be protected as a dinitrophenyl derivative.

These methods of blocking the α-amino groups and reactive functional groups are standard and most of the protected amino acids are available commercially.

The reaction between the N-protected amino acid and the trialkylsilyl chloride, preferably trimethylsilyl chloride or t-butyldimethyl silyl chloride, is effected in an inert solvent, preferably an aprotic solvent such as ether, for example diethyl ether or tetrahydrofuran or in dimethyl formamide. The solvent should be carefully dried prior to use.

Most conveniently, the trialkylsilyl chloride, neat or dissolved in the solvent, is added dropwise over a period of time to a solution of the N-t-Boc blocked amino acid in a reaction vessel chilled in a cold water or water-ice bath. The reaction medium preferably contains a tertiary amine, e.g. triethyl amine, pyridine or imidazole, to serve as a scavenger for the hydrogen chloride formed during the course of the reaction.

After the trialkylsilyl chloride has been added, the reaction mixture is generally allowed to stand for about an hour to permit the reaction to reach completion. If the reaction mixture had been cooled, it may at this stage be permitted to warm to room temperature.
The solid tertiary amine hydrochloride, formed during the course of the reaction, may then be separated, most conveniently by filtration. Removal of the solvent from the filtrate under reduced pressure gives an almost theoretical yield of highly pure trialkylsilyl ester of the N-protected amino acid.

The silylation reaction is preferably carried out in an aprotic solvent in the presence of one equivalent of base (triethylamine, pyridine or imidazole). For the preparation of the trimethylsilyl esters, diethyl ether is the preferred solvent. Dimethylformamide is preferred for production of the t-butyldimethylsilyl esters. The reaction is generally complete in one hour at room temperature for the trimethylsilyl esters and in 2-8 hours for the t-butyldimethyl silyl esters.

In the case where the N-blocking group is t-butoxycarbonyl, to prepare the N-unblocked trialkylsilyl ester, the intermediate N-t-Boc blocked ester may be dissolved in dry ether and dry hydrogen chloride passed through the solution, preferably chilled to about 0-5°C, for about 30 minutes. The product can be isolated either by filtration or by evaporation of the solvent.
Using the procedure described above, the following trialkylsilyl esters of L-\(\alpha\)-amino acids were prepared:

- Tyr (2-Br-Z) - trimethylsilyl ester
- Ala - trimethylsilyl ester
- Phe - trimethylsilyl ester
- Leu - trimethylsilyl ester
- Pro - trimethylsilyl ester
- Lys (2-Cl-Z) - trimethylsilyl ester
- Gly - trimethylsilyl ester
- Met - trimethylsilyl ester
- Arg(Tos) - trimethylsilyl ester
- Glu(OBz) - trimethylsilyl ester
- Ile - trimethylsilyl ester
- Ser(Bz1) - trimethylsilyl ester
- Thr(Bz1) - trimethylsilyl ester
- Trp(Formyl) - trimethylsilyl ester
- Gln - trimethylsilyl ester
- Asn - trimethylsilyl ester
- Cys(4-Methyl Bz1) - trimethylsilyl ester
- Asp(OBz1) - trimethylsilyl ester
- Val - trimethylsilyl ester
- His(DNP) - trimethylsilyl ester

from
t-Boc - Tyr(2-Br-Z) - trimethylsilyl ester
  t-Boc - Ala - trimethylsilyl ester
  t-Boc - Phe - trimethylsilyl ester
  t-Boc - Leu - trimethylsilyl ester
  t-Boc - Pro - trimethylsilyl ester
  t-Boc - Lys (2-Cl-Z) - trimethylsilyl ester
  t-Boc - Gly - trimethylsilyl ester
  t-Boc - Met - trimethylsilyl ester
  t-Boc - Arg(Tos) - trimethylsilyl ester
  t-Boc - Glu(OBz) - trimethylsilyl ester
  t-Boc - Ile - trimethylsilyl ester
  t-Boc - Ser(Bzl) - trimethylsilyl ester
  t-Boc - Thr(Bzl) - trimethylsilyl ester
  t-Boc - Trp(Formyl) - trimethylsilyl ester
  t-Boc - Gln - trimethylsilyl ester
  t-Boc - Asn - trimethylsilyl ester
  t-Boc - Cys(4-Methyl Bzl) - trimethylsilyl ester
  t-Boc - Asp(OBz) - trimethylsilyl ester
  t-Boc - Val - trimethylsilyl ester
  t-Boc - His(DNP) - trimethylsilyl ester

Trp(Formyl), His(DNP) Arg(Tos) and Lys(2-Cl-Z) trimethyl silyl esters were found to be unstable on prolonged storage at room temperature due to their hygroscopic nature.

This problem was however circumvented by substituting a more stable t-butyldimethylsilyl moiety in place of trimethylsilyl group. N-t-Boc and Trt protected t-butyldimethyl silyl esters of alpha amino acids were prepared in N,N-dimethylformamide using imidazole as a base.

Using this methodology the esters specified in Table 3 (below) were prepared.

To prepare a peptide by the liquid phase procedure of the present invention (Scheme 1), the carboxylic acid group of an N-blocked amino acid is activated for coupling by treatment with an activator such as dicyclohexylcarbodiimide.
Then a trialkylsilyl ester of the next amino acid in the peptide chain being formed is added. The amino group of the amino acid moiety of the trialkylsilyl ester then couples with the activated carboxylic acid group of the N-blocked amino acid in solution.

The trialkylsilyl ester of the intermediate peptide precursor is readily cleaved with methanol or methanol/acetic acid and the liberated carboxylic acid group activated for coupling with a conventional activator such as DCC/HOBt. This sequence is repeated until a peptide chain of the required structure is formed. Then, the trialkylsilyl ester at the C-terminal end of the chain is cleaved and the N-blocking group removed with hydrochloric acid/diethyl ether to liberate the peptide. In order to prepare a pure peptide product, it is generally necessary to isolate and/or separate unreacted reagents from one or more of the intermediate peptide precursors produced during the course of the synthesis.

Tyr-Ala-Ala-Phe-Leu-OH and Ala-Ala-OH were prepared using the liquid phase procedure described above.

In the solid phase process of the present invention unlike that of the Merrifield procedure wherein an N-tBoc blocked amino acid is coupled via its carboxylic acid group to the resin, the trialkysilyl ester of the initial amino acid in the peptide chain to be formed is bound via its amino group to the insoluble resin. Most conveniently, the resin is a polystyrene resin bearing a -CH₂COCl substituent, e.g.

\[
\text{(Alk)}_3\text{SiOOCCHRNH}_2 + \text{ClOOCOCH}_2\text{-Resin} \xrightarrow{\text{Alk}} \text{(Alk)}_3\text{SiOOCCHRNOOCOCH}_2\text{-Resin}
\]

The carboxyl group is then deprotected by cleaving the trialkylsilyl ester with methanol or methanol/acetic acid, and the thus liberated carboxyl group activated with DCC/HOBt. A trialkylsilyl ester of a second amino acid is added, and the amino group of the second amino acid couples with the activated carboxyl group of the first amino acid, which remains bound to the resin.
This sequence of reactions is repeated until a peptide chain of
the desired structure is formed. Finally, the last trialkylsilyl ester
is cleaved with methanol or methanol/acetic acid, and the peptide is
liberated from the resin under acidic conditions e.g. HBr/acetic acid,
trifluoromethanesulphonic acid (TFMSA) in trifluoracetic acid (TFA) and
HF.

When preparing peptides using the process of the present
invention, lesser amounts of reagents are required in each of the cycles
in which an amino acid residue is added to the peptide chain, and the
cost of reagents and the overall time required to complete the synthesis
are reduced.

The reagents and reaction condition utilized are milder than
those customarily utilized (in the present process, methanol may be used
to cleave the protecting group; (c.f. the Merrifield procedure:
trifluoroacetic acid is used to remove the N-t-Boc blocking group and
Sheppard's Fmoc system: the base piperidine is used to remove the
N-terminal blocking group).

Accordingly, there is less damage to the resin matrix in solid
phase procedures or risk of adverse reactions with the constituent amino
acid in the peptide being formed. The reaction of trialkylsilyl chloride
with amino acid provides stable C-blocked amino acids, convenient to use
as intermediates in the synthesis of peptides, and the blocking group is
readily removable when the desired peptide chain has been prepared.

A further advantage of the method of the invention is that it is
possible to test for completion of the individual reaction stages by
measuring the conductivity of the reaction medium.
The following examples illustrate the invention

**EXAMPLE 1**

**Preparation of t-Butyloxycarbonyl (tBOC) Amino Acid Trimethylsilyl Esters**

1.1 **t-Boc-L-Alanine-Trimethylsilyl Ester**

The trimethylsilyl ester of L-alanine was prepared by the following procedure.

N-t-Boc-L-alanine (10 m.moles) was dissolved in anhydrous diethyl ether (40 ml) and pyridine (10 m.moles) was added. The solution was cooled to 0°C on an ice bath and stirred.

Trimethylsilyl chloride (11 m.moles) was then added dropwise. Immediately a white precipitate began to form (pyridine-HCl). The mixture was stirred for 1 hour and then allowed to warm to room temperature. The white solid was removed by filtration and the organic layer was evaporated under reduced pressure to give N-t-Boc-L-alaninetricmethysilyl ester as a liquid in a yield of 94%, which was stored under nitrogen. All operations were carried out under anhydrous conditions.

The product was characterised by its infra-red (IR) spectrum, $^1$H Nuclear Magnetic Resonance (NMR) spectrum and by thin layer chromatography (tlc) analysis. The $^1$H NMR spectrum were recorded on a R-1500 Hitachi (60 MHz) instrument using tetramethylsilane (TMS) as the internal standard.

The Infra-red spectrum showed bands at max (thin film):

- 760 (st.), 840 (st.), 1695 (st.), 1740 (st.) and 3400 cm$^{-1}$.
- (st. refers to a strong band).

The $^1$H NMR spectrum ($CDCl_3$) showed the following δ values:

- δ 0.25 (s., 9H), 1.45 (s., 9H), 4.0-4.25 (t., 1H)
- and 5.2 (br., 1H).

Thin layer chromatography on a silica plate using 70% diethyl ether-light petroleum (60-80°C) as solvent gave a Rf value of 0.31.
1.2-20 Trimethylsilyl Esters of (2) t-Boc Glycine,
   (3) t-Boc L-Valine, (4) t-Boc L-Phenylalanine,
   (5) t-Boc L-Isoleucine, (6) T-Boc L-Leucine,
   (7) t-Boc (2-Cl-Z)-L-Lysine, (8) t-Boc L-Methionine,
   (9) t-Boc L-Asparagine, (10) t-Boc L-Proline,
   (11) t-Boc (4-MBzl)-L-Cysteine,
   (12) t-Boc (2-Br-Z)-L-Tyrosine,
   (13) t-Boc (Bzl)-L-Threonine,
   (14) t-Boc (0-Bzl)-L-Glutamic acid,
   (15) t-Boc (0-Bzl)-L-Aspartic acid,
   (16) t-Boc L-Glutamine, (17) t-Boc (Tos)-L-Arginine,
   (18) t-Boc (Formyl)-L-Tryptophan,
   (19) t-Boc (DNP)-L-Histidine and
   (20) t-Boc (Bzl)-L-Serine

Trimethylsilyl esters of the title amino acids and protected
amino acids were prepared by the procedure described in Example 1.1
above, but substituting the following amino acids and protected amino
acids for N-t-Boc-L-alanine: t-Boc-glycine, L-valine, L-phenylalanine,
L-isoleucine, L-leucine, N-2-Cl-Z-L-lysine, L-methionine, L-asparagine,
L-proline, S-4-MBzl-L-cysteine, O-2-Br-Z-L-tyrosine, O-Bzl-L-threonine,
O-Bzl-L-glutamic acid, O-Bzl-L-aspartic acid, L-glutamine,
N-Tos-L-arginine, N-formyl-L-tryptophan, N-DNP-L-histidine and
O-Bzl-L-serine.

Diethylether was used as solvent in all cases except for Met,
Asp, Glu, Arg and His for which dichloromethane was used.

The trimethylsilyl esters were obtained in the yields reported in
Table 1.
<table>
<thead>
<tr>
<th>t-Boc-amino Acid</th>
<th>Side-chain Protection</th>
<th>Solvent</th>
<th>Rf</th>
<th>Nature of Product</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 L-Alanine</td>
<td>-</td>
<td>DEE</td>
<td>0.31</td>
<td>Liquid</td>
<td>94</td>
</tr>
<tr>
<td>2 Glycine</td>
<td>-</td>
<td>DEE</td>
<td>0.30</td>
<td>Liquid</td>
<td>95</td>
</tr>
<tr>
<td>3 L-Valine</td>
<td>-</td>
<td>DEE</td>
<td>0.28</td>
<td>Liquid</td>
<td>90</td>
</tr>
<tr>
<td>4 L-Phenylamine</td>
<td>-</td>
<td>DEE</td>
<td>0.29</td>
<td>Liquid</td>
<td>90</td>
</tr>
<tr>
<td>5 L-Isoleucine</td>
<td>-</td>
<td>DEE</td>
<td>0.3</td>
<td>Liquid</td>
<td>90</td>
</tr>
<tr>
<td>6 L-Leucine</td>
<td>-</td>
<td>DEE</td>
<td>0.32</td>
<td>Liquid</td>
<td>92</td>
</tr>
<tr>
<td>7 L-Lysine</td>
<td>2-Cl-Z</td>
<td>DEE</td>
<td>0.29</td>
<td>Liquid</td>
<td>92</td>
</tr>
<tr>
<td>8 L-Methionine</td>
<td>-</td>
<td>DCM</td>
<td>-</td>
<td>Liquid</td>
<td>94</td>
</tr>
<tr>
<td>9 L-Asparagine</td>
<td>-</td>
<td>DCM</td>
<td>0.28</td>
<td>Liquid</td>
<td>95</td>
</tr>
<tr>
<td>10 L-Proline</td>
<td>-</td>
<td>DEE</td>
<td>0.31</td>
<td>Liquid</td>
<td>90</td>
</tr>
<tr>
<td>11 L-Cysteine</td>
<td>4-MBzl</td>
<td>DEE</td>
<td>0.28</td>
<td>Liquid</td>
<td>92</td>
</tr>
<tr>
<td>12 L-Tyrosine</td>
<td>2-BrZ</td>
<td>DEE</td>
<td>0.26</td>
<td>Liquid</td>
<td>94</td>
</tr>
<tr>
<td>13 L-Threonine</td>
<td>Bz1</td>
<td>DEE</td>
<td>0.4</td>
<td>Liquid</td>
<td>90</td>
</tr>
<tr>
<td>14 L-Glutamic acid</td>
<td>O-Bzl</td>
<td>DEE</td>
<td>0.27</td>
<td>Liquid</td>
<td>91</td>
</tr>
<tr>
<td>15 L-Aspartic acid</td>
<td>O-Bzl</td>
<td>DEE</td>
<td>0.3</td>
<td>Liquid</td>
<td>90</td>
</tr>
<tr>
<td>16 L-Glutamine</td>
<td>-</td>
<td>DCM</td>
<td>0.27</td>
<td>Liquid</td>
<td>91</td>
</tr>
<tr>
<td>17 L-Arginine</td>
<td>Tos</td>
<td>DCM</td>
<td>0.25</td>
<td>(hygroscopic liquid)</td>
<td>95</td>
</tr>
<tr>
<td>18 L-Tryptophan</td>
<td>Formyl (CHO)</td>
<td>DEE</td>
<td>-</td>
<td>(hygroscopic liquid)</td>
<td>90</td>
</tr>
<tr>
<td>19 L-Histidine</td>
<td>DNP</td>
<td>DCM</td>
<td>-</td>
<td>(hygroscopic liquid)</td>
<td>91</td>
</tr>
<tr>
<td>20 L-Serine</td>
<td>Bz1</td>
<td>DEE</td>
<td>0.39</td>
<td>Liquid</td>
<td>95</td>
</tr>
</tbody>
</table>

2-Cl-Z = 2-chlorobenzylxloxy carbonyl
4-M-Bzl = 4-Methylbenzyl
2-Br-Z = 2-Bromobenzylxloxy carbonyl
Bz1 = Benzyl
O-Bzl = Benzyl ester
Tos = p-Toluenesulphonyl
CHO = Formyl
DNP = 2,4-dintrophenyl
DEE = Diethyl ether
DCM = Dichloromethane
EXAMPLE 2

Cleavage of t-Butyloxy carbonyl (tBOC) Amino Acid Trimethylsilyl Esters to Yield Amino Acid Trimethylsilyl Esters

2.1 L-Alanine-Trimethylsilyl Ester Hydrochloride.

N-t-Boc-L-alanine trimethyl silyl ester (10 mmoles) prepared as described in Example 1.1 was dissolved in anhydrous diethyl ether (50 ml). Dry hydrogen chloride gas was then bubbled into the solution until saturation. A white precipitate formed which was filtered, washed with diethyl-ether to remove traces of HCl and was dried under reduced pressure. The solid was then stored under inert atmosphere (N₂).

The resulting L-alanine-trimethyl silyl ester hydrochloride was characterised by its Infra-red spectrum, and by melting point determination.

Infra-red spectrum (Nujol mull) showed bands at max 760 (st.), 845-860 (st.), 1175 (st.), 1200 (st.), 1755 (st.), 2985 and 3400 cm⁻¹.

2.2-20 Trimethylsilyl Ester Hydrochlorides of (2) Glycine, (3) L-Valine,
(4) L-Phenylalanine, (5) L-Isoleucine, (6) L-Leucine,
(7) 2-Cl-z-L-Lysine, (8) L-Methionine, (9) L-Asparagine,
(10) L-Proline, (11) (4-MBzl)-L-Cysteine,
(12) (2-Br-Z)-L-Tyrosine, (13) (Bz1)-L-Threonine,
(14) (0-Bz1)-L-Glutamic acid,
(15) (0-Bz1)-L-Aspartic acid,
(16) L-Glutamine, (17) (Tos)-L-Arginine,
(18) (Formyl)-L-Tryptophan, (19) (DNP)-L-Histidine and
(20) (Bz1)-L-Serine

Trimethylsilyl esters of the title amino acids and protected amino acids were prepared by the procedure described in Example 2.1 above, but substituting the corresponding t-Boc amino acid and t-Boc protected amino acid trimethylsilyl esters (2) to (20) referred to in Table 1 for t-Boc L-alanine trimethylsilyl ester.

The trimethylsilyl ester hydrochlorides were obtained in the yields reported in Table 2.
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Side-chain Protection</th>
<th>Melting Point (Uncorrected) °C</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 L-Alanine</td>
<td>-</td>
<td>225-228</td>
<td>85</td>
</tr>
<tr>
<td>2 Glycine</td>
<td>-</td>
<td>165-167</td>
<td>75</td>
</tr>
<tr>
<td>3 L-Valine</td>
<td>-</td>
<td>230-233</td>
<td>80</td>
</tr>
<tr>
<td>4 L-Phenylalanine</td>
<td>-</td>
<td>227-229</td>
<td>87</td>
</tr>
<tr>
<td>5 L-Isoleucine</td>
<td>-</td>
<td>176-177</td>
<td>78</td>
</tr>
<tr>
<td>6 L-Leucine</td>
<td>-</td>
<td>232-234</td>
<td>81</td>
</tr>
<tr>
<td>7 L-Lysine</td>
<td>2-Cl-Z</td>
<td>205-208</td>
<td>81</td>
</tr>
<tr>
<td>8 L-Methionine</td>
<td>-</td>
<td>222-223</td>
<td>89</td>
</tr>
<tr>
<td>9 L-Asparagine</td>
<td>-</td>
<td>162-164</td>
<td>77</td>
</tr>
<tr>
<td>10 L-Proline</td>
<td>-</td>
<td>125</td>
<td>75</td>
</tr>
<tr>
<td>11 L-Cysteine</td>
<td>4-M-Bzl</td>
<td>210-212</td>
<td>75</td>
</tr>
<tr>
<td>12 L-Tyrosine</td>
<td>2-Br-Z</td>
<td>221-222</td>
<td>88</td>
</tr>
<tr>
<td>13 L-Arginine</td>
<td>Tos</td>
<td>hygroscopic</td>
<td>72</td>
</tr>
<tr>
<td>14 L-Threonine</td>
<td>Bzl</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>15 L-Glutamic acid</td>
<td>O-Bzl</td>
<td>145-148</td>
<td>75</td>
</tr>
<tr>
<td>16 L-Aspartic acid</td>
<td>O-Bzl</td>
<td>186-188</td>
<td>78</td>
</tr>
<tr>
<td>17 L-Glutamine</td>
<td>-</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>18 L-Tryptophan</td>
<td>CHO</td>
<td>-</td>
<td>72</td>
</tr>
<tr>
<td>19 L-Histidine</td>
<td>DNP</td>
<td>hygroscopic</td>
<td>70</td>
</tr>
<tr>
<td>20 L-Serine</td>
<td>Bzl</td>
<td>-</td>
<td>81</td>
</tr>
</tbody>
</table>

(Abbreviations as in Table 1)
EXAMPLE 3

Preparation of t-Butyloxycarbonyl (tBOC) Amino Acid t-Butyl-Dimethylsilyl Esters

3.1 t-Boc-L-Alanine-t-Butyldimethylsilyl Ester

t-Boc-L-alanine-t-butyltrimethylsilyl ester was prepared by the following procedure.

N-t-Boc-L-alanine (10 m.moles) was dissolved in N,N-dimethylformamide (20 ml) and imidazole (20 m.moles) added to act as base and catalyst.

The solution was stirred at room temperature and then t-butyltrimethylsilyl chloride (11 m.moles, 1.6 g) was added slowly. The reaction mixture was stirred for a further period of 2 hours at room temperature and a precipitate of imidazole hydrochloride formed.

The mixture was diluted with diethyl ether (60 ml) and washed with 10% sodium bicarbonate (1 x 20 ml), water (1 x 20 ml), 0.1 M hydrochloric acid (1 x 20 ml), and water (2 x 20 ml). The organic layer was then dried over anhydrous sodium sulphate and was evaporated under reduced pressure to give N-t-Boc-L-alanine-t-butyldimethyl silyl ester as an oil.

The N-t-Boc-L-alanine-t-butyldimethyl silyl ester was characterised by its Infra-red spectrum, $^1$H nuclear magnetic resonance spectrum and by thin layer chromatography (tlc).

Infra-red spectra showed bands at max. (thin film) 760-780 (st.), 845 (st.), 1180 (st.), 1260 (st.), 1695 (st.), 1720 (st.), 2960, and 3400 cm$^{-1}$.

$^1$H n.m.r. (CDCl$_3$) δ 0.21 (s., 6H), 0.9 (s., 9H), 1.45 (s., 9H)
3.2-11 t-Butyl-Dimethyldimethylsilyl Esters of (2) t-Boc Phenylalanine
(3) t-Boc (O-Bz1)-L-Aspartic acid,
(4) t-Boc L-Leucine,
(5) t-Boc (2-Br-Z)-L-Tyrosine, (6) t-Boc L-proline
(7) t-Boc L-Valine, (8) t-Boc (DNP)-L-Histidine and
(9) t-Boc L-Isoleucine, (10) t-Boc L-Glycine,
(11) t-Boc (Tos)-L-Arginine,

t-Butyl-dimethyldimethylsilyl esters of the title t-Boc amino acids and
protected amino acids were prepared by the procedure described in Example
3.1 above, but substituting the following t-Boc amino acids and protected
amino acids for N-t-Boc-alanine:
t-Boc Phenylalanine t-Boc 0-Bz1-L-Aspartic acid, t-Boc
L-Leucine, t-Boc 2-Br-Z-L-Tyrosine, t-Boc L-proline t-Boc L-Valine,
t-Boc DNP-L-Histidine and t-Boc L-Isoleucine, t-Boc L-Glycine,
t-Boc Tos-L-Arginine.

Dimethylformamide was used as solvent in all cases.

The t-butyl-dimethyldimethylsilyl esters were obtained in the yields
reported in Table 3.

Cys(4-methylBz1)-t-butyl-dimethyldimethylsilyl ester and Lys(2-Cl-Z)-
t-butyl-dimethyldimethylsilyl ester have also been prepared by the above
procedure.
### TABLE 3
**t-BOC-AMINO ACID t-BUTYLDIMETHYLSIYL ESTERS**

<table>
<thead>
<tr>
<th>t-Boc-Amino Acid</th>
<th>Side-chain Protection</th>
<th>Solvent (reaction)</th>
<th>Nature of Product</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>60</td>
</tr>
<tr>
<td>L-Phenylalaine</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>55</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>O-Bzl</td>
<td>DMF</td>
<td>Liquid</td>
<td>45</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>60</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>2-Br-Z</td>
<td>DMF</td>
<td>Liquid</td>
<td>50</td>
</tr>
<tr>
<td>L-Proline</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>45</td>
</tr>
<tr>
<td>L-Valine</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>50</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>DNP</td>
<td>DMF</td>
<td>Liquid</td>
<td>50</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>55</td>
</tr>
<tr>
<td>Glycine</td>
<td>-</td>
<td>DMF</td>
<td>Liquid</td>
<td>45</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>Tos</td>
<td>DMF</td>
<td>Liquid</td>
<td>51</td>
</tr>
</tbody>
</table>

*(Abbreviations as in Table 1)*
EXAMPLE 4
Cleavage of t-Boc-group - Preparation of Amino
Acid t-Butyldimethylsilyl Esters

4.1 L-Alanine t-Butyldimethylsilyl Ester

t-Boc-L-alanine t-butyldimethylsilyl ester (10 m.moles) was treated with
25% trifluoroacetic acid in dichloromethane (25 ml) for 30 minutes at
room temperature. The solvents (i.e. dichloromethane, trifluoroacetic
acid) were then removed under reduced pressure at room temperature and an
oil was obtained in high yield.

The resulting L-alanine t-butyldimethylsilyl ester was characterised by
its Infra-red spectrum and $^1H$ n.m.r. spectrum.

Infra-red spectrum (thin film) showed bands at max. 700 (st.),
800-860 (st.), 1200 (st.), 1740-1790 (st.), 2960, 3050
and 3240 cm$^{-1}$.

$^1H$ n.m.r. (DMSO-$d_6$) $\delta$ 0.19 (s., 6H), 0.9 (s., 9H).

4.2 Cleavage of t-Boc-group - Preparation of
t-Butyldimethylsilyl Esters of (2) Phenylalanine
(3) (O-Bzl)-L-Aspartic acid, (4) L-Leucine,
(5) (2-Br-Z)-L-Tyrosine, (6) L-proline
(7) L-Valine, (8) (DNP)-L-Histidine,
(9) L-Isoleucine, (10) L-Glycine, and
(11) (Tos)-L-Arginine,

The t-Boc t-butyldimethylsilyl esters of the title t-Boc amino
acids and protected amino acids may be cleaved by the procedure described
in Example 4.1 above.
EXAMPLE 5
Production of Resin

A resin containing -(CH₂O)₂CO.C₁ groups was prepared from a standard, commercially available "Merrifield" resin (bearing -(CH₂Cl groups) by the following procedure (see Scheme 2).

Merrifield resin (25g) was reacted with sodium acetate in dimethoxyethane for 48 hours at 80°C to give the ester in high yield. The identity of the ester was confirmed by its infra-red spectrum (1730 cm⁻¹ and an undetectable amount of chlorine. Reduction of the ester with LiAlH₄ in diethylether gave after the usual work-up procedure the methylol resin. The methylol resin was then treated with phosgene in toluene at room temperature and afforded the desired chloroformate resin in high yield. Chlorine analysis indicated that there was 0.9 mmoles/g of Cl on the resin.

EXAMPLE 6
Production of Peptides
Solid Phase Synthesis of Tetrapeptide

The tetrapeptide H-Leu-Ala-Gly-Val-OH was synthesised manually by the following procedure. All amino acids were of L-configuration. The reaction sequence is outlined in Schemes 3 and 4.

The resin as prepared in Example 5 was placed in a reaction vessel and washed with dimethylacetamide (DMA) (3 x 10 ml).

Leu t-butyl dimethysilyl ester was added in DMA (10 ml) followed by triethylamine and the reaction vessel was shaken at room temperature for 2 hours. The liquid phase was drained out and the resin washed with DMA (2 x 10 ml). Estimation of unreacted Leu derivative indicated that 0.61 mmol/g had been bound to the resin. The remaining free chloroformate on the resin was capped with diethylamine in DMA.

The t-butyldimethysilyl Leu resin was treated with warm (40°C.) methanol (10 ml) for 30 minutes to remove the t-butyl dimethysilyl group. The resin was washed with DMA (3 x 10 ml) and was shaken with H-Ala-OSi-(Me)₂t-Bu in DMA in the presence of DCC/HOBt for 45 minutes.
The solvents were drained and the resin washed with DMA (3 x 10 ml). 100 mg resin was withdrawn from the reaction vessel. The resin was then coupled as described above to H-Gly-OSi(Me)$_2$-t-Bu and 100 mg of

Resin-$(C_6H_5)_2CH2O-CO-NH-Leu-Ala-Gly-OSi(Me)$_2$-tBu

was withdrawn as usual. It was then coupled to H-Val-OSi(Me)$_2$-tBu in a similar manner and the desired resin peptide was obtained. The peptides Leu-Ala, Leu-Ala-Gly, and Leu-Ala-Gly-Val were released from the resin by the standard HF cleavage procedure.

These peptides were also synthesised by Merrifield solid phase procedure. Comparative TLC and HPLC have shown that di-, tri-, and tetrapeptides synthesised by both methods are identical. There was no indication of any racemisation during solid phase silyl methodology in the synthesis of these peptides.
1. A process for producing a peptide of the formula

\[
\text{H-} \overline{\text{NH.A.CO}} \overline{\text{OH}}^{n+x+1} \quad (\text{XXIV})
\]

which comprises the steps of

(A) reacting a solid phase reactant comprising a solid support-bound amino acid or a solid support-bound peptide, said solid phase reactant having the formula

\[
\text{Support-} \overline{\text{NH.A.CO}} \overline{\text{OH}}^{n} \quad (\text{XXV})
\]

with a carboxyl group activating agent to form an activated solid phase reactant of the formula

\[
\text{Support-} \overline{\text{NH.A.CO}} \overline{\varepsilon}^{n} \quad (\text{XXVI})
\]

(B) reacting the activated solid phase reactant from step (A) with a carboxy-protected amino acid of formula

\[
\text{H.NH.A.CO.Prot} \quad (\text{XXVII})
\]

to form a chain-extended product of formula

\[
\text{Support-} \overline{\text{NH.A.CO}} \overline{\text{O.Prot}}^{n+1} \quad (\text{XXVIII})
\]

(C) removing said protective group Prot,
(D) optionally repeating said steps (A), (B) and (C) x times, and
(E) cleaving the resulting peptide of formula

\[ \text{H} - \left[ \text{NH.A.CO} \right]_{n+x+1} \text{OH} \]

from the support.

wherein n is a positive integer,
x is 0 or a positive integer,
\( \epsilon \) is a leaving group,
Prot is a silyl group.
each A, which may be the same or different, either represents the residue of an amino acid or the structure NH.A.CO is the residue \( .NKA \) of an imino acid HN\( \epsilon \)ACO\( \epsilon \)H
and wherein any reactive side chains on residues A are protected and are subsequently deprotected, and said steps (A) and (B) are carried out successively or in a single operation.

2. A process according to Claim 1 comprising the following steps:
(a) reacting a solid phase reactant comprising a solid support-bound amino acid or a solid support-bound peptide, said solid phase reactant having the formula

\[ \text{Support} - \left[ \text{NH.A.CO} \right]_{n} \text{OH} \quad (XXV) \]

simultaneously with a carboxyl group activating agent and with a carboxy-protected amino acid of formula

\[ \text{H.NH.A.CO.Prot} \quad (XXVII) \]

to form a chain-extended product of formula

\[ \text{Support} - \left[ \text{NH.A.CO} \right]_{n+1} \text{0.Prot} \quad (XXVIII) \]
(b) removing said protective group Prot,
(c) optionally repeating said steps (A), (B) and (C) x times, and
(d) cleaving from the support the resulting peptide of formula

\[
H\left[\text{NH.A.CO}\right]_{n+x+1} \text{OH} \quad (XXIX)
\]

n, x, ε, Prot and A being as defined in Claim 1.

3. A process according to Claim 1 wherein said solid phase reactant

\[
\text{Support} \left[\text{NH.A.CO}\right]_{n} \text{OH} \quad (XXV)
\]

wherein n is 1 is produced by reacting an amino acid silyl ester of formula

\[
H\text{.NH.A.CO.OProt} \quad (XXVII)
\]

wherein Prot is a silyl group with a support material having groups capable of forming a covalent bond with the amino or imino group of the amino acid silyl ester, and removing said group Prot.

4. A process according to Claim 3 wherein said groups capable of forming a covalent bond are \(-\text{CH}_2\text{OCOCl}\) groups.

5. A process according to any preceding claim wherein said silyl group has the formula

\[
\text{Si}(R_1,R_2,R_3) \quad (XXXI)
\]

wherein \(R_1\), \(R_2\) and \(R_3\), which may be the same or different represent hydrocarbyl groups containing 1 to 20 carbon atoms.

6. A process according to Claim 5 wherein said silyl group has the formula

\[
\text{Si}(R_1,R_2,R_3) \quad (XXXII)
\]

wherein \(R_1\), \(R_2\) and \(R_3\), which may be the same or different represent \(C_{1-4}\) alkyl groups.
7. A process according to Claim 6 wherein said silyl group is a trimethyl silyl group or a t-butyldimethyl silyl group.

9. A process according to any preceding claim wherein said group ε represents an electron-withdrawing group.

10. A process according to any preceding claim in which leaving group ε is selected from Cl, Br, I, C<sub>1-4</sub> alkoxy, sec-amino and disilazano.

11. A process according to any of Claims 1 to 9 wherein leaving group ε is selected from groups having the structures:

\[ R^5\text{NH-C=N-R}^6 \] (a)

where \( R^5 \) and \( R^6 \) which may be the same or different represent C<sub>1-10</sub> hydrocarbyl groups;

pentafluorophenoxy, i.e.

![Diagram](b)

and the group

![Diagram](c)

12. A process for producing a peptide of the formula

\[
\text{H-}[\text{NH.A.CO}]_{n+x+1}\text{-OH} \quad (\text{XXIX})
\]

which comprises the steps of
(A) reacting a carboxy-activated reactant comprising an \( \text{NH}_2 \)-protected amino acid or N-terminal \( \text{NH}_2 \)-protected peptide, said carboxy-activated reacting having the formula

\[ \text{Prot}'\left[\text{NH.A.CO} \right]_n \epsilon \]  

(XXXII)

wherein \( \text{Prot}' \) represents an \( \text{-NH}_2 \) protecting group with a carboxy protected amino acid of formula

\[ \text{H.NH.A.CO.OProt} \]

wherein \( \text{Prot} \) is a silyl group to form a chain-extended product of formula

\[ \text{Prot}'\left[\text{NH.A.CO} \right]_{n+1} \text{O.Prot} \]

(B) removing said protecting group \( \text{Prot} \),
(C) optionally repeating steps (A) and (B) \( x \) times, and
(D) removing the \( \text{-NH}_2 \) protecting groups \( \text{Prot}' \).

wherein \( n \) is a positive integer,
\( x \) is 0 or a positive integer,
\( \epsilon \) is a leaving group,
\( \text{Prot} \) is a silyl group,
each \( A \), which may be the same or different, either represents the residue of an amino acid or the structure \( \text{NH.A} \) is the residue \( \text{N}<\text{A} \) of an imino acid \( \text{HN}<\text{ACOOH} \)

and

wherein any reactive side chains on residues \( A \) are protected and are subsequently deprotected.

13. A process according to Claim 12 wherein \( \text{Prot} \) and \( \epsilon \) are as defined in any of Claims 5 to 11.

14. A silyl ester of an L-amino acid selected from, L-valine, L-isoleucine, L-serine, L-threonine, L-aspartic acid, L-asparagine, L-lysine and L-methionine, said ester being substantially free of the corresponding D-isomer.
7. A silyl ester of a protected amino acid being:
(a) an 0-protected amino acid selected from 0-protected serine, 0-protected threonine and 0-protected tyrosine,
(b) a β- and γ-carboxy-protected amino acid selected from β-carboxy protected glutamic acid and γ-carboxy protected aspartic acid,
(c) ε-amino protected lysine and guanidino-protected arginine,
(d) indol-protected tryptophan,
(e) thio-protected cysteine and
(f) imidazole-protected histidine.

16. A silyl ester of an α-NH₂-protected amino acid selected from α-amino protected glycine, α-amino protected alanine, α-amino protected valine, α-amino protected leucine, α-amino protected isoleucine, α-amino protected serine, α-amino protected threonine, α-amino protected aspartic acid, α-amino protected asparagine, α-amino protected glutamic acid, α-amino protected glutamine, α-amino protected lysine, α-amino protected histidine, α-amino protected arginine, α-amino protected phenylalanine, α-amino protected tyrosine, α-amino protected tryptophan, α-amino protected cysteine, α-amino protected methionine and α-amino protected proline, characterised in that said α-NH₂-protected group is other than a silyl group.

17. An ester as claimed in claim 16 wherein said α-NH₂-protective group is a t-butoxycarbonyl group, a fluorenylmethoxycarbonyl group, or a triphenylmethyl group.

18. A trialkyl silyl ester of an amino acid of the formula

\[ \text{H.NH.A.CO.0.Si(R}_1\text{R}_2\text{R}_3 \] 

wherein \( R_1, R_2 \) and \( R_3 \), which may be the same or different represent alkyl groups having 1-20 carbon atoms, and H.NH.A.CO.0 is the residue of an α-amino acid selected from glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, asparagine, glutamic acid, glutamine, lysine, histidine, arginine, phenylalanine, tyrosine, tryptophan, cystein, methionine and proline, with the proviso that where the amino acid is other than lysine, aspartic acid or glutamic acid, at least one of \( R_1, R_2 \) and \( R_3 \) is other than methyl.
A silyl ester of an amino acid according to any of Claims 15 to 18 being in the L-configuration.

20. A kit of reagents for use in the synthesis of peptides comprising supplies of silyl esters of amino acids, and a supply of a reagent for activating carboxyl groups to convert them to activated carboxyl groups capable of reacting with amino groups to form peptide bonds.
SCHEME 1

\[ \text{t.Boc.NH}_2\text{.COOH} \rightarrow \text{t.Boc.NH}_2\text{.COE} \]

\[ \text{t.Boc.NH}_2\text{.COE} + \text{NH}_2\text{.A.CO Silyl} \]

\[ \text{CH}_2\text{Cl}_2 \]

\[ \text{t.Boc.NH}_2\text{.CO.NH}_2\text{.COO Silyl} \]

SCHEME 2

Resin \( \text{CH}_2\text{Cl} \) \rightarrow Resin \( \text{CH}_2\text{O.CO.CH}_3 \)

Merrifield Resin

Resin \( \text{CH}_2\text{OH} \) \rightarrow Resin \( \text{CH}_2\text{O.CO.Cl} \)

SUBSTITUTE SHEET
SCHEME 3

\[
\text{Silyl-}O\text{-CO.A.NH}_2 + \text{Cl-}COO\text{CH}_2\text{-Resin} \rightarrow \text{Silyl-}O\text{-CO.A.NH.COOCOCH}_2\text{-Resin}
\]

\[\text{Resin} \quad \text{NH.A.COOSilyl} \quad \text{MeOH}\]
\[\text{Resin} \quad \text{NH.A.COOH} \quad \text{Activation (e.g. DCC)}\]
\[\text{Resin} \quad \text{NH.A.COE} \quad \text{NH}_2\text{.A}^{1}\text{.COO Silyl}\]
\[\text{Resin} \quad \text{NH.A.CO.NH.A}^{1}\text{.COOSilyl}\]

(1) MeOH

(2) HF cleavage or TFMSA

Peptide

SUBSTITUTE SHEET
SCHEME 4

Resin - CH₂OCOC₁ + H₂N-Leu-OSi-Me + Bu →

Resin - CH₂OCONH-Leu.OSi-Me →

Resin - CH₂OCONH Leu.OH

DCC/HOBT →

Resin - CH₂OCONH Leu.Ala.OSi-Me →

Resin - CH₂OCONH. Leu.Ala.Gly.Val.OH

LeuAlaGlyVal →

HF or TFMSA

SUBSTITUTE SHEET
I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

**IPC 5:** C 07 K 1/04, C 07 K 1/08

II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
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<tr>
<td>IPC 5</td>
<td>C 07 K</td>
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Documentation searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>Chemische Berichte, vol. 100, 1967 Verlag Chemie GmbH (Weinheim/Bergstr. DE) J. Hils et al.: &quot;Die Umsetzung von Aminosäuren mit Trimethylchlo... pages 1638-1645 see the whole article esp. the 5th compound on table 1, page 1640</td>
<td>18,19</td>
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</tbody>
</table>

* Special categories of cited documents: 10

**A** document defining the general state of the art which is not considered to be of particular relevance

**E** earlier document but published on or after the international filing date

**L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

**O** document referring to an oral disclosure, use, exhibition or other means

**P** document published prior to the international filing date but later than the priority data claimed

**T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

**X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

**Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

**A** document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

5th February 1990

Date of Mailing of this International Search Report

01.03.90

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

T.K. WILLIS

Form PCT/ISA/210 (second sheet) (January 1985)