Organic Syntheses Employing Supercritical Carbon Dioxide as a Reaction Solvent

Inventors: Leon E. Barstow; Glen D. Ward; Milan Bier, all of Tucson, Ariz.


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Related U.S. Application Data

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ABSTRACT

Chemical reactions are readily carried out using supercritical carbon dioxide as the reaction medium. Supercritical carbon dioxide is of special value as a reaction medium in reactions for synthesizing polypeptides, for sequencing polypeptides, or for amino acid analysis.

4 Claims, No Drawings
ORGANIC SYNTHESSES EMPLOYING 
SUPERCritical CARBON DIOXIDE AS A 
REACTION SOLVENT

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under SBIR Contract No. NAS 2-12563 awarded by the 
National Aeronautics and Space Administration 
(NASA). The Government has certain rights in this 
invention.

This application is a division of application Ser. No. 
167,201, filed Mar. 11, 1988, and now U.S. Pat. No. 
5,001,224.

This invention pertains to a new procedure for con-
ducting chemical reactions in a less expensive manner 
that in many cases may yield a reaction product in a 
purer form than heretofore possible. It has particular 
applicability to processes for forming synthetic poly-
peptides and amino acid derivatives by reactions at 
carbon and nitrogen to form esters and amides.

BACKGROUND OF THE INVENTION

It is well known that for many chemical reactions to 
occur at practical rates at least one of the reactants must 
be dissolved in an inert solvent. For many organic reac-
tions, those solvents are liquid hydrocarbons or haloge-
nated hydrocarbons, such as chloroform, methylene 
chloride and chloromethane.

In many cases, the use of such solvents give satisfac-
tory yield of product but presents problems and expense 
in solvent costs, manipulation, recovery and disposal. 
There are certain reactions important to biochemistry, 
such as polypeptide syntheses, in which such solvents 
pose certain problems. Polypeptides are frequently pre-
bared by sequential addition of protected amino acids to 
a growing peptide chain attached to a solid substrate, 
such as a polystyrene or porous glass substrate. As pres-
ently practiced, these reactions do not go 100% to com-
pletion.

For example, conventional techniques using solid 
supports sometime suffer from incomplete reaction be-
cause of the solvent’s inability to penetrate the resin. As 
a consequence, the final product often is a complex 
mixture of polypeptides which is difficult to purify. The 
synthesis is further complicated by the presence of or-
ganic reaction solvents which also must be removed. 
Moreover, such solvents tend to be fairly expensive and 
organize facilities making it a very desirable reaction sol-
vent. It is inert under a wide variety of organic reaction 
conditions. It has a high diffusivity and is readily able to 
penetrate solid phase supports. Since it is inexpensive and 
non-toxic, it can be vented to the atmosphere for 
disposal. It is easily used where anhydrous conditions 
must be maintained. It minimizes problems in recovery 
of reaction product because the solvent is removed 
simply by reducing pressure to subcritical, thereby 
avoiding the need for repeated washings to remove 
spent reagents, side products and other reaction sol-
vents.

Supercritical carbon dioxide may be used in any 
chemical reaction in which a chemical reaction product 
is obtained by contacting two or more reactive chemi-
cal moieties in the presence of an inert reaction solvent, 
causing combination of the moieties to form the reac-
tion product, under the conditions needed to maintain 
supercritical carbon dioxide. The moieties may be the 
same or different. The moieties each may have a single 
reactive site, or they may have more than one. The 
reaction may be a conventional reaction to form a low 
molecular weight chemical, such as an ester, an ether, 
an amide, etc., or it may be a dimerization, trimeriza-
tion, oligomerization or polymerization.

Supercritical carbon dioxide may be the sole solvent, 
but other solvents may be present and still achieve the 
benefits attributable to supercritical carbon dioxide. 
Thus, if reactants are obtained in the form of solutions, 
the solution may be used without first separating the 
reactant from the solvent.

The reactions may be carried out at any temperature 
above the critical temperature of carbon dioxide, and 
any pressure above its critical pressure, consistent with 
the stability of reactants and reaction products, and 
with the desired rates of the desired reaction and unde-
sired side reactions with carbon dioxide. It has been 
found that many reactions readily occur at temperatures 
from about 90° F. to about 150° F., and at pressures 
from about 1073 psi up to 4000 psi or even higher.

The use of supercritical carbon dioxide is of special 
importance in carrying out reactions used in peptide 
synthesis, such as acylation, e.g., acetylation of primary 
and secondary amines with various acylating reagents 
such as acylimidazoles, e.g., acetylimidazole, acyl chlo-
rdes, e.g., acetylchloride, or acyl anhydrides, e.g. 
acetic anhydride and esterification of alcohols with re-
agents such as acylimidazoles, acyl anhydrides and acyl 
chlorides.

For example, a common solid-phase process for the 
synthesis of synthetic polypeptides, known as the 
Merrifield method, starts with an amino acid coupled to 
a solid support, commonly a polystyrene resin, through 
the carboxyl group of the amino acid. In addition, the 
E-amino group is protected against undesired reactions 
by a t-butoxycarbonyl (BOC) protecting group. The 
conventional polypeptide synthesis proceeds by remov-
ing the BOC protecting group (deprotection) by treat-
ment with a mild acid such as trifluoroacetic acid 
(TFA) in dichloromethane, washing with dichloro-
methane to remove all traces of TFA, neutralizing with 
triethylamine (TEA) in dichloromethane, washing with 
dichloromethane to remove excess TEA and its salts, 
reacting the deprotected resin-amino acid with a BOC-

term “supercritical carbon dioxide”, as used herein, 
refers to carbon dioxide at a temperature greater than 
88° F. and a pressure greater than 1073 psi. 
Carbon dioxide in its supercritical state has a number 
of properties making it a very desirable reaction sol-
vent. It is inert under a wide variety of organic reaction 
conditions. It has a high diffusivity and is readily able to 
penetrate solid phase supports. Since it is inexpensive and 
non-toxic, it can be vented to the atmosphere for 
disposal. It is easily used where anhydrous conditions 
must be maintained. It minimizes problems in recovery 
of reaction product because the solvent is removed 
simply by reducing pressure to subcritical, thereby 
avoiding the need for repeated washings to remove 
spent reagents, side products and other reaction sol-
vents.

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protected amino acid dissolved in dichloromethane in
the presence of a carboxyl activating agent such as
dicyclohexyl carbodiimide (DCC), washing with di-
chloromethane to remove soluble reagents and by-pro-
ducts, washing with methanol to remove dicyclohexyl
urea by-product which is insoluble in dichloromethane,
and washing with dichloromethane to remove metha-
nol. The sequence may be represented as follows:

1) RESIN-OC-CH-NH-BOC \[ \rightarrow \] RESIN-OC-CH-NH$_2$$^\oplus$
2) Wash.
3) RESIN-OC-CH-NH$_2$$^\oplus$ \[ \rightarrow \] RESIN-OC-CH-NH$_2$
4) Wash.
5) RESIN-OC-CH-NH$_2$ + HOC-CH-NH-BOC \[ \rightarrow \] RESIN-OC-CH-NH$_2$
6) Dichloromethane wash.
7) Methanol wash.
8) Dichloromethane wash.

Since most wash steps are performed two or three times
each, the procedure is very time consuming, with a 45
complete cycle requiring up to two hours for each
amino acid incorporated into the peptide chain. This
procedure has several drawbacks, including the exten-
sive use of chlorinated solvents, frequent incomplete
couplings, reduced yields due to trifluoroacetylation of
the amino group during repetitive deprotection steps,
reactions with the side chain of the amino acid, and
premature cleavage of the peptide chain from the sup-
port. As a consequence, the final product is inhomoge-
neous, and may even contain peptides which have activ-
ity antagonistic to the activity of the desired peptide
hormone.

An alternate solid phase synthesis employs the base
liable fluorenlymethoxycarbonyl (FMOC) protecting
group on the $\alpha$-amino group. This group is removed by
piperidine (Pip) or other organic base dissolved in dime-
ethylformamide (DMF), the resin is washed with DMF to
remove excess and spent FMOC reagents, piperidine
and its salts, the deprotected resin is reacted with an
activated FMOC-protected amino acid, such as penta-
fluorophenyl ester of the acid, and the resin is washed
with DMF to remove by-products and excess ester.
This sequence may be illustrated as follows:

7) Methanol wash.
8) Dichloromethane wash.
40 While this scheme has fewer steps than the BOC
scheme, it still has numerous washings and suffers from
some of the same problems with respect to yield and
formation of an inhomogeneous product.

Prior to the development of solid phase techniques,
peptides had been synthesized in solution by reacting an
amino acid with a reactive amino acid derivative such
as the N-carboxyloxalidhydride (NCA) followed by re-
moval of carbon dioxide, as follows:

4) Wash.

While this procedure is quite simple because the amino
acid NCA reacts directly with the free $\alpha$-amino group
without activation, and the product is a peptide carba-
mate which acts as a protecting group, it has not been
widely used because of low yields and it usually gives
an impure product. The carbamate group is unstable in
solutions below pH of 11, and decarboxylation occurs leaving free amino groups which cause polymerization or over-reaction. If the pH is increased above 11, other competing reactions occur as a result of hydrolyses or the formation of hydantoic acid. As a consequence, coupling efficiencies normally do not exceed 90-95%, and the practical value of this technique is limited to large-scale production of small peptides.

Supercritical carbon dioxide can be used as a reaction solvent for processes for polymerizing N-carboxyanhydride or its derivatives to form peptide homopolymer and copolymers having repeating α-amino acid units.

In the second reaction, the "R" side-chain will vary depending upon R1 or R2 and the relative proportions of co-monomers. Terpolyamides and higher polymers can be obtained through use of three or more starting anhydride monomers.

In the above-mentioned reaction schemes, R and R1 represent hydrogen or α-amino acid side chains, such as hydrocarbon side chains of α-amino acids such as alanine, valine, leucine, isoleucine, and phenylalanine, and substituted hydrocarbon side chains of amino acids such as tyrosine, threonine, serine, tryptophane, methionine, cysteine, lysine, arginine, histidine, aspartic acid, and glutamic acid. In addition, α-amino acids in which the side chain combines with the α-amino group to form a heterocyclic ring, such as proline and hydroxyproline, may be employed. This invention is not, of course, limited to the naturally-occurring amino acids, or to the specific syntheses specified herein. On the contrary, it can be employed advantageously in any procedure for producing a peptide oligomer or polymer.

Use of supercritical carbon dioxide as a solvent is of immense value in the preparation of peptides. It may be used in solid phase syntheses, and is of particular value where the support is a controlled pore glass (CPG) bed. The high diffusibility of supercritical carbon dioxide assures penetration of the pores of the substrate. Further, volatile reagents may be removed simply by venting the compressed fluid, yielding a dry powder, thereby avoiding the need to dispose of or recycle organic solvents. The time and expense saved can be enormous when synthesizing polypeptides with, e.g., 20 to 40 amino acids, such as calcitonin. Anyhydroxyl groups are required for some reactions to give a 100% coupling yield, and such reactions are readily carried out with supercritical carbon dioxide. Use of carbon dioxide also reduces the cost of the procedure because it is considerably cheaper than the chlorinated hydrocarbons normally used as solvents, and because it is nontoxic, it can be vented to the atmosphere. Moreover, by avoiding the need for large volumes of liquid solvents, use of supercritical carbon dioxide can permit the construction of low cost commercial scale production equipment for the manufacture of polypeptides that is only slightly larger than laboratory-size equipment.

The use of supercritical carbon dioxide as a reaction solvent is also of benefit for the liquid phase synthesis using amino carboxy anhydrides (NCA) as reagents, in that it may stabilize the carbamate protecting group and facilitate the separation of the reaction products from solvents before the next chemical reaction.

Supercritical carbon dioxide may also be useful as a reaction solvent in the analysis of amino acid from proteins. In conventional amino acid analyses, a protein is hydrolyzed to its individual amino acid components, and the amino acids are separated, identified and quantitated. Detection of the amino acids depends on producing a derivative that can be measured by ultra-violet absorption, color or fluorescence. The first automated method for amino acid analysis, which was introduced by Moore and Stein in 1951, uses ion exchange columns to separate the amino acids. A colored derivative is made of each amino acid by the reaction of ninhydrin with the alpha-amino group of the amino acid. These colored compounds are then detected using a colorimeter and, when compared with standard concentrations and retention times, give both a qualitative and quantitative analysis. The Moore and Stein technique is the primary example of a post-column derivatization method for amino acid analysis.

Recently, several pre-column methods have been developed, in which derivatives of the amino acids are prepared in the crude hydrolyzed mixture. The resulting mixture of amino acids is then separated by reverse phase HPLC chromatography and the amino acids are identified qualitatively by retention time and quantitatively by comparison of the color adsorption with a standard. There are several reagents that have been used for preparing derivatives in the pre-column methods, including phenylisothiocyanate, orthophthalaldehyde and dansyl chloride.

Use of supercritical carbon dioxide as the solvent for these reactions is of great importance because many proteins of biological interest are normally found in very minute quantities. It is important, therefore, to have very sensitive quantitative analytical procedures.

Supercritical carbon dioxide is also of value in protein sequencing techniques for determining the primary structure of proteins. In the basic sequencing technique, known as the Edman Degradation [Cf. Edman, P., Acta. Chem. Scand. Iv. 283 (1950)], phenylisothiocyanate is used to make a derivative of the N-terminal alpha-amino acid. This derivative, when treated with an anhydrous organic acid such as trifluoroacetic acid, cyclizes to remove the N-terminal alpha acid and produce an anilinothiazolone amino acid derivative. This somewhat unstable derivative is then converted on treatment with strong acid to phenylthiohydantoin. The reaction sequence may be illustrated as follows:
The phenylthiohydantoin amino acid (PTC amino acid) is identified by chromatography and quantified using a comparison of its fluorescence adsorption with a standard. The Edman technique can be done manually, however, it is a tedious job requiring highly skilled technicians.

A number of groups have developed automated Edman techniques including the liquid phase spinning cup sequencer covered by U.S. Pat. No. 3,725,010, the solid phase/liquid sequencer developed by Laursen, R. A., Eur. J. Biochem. 20 (1971), and the gas phase sequencer covered by U.S. Pat. No. 4,065,412. The gas phase sequencer has been a significant commercial success because it allows the sequencing of very small amounts of protein.

Use of supercritical carbon dioxide as a substitute for the organic solvents involved in any of the above modifications of the Edman procedure not only improves the chemical yield, but also significantly reduces the mechanical losses due to wash-out by the liquid solvent. In addition to the improved yield, the analysis of the resulting derivative is more effective because the carbon dioxide solvent can be removed by routing the supercritical carbon dioxide containing the amino acid derivative through an HPLC injection valve and the carbon dioxide is removed simply by reducing the pressure to sub-critical. The analysis of the derivative is then performed with a standard HPLC reverse phase system. A much more sensitive analysis is obtained by depositing the derivative in the inlet port of a mass spectrometer by reducing the pressure to sub-critical. The combination of improved chemistry, reduced mechanical yields and improved delivery to the analytical system very significantly increases the sensitivity and allows the determination of the secondary structure of proteins on sub-picomole quantities.

The phenylisothiocyanate reaction described above takes place with the α-amino group of the protein at moderate pH (e.g., 7-9); however, at higher pH the reaction also takes place on the basic side chains of amino acids like lysine, ornithine and arginine.

The use of supercritical carbon dioxide as a reaction solvent is also beneficial in the analysis of individual α-amino acids, such as a mixture of, α-amino acids obtained by hydrolysis of a protein or a polypeptide. In such analyses, a derivatizing agent is added to the amino acid or mixture of amino acids, and allowed to react to form a derivative. Suitable derivatizing agents include phenylisothiocyanate, dansyl chloride, orthophthalaldehyde, ninhydrin, dinitrobenzene and fluorescamine. These protein derivatives may be made in supercritical carbon dioxide to increase the sensitivity of the analysis by improving the yield of the chemical reaction, decreasing the amount of side reaction(s) and improving the delivery to the analytical system.

Amino acids must be derivatized to make them chromogenic or fluorogenic before they can be detected on minute quantities by standard analytical spectrometers. The same derivatives described for protein analysis above are useful for amino acid analysis and may be made in supercritical carbon dioxide. This approach may give much improved sensitivity over current solution methods.

The following examples are illustrative. All reactions were carried out in a Super Critical Fluid System made by Milton Roy, of Ivyland, Pa., employing as a reaction vessel a 55 ml. pressure vessel rated to 5000 psi, provided with a bottom inlet for compressed CO₂ and a top outlet for venting the vessel. As a general rule, liquid reactants were adsorbed on a filter medium while solid reactants were placed in an envelope of folded filter paper. The reactant which was more soluble in supercritical CO₂ was placed in the chamber nearer the CO₂ inlet, so that it had to be extracted by the CO₂ stream before it contacted the less soluble reactant.

**EXAMPLE I**

A filter medium containing 1 ml. of liquid n-butanol was placed in the vessel adjacent the CO₂ inlet, and 550 mg. solid acetylmidazole was placed above the filter medium. The system was then charged with carbon dioxide at 96°C and 3000 psi and allowed to react for one hour. The system was reduced to subcritical conditions and vented to the atmosphere. The liquid reaction product was drained out of the reaction vessel and was found by gas chromatography and mass spectroscopy to be butyl acetate in 57% yield.

**EXAMPLE II**

Using similar procedures, 660 mg. of solid acetylmidazole was reacted with 516 mg. of liquid 5-amino-1-pentanol. The system was charged with carbon dioxide at 3000 psi and 154°F and allowed to react for 4 hours. The system was vented and disassembled, and the filter media were soaked in 5 ml. chloroform for about 5 minutes to extract reaction products. The chloroform solution was analyzed by gas chromatography and mass spectroscopy and found to contain, in addition to the two starting materials, N,N-diacyl-5-amino-pentanol, N-acetyl-5-amino-1-pentanol and O-acetyl-5-amino-1-pentanol.

**EXAMPLE III**

Using procedures similar to those described in Example I, 1 ml. of liquid acetic acid anhydride was reacted
with 150 mg. of BOC-Valine Merrifield resin which had been deprotected by removal of the t-butoxycarbonyl protecting group by the method of Coenen, et al., *German Chemical Engineering*, 7,335 (1984). The vessel was charged with carbon dioxide at 3000 psi and 94° F. and allowed to react for 20 minutes. The system was reduced to subcritical conditions and vented to the atmosphere. Acetylation of the deprotected valine residue was greater than 99.4% complete by ninhydrin analysis using the Kaiser procedure described in McHugh, et al., *Supercritical Fluid Extraction*, Butterworth & Co., Ltd. (1986).

**EXAMPLE IV**

Using procedures similar to those described in Example I, 100 mg. of commercially-available long chain alkyl amine controlled pore glass (LCAA-CPG) resin was reacted with 200 mg. of solid acetylimidazole. Both reagents were placed in filter paper envelopes, with the envelope containing acetylimidazole being placed in the vessel below the envelope containing the LCAA-CPG. The system was charged with carbon dioxide at 3000 psi and 95° F., and the reaction was allowed to proceed for one hour. The vessel was then reduced to subcritical conditions and vented to the atmosphere. Analysis of a small sample of the resin showed that coupling of the LCAA-CPG resin was complete. Employing procedures similar to those described in Examples III-V, it appears that LCAA-CPG resin is a better support when a solid acylating agent is employed, while both the LCAA-CPG resin and the polystyrene-based Merrifield resin can be employed when a liquid acylating agent is employed.

**EXAMPLE V**

Employing procedures similar to those described in Examples I, III and IV, 200 mg. of BOC-Valine Merrifield resin was deprotected and the deprotected resin was reacted with 220 mg. of solid acetylimidazole for 2 hours in the presence of carbon dioxide at 100° F. and 3000 psi. Analysis of small samples of the resin with the Kaiser ninhydrin test showed acetylation was less than 74% complete. Based upon the results of Examples III-V, it appears that LCAA-CPG resin is a better support when a solid acylating agent is employed, while both the LCAA-CPG resin and the polystyrene-based Merrifield resin can be employed when a liquid acylating agent is employed.

**EXAMPLE VI**

Using procedures similar to those described in Example I, 100 mg. of LCAA-CPG resin was reacted with 272 mg. of solid N-hydroxysuccinimide ester of BOC-glycine for 5 hours in carbon dioxide at 3000 psi and 96° F. It was found that 240 mg. of the BOC-glycine ester remained in the envelope, indicating that only 32 mg. had dissolved in the carbon dioxide. Analysis of a small sample of the resin with Kaiser ninhydrin indicated that reaction of the protected glycine with the LCAA-CPG resin was about 94% complete.

**EXAMPLE VII**

Employing procedures similar to those described in Example IV, a solution containing 1.0 mM of the symmetric anhydride of BOC-glycine was reacted with 100 mg. of LCAA-CPG for 105 minutes in carbon dioxide at 3500 psi and 100° F. Analysis of a small sample of the resin with Kaiser ninhydrin reagent demonstrated that coupling of BOC-glycine with the LCAA-CPG was complete.

**EXAMPLE VIII**

Employing procedures similar to those described in Example IV, 87 mg. of a Gly-LCAA-CPG resin made by deprotecting the product of Example VII by standard techniques, was reacted with 552 mg. of the BOC-leucine p-nitrophenyl ester for about 16 hours (overnight) in carbon dioxide at 3100 psi and 101° F. Analysis of a small sample of the resin product with Kaiser ninhydrin reagent demonstrated that the coupling with BOC-leucine was complete to form a BOC-Leu-Gly-LCAA-CPG resin.

**EXAMPLE IX**

Employing procedures similar to those described in Example VIII, the product of Example VIII was deprotected and reacted with 0.5 mM of the symmetric anhydride of BOC-glycine for 90 minutes in carbon dioxide at 3000 psi and 105° F. Analysis of a small sample of the resin showed that coupling of the BOC-glycine to produce a BOC-Gly-Leu-Gly-LCAA-CPG resin was complete.

**EXAMPLE X**

Using procedures similar to those described in Example VIII, the product of Example IX was deprotected and the deprotected product was reacted with 0.5 mM of the symmetric anhydride of BOC-alanine for 90 minutes in carbon dioxide at 3000 psi and 105° F. Analysis of a small sample of the resin showed that coupling of the BOC-alanine to produce BOC-Ala-Gly-Leu-LCAA-CPG resin was complete. The thus-produced resin was lyophilized with 0.5 ml. concentrated hydrochloric acid/propanionic acid (1:1, V/V) overnight at 110° C. The resulting mixture was evaporated to dryness and the solid resin was mixed with 1 ml. water. The resulting aqueous solution was drawn into a syringe fitted with a 0.22 um filter, the water was evaporated and the tube was sealed for amino acid analysis by standard procedures on a Beckman 890 amino acid analyzer. Only glycine, alanine and leucine were found present in a molar ratio of 2:1:1.

**EXAMPLE XI**

Using procedures similar to those described in Example IV, 250 mg. of an LCAA-CPG resin to which 4-hydroxybenzylbenzoic acid had been attached as a base-labile link, using standard symmetric coupling techniques, was reacted with 0.25 mM of the symmetric anhydride of BOC-valine for 1 hour in carbon dioxide at 2800 psi and 103° F. Analysis of a small portion of the resin by the Kaiser ninhydrin reagent demonstrated the coupling reaction to form BOC-Val-HMBA-LCAA-CPG resin was complete.

**EXAMPLE XII**

Using procedures similar to those described in Example IV, all of the product of Example XI was deprotected and reacted with 0.25 mM of the symmetric anhydride of BOC-glycine for 1 hour in carbon dioxide at 2800 psi and 103° F. Analysis of a small portion of the resin by the Kaiser ninhydrin reagent demonstrated the coupling reaction to form BOC-Gly-Val-HMBA-LCAA-CPG resin was complete.

**EXAMPLE XIII**

Using procedures similar to those described in Example IV, all of the product of Example XII was de-
protected and reacted with 0.25 mM of the symmetric anhydride of BOC-alanine for 1 hour in carbon dioxide at 2800 psi and 103°F. Analysis of a small portion of the resin by the Kaiser ninhydrin reagent demonstrated the coupling reaction to form BOC-Ala-Gly-Val-HMBA-LCAA-CPG resin was complete.

EXAMPLE XIV

Using procedures similar to those described in Example IV, all of the product of Example XII was deprotected and reacted with 0.25 mM of the symmetric anhydride of BOC-leucine for 1 hour in carbon dioxide at 2800 psi and 103°F. Analysis of a small portion of the resin by the Kaiser ninhydrin reagent demonstrated the coupling reaction to form BOC-Leu-Ala-Gly-Val-HMBA-LCAA-CPG resin was complete.

EXAMPLE XV

A 2-3 ml. portion of 40% trifluoroacetic acid in dichloromethane was placed in the vessel and 50 mg. of the product of Example IV, contained in a filter paper envelope was placed in the vessel. The vessel was charged with carbon dioxide at 2800 psi and 120°F., and the reaction was allowed to proceed for 1 hour. After reducing the system to subcritical conditions, venting to the atmosphere, removal of the resin and neutralization, analysis of a small sample of the product with Kaiser ninhydrin reagent showed greater than 95% removal of the BOC protecting group to leave a free amino group.

EXAMPLE XVI

Employing procedures similar to those described in Examples IV, Merrifield's peptide was prepared by reacting, in turn, protected valine, protected glycine, protected alanine and protected leucine with long chain aliphatic amine controlled pore glass beads, using supercritical carbon dioxide at 95°F. and 3000 psi as the reaction solvent.

A 100-mg. sample of this was reacted with 1.0 ml. of a 5% solution of phenylisothiocyanate in hexane for 1 hour in carbon dioxide at 3300 psi and 100°F., to form the phenyl thiocarbamyl. This product was treated with 2.0 ml. anhydrous trifluoroacetic acid, and the resulting trifluoroacetic acid solution was analyzed in a high pressure liquid chromatography system on a standard Beckman 990 Spinning Cup Sequencer. The predominant peak was phenylisothiocyanate leucine hydantoin.

What is claimed is:

1. In a process for producing a chemical reaction product by contacting at least two reacting moieties in the presence of at least one inert reaction solvent, one of the reacting moieties being an amino acid or polypeptide, and another of the reacting moieties being an N-carboxyl anhydride, an improvement which comprises using supercritical carbon dioxide as the principal inert reaction solvent.

2. In a process for producing a chemical reaction product, wherein monomeric units of at least one N-carboxyl anhydride are polymerized to form a polymer of amino acid units, an improvement which comprises using supercritical carbon dioxide as the principal reaction solvent.

3. A process according to claim 2 wherein monomeric units of an N-carboxyl anhydride are polymerized to form a homopolymer of repeating amino acid units.

4. A process according to claim 2 wherein monomeric units of at least two different N-carboxyl anhydrides are polymerized to form a copolymer of at least two different amino acid units.