A One-Pot Peptide Synthesis via Se-phenyl Carboselenoate in Mixed Aqueous/Organic Solvent System

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(Received October 23, 1992)

Synopsis. A one pot synthesis of peptides with free C-terminal residues has been accomplished via the active Se-phenyl carboselenoate using diphenyl diselenide, tributylphosphine, and *N*-methylmorpholine *N*-oxide in an acetonitrile– water mixed solvent system. Free amino acids and peptides have been used as the amine component without pH adjustment.

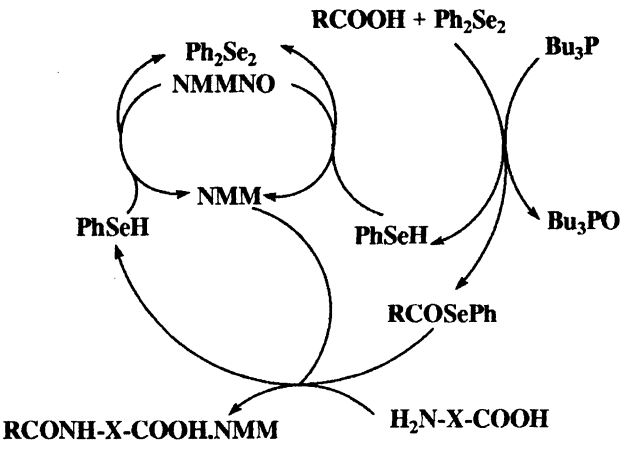
Although a large number of methods[1] have been developed for the synthesis of peptides, relatively little attention has been given to the synthesis of peptides with a free C-terminal residue using free amino acids and peptides as the amine component. This is because, in addition to the usual problems[2] of peptide synthesis, there are other complications such as i) the need for two steps: preparation of different active esters followed by condensation, ii) poor solubility of the amino acids and peptides in common organic solvents which necessitates polar co-solvents like water, iii) adjustment of pH, and iv) sensitivity of the active carboxyl component towards hydrolysis.

Recently, we have reported[3] a new approach to peptide synthesis based on redox reactions using tributylphosphine, diphenyl diselenide, and *N*-methylmorpholine *N*-oxide. We have shown that the active Se-phenyl carboselenoate is the sole acylating agent and have synthesized linear peptides from N-protected amino acids/peptides and amino acid/peptide ester hydrochlorides by stepwise as well as segment condensations. In a subsequent report,[4] we have further shown the power of this approach by synthesizing peptides using azido esters as a direct source of the amine component. These results together with the properties of Se-phenyl carboselenoates, such as a high rate of aminolysis relative to its hydrolysis,[5] stimulated us to expand our new approach to the synthesis of peptides with a free C-terminal residue using amino acids and peptides as the amine component in one pot. This report describes our results for this newly developed protocol.

Addition of tributylphosphine to a solution of an N-protected amino acid (RCOOH) and diphenyl diselenide in acetonitrile generates the Se-phenyl carboselenoate (RCOSePh) and benzeneselenol. Treatment with *N*-methylmorpholine *N*-oxide (NMMNO) oxidizes the benzeneselenol to diselenide. The reduced compound, *N*-methylmorpholine (NMM), forms the carboxylate salt of the amino acid/peptide (H_2N–X–COOH) in the acetonitrile–water mixed solvent system, which on subsequent condensation with the active Se-phenyl carboselenoate gives the desired peptide (RCONH–X–COO^- $NMMH^+$). The benzeneselenol, that is generated, again reacts with NMMNO and the above sequence of reactions is repeated, (Scheme 1) thus constituting a self regulated one-pot peptide synthesis in a mixed aqueous organic solvent system.

To measure the capability of this protocol in preserving chiral integrity, we have synthesized the model peptide methyl benzyloxycarbonylglycylphenylalanylalaninate (Z–Gly–Phe–Ala–OMe) by condensing benzyloxycarbonylglycylphenylalanine (Table 1, Entry 3) with alanine followed by esterification with diazomethane. For direct comparison purposes, we have also prepared this model peptide by condensation of benzyloxycarbonylglycylphenylalanine *N*-hydroxysuccinimide (Z–Gly–Phe–OSu) and pentachlorophenyl (Z–Gly–Phe–OPcp) esters with the triethylammonium salt of alanine (Ala·Et_3N) under our reaction conditions followed by esterification (Table 1, Entries 1 and 2). The extent of racemization was measured by ^1H NMR as reported by Weinstein and co-workers.[6] The percentage of racemized product (D–L) in our method is 10% while the above active esters gave 15 and 40% respectively. This suggests that our new protocol is better in terms of preservation of optical purity of the products compared to the usual active ester methods. This is probably due to the low concentration of the tertiary base (NMM), whose generation is controlled by the self regulated reaction.

This methodology has also been successfully applied with different N-protected amino acids and amino acid or peptide segments listed in the Table 1. The acylation of amino acids and peptides, which have a func-



Scheme 1.

Table 1. List of Peptides[a)]

Entry	Carboxyl component	Amine component	Product	Yield %	Mp/°C	$[\alpha]_D^{27}$	(c, solv.)	Ref.
1)	Z–Gly–Phe–OSu	Ala. Et_3N	Z–Gly–Phe–Ala [b)]	46				
2)	Z–Gly–Phe–OPcp	Ala. Et_3N	Z–Gly–Phe–Ala [b)]	34				
3)	Z–Gly–Phe	Ala	Z–Gly–Phe–Ala [b)]	61				
4)	Z–Gly	Phe	Z–Gly–Phe	87 [c)]	126—127	+38	(2, EtOH)	7
5)	Z–Phe	Pro	Z–Phe–Pro	60	106—108	−65	(2, Py)	8
6)	Z–Met	Gly	Z–Met–Gly	68	132—134	−19.4	(1.2, MeOH)	9
7)	Z–Gly	Trp	Z–Gly–Trp	93	141—142	+32.2	(2.3, EtOH)	10
8)	Z–Phe	Leu	Z–Phe–Leu	94 [c)]	141—142	−20	(1.5, MeOH)	11
9)	Z_2–Orn	β-Ala	Z_2–Orn–β-Ala	64 93 [c)]	163—164	−6.1	(1, MeOH)	12
10)	Z–Pro	Gly–Phe–Gly	Z–Pro–Gly–Phe–Gly	65	176—178	−27.4	(2, dioxan)	10
11)	Z–Pro	Gly–Tyr	Z–Pro–Gly–Tyr	46	100—102	−11.8	(1, EtOH)	13
12)	Z–Asn	Gly	Z–Asn–Gly	79	170—172	−4	(1, DMF)	14
13)	Z–Ala	Ser	Z–Ala–Ser	67	203—205	+27	(1, DMF)	15
14)	Z–Tyr	Arg	Z–Tyr–Arg	85	161—164	−151	(1, DMF)	16
15)	Z–Phe	His	Z–Phe–His	60	204—206	−206	(1, DMF)	17

a) All amino acids used are of L configuration; b) Characterized by ^{1}H NMR as methyl esters, see Ref. 6; c) Yield refers to the experiment where triethylamine was used as an additive.

tional group in their side chain such as tyrosine, serine, arginine, histidine, and asparagine shows only α-acylation product without any side reactions and no pH adjustment is required. Synthesis of this type of peptide in aqueous phase is accomplished with rigorous pH control.[18)] Tryptophan and methionine, which are sensitive to oxidation conditions also give the expected products without any side reactions. In our reaction conditions (combination of solvents, temperature, and time) the reaction between Z–Gly–Phe–OSu or Z–Gly–Phe–OPcp, and Ala·Et_3N does not get completed resulting in poor yield of the product peptide (Table 1, Entries 1 and 2). The low yield of Z–Pro–Gly–Tyr (Table 1, Entry 11) is probably due to poor solubility of the amine component i.e. Gly–Tyr in the reaction medium. The yield of the other peptides as shown in the Table 1 is reasonably good. In many cases small amount of the unreacted carboxyl component is isolated as its Se-phenyl carboselenoate derivative along with other neutral by-products during workup (vide Experimental) and the unreacted amine component is lost. However, the yield of peptides increased dramatically (Table 1, Entries 4, 8, and 9) when one equivalent of triethylamine is used as an additive without detectable loss of optical purity. This is likely due to the speed up of acylation at higher pH and also the presence of base perhaps increases the solubility of the amino acids/peptides in the reaction medium. The isolated peptides are pure and no products are observed (TLC) due to multiple incorporation of amine components which are expected to have relatively lower R_f compared to product peptides. This is because, during condensation process the reaction medium is free from tributylphosphine and the intermediate active carboselenoate derivative is rather unlikely to exchange carboxyl activation to the product peptide or the amine component. The result of the racemization test also suggests that this one-pot self-regulated method, which goes via an active carboselenoate intermediate is as useful as the existing two-pot active ester methodologies wherein stepwise condensations using N-protected amino acids with urethane type protecting groups are involved. During workup, the removal of unreacted carboselenoate derivative, tributylphosphine oxide and the regenerated diphenyl diselenide is easily achieved by solvent extraction. Furthermore, the diselenide thus recovered may be recycled.

Experimental

Amino acids and peptides were purchased from Aldrich/Sigma Chemical Co. Benzyloxycarbonyl protected amino acids were prepared following the literature[19)] procedure. The active esters, Z–Gly–Phe–OSu[20)] and Z–Gly–Phe–OPcp[21)] were prepared from Z–Gly–Phe as described in the literature. Tributylphosphine was distilled before use and the tributylphosphine content was estimated from ^{31}P NMR. *N*-Methylmorpholine *N*-oxide monohydrate was purchased from Merck and used as such. Melting points were checked using a Fischer–John apparatus and are uncorrected. Optical rotations were measured in a Perkin–Elmer (Model 241) spectrometer using the sodium-D line. IR spectra were obtained using a Perkin–Elmer (Model 283) grating spectrometer. ^{1}H NMR spectra were obtained using a Bruker 500 MHz or 200 MHz spectrometers.

Typical Procedure for the Synthesis of Peptides. Tributylphosphine (95%) (0.55 ml, 2.1 mmol) was added dropwise to a stirred solution of benzyloxycarbonylphenylalanine (598 mg, 2 mmol) and diphenyl diselenide (655 mg, 2.1 mmol) in acetonitrile (6 ml) under nitrogen at room temperature (27 °C). After 1 h, proline (240 mg, 2.08 mmol) was added, followed by a solution of *N*-methylmorpholine *N*-oxide monohydrate (285 mg, 2.1 mmol) in water (6 ml) and the mixture was stirred overnight (15 h). The acetonitrile

was evaporated under reduced pressure, diluted with water (10 ml) and extracted with ether (2×10 ml) to remove the by-products such as diphenyl diselenide, tributylphosphine oxide and the unreacted carboselenoate derivative of Z–Phe. The ether layer was extracted once with saturated sodium hydrogencarbonate solution (10 ml). The combined aqueous phase was cooled (ice-bath), acidified with HCl (6 M, 1 M=1 mol dm^{-3}) and extracted with ethyl acetate (3×15 ml). The organic phase was washed with water and with brine, dried (anhydrous Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, $CHCl_3$–MeOH–AcOH; 97 : 2 : 1) to give benzyloxycarbonylphenylalanylproline (475 mg, 60%); mp 106—108 °C (ethyl acetate–hexane) (lit,[8] 105—106 °C); $[\alpha]_{27}^{D}$ −65.0 (*c* 2, pyridine) (lit,[8] $[\alpha]_{D}^{21.5}$ −64.3 (*c* 2.6, pyridine).

The peptides in Entries 3—11 (Table 1) are prepared following above procedure. For Z–Asn–Gly (Table 1, Entry 12) the solvent (acetonitrile–water) was evaporated under vacuum and the residue was triturated with ether. The ether solution was decanted and the residue was purified by chromatography (SiO_2, $CHCl_3$: MeOH : AcOH 70 : 28 : 2). For Z–Ala–Ser (Table 1, Entry 13), acidification of the aqueous phase gave the product as a precipitate which was collected by filtration and crystallized from ethanol. For the peptides in Entries 14 and 15 (Table 1), following the removal of the acetonitrile, the product was collected by filtration. The residue was washed with several portions of ether to remove the by-products (Ph_2Se_2 and Bu_3PO) and then crystallized. In experiments where triethylamine (1 equiv) was used as an additive, it was added together with the amine component and NMMNO. For the preparation of Z–Gly–Phe–Ala–OMe, the crude product was esterified with ethereal diazomethane and purified by column chromatography (SiO_2, $CHCl_3$–MeOH; 97 : 3).

Typical Procedure for the Preparation of Z–Gly–Phe–Ala–OMe Using Z–Gly–Phe–OSu or Z–Gly–Phe–OPcp Active Esters. The active ester (2 mmol) was added to a stirred solution of alanine (187 mg, 2.1 mmol) and triethylamine (0.295 ml, 2.1 mmol) in acetonitrile–water (1 : 1) (12 ml) at room temperature and the mixture stirred overnight. The acetonitrile was evaporated, water (10 ml) was added and extracted with ethyl acetate (2×10 ml). The ethyl acetate layers were extracted with saturated sodium hydrogencarbonate solution (10 ml). The combined aqueous phase was cooled (ice-bath), acidified with HCl (6 M) and extracted with ethyl acetate (3×15 ml). The extract was washed with water and with brine, dried (anhydrous Na_2SO_4) and evaporated under reduced pressure. The residue was esterified with ethereal diazomethane and purified by column chromatography (SiO_2, $CHCl_3$–MeOH; 97 : 3) which gave the peptide Z–Gly–Phe–Ala–OMe.

We are thankful to Dr. K. Nagarajan, Searle R&D Centre, Bombay for optical rotations. The authors (U.S. and M.S.C) gratefully acknowledge the support of DST, New Delhi.

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