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Solid-phase synthesis of C-terminal peptide amides using a photoremovable α-methylphenacylamido anchoring linkage

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Abstract. Polymer-supported solid-phase synthetic procedures have been developed for the synthesis of C-terminal peptide amides using a new photolytically removable α-methylphenacylamido anchoring linkage between the polymeric support and the growing peptide. The preparation of this new polymeric support involves a four-step polymer-analogous reaction starting from 2%-divinylbenzene-crosslinked polystyrene resin. The steps involved are (i) Friedel–Crafts reaction with 2-bromopropionyl chloride to give the 2-bromopropionyl resin, (ii) reaction of the 2-bromopropionyl resin with potassium phthalimide to give the phthalimidomethyl resin, (iii) hydrolysis with alcoholic potash to give the partially hydrolysed phthalamido resin and (iv) treatment with alcoholic HCl to give the 2-aminopropionyl resin. N-protected amino acids undergo coupling with this amino resin by the dicyclohexylcarbodiimide-mediated coupling. The acylated resins on irradiation at 350 nm in DMF released the attached carboxyl function in the C-terminal amide form. The mechanism of the photolytic deprotection involves a radical scission of the amide linkage adjacent to the phenacyl group. The synthetic utility of the new resin has been illustrated by the preparation of several N-protected amino acid amides and the C-terminal peptide amides, Boc-Pro-Val-NH_2, Boc-Gly-Phe-Pro-NH_2 and Boc-Leu-Ala-Gly-Val-NH_2 in 70–74% yield.

Keywords. Peptide synthesis; solid-phase organic synthesis; photosensitive polymeric supports; phenacyl anchoring group.

1. Introduction

Chemical synthesis of C-terminal peptide amides is important for the structure–activity studies of biologically active peptide amides (Bodansky and Sheehan 1966; Takashima *et al* 1968; Schally *et al* 1971; Rivier *et al* 1972) and for the conformational studies of model peptides (Pillai and Mutter 1981; Maser *et al* 1984). In Merrifield's method of polymer-supported solid-phase peptide synthesis (Merrifield 1963), these C-terminal peptide amides are usually prepared by the ammonolysis of the polymer–peptide ester linkage (Manning 1968). Another variation of the synthetic approach to peptide amides is the use of resins, containing amino groups, which facilitate the coupling of the C-terminal amino acid of the desired peptide through an amide linkage and the final release of the synthesised

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peptide in the C-terminal amide form by acid-cleavage (Matsueda and Stewart 1970; Pietta *et al* 1974; Orlowski *et al* 1976; Penke and Rivier 1987). For the selective ammonolysis of the peptide–resin ester linkage or for the acid cleavage, it is necessary that the side-chain ester protecting groups are sufficiently stable so that they are not affected in the ammonolysis procedure or in the acid cleavage. Introduction of a photosensitive 2-nitrobenzamido anchoring linkage between the polymer support and the peptide has been shown to permit the release of the peptide amides under neutral conditions at room temperature, without affecting the side-chain protecting groups or the N-protecting groups (Rich and Gurwara 1973, 1975). This approach has been used in the synthesis of a number of model peptide amides using different polymer-supported approaches (Pillai *et al* 1979, 1980; Pillai and Mutter 1981; Pillai 1987).

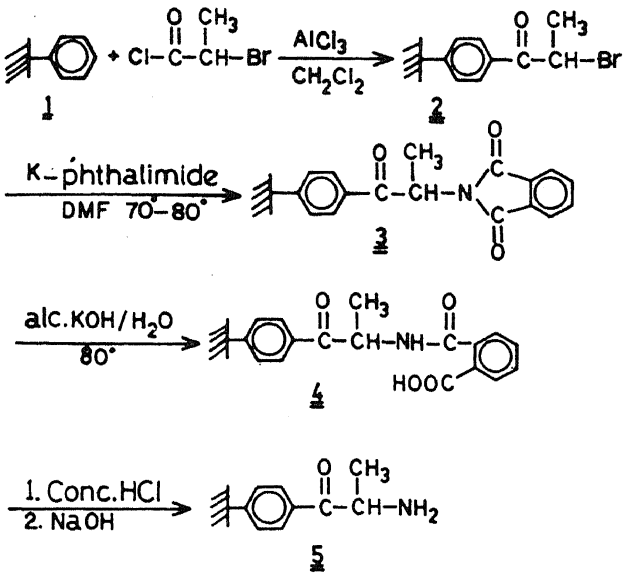
In this paper, we report on the design and use of a new photolytically cleavable polystyrene resin with alpha-methylphenacylamino function for the solid-phase synthesis of fully protected C-terminal peptide amides. Our starting point is the reported use of polymers containing α-methyl phenacyl ester anchoring linkage and other phenacyl-type handles for the solid-phase and liquid-phase synthesis of protected C-terminal peptide fragments which can be subsequently used for the segment condensation method (Wang 1976; Tam *et al* 1980; Tjoeng and Heavner 1983; Bellof and Mutter 1985). The present approach combines the advantages of the photochemical deprotection of the α-methylphenacyl carboxyl protecting group, the solid-phase polymer-supported peptide synthesis and the polymer-analogous functionalization procedures to obtain the C-terminal peptide amides under mild conditions.

2. Results and discussion

2.1 *Synthesis of 2-aminopropionyl polystyrene resin*

α-Bromopropionyl polystyrene resin 2 was prepared by the Friedel–Crafts reaction of copolystyrene–2% divinylbenzene beads (200–400 mesh) with 2-bromopropionyl chloride in the presence of anhydrous $AlCl_3$. The product resin contained 2·4 m mol of Br/g. The IR spectrum of resin 2 showed a strong absorption band at 1685 cm^{-1}. The bromopropionyl resin 2 was converted to the α-aminopropionyl polystyrene resin by a three-step polymer-analogous reaction. The resin 2 was first treated with potassium phthalimide to give the phthalimidopropionyl resin 3. This resin was partially hydrolysed to resin 4 by the reaction with alcoholic potash at 80°C. The partially hydrolysed phthalamidopropionyl resin thus obtained was stirred with a mixture of conc. HCl–EtOH (1:1) at 70°C to give the hydrochloride of the aminopropionyl resin 5. The neutralised resin contained 2 m mol NH_2/g as determined by the picric acid titration method (Gisin 1972). The IR spectrum of resin 5 showed absorption bands at 3400–3500 cm^{-1} (NH_2) and at 1685 cm^{-1} (−C=O).

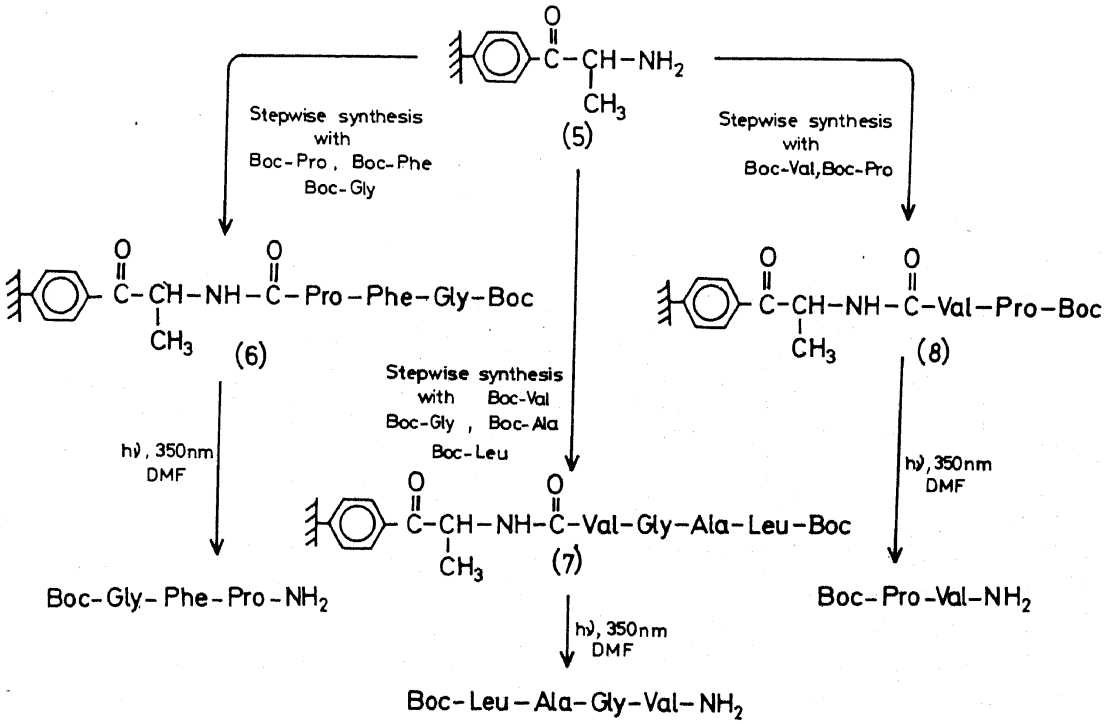
The usual hydrazinolysis in the Gabriel's phthalimide synthesis is not applicable to the phthalimidopropionyl resin 3. During the hydrazinolysis, the carbonyl group of resin 3 reacts with hydrazine hydrate resulting in the formation of the corresponding hydrazone.



Scheme 1. Preparation of α-aminopropionyl polystyrene resin.

The IR spectrum of the hydrazinolysis product of resin 3 is well-indicative of this side reaction. The carbonyl absorption band at 1685 cm^{-1} of resin 3 was totally absent in the product after the hydrazinolysis.

Since the α-aminopropionyl resin 5 contained both a carbonyl and an amino group there is some possibility of intramolecular Schiff's base formation. This is indicated by the slight reduction of amino group capacity on prolonged keeping of resin 5. In order to avoid this side reaction we kept the resin 5 as its hydrochloride and neutralised it just before use by a 10% solution of Et_3N in CH_2Cl_2.



Scheme 2. Solid-phase synthesis of peptide amides via photocleavable-methylphenacylamido anchoring linkage.

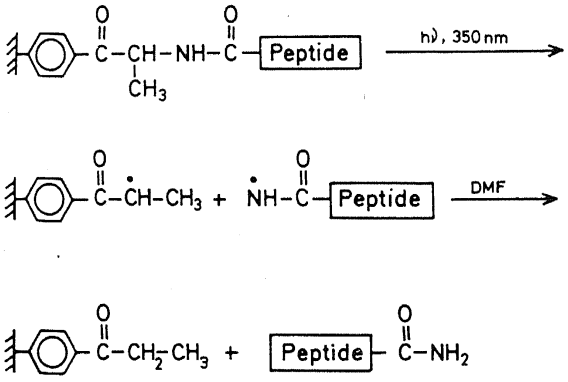
2.2 *Incorporation of amino acid units and solid phase synthesis*

Preliminary investigations on the use of resin 5 as a photo-cleavable support were carried out by incorporating *t*-butyloxycarbonyl (Boc) amino acids and cleaving them in the C-terminal amide forms by the photolytic deprotection method. All the Boc amino acids were coupled to the resin 5 by the symmetric anhydride double coupling procedure. Boc-Val-NH_2, Boc-Gly-NH_2, Boc-Pro-NH_2 and Boc-Leu-NH_2 were prepared in 75–85% yield.

The use of the resin 5 in solid-phase peptide synthesis was illustrated by the synthesis of Boc-Pro-Val-NH_2, Boc-Gly-Phe-Pro-NH_2 and Boc-Leu-Ala-Gly-Val-NH_2 (scheme 2). The peptides were synthesized on the resin 5 by the stepwise incorporation of the Boc amino acids by the symmetric anhydride procedure.

2.3 *Photolytic cleavage of amino acid and peptide amides*

The synthesized peptides were removed in the form of the C-terminal amides by suspending the peptide resin in DMF and irradiating at 350 nm using a Philips HPK mercury lamp. The crude peptides were purified by chromatography and crystallization. The mechanism of the photolytic cleavage of the α-methylphenacyl-amide linkage is analogous to that of the α-methylphenacyl ester bond which involves a radical scission of the carbon–oxygen ester bond adjacent to the phenacyl group (Sheehan and Umezawa 1973). Because of the interaction between the electrons of the carbonyl group and the phenyl ring, the phenacyl group has low-lying excited states. Such interaction makes the phenacylamido linkage photolytically cleavable (scheme 3). The presence of the α-methyl group stabilizes the resulting radical and thereby enhances the possibility of photolytic cleavage.



Scheme 3. Mechanism of the photolytic release of peptide amides from the support.

2.4 *Conclusion and outlook*

The approach combines the advantages of both the solid-phase synthesis and photolytic deprotection methods. The presence of the amino group in the resin 5 facilitates easy incorporation of the first amino acid. The possibility of obtaining the attached peptide in the C-terminal amide form, avoiding rigorous chemical cleavage conditions, is one of the attractive alternatives in solid-phase peptide synthesis. This method has potential applications in the synthesis of biologically active peptide amides particularly with hindered C-terminal amino acids like valine.

3. Experimental section

3.1 *General*

Melting points are uncorrected. The solvents were distilled and purified by standard procedures. IR spectra were recorded on a Pye-Unicam sp-3-300 spectrophotometer using KBr pellets. 2-Bromopropionyl chloride was purchased from Aldrich. Polystyrene beads (2%-divinylbenzene crosslinked, 200–400 mesh) were purchased from Fluka.

3.2 *Friedel–Crafts reaction of crosslinked polystyrene with 2-bromopropionyl chloride. Preparation of α-bromopropionyl polystyrene resin (2)*

Anhydrous $AlCl_3$ (21 g, 157·4 m mol) was placed in CH_2Cl_2 (50 ml) and 2-bromopropionyl chloride (13·5 g, 78·1 m mol) was added slowly with gentle stirring. The light brown liquid thus obtained was added carefully to a suspension of the polystyrene beads (10 g), swelled in CH_2Cl_2 (50 ml). The reaction mixture was stirred for 18 h. The acylated resin thus obtained (13·8 g) was collected by filtration and washed successively with CH_2Cl_2, 4N HCl–dioxane (1:1), dioxane–H_2O (1:1); H_2O, MeOH and dried *in vacuo*. IR (KBr): 1685 cm^{-1} (C=O); Br, 2·4 m mol/g.

3.3 *Phthalimide synthesis with resin 2. Preparation of phthalimidopropionyl resin (3)*

α-Bromopropionyl resin (10 g, 2·4 m mol Br/g.) was swelled in DMF (200 ml) for 30 min. Potassiumphthalimide (8·9 g, 48 m mol) was added and the reaction mixture was stirred magnetically at 110–120°C for 12 h. The resin was collected by filtration and washed thoroughly with DMF, DMF–H_2O (1:1), H_2O, H_2O–dioxane (1:1), dioxane, EtOH, MeOH and dried under vacuum to give the resin 3 (11·4 g); IR (KBr): 1790, 1725 and 1410 cm^{-1} (phthalimide), 1685 cm^{-1} (C=O).

3.4 *Hydrolysis of resin 3. Preparation of partially hydrolysed phthalamidopropionyl resin (4)*

Resin 3 (10 g) was added to a solution of KOH (6·75 g) in EtOH–H_2O (1:1, 90 ml). The reaction mixture was stirred and heated at 80°C for 10 h. The partially hydrolysed phthalamido-propionyl resin thus obtained was filtered and washed several times with H_2O, EtOH and MeOH. The product resin was dried *in vacuo*. IR (KBr): 1700 (phthalamide), 1685 (C=O), 3400 cm^{-1} (NH).

3.5 *Preparation of α-aminopropionyl resin (5)*

Resin 4 (10 g) was added to a mixture of EtOH (80 ml) and conc. HCl (80 ml). The suspension was stirred at 70°C for 12 h. The reaction mixture was then cooled and diluted with H_2O. The resin was collected by filtration and washed successively with H_2O, EtOH and MeOH. This resin was stirred with IN NaOH for 3 h, filtered, washed with H_2O, EtOH and finally MeOH to give resin 5. The amino group capacity was determined by the picric acid titration method. N, 2·84% (2·02 m mol NH_2/g). IR (KBr): 3400 cm^{-1} ($-NH_2$); 1685 cm^{-1} ($-C=O$).

3.6 *Hydrazinolysis of resin* 3

Resin 3 (1 g) in EtOH (40 ml) and hydrazine hydrate (>95%, 0·6 ml, 18 m mol) were heated with refluxing for 3 h. The resin was filtered, washed with hot EtOH, DMF, DMF–H_2O (1:1), H_2O, EtOH and MeOH to give an orange red resin. The IR (KBr) absorption band at 1685 cm^{-1} in the starting resin completely disappeared.

3.7 *Coupling of Boc amino acids: general procedure*

Boc amino acids were incorporated into the resin 5 by the symmetric anhydride method. In a typical procedure a 3-fold molar excess of the Boc-amino acid in CH_2Cl_2 and a 1·5-fold molar excess of DCC were stirred together at 0°C for 1 h. In the case of Boc-Pro-OH a 7-fold molar excess was used. This solution containing the symmetric anhydride of the Boc amino acid was filtered directly into the resin 5 (1 g) and shaken for 3 h at room temperature. A second coupling was performed for 1 h to ensure maximum coupling. The extent of coupling was monitored by the semiquantitative ninhydrin test (Moore *et al* 1958). The Boc group was deprotected with 4N HCl–dioxane (20 ml/g of resin) for 30 min. A 10% solution of Et_3N in CH_2Cl_2 was used for neutralization.

3.8 *General procedure of photolysis*

The peptide resin (1 g) was suspended in DMF (100 ml) in a water-cooled immersion type photochemical reactor. The dissolved oxygen was removed by bubbling dry N_2 gas for 1 h. The suspension was irradiated at 350 nm with a Philips HPK 125 W mercury lamp for 48 h at room temperature with gentle magnetic stirring. Saturated $CuSO_4$ solution was used to filter out wavelengths below 320 nm. The spent polymer was separated by filtration and washed with DMF (10 ml × 3 × 2 min). The solvent was removed from the combined filtrate and washings in a vacuum rotary evaporator.

3.9 *Coupling of Boc-Val to the resin* 5 *and cleavage as Boc-Val-*NH_2

The symmetric anhydride of Boc-Val-OH (1·32 g, 6·1 m mol) in CH_2Cl_2 (10 ml) was prepared using DCC (0·62 g, 3·05 m mol) as described in the general procedure and added to the resin 5 (1 g) swelled in CH_2Cl_2 (5 ml). After shaking for 3 h the resin was filtered and washed with CH_2Cl_2. This resin was recoupled with a solution of the symmetric anhydride of Boc-Val-OH (3·05 m mol) for 1 h. The resin was collected by filtration, washed with CH_2Cl_2 (10 ml × 3 × 3 min) MeOH (10 ml × 2 × 2 min) and dried *in vacuo*. Amino acid analysis indicated the presence of 1·42 m mol of Val/g. The Boc-Val resin (1 g) was suspended in DMF (100 ml) and photolysed according to the general procedure. The crude product was crystallised from MeOH–petroleum ether; m.p. 153–154°C.

Boc-Gly-NH_2 (m.p. 141–143°C), Boc-Pro-NH_2 (m.p. 172–174°C) and Boc-Leu-NH_2 (m.p. 78–80°C) were prepared similarly in 75–85% yield.

3.10 *Synthesis of Boc-Pro-Val-*NH_2

Boc-Val-resin (1 g, 1.42 m mol Val/g) was deprotected with 4N HCl–dioxane (20 ml, 30 min), neutralized (10% Et_3N, 10 min) and washed with CH_2Cl_2

(10 ml × 3 × 3 min). The symmetric anhydride of Boc-Pro-OH (2.1 g, 9·9 m mol) was added and shaken for 3 h. A second coupling with Boc-Pro-OH (1 g) was done for 1 h. The resin was filtered, washed with CH_2Cl_2 (10 ml × 3 × 3 min), MeOH (10 ml × 2 × 2 min) and CH_2Cl_2 (10 ml × 2 × 2 min). This resin was suspended in DMF (100 ml) and photolysed as described in the general procedure. After purification by chromatography the Boc-Pro-Val-NH_2 was obtained in 74% yield; m.p. 82°C. Amino acid analysis: Pro, 0·91; Val, 1·0.

3.11 *Solid-phase synthesis of Boc-Gly-Phe-Pro-NH_2*

Boc-Pro-resin (1 g) was deprotected with 4N HCl–dioxane (20 ml) for 30 min. After neutralization with 10% Et_3N in CH_2Cl_2 the resin was washed with CH_2Cl_2 (10 ml × 3 × 2 min). A solution of the symmetric anhydride of Boc-Phe-OH (1·19 g, 4·5 m mol) was added and the resin was shaken for 3 h. The filtered resin was washed with CH_2Cl_2 (10 ml × 3 × 3 min), MeOH (10 ml × 3 × 3 min) and CH_2Cl_2 (10 ml × 2 × 2 min). After a second coupling with 0·6 g of Boc-Phe-OH for 1 h, the resin was collected by filtration and washing. After deprotection of the Boc-group, Boc-Gly-OH (0·78 g, 4·5 m mol) was incorporated following the general coupling procedure. The Boc-Gly-Phe-Pro-NH_2 was removed from the peptide resin by photolysis. The crude product after crystallization from EtOAc/Et_2O afforded the peptide amide in 80% yield; m.p. 80–82°C. Amino acid analysis: Gly, 0·96; Phe, 1·0; Pro, 0·98.

3.12 *Solid-phase synthesis of Boc-Leu-Ala-Gly-Val-NH_2*

Boc-Gly-OH (0·78 g, 4·5 m mol), Boc-Ala-OH (0·85 g, 4·5 m mol) and Boc-Leu-OH (1·03 g, 4·5 m mol) were incorporated into Boc-Val-resin (1 g) by the symmetric anhydride procedure as described in the general procedure of coupling. After the solid-phase assembly, the peptide was cleaved from the support by photolysis. The crude peptide after chromatography and crystallization from EtOAc/Et_2O afforded the peptide amide in 71% yield, m.p. 136–138°C. Amino acid analysis: Leu, 1·00; Ala, 0·95; Gly, 1·02; Val 0·97.

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