Tetrahedron Letters,Vol.23,No.43,pp 4439-4442,1982 0040-4039/82/434439-04$03.00/0
Printed in Great Britain

4-(2-CHLOROPROPIONYL)PHENYLACETOXY-POLYETHYLENE GLYCOL: A NEW PHOTOLABILE SUPPORT FOR LIQUID PHASE PEPTIDE SYNTHESIS

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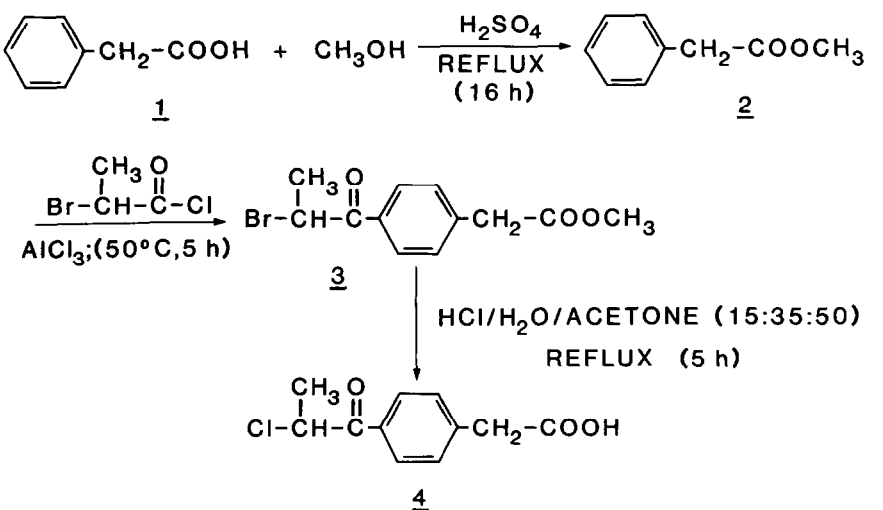
Summary: The synthesis of a photolabile 4-(2-Chloropropionyl)phenylacetoxy-polyethylene glycol support and its application in liquid phase peptide synthesis is described. A protected pentapeptide is prepared and removed quantitatively from the support by irradiation at 350 nm.

The liquid phase method of peptide synthesis on polyethylene glycol (PEG) has been successfully applied for the preparation of biologically active peptides[1]. Its major disadvantage, low yields of final peptide obtained by cleavage under drastic conditions such as saponification or hydrazinolysis, was overcome by the introduction of a 3-nitro-4-bromomethyl-benzoyl-polyethylene glycol support[2,3]. Protected or free peptide acids prepared on this support can be efficiently cleaved by photolysis and catalytic hydrogenation[4,5]. Recently we used 4-(2-chloropropionyl)phenylacetic acid 4 as a photolabile handle in the preparation of a new support for solid phase peptide synthesis[6]. One advantage of using a handle is that it can be adapted to various types of supports. In this paper we wish to describe the application of this handle in the preparation of a new photolabile 4-(2-chloropropionyl)phenylacetoxy-polyethylene glycol 7 for liquid phase peptide synthesis.

Starting from phenylacetic acid 1, as outlined in scheme 1, 4-(2-chloropropionyl)phenylacetic acid 4 was obtained in three steps with an 82 percent overall yield[6].

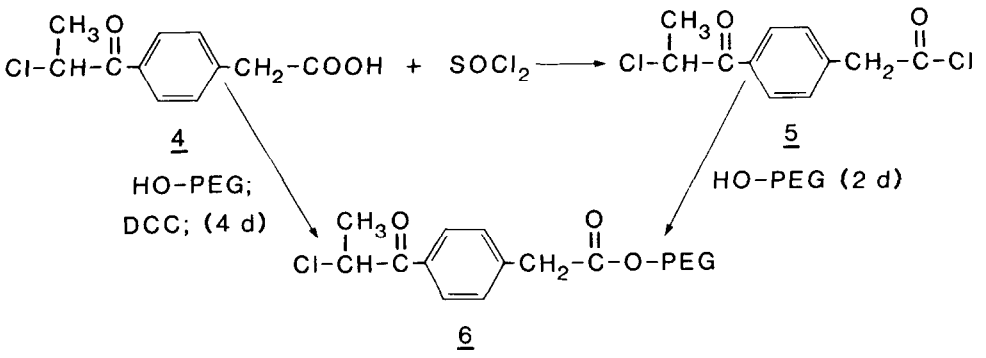
scheme 1



During the acid hydrolysis of the ester 3, the bromine residue was replaced by chlorine to give the acid 4.

The new polyethylene glycol support 6 was obtained by coupling 4-(2-chloropropionyl)-phenylacetic acid 4 (3 eq.) in the presence of dicyclohexylcarbodiimide (3 eq.) to a bifunctional polyethylene glycol (Mr = 6000; 0.33 mmol OH groups per gram of support) in dichloromethane (scheme 2). Alternatively, 4-(2-chloropropionyl)phenylacetic acid 4 (5 eq.) was converted to its acid chloride 5 with thionyl chloride in chloroform. After removal of excess thionyl chloride, the crude acid chloride was then reacted with polyethylene glycol in refluxing toluene. The products obtained from both methods were purified by crystallization from dichloromethane/ether to give a substitution of 0.21 mmol Cl per gram of support (64 percent yield).

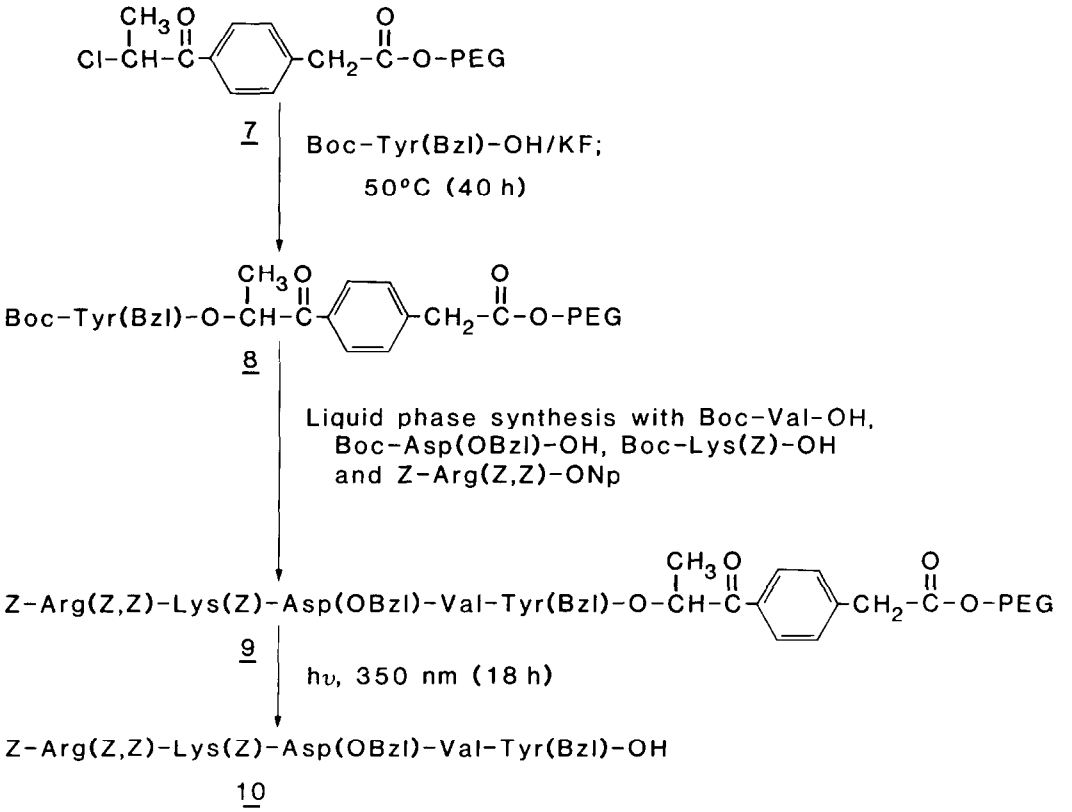
scheme 2



To test the new support we have prepared by the liquid phase method[1,3] (scheme 3) a protected pentapeptide, Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)-OH, which corresponds to the active segment 32-36 of Thymopoietin II[7], a peptide isolated from bovine thymus.

Initially, BOC-Tyr(Bzl)-OH (6 eq.) was esterified to the support via its diisopropylethyl-ammonium salt in ethyl acetate[3]. The substitution of Tyr was only 0.05 mmol per gram of support. A more efficient and mild approach is to use potassium fluoride in the esterification reaction[8]: 4-(2-chloropropionyl)phenylacetoxy-polyethylene glycol 6 (3 g; 0.63 mmol Cl) was dissolved in DMF (15 ml) and potassium fluoride (0.58 g; 10 mmol) and BOC-Tyr(Bzl)-OH (1.85 g; 5 mmol) were added slowly. The mixture was stirred at 50°C for 40 h and filtered. Ether was added to the filtrate and the product was collected by filtration. Recrystallization from methanol gave 2.8 g of pure product (0.18 mmol Tyr per g support; 86 percent yield based on the available Cl sites). Thin layer chromatography of the sample showed no traces of free tyrosine derivatives (Silica Gel 254 F_{60}; 200 microns; n-butanol:acetic acid:water = 3:1:1).

scheme 3



After removal of the BOC-group with 50 percent trifluoroacetic acid/dichloromethane (v/v; 1/2 h), the pentapeptide was assembled stepwise by the liquid phase method. All couplings were made via the in situ symmetrical anhydride method, using a 4-fold molar excess of BOC amino acid and a 2-fold molar excess of dicyclocarbodiimide, except for Arg, which was coupled via its p-nitrophenyl ester (5 eq.) in dimethylformamide. The BOC amino acid was dissolved in 15 ml of dichloromethane, and the solution was cooled to 0° C. A solution of dicyclocarbodiimide in 15 ml of dichloromethane was prepared and cooled to 0°C.Both solutions were combined and allowed to stand at 0°C for 1 h. This solution containing the symmetrical anhydride of the BOC amino acid was filtered directly into a flask containing the deprotected peptide support dissolved in

7-10 ml of dichloromethane per gram of peptide support. N-methyl morpholine was added to neutralized the trifluoroacetate salt (pH 7.5-8.0 as measured on moistened indicator paper). After stirring the reaction mixture for 1 h at room temperature, the pH was readjusted to 7.5-8.0 if necessary by further addition of N-methyl morpholine and the reaction mixture was stirred for an additional 1-3 h. The solution was reduced to a small volume by evaporation in vacuo. The peptide-PEG support was precipitated by the slow addition of anhydrous ether, filtered, washed with ether, and dried in vacuo. The extent of coupling was monitored by a qualitative ninhydrin test[3].

The protected pentapeptide 10 was released from the support by photolysis. A solution of peptide support in dry dimethylformamide (1.5 g in 40 ml) was placed in a screw-capped glass vial and was bubbled with argon for 1 h and irradiated in a Rayonet Photochemical reactor RPR-100 equipped with 3500 Å lamps at 37°C for 18 h. The solvent was removed under reduced pressure and the residue was thoroughly washed with dichloromethane and collected by filtration. Recrystallization from dimethylformamide/ethyl acetate gave 330 mg of protected peptide (92 percent yield); m.p. 192°-195°C; amino acid analysis: Asp, 1.02; Val, 1.00; Tyr, 0.95; Lys, 1.01; Arg, 0.94. Acid hydrolysis of the photolyzed peptide-support showed no peptide remaining on the support, indicating that the peptide had been cleaved quantitatively.

The easy availability of 4-(2-chloropropionyl)phenylacetic acid 4 and high photolytic cleavage yield should make 4-(2-chloropropionyl)phenylacetoxy-polyethylene glycol 6 a suitable support for liquid phase peptide synthesis.

Acknowledgement: The authors wish to thank Mrs. A. Gerelus for performing the amino acid analyses. We also wish to thank Mrs. M. Sanders for editing and Mrs. N. Geshner for typing the manuscript.

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(Received in USA 20 July 1982)