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REMOVAL OF C-TERMINAL PEPTIDE AMIDES FROM A 3-NITRO-4-AMINOMETHYL-BENZOYL AMIDE RESIN BY PHOTOLYSIS

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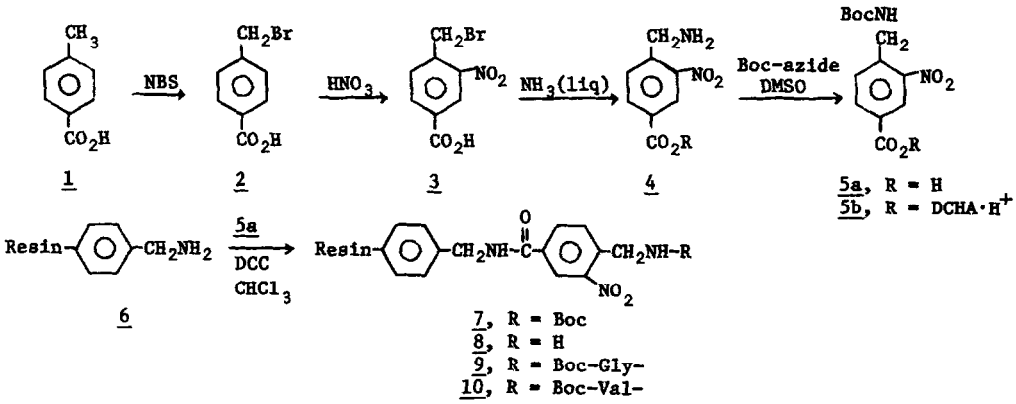
The C-terminal amide group is present in several biologically active peptides including deamino-oxytocin,[1] LH-RH,[2,3] and secretin.[4] These peptides have been synthesized by solid phase methods[5] in which the C-terminal amide of the protected peptide was removed from the resin by amminolysis or transesterification.[6] However, these conditions necessitate the use of side chain ester protecting groups which are resistent to amminolysis or transesterification, and, therefore, restrict the type of acid labile protecting groups which can be used to synthesize the peptide. Furthermore, peptides with hindered C-terminal residues, such as the valine in secretin, can be difficult to remove from the resin.[4] Thus new methods for preparing and removing protected peptides from a solid phase as the C-terminal amides would be useful. We report here the synthesis of the 3-nitro-4-aminomethylbenzoylamide polystyrene resin 7. Protected peptides can be removed from resin 7 as the C-terminal amide by photolysis under conditions which do not destroy acid or base labile protecting groups nor aromatic amino acids. The synthesis of LH-RH is presented to illustrate the method.

Resin 7 was prepared as shown in Scheme I. Bromination of p-toluic acid (1) with N-bromosuccinimide in refluxing benzene gave α-bromo-p-toluic acid (2) in 81% yield.[7,8] Nitration of 2 in 90% nitric acid at -10°C for 2.5 hr gave 3-nitro-4-bromomethylbenzoic acid (3) in 85% yield,[8] which upon reaction in liquid ammonia for 48 hr at 4°C in a pressure bottle gave 3-nitro-4-aminomethylbenzoic acid (4) (mp 235-237°C) in 72% yield. Amino acid 4 was converted to its Boc-derivative 5a by reaction with Boc-azide in DMSO[8] and characterized as the dicyclohexylamine salt 5b (mp 205-207°C) (85%). Resin 7 (0.23 mmol/g Boc-NH) was prepared by coupling the Boc-nitro acid 5a in DMF using DCC with the aminomethyl

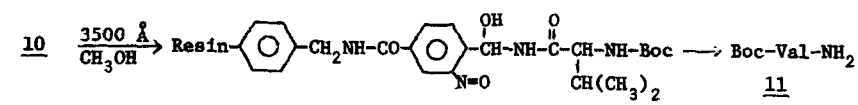
resin 6 (0.23 mmol/g NH_2), which was prepared by amination of chloromethylated polystyrene (0.23 mmol/g Cl, 1% divinyl benzene) with liquid ammonia in methylene chloride.[7,8] Resin 8 was prepared by treating resin 7 with 50% TFA/CH_2Cl_2 followed by neutralization with triethylamine in CH_2Cl_2. Resins 9 and 10 were prepared by coupling Boc-Gly and Boc-Val with resin 8 in DMF using DCC.

Scheme I



Protected amino acid amides were formed upon photolysis of resins 9 and 10 (Scheme II). The resin was suspended in methanol and irradiated at 3500 Å in the absence of oxygen. A 40% $CuSO_4$ solution was used to filter out wavelengths below 3200 Å. Boc-Gly-NH_2 and Boc-Val-NH_2 (11)[9] were isolated in quantitative yield, which established that hindered amino acid derivatives can be removed efficiently from resin 10 under mild conditions. In addition to the Boc group, the O-benzyl group (on Ser, Tyr, Asp and Glu), and the tosyl group (on Arg and His) are not removed by photolysis.

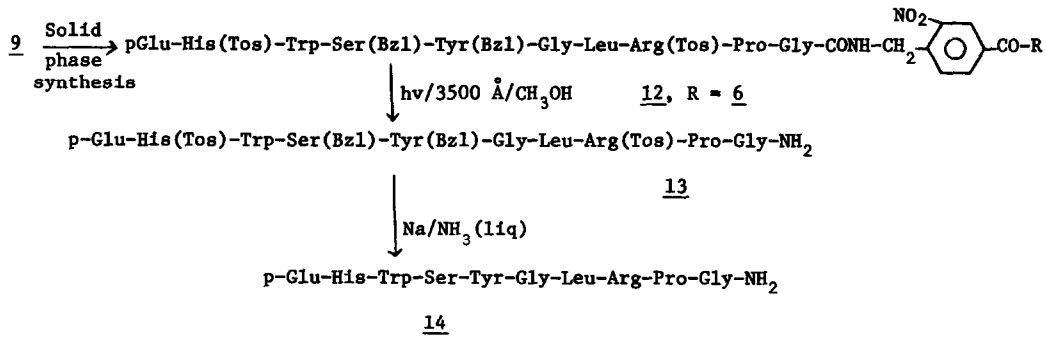
Scheme II



The C-terminal peptide amide, LH-RH 14 was synthesized using resin 9 (Scheme III). Boc-amino acids were coupled stepwise with resin 9 with DCC following the procedure of Merrifield.[4] The protected decapeptide resin (12) was photolyzed as described and after chromatography and crystallization the protected decapep-

tide amide 13 was isolated in analytically pure form in 65% yield based on Boc-Gly-resin 9.[8]

Scheme III



Decapeptide 13 was converted to LH-RH 14 by reaction with sodium in liquid ammonia as described.[10] The LH-RH (14) was isolated in 46% yield (which is comparable to the yields we obtain for this reaction on LH-RH prepared using standard solid phase resins) and shown to be identical with authentic LH-RH.[8] The protected heptapeptide Boc-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(Tos)-Pro-Gly-NH_2 also prepared by this method was isolated in 56% yield (182 mg),[8] based on starting Boc-Gly-resin 9, and shown to be identical with a sample prepared by standard solid phase synthesis.

These results establish that resin 7 can be used to synthesize protected peptides as the C-terminal amides in good yield on a preparative scale. Hindered C-terminal amino acids, e.g. Val, Leu, Phe, are removed under normal photolysis conditions. Thus resin 7 can be useful for the synthesis of C-terminal peptide amide which is difficult to remove from the Merrifield resin under mild conditions.

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3. Abbreviations used are: LH-RH, luteinizing hormone-releasing hormone; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; Tos, tosyl; Boc, tert-butoxy carbonyl; TEA, triethylamine.

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