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SOLID PHASE PEPTIDE SYNTHESIS. I. A MILD METHOD OF SOLID PHASE PEPTIDE SYNTHESIS EMPLOYING AN ENAMINE NITROGEN PROTECTING GROUP AND A BENZHYDRYL RESIN AS A SOLID SUPPORT.

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Solid phase peptide synthesis as introduced by Merrifield[1] and modified by others[2] is proving to be a valuable addition to the tools of the peptide chemist. However, the method employs moderately severe conditions for nitrogen deblocking and resin cleavage that may limit its usefulness for the synthesis of large peptides. The purpose of this report is to describe a new method of solid phase synthesis using the N-(2-benzoyl-1-methylvinyl) nitrogen protecting group and a benzhydryl resin that permit the use of mild deblocking and cleavage conditions as well as a wider choice of side chain protecting groups that do not rely on severe reagents such as HF or sodium liquid NH_3 for removal.

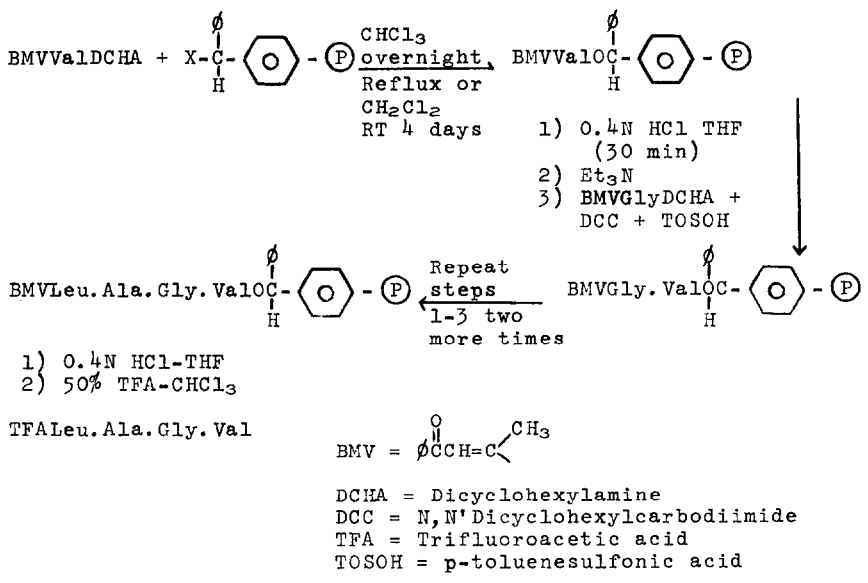
The benzhydryl resin was prepared for peptide synthesis by benzoylation of 2% cross-linked polystyrene, reduction to the carbinol with $NaBH_4$, and halogenation with anhydrous HCl or HBr.

Amino acids were converted to their N-(2-benzoyl-1-methylvinyl) BMV derivatives and utilized as dicyclohexyl ammonium salts in preference to their potassium salts[6] in order to enhance their solubility in organic solvents, a requirement for solid phase synthesis. The products are crystalline, high melting solids, possessing good shelf stability at room temperature of at least two years with no change in melting point or optical rotation. No racemization of the amino acids occurred during the synthesis of the BMV derivatives. This was shown by comparing the specific rotation of the starting amino acids and the amino acids obtained by hydrolytic removal of the BMV group.

The excellent solubility of BMV amino acids as dicyclohexylammonium salts permitted easy attachment to the resin by refluxing overnight in chloroform with benzhydryl halide resin. More recently other methods of attachment to the resin that give greater substitution levels have been found. These procedures will be detailed in future publications.

The BMV group can readily be removed from an amino acid attached to the resin by mild acid hydrolysis employing 0.4N (aq.) HCl-tetrahydrofuran (THF) or 1N p-toluenesulfonic acid monohydrate-THF. The 0.4N HCl solution is made by diluting 1 ml 6N HCl to 15 ml with THF. After subsequent couplings the same conditions are employed for BMV removal. These conditions are much milder than 1N HCl-acetic acid or dioxane required for BOC cleavage and 30% HBr/acetic acid for carbobenzoxy cleavage. Cleavage of the peptide from the benzhydryl resin is achieved with 5 to 50% trifluoroacetic acid in chloroform (30 min). These conditions are milder than HBr-trifluoroacetic acid[1a,c,d,e,f], anhydrous hydrogen fluoride[4], or hydrogen fluoride in trifluoroacetic acid[1b] used for the benzyl resin.

Figure I



BMV = $\phi C(=O)CH=C(CH_3)$

DCHA = Dicyclohexylamine
DCC = N,N'Dicyclohexylcarbodiimide
TFA = Trifluoroacetic acid
TOSOH = p-toluenesulfonic acid

The general scheme of peptide synthesis is shown in Figure I and is illustrated with the synthesis of the tetrapeptide L-leucyl-L-alanyl-glycyl-L-valine.

The dicyclohexylammonium salt of N-(2-benzoyl-1-methylvinyl)-L-valine was refluxed overnight in chloroform with benzhydrylbromide resin to give the ester. The BMV group was removed with 6N (aq.) HCl-THF (1:14) at 25° for 30 min and the resulting hydrochloride was neutralized with triethylamine in chloroform. The free base was then coupled overnight in methylene chloride with five equivalents of the dicyclohexylammonium salt of BMV-glycine and five equivalents each of anhydrous p-toluenesulfonic acid and dicyclohexylcarbodiimide to give BMV glycyl-L-valyl resin. Excess reagents were removed from the resin by successive washes with DMF, methylene chloride, methanol, and THF. The cycle of BMV removal, liberation of free base, and coupling was repeated in exactly the same way with the dicyclohexylammonium salts of BMV-L-alanine and BMV-L-leucine. The ratios of amino acids in the peptides at each coupling step were determined by hydrolysis of the resin peptide with 6N HCL[1d] and analysis of the hydrolysate by the method of Moore, Spackman and Stein[5].

The synthesis on the resin appeared to proceed quantitatively, and the absolute amounts of amino acids found indicated that none of the growing peptide chain was cleaved from the resin by the deblocking procedure. The peptide was cleaved from the resin with 50% trifluoroacetic acid-chloroform (30 minutes at room temperature), and after precipitation with ether, 300 mg of a white solid was collected as the trifluoroacetate. The product was purified chromatographically on a column of Dowex 50X-4 by elution with 0.1M pyridine-acetate, pH 4.0. Ninhydrin analysis of the effluent indicated emergence of a minor fraction followed by a major fraction containing the bulk of material. No other ninhydrin-positive fractions were eluted. Lyophilization of the latter fraction gave 139.3 mg (76%) of peptide. Chromatography on paper in n-butanol-acetic acid-water (3:1:1) gave a single spot with an Rf of 0.72. Ratios of amino acids found in an acid hydrolysate and an aminopeptidase M digest[e] are shown in Table I.

Table I

	Leu	Ala	Gly	Val	Average % Recovery
Acid hydrolysate	0.92	1.04	1.00	1.00	93
APM	0.98	0.98	0.95	1.08	90

A sample of the tetrapeptide monohydrate was precipitated from ethanol with ether to give 99 mg of material, $[\alpha]_D^{25}$ + 20.25 (C, 2 EtOH), reported $[\alpha]_D^{20}$ + 22.5 (C, 1-2 EtOH)[7] and $[\alpha]_D^{21}$ + 18.0 (C, 2 EtOH)[1a]. Anal. Calcd for $C_{16}H_{32}N_4O_6$: C, 51.4; H, 8.5; N, 14.9. Found: C, 52.0; H, 8.5; N, 14.4.

The substituted benzhydryl resins in combination with amino acids having the amino function protected by the enamine and other acid labile nitrogen blocking groups appear to offer a generally useful method for the synthesis of peptides. The general utility of this method including its use with multifunctional amino acids bearing easily cleavable side chain protecting groups will be the subject of future publications.

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