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DESIGN AND SYNTHESIS OF A MULTI-DETACHABLE BENZHYDRYLAMINE-RESIN FOR SOLID PHASE PEPTIDE SYNTHESIS

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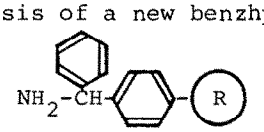
Summary: Peptides with C-terminal α-carboxamides were synthesized from a multi-detachable benzhydrylamine-resin containing a Boc-(4-acetoxy)benzhydrylamine handle of unambiguous origin. The peptides bound to the new resin are stable to trifluoroacetic acid, but are cleavable by hydrogen fluoride, base and nucleophiles to give unprotected or protected peptide fragments.

In the synthesis of many naturally occurring and bioactive peptides that have C-terminal α-carboxamides, the use of a benzhydrylamine-resin support 1 has been found to be most convenient[1]. Usually the benzhydrylamine is formed by direct derivatization of the resin-support, under conditions that are difficult to control[2] and which often result in benzhydrylamine being only one of several functionalized resin-products. Thus, low and unsatisfactory yields of α-carboxamides synthesized from benzhydrylamine-resins have been reported[3]. It has been recognized for some time that better yields and greater purity of peptide products can often be obtained from improved preparations of the resin-supports[4,5]. Recently, the use of benzhydrylamine handles for the benzhydrylamide-resin synthesis have been described[6]. Here we wish to report the synthesis of a new benzhydrylamine-resin 2 for solid phase peptide synthesis.



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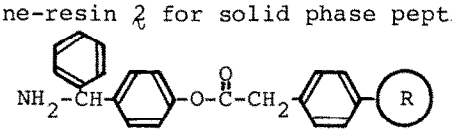

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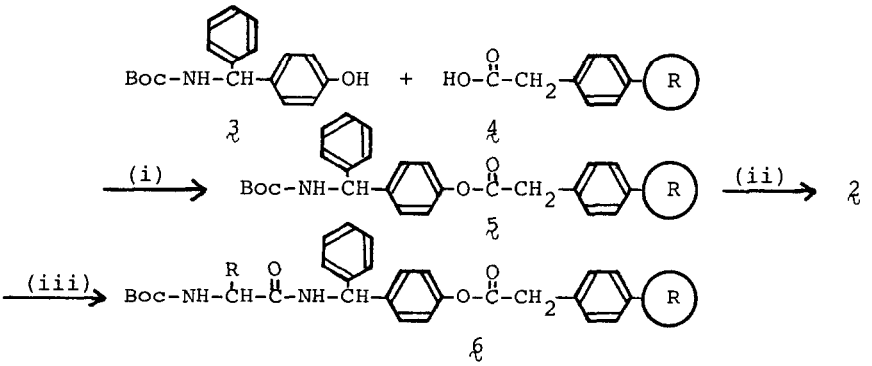
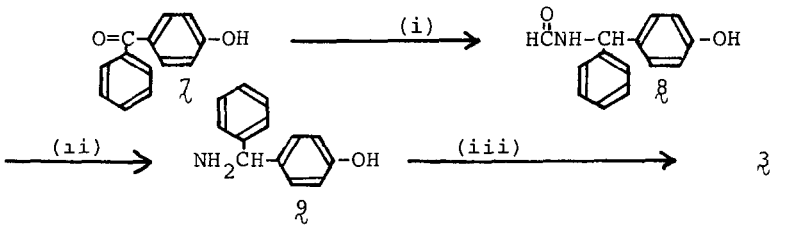
The new benzhydrylamine-resin 2 is designed with two specific improvements: (1) it is made from a chemically defined benzhydrylamine handle which is purified and then attached to the resin support, and (2) it is a multi-detachable resin[5,7], which is suitable for the preparation of protected peptide fragments. The synthesis was achieved (Schemes 1 and 2) by esterification of purified N-Boc-(4-hydroxy)benzhydrylamine 3 to carboxymethyl-copoly(styrene-1%-divinylbenzene) resin 4 to form N-Boc-benzhydrylamine-4-oxycarbonylmethyl-resin 5. After removal of the Boc-group by trifluoroacetic acid, the new benzhydrylamine-resin, 2 can be coupled with an activated Boc-amino acid to give the Boc-aminoacyl-benzhydrylamine-4-oxycarbonylmethyl-resin 6 (Scheme 1), which can then be lengthened by stepwise coupling to the desired peptide-resin.

Starting from 4-hydroxybenzophenone 7 (Scheme 2) Boc-(D,L-4-hydroxy)-benzhydrylamine 3 was prepared in three steps in 37% overall yield. The



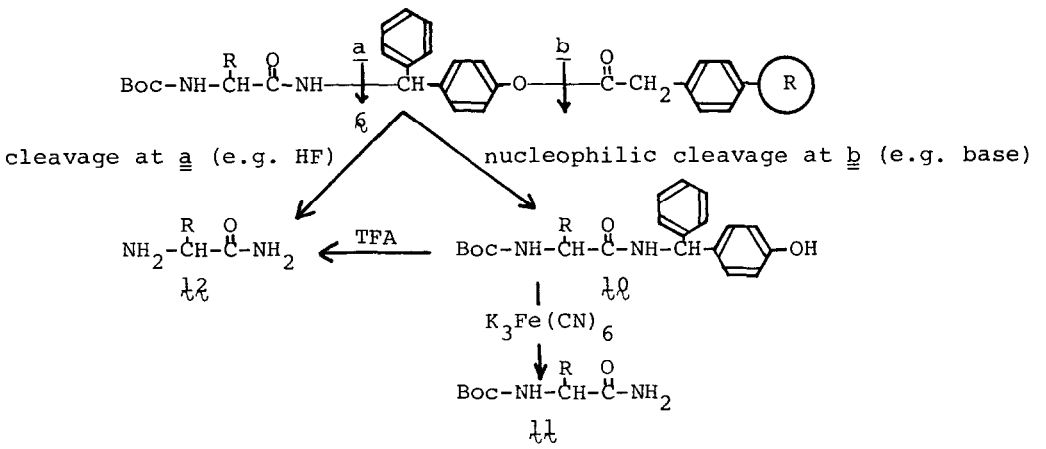
Scheme 1. Preparation of Boc-amino acyl-benzhydrylamine-4-oxycarbonylmethyl-resins 6. (i) Dicyclohexylcarbodiimide (DCC) and N,N-dimethylaminopyridine (DMAP), CH_2Cl_2, 14h (ii) Trifluoroacetic acid (TFA)/CH_2Cl_2 (1:1, v/v), 0.5h (iii) Boc-amino acid, DCC/DMAP, CH_2Cl_2, 12h.

formylated intermediate 8 resulting from the Leukart reaction[8] was found to be very acid labile and deaminated readily in strong aqueous acidic solution. A modified but milder acid hydrolysis was necessary to obtain a good yield of 9, which was immediately converted to the crystalline racemic Boc-derivative 3 (m.p. 138-140°C). The carboxymethyl-resin 4 was obtained in two steps from commercially available chloromethyl-resin by the displacement of chloride by cyanide in DMSO/DMF, and subsequent aqueous acid hydrolysis of the nitrile.[9]



Scheme 2. Preparation of Boc-(4-hydroxy)benzhydrylamine. (i) $HCO_2H/HCONH_2$ (1:4), 168°C, 4h (ii) 12 N HCl in HCO_2H-H_2O (1:1:1), 75°C, 0.7h (iii) $(Boc)_2O$, NaOH, Bu^tOH, 12 hr.

Boc-aminoacyl-benzyhydrylamine-4-oxycarbonylmethyl-resin 6 is by design a multi-detachable resin (Scheme 3)[5,7]. It consists of two cleavable points: the benzhydrylamide linkage a and the phenyl ester linkage b, connected by a spacer. In strong acid, a peptide amide (usually free and unprotected) is expected from the cleavage at point a. Indeed, as shown in Table 1, treatment with HF/p-cresol (9:1, v/v)[10] at 0° C for 1 h provided the desired amino acid or peptide derivative in excellent yields, even with a problematic residue such as phenylalanine α-carboxamide.[3] Pentagastrin, containing a sequence of difficult amino acids was obtained in 85% yield and gave one spot in several TLC systems. The electronic withdrawing effect of the 4-acetoxy group was expected to render the new resin 6 quite stable to acidolytic loss of peptide during the sequential TFA



Scheme 3. Design of a Multi-detachable Benzhydrylamine-Resin

treatments. Preliminary kinetics in 50% TFA at 25° C indicated that Boc-Gly-resin 6 and Boc-Phe-resin 6 were 60-120 times more stable than in the usual Boc-Gly-OCH_2-resin containing a benzyl ester, with losses of peptide chain of less than 0.014% per 30-min acidic deprotection cycle.

Peptides can also be removed from the multi-detachable resin without the use of HF, thus avoiding the undesirable side reactions sometimes produced by strong acids. For example, Pro-Leu-Gly-NH_2 12 was obtained in 89% yield first by treatment with 5% hydrazine/DMF to give Boc-Pro-Leu-Gly-(4-hydroxy)-benzhydryl-amide 10 and then by removal of the 4-hydroxybenzhydryl and Boc groups by TFA/p-cresol (9:1, v/v) for 1 hr (Scheme 3). This is possible because the 4-hydroxy-benzhydryl amine peptide such as 10 has lost the acid-stabilizing effect of the 4-acetoxy group of compound 6.

A distinctive feature of this new resin is that the ester linkage b is not a phenyl ester of the peptide component, but a retro-phenyl ester in which the carboxyl group is part of the resin component. This design confers the electronic properties required for the TFA stability and HF lability of the benzhydrylamide moiety. In addition a flexible cleavage pattern is achieved because the lability of the ester bond to a variety of nucleophiles leads to fully protected peptide products. 4-Hydroxybenzhydryl-peptide 10 is produced whether the reagent be hydrazine, cyanide or peroxide anion, and under the conditions of the hydrazinolysis this product is stable and isolable in good yield. The protected peptide 10 can be purified and characterized and then reattached to the solid support 4 for further stepwise or fragment synthesis. Finally, the 4-hydroxybenzhydryl group can be removed by a 1,6-elimination reaction in the presence of aqueous bisulfite or oxidatively with $K_3Fe(CN)_6$ in aqueous ethanol to give 11 (Scheme 3)[7]. This degree of chemical flexibility is not available in any previously reported benzhydrylamine resin.

Table I. Cleavage Methods for Multidetachable Benzhydrylamine Resin

amino acid and peptide attached to resin % [a]	cleavage yields % [b] HF [c]	OH^-/OOH [d]	NH_2NH_2 [d]	KCN/MeOH [d]
Boc-Gly	92	89	95	90
Boc-Leu	90	87	90	80
Boc-Val	88	80	82	82
Boc-Phe	90	85	91	78
Boc-Leu-Phe	90	80	86	80
Boc-Pro-Leu-Gly	92	86	92	81
Boc-Arg(Tos)-Phe(4-F)-Phe	91	86	95	80
pGlu-His(Tos)Pro	90	89	95	90
Boc-Gly-Lys(2-Cl-Z)-Pro-Val	85	80	85	82
Boc-Gly-Trp(For)-Met-Asp(OcHex)-Phe	85 [e]	-	82	-

a. 0.26 mmol/g substitution b. Yield based on amino acid analysis, products as α-carboxamides. c. HF/p-cresol (9:1, v/v), 0° C, 1h. d. followed by basic oxidative work up. e. HF/p-cresol/p-thiocresol (9:0.5:0.5, v/v/v), 0° C, 1 hr.[10]

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