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CLEAVAGE OF PEPTIDES FROM BENZHYDRYLAMINE RESINS USING TRIFLUOROMETHANESULFONIC ACID

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Abstract A general method is described for forming peptide amides by cleavage from benzhydrylamine resin using a mixture of trifluoromethanesulfonic acid, thioanisole and trifluoroacetic acid. This procedure gives comparable results to cleavage by liquid hydrogen fluoride.

The necessity for using liquid HF to cleave peptides from benzhydrylamine resins has been a limiting factor in the scale-up of C-terminal amide containing peptides prepared by solid phase synthesis. While other acidic techniques are applicable for cleaving peptides attached via ester linkages, hydrogen fluoride remains the standard cleavage technique for forming amides where ammonolysis is not possible. Trifluoromethanesulfonic acid (TFMSA) has been used to remove a variety of protecting groups used in peptide synthesis including N^G-tosyl, N-benzyloxycarbonyl, benzyl ethers, methyl ethers and benzylesters[1-6]. It has also been used to remove peptides from the standard Merrifield resin joined by an ester linkage[7]. We wish to report the use of TFMSA to remove peptide amides from benzhydrylamine resins. In comparative studies we have found that of a mixture of TFMSA, thioanisole and trifluoroacetic acid gave similar yields to those obtained by hydrogen fluoride cleavage.

For comparison we chose the test pentapeptide Asp-Ile-Arg-Lys-Phe-NH_2 for three reasons: (i) this peptide resin contained a several different protecting groups in its protected form (TFA Asp(Bzl)-Ile-Arg(Tos)-Lys(Z)-Phe-benzhydrylamine resin), (ii) it had the possibility of α to β rearrangement of aspartic acid and (iii) it was attached to the resin by a phenylalanine, an attachment which has been difficult for us to completely remove from benzhydrylamine resins using the standard one hour liquid HF cleavage at 0°. Although peptides are more readily removed from the p-methylbenzhydrylamine resin, we chose to use the unsubstituted benzhydrylamine resin as a more difficult test.

The peptide was synthesized on a benzhydrylamine resin (10 g 0.68 meq/g) using BOC-amino acids coupled by DCC/HOBt. The final protected peptide resin amide (16 g) was divided equally. Using the following procedures, one half was cleaved with anhydrous HF and anisole while the second half was cleaved with a mixture of TFMSA, thioanisole and trifluoroacetic acid.

Hydrogen Fluoride Cleavage: The protected pentapeptide resin amide (8.0 g) was treated with a mixture of anisole (11.2 ml) and liquid hydrogen fluoride (80 ml) for 2 hr at 0°. The hydrogen fluoride was removed under reduced pressure at 0° and the residue washed with diethylether. The peptide was extracted from the residue with 33 percent aqueous acetic acid. Lyophilization gave 1.50 g of crude peptide.

Trifluoromethanesulfonic Acid Cleavage: The protected pentapeptide resin amide (8.0 g), trifluoroacetic acid (40 ml) and thioanisole (13.2 ml) were stirred under a dry nitrogen atmosphere for five minutes in a round bottom flask. Trifluormethanesulfonic acid (4.8 ml) was added and resulting mixture stirred for 6 hr at 23°C. The mixture was filtered and the resin washed with trifluoroacetic acid. The combined filtrate and washes were evaporated under reduced pressure. The residue was triturated with dichloromethane (2 x 50 ml) and diethylether (3 x 50 ml) to give 4.2 g of crude peptide.

The crude peptide was purified by preparative HPLC on a Whatman M-20 (20 x 500 mM OSD-3) column with 0.01M NH_4OAc, pH 5.0, 15 percent acetonitrile, 7.5 ml/min, 220 nm detection. The acetonitrile was removed under reduced pressure and the aqueous solution lyophilized. Analytical HPLC was run with the same solvent system on a Waters μbondapak 4.5 x 300 mm 0.75 ml/min.

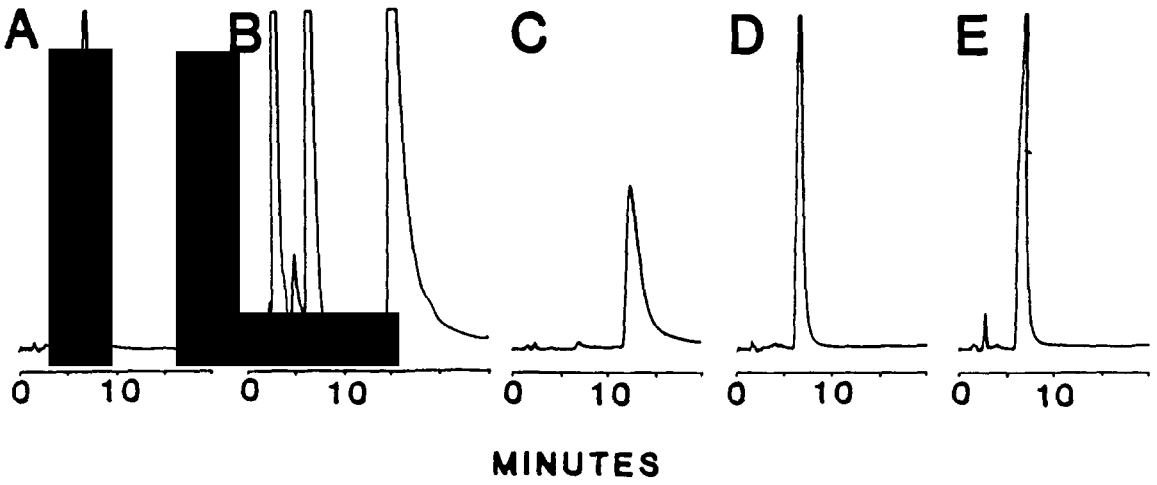

Figure 1 A- Crude HF cleaved, B- Crude TFMSA cleaved product, C- ß-Asp-Ile-Arg-Lys- Phe-NH_2, D- purified HF cleaved product, E- purified TFMSA cleaved product

Figures 1A and 1B are the crude peptides from HF and TFMSA cleavages respectively. Figure 1C is ß-Asp-Ile-Arg-Lys-Phe-NH_2 synthesized and purified independently. Figures 1D and 1E are the purified peptides from HF and TFMSA cleavage respectively.

Table I compares the results of the two cleavage techniques. Both the TFMSA and extended (2 hr)

HF cleavage gave comparable removal from the resin, as determined by amino acid analysis, and equal yields of purified material. The weight of the crude TFMSA product was substantially greater than that obtained from HF cleavage but the presence of this uncharacterized nonpeptidic material did not interfere with purification or affect the isolated yield. The presence of 1.1 percent of β-aspartic acid product was detected in the crude TFMSA cleaved material and is consistent with previous observations on α to β rearrangement with TFMSA[1].

TABLE I.

Comparison of hydrogen fluoride and trifluoromethanesulfonic acid cleavage of TFA Asp(Bzl)-Ile-Arg(Tos)-Lys(Z)-Phe-benzhydrylamine resin. * from amino acid analysis, ± from HPLC (area percent)

	HF cleavage	TFMSA cleavage
Weight of peptide resin	8.0 g	8.0 g
Weight of crude peptide	1.5 g	4.2 g
Weight of resin after cleavage	5.0 g	6.1 g
Peptide remaining on resin*	0.06 meq/g	0.05 meq/g
Percent peptide of crude product*	59.3	27.6
Amino acid ratios of purified product* (Asp/Ile/Arg/Lys/Phe)	0.99/0.92/1.02/ 1.01/0.98	0.99/0.92/1.01/ 1.02/0.98
Percent peptide in purified product*	68.6	64.4
Theoretical yield	2.3 g	2.3 g
Isolated yield	0.75 g (33 percent)	0.73 g (32 percent)
β-Asp content±	0 percent	1.1 percent

This cleavage technique has been applied to a number of peptides including Arg-His- Gly-NH_2, Lys-Tyr-Gly-NH_2, Lys-His-Ala-NH_2, Lys-Ala-Pro-Asp-Val-Phe-NH_2, Phe-Met-Arg-Phe-NH_2 and Arg-Pro-Asp-Val-Phe-NH_2. Because of the lack of a requirement for special handling equipment, the technique is applicable for large scale cleavage. We have used this technique on 100 g of peptide resin amides with isolated yields of 30-40 percent based on initial benzhydrylamine resin substitution. On a smaller scale the same resins gave identical isolated yields when cleaved with HF.

The trifluoromethanesulfonic acid, thioanisole, trifluoroacetic acid cleavage techniques is

generally applicable to benzhydrylamine resin peptide cleavage and comparable to HF in the degree of removal of the peptide from the resin, isolated yield and purity of final product.

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