Cleavage of Single Amino Acid Residues from Merrifield Resin with Hydrogen Chloride and Hydrogen Fluoride

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Anhydrous HF-containing anisole is being used for the removal of the resin and side-chain blocking groups in the final step of Merrifield solid-phase peptide synthesis,[1c,2,3] and HCl hydrolysis is being used for the preparation of samples for amino acid analysis during the course of Merrifield solid-phase peptide synthesis.[1c,3-5] We have performed some experiments in order to determine the optimal conditions for these two reactions. The determined conditions have been used routinely in our laboratory for the past 2 years during several hundred cycles of Merrifield solid-phase peptide synthesis with good results.

HF Reaction.—For it we use 5000 μmol of anisole in 5 ml of anhydrous HF at 0° for 0.50 hr. Trifluoroacetic acid or toluene decreases the overall recovery of the single amino acids, and the use of only 500 μmol of anisole in 5 ml of anhydrous HF decreases the recovery of the amino acids even if the total amino acids to be recovered in the reaction mixture is less than 20 μmol. The resin itself serves as a scavenger for blocking groups but is not usually present in high enough concentration to prevent attack on the amino acid side chains themselves. Recoveries without any anisole are about two-thirds complete for most amino acids but are very low for Tyr, Trp, Phe, and Met.

AsN and GIN are deamidated in some peptides during this procedure.[6]

HCl Reaction.—For it we use an "anaerobic" mixture of 0.5 ml of 6 *N* HCl in H_2O and 0.5 ml of propionic acid at 130° for 2 hr (see Table I). We use 10–50 mg of resin–peptide. This mixture gives more clean, consistent, and complete recoveries of the 20 amino acids than do similar mixtures in which propionic acid has been substituted by H_2O, dioxane, dimethyl sulfoxide, dimethylformamide, formic acid, or acetic acid. Valeric acid and propionic acid give the same results, but propionic acid is more pleasant. This procedure has been used extensively in our laboratory with resin–peptides with good results.

In addition, our rate experiments have shown that the effect of improper solvation of the Merrifield resin is to close off completely the less accessible sites on the resin, rather than generally to decrease rates of reaction at all the sites by similar amounts. This is illustrated by Figure 1, which shows the rates of cleavage of ε-CBZ-*t*-BOC-lysine from Merrifield resin in the mixture reacted with HCl–H_2O–acetic acid and HCl–H_2O–propionic acid. This closing off is responsible for the fact that Merrifield synthesis itself yields such excellent products even when quantitative analysis during the synthesis is sometimes discouraging. During the synthesis, of course, the particular structure of the peptide being synthesized also has an important effect on solvation of the resin–peptide surface.

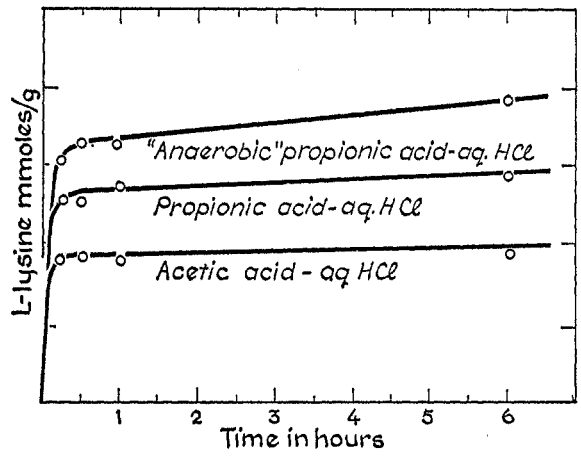

Figure 1.—Recovery of L-lysine from the hydrolysis mixture *vs.* time. Variations in solvent change the total accessible resin sites.

TABLE I
RECOVERY OF AMINO ACIDS BY HCl AND HF

Compd	"Anaerobic" HCl—H_2O–propionic acid, mmol/g: 2 hr at 130°[a]	Maximum recovery at 130° (hr)	5 ml of HF-5000, μmol of anisole, 30 min at 0°
BOC-L-Ala-resin	0.48	0.48 (2)	0.48
BOC-nitro-L-Arg-resin	0.25[b]	0.26 (12)	0.16
BOC-β-BZL-L-Asp-resin + BOC-L-AsN-resin	0.41	0.41 (2)	0.43
BOC-S-*p*-methoxybenzyl-L-Cys-resin	0.11	0.13 (1)	0.07
BOC-γ-L-Glu-resin + BOC-L-GIN-resin	0.47	0.47 (2)	0.43
BOC-Gly-resin	0.45	0.45 (2)	0.38
BOC-L-Lleu-resin	0.48	0.48 (2)	0.53
BOC-L-Leu-resin	0.47	0.47 (2)	0.44
BOC-ε-CBZ-L-Lys-resin	0.32	0.39 (12)	0.43
BOC-L-Met-resin	0.29	0.29 (2)	0.28
BOC-L-Phe-resin	0.47	0.47 (2)	0.44
BOC-L-Pro-resin	0.48	0.50 (1)	(0.7 ± 0.3)[c]
BOC-O-BZL-L-Ser-resin	0.35	0.39 (1)	0.50
BOC-O-BZL-L-Thr-resin	0.44	0.44 (2)	0.46
BOC-L-Trp-resin	0.06[d]		0.10
BOC-O-BZL-L-Tyr-resin	0.34	0.35 (1)	0.34
BOC-L-Val-resin	0.46	0.47 (12)	0.49

[a] Each value in the table is an average for ten rate experiments like that illustrated in Figure 1. Error of each analysis was ±7%, hence the table values are ±3%. [b] Recovery is of Arg. Very little ornithine and nitroarginine are observed with the propionic acid hydrolysis procedure. [c] The modified amino acid analyses (K. Dus and R. Smith) used for these experiments gave very poor analysis of Pro. [d] Trp is destroyed by hydrolysis. Probably a few drops of some reducing agent would prevent this.

Experimental Section

Copolystyrene–2% divinylbenzene was obtained from Bio-Rad Laboratories and was washed and chloromethylated in the usual way.[1c] Volhard titration after boiling in pyridine for 1 hr

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showed 1.15 mmol of chloride per gram of resin. BOC[7] derivatives of L-Ala, nitro-L-Arg, β-BZL-L-Asp, S-*p*-methoxy-BZL-L-Cys, γ-BZL-L-Glu, L-Gly, L-Ileu, L-Leu, ε-CBZ-L-Lys, L-Met, L-Phe, L-Pro, O-BZL-L-Ser, O-BZL-L-Thr, L-Trp, O-BZL-L-Tyr, and L-Val were esterified to the resin by refluxing 20 g of chloromethylated resin, 20 mmol of derivative, 18 mmol of triethylamine, and 50 ml of ethanol for 46 hr. An equal weight mixture of all of the resin-derivative preparations was treated with acetic acid–anhydrous HCl (1 *M*) for 30 min and washed extensively and dried from ethanol. This mixture was used for all experiments, so that a representative collection of blocking groups was always present. The hydrolyses were performed in sealed glass tubes which were frozen by liquid nitrogen and thawed several times on a vacuum line before sealing. These "anaerobic" conditions seem to give higher recoveries of all of the amino acids. HF reactions were carried out in the apparatus described by Robinson and Kamen[8] which is similar to that described by Sakakibara, *et al.*[9]

Registry No.—Hydrogen chloride, 7647-01-0; hydrogen fluoride, 7664-39-3.

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(7) Abbreviations: BOC = *t*-butyloxycarbonyl, CBZ = carbobenzyloxy, BZL = benzyl (ethers and esters).

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Synthesis of *trans*-3,*cis*-5-Tetradecadienoic Acid (Megatomoic Acid), the Sex Attractant of the Black Carpet Beetle, and Its Geometric Isomers[1]

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The sex attractant of the black carpet beetle, *Attagenus megatoma* (Fabricius), was identified[2] as *trans*-3,*cis*-5-tetradecadienoic acid (I), to which we assign the trivial name, megatomoic acid. The synthesis of megatomic acid and its geometric isomers, *cis*-3,*cis*-5- (II), *cis*-3,*trans*-5- (III), and *trans*-3,*trans*-5-tetradecadienoic acid (IV) is described herein.

$C_8H_{17}CH{=}CH{-}CH{=}CHCH_2COOH$

trans-3,*cis*-5 (I)
cis-3,*cis*-5 (II)
cis-3,*trans*-5 (III)
trans-3,*trans*-5 (IV)

Megatomic acid was synthesized by the sequence shown in Scheme I based on the procedure described by Celmer and Solomons.[3] The *cis*-3,*cis*-5 isomer (II) also resulted.

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The sequence in Scheme II produced *cis*-3,*cis*-5-tetradecadienoic acid (II) and *cis*-3,*trans*-5-tetradecadienoic acid (III).

trans-3,*trans*-5-Tetradecadienoic acid (IV) was prepared by isomerization with iodine[3] of the *trans*-3,*cis*-5- or *cis*-3,*trans*-5-methyl esters, followed by hydrolysis.

Since none of the isomers (II–IV) nor the by-products resulting from the synthesis of megatomic acid masked its attractiveness, the crude mixture was submitted for large-scale field testing. Analytical samples of all the isomeric acids were prepared by mild alkaline hydrolysis of the corresponding methyl esters, which were isolated by gas chromatography and shown to be homogeneous on several substrates of different polarities. The acids were re-esterified with diazomethane to verify that only a negligible amount of isomerization occurred during the hydrolysis.

Experimental Section

The spectra were recorded on the following instruments unless otherwise noted: ir, Perkin-Elmer 137; uv, Perkin-Elmer 202; mass, CEC 103; nmr, Varian T 60 (60 Mc). The nmr spectra were obtained in CCl_4 and the chemical shifts are in τ values using TMS as an internal standard. The abbreviations "s, d, q, and m" denote "singlet, doublet, quartet, and multiplet," respectively. Gas chromatography (glc) was done on a Varian Aerograph 205 equipped with a hydrogen flame detector; a 1:20 splitter and N_2 make-up gas were used for preparative runs. Glc substrates were obtained from Applied Science Laboratory, Inc., State College, Pa.

1-*n*-Tridecen-4-yn-3-ol (V).—This compound was prepared in 56% yield from 1-decyne and acrolein according to the procedure of Celmer and Solomons.[3] The crude product was distilled at 114–117° (0.9 mm). Ir (λ^{film}, μ) 3.0 (OH), 3.25 (olefinic CH) 4.45 (C≡C), 6.03 (C=C), 9.8 (C—OH), 10.1 and 10.8 (vinyl).

Anal. Calcd for $C_{13}H_{22}O$: C, 80.35; H, 11.4. Found: C, 80.0; H, 11.5.

1-Bromo-*cis*- and -*trans*-2-tridecen-4-yne (VI).—A mixture of 3-bromo-1-tridecen-4-yne and 1-bromo-2-tridecen-4-yne resulted from the reaction of V with phosphorus tribromide according to the procedure of Celmer and Solomons.[3] Ir (λ^{film}, μ) 4.5 (C≡C), 10.15 and 10.8 (vinyl), 10.5 (*trans* -CH=CH-).

The 3-bromo compound was converted to VI when the mixture was heated under nitrogen at 117° for 75 min. Distillation though a Claisen head at 0.15 mm (bath temp 95–110°) afforded a 75% yield of VI.

Anal. Calcd for $C_{13}H_{21}Br$: C, 60.7; H, 8.2. Found: C, 61.0; H, 8.5.

The *cis* and *trans* isomers of VI were obtained in a ratio of 1:2 by glc fractionation (SE 30, 4% on Chromosorb G, 60–80 mesh, 0.9 m × 7 mm i.d. Pyrex, 160°, 50 cm³ He/min) with fractions collected: at 20 min ir (λ^{film}, μ) 4.50 (C≡C), 8.3, 13.1 (*cis*-CH=CH-); and 25 min ir (λ^{film}, μ) 4.50 (C≡C), 8.3 and 10.5 (*trans* -CH=CH-).

1-Cyano-2-*cis*- and -*trans*-tridecen-4-yne (VII).—A solution of 140 g (0.54 mol) of VI in 70 ml of dimethyl sulfoxide (dried over Linde 4X molecular sieves) was added dropwise over 20 min to a stirred suspension of 55.7 g (0.62 mol) of cuprous cyanide in 300 ml of dimethyl sulfoxide. The reaction mixture was stirred without external heating for 1 hr, at 40° for 1 hr, and finally at 85° for 2 hr. After cooling, the mixture was diluted with water and extracted with hexane. The extract was washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was distilled through a Claisen head at 0.1 mm (bath temp 110–130°) to give 72 g (66% yield) of VII.

Anal. Calcd for $C_{14}H_{21}N$: N, 6.9. Found: N, 6.9.

The *cis* and *trans* isomers of VII were obtained in a ratio of 1:2 by glc fractionation (Carbowax 20M, 10% on Gas Chrom Q, 60–80 mesh, 0.6 m × 8 mm i.d. Pyrex, 170°, 100 cm³ He/min) with fractions collected at 45 min and 100 min. The 45 min peak showed: ir (λ^{film}, μ) 4.44 and 4.51 (C≡C) and 13.7 (broad. *cis* -CH=CH-); nmr 4.3 (m, CH=CH) and 6.7 (d, $-CH_2CN$).

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