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A PRACTICAL METHOD FOR THE PREPARATION OF PROTECTED PEPTIDE FRAGMENTS USING THE KAISER OXIME RESIN†

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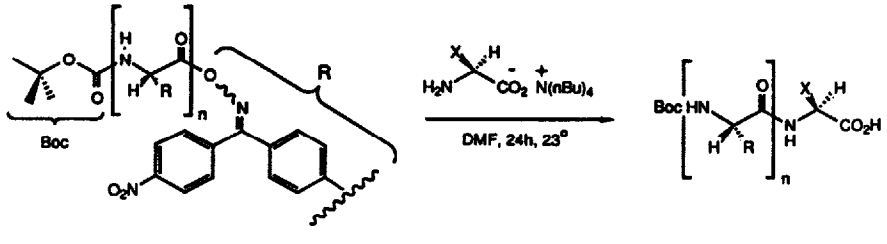
Summary: Resin-bound peptides can be cleaved from the Kaiser oxime resin using the tetra-n-butylammonium salts of side-chain protected amino acids to directly provide the α-amino-protected (Boc), side-chain protected peptide. Complete experimental details are provided. The protected peptide fragments can be purified and used for fragment coupling synthetic strategies.

The oxime-functionalized polystyrene resin (R), which was developed by Kaiser,[1,2,15] offers an advantage over Merrifield-type resins[3] in that the peptide can be cleaved from the Kaiser resin under nucleophilic conditions which do not effect deprotection of the amino acid side-chains[12] or the α-amino group (t-Boc).[1,2,4-8] Protected peptides of up to ten amino acids can be synthesized in high yield on the Kaiser oxime resin,[13] cleaved, and purified to homogeneity by reversed-phase (RP) HPLC.[1,6,9] The pure fragments can then be coupled on the Kaiser resin[4,6,8] or in solution[6,9] to provide synthetic proteins which are free from impurities resulting from a single amino acid deletion. Deletion impurities, which accumulate in every stepwise synthesis, can be extremely difficult to separate from the desired peptide. The acyl oxime resin-peptide bond can be cleaved by treatment with hydrazine (to afford the peptide hydrazide)[2], an alkyl amine (to afford the C-terminal amide)[7], N-hydroxypiperidine (to afford the C-terminal hydroxypiperidyl ester, which can be reductively cleaved using Zn/acetic acid to afford the free acid)[5], and an amino acid alkyl ester (to afford the fully protected peptide).[2]

We have developed a method for directly producing the desired free-carboxyl protected fragments by cleavage from the Kaiser resin using amino acid tetra-n-butylammonium salts. This method allows us to introduce isotopically-labelled or unnatural amino acids at a late stage in the synthesis via cleavage of large resin-bound peptides with the appropriate amino acid salt. The tetra-n-butylammonium salts of several amino acids, including some side-chain protected residues, have been prepared (see experimental section)[10] and used for cleavages.



† This paper is dedicated to the memory of Professor E. Thomas Kaiser which continues to inspire our research.

A partial list of protected peptides which have been prepared by this method is found in Table I. In every case, acid hydrolysis (1:1 HCl-propionic acid, 130°, 3h) followed by amino acid analysis of resin-bound peptides before and after cleavage showed that less than 10% of the peptide-oxime starting material remained uncleaved. The purified yields shown in Table I are typical for protected fragments synthesized on the Kaiser resin and cleaved by any of the available methods.

Table I

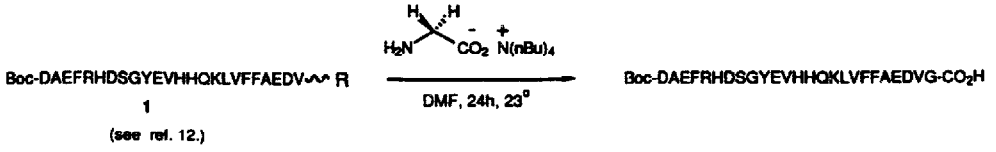
Resin-bound peptide[12]	Salt[10]	Protected Fragment[12,a]	Yield[b]
Boc-FAKLNC~**R**	Arg(NO_2)	Boc-FAKLNCR-CO_2H	45%/33%
Boc-LY~**R**	Arg(NO_2)	Boc-LYR-CO_2H	88%/88%
Boc-FKAKNC~**R**	Arg(NO_2)	Boc-FKAKNCR-CO_2H	60%/52%
Boc-DAEFRHDS~**R**	Gly	Boc-DAEFRHDSG-CO_2H	15%/6%[c]
Boc-VQIYPVAA~**R**	Leu	Boc-VQIYPVAAL-CO_2H	60%/36%
Boc-YAVE~**R**	Ser(OBzl)	Boc-YAVES-CO_2H	n.a./40%

a All protected fragments have been characterized by amino acid analysis, mass spectral analysis, and, in most cases, ^1H NMR.

b The first number represents the HPLC-purified yield as calculated from resin-bound peptide. The second number represents the purified yield from the C-terminal resin-bound amino acid. The difference is caused by non-specific loss of material from the resin during the solid-phase synthesis.

c The low yield of this fragment is due to poor recovery off the C-4 RPHPLC column.

In the course of our ongoing total synthesis of the amyloid proteins of Alzheimer's disease[11] we have prepared the resin-bound 24-mer **1** by a series of solid-phase fragment couplings.[6,8] Cleavage of the crude peptide from resin using tetra-n-butylammonium glycinate proceeded in 99% yield (by hydrolysis/amino acid analysis of resin before and after cleavage) to afford *ca.* 150 mg of crude protected peptide. The starting material, the crude cleavage product and the uncleaved product had very similar amino acid analyses after hydrolysis, indicating that selective cleavage of shorter impurities is not occurring. Purification and characterization of the 25-mer product are in progress.



Although the nucleophilic cleavage of protected peptides from the Kaiser oxime resin is not always a high-yield reaction, presumably due to aggregation of resin-bound protected peptide, we have yet to encounter a situation where the salt cleavage is significantly less effective than previously described methods.[2,5,7] In principle, diastereomeric impurities may arise from epimerization of the amino acid salt or the resin-bound amino acid, however, these minor side-products should be easily removed by preparative HPLC. We are in the process of investigating the extent of epimerization at both α positions during the formation of a series of protected dipeptides.

EXPERIMENTAL

Preparation of Amino Acid Tetra-n-butylammonium Salt:

To a suspension of amino acid or side-chain protected amino acid in 1:1 methanol-water (100 ml/30 mmoles amino acid) at 23° was added aqueous tetra-n-butyl-ammonium hydroxide (0.95 equiv, 1.54 M). The suspension cleared within minutes. After stirring for 1h at 23°, methanol was removed by evaporation and the resultant aqueous solution was lyophilized to afford a clear gel. Complete removal of water was not necessary. When stored at -20° this gel beame a waxy solid which could be easily handled.

Cleavage of Resin-Bound Peptide:

Resin-bound peptide (0.2-0.4 mmol/g) was moistened with methylene chloride (to swell the polystyrene beads) and a solution of amino acid tetra-n-butylammonium salt (2-5 equiv) in DMF (0.1 M salt) was added. The reaction mixture was stirred for 24h at 23°, then filtered to remove resin. The resin was washed with methylene chloride, methanol, and DMF and the combined filtrate was concentrated to give a yellow oil. (In the case of extremely hydrophobic fragments, it was necessary to wash resin repeatedly with DMSO. The filtrate was isolated by centrifugation and concentrated.) The oil was dissolved in a minimum amount of DMF (*ca.* 0.5 ml/0.1 mmol peptide) and 10% aqueous citric acid (1.5 ml/0.1 mmol peptide) was added to precipitate protected peptide. The fluffy white precipitate was isolated by centrifugation and washed thoroughly with water. The resultant white powder was directly purified by RPHPLC (C-4, Waters Delta-Pak®, H_2O-CH_3CN, 0.1% acetic acid). Quantitative amino acid analysis of the recovered resin showed that over 90% of the peptide had been cleaved.

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10. In addition to the salts shown in Table I, the salts of 4-methyl-S-benzyl cysteine and alanine have been successfully prepared and used for cleavages. Attempted preparation of the tetra-n-butylammonium salts of benzylglutamate and benzylaspartate have failed due to loss of benzyl ester, presumably via intramolecular anhydride formation.

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12. The single letter code is utilized in the interest of space. The following side-chain protecting groups are utilized in conjunction with the Kaiser oxime resin: K(Lys)-2-chlorobenzyl carbamate, C(Cys)-4- methyl benzyl thioether, R(Arg)-N^{g}-nitro, Y(Tyr)-2,6-dichlorobenzyl ether, D(Asp)-benzyl ester, E(Glu)-benzyl ester, S(Ser)-benzyl ether, H(His)-N^{π}-benzyloxymethyl.

13. The Kaiser oxime resin is now commercially available from Chemical Dynamics Corp., P. O. Box 395, South Plainfield, NJ 07080.

14. Some side-chain protected free amino acids are commercially available while others are prepared by treatment of the Boc-protected, side chain-protected amino acid with trifluoroacetic acid (0°, 1h) followed by repeated lyophilization.

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