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Cyanogen Bromide as a Cleavage Procedure in Solid Phase Peptide Synthesis

Sir:

One disadvantage of the Merrifield solid phase method has been the vigorous conditions used for removal of the completed peptide from the support.[1] Several workers have attempted to solve this problem by attachment of the peptide to the support with a stable linkage which can be made labile at the end of the synthesis to allow facile removal of the product. Despite the attractiveness of this approach, several practical details prevent its routine use, e.g., preparation of the solid support often requires long syntheses,[2,3] or activation of the stable linkage can involve destructive reaction conditions.[4-7]

Cyanogen bromide has several ideal properties for use as a specific agent for the cleavage of peptides and has found wide use in the determination of the amino acid sequence of proteins. Cyanogen bromide was shown to react specifically with methionine, with cleavage of the peptide chain in high yield at the carboxyl end of methionine.[8] Furthermore, the by-products and excess reagent are volatile and readily removed from the product. For these reasons, we propose the use of cyanogen bromide for cleavage of the peptide from the resin at the completion of a solid phase synthesis. In Figure 1 the proposed strategy is outlined in Scheme A, in which the desired peptide is synthesised with methionine added to the C-terminus. At the end of the synthesis, the peptide-resin is treated with cyanogen bromide and the peptide with homoserine at the C-terminus is released from the resin. The homoserine is then removed with carboxypeptidase A yielding the desired peptide. Carboxypeptidase A readily cleaves the peptide bond adjacent to a C-terminal homoserine, but not adjacent to lysine or arginine.[9]

The mild conditions used in this new cleavage procedure should allow the preparation of acid sensitive peptides that cannot be readily obtained by the normal cleavage methods, e.g., HBr–trifluoroacetic acid or anhydrous HF. Also the method has allowed the preparation of protected peptide fragments which can be used in further synthetic operations. Furthermore, the simplicity of the new cleavage procedure allows it to be carried out in the vessel used for the synthesis. The cleavage reaction can now be incorporated into an automated synthetic program.

The cleavage reaction was found to occur more readily if the methionine residue was at least two amino acids removed from the point of attachment to the resin.[10] This result is presumably due to a decrease in steric hindrance at a greater distance from the interior of the resin. Before the desired peptide was synthesized, therefore, the sequence methionylglycylglycine was added to the resin. After the peptide had been assembled using the normal solid phase

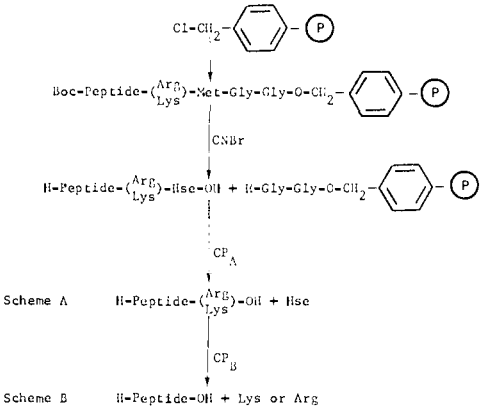

Figure 1. The strategy for the use of cyanogen bromide as a cleavage reagent. Carboxypeptidase A and B are represented by CP_A and CP_B, respectively.

Table I. Yields of Protected Amino Acids and Peptides Prepared by Cyanogen Bromide and Carboxypeptidase A Cleavage

Peptide	CNBr cleavage[a,b] yield mol %	Carboxypeptidase A[b] cleavage yield mol %
Glu(OBzl)-Arg(NO_2)[c,d]	90	83
Arg(NO_2)-Arg(NO_2)[c,e]	65	90
H-Ser(Bzl)-Thr(Bzl)-Ile-Glu(OBzl)-Glu(OBzl)-Arg(NO_2)-OH[c]	55	80
Arg(NO_2)[c,f]	95	98
Ala-Lys(Z)[c,g]	64	95
[a]Glu(OBzl)[c,h]	98	98

[a] The yield is based on the amount of methionine present in the peptide–resin. The peptide–resin was hydrolyzed with HCl–propionic acid at 130° for 2 hr,[12] and the methionine was quantitated by amino acid analysis. [b] The amount of peptide was determined by amino acid analysis.[16] [c] The products had amino acid analysis which were consistent with the structures shown. [d] Mp 208–211° dec. [e] This peptide was identified by conversion to Arg-Arg, mp 287–289° (lit. mp 289–290°).[17] [f] Mp 250–253° (lit. mp 255° dec).[18] [g] The hydrogenation product Ala-Lys was identified by comparison with an authentic sample supplied by Bachem. [h] Mp 171–173° (lit. mp 169–170°).[19]

method,[11] the cleavage was carried out by the addition of cyanogen bromide dissolved in propionic acid. The choice of propionic acid was promoted by its use, in combination with hydrochloric acid, for hydrolysis of peptide–resin samples for amino acid analysis.[12] Propionic acid was found to be superior to acetic acid, and formic acid for the cleavage reaction. A large excess of cyanogen bromide (50-fold) and long reaction times (20–40 hr) were used to ensure a high cleavage yield.[13]

As is shown in Table I, several peptides have been cleaved from the resin in high yield. In each case, the crude products had satisfactory amino acid analyses and showed only one major component on gel filtration, high voltage electrophoresis, and thin layer chromatography (TLC). Treatment of these peptides with carboxypeptidase A released only homoserine, and the desired peptide was then isolated by gel filtration. The resistance to cleavage of the protected forms of Arg and Lys may possibly be explained by retained charge (Arg), and by steric interaction between the hydrophobic pocket of carboxypepidase and the increased bulk of the protected side chain (Lys).

The BOC group was found to be labile in the presence of propionic acid and cyanogen bromide, but side chain protecting groups such as the γ-benzyl ester of glutamic acid, carbobenzoxy group of lysine, and N^G-nitro group of arginine are all retained. Although the cleaved peptide had lost the BOC group, either the NH_2- or -COOH function can

be readily blocked yielding a protected peptide suitable for further synthetic operations.

The preparation of the peptide γ-benzylglutaminyl-N^G-nitroarginine is described here to serve as an example of the reaction conditions used in the cleavage procedure. A solution of cyanogen bromide (1.85 g, 18.5 mmol) in propionic acid (7 ml) was added to the peptide-resin (0.37 mmol) and the mixture was shaken at room temperature for 24 hr. The propionic acid solution was collected and the resin was washed with the following solvents (each with three washes of 10 ml), trifluoroacetic acid, ethanol, and chloroform. The cleaved peptide (0.34 mmol) was then obtained by removal of solvent and excess reagent under reduced pressure. After purification by gel filtration on a polyacrylamide column (P_2), the peptide (0.28 mmol; amino acid analysis: $Arg_{1.0}$, Glu and $Hse_{1.8}$ Hse $lactone_{0.18}$) was digested with carboxypeptidase A (0.2 mg) dissolved in 5 ml of 0.2 *M* *N*-ethylmorpholine acetate, pH 8.5 at 31°. The digestion was followed by high voltage electrophoresis and showed that the release of homoserine with conversion of tripeptide into the desired dipeptide was complete after 2 hr. No other products could be detected even after a 24-hr digestion. The dipeptide (0.3 mmol) was purified by gel filtration and was shown to be homogeneous by high voltage electrophoresis at pH 2.1 and 5.6 (R_f 0.48 and 1.0, respectively, Gly = R_f 1.0), TLC (R_f 0.81, 1-butanol-acetic acid-diethylamine-H_2O (10:10:2:5), 0.58, 1-butanol-acetic acid-water (4:1:1)), and amino acid analysis (Arg, 1.0; Glu, 0.95).

These results show that a cyanogen bromide cleavage reaction allows the convenient preparation of peptides protected by many of the more stable groups such as carbobenzoxy, nitro, benzyl, etc., which should prove very useful in the semisynthesis of proteins.[14] At present the cleavage method is limited to peptides with C-terminal amino acids which are not readily cleaved by carboxypep idase A. It is anticipated that peptides containing methionine can be synthesized by this approach by incorporation of the methionine residue protected as the sulfoxide which is resistant to cyanogen bromide. Current investigations of Scheme B indicate that a peptide containing any C-terminal amino acid can be isolated in a homogeneous state by utilizing the marked specificity of carboxypeptidase B for basic residues (ref 15, see Scheme B, Figure 1).

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Heterogenized Rhodium Chloride Catalyst for Ethylene Dimerization

Sir:

A new catalyst system, rhodium chloride supported on silica gel, with high activity for ethylene dimerization has been found. As is well known, rhodium chloride is an active homogeneous catalyst for ethylene dimerization.[1-3] The new heterogeneous catalyst is more active than homogeneous rhodium chloride. Here, some characteristics of the catalyst are reported.

Carrier (5 g) (calcined at 300°C for 3 hr in vacuo before use) was impregnated with 20 ml of a methanol solution of rhodium trichloride trihydrate (rhodium content = 25%) (catalysts 1 and 2 in Table I, concentration 0.019 mol/l., catalysts 3-6, concentration 0.025 mol/l., respectively) for 2.5 hr at room temperature, followed by evacuation at 35°C for 6 hr without decantation. The solid was washed with 300 ml of methanol on a glass filter, and then evacuated at 35°C for 6 hr. The rhodium content of the catalyst was found by atomic absorption spectrophotometric analysis of rhodium from catalyst dissolved in hydrofluoric acid. Ethylene dimerization was carried out in a fixed bed type glass-made apparatus (200 ml). Reacting gas was circulated through the catalyst bed. A small amount of reacting gas was collected periodically through a sampling stopcock, and analyzed by gas chromatography. Propene hydrogenation was also carried out in the same apparatus with an equimolar mixture of propene and hydrogen at 25°C.

Preliminary experiments showed that hydrogen chloride remarkably enhanced the catalytic activity for ethylene dimerization, as is known in the case of homogeneous catalysis. In the absence of hydrogen chloride, the initial rate of butene formation with catalyst 1 was pretty low, 0.1×10^{-4} mol/(g-catalyst min). Thus, the dimerization was carried out in the presence of hydrogen chloride (2×10^{-4} mol). The activity was independent of hydrogen chloride amount as long as it exceeds 0.6×10^{-4} mol. 1-Butene was formed predominantly at the initial stage. The molar fraction of 1-butene decreases with time as it isomerizes to 2-butene. The molar ratio of *trans*- to *cis*-2-butene was 2.9. The rhodium content of the catalysts and the initial rate of butene formation are summarized in Table I with those of the other rhodium catalysts reported. Under the same conditions of catalyst preparation, the amount of rhodium supported on the carriers depended on the sort of carrier and increased in the order, silica gel (catalyst 3) < silica-alumina (catalysts 4,5) < alumina (catalyst 6). However, the dimerization activity per unit weight of catalyst increased in the reverse order, silica gel > silica-alumina > alumina. The catalytic activities per unit amount of rhodium are also summarized and