A New Reagent for the Cleavage of Fully Protected Peptides synthesised on 2-Chlorotrityl Chloride Resin

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A mixture of hexafluoroisopropanol–dichloromethane (1 : 4 v/v) acts as a fast, effective and convenient reagent for cleaving protected peptide fragments with a minimal amount of racemization from 2-chlorotrityl chloride resin.

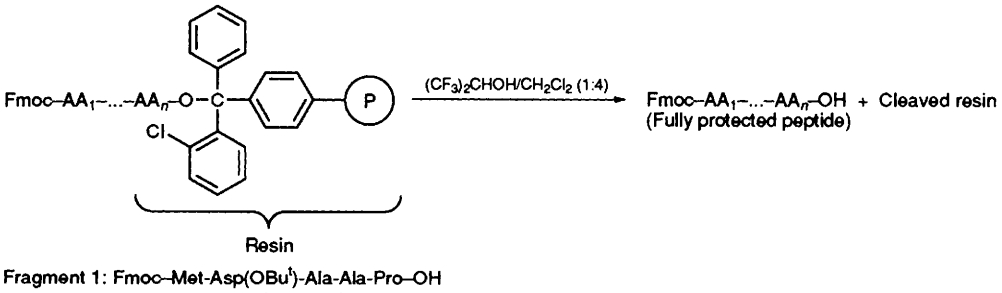
The development of the very acid-labile 2-chlorotrityl chloride resin for use in solid-phase peptide[1,2] and protein[3] synthesis has shown many advantages, in particular regarding segment condensation procedures that are generally carried out with Pro or Gly at the C-terminus to minimize or avoid racemization. The best results in terms of cleavage rate, yield and selectivity in the presence of protecting groups of the *tert*-butyl and Trt type, differing in acid lability, have been reported with mixtures of glacial acetic acid (AcOH)–trifluoroethanol (TFE)–dichloromethane (DCM) of different compositions.[4]

Nonpurified, N-terminal and side-chain protected peptide fragments containing 3–10 amino acid residues in their peptide chain are often obtained by this cleavage method at greater than 95% purity. Therefore, such peptides in principle might be applied without further purification for the preparation of cyclic peptides or for fragment condensation reactions. A successful preparation of large or cyclic peptides by this method requires the strict exclusion of carboxylic acids originating from the deprotection medium. The cleavage conditions mentioned above, however, exhibit the distinct disadvantage that traces of remaining carboxylic acids have to be removed by applying extended work-up procedures. In order to overcome this disadvantage a new, fast and very simple cleavage method has been developed which allows one to use extremely mild conditions. This method provides protected peptides in high yield and excellent purity which can be used for segment condensation reactions without the necessity of removing carboxylic acids beforehand.

The peptide fragments investigated (Fig. 1), which were synthesized by the mild, well-established Fmoc/*tert*-butyl method and on 2-chlorotrityl chloride resin are cleaved as illustrative examples by hexafluoroisopropanol (HFIP)–dichloromethane (1 : 4 v/v). Under these conditions, HFIP may act as a very weak acid, but is sufficiently strong to facilitate the solvolytic cleavage, though not strong enough to cause deprotection of the peptide under the chosen conditions. Only in the special case of the very acid-labile Trt side-chain protection of Ser, Thr and N^{im} of His, minor amounts of deprotected peptides are observable upon cleaving, which depend on the chosen reaction time. It is thus advisable to omit this type of protection for the indicated amino acids. The HFIP–DCM solvent mixture exhibits superior properties, dissolving even peptides with preferentially hydrophobic properties and can be easily evaporated *in vacuo*. The cleavage products are characterized by fast atom bombardment mass spectrometry (FAB-MS).† The corresponding results clearly indicate that the peptides remain protected. The cleavage performed at room temperature in the presence of atmospheric oxygen is quantitative after 3 min as indicated by HPLC and gravimetric product determinations. During cleavage the resin shows a typical red-violet or red colour which sometimes disappears later. Besides some Met oxidation, detailed HPLC studies of the crude HFIP–DCM cleaved products (Fig. 2) indicate that the protected peptides are characterized by a high degree of purity already after evaporation of the cleaving medium. In addition, the peptides obtained according to this new method are indistinguishable from those split by acetic acid treatment according to the standard procedure hitherto applied.[4]

Although no racemization as a consequence of the very mild splitting procedure reported here is expected, a detailed test has been carried out on model peptides with C-terminal His and Phe which tend to exhibit strong racemization. The peptides H–Leu-His(Trt)–OH **A** and the reference sample H–Leu-D-His(Trt)–OH **B** as well as H–Leu-Phe–OH **C** and H–Leu-D-Phe–OH **D**, accordingly, are synthesized by employing the standard method mentioned above, and are obtained after HFIP–DCM cleavage. The amount of racemization is analysed by HPLC diastereoisomer separation‡ of the product and determined from the corresponding peak areas. The racemization degree of both C-terminal amino acids is found to be less than 0.1%. According to these results, no significant racemization of the amino acids tested is caused by this new cleavage procedure.

In summary, these results clearly show that HFIP–DCM mixtures are ideally suited for cleaving protected peptide fragments from 2-chlorotrityl resin and represent a development of practical importance in solid-phase peptide synthesis.



Fragment 1: Fmoc–Met-Asp(OBu^t)-Ala-Ala-Pro–OH
Fragment 2: Fmoc–Gly-Tyr(Bu^t)-Tyr(Bu^t)-Leu-Ile-Gln(Trt)-Met-Tyr(Bu^t)-Ile-Pro–OH
Fragment 3: Fmoc–Ser(Trt)-Asp(OBu^t)-Ala-Ala–OH
Fragment 4: Fmoc–Leu-Gly-Cys(Trt)-Asn-Ser(Trt)-Phe-Arg(Pmc)–OH
Fragment 5: Fmoc–Ile-Thr(Bu^t)-Thr(Bu^t)-Lys(Boc)-Asp(OBu^t)-Leu-Lys(Boc)-Glu(OBu^t)-Lys(Boc)–OH

Fig. 1 Schematic reaction scheme and synthesized peptide fragments (Fmoc = 9-fluorenylmethoxycarbonyl, Trt = triphenylmethyl, Boc = *tert*-butyloxycarbonyl, Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl)

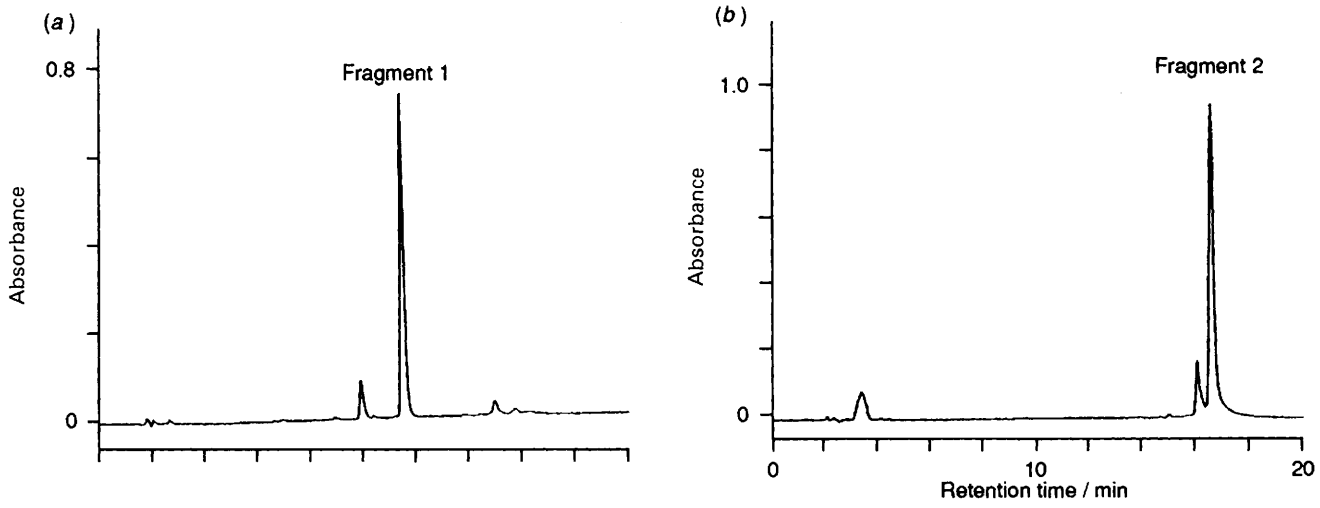


Fig. 2 Analytical HPLC chromatogram of crude fragments 1(*a*) and 2(*b*); column, Lichrospher 100 CN (5 μm, 4 × 250 mm, Merck); solvents, **A** 0.01% TFA in water, **B** 0.01% TFA in MeCN; flow rate, 1.5 ml min^{-1}; 1 min isocratic at 10% **B** followed by a linear gradient from 10% **B** to 90% **B** in 20 min; absorption detector wavelength = 215 nm. HPLC separation of crude fragments 1 and 2 and subsequent FAB-MS investigation of the fractions indicated that the intense main peaks can be assigned to the corresponding pure fragments (data *cf.* text) whereas the low intensity side peaks at 9.96 min in (*a*) (*m/z* 820.5 $[M + Na]^+$, 798.5 $[M + H]^+$) and at 16.09 min (*b*) (*m/z* 1932 $[M + Na]^+$) are assigned to the oxidized form of Met in the corresponding peptides.

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Footnotes

† Fragment 1: *m/z* 804.4 $[M + Na]^+$ and 782.4 $[M + H]^+$. Fragment 2: *m/z* 1916 $[M + Na]^+$. Fragment 3: *m/z* 889 $[M + Li]^+$. Fragment 4: *m/z* 1791 $[M + Na]^+$ and 1769 $[M + H]^+$. Fragment 5 according to ref. 3.

‡ Isocratic elution from a prepacked analytical Hibar column [LiChrospher 100 RP-18 (5 μm), 250 × 4 mm] with H_2O–MeCN–MeOH (67 : 28 : 5, v/v/v) containing 0.1% TFA and 0.5 mmol dm^{-3} triethylamine exhibits retention times for **A** and **B** of 22.8 and 28.1 min respectively (1.5 ml min^{-1}, 205 nm) and with H_2O–MeCN (67 : 33, v/v) containing 0.1% TFA of 4.3 and 5.9 min (1 ml min^{-1}, 218 nm) for **C** and **D** respectively.

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