Improved Deprotection in Solid Phase Peptide Synthesis: Deprotection of N^{i}-Formyl-tryptophan to Tryptophan in Low Concentrations of HF in Me_2S–*p*-Thiocresol Mixtures

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The N^{i}-formyl protecting group of tryptophan in solid phase peptide synthesis was found to be removed efficiently and quantitatively by a new reagent, HF–Me_2S–*p*-thiocresol–*p*-cresol (25 : 65 : 5 : 5, v/v/v/v) without detectable side reaction.

During repetitive peptide synthesis, the sequential mild acid treatments to remove the α-amino-protecting group often lead to electrophilic alkylation of the unprotected tryptophan residue.[1,2] This alkylation has recently been recognized to be more serious than that found in the final strong-acid cleavage step,[3] *e.g.*, the acidolytic removal of the N^{α}-t-butoxycarbonyl group by CF_3CO_2H results in *ca.* 30% of t-butylated tryptophan side products.

Although the seriousness of the alkylation can be reduced by the presence of appropriate scavengers during the acid treatment, it is best to prevent the alkylation side reaction by protecting the indole moiety against electrophiles during the synthesis. This has been achieved by using the indole-formyl (N^{1}-For) protecting group.[4,5] N^{1}-Formyl-tryptophan is stable to strong acid, including HF and sulphonic acids, and is usually removed by nucleophiles or aqueous base after strong-acid deprotection of side chain protecting groups.

Recently, we have reported an improved HF deprotection method for the cleavage of benzyl alcohol-derived protecting groups by an S_N2 mechanism, in which HF is maintained at low concentration (25% by volume) and is complexed with Me_2S (75% by volume) in a 1 : 1 molar ratio.[6–8] The low concentration HF–Me_2S (1 : 3, v/v) deprotecting reagent removes the chronic problem of alkylation of nucleophilic side chains of amino-acid residues such as tyrosine and methionine. The low acidity function of this reagent also prevents dehydration of the side chain carboxy-groups of glutamic and aspartic acids. Here, we report a convenient procedure for the deprotection of the N^{1}-formyl group of Trp(For) with a low concentration of HF in Me_2S by the addition of a thiol to the mixture. In this way it is possible to remove in a single manipulation the N^{1}-formyl group as well as the commonly used benzyl protecting groups from synthetic peptides.

In our investigation of 'deactivated' high HF-concentration solution (HF *ca.* 90%) with nucleophilic scavengers to reduce HF catalysed side reactions, we found that when 10% *p*-thiocresol alone or in mixture with *p*-cresol was added as a scavenger, Trp(For) was partially deprotected to Trp.

Table 1. Deprotection of N^{i}-formyl-tryptophan and N^{i}-formyl-tryptophan-containing peptides in HF–Me_2S–*p*-thiocresol mixtures.

	Composition (volume %)[a]				Products (mol%)[b]		
Sample	HF	Me_2S	Thiol	*p*-Cresol	Trp	Trp(For)	Side product
Boc-Trp (For)	90	5	0	5	2	98	0
	90	0	5[c]	5	56	44	0
	90	0	5[d]	5	29	71	0
	90	0	5[e]	5	36	32	32
	75	15	5[e]	5	78	19	3
	25	65	5[e]	5	85	15	0
	25[f]	65	5[e]	5	95	5	0
Boc-Lys(Z)-Trp(For)-Lys(Z)-OCH_2- resin[g]	25	65	5[e]	5	100	0	0
Boc-Gly-Trp(For)-Met(O)-Asp(OBzl)-Phe-NH-resin[g]	25	65	5[e]	5	100	0	0

[a] At 0° C, 1 h unless otherwise specified. [b] Analysed by ion-exchange chromatography. [c] Ethanethiol. [d] Ethanedithiol. [e] *p*-Thiocresol. [f] Reaction carried out at 0 °C for 2 h. [g] Peptide was first treated with low HF concentration at 0 °C for 2 h and then HF concentration was raised to 90% and the reaction continued for 1 h at 0 °C to remove peptide from resin.

Similarly, other thiols such as thiophenol, ethanethiol, and ethanedithiol also led to some deprotection (Table 1). When HF was maintained at 90% by volume and 5—10% of aromatic thiol was added to the strong acid mixture, several side products were also observed; these are probably thiolytic addition products of the Trp residue. With free, unprotected Trp no side reactions were observed. Alkyl thiols such as ethanedithiol were found to polymerize extensively in HF and gave poor recovery yields of Trp-containing peptides. This was also found when CF_3SO_3H–CF_3CO_2H was used as the acid. Me_2S or MeSPh were not effective as deprotecting agents for the N^{i}-formyl group. However, when 2.5—10% of thiol was added to the low concentration HF–Me_2S mixture, near quantitative removal of N^{i}-formyl from Trp(For) was observed (Table 1). Furthermore the side products were not observed, which further confirmed that the thiol addition side reaction with Trp(For) occurred only in high HF concentrations. Thus, when Trp(For) was completely deprotected in the low HF–Me_2S–thiol mixture, further exposure to high concentrations of HF in the presence of an aromatic thiol did not produce this side reaction. The best thiolytic deprotecting mixture was found to be HF–Me_2S–*p*-thiocresol–*p*-cresol (25:65:5:5, v/v/v/v). A phenolic compound such as *p*-cresol was added to promote resin swelling and also to act as a scavenger.[6] In this mixture, Trp(For) or Trp(For)-containing peptides were quantitatively converted into Trp or Trp-containing peptides. No t-butylated tryptophan products were observed when a t-butyl cation source such as Boc-Trp(For) was present.

The difference in the mechanism of thiolytic deprotection of Trp(For) in high and low concentrations of HF is not immediately clear, however, this observation is consistent with our other results which show that the deprotection of Ser(Bzl),[6] reduction of Met(O),[7] and the deprotection of Tyr(Bzl)[8] are all dependent on the HF concentration and the reaction mechanism. At high HF concentrations weak bases such as Me_2S are largely protonated and therefore are ineffective as nucleophiles. Thus, at high HF–Me_2S ratio (9:1, v/v) the deprotection of benzyl alcohol-derived protecting groups is by an S_N1 mechanism and is rapid, but at low HF–Me_2S (1:3, v/v) the reaction is largely by an S_N2 mechanism and is slower.[6] In addition, methionine sulphoxide is stable at the high HF–Me_2S ratio but is reduced to methionine at low HF–Me_2S (1:3, v/v).[7] Similarly, the low HF–Me_2S–thiol deprotection mixture, where the HF was maintained between 20 and 40%, was found to be of near optimal acidity for the removal of the N^{i}-formyl group and to prevent the formation of side products (Table 1). With HF concentrations above or below this range, either the deprotection was too slow or side reactions occurred. When the weaker CF_3CO_2H was substituted for HF, the deprotection of Trp(For) was found to be slow ($t_{1/2}$ >24 h).

Using our new deprotecting method, we have synthesized the *C*-terminal pentagastrin amide (H-Gly-Trp-Met-Asp-Phe-NH_2) on a multidetachable benzhydrylamine resin[9] by the solid phase method. This difficult sequence entails the alkylation of Trp and Met, aspartimide formation, and low cleavage from the amide resin. The fully protected peptide-resin in which Trp was incorporated as Trp(For) and Met as Met(O) was deprotected first by the low concentration HF solution (HF–Me_2S–*p*-thiocresol–*p*-cresol, 25:65:5:5, v/v/v/v) for 2 h at 0 °C, followed by further treatment in high HF at 0 °C for 1 h, in which the HF concentration was raised to 90%. This provided pentagastrin amide in 90% yield. Also, in this single procedure the N^{i}-formyl on tryptophan was removed and the sulphoxide of methionine was reduced. 95% of the crude peptide emerged in reverse-phase h.p.l.c. as a single major peak of pure pentagastrin amide. By comparison, the same peptide-resin treated with the usual condition of HF–anisole (9:1, v/v, 1 h, 0 °C) gave a similar cleavage yield but, after an aqueous basic deformylation procedure and thiolytic reduction of sulphoxide, gave several major peaks in the same chromatographic analysis. Thus, the low concentration HF–Me_2S–thiol–cresol deprotection procedure for this Trp(For)-containing peptide was a significant improvement over the strong acid procedure.

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