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One-pot full peptide deprotection in Fmoc-based solid-phase peptide synthesis: methionine sulfoxide reduction with Bu_4NBr

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Abstract—The reduction of methionine sulfoxide with Bu_4NBr in TFA is reported. The use of this reagent in conjunction with Fmoc chemistry-based acidolytic cocktails and the short reaction times that are needed for sulfoxide reduction (5 min) are the main advantages of this method, which is compatible with peptides containing aromatic amino acids. Cysteine is also stable under these conditions although, if desired, simultaneous disulfide formation can be achieved in the absence of thiols.

Acid-promoted full peptide deprotection constitutes one of the key steps in solid-phase peptide synthesis.[1] In general, protecting groups are removed under SN_1 and/or SN_2 conditions, but a redox process is required for protection of Met as its sulfoxide.[2] While Me_2S[3] has proved to be efficient for sulfoxide reduction under the acidic conditions that are used in the Boc/Bzl strategy (HF),[4] a general protocol for Fmoc/*t*Bu chemistry (TFA) has yet to be established. In fact, the deprotection of Met has usually been carried out after isolation or even purification of the crude peptide resulting from the acidolytic treatment.[5] Our interest in seeking a suitable methodology for the reduction of Met(O) that is compatible with the Fmoc strategy prompted us to explore the use of halides as reducing agents under mild conditions. Thus, we have recently reported a study on the use of NH_4I in TFA for sulfoxide reduction.[6] We now present the Bu_4NBr/TFA system as a useful reagent for the deprotection of Met under peptide cleavage conditions.

Reduction of Met(O) with halides in peptides has been restricted to the use of iodides[7] and TMSBr.[8] Experimental results support a general mechanism that involves nucleophilic attack of the halide at the sulfur atom of the protonated sulfoxide to afford a tetracovalent sulfur intermediate, which undergoes release of water after further protonation to give a halosulfonium ion as the rate determining process. Sulfide is formed from these species with the obtention of I_2 as a by-product.[9] A similar reaction pathway has been proposed for TMSBr, where bromide is formed in situ through trimethylsililation of the sulfoxide.[8]

Solid-phase peptide synthesis based on the Fmoc strategy involves, for full peptide deprotection, the use of TFA together with several chemicals to trap the carbocations that are produced under these acidic conditions. A variety of cleavage cocktails have been reported in the literature, being among the most used additives thiols, sulfides, phenyl ethers, silanes or water.[10a] Very recently, the use of NH_4I to avoid Met oxidation during the acidolytic treatment has been reported.[10b] The fact that bromine is obtained as a by-product of the redox reaction when bromide is used as the reducing agent,[9] prompted us to use scavengers to trap the halogen in order to avoid undesired reactions with aromatic amino acids or Cys oxidation. In this context, the use of thioanisole and 1,2-ethanedithiol as bromine scavengers has been reported in the literature.[8a,b] According to these reports, reagent R (TFA 90%, thioanisole 5%, anisole 2%, 1,2-ethanedithiol 3%),[11] one of the cocktails of wide ranging applicability, seemed to fit the requirements discussed above.

The study was performed with the peptide sequence Gly-Ile-Xxx-Pro-Leu-Abu-Met in order to explore the scope of application of the method (Xxx = Gly, Phe, His, Tyr, Trp and Cys). Peptide chains were grown using the base-labile Fmoc α-amino protecting group and the AB anchoring linkage (Scheme 1). Side-chain protection for trifunctional amino acids was achieved with Trt (Cys and His), *t*Bu (Tyr), Boc (Trp) and sulfoxide (Met). A commercially available MBHA resin was used as the polymeric support. Fmoc amino acids

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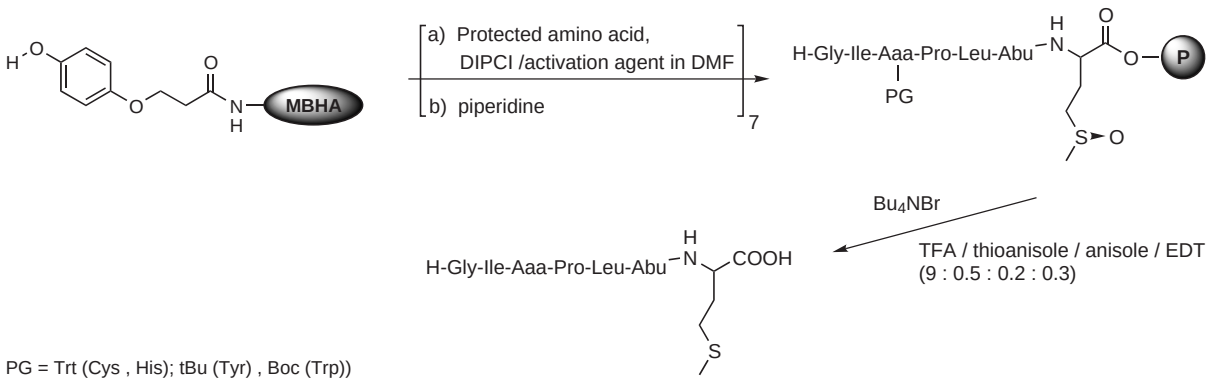

Scheme 1.

were coupled manually using DIPCDI/HOBt activation in DMF with the exception of the first amino acid, which was anchored with DIPCDI/DMAP.[12]

In general, 5–30 mg batches of the peptidyl resins were suspended in 200–500 μl of reagent R for 2 h at room temperature after removal of the N-terminal Fmoc group with piperidine. The crude peptides were precipitated with EtO*t*Bu, filtered and lyophilized in aqueous 10% AcOH. The products were characterized by HPLC/ESI-MS, ESI-MS and MALDI/TOF-MS. Treatment of the peptidyl resins with reagent R in the absence of reducing agent afforded the corresponding peptides containing Met as its sulfoxide. The resulting chromatographic profiles showed the desired peptide without any evidence of sulfoxide reduction.[13]

Reductions were performed under conditions similar to those described above (Scheme 1). In a control experiment, the peptide containing Gly resin was treated with reagent R for 2 h at room temperature when an excess of Bu_4NBr (30 equiv.) was added to the suspension. HPLC analysis of the reaction mixture did not show the oxidized peptide after 5 min and a new product, with a longer retention time, was detected instead. This new product was found by HPLC/ES-MS analysis to be the desired reduced peptide. It is interesting to note that the addition of Bu_4NBr just before the end of the cleavage treatment (5 min) allows the exposure time of the reducing agent to the peptide to be minimized and thus prevents undesired processes. When this experimental protocol was used with the other peptide resins, the corresponding reduced products were obtained, and these proved to be fairly stable even after 2 h under the reducing conditions.[14] Reduction was also achieved with NH_4I and TMSBr, but Bu_4NBr was more soluble in TFA than the former and the reaction crudes obtained with Bu_4NBr were cleaner than those afforded by the latter.

It should be noted that special attention must be paid to the Cys-containing peptide. For example, treatment of the corresponding peptide resin with Bu_4NBr under the conditions mentioned above (see footnote in Table 1) afforded the desired Met unprotected peptide without any detectable disulfide formation when reagent R was used. However, the homodimer was obtained almost quantitatively when the reduction was carried out in TFA/anisole (95:5) (entry a, compare both cocktails of cleavage).[15] The efficiency of EDT to prevent Cys oxidation during the Met(O) deprotection step could explain these results. On the other hand, while TMSBr behaved in a similar way than Bu_4NBr when used in the absence of EDT (entry b, Table 1), interestingly, iodide (NH_4I) afforded a different result. Thus, treatment for a short period of time with this reducing agent in TFA/anisole (95:5) (5 min) yielded mixtures of both products (monomer and dimer), whereas longer reaction times were required to obtain the dimer in high yields (2 h) (entry c, Table 1), which indicates a higher efficiency of bromine as an oxidant under these conditions in comparison to iodine.

In summary, we have presented Bu_4NBr as an efficient reagent for the reduction of Met(O) to be used in solid-phase peptide synthesis involving Fmoc chemistry. A novel methodology that involves the deprotection of this amino acid and peptide cleavage from the resin in a one-pot process has been developed. The reduction is carried out in a short period of time and is compatible with sensitive amino acids such as Phe, Tyr, His and Trp. Cys is also stable under these conditions although, if desired, disulfide formation can be achieved in the absence of thiol. The high solubility in TFA and the full compatibility with the scavengers used as additives in the cleavage cocktail are other advantages of Bu_4NBr over NH_4I and TMSBr, respectively. Bu_4NBr could also be useful for the reduction of other sulfoxides under the conditions described above.

Table 1. Monomer/dimer ratio resulting from treatment of the Cys containing peptide resin with different reducing agents under peptide cleavage conditions[a]

		Cocktail of cleavage	
Entry	Reducing agents	Reagent R	TFA/anisole (95:5)
a	Bu_4NBr	9.3:0.7	0.4:9.6
b	TMSBr	9.9:0.1	0.6:9.4
c	NH_4I	9.6:0.4	6.7:3.3 (0.1: 9.9[b])

[a](i) Cocktail of cleavage, 2 h; (ii) reducing agent, 5 min ([b]2 h). The ratios were determined by HPLC.

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3. AB, 3-(4-hydroxy-methylphenoxy)propionic acid; Abu, 2-aminobutyric acid; Boc, *tert*-butoxycarbonyl; Bu_4NBr, tetrabutylammonium bromide; Bzl, benzyl; DIPCDI, *N*,*N*′-diisopropyl-carbodiimide; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide, EDT, 1,2-ethanedithiol; ESI–MS, electrospray ionization–mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydro-xybenzotriazole; HPLC, high performance liquid chromatography; MALDI/TOF, matrix-assisted laser desorption/ionization time-of-flight; MBHA, 4-methylbenzhydrylamine (polystyrene resin); Me_2S, dimethyl sulfide; Met(O), sulfoxide on methionine thioether; *t*Bu, *tert*butyl; TFA, trifluoroacetic acid; TMSBr, trimethylsilylbromide; Trt, trityl.
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13. Aaa = Gly, HPLC: rt, 11.9 min; MS *m*/*z* 689.1 $[M+1]^+$, $C_{30}H_{53}N_7O_9S$ requires 687.9. Aaa = Phe, HPLC: rt, 14.2 min; MALDI/TOF-MS *m*/*z* 779.1 $[M+1]^+$, $C_{37}H_{59}N_7O_9S$ requires 778.0. Aaa = Tyr, HPLC: rt, 11.5 min; MS *m*/*z* 794.6 $[M+1]^+$, $C_{37}H_{59}N_7O_{10}S$ requires 794.0. Aaa = His, HPLC: rt, 9.4 min; MS *m*/*z* 769.0 $[M+1]^+$, $C_{34}H_{57}N_9O_8S$ requires 768.0. Aaa = Trp, HPLC: rt, 15.1 min; MS *m*/*z* 817.5 $[M+1]^+$, $C_{39}H_{60}N_8O_8S$ requires 817.0. Aaa = Cys, HPLC: rt, 12.0 min; MS *m*/*z* 734.8 $[M+1]^+$, $C_{31}H_{55}N_7O_8S_2$ requires 733.4. HPLC was carried out with a Nucleosil C_{18} reverse-phase column (35×0.5 cm, 5 μm), using a flow rate of 1 mL/min (A: H_2O, 0.045% of TFA; B: CH_3CN, 0.035% of TFA; 10–100% of B over 30 min; detection at 220 nm). Mass spectra were performed in a MALDI/TOF apparatus (CH_3CN/H_2O (1:1) with 0.1% TFA; matrix, 2,5-dihydroxybenzoic acid).
14. Aaa = Gly, HPLC: rt, 14.2 min; MS *m*/*z* 673.1 $[M+1]^+$, $C_{30}H_{53}N_7O_8S$ requires 671.9. Aaa = Phe, HPLC: rt, 16.2 min; MS *m*/*z* 763.1 $[M+1]^+$, $C_{37}H_{59}N_7O_8S$ requires 762.0. Aaa = Tyr, HPLC: rt, 13.6 min; MS *m*/*z* 779.7 $[M+1]^+$, $C_{37}H_{59}N_7O_9S$ requires 778.0. Aaa = His, HPLC: rt, 11.6 min; MS *m*/*z* 752.9 $[M+1]^+$, $C_{34}H_{57}N_9O_8S$ requires 752.0. Aaa = Trp, HPLC: rt, 17.0 min; MS *m*/*z* 802.1 $[M+1]^+$, $C_{39}H_{60}N_8O_8S$ requires 801.0. Aaa = Cys, HPLC: rt, 14.2 min; MS *m*/*z* 718.5 $[M+1]^+$, $C_{31}H_{55}N_7O_8S_2$ requires 717.4. See Ref. 13 for experimental conditions.
15. Dimer, HPLC: rt, 15.6 min; MS *m*/*z* 1434.4 $[M+1]^+$, $C_{60}H_{104}N_{14}O_{18}S_2$ requires 1432.8. See Ref. 13 for experimental conditions.