Notes

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Studies on Peptides. CXXX.[1,2] Convenient Procedure for the Reduction of Methionine Sulfoxide

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Selenophenol was found to be an effective reducing reagent for Met(O) in protected peptides. Trimethylphenylthiosilane and its combination with tetrabutylammonium bromide were also found to have a powerful ability to reduce Met(O).

Keywords——Met(O) reduction; selenophenol; trimethylphenylthiosilane; catalyst tetrabutylammonium bromide; thiophenol reduction

The sulfoxide of methionine, Met(O),[3] is a useful compound for peptide synthesis, particularly for the synthesis of large peptides, in order to avoid *S*-alkylation during the N^{α}-deprotection and partial air-oxidation involved in the manipulation. Usually, in the final step of the peptide synthesis, Met(O), after deprotection, is reduced back to methionine by treatment with thiol compounds, such as mercaptoethanol or dithiothreitol.[4] Houghten and Li[5] recommended the use of *N*-methylmercaptoacetamide for this purpose. Recently, it has been shown that thioanisole mediated deprotection with TFMSA[6] or HF deprotection in the presence of 2-mercaptopyridine[7] or dimethylsulfide[8] can also partially reduce Met(O). However, after deprotection, thiol treatment is still required to complete the reduction. From a practical standpoint, incubation of deprotected peptides containing Met(O) in water with any thiol compounds usually takes a rather long time, for example 12 to 18 h at 37 °C.

In order to eliminate such a time-consuming reduction step, we considered the reduction of Met(O) before deprotection, since the thioanisole-mediated deprotection with TFMSA which we currently employ has adequate ability to prevent *S*-alkylation and air oxidation of the Met residue, even if it is unprotected. We found that selenophenol is a more effective reducing reagent of Met(O) than thiophenol.[9] Z(OMe)–Met(O)–OH was quantitatively reduced back to Z(OMe)–Met–OH by incubation with selenophenol (10 eq) at 40 °C within 3 h, while thiophenol required a higher temperature (90 °C, 3 h).

Trimethylphenylthiosilane was reported to deoxygenate alkyl phenyl sulfoxide slowly but smoothly in the presence of a catalyst, tetrabutylammonium bromide.[10] This reagent was found to have a powerful ability to reduce Met(O), even in the absence of a catalyst. Reduction of Z(OMe)–Met(O)–OH was completed within 15 min at room temperature. If peptides do not possess Ser, Thr and Tyr, which suffer trimethylsilylation at their functional group, this reagent should be an attractive reducing reagent for protected peptides containing Met(O).

Two model peptides, Z(OMe)–Leu–Met(O)–NH_2,[11] and Z(OMe)–Phe–Met(O)–NH_2,[1] were smoothly reduced back to the parent dipeptides in DMF by incubation with seleno-

phenol. For practical purposes, this reagent may be applied to the synthesis of more complex Met-containing peptides and to the reduction of *S*-substituted cysteine sulfoxides as well.

In 1982, Bodanszky and Bednarek[12] suggested the protection of the thioether of methionine with methyl *p*-toluenesulfonate. The product can be smoothly converted, before deprotection, to Met residues by thiolysis in organic solvent. However, *S*-sulfonium compounds are known to be decomposed even by treatment with methanol. Thus, as mentioned by the authors, the possible usefulness of this approach in peptide synthesis remains to be established.

Experimental

Thin-layer chromatography (TLC) was performed on silica gel (Kieselgel G, Merck) using $CHCl_3$–MeOH–AcOH (9 : 1 : 0.5).

Reduction of Z(OMe)–Met(O)–OH in DMF——Z(OMe)–Met(O)–OH (100 mg, 0.44 mmol) in DMF (1 ml) was incubated at 40 °C with various reducing reagents (10 eq each): thiophenol (0.45 ml), selenophenol (Wako, purity 80%. 0.86 ml), trimethylphenylthiosilane (0.83 ml) and trimethylphenylthiosilane (0.83 ml) plus tetrabutylammonium bromide (28 mg, 0.2 eq). Periodically, an aliquot was examined by TLC and the spots (stained with ninhydrin) of Z(OMe)–Met–OH (*Rf* 0.63) and the starting material (*Rf* 0.27) were determined quantitatively with a Shimadzu CS-900 dual-wavelength TLC scanner. The results are shown in Table I.

Reduction of Protected Dipeptide Amides——Z(OMe)–X–Met(O)–NH_2 (X = Leu,[11] Phe,[1] 101 μmol each) in DMF (2 ml) was incubated with selenophenol (198 μl, 10 eq) at 40 °C and the progress of the reduction was monitored with the dual-wavelength TLC scanner as described above. *Rf*: Z(OMe)–Leu–Met(O)–NH_2 0.33, Z(OMe)–Leu–Met–NH_2 0.71, Z(OMe)–Phe–Met(O)–NH_2 0.37, Z(OMe)–Phe–Met–NH_2 0.77. The results are shown in Table II.

Table I. Reduction of Z(OMe)–Met(O)–OH in DMF (%)

	min	15	30	60	120	180
1. PhSH		0	8.3	11.8	14.7	30.4
2. PhSeH		0	84.0	86.0	89.5	93.2
3. $PhSSiMe_3$		100	100	100	100	100
4. 3 + Bu_4NBr		100	100	100	100	100

Table II. Reduction of Protected Dipeptide Amides (%)

	min	60	120	180
Z(OMe)–Leu–Met(O)–NH_2		93.4	95.7	98.2
Z(OMe)–Phe–Met(O)–NH_2		92.2	93.3	98.2

References and Notes

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