Silver Trifluoromethanesulphonate as an S-Deprotecting Reagent for the Synthesis of Cystine Peptides

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The acetamidomethyl (Acm) group attached at the thiol function of cysteine was found to be cleaved quantitatively by treatment with silver trifluoromethanesulphonate in trifluoroacetic acid, this reagent being more effective than silver trifluoroacetate and cleaved the S-benzamidomethyl and the S-*p*-methoxybenzyl groups as well; this new S-deprotecting reagent has been used in the syntheses of oxytocin and chicken calcitonin.

Monovalent silver ion in the form of the nitrate has been used under basic conditions for the removal of certain thiol-protecting groups from cysteine, *e.g.*, S-trityl,[1] or S-ethylcarbamoyl.[2] However, as the acetate, silver is not effective in removing the S-*p*-methoxybenzyl (MBzl) group.[3] Currently, this soft acid metal is not used in practical peptide synthesis. We have found that silver trifluoromethanesulphonate (AgOTf) in trifluoroacetic acid (TFA) is able to cleave not only the S–MBzl group, but also the acetamidomethyl (Acm) group,[4] which would normally require oxidative cleavage with iodine[5] or treatment with toxic reagents, *e.g.*, mercury(II) acetate[4] or thallium trifluoroacetate.[6]

Boc-Cys(Acm)-OH in TFA was treated with AgOTf (5 equiv.) in the presence of anisole (2 equiv.) in an ice-bath for 60 min. After treatment with dithiothreitol (DTT) at room temperature for 30 min, the regenerated cysteine was quantified by an amino acid analyser. Under identical conditions two other S-protecting groups, benzamidomethyl (Bam)[7] and MBzl, were also cleaved quantitatively, but S-1-adamantyl (Ad),[8] S-t-butyl (Bu^t),[9] and 4-methylbenzyl (4-Me-Bzl),[10] were not affected (Table 1). The results indicate that the cysteine sulphur atom adjacent to the Acm and the Bam groups is very sensitive to silver ion in the form of the trifluoromethanesulphonate ion. Of the acid-labile S-protecting groups tested, the relatively TFA-labile MBzl group is the only one found to be cleavable by AgOTf. Other sensitive amino acids, such as Met, Trp, and Tyr, remained intact during the above AgOTf–DTT treatment. Silver trifluoroacetate (AgTFA) was also examined, the Acm group being cleaved quantitatively within 60 min, when the reaction was carried out at room temperature and the amount of the reagent was increased from 5 to 10 equiv. In view of its greater reactivity, AgOTf is a more attractive reagent than AgTFA.

In order to examine the usefulness of AgOTf as an S-deprotecting reagent for the synthesis of cystine-containing peptides, two model peptides, oxytocin[11] and chicken calcitonin (c-CT),[12] were synthesized.

Protected oxytocin, Boc-Cys(R)-Tyr-Ile-Gln-Asn-Cys(R)-Pro-Leu-Gly-NH_2 (R = Acm or MBzl), in TFA was treated with AgOTf (10 equiv.) in the presence of anisole (10 equiv.) in an ice-bath for 60 min. The Ag salt, which precipitated upon the addition of ether, was treated with DTT (50 equiv.) in 50% AcOH at room temperature for 2 h. After centrifugation, the supernatant was gel-filtered on Sephadex G-15 using 1 M AcOH as eluant. The desired eluate, after being diluted with water, was subjected to air-oxidation at pH 7.5. The reaction mixture was examined by h.p.l.c. and compared with an authentic sample of oxytocin (purchased from the Protein Research Foundation, Osaka, Japan). The product was purified by absorption chromatography on Diaion HP-20 (Mitubishi Chem. Co.) using 35% MeCN in 0.2 M AcOH as the eluant (yield 61% from the Acm derivative and 63% from the MBzl derivative). When the Acm derivative was treated with AgTFA (20 equiv.) at room temperature for 2 h, the yield of oxytocin was 61%.

Next, a Cys(Acm)-form of c-CT was prepared by a Boc-based solid phase procedure,[13] starting with a 4-methylbenzhydrylamine (MBHA)-resin,[14] followed by deprotection with 1 M trimethylsilyl trifluoromethanesulphonate (TMSOTf)-thioanisole/TFA.[15] After gel-filtration on Sephadex G-25, the product was dissolved in TFA and treated with AgOTf (10 equiv.) in the presence of anisole (30 equiv.),

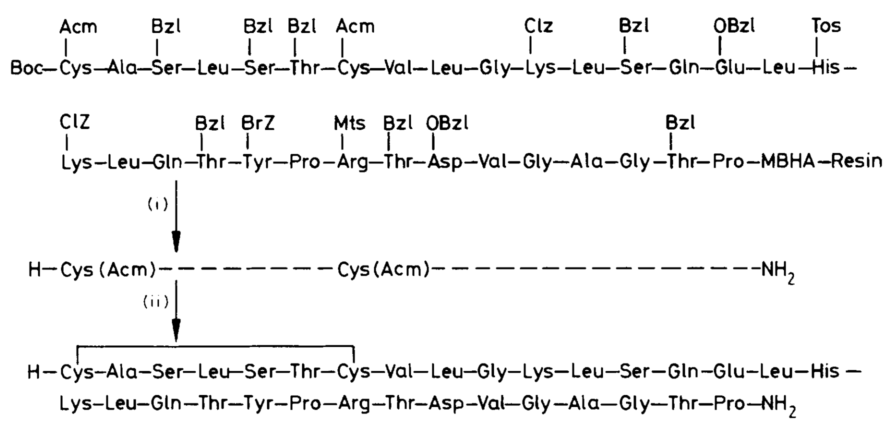

Figure 1. Synthetic scheme for chicken calcitonin. (i) 1 M TMSOTf–thioanisole/TFA, *m*-cresol. (ii) AgOTf/TFA; dithiothreitol; air-oxidation at pH 7.5. *Abbreviations*: Bzl = benzyl, ClZ = 2-chlorobenzyloxycarbonyl, BrZ = 4-bromobenzyloxycarbonyl, Tos = toluene-*p*-sulphonyl, Mts = mesitylenesulphonyl.

Table 1. Regeneration of cysteine (%) from Cys(R) after treatment (0 °C for 60 min) with AgOTf (5 equiv.).

R	Acm	Bam	MBzl	Ad	Bu^t	4-Me-Bzl
10 min	90.5	52.7	44.7	0	0	0
30 min	99.0	91.6	90.3	0	0	0
60 min	99.1	100	100	0	0	0

followed by treatment with DTT as before (Figure 1). After air-oxidation at pH 7.5, the product was purified by h.p.l.c., on a Cosmosil 5C18 column using a gradient of MeCN (30—50%) in 0.1% TFA; yield 28%, based on the first Pro unit loaded onto the resin, $[\alpha]_D^{16}$ −90.3° (*c* 0.1, H_2O). Mass spectrometry (fast atom bombardment) of the synthetic c-CT gave a result in agreement with the theoretical value; *m/z*: 3372.77 $(M + H)^+$; calc. for $C_{145}H_{240}N_{42}O_{46}S_2$ 3372.83. Leucine amino peptidase digestion of the synthetic c-CT gave a good recovery of cystine; Asp 1.00 (1), Thr + Gln 5.17 (4 + 2), Ser 2.82 (3), Glu 1.12 (1), Pro 1.89 (2), Gly 2.96 (3), Ala 2.00 (2), Cys 0.82 (1), Val 1.78 (2), Leu 4.75 (5), Tyr 0.96 (1), Lys 1.94 (2), His 0.92 (1), Arg 1.02 (1), recovery of Ala 81.9%.

X-Ray energy spectroscopy detected no silver contamination in the purified synthetic peptides. Our present AgOTf-deprotecting procedure is clearly a useful tool for the preparation of cystine-peptides which are free from toxic Hg contamination, when Acm or Bam protection is employed. This technique may prove to be useful for the synthesis of peptides containing several disulphide bonds, since one such bond can be established between two cystine residues protected by Acm, Bam, or MBzl groups, while leaving other S-protected residues, Cys(R) (R = Ad, Bu^t, 4-Me-Bzl), intact.

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