New Procedure for the Synthesis of Cystine-Peptides by Oxidation of S-Substituted Cysteine-Peptides with Thallium(III) Trifluoroacetate

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Thallium(III) trifluoroacetate cleaves various S-protecting groups of cysteine in trifluoroacetic acid forming cystine; as examples, three model peptides, oxytocin, urotensin II, and a human calcitonin gene-related peptide, were prepared by direct oxidative conversion of the respective S-substituted cysteine-peptides.

Iodine has been used as an oxidant to convert directly Cys(Tri)-peptides and Cys(Acm)-peptides† into cystine-peptides,[1] with particular care on iodination of some amino acids such as Tyr, His, Met, and Trp.[2] We have found that thallium(III) trifluoroacetate in trifluoroacetic acid (TFA) can cleave various S-protecting groups of cysteine, including the above two groups, to form cystine. The $(CF_3COO)_3Tl$ in TFA acts as a soft acid,[3] and then as an oxidant[4] (Figure 1).

Each Cys-derivative dissolved in TFA was treated with $(CF_3COO)_3Tl$ (1 equiv.) in an ice-bath for 60 min, then part of the solution was subjected to amino acid analysis. Anisole (*ca.* 2 equiv.) was used to trap alkyl cations. Except for Cys(Bzl),[5] other S-protecting groups so far examined here (MBzl,[6] Bu^t,[7] Ad,[8] Acm,[9] Tri,[10] and Dbs,[11])† were cleaved to form cystine as the sole product. Cys(4-MeBzl)[12] generated cystine, but a small amount of a by-product was detected. After incubation of each cleaved sample with ethanedithiol (10 equiv.) at 40 °C for 5 h, cysteine was regenerated quantitatively (Table 1). Unmasked Trp gave several unidentified products (recovery of Trp, 38%). Met was partially oxidized to the sulphoxide (34%), but not the sulphone. Amino acids, including His and Tyr, and their derivatives, Trp(Mts),[13] and Met(O),[14] were recovered unchanged after the above $(CF_3COO)_3Tl$ treatment.

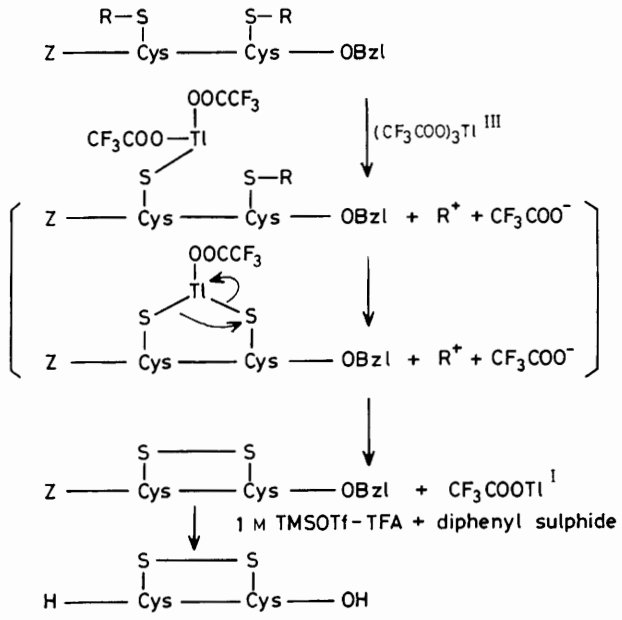
In order to examine the usefulness of $(CF_3COO)_3Tl$ for the intra-molecular disulphide bond formation, three model

Table 1. Oxidative cleavage of various S-protecting groups of cysteine by $(CF_3COO)_3Tl$.

	Cystine formed/%[a]	Cysteine regenerated after reduction/%[b]
Cys(MBzl)	86.7	98.5
Cys(Bu^t)	80.5	96.9
Cys(Ad)	83.0	89.3
Cys(Acm)	81.0	95.3
Cys(Tri)	80.2	93.6
Cys(Dbs)	81.1	87.0
Cys(4-MeBzl)	74.5[c]	89.7
Cys(Bzl)	0[c]	0

[a] Owing to poor solubility of cystine in buffers, quantitative data were not obtained, but no cysteic acid, as well as no starting materials, were detected. Cysteic acid was detected when an aqueous solution of the treated sample was kept in the presence of the Tl salt. [b] Ethanedithiol reduction was conducted at pH 7.5 (adjusted with 5% NH_4OH). [c] Small amount of a by-product, presumably the sulphoxide, was detected.

† Abbreviations used: Tri = triphenylmethyl, Acm = acetamidomethyl, Bzl = benzyl, MBzl = *p*-methoxybenzyl, Bu^t = t-butyl, Ad = 1-adamantyl, Dbs = dibenzosuberyl, 4-MeBzl = 4-methylbenzyl, Mts = mesitylenesulphonyl, Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl.



Example: hCGRP

H–Ala–Cys–Asp–Thr–Ala–Thr–Cys–Val–Thr–His–Arg–Leu–Ala–Gly–Leu–Leu–Ser–Arg–Ser–Gly–Gly–Val–Val–Lys–Asn–Asn–Phe–Val–Pro–Thr–Asn–Val–Gly–Ser–Lys–Ala–Phe–NH_2

Figure 1. Synthetic scheme for cystine-peptides by oxidation with $(CF_3COO)_3Tl^{III}$.

cystine-peptides, oxytocin,[15] urotensin II,[16] and a human calcitonin gene-related peptide (hCGRP),[17] were prepared according to the general procedure (Figure 1) and compared with authentic samples by h.p.l.c. By using this procedure, the time-consuming air oxidation step for disulphide bond formation in highly dilute aqueous media was eliminated.

Oxytocin was obtained as a sole product, when Z(OMe)–Cys(MBzl)–Tyr–Ile–Gln–Asn–Cys(MBzl)–Pro–Leu–Gly–NH_2 in TFA was treated with $(CF_3COO)_3Tl$ (1.1 mol. equiv.) in an ice-bath for 60 min. The reaction mixture was examined analytically by h.p.l.c., comparing it to an authentic sample of oxytocin (purchased from Protein Research Foundation, Osaka, Japan). The product was isolated (45% yield) by gel-filtration on Sephadex G-15, followed by h.p.l.c. on a Nucleosil 5C18 column.

As an example of Trp-containing peptide, urotensin II, a caudal neurosecretory hormone of the teleost fish, was prepared. Z–Ala–Gly–Thr–Ala–Asp(OBzl)–Cys(MBzl)–Phe–Trp(Mts)–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl in TFA was first treated with $(CF_3COO)_3Tl$ as stated above, then with 1 M trimethylsilyl trifluoromethanesulphonate (TMSOTf)–TFA[18] in the presence of diphenyl sulphide and Met rather than thioanisole, in an ice-bath for 120 min to remove other protecting groups. This did not affect the disulphide bond or the indole moiety of Trp. After gel-filtration and preparative h.p.l.c. purification, the product, identical with an authentic sample,[19] was obtained in 34% yield. The yield of the authentic sample prepared by the air-oxidation procedure was 16%.

Using the present method, the final step of our previous synthesis of hCGRP[20] has been simplified. The protected 37-residue peptide (Figure 1) was first treated with $(CF_3COO)_3Tl$ to remove the S-Ad group and spontaneously form the disulphide bond, then with 1 M TMSOTf–TFA in the presence of diphenyl sulphide to remove the other protecting groups (Mts from Arg, Bzl from Ser, and Z from Lys). After purification by gel-filtration, followed by h.p.l.c., the product was obtained in a somewhat better yield (11%) than before (8%).

No thallium contamination occurred in the synthetic peptides (confirmed by *X*-ray spectroscopy). An attractive feature of this mild oxidant is that disulphide bond formation can be carried out in TFA without any solubility problems, since TFA dissolves most of the peptides freely. We have found that the combination of ammonium iodide[21] and dimethyl sulphide in TFA was effective to reduce Met(O) to Met, without affecting the cystine disulphide bond.

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