(*S*)-9-Fluorenylmethyl-L-cysteine, a Useful HF-stable Derivative for Peptide Synthesis

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The reliability of the (*S*)-9-fluorenylmethyl-L-cysteine, a completely HF-stable cysteine derivative, has been tested on a solid phase synthesis of oxytocin.

The solid phase synthesis of disulphide bridge containing peptides is complicated by the fact that most common benzyl-type cysteine protecting groups are not stable to HF cleavage conditions, which leads to the formation of substantial amounts of polymeric materials[1—3] of difficult reduction.†[4]

One possible alternative to this problem is the use of the (*S*)-acetamidomethyl protecting group,[5] which is stable to HF deprotection conditions. However, Van Rietschoten *et al.*[6] have estimated 20% deprotection during the synthesis and HF cleavage of tetra-(*S*)-acetamidomethylapamin.

In this communication, we propose the use of the recently described 9-fluorenylmethyl group for cysteine side-chain protection[7] in solid phase peptide synthesis. The synthesis of (*S*)-9-fluorenylmethyl-L-cysteine was described by Bodanszky *et al.* in 1982 and, to the best of our knowledge, its use in solid phase peptide synthesis has never been reported. As shown below, this group is completely stable to solid phase reaction conditions, including the HF cleavage step. This allows the purification of the (*S*)-9-fluorenylmethyl-L-cysteine peptides, without the complications arising from the presence of free thiol groups, and its further conversion into disulphide at the end of the synthesis.

We have tested the stability of the (*S*)-9-fluorenylmethyl-L-cysteine under several conditions representative of solid phase peptide synthesis. Our results show that (i) (*S*)-9-fluorenylmethyl-L-cysteine is completely deprotected by 50% piperidine–dimethylformamide (DMF) in 2 h, or 10% piperidine–DMF overnight,‡ (ii) only traces of deprotected product

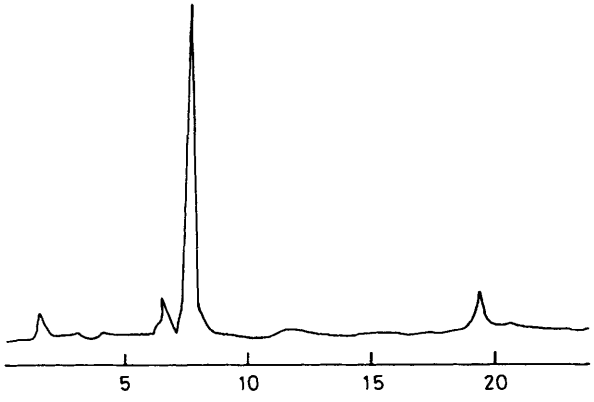

Figure 1. Analytical h.p.l.c. of crude protected oxytocin on Spherisorb C_{18} (30 × 0.39 cm) column. A: 90% water, 10% acetonitrile, 0.45% trifluoroacetic acid; B: 20% water, 80% acetonitrile, 0.45% trifluoroacetic acid. H.p.l.c. conditions: linear gradient of 10% to 90% B over 15 min at 1.5 ml/min. The effluent was monitored at 220 nm.

† Recently, a good recovery yield of cysteine from 4-methylbenzyl protection when treated with 90% HF–5% *p*-cresol–5% thiocresol has been reported (R. B. Merrifield, personal communication).

‡ The product of deprotection is cystine. No free cysteine has been detected. The deprotection was followed by t.l.c.

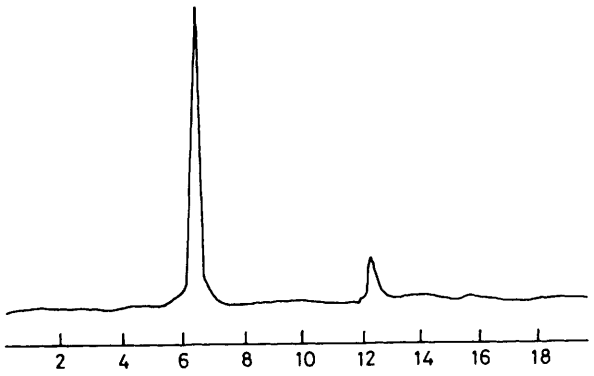

Figure 2. H.p.l.c. of crude oxytocin. Column and solvents same as in Figure 1. Linear gradient of 60% to 80% B over 20 min at 1.5 ml/min. The effluent was monitored at 220 nm.

are detectable after treatment overnight with a 10% solution of benzylamine or di-isopropylethylamine in DMF, and (iii) this derivative is totally stable to both 95% HF–5% anisole for 1 h at 0 °C and 0.1 M iodine in DMF.

The use of N^{α}-t-butoxycarbonyl-(*S*)-9-fluorenylmethyl-L-cysteine in solid phase peptide synthesis is further illustrated with the synthesis of oxytocin (**1**), which contains two residues of cysteine in positions 1 and 6.

H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
S–S
(**1**)

The synthesis was carried out on a benzhydrylamine resin using t-butoxycarbonyl N^{α}-amino protection and 2,6-dichlorobenzyl ether for tyrosine side-chain protection. Single couplings were performed with a 2.5-fold excess of amino acid and *N*,*N*′-dicyclohexylcarbodiimide in dichloromethane for 90 min. For the incorporation of Asn, Gln, and Ile, 1-hydroxybenzotriazole (2.5-fold excess) was added to prevent the formation of cyano derivatives and pyroglutamic acid. In all cases, the ninhydrin test[8] gave negative results after single coupling. Deprotection conditions were 30% trifluoroacetic acid in dichloromethane (1 × 30 min) followed by washings with dichloromethane (3 × 1 min) and neutralisation with 5% di-isopropylethylamine in dichloromethane (3 × 1 min).

The peptide was cleaved from the resin with 90% *p*-cresol for 1 h at 0 °C in 62% yield. H.p.l.c. of the crude product (Figure 1) showed a major peak (Asp: 0.93, Glu: 0.99, Gly: 1.07, Ile: 0.92, Pro: 0.86, Leu: 1.08, Tyr: 0.74, Cys: not determined§) which had the characteristic absorption of the 9-fluorenylmethyl group.

Treatment of the crude product with 50% piperidine–DMF (0.5 μmol/ml) gave, after evaporation, a main product (Asp: 1.01, Glu: 1.04, Gly: 0.99, Ile: 0.91, Leu: 0.91, Tyr: 0.87, Cys: 1.40, Pro: 0.95) whose retention time is identical to that of an authentic oxytocin sample (Figure 2). The minor peak in Figure 2 did not contain peptide material. For preparative purposes, pure oxytocin (20 μmol) was obtained after medium pressure reverse phase liquid chromatography on Vydac C-18 silica with a combined (deprotection–cyclisation and chromatography), non-optimized yield of 40%.

These results probe the usefulness of (*S*)-9-fluorenylmethyl sulphide protection of cysteine and suggest that, in conjunction with another protecting group stable to piperidine, this could constitute an excellent strategy for stepwise disulphide formation in molecules with more than one disulphide bridge.

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§ (*S*)-9-Fluorenylmethyl-L-cysteine is stable to HCl hydrolysis.[7]