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# Synthesis of asymmetric disulfides as potential alternative substrates for trypanothione reductase and glutathione reductase: Part 2

**R. Jaouhari, T. Besheya, J. H. McKie,** and **K. T. Douglas**

Pharmacy Department, University of Manchester, Manchester, United Kingdom

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**Summary.** The synthesis of asymmetrical disulfides, based on Zervas' intermediate, monocarbobenzoxy-L-cystine, has been developed. A series of substrate analogues of trypanothione disulfide (TSST) and glutathione disulfide (GSSG) are described, where the spermidine ring of (TSST) has been replaced by 3-dimethylaminopropylamine (DMAPA). The free amino group in Zervas' product was condensed with phenylalanyl, tryptophanyl or glutamyl residues, while the carbobenzoxy group was unaffected under the reaction conditions employed. The same synthetic approach was applied in the design of analogues of glutathione disulfide (GSSG).

**Keywords:** Amino acids – Synthesis – Asymmetrical disulfide – Trypanothione reductase – Glutathione reductase

## Introduction

In the preceding paper (Jaouhari et al., 1995), an investigation was undertaken with the primary purpose of developing a route for the preparation of symmetrical disulfides as potential substrate anologues of trypanothione reductase (TR) and glutathione reductase (GR). Further modifications which have been carried out in this laboratory include the replacement of the diionic $\gamma$-Glu groups of trypanothione analogues by nonpolar substituents such as the "Z" (benzyloxycarbonyl) group. The ZCysGlyDMAPA disulfide was found to be a good substrate for trypanothione reductase (El-Waer et al., 1992), which implies that one or both Z groups are involved in favorable binding interactions. Modelling this substrate in the active-site of TR suggested that one of the Z groups (on the T(II) side of the enzyme) was located in the hydrophobic pocket, in close proximity to the $\gamma$-Glu binding site in the region of phenylalanine F-396. Modelling studies suggested that substituting the Z group by a phenylalanine residue would maintain this hydrophobic interaction and, in addition, allow the free amino group (of Phe on the substrate

analogue) to form a charge interaction with E′466′ and E′467′, in a similar way to the γ-Glu moiety of TSST. The second Z group was not considered to be involved in significant favourable interactions. It was considered that maintaining the γ-Glu moiety at this site would provide an insight into the relative binding interactions at the two sites. Modification of the symmetric substrates to produce mixed disulfides would allow the asymmetry of the active-site of TR to be studied in a rational manner.

The goal of this study was to design eventual peptide inhibitors which would incorporate the key interactions mapped out by the mixed disulfide substrates. A route for the synthesis of these novel potential substrate analogues of trypanothione reductase and glutathione reductase is reported.

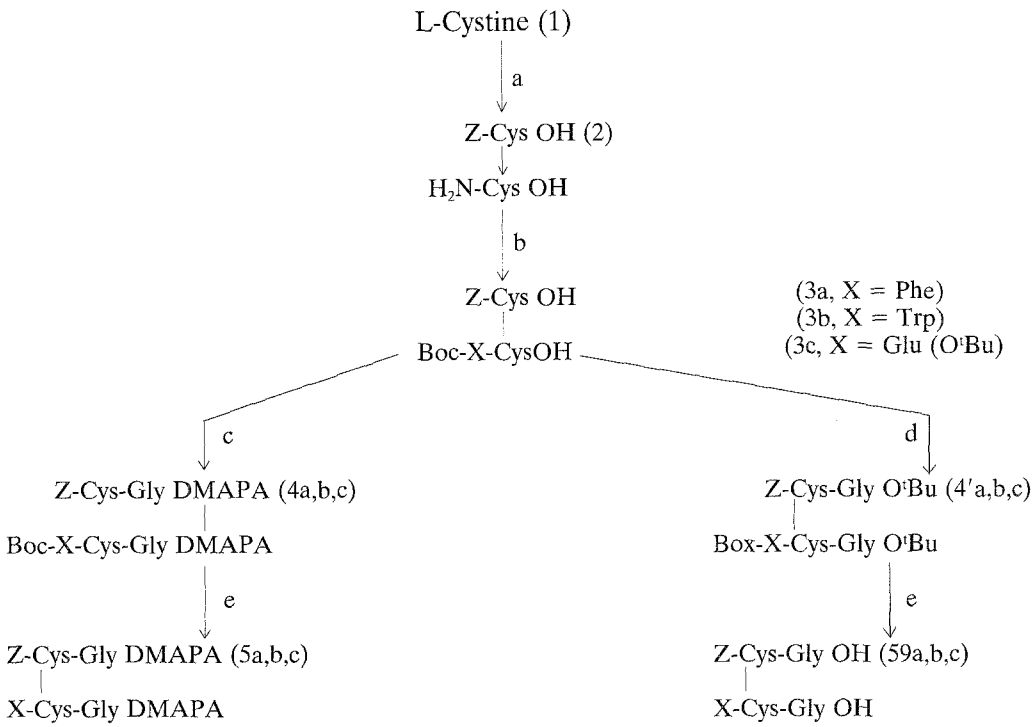
## Materials and methods

All the experimental procedures, chemicals used and analytical instruments were described in the previous paper (Jaouhari et al., 1995). The reactions were carried out under an inert atmosphere (argon or nitrogen) and the solvents dried according to standard methods.

## Discussions and results

The preparation of asymmetrical peptides presents a major problem as the peptide contains two or more cysteine residues and disulfide interchange is likely to occur, during the various steps of blocking, coupling or de-blocking (Hiskey et al., 1967). The first synthesis of oxytocin, vasopressin and analogues provided unsymmetrical peptides with the disulfide linkage in a cyclic structure (Du Vigneaud et al., 1954). Hiskey established elegant methodology for this kind of preparation, either *via* a sulphenyl thiocyanate (Hiskey et al., 1962) or a tetrahydropyranyl thioether and related hemiacetals (Hiskey et al., 1962). However, these syntheses do not provide for total de-blocking at C and N terminals, which would be invaluable for studies binding to the active sites of enzymes. If this de-blocking occurs in acidic medium, disulfide interchange takes place slowly.

Our synthesis strategy was to elaborate Zervas' product, monocarbobenzoxy-L-cystine (Zervas et al., 1959), formed by the interaction of excess of L-cystine with carbobenzoxy chloride, under Schotten-Baumann conditions (Scheme 1). Monocarbobenzoxy-L-cystine was mixed with BocXOSu (X = Phe, Trp, Glu(O$^t$Bu)) in a mixture of dioxane-water, in the presence of an organic base. After stirring at room temperature for an appropiate time, the aqueous layer was basified exactly to pH 8.1, then extracted quickly with ethyl acetate, before being acidified to pH 3.2, and kept at 4°C overnight to deposit the diprotected L-cystine. Derivative (3) was then coupled with either H-GlyDMAPA (El-Waer et al., 1992) or H-Gly-O$^t$Bu, using N-hydroxybenzotriazole, and N,N′-dicyclohexylcarbodiimide. The full protected L-cystine derivative (4) was obtained pure after flash chromatography (neutral alumina gel). Removal of the blocking groups (Boc and tBu




**Scheme 1.** General route of preparation of asymmetric disulfide: Substrate analogues of trypanothione reductase and glutathione, reductase. *a* Benzylchloroformate (Z-Cl), aqueous 20% NaOH, rt, 2 h. *b* Boc-X-OSu (X 5 Phe, Glu(O$^t$Bu), Trp), $Et_3N$, Dioxane/water, rt, 16 h. *c* *H*-Gly DMAPA, DCC, N-hydroxybenzotriazole, rt, 5 h. *d* H-Gly? O$^t$Bu, HCl, $Et_3N$, DCC, N-hydroxybenzotriazole, rt, 16 h. *e* $Et_3SiH/TFA/CH_2Cl_2$, rt, 45 minutes.

ester) occurred smoothly in a mixture of triethylsilane/trifluoroacetic acid/dichloromethane (Mehta et al., 1992).

Monocarbobenzoxy-L-cystine hydrochloride (2) was prepared according to a published method (Zevras et al., 1959), by the interaction of excess L-cystine (1) with carbobenzoxy chloride (Z-Cl) (20% aq. NaOH, under Schotten Baumann conditions, after which the pH of the reaction mixture had to be adjusted to 6, the highly insoluble excess cystine being removed by filtration and the pH of the filtrate readjusted to 3.2 to precipitate (2) as the hydrochloride salt.

$^1$H nmr (DMSO-$d_6$): 4.85–4.9(m, 1H, CH-NH), 5–5.1(m, 1H, CH-$NH_3$), 5.25(s, 2H, $CH_2$-O), 7.1–7.4(m, 5H, aromatic).

General procedure for the preparation of compounds (3)

Monocarbobenzoxy-L-cystine hydrochloride (2) (1 mmol) and triethylamine (4 mmol) in 10 ml water/dioxane (1:1, by vol) were stirred at room temperature for 10 minues. BocXOSu (0.9 mmol) was added in small portions with vigorous mixing under a nitrogen atmosphere and the whole solution stirred rapidly until reaction was complete (10–16 hours, determined by TLC), during which period the reaction mixture remained turbid. The mixture was

filtered, most of the solvent evaporated, the residue dissolved in water (10 ml) and the solution cooled in an ice-bath. Adjustment to pH 3 resulted in a white precipitate which was isolated by filtration and dried to give product.

(3a, yield: 65%, m.p. 78–81°C), (3b, yield: 56%, m.p. 112–115°C), (3c, yield: 45%, m.p. 134–136°C).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-phenylalanyl-L-cystine (3a)
$^1$H nmr ($CDCl_3$): 1.30(s, 9H, $^t$Bu), 2.85–3.65(m, 6H, 2$C_\beta H_2$-Cys and $C_\beta H_2$Phe), 4.2–4.54 and 4.6–4.87(2m, 3H, 2$C_\alpha$H-Cys and $C_\alpha$H-Phe), 5.15(d, J = 2.9 Hz, 1H, NH)*, 5.35(s, 2H, $CH_2$-O), 6.78(d, J = 3.1 Hz, 1H, NH)*, 7.2–7.4(m, 5H, Phe).

$^{13}$C nmr: 29.75($CH_3$-O$^t$Bu), 38.02($C_\beta H_2$-Trp), 40.21 and 40.35($C_\beta H_2$-Cys), 54.25(C-O$^t$Bu), 55.34 and 56.56($C_\alpha$H-Cys), 56.74($C_\alpha$H-Phe), 65.62($CH_2$-O), 124.55, 127.43, 128.01, 128.29, 129.67, 130.23, 133.52, 135.79, (aromatic C-Phe), 162.51(CO-Boc), 169.95(CO-Phe), 175.61 and 177.21(CO-Cys).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-tryptophanyl-L-cystine (3b)
$^1$H nmr ($CDCl_3$): 1.30(s, 9H, $^t$Bu), 2.95–3.4(m, 6H, 2x$C_\beta H_2$-Cys and $C_\beta H_2$-Trp), 4.62(m, 2H, 2x$C_\alpha$H-Cys and $C_\alpha$H-Trp), 5.15(s, 2H, $CH_2$-O) 5.4(d, J = 3.5 Hz, 1H, NH)*, 7.1(t, J = 6.3 Hz, 1H, NH)*, 7.2(m, 3H, Trp), 739(d, J = 8.4 Hz, 1H, Trp), 7.6(d, J = 8.4 Hz, 1H, Trp), 8.55 and 8.65(m, 2H, 2xNH)*.

$^{13}$C nmr: 26.71($CH_3$-O$^t$Bu), 38.23($C_\beta H_2$-Trp), 40.52 and 41.65($C_\beta H_2$-Cys), 43.81($C_\beta H_2$-Trp), 54.50(C-O$^t$Bu), 56.12($C_\alpha$H-Trp), 58.11 and 59.43(2x$C_\alpha$H-Cys), 64.42($CH_2$-O), 109.80, 110.12, 111.82, 118.32, 118.90, 119.81, 121.52, 122.42, 127.25, 128.53, 133.44, 136.32(Trp-Phe), 168.52(CO-Boc), 170.12, 171.2, 173.85(2xCO-Cys or Trp).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-glutamyl-($\alpha$ $^t$butyl)-L-cystine (3c)
$^1$H nmr ($CDCl_3$): 1.44, 1.47(2s, 18H, 2x$^t$Bu), 1.8–2.1(m, 2H, $C_\beta H_2$-Glu), 2.28–2.45(2m, 2H, $C_\beta H_2$-Glu), 2.75–2.94(H, 2x$C_\beta H_2$-Cys), 3.94–4.1(m, 1H, $C_\alpha$H-Glu), 4.56–4.65(m, 2H, 2$C_\alpha$H-Cys), 5.24(m, 2H), 5.15(s, 2H, $CH_2$-O), 5.2(d, J = 3.5 Hz, 1H, NH)*, 6.1(t, J = 6.3 Hz, 1H, NH)*, 7–7.35(m, 5H, Phe).

$^{13}$C nmr: 29.72, 29.76(2x$CH_3$-O$^t$Bu), 40.52, 41.65, 43.80, 53.12, 54.11, 55.98, 56.22(2x$C_\alpha$H-Cys, 2x$C_\beta H_2$-Cys, $C_\alpha$H-Glu, $C_\beta H_2$-Glu, C$\gamma H_2$-Glu), 40.52 and 41.65($C_\beta H_2$-Cys), 43.81($C_\beta H_2$-Trp), 54.67(C-O$^t$Bu), 65.21($CH_2$-O), 111.87, 121.50, 128.52, 133.47(Phe), 158.83(CO-Boc), 174.33, 174.46 and 175.42(CO-O$^t$Bu, 2xCO-Cys).

General procedure for the preparation of compounds (4) and (5)
Glycine-3-dimethylaminopropylamide (2 mmol) or glycine t-butyl ester hydrochloride (2 mmol) was added to anhydrous DMF (30 ml) under argon, followed by triethylamine (6.1 mmol, in the case of glycine t-butyl hydrochloride, 4.05 mmol in the case of glycine-3-dimethylaminopropylamide), N-hydroxybenzotriazole (4 mmol) and finally N,N′-dicyclohexylcarbodiimide (4 mmol). The mixture was stirred for 5 to 15 hours at room temperature (monitored by TLC (chloroform)). On completion of reaction, the solvent was removed under high vacuum without heat, the residue dissolved in

tetrahydrofuran and the dicyclohexylurea filtered off. The filtrate was evaporated to dryness and purified by flash chromatography on neutral alumina (elution: chloroform) to give product.

(4a), yellowish oily solid, yield: 46%, (4b), yellowish oil, yield: 37%, (4c), colourless oil, 39%, (6a), colourless oil, yield: 51%, (6b), sticky solid, yield: 47%, (6c), sticky solid, yield: 31%.

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-phenylalanyl-L-cysteinylglycyl-3-dimethylaminopropylamide (4a). [DMAPA = dimethylaminopropylamine: $(CH_3)_2NH\text{-}C^1H_2\text{-}C^2H_2\text{-}C^3H_2\text{-}N$]

$^1$H nmr ($CDCL_3$): 1.30(s, 9H, $^t$Bu),1.56(p, J = 7.4 Hz, 2H, $C^2H_2$-DMAPA), 2.25(s, 6H, 2x$CH_3$-DMAPA), 2.56(t, J = 7.7 Hz, 2H, $C^1H_2$-DMAPA), 2.87–3.05(m, 4H, $C_\beta H_2$-Cys and $C_\beta H_2$-Phe), 3.75–4.1(AB(X), $J_{AB}$ = 16.3 Hz, $J_{NH}$ = 4.7 Hz, 1H, $CH_2$-Gly), 4.45(q, J = 6.2 Hz, 2H, $C^3H_2$-DMAPA), 4.62(m, 2H, $C_\alpha$H-Cys and $C_\alpha$H-Phe), 5.1(d, J = 3.1 Hz, 1H, NH)*, 5.24(s, 2H, $CH_2$-O), 6.1(bs, 1H, NH)*, 7.2–7.65(m, 10H, Phe).

$^{13}$C nmr: 26.34 and 26.65($C^2$-DMAPA), 29.75($CH_3$-O$^t$Bu), 37.52 and 37.82($C^1$-DMAPA), 38.24($C_\beta H_2$-Phe), 39.81 and 40.35($C_\beta H_2$-Cys), 43.81 and 43.37($CH_2$-Gly), 45.0 and 45.23($CH_3$-DMAPA), 54.25(C-O$^t$Bu), 56.4 and 56.54($C_\beta$H-Cys), 56.74($C_\alpha$H-Phe), 57.12 and 57.34($C^3$-DMAPA), 65.45($CH_2$-O), 67.46 and 68.53(2xC-O$^t$Bu), 112.31, 118.54, 123.50, 127.43, 129.45, 129.12, 134.51, 135.79 (aromatic C, Phe), 158.51(CO-Boc), 164.41(CO-Z), 68.2 and 168.58(CO-Gly), 169.95(CO-Phe), 171.61 and 172.21(2xCO-Cys).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-tryptophanyl-L-cysteinylglycyl-3-dimethylaminopropylamide (4b)

$^1$H nmr ($CDCl_3$): 1.30(s, 9H, $^t$Bu), 1.65 and 1.7(2xp (overlapped), J = 7.7 Hz, 4H, $C^2H_2$-DMAPA), 2.25 and 2.28(s, 12H, 2x$CH_3$-DMAPA), 2.33 and 2.38(2xt (overlapped), J = 8.1 Hz, 4H, $C^1H_2$-DMAPA), 2.95–3.4(m, 4H, $C_\beta H_2$-Cys and $C_\beta H_2$-Trp), 3.75–4.0(AB(X), $J_{AB}$ = 16.3 Hz, $J_{NH}$ = 4.8 Hz, 4H, $CH_2$-Gly, 4.25 and 4.3(2xq (overlapped), J = 6.2 Hz, 4H, $C^3H_2$-DMAPA), 4.52(m, 2H, $C_\alpha$H-Cys and $C_\alpha$H-Trp), 5.15(s, 2H, $CH_2O$), 6.3(d, J = 3.5 Hz, 1H, NH)*, 6.9(t, J = 6.3 Hz, 1H, NH)*, 7–7.3(m, 7H, Trp and Phe), 7.39(d, J = 8.2 Hz, 1H, Trp), 7.6(d, J = 8.4 Hz, 1H, Trp), 8.12 and 8.34(m, 2H, 2xNH)*.

$^{13}$C nmr: 26.51 and 26.83($C^2$-DMAPA), 29.93($CH_3$-O$^t$Bu), 38.23 and 38.50($C^1$-DMAPA), 38.98($C_\beta H_2$-Trp), 40.01 and 40.13($C_\beta H_2$-Cys), 40.75 and 40.88($C_\beta H_2$-Trp), 43.21 and 43.56($CH_2$-Gly), 45.22 and 45.46($CH_3$-DMAPA), 54.59(C-O$^t$Bu), 56.15($C_\alpha$H-Trp), 56.57($C_\alpha$H-Cys), 57.26 and 57.37($C^3$-DMAPA), 65.40($CH_2$-O), 109.82, 110.12, 110.56, 111.46, 111.84, 118.96, 119.88, 120.13, 121.50, 122.81, 123.60 and 123.72, 127.36, 135.15, 136.58(Trp and Phe), 162.0(CO-Z), 169.21(CO-Boc), 170.14, 172.81, 173.26(CO-Cys or Trp or Gly).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-glutamyl($\alpha$-$^t$butyl)-L-cysteinylglycyl-3-dimethylaminopropyl-amide disulfide (4c)

$^1$H nmr ($CDCl_3$): 1.44(s, 18H, 2x$^t$Bu), 1.8–2.1(m, 2H, $C_\beta H_2$-Glu), 1.55 and 1.76(2xp (overlapped), J = 7.3 Hz, 4H, $C^2H_2$-DMAPA), 2.25 and 2.28(s, 12H, 2x$CH_3$-DMAPA), 2.32–2.45(2m, 2H, $C_\beta H_2$-Glu) 2.75–2.94(H, 2$C_\beta H_2$-Cys),

3.75–4.0(AB(X), $J_{AB}$ = 16.3 Hz, $J_{NH}$ = 4.8 Hz, 4H, $CH_2$-Gly), 4.25 and 4.3(2xq (overlapped), J = 6.2 Hz, 4H, $C^3H_2$-DMAPA), 2.75–2.94H, 2$C_\beta H_2$-Cys), 3.94–4.1(m, 1H, $C_\alpha$H-Glu), 4.5–4.65(m, 2H, 2$C_\alpha$H-Cys), 5.24(m, 2H, 5.1(s, 2H, $CH_{2-}$ O), 5.2(d, J = 3.5 Hz, 1H, NH)*, 6.6(t, J = 6.3 Hz, 1H, NH)*, 71–7.3(m, 5H, Phe).

$^{13}$C nmr: 26.52 and 26.80($C^2$-DMAPA), 29.91($CH_3$-O$^t$Bu), 38.22 and 38.50($C^1$-DMAPA), 33.26, 33,8, 35.38, 53.12, 54.11, 54.56, 55.67, 56.20(2x$C_\alpha$H-Cys, 2x$C_\beta H_2$-Cys, $C_\alpha$H-Glu, $C_\beta H_2$-Gly, $C_\gamma H_2$-Glu, C-O$^t$Bu), 57.21 and 57.36($C^3$-DMAPA), 65.44($CH_2$-O), 111.83, 121.57, 128.52, 133.44(Phe), 158.83(CO-Boc), 164.51(CO-Z), 174.35, 174.47 and 175.48(CO-O$^t$Bu, 2xCO-Cys).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-phenylalanyl-L-cysteinylglycine O$^t$butyl ester disulfide (4′a)

$^1$H nmr ($CDCl_3$): 1.43 and 1.46(2s, 18H, 2x$^t$Bu), 2.75–2.94(m, 4H, 2x$C_\beta H_2$-Cys), 3.8(AB(X), $J_{AB}$ = 15.9 Hz, $J_{NH}$ = 3.6 Hz , 4H, $CH_2$-Gly), 4.5–4.65(m, 2H, 2x$C_\alpha$H-Cys), 5.1(s, 2H, $CH_2$-O), 5.2(d, J = 3.4 Hz, 1H, NH)*, 6.3(t, J = 6.23 Hz, 1H, NH)*, 7.1–7(m, 5H, Phe).

$^{13}$C nmr: 29.12 and 29.91($CH_3$-O$^t$Bu), 38.65($C_\beta H_2$-Phe), 39.81 and 40.12($C_\beta H_2$-Cys), 42.23 and 42.62($CH_2$-Gly), 54.37 and 54.65(C-O$^t$Bu), 56.44 and 56.53($C_\alpha$H-Cys), 56.74($C_\alpha$H-Phe), 65.60($CH_2$-O), 111.66, 117.94, 123.78, 126.46, 129.11, 129.29, 134.53, 134.79(aromatic C, Phe), 159.56(CO-Boc), 163.71(CO-Z), 168.12 and 168(CO-Gly), 170.14(CO-Phe), 172.65 and 173.41(2xCO-Cys).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-tryptophanyl-L-cysteinylglycine O$^t$butyl ester disulfide (4′b)

$^1$H nmr ($CDCl_3$): 1.43 and 1.46(2s, 18H, 2x$^t$Bu), 2.75–2.94(m, 4H, 2x$C_\beta H_2$-Cys), 3.45(AB(X), $J_{AB}$ = 15.2 Hz, $J_{NH}$ = 4.8 Hz, 4H, $CH_2$-Gly), 4.5–4.65(m, 2H, 2x$C_\alpha$H-Cys), 5.1(s, 2H, $CH_2$-O) 5.2(d, J = 3.5 Hz, 1H, NH)*, 6.3(t, J = 6.3 Hz, 1H, NH)*, 7.2–7.55(m, 7H, Trp and Phe), 7.75(d, J = 8.1 Hz, 1H, Trp), 7.9(d, J = 7.8 Hz, 1H, Trp), 8.2 and 8.25(m, 2H, 2xNH)*.

$^{13}$C nmr: 29.12 and 29.93($CH_3$-O$^t$Bu), 37.53($C_\beta H_2$-Trp), 41.51 and 41.30($C_\beta H_2$-Cys), 41.77 and 41.89($C_\beta H_2$-Trp), 43.21 and 43.50($CH_2$-Gly), 54.52(C-O$^t$Bu), 56.12($C_\alpha$H-Trp), 56.58($C_\alpha$H-Cys), 65.47($CH_2$-O), 109.81, 110.87, 111.48, 111.89, 118.95, 119.87, 120.18, 121.59, 122.86, 123.68, 123.71, 127.34, 135.11, 136.50(Trp and Phe), 165.12(CO-Z), 168.12(CO-Boc), 171.63, 173.87, 174.26(CO-Cysor Trp or Gly).

N-Carbobenzoxy-N′-$^t$butoxycarbonyl-L-$\gamma$-glutamyl($\alpha$-$^t$butyl-L-cysteinylglycine O$^t$butyl ester disulfide (4′c)

$^1$H nmr ($CDCl_3$): 1.43 and 1.46(2s, 18H, 2x$^t$Bu), 1.7–2.2(m, 2H, $C_\beta H_2$-Glu), 2.25–2.45(2m, 2H, $C_\gamma H_2$-Clu), 2.75–2.94(m, 4H, 2x$C_\beta H_2$-Cys), 3.86(AB(X), $J_{AB}$ = 16.2 Hz, $J_{NH}$ = 3.8 Hz 4H, $CH_2$-Gly), 3.94–4.1(m, 1H, $C_\alpha$H-Clu), 4.5–4.65(m, 2H, 2x$C_\alpha$H-Cys), 5.1(s, 2H, $CH_2$-O), 5.2(d, J = 3.5 Hz, 1H, NH)*, 6.3(t, J = 6.3 Hz, 1H, NH)*, 7.1–7.45(m, 5H, Phe).

$^{13}$C nmr: 28.78 and 29.91($CH_3$-O$^t$Bu), 33.26, 33.81, 35.38, 41.94, 53.12, 54.11, 55.34, 55.98, 56.2(2x$C_\alpha$-Cys, 2x$C_\beta H_2$-Cys, $C_\alpha$H-Glu, $C_\beta H_2$-Glu, $C_\gamma H_2$-

Glu, $CH_2$ Gly, C-O$^t$Bu), 64.42($CH_2$-O), 111.81, 121.52, 128.51, 133.46(Phe), 159.83(CO-Boc), 166.52(CO-Z), 173.12, 174.52 and 176.17(CO-O$^t$Bu, 2xCO-Cys).

General procedure for the preparation of compounds (5) and (5′)
The protected peptide (4) or (4′) (100 mmol) was dissolved in freshly distilled trifluoroacetic acid (3 ml) and dichloromethane (4 ml). Triethylsilane (200 mmol) was added quickly and the reaction mixture stirred at room temperature for 35 minutes for compounds (4a) and (4b), 1 hour for compound (4c), and for 2.5 h for compounds (4′a), (4′b) and (4′c). After complete removal of volatile material under high vacuum, the residue was triturated with Na-dried $Et_2O$. The solid thus obtained was washed several times by suspension in dry ether and decantation of the ether filtrate. The product was further purified by partitioning between ethyl acetate (60 ml) and water (100 ml). The aqueous layer was separated and lyophilised to give 5a, (yield: 32%) as an off-white solid (m.p. 98–101°C). 5b, (yield: 16%) as a yellowish solid (m.p. 123–127°C). 5c, (yield: 26%) as a white solid (m.p. 156–160°C). 5′a, (yield: 45%) as a white solid (m.p. 76–79°C). 5′b, (yield: 32%) as a off-white solid (m.p. 150–154°C) and 5′c, (yield: 45%) as a white solid (m.p. 210–213°C).

All the salts were obtained as trifluoroacetate salts (as demonstrated by $^{19}$F nmr) and were highly hygroscopic crystalline solids, which moved as single spots on thin layer chromatography (2 solvent systems).

N-Carbobenzoxy-N′-L-phenylalanyl-L-cysteinylglycyl-3-dimethylaminopropylamide disulfide (5a)
$^1$H nmr ($D_2O$): 1.30(s, 9H, $^t$Bu),1.56(p, J = 7.4 Hz, 2H, $C^2H_2$-DMAPA), 2.25(s, 6H, 2x$CH_3$-DMAPA), 2.56(t, J = 7.7 Hz, 2H, $C^1H_2$-DMAPA), 2.87–3.05(m, 4H, $C_\beta H_2$-Cys and $C_\beta H_2$-Phe), 3.75–4.1(AB(X), $J_{AB}$ = 16.3 Hz, $J_{NH}$ = 4.7 Hz, 1H, $CH_2$-Gly), 4.45(q, J = 6.2 Hz, 2H, $C^3H_2$-DMAPA), 4.62(m, 2H, $C_\alpha$H-Cys and $C_\alpha$H-Phe), 5.1(d, J = 3.1 Hz, 1H, NH)*, 5.24(s, 2H, $CH_2$-O).6.1(bs, 1H, NH)*, 7.2–7.65(m, 10H, Phe).

$^{13}$C nmr: 26.34 and 26.65($C^2$-DMAPA), 29.75($CH_3$-O$^t$Bu), 37.56 and 37.82($C^1$-DMAPA), 38.23($C_\beta H_2$-Phe), 39.81 and 40.35($C_\beta H_2$-Cys), 43.12 and 43.31($CH_2$-Gly), 45.0 and 45.23($CH_3$-DMAPA), 54.25(C-O$^t$Bu), 56.42 and 56.51($C_\alpha$H-Cys), 56.74($C_\alpha$H-Phe), 57.12 and 57.34($C^3$-DMAPA), 65.16($CH_2$-O), 112.32, 118.54, 123.51, 127.43, 129.98, 129.12, 134.51, 135.79(aromatic C, Phe), 158.51(CO-Boc), 164.41(CO-Z), 68.25 and 168.58(CO-Gly), 169.95(CO-Phe), 171.61 and 172.21(2xCO-Cys).

N-Carbobenzoxy-N′-L-tryptophanyl-L-cysteinylglycyl-3-dimethylaminopropylamide disulfide (5b)
$^1$H nmr ($D_2O$): 1.30(s, 9H, $^t$Bu), 1.65 and 1.7(2xp (overlapped), J = 7.7 Hz, 4H, $C^2H_2$-DMAPA), 2.25 and 2.28(s, 12H, 2x$CH_3$-DMAPA), 2.33 and 2.38(2xt (overlapped), J = 8.1 Hz, 4H, $C^1H_2$-DMAPA), 2.95–3.4(m, 4H, $C_\beta H_2$-Cys and $C_\beta H_2$Trp), 3.75–4.0(AB(X), $J_{AB}$ = 16.3 Hz, $J_{NH}$ = 4.8 Hz, 4H, $CH_2$-Gly), 4.25 and 4.3(2xq (overlapped), J = 6.2 Hz, 4H, $C^3H_2$-DMAPA), 4.52(m, 2H, $C_\alpha$H-

Cys and $C_\alpha$H-Trp), 5.15(s, 2H, $CH_2O$), 63(d, J = 3.5 Hz, 1H, NH)*, 6.9(t, J = 6.3 Hz, 1H, NH)*, 7–7.3(m, 7H, Trp and Phe), 7.39(d, J = 8.2 Hz, 1H, Trp), 7.6(d, J = 8.4 Hz, 1H, Trp), 8.12 and 8.34(m, 2H, 2xNH)*.

$^{13}$C nmr: 26.52 and 26.81($C^2$-DMAPA), 29.94($CH_3$-O$^t$Bu), 38.21 and 38.57($C^1$-DMAPA), 38.71($C_\beta H_2$-Trp), 40.0 and 40.22($C_\beta H_2$-Cys), 40.81 and 40.89($C_\beta H_2$-Trp), 43.22 and 43.58($CH_2$-Gly), 45.21 and 45.45($CH_3$-DMAPA), 54.51(C-O$^t$Bu), 56.17($C_\alpha$H-Trp), 56.54($C_\alpha$H-Cys), 57.23 and 57.39($C^3$-DMAPA), 64.41($CH_2$-O), 109.84, 110.12, 110.67, 111.42, 111.84, 118.91, 119.82, 120.11, 121.57, 122.8, 123.6 and 123.7, 127.3, 135.1, 136.5(Trp and Phe), 162(CO-Z), 169.21(CO-Boc), 170.1, 172.8, 173.22(CO-Cys or Trp or Gly).

N-Carbobenzoxy-N′-L-$\gamma$-glutamyl($\alpha$-$^t$butyl)-L-cysteinylglycyl-3-dimethylaminopropylamide disulfide (5c)

$^1$H nmr ($D_2O$): 1.44(s, 18H, 2$^t$Bu), 1.8–2.1(m, 2H, $C_\beta H_2$-Glu), 1.55 and 1.76(2xp (overlapped), J = 7.3 Hz, 4H, $C^2H_2$-DMAPA), 2.25 and 2.28(s, 12H, 2x$CH_3$-DMAPA), 2.32–2.45(2m, 2H, $C_\gamma H_2$-Glu) 2.75–2.9, 4H, 2$C_\beta H_2$-Cys), 3.75–4.0(AB(X), $J_{AB}$ = 16.3 Hz, $J_{NH}$ = 4.8 Hz, 4H, $CH_2$-Gly), 4.25 and 4.3(2xq (overlapped), J = 6.2 Hz, 4H, $C^3H_2$-DMAPA), 3.94–4.1(m, 1H, $C_\alpha$H-Glu), 4.5–4.65(m, 2H, 2$C_\alpha$H-Cys), 5.24(m, 2H, 5.1(s, 2H, $CH_2$-O), 5.2(d, J = 3.5 Hz, 1H, NH)*, 6.6(t, J = 6.3 Hz, 1H, NH)*, 71–7.3(m, 5H, Phe).

$^{13}$C nmr: 26.52 and 26.81($C^2$-DMAPA), 33.21($CH_3$-O$^t$Bu), 38.23 and 38.5($C^1$-DMAPA), 33.87, 35.38, 53.12, 54.11, 55.71, 55.98, 56.24(2x$C_\alpha$-Cys, 2x$C_\beta H_2$Cys, $C_\alpha$H-Glu, $C_\gamma H_2$-Glu, C-O$^t$Bu), 57.22 and 57.31($C^3$-DMAPA), 64.67($CH_2$-O), 111.81, 121.5, 128.5, 133.45(Phe), 158.82(CO-Boc), 164.5(CO-Z), 174.31, 174.43 and 175.42(CO-O$^t$Bu, 2xCO-Cys).

N-Carbobenzoxy-N′-L-phenylalanyl-L-cysteinylglycine disulfide (5′a)

$^1$H nmr ($D_2O$): 2.6–2.84(dd, J = 8.4 Hz, 4H, 2$C_\beta H_2$-Cys), 2.85–3.10(dd, J = 6.4 Hz, 2H, $C_\beta H_2$-Phe), 3.9 and 4.1(2s, 4H, $CH_7$-Gly), 4.14(t, J = 7.2 Hz, 2H, $C_\alpha$H-Phe), 5–4.65(m, 2H, 2$C_\alpha$H-Cys), 5.1(s, 2H, $CH_2O$), 6.8–7.2(m, 5H, Phe).

$^{13}$C nmr: 38.23($C_\beta H_2$-Phe), 39.81 and 40.54($C_\beta H_2$-Cys), 44.12 and 44.65($CH_2$-Gly), 64.41($CH_2O$), 55.91 and 56.50($C_\alpha$H-Cys), 57.74($C_\alpha$H-Phe), 64.93($CH_2$-O), 68.55 and 69.11(2xC-O$^t$Bu), 112.35, 117.82, 124.75, 126.24, 129.11, 129.35, 133.50, 133.79(aromatic C, Phe), 159.54(CO-Boc), 164.76(CO-Z), 168.12 and 168.44(CO-Gly), 170.0(CO-Phe), 172.63 and 173.65(2xCO-Cys).

N-Carbobenzoxy-N′-L-trptophanyl-L-cysteinylglycine disulfide (5′b)

$^1$H nmr ($D_2O$): 2.75–2.94(m, 4H, 2$C_\beta H_2$-Cys), 3.3(bd, 2H, $C_\beta H_2$-Trp), 3.5 and 3.6(2s, 4H, $CH_2$-Gly), 4.1(m, 1H, $C_\alpha$H-Trp), 4.42(m, 2H, 2$C_\alpha$H-Cys), 5.1(s, 2H, $CH_2$-O), 7.2–7.55(m, 7H, Trp and Phe), 7.6(d, J = 7.87 Hz, 1H, Trp), 8.21(d, J = 8.1 Hz, 1H, Trp).

$^{13}$C nmr: 37.42($C_\beta H_2$-Trp), 41.50, 41.62, 41.71, 41.82($C_\beta H_2$-Trp, and $C_\beta H_2$-Cys), 43.22 and 43.51($CH_2$-Gly), 54.56(C-O$^t$Bu), 56.12($C_\alpha$H-Trp), 56.5($C_\alpha$H-Cys), 65.31($CH_2$-O), 110.82, 111.34, 111.48, 111.81, 118.94, 119.87, 120.52, 121.57, 122.47, 127.34, 135.13, 136.52(Trp+Phe), 16.52(CO-Z), 168.21(CO-Boc), 171.62, 173.8, 174.21(CO-Cys or Trp or Gly).

N-Carbobenzoxy-N′-L-glutamyl ($\alpha$-$^t$butyl)-L-cysteinylglycine disulfide (5′c)

$^1$H nmr ($D_2O$): 1.7–2.2(m, 2H, $C_\beta H_2$-Glu), 2.25–2.45(2m, 2H, $C_\gamma H_2$-Glu), 2.75–2.94(m, 4H, $2C_\beta H_2$-Cys), 3.86(2s, 4H, $CH_2$-Gly), 3.64–3.82(m, 1H, $C_\alpha$H-Glu), 4.5–4.65(m, 2H, $2C_\alpha$H-Cys), 5.2(s, 2H, $CH_2$-O), 7.15–7.35(m, 5H, Phe) $^{13}$C nmr: 28.51($CH_2$-Glu). 33.26, 33.82, 35.38, 53.12, 54.11, 55.98, 56.2(2xC-Cys, 2x$CH_2$-Cys, CH-Glu), $C_\alpha H_2$-Glu, C-O$^t$Bu), 65.84($CH_2$-O), 112.1, 120.56, 128.53, 134.42(Phe), 164.50(CO-Z), 174.31, 174.44, 175.61(3xCO).

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**Authors' address:** Dr. R. Jaouhari, QUCHEM, School of Chemistry, The Queen's University of Belfast, David Keir Building, Belfast BT9 5AG Northern Ireland, UK.

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