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On the stability of *S*-(alkylsulfanyl)cysteine derivatives during solid-phase synthesis

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Abstract. *N*-Acetyl-*S*-(alkylsulfanyl)cysteine benzyl esters were synthesized as models for *N*-acylated *S*-(alkylsulfanyl)cysteine residues linked via an ester bond to a solid phase. The S-protections involved were: the ethylsulfanyl, the well-established *tert*-butylsulfanyl and the newly developed tritylsulfanyl group. We investigated the chemical behaviour of the disulfides in reagents commonly applied in solid-phase peptide synthesis using the Fmoc strategy (Fmoc SPPS). It was found that the tritylsulfanyl group as a thiol protection is comparable with the *tert*-butylsulfanyl group in these respects. It is stable in trifluoroacetic acid and is rapidly reduced by thiols and phosphines. For all three cysteine esters rapid racemization was observed in piperidine (25%) in DMF, the amides being chirally stable. The demonstrated chiral instability of cysteine esters has consequences for the solid-phase synthesis of peptides using the Fmoc protocol.

Introduction

Since 1970, alkylsulfanyl groups (RS-)[1,2] have been used as protection for the thiol group of cysteine during the synthesis of cysteinyl and cystinyl peptides[3,4,5,6]. This type of thiol protection of cysteine can be introduced directly into cysteine using alkoxycarbonylsulfanyl activation[7]. Using this method, we have synthesized *S*-(ethylsulfanyl)-, *S*-(*tert*-butylsulfanyl)-, and the previously undescribed *S*-(tritylsulfanyl)cysteine.

The *N*-(9-fluorenylmethyloxycarbonyl) derivative of *S*-(*tert*-butylsulfanyl)cysteine [Fmoc-Cys(S^{t}Bu)-OH] has been widely used in solid-phase synthesis employing the Fmoc strategy (Fmoc SPPS)[4,5,6]. This compound is well suited for the purpose because it largely fulfills the demands of orthogonal protection of the cysteine side-chain. The S^{t}Bu function is reported to be stable to trifluoroacetic acid (TFA)[5] and amines[1] and it is removed by reduction with phosphines[5] and thiols[6].

However, some specific problems associated with protected cysteine residues linked to a solid phase via a C-terminal ester bond have largely been overlooked. *Eritja* et al.[6] found repeatedly the formation of an undesired product comprising an unidentified modification of the C-terminal cysteine residue in Fmoc SPPS starting from resin-linked Fmoc-Cys(S^{t}Bu)-OH. In addition, *Atherton* et al.[8] showed that 36% of Boc-Cys(S^{t}Bu)-OH esterified to a resin had been converted to the D-isomer after a 4-hour treatment with piperidine 20% in DMF. In the performance of a solid-phase synthesis, the situation will probably be worse, since the cysteinyl residue, following its acylation, is no longer blocked with an urethane-type function which protects against racemization more effectively than an (amino)acyl function. Enolization of a carbonyl function has been observed in esters and active esters[9a,b].

Hence, we consider *N*-acetyl-*S*-(alkylsulfanyl)cysteine benzyl esters to be suitable mimics of a C-terminal Cys(SR) residue esterified to a 4-alkoxybenzyl-alcohol resin[10] (R = ethyl, *tert*-butyl or trityl). We synthesized the three benzyl esters as well as the protected dipeptides X-Cys(STrt)-Gly-OBzl (X = Boc, Ac). Then we investigated the stability of these compounds with respect to reagents commonly applied in Fmoc SPPS. Some properties of the *S*-tritylsulfanyl function could be established simultaneously and compared with those of the two better known protecting groups.

Results and discussion

Synthesis of derivatives of H-Cys(SR)-OH

The chiral and chemical stability of the three disulfides was investigated following their conversion into *N*-acetyl benzyl esters Ac-Cys(SR)-OBzl (R = Et, ^{t}Bu, Trt [a]) by mild and non-racemizing standard methods. Additionally, the derivatives Boc-Cys(STrt)-OH (and its DCA-salt), Boc-Cys(STrt)-Gly-OBzl and Ac-Cys(STrt)-Gly-OBzl were synthesized by standard procedures in peptide chemistry (see Experimental). The two protected dipeptides were used as chirally stable reference compounds in the racemization studies.

[a] Trt = trityl = triphenylmethyl = Ph_3C.

The ease of the described conversions of H-Cys(STrt)-OH and derivatives contributes to the suitability of the new thiol protection method for peptide synthesis.

Stability tests

The three model compounds Ac-Cys(SR)-OBzl were subjected to the conditions applied during solid-phase synthesis. They were examined by TLC and polarimetry.
Each of the cysteine derivatives was soluble in the relevant organic solvents ensuring optimal exposure to the reagents specified below.

(1) Acidolysis using trifluoroacetic acid (TFA). Applying the Fmoc method cleavage of the anchoring bond of a peptide to the support and concomitant acidolytic deprotection of side-chain functions is commonly performed with TFA[11]. We confirm the reported stability of the disulfanyl-type side-chain protection during mild acidolysis[5,6], since the acetylated compounds underwent no significant decomposition when kept in pure TFA for at least 5 hours, at room temperature. Standing for longer periods caused the appearance of small amounts of Ac-Cys(H)-OBzl on TLC. Simultaneously, traces of a trityl-like compound could be visualized, obviously detached from Ac-Cys(STrt)-OBzl.
The same experiment was carried out with TFA containing some commonly used scavengers against alkylating and sulfonating cations. Using TFA/ethanedithiol/water 95 : 2.5 : 2.5 (v/v/v) the thiol deprotection of the cysteine derivatives was slightly larger than in pure TFA, but was still insignificant within periods shorter than 4 hours. This observation is in agreement with the finding that thiol-type scavengers do not attack disulfide bonds in peptides during deprotection in TFA[12]. Furthermore, the observed stability against TFA for at least 4 hours is compatible with the maximal period required for full acidolytic deprotection of Ser('Bu) and Arg(Pmc) residues[13].

(2) Reduction with thiols or phosphines. Thiols[6] (*e.g.* 2-sulfanylethanol or dithiothreitol [b]) and phosphines[5] (trialkyl- or triarylphosphines) were found to be effective for mild reductive removal of the *tert*-butylsulfanyl moiety on the sulfur atom of cysteine.
We confirm that treatment of the three derivatives Ac-Cys(SR)-OBzl with excess 2-sulfanylethanol or dithiothreitol in DMF, or with tributylphosphine in appropriate media gives quantitative conversion to the thiol Ac-Cys(H)-OBzl within less than 10 minutes. TLC did not indicate any difference in the reaction rates of the cysteine derivatives involved. The chromatographic and spectrophotometric detectability of by-products of the trityl type proved to be an analytical advantage.
Since it has been demonstrated that reduction of disulfide bonds with thiols is insignificant in TFA (see above), but quantitative in neutral media, the use of an alkylsulfanyl group for protection of a thiol function is recommended.

(3) Stability towards piperidine in dimethylformamide (DMF). Piperidine in DMF is widely applied as the reagent for removal of the Fmoc group in SPPS[11], but the concomitant racemization of C-terminally located cysteine residues is a serious problem under these conditions.
We studied the problem by following the change in observed rotation of 1% (10 mg/ml) solutions of each of the three derivatives Ac-Cys(SR)-OBzl in piperidine/DMF 1 : 3 (v/v) at room temperature. The absolute value of the rotation was found to fall to zero quite rapidly. Plotting the course of this parameter against time gave the curves illustrated in Figure 1.

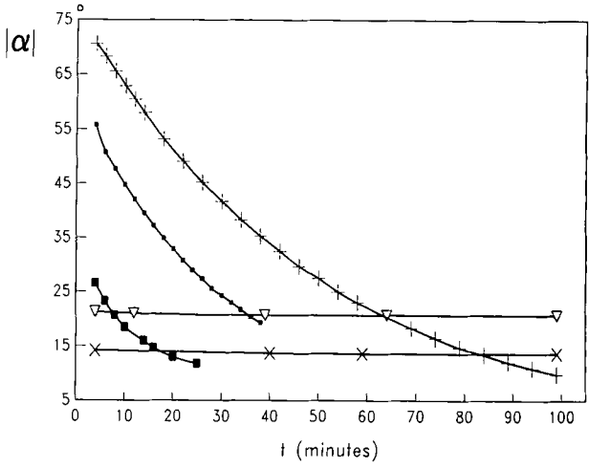

Figure 1. The course of the absolute values of the observed rotations of the substrates Ac-Cys(SR)-OBzl (initial concentration c 1.0), Boc-Cys(STrt)-Gly-OBzl (c 0.37) and Ac-Cys(STrt)-Gly-OBzl (c 0.32) in piperidine/DMF (at 25°C and at the sodium D line), versus time.
+ Ac-Cys(S'Bu)-OBzl ■ Ac-Cys(SEt)-OBzl ▪ Ac-Cys(STrt)-OBzl
▽ Boc-Cys(STrt)-Gly-OBzl × Ac-Cys(STrt)-Gly-OBzl

The intense green colour developed by the solutions during the racemization experiments, by itself indicative of a chemical change, is noteworthy. We did not analyse extensively the composition of the mixture obtained after the experiment. However, TLC after the reaction of Ac-Cys(STrt)-OBzl with piperidine indicated that part of the starting compound was unchanged (chemically, not optically) and part had been converted into other compounds, among which were fast-running products stemming from detachment of the trityl moiety. The dipeptides Ac-Cys(STrt)-Gly-OBzl and Boc-Cys(STrt)-Gly-OBzl did not show any significant decrease in the absolute value of optical rotation after dissolution to a known concentration in the piperidine reagent. Nor did the solutions attain a colour.
A plausible mechanism[14] for the observed loss of optical activity of the esters might be base-induced formation of the carbanion at the chiral centre, causing complete racemization and subsequent elimination of a part of the alkyl disulfide moiety. Nucleophilic addition of piperidine or an alkyl sulfide anion to the dehydroalanine derivative formed will result in the formation of new but also racemic products.
The measurements with the dipeptides suggest that amides of *N*-acyl and *N*-(alkoxycarbonyl)cysteine *S*-sulfides are chemically and optically stable under these conditions. These findings are in accordance with the well-known fact that enolate-stabilized carbanions are readily formed by α-proton abstraction from esters but not from amides. The occurrence of the 'anomalous' products found by *Eritja* et al.[6] may be explained by elimination of the *tert*-butyl disulfide anion and the subsequent addition of piperidine during the Fmoc deprotection. In any case, it is clear that rapid racemization and/or decomposition occurs when *N*-acyl-*S*-(alkylsulfanyl)cysteine esters are subjected to a 25% piperidine solution. This tendency must be taken into account when one intends to synthesize (even small) peptides with a C-terminal cysteine residue by the Fmoc strategy. The danger is non-existent when the cysteinyl carboxylic function is linked to the next residue (or solid-phase linker) via a normal peptide (amide) bond (Figure 2). It is possible that the use of a different base or a lower base concentration for deprotec-

[b] Dithiothreithol = 1,4-disulfanylbutane-2,3-diol.

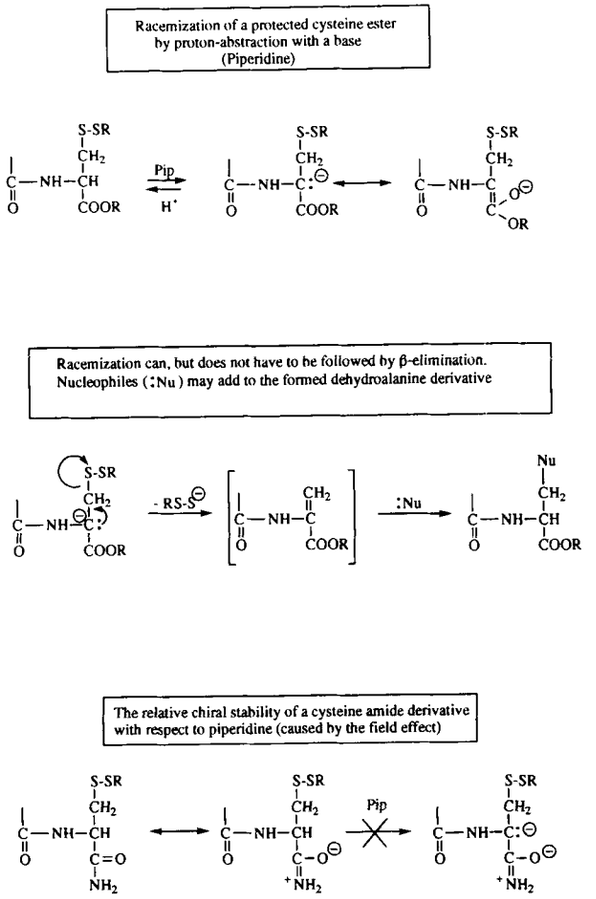

Figure 2. Proposed mechanism for the racemization of N-acylcysteine benzyl esters in piperidine / DMF.

tion may change the rate of racemization, as has been shown with respect to a C-terminally esterified Cys(Trt) residue[15].

Experimental

Materials and methods

Melting points were determined on a Tottoli capillary melting-point apparatus from Büchi and are uncorrected. Optical rotations were measured in a Perkin–Elmer polarimeter, model 241. Elemental analyses were performed using the Carlo Erba elemental analyser 1108. Thin-layer chromatography (TLC) was carried out on Merck $60F_{254}$ silica-coated glass plates. The spots were visualized by: (1) UV-fluorescence quenching, (2) chlorine/TDM [c][16], (3) ninhydrin, (4) sodium nitroprusside in water, (5) concentrated-sulfuric-acid/methanol 1:1 (v/v). Spraying with reagent (4) reveals free thiols, *e.g.* Ac-Cys(H)-OBzl, by a red colour. Spraying with reagent (5), eventually followed by heating, reveals trityl-containing compounds by a yellow colour. NMR spectra were recorded on a 100-MHz FT Bruker AC 100 or on a 400-MHz FT Bruker AM 400 apparatus. A double-focussing VG 7070E mass spectrometer was used to obtain mass spectra. All reagents and solvents were of the highest purity available. Compounds H-Cys(SR)-OH (R = Et, ^{t}Bu, Trt; optically pure L-form) were prepared from L-cysteine using methoxycarbonylsulfanyl chioride as a thiol-activating agent[7]. *N*-Succinimidyl acetate (Ac-ONSu) was obtained from Sigma, di-*tert*-butyl dicarbonate (Boc-O-Boc) was from Fluka, and tributylphosphine was from Aldrich.

[c] TDM = *N,N,N',N'*-tetramethyl-4,4'-diaminodiphenylmethane.

Synthesis

N-*Acetyl*-S-*(ethylsulfanyl)cysteine, Ac-Cys(SEt)-OH.* This compound was prepared from H-Cys(SEt)-OH and acetic anhydride analogously to *Schnabel's* method for the preparation of Boc-Ile-OH from H-Ile-OH and Boc-N_3[17]. The reaction was conducted in water at pH 6.0 and at 0°C. After evaporation of the final ethyl-acetate extract, the product was obtained as an oil which was taken up in hexane. On slow evaporation of the hexane at room temperature, the product crystallized in colourless crystals, yield 98%; m.p. 98°C $[\alpha]_D^{25}$ −132.4° (*c* 1.0, 1.5N HCl). TLC: R_f 0.39 ($CHCl_3$/MeOH/HOAc 95/20/3). ^{1}H NMR (D_2O) δ (ppm): 1.05 (3H, t, *J* 7.3 Hz; ethyl CH_3), 1.82 (3H, s, acetyl CH_3), 2.51 (2H, q, *J* 7.3 Hz; ethyl CH_2), 2.77 (1H, dd; *J* 8.9 and 14.2 Hz; β-CH_2), 3.06 (1H, dd, *J* 4.5 and 14.2 Hz; β-CH_2), 4.46 (1H, dd, *J* 4.5 and 8.9 Hz; α-CH). MS (EI) *m/e* (fragment, %): 223 (M^+, 30), 178 ($M^+ - CHO_2$, 24), 149 ($M^+ - CHO_2 - C_2H_5$, 100).

N-*Acetyl*-S-*(*tert*-butylsulfanyl)cysteine, Ac-Cys(S^{t}Bu)-OH.* This compound was prepared from H-Cys(S^{t}Bu)-OH and Ac-ONSu using a method similar to that used for the introduction of the Fmoc group with Fmoc-ONSu[18]. After acidification of the reaction mixture, part of the product precipitated as a crystalline solid. It was collected by filtration and washed with water. The combined mother liquor and washings were evaporated *in vacuo* and the white residue was extracted with acetone. The extract was filtered and evaporated affording a second batch of product which was also dried in *in vacuo* and was homogeneous as judged by TLC and NMR. Yield 89%. Colourless crystals, m.p. 168–171°C (dec.); $[\alpha]_D^{25}$ +33.1° (*c* 1.0, methanol). TLC: R_f 0.48 ($CHCl_3$/MeOH/HOAc 95:20:3). ^{1}H NMR (CD_3OD) δ (ppm): 1.33 (9H, s; $(CH_3)_3C$), 2.06 (3H, s; acetyl CH_3), 3.20 (2H, d, *J* 5.3 Hz; β-CH_2), 4.78 (1H, t, *J* 5.3 Hz; α-CH). MS (EI) *m/e* (fragment, %): 252 (M^+, 17), 209 ($M^+ - C_2H_3O$, 6) 195 ($M^+ - C_4H_9$, 37), 57 ($C_4H_9^+$, 100), 43 ($C_2H_3O^+$, 72). Anal. calcd.: C 43.00, H 6.82, N 5.57, S 25.51; found: C 42.89, H 6.71, N 5.57, S 25.94%.

N-*Acetyl*-S-*(tritylsulfanyl)cysteine, Ac-Cys(STrt)-OH.* This compound was prepared from H-Cys(STrt)-OH and acetic anhydride using a method analogous to 'method B' for the introduction of the Boc group with Boc-O-Boc by *Moroder* et al.[19]. Recrystallization from toluene afforded the product as a colourless solid that was homogeneous as judged by TLC and NMR. Yield 81%; m.p. 84°C; $[\alpha]_D^{25}$ +4.4° (*c* 1.0, chloroform). TLC: R_f 0.52 ($CHCl_3$/MeOH/HOAc 95:20:3). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.97 (3H, s; CH_3), 2.13 (2H, d, *J* 5.0 Hz; β-CH_2), 4.45 (1H, d t, *J* 7.5 and 5.0 Hz; α-CH), 6.22 (1H, d, *J* 7.5 Hz; NH), 7.2–7.4 (15H, m; ArH). MS (FAB) *m/e* (fragment, %): 460 (MNa^+, 4), 243 $(C_6H)_3C^+$, 100).

N-*Acetyl*-S-*(ethylsulfanyl)cysteine benzyl ester, Ac-Cys(SEt)-OBzl.* This compound was synthesized from Ac-Cys(SEt)-OH and benzyl bromide according to the method described by *Nefkens* and *Nivard* for esterification of glutamic acid derivatives[20]. Evaporation of the final organic extract *in vacuo* gave the product as a yellowish oil that was homogeneous as judged by TLC and NMR. Yield 63%; $[\alpha]_D^{25}$ +57.6° (*c* 1.14, chloroform); $[\alpha]_D^{25}$ −50.6° (*c* 1.0, DMF). TLC: R_f 0.72 ($CHCl_3$/MeOH/HOAc 95:20:3); see also Table I. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.28 (3H, t, *J* 7.3 Hz; ethyl CH_3), 2.05 (3H, s; acetyl CH_3), 2.65 (2H, q, *J* 7.3 Hz; ethyl CH_2), 3.20 (2H, d, *J* 5.0 Hz; β-CH_2), 4.93 (1H, d t, *J* 7.6 and 5.0 Hz; α-CH), 5.20 (2H, s; benzyl-CH_2), 6.40 (2H, br d; NH), 7.2–7.4 (5H, m; ArH). MS (EI) *m/e* (fragment, %): 313 (M^+, 10), 91 ($C_6H_5CH_2^+$, 100).

N-*Acetyl*-S-*(tert-butylsulfanyl)cysteine benzyl ester, Ac-Cys(S^{t}Bu)-OBzl.* This compound was synthesized from Ac-Cys(S^{t}Bu)-OH and benzyl bromide according to the method described by *Nefkens* and *Nivard*[20]. The final organic extract was evaporated *in vacuo* and the yellow oily residue was diluted with petr. ether (40–65). The solution was filtered and the filtrate was evaporated *in vacuo* at room temperature, yielding the product as a white amorphous semi-solid, homogeneous as judged by TLC and NMR. Yield 52%; $[\alpha]_D^{25}$ −78.8° (*c* 1.0, DMF). TLC: R_f 0.75 ($CHCl_3$/MeOH/HOAc 95:20:3); see also Table I. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.30 (9H, s; $C(CH_3)_3$), 2.05 (3H, s; acetyl CH_3), 3.21 (2H, d, *J* 4.8 Hz; β-CH_2), 4.92 (1H, d t, *J* 7.6 and 4.8 Hz; α-CH), 5.20 (2H, s; benzyl CH_2), 6.42 (2H, br d; NH), 7.2–7.4 (5H, m; ArH). MS (EI) *m/e* (fragment, %): 341 (M^+, 19), 285 ($M^+ - C_4H_9$, 23), 91($C_6H_5CH_2^+$, 100), 57($C_4H_9^+$, 39), 43($C_2H_3O^+$, 29). Anal. calcd.: C 56.28, H 6.78, N 3.62; found: C 56.69, H 6.93, N 4.10%.

N-*Acetyl*-S-*(tritylsulfanyl)cysteine benzyl ester, Ac-Cys(STrt)-OBzl.* This compound was synthesized from Ac-Cys(STrt)-OH and benzyl bromide according to the method described by *Nefkens* and *Nivard*[20]. Evaporation of the final organic extract *in vacuo* gave an oil which was subjected to low pressure chromatography on silica using the eluent chloroform/methanol, 99:1 (v/v). After evaporation of the product-containing fractions, an oil was obtained that was triturated with pentane affording a white semi-solid that was homogeneous as judged by TLC and NMR. Yield 53%; $[\alpha]_D^{25}$ −62.3° (*c* 1.0, DMF). TLC: R_f 0.80 ($CHCl_3$/MeOH/HOAc 95:20:3); see also Table I. ^1H NMR ($CDCl_3$) δ (ppm): 1.98 (3H, s; CH_3), 2.09 (2H, d, *J* 4.5 Hz; β-CH_2), 4.58 (1H, d t, *J* 7.5 and 4.5 Hz; α-CH), 5.07 (2H, s; benzyl CH_2), 6.15 (1H, d, *J* 7.5 Hz; NH), 7.2–7.5 (20H, m; ArH). MS (FAB) *m/e* (fragment, %): 243 [$(C_6H_5)_3C^+$, 55], 91 ($C_6H_5CH_2^+$, 100); molecular ion not observed.

N-*(*tert*-Butyloxycarbonyl)*-S-*(tritylsulfanyl)cysteine, Boc-Cys(STrt)-OH.* This compound was prepared from H-Cys(STrt)-OH and Boc-O-Boc according to 'method B' described by *Moroder* et al.[19]. Evaporation of the final organic extract gave an oil which was taken up in diisopropyl ether; insoluble contaminants were filtered off. The product was precipitated and triturated with petr. ether to afford Boc-Cys(STrt)-OH as a white amorphous powder. Yield 62%; $[\alpha]_D^{25}$ −3.84° (*c* 0.37, chloroform). TLC: R_f 0.74 ($CHCl_3$/MeOH/HOAc 95:20:3); R_f 0.37 ($CHCl_3$/MeOH 7:1). ^1H NMR ($CDCl_3$) δ (ppm): 1.40 (9H, s, $C(CH_3)_3$), 2.10 (2H, d, *J* 4.8 Hz, CH_2), 4.24 (1H, d t, *J* 7.3 and 4.8 Hz, α-CH), 5.10 (2H, d, *J* 7.3 Hz, NH), 7.2–7.4 (15H, m, ArH). MS (FAB) *m/e* (fragment, %): 518 (MNa^+, 8.5), 307 [$(C_6H_5)_3CS_2^+$, 3], 275 [$(C_6H_5)_3CS^+$, 12], 243 [$(C_6H_5)_3C^+$, 100].
A part of the batch was converted to the DCA salt for additional characterization[21].

Dicyclohexylammonium salt of N-*(*tert*-butyloxycarbonyl)*-S-*(tritylsulfanyl)-cysteine, Boc-Cys(STrt)-OH.DCA.* This salt was prepared from Boc-Cys(STrt)-OH and dicyclohexylamine according to the method of *Klieger* et al.[21]. Colourless crystals, m.p. > 167°C (dec); $[\alpha]_D^{25}$ −10.4° (*c* 1.0, chloroform). MS (FAB) *m/e* (fragment, %): 677 (MH^+, 7), 243 [$(C_6H_5)_3C^+$, 100], 182 [$(C_6H_{12})_2NH_2^+$, 100]. Anal. calcd: C 69.19, H 7.74, N 4.14, S 9.47; found: C 68.61, H 7.82, N 4.32, S 8.95%.

N-*[*N-*(*tert*-Butyloxycarbonyl)*-S-*(tritylsulfanyl)cysteinyl]glycine benzyl ester, Boc-Cys(STrt)-Gly-OBzl.* The active ester Boc-Cys(STrt)-ONSu was prepared from Boc-Cys(STrt)-OH and *N*-hydroxysuccinimide (HONSu) according to the method of *Anderson* et al.[22], and was reacted with H-Gly-OBzl.HOTos. Thus, 147 mg (0.44 mmol) of the latter and 258 mg (0.44 mmol) of Boc-Cys(STrt)-ONSu were dissolved in 10 ml DMF. The apparent pH was raised to 7 by the addition of diisopropylethylamine and the solution was left standing for 10 min at room temperature. On the addition of 50 ml water, an emulsion formed. The mixture was extracted with chloroform until the aqueous layer remained clear. The combined chloroform extracts were dried over sodium sulfate and evaporated *in vacuo*. The product was purified by column chromatography on silica using the eluent chloroform/methanol 19:1 (v/v). Yield 201 mg (72%) of an amorphous white solid, homogeneous as judged by TLC and NMR; $[\alpha]_D^{25}$ −57.5° (*c* 1, DMF). TLC: R_f 0.90 (chloroform/methanol 19:1). ^1H NMR ($CDCl_3$) δ (ppm): 1.41 (9H, s; $C(CH_3)_3$), 2.00 (2H, d, *J* 6.6 Hz; β-CH_2 of Cys), 4.01 (2H, d, *J* 5.2 Hz; α-CH_2 of Gly), 5.00 (1H, d, *J* 8.1 Hz; urethane NH), 5.16 (2H, s; benzyl CH_2), 6.66 (1H, t, *J* 5.2 Hz; amide NH), 7.2–7.5 (20H, m; benyzl + trityl ArH); α-CH signal of Cys largely obscured by α-CH_2 signal of Gly. MS (FAB) *m/e* (fragment, %): 665 (MNa^+, 10), 643 (MH^+, 15), 275 [$(C_6H_5)_3CS^+$, 55], 243 [$(C_6H_5)_3C^+$, 100], 91 [$(C_6H_5)CH_2^+$, 45].

N-*[*N-*Acetyl*-S-*(tritylsulfanyl)cysteinyl]glycine benzyl ester, Ac-Cys(STrt)-Gly-OBzl.* Compound Boc-Cys(STrt)-Gly-OBzl (50 mg, 0.076 mmol) was dissolved in 2 ml trifluoroacetic acid (TFA) at room temperature. The yellow solution was left standing for 10 min to remove the Boc group. Then the TFA was evaporated *in vacuo* to leave a yellow syrup, which was dissolved in 5 ml DMF. The acidic solution was neutralized with diisopropylethylamine to apparent pH 5. Next, 0.5 ml Ac_2O and 1 ml pyridine were added and the solution was left standing at room temperature overnight for complete acetylation. The mixture was partitioned between ethyl acetate and water. The aqueous phase was extracted twice with ethyl acetate. The organic layers were combined, washed with water, dried over sodium sulfate, and evaporated in vacuo. The oily residue was triturated with ether and dried in vacuo affording a yellowish glass, homogeneous by TLC and NMR. Yield 90%. $[\alpha]_D^{25}$ +44.4° (*c* 0.32, DMF). TLC: R_f 0.80 (chloroform/methanol 7:1). ^1H NMR ($CDCl_3$) δ (ppm): 1.87 (3H, s, CH_3), 1.90 (2H, d, *J* 6.0 Hz; β-CH_2 of Cys), 3.92 (2H, d, *J* 5.5 Hz; α-CH_2 of Gly), 4.33 (1H, d t, *J* 7.5 and 6.0 Hz; α-CH of Cys), 5.08 (2H, s, benzyl CH_2), 5.97 (1H, d, *J* 7.5 Hz; amide NH), 6.64 (1H, t, *J* 5.4 Hz; urethane NH), 7.2–7.5 (20H, m, ArH).

Table I R_f *values of compounds Ac-Cys(SR)-OBzl and decomposition products on TLC.*

Compound	R_f value ($CHCl_3$/MeOH 38:1)
Ac-Cys(SEt)-OBzl	0.43
Ac-Cys(S*t*Bu)-OBzl	0.49
Ac-Cys(STrt)-OBzl	0.51
Ac-Cys(H)-OBzl	0.32
Trt-OH	0.82
Trt-SH	0.88

Stability towards trifluoroacetic acid (TFA)

Each of the benzyl esters Ac-Cys(SR)-OBzl was used to prepare a solution (10 mg/ml) in pure TFA. The trityl derivative (R = Trt) gave a yellow colour upon dissolution, probably caused by the formation of a small amount of trityl cations. The three clear solutions were left standing at room temperature. At intervals of 30 min, aliquots of 2 μl were spotted onto TLC plates and chromatographed using the eluent chloroform/methanol, 38:1 (v/v). No decomposition was observed, during 5 h of standing. After this period, gradual decomposition of the substrate was indicated by the appearance of new spots on TLC. The R_f values of the starting compounds and identified decomposition products are given in Table I.
The stability experiment was repeated with the cocktail TFA/EDT/H_2O, 95:2.5:2.5 (v/v/v). In this case, decomposition was observed to begin after 4 h standing.

Reduction by thiols and phosphines

The following three reducing reagents were prepared:
1) 2-sulfanylethanol/DMF 1:10 (v/v);
2) 0.17 ml tributylphosphine in a mixture of trifluoroethanol (1 ml) and water (0.02 ml);
3) 3.4 ml tributylphosphine in 20 ml acetic acid/water, 4:1 (v/v) containing 0.87 g ammonium acetate.

Of each of the three derivatives Ac-Cys(SR)-OBzl, solutions (10 mg/ml) were prepared in each of the three reducing solutions. TLC, after 10 min standing at room temperature, indicated quantitative deprotection of the thiol function, invariably yielding the derivative Ac-Cys(H)-OBzl (Table I).

Stability towards piperidine in DMF

Polarimetric experiments on the behavior of the L-cysteine derivatives in piperidine 25% in DMF were performed as described under 'Results and discussion'. The results are depicted in Figure 1.

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