(ether/hexane, 1:5), affording 66 mg (87%) of the title compound, $[\alpha]^{23}_D$ = -20.1° (c = 1, EtOAc). That obtained from (-)-erythromycin[16] had $[\alpha]^{23}_D$ = -26.0° (c = 1, EtOAc). IR ($CHCl_3$): 3600-3350, 2980, 2940, 2840, 1450, 1370, 1160, 1055, 1000, 920 cm^{-1}. NMR: δ 4.48 (dd, J = 2, 10 Hz, 1 H), 3.58 (dq, J = 9, 7 Hz, 1 H), 3.36 (s, 3 H), 3.23 (s, 3 H), 2.95 (dd, J = 9, 9 Hz, 1 H), 2.23 (dd, J = 2, 14 Hz, 1 H), 2.10 (d, J = 9 Hz, OH), 1.36 (dd, J = 10, 14 Hz, 1 H), 1.30 (d, J = 7, 3 H), 1.21 (s, 3 H). MS, chemical ionization m/e (rel intensity): 191 (M^+ + 1, 11), 159 (100), 127 (20), 109 (4).

(-)-β-Methyl Mycaroside (17). (-)-14 (0.27 g, 1.1 mmol) prepared from 11 was reduced (75 mL of liquid NH_3, 0.3 g of Li, 6 mL of t-BuOH, 20 mL of THF) and acidified (CH_3OH, PPTS) as described in the preparation of methyl (-)-cladinoside to yield 0.16 g (82%) of 17, $[\alpha]^{25}_D$ = -52.8° (c = 1, $CHCl_3$) (lit.[18] +54° for naturally derived material). NMR: δ 4.62 (dd, J = 2.1, 9.4 Hz, 1 H), 3.58 (dq, J = 9, 7 Hz, 1 H), 3.42 (s, 3 H), 3.02 (br d, J = 9 Hz, 1 H), 2.50, 2.30 (br s, OH), 1.96 (dd, J = 2.1, 14 Hz, 1 H), 1.55 (dd, J = 9.4, 14 Hz, 1 H), 1.30 (d, J = 7 Hz, 1 H), 1.24 (s, 3 H).

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Acknowledgment. This work was supported by a grant (GM37066) from the National Institutes of Health, for which we are grateful.

Registry No. **1a**, 69016-02-0; **1b**, 120313-15-7; **2a**, 120313-17-9; **2b**, 120313-18-0; **2c**, 120313-19-1; **2d**, 120313-20-4; **3d**, 120313-21-5; **4a**, 120313-16-8; **4b**, 120313-14-6; **5a**, 120313-22-6; **5b**, 120313-24-8; **6a**, 120313-23-7; **6b**, 120313-25-9; **6c**, 120313-26-0; **6d**, 120313-27-1; **7**, 120313-28-2; **8**, 106470-99-9; **9a**, 120313-31-7; **9b**, 120313-29-3; **10a**, 120313-32-8; (±)-**11**, 120313-30-6; (+)-**11**, 120313-35-1; (+)-**11** aldose derivative, 120313-36-2; **12a**, 120313-34-0; **12b**, 120313-33-9; (*E*)-**13**, 120313-38-4; (*Z*)-**13**, 120313-37-3; **14**, 120313-39-5; (-)-**14**, 120313-41-9; **15**, 120313-40-8; **16**, 57794-93-1; **17**, 38411-52-8; PhCHO, 100-52-7; $(CH_3)_2CHCHO$, 78-84-2; CH_3CHO, 75-07-0; *trans*-$CH_3CH{=}CHCHO$, 123-73-9; $CH_2{=}CHCHO$, 107-02-8; $Ph_2POCHLiOMe$, 83532-12-1; phenoxyacetic acid, 122-59-8; *N,N*-diisopropyloxamate, 120313-13-5; 2-furancarboxaldehyde, 98-01-1.

Supplementary Material Available: Tables of crystal structure data, atomic coordinates, bond lengths, bond angles, anisotropic parameters, and hydrogen atom coordinates for **9a** (11 pages). Ordering information is given on any current masthead page.

Synthesis of Peptides Containing *S*-(*N*-Alkylcarbamoyl)cysteine Residues, Metabolites of *N*-Alkylformamides in Rodents and in Humans

Michael D. Threadgill*,[1] and Adrian P. Gledhill

Cancer Research Campaign Experimental Chemotherapy Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, U.K.

Received August 24, 1988

Hydrochloride salts of *S*-(*N*-methylcarbamoyl), *S*-(*N*-ethylcarbamoyl), and *S*-(*N,N*-dimethylcarbamoyl) derivatives of cysteine, *N*-acetylcysteine, and cysteinylglycine have been prepared. *S*-(*N*-Methylcarbamoyl)glutathione hydrochloride has also been synthesized. Protecting groups for amino and carboxylic acid functions were selected for their ability to solubilize the peptides in dichloromethane in which solvent the thiols were treated with alkyl isocyanates and with *N,N*-dimethylcarbamoyl chloride. Removal of *S*-(amidomethyl) protecting groups using mercury(II) acetate was found to cause some loss of *N*-(*tert*-butoxycarbonyl) groups. Elimination of disulfide was evident during coupling of disulfide derivatives of cysteine using mixed anhydride methods but not with a carbodiimide coupling agent. Mixed disulfide protections were reductively cleaved by propane-1,3-dithiol. Many of the deprotected *S*-carbamoyl amino acids and peptides are metabolites of the corresponding *N*-alkylformamides in rodents and in humans.

N-Substituted formamides have a variety of biological activities, both beneficial and adverse. *N*-Methylformamide (NMF; **1a**) has been found to be an antitumor agent in experimental systems[2] and also to be an hepatotoxin,[3,4] whereas *N*-ethylformamide (**1b**) has little or no anticancer activity[2] but is also toxic to the liver.[5] *N,N*-Dimethylformamide (DMF; **1c**), however, displays both of these effects only weakly in rodents.[2,5,7] We have recently shown that the two secondary amides are metabolized to the corresponding mercapturic acids **5a,b** in mice[8] and that this metabolic pathway (Scheme I) is implicated[5,8,9] in the hepatotoxicity of **1a,b**. A mass spectrometric study[9] has also enabled the characterization of the glutathione derivative **2a** as a metabolite of **1a** in mice. *N*-Acetyl-*S*-(*N*-methylcarbamoyl)cysteine (**5a**) has also been detected[7] in the urine of mice and humans exposed to **1c**, with an apparent parallel between the amount excreted and the extent of hepatotoxicity. Selective oxidation of the formyl group of *N*-methylformamide has been reported rarely in

(1) Author to whom correspondence should be addressed at: Medical Research Council Radiobiology Unit, Chilton, Didcot, Oxfordshire OX11 0RD, U.K.

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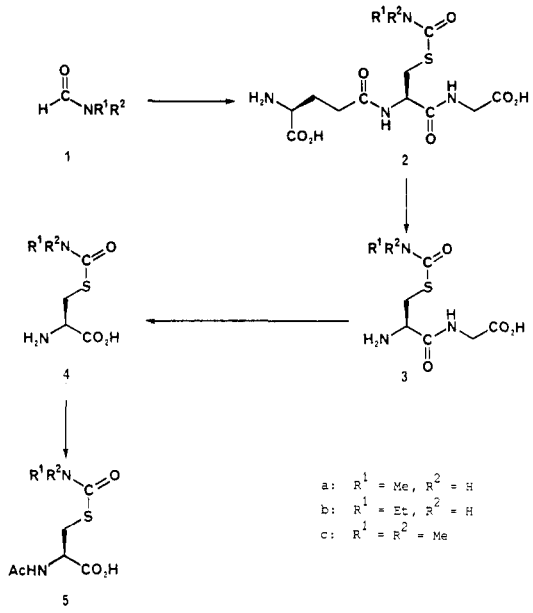
Scheme I. Partial Proposed Routes of Metabolism of Formamides in the Mouse



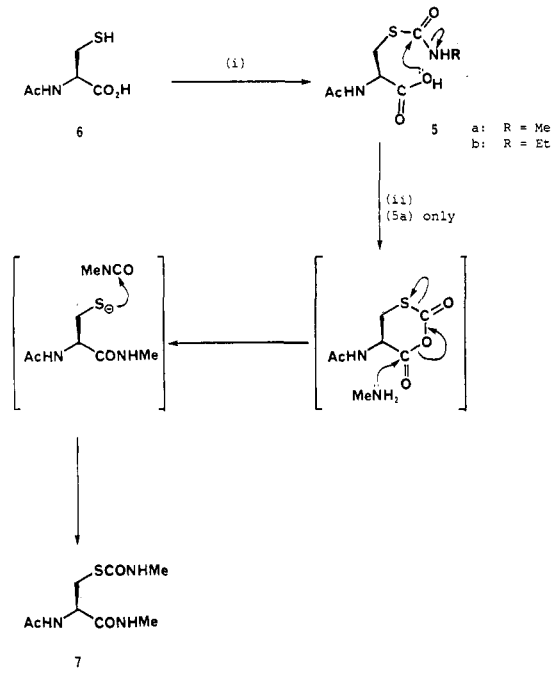
[a] Compounds **2a** and **5a,b** have been shown to be metabolites.

the chemical literature, oxidants being limited to transition metal ions,[12] ketones (under drastic conditions in the Leukart reaction),[13] and elemental selenium;[14] the latter gives a carbamoylating intermediate possibly analogous to that formed in vivo. The synthesis of the proposed metabolic intermediates and the *N,N*-dimethyl analogues **2–5** was therefore of importance for the study of their chemical, biochemical, and toxicological properties.

The preparation of *S*-(*N*-ethylcarbamoyl)cysteine (**4b**) has been reported by Guttmann[15] who used it as an S-protected cysteine during syntheses of glutathione (**21**) and oxytocin. In our laboratory, however, neither cysteine nor its N-acetylated analogue **6** reacted smoothly with methyl isocyanate in DMF according to this method.[15] Treatment of *N*-acetylcysteine (**6**) with methyl isocyanate or with ethyl isocyanate in anhydrous pyridine at 0 °C gave **5a,b**, respectively, in good yield.[8] Higher temperatures (>15 °C) led to exclusive formation of the corresponding *N*-methylamide **7** from **6** and methyl isocyanate. A route of the type shown in Scheme II is likely to be involved, although direct formation of N-substituted amides by treatment of carboxylic acids with isocyanates has been reported.[16]

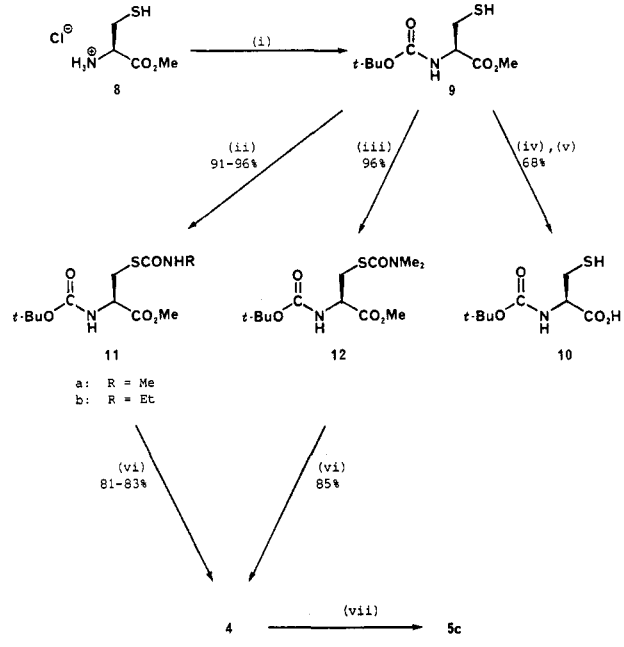
Owing to the reactivity of isocyanates and dimethylcarbamoyl chloride with polar solvents, protecting groups for amino and carboxyl functions of **6** and cysteine were sought, which would confer good solubility in the less polar and nonnucleophilic organic solvents. Facile removal upon mild treatment with acid was also required in view of the lability of thiocarbamates to base.[8,15] A suitably protected cysteine derivative **9** was prepared in high yield by the selective *tert*-butoxycarbonylation of cysteine methyl ester **8** with di-*tert*-butyl dicarbonate in dichloromethane in the presence of a tertiary amine base (Scheme III). No reaction of the thiol was observed under these conditions.

Scheme II. Reactions of *N*-Acetylcysteine (6) with Alkyl Isocyanates in Pyridine[a]



[a] (i) RNCO/pyridine/0 °C; (ii) MeNCO/pyridine/25 °C.

Scheme III. Syntheses of *S*-(*N*-Alkylcarbamoyl)cysteines 4 and the *N*-Acetyl Analogue 5c[a]



[a] (i) $(Bu^tOCO)_2O/Et_3N/CH_2Cl_2$; (ii) RNCO/($Pr^i_2NEt$ or Et_3N)/CH_2Cl_2; (iii) Me_2NCOCl/pyridine/CH_2Cl_2; (iv) NaOH/MeOH; (v) H^+; (vi) 9 M aqueous HCl; (vii) Ac_2O/pyridine (**4c** only).

The resulting N-protected ester **9** could be hydrolyzed smoothly with methanolic sodium hydroxide to *N*-(*tert*-butoxycarbonyl)cysteine (**10**). Now suitably protected and solubilized, **9** was carbamoylated smoothly by using the appropriate isocyanate or dimethylcarbamoyl chloride in dichloromethane. Deprotection of the resulting *S*-carbamoyl compounds **11a,b** and **12** to the desired cysteine derivatives **4** was effected by dissolution in concentrated hydrochloric acid for a prolonged period, a procedure to which the thiocarbamate proved largely inert. Subsequent acetylation of the *N,N*-dimethyl compound **4c** with acetic

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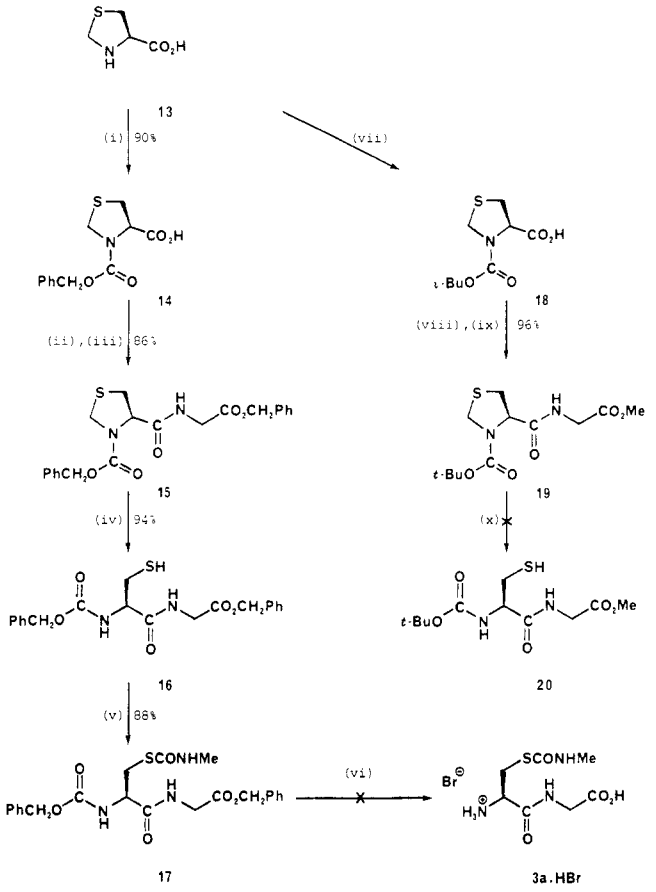
Scheme IV. Attempted Approaches to *S*-(*N*-Alkylcarbamoyl)cysteinylglycines from 1,3-Thiazolidine-4-carboxylic Acid (13)[a]



[a] (i) $PhCH_2OCOCl/KOH/H_2O$; (ii) dicyclohexylcarbodiimide/CH_2Cl_2; (iii) $GlyOCH_2Ph{\cdot}HOTs/Pr^i_2NEt/CH_2Cl_2$; (iv) $Hg(OAc)_2$/$HOAc/H_2O$/60 °C; (v) $MeNCO/Pr^i_2NEt/CH_2Cl_2$; (vi) HBr/HOAc; (vii) $(Bu^tOCO)_2O/NaOH/Et_2O/H_2O$; (viii) Bu^tOCOCl/Et_3N/CH_2Cl_2; (ix) $GlyOMe{\cdot}HCl/Et_3N/CH_2Cl_2$; (x) $Hg(OAc)_2$/various conditions.

anhydride in pyridine gave the mercapturic acid **5c**.

For the syntheses of the dipeptide series of compounds **3**, initial approaches to a suitable amino- and carboxy-protected and solubilized cysteinylglycine centered on the preparation of the *N*-(benzyloxycarbonyl)cysteinylglycine esters **16** and **17**. Protection of the thiol moiety with an amidomethyl group was an attractive proposition as such groups are reported[17] to be labile to mild acid in the presence of mercuric or cadmium ions, conditions to which *N*-(benzyloxycarbonyl) groups and carboxylic esters are stable. An intramolecular version of an *S*-(amidomethyl)-protected cysteine is available via acylation of 1,3-thiazolidine-4-carboxylic acid (thiaproline; **13**) (Scheme IV). Both *N*-(benzyloxycarbonyl)thiazolidinecarboxylic acid **14** and the *N*-(*tert*-butoxycarbonyl) analogue **18** proved to be excellent substrates for the carbodiimide method of peptide coupling; *N*-[[*N*-(benzyloxycarbonyl)-1,3-thiazolidin-4-yl]carbonyl]glycine benzyl ester (**15**) and the methyl ester **19** of the corresponding *N*-(*tert*-butoxycarbonyl) dipeptide were prepared in high yield. Treatment of the benzyl compound **15** with mercury(II) ions in warm aqueous acetic acid caused deprotection at sulfur to give the thiol **16**, whereas the *N*-(*tert*-butoxycarbonyl) group of **19** was not stable to any

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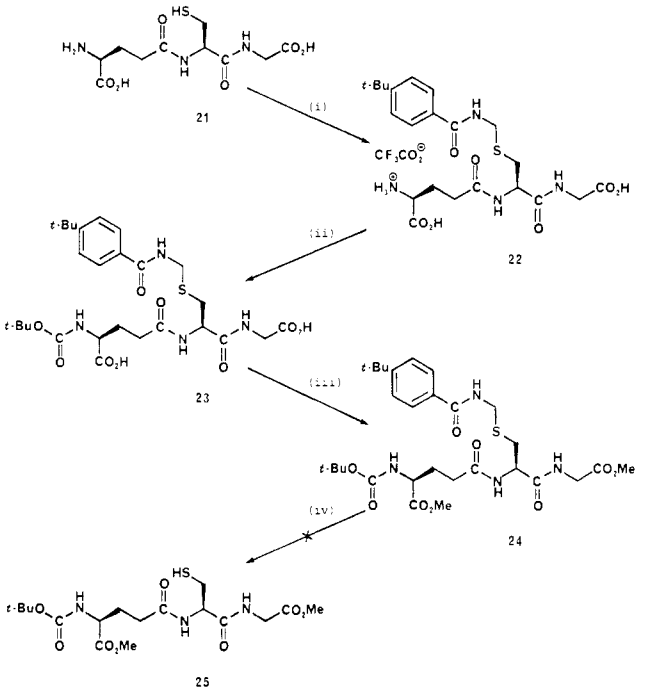
Scheme V. Loss of the *N*-(*tert*-Butoxycarbonyl) Group during Removal of an *S*-(Amidomethyl) Group from Glutathione[a]



[a] (i) 4-*tert*-Butyl-*N*-(hydroxymethyl)benzamide/CF_3CO_2H;[18] (ii) $(Bu^tOCO)_2O/Et_3N/Et_2O/H_2O$; (iii) CH_2N_2/Et_2O; (iv) $Hg(OAc)_2$/MeOH or $HOAc/H_2O$.

similar conditions which would hydrolyze the "*S*-amidomethyl" function; thus **20** is unavailable by this route (Scheme IV). The dibenzyl compound **16** reacted with methyl isocyanate to give the *S*-(*N*-methylcarbamoyl)-protected dipeptide **17** (Scheme IV). However, the *S*-carbamoyl moiety was found not to be stable to hydrogen bromide in acetic acid in this case, in contrast to the analogous deprotections reported to be useful by Guttmann.[15] Only traces of the correct *S*-(*N*-methylcarbamoyl)cysteinylglycine (**3a**) could be detected by mass spectrometry (cesium ion liquid secondary ionization MS) in the crude product mixtures. In the tripeptide series, a sample[18] of the S-protected glutathione **22** was *tert*-butyloxycarbonylated at nitrogen, giving **23**, and the dimethyl ester **24** was formed (Scheme V). Again, no conditions could be found in which the *N*-BOC function outlasted the *S*-amidomethyl group. Alternative methods of removal of amidomethyl protecting groups either destroyed the *N*-BOC group (Cd^{2+}/acetic acid) or were considered to be inappropriate.[19,20]

A conceptually different form of temporary inactivation of thiols as irreversible nucleophiles is to form either symmetrical or mixed disulfides from which the thiol can be unmasked by reduction. Owing to some difficulties in separating the coformed *N*,*N*′-dicyclohexylurea from the products from the carbodiimide couplings above, a cleaner coupling system was investigated for the reactions of the symmetrical disulfides, *N*,*N*′-bis(*tert*-butoxycarbonyl)-cystine (**26b**) and *N*,*N*′-bis(benzyloxycarbonyl)cystine (**26c**), and the unsymmetrical disulfide *N*-(*tert*-butoxy-

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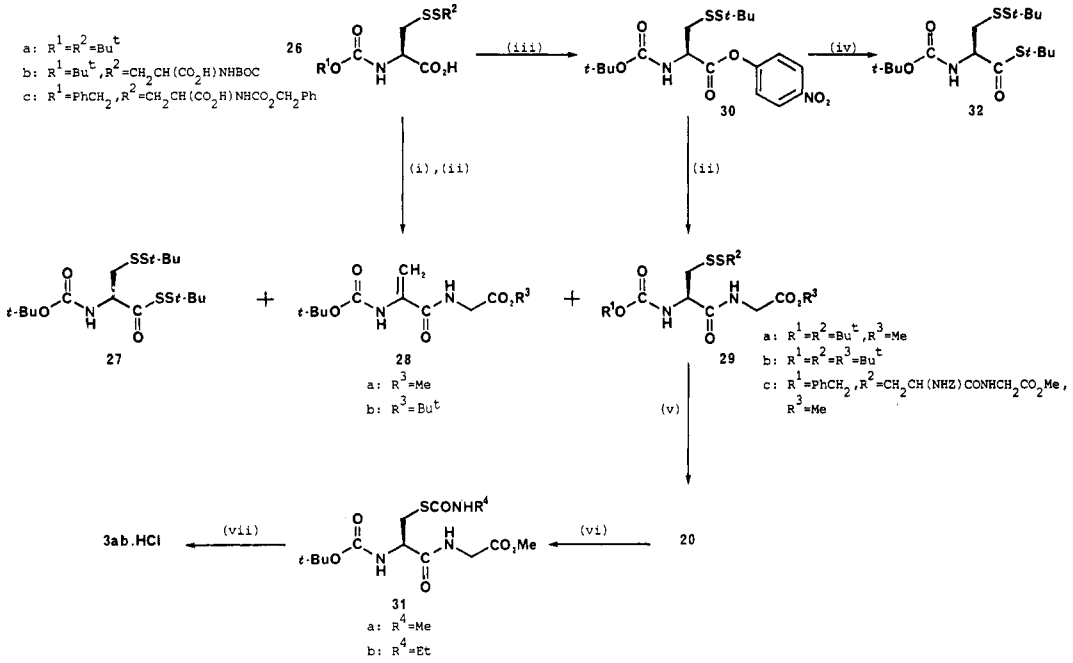
Scheme VI. Preparation of *S*-(*N*-Alkylcarbamoyl)cysteinylglycines 3 and Elimination of RSS⁻ during Mixed-Anhydride Coupling[a]



[a] (i) $Bu^iOCOCl/(Pr^i_2NEt$ or $Et_3N)/CH_2Cl_2$; (ii) $X^-H_3N^+CH_2CO_2R^3/Pr^i_2NEt$ or Et_3N/CH_2Cl_2; (iii) dicyclohexylcarbodiimide/4-nitrophenol/CH_2Cl_2 (**26a** only); (iv) $Bu^tSH/Pr^i_2NEt/CH_2Cl_2$; (v) $HSCH_2CH_2CH_2SH/Pr^i_2NEt$/tetrahydrofuran; (vi) $R^4NCO/Pr^i_2NEt/CH_2Cl_2$; (vii) 9 M aqueous HCl.

carbonyl)-*S*-(*tert*-butylthio)cysteine (**26a**) (Scheme VI). The "mixed-anhydride" method has been widely used to form amide bonds and mixed carboxylic–carbonic anhydrides derived from isobutyl chloroformate have been reported[19] to be particularly effective. In the present case, *N*,*N*′-di-BOC-cystine **26b** was treated with isobutyl chloroformate and glycine methyl ester at ambient temperature. The sole isolable product (in moderate yield) was characterized as the protected dehydroalanylglycine **28a**. This elimination also took place to a minor extent (giving **28b**) during the coupling of the unsymmetrical disulfide **26a** with glycine *tert*-butyl ester, as shown by NMR of the crude product. Unwanted elimination of an activated sulfur group from a cysteinyl peptide has been reported[22] to occur, for example, when the *S*-(dimethylthionophosphino) protecting group is employed. However, the elimination of an apparently unactivated disulfide during a mixed-anhydride coupling procedure is undocumented. The mechanism of the process is unclear. Also formed during the reaction of **26a** with glycine *tert*-butyl ester, isobutyl chloroformate, and tertiary amine base were the expected product **29b** and a low yield of an unstable oily material tentatively characterized as the *S*-(*tert*-butylthio) thioester **27**. This compound has similar, but distinct, spectroscopic properties from the analogous *tert*-butyl thioester **32** prepared from the 4-nitrophenyl ester **30** (see below). Thioester **27** may well result from acylation of *tert*-butyl disulfide anion by the mixed anhydride, and its formation implies that the elimination of Bu^tSS^- is taking place while the anhydride is still unreacted with the glycine ester and possibly before the amino acid ester is added. In contrast, the coupling of **26a** and *N*,*N*′-bis(benzyloxycarbonyl)cystine (**26c**) with glycine methyl ester by this mixed-anhydride method gave only the protected dipeptides **29a**,**c**. During some experiments, however, the crude products were shown by NMR to be contaminated with the corresponding 2-methylprop-1-yl esters. Compound **29c** had properties identical with those described by Zahn and Schmidt[23] and by Zervas et al.[24] but not to those claimed by Dadič et al.[25] for this material. A more reliable synthesis of the potentially useful **29a**, with *N*-, S-, and O-protecting groups separately removable under conditions of anhydrous acid, reduction, and aqueous base, was therefore sought.

N-BOC-*S*-(*tert*-butylthio)cysteine (**26a**) was coupled in good yield with 4-nitrophenol by the carbodiimide method to give the "active" ester **30** (Scheme VI). This ester reacted smoothly with 2-methylpropane-2-thiol to give the thioester **32** alluded to above. The reaction with glycine esters was similarly rapid and facile, affording the protected dipeptide methyl and *tert*-butyl esters **29a**,**b** in almost quantitative yield. Of these dipeptide esters, **29a** is the more synthetically useful, being the precursor of S-substituted cysteinylglycines and, after further elaboration, of *S*-(*N*-methylcarbamoyl)glutathione (**2a**).

In the approach to **3a**,**b**, selective reduction of the unsymmetrical disulfide moiety of **29a** was required. The reductive removal of mixed disulfide protecting groups during peptide synthesis has been reported to be effected by sodium borohydride (although no details of yield or method were given)[26] and by treatment with thiophenol.[27] In the present work, reduction of **29a** by treatment with ethanolic sodium borohydride was not successful and proceeded only very poorly with thiophenol. A dithiol, such as propane-1,3-dithiol, should be a more effective reagent for this purpose. After initial intermolecular di-

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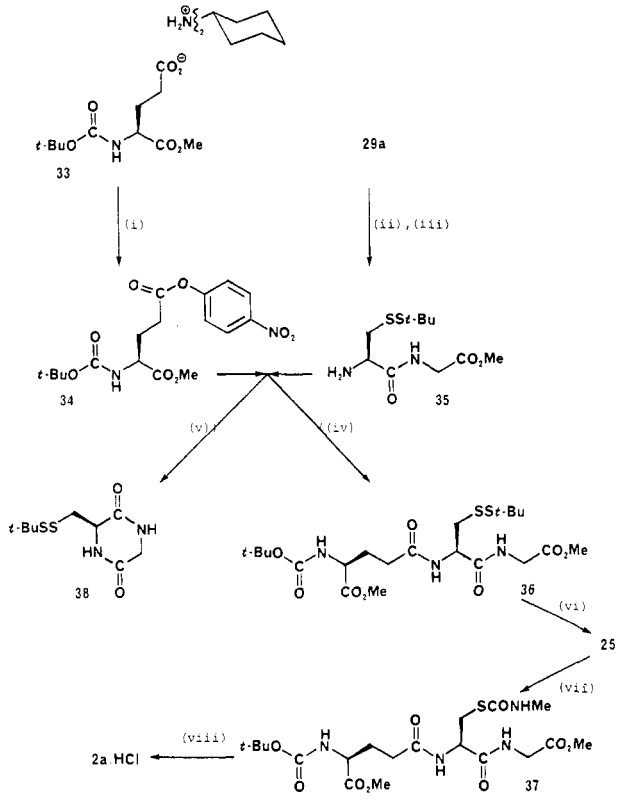
Scheme VII. Synthesis of *S*-(*N*-Methylcarbamoyl)glutathione (2a)[a]



[a] (i) Dicyclohexylcarbodiimide/4-nitrophenol/CH_2Cl_2; (ii) CF_3CO_2H; (iii) base; (iv) Pr^i_2NEt/4-(dimethylamino)pyridine/tetrahydrofuran; (v) Pr^i_2NEt/tetrahydrofuran; (vi) $HSCH_2CH_2CH_2SH/Pr^i_2NEt$/tetrahydrofuran; (vii) MeNCO/Et_3N/CH_2Cl_2; (viii) 9 M aqueous HCl.

sulfide exchange (with exogenous thiolate presumably attacking the less sterically hindered cysteinyl sulfur rather than that adjacent to the *tert*-butyl group), a subsequent intramolecular disulfide exchange would lead to the required *N*-BOC-cysteinylglycine with a free thiol for further reaction with carbamoylating agents. This process was, indeed, found to be satisfactory, in that treatment of the protected dipeptide **29a** with an excess of propane-1,3-dithiol in tetrahydrofuran gave the dipeptide thiol **20** in good yield. The rate of the process was considerably enhanced by addition of a hindered tertiary amine to aid intermediate formation of the appropriate thiolate anions. The byproduct, 1,2-dithiacyclopentane, has been reported[28-30] to be unstable and to give rise to intractable insoluble polymers, but no such degradation was evident during any of these reductive deprotection steps. The dipeptide thiol **20** reacted with methyl and ethyl isocyanates in the presence of catalytic tertiary amine base to give the thiocarbamates **31a,b**, respectively. Deprotection of the N- and C-terminals of these dipeptides was achieved in a "one-pot" process involving dissolution of **31** in concentrated aqueous hydrochloric acid. Rapid evolution of gaseous carbon dioxide and 2-methylpropene indicated that the removal of the *tert*-butoxycarbonyl group was complete within 5 min, whereas hydrolysis of the methyl ester took several hours (as shown by proton NMR monitoring of an experiment with 35% deuterium chloride in deuterium oxide as the hydrolytic medium). Evaporation of the reagents gave the unprotected *S*-(*N*-alkylcarbamoyl)cysteinylglycines (**3a,b**) as the hydrochloride salts.

The selective deprotection (using trifluoroacetic acid) of the amino function of **29a** was exploited in the successful synthetic approach to the glutathione derivative **2a** (Scheme VII). The evaporation residue comprised the trifluoroacetate salt of the amine **36** from which the free nucleophile could be liberated for reaction with an appropriate *N*- and α-carboxyl-protected glutamic acid bearing an activated γ-carboxyl function. Following the success of the corresponding protection and activation strategies in the dipeptide series, the novel *N*-(*tert*-butoxycarbonyl)glutamic acid α-methyl γ-(4-nitrophenyl) diester **34** was the chosen electrophile for the coupling reaction and was synthesized as follows. *N*-BOC-glutamic acid α-methyl ester dicyclohexylammonium salt (**33**) was prepared generally by the method of Schröder and Klieger.[31] The γ-carboxylic acid moiety was then coupled with 4-nitrophenol by the dicyclohexylcarbodiimide method, giving the diester **34** in excellent yield after chromatography. The correct regioisomeric identity of **34** as being the α-methyl γ-(4-nitrophenyl) diester was confirmed, after satisfactory elemental and spectroscopic analyses, by the significant difference between the melting point of this material and the reported[32] melting point of *N*-(*tert*-butyloxycarbonyl)glutamic acid α-(4-nitrophenyl) γ-methyl diester. The reaction of the "active ester" **34** with **35** was efficient but was markedly slower than the acylation of glycine esters by the protected cysteine 4-nitrophenyl ester **30**, probably owing to the bulky nature of the dipeptide nucleophile. Catalysis by 4-(dimethylamino)-pyridine was required, since its absence led to lower yields of **36** and formation of the diketopiperazine **38**. Reductive deprotection of this tripeptide **36** with propane-1,3-dithiol was again effective, and the thiol **25** was obtained in good yield. As in the case of protected cysteine **9** and the protected cysteinylglycine **20**, treatment with methyl isocyanate in dichloromethane in the presence of *N*,*N*-diisopropylethylamine gave high yields of the corresponding *N*-BOC-*S*-(*N*-methylcarbamoyl)glutathione dimethyl ester **37**. Deprotection of the amino group and the carboxylic acids was again carried out in high yield with aqueous acid. Using deuterated solvent and reagent, the reaction of the *S*-(*N*-methylcarbamoyl)-protected glutathione **37** with acid was monitored by ^1H NMR spectroscopy, revealing that, as expected, the *tert*-butyl and *N*-carboxy groups were eliminated immediately upon dissolution. The hydrolyses of the methyl esters were seen to be almost complete in 20 min and 20 h, respectively, although it is not clear which ester of the intermediate protonated S-substituted glutathione diester is the more labile.

Optical rotations were measured for 12 representative compounds. In no case was complete racemization observed during the various chemical transformations. Significant partial loss of stereochemical integrity is also unlikely, as there was no evidence of other diastereoisomers in the NMR spectra of the substituted glutathiones **2a**, **25**, and **36**.

Evaluation of the biochemical and biological properties of the synthetic *S*-(*N*-alkylcarbamoyl)cysteines and peptides is in progress and will be described elsewhere. Interestingly, the free base of *S*-(*N*-ethylcarbamoyl)cysteine together with the analogous *S*-[*N*-(2-chloroethyl)carbamoyl]cysteine have been reported to have antitumor,[33]

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antimicrobial,[34] and cytotoxic[33] properties. In addition to the preparation of the thiocarbamates **2a**, **3a,b**, **4**, and **5**, the successful synthetic route described above involves the preparation, in good yield, of the glutathione derivative **40**, with separately removable protecting groups for amino, thiol, and carboxyl functions, and furnishes a potentially highly useful intermediate in the chemical synthesis of of S-substituted glutathiones. These compounds are involved[35-38] in the metabolic activation or detoxification of many xenobiotic organic compounds.

Experimental Section

IR spectra were recorded as liquid films (except where noted) with Perkin–Elmer 1310 or Philips PU9516 spectrometers. NMR spectra were obtained (in $CDCl_3$ except where noted otherwise) at 60 MHz with Varian EM360A or JEOL PMX60SI spectrometers, at 300 MHz with Varian XL300 or Bruker AC300 spectrometers, and at 400 MHz with a Bruker WH400 instrument. Optical rotations were measured with an Optical Activity Ltd. AA-10 polarimeter. Electron impact mass spectra were furnished by VG Micromass 12B and ZAB-E instruments. Cesium ion promoted liquid matrix secondary ionization mass spectra (LSIMS) were obtained with a Kratos MS-50S mass spectrometer equipped with a 23-kG magnet and a postacceleration detector operating at −10 kV. Samples were dissolved in a glycerol matrix containing HCl (to increase the intensity of MH^+ species), and ionization was achieved by bombardment with a 1.0-μA primary beam of Cs^+ ions.[39] Melting points are corrected. Reactions were carried out at ambient temperature except where indicated otherwise. Organic solutions were dried by treatment with anhydrous Na_2SO_4 and filtration. Solvents were evaporated under reduced pressure. All chiral amino acids were of the L configuration. THF refers to tetrahydrofuran.

***S*-(*N*-Methylcarbamoyl)glutathione Hydrochloride (2a).** Compound **25** (435 mg, 1 mmol) was stirred for 3 days with methyl isocyanate (285 mg, 5 mmol) and *N,N*-diisopropylethylamine (129 mg, 1 mmol) in CH_2Cl_2 (10 mL). The solution was washed with ice-cold aqueous H_2SO_4 (1 M), H_2O, and saturated aqueous NaCl before being dried. Evaporation of the solvent afforded **37** (400 mg, 81%) as a colorless gum: IR 3300, 1720, 1700, 1680 cm^{-1}; NMR δ 1.43 [9 H, s, $C(CH_3)_3$], 1.9–2.5 (4 H, m, Glu β-CH_2 and Glu γ-CH_2), 2.83 (3 H, d, J = 5 Hz, NCH_3), 3.3 (2 H, m, Cys β-CH_2), 3.74 (6 H, s, 2 OCH_3), 4.00 (2 H, d, J = 5 Hz, Gly CH_2), 4.24 (1 H, m, Glu α-H), 4.75 (1 H, m, Cys α-H), 5.75 (1 H, d, J = 7 Hz, Glu NH or Cys NH), 7.3–7.7 (2 H, m, 2 NH), and 8.41 (1 H, ca. q, J = ca. 5 Hz, N*H*CH_3); mass spectrum, m/z 335 [$(M - C_4H_8 - CO_2 - MeNCO)^+$], 304 [$(M - C_4H_8 - CO_2 - NHCH_2CO_2CH_3)^+$], 247, 57 (100%). This material (246 mg, 0.5 mmol) was treated with 35% DCl in D_2O (2 mL) at ambient temperature for 4 days before excess reagent was evaporated. H_2O (5 mL) was added. The gummy evaporation residue was triturated with acetone and then with anhydrous THF to afford **2a** (160 mg, 65%) as a very hygroscopic pale buff solid of indefinite melting point (dec) which did not give a satisfactory microanalysis but was shown to be >95% pure by NMR: IR (Nujol) 2900–2600, 1710, 1690 cm^{-1}; NMR (D_2O) δ 2.10 (2 H, m, Glu β-CH_2), 2.45 (2 H, m, Glu γ-CH_2), 2.75 (3 H, s, NCH_3), 3.15 (1 H, dd, J = 14 Hz, J = 7 Hz) and 3.40 (1 H, dd, J = 14 Hz, J = 4 Hz) (Cys β-CH_2), 3.75 (1 H, t, J = 7 Hz, Glu α-H), 3.95 (2 H, s, Gly CH_2), and 4.50 (1 H, m, Cys α-H).

***N*-[*S*-(*N*-Methylcarbamoyl)cysteinyl]glycine Hydrochloride (3a).** Compound **31a** (200 mg, 573 μmol) was treated with aqueous HCl (9 M) for 1 week. Trituration of the gummy evaporation residue with acetone afforded **3a** (143 mg, 92%) as an hygroscopic white powder which decomposed on heating at >70 °C: NMR (D_2O) δ 2.70 (3 H, s, NCH_3), 3.2–3.35 (2 H, m, Cys β-CH_2), 4.00 (2 H, s, Gly CH_2), and 4.15 (1 H, m, Cys α-H); mass spectrum (FAB), m/z 236 [$(M + H)^+$]. Anal. Calcd for $C_7H_{14}ClN_3O_4S$: C, 30.95; H, 5.2; N, 17.45. Found: C, 30.65; H, 5.5; N, 17.25.

***N*-[*S*-(*N*-Ethylcarbamoyl)cysteinyl]glycine Hydrochloride (3b).** Compound **31b** was treated with aqueous HCl, as for the preparation of **3a** above, to give **3b** (83%) as an hygroscopic white powder which decomposed on heating at >70 °C: NMR (D_2O) δ 1.15 (3 H, t, J = 7 Hz, CH_2CH_3), 2.90 (2 H q, J = 7 Hz, NCH_2CH_3), 3.2–3.4 (2 H, m, Cys β-CH_2), 4.00 (2 H, s, Gly CH_2), and 4.20 (1 H, m, Cys α-H); mass spectrum (FAB), m/z 250 [$(M + H)^+$]. Anal. Calcd for $C_8H_{16}ClN_3O_4S$: C, 33.65; H, 5.65; N, 14.7. Found: C, 33.35; H, 5.9; N, 14.5.

***S*-(*N*-Methylcarbamoyl)cysteine Hydrochloride (4a).** Ester **11a** (1.20 g, 4.1 mmol) was treated with aqueous HCl (9 M; 25 mL) for 1 week. The gummy evaporation residue was triturated with propan-2-ol (30 mL) to give a white solid (100 mg, 15%), which was identified as cysteine hydrochloride. The solvent was evaporated from the supernatant solution to give a gum. Trituration with acetone gave **4a** (730 mg, 83%) as a white powder: mp 186–189 °C dec; IR (Nujol) 3325, 2750 br, 1720, 1660, 1565 cm^{-1}; NMR (400 MHz; D_2O) δ 2.70 (3 H, s, NCH_3), 3.33 (1 H, dd, J = 15.4 Hz, J = 6.4 Hz) and 3.50 (1 H, dd, J = 15.4 Hz, J = 4.0 Hz) ($CHCH_2S$), 4.24 (1 H, dd, J = 6.4 Hz, J = 4.0 Hz, $CHCH_2$), and 4.75 (5 H, br s, HOD); mass spectrum (LSIMS), m/z 179 [$(M + H)^+$]. Anal. Calcd for $C_5H_{11}ClN_2O_3S$: C, 28.0; H, 5.15; N, 13.05. Found: C, 27.7; H, 5.2; N, 12.75.

***S*-(*N*-Ethylcarbamoyl)cysteine Hydrochloride (4b).** Ester **11b** was hydrolyzed with aqueous HCl, as for the preparation of **4a** above, to give **4b** (81%) as a white powder: mp 177–180 °C dec; optical rotation (c = 354 mM in H_2O) $[\alpha]^{21}_{589}$ −56.2°, $[\alpha]^{21}_{578}$ −58.2°; IR (Nujol) 3300, 2750 br, 1725, 1665 cm^{-1}; NMR (60 MHz; D_2O) δ 1.10 (3 H, t, J = 7 Hz, CH_2CH_3), 3.25 (2 H, q, J = 7 Hz, NCH_2), 3.45 (1 H, d, J = 6 Hz) and 3.50 (1 H, d, J = 4 Hz) ($CHCH_2S$), 4.35 (1 H, dd, J = 6 Hz, J = 4 Hz, $CHCH_2$), 4.9 (5 H, br s, HOD); NMR [300 MHz; $(CD_3)_2SO$] δ 1.04 (3 H, t, J = 7.4 Hz, CH_2CH_3), 3.15 (2 H, dq, J = 6.4 Hz, J = 7.4 Hz, NCH_2), 3.27 (1 H, dd, J = 14.8 Hz, J = 5.6 Hz) and 3.37 (1 H, dd, J = 14.8 Hz, J = 4.6 Hz) ($CHCH_2S$), 4.10 (1 H, ca. t, J = ca. 5 Hz, $CHCH_2$), 8.39 (1 H, ca. t, J = ca. 6 Hz, $NHCH_2$), 8.5 (3 H, br, CHN^+H_3); mass spectrum (LSIMS), m/z 193 (100%) [$(M + H)^+$]. Anal. Calcd for $C_6H_{13}ClN_2O_3S$: C, 31.5; H, 5.75; N, 12.25. Found: C, 31.2; H, 5.4; N, 12.0.

***S*-(*N*,*N*-Dimethylcarbamoyl)cysteine Hydrochloride (4c).** Compound **12** was hydrolyzed with aqueous HCl, as for the preparation of **4a** above, to give **4c** a white powder: mp 146–150 °C dec; NMR (D_2O) 3.00 [6 H, s, $N(CH_3)_2$], 3.2–3.4 (2 H, m, $CHCH_2S$), 3.50 (1 H, dd, J = 6 Hz, J = 4 Hz, $CHCH_2$), and 4.7 (5 H, br s, HOD); mass spectrum (LSIMS), m/z 193 (100%) [$(M + H)^+$]. Anal. Calcd for $C_6H_{13}ClN_2O_3S$: C, 31.5; H, 5.75; N, 12.25. Found: C, 31.5; H, 5.45; N, 11.95.

***N*-Acetyl-*S*-(*N*,*N*-dimethylcarbamoyl)cysteine (5).** Compound **4c** (228 mg, 1 mmol) was treated with acetic anhydride (2 mL) and pyridine (100 mg, 1.3 mmol) for 4 h. Evaporation of the excess reagents gave a gum which, after preparative TLC (silica gel; $CHCl_3$/MeOH, 7:1), afforded **5** (140 mg, 60%) as a colorless gum: IR 3300, 2700 br, 1730, 1680, 1660 cm^{-1}; NMR [$(CD_3)_2SO$] δ 2.00 (3 H, s, $COCH_3$), 3.05 [6 H, s, $N(CH_3)_2$], 3.2 (2 H, m, $CHCH_2S$), 4.30 (1 H, m, $CHCH_2$), 7.1 (1 H, br d, J = 6 Hz, NH); mass spectrum, m/z 234 (M^+). Anal. Calcd for $C_8H_{14}N_2O_4S$: C, 41.0; H, 6.0; N, 11.95. Found: C, 40.85; H, 6.25; N, 11.65.

***N*-Acetyl-*S*-(*N*-methylcarbamoyl)cysteine *N*-Methylamide (7).** *N*-Acetylcysteine (**6**) (4.1 g, 25 mmol) was stirred with methyl isocyanate (2.0 g, 35 mmol) in pyridine at 25 °C for 2 days before evaporation of the volatile materials. Trituration with ether gave **7** (4.31 g, 74%) as a white solid: mp 195 °C; optical rotation (c = 45.1 mM in H_2O) $[\alpha]^{21}_{589}$ −23.8°, $[\alpha]^{21}_{578}$ −24.1°, $[\alpha]^{21}_{546}$ −33.3°, $[\alpha]^{21}_{436}$ −53.4°; IR 3300, 3220, 3080, 1675, 1640 cm^{-1}; NMR [$(CD_3)_2SO$] δ 1.90 (3 H, s, $COCH_3$), 2.60 (3 H, d, J = 6 Hz, NCH_3)

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(becomes s on decoupling at δ 7.8), 2.68 (3 H, d, J = 6 Hz, NCH_3) (becomes s on decoupling at δ 7.8), 3.06 (1 H, d, J = 8 Hz) and 3.12 (1 H, s, J = 5 Hz) ($CHCH_2S$), 4.30 (1 H, dt, J = 5 Hz, J = 8 Hz, $CHCH_2$) (becomes d, J = 8 Hz on decoupling at δ 3.1), 7.8 (3 H, br m, NH); mass spectrum, m/z 234 (M^+). Anal. Calcd for $C_8H_{15}N_3O_3S$: C, 41.2; H, 6.5; N, 18.0. Found: C; 41.15; H, 6.25; N, 17.6.

***N*-(*tert*-Butoxycarbonyl)cysteine Methyl Ester (9).** Et_3N (1.01 g, 10 mmol) was added to a well stirred slurry of cysteine methyl ester hydrochloride (8) (1.72 g, 10 mmol) in CH_2Cl_2 (20 mL), followed after 10 min by di-*tert*-butyl dicarbonate (2.18 g, 10 mmol). The mixture was stirred for 16 h, washed with H_2O, and dried. Evaporation of the solvent gave 9 (2.29 g, 97%) as a colorless oil: optical rotation (c = 318 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ +28.5°, $[\alpha]^{21}_{578}$ +29.6°, $[\alpha]^{21}_{546}$ +33.2°, $[\alpha]^{21}_{436}$ +57.6°; IR 3360, 2560, 1745, 1710 cm^{-1}; NMR δ 1.45 [9 H, s, $C(CH_3)_3$], 1.55 (1 H, br, SH), 2.85 (1 H, d, J = 4 Hz) and 3.00 (1 H, d, J = 4 Hz) ($CHCH_2S$), 3.75 (3 H, s, OCH_3), 4.55 (1 H, dt, J = 8 Hz, J = 4 Hz, $CHCH_2$), 5.60 (1 H, d, J = 8 Hz, NH); mass spectrum, m/z 235 (M^+). Anal. Calcd for $C_9H_{17}NO_4S$: C, 45.95; H, 7.3; N, 5.95. Found: C, 46.1; H, 7.3; N, 6.0.

***N*-(*tert*-Butoxycarbonyl)cysteine (10).** Ester 9 (235 mg, 1 mmol) and NaOH (100 mg, 2.5 mmol) were stirred in MeOH (5 mL) under N_2 for 16 h before evaporation of the solvent. Aqueous HCl (1 M; 2.5 mL) was added to the residue to give a solution with final pH = 6.0, which was extracted twice with EtOAc. The combined extracts were washed with saturated aqueous NaCl and were dried. Evaporation of the solvent furnished 10 (150 mg, 68%) as a colorless gum, which was shown to be >97% pure by NMR but would not give a satisfactory microanalysis: IR 3340, 2900–2600, 1710 cm^{-1}; NMR δ 1.40 [9 H, s, $C(CH_3)_3$], 1.50 (1 H, br, SH), 2.9 (2 H, m, $CHCH_2S$), 4.55 (1 H, dt, J = 8 Hz, J = 4 Hz, $CHCH_2$), 5.60 (1 H, d, J = 8 Hz, NH), 8.0 (1 H, br, CO_2H); mass spectrum, m/z 221 (M^+). A small sample was oxidized with I_2 to given *N*,*N*′-bis(*tert*-butoxycarbonyl)cystine as a white solid: mp 141–143 °C, identical with a commercial sample.

***N*-(*tert*-Butoxycarbonyl)-*S*-(*N*-methylcarbamoyl)cysteine Methyl Ester (11a).** Compound 19 (2.20 g, 9.4 mmol), methyl isocyanate (4 mL), and *N*,*N*-diisopropylethylamine (0.5 mL) were stirred together in CH_2Cl_2 (20 mL) for 3 days after which time the solvent and excess reagents were evaporated. The residue, in CH_2Cl_2, was washed with H_2O and with saturated aqueous NaCl and was dried. The solvent was evaporated to furnish 11a (2.35 g, 96%) as white needles: mp 45–46 °C; IR 3350, 1735, 1700, 1665 cm^{-1}; NMR δ 1.45 [9 H, s, $C(CH_3)_3$], 2.95 (3 H, d, J = 7 Hz, NCH_3), 3.35 (2 H, d, J = 6 Hz, $CHCH_2S$), 3.70 (3 H, s, OCH_3), 4.45 (1 H, ca. q. J = ca. 6 Hz, $CHCH_2$), 5.85 (1 H, d, J = 7 Hz, OCONH), 6.20 (1 H, br q, J = 7 Hz, MeNH); mass spectrum, m/z 292 (M^+). Anal. Calcd for $C_{11}H_{20}N_2O_5S$: C, 45.15; H, 6.9; N, 9.65. Found: C, 45.45; N, 7.0; N, 9.4.

***N*-(*tert*-Butoxycarbonyl)-*S*-(*N*-ethylcarbamoyl)cysteine Methyl Ester (11b).** Compound 9 was treated with ethyl isocyanate and Et_3N in CH_2Cl_2 as for the preparation of 11a above, to yield 11b (91%) as a colorless oil: IR 3300, 1735, 1700, and 1670 cm^{-1}; NMR δ 1.15 (3 H, t, J = 7 Hz, CH_2CH_3), 1.45 [9 H, s, $C(CH_3)_3$], 3.30 (2 H, quintet, J = 7 Hz, $NHCH_2CH_3$), 3.35 (2 H, d, J = ca. 6 Hz, $CHCH_2S$), 3.70 (3 H, s, OCH_3), 4.45 (1 H, ca. q, J = 6 ca. Hz, $CHCH_2$), 5.55 (1 H, d, J = 7 Hz, OCONH), and 6.00 (1 H, br t, J = 7 Hz, EtNH); mass spectrum, m/z 306 (M^+). Anal. Calcd for $C_{12}H_{22}N_2O_5S$: C, 47.05; H, 7.25; N, 9.15. Found: C, 47.1; H, 7.05; N, 9.0.

***N*-(*tert*-Butoxycarbonyl)-*S*-(*N*,*N*-dimethylcarbamoyl)cysteine Methyl Ester (12).** Ester 9 (7.05 g, 30 mmol), dimethylcarbamoyl chloride (3.3 g, 30.7 mmol), and pyridine (5.0 g, 63.3 mmol) were stirred together in CH_2Cl_2 (120 mL) for 3 days before the mixture was washed with aqueous H_2SO_4 (1 M) and H_2O. The solution was dried, and the solvent was evaporated to give 12 (2.94 g, 96%) as a colorless oil: optical rotation (c = 251 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ +10.9°; IR 3350, 1740, 1705, 1680 cm^{-1}; NMR 1.40 [9 H, s, $C(CH_3)_3$], 3.05 (3 H, s) and 3.15 (3 H, s) [$CON(CH_3)_2$], 3.1 (2 H, m, $CHCH_2S$), 3.73 (3 H, s, OCH_3), 4.5 (1 H, m, $CHCH_2$), 5.50 (1 H, d, J = 7 Hz, NH); mass spectrum, m/z 306 (M^+). Anal. Calcd for $C_{12}H_{22}N_2O_5S$: C, 47.05; H, 7.25; N, 9.15. Found: C, 47.2; H, 7.1; N, 9.25.

***N*-(Benzyloxycarbonyl)-1,3-thiazolidine-4-carboxylic Acid (14).** Benzyl chloroformate (3.4 g, 20 mmol) was added in one portion to 1,3-thiazolidine-4-carboxylic acid (13) (2.66 g, 20 mmol) and KOH (2.55 g, 45.5 mmol) in H_2O (30 mL), and the whole was stirred vigorously for 16 h. The solution was washed with CH_2Cl_2 and acidified with aqueous HCl (9 M) before being extracted twice with CH_2Cl_2. The combined extracts were dried. The solvent was evaporated to give 14 (4.81 g, 90%); as a colorless gum: NMR δ 3.20 (2 H, d, J = 5 Hz, $CHCH_2S$), 4.45 (1 H, d, J = 9 Hz) and 4.55 (1 H, d, J = 9 Hz) (NCH_2S), 4.85 (1 H, m, $NCHRCO_2H$), 5.20 (2 H, s, OCH_2Ph), 7.30 (5 H, s, ArH), and 9.65 (1 H, s, CO_2H); mass spectrum, m/z 267 (M^+), 221, 91 (100%). A sample was converted to the diisopropylamine salt: NMR δ 1.20 [12 H, d, J = 7 Hz, 2 $CH(CH_3)_2$], 3.17 (2 H, septet, J = 7 Hz, 2 $CH(CH_3)_2$], 3.25 (2 H, m, thiazolidine 5-CH_2), 4.40 (1 H, d, J = 8 Hz) and 4.65 (1 H, d, J = 8 Hz) (thiazolidine 2-CH_2), 4.75 (1 H, m, thiazolidine 4-H), 5.10 (2 H, s, $PhCH_2$), 7.25 (5 H, s, ArH), 8.1 (2 H, br, N^+H_2). Anal. Calcd for $C_{18}H_{28}N_2O_4S$: C, 58.65; H, 7.65; N, 7.6. Found: C, 58.9; H, 7.6; N, 7.65.

***N*-[[*N*-(Benzyloxycarbonyl)-1,3-thiazolidin-4-yl]carbonyl]glycine Benzyl Ester (15).** Acid 14 (1.33 g, 5 mmol) was added to *N*,*N*′-dicyclohexylcarbodiimide (1.03 g, 5 mmol) in CH_2Cl_2 (20 mL). After 10 min, a mixture of glycine benzyl ester 4-methylbenzenesulfonic acid salt (1.68 g, 5 mmol), *N*,*N*-diisopropylethylamine (650 mg, 5 mmol), and CH_2Cl_2 (20 mL) was added, and the whole was stirred for 4 days. The evaporation residue was extracted with Et_2O (2 × 50 mL). The combined extracts were washed with H_2O (50 mL), aqueous HCl (2 M; 2 × 50 mL), and saturated aqueous $NaHCO_3$ (2 × 50 mL) before being dried. The filtrate was cooled to 4 °C for 3 days and filtered again. Evaporation of the solvent afforded 15 (1.78 g, 86%) as a colorless oil: IR 3360, 1735, 1680 cm^{-1}; NMR δ 3.30 (1 H, dd, J = 12 Hz, J = 6 Hz) and 3.40 (1 H, dd, J = 12 Hz, J = 4 Hz) (thiazolidine 5-CH_2), 4.00 (2 H, d, J = 5.5 Hz, Gly CH_2), 4.40 (1 H, d, J = 9 Hz) and 4.60 (1 H, d, J = 9 Hz) (thiazolidine 2-CH_2), 4.75 (1 H, dd, J = 6 Hz, J = 4 Hz, thiazolidine 4-H), 5.15 (4 H, s, 2 $PhCH_2O$), 6.7 (1 H, br, NH) and 7.15 (10 H, s, ArH); mass spectrum, m/z 414.1256 (M^+) ($C_{21}H_{22}N_2O_5S$ requires 414.1249), 413, 279, 220, 91 (100%).

***N*-[*N*-(Benzyloxycarbonyl)cysteinyl]glycine Benzyl Ester (16).** Compound 15 (1.0 g, 2.4 mmol) was stirred with $Hg(OAc)_2$ (1.0 g, 3.1 mmol) in AcOH (30 mL) and H_2O (12 mL) at 60 °C for 2 h before being cooled to ambient temperature. A steady stream of H_2S was passed through the solution for 20 min, and the black precipitate of HgS was removed by filtration through diatomaceous earth. Evaporation of the filtrate and AcOH washings gave 16 (903 mg, 94%) as a colorless gum: NMR (400 MHz) δ 1.65 (1 H, dd, J = 7.5 Hz, J = 10.7 Hz, SH), 2.71 (1 H, ddd, J = 6.1 Hz, J = 10.7 Hz, J = 14.0 Hz) and 3.13 (1 H, ddd, J = 4.2 Hz, J = 7.5 Hz, J = 14.0 Hz) (Cys β-CH_2), 4.08 (2 H, d, J = 5 Hz, Gly CH_2), 4.48 (1 H, m, Cys α-H), 5.12 (2 H, s, OCH_2Ph), 5.17 (2 H, s, OCH_2Ph), 5.80 (1 H, br d, J = 7.8 Hz, Cys NH), 6.83 (1 H, br t, J = 5 Hz, Gly NH), 7.35 (10 H, s, ArH); mass spectrum, m/z 402.1246 ($C_{20}H_{22}N_2O_5S$ requires 402.1210), 91 (100%).

***N*-[*N*-(Benzyloxycarbonyl)-*S*-(*N*-methylcarbamoyl)cysteinyl]glycine Benzyl Ester (17).** Methyl isocyanate (2.0 mL) and *N*,*N*-diisopropylethylamine (1 mL) were added to 16 (470 mg, 1.17 mmol) in CH_2Cl_2 (10 mL), and the whole was stirred for 16 h. The evaporation residue, in CH_2Cl_2, was washed with aqueous HCl (2 M) and H_2O before being dried. Evaporation of the solvent afforded 17 (470 mg, 88%) as a colorless gum: IR 3350, 1725, 1660, 1535 cm^{-1}; NMR (400 MHz) δ 2.83 (3 H, d, J = 4.9 Hz, NCH_3), 3.22 (1 H, dd, J = 14.8 Hz, J = 8.6 Hz) and 3.36 (1 H, dd, J = 14.8 Hz, J = 4.1 Hz) (Cys β-CH_2), 4.05 (1 H, br d, J = 4.7 Hz) and 4.08 (1 H, br d, J = 5.6 Hz) (Gly CH_2), 4.42 (1 H, m, Cys α-H), 5.11 (1 H, d, J = 12.3 Hz) and 5.13 (1 H, d, J = 12.3 Hz) (OCH_2Ph), 5.17 (2 H, s, OCH_2Ph), 5.47 (1 H, br, NH), 6.21 (1 H, br d, J = ca. 6 Hz, Cys-NH), 7.02 (1 H, br, NH), 7.35 (10 H, m, ArH); mass spectrum, m/z 311 [($M - PhCH_2 - MeNCO$)$^+$], 294, 267, 91 (100%). Anal. Calcd for $C_{22}H_{25}N_3O_6S$: C, 57.5; H, 5.5; N, 9.15. Found: C, 57.2; H, 5.7; N, 8.85.

***N*-[[*N*-(*tert*-Butoxycarbonyl)-1,3-thiazolidin-4-yl]carbonyl]glycine Methyl Ester (19).** Et_3N (520 mg, 5.15 mmol) was added to 18[38] (1.16 g, 5.0 mmol) in CH_2Cl_2 (20 mL), followed after 20 min by isobutyl chloroformate (680 mg, 5.0 mmol), and the mixture was stirred for 1 h. A mixture of glycine methyl ester

hydrochloride (650 mg, 5.2 mmol), Et_3N (540 mg, 5.35 mmol), and CH_2Cl_2 (20 mL) was then added, causing vigorous effervescence. After a further 2 h, the mixture was washed with H_2O, aqueous H_2SO_4 (2 M), aqueous K_2CO_3 (2 M), and H_2O and was dried. The solvent was evaporated to afford **19** (1.46 g, 96%) as a colorless oil: optical rotation (c = 43.6 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ −120.8°, $[\alpha]^{21}_{578}$ −126.9°, $[\alpha]^{21}_{546}$ −145.0°; NMR δ 1.50 [9 H, s, $C(CH_3)_3$], 3.30 (1 H, dd, J = 12 Hz, J = 6 Hz) and 3.40 (1 H, dd, J = 12 Hz, J = 4 Hz) (thiazolidine 5-CH_2), 3.70 (3 H, s, OCH_3), 4.05 (2 H, d, J = 5.5 Hz, Gly CH_2), 4.40 (1 H, d, J = 9 Hz) and 4.65 (1 H, d, J = 9 Hz) (thiazolidine 2-CH_2), 4.70 (1 H, dd, J = 6 Hz, J = 4 Hz, thiazolidine 4-H), 6.9 (1 H, br, NH); mass spectrum, m/z 304 (M^+). Anal. Calcd for $C_{12}H_{20}N_2O_5S$: C, 47.35; H, 6.6; N, 8.95. Found: C, 47.5; H, 6.6; N, 8.95.

N-[N-(*tert*-Butoxycarbonyl)cysteinyl]glycine Methyl Ester (20). Compound **29a** (810 mg, 2.13 mmol) was boiled under reflux with propane-1,3-dithiol (1.6 g, 23 mmol) and *N,N*-diisopropylethylamine (700 mg, 5.4 mmol) in anhydrous THF (20 mL) for 2 days. The evaporation residue, in CH_2Cl_2, was washed twice with ice-cold aqueous H_2SO_4 (1 M) and with saturated aqueous NaCl and was dried. Column chromatography of the evaporation residue (silica gel; $CHCl_3$) afforded **20** (590 mg, 95%) as a colorless oil with a distinctive odor: IR 3320, 2560, 1745, 1700, 1660 cm^{-1}; NMR δ 1.45 [9 H, s, $C(CH_3)_3$], 1.72 (1 H, t, J = 8 Hz, SH), 2.7–3.1 (2 H, m, Cys β-CH_2), 3.72 (3 H, s, OCH_3), 4.02 (2 H, d, J = 5 Hz, Gly CH_2), 4.45 (1 H, ddd, J = 8 Hz, J = 7 Hz, J = 5 Hz, Cys α-H), 5.80 (1 H, d, J = 8 Hz, Cys NH), and 7.37 (1 H, t, J = 5 Hz, Gly NH); mass spectrum, m/z 236 [$(M - C_4H_8)^+$] and 220. Anal. Calcd for $C_{11}H_{20}N_2O_5S$: C, 45.2; H, 6.9; N, 9.6. Found: C, 45.5; H, 7.2; N, 9.3.

Experiment to determine the relative lability of *S*-(amidomethyl)- and *N*-(*tert*-butoxycarbonyl) protecting groups. Di-*tert*-butyl dicarbonate (1.09 g, 5 mmol) in Et_2O (10 mL) was added to *S*-[(4-*tert*-butylbenzamido)methyl]glutathione trifluoroacetate salt[18] (**22**) (610 mg, 1 mmol) and Et_3N (1 mL) in H_2O (5 mL), and the whole was stirred vigorously for 3 days. The resulting aqueous solution was washed twice with CH_2Cl_2, and the solvent and excess reagents were removed by freeze-drying to give crude **23** (600 mg) as a gummy white solid: NMR [400 MHz; $(CD_3)_2SO$] δ 1.30 [9 H, s, $ArC(CH_3)_3$], 1.39 [9 H, s, $OC(CH_3)_3$], 2.10 (1 H, m) and 2.18 (1 H, m) (Glu β-CH_2), 2.36 (2 H, m, Glu γ-CH_2), 2.86 (1 H, dd, J = 14.7 Hz, J = 7.3 Hz) and 3.15 (1 H, dd, J = 14.7 Hz, J = 3.6 Hz) (Cys β-CH_2), 3.81 (1 H, d, J = 3.7 Hz) and 3.82 (1 H, d, J = 4.4 Hz) (Gly CH_2), 4.13 (1 H, ca. q, J = ca. 6 Hz, Glu α-CH), 4.59 (1 H, dd, J = 13.5 Hz, J = 6.2 Hz) and 4.66 (1 H, dd, J = 13.5 Hz, J = 6.0 Hz) (NCH_2S), 4.65 (1 H, m, Cys α-CH), 5.61 (1 H, d, J = 6.9 Hz, Glu NH or Cys NH), 7.32 (1 H, ca. t, J = ca. 4 Hz, Gly NH), 7.38 (1 H, d, J = 6.8 Hz, Cys NH or Glu NH), 7.44 (2 H, d, J = 8.4 Hz) and 7.88 (2 H, d, J = 8.4 Hz) (ArH), 8.50 (1 H, ca. t, J = ca. 6 Hz, SCH_2NH). This material (500 mg) was treated with excess CH_2N_2 in Et_2O for 24 h. Careful evaporation of the solvent and excess reagent furnished **24** (500 mg) as a colorless oil of sufficient purity for the next stage: NMR [400 MHz, $(CD_3)_2SO$] δ 1.31 [9 H, s, $ArC(CH_3)_3$], 1.42 [9 H, s, $OC(CH_3)_3$], 2.10–2.20 (2 H, m, Glu β-CH_2), 2.35 (2 H, m, Glu γ-CH_2), 2.85 (1 H, dd, J = 14.5 Hz, J = 7.5 Hz) and 3.15 (1 H, dd, J = 14.5 Hz, J = 3.3 Hz) (Cys β-CH_2), 3.75 (3 H, s, OCH_3), 3.78 (3 H, s, OCH_3), 3.83 (1 H, d, J = 3.8 Hz) and 3.84 (1 H, d, J = 4.3 Hz) (Gly CH_2), 4.15 (1 H, ca. q, J = ca. 6 Hz, Glu α-H), 4.60 (1 H, dd, J = 13.5 Hz, J = 6.2 Hz) and 4.66 (1 H, dd, J = 13.5 Hz, J = 6.0 Hz) (NCH_2S), 4.68 (1 H, m, Cys α-H), 5.73 (1 H, br, NH), 7.31 (1 H, br, NH), 7.39 (1 H, br, NH), 7.45 (2 H, d, J = 8.6 Hz) and 7.91 (2 H, d, J = 8.6 Hz) (ArH), and 8.60 (1 H, ca. t, J = ca. 6 Hz, SCH_2NH). Treatment of a sample (100 mg) of this material with $Hg(OAc)_2$ in 50% aqueous AcOH at 25 °C was without effect during 1 h, but warming to 45 °C for 10 min gave material which had lost the BOC protecting group (primary amine shown by color reaction with indan-1,2,3-trione) whereas no thiol was evident by TLC (comparison with authentic **25**).

N-(*tert*-Butoxycarbonyl)glutathione Dimethyl Ester (25). Compound **36** (800 mg, 1.53 mmol) was boiled under reflux with propane-1,3-dithiol (1.2 g, 8.0 mmol) and *N,N*-diisopropylethylamine (400 mg, 3.1 mmol) in anhydrous THF (10 mL) under N_2 for 2 days. The evaporation residue, in CH_2Cl_2, was washed with ice-cold aqueous H_2SO_4 (2 M) and H_2O and was dried. Chromatography (silica gel; $CHCl_3$/MeOH, 50:1) of the yellow oily evaporation residue gave unreacted disulfide **36** (260 mg, 32%) and **25** (330 mg, 50%) as a white solid: mp 94–95 °C; NMR (400 MHz) δ 1.43 [9 H, s, $OC(CH_3)_3$], 1.82 (1 H, dd, J = 10.0 Hz, J = 7.9 Hz, SH), 1.96 (1 H, ca. dq, J = ca. 14 Hz, J = 7 Hz) and 2.21 (1 H, ca. dq, J = 14 Hz, J = 7 Hz) (Glu β-CH_2), 2.37 (2 H, t, J = 7.2 Hz, Glu γ-CH_2), 2.78 (1 H, m) and 3.13 (1 H, ddd, J = 14.0 Hz, J = 7.9 Hz, J = 4.6 Hz) (Cys β-CH_2), 3.74 (3 H, s, OCH_3), 3.75 (3 H, s, OCH_3), 4.00 (1 H, dd, J = 18.1 Hz, J = 5.4 Hz) and 4.08 (1 H, dd, J = 18.1 Hz, J = 5.8 Hz) (Gly CH_2), 4.34 (1 H, m, Glu α-H), 4.68 (1 H, ddd, J = 8.5 Hz, J = 6.0 Hz, J = 4.6 Hz, Cys α-H), 5.29 (1 H, d, J = 8.5 Hz, Cys NH), 6.86 (1 H, d, J = 7.0 Hz, Glu NH), 6.97 (1 H, ca. t, J = ca. 5.5 Hz, Gly NH); mass spectrum, m/z 435 (M^+), 335, 144, 84 (100%), 57. Anal. Calcd for $C_{17}H_{29}N_3O_8S$: C, 46.9; H, 6.7; N, 9.65. Found: C, 46.6; H, 6.5; N, 9.4.

Attempted Coupling of N-(*tert*-Butoxycarbonyl)-S-(*tert*-butylthio)cysteine (26a) with Glycine *tert*-Butyl Ester by the Mixed-Anhydride Method. Compound **26a** (309 mg, 1 mmol) was stirred with Et_3N (120 mg, 1.2 mmol) and isobutyl chloroformate (137 mg, 1 mmol) in CH_2Cl_2 (10 mL) for 20 min before addition of a mixture of CH_2Cl_2 (5 mL), Et_3N (110 mg, 1.1 mmol), and glycine *tert*-butyl ester hydrochloride (170 mg, 1 mmol). After a further 15 min, the mixture was washed with H_2O, aqueous H_2SO_4 (2 M), and H_2O and was dried. Chromatography of the evaporation residue (silica gel; $CHCl_3$) yielded **27** (60 mg, 14%) as an unstable colorless gum, which did not give a satisfactory microanalysis but appeared to be >95% pure by NMR; IR 3350, 1700, 1680 cm^{-1}; NMR δ 1.30 [9 H, s, $SC(CH_3)_3$], 1.33 [9 H, s, $SC(CH_3)_3$], 1.47 (9 H, s, $OC(CH_3)_3$], 3.10 (2 H, br d, J = 6 Hz, CH_2S), 4.70 (1 H, ca. q, J = ca. 6 Hz, CH), 5.40 (1 H, br d, J = ca. 7 Hz, NH). From later running fractions was obtained a colorless gum (230 mg), which was shown by NMR to comprise 85 mol % **29b**, identical with the material described below and 15 mol % **28b**: NMR δ 1.45 [18 H, s, 2 $C(CH_3)_3$], 3.95 (2 H, d, J = 5 Hz, Gly CH_2), 5.20 (1 H, m) and 6.00 (1 H, d, J = 1.5 Hz) (propenoyl 3-CH_2), 6.5 (1 H, br, Gly NH), 7.2 (1 H, ca. d, J = ca. 2 Hz, NH).

Attempted Coupling of N,N'-Bis(*tert*-butoxycarbonyl)-cystine (26b) with Glycine Methyl Ester by the Mixed-Anhydride Method. Compound **26b** (1.76 g, 4 mmol) was treated with Et_3N (810 mg, 8 mmol) and isobutyl chloroformate (1.09 g, 8 mmol) in CH_2Cl_2 (10 mL) for 20 min before addition of Et_3N (810 mg, 8 mmol) and glycine methyl ester hydrochloride (1.0 g, 8 mmol) in CH_2Cl_2 (20 mL). After a further 45 min, the mixture was washed with H_2O and aqueous H_2SO_4 (2 M) and was dried. Chromatography of the evaporation residue (silica gel; $CHCl_3$) yield *N*-[2-[(*tert*-butoxycarbonyl)amino]propenoyl]glycine methyl ester (**28a**) (490 mg, 24%) as an unstable colorless gum: IR 3300, 1720, 1690 cm^{-1}; NMR (400 MHz) δ 1.46 [9 H, s, $C(CH_3)_3$], 4.10 (2 H, d, J = 6 Hz, Gly CH_2), 5.13 (1 H, t, J = 1.7 Hz becomes d, J = 1.7 Hz on decoupling at δ 7.3) and 6.04 (1 H, d, J = 1.7 Hz) (propenoyl 3-CH_2), 7.3 (1 H, ca. d, J = ca. 2 Hz, NH); mass spectrum, m/z 258.1215 (M^+) ($C_{11}H_{18}N_2O_5$ requires 258.1216), 202, 185, 57 (100%).

N-[N-(Benzyloxycarbonyl)cysteinyl]glycine Methyl Ester Disulfide (29c). *N,N'*-Bis(benzyloxycarbonyl)cystine (**26c**) (508 mg, 1 mmol) was treated with isobutyl chloroformate, Et_3N, and glycine methyl ester hydrochloride, as for the reaction of **26b** above, to give a gum comprising mixed methyl and 2-methylprop-1-yl esters of *N*-[*N*-(benzyloxycarbonyl)cysteinyl]glycine disulfide as determined by NMR. This mixture was stirred for 2 days with MeOH (30 mL) and Et_3N (1 mL). Chromatography (silica gel; $CHCl_3$) furnished **29c** (430 mg, 66%) as a white powder: mp 168–171 °C (lit.[40] mp 170–171 °C); NMR δ 3.2 (4 H, m, 2 Cys β-CH_2), 3.70 (6 H, s, 2 OCH_3), 4.00 (4 H, d, J = 6 Hz, 2 Gly CH_2), 4.55 (2 H, m, 2 Cys α-H), 5.10 (4 H, s, 2 $PhCH_2$), 5.8 (4 H, br, 4 NH), 7.30 (10 H, ArH).

N-[N-(*tert*-Butoxycarbonyl)-S-(*tert*-butylthio)cysteinyl]glycine Methyl Ester (29a). Compound **30** (320 mg, 0.74 mmol) was stirred with glycine methyl ester hydrochloride (251 mg, 2 mmol) and *N,N*-diisopropylethylamine (600 mg, 4.5 mmol) in CH_2Cl_2 (6 mL) for 4 h. The mixture was washed with aqueous NaOH (2 M), H_2O (thrice), aqueous H_2SO_4 (2 M; twice), and H_2O and was dried. Evaporation of the solvent afforded **29a** (270 mg, 96%) as a white solid: mp 105–107 °C; optical rotation (c = 279 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ −56.7°, $[\alpha]^{21}_{578}$ −59.4°, $[\alpha]^{21}_{546}$ −68.0°; IR

3350, 1725, 1685 cm^{-1}; NMR (300 MHz) δ 1.24 [9 H, s, SC(CH$_3$)$_3$], 1.46 [9 H, s, OC(CH$_3$)$_3$], 3.08 (2 H, m, Cys β-CH$_2$), 3.75 (3 H, s, OCH$_3$), 4.03 (1 H, dd, J = 18.3 Hz, J = 5.2 Hz) and 4.08 (1 H, dd, J = 18.3 Hz, J = 5.2 Hz) (Gly CH$_2$), 4.43 (1 H, ca. q. J = ca. 6 Hz, Cys α-CH), 5.29 (1 H, d, J = 7 Hz, Cys NH), 6.82 (1 H, br t, J = ca. 5 Hz, Gly NH); mass spectrum, m/z 381.1512 [(M + H)$^+$] ($C_{15}H_{29}N_2O_5S_2$ requires 381.1518), 380.1431 (M$^+$) ($C_{15}H_{28}N_2O_5S_2$ requires 380.1440), 325, 268, 224, 57 (100%). Anal. Calcd for $C_{15}H_{28}N_2O_5S_2$: C, 47.35; H, 7.4; N, 7.35. Found: C, 47.5; H, 7.7; N, 7.4. A sample (30 mg) was treated with 35% DCl in D_2O for 4 days to give a solution of *N*-[*S*-(*tert*-butylthio)cysteinyl]glycine deuteriochloride: NMR (DCl/D_2O) δ 1.40 [9 H, s, SC(CH$_3$)$_3$], 3.40 (2 H, d, J = 6 Hz, cys β-CH$_2$), 4.20 (2 H, s, Gly CH$_2$), 4.62 (1 H, t, J = 6 Hz, Cys α-H).

***N*-[*N*-(*tert*-Butoxycarbonyl)-*S*-(*tert*-butylthio)cysteinyl]glycine Methyl Ester (29a) by the Mixed-Anhydride Method.** Compound **26a** was treated with isobutyl chloroformate, Et_3N, and glycine methyl ester hydrochloride, as for the reaction of **26b** above, to give, after chromatography, **29a** (44%) as a white solid identical with the material described above.

***N*-[*N*-(*tert*-Butoxycarbonyl)-*S*-(*tert*-butylthio)cysteinyl]glycine *tert*-Butyl Ester (29b).** Ester **30** was treated with glycine *tert*-butyl ester hydrochloride and *N*,*N*-diisopropylethylamine, as for the preparation of **29a** above, to furnish **29b** (240 mg, 95%) as a colorless gum: optical rotation (c = 57.0 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ −11.6°; IR 3320, 1730, 1670 cm^{-1}; NMR δ 1.28 [9 H, s, SC(CH$_3$)$_3$], 1.41 [18 H, s, 2 OC(CH$_3$)$_3$], 3.04 (2 H, ca. d, J = ca. 6 Hz, becomes s on decoupling at δ 4.40, Cys β-CH$_2$), 3.86 (2 H, d, J = 5 Hz, Gly CH$_2$), 4.40 (1 H, ca. q, J = ca. 7 Hz, becomes ca. t, J = ca. 6 Hz on decoupling at δ 5.60, becomes d, J = 7 Hz on decoupling at δ 3.04, Cys α-H), 5.60 (1 H, d, J = 7 Hz, Cys NH), and 6.99 (1 H, ca. t, J = ca. 5 Hz, becomes s on decoupling at δ 3.86, Gly NH). Anal. Calcd for $C_{18}H_{34}N_2O_5S_2$: C, 51.15; H, 8.1; N, 6.65. Found: C, 51.05; H, 8.0; N, 6.5.

***N*-(*tert*-Butoxycarbonyl)-*S*-(*tert*-butylthio)cysteine 4-Nitrophenyl Ester (30).** *N*-(*tert*-Butoxycarbonyl)-*S*-(*tert*-butylthio)cysteine (**26a**) (309 mg, 1 mmol) was stirred with dicyclohexylcarbodiimide (206 mg, 1 mmol) and 4-nitrophenol (139 mg, 1 mmol) in CH_2Cl_2 (5 mL) for 2 h before being filtered. Chromatography of the evaporation residue (silica gel; $CHCl_3$) afforded **30** (330 mg, 77%) as white needles: mp 102–103 °C; optical rotation (c = 146 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ +25.2°, $[\alpha]^{21}_{578}$ +27.3°, $[\alpha]^{21}_{546}$ +31.1°; IR 1750, 1690, 1510, 1330 cm^{-1}; NMR δ 1.35 [9 H, s, SC(CH$_3$)$_3$], 1.48 [9 H, s, OC(CH$_3$)$_3$], 3.28 (2 H, d, J = 6 Hz, β-CH$_2$), 4.78 (1 H, m, α-CH), 5.65 (1 H, d, J = 8 Hz, NH), 7.30 (2 H, d, J = 9 Hz, Ar 2,6-H), and 8.20 (2 H, d, J = 9 Hz, Ar 3,5-H); mass spectrum, m/z 318 [(M − 2 C$_4$H$_8$)$^+$], 301, 274, 208, 57 (100%). Anal. Calcd for $C_{18}H_{26}N_2O_6S_2$: C, 50.25; H, 6.1; N, 6.5. Found: C, 50.55; H, 6.05; N, 6.25.

***N*-[*N*-(*tert*-Butoxycarbonyl)-*S*-(*N*-methylcarbamoyl)cysteinyl]glycine Methyl Ester (31a).** Compound **20** (270 mg, 0.92 mmol) was stirred with methyl isocyanate (570 mg, 10 mmol) and *N*,*N*-diisopropylethylamine (300 mg, 2.3 mmol) in CH_2Cl_2 (12 mL) for 3 days before the mixture was washed twice with ice-cold aqueous H_2SO_4 (1 M), with H_2O and with saturated aqueous NaCl. The solution was dried, and the solvent and excess isocyanate were evaporated to furnish **31a** (244 mg, 70%) as an unstable colorless oil, which did not give a satisfactory microanalysis but was shown to be >97% pure by NMR: IR 3350, 1735, 1665 cm^{-1}; NMR δ 1.43 [9 H, s, C(CH$_3$)$_3$], 2.95 (3 H, d, J = 5 Hz, NCH$_3$), 3.2 (2 H, m, Cys β-CH$_2$), 3.72 (3 H, s, OCH$_3$), 4.05 (2 H, d, J = 5 Hz, Gly CH$_2$), 4.35 (1 H, m, Cys α-H), 6.08 (1 H, d, J = 7 Hz, Cys NH), 6.90 (1 H, t, J = 5 Hz, Gly NH), and 7.20 (1 H, q, J = 5 Hz, NHCH$_3$); mass spectrum, m/z 236 [(M − C$_4$H$_8$ − MeNCO)$^+$] and 192.

***N*-[*N*-(*tert*-Butoxycarbonyl)-*S*-(*N*-ethylcarbamoyl)cysteinyl]glycine Methyl Ester (31b).** Compound **20** (270 mg, 0.92 mmol) was treated with ethyl isocyanate (600 mg, 8.5 mmol), as for the preparation of **31a** above, to give **31b** (235 mg, 70%) as an unstable colorless gum: NMR δ 1.14 (3 H, t, J = 7 Hz, CH$_2$CH$_3$), 1.43 [9 H, s, C(CH$_3$)$_3$], 3.1–3.4 (4 H, m, Cys β-CH$_2$ + NCH$_2$CH$_3$), 3.71 (3 H, s, OCH$_3$), 4.02 (2 H, d, J = 5 Hz, Gly CH$_2$), 4.3 (1 H, m, Cys α-H), 5.98 (1 H, d, J = 7 Hz, Cys NH), 6.73 (1 H, t, J = 5 Hz, NHEt or Gly NH), and 7.39 (1 H, t, J = 5 Hz, Gly NH or NHEt); mass spectrum, m/z 236 [(M − C$_4$H$_8$ − EtNCO)$^+$] and 192.

***N*-(*tert*-Butoxycarbonyl)-*S*-(*tert*-butylthio)cysteine *tert*-Butyl Thioester (32).** Ester **30** (430 mg, 1 mmol) was stirred with *N*,*N*-diisopropylethylamine (516 mg, 4 mmol) in 2-methylpropane-2-thiol (5 mL) for 16 h under reflux. Evaporation of excess reagent gave an oil which, in CH_2Cl_2, was washed with ice-cold aqueous H_2SO_4 (1 M) and with saturated aqueous NaCl and was dried. Chromatography of the evaporation residue (silica gel; EtOAc/hexane, 1:4) afforded **32** (156 mg, 41%) as a colorless gum, which would not give a satisfactory microanalysis but appeared by NMR and TLC to be >96% pure: NMR δ 1.35 [18 H, s, 2 SC(CH$_3$)$_3$], 1.45 [9 H, s, OC(CH$_3$)$_3$], 3.25 (2 H, d, J = 6 Hz, β-CH$_2$), 4.68 (1 H, m, α-H), and 6.10 (1 H, br, NH).

***N*-(*tert*-Butoxycarbonyl)glutamic Acid α-Methyl γ-(4-Nitrophenyl) Diester (34).** *N*-(*tert*-Butoxycarbonyl)glutamic acid α-methyl ester dicyclohexylammonium salt[31] (**33**) (optical rotation (c = 53.5 m*M* in $CHCl_3$) $[\alpha]^{21}_{589}$ −2.0°) (2.87 g, 6.5 mmol), in CH_2Cl_2 (80 mL), was washed with aqueous H_2SO_4 (2 M; 2 × 50 mL) at 0 °C and was dried. The solution was then stirred with dicyclohexylcarbodiimide (1.34 g, 6.5 mmol) and 4-nitrophenol (904 mg, 6.5 mmol) for 24 h before being filtered. Chromatography of the evaporation residue (silica gel; EtOAc/hexane, 1:4) gave **34** (1.93 g, 78%) as a white solid: mp 53–54 °C; IR (Nujol) 3380, 1760, 1735, 1680, 1520, 1350 cm^{-1}; NMR (300 MHz) δ 1.43 [9 H, s, C(CH$_3$)$_3$], 2.01 (1 H, ca. dq, J = 15 Hz, J = 7 Hz) and 2.32 (1 H, ca. dq, J = 15 Hz, J = 7 Hz) (β-CH$_2$), 2.69 (1 H, dt, J = 17.2 Hz, J = 6.8 Hz) and 2.72 (1 H, dt, J = 17.2 Hz, J = 7.5 Hz) (γ-CH$_2$), 3.76 (3 H, s, OCH$_3$), 4.45 (1 H, ca. q, J = ca. 7 Hz, α-H), 5.14 (1 H, d, J = 7.8 Hz, NH), 7.29 (2 H, d, J = 9.2 Hz, Ar 2,6-H), 8.26 (2 H, d, J = 9.2 Hz, Ar 3,5-H); mass spectrum, m/z 382 (M$^+$), 325, 309, 139, 57 (100%). Anal. Calcd for $C_{17}H_{22}N_2O_8$: C, 53.4; H, 5.8; N, 7.35. Found: C, 53.5; H, 5.85; N, 7.4.

***N*-(*tert*-Butoxycarbonyl)-*S*-(*tert*-butylthio)glutathione Bis(methyl ester) (36).** Compound **29a** (1.14 g, 3 mmol) was stirred with CF_3CO_2H (10 mL) for 16 h before CH_2Cl_2 (30 mL) was added. This solution was washed with saturated aqueous $NaHCO_3$ and dried. Evaporation of the solvent gave crude **35** (800 mg, 95%) as a colorless oil: NMR δ 1.30 [9 H, s, SC(CH$_3$)$_3$], 2.55 (2 H, br, NH$_2$), 2.70 (1 H, dd, J = 12 Hz, J = 9 Hz) and 3.30 (1 H, dd, J = 12 Hz, J = 3 Hz) (Cys β-CH$_2$), 3.67 (1 H, dd, J = 9 Hz, J = 3 Hz, Cys α-H), 3.70 (3 H, s, OCH$_3$), 4.02 (2 H, d, J = 5 Hz, Gly CH$_2$), and 7.9 (1 H, ca. t, J = ca. 5 Hz, NH). This amine was boiled under reflux with **34** (764 mg, 2 mmol), *N*,*N*-diisopropylethylamine (400 mg, 3.1 mmol), and 4-(dimethylamino)pyridine (20 mg) in anhydrous THF (20 mL) for 30 h. The evaporation residue, in CH_2Cl_2, was washed with H_2O (twice), aqueous H_2SO_4 (2 M) (twice), 2 M aqueous NaOH (twice), and H_2O. The solution was dried, and the solvent was evaporated to afford **36** (878 mg, 84%) as a colorless gum: optical rotation (c = 24.3 mM in $CHCl_3$) $[\alpha]^{21}_{589}$ −47.2°, $[\alpha]^{21}_{578}$ −50.4°, $[\alpha]^{21}_{546}$ −56.7°, $[\alpha]^{21}_{436}$ −103.9°; IR 3320 (br), 1750, 1710–1640 (br) cm^{-1}; NMR (400 MHz) δ 1.32 [9 H, s, SC(CH$_3$)$_3$], 1.42 [9 H, s, OC(CH$_3$)$_3$], 1.93 (1 H, dq, J = 15 Hz, J = 7.5 Hz) and 2.17 (1 H, J = 15 Hz, J = 7.5 Hz) (Glu γ-CH$_2$), 2.36 (2 H, m, Glu β-CH$_2$), 3.09 (1 H, dd, J = 14.0 Hz, J = 6.1 Hz) and 3.13 (1 H, dd, J = 14.0 Hz, J = 6.9 Hz) (Cys CH$_2$), 3.728 (3 H, s, OCH$_3$), 3.734 (3 H, s, OCH$_3$), 4.00 (1 H, dd, J = 18.1 Hz, J = 5.3 Hz) and 4.05 (1 H, dd, J = 18.1 Hz, J = 5.6 Hz) (Gly CH$_2$), 4.37 (1 H, m, Glu α-H), 4.73 (1 H, ca. q, J = ca. 7 Hz, Cys α-H), 5.35 (1 H, d, J = 7.3 Hz, Cys NH or Glu NH), 6.80 (1 H, d, J = 7.2 Hz, Glu NH or Cys NH), and 7.09 (1 H, ca. t, J = ca. 5.5 Hz, Gly NH); mass spectrum, m/z 523 (M$^+$), 467, 450, 434, 367 (100%), 224, 90, 57, 136.72 (M*: 367 → 224). Anal. Calcd for $C_{21}H_{37}N_3O_8S_2$: C, 48.15; H, 7.1; N, 8.0. Found: C, 47.85; H, 7.1; N, 7.7. From one experimental run in the absence of 4-(dimethylamino)pyridine, a gum was obtained which was subjected to column chromatography (silica gel; $CHCl_3$). From the slowest running fraction was isolated 3-[[(*tert*-butylsulfenyl)thio]methyl]-2,5-dioxopiperazine (**38**) (6%) as a white solid: mp 210–214 °C dec; IR (Nujol) 3200, 3050, 1660 cm^{-1}; NMR [$CDCl_3$/$(CD_3)_2SO$; 1:1] δ 1.35 [9 H, s, SC(CH$_3$)$_3$], 3.22 (2 H, d, J = 5 Hz, SCH$_2$CH), 3.84 (2 H, m, NHCH$_2$CO), 4.14 (1 H, dt, J = 2 Hz, J = 5 Hz, NHCHCH$_2$) (becomes t, J = 5 Hz on decoupling at δ 8.10), and 8.1 (2 H, br, 2 NH). From other fractions were obtained **36** (59%) and **35** (14%).

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Registry No. **2a**, 120033-44-5; **3a**, 120033-45-6; **3b**, 120033-68-3; **4a**, 120033-46-7; **4b**, 120033-69-4; **4c**, 120033-70-7; **5c**, 120033-71-8; **6**, 616-91-1; **7**, 120033-47-8; **8**, 18598-63-5; **9**, 55757-46-5; **10**, 20887-95-0; **11a**, 120033-48-9; **11b**, 120144-33-4; **12**, 120033-49-0; **13**, 34592-47-7; **14**, 96402-64-1; **14**·$(i\text{-Pr})_2$NH, 120033-72-9; **15**, 120033-50-3; **16**, 120033-51-4; **17**, 120033-52-5; **18**, 51077-16-8; **19**, 120033-53-6; **20**, 120033-54-7; **22**, 120033-55-8; **23**, 120033-56-9; **24**, 120033-57-0; **25**, 120033-58-1; **26a**, 30044-61-2; **26b**, 10389-65-8; **26c**, 6968-11-2; **27**, 120033-59-2; **28a**, 120033-60-5; **28b**, 120033-73-0; **29a**, 120033-61-6; **29b**, 120033-74-1; **29c**, 24948-53-6; **30**, 58036-46-7; **31a**, 120033-62-7; **31b**, 120033-75-2; **32**, 120033-63-8; **33**, 82152-24-7; **34**, 120033-64-9; **35**, 120033-65-0; **36**, 120033-66-1; **37**, 118448-76-3; **38**, 120033-67-2; MeNCO, 624-83-9; H-Cys-OH·HCl, 52-89-1; EtNCO, 109-90-0; Me_2NCOCl, 79-44-7; $PhCH_2OCOCl$, 501-53-1; H-Gly-OCH_2Ph·TsOH, 1738-76-7; H-Gly-OMe·HCl, 5680-79-5; H-Gly-OBu-*t*·HCl, 27532-96-3; H-Cys(SBu-*t*)-Gly-OH·DCl, 120033-76-3; *t*-BuSH, 75-66-1.

A New Synthetic Method for the 2-Substitution of N-Unsubstituted Benzimidazoles: Formaldehyde as a Versatile Protecting Agent for Heterocyclic NH[1]

Alan R. Katritzky* and Kunihiko Akutagawa

Department of Chemistry, University of Florida, Gainesville, Florida 32611

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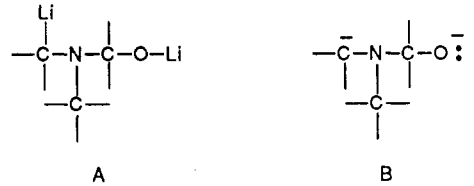
N-Unsubstituted benzimidazoles **1** are readily converted in a one-pot sequence into 2-substituted derivatives **2** with good overall yields. N-Protection with formaldehyde and lithiation with lithium *N,N*-diisopropylamide (LDA), *n*-butyllithium, or *tert*-butyllithium gives the dilithiohemiaminals **6**, which readily react with a range of electrophiles at the 2-carbon. The 2-substituted 1-(lithioxymethyl)benzimidazoles **7** undergo smooth acid-catalyzed dehydroxymethylation under mild conditions to give N-unsubstituted 2-substituted benzimidazoles **2**.

In connection with our investigations of methodologies for the protection of amines and alcohols during functionalization using carbon dioxide as the source of protecting group,[2] we have been seeking alternative protecting groups that satisfy our criterion that both protection and deprotection should work well under conditions mild enough not to damage sensitive functionalities. We focused our search for a group that could be used for systems when the carbon dioxide method failed, as for heterocyclic NH in rings containing more than one heteroatom.[3] We now report a solution to this problem.

Hemiaminals are well known as unstable intermediates in the reactions of aldehydes and ketones with amines and some are stable enough to isolate.[4] Most stable hemiaminals contain electron-withdrawing groups attached to the nitrogen atom as in those derived from benzotriazole,[5] phthalimide,[6] and succinimide.[7] The chemistry of hemiaminals is for the most part unexplored although recent publications from our laboratory have described a series of benzotriazole hemiaminals.[8]

We anticipated that hemiaminal formation could provide potential protection for the functionalization of certain NH compounds since some hemiaminals are readily prepared from the nitrogen compound and aldehyde and are readily converted back into the NH derivative under mild acidic conditions at low temperature.[9] Provided a formal hemiaminal oxyanion could survive in the presence of a strong base such as *n*-butyllithium, it seems likely that the subsequent lithiation would occur to afford A, which should be stable, cf. B. Although elimination might be



(1) Paper 1 of our new series Formaldehyde: A Reagent for the Simultaneous Protection of Nucleophilic Centers and the Activation and Stabilization of Alternative Locations to Electrophilic Attack.

(2) Cf. our series Carbon Dioxide: A Reagent for the Simultaneous Protection of Nucleophilic Centers and the Activation of Alternative Locations to Electrophilic Attack. (a) Part 1: Katritzky, A. R.; Akutagawa, K. *Tetrahedron Lett.* **1985**, *26*, 5935. Part 2: Katritzky, A. R.; Akutagawa, K. *Tetrahedron* **1986**, *42*, 2571. Part 3: Katritzky, A. R.; Fan, W.; Akutagawa, K. *Tetrahedron* **1986**, *42*, 4027. Part 4: Katritzky, A. R.; Fan, W.; Akutagawa, K. *Synthesis* **1987**, 415. Part 5: Katritzky, A. R.; Akutagawa, K. *J. Am. Chem. Soc.* **1986**, *108*, 6808 and subsequent papers.

(3) E.g., for benzimidazole, the expected carbamic acid lithium salt is formed by the successive action of *n*-butyllithium and carbon dioxide, but on further treatment with strong base does not undergo 2-metalation, probably because of alkyllithium attack on the carbonyl group of the carbamic anion.

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