The Formation and Metabolism of *N*-Hydroxymethyl Compounds. Part 6.[1] The Synthesis of *S*-Amidomethyl-, *S*-Ureidomethyl-, and *S*-(1,3,5-Triazin-2-ylaminomethyl)-glutathione Derivatives

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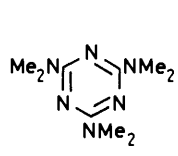
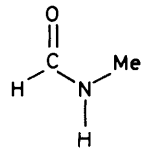
Treatment of *N*-hydroxymethyl and *N*-alkoxymethyl compounds with glutathione or *N*-acetylcysteine in trifluoroacetic acid affords the corresponding glutathione or *N*-acetylcysteine derivatives in high yield. Alkoxymethylureas are formed by the condensation of ureas with formaldehyde and alcohols under basic conditions; the implications of this observation are discussed with reference to possible biochemical mechanisms.

N-Hydroxymethyl compounds are produced by the metabolism of drugs and other materials containing *N*-methyl groups, by preparations of murine liver.[2] It has been reported that, for example, the antitumour agents hexamethylmelamine (**1**)[3] and *N*-methylformamide (**2**)[4] and the herbicide Monuron [*N*-(4-chlorophenyl)-*N'*,*N'*-dimethylurea] (**3**)[5] are hydroxylated by this route which involves cytochrome P450. These hepatic metabolites containing the carbinolamine moiety may act as electrophiles, either through the intermediacy of a small equilibrium concentration of the corresponding iminium ion (a dehydration product) or through biological derivatisation of the alcohol (*e.g.* sulphation, acetylation) which increases its leaving group ability. As electrophiles, they may be predicted to be conjugated *in vivo* (either enzymatically or chemically) to glutathione (γ-glutamylcysteinylglycine)[6] and hence be excreted as the glutathione conjugate (**4**) itself or as the corresponding mercapturic acid (**5**). Any or all of these compounds may be electrophiles in their own right (with RS^- as a leaving group), or may act as transport forms of formaldehyde and thus be present as toxic or carcinogenic metabolites. We sought therefore to prepare a range of such *S*-aminomethyl compounds.



(**1**)



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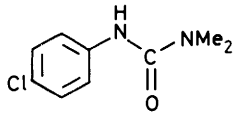

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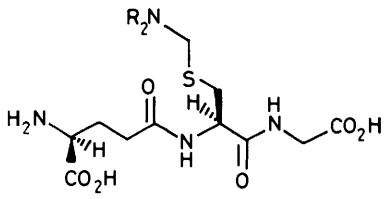

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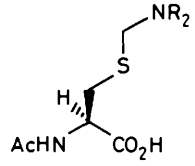
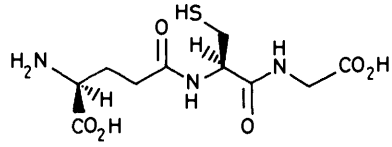


(**5**)

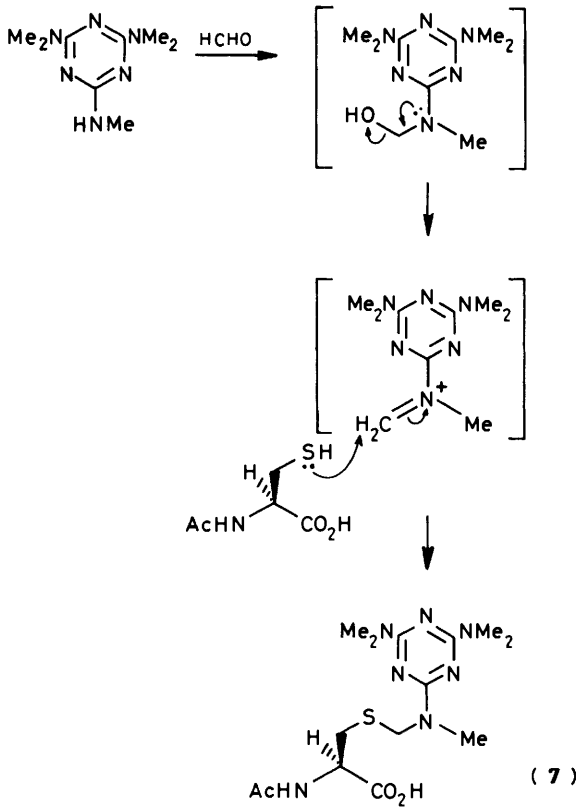


(**6**)

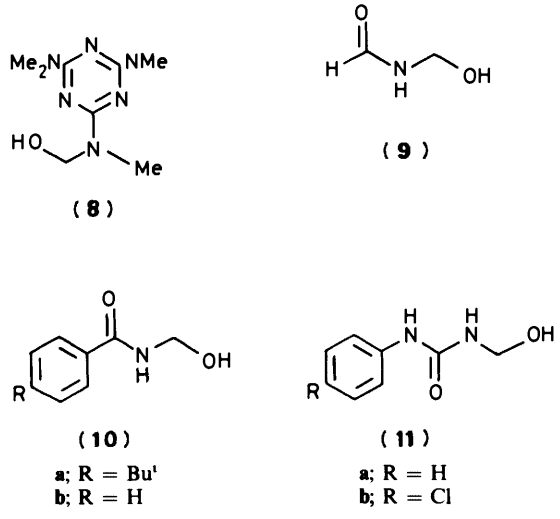
The synthesis of one such *S*-aminomethylglutathione derivative has been reported recently,[7] albeit in low yield, from the Mannich-type condensation of 4-aminoazobenzene with formaldehyde and glutathione (**6**) in an aqueous medium. A modification of this technique enabled us to prepare the *N*-acetylcysteine–melamine adduct (**7**) in good yield. Glutathione is insoluble in the aqueous methanol employed and, not surprisingly, failed to react under these conditions. The mechanism presumably involves a methylene-iminium ion (Scheme 1). It is interesting to note that benzamide did not condense with *N*-acetylcysteine and formaldehyde under these conditions, the starting materials being recovered; thus any *N*-hydroxymethylbenzamide formed is not in equilibrium with sufficient imine or iminium species to effect condensation.

However, dissolution of equimolar amounts of preformed *N*-hydroxymethyl-*N*,*N'*,*N'*,*N''*,*N''*-pentamethylmelamine (**8**) or *N*-hydroxymethylamides (**9**) and (**10**) in *ca.* 0.7M-solutions of glutathione in anhydrous trifluoroacetic acid (TFA), followed immediately by evaporation of the solvent under reduced pressure, gave the condensation products in consistently high yields. The use of TFA is apposite in that it acts both as a good solvent for the otherwise troublesome glutathione and as an acid catalyst of low nucleophilicity for the generation of iminium ions for capture by the nucleophilic thiol. The condensation of *N*-acetylcysteine with *N*-hydroxymethyl-4-t-butylbenzamide was similarly effected. It proved impossible to generate the methylene-iminium ion under these conditions by elimination of water in the reverse sense from 4-chloro-*N*-methylbenzohydroxamic acid[8] (**12**) as shown in Scheme 2.

The *N*-hydroxymethylamides (**9**) and (**10**) and *N*-hydroxymethyl(pentamethyl)melamine (8 g) used above were prepared without difficulty in the usual way from the corresponding NH compound, formaldehyde, and base in an appropriate solvent. However, on attempting to prepare the *N*-hydroxymethylureas (**11**), we were unable to repeat the work of Zigeuner *et al*[9] who warmed phenylurea (**13a**) with paraformaldehyde and sodium



Scheme 1. Proposed mechanism of formation of compound (7) *via* the methylene-iminium ion

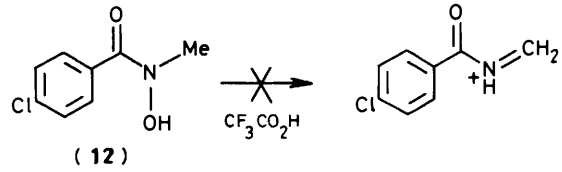


(8) (9) (10) (11)

(10)
a; R = Bu^t
b; R = H

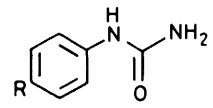
(11)
a; R = H
b; R = Cl

hydroxide in methanol. In our hands, the sole isolable product was *N*-methoxymethyl-*N'*-phenylurea in very low yield. Increasing the reaction time and temperature enabled the three representative alkoxymethylureas (**14a**—**c**) to be synthesised smoothly in the appropriate alcohols, as in Scheme 3. Alkoxymethylureas are reported[9] to be formed when *N*-hydroxymethylureas are treated with an alcoholic hydrogen chloride solution, thus favouring the necessary iminium ion formation; nevertheless, a small but significant equilibrium concentration of arylurea methylene-iminium or methylene-

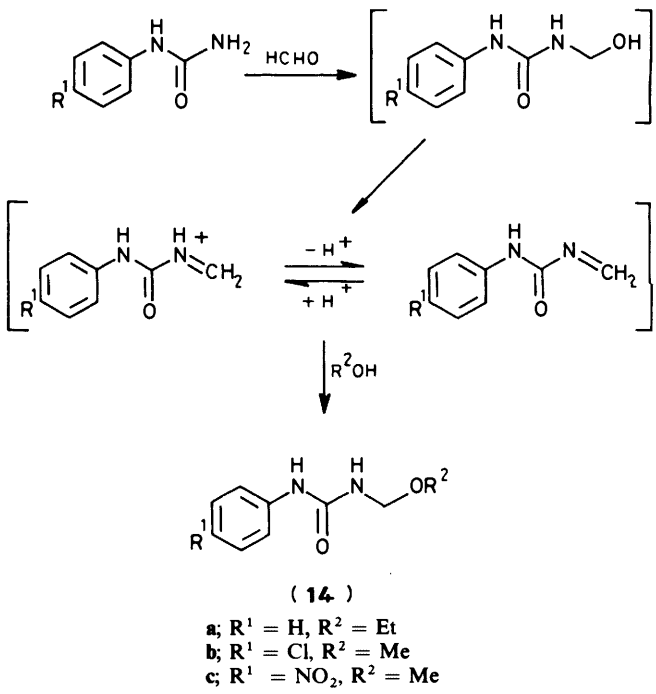


(12)

Scheme 2. [4-Chloro-*N*-methylbenzohydroxamic acid (12) is unreactive towards nucleophiles in CF_3CO_2H.]



(13)
a; R = H
b; R = Cl
c; R = NO_2



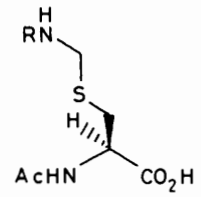
(14)
a; R^1 = H, R^2 = Et
b; R^1 = Cl, R^2 = Me
c; R^1 = NO_2, R^2 = Me

Scheme 3. Formation of the alkoxymethylureas (**14a**—**c**)

imine moieties must be present even under the mildly basic conditions of our experiments.

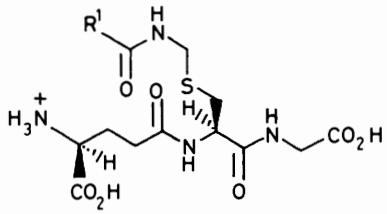
The alkoxymethylureas (**14a**) and (**14b**) are found to react with glutathione, and (**14a**) with *N*-acetylcysteine, as readily as the *N*-hydroxymethylamides. Direct ^{1}H n.m.r. monitoring of these reaction mixtures reveals that the condensation is complete within 2 min, but that only after 20 min has all the methanol or ethanol released been esterified by the trifluoroacetic acid; this implies that the rate of the reversible methylene-iminium ion formation is unaffected by the rate at which the alcohol is irreversibly sequestered.

The structural assignment of the synthetic conjugates is based on spectroscopic data. The characteristic feature of the ^{1}H n.m.r. spectrum of compounds (**15**) and (**16**) [$(CD_3)_2SO$; 220 MHz] is the resonance of the NCH_2S moiety which appears as two separate sets of signals, indicating that the prochiral



(15)

a; R = 4-Bu^{t}C$_6$H$_4$CO
b; R = PhNHCO



(16)

a; R^1 = H, R^2 = $CF_3CO_2^-$
b; R^1 = 4-Bu^{t}C$_6$H$_4$, R^2 = picrate$^-$
c; R^1 = Ph, R^2 = $CF_3CO_2^-$
d; R^1 = PhNH, R^2 = $CF_3CO_2^-$
e; R^1 = 4-ClC$_6$H$_4$NH, R^2 = $CF_3CO_2^-$

methylene group is in an asymmetric environment. This effect is well illustrated in the ^{1}H n.m.r. spectrum of compound (**16b**) in which the NCH_2S resonances appear at δ 4.46 (1 H, dd, *J* 13 and 6.5 Hz) and 4.57 (1 H, dd, *J* 13 and 6.5 Hz). Treatment with deuterium oxide removes the corresponding NH triplet at δ 9.16 and its 6.5 Hz coupling, leaving the 13 Hz geminal coupling typical of an asymmetric methylene group; this contrasts with the corresponding 2 H singlet in the spectrum of the achiral substrate (**10a**). As expected, the cysteine β-CH_2 is prochiral. The coupling constants in the spectrum of the benzamidomethyl glutathione compound (**16c**) are typical, with geminal coupling constants $J_{\beta1.\beta2}$ 14 Hz, $J_{\beta1.\alpha}$ 10.5 Hz, and $J_{\beta2.\alpha}$ 4 Hz. From a simplified Karplus analysis, it can be deduced that the molecule adopts one of the staggered conformations about the C_α–C_β bond shown in the Figure. As predicted from steric considerations, the benzamidomethylthio group is *gauche* to one of the peptide links and *trans* to the other (Figure). The ^{1}H n.m.r. spectrum of the formamide (**16a**) was more complex, indicating approximately equal populations of two rotamers about the formamide carbonyl–nitrogen bond.

Two main conclusions can be drawn from these results. Firstly, since alkoxymethylureas are formed under basic conditions and are stable under such conditions, there must be a small but significant equilibrium proportion of the corresponding iminium ions or imines under these very basic conditions. It is therefore reasonable to postulate that an equal or higher equilibrium proportion of iminium ions or imines is present under the much less basic physiological conditions (pH 7.4). Hence it is feasible that the methylene-iminium ions formed directly from the dehydration of *N*-(4-chlorophenyl)-*N'*-hydroxymethylurea, a known metabolite of Monuron,[5] or from *N*-hydroxymethyl(pentamethyl)melamine may, as proposed[10] for *N*-hydroxymethylamines (in which iminium ion formation is more favoured), be the actual electrophile responsible for biological activity (mutagenic,[11] antineoplastic, or antibacterial[10] respectively). Secondly, it is shown here that a rapid, facile

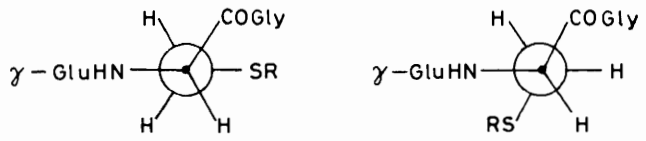


Figure. Newman projections of the C_α–C_β bond of the *S*-benzamidomethylglutathione (**16c**) conformers established by ^{1}H n.m.r. spectroscopy (R = $PhCONHCH_2$)

synthesis of glutathione conjugates, putative metabolites of some xenobiotic *N*-methyl compounds, is available. Since conjugation to glutathione is a common fate of hepatically generated electrophiles, it is important to have such authentic material for chemical and biochemical study. No attempt has been made to prepare the free glutathione forms of the glutathione derivatives from the salts, since the former would be expected to be released upon dissolution of the salts in the buffered aqueous media required for biochemical experiments.

Experimental

I.r. spectra were determined as Nujol mulls, except where otherwise stated. ^{1}H N.m.r. spectra were obtained at 60 MHz using a Varian EM360A spectrometer and at 220 MHz using a Perkin-Elmer R34 instrument and ^{13}C n.m.r. spectra were obtained with a Bruker WH-180, using tetramethylsilane as internal standard. M.p.s are uncorrected.

N-(4-*Chlorophenyl*)*urea* (**13b**).—This compound was prepared in 81% yield according to the general method of Furniss *et al.*[12] and had m.p. 208—210 °C (lit.,[13] 204—206 °C).

N-(4-*Nitrophenyl*)*urea* (**13c**).—4-Nitrobenzoic acid (8.35 g, 50 mmol) and phosphorus pentachloride (10.4 g, 50 mmol) were heated together at 120 °C until gas evolution ceased. Toluene (15 ml) was added and the mixture was heated to 205 °C during which process all volatile materials were distilled off (mainly toluene and phosphorus oxychloride). On being cooled, the crystalline residue was dissolved in acetone (200 ml) and was added to sodium azide (10.0 g, 154 mmol) and sodium hydrogen carbonate (1.0 g) in water (40 ml). This mixture was stirred for 2 h before being extracted with dichloromethane (2 × 200 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and the solvents evaporated under reduced pressure to give almost pure 4-nitrobenzoyl azide as pale yellow prisms ($\nu_{max.}$ 2 180, 2 120, and 1 675 cm^{-1}). This azide, in toluene (60 ml), was boiled under reflux for 10 min after which a small evaporated sample showed $\nu_{max.}$ 2 250 cm^{-1}, corresponding to 4-nitrophenyl isocyanate. The cooled toluene solution was added to a large excess of ethereal ammonia giving an immediate yellow precipitate. Recrystallisation from aqueous methanol yielded the urea (**13c**) (3.30 g, 37%) as lemon yellow needles, m.p. 214—215 °C (lit.,[14] 215 °C) δ_H [60 MHz; $CDCl_3$–$(CD_3)_2SO$; 1:3] 6.1 (2 H, br, NH_2), 7.65 (2 H, d, *J* 9 Hz, ArH), 8.12 (2 H, d, *J* 9 Hz, ArH), and 9.2 (1 H, br, NH).

N'-*Ethoxymethyl*-N-*phenylurea* (**14a**).—Paraformaldehyde (2 g, 66.7 mmol of HCHO) was added to phenylurea (2.72 g, 20 mmol) and sodium hydroxide (100 mg, 2.5 mmol) in a mixture of ethanol (50 ml) and water (1 ml). The resulting suspension was boiled under reflux for 1.5 h before evaporation of the solvent under reduced pressure. Recrystallisation of the residue from aqueous ethanol furnished the *ethoxymethylurea* (**14a**) (3.41 g, 84%) as white needles, m.p. 105—107 °C (Found: C, 61.7; H, 7.1; N, 14.6. $C_{10}H_{14}N_2O_2$ requires C, 61.85; H, 7.25; N, 14.4%); $\nu_{max.}$ 3 250 and 1 660 cm^{-1}; δ_H (60 MHz; $CDCl_3$) 1.12 (3

H, t, *J* 7 Hz, CH_2CH_3), 3.48 (2 H, q, *J* 7 Hz, OCH_2CH_3), 4.60 (2 H, d, *J* 7 Hz, NCH_2O), 6.86 (1 H, t, *J* 7 Hz, $CONHCH_2$), 7.3 (5 H, m, ArH), and 8.1 (1 H, s, ArNH).

N-(4-*Chlorophenyl*)-N'-*methoxymethylurea* (**14b**).—Paraformaldehyde (3.0 g, 100 mmol of HCHO) and aqueous sodium hydroxide (10% w/v; 1.5 ml, 3.75 mmol) were added to *N*-(4-chlorophenyl)urea (**15b**) (5.12 g, 30 mmol) in methanol (60 ml). This suspension was boiled under reflux for 5 h before being cooled to 0 °C for 16 h. The solids were isolated by filtration and washed with a small volume of cold methanol to give the *methoxymethylurea* (**14b**) as white needles (5.08 g, 78%), m.p. 126—128 °C (Found: C, 50.35; H, 5.3; N, 12.8. $C_9H_{11}ClN_2O_2$ requires C, 50.35; H, 5.15; N, 13.05%); $\nu_{max.}$ 3 400, 3 300, and 1 630 cm^{-1}; δ_H (60 MHz; $CDCl_3$) 3.30 (3 H, s, OMe), 4.60 (2 H, d, *J* 7 Hz, NCH_2O), 6.93 (1 H, t, *J* 7 Hz, $NHCH_2O$), 7.22 (2 H, d, *J* 9 Hz, ArH), 7.45 (2 H, d, *J* 9 Hz, ArH), and 8.7 (1 H, br, ArNH).

N-(4-*Nitrophenyl*)-N'-*methoxymethylurea* (**14c**).—Aqueous formaldehyde solution (37% w/v; 6.0 ml, 74 mmol) and paraformaldehyde (2.7 g, 90 mmol of HCHO) were added to *N*-(4-nitrophenyl)urea (**13c**) (530 mg, 2.9 mmol) and potassium hydroxide (640 mg, 11.4 mmol) in methanol (50 ml). This suspension was boiled under reflux for 3 h before evaporation of the solvent under reduced pressure. Recrystallisation of the residue from methanol afforded the *methoxymethylurea* (**14c**) (310 mg, 44%) as pale yellow needles, m.p. 163.5 °C (decomp.) (Found: C, 48.1, H, 5.1; N, 18.5. $C_9H_{11}N_3O_4$ requires C, 48.0; H, 4.9; N, 18.65%); $\nu_{max.}$ 3 250 and 1 675 cm^{-1}; δ_H [60 MHz; $CDCl_3$–$(CD_3)_2SO$, 20:1] 3.37 (3 H, s, OMe), 4.70 (2 H, d, *J* 7 Hz, NCH_2O), 6.9 (1 H, br, NH), 7.70 (2 H, d, *J* 9 Hz, ArH), 8.20 (2 H, d, *J* 9 Hz, ArH), and 9.1 (1 H, br, NH).

N-*Acetyl*-S-(4-*t-butylbenzamidomethyl*)*cysteine* (**15a**).—*N*-Acetyl-L-cysteine (326 mg, 2 mmol) in trifluoroacetic acid (3 ml) was added to *N*-hydroxymethyl-4-t-butylbenzamide (**10a**)[1] (414 mg, 2 mmol). This mixture was stirred for 5 min at 20 °C before evaporation of the solvent at 35 °C and 1 Torr. The residue, in dichloromethane (20 ml), was washed with water (10 ml). The solution was dried (Na_2SO_4), filtered and the solvent evaporated to give a colourless gum. Column chromatography (silica gel; $CHCl_3$ with MeOH increasing from 0 to 15%) gave 4-t-butylbenzamide (90 mg, 26%) as a white powder identical with an authentic sample.[15] Evaporation of the solvents from later eluates afforded the *cysteine derivative* (**15a**) (413 mg, 59%) as a white powder which decomposed on gentle heating (Found: C, 57.6; H, 6.7; N, 7.7. $C_{17}H_{24}N_2O_4S$ requires C, 57.95; H, 6.85; N, 7.95%); $\nu_{max.}$ 3 300, 3 100, 1 715, and 1 665 cm^{-1}; δ_H [220 MHz; $(CD_3)_2SO$] 1.30 (9 H, s, CMe_3), 1.87 (3 H, s, Ac), 2.95 (1 H, dd, *J* 7 and 13 Hz), and 3.16 (1 H, dd, *J* 4 and 13 Hz) (cysteine CH_2), 3.5 (1 H, br, CO_2H), 4.35 (1 H, dt, *J* and 7 Hz, cysteine α-H), 4.48 (1 H, dd, *J* 6 and 13 Hz) and 4.52 (1 H, dd, *J* 6 and 13 Hz) (NCH_2S), 7.56 (2 H, d, *J* 8 Hz, ArH), 7.83 (1 H, d, *J* 7 Hz, AcN*H*Cys), 7.94 (2 H, d, *J* 8 Hz, ArH), and 9.28 (1 H, t, *J* 6 Hz, $ArCONHCH_2$); *m/z* 352 (M^+) and 190.

N-*Acetyl*-S-(N'-*phenylureidomethyl*)*cysteine* (**15b**).—*N'*-Ethoxymethyl-*N*-phenylurea (**14a**) (970 mg, 5 mmol) was added to *N*-acetyl-L-cysteine (815 mg, 5 mmol) in trifluoroacetic acid (6 ml). The mixture was stirred at ambient temperature for 15 min before the solvent was evaporated at 2 Torr. Column chromatography of the residue (silica gel; $CHCl_3$—MeOH, 7:1) gave the *cysteine derivative* (**15b**) as a colourless gum (913 mg, 59%) which could not be crystallised. A satisfactory microanalysis could not be obtained, but the sample appeared to be pure by t.l.c. and n.m.r. analysis. $\nu_{max.}$ (liquid film) 3 150, 1 705, and 1 660 cm^{-1}; δ_H [220 MHz; $(CD_3)_2SO$] 2.10 (3 H, s, Ac), 2.89 (1 H, dd, *J* 8.5 and 13.5 Hz) and 3.07 (1 H, dd, *J* 5 and 13.5 Hz) (cysteine CH_2), 3.6 (1 H, br, CO_2H), 4.41 (1 H, d, *J* 6.5 Hz) and 4.42 (1 H, d, *J* 6.5 Hz) (NCH_2S), 4.51 (1 H, dt, *J* 5 and 8.5 Hz, cysteine α-H), 6.86 (1 H, t, *J* 6.5 Hz, $CONHCH_2S$), 6.98 (1 H, t, *J* 8 Hz, ArH), 7.31 (2 H, t, *J* 8 Hz, ArH), 7.48 (2 H, d, *J* 8 Hz, ArH), 8.32 (1 H, d, *J* 8.5 Hz, AcNH), and 8.72 (1 H, s, PhNHCO); *m/z* 311 (M^+), 149.

N-*Acetyl*-S-{N-[4,6-*bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-N-*methylaminomethyl*}*cysteine* (**7**).—A mixture of aqueous formaldehyde (37% w/v; 3 ml, 37 mmol), methanol (20 ml), *N*-acetyl-L-cysteine (1.0 g, 6.1 mmol) and 2,4-bis(dimethylamino)-6-methylamino-1,3,5-triazine[16] (980 mg, 5 mmol) was stirred at 37 °C for 2 h before being cooled to 0 °C for 1 h. The precipitate was filtered off and washed with a small volume of cold methanol to give the *cysteine derivative* (**7**) as a white powder (1.30 g; 70% based on the pentamethyl melamine) which decomposed without melting at < 60 °C (Found C, 45.55; H, 6.9; N, 26.1. $C_{14}H_{25}N_7O_3S$ requires C, 45.25; H, 6.8; N, 26.4%); $\nu_{max.}$ 3 230, 1 700, and 1 610 cm^{-1}; δ_H [220 MHz; $CDCl_3$–$(CD_3)_2SO$, 2:1] 1.99 (3 H, s, Ac), 3.10 (1 H, dd, *J* 7 and 13.5 Hz, cysteine β-H), 3.12 (12 H, s, NMe_2), 3.14 (3 H, s, melamine-$NRCH_3$), 3.24 (1 H, dd, *J* 4.5 and 13.5 Hz, cysteine β-H), 3.76 (1 H, m, cysteine α-H), 4.88 (1 H, d, *J* 14 Hz) and 5.07 (1 H, d, *J* 14 Hz) (NCH_2S), and 6.92 (1 H, d, *J* 8 Hz, NH); δ_C [$(CD_3)_2SO$] 22.17, 32.00, 32.44, 35.39, 40.35, 40.79, 51.92, 164.97, 169.06, and 172.10 p.p.m. *m/z* 371.1737 ($C_{14}H_{25}N_7O_3S$ requires 371.1734) (M^+), 209 (100%).

S-[N'-(4-*Chlorophenyl*)*ureidomethyl*]*glutathione Trifluoroacetate Salt Hydrate* (**16e**).—*N*-(4-Chlorophenyl)-*N'*-methoxymethylurea (**14b**) (429 mg, 2 mmol) was added to glutathione (614 mg, 2 mmol) in trifluoroacetic acid (3 ml). The mixture was stirred for 5 min before evaporation of the solvent under reduced pressure. The oily residue was triturated with diethyl ether to give a white powder. Dissolution of this material in acetone followed by reprecipitation on addition of diethyl ether and filtration furnished the *glutathione derivative* (**16e**) (1.09 g, 88%) as a slightly hygroscopic white powder without a definite m.p. but which decomposed on gentle heating (Found: C, 38.3; H, 4.7; N, 11.0. $C_{20}H_{27}ClF_3N_5O_{10}S$ requires C, 38.6; H, 4.4; N, 11.25%); $\nu_{max.}$ 3 150, 1 705, and 1 640 cm^{-1}; δ_H [$(CD_3)_2SO$] 2.07 (2 H, m, glutamyl β-CH_2), 2.39 (2 H, m, glutamyl γ-CH_2), 2.74 (1 H, m, cysteine β-H), 3.05 (1 H, dd, *J* 4.5 and 14 Hz, cysteine β-H), 3.83 (2 H, br, glycine CH_2), 4.00 (1 H, m, glutamyl α-H), 4.36 (1 H, dd, *J* 7 and 13 Hz) and 4.44 (1 H, dd, *J* 7 and 13 Hz) (NCH_2S), 4.63 (1 H, m, cysteine α-H), 7.35 (2 H, d, *J* 8 Hz, ArH), 7.53 (2 H, d, *J* 8 Hz, ArH), and 8.4 (9 H, m, NH and OH).

S-*Formamidomethylglutathione Trifluoroacetate Salt Dihydrate* (**16a**).—This compound was prepared from *N*-hydroxymethyl formamide[17] (**9**) (150 mg, 2 mmol) and glutathione (614 mg, 2 mmol) according to the method for (**13e**) above, giving the *glutathione derivative* (**16a**) (966 mg, 94%) as a hygroscopic white solid without a definite m.p. (Found: C, 32.4; H, 5.2; N, 10.6. $C_{14}H_{25}F_3N_4O_{11}S$ requires C, 32.8; H, 4.9; N, 11.0%); $\nu_{max.}$ 3 200, 1 710, and 1 660 cm^{-1}; δ_H [220 MHz; $(CD_3)_2SO$] 2.07 (2 H, m, glutamyl β-CH_2), 2.40 (2 H, m, glutamyl γ-CH_2), 2.6—3.1 (2 H, m, cysteine CH_2), 3.85 (2 H, br s, glycine CH_2), 4.00 (1 H, m, glutamyl α-H), 4.32 (0.5 H, dd, *J* 6 and 13 Hz) and 4.38 (0.5 H, dd, *J* 6 and 13 Hz) (NCH_2S of rotamer A), 4.47 (0.5 H, d, *J* 13 Hz, NCHS of rotamer B), 4.64 (1.5 H, m, cysteine α-H and NCHS of rotamer B), 8.17 (0.5 H, s, formyl H), 8.34 (0.5 H, s, formyl H), 8.42 (5.5 H, m, NH and OH), 8.75 (0.5 H, *ca.* t, *J* 6 Hz, $HCONHCH_2$ of one rotamer), and 9.5 (2 H, br, NH and OH).

S-(4-*t-Butylbenzamidomethyl*)*glutathione Picrate* (**16b**).—*N*-Hydroxymethyl-4-t-butylbenzamide[1] (414 mg, 2 mmol) was

added to glutathione (614 mg, 2 mmol) in trifluoroacetic acid (3 ml). The mixture was stirred at ambient temperature for 10 min before the solvent was evaporated at 2 Torr. The gummy residue was dissolved in acetone (20 ml) and 2,4,6-trinitrophenol (458 mg, 2 mmol) in methanol (4 ml) was added. The solvents were evaporated from this mixture under reduced pressure. The residue was precipitated from acetone solution by addition of diethyl ether to give the *glutathione derivative* (**16b**) (1.351 g, 93%) as a slightly hygroscopic bright yellow powder without definite m.p. (Found: C, 46.0; H, 5.0; N, 13.3. $C_{28}H_{35}N_7O_{14}S$ requires C, 46.35; H, 4.85; N, 13.5%); $[\alpha]_D^{23}$ −21.7° (*c* 17.5% w/v in dimethyl sulphoxide); $\nu_{max.}$ 3 150, 1 705, 1 650, 1 515, and 1 345 cm^{-1}; δ_H [$(CD_3)_2SO$] 1.31 (9 H, s, CMe_3), 2.05 (2 H, m, glutamyl β-CH_2), 2.40 (2 H, m, glutamyl γ-CH_2), 2.78 (1 H, dd, *J* 9 and 14 Hz) and 3.10 (1 H, dd, *J* 4 and 9 Hz) (cysteine CH_2), 3.7 (5 H, br, CO_2H and RN^+H_3), 3.84 (2 H, d, *J* 5.5 Hz, glycine CH_2), 4.00 (1 H, t, *J* 7 Hz, glutamyl α-H), 4.46 (1 H, dd, *J* 6.5 and 13 Hz) and 4.57 (1 H, dd, *J* 6.5 and 13 Hz) (NCH_2S), 4.68 (1 H, dt, *J* 4 and 9 Hz, cysteine α-H), 7.57 (2 H, d, *J* 8.5 Hz, benzamide ArH), 7.89 (2 H, d, *J* 8.5 Hz, benzamide ArH), 8.35 (1 H, t, *J* 5.5 Hz, glycine NH), 8.40 (1 H, d, *J* 9 Hz, cysteine NH), 8.67 (2 H, s, picrate ArH), and 9.16 (1 H, t, *J* 6.5 Hz, ArCONH).

S-*Benzamidomethylglutathione Trifluoroacetate Salt Hydrate* (**16c**).—*N*-Hydroxymethylbenzamide[1] (302 mg, 2 mmol) and glutathione (614 mg, 2 mmol) were treated as for the preparation of (**16e**). This method afforded the *glutathione derivative* (**16c**) (1.929 g, 90%) as a hygroscopic white powder without definite m.p. (Found: C, 40.7; H, 4.6; N, 9.2. $C_{20}H_{29}F_3N_4O_{11}S$ requires C, 40.7; H, 4.95; N, 9.5%); $[\alpha]_D^{23}$ −16.2° (*c* 39% w/v in water); $\nu_{max.}$ 3 200 and 1 690 cm^{-1}; δ_H [220 MHz; $(CD_3)_2SO$] 2.06 (2 H, m, glutamyl β-CH_2), 2.38 (1 H, dt, *J* 14 and 7 Hz), and 2.43 (1 H, dt, *J* 14 and 7 Hz) (glutamyl γ-CH_2), 2.89 (1 H, dd, *J* 10.5 and 14 Hz) and 3.11 (1 H, dd, *J* 4 and 14 Hz) (cysteine CH_2), 3.83 (2 H, d, *J* 7 Hz, glycine CH_2), 4.01 (1 H, m, glutamyl α-H), 4.48 (1 H, dd, *J* 6 and 13.5 Hz) and 4.55 (1 H, dd, *J* 6 and 13.5 Hz) (NCH_2S), 4.69 (1 H, ddd, *J* 4, 6, and 10.5 Hz, cysteine α-H), 6.0 (1 H, br, NH or OH), 7.57 (2 H, t, *J* 7 Hz, ArH), 7.62 (1 H, t, *J* 7 Hz, ArH), 7.94 (2 H, d, *J* 7 Hz, ArH), 8.38 (6 H, m, NH ± OH), and 9.24 (1 H, t, *J* 6 Hz, PhCONH).

S-(N′-*Phenylureidomethyl*)*glutathione Trifluoroacetate Salt Dihydrate* (**16d**).—This compound was prepared from *N*-ethoxymethyl-*N*′-phenylurea (**14a**) (388 mg, 2 mmol) and glutathione according to the method for (**16e**) above. The *glutathione derivative* (**16d**) (1.031 g, 90%) was obtained as a slightly hygroscopic white powder without a definite m.p. (Found: C, 39.8; H, 4.7; N, 11.5. $C_{20}H_{30}F_3N_5O_{11}$ requires C, 39.65; H, 5.0; N, 11.55%); $[\alpha]_D^{23}$ −31.0° (*c* 25% w/v in water); $\nu_{max.}$ 3 150 and 1 685 cm^{-1}; δ_H [220 MHz; $(CD_3)_2SO$] 2.08 (3 H, m, glutamyl β-CH_2 and cysteine NH), 2.40 (2 H, m, glutamyl γ-CH_2), 2.76 (1 H, dd, *J* 10 and 14 Hz) and 3.04 (1 H, dd, *J* 4 and 14 Hz) (cysteine CH_2), 3.81 (2 H, d, *J* 7 Hz, glycine CH_2), 4.01 (1 H, m, glutamyl α-H), 4.37 (1 H, dd, *J* 7 and 13.5 Hz) and 4.46 (1 H, dd, *J* 7 and 13.5 Hz) (NCH_2S), 4.46 (1 H, m, cysteine α-H), 6.99 (1 H, t, *J* 8 Hz, ArH), 7.20 (1 H, t, *J* 7 Hz, glycine NH or CON*H*CH_2S), 7.31 (2 H, t, *J* 8 Hz, ArH), 7.50 (2 H, d, *J* 8 Hz, ArH), 8.4 (5 H, br, NH and OH), and 9.02 (1 H, s, PhNH).

S-{N-[4,6-*Bis*(*dimethylamino*)-1,3,5-*triazin*-2-*yl*]-N-*methylaminomethyl*}*glutathione Trifluoroacetate Salt*.—This compound was prepared from 2,4-bis(dimethylamino)-6-(*N*-hydroxymethyl-*N*-methylamino)-1,3,5-triazine[16] (452 mg, 2 mmol) and glutathione (614 mg, 2 mmol) as for (**13e**) above; the *glutathione derivative* (520 mg, 51%) could not be purified without decomposition. ^1H N.m.r. spectroscopy indicated this white non-hygroscopic powder to be *ca.* 85% pure; $\nu_{max.}$ 3 300 and 1 660 cm^{-1}; δ_H [220 MHz; $(CD_3)_2SO$] 2.06 (2 H, m, glutamyl β-CH_2), 2.39 (2 H, m, glutamyl γ-CH_2), 2.74 (1 H, m) and 3.03 (1 H, m) (cysteine CH_2), 3.06 (12 H, s, NMe_2), 3.16 (3 H, br, s, melamine-NRCH_3), 3.80 (2 H, br s, glycine CH_2), 3.99 (1 H, m, glutamyl α-H), 4.58 (1 H, m, cysteine α-H), 4.74 (1 H, d, *J* 13 Hz) and 5.08 (1 H, d, *J* 13 Hz) (NCH_2S), and 8.4 (7 H, m, NH and OH).

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