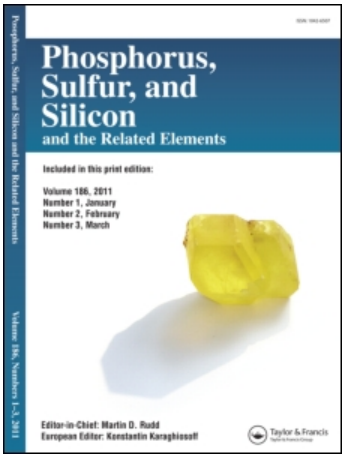

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THE CHEMISTRY OF 1,1'-THIOBIS(2-CHLOROETHANE) (SULPHUR MUSTARD) PART II.[1] THE SYNTHESIS OF SOME CONJUGATES WITH CYSTEINE, *N*-ACETYLCYSTEINE AND *N*-ACETYLCYSTEINE METHYL ESTER

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THE CHEMISTRY OF 1,1′-THIOBIS(2-CHLOROETHANE) (SULPHUR MUSTARD) PART II.[1] THE SYNTHESIS OF SOME CONJUGATES WITH CYSTEINE, *N*-ACETYLCYSTEINE AND *N*-ACETYLCYSTEINE METHYL ESTER

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The syntheses of a number of conjugates of 1,1′-thiobis(2-chloroethane) ("sulphur mustard") and its simple derivatives with cysteine, *N*-acetylcysteine and *N*-acetylcysteine methyl ester are described. These compounds were synthesised for use as reference compounds to support metabolite identification in metabolic studies and, in some cases, to provide standards for analytical procedures being developed for the retrospective confirmation of exposure to sulphur mustard.

Key words: Thiobis(2-chloroethane) and conjugates with cysteine, *N*-acetylcysteine and *N*-acetylcysteine methyl ester; metabolic studies.

INTRODUCTION

Sulphur mustard, 1,1′-thiobis(2-chloroethane), has posed a threat as a chemical warfare agent since its first use in 1917, due to its potent cytotoxic and vesicant properties plus ease of synthesis. The biological activity of sulphur mustard is generally believed to stem from its ability to function as an alkylating agent and, in particular, its ability to interact with RNA or DNA in a bidentate fashion to produce cross-linked chains.[2] Protein molecules have also been identified as likely targets for alkylation although the physiological consequences of such alkylations are unknown.[2] We are interested in developing forensic methods for the retrospective confirmation of exposure to sulphur mustard by the identification of urinary metabolites, alkylated proteins or any other indicators of poisoning.

Previous studies into the *in vivo* metabolism of sulphur mustard in animals and man indicated, predictably, that conjugation with glutathione is an important primary metabolic process leading to the excretion in the urine of glutathione, cysteine or, more probably, *N*-acetylcysteine conjugates.[3,4] With two electrophilic sites available for substitution, the formation of both mono- and bis-conjugates must be considered and, in the case of the former, the biotransformation of the second chlorine via either elimination or substitution reactions is important. In all cases, modification of the oxidation state at sulphur from sulphide to sulphoxide or sulphone greatly increases the number of putative metabolites.

Results[5] of more recent metabolic studies in these laboratories have confirmed the earlier general observations concerning the primary role of glutathione in the metabolism of sulphur mustard in the rat. However, the fate of these glutathione

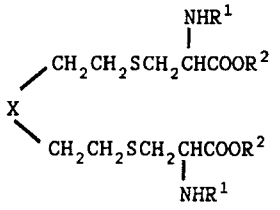
conjugates is quite complex and further bio-transformations occur, leading to mono- and bis-*N*-acetyl cysteine conjugates and methylthio/methylsulphinyl derivatives, prior to excretion in the urine. A larger number of metabolites are present in the urine of which a substantial number were isolated by HPLC and identified by mass spectrometry.[5] In support of these studies, and taking note of conclusions of earlier work, some mono- and bis-conjugates of sulphur mustard and its simple derivatives were synthesised for use as reference compounds in metabolic and analytical studies. This paper reports these syntheses with full spectral characterisation of the products.

RESULTS AND DISCUSSION

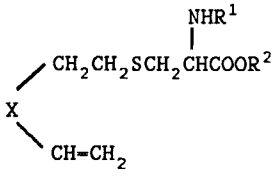
The majority of compounds reported in this paper were synthesized by a simple common approach, employing the base-catalysed addition of cysteine, *N*-acetylcysteine or *N*-acetylcysteine methyl ester to an appropriate mustard derivative, usually in aqueous acetonitrile solution. Some reactions produced a number of products and it is worth noting that all reaction mixtures were complicated to a greater or lesser extent, by the formation of the appropriate cystine dimer (depending on the cysteine derivative used). In addition, those reactions in which mustard or half-mustard was used produced unwanted by-products, mainly thiodiglycol and 1,2-bis(2-hydroxyethylthio)ethane,[6] derived from hydrolysis reactions.

The compounds prepared are shown in the Figure. Most of the reactions occurred under very mild conditions using weak organic or inorganic bases. However, whilst experimental procedures were broadly similar, the mechanism of substitution of chlorine by nucleophiles in sulphides is widely disparate from that operative in structurally similar sulphoxides and sulphones. It is well understood that substitution in 2-chloroethylsulphides proceeds via an intermediate cyclic sulphonium ion,[6] whilst for sulphoxides and sulphones, substitution occurs as a result of an elimination-addition mechanism.[7] Sulphoxides were significantly less reactive than analogous sulphones[7] under these conditions (reflecting the substantially lower acidity of the respective α protons) and in some instances gave unexpectedly complex reaction mixtures and poor yields of products. In general, the chemistry described is frequently of a capricious character whose outcome is dependent on the precise reaction conditions.

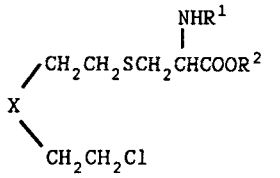
The reaction of mustard with cysteine in aqueous sodium bicarbonate, with vigorous stirring, gave the bis-cysteine adduct **1** in good yield. The extreme insolubility of the product in all common solvents under neutral conditions made purification difficult and precluded simple recrystallisation. Purification was achieved by extensive washing of the product with solvents and precipitation from hydrochloric acid solution by the careful addition of aqueous ammonia. This reaction was originally examined by Hartwell[8] and subsequently by Roberts and Warwick[4] but yield, melting point and analytical data were not reported in either case. The reaction of mustard with *N*-acetylcysteine using triethylamine as base readily gave the bis-adduct **2**, again in good yield. Similar reactions with mustard sulphone proceeded readily to afford the required bis-cysteine adduct **6** (previously reported by Hartwell[8] and Ford-Moore *et al.*[9]) and bis-*N*-acetylcysteine adduct **7** in 72%



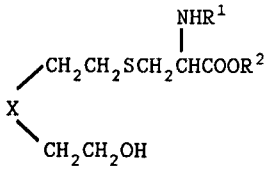
1 X=S; R^1=H; R^2=H.
2 X=S; R^1=Ac; R^2=H.
3 X=S; R^1=Ac; R^2=CH_3.
4 X=SO; R^1=H; R^2=H.
5 X=SO; R^1=Ac: R^2=H.
6 X=SO_2; R^1=H; R^2=H.
7 X=SO_2; R^1=Ac; R^2=H.
8 X=SO_2; R^1=Ac; R^2=CH_3.



14 X=SO_2; R^1=Ac; R^2=H
15 X=SO_2; R^1=Ac; R^2=CH_3



9 X=S; R^1=Ac; R^2=CH_3
10 X=SO; R^1=Ac; R^2=H.
11 X=SO; R^1=Ac; R^2=CH_3.
12 X=SO_2; R^1=Ac; R^2=H.
13 X=SO_2; R^1=Ac: R^2=CH_3.



16 X=S; R^1=H; R^2=H
17 X=S; R^1=H; R^2=CH_3
18 X=S; R^1=Ac; R^2=CH_3
19 X=SO; R^1=H; R^2=H

and 70% yields, respectively. In marked contrast, the corresponding reactions with mustard sulphoxide to give sulphinyl derivatives **4** and **5** were less than satisfactory. None of the required products could be isolated using mustard sulphoxide as starting material. The use of divinyl sulphoxide as starting material and conjugate addition was more successful although yields of the required adducts were still low. A similar procedure reported by Ford-Moore[10] for the synthesis of **4** gave a product of lower melting point with no yield quoted.

Predictably, attempts to prepare mono-conjugates from dihalogenated precursors gave on the whole more complicated reaction mixtures, often containing bis-adducts in addition to the required products, and products derived from elimination reactions when using sulphoxide and sulphone derivatives. Thus, the reaction of mustard sulphone with *N*-acetylcysteine methyl ester and sodium bicarbonate in aqueous acetonitrile solution gave a number of products including bis-adduct **8**, mono-conjugate **13** and a compound tentatively identified as the unsaturated mono-conjugate **15**. Clearly, **13** and **15** are probably intermediates on the reaction pathway to **8**. The reaction of mustard with *N*-acetylcysteine methyl ester under comparable

conditions gave mono-adduct **9** and bis-adduct **3**. As might be anticipated, no unsaturated product (analogous to **15**) was isolated reflecting the much lower susceptibility of 2-chloroethylsulphides to elimination reactions and the fundamental difference in reaction mechanism from that operative with mustard sulphone.

The synthesis of mono-conjugate **10** was achieved in 58% yield from mustard sulphoxide and *N*-acetylcysteine, together with a small amount of an unidentified vinyl conjugate. Preparative HPLC was used to separate and purify the products. Further HPLC analysis using high resolution columns (two 3μ Hypersil C-18 columns, 4.6 mm i.d. × 15 cm, linked in series) was used to verify (with some difficulty) that **10** consisted of a pair of diastereoisomers. These were shown by liquid chromatography-mass chromatography to be comparable with two compounds isolated as urinary metabolites from the urine of rats to which [^{35}S]-mustard had been administered.[5] The diastereoisomers of **10** were not separated preparatively. The corresponding methyl ester **11** was synthesised in poor yield (18%) using *N*-acetylcysteine methyl ester. Although **11** was almost certainly formed as a pair of diastereoisomers, it was not possible to demonstrate this fact by HPLC using a variety of stationary phases or by careful examination of the NMR spectrum for multiple signals. An unidentified unsaturated conjugate was also isolated from the reaction in low yield.

The 2-chloroethyl mono-conjugate **12** and vinyl mono-conjugate **14** were prepared from mustard sulphone and *N*-acetyl cysteine in aqueous sodium bicarbonate solution. Using weakly basic conditions and monitoring the reaction closely by HPLC, it was possible to isolate (by HPLC) good yields of the 2-chloroethyl mono-conjugate **12**. At higher bicarbonate concentration, **12** was present only in small amounts with **14** being the preferred product. Concommitant formation of bis-conjugate **7** was always observed during the preparation of the latter. Again, products were conveniently isolated and purified by preparative HPLC.

The fact that mustard reacts readily with sulphur containing nucleophiles suggested that a likely site of alkylation in a readily isolable protein, such as haemoglobin in blood, would be the SH group on a cysteine residue. Hydrolysis of such an alkylated protein to individual amino acids should result in the liberation of mono-cysteine adduct **16**. Using the method of Kinsey and Grant,[11] **16** was conveniently synthesised in good yield from half-mustard and cysteine maintaining the correct pH by the addition of aliquots of sodium hydroxide solution. It is of interest to note that in aqueous acetonitrile solution using sodium bicarbonate as base, attempts to react half-mustard with cysteine gave thiodiglycol as the major isolable product. However, the use of cysteine methyl ester gave a complex array of products from which the required adduct **17** could be isolated with some difficulty in 25% yield. Subsequent hydrolysis with dilute sodium hydroxide gave the adduct **16** in only moderate yield.

Alternative routes for the synthesis of **16** were sought in order to establish methods for synthesising deuterium labelled **16** for use in analysis as an internal standard. Reaction of 2-mercaptoethanol with the half-mustard, *S*-(2-chloroethyl)cysteine, gave **16** directly but again in very modest yield. Preparation of sulphoxide mono-conjugate **19** was achieved in good yield from 1-(ethenylsulphinyl)-2-hydroxyethane[1] and cysteine. As with the other asymmetrically substituted sulphoxides containing the *L*-cysteine residue, **19** would be formed as a

pair of diastereoisomers but it was not possible to show this either by HPLC analysis or by analysis of the poorly resolved NMR spectrum. Indeed, the presence of diastereoisomers may well contribute to the latter and to the lack of crystallinity of the compound. Reduction of **19** with titanium trichloride[12] in dilute hydrochloric acid solution at low pH provided a convenient route to **16** in high yield. Whilst all these procedures worked satisfactorily, it should be noted that pure **16** could be isolated from reaction mixtures only by using preparative HPLC.

The products reported were all characterised by IR, NMR and MS. Thermospray ionisation with discharge (Plasmaspray ionisation) or desorption chemical ionisation (DCI) with ammonia as reagent gas was employed for mass spectral characterisation. However, even using these very soft ionisation conditions some compounds gave weak or no quasi-molecular ions, particularly some of the bis-adducts. Some compounds were found to give very variable mass spectra presumably due to thermal decomposition in the ion source or heated thermospray interface. The S atom on the cysteinyl residue appears to promote extensive fragmentation or thermal cleavage of the C—S bonds and in many cases the predominant fragments carrying the positive charge were associated with elimination products derived from the cysteine moiety. In general, the more polar the compounds, such as the sulphinyl and sulphonyl bis-cysteinyl conjugates, the more fragmentation or decomposition was observed. Some of the sulphoxides, e.g. **4** and **5**, which are the most polar of the compounds reported, gave particularly poor and variable mass spectra.

EXPERIMENTAL

General procedures are reported in Part I.[1] "Cysteine" refers to *L*-cysteine throughout. Analytical HPLC was performed under isocratic conditions on columns that were 4.6 mm i.d. × 25 cm at a flow rate of 1.0 ml/min. Preparative separations were carried out on columns that were 21.4 mm i.d. × 25 cm at a flow rate of 21.6 ml/min, using the same stationary phase and eluant as for analysis. Peaks were observed using a UV detector at 225 nm.

Mass spectra were obtained using a VG7070 EQ double focussing mass spectrometer interfaced to an 11/250 data system and fitted with a standard VG thermospray-Plasmaspray™ interface. Plasmaspray spectra (glow discharge as the ionisation source) were obtained using a loop injector, aqueous methanol or aqueous acetonitrile as solvent, flow rate ca 0.8 ml/min, usually in the presence of ammonium acetate buffer, unless otherwise stated; 0.5 M ammonium acetate was added at 0.2 ml/min via a T-piece inserted before the probe. The accelerating voltage was 6 kV and the plasmaspray discharge voltage was 300–400 V. The vaporiser tip temp. was typically 250°C and the source temp. 220°C. The desolvation chamber was held at 1.5 A. The scan range was typically 750–120 amu at a scan rate of 1 s/decade. DCI mass spectra were obtained using ammonia as reagent gas (source pressure ca 0.3 Torr). Typically 0.5–1 μl of a concentrated solution of the compound in methanol was loaded onto a platinum filament and the solvent allowed to evaporate. After insertion into the ion source the filament was heated at a rate of 50°C/s using a current from 0–1.8 A. The source temp. was typically held at around 220°C, the scan range was 40–750 amu at 1 s/decade with 0.2 sec interscan time. Mass resolution was nominal and structural assignments shown for fragment ions are thereafter tentative, although sulphur isotope ratios assisted assignment in some cases.

CAUTION. Sulphur mustard is a potent vesicant and carcinogen and should be handled only by suitably qualified and protected individuals using a well ventilated fume hood. See Part I.

1,1'-Thiobis[2-(S-cysteinyl)ethane] (**1**). Mustard (1.3 g, 0.0082 mole) was added to a vigorously stirred solution of cysteine hydrochloride (3.0 g, 0.0019 mole) and sodium bicarbonate (3 g) in water (30 ml). After 3 h, the white precipitate was filtered off and washed thoroughly with water. The product was taken up in dilute hydrochloric acid, re-precipitated by the careful addition of ammonia solution (S.G. 0.88) and washed with water and acetone to give the bis-cysteinyl adduct **1** (1.83 g, 66%) as an extremely insoluble white solid, m.p. 255–258°C. $C_{10}H_{20}O_4N_2S_3$: Calcd: C, 36.75; H, 6.14; N, 8.53. Found: C, 36.92; H, 5.94; N, 8.53. NMR: ^{1}H: δ (D_2O) 2.88 (8H, s, $C\underline{H}_2C\underline{H}_2SC\underline{H}_2C\underline{H}_2$), 3.09 and 3.16 (both 2H, both dd, A and B parts of ABX system, $SC\underline{H}_2CH$) and 4.38 (2H, dd, X part of ABX system, $SCH_2C\underline{H}$);

^{13}C: δ 19.71, 20.39 and 21.08 (methylene C), 41.57 (SCH_2CH) and 159.58 (COOH); IR: ν_{max} 3350, 2940, 1615, 1585, 1515, 1420, 1390, 1315, 1190, 1135, 665 and 540; MD (PS): m/z 329 (MH^+, 20%), 311 ($MH^+—H_2O$, 7), 285 ($MH^+—CO_2$, 100), 266 (15), 242 ($MH^+—CH_2{=}C[NH_2]CO_2H$, 100), 222 (29), 198 ($MH^+—CH_2{=}C[NH_2]CO_2H—CO_2$, 65), 182 ($HSC_2H_4SC_2H_3[NH_3]CO_2H^+$, 11), 164 (8), 148 ($C_2H_3SC_2H_3[NH_3]CO_2H^+$, 10).

1,1'-Thiobis[2-S-(N-acetylcysteinyl)ethane] (**2**). Triethylamine was added to a stirred mixture of mustard (1.1 g, 0.007 mole) and *N*-acetylcysteine (3.0 g, 0.007 mole) in water (20 ml) until the pH was between 9 and 10. Stirring was continued for 3.5 h, when no starting material remained. The mixture was concentrated to ca 10 ml and the pH adjusted to 3 with dilute hydrochloric acid. On standing, the product precipitated and was filtered off. Recrystallisation from water gave the bis-*N*-acetylcysteine adduct **2** (2.1 g, 72%) as a colourless solid, m.p. 173–174°C. $C_{14}H_{24}O_6N_2S_3$: Calcd: C, 40.76; H, 5.86; N, 6.79. Found: C, 40.69; H, 5.78; N, 6.71. NMR: ^1H: δ (D_2O) 1.81 (6H, s, $NHCOCH_3$), 2.83 (8H, broad s, $CH_2CH_2SCH_2CH_2$), 3.02 and 3.09 (both 2H, both dd, A and B parts of ABX system, SCH_2CH), 4.36 (2H, dd, X part of ABX system, SCH_2CH); ^{13}C: δ 22.3 ($COCH_3$), 31.1, 31.8 and 32.8 (methylene C), 52.1 (SCH_2CH), 169.1 and 171.9 (COOH and $COCH_3$); IR: ν_{max} 3425, 3390, 2940, 1720, 1615, 1540, 1430, 1260, 1220 and 895; MS (PS): m/z 413 (MH^+, 30%), 301 ($M + NH_4^+—CH_2{=}C[NHAc]CO_2H$, 47), 284 ($MH^+—CH_2{=}C[NHAc]CO_2H$, 100), 147 ($CH_2{=}C[NHAc]CO_2H + NH_4^+$, 32).

1,1'-Sulphinylbis[2-(S-cysteinyl)ethane] (**4**). Divinyl sulphoxide (3.0 g, 0.029 mole) was added to a solution of cysteine hydrochloride (7.1 g, 0.045 mole) and sodium bicarbonate (7.5 g) in water (70 ml). The mixture was stirred at 50–55°C for 30 min. After standing overnight at room temperature, the solution was neutralised with hydrochloric acid and carefully evaporated to dryness. The residual solid was washed twice with boiling ethanol and then taken up in boiling water. The solution was filtered, sufficient ethanol added to produce a slight turbidity and the mixture allowed to crystallise overnight in the refrigerator. The crystals were filtered off, dried and boiled with ethanol (100 ml) to which water was added until a clear solution was obtained. This solution was treated with decolourising charcoal, filtered and cooled. On standing, colourless crystals of the bis-cysteinyl adduct **4** (3.6 g, 35%) were deposited, m.p. 235–237°C, lit[10] 226–227°C. $C_{10}H_{20}O_5N_2S_3$: Calcd: C, 34.87; H, 5.85; N, 7.92. Found: C, 34.51; H, 5.60; N, 8.13. NMR: ^1H: δ (D_2O) 2.99 (4H, m, SCH_2CH_2) overlapping 3.08 (4H, m, $SOCH_2$), 2.85 and 2.91 (both 2H, both dd, A and B parts of ABX system, J_{AB} = 15.2, J_{AX} = 5.6, J_{BX} = 8.8, SCH_2CH), 3.83 (2H, dd, X part of ABX system, J_{XA} = 5.6 and J_{XB} = 8.8, SCH_2CH); ^{13}C: δ 27.2 (SCH_2CH_2), 34.7 (SCH_2CH), 53.0 and 56.22 ($SOCH_2$ and SCH_2CH) and 175.42 (COOH); IR: ν_{max} 3390, 1590, 1490, 1380, 1335, 1295, 1130 and 1025; MS (PS): m/z 283 ($MH^+—CO_2—H_2O$, 11), 257 ($MH^+—2CO_2$, 32), 240 (23), 214 (74), 198 ($HSOC_2H_4SC_2H_3[NH_3]CO_2H^+$, 27), 169 ($SC_2H_4SOC_2H_4SH^+$, 100).

1,1'-Sulphinylbis[2-S-(N-acetylcysteinyl)ethane] (**5**). A mixture of divinyl sulphoxide (0.63 g, 0.006 mole) and *N*-acetylcysteine (2.0 g, 0.013 mole) in aqueous saturated sodium bicarbonate solution (10 ml) were stirred at room temperature for 8 h. The water was removed under reduced pressure and the residue passed down an Amberlite IR 120 ion-exchange resin (H^+ form) eluted with water. Removal of the solvent gave a brown gum that was taken up in hot water. On cooling, the sulphinyl bis-*N*-acetylcysteine adduct **5** was slowly deposited as white crystals (0.26 g, 10%), m.p. 168°C. $C_{14}H_{24}O_7N_2S_3$: Calcd: C, 39.24; H, 5.64; N, 6.54. Found: C, 38.92; H, 5.60; N, 6.59. NMR: ^1H: δ (D_2O) 2.05 (6H, s, $COCH_3$), 2.82 (8H, broad s, $SOCH_2CH_2$), 2.92 and 3.04 (both 2H, both dd, A and B parts of ABX system, SCH_2CH) and 4.6 (2H, dd, X part of ABX system, SCH_2CH); ^{13}C: δ 23.62 ($COCH_3$), 34.05 and 34.73 (CH_2SCH_2), 35.84 ($SOCH_2$), 54.75 (SCH_2CH) and 174.48 and 174.84 (COOH and $COCH_3$); IR: ν_{max} 3425, 3370, 2940, 1725, 1615, 1540, 1430, 1390, 1315, 1260, 1230, 935, 650, 605 and 580; MS: PS and DCI spectra poor and variable; major ions m/z 190 ($CH_2{=}CHSCH_2CH[NH_3]CO_2H^+$) and 130 ($CH_2{=}C[NHAc]CO_2H + H^+$).

1,1'-Sulphonylbis(S-2-cysteinylethane) (**6**). A solution of cysteine hydrochloride (2.5 g, 0.016 mole) in water (10 ml) was added to a solution of divinyl sulphone in aqueous sodium bicarbonate solution (3%, 10 ml) and the mixture stirred at room temperature for 30 min. The crude product was filtered off and recrystallised from water to give the sulphonyl bis-cysteinyl adduct **6** (1.81 g, 72%), m.p. 260°C, lit[9] 251–252°C. $C_{10}H_{20}O_6N_2S_3$: Calcd: C, 33.32; H, 5.59; N, 7.77. Found: C, 33.11; H, 5.59; N, 7.89. NMR: ^1H: δ (D_2O) 3.1 (4H, m, $SO_2CH_2CH_2S$), 3.2 (4H, dd, A and B parts of ABX system, SCH_2CH), 3.63 (4H, m, SO_2CH_2) and 4.41 (2H, dd, X part of ABX system SCH_2CH); ^{13}C: δ 25.97 and 33.92 (CH_2SCH_2), 54.6 and 55.1 (SO_2CH_2 and SCH_2CH) and 172.52 (COOH); IR: ν_{max} 3390, 2940, 1740, 1480, 1220, 1190, 1135, 1110, 1050, 940 and 810; MS (PS): m/z 274 ($MH^+—CH_2{=}C[NH_2]CO_2H$, 10%), 246 (25), 230 ($MH_+—CH_2{=}C[NH_2]CO_2H—CO_2$, 47), 204 (31), 170 ($O_2S(C_2H_4)_2S + NH_4^+$, 100), 154 (28), 136 ($(O_2S(C_2H_3)_2 + NH_4^+$, 85).

1,1'-Sulphonylbis[2-S-(N-acetylcysteinyl)ethane] (**7**). Triethylamine was added to a solution of mustard sulphone (1.0 g, 0.0052 mole) and *N*-acetylcysteine (1.8 g, 0.011 mole) in water (10 ml) until the pH

was between 9 and 10. The mixture was stirred for 2 h and then concentrated to ca 5 ml. Acidification to pH 3 with dilute hydrochloric acid followed by cooling gave a solid that was filtered off and recrystallised from water to give the sulphonyl bis-*N*-acetylcysteinyl adduct **7** (1.65 g, 70%) as white crystals, m.p. 205°C. $C_{14}H_{24}N_2O_8S_3$: Calcd: C, 37.83; H, 5.44; N, 6.30. Found: C, 38.19; H, 5.44; N, 6.47. NMR: ^{1}H: δ (D_2O) 1.96 (6H, s, $COCH_3$), 2.89 (4H, m, $SO_2CH_2CH_2$) overlapping 2.95 and 2.99 (4H, m, SCH_2CH), 3.41 (4H, m, SO_2CH_2) and 4.4 (2H, dd, X part of ABX system, J_{XA} = 6.9, J_{XB} = 9.8, SCH_2CH); ^{13}C: δ 22.2 ($COCH_3$), 23.3 ($SO_2CH_2CH_2$), 32.8 (SCH_2CH), 51.7 (SCH_2CH), 52.1 (SO_2CH_2) and 169.1 and 171.8 (COOH and $COCH_3$); IR: ν_{max} 3425, 3345, 2940, 1725, 1615, 1540, 1430, 1390, 1315, 1275, 1235, 1230, 1175, 1150, 1120, 945, 650, 520 and 485; MS (PS): m/z 333 ($M + NH_4^+$—CH_2=C[NHAc]CO_2H, 6), 330 (4), 316 (MH^+—CH_2=C[NHAc]CO_2H, 17), 246 (5), 224 (10), 204 (8), 181 (HSC_2H_3[NHAc]$CO_2H + NH_4^+$, 19), 164 (HSC_2H_3[NHAc]$CO_2H + H^+$, 55), 147 (21), 136 (48) (spectrum variable).

1,1'-Sulphonylbis[2-S-(N-acetylcysteinyl)ethane] dimethyl ester (**8**) *and 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphonyl)ethane methyl ester* (**13**). A saturated solution of sodium bicarbonate in water (3 ml) was added to a solution of mustard sulphone (0.225 g, 0.0012 mole) and *N*-acetylcysteine methyl ester (0.20 g, 0.0011 mole) in acetonitrile (8 ml). After stirring at room temperature for 4 h, no starting material remained and several product spots were apparent. The mixture was evaporated to dryness and the residue chromatographed with chloroform-methanol 19:1 to give firstly a mixed fraction and secondly, the sulphonyl bis-*N*-acetylcysteine ester **8** (rf 0.5), which was isolated as a colourless solid and recrystallised from a small volume of ethanol to give **8** (0.11 g, 20%), m.p. 132–133°C. $C_{16}H_{28}N_2O_8S_3$: Calcd: C, 40.66; H, 5.97; N, 5.92. Found: C, 40.97; H, 6.06; N, 5.44. NMR: ^{1}H: δ 2.03 (6H, s, $NHCOCH_3$), 3.02 and 3.18 (both 2H, both dd, A and B parts of ABX system, SCH_2CH) overlapping 3.05 (4H, m, $SO_2CH_2CH_2$), 3.39 (4H, m, SO_2CH_2), 3.85 (6H, s, $COOCH_3$), 4.73 (2H, dd, X part of ABX system, SCH_2CH), and 6.68 (2H, s, NH); ^{13}C: δ 23.09 and 24.56 ($NHCOCH_3$ and $SO_2CH_2CH_2$), 34.32 (SCH_2CH), 52.22, 52.98 and 53.54 (SO_2CH_2, SCH_2CH and $COOCH_3$) and 170.27 and 171.06 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3280, 1725, 1640, 1545, 1430, 1420, 1370, 1315, 1265, 1220, 1165, 1105, 1055, 945, 800, 790 and 760; MS (DCI): m/z 473 (MH^+, 17%), 330 (MH^+—CH_2=C[NHAc]CO_2CH_3, 7), 296 (9), 204 ($C_2H_3SC_2H_4$[NHAc]$CO_2CH_3 + H^+$, 30), 195 (19), 178 (HSC_2H_3[NHAc]$CO_2CH_3 + H^+$, 52), 161 (CH_2=CH[NHAc]$CO_2CH_3 + NH_4^+$, 30), 144 (CH_2=CH[NHAc]$CO_2CH_3 + H^+$, 100), 112 (15), 102 (14).

The mixed fractions having a greater rf than **8** were bulked and rechromatographed with petrol-acetone, 1:1 to afford firstly the suphonyl mono adduct **13** (rf 0.40) (0.088 g, 24%), m.p. 118–120°C. $C_{10}H_{18}ClNO_5S_2$: Calcd: C, 36.19; H, 5.47; N, 4.22. Found: C, 36.54; H, 5.56; N, 4.33. NMR: ^{1}H: δ 2.04 (3H, s, $NHCOCH_3$), 3.01 and 3.32 (4H, t, $SO_2CH_2CH_2S$) overlapping 3.03 and 3.14 (each 1H, each dd, AB portion of ABX system, SCH_2CH) overlapping 3.48 and 3.92 (each 2H, each t, $SO_2CH_2CH_2Cl$) overlapping 3.88 (3H, s, $COOCH_3$), 4.84 (1H, dd, SCH_2CH) and 6.42 (NH); ^{13}C: δ 23.12 ($NHCOCH_3$), 24.31 (SCH_2CH), 34.27 ($SO_2CH_2CH_2S$), 35.84 (CH_2Cl), 52.10, 52.99, 54.46 and 55.8 ($CH_2SO_2CH_2$ and $CHNHCOCH_3$) and 170.17 and 170.99 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3350, 1740, 1650, 1525, 1370, 1325, 1305, 1215, 1110, 590, 510 and 500; MS (NH_3 DCI): m/z 351 (2), 349 ($M + NH_4^+$, 6%), 334 (39), 332 (MH^+, 89), 313 ($M + NH_4^+$—HCl, 12), 296 (MH^+—HCl, 100), 254 (9), 204 (16), 176 (5), 162 (5), 144 (34): secondly, a small quantity of a compound whose spectroscopic properties were consistent with the unsaturated mono-ester **15** (rf, 0.45) (44 mg, 14%). (However, a satisfactory microanalysis could not be obtained for this compound.) NMR: ^{1}H: δ 2.03 (3H, s, $NHCOCH_3$), 2.86 (2H, t, $SO_2CH_2CH_2S$), 2.91 and 3.04 (2H, both dd, AB part of ABX system, SCH_2CH), 3.32 (2H, t, $SO_2CH_2CH_2S$), 3.86 (3H, s, $COOCH_3$), 4.83 (1H, dd, X part of ABX system, SCH_2CH), 6.21 and 6.40 (2H, d, SO_2CH=CH_2), 6.42 (1H, broad s, NH) and 6.72 (1H, dd, SO_2CH=CH_2); ^{13}C: δ 23.06 ($NHCOCH_3$), 24.60 ($SO_2CH_2CH_2S$), 34.20 (SCH_2CH), 52.07, 52.91 and 54.07 ($SO_2CH_2CH_2S$ and $CHCOCH_3$) 131.46 (SO_2CH=CH_2), 135.93 (SO_2CH=CH_2), 170.21 and 171.02 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3330, 1754, 1665, 1540, 1450, 1370, 1315, 1290, 1220, 1135, 1115, 985, 805, 775, 640, 520 and 495.

1,1'-Thiobis[2-S-(N-acetylcysteinyl)ethane] dimthyl ester (**3**) *and 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphonyl)ethane methyl ester* (**9**). A solution of aqueous saturated sodium bicarbonate solution (2.5 ml) was added to a stirred solution of mustard (0.325 g, 0.002 mole) and *N*-acetylcysteine methyl ester (0.35 g, 0.002 mole) in acetonitrile (10 ml). After 3.5 h, precipitated sodium chloride was filtered off and the solvent removed under reduced pressure. The residual oil was chromatographed with acetone-cyclohexane 4:6, to afford three major components. Firstly, the mono adduct **9** (rf 0.55) (0.11 g, 19%), m.p. 96°C. $C_{10}H_{18}ClNO_3S_2$: Calcd: C, 40.06; H, 6.05; N, 4.67. Found: C, 39.84; H, 6.10; N, 4.70. NMR: ^{1}H: δ 2.03 (3H, s, $NHCOCH_3$), 2.74 (4H, broad s, SCH_2CH_2S), 2.88 (2H, t, J = ca 12, SCH_2CH_2Cl), 3.02 and 3.09 (2H, dd, AB part of ABX system, SCH_2CH), 3.66 (2H, t, J = ca 12, CH_2Cl), 3.82 (3H, s, $COOCH_3$) and 4.68 (1H, dd, X part of ABX system, SCH_2CH), 6.64 (1H, broad s, NH); ^{13}C: δ 23.09 ($NHCOCH_3$), 32.18, 32.65, 34.16 and 34.28 ($CH_2SCH_2CH_2SCH_2$), 43.11 (CH_2Cl), 51.73 (SCH_2CH), 52.78 ($COOCH_3$) and 170.01 and 171.23 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3335,

2940, 1740, 1640, 1540, 1440, 1380, 1316, 1220, 1145, 1030, 990, 915, 860, 800, 705, 690, 680, 590, 530 and 500; MS (NH_3 DCI): m/z 319 (3), 317 ($M + NH_4^+$, 10%), 302 (40), 300 (MH^+, 100), 204 (8), 178 (4), 160 (4), 144 (12), 123 (5).

Secondly, the bis adduct **3** (rf 0.25) (0.051 g, 6%). $C_{16}H_{28}N_2O_6S_3$: Calcd: C, 43.62; H, 6.41; N, 6.36. Found: C, 43.85; H, 6.30; N, 6.31. NMR: ^1H: δ 2.07 (6H, s, $NHCOCH_3$), 2.74 (8H, broad s, $CH_2CH_2SCH_2CH_2$), 3.02 and 3.08 (each 2H, each dd, AB part of ABX system, SCH_2CH), 3.76 (6H, s, $COOCH_3$) and 4.78 (2H, dd, X part of ABX system, CH_2CH); ^{13}C: δ 23.05 ($NHCOCH_3$), 31.84, 32.71 and 34.01 (methylene C), 51.89 and 52.77 ($CHCOOCH_3$) and 170.13 and 171.27 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3300, 2940, 1755, 1665, 1540, 1440, 1380, 1220, 1135, 1040, 1010, 835, 725 and 595; MS (PS): 441 (MH^+, 100), 264 (MH^+—$HSC_2H_3[NHAc]CO_2CH_3$, 7), 204 (12), 195 (7), 178 ($HSC_2H_3[NHAc]CO_2CH_3 + H^+$, 15), 163 (8), 161 (8).

Thirdly, *N*-acetylcysteine dimer dimethyl ester (rf 0.2) (0.125 g), m.p. 128°C.

1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphinyl)ethane (**10**). A mixture of mustard sulphoxide (0.35 g, 0.002 mole) and *N*-acetyl cysteine (0.170 g, 0.001 mole) in aqueous potassium carbonate (5% w/v, 10 ml) was heated at 60°C for 1.5 h when HPLC analysis (Dynamax C-18 8μ column eluted with 0.1% TFA/water) showed only a small amount of starting material. The reaction mixture was acidified to pH 2 with TFA, concentrated to ca 2 ml and purified by preparative HPLC (Dynamamx C-18 8μ column, 21.4 mm by 25 cm eluted with 0.1% TFA/10% acetonitrile/90% water) to give a mixture that was mainly **10**. Further preparative HPLC (Dynamax phenyl bonded-phase 8μ column eluted with 0.1% TFA/20% acetonitrile/80% water) gave firstly, as a mixture of diastereoisomers, the sulphinyl mono-conjugate **10** (retention time 6 min) as a viscous oil (0.175 g, 58%). NMR: ^1H: δ (D_2O) 2.06 (3H, s, $NHCOCH_3$), 2.98 and 3.04 (2H, each dd, AB part of ABX system, SCH_2CH), 3.16 and 3.24 (each 2H, m, $SOCH_2CH_2S$), 3.30 (2H, t, $SOCH_2CH_2Cl$), 3.86 (2H, t, $SOCH_2CH_2Cl$), 4.58 (1H, dd, X part of ABX system, SCH_2CH); ^{13}C: δ 24.42 ($NHCOCH_3$), 27.57 (SCH_2CH), 35.32 ($SOCH_2CH_2S$), 39.86 (CH_2Cl), 53.55, 55.11 and 56.05 (CH_2SOCH_2 and SCH_2CH) and 176.39 and 176.94 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3585, 3390, 3315, 3270, 3185, 2980, 2935, 1756, 1732, 1716, 1650, 1645, 1555, 1540, and 1210; MS (PS): m/z 304 (3), 302 (MH^+, 8%), 266 (MH^+—HCl, 21), 190 ($C_2H_3SCH_2CH[NH_3]CO_2H^+$, 100), 178 (4), 164 (5), 155(4), 148 (9), 137 ($OS[C_2H_4]_2S + H^+$, 77), 130 ($CH_2{=}C[NHAc]CO_2H + H^+$, 43); a second unsaturated component (0.020 g, 8%) (retention time 7 min) could not be identified.

1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphinyl)ethane methyl ester (**11**). To a vigorously stirred solution of 1,1′-sulphinylbis(2-chloroethane) (0.3 g, 0.0017 mole) and *N*-acetylcysteine methyl ester (0.32 g, 0.0018 mole) in acetonitrile (7 ml) was added a saturated aqueous solution of sodium bicarbonate (2 ml). After 4 h, the inorganic solids were filtered off and the solvent removed under reduced pressure. The residue was chromatographed with chloroform-methanol 38:1 to afford the required sulphinyl mono-conjugate ester **11** (rf 0.3) (0.096 g, 18%), m.p. 92°C. $C_{10}H_{18}ClNO_4S_2$: Calcd: C, 38.08; H, 5.74; N, 4.44. Found: C, 37.66; H, 5.67; N, 3.90. NMR: ^1H: δ 2.03 (3H, s, $NHCOCH_3$), 2.98 overlapping 3.02 (each 2H, m, $SOCH_2CH_2S$), 3.02 and 3.09 (2H, each dd, AB part of ABX system, SCH_2CH) overlapping 3.04 (2H, t, $SOCH_2CH_2Cl$), 3.86 (3H, s, $COOCH_3$), 3.98 (2H, t, CH_2Cl), 4.92 (1H, dd, X part of ABX system, SCH_2CH) and 6.9 (1H, br s, NH); ^{13}C: δ 23.05 ($NHCOCH_3$), 25.62 ($SOCH_2CH_2S$), 34.40 (SCH_2CH), 36.86 (CH_2Cl), 52.05, 52.29, 52.86 and 54.87 (CH_2SOCH_2 and $CHCOOCH_3$) and 170.24 and 171.13 ($NHCOCH_3$ and $COOCH_3$); IR: ν_{max} 3335, 1755, 1655, 1540, 1440, 1390, 1230, 1170, 1150, 1025, 720, 670 and 615; MS (DCI): m/z 318 (40), 316 (MH^+, 100), 280 (MH^+—HCl, 8), 204 (41), 176 (53), 162 (8), 144 (37). Further elution yielded *N*-acetylcysteine dimer dimethyl ester (rf 0.2) (0.132 g).

1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphonyl)ethane (**12**). A solution of 1,1′-sulphonylbis(2-chloroethane) (0.192 g, 0.001 mole) in acetonitrile (1 ml) was added to a solution of *N*-acetylcysteine (0.164 g, 0.001 mole) in water (7 ml) and saturated sodium bicarbonate solution (2 ml). The course of the reaction was monitored by HPLC (Dynamax C-18 8μ column eluted with 0.1% TFA/20% acetonitrile/80% water). After 2 h, the reaction mixture was acidified to pH 2 with TFA and the product isolated by preparative HPLC in two 5 ml aliquots to give the sulphonyl mono-conjugate **12** (retention time, 6.08 min) as an oil that solidified on standing (0.21 g, 65%), m.p. 128–129°C (from propan-2-ol). $C_9H_{16}ClNO_5S_2$: Calcd: C, 34.01; H, 5.07; N, 4.41. Found: C, 34.04; H, 4.96; N, 4.31. NMR: ^1H δ 1.92 (3H, s, $NHCOCH_3$), 2.74 and 2.89 (2H, dd, AB part of ABX system, $J_{AB} = 14.2$, $J_{AX} = 4.8$, $J_{BX} = 8.35$, SCH_2CH), 2.79 (2H, t, $J = 6.9$, $SO_2CH_2CH_2S$), 3.36 (2H, t, $J = 6.9$, $SO_2CH_2CH_2S$), 3.44 (2H, t, $J = 7.42$, $SO_2CH_2CH_2Cl$), 3.72 (2H, t, $J = 7.42$, CH_2Cl), 4.35 (1H, dd, X part of ABX system, $J_{AX} = 4.8$, $J_{BX} = 8.35$, SCH_2CH) and 8.3 ($NHCOCH_3$); ^{13}C: δ 22.3 and 23.5 ($NHCOCH_3$ and $SO_2CH_2CH_2S$), 32.3 (SCH_2CH), 36.4 (CH_2Cl), 51.87, 53.28 and 54.15 ($CH_2SO_2CH_2$ and SCH_2CH) and 169.3 and 171.9 ($NHCOCH_3$ and COOH); IR: ν_{max} 3450, 3385, 1725, 1615, 1540, 1430, 1325, 1300, 1265, 1250, 1110, 1040, 945, 900, 820, 655, 600, 570, 545, 515 and 485; MS (PS without NH_4OAc): m/z 320 (10), 318 (MH^+, 28%), 282 (MH^+—HCl, 100), 190 (5), 164 (5), 130 (7).

1-[S-(N-acetylcysteine)]-2-(2-ethenylsulphonyl)ethane (**14**). A mixture of 1,1'-sulphonylbis(2-chloroethane) (0.192 g, 0.001 mole), *N*-acetylcysteine (0.164 g, 0.001 mole), saturated sodium bicarbonate solution (5 ml) and water (5 ml) were heated to 60°C (when all the sulphone was in solution) and maintained at this temperature for 2 h. The reaction mixture was acidified to pH 2 with TFA and separation of the products by preparative HPLC (Dynamax phenyl bonded-phase column eluted with 0.1% TFA/10% acetonitrile/90% water) gave firstly the sulphonyl bis-conjugate **7** (0.040 g, 9%) (retention time 12.13 min) and secondly the unsaturated mono-conjugate **14** (0.154 g, 55%) (retention time 16.01 min), m.p. 127–128°C (from propan-2-ol). $C_9H_{15}NO_5S_2$: Calcd: C, 38.42; H, 5.37; N, 4.97. Found: C, 38.61; H, 5.33; N, 4.94. NMR: ^{1}H δ (D_2O) 2.01 (3H, s, $NHCOCH_3$), 2.96 (2H, t, J = 7.8, $SO_2CH_2CH_2S$), 2.99 and 3.12 (each 1H, each dd, AB part of ABX system, J_{AB} = 14.4, J_{AX} = 5.8, J_{BX} = 8.7, SCH_2CH), 3.52 (2H, t, J = 7.8, SO_2CH_2), 4.61 (1H, dd, X part of ABX system, J_{AX} = 5.8, J_{BX} = 8.7, SCH_2CH), 6.39 (1H, d, J_{cis} = 10.4, $SO_2CH{=}CH_2$), 6.48 (1H, d, J_{trans} = 16.84, $SO_2CH{=}CH_2$) and 6.84 (1H, dd, J_{cis} = 10.4, J_{trans} = 16.84, $SO_2CH{=}CH_2$); ^{13}C: δ 22.42 ($NHCOCH_3$), 25.47 (SCH_2CH), 34.46 ($SO_2CH_2CH_2S$), 53.52 (SCH_2CH), 54.93 (SO_2CH_2), 131.65 ($SO_2CH{=}CH_2$), 137.56 ($SO_2CH{=}CH_2$) and 173.34 and 173.40 ($NHCOCH_3$ and $COOH$); IR: ν_{max} 3340, 1700, 1615, 1565, 1425, 1320, 1255, 1225, 1140 and 1115; MS (PS): 299 ($M + NH_4^+$, 82), 282 (MH^+, 100), 190 (10), 181 (22), 164 (21).

2-[2-(S-cysteinyl)ethylthio]ethanol (**16**).

(i) By hydrolysis of ester **17**. A solution of mono-ester **17** (0.30 g, 0.0012 mole) in a mixture of aqueous sodium hydroxide (1.5% w/v, 1 ml) and methanol (1 ml) was stirred at room temperature for 6 h. The reaction mixture was evaporated to ca half volume, acidified to pH 2 with TFA and chromatographed (Dynamax phenyl bonded-phase 8μ column eluted with 0.1% TFA/6% acetonitrile/94% water, retention time, 5.6 min) to give the hydroxyethyl mono-acid **16** (0.15 g, 45%) as the semi-crystalline TFA salt. The salt was taken up in ethyl acetate containing 2% hydrogen chloride gas; on standing, the acid **16** slowly precipitated out as the crystalline hydrochloride salt, m.p. 125–126°C. NMR: ^{1}H: δ (D_2O) 2.73 (2H, t, J = 6.2, SCH_2CH_2OH), 2.80 (4H, broad s, SCH_2CH_2S), 3.17 and 3.23 (each 1H, each dd, AB part of ABX system, J_{AB} = 15.23, J_{AX} = 7.24, J_{BX} = 4.32, SCH_2CH), 3.74 (2H, t, J = 6.2, CH_2OH and 4.24 (1H, dd, X part of ABX system, J_{AX} = 7.24, J_{BX} = 4.32, SCH_2CH); ^{13}C: δ 33.70, 34.10, 34.38 and 36.15 ($CH_2SCH_2CH_2SCH_2$), 55.42 (SCH_2CH), 63.12 (CH_2OH) and 173.70 ($COOH$); IR: ν_{max} 3270, 3200, 3160, 2950, 2915, 2850, 1725, 1485, 1260, 1220, 1195, 1060 and 1005; MS (DCI): m/z 226 (MH^+, 100%).

(ii) From *S*-(2-chloroethyl)cysteine and 2-mercaptoethanol. A solution of *S*-(2-chloroethyl)cysteine (0.20 g, 0.00091 mole) in water (5 ml) containing sodium bicarbonate (0.15 g) was treated with 2-mercaptoethanol (0.45 g, 0.0058 mole) at room temperature for 30 min when HPLC analysis (above) showed an absence of starting material. Work up and isolation of the product by HPLC (as above) gave the acid **16** as the TFA salt (0.16 g, 51%).

(iii) From titanium trichloride reduction of sulphoxide **19**. An aqueous solution of tianium trichloride (15% w/w in aqueous hydrochloric acid, 2 ml) was added to a solution of sulphoxide mono-conjugate (0.50 g, 0.002 mole) in water (2 ml) and the mixture heated at 60°C for 30 min when HPLC analysis showed no **19** to be present. Preparative HPLC (Dynamax C-18 8μ column eluted with 0.1% TFA/5% methanol/95% water, retention time 6.2 min) gave the mono-conjugate acid **16** as the TFA salt (0.4 g, 86%).

2-[2-(S-cysteinyl)ethylthio]ethanol methyl ester (**17**). A mixture of cysteine methyl ester hydrochloride (1.0 g, 0.0056 mole), 2-(2-chloroethylthio)ethanol (1.0 g, 0.007 mole) and potassium carbonate in acetonitrile-water solution (3:1, 16 ml) was stirred at 60°C for 12 h. The solvent was removed and the residue chromatographed with chloroform-methanol 19:1. From among the many products present in the reaction mixture, the fraction with rf 0.2 provided a sample that was mainly **17**. Further purification by HPLC (Dynamax phenyl bonded-phase 8μ column eluted with 0.1% TFA/12.5% acetonitrile/87.5% water) gave the pure hydroxyethyl mono-conjugate **17** (0.34 g, 25%) as an oil. NMR: ^{1}H: δ (D_2O) 2.76 (2H, t, SCH_2CH_2OH), 2.86 (4H, broad s, SCH_2CH_2S), 3.19 and 3.29 (2H, dd, AB part of ABX system, SCH_2CH), 3.87 (2H, t, CH_2OH), 3.96 (3H, s, $COOCH_3$) and 4.46 (1H, dd, X part of ABX system, SCH_2CH); ^{13}C: δ 33.9, 34.1, 34.7 and 34.9 ($CH_2SCH_2CHSCH_2$), 53.5 and 53.8 ($CHCOOCH_3$), 63.11 (CH_2OH) and 170.13 ($COOCH_3$); IR: ν_{max} 2920, 2850, 1750, 1680, 1205, 1185 and 1140; MS (DCI): m/z 240 (MH^+, 100%), 208 (MH^+—CH_3OH, 6), 196 (MH^+—C_2H_4O, 7), 162 (MH^+—HSC_2H_4OH, 14), 136 (9), 105 ($C_2H_4SC_2H_4OH^+$, 10), 102 ($CH_2{=}C[NH_3]Co_2CH_3^+$, 23).

2-[2-S-(N-acetylcysteinyl)ethylthio]ethanol methyl ester (**18**). A solution of saturated aqueous sodium bicarbonate (3.0 ml) was added to a solution of 2-(2-chloroethylthio)ethanol (0.3 g, 0.0021 mole) and *N*-acetylcysteine methyl ester (0.36 g, 0.0022 mole) in acetonitrile (10 ml). The mixture was stirred vigorously for 4 h at room temperature. Most of the solvent was removed under reduced pressure, water was added and the product extracted into chloroform. The extract was dried and concentrated

and the residue chromatographed (cyclohexane-acetone 6:4) to give the hydoxyethyl conjugate **18** (0.19 g, 34%) as a low melting solid, m.p. 37°C. $C_8H_{17}NO_3S_2$: Calcd: C, 42.69; H, 6.81; N, 4.98. Found: C, 42.29; H, 6.84; N, 4.74. NMR: ^{1}H: δ 2.06 (3H, s, $NHCOCH_3$), 2.46 (1H, broad s, OH), 2.74 (2H, t, SCH_2CH_2OH), 2.80 (4H, broad s, SCH_2SCH_2S), 2.99 and 3.06 (each 1H, each dd, AB part of ABX system, SCH_2CH), 3.80 (2H, t, CH_2OH), 3.84 (3H, s, $COOCH_3$), 4.86 (1H, dd, X part of ABX system, SCH_2CH) and 6.42 (1H, broad s, $NHCOCH_3$); ^{13}C: δ 22.98 ($NHCOCH_3$), 30.99, 31.75, 32.53 and 34.14 ($CH_2SCH_2CH_2SCH_2$), 51.88 (SCH_2CH), 52.77 ($COOCH_3$), 61.08 (CH_2OH) and 170.55 and 171.31 ($COOCH_3$ and $NHCOCH_3$); IR: ν_{max} 3295, 1745, 1655, 1545, 1435, 1375, 1270, 1215 and 1175; MS (NH_3 DCI): m/z 282 (MH^+, 100%), 264 (MH^+—H_2O, 17), 236 (11), 204 ($C_2H_3SC_2H_3$[NHAc]CO_2CH_3 + H^+, 27), 178 (11), 176 (11), 161 (6), 144 (52), 105 (16).

2-[2-(S-cysteinylethyl)sulphinyl]ethanol (**19**). A solution of 2-(2-chloroethylsulphinyl)ethanol (0.5 g, 0.0029 mole) in aqueous potassium carbonate solution (10% w/v, 5 ml) was heated at 60°C until virtually no starting material remained and the solution contained essentially 2-[2-(ethylenylsulphinyl)]ethanol only (HPLC analysis, Microsorb C-18 5μ column eluted with water only). This solution was cooled to room temperature and cysteine hydrochloride (0.75 g, 0.0048 mole) added. The pH was adjusted to 9 with aqueous potassium carbonate solution and the reaction mixture stored overnight at room temperature. After acidification to pH 2 with TFA, the mixture was chromatographed in 2 ml aliquots, collecting the peak with retention time 6 min to give the mono-conjugate **19** (0.505 g, 70%) as a clear viscous liquid. NMR: ^{1}H: δ (D_2O) 2.97–3.31 (8H, overlapping m, $CH_2SOCH_2CH_2SCH_2$), 3.99 (2H, m, CH_2OH) and 4.27 (1H, m, SCH_2CH); ^{13}C: δ 26.15 ($SOCH_2CH_2S$), 33.03 (SCH_2CH), 52.57, 52.61 and 53.48 (CH_2SOCH_2 and SCH_2CH), 55.82 (CH_2OH) and 170.55 (COOH); IR: ν_{max} 3450, 2940, 1740, 1680, 1540, 1430, 1205, 1150, 1065, 1020, 990, 840, 800 and 730; MS (PS): 242 (MH^+, 92%), 224 (MH^+—H_2O, 24), 198 (MH^+—C_2H_4O, 80), 155 (MH^+—CH_2=C[NH_2]CO_2H, 100).

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