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APPENDIX

Synthesis of N-acylglutathione derivatives by dimethylaminopyridine catalysis

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A general procedure, involving 4-dimethylaminopyridine-catalysed acylation of the amino groups in the appropriate S-blocked glutathione, is reported for the preparation of N-blocked derivatives of glutathiones.

Glutathione (GSH), ubiquitous in biochemistry, has many roles ranging from those as coenzyme or co-substrate through transport processes, detoxification, protein SH maintenance to primitive chemoreceptor activity (Jocelyn, 1972). We have been studying GSH-dependent enzymes from a variety of angles, including the use of chemically modified GSH analogues, e.g. photoaffinity labels based on GSH (Seddon & Douglas, 1980; Seddon *et al.*, 1980; D'Silva *et al.*, 1981). In the course of such work we required a range of N-modified analogues of glutathione and experienced considerable difficulty in simple Schotten–Baumann acylation, either under aqueous or non-aqueous conditions. In spite of their apparent simplicity, such derivatives have only rarely been described by routes starting with GSH (Sokolovsky *et al.*, 1964a,b; Utzinger *et al.*, 1963; Fujihara *et al.*, 1972; Vince *et al.*, 1971), and are usually the results of classical solution total peptide syntheses of GSH analogues (Goldschmidt & Jutz, 1953; Amiard *et al.*, 1958; Wieland & Wedenmuller, 1955; Kovacs *et al.*, 1972). We now

report a successful and convenient application of the catalyst 4-dimethylaminopyridine (Steglich & Vorbrüggen, 1978) to solve this problem of the low reactivity of the glutathione amino group. We also report purification procedures for such derivatives (2–7):

- (1) $R^1 = R^2 = \text{COOCH}_2\text{C}_6\text{H}_5$
- (2) $R^1 = \text{COPh}$; $R^2 = \text{CH}_2\text{C}_6\text{H}_4m\text{CF}_3$
- (3) $R^1 = \text{COC}_6\text{H}_4p\text{N}_3$; $R^2 = \text{CH}_2\text{C}_6\text{H}_4m\text{CF}_3$
- (4) $R^1 = \text{COCH}_3$; $R^2 = \text{CH}_2\text{C}_6\text{H}_4p\text{Br}$
- (5) $R^1 = \text{COC}(\text{CH}_3)_3$; $R^2 = \text{CH}_2\text{C}_6\text{H}_4p\text{Br}$
- (6) $R^1 = \text{COC}_6\text{H}_5$; $R^2 = \text{CH}_2\text{C}_6\text{H}_4p\text{Br}$
- (7) $R^1 = \text{COOCH}_2\text{C}_6\text{H}_5$; $R^2 = \text{CH}_2\text{C}_6\text{H}_4p\text{Br}$

Experimental

Elemental analyses were performed by the Micro-analytical Laboratory, Department of Chemistry,

Abbreviation used: GSH, reduced glutathione.

University of Manchester, Manchester, U.K. Precursors and solvents were purchased commercially and purified appropriately. Dioxan was routinely freed from peroxides by passage over neutral alumina; peroxides were tested for with KI solution.

U.v. and visible spectra were recorded by means of Pye– Unicam SP.8-100 and Carlo Erba Spectra-comp 601 spectrophotometers at 25°C. ^1H n.m.r. spectra were obtained by using a Varian EM 360 instrument and ^{13}C n.m.r. (Fourier-transform) spectra recorded at 20.15 MHz on a Bruker WP 805 Y spectrometer. ^1H and ^{13}C chemical shifts (δ) are in p.p.m. from tetramethylsilane; resonances marked with an asterisk disappeared on addition of $^2\text{H}_2\text{O}$; spectra were run in $[\text{}^2\text{H}_6]\text{dimethyl sulphoxide}$. T.l.c. was performed on 0.2 mm-thick precoated plates (Merck DC-Plastikfolien Kieselgel 60 F₂₅₄). Solvents used were: A, butan-1-ol/acetic acid/water (7:1:2, by vol.); B, phenol/water (4:1, v/v); C, water/butan-1-ol/ethanol (5:4:1, by vol.); D, propan-1-ol/acetic acid/water (7:1:2, by vol.). Spots were made visible by u.v. illumination and ninhydrin/acetone spray. Tests for completion of *N*-blocking were carried out by means of ninhydrin reagent. Melting points (uncorrected) were determined with a m.p. microscope (Reichart).

4-Azidobenzoyl chloride (Hixson & Hixson, 1975), *S*-(4-bromobenzyl)glutathione (Vince *et al.*, 1971) and *S*-(3-trifluoromethylbenzyl)glutathione (m.p. 201–202°C) were prepared by literature procedures or modifications thereof. During all syntheses involving derivatives with azido groups the systems were protected from light, usually by metal foil.

N,S-Bis(benzyloxycarbonyl)glutathione (1)

This was prepared by a modification of the method of Sokolovsky *et al.* (1964b). It differed from that procedure in that the product on acidification of the reaction mixture was extracted into ethyl acetate; the extracts were washed with water, dried (over anhydrous Na_2SO_4), the solvent was removed, and the residue recrystallized from ethyl acetate/ethanol to give a colourless solid. Yield (68.6%); m.p. 167–170°C [literature values 105–107 and 141–143°C (both Sokolovsky *et al.*, 1964b)]. [Found: C, 54.4; H, 5.1; N, 7.4; S, 5.6. $\text{C}_{26}\text{H}_{29}\text{N}_3\text{O}_{10}\text{S}$ (mol.wt. 576.4) calc. for C, 54.25; H, 5.1; N, 7.3; S, 5.6%]; δ_{H} (p.p.m.) [(C^2H_5)₂SO] 8.1* (2H, m, 2 × NH), 7.4* (1H, m, N-H), 7.25 (10H, s, Ar-H), 5.1 (2H, s, $\text{S-CO}_2\text{CH}_2\text{-Ph}$), 4.9 (2H, s, $\text{N-CO}_2\text{-CH}_2\text{-Ph}$), 4.5 (1H, m, $-\text{CH}$), 3.7 (2H, d, $-\text{CH}_2\text{-S}$), 3.2 (1H, m, $-\text{CH}$), 2.4–2.0. (6H, m, Gly-CH_2 , $\text{Glu-CH}_2\text{CH}_2$).

General acylation procedure

Derivatives (2)–(7) were prepared by the following general procedure, which involves dimethylaminopyridine-catalysed acylation of the appro-

priate *S*-blocked glutathione. Usually a second treatment with dimethylaminopyridine plus acyl chloride (1:1 molar ratio in diethyl ether) was found to be necessary. In a typical procedure, *S*-blocked glutathione (1 equiv.) was dissolved in 2M-NaOH with an equal volume of dioxan (final pH_{app} 9–10) and then a mixture of acyl chloride (1–1.5 equiv.) plus dimethylaminopyridine (1 equiv.), pre-mixed in diethyl ether, added. The solution was then stirred for 6–24 h at room temperature with the exception of compounds (6) and (7), for which 35°C was used. A further portion of acyl chloride plus dimethylaminopyridine was then added and the stirring process repeated. The solution was then adjusted to pH_{app} 4.5, extracted with diethyl ether, then adjusted to pH_{app} 2.0 and the product extracted into ethyl acetate (propan-1-ol being added for solubilization if necessary). The organic layer was then washed with water, dried over anhydrous MgSO_4 , filtered, evaporated and the residue purified by the appropriate technique.

N-(Benzoyl)-*S*-(3-trifluoromethylbenzyl)glutathione (2)

This compound was prepared via the general procedure from *S*-(3-trifluoromethylbenzyl)glutathione (2.5 g; 5.37 mmol). The residue obtained was recrystallized twice from ethyl acetate/methanol to give a colourless solid (1.5 g, 49.0%). The compound (0.7 g) was further purified by LH-20 Sephadex (1.1 cm × 44 cm column) chromatography, with methanol as eluant, to recover (0.4 g, 55.5%) of material of m.p. 199–201°C. [Found: C, 52.6; H, 4.55; N, 7.2. $\text{C}_{25}\text{H}_{26}\text{N}_3\text{O}_7\text{SF}_3$ (mol.wt. 569.56) requires C, 52.7; H, 4.6; N, 7.4%]; δ_{H} (p.p.m.) [(C^2H_5)₂SO] 8.7* (2H, d, 2 × NH), 8.4* (1H, m, NH), 8.0–7.7 (9H, m, Ar-H), 4.8 (2H, m, 2 × CH), 4.1 (4H, m, $\text{S-CH}_2\text{-Ar}$, $\text{S-CH}_2\text{-}$), 3.0–2.6 (6H, m, Gly-CH_2 , $\text{Glu-CH}_2\text{-CH}_2$); t.l.c. R_F (solvent A) 0.66; R_F (solvent C), 0.38.

N-(4-Azidobenzoyl)-*S*-(3-trifluoromethylbenzyl)glutathione (3)

This was prepared via the general procedure from *S*-(3-trifluoromethylbenzyl)glutathione (2.5 g; 5.37 mmol). The residue obtained was recrystallized from ethyl acetate/methanol to give an off-white solid (1.7 g; 51.8%), m.p. 162–164°C with decomposition. The compound (0.7 g) was further purified by LH-20 Sephadex (1.1 cm × 42 cm column) chromatography with methanol as eluant, to recover (0.25 g, 35.7%) of material of m.p. 161–163°C with decomposition [found: C, 49.2; H, 4.1; N, 13.5; $\text{C}_{25}\text{H}_{25}\text{N}_6\text{O}_7\text{SF}_3$ (mol.wt. 610.6) requires C, 49.2; H, 4.1; N, 13.8%]; δ_{H} (p.p.m.) [(C^2H_5)₂SO] 8.57* (1H, d, N-H), 8.15* (2H, d, N-H), 7.8 (2H, d, Ar-H), 7.4 (4H, s, Ar-H), 7.0 (2H, d, Ar-H), 4.3 (2H, m, 2 × CH), 3.7 (4H, s, 2 × CH_2), 2.25 (6H, m,

$3 \times \text{CH}_2$); t.l.c., R_F (solvent A) 0.67; R_F (solvent C) 0.33 [*S*-(3-trifluoromethylbenzyl)glutathione: R_F (A) 0.35; R_F (C) 0.22]; λ_{max} . (0.83% dimethyl sulphoxide/0.05 M- KH_2PO_4 buffer, pH 6.60) 271 nm ($\log \epsilon$ 4.38).

N-Acetyl-*S*-(4-bromobenzyl)glutathione (4)

This was prepared via the general procedure from *S*-(4-bromobenzyl)glutathione (1.5 g; 3.15 mmol). The residue obtained was twice recrystallized from ethanol/ethyl acetate to give a colourless solid (0.66 g; 40.4%), m.p. 176–178°C. (Literature value 128–130°C; Vince *et al.*, 1971). [Found: C, 44.1; H, 4.8; N, 7.9. $\text{C}_{19}\text{H}_{24}\text{N}_3\text{O}_7\text{SBr}$ (mol.wt. 518.4) calc. for C, 44.0; H, 4.7; N, 8.1%]; δ_{H} (p.p.m.) [(C^2H_3)₂SO] 8.3* (3H, m, $3 \times \text{NH}$), 7.7 (4H, m, Ar-H), 5.0 (1H, m, CH), 4.8 (1H, m, CH), 4.3 (4H, d, $2 \times \text{CH}_2\text{-S}$), 3.2–2.7 (6H, m, Gly- CH_2 , Glu- CH_2CH_2), 2.4 (3H, s, CO- CH_3); δ_{C} (p.p.m.) [(C^2H_3)₂SO] 22.3 (CH_3), 27.1, 31.7, 33.16, 34.3 ($4 \times \text{CH}_2$), 51.5, 51.78 ($2 \times \text{C}_\alpha$), 119.8, 131.06, 131.1, 137.96 ($4 \times \text{C-Ar}$), 169.3, 170.5, 170.8, 171.5, 173.3 ($5 \times \text{C=O}$); t.l.c.: R_F (solvent A) 0.7; R_F (solvent C) 0.31.

N-Pivaloyl-*S*-(4-bromobenzyl)glutathione (5)

This was prepared via the general procedure from *S*-(4-bromobenzyl)glutathione (2.5 g; 5.25 mmol). An oily residue was obtained (1.78 g) of which (0.5 g) was dissolved in methanol and purified by LH-20 Sephadex (1.1 cm $\times$ 44 cm column) chromatography (two passages) with methanol as eluant, to recover 0.21 g (42%) of material as a colourless solid, m.p. 85°C. [Found: C, 46.1; H, 5.5; N, 7.2. $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_7\text{SBr}$ (mol.wt. 564.4) requires C, 45.9; H, 5.4; N, 7.5%]; δ_{H} (p.p.m.) [(C^2H_3)₂SO] 8.5* (2H, m, $2 \times \text{NH}$), 7.8 (4H, m, Ar-H); 5.0 (2H, m, $2 \times \text{CH}$), 4.1 (4H, d, $2 \times \text{CH}_2\text{-S}$), 3.0–2.6 (6H, m, Gly- CH_2 , Glu- CH_2CH_2), 1.5 [9H, s, CO-(CH_3)₃]; t.l.c.: R_F (solvent A) 0.61; R_F (solvent C) 0.39.

N-Benzoyl-*S*-(4-bromobenzyl)glutathione (6)

This was prepared via the general procedure from *S*-(4-bromobenzyl)glutathione (1.9 g, 4 mmol). The residue obtained was recrystallized from ethanol to give a colourless solid (1.45 g). The compound (0.7 g) was further purified by LH-20 Sephadex (1.1 cm $\times$ 44 cm column) chromatography (three passages) with methanol as eluant to recover 1.08 g (15.5%) of material of m.p. 190–192°C. [Found: C, 49.4; H, 4.5; N, 7.0. $\text{C}_{24}\text{H}_{26}\text{N}_3\text{O}_7\text{SBr}$ (mol. wt. 580.45) requires C, 49.7; H, 4.5; N, 7.2%]; δ_{H} (p.p.m.) [(C^2H_3)₂SO] 8.6* (1H, d, N-H), 8.2* (2H, m, N-H), 7.85 (2H, m, Ar-H), 7.4 (6H, m, Ar-H), 4.5 (2H, m, $2 \times \text{CH}$), 3.75 (4H, d, $2 \times \text{CH}_2$), 2.4 (6H, m, $3 \times \text{CH}_2$); t.l.c.: R_F (solvent A) 0.7.

N-Benzyloxycarbonyl-*S*-(4-bromobenzyl)glutathione (7)

This compound was prepared via the general procedure from *S*-(4-bromobenzyl)glutathione (2.3 g; 4.84 mmol). The residue obtained was thrice recrystallized from ethanol/ethyl acetate to give a colourless solid (1.32 g, 43%), m.p. 199–201°C. [Found: C, 49.2; H, 4.6; N, 6.7. $\text{C}_{25}\text{H}_{28}\text{N}_3\text{O}_8\text{SBr}$ (mol.wt. 610.5) requires C, 49.1; H, 4.8; N, 6.9%]. δ_{H} (p.p.m.) [(C^2H_3)₂SO] 8.1 (3H, m, N-H), 7.2 (9H, m, Ar-H), 4.9 (2H, s, CO₂-CH₂-Ph) 4.4 (1H, m, CH), 3.8 (1H, m, CH), 3.6 (4H, m, $2 \times \text{CH}_2\text{-S}$), 2.3 (6H, m, $3 \times \text{CH}_2$); t.l.c.: R_F (solvent A) 0.65; R_F (solvent C) 0.37; R_F (solvent D) 0.7.

Results and discussion

The procedure described involves the catalysis of *N*-acylation of the terminal NH_2 group of glutathione by *NN*-dimethylaminopyridine and is considerably more effective than direct acylation with the acid halide under aqueous or non-aqueous Schotten–Baumann conditions. The major problems experienced in synthetic reactions of the NH_2 group of GSH are the rather unreactive nature of this group and purification of the products. The catalysis used overcomes the former and we have found the latter to be resolved usually by means of Sephadex LH-20 column chromatography, although in a number of instances recrystallization was satisfactory.

In the case of *N,S*-bis(benzyloxycarbonyl)glutathione (1), a number of values are reported in the literature (Sokolovsky *et al.*, 1964*b*) for the melting point (namely 105–107°C and 141–143°C) in addition to the value we find of 167–170°C. This is presumably caused by the existence of different crystalline forms. A similar phenomenon presumably explains the difference between the literature melting-point value (128–130°C; Vince *et al.*, 1971) for *N*-acetyl-*S*-(*p*-bromobenzyl)glutathione and the value that we observe (176–178°C).

The availability of such *N,S*-blocked glutathione analogues has already provided some novel information of GSH binding sites on biological macromolecules. Such derivatives are competitive inhibitors of yeast glyoxalase I [see the main paper (Douglas *et al.*, 1982)] and may be used to map the binding site for GSH. We have found evidence, using such derivatives, of a hitherto-unsuspected GSH-binding site on ribonucleotide reductase from *Escherichia coli* as well as on glutaredoxin (Höög *et al.*, 1982).

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