S-(N-Aryl-N-hydroxycarbamoyl)glutathione Derivatives Are Tight-Binding Inhibitors of Glyoxalase I and Slow Substrates for Glyoxalase II

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S-(*N*-Aryl-*N*-hydroxycarbamoyl)glutathione derivatives are powerful competitive inhibitors of the anticancer target enzyme glyoxalase I. Indeed, the *N*-*p*-bromophenyl derivative is the strongest inhibitor of the enzyme from human erythrocytes yet reported ($K_i = 1.4 \times 10^{-8}$ M). Structure–activity correlations indicate that the high affinities of the derivatives for both human and yeast glyoxalase I are due to the fact that the derivatives are hydrophobic analogs of the enediol(ate) intermediate associated with the glyoxalase I reaction. The derivatives also proved to be slow substrates for the thioester hydrolase glyoxalase II (bovine liver). Compounds of this type are of interest as potential tumor-selective anticancer agents, based on the abnormally low levels of glyoxalase II activity in some types of cancer cells.

Introduction

The glyoxalase pathway has been the subject of renewed interest as a possible anticancer target.[1,2] The apparent physiological function of this elementary detoxification pathway is to remove cytotoxic methylglyoxal from cells as D-lactate, as outlined in Scheme 1.[3] Methylglyoxal appears to arise as an unavoidable byproduct of normal cellular metabolism.[4] Long ago, Vince and Daluge proposed that inhibitors of glyoxalase I might function as anticancer agents, on the basis of the potential ability of these inhibitors to induce elevated levels of methylglyoxal in cells.[5] Indeed, Lo and Thornalley recently reported that simple competitive inhibitors of human erythrocyte glyoxalase I, like *S*-(*p*-bromobenzyl)glutathione ($K_i = 0.27$ μM),[6] arrest the growth of human leukemia 60 (HL 60) cells when delivered into these cells as the diethyl esters.[1] However, these compounds showed limited ability to preferentially inhibit HL 60 cells versus fully differentiated neutrophils, the corresponding normal cell type. Thus, simple competitive inhibitors of glyoxalase I appear to have limited value as tumor-selective anticancer agents.

Recently, we suggested that inhibitors of glyoxalase I might preferentially inhibit tumor cells, if the inhibitors were also designed to serve as substrates for the thioester hydrolase glyoxalase II.[2] This follows from the observation that glyoxalase II activity is abnormally low in many types of cancer cells versus normal cells.[7,8] Thus, tumor-selectivity could arise from the reduced ability of cancer cells to hydrolyze the inhibitors. We also described the synthesis and characterization of the prototypical inhibitor *S*-(*N*-methyl-*N*-hydroxycarbamoyl)glutathione (**2**), a moderately strong competitive inhibitor of yeast glyoxalase I (K_i = 68 μM) and a slow substrate for bovine liver glyoxalase II. Inhibitors of this type might have practical utility as tumor-selective anticancer agents, provided that tighter binding derivatives can be synthesized.

In this report, we describe the synthesis and characterization of *S*-(*N*-aryl-*N*-hydroxycarbamoyl)glutathione derivatives **6a–c** that are among the strongest competitive inhibitors of glyoxalase I yet reported and that also serve as substrates for glyoxalase II (Scheme 2). Also described is an analysis of the structural basis of tight-binding inhibition by these compounds.

Chemistry and Enzymology

The *S*-(*N*-aryl-*N*-hydroxycarbamoyl)glutathione derivatives **6a–c** were prepared by the reaction of GSH and the 4-chlorophenyl esters of the corresponding *N*-aryl-*N*-hydroxycarbamates **5a–c**, as outlined in Scheme 2. The crude products were purified to apparent homogeneity by reverse-phase HPLC. NMR spectral assignments were based on comparisons with previously published NMR studies of GSH and its derivatives.[9] The acylating reagents (**5a–c**) were prepared by a modification of a general procedure.[10] The hydroxylamines were prepared by reduction of the corresponding para-substituted nitrobenzenes (**3a–c**) using published methods: *N*-phenylhydroxylamine (**4a**),[11] *N*-(*p*-chlorophenyl)hydroxylamine (**4b**),[12] and *N*-(*p*-bromophenyl)hydroxylamine (**4c**).[13] *S*-(*N*-Methylcarbamoyl)glutathione was synthesized by the reaction of GSH with methyl isocyanate, as described elsewhere.[14] *S*-Propionylglutathione was prepared by an acyl-interchange reaction between GSH and *S*-propionylthiophenol following the general method of Wieland and Koppe.[15]

The preparations of yeast glyoxalase I and bovine liver glyoxalase II were purchased from Sigma Chemical Co. Human erythrocyte glyoxalase I was purified to homogeneity from outdated human blood by a published procedure.[16] The magnitudes of the kinetic constants of **6a–c** with glyoxalase I and glyoxalase II were determined as previously described.[2] Standard errors for V_m and K_m were obtained by analysis of the initial rate data using the HYPER program of Cleland.[17]

Results and Discussion

The design of tight-binding inhibitors of glyoxalase I that also serve as substrates for glyoxalase II is a challenging problem because thioester derivatives of GSH generally bind weakly to the active site of glyoxalase I. Although simple *S*-alkyl and *S*-aryl derivatives of GSH are known to bind tightly to the active site of glyoxalase I, primarily

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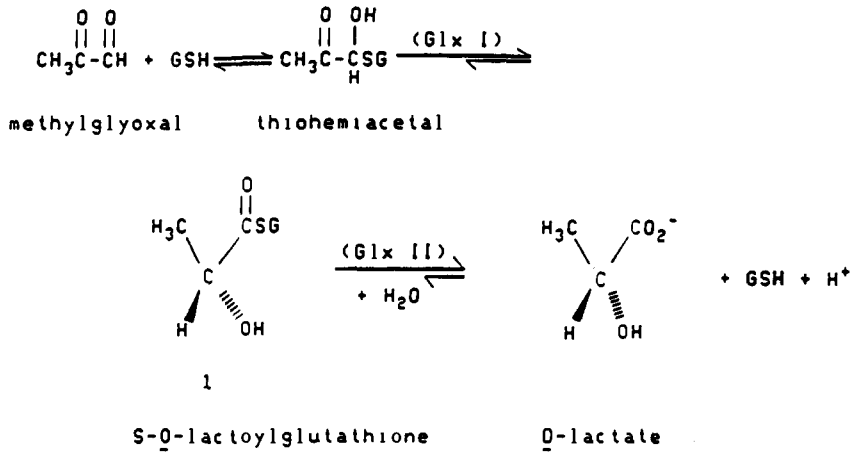
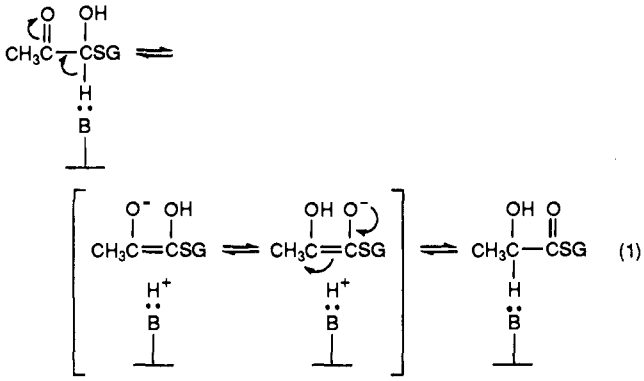
Scheme 1. Glyoxalase Pathway[a]



[a] Symbols: GSH, glutathione (γ-L-Glu-L-CysGly); Glx I, glyoxalase I (EC 4.4.1.5); Glx II, glyoxalase II (EC 3.1.2.6).

through hydrophobic interactions,[18,19] the introduction of a thioester function generally decreases binding affinity. For example, the inhibition constant of *S*-propionylglutathione with yeast glyoxalase I is about 2 mM, approximately 10-fold higher than that of *S*-propylglutathione.[18]

However, the *N*-hydroxycarbamoyl esters of GSH depicted in Scheme 2 are potential exceptions to the general rule of weak binding by thioesters. Several observations now indicate that the glyoxalase I reaction involves the formation of a enediol(ate) intermediate,[20,21] possibly having the cis configuration,[22] eq 1.



Thus, the *N*-hydroxycarbamoyl esters would be predicted to bind tightly to glyoxalase I on the basis of (a) their stereoelectronic similarity to the planar enediol(ate) intermediate[23] and (b) the theory that active sites preferentially bind intermediates/transition states versus substrates and products.[24] Indeed, we previously reported that the inhibition constant of **2** with yeast glyoxalase I (K_i = 68 μM) is about 30-fold lower than that of *S*-D-lactoylglutathione and about 7-fold lower than the K_m of the substrate GSH-methylglyoxal thiohemiacetal.[2] Similarly, we now report that compound **2** is also a tight-binding inhibitor of human erythrocyte glyoxalase I (K_i = 1.7 μM).

Inhibition Studies with Glyoxalase I. In order to explore the structural basis of tight binding by **2**, the inhibition constants with yeast glyoxalase I of *S*-(*N*-methylcarbamoyl)glutathione (**2a**) and *S*-propionylglutathione (**1a**) were determined and compared with those of the enediol analog **2** and *S*-D-lactoylglutathione (**1**) (Scheme 3). Apparently, the N-OH function of **2** makes an important contribution to binding (relative to an N-H group) because **2** binds 80-fold (~2.6 kcal/mol) more tightly to the active site of glyoxalase I than its deoxy analog (**2a**). In contrast, the lactoyl C-OH function of *S*-D-lactoylglutathione contributes little to net binding (relative to a C-H group) because *S*-D-lactoylglutathione (**1**) and its deoxy analog (**1a**) bind to the enzyme with similar affinities. Thus, preferential binding of the enediol analog by the enzyme can be partly attributed to preferential binding of the N-OH function.[25]

The glycyl-CO_2^- of the enediol analog also appears to contribute significantly to the preferential binding of **2** versus **1**, based on the reported 10-fold higher affinity of the enzyme for **2** versus its glycyl ethyl ester (**2**(Et)) and the similar affinities of the enzyme for **1** and its glycyl ethyl ester (**1**(Et)).[26] Moreover, the glycyl-CO_2^- of the substrate makes a similar contribution to transition-state stabilization during catalysis, as evidenced by the finding that k_{cat}/K_m for GSH-methylglyoxal thiohemiacetal is 10-fold larger than that of its glycyl ethyl ester.[26] *Taken together, these observations indicate that the high affinity of glyoxalase I for the enediol analog results from preferential binding of functional groups that are both near to and far from the reaction center.*[27]

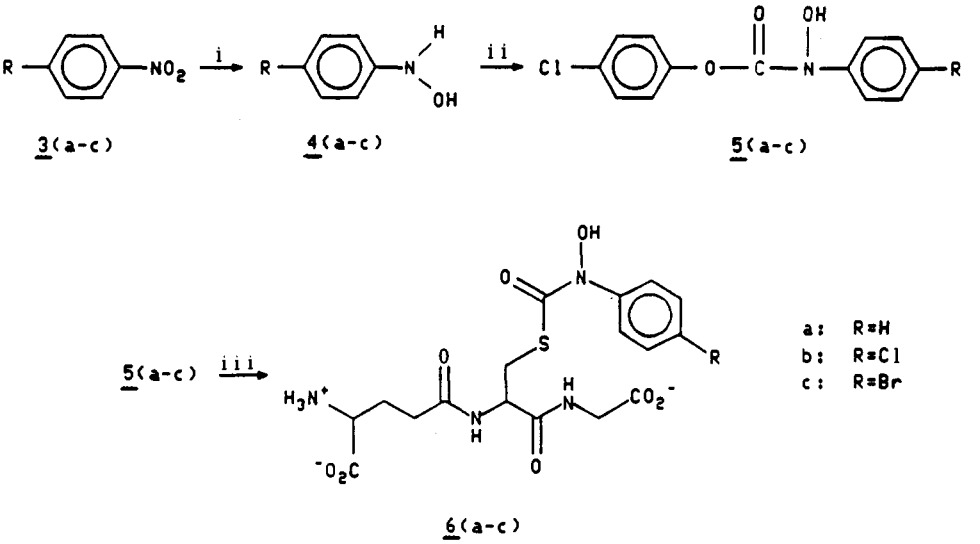
The three *N*-aryl derivatives (**6a–c**) shown in Scheme 2 were synthesized on the theory that they would bind more tightly than **2** to glyoxalase I because of the presence of a hydrophobic binding pocket in the active site of the enzyme. This was based on published structure–activity correlations indicating that hydrophobic interactions contribute to the binding of *S*-alkyl and *S*-aryl derivatives of GSH to yeast glyoxalase I.[18,19] In accordance with this prediction, the *N*-aryl derivatives proved to be linear competitive inhibitors of both yeast and human erythrocyte glyoxalase I, with K_i values much lower than those of **2** (Table 1). Hydrophobic interactions contribute to binding, on the basis that the magnitudes of the K_i values decrease with increasing hydrophobicity of the N-substituents, as shown in eqs 2 and 3.

$$\text{yeast glyoxalase I: } \log K_i = -0.58\pi - 3.82, \quad r = -0.976 \quad (2)$$

$$\text{human glyoxalase I: } \log K_i = -0.70\pi - 5.38, \quad r = -0.985 \quad (3)$$

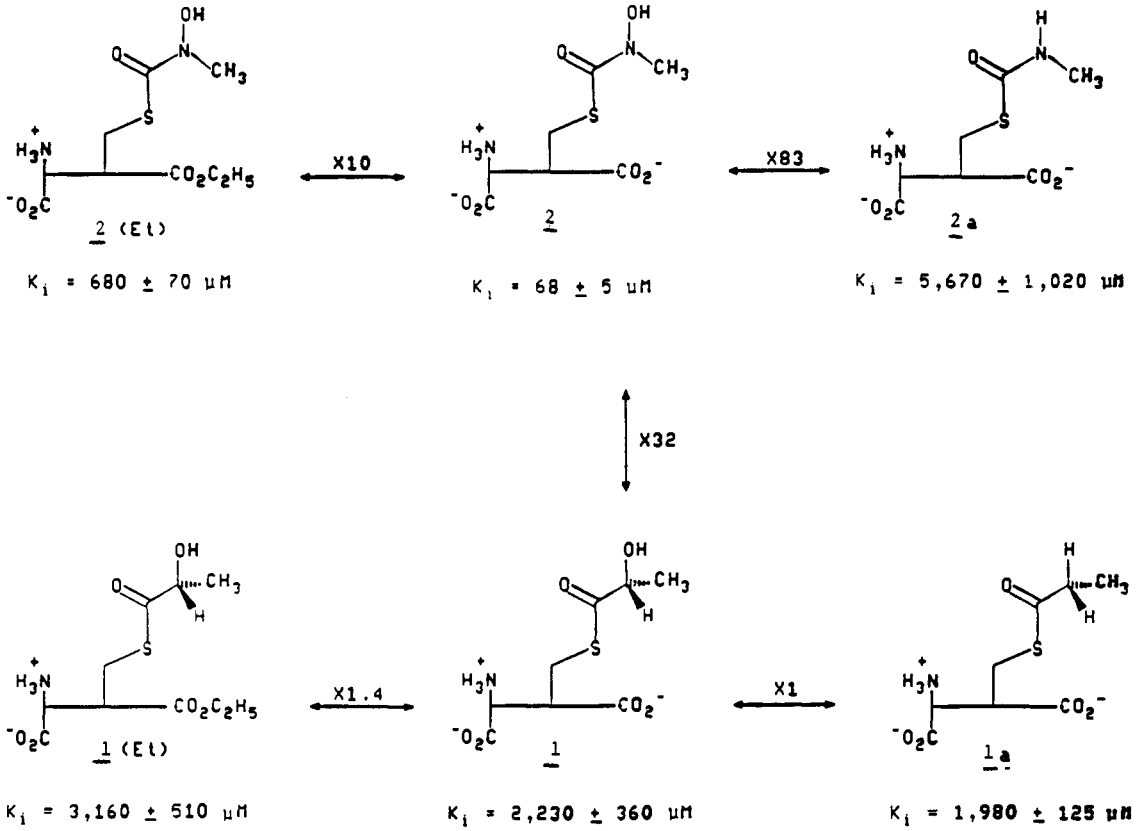
where π = Hansch hydrophobicity constant.[28] The similar magnitudes of the slope terms for the two equations indicate that the active sites of the yeast and human enzymes have similar hydrophobicities (~58% and 70%

Scheme 2. Synthesis of S-(N-Aryl-N-hydroxycarbamoyl)glutathiones[a]



[a] Reagents and conditions: (i) e.g., N_2H_4/5% Rh on carbon/THF; (ii) 4-chlorophenyl chloroformate/K_2CO_3/EtOH; (iii) GSH/EtOH–H_2O.

Scheme 3. Effect of Functional Group Substitutions on the Inhibition Constants of S-(N-Methyl-N-hydroxycarbamoyl)glutathione (2) and S-D-Lactoylglutathione (1) with Yeast Glyoxalase I[a]



[a] In the above structures, the peptide backbone of the glutathione moiety is symbolized by a horizontal line. Inhibition constants were determined in phosphate buffer (50 mM, pH 7), 25 °C.

that of *n*-octanol). It is not clear why the K_i values for the human enzyme are 40–86-fold lower than those for the yeast enzyme (Table 1). This difference appears to be due to stronger polar interactions between the bound enediol analogs and the active site of the human enzyme, judging from the different intercept terms of eqs 2 and 3. Compound **6c** is the strongest competitive inhibitor of human erythrocyte glyoxalase I yet reported, having an inhibition constant at least an order of magnitude lower than that determined in our laboratory for S-(*p*-bromobenzyl)glutathione (K_i = 0.17 μM). Heretofore, the latter compound was the strongest known competitive inhibitor of human erythrocyte glyoxalase I, with reported K_i values of 0.27[6] and 0.08 μM.[29]

Substrate Studies with Glyoxalase II. The *N*-hydroxycarbamoyl esters also proved to be slow substrates for a crude commercial preparation of bovine liver glyoxalase II (Table 2). In order to confirm that the catalyzed hydrolysis of these compounds was due to glyoxalase II, each compound was tested as a competitive inhibitor of the hydrolysis of S-D-lactoylglutathione by the enzyme preparation. The similarity of the observed

Table 1. Enediol Analogs (GSC(O)N(OH)R') Are Strong Competitive Inhibitors of Yeast and Human Erythrocyte Glyoxalase I, with K_i Values That Decrease with Increasing Hydrophobicity (π) of R[a]

compd	R'	π[b]	K_i (μM) yeast	K_i (μM) erythrocytes	K_i (yeast)/K_i (eryth)
2	CH_3	0.5	68 ± 5[c]	1.7 ± 0.1	40
6a	C_6H_5	2.13	11 ± 1	0.16 ± 0.1	69
6b	C_6H_4Cl	3.04	3.6 ± 0.3	0.046 ± 0.004	78
6c	C_6H_4Br	3.25	1.2 ± 0.2	0.014 ± 0.001	86

[a] Phosphate buffer (50 mM, pH 7), 25 °C. [b] Hansch hydrophobicity constants obtained from ref 28. [c] From ref 2.

Table 2. Enediol Analogs (GSC(O)N(OH)R') Are Slow Substrates for Bovine Liver Glyoxalase II[a]

compd	R'	V_m (rel)[b] × 10^5	K_m (μM)	K_i (μM)[c]
2	CH_3	1.7 ± 0.1	478 ± 75	426 ± 77
6a	C_6H_5	0.46 ± 0.01	13 ± 2	11 ± 1.2
6b	C_6H_4Cl	1.5 ± 0.3	*d*	3.4 ± 0.8
6c	C_6H_4Br	2.1 ± 0.4	*d*	1.2 ± 0.4

[a] HEPES buffer (100 mM, pH 7.4), 25 °C. [b] Listed values are relative to V_m for *S*-D-lactoylglutathione. [c] Inhibition constants obtained from using the enediol analogs as competitive inhibitors of the hydrolysis of *S*-D-lactoylglutathione by glyoxalase II. [d] Could not be measured because accurate initial rates could not be obtained at substrate concentrations less than 3 μM.

inhibition constants of **2** and **6a** to their respective K_m values as substrates indicates that a single enzyme species is responsible for the hydrolysis of *S*-D-lactoylglutathione and the carbamoyl esters.[30] As in the case of glyoxalase I, hydrophobic interactions contribute significantly to active site binding (eq 4):

$$\log K_i = -0.88\pi - 2.96, r = 0.993 \quad (4)$$

The V_{max} values of the carbamoyl esters are small in comparison to that of *S*-D-lactoylglutathione, perhaps reflecting the greater intrinsic stability of the resonance-stabilized carbamoyl ester function. The thioester *S*-[(benzyloxy)carbonyl]glutathione ($GSC(O)OCH_2C_6H_5$) has also been reported to bind tightly to the active site of bovine liver glyoxalase II (K_i = 13 μM).[31] However, this compound is reported not to be a substrate for the enzyme.[31,32]

Conclusions

S-(*N*-Aryl-*N*-hydroxycarbamoyl)glutathione derivatives are a new class of tight-binding inhibitors of glyoxalase I that also serve as substrates for the hydrolase glyoxalase II. Their ability to bind tightly to the active site of glyoxalase I results from a combination of their stereoelectronic similarity to the tightly bound enediol(ate) intermediate that forms along the reaction pathway of the enzyme and their ability to interact with a hydrophobic binding pocket in the active site. Given the role of the glyoxalase system in removing cytotoxic methylglyoxal from cells and the observation that glyoxalase II activity is abnormally low in many types of tumor cells, these compounds are currently being tested as possible tumor-selective anticancer agents.

Experimental Section

Synthetic methods are outlined in Scheme 2. Melting points were determined on a Mel-Temp apparatus and are uncorrected. All analytical samples were homogeneous by TLC. NMR spectra were taken on a GE QE-300 NMR spectrometer. Mass spectral data were obtained at the Midwest Center for Mass Spectrometry, University of Nebraska-Lincoln. Elemental analyses were obtained at Atlantic Microlabs, Inc., Norcross, GA, and are within ±0.4% of the calculated values unless otherwise indicated.

***N*-Hydroxy-*N*-phenylcarbamate 4-Chlorophenyl Ester (5a).** A solution of 4-chlorophenyl chloroformate (3.5 g, 18 mmol) in diethyl ether (20 mL) was added dropwise to an ice-cold stirring mixture of *N*-phenylhydroxylamine (2.02 g, 18.5 mmol), K_2CO_3 (1.28 g, 9.3 mmol), diethyl ether (20 mL), and water (0.5 mL) over a period of 20 min. The reaction mixture was allowed to come to room temperature and followed to completion (~1.5 h) by silica gel TLC (diethyl ether:hexane (1:1)): product R_f = 0.42 ($FeCl_3$ stain). The ether layer was removed and washed with water (2 × 20 mL) and the solvent removed in vacuo. The residue was twice recrystallized from ethanol/water to give the final product as white needlelike crystals. Yield: 91%. Mp: 116–117 °C. IR (KBr, 1%): 3300, 1680, 1480, 1360, 1200, 860, 750, 690 cm^{-1}. 300-MHz ^1H NMR ($CDCl_3$, TMS): δ 7.10 (d, *J* = 8.7 Hz, 4-chlorophenyl ring meta-H_2), 7.20 (br s, OH), 7.26 (t, *J* = 7.5 Hz, *N*-phenyl ring para-H), 7.34 (d, *J* = 8.7 Hz, 4-chlorophenyl ring ortho-H_2), 7.40 (t, *J* = 7.8 Hz, *N*-phenyl ring meta-H_2), 7.54 (d, *J* = 8.1 Hz, *N*-phenyl ring ortho-H_2). HR EI-MS consistent with $ClC_{13}H_{10}NO_3$. Anal. ($ClC_{13}H_{10}NO_3$) C, H, N.

***N*-Hydroxy-*N*-(4-chlorophenyl)carbamate 4-Chlorophenyl Ester (5b).** This compound was prepared by the same general procedure used to prepare **5a**. The crude product was recrystallized from ethanol/water to give the final product as a tan solid. Yield: 65%. Mp: 136–138 °C. Silica gel TLC (diethyl ether:hexane (1:1)): R_f = 0.42 ($FeCl_3$ stain). IR (KBr, 1%): 3240, 1710, 1580, 1470, 1330, 1290 cm^{-1}. 300-MHz ^1H NMR ($CDCl_3$, TMS): δ 6.88 (br s, OH), 7.12 (d, *J* = 9.0 Hz, 4-chlorophenyl ring ortho-H_2), 7.36 (d, *J* = 9.0 Hz, 4-chlorophenyl ring meta-H_2), 7.37 (d, *J* = 9.0 Hz, *N*-phenyl ring ortho-H_2), 7.52 (d, *J* = 8.7 Hz, *N*-phenyl ring meta-H_2). HR EI-MS consistent with $Cl_2C_{13}H_9NO_3$. Anal. ($Cl_2C_{13}H_9NO_3$) C, H, N.

***N*-Hydroxy-*N*-(4-bromophenyl)carbamate 4-Chlorophenyl Ester (5c).** This compound was prepared by the same general procedure used to prepare **5a**. However, isolation of the desired product was complicated by the presence of significant amounts of side products. A solution of 4-chlorophenyl chloroformate (0.47 g, 2.46 mmol) in diethyl ether (2 mL) was added dropwise to an ice-cold stirring mixture of *N*-(4-bromophenyl)-hydroxylamine (0.46 g, 2.45 mmol), K_2CO_3 (0.17 g, 1.23 mmol), diethyl ether (5 mL), and water (0.2 mL) over a period of 20 min. The reaction mixture was then stirred at room temperature for 20 min. Silica gel TLC (diethyl ether:hexane (1:1)) of the reaction mixture indicated the absence of starting materials and the presence of at least two product species (R_f = 0.4, 0.6). The reaction mixture was partitioned between diethyl ether and water, the ether phase was dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give an orange solid as a crude product (~0.8 g). The crude product was dissolved in a minimum of warm methanol and cooled to 4 °C and the undesired crystalline precipitate (~0.27 g) removed by filtration. The filtrate was concentrated in vacuo, brought to a cloud by the addition of water, and allowed to stand at room temperature for ~1 day. The resulting yellow needlelike crystals were isolated by filtration and further purified by silica gel column chromatography (silica gel 60, EM Science ASTM 70–30 mesh, 2 × 10 cm), using $CHCl_3$ as an eluting solvent. The peak fractions were pooled, and the solvent was removed in vacuo to give a light yellow solid as the final product, having an R_f of 0.34 by silica gel TLC ($CHCl_3$:ethyl acetate (9:1)). Yield: 20%. Mp: 160–162 °C. IR (KBr, 1%): 3295, 1680, 1480, 1360, 1190, 860, 740 cm^{-1}. 300-MHz ^1H NMR ($CDCl_3$, TMS): δ 6.79 (1H, s, OH), 7.11–7.38 (4H, m, 4-chlorophenyl), 7.45–7.54 (4H, m, 4-bromophenyl). HR EI-MS consistent with $BrClC_{13}H_9NO_3$. Anal. ($BrClC_{13}H_9NO_3$) C, H, N.

***S*-(*N*-Hydroxy-*N*-phenylcarbamoyl)glutathione (6a).** A stirring solution of GSH (307 mg, 1 mmol) in degassed and argon-saturated 1 N NaOH (2 mL) under an argon atmosphere was brought nearly to a cloud point by the addition of ethanol (2 mL). This was followed by the dropwise addition of a solution of **5a** (264 mg, 1 mmol) in ethanol (2 mL) over a period of ~5 min. The reaction vessel was sealed under argon, enclosed in foil, and stirred at room temperature until little GSH remained (88 h), as judged by silica gel TLC (*n*-propanol:acetic acid:water (10:1:5)): product R_f = 0.57 ($FeCl_3$ stain, purple spot; ninhydrin

stain, maroon spot). The reaction mixture was brought to pH 3.5 with HCl and the solvent removed in vacuo. The crude residue was suspended in water (10 mL) and extracted with diethyl ether (3 × 10 mL) to remove 4-chlorophenol and unreacted **5a**. The combined ether phases were then back-extracted with water (10 mL). The combined aqueous phases were reduced to a minimum volume in vacuo, and the residue was purified by reverse-phase HPLC (Whatman Partisil C_{18}, 2.2 × 50 cm), using 0.25% acetic acid and 30% methanol in water as an eluting solvent (retention volume: ~500 mL). The peak fractions were lyophilized to dryness to give the final product as a white powder. Yield: ~50%. UV (water): λ_{max} = 253.5 nm: $\epsilon_{253.5}$ = 11 800 M^{-1} cm^{-1}. 300-MHz ^1H NMR (D_2O, pD 3.7, DSS): δ 2.15 (m, Glu-$C_\beta H_2$), 2.51 (m, Glu-$C_\gamma H_2$), 3.19 (q, J = 8.3, 14.6 Hz, Cys-$C_\beta H_a$), 3.44 (q, J = 5.0, 14.6 Hz, Cys-$C_\beta H_b$), 3.79 (t, J = 6.3 Hz, Glu-$C_\alpha H$), 3.96 (s, Gly-$C_\alpha H_2$), 4.70 (q, J = 5.0, 8.3 Hz, Cys-$C_\alpha H$), 7.37 (m, aromatic 1H), 7.52 (m, aromatic 4H). HR FAB-MS consistent with $C_{17}H_{22}N_4O_8S$. Anal. ($C_{17}H_{22}N_4O_8S \cdot 1.5H_2O$) C, H, N.

***S*-[*N*-Hydroxy-*N*-(4-chlorophenyl)carbamoyl]glutathione (6b).** To a stirring solution of **5b** (37 mg, 0.12 mmol) in ethanol (2 mL) was added a solution of GSH (191 mg, 0.62 mmol) in argon-saturated water (2.0 mL), pH 9.1. The reaction vessel was sealed under argon, enclosed in foil, and stirred at room temperature until little **5b** remained (27 h), as judged by silica gel TLC (diethyl ether:hexane (1:1)). The reaction mixture was worked up as described for **6a**. The crude product was purified by reverse-phase HPLC (Waters μBondapak C_{18}, 0.78 × 30 cm), using 0.25% acetic acid and 35% methanol in water as an eluting solvent (retention volume: ~112 mL). The peak fractions were lyophilized to dryness to give the final product as a white powder. Yield: 34%. UV (water): λ_{max} = 259.5 nm; $\epsilon_{259.5}$ = 13 489 M^{-1} cm^{-1}. 300-MHz ^1H NMR (D_2O, pD 3.55, reference to Gly-$C_\alpha H_2$ peak): δ 2.23 (m, Glu-$C_\beta H_2$), 2.61 (m, Glu-$C_\gamma H_2$), 3.28 (q, J = 8.1, 14.4 Hz, Cys-$C_\beta H_a$), 3.54 (q, J = 5.1, 14.4 Hz, Cys-$C_\beta H_b$), 3.86 (t, J = 6.3 Hz, Glu-$C_\alpha H$), 3.98 (s, Gly-$C_\alpha H_2$), 7.57 (d, J = 9.0 Hz, aromatic ring 2H, meta to Cl), 7.62 (d, J = 9.3 Hz, aromatic ring 2H, ortho to Cl). HR FAB-MS consistent with $ClC_{17}H_{21}N_4O_8S$. The monosodium salt of **6b** was prepared by neutralization of an aqueous solution of **6b** to pH 7 with NaOH followed by removal of the solvent in vacuo. Anal. ($C_{17}H_{20}ClN_4O_8SNa \cdot 0.5H_2O$): C: calcd, 40.20; found, 40.88. H: calcd, 4.17; found, 4.59. N: calcd, 11.03; found, 10.50.

***S*-[*N*-Hydroxy-*N*-(4-bromophenyl)carbamoyl]glutathione (6c).** This compound was prepared by the same general procedure used to prepare **6a**. The crude product was purified by reverse-phase HPLC (Whatman, Partisil C_{18}, 2.2 × 50 cm), using 0.25% acetic acid and 40% methanol in water as an eluting solvent (retention volume: ~665 mL). Yield: ~5%. UV (water): λ_{max} = 257 nm: ϵ_{257} = 18 067 M^{-1} cm^{-1}. 300-MHz ^1H NMR (D_2O, pD 3.7, DSS): δ 2.14 (m, Glu-$C_\beta H_2$), 2.51 (m, Glu-$C_\gamma H_2$), 3.18 (q, J = 8.4, 14.7 Hz, Cys-$C_\beta H_a$), 3.45 (q, J = 4.8, 14.7 Hz, Cys-$C_\beta H_b$), 3.76 (t, J = 6.0 Hz, Glu-$C_\alpha H$), 3.88 (s, Gly-$C_\alpha H_2$), 7.64 (d, J = 8.1 Hz, aromatic ring 2H, ortho to Br), 7.47 (d, J = 8.1 Hz, aromatic ring 2H, meta to Br). HR FAB-MS consistent with $BrC_{17}H_{21}N_4O_8S$. Anal. ($C_{17}H_{21}BrN_4O_8S \cdot 2H_2O$) C, N; H: calcd, 4.52; found, 3.95.

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