1, CH), 3.68 (s, 2, CH_2), 3.48 (s, 1, Ar OH), 2.96 (m, 1, C_3H_a), 2.61 (m, 1, C_3H_b), 2.42 (s, 3, NCH_3), 1.25 (s, 1, NH). Anal. ($C_{16}H_{17}$-BrClNO) C, H, N.

Pharmacology. The procedure for the dopamine-stimulated rat retinal adenylate cyclase assay was as follows: Rat retinas were homogenized in 150 vol/wt of 2.0 mM Tris·HCl, pH 7.4, with 2 mM EDTA with a Teflon–glass homogenizer. Each reaction mixture contained the following final concentrations in a volume of 0.2 mL: 2 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM EGTA, 1 mM IBMX, 0.01 mM GTP, 80 mM Tris·HCl (pH 7.4), 0.5 mM ATP with approximately 5×10^6 dpm [^{32}P]ATP and 20–30 μg of retinal homogenate protein. Following an incubation of 20 min at 30 °C, the reaction was terminated by adding 200 μL of a solution containing 1% SDS, 20 mM ATP, 0.7 mM cyclic AMP with 1.0×10^4 dpm [^{3}H]cyclic AMP in 80 mM Tris·HCl pH 7.4 and heating to 85 °C for 2 min. Cyclic AMP was isolated from the mixture by using the column chromatographic technique of Salomon.[22]

Acknowledgment. This work was supported by NIH Grants HL-31106 and MH-42705.

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Prodrugs of L-Cysteine as Protective Agents against Acetaminophen-Induced Hepatotoxicity. 2-(Polyhydroxyalkyl)- and 2-(Polyacetoxyalkyl)thiazolidine-4(*R*)-carboxylic Acids

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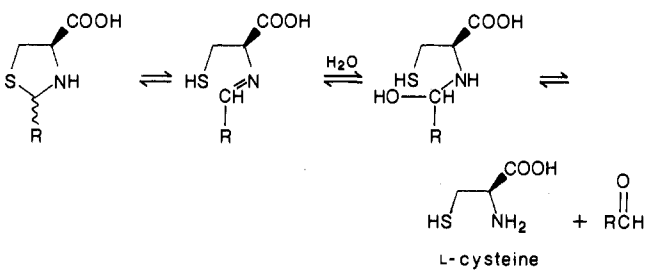
Eight prodrugs of L-cysteine (**1a–h**) were synthesized by the condensation of the sulfhydryl amino acid with naturally occurring aldose monosaccharides containing three, five, and six carbon atoms. The resulting 2-(polyhydroxyalkyl)thiazolidine-4(*R*)-carboxylic acids (TCAs) are capable of releasing L-cysteine and the sugars by nonenzymatic ring opening and hydrolysis. Thus, when added to rat hepatocyte preparations in vitro, these TCAs (1.0 mM) raised cellular glutathione (GSH) levels 1.2–2.1-fold relative to controls. On the basis of this finding, the cysteine prodrugs were tested as protective agents against acetaminophen-induced hepatotoxicity in a mouse model. The TCA derived from D-ribose and L-cysteine (RibCys, **1d**) showed the greatest therapeutic promise of the series, with a 100% (12/12) survival profile compared to 17% without treatment. However, the degree of stimulation of GSH production in rat hepatocytes by these prodrugs did not correlate with the extent of protection afforded in mice, suggesting that pharmacokinetic parameters must supervene in vivo. To evaluate the effect of increased lipid solubility, we prepared prodrugs **2a–c** by using peracetylated aldehydic sugars in the condensation reaction. These compounds, however, displayed acute toxicity to mice, possibly due to liberation of the acetylated sugars themselves. Nevertheless, the efficacy of the unacetylated TCAs, and RibCys (**1d**) in particular, suggests that the prodrug approach for the delivery of L-cysteine to the liver represents a viable means of augmenting existing detoxication mechanisms in protecting cells against xenobiotic substances that are bioactivated to toxic, reactive metabolites.

In previous studies,[1] it was shown that 2-substituted thiazolidine-4(*R*)-carboxylic acids (TCAs) can protect against the hepatotoxicity elicited by high doses of acetaminophen in mice. These TCAs function as prodrug forms of L-cysteine, liberating this sulfhydryl amino acid by nonenzymatic ring opening and hydrolysis (Scheme I). It was hypothesized that delivery of L-cysteine to the liver would elevate the intracellular levels of glutathione (GSH) by supplying this biochemical amino acid precursor of GSH to the cell. GSH is the coenzyme that mediates the protection against the reactive electrophilic species generated during the oxidative metabolism of acetaminophen by the hepatic cytochrome P-450 system.[2]

The TCAs alluded to were prepared by condensation of L-cysteine with aliphatic or aromatic aldehydes.[3] Thus, the release of an equimolar quantity of the aldehyde used in prodrug synthesis would be expected by dissociation, along with the desired therapeutic agent, L-cysteine. To avoid any possible toxicity associated with the liberation in vivo of potentially reactive aldehydes, albeit in small amounts, the present study utilized naturally occurring aldose monosaccharides of three, five, and six carbon atoms as the aldehyde moiety in thiazolidine construction.[4] The

Scheme I



aldoses used for synthesis were glyceraldehyde (Glyc), arabinose (Ara), lyxose (Lyx), ribose (Rib), xylose (Xyl),

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(1) (a) Nagasawa, H. T.; Goon, D. J. W.; Zera, R. T.; Yuzon, D. L. *J. Med. Chem.* **1982**, *25*, 489. (b) Nagasawa, H. T.; Goon, D. J. W.; Muldoon, W. P.; Zera, R. T. *J. Med. Chem.* **1984**, *27*, 591.
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(3) (a) Schubert, M. P. *J. Biol. Chem.* **1936**, *114*, 341. (b) Ratner, S.; Clarke, H. T. *J. Am. Chem. Soc.* **1937**, *59*, 200. (c) Schmolka, I. R.; Spoerri, P. E. *J. Org. Chem.* **1957**, *22*, 943. (d) Riemschneider, R.; Hoyer, G.-A. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1963**, *18B*, 25. (e) Kallen, R. G. *J. Am. Chem. Soc.* **1971**, *93*, 6227.

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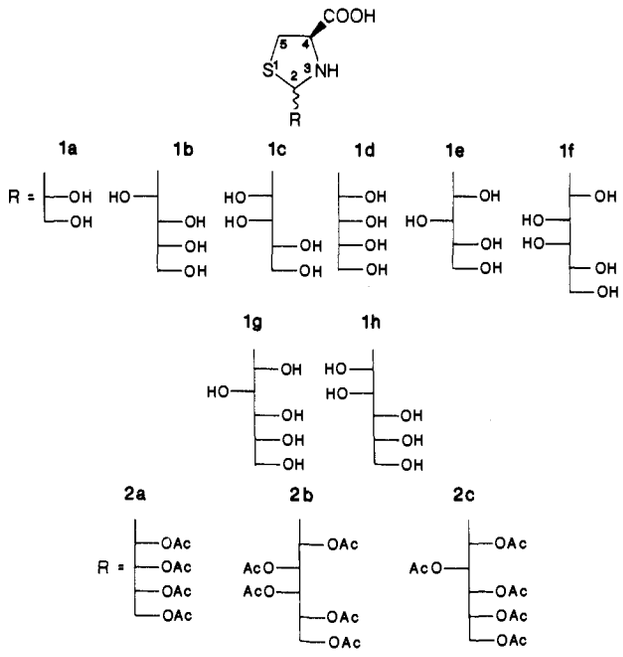
Chart I



galactose (Gal), glucose (Glc), and mannose (Man), all of D absolute configuration. Condensation with L-cysteine produced the corresponding thiazolidine-4(*R*)-carboxylic acids (**1a–h**, respectively, Chart I).

The multiple hydroxyl groups present in this series imparted high polarity and water solubility to these prodrugs. In an attempt to increase the duration of action of these agents by increasing their lipophilicity, we used peracetylated derivatives of selected sugars (Rib, Gal, and Glc) to synthesize a second set of L-cysteine prodrugs (**2a–c**, respectively, Chart I).

Chemistry

2-Polyhydroxyalkyl TCAs. Formation of the thiazolidine ring system by condensation of L-cysteine with simple aldehydic sugars generally followed known reaction conditions,[4] but optimum conditions varied slightly depending on the sugar used as the aldehyde donor. GlycCys (**1a**) and RibCys (**1d**) were prepared in aqueous medium under nitrogen, with absolute ethanol added to precipitate the desired condensation product. The remaining six prodrugs formed best in refluxing methanol, resulting in spontaneous precipitation of the products as they formed. All reactions were carried out in the presence of 1 equiv of pyridine, which was not necessary for product formation but increased the overall yields somewhat. This is probably due to an increase in solution pH, which shifts the equilibrium slightly more in favor of the thiazolidine.[3a,4d] All products (**1a–h**) consisted of a pair of C-2 epimers, which were, however, not separated. These 2-polyhydroxyalkyl TCAs were readily soluble in water, but virtually insoluble in organic solvents. Yields generally ranged from 75% to 95%.

Physical characterization of the products relied mainly on elemental analyses, as well as on consistent optical

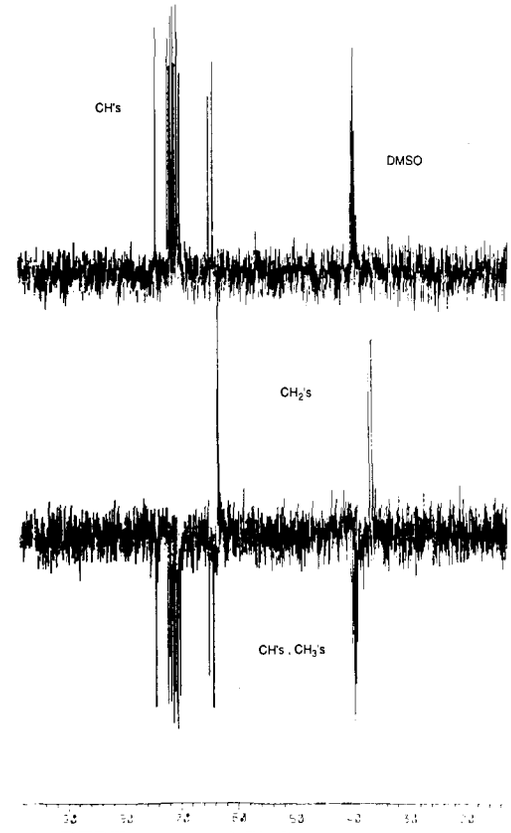


Figure 2. DEPT spectra of GlcCys (**1g**).

rotation and melting point values. Although Schubert[4a] has reported that the latter can fluctuate as much as 10 °C depending on moisture conditions, such wide discrepancies were generally not observed. TLC analyses invariably showed the presence of L-cysteine starting material, presumably due to dissociation at the surface of the TLC plates. Recrystallization attempts resulted in dramatic loss of product. Mutarotation suggestive of C-2 epimerization[5] was observed; therefore, all measurements of specific rotation were made after a 3-min mixing interval. Consistent with these results, the NMR spectra of compounds **1a–h** usually showed trace amounts of free L-cysteine, which can also form under the conditions of spectral measurement. All spectra were very complex, but exhibited two clear doublets at ~5 ppm, due to the epimeric C-2 protons. The doublet due to the cis isomer resonated downfield from the trans isomer by 0.1–0.2 ppm, and most compounds exhibited cis:trans ratios of around 1:2. Also, the characteristic A_2B pattern of the methylene group in free L-cysteine became a more complex ABX system when incorporated into the thiazolidine ring structure.[1b,6]

All of the thiazolidine prodrugs exhibited broad infrared bands in the 2500–3200 cm^{-1} range for overlapping carboxyl and hydroxyl absorbances. No absorption attributable to asymmetric NH stretching was observed, and a strong carboxyl anion band appeared at around 1610 cm^{-1}. Depending on the methods of crystallization and isolation, however, absorptions in the carbonyl range of 1700–1750 cm^{-1} were found. When this occurred, a sharp NH stretching band was also evident at around 3250 cm^{-1}.

The thiazolidine structure of a representative prodrug derived from L-cysteine and an aldose monosaccharide was established by analysis of the ^{13}C NMR spectra of GlcCys

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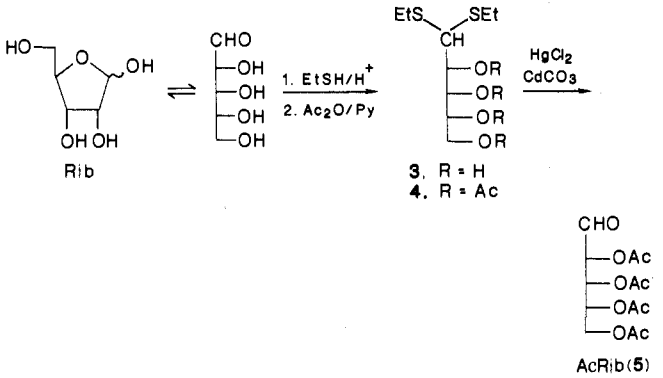
Scheme II



(**1g**) and its ^{13}C-enriched product ([^{13}C]-**1g**). The spectrum of **1g** (Figure 1, supplementary material) clearly showed eighteen peaks, indicating the presence of two epimers. The spectrum was complicated by the overlapping signals of most of the sugar and ring carbons, but the assignment of the positions of the CH_2O carbons was readily made by DEPT (distortionless enhancement by polarization transfer) analysis[7] (Figure 2). The NMR spectrum of a sample of GlcCys (**1g**) enriched with ^{13}C at C-2 identified this epimeric carbon (70.18, 71.76 ppm, Figure 3, supplementary material), the peaks of which were found upfield from that of anomeric carbon of D-glucose itself (92.34 ppm), thus verifying the structural change from a hemiacetal to the thiazolidine ring form.

2-Polyacetoxyalkyl TCAs. The thiazolidine prodrugs prepared above (**1a–h**) were highly polar, water-soluble compounds owing to the presence of multiple hydroxyl groups. The possibility therefore existed that they might be rapidly excreted by the kidneys in essentially the first pass, and it was thus desirable to acetylate the sugar hydroxyl groups to enhance lipid solubility and to delay urinary excretion. Since direct acetylation of the sugar hydroxyl groups in the intact TCAs would also acetylate the ring nitrogen, the sugars were peracetylated before incorporation into the thiazolidine structure.

The synthetic procedure paralleled that of Wolfrom et al.[8] and Zinner,[9] patterned after that of Fischer,[10] and is illustrated for D-ribose in Scheme II. The aldehyde functionality was protected as the diethyl dithioacetal **3**, and acetylation of the hydroxyl groups was then accomplished with Ac_2O/pyridine, yielding **4**. Deprotection gave the acetylated sugar **5**, which was then available for thiazolidine ring formation.

Three peracetylated sugar derivatives were synthesized by using this scheme, namely, peracetylated ribose (AcRib, **5**), peracetylated galactose (AcGal, **6**), and peracetylated glucose (AcGlc, **7**), and these were used to prepare prodrugs **2a–c**, respectively. Condensation to their respective thiazolidines was accomplished in hot water under a nitrogen atmosphere, but only AcGalCys (**2b**) was a truly crystalline compound. AcRibCys (**2a**) and AcGlcCys (**2c**) were amorphous solids with no specific melting points.

These sugar-acetylated TCAs appeared to be more stable than their unacetylated analogues as TLC analyses showed no detectable free L-cysteine. The IR spectra of all three acetylated prodrugs showed the presence of strong ester carbonyl bands at 1750 cm^{-1}. The acetyl CH_3 groups were also clearly visible in the NMR spectra, with a general downfield shift of the sugar side chain protons relative to that of the unacylated analogues due to the presence of the more strongly deshielding acetyl groups.

Table I. Increased GSH Content of Rat Hepatocytes after Incubation with L-Cysteine Prodrugs

prodrug	concn, mM	[GSH] ± SE, nmol/10^6 cells	[GSH] rel to controls
none (control)		35.4 ± 0.78	1.0
GlcCys (**1g**)	1.0	75.2 ± 2.15	2.1
RibCys (**1d**)	1.0	61.2 ± 1.52	1.7
XylCys (**1e**)	1.0	58.3 ± 0.99	1.6
GalCys (**1f**)	1.0	58.0 ± 2.00	1.6
ManCys (**1h**)	1.0	57.8 ± 0.87	1.6
GlycCys (**1a**)	1.0	46.1 ± 1.10	1.3
LyxCys (**1c**)	1.0	45.9 ± 1.95	1.3
AraCys (**1b**)	1.0	42.9 ± 2.17	1.2
N-acetyl-L-cysteine (NAC)	2.5	45.8 ± 1.27	1.3

Table II. Inhibitory Effect of Buthionine Sulfoximine (BSO) on GSH Elevation Elicited by L-Cysteine Prodrugs in Rat Hepatocytes

prodrug (1.0 mM)	BSO (0.2 mM)	[GSH] ± SE, nmol/10^6 cells	[GSH] rel to controls
none (control)	–	35.4 ± 0.78	1.0
none	+	18.4 ± 2.08	0.5
GlcCys (**1g**)	+	25.4 ± 0.99	0.7
RibCys (**1d**)	+	16.2 ± 3.60	0.5
XylCys (**1e**)	+	22.6 ± 1.84	0.8
GalCys (**1f**)	+	23.2 ± 2.56	0.7
ManCys (**1h**)	+	18.9 ± 1.87	0.5
GlycCys (**1a**)	+	18.3 ± 6.41	0.5
LyxCys (**1c**)	+	22.4 ± 1.98	0.6
AraCys (**1b**)	+	19.5 ± 3.75	0.6
N-acetyl-L-cysteine (NAC, 2.5 mM)	+	25.5 ± 1.59	0.7

Biological Results

Stimulation of GSH Biosynthesis in Vitro and Inhibition by Buthionine Sulfoximine. Compounds **1a–h** were incubated in vitro with rat hepatocytes,[11] and the GSH levels were measured after 4 h. As can be seen from Table I, all eight prodrugs elevated GSH levels 1.2–2.1-fold relative to controls in these hepatocytes. GlcCys (**1g**) was the most effective compound in this series, elevating GSH levels to over twice those seen for the controls. *N*-Acetyl-L-cysteine (NAC), the drug of choice for the clinical treatment of acetaminophen overdoses,[12] also raised GSH levels by 30% in this system, but required 2.5 times the concentration of the thiazolidine prodrugs for comparable elevation. That GSH biosynthesis was actually stimulated by liberation of its biochemical precursor, L-cysteine, from the prodrugs was indicated by experiments conducted in the presence of 0.20 mM buthionine sulfoximine (BSO). BSO is a specific inhibitor of γ-glutamylcysteine synthetase, the enzyme responsible for catalyzing the first step in GSH biosynthesis.[13] GSH levels were decreased by this inhibitor even in the presence of the prodrugs (Table II),

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(13) Griffith, O. W.; Meister, A. *J. Biol. Chem.* **1979**, *254*, 7558.

Table III. Protection against Acetaminophen-Induced Hepatotoxicity in Mice by Prodrugs of L-Cysteine

compounds[a]	survival (48 h)		no. of animals with necrosis				
	number	%	4+	3+	2+	1+	0
acetaminophen (A)	2/12	17	11	0	1	0	0
A + RibCys (**1d**)	12/12	100	0	0	6	2	4
A + XylCys (1e)	6/10	60	5	0	0	2	3
A + GalCys (**1f**)	6/10	60	4	0	2	3	1
A + AraCys (**1b**)	4/10	40	7	0	2	1	0
A + ManCys (**1h**)	4/10	40	6	0	1	0	3
A + GlycCys (**1a**)	3/10	30	7	2	1	0	0
A + LyxCys (**1c**)	3/10	30	9	1	0	0	0
A + GlcCys (**1g**)	3/10	30	7	1	0	2	0
A + NAC[b]	17/18	94	1	1	0	3	13

[a] Dose of acetaminophen: 750 mg/kg (4.97 mmol/kg) ip. Dose of prodrugs: 2.45 mmol/kg ip. Protocol described in the Experimental Section. [b] Data from ref 1a; dose of NAC: 2.45 mmol/kg ip.

thus providing evidence that the increased levels of GSH observed (Table I) were indeed due to its de novo biosynthesis from the L-cysteine provided by the thiazolidine prodrugs.

Protection against Acetaminophen-Induced Hepatotoxicity in Mice. Compounds **1a–h** were evaluated in a mouse model for their ability to protect against the hepatotoxic action of acetaminophen (Table III). The experimental protocol involved the administration of an LD_{90} dose of acetaminophen, followed 30 min later by the prodrug as potential protective agent. Toxicity was assessed on the basis of overall survival data at 48 h, as well as histological criteria of hepatocellular damage using the rating system of Mitchell.[2a]

While all the prodrugs (**1a–h**) increased the number of survivors over untreated controls, the thiazolidine prodrug derived from the condensation of D-ribose and L-cysteine (RibCys, **1d**) showed the greatest therapeutic promise, with 100% survival at 48 h. RibCys also showed the best histopathological profile, with all sample liver specimens showing a necrosis rating of 2+ or below. Data for NAC[1a] is supplied for comparison.

When compounds **2a–c** were tested in mice by using the same in vivo protocol, it became immediately apparent that these compounds were extremely toxic, i.e., the majority of animals died within 8 h. Further investigation ruled out the vehicle itself and pointed to toxicity of the acetylated aldehydic sugars themselves (data not shown). Although survivors of this group did not manifest histological evidence of *hepato*toxicity, other toxicity considerations being overriding, further evaluation of these sugar-acetylated thiazolidine derivatives did not appear warranted.

Discussion

The prodrugs derived from L-cysteine and the aldose monosaccharides were capable of being assimilated by the cell. The increase in cellular levels of GSH elicited by these prodrugs (Table I) and inhibition of this effect by BSO (Table II) in rat hepatocyte preparations suggest that the free L-cysteine liberated was stimulating the biosynthesis of this coenzyme. In vivo, all these prodrugs (**1a–h**) increased to some degree the survival of animals given LD_{90} doses of acetaminophen (Table III).

Although GlcCys (**1g**) produced the greatest elevation of GSH in vitro (Table I), this compound did not appreciably protect against acetaminophen-induced hepatotoxicity in vivo. Differences in absorption, distribution, excretion, and metabolic disposition would be expected among the compounds represented by this series and may account for these differential efficacies in vivo.

RibCys (**1d**) stood out as the best hepatoprotective agent of the series, with 100% survival of the acetaminophen-treated mice at 48 h, the time at which the overall results were assessed. Histological evaluation of the extent of hepatic damage also verified the protection afforded by RibCys (**1d**). Its efficacy was comparable to that of other prodrugs of L-cysteine, viz., the 2-alkyl-substituted TCAs,[1b] 2-oxothiazolidine-4-carboxylic acid,[14] and NAC.[1a] All of these agents offer improved protection over that of L-cysteine itself and underscore the importance of the prodrug approach to drug design.[15] Indeed, RibCys (**1d**) was able to deliver masked L-cysteine to the cell and liberate this sulfhydryl amino acid intracellularly, thereby stimulating the biosynthesis of GSH.

The prodrugs of L-cysteine derived from acetylated sugars (**2a–c**), on the other hand, were acutely toxic to mice. This is possibly due to their premature dissociation to the respective acetylated sugars (**5**, **8**, **11**) before the expected deacetylation took place.

Conclusions

The thiazolidine prodrugs of L-cysteine prepared by the condensation of the sulfhydryl amino acid with aldose monosaccharides were all capable of elevating GSH levels in isolated rat hepatocytes. These prodrugs also increased, to varying degrees relative to untreated controls, the survival of mice given LD_{90} doses of acetaminophen. The degree of stimulation of GSH production in rat hepatocytes in vitro, however, did not correlate with the extent of hepatoprotection afforded in mice, suggesting that pharmacokinetic parameters very likely play a role in vivo.

The TCA prodrug derived from D-ribose and L-cysteine (RibCys, **1d**) was the best protective agent of this series, with activity comparable to that of other cysteine prodrugs such as NAC, 2-alkyl-substituted TCAs, and L-2-oxothiazolidine-4-carboxylic acid. Thus, the prodrug approach for delivering L-cysteine to the liver appears to be a viable means for augmenting the endogenous protective mechanism mediated by GSH, and hepatoprotection by these cysteine prodrugs may be universally applicable to a wide variety of xenobiotic substances that are metabolized by the hepatic cytochrome P-450 enzymes to toxic, reactive electrophilic species.

Experimental Section

Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. Optical activities were measured on a Perkin-Elmer 141 or Autopol III polarimeter, and IR spectra were obtained on a Perkin-Elmer 281 IR spectrophotometer. Proton NMR spectra were obtained on a JEOL FX90 or Nicolet 300 spectrometer with tetramethylsilane (TMS) or

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(15) (a) Albert, A. *Nature (London)* **1958**, *182*, 421. (b) Sinkula, A. A. *Annu. Rep. Med. Chem.* **1975**, *10*, 306. (c) Stella, V. J.; Naringrekar, V. J.; Charman, W. N. A. *Pharm. Int.* **1984**, *5*, 276.

2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. The 300-MHz spectrometer was also used for carbon-13 NMR analyses with DMSO-d_6 as solvent and internal standard. Unless otherwise indicated, Analtech silica gel HF plates were used for TLC analyses, with visual detection of spots by iodine vapor, ninhydrin, or Ellman's reagent. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory (Woodside, NY) or Galbraith Laboratories (Knoxville, TN). Chemicals were purchased from the following commercial vendors: Glyc, Ara, Rib, Lyx, Xyl, Gal, Man, all of D configuration, and L-cysteine, Sigma Chemical Co. (St. Louis, MO); acetaminophen, Aldrich Chemical Co. (Milwaukee, WI); D-Glc, Mallinckrodt Chemical Co. (St. Louis, MO); $CdCO_3$, Fluka Chemical Corp. (Hauppauge, NY);and [1-^{13}C]-D-Glc, KOR Isotopes (Cambridge, MA). Male Swiss-Webster mice were purchased from Biolab (St. Paul, MN). All animal studies were performed in adherence with guidelines established in the "Guide for the Care and Use of Laboratory Animals", published by the U.S. Department of Health and Human Resources (NIH Publication 85-23, revised, 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the research protocol was approved by the Animal Study Subcommittee of the Minneapolis VA Medical Center.

2(*RS*)-D-*glycero*-(1′,2′-Dihydroxyethyl)thiazolidine-4-(*R*)-carboxylic Acid (GlycCys, 1a). This compound was prepared by the procedure of Korber et al.[4c] with the following modifications. The reaction was run under a N_2 atmosphere to reduce the possibility of oxidation of L-cysteine in aqueous medium. The Glyc used in the reaction was purchased commercially.[16] The resulting precipitate was collected and dried in vacuo overnight to give 0.47 g (40.5% yield) of pale yellow crystalline product: mp 126–129 °C dec; $[\alpha]^{25}_D$ −130.6° (*c* 1.00, H_2O); IR (KBr) ν 3280 (br, OH, COO^-), 1630 cm^{-1} (COO^-). Anal. ($C_6H_{11}NO_4S$) C, H, N.

2(*RS*)-D-*arabino*-(1′,2′,3′,4′-Tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic Acid (AraCys, 1b). This compound was synthesized by the method of Weitzel et al.[4b] in MeOH, a procedure that was superior to the aqueous system of Schubert[4a] or of Bognar et al.[4d,f] The solids were collected and dried in vacuo overnight to give 1.10 g (86.6% yield) of white crystalline product: mp 163–165 °C dec (lit.[4a] mp 153 °C dec, lit.[4d] mp 161–162 °C dec); $[\alpha]^{25}_D$ −128.8° (*c* 1.04, H_2O); IR (KBr) ν 3460 (NH), 3255 (br, OH, COO^-), 1625 cm^{-1} (COO^-). Anal. ($C_8H_{15}NO_6S$) C, H, N.

2(*RS*)-D-*lyxo*-(1′,2′,3′,4′-Tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic Acid (LyxCys, 1c). The procedure for the synthesis of **1b** was followed on a 20-mmol scale in 150 mL of MeOH to give 4.16 g (82.2% yield) of **1c** as colorless solids: mp 172–175 °C dec (lit.[4d] mp 150–151 °C dec); $[\alpha]^{25}_D$ −98.9° (*c* 1.00, H_2O); IR (KBr) ν 3245 (br, OH, COO^-), 1610 cm^{-1} (COO^-). Anal. ($C_8H_{15}NO_6S$) C, H, N.

2(*RS*)-D-*ribo*-(1′,2′,3′,4′-Tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic Acid (RibCys, 1d). This compound was synthesized as described.[4d] The product was collected to give 4.71 g (92.2% yield) of pale yellow material: mp 149–151 °C dec (lit.[4d] mp 152–155 °C dec); $[\alpha]^{25}_D$ −103.1° (*c* 0.52, H_2O); IR (KBr) ν 3220 (br, OH, COO^-), 1610 cm^{-1} (COO^-). Anal. ($C_8H_{15}NO_6S$) C, H, N.

2(*RS*)-D-*xylo*-(1′,2′,3′,4′-Tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic Acid (XylCys, 1e). This compound was prepared according to Weitzel[4b] to give 0.95 g (74.8% yield) of colorless product: mp 119–123 °C dec (lit.[4a] mp 133 °C dec, lit.[4d] 152 °C dec); $[\alpha]^{25}_D$ −97.8° (*c* 1.01, H_2O); IR (KBr) ν 3260 (br, OH, COO^-), 1640 cm^{-1} (COO^-). Anal. ($C_8H_{15}NO_6S{\cdot}0.5H_2O$) C, H, N.

2(*RS*)-D-*galacto*-(1′,2′,3′,4′,5′-Pentahydroxypentyl)thiazolidine-4(*R*)-carboxylic Acid (GalCys, 1f). The synthetic procedure[4b] for **1b** was followed on a 20-mmol scale in 340 mL of MeOH to give 4.38 g (93.5% yield) of colorless product: mp 148 °C dec (lit.[4a] mp 138 °C dec, lit.[4d] 148–149 °C dec); $[\alpha]^{25}_D$ −66.9° (*c* 0.49, H_2O); IR (KBr) ν 3440 (NH), 3260 (br, OH, COO^-), 1630 cm^{-1} (COO^-). Anal. ($C_9H_{17}NO_7S{\cdot}0.5H_2O$) C, H, N.

2(*RS*)-D-*gluco*-(1′,2′,3′,4′,5′-Pentahydroxypentyl)thiazolidine-4(*R*)-carboxylic Acid (GlcCys, 1g). The procedure[4b] for the synthesis of **1b** was followed to give 1.12 g (78.9% yield) of product: mp 165 °C dec (lit.[4a] mp 167 °C dec, lit.[4d] mp 169 °C dec); $[\alpha]^{25}_D$ −89.7° (*c* 0.49, H_2O); IR (KBr) ν 3340 (br, OH, COO^-), 1610 cm^{-1} (COO^-); ^{13}C NMR (DMSO) δ 36.46, 37.01 (C-5), 63.46, 63.57 (C-5′), 70.18, 71.76 (C-2), 64.39, 65.15, 70.64, 70.71, 70.73, 71.11, 71.39, 72.33, 74.46 (C-1′-4′; C-4), 172.78, 174.67 (COOH). Anal. ($C_9H_{17}NO_7S$) C, H, N.

2(*RS*)-[2-^{13}C]-D-*gluco*-(1′,2′,3′,4′,5′-Pentahydroxypentyl)-thiazolidine-4(*R*)-carboxylic Acid ([^{13}C]GlyCys, [^{13}C]-1g). [1-^{13}C]-D-Glc (0.090 g, 0.50 mmol) was dissolved in hot MeOH (40 mL), and L-cysteine (0.67 g, 5.5 mmol) was added. After the mixture was heated under reflux for 2 min, unlabeled D-Glc (0.90 g, 5.0 mmol) was added, followed by pyridine (0.5 mL) 5 min later. Additional MeOH (20 mL) was added after 5 min to aid dissolution, and the solution was heated under reflux for 4 h. White fluffy material began to accumulate after 1 h. After cooling to room temperature, this product was collected to give 1.17 g (75.0% yield) of product: mp 161–164 °C dec; ^{13}C NMR (DMSO-d_6) δ 36.46, 37.01 (C-5), 63.46, 63.57 (C-5′), 70.18, 71.76 (enhanced C-2), 64.39, 65.15, 70.64, 70.71, 70.73, 71.11, 71.39, 72.33, 74.46 (C-1′-4′; C-4), 172.78, 174.67 (*C*OOH).

2(*RS*)-D-*manno*-(1′,2′,3′,4′,5′-Pentahydroxypentyl)thiazolidine-4(*R*)-carboxylic Acid (ManCys, 1h). Weitzel's procedure[4b] was followed to give 1.35 g (95.1% yield) of white product: mp 172–176 °C dec (lit.[4a] mp 171 °C dec, lit.[4d] mp 173.5 °C dec); $[\alpha]^{25}_D$ −64.0° (*c* 0.49, H_2O); IR (KBr) ν 3340 (br, OH, COO^-), 1615 cm^{-1} (COO^-). Anal. ($C_9H_{17}NO_7S$) C, H, N.

D-Ribose diethyl dithioacetal (3) was prepared by combining the procedures of Zinner[9] and Wolfrom and Thompson[8b] as follows. D-Rib (37.5 g, 0.250 mol) was dissolved in concentrated HCl (105 mL) at room temperature. EtSH (37.8 g, 45 mL, 0.610 mol) was added all at once, and the mixture was stirred vigorously for 15 min, with the temperature maintained between 25 and 30 °C by addition of small amounts of ice and cold H_2O to the reaction mixture and by immersion of the reaction flask in an ice bath. Neutralization with Na_2CO_3 caused the entire volume to solidify to a white mass. Two recrystallizations of the product from C_6H_6 (500 mL) yielded 27.3 g (42.5% yield) of white needles: mp 72–73 °C (lit.[9] mp 83.5–84 °C); TLC, R_f 0.28 (EtOAc); IR (KBr) ν 3350 cm^{-1} (br, OH); NMR (MeOH-d_4) δ 1.17 (t, CH_2CH_3), 2.62 (m, CH_2CH_3), 3.23 (m, *H*-5a,5b), 3.40–3.90 (m, *H*-2,3,4), 4.16 (d, *H*-1). Anal. ($C_9H_{20}O_4S_2{\cdot}0.25H_2O$) C, H.

2,3,4,5-Tetra-*O*-acetyl-D-ribose diethyl dithioacetal (4) was prepared as for the Glc analogue (vide infra). The brown oil that formed was repeatedly triturated with H_2O until crystals formed. The product was recrystallized twice from MeOH (150 mL) by adding H_2O to turbidity to give 21.9 g (64.6% yield) of colorless plates: mp 43–44 °C; TLC, R_f 0.76 (EtOAc); IR (KBr) ν 1750 cm^{-1} (CO); NMR ($CDCl_3$) δ 1.22, 1.24 (2 t, CH_2CH_3), 2.03, 2.07, 2.16 (3 s, CH_3CO), 2.63 (m, CH_2CH_3), 3.95 (d, *H*-1), 4.26 (m, *H*-5a,5b), 5.24–5.70 (m, *H*-2,3,4). Anal. ($C_{17}H_{28}O_8S_2$) C, H.

2,3,4,5-Tetra-*O*-acetyl-*aldehydo*-D-ribose (AcRib, 5) was prepared by following the procedure of Wolfrom and Thompson[8b] for the Glc analogue. The precipitated material was collected, giving 1.35 g (68.1% yield) of white needles: mp 93–94 °C; TLC, R_f 0.65 (EtOAc); IR (KBr) ν 1750 cm^{-1} (CO); NMR ($CDCl_3$) δ 2.01, 2.08, 2.10, 2.20 (4 s, CH_3CO), 4.28 (m, *H*-5a,5b), 5.17–5.62 (m, *H*-2,3,4), 9.52 (s, *H*-1). Anal. ($C_{13}H_{18}O_9$) C, H.

2(*RS*)-D-*ribo*-(1′,2′,3′,4′-Tetraacetoxybutyl)thiazolidine-4(*R*)-carboxylic Acid (AcRibCys, 2a). 2,3,4,5-Tetra-*O*-acetyl-*aldehydo*-D-ribose (AcRib, **5**) (0.64 g, 2.0 mmol) was dissolved in boiling H_2O (5 mL) under N_2. L-Cysteine (0.24 g, 2.0 mmol) was then added, and the flask was manually swirled until cool and then refrigerated overnight. The solution was concentrated to approximately 1 mL, and the white amorphous product was isolated in two crops to give 0.48 g (combined, 57.4% yield): $[\alpha]^{25}_D$ −43.3° (*c* 1.02, $CHCl_3$); TLC, R_f 0.41, 0.48 (*n*-BuOH/AcOH/H_2O, 50:11:25); IR (KBr) ν 3445 (NH), 3200 (br, COO^-), 1745 (CO), 1624 cm^{-1} (COO^-); NMR (acetone-d_6) δ 2.01, 2.03, 2.04 (3 s, CH_3CO), 3.01–3.54 (m, *H*-5a,5b), 3.65–4.50 (m, *H*-4,4′a,4′b), 4.63–5.67 (m, *H*-2,1′,2′,3′). Anal. ($C_{16}H_{23}NO_{10}S{\cdot}0.5H_2O$) C, H, N.

D-Galactose Diethyl Dithioacetal (6). The procedure of Zinner[9] for the synthesis of **3**, as modified by Wolfrom and Thompson[8b] for Glc, was followed here. After 15 min of reaction time, the entire reaction mixture solidified to a white mass. After standing in an ice/salt bath for an additional 30 min, the white

(16) The Glyc available commercially is a viscous oil that can only be purchased as an aqueous solution reported to be "70% pure by TLC".

solids were collected, washed with ice cold H_2O, and immediately recrystallized from hot H_2O (1500 mL) to which a small amount of $NaHCO_3$ had been added. The product was collected to give 48.3 g (67.4% yield) of long white needles: mp 135–136 °C (lit.[8a,10] mp 140–142 °C); TLC, R_f 0.68 (*n*-BuOH/AcOH/H_2O, 75:15:10); IR (KBr) ν 3320 cm^{-1} (OH); NMR (MeOH-d_4) δ 1.20, 1.21 (2 t, CH_2CH_3), 2.70 (m, CH_2CH_3), 3.26 (m, *H*-6a,6b), 3.50–4.21 (m, *H*-1,2,3,4,5). Anal. ($C_{10}H_{22}O_5S_2$) C, H.

2,3,4,5,6-Penta-*O*-acetyl-D-galactose Diethyl Dithioacetal (7). The procedure of Wolfrom[8a] was followed on an 80-mmol scale. Colorless crystalline material precipitated out of the reaction mixture. This product was washed with H_2O and recrystallized from MeOH (15 mL) by adding H_2O to turbidity to give 1.59 g (91.4% yield) of white product: mp 56–57 °C (lit.[8a] mp 77.5–78.5 °C); TLC, R_f 0.75 (EtOAc); IR (KBr) ν 1750 cm^{-1} (CO); NMR ($CDCl_3$) δ 1.20, 1.22 (2 t, CH_2CH_3), 2.05, 2.12, 2.15 (3 s, CH_3CO), 2.65 (m, CH_2CH_3), 4.05 (m, *H*-1,6a,6b), 5.10–5.90 (m, *H*-2,3,4,5). Anal. ($C_{20}H_{32}O_{10}S_2$) C, H.

2,3,4,5,6-Penta-*O*-acetyl-*aldehydo*-D-galactose (AcGal, 8). The procedure of Wolfrom[8a] was followed on a 10-mmol scale to give 0.30 g (38.5% yield) of white product: mp 106–110 °C (lit.[8a] mp 107–110 °C); TLC, R_f 0.84 (*n*-BuOH/AcOH/H_2O, 75:15:10), 0.67 (EtOAc), 0.22 (C_6H_6/Et_2O, 3:1); IR (KBr) ν 1750 cm^{-1} (CO); NMR ($CDCl_3$) δ 2.02, 2.10, 2.13, 2.20, (4 s, CH_3CO), 4.10 (m, *H*-6a,6b), 5.21–5.72 (m, *H*-2,3,4,5), 9.25 (s, *H*-1). Anal. ($C_{16}H_{22}O_{11}$) C, H.

2(*RS*)-D-*galacto*-(1′,2′,3′,4′,5′-Pentaacetoxypentyl)thiazolidine-4(*R*)-carboxylic acid (AcGalCys, 2b) was prepared by the method of Bognar et al.[4d] to give 0.39 g (39.4% yield) of the desired product in two combined crops: mp 182–184 °C (lit.[4d] mp 177–179 °C, lit.[4e] mp 179 °C); $[\alpha]^{25}_D$ −6.7° (*c* 0.96, pyridine); TLC, R_f 0.46 (*n*-BuOH/AcOH/H_2O, 75:15:10), 0.57, 0.64 (*n*-BuOH/AcOH/H_2O, 50:11:25); IR (KBr) ν 3460 (NH), 3290 (br, COO^-), 1750 (CO), 1645 cm^{-1} (COO^-); NMR (pyridine-d_5) δ 1.98, 2.08, 2.10, 2.14, 2.17 (5 s, CH_3CO), 3.22 (m, *H*-5a,5b), 3.94 (m, *H*-4), 4.42 (m, *H*-5′a,5′b), 5.05 (d, *H*-2), 5.57–6.12 (m, *H*-1′,2′,3′,4′). Anal. ($C_{19}H_{27}NO_{12}S$) C, H, N.

D-Glucose Diethyl Dithioacetal (9). The synthetic procedure of Wolfrom and Thompson[8b] was followed. The product (long colorless needles) was collected and dried in air to give 41.6 g (58.1% yield): mp 123–124 °C (lit.[8b] mp 127 °C, lit.[10] mp 127–128 °C); TLC, R_f 0.65 (*n*-BuOH/AcOH/H_2O, 75:15:10); IR (KBr) ν 3320 cm^{-1} (br, OH); NMR (D_2O) δ 1.20, 1.22 (2 t, CH_2CH_3), 2.66 (m, CH_2CH_3), 3.71 (m, *H*-6a,6b), 3.90–4.34 (m, *H*-1,2,3,4,5). Anal. ($C_{10}H_{22}O_5S_2$) C, H.

2,3,4,5,6-Penta-*O*-acetyl-D-glucose Diethyl Dithioacetal (10). The method of Wolfrom and Thompson[8b] was followed except that the crude product was isolated as a white syrup rather than as crystals. This was washed several times by triturating with H_2O and decanting the supernatant. The residue was then covered with H_2O and allowed to stand at 4 °C. Since crystallization did not take place, the material was dried in vacuo to give 1.12 g (64.4% yield) of a thick white gum (lit.[8b] mp 45–47 °C): TLC, R_f 0.80 (EtOAc); IR (neat) ν 1750 cm^{-1} (CO); NMR ($CDCl_3$) δ 1.25 (m, CH_2CH_3), 2.04, 2.10, 2.12 (3 s, CH_3CO), 2.60 (m, CH_2CH_3), 4.10 (m, *H*-6a,6b), 4.15 (d, *H*-1), 4.90–5.85 (m, *H*-2,3,4,5). Anal. ($C_{20}H_{32}O_{10}S_2$) C, H.

2,3,4,5,6-Penta-*O*-acetyl-*aldehydo*-D-glucose (AcGlc, 11). The procedure of Wolfrom and Thompson[8b] was followed on a 20-mmol scale, giving 0.97 g (62.2% yield) of white product: mp 116–117 °C (lit.[8b] mp 119.5–120.5 °C); TLC, R_f 0.60 (EtOAc); IR (KBr) ν 1750 cm^{-1} (CO); NMR ($CDCl_3$) δ 2.10, 2.12, 2.20 (3 s, CH_3CO), 4.15 (m, *H*-6a,6b), 4.96–5.70 (m, *H*-2,3,4,5), 9.52 (s, *H*-1). Anal. ($C_{16}H_{22}O_{11}$) C, H.

2(*RS*)-D-*gluco*-(1′,2′,3′,4′,5′-Pentaacetoxypentyl)thiazolidine-4(*R*)-carboxylic Acid (AcGlcCys, 2c). The procedure for **2a** was followed by using the glucose analogue **11** to give 0.85 g (85.9% yield) of **2c** as a white amorphous solid in two crops (combined): $[\alpha]^{25}_D$ −29.3° (*c* 1.07, $CHCl_3$); TLC, R_f 0.45, 0.57 (*n*-BuOH/AcOH/H_2O, 50:11:25), 0.64, 0.68 (*n*-BuOH/AcOH/H_2O, 75:15:10); IR (KBr) ν 3470 (NH), 3150 (br, COO^-), 1745 (CO), 1635 cm^{-1} (COO^-); NMR (acetone-d_6) δ 2.01, 2.03, 2.05, 2.06, 2.08 (5 s, CH_3CO), 3.14 (m, *H*-5a,5b), 3.87 (m, *H*-4), 4.00–4.37 (m, *H*-5′a,5′b), 4.78 (d, *H*-2, 4.93–5.59 (m, *H*-1′,2′,3′,4′). Anal. ($C_{19}H_{27}NO_{12}S$) C, H, N.

Stimulation of Glutathione Biosynthesis in Isolated Rat Hepatocytes by L-Cysteine Prodrugs and Inhibition by Buthionine Sulfoximine (BSO). Rat hepatocytes were isolated by following the methods of Seglen.[11] After final plating, the hepatocytes were maintained in culture for 24 h prior to use. Only primary cultures were used throughout the studies. The hepatocytes were incubated with the cysteine prodrugs for a 4-h period, and after removal of media by aspiration, the cells were rinsed with cold phosphate-buffered saline and deproteinized with 5% sulfosalicylic acid. Total GSH content (GSH + GSSG) was determined by a modification of the DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] glutathione reductase recycling method.[17] The GSH concentration in the sample was quantified by determining the cycling rate (ΔOD at 412 nm/min) of the sample. For the inhibition studies with BSO, the cells were preexposed to BSO (0.20 mM) before treatment with the L-cysteine prodrugs.

Protection of Mice against Acetaminophen-Induced Hepatotoxicity. The prodrugs of L-cysteine were examined for their ability to protect against acetaminophen-induced hepatotoxicity in mice essentially as described previously.[1] Male, Swiss-Webster mice (19–31 g) were fasted overnight but given free access to water. An LD_{90} dose of acetaminophen (750 mg/kg, 4.97 mmol/kg, ip) was then administered, followed 30 min later by a 2.45 mmol/kg ip dose of the L-cysteine prodrug. All injection solutions were freshly prepared in sterile water, except where solubility problems required the use of sterile 0.1 N aqueous $NaHCO_3$ or a slurry of the prodrug in 2% aqueous (carboxymethyl)cellulose (CMC). The animals were then given food and water ad libitum and were observed over a 48-h period.

Representative sections from the central lobe of the liver were obtained from those animals already found dead when checked at 12, 24, and 48 h. Survivors at 48 h were sacrificed by cervical dislocation, and the liver sections were similarly excised and sectioned. All specimens were fixed in 10% buffered formalin or in Form-a-Less and stained with hematoxylin and eosin. The extent of hepatic necrosis was evaluated histologically by one author (R.T.Z.), who had no knowledge of the experimental protocols or sample identity.

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Supplementary Material Available: Figures 1 and 3, ^{13}C NMR spectra of GlcCys (**1g**) and GlcCys enriched with ^{13}C at C-2 ([^{13}C]-**1g**), respectively (2 pages). Ordering information is given on any current masthead page.

(17) Tietze, F. *Anal. Biochem.* **1969**, *27*, 502.